MECHANISTIC STUDIES OF TWO STEROIDOGENIC CYTOCHROMES P450, CYP17A1 AND CYP21A2

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DEDICATION

Graduate school has been a wild adventure for me. I traveled to three different states, three different graduate schools and had three different PhD advisors.

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MECHANISTIC STUDIES OF TWO STEROIDOGENIC CYTOCHROMES P450, CYP17A1 AND CYP21A2

by

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Human CYP17A1 (P450c17 or 17α-hydroxylase/17,20-lyase) is a member of the P450 superfamily of proteins. This enzyme is responsible for hydroxylating the 17-position of pregnenolone or progesterone and cleaving the 17,20-carbon,carbon-bond of its hydroxylated products, which leads to the formation of androgens. The latter activity makes the inhibition of this enzyme a target for the treatment of prostate cancer. Human CYP21A2 (P450c21 or 21-hydroxylase) hydroxylates the 21-position of progesterone and 17-

hydroxyprogesterone – deficiency in this enzyme leads to congenital adrenal hyperplasia.

Intermolecular and intramolecular kinetic isotope effects were determined for both enzymes, which required the synthesis of regioselectively deuterated steroids: 17α-[^{2}H]-progesterone, 17α-[^{2}H]-pregnenolone, 21,21,21-[$^{2}H_{3}$]-progesterone, 21-[^{2}H]-progesterone, 16α-[^{2}H]-progesterone and 21,21,21-[$^{2}H_{3}$]-17-hydroxyprogesterone. Based on the calculated isotope effects the C-H abstraction step was determined to be partially rate-limiting in the overall hydroxylation process. Moreover, novel 21-hydroxylase activity and 16-hydroxylase activity on progesterone was observed for CYP17A1 and CYP21A2, respectively. CYP17A1 catalyzed hydroxylation of progesterone on the 21:17:16 positions in a 3.4:76.3:20.3 ratio, and this ratio changed to 5.5:87.4:7.1 when 16α-[^{2}H]-progesterone was used as the substrate. Meanwhile, CYP21A2 hydroxylated the 21:16 positions in a 99.6:0.4 ratio and this ratio changed to 94.3:5.7 when 21,21,21-[$^{2}H_{3}$]-progesterone was used as the substrate. Kinetic isotope effects with 17α-[^{3}H]-pregnenolone were calculated for CYP17A1.

Olefinated progesterone analogs were synthesized to probe possible epoxidase activity of these steroidogenic enzymes. CYP17A1 hydroxylated the 21-position and epoxidized the 16α,17α-position of 16,17-dehydroprogesterone while CYP21A2 was found to only hydroxylate the 21-position of the same

substrate. Both CYP17A1 and CYP21A2 reduced the 21,22-alkene of 21-homo-21,22-dehydroprogesterone and further hydroxylated the metabolite.

Other potential substrates such as cyclopropyl and halogenated progesterone analogs were also synthesized to study the enzymatic reactivity towards these substrates. CYP17A1 was found to metabolize 17β-cyclopropylmethylandrostenone and CYP21A2 was found to metabolize 17-fluoroprogesterone. Both enzymes were found to metabolize 20-desoxoprogesterone – each enzyme yielded a different product with this substrate. Exploring the mechanistic behavior of these enzymes towards various steroid analogs enhances our understanding of their reactivities and structural properties. This research ultimately provides insights on more detailed roles of these enzymes in human disease and may help us to design better inhibitors.

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activities and transition state properties of the human steroid hydroxylases cytochromes P450c17 and P450c21from reactions occurred with isotopically-labeled substrates."

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3β-HSD:	3beta-hydroxysteroid dehydrogenase
17β-HSD:	17βeta-hydroxysteroid dehydrogenase
AN:	Androsterone
AR:	Androgen receptor
β-ram:	Beta-Radioactive monitor
CAH:	Congenital adrenal hyperplasia
CYP:	Cytochrome P450
CYP17A1:	17α-hydroxylase or 17,20-lyase
CYP21A2:	21-hydroxylase
DBU:	1,8-Diazabicyclo[5.4.0]undec-7-ene
DHEA:	Dehydroepiandrosterone
DHT:	Dihydrotestosterone
DIBAL:	Diisobutylaluminum hydride
DIPA:	Diisopropylamine
DMP:	Dess-Martin periodinane
DOC:	11-Deoxycorticosterone
GR:	Glucocorticoid receptor
HPLC:	High performance liquid chromatography
Hz:	Hertz
IC_{50}:	Half maximal inhibitory concentration

K:	See K_M
k_{cat}:	Overall enzymatic catalytic rate
k_D:	Reaction rate for the deuterium isotope
k_H:	Reaction rate for the hydrogen isotope
KIE:	Kinetic isotope effect
K_M:	Michaelis constant (substrate concentration where reaction rate is half of V_{max})
LC/MS:	Liquid chromatography/mass spectrometry
MR:	Mineralocorticoid receptor
NADPH:	Nicotinamide adenine dinucleotide phosphate reduced
NBS:	N-bromosuccinimide
NCS:	N-chlorosuccinimide
NMR:	Nuclear magnetic resonance
NTD:	N-terminal domain
POR:	P450 oxidoreductase
PR:	Progesterone receptor
Preg:	Pregnenolone
Prog:	Progesterone
RT:	Room temperature
S:	Substrate concentration
SAR:	Structure activity relationship

SGRM:	Selective glucocorticoid receptor modulator
SULT2A1:	Sulfotransferase family, cytosolic, 2A, dehydroepiandrosterone preferring, member 1
TBDMS:	Tert-butyldimethylsilyl
THF:	Tetrahydrofuran
TLC:	Thin layer chromatography
UV:	Ultra-violet
UV/Vis:	Ultra-violet/visual
V:	Velocity (reaction rate)
WT:	Wild-type

CHAPTER ONE
Introduction

1.1. HUMAN STEROIDOGENESIS

1.1.1. The Chemistry of Human Steroid Biosynthesis

Steroid biosynthesis has three major types of enzymes: hydroxysteroid dehydrogenases (HSDs), 5α-reductases (3-oxo-5α-steroid 4-dehydrogenase) and cytochromes P450 [1]. HSDs are oxidoreductases, which catalyze the oxidation and reduction of steroidal alcohols and ketones using nicotinamide adenine dinucleotide cosubstrate (NAD[P][H]) as redox cofactors. Cytochromes P450 (CYPs) catalyze the hydroxylation of C-H bonds and cleave carbon-carbon bonds in the steroid biosynthesis pathway, and human steroidogenesis incorporates 6 CYP enzymes. The pathway begins with cleavage of the cholesterol side chain by CYP11A1 to yield pregnenolone, the first 21-carbon steroid found in the pathway. Pregnenolone can undergo either (1) hydroxylation at the 17-carbon by CYP17A1 to yield 17-hydroxypregnenolone or (2) transformation by 3β-hydroxysteroid dehydrogenase/isomerase (3βHSD) via oxidation of the 3-hydroxy group and isomerization of the $\Delta^{5,6}$-olefin to the $\Delta^{4,5}$-position to yield progesterone. This first branchpoint is followed by additional nodes, where committed pathways to the various classes of steroid hormones emerge, depending on the presence of

specific enzymes required to make each type of steroid. There are classical and "backdoor" pathways in humans, the latter is relevant for the alternative formation of dihydrotestosterone through direct reduction of the $\Delta^{4,5}$-olefin of 17-hydroxyprogesterone by 5α-reductase to form 17-hydroxydihydroprogesterone, which in turn yields 5α-reduced androsterone directly [2, 3]. CYP17A1 (steroid 17-hydroxylase/17,20-lyase, P450c17) and CYP21A2 (steroid 21-hydroxylase, P450c21) are both found near the beginning of steroidogenesis, and their activities are critical determinants of the types of steroid hormones produced by a given cell. This thesis is a compilation of our mechanistic studies of the reactions catalyzed by the human steroid hydroxylases CYP17A1 and CYP21A2, using techniques based in synthetic organic chemistry and physical biochemistry.

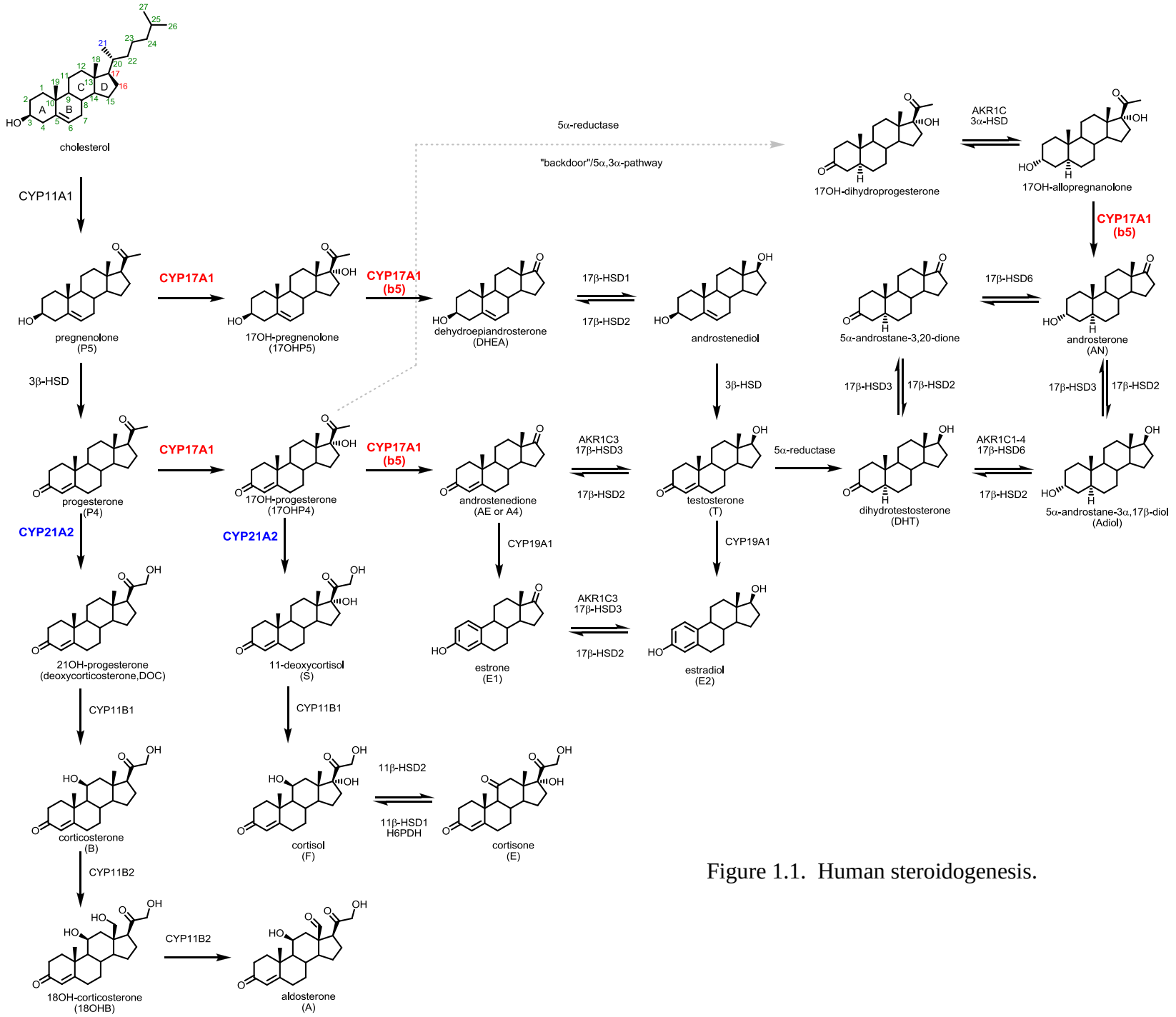


Figure 1.1. Human steroidogenesis.

1.1.2. Steroid Hormone Classes and Signaling

Steroid hormones are classified by their biologic activities, which are in turn determined by their structural features. The five fundamental classes of steroid hormones include:

1. Progestins: Progestins are 21-carbon steroids, whose function is to prepare the uterus for implantation and to support pregnancy. Progesterone, the major endogenous progestin in human beings, rises markedly during the luteal phase of the menstrual cycle and more dramatically during pregnancy. A variety of synthetic progestins are found commonly in oral contraceptives.

2. Mineralocorticoids: Mineralocorticoids are 21-carbon steroids, generally hydroxylated on the 21-position, which promote salt and water retention and potassium excretion. Aldosterone is the endogenous mineralocorticoid, which is regulated by the renin-angiotensin system of the kidney. Primary aldosteronism, in which excess aldosterone is produced despite suppressed renin, accounts for up to 8% of hypertension. Besides aldosterone, 11-deoxycorticosterone (DOC), corticosterone, and cortisol are all endogenous mineralocorticoids. Fludrocortisone is a synthetic mineralocorticoid with minor glucocorticoid properties, and spironolactone and eplerenone are mineralocorticoid antagonists used to treat hypertension and heart failure.

3. Glucocorticoids: Glucocorticoids are 21-carbon steroids hydroxylated on the 11- and 21-positions, which stimulate gluconeogenesis and glycolysis, suppress the immune system, and orchestrate the acute stress response. Cortisol is the major endogenous glucocorticoid in human beings, while rodent adrenals produce corticosterone instead. Many synthetic glucocorticoids are used for their anti-inflammatory properties. Cortisol excess is known as the Cushing syndrome, and mifepristone, which is an effective glucocorticoid antagonist at high doses, has been recently approved by the FDA for treatment of Cushing syndrome.

4. Androgens: Androgens are 19-carbon steroids responsible for male sexual differentiation and reproductive function. Testosterone and dihydrotestosterone are the endogenous androgens with distinct biological functions, such that only dihydrotestosterone enables formation of the male external genitalia and prostate formation in fetal life. Prostate cancer and hyperplasia are common disorders of adult males, and various treatments are used to block androgen action in the treatment of these disorders (see below).

5. Estrogens: Estrogens are 18-carbon steroids produced by the activity of CYP19A1 (aromatase), whose crystal structure has recently been reported [4]. Estrogens are responsible for female secondary sexual characteristics, accrual of

bone mass, female reproduction, and multiple other biologic activities. Synthetic and equine estrogens are commonly found in oral contraceptive pills and postmenopausal hormone replacement regimens, respectively. Breast cancer, which afflicts 1 in 9 women is often estrogen-dependent and treated with the anti-estrogens tamoxifen and fulvestrant or with aromatase inhibitors (anastrozole, letrozole, exemestane).

Steroid hormones primarily signal by acting as ligands to their cognate protein receptors, and nuclear hormone signaling has been the major thrust of steroid research for the past 2-3 decades. Receptors related to steroids include: the progesterone receptor (existing in A and B forms), the mineralocorticoid receptor, the glucocorticoid receptor (one gene, α and β forms), the androgen receptor, and estrogen receptors (α and β, two genes). Steroid hormone receptors are transcription factors and have implications in cancer [5], stress response, fertility [6] and salt/blood volume homeostasis. Steroid hormone receptors possess a zinc finger DNA binding domain (DBD) and a less conserved ligand binding domain (LBD) on the carboxy terminus and a divergent amino terminus (N-terminal domain, NTD) [7]. Liganded receptors interact with co-activator or co-repressor proteins at hormone response elements (HREs, direct or inverted repeat sequences) on DNA to recruit the basic transcriptional machinery, which alters expression of target genes. Although these proteins are transcription

factors, there are non-genomic signaling activities associated with some steroid receptors and with ion channels (i.e.: corticosteroid signaling with MR and GR in the brain) [8].

1.2. CYP17A1 AND CYP21A2 IN HUMAN BIOLOGY AND DISEASE

CYP17A1 and CYP21A2 are both found in the adrenal glands where both are required for cortisol biosynthesis, but only CYP17A1 is expressed in the gonad (Figure 1.2). Both enzymes are microsomal CYPs, meaning they are located in the smooth endoplasmic reticulum and receive electrons via the flavoprotein P450-oxidoreductase (POR). In addition, cytochrome b_5 (b5), a small membrane-bound hemoprotein, stimulates the 17,20-lyase activity of CYP17A1 but not other activities of either enzyme. Because of their critical positioning in the steroidogenic pathways, both enzymes are highly relevant to human biology and disease, as is briefly reviewed below.

1.2.1. CYP17A1: Combined 17α-Hydroxylase/17,20-Lyase Deficiency

Amazingly, a single amino acid residue mutation in CYP17A1 can drastically change the activity of the enzyme and in turn can affect the phenotype of the individual. Various types of mutations in the gene have been reported to appear in homozygous or more commonly compound heterozygous forms. In addition to missense and nonsense mutations, intronic mutations that disrupt splicing of the *CYP17A1* gene have also been reported [9]. Because CYP17A1 is

required for androgen and estrogen synthesis and for the removal of steroids from the mineralocorticoid biosynthesis pathway, the phenotype of severe CYP17A1 deficiency is sexual infantilism with mineralocorticoid excess. If untreated, the deficiency in CYP17A1 activity (17-hydroxylase deficiency, 17OHD) leads to the rise in 17-deoxysteroids (DOC, 18-hydroxyDOC, corticosterone, 18-hydroxycorticosterone), which results in hypertension, hypokalemia, and renin and aldosterone suppression [10]. Both genetic boys and girls (46,XY and 46,XX, respectively) are raised as females and given estrogen replacement at the time of expected puberty to induce secondary sexual characteristics. The hypertension is managed with mineralocorticoid antagonist (spironolactone) and/or glucocorticoid replacement, the latter to lower DOC production. Incomplete deficiency can give a milder phenotype, but normal sexual development and fertility still do not occur. Examples of *CYP17A1* mutations are given in Table 1.1.

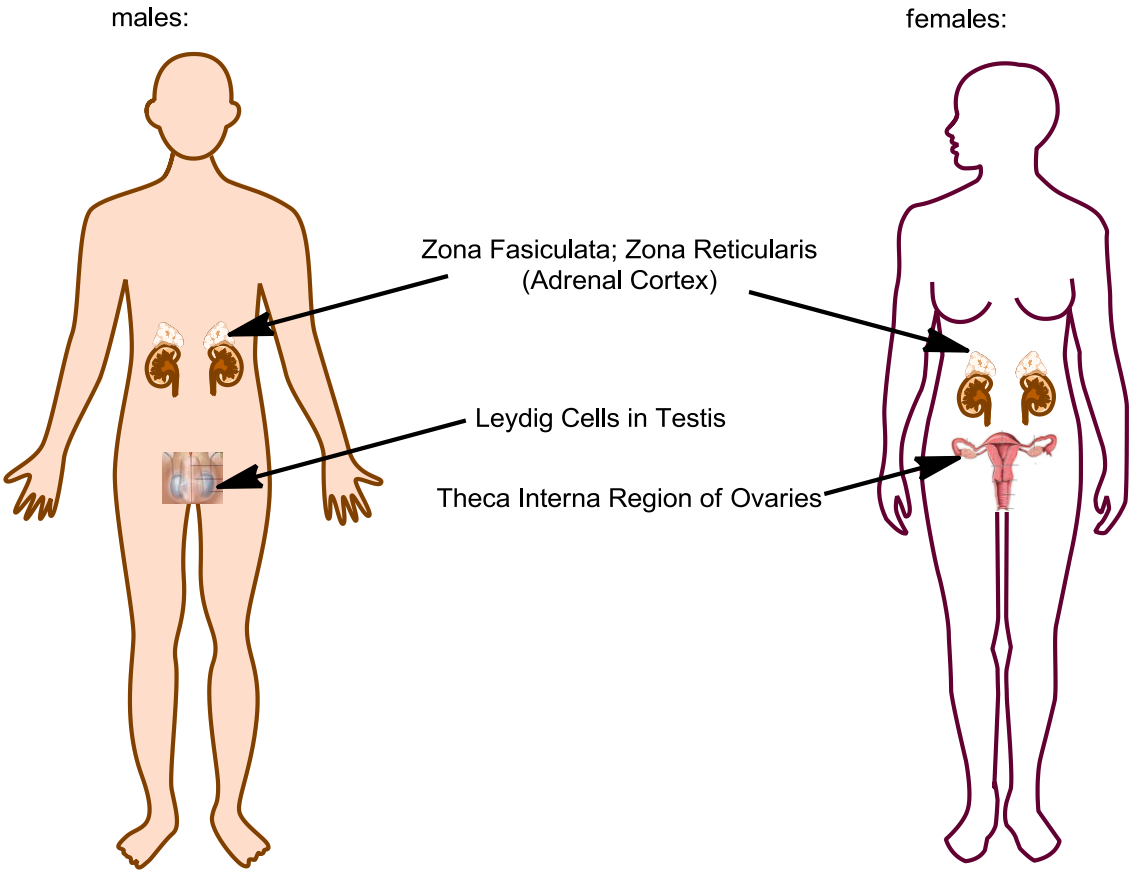


Figure 1.2. Location of CYP17A1 (adrenal cortex, testis, and ovaries) and CYP21A2 (adrenal cortex) in the human body.

Case	Mutation (DNA)	Mutation (Protein)	Result	Reference
1	g.417 C>A (het)	A82D	17OHD	[11]
1	g.4869 T>A, 4871del (het)	Y329fs		[11]
2	g. 2045 C>T (het)	R125X	17OHD	[11]
2	g. 6457 T>C (het)	C442R		[11]
3	g. TAC329AA	Y329K	partial 17OHD	[12]
3	-	A398V		[12]
4	C327dupT	premat. stop codon	17OHD	[13]
4	C362G 1 A	W121X (prem. SC)		[13]
5	CGG > TGG	R96W	17OHD	[14, 15]
6	c.1388T>C	W406R	17OHD	[16]
7	c.1256C>T	R362C		[16]
8	(TCA→TAA) in exon 5	S288X	17OHD	[17]
9	exon 2	R125Q	17OHD	[18]
9	exon 8	R416H		[18]
10	exon 4 c.775C>T (het)	R239X	17OHD	[19]
10	exon 6 C>A (het)	Pro342Thre		[19]
11	exon 1 458C>T (het)	R96W	17OHD	[20]
11	exon 6 5001C>G (het)	H373D		[20]
12	exon 1 TAC>TAA (homo)	Y27X	17OHD	[21]
13	exon 4 CTT>CT: 247delT (het)	frameshift	17OHD	[22]
13	exon 6 CAC>CTC (het)	H373L		[22]

Table 1.1. *CYP17A1* mutations causing combined 17-hydroxylase/17,20-lyase deficiency.

1.2.2. CYP17A1: Male Pseudohermaphroditism 46,XY Disorder of Sex Development = 46,XY DSD) from Isolated 17,20-Lyase Deficiency

Since CYP17A1 catalyzes both the 17-hydroxylase and 17,20-lyase reactions, it is theoretically possible that point mutations might preferentially disrupt one of these activities. Despite clinical reports of patients with biochemical evidence of isolated 17,20-lyase deficiency, the genetic basis was not defined until 1997. In this study, a set of two patients were mis-assigned to the female gender at birth, and found to have isolated 17,20-lyase deficiency without 17α-hydroxylase deficiency [23]. Interestingly, both patients had homozygous mutations involving arginine residues – one patient had R347H and the other patient had R358Q. When the vectors encoding for these mutant CYP17A1 cDNAs were transiently transfected into COS-1 cells, these mutations were both found to retain 65% 17α-hydroxylase activity but <5% 17,20-lyase activity relative to the wild type. These mutations map not to the active site but rather to the surface where CYP17A1 interacts with electron transfer proteins [24]. Subsequently, another report described a kindred with the E305G mutation in CYP17A1, which led to isolated 17,20-lyase deficiency by impairing both 17-hydroxypregnenolone binding and turnover [25]. Site-directed mutations have also been used to probe key residues for 17,20-lyase activity. Lee-Robichaud and colleagues showed that R449A leads to disrupted 17,20-lyase activity with preserved 17-hydroxylation [26].

Alternatively, isolated 17,20-lyase deficiency can occur when there is a mutation on the genes encoding either POR or b5, which are both particularly critical for this activity. POR mutation G539R in homozygous form appears clinically as isolated 17,20-lyase deficiency, because that activity is much more impaired than either 17- or 21-hydroxylase activities. A homozygous W27X mutation in the *CYB5* gene leads to a truncation of the b5 protein and causes isolated 17,20-lyase deficiency [27]. Similarly, homozygous mutation H44L, which eliminates one of the heme-liganding histidines of b5, causes isolated 17,20-lyase deficiency.

1.2.3. CYP17A1 and Prostate Cancer

Recognizing that prostate formation and growth requires dihydrotestosterone, adrogen deprivation therapy (ADT) has been used for decades to treat prostate cancer [28], originally by surgical orchiectomy. Estrogens, progestins, and later long-acting gonadotropin-releasing hormone (GnRH) agonists were then employed to suppress testicular testosterone synthesis pharmacologically, but this approach neglects the adrenal contribution to 19-carbon steroids in the circulation, which are accessible to the prostate cancer cells. Androgen receptor antagonists (flutamide, bicalutamide, and nilutimide) have been used when ADT fails and the disease reaches the “castration-resistant” state (CRPC), but responses to these drugs are seldom adequate. Because all androgen

production requires the 17,20-lyase activity of CYP17A1, this activity has become a target to treat prostate cancer. Ketoconazole, which inhibits CYP17A1 at doses higher than used to treat fungal infections, has been used off-label to treat prostate cancer upon progression during ADT, but liver and gastrointestinal toxicities limits its use. Abiraterone acetate, a potent and specific CYP17A1 inhibitor (K_i ~4 nM) has recently emerged as an agent effective in treating CRPC in combination with prednisone, which prevents the hypertension and hypokalemia from acquired 17-hydroxylase deficiency. Nevertheless, abiraterone resistance has been found to occur in some CRPC patients, and Montgomery and co-workers suggested that this resistance is due both to upregulation of CYP17A1 and expression of androgen receptor variants that are activated independent of ligand binding in the prostate cancer cells themselves [29]. Whether abiraterone primarily acts by inhibiting adrenal CYP17A1 or intratumoral CYP17A1 is controversial. For example, Jeong et. al have shown that neither CYP17A1 protein nor RNA is detectable in the prostate cancer cell lines they tested [30].

1.2.4. CYP21A2: Congenital Adrenal Hyperplasia

Over 90% of cases of congenital adrenal hyperplasia (CAH) are due to 21-hydroxylase deficiency (21OHD) from mutations in the *CYP21A2* gene, which arise primarily from gene conversion with the adjacent *CYP21A1P* pseudogene [31] (Table 1.2). There are different forms of 21OHD: Non-classical (NCAH)

and classical, the latter of which is associated with salt-wasting (SW) or without salt-wasting, called simple virilizing (SV) 21OHD. The SW form is due to complete loss of 21-hydroxylase activity, which eliminates all detectable mineralocorticoid and glucocorticoid production (deletions, cluster mutations), whereas mutations causing the SV form, most commonly I172N, retain a trace of activity and allow minimal aldosterone production [32, 33], which prevents salt wasting in well newborns but not during significant illness. While classical 21OHD occurs in 1:16,000 births worldwide, NCAH is ten or more times more common and is particularly prevalent in Ashkenazi Jews and Yupik Eskimos. NCAH presents in girls and young women as androgen excess, oligomenorrhea, and infertility, but many women and most males with NCAH are never ascertained.

Boys born with classical 21OHD appear normal at birth but develop failure to thrive and salt-wasting crisis during the first two weeks of life or at the first significant illness. Girls with classical 21OHD are generally ascertained at birth, because they are affected with the most common form of 46,XX DSD. The block at 21-hydroxylation not only prevents mineralocorticoid and glucocorticoid synthesis, but the only pathway available to upstream steroids is CYP17A1-catalyzed androgen synthesis. Consequently, excess androgens are formed during the window of time when the external genitalia form in the fetus, and the girls are born virilized to varying degrees. Dexamethasone has been used to treat 21OHD

in affected fetuses [34] to prevent genital virilization in females [35], but this treatment is considered experimental. Nevertheless, treatment of 21OHD involves replacing both glucocorticoid (typically hydrocortisone = cortisol, in divided doses) and mineralocorticoid (with fludrocortisone), to both replace the deficiencies and to prevent androgen excess and the premature growth and physical changes induced by androgens. Unfortunately, in order to adequately suppress androgen production, supraphysiologic glucocorticoid doses are required, which causes iatrogenic Cushing syndrome and long-term health consequences.

CYP21A2 genetic mutations

***CYP21A1 De Novo* Mutations**

Case	Mutation (DNA)	Mutation (Prot)	Result	Ref.
1	intron 2 g.868A/C>G (het)		21OHD	[36]
1	exon 7 g. 1901C>A (het)	H282N		[36]
2	exon 7 (het)	V281L	21OHD	[36]
2	exon 5 g.1358T>C (het)	Y191H		[36]
3	intron 9 IVS9-9C>A		21 OHD classic CAH	[37]
4		T295N	5% activity 17OHP	[33]
			0.8% activity P	[33]
5		C147R	4% activity	[33]
6	exon 1 (codon 38)	H38L		[38]
6	c.290-13A/C>G			[38]
7	g.1004T>A	I172N	1% activity (SV)	[39]

***CYP21A1P* Pseudogene Mutations [40]**

Case	Mutation (DNA)	Mutation (Prot)	Result	Ref.
1	89C>T	P30L	60%/30%	[41]
2	655A/C>G	Splicing defect	Weak activity	[42, 43]
3	707_714delGAGACTAC	G110Frameshift		[44]
4	999T>A	I172N	<2%	[45]
5	1380T>A	I236N	1%/2.4%	[44, 46]
6	1383T>A	V237E	0%/0.1%	[44, 46]
7	1389T>A	M239K	95%/98%	[44, 46]
8	1683G>T	V281L	50%/20%	[47, 48]
9	1762_1763insT	L307Frameshift		[44]
10	1994C>T	Q318Stop		[49]
11	2108C>T	R356W	No activity	[50]
12	2578C>T	P453S	50-68%/20-46%	[51-53]

Table 1.2. Reported CYP21A2 mutations that lead to 21OHD.

1.3. CYTOCHROMES P450

Cytochromes P450 are enzymes, which contain prosthetic heme groups, found in plants, mammals, bacteria, fungi, protists and viruses. These enzymes use molecular oxygen and electrons from NADPH to catalyze monoxygenation reaction, most characteristically to hydroxylate alkanes into corresponding alcohols:

$$R\text{-}H + O_2 + 2e^- + 2H^+ \rightarrow R\text{-}OH + H_2O$$

Humans have a total of 57 genes that encode P450 enzymes – 7 of which are found in mitochondria [54], and 50 are located in the endoplasmic reticulum [55]; moreover, the human genome contains 46 pseudogenes [56]. Prokaryotic cytochromes P450 are soluble [57], and eukaryotic P450s are all membrane bound. The functional eukaryotic cytochrome P450 enzyme system is comprised of multiple proteins. Mitochondrial P450s require the flavoprotein ferredoxin reductase and the iron-sulfur protein ferredoxin to shuttle electrons from NADPH to the P450 enzyme. Microsomal (endoplasmic reticulum) P450s receive electrons from POR, which contains two flavins, and b5 participates in some of these reactions, either as an electron transfer protein or an allosteric regulator. Nuclear magnetic resonance (NMR), computational modeling, X-ray crystallography, substrate analogs, site-directed mutagenesis, CO flash photolysis [58], and mass spectrometry have been used to study the structures of cytochromes P450 [59].

Recently the crystal structures of CYP17A1 [60] and CYP21A2 [61] have been reported. CYP17A1 structure had the known inhibitor, abiraterone, bound and CYP21A2 structure had two 17α-hydroxyprogesterone substrates bound.

1.3.1. Structural Features of Cytochromes P450

Poulos and co-workers revealed the first crystal structure of a cytochrome P450 (P450-cam or CYP101A1) bound to its substrate, camphor [62]. The overall structure of P450 enzymes resembles a triangular prism, with an alpha-helical domain containing a four-helix bundle and a beta-sheet domain. Deisenhoefer and co-workers compared the crystal structures of $P450_{cam}$ (CYP101), $P450_{terp}$ and $P450_{BM-3}$ and observed structural similarities in the three different proteins: 13 α-helices (A, B, B', and C-L) and 5 β-sheets (β1-β5) [63]. The active site of cytochrome P450 contains a prosthetic heme group, with an iron center coordinated with protoporyphrin IX and a cysteine sulfur axial ligand. This cysteine residue is the only residue that is absolutely conserved among all P450s. The K-helix contains an EXXR motif that is generally conserved in P450s. The glutamic acid (E) and arginine (R) residues seem to interact with a histidine, arginine or asparagine in the meander region to form a salt bridge interaction and contribute to the tertiary structure of these enzymes [64]. Mutation of this R (R362C) in CYP17A1 is a common cause of 17OHD in Brazil.

1.3.2. Mechanism of C-H Abstraction (i.e.: Catalytic Cycle of Cytochrome P450)

In the resting state of a cytochrome P450 enzyme, the porphyrin ligand has a -2 charge, and the axial cysteine side chain has a -1 charge to give a net +3 oxidation state on the iron center. The first step of the reaction cycle of P450s involves the substrate binding, which lowers the redox potential and/or changes the conformation of the enzyme. Substrate binding typically transitions the iron from low-spin to high-spin state, which can be monitored as a shift in the visible spectrum [65]. A single electron reduction of the iron, derived from NAD(P)H and transferred by a redox partner protein, leads to a one-electron reduction of the iron and results in a Fe^{+2} center [66], which binds one molecule of iron. A second electron reduces the iron-porphyrin center of the oxyferrous P450, and for most P450s, the weight of the evidence suggest that the O-O bond is broken after a protonation step, to form water and a species best described as an iron oxene with unpaired electron character (compound I). The first chemical step involving substrate next occurs, with hydrogen atom abstraction from a C-H bond, forming a carbon-based substrate radical and the equivalent of an iron-bound hydroxyl radical. Radical recombination described as “oxygen rebound” follows to form the alcohol product. At the end of the reaction, the product is released from the enzyme to restore the resting state of the enzyme and the starting point of the

cycle. The work in this thesis is particularly focused on studying the C-H bond-breaking step in the reactions catalyzed by human CYP17A1 and CYP21A2.

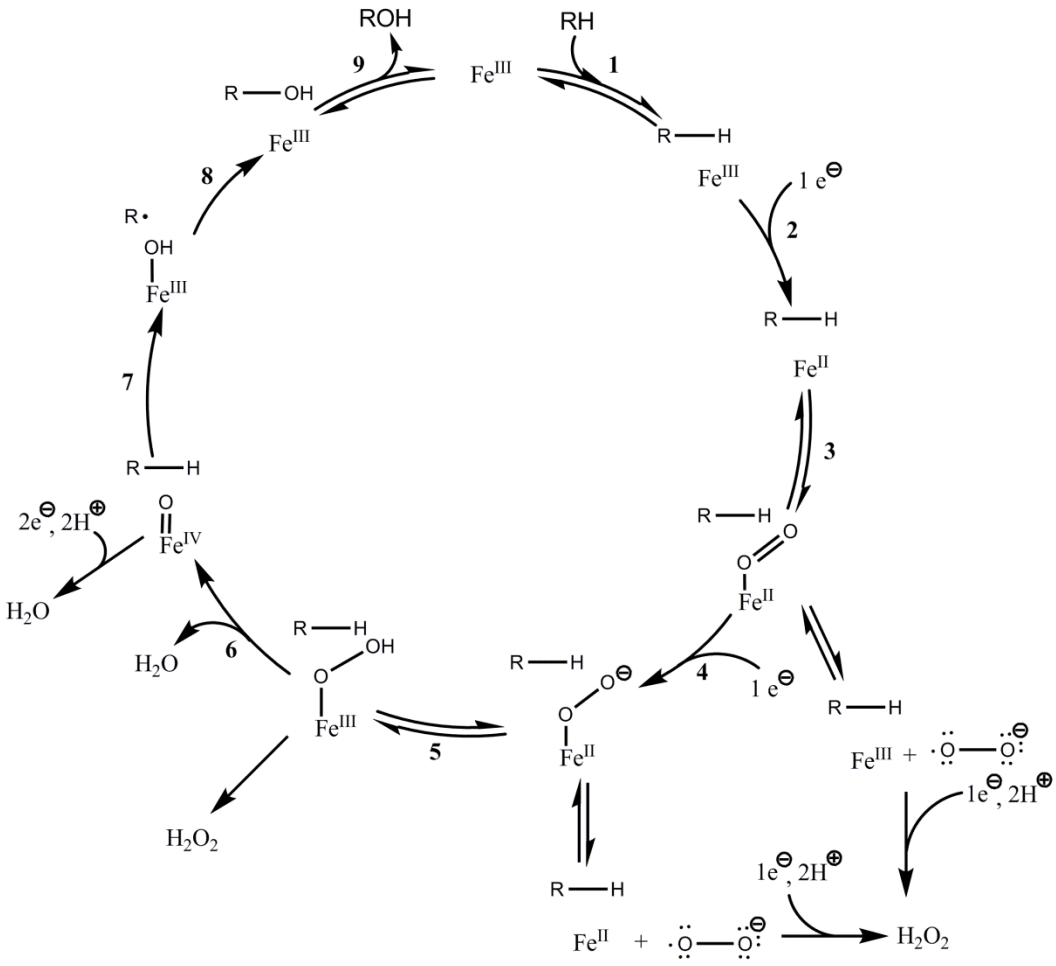


Figure 1.3. P450 catalytic cycle.

1.3.3. CYP17A1 and CYP21A2 as Cytochrome P450 Enzymes

Human CYP17A1 (steroid 17-hydroxylase/17,20-lyase) is a P450 enzyme containing 508 amino acids, which is found in gonads and adrenal glands [67].

Hall and colleagues originally purified CYP17A1 from neonatal porcine testes while Kominami and co-workers isolated CYP17A1 from guinea pig adrenals soon afterwards [68]. This enzyme is responsible for introducing a hydroxyl group on the 17α-position of pregnenolone and progesterone, and human CYP17A1 performs the 17,20-lyase reaction ~50 times more efficiently with 17-hydroxypregnenolone than with 17-hydroxyprogesterone. In addition to hydroxylating the 17α-position of progesterone, human CYP17A1 also hydroxylates the 16α-position 25-35% of the time [69]. Swart et al. aligned several mammalian CYP17A1 isoenzymes with variable 16α-hydroxylase activities and, through site directed mutagenesis, showed that the alanine 105 residue is responsible for the 16α-hydroxylase activity [69, 70]. This group showed that the A105L mutation increased the ratio of progesterone 17α- to 16α-hydroxylation from ~3:1 to ~9:1.

Interaction between the b5 protein and CYP17A1 stimulates the cleavage of the 17,20-carbon-carbon bond of 17-hydroxypregnenolone and 17-hydroxyprogesterone to form dehydroepiandrosterone (DHEA) and androstenedione, respectively, by a factor of 10 (Figure 1.3). Residues E48 and E49 of b5 are essential for stimulating the 17,20-lyase reaction but not for the function of b5 as an electron-transfer protein. In addition, it has been suggested

that the 17,20-lyase activity is promoted by a post-translational modification via phosphorylation by a cAMP-dependent protein kinase [71, 72].

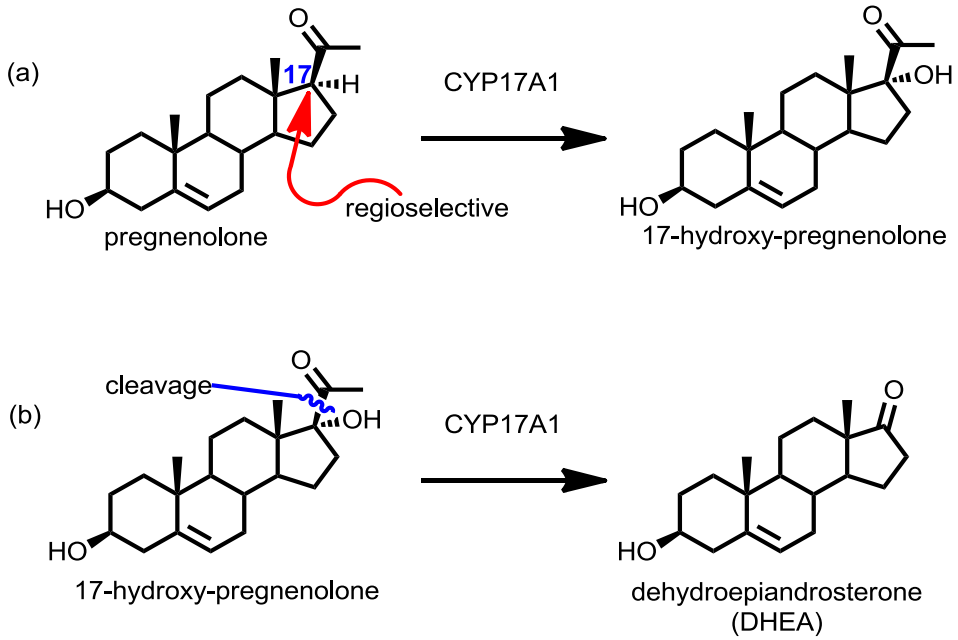


Figure 1.4. CYP17A1 (a) hydroxylates the 17-position of pregnenolone and (b) cleaves the 17,20-carbon carbon bond of 17-hydroxypregnenolone. CYP17A1 also hydroxylates progesterone at the 17α- and 16α-positions and cleaves the 17,20-carbon carbon bond of 17-hydroxyprogesterone, albeit far less efficiently than DHEA formation.

CYP21A2 (steroid 21-hydroxylase) is a P450 enzyme containing 495 amino acids and is found exclusively in the adrenal glands. The 21-hydroxylase reaction is the first chemical reaction ascribed to a cytochrome P450 by Estabrook's group. The preferred substrate for human CYP21A2 is 17-hydroxyprogesterone, but progesterone is also a good substrate for CYP21A2 (Figure 1.4). Hepatic P450 enzymes perform the 21-hydroxylase reaction with progesterone but not 17-hydroxyprogesterone, and Miller and co-workers quantitated the 21-hydroxylase activity of two human hepatic cytochromes P450, CYP2C19 and CYP3A4 [73]. The 21-hydroxylase reaction is unusual in that an electron-deficient primary carbon radical is formed after hydrogen atom abstraction, which is kinetically disfavored compared to formation of more stable secondary or tertiary carbon radicals at adjacent sites in the steroid. The hydrophobic side chain of residue V359 is critical for positioning progesterone to present only the 21-hydrogen atoms to the ferryl oxene species. Progressive removal of bulk in V359 introduces 16α-hydroxylase activity to human CYP21A2, as mutations V359A and V359G hydroxylate progesterone at both the 21- and 16α-positions in ratios of 3:2 and 1:9, respectively. Kinetic studies published on CYP21A2 by the Kominami group [74] concluded that the rate-determining step of this enzyme-catalyzed 21-hydroxylation was the product dissociation from the enzyme.

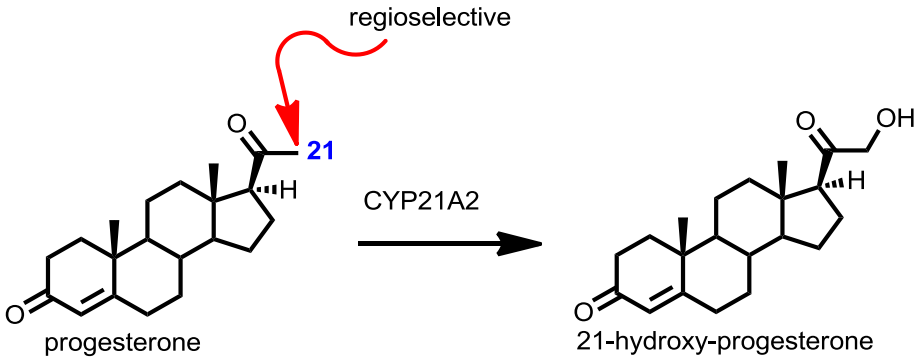


Figure 1.5. CYP21A2 hydroxylates the 21-position of progesterone. CYP21A2 also 21-hydroxylates 17-hydroxyprogesterone.

Both human CYP17A1 and human CYP21A2 have similar protein sequences (Figure 1.5) but have different chemical reactivity. In addition to cleaving the 17,20-carbon carbon bond of 17-hydroxypregnenolone, 17-hydroxyprogesterone, and 5α-pregnan-3α,17α-diol-20-one [75], CYP17A1also hydroxylates several pregnanes on the 17α-position and additionally hydroxylates the 16α-position of progesterone. Although 16α-hydroxyprogesterone has been studied as an agonist for both isoforms of the progesterone receptor [76], the biologic function of 16α-hydroxylase activity is not known. Nevertheless, the capacity of CYP17A1 to 16α-hydroxylate progesterone can be exploited to provide clues to its structure and mechanism. The chemical reactivity of CYP17A1 with progesterone substrate follows a pattern predicted for a reaction cycle that generates a carbon-centered radical in the area of the steroid D-ring and side chain: the major reaction is 17α-hydroxylation, which forms a tertiary carbon

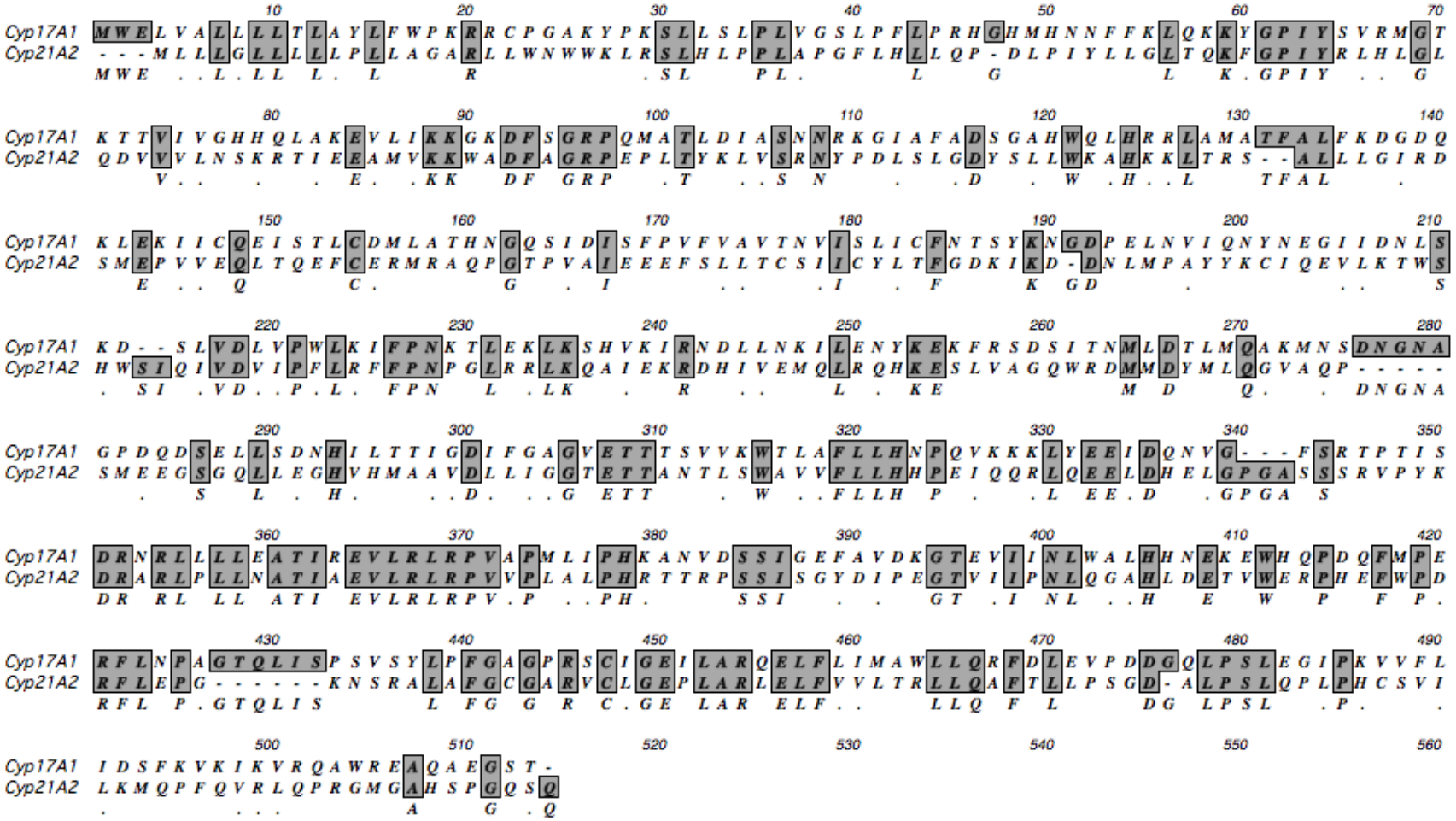


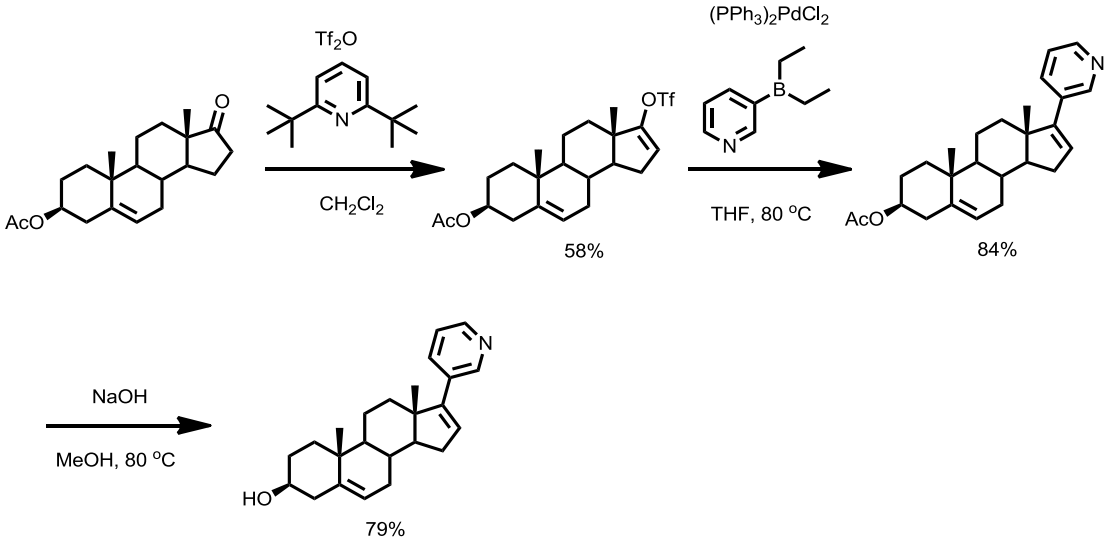
Figure 1.6. Alignment of CYP17A1 and CYP21A2 protein sequences using MacVector.

radical; 16α-hydroxylation, which generates a secondary carbon radical, is a minor reaction, and 21-hydroxylation, which involves a primary carbon radical, is not observed. CYP21A2, on the other hand, exclusively 21-hydroxylates progesterone and 17-hydroxyprogesterone, which is the least favorable reaction for that same region of these 3-keto-$\Delta^{4,5}$-steroids. To understand why these enzymes metabolize progesterone differently, we sought to probe the reaction cycle of these enzymes and site-directed mutations with different product distributions using substrate analogs and isotopically-labeled steroids. A better understanding of the detailed chemical reactivity of each enzyme can help us explain why such similar enzymes have different activities.

1.3.4. CYP17A1 Inhibitors as Drugs

Because the 17,20-lyase activity of CYP17A1 is required for androgen biosynthesis, inhibitors of this enzyme have been developed for the treatment in prostate cancer. Inhibitors of cytochromes P450 usually possess a Lewis basic nitrogen that can coordinate to the iron in the heme active site. Thus, azole-containing compounds are often used to inhibit P450s [77]; however, alkynes have also been used as mechanism-based inactivators of P450s [78]. The acetylenic inhibitors form electrophilic ketene intermediates upon P450 oxygenation and subsequently form covalent adducts with the enzyme. Recently, Shumyantseva and co-workers have reported on a method to screen potential

CYP17A1 substrates and inhibitors using electrochemical methods [79]. Abiraterone [80, 81], a potent inhibitor of CYP17A1 now FDA-approved for the treatment of prostate cancer as the acetate, bears a pyridyl ring substituent on the D-ring of the steroid backbone and $\Delta^{16,17}$-double bond (Scheme 1.1). The 16,17-saturated analog of abiraterone is ~10-fold less potent than abiraterone [80], and its mode of inhibition becomes reversible [82]. Although abiraterone potently shuts down 17,20-lyase activity and thus androgen synthesis, the compound inhibits 17α-hydroxylase activity as well, which in turn inhibits the production of cortisol and increases mineralocorticoid production as in genetic 17OHD. For this reason, abiraterone is administered with prednisone to block the rise in DOC and subsequent hypertension and hypokalemia. To avoid the consequences of mineralocorticoid excess, efforts have been extended to synthesize a compound that selectively inhibits the 17,20-lyase activity over the hydroxylase activity of CYP17A1, but no clearly superior compounds have been described [83]. This selective inhibition of one of two activities of the same enzyme is particularly an intriguing concept. Abiraterone is metabolized in the body to abiraterone-sulfate and abiraterone-sulfate N-oxide – CYP3A4 is responsible for oxidizing the nitrogen and SULT2A1 introduces the sulfate [84] (Figure 1.6).



Scheme 1.1. Synthesis of abiraterone from DHEA acetate as published by Potter *et. al* (1995) [80].

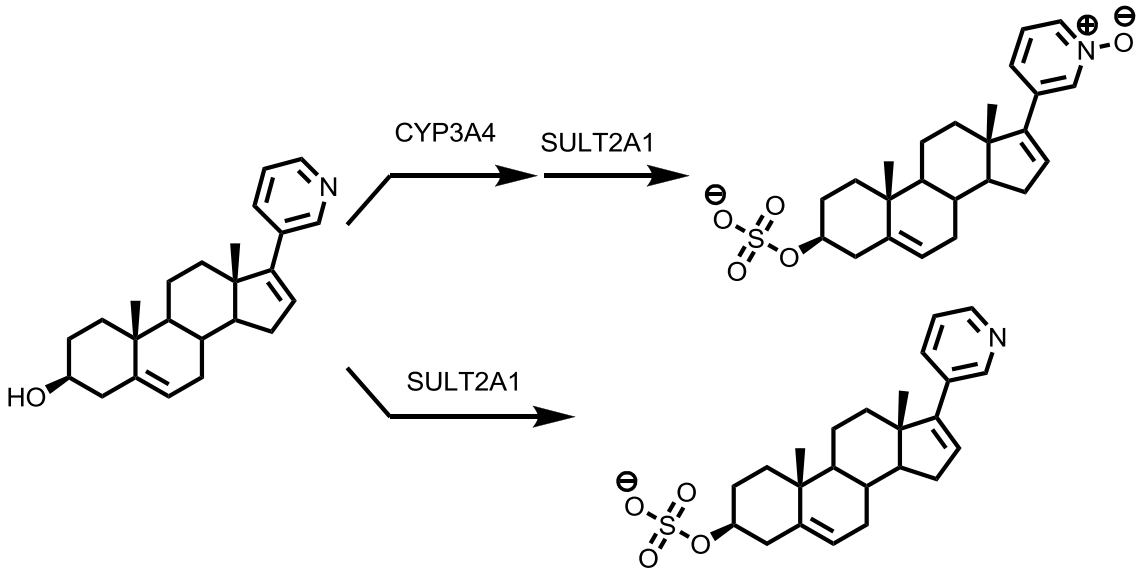


Figure 1.7. Abiraterone becomes metabolized into inactive forms by two liver enzymes, CYP3A4 and SULT2A1 [85].

For CYP17A1, docking studies have been performed to synthesize better inhibitors [86, 87]. A transition state inhibitor of CYP17A1 also has been synthesized where the authors have introduced an olefin at the 17,20-carbon-carbon bond position [88]. Oxazoline-substituted steroids have also been synthesized as potential inhibitors of CYP17A1 [89]. Njar and co-workers have reported on an abiraterone analog with a benzimidazole substituent on the D-ring [90], and this drug (TOK-001) is now in clinical trials for treatment of prostate cancer (Figure 1.7). Furthermore, the enantiomer of progesterone was shown to inhibit CYP17A1 and CYP21A2, which exemplifies the considerably more structural tolerance than generally assumed for these biosynthetic enzymes [91].

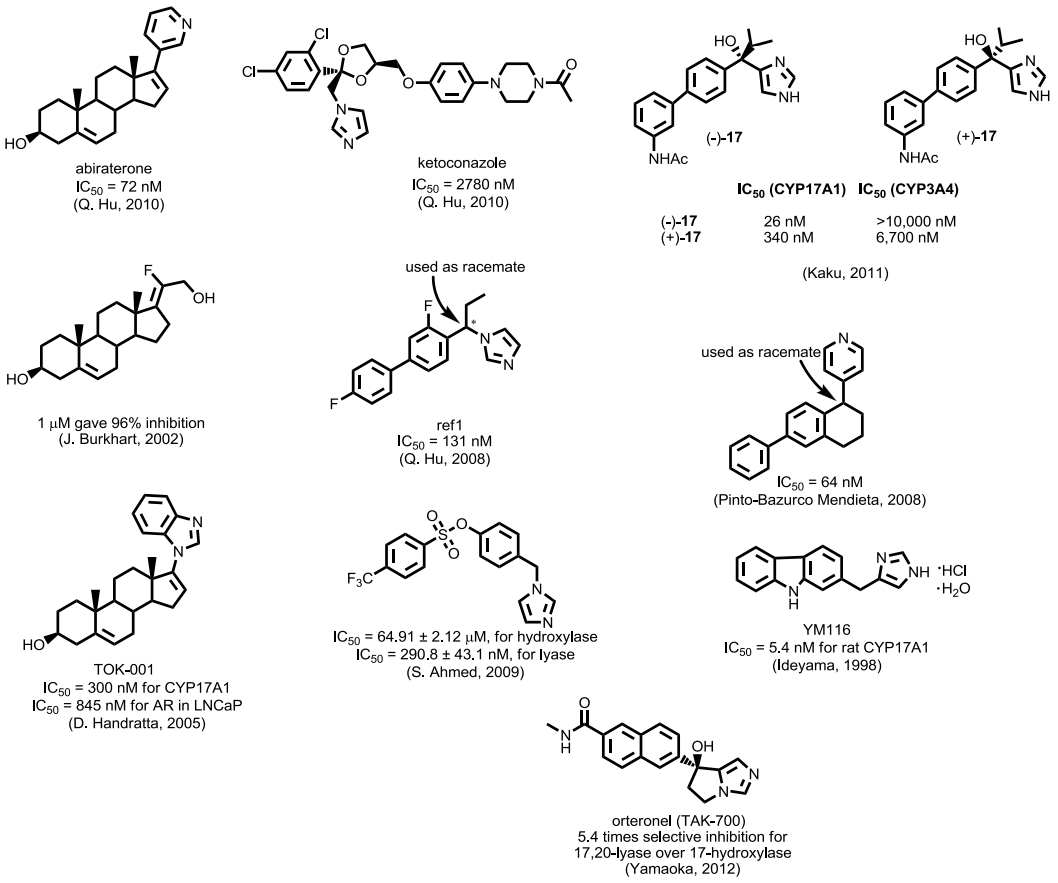


Figure 1.8. Various synthetic compounds and respective IC_{50} values for CYP17A1 unless otherwise specified. The IC_{50} values are each determined by a different method for each reference and are not comparable amongst references [83, 88, 90, 92-96].

1.3.5. Non-steroidal inhibitors of CYP17A1

Non-steroidal inhibitors of CYP17A1 are desirable because the steroid backbone of a potential drug might allow interaction with steroid receptors, leading to unwanted side effects [95, 96]. In this regard, Hartmann and co-workers have reported on the synthesis of some fluorinated imidazole inhibitors of CYP17A1[93, 94]. Recent efforts by Kaku et al. have resulted in the enantioselective synthesis of imidazole-containing compounds, which show selective inhibition of human CYP17A1 over human CYP3A4, an important liver enzyme responsible for detoxification of xenobiotics [97]. In addition, the two enantiomers had different inhibitory activity in that one enantiomer demonstrated greater selective and potency towards CYP17A1 [92]. Moreover, TAK-700 has been reported to selectively inhibit the 17,20-lyase activity over the 17-hydroxylase activity [98, 99]. An alternative approach to inhibit the enzyme would be to inhibit the interaction with redox partner POR and/or b5. An inhibitor that blocks the action of b5 might lead to selective 17,20-lyase inhibition without impairing 17-hydroxylase activity. Friedman and co-workers have disclosed such an approach by inhibiting CYP2B1by using peptides that mimic the binding surface between the enzyme and the redox partner POR [100].

1.4. Mechanistic Studies of CYP17A1 and CYP21A2

Chapter 2 of this thesis describes the detail of chemical syntheses required to make compounds used to study the enzymes, CYP17A1 and CYP21A2. The syntheses involved structural modifications on progesterone and pregnenolone. This chapter includes the synthesis of halogenated, deuterated, olefinated, and oxygenated progesterone analogs as well as steroids bearing a cyclopropane ring.

Chapter 3 includes enzymology studies of CYP17A1 and CYP21A2 with our progesterone and pregnenolone analogs.

Chapter 4 involves the kinetic isotope effect studies with CYP17A1 and CYP21A2.

Chapter 5 describes our experimental set up to determine any tunneling contributions in the C-H abstraction step.

Finally, chapter 6 summarizes the overall results and suggests future experiments to improve our understanding of these two steroidogenic enzymes.

CHAPTER TWO

Synthesis of Chemical Probes for CYP17A1 and CYP21A2

2.1. INTRODUCTION

As discussed in the introduction, cytochromes P450 are ubiquitous enzymes and have been found to catalyze a wide variety of chemical reactions in many domains of life. As a synthetic chemist's approach to understanding the mechanism of CYP17A1 and CYP21A2, one can synthesize different analogs and observe the structural tolerance and various reactivities of these enzymes by using the analogs as potential substrates. In other words, using different steroid substrates by varying the sterics and electronics of the substrates allows one to probe the enzyme's substrate binding site. In addition, radical clock studies have been performed to measure the lifetime of the radical intermediate in cytochromes P450. The fluorine atom is closest in size to the hydrogen atom on the periodic table of elements, and the greater strength of the C-F bond versus the C-H bond renders sites of fluorination less reactive than the protonated congener. Therefore, fluorinated steroid substrates are potentially useful chemical probes. For example, fluorinated compounds have been used to study the structure of cytochromes P450 through NMR [101, 102]. Fluorinated substrates and inhibitors have also been used to study other enzyme systems [103].

Further analog-activity studies might lead to an improvement in the understanding of the enzyme-substrate complex. Linear free energy relationship plots are used to probe the stereoelectronic structure of the binding site. The idea of using substituted substrate analogs to study the linear free energy relationships to predict the reaction rates of cytochromes P450 has been reported before [104, 105]. Other informative substrates are isotopically-labeled compounds (ie: deuterium or tritium labeled), in order to measure possible kinetic isotope effects of the C-H abstraction step.

In summary, we synthesized various steroid analogs to study potential reactivities with CYP17A1 and CYP21A2. The substrates include: (1) halogenated substrates, (2) deuterated substrates, (3) olefinated substrates, (4) cyclopropyl substrates, (5) various alkyl substrates and (6) oxygenated steroids to prove the identity of potential hydroxylated metabolites (Figure 2.1).

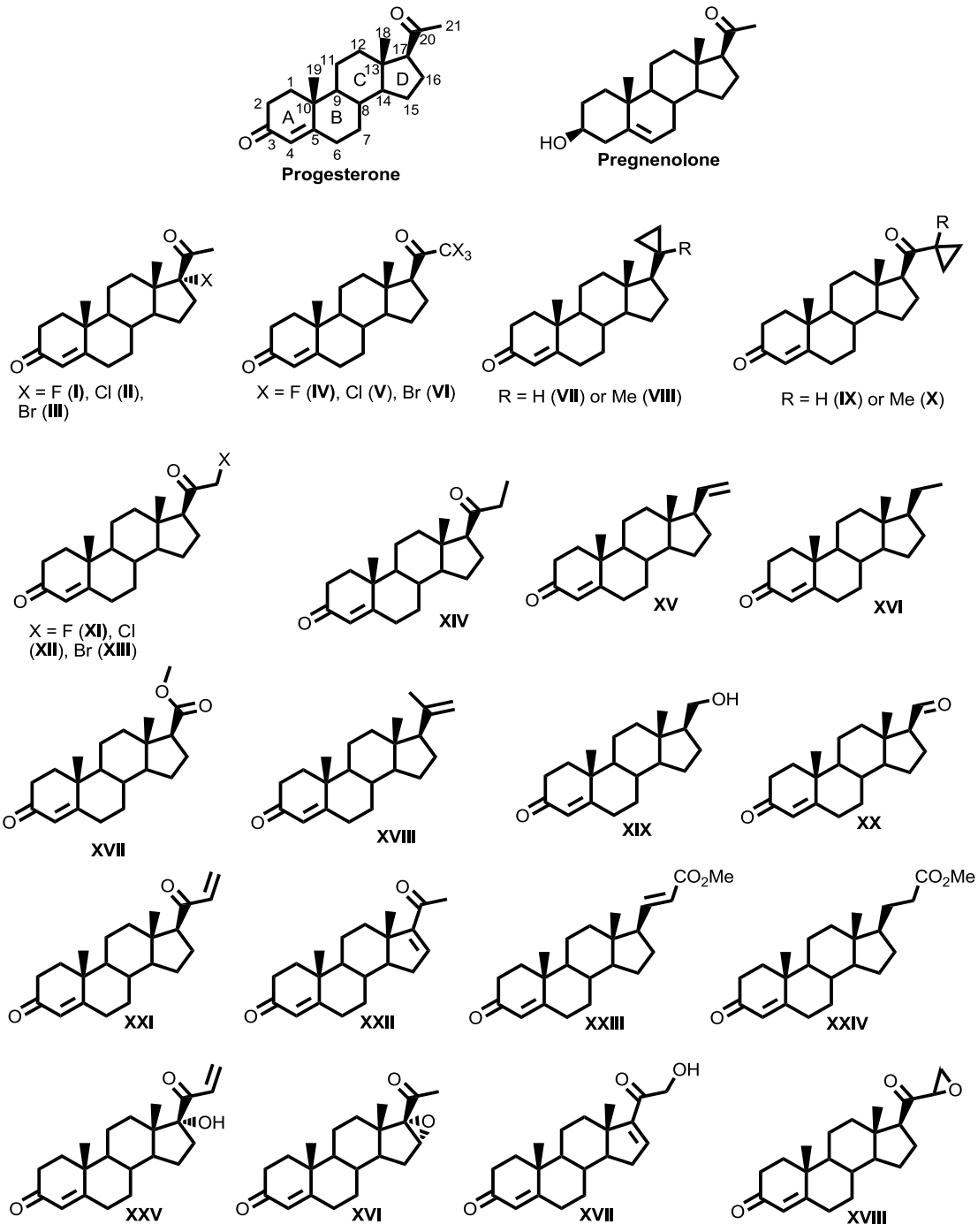


Figure 2.1. Most of the substrate analogs and products synthesized are shown above.

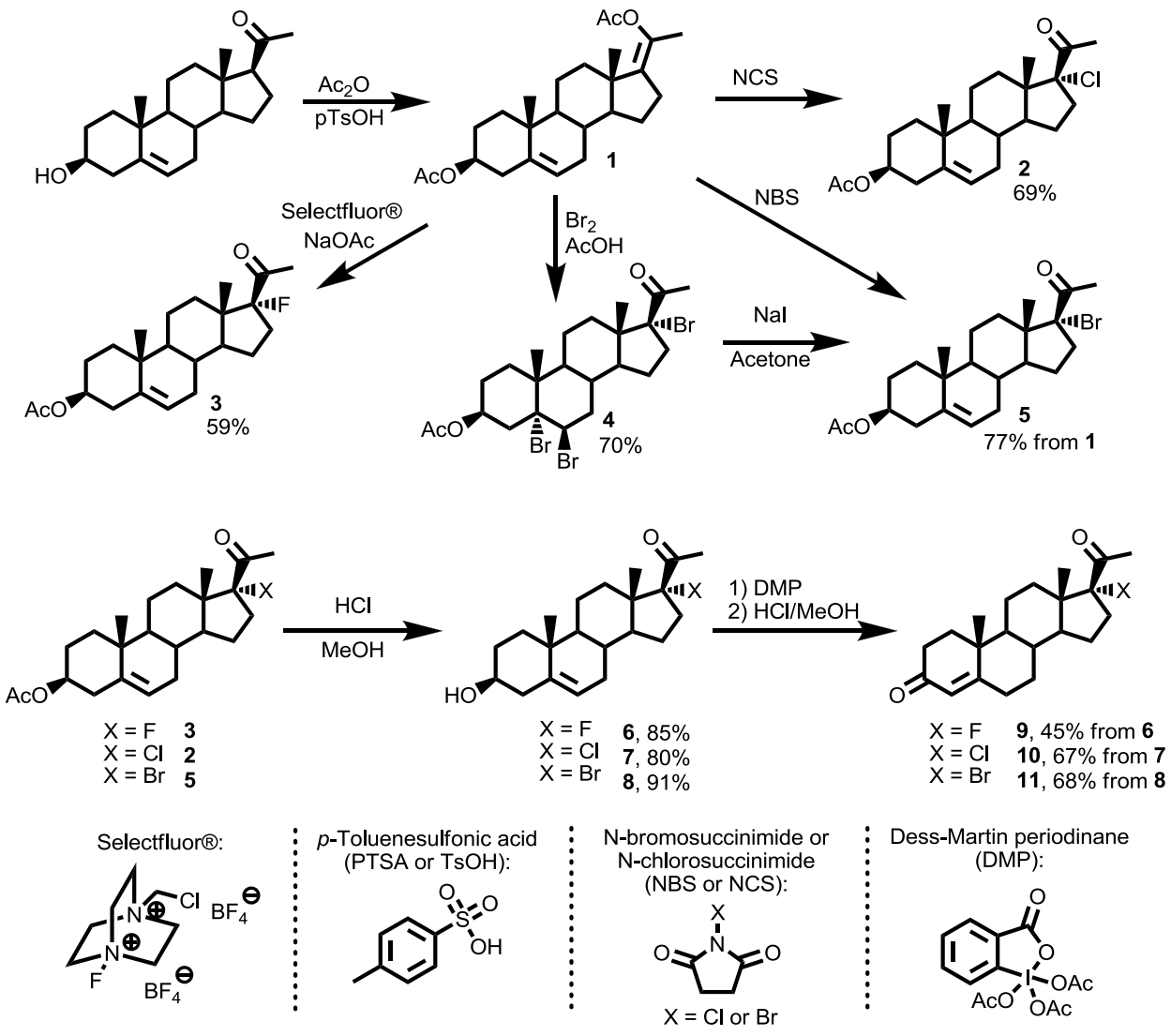
2.2. HALOGENATED SUBSTRATES

The brominated compounds were desired for access to deuterated compounds – however, the methods developed to synthesize brominated steroids are applicable to accessing other halogenated steroids. Other possible substituents would include: fluoride and chloride groups – iodide groups were also possible, however, because of the high reactivity of organic iodides, these halides tend to be too labile for the enzymatic incubations in aqueous buffers. Exploration of the syntheses of new halogenated steroid substrates would lead new chemical reactions and potential substrates for CYP17A1 and CYP21A2 to probe the enzyme active site.

2.2.1. 17-Halogenated Substrates

The 17-monohalo compounds were afforded from a common intermediate – the 17,20-enol acetate, obtained in literature precedence [106] by refluxing pregnenolone in acetic anhydride and catalytic p-toluenesulfonic acid. The 17,20-enol acetate was a highly versatile intermediate, and we were able to use Selectfluor to fluorinate [107], NCS to chlorinate, and NBS to brominate the 17-position and access compounds **6**-**8** and **9**-**11**. As an alternative to NBS, we could repeat the reported protocol of bromine in acetic acid [106], but the alkene on the 5-position is dibrominated, adding an additional step in reforming the Δ^5-alkene **5** with sodium iodide (Scheme 2.1). Presence of the 17-halogen did not complicate the facile oxidation and isomerization of pregnenolone halides to the

corresponding progesterone series with Dess-Martin periodinane, followed by mild acid or spontaneous isomerization during purification on silica gel chromatography.

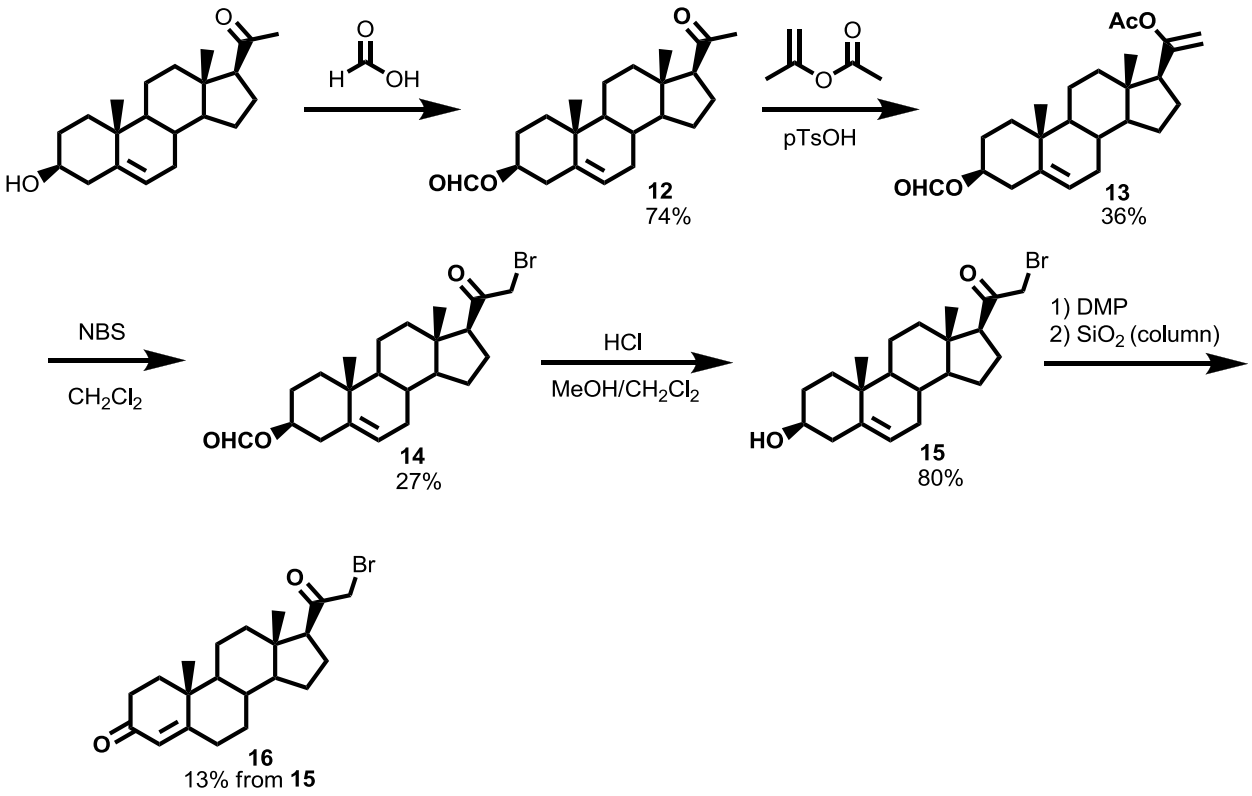


Scheme 2.1. Synthesis of 17-monohalo-pregnenolone (**6**-**8**) and –progesterone analogs (**9**-**11**).

2.2.2. 21-Halogenated Substrates

2.2.2.1. 21-Monohalogenated Substrates

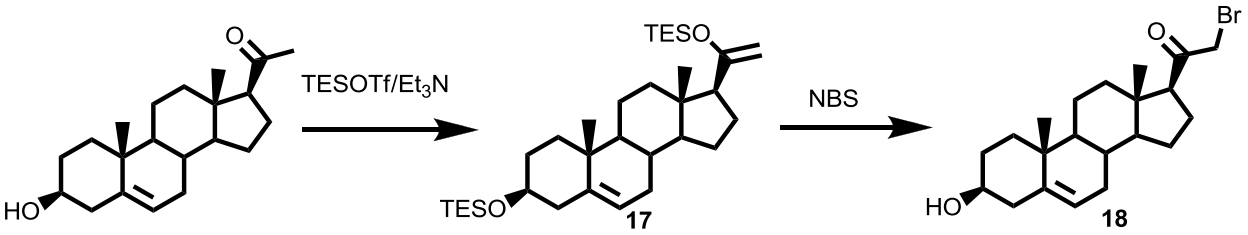
Similarly, the 20,21-enol acetate was a suitable precursor to regioselectively monohalogenating the 21-position. Selectfluor did not yield the 21-fluoro-product, but iodination followed by nucleophilic substitution with AgF would lead to the desired 21-fluoro-product. Pregnenolone-3-formate **12** was refluxed in isopropenyl acetate and catalytic p-toluenesulfonic acid to afford the 20,21-enol acetate **13** (Scheme 2.2).



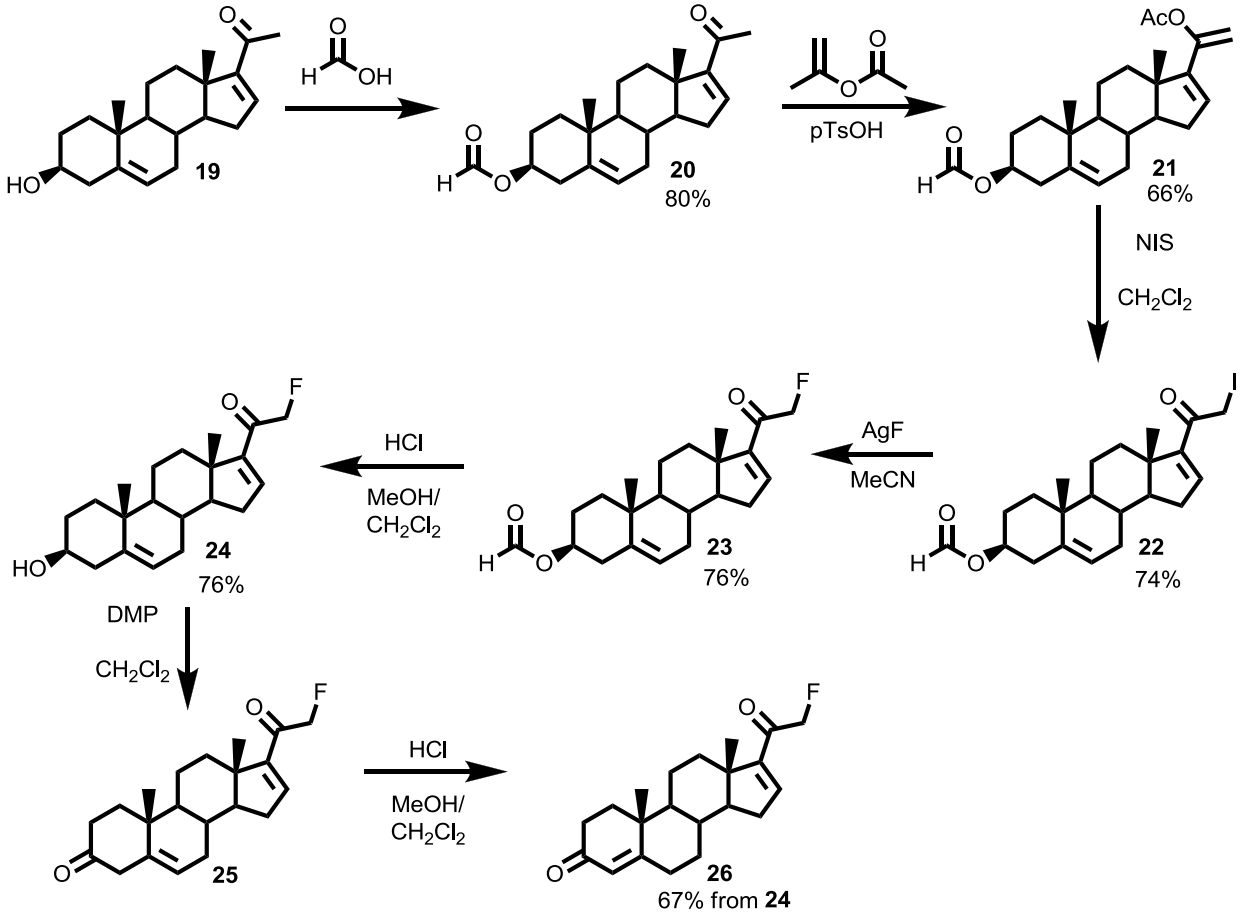
Scheme 2.2. Synthesis of 21-monobromo-pregnenolone (**15**) and –progesterone (**16**) analogs via enol acetate **13**.

Because the 17,20-enol acetate formation had competed with the 20,21-enol acetate formation, we opted to use a more robust method in forming the kinetic enolate. The alternative route involved the use of triethylsilyl trifluoromethanesulfonate in the presence of triethylamine base. These reaction conditions yielded the silyl enol ether **17** in quantitative yield (Scheme 2.3). Trimethylsilyl trifluoromethanesulfonate was also used, but this silyl group was

more labile compared to the triethyl silyl case. A modified route via the enol acetate gave the 21-monohalo-$\Delta^{4,16}$-steroids (Scheme 2.4).



Scheme 2.3. Synthesis of 21-monobromopregnenolone via silyl enol ether.



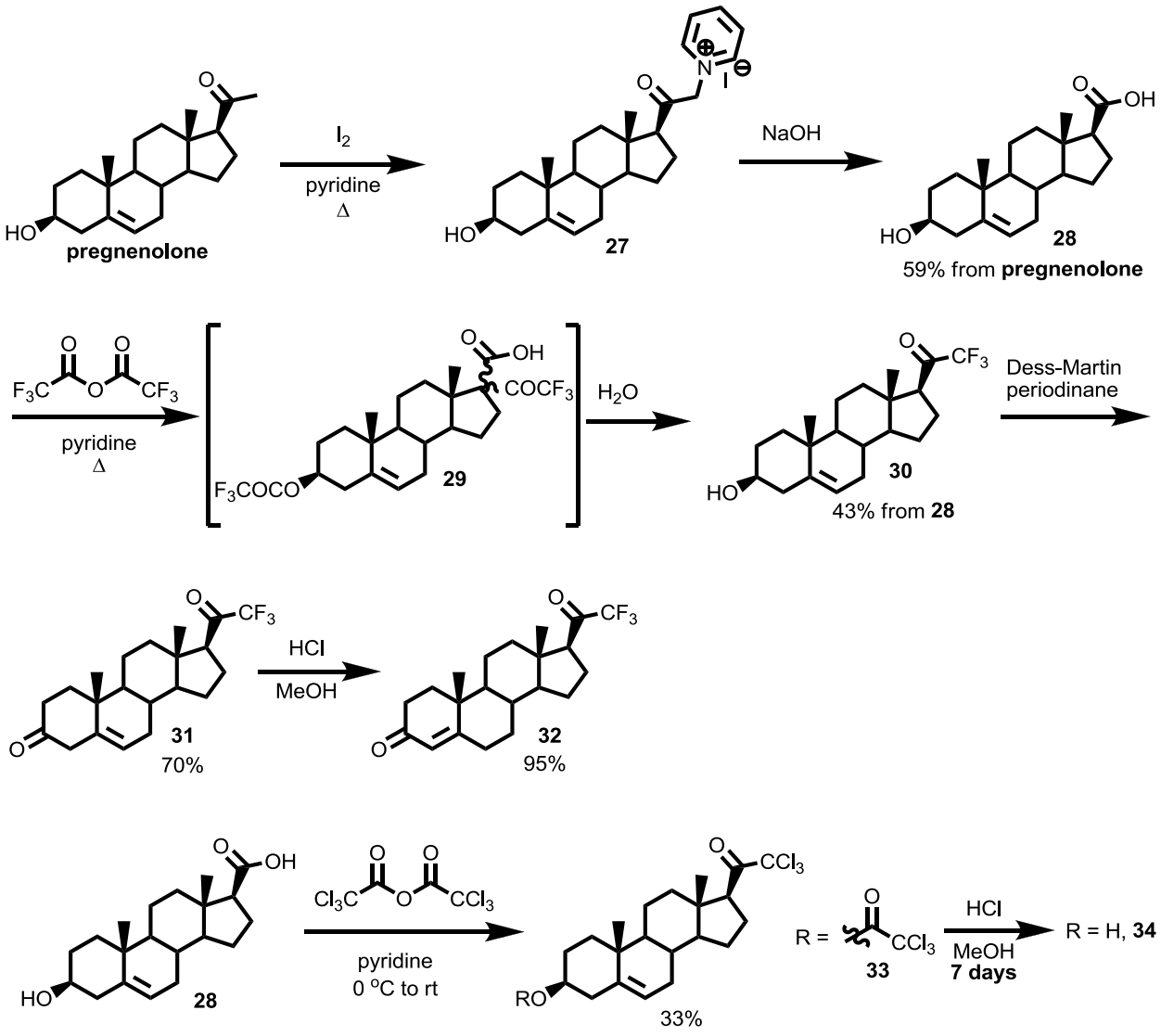
Scheme 2.4. Synthesis of 21-monofluoro-$\Delta^{4,16}$-progesterone (**26**) analog.

2.2.2.2. 21,21,21-Trihalogenated Substrates

The first approach to 21,21,21-trihalo compounds (Scheme 2.5) used the 20-carbon carboxylic acid **28**, derived from the pyridinium salt **27**, to attempt Zard's protocol of transforming carboxylic acids to trifluoromethyl-ketones [108]. Trifluoroacetic anhydride and pyridine converted the carboxylic acid **28** to the trifluoromethyl ketone **30** upon addition of water and heating. The β-kethyl acetateid intermediate **29** is observable by NMR before the addition of water, and the decarboxylation proceeds with the desired stereochemistry at C-17, probably due to torsional strain in the protonation step (Figure 2.2). The stereochemistry at C-17 was confirmed when we subjected 21,21,21-trifluoropregnenolone (**30**) to zinc and deuterated acetic acid under refluxing conditions to afford 21,21,21-[$^{2}H_{3}$]-pregnenolone. Moreover, the reported ^{1}H NMR by Njar and colleagues matched with our chemical shifts we obtained with our compound [109].

Interestingly, the trichloromethyl ketone **33** was also obtained in a similar manner by using either trichloroacetic anhydride or trichloroacetic acid chloride, except we found that the reaction must be conducted at 0 ^{o}C due to the reactive chloride leaving groups and that the product contained an incidental 3-trichloroacetate group (Scheme 2.5). Moreover, in the trichloro- case, the β-kethyl acetateid decarboxylation occurred spontaneously, without the addition of water. The base-labile trichloroacetyl group mandated the deprotection of the 3-trichloroacetate

group under acidic conditions in methanol, which took a week to complete and prompted investigation of alternative approaches. Interestingly, trichloroacetate **33** was subjected to zinc and deuterated acetic acid in ether to afford 21,21,21-[$^{2}H_{3}$]-pregnenolone-3-trideuteroacetate at room temperature. In a similar manner to the trichloromethyl ketone case, we attempted to use tribromoacetyl chloride to form the tribromomethyl ketone, but too many side reactions were observed.



Scheme 2.5. Preparation of 21,21,21-trihalosteroids using Zard's method.

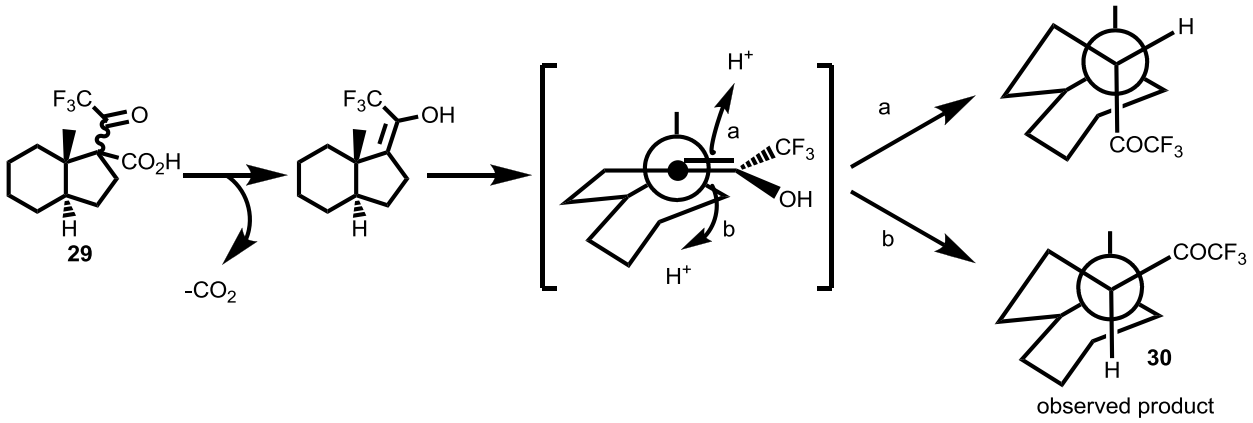


Figure 2.2. Explanation of C-17 stereochemistry of 21,21,21-F_3-pregnenolone **30**– AB ring system omitted for clarity.

The stereochemistry of 21,21,21-trifluoropregnenolone obtained from the Zard methodology is based on multiple pieces of evidence:

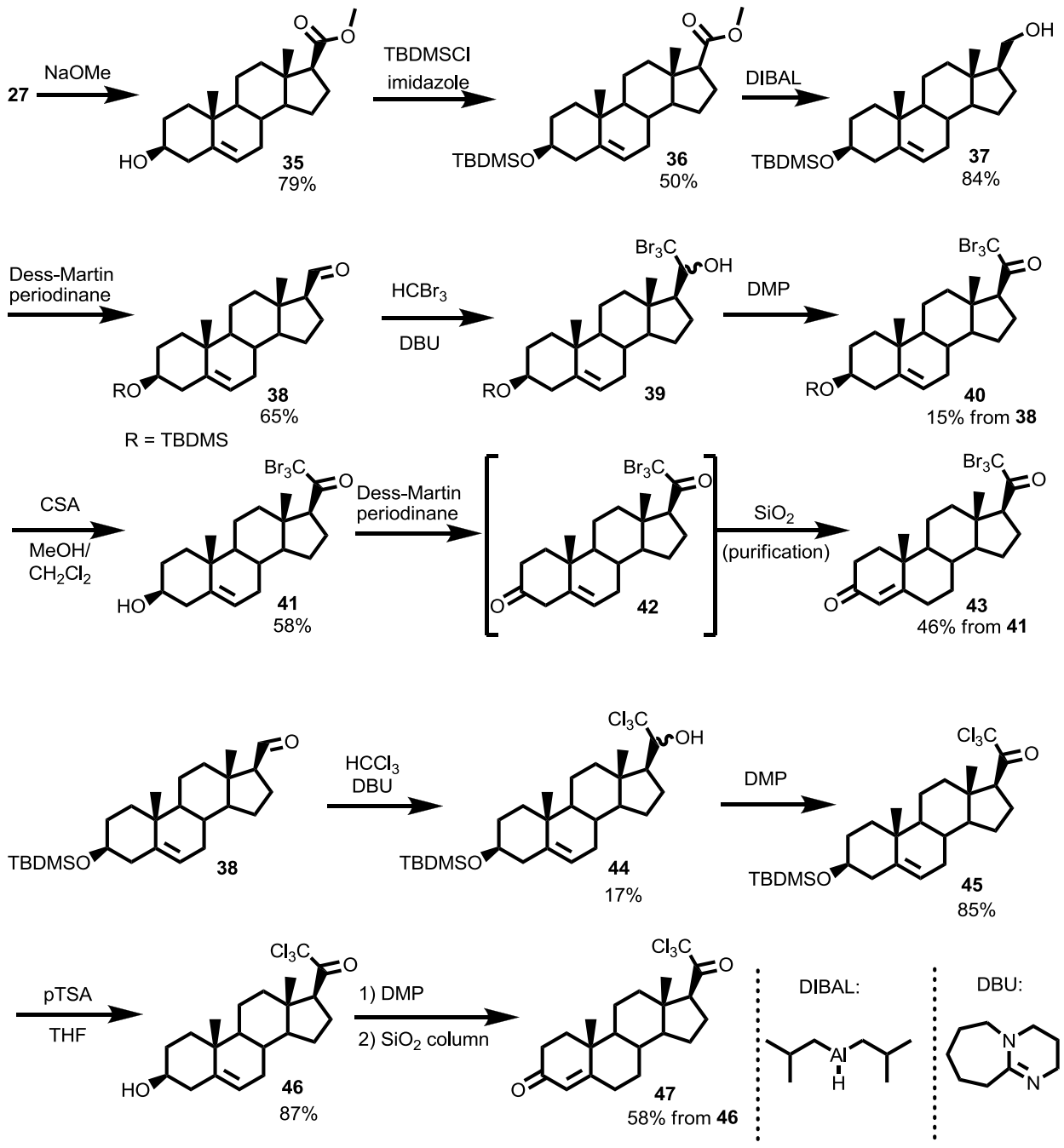
A) Subjecting the compound to refluxing zinc in deuterated acetic acid, which afforded the 21,21,21-[$^2H_3$]-pregnenolone compound.

B) NMR of the compound with that reported by Njar and co-workers, who have synthesized this compound by the addition of TMS-CF_3 nucleophile on the aldehyde nucleophile bearing the same β-stereochemistry at C-17 [109].

C) The similar Zard methodology was applied to form 3-trichloroacetoxy-21,21,21-trichloropregnenolone, which also matched our other approach to synthesize 21,21,21-trichloropregnenolone by addition of chloroform nucleophile on the appropriate aldehyde.

Due to difficulties with the trichloromethyl and tribromomethyl cases, we pursued a second route, which involved the addition of a trihalomethyl anion onto

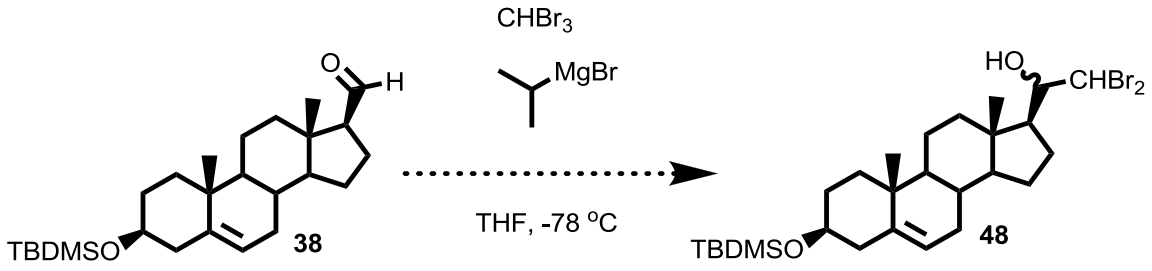
the 20-carbon aldehyde **27** (Scheme 2.6). One equivalent of DBU and excess bromoform was used to optimize yield and to prevent solidification of the reaction mixture [110]. Aldehyde **27** also served as a versatile intermediate to make alkyl substituents at the 21-position in addition to making the 21,21,21-trihalo compounds, including the 21,21,21-trichloroketone **47** by using the chloroform anion as the nucleophile (Scheme 2.6). If 3-ketosteroids are sought, the TBDMS ether can be removed before the Dess-Martin periodinane-mediated oxidation step, and the resulting 3,20-diketones are isomerized to the Δ^4-3-ketosteroids under mildly acidic conditions, which avoids the harsher Oppenauer oxidation protocol when the compounds contain labile or reactive groups.



Scheme 2.6. Synthesis of 21,21,21-tribromo- and 21,21,21-trichloro-steroids (**41**,**43**,**46**,**47**).

2.2.2.3. 21,21-Dihalogenated Substrates

We were able to introduce the 21,21-dibromomethyl moiety from the aldehyde precursor using bromoform and isopropylmagnesium bromide in a dry ice bath (Scheme 2.7). The -78 ^{o}C temperature was important for the metal halogen exchange process; otherwise, gas forms violently.



Scheme 2.7. Synthesis of 21,21-dibromosteroid analog.

The resulting dibromomethyl carbinol can be oxidized with Dess-Martin periodinane to yield 21,21-dibromopregnenolone-3-tert-butyl-dimethylsilyl ether. The TBDMS-group was cleaved in the presence of camphor-sulfonic acid (CSA) in methanol, and the resulting alcohol was oxidized and isomerized with Dess-Martin periodinane and mild acid conditions to afford 21,21-dibromoprogesterone.

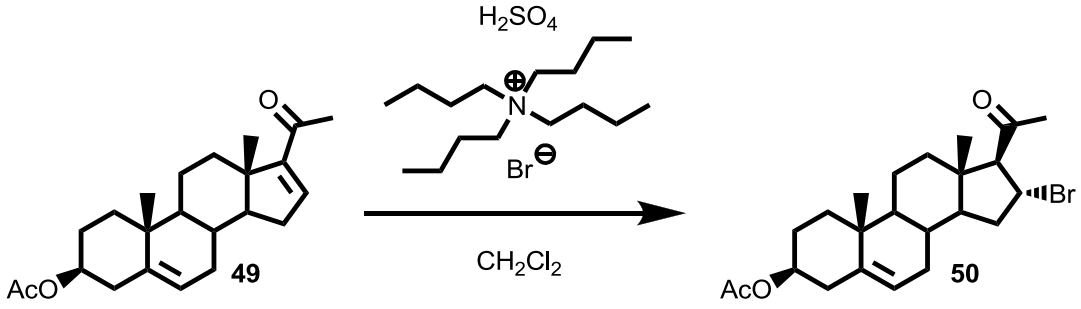
2.2.3. 16-Halogenated Substrates

Previous reports describe syntheses of 16-halogenated steroids from the addition of HX (-X = -Br or -Cl) onto 16,17-dehydropregnenolone-3-acetate; however, these conditions required hazardous HBr or HCl gas [111-114]. Moreover, these references lacked NMR spectra to confirm product identities, and we found these results difficult to reproduce.

We initially attempted to cleave the 3-acetate from the 16-halogenated product using sodium hydroxide and tetrabutylammonium iodide in THF, which took several days. The reaction mixture was directly loaded on a silica gel column without neutralization or concentration of the reaction mixture. We obtained the 16-iodo-compound, but only 50% of the acetate was cleaved based on ^{1}H NMR spectra. We attempted to reproduce these results; however, we only isolated the olefin product, 16,17-dehydropregnenolone. We concluded that the 16-iodide is labile and readily undergoes β-elimination, which reforms the 16,17-olefin.

Therefore, we attempted to halogenate the 16-position of 16,17-dehydropregnenolone-3-acetate **49** using tetrabutylammonium bromide and sulfuric acid (Scheme 2.8). The sulfuric acid presumably activates the β-position as a Lewis base, through protonation of the carbonyl oxygen, facilitating bromide delivery to the 16α-position by Michael addition.

The 16α-bromide is labile and readily eliminated HBr when the crude reaction mixture was directly concentrated; consequently, upon basic workup and concentration of the organic extracts, we were unable to detect 16α-bromopregnenolone-3-acetate by ^{1}H NMR. The 16-bromo-pregnenolone-3-acetate product could not be resolved from the 16,17-dehydropregnenolone-3-acetate, starting material by TLC, adding to the difficulties we encountered. Nevertheless, we found that loading the reaction mixture directly on a silica gel column followed by concentration under reduced pressure yielded the 16α-bromo compound **50**. With prolonged reaction times (2 days) and excess tetrabutylammonium bromide (~10 mol equivalents), the $\Delta^{5,6}$-double bond becomes dibrominated, but the $\Delta^{5,6}$-double bond of this intermediate can be reformed by stirring the compound with stoichiometric sodium iodide in acetone.



Scheme 2.8. Synthesis of 16α-monobromopregenolone-3-acetate.

The stereochemistry was verified when we deuterated the 16α-bromocompound as described in the following section of this chapter (Deuterated

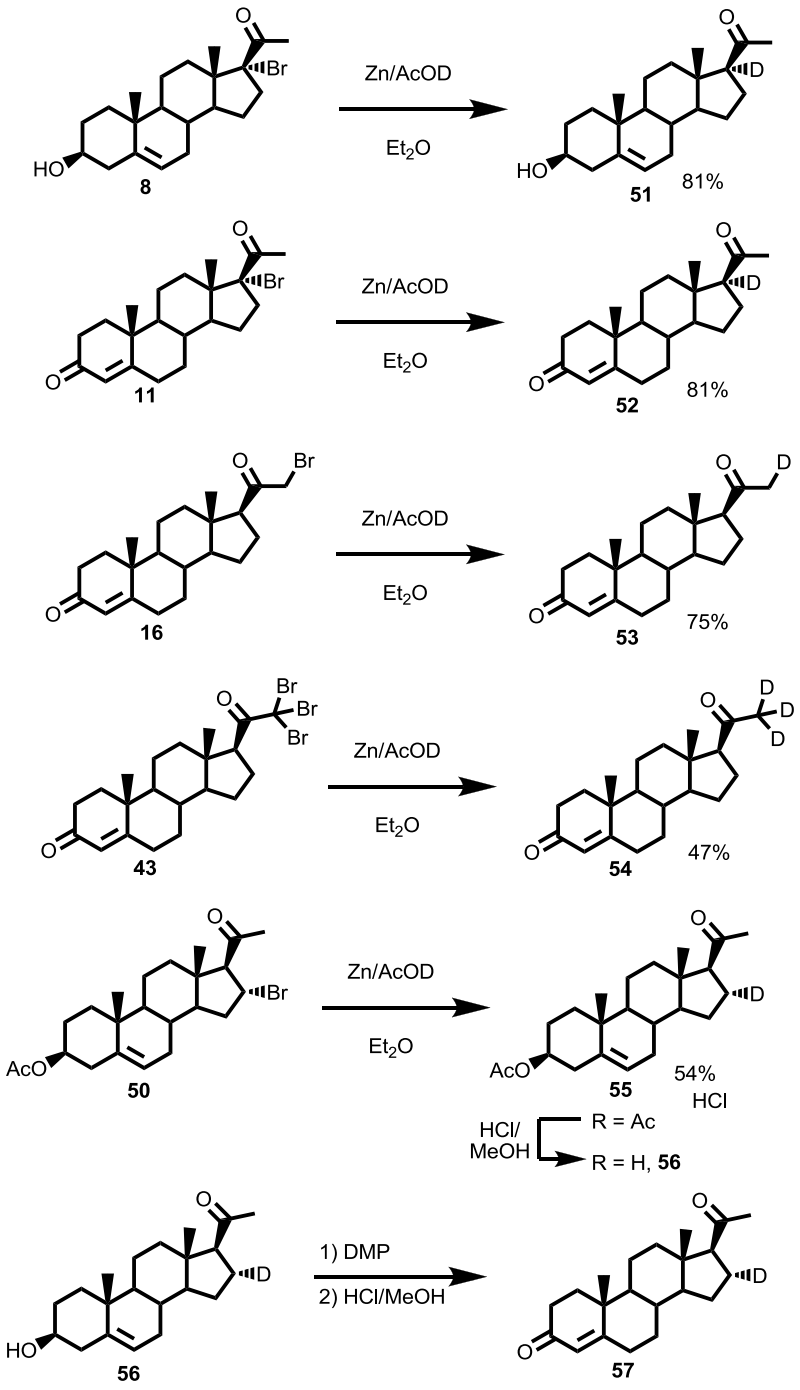
Substrates). In addition, when we attempted to displace the 16α-bromide with AgF, 16,17-dehydropregnenolone-3-acetate was reformed.

2.3. DEUTERATED SUBSTRATES

The key feature of synthesizing deuterated substrates was the use of a Reformatzky-type reduction of a brominated precursor (**8**, **11**, **16**, **43**, **50)**, which involved subjecting the substrate in zinc and deuterated acetic acid (CH_3COO^2H) (Scheme 2.9). The main challenge was to access the brominated precursors, as described in the previous section. Since CYP17A1 oxygenates the 16α- and 17-positions of progesterone, we attempted to deuterate a16α-bromo- and 17-bromosteroid precursors. Similarly, CYP21A2 hydroxylates the 21-position of progesterone and 17-hydroxyprogesterone, so the 21-brominated (mono- and poly-) steroid precursors were needed. The precursors were halogenated with bromine atoms as opposed to iodine, because iodides are relatively labile. Analogously, fluorides were not used because they are not as reactive as bromides in the zinc insertion process, because the C-F bond is much stronger than the C-Br bond.

Although the 16α-bromide eliminated when we attempted to displace the bromide with AgF in 16α-bromopregnenolone-3-acetate **50**, zinc had readily exchanged with the bromide in the presence of deuterated acetic acid to form 16α-[^{2}H]-pregnenolone-3-acetate. Alternatively, the 16α-[^{2}H]-pregnenolone

compound was accessed from 16,17-dehydropregnenolone directly via selective reduction of the $\Delta^{16,17}$-double bond with deuterium gas (catalyzed by palladium on $BaSO_4$) followed by Oppenauer oxidation; however, this route yielded 80% deuterium incorporation on the 16α-position (determined by ^{1}H NMR).



Scheme 2.9. Synthesis of selectively deuterium-labeled steroids.

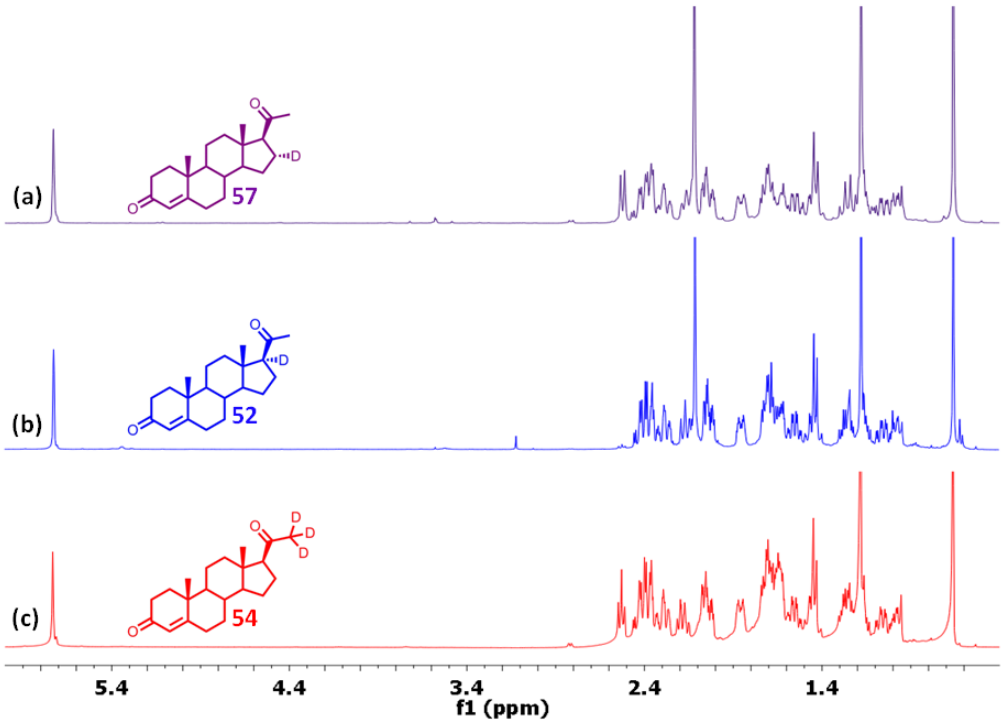
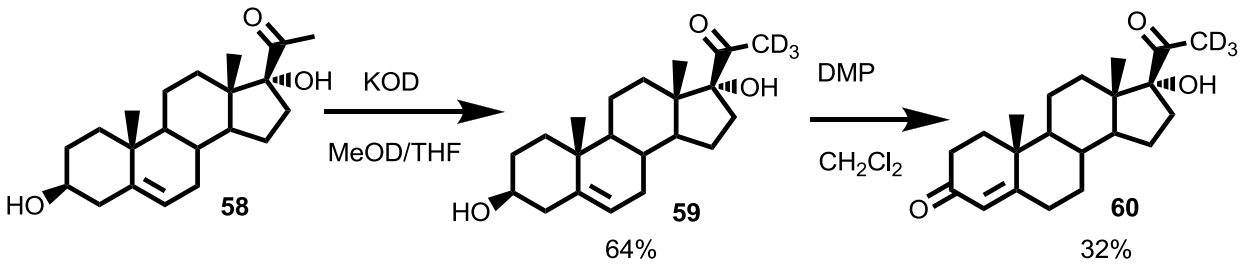


Figure 2.3. ^{1}H NMR spectra of deuterated compounds: **57** (a), **52** (b) and **54** (c).

Moreover, because CYP21A2 hydroxylates the 21-position of 17-hydroxyprogesterone to furnish 11-deoxycortisol (17,21-dihydroxyprogesterone), we deuterated the 21-position of 17-hydroxypregnenolone **58** with KO^{2}H in $CH_3O^{2}H$ and oxidized the 3-hydroxy group using the mild Dess-Martin periodinane protocol to yield 17-hydroxy-21,21,21-[$^{2}H_3$]-progesterone **60** (Scheme 2.10).



Scheme 2.10. Synthesis of 21,21,21-[$^2H_3$]-17-hydroxyprogesterone (**60**).

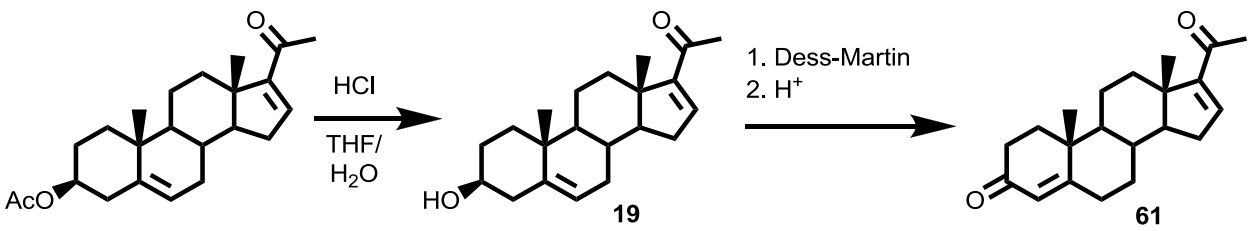
2.4. OLEFIN-CONTAINING SUBSTRATES

Access to unsaturated steroid analogs was desired, because olefins are known to undergo epoxidation as substrates of cytochromes P450. For example, in cockroaches methyl farnesoate is the substrate of CYP15A1, which epoxidizes the alkene to juvenile hormone [115]. In addition, alkenes are synthons for cyclopropanation chemistry, which might lead to other potential chemical probes (see next section on Cyclopropyl Substrates).

2.4.1. 16,17-dehydroprogesterone

The synthesis of 16,17-dehydroprogesterone started with commercially available 16,17-dehydropregnenolone-3-acetate. The 16-position was found to be electrophilic in acidic conditions with a Lewis basic solvent such as methanol. Therefore, the 3-acetate was cleanly deprotected by stirring in a 1:1 mixture of THF:water in the presence of concentrated HCl for 7 days.

Oxidation of the resulting 3-hydroxy group of **19** using Dess-Martin periodinane followed by isomerization of the double bond ($\Delta^{5,6}$ to $\Delta^{4,5}$) yielded 16,17-dehydroprogesterone **61** (Scheme 2.11). This compound would be tested as a substrate for CYP17A1, which might epoxidize the 16,17-olefin.



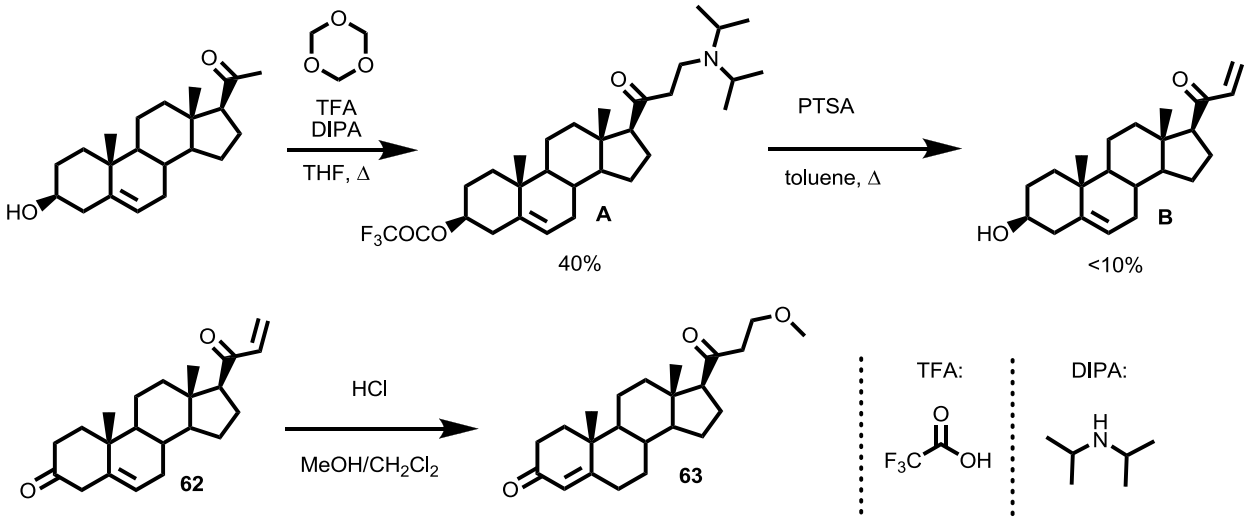
Scheme 2.11. Synthesis of 16,17-dehydroprogesterone **61**.

2.4.2. 21-homo-21,22-dehydroprogesterone

A similar olefinated substrate was 21-homo-21,22-dehydroprogesterone, which might be epoxidized by CYP21A2. We originally attempted a one-step procedure from pregnenolone, using diisopropylamine and paraformaldehyde in the presence of trifluoroacetic acid to introduce the alkene (Scheme 2.12)[116]. Although this reaction worked, the resulting product was so electrophilic that Michael adducts would readily form even under mild conditions (ie: N-diisopropylamine-adduct on the β-position, or methanol adduct). Heating the Michael adducts in p-toluenesulfonic acid in toluene successfully eliminated the

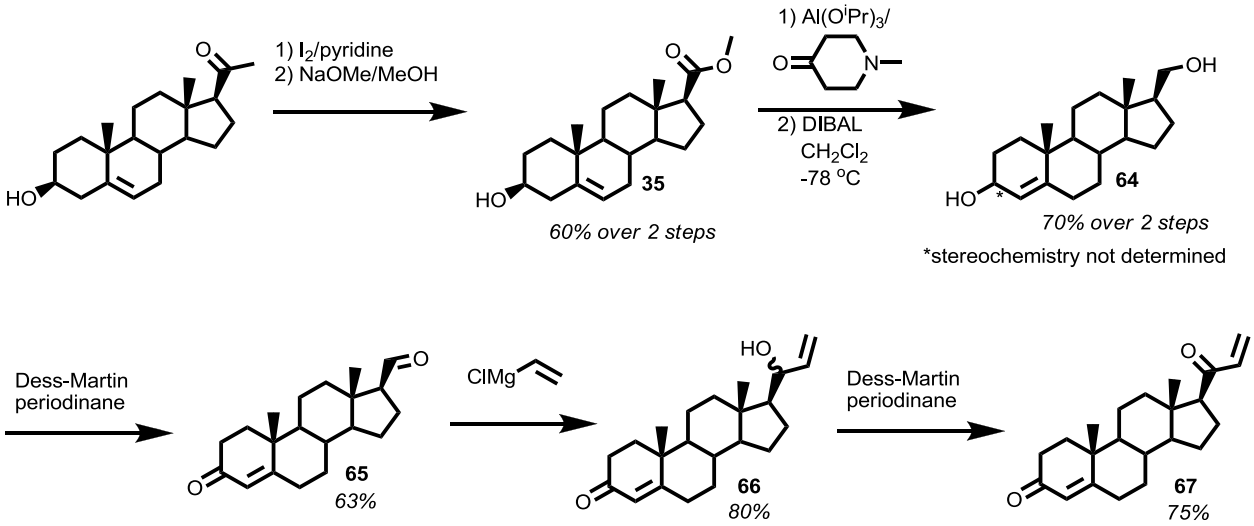
Michael adducts to re-form the desired α,β-unsaturated ketone; however, the yields were low (~10%).

Therefore, we used vinylmagnesium bromide with 17-carboxaldehyde to introduce the alkene. Because the 21,22-α,β-unsaturated ketone moiety was so reactive at the β-position, we decided to form the $\Delta^{4,5}$-double bond first, to avoid having to isomerize this olefin at the last step. In other words, when we had isomerized the $\Delta^{5,6}$-double bond in the final step, we obtained the methanol conjugate adduct **63** (Scheme 2.12). The methoxy group was eliminated upon heating with p-toluenesulfonic acid and toluene; however, we found that the yield was again low (<10%).



Scheme 2.12. 22-methoxy adduct formed with 21-homo-21,22-dehydroprogesterone.

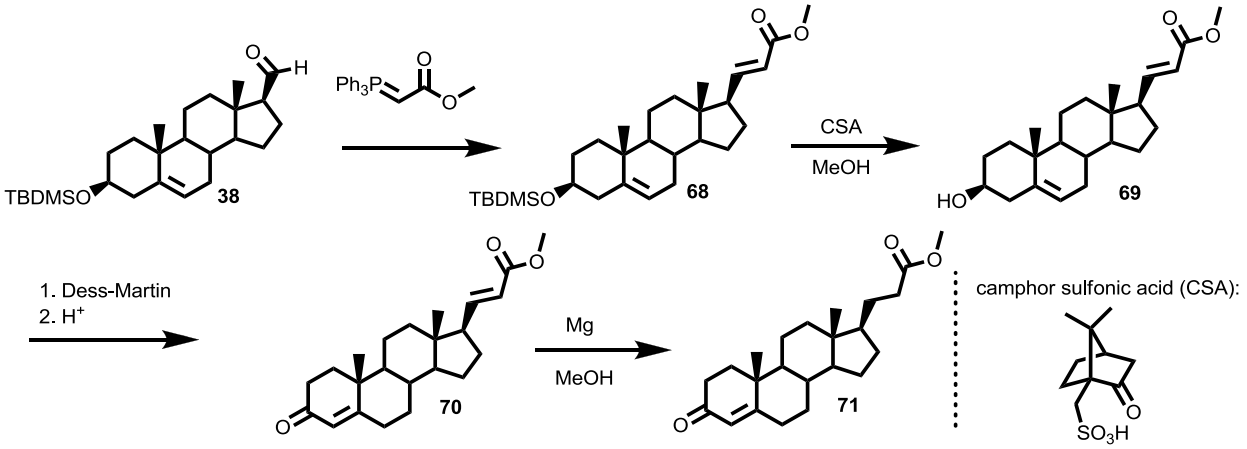
The methyl ester **35** was subjected to the Oppenauer oxidation protocol, which oxidized the 3-position and isomerized the olefin as expected (Scheme 2.13). The resulting 3-keto-$\Delta^{4,5}$-methyl ester was reduced with excess DIBAL to furnish the $\Delta^{4,5}$-3,20-diol **64**. Dess-Martin periodinane was used to oxidize the diol to aldehyde **65**. The reaction was monitored by NMR, and interestingly the secondary alcohol was more reactive than the primary alcohol during the reaction conditions. The secondary alcohol reactivity was probably attributed to the more nucleophilic character towards the hypervalent iodide (ie: $3^{o} > 2^{o} > 1^{o}$ in the nucleophilicity trend). The vinylmagnesium chloride Grignard reagent was used to introduce the alkene group selectively onto the aldehyde. The resulting allylic alcohol **66** was oxidized with Dess-Martin periodinane to yield the desired dienedione **67**.



Scheme 2.13. Synthesis of 21-homo-21,22-dehydroprogesterone.

2.4.3. 17β-(2-propenoic methyl ester)-androsten-3β-one

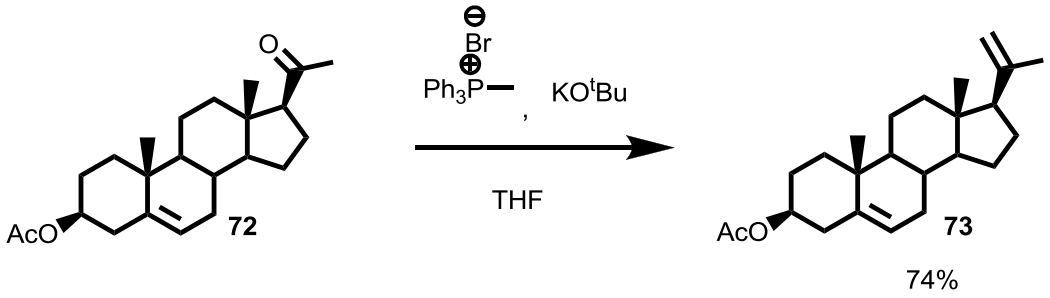
The protected 17β-carboxaldehyde **38** was also a good Wittig substrate, which reacted with the ylide from methyl iodoacetate to form **68** with the double bond on the 20,21-position. Furthermore, it is possible to reduce the enoate-double bond using magnesium in methanol at room temperature [117] (Scheme 2.14).



Scheme 2.14. Wittig reaction to homologate progesterone at 21-position.

2.4.4. 17β-(isopropenyl)-androstenone

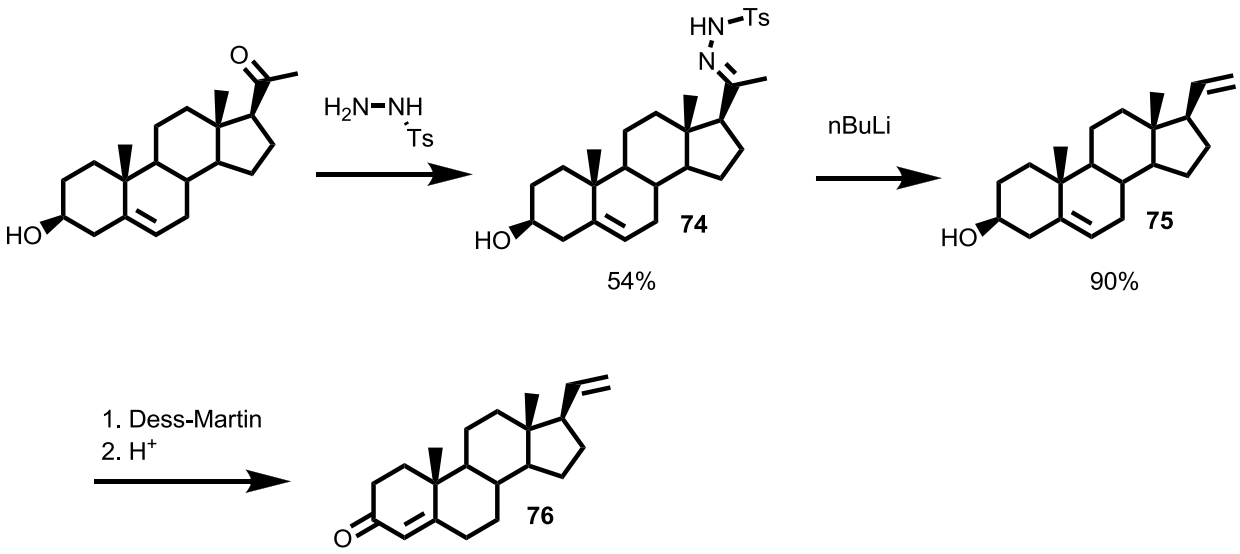
Pregnenolone-3-acetate was used as the starting material for introducing a methylene group on the 20-position to form 17β-(isopropenyl)-androstenol-3-acetate **73** via a Wittig olefination [118] (Scheme 2.15). The resulting 3-hydroxy-$\Delta^{5,6}$-alkene was oxidized/isomerized to afford the 3-keto-$\Delta^{4,5}$-steroid backbone (not shown).



Scheme 2.15. Wittig reaction to form 20-methylene prenenolone and progesterone analogs.

2.4.5. 17β-(ethenyl)-androstenone

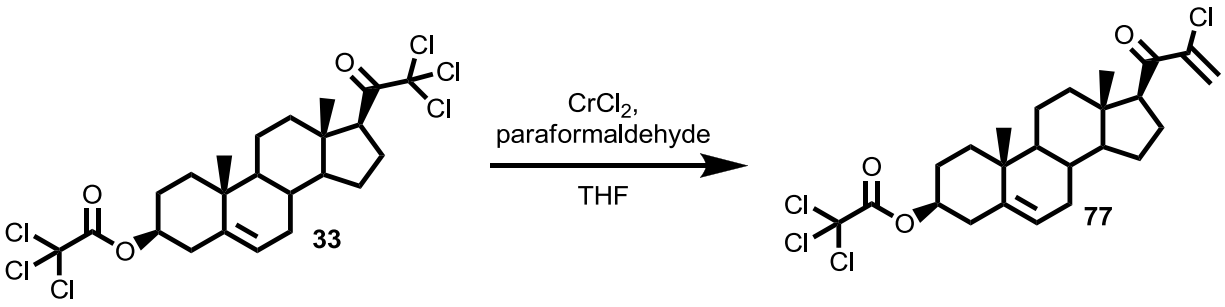
The 20-keto group of pregnenolone was converted to the tosyl hydrazone **74** and isolated after purification [119] (Scheme 2.16). The tosyl hydrazone was then eliminated with n-BuLi to afford the 20,21-alkene **75** and oxidized/isomerized to give the progesterone analog **76**.



Scheme 2.16. Preparation of 20-deoxy-vinylprogesterone analog.

2.4.6. 21-Chloro-21-homo-21,22-dehydropregnenolone-3-(2-chlorovinyl-acetate)

Falck and co-workers have reported on a method to convert trichloroketones to vinyl chlorides using an aldehyde 1-carbon source and a chromium (II) chloride additive [120]. We endeavored to convert 21,21,21-trichloropregnenolone-3-trichloroacetate to the vinyl chloride **77** in this manner (Scheme 2.17). This reaction required 20 mol equivalents of the chromium reagent – when we used 10 mol equivalents, we noticed the presence of the non-eliminated alcohol product.



Scheme 2.17. Synthesis of progesterone vinyl chloride analog **77**.

2.5. CYCLOPROPYL SUBSTRATES

Methylcyclopropyl substrates were designed as potential substrates for CYP17A1 and CYP21A2. The key feature of these substrates would be to form a radical methylcyclopropane intermediate as the enzyme abstracts hydrogen from a C-H bond adjacent to the cyclopropane (C17 or C21). This radical intermediate

can be hydroxylated if oxygen rebound is fast relative to rearrangement, but the intermediate can also undergo either a ring expansion C-C bond migration to a cyclobutene or a ring-opening rearrangement to form an allylic radical (Figure 2.3), which in turn form rearranged radicals, which are hydroxylated by the enzyme. Since the rates of these rearrangement reactions are known from model systems, this experiment is used to estimate the rate of the oxygen rebound step.

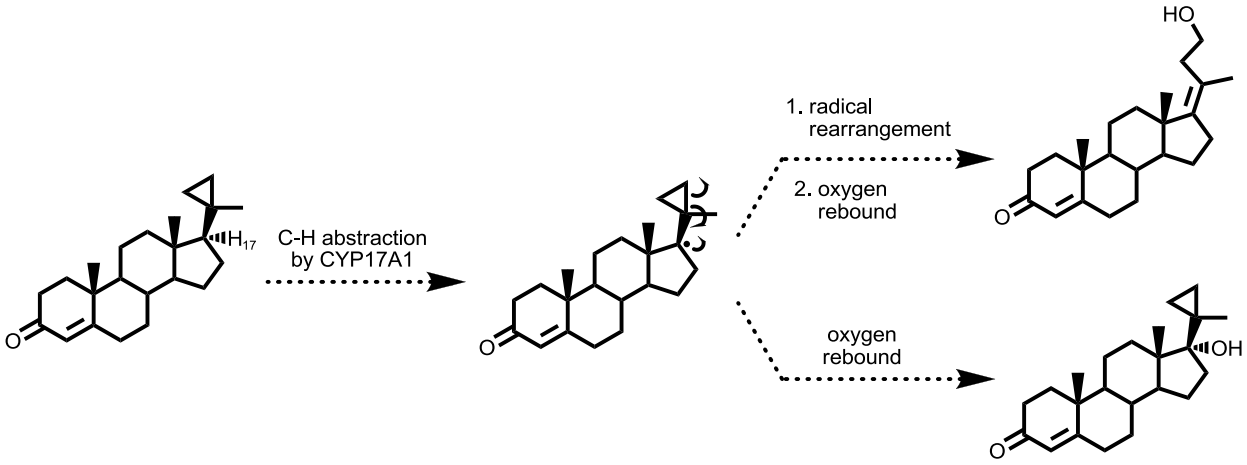
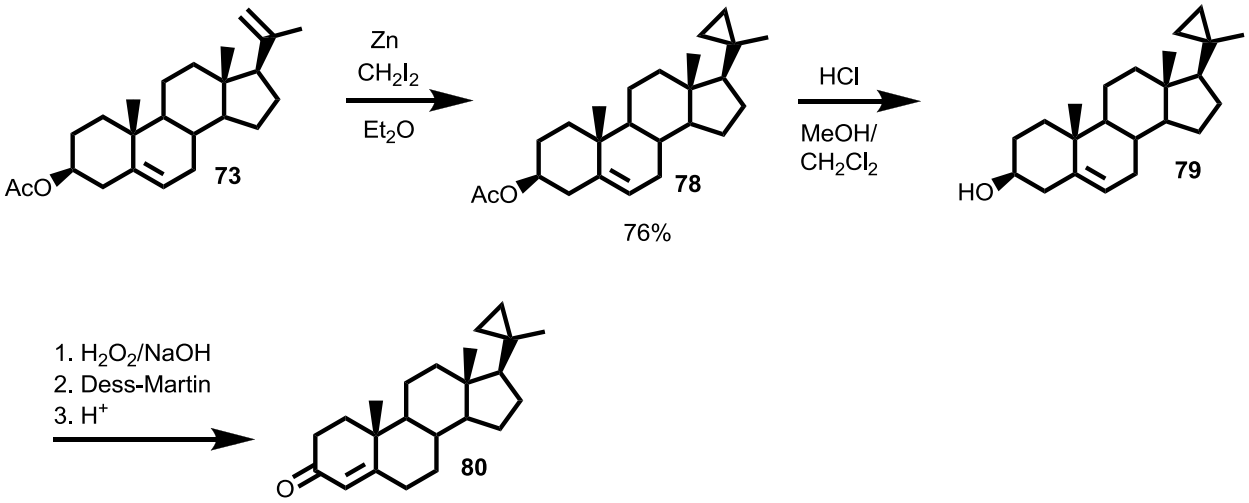


Figure 2.4. Illustration of a radical clock experiment with CYP17A1 and a possible cyclopropane substrate.

2.5.1. 20-cyclopropylprogesterone

The classical cyclopropane-forming reaction is the Simmons-Smith cyclopropanation with Zn and a carbene source. This reaction proved useful in introducing the cyclopropane group on the 20-position of the steroid backbone from the 17β-isopropenyl androsten-3β-acetate starting material **73**. The

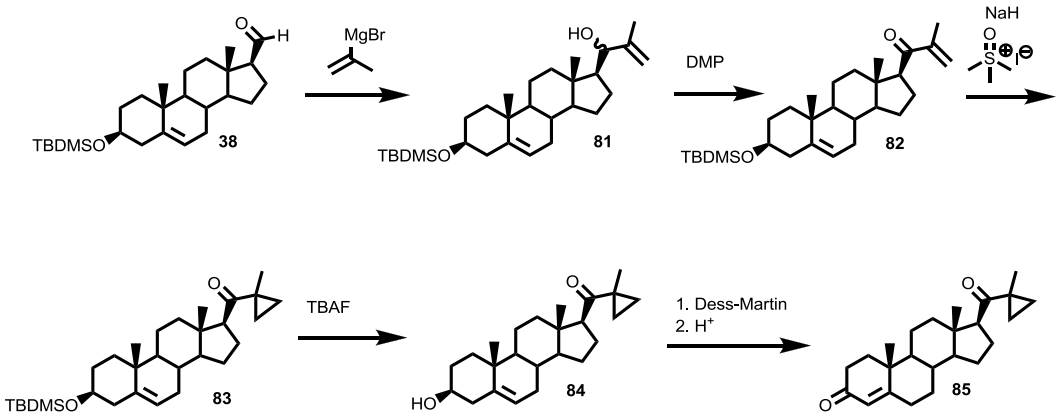
sterically hindered trisubstituted $\Delta^{5,6}$-olefin remained unreactive in these conditions (Scheme 2.18).



Scheme 2.18. Synthesis of progesterone cyclopropane analog **80**.

2.5.2. 21-cyclopropyl-21-methylprogesterone

Another cyclopropane-forming reaction we employed was the Corey-Chaykovsky reaction, which makes use of a sulfur ylide and an electrophilic alkene. We synthesized an isopropenyl ketone, which came from the aldehyde precursor. The sulfur ylide formed the cyclopropane and consumed the starting material completely. Not surprisingly, the cyclopropane was sensitive to Dess-Martin periodinane treatment, and we found it necessary to add a stoichiometric amount of the hypervalent iodine oxidant in small portions (Scheme 2.19).



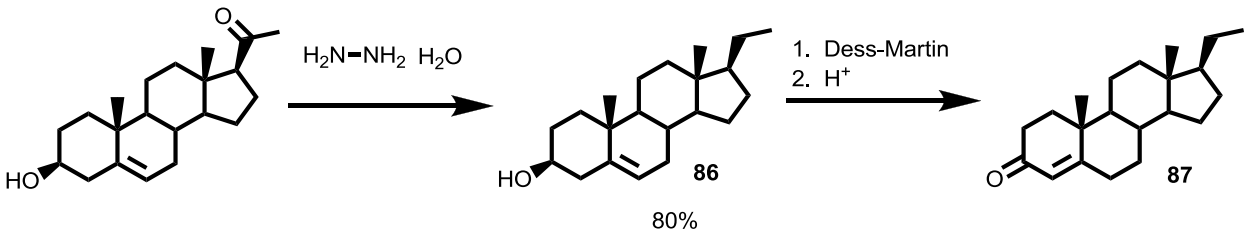
Scheme 2.19. Synthesis of methylcyclopropyl progesterone analog **85**.

2.6. OTHER VARIOUS ALKYL SUBSTRATES

Many structures of alkyl analogs of pregnenolone and progesterone can be envisioned, and a few selected examples are shown here. By testing as substrates and inhibitors, these analogs serve as probes for the active sites of CYP17A1 and CYP21A2.

2.6.1. 20-desoxyprogesterone

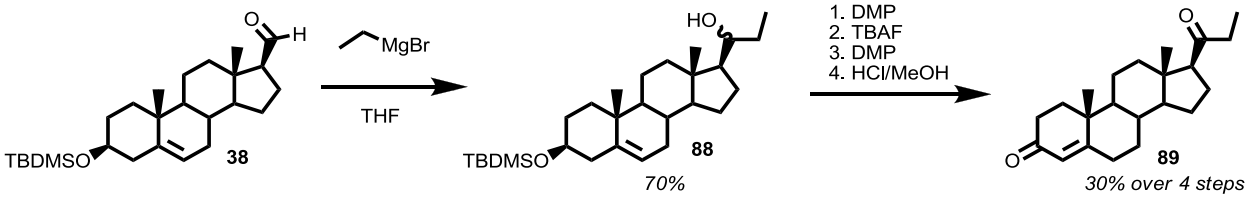
Substrate analogs might be generated by the addition of a methyl group on either the 16-, 17- or 21-positions. Moreover, 20-desoxoprogesterone **87** was accessed from a Wolff-Kishner reduction [121] of pregnenolone followed by the usual 3-hydroxy oxidation/$\Delta^{5,6}$ to $\Delta^{4,5}$-isomerization protocol with Dess-Martin periodinane and mild acidic conditions (Scheme 2.20).



Scheme 2.20. Synthesis of 20-desoxy (20-deoxy) progesterone **87**.

2.6.2. 21-homomethylprogesterone

The synthesis of 21-homomethylprogesterone **89** was achieved from the ethylmagnesium bromide addition onto the aldehyde precursor **38** (Scheme 2.21).



Scheme 2.21. Synthesis of 21-homomethylprogesterone **89**.

2.7. OXYGENATED STANDARDS

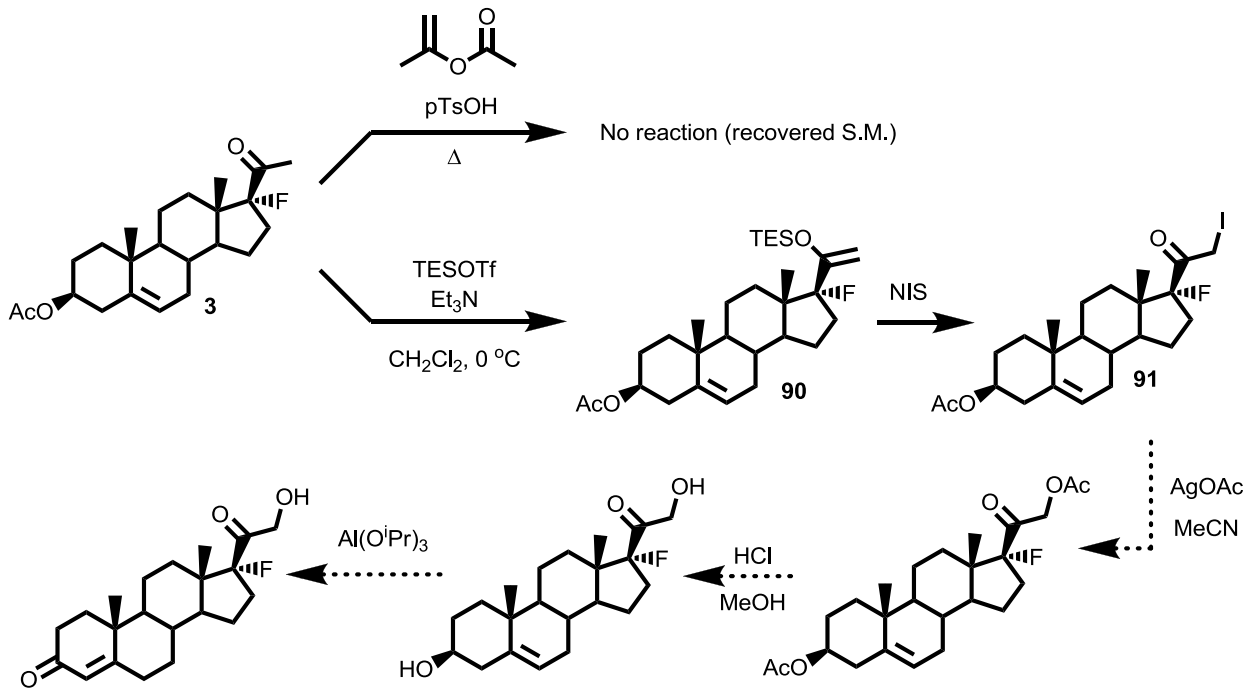
2.7.1. 17-fluoro-21-hydroxyprogesterone

The synthesis of 21-hydroxy-17-fluoroprogesterone was required to verify the metabolite in the incubation of 17-fluoroprogesterone with CYP21A2. Initially, we attempted to form the 20,21-enol acetate from 17-fluoropregnenolone-3-acetate using the same conditions to synthesize the des-

fluoro compound (compound **13**); however, we found that these conditions yielded no reaction. This poor reactivity was probably due to the 17α-fluoro group, which rigidified the 17β-acetyl group syn to the axial 18-methyl substituent located on the C-D ring junction (Scheme 2.22).

A different approach involved the use of triethylsilyl trifluoromethanesulfonate to form the silyl enol ether **90**. This reaction cleanly yielded the silyl enol ether, which was subjected to halogenations with N-iodosuccinimide to yield 17-fluoro-21-iodo-pregnenolone-3-acetate **91** (Scheme 2.22). The resulting 21-iodide was displaced with AgOAc. The resulting 3,21-diacetate was cleaved to the 3,21-diol, and the 3-hydroxy group was selectively oxidized with the Oppenauer oxidation protocol, which also isomerized the intermediate to the progesterone enone system.

In the ^{1}H NMR spectrum of the silyl enol ether intermediate, the diagnostic vinyl C21-protons appeared to verify the product. The iodide formed by addition of N-iodosuccinimide displayed a ^{1}H NMR spectrum with a slight upfield change in the chemical shift of the C21-protons, which both appeared as doublet of doublets due to geminal coupling plus the four-bond coupling from the 17-fluoro group.

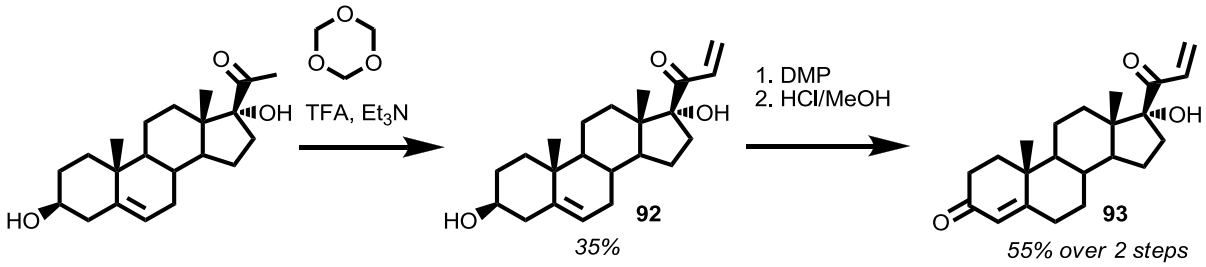


Scheme 2.22. Synthesis of 17-fluoro-21-hydroxyprogesterone.

2.7.2. 17-hydroxy-21-homo-21.22-dehydroprogesterone

Because CYP17A1 hydroxylates the 17-position of progesterone, we synthesized 21-homomethyl-17-hydroxy-progesterone as a possible product from incubations with 21-homo-21,22-dehydroprogesterone. The one-carbon homologation protocol using paraformaldehyde and the trifluoroacetic acid salt of DIPA came in useful for this route [122]. Unlike the 17-deoxy case, we observed that the Michael adducts are not facile with the 17-hydroxy-21,22-α,β-unsaturated ketone **92** (Scheme 2.23). This inertness is probably due to the 18-methyl

substituent, similar to the effect observed in the attempted formation of the 17-fluoro-20,21-enol-acetate.

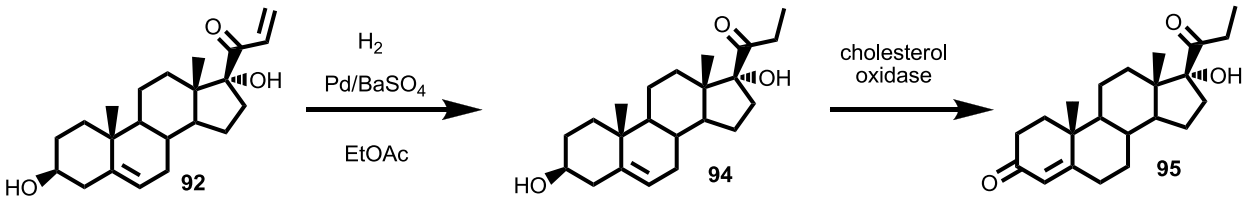


Scheme 2.23. Synthesis of 17-hydroxy-21-homo-21,22-dehydroprogesterone.

Interestingly, when we attempted to epoxidize the 21,22-position using basic conditions in the presence of hydrogen peroxide, the substituent on the D-ring was cleaved to afford DHEA [123].

2.7.3. 17-hydroxy-21-homomethylprogesterone

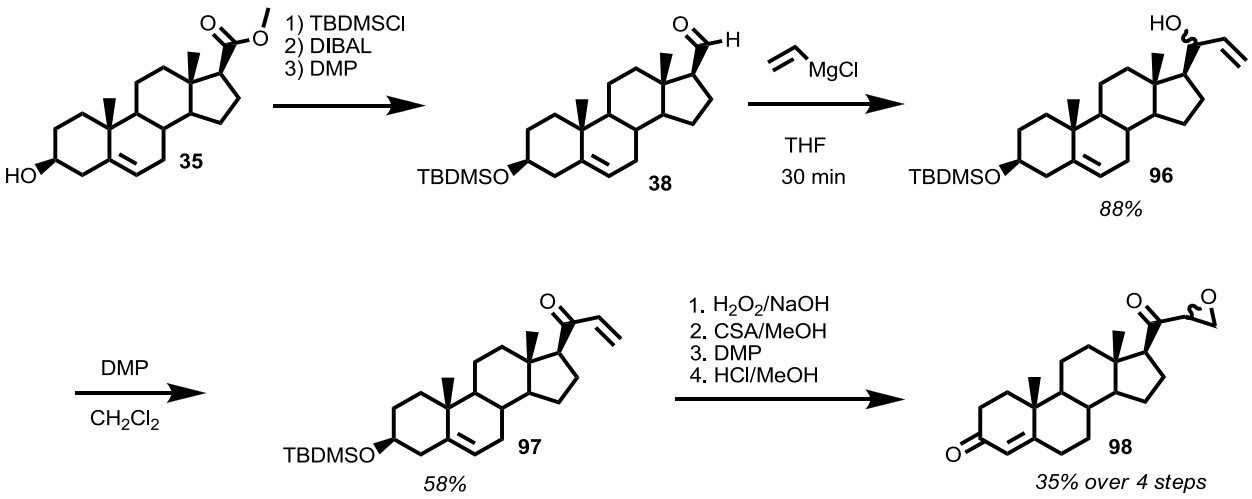
The synthesis of 17-hydroxy-21-homomethylprogesterone **95** was enabled through the selective reduction of the 21,22-alkene of 17-hydroxy-21-homodehydropregnenolone using $Pd/BaSO_4$ in the presence of hydrogen gas. The reaction must be monitored carefully to avoid reduction of the $\Delta^{5,6}$-double bond as well; the activated 21,22-olefin was hydrogenated within a few hours (Scheme 2.24). To convert the 3-hydroxy-$\Delta^{5,6}$-backbone to the 3-keto-$\Delta^{4,5}$-steroid, we used mild conditions with cholesterol oxidase on a milligram scale (see following chapter 3 on enzymology).



Scheme 2.24. Synthesis of 17-hydroxy-21-homomethylprogesterone.

2.7.4. 21,22-epoxy-21-homomethyleneprogesterone

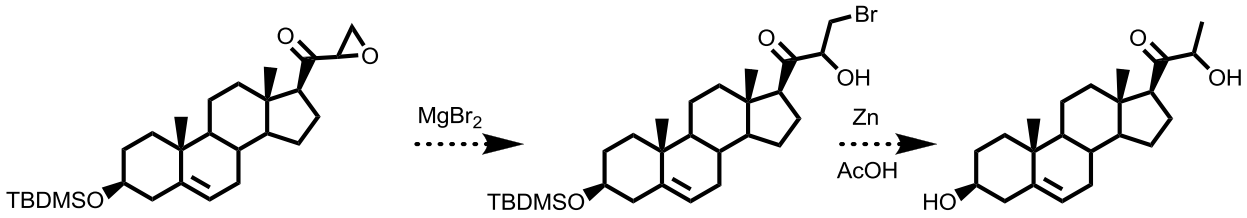
α,β-Epoxyketones have been accessed through nucleophilic epoxidation from α,β- enones using hydrogen peroxide and base. This approach stereoselectively introduced the 16α,17α-epoxy moiety to 16,17-dehydropregnenolone; however, when the 21-homo-21,22-dehydropregnenolone substrate was used, the 21,22-epoxide **98** was formed as an epimeric mixture, which was separable by silica column chromatography. We found that protection or oxidation of the 3-hydroxy group improved the separation of the 21,22-epoxide epimers (Scheme 2.25).



Scheme 2.25. Synthesis of 21,22-epoxy-21-homomethyleneprogesterone epimers **98**.

2.7.5. 21-hydroxy-21-homomethylprogesterone

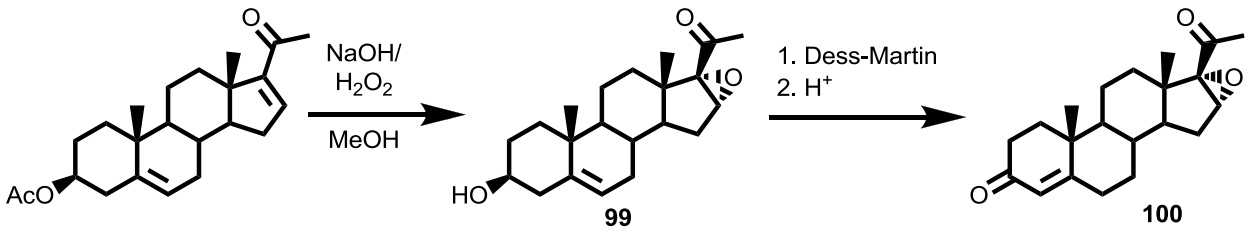
The possibility of CYP21A2 hydroxylating the 21-position of 21-homomethylprogesterone prompted us to synthesize 21-hydroxy-21-homomethylprogesterone. This compound can be accessed from the ring opening of the 21,22-epoxide using a bromide and reducing the resulting halogen with zinc in acetic acid (Scheme 2.26).



Scheme 2.26. Synthesis of 21-hydroxy-21-homomethylprogesterone.

2.7.6. 16α,17α-epoxyprogesterone

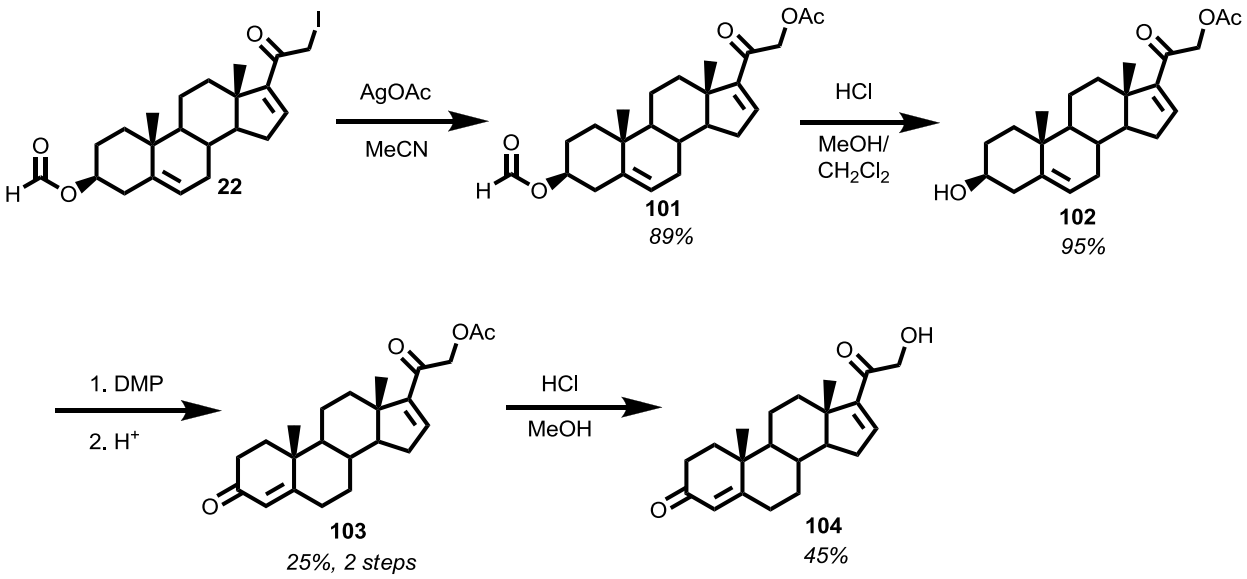
The synthesis of 16α,17α-epoxyprogesterone was achieved by epoxidizing 16,17-dehydropregnenolone-3-acetate with basic hydrogen peroxide, which simultaneously epoxidized the $\Delta^{16,17}$-olefin and cleaved the 3-acetate [124]. The resulting 3-hydroxy group was oxidized and $\Delta^{5,6}$-alkene was isomerized under mildly acidic conditions to yield 16α,17α-epoxyprogesterone **100** (Scheme 2.27).



Scheme 2.27. Synthesis of 16α,17α-epoxyprogesterone **100**.

2.7.7. 21-hydroxy-16,17-dehydroprogesterone

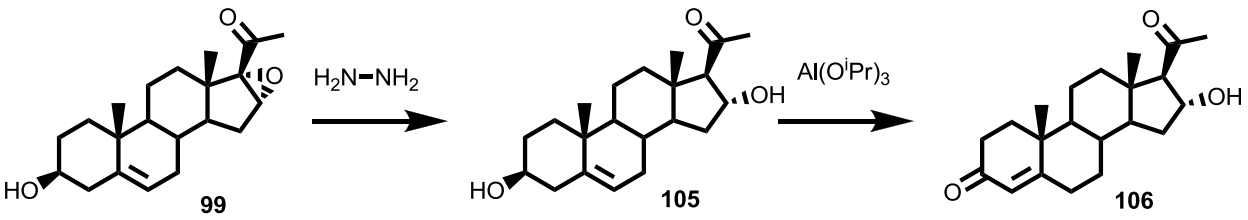
The synthesis of 21-hydroxy-16,17-dehydroprogesterone was accomplished by treating 21-iodo-16,17-dehydropregnenolone-3-formate **22** with AgOAc in acetonitrile. The 3-formate was selectively methanolyzed with careful monitoring, as the 3-formate is more reactive than the 21-acetate. The 3-hydroxy moiety was oxidized with Dess-Martin periodinane, and under acidic conditions, the 21-acetate was cleaved and the double bond was subsequently isomerized to yield 21-hydroxy-16,17-dehydroprogesterone **104** (Scheme 2.28).



Scheme 2.28. Synthesis of 21-hydroxy-16,17-dehydroprogesterone.

2.7.8. 16α-hydroxyprogesterone

Based on literature precedence, we converted 16α,17α-epoxypregnenolone to 16α-hydroxypregnenolone **105** via the treatment with hydrazine [125]. Careful purification and separation of impurities indicated the formation of the Wolff-Kishner product, 20-desoxy-16α-hydroxypregnenolone, as well. Oppenauer oxidation was used to convert 16α-hydroxypregnenolone to 16α-hydroxyprogesterone – interestingly, this oxidation procedure selectively oxidizes the 3-hydroxy group over the 16α-hydroxy group.

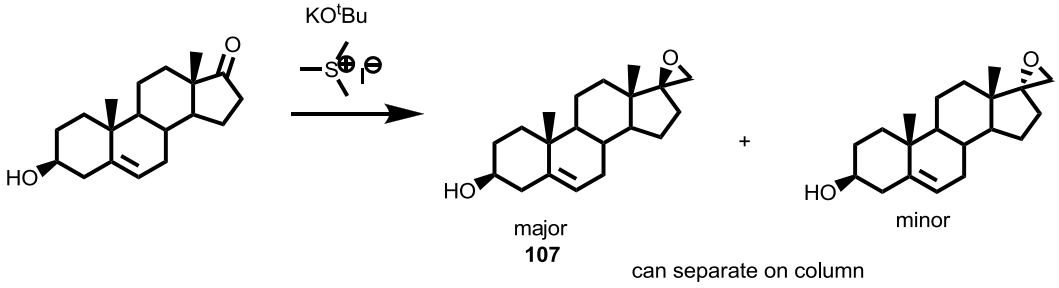


Scheme 2.29. Synthesis of 16α-hydroxyprogesterone **106**.

2.7.9. 17-epoxide

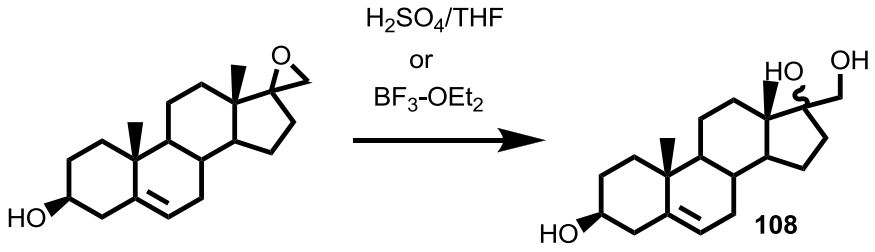
Since the 17β-carboxaldehyde precursor **38** proved to be a useful intermediate in accessing progesterone analogs with R-groups on the 21-position, we explored the synthesis of 17α-hydroxy-17β-carboxaldehyde in order to obtain 17α-hydroxyprogesterone analogs with R-groups on the 21-position.

We took advantage of the Corey-Chaykovsky reaction to synthesize the epimeric 17-epoxides from DHEA [126] (Scheme 2.30). The epoxide was heated with water and THF in the presence of catalytic amounts of sulfuric acid to form the triol.



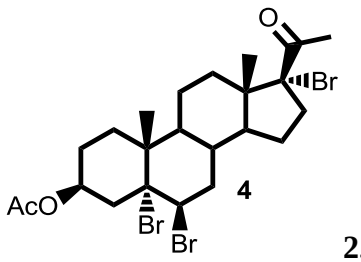
Scheme 2.30. Synthesis of spiro[androst-5-ene-17,2'-oxiran]-3-ol **107**.

Interestingly, when we attempted to convert the epoxide to the corresponding aldehyde using the Meinwald rearrangement using BF_3-OEt_2 [127], we obtained the triol **108** as the major product and the expected aldehyde as the minor product. It is possible that our BF_3 etherate reagent bottle had water as a contaminant, which would explain the triol product (Scheme 2.31). Alternatively, we can subject the epoxide to acidic conditions and form the diol.



Scheme 2.31. Synthesis of 17-(hydroxymethyl)-androst-5-ene-3,17-diol **108.**

2.8. EXPERIMENTAL

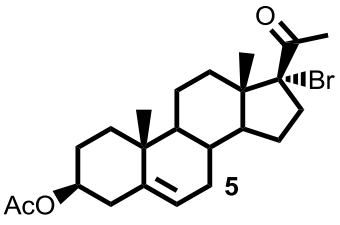


2.8.1. 5,6,17-tribromo-20-oxo-pregn-5-ene-3β-yl acetate, compound **4**

Pregnenolone (4.0 g, 12.6 mmol) and p-toluenesulfonic acid (2.4 g, 14.0 mmol, 1.1 mol eq) were refluxed in acetic anhydride (45 mL, 25 mol eq) until 20 mL of solvent was condensed out of the reaction via a Dean-Stark trap and a reflux condenser. The reaction was cooled to room temperature and then maintained at 0 ^{o}C for 1 h. The cold reaction solution was diluted with diethyl ether (25 mL) and washed with $NaHCO_3$ (sat. aqueous solution, 3 x 25 mL). The organic layer was concentrated via reduced pressure to yield enol acetate **1** as a brown solid, which was used without purification.

The crude enol acetate **1** (5.0 g, 12.5 mmol) in acetic acid (20 mL) with sodium acetate (2.0 g, 24.3 mmol, 1.9 mol eq) was treated with a mixture of bromine (1.3 mL, 25.4 mmol, 2.0 mol eq) in 5 mL H_2O, and the reaction was stirred for 1 h at room temperature. The reaction was quenched with saturated aqueous $Na_2S_2O_3$ (30 mL), extracted with ethyl acetate (3 x 15 mL), and concentrated under

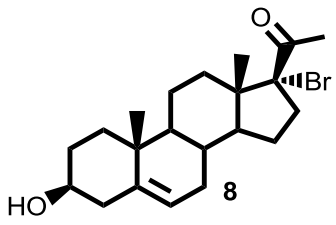
reduced pressure. The crude material was purified via flash column chromatography (10% ethyl acetate in hexanes) to yield the tribromide **4** as a white solid (5.2 g, 8.8 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.41-5.49 (m, 1H), 4.84 (broad s, 1H), 3.04 (ddd, J_1 = 16.0, J_2 = 12.0, J_3 = 3.6 Hz, 1H), 2.76 (ddd, J_1 = 15.6, J_2 = 12.4, J_3 = 4.0 Hz, 1H), 2.65-2.56 (m, 1H), 2.35 (s, 3H), 2.23-2.31 (m, 2H), 2.04-2.15 (m, 2H), 2.03 (dd, J_1 = 14.0, J_2 = 10.4 Hz, 1H), 2.02 (s, 3H), 1.78-1.99 (m, 5H), 1.69-1.75 (m, 2H), 1.56-1.65 (m, 3H), 1.44 (s, 3H), 1.21-1.32 (m, 2H), 0.78 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.3, 170.4, 87.4, 85.8, 71.9, 55.6, 49.7, 47.3, 46.6, 41.94, 41.91, 37.1, 36.6, 36.1, 35.4, 31.4, 27.5, 26.2, 22.8, 21.5, 21.4, 20.3, 14.4.



2.8.2. 17-bromopregnenolone-3-acetate (17-bromo-20-oxo-pregn-5-ene-3β-yl acetate, compound **5**)

Tribromide **4** (2.5 g, 4.2 mmol) and sodium iodide (1.5 g, 10 mmol, 2.4 mol eq) were dissolved in acetone (30 mL), and the reaction was stirred for 1 h. The reaction was quenched with $Na_2S_2O_3$ (20 mL sat. aqueous), mixed with brine (10 mL), and extracted with diethyl ether (3 x 25 mL). The organic extracts were combined and concentrated via reduced pressure, and the crude material was

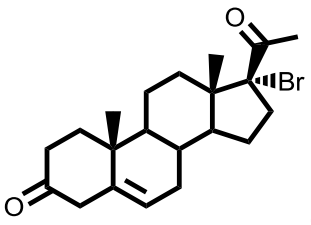
purified via silica column chromatography (10% ethyl acetate in hexanes) to yield **5** (1.4 g, 3.3 mmol, 77%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.37 (d, J = 5.2 Hz, 1H), 4.55-4.63 (m, 1H), 3.06 (ddd, J_1 = 16.0, J_2 = 12.0, J_3 = 4.0 Hz, 1H), 2.37 (s, 3H), 2.23-2.34 (m, 3H), 2.02, (s, 3H), 1.93-2.00 (m, 2H), 1.82-1.90 (m, 5H), 1.36-1.69 (m, 5H), 1.22-1.36 (m, 1H), 1.12-1.20 (m, 1H), 1.06 (dd, J_1 = 12.4, J_2 = 4.8 Hz, 1H), 1.01 (s, 3H), 0.76 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.6, 170.6, 139.7, 122.3, 86.2, 73.9, 51.1, 49.3, 47.0, 38.1, 37.0, 36.6, 36.1, 35.5, 32.4, 31.2, 27.8, 27.6, 23.1, 21.5, 21.2, 19.4, 14.0.



2.8.3. 17-bromo-3β-hydroxy-pregn-5-en-20-one (17-bromopregnenolone, compound **8**)

To a solution of acetate **5** (0.55 g, 1.26 mmol) dissolved in 5 mL CH_2Cl_2 and 45 mL methanol was added 0.2 mL of 12 M HCl, and the reaction was stirred overnight. The reaction mixture was concentrated via reduced pressure, and the crude product was purified via flash column chromatography (20% ethyl acetate in hexanes) to yield **8** (0.45 g, 1.14 mmol, 91%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.36 (d, J = 8.5 Hz, 1H), 3.48-3.59 (m, 1H), 3.08 (ddd, J_1 = 19.0, J_2 = 7.5, J_3 = 5.5 Hz, 1H), 2.38 (s, 3H), 2.23-2.34 (m, 3H), 2.03-2.07 (m, 1H),

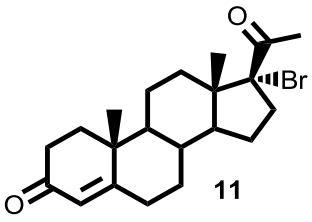
1.97-2.02 (m, 2H), 1.82-1.94 (m, 4H), 1.39-1.71 (m, 3H), 1.04-1.36 (m, 5H), 1.01 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.6, 140.9, 121.1, 86.3, 71.5, 51.1, 49.3, 46.9, 42.2, 37.3, 36.4, 36.0, 35.5, 32.3, 31.6, 31.5, 27.5, 23.0, 21.1, 19.5, 13.9.



2.8.4. 17α-bromo-pregn-5-ene-3,20-dione (17-bromo-Δ^5-progesterone)

Alcohol **6** (0.51 g, 1.28 mmol) and Dess-Martin periodinane (0.52 g, 1.21 mmol, 0.9 mol eq) were dissolved in CH_2Cl_2 (5 mL) and stirred for 30 min at room temperature, then concentrated via reduced pressure and purified via flash column chromatography (10% ethyl acetate in hexanes) to yield the 3-ketosteroid intermediate (0.36 g, 0.92 mmol, 72%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.30 (apparent t, J = 2.8 Hz, 1H), 3.23 (dd, J_1 = 16.4, J_2 = 3.6 Hz, 1H), 3.02, (ddd, J_1 = 16.4, J_2 = 12.0, J_3 = 4.0 Hz, 1H), 2.78 (dd, J_1 = 16.4, J_2 = 2.0, Hz, 1H), 2.44 (ddd, J_1 = 13.6, J_2 = 13.6, J_3 = 6.0 Hz, 1H), 2.34 (s, 3H), 2.20-2.29 (m, 2H), 1.98-2.05 (m, 2H), 1.79-1.96 (m, 4H), 1.60-1.70 (m, 2H), 1.41-1.56 (m, 3H), 1.18-1.29 (m, 1H), 1.14 (s, 3H), 1.06-1.11 (m, 1H), 0.76 (s, 3H); ^{13}C NMR (126

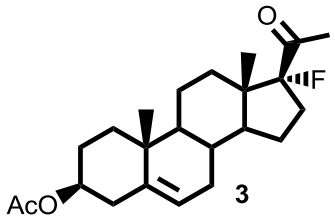
MHz, $CDCl_3$) δ 210.0, 201.4, 138.6, 122.4, 86.0, 51.0, 48.4, 48.3, 47.0, 37.6, 36.9, 36.8, 36.1, 35.4, 32.4, 31.5, 27.5, 23.0, 21.4, 19.3, 14.0.



11

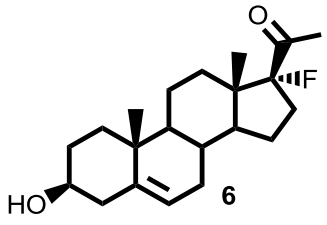
2.8.5. 17α-bromo-pregn-4-ene-3,20-dione (17-bromo-progesterone, compound **11**)

17-Bromo-pregn-5-ene-3,20-dione (0.36 g, 0.92 mmol) was dissolved in 1:1 CH_2Cl_2:methanol (30 mL), and 0.1 mL of 12 M HCl was added. After stirring at RT for 1h, the reaction mixture was concentrated via reduced pressure, and the crude material was purified via flash column chromatography (hexanes to 10% ethyl acetate in hexanes) to yield **11** [106] as a white solid (0.34 g, 0.87 mmol, 94%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.75 (s, 1H), 3.07 (ddd, J_1 = 17, J_2 = 12, J_3 = 4 Hz, 1H), 2.38 (s, 3H), 2.29-2.48 (m, 6H), 2.40 (s, 3H), 1.86-2.08 (m, 3H), 1.67-1.79 (m, 2H), 1.54-1.62 (m, 1H), 1.21 (s, 3H), 1.12-1.18 (m, 2H), 1.06 (ddd, J_1 = 10.0, J_2 = 10.0, J_3 = 3.2 Hz, 1H), 0.83 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ201.3, 199.4, 170.6, 124.1, 85.9, 52.9, 50.4, 47.0, 38.6, 36.05, 36.02, 35.7, 35.4, 34.0, 32.8, 31.7, 27.5, 22.9, 21.1, 17.5, 14.1.



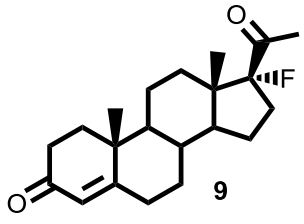
2.8.6. 17-fluoro-20-oxo-pregn-5-en-3β-yl acetate (17-fluoropregnenolone-3-acetate, compound **3**)

Enol acetate **1** (0.10 g, 0.25 mmol), Selectfluor (0.25 g, 0.71 mmol, 2.8 mol eq) and sodium acetate (0.05 g) were weighed in a screw-cap vial and dissolved in acetonitrile (3 mL). The reaction was stirred at 60 ^{o}C for 2 h, then concentrated under reduced pressure and purified via flash column chromatography (10% to 50% ethyl acetate in hexanes) to afford fluoride **3** (56 mg, 0.15 mmol, 59%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.37 (m, 1H), 4.63-4.55 (m, 1H), 2.64-2.49 (m, 1H), 2.37-2.28 (m, 1H), 2.21 (d, J_{HF} = 8 Hz, 3H), 2.01 (s, 3H), 1.98-1.93 (m, 1H), 1.20-1.10 (m, 1H), 1.00 (s, 3H), 0.64 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.7 (d, J_{CF} = 132 Hz), 170.6, 139.7, 122.4 (d, J_{CF} = 60 Hz), 112.2, 110.3, 73.9, 51.6, 49.5, 38.2, 37.1, 36.7, 32.0, 31.3, 30.7, 27.7, 23.9, 21.5, 20.6, 19.4, 14.1 (d, J_{CF} = 20 Hz).



2.8.7. 17-fluoro-3β-hydroxy-pregn-5-en-20-one (17-fluoropregnenolone, compound **6**)

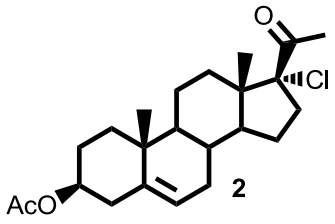
Methanolysis of acetate **3** as described in section 2.2.19, yielding compound **6** (20 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.37-5.34 (m, 1H), 3.58-3.50 (m, 1H), 2.66-2.49 (m, 1H), 2.36-2.23 (m, 1H), 2.23 (d, J_{HF} = 5.2 Hz, 3H), 2.09-1.95 (m, 2H), 1.90-1.72 (m, 3H), 1.72-1.60 (m, 2H), 1.35-1.25 (m, 1H), 1.15-1.05 (m, 1H), 1.01 (s, 3H), 0.67 (s, 3H).



9

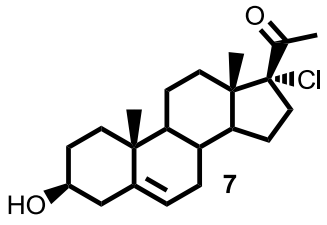
2.8.8. 17-fluoro-pregn-4-ene-3,20-dione (17-fluoroprogesterone, compound **9**)

The procedures for oxidation and isomerization of **6** to **9** [128] were followed as described in sections 2.2.20 and 2.2.21 (10 mg, 45%, 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.74 (broad s, 1H), 2.59 (dddd, J_{HF} = 40, J_1 = 12, J_2 = 4, J_3 = 2 Hz, 1H), 2.48-2.26 (m, 1H), 2.23 (d, J_{HF} = 5 Hz, 3H), 2.05-2.01 (m, 1H), 1.92-1.62 (m, 9H), 1.19 (s, 3H), 1.08-0.97 (m, 1H), 0.70 (s, 3H); additional resonances from impurities appeared in the 2.25-2.50 ppm region of the spectrum.



2.8.9. 17-chloro-20-oxo-pregn-5-en-3β-yl acetate (17-chloropregnenolone-3-acetate, compound **2**)

To enol acetate **1** (90 mg, 0.22 mmol) in CH_2Cl_2 (5 mL) in a screw cap vial was added N-chlorosuccinimide (46 mg, 0.34 mmol, 1.5 mol eq). The reaction was stirred at 55 ^oC for 2 h, concentrated under reduced pressure, and purified via flash column chromatography (10% to 50% ethyl acetate in hexanes) to afford chloride **2** (60 mg, 0.15 mmol, 69%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 5.39-5.38 (m, 1H), 4.67-4.56 (m, 1H), 3.09-2.98 (m, 1H), 2.32 (s, 3H), 2.10-1.95 (m, 3H), 2.04 (s, 3H), 1.95-1.84 (m, 4H), 1.75-1.61 (m, 3H), 1.30-1.05 (m, 4H), 1.02 (s, 3H), 0.74 (s, 3H).

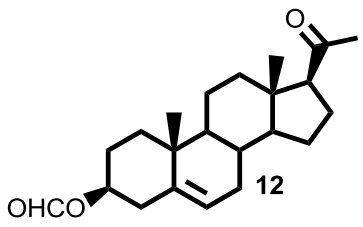


2.8.10. 17-chloro-3β-hydroxy-pregn-5-en-20-one (17-chloropregnenolone, compound **7**) and 17α-chloro-pregn-4-ene-3,20-dione (17-chloroprogesterone, compound **10**)

Using procedures described in sections 2.2.19-2.2.21, methanolysis of acetate **2** gave **7** (15 mg, 80%), and oxidation/isomerization of **7** gave **10** [129] (10 mg,

67%, 2 steps). ^{1}H NMR of compound **7** (400 MHz, $CDCl_3$) δ 5.37-5.35 (m, 1H), 3.57-3.50 (m, 1H), 3.01 (ddd, J_1 = 15.4, J_2 = 11.5, J_3 = 2.9 Hz, 1H), 2.32 (s, 3H), 2.32-2.15 (m, 2H), 2.06-1.93 (m, 3H), 1.92-1.80 (m, 4H), 1.79-1.74 (m, 1H), 1.72-1.64 (m, 2H), 1.60-1.37 (m, 3H), 1.15-1.02 (m, 2H), 1.01 (s, 3H), 0.74 (s, 3H).

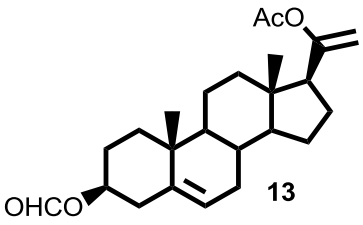
^{1}H NMR of compound **10** (400 MHz, $CDCl_3$) δ 5.75 (s, 1H), 3.07-2.96 (m, 1H), 2.45-2.26 (m, 3H), 2.32 (s, 3H), 2.09-1.66 (m, 11H), 1.19 (s, 3H), 1.06-0.97 (m, 2H), 0.91-0.79 (m, 3H), 0.77 (s, 3H).



2.8.11. 20-oxo-pregn-5-en-3β-yl formate (pregnenolone-3-formate, compound **12**)

Pregnenolone (6.0 g, 19.0 mmol) and formic acid (60 mL) were stirred for 30 min at 70 ^{o}C. The reaction was cooled to RT, diluted with diethyl ether (200 mL), and washed sequentially with water (50 mL) and $NaHCO_3$ (2 x 50 mL, saturated aqueous solution). The organic phase was concentrated and crystallized with acetone and diethyl ether to yield formate **12** (5.0 g, 14.0 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 5.38 (m, 1H), 4.77-4.67 (m, 1H), 2.52 (apparent t, J = 9 Hz, 1H), 2.36 (m, 2H), 2.21-2.10 (m, 3H), 2.10 (s, 3H), 2.05-

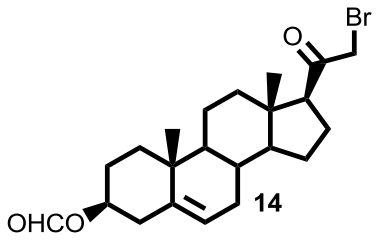
1.95 (m, 2H), 1.90-1.86 (m, 2H), 1.71-1.57 (m, 5H), 1.51-1.39 (m, 4H), 1.01 (s, 3H), 0.61 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.57, 160.7, 139.4, 122.8, 73.9, 63.8, 56.9, 50.0, 44.1, 38.9, 38.1, 37.0, 36.7, 31.9, 31.8, 31.6, 27.8, 24.6, 22.9, 21.1, 19.4, 13.3.



13

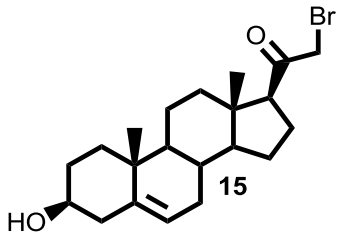
2.8.12. pregna-5,20-diene-3β,20-diyl 20-acetate 3-formate, compound **13**

To formate **12** (1.0 g, 2.8 mmol) in isopropenyl acetate (50 mL) with p-toluenesulfonic acid monohydrate (0.1 g) was refluxed for 20 h as water was removed with a Dean Stark trap. Longer times (>24 h) produced isomerization to the 17,20-enol acetate. The reaction was diluted with diethyl ether (50 mL) and washed with $NaHCO_3$ (3 x 40 mL, saturated aqueous solution). The combined organic extracts were concentrated and purified via flash column chromatography (hexanes to 50% ethyl acetate in hexanes) to afford enol acetate **13** (0.4 g, 1.0 mmol, 36%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 5.38 (d, J = 4 Hz, 1H), 4.69 (s, 2H), 4.76-4.65 (m, 1H), 2.40-2.33 (m, 2H), 2.10 (s, 3H), 1.93-1.84 (m, 4H), 1.30-1.02 (m, 5H), 1.01 (s, 3H), 0.66 (s, 3H).



2.8.13. 21-bromo-20-oxo-pregn-5-en-3β-yl formate (21-bromopregnenolone-3-formate, compound **14**)

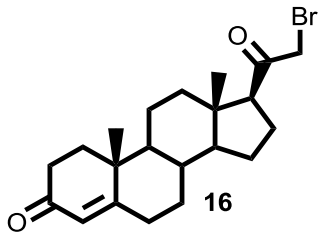
A solution of enol acetate **13** (135 mg, 0.35 mmol) and N-bromosuccinimide (0.062 g, 0.350 mmol, 1 mol eq) in methylene chloride (10 mL) was refluxed for 2 h. The reaction mixture was cooled, concentrated, and purified via flash column chromatography (hexanes to 50% ethyl acetate in hexanes) to yield 21-bromide **14** (40 mg, 0.10 mmol, 27%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1H), 5.40 (m, 1H), 4.77-4.68 (m, 1H), 3.93 (d, J = 12 Hz, 1H), 3.90 (d, J = 12 Hz, 1H), 2.83 (apparent t, J = 8 Hz, 1H), 1.35-1.11 (m, 8H), 1.02 (s, 3H), 0.66 (s, 3H).



2.8.14. 21-bromo-pregn-5-en-3β-ol-20-one (21-bromopregnenolone, compound **15**)

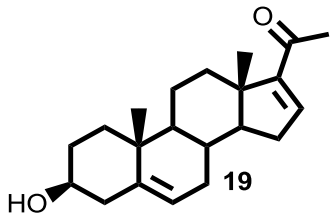
Formate **14** (40 mg, 0.1 mmol) was dissolved 5 mL each methanol and CH_2Cl_2, and 2 drops of 12 M HCl was added. The reaction was stirred for 1 h and directly

purified via flash column chromatography (10% to 50% ethyl acetate in hexanes) to afford **15** [130] (30 mg, 0.08 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ5.35 (m, J = 8 Hz, 1H), 3.94 (d, J = 12 Hz, 1H), 3.90 (d, J = 12 Hz, 1H), 3.57-3/47 (m, 1H), 2.83 (apparent t, J = 8 Hz, 1H), 2.31-2.25 (m, 3H), 2.05-1.95 (m, 2H), 1.80-1.70 (m, 4H), 1.35-1.06 (m, 5H), 1.01 (s, 3H), 0.66 (s, 3H).



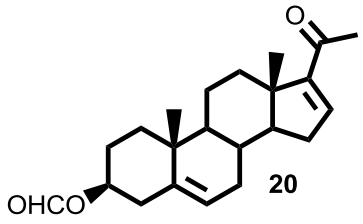
2.8.15. 21-bromo-pregn-4-ene-3,20-dione (21-bromoprogesterone, compound **16**)

To a solution of **15** (15.6 mg, 0.04 mmol) in CH_2Cl_2 (20 mL) was added Dess-Martin periodinane (17 mg, 0.04 mmol, 1 mol eq). The reaction was stirred for 2 h, and the mixture was directly purified via flash column chromatography (hexanes to 50% ethyl acetate in hexanes) to yield **16** [131] (2.0 mg, 13%). The Δ^5-olefin appeared to isomerize to the Δ^4-isomer on the silica column. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.74 (broad s, 1H), 3.93 (d, J = 12 Hz, 1H), 3.89 (d, J = 12 Hz, 1H), 2.84 (apparent t, J = 8 Hz, 1H), 2.49-2.17 (m, 6H), 1.19 (s, 3H), 1.12-0.85 (m, 5H), 0.70 (s, 3H).



2.8.16 16,17-dehydropregnenolone (compound **19**)

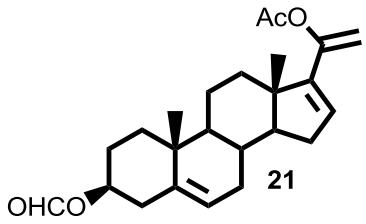
16,17-dehydropregnenolone-3-acetate (4.65 g, 13.0 mmol) was dissolved in THF (50 mL), and H_2O (10 mL) and HCl (12 N, 5 mL) was added. After stirring at RT for 3 d, the reaction was filtered through a cotton-plugged funnel, and the white solid was washed with hexanes. The solid residue was collected and was further concentrated under vacuum to yield alcohol **19** (3.5 g, 11.1 mmol, 85%) as the product.



2.8.17. 20-oxo-pregna-5,16-dien-3β-yl formate, compound **20**

Formic acid (50 mL) and 16,17-dehydropregnenolone (5.0 g, 19.2 mmol) were stirred at reflux for 30 min, cooled to RT, washed with H_2O, and extracted with diethyl ether (2 x 100 mL). The resulting mixture was crystallized with acetone and diethyl ether to yield formate **20** (5.46 g, 16.0 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 5.37 (m, 1H), 4.77-4.65 (m, 1H), 2.52 (apparent t, J = 9 Hz, 1H), 2.40-2.32 (m, 2H), 2.23-2.10 (m, 3H), 2.10 (s, 3H), 2.05-1.82 (m,

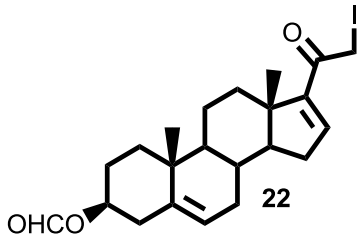
4H), 1.75-1.40 (m, 5H), 1.25-1.10 (m, 3H), 1.01 (s, 3H), 0.61 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.6, 160.7, 139.4, 122.8, 63.8, 56.9, 50.0, 44.0, 38.9, 38.1, 37.0, 36.7, 31.9, 31.8, 27.8, 24.6, 22.9, 21.1, 19.4, 13.3.



2.8.18. pregna-5,16,20-triene-3β,20-diyl 20-acetate 3-formate, compound **21**

Ketone **20** (1.22 g, 3.56 mmol) and p-toluenesulfonic acid monohydrate (0.15 g, 0.8 mmol, 0.2 mol eq) were refluxed in isopropenyl acetate (50 mL) for 20 h as water was removed with a Dean-Stark apparatus. The reaction was cooled to RT and diluted with diethyl ether (100 mL). The solution was washed with $NaHCO_3$ (saturated aqueous solution, 2 x 50 mL), and the organic layer was washed with brine (2 x 25 mL), dried with $MgSO_4$, and concentrated via reduced pressure. The solid formed during concentration was washed with ice-cold methanol to yield the enol acetate **21** [132] (0.90 g, 2.34 mmol, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1H), 5.82 (m, 1H), 5.40 (m, 1H), 5.06 (s, 1H), 4.78 (s, 1H), 4.78-4.65 (m, 1H), 2.39-2.36 (m, 2H), 2.18 (s, 3H), 2.15-2.10 (m, 1H), 2.04-1.99 (m, 1H), 1.95-1.86 (m, 3H), 1.70-1.47 (m, 8H), 1.19-1.13 (m, 1H), 1.07 (s, 3H), 0.98 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.2, 160.7, 149.7, 148.2, 139.7,

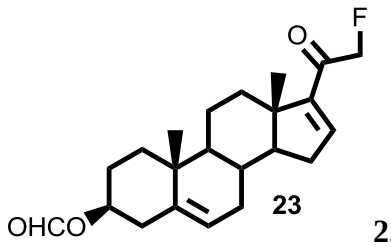
129.8, 122.7, 104.8, 102.3, 73.9, 57.0, 50.1, 46.1, 38.1, 36.9, 35.3, 31.6, 31.0, 30.2, 27.8, 21.0, 20.9, 19.3, 15.9.



22

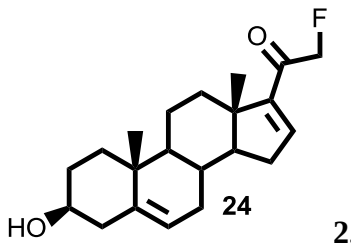
2.8.19. 21-iodo-20-oxo-pregna-5,16-dien-3β-yl formate, compound **22**

To enol acetate **21** (0.33 g, 0.86 mmol) in CH_2Cl_2 (50 mL) was added N-iodosuccinimide (0.29 g, 1.29 mmol, 1.5 mol eq). The reaction was stirred at RT for 1 h and directly purified via flash column chromatography (hexanes to 50% ethyl acetate in hexanes) to afford iodide **22** (0.30 g, 0.64 mmol, 74%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (s, 1H), 6.79 (s, 1H), 5.39 (broad s, 1H), 4.70-4.65 (m, 1H), 4.07 (d, J =12 Hz, 1H), 4.02 (d, J = 12 Hz, 1H), 2.39-2.30 (m, 5H), 2.05-1.98 (m, 1H), 1.92-1.80 (m, 2H), 1.76-1.53 (m, 5H), 1.50-1.43 (m, 1H), 1.37 (ddd, J_1 = 12, J_2 = 12, J_3 = 5 Hz, 1H), 1.14 (apparent t, J = 12 Hz, 1H), 1.06 (s, 3H), 0.96 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.2, 160.7, 151.7, 145.7, 140.0, 122.4, 73.9, 56.1, 50.4, 46.5, 38.2, 36.9, 36.8, 34.4, 32.7, 31.6, 30.2, 27.8, 20.7, 19.3, 15.6, 3.8.



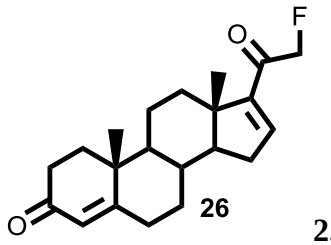
2.8.20. 21-fluoro-20-oxo-pregna-5,16-dien-3β-yl formate, compound **23**

A solution of iodide **22** (0.70 g, 1.45 mmol) in acetonitrile (50 mL) with AgF (0.54 g, 4.26 mmol, 3.4 mol eq) was stirred at RT for 36 h to complete the reaction. After addition of water, the mixture was extracted with ethyl acetate (2 x 25 mL), and the combined organic extracts were concentrated via reduced pressure. The crude material was purified via flash column chromatography (hexanes to 50% ethyl acetate in hexanes) to yield fluoride **23** [133] (0.39 g, 1.07 mmol, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (s, 1H), 6.78 (s, 1H), 5.4 (broad s, 1H), 5.16 (dd, J_{HF} = 24, J_1 = 8 Hz, 1H), 5.04 (dd, J_{HF} = 24, J_1 = 8 Hz, 1H), 4.80-4.65 (m, 1H), 2.48-2.29 (m, 5H), 2.20-1.95 (m, 4H), 1.95-1.80 (m, 4H), 1.07 (s, 3H), 0.96 (s, 3H).



2.8.21. 21-fluoro-3β-hydroxy-pregna-5,16-dien-20-one (16,17-dehydro-21-fluoropregnenolone, compound **24**)

Formate **23** (0.39 g, 1.07 mmol) was methanolyzed with 12 M HCl as in section **2.8.14.** to give **24** (0.27 g, 0.81 mmol, 76% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.77 (s, 1H), 5.35 (m, 1H), 5.13 (dd, J_{HF} = 24, J_1 = 5 Hz, 1H), 5.04 (dd, J_{HF} = 24, J_1 = 5 Hz, 1H), 3.54-3.48 (m, 1H), 2.42-2.20 (m, 5H), 2.15-1.95 (m, 2H), 1.88-1.80 (m, 2H), 1.56-1.46 (m, 1H), 1.45-1.32 (m, 2H), 1.12-1.01 (m, 3H), 1.04 (s, 3H), 0.95 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.1 (d, J_{CF} = 16 Hz), 151.6, 145.4 (d, J_{CF} = 38 Hz), 141.5, 121.0, 83.4 (d, J_{CF} = 181 Hz), 71.8, 56.0, 50.6, 46.9, 42.4, 37.2, 36.8, 34.5, 33.0, 32.4, 31.7, 30.2, 20.8, 19.4, 16.0.

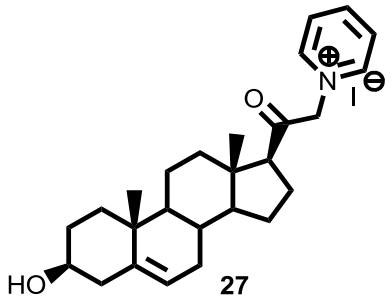


26

2.8.22. 21-fluoro-pregna-4,16-diene-3,20-dione (16,17-dehydro-21-fluoroprogesterone, compound **26**)

Using the procedure in section **2.8.4.**, **24** (0.27 g, 0.81 mmol) was oxidized to **25**, followed by isomerization as in section **2.8.5.** to yield **26** (0.18 g, 0.54 mmol, 67%, 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.78 (s, 1H), 5.74 (broad s, 1H), 5.15 (dd, J_{HF} = 24, J_1 = 12 Hz, 1H), 5.05 (dd, J_{HF} = 24, J_1 = 12 Hz, 1H), 2.50-2.25 (m, 5H), 2.16-2.10 (m, 1H), 2.06-2.00 (m, 1H), 1.92-1.85 (m, 1H), 1.82-1.50 (m, 6H), 1.46-1.33 (m, 3H), 1.22 (s, 3H), 0.98 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 210.2, 192.1 (d, J_{CF} = 16 Hz), 151.5, 145.3 (d, J_{CF} = 5 Hz), 139.3, 122.3, 104.9,

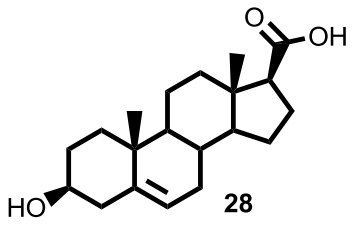
83.4 (d, J_{CF} = 183 Hz), 55.8, 49.7, 48.5, 47.0, 37.7, 37.2, 34.4, 33.0, 31.6, 30.3, 21.0, 19.2, 16.0.



27

2.8.23. pregn-5-ene-21-N-pyridyl-3β-ol-20-one iodide (21-N-pyridinyl-pregnenolone iodide, compound **27**)

Iodine (6.0 g, 23.6 mmol, 1.3 mol eq) was added to a heated stirring solution of pregnenolone (6.0 g, 19.0 mmol) in pyridine, and the reaction was stirred at reflux for 2 h under a N_2 atmosphere. Additional iodine (3.0 g, 11.8 mmol) was added, and the resulting mixture was refluxed overnight. The reaction mixture was cooled to room temperature, diluted with ethyl acetate (100 mL), filtered through cotton, and dried under reduced pressure to afford the crude pyridinium salt **27** as a yellow powder, which was used without purification. ^{1}H NMR (500 MHz, CD_3OD) δ 8.78 (d, J = 5.5 Hz, 1H), 8.64 (t, J = 8.0 Hz, 1H), 8.14 (apparent t, J = 7.0 Hz, 2H), 5.76 (d, J = 17.5 Hz, 1H), 5.61 (d, J = 17.5 Hz, 1H), 5.34 (d, J = 5.5 Hz, 1H), 3.39 (ddd, J_1 = 15.5, J_2 = 10.5, J_3 = 4.5 Hz, 1H), 2.83 (apparent t, J = 9 Hz, 1H), 2.25-2.16 (m, 3H), 2.04-1.98 (m, 2H), 1.90-1.85 (m, 2H), 1.79-1.77 (m, 2H), 1.72-1.68 (m, 1H), 1.63-1.43 (m, 4H), 1.31-1.24 (m, 3H), 1.15-1.00 (m, 2H), 1.02 (s, 3H), 0.88-0.83 (m, 1H), 0.74 (s, 3H).

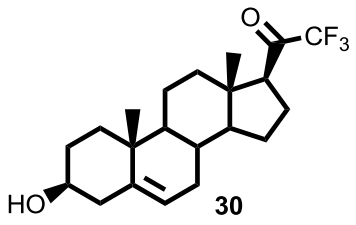


28

2.8.24. 3β-hydroxy-androst-5-ene-17β-carboxylic acid (etienic acid, compound **28**)

To the crude pyridinium salt **27** dissolved in THF (40 mL), NaOH pellets (5.2 g) were added, and the reaction was stirred at reflux. After 10 h, the reaction was cooled to RT, diluted with methanol (4 mL) and CH_2Cl_2 (30 mL), and washed with saturated aqueous $NaHCO_3$ solution to submerge the carboxylic acid in the aqueous layer (50 mL). The aqueous layer was separated and concentrated via reduced pressure. The resultant solid was filtered through a cotton-plugged funnel with CH_2Cl_2 (100 mL), and the sodium salt **28a** was collected as a white powder (3.8 g, 11.2 mmol, 59% over two steps). The carboxylic acid **28** [134] is obtained by dispersing **28a** in diethyl ether, washing with 10% HCl, and concentrating the organic extracts. ^{1}H NMR of carboxylic acid **20** (300 MHz, $CDCl_3$) δ 5.35 (d, J = 5.1 Hz, 1H), 3.53 (ddd, J_1 = 16.5, J_2 = 10.8, J_3 = 5.1 Hz, 1H), 2.40 (apparent t, J = 8.7 Hz, 1H), 2.34-2.23 (m, 2H), 2.11-1.94 (m, 3H), 1.90-1.82 (m, 3H), 1.47-1.80 (m, 7H), 1.34-1.23 (m, 2H), 1.23-1.07 (m, 1H), 1.02 (s, 3H), 1.04-0.94 (m, 1H), 0.75 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 177.8,

142.4, 122.4, 72.5, 57.7, 56.5, 51.8, 44.9, 43.1, 39.5, 38.7, 37.9, 33.5, 33.1, 32.4, 25.7, 24.8, 22.3, 20.1, 13.8.



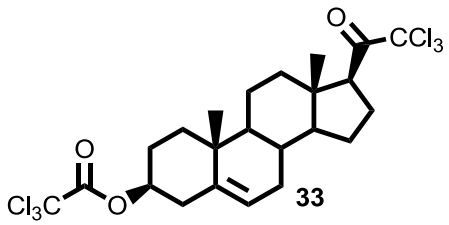
30

2.8.25. 21,21,21-trifluoro-3β-hydroxy-pregn-5-en-20-one (21,21,21-trifluoropregnenolone, compound **30**) and 21,21,21-trifluoro-pregn-4-ene-3,20-dione (21,21,21-trifluoroprogesterone, compound **32**)

The carboxylic acid **28** (0.2 g, 0.6 mmol, 1.0 mol eq) in dry toluene (20 mL) was cooled to 0 ^{o}C. Pyridine (1.0 mL, 8.6 mmol, 14 mol eq) was added via syringe under a N_2 atmosphere followed by trifluoroacetic anhydride (1.2 mL, 8.6 mmol, 13.7 mol eq). The reaction was stirred at reflux for 16 h, and a second portion of trifluoroacetic anhydride (1.0 mL) was added via syringe. Once the trifluoroketone was verified by NMR and TLC, water (0.2 mL) was added, and the reaction continued to stir at reflux for 2 h. The reaction was cooled to room temperature, washed with H_2O (20 mL), and the aqueous layer was back-extracted with ethyl acetate (2 x 20 mL). The combined organic extracts were concentrated, and the crude material was purified via flash column chromatography (hexanes to 50% ethyl acetate in hexanes) to yield **30** as a light yellow solid (0.1 g, 0.27 mmol, 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.32 (d, J =

5.2 Hz, 1H), 3.50 (ddd, J_1 = 15.6, J_2 = 11.2, J_3 = 4.4 Hz, 1H), 2.93 (apparent t, J = 9.2 Hz, 1H), 2.30-2.10 (m, 3H), 2.02-1.93 (m, 3H), 1.89-1.71 (m, 4H), 1.63-1.41 (m, 5H), 1.38-1.18 (m, 2H), 1.10-1.02 (m, 1H), 1.00-0.93 (m, 1H), 0.98 (s, 3H), 0.72 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 193.9 (q, J_{CF} = 33.9 Hz), 140.9, 121.2, 115.5 (q, J_{CF} = 174.7), 71.6, 57.3, 56.7, 49.8, 47.1, 42.2, 38.04, 38.03, 37.3, 36.5, 32.1, 31.8, 31.6, 24.7, 21.1, 19.4, 13.5.

Compound **32** (21,21,21-trifluoroprogesterone) is obtained by Oppenauer oxidation of compound **30** as previously described [109] or the by the procedure in sections 2.2.20 and 2.2.21.

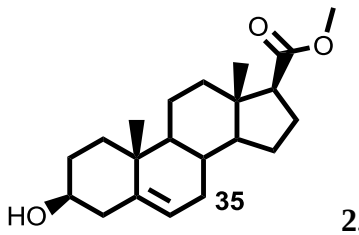


33

2.8.26. 21,21,21-trichloro-3β-trichloroacetoxy-pregn-5-en-20-one (21,21,21-trichloropregnenolone-3-trichloroacetate, compound **33**)

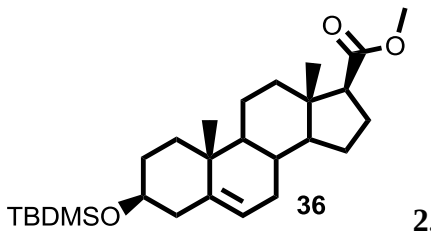
To carboxylic acid **28** (1.09 g, 3.5 mmol) in CH_2Cl_2 (100 mL) was added pyridine (1.5 mL, 18.6 mmol, 5.4 mol eq) and trichloroacetic anhydride (1.0 mL, 8.9 mmol, 2.6 mol eq) under a N_2 atmosphere at 0 ^{o}C. The reaction was warmed to RT and stirred for 18 h. The resulting reaction mixture was concentrated via reduced pressure and purified via flash column chromatography (hexanes to 50%

ethyl acetate in hexanes) to yield trichloroketone **33** (0.65 g, 1.2 mmol, 33%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.42 (m, 1H), 4.80-4.71 (m, 1H), 3.22 (apparent t, J = 8 Hz, 1H), 2.51-2.39 (m, 2H), 2.22-2.11 (m, 1H), 2.07-1.89 (m, 6H), 1.82-1.71 (m, 2H), 1.39-1.31 (m, 1H), 1.27-1.14 (m, 2H), 1.06 (s, 3H), 0.93 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.9, 161.5, 138.9, 123.3, 79.7, 56.9, 54.2, 49.8, 47.3, 38.7, 37.4, 36.9, 36.7, 32.1, 32.0, 31.4, 27.2, 25.4, 21.1, 19.5, 13.6.



2.8.27. methyl 3β-hydroxy-androst-5-ene-17β-carboxylate (etienic acid methyl ester, compound **35**)

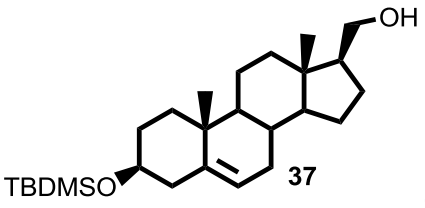
To pyridinium iodide **27** (6.5 g, 12.5 mmol) dissolved in methanol (10 mL) was added sodium methoxide solution (5.4 M, 10 mL). The reaction was stirred for 48 h and loaded directly on a silica gel column (hexanes to 50% ethyl acetate in hexanes), and methyl ester **35** (3.3 g, 9.9 mmol, 79%) was purified as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.36-5.34 (m, 1H), 3.67 (s, 3H), 3.56-3.49 (m, 1H), 2.37-2.24 (m, 3H), 2.21-2.09 (m, 2H), 2.00-1.94 (m, 2H), 1.89-1.65 (m, 4H), 1.53-1.38 (m, 4H), 1.35-1.23 (m, 3H), 1.15-1.05 (m, 2H), 1.00 (s, 3H), 0.67 (s, 3H).



2.8.28. methyl 3β-(t-butyl)dimethyl-siloxy-androst-5-ene-17β-carboxylate (etienic acid methyl ester-3-TBDMS-ether, compound **36**)

Solid t-butyldimethylsilyl chloride (0.61 g, 4.0 mmol, 1.3 mol eq) followed by imidazole (0.28 g, 4.2 mmol, 1.3 mol eq) was added to a stirring solution of methyl ester **35** (1.05 g, 3.2 mmol) in dimethylformamide (50 mL). The reaction was stirred for 1 h, and the resulting mixture was diluted with ethyl acetate (50 mL) and washed with water (at least 2 x 30 mL). The combined organic extracts were concentrated via reduced pressure and purified via flash column chromatography (hexanes to 50% ethyl acetate in hexanes), which also removed residual dimethylformamide and yielded TBDMS-ether **36** (0.70 g, 1.6 mmol, 50%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.31 (broad s, 1H), 3.66 (s, 3H), 3.50-3.44 (m, 1H), 2.34 (apparent t, J = 12 Hz, 1H), 2.30-2.19 (m, 2H), 2.19-2.09 (m, 2H), 2.05-1.94 (m, 2H), 1.85-1.65 (m, 5H), 1.60-1.37 (m, 4H), 1.32-1.22 (m, 2H), 1.14-1.04 (m, 2H), 0.99 (s, 3H), 1.00-0.89 (m, 2H), 0.88 (s, 12H), 0.66 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 141.7, 121.0, 72.7, 61.5, 56.3,

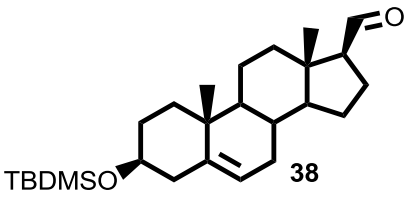
55.3, 51.4, 50.3, 44.1, 42.9, 38.4, 37.5, 36.8, 32.2, 32.0, 26.1, 24.7, 23.8, 21.0, 19.6, 18.4, 13.5, 4.5.



2.8.29. 3β-(t-butyl)dimethyl-siloxy-androst-5-ene-17β-methanol, compound **37**

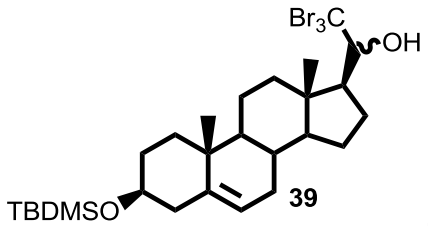
To methyl ester **36** (1.56 g, 3.5 mmol) in CH_2Cl_2 (100 mL) was added di-isobutyl aluminum hydride (3.0 mL, 16.8 mmol, 4.8 mol eq, neat) at -78 ^oC under a N_2 atmosphere. The reaction was stirred for 1 h, then ethyl acetate (10 mL) and a saturated aqueous solution of Rochelle's salt (10 mL) were added. The reaction mixture warmed to RT and stirred until the two layers separated (~1-2 h). The aqueous layer was extracted with ethyl acetate (3 x 40 mL), and the combined organic extracts were dried with $MgSO_4$ and concentrated under reduced pressure. The crude material was purified via flash column chromatography (hexanes to 50% ethyl acetate in hexanes) to yield alcohol **37** (1.28 g, 3.1 mmol, 84%). ^1H NMR (400 MHz, $CDCl_3$) δ 5.31 (broad s, 1H), 3.71 (dd, J_1 = 12.0, J_2 = 8.0 Hz, 1H), 3.53 (dd, J_1 = 12.0, J_2 = 8.0 Hz, 1H), 3.53-3.44 (m, 1H), 2.30-2.25 (m, 1H), 2.25-2.15 (m, 1H), 2.05-1.95 (m, 1H), 1.85-1.75 (m, 3H), 1.75-1.40 (m, 10H), 1.00 (s, 3H), 0.89 (s, 9H), 0.66 (s, 3H), 0.06 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$)

δ 141.7, 121.2, 72.7, 64.8, 56.4, 53.1, 50.6, 43.0, 41.8, 38.8, 37.5, 36.8, 32.2, 32.1, 31.8, 26.1, 25.7, 24.8, 20.9, 19.6, 18.4, 12.6, -4.4.



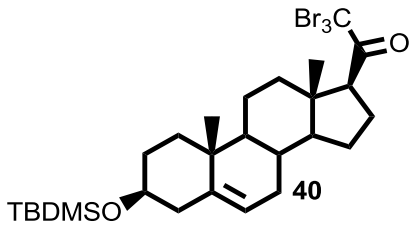
2.8.30. 3β-(t-butyl)dimethyl-siloxy-androst-5-ene-17β-carboxaldehyde, compound **38**

Alcohol **37** (1.03 g, 2.5 mmol) in CH_2Cl_2 (100 mL) was treated at RT with Dess-Martin periodinane (1.53 g, 3.6 mmol, 1.4 mol eq), and the reaction was stirred for 2 h. The reaction mixture was washed with saturated aqueous $NaHCO_3$ and extracted with ethyl acetate. The combined organic extracts were dried with $MgSO_4$ and concentrated under reduced pressure. The crude material was purified via flash column chromatography (hexanes to 50% ethyl acetate in hexanes) to yield aldehyde **38** (0.66 g, 1.6 mmol, 65%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.77 (d, J = 4 Hz, 1H), 5.31 (broad s, 1H), 3.51-3.43 (m, 1H), 2.38-1.97 (m, 9H), 1.85-1.71 (m, 6H), 1.35-1.21 (m, 3H), 1.00 (s, 3H), 0.88 (s, 9H), 0.76 (s, 3H), 0.05 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.2, 141.7, 120.9, 72.6, 63.0, 56.6, 50.3, 44.9, 42.9, 38.5, 37.5, 36.8, 32.2, 32.0, 31.6, 26.1, 26.0, 25.1, 21.2, 20.7, 19.6, 18.4, 13.9.



2.8.31. 21,21,21-tribromo-pregn-5-ene-3β-(t-butyl)dimethylsiloxy-20-ol, compound **39**

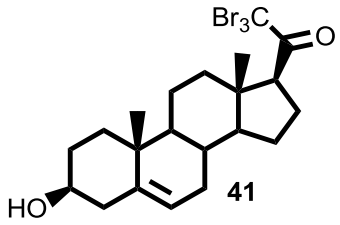
To a flask containing aldehyde **38** (0.78 g, 1.9 mmol) was added bromoform (1.4 mL, 16.0 mmol, 8.5 mol eq) followed by DBU (0.32 mL, 2.1 mmol, 1.1 mol eq). The reaction was stirred at RT for 24 h and partially purified via flash column chromatography (hexanes to 30% ethyl acetate in hexanes) to yield a mixture of the 20α- and 20β-carbinols **39** with unreacted **38** (0.56 g total weight), which was used directly for the next step.



2.8.32. 21,21,21-tribromo-pregn-5-ene-3β-(t-butyl)dimethylsiloxy-20-one, compound **40**

To the mixture of 20α- and 20β-alcohols **39** with unreacted aldehyde **38** (0.56 g, 1.0 mmol) in CH_2Cl_2 (50 mL) was added Dess-Martin periodinane (0.60 g, 1.4 mmol, 1.3 mol eq). The reaction was stirred for 2 h and purified via flash column chromatography (hexanes to 50% ethyl acetate in hexanes) directly to yield

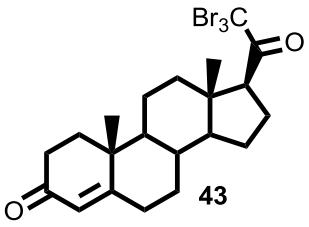
ketone **40** (0.19 g, 0.28 mmol, 27%, 15% over two steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.31 (broad s, 1H), 3.53-3.44 (m, 1H), 3.37 (apparent t, J = 8 Hz, 1H), 2.32-2.11 (m, 2H), 2.10-1.94 (m, 2H), 1.86-1.75 (m, 2H), 1.65-1.35 (m, 5H), 1.25 (s, 3H), 1.02 (s, 3H), 0.96 (s, 3H), 0.88 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (100 MHz, CD Cl_3) δ 192.1, 141.8, 120.9, 72.6, 56.9, 52.9, 50.0, 47.4, 42.9, 39.3, 37.5, 36.8, 33.0, 32.2, 32.1, 29.9, 26.1, 25.6, 21.2, 19.6, 18.4, 13.7, 9.9, -4.4.



2.8.33. 21,21,21-tribromo-pregn-5-en-3β-ol-20-one (21,21,21-tribromopregnenolone, compound **41**)

To a solution of **40** (0.19 g, 0.28 mmol) in CH_2Cl_2 (10 mL) and methanol (5 mL) was added camphorsulfonic acid (0.2 mg, 0.85 μmol, 0.003 mol eq). The reaction was stirred for 12 h and directly purified via flash column chromatography (hexanes to 50% ethyl acetate in hexanes) to afford tribromide **41** (90 mg, 0.16 mmol, 58%) as a white solid. Deprotection using p-toluenesulfonic acid was also successful, but longer reaction times led to formation of the dibromide (21,21-dibromopregnenolone) by mono-dehalogenation. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.36 (m, 1H), 3.58-3.48 (m, 1H), 3.37 (apparent t, J = 8 Hz, 1H), 2.35-2.20 (m,

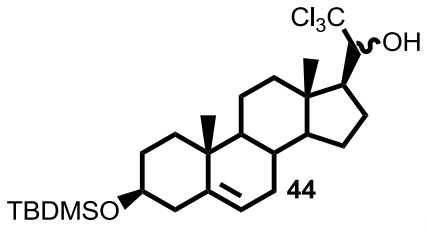
3H), 2.13-1.93 (m, 3H), 1.90-1.75 (m, 3H), 1.65-1.35 (m, 9H), 1.15-0.95 (m, 1H), 1.03 (s, 3H), 0.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.1, 141.0, 121.4, 71.8, 56.9, 52.9, 50.1, 47.4, 42.3, 39.3, 37.4, 36.7, 33.0, 32.2, 32.0, 31.7, 25.6, 21.2, 19.6, 13.7.



43

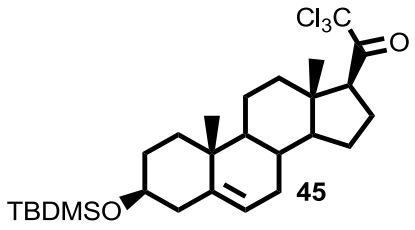
2.8.34. 21,21,21-tribromo-pregn-4-ene-3,20-dione (21,21,21-tribromoprogesterone, compound **43**)

To a solution of 21,21,21-tribromopregnenolone **41** (70 mg, 0.13 mmol) in CH_2Cl_2 (15 mL) was added Dess-Martin periodinane (55 mg, 0.13 mmol, 1.0 mol eq). After stirring for 3 h at RT, the reaction mixture was directly purified via flash column chromatography (hexanes to 50% ethyl acetate in hexanes) to yield 21,21,21-tribromoprogesterone **43** (32 mg, 0.06 mmol, 46%). The Δ^5-olefin isomerized to the Δ^4-isomer on the silica column. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73 (s, 1H), 3.38 (apparent t, J = 8 Hz, 1H), 2.55-2.20 (m, 7 H), 2.10-1.95 (m, 4H), 1.92-1.80 (m, 3H), 1.55-1.35 (m, 2H), 1.30-1.15 (m, 2H), 1.17 (s, 3H), 0.99 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.6, 191.9, 170.9, 124.1, 56.0, 53.6, 52.7, 49.8, 47.3, 39.1, 38.7, 35.82, 35.78, 34.1, 32.87, 32.85, 32.1, 25.4, 21.1, 17.5, 13.8.



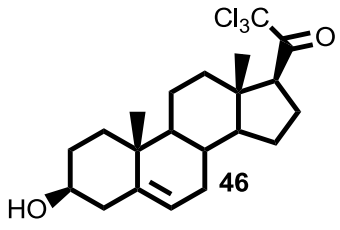
2.8.35. 21,21,21-trichloro-pregn-5-ene-3β-(t-butyl)dimethylsilyloxy-20-ol, compound **44**

To a flask containing aldehyde **38** (0.60 g, 1.4 mmol) was added chloroform (20 mL) followed by DBU (0.50 mL, 3.7 mmol, 2.6 mol eq). The reaction was stirred at RT for 72 h and directly purified via flash column chromatography (hexanes to 50% ethyl acetate in hexanes) to afford epimeric trichlorocarbinols **44** [110] (0.13 g, 17%) as a white solid. ^{1}H NMR (less polar 20-epimer, 400 MHz, $CDCl_3$) δ 5.31 (broad s, 1H), 4.27 (d, J = 4 Hz, 1H), 3.55-3.41 (m, 1H), 2.80 (d, J = 4 Hz, 1H), 2.39 (dd, J_1 = 8, J_2 = 4 Hz, 1H), 2.30-2.23 (m, 1H), 2.20-1.96 (m, 4H), 1.90-1.80 (m, 2H), 1.76-1.70 (m, 3H), 1.35-1.19 (m, 5H), 1.01 (s, 3H), 0.89 (s, 9H), 0.86 (s, 3H), 0.06 (6H); ^{1}H NMR (more polar 20-epimer, 400 MHz, $CDCl_3$) δ 5.32 (broad s, 1H), 4.02 (dd, J_1 = 9, J_2 = 6 Hz, 1H), 3.55-3.43 (m, 1H), 2.69 (d, J = 6 Hz, 1H), 2.32-2.14 (m, 5H), 2.05-1.91 (m, 4H), 1.85-1.65 (m, 8H), 1.35-1.15 (m, 3H), 0.89 (s, 9H), 0.86 (s, 3H), 0.06 (s, 6H).



2.8.36. 21,21,21-trichloro-pregn-5-ene-3β-(t-butyl)dimethylsilyloxy-20-one, compound **45**

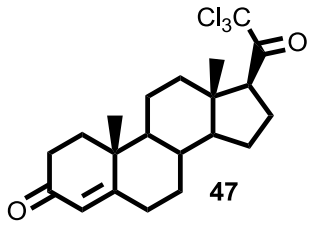
Compound **45** was obtained from **44** in the similar fashion to section 2.2.10 (110 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.32 (broad s, 1H), 3.52-3.44 (m, 1H), 3.22 (apparent t, J = 12 Hz, 1H), 2.32-2.10 (m, 4H), 2.10-1.95 (m, 2H), 1.90-1.65 (m, 6H), 1.65-1.40 (m, 6H), 1.01 (s, 3H), 0.92 (s, 3H), 0.88 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.0, 141.8, 120.8, 72.6, 57.0, 54.2, 50.0, 47.3, 42.9, 38.8, 37.5, 36.8, 32.18, 32.15, 32.06, 31.4, 26.1, 25.5, 21.2, 19.6, 18.4, 13.6, -4.4.



2.8.37. 21,21,21-trichloro-pregn-5-en-3β-ol-20-one (21,21,21-trichloropregnenolone, compound **46**)

To a solution of **45** (104 mg, 0.19 mmol) in THF (25 mL) was added p-toluenesulfonic acid monohydrate (66 mg, 0.35 mmol, 1.8 mol eq). The reaction was stirred for 2 h, and the product was purified via flash column chromatography

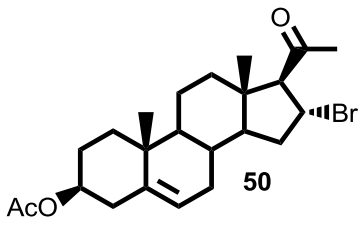
(hexanes to 70% ethyl acetate in hexanes) to yield **46** (71 mg, 0.17 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.36 (broad s, 1H), 3.60-3.45 (m, 1H), 3.22 (apparent t, J = 12 Hz, 1H), 2.35-2.10 (m, 4H), 1.40-1.20 (m, 5H), 1.00 (s, 3H), 0.93 (s, 3H).



47

2.8.38. 21,21,21-trichloro-pregn-4-ene-3,20-dione (21,21,21-trichloroprogesterone, compound **47**)

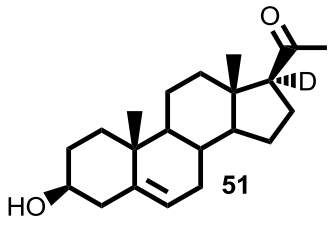
The procedure to oxidize **46** was followed as in section **2.8.4.** with isomerization on the silica gel column to yield **5** [135] (7 mg, 58%, over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73 (broad s, 1H), 3.22 (apparent t, J = 12 Hz, 1H), 2.49-2.27 (m, 5H), 2.21-2.11 (m, 1H), 2.08-1.96 (m, 1H), 1.95-1.75 (m, 4H), 1.70-1.56 (m, 4H), 1.55-1.25 (m, 2H), 1.19 (s, 3H), 0.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.6, 191.8, 170.8, 124.1, 56.1, 53.6, 53.0, 47.1, 38.6, 35.84, 35.77, 34.0, 32.9, 32.1, 31.3, 25.3, 21.1, 17.5, 13.7.



2.8.39. 16-bromopregnenolone-3-acetate (compound **50**)

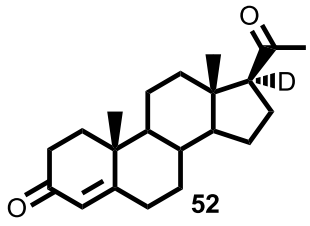
To a solution of tetrabutylammonium bromide (2.0 g, 6.2 mmol) and 16,17-dehydropregnenolone-3-acetate (1.21 g, 3.4 mmol) in CH_2Cl_2 (20 mL) was added concentrated sulfuric acid (1.0 mL). The reaction was stirred for 2 h and the reaction mixture was directly loaded onto a silica gel column and purified (100% hexanes to 50% hexanes in ethyl acetate) to afford bromide **50** (0.5 g, 1.1 mmol, 32%) in addition to the more polar 5,6,16-tribromopregnenolone-3-acetate (0.1 g, 0.2 mmol). ^{1}H NMR of **50** (400 MHz, $CDCl_3$) δ 5.38 (broad s, 1H), 4.82 (apparent t, J = 9.0 Hz, 1H), 4.66-4.55 (m, 1H), 3.11 (d, J = 7.4 Hz, 1H), 2.33-2.30 (m, 2H), 2.18 (s, 3H), 2.15-2.10 (m, 1H), 2.03 (s, 3H), 2.04-1.85 (m, 4H), 1.80-1.42 (m, 7H), 1.24-1.05 (m, 2H), 1.01 (s, 3H), 0.61 (s, 3H).

^{1}H NMR of 5,6,16-tribromopregnenolone-3-acetate (400 MHz, $CDCl_3$) δ 5.54-5.46 (m, 1H), 4.81-4.76 (m, 1H), 3.13 (d, J = 7.5 Hz, 1H), 2.32 (dd, J_1 = 13.0 Hz, J_2 = 4.9 Hz, 1H), 2.16 (s, 3H), 2.14-1.94 (m, 4H), 2.02 (s, 3H), 1.89-1.48 (m, 5H), 1.08 (s, 3H), 0.57 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.4, 170.5, 87.4, 75.5, 71.3, 54.1, 47.6, 46.5, 46.2, 42.4, 41.3, 38.6, 37.4, 34.0, 33.0, 31.9, 31.7, 27.3, 26.5, 22.8, 21.4, 15.4, 14.0.



2.8.40. 17α-[^{2}H]-pregnenolone (compound **51**)

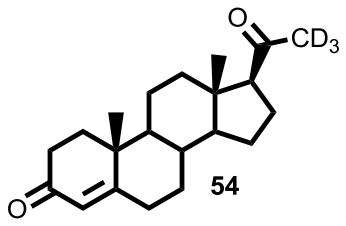
A solution of 17-bromopregnenolone **8** (61 mg, 0.16 mmol) in diethyl ether (1.0 mL) was added to a stirring solution of D_2O (0.3 mL, 15.0 mmol) and acetic anhydride (0.3 mL, 3.2 mmol) in diethyl ether (0.5 mL) (D_2O was pre-stirred with acetic anhydride for 5 min in diethyl ether). Zinc dust (160 mg, 2.5 mmol) was added, and the reaction was stirred at RT for 1 h under a N_2 atmosphere. The reaction was diluted with diethyl ether (10 mL) and washed with D_2O (1 mL). The organic layer was concentrated via reduced pressure and quickly purified to remove the grease from zinc dust (to avoid any deuteron-proton exchange on the silica gel column) via flash column chromatography (100% hexanes to 50% hexanes/ethyl acetate) to afford 17α-[^{2}H]-pregnenolone **51** (40 mg, 0.13 mmol, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35-5.34 (m, 1H), 3.55-3.49 (m, 1H), 2.30 (ddd, J_1 = 7.2 Hz, J_2 = 5.2 Hz, J_3 = 2.30 Hz, 1H), 2.27-2.19 (m, 1H), 2.19-2.14 (m, 1H), 2.12 (s, 3H), 2.08-1.97 (m, 2H), 1.88-1.81 (m, 2H), 1.74-1.42 (m, 4H), 1.28-1.06 (m, 3H), 1.00 (s, 3H), 0.98 (dd, J_1 = 11.5 Hz, J_2 = 4.8 Hz, 1H), 0.63 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 209.8, 140.9, 121.5, 71.8, 57.1, 50.1, 44.1, 42.4, 38.9, 37.4, 36.7, 32.0, 31.9, 31.8, 31.7, 24.6, 22.9, 21.2, 19.5, 13.4.



52

2.8.41. 17α-[^{2}H]-progesterone (compound **52**)

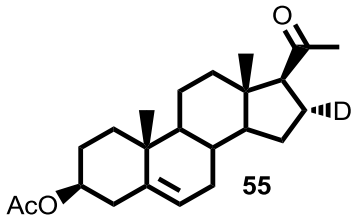
The procedure for synthesizing 17α-[^{2}H]-pregnenolone **51** was followed: Acetic anhydride (0.30 mL, 3.18 mmol) was added to a vial containing D_2O (0.30 mL, 6.00 mmol) and diethyl ether (1.0 mL) under a N_2 atmosphere and the reaction was stirred for 3 min. 17-Bromoprogesterone **11** (62 mg, 0.16 mmol) was added as a solid, and the reaction was stirred for 1 min under inert atmosphere. Zinc dust (0.16 g, 2.45 mmol) was added, and the reaction was stirred for 1 h. The reaction mixture was purified via flash column chromatography (100% hexanes to 50% hexanes in ethyl acetate) to afford 17α-[^{2}H]-progesterone **52** (40 mg, 0.13 mmol, 81%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.72 (s, 1H),2.47-2.35 (m, 2H), 2.20-2.12 (m, 1H), 2.11 (s, 3H), 2.06-2.01 (m, 2H), 1.86-1.83 (m, 1H), 1.76-1.62 (m, 4H), 1.58-1.50 (m, 1H), 1.50-1.41 (m, 2H), 1.31-1.24 (m, 2H), 1.18 (s, 3H), 1.21-1.14 (m, 1H), 1.09-0.95 (m, 2H), 0.66 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 209.5, 199.6, 171.1, 124.0, 56.1, 53.8, 44.0, 38.75, 38.70, 35.9, 35.7, 34.1, 32.9, 32.0, 31.6, 24.5, 22.9, 21.1, 17.5, 13.5.



54

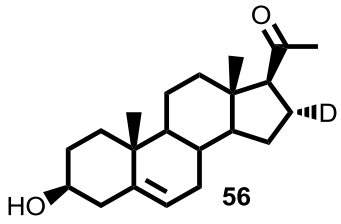
2.8.42. 21,21,21-[$^2H_3$]-progesterone (compound **54**)

To a stirring solution of D_2O (0.2 mL, 10.0 mmol) and acetic anhydride (0.20 mL, 2.12 mmol) was added 21,21,21-tribromoprogesterone **43** (63 mg, 0.11 mmol) in diethyl ether (5 mL). Zinc dust (0.20 g, 3.06 mmol) was added, and the reaction was stirred at RT under a N_2 atmosphere for 1 h and directly purified on a silica gel column (100% hexanes to 50% hexanes in ethyl acetate) to afford 21,21,21-[$^2H_3$]-progesterone **54** (17 mg, 0.05 mmol, 47%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73 (s, 1H), 2.53 (apparent t, J_1 = 9.0 Hz, 1H), 2.46-2.35 (m, 2H), 2.33-2.25 (m, 1H), 2.21-2.15 (m, 1H), 2.08-2.01 (m, 2H), 1.85-1.83 (m, 1H), 1.76-1.62 (m, 4H), 1.57-1.52 (m, 1H), 1.50-1.42 (m, 2H), 1.28-1.22 (m, 2H), 1.18 (s, 3H), 1.16-1.13 (m, 1H), 1.10-0.95 (m, 2H), 0.66 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.6, 171.1, 124.1, 63.6, 56.2, 53.8, 44.1, 38.8, 38.7, 35.9, 35.7, 34.1, 32.9, 32.0, 24.5, 22.9, 21.1, 17.5, 13.5. The resonances for carbons 20 and 21 in the ^{13}C-NMR were obscured by C-D dipolar and quadrupolar couplings.



2.8.43. 16α-[^{2}H]-pregnenolone-3-acetate (compound **55**)

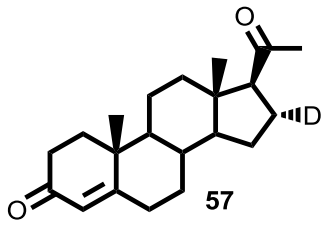
16-bromopregnenolone-3-acetate **50** (77 mg, 0.177 mmol) in diethyl ether (2 mL) was added to a stirring solution of acetic anhydride (0.2 mL) and D_2O (0.2 mL). Zinc dust (0.20 g) was added and the reaction was stirred for 1 h under a N_2 atmosphere and purified directly on a silica gel column (100% hexanes to 50% hexanes in ethyl acetate) to afford 16α-[^{2}H]-pregnenolone-3-acetate **55** (34 mg, 0.095 mmol, 54%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.37 (broad s, 1H), 4.68-4.54 (m, 1H), 2.53 (d, J = 8.8 Hz, 1H), 2.38-2.41 (m, 1H), 2.21-2.15 (m, 1H), 2.13 (s, 3H), 2.05-1.95 (m, 1H), 2.04 (s, 3H), 1.73-1.55 (m, 2H), 1.50-1.43 (m, 2H), 1.91-1.83 (m, 2H), 1.28-1.12 (m, 2H), 1.02 (s, 3H), 0.63 (s, 3H).



2.8.44. 16α-[^{2}H]-pregnenolone (**56**)

To a solution of 16α-[^{2}H]-pregnenolone (34 mg, 0.09 mmol) in CH_2Cl_2 (5 mL) and Methanol (2 mL) was added 0.1 mL of 12 M HCl. The reaction was stirred for 10 h and washed with saturated aqueous $NaHCO_3$ solution (2 x 10 mL). The

aqueous layer was extracted with ethyl acetate (2 x 15 mL) and the combined organic extracts were concentrated via reduced pressure. The crude material was purified via flash column chromatography (100% hexanes to 50% hexanes in ethyl acetate) to afford 16α-[^{2}H]-pregnenolone (10 mg, 0.05 mmol, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.36-5.35 (m, 1H), 3.57-3.49 (m, 1H), 2.52 (d, J = 9.0 Hz, 1H), 2.33-2.25 (m, 2H), 2.22-2.14 (m, 1H), 2.12 (s, 3H), 2.07-1.97 (m, 2H), 1.75-1.43 (m, 7H), 1.27-1.03 (m, 5H), 1.01 (s, 3H), 0.99-0.98 (m, 1H), 0.63 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 209.8, 140.9, 121.6, 71.9, 63.8, 57.1, 50.1, 44.2, 42.4, 39.0, 37.4, 36.7, 32.0, 31.9, 31.8, 31.7, 24.5, 21.2, 19.5, 13.4.

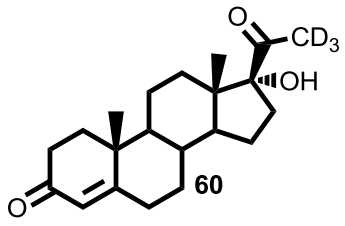


57

2.8.45. 16α-[^{2}H]-progesterone (compound **57**)

Dess-Martin periodinane (12 mg) was added to 16α-[^{2}H]-pregnenolone (10 mg) in CH_2Cl_2 (3 mL) and the reaction was stirred for 1 h. The reaction was washed with saturated $NaHCO_3$ aqueous solution (2 x 15 mL) and extracted with ethyl acetate (2 x 15 mL). The combined organic extracts were concentrated via reduced pressure and purified via flash column chromatography (100% hexanes to 50% hexanes in ethyl acetate) to afford 16α-[^{2}H]-$\Delta^{5,6}$-progesterone (5 mg, 50%). 16α-[^{2}H]-$\Delta^{5,6}$-progesterone was dissolved in Methanol (2 mL) and CH_2Cl_2 (1 mL)

and 10 μL of 12 M HCl was added the reaction was purified directly on a silica gel column (100% hexanes to 40% hexanes in ethyl acetate) to afford 16α-[^{2}H]-progesterone **57** (2 mg, 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73 (s, 1H), 2.52 (d, J = 9.1 Hz, 1H), 2.47-2.34 (m, 3H), 2.34-2.25 (m, 1H), 2.19-2.15 (m, 1H), 2.12 (s, 3H), 2.07-1.98 (m, 2H), 1.88-1.83 (m,1H), 1.75-1.61 (m, 3H), 1.57-1.51 (m, 1H), 1.47-1.39 (m, 2H), 1.30-1.21 (m, 2H), 1.20-1.15 (m, 1H), 1.18 (s, 3H), 1.15-0.95 (m, 3H), 0.66 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 209.5, 199.6, 171.1, 124.1, 63.6, 56.1, 53.8, 44.1, 38.8, 38.7, 35.8, 35.7, 34.1, 32.9, 32.0, 31.7, 24.4, 21.1, 17.5, 13.5.

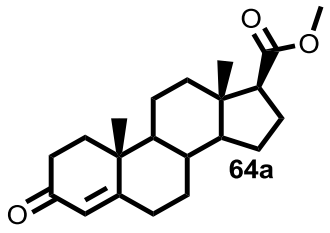


2.8.46. 17-hydroxy-21,21,21-[$^2H_3$]-progesterone (compound **60**)

To a stirring solution of 17-hydroxypregnenolone (248 mg, 0.747 mmol) in CH_3OD (5 mL) and THF (1 mL) was added KOD (40% wt/v, 0.5 mL), and the reaction was stirred for 7 days. The reaction was stopped and purified directly on a silica gel column to afford 17-hydroxy-21,21,21-[$^2H_3$]-pregnenolone (160 mg, 0.478 mmol, 64%), and the deuteration was confirmed through the loss of the C21-methyl singlet in ^{1}H NMR. Dess-Martin periodinane (200 mg, 0.472 mmol)

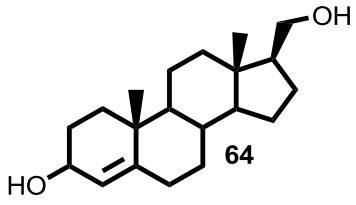
was added to a stirring solution of 17-hydroxy-21,21,21-[$^{2}H_3$]-pregnenolone (160 mg, 0.478 mmol), and the reaction was stirred for 1.5 h. The reaction was purified directly on a silica gel column (100% hexanes to 50% hexanes in ethyl acetate) to afford 17-hydroxy-21,21,21-[$^{2}H_3$]-$\Delta^{5,6}$-progesterone (40 mg, 0.120 mmol, 26%) and 17-hydroxy-21,21,21-[$^{2}H_3$]-progesterone **60** (50 mg, 0.150 mmol, 32%).

17-hydroxy-21,21,21-[$^{2}H_3$]-$\Delta^{5,6}$-progesterone: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.33 (broad s, 1H), 3.27 (d, J = 16.4 Hz, 1H), 2.81 (d, J = 16.4 Hz, 1H), 2.70-2.64 (m, 1H), 2.45 (ddd, J_1 = 19.7 Hz, J_2 = 14.1 Hz, J_3 = 5.8 Hz, 1H), 2.31-2.22 (m, 1H), 2.06-2.01 (m, 3H), 1.85-1.41 (m, 9H), 1.36-1.29 (m, 2H), 1.17 (s, 3H), 1.07 (ddd, J_1 = 16.0 Hz, J_2 = 12.2 Hz, J_3 = 4.7 Hz, 1H), 0.73 (s, 3H).
17-hydroxy-21,21,21-[$^{2}H_3$]-progesterone **60**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.73 (s, 1H), 2.81 (s, 1H), 2.67 (apparent t, J = 12.0 Hz, 1H), 2.45-2.25 (m, 4H), 1.90-1.79 (m, 2H), 1.75-1.56 (m, 7H), 1.45-1.35 (m, 3H), 1.18 (s, 3H), 1.11 (ddd, J_1 = 16.8 Hz, J_2 = 13.1 Hz, J_3 = 3.6 Hz, 1H), 0.97 (ddd, J_1 = 15.7 Hz, J_2 = 11.4 Hz, J_3 = 3.5 Hz, 1H), 0.75 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.7, 171.1, 124.1, 89.9, 53.4, 50.1, 48.4, 38.7, 35.8, 35.6, 34.1, 33.6, 32.9, 32.1, 30.1, 24.1, 20.6, 17.5, 15.6.



2.8.47. 17β-methyl ester-androst-4-en-3-one (compound **64a**)

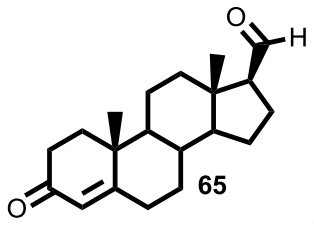
A reaction flask containing methyl ester **35** (0.8 g, 2.4 mmol) in toluene (40 mL) and N-methylpiperidone (2 mL) was fitted with a Dean-Stark trap and heated to 160 ^{o}C for 1 h until 2 mL of liquid was distilled off. $Al(O^{i}Pr)_3$ (2 mol eq) was added, and the reaction was stirred at reflux for 12 h. The reaction mixture was washed with HCl (1 M, 10 mL) and extracted with ethyl acetate (3 x 20 mL). The combined organic extracts were concentrated via reduced pressure and purified via flash column chromatography (100% hexanes to 50% hexanes/ethyl acetate) to afford ketone **64a** (0.5 g, 1.5 mmol, 63%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.73 (s, 1H), 3.67 (s, 3H), 2.97-2.90 (m, 1H), 2.75-2.67 (m, 1H), 2.55-2.23 (m, 4H), 2.21-1.55 (m, 5H), 1.18 (s, 3H), 1.15-0.90 (m, 2H), 0.70 (s, 3H).



2.8.48. 17β-(1-hydroxymethyl)-androst-4-ene-3-ol (compound **64**)

DIBAL (5 mL) was added to 17β-methyl ester-androst-4-en-3-one **64a** (1.3 g, 3.9 mmol) in CH_2Cl_2 (50 mL) at -78 ^{o}C under a N_2 atmosphere. The reaction was

stirred for 2 h and quenched with Rochelle's salt (sat. aqueous solution, 20 mL) with stirring until 2 clear layers formed. The organic layer was removed, and the aqueous layer was extracted with ethyl acetate (2 x 30 mL). The combined organic extracts were concentrated via reduced pressure. The crude material was purified via flash column chromatography (100% hexanes to 30% hexanes in ethyl acetate) to afford the diol **64** as a white solid (0.8 g, 2.4 mmol, 62%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.27 (s, 1H), 3.75-3.65 (m, 1H), 3.57-3.47 (m, 1H), 3.46-3.37 (m, 1H), 2.30-2.15 (m, 1H), 2.05-1.90 (m, 2H), 1.90-1.75 (m, 2H), 1.75-1.55 (m, 3H), 1.55-1.40 (m, 3H), 1.33-1.10 (m, 5H), 1.01 (s, 3H), 0.66 (s, 3H).

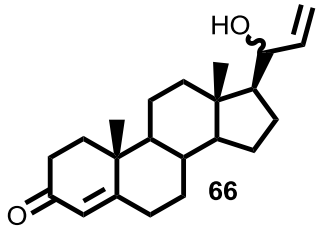


2.8.49. 17β-carboxaldehyde-androsten-3-one (compound **65**)

The diol **64** was dissolved in CH_2Cl_2, and Dess-Martin periodinane (1.6 g, 3.8 mmol,) was added. The reaction was stirred for 1 h and purified directly via flash column chromatography (100% hexanes to 50% hexanes/ethyl acetate) to yield aldehyde **65** (0.42 g) and the 3-keto-$\Delta^{4,5}$-20-alcohol (0.1 g).

Aldehyde **65**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.72 (s, 1H), 5.68 (s, 1H), 2.40-2.20 (m, 4H), 2.15-1.95 (m, 3H), 1.90-1.50 (m, 6H), 0.45-0.35 (m, 2H), 1.31-1.21 (m, 1H), 1.11 (s, 3H), 1.15-0.90 (m, 3H), 0.75 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.7, 199.6, 171.0, 123.9, 62.6, 55.6, 53.7, 44.6, 38.1, 35.7, 35.`, 33.9, 32.8, 31.9, 24.7, 21.0, 20.5, 17.4, 13.8.

20-Alcohol (more polar compound): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.73 (s, 1H), 3.72 (dd, J_1 = 10.6 Hz, J_2 = 7.1 Hz, 1H), 3.57 (dd, J_1 = 10.6 Hz, J_2 = 7.5 Hz, 1H), 2.50-2.33 (m, 2H), 2.32-2.26 (m, 1H), 2.07-1.98 (m, 1H), 1.74-1.37 (m, 6H), 1.36-1.26 (m, 1H), 1.25-1.14 (m, 1H), 1.19 (s, 3H), 1.10-0.92 (m, 3H), 0.70 (s, 3H).

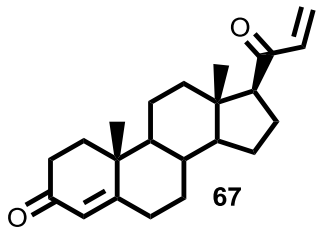


66

2.8.50. 17β-(prop-2-en-1-ol)-androsten-3-one (compound **66**)

Vinylmagnesium chloride (1.6 M, 1.1 mL, 1.79 mmol, 1.1 mol eq) was added to aldehyde **65** (0.49 g, 1.63 mmol) in THF (50 mL). The reaction was stirred for 30 min and washed with H_2O (10 mL) and extracted with ethyl acetate (20 mL). The organic layer was concentrated via reduced pressure, and the crude material was

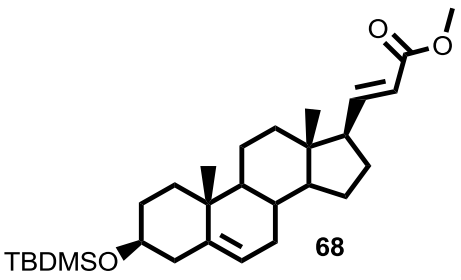
purified via flash column chromatography (100% hexanes to 50% hexanes/ethyl acetate) to afford alcohol **66** (0.16 g, 0.48 mmol). The following reported chemical shifts are of the epimeric mixture at C20: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.91-5.79 (m, 1H), 5.71 (broad s, 1H), 5.24-5.15 (m, 1H), 5.12-5.01 (m, 1H), 4.02-3.96 (m, 1H), 2.42-2.22 (m, 8H), 2.02-1.30 (m, 10H), 1.25-0.85 (m, 3H), 0.82 (s, 3H), 0.71 (s, 3H).



2.8.51. 21-homo-21,22-dehydroprogesterone (compound **67**)

Dess-Martin periodinane (0.21 g, 0.5 mmol) was added to alcohol **66** (0.16 g, 0.48 mmol) in CH_2Cl_2 (20 mL), and the reaction was stirred for 20 min at RT. The reaction mixture was washed with $NaHCO_3$ (saturated aqueous solution, 10 mL), and the concentrated organic phase was purified via flash column chromatography (100% hexanes to 50% hexanes/ethyl acetate) to afford diene-dione **67** (50 mg, 0.15 mmol, 32%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.42 (dd, J_1 = 17.4 Hz, J_2 = 10.4 Hz, 1H), 6.21 (d, J = 17.3 Hz, 1H), 5.74 (s, 1H), 5.69 (d, J = 10.6 Hz, 1H), 2.80 (apparent t, J = 9.0 Hz, 1H), 2.49-2.35 (m, 2H), 2.35-2.25 (m, 2H), 2.07-2.00

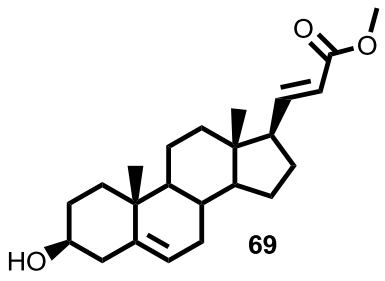
(m, 1H), 1.98-1.95 (m, 1H), 1.91-1.83 (m, 1H), 1.79-1.65 (m, 2H), 1.56 (s, 3H), 1.35-1.22 (m, 2H), 1.18 (s, 3H), 1.05-0.88 (m, 2H), 0.64 (s, 3H).



68

2.8.52. 17β-(ethen-2-oic acid methyl ester)-pregnenolone-3-tert-butyldimethylsilyl ether (compound **68**)

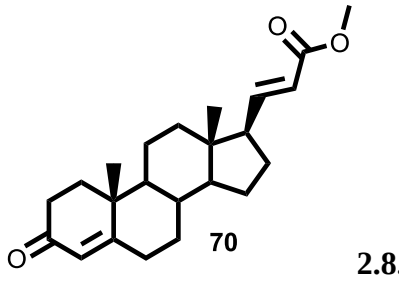
Aldehyde **38** (0.34 g, 0.82 mmol) and carbomethoxymethylene triphenylphosphorane (ie: methyl(triphenylphosphoranylidene)acetate, CAS #: [2605-67-6]) (0.49 g, 1.47 mmol, 1.8 mol eq) were dissolved in CH_2Cl_2 (3 mL) and toluene (3 mL). The reaction was refluxed for 2 h and purified via flash column chromatography (100% hexanes to 50% hexanes/ethyl acetate) to afford methyl ester **68** (0.30 g, 0.64 mmol, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.96 (dd, J_1 = 15.6 Hz, J_2 = 4.0 Hz, 1H), 5.79 (d, J = 15.6 Hz, 1H), 5.31 (broad s, 1H), 3.73 (s, 3H), 3.51-3.49 (m, 1H), 2.26 (apparent t, J = 11.7 Hz, 1H), 2.18-2.10 (m, 2H), 2.04-1.96 (m, 1H), 1.85-1.77 (m, 2H), 1.76-1.37 (m, 10H), 1.15-1.05 (m, 3H), 1.00 (s, 3H), 0.88 (s, 9H), 0.65 (s, 3H), 0.05 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.3, 151.2, 141.8, 121.1, 121.0, 72.7, 56.3, 54.0, 51.6, 50.5, 44.9, 42.9, 37.6, 36.8, 32.1, 27.2, 26.1, 25.2, 20.8, 19.6, 18.4, 13.2, -4.4.



69

2.8.53. 17β-(ethen-2-oic acid methyl ester)-pregnenolone **69**

Camphorsulfonic acid (0.01 g) and TBDMS-ether (0.30 g, 0.64 mmol) were stirred in CH_2Cl_2 (5 mL) and Methanol (10 mL) for 2 h. The reaction mixture was directly loaded on a silica gel column and purified (100% hexanes to 50% hexanes/ethyl acetate) to yield alcohol **69** (0.20 g, 0.56 mmol, 87%).[1]H NMR (500 MHz, $CDCl_3$) δ 6.95 (dd, J_1 = 15.6 Hz, J_2 = 4.0 Hz, 1H), 5.79 (d, 15.6 Hz, 1H), 5.35 (broad s, 1H), 3.57-3.47 (m, 1H), 2.35-2.20 (m, 2H), 2.16-2.06 (m, 1H), 2.05-1.96 (m, 1H), 1.90-1.78 (m, 3H), 1.76-1.38 (m, 9H), 1.32-1.21 (m, 1H), 1.16-1.05 (m, 3H), 1.00 (s, 3H), 0.65 (s, 3H).



70

2.8.54. 17β-(ethen-2-oic acid methyl ester)-progesterone (compound **70**)

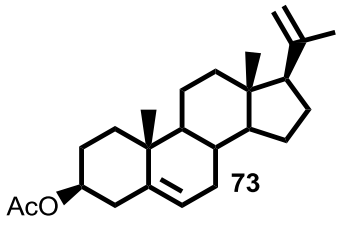
Dess-Martin periodinane (0.14 g, 0.33 mmol) was added to alcohol **69** (0.10 g, 0.29 mmol) in CH_2Cl_2 (5 mL). The reaction was stirred for 30 min, diluted with

ethyl acetate (50 mL), and washed with $NaHCO_3$ (saturated aqueous solution, 3 x 30 mL). The organic layer was concentrated via reduced pressure and used directly for the next step. The crude 3-keto-$\Delta^{5,6}$-steroid was dissolved in methanol (10 mL), and HCl (12 M, 2 drops) was added. The reaction was loaded directly on a silica gel column (100% hexanes to 50% hexanes/ethyl acetate) to afford 3-keto-$\Delta^{4,5}$-product **70** (70 mg, 0.20 mmol, 69%) and 3,3-dimethoxy acetal (less polar) as a side product (10 mg).

3-keto-$\Delta^{5,6}$-product: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.95 (dd, J_1 = 15.6 Hz, J_2 = 8.0 Hz, 1H), 5.79 (d, J = 15.6 Hz, 1H), 5.34-5.33 (m, 1H), 3.71 (s, 3H), 3.27 (d, J = 16.4 Hz, 1H), 2.82 (d, J = 16.4 Hz, 1H), 2.47 (ddd, J_1 = 14 Hz, J_2 = 14 Hz, J_3 = 5.7 Hz, 1H), 2.29 (d, J = 15.1 Hz, 1H), 2.13 (dd, J_1 = 17.4 Hz, J_2 = 8.6 Hz, 1H), 2.08-1.96 (m, 2H), 1.89-1.80 (m, 1H), 1.80-1.72 (m, 2H), 1.68-1.56 (m, 3H), 1.58-1.42 (m, 3H), 1.35-1.23 (m, 2H), 1.18 (s, 3H), 1.12-1.01 (m, 2H), 0.68 (s, 3H).

3-keto-$\Delta^{4,5}$-product **70**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.94 (dd, J = 15.6, 8.0 Hz, 1H), 5.78 Hz (d, J = 15.6 Hz, 1H), 5.71 (s, 1H), 3.71 (s, 3H), 2.47-2.35 (m, 3H), 2.32-2.23 (m, 1H), 2.12 (dd, J_1 = 17.6 Hz, J_2 = 8.8 Hz, 1H), 2.06-1.97 (m, 2H), 1.90-1.80 (m, 2H), 1.77-1.63 (m, 4H), 1.60-1.52 (m, 2H), 1.39 (ddd, J_1 = 16.9 Hz, J_2 = 13.0 Hz, J_3 = 3.9 Hz, 1H), 1.32-1.25 (m, 2H), 1.17 (s, 3H), 1.14-1.00 (m,

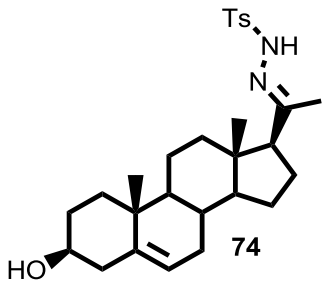
3H), 0.94 (ddd, J_1 = 15.4 Hz, J_2 = 11.7 Hz, J_3 = 4.2 Hz, 1H), 0.68 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.6, 171.2, 167.1, 150.6, 124.0, 121.2, 55.3, 53.8, 51.5, 44.8, 38.8, 37.3, 35.8, 35.7, 34.1, 33.0, 32.1, 31.7, 27.1, 25.0, 20.7, 17.5, 13.3.



2.8.55. 17β-isopropenylandrosten-3β-acetate (compound **73**):

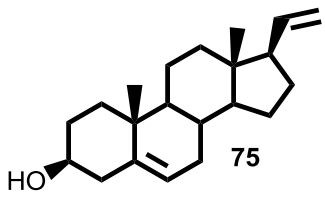
Methyltriphenlyphosphonium bromide (3.09 g, 8,65 mmol, 3.10 mol eq) and potassium tert-butoxide (0.98 g, 8.70 mmol, 3.12 mol eq) were stirred at RT in THF (20 mL). After 15 min, the solution turned orange, and pregnenolone-3-acetate (1.00 g, 2.79 mmol) in THF (5 mL) was added. The reaction was stirred overnight for 9 h at RT and then heated to 50 ^oC for 3 h. The mixture was washed with water (2 x 10 mL) and extracted with ethyl acetate (2 x 30 mL). The combined organic extracts were dried under reduced pressure, and the crude material was purified via flash column chromatography (100% hexanes to 50% hexanes/50% ethyl acetate) to yield 20-alkene **73** as a white solid (R_f 0.9 in 30% Ethyl acetate/hexanes, higher than starting material on TLC, 0.74 g, 74%). ^1H NMR (500 MHz, $CDCl_3$) δ 5.36 (m, 1H), 4.84 (s, 1H), 4.70 (s, 1H), 4.65-4.53 (m, 1H), 2.33-2.28 (m, 2H), 2.02 (s, 3H), 1.91-1.82 (m, 3H), 1.75 (s, 3H), 1.74-1.62

(m, 2H), 1.61-1.52 (m, 2H), 1.50-1.36 (m, 2H), 1.25-1.07 (m, 4H), 1.01 (s, 3H), 0.96 (dd, J_1 = 10.0 Hz, J_2 = 5.0 Hz, 1H), 0.57 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 145.6, 139.8, 133.9, 122.6, 110.8, 74.0, 57.3, 56.5, 50.3, 43.2, 38.7, 38.2, 37.1, 36.7, 32.3, 31.9, 31.7, 27.9, 25.5, 25.4, 24.8, 24.4, 21.5, 21.2, 19.4, 12.8.



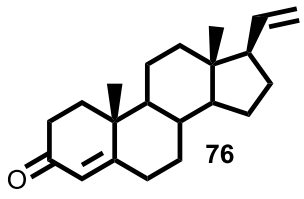
2.8.56. 20-tosylhydrazone-pregnen-3β-ol (compound **74**)

Pregnenolone (2.21 g, 6.98 mmol) and tosylhydrazide (1.30 g, 7.00 mmol) were dissolved in methanol, and the reaction was heated to 50 ^{o}C. The reaction mixture was concentrated and purified via flash column chromatography to afford tosylhydrazone **74** (1.86 g, 4.08 mmol, 58%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85-7.83 (m, 2H), 7.30-7.27 (m, 2H), 5.34 (broad s, 1H), 3.55-3.49 (m, 1H), 2.43 (s, 3H), 1.94 (s, 3H), 0.82 (s, 3H).



2.8.57. 17β-ethenylandrosten-3β-ol (compound **75**)

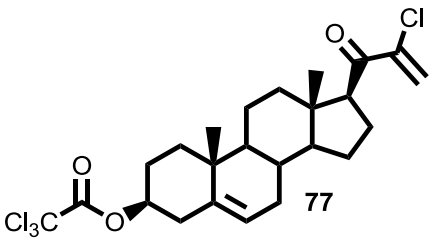
Tosylhydrazone **74** (1.86 g, 4.08 mmol) was dissolved in diethyl ether (15 mL), and a solution of n-butyllithium (2.9 M in hexanes, 10.5 mL, 31 mmol, 7.5 mol eq) was added at -78 ^{o}C under a N_2 atmosphere. The reaction was warmed to RT, and after 24 h, water (10 mL) was added at 0 ^{o}C (exothermic). The reaction mixture was extracted with ethyl acetate (3 x 20 mL), and the combined organic extracts were dried under reduced pressure. The crude material was purified via flash column chromatography (100% hexanes to 50% hexanes/ethyl acetate) to afford alkene **75** (0.2 g, 0.67 mmol, 17%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.79-5.70 (m, 1H), 5.34 (broad s, 1H), 4.98-4.93 (m, 2H), 3.57-3.35 (m, 1H), 2.40-2.14 (m, 3H), 2.10-1.31 (m, 20H), 0.99 (s, 3H), 0.59 (s, 3H).



2.8.58. 17β-ethenylandrosten-3-one (compound **76**)

Compound **76** (0.12 g, 0.40 mmol, 56%) was obtained from the Dess-Martin periodinane protocol as in section **2.8.15**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.77 (dd, J_1 = 9.2 Hz, J_2 = 8.0 Hz, 1H), 5.72 (s, 1H), 4.99 (broad s, 1H), 4.96 (d, J = 9.7

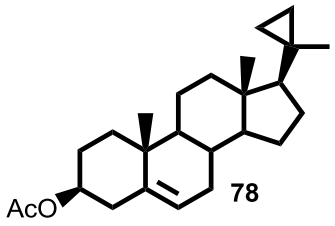
Hz, 1H), 2.48-2.35 (m, 2H), 2.32-2.22 (m, 1H), 2.05-2.00 (m, 1H), 1.97 (dd, J_1 = 17.5 Hz, J_2 = 8.8 Hz, 1H), 1.88-1.76 (m, 2H), 1.76-1.65 (m, 3H), 1.62-1.51 (m, 2H), 1.40 (ddd, J_1 = 22.5 Hz, J_2 = 9.4 Hz, J_3 = 3.8 Hz, 1H), 1.23 (dd, J_1 = 12.1 Hz, J_2 = 5.7 Hz, 1H), 1.82 (s, 3H), 1.10-0.98 (m, 2H), 0.95 (ddd, J_1 = 15.7 Hz, J_2 = 12.0 Hz, J_1 = 4.0 Hz, 1H), 0.64 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.7, 171.6, 139.6, 123.9, 114.9, 55.3, 55.1, 54.2, 43.5, 38.8, 35.8, 34.1, 33.1, 32.2, 27.3, 24.9, 20.8, 17.5, 13.0.



2.8.59. 21-chloro-21-homodehydropregnenolone-3-trichloroacetate (compound **77**)

$CrCl_2$ (0.36 g, 2.93 mmol, 21.4 mol eq) was added to a solution of trichloroketone **33** (77 mg, 0.14 mmol, 1.0 mol eq) and praformaldehyde (0.55 g, 18.2 mmol, 13.3 mol eq) in THF (10 mL). The reaction was stirred for 15 min at RT, then diluted with ethyl acetate (20 mL) and washed with brine (10 mL). The aqueous layer was extracted with ethyl acetate (3 x 20 mL), and the combined organic extracts were dried under reduced pressure. The crude material was purified via flash column chromatography (100% hexanes to 50% hexanes in ethyl acetate) to afford vinyl chloride **77** (65 mg, 0.13 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$)

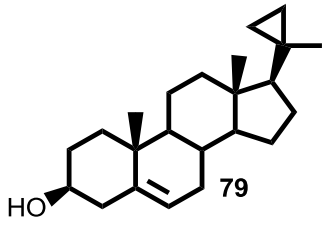
δ 5.91 (s, 1H), 5.84 (s, 1H), 5.42-5.40 (m, 1H), 3.00 (apparent t, J = 9.3 Hz, 1H), 2.42-2.40 (m, 2H), 2.24-2.12 (m, 1H), 2.10-1.85 (m, 4H), 1.84-1.15 (m, 11H), 1.03 (s, 3H), 0.75 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 197.5, 184.2, 164.1, 139.0, 123.1, 77.6, 70.3, 64.7, 57.4, 56.9, 49.7, 45.9, 38.6, 37.5, 36.9, 36.7, 32.0, 31.9, 27.3, 26.3, 24.9, 21.1, 19.4, 13.7.



2.8.60. 20-cyclopropylpregnenolone-3-acetate (compound **78**)

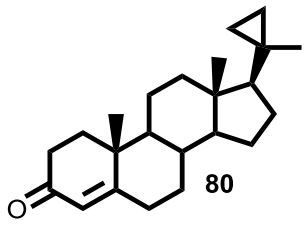
To a solution of 20-alkene **73** (1.19 g, 3.22 mmol) in diethyl ether (100 mL) was added diiodomethane (4.20 g), CuCl (2.00 g), and Zn dust (1.93 g). The reaction was stirred at reflux for 24 h. The reaction was monitored by ^{1}H NMR, and if needed, excess diiodomethane and zinc were added. The reaction mixture was concentrated and purified via flash column chromatography (100% hexanes to 50% hexanes/ethyl acetate) to yield cyclopropane **78** (0.9 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34-5.37 (m, 1H), 4.68-4.44 (m, 1H), 2.32-2.30 (m, 2H), 2.03 (s, 3H), 2.00-1.91 (m, 1H), 1.89-1.83 (m, 2H), 1.64-1.37 (m, 5H), 1.06 (s, 3H), 1.02 (s, 3H), 0.90-0.84 (m, 1H), 0.68 (s, 3H), 0.48-0.43 (m, 1H), 0.30-0.22 (m, 1H), 0.16 (ddd, J_1 = 8.0 Hz, J_2 = 4.0 Hz, J_3 = 4.0 Hz, 1H), 0.02--0.02 (m, 1H); ^{13}C

NMR (100 MHz, $CDCl_3$) δ 170.7, 139.8, 122.7, 74.1, 56.5, 56.0, 50.3, 44.0, 39.7, 38.3, 37.1, 36.8, 32.0, 31.7, 27.9, 25.3, 24.1, 23.6, 21.6, 21.0, 19.5, 14.8, 13.5, 11.1, 11.0.



2.8.61. 20-cyclopropylpregnenolone (compound **79**)

Acetate **78** (0.9 g) was dissolved in methanol (40 mL), and HCl (12 M, 2 drops) was added. The reaction was stirred for 10 h and purified via flash column chromatography to yield the alcohol **79** (0.72 g, 2.19 mmol, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35-5.34 (m, 1H), 3.60-3.43 (m, 1H), 2.35-2.17 (m, 2H), 2.08-1.92 (m, 2H), 1.90-1.80 (m, 2H), 1.63-1.40 (m, 5H),1.20-1.10 (m, 2H), 1.06 (s, 3H), 1.01 (s, 3H), 0.74 (s, 3H), 0.74-0.68 (m, 1H), 0.48-0.43 (m, 1H), 0.29-0.23 (m, 1H), 0.18-0.15 (m, 1H), 0.02--0.04 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.9, 121.8, 72.0, 56.6, 56.1, 50.4, 44.0, 42.4, 39.7, 37.4, 36.7, 32.0, 31.8, 25.3, 24.1, 23.6, 21.1, 19.6, 14.8, 13.5, 11.1, 11.0.

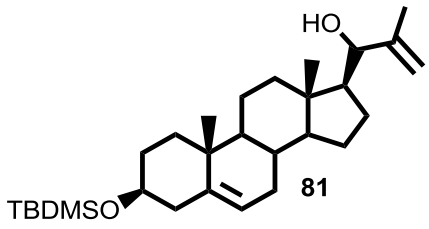


2.8.62. 20-cyclopropylprogesterone (compound **80**)

Alcohol **79** (0.22 g, 0.67 mmol) was dissolved in dichloromethane (20 mL), and Dess-Martin periodinane was added (0.29 g, 0.68 mmol, 1.02 mol eq). The reaction was stirred for 10 h and was purified via flash column chromatography (100% hexanes to 50% hexanes/ethyl acetate) to afford 3-keto product (R_f = 0.6 at 20% ethyl acetate/hexanes, 0.12 g, 0.37 mmol, 55%). The 3-keto-$\Delta^{5,6}$-product was dissolved in dichloromethane, and methanol with HCl (10% 12 M HCl in methanol, 0.1 mL) was added. The reaction was stirred for 10 min, concentrated, and purified via flash column chromatography (100% hexanes to 50% ethyl acetate in hexanes) to afford the isomerized product **80** (0.1 g, 0.31 mmol, 83%). 3-keto-$\Delta^{5,6}$-product: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.32-5.31 (m, 1H), 3.27 (ddd, J_1 = 16.0 Hz, J_2 = 5.6 Hz, J_3 = 3.6 Hz,1H), 2.80 (dd, J_1 = 16.0 Hz, J_2 = 4.8 Hz, 1H), 2.47 (ddd, J_1 = 14.0 Hz, J_2 = 14.0 Hz, J_3 = 5.6 Hz, 1H), 2.32-2.16 (m, 1H), 2.0-1.92 (m, 3H), 1.18 (s, 3H), 1.06-0.97 (m, 2H), 0.76 (s, 3H), 0.45 (ddd, J_1 = 9.2 Hz, J_2 = 4.8 Hz, J_3 = 4.8 Hz, 1H), 0.26 (ddd, J_1 = 9.2 Hz, J_2 = 4.4 Hz, J_3 = 4.4 Hz, 1H), 0.16 (ddd, J_1 = 9.2 Hz, J_2 = 4.4 Hz, J_3 = 4.4 Hz, 1H), -0.003 (ddd, J_1 = 9.2 Hz, J_2 = 4.0 Hz, J_3 = 4.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 210.4, 138.7, 122.9, 56.4, 56.0, 49.5, 49.4, 48.5, 44.0, 39.6, 37.8, 37.1, 37.0, 31.8, 25.3, 24.1, 23.5, 21.3, 19.3, 14.7, 13.5, 11.0, 10.9.

3-keto-$\Delta^{4,5}$-product **80**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.72 (s, 1H), 2.48-2.34 (m, 2H), 2.33-2.22 (m, 1H), 2.10-1.98 (m, 2H), 1.87-1.77 (m, 1H), 1.75-1.65 (m, 1H),

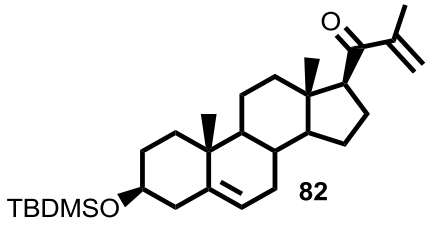
1.64-1.57 (m, 1H), 0.55-0.42 (m, 3H), 1.18 (s, 3H), 1.07 (s, 3H), 1.06-0.98 (m, 1H), 0.97-0.88 (m, 1H), 0.77 (s, 3H), 0.47 (ddd, J_1 = 9.6 Hz, J_2 = 4.8 Hz, J_3 = 0.0 Hz, 1H), 0.27 (ddd, J_1 = 9.2 Hz, J_2 = 4.0 Hz, J_3 = 4.0 Hz, 1H), 0.18 (ddd, J_1 = 9.2 Hz, J_2 = 4.4 Hz, J_3 = 4.4 Hz, 1H), 0.01(ddd, J_1 = 9.2 Hz, J_2 = 5.2 Hz, J_3 = 5.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.8, 171.8, 123.9, 55.9, 55.7, 54.0, 44.0, 39.5, 38.7, 35.8, 35.4, 34.1, 33.1, 32.1, 25.3, 24.0, 23.5, 21.0, 17.5, 14.7, 13.5, 11.0, 10.9.



2.8.63. 17β-(2-methyl-prop-2-en-1-ol)-androsten-3β-tertbutyldimethylsilyl ether (compound **81**)

Isopropenyl magnesium bromide (0.5 M in THF, 1.69 mL, 0.84 mmol, 1.5 mol eq) was added to a stirring solution of aldehyde **38** (0.26 g, 0.63 mmol) in THF (100 mL) at 0 ^{o}C under a N_2 atmosphere. The reaction was stirred for 16 h, washed with NH_4Cl (saturated aqueous solution, 5 mL), and extracted with ethyl acetate (2 x 20 mL). The combined organic extracts were concentrated via reduced pressure and purified via flash column chromatography (100% hexanes to 50% hexanes/ethyl acetate) to afford allylic alcohol **81** (0.18 g, 0.39 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.32-5.31 (m, 1H), 4.95 (s, 1H), 4.84 (s, 1H), 4.02

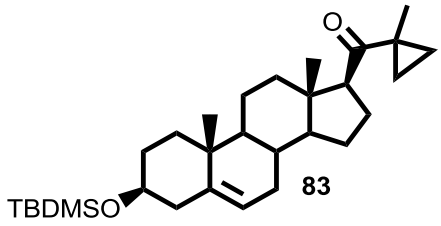
(d, J = 8.0 Hz, 1H), 3.52-3.43 (m, 1H), 2.32-2.22 (m, 1H), 2.16 (ddd, J_1 = 13.6 Hz, J_2 = 5.2 Hz, J_3 = 2.4 Hz, 1H), 2.05-1.85 (m, 2H), 1.81-1.25 (m, 10H), 1.75 (s, 3H), 0.98 (s, 3H), 0.88 (s, 9H), 0.69 (s, 3H), 0.05 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.6, 141.7, 121.2, 113.3, 78.9, 72.7, 56.8, 52.6, 50.4, 42.9, 41.7, 37.7, 37.5, 36.8, 31.8, 26.1, 24.2, 20.8, 19.6, 18.4, 16.9, 12.0, -4.4.



2.8.64. 17β-(2-methyl-prop-2-en-1-oxo)-androsten-3β-tertbutyldimethylsilyl ether (compound **82**)

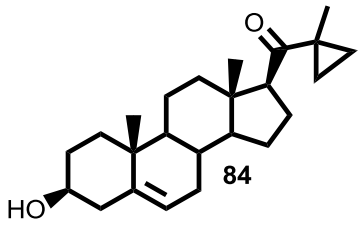
Dess-Martin periodinane (0.25 g, 0.59 mmol, 1.5 mol eq) was added to a stirring solution of alcohol **81** in CH_2Cl_2 (50 mL). The reaction was stirred for 4 h and quenched with $Na_2S_2O_3$ (saturated aqueous solution, 5 mL). The reaction mixture was washed with $NaHCO_3$ (saturated aqueous solution, 2 x 15 mL), and the organic layer was dried with $MgSO_4$ and concentrated via reduced pressure to afford enone **82** (0.15 g, 0.33 mmol, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.83 (s, 1H), 5.72 (s, 1H), 5.32 (broad s, 1H), 3.52-3.45 (m, 1H), 3.22 (apparent t, J = 8.0 Hz, 1H), 2.36-2.22 (m, 2H), 2.20-2.12 (m, 1H), 2.05-1.98 (m, 1H), 1.88 (s, 3H), 1.85-1.15 (m, 8H), 0.98 (s, 3H), 0.88 (s, 9H), 0.56 (s, 3H), 0.05 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.4, 184.4, 146.6, 141.7, 124.1, 121.1, 72.7, 57.5,

56.3, 50.3, 44.7, 42.9, 39.5, 37.5, 36.8, 32.21, 32.19, 32.1, 26.1, 24.9, 24.1, 21.3, 19.6, 18.44, 18.43, 13.8, -4.4.



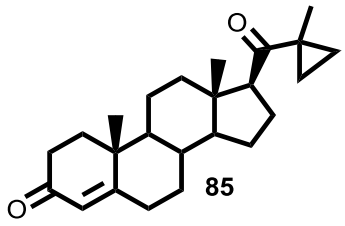
2.8.65. 21-cyclopropyl-21-methylpregnenolone-3-tert-butyldimethylsilyl ether (compound **83**)

Sodium hydride (0.034 g, 0.85 mmol) was added to a solution of trimethylsulfoxonium iodide (0.16 g, 0.73 mmol). After 10 min, a solution of enone **82** (0.28 g, 0.66 mmol) in THF was added, and the reaction was stirred for 1 h at RT. The reaction was washed with NH_4Cl (saturated aqueous solution, 10 mL) and diluted with ethyl acetate (20 mL). The reaction mixture was washed with brine (10 mL), and the organic layer was dried with $MgSO_4$ and concentrated via reduced pressure. The crude material was purified via flash column chromatography (100% hexanes to 50% hexanes/ethyl acetate) to yield cyclopropane **83** as a white solid (0.28 g, 0.6 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.32-5.31 (m, 1H), 3.51-3.45 (m, 1H), 2.89 (apparent t, J = 8.0 Hz, 1H), 2.35-1.89 (m, 3H), 1.8-1.45 (m, 4H), 1.40-1.10 (m, 4H), 1.36 (s, 3H), 0.99 (s, 3H), 0.89 (s, 9H), 0.66-0.56 (m, 1H), 0.63 (s, 3H), 0.05 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.6, 141.7, 121.1, 72.7, 57.2, 56.4, 50.3, 45.6, 42.9, 39.4, 36.8, 32.3, 32.1, 27.4, 26.1, 25.5, 25.1, 21.2, 20.8, 19.6, 18.4, 16.7, 13.8, -4.4.



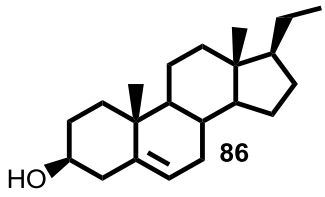
2.8.66. 21-cyclopropyl-21-methylpregnenolone (compound **84**)

TBAF (1 M, 0.6 mL, 0.6 mmol) was added to a solution of TBDMS ether **83** (0.21 g, 0.45 mmol) in THF (50 mL). The reaction was stirred for 20 h and diluted with H_2O (20 mL). The reaction mixture was extracted with ethyl acetate (2 x 30 mL), and the combined organic extracts were dried under reduced pressure. The crude material was purified via flash column chromatography (100% hexanes to 30% hexanes/ethyl acetate) to afford alcohol **84** (0.14 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35 (broad s, 1H), 3.53-3.45 (m, 1H), 2.89 (apparent t, 12.0 Hz, 1H), 2.35-2.17 (m, 2H), 2.18-1.95 (m, 2H), 1.94-1.82 (m, 3H), 1.76-1..40 (m, 4H), 1.35 (s, 3H), 1.34-1.05 (m, 3H), 1.00 (s, 3H), 0.67-0.55 (m, 1H), 0.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.6, 140.9, 121.6, 71.8, 57.2, 56.3, 50.2, 45.5, 42.4, 37.4, 36.7, 32.2, 32.1, 31.7, 27.4, 25.5, 25.0, 21.2, 20.7, 19.5, 18.4, 16.7, 13.8.

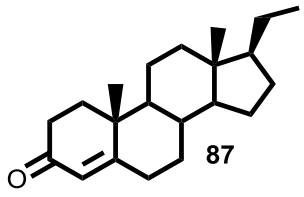


2.8.67. 21-cyclopropyl-21-methylprogesterone (compound **85**)

Dess Martin periodinane (80 mg, 0.19 mmol, 1.2 mol eq) was added to alcohol **84** (56 mg, 0.16 mmol) in CH_2Cl_2 (15 mL). The reaction mixture was loaded directly on a silica gel column, and 21-cyclopropyl-21-methyl-$\Delta^{5,6}$-progesterone (20 mg, 0.06 mmol, 38%) was isolated. For optimal yield, workup should be avoided, and DMP should be added in portions totalling a little less than 1 mol equivalent. The 3-keto-$\Delta^{5,6}$-steroid (11 mg) was dissolved in methanol/CH_2Cl_2 (2 mL/2 mL), and HCl (12 M, 1 drop) was added. After stirring for 30 min, the reaction mixture was purified via flash column chromatography to afford 21-cyclopropyl-21-methylprogesterone **85** (10 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.74 (s, 1H), 2.90 (apparent t, J = 9.0 Hz, 1H), 2.51-2.26 (m, 2H), 2.15-1.80 (m, 3H), 0.67 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.7, 171.2, 124.0, 56.3, 56.2, 53.9, 45.5, 39.1, 29.9, 25.5, 24.9, 21.2, 20.7, 13.9, 11.6.

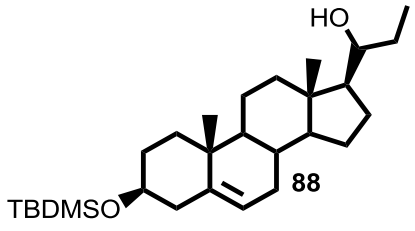


2.8.68. 20-desoxypregnenolone (compound **86**): Pregnenolone (0.25 g, 0.79 mmol), diethylene glycol (5 mL), n-butanol (2 mL), and hydrazine hydrate (0.63 mL, 12.64 mmol, 16.0 mol eq) were refluxed (200 ^{o}C) in a 100 mL round bottom flask equipped with stirrer for 12 h. The reaction was cooled to 80 ^{o}C and KOH (0.62 g) was added. The reaction was refluxed again for 3 h. After cooling to RT, the water was added (10 mL), and the reaction mixture was diluted with diethyl ether (20 mL). The aqueous layer was extracted with diethyl ether (3 x 30 mL). The combined organic extracts were dried with $MgSO_4$ and purified via flash column chromatography (100% hexanes to 50% ethyl acetate/dichloromethane) to yield 20-desoxypregnenolone **86** as a white solid (0.19 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34-5.33 (m, 1H), 3.53-3.47 (m, 1H), 2.34-2.15 (m, 2H), 2.14-1.95 (m, 1H), 1.92-1.80 (m, 4H), 1.79-1.74 (m, 1H), 1.65-1.55 (m, 1H), 1.55-1.40 (m, 5H), 1.20-1.06 (m, 5H), 1.00 (s, 3H), 0.86 (t, J = 8.0 Hz, 3H), 0.56 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.9, 121.8, 71.8, 56.3, 50.7, 42.4, 42.0, 38.0, 37.4, 36.7, 32.1, 32.0, 31.7, 28.2, 24.7, 23.1, 21.0, 19.5, 13.5, 12.5.



2.8.69. 20-desoxyprogesterone (compound **87**):

Dess-Martin periodinane (34.6 mg, 0.08 mmol, 0.8 mol eq) was added to a solution of 20-desoxypregnenolone (31.7 mg, 0.10 mmol) in CH_2Cl_2 (15 mL). After 1 h, the reaction mixture was directly loaded on a silica gel column and purified (100% hexanes to 50% hexanes/ethyl acetate) to yield 20-desoxy-$\Delta^{5,6}$-progesterone (15.1 mg, 44%). The $\Delta^{5,6}$-olefin was isomerized using hydrochloric acid and methanol to afford 20-desoxyprogesterone **87**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.72 (s, 1H), 2.48-2.33 (m, 2H), 2.38-2.24 (m, 1H), 2.05-1.99 (m, 1H), 1.90-1.83 (m, 1H), 1.82-1.75 (m, 1H), 1.75-1.59 (m, 2H), 1.58-1.50 (m, 1H), 1.45-1.34 (m, 2H), 1.18 (s, 3H), 1.15-0.95 (m, 5 H), 0.89 (t, J = 15.0 Hz, 3H), 0.63 (s, 3H).



2.8.70. 17β-(1-hydroxypropyl)-androstenol-3-tert-butyldimethylsilyl ether (compound **88**):

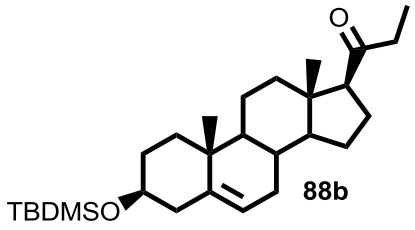
To aldehyde **38** (0.1515 g, 0.36 mmol) in THF (15 mL) was added ethylmagnesium bromide (3.0 M, 0.16 mL, 0.48 mmol, 1.40 mol eq) at 0 ^{o}C under

a N_2 atmosphere. The reaction was warmed to RT and stirred for 20 h. The reaction mixture was washed with NH_4Cl (saturated aqueous solution, 10 mL) and extracted with ethyl acetate (2 x 30 mL). The combined organic extracts were dried under reduced pressure and purified via flash column chromatography (100% hexanes to 50% hexanes/ethyl acetate) to afford the Grignard adduct **88** (0.15 g, 0.34 mmol, 94% combined epimeric mixture, upper spot with higher R_f value was the minor adduct). The epimeric mixture was separable on column, but the epimers were combined for the next step.

Less polar epimer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.30 (broad s, 1H), 3.53-3.44 (m, 1H), 2.32-2.22 (m, 1H), 2.20-2.06 (m, 2H), 2.01-1.95 (m, 1H), 1.85-1.77 (m, 1H), 1.61-1.35 (m, 6H), 1.31-1.11 (m, 4H), 1.06 (dd, J_1 = 12.0 Hz, J_2 = 4.0 Hz, 1H), 1.01 (s, 3H), 0.95 (t, J = 8.0 Hz, 3H), 0.88 (s, 9H), 0.77 (s, 3H), 0.05 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.8, 121.1, 75.6, 72.8, 56.4, 50.4, 42.5, 40.1, 37.5, 36.8, 32.2, 31.9, 29.5, 26.1, 25.5, 24.7, 21.1, 19.6, 18.4, 12.5, 9.6, -4.4.

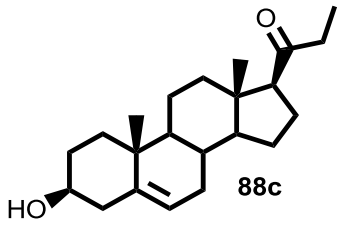
More polar epimer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.30 (broad s, 1H), 3.55-3.44 (m, 1H), 2.30-2.20 (m, 1H), 2.18-2.11 (m, 1H), 2.02-1.95 (m, 1H), 1.90-1.75 (m, 2H), 1.75-1.33 (m, 10H), 1.18-1.12 (m, 2H), 1.05 (dd, . J_1 = 12.0 Hz, J_2 = 4.0 Hz, 1H), 95 (t, J = 8.0 Hz, 3H), 0.88 (s, 9H), 0.69 (s, 3H), 0.04 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.7, 121.2, 74.7, 72.7, 56.7, 55.9, 50.4, 42.9, 41.8, 38.9, 37.5,

36.8, 32.2, 32.0, 31.7, 29.6, 26.1, 25.0, 24.3, 20.9, 19.6, 18.4, 12.8, 9.9, -4.4.



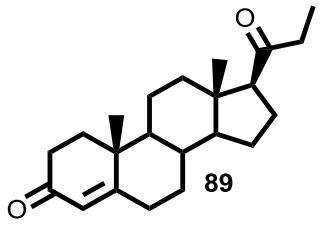
2.8.71. 21-homomethylpregnenolone-3-tert-butyldimethylsilyl ether:

Dess-Martin periodinane (0.20 g, 0.47 mmol, 1.4 mol eq) was added to alcohol **88** (0.15 g, 0.34 mmol) in CH_2Cl_2 (100 mL). The reaction mixture was washed with $Na_2S_2O_3$ (saturated aqueous solution, 10 mL) and $NaHCO_3$ (saturated aqueous solution, 20 mL). The organic layer was washed with brine, and the organic phase was dried with $MgSO_4$. The ketone was obtained as a white solid (0.12 g, 80%) and used directly for the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.31 (broad s, 1H), 3.60-3.40 (m, 1H), 2.51 (apparent t, J = 8.0 Hz, 1H), 2.48-2.31 (m, 1H), 2.30-2.15 (m, 2H), 2.12-1.95 (m, 1H), 1.83-1.75 (m, 1H), 1.74-1.40 (m, 4H), 1.25-1.10 (m, 2H), 1.02 (t, J = 8.0 Hz, 3H), 0.98 (s, 3H), 0.88 (s, 9H), 0.60 (s, 3H), 0.04 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.1, 141.6, 121.0, 72.7, 62.8, 57.1, 50.2, 44.3, 42.9, 37.5, 36.7, 32.2, 32.0, 31.9, 26.1, 24.7, 23.1, 21.2, 19.5, 18.4, 13.5, 7.9, -4.5.



2.8.72. 21-homomethylpregnenolone

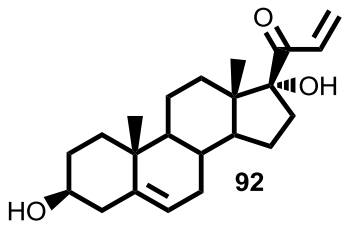
TBAF (1.0 M, 0.5 mL, 0.5 mmol, 1.5 mol eq) was added to a solution of TBDMS-ether **88b** (0.15 g, 0.34 mmol) in THF (50 mL). The reaction was stirred for 4h, and TBAF (0.5 mL, 0.5 mmol) was added followed by a second addition of TBAF (1 mL, 1 mmol) after 1h. The reaction was diluted with H_2O (10 mL) and extracted with ethyl acetate (2 x 20 mL). The combined organic layers were concentrated via reduced pressure and purified via flash column chromatography to afford 21-homomethylpregnenolone **88c** as a white solid (70 mg, 0.21 mmol, 63%). Alternatively, CSA in methanol protocol was also used to cleave the TBDMS group. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.33 (broad s, 1H), 3.60-3.45 (m, 1H), 2.50 (apparent t, J = 8.0 Hz, 1H), 2.45-2.35 (m, 2H), 2.32-2.15 (m, 3H), 2.06-1.94 (m, 2H), 1.90-1.77 (m, 3H), 1.18-1.06 (m, 2H), 1.01 (t, J = 8.0 Hz, 3H), 0.98 (s, 3H), 0.59 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.2, 140.9, 121.5, 71.7, 62.8, 57.0, 50.1, 44.2, 42.3, 39.0, 37.5, 37.4, 36.6, 32.0, 31.9, 31.4, 24.7, 23.1, 21.0, 19.5, 13.5, 7.9.



2.8.73. 21-homomethylprogesterone (compound **89**)

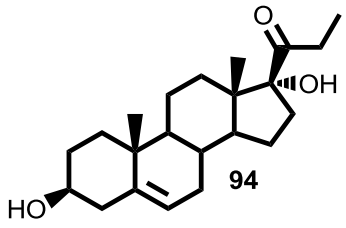
Dess-Martin periodinane (100 mg, 0.24 mmol, 1.9 mol eq) was added to a stirring solution of 21-homomethylpregnenolone (400 mg, 0.12 mmol) in CH_2Cl_2 (40 mL). The reaction was stirred for 1 h and washed with $Na_2S_2O_3$ (saturated aqueous solution, 10 mL) and $NaHCO_3$ (saturated aqueous solution, 2 x 10 mL). The organic layer was concentrated via reduced pressure, and the crude product 21-homomethyl-$\Delta^{5,6}$-progesterone was dissolved in methanol/CH_2Cl_2 (5 mL/5 mL) and treated with HCl (12 M, 1 drop). The reaction was stirred for 30 min and concentrated via reduced pressure. The crude material was purified via flash column chromatography (100% hexanes to 30% hexanes/ethyl acetate) to yield 21-homomethylprogesterone **89** (60 mg, 60%) as the product.

3-keto-$\Delta^{5,6}$-product: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.33 (broad s, 1H), 3.27 (dq, J_1 = 16.0 Hz, J_2 = 4.0 Hz, 1H), 2.82 (dd, J_1 = 16.0 Hz, J_2 = 4.0 Hz, 1H), 2.52 (apparent t, J = 8.0 Hz, 1H), 2.56-2.15 (m, 6H0, 2.10-0.95 (m, 3H), 1.75-1.35 (m, 6H), 1.35-1.2 (m, 2H), 1.17 (s, 3H), 1.02 (t, J = 8.0 Hz, 3H), 0.63 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.1, 210.3, 138.6, 122.7, 62.7, 56.8, 49.2, 48.4, 44.2, 38.9, 37.7, 37.5, 37.0, 32.0, 31.8, 24.6, 23.2, 21.1, 19.3, 13.6, 7.9.



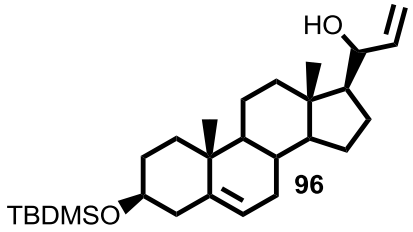
2.8.74. 17-hydroxy-21-homo-21,22-dehydropregnenolone (compoundd **92**)

To a solution of 17-hydroxypregnenolone (0.28 g, 0.84 mmol) and paraformaldehyde (1.4 g) in THF (20 mL) was added diisopropylamine (2.0 mL) and trifluoroacetic acid (4.0 mL), and the reaction was heated to 60 ^{o}C at reflux for 2 d. The reaction was cooled to RT and diluted with diethyl ether (50 mL), then washed with NH_4Cl (saturated aqueous solution, 50 mL) followed by $NaHCO_3$ (saturated aqueous solution, 50 mL). The organic layer was dried under reduced pressure and purified via flash column chromatography (100% hexanes to 30% hexanes in ethyl acetate) to afford enone **92** (0.10 g, 0.29 mmol, 35%) as a white solid. R_f = 0.50 in 50% hexanes/ethyl acetate (starting material R_f = 0.45); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.93 (dd, J_1 = 13.6 Hz, J_2 = 8.0 Hz, 1H), 6.38 (d, J = 13.6 Hz, 1H), 5.70 (d, J = 8.3 Hz, 1H), 5.36-5.35 (m, 1H), 3.58-3.45 (m, 1H), 2.82 (broad s, 1H), 2.76 (ddd, J_1 = 11.6 Hz, J_2 = 5.5 Hz, 2.0 Hz, 1H), 2.35-2.28 (m, 1H), 2.25-2.19 (m, 1H), 2.25-1.95 (m, 1H), 1.90-1.80 (m, 2H), 1.78-1.70 (m, 2H), 1.70-1.33 (m, 10H), 1.15-1.12 (m, 1H), 1.00 (s, 3H), 0.72 (s, 3H).



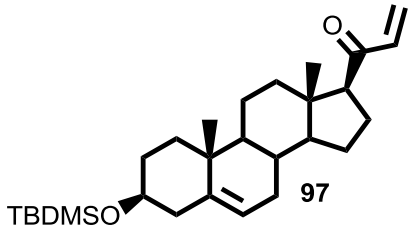
2.8.75. 17-hydroxy-21-homomethylpregnenolone (compound **94**)

To a solution of 17-hydroxy-21-homo-21,22-dehydropregnenolone **92** (115 mg, 0.34 mmol) in ethyl acetate (20 mL) was added Pd on barium sulfate (20 mg). The flask was evacuated and backfilled with H_2 gas. The reaction was stirred for 24h and checked by NMR to indicate completion with loss of the vinyl enone proton peaks (C21-C22). The reaction mixture was directly loaded on a silica gel column and purified to afford 17-hydroxy-21-homomethylpregnenolone **94** (50 mg, 0.15 mmol, 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.37-5.35 (m, 1H), 3.61-3.45 (m, 1H), 2.83-2.72 (m, 1H), 2.71-2.63 (m, 1H), 2.52-2.45 (m, 1H), 2.35-2.18 (m, 2H), 2.07-1.97 (m, 1H), 1.88-1.78 (m, 2H), 1.75-1.30 (m, 10H), 1.06 (t, J = 7.2 Hz, 3H), 1.00 (s, 3H), 0.70 (s, 3H).



2.8.76. 17β-(prop-2-en-1-ol)-androstene-3β-tert-butyldimethylsilyl ether (compound **96**)

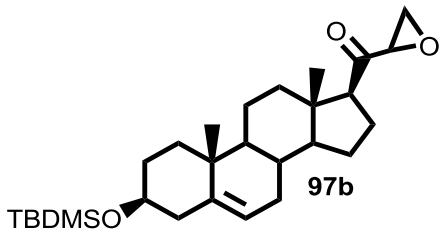
To a solution of aldehyde **38** (1.13 g, 2.7 mmol) in THF (25 mL) at 0 ^{o}C was added vinyl magnesium chloride (1.6 M in THF, 1.9 mL, 3 mmol). The reaction was warmed to RT and stirred for 30 min, then quenched with NH_4Cl (saturated aqueous solution, 5 mL). The reaction mixture was diluted with ethyl acetate (100 mL), and the resulting solution was washed with brine (50 mL). The combined organic extracts were dried under reduced pressure, and the crude material was purified via flash column chromatography (100% hexanes to 50% hexanes/ethyl acetate) to afford allylic alcohol **96** (1.00 g, 2.3 mmol, 83%). The C20-epimeric mixture was used directly for the next step (oxidation with Dess-Martin periodinane).



2.8.77. 21-homo-21,22-dehydropregnenolone-3-tert-butyldimethylsilyl ether (compound **97**)

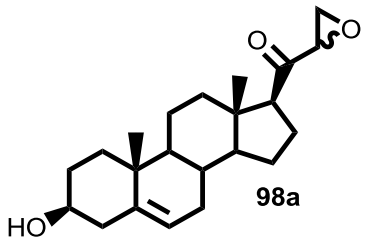
To a solution of allylic alcohol **96** (0.28 g, 0.63 mmol) in CH_2Cl_2 (10 mL) was added Dess-Martin periodinane (0.29 g). The reaction was stirred for 1 h and purified directly on a silica gel column (100% hexanes to 50% hexanes/ethyl acetate) to afford enone **97** (0.16 g, 0.36 mmol, 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.42 (dd, J_1 = 17.4 Hz, J_2 = 10.4 Hz, 1H), 6.20 (d, J = 17.3 Hz, 1H),

5.67 (d, J = 10.5 Hz, 1H), 5.31-5.30 (m, 1H), 3.55-3.41 (m, 1H), 2.81 (apparent t, J = 9.0 Hz, 1H), 2.30-2.15 (m, 2H), 2.12-1.95 (m, 2H), 1.83-1.40 (m, 8H), 0.99 (s, 3H),0.88 (s, 9H), 0.60 (s, 3H), 0.05 (s. 6H).



2.8.78. 21,22-epoxy-pregnenolone-3-tert-butyldimethylsilyl-ether (compound **97b**)

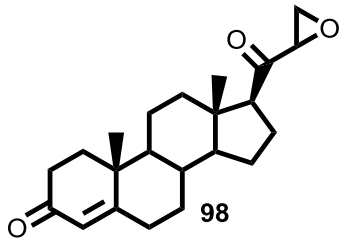
To 21-homo-21,22-dehydropregnenolone-3-tert-butyldimethylsilyl ether **97** (0.16 g) in methanol (5 mL) and hydrogen peroxide (30% aqueous, 1 mL) was added sodium hydroxide (0.1 g), and the reaction was stirred for 30 min. The reaction was extracted with ethyl acetate, and the combined organic layers were dried under reduced pressure. The crude material was purified via flash column chromatography (100% hexanes to 50% hexanes/ethyl acetate) to afford the epoxide **97b** as an epimeric mixture (61 mg). The epimeric mixture could be separated on the a silica gel column, but the separation was better when converted to the 3-keto-$\Delta^{4,5}$-compounds and best when the 3-hydroxy group was protected as the silyl ethers. The NMR is not reported due to the complex mixture of the 21-epimers – see supporting information for NMR spectrum.



98a

2.8.79. 21,22-epoxy-pregnenolone (compound **98a**)

Camphor-sulfonic acid (4 mg) was added to a solution of TBDMS ether **97** (61 mg, 0.13 mmol) in methanol (5 mL), and the solution was stirred for 30 min. The reaction was purified directly on a silica gel column (100% hexanes to 30% hexanes in ethyl acetate) to afford alcohols **98a** (30 mg, 0.09 mmol, 67%) as an epimeric mixture at C21. The NMR is not reported due to the complex mixture of the 21-epimers – see supporting information for NMR spectrum.



98

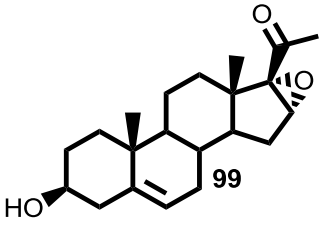
2.8.80. 21,22-epoxyprogesterone (compound **98**)

Dess-Martin periodinane was added to alcohol **98a** (epimeric mixture at C21) in CH_2Cl_2, and the mixture was stirred for 30 min. Isomerization of the $\Delta^{5,6}$-olefin to the $\Delta^{4,5}$-alkene was achieved using catalytic camphor-sulfonic acid in methanol as HCl in methanol appeared to open the epoxide and form the 21,22-diol.

Less polar epimer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.74 (s, 1H), 3.46 (s, 1H), 2.98-2.96 (m, 1H), 2.78-2.75 (m, 1H), 2.73 (apparent t, J = 9.1 Hz, 1H), 2.50-2.32

(m, 2H), 2.32-2.15 (m, 2H), 2.05-1.95 (m, 2H), 1.90-1.50 (m, 6H), 1.42-1.38 (m, 1H), 1.35-1.21 (m, 3H), 1.19 (s, 3H), 1.15-0.95 (m, 2H), 0.68 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 207.6, 200.0, 170.9, 124.1, 58.4, 56.3, 53.7, 53.5, 46.1, 45.7, 38.8, 38.7, 35.83, 35.80, 34.1, 32.9, 32.0, 24.7, 22.9, 21.1, 17.5, 14.0.

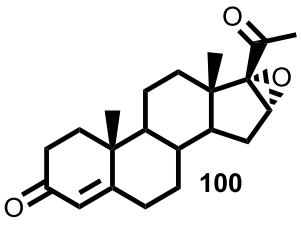
More polar epimer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.74 (s, 1H), 3.51-3.50 (m, 1H), 2.95-2.88 (m, 1H), 2.73 (apparent t, J = 10.6 Hz), 2.48-2.35 (m, 1H), 2.34-1.98 (m, 3H), 1.92-1.85 (m, 1H), 1.75-1.45 (m, 4H), 1.19 (s, 3H), 1.15-0.80 (m, 3H), 0.75 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 207.0, 199.6, 170.9, 124.1, 60.7, 56.2, 53.7, 52.6, 47.3, 45.3, 38.9, 38.7, 35.9, 35.7, 34.1, 32.9, 32.0, 24.6, 23.2, 21.1, 17.5, 14.0.



2.8.81. 16α,17α-epoxypregnenolone (compound **99**)

To a stirring solution of 16,17-dehydropregnenolone acetate (1.0 g, 2.8 mmol in methanol/CH_2Cl_2 (20 mL/5 mL) was added sodium hydroxide (0.5 g, 12 mmol, 4.4 mol eq) at 0 ^{o}C. Hydrogen peroxide (35%, 2.5 mL) was added, and the reaction was stirred for 48 h at RT. The resulting mixture was diluted with ethyl acetate (100 mL) and washed with brine. The combined organic extracts were

dried with $MgSO_4$ and concentrated via reduced pressure. The crude material was used for the next steps (either oxidation of 3-hydroxy or 16α-hydroxypregnenolone synthesis). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34-5.32 (m, 1H), 3.68 (s, 1H), 3.52-3.45 (m, 1H), 2.32-2.17 (m, 2H), 2.06-2.01 (m, 1H), 2.03 (s, 3H), 2.0-1.9 (m, 2H), 1.9-1.8 (m, 2H), 1.70-1.40 (m, 6H), 1.32 (apparent t, J = 12.0 Hz, 1H), 1.2-0.9 (m, 2H), 1.04 (s, 3H), 1.02 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.1, 141.3, 121.2, 71.8, 71.2, 60.7, 50.4, 45.7, 42.4, 41.7, 37.3, 36.8, 31.7, 31.6, 31.5, 29.9, 27.7, 26.2, 20.6, 19.5, 15.4.



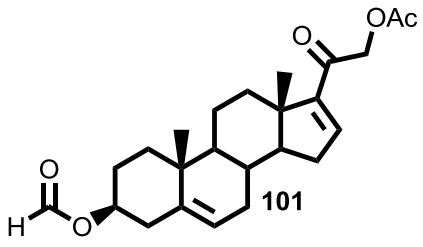
2.8.82. 16α,17α-epoxyprogesterone (compound **100**)

Alcohol **99** (2.0 g, 6.0 mmol) was weighed in an oven-dried 100 mL round bottom flask equipped with a stirrer. Dichloromethane (50 mL) was added to dissolve the alcohol, and Dess-Martin periodinane (2.8 g, 6.60 mmol, 1.1 mol eq) was added to the stirring solution. The reaction was stirred at RT and monitored by TLC. Upon completion, the mixture was diluted with ether (100 mL) and washed with water (50 mL) and $NaHCO_3$ (saturated aqueous solution, 3 x 50 mL). The organic layer was concentrated, and the crude material was purified via flash column chromatography (100% hexanes to 50% hexanes/ethyl acetate) to

afford the less polar 3-keto-$\Delta^{5,6}$-product (0.4 g, 1.2 mmol, 20%) and the isomerized (more polar) 3-keto-$\Delta^{4,5}$-product **100** (0.6 g, 1.8 mmol, 30%).

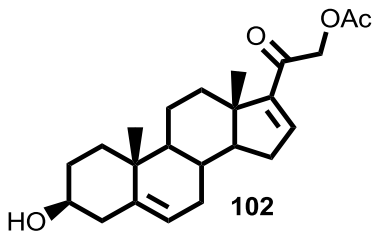
3-keto-$\Delta^{5,6}$-product: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.32-5.31 (m, 1H), 3.69 (s, 1H), 3.30-3.24 (m, 1H), 2.85-2.78 (m, 1H), 2.46 (ddd, J_1 = 12.0 Hz, J_2 = 12.0 Hz, J_3 = 4.0 Hz, 1H), 2.33-2.25 (m, 1H), 2.13-2.00 (m, 2H), 2.02 (s, 3H), 1.99-1.94 (m, 2H), 1.70-1.55 (m, 3H), 1.52-1.40 (m, 2H), 1.38-1.30 (m, 1H), 1.23-1.15 (m, 1H), 1.19 (s, 3H), 1.07 (s, 3H) 1.11-1.02 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 210.1, 205.0, 139.0, 122.3, 71.1, 60.6, 49.5, 48.5, 45.6, 41.7, 37.7, 37.2, 37.0, 31.5, 31.4, 29.9, 27.6, 26.1, 20.8, 19.2, 15.4.

3-keto-$\Delta^{4,5}$-product **100**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.68-5.67 (m, 1H), 3.67 (s, 1H), 2.43-2.21 (m, 4H), 2.1-1.9 (m, 5H), 1.98 (s, 3H), 1.79-1.70 (m, 1H), 1.70-1.55 (m, 3H), 1.50-1.28 (m, 3H), 1.16 (s, 3H), 1.04 (s, 3H), 0.95-0.86 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 204.8, 199.5, 170.7, 124.1, 70.7, 60.5, 53.9, 44.9, 41.6, 38.7, 35.6,34.0, 33.3, 32.7, 31.6, 31.2, 27.4, 26.0, 20.4, 17.3, 15.3.



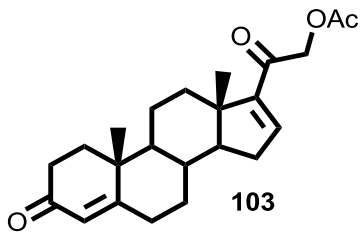
2.8.83. 16,17-dehydropregnenolone-3-formate-21-acetate (compound **101**)

Formate **22** (92 mg, 0.20 mmol) was dissolved in acetonitrile (5 mL) in a 100 mL round bottom flask equipped with stirrer, and AgOAc (60 mg, 0.36 mmol, 1.8 mol eq) was added. The reaction was stirred for 24 h at RT, and the reaction mixture was directly loaded on a silica gel column for purification (100% hexanes to 50% Ethyl acetate/hexanes) to yield acetate **101** (70 mg, 0.18 mmol, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1H), 6.75 (s, 1H), 5.35 (m, 1H), 4.99, 4.84 (ABq, 2H, J_{AB} = 16.0 Hz), 4.65-4.78 (m, 1H), 2.31-2.38 (m, 4H), 2.18 (s, 3H), 2.13-2.00 (m, 2H), 1.90-1.87 (m, 2H), 1.71-1.59 (m, 4H), 1.47-1.34 (m, 2H), 1.18-1.11 (m, 1H), 1.06 (s, 3H), 0.92 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 190.5, 170.4, 160.6, 151.9, 144.1, 139.9, 122.2, 104.8, 73.7, 65.6, 55.9, 50.3, 46.7, 38.0, 36.8, 36.7, 34.3, 32.7, 31.5, 30.0, 27.7, 20.6, 19.2, 15.8.



2.8.84. 16,17-dehydropregneonlone-21-acetate (compound **102**)

To a stirring solution of formate **101** (16 mg) in methanol/CH_2Cl_2 (10 mL:10 mL) was added HCl (12 N, 0.2 mL). The reaction was stirred for 1 h and purified via flash column chromatography (100% hexanes to 50% Ethyl acetate/hexanes) to yield 3-alcohol **102** as a white solid (14 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.75 (s, 1H), 5.35 (broad s, 1H), 5.03, 4.88 (ABq, 2H, J_{AB} = 16.0 Hz), 3.60-3.49 (m, 1H), 2.42-2.22 (m, 2H), 2.18 (s, 3H), 2.20-1.85 (m, 2H), 0.90-0.80 (m, 2H), 1.04 (s, 3H), 0.94 (s, 3H).

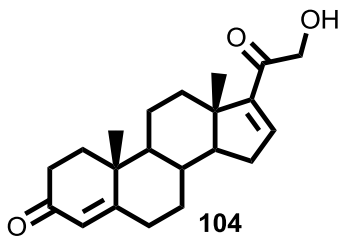


103

2.8.85. 21-acetoxy-16,17-dehydroprogesterone (compound **103**)

Dess-Martin periodinane (0.43 g, 1.0 mmol) was added to a solution of alcohol **102** (0.27 g, 0.72 mmol) in CH_2Cl_2 (20 mL) and the reaction was stirred for 1 h followed by direct purification via flash column chromatography (100% hexanes to 50% ethyl acetate in hexanes) with isomerization of the 5,6-double bond to the 4,5-double bond occurring on the column yielding ketone **103** (0.13 g, 0.35 mmol, 49%) as a white solid. 21-acetoxy-16,17-dehydroprogesterone **103**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.74 (s, 1H), 5.74 (s, 1H), 5.03, 4.87 (ABq, 2H, J_{AB} = 16.0 Hz), 2.48-2.25 (m, 6H), 2.18 (s, 3H), 2.12 (dd, J_1 = 17.0 Hz, J_2 = 13.4 Hz, 1H),

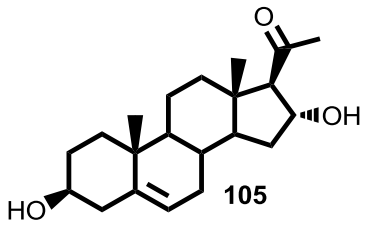
2.03-2.00 (m, 1H), 1.88-1.85 (m, 1H), 1.80-1.60 (m, 3H), 1.53 (ddd, J_1 = 17.0 Hz, J_2 = 13.5 Hz, J_3 = 4.0 Hz, 1H), 1.46-1.32 (m, 2H), 1.21 (s, 3H), 1.13 (ddd, J_1 = 16.5 Hz, J_1 = 13.0 Hz, J_3 = 4.0 Hz, 1H), 1.01 (ddd, J_1 = 15.5 Hz, J_2 = 12.0 Hz, J_3 = 5.0 Hz, 1H), 0.96 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.6, 190.6, 170.8, 170.6, 151.9, 143.9, 124.2, 65.7, 55.4, 54.2, 46.8, 38.8, 35.7, 34.3, 34.1, 33.9, 32.8, 32.7, 31.9, 20.8, 20.7, 17.3, 16.1.



104

2.8.86. 21-hydroxy-16,17-dehydroprogesterone **104**: To a flask containing 21-acetate **103** (25 mg, 0.068 mmol) was added methanol (5 mL) and HCl (12 N, 0.1 mL). The reaction was stirred for 10 h and concentrated via reduced pressure, then purified via flash column chromatography (gradient from 100% hexanes to 50% Ethyl acetate in hexanes); 21-hydroxy-16,17-dehydroprogesterone **104** (10 mg, 0.030 mmol, 45%) was isolated as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.74 (dd, J = 3.6, 1.6 Hz, 1H), 5.74 (broad s, 1H), 4.54 (dd, J_1 = 18.0 Hz, J_2 = 4.8 Hz, 1H), 4.31 (dd, J_1 = 18.0 Hz, J_2 = 4.8 Hz, 1H), 3.28 (apparent t, J = 4.8 Hz, 1H), 2.29-2.49 (m, 5H), 2.13 (ddd, J_1 = 16.0 Hz, J_2 = 8.0 Hz, J_3 = 1.6 Hz, 1H), 2.06-2.00 (m, 1H), 1.92-1.85 (m, 1H), 1.80-1.61 (m, 3H), 1.55-1.41 (m, 1H), 1.39-1.35 (m, 1H), 1.28-1.24 (m, 1H), 1.23 (s, 3H),

1.20-1.12 (m, 1H), 1.09-1.00 (m, 1H), 0.98 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.61, 199.58, 170.8, 151.4, 144.6, 124.2, 65.2, 54.2, 46.7, 38.8, 35.7, 34.4, 34.1, 34.0, 33.9, 32.8, 32.7, 31.9, 20.8, 17.3, 16.2.

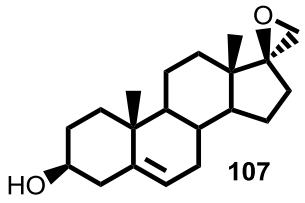


105

2.8.87. 16α-hydroxypregnenolone (compound **105**)

Hydrazine hydrate (2.5 mL, 80 mmol, 40 mol eq) was added to epoxide **99** (1.00 g, 2.71 mmol) in methanol (40 mL). The reaction was stirred for 48 h and diluted with CH_2Cl_2 (40 mL). The organic layer was concentrated and purified via flash column chromatography (100% hexanes to 30% hexanes/ethyl acetate) to afford 16α-hydroxypregnenolone (0.40 g, 1.2 mmol, 44%).

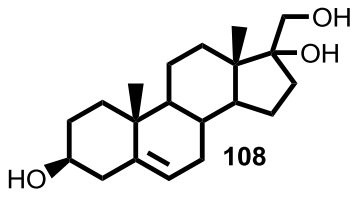
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35-5.34 (m, 1H), 4.86 (ddd, J_1 = 8.6 Hz, J_2 = 6.8 Hz, J_3 = 1.8 Hz, 1H), 3.60-3.47 (m, 1H), 2.52 (d, J = 6.5 Hz, 1H), 2.35-2.15 (m, 4H), 2.18 (s, 3H), 2.05-1.93 (m, 2H), 1.91-1.80 (m, 2H), 1.75-1.45 (m, 6H), 1.15-1.05 (m, 1H), 1.00 (s, 3H), 0.64 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 208.8, 140.9, 121.4, 73.9, 72.3, 71.8, 54.6, 50.0, 45.0, 42.3, 38.9, 37.2, 36.6, 35.4, 31.9, 31.7, 31.5, 20.8, 19.5, 14.5.



2.8.88. 17,20-epoxy-androst-5-en-3β-ol (compound **107**)

Trimethylsulfonium iodide (4.2 g, 20.6 mmol, 2.0 mol eq) and potassium tert-butoxide (2.2 g, 19.6 mmol, 1.9 mol eq) were dissolved in DMF (50 mL), and the reaction was stirred for 15 min. DHEA (3.0 g, 10.4 mmol) was added at 0 ^{o}C, and the reaction was stirred for 12 h under a N_2 atmosphere. The reaction was concentrated and purified via flash column chromatography (100% hexanes to 50% hexanes/ethyl acetate) to afford epoxide **107** (1.0 g, 3.3 mmol, 32%).

Less polar epimer (major product): R_f = 0.6 in 50% hexanes/ethyl acetate; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34-5.33 (m, 1H), 3.58-3.43 (m, 1H), 2.88 (d, J = 5.1 Hz, 1H), 2.59 (d, J = 5.1 Hz, 1H), 2.33-2.15 (m, 2H), 2.00-1.91 (m, 2H), 1.85-1.78 (m, 2H), 1.75-1.68 (m, 1H), 1.61-1.55 (m, 3H), 1.50-1.35 (m, 4H), 1.30-1.20 (m, 1H), 1.10-1.05 (m, 1H), 1.01-0.96 (m, 1H), 0.99 (s. 3H), 0.87 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 141.3, 121.3, 71.7, 70.7, 53.7, 53.2, 50.2, 42.3, 40.0, 37.3, 36.7, 33.9, 32.1, 31.6, 31.5, 29.1, 23.7, 20.5, 19.5, 14.3.



2.8.89. 17-hydroxy-17-(hydroxy methyl)-androstenol (compound **108**)

BF_3-OEt_2 (0.126 mL, 0.99 mmol, 0.5 mol eq) was added to a stirring solution of epoxide **107** (0.60 g, 1.99 mmol, 1.0 mol eq) in THF (100 mL) at 0 ^oC under a N_2 atmosphere. The reaction was stirred for 12 h, and BF_3-OEt_2 (0.15 mL) was added to the reaction. After 1 h, the reaction was quenched with water (2 mL), and the reaction mixture was extracted with ethyl acetate (20 mL). The combined organic extracts were concentrated under reduced pressure, and the crude material was purified via flash column chromatography (100% hexanes to 20% hexanes in ethyl acetate) to afford triol **108** (0.40 g, 1.26 mmol, 63%). R_f = 0.34 in 50% hexanes/ethyl acetate; ^1H NMR (400 MHz, $CDCl_3$) δ 5.41-5.40 (m, 1H), 3.60-3.45 (m, 1H), 3.40 (d, J = 10.4 Hz, 1H), 3.30 (d, J = 10.4 Hz, 1H), 2.35-0.71 (m, 10H), 1.35-1.25 (m, 2H), 1.18-1.08 (m, 2H), 1.05-0.98 (m, 2H), 0.99 (s, 6H).

2.8.90. 17α-[^3H]-pregnenolone (compound **109**)

The procedure in 2.8.40 was followed except that ^3H_2O (1Ci/0.2 mL) was used instead of D_2O and the reaction was stirred in a disposable glass vial. The following workup procedure was used: sodium sulfate was added to the reaction mixture after 2 h, and the reaction mixture was dissolved in diethyl ether (0.5

mL). The reaction mixture was dried over the weekend in the fumehood and the reaction mixture was dissolved in dichloromethane and purified via flash column chromatography (100% hexanes to 10% ethyl acetate in hexanes to 25% ethyl acetate in hexanes to 50% ethyl acetate in hexanes). Phosphorimager screen was used to visualize the radioactive material on TLC and the tubes containing 17α-[^{3}H]-pregnenolone were collected and dried in a tared vial under gentle nitrogen flow. The resulting solid was dissolved in ethanol.

CHAPTER THREE

Halogenated, Olefinic, and Homologated Pregnanes as Mechanistic Probes of Human Steroid Hydroxylases CYP17A1 and CYP21A2

3.1. BACKGROUND

The human steroidogenic cytochromes P450 CYP17A1 (P450c17, 17α-hydroxylase/17,20-lyase) and CYP21A2 (P450c21, 21-hydroxylase) oxygenate the 17-position and 21-positions of progesterone, respectively. The hydroxylase activity of CYP17A1 leads to the production of the glucocorticoid cortisol, while the activity of CYP21A2 leads to the production of both mineralocorticoids and glucocorticoids. Consequently, these enzymes are critically important in human physiology and disease, and a better understanding of their chemistries might lead to better treatments of rare and common diseases. Interestingly, when progesterone is the substrate, CYP17A1, both 17α- and 16α-hydroxylates in a 3:1 ratio [136]; however when pregnenolone is used as a substrate, 17-hydroxypregnenolone is the only hydroxylation product. CYP21A2 exclusively 21-hydroxylates progesterone and 17-hydroxyprogesterone, but we recently showed that the rationally designed CYP21A2 mutation V359G is primarily a progesterone 16α-hydroxylase [137]. Consequently, these biosynthetic enzymes, known for their high fidelity, also demonstrate intrinsic and latent catalytic plasticity. Until recently, insight to structure-function relationships derives largely

from computer modeling [138], site-directed mutagenesis [139], human genetics [140], and substrate analog studies. Halogenated substrate analogs have been employed as probes of cytochrome P450 function and as a strategy to prevent drug metabolism for many years. Furthermore, many cytochromes P450 are known to epoxidize unsaturated substrates, but natural-occurring steroids do not contain carbon-carbon double bonds in the vicinity of the sites where CYP17A1 and CYP21A2 perform oxygenation. Limited data exist on how steroidogenic P450 enzymes handle halogenated and olefinic substrates, and few efforts have comprehensively pursued their synthesis and characterization. Consequently, we tested these halogenated and unsaturated steroids as substrates and inhibitors for CYP17A1 and CYP21A2.

3.2. EXPERIMENTAL PROCEDURES

3.2.1. CYP17A1 and CYP21A2 Assays

Enzyme assays used human CYP21A2 and CYP17A1 with P450-oxidoreductase in microsomes from yeast expressing the recombinant proteins as described [141]. Test steroid (20-50 μM, delivered in <5 μL ethanol) and microsomes (5-10 μL, ~10 pmol P450) were incubated in 1 mL 50 mM potassium phosphate (pH 7.4) with 1 mM NADPH for 2-5 hours at 37 ^{o}C. For control incubations with ketoconazole to inhibit P450 activity, microsomes and 100 mM ketoconazole (or 20 μM abiraterone) were preincubated at room temperature for

15 min before the addition of steroid followed by NADPH. The incubations were terminated with the addition of CH_2Cl_2 (1 mL), extraction, and centrifugation. The organic extract was dried under nitrogen flow, dissolved in 50% aqueous methanol (70 -90 mL), and analyzed by high performance liquid chromatography (HPLC).

Superoxide dismutase (4,500 units/mg) and catalase from bovine liver (2000-5000 units/mg, Product # C1345) were purchased from Sigma Aldrich. Two sets of incubations were run in triplicate. One set involved 6 µM 16,17-dehydroprogesterone and CYP17A1 microsomes (10 µL) with SOD (4,500 units/mL, 10 µL) and catalase (300 units/mL, 10µL) in 1 mL50 mM potassium phosphate buffer with 1 mM NADPH for 45 min and terminated with the addition of CH_2Cl_2 (1 mL). The second set was the same except SOD (4,500 units/mL, 10 µL) and catalase (300 units/mL, 10µL) were added at the end of 45 min followed by extraction with CH_2Cl_2 (1 mL).

3.2.2. Cholesterol Oxidase Treatment

For analysis of incubations with Δ^5,3β-hydroxy-steroids, substrates and products were converted to their Δ^4,3-ketosteroid congeners to generate a UV chromophore. The dried extracts were dissolved in 80 mL of ethanol and diluted in 0.2 mL of 50 mM potassium phosphate (pH 7.4) solution. To this solution, 1 unit of cholesterol oxidase in 40 mL water was added, and the incubation was

shaken at 200 rpm and 30 ^{o}C for 5-10 h. The reaction was extracted with 1 mL of CH_2Cl_2, and the dried extracts were dissolved in 50% methanol for HPLC analysis.

3.2.3. HPLC Analyses

Steroids were resolved using a Waters Breeze 1525 HPLC equipped with autosampler and dual-wavelength UV-visible detector set at 254 nm or an Agilent 1260 Infinity HPLC system with UV detector and β-RAM4 in-line scintillation counter (LabLogic, Brandon, FL). The stationary phase was a Symmetry 3.1 x 150 mm, 3.5 mm C_{18} (Waters) or either Kinetex 2.1 x 100 mm, 2.6 mm C_{18} or 2.1-3.0 x 50 mm, 2.6 mm, C_8 (Phenomenex) reverse-phase columns maintained at 40 ^{o}C (30 ^{o}C for Kinetex 3.0 x 50 mm), and methanol-water gradients at 0.4 mL/min (0.6 mL/min for Kinetex 3.0 x 50 mm) comprised the mobile phase. For the Kinetex 3.0 x 50 mm C_8 column in each run, the gradient went from 27% methanol from 0 to 0.5 min, 39% methanol at 0.51 min, 44% methanol to 16 min, 60% methanol to 20 min, 71% methanol to 22 min, 75% methanol to 30 min, and back to 27% methanol to re-equilibrate for 3 min. Authentic standards and starting substrates were chromatographed before and/or after incubation products with each experiment.

3.3. RESULTS

3.3.1. Halogenated Steroids as Substrates for Human CYP17A1 and CYP21A2

Fluorinated small molecules have importance in the field of medicine to probe the biological function of compounds in vivo [142]. Consequently, we screened how the incorporation of fluorine atom(s) on either the 17-position or the 21-position of progesterone or pregnenolone altered metabolism by CYP17A1 or CYP21A2.

17-fluorosteroids as substrates

When yeast microsomes containing CYP17A1 and P450-oxidoreductase were incubated with 17-fluoroprogesterone, under conditions where progesterone is largely metabolized to 17α- and 16α-hydroxylated products, no metabolism of 17-fluoroprogesterone **1** could be detected despite prolonged incubations (not shown). Similarly, CYP17A1-containing microsomes did not metabolize 17-fluoropregnenolone **10** to any demonstrable products, as assessed after conversion via cholesterol oxidase to their cognate Δ^4-steroids. In both experiments, which were repeated 3 or more times, only 17-fluoroprogesterone was recovered, without any convincing evidence of product formation (not shown). These results demonstrate that blocking metabolism at the 17-position of both pregnenolone and progesterone by fluorine replacement does not result in metabolic switching

to an alternative C-H bond, such as the 16α-position, as might be predicted from the known progesterone 16α-hydroxylase activity of human CYP17A1 [136].

In contrast, when yeast microsomes containing CYP21A2 and P450-oxidoreductase were incubated with 17-fluoroprogesterone, under conditions where progesterone is metabolized to 11-deoxycorticosterone via 21-hydroxylation, comparable amounts of 17-fluoroprogesterone were reproducibly converted to an earlier-eluting peak, consistent with 21-hydroxylation (Figure 3.1). This metabolism of progesterone and 17-fluoroprogesterone was comparably inhibited by ketoconazole, consistent with P450-mediated oxygenation (Figure 3.1). This result suggests that 17-fluorination does not significantly impede substrate binding or alter catalysis by CYP21A2.

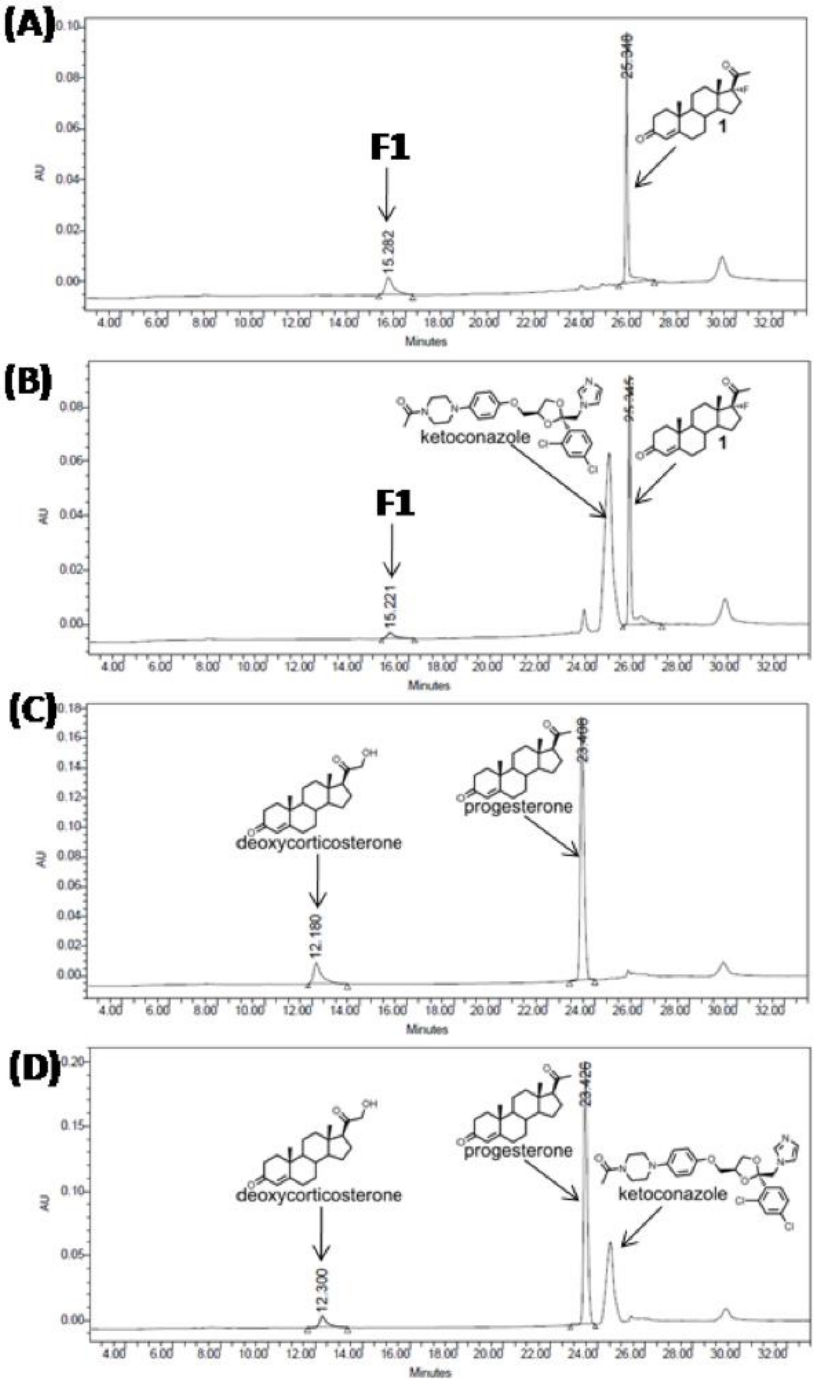


Figure 3.1. *In vitro* studies of 17-fluoroprogesterone as a substrate for CYP21A2. HPLC traces of incubations: (A) 17-fluoroprogesterone (**1**) incubation with CYP21A2, (B) 17-fluoroprogesterone (**1**) incubation with CYP21A2 and ketoconazole, (C) progesterone incubation with CYP21A2, (D) progesterone

incubation with CYP21A2 and ketoconazole. The formation and migration of compound **F1** is consistent with 17-fluoro-21-hydroxyprogesterone. Conversion of 17-fluoroprogesterone to **F1** was inhibited 65% by pre-incubation with ketoconazole, and conversion of progesterone to 11-deoxycorticosterone was inhibited 40% by ketoconazole using the same conditions. The peak at 30 min is due to the change in solvent gradient.

21,21,21-trifluorosteroids as substrates

When 21,21,21-trifluoroprogesterone **4** was incubated with yeast microsomes containing CYP17A1 or CYP21A2, two earlier-eluting peaks were variably observed, but their formation was only partially inhibited by ketoconazole. The same pattern of product formation was observed from incubations with CYP17A1-containing microsomes and 21,21,21-trifluoropregnenolone **13**, followed by cholesterol oxidase treatment (Figure 3.2). Consequently, we are unable to conclude that the 21,21,21,-trifluorosteroids are substrates for CYP17A1 and CYP21A2.

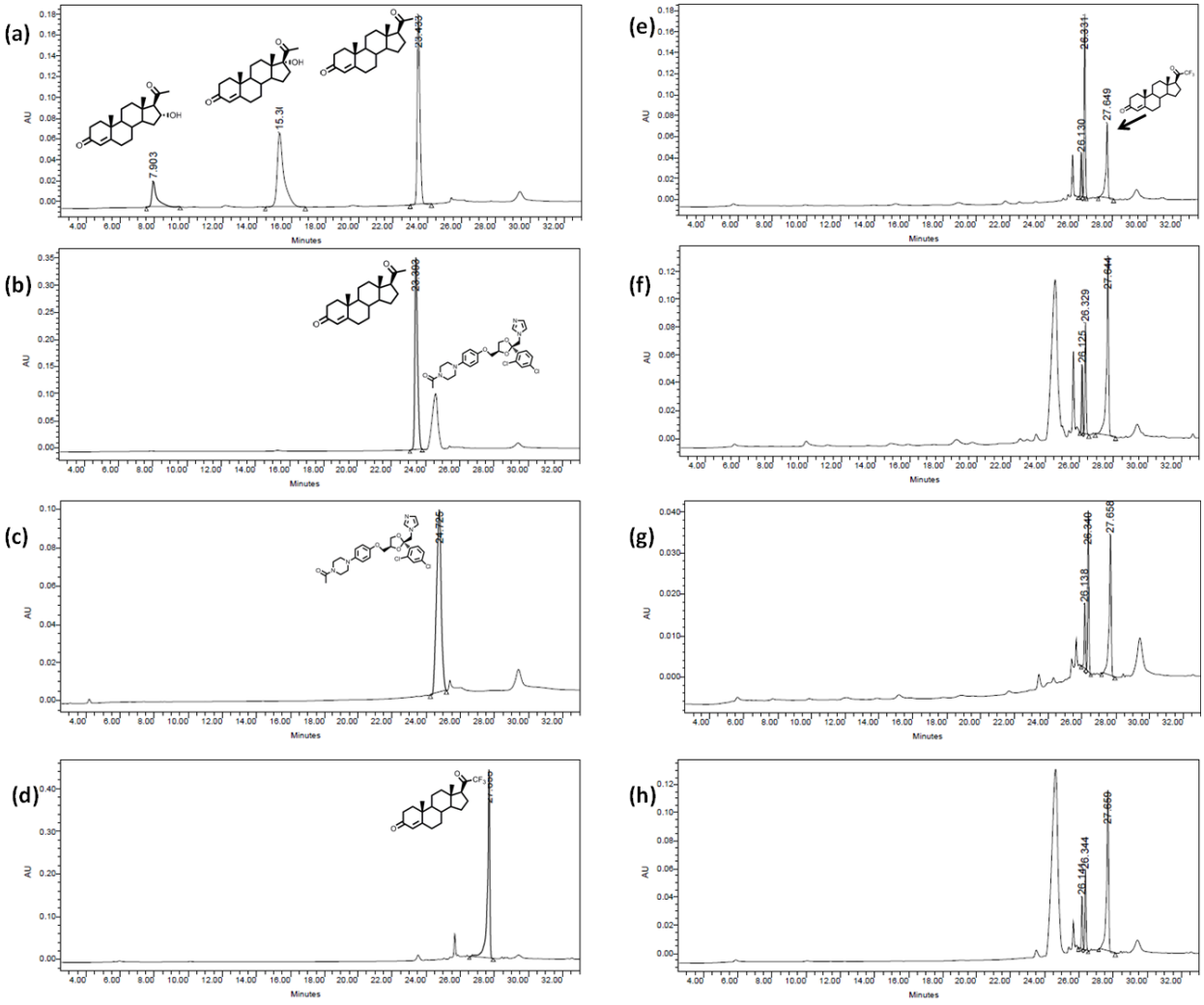


Figure 3.2. HPLC traces. (a) CYP17A1 incubation with progesterone. (b) CYP17A1 incubation with progesterone and ketoconazole inhibitor. (c) ketoconazole standard. (d) 21,21,21-trifluoroprogesterone standard. (e) CYP17A1 incubation with 21,21,21-trifluoroprogesterone. (f) CYP17A1 incubation with 21,21,21-trifluoroprogesterone and ketoconazole inhibitor. (g) CYP17A1 incubation with 21,21,21-trifluoropregnenolone followed by cholesterol oxidase treatment. (h) CYP17A1 incubation with 21,21,21-trifluoropregnenolone and ketoconazole.

3.3.2. Epoxidation/Reduction of C=C Double Bonds

Besides hydroxylation activity and carbon-carbon bond cleavage reactions, cytochromes P450 catalyze a variety of other oxidative reactions including: epoxidation, Wagner-Meerwein type rearrangements[2] [143], and more. Although reductive reactions catalyzed by these enzymes are rare, some examples have also been observed. Moreover, a non-redox reaction – a Paterno-Buchi type cyclization – has recently been reported by Cheng and co-workers [144].

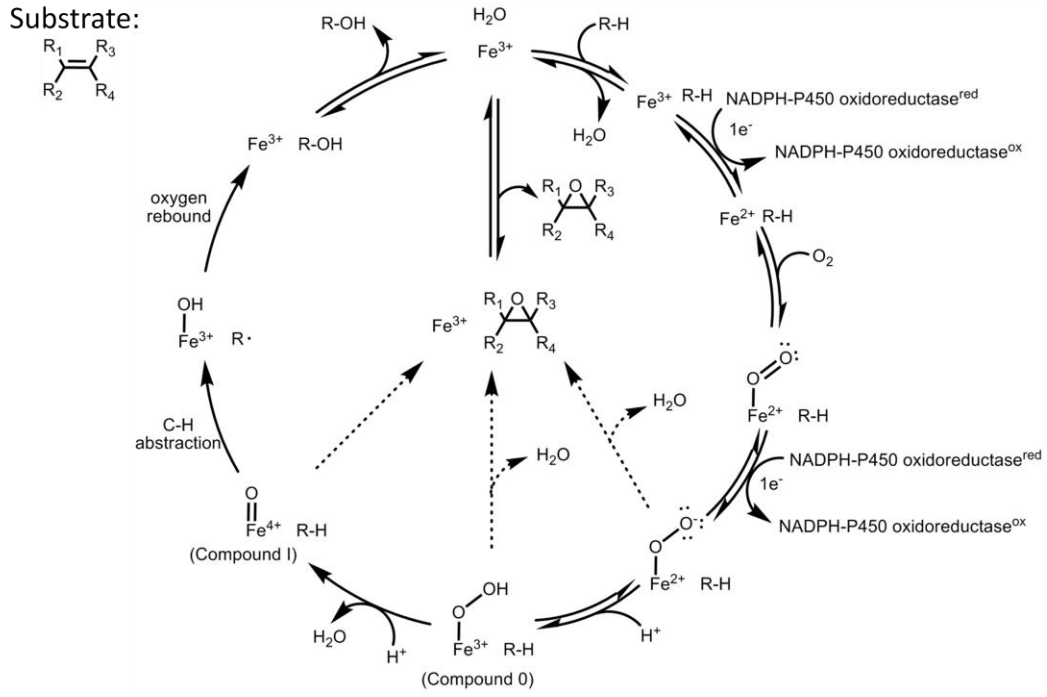


Figure 3.3. P450 catalytic cycle involving epoxidation of an olefinated substrate.

Here we tested unsaturated steroid analogs to study the active oxygen species in the cytochrome P450 catalytic cycle of CYP17A1 and CYP21A2. In other words, we have introduced an alkene in the position of the steroid substrate where these enzymes normally hydroxylate to probe for epoxidation activity. For CYP17A1, the substrate analog contained a double bond between carbons 16 and 17, and for CYP21A2, the analog was a homologated 22-carbon pregnane with a double bond between carbons 21 and 22. In addition, we employed mutations with different product distributions, CYP17A1-A105L and CYP21A2-V359A, to determine the influence of regiochemical preferences on epoxidase activity.

The incubation of 16,17-dehydroprogesterone with wild-type CYP17A1 led to the formation of two new unidentified peaks in the HPLC trace in a ~1:1 ratio. Interestingly, when we used the A105L mutation, we observed that the ratio of these peaks changed to 8:1. We predicted the identity of one of the peaks to be 16α,17α-epoxyprogesterone **100** and confirmed the identity of the later-eluting product using authentic standard (Figure 3.4). Furthermore, we determined the second, earlier-eluting product to be 21-hydroxy-16,17-dehydroprogesterone **104**, a type of metabolic switching introduced by blocking hydroxylation chemistry at C16 and C17. Because mutation A105L has poor progesterone 16α-hydroxylase activity, this result suggests that epoxidation activity at the C16-C17 olefin derives primarily from the capacity of the enzyme to perform 16α-hydroxylation.

In addition, this result indicates that CYP17A1 has intrinsic 21-hydroxylase activity, suggesting that 21-hydroxylation is normally suppressed by the presence of more reactive C-H bonds at C16 and C17. The formation of these newly identified peaks were inhibited in the presence of ketoconazole and abiraterone, known inhibitors of CYP17A1 (Figure 3.5).

Because pregnenolone is a substrate for CYP17A1, we also performed incubations of 16,17-dehydropregnenolone with CYP17A1. In order to inspect the products on the HPLC, we converted the 17α-hydroxylase incubation products to the 3-keto-Δ^4-steroids by treatment with cholesterol oxidase. This substrate was metabolized to both the 21-hydroxylated product and the 16α,17α-epoxyproduct with this enzyme, however, a third minor unidentified peak had appeared, which appeared to be more polar than the other two products.

We attempted to rule out the shunt pathway for the formation of the epoxide product because we did notice that the presence of excess hydrogen peroxide (<100 mol equivalents vs. 16,17-dehydroprogesterone) in the absence of enzyme did form the 16α,17α-epoxyproduct. The 16,17-dehydroprogesterone was incubated as usual with CYP17A1 with the presence of super oxide dismutase and catalase. The control incubation involved the addition of SOD and catalase after 45 minutes of incubation and termination of both sets of incubations followed by HPLC analysis resulted in the same ratio of 21-hydroxylated: 16α,17α-epoxidized products.

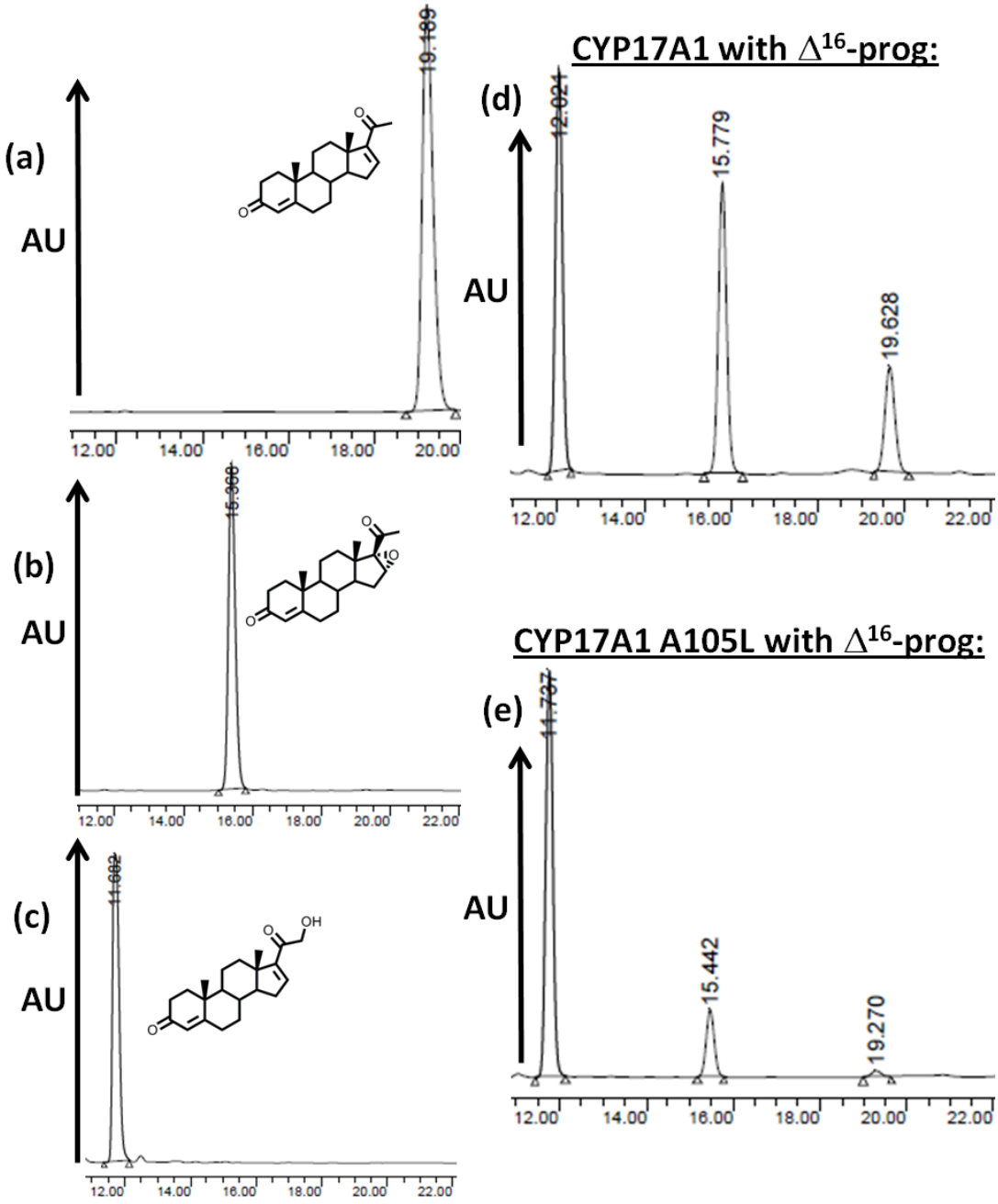


Figure 3.4. HPLC traces. (a) synthetic 16,17-dehydroprogesterone standard. (b) synthetic 16α,17α-epoxyprogesterone standard. (c) synthetic 16,17-dehydro-21-hydroxyprogesterone standard. (d) CYP17A1 incubation with 16,17-dehydroprogesterone. (e) CYP17A1 A105L incubation with 16,17-dehydroprogesterone.

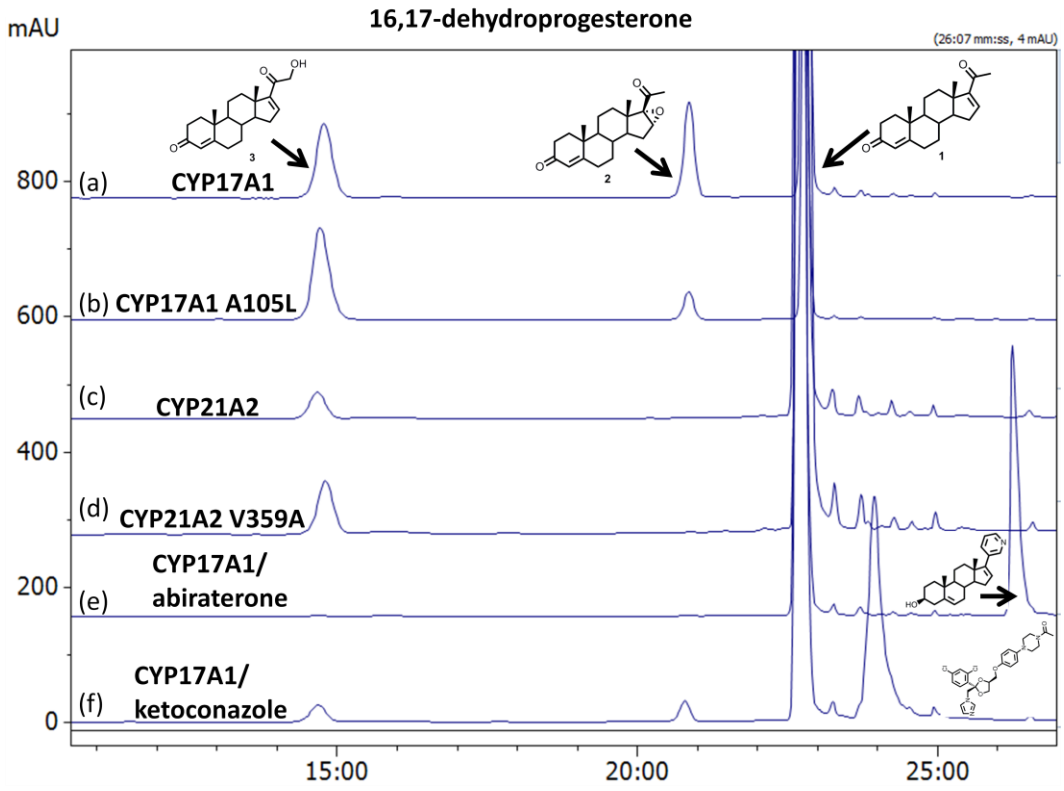


Figure 3.5. HPLC chromatograms of CYP17A1 incubation with 16,17-dehydroprogesterone as the substrate. (a) CYP17A1 incubation with 16,17-dehydroprogesterone. (b) CYP17A1 A105L incubation with 16,17-dehydroprogesterone. (c) CYP21A2 incubation with 16,17-dehydroprogesterone. (d) CYP21A2 V359A with 16,17-dehydroprogesterone. (e) CYP17A1 incubation with 16,17-dehydroprogesterone and abiraterone (20 μM). (f) CYP17A1 incubation with 16,17-dehydroprogesterone and ketoconazole (50 μM).

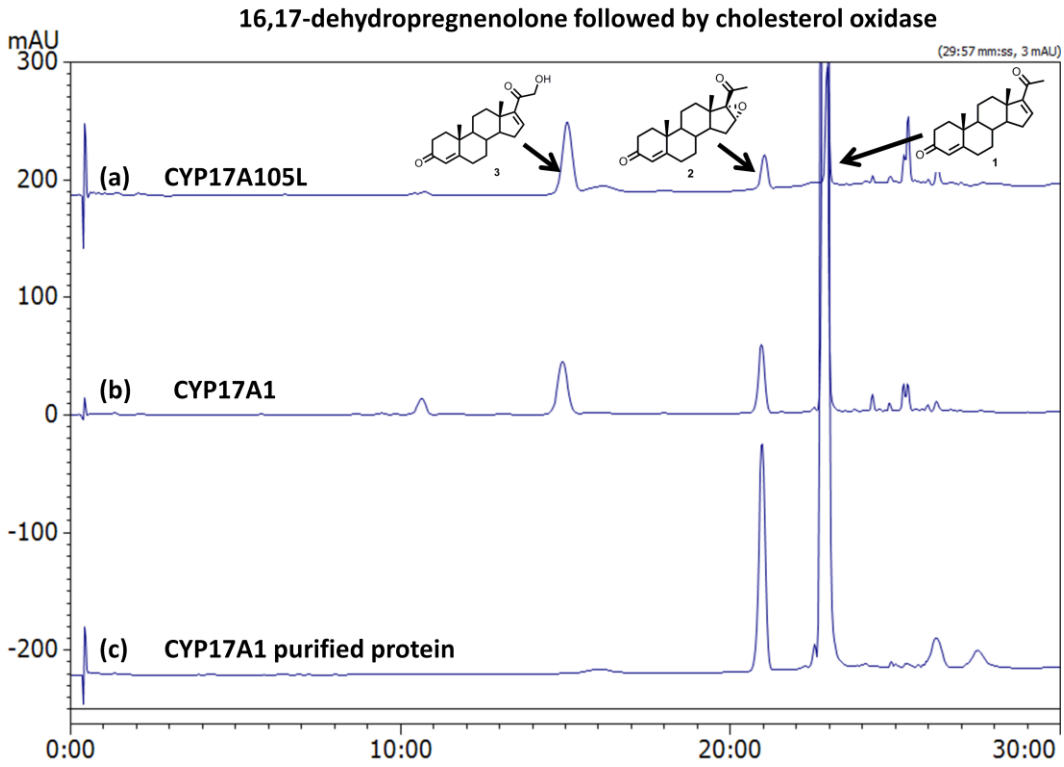


Figure 3.6. HPLC chromatograms of CYP17A1 incubation with 16,17-dehydropregnenolone as the substrate followed by cholesterol oxidase transformation. (a) CYP17A1 A105L incubation with 16,17-dehydropregnenolone. (b) CYP17A1 incubation with 16,17-dehydropregnenolone. (c) CYP17A1 purified protein incubation with 16,17-dehydropregnenolone.

Although 16,17-dehydroprogesterone was a suitable substrate to show epoxidase activity in CYP17A1, we observed only 21-hydroxylase activity when we used this substrate for CYP21A2. Additionally, when we used 16,17-dehydroprogesterone as the substrate for the site-directed mutant, CYP21A2 V359A, we only observed the 21-hydroxylated product. Thus, we were able to conclude that the 16α,17α-epoxidation activity was only present when the enzyme acted on both the 16α- and 17-positions.

We designed 21-homo-21,22-dehydroprogesterone as a potential epoxidation substrate for CYP21A2. However, when we used 21-homo-21,22-dehydroprogesterone as the substrate for CYP21A2, we did not observe the expected epoxidation product, 21-homo-21,22-epoxyprogesterone. Instead, we observed a later-eluting product compared to the starting material, in addition to an earlier-eluting product, which did not match the retention time of the synthetic 21-homo-21,22-epoxyprogesterone standards (Figure 3.5). Because of precedent for P450 enzymes to perform olefin reduction reactions, we predicted that the late-eluting peak is a non-hydroxylated product, and hence we hypothesized the identity of the peak to be 21-homomethylprogesterone. Consistent with this prediction, the synthetic standard, 21-homomethylprogesterone, has a retention time that matches that of the late-eluting product. Consequently, we suspected that the early-eluting product was a metabolite of the reduced compound, 21-homomethylprogesterone. Incubations using CYP21A2 with synthetic 21-

homomethylprogesterone yielded a product, whose retention time matched the early-eluting product derived from incubations with 21-homo-21,22-dehydroprogesterone as the substrate.

Besides CYP21A2, CYP17A1 and the respective mutants also seemed to metabolize 21-homo-21,22-dehydroprogesterone (Figure 3.6).

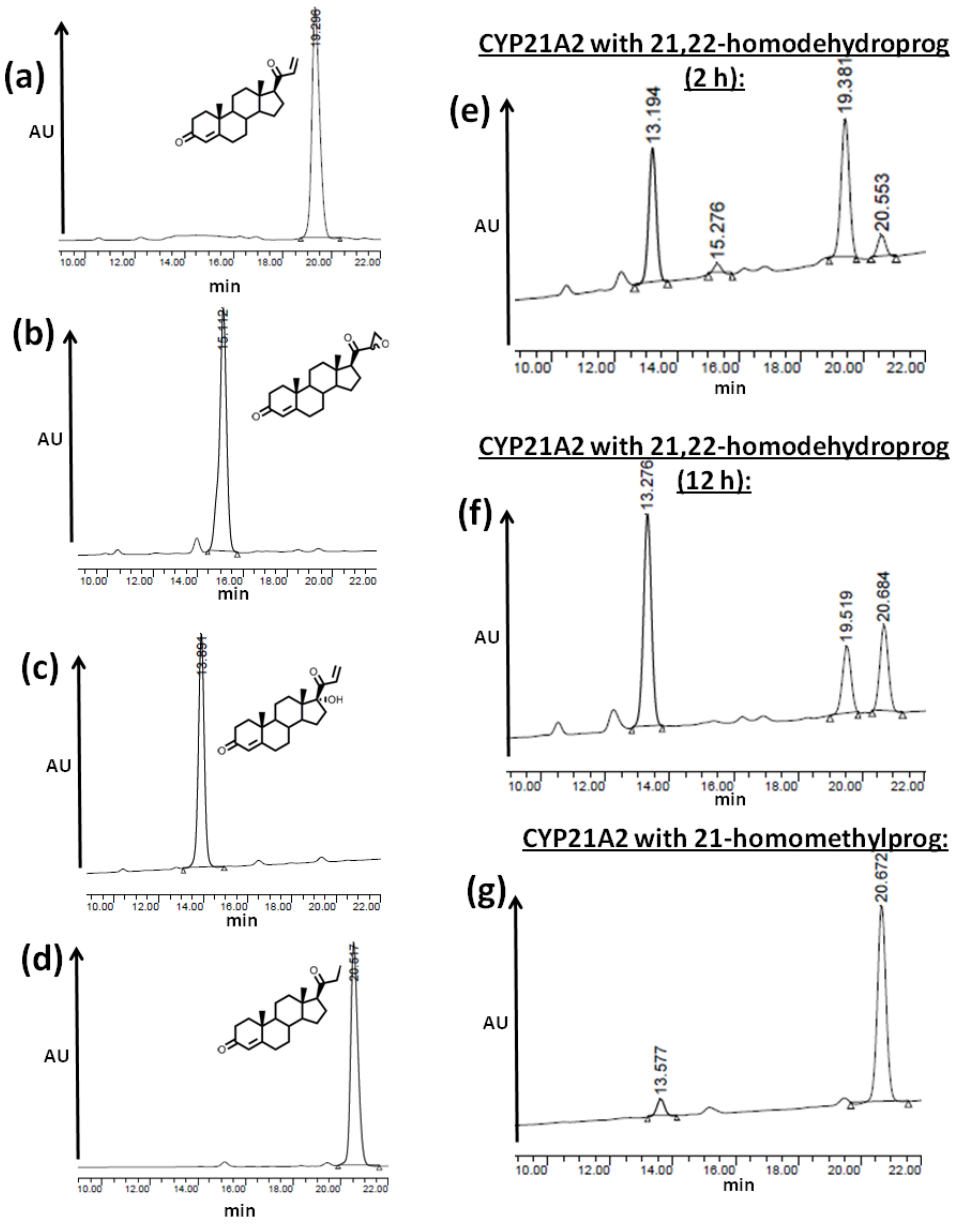


Figure 3.7. HPLC traces. (a) synthetic 21-homo-21,22-dehydroprogesterone standard. (b) synthetic 21,22-epoxyprogesterone standard (epimeric mixture at C21). (c) synthetic 21-homo-21,22-dehydro-17-hydroxyprogesterone standard. (d) synthetic 21-homomethylprogesterone. (e) CYP21A2 incubation with 21-homo-21,22-dehydroprogesterone, 2 hour time point. (f) CYP21A2 incubation with 21-homo-21,22-dehydroprogesterone, 12 hour time point. (g) CYP21A2 incubation with 21-homomethylprogesterone incubation.

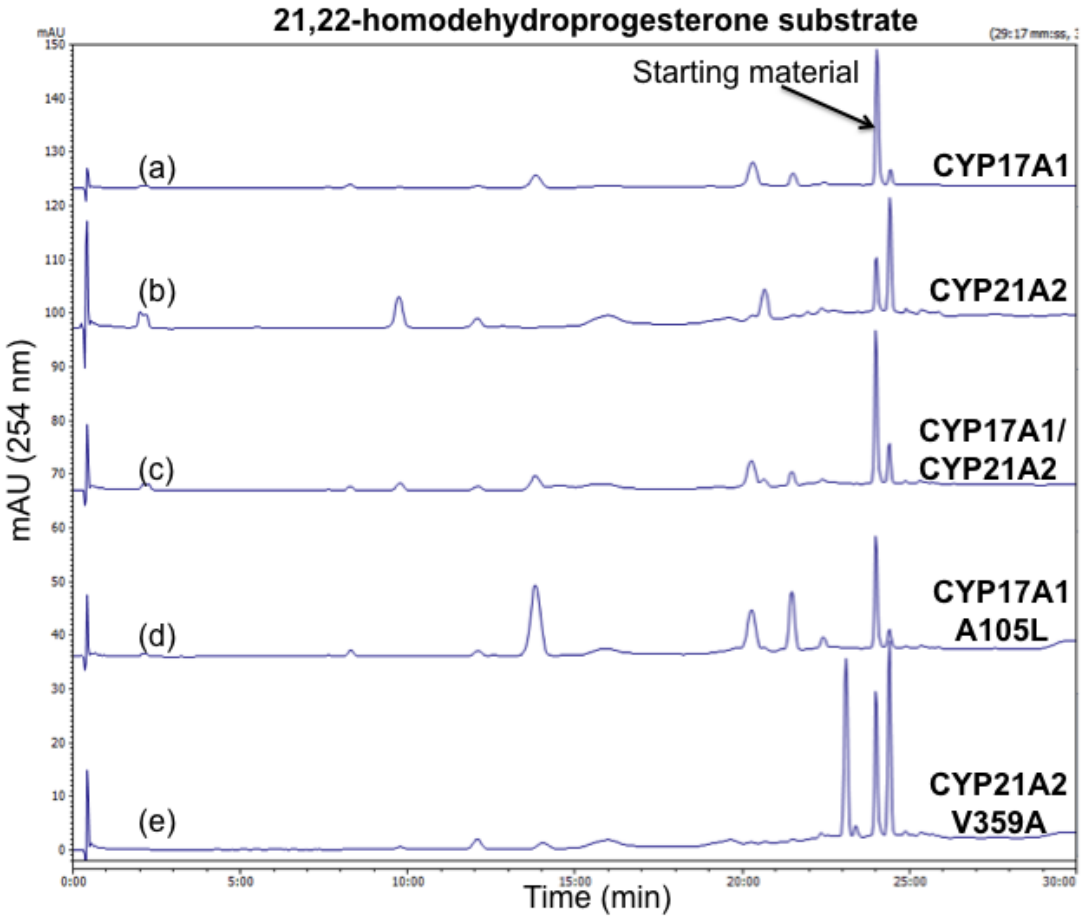


Figure 3.8. HPLC chromatograms. (a) CYP17A1 incubation with 21-homo-21,22-dehydroprogesterone substrate. (b) CYP21A2 incubation with 21-homo-21,22-dehydroprogesterone substrate. (c) CYP17A1 and CYP21A2 incubations combined and injected on HPLC. (d) CYP17A1 A105L incubation with 21-homo-21,22-dehydroprogesterone substrate. (e) CYP21A2 V359A incubation with 21-homo-21,22-dehydroprogesterone substrate.

The enone substrate, 21-homo-21,22-dehydroprogesterone, possesses an electrophilic carbon on the 22-β-position. This electrophilic quality might render this substrate an inhibitor of this cytochrome P450. The Dewar-Chatt-Duncanson model suggests interaction between alkenes and a transition metal – the π* orbital of the alkene accepting electron density from the transition metal d-orbital and the p-orbital of the alkene donating electron density to the transition metal d-orbital (Figure 3.7).

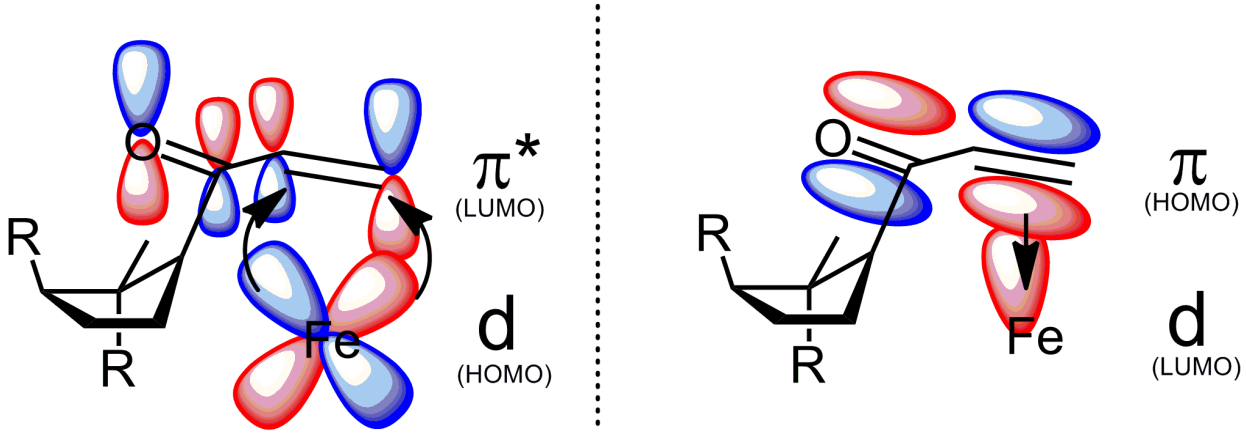


Figure 3.9. Dewar-Chatt-Duncanson model illustrating the frontier molecular orbital interaction between 21-homo-21,22-dehydroprogesterone and the iron heme center – a possible explanation of the reduction product observed when 21-homo-21,22-dehydroprogesterone was used as the substrate for CYP21A2.

3.3.3. Other Steroid Analogs

Cyclopropyl rings are probes for measuring the radical intermediate lifetime. We used the 20-cyclopropyl analog of progesterone to determine whether CYP17A1 could metabolize this substrate. The appearance of a new peak with faster retention time compared to the starting material (more polar compound than starting material) was observed in the incubation extract; however, we have not determined the structure of the metabolite (Figure 3.8). The structure might be determined through synthesis, but the first step is to obtain a mass spectrum of the product. From a large-scale incubation, we could purify and isolate the metabolite, then identify the structure through NMR in order to determine if any cyclopropane rearrangement or direct hydroxylation occured. This substrate is a promising chemical probe to measure the lifetime of the radical intermediate.

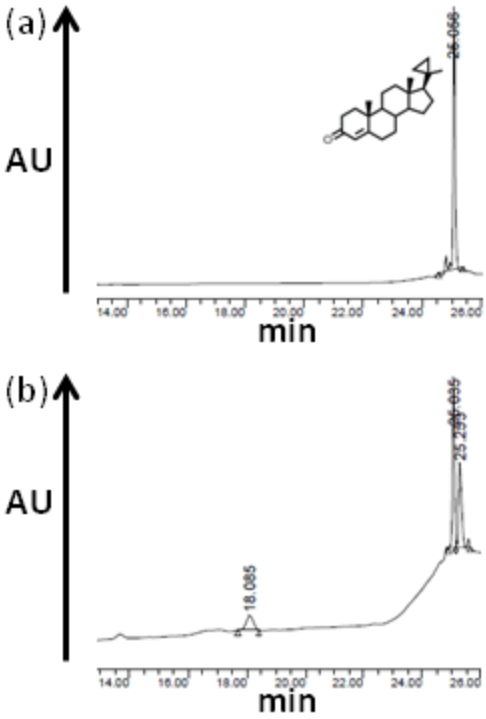


Figure 3.10. HPLC traces. (a) synthetic 20-cyclopropyl-20-methylprogesterone standard. (b) CYP17A1 incubation with 20-cyclopropyl-20-methylprogesterone.

We did not observe any metabolism of 21-cyclopropyl-21-methyl-progesterone nor the 20-cyclopropyl-20-methylprogesterone compounds by CYP21A2. Although the 20-cyclopropyl-20-methylprogesterone substrate did not appear to be metabolized by CYP21A2, it would be worthwhile to repeat this incubation again with larger amounts of protein because the enzyme seemed to tolerate 20-desoxoprogesterone.

When we used 21-homomethylprogesterone as a substrate for CYP17A1, we found that the enzyme was able to metabolize the substrate to a more polar compound, which suggests hydroxylation of the substrate (Figure 3.9). Interestingly, unlike the natural progesterone case where we see a 3:1 distribution of 17-hydroxy- to 16α-hydroxy-products, when we use this ethyl ketone analog, we only see one major product peak. The product peak did not match the retention time of 21-homomethyl-17-hydroxyprogesterone, suggesting that CYP17A1 had either hydroxylated the 16α-position or the 21-position of the 21-homomethylprogesterone substrate.

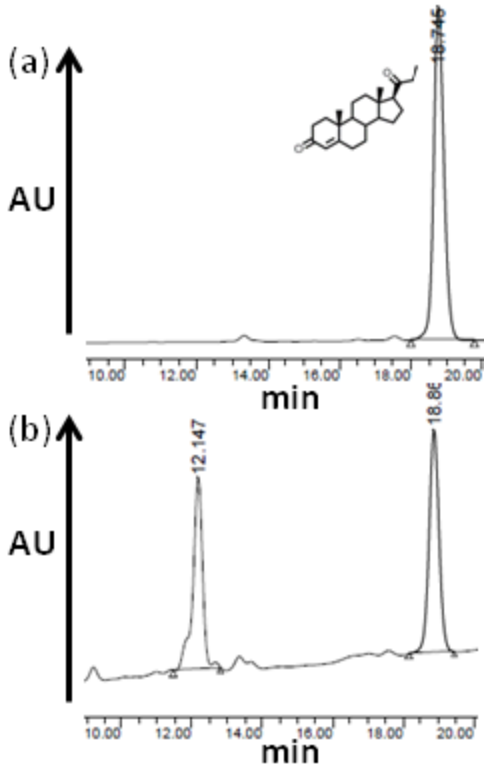


Figure 3.11. HPLC traces. (a) synthetic 21-homomethylprogesterone standard. (b) CYP17A1 incubation with 21-homomethylprogesterone.

We found that 21-homomethylprogesterone is also a substrate for CYP21A2, affording a different product than CYP17A1 (see Figure 3.11 for overlay), neither of which is 21-homomethyl-17-hydroxyprogesterone (Figure 3.10). Based on results with 16,17-dehydroprogesterone, we suspect that CYP17A1 and CYP21A2 both hydroxylate 21-homomethylprogesterone on the ethyl side chain, either the 21 or 22 position. Because the hydrogens at C21 are prochiral, identification of these products might require the stereoselective

synthesis of both 21-hydroxylated epimers. Knowledge of the stereoselectivities for both enzymes could help us understand the steric environment in the binding pockets for each enzyme.

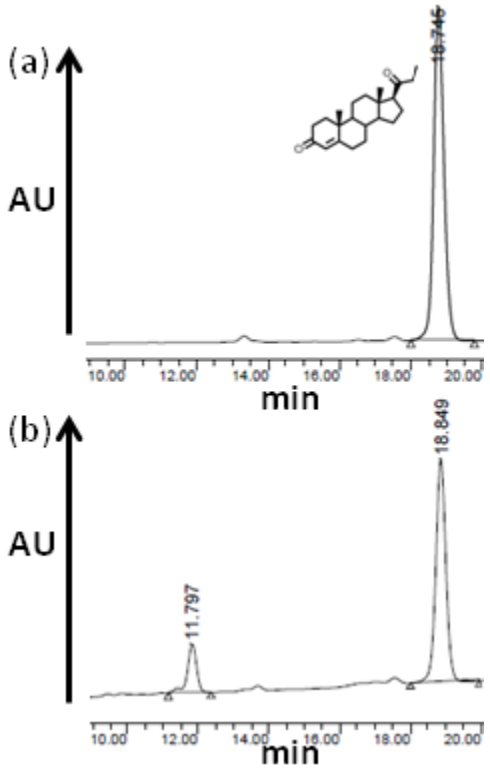


Figure 3.12. HPLC traces. (a) synthetic 21-homomethylprogesterone standard. (b) CYP21A2 incubation with 21-homomethylprogesterone substrate.

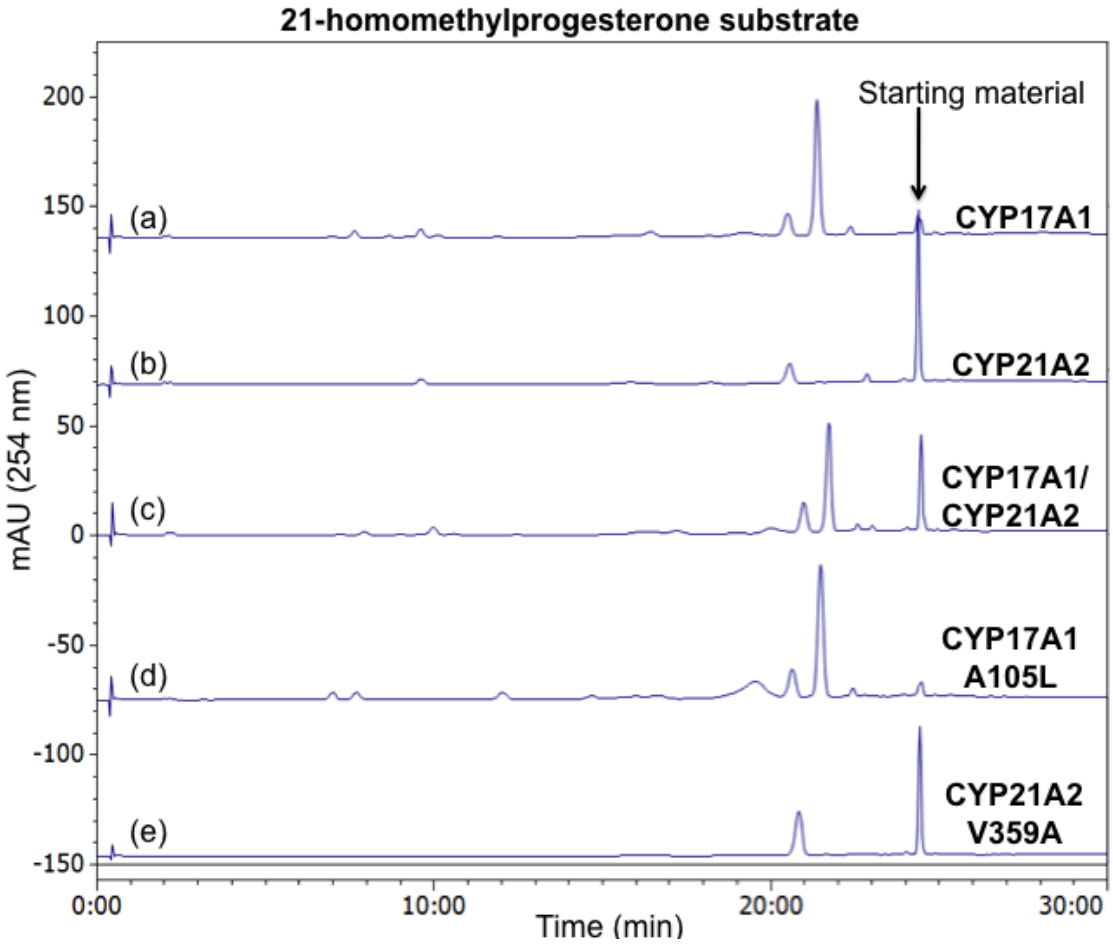


Figure 3.13. Enzyme incubations with 21-homomethylprogesterone substrate. (a) CYP17A1 incubation with 21-homomethylprogesterone. (b) CYP21A2 incubation with 21-homomethylprogesterone. (c) CYP17A1 and CYP21A2 incubation extracts combined and injected on HPLC. (d) CYP17A1 A105L incubation with 21-homomethylprogesterone. (d) CYP21A2 V359A incubation with 21-homomethylprogesterone.

We also found that 20-desoxo-progesterone is a substrate for CYP17A1. It is likely that the product is 17-hydroxy-20-desoxo-progesterone, but the identity of this product has not been determined conclusively (Figure 3.12).

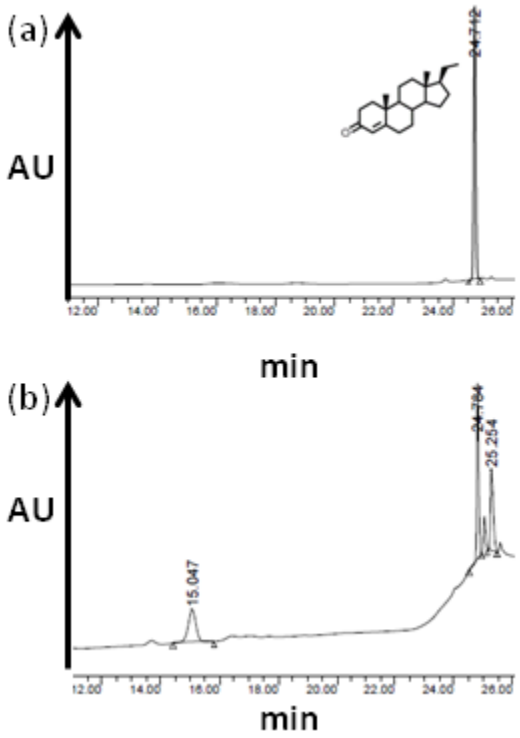


Figure 3.14. HPLC traces. (a) synthetic 20-desoxoprogesterone standard. (b) CYP17A1 incubation with 20-desoxoprogesterone.

When we used 20-desoxoprogesterone as a substrate for CYP21A2, small amounts of two early-eluting metabolites were observed, distinct from the product obtained with CYP17A1. We suspect that the major product is 21-hydroxy-20-desoxoprogesterone (Figure 3.13).

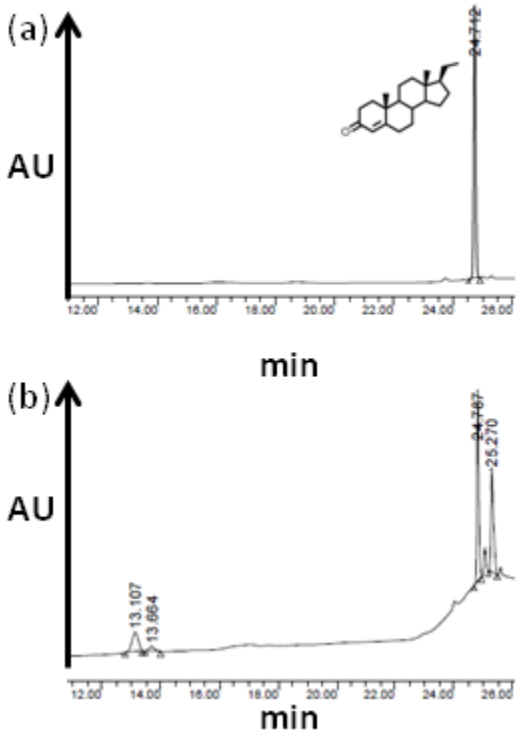


Figure 3.15. HPLC traces. (a) synthetic 20-desoxoprogesterone standard. (b) CYP21A2 incubation with 20-desoxoprogesterone.

Moreover, when we used 17β-(2-propenoic methyl ester)-androst-4-en-3-one as a substrate for CYP21A2, we observed a later-eluting product, which suggested a possible reduction of the double bond (Figure 3.14).

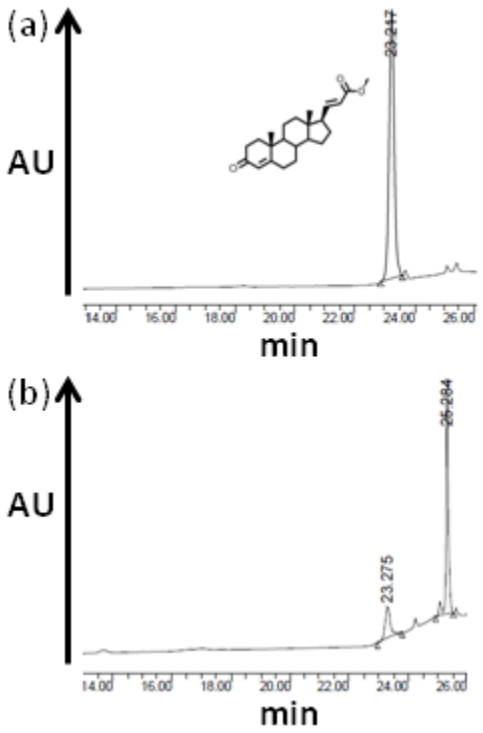


Figure 3.16. HPLC trace. (a) synthetic 17β-(2-propenoic methyl ester)-androsten-3-one standard. (b) CYP21A2 incubation with 17β-(2-propenoic methyl ester)-androsten-3-one.

3.4. DISCUSSION

3.4.1. Fluorinated substrates

Njar and colleagues have reported the synthesis of 21-trifluoropregnenolone through the addition of trifluoromethyl silane onto an aldehyde intermediate and have shown that 21-trifluoropregnanes are inhibitors of CYP17A1 [109]. We found some evidence that 21-trifluorinated substrates might also be metabolized by CYP17A1 and CYP21A2, but the inherent difficulties in these experiments precluded unambiguous conclusions. One explanation for these results is that the electron-withdrawing trifluoromethyl group shifts the hydration equilibrium from the C20 ketone of compound **4** to its geminal diol, which is too bulky to bind to either active site. In contrast, we reproducibly demonstrated that CYP21A2 but not CYP17A1 metabolized 17-fluoroprogesterone to a single ketoconazole-inhibited product, possibly 17-fluoro-21-hydroxyprogesterone. These data demonstrate that steroid halogenation might block normal sites of P450 oxygenation but not preclude substrate binding and turnover.

By varying the location and number of halogen atoms, as well as the specific halide employed, variations in steric, electronic, and reactivity properties are introduced in the steroid near the sites of reactivity. Although the classical cytochrome P450 reaction is the hydroxylation of C-H bonds in alkanes, these substituted pregnanes are also reagents to test the capacity of CYP17A1 and

CYP21A2 to catalyze halide reductions, as described for other cytochromes P450 [145].

In addition, halogenated steroids have been successfully employed for a variety of purposes in science and medicine. As suggested above, halogenated steroids might be employed as reversible [146] or irreversible [147] enzyme inhibitors, by blocking catalysis and/or increasing affinity for the steroid. Halogenated steroids are also in use as agonists and antagonists of steroid hormone receptors, and halogenation also blocks positions of metabolism by hepatic enzymes such as CYP3A4. This modification increases the half-life of the potential drug, as seen in the potent glucocorticoid dexamethasone (9α-fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione), with a half-life of over 36 hours. Furthermore, fluorinated [^{18}F-] steroids are used as imaging agents for positron-emitted tomography (PET) studies [148, 149], by binding to receptors for these steroids. Consequently, improved methods to selective halogenation of steroids might facilitate advances in several disciplines.

3.4.2. Other steroid analogs

The introduction of double bonds in our substrate analogs yielded novel reactivity in our enzyme incubations. CYP17A1 was found to epoxidize 16,17-dehydroprogesterone and 16,17-dehydropregnenolone. Reactive oxygen species

such as hydrogen peroxide formed in the shunt pathway of P450 enzymes are sometimes responsible for epoxidizing small molecules.

Moreover, identification of the different metabolites reveals important steric and electronic information about the active site of the enzymes. A more detailed investigation in determining the structures of these products is required.

ACKNOWLEDGEMENTS

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CHAPTER FOUR

Minor activities and transition state properties of CYP17A1 and CYP21A2 observed with isotopically-labeled substrates

4.1. BACKGROUND

Although the steroidogenic P450s have been known and studied for many years, several mysteries about their activities remain unsolved, despite the recent x-ray crystal structures of modified bovine CYP21A2 [150] and human CYP17A1 [60]. First, CYP21A2 oxygenates a methyl group adjacent to other more easily oxidized carbon atoms. Second, CYP17A1 performs not only the 17α-hydroxylase reaction but also the 16α-hydroxylase reaction with progesterone as substrate in a 3:1 ratio [136], and the small side chain of A105 allows 16α-hydroxylation [151] (Figure 4.1). Furthermore, CYP17A1 performs the 17,20-lyase reaction, involving the oxidative cleavage of a carbon-carbon bond. Only a few P450 enzymes incorporate carbon-carbon cleavages in their physiologic functions, including the steroidogenic enzymes CYP11A1 (P450scc, the cholesterol side chain cleavage enzyme), CYP17A1, and CYP19A1 (P450aro, aromatase) as well as CYP51A1 (lanosterol demethylase) [152, 153]. Common catalytic mechanisms or themes for these enzymes have not emerged from the literature, and debate continues for the mechanisms of individual reactions. The participation of cytochrome b_5 in the 17,20-lyase reaction has been extensively documented [154-157] and physiologically validated by patients with isolated

17,20-lyase deficiency due to *CYB5* mutations [27, 158], yet the mechanism of this stimulation is not yet resolved [159]. Finally, the steroidogenic P450s are very slow catalysts, with turnover numbers <10 min^{-1}, compared to well-characterized members of the superfamily such as P450cam and P450BM3, which catalyze thousands of turnovers per second. Consequently, the fundamental assumptions regarding the catalytic cycle and rate-determining steps gleaned from prokaryotic P450 enzymes might apply differently to the steroid hydroxylases. Interestingly, the C-H abstraction step was not found to be rate-limiting in the case of CYP7A1 [160].

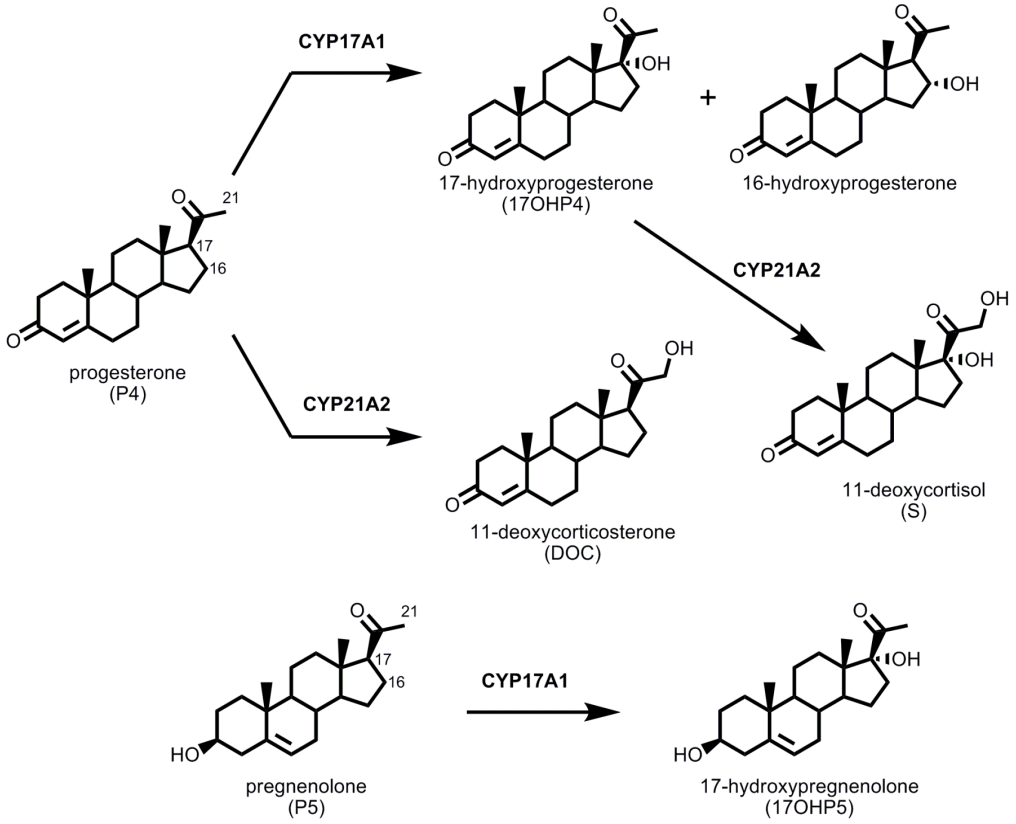

Figure 4.1. Hydroxylase activity of CYP17A1 and CYP21A2.

The available evidence supports a model in which the first chemical step for cytochrome P450 hydroxylations involving substrate is hydrogen atom abstraction from a C-H bond using a highly reactive oxygenated heme species resembling a ferryl oxene with radical (odd-electron) character [161]. For several P450 enzymes, the C-H abstraction step has been studied in detail by measuring the kinetic isotope effects (KIEs) in order to determine the contribution of this step to the reaction rate relative to the other steps associated with hydroxylation [162-167]. Depending on the specific cytochrome P450, the contribution of C-H bond cleavage to the overall rate varies – and it is not clear what properties of the proteins determine these kinetic features. Furthermore, for P450-catalyzed reactions yielding two or more products from a common E•S complex, intramolecular KIE experiments have been employed to deduce the contribution of C-H bond breakage to product partitioning [163, 165]. For some P450 enzymes, anomalously large intramolecular KIEs over 10 have been observed [162], suggesting that proton-coupled electron transfer or hydrogen atom tunneling rather than classical reaction mechanics best describes these reactions [168]. For the human steroid hydroxylases, however, few comprehensive KIE studies have been reported [160]. Therefore, we conducted a series of intramolecular and intermolecular KIE experiments to probe the mechanisms of human CYP17A1 and CYP21A2.

4.2. EXPERIMENTAL PROCEDURES

4.2.1. Site-Directed Mutagenesis

Oligonucleotides were obtained from Integrated DNA Technologies (Coralville, IA). Cholesterol oxidase was purchased from Sigma Aldrich (*Brevibacterium* sp., product C8868-100UN). Site-directed mutagenesis employed the primers for CYP17A1-A105L as reported [151] hCYP17A105L_S: 5'-TCA AAT GGC AAC TCT AGA CAT CCT GTC CAA CAA C-3' and hCYP17A105L_AS: 5'-GAT GTC TAG AGT TGC CAT TTG AGG CCG CC-3'; primers for CYP21A2 V359A [169] were C21V359A_S: 5'-CCC GTT GCG CCC TTA GCC TTG-3' and C21V359_AS: 5'-AAG GCT AAG GGC GCA ACG GGC C-3'. Constructs were sequenced to assure accurate mutagenesis.

4.2.2. Microsomal Enzyme Incubations

Enzyme preparations included microsomes from yeast cells expressing native human P450-oxidoreductase (POR) plus the P450 [141]: CYP17A1, CYP17A1 mutation A105L, CYP21A2 or CYP21A2 mutation V359A. The primary H/D intramolecular KIEs were measured by incubation of the enzyme (1-10 pmol P450, 20-200 μg protein) in 1 mL of 50 mM potassium phosphate (pH 7.4) with 20-40 mM deuterium-labeled or unlabeled steroid. For competitive intermolecular KIEs, incubations included deuterium-labeled steroid plus tracer amounts of steroid with tritium label distant from the reaction sites (1,2,6,7-[$^{3}H_{4}$]-

progesterone, 90 Ci/mmol 7-[^{3}H]-pregnenolone, 25 Ci/mmol [both PerkinElmer NEN, Waltham, MA] or 1,2-[$^{3}H_2$]-17-hydroxyprogesterone, 50 Ci/mmol [American Radiolabeled Chemicals, St. Louis, MO], 0.2-0.6 nCi, 1-4 nM). One set of incubations replaced the 1,2,6,7-[$^{3}H_4$]-progesterone with 4-[^{14}C]-progesterone (55 mCi/mmol, PerkinElmer NEN, 120 nCi, 2.2 mM). The reaction was started by addition of 10 mM NADPH at 37°C and continued for 20-60 min, then terminated by extracting the steroids 1 mL methylene chloride.

4.2.3. Expression and Purification of Enzymes

Cytochrome P450c17 was expressed in *E. coli* JM109 cells and purified to homogeneity as described [170]. GroEL/ES chaperones (pGro7 plasmid) were co-expressed with the P450c17 to increase the level of expression. P450 NADPH-reductase was expressed in E. coli C41(DE3) cells and purified according to a previously published procedure [171] except that the bound protein was eluted in buffer containing 200 mM imidazole.

4.2.4. Reconstituted Enzyme Incubations

Incubations with CYP17A1 were repeated with reconstituted recombinant modified P450 and POR – the regioselectivity of 17- to 16-hydroxylation of the reconstituted system went to 9:1. 1,2-Didodecanoyl-sn-glycero-3-phosphocholine (CAS#: [18194-25-7]) was dissolved in water (2 mg/mL) and was sonicated for

10 min under ice prior to use for incubation. CYP17A1 (30 pmol, 8 pmol/mL), hPOR (170 pmol, 100 pmol/mL) and 1,2-didodecanoyl-sn-glycero-3-phosphocholine (10 mg) were added to 2 mL microcentrifuge tube for a duplicate 200 mL total reaction volume. The contents were gently swirled (tube was tapped with finger) and stood at room temperature for 20 min. The mixture was dissolved in 50 mM potassium phosphate buffer (pH = 7.4, 0.1 M), 6 mM potassium acetate (0.1 M), 10 mM $MgCl_2$ (0.1 M), 1 mM glutathione (0.1 M), 1 mM progesterone, 1,2,6,7-[$^3H_4$]-progesterone (90 Ci/mmol) (1:10 dilution from commercially available source ~100,000 cpm/mL), and glycerol (20% of reaction volume). The resulting mixture was swirled gently (vortex – level 3 setting) and stood at room temperature for 3 min. NADPH (10 mM) was added and the incubation was started at 37 C for 90 min. The incubation was extracted with methylene chloride, and the organic phase was dried under nitrogen flow. The dried extracts were ready for HPLC analysis.

4.2.5. Cholesterol Oxidase Transformation

For incubations involving the pregnenolone substrate, the dried incubation extracts were dissolved in methanol (70 mL) and suspended in potassium phosphate buffer (100 mL) and water (100 mL) and cholesterol oxidase (70 mL, 28 units/mL) was added. The resulting solution was shaken at 30 ^oC at 200 rpm

for 6 h. The reaction was stopped by the addition of methylene chloride (1 mL). The mixture was extracted and the organic layer was dried under N_2 flow.

4.2.6. Chromatography and Data Acquisition

Reaction products were analyzed using either a Breeze 1525 high-performance liquid chromatography (HPLC) system equipped with in-line UV detector set to 254 nm (Waters, Woburn, MA) and β-RAM3 in-line scintillation counter or an Agilent 1260 Infinity HPLC system with UV detector and β-RAM4 in-line scintillation counter (LabLogic, Brandon, FL). Steroids were dissolved in 20 µL of methanol, and 5 µL injections were resolved with a C_{18} Kinetex column (50 x 2.1 mm, 2.6 µm, Phenomenex, Torrance, CA), equipped with a guard column, at a flow rate of 0.4 mL/min and a methanol/water linear gradients: 27% methanol from 0 to 0.5 min, 39% to 16 min, 44% to 20 min, 60% to 22 min, 71% to 30 min, 75% to 30.5 min, 27% to 33 min. Products were identified by retention times of external standards chromatographed at the beginning and ends of the experiments. The flow rate of the scintillation cocktail (Bio-SafeII, Research Products International, Mount Prospect, IL) was 1.2 mL/min, and the data were processed with Laura4 software (LabLogic). Protein determinations used the Coomassie Plus Reagent (Pierce, Rockford, IL). The method setting was adjusted under the channel parameters to either detect for ^{14}C or ^{3}H radioisotope.

4.2.7. Mass Spectrometry

The products from a subset of reactions were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The dried incubation extracts were diluted in acetonitrile (1:1000 dilution) and were ready for injection. The reaction products were injected into an Agilent 1290 HPLC coupled to a 6490 triple-quadrupole mass spectrometer and resolved using a Kinetex C_8 column with methanol-10 mM ammonium acetate gradients as follows at a flow rate of 0.3 mL/min: 20% methanol in 10 mM ammonium acetate from 0 to 1 min, to 50% methanol in 10 mM ammonium acetate to 2 min, 100% methanol to 6 min, and to 20% methanol in 10 mM ammonium acetate to 8 min to equilibrate for 2 min. Replicate injections from duplicate incubations were analyzed in multiple reaction monitoring (MRM) mode with differential gating of precursor ions to discriminate and quantify deuterium-retained and deuterium-removed products relative to a second product of uniform isotopic composition. For example, starting with 21-[^{2}H]-progesterone, the MRM parameters for the 21-hydroxylation product (11-deoxycorticosterone) are *m/z* 331.2/97.1 for natural-abundance (deuterium-removed) and *m/z* 332.2/97.1 for deuterium-retained species. The signal from 11-deoxycorticosterone is normalized to that from a second product (17- or 16α-hydroxyprogesterone) to correct for losses and inconsistencies.

4.2.8. KIE Calculations

The intramolecular competitive kinetic isotope experiments involve the use of one substrate in each incubation to measure the intramolecular kinetic isotope effect (ie: there are two or more possible hydroxylation sites in the substrate) [172]. The KIE equals the ratio of hydroxylation products in the deuterated vs. the non-deuterated substrates, and these ratios can be given by integration of the peaks in the output of the UV detector. For example, the *competitive intramolecular KIE* of the 17-position can be calculated from data obtained from two separate incubations:

$$k_H/k_D = ([17OHProg]_{17H}/(\sum[21OH\text{-}Prog]_{17H}+[16OH\text{-}Prog]_{17H}))\div ([17OHProg]_{17D}/(\sum[21OH\text{-}Prog]_{17D}+[16OH\text{-}Prog]_{17D})) \quad (1)$$

where [17OHProg] is the amount of 17α-hydroxyprogesterone formed, and $(\sum[21OH\text{-}Prog]_{17D}+[16OH\text{-}Prog]_{17D})$ is the sum of all other hydroxylated products, which includes: 16α-hydroxyprogesterone, and 21-hydroxyprogesterone (11-deoxycorticosterone, if any). The qualifiers 17H and 17D indicate whether the substrate contained H or D at H-17α.

In a competitive intermolecular KIE experiment, the incubation contains both the substrate selectively deuterated at the site of reaction (ie: 17α-[^{2}H]-progesterone) and substrate bearing hydrogen at the site of reaction but

incorporating a radiolabel distant from the site of reaction (ie: 1,2,6,7-[$^{3}H_4$]-progesterone). The incubation is analyzed via HPLC coupled to both a UV detector and in-line scintillation counter. The radioactivity detector only measures the tritiated compound, reflecting product partitioning of the natural-abundance substrate; this product distribution was verified using separate incubations with unlabeled steroid and UV detection of products. The UV detector measures only the deuterium-labeled steroid, because the concentration of the tritiated compound used is >1000 times lower than the non-radioactive compound, and its UV absorbance is negligible. In order to calculate the *competitive intermolecular KIE*, one begins with the relationship derived by Northrop [173] between initial velocity and substrate concentration.

$$^{D}V/K = \ln(1-f_H)/\ln(1-f_D) \quad (2)$$

As shown in equation 2, the kinetic isotope effect is dependent on f, the fractional conversion of the substrate to the product. The equation to calculate the competitive kinetic isotope effect for the 17-position with CYP17A1 and 17α-[^{2}H]-progesterone following Northrop's derivation is given by:

$$^{D}V/K = \ln\{1-([17OHProg^*]/[16OHProg^*+17OHProg^*+21OHProg^*+Prog^*])\} \div \ln\{1-([17OHProg]/[16OHProg+17OHProg+21OHProg+Prog])\} \quad (3)$$

where the "*" denotes non-deuterated compound tracked with the radioactivity measurements. Under this experimental paradigm, we calculated the competitive intermolecular KIE values ^{D}V at V_{max} conditions from the ratio of product formation rates derived from the non-deuterated substrate compared to the product formation rate derived from the deuterated substrate. For example, using 17α-[^{2}H]-pregnenolone plus 7-[^{3}H]-pregnenolone incubated with CYP17A1 in which products other than 17-hydroxylation are negligible, ^{D}V is given by simply (after cholesterol oxidase conversion) the fractional conversion to 17-hydroxyprogesterone from radiochemical detection divided by the fractional conversion to 17-hydroxyprogesterone from UV detection.

$$^{D}V = [17OHProg^*]/[Prog^*+17OHProg^*] \div [17OHProg]/[Prog+17OHProg] \quad (4)$$

For other reactions with multiple products, the denominators in equation 4 are replaced by the sum of remaining substrate plus all products, or the ratio of percent conversion to the product hydroxylated at the site of deuterium labeling.

4.3. RESULTS

4.3.1. KIE for CYP17A1 at H-17α and H-16α, and H-21

When incubated with pregnenolone, human CYP17A1 essentially only hydroxylates the 17α-position, whereas for progesterone substrate, 16α-hydroxyprogesterone accounts for approximately 25% of the products [136]. In our conditions we found that there is no formation of the 17,20-lyase product (androstenedione, which is DHEA prior to the cholesterol oxidase transformation); however, it is possible to form the cleaved product if we use more volume of yeast microsomes (from 3 μL/1 mL incubation to 10 μL/1 mL incubation). Swart and colleagues used phylogenetic analysis to identify A105 as a key residue enabling 16α-hydroxylase activity, and mutation A105L in human CYP17A1 reduces 16α-hydroxyprogesterone to 10% of the products [151]. Consequently, we measured intramolecular and intermolecular KIEs at the 17α and 16α positions for progesterone and at the 17α position for pregnenolone.

Intramolecular (intrinsic) KIEs

When yeast microsomes with CYP17A1 and POR are incubated with pregnenolone, 17-hydroxypregnenolone is the exclusive hydroxylation product detectable, but with 17α-[^{2}H]-pregnenolone, a trace amount of 16α-hydroxypregnenolone appeared to be formed in amounts too low to use for

calculating an intramolecular KIE (Figure 4.2). Given the known product partitioning for progesterone substrate during CYP17A1 catalysis, we studied progesterone hydroxylation in detail, first measuring the intramolecular KIEs at the 17α- and 16α- positions. Incubations with 17α-[^{2}H]-progesterone suppressed 17-hydroxylation, affording an intramolecular KIE of $k_H/k_D = 4.13 \pm 0.20$ (Figure 4.2, Table 4.1). Similarly, incubations with 16α-[^{2}H]-progesterone suppressed 16α-hydroxylation, affording an intramolecular KIE of $k_H/k_D = 2.86 \pm 0.24$ (Table 4.1). Experiments with reconstituted, purified CYP17A1 and POR gave comparable results (not shown).

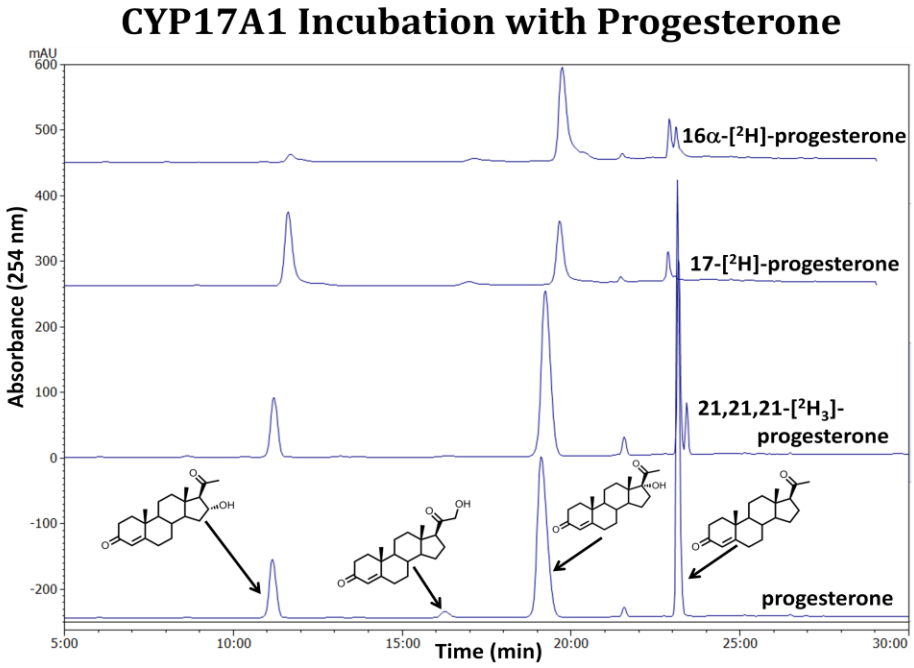

Figure 4.2. CYP17A1 incubations with different progesterone substrates-intramolecular KIEs and metabolic switching.

To further validate these results, we performed intramolecular KIE experiments with CYP17A1 mutation A105L, which demonstrates suppresses 16α-hydroxylation to 10% of turnover. We confirmed that the altered product distribution reported using transfected COS-7 cells [151] is similarly observed with recombinant enzyme in yeast microsomes, with one exception. We also observed an additional peak in the HPLC chromatogram, which co-chromatographs with 21-hydroxyprogesterone in a ~1:1 ratio to 16α-hydroxyprogesterone, both by HPLC (Figure 4.3) and TLC (not shown). Nevertheless, incubations of CYP17A1-A105L with 17α-[^{2}H]-progesterone

afforded an intramolecular KIE value of $k_H/k_D = 3.83 \pm 0.20$, similar to that observed with wild-type CYP17A1. In contrast, the intramolecular KIE observed with mutation A105L using 16α-[^{2}H]-progesterone substrate was 5.10 ± 0.20, considerably higher than observed with wild-type CYP17A1 (Figure 4.3, Table 4.1). Because we also observed the 21-hydroxylation product, we conducted experiments with 21,21,21-[$^2H_3$]-progesterone, and we found an intramolecular KIE of $k_H/k_D = 3.31 \pm 0.72$, similar to those observed at H-17 for both wild-type CYP17A1 or the A105L mutation (Table 4.1). We also observed a trace of 16α-hydroxypregnenolone from incubations with 17α-[^{2}H]-pregnenolone (Figure 4.3).

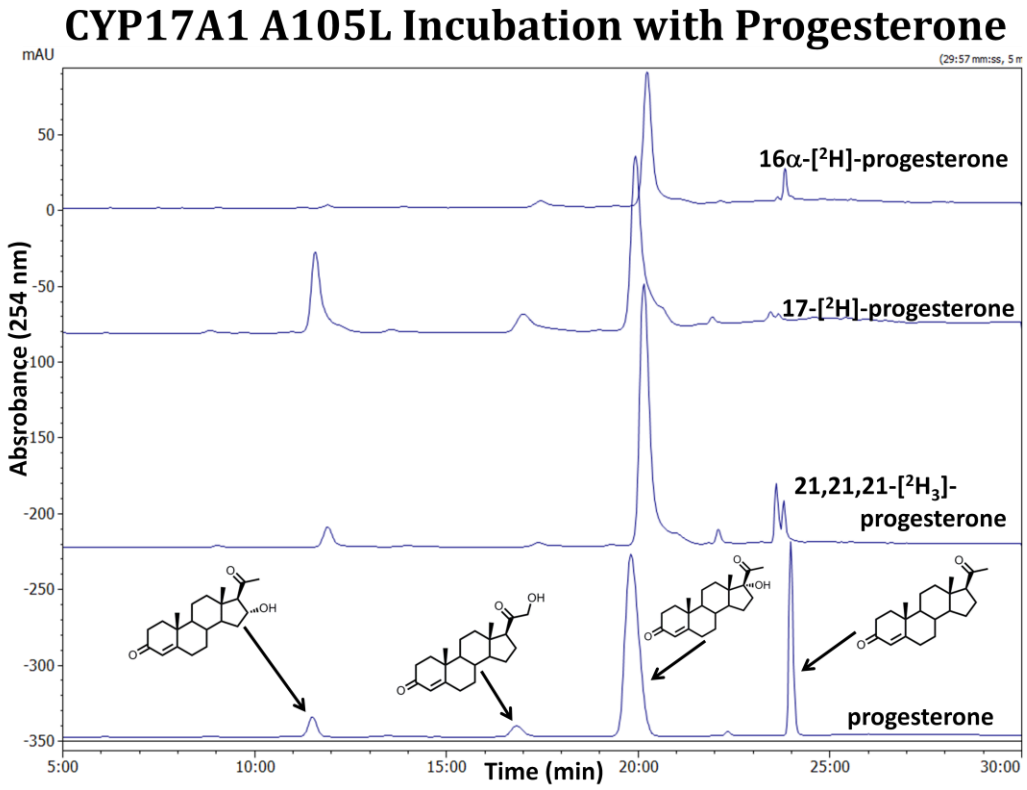

Figure 4.3. CYP17A1-A105L incubations with different progesterone substrates-intramolecular KIEs and metabolic switching.

Because we observed 21-hydroxylation for mutation A105L, we reviewed chromatograms with exhaustive incubations using wild-type CYP17A1 and progesterone, and a small peak of 21-hydroxylated product, comprising 3% of the total products, was consistently found, and this proportion was increased to 4-5% for incubations with 17α-[^{2}H]- or 16α-[^{2}H]-progesterone due to metabolic switching (Figure 4.2). The identity as the 21-hydroxylation product (11-deoxycorticosterone was confirmed by mass spectrometry (not shown). We

pursued this observation by conducting incubations with 21,21,21-[$^2H_3$]-progesterone and wild-type CYP17A1, and the deuterium label suppressed 21-hydroxylation with this substrate to <1% (Figure 4.2). We calculated an intramolecular KIE of $k_H/k_D = 4.00 \pm 0.64$, similar to the KIE at H-21 for the A105L mutation; however, the calculated KIE values at H-21 might be distorted by the very small peak areas for this metabolite, consistent with the large standard deviations. These results demonstrate modest intrinsic primary KIEs on C-H abstraction for CYP17A1 catalysis at H-17α, H-16α, and H-21.

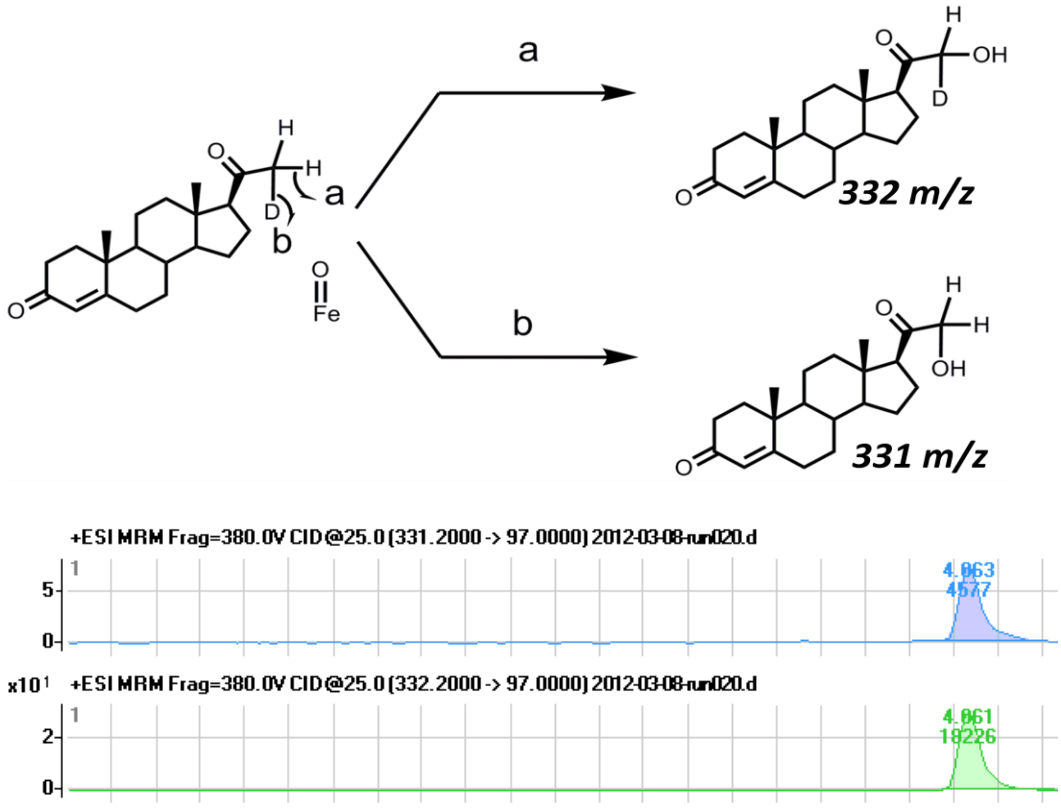

Figure 4.4. Non-competitive intramolecular KIE calculation of CYP21A2 using 21-[^{2}H]-progesterone.

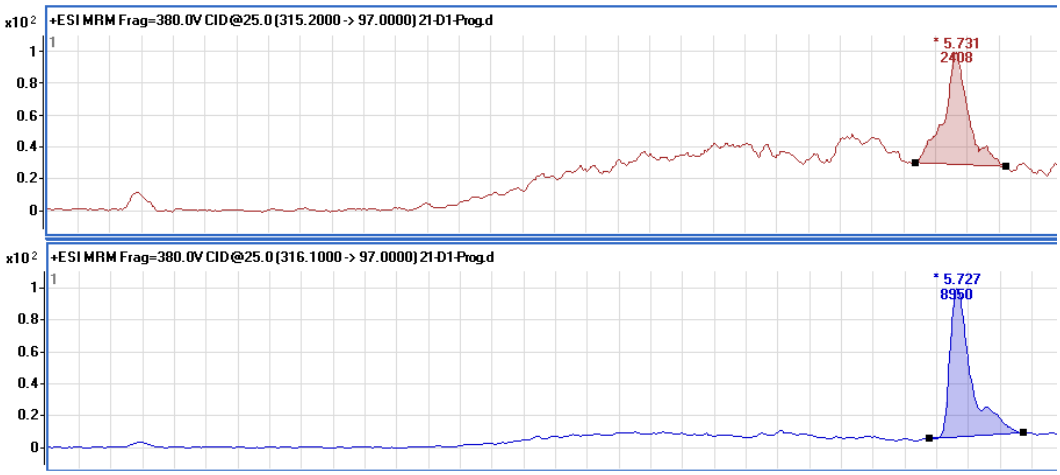

Figure 4.5. Starting material contained non-deuterated progesterone impurity (21-[^{2}H]-progesterone).

Finally, we characterized the composition of 16α-hydroxyprogesterone derived from incubations with 16α-[^{2}H]-progesterone. For wild-type CYP17A1 deuterium-retained product (ie: 16β-[^{2}H]-16-hydroxyprogesterone, product **A**) was found in mass spectrometry analyses (Figure 4.6), indicating that the 16β-hydrogen is abstracted and the resulting radical intermediate is hydroxylated in the α-configuration at C-16 (Figure 4.7). The isotope effect (k_H/k_D) was calculated to be 4.61 ± 0.42.

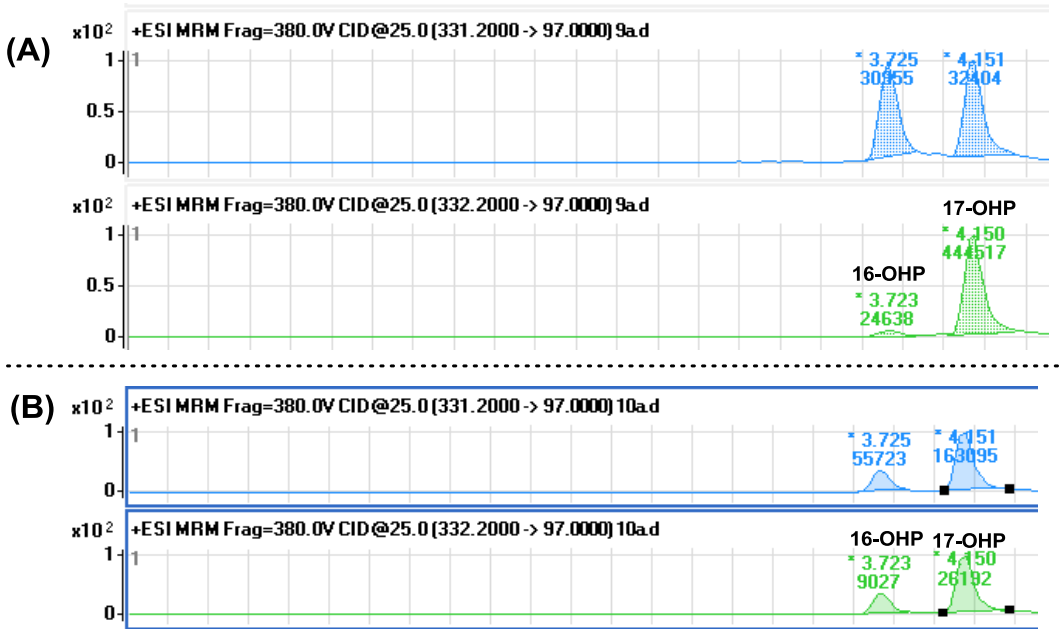

Figure 4.6. MRM transition for incubation of CYP17A1 with 16α-[^{2}H]-progesterone (A) and progesterone (B). The peak with eluting at 3.73 min is 16-hydroxyprogesterone and the peak eluting at 4.15 min is 17-hydroxyprogesterone.

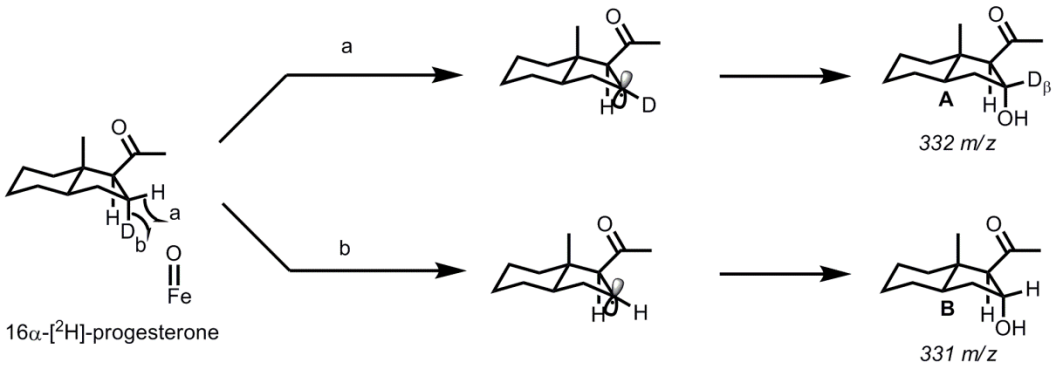

Figure 4.7. Illustration of 16α-[^{2}H]-progesterone as the substrate for CYP17A1 (AB-ring system of the steroid is omitted for clarity). Using mass spectrometry 16β-[^{2}H]-16-hydroxyprogesterone product was observed.

Intermolecular KIEs

In addition, we measured intermolecular competitive KIEs ^{D}V and calculated $^{D}V/K$ using Northrop's equations for CYP17A1 and mutation A105L at H-17α, H-16α, and H-21 of progesterone (Figures 4.9 and 4.10) and at H-17α of pregnenolone. For 17α-[^{2}H]-pregnenolone, which has negligible product partitioning to complicate analysis, we found a competitive KIE at H-17α of ^{D}V = 1.99 ± 0.40 and $^{D}V/K$ = 2.30 ± 0.45 for wild-type CYP17A1 (Figure 4.8) and ^{D}V = 2.29 ± 0.41 and $^{D}V/K$ = 2.67 ± 0.60 for mutation A105L (Figure 4.8). Using 17α-[^{2}H]-progesterone, we found a competitive KIE at H-17α of ^{D}V = 1.74 ± 0.16 and $^{D}V/K$ = 2.11 ± 0.36 for wild-type CYP17A1 and ^{D}V = 1.35 ± 0.02 and $^{D}V/K$ = 1.98 ± 0.04 for mutation A105L (Figures 4.9 and 4.10, Table 4.1). Using 16α-[^{2}H]-progesterone, we obtained KIE values of ^{D}V = 2.96 ± 0.05 and $^{D}V/K$ = 3.19 ± 0.06 at H-16α for wild-type CYP17A1 and ^{D}V = 5.08 ± 0.32 and $^{D}V/K$ = 5.10 ± 0.32 for mutation A105L, respectively. These values are considerably higher than the intermolecular KIE values at H-17α. Using 21,21,21-[$^{2}H_3$]-progesterone substrate, we obtained intermolecular KIE values at H-21 of ^{D}V = 5.77 ± 0.77 and $^{D}V/K$ = 5.84 ± 0.58 for wild-type CYP17A1 and ^{D}V = 3.85 ± 0.90 and $^{D}V/K$ = 3.91 ± 0.92 for mutation A105L (Table 4.1). These values, which are also higher than the intramolecular KIE values at H-17α, are prone to higher error due to low fractional conversion but are internally consistent and reproducible. Although the

concentrations of the deuterated and non-deuterated substrates in these incubations were very dissimilar, increasing the [^{3}H]-labeled, non-deuterated progesterone substrate concentration by a factor of 10 or substituting [^{14}C]-labeled, non-deuterated progesterone at 1000-fold higher concentration gave equivalent results (Table 4.1). Our data indicate an intermolecular KIE significantly >1 at all sites of oxygenation, suggesting that C-H bond breaking is partially rate-limiting in the CYP17A1 reaction cycle, and least contributory for the dominant oxygenation at H-17α.

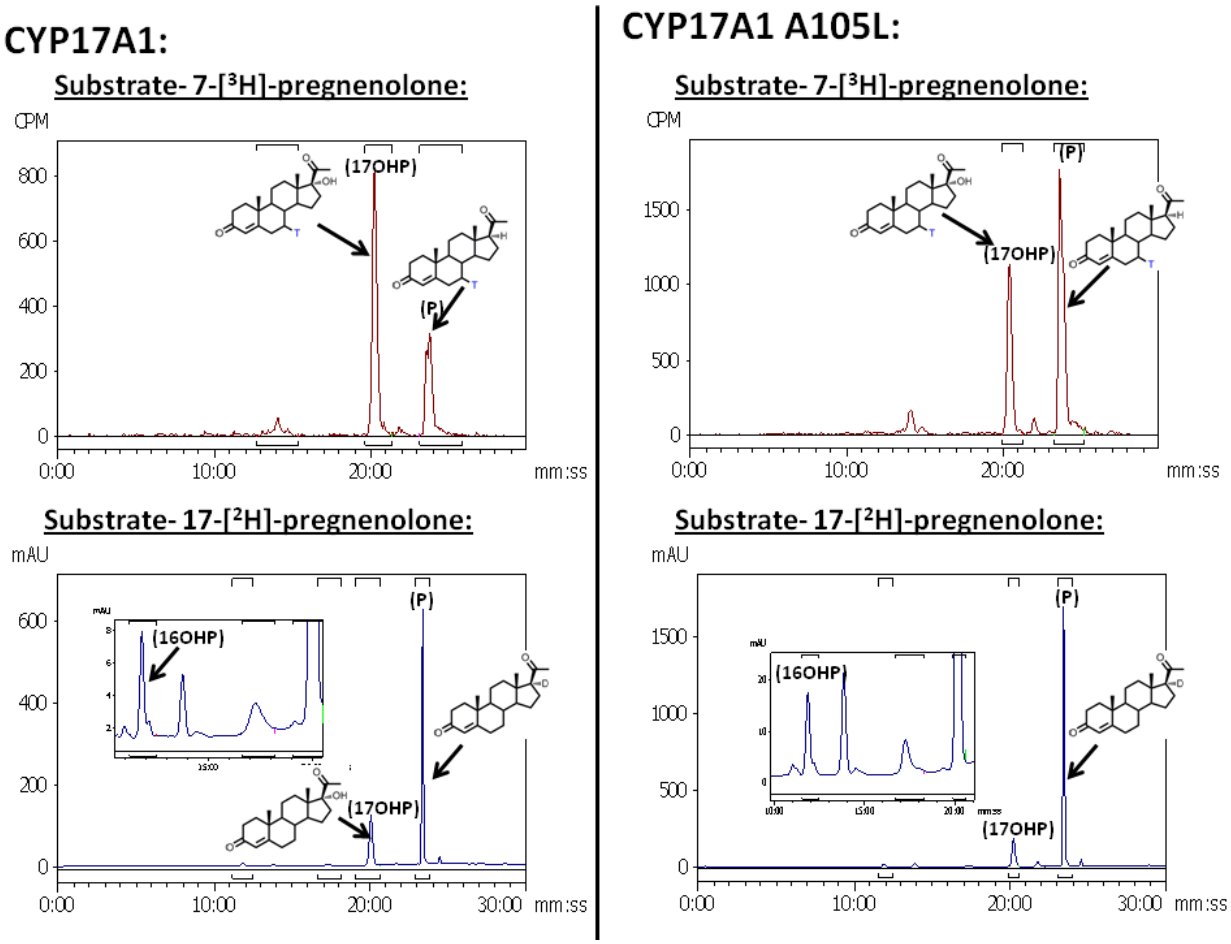

Figure 4.8. CYP17A1 incubations with pregnenolone

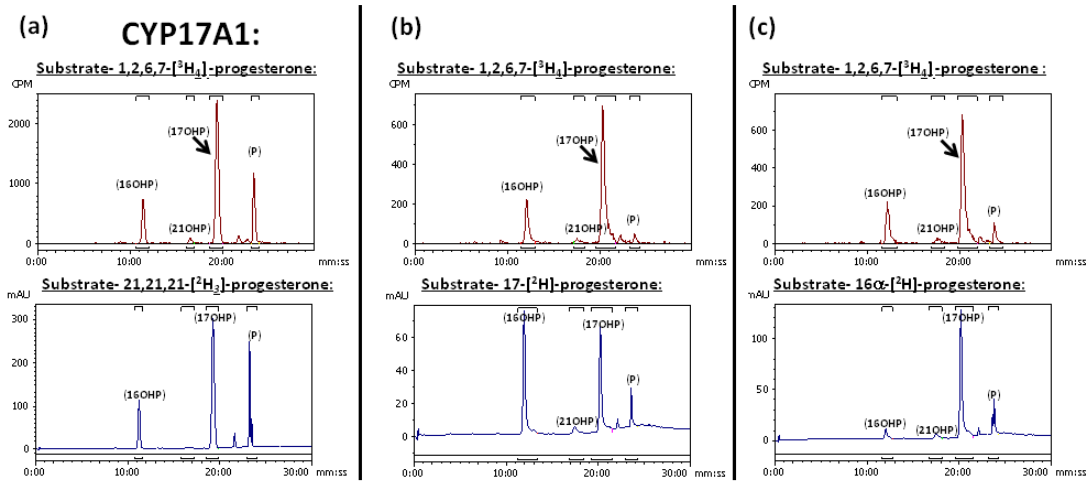

Figure 4.9. CYP17A1 incubations with different progesterone substrates-intermolecular KIEs (a-c).

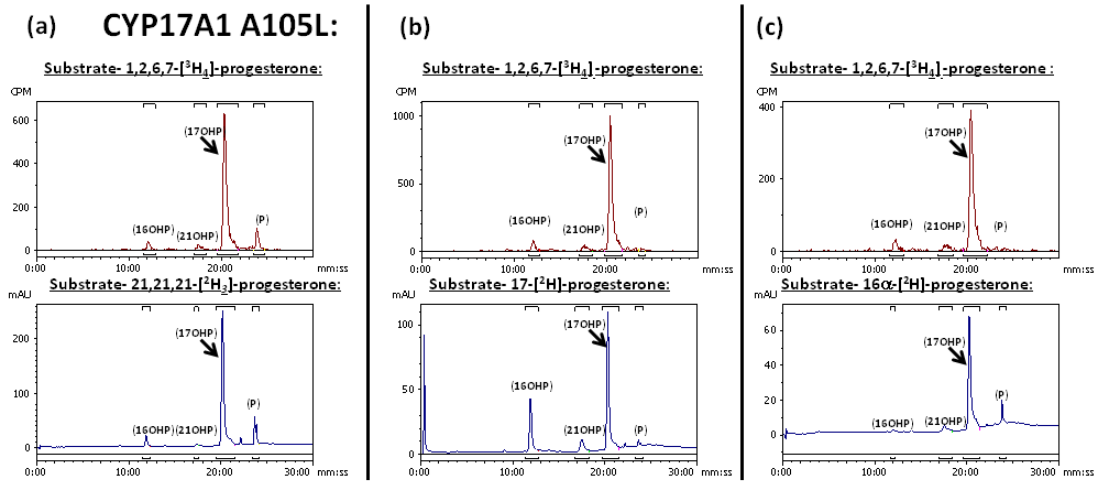

Figure 4.10. CYP17A1 mutation A105L incubations with different progesterone substrates-intermolecular KIEs.

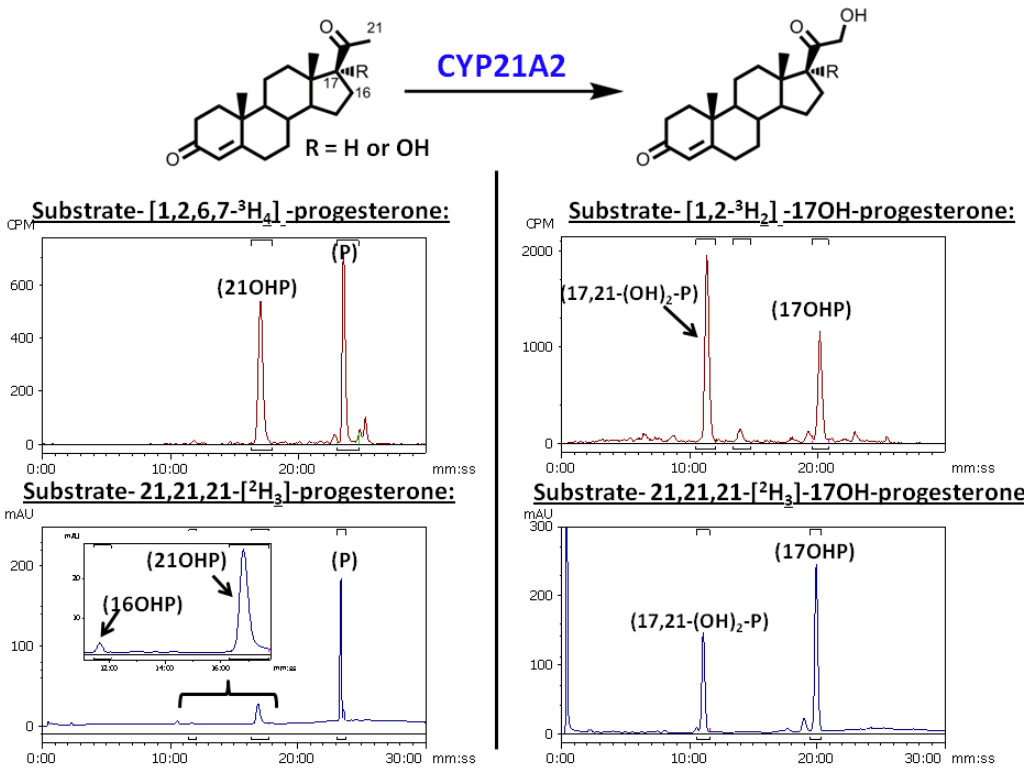

Figure 4.11. CYP21A2 incubation with progesterone and 17-hydroxyprogesterone substrates.

4.3.2. KIE for CYP21A2 at H-21 and H-16α

Intramolecular (intrinsic) KIEs

Upon incubation of yeast microsomes containing CYP21A2 and POR with either progesterone or 17-hydroxyprogesterone, the 21-hydroxylation products 21-hydroxyprogesterone (11-deoxycorticosterone) and 11-deoxycortisol, respectively, are observed. Incubations with 21,21,21-[$^{2}H_{3}$]-progesterone, however, consistently afforded an additional product corresponding to 16α-

hydroxyprogesterone (Figure 4.11). This product was not observed in control experiments omitting enzyme or using enzyme inhibited with ketoconazole, and the identity of the product was confirmed by mass spectrometry (not shown). Further examination of chromatograms from incubations with progesterone and CYP21A2 also consistently demonstrated a trace of 16α-hydroxyprogesterone (Figure 4.11), and from these tracings we estimated an intramolecular KIE of 17.2 ± 3.5, much larger than those for CYP17A1. Experiments using 21-[^{2}H]-progesterone afforded an intramolecular **non-competitive** KIE of 2.40 ± 0.01 for CYP21A2.

To better quantify the intramolecular KIEs for CYP21A2 at H-21 and H-16α, we used mutation V359A, which we have shown yields 60% 21-hydroxylation and 40% 16α-hydroxylation [169] and thus leads to more accurately measured changes in product distributions. Using 21,21,21-[$^2H_3$]-progesterone substrate, we calculated an intramolecular KIE at H-21 of k_H/k_D = 6.24 ± 0.95; using 16α-[^{2}H]-progesterone substrate, we calculated an intramolecular KIE at H-16α of k_H/k_D = 3.75 ± 0.79 (Figure 4.12, Table 4.1).

Intermolecular KIEs

Using similar conditions to those employed with CYP17A1, we observed intermolecular competitive KIE values at H-21 for wild-type CYP21A2 of ^DV = 1.86 ± 0.20 and $^DV/K$ = 2.00 ± 0.22 with 21,21,21-[$^2H_3$]-progesterone and ^DV = 2.25 ± 0.27 and $^DV/K$ = 2.38 ± 0.32 with 21,21,21-[$^2H_3$]-17-hydroxyprogesterone substrates (Figures 4.11 and 4.13, Table 4.1). The intermolecular KIE values with mutation V359A were slightly larger at H-21, ^DV = 2.97 ± 0.56 and $^DV/K$ = 3.10 ± 0.66 with 21,21,21-[$^2H_3$]-progesterone and ^DV = 2.39 ± 0.14 and $^DV/K$ = 3.78 ± 0.65 with 21,21,21-[$^2H_3$]-17-hydroxyprogesterone. Additionally, we obtained the intermolecular KIE at H-16α using 16α-[^{2}H]-progesterone, ^DV = 2.76 ± 0.26 and $^DV/K$ = 3.03 ± 0.40 (Figures 4.12, Table 4.1). These data demonstrate that C-H bond breakage is also partially rate-limiting for the reactions catalyzed by CYP21A2.

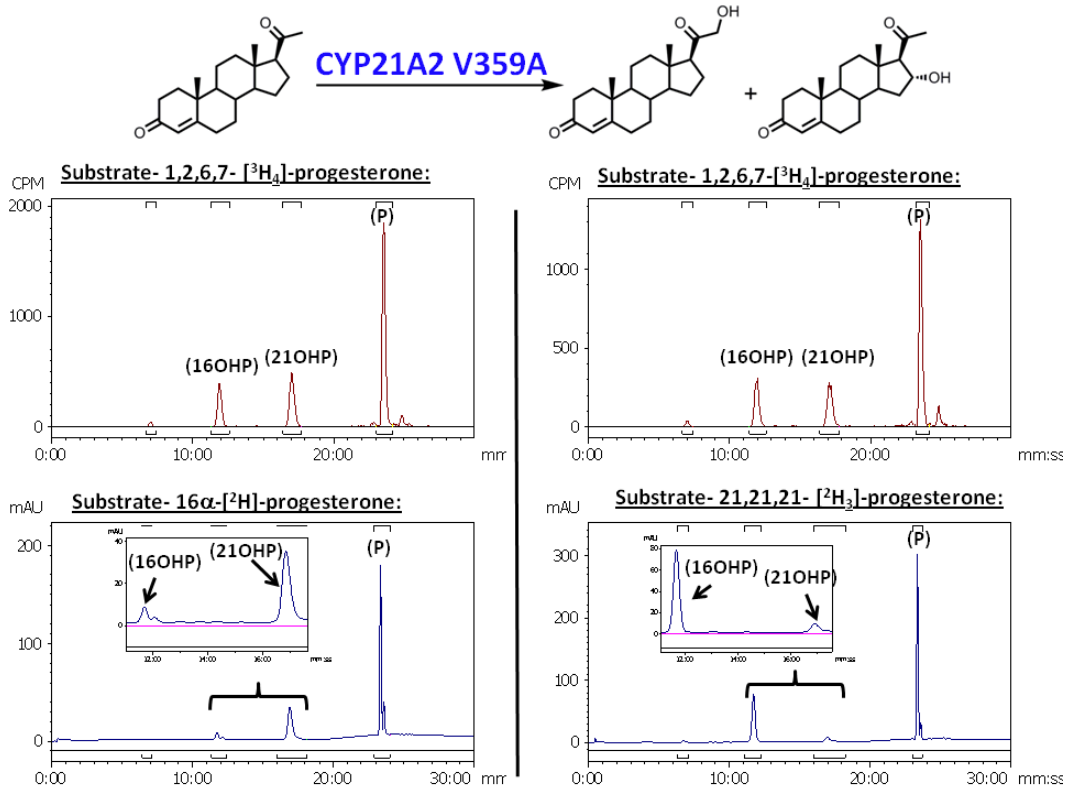

Figure 4.12. CYP21A2-V3359A incubation with progesterone substrates.

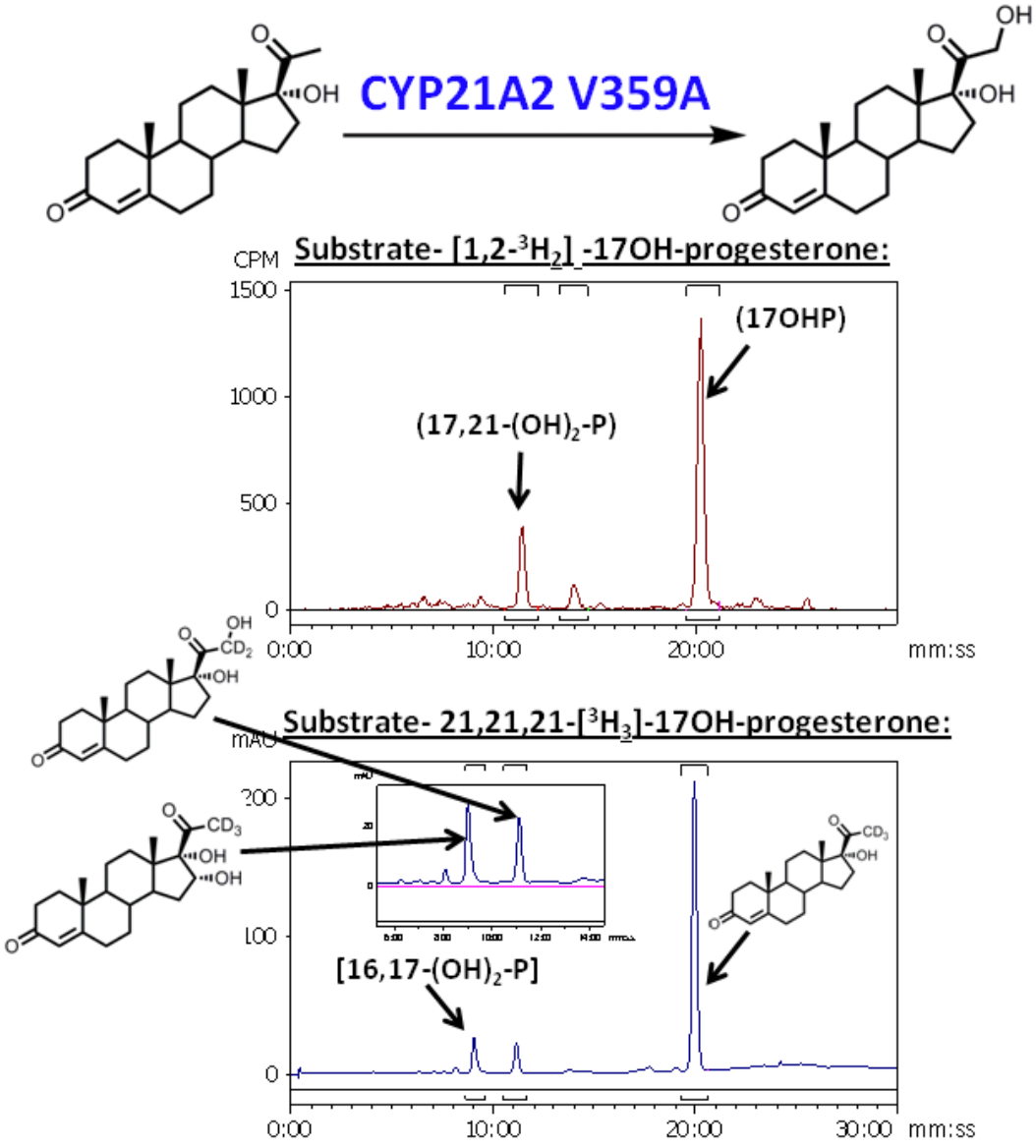

Figure 4.13. CYP21A2-V359A intermolecular KIE with 17-hydroxyprogesterone.

System and substrate	C-H Bond	Intramolecular k_H/k_D (n)[a]	Intermolecular ^DV (n)[b]	Intermolecular $^DV/K$ (n)
CYP17A1				
16α-[^{2}H]-progesterone	C16	2.86 ± 0.24 (3)	2.96 ± 0.05 (3)	3.19 ± 0.06 (3)
17α-[^{2}H]-progesterone	C17	4.13 ± 0.20 (3)	1.74 ± 0.16 (11)	2.11 ± 0.36 (11)
17α-[^{2}H]-progesterone [using ^{14}C]	C17	not applicable	2.17 ± 0.30 (3)	1.80 ± 0.91 (3)
17α-[^{2}H]-progesterone [using 10x ^{3}H]	C17	not applicable	1.99 ± 0.44 (3)	2.26 ± 0.07 (3)
17α-[^{2}H]-pregnenolone	C17	not determinable	1.99 ± 0.40 (6)	2.30 ± 0.45 (6)
21,21,21-[$^2H_3$]-progesterone	C21	4.00 ± 0.64 (3)	5.77 ± 0.77 (3)	5.84 ± 0.58 (3)
CYP17A1-A105L				
16α-[^{2}H]-progesterone	C16	5.10 ± 0.20 (3)	5.08 ± 0.32 (3)	5.10 ± 0.32 (3)
17α-[^{2}H]-progesterone	C17	3.83 ± 0.20 (3)	1.35 ± 0.02 (3)	1.98 ± 0.04 (3)
17α-[^{2}H]-pregnenolone	C17	not determinable	2.29 ± 0.41 (3)	2.67 ± 0.60 (3)
21,21,21-[$^2H_3$]-progesterone	C21	3.31 ± 0.72 (3)	3.85 ± 0.90 (3)	3.91 ± 0.92 (3)
CYP21A2				
21-[^{2}H]-progesterone	C21	2.40 ± 0.01 (2)		
21,21,21-[$^2H_3$]-progesterone	C21	17.2 ± 3.46 (3)	1.86 ± 0.20 (10)	2.00 ± 0.22 (10)
21,21,21-[$^2H_3$]-17-hydroxyprogesterone	C21	none	2.25 ± 0.27 (7)	2.38 ± 0.32 (7)
CYP21A2-V359A				
16α-[^{2}H]-progesterone	C16	3.75 ± 0.79 (4)	2.76 ± 0.26 (7)	3.03 ± 0.40 (7)
21,21,21-[$^2H_3$]-progesterone	C21	6.24 ± 0.95 (4)	2.97 ± 0.56 (4)	3.10 ± 0.66 (4)
21,21,21-[$^2H_3$]-17-hydroxyprogesterone	C21	not determinable	2.39 ± 0.14 (4)	3.78 ± 0.65 (4)

Table 4.1. Summary of kinetic isotope effects. Data are given as means ± standard deviations for n experiments. [a] Ratio of relative yield between probed hydroxylated product to the sum of other hydroxylated products. [b] Ratio of probed hydroxylated products.

4.4. DISCUSSION

4.4.1. Significance of KIE values

For an enzyme-catalyzed C-H bond cleavage reaction following classical mechanics without proton-coupled electron transfer (hydrogen atom tunneling), the magnitude of the kinetic isotope effects is indicative of the transition state structure [173]. Generally in the P450 C-H abstraction step that lacks tunneling, the calculated kinetic isotope effect is higher for a more symmetrical transition state (ie: the distance between the hydrogen atom donor and the hydrogen and the distance between the hydrogen and the hydrogen atom acceptor become equal as the KIE values are higher). Assuming that there is no tunneling involved, the magnitudes of the kinetic isotope effects is indicative of the transition state structure [174]. In our intramolecular KIE calculations we are comparing the C-H abstraction steps on two adjacent carbon atoms within the same molecule and substrate release is not involved in differentiating the two different oxygenated products (16α-hydroxyprogesterone and 17-hydroxyprogesterone).

A more symmetrical or linear transition state would lead to a higher KIE value as stated by Westheimer [175] and a non-linear or bent transition state yields lower KIE values [176]. The kinetic isotope effect originates from the difference between the zero point energies of the C-D and the C-H bond in the ground state (~1.15 kcal/mol)[177].

(a) C-H-O (b) C-H-O

Figure 4.14: Bond stretching vibrations between the hydrogen atom donor and the hydrogen atom acceptor. (a) symmetric vibration representing the transition state. (b) anti-symmetric vibration.

The maximum KIE value (the theoretical limit excluding tunneling effects: k_H/k_D ~9 at 37 ^oC [178] and ~7 at 50 ^oC [179]) stems from the fact that the transition state energy levels of the C-D case and the C-H case are identical in a symmetrical transition state complex, C-D-O or C-H-O, where C is the deuterium or hydrogen donor, D is the deuterium atom, H is the hydrogen atom and O is deuterium or hydrogen acceptor. The distance between hydrogen donor (C) and hydrogen atom (H) is the same as the distance between the hydrogen atom (H) and hydrogen acceptor (O) in the symmetrical transition state and in the zero point energy level the mass of the hydrogen atom is negligible because the hydrogen atom remains stationary in the vibrational motion. In a non-symmetrical transition state complex, C-H-O, the distance between hydrogen donor (C) and hydrogen atom (H) is different from the distance between hydrogen atom (H) and the hydrogen acceptor (O). This difference in distances lowers the zero point vibrational energy of the C-D-O complex relative to the C-H-O

complex **because the deuterium or hydrogen atom is not in the center of the transition state resulting in an asymmetric transition state.**

The symmetrical stretching vibration where both C and O are moving away from each other represents the zero point energy level of the transition state (the anti-symmetrical stretching vibration is the imaginary vibrational mode representing the motion leading to a reaction). Since the deuterium would lower the zero point energy of the transition state in the case where the distances between C-H and H-O are not the same (ie: the transition state structure is either product-like or reactant-like), the k_H/k_D values would diminish.

A symmetrical, linear transition state leads to a high KIE value, whereas a bent or asymmetric transition state yields lower KIE values [180]. Asymmetric transition states for C-H bond cleavage might be not only non-linear or angled but also "early" or "late," meaning that the C-H bond resembles more the reactant (C-H bond mostly formed) or the product (C-H bond mostly broken) in the transition state. The magnitude of an intermolecular KIE is also reduced or "masked" by other steps in the catalytic cycle if C-H bond cleavage is not substantially rate-limiting. Masking might complicate intermolecular KIE experiments in which two different molecules compete for metabolism in a single incubation, limiting the information obtained. Many cytochrome P450 reactions, however, demonstrate incomplete regiochemical selectivity, executing hydroxylation at more than one site. This relaxed catalytic selectivity leads to the phenomenon of

metabolic switching, where oxygenation shifts to an alternate site upon deuterium substitution at the principal site of reactivity. Metabolic switching permits the calculation of intramolecular KIEs, reflecting the intrinsic KIE for the C-H bond breakage step, independent of masking from slower steps in the reaction cycle. In the present study, we used intramolecular KIEs to compare transition state features for steroid hydroxylations at H-16α, H-17α, and H-21 catalyzed by CYP17A1 and CYP21A2.

We found relatively modest intramolecular KIEs for wild-type CYP17A1 and mutation A105L at H-17α, the principal site of steroid hydroxylation. The intramolecular KIE at H-16α was lower than at H-17α for wild-type CYP17A1 but slightly higher for mutation A105L. The KIEs well below 9 indicate that the transition state for hydrogen atom abstraction is either somewhat bent or asymmetrical, particularly for 16α-hydroxylation by wild-type CYP17A1 (KIE = 2.86). Computer modeling studies of CYP17A1 predict that steroids bind with the cyclopentanophenanthrene nucleus parallel to the plane of the heme with H17α very close to the iron-oxygen complex [181], although the recent x-ray crystal structure of human CYP17A1 with bound abiraterone (not substrate) suggests that other orientations are possible [60]. If the transition state geometry mimics that predicted from computational studies, then the C—H—O(Fe) alignment should not significantly deviate from linearity, and the most plausible explanation for low KIEs would be an early or late transition state. The bond

energy of the C-H17α bond, a methine adjacent to a carbonyl, is predicted to be weaker (82 kcal/mol) than a carboxymethyl C-H21 (85 kcal/mol) or methylene C-H16α (99 kcal/mol). Based on this analysis, we favor an early transition state for C-H17α bond cleavage, which reduces the observed intramolecular KIE to less than half of theoretical maximum. Transition state geometry the secondary sites of reactivity H-16α and H-21 are likely to be more distorted than for H-17α, but the similar KIE values observed at H-21 suggest that the transition state for C-H bond cleavage is more symmetrical than for H-17α, possibly due to the stronger C-H bonds. It is not obvious why the intramolecular KIE for mutation A105L at H-16α is larger than at H-17α—the largest intramolecular KIE observed in our studies with CYP17A1—whereas the converse is true for wild-type CYP17A1, but even this value at 5.1 is small compared to values of 10 or higher documented for other P450 oxygenations.

For CYP21A2, intramolecular KIEs were best obtained for mutation V359A with progesterone, which gave values of 4-5 at both H-16α and H-21. Computer modeling studies suggested that progesterone binds to CYP21A2 with the steroid nucleus perpendicular to the plane of the heme ring with the C-21 methyl group dangling over the iron-oxygen complex [169]. In mutation V359A, the larger steroid-binding pocket enables trajectories with the steroid tipping on its long axis to present the more reactive H-16α in addition to H-21, yielding both

products [169]. The crystal structure of bovine CYP21A2 with 17-hydroxyprogesterone bound confirms the orthogonal orientation of heme and steroid, plus a second apparently structural steroid distant from the steroid-binding pocket [150]. Based on our results, the transition states for these two hydrogen atom abstractions must share considerable similarities. For wild-type CYP21A2, an anomalously large KIE of 17.2 was observed; however, the very low fraction of 16α-hydroxylation renders these values prone to error and suggests proton-coupled electron transfer (hydrogen atom tunneling) in the reaction mechanism [168].

In addition, experiments with deuterium-labeled substrates have provided compelling evidence for additional hydroxylase activities of wild-type human CYP17A1 and CYP21A2. In addition to both progesterone 17α- and 16α-hydroxylase activities, human CYP17A1 catalyzes progesterone 21-hydroxylation. This activity is augmented with either the 17-[^{2}H]-progesterone substrate or 16-[^{2}H]-progesterone substrate and accentuated in mutation A105L. The 21-hydroxylation comprises only 3% of the products; nonetheless, this trace of activity might be clinically significant in classical 21-hydroxylase deficiency with null *CYP21A2* alleles (salt-wasting phenotype) and might explain some of the discrepancies observed between phenotype and genotype in this disease. We also reproducibly observed a trace of pregnenolone 16α-hydroxylation using 17α-[^{2}H]-pregnenolone substrate but not unlabeled pregnenolone. Analogously,

CYP21A2 is a progesterone 16α-hydroxylase, accounting for <1% of the products but augmented with 21,21,21-[$^{2}H_3$]-progesterone and markedly increased with mutations that reduce the bulk of V359.

4.4.2. Metabolic Switching

The phenomenon of "metabolic switching" occurs when a common E•S complex breaks down to form two (or more) products, P1 and P2, and the partitioning is altered via isotopic substitution at one reaction site. In the absence of secondary isotope effects, the rate of reaction at the unlabeled site should remain constant, while the rate for reaction at the substituted (deuterated) site slows by an amount equal to the intramolecular KIE. For chemical reactions best described by classical mechanics, the limiting KIE at 37C is 9, meaning that the proportion of minor product can only increase by a factor of 10 or less. Depending on the sensitivity of the assay, new products might seem to "appear" due to metabolic switching, but in fact these "new" products must be present in at least trace amounts in reactions with unlabeled substrate. We observed this phenomenon several times in our studies; we found that CYP17A1 16α-hydroxylates pregnenolone and 21-hydroxylates progesterone and that CYP21A2 16α-hydroxylates progesterone. These products were recognized from reactions with deuterium-labeled substrates and site-directed mutations, but our findings prompted us to identify these products in reactions with wild-type enzymes and

natural abundance substrates. We could not confidently identify 16α-hydroxypregnenolone from reactions with CYP17A1 and unlabeled pregnenolone, possibly because the reaction was coupled to cholesterol oxidase treatment to generate a UV-chromophore.

We also observed metabolic switching with 21,21,21-[$^2H_3$]-17-hydroxyprogesterone for both wild-type CYP21A2 and mutation V359A, but we could only partially characterize these products. CYP21A2 mutation V359A metabolizes 17-hydroxyprogesterone to 11-deoxycortisol and one additional unidentified product, which is not 16α,17α-dihydroxyprogesterone (pregn-4-ene-16α,17α-diol-3,20-dione) [169]. Upon incubation with 21,21,21-[$^2H_3$]-17-hydroxyprogesterone, however, a product co-eluting with 16α,17α-dihydroxyprogesterone appeared, indicative of metabolic switching. Inspection of chromatograms obtained from wild-type CYP21A2 incubations with 17-hydroxyprogesterone reveals a trace of the same unknown product, and its formation is also suppressed with 21,21,21-[$^2H_3$]-17-hydroxyprogesterone. Therefore, we speculate that this unknown compound is further oxygenated at C-21, possibly a gem-diol of 21-formyl-17-hydroxyprogesterone, but we could not calculate KIE values at H-21 for 17-hydroxyprogesterone.

4.4.3. Intramolecular KIE vs. Intermolecular KIE

Competitive intermolecular KIE experiments gave predominantly values of 1.3-2.7 at the major sites of CYP17A1 and CYP21A2 hydroxylations. These KIE values are unusually high for a P450 reaction, for which the second electron transfer step is traditionally assumed to be the rate-limiting step, based on studies of $P450_{cam}$. The turnover number for the steroid hydroxylation reactions catalyzed by CYP17A1 and CYP21A2, however, are many orders of magnitude slower than those of bacterial P450s [153], and different steps might become at least partially rate-limiting in this context. Our data indicate that C-H bond cleavage is partially rate-limiting for all reactions catalyzed by these two enzymes, which we studied. The largest intramolecular KIE values were obtained for progesterone 21-hydroxylation by CYP21A2 and mutation V359A, suggesting that C-H bond breakage is most rate-limiting for this reaction of those studied in our present experiments. For bovine CYP21A2, pre-steady state kinetic experiments demonstrated that product release was rate-limiting for progesterone 21-hydroxylation but not for the more rapid 21-hydroxylation of 17-hydroxyprogesterone [182]. The design of our experiments, which incorporates tracer tritium label at distant sites to monitor both species in the same incubation, controls for several variables that can confound intermolecular KIE determinations. Varying the ratio of deuterium-labeled to undeuterated steroid or substitution of [^{14}C]-tracer for [^{3}H]-tracer did not influence the results,

strengthening our conclusions. Consequently, the rates of C-H bond cleavage and product release for CYP21A2-catalyzed progesterone 21-hydroxylation are likely very similar. The slow rate for this C-H bond cleavage reaction probably explains why the intramolecular KIE at H-21 is larger than for other reactions studied herein.

Intramolecular KIEs have been determined for several P450-catalyzed reactions. KIE values of 9-11 have been obtained for reactions catalyzed CYP2E1 and CYP2B4, including reactions at methyl groups [162]. In contrast, the non-competitive KIE for CYP3A4-catalyzed testosterone-6β-hydroxylation of an allylic C-H bond is only 2-3 [166]. Holland and colleague have reported on the intermolecular kinetic isotope effect of the 19-methyl position on testosterone with aromatase (CYP19A1) with a k_H/k_D of 2.3 for 19-[^{2}H]-testosterone and 3.2 for 19,19,19-[$^2H_3$]-testosterone substrates [183]. This wide range in observed KIE values suggests that, although all cytochrome P450 hydroxylation reactions might incorporate hydrogen atom abstraction in their catalytic cycles, the structural features of these transition states are considerably variable. Although some characteristics are undoubtedly common to all mammalian P450-catalzyed reactions, the steroid hydroxylases CYP17A1 and CYP21A2 are likely to require some unique properties to execute their enzymatic functions, which serve to support reproduction, response to stress, and fluid balance. Further experiments, which directly measure the rates of individual steps for these reactions, will

provide further insight to the mechanism of catalysis for these important enzymes in human physiology and disease.

ACKNOWLEDGEMENTS

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CHAPTER FIVE

Measuring Hydrogen Tunneling Contributions in the C-H Abstraction Step

5.1. INTRODUCTION

Hydrogen atom tunneling is thought to occur in all C-H bond cleavage reactions in biological systems, and this chemical transformation occurs in 50% of all biological reactions [184]. The methods used to probe tunneling in enzyme catalyzed reactions include: (1) varying the temperature and measuring the kinetic isotope effects and (2) measuring the kinetic isotope effects and comparing a tritiated, deuterated and protiated substrate (ie: Swain-Schaad relationship [185]).

5.2. METHODS

Enzyme assays used human CYP21A2 and CYP17A1 with P450-oxidoreductase in microsomes from yeast expressing the recombinant proteins as described [141]. Test steroid (20-50 μM, delivered in <5 μL ethanol) and microsomes (5-10 μL, ~10 pmol P450) were incubated in 50 mM potassium phosphate (pH 7.4) with 1 mM NADPH at given temperature (either: 20, 26, 30, 37, 45 °C) and 2 mL total volume. Aliquots leaving half incubation volume behind were taken after 45 min and incubation was left for another 45 min. The incubations were terminated with the addition of CH_2Cl_2 (1 mL), extraction, and

centrifugation. The organic extract was dried under nitrogen flow, dissolved in 50% aqueous methanol (70 -90 mL), and analyzed by high performance liquid chromatography (HPLC). See Chapter 4 for cholesterol oxidase transformation and HPLC method. In the case of 17-[^{3}H]-pregnenolone, it was possible to mix the incubation with 17-[^{2}H]-pregnenolone or independently incubate 17-[^{2}H]-pregnenolone with the enzyme.

5.3. TEMPERATURE DEPENDENCE

We determined the temperature dependence of the kinetic isotope effects. The experimental temperature ranged from 20 °C to 55 °C. Interestingly, CYP17A1 seemed to tolerate the highest temperature (55 °C) but CYP21A2 did not; in other words, we could not analyze the incubation data of the CYP21A2 wild-type and the CYP21A2 V359A mutant for the incubations run at 55 °C due to the low conversion to products at this temperature. In the case of CYP17A1 the data did not fit the trend when the 55 °C temperature point was included in the CYP17A1 data set. For example, the sign of the slope would change from positive to negative if this data point was included in the case of CYP17A1 and 17-[^{2}H]-progesterone. Therefore, this temperature point (55 °C) was omitted in most of the Arrhenius plots in order to maintain consistency (ie: if the trend line was completely off scale due to this point). The temperature points generally used were: 20 °C, 26 °C, 30 °C, 37 °C and 45 °C.

The purpose of the Arrhenius plots was to measure: (a) the difference in activation energy between the non-deuterated substrate and the deuterated substrate ($\Delta Ea_{(D-H)}$) and (b) to determine the Arrhenius prefactor (A_H/A_D). Equipped with this knowledge, we can determine the tunneling contributions in the C-H abstraction step of these enzyme catalyzed reactions .

$$k = A\exp[-Ea/(RT)] \quad (1)$$

$$\ln k = (-Ea/R)(1/T) + \ln A \quad (2)$$

$$y = mx + b \quad (3)$$

$$\ln(k_H/k_D) = [(Ea_D-Ea_H)/R]*(1/T) + \ln(A_H/A_D) \quad (4)$$

As shown in equation (4) above, the natural log of the kinetic isotope effect was plotted on the y-axis and the inverse temperature (Kelvin^{-1} units) is plotted on the x-axis. The slope corresponds to the difference in activation energies, $\Delta Ea_{(D-H)}$, and the y-intercept is related to the Arrhenius prefactor ratio, A_H/A_D. The Arrhenius prefactor ratio is thought to suggest evidence of tunneling when this ratio is distinct from 1 [186, 187]. The Arrhenius constant, A, is interpreted as the number of collisions per unit time and $e^{-Ea/(RT)}$ is the probability that a collision will lead to a reaction. R is the gas constant (1.9858775×10^{-3} kcal/mol/K).

Enzyme and Substrate	^{D}V or $^{D}V/K$	$\Delta Ea_{(D-H)}$ (kcal/mol)	A_H/A_D
CYP17A1			
21,21,21-[$^{2}H_3$]-progesterone	^{D}V	2.30	0.15
21,21,21-[$^{2}H_3$]-progesterone	$^{D}V/K$	9.05	3.12×10^{-6}
17-[^{2}H]-progesterone	^{D}V	0.77	0.51
17-[^{2}H]-progesterone	$^{D}V/K$	0.29	0.05
16α-[^{2}H]-progesterone	^{D}V	0.86	0.74
16α-[^{2}H]-progesterone	$^{D}V/K$	1.41	0.33
CYP17A1-A105L			
21,21,21-[$^{2}H_3$]-progesterone	^{D}V	0.28	2.42
21,21,21-[$^{2}H_3$]-progesterone	$^{D}V/K$	6.61	9.66×10^{-5}
17-[^{2}H]-progesterone	^{D}V	-0.76	5.03
17-[^{2}H]-progesterone	$^{D}V/K$	-1.44	23.6
16α-[^{2}H]-progesterone	^{D}V	4.56	2.64×10^{-3}
16α-[^{2}H]-progesterone	$^{D}V/K$	5.31	8.32×10^{-4}
CYP21A2			
21,21,21-[$^{2}H_3$]-progesterone	^{D}V	-0.38	3.39
21,21,21-[$^{2}H_3$]-progesterone	$^{D}V/K$	-1.03	11.1
CYP21A2-V359A			
21,21,21-[$^{2}H_3$]-progesterone	^{D}V	7.63	1.42×10^{-5}
21,21,21-[$^{2}H_3$]-progesterone	$^{D}V/K$	7.73	1.25×10^{-5}
16α-[^{2}H]-progesterone	^{D}V	6.72	4.92×10^{-5}
16α-[^{2}H]-progesterone	$^{D}V/K$	6.71	5.37×10^{-5}
CYP17A1 (purified)			
17-[^{2}H]-progesterone	^{D}V	0.90	0.30
17-[^{2}H]-progesterone	$^{D}V/K$	0.85	0.33
16α-[^{2}H]-progesterone	^{D}V	-0.39	4.80
16α-[^{2}H]-progesterone	$^{D}V/K$	-0.50	5.98

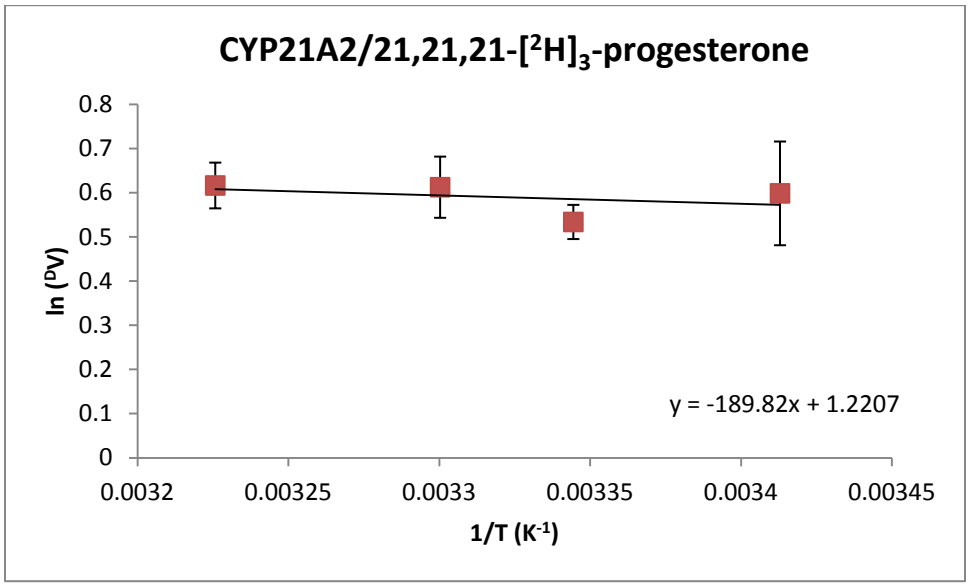
Table 5.1. List of $\Delta Ea_{(D-H)}$ (kcal/mol) and A_H/A_D values from isotope effects (^{D}V or $^{D}V/K$) derived from intermolecular kinetic isotope effects (ie: deuterated substrate was incubated in the presence of tritiated progesterone substrate possessing hydrogens at the site of reactivity).

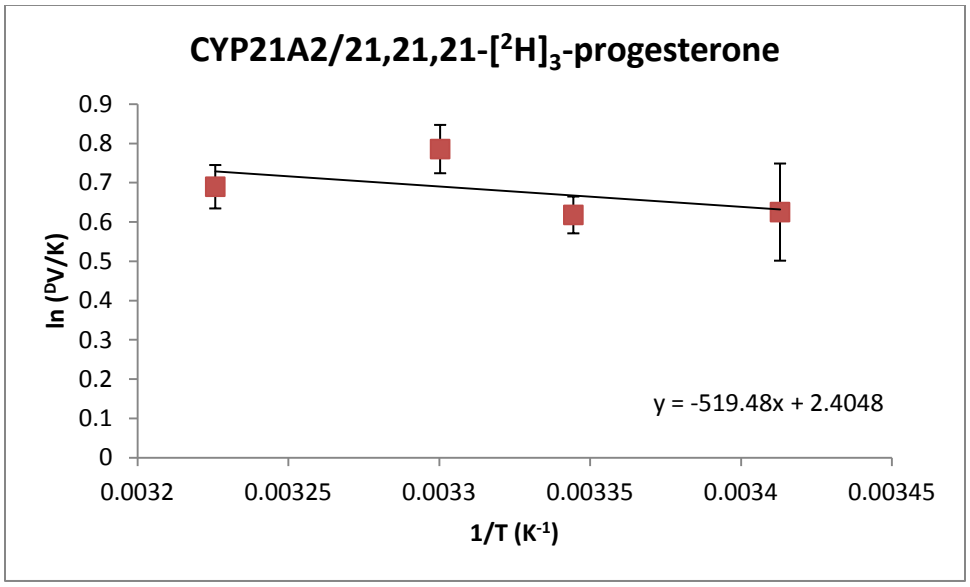
5.4. INTERPRETATION

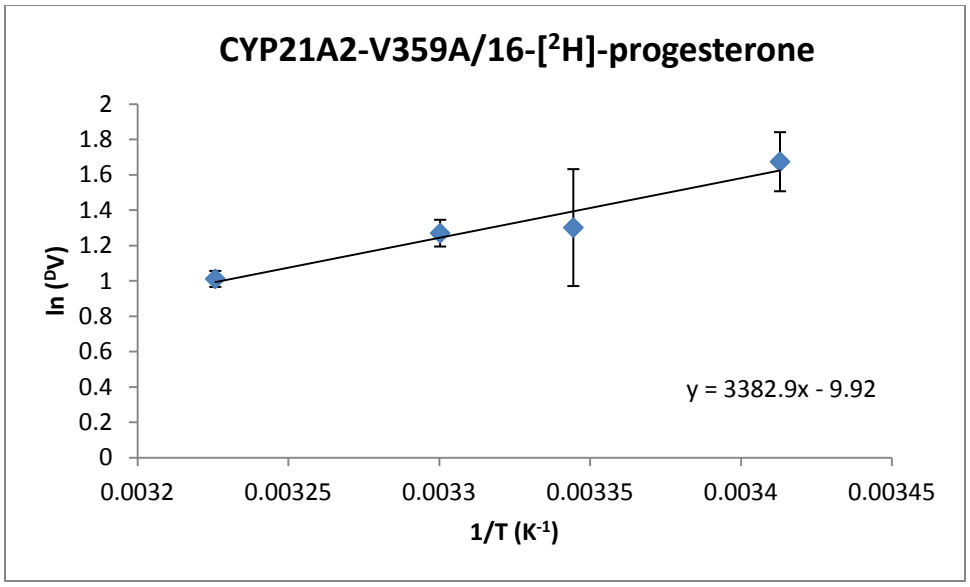
The values for $\Delta Ea_{(D-H)}$ and A_H/A_D were obtained from the intermolecular kinetic isotope effects (ie: not intramolecular KIE). In general, similar magnitudes of $\Delta Ea_{(D-H)}$ and A_H/A_D were calculated when either using ^{D}V or $^{D}V/K$ values (Table 5.1). As mentioned in Chapter four, the kinetic isotope effect is thought to arise from the difference in zero point energies between the C-D and C-H bonds. The zero point energy level difference is calculated to be about 1.15 kcal/mol .

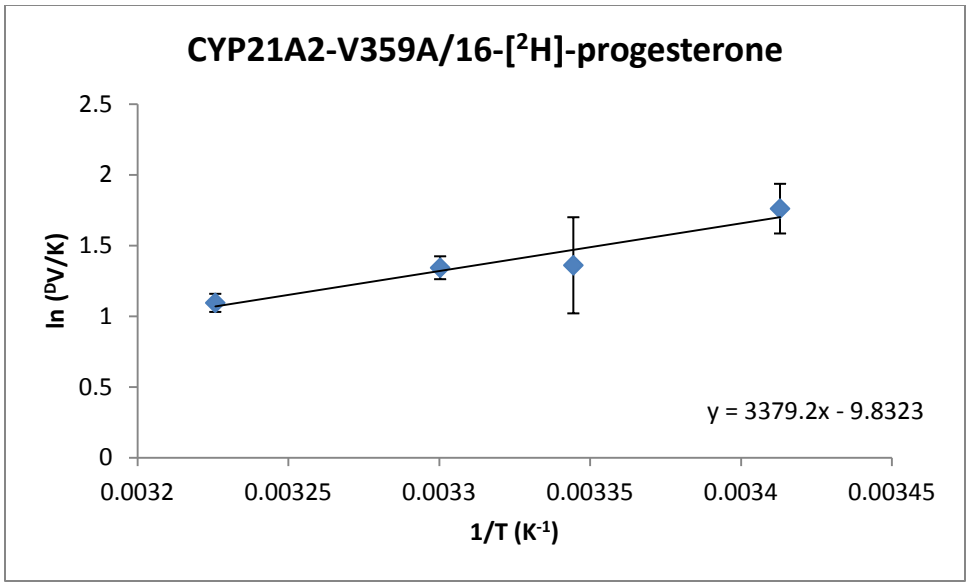
The 21,21,21-[$^{2}H_3$]-progesterone substrate seemed to yield a relatively large difference in activation energy between C-H and C-D bond cleavage when comparing to the other substrates used within an enzyme system. This observation is consistent with the secondary kinetic isotope effect – the presence of two additional deuteriums on the C21-position increase the C-D bond strength. Moreover, the negative values of $\Delta Ea_{(D-H)}$ such as in the case of CYP21A2 with 21,21,21-[$^{2}H_3$]-progesterone could be explained by other steps in the reaction cycle such as substrate binding and product release being affected by the temperature change. In other words, the kinetic isotope effects could be "masked" by other steps in the reaction cycle (ie: there is kinetic complexity) and therefore, it would be worthwhile to measure the Arrhenius prefactor ratios and $\Delta Ea_{(D-H)}$ values from intramolecular KIEs. Another possibility could be due to the denaturation of the protein at different temperatures.

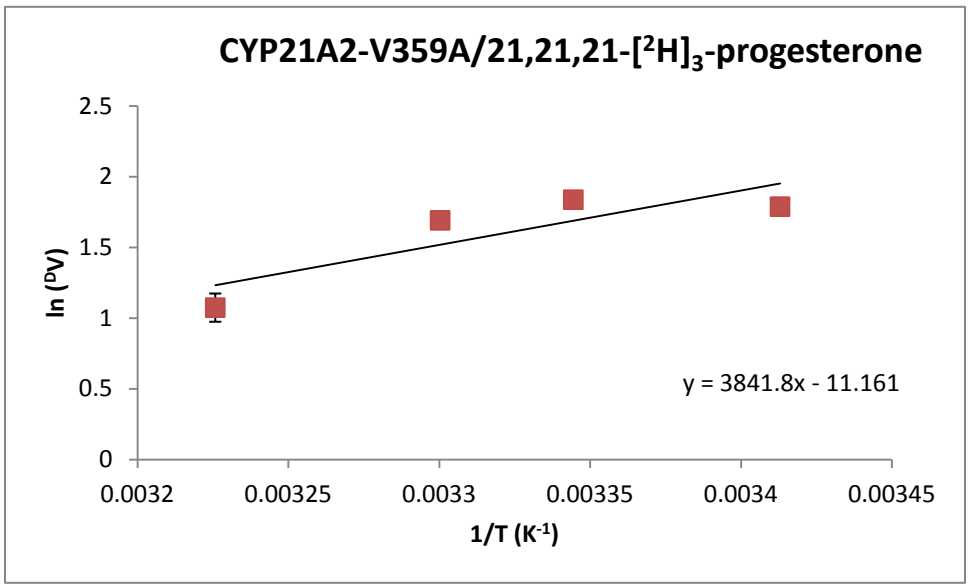
The following graphs are the Arrhenius plots obtained for the indicated enzyme/substrate system in this chapter:

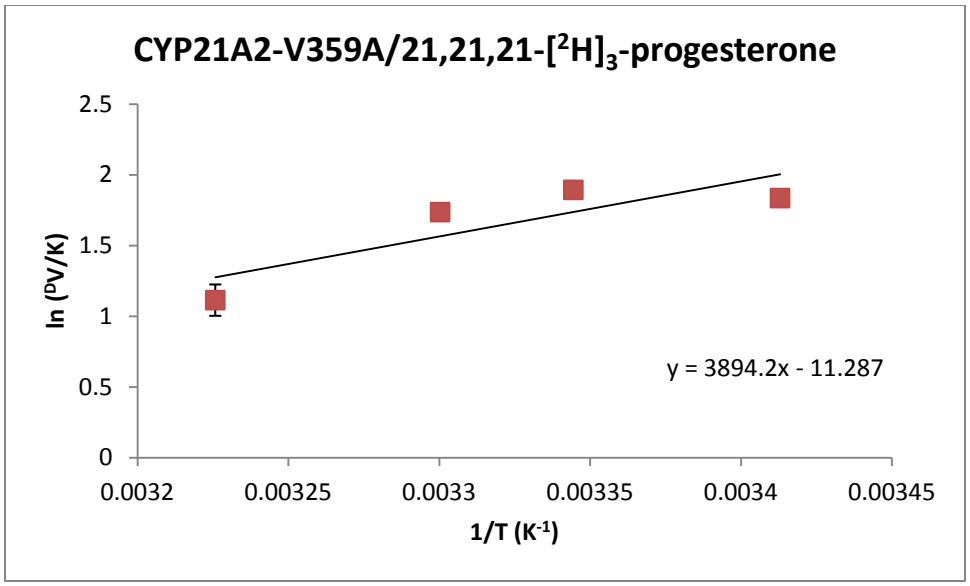


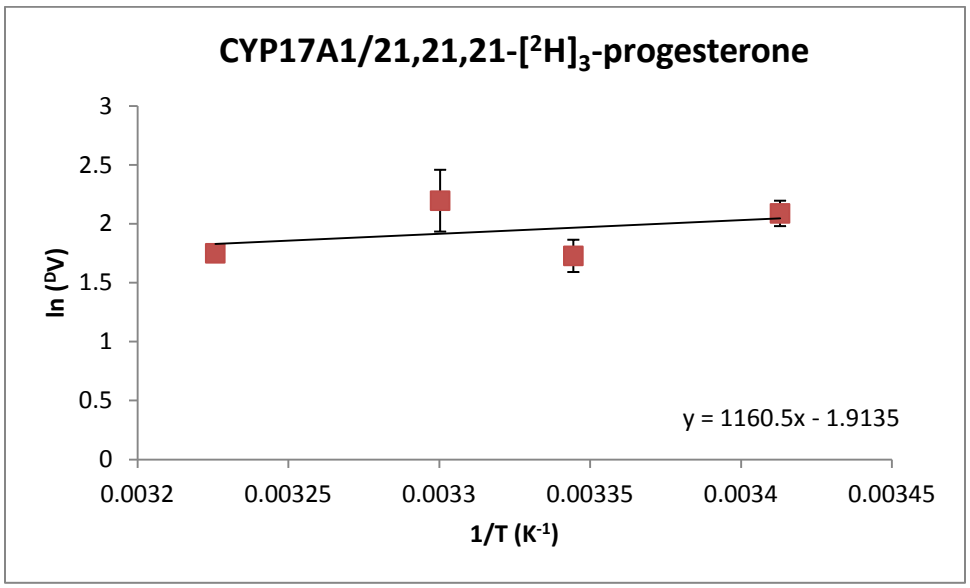


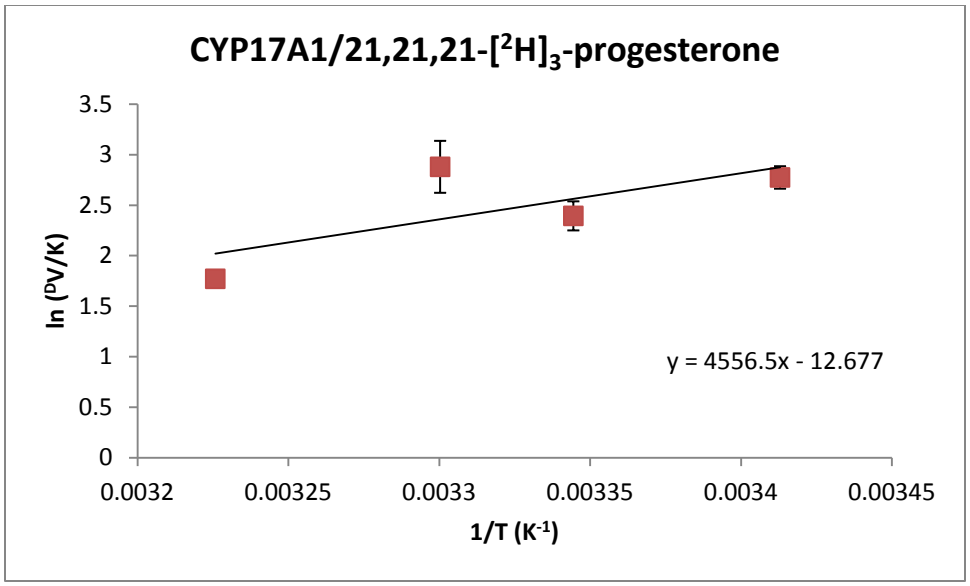

CYP21A2-V359A/16-[2H]-progesterone
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
ln (PV)
y = 3382.9x - 9.92
0.0032
0.00325
0.0033
0.00335
0.0034
0.00345
1/T (K-1)

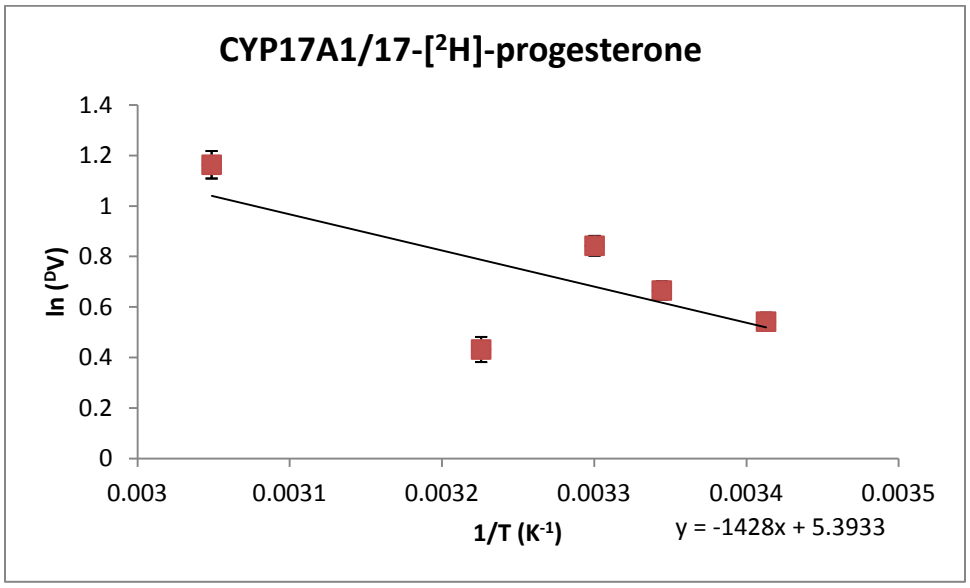

CYP21A2-V359A/16-[2H]-progesterone
2.5
2
1.5
1
0.5
0
ln (DV/K)
y = 3379.2x - 9.8323
0.0032
0.00325
0.0033
0.00335
0.0034
0.00345
1/T (K-1)

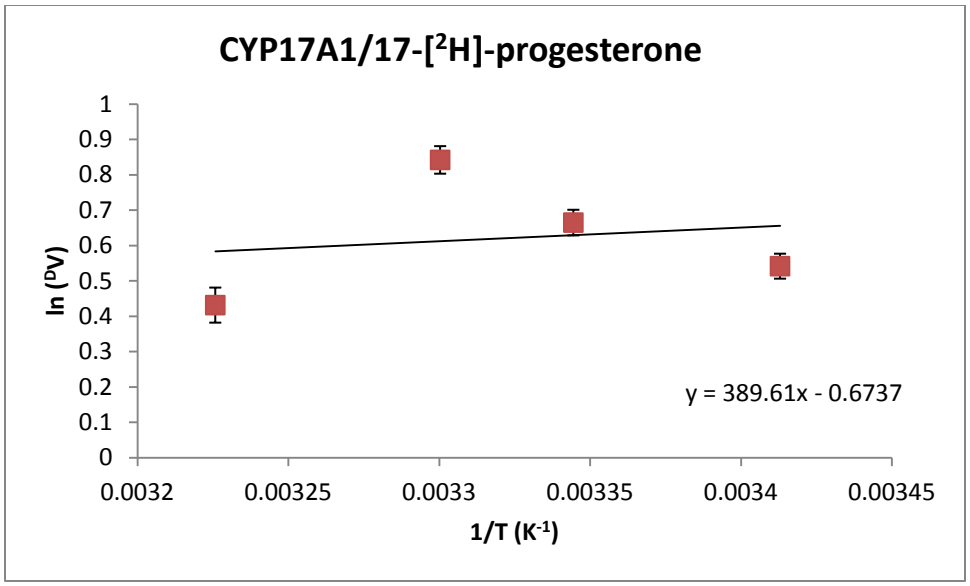

CYP21A2-V359A/21,21,21-[2H]3-progesterone
2.5
2
1.5
1
0.5
0
ln (DV)
y = 3841.8x - 11.161
0.0032
0.00325
0.0033
0.00335
0.0034
0.00345
1/T (K-1)

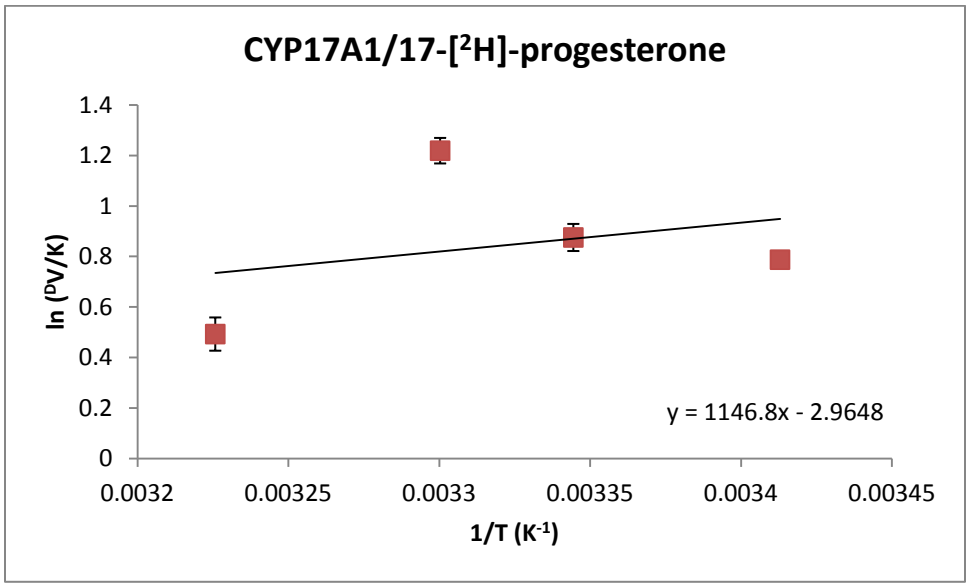

CYP21A2-V359A/21,21,21-[2H]3-progesterone
ln (DV/K)
2.5
2
1.5
1
0.5
0
y = 3894.2x - 11.287
0.0032
0.00325
0.0033
0.00335
0.0034
0.00345
1/T (K-1)

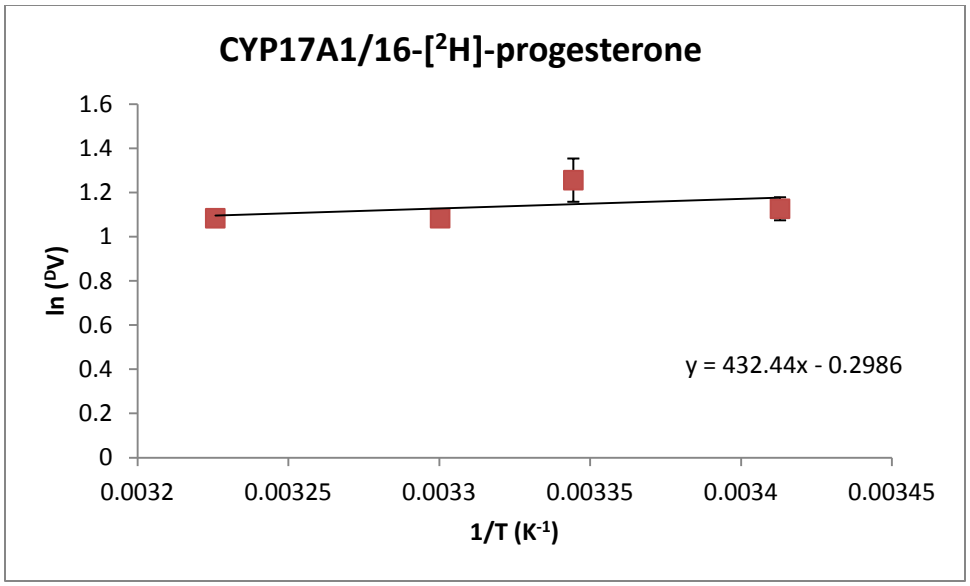

CYP17A1/21,21,21-[2H]3-progesterone
ln (DV)
3
2.5
2
1.5
1
0.5
0
y = 1160.5x - 1.9135
0.0032
0.00325
0.0033
0.00335
0.0034
0.00345
1/T (K-1)

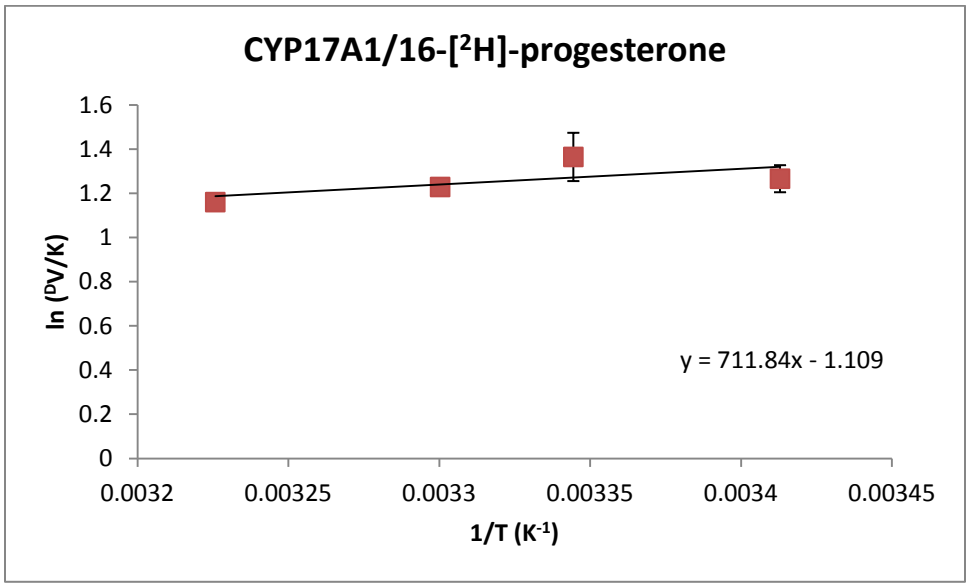

CYP17A1/21,21,21-[2H]3-progesterone
ln (PV/K)
3.5
3
2.5
2
1.5
1
0.5
0
0.0032
0.00325
0.0033
0.00335
0.0034
0.00345
1/T (K-1)
y = 4556.5x - 12.677

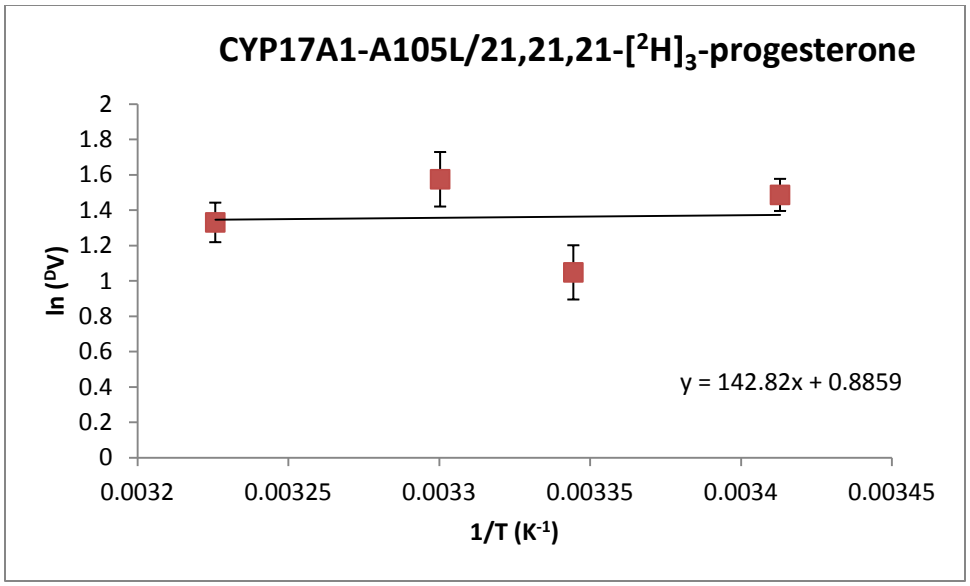

CYP17A1/17-[2H]-progesterone
ln (PV)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.003
0.0031
0.0032
0.0033
0.0034
0.0035
1/T (K-1)
y = -1428x + 5.3933

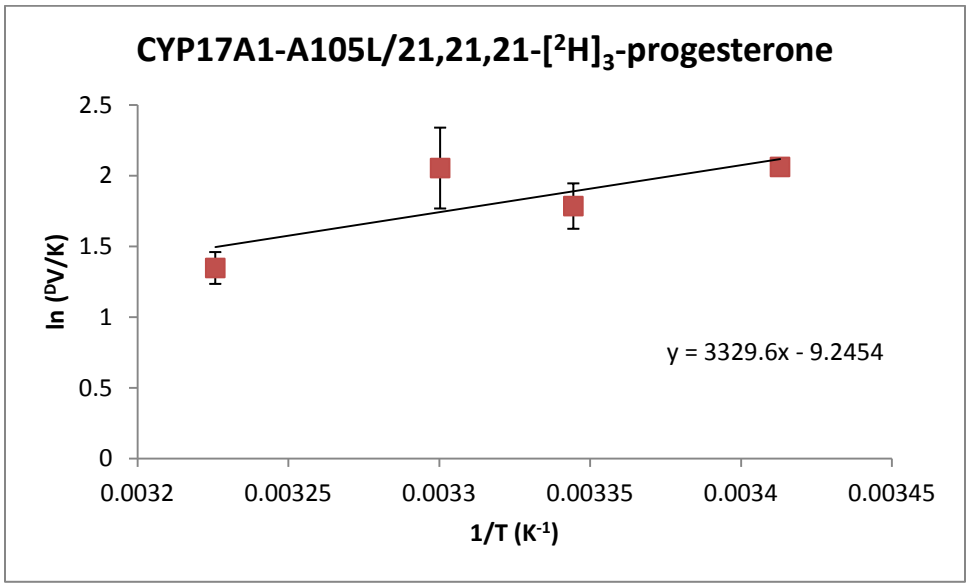

CYP17A1/17-[2H]-progesterone
ln (DV)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
y = 389.61x - 0.6737
0.0032
0.00325
0.0033
0.00335
0.0034
0.00345
1/T (K-1)

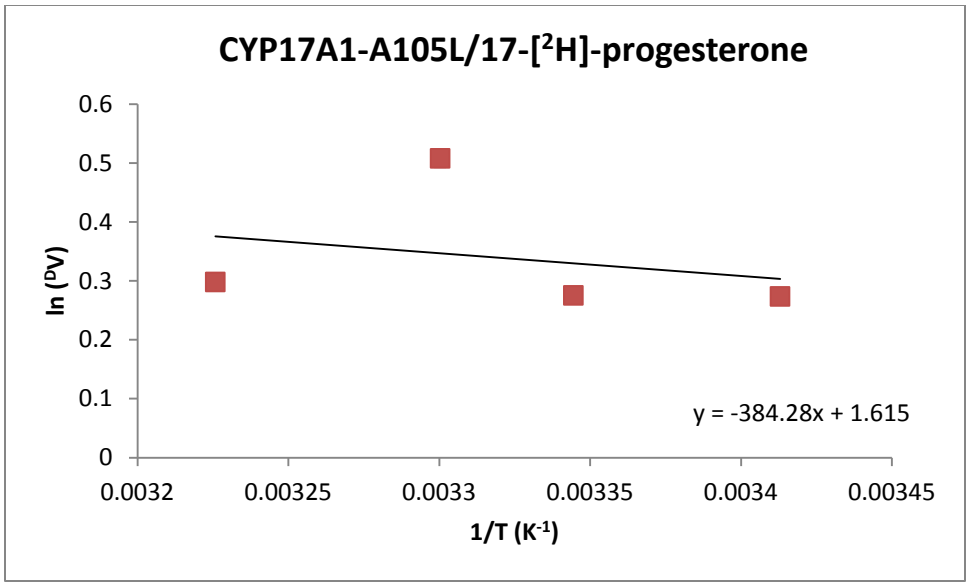

CYP17A1/17-[2H]-progesterone
ln (DV/K)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
y = 1146.8x - 2.9648
0.0032
0.00325
0.0033
0.00335
0.0034
0.00345
1/T (K-1)

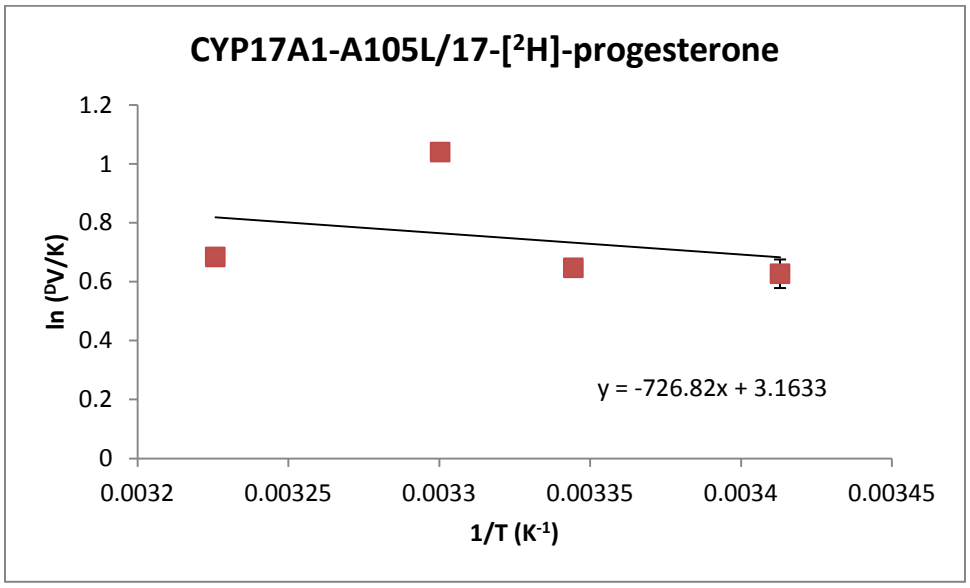

CYP17A1/16-[2H]-progesterone
ln (DV)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.0032
0.00325
0.0033
0.00335
0.0034
0.00345
1/T (K-1)
y = 432.44x - 0.2986

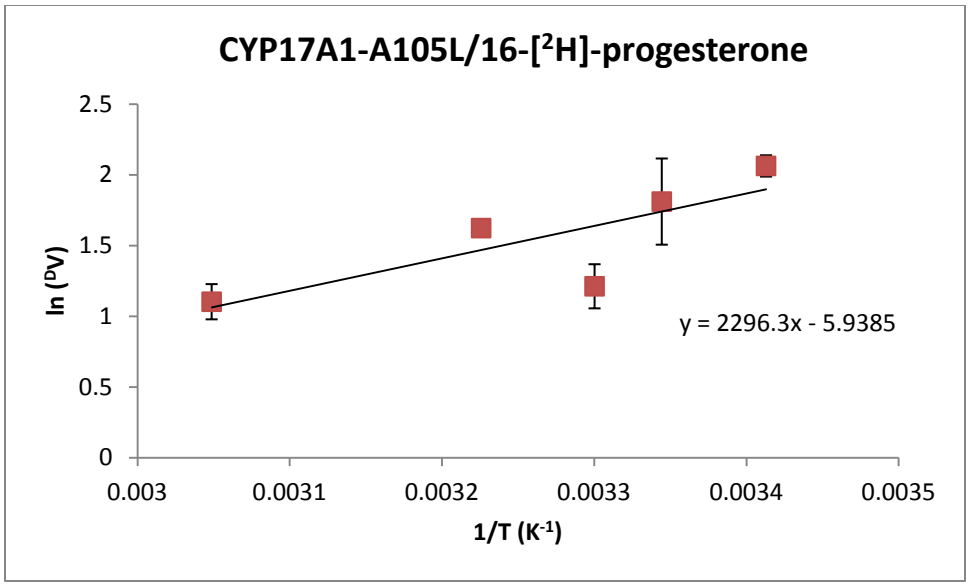

CYP17A1/16-[2H]-progesterone
ln (DV/K)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.0032
0.00325
0.0033
0.00335
0.0034
0.00345
1/T (K-1)
y = 711.84x - 1.109

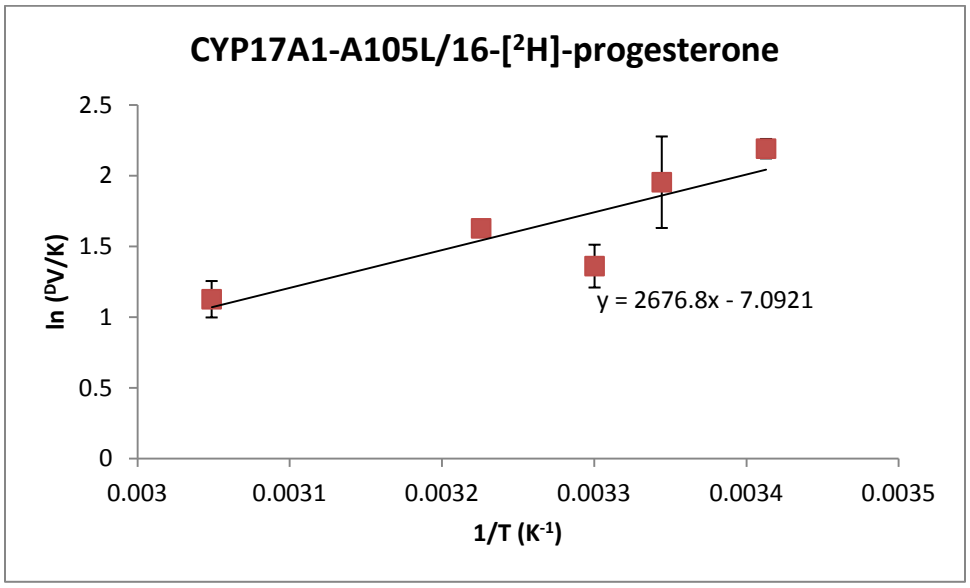

CYP17A1-A105L/21,21,21-[2H]3-progesterone
ln (PV)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
y = 142.82x + 0.8859
0.0032
0.00325
0.0033
0.00335
0.0034
0.00345
1/T (K-1)

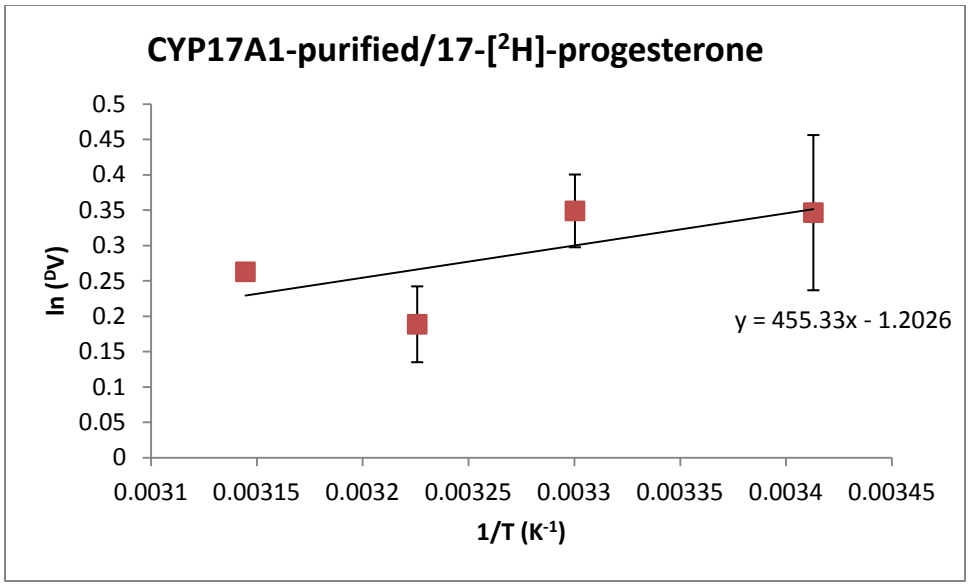

CYP17A1-A105L/21,21,21-[2H]3-progesterone
ln (PV/K)
2.5
2
1.5
1
0.5
0
y = 3329.6x - 9.2454
0.0032
0.00325
0.0033
0.00335
0.0034
0.00345
1/T (K-1)

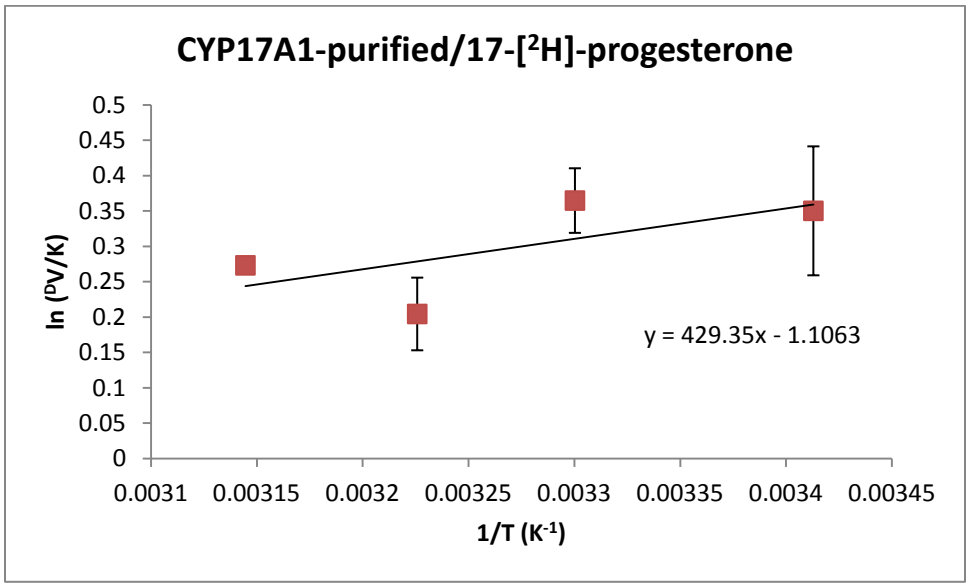

CYP17A1-A105L/17-[2H]-progesterone
ln (DV)
0.6
0.5
0.4
0.3
0.2
0.1
0
y = -384.28x + 1.615
0.0032
0.00325
0.0033
0.00335
0.0034
0.00345
1/T (K-1)

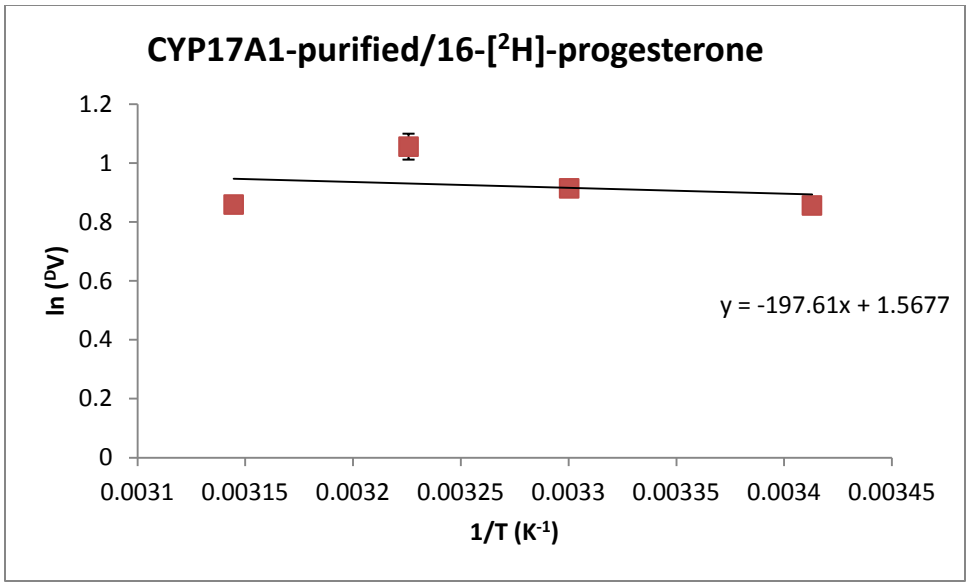

CYP17A1-A105L/17-[2H]-progesterone
ln (DV/K)
1.2
1
0.8
0.6
0.4
0.2
0
y = -726.82x + 3.1633
0.0032
0.00325
0.0033
0.00335
0.0034
0.00345
1/T (K-1)

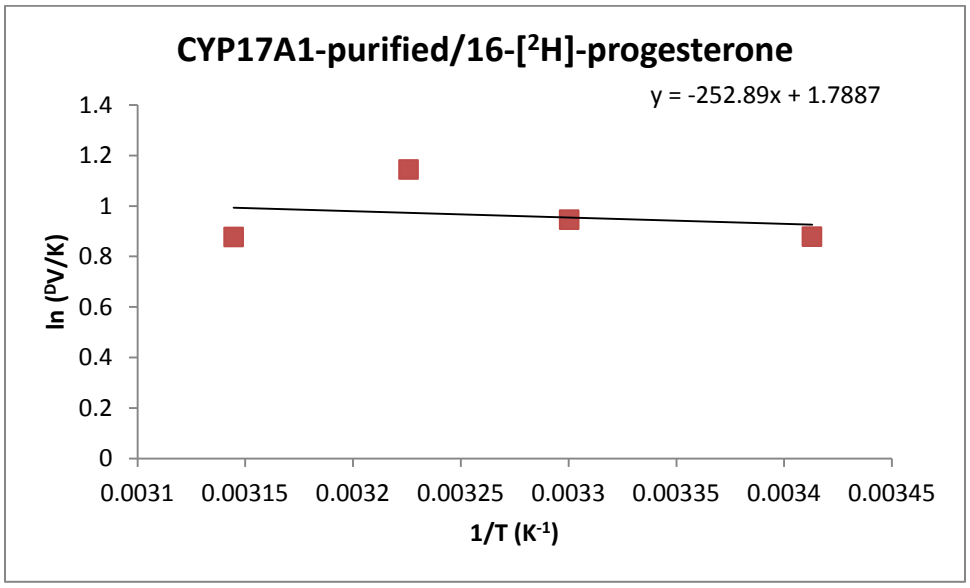

CYP17A1-A105L/16-[2H]-progesterone
2.5
2
1.5
1
0.5
0
ln (DV)
y = 2296.3x - 5.9385
0.003
0.0031
0.0032
0.0033
0.0034
0.0035
1/T (K-1)

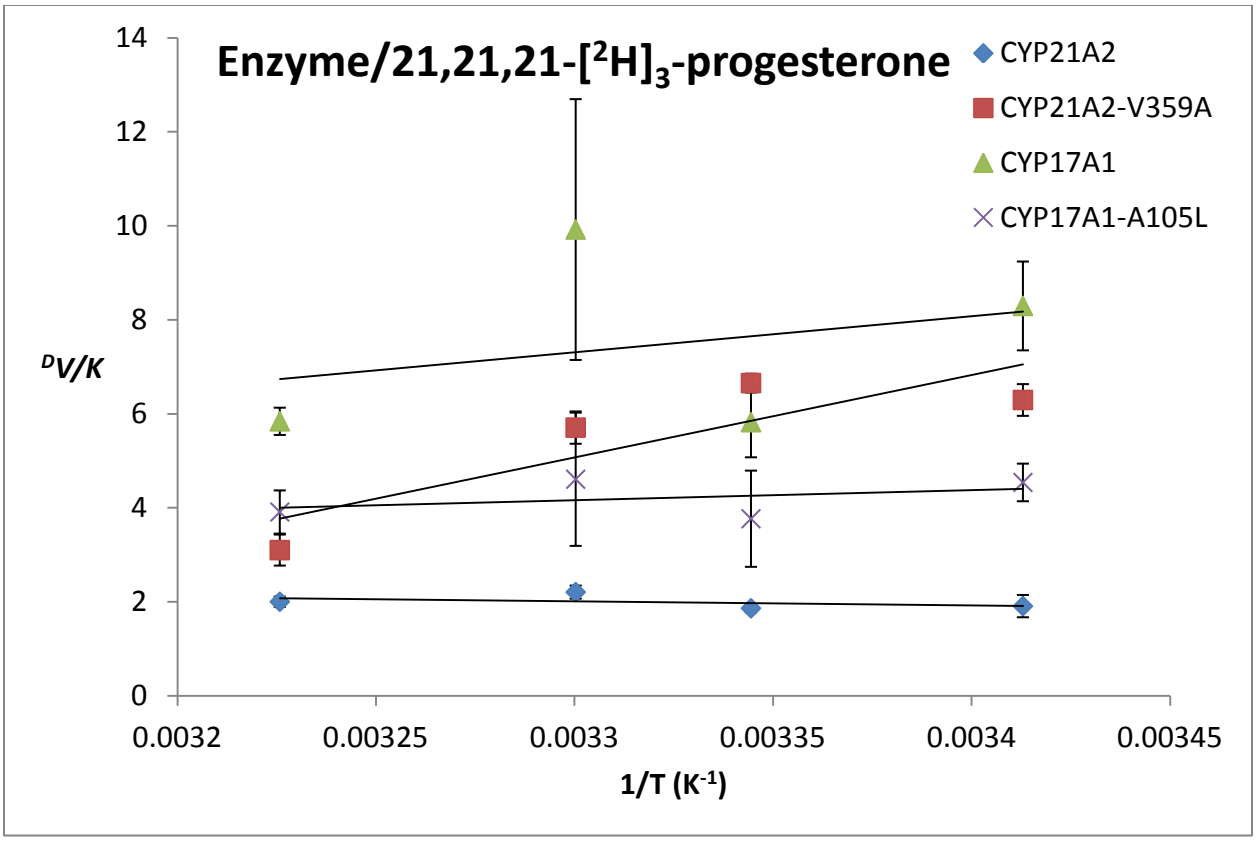

CYP17A1-A105L/16-[2H]-progesterone
2.5
2
1.5
1
0.5
0
ln (DV/K)
y = 2676.8x - 7.0921
0.003
0.0031
0.0032
0.0033
0.0034
0.0035
1/T (K-1)

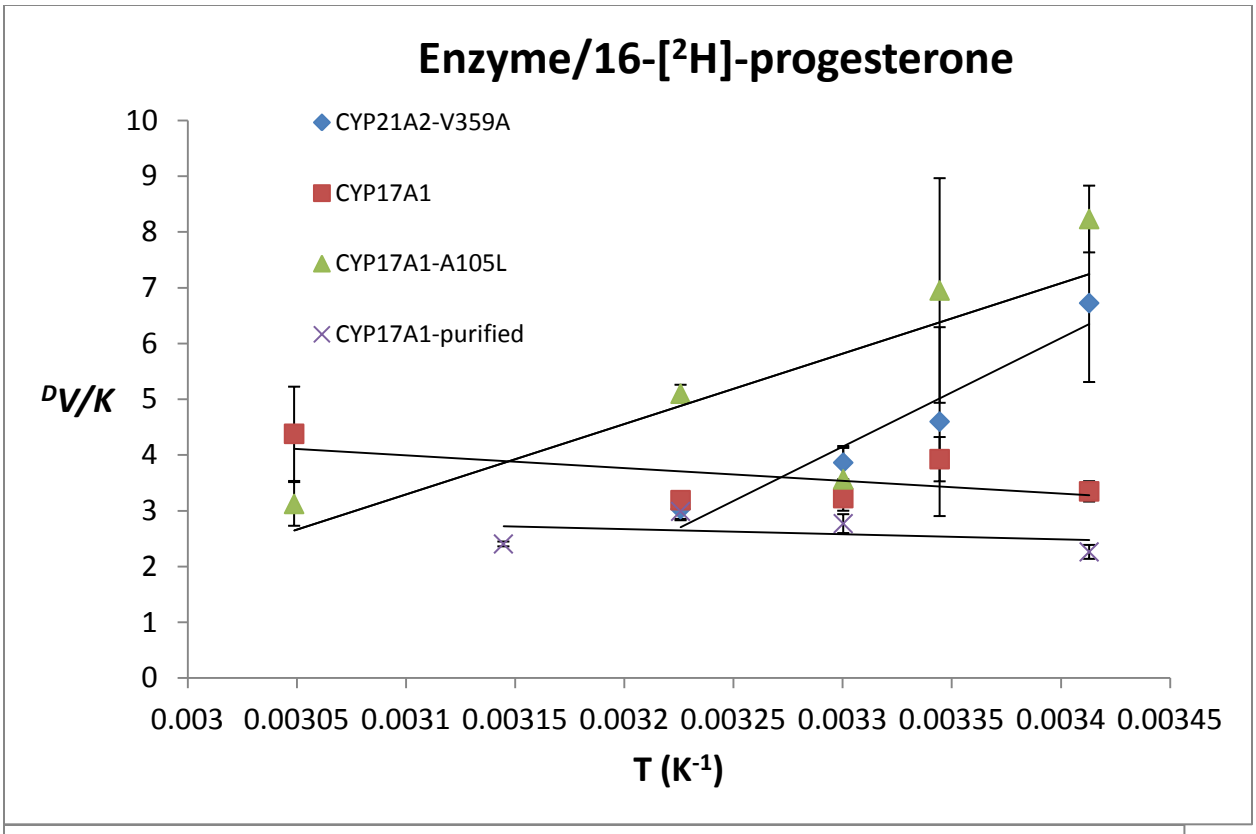

CYP17A1-purified/17-[2H]-progesterone
ln (DV)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.0031
0.00315
0.0032
0.00325
0.0033
0.00335
0.0034
0.00345
1/T (K-1)
y = 455.33x - 1.2026

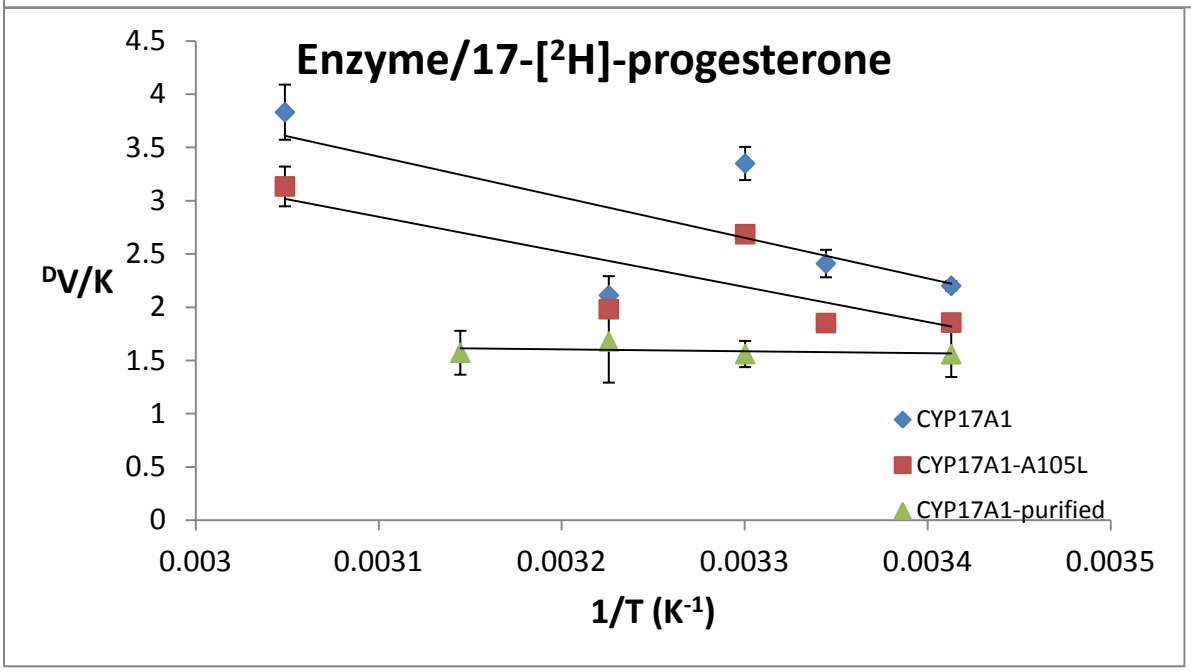

CYP17A1-purified/17-[2H]-progesterone
ln (DV/K)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.0031
0.00315
0.0032
0.00325
0.0033
0.00335
0.0034
0.00345
1/T (K-1)
y = 429.35x - 1.1063


CYP17A1-purified/16-[2H]-progesterone
y = -197.61x + 1.5677
ln (DV)
1/T (K-1)
1.2
1
0.8
0.6
0.4
0.2
0
0.0031
0.00315
0.0032
0.00325
0.0033
0.00335
0.0034
0.00345


CYP17A1-purified/16-[2H]-progesterone
y = -252.89x + 1.7887
ln (DV/K)
1/T (K-1)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.0031
0.00315
0.0032
0.00325
0.0033
0.00335
0.0034
0.00345


Enzyme/21,21,21-[2H]3-progesterone
CYP21A2
CYP21A2-V359A
CYP17A1
CYP17A1-A105L
DV/K
14
12
10
8
6
4
2
0
0.0032
0.00325
0.0033
0.00335
0.0034
0.00345
1/T (K-1)


Enzyme/16-[2H]-progesterone
CYP21A2-V359A
CYP17A1
CYP17A1-A105L
CYP17A1-purified
DV/K
T (K-1)
0
1
2
3
4
5
6
7
8
9
10
0.003
0.00305
0.0031
0.00315
0.0032
0.00325
0.0033
0.00335
0.0034
0.00345


Enzyme/17-[2H]-progesterone
DV/K
1/T (K-1)
CYP17A1
CYP17A1-A105L
CYP17A1-purified
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
0.003
0.0031
0.0032
0.0033
0.0034
0.0035

5.5. TRITIUM AS A TUNNELING PROBE

We also measured the isotope effect of our tritiated substrate, 17-[^{3}H]-pregnenolone, for CYP17A1. Because the tritium incorporation is trace in our synthesized 17-[^{3}H]-pregnenolone substrate, we are actually running an intermolecular incubation between pregnenolone and 17-[^{3}H]-pregnenolone when we use this tritiated substrate. A better probe probably would have been to label both the 17-position and a different position (ie: the 7-position) on pregnenolone with tritiums in order to measure the amount of 17-hydroxy-product directly. The 7,17-[$^3H_2$]-pregnenolone substrate could be synthesized from the 7,17-dibromopregnenolone precursor followed by reduction with zinc in tritiated acetic acid.

Although the 16-hydroxypregnenolone product is minor in the natural abundance case (~200:1, 17OH:16OH ratio at room temperature), we opted to compare the intramolecular kinetic isotope effects of 17-[^{3}H]-pregnenolone, 17-[^{2}H]-pregnenolone and pregnenolone with CYP17A1 because the 16-hydroxy product becomes more apparent with the deuterated case (ie: ~50:1, 17OH:16OH ratio at room temperature) and even more so in the tritiated case (ie: ~13:1, 17OH:16OH ratio at room temperature).

The calculation of the ratios of the 16- to the 17-hydroxy products were straightforward for both the 17-[^{2}H]-pregnenolone and pregnenolone substrates because both products were detected in the UV-chromatogram. The challenge in

measuring the ratio of the 16- to the 17-hydroxy products using the 17-[^{3}H]-pregnenolone substrate was the detection of the 17-hydroxyproduct. The 17-hydroxy product was measured by comparing the ratio of the area measured under the β-RAM to the area measured by the UV-detector for the non-reacted material:

$$(\beta_1)/(UV_1) = (\beta_{prog2}+\beta_{17OHprog2}+\beta_{16OHprog2})/(UV_2) \qquad (5)$$

$$\beta_{17OHprog2} = [(UV_2)*(\beta_1/UV_1)]-\beta_{prog2}-\beta_{16OHprog2} \qquad (6)$$

The equation (5) above assumes that there is no radioactivity lost from the 17-[^{3}H]-pregnenolone substrate besides from the direct removal of tritium from the 17-hydroxylase activity of the enzyme. Moreover, the equations above are the output of the cholesterol oxidase transformation of either the 17-[^{3}H]-pregnenolone substrate with no incubation with CYP17A1 (subscript of 1) or after incubation with CYP17A1 (subscript of 2). In the above equations (5) and (6): β_1 is the area under the progesterone peak detected by β-RAM of the control with no CYP17A1 incubation (ie: 17-[^{3}H]-pregnenolone subjected to cholesterol oxidase treatment), UV_1 is the area under the progesterone peak detected by UV-absorption of the control with no CYP17A1 incubation, β_{prog2} and $\beta_{16OHprog2}$ correspond to the area under the progesterone and 16-hydroxyprogesterone peaks detected by β-RAM after incubation with CYP17A1 and cholesterol oxidase, UV_2 is the sum of the area under the peaks (16-hydroxyprogesterone, 17-hydroxyprogesterone and progesterone) detected by UV-absorption after incubation with CYP17A1 and cholesterol oxidase. Finally, $\beta_{17OHprog2}$ is the

theoretical amount of 17-hydroxyprogesterone that arises from only the tritiated pregnenolone compound.

We also ran incubations with CYP17A1 where we used the 17-[^{3}H]-pregnenolone substrate in the presence of 17-[^{2}H]-pregnenolone substrate. The rate of 16-hydroxy product formed from the deuterated compound could be extrapolated by two steps: (1) compare the 16-hydroxyprogesterone:progesterone ratios detected by β-RAM in the incubation with just the 17-[^{3}H]-pregnenolone and in the incubation where both 17-[^{3}H]-pregnenolone and 17-[^{2}H]-pregnenolone were used and (2) subtract out the contribution of pregnenolone (natural abundance) in the peaks detected by UV-absorption where 17-[^{2}H]-pregnenolone was used (20, 26, and 30 °C temperature points). Alternatively, we could have run the incubation with just 17-[^{2}H]-pregnenolone and measure the intramolecular kinetic isotope effect directly without having to subtract out the contribution from 17-[^{3}H]-pregnenolone (37 °C temperature point).

In the Arrhenius plots from 17-[^{2}H]-pregnenolone or 17-[^{3}H]-pregnenolone incubated with CYP17A1, we obtained a negative slope (Figure 5.2). **This negative slope is not what we would expect** as mentioned in section 5.3. The explanation for a negative slope is that there are two sites of hydroxylase activity: the 17- and 16-positions are hydroxylated by the enzyme. The way we obtained the intramolecular kinetic isotope effect, k_H/k_D, was from the ratio of hydroxylation products comparing the non-deuterated to deuterated cases:

$[17OH:16OH]_H/[17OH:16OH]_D$ where the subscript "H" denotes the non-deuterated substrate (pregnenolone) and the subscript "D" denotes the deuterated substrate (17-[^{2}H]-pregnenolone). To clarify the situation we can look at the ratio of the 17-hydroxy product to the 16-hydroxy product at 20 °C and 37 °C for both substrates. At 20 °C the ratio of 17-hydroxy product to 16-hydroxy product for pregnenolone was ~200:1 and at 37 °C, this ratio stayed the same. However, in the case of 17-[^{2}H]-pregnenolone as the substrate, at 20 °C the ratio was ~45:1 and at 37 °C the ratio was ~25:1, and this decrease in ratio at increased temperature (45:1 to 25:1) led to a higher calculated k_H/k_D value at higher temperature because the ratio of $[17OH:16OH]_H/[17OH:16OH]_D$ goes from 200/45 at 20 °C to 200/25 at 37 °C.

The Swain-Schaad relationship [188] relates the reduced masses of the three isotopes (μ_H, μ_D, μ_T) [185] to an exponent value:

$$\ln(k_H/k_T)/\ln(k_D/k_T) = EXP = \{(\mu_H)^{-1/2}-(\mu_T)^{-1/2}\} \div \{(\mu_D)^{-1/2}-(\mu_T)^{-1/2}\} \quad (7)$$

$$\mu = (m_A m_B)/(m_A+m_B) \quad (8)$$

The reduced mass is given by the two product of the two masses, m_A and m_B, divided by the sum of the two masses, m_A and m_B where m_A is the mass of carbon and m_B is the mass of either the hydrogen, deuterium or tritium atom. The exponent value of 3.34 is the upper semiclassical limit for tunneling ($\ln(k_H/k_T)=\ln(k_D/k_T)^{exponent}$); if an experimental value greater than 3.34 is obtained, then that is evidence for tunneling. As shown in Table 5.2, the exponents

calculated for pregnenolone and CYP17A1 were within the 3.34 upper limit (calculated exponents: 1.51-2.81). Interestingly, as the temperature increased, the exponent decreased. However, there seems to be an optimum temperature for the exponent to reach the maximum value at 2.81 when the temperature is 26 °C (Table 5.2).

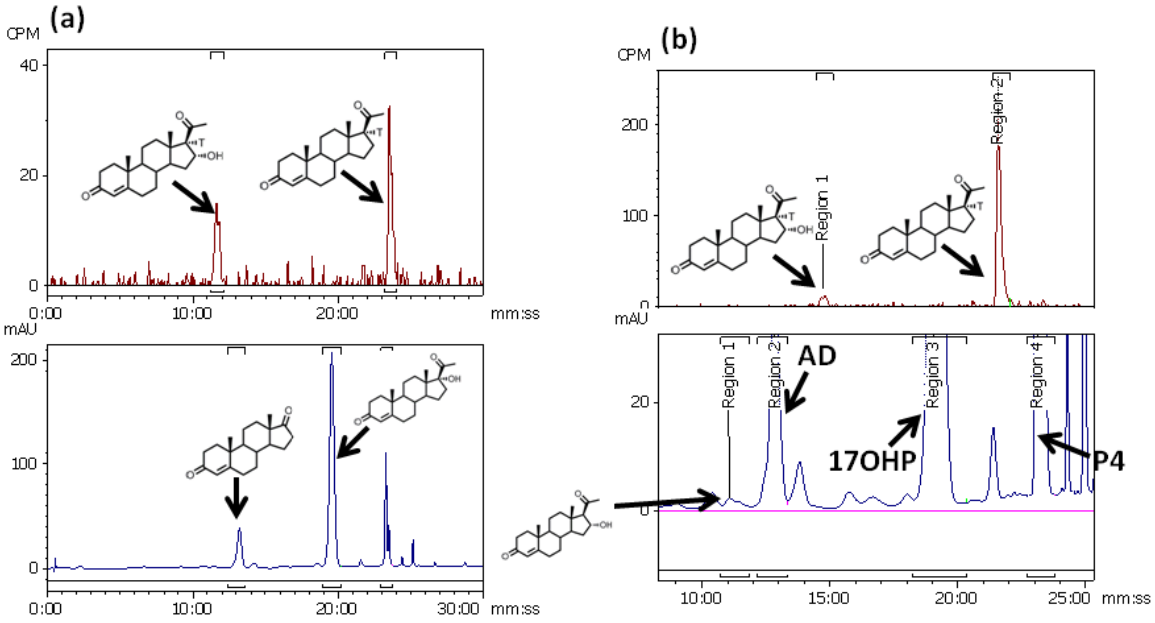


Figure 5.1. Chromatograms of CYP17A1 incubation with 17-[^{3}H]-pregnenolone: (a) and (b) (2 separate incubations (a) and (b)). 16-Hydroxy product is detectable on UV absorption (254 nm) when the chromatogram is zoomed in (b).

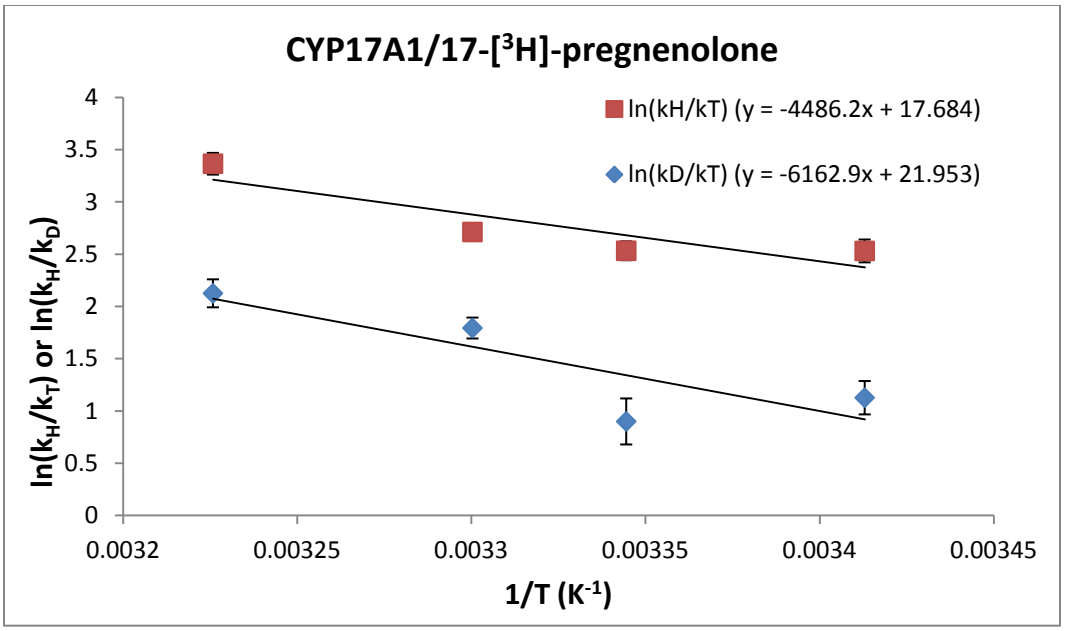


Figure 5.2. Arrhenius plots of CYP17A1 and 17-[^{3}H]-pregnenolone and 17-[^{2}H]-pregnenolone and pregnenolone.

$T(K^{-1})$	$\ln(k_H/k_T)$	$\ln(k_D/k_T)$	$\ln(k_H/k_T)/\ln(k_D/k_T)$
293	2.53	1.13	2.25
299	2.53	0.90	2.81
303	2.71	1.79	1.51
310	3.37	2.13	1.58

Table 5.2. Swain-Schaad relationship calculated for CYP17A1 and 17-[^{3}H]-pregnenolone and 17-[^{2}H]-pregnenolone and pregnenolone.

5.6. CONCLUSION

In conclusion, by varying the temperature range of the incubations and using regioselectively tritiated and deuterated substrates, we were able to determine any tunneling contributions in the C-H abstraction step. The efficiency of the enzyme (ie: how much the enzyme tunnels) depends on the isotope (tritium vs. deuterium vs. hydrogen) and the reaction conditions (ie: temperature, intermolecular, intramolecular KIE, location of the isotope label, enzyme mutant, etc.).

CHAPTER SIX

Conclusions and Future Directions

6.1. SUMMARY

The research opportunities when combining chemical synthesis and enzymology seem limitless. What we have done by synthesizing potential analogs and showing that they are substrates for CYP17A1 and CYP21A2 have shown the steric and electronic flexibilities in the active site of the enzymes.

The analogs have also revealed novel reactivities of these two enzymes, which help us improve our understanding of the dynamic behavior of these proteins, and in turn help us design better inhibitors. For example, knowing that these enzymes metabolize one-carbon homologated progesterone analogs (ie: 21-homo-21,22-dehydroprogesterone and 21-homomethylprogesterone) informs us that a potential ligand with an extended alkyl chain on the C21 position of progesterone can be designed. In addition, it would be worth incubating CYP17A1 with 21-homo-21,22-dehydroprogesterone under anaerobic conditions so that we can confirm the identity of the product, 21-homomethylprogesterone and it mechanism of reduction.

The radical clock studies are worth pursuing especially since we found that 20-cyclopropyl-20-methylprogesterone is a substrate for CYP17A1. This task requires the synthesis of the potential product standards to match the identity of the products from the incubation. The compound 20-cyclopropyl-20-methyl-

17-hydroxyprogesterone, which might be the product of CYP17A1 metabolism of 20-cyclopropyl-20-methyl-progesterone, might be a radical clock substrate for CYP21A2, because 17-hydroxylated steroids are betters substrates for CYP21A2 than the 17-deoxysteroids.

6.2. A MODIFIED RADICAL CLOCK SUBSTRATE

We can also design better radical clock substrates, which might be faster-rearranging probes. For example, we have considered the substrate where the cyclopropyl ring is fused in the D-ring of progesterone. The 3,4-bicyclic ring system is likely to rearrange more rapidly than the 20-cyclopropyl-20-methyl substrate, due to a larger ring strain in the bicyclic case (Figure 6.1).

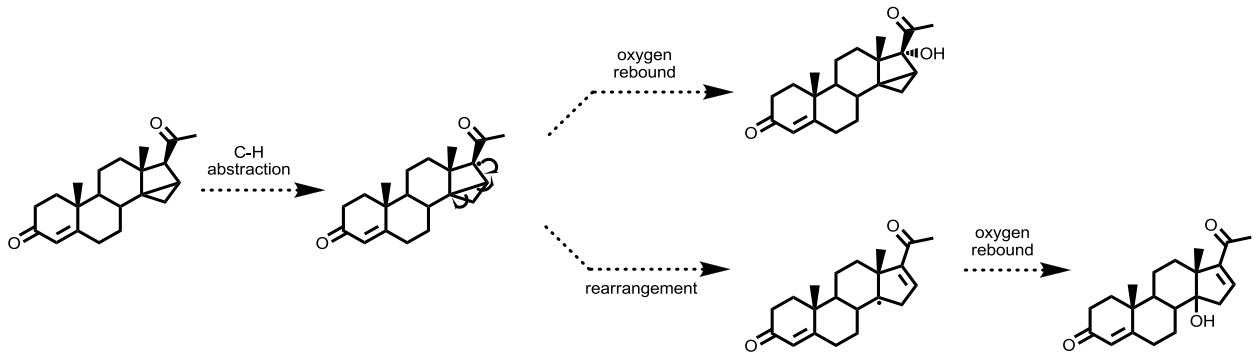


Figure 6.1. A possible radical clock substrate probe for CYP17A1 with a fused 3,4-bicycle.

This 3,4-bicyclic compound can be accessed from a [2+2] photocycloaddition from the seco-D-steroid diene precursor (Figure 6.2). The [2+2] reaction was based on literature precedence where an intramolecular [2+2] photocycloaddition occurred between an allene and an enone moiety to form the bicyclo[2.1.0]pentane [189]. If this reaction would not work there are other approaches which can be considered to form this 3,4-bicyclic ring system such as the cyclopropanation of a cyclobutene. Alternatively, instead of the enone in the [2+2] reaction we can use a ketene precursor, which can form the 3,4-bicyclic ring as the cyclobutanone followed by a Wittig olefination to introduce the extended carbonyl unit.

Not only would achieving this target provide for a suitable radical clock substrate for CYP17A1, but we would also be exploring novel synthetic chemistry.

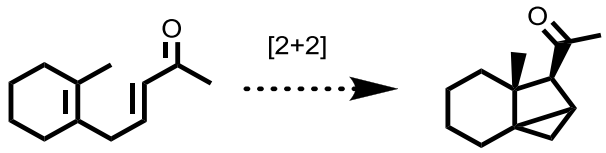


Figure 6.2. The fused 3,4-bicyclic compound can likely be accessed through a [2+2] cycloaddition of the diene precursor.

6.3. SECONDARY KINETIC ISOTOPE EFFECT

Since there are three deuteriums on the 21,21,21-[$^2H_3$]-progesterone substrate, it is worth synthesizing and incubating 21,21-[$^2H_2$]-progesterone and 21-[^2H]-progesterone with CYP21A2 to interrogate for a secondary kinetic isotope effect, which arises when deuteriums not involved directly in the C-D cleavage affect on the rate of hydroxylation. We can access 21-[^2H]-progesterone by subjecting 21-monobromoprogesterone to Zn dust in deuterated acetic acid. In order to differentiate the hydrogen vs. deuterium abstraction for these particular incubations, we will have to characterize the products using LC-MS/MS as opposed to the UV/Vis method. The mass transitions detected for the 21-[^2H]-progesterone substrate will correspond to: 21-hydroxy-21-[^2H]-progesterone and 21-hydroxyprogesterone. For the 21,21-[$^2H_2$]-progesterone substrate, the mass transitions detected will correspond to: 21-hydroxy-21-[^2H]-progesterone and 21-hydroxy-21,21-[$^2H_2$]-progesterone. It is worth noting that one of the 21-hydroxylated products in both cases leads to a new chiral carbon center on 21-position due to the prochiral 21-carbon in the starting material (Figure 6.3). The determination of the diastereomeric excess might require the stereoselective synthesis of a single C-21 epimer (21-hydroxy-21-[^2H]-progesterone) and comparing the NMR of the enzymatic product with the synthesized product. The 21-hydroxylated product has a potential hydrogen bond between the 21-hydroxy proton and the 20-keto oxygen, which might restrict rotation and differentiate the

chemical environment of the two C-21 epimers. Alternatively, the use of lanthanide chemical shift reagents such as $Eu(FOD)_3$ or the synthesis of esters with one isomer of mandelic acid or other chiral acid might be necessary to resolve the diastereotopic protons in the NMR spectrum.

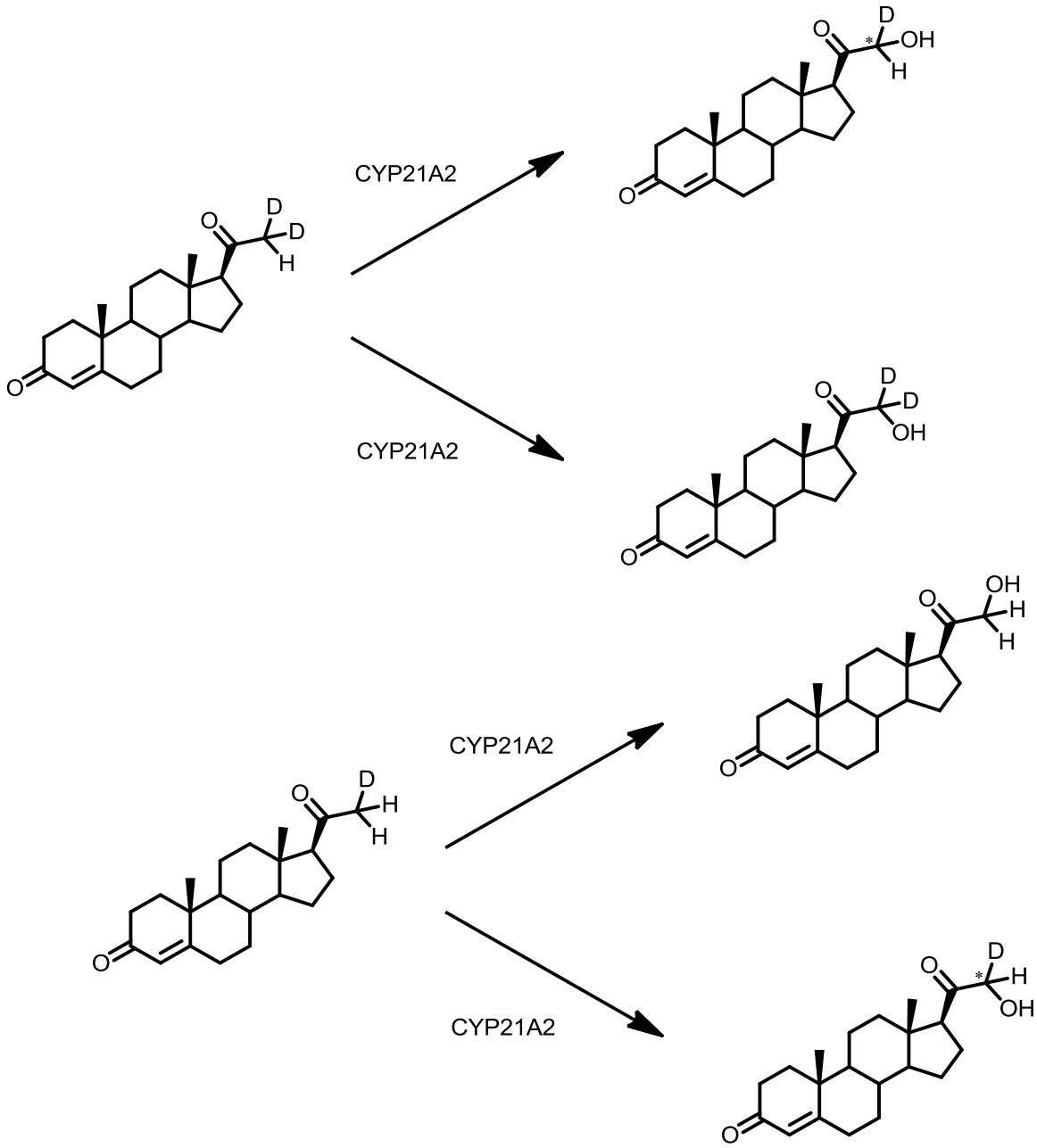


Figure 6.3. 21,21-[$^2H_2$]-progesterone (top) and 21-[^2H]-progesterone (bottom) are potential kinetic isotope effect probes for CYP21A2.

6.4. TEMPERATURE DEPENDENCE ON INTRAMOLECULAR KIEs

Moreover, we have begun to determine the temperature dependence of the isotope effect using steroids labeled with [^{2}H] and [^{3}H] at the 17-position for CYP17A1 and the 21-position for CYP21A2. Our data includes the intermolecular kinetic isotope effects to calculate the $\Delta Ea_{(D-H)}$ and the Arrhenius prefactor ratio, A_H/A_D; however, it would be interesting to extend the study by using intramolecular kinetic isotope effects. Of particular interest would be to use the mass spectral data we can obtain using our deuterated probes (21-[^{2}H]-progesterone for CYP21A2 and 16α-[^{2}H]-progesterone for CYP17A1).

Since the kinetics of reactions involving hydrogen atom tunneling are very sensitive to distance and minimally sensitive to temperature, a small slope for the Arrhenius plot is strong evidence for hydrogen atom tunneling. Performing the incubations at lower temperatures will require purified proteins, which allows the use of more enzyme to accurately measure low turnover rates.

6.5. SITE-DIRECTED MUTAGENESIS

Further site-directed mutagenesis studies can also be performed to potentially change the regiochemistry of the enzymes. We found that CYP17A1 A105L mutation has significant 21-hydroxylase activity. Therefore, it would be informative to construct the CYP17A1-A105F mutation and to determine if the bulkier phenylalanine side chain further increases 21-hydroxylase activity.

Conversely, the less bulky CYP17A1-A105G mutation might lead to an increase in 16α-hydroxylase activity relative to 17-hydroxylation.

6.6. CYTOCHROME B5 RELATIONSHIP WITH CYP17A1 REACTIVITY

Interestingly, our approach with studying CYP17A1 enzymology has involved the P450 with POR but without the b5 protein interaction, since enzyme incubations were conducted without adding b5 protein. Therefore, we can extend our structure-activity relationships (SAR) studies by running the incubations with our analogs in the presence of the b5 protein. Although b5 stimulates the steady-state rate of the 17,20-lyase reaction but not the 16α- or 17-hydroxylase reactions, b5 might influence the product distribution or KIE values via allosteric mechanisms.

6.7. OTHER PROGESTERONE ANALOGS

Other progesterone analogs worth pursuing include 17-alkylprogesterone analogs. We know that a hydroxyl moiety and a fluoride group on the 17α-position are both tolerated by CYP21A2. It would be interesting to see if a less electronegative atom such as carbon (as in 17α-methylprogesterone) would be hydroxylated by CYP21A2.

6.8. LINEAR FREE ENERGY RELATIONSHIPS

Interestingly we did notice some metabolism of 16α-hydroxyprogesterone by CYP21A2, probably to furnish 16α,21-dihydroxyprogesterone. Perhaps other substituents on the 16-positions would be suitable substrates for CYP21A2. Some common synthetic steroids such as dexamethasone contain 16α-methyl substituents, increasing the relevance of this work. When equipped with the incubation data of many different progesterone analogs, we can plot a graph relating the electronic free energy relationships with the steric and electronic effects in order to predict the reactivity of these enzymes [190].

The goal of the LFER study is to see if it is possible to determine the mechanism of these enzymes using analogs [104, 191]. We already know that some analogs (ie: 21-methylprogesterone analog) are metabolized by these enzymes just as well as the natural substrate progesterone. If we plot log Vmax (y-axis) vs. the polar substituent constants σ (x-axis) then we can determine the electronic features of the transition state. Obtaining a positive slope would mean that the transition state is stabilized by electron withdrawing groups and hence the transition state is negatively charged. A negative slope would be the opposite situation and electron donating groups would stabilize the transition state and the transition state is positively charged.

6.9. DENSITY FUNCTIONAL THEORY CALCULATIONS

Moreover, density functional theory (using the program Gaussian) could be used to understand the transition state structures for the C-H abstraction step in each enzyme. For other examples of using DFT to calculate the transition state structure of an enzyme catalyzed reaction refer to cited references from reports by Schramm and co-workers [192-194]. Two characteristics in density functional theory are important: the basis set and the method. The basis set is the set of atomic orbital parameters, which the program (Gaussian03) uses to make a linear combination to represent molecular bonding orbitals. The selection of the basis set in using Gaussian03 is important in this enzyme-catalyzed reaction because of the transition metal (iron). The Pople basis set of 6-31G(d,p) is a popular choice; however, because iron is present, this basis set might complicate the simulations, ultimately causing Gaussian calculations to fail due to too many possibilities involved when locating the transition state. In preliminary experiments, the minima (ground state starting material and radical intermediate) were successfully located using just the 6-31G(d,p) basis set and the B3LYP method (Figure 6.4). We found it necessary to use the 6-31G(d,p) basis set to represent the non-iron atoms (such as carbon, nitrogen, hydrogen, sulfur and oxygen atoms) and a different basis set for the iron atom, LANL2DZ (Los Alamos National Laboratory 2-double Z), which averages the smaller atomic orbitals in larger orbitals to simplify calculations, known as an effective core potential. Knowledge of the

transition state structures can also help us in designing transition state inhibitors for these enzymes.

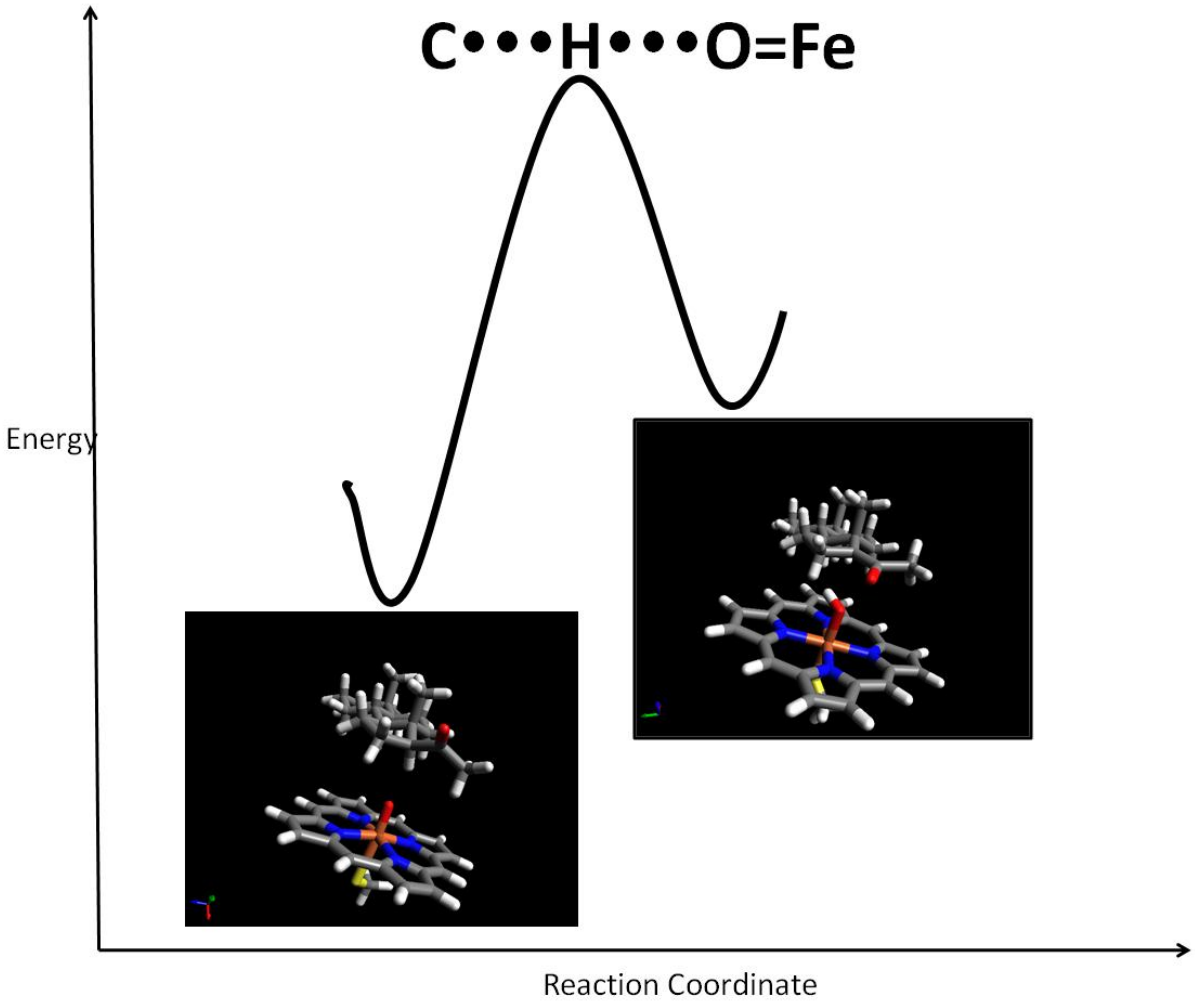


Figure 6.4. Gaussian03 calculated minima of C-H abstraction (ground state: iron oxene with progesterone, intermediate state: protonated iron oxene, Fe-OH, with C17-radical at progesterone). Geometries of minimized structures of progesterone and heme were obtained using Gaussian03 at the B3LYP/6-31G(d,p) level.

6.10. CONCLUSION

In conclusion, we can learn a lot about enzymes through SAR studies. CYP17A1 and CYP21A2 both have important roles in human health. Interestingly, selective CYP17A1 inhibition of the lyase activity is desired to treat prostate cancer; on the other hand, enhancement of 21-hydroxylase activity would be a desired treatment for congenital adrenal hyperplasia. X-ray crystallography and nuclear magnetic resonance yield important structural data; however, structural information does not allow us to completely predict the reactivity of these enzymes. Therefore, the knowledge gained through our research will allow us to improve our understanding these steroidogenic enzymes and allow us to design better inhibitors. In addition, the induction of latent activities, such as the 21-hydroxylase activity of CYP17A1, might be harnessed in the treatment of CAH due to 21-hydroxylase deficiency. Furthermore, this type of research in combining synthetic chemistry with enzymology to reveal novel enzymatic reactivity can be extended to other enzyme systems.

APPENDIX

Derivation of Competitive Kinetic Isotope Effect

$V_H/V_D = ([V_H/K_H][S_H])/([V_D/K_D][S_D])$ (1)

$[V/K][S] = d[S]/dt$ (2)

$[V/K]dt = d[S]/[S]$ (3)

$[V/K](t-0) = \ln[S]_t - \ln[S]_0$ (4)

$[V/K](t) = \ln[S]_t - \ln[S]_0$ (5)

$kH = [V_H/K_H]t = \ln[S_H]_t - \ln[S_H]_0$ (6)

$kD = [V_D/K_D]t = \ln[S_D]_t - \ln[S_D]_0$ (7)

$[S]_t = [S]_0(1-f)$ (8)

$[V_H/K_H]t = \ln([S_H]_0(1-f_H))/\ln[S_H]_0$ (9)

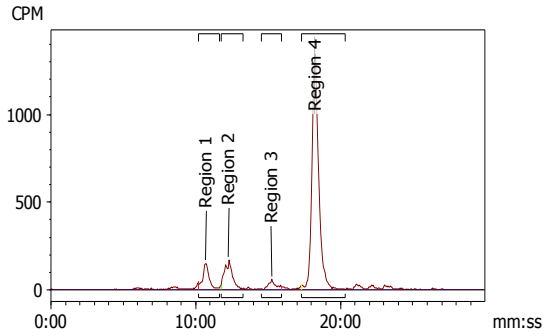
$k_H/k_D = \ln(1-f_H)/\ln(1-f_D)$ (10)

$^{D}V/K = [V_H/K_H]/[V_D/K_D]$

Enzyme and substrate	21OH product (n)	17OH product (n)	16OH product (n)
CYP17A1			
1,2,6,7-[$^{3}H_{4}$]-progesterone	3.37 ± 0.18% (3)	76.34 ± 0.26% (3)	20.28 ± 0.33% (3)
21,21,21-[$^{2}H_{3}$]-progesterone	0.61 ± 0.04% (3)	78.31 ± 0.35% (3)	21.08 ± 0.34% (3)
17-[^{2}H]-progesterone	4.45 ± 0.39% (3)	42.46 ± 0.86% (3)	53.09 ± 0.81% (3)
16α-[^{2}H]-progesterone	5.26 ± 0.57% (3)	86.52 ± 0.39% (3)	8.22 ± 0.42% (3)
CYP17A1 A105L			
1,2,6,7-[$^{3}H_{4}$]-progesterone	5.46 ± 0.32% (3)	87.45 ± 0.33% (3)	7.09 ± 0.38% (3)
21,21,21-[$^{2}H_{3}$]-progesterone	1.31 ± 0.26% (3)	92.59 ± 0.31% (3)	6.10 ± 0.05% (3)
17-[^{2}H]-progesterone	9.11 ± 0.56% (3)	64.90 ± 1.28% (3)	25.99 ± 0.75% (3)
16-[^{2}H]-progesterone	7.08 ± 0.36% (3)	91.33 ± 0.33% (3)	1.59 ± 0.05% (3)
CYP17A1 reconstituted			
1,2,6,7-[$^{3}H_{4}$]-progesterone	2.57 ± 0.09% (3)	84.84 ± 0.70% (3)	12.59 ± 0.66% (3)
17-[^{2}H]-progesterone*		73.06 ± 1.02% (3)	26.94 ± 1.02% (3)
16α-[^{2}H]-progesterone*		93.40 ± 0.14% (3)	6.60 ± 0.14% (3)
CYP21A2			
progesterone	99.57 ± 0.07% (3)	none	0.43 ± 0.07% (3)
21,21,21-[$^{2}H_{3}$]-progesterone	94.29 ± 1.88% (5)	none	5.71 ± 1.88% (5)
CYP21A2 V359A			
1,2,6,7-[$^{3}H_{4}$]-progesterone	51.21 ± 1.46% (3)	none	48.79 ± 1.46% (3)
21,21,21-[$^{2}H_{3}$]-progesterone	18.03 ± 1.94% (3)	none	81.97 ± 1.94% (3)
16α-[^{2}H]-progesterone	81.65 ± 0.38% (3)	none	18.35 ± 0.38% (3)

Table A.1: Illustrating the % hydroxylation on each position (21-, 17-, or 16-positions) based on the substrate and the enzyme used at 37 °C. *An impurity overlapped with 21-hydroxyprogesterone so the quantity of 21-hydroxyprogesterone could not be determined.

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:12	11:39	10:42	4662	8.16	7.26
Region 2	11:46	13:16	12:16	6480	11.35	10.09
Region 3	14:34	15:55	15:14	2054	3.60	3.20
Region 4	17:20	20:20	18:14	43914	76.89	68.36
4 Peaks				57110	100.00	88.90

Total Area: 64240 CPM
Average Background: 0 CPM

FYX-037 16D.prog(20uM).C17A105L.5uL.RT(22 C).6A1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 11:43:09 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:56:56 PM

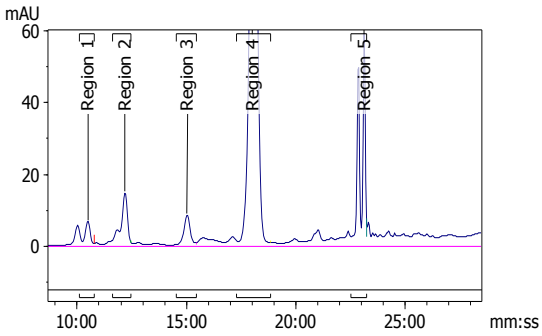
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 56
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:07	10:48	10:29	44	0.93	0.57
Region 2	11:36	12:27	12:11	250	5.27	3.22
Region 3	14:31	15:28	15:01	142	3.00	1.84
Region 4	17:16	18:51	18:02	3703	78.04	47.72
Region 5	22:32	23:13	23:07	605	12.76	7.80
5 Peaks				4744	100.00	61.14

Total Area: 7759 mAU
Average Background: N/A mAU

FYX-037 16D.prog(20uM).C17A105L.5uL.RT(22 C).6A1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 11:43:09 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:56:56 PM

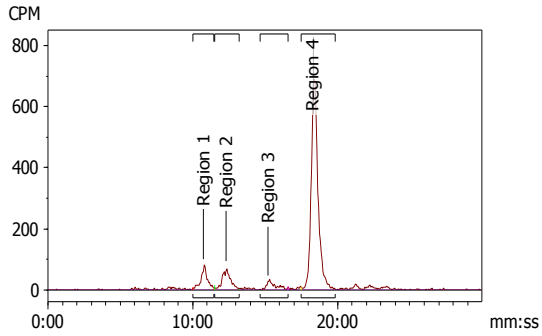
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 56
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:02	11:28	10:45	2477	8.39	7.52
Region 2	11:32	13:12	12:20	2797	9.47	8.49
Region 3	14:38	16:36	15:13	1398	4.74	4.24
Region 4	17:30	19:52	18:20	22858	77.41	69.36
4 Peaks				29530	100.00	89.60

Total Area: 32957 CPM
Average Background: 0 CPM

FYX-037 16D.prog(20uM).C17A105L.5uL.RT(22 C).6A2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 12:18:02 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:58:44 PM

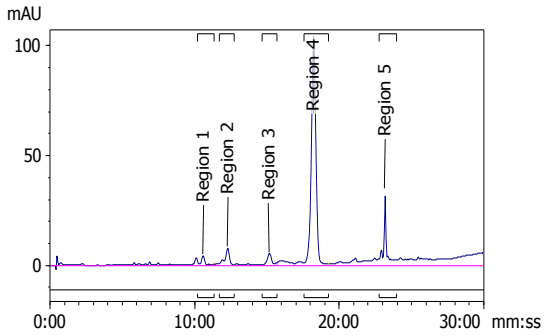
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 57
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:12	11:21	10:34	26	1.00	0.51
Region 2	11:42	12:44	12:16	125	4.76	2.41
Region 3	14:39	15:40	15:08	78	2.95	1.50
Region 4	17:33	19:16	18:12	2186	83.04	42.05
Region 5	22:46	23:57	23:08	217	8.25	4.18
5 Peaks				2633	100.00	50.63

Total Area: 5200 mAU
Average Background: N/A mAU

FYX-037 16D.prog(20uM).C17A105L.5uL.RT(22 C).6A2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 12:18:02 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:58:44 PM

Run Length: 30m
Dwell: 1s

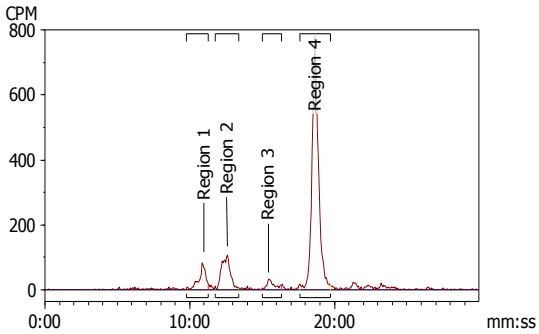
Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %

Off

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 57
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	9:48	11:16	10:59	2621	8.15	7.15
Region 2	11:47	13:22	12:36	4470	13.91	12.19
Region 3	15:03	16:21	15:26	1229	3.82	3.35
Region 4	17:38	19:44	18:40	23827	74.12	64.96
4 Peaks				32147	100.00	87.65

Total Area: 36678 CPM
Average Background: 0 CPM

FYX-037 16D.prog(20uM).C17A105L.5uL.RT(22 C).6A3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 12:52:54 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:00:43 AM

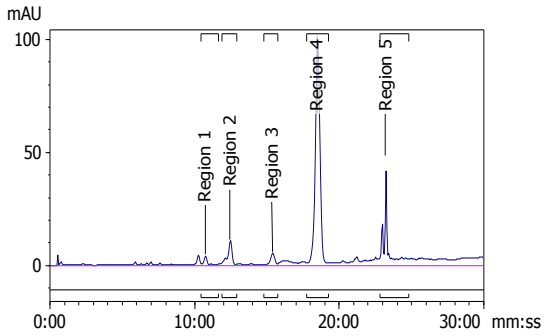
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 58
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:25	11:38	10:44	35	1.23	0.68
Region 2	11:54	12:56	12:27	176	6.17	3.41
Region 3	14:46	15:45	15:22	86	3.03	1.67
Region 4	17:43	19:16	18:28	2168	76.04	41.98
Region 5	22:48	24:48	23:12	386	13.53	7.47
5 Peaks				2852	100.00	55.22

Total Area: 5165 mAU
Average Background: N/A mAU

FYX-037 16D.prog(20uM).C17A105L.5uL.RT(22 C).6A3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 12:52:54 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:00:43 AM

Run Length: 30m
Dwell: 1s

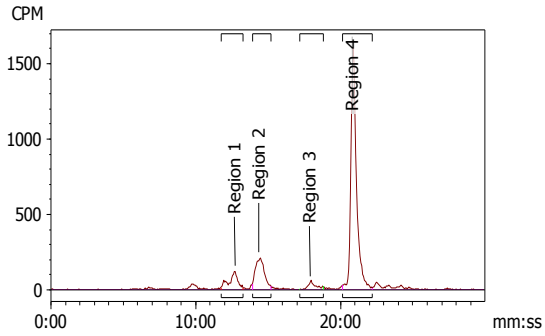
Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %

Off

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 58
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:47	13:16	12:42	5229	8.66	7.45
Region 2	13:55	15:12	14:22	9315	15.42	13.28
Region 3	17:13	18:50	17:55	2214	3.67	3.16
Region 4	20:09	22:12	20:50	43642	72.25	62.20
4 Peaks				60400	100.00	86.09

Total Area: 70163 CPM
Average Background: 0 CPM

FYX-037 16D.prog(20uM).C17A105L.10uL.(26 C).6B1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 11:25:12 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:59:55 PM

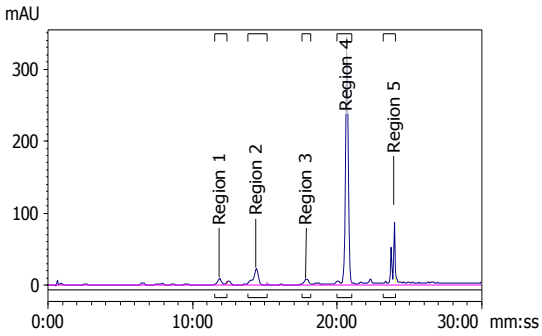
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 83
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:30	12:24	11:50	52	0.92	0.58
Region 2	13:50	15:09	14:23	384	6.85	4.27
Region 3	17:34	18:09	17:50	116	2.07	1.29
Region 4	19:57	21:01	20:38	4304	76.81	47.92
Region 5	23:10	24:02	23:55	748	13.34	8.32
5 Peaks				5604	100.00	62.39

Total Area: 8982 mAU
Average Background: N/A mAU

FYX-037 16D.prog(20uM).C17A105L.10uL.(26 C).6B1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 11:25:12 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:59:55 PM

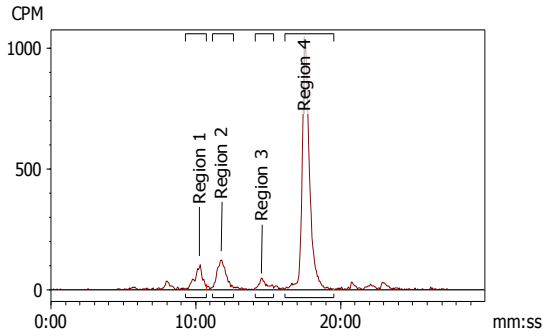
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 83
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	9:17	10:43	10:16	3773	8.07	7.07
Region 2	11:09	12:36	11:46	5286	11.31	9.91
Region 3	14:07	15:22	14:33	1747	3.74	3.28
Region 4	16:09	19:34	17:30	35949	76.89	67.41
4 Peaks				46755	100.00	87.67

Total Area: 53331 CPM
Average Background: 0 CPM

FYX-037 16D.prog(20uM).C17A105L.10uL.(26 C).6B3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, March 07, 2012 8:50:39 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 1:01:23 PM

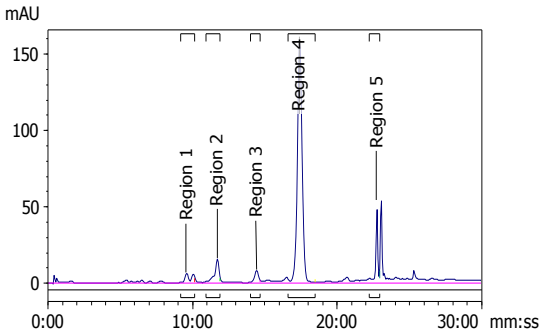
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 85
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:11	10:09	9:34	84	2.03	1.13
Region 2	10:56	11:54	11:41	219	5.33	2.97
Region 3	13:59	14:39	14:24	118	2.86	1.60
Region 4	16:36	18:30	17:22	3464	84.22	47.00
Region 5	22:12	22:55	22:42	228	5.55	3.10
5 Peaks				4113	100.00	55.80

Total Area: 7371 mAU
Average Background: N/A mAU

FYX-037 16D.prog(20uM).C17A105L.10uL.(26 C).6B3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, March 07, 2012 8:50:39 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 1:01:23 PM

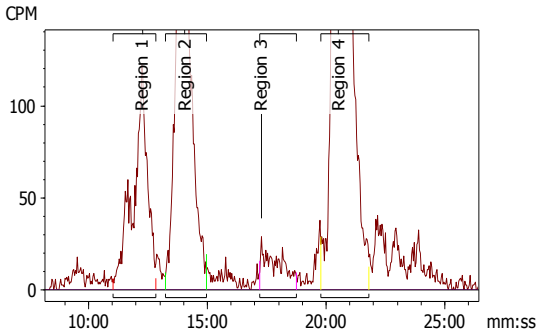
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 85
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:03	12:51	12:15	5024	9.50	8.30
Region 2	13:15	14:57	14:05	9360	17.69	15.46
Region 3	17:13	18:45	17:16	1312	2.48	2.17
Region 4	19:46	21:49	20:31	37213	70.33	61.47
4 Peaks				52909	100.00	87.40

Total Area: 60538 CPM
Average Background: 0 CPM

FYX-037 C17A105L(5uL).16Dprog(20 uM).30C.6C1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 7:03:23 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:22:15 PM

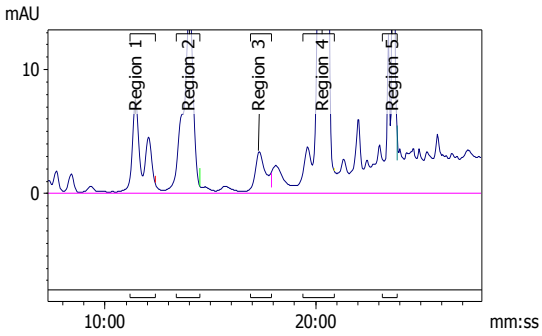
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 46
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:11	12:22	11:26	170	3.71	2.35
Region 2	13:22	14:30	13:59	387	8.48	5.36
Region 3	16:55	17:54	17:18	53	1.16	0.74
Region 4	19:25	20:53	20:19	3553	77.83	49.23
Region 5	23:11	23:52	23:40	403	8.82	5.58
5 Peaks				4566	100.00	63.26

Total Area: 7217 mAU
Average Background: N/A mAU

FYX-037 C17A105L(5uL).16Dprog(20 uM).30C.6C1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 7:03:23 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:22:15 PM

Run Length: 30m
Dwell: 1s

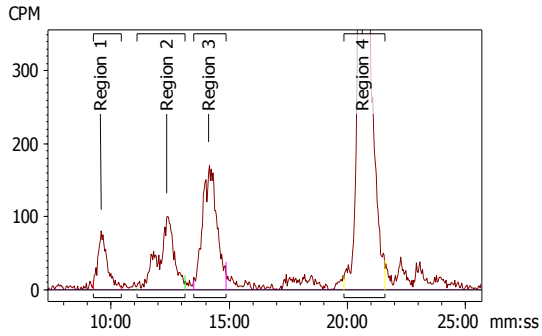
Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %

Off

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 46
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	9:15	10:26	9:35	2240	4.82	4.10
Region 2	11:05	13:08	12:22	4717	10.14	8.62
Region 3	13:31	14:51	14:08	7114	15.30	13.01
Region 4	19:51	21:34	20:39	32426	69.74	59.28
4 Peaks				46496	100.00	85.01

Total Area: 54698 CPM
Average Background: 0 CPM

FYX-037 C17A105L(5uL).16Dprog(20 uM).30C.6C2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 7:38:15 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:23:52 PM

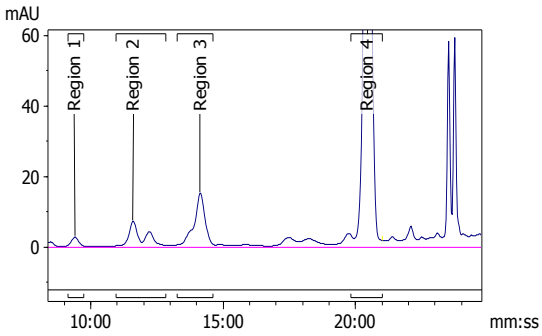
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 47
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:09	9:44	9:24	39	1.02	0.52
Region 2	10:58	12:50	11:35	227	6.01	3.08
Region 3	13:16	14:36	14:08	390	10.31	5.29
Region 4	19:50	21:01	20:25	3127	82.66	42.42
4 Peaks				3783	100.00	51.32

Total Area: 7371 mAU
Average Background: N/A mAU

FYX-037 C17A105L(5uL).16Dprog(20 uM).30C.6C2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 7:38:15 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:23:52 PM

Run Length: 30m
Dwell: 1s

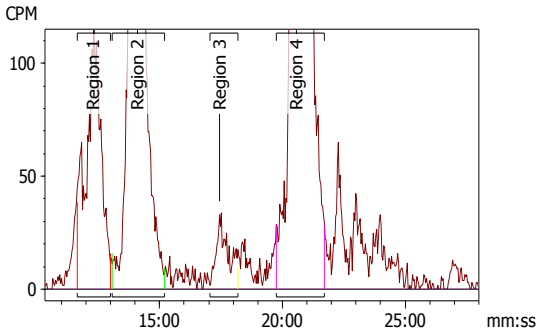
Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %

Off

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 47
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:38	13:01	12:19	4954	8.56	7.37
Region 2	13:05	15:12	14:07	10822	18.71	16.11
Region 3	17:03	18:11	17:27	1104	1.91	1.64
Region 4	19:46	21:43	20:36	40973	70.82	61.00
4 Peaks				57853	100.00	86.13

Total Area: 67171 CPM
Average Background: 0 CPM

FYX-037 C17A105L(5uL).16Dprog(20 uM).30C.6C3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 8:13:06 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:29:10 PM

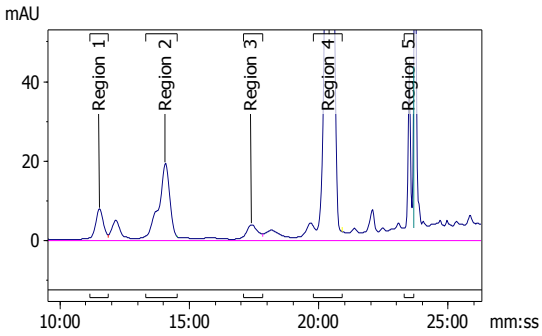
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 48
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:10	11:52	11:31	123	2.73	1.41
Region 2	13:19	14:31	14:04	509	11.33	5.83
Region 3	17:05	17:50	17:23	57	1.27	0.65
Region 4	19:48	20:55	20:22	3895	86.73	44.57
Region 5	23:18	23:41	23:29	-93	-2.07	-1.06
5 Peaks				4491	100.00	51.39

Total Area: 8738 mAU
Average Background: N/A mAU

FYX-037 C17A105L(5uL).16Dprog(20 uM).30C.6C3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 8:13:06 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:29:10 PM

Run Length: 30m
Dwell: 1s

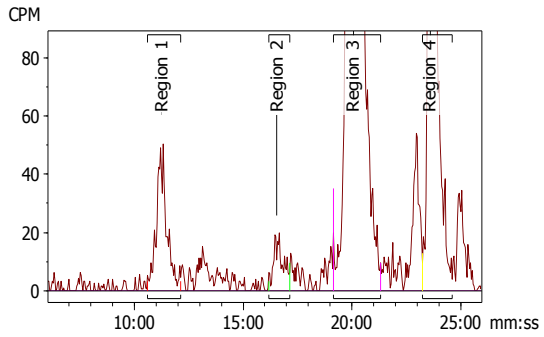
Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %

Off

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 48
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:37	12:08	11:15	1590	5.44	4.67
Region 2	16:10	17:08	16:33	570	1.95	1.67
Region 3	19:08	21:19	20:03	18893	64.60	55.46
Region 4	23:15	24:36	23:36	8195	28.02	24.06
4 Peaks				29248	100.00	85.85

Total Area: 34067 CPM
Average Background: 0 CPM

FYX-037 C17A105L(5uL).16Dprog(20 uM).55C.6D1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 2:04:12 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:36:42 PM

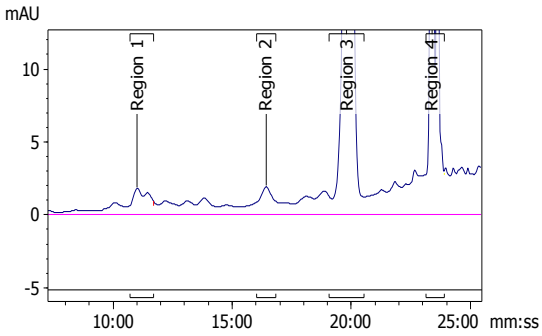
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 73
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:42	11:42	11:01	33	1.42	0.71
Region 2	16:02	16:51	16:25	24	1.02	0.50
Region 3	19:05	20:32	19:50	1246	53.02	26.25
Region 4	23:09	23:54	23:22	1047	44.54	22.05
4 Peaks				2350	100.00	49.51

Total Area: 4747 mAU
Average Background: N/A mAU

FYX-037 C17A105L(5uL).16Dprog(20 uM).55C.6D1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 2:04:12 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:36:42 PM

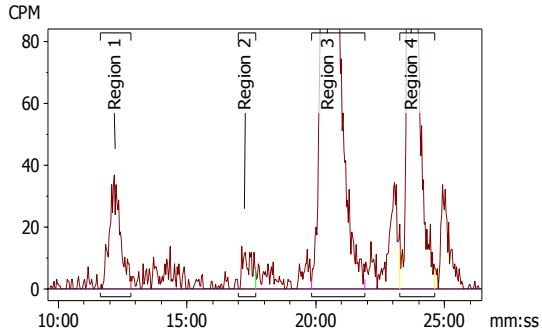
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 73
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:39	12:50	12:12	1046	4.87	4.18
Region 2	17:00	17:41	17:14	304	1.42	1.21
Region 3	19:50	21:54	20:28	13603	63.35	54.31
Region 4	23:16	24:38	23:44	6518	30.36	26.03
4 Peaks				21472	100.00	85.73

Total Area: 25046 CPM
Average Background: 0 CPM

FYX-037 C17A105L(5uL).16Dprog(20 uM).55C.6D2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 2:39:06 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:40:43 PM

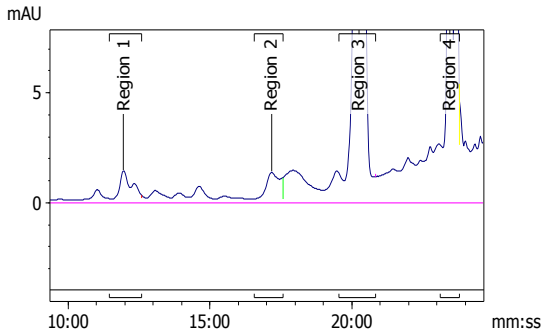
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 74
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:27	12:35	11:57	28	1.59	0.75
Region 2	16:33	17:35	17:10	10	0.55	0.26
Region 3	19:33	20:50	20:14	955	54.49	25.69
Region 4	23:06	23:47	23:26	760	43.37	20.44
4 Peaks				1752	100.00	47.14

Total Area: 3716 mAU
Average Background: N/A mAU

FYX-037 C17A105L(5uL).16Dprog(20 uM).55C.6D2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 2:39:06 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:40:43 PM

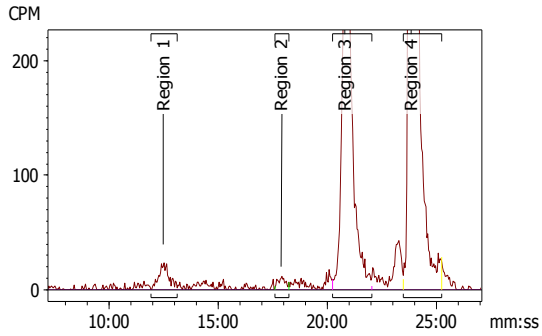
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 74
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:55	13:08	12:30	771	1.98	1.80
Region 2	17:35	18:15	17:54	298	0.77	0.69
Region 3	20:15	22:02	20:48	10883	27.99	25.38
Region 4	23:28	25:14	23:52	26928	69.26	62.80
4 Peaks				38880	100.00	90.67

Total Area: 42880 CPM
Average Background: 0 CPM

FYX-037 C17A105L(5uL).16Dprog(20 uM).55C.6D3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 3:14:01 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:43:22 PM

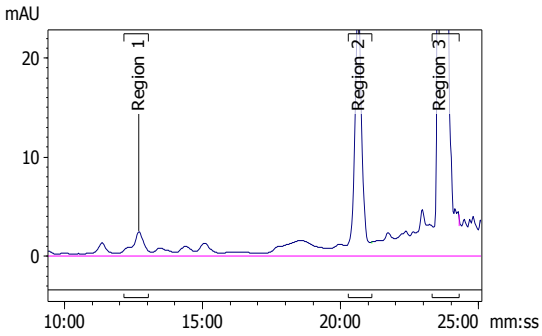
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 75
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	12:10	13:02	12:41	37	0.85	0.55
Region 2	20:17	21:07	20:38	384	8.73	5.67
Region 3	23:19	24:17	23:35	3978	90.42	58.69
3 Peaks				4399	100.00	64.91

Total Area: 6778 mAU
Average Background: N/A mAU

FYX-037 C17A105L(5uL).16Dprog(20 uM).55C.6D3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 3:14:01 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:43:22 PM

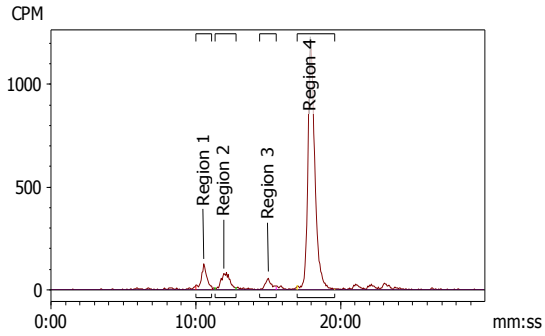
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 75
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:02	11:06	10:32	3306	6.80	6.01
Region 2	11:22	12:46	11:55	3414	7.02	6.21
Region 3	14:24	15:35	15:00	1741	3.58	3.17
Region 4	17:01	19:35	17:55	40154	82.60	73.03
4 Peaks				48614	100.00	88.41

Total Area: 54986 CPM
Average Background: 0 CPM

FYX-037 21.21.21.D3.prog(20uM).C17A105L.5uL.RT(22 C).5A1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 9:58:32 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:48:24 PM

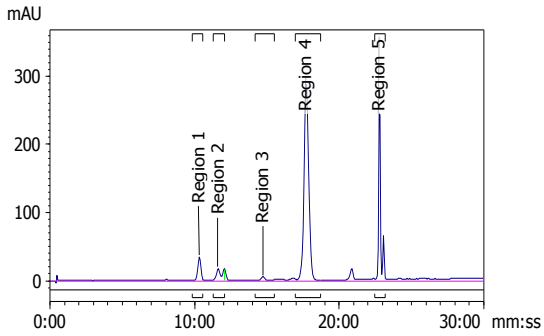
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 53
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:51	10:33	10:18	481	4.94	3.73
Region 2	11:18	12:05	11:36	87	0.90	0.68
Region 3	14:12	15:31	14:42	75	0.77	0.58
Region 4	16:59	18:42	17:40	6438	66.15	49.91
Region 5	22:26	23:11	22:45	2651	27.24	20.55
5 Peaks				9733	100.00	75.45

Total Area: 12899 mAU
Average Background: N/A mAU

FYX-037 21.21.21.D3.prog(20uM).C17A105L.5uL.RT(22 C).5A1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 9:58:32 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:48:24 PM

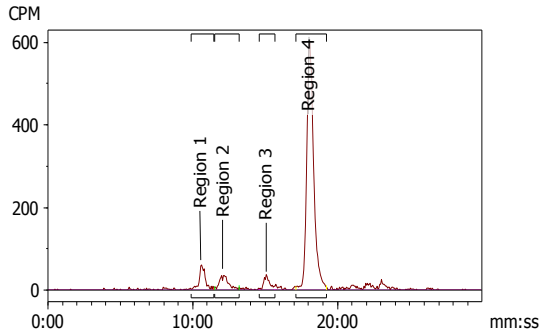
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 53
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	9:55	11:26	10:32	1805	7.35	6.47
Region 2	11:30	13:12	12:05	1581	6.44	5.67
Region 3	14:36	15:42	15:04	1050	4.27	3.76
Region 4	17:09	19:13	18:01	20125	81.94	72.15
4 Peaks				24560	100.00	88.06

Total Area: 27891 CPM
Average Background: 0 CPM

FYX-037 21.21.21.D3.prog(20uM).C17A105L.5uL.RT(22 C).5A2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 10:33:24 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:50:39 PM

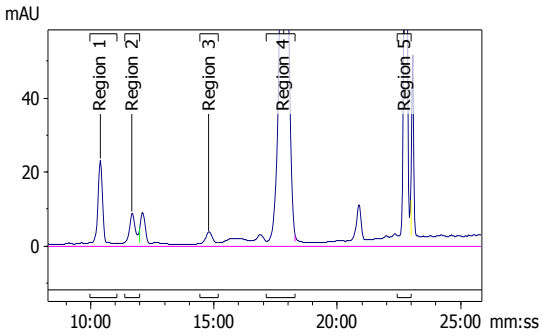
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 54
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:58	11:04	10:23	278	4.36	2.88
Region 2	11:23	12:00	11:41	61	0.96	0.64
Region 3	14:26	15:10	14:48	53	0.83	0.55
Region 4	17:07	18:18	17:49	3493	54.91	36.26
Region 5	22:25	22:59	22:45	2477	38.94	25.71
5 Peaks				6361	100.00	66.04

Total Area: 9632 mAU
Average Background: N/A mAU

FYX-037 21.21.21.D3.prog(20uM).C17A105L.5uL.RT(22 C).5A2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 10:33:24 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:50:39 PM

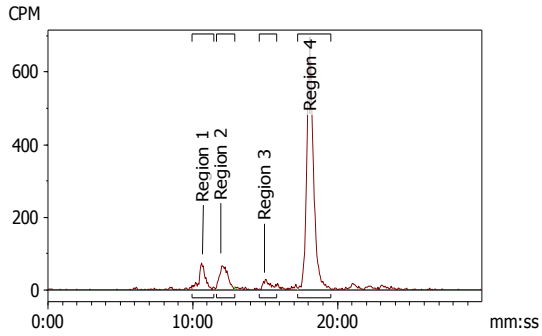
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 54
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	9:58	11:28	10:42	2282	8.25	7.27
Region 2	11:39	12:55	11:57	2730	9.87	8.70
Region 3	14:36	15:47	14:59	1040	3.76	3.31
Region 4	17:16	19:32	18:07	21613	78.13	68.88
4 Peaks				27664	100.00	88.17

Total Area: 31376 CPM
Average Background: 0 CPM

FYX-037 21.21.21.D3.prog(20uM).C17A105L.5uL.RT(22 C).5A3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 11:08:17 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:53:24 PM

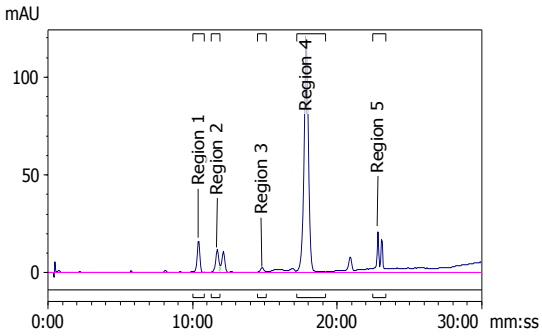
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 55
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:00	10:50	10:23	205	6.63	3.75
Region 2	11:18	11:55	11:40	126	4.07	2.30
Region 3	14:29	15:04	14:47	32	1.04	0.59
Region 4	17:14	19:11	17:49	2475	79.95	45.27
Region 5	22:26	23:23	22:47	257	8.31	4.71
5 Peaks				3096	100.00	56.62

Total Area: 5468 mAU
Average Background: N/A mAU

FYX-037 21.21.21.D3.prog(20uM).C17A105L.5uL.RT(22 C).5A3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 11:08:17 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:53:24 PM

Run Length: 30m
Dwell: 1s

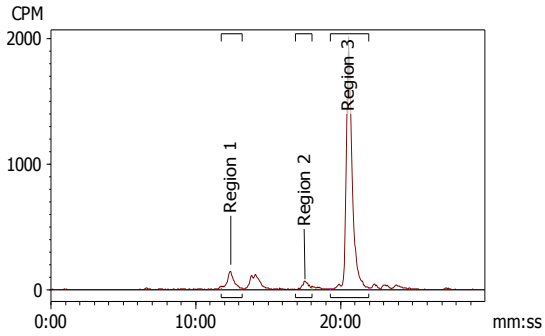
Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %

Off

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 55
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:47	13:12	12:24	5011	7.90	6.44
Region 2	16:53	18:02	17:32	2218	3.49	2.85
Region 3	19:19	21:57	20:34	56237	88.61	72.23
3 Peaks				63466	100.00	81.51

Total Area: 77859 CPM
Average Background: 0 CPM

FYX-037 21.21.21D3.prog(20uM).C17A105L.10uL.(26 C).5B1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 9:40:36 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:55:56 PM

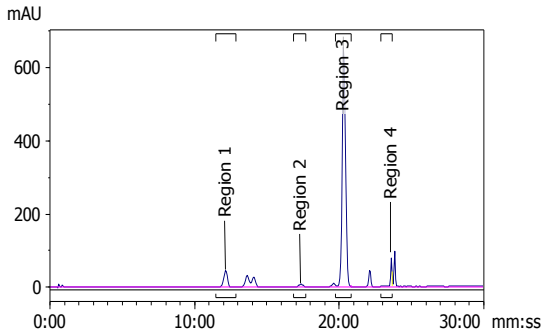
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 80
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:29	12:50	12:07	812	7.45	4.92
Region 2	16:51	17:41	17:19	165	1.52	1.00
Region 3	19:43	20:49	20:16	10347	94.98	62.62
Region 4	22:54	23:38	23:34	-431	-3.95	-2.61
4 Peaks				10893	100.00	65.93

Total Area: 16522 mAU
Average Background: N/A mAU

FYX-037 21.21.21D3.prog(20uM).C17A105L.10uL.(26 C).5B1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 9:40:36 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:55:56 PM

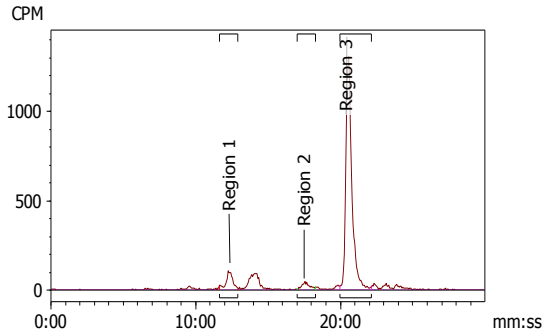
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 80
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:38	12:55	12:21	3482	8.06	6.39
Region 2	17:02	18:16	17:30	1744	4.04	3.20
Region 3	19:59	22:10	20:27	37962	87.90	69.63
3 Peaks				43187	100.00	79.21

Total Area: 54522 CPM
Average Background: 0 CPM

FYX-037 21.21.21D3.prog(20uM).C17A105L.10uL.(26 C).5B2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 10:15:29 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:57:50 PM

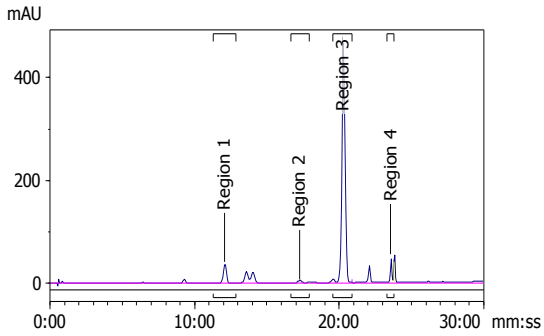
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 81
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:17	12:50	12:04	577	7.91	4.87
Region 2	16:40	17:55	17:15	83	1.14	0.70
Region 3	19:33	20:53	20:15	6814	93.30	57.47
Region 4	23:17	23:46	23:33	-171	-2.34	-1.44
4 Peaks				7304	100.00	61.60

Total Area: 11857 mAU
Average Background: N/A mAU

FYX-037 21.21.21D3.prog(20uM).C17A105L.10uL.(26 C).5B2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 10:15:29 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:57:50 PM

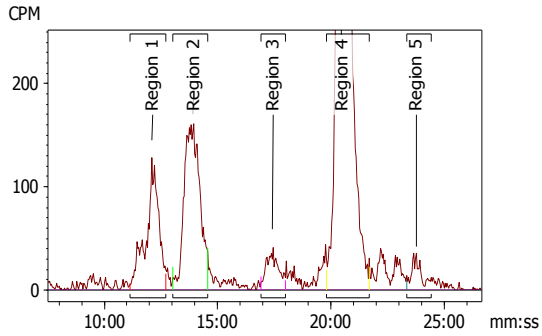
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 81
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:08	12:43	12:06	4858	9.05	7.98
Region 2	13:02	14:34	13:56	7843	14.61	12.89
Region 3	16:55	18:00	17:25	1434	2.67	2.36
Region 4	19:47	21:42	20:29	38560	71.83	63.35
Region 5	23:21	24:26	23:45	986	1.84	1.62
5 Peaks				53680	100.00	88.19

Total Area: 60870 CPM
Average Background: 0 CPM

FYX-037 C17A105L(5uL).21.21.21.D3prog(20 uM).30C.5C1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 5:18:47 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:17:00 PM

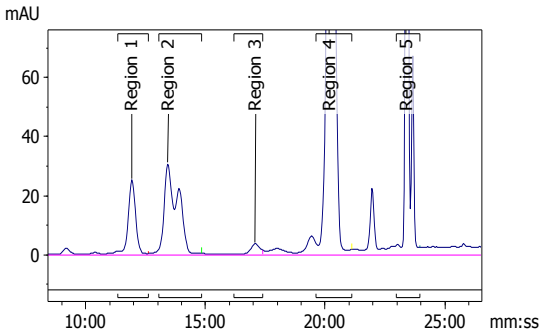
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 43
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:21	12:37	11:55	482	4.87	3.82
Region 2	13:04	14:51	13:25	1042	10.53	8.25
Region 3	16:11	17:23	17:04	41	0.42	0.33
Region 4	19:36	21:07	20:12	5471	55.28	43.31
Region 5	22:59	23:58	23:24	2860	28.90	22.64
5 Peaks				9897	100.00	78.34

Total Area: 12632 mAU
Average Background: N/A mAU

FYX-037 C17A105L(5uL).21.21.21.D3prog(20 uM).30C.5C1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 5:18:47 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:17:00 PM

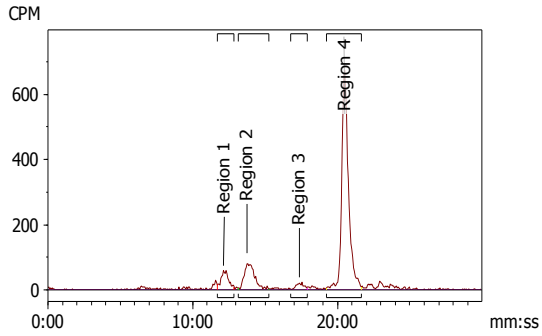
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 43
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:42	12:51	12:08	2182	7.48	6.41
Region 2	13:09	15:16	13:45	4163	14.27	12.22
Region 3	16:47	17:56	17:21	822	2.82	2.41
Region 4	19:14	21:38	20:28	22010	75.43	64.61
4 Peaks				29178	100.00	85.66

Total Area: 34064 CPM
Average Background: 0 CPM

FYX-037 C17A105L(5uL).21.21.21.D3prog(20 uM).30C.5C2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 5:53:38 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:18:26 PM

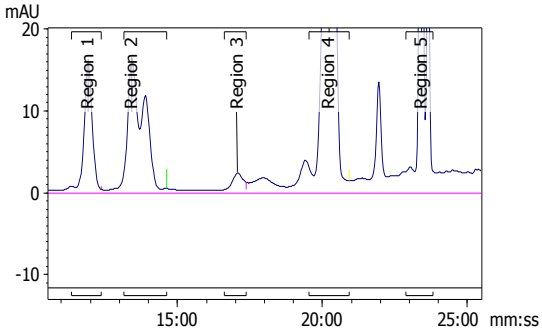
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 44
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:21	12:23	11:55	278	5.35	3.71
Region 2	13:10	14:37	13:25	456	8.77	6.09
Region 3	16:37	17:22	17:04	29	0.55	0.38
Region 4	19:33	20:56	20:12	3207	61.68	42.81
Region 5	22:52	23:49	23:24	1229	23.64	16.40
5 Peaks				5198	100.00	69.40

Total Area: 7491 mAU
Average Background: N/A mAU

FYX-037 C17A105L(5uL).21.21.21.D3prog(20 uM).30C.5C2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 5:53:38 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:18:26 PM

Run Length: 30m
Dwell: 1s

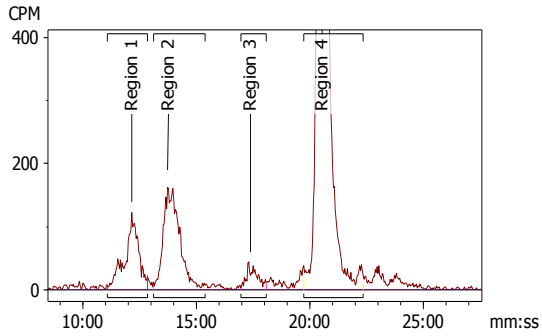
Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %

Off

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 44
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:03	12:50	12:08	4976	9.31	8.31
Region 2	13:07	15:22	13:44	8144	15.23	13.59
Region 3	16:57	18:05	17:22	1366	2.56	2.28
Region 4	19:44	22:21	20:31	38973	72.90	65.05
4 Peaks				53459	100.00	89.23

Total Area: 59910 CPM
Average Background: 0 CPM

FYX-037 C17A105L(5uL).21.21.21.D3prog(20 uM).30C.5C3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 6:28:31 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:20:40 PM

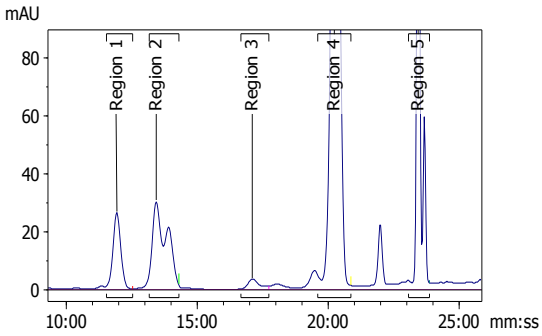
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 45
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:32	12:32	11:56	513	5.35	4.00
Region 2	13:10	14:18	13:26	888	9.26	6.92
Region 3	16:40	17:44	17:06	71	0.74	0.56
Region 4	19:36	20:52	20:13	5888	61.36	45.86
Region 5	23:04	23:52	23:25	2235	23.29	17.40
5 Peaks				9595	100.00	74.74

Total Area: 12839 mAU
Average Background: N/A mAU

FYX-037 C17A105L(5uL).21.21.21.D3prog(20 uM).30C.5C3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 6:28:31 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:20:40 PM

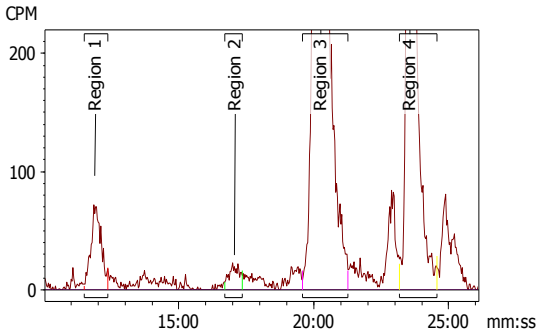
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 45
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:30	12:22	11:53	1923	4.35	3.63
Region 2	16:42	17:20	17:04	563	1.27	1.06
Region 3	19:35	21:16	20:14	26442	59.78	49.97
Region 4	23:11	24:33	23:33	15306	34.60	28.93
4 Peaks				44234	100.00	83.60

Total Area: 52912 CPM
Average Background: 0 CPM

FYX-037 C17A105L(5uL).21.21.21.D3prog(20 uM).55C.5D1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 11:19:12 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:29:17 PM

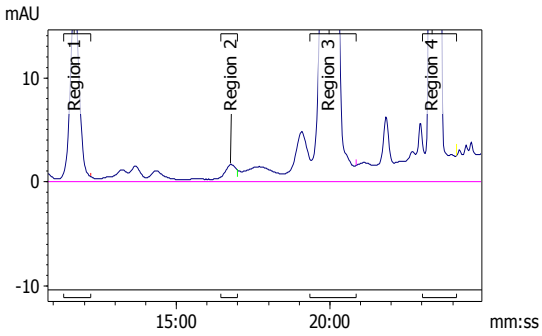
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 70
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:20	12:12	11:41	325	4.25	3.15
Region 2	16:27	16:59	16:46	13	0.17	0.13
Region 3	19:21	20:52	19:57	4160	54.27	40.31
Region 4	23:01	24:08	23:19	3167	41.31	30.69
4 Peaks				7666	100.00	74.28

Total Area: 10321 mAU
Average Background: N/A mAU

FYX-037 C17A105L(5uL).21.21.21.D3prog(20 uM).55C.5D1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 11:19:12 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:29:17 PM

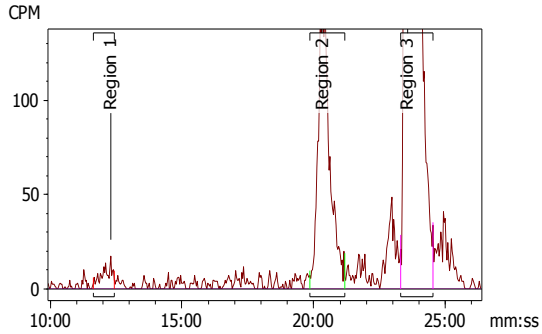
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 70
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:39	12:26	12:18	368	1.01	0.89
Region 2	19:52	21:10	20:21	4762	13.02	11.45
Region 3	23:18	24:32	23:34	31453	85.98	75.65
3 Peaks				36582	100.00	87.99

Total Area: 41578 CPM
Average Background: 0 CPM

FYX-037 C17A105L(5uL).21.21.21.D3prog(20 uM).55C.5D2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 11:54:06 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:32:22 PM

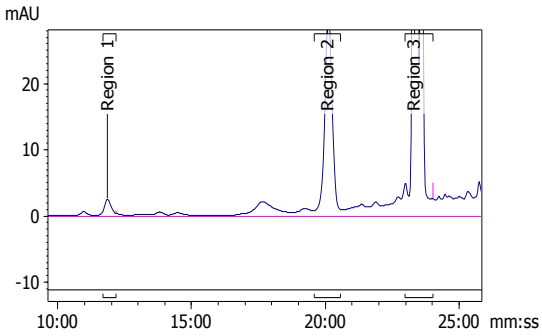
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 71
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:42	12:10	11:52	24	0.39	0.29
Region 2	19:35	20:34	20:06	562	8.93	6.69
Region 3	22:59	24:01	23:20	5707	90.68	67.87
3 Peaks				6294	100.00	74.84

Total Area: 8410 mAU
Average Background: N/A mAU

FYX-037 C17A105L(5uL).21.21.21.D3prog(20 uM).55C.5D2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 11:54:06 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:32:22 PM

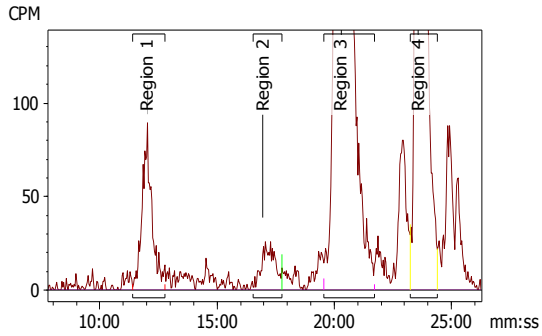
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 71
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:24	12:46	12:01	2474	4.75	4.07
Region 2	16:32	17:47	16:58	1014	1.95	1.67
Region 3	19:33	21:42	20:18	29094	55.84	47.86
Region 4	23:14	24:23	23:35	19523	37.47	32.12
4 Peaks				52106	100.00	85.71

Total Area: 60790 CPM
Average Background: 0 CPM

FYX-037 C17A105L(5uL).21.21.21.D3prog(20 uM).55C.5D3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 12:28:58 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:34:57 PM

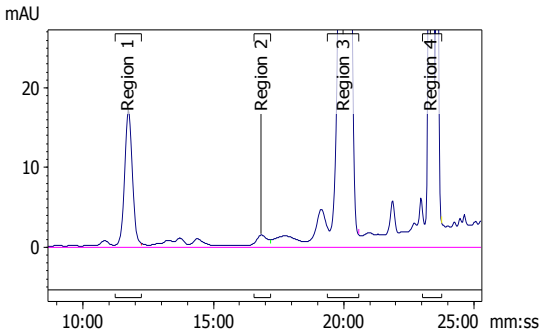
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 72
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:13	12:15	11:43	338	4.04	3.07
Region 2	16:33	17:11	16:50	17	0.20	0.15
Region 3	19:23	20:34	20:00	4154	49.55	37.71
Region 4	23:02	23:46	23:20	3873	46.21	35.17
4 Peaks				8382	100.00	76.11

Total Area: 11013 mAU
Average Background: N/A mAU

FYX-037 C17A105L(5uL).21.21.21.D3prog(20 uM).55C.5D3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 12:28:58 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:34:57 PM

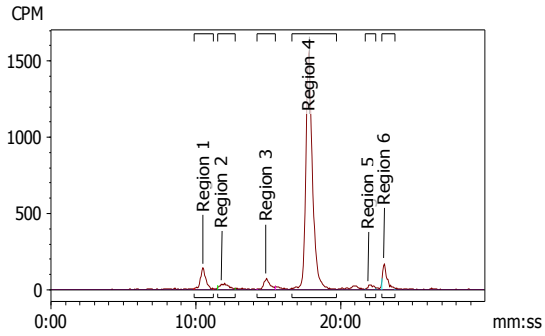
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 72
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	9:55	11:14	10:30	3850	6.15	5.69
Region 2	11:30	12:42	11:46	1802	2.88	2.66
Region 3	14:13	15:30	14:51	2365	3.78	3.50
Region 4	16:40	19:44	17:50	50058	79.92	73.99
Region 5	21:42	22:28	21:55	966	1.54	1.43
Region 6	22:51	23:46	23:00	3594	5.74	5.31
6 Peaks				62634	100.00	92.58

Total Area: 67654 CPM
Average Background: 0 CPM

FYX-037 17Dprog(20uM).C17A105L.5uL.RT(22 C).4A1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 8:13:56 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:43:05 PM

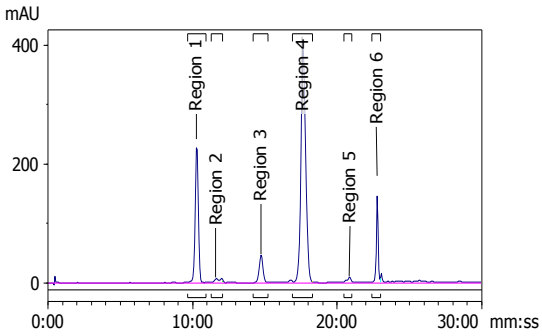
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 50
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:38	10:57	10:15	3438	23.38	19.49
Region 2	11:19	12:06	11:36	143	0.97	0.81
Region 3	14:12	15:13	14:42	843	5.73	4.78
Region 4	16:53	18:17	17:35	9012	61.28	51.10
Region 5	20:28	20:59	20:50	98	0.67	0.56
Region 6	22:25	22:59	22:44	1174	7.98	6.66
6 Peaks				14707	100.00	83.39

Total Area: 17636 mAU
Average Background: N/A mAU

FYX-037 17Dprog(20uM).C17A105L.5uL.RT(22 C).4A1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 8:13:56 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:43:05 PM

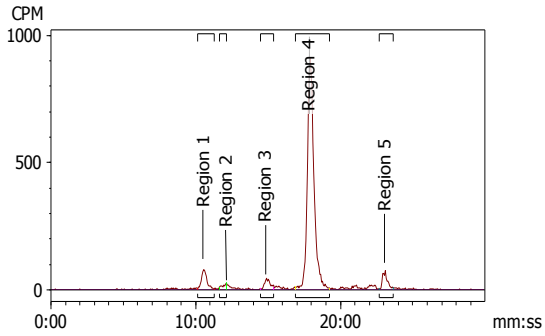
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 50
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:07	11:17	10:31	2163	6.22	5.51
Region 2	11:38	12:09	12:07	566	1.63	1.44
Region 3	14:29	15:22	14:50	1254	3.61	3.20
Region 4	16:55	19:15	17:51	28970	83.35	73.81
Region 5	22:40	23:38	23:03	1805	5.19	4.60
5 Peaks				34758	100.00	88.56

Total Area: 39248 CPM
Average Background: 0 CPM

FYX-037 17Dprog(20uM).C17A105L.5uL.RT(22 C).4A2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 8:48:48 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:44:44 PM

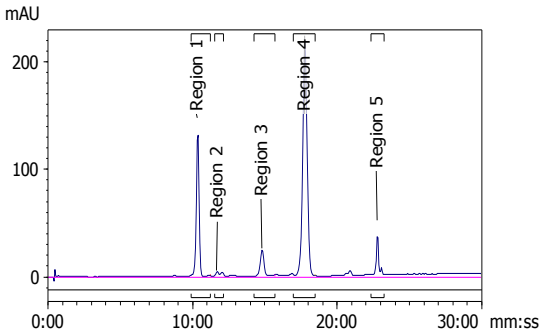
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 51
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:55	11:12	10:19	1727	23.92	17.77
Region 2	11:31	12:09	11:40	60	0.83	0.62
Region 3	14:15	15:43	14:47	415	5.75	4.27
Region 4	16:56	18:29	17:43	4597	63.68	47.31
Region 5	22:21	23:14	22:45	420	5.82	4.32
5 Peaks				7219	100.00	74.30

Total Area: 9717 mAU
Average Background: N/A mAU

FYX-037 17Dprog(20uM).C17A105L.5uL.RT(22 C).4A2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 8:48:48 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:44:44 PM

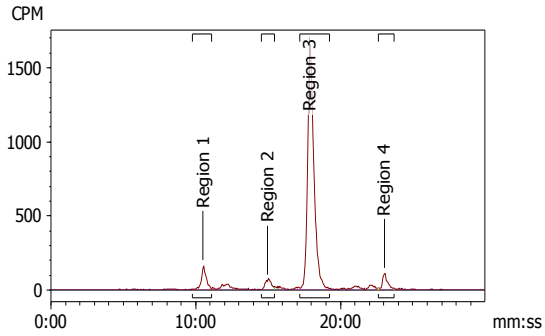
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 51
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	9:48	11:06	10:31	3894	6.57	5.76
Region 2	14:31	15:28	14:58	2285	3.85	3.38
Region 3	17:13	19:13	17:52	50243	84.77	74.34
Region 4	22:38	23:42	23:02	2848	4.81	4.21
4 Peaks				59270	100.00	87.70

Total Area: 67584 CPM
Average Background: 0 CPM

FYX-037 17Dprog(20uM).C17A105L.5uL.RT(22 C).4A3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 9:23:41 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:46:13 PM

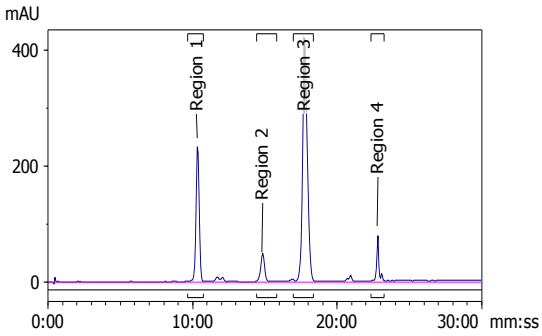
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 52
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:41	10:45	10:18	3607	24.97	20.54
Region 2	14:24	15:48	14:49	850	5.88	4.84
Region 3	16:57	18:20	17:42	9196	63.66	52.37
Region 4	22:19	23:14	22:46	793	5.49	4.52
4 Peaks				14446	100.00	82.26

Total Area: 17562 mAU
Average Background: N/A mAU

FYX-037 17Dprog(20uM).C17A105L.5uL.RT(22 C).4A3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 9:23:41 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:46:13 PM

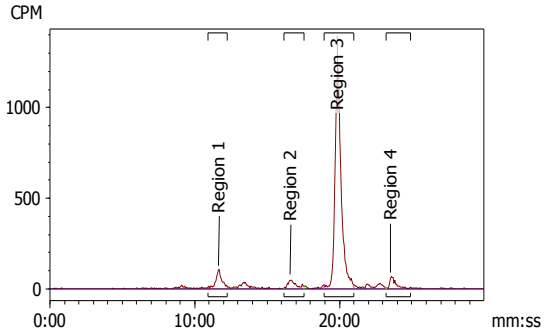
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 52
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:56	12:15	11:39	3139	7.05	6.14
Region 2	16:09	17:34	16:36	2013	4.52	3.93
Region 3	18:55	20:58	19:51	37280	83.68	72.87
Region 4	23:12	24:56	23:32	2118	4.76	4.14
4 Peaks				44550	100.00	87.08

Total Area: 51162 CPM
Average Background: 0 CPM

FYX-037 17D.prog(20uM).C17A105L.10uL.(26 C).4B1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 7:55:57 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:51:24 PM

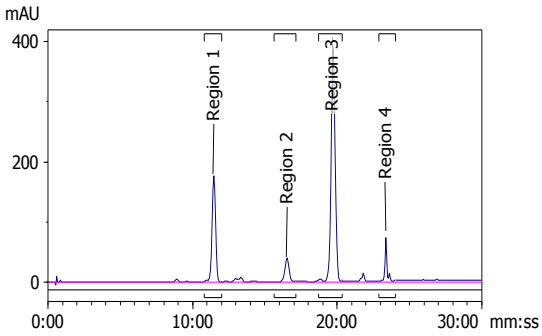
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 77
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:48	12:02	11:26	2972	25.14	19.63
Region 2	15:39	17:07	16:30	782	6.61	5.17
Region 3	18:42	20:22	19:40	7460	63.12	49.29
Region 4	22:54	24:00	23:20	605	5.12	4.00
4 Peaks				11818	100.00	78.09

Total Area: 15134 mAU
Average Background: N/A mAU

FYX-037 17D.prog(20uM).C17A105L.10uL.(26 C).4B1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 7:55:57 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:51:24 PM

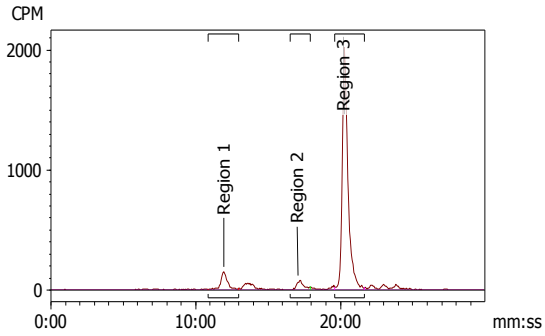
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 77
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:52	12:59	11:57	5110	8.06	6.89
Region 2	16:32	17:55	17:04	2790	4.40	3.76
Region 3	19:38	21:38	20:13	55478	87.53	74.83
3 Peaks				63379	100.00	85.48

Total Area: 74144 CPM
Average Background: 0 CPM

FYX-037 17D.prog(20uM).C17A105L.10uL.(26 C).4B2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 8:30:50 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:52:41 PM

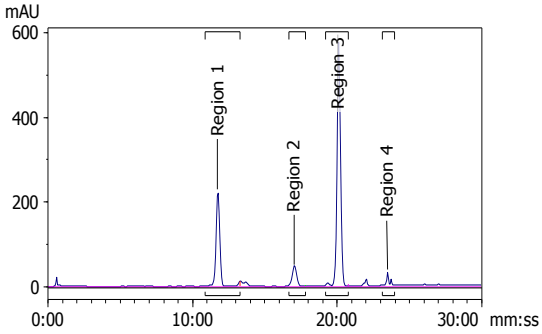
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 78
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:52	13:16	11:43	3222	22.94	16.86
Region 2	16:40	17:48	17:01	870	6.20	4.55
Region 3	19:12	20:45	20:03	9611	68.42	50.29
Region 4	23:07	23:58	23:28	344	2.45	1.80
4 Peaks				14048	100.00	73.51

Total Area: 19112 mAU
Average Background: N/A mAU

FYX-037 17D.prog(20uM).C17A105L.10uL.(26 C).4B2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 8:30:50 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:52:41 PM

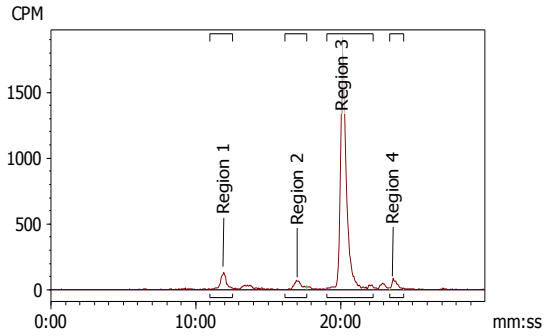
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 78
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:00	12:34	11:54	3936	6.41	5.76
Region 2	16:11	17:41	17:01	2659	4.33	3.89
Region 3	19:04	22:16	20:08	52678	85.75	77.04
Region 4	23:24	24:22	23:36	2157	3.51	3.15
4 Peaks				61430	100.00	89.84

Total Area: 68378 CPM
Average Background: 0 CPM

FYX-037 17D.prog(20uM).C17A105L.10uL.(26 C).4B3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 9:05:43 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:53:56 PM

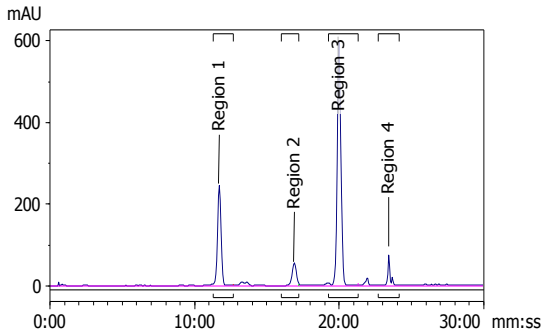
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 79
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:17	12:42	11:40	4025	25.25	20.47
Region 2	15:58	17:13	16:52	1033	6.48	5.25
Region 3	19:16	21:19	19:56	10147	63.65	51.61
Region 4	22:42	24:09	23:24	736	4.62	3.74
4 Peaks				15941	100.00	81.08

Total Area: 19661 mAU
Average Background: N/A mAU

FYX-037 17D.prog(20uM).C17A105L.10uL.(26 C).4B3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 9:05:43 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:53:56 PM

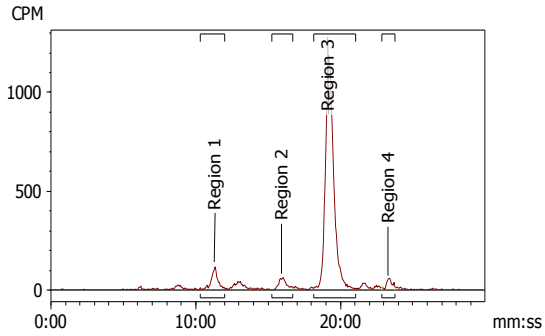
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 79
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:18	12:02	11:18	3741	6.79	5.93
Region 2	15:14	16:43	15:56	2432	4.41	3.85
Region 3	18:08	21:05	19:08	47229	85.72	74.81
Region 4	22:51	23:47	23:19	1693	3.07	2.68
4 Peaks				55094	100.00	87.27

Total Area: 63130 CPM
Average Background: 0 CPM

FYX-037 C17A105L 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.30C.4C1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Sunday, March 11, 2012 2:06:38 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:25:55 PM

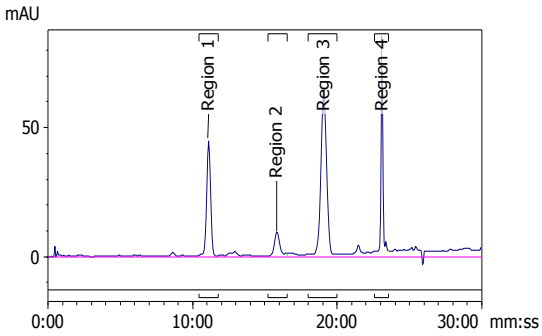
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 44
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:26	11:47	11:04	830	24.52	15.57
Region 2	15:11	16:34	15:48	201	5.93	3.77
Region 3	18:01	19:59	19:02	1709	50.50	32.06
Region 4	22:33	23:34	23:04	645	19.05	12.09
4 Peaks				3385	100.00	63.49

Total Area: 5332 mAU
Average Background: N/A mAU

FYX-037 C17A105L 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.30C.4C1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Sunday, March 11, 2012 2:06:38 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:25:55 PM

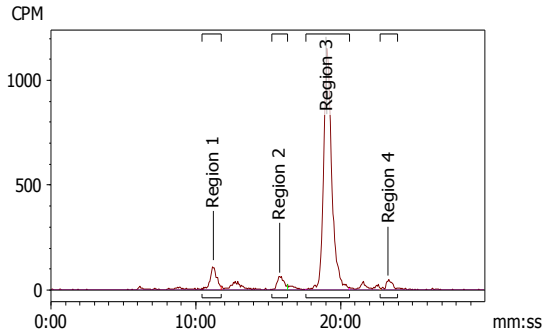
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 44
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:27	11:45	11:13	3606	6.91	6.06
Region 2	15:16	16:22	15:48	2275	4.36	3.82
Region 3	17:38	20:39	19:02	44755	85.69	75.15
Region 4	22:46	23:58	23:18	1590	3.05	2.67
4 Peaks				52227	100.00	87.69

Total Area: 59558 CPM
Average Background: 0 CPM

FYX-037 C17A105L 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.30C.4C3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Sunday, March 11, 2012 3:16:22 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:30:02 PM

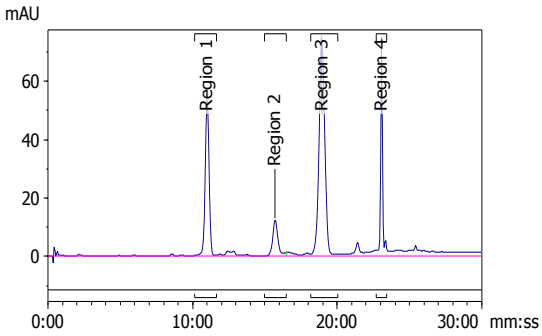
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 46
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:07	11:38	10:58	991	25.92	18.26
Region 2	15:00	16:27	15:40	265	6.92	4.87
Region 3	18:11	20:01	18:54	2013	52.65	37.08
Region 4	22:41	23:25	23:02	555	14.51	10.22
4 Peaks				3824	100.00	70.43

Total Area: 5429 mAU
Average Background: N/A mAU

FYX-037 C17A105L 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.30C.4C3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Sunday, March 11, 2012 3:16:22 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:30:02 PM

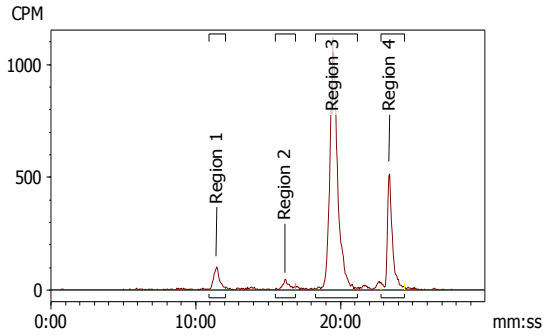
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 46
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:54	12:05	11:25	2906	5.03	4.64
Region 2	15:32	16:54	16:09	1523	2.64	2.43
Region 3	18:15	21:09	19:28	40822	70.73	65.21
Region 4	22:49	24:26	23:21	12467	21.60	19.91
4 Peaks				57718	100.00	92.19

Total Area: 62605 CPM
Average Background: 0 CPM

FYX-037 C17A105L 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.55C.4D1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 12, 2012 3:24:28 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:39:54 PM

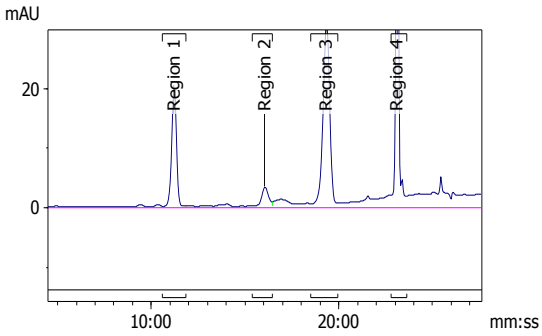
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 50
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:34	11:50	11:12	357	15.93	8.97
Region 2	15:22	16:27	16:03	56	2.52	1.42
Region 3	18:31	19:57	19:20	725	32.38	18.23
Region 4	22:47	23:38	23:06	1102	49.17	27.68
4 Peaks				2241	100.00	56.30

Total Area: 3980 mAU
Average Background: N/A mAU

FYX-037 C17A105L 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.55C.4D1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 12, 2012 3:24:28 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:39:54 PM

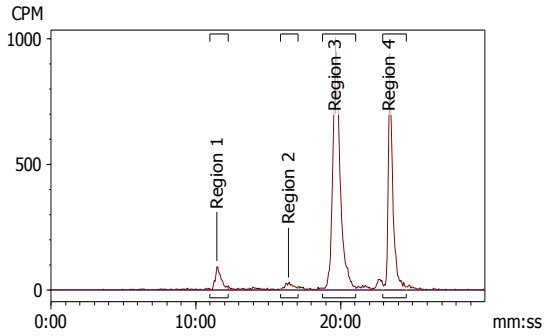
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 50
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:57	12:13	11:29	2688	4.71	4.34
Region 2	15:53	17:06	16:25	1197	2.10	1.93
Region 3	18:46	21:04	19:40	34298	60.07	55.32
Region 4	22:55	24:32	23:23	18915	33.13	30.51
4 Peaks				57098	100.00	92.09

Total Area: 62003 CPM
Average Background: 0 CPM

FYX-037 C17A105L 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.55C.4D2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 12, 2012 3:59:20 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:41:27 PM

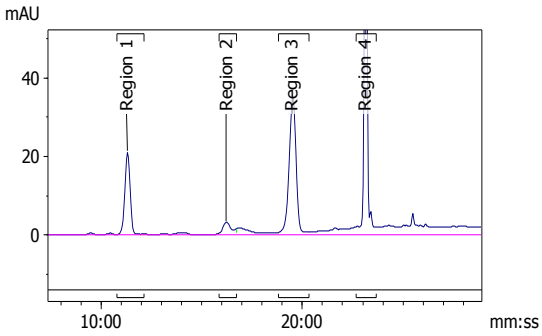
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 51
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:48	12:07	11:18	378	13.38	8.48
Region 2	15:51	16:43	16:13	48	1.68	1.07
Region 3	18:48	20:21	19:30	801	28.33	17.95
Region 4	22:41	23:40	23:08	1601	56.61	35.87
4 Peaks				2828	100.00	63.37

Total Area: 4462 mAU
Average Background: N/A mAU

FYX-037 C17A105L 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.55C.4D2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 12, 2012 3:59:20 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:41:27 PM

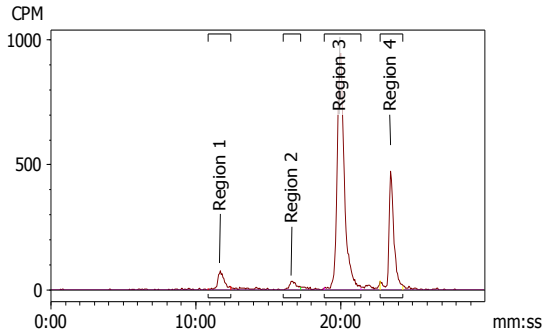
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 51
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:53	12:26	11:40	2499	5.20	4.79
Region 2	16:03	17:17	16:37	1309	2.73	2.51
Region 3	18:52	21:24	19:58	32883	68.47	63.05
Region 4	22:46	24:19	23:27	11334	23.60	21.73
4 Peaks				48026	100.00	92.08

Total Area: 52157 CPM
Average Background: 0 CPM

FYX-037 C17A105L 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.55C.4D3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 12, 2012 4:34:13 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:42:59 PM

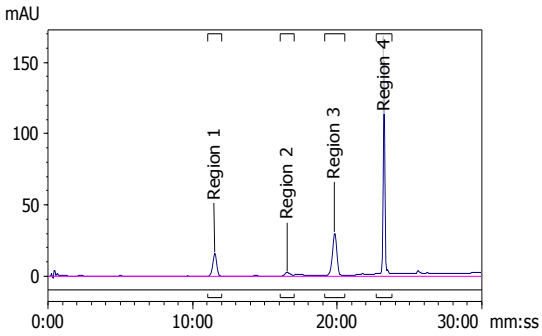
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 52
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:01	12:01	11:30	295	13.91	8.32
Region 2	16:03	17:01	16:31	47	2.21	1.32
Region 3	19:09	20:31	19:47	619	29.18	17.45
Region 4	22:41	23:48	23:12	1161	54.71	32.71
4 Peaks				2123	100.00	59.79

Total Area: 3550 mAU
Average Background: N/A mAU

FYX-037 C17A105L 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.55C.4D3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 12, 2012 4:34:13 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:42:59 PM

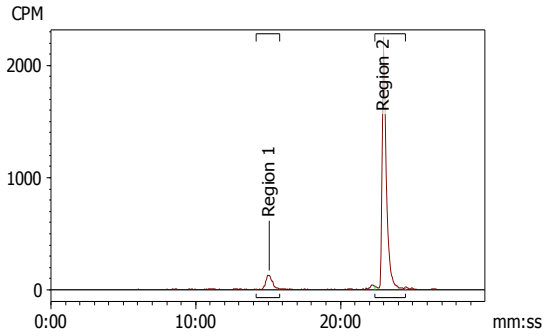
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 52
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	14:09	15:47	15:04	4368	8.94	8.12
Region 2	22:23	24:28	22:58	44515	91.06	82.76
2 Peaks				48883	100.00	90.89

Total Area: 53786 CPM
Average Background: 0 CPM

FYX-037 21.21.21D3.prog(20uM).C21A2WT.10uL.RT(22 C).7A1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 8:04:29 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:04:10 AM

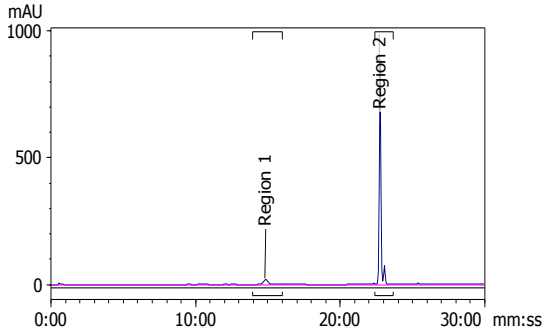
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 59
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	13:57	16:00	14:49	467	6.16	4.73
Region 2	22:22	23:38	22:43	7111	93.84	72.00
2 Peaks				7578	100.00	76.73

Total Area: 9876 mAU
Average Background: N/A mAU

FYX-037 21.21.21D3.prog(20uM).C21A2WT.10uL.RT(22 C).7A1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 8:04:29 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:04:10 AM

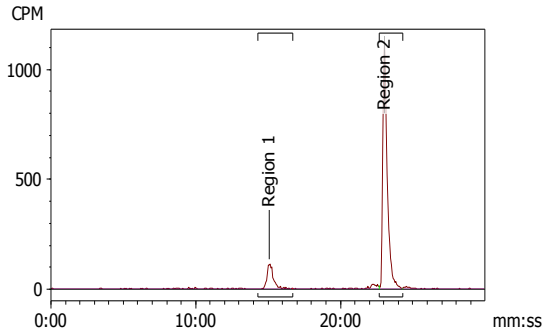
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 59
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	14:17	16:42	15:04	3610	12.94	11.74
Region 2	22:40	24:18	23:02	24275	87.06	78.92
2 Peaks				27885	100.00	90.66

Total Area: 30758 CPM
Average Background: 0 CPM

FYX-037 21.21.21D3.prog(20uM).C21A2WT.10uL.RT(22 C).7A2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 8:39:24 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:06:21 AM

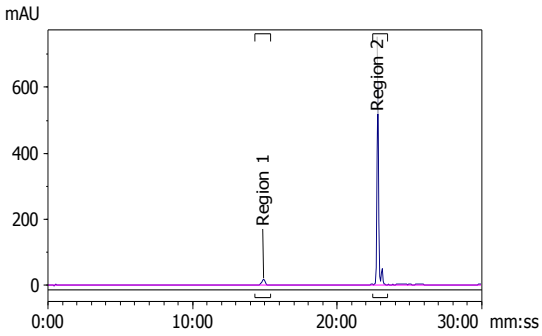
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 60
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	14:20	15:24	14:53	312	5.58	4.17
Region 2	22:28	23:28	22:46	5275	94.42	70.52
2 Peaks				5587	100.00	74.69

Total Area: 7480 mAU
Average Background: N/A mAU

FYX-037 21.21.21D3.prog(20uM).C21A2WT.10uL.RT(22 C).7A2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 8:39:24 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:06:21 AM

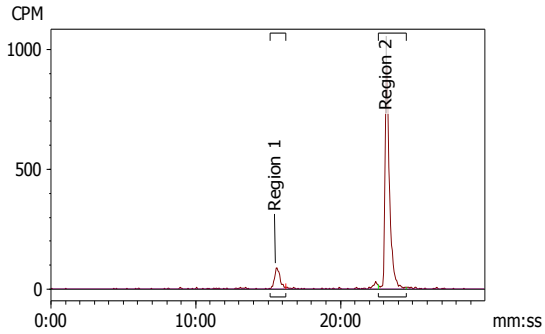
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 60
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	15:09	16:14	15:30	2438	10.63	9.49
Region 2	22:37	24:35	23:09	20493	89.37	79.76
2 Peaks				22931	100.00	89.25

Total Area: 25693 CPM
Average Background: 0 CPM

FYX-037 21.21.21D3.prog(20uM).C21A2WT.10uL.RT(22 C).7A3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 9:14:16 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:07:59 AM

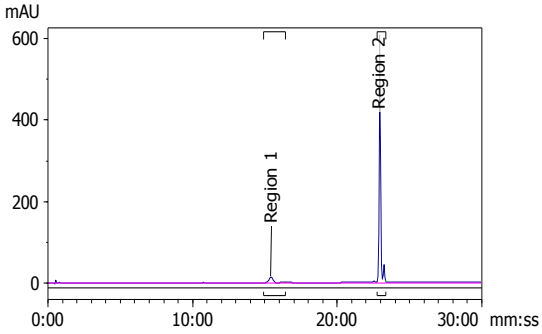
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 61
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	14:55	16:25	15:24	249	5.94	3.97
Region 2	22:46	23:21	22:55	3935	94.06	62.85
2 Peaks				4183	100.00	66.82

Total Area: 6261 mAU
Average Background: N/A mAU

FYX-037 21.21.21D3.prog(20uM).C21A2WT.10uL.RT(22 C).7A3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 9:14:16 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:07:59 AM

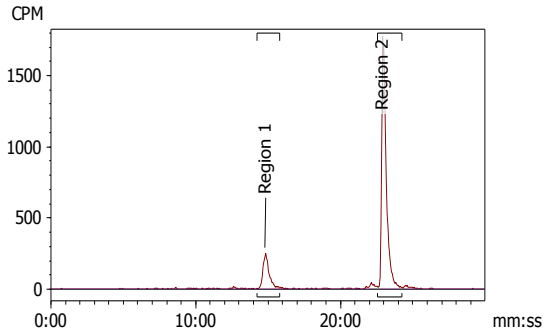
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 61
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	14:15	15:50	14:48	7475	16.15	14.52
Region 2	22:32	24:14	22:55	38810	83.85	75.40
2 Peaks				46285	100.00	89.93

Total Area: 51469 CPM
Average Background: 0 CPM

FYX-037 21.21.21.D3.prog(20uM).C21WT. 10uL.(26 C).7B1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, March 07, 2012 9:25:30 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 1:04:30 PM

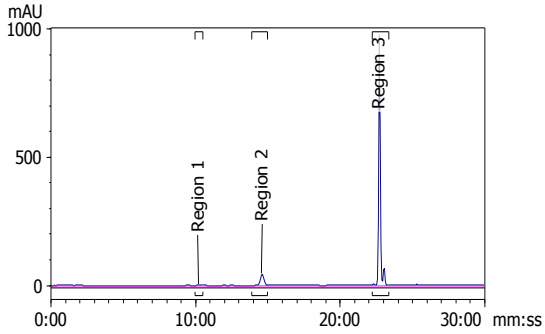
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 86
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:58	10:29	10:12	29	0.36	0.31
Region 2	13:54	14:58	14:34	787	9.67	8.44
Region 3	22:12	23:23	22:40	7321	89.98	78.55
3 Peaks				8137	100.00	87.30

Total Area: 9321 mAU
Average Background: N/A mAU

FYX-037 21.21.21.D3.prog(20uM).C21WT. 10uL.(26 C).7B1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, March 07, 2012 9:25:30 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 1:04:30 PM

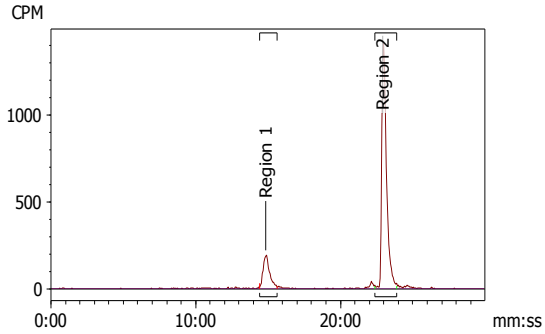
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 86
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	14:26	15:39	14:51	6205	16.01	14.31
Region 2	22:23	23:55	22:56	32550	83.99	75.06
2 Peaks				38755	100.00	89.37

Total Area: 43366 CPM
Average Background: 0 CPM

FYX-037 21.21.21.D3.prog(20uM).C21WT. 10uL.(26 C).7B2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, March 07, 2012 10:00:21 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 1:06:39 PM

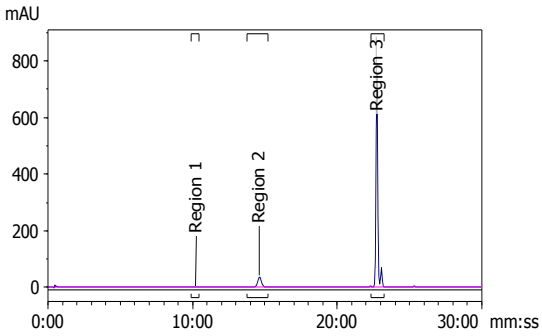
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 87
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:55	10:26	10:13	29	0.39	0.31
Region 2	13:45	15:12	14:36	684	9.31	7.54
Region 3	22:21	23:14	22:42	6642	90.31	73.18
3 Peaks				7355	100.00	81.04

Total Area: 9076 mAU
Average Background: N/A mAU

FYX-037 21.21.21.D3.prog(20uM).C21WT. 10uL.(26 C).7B2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, March 07, 2012 10:00:21 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 1:06:39 PM

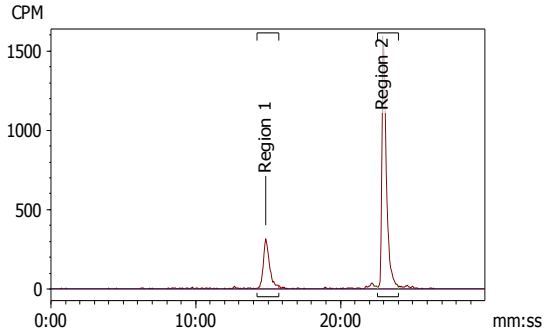
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 87
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	14:14	15:43	14:50	9482	21.98	19.66
Region 2	22:32	23:59	22:55	33654	78.02	69.80
2 Peaks				43136	100.00	89.46

Total Area: 48218 CPM
Average Background: 0 CPM

FYX-037 21.21.21.D3.prog(20uM).C21WT. 10uL.(26 C).7B3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, March 07, 2012 10:35:13 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 1:08:27 PM

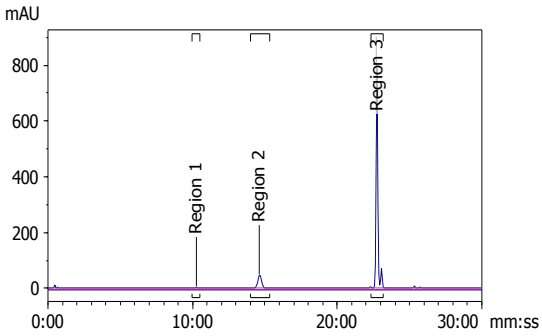
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 88
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:59	10:29	10:14	36	0.48	0.39
Region 2	13:59	15:20	14:36	858	11.33	9.19
Region 3	22:19	23:10	22:42	6677	88.20	71.57
3 Peaks				7571	100.00	81.15

Total Area: 9330 mAU
Average Background: N/A mAU

FYX-037 21.21.21.D3.prog(20uM).C21WT. 10uL.(26 C).7B3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, March 07, 2012 10:35:13 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 1:08:27 PM

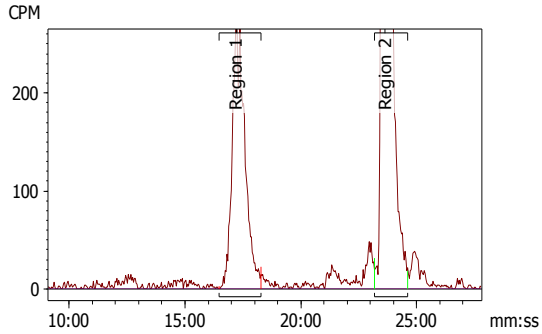
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 88
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	16:28	18:16	17:13	10400	23.87	20.60
Region 2	23:11	24:36	23:38	33168	76.13	65.70
2 Peaks				43568	100.00	86.30

Total Area: 50483 CPM
Average Background: 0 CPM

FYX-037 C21WT(10uL).21.21.21.D3prog(20 uM).30C.7C1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 8:47:59 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:34:00 PM

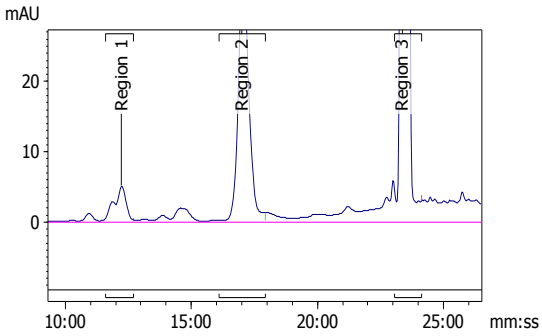
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 49
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:36	12:43	12:14	137	1.74	1.36
Region 2	16:06	17:57	17:02	998	12.72	9.96
Region 3	23:04	24:07	23:21	6714	85.54	66.96
3 Peaks				7849	100.00	78.28

Total Area: 10027 mAU
Average Background: N/A mAU

FYX-037 C21WT(10uL).21.21.21.D3prog(20 uM).30C.7C1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 8:47:59 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:34:00 PM

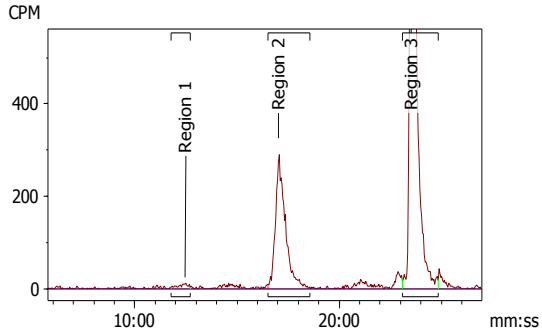
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 49
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:47	12:43	12:28	397	1.01	0.89
Region 2	16:32	18:33	17:03	9725	24.76	21.89
Region 3	23:07	24:52	23:33	29152	74.23	65.62
3 Peaks				39274	100.00	88.40

Total Area: 44429 CPM
Average Background: 0 CPM

FYX-037 C21WT(10uL).21.21.21.D3prog(20 uM).30C.7C2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 9:22:51 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:36:51 PM

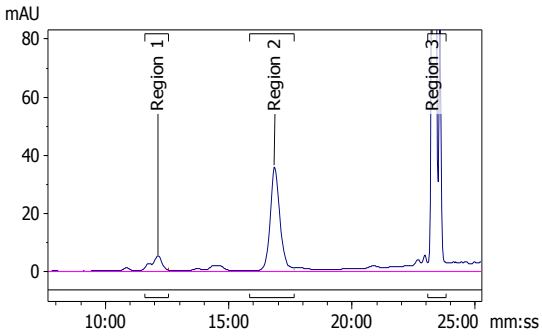
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 50
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:35	12:33	12:07	111	1.30	1.03
Region 2	15:51	17:39	16:51	907	10.66	8.42
Region 3	23:04	23:49	23:17	7488	88.04	69.54
3 Peaks				8506	100.00	78.99

Total Area: 10768 mAU
Average Background: N/A mAU

FYX-037 C21WT(10uL).21.21.21.D3prog(20 uM).30C.7C2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 9:22:51 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:36:51 PM

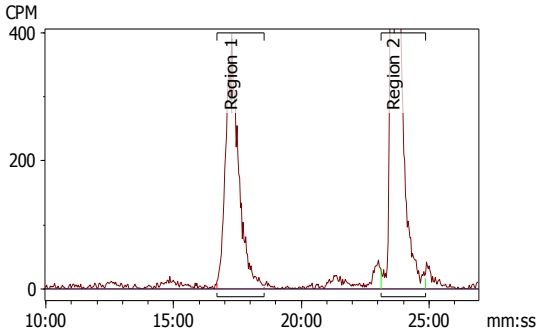
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 50
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	16:41	18:33	17:16	13504	30.07	26.25
Region 2	23:07	24:51	23:37	31398	69.93	61.04
2 Peaks				44902	100.00	87.29

Total Area: 51440 CPM
Average Background: 0 CPM

FYX-037 C21WT(10uL).21.21.21.D3prog(20 uM).30C.7C3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 9:57:44 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:39:57 PM

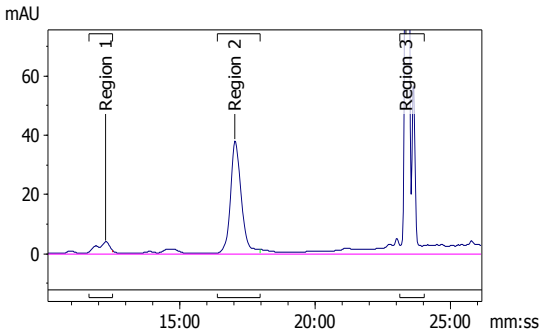
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 51
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:38	12:30	12:15	79	1.28	0.96
Region 2	16:22	17:57	17:02	977	15.75	11.80
Region 3	23:07	24:01	23:22	5148	82.97	62.14
3 Peaks				6205	100.00	74.89

Total Area: 8285 mAU
Average Background: N/A mAU

FYX-037 C21WT(10uL).21.21.21.D3prog(20 uM).30C.7C3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 9:57:44 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:39:57 PM

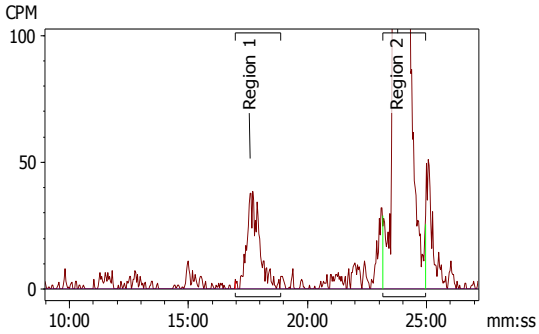
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 51
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	16:58	18:52	17:35	1386	5.08	4.49
Region 2	23:10	24:58	23:46	25872	94.92	83.79
2 Peaks				27258	100.00	88.28

Total Area: 30877 CPM
Average Background: 0 CPM

FYX-037 C21WT(10uL).21.21.21.D3prog(20 uM).55C.7D2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 4:23:47 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:46:56 PM

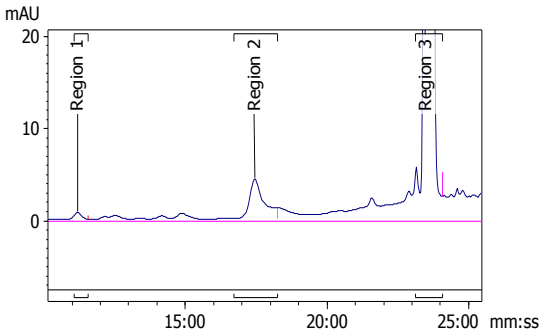
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 77
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:04	11:34	11:11	5	0.09	0.07
Region 2	16:42	18:16	17:27	94	1.60	1.17
Region 3	23:07	24:05	23:29	5754	98.31	71.87
3 Peaks				5853	100.00	73.11

Total Area: 8006 mAU
Average Background: N/A mAU

FYX-037 C21WT(10uL).21.21.21.D3prog(20 uM).55C.7D2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 4:23:47 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:46:56 PM

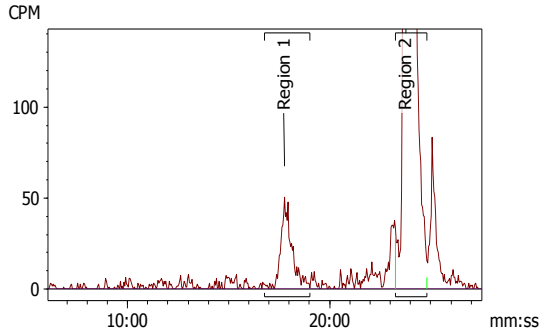
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 77
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	16:46	19:02	17:46	1850	5.42	4.74
Region 2	23:16	24:50	23:46	32275	94.58	82.77
2 Peaks				34125	100.00	87.52

Total Area: 38992 CPM
Average Background: 0 CPM

FYX-037 C21WT(10uL).21.21.21.D3prog(20 uM).55C.7D3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 4:58:40 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:49:20 PM

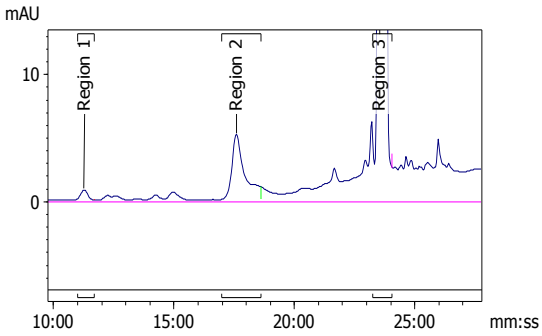
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 78
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:00	11:42	11:16	14	0.23	0.17
Region 2	16:58	18:36	17:35	136	2.27	1.66
Region 3	23:16	24:04	23:32	5831	97.50	71.31
3 Peaks				5980	100.00	73.14

Total Area: 8177 mAU
Average Background: N/A mAU

FYX-037 C21WT(10uL).21.21.21.D3prog(20 uM).55C.7D3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 4:58:40 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:49:20 PM

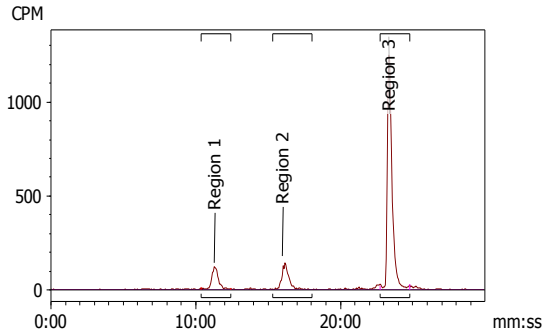
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 78
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:21	12:26	11:17	4125	10.95	9.99
Region 2	15:21	18:01	16:01	4890	12.98	11.85
Region 3	22:45	24:47	23:21	28662	76.07	69.44
3 Peaks				37677	100.00	91.28

Total Area: 41277 CPM
Average Background: 0 CPM

FYX-037 21.21.21D3.prog(20uM).C21V359A.10uL.RT(22 C).8A1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 10:27:31 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:09:56 AM

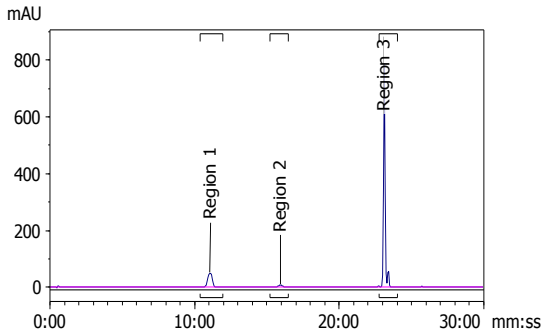
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 62
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:24	11:57	11:04	1112	15.36	13.42
Region 2	15:11	16:29	15:55	178	2.46	2.15
Region 3	22:47	24:01	23:05	5948	82.17	71.77
3 Peaks				7238	100.00	87.34

Total Area: 8288 mAU
Average Background: N/A mAU

FYX-037 21.21.21D3.prog(20uM).C21V359A.10uL.RT(22 C).8A1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 10:27:31 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:09:56 AM

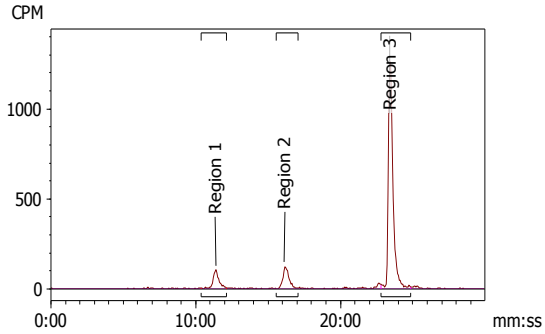
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 62
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:24	12:07	11:23	3133	8.84	8.02
Region 2	15:34	17:06	16:08	3731	10.53	9.55
Region 3	22:50	24:52	23:25	28573	80.63	73.13
3 Peaks				35437	100.00	90.70

Total Area: 39069 CPM
Average Background: 0 CPM

FYX-037 21.21.21D3.prog(20uM).C21V359A.10uL.RT(22 C).8A2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 11:02:25 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:11:36 AM

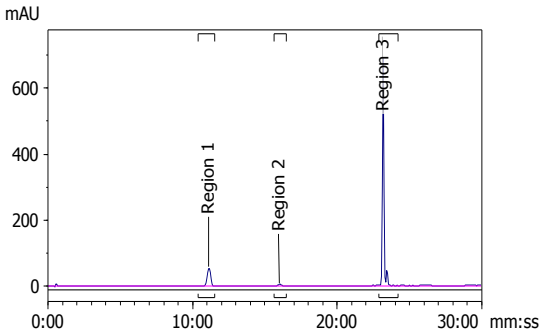
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 63
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:24	11:30	11:06	921	15.50	11.50
Region 2	15:37	16:30	15:59	103	1.73	1.28
Region 3	22:51	24:11	23:08	4918	82.77	61.41
3 Peaks				5942	100.00	74.19

Total Area: 8009 mAU
Average Background: N/A mAU

FYX-037 21.21.21D3.prog(20uM).C21V359A.10uL.RT(22 C).8A2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 11:02:25 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:11:36 AM

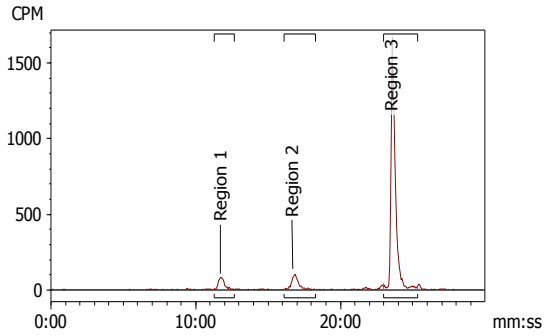
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 63
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:18	12:39	11:43	2646	6.65	6.10
Region 2	16:08	18:18	16:42	3626	9.11	8.36
Region 3	23:00	25:19	23:35	33507	84.23	77.24
3 Peaks				39779	100.00	91.69

Total Area: 43382 CPM
Average Background: 0 CPM

FYX-037 21.21.21D3.prog(20uM).C21V359A.10uL.RT(22 C).8A3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 11:37:17 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:17:45 AM

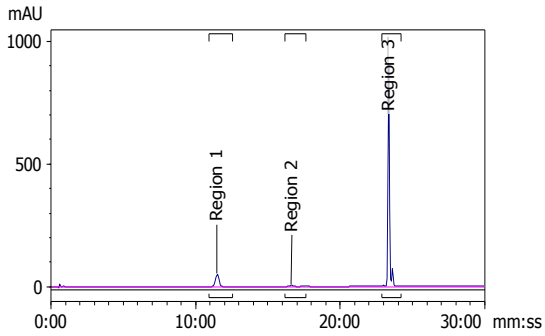
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 64
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:55	12:32	11:28	876	11.60	9.25
Region 2	16:11	17:37	16:36	104	1.37	1.09
Region 3	22:51	24:14	23:19	6570	87.03	69.36
3 Peaks				7549	100.00	79.70

Total Area: 9472 mAU
Average Background: N/A mAU

FYX-037 21.21.21D3.prog(20uM).C21V359A.10uL.RT(22 C).8A3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 11:37:17 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:17:45 AM

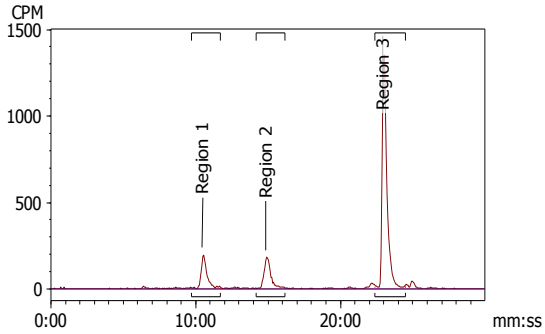
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 64
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	9:42	11:44	10:27	5648	13.14	11.72
Region 2	14:12	16:09	14:52	6077	14.13	12.61
Region 3	22:24	24:30	22:57	31274	72.73	64.89
3 Peaks				42998	100.00	89.21

Total Area: 48198 CPM
Average Background: 0 CPM

FYX-037 21.21.21.D3.prog(20uM).C21V359A. 10uL.(26 C).8B1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, March 07, 2012 11:10:05 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 1:09:55 PM

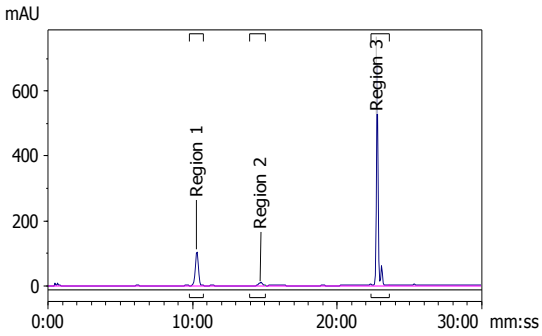
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 89
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:48	10:46	10:16	1550	20.90	16.55
Region 2	13:58	15:03	14:41	179	2.41	1.91
Region 3	22:20	23:37	22:43	5687	76.68	60.71
3 Peaks				7416	100.00	79.17

Total Area: 9367 mAU
Average Background: N/A mAU

FYX-037 21.21.21.D3.prog(20uM).C21V359A. 10uL.(26 C).8B1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, March 07, 2012 11:10:05 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 1:09:55 PM

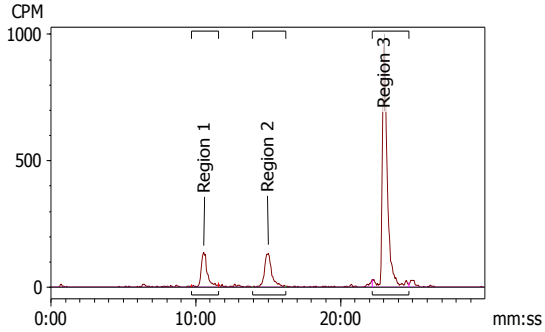
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 89
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	9:44	11:35	10:34	4067	12.59	11.42
Region 2	13:57	16:13	15:00	4726	14.63	13.27
Region 3	22:12	24:45	23:01	23510	72.78	66.02
3 Peaks				32304	100.00	90.72

Total Area: 35610 CPM
Average Background: 0 CPM

FYX-037 21.21.21.D3.prog(20uM).C21V359A. 10uL.(26 C).8B2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, March 07, 2012 11:45:01 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 1:24:45 PM

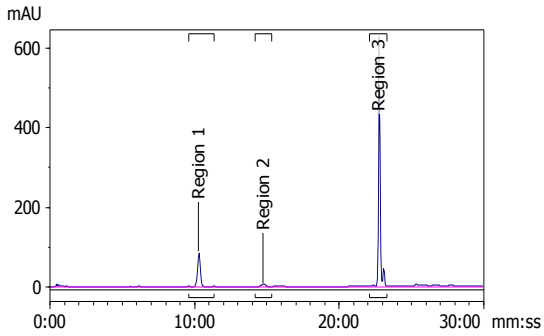
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 90
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:37	11:21	10:16	1061	18.29	13.10
Region 2	14:11	15:21	14:44	129	2.22	1.59
Region 3	22:07	23:19	22:44	4609	79.48	56.91
3 Peaks				5798	100.00	71.60

Total Area: 8098 mAU
Average Background: N/A mAU

FYX-037 21.21.21.D3.prog(20uM).C21V359A. 10uL.(26 C).8B2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, March 07, 2012 11:45:01 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 1:24:45 PM

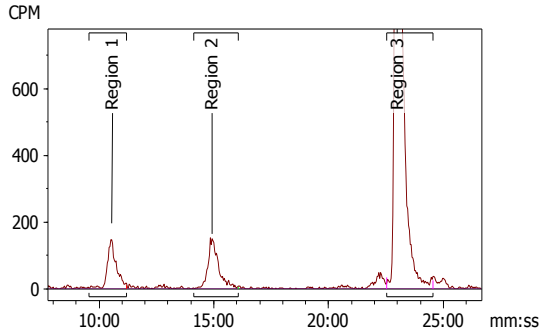
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 90
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	9:34	11:12	10:33	4026	7.44	6.78
Region 2	14:07	16:04	14:55	4925	9.10	8.29
Region 3	22:33	24:32	23:00	45152	83.46	76.02
3 Peaks				54102	100.00	91.09

Total Area: 59392 CPM
Average Background: 0 CPM

FYX-037 21.21.21.D3.prog(20uM).C21V359A. 10uL.(26 C).8B3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, March 07, 2012 12:19:55 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 3:09:59 PM

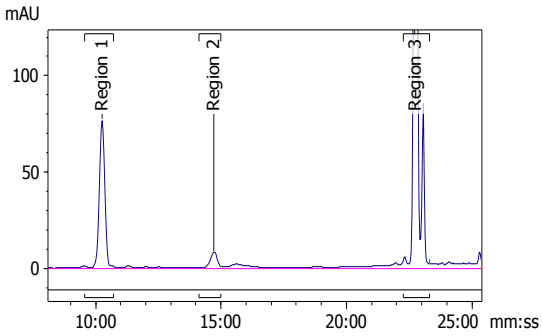
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 91
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:33	10:43	10:15	1078	11.79	9.72
Region 2	14:07	14:59	14:42	130	1.42	1.17
Region 3	22:16	23:19	22:44	7942	86.79	71.59
3 Peaks				9150	100.00	82.48

Total Area: 11094 mAU
Average Background: N/A mAU

FYX-037 21.21.21.D3.prog(20uM).C21V359A. 10uL.(26 C).8B3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, March 07, 2012 12:19:55 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 3:09:59 PM

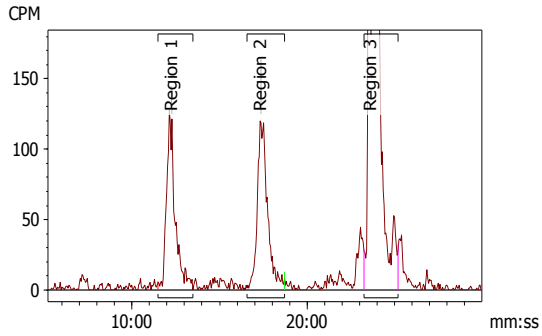
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 91
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:30	13:28	12:15	4272	8.99	8.12
Region 2	16:33	18:43	17:22	4928	10.37	9.37
Region 3	23:16	25:11	23:37	38317	80.64	72.87
3 Peaks				47517	100.00	90.37

Total Area: 52579 CPM
Average Background: 0 CPM

FYX-037 C21V359A(10uL).21.21.21.D3prog(20 uM).30C.8C1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 10:32:37 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:42:13 PM

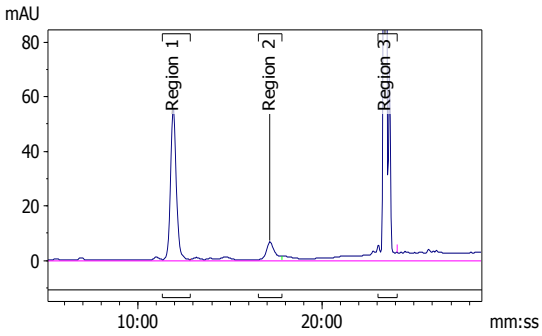
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 52
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:18	12:50	11:53	1190	15.52	12.04
Region 2	16:31	17:49	17:08	151	1.97	1.53
Region 3	23:01	24:04	23:23	6326	82.52	64.04
3 Peaks				7667	100.00	77.61

Total Area: 9879 mAU
Average Background: N/A mAU

FYX-037 C21V359A(10uL).21.21.21.D3prog(20 uM).30C.8C1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 10:32:37 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:42:13 PM

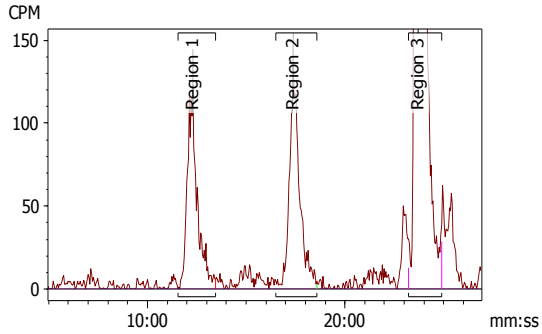
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 52
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:32	13:27	12:17	4211	9.02	7.88
Region 2	16:31	18:36	17:22	4739	10.15	8.87
Region 3	23:13	24:55	23:41	37718	80.82	70.59
3 Peaks				46669	100.00	87.33

Total Area: 53437 CPM
Average Background: 0 CPM

FYX-037 C21V359A(10uL).21.21.21.D3prog(20 uM).30C.8C2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 11:07:29 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:44:48 PM

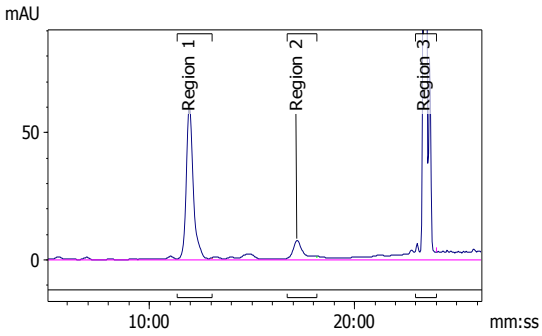
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 53
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:22	13:04	11:56	1394	16.16	12.61
Region 2	16:44	18:09	17:11	178	2.06	1.61
Region 3	23:00	23:59	23:24	7056	81.78	63.83
3 Peaks				8627	100.00	78.04

Total Area: 11054 mAU
Average Background: N/A mAU

FYX-037 C21V359A(10uL).21.21.21.D3prog(20 uM).30C.8C2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 11:07:29 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:44:48 PM

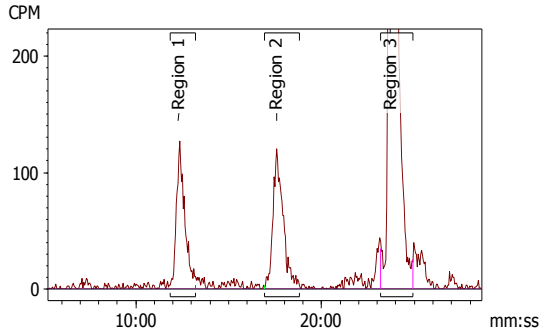
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 53
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:51	13:14	12:17	3942	9.70	8.59
Region 2	16:56	18:48	17:34	4531	11.15	9.87
Region 3	23:12	24:55	23:43	32163	79.15	70.07
3 Peaks				40637	100.00	88.53

Total Area: 45901 CPM
Average Background: 0 CPM

FYX-037 C21V359A(10uL).21.21.21.D3prog(20 uM).30C.8C3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 11:42:20 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:46:48 PM

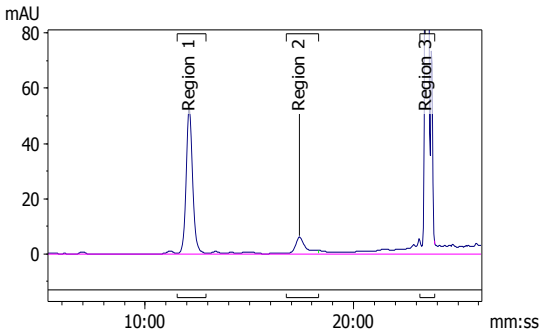
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 54
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:33	12:55	12:05	1120	13.67	11.07
Region 2	16:47	18:19	17:23	148	1.81	1.47
Region 3	23:10	23:54	23:28	6927	84.52	68.46
3 Peaks				8195	100.00	80.99

Total Area: 10118 mAU
Average Background: N/A mAU

FYX-037 C21V359A(10uL).21.21.21.D3prog(20 uM).30C.8C3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 11:42:20 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:46:48 PM

Run Length: 30m
Dwell: 1s

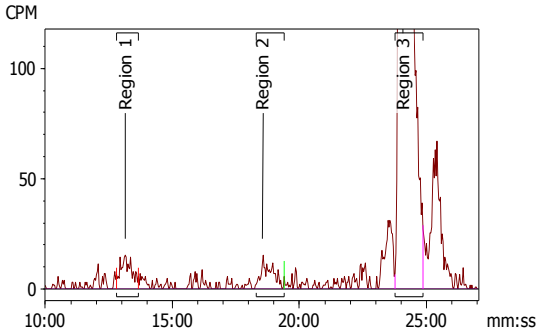
Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %

Off

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 54
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	12:49	13:40	13:09	454	1.99	1.65
Region 2	18:19	19:24	18:33	432	1.89	1.57
Region 3	23:47	24:53	24:05	21939	96.12	79.83
3 Peaks				22826	100.00	83.06

Total Area: 27482 CPM
Average Background: 0 CPM

FYX-037 C21V359A(10uL).21.21.21.D3prog(20 uM).55C.8D1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 5:33:33 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:52:49 PM

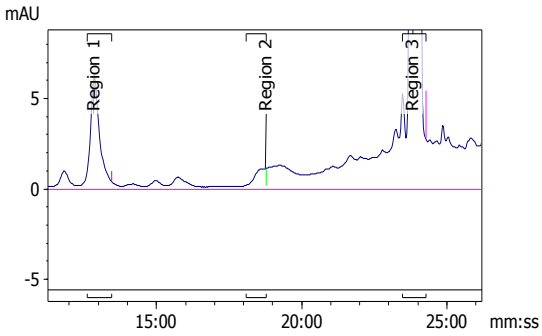
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 79
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	12:37	13:27	12:51	99	2.00	1.41
Region 2	18:06	18:46	18:45	4	0.08	0.06
Region 3	23:28	24:16	23:48	4818	97.91	68.75
3 Peaks				4921	100.00	70.22

Total Area: 7008 mAU
Average Background: N/A mAU

FYX-037 C21V359A(10uL).21.21.21.D3prog(20 uM).55C.8D1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 5:33:33 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:52:49 PM

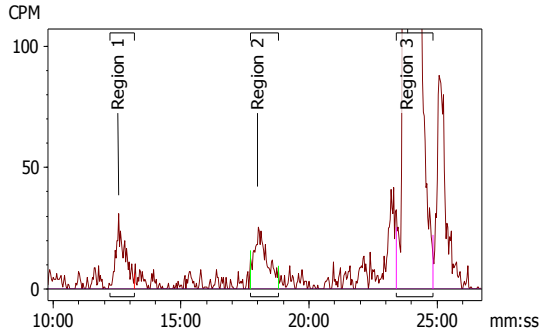
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 79
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	12:13	13:11	12:33	704	2.30	1.95
Region 2	17:42	18:49	18:00	890	2.91	2.47
Region 3	23:25	24:50	23:51	29014	94.79	80.54
3 Peaks				30608	100.00	84.96

Total Area: 36026 CPM
Average Background: 0 CPM

FYX-037 C21V359A(10uL).21.21.21.D3prog(20 uM).55C.8D2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 6:08:24 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:57:51 PM

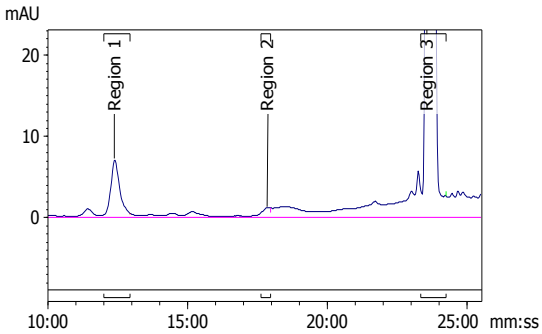
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 80
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:59	12:57	12:23	139	2.49	1.77
Region 2	17:37	17:58	17:51	3	0.06	0.04
Region 3	23:20	24:14	23:34	5437	97.45	69.31
3 Peaks				5579	100.00	71.12

Total Area: 7844 mAU
Average Background: N/A mAU

FYX-037 C21V359A(10uL).21.21.21.D3prog(20 uM).55C.8D2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 6:08:24 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:57:51 PM

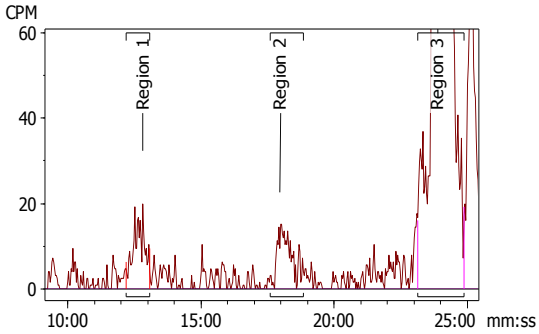
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 80
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	12:11	13:04	12:49	531	2.02	1.74
Region 2	17:35	18:51	17:58	570	2.16	1.87
Region 3	23:08	24:53	23:52	25222	95.82	82.83
3 Peaks				26323	100.00	86.44

Total Area: 30451 CPM
Average Background: 0 CPM

FYX-037 C21V359A(10uL).21.21.21.D3prog(20 uM).55C.8D3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 6:43:17 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:59:50 PM

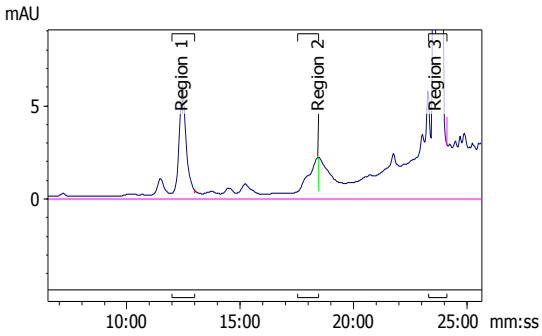
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 81
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	12:00	12:58	12:26	113	1.87	1.32
Region 2	17:31	18:26	18:25	-2	-0.03	-0.02
Region 3	23:19	24:07	23:35	5931	98.16	69.39
3 Peaks				6043	100.00	70.69

Total Area: 8548 mAU
Average Background: N/A mAU

FYX-037 C21V359A(10uL).21.21.21.D3prog(20 uM).55C.8D3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 6:43:17 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:59:50 PM

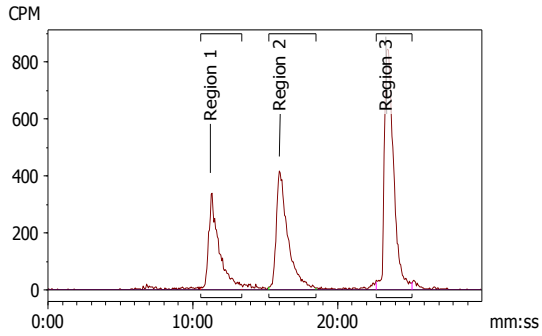
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 81
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:33	13:25	11:15	17066	21.88	20.34
Region 2	15:14	18:33	16:01	23338	29.92	27.81
Region 3	22:41	25:09	23:21	37587	48.19	44.79
3 Peaks				77990	100.00	92.94

Total Area: 83917 CPM
Average Background: 0 CPM

FYX-037 21.21.16D.prog(20uM).C21V359A.10uL.RT(22 C).9A2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 1:31:34 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:22:48 PM

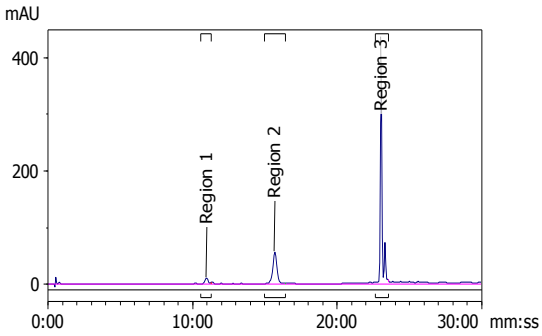
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 66
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:33	11:16	10:56	118	2.79	1.71
Region 2	15:00	16:24	15:39	1096	25.96	15.92
Region 3	22:37	23:31	23:00	3008	71.25	43.68
3 Peaks				4222	100.00	61.31

Total Area: 6887 mAU
Average Background: N/A mAU

FYX-037 21.21.16D.prog(20uM).C21V359A.10uL.RT(22 C).9A2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 1:31:34 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:22:48 PM

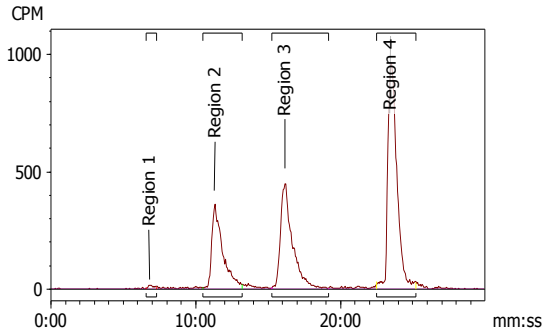
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 66
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	6:35	7:18	6:49	528	0.60	0.56
Region 2	10:31	13:12	11:17	19062	21.63	20.36
Region 3	15:15	19:11	16:10	25926	29.42	27.70
Region 4	22:30	25:13	23:28	42605	48.35	45.51
4 Peaks				88122	100.00	94.13

Total Area: 93613 CPM
Average Background: 0 CPM

FYX-037 21.21.16D.prog(20uM).C21V359A.10uL.RT(22 C).9A3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 2:06:29 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:25:26 PM

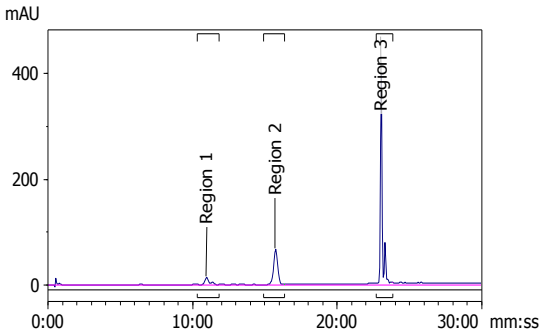
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 67
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:19	11:50	10:56	249	5.03	3.21
Region 2	14:54	16:23	15:43	1296	26.22	16.74
Region 3	22:40	23:50	23:00	3397	68.75	43.88
3 Peaks				4941	100.00	63.82

Total Area: 7741 mAU
Average Background: N/A mAU

FYX-037 21.21.16D.prog(20uM).C21V359A.10uL.RT(22 C).9A3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 2:06:29 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:25:26 PM

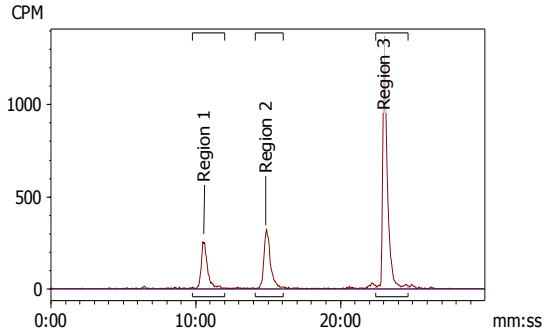
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 67
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	9:47	12:00	10:34	8000	16.73	15.36
Region 2	14:08	16:02	14:51	10198	21.33	19.58
Region 3	22:28	24:41	23:02	29622	61.94	56.86
3 Peaks				47821	100.00	91.79

Total Area: 52096 CPM
Average Background: 0 CPM

FYX-037 16Dprog(20uM).C21V359A. 10uL.(26 C).9B1 Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, March 07, 2012 12:54:48 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 3:12:39 PM

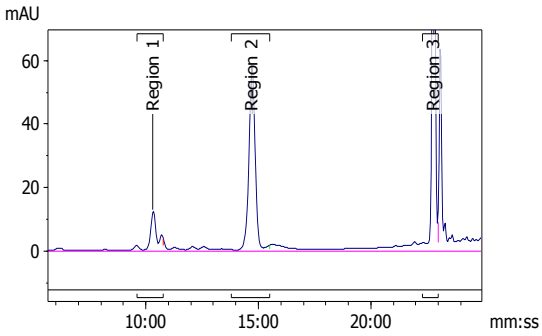
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 92
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:37	10:47	10:19	72	2.13	1.09
Region 2	13:48	15:31	14:42	1042	30.85	15.85
Region 3	22:18	22:59	22:47	2264	67.02	34.42
3 Peaks				3379	100.00	51.36

Total Area: 6578 mAU
Average Background: N/A mAU

FYX-037 16Dprog(20uM).C21V359A. 10uL.(26 C).9B1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, March 07, 2012 12:54:48 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 3:12:39 PM

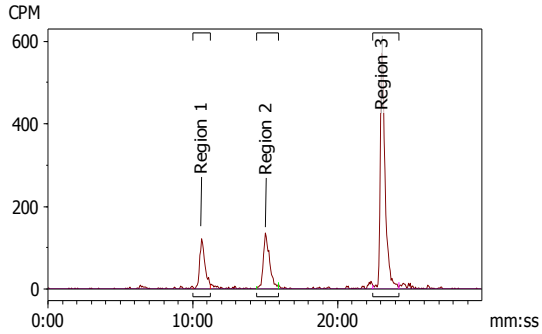
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 92
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:02	11:15	10:35	3101	14.96	13.26
Region 2	14:25	15:55	15:03	4074	19.65	17.42
Region 3	22:27	24:15	23:05	13552	65.39	57.95
3 Peaks				20726	100.00	88.63

Total Area: 23386 CPM
Average Background: 0 CPM

FYX-037 16Dprog(20uM).C21V359A. 10uL.(26 C).9B2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, March 07, 2012 3:54:27 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 3:22:29 PM

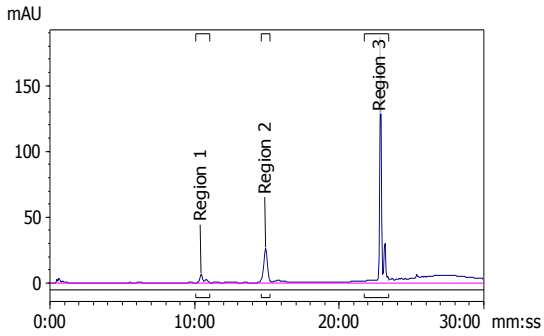
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 93
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:05	11:02	10:25	109	5.53	2.32
Region 2	14:35	15:11	14:53	389	19.75	8.30
Region 3	21:45	23:27	22:49	1471	74.72	31.39
3 Peaks				1968	100.00	42.02

Total Area: 4684 mAU
Average Background: N/A mAU

FYX-037 16Dprog(20uM).C21V359A. 10uL.(26 C).9B2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, March 07, 2012 3:54:27 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 3:22:29 PM

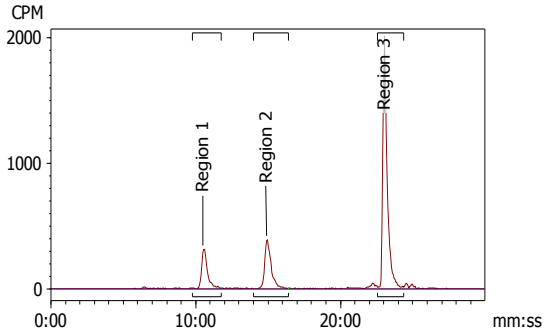
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 93
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	9:47	11:46	10:29	9213	14.44	13.20
Region 2	13:59	16:23	14:54	12288	19.25	17.61
Region 3	22:32	24:22	23:01	42317	66.31	60.65
3 Peaks				63818	100.00	91.46

Total Area: 69773 CPM
Average Background: 0 CPM

FYX-037 16Dprog(20uM).C21V359A. 10uL.(26 C).9B3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, March 07, 2012 2:44:38 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 3:18:20 PM

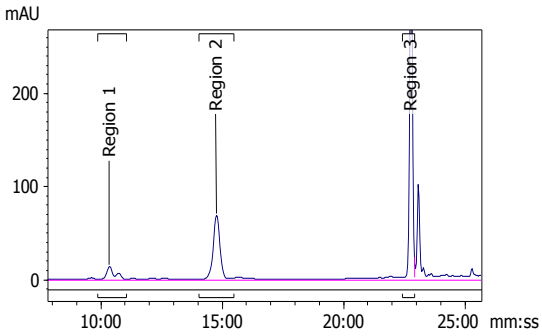
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 94
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:51	11:03	10:21	309	6.18	3.43
Region 2	14:02	15:29	14:45	1289	25.73	14.30
Region 3	22:26	22:55	22:46	3411	68.09	37.83
3 Peaks				5010	100.00	55.56

Total Area: 9018 mAU
Average Background: N/A mAU

FYX-037 16Dprog(20uM).C21V359A. 10uL.(26 C).9B3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, March 07, 2012 2:44:38 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 3:18:20 PM

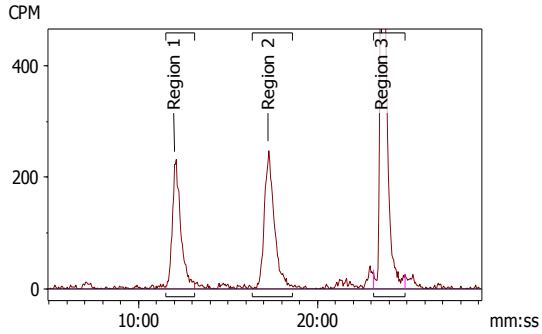
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 94
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:31	13:08	12:00	7610	19.43	17.08
Region 2	16:23	18:35	17:15	9478	24.21	21.28
Region 3	23:08	24:54	23:37	22067	56.36	49.54
3 Peaks				39155	100.00	87.91

Total Area: 44541 CPM
Average Background: 0 CPM

FYX-037 C21V359A(10uL).16Dprog(20 uM).30C.9C1 Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 12:17:13 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:57:20 PM

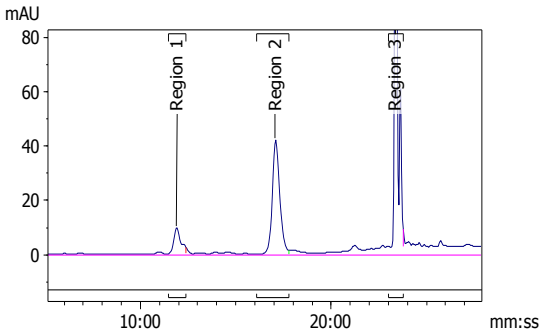
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 55
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:29	12:24	11:53	181	4.91	2.85
Region 2	16:06	17:47	17:04	1069	28.98	16.83
Region 3	23:00	23:47	23:22	2438	66.10	38.38
3 Peaks				3688	100.00	58.06

Total Area: 6351 mAU
Average Background: N/A mAU

FYX-037 C21V359A(10uL).16Dprog(20 uM).30C.9C1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 12:17:13 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:57:20 PM

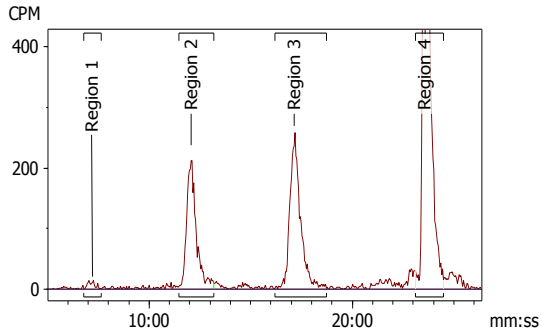
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 55
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	6:48	7:39	7:12	323	0.76	0.68
Region 2	11:28	13:10	12:05	7053	16.65	14.77
Region 3	16:11	18:44	17:09	9242	21.81	19.35
Region 4	23:08	24:29	23:33	25750	60.78	53.91
4 Peaks				42368	100.00	88.70

Total Area: 47763 CPM
Average Background: 0 CPM

FYX-037 C21V359A(10uL).16Dprog(20 uM).30C.9C2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 12:52:05 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:59:57 PM

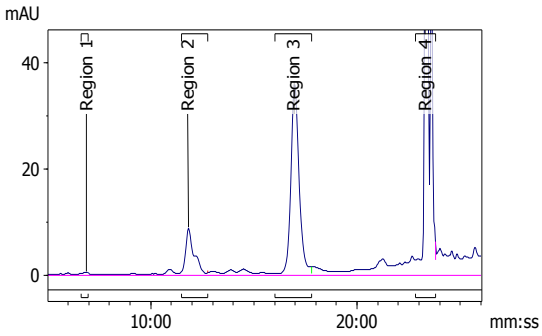
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 56
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	6:37	6:57	6:53	1	0.03	0.02
Region 2	11:30	12:46	11:49	207	5.56	3.27
Region 3	16:02	17:49	16:57	909	24.47	14.39
Region 4	22:51	23:48	23:20	2597	69.93	41.13
4 Peaks				3713	100.00	58.82

Total Area: 6313 mAU
Average Background: N/A mAU

FYX-037 C21V359A(10uL).16Dprog(20 uM).30C.9C2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 12:52:05 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:59:57 PM

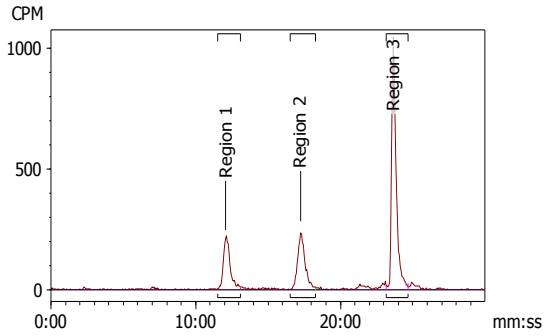
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 56
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:32	13:05	12:05	7251	18.79	16.28
Region 2	16:32	18:16	17:14	8541	22.13	19.17
Region 3	23:10	24:41	23:40	22797	59.08	51.17
3 Peaks				38589	100.00	86.61

Total Area: 44554 CPM
Average Background: 0 CPM

FYX-037 C21V359A(10uL).16Dprog(20 uM).30C.9C3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 1:26:57 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:01:43 PM

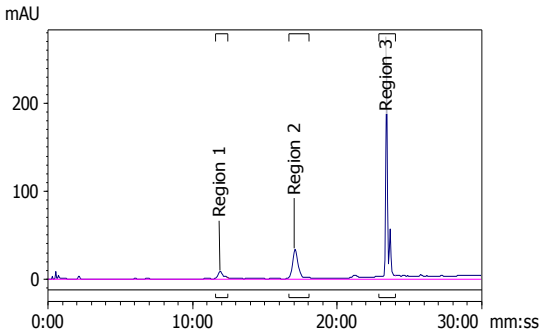
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 57
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:34	12:25	11:52	155	4.93	2.65
Region 2	16:38	18:03	17:03	785	24.90	13.37
Region 3	22:54	24:00	23:22	2212	70.17	37.68
3 Peaks				3153	100.00	53.71

Total Area: 5870 mAU
Average Background: N/A mAU

FYX-037 C21V359A(10uL).16Dprog(20 uM).30C.9C3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 1:26:57 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:01:43 PM

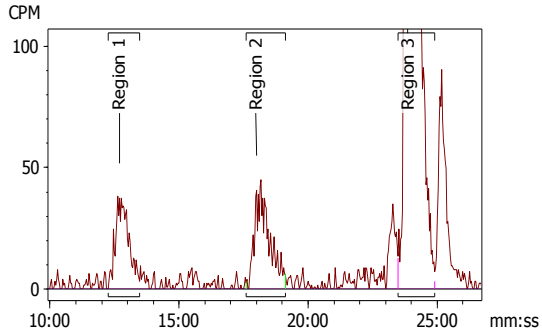
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 57
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	12:16	13:29	12:41	1363	5.12	4.37
Region 2	17:35	19:08	18:00	1709	6.42	5.48
Region 3	23:29	24:55	23:53	23549	88.46	75.47
3 Peaks				26621	100.00	85.31

Total Area: 31203 CPM
Average Background: 0 CPM

FYX-037 C21V359A(10uL).16Dprog(20 uM).55C.9D1 Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 7:18:09 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:09:28 PM

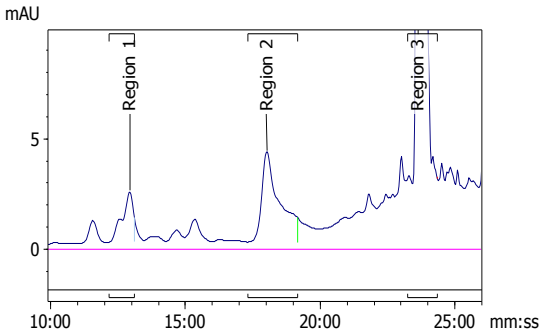
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 82
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	12:11	13:07	12:56	32	1.06	0.57
Region 2	17:19	19:10	18:01	124	4.12	2.23
Region 3	23:15	24:21	23:38	2859	94.82	51.36
3 Peaks				3015	100.00	54.16

Total Area: 5567 mAU
Average Background: N/A mAU

FYX-037 C21V359A(10uL).16Dprog(20 uM).55C.9D1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 7:18:09 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:09:28 PM

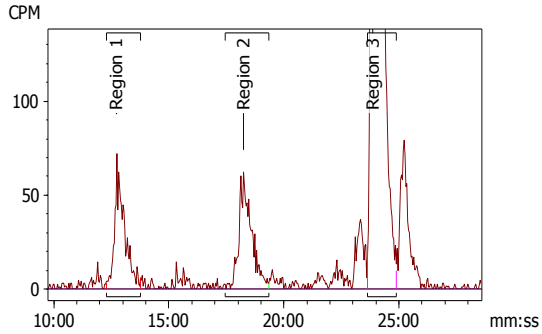
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 82
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	12:17	13:47	12:44	2022	6.62	5.63
Region 2	17:27	19:20	18:15	2275	7.45	6.33
Region 3	23:39	24:53	23:53	26246	85.93	73.06
3 Peaks				30544	100.00	85.03

Total Area: 35923 CPM
Average Background: 0 CPM

FYX-037 C21V359A(10uL).16Dprog(20 uM).55C.9D2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 7:52:59 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:11:05 PM

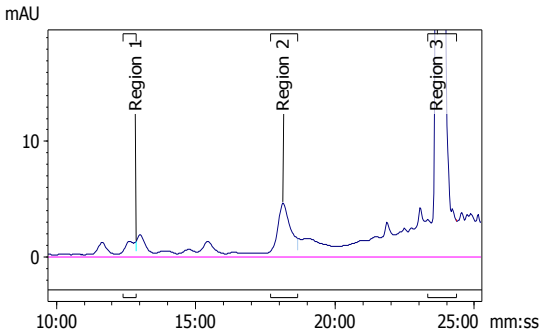
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 83
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	12:23	12:52	12:51	6	0.20	0.11
Region 2	17:42	18:40	18:08	89	3.11	1.63
Region 3	23:19	24:22	23:40	2775	96.69	50.68
3 Peaks				2870	100.00	52.42

Total Area: 5474 mAU
Average Background: N/A mAU

FYX-037 C21V359A(10uL).16Dprog(20 uM).55C.9D2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 7:52:59 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:11:05 PM

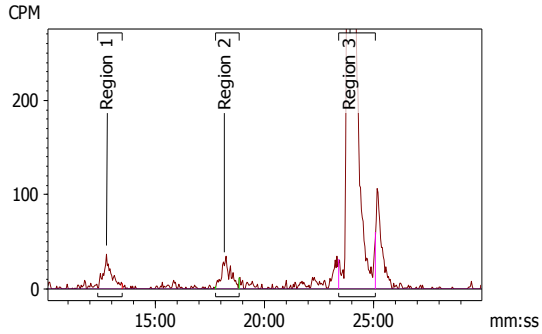
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 83
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	12:21	13:28	12:45	909	3.51	2.98
Region 2	17:45	18:50	18:09	928	3.58	3.05
Region 3	23:23	25:04	23:54	24074	92.91	79.00
3 Peaks				25910	100.00	85.03

Total Area: 30474 CPM
Average Background: 0 CPM

FYX-037 C21V359A(10uL).16Dprog(20 uM).55C.9D3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 8:27:49 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:14:44 PM

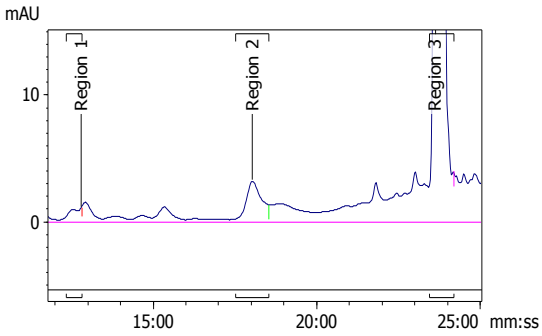
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 84
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	12:20	12:49	12:48	3	0.11	0.05
Region 2	17:32	18:33	18:02	58	2.27	1.15
Region 3	23:27	24:11	23:38	2507	97.62	49.24
3 Peaks				2568	100.00	50.45

Total Area: 5090 mAU
Average Background: N/A mAU

FYX-037 C21V359A(10uL).16Dprog(20 uM).55C.9D3Method: Halogens_Test1

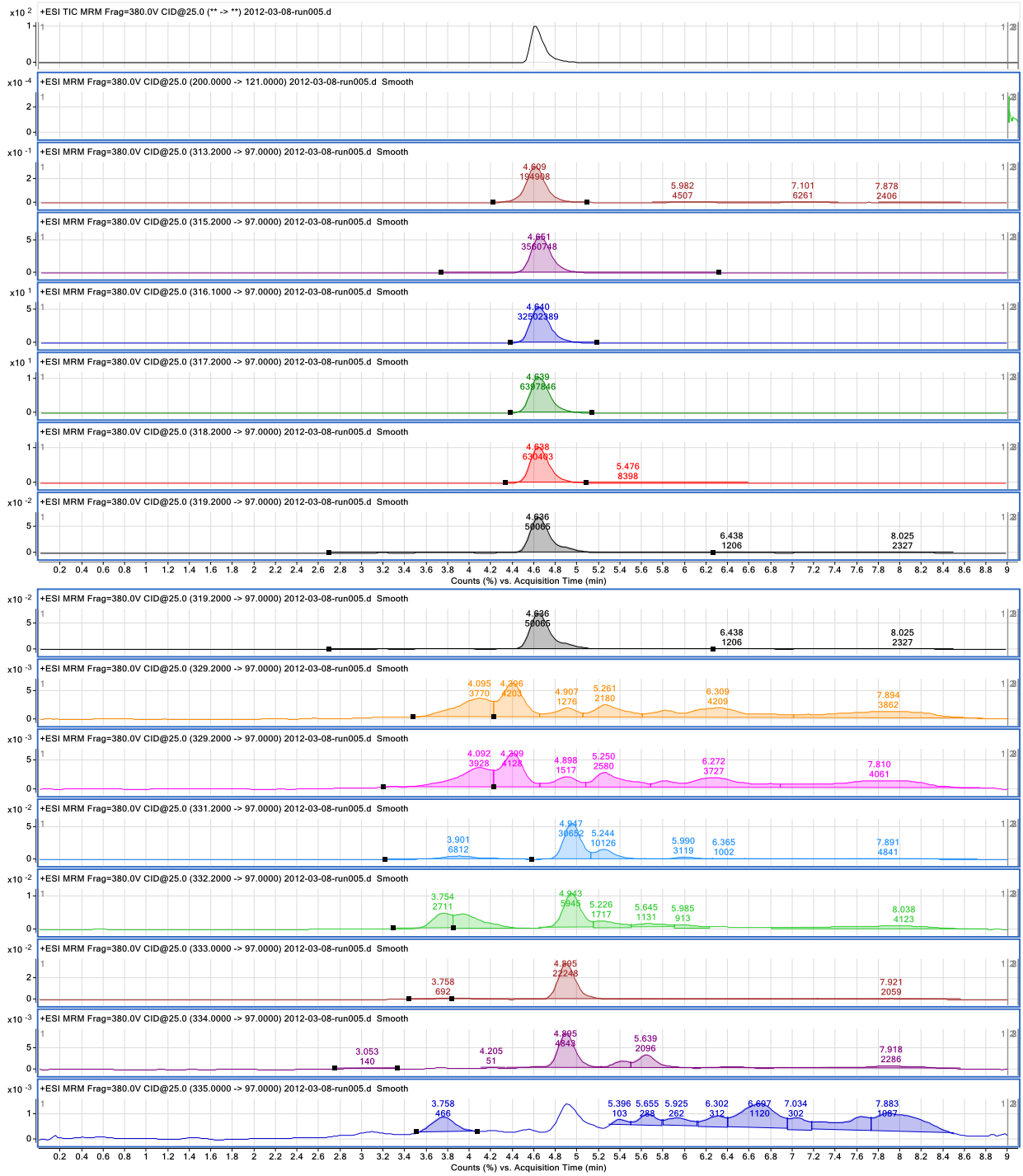
Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 8:27:49 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:14:44 PM

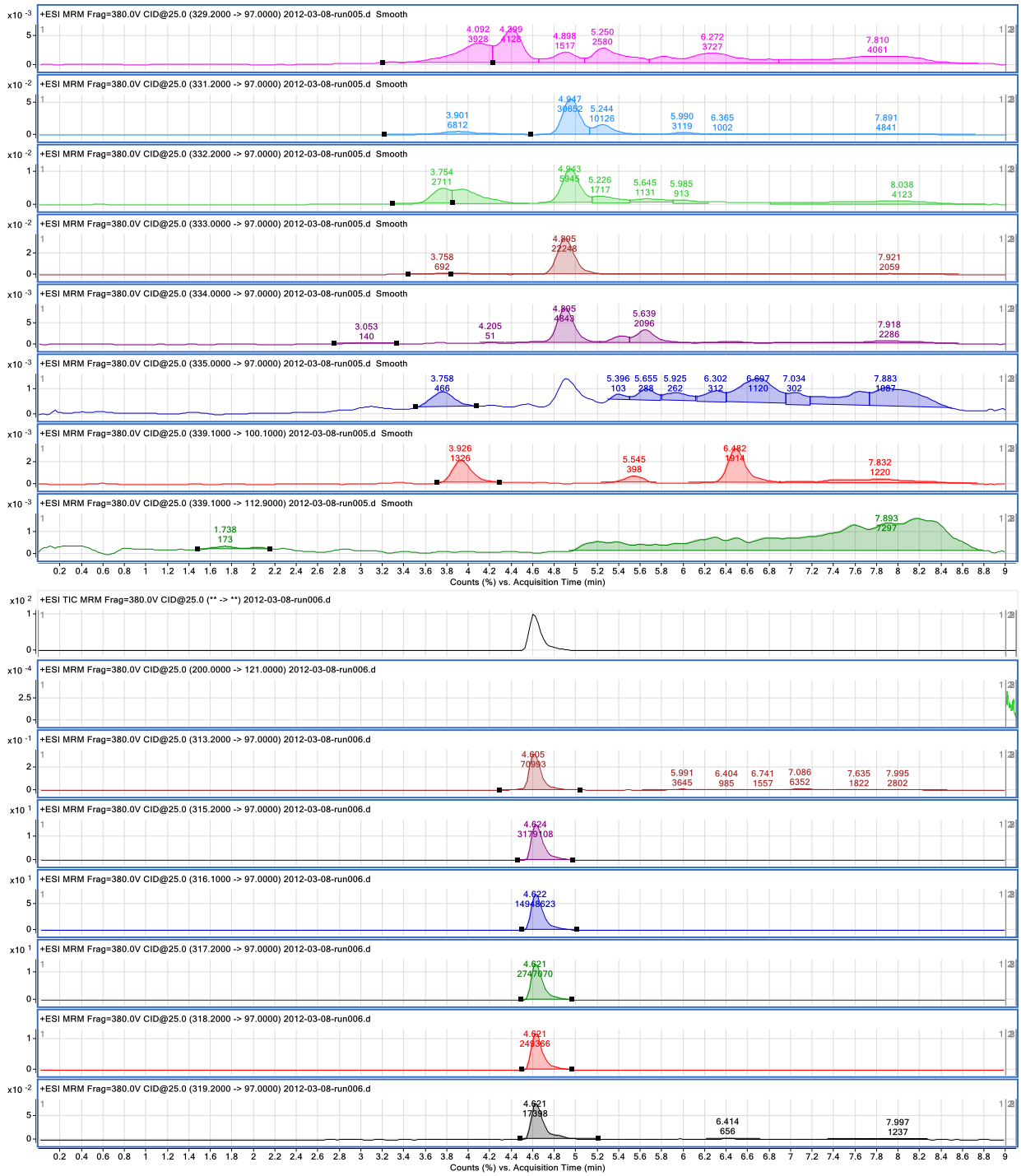
Run Length: 30m
Dwell: 1s

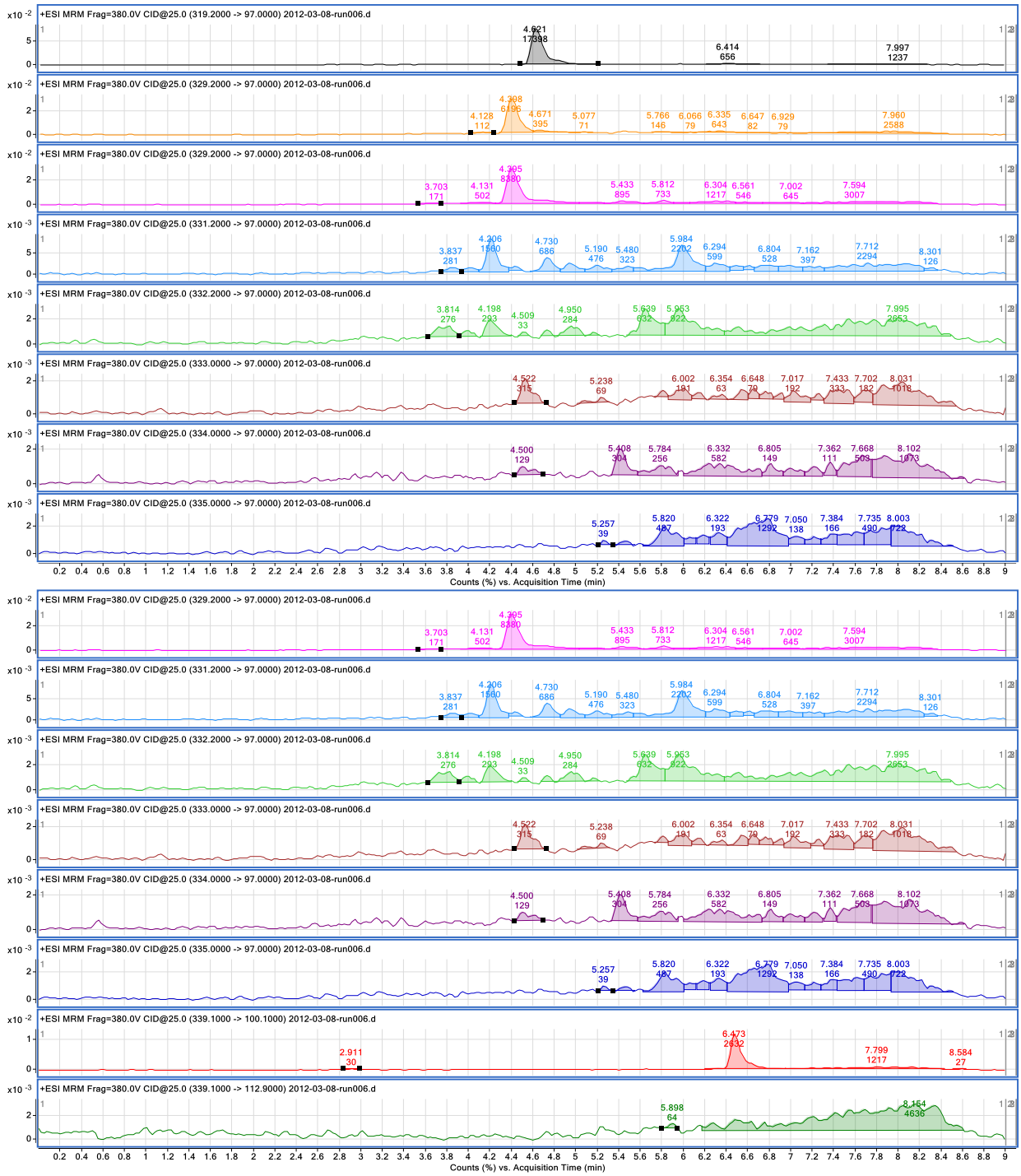
Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

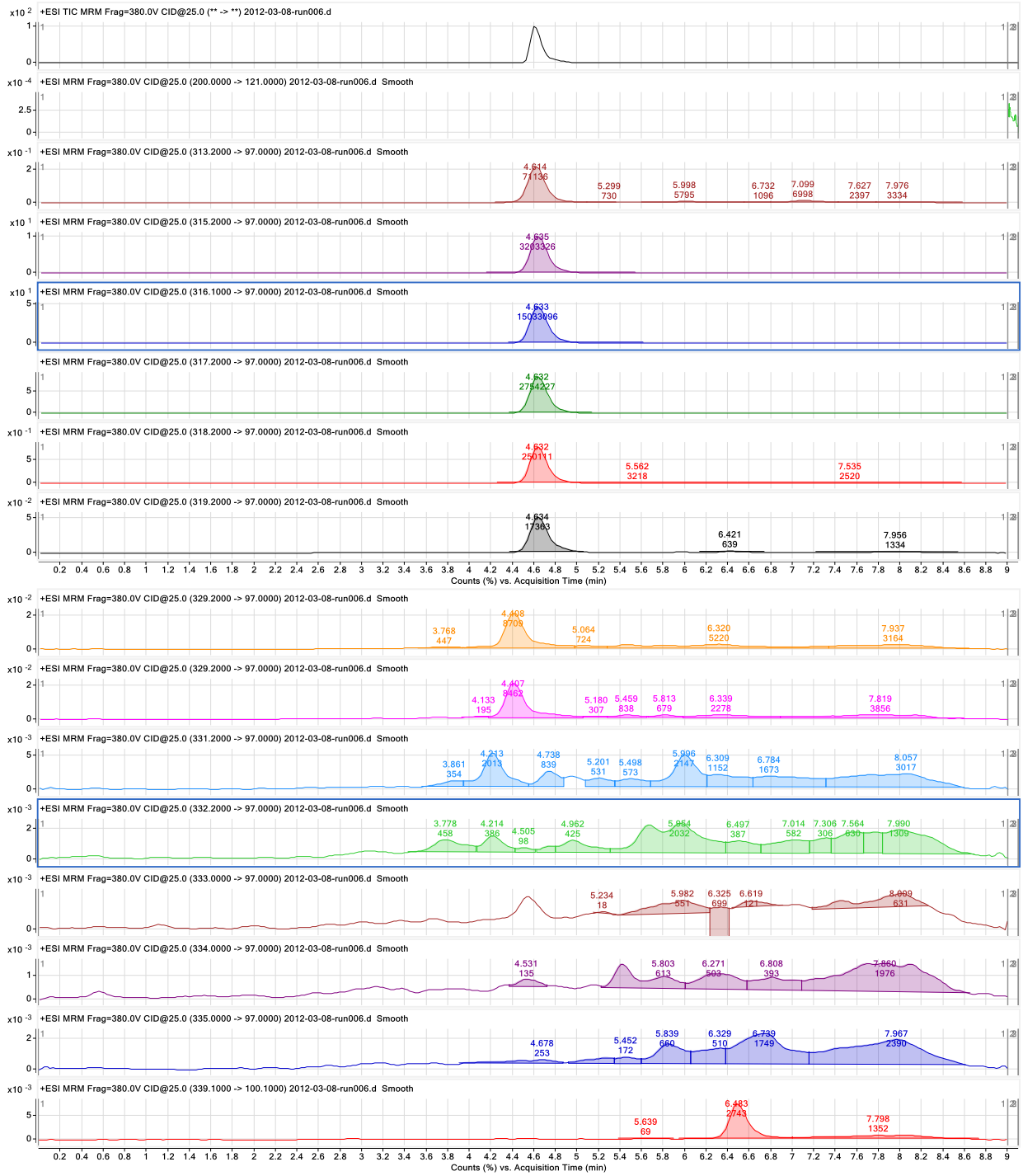
Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 84
Injection Volume: 5 µL

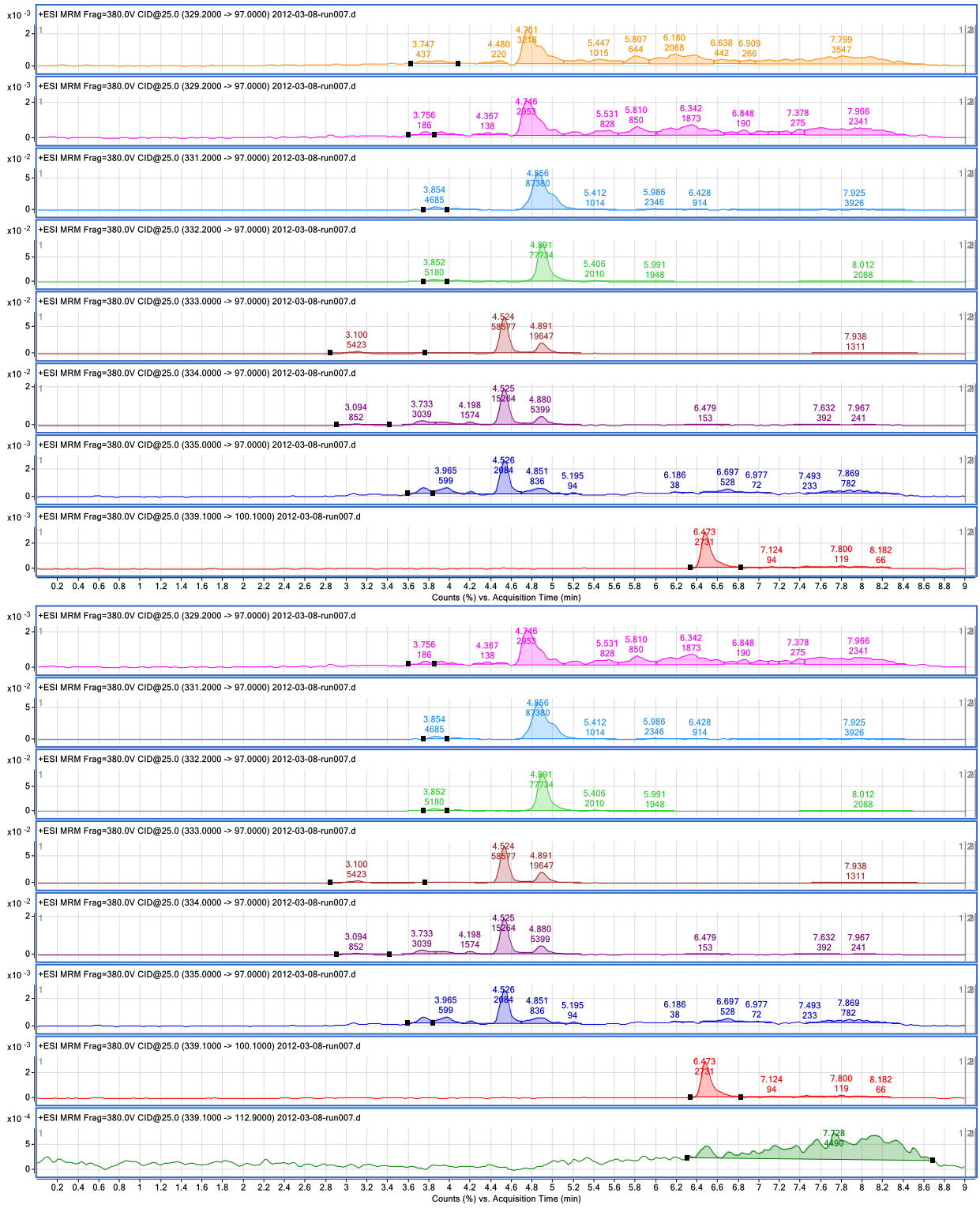
Comments:

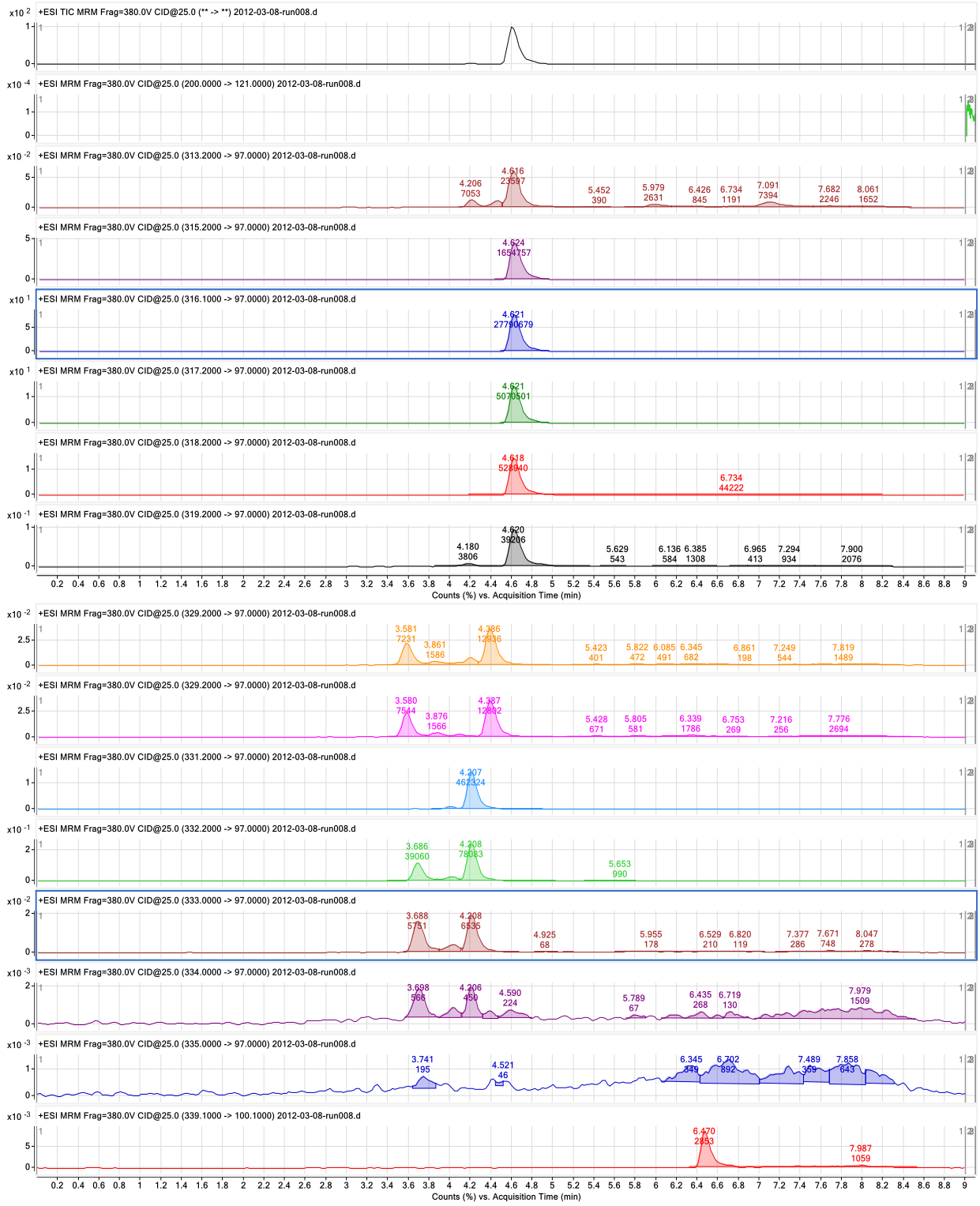

x10 2 +ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run005.d
x10 -4 +ESI MRM Frag=380.0V CID@25.0 (200.0000 -> 121.0000) 2012-03-08-run005.d Smooth
x10 -1 +ESI MRM Frag=380.0V CID@25.0 (313.2000 -> 97.0000) 2012-03-08-run005.d Smooth
4.609 194998
5.982 4507
7.101 6261
7.878 2406
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run005.d Smooth
4.651 3560748
x10 1 +ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run005.d Smooth
4.640 32502389
x10 1 +ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run005.d Smooth
4.639 6387846
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run005.d Smooth
4.638 630483
5.476 8398
x10 -2 +ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run005.d Smooth
4.636 58065
6.438 1206
8.025 2327
Counts (%) vs. Acquisition Time (min)
x10 -2 +ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run005.d Smooth
4.636 58065
6.438 1206
8.025 2327
x10 -3 +ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run005.d Smooth
4.095 3770
4.396 4203
4.907 1276
5.261 2180
6.309 4209
7.894 3862
x10 -3 +ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run005.d Smooth
4.092 3928
4.399 4128
4.898 1517
5.250 2580
6.272 3727
7.810 4061
x10 -2 +ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run005.d Smooth
4.947 30632
3.901 6812
5.244 10126
5.990 3119
6.365 1002
7.891 4841
x10 -2 +ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run005.d Smooth
3.754 2711
4.943 5945
5.226 1717
5.645 1131
5.985 913
8.038 4123
x10 -2 +ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run005.d Smooth
4.895 22248
3.758 692
7.921 2059
x10 -3 +ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run005.d Smooth
4.895 4843
5.639 2096
3.053 140
4.205 51
7.918 2286
x10 -3 +ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run005.d Smooth
3.758 466
5.396 103
5.655 288
5.925 262
6.302 312
6.697 1120
7.034 302
7.883 1087
Counts (%) vs. Acquisition Time (min)

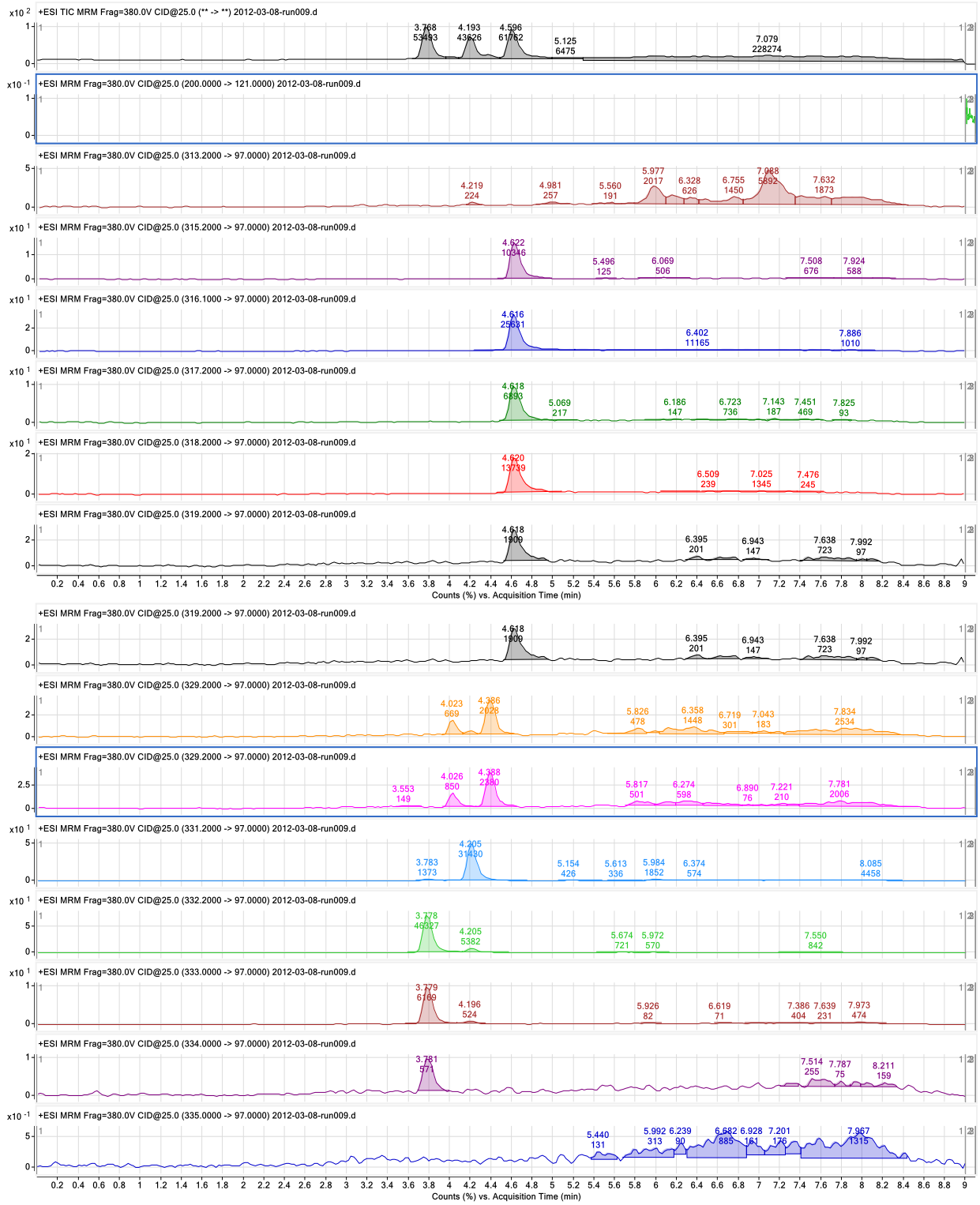

+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run005.d Smooth
4.092 3928
4.399 4128
4.898 1517
5.250 2580
6.272 3727
7.810 4061
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run005.d Smooth
3.901 6812
4.947 30652
5.244 10126
5.990 3119
6.365 1002
7.891 4841
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run005.d Smooth
3.754 2711
4.943 5945
5.226 1717
5.645 1131
5.985 913
8.038 4123
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run005.d Smooth
3.758 692
4.895 22248
7.921 2059
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run005.d Smooth
3.053 140
4.205 51
4.895 4843
5.639 2096
7.918 2286
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run005.d Smooth
3.758 466
5.396 103
5.655 288
5.925 262
6.302 312
6.697 1120
7.034 302
7.883 1667
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 100.1000) 2012-03-08-run005.d Smooth
3.926 1326
5.545 398
6.482 1914
7.832 1220
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 112.9000) 2012-03-08-run005.d Smooth
1.738 173
7.893 7297
Counts (%) vs. Acquisition Time (min)
+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run006.d
+ESI MRM Frag=380.0V CID@25.0 (200.0000 -> 121.0000) 2012-03-08-run006.d
+ESI MRM Frag=380.0V CID@25.0 (313.2000 -> 97.0000) 2012-03-08-run006.d
4.605 70993
5.991 3645
6.404 985
6.741 1557
7.086 6352
7.635 1822
7.995 2802
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run006.d
4.624 3179108
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run006.d
4.622 14943623
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run006.d
4.621 2747070
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run006.d
4.621 249366
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run006.d
4.621 17398
6.414 656
7.997 1237
Counts (%) vs. Acquisition Time (min)

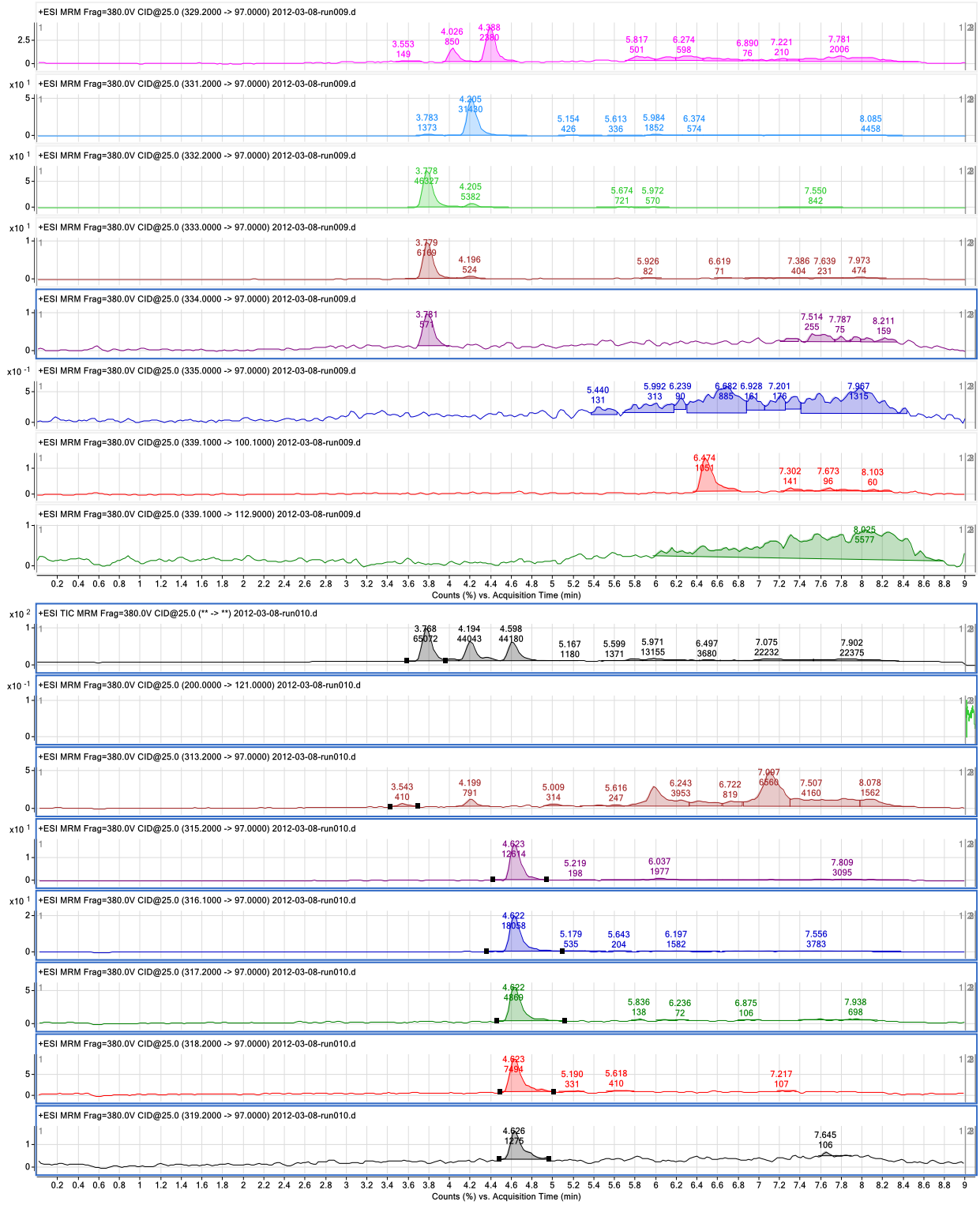

+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run006.d
4.921 17398
6.414 656
7.997 1237
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run006.d
4.128 112
4.308 6196
4.671 395
5.077 71
5.766 146
6.066 79
6.335 643
6.647 82
6.929 79
7.960 2588
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run006.d
3.703 171
4.131 502
4.395 8180
5.433 895
5.812 733
6.304 1217
6.561 546
7.002 645
7.594 3007
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run006.d
3.837 281
4.206 1500
4.730 686
5.190 476
5.480 323
5.984 2702
6.294 599
6.804 528
7.162 397
7.712 2294
8.301 126
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run006.d
3.814 276
4.198 293
4.509 33
4.950 284
5.639 632
5.953 922
7.995 2953
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run006.d
4.522 315
5.238 69
6.002 191
6.354 63
6.648 79
7.017 192
7.433 333
7.702 182
8.031 1018
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run006.d
4.500 129
5.408 304
5.784 256
6.332 582
6.805 149
7.362 111
7.668 503
8.102 1473
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run006.d
5.257 39
5.820 487
6.322 193
6.779 1292
7.050 138
7.384 166
7.735 490
8.003 722
Counts (%) vs. Acquisition Time (min)
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run006.d
3.703 171
4.131 502
4.395 8180
5.433 895
5.812 733
6.304 1217
6.561 546
7.002 645
7.594 3007
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run006.d
3.837 281
4.206 1500
4.730 686
5.190 476
5.480 323
5.984 2702
6.294 599
6.804 528
7.162 397
7.712 2294
8.301 126
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run006.d
3.814 276
4.198 293
4.509 33
4.950 284
5.639 632
5.953 922
7.995 2953
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run006.d
4.522 315
5.238 69
6.002 191
6.354 63
6.648 79
7.017 192
7.433 333
7.702 182
8.031 1018
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run006.d
4.500 129
5.408 304
5.784 256
6.332 582
6.805 149
7.362 111
7.668 503
8.102 1473
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run006.d
5.257 39
5.820 487
6.322 193
6.779 1292
7.050 138
7.384 166
7.735 490
8.003 722
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 100.1000) 2012-03-08-run006.d
2.911 30
6.473 2632
7.799 1217
8.584 27
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 112.9000) 2012-03-08-run006.d
5.898 64
8.164 4636
Counts (%) vs. Acquisition Time (min)

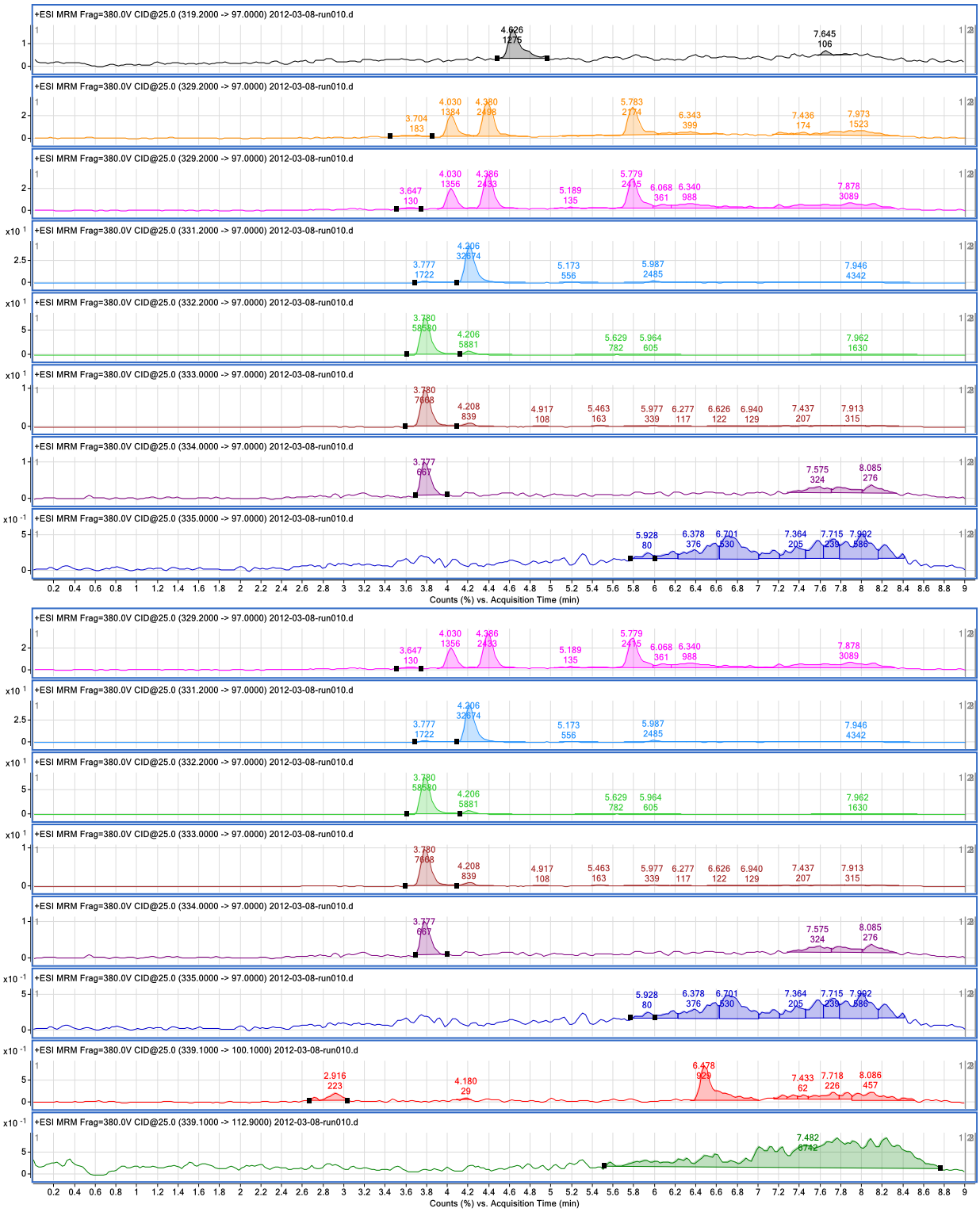

+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run006.d
+ESI MRM Frag=380.0V CID@25.0 (200.0000 -> 121.0000) 2012-03-08-run006.d Smooth
+ESI MRM Frag=380.0V CID@25.0 (313.2000 -> 97.0000) 2012-03-08-run006.d Smooth
4.614 71136
5.299 730
5.998 5795
6.732 1096
7.099 6998
7.627 2397
7.976 3334
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run006.d Smooth
4.635 3203326
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run006.d Smooth
4.633 15033096
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run006.d Smooth
4.632 2754227
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run006.d Smooth
4.632 250111
5.562 3218
7.535 2520
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run006.d Smooth
4.634 17383
6.421 639
7.956 1334
Counts (%) vs. Acquisition Time (min)
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run006.d Smooth
3.768 447
4.408 8709
5.064 724
6.320 5220
7.937 3164
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run006.d Smooth
4.133 195
4.407 8462
5.180 307
5.459 838
5.813 679
6.339 2278
7.819 3856
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run006.d Smooth
3.861 354
4.213 2013
4.738 839
5.201 531
5.498 573
5.996 2147
6.309 1152
6.784 1673
8.057 3017
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run006.d Smooth
3.778 458
4.214 386
4.505 98
4.962 425
5.954 2032
6.497 387
7.014 582
7.306 306
7.564 630
7.990 1309
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run006.d Smooth
5.234 18
5.982 551
6.325 699
6.619 121
8.009 631
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run006.d Smooth
4.531 135
5.803 613
6.271 503
6.808 393
7.860 1976
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run006.d Smooth
4.678 253
5.452 172
5.839 660
6.329 510
6.739 1749
7.967 2390
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 100.1000) 2012-03-08-run006.d Smooth
5.639 69
6.483 2743
7.798 1352
Counts (%) vs. Acquisition Time (min)

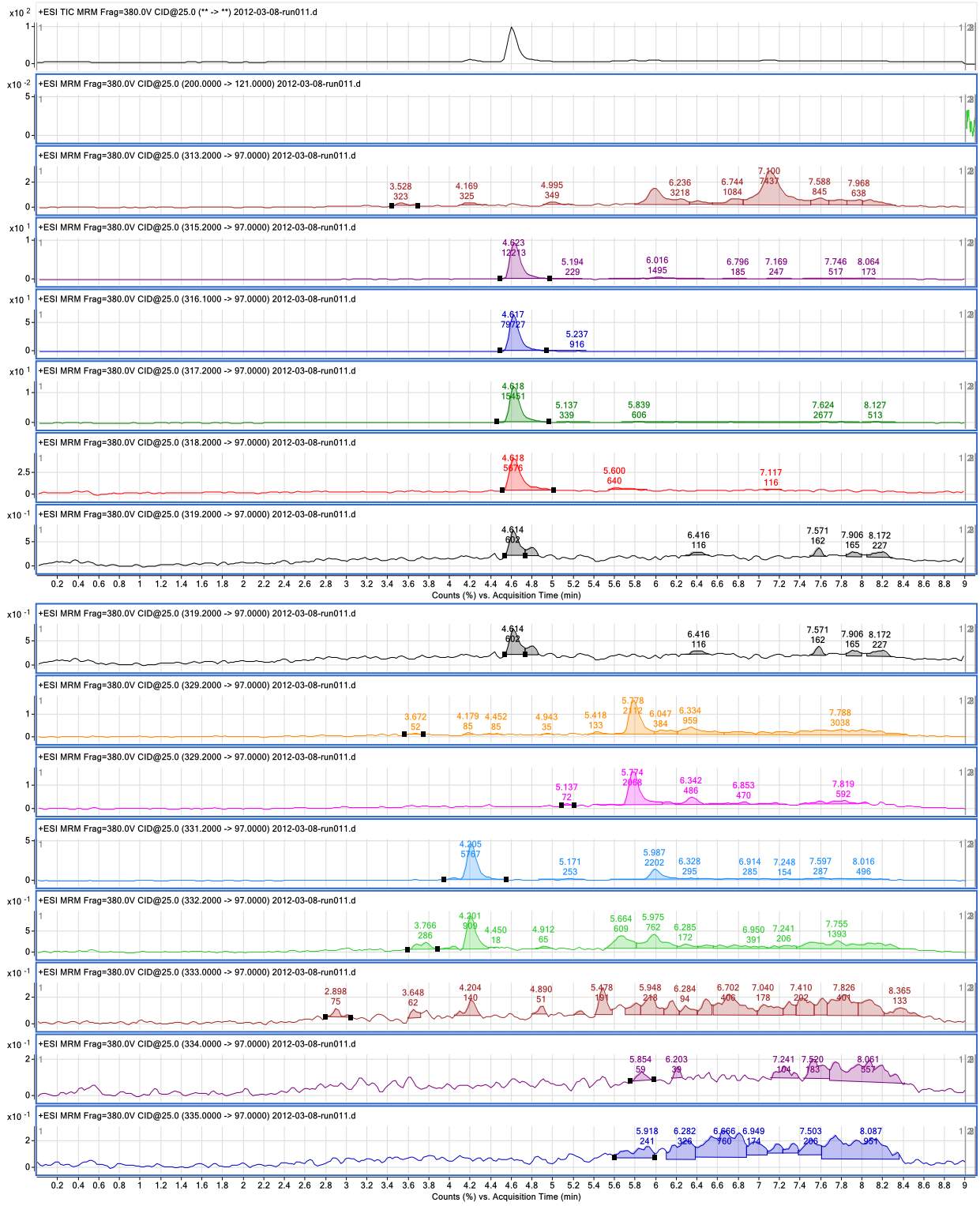

+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run007.d
3.747 437
4.480 220
4.751 3218
5.447 1015
5.807 644
6.180 2068
6.638 442
6.909 266
7.799 3547
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run007.d
3.756 186
4.367 138
4.746 2953
5.531 828
5.810 850
6.342 1873
6.848 190
7.378 275
7.966 2341
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run007.d
3.854 4685
4.856 87380
5.412 1014
5.986 2346
6.428 914
7.925 3926
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run007.d
3.852 5180
4.901 77734
5.406 2010
5.991 1948
8.012 2088
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run007.d
3.100 5423
4.524 58577
4.891 19647
7.938 1311
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run007.d
3.094 852
3.733 3039
4.198 1574
4.525 15264
4.880 5399
6.479 153
7.632 392
7.967 241
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run007.d
3.965 599
4.526 2084
4.851 836
5.195 94
6.186 38
6.697 528
6.977 72
7.493 233
7.869 782
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 100.1000) 2012-03-08-run007.d
6.473 2731
7.124 94
7.800 119
8.182 66
Counts (%) vs. Acquisition Time (min)
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run007.d
3.756 186
4.367 138
4.746 2953
5.531 828
5.810 850
6.342 1873
6.848 190
7.378 275
7.966 2341
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run007.d
3.854 4685
4.856 87380
5.412 1014
5.986 2346
6.428 914
7.925 3926
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run007.d
3.852 5180
4.901 77734
5.406 2010
5.991 1948
8.012 2088
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run007.d
3.100 5423
4.524 58577
4.891 19647
7.938 1311
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run007.d
3.094 852
3.733 3039
4.198 1574
4.525 15264
4.880 5399
6.479 153
7.632 392
7.967 241
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run007.d
3.965 599
4.526 2084
4.851 836
5.195 94
6.186 38
6.697 528
6.977 72
7.493 233
7.869 782
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 100.1000) 2012-03-08-run007.d
6.473 2731
7.124 94
7.800 119
8.182 66
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 112.9000) 2012-03-08-run007.d
7.728 4490
Counts (%) vs. Acquisition Time (min)

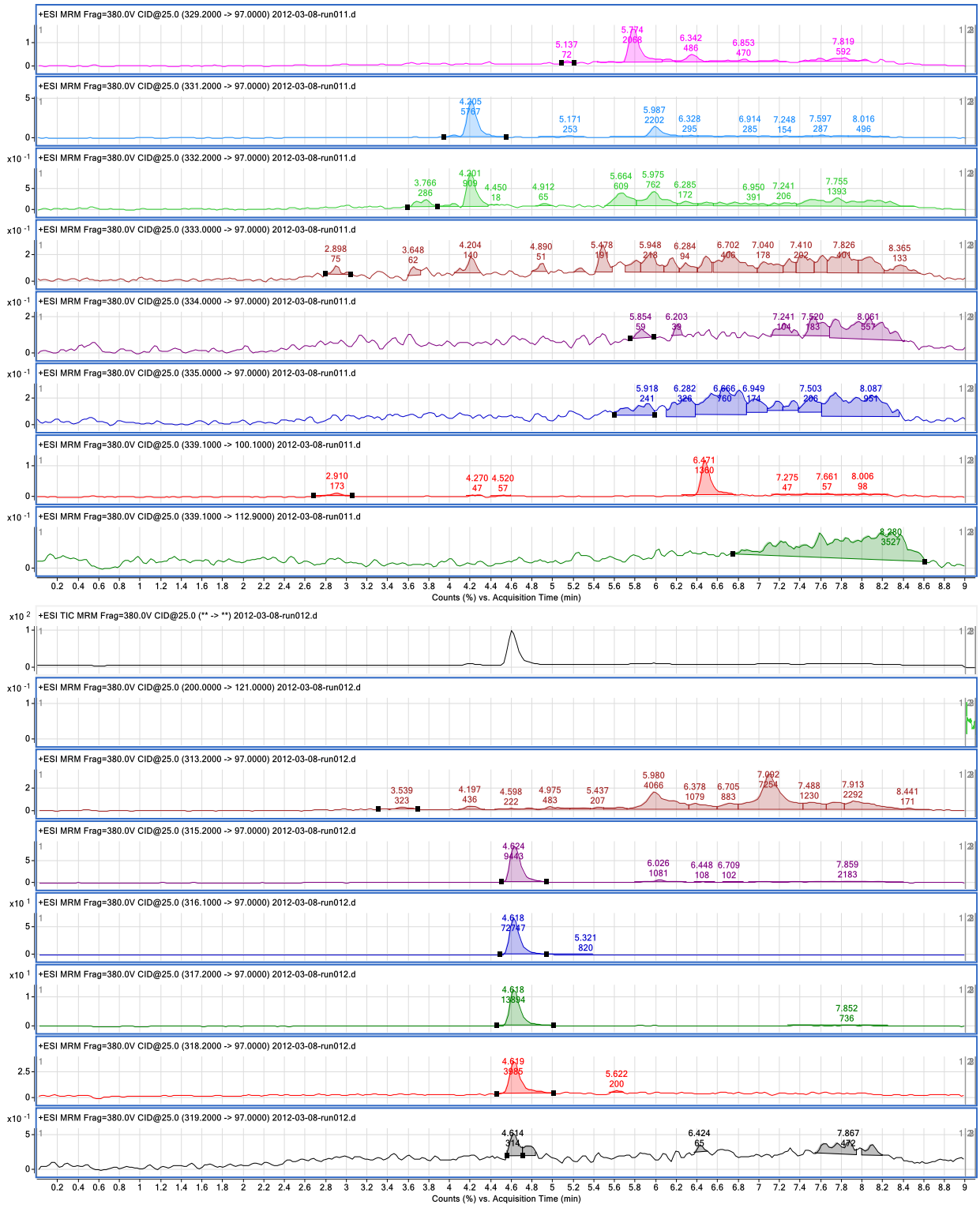

+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run008.d
+ESI MRM Frag=380.0V CID@25.0 (200.0000 -> 121.0000) 2012-03-08-run008.d
+ESI MRM Frag=380.0V CID@25.0 (313.2000 -> 97.0000) 2012-03-08-run008.d
4.206 7053
4.616 23587
5.452 390
5.979 2631
6.426 845
6.734 1191
7.091 7394
7.682 2246
8.061 1652
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run008.d
4.624 1654757
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run008.d
4.621 27790679
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run008.d
4.621 5070501
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run008.d
4.618 528940
6.734 44222
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run008.d
4.180 3806
4.620 39206
5.629 543
6.136 584
6.385 1308
6.965 413
7.294 934
7.900 2076
Counts (%) vs. Acquisition Time (min)
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run008.d
3.581 7231
3.861 1586
4.386 12936
5.423 401
5.822 472
6.085 491
6.345 682
6.861 198
7.249 544
7.819 1489
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run008.d
3.580 7544
3.876 1566
4.387 12882
5.428 671
5.805 581
6.339 1786
6.753 269
7.216 256
7.776 2694
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run008.d
4.207 462324
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run008.d
3.686 39060
4.208 78083
5.653 990
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run008.d
3.688 5751
4.208 6535
4.925 68
5.955 178
6.529 210
6.820 119
7.377 286
7.671 748
8.047 278
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run008.d
3.698 566
4.206 450
4.590 224
5.789 67
6.435 268
6.719 130
7.979 1509
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run008.d
3.741 195
4.521 46
6.345 349
6.702 892
7.489 359
7.858 643
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 100.1000) 2012-03-08-run008.d
6.470 2053
7.987 1059
Counts (%) vs. Acquisition Time (min)

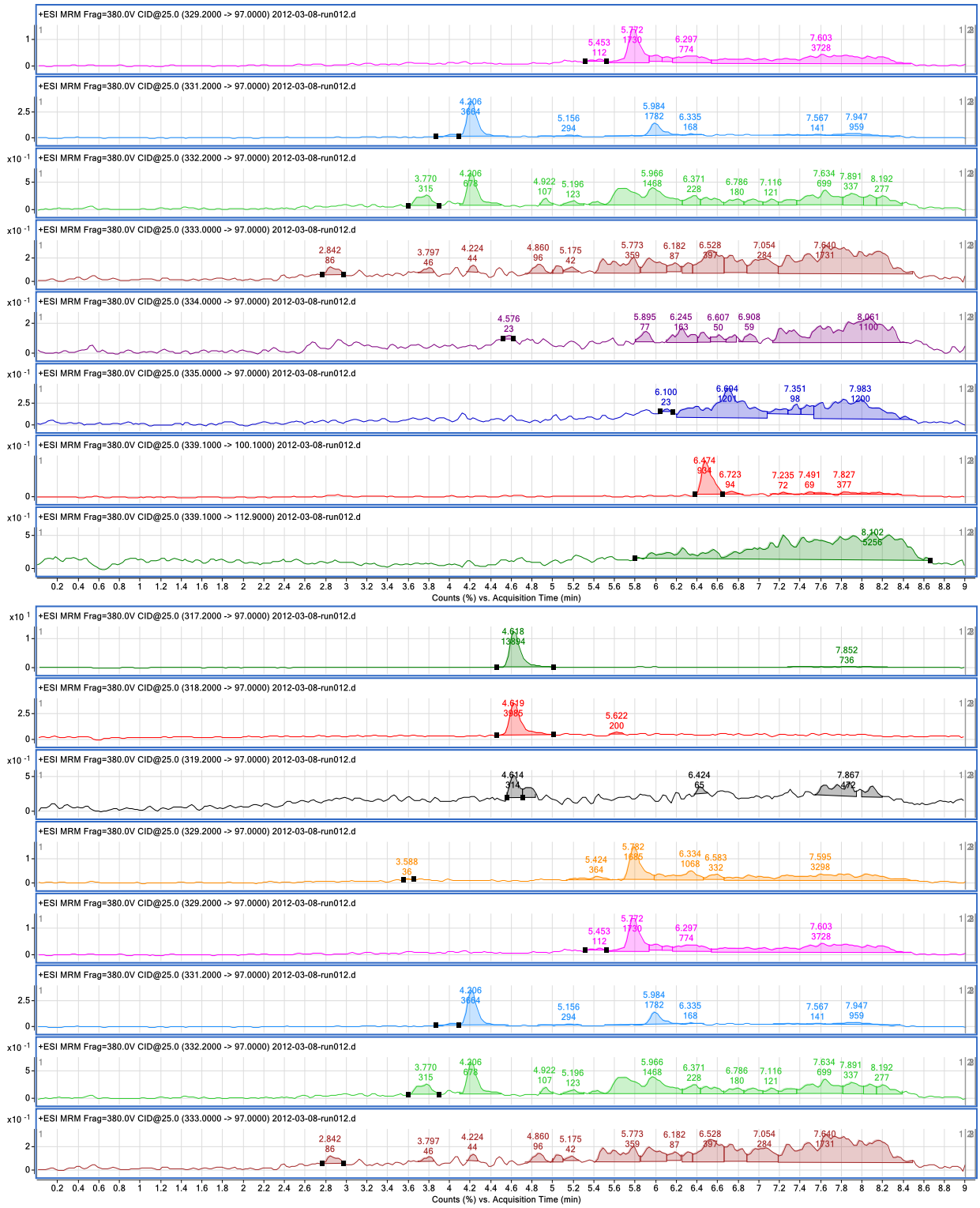

+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run009.d
3.768 53493
4.193 43626
4.596 61752
5.125 6475
7.079 228274
+ESI MRM Frag=380.0V CID@25.0 (200.0000 -> 121.0000) 2012-03-08-run009.d
+ESI MRM Frag=380.0V CID@25.0 (313.2000 -> 97.0000) 2012-03-08-run009.d
4.219 224
4.981 257
5.560 191
5.977 2017
6.328 626
6.755 1450
7.088 5692
7.632 1873
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run009.d
4.622 10346
5.496 125
6.069 506
7.508 676
7.924 588
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run009.d
4.616 25631
6.402 11165
7.886 1010
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run009.d
4.618 6893
5.069 217
6.186 147
6.723 736
7.143 187
7.451 469
7.825 93
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run009.d
4.620 13739
6.509 239
7.025 1345
7.476 245
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run009.d
4.618 1909
6.395 201
6.943 147
7.638 723
7.992 97
Counts (%) vs. Acquisition Time (min)
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run009.d
4.618 1909
6.395 201
6.943 147
7.638 723
7.992 97
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run009.d
4.023 669
4.386 2028
5.826 478
6.358 1448
6.719 301
7.043 183
7.834 2534
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run009.d
3.553 149
4.026 850
4.388 2280
5.817 501
6.274 598
6.890 76
7.221 210
7.781 2006
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run009.d
3.783 1373
4.205 31430
5.154 426
5.613 336
5.984 1852
6.374 574
8.085 4458
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run009.d
3.778 46327
4.205 5382
5.674 721
5.972 570
7.550 842
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run009.d
3.779 6189
4.196 524
5.926 82
6.619 71
7.386 404
7.639 231
7.973 474
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run009.d
3.781 571
7.514 255
7.787 75
8.211 159
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run009.d
5.440 131
5.992 313
6.239 90
6.682 885
6.928 161
7.201 176
7.967 1315
Counts (%) vs. Acquisition Time (min)

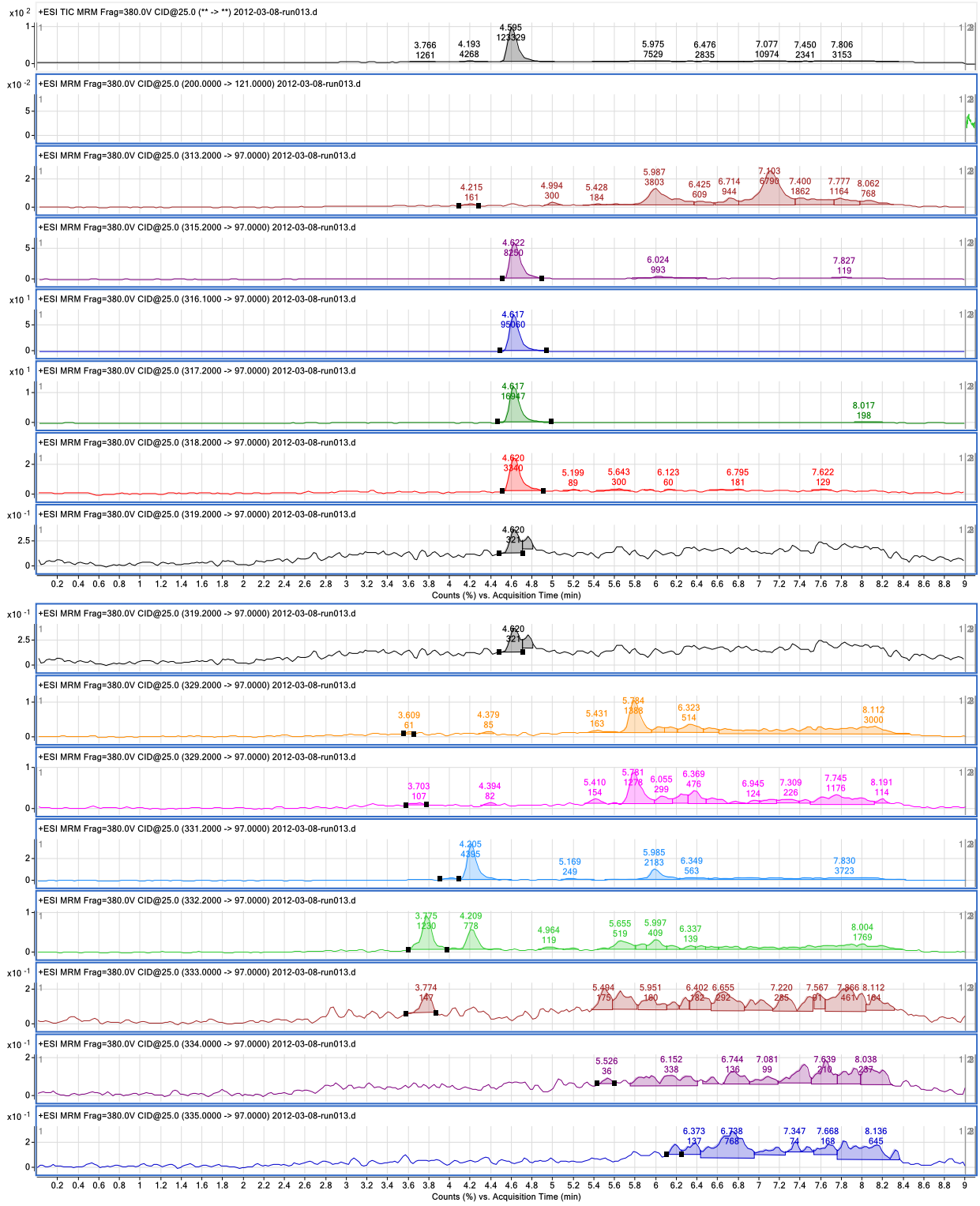

+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run009.d
3.553 149
4.026 850
4.388 2390
5.817 501
6.274 598
6.890 76
7.221 210
7.781 2006
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run009.d
3.783 1373
4.205 31430
5.154 426
5.613 336
5.984 1852
6.374 574
8.085 4458
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run009.d
3.778 46327
4.205 5382
5.674 721
5.972 570
7.550 842
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run009.d
3.779 6189
4.196 524
5.926 82
6.619 71
7.386 404
7.639 231
7.973 474
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run009.d
3.781 571
7.514 255
7.787 75
8.211 159
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run009.d
5.440 131
5.992 313
6.239 90
6.682 885
6.928 161
7.201 176
7.967 1315
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 100.1000) 2012-03-08-run009.d
6.474 1051
7.302 141
7.673 96
8.103 60
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 112.9000) 2012-03-08-run009.d
8.025 5577
Counts (%) vs. Acquisition Time (min)
+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run010.d
3.768 65072
4.194 44043
4.598 44180
5.167 1180
5.599 1371
5.971 13155
6.497 3680
7.075 22232
7.902 22375
+ESI MRM Frag=380.0V CID@25.0 (200.0000 -> 121.0000) 2012-03-08-run010.d
+ESI MRM Frag=380.0V CID@25.0 (313.2000 -> 97.0000) 2012-03-08-run010.d
3.543 410
4.199 791
5.009 314
5.616 247
6.243 3953
6.722 819
7.007 6560
7.507 4160
8.078 1562
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run010.d
4.623 12614
5.219 198
6.037 1977
7.809 3095
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run010.d
4.622 18058
5.179 535
5.643 204
6.197 1582
7.556 3783
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run010.d
4.622 4869
5.836 138
6.236 72
6.875 106
7.938 698
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run010.d
4.623 7494
5.190 331
5.618 410
7.217 107
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run010.d
4.626 1276
7.645 106
Counts (%) vs. Acquisition Time (min)

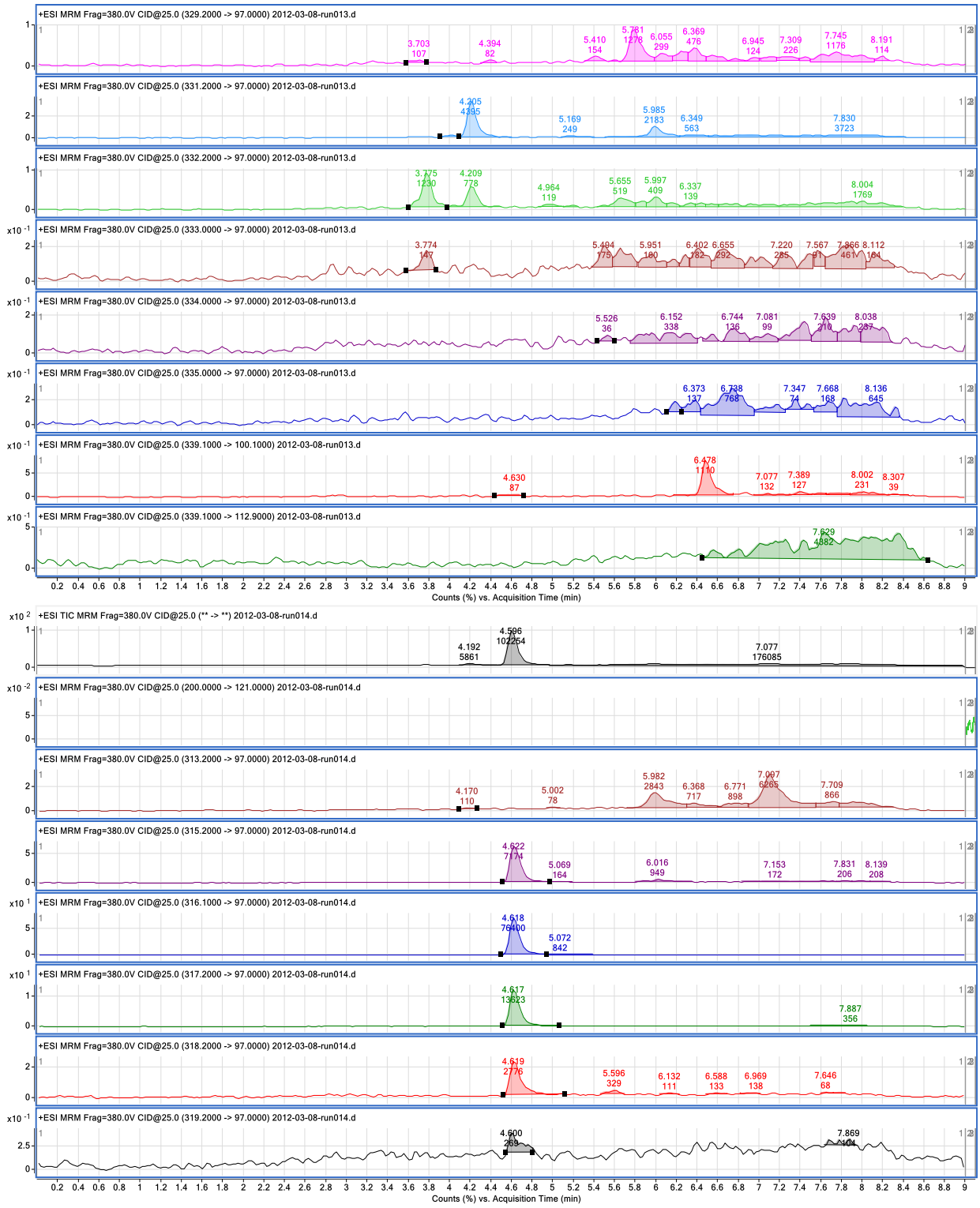

+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run010.d
4.626 1275
7.645 106
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run010.d
3.704 183
4.030 1384
4.380 2498
5.783 2174
6.343 399
7.436 174
7.973 1523
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run010.d
3.647 130
4.030 1356
4.386 2433
5.189 135
5.779 2415
6.068 361
6.340 988
7.878 3089
x10 1
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run010.d
3.777 1722
4.206 32674
5.173 556
5.987 2485
7.946 4342
x10 1
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run010.d
3.780 58580
4.206 5881
5.629 782
5.964 605
7.962 1630
x10 1
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run010.d
3.780 7668
4.208 839
4.917 108
5.463 163
5.977 339
6.277 117
6.626 122
6.940 129
7.437 207
7.913 315
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run010.d
3.777 667
7.575 324
8.085 276
x10 -1
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run010.d
5.928 80
6.378 376
6.701 530
7.364 205
7.715 239
7.992 586
0.2 0.4 0.6 0.8 1 1.2 1.4 1.6 1.8 2 2.2 2.4 2.6 2.8 3 3.2 3.4 3.6 3.8 4 4.2 4.4 4.6 4.8 5 5.2 5.4 5.6 5.8 6 6.2 6.4 6.6 6.8 7 7.2 7.4 7.6 7.8 8 8.2 8.4 8.6 8.8 9
Counts (%) vs. Acquisition Time (min)
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run010.d
3.647 130
4.030 1356
4.386 2433
5.189 135
5.779 2415
6.068 361
6.340 988
7.878 3089
x10 1
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run010.d
3.777 1722
4.206 32674
5.173 556
5.987 2485
7.946 4342
x10 1
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run010.d
3.780 58580
4.206 5881
5.629 782
5.964 605
7.962 1630
x10 1
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run010.d
3.780 7668
4.208 839
4.917 108
5.463 163
5.977 339
6.277 117
6.626 122
6.940 129
7.437 207
7.913 315
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run010.d
3.777 667
7.575 324
8.085 276
x10 -1
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run010.d
5.928 80
6.378 376
6.701 530
7.364 205
7.715 239
7.992 586
x10 -1
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 100.1000) 2012-03-08-run010.d
2.916 223
4.180 29
6.478 929
7.433 62
7.718 226
8.086 457
x10 -1
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 112.9000) 2012-03-08-run010.d
7.482 6742
0.2 0.4 0.6 0.8 1 1.2 1.4 1.6 1.8 2 2.2 2.4 2.6 2.8 3 3.2 3.4 3.6 3.8 4 4.2 4.4 4.6 4.8 5 5.2 5.4 5.6 5.8 6 6.2 6.4 6.6 6.8 7 7.2 7.4 7.6 7.8 8 8.2 8.4 8.6 8.8 9
Counts (%) vs. Acquisition Time (min)

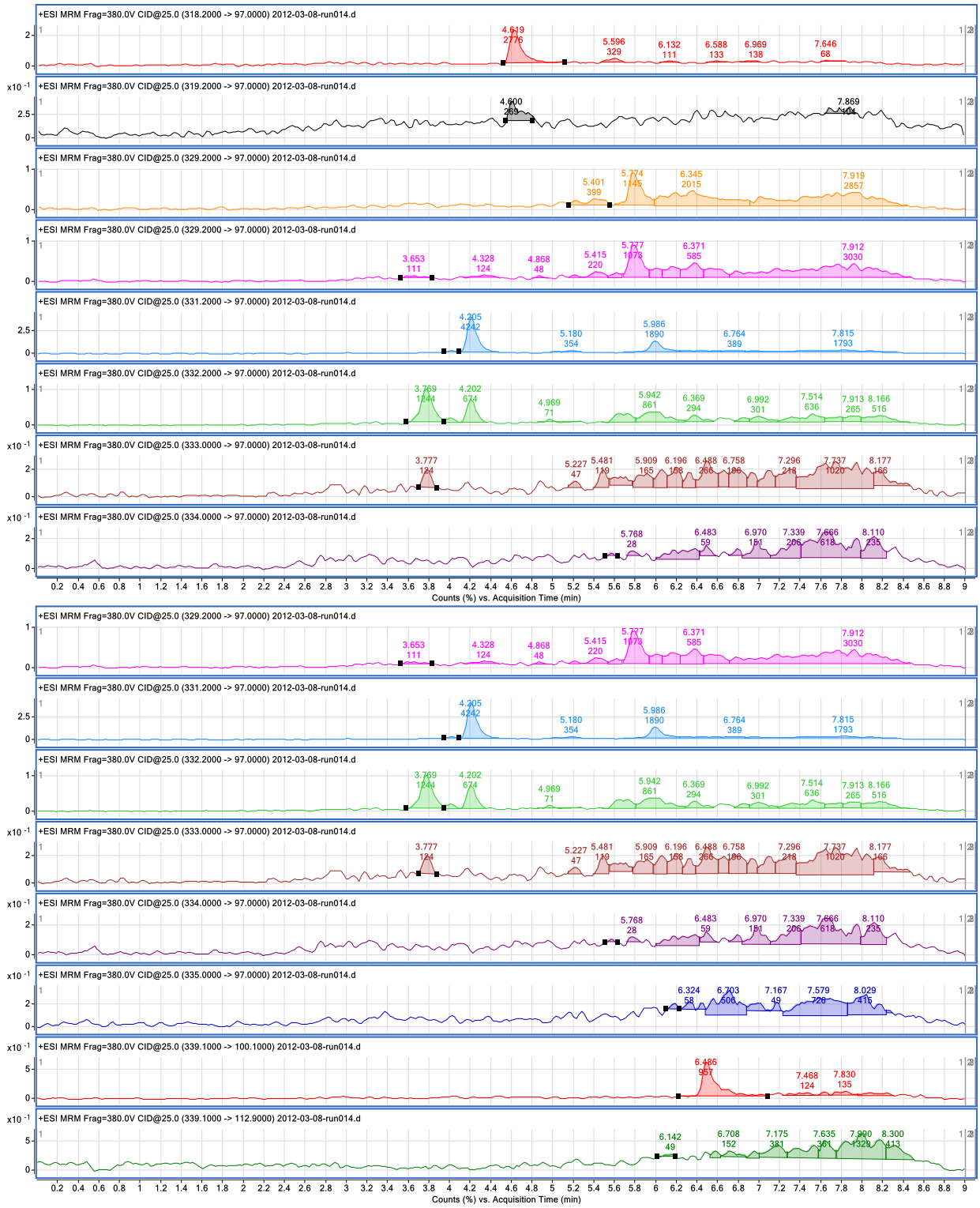

+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run011.d
+ESI MRM Frag=380.0V CID@25.0 (200.0000 -> 121.0000) 2012-03-08-run011.d
+ESI MRM Frag=380.0V CID@25.0 (313.2000 -> 97.0000) 2012-03-08-run011.d
3.528 323
4.169 325
4.995 349
6.236 3218
6.744 1084
7.100 7437
7.588 845
7.968 638
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run011.d
4.623 12213
5.194 229
6.016 1495
6.796 185
7.169 247
7.746 517
8.064 173
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run011.d
4.617 79727
5.237 916
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run011.d
4.618 15451
5.137 339
5.839 606
7.624 2677
8.127 513
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run011.d
4.618 5676
5.600 640
7.117 116
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run011.d
4.614 602
6.416 116
7.571 162
7.906 165
8.172 227
Counts (%) vs. Acquisition Time (min)
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run011.d
4.614 602
6.416 116
7.571 162
7.906 165
8.172 227
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run011.d
3.672 52
4.179 85
4.452 85
4.943 35
5.418 133
5.778 2112
6.047 384
6.334 959
7.788 3038
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run011.d
5.137 72
5.774 2068
6.342 486
6.853 470
7.819 592
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run011.d
4.205 5787
5.171 253
5.987 2202
6.328 295
6.914 285
7.248 154
7.597 287
8.016 496
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run011.d
3.766 286
4.201 909
4.450 18
4.912 65
5.664 609
5.975 762
6.285 172
6.950 391
7.241 206
7.755 1393
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run011.d
2.898 75
3.648 62
4.204 140
4.890 51
5.478 191
5.948 248
6.284 94
6.702 406
7.040 178
7.410 292
7.826 401
8.365 133
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run011.d
5.854 59
6.203 39
7.241 104
7.520 183
8.061 557
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run011.d
5.918 241
6.282 326
6.666 760
6.949 174
7.503 206
8.087 951
Counts (%) vs. Acquisition Time (min)

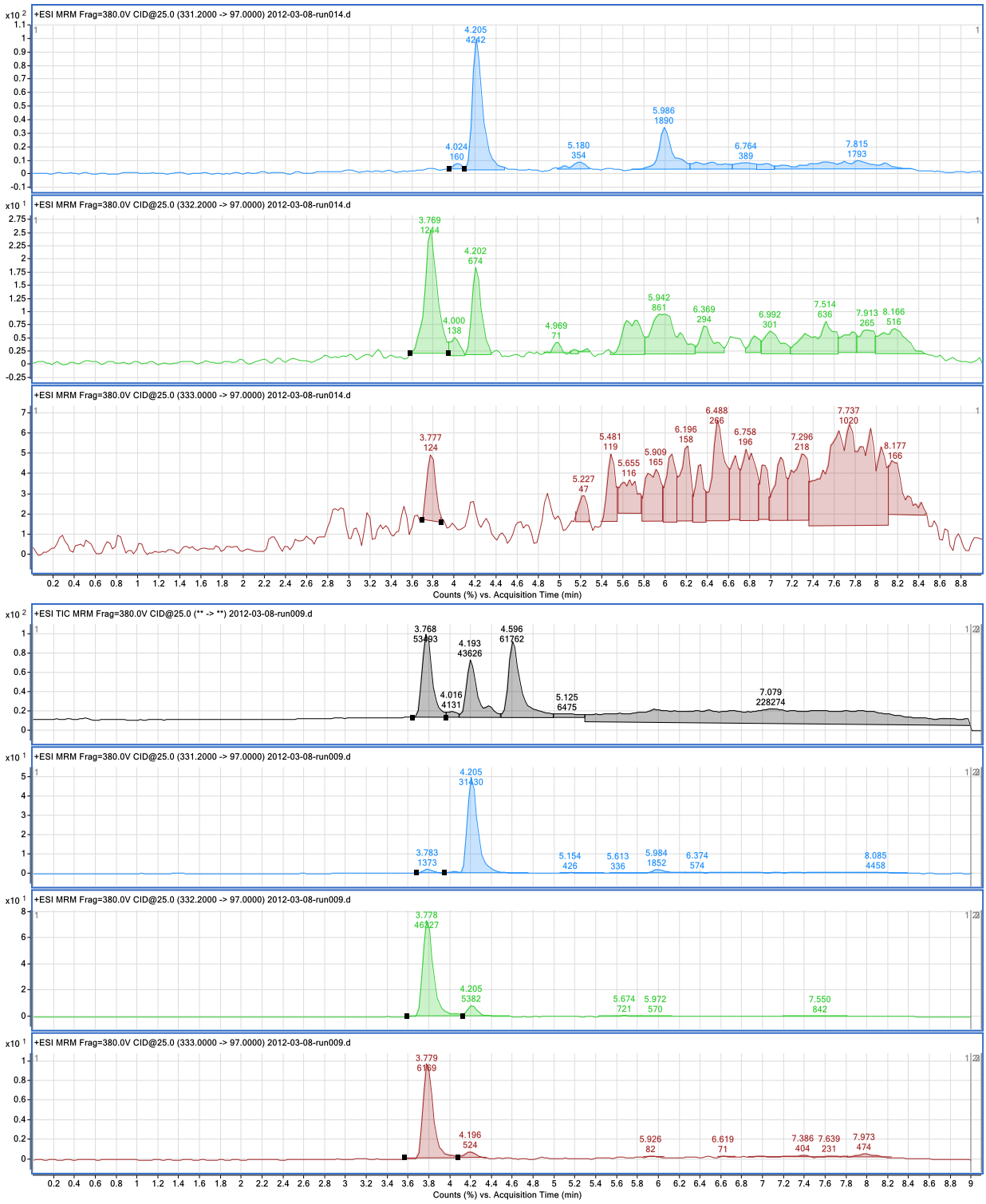

+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run011.d
5.137 72
5.774 2088
6.342 486
6.853 470
7.819 592
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run011.d
4.205 5767
5.171 253
5.987 2202
6.328 295
6.914 285
7.248 154
7.597 287
8.016 496
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run011.d
3.766 286
4.201 909
4.450 18
4.912 65
5.664 609
5.975 762
6.285 172
6.950 391
7.241 206
7.755 1393
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run011.d
2.898 75
3.648 62
4.204 140
4.890 51
5.478 191
5.948 218
6.284 94
6.702 406
7.040 178
7.410 292
7.826 901
8.365 133
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run011.d
5.854 59
6.203 39
7.241 104
7.520 183
8.061 557
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run011.d
5.918 241
6.282 326
6.666 760
6.949 174
7.503 206
8.087 951
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 100.1000) 2012-03-08-run011.d
2.910 173
4.270 47
4.520 57
6.471 1380
7.275 47
7.661 57
8.006 98
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 112.9000) 2012-03-08-run011.d
8.280 3527
Counts (%) vs. Acquisition Time (min)
+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run012.d
+ESI MRM Frag=380.0V CID@25.0 (200.0000 -> 121.0000) 2012-03-08-run012.d
+ESI MRM Frag=380.0V CID@25.0 (313.2000 -> 97.0000) 2012-03-08-run012.d
3.539 323
4.197 436
4.598 222
4.975 483
5.437 207
5.980 4066
6.378 1079
6.705 883
7.092 7254
7.488 1230
7.913 2292
8.441 171
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run012.d
4.624 9443
6.026 1081
6.448 108
6.709 102
7.859 2183
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run012.d
4.618 72747
5.321 820
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run012.d
4.618 13894
7.852 736
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run012.d
4.619 3985
5.622 200
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run012.d
4.614 311
6.424 65
7.867 472
Counts (%) vs. Acquisition Time (min)


+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run012.d
5.453 112
5.772 1730
6.297 774
7.603 3728
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run012.d
4.206 3604
5.156 294
5.984 1782
6.335 168
7.567 141
7.947 959
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run012.d
3.770 315
4.206 678
4.922 107
5.196 123
5.966 1468
6.371 228
6.786 180
7.116 121
7.634 699
7.891 337
8.192 277
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run012.d
2.842 86
3.797 46
4.224 44
4.860 96
5.175 42
5.773 359
6.182 87
6.528 397
7.054 284
7.640 1731
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run012.d
4.576 23
5.895 77
6.245 163
6.607 50
6.908 59
8.061 1100
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run012.d
6.100 23
6.694 1201
7.351 98
7.983 1200
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 100.1000) 2012-03-08-run012.d
6.474 994
6.723 94
7.235 72
7.491 69
7.827 377
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 112.9000) 2012-03-08-run012.d
8.102 5256
Counts (%) vs. Acquisition Time (min)
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run012.d
4.618 13894
7.852 736
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run012.d
4.619 3985
5.622 200
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run012.d
4.614 314
6.424 65
7.867 472
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run012.d
3.588 36
5.424 364
5.782 1685
6.334 1068
6.583 332
7.595 3298
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run012.d
5.453 112
5.772 1730
6.297 774
7.603 3728
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run012.d
4.206 3604
5.156 294
5.984 1782
6.335 168
7.567 141
7.947 959
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run012.d
3.770 315
4.206 678
4.922 107
5.196 123
5.966 1468
6.371 228
6.786 180
7.116 121
7.634 699
7.891 337
8.192 277
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run012.d
2.842 86
3.797 46
4.224 44
4.860 96
5.175 42
5.773 359
6.182 87
6.528 397
7.054 284
7.640 1731
Counts (%) vs. Acquisition Time (min)


+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run013.d
3.766 1261
4.193 4268
4.595 123329
5.975 7529
6.476 2835
7.077 10974
7.450 2341
7.806 3153
+ESI MRM Frag=380.0V CID@25.0 (200.0000 -> 121.0000) 2012-03-08-run013.d
+ESI MRM Frag=380.0V CID@25.0 (313.2000 -> 97.0000) 2012-03-08-run013.d
4.215 161
4.994 300
5.428 184
5.987 3803
6.425 609
6.714 944
7.103 6790
7.400 1862
7.777 1164
8.062 768
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run013.d
4.622 8250
6.024 993
7.827 119
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run013.d
4.617 95060
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run013.d
4.617 16947
8.017 198
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run013.d
4.620 3340
5.199 89
5.643 300
6.123 60
6.795 181
7.622 129
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run013.d
4.620 321
Counts (%) vs. Acquisition Time (min)
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run013.d
4.620 321
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run013.d
3.609 61
4.379 85
5.431 163
5.784 1888
6.323 514
8.112 3000
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run013.d
3.703 107
4.394 82
5.410 154
5.761 1278
6.055 299
6.369 476
6.945 124
7.309 226
7.745 1176
8.191 114
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run013.d
4.205 4395
5.169 249
5.985 2183
6.349 563
7.830 3723
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run013.d
3.775 1230
4.209 778
4.964 119
5.655 519
5.997 409
6.337 139
8.004 1769
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run013.d
3.774 147
5.494 175
5.951 180
6.402 182
6.655 292
7.220 285
7.567 91
7.866 461
8.112 184
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run013.d
5.526 36
6.152 338
6.744 136
7.081 99
7.639 210
8.038 237
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run013.d
6.373 137
6.738 768
7.347 74
7.668 168
8.136 645
Counts (%) vs. Acquisition Time (min)


+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run013.d
3.703 107
4.394 82
5.410 154
5.761 1278
6.055 299
6.369 476
6.945 124
7.309 226
7.745 1176
8.191 114
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run013.d
4.205 4395
5.169 249
5.985 2183
6.349 563
7.830 3723
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run013.d
3.775 1230
4.209 778
4.964 119
5.655 519
5.997 409
6.337 139
8.004 1769
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run013.d
3.774 147
5.494 175
5.951 180
6.402 182
6.655 292
7.220 285
7.567 91
7.866 461
8.112 164
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run013.d
5.526 36
6.152 338
6.744 136
7.081 99
7.639 210
8.038 287
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run013.d
6.373 137
6.738 768
7.347 74
7.668 168
8.136 645
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 100.1000) 2012-03-08-run013.d
4.630 87
6.478 1110
7.077 132
7.389 127
8.002 231
8.307 39
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 112.9000) 2012-03-08-run013.d
7.629 4882
Counts (%) vs. Acquisition Time (min)
+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run014.d
4.192 5861
4.596 102254
7.077 176085
+ESI MRM Frag=380.0V CID@25.0 (200.0000 -> 121.0000) 2012-03-08-run014.d
+ESI MRM Frag=380.0V CID@25.0 (313.2000 -> 97.0000) 2012-03-08-run014.d
4.170 110
5.002 78
5.982 2843
6.368 717
6.771 898
7.007 6265
7.709 866
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run014.d
4.622 7174
5.069 164
6.016 949
7.153 172
7.831 206
8.139 208
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run014.d
4.618 76400
5.072 842
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run014.d
4.617 13623
7.887 356
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run014.d
4.619 2776
5.596 329
6.132 111
6.588 133
6.969 138
7.646 68
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run014.d
4.600 269
7.869 404
Counts (%) vs. Acquisition Time (min)


+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run014.d
4.619 2776
5.596 329
6.132 111
6.588 133
6.969 138
7.646 68
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run014.d
4.600 269
7.869 404
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run014.d
5.401 399
5.774 1145
6.345 2015
7.919 2857
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run014.d
3.653 111
4.328 124
4.868 48
5.415 220
5.777 1073
6.371 585
7.912 3030
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run014.d
4.205 4242
5.180 354
5.986 1890
6.764 389
7.815 1793
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run014.d
3.769 1244
4.202 674
4.969 71
5.942 861
6.369 294
6.992 301
7.514 636
7.913 265
8.166 516
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run014.d
3.777 124
5.227 47
5.481 119
5.909 165
6.196 168
6.488 266
6.758 186
7.296 218
7.737 1020
8.177 166
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run014.d
5.768 28
6.483 59
6.970 151
7.339 206
7.666 618
8.110 235
Counts (%) vs. Acquisition Time (min)
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run014.d
3.653 111
4.328 124
4.868 48
5.415 220
5.777 1073
6.371 585
7.912 3030
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run014.d
4.205 4242
5.180 354
5.986 1890
6.764 389
7.815 1793
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run014.d
3.769 1244
4.202 674
4.969 71
5.942 861
6.369 294
6.992 301
7.514 636
7.913 265
8.166 516
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run014.d
3.777 124
5.227 47
5.481 119
5.909 165
6.196 168
6.488 266
6.758 186
7.296 218
7.737 1020
8.177 166
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run014.d
5.768 28
6.483 59
6.970 151
7.339 206
7.666 618
8.110 235
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run014.d
6.324 58
6.703 506
7.167 49
7.579 726
8.029 415
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 100.1000) 2012-03-08-run014.d
6.486 957
7.468 124
7.830 135
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 112.9000) 2012-03-08-run014.d
6.142 49
6.708 152
7.175 381
7.635 361
7.990 1329
8.300 413
Counts (%) vs. Acquisition Time (min)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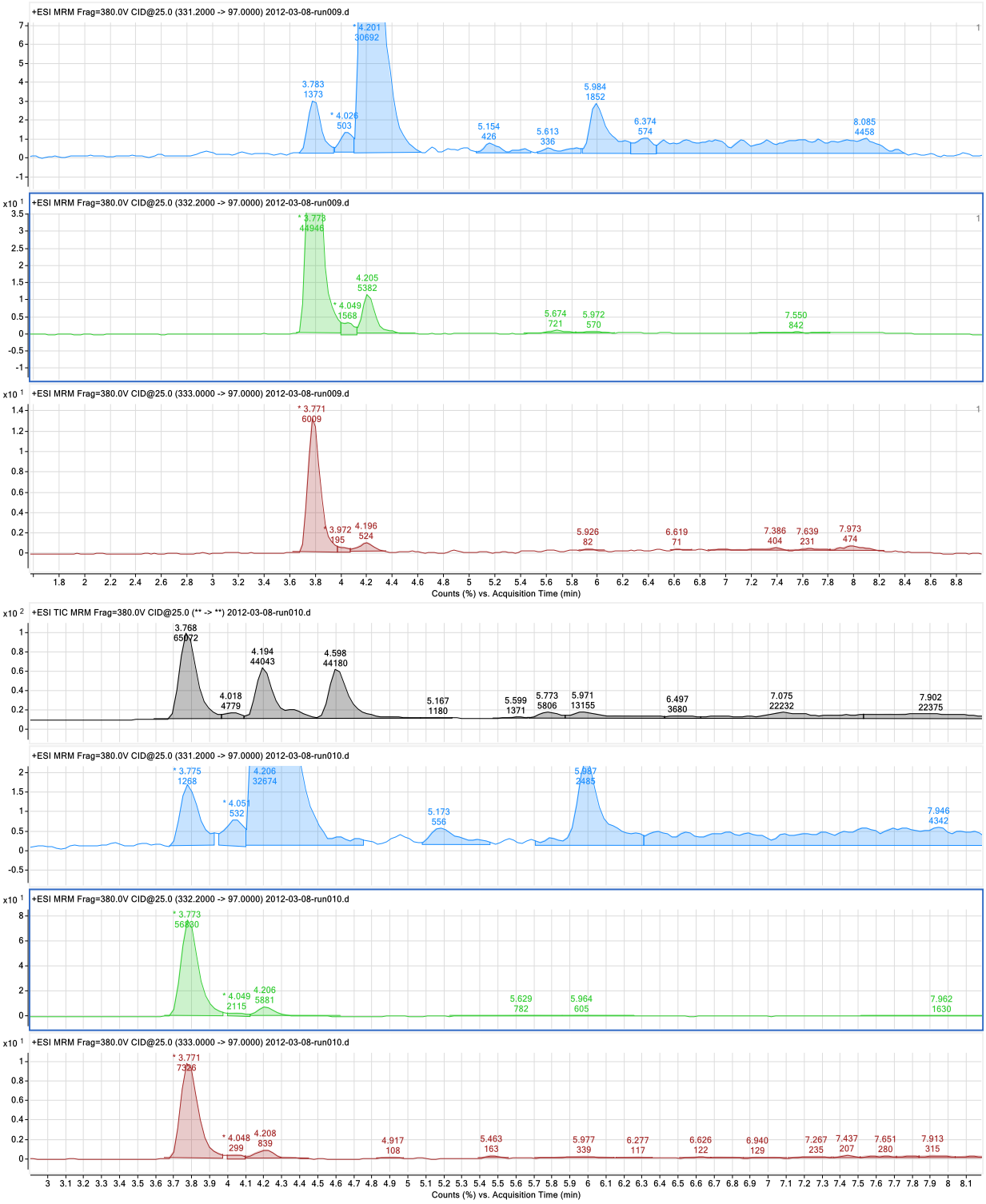
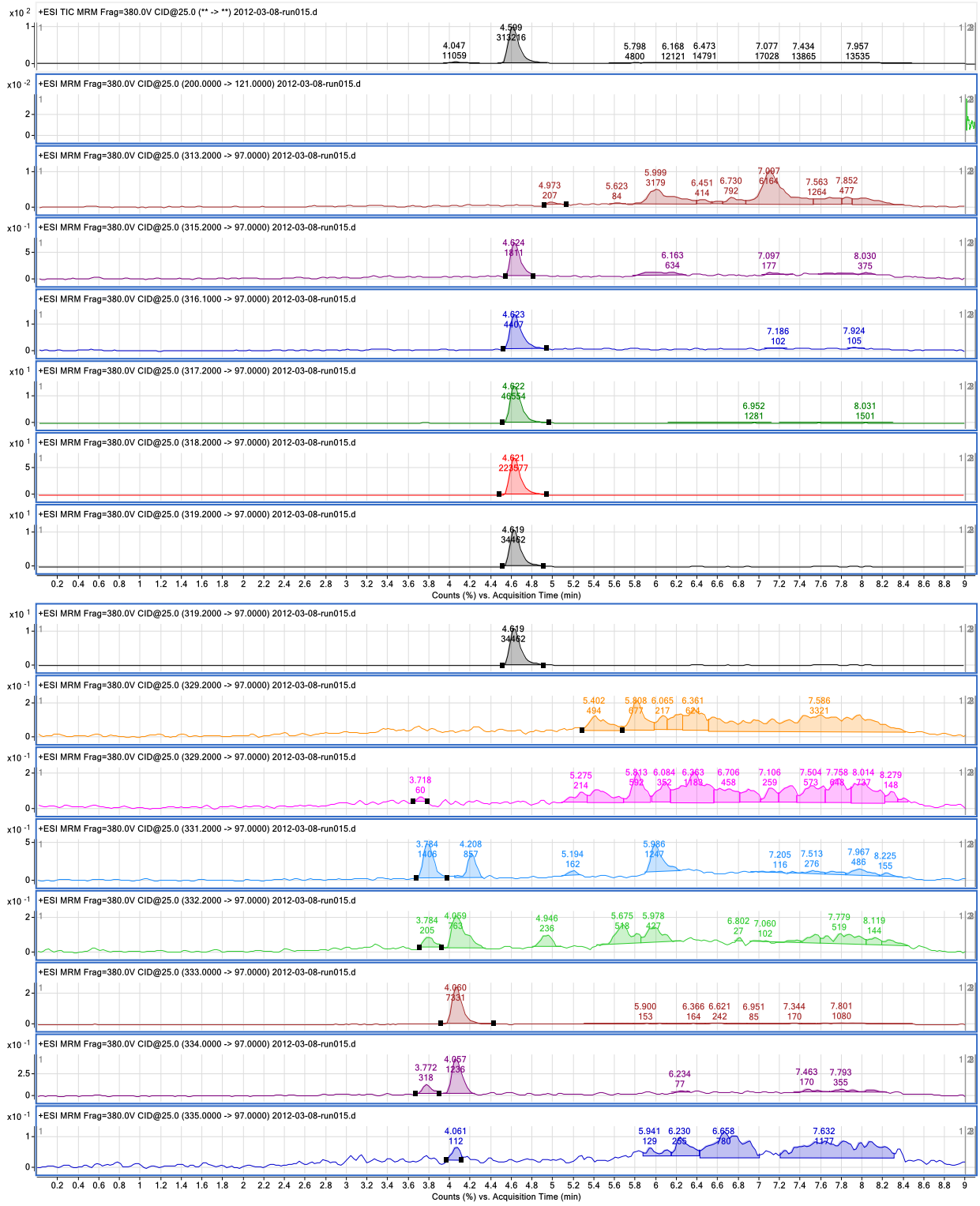
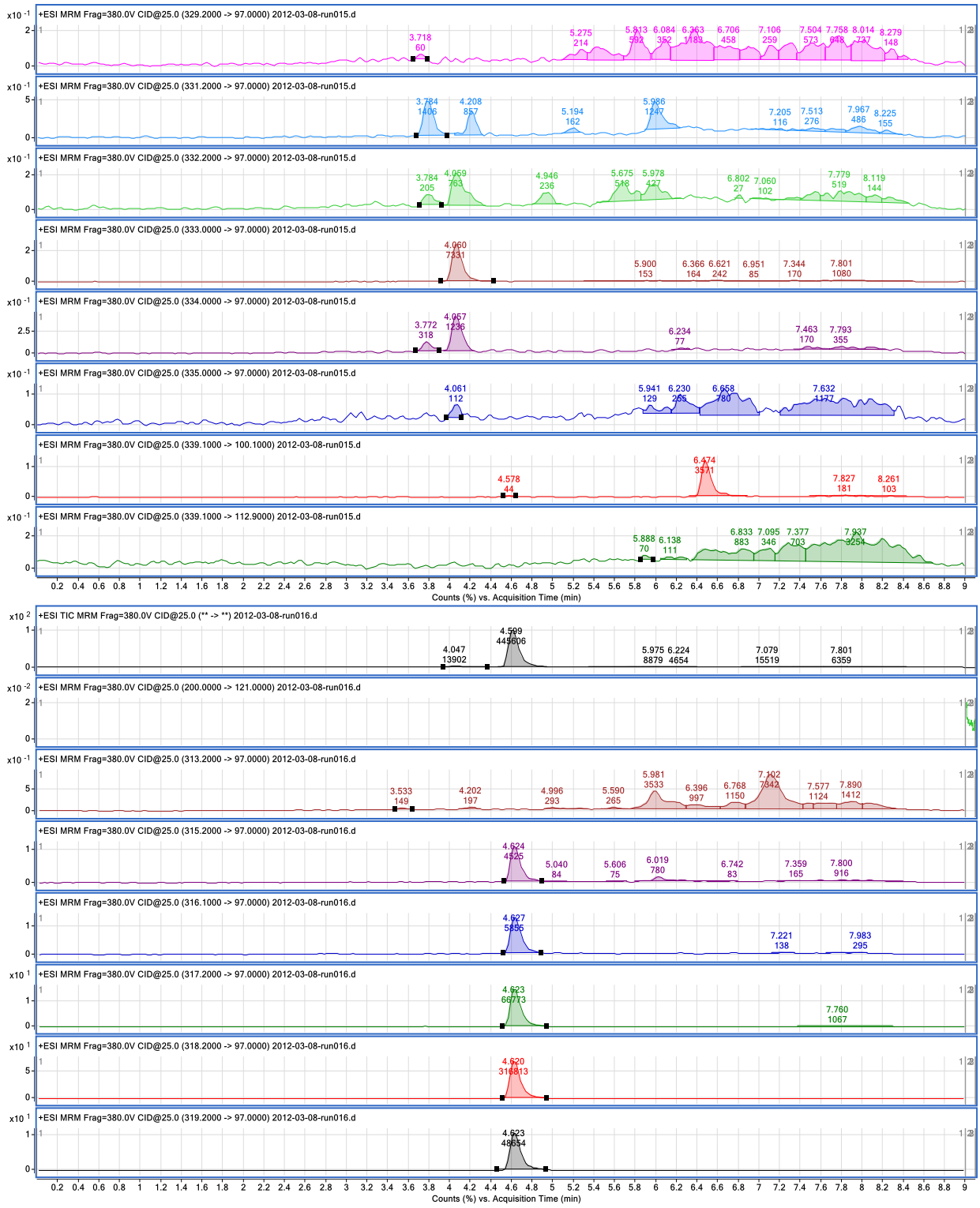
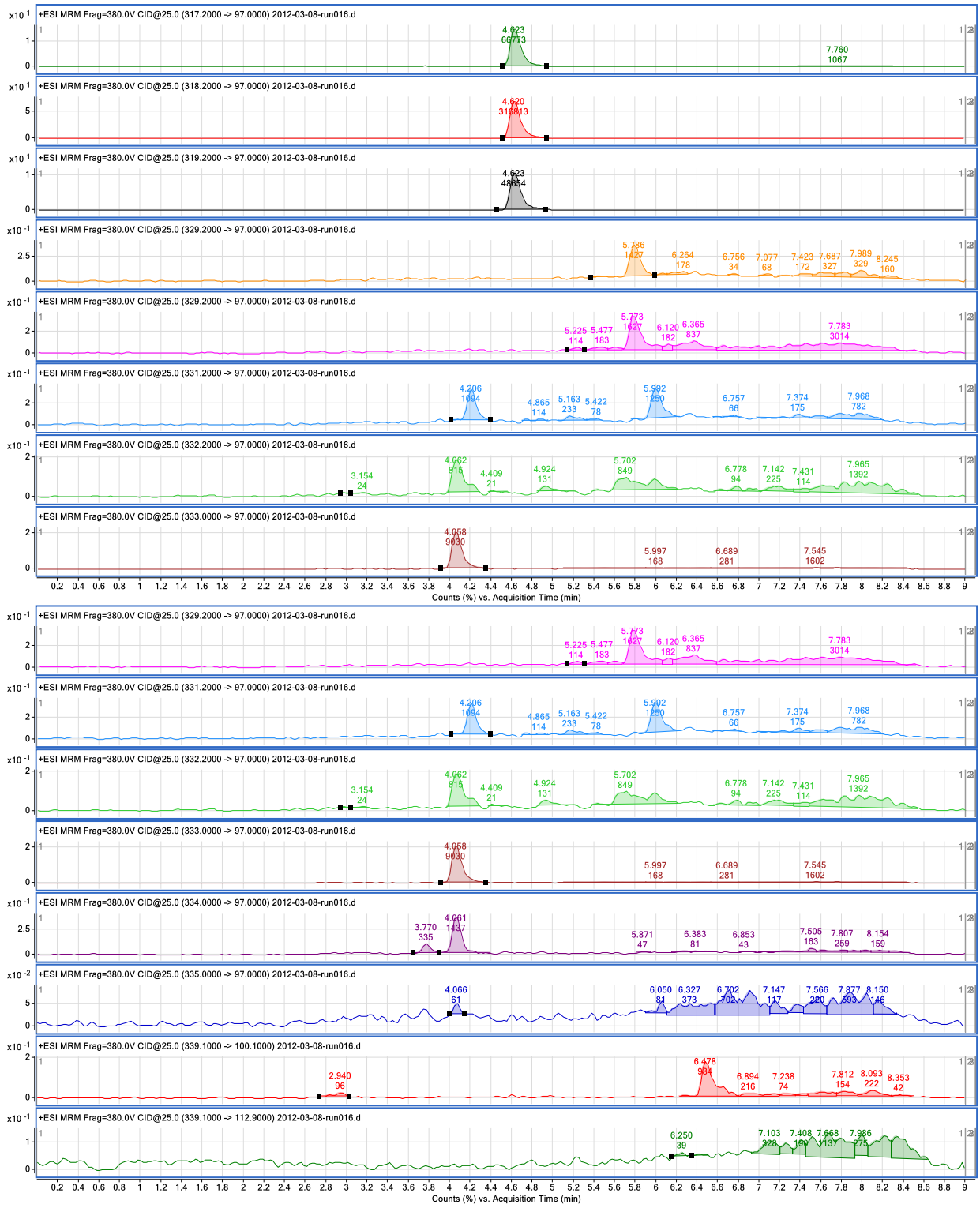
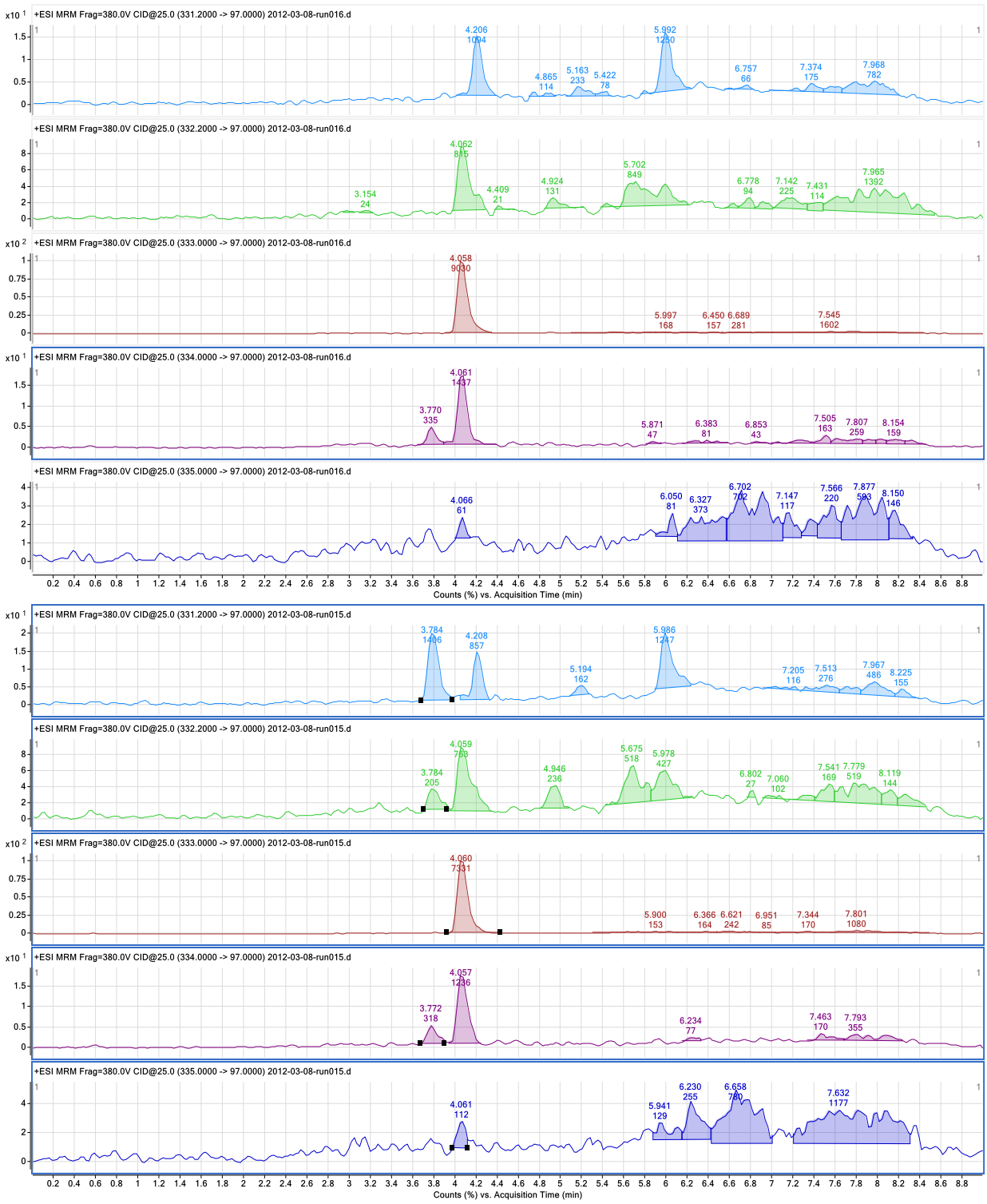
4.205 4242
4.024 160
5.180 354
5.986 1890
6.764 389
7.815 1793
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run014.d
3.769 1244
4.202 674
4.000 138
4.969 71
5.942 861
6.369 294
6.992 301
7.514 636
7.913 265
8.166 516
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run014.d
3.777 124
5.227 47
5.481 119
5.655 116
5.909 165
6.196 158
6.488 266
6.758 196
7.296 218
7.737 1020
8.177 166
Counts (%) vs. Acquisition Time (min)
+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run009.d
3.768 53493
4.193 43626
4.596 61762
4.016 4131
5.125 6475
7.079 228274
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run009.d
4.205 31430
3.783 1373
5.154 426
5.613 336
5.984 1852
6.374 574
8.085 4458
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run009.d
3.778 46327
4.205 5382
5.674 721
5.972 570
7.550 842
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run009.d
3.779 6169
4.196 524
5.926 82
6.619 71
7.386 404
7.639 231
7.973 474
Counts (%) vs. Acquisition Time (min)

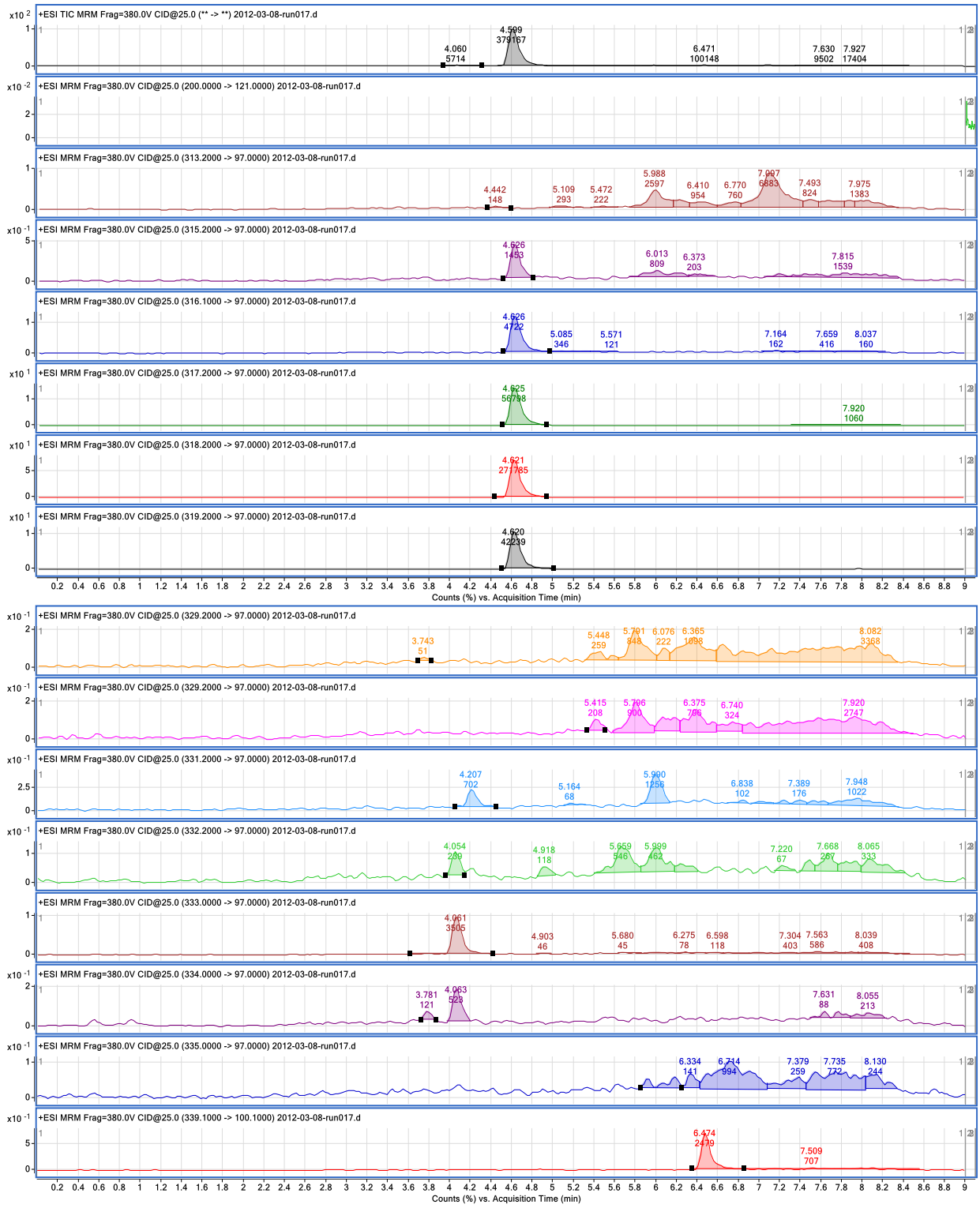

+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run009.d
* 4.201 30692
3.783 1373
* 4.026 503
5.154 426
5.613 336
5.984 1852
6.374 574
8.085 4458
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run009.d
* 3.778 44946
4.205 5382
* 4.049 1568
5.674 721
5.972 570
7.550 842
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run009.d
* 3.771 6009
3.972 195
4.196 524
5.926 82
6.619 71
7.386 404
7.639 231
7.973 474
Counts (%) vs. Acquisition Time (min)
+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run010.d
3.768 65072
4.018 4779
4.194 44043
4.598 44180
5.167 1180
5.599 1371
5.773 5806
5.971 13155
6.497 3680
7.075 22232
7.902 22375
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run010.d
* 3.775 1268
* 4.051 532
4.206 32674
5.173 556
5.987 2485
7.946 4342
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run010.d
* 3.773 56330
* 4.049 2115
4.206 5881
5.629 782
5.964 605
7.962 1630
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run010.d
* 3.771 7326
* 4.048 299
4.208 839
4.917 108
5.463 163
5.977 339
6.277 117
6.626 122
6.940 129
7.267 235
7.437 207
7.651 280
7.913 315
Counts (%) vs. Acquisition Time (min)


+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run015.d
4.047 11059
4.509 313216
5.798 4800
6.168 12121
6.473 14791
7.077 17028
7.434 13865
7.957 13535
+ESI MRM Frag=380.0V CID@25.0 (200.0000 -> 121.0000) 2012-03-08-run015.d
+ESI MRM Frag=380.0V CID@25.0 (313.2000 -> 97.0000) 2012-03-08-run015.d
4.973 207
5.623 84
5.999 3179
6.451 414
6.730 792
7.097 6164
7.563 1264
7.852 477
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run015.d
4.624 1811
6.163 634
7.097 177
8.030 375
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run015.d
4.623 4407
7.186 102
7.924 105
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run015.d
4.622 46554
6.952 1281
8.031 1501
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run015.d
4.621 223577
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run015.d
4.619 34462
Counts (%) vs. Acquisition Time (min)
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run015.d
4.619 34462
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run015.d
5.402 494
5.808 677
6.065 217
6.361 621
7.586 3321
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run015.d
3.718 60
5.275 214
5.813 592
6.084 352
6.363 1189
6.706 458
7.106 259
7.504 573
7.758 648
8.014 737
8.279 148
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run015.d
3.784 1406
4.208 857
5.194 162
5.986 1247
7.205 116
7.513 276
7.967 486
8.225 155
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run015.d
3.784 205
4.059 763
4.946 236
5.675 548
5.978 427
6.802 27
7.060 102
7.779 519
8.119 144
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run015.d
4.060 7331
5.900 153
6.366 164
6.621 242
6.951 85
7.344 170
7.801 1080
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run015.d
3.772 318
4.057 1236
6.234 77
7.463 170
7.793 355
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run015.d
4.061 112
5.941 129
6.230 265
6.658 780
7.632 1127
Counts (%) vs. Acquisition Time (min)

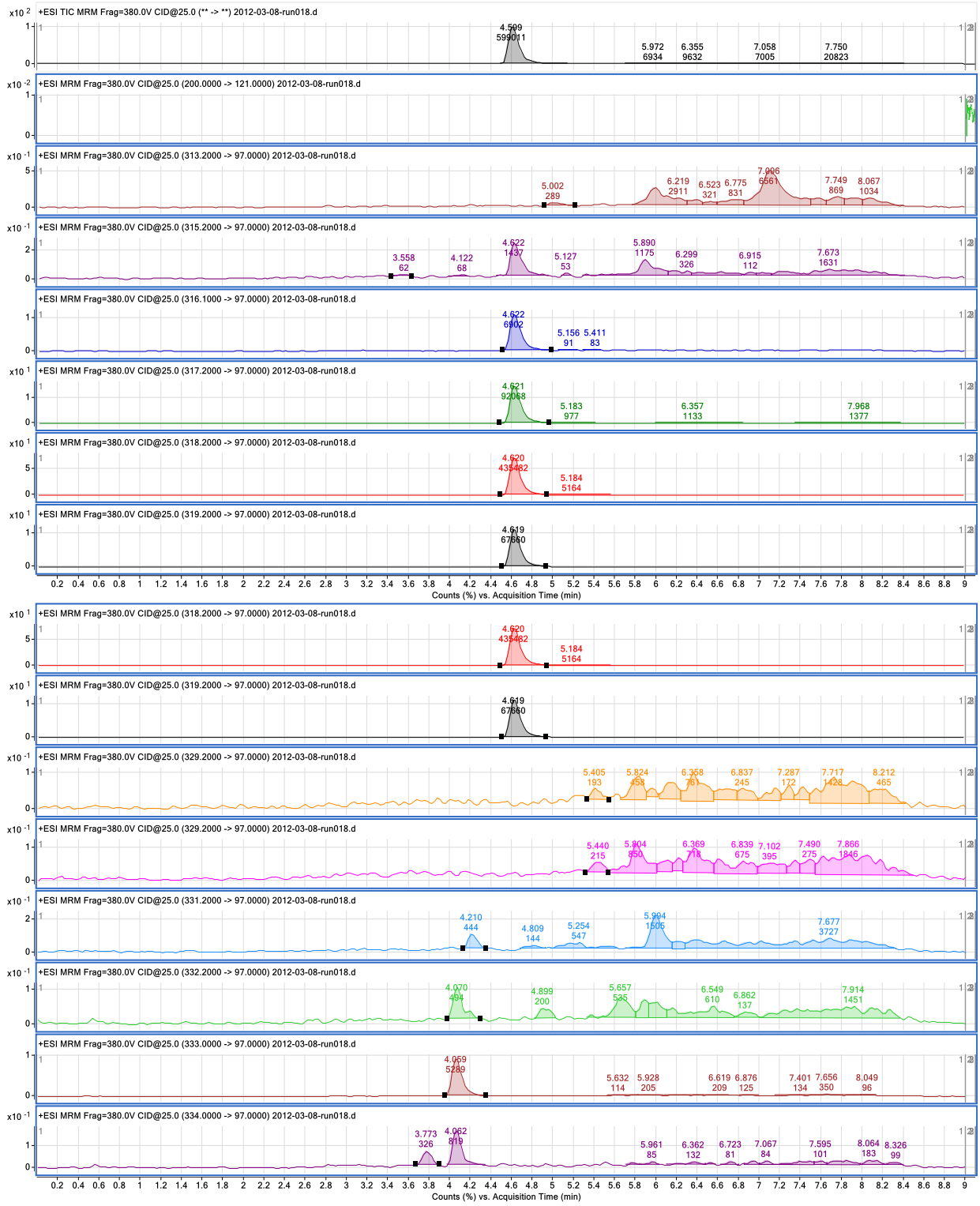

+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run015.d
3.718 60
5.275 214
5.813 592
6.084 352
6.263 1189
6.706 458
7.106 259
7.504 573
7.758 968
8.014 737
8.279 148
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run015.d
3.784 1406
4.208 857
5.194 162
5.986 1247
7.205 116
7.513 276
7.967 486
8.225 155
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run015.d
3.784 205
4.959 763
4.946 236
5.675 548
5.978 427
6.802 27
7.060 102
7.779 519
8.119 144
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run015.d
4.060 7331
5.900 153
6.366 164
6.621 242
6.951 85
7.344 170
7.801 1080
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run015.d
3.772 318
4.957 1236
6.234 77
7.463 170
7.793 355
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run015.d
4.061 112
5.941 129
6.230 255
6.658 780
7.632 1177
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 100.1000) 2012-03-08-run015.d
4.578 44
6.474 3511
7.827 181
8.261 103
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 112.9000) 2012-03-08-run015.d
5.888 70
6.138 111
6.833 883
7.095 346
7.377 703
7.937 3254
Counts (%) vs. Acquisition Time (min)
+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run016.d
4.047 13902
4.599 445606
5.975 8879
6.224 4654
7.079 15519
7.801 6359
+ESI MRM Frag=380.0V CID@25.0 (200.0000 -> 121.0000) 2012-03-08-run016.d
+ESI MRM Frag=380.0V CID@25.0 (313.2000 -> 97.0000) 2012-03-08-run016.d
3.533 149
4.202 197
4.996 293
5.590 265
5.981 3533
6.396 997
6.768 1150
7.102 7342
7.577 1124
7.890 1412
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run016.d
4.624 4525
5.040 84
5.606 75
6.019 780
6.742 83
7.359 165
7.800 916
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run016.d
4.627 5856
7.221 138
7.983 295
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run016.d
4.623 66773
7.760 1067
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run016.d
4.620 316813
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run016.d
4.623 48654
Counts (%) vs. Acquisition Time (min)

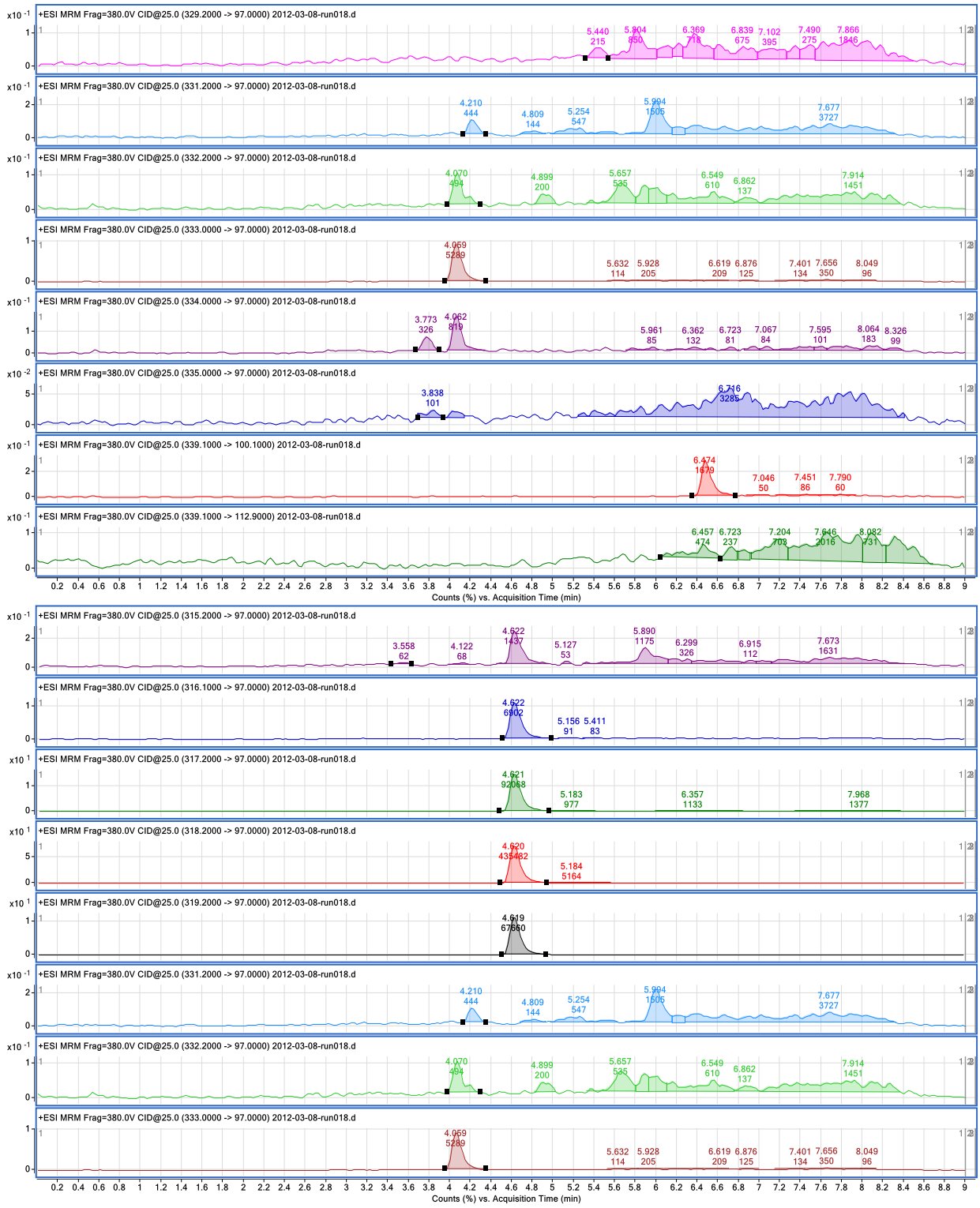

+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run016.d
4.623 66773
7.760 1067
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run016.d
4.620 316813
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run016.d
4.623 48654
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run016.d
5.786 1427
6.264 178
6.756 34
7.077 68
7.423 172
7.687 327
7.989 329
8.245 160
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run016.d
5.225 114
5.477 183
5.773 1427
6.120 182
6.365 837
7.783 3014
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run016.d
4.206 1094
4.865 114
5.163 233
5.422 78
5.992 1250
6.757 66
7.374 175
7.968 782
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run016.d
3.154 24
4.062 815
4.409 21
4.924 131
5.702 849
6.778 94
7.142 225
7.431 114
7.965 1392
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run016.d
4.058 9030
5.997 168
6.689 281
7.545 1602
Counts (%) vs. Acquisition Time (min)
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run016.d
5.225 114
5.477 183
5.773 1427
6.120 182
6.365 837
7.783 3014
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run016.d
4.206 1094
4.865 114
5.163 233
5.422 78
5.992 1250
6.757 66
7.374 175
7.968 782
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run016.d
3.154 24
4.062 815
4.409 21
4.924 131
5.702 849
6.778 94
7.142 225
7.431 114
7.965 1392
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run016.d
4.058 9030
5.997 168
6.689 281
7.545 1602
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run016.d
3.770 335
4.061 1437
5.871 47
6.383 81
6.853 43
7.505 163
7.807 259
8.154 159
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run016.d
4.066 61
6.050 81
6.327 373
6.702 702
7.147 117
7.566 220
7.877 593
8.150 146
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 100.1000) 2012-03-08-run016.d
2.940 96
6.478 984
6.894 216
7.238 74
7.812 154
8.093 222
8.353 42
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 112.9000) 2012-03-08-run016.d
6.250 39
7.103 328
7.408 1137
7.668 1137
7.986 275
Counts (%) vs. Acquisition Time (min)

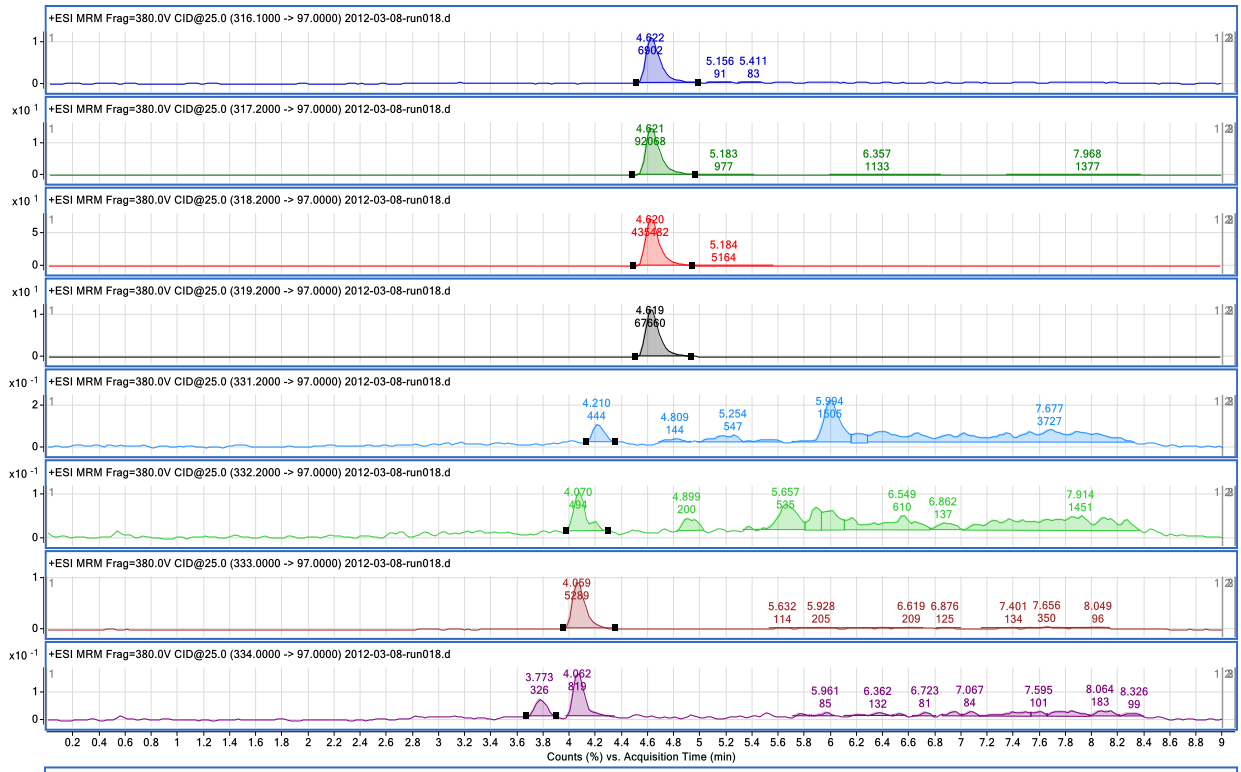

+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run016.d
4.206 1004
4.865 114
5.163 233
5.422 78
5.992 1250
6.757 66
7.374 175
7.968 782
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run016.d
3.154 24
4.062 815
4.409 21
4.924 131
5.702 849
6.778 94
7.142 225
7.431 114
7.965 1392
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run016.d
4.058 9030
5.997 168
6.450 157
6.689 281
7.545 1602
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run016.d
3.770 335
4.061 1437
5.871 47
6.383 81
6.853 43
7.505 163
7.807 259
8.154 159
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run016.d
4.066 61
6.050 81
6.327 373
6.702 702
7.147 117
7.566 220
7.877 593
8.150 146
Counts (%) vs. Acquisition Time (min)
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run015.d
3.784 1406
4.208 857
5.194 162
5.986 1247
7.205 116
7.513 276
7.967 486
8.225 155
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run015.d
3.784 205
4.059 768
4.946 236
5.675 518
5.978 427
6.802 27
7.060 102
7.541 169
7.779 519
8.119 144
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run015.d
4.060 7331
5.900 153
6.366 164
6.621 242
6.951 85
7.344 170
7.801 1080
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run015.d
3.772 318
4.057 1236
6.234 77
7.463 170
7.793 355
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run015.d
4.061 112
5.941 129
6.230 255
6.658 780
7.632 1177
Counts (%) vs. Acquisition Time (min)


+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run017.d
4.060 5714
4.509 379167
6.471 100148
7.630 9502
7.927 17404
+ESI MRM Frag=380.0V CID@25.0 (200.0000 -> 121.0000) 2012-03-08-run017.d
+ESI MRM Frag=380.0V CID@25.0 (313.2000 -> 97.0000) 2012-03-08-run017.d
4.442 148
5.109 293
5.472 222
5.988 2597
6.410 954
6.770 760
7.997 6883
7.493 824
7.975 1383
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run017.d
4.626 1453
6.013 809
6.373 203
7.815 1539
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run017.d
4.626 4722
5.085 346
5.571 121
7.164 162
7.659 416
8.037 160
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run017.d
4.625 56798
7.920 1060
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run017.d
4.621 27785
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run017.d
4.620 42239
Counts (%) vs. Acquisition Time (min)
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run017.d
3.743 51
5.448 259
5.701 945
6.076 222
6.365 1698
8.082 3368
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run017.d
5.415 208
5.706 900
6.375 706
6.740 324
7.920 2747
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run017.d
4.207 702
5.164 68
5.990 1256
6.838 102
7.389 176
7.948 1022
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run017.d
4.054 209
4.918 118
5.659 546
5.999 462
7.220 67
7.668 267
8.065 333
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run017.d
4.061 3505
4.903 46
5.680 45
6.275 78
6.598 118
7.304 403
7.563 586
8.039 408
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run017.d
3.781 121
4.063 528
7.631 88
8.055 213
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run017.d
6.334 141
6.714 994
7.379 259
7.735 772
8.130 244
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 100.1000) 2012-03-08-run017.d
6.474 2479
7.509 707
Counts (%) vs. Acquisition Time (min)


+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run017.d
5.415 208
5.706 900
6.375 786
6.740 324
7.920 2747
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run017.d
4.207 702
5.164 68
5.900 1256
6.838 102
7.389 176
7.948 1022
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run017.d
4.054 280
4.918 118
5.659 946
5.999 462
7.220 67
7.668 267
8.065 333
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run017.d
4.061 3505
4.903 46
5.680 45
6.275 78
6.598 118
7.304 403
7.563 586
8.039 408
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run017.d
3.781 121
4.063 528
7.631 88
8.055 213
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run017.d
6.334 141
6.714 994
7.379 259
7.735 772
8.130 244
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 100.1000) 2012-03-08-run017.d
6.474 2479
7.509 707
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 112.9000) 2012-03-08-run017.d
7.953 4523
Counts (%) vs. Acquisition Time (min)
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run017.d
4.626 1453
6.013 809
6.373 203
7.815 1539
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run017.d
4.626 4722
5.085 346
5.571 121
7.164 162
7.659 416
8.037 160
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run017.d
4.625 56798
7.920 1060
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run017.d
4.621 271785
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run017.d
4.620 42239
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run017.d
4.207 702
5.164 68
5.900 1256
6.838 102
7.389 176
7.948 1022
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run017.d
4.054 280
4.918 118
5.659 946
5.999 462
7.220 67
7.668 267
8.065 333
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run017.d
4.061 3505
4.903 46
5.680 45
6.275 78
6.598 118
7.304 403
7.563 586
8.039 408
Counts (%) vs. Acquisition Time (min)


+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run018.d
4.509 599011
5.972 6934
6.355 9632
7.058 7005
7.750 20823
+ESI MRM Frag=380.0V CID@25.0 (200.0000 -> 121.0000) 2012-03-08-run018.d
+ESI MRM Frag=380.0V CID@25.0 (313.2000 -> 97.0000) 2012-03-08-run018.d
5.002 289
6.219 2911
6.523 321
6.775 831
7.006 6561
7.749 869
8.067 1034
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run018.d
3.558 62
4.122 68
4.622 1437
5.127 53
5.890 1175
6.299 326
6.915 112
7.673 1631
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run018.d
4.622 6902
5.156 91
5.411 83
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run018.d
4.621 92068
5.183 977
6.357 1133
7.968 1377
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run018.d
4.620 435482
5.184 5164
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run018.d
4.619 67660
Counts (%) vs. Acquisition Time (min)
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run018.d
4.620 435482
5.184 5164
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run018.d
4.619 67660
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run018.d
5.405 193
5.824 458
6.358 761
6.837 245
7.287 172
7.717 1428
8.212 465
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run018.d
5.440 215
5.804 950
6.369 718
6.839 675
7.102 395
7.490 275
7.866 1846
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run018.d
4.210 444
4.809 144
5.254 547
5.994 1505
7.677 3727
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run018.d
4.070 494
4.899 200
5.657 535
6.549 610
6.862 137
7.914 1451
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run018.d
4.059 5289
5.632 114
5.928 205
6.619 209
6.876 125
7.401 134
7.656 350
8.049 96
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run018.d
3.773 326
4.062 819
5.961 85
6.362 132
6.723 81
7.067 84
7.595 101
8.064 183
8.326 99
Counts (%) vs. Acquisition Time (min)


+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run018.d
5.440 215
5.904 650
6.369 718
6.839 675
7.102 395
7.490 275
7.866 1846
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run018.d
4.210 444
4.809 144
5.254 547
5.994 1505
7.677 3727
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run018.d
4.070 494
4.899 200
5.657 535
6.549 610
6.862 137
7.914 1451
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run018.d
4.059 5289
5.632 114
5.928 205
6.619 209
6.876 125
7.401 134
7.656 350
8.049 96
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run018.d
3.773 326
4.062 819
5.961 85
6.362 132
6.723 81
7.067 84
7.595 101
8.064 183
8.326 99
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run018.d
3.838 101
6.716 3285
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 100.1000) 2012-03-08-run018.d
6.474 1670
7.046 50
7.451 86
7.790 60
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 112.9000) 2012-03-08-run018.d
6.457 474
6.723 237
7.204 703
7.646 2016
8.082 731
Counts (%) vs. Acquisition Time (min)
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run018.d
3.558 62
4.122 68
4.622 1437
5.127 53
5.890 1175
6.299 326
6.915 112
7.673 1631
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run018.d
4.622 6902
5.156 91
5.411 83
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run018.d
4.621 92068
5.183 977
6.357 1133
7.968 1377
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run018.d
4.620 435482
5.184 5164
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run018.d
4.619 67660
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run018.d
4.210 444
4.809 144
5.254 547
5.994 1505
7.677 3727
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run018.d
4.070 494
4.899 200
5.657 535
6.549 610
6.862 137
7.914 1451
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run018.d
4.059 5289
5.632 114
5.928 205
6.619 209
6.876 125
7.401 134
7.656 350
8.049 96
Counts (%) vs. Acquisition Time (min)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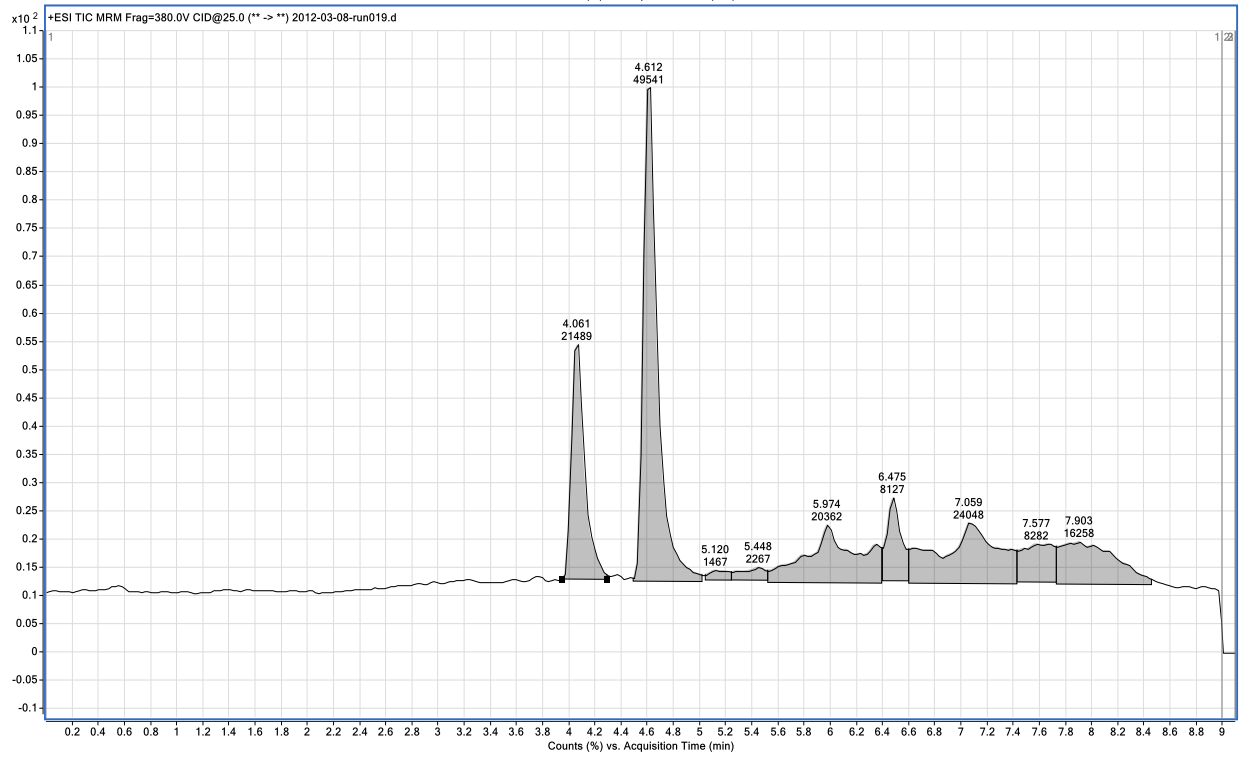
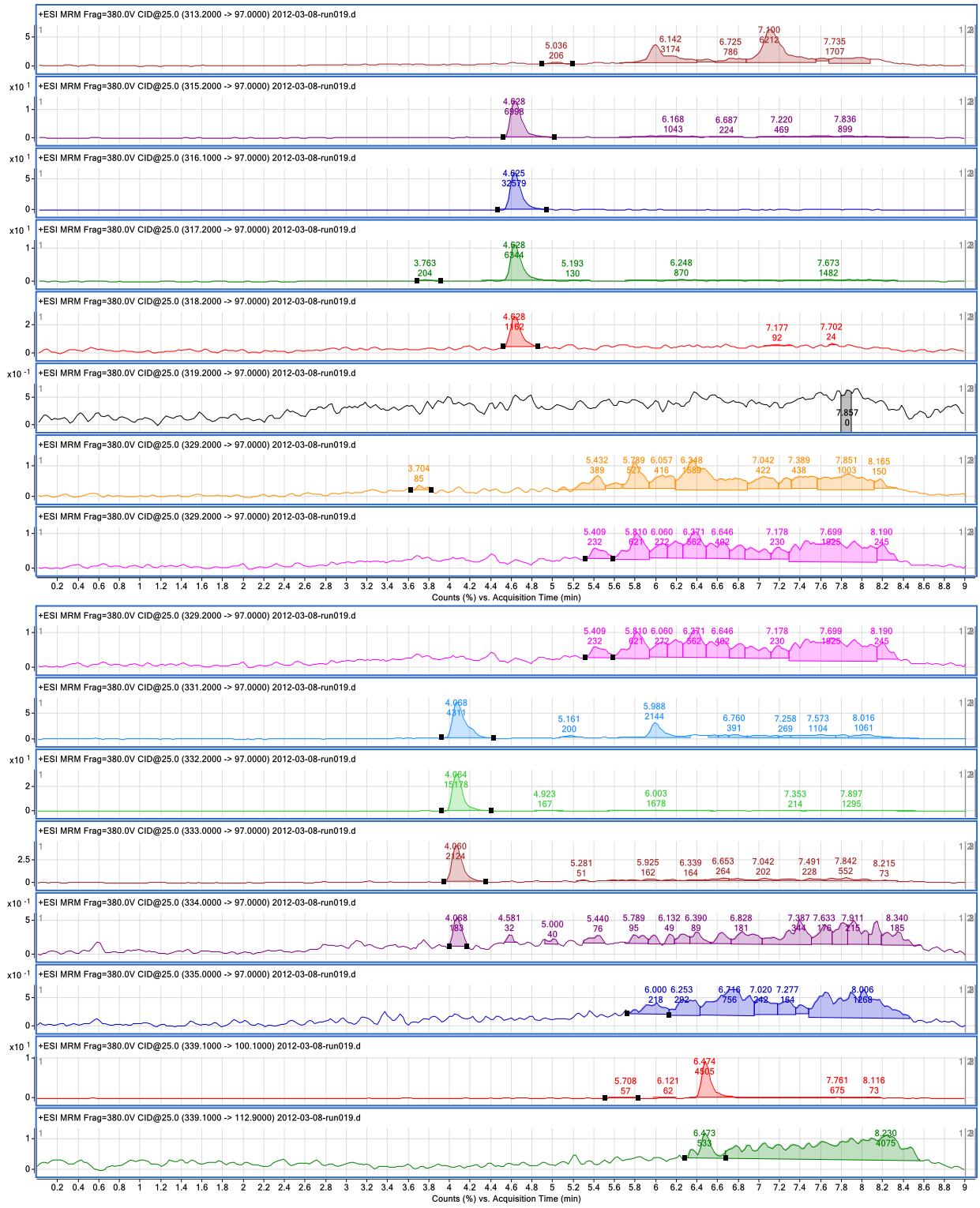
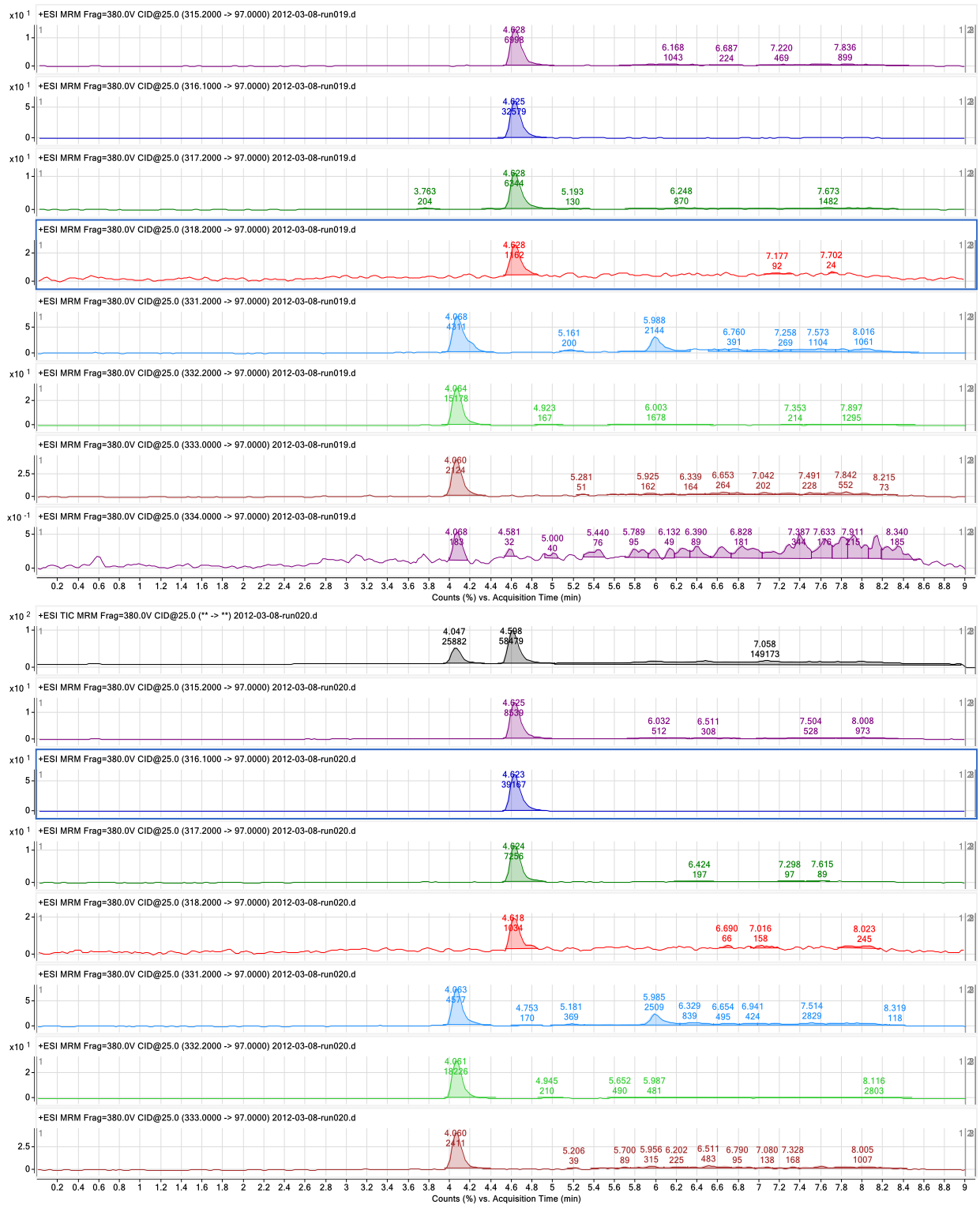
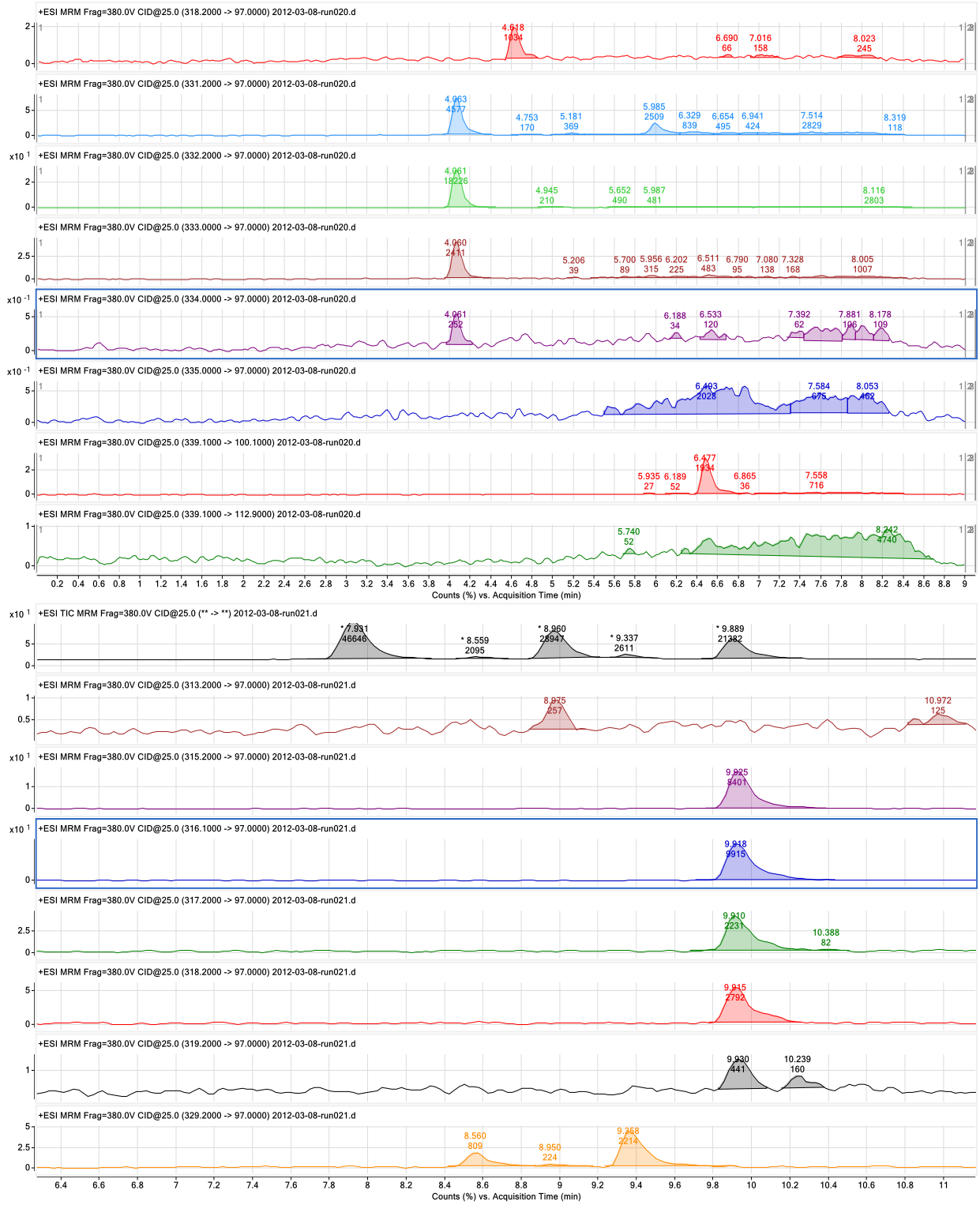
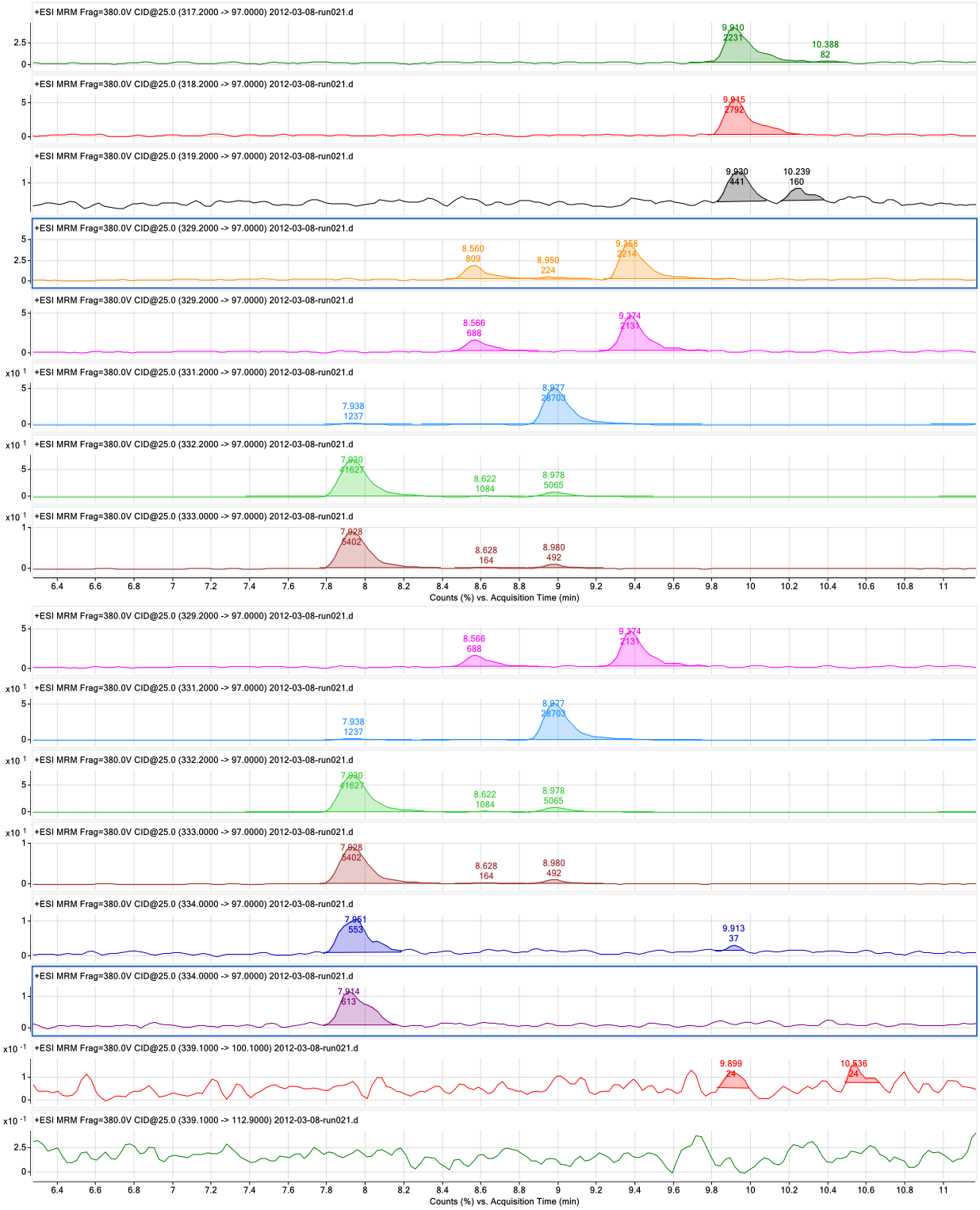
4.622 6902
5.156 91
5.411 83
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run018.d
4.621 92068
5.183 977
6.357 1133
7.968 1377
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run018.d
4.620 435482
5.184 5164
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run018.d
4.619 67660
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run018.d
4.210 444
4.809 144
5.254 547
5.994 1506
7.677 3727
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run018.d
4.070 494
4.899 200
5.657 535
6.549 610
6.862 137
7.914 1451
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run018.d
4.059 5289
5.632 114
5.928 205
6.619 209
6.876 125
7.401 134
7.656 350
8.049 96
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run018.d
3.773 326
4.062 819
5.961 85
6.362 132
6.723 81
7.067 84
7.595 101
8.064 183
8.326 99
Counts (%) vs. Acquisition Time (min)

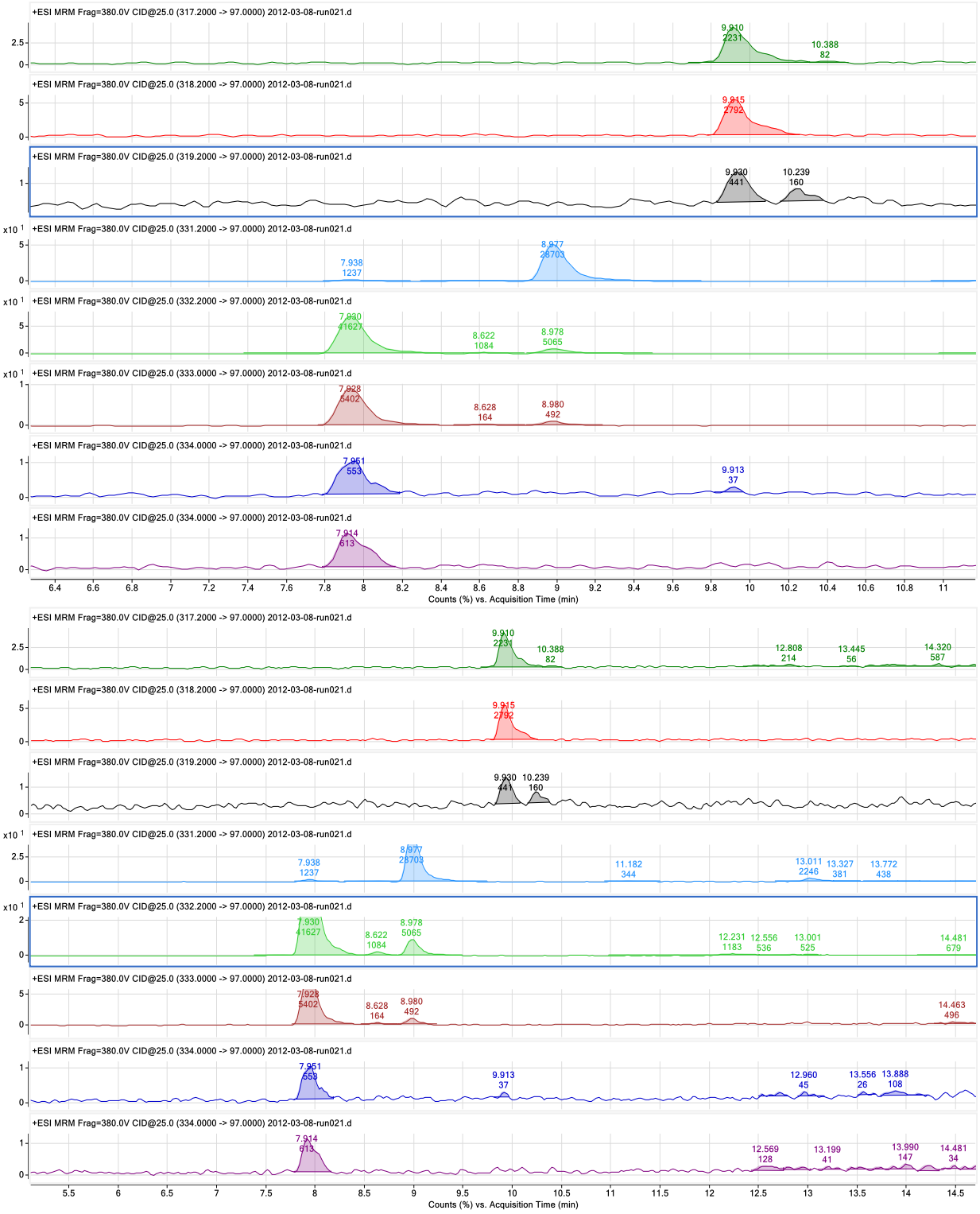

+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run019.d
4.612 49541
4.061 21489
5.120 1467
5.448 2267
5.974 20362
6.475 8127
7.059 24048
7.577 8282
7.903 16258
Counts (%) vs. Acquisition Time (min)

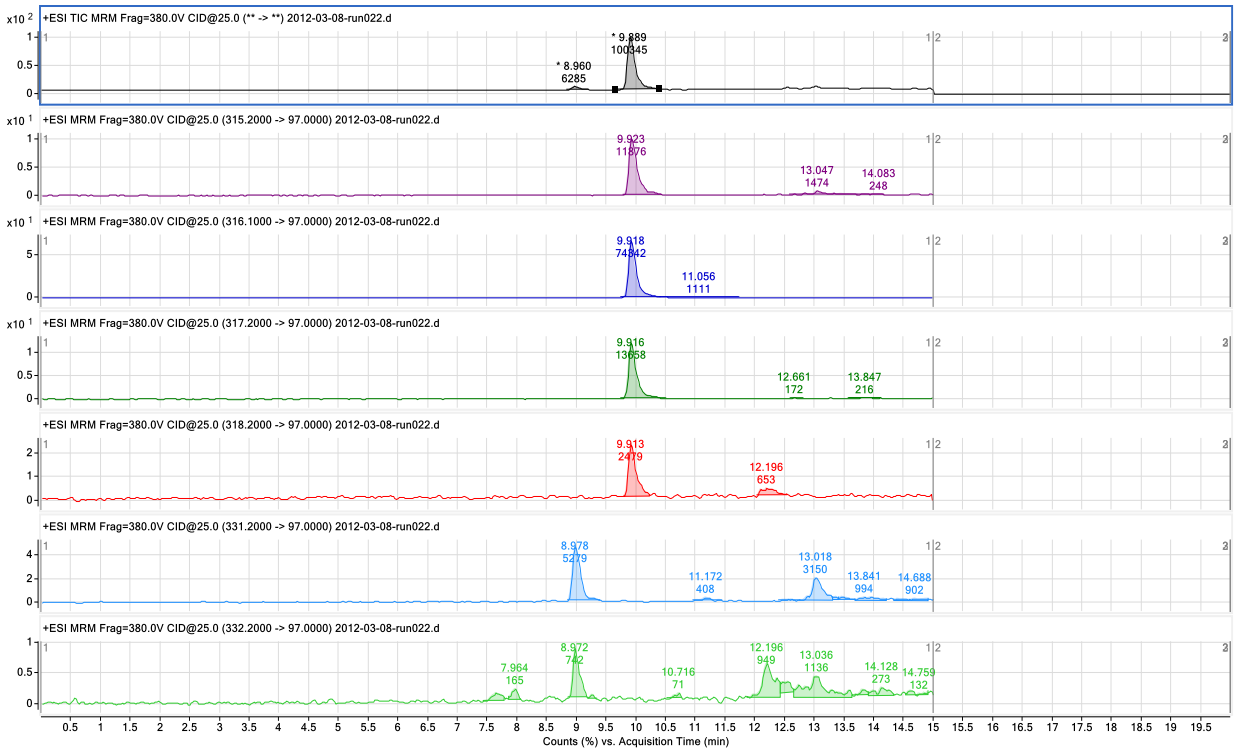

+ESI MRM Frag=380.0V CID@25.0 (313.2000 -> 97.0000) 2012-03-08-run019.d
5.036 206
6.142 3174
6.725 786
7.100 6212
7.735 1707
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run019.d
4.628 6998
6.168 1043
6.687 224
7.220 469
7.836 899
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run019.d
4.625 32579
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run019.d
3.763 204
4.628 6144
5.193 130
6.248 870
7.673 1482
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run019.d
4.628 1162
7.177 92
7.702 24
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run019.d
7.857 0
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run019.d
3.704 85
5.432 389
5.789 527
6.057 416
6.348 1589
7.042 422
7.389 438
7.851 1003
8.165 150
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run019.d
5.409 232
5.810 621
6.060 272
6.371 562
6.646 402
7.178 230
7.699 1825
8.190 245
Counts (%) vs. Acquisition Time (min)
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run019.d
5.409 232
5.810 621
6.060 272
6.371 562
6.646 402
7.178 230
7.699 1825
8.190 245
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run019.d
4.068 4311
5.161 200
5.988 2144
6.760 391
7.258 269
7.573 1104
8.016 1061
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run019.d
4.064 15178
4.923 167
6.003 1678
7.353 214
7.897 1295
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run019.d
4.060 2124
5.281 51
5.925 162
6.339 164
6.653 264
7.042 202
7.491 228
7.842 552
8.215 73
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run019.d
4.068 183
4.581 32
5.000 40
5.440 76
5.789 95
6.132 49
6.390 89
6.828 181
7.387 344
7.633 176
7.911 215
8.340 185
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run019.d
6.000 218
6.253 282
6.716 756
7.020 342
7.277 164
8.006 1668
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 100.1000) 2012-03-08-run019.d
5.708 57
6.121 62
6.474 4505
7.761 675
8.116 73
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 112.9000) 2012-03-08-run019.d
6.473 503
8.230 4075
Counts (%) vs. Acquisition Time (min)

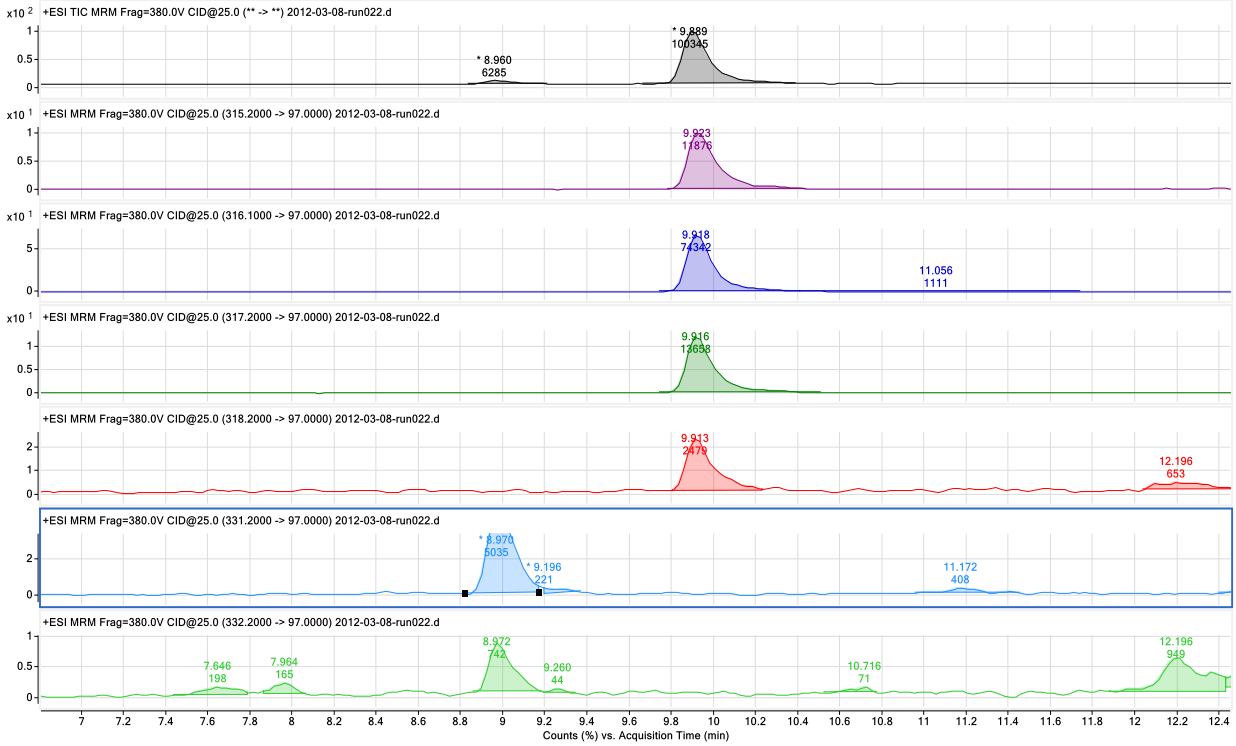

+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run019.d
4.628 6998
6.168 1043
6.687 224
7.220 469
7.836 899
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run019.d
4.625 32579
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run019.d
3.763 204
4.628 6344
5.193 130
6.248 870
7.673 1482
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run019.d
4.628 1162
7.177 92
7.702 24
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run019.d
4.068 4311
5.161 200
5.988 2144
6.760 391
7.258 269
7.573 1104
8.016 1061
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run019.d
4.064 15178
4.923 167
6.003 1678
7.353 214
7.897 1295
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run019.d
4.060 2124
5.281 51
5.925 162
6.339 164
6.653 264
7.042 202
7.491 228
7.842 552
8.215 73
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run019.d
4.068 183
4.581 32
5.000 40
5.440 76
5.789 95
6.132 49
6.390 89
6.828 181
7.387 344
7.633 176
7.911 215
8.340 185
Counts (%) vs. Acquisition Time (min)
+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run020.d
4.047 25882
4.598 58479
7.058 149173
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run020.d
4.625 8539
6.032 512
6.511 308
7.504 528
8.008 973
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run020.d
4.623 39167
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run020.d
4.624 7256
6.424 197
7.298 97
7.615 89
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run020.d
4.618 1034
6.690 66
7.016 158
8.023 245
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run020.d
4.063 4537
4.753 170
5.181 369
5.985 2509
6.329 839
6.654 495
6.941 424
7.514 2829
8.319 118
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run020.d
4.061 18226
4.945 210
5.652 490
5.987 481
8.116 2803
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run020.d
4.060 2411
5.206 39
5.700 89
5.956 315
6.202 225
6.511 483
6.790 95
7.080 138
7.328 168
8.005 1007
Counts (%) vs. Acquisition Time (min)

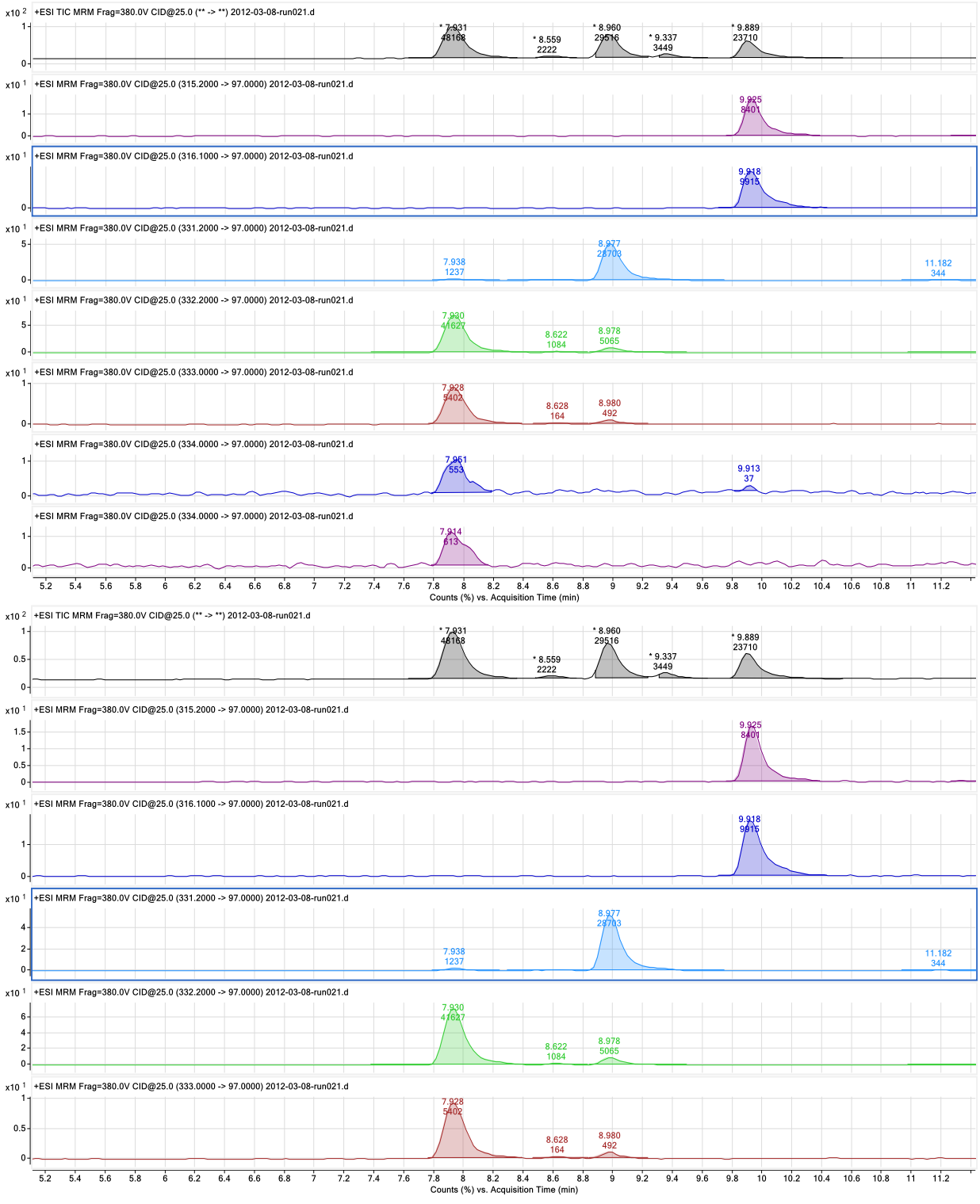

+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run020.d
4.618 1634
6.690 66
7.016 158
8.023 245
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run020.d
4.963 4577
4.753 170
5.181 369
5.985 2509
6.329 839
6.654 495
6.941 424
7.514 2829
8.319 118
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run020.d
4.961 18226
4.945 210
5.652 490
5.987 481
8.116 2803
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run020.d
4.960 2411
5.206 39
5.700 89
5.956 315
6.202 225
6.511 483
6.790 95
7.080 138
7.328 168
8.005 1007
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run020.d
4.961 252
6.188 34
6.533 120
7.392 62
7.881 106
8.178 109
+ESI MRM Frag=380.0V CID@25.0 (335.0000 -> 97.0000) 2012-03-08-run020.d
6.493 2028
7.584 675
8.053 462
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 100.1000) 2012-03-08-run020.d
6.477 1934
5.935 27
6.189 52
6.865 36
7.558 716
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 112.9000) 2012-03-08-run020.d
5.740 52
8.242 4740
Counts (%) vs. Acquisition Time (min)
+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run021.d
* 7.931 46646
* 8.559 2095
* 8.960 23947
* 9.337 2611
* 9.889 21382
+ESI MRM Frag=380.0V CID@25.0 (313.2000 -> 97.0000) 2012-03-08-run021.d
8.975 257
10.972 125
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run021.d
9.925 8401
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run021.d
9.918 9915
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run021.d
9.910 2231
10.388 82
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run021.d
9.915 2792
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run021.d
9.930 441
10.239 160
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run021.d
8.560 809
8.950 224
9.358 2214
Counts (%) vs. Acquisition Time (min)

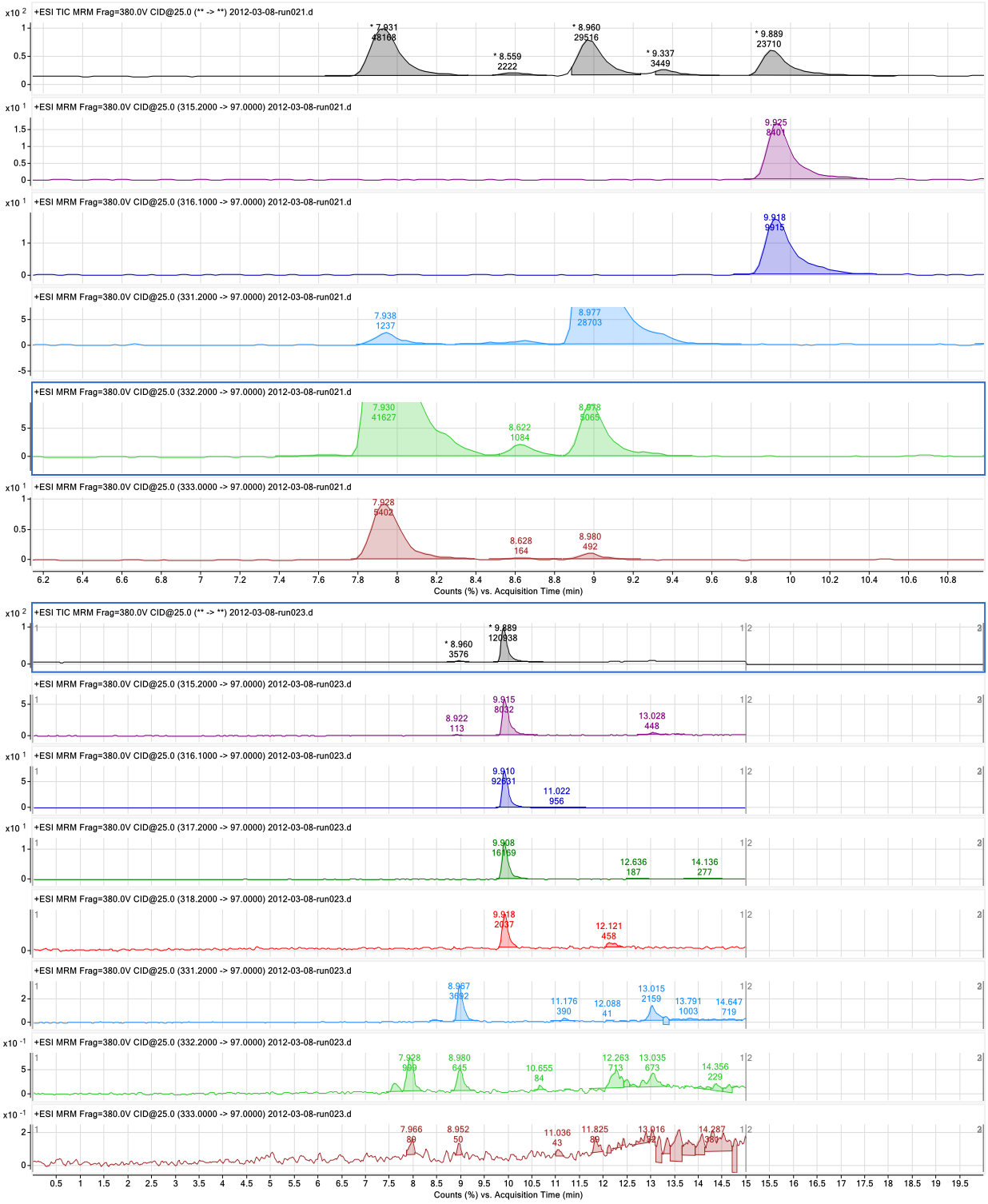

+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run021.d
9.910
2231
10.388
82
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run021.d
9.915
2792
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run021.d
9.930
441
10.239
160
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run021.d
8.560
809
8.950
224
9.258
2214
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run021.d
8.566
688
9.274
2131
x10 1
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run021.d
7.938
1237
8.977
26703
x10 1
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run021.d
7.930
41627
8.622
1084
8.978
5065
x10 1
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run021.d
7.928
5402
8.628
164
8.980
492
6.4 6.6 6.8 7 7.2 7.4 7.6 7.8 8 8.2 8.4 8.6 8.8 9 9.2 9.4 9.6 9.8 10 10.2 10.4 10.6 10.8 11
Counts (%) vs. Acquisition Time (min)
+ESI MRM Frag=380.0V CID@25.0 (329.2000 -> 97.0000) 2012-03-08-run021.d
8.566
688
9.274
2131
x10 1
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run021.d
7.938
1237
8.977
26703
x10 1
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run021.d
7.930
41627
8.622
1084
8.978
5065
x10 1
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run021.d
7.928
5402
8.628
164
8.980
492
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run021.d
7.951
553
9.913
37
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run021.d
7.914
513
x10 -1
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 100.1000) 2012-03-08-run021.d
9.899
24
10.536
24
x10 -1
+ESI MRM Frag=380.0V CID@25.0 (339.1000 -> 112.9000) 2012-03-08-run021.d
6.4 6.6 6.8 7 7.2 7.4 7.6 7.8 8 8.2 8.4 8.6 8.8 9 9.2 9.4 9.6 9.8 10 10.2 10.4 10.6 10.8 11
Counts (%) vs. Acquisition Time (min)

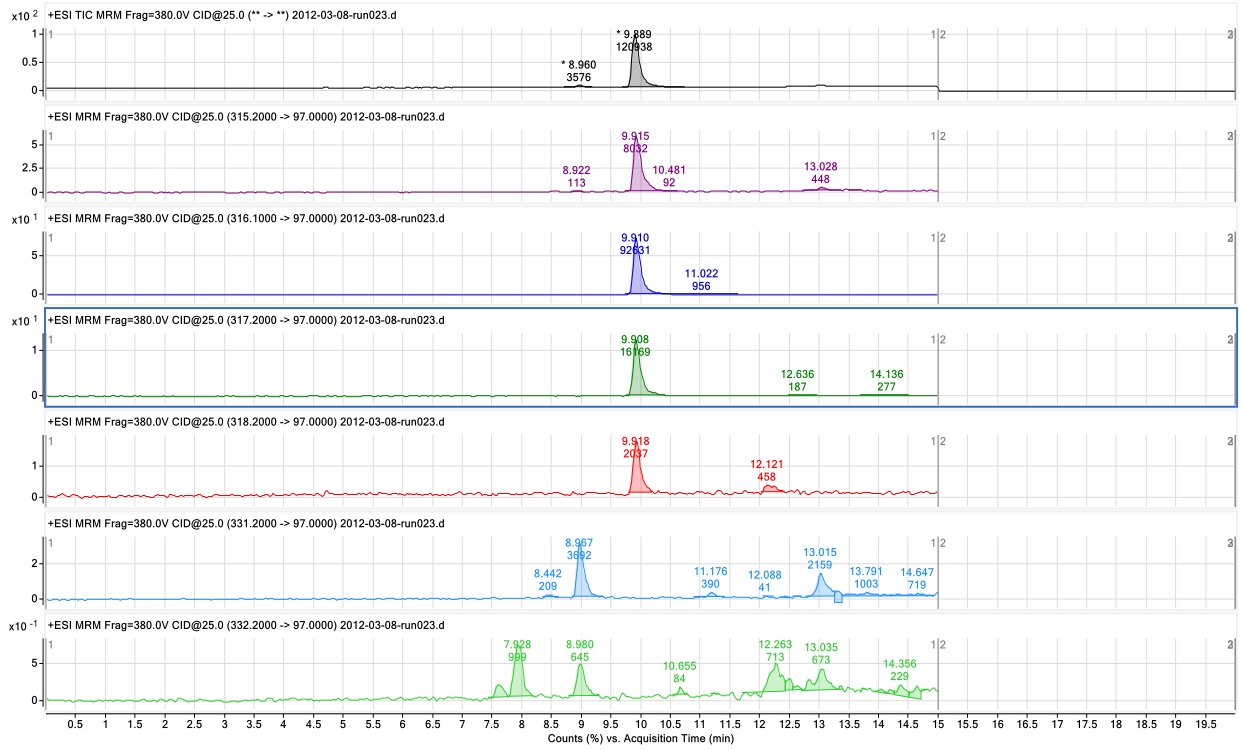

+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run021.d
9.910 2231
10.388 82
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run021.d
9.915 2792
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run021.d
9.930 441
10.239 160
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run021.d
7.938 1237
8.977 26703
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run021.d
7.930 41627
8.622 1084
8.978 5065
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run021.d
7.928 5402
8.628 164
8.980 492
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run021.d
7.951 553
9.913 37
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run021.d
7.914 613
Counts (%) vs. Acquisition Time (min)
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run021.d
9.910 2231
10.388 82
12.808 214
13.445 56
14.320 587
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run021.d
9.915 2792
+ESI MRM Frag=380.0V CID@25.0 (319.2000 -> 97.0000) 2012-03-08-run021.d
9.930 441
10.239 160
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run021.d
7.938 1237
8.977 26703
11.182 344
13.011 2246
13.327 381
13.772 438
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run021.d
7.930 41627
8.622 1084
8.978 5065
12.231 1183
12.556 536
13.001 525
14.481 679
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run021.d
7.928 5402
8.628 164
8.980 492
14.463 496
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run021.d
7.951 553
9.913 37
12.960 45
13.556 26
13.888 108
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run021.d
7.914 613
12.569 128
13.199 41
13.990 147
14.481 34
Counts (%) vs. Acquisition Time (min)

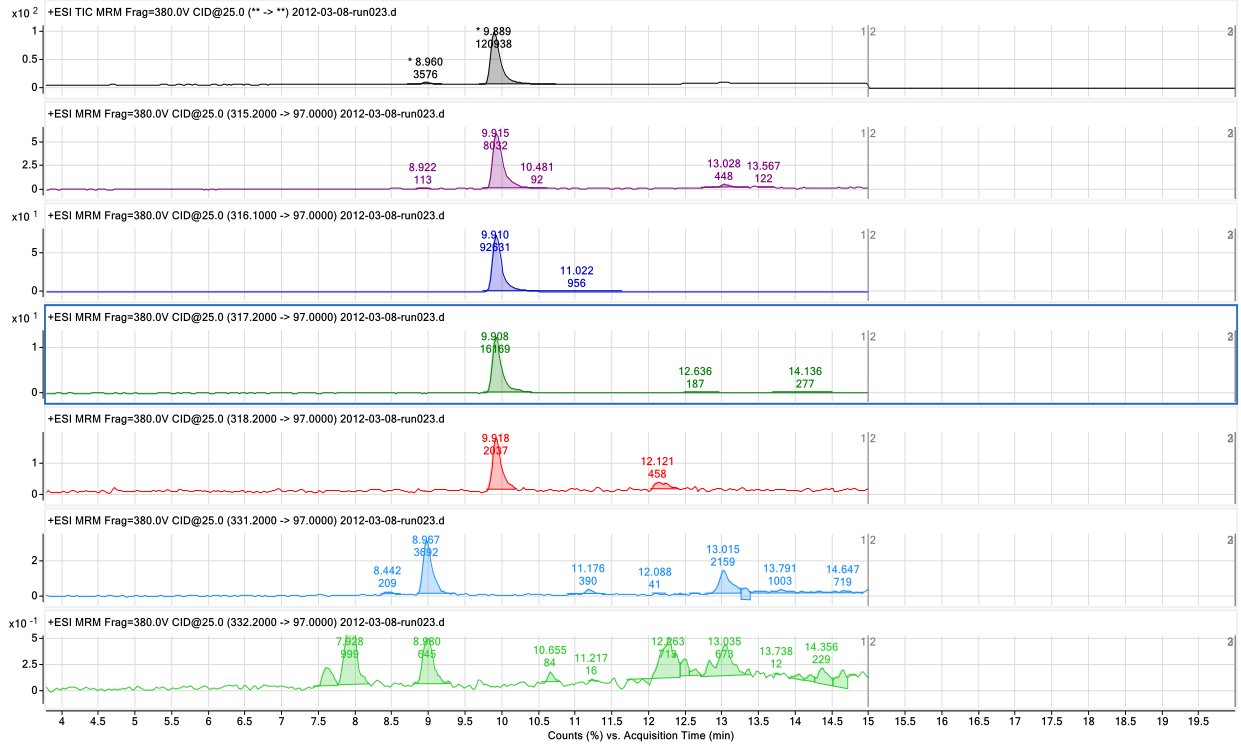

+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run022.d
* 8.960 6285
* 9.889 100345
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run022.d
9.923 11876
13.047 1474
14.083 248
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run022.d
9.918 74342
11.056 1111
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run022.d
9.916 13658
12.661 172
13.847 216
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run022.d
9.913 2479
12.196 653
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run022.d
8.978 5279
11.172 408
13.018 3150
13.841 994
14.688 902
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run022.d
7.964 165
8.972 742
10.716 71
12.196 949
13.036 1136
14.128 273
14.759 132
Counts (%) vs. Acquisition Time (min)

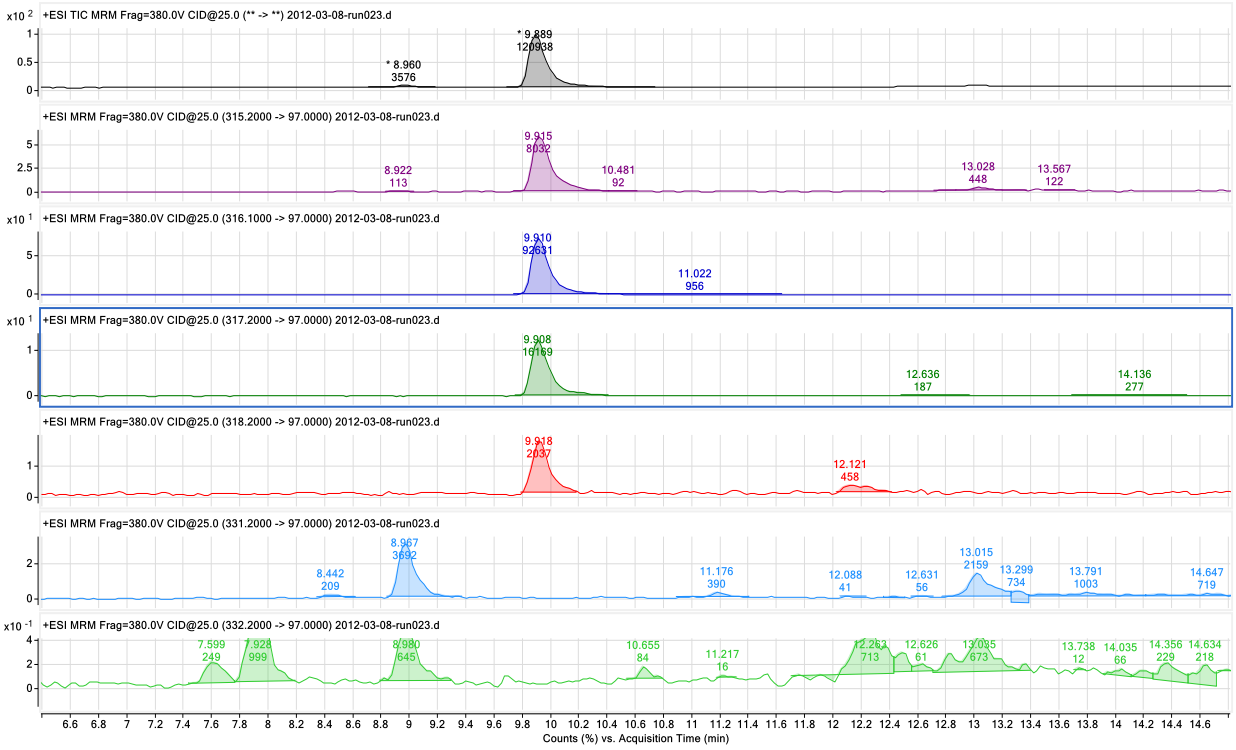

+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run022.d
* 8.960 6285
* 9.889 100345
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run022.d
9.923 11876
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run022.d
9.918 74342
11.056 1111
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run022.d
9.916 13658
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run022.d
9.913 2479
12.196 653
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run022.d
* 8.970 5035
* 9.196 221
11.172 408
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run022.d
7.646 198
7.964 165
8.972 742
9.260 44
10.716 71
12.196 949
Counts (%) vs. Acquisition Time (min)

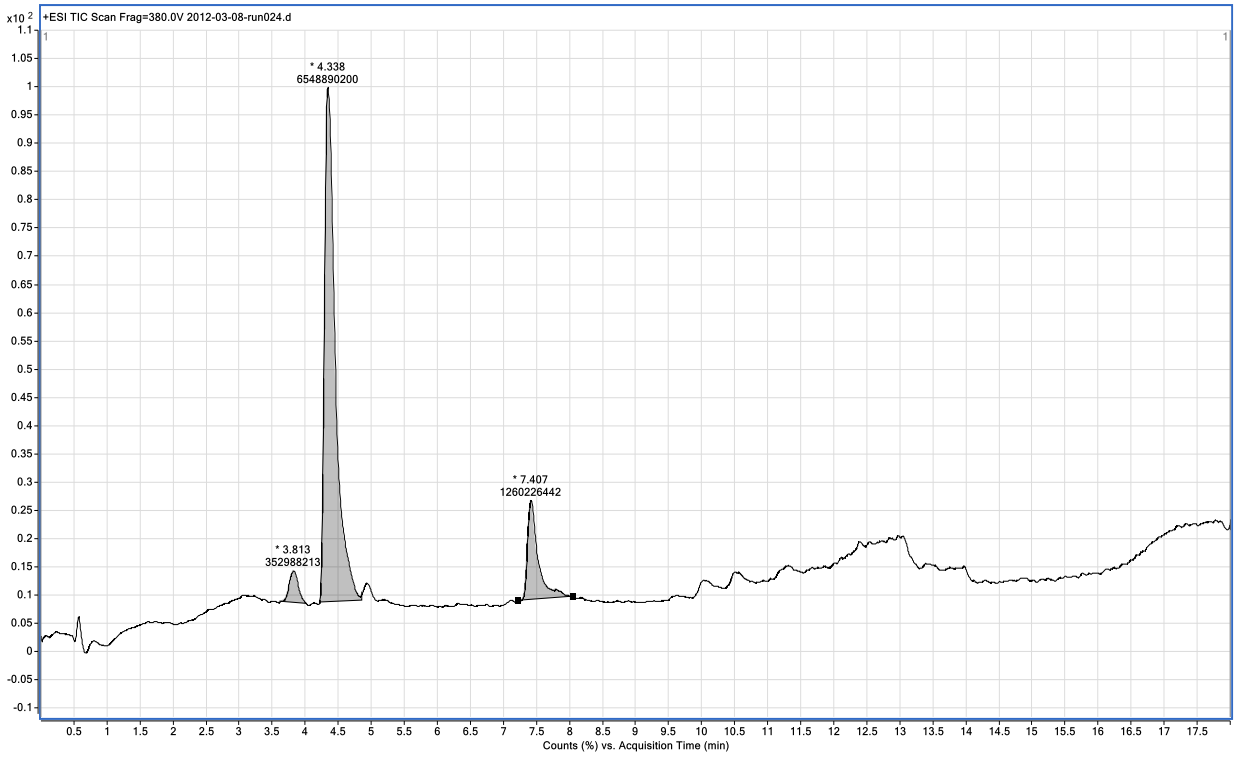

+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run021.d
* 7.931 48168
* 8.559 2222
* 8.960 29516
* 9.337 3449
* 9.889 23710
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run021.d
9.925 8401
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run021.d
9.918 9915
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run021.d
7.938 1237
8.977 28703
11.182 344
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run021.d
7.930 47627
8.622 1084
8.978 5065
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run021.d
7.928 5402
8.628 164
8.980 492
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run021.d
7.951 553
9.913 37
+ESI MRM Frag=380.0V CID@25.0 (334.0000 -> 97.0000) 2012-03-08-run021.d
7.914 613
Counts (%) vs. Acquisition Time (min)
+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run021.d
* 7.931 48168
* 8.559 2222
* 8.960 29516
* 9.337 3449
* 9.889 23710
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run021.d
9.925 8401
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run021.d
9.918 9915
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run021.d
7.938 1237
8.977 28703
11.182 344
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run021.d
7.930 47627
8.622 1084
8.978 5065
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run021.d
7.928 5402
8.628 164
8.980 492
Counts (%) vs. Acquisition Time (min)

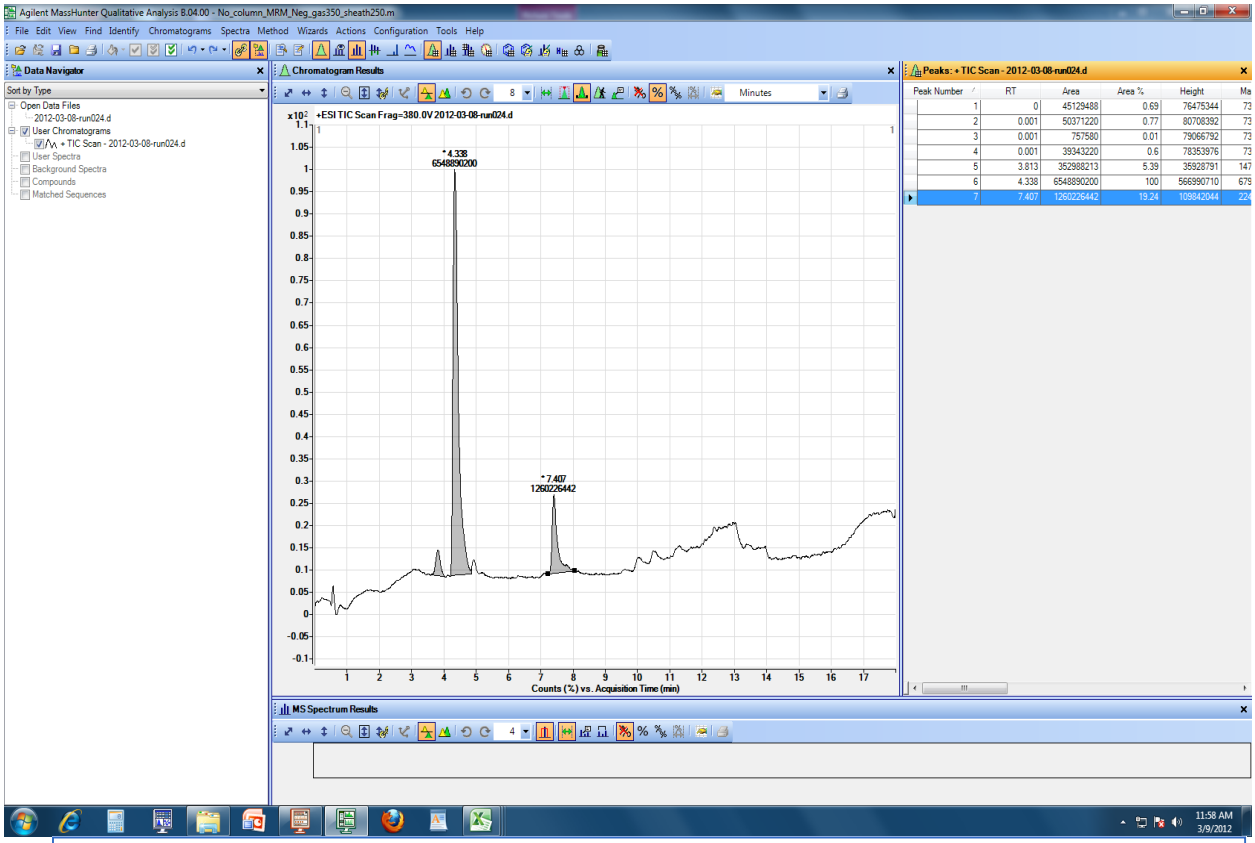

+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run021.d
* 7.931 48188
* 8.559 2222
* 8.960 29516
* 9.337 3449
* 9.889 23710
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run021.d
9.925 8401
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run021.d
9.918 9915
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run021.d
7.938 1237
8.977 28703
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run021.d
7.930 41627
8.622 1084
8.978 5065
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run021.d
7.928 5402
8.628 164
8.980 492
Counts (%) vs. Acquisition Time (min)
+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run023.d
* 8.960 3576
* 9.889 120938
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run023.d
8.922 113
9.915 8032
13.028 448
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run023.d
9.910 92631
11.022 956
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run023.d
9.908 16169
12.636 187
14.136 277
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run023.d
9.918 2037
12.121 458
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run023.d
8.967 3692
11.176 390
12.088 41
13.015 2159
13.791 1003
14.647 719
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run023.d
7.928 999
8.980 645
10.655 84
12.263 713
13.035 673
14.356 229
+ESI MRM Frag=380.0V CID@25.0 (333.0000 -> 97.0000) 2012-03-08-run023.d
7.966 80
8.952 50
11.036 43
11.825 80
13.016 133
14.287 389
Counts (%) vs. Acquisition Time (min)

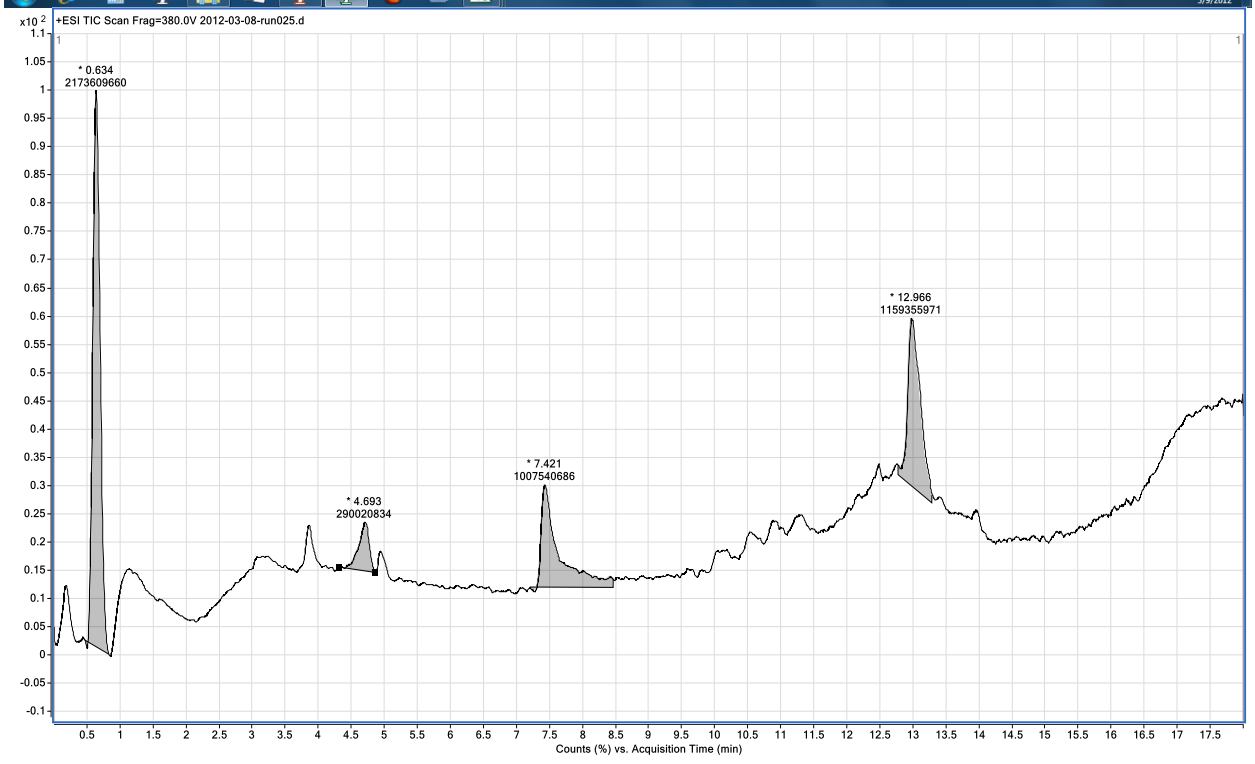

x10 2 +ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run023.d
* 9.889 120938
* 8.960 3576
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run023.d
9.915 8032
8.922 113
10.481 92
13.028 448
x10 1 +ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run023.d
9.910 92631
11.022 956
x10 1 +ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run023.d
9.908 16169
12.636 187
14.136 277
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run023.d
9.918 2037
12.121 458
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run023.d
8.967 3692
8.442 209
11.176 390
12.088 41
13.015 2159
13.791 1003
14.647 719
x10 -1 +ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run023.d
7.928 999
8.980 645
10.655 84
12.263 713
13.035 673
14.356 229
Counts (%) vs. Acquisition Time (min)

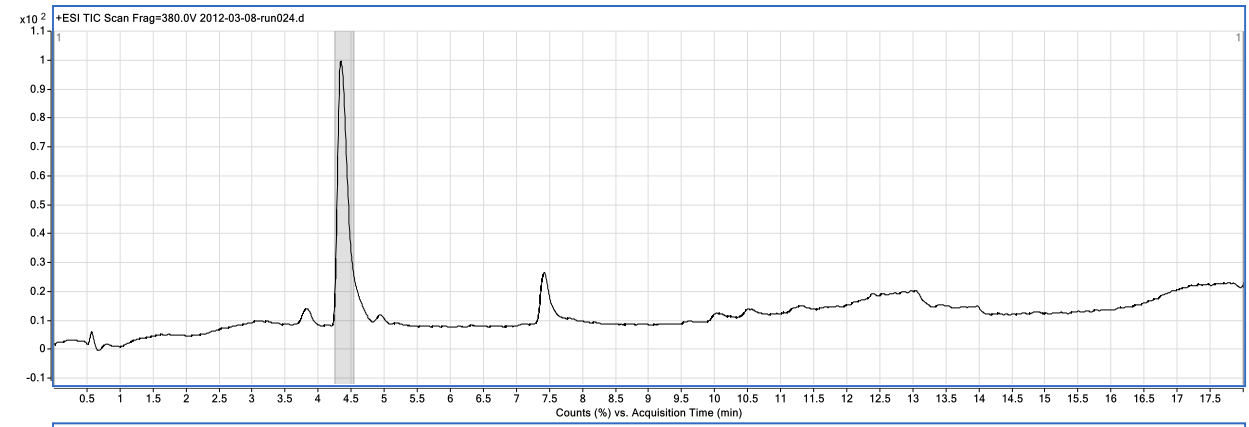

x10 2 +ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run023.d
* 9.889 120938
* 8.960 3576
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run023.d
9.915 8032
8.922 113
10.481 92
13.028 448
13.567 122
x10 1 +ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run023.d
9.910 92631
11.022 956
x10 1 +ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run023.d
9.908 16169
12.636 187
14.136 277
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run023.d
9.918 2037
12.121 458
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run023.d
8.967 3692
8.442 209
11.176 390
12.088 41
13.015 2159
13.791 1003
14.647 719
x10 -1 +ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run023.d
7.928 999
8.980 645
10.655 84
11.217 16
12.263 713
13.035 673
13.738 12
14.356 229
Counts (%) vs. Acquisition Time (min)

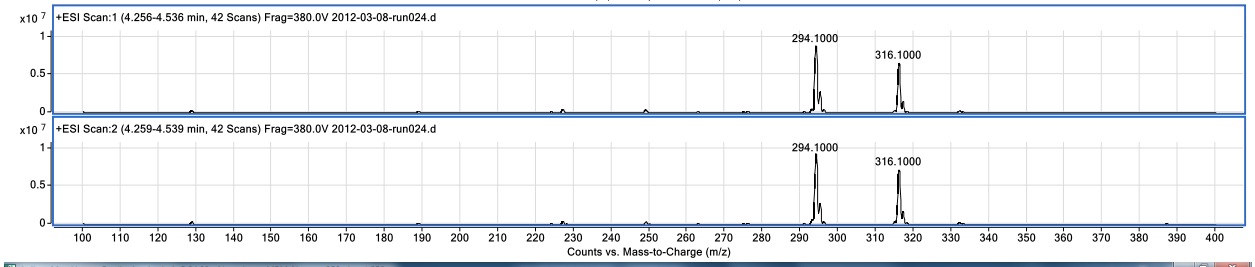

+ESI TIC MRM Frag=380.0V CID@25.0 (** -> **) 2012-03-08-run023.d
* 8.960
3576
* 9.889
120938
+ESI MRM Frag=380.0V CID@25.0 (315.2000 -> 97.0000) 2012-03-08-run023.d
8.922
113
9.915
8032
10.481
92
13.028
448
13.567
122
+ESI MRM Frag=380.0V CID@25.0 (316.1000 -> 97.0000) 2012-03-08-run023.d
9.910
92631
11.022
956
+ESI MRM Frag=380.0V CID@25.0 (317.2000 -> 97.0000) 2012-03-08-run023.d
9.908
16169
12.636
187
14.136
277
+ESI MRM Frag=380.0V CID@25.0 (318.2000 -> 97.0000) 2012-03-08-run023.d
9.918
2037
12.121
458
+ESI MRM Frag=380.0V CID@25.0 (331.2000 -> 97.0000) 2012-03-08-run023.d
8.442
209
8.967
3692
11.176
390
12.088
41
12.631
56
13.015
2159
13.299
734
13.791
1003
14.647
719
+ESI MRM Frag=380.0V CID@25.0 (332.2000 -> 97.0000) 2012-03-08-run023.d
7.599
249
7.928
999
8.980
645
10.655
84
11.217
16
12.263
713
12.626
61
13.035
673
13.738
12
14.035
66
14.356
229
14.634
218
Counts (%) vs. Acquisition Time (min)

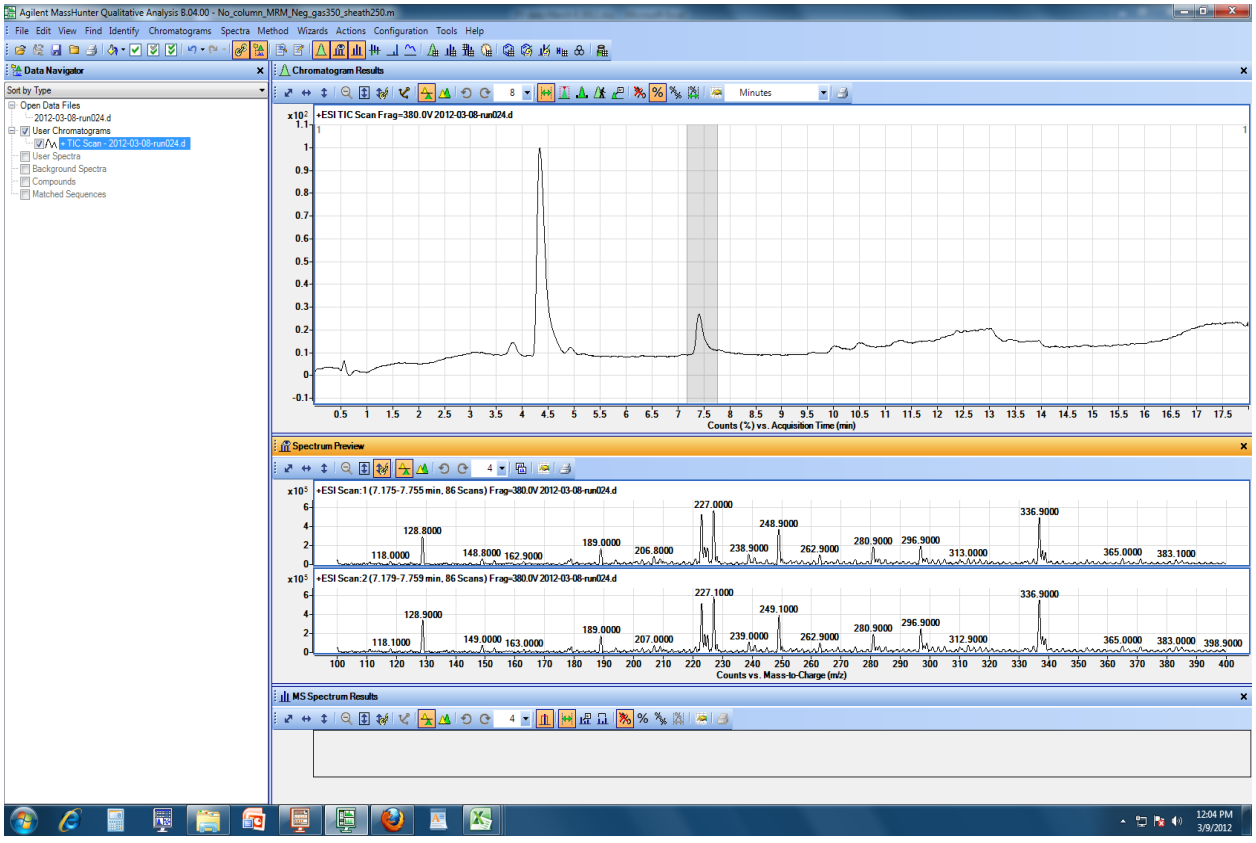

+ESI TIC Scan Frag=380.0V 2012-03-08-run024.d
* 4.338
6548890200
* 7.407
1260226442
* 3.813
352988213
Counts (%) vs. Acquisition Time (min)

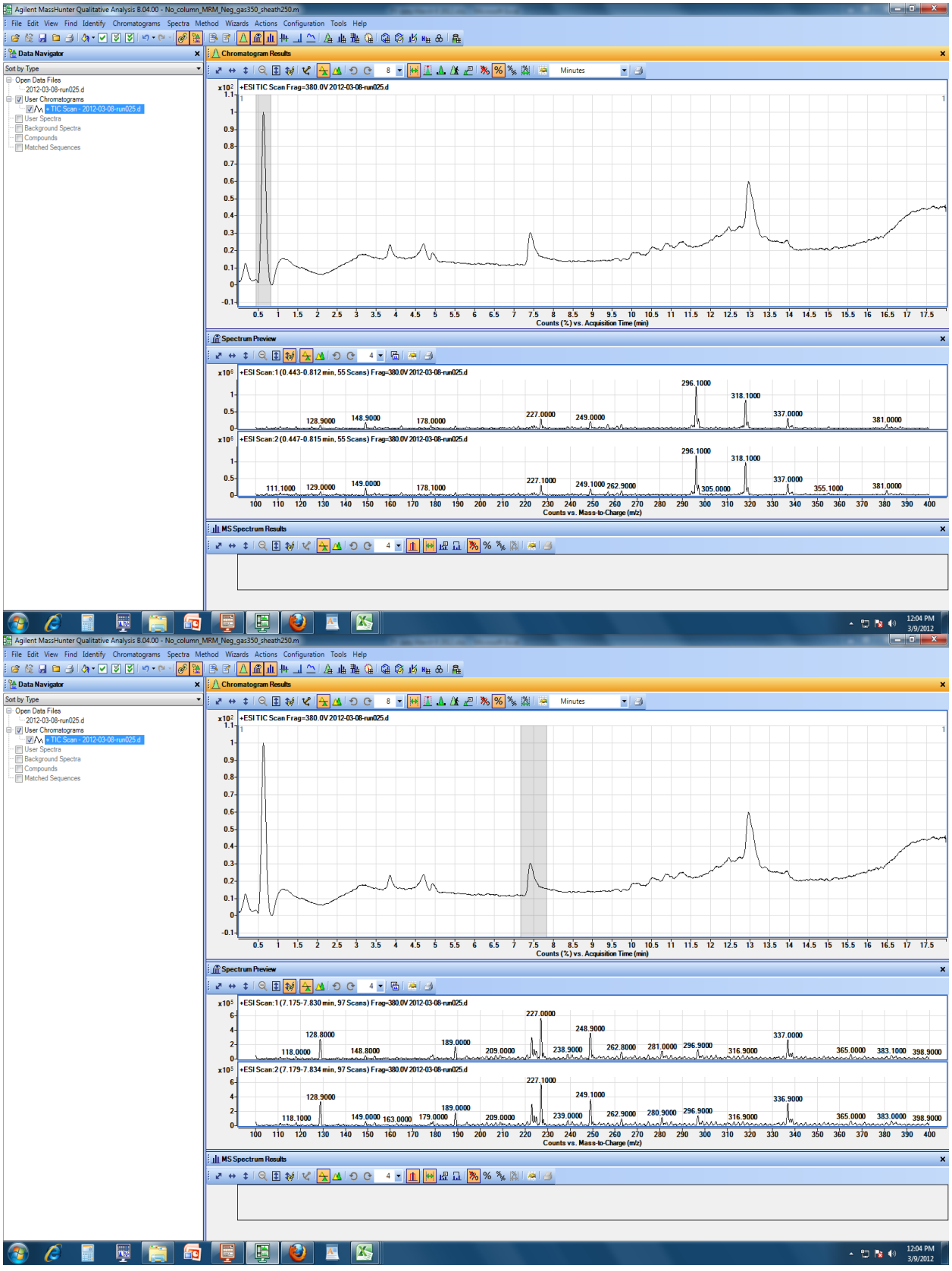

Agilent MassHunter Qualitative Analysis B.04.00 - No_column_MRM_Neg_gas350_sheath250.m
File Edit View Find Identify Chromatograms Spectra Method Wizards Actions Configuration Tools Help
Data Navigator
Sort by Type
Open Data Files
2012-03-08-run024.d
User Chromatograms
+ TIC Scan - 2012-03-08-run024.d
User Spectra
Background Spectra
Compounds
Matched Sequences
Chromatogram Results
Minutes
x10^2 +ESI TIC Scan Frag=380.0V 2012-03-08-run024.d
* 4.338
6548890200
* 7.407
1260226442
Counts (%) vs. Acquisition Time (min)
Peaks: + TIC Scan - 2012-03-08-run024.d
Peak Number RT Area Area % Height
1 0 4529488 0.69 76475344
2 0.001 50371220 0.77 80708392
3 0.001 757580 0.01 79066792
4 0.001 39343220 0.6 78353976
5 3.813 352988213 5.39 35928791
6 4.338 6548890200 100 566990710
7 7.407 1260226442 19.24 109842044
MS Spectrum Results
11:58 AM
3/9/2012

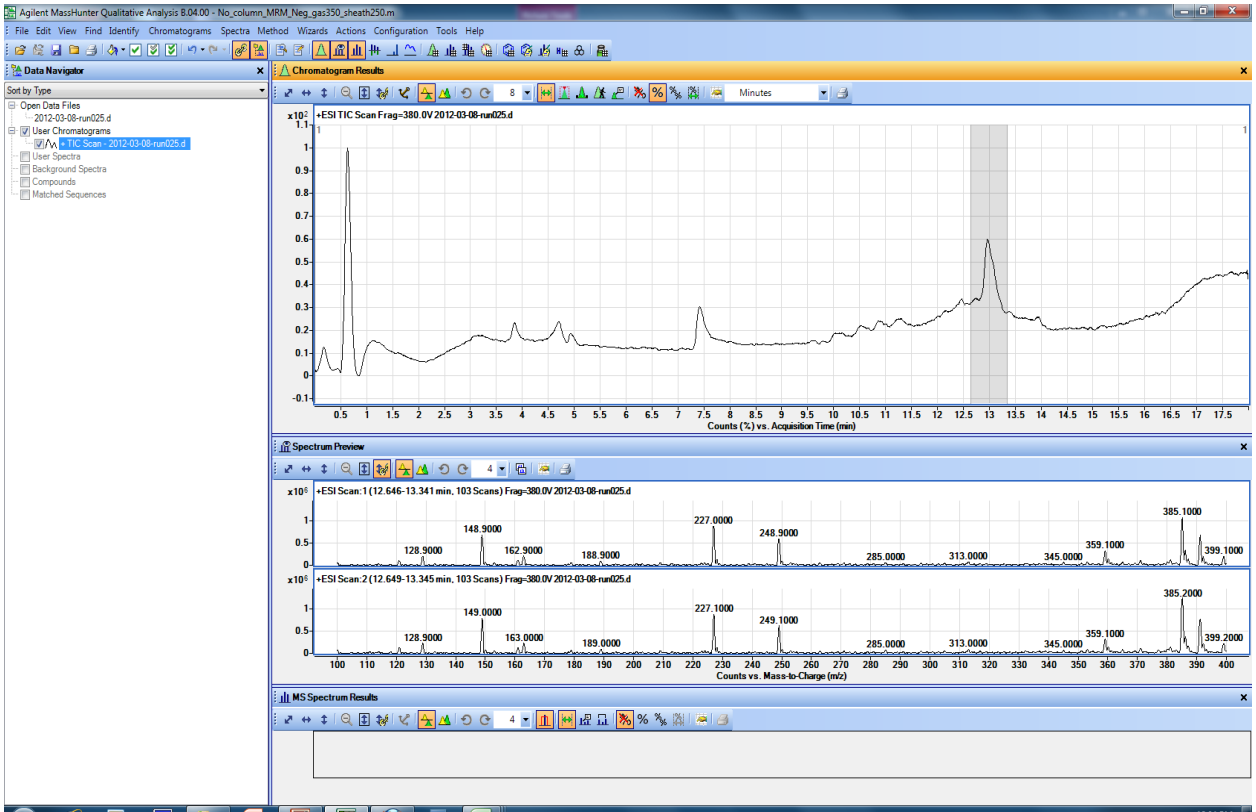

x10^2 +ESI TIC Scan Frag=380.0V 2012-03-08-run025.d
* 0.634
2173609660
* 4.693
290020834
* 7.421
1007540686
* 12.966
1159355971
Counts (%) vs. Acquisition Time (min)

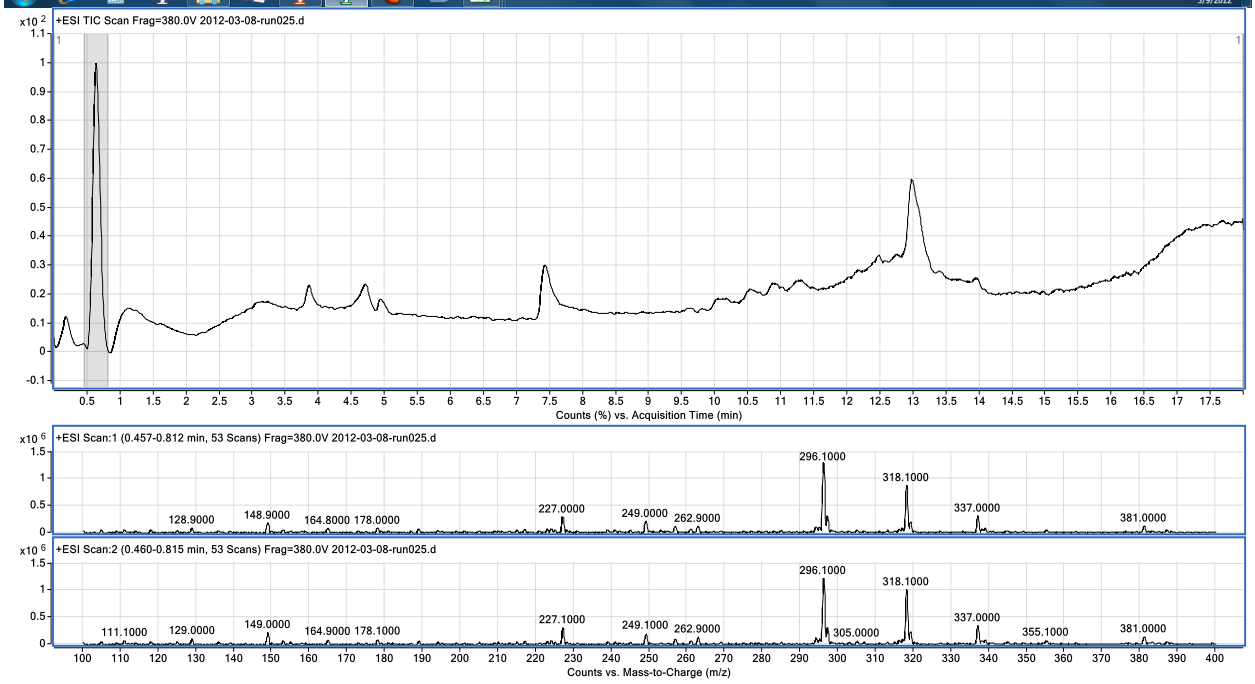

+ESI TIC Scan Frag=380.0V 2012-03-08-run024.d
Counts (%) vs. Acquisition Time (min)

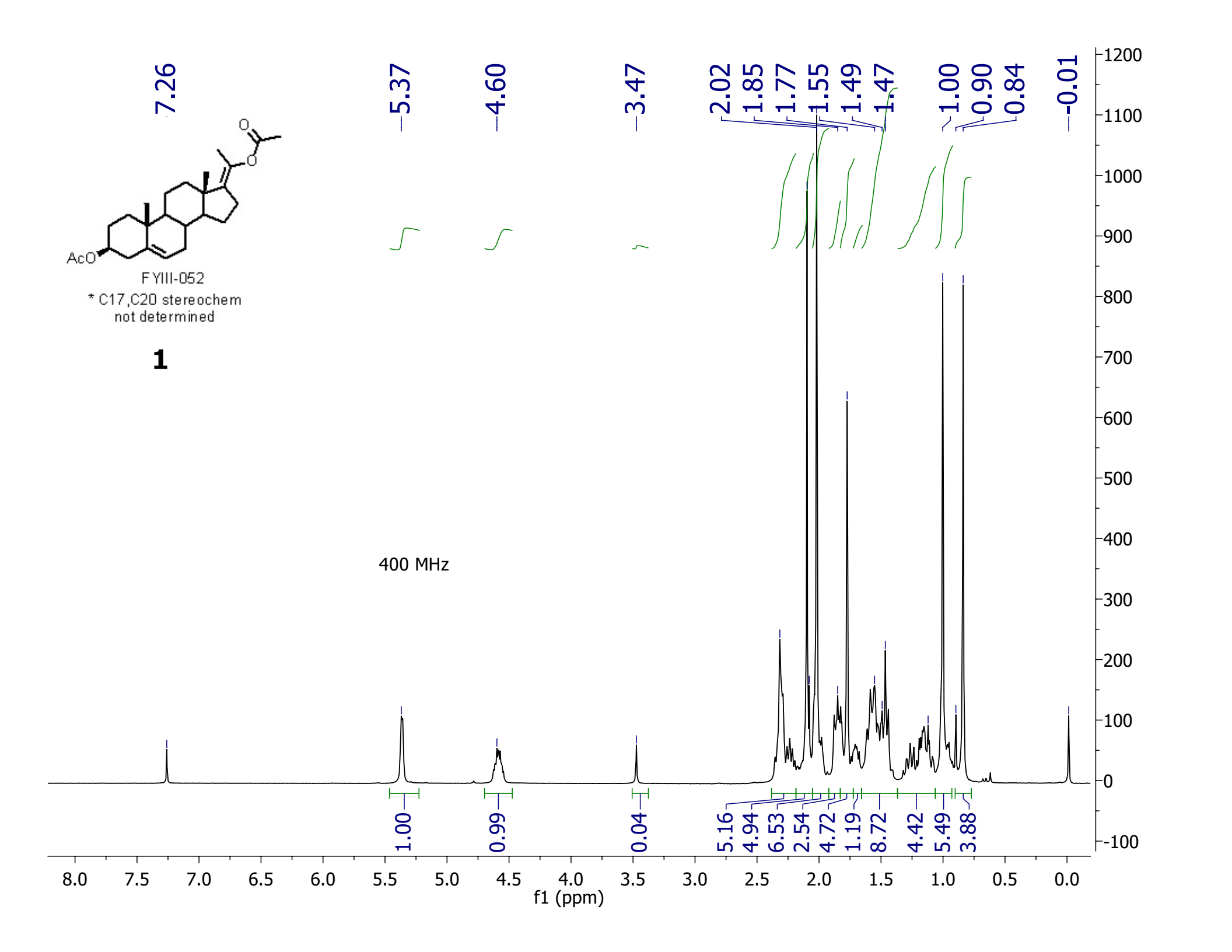

+ESI Scan:1 (4.256-4.536 min, 42 Scans) Frag=380.0V 2012-03-08-run024.d
294.1000
316.1000
+ESI Scan:2 (4.259-4.539 min, 42 Scans) Frag=380.0V 2012-03-08-run024.d
294.1000
316.1000
Counts vs. Mass-to-Charge (m/z)

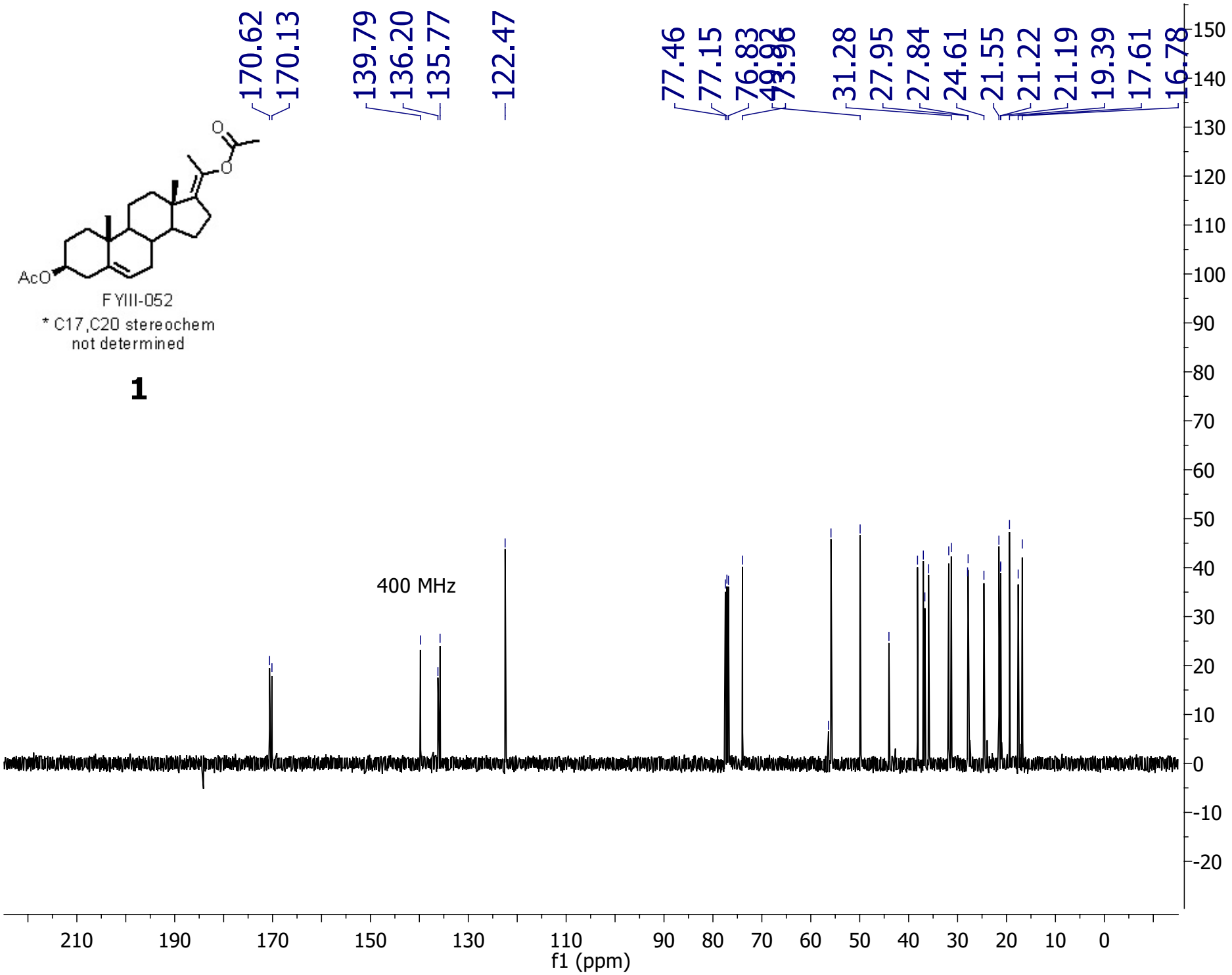

Agilent MassHunter Qualitative Analysis B.04.00 - No_column_MRM_Neg_gas350_sheath250.m
Data Navigator
Chromatogram Results
+ESI TIC Scan Frag=380.0V 2012-03-08-run024.d
Counts (%) vs. Acquisition Time (min)
Spectrum Preview
+ESI Scan:1 (7.175-7.755 min, 86 Scans) Frag=380.0V 2012-03-08-run024.d
+ESI Scan:2 (7.179-7.759 min, 86 Scans) Frag=380.0V 2012-03-08-run024.d
Counts vs. Mass-to-Charge (m/z)
MS Spectrum Results

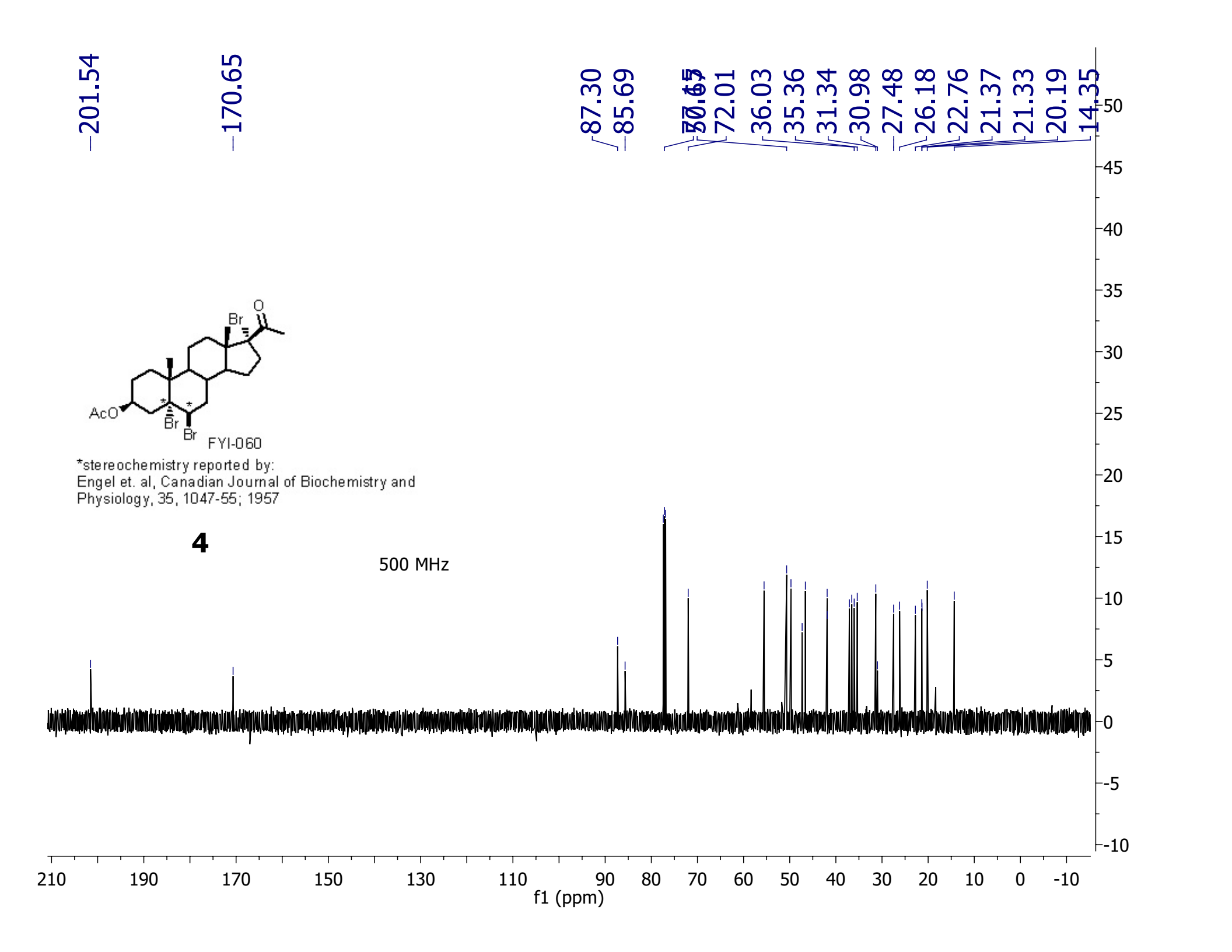

Agilent MassHunter Qualitative Analysis B.04.00 - No_column_MRM_Neg_gas350_sheath250.m
File Edit View Find Identify Chromatograms Spectra Method Wizards Actions Configuration Tools Help
Data Navigator
Sort by Type
Open Data Files
2012-03-08-run025.d
User Chromatograms
TIC Scan - 2012-03-08-run025.d
User Spectra
Background Spectra
Compounds
Matched Sequences
Chromatogram Results
Minutes
+ESI TIC Scan Frag=380.0V 2012-03-08-run025.d
Counts (%) vs. Acquisition Time (min)
Spectrum Preview
+ESI Scan:1 (0.443-0.812 min, 55 Scans) Frag=380.0V 2012-03-08-run025.d
+ESI Scan:2 (0.447-0.815 min, 55 Scans) Frag=380.0V 2012-03-08-run025.d
Counts vs. Mass-to-Charge (m/z)
MS Spectrum Results
12:04 PM
3/9/2012
Agilent MassHunter Qualitative Analysis B.04.00 - No_column_MRM_Neg_gas350_sheath250.m
File Edit View Find Identify Chromatograms Spectra Method Wizards Actions Configuration Tools Help
Data Navigator
Sort by Type
Open Data Files
2012-03-08-run025.d
User Chromatograms
TIC Scan - 2012-03-08-run025.d
User Spectra
Background Spectra
Compounds
Matched Sequences
Chromatogram Results
Minutes
+ESI TIC Scan Frag=380.0V 2012-03-08-run025.d
Counts (%) vs. Acquisition Time (min)
Spectrum Preview
+ESI Scan:1 (7.175-7.830 min, 97 Scans) Frag=380.0V 2012-03-08-run025.d
+ESI Scan:2 (7.179-7.834 min, 97 Scans) Frag=380.0V 2012-03-08-run025.d
Counts vs. Mass-to-Charge (m/z)
MS Spectrum Results
12:04 PM
3/9/2012

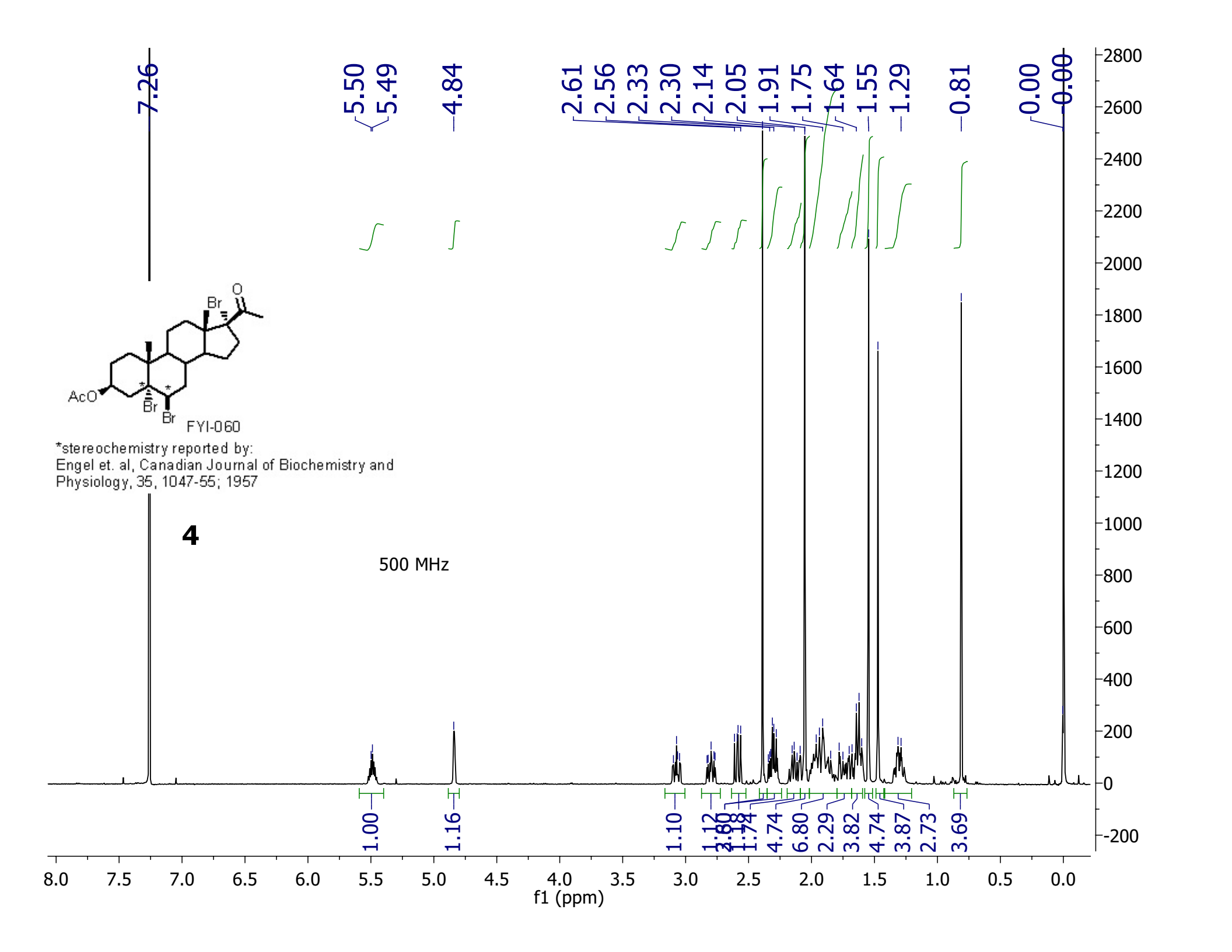

Agilent MassHunter Qualitative Analysis B.04.00 - No_column_MRM_Neg_gas350_sheath250.m
File Edit View Find Identify Chromatograms Spectra Method Wizards Actions Configuration Tools Help
Data Navigator
Sort by Type
Open Data Files
2012-03-08-run025.d
User Chromatograms
+ TIC Scan - 2012-03-08-run025.d
User Spectra
Background Spectra
Compounds
Matched Sequences
Chromatogram Results
Minutes
x10 2 +ESI TIC Scan Frag=380.0V 2012-03-08-run025.d
Counts (%) vs. Acquisition Time (min)
Spectrum Preview
x10 6 +ESI Scan:1 (12.646-13.341 min, 103 Scans) Frag=380.0V 2012-03-08-run025.d
128.9000 148.9000 162.9000 188.9000 227.0000 248.9000 285.0000 313.0000 345.0000 359.1000 385.1000 399.1000
x10 6 +ESI Scan:2 (12.649-13.345 min, 103 Scans) Frag=380.0V 2012-03-08-run025.d
128.9000 149.0000 163.0000 189.0000 227.1000 249.1000 285.0000 313.0000 345.0000 359.1000 385.2000 399.2000
Counts vs. Mass-to-Charge (m/z)
MS Spectrum Results
12:04 PM
3/9/2012

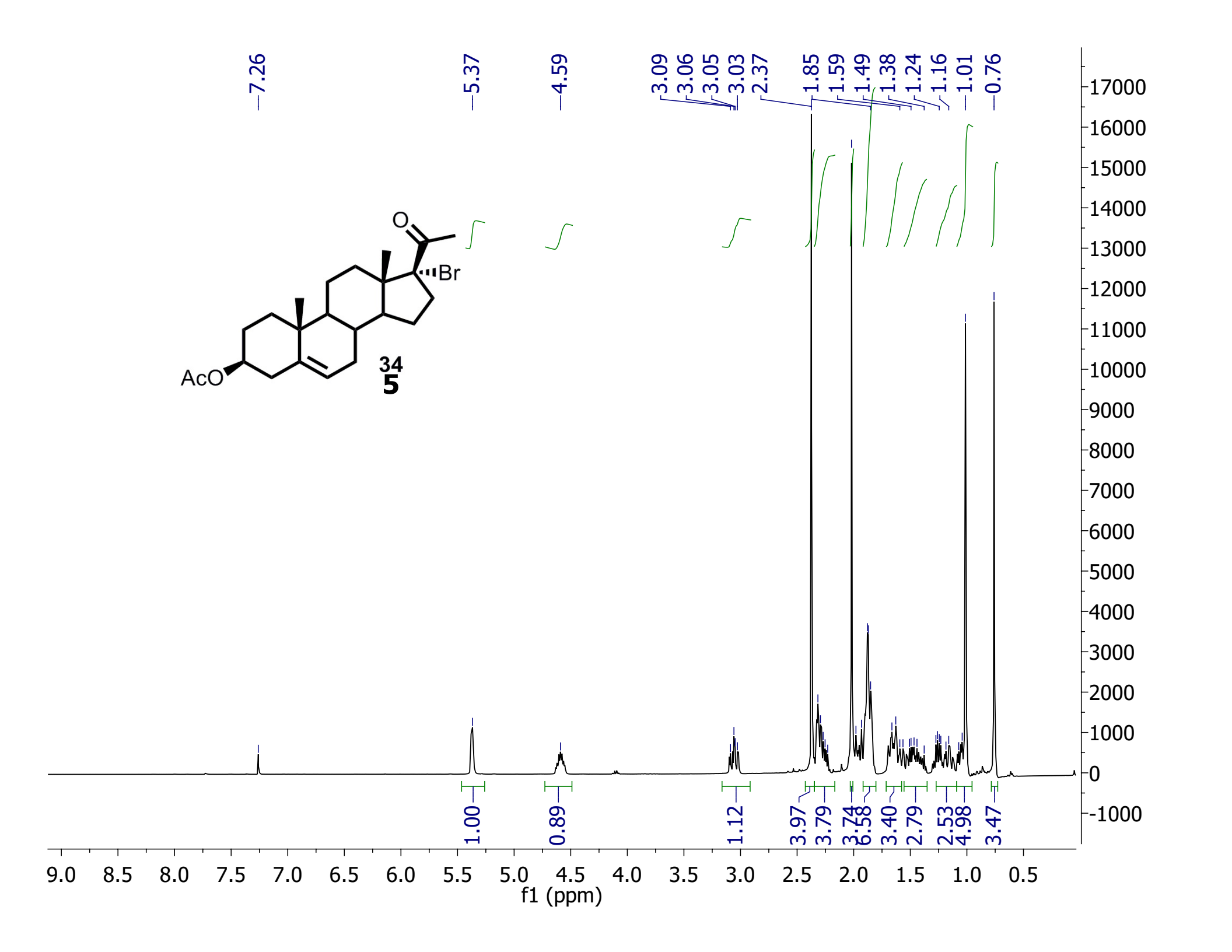

x10 2 +ESI TIC Scan Frag=380.0V 2012-03-08-run025.d
Counts (%) vs. Acquisition Time (min)
x10 6 +ESI Scan:1 (0.457-0.812 min, 53 Scans) Frag=380.0V 2012-03-08-run025.d
128.9000 148.9000 164.8000 178.0000 227.0000 249.0000 262.9000 296.1000 318.1000 337.0000 381.0000
x10 6 +ESI Scan:2 (0.460-0.815 min, 53 Scans) Frag=380.0V 2012-03-08-run025.d
111.1000 129.0000 149.0000 164.9000 178.1000 227.1000 249.1000 262.9000 296.1000 305.0000 318.1000 337.0000 355.1000 381.0000
Counts vs. Mass-to-Charge (m/z)

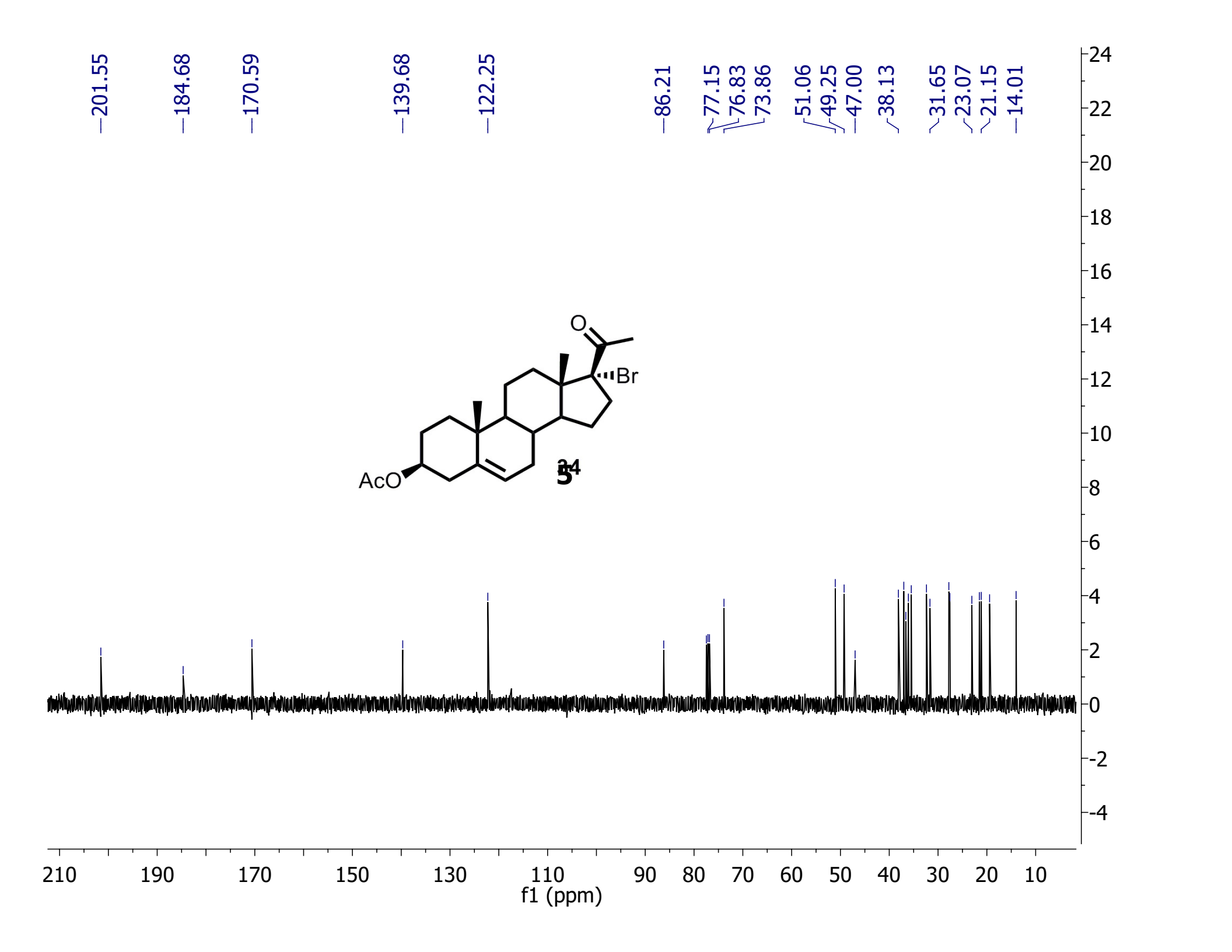

7.26
5.37
4.60
3.47
2.02
1.85
1.77
1.55
1.49
1.47
1.00
0.90
0.84
-0.01
AcO
O
O
FYIII-052
* C17,C20 stereochem
not determined
1
400 MHz
1.00
0.99
0.04
5.16
4.94
6.53
2.54
4.72
1.19
8.72
4.42
5.49
3.88
f1 (ppm)

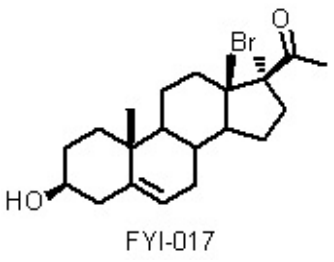

170.62
170.13
139.79
136.20
135.77
122.47
77.46
77.15
76.83
49.82
43.96
31.28
27.95
27.84
24.61
21.55
21.22
21.19
19.39
17.61
16.78
AcO
O
O
FYIII-052
* C17,C20 stereochem
not determined
1
400 MHz
f1 (ppm)


201.54
170.65
87.30
85.69
72.01
36.03
35.36
31.34
30.98
27.48
26.18
22.76
21.37
21.33
20.19
14.35
Br
O
AcO
Br
Br
FYI-060
*stereochemistry reported by:
Engel et. al, Canadian Journal of Biochemistry and Physiology, 35, 1047-55; 1957
4
500 MHz
f1 (ppm)


7.26
5.50
5.49
4.84
2.61
2.56
2.33
2.30
2.14
2.05
1.91
1.75
1.64
1.55
1.29
0.81
0.00
-0.00
Br
O
AcO
Br
Br
FYI-060
*stereochemistry reported by:
Engel et. al, Canadian Journal of Biochemistry and Physiology, 35, 1047-55; 1957
4
500 MHz
1.00
1.16
1.10
1.12
3.80
1.18
1.74
4.74
6.80
2.29
3.82
4.74
3.87
2.73
3.69
f1 (ppm)


7.26
5.37
4.59
3.09
3.06
3.05
3.03
2.37
1.85
1.59
1.49
1.38
1.24
1.16
1.01
0.76
O
Br
AcO
34
5
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
1.00
0.89
1.12
3.97
3.79
3.74
6.58
3.40
2.79
2.53
4.98
3.47
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


201.55
184.68
170.59
139.68
122.25
86.21
77.15
76.83
73.86
51.06
49.25
47.00
38.13
31.65
23.07
21.15
14.01
O
Br
AcO
5
24
22
20
18
16
14
12
10
8
6
4
2
0
-2
-4
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
f1 (ppm)



FYI-017

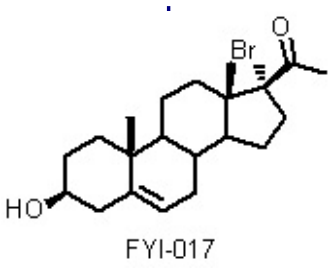
8

5.30 3.47 3.02 2.34 2.21 1.83 1.79 1.61 1.47 1.45 1.24 1.06 0.96 0.72

500 MHz

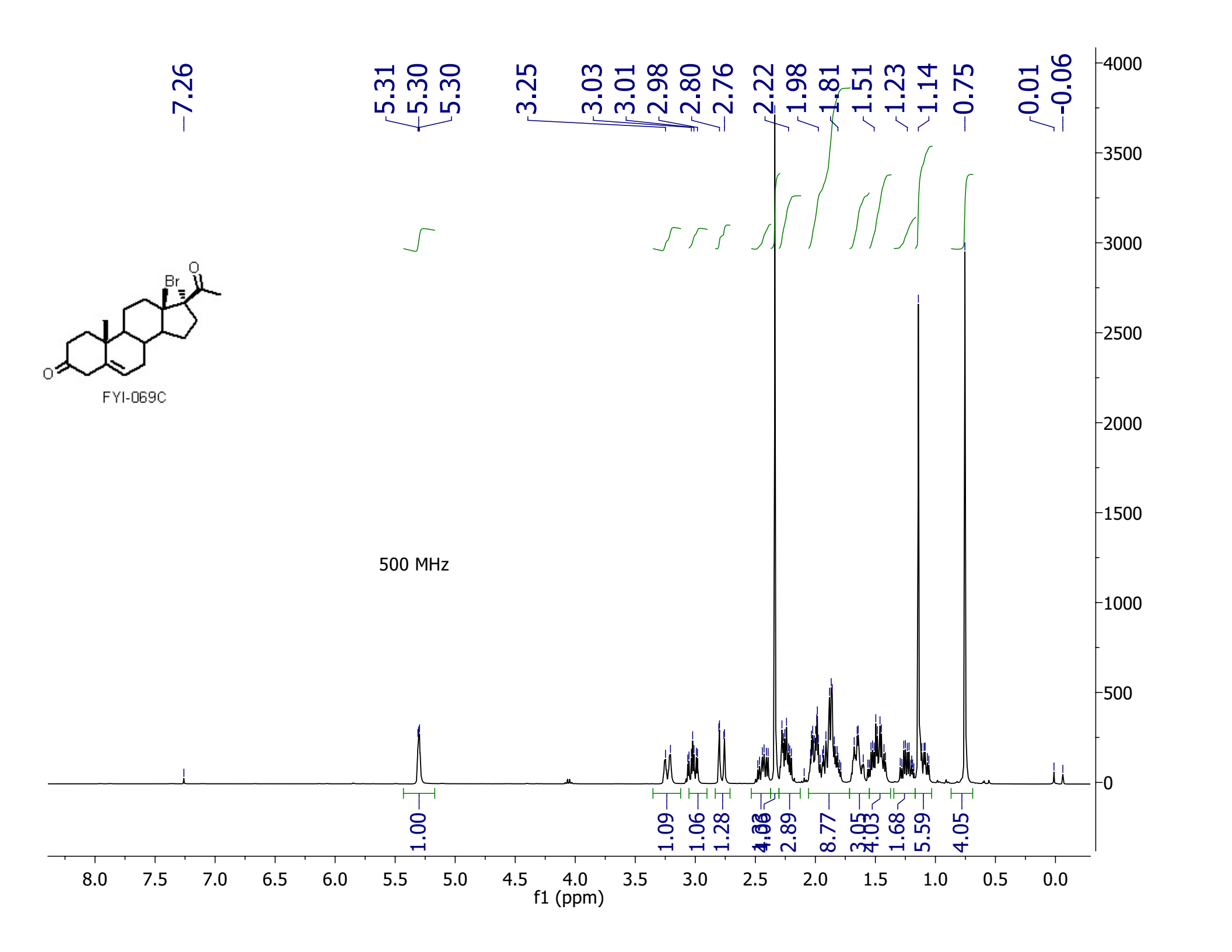
1.00 1.11 1.11 1.29 4.65 4.35 10.44 3.29 4.46 1.59 7.35 4.08

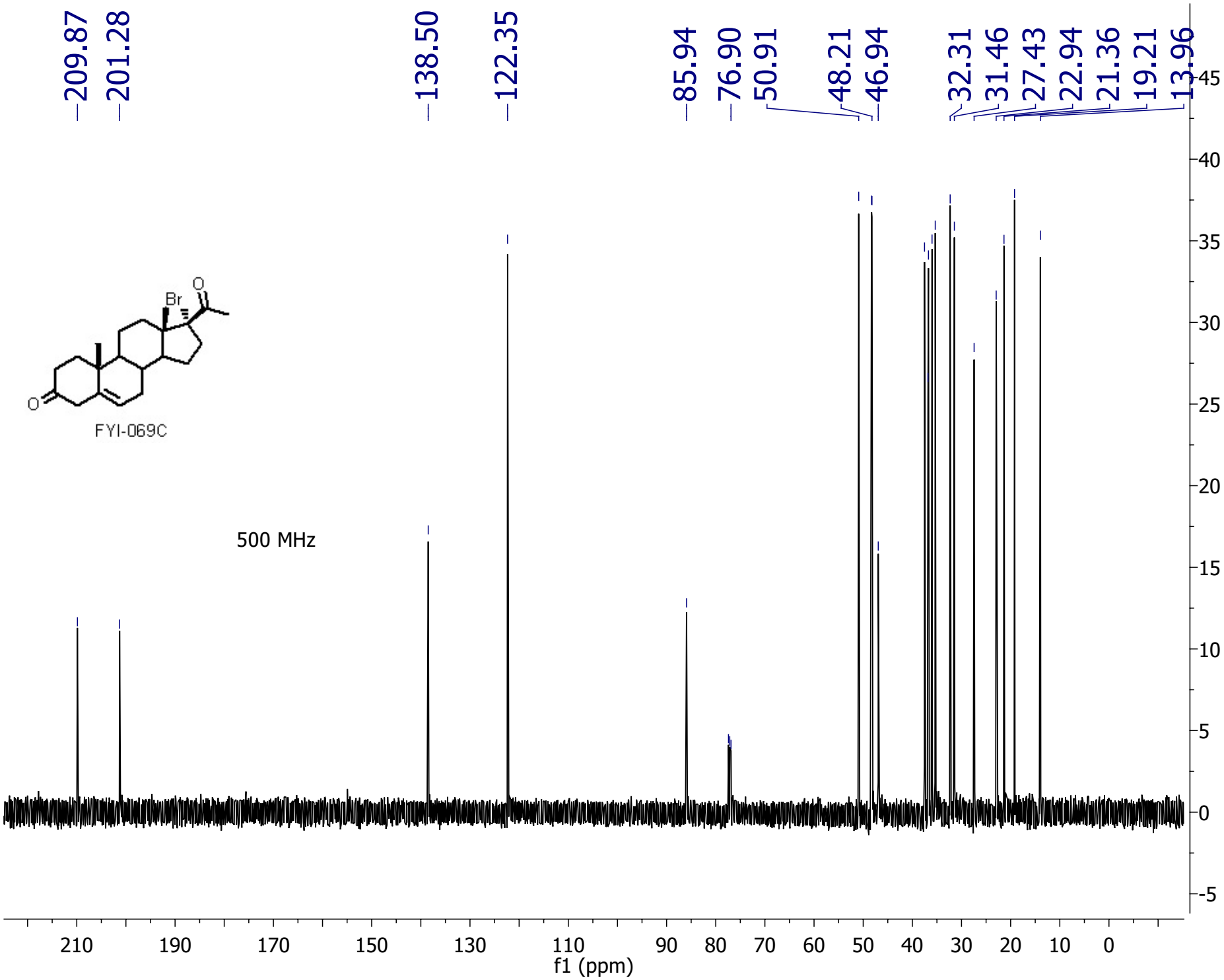
f1 (ppm)

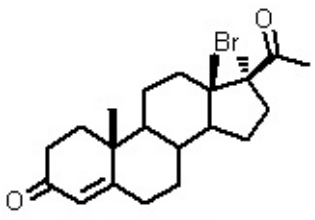

Br
O
HO
FYI-017

8

140.78
121.03
86.18
71.40
51.00
35.96
35.40
32.27
31.54
31.45
27.45
22.94
21.08
19.42
13.88
500 MHz
f1 (ppm)


7.26
5.31
5.30
5.30
3.25
3.03
3.01
2.98
2.80
2.76
2.22
1.98
1.81
1.51
1.23
1.14
0.75
0.01
-0.06
Br
O
O
FYI-069C
500 MHz
1.00
1.09
1.06
1.28
2.89
8.77
1.68
5.59
4.05
f1 (ppm)


209.87
201.28
138.50
122.35
85.94
76.90
50.91
48.21
46.94
32.31
31.46
27.43
22.94
21.36
19.21
13.96
Br
O
O
FYI-069C
500 MHz
f1 (ppm)



FYI-071D

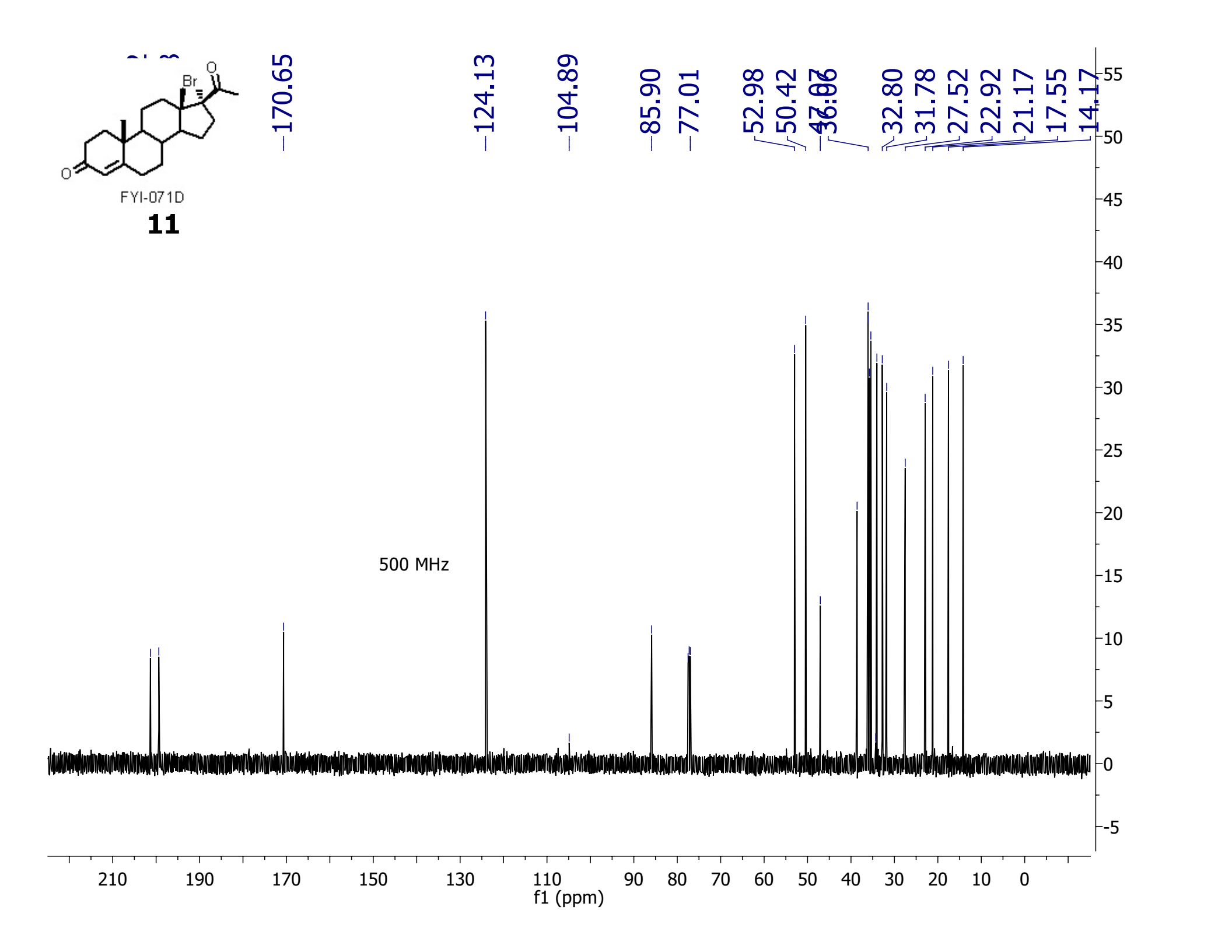
11

5.75 3.60 3.07 3.04 2.40 2.28 1.97 1.87 1.69 1.35 1.17 0.81 0.00 -0.00 -0.01 -0.01

500 MHz

1.00 1.20 8.80 3.83 9.58 2.59 3.21 6.50 1.54 4.55

f1 (ppm)


Br
O
O
FYI-071D
11
170.65
124.13
104.89
85.90
77.01
52.98
50.42
47.07
36.06
32.80
31.78
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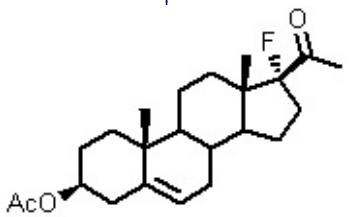
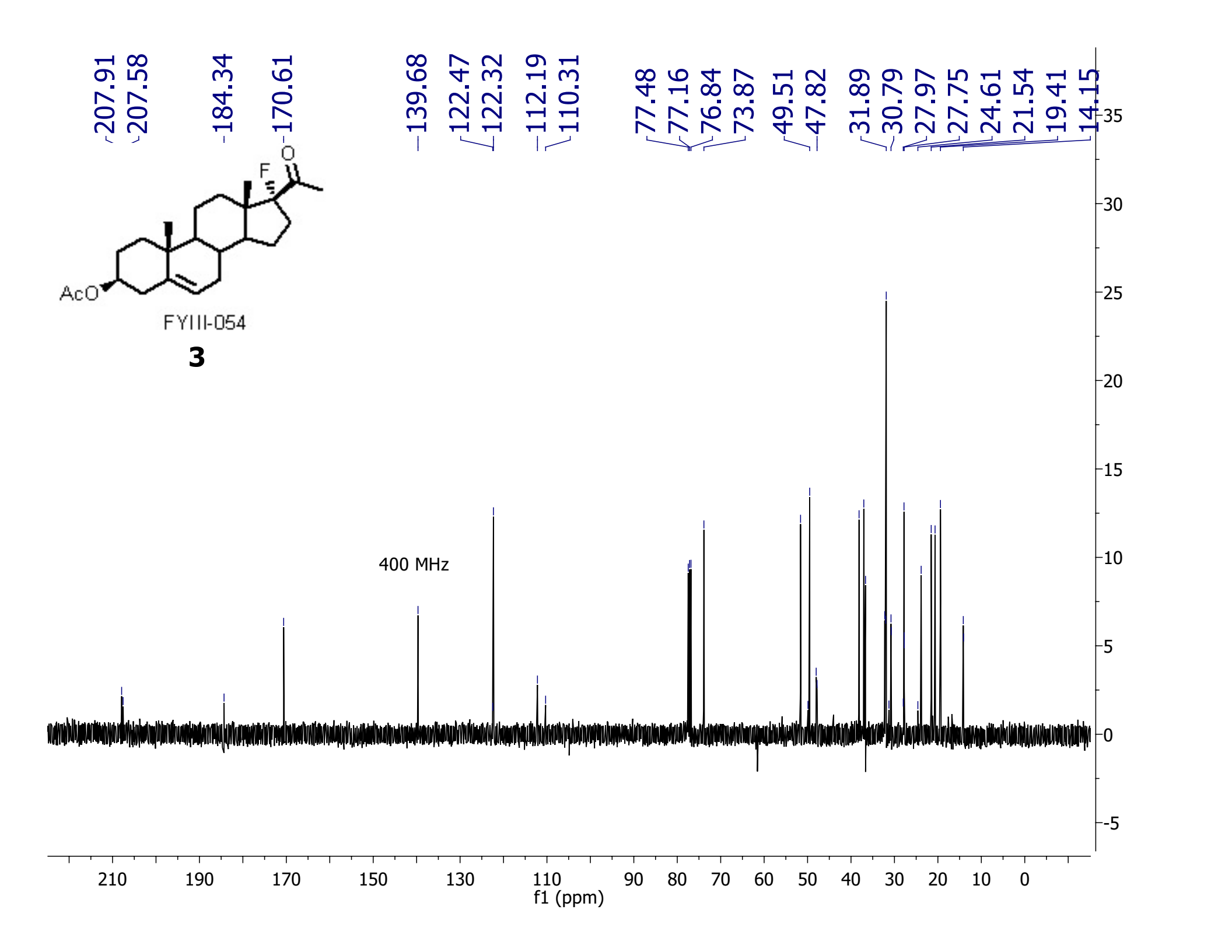
21.17
17.55
14.17
500 MHz
f1 (ppm)

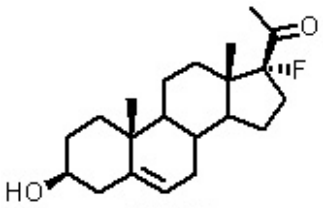

O
F
AcO

FYIII-054

3

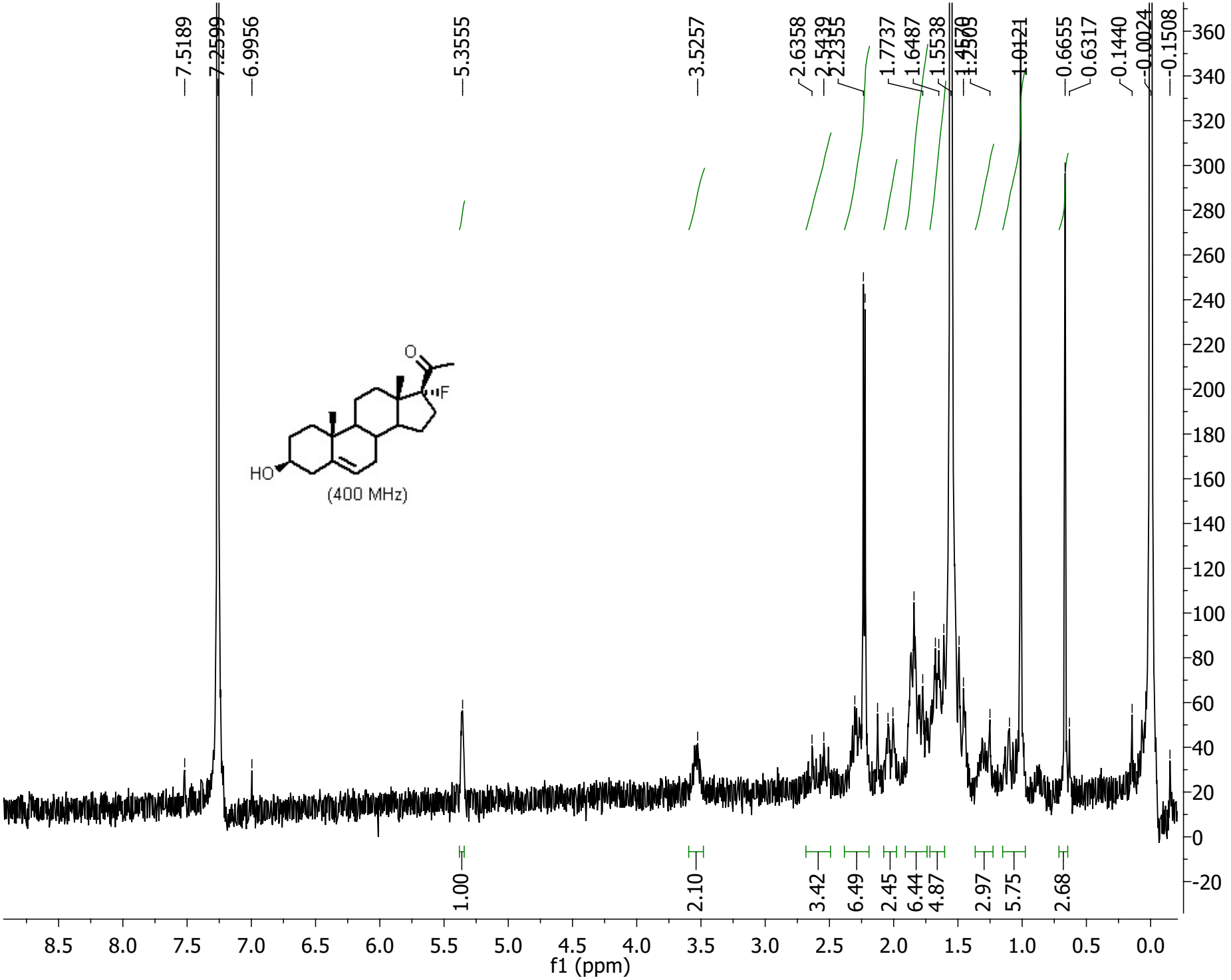
7.26
5.37
4.60
2.01
1.84
1.77
1.71
1.66
1.56
1.48
1.36
1.28
1.14
1.00
0.83
0.64
-0.02
400 MHz
1.52
1.25
1.00
3.49
8.96
23.64
2.70
1.88
8.02
4.78
f1 (ppm)

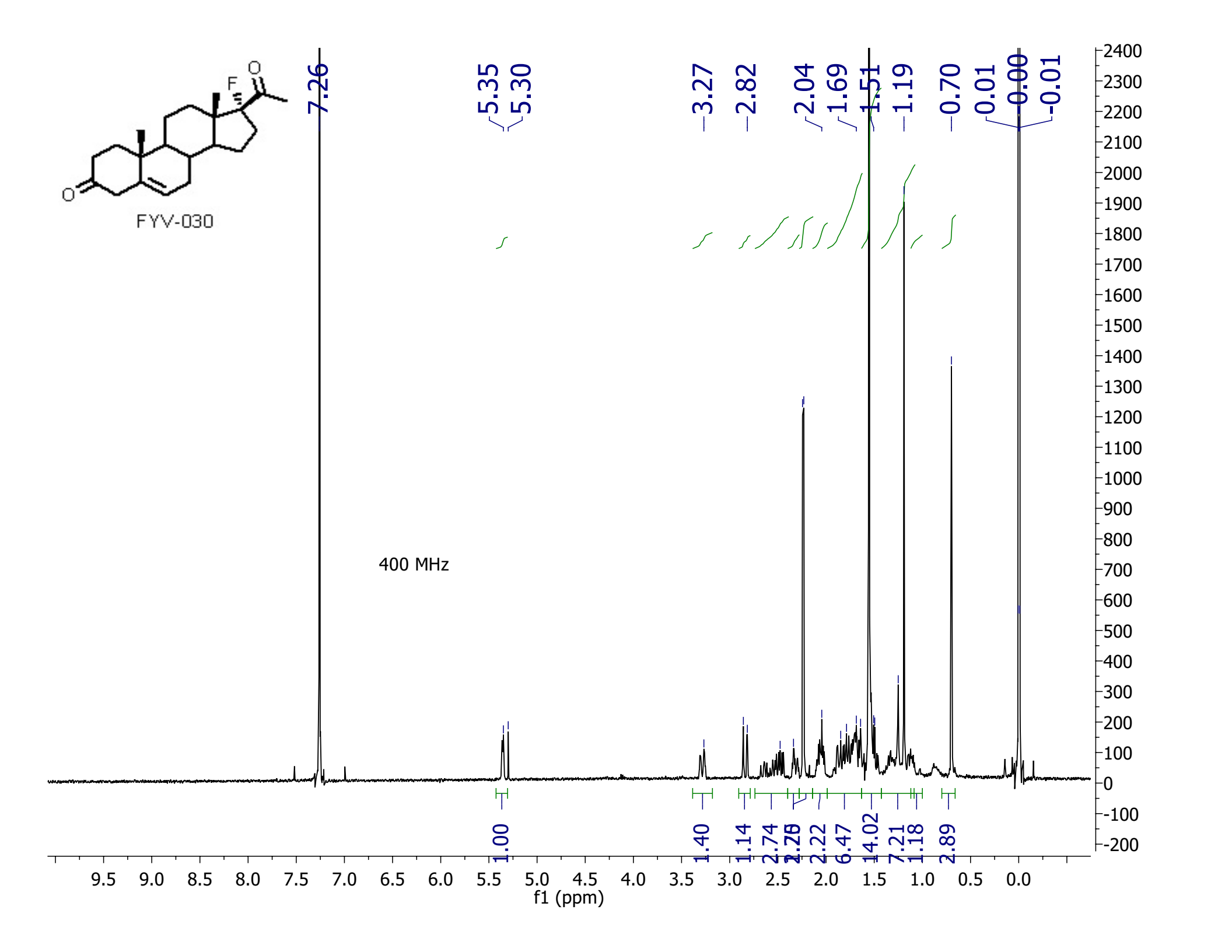

207.91
207.58
184.34
170.61
139.68
122.47
122.32
112.19
110.31
77.48
77.16
76.84
73.87
49.51
47.82
31.89
30.79
27.97
27.75
24.61
21.54
19.41
14.15
O
F
AcO
FYIII-054
3
400 MHz
f1 (ppm)

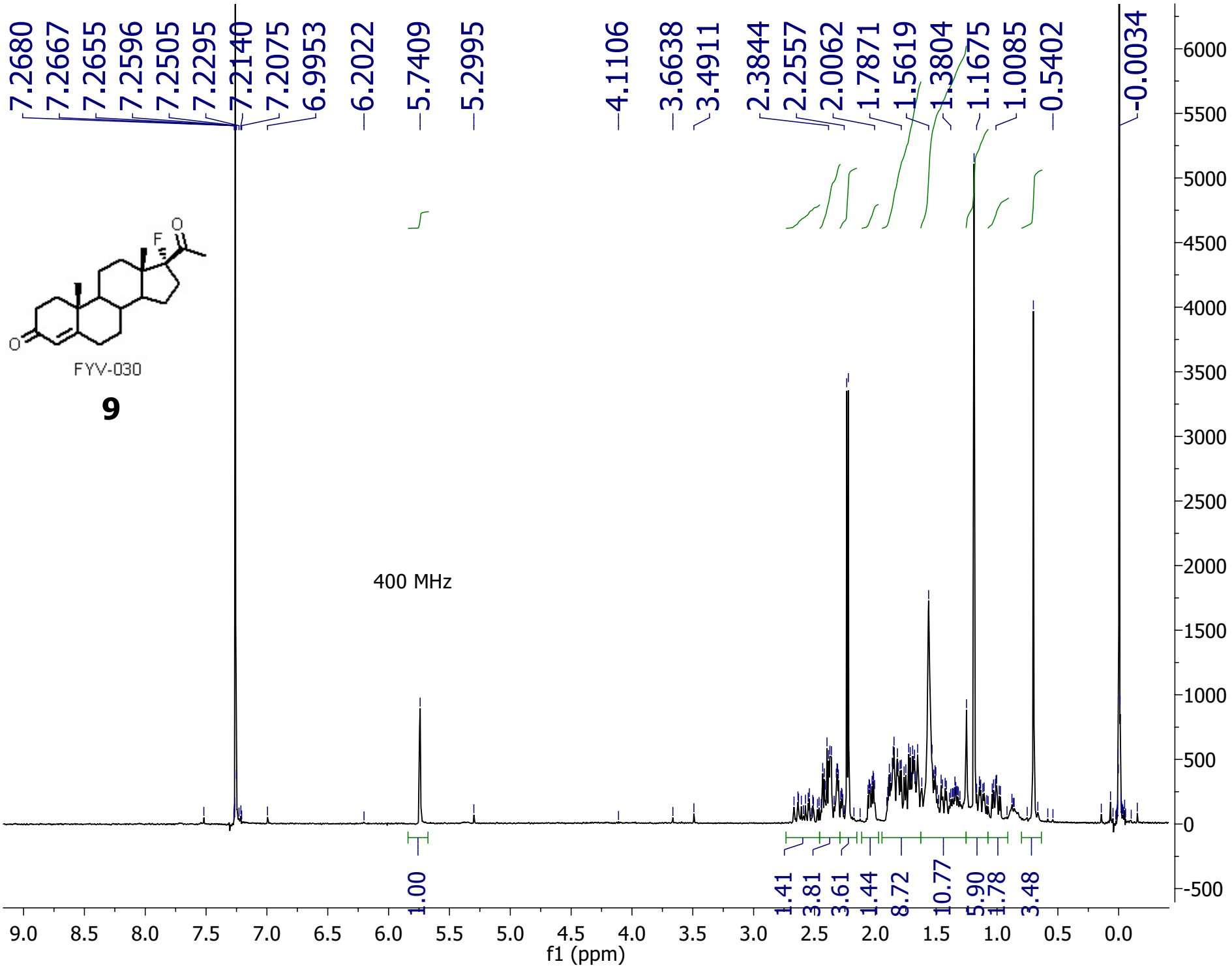


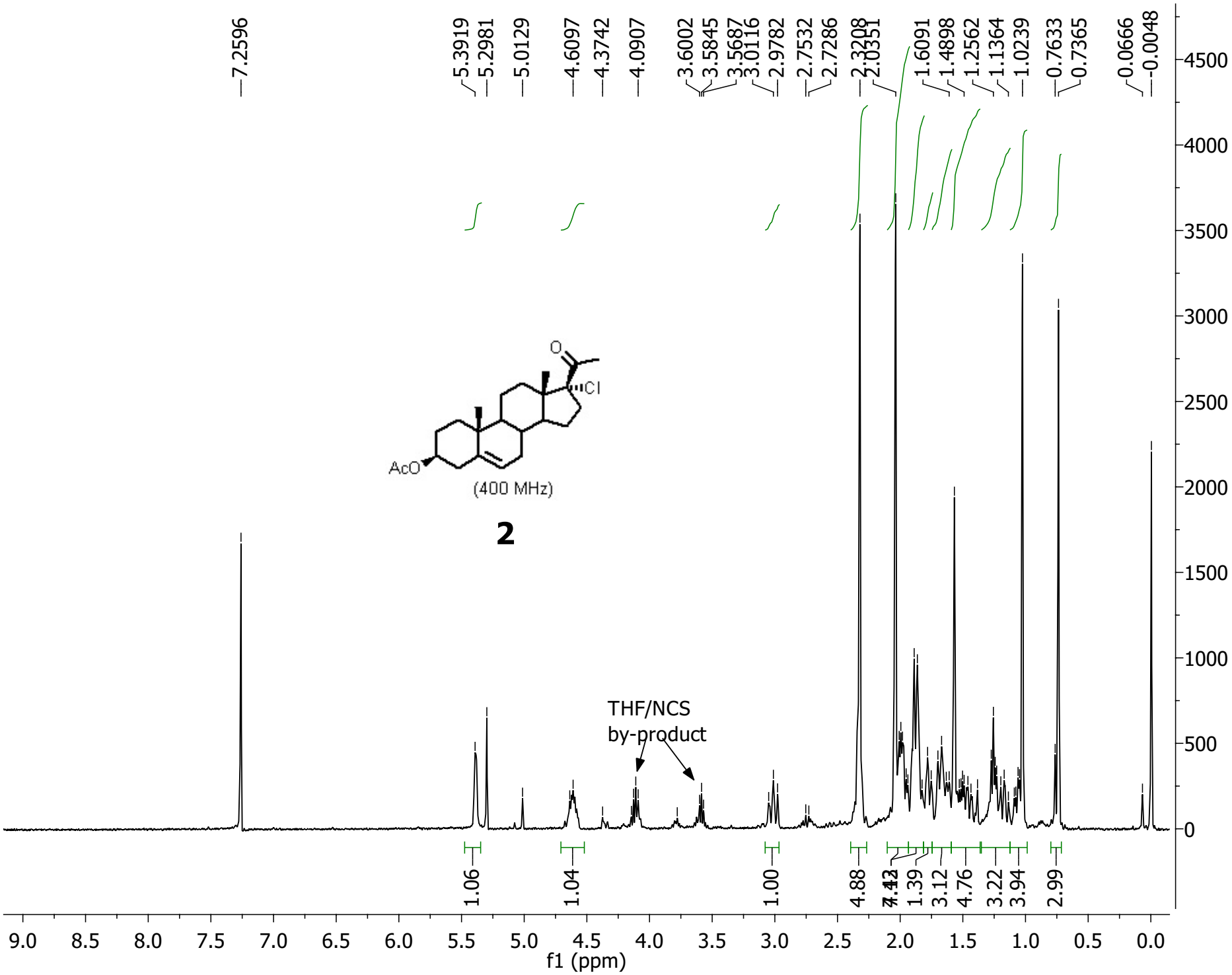
DATA 2
(400 MHz)

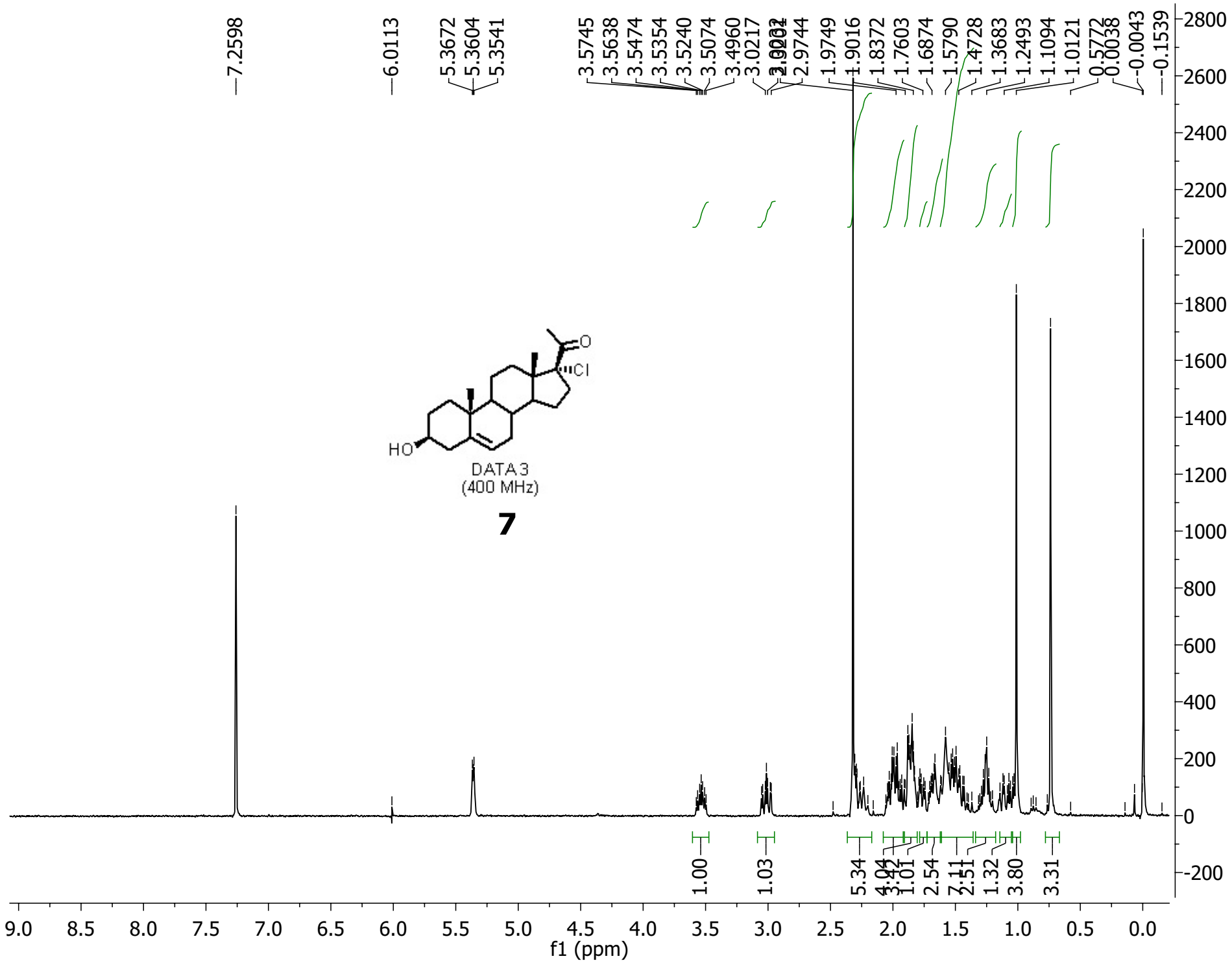
6

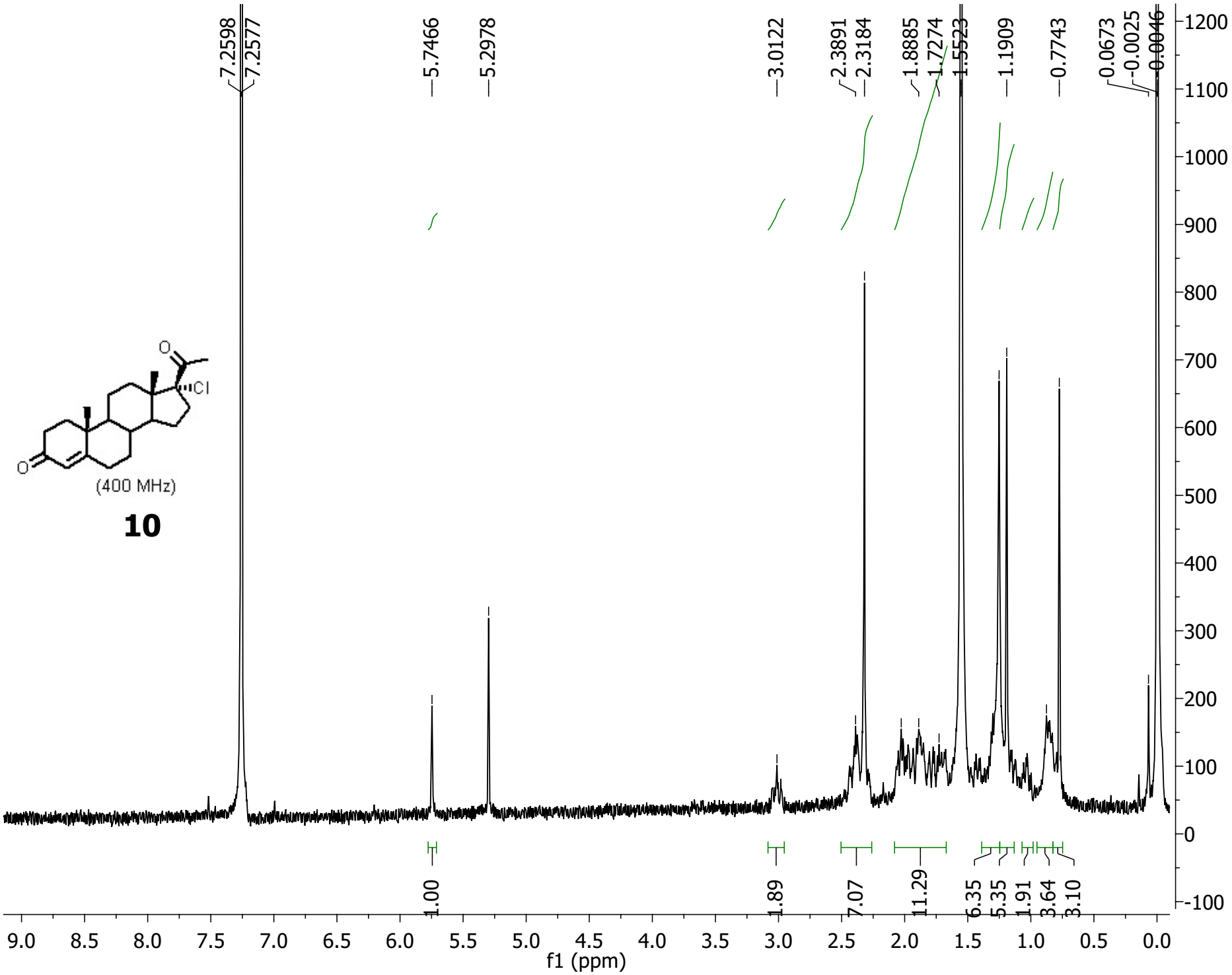

7.5189
7.2599
6.9956
5.3555
3.5257
2.6358
2.5439
2.2355
1.7737
1.6487
1.5538
1.4570
1.2503
1.0121
0.6655
0.6317
0.1440
-0.0024
-0.1508
O
F
HO
(400 MHz)
1.00
2.10
3.42
6.49
2.45
6.44
4.87
2.97
5.75
2.68
f1 (ppm)

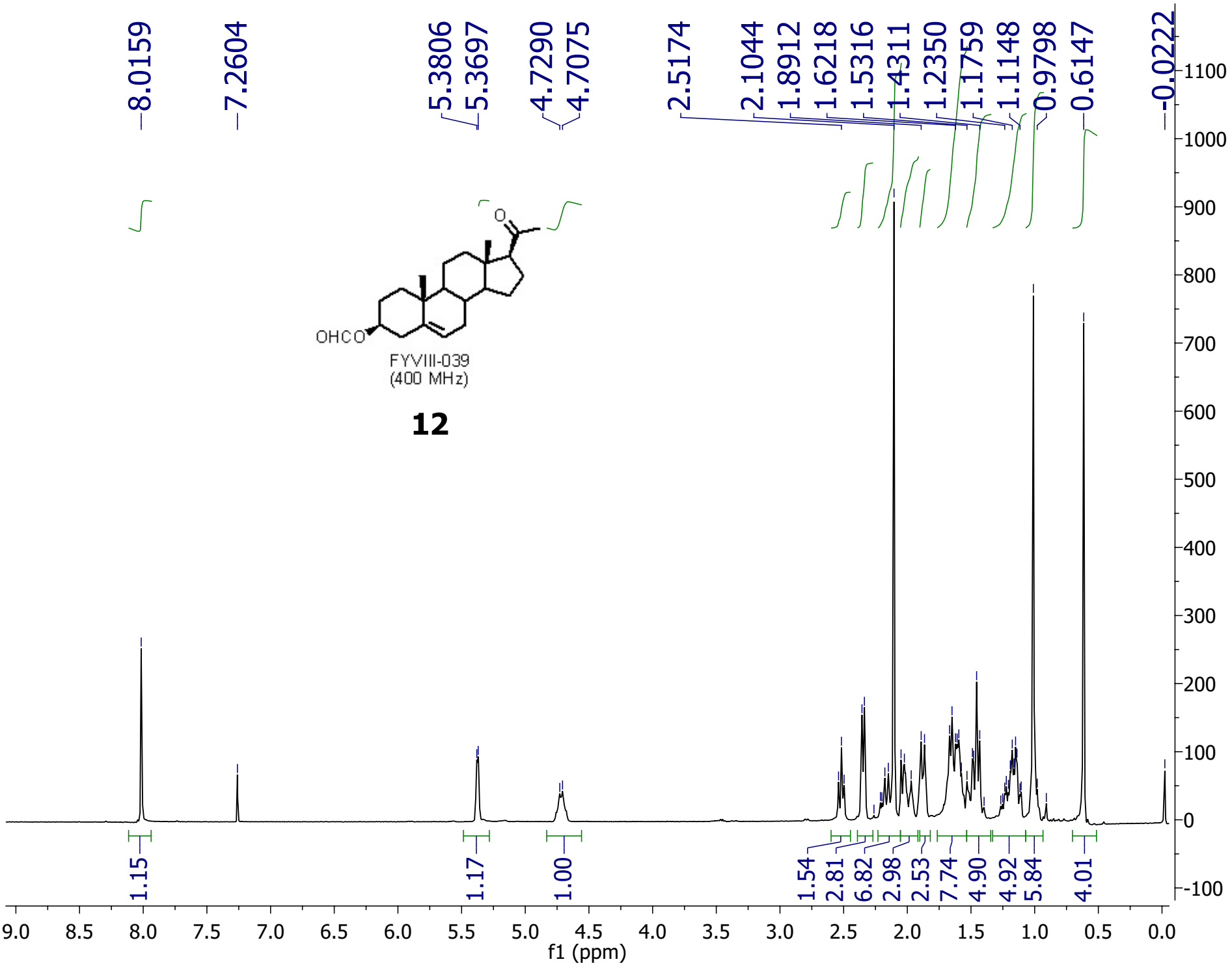

FYV-030
400 MHz
7.26
5.35
5.30
3.27
2.82
2.04
1.69
1.51
1.19
0.70
0.01
0.00
-0.01
1.00
1.40
1.14
2.74
2.22
6.47
14.02
7.21
1.18
2.89
f1 (ppm)

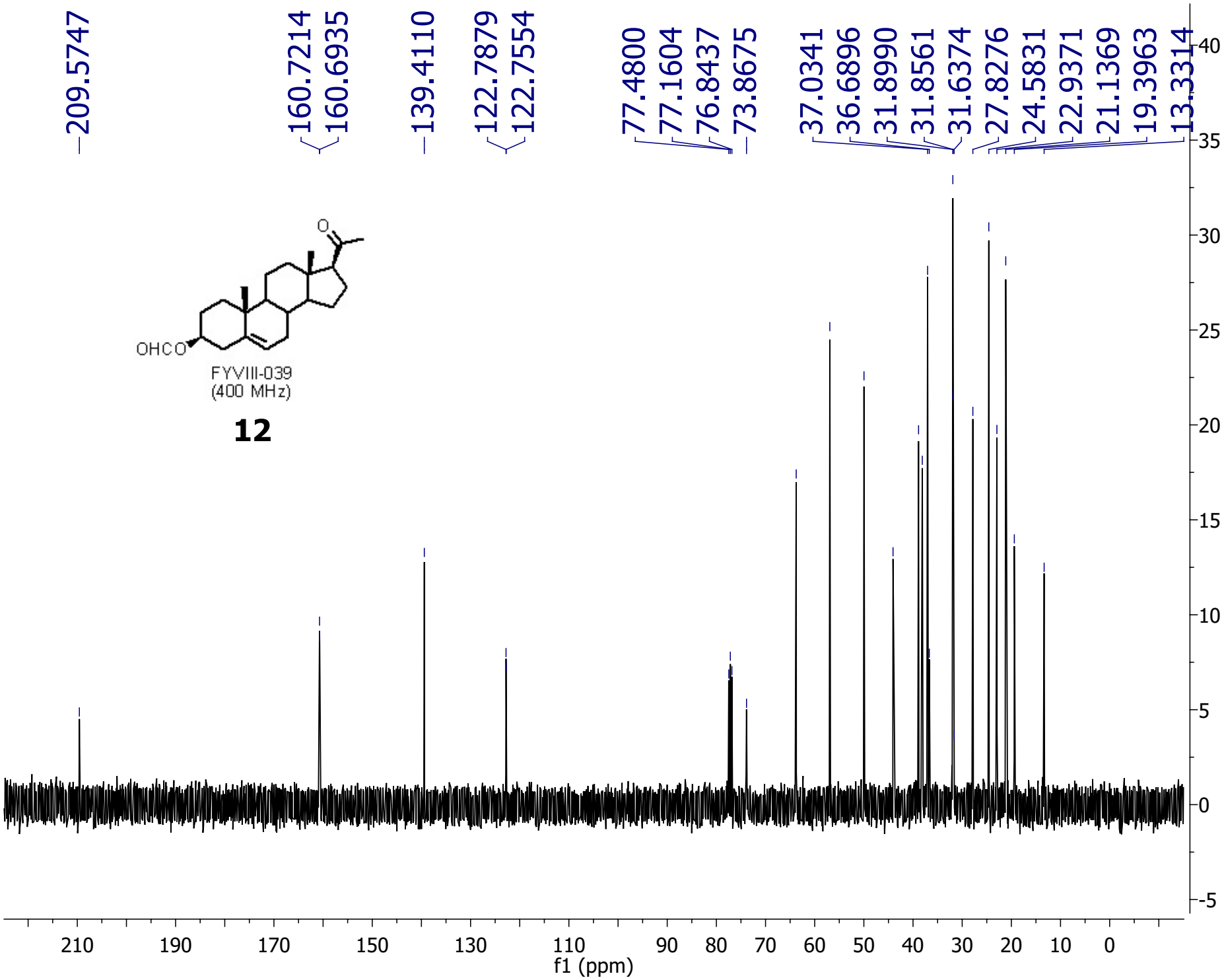

7.2680
7.2667
7.2655
7.2596
7.2505
7.2295
7.2140
7.2075
6.9953
6.2022
5.7409
5.2995
4.1106
3.6638
3.4911
2.3844
2.2557
2.0062
1.7871
1.5619
1.3804
1.1675
1.0085
0.5402
-0.0034
F
O
O
FYV-030
9
400 MHz
1.00
1.41
3.81
3.61
1.44
8.72
10.77
5.90
1.78
3.48
f1 (ppm)

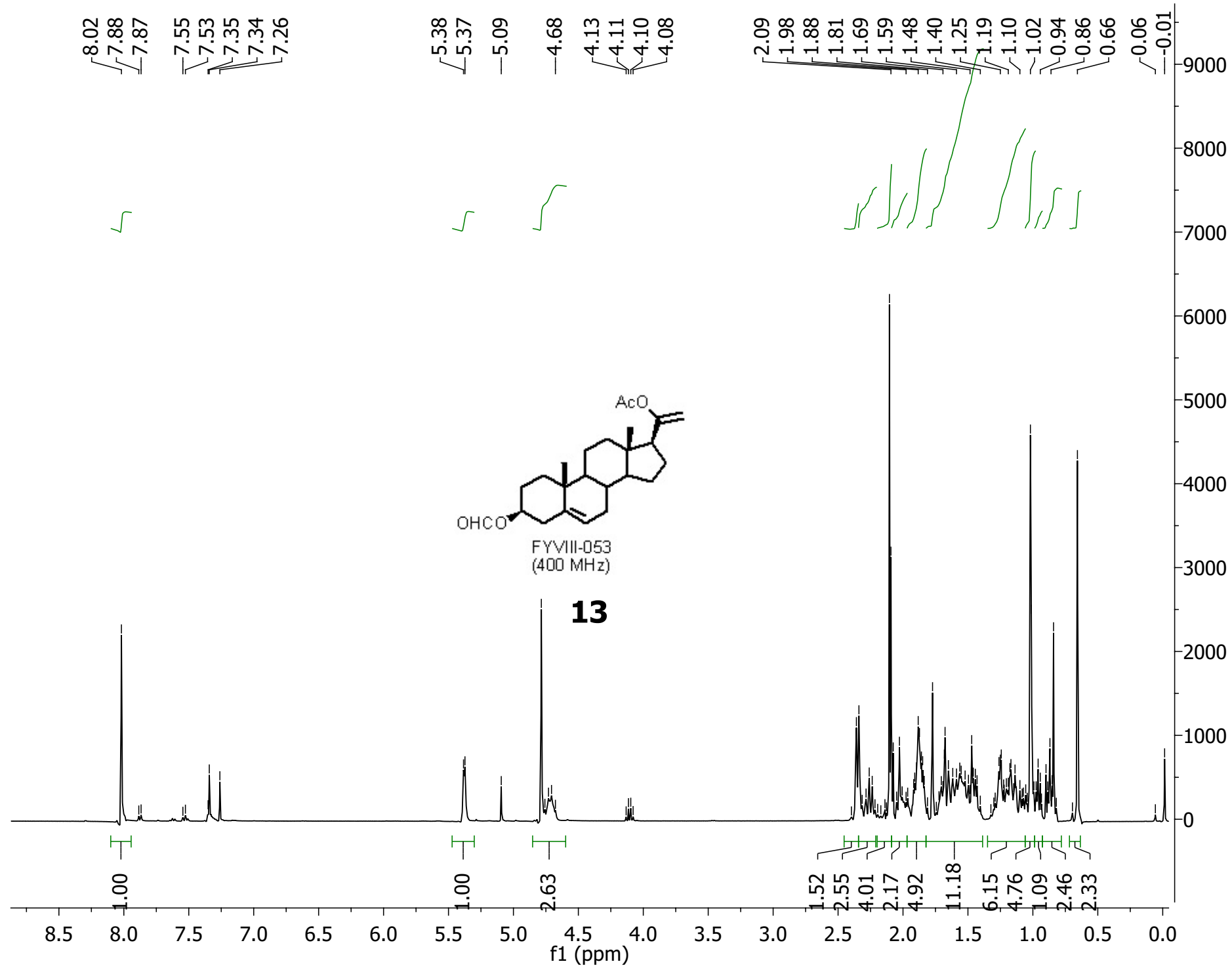

7.2596
5.3919
5.2981
5.0129
4.6097
4.3742
4.0907
3.6002
3.5845
3.5687
3.0116
2.9782
2.7532
2.7286
2.3208
2.0351
1.6091
1.4898
1.2562
1.1364
1.0239
0.7633
0.7365
0.0666
-0.0048
O
Cl
AcO
(400 MHz)
2
THF/NCS
by-product
1.06
1.04
1.00
4.88
1.39
3.12
4.76
3.22
3.94
2.99
f1 (ppm)

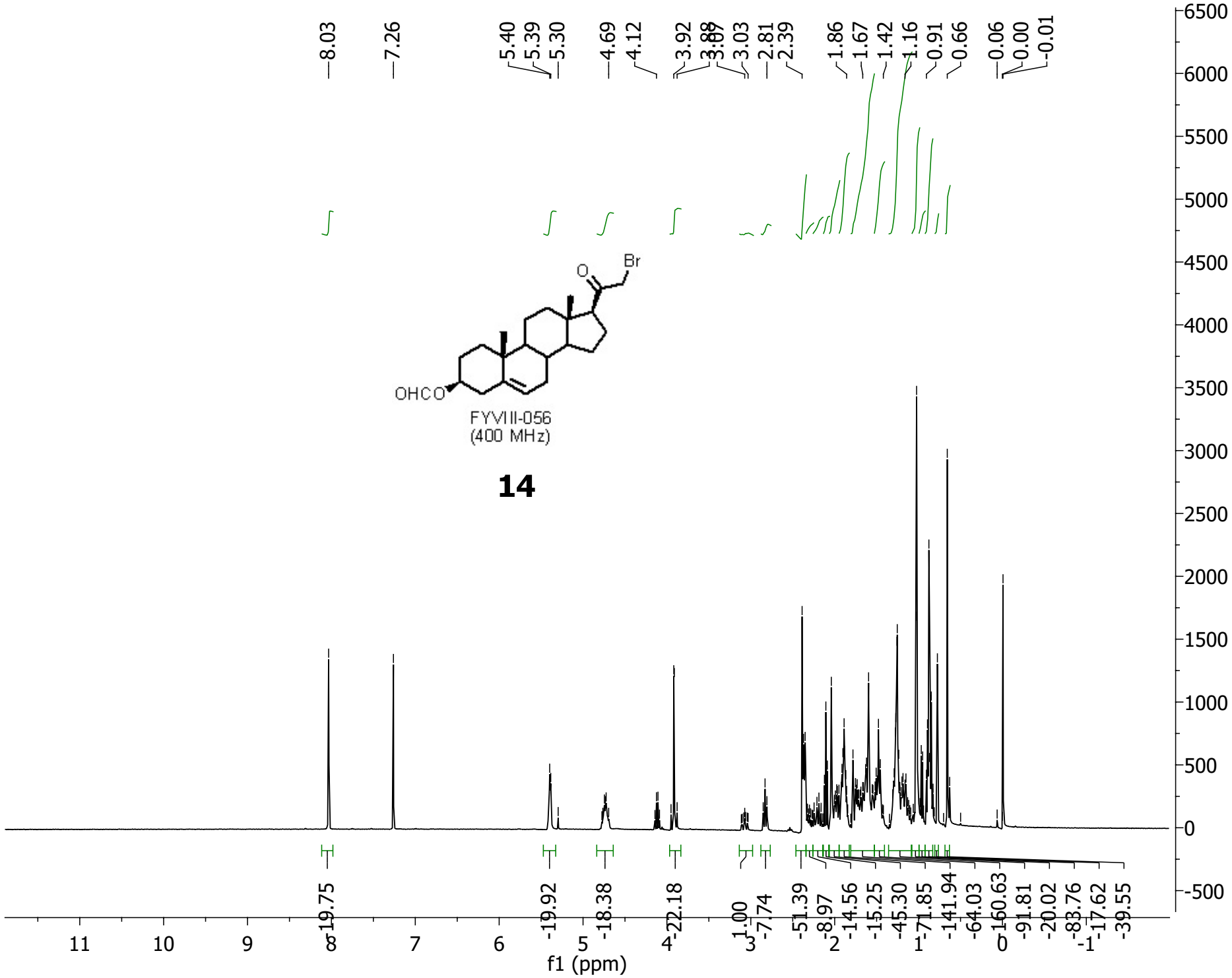

7.2598
6.0113
5.3672
5.3604
5.3541
3.5745
3.5638
3.5474
3.5354
3.5240
3.5074
3.4960
3.0217
2.9744
1.9749
1.9016
1.8372
1.7603
1.6874
1.5790
1.4728
1.3683
1.2493
1.1094
1.0121
0.5772
0.0038
-0.0043
-0.1539
O
Cl
HO
DATA3
(400 MHz)
7
1.00
1.03
5.34
4.04
3.42
1.01
2.54
2.11
2.51
1.32
3.80
3.31
f1 (ppm)

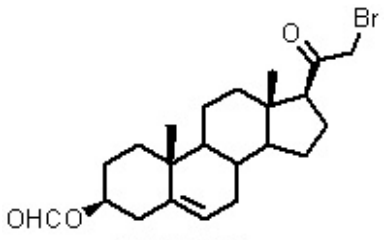

7.2598
7.2577
5.7466
5.2978
3.0122
2.3891
2.3184
1.8885
1.7274
1.5523
1.1909
0.7743
0.0673
-0.0025
-0.0046
O
Cl
O
(400 MHz)
10
1.00
1.89
7.07
11.29
6.35
5.35
1.91
3.64
3.10
f1 (ppm)


8.0159
7.2604
5.3806
5.3697
4.7290
4.7075
2.5174
2.1044
1.8912
1.6218
1.5316
1.4311
1.2350
1.1759
1.1148
0.9798
0.6147
-0.0222
OHCO
O
FYVIII-039
(400 MHz)
12
1.15
1.17
1.00
1.54
2.81
6.82
2.98
2.53
7.74
4.90
4.92
5.84
4.01
f1 (ppm)


209.5747
160.7214
160.6935
139.4110
122.7879
122.7554
77.4800
77.1604
76.8437
73.8675
37.0341
36.6896
31.8990
31.8561
31.6374
27.8276
24.5831
22.9371
21.1369
19.3963
13.3314
OHCO
FYVIII-039
(400 MHz)
12
f1 (ppm)

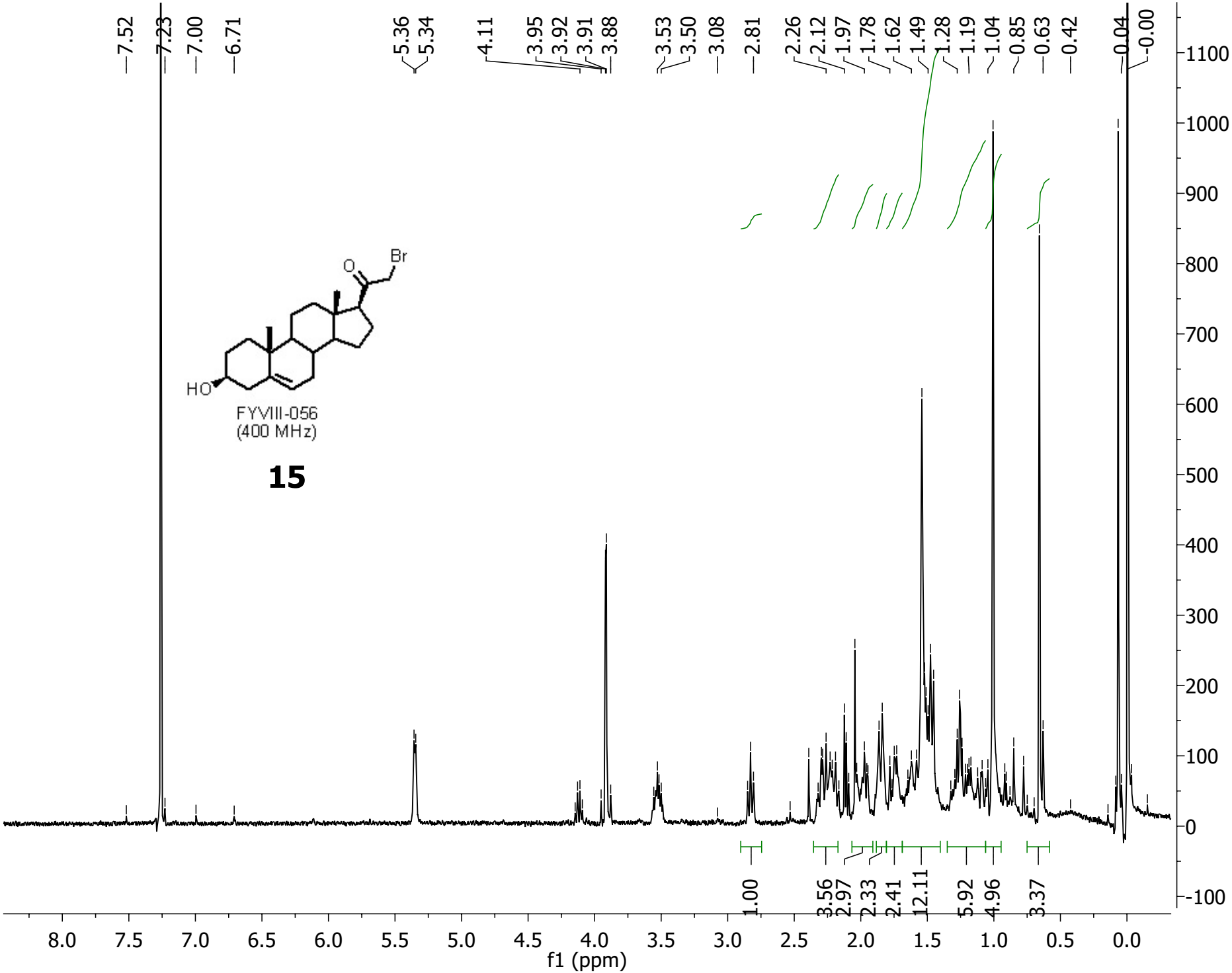

8.02
7.88
7.87
7.55
7.53
7.35
7.34
7.26
5.38
5.37
5.09
4.68
4.13
4.11
4.10
4.08
2.09
1.98
1.88
1.81
1.69
1.59
1.48
1.40
1.25
1.19
1.10
1.02
0.94
0.86
0.66
0.06
-0.01
AcO
OHCO
FYVIII-053
(400 MHz)
13
1.00
1.00
2.63
1.52
2.55
4.01
2.17
4.92
11.18
6.15
4.76
1.09
2.46
2.33
f1 (ppm)

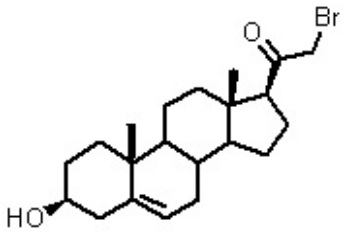

8.03
7.26
5.40
5.39
5.30
4.69
4.12
3.92
3.88
3.87
3.03
2.81
2.39
1.86
1.67
1.42
1.16
0.91
0.66
0.06
0.00
-0.01
O
Br
OHCO
FYVIII-056
(400 MHz)
14
19.75
19.92
18.38
22.18
1.00
7.74
51.39
8.97
14.56
15.25
45.30
71.85
141.94
64.03
160.63
91.81
20.02
83.76
17.62
39.55
f1 (ppm)



FYVIII-056
(400 MHz)

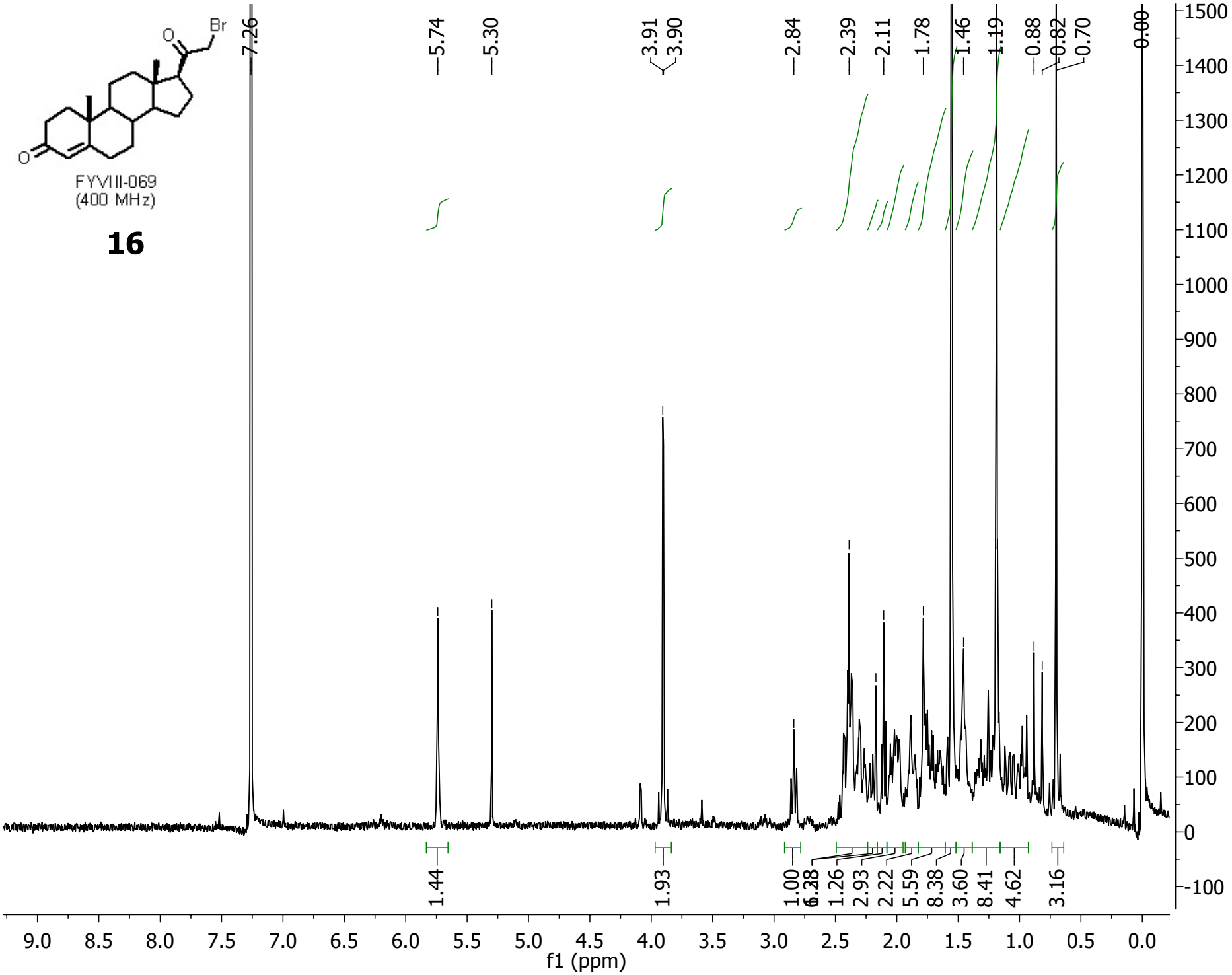
14

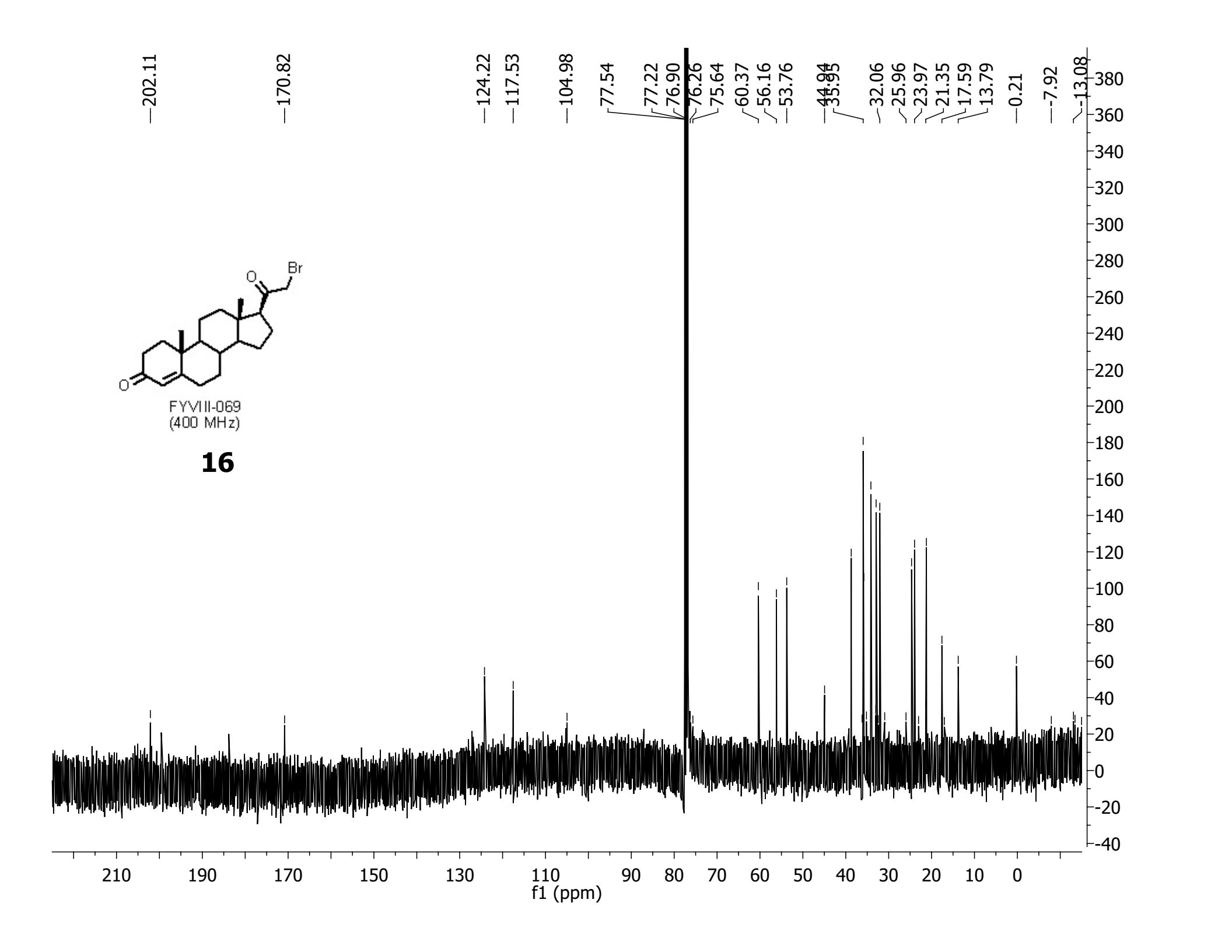

7.52
7.23
7.00
6.71
5.36
5.34
4.11
3.95
3.92
3.91
3.88
3.53
3.50
3.08
2.81
2.26
2.12
1.97
1.78
1.62
1.49
1.28
1.19
1.04
0.85
0.63
0.42
0.04
-0.00
Br
O
HO
FYVIII-056
(400 MHz)
15
1.00
3.56
2.97
2.33
2.41
12.11
5.92
4.96
3.37
f1 (ppm)

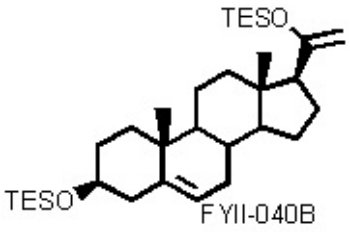


FYVIII-056
(400 MHz)

15


Br
O
O
FYVIII-069
(400 MHz)
16
7.26
5.74
5.30
3.91
3.90
2.84
2.39
2.11
1.78
1.46
1.19
0.88
0.82
0.70
0.00
1.44
1.93
1.00
6.38
1.26
2.93
2.22
5.59
8.38
3.60
8.41
4.62
3.16
f1 (ppm)

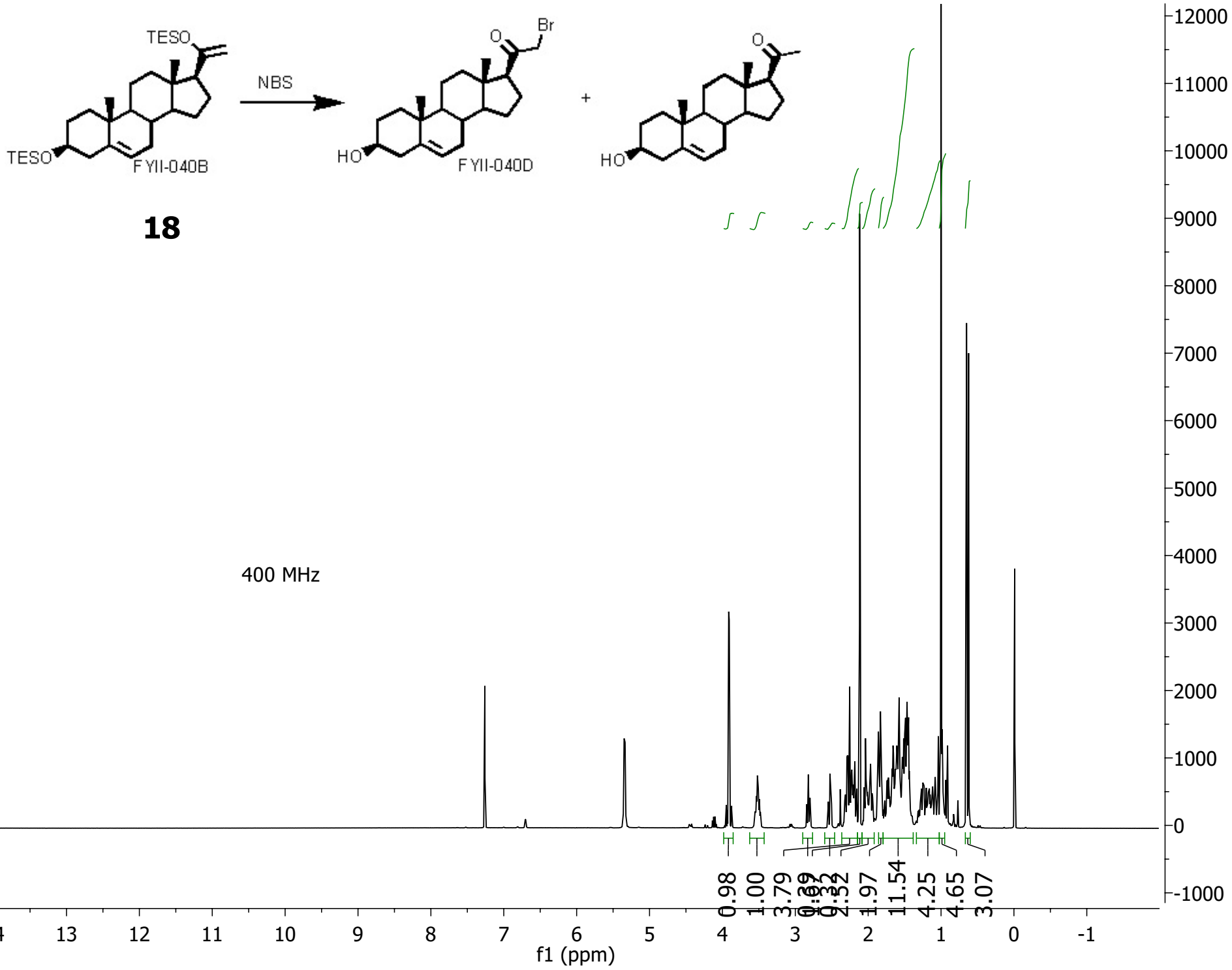

202.11
170.82
124.22
117.53
104.98
77.54
77.22
76.90
76.26
75.64
60.37
56.16
53.76
44.94
35.95
32.06
25.96
23.97
21.35
17.59
13.79
0.21
-7.92
-13.08
Br
O
O
FYVIII-069
(400 MHz)
16
f1 (ppm)

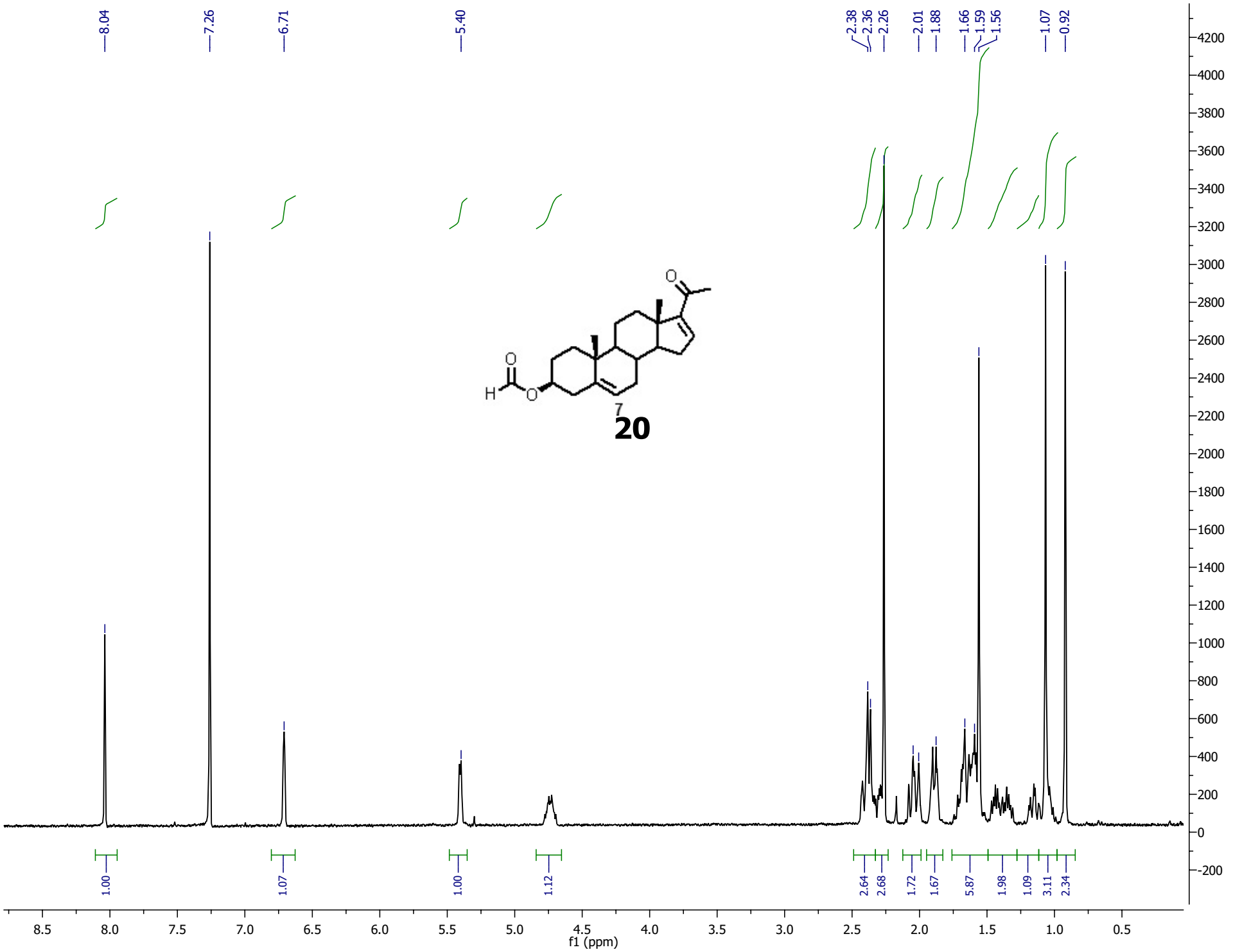


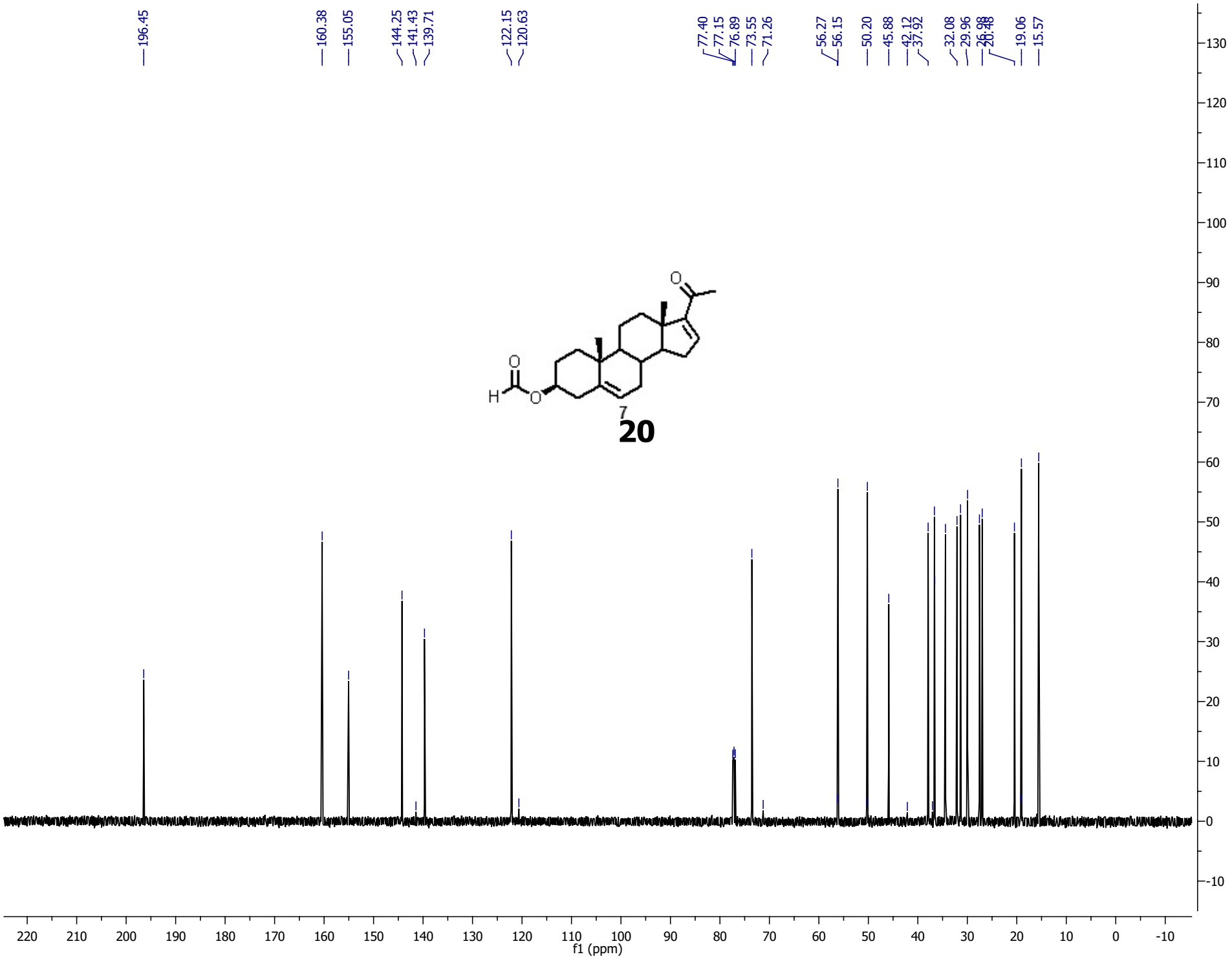
17

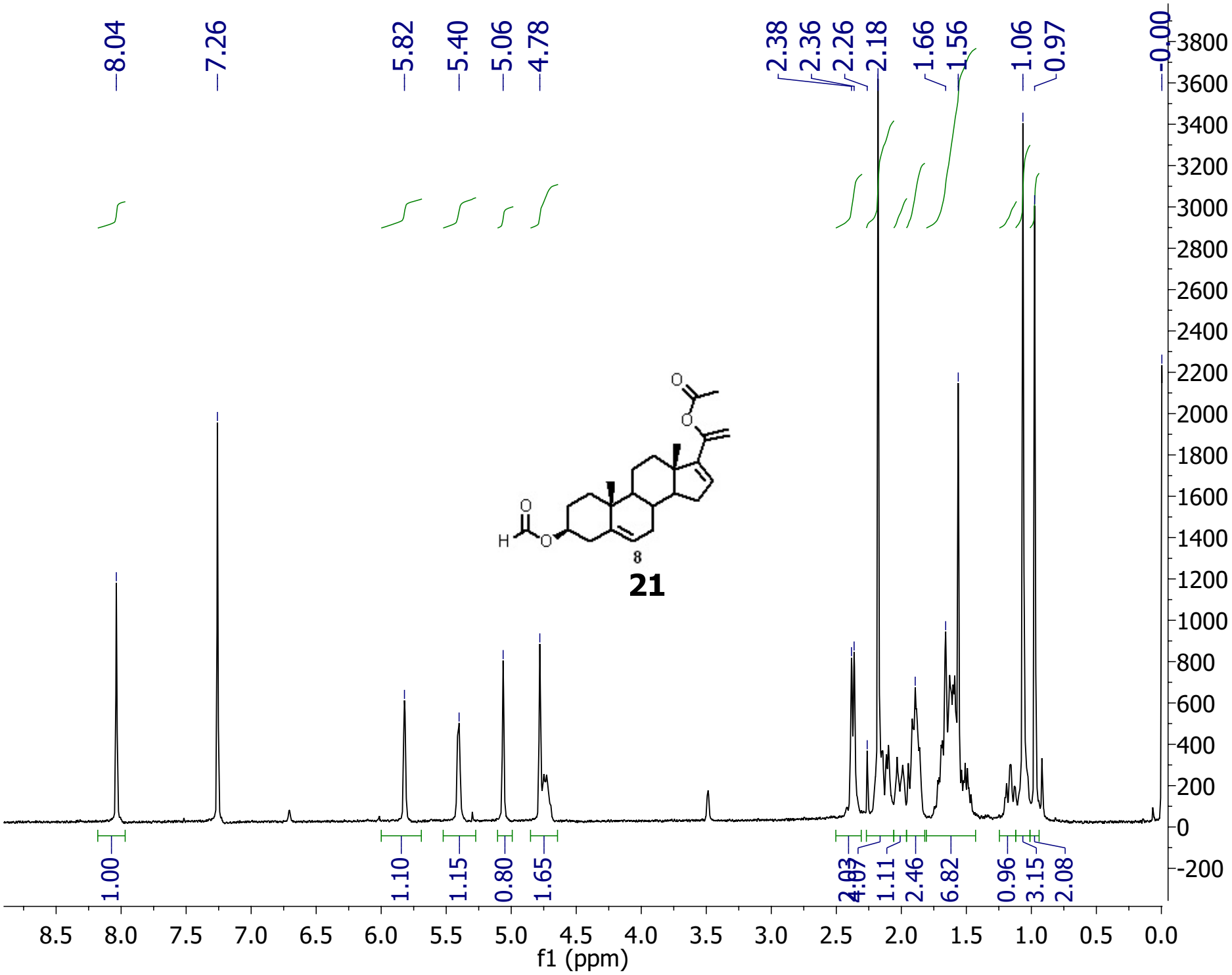
400 MHz

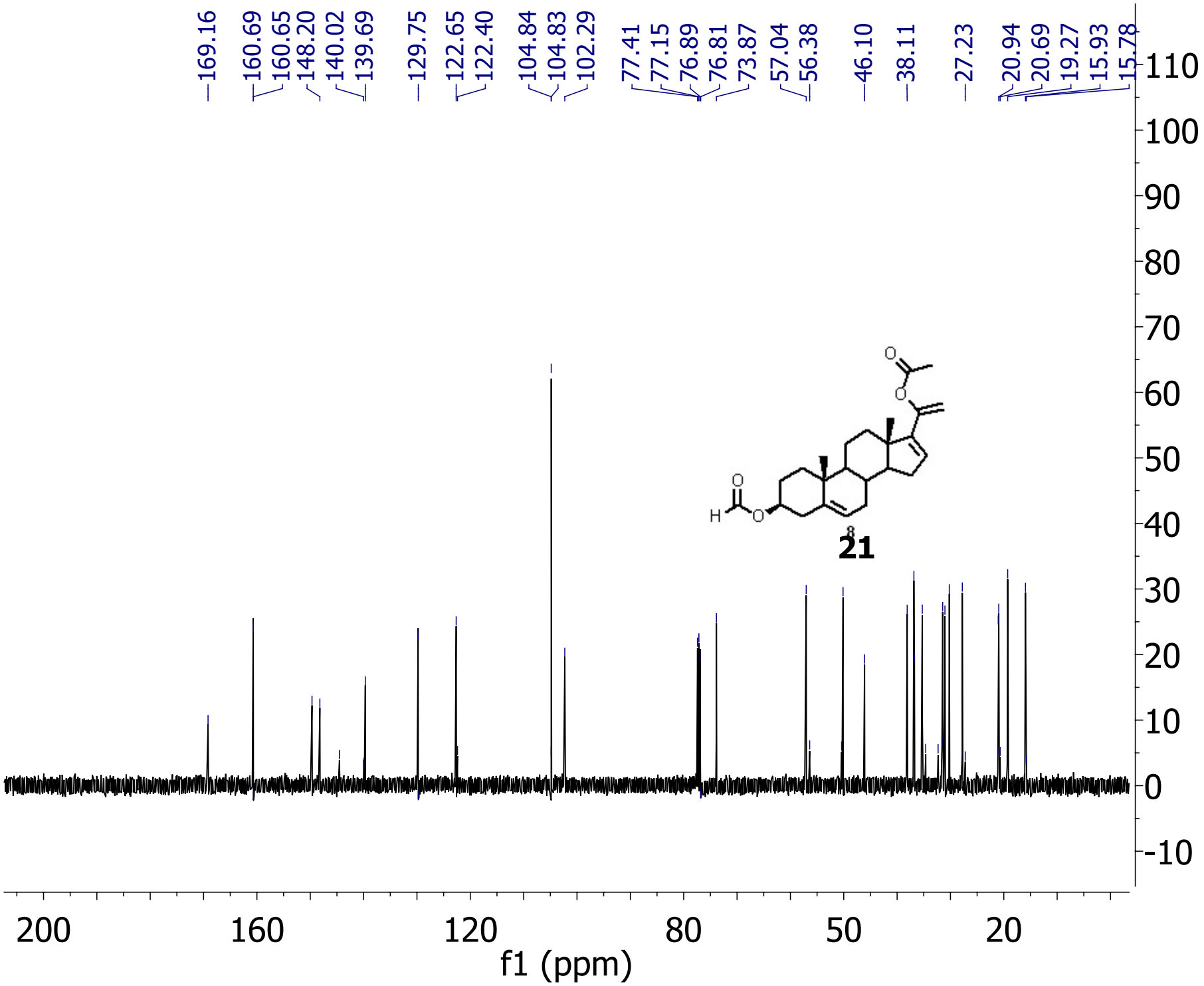
f1 (ppm)

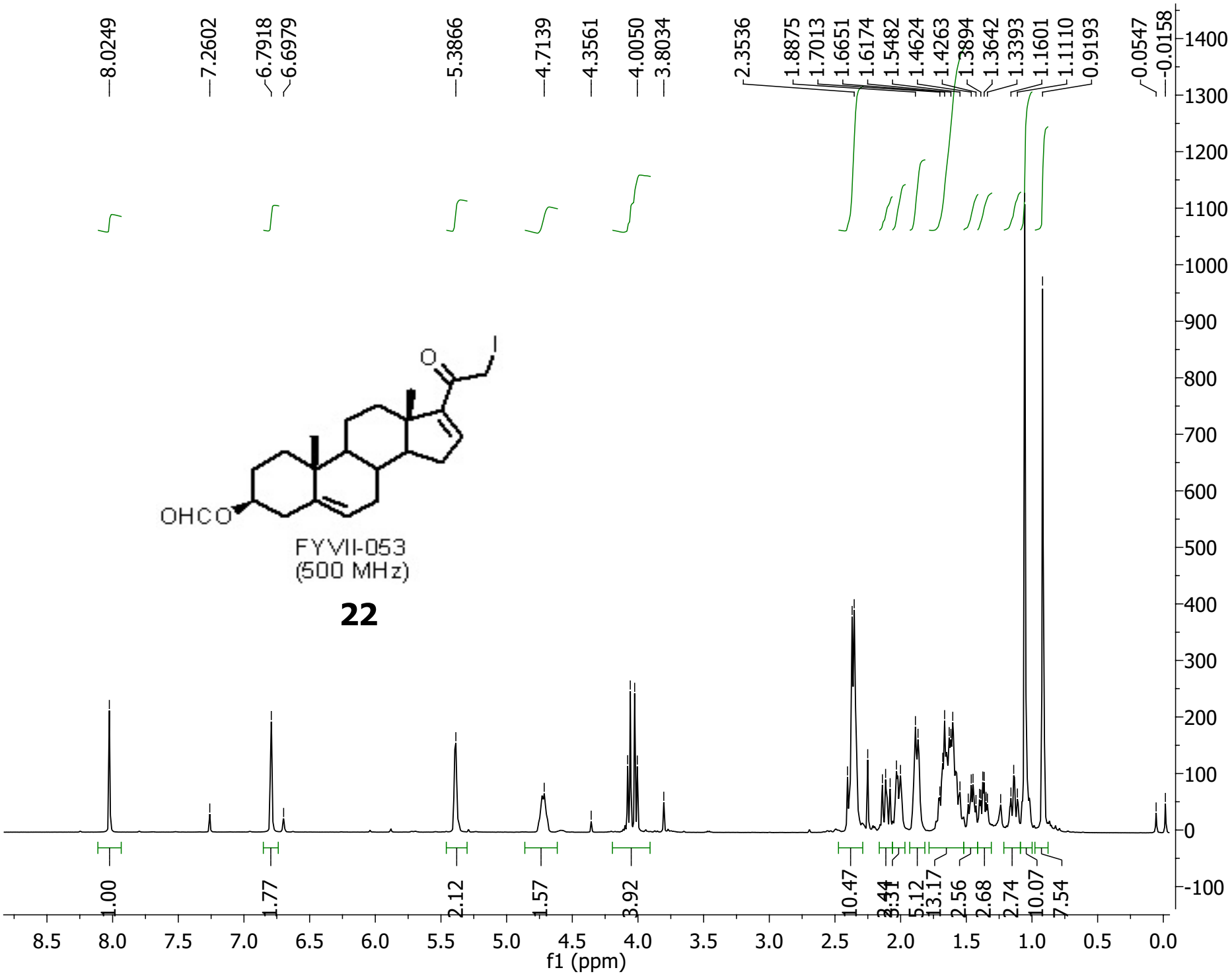

TESO
TESO
FYII-040B
NBS
Br
O
HO
FYII-040D
+
O
HO
18
400 MHz
0.98
1.00
3.79
2.52
1.97
11.54
4.25
4.65
3.07
f1 (ppm)

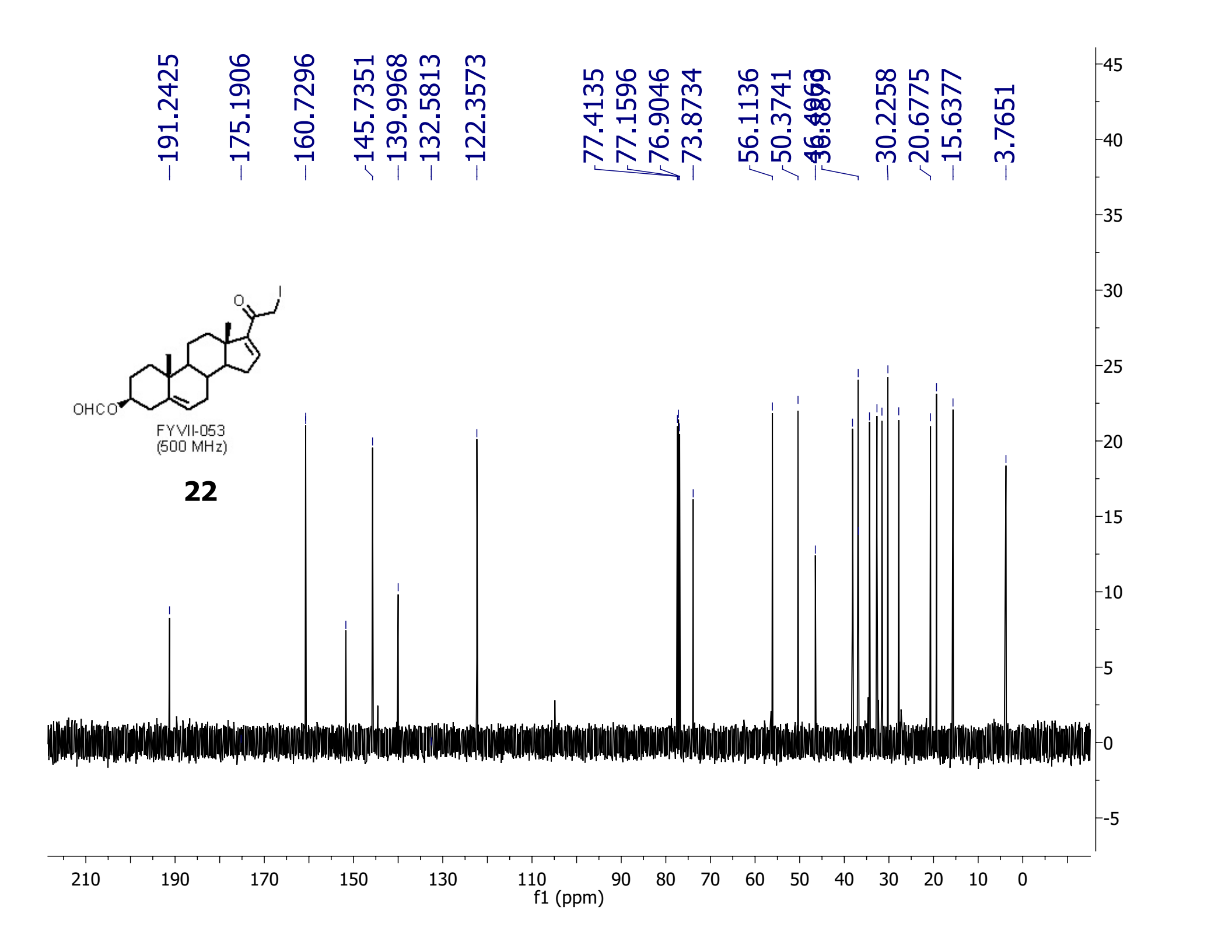

8.04
7.26
6.71
5.40
2.38
2.36
2.26
2.01
1.88
1.66
1.59
1.56
1.07
0.92
O
H
O
O
7
20
1.00
1.07
1.00
1.12
2.64
2.68
1.72
1.67
5.87
1.98
1.09
3.11
2.34
f1 (ppm)

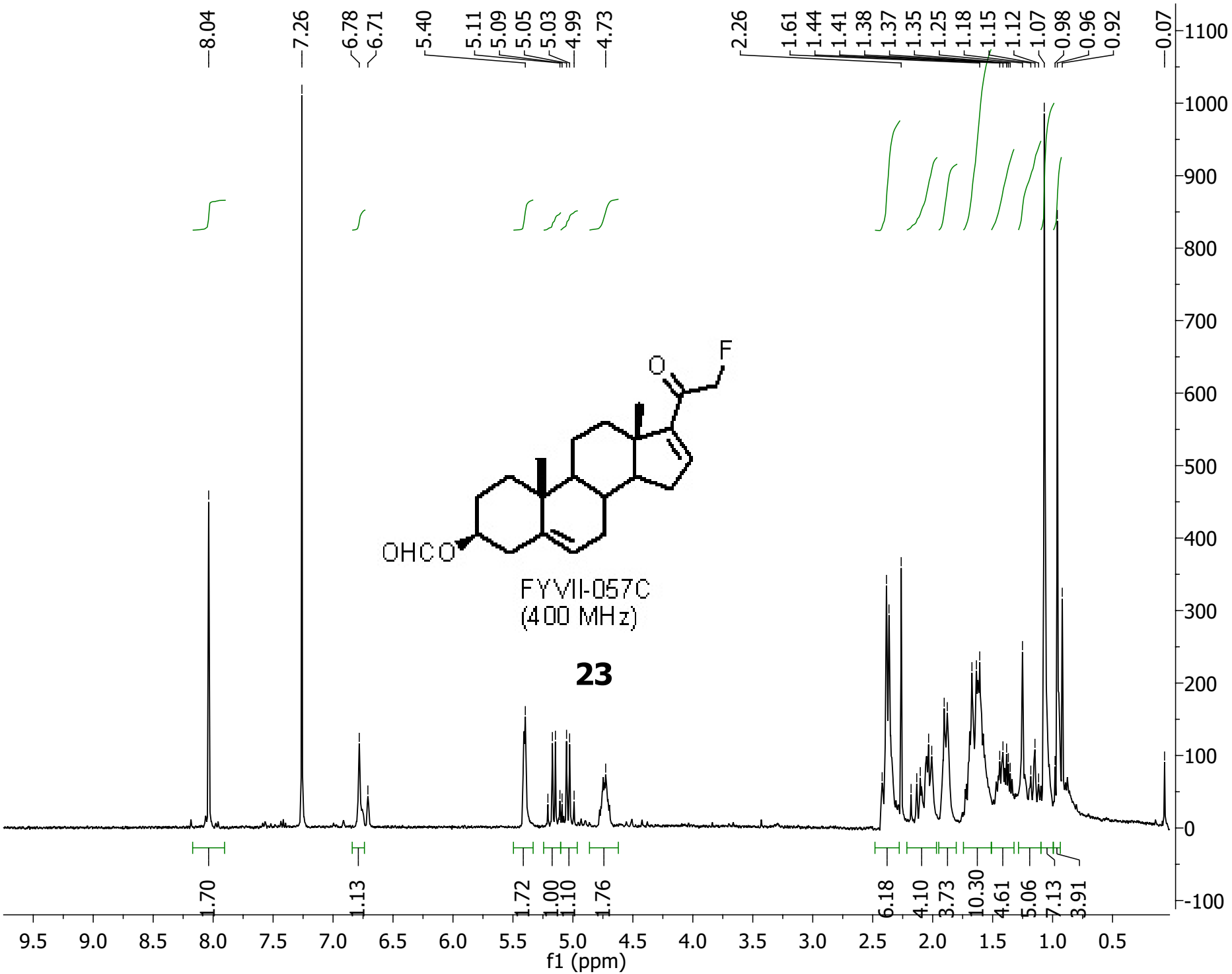

196.45
160.38
155.05
144.25
141.43
139.71
122.15
120.63
77.40
77.15
76.89
73.55
71.26
56.27
56.15
50.20
45.88
42.12
37.92
32.08
29.96
26.98
20.48
19.06
15.57
O
O
H
O
7
20
f1 (ppm)

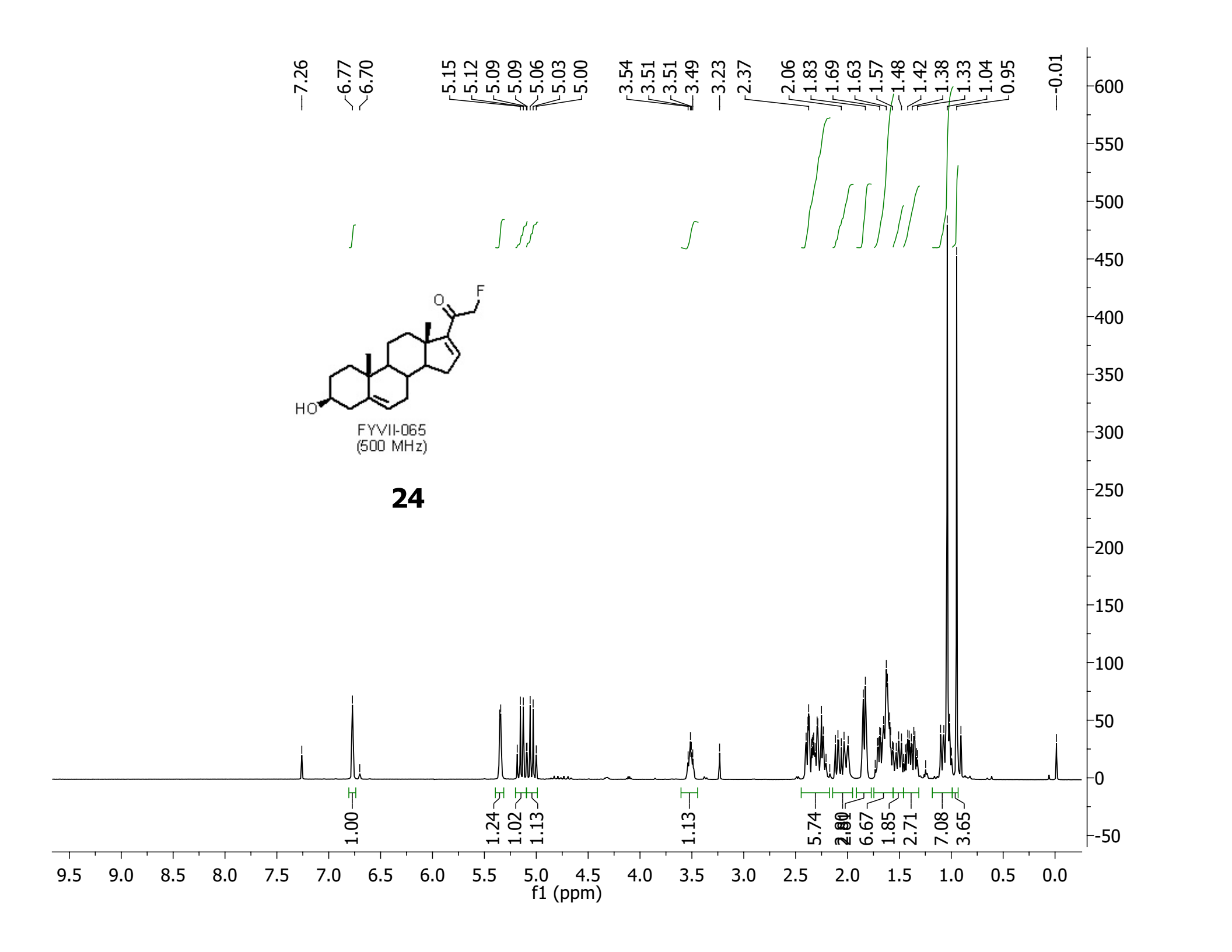

8.04
7.26
5.82
5.40
5.06
4.78
2.38
2.36
2.26
2.18
1.66
1.56
1.06
0.97
0.00
1.00
1.10
1.15
0.80
1.65
2.03
4.07
1.11
2.46
6.82
0.96
3.15
2.08
f1 (ppm)
21

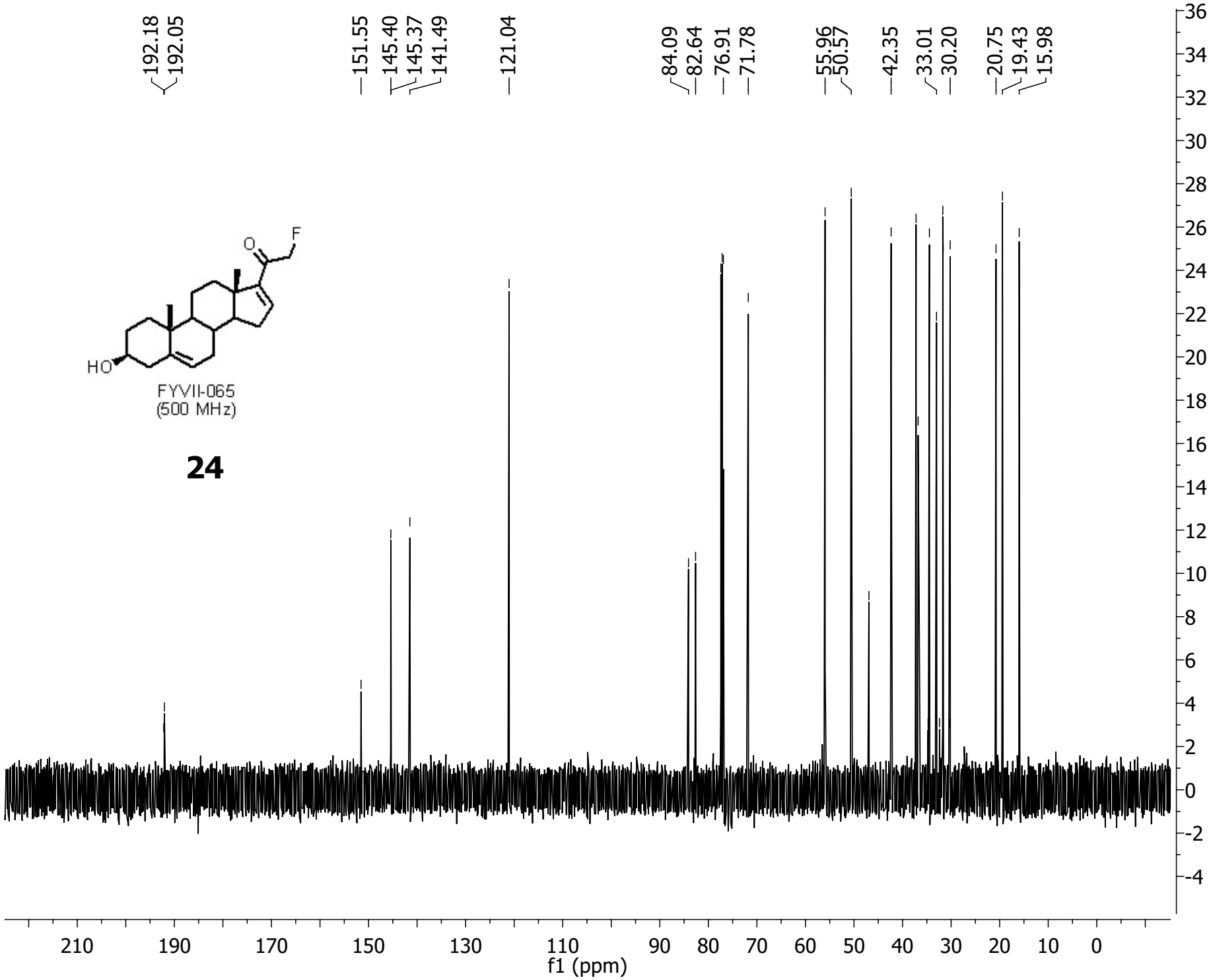

169.16
160.69
160.65
148.20
140.02
139.69
129.75
122.65
122.40
104.84
104.83
102.29
77.41
77.15
76.89
76.81
73.87
57.04
56.38
46.10
38.11
27.23
20.94
20.69
19.27
15.93
15.78
110
100
90
80
70
60
50
40
30
20
10
0
-10
200
160
120
80
50
20
f1 (ppm)
21

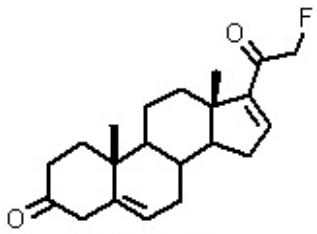

8.0249
7.2602
6.7918
6.6979
5.3866
4.7139
4.3561
4.0050
3.8034
2.3536
1.8875
1.7013
1.6651
1.6174
1.5482
1.4624
1.4263
1.3894
1.3642
1.3393
1.1601
1.1110
0.9193
0.0547
-0.0158
OHCO
O
FYVII-053
(500 MHz)
22
1.00
1.77
2.12
1.57
3.92
10.47
3.44
3.31
5.12
13.17
2.56
2.68
2.74
10.07
7.54
f1 (ppm)


191.2425
175.1906
160.7296
145.7351
139.9968
132.5813
122.3573
77.4135
77.1596
76.9046
73.8734
56.1136
50.3741
46.4063
30.8879
30.2258
20.6775
15.6377
3.7651
O
OHCO
FYVII-053
(500 MHz)
22
45
40
35
30
25
20
15
10
5
0
-5
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


8.04
7.26
6.78
6.71
5.40
5.11
5.09
5.05
5.03
4.99
4.73
2.26
1.61
1.44
1.41
1.38
1.37
1.35
1.25
1.18
1.15
1.12
1.07
0.98
0.96
0.92
0.07
O
F
OHCO
FYVII-057C
(400 MHz)
23
1.70
1.13
1.72
1.00
1.10
1.76
6.18
4.10
3.73
10.30
4.61
5.06
7.13
3.91
f1 (ppm)

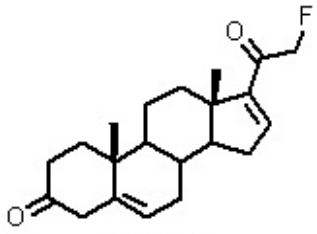

7.26
6.77
6.70
5.15
5.12
5.09
5.09
5.06
5.03
5.00
3.54
3.51
3.51
3.49
3.23
2.37
2.06
1.83
1.69
1.63
1.57
1.48
1.42
1.38
1.33
1.04
0.95
-0.01
O
F
HO
FYVII-065
(500 MHz)
24
1.00
1.24
1.02
1.13
1.13
5.74
6.67
1.85
2.71
7.08
3.65
f1 (ppm)


192.18
192.05
151.55
145.40
145.37
141.49
121.04
84.09
82.64
76.91
71.78
55.96
50.57
42.35
33.01
30.20
20.75
19.43
15.98
O
F
HO
FYVII-065
(500 MHz)
24
f1 (ppm)



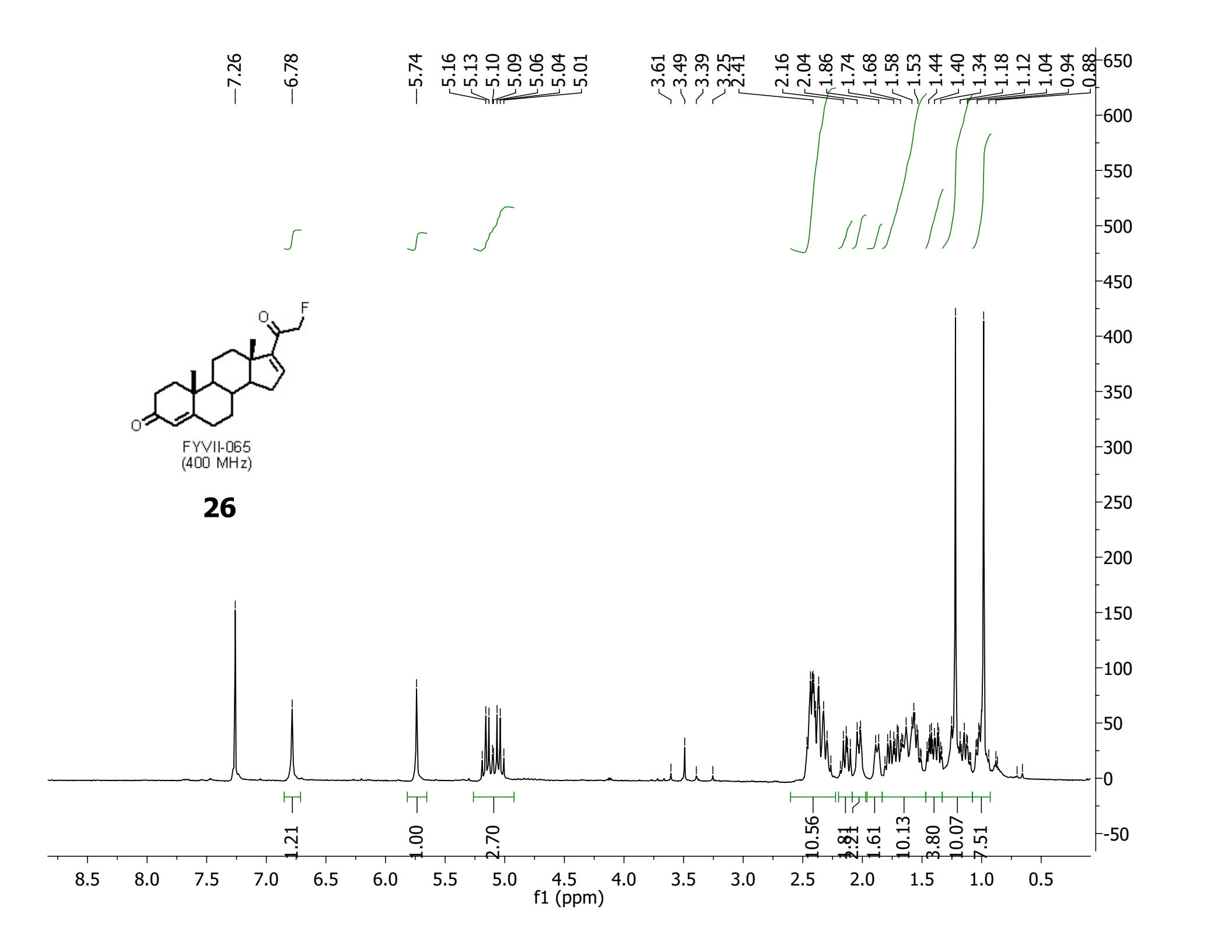
FYVII-064
(400 MHz)

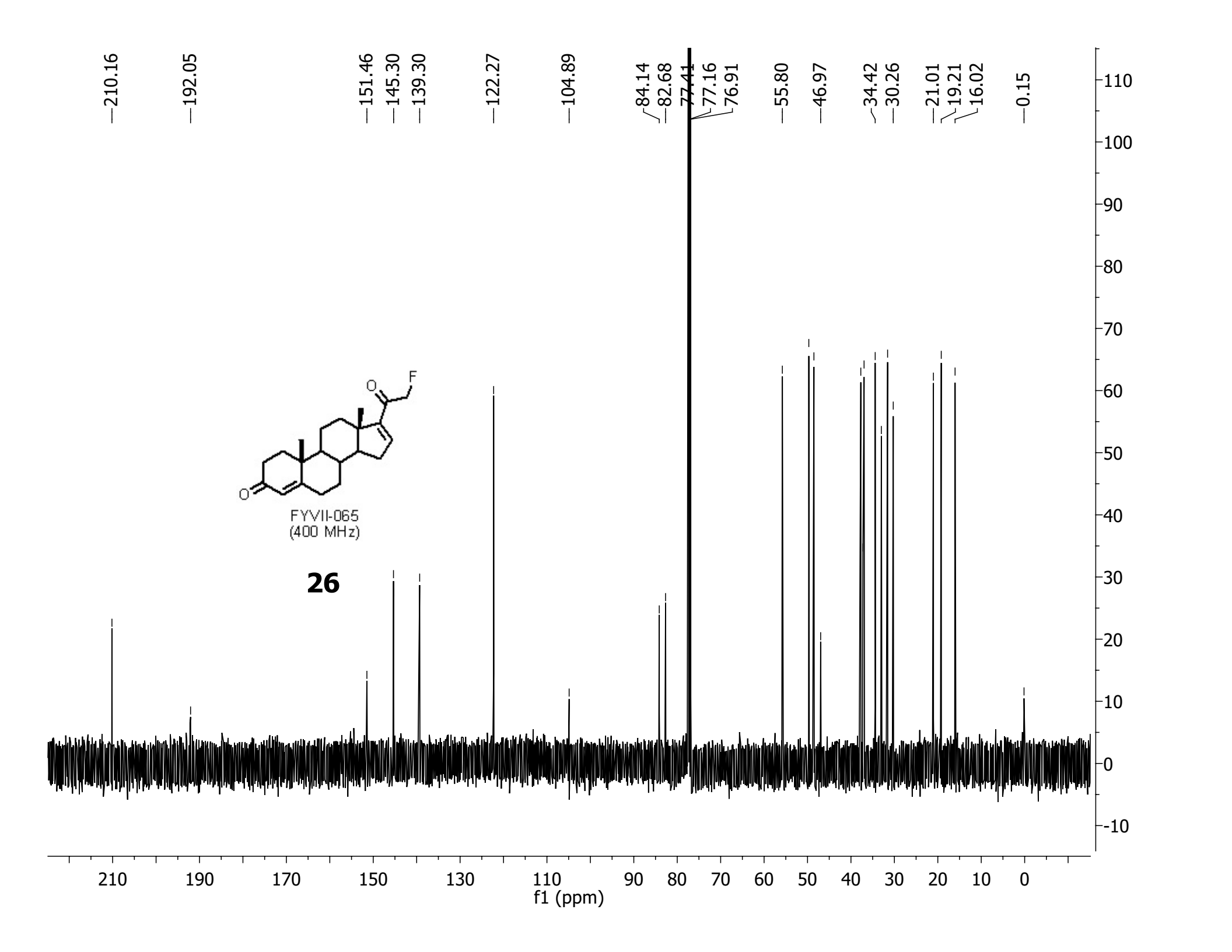
25

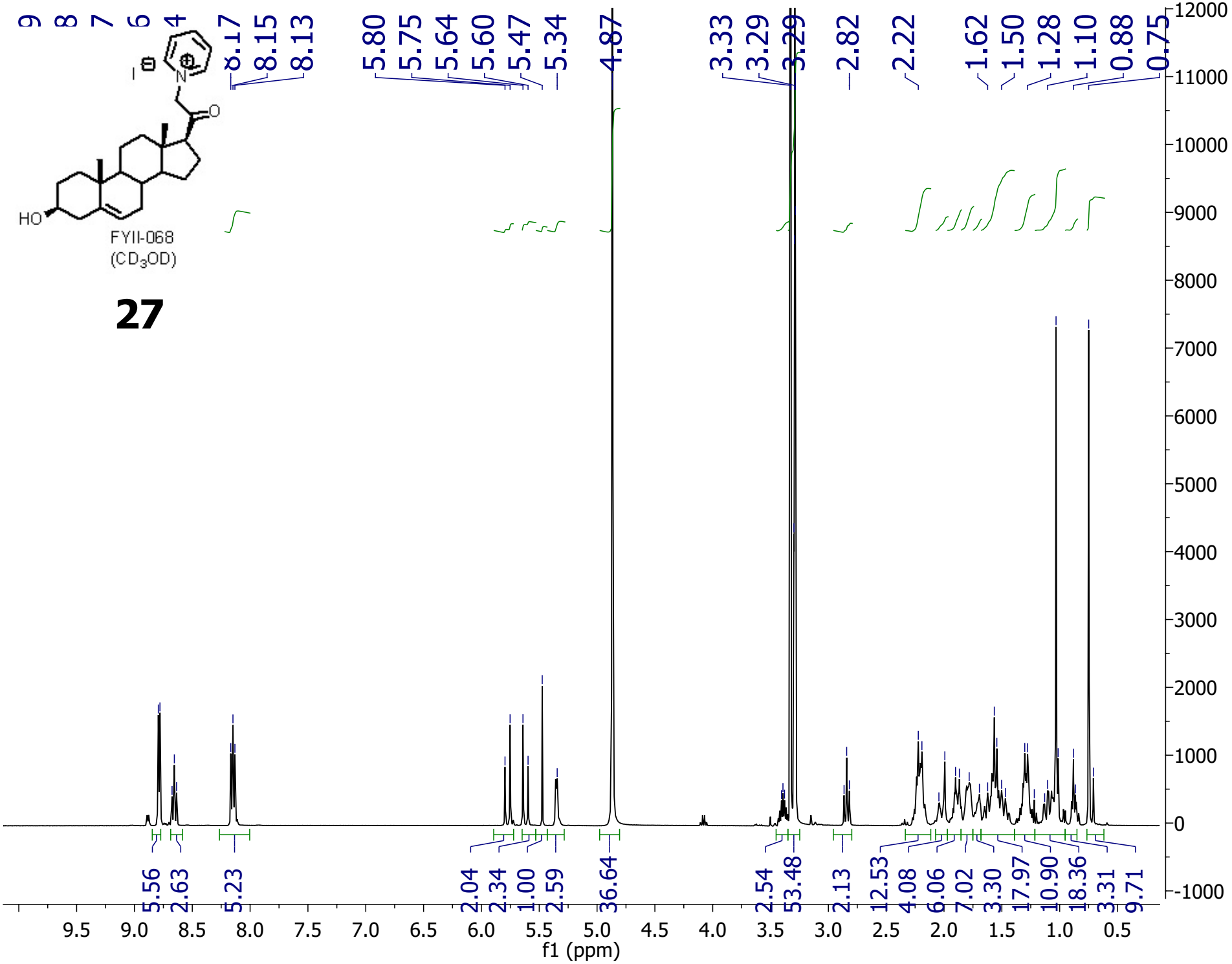


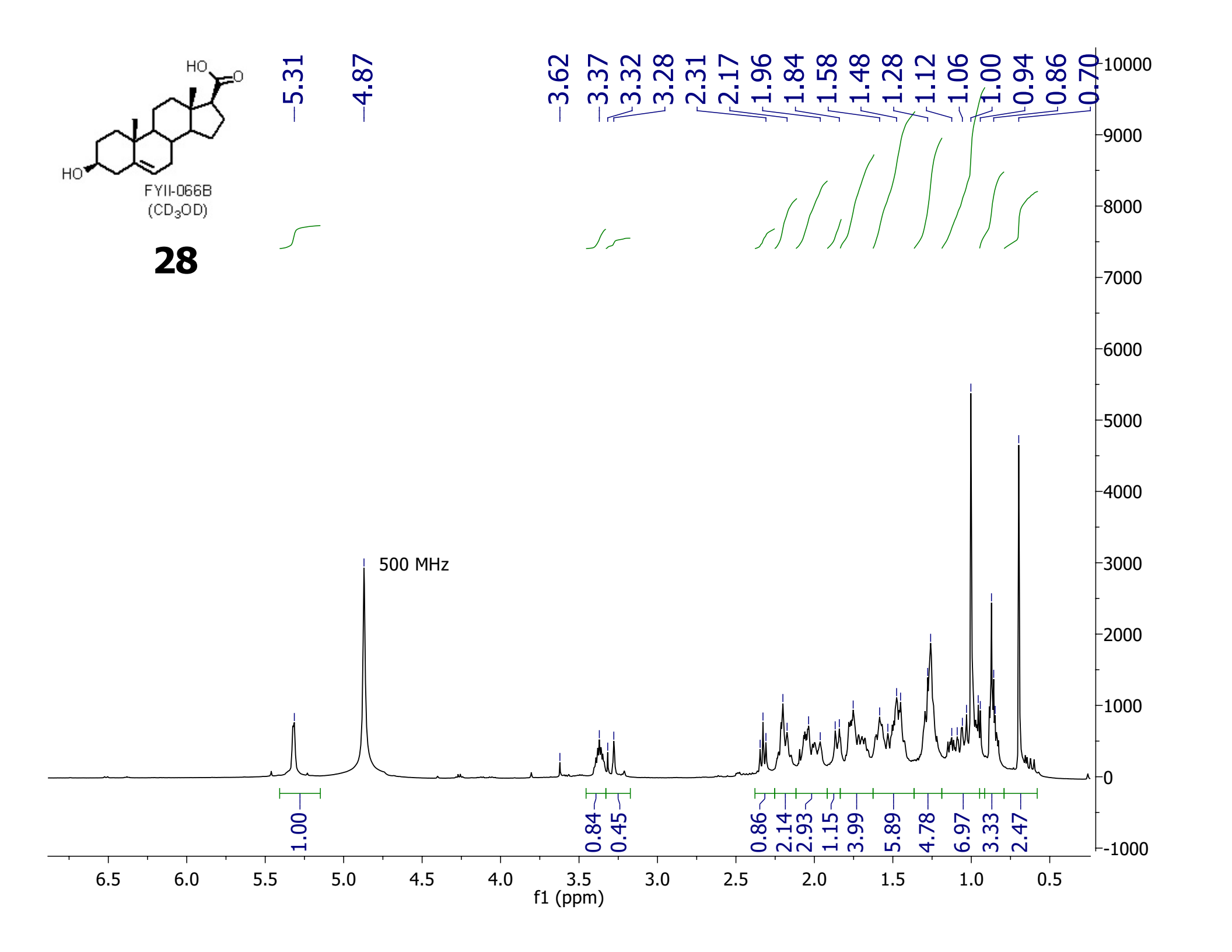
FYVII-065
(500 MHz)
^{13}C NMR

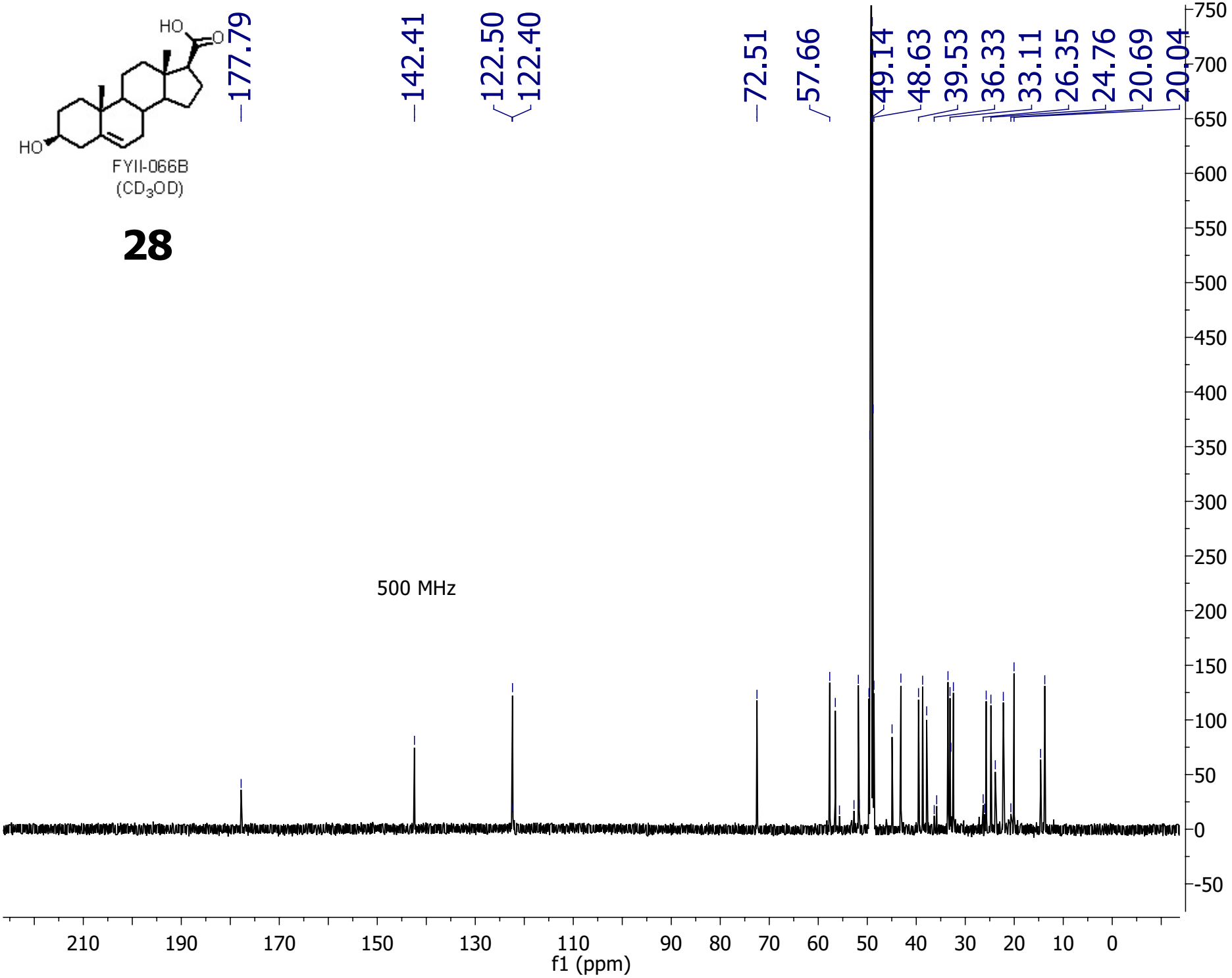
25

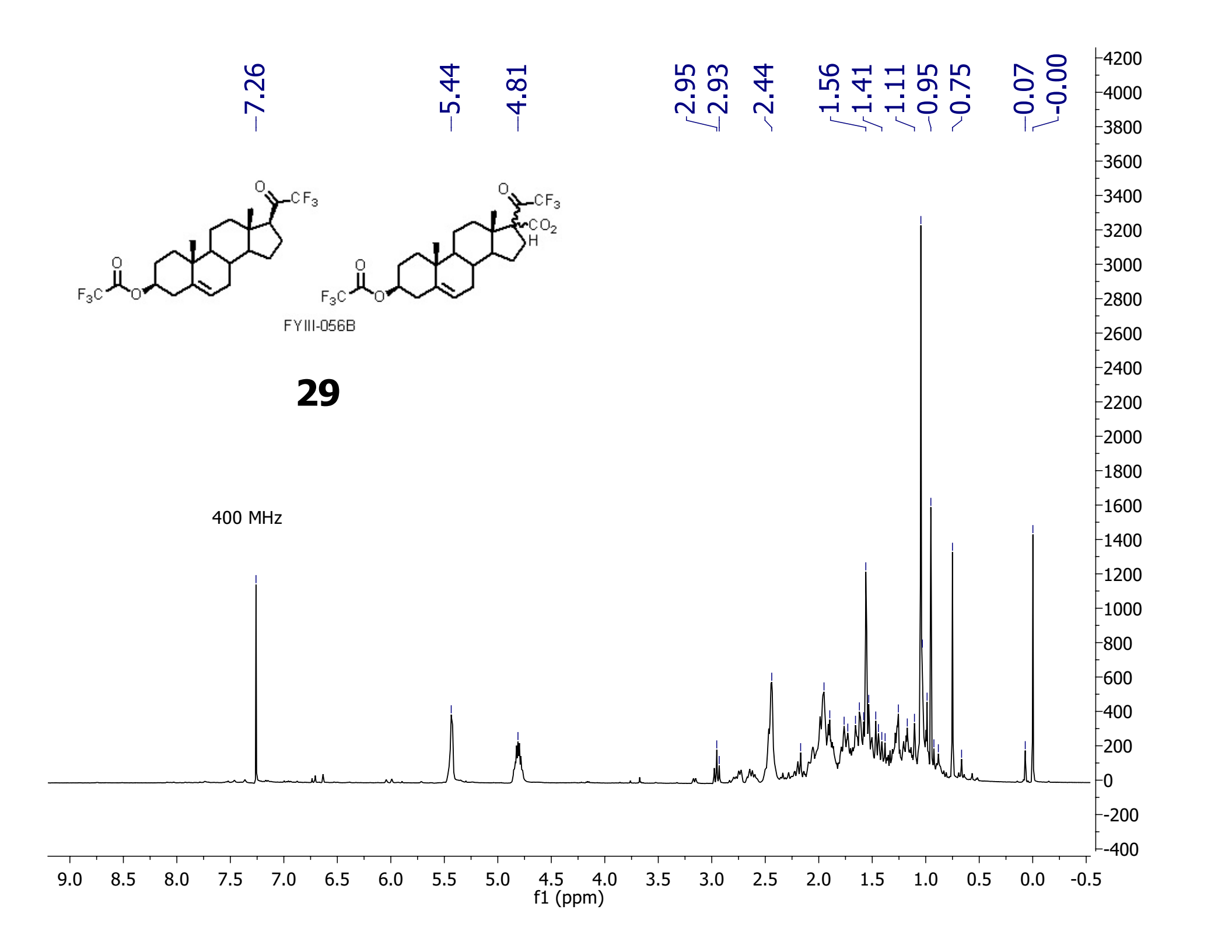

7.26
6.78
5.74
5.16
5.13
5.10
5.09
5.06
5.04
5.01
3.61
3.49
3.39
3.25
2.41
2.16
2.04
1.86
1.74
1.68
1.58
1.53
1.44
1.40
1.34
1.18
1.12
1.04
0.94
0.88
O
F
O
FYVII-065
(400 MHz)
26
1.21
1.00
2.70
10.56
1.81
2.21
1.61
10.13
3.80
10.07
7.51
f1 (ppm)

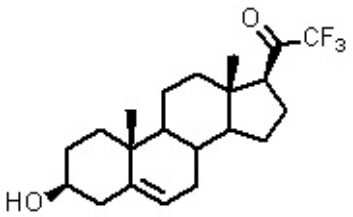

210.16
192.05
151.46
145.30
139.30
122.27
104.89
84.14
82.68
77.41
77.16
76.91
55.80
46.97
34.42
30.26
21.01
19.21
16.02
0.15
O
F
O
FYVII-065
(400 MHz)
26
f1 (ppm)


FYII-068
(CD3OD)
27
f1 (ppm)


HO
O
HO
FYII-066B
(CD3OD)
28
5.31
4.87
3.62
3.37
3.32
3.28
2.31
2.17
1.96
1.84
1.58
1.48
1.28
1.12
1.06
1.00
0.94
0.86
0.70
500 MHz
1.00
0.84
0.45
0.86
2.14
2.93
1.15
3.99
5.89
4.78
6.97
3.33
2.47
f1 (ppm)


HO
O
HO
FYII-066B
(CD3OD)
28
177.79
142.41
122.50
122.40
72.51
57.66
49.14
48.63
39.53
36.33
33.11
26.35
24.76
20.69
20.04
500 MHz
750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


7.26
5.44
4.81
2.95
2.93
2.44
1.56
1.41
1.11
0.95
0.75
0.07
-0.00
FYIII-056B
29
400 MHz
f1 (ppm)



FYII-060B

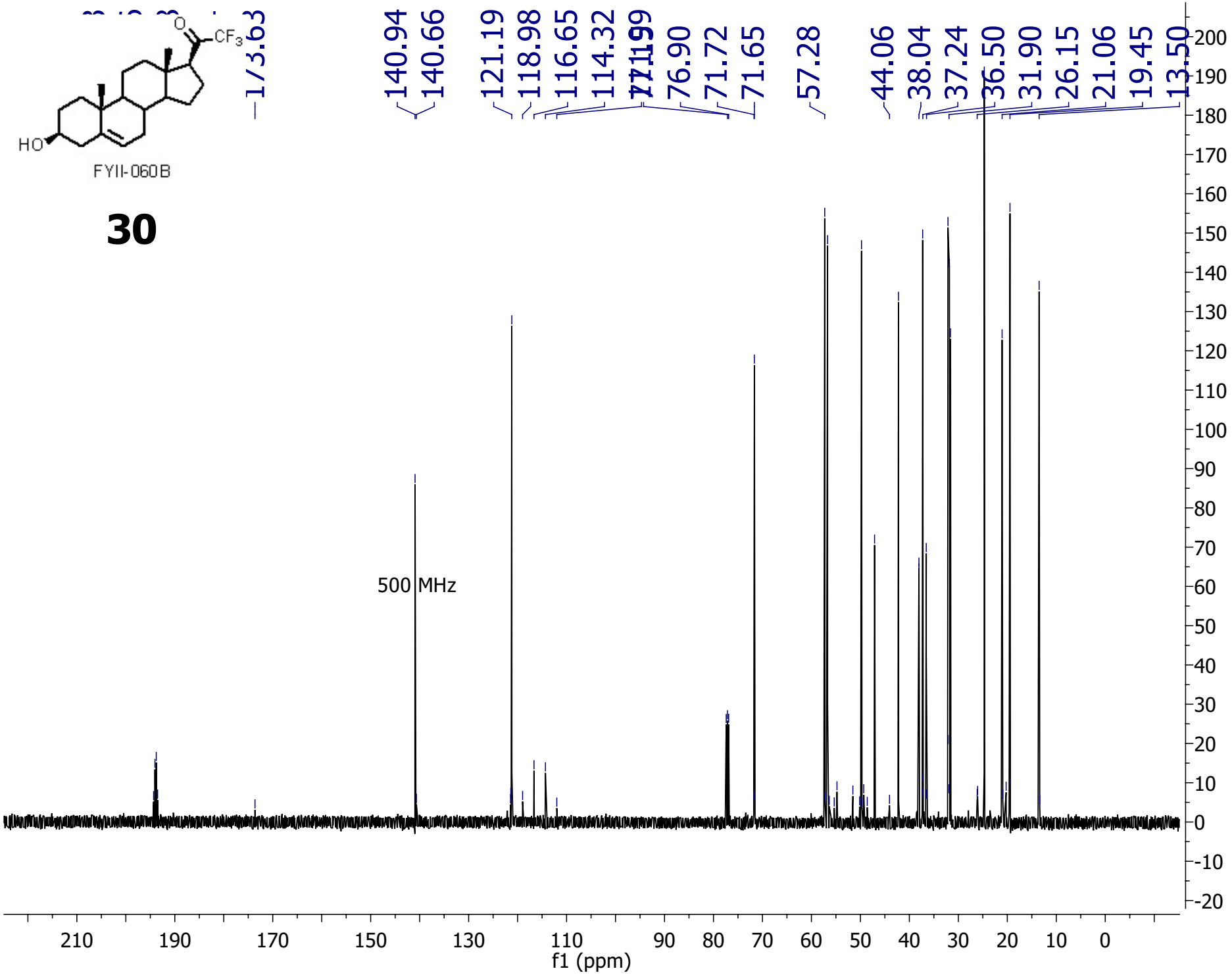
30

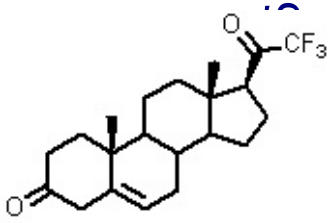
5.31 3.49 3.48 3.47 2.92 2.91 1.99 1.88 1.77 1.58 1.49 1.34 1.29 1.21 1.03 0.98

500 MHz

1.17 1.00 1.05 4.21 4.34 5.74 8.59 1.60 1.45 1.52 5.75 3.66

f1 (ppm)


HO
O
CF3
FYII-060B
30
173.63
140.94
140.66
121.19
118.98
116.65
114.32
76.90
71.72
71.65
57.28
44.06
38.04
37.24
36.50
31.90
26.15
21.06
19.45
13.50
500 MHz
f1 (ppm)

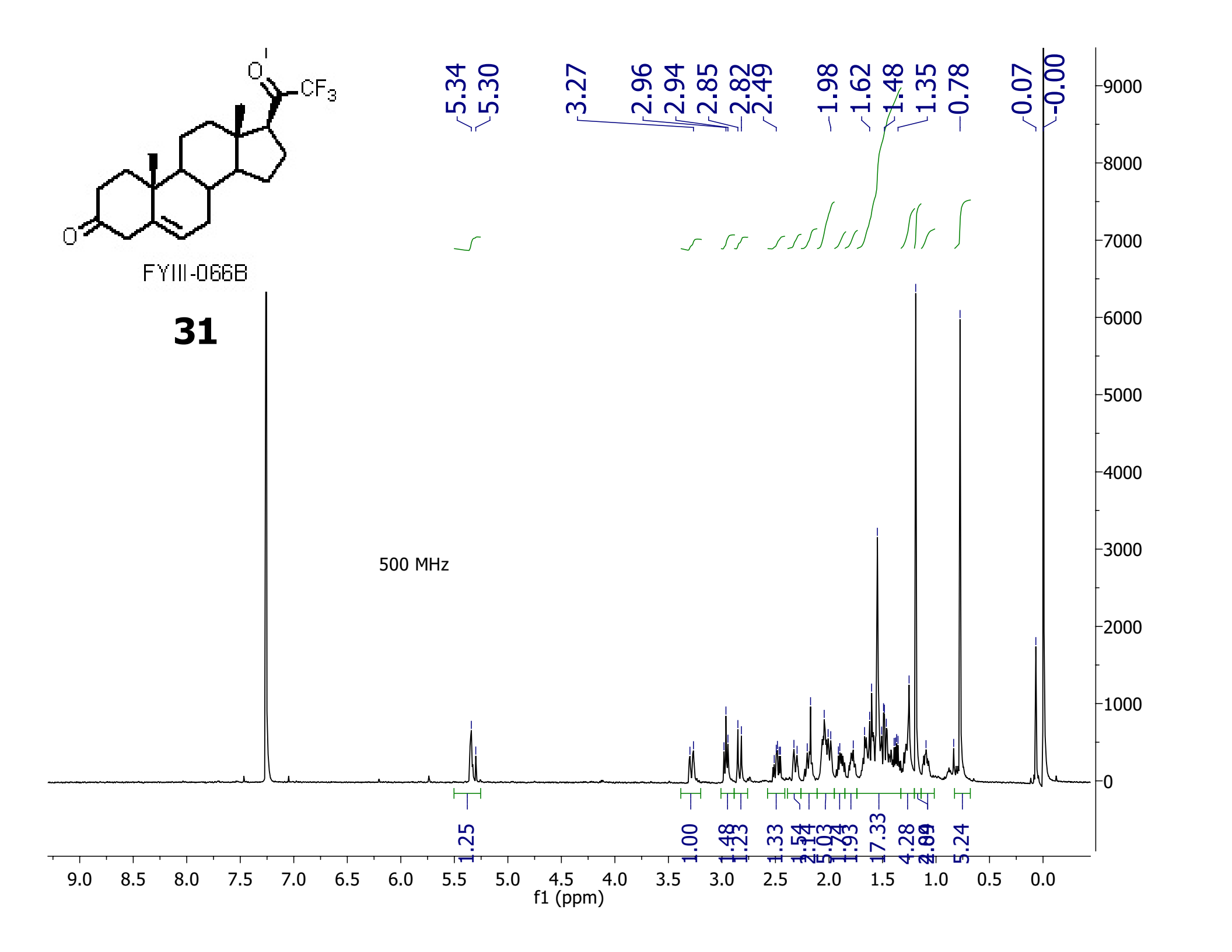


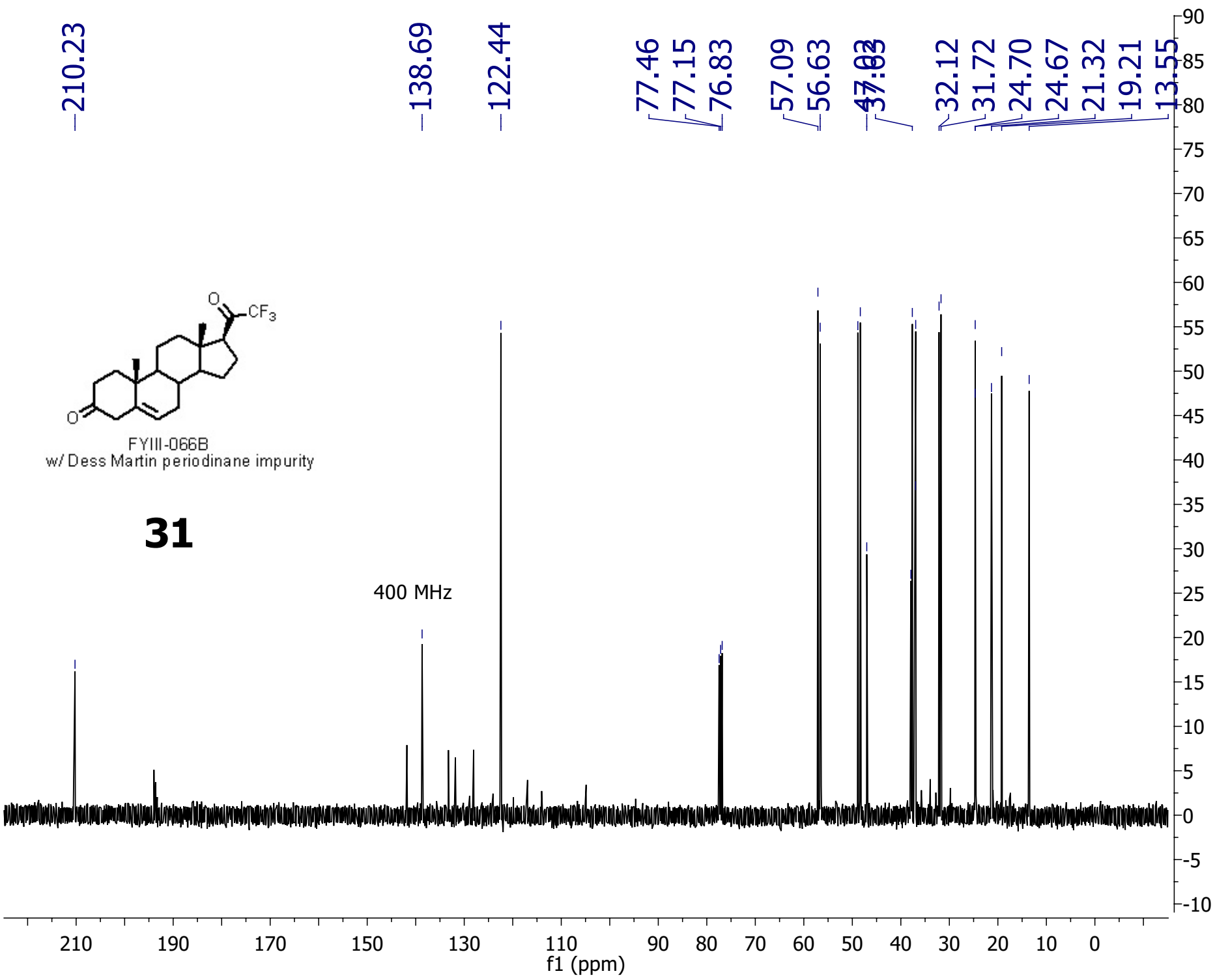
FYIII-066B
w/ Dess Martin periodinane impurity

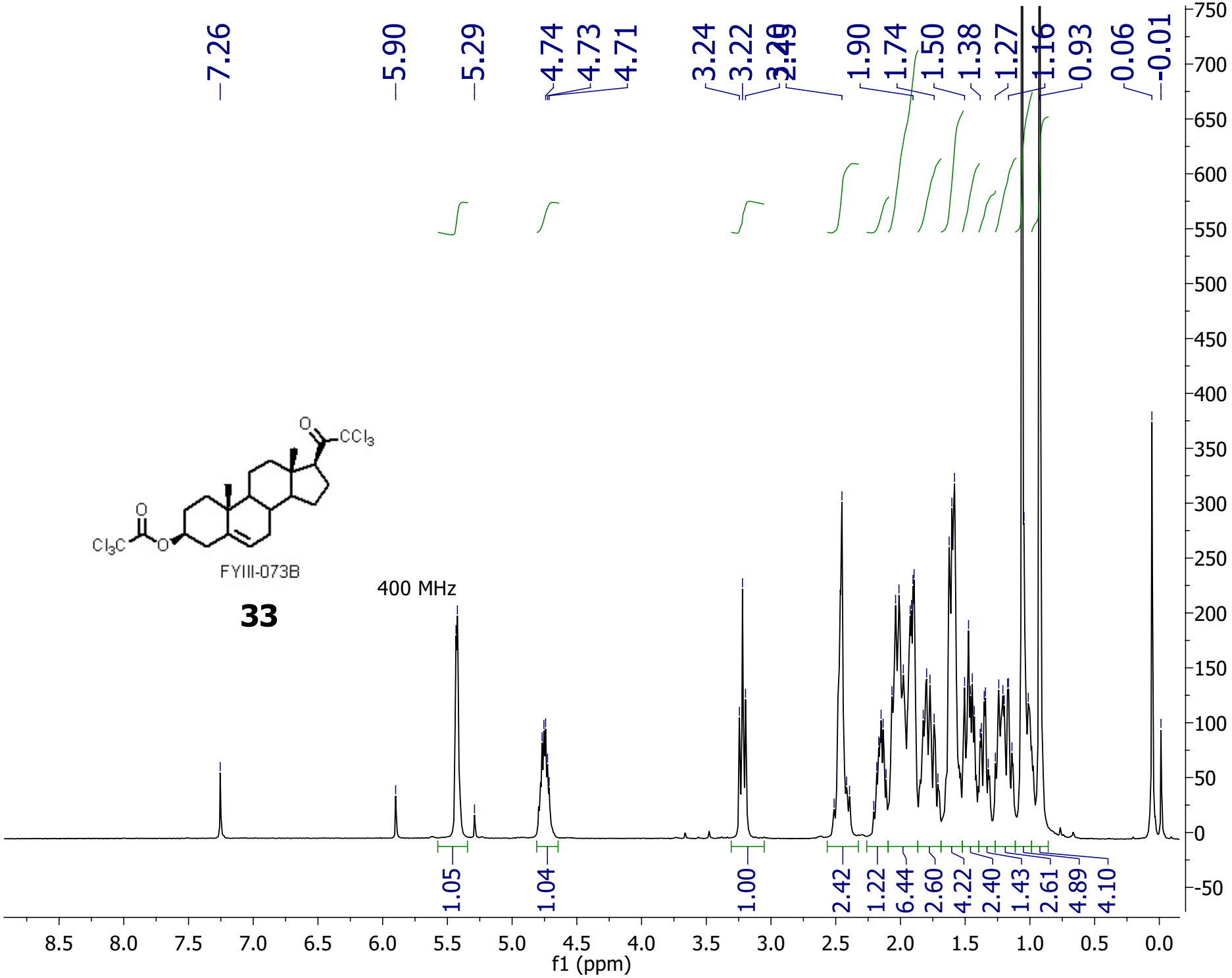
31

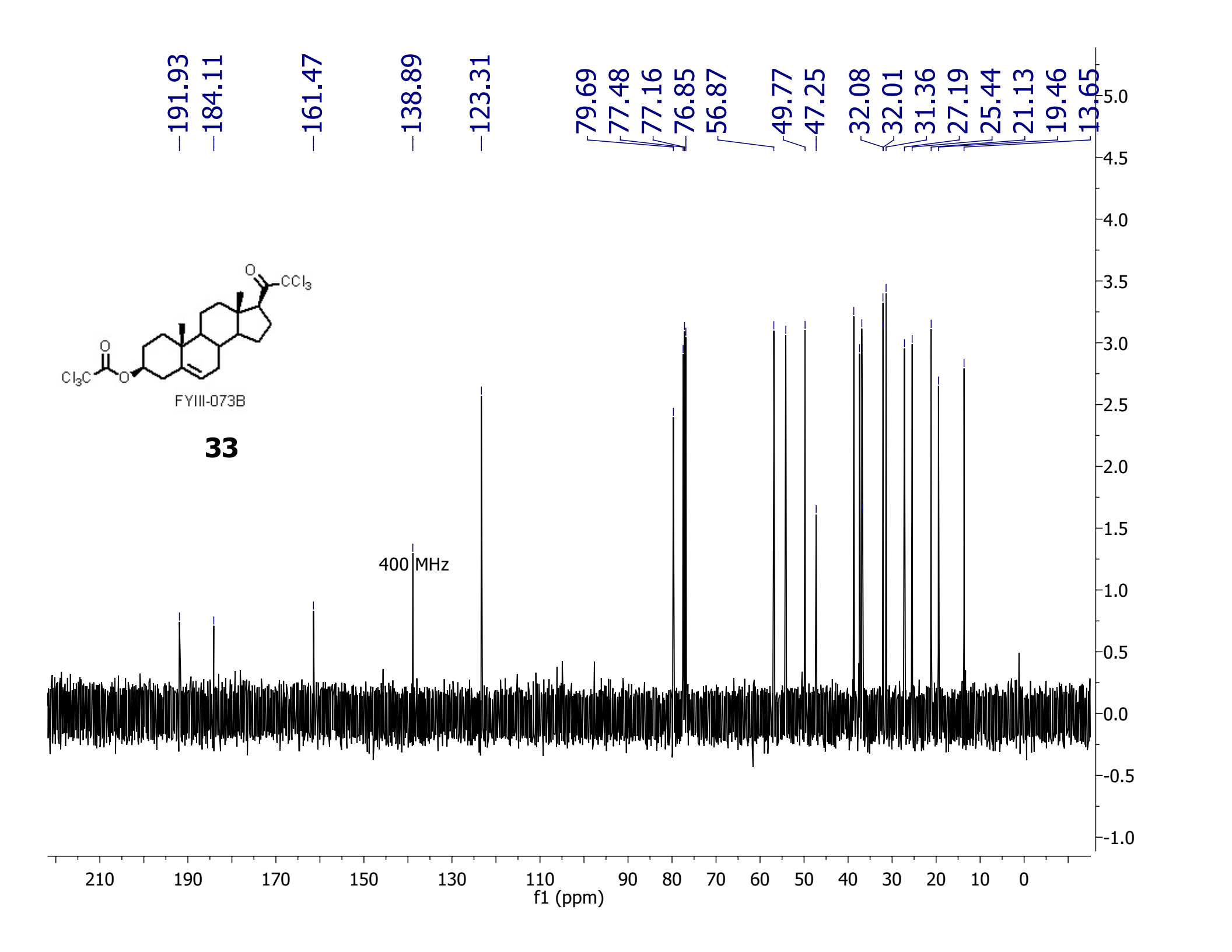
400 MHz

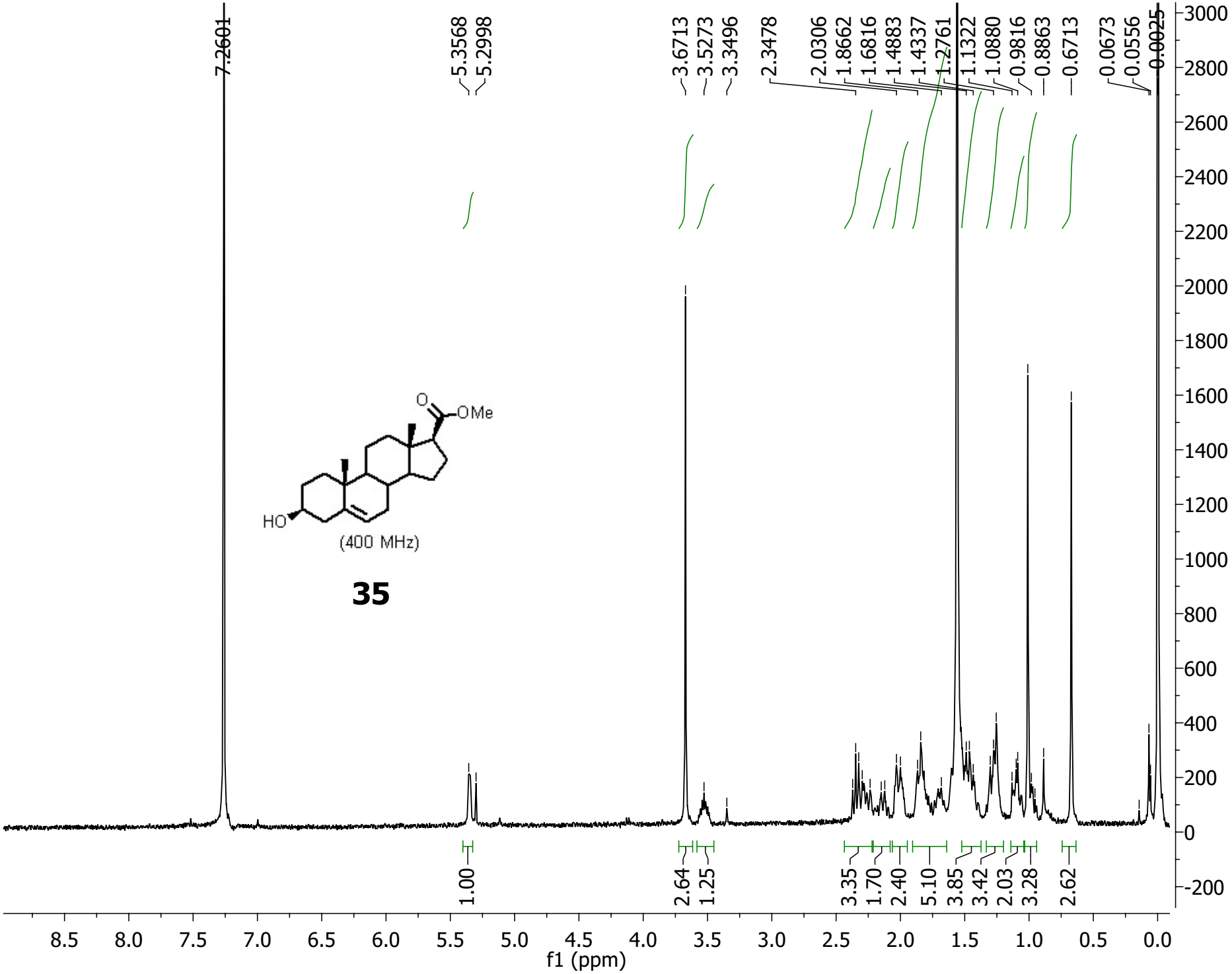
f1 (ppm)

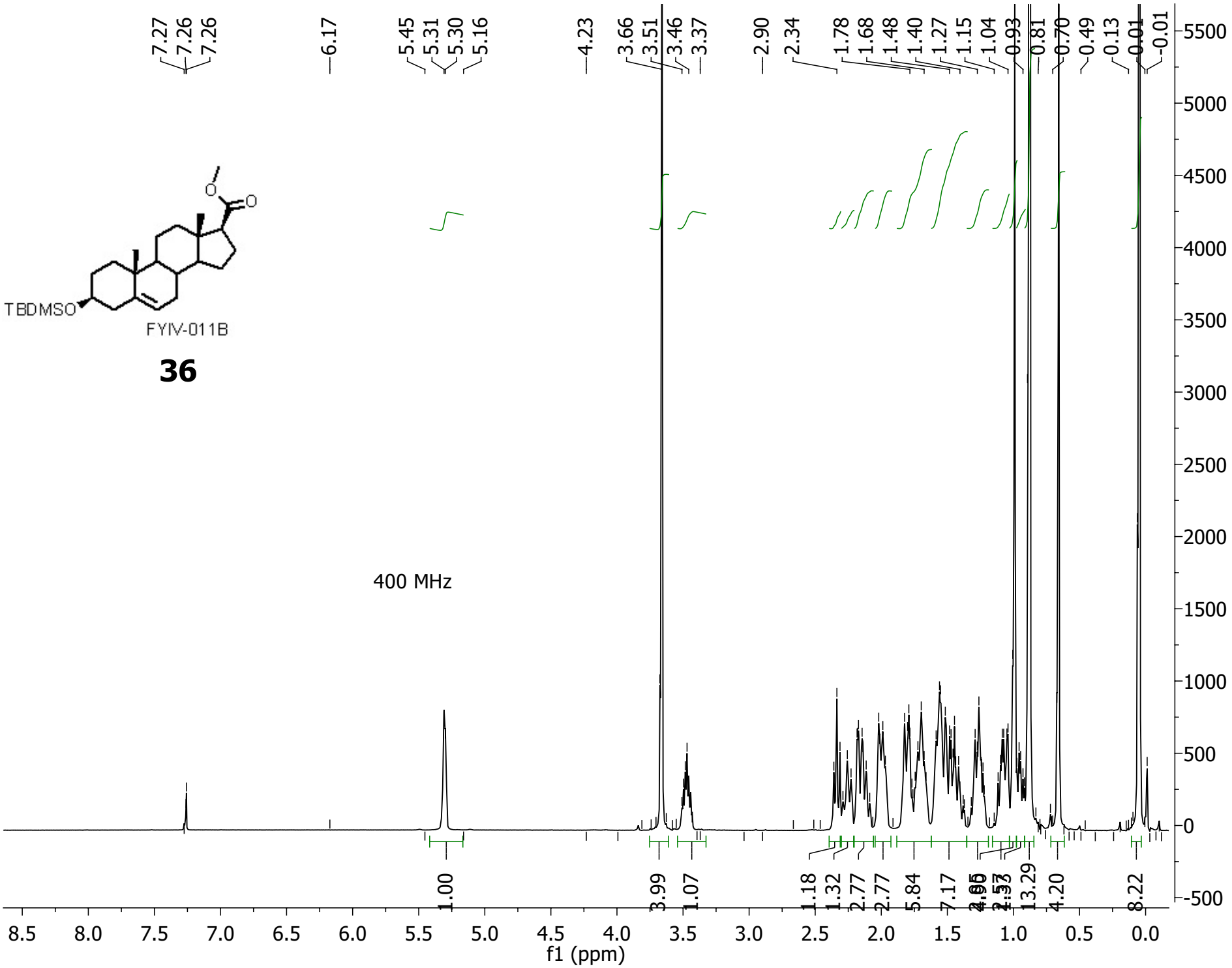

O
O
CF3
FYIII-066B
31
500 MHz
5.34
5.30
3.27
2.96
2.94
2.85
2.82
2.49
1.98
1.62
1.48
1.35
0.78
0.07
-0.00
1.25
1.00
1.48
1.23
1.33
17.33
4.28
5.24
f1 (ppm)

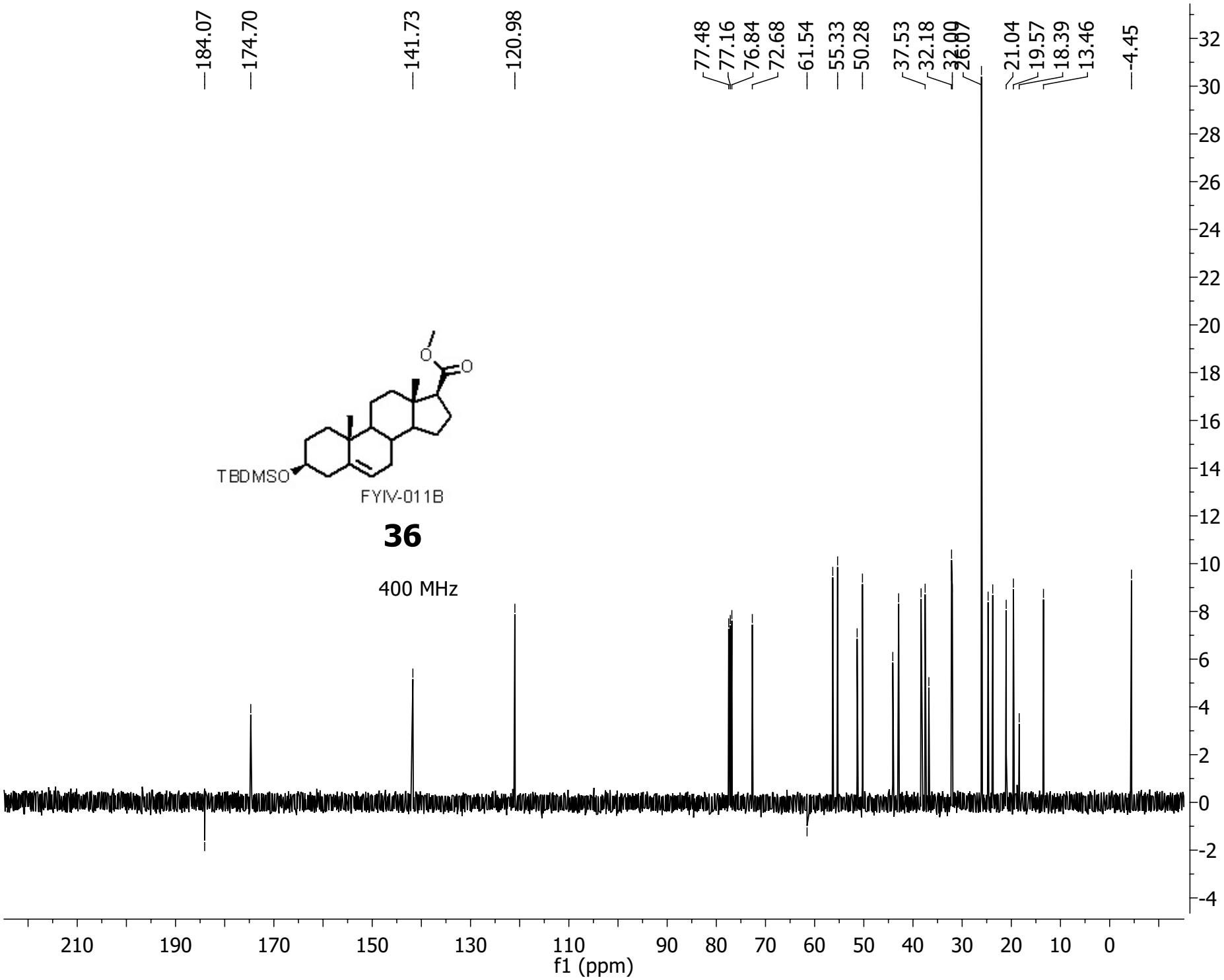

210.23
138.69
122.44
77.46
77.15
76.83
57.09
56.63
47.03
37.03
32.12
31.72
24.70
24.67
21.32
19.21
13.55
O
CF3
O
FYIII-066B
w/ Dess Martin periodinane impurity
31
400 MHz
f1 (ppm)

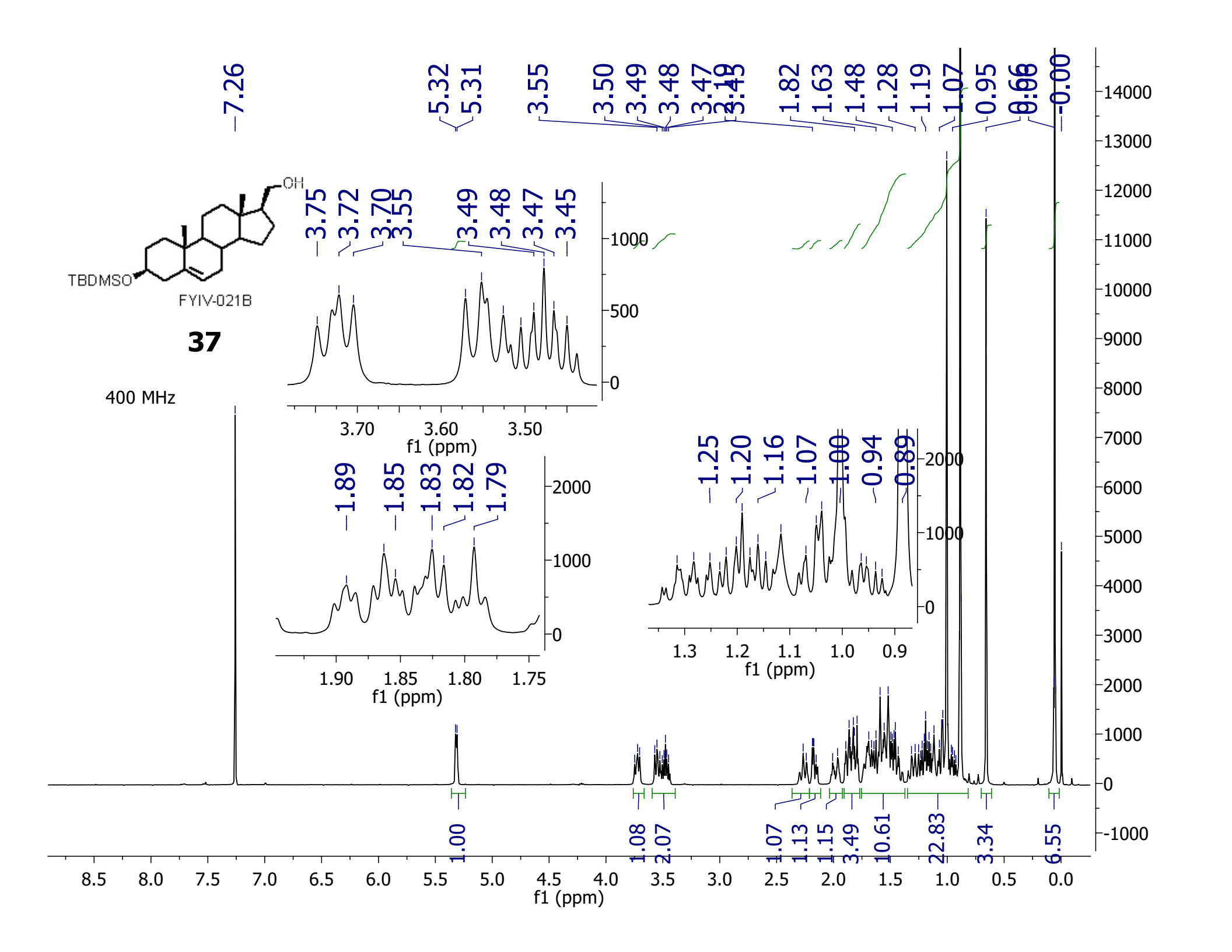

7.26
5.90
5.29
4.74
4.73
4.71
3.24
3.22
1.90
1.74
1.50
1.38
1.27
1.16
0.93
0.06
-0.01
FYIII-073B
33
400 MHz
1.05
1.04
1.00
2.42
1.22
6.44
2.60
4.22
2.40
1.43
2.61
4.89
4.10
f1 (ppm)

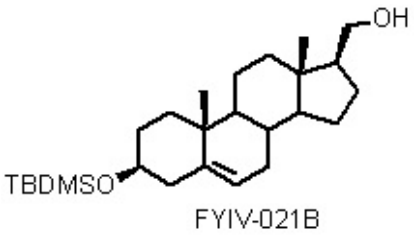

191.93
184.11
161.47
138.89
123.31
79.69
77.48
77.16
76.85
56.87
49.77
47.25
32.08
32.01
31.36
27.19
25.44
21.13
19.46
13.65
FYIII-073B
33
400 MHz
f1 (ppm)


7.2601
5.3568
5.2998
3.6713
3.5273
3.3496
2.3478
2.0306
1.8662
1.6816
1.4883
1.4337
1.2761
1.1322
1.0880
0.9816
0.8863
0.6713
0.0673
0.0556
0.0025
O
OMe
HO
(400 MHz)
35
1.00
2.64
1.25
3.35
1.70
2.40
5.10
3.85
3.42
2.03
3.28
2.62
f1 (ppm)


TBDMSO
O
O
FYIV-011B
36
400 MHz
7.27
7.26
7.26
6.17
5.45
5.31
5.30
5.16
4.23
3.66
3.51
3.46
3.37
2.90
2.34
1.78
1.68
1.48
1.40
1.27
1.15
1.04
0.93
0.81
0.70
0.49
0.13
0.01
-0.01
1.00
3.99
1.07
1.18
1.32
2.77
2.77
5.84
7.17
13.29
4.20
8.22
f1 (ppm)

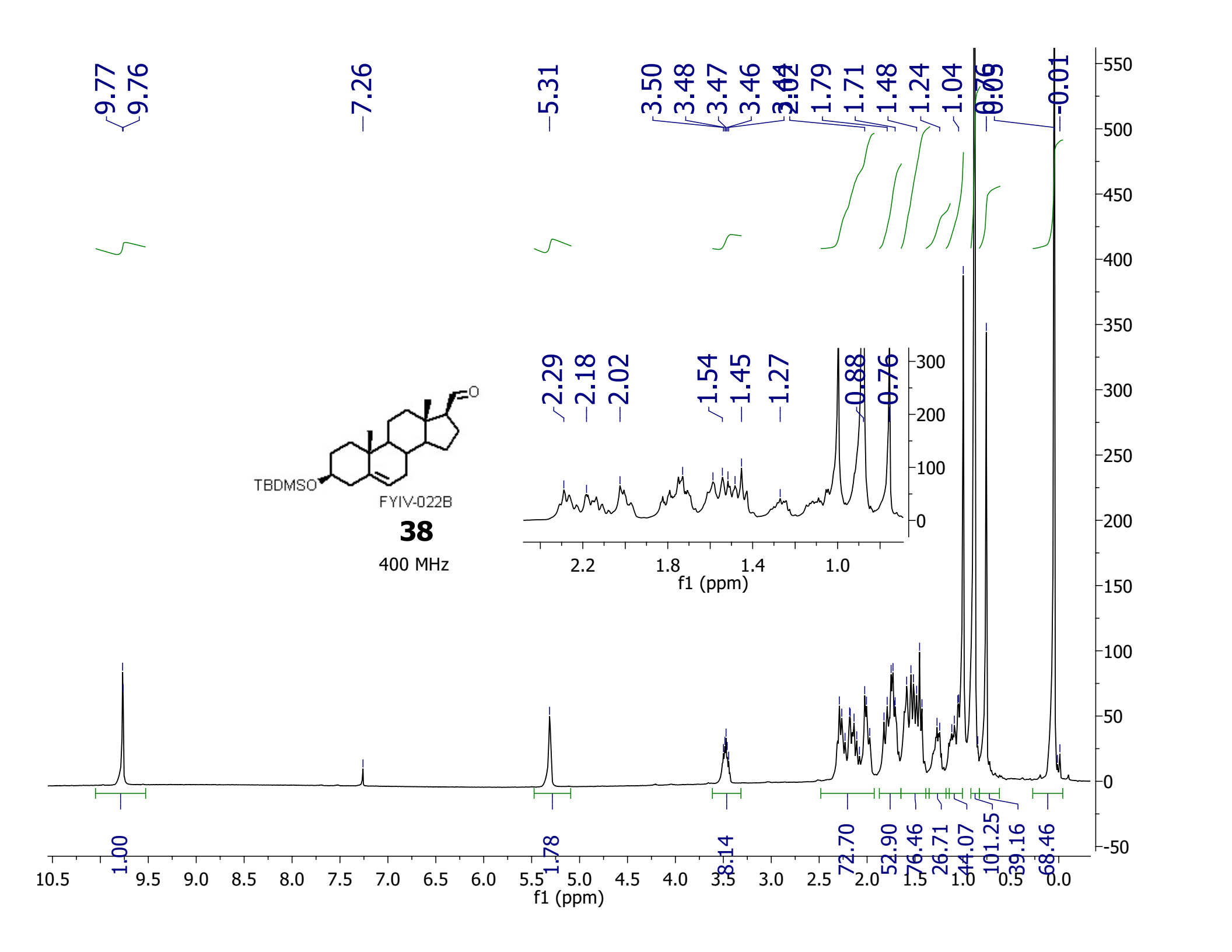

184.07
174.70
141.73
120.98
77.48
77.16
76.84
72.68
61.54
55.33
50.28
37.53
32.18
32.00
26.07
21.04
19.57
18.39
13.46
-4.45
TBDMSO
O
O
FYIV-011B
36
400 MHz
f1 (ppm)

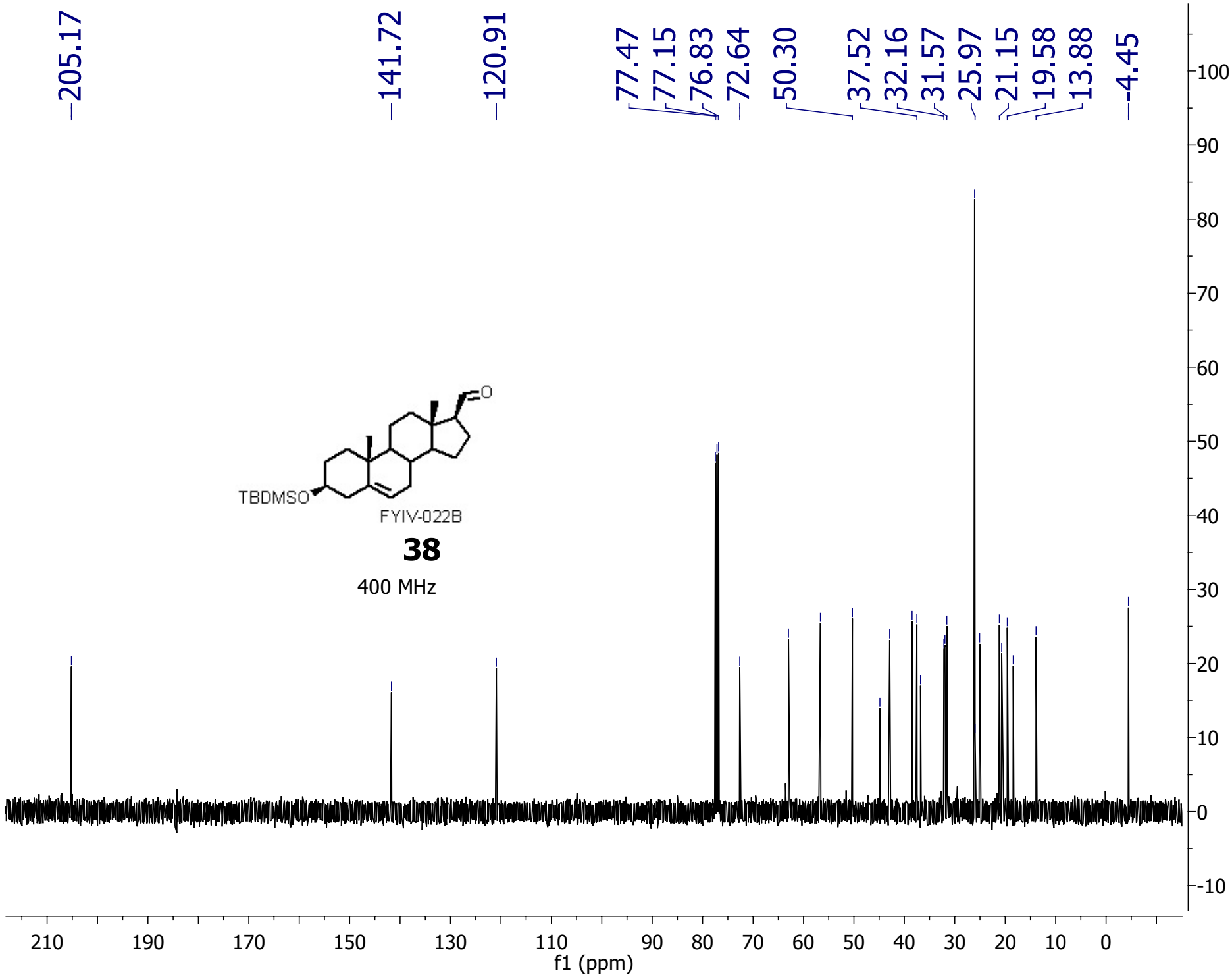

TBDMSO
OH
FYIV-021B
37
400 MHz
f1 (ppm)

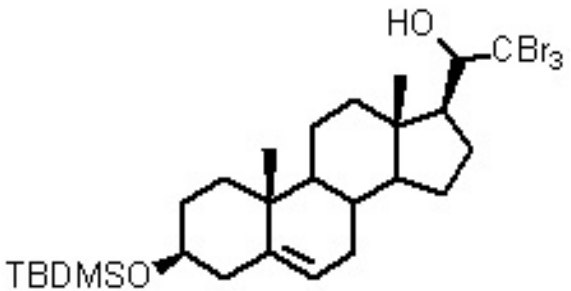

OH
TBDMSO
FYIV-021B

37

141.74
121.16
77.47
77.15
76.83
72.73
64.79
50.57
42.95
32.10
26.08
24.81
19.61
12.57
-4.44
400 MHz
90
80
70
60
50
40
30
20
10
0
-10
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


9.77
9.76
7.26
5.31
3.50
3.48
3.47
3.46
1.79
1.71
1.48
1.24
1.04
0.05
-0.01
TBDMSO
O
FYIV-022B
38
400 MHz
2.29
2.18
2.02
1.54
1.45
1.27
0.88
0.76
f1 (ppm)
f1 (ppm)
1.00
1.78
3.14
72.70
52.90
76.46
26.71
44.07
101.25
39.16
68.46


205.17
141.72
120.91
77.47
77.15
76.83
72.64
50.30
37.52
32.16
31.57
25.97
21.15
19.58
13.88
-4.45
TBDMSO
O
FYIV-022B
38
400 MHz
f1 (ppm)



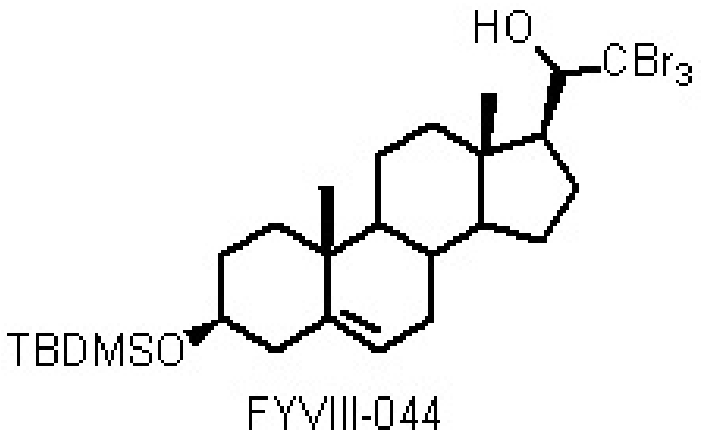
FYV-045C.Br

39

400 MHz

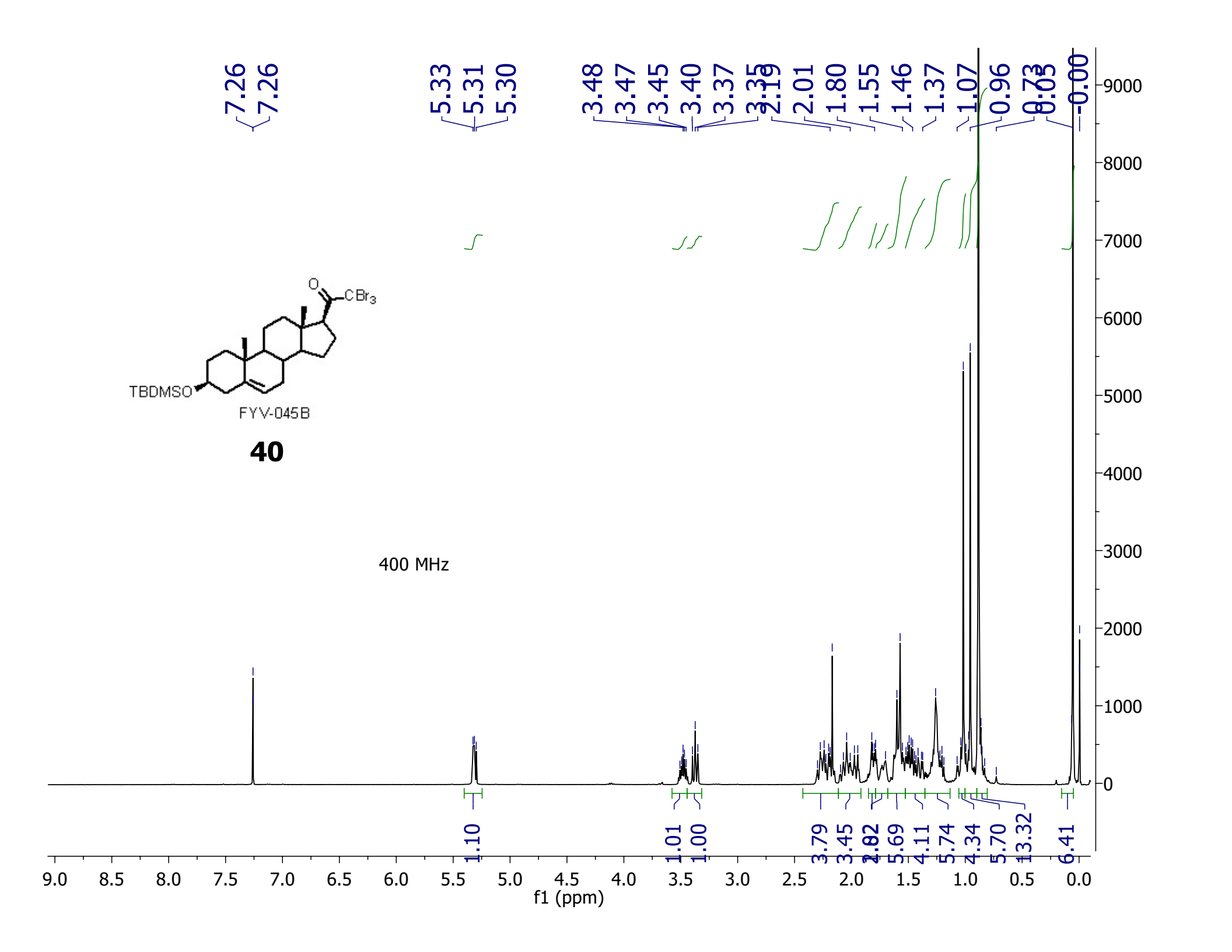
0.15 1.00 0.32 0.76 0.28 3.22 1.55 4.24 8.59 2.69 5.63 9.84 3.21 5.16

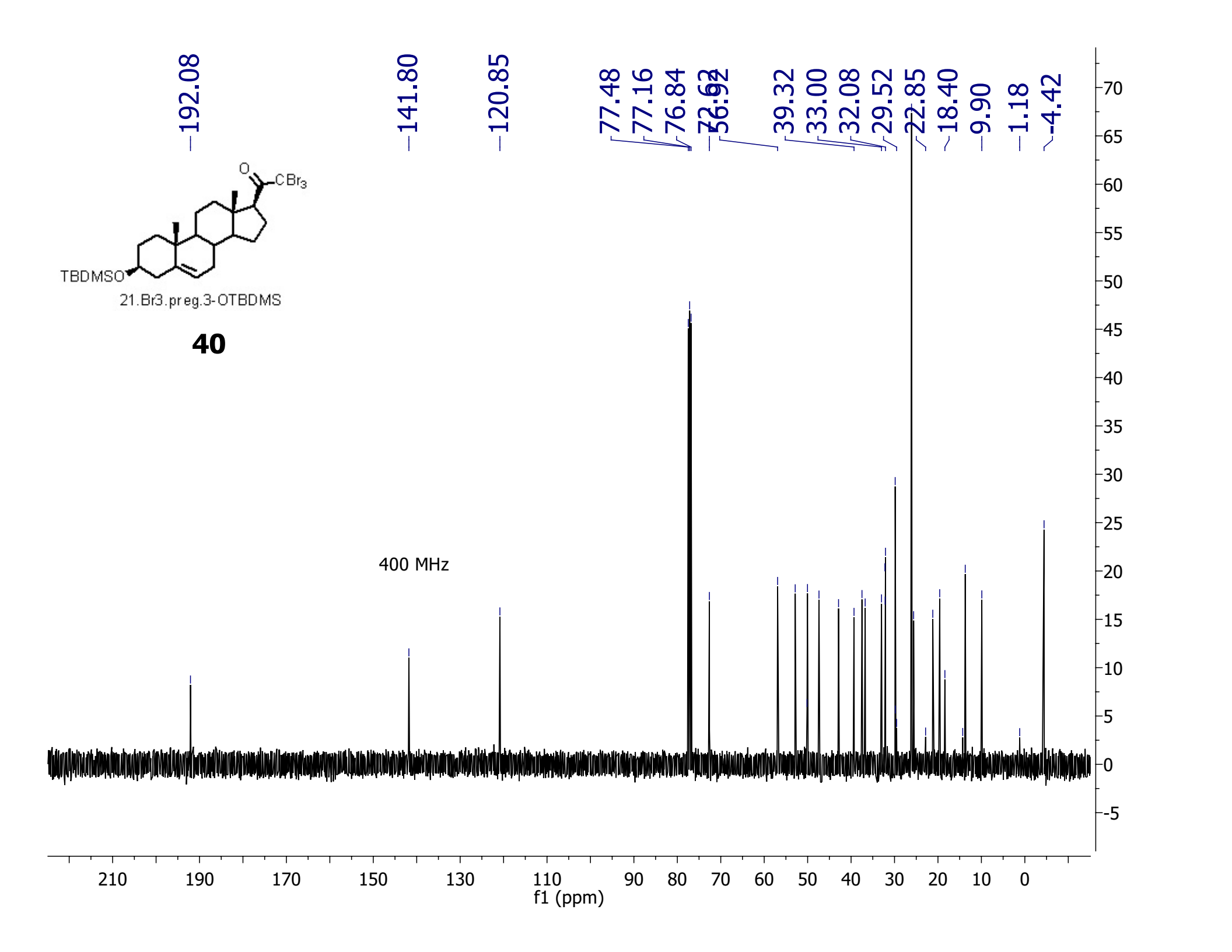
f1 (ppm)

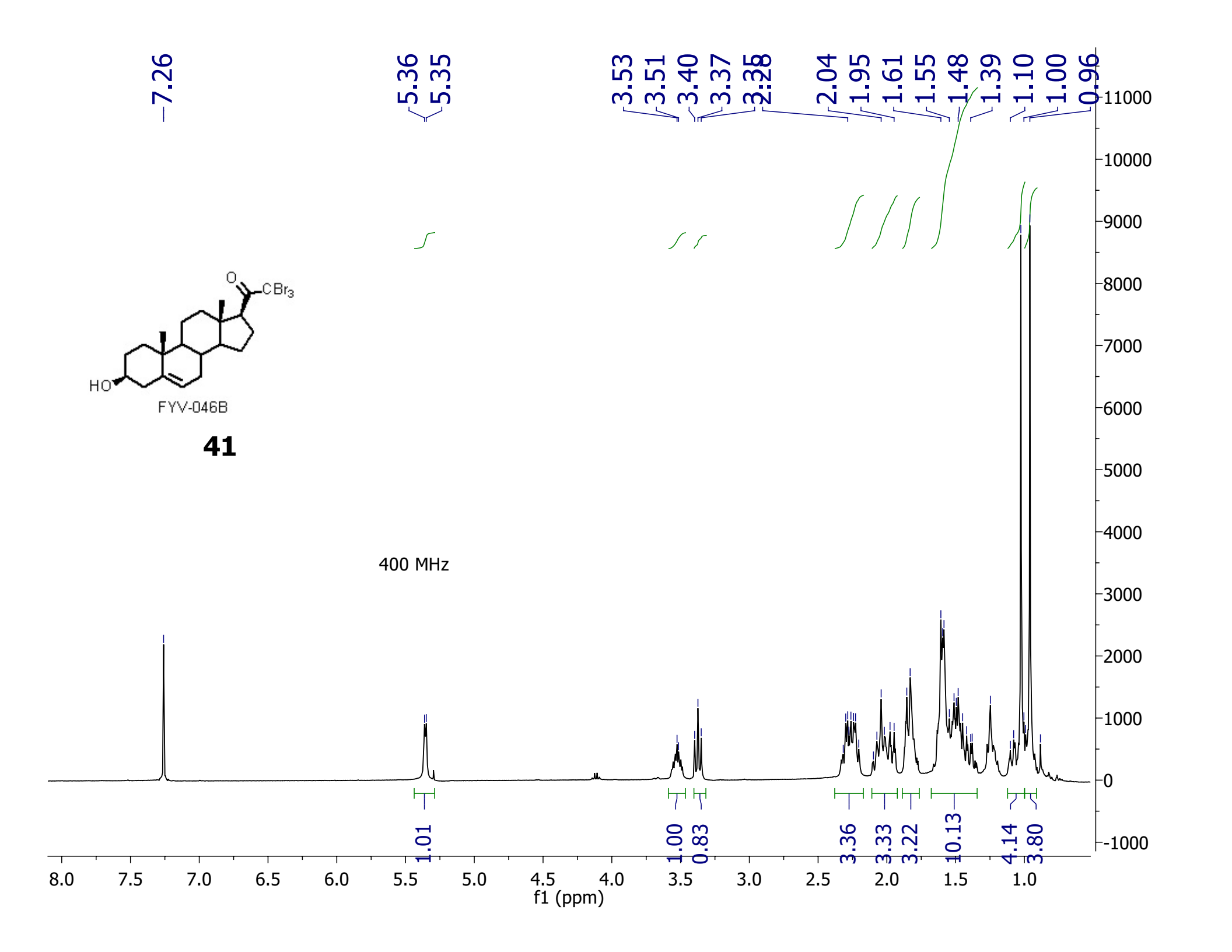


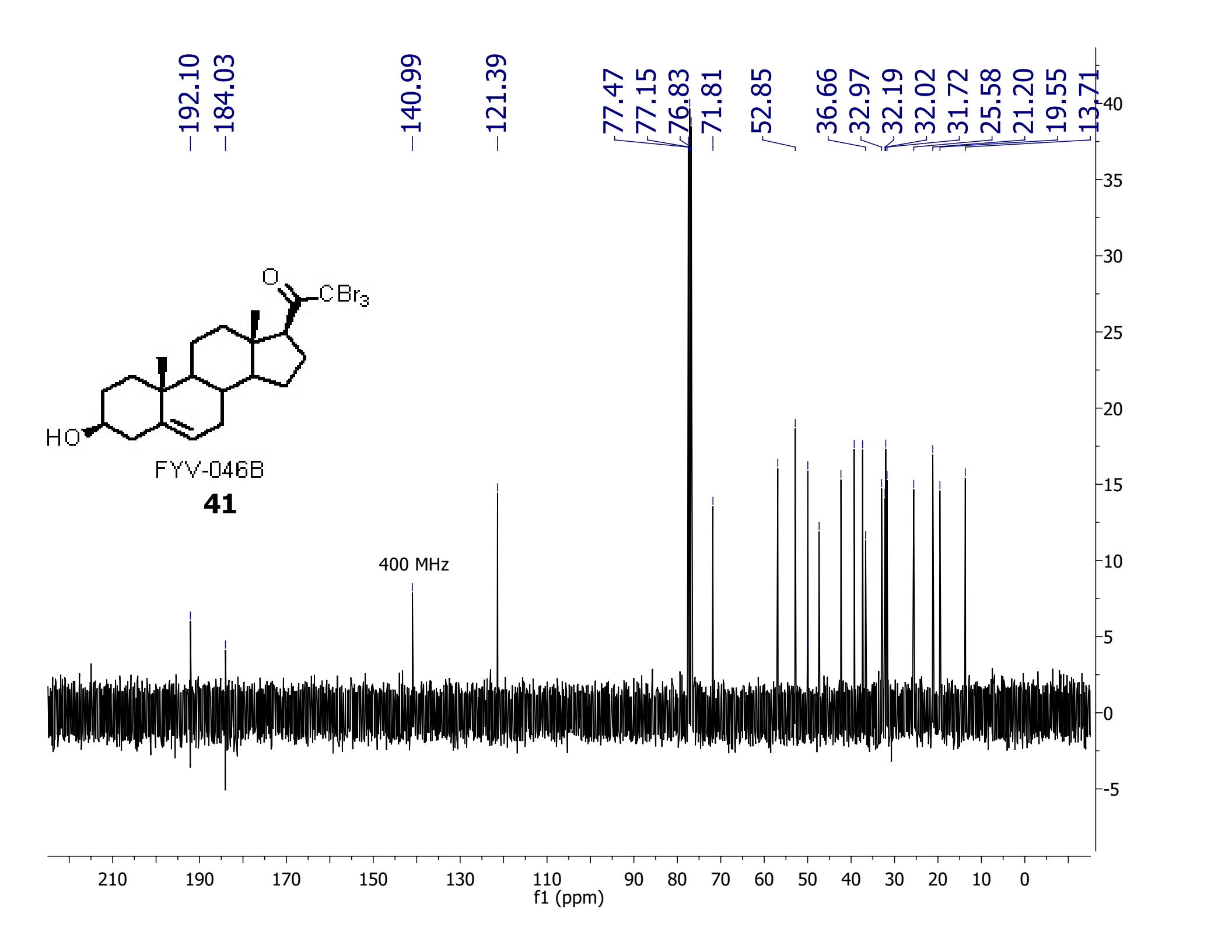
FYVIII-044

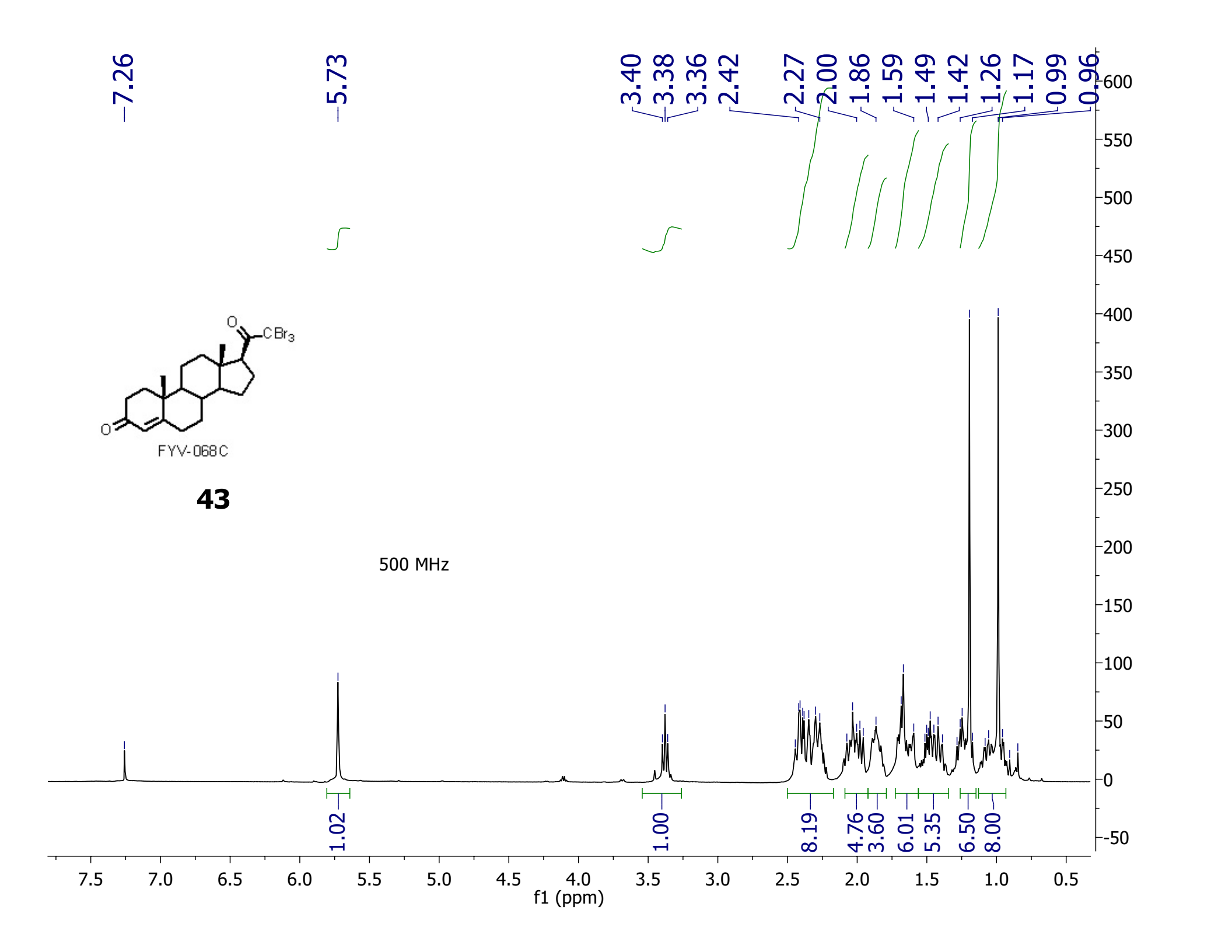
39

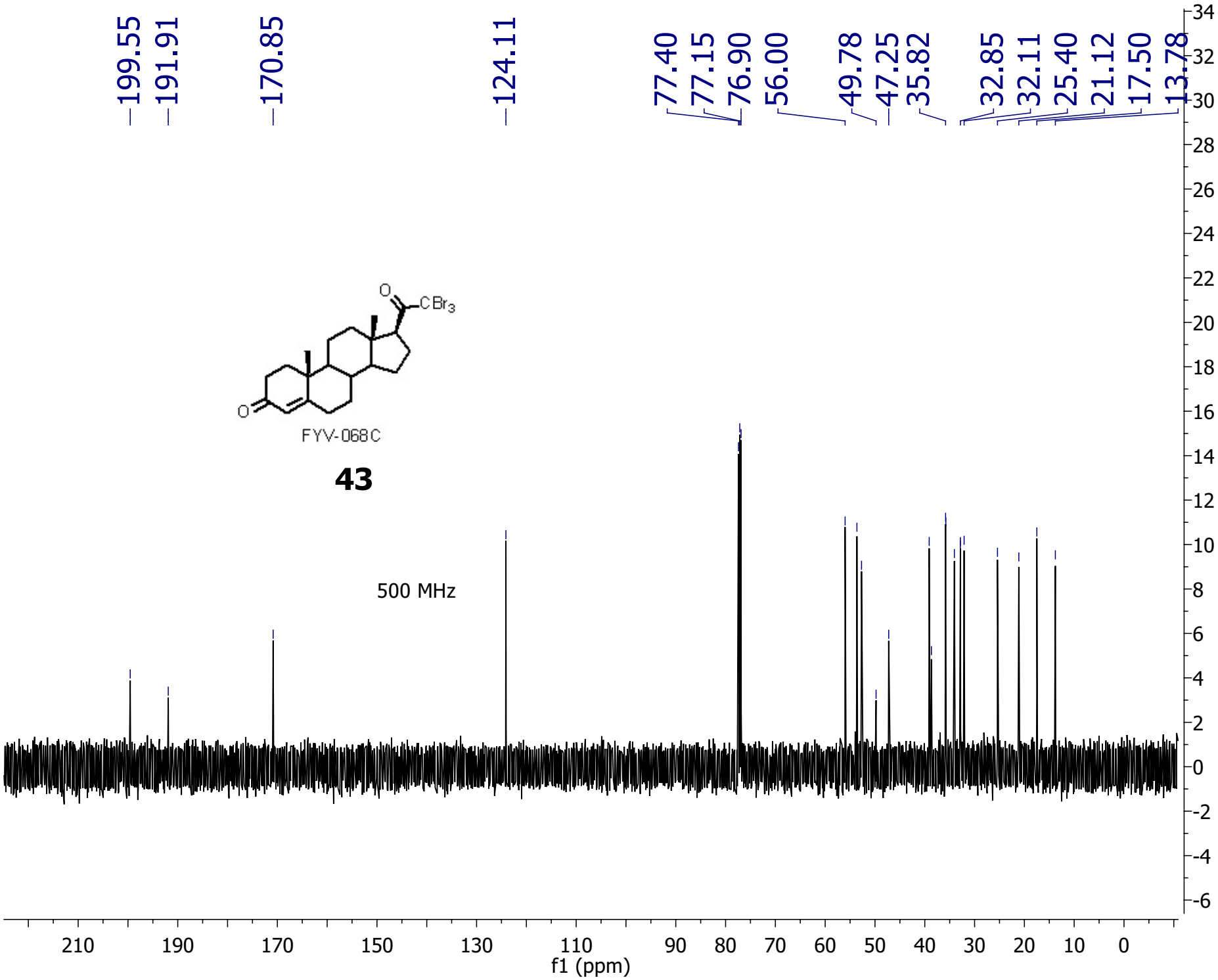

7.26
7.26
5.33
5.31
5.30
3.48
3.47
3.45
3.40
3.37
3.35
2.19
2.01
1.80
1.55
1.46
1.37
1.07
0.96
0.73
0.03
0.00
O
CBr3
TBDMSO
FYV-045B
40
400 MHz
1.10
1.01
1.00
3.79
3.45
5.69
4.11
5.74
4.34
5.70
13.32
6.41
f1 (ppm)

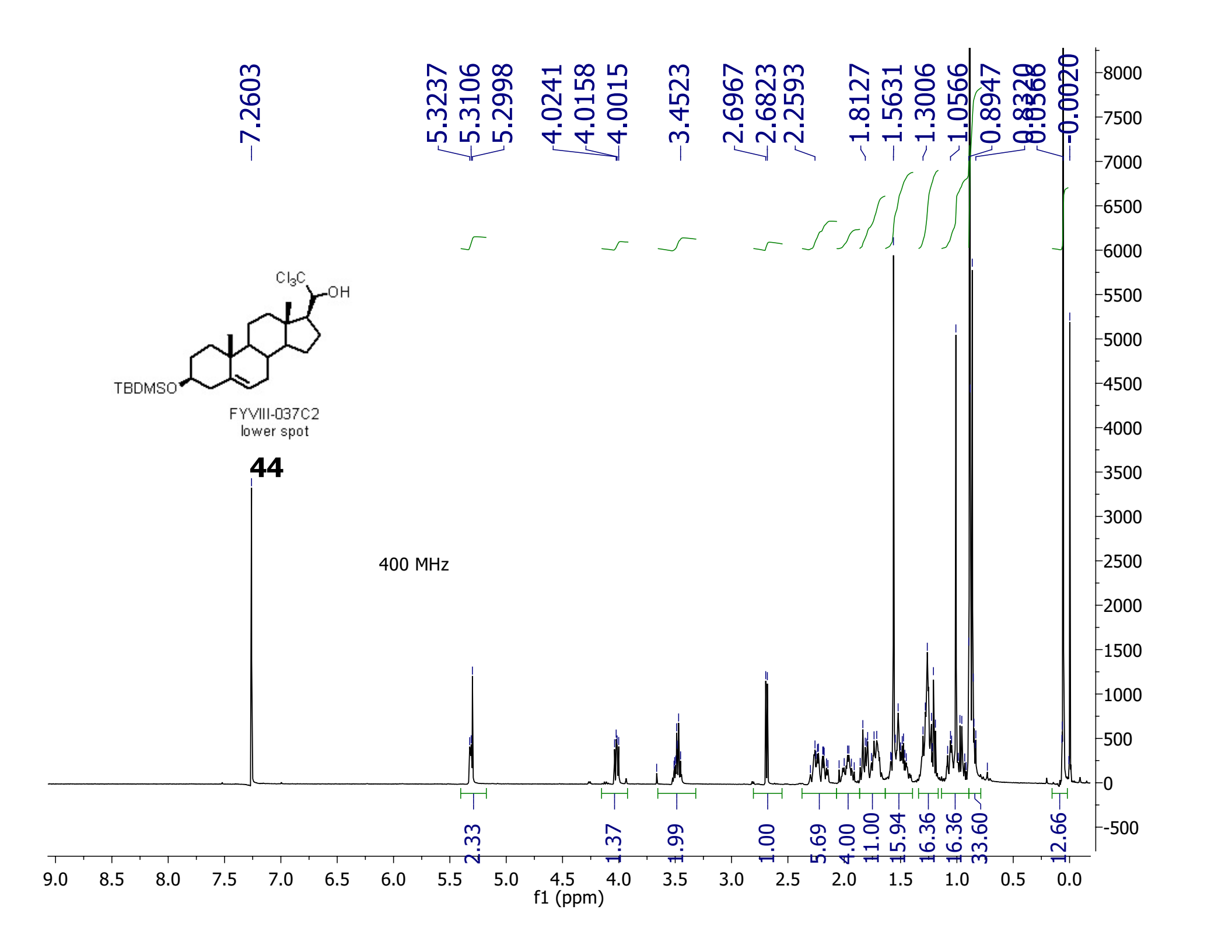

192.08
141.80
120.85
77.48
77.16
76.84
72.62
56.92
39.32
33.00
32.08
29.52
22.85
18.40
9.90
1.18
-4.42
O
CBr3
TBDMSO
21.Br3.preg.3-OTBDMS
40
400 MHz
f1 (ppm)

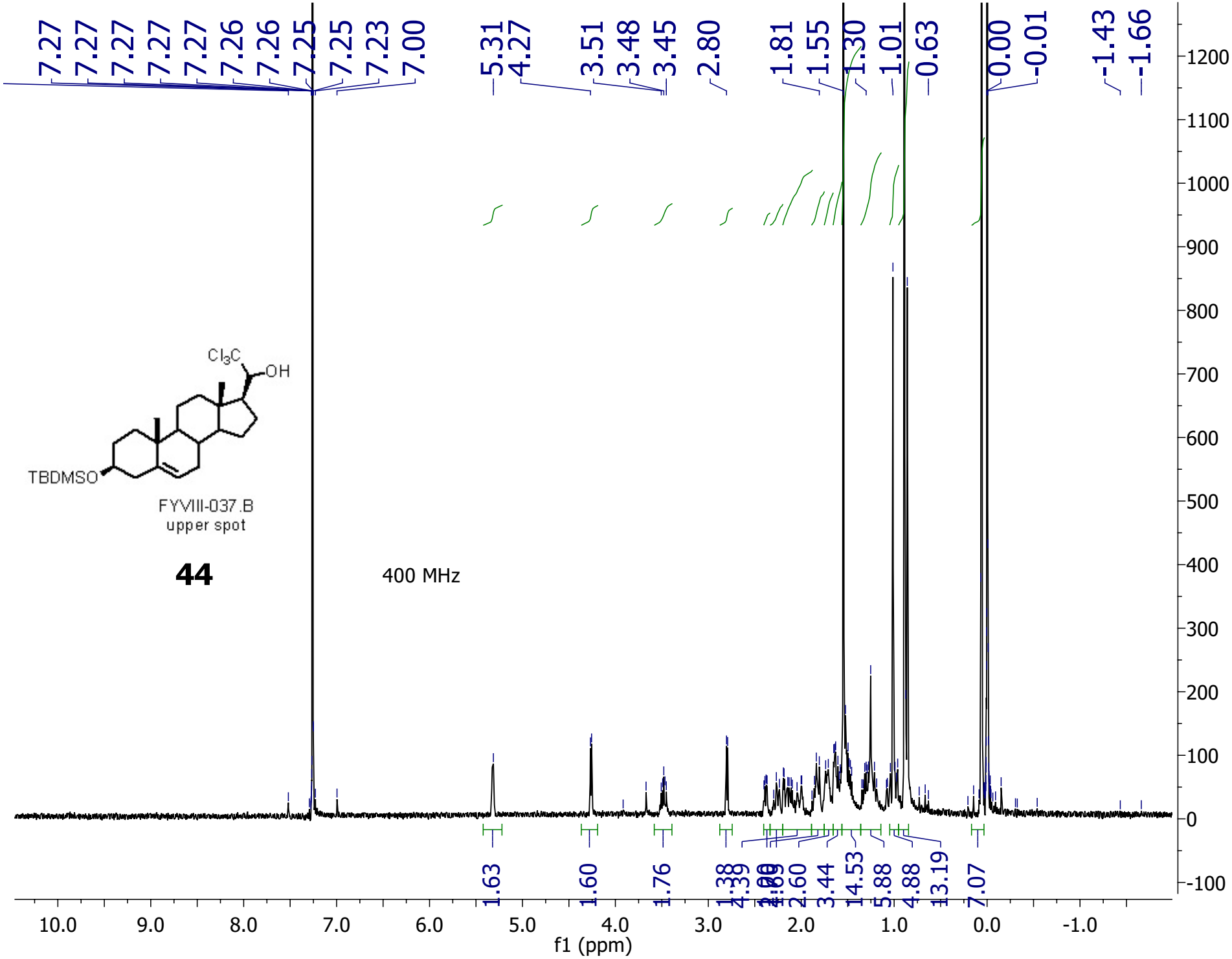

7.26
5.36
5.35
3.53
3.51
3.40
3.37
3.35
3.28
2.04
1.95
1.61
1.55
1.48
1.39
1.10
1.00
0.96
O
CBr3
HO
FYV-046B
41
400 MHz
1.01
1.00
0.83
3.36
3.33
3.22
10.13
4.14
3.80
f1 (ppm)

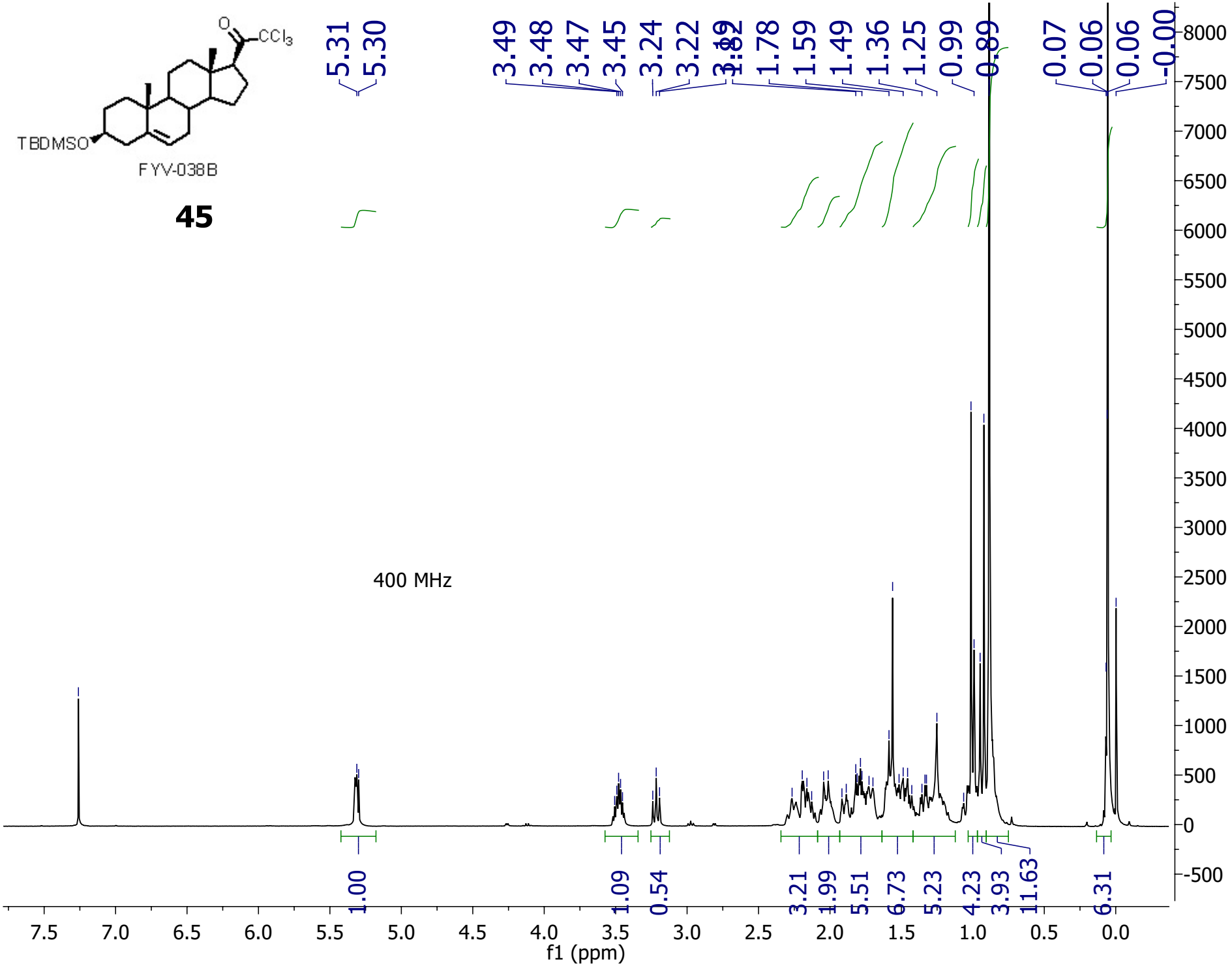

192.10
184.03
140.99
121.39
77.47
77.15
76.83
71.81
52.85
36.66
32.97
32.19
32.02
31.72
25.58
21.20
19.55
13.71
O
CBr3
HO
FYV-046B
41
400 MHz
f1 (ppm)

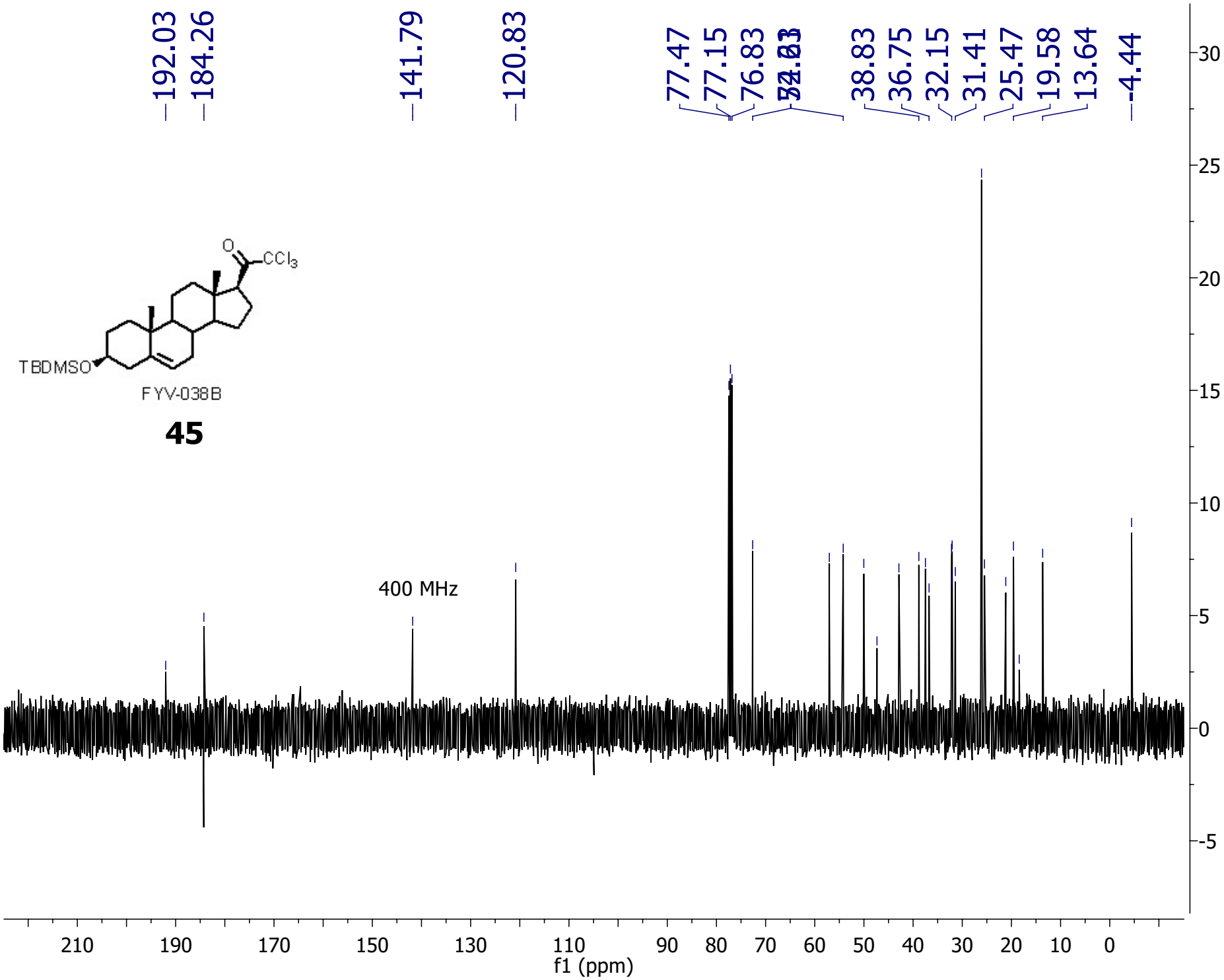

7.26
5.73
3.40
3.38
3.36
2.42
2.27
2.00
1.86
1.59
1.49
1.42
1.26
1.17
0.99
0.96
O
CBr3
O
FYV-068C
43
500 MHz
1.02
1.00
8.19
4.76
3.60
6.01
5.35
6.50
8.00
f1 (ppm)

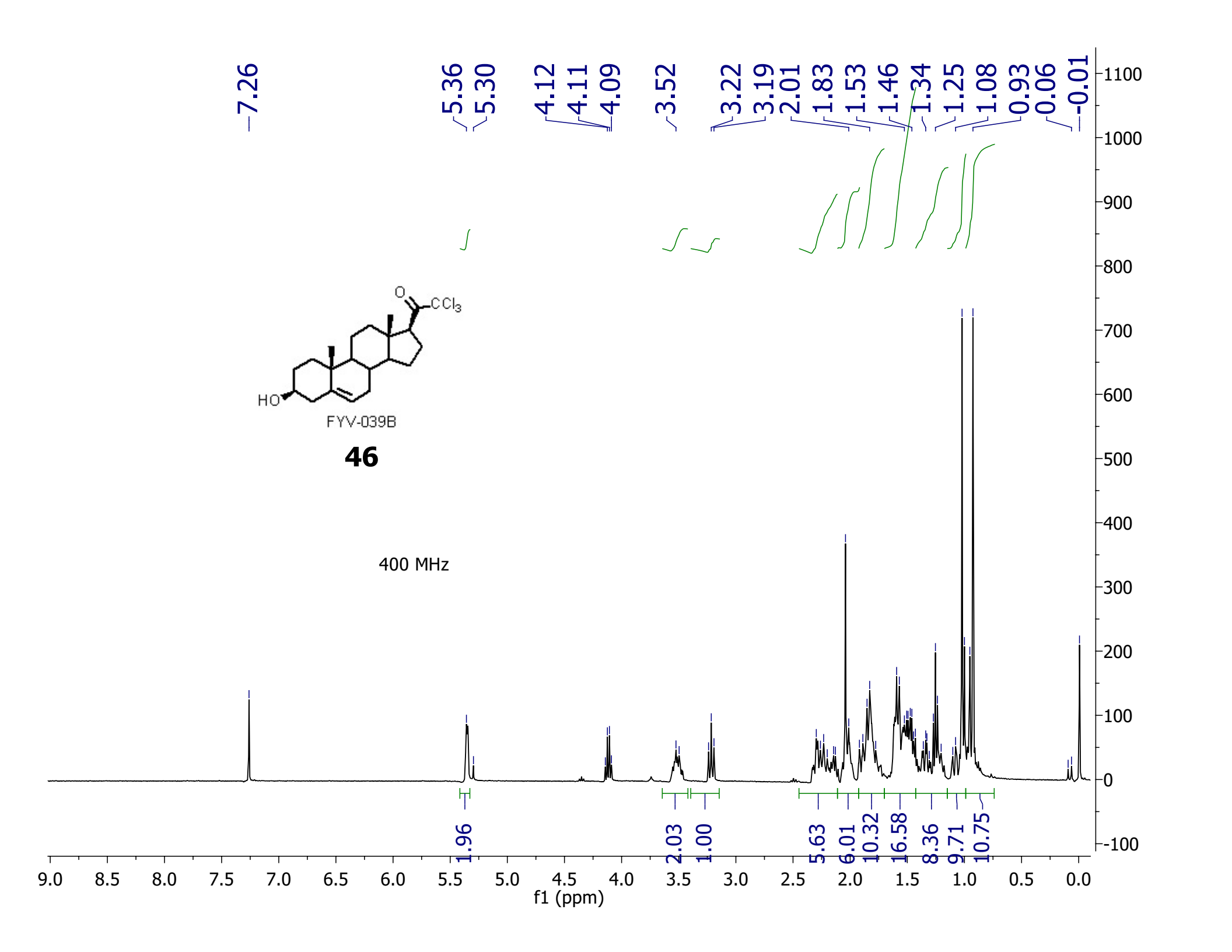

199.55
191.91
170.85
124.11
77.40
77.15
76.90
56.00
49.78
47.25
35.82
32.85
32.11
25.40
21.12
17.50
13.78
O
CBr3
O
FYV-068C
43
500 MHz
f1 (ppm)

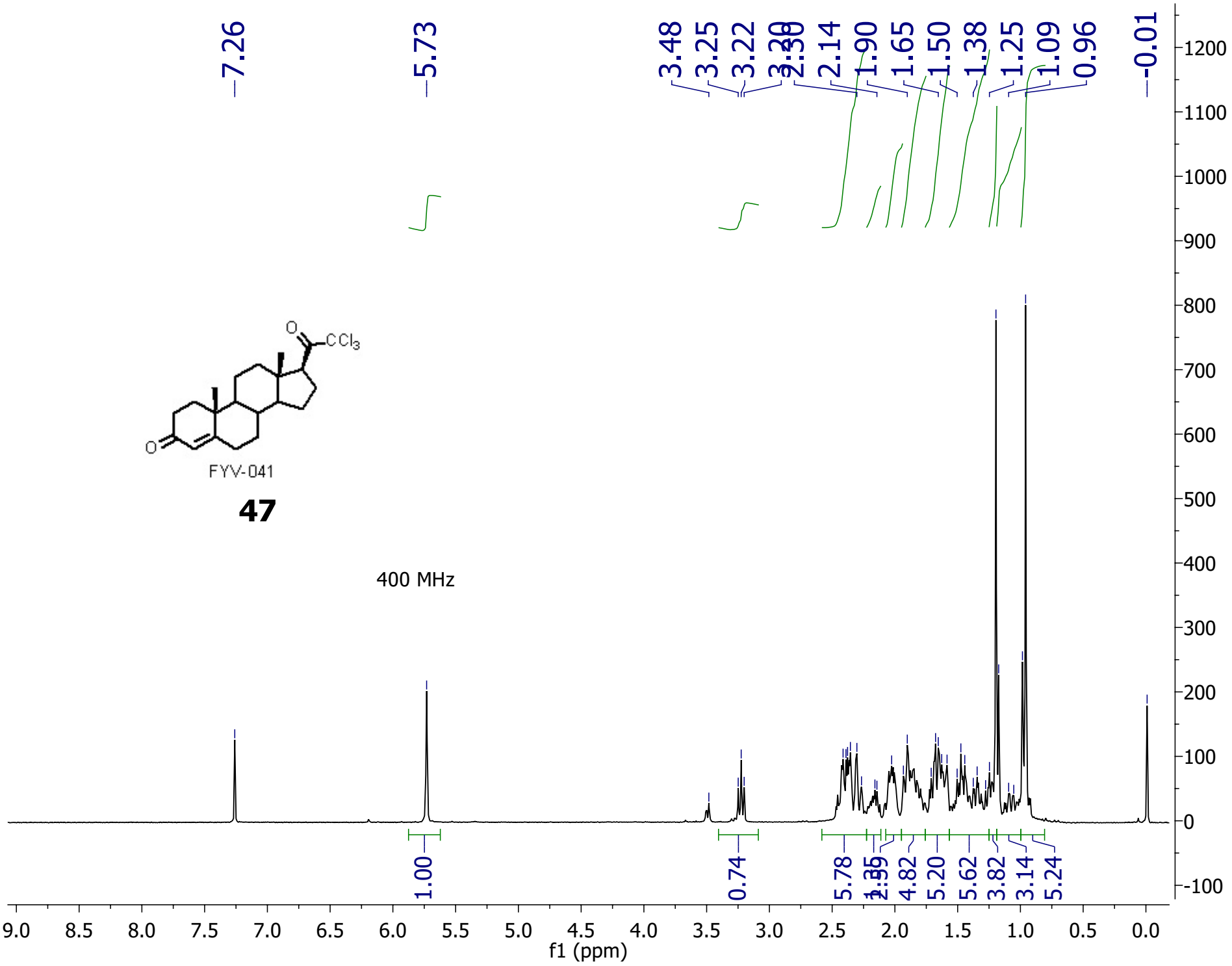

7.2603
5.3237
5.3106
5.2998
4.0241
4.0158
4.0015
3.4523
2.6967
2.6823
2.2593
1.8127
1.5631
1.3006
1.0566
0.8947
0.0020
Cl3C
OH
TBDMSO
FYVIII-037C2
lower spot
44
400 MHz
2.33
1.37
1.99
1.00
5.69
4.00
11.00
15.94
16.36
16.36
33.60
12.66
f1 (ppm)

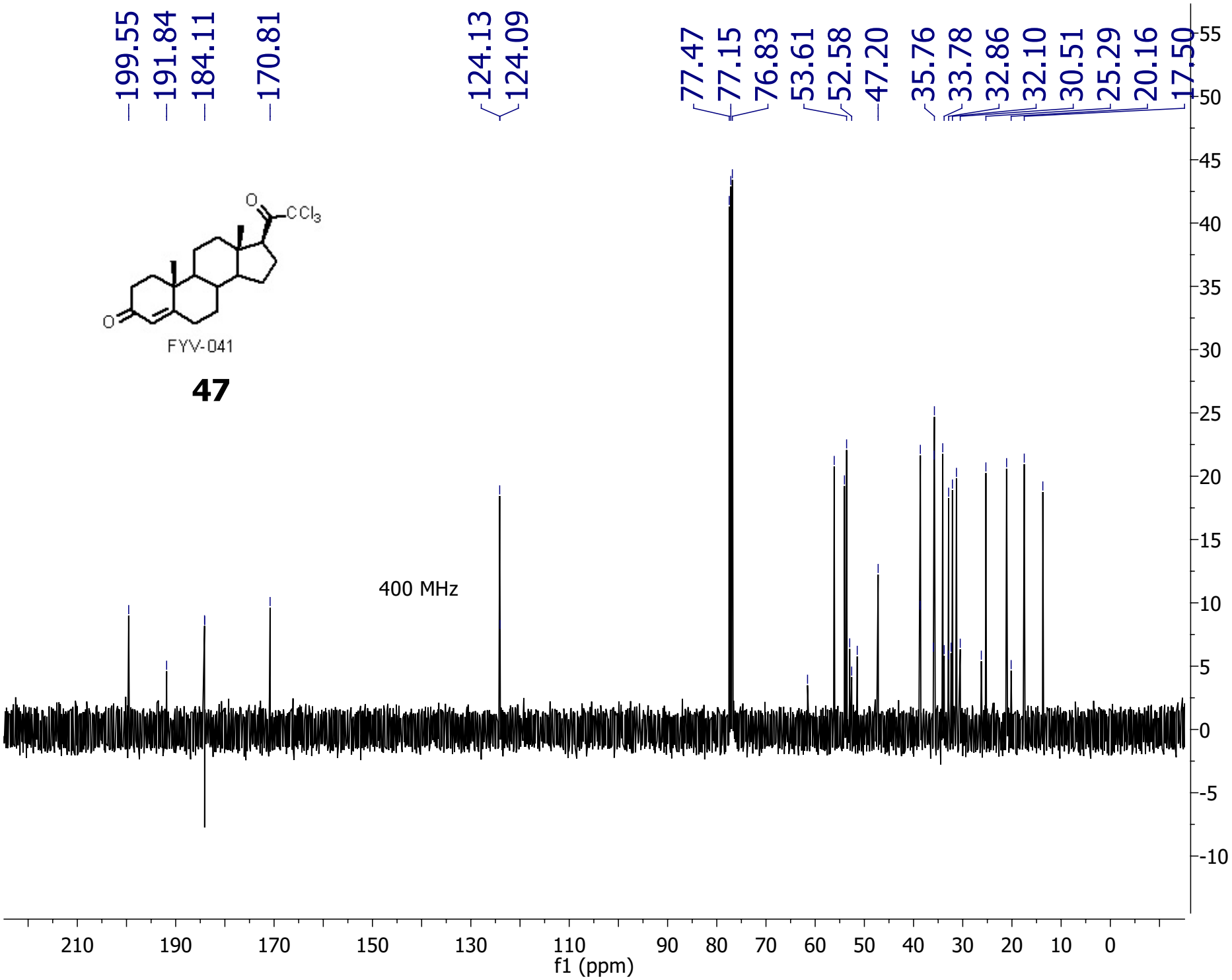

7.27
7.27
7.27
7.27
7.27
7.26
7.26
7.25
7.25
7.23
7.00
5.31
4.27
3.51
3.48
3.45
2.80
1.81
1.55
1.30
1.01
0.63
0.00
-0.01
-1.43
-1.66
Cl3C
OH
TBDMSO
FYVIII-037.B
upper spot
44
400 MHz
1.63
1.60
1.76
1.38
2.39
2.60
3.44
14.53
5.88
4.88
13.19
7.07
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
f1 (ppm)
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100

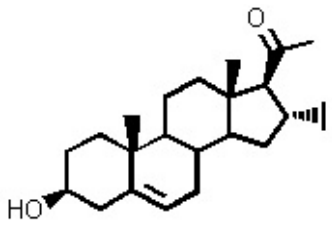

TBDMSO
O
CCl3
FYV-038B
45
400 MHz
5.31
5.30
3.49
3.48
3.47
3.45
3.24
3.22
1.78
1.59
1.49
1.36
1.25
0.99
0.89
0.07
0.06
0.06
-0.00
1.00
1.09
0.54
3.21
1.99
5.51
6.73
5.23
4.23
3.93
11.63
6.31
f1 (ppm)


192.03
184.26
141.79
120.83
77.47
77.15
76.83
38.83
36.75
32.15
31.41
25.47
19.58
13.64
-4.44
O
CCl3
TBDMSO
FYV-038B
45
400 MHz
f1 (ppm)


7.26
5.36
5.30
4.12
4.11
4.09
3.52
3.22
3.19
2.01
1.83
1.53
1.46
1.34
1.25
1.08
0.93
0.06
-0.01
O
CCl3
HO
FYV-039B
46
400 MHz
1.96
2.03
1.00
5.63
6.01
10.32
16.58
8.36
9.71
10.75
f1 (ppm)


7.26
5.73
3.48
3.25
3.22
2.14
1.90
1.65
1.50
1.38
1.25
1.09
0.96
-0.01
O
CCl3
O
FYV-041
47
400 MHz
1.00
0.74
5.78
4.82
5.20
5.62
3.82
3.14
5.24
f1 (ppm)

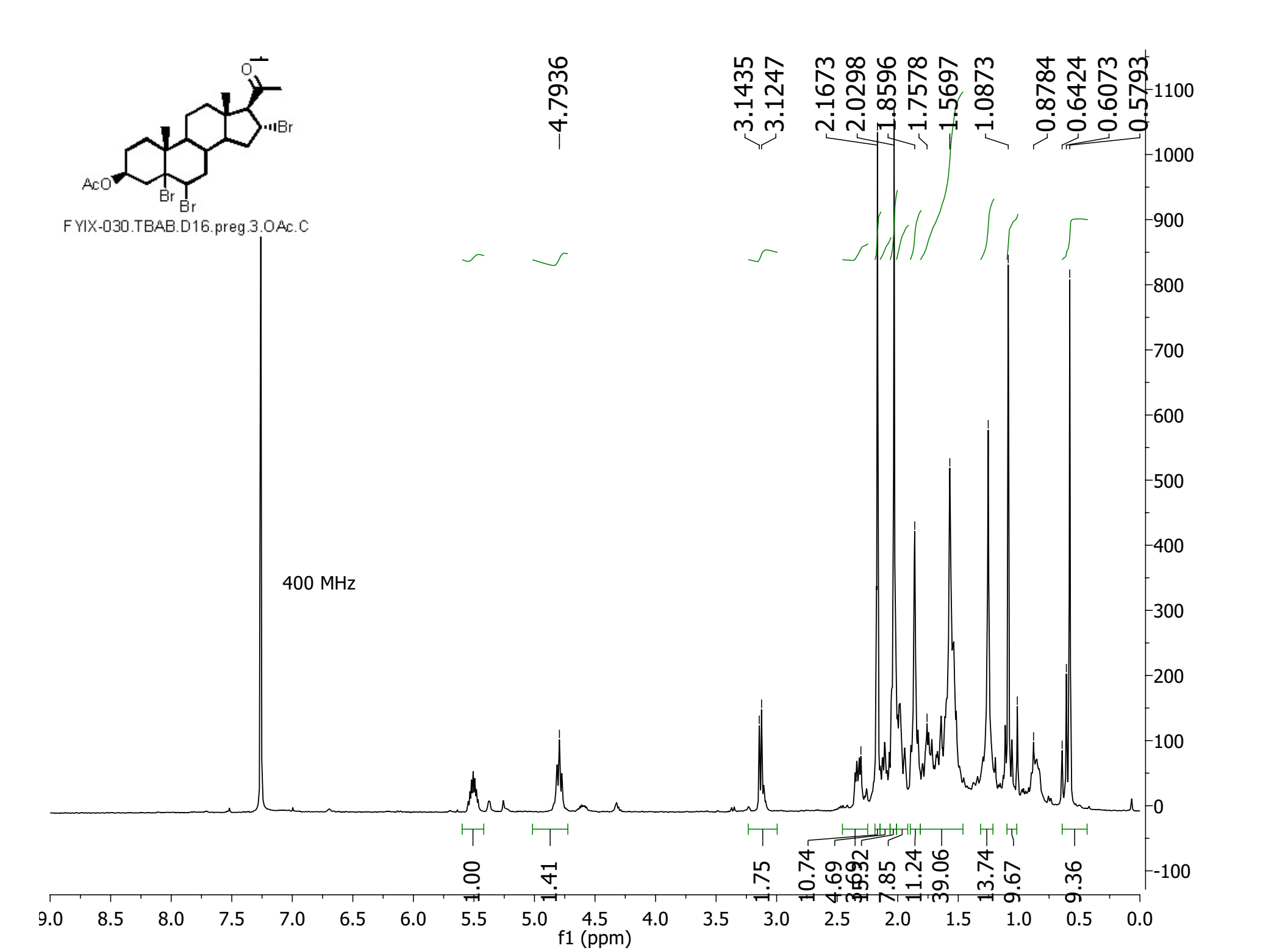

199.55
191.84
184.11
170.81
124.13
124.09
77.47
77.15
76.83
53.61
52.58
47.20
35.76
33.78
32.86
32.10
30.51
25.29
20.16
17.50
O
CCl3
O
FYV-041
47
400 MHz
55
50
45
40
35
30
25
20
15
10
5
0
-5
-10
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

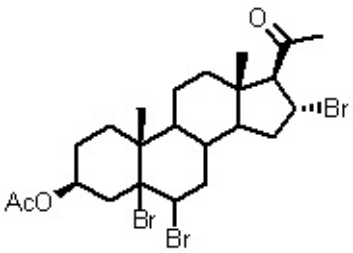


FYVI-062
(mislabeled 16OHpreg.
TBAI with 16-dehydropregnenolone-3-acetate)

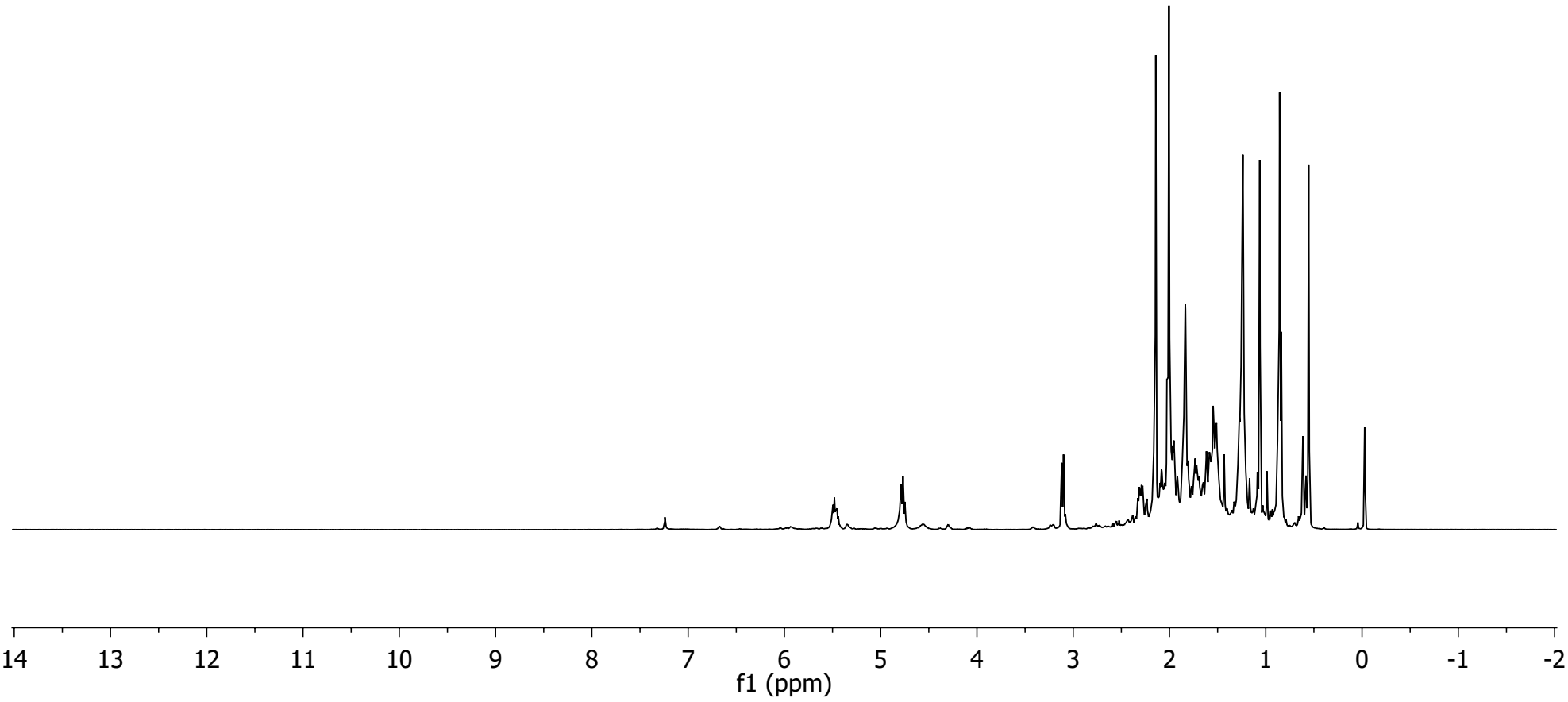
400 MHz

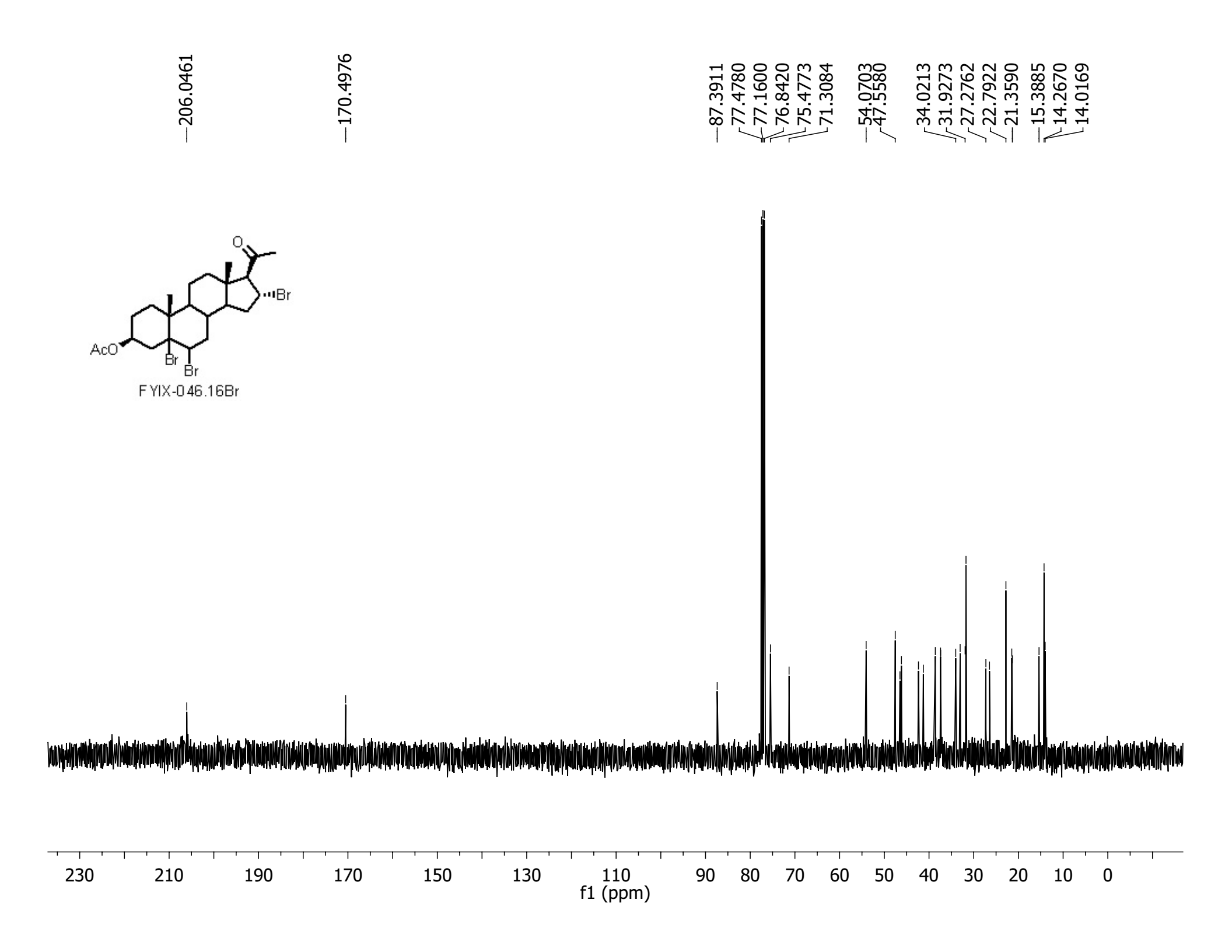
f1 (ppm)

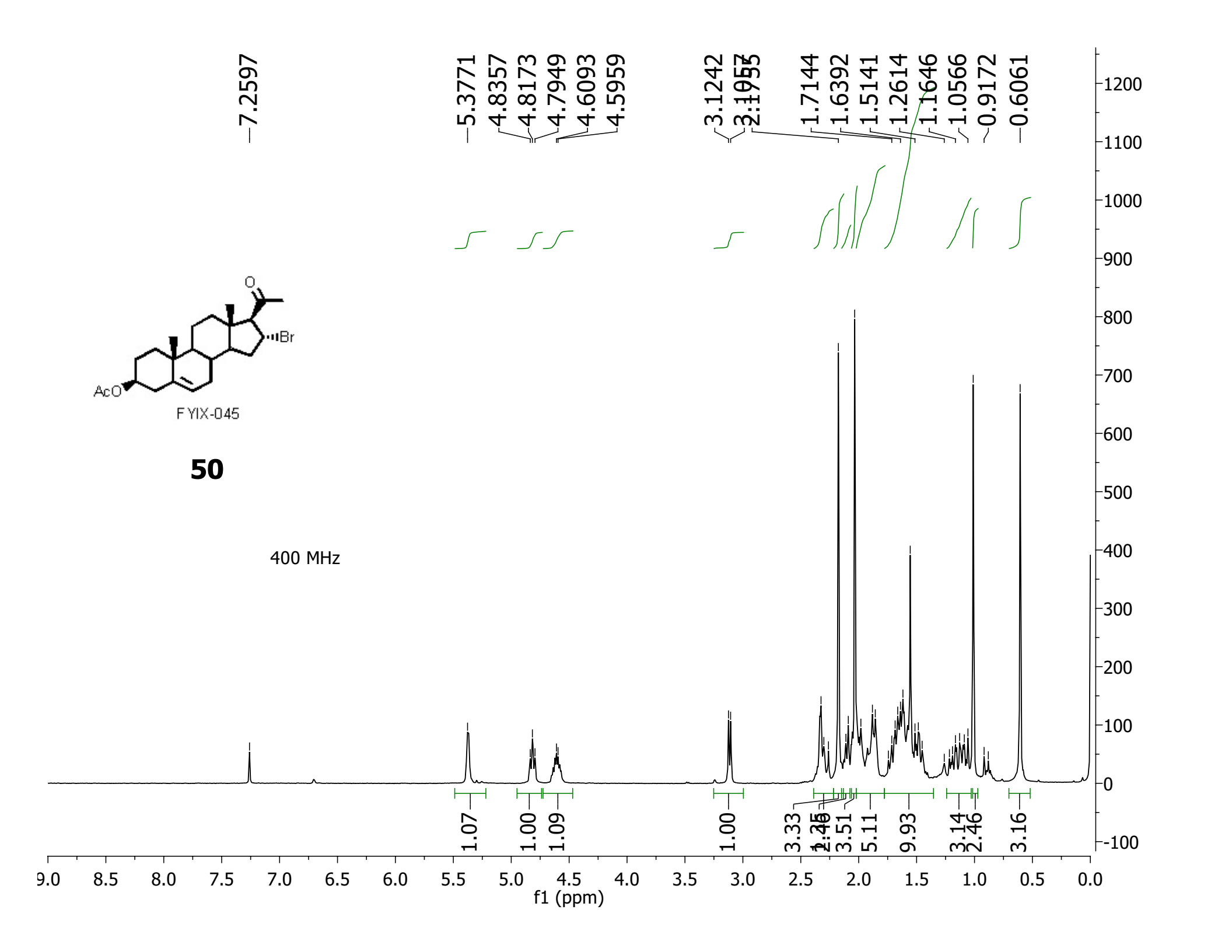

AcO
Br
Br
Br
FYIX-030.TBAB.D16.preg.3.OAc.C
400 MHz
4.7936
3.1435
3.1247
2.1673
2.0298
1.8596
1.7578
1.5697
1.0873
0.8784
0.6424
0.6073
0.5793
1.00
1.41
1.75
10.74
4.69
7.85
11.24
39.06
13.74
9.67
9.36
f1 (ppm)

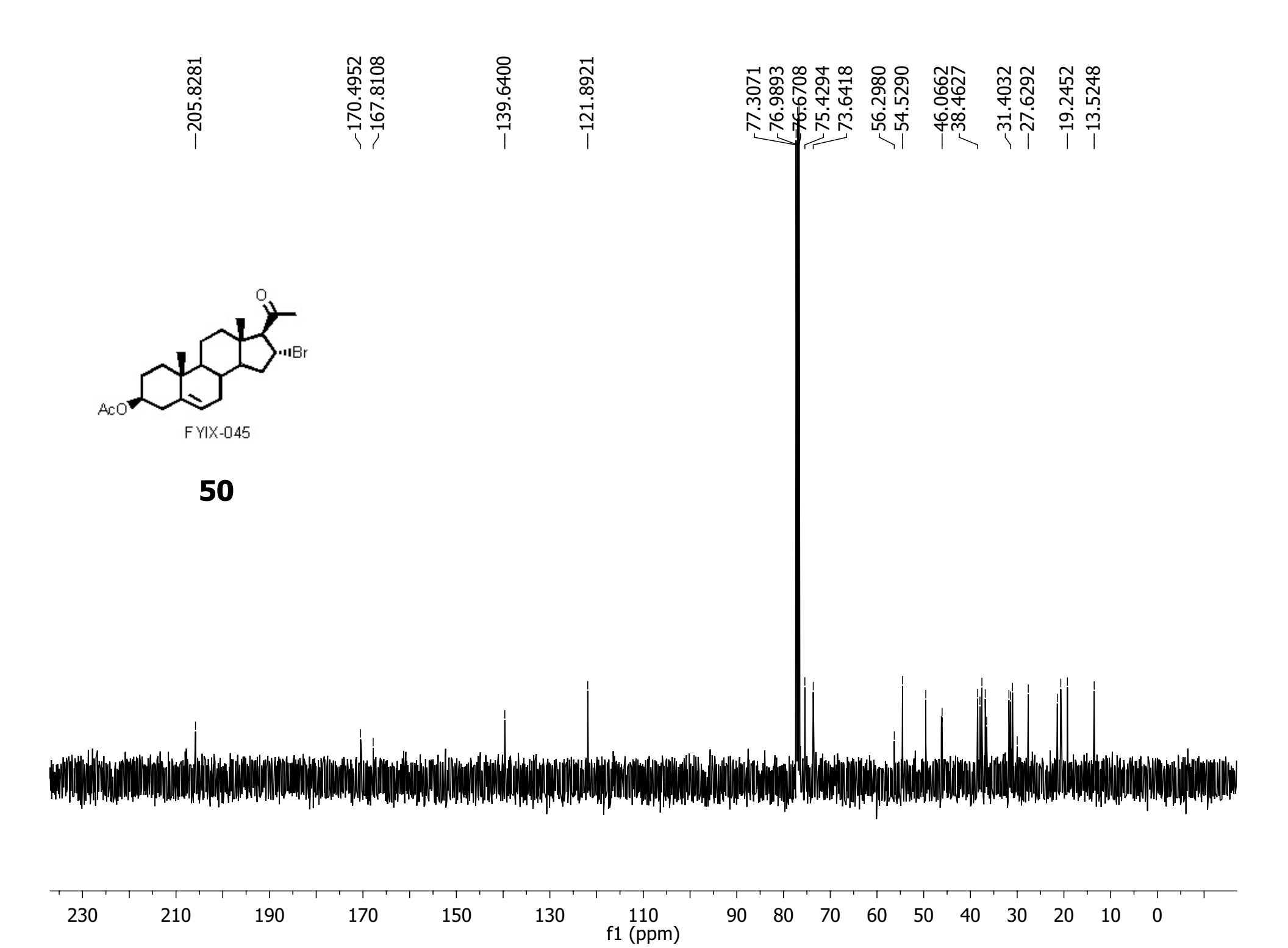


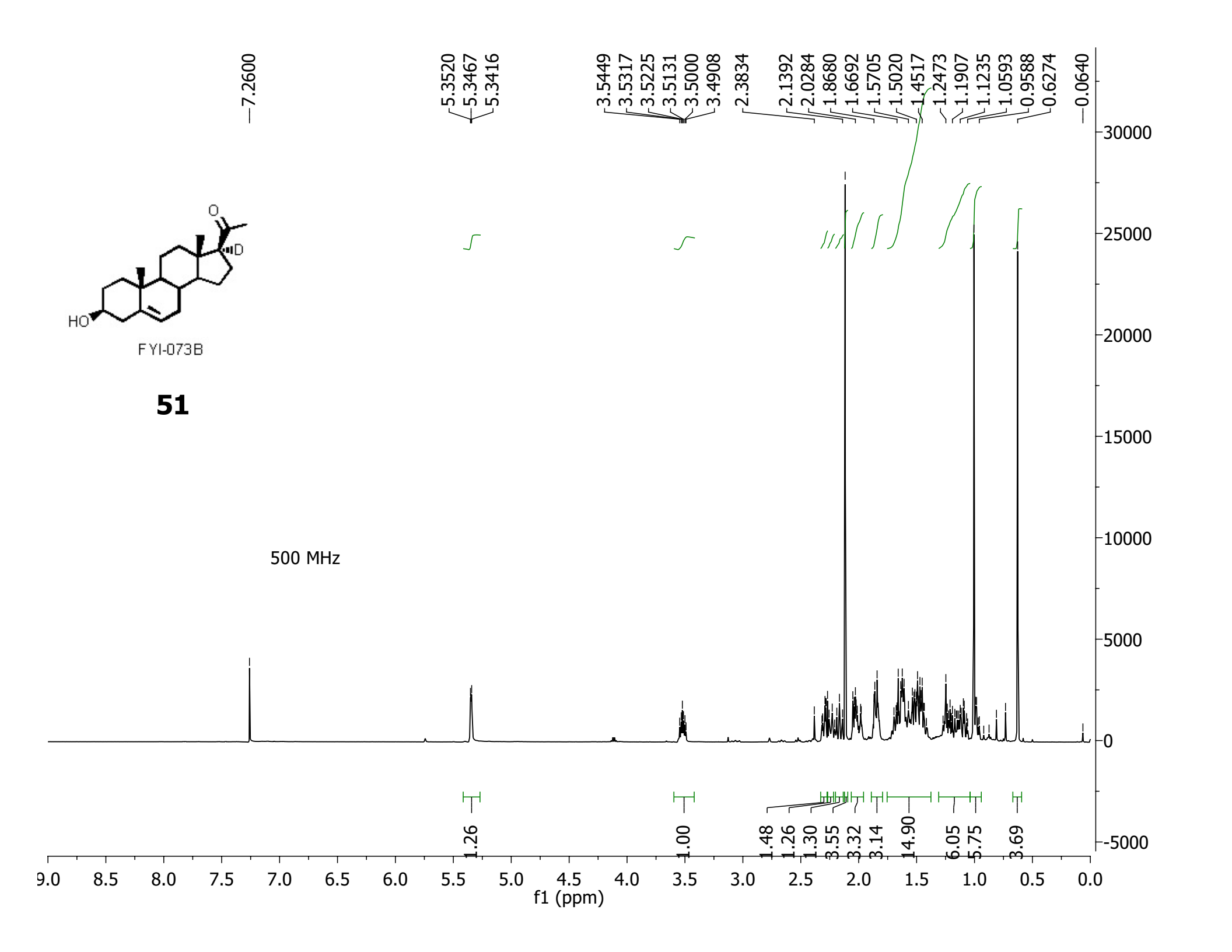
FYIX-046.16Br

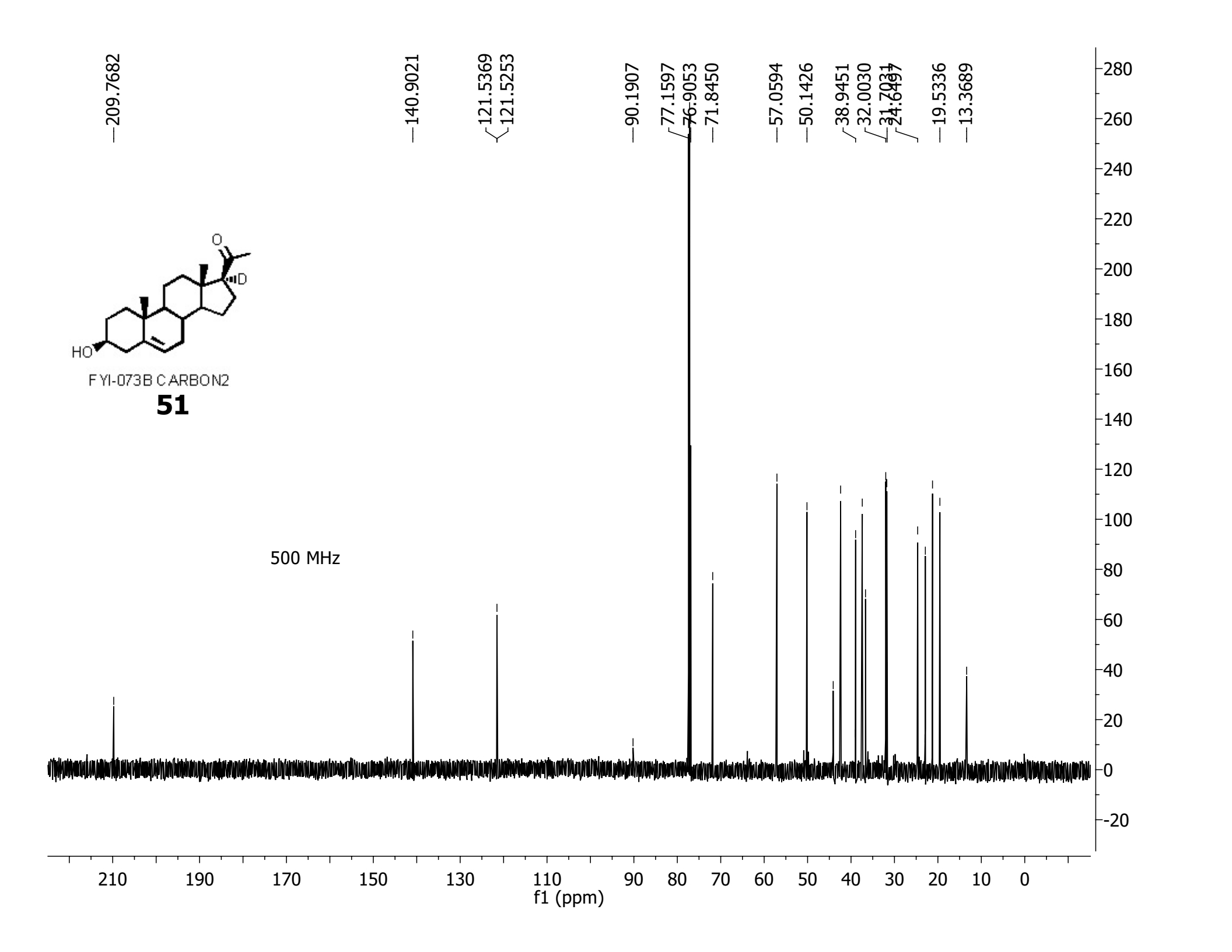


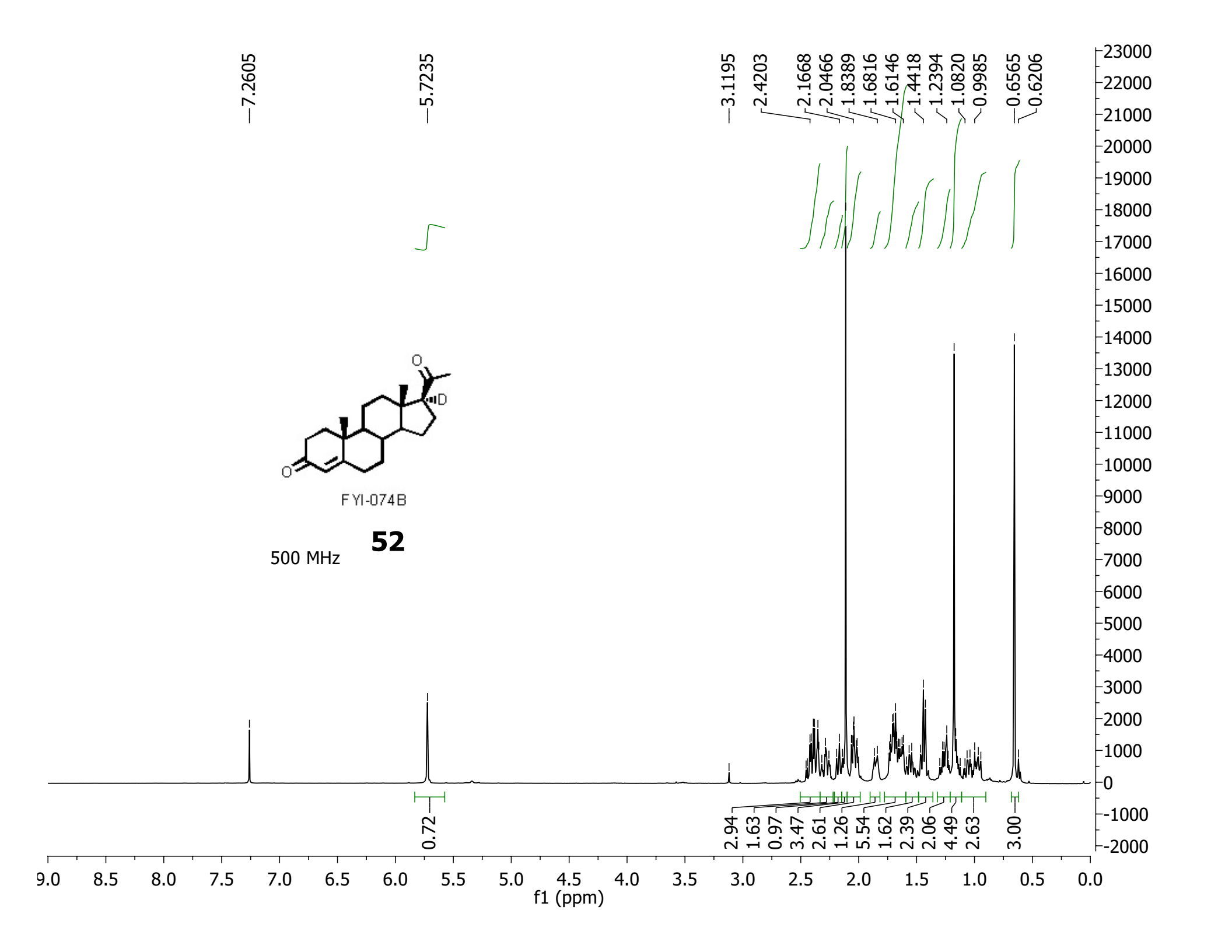

206.0461
170.4976
87.3911
77.4780
77.1600
76.8420
75.4773
71.3084
54.0703
47.5580
34.0213
31.9273
27.2762
22.7922
21.3590
15.3885
14.2670
14.0169
O
Br
AcO
Br
Br
FYIX-046.16Br
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

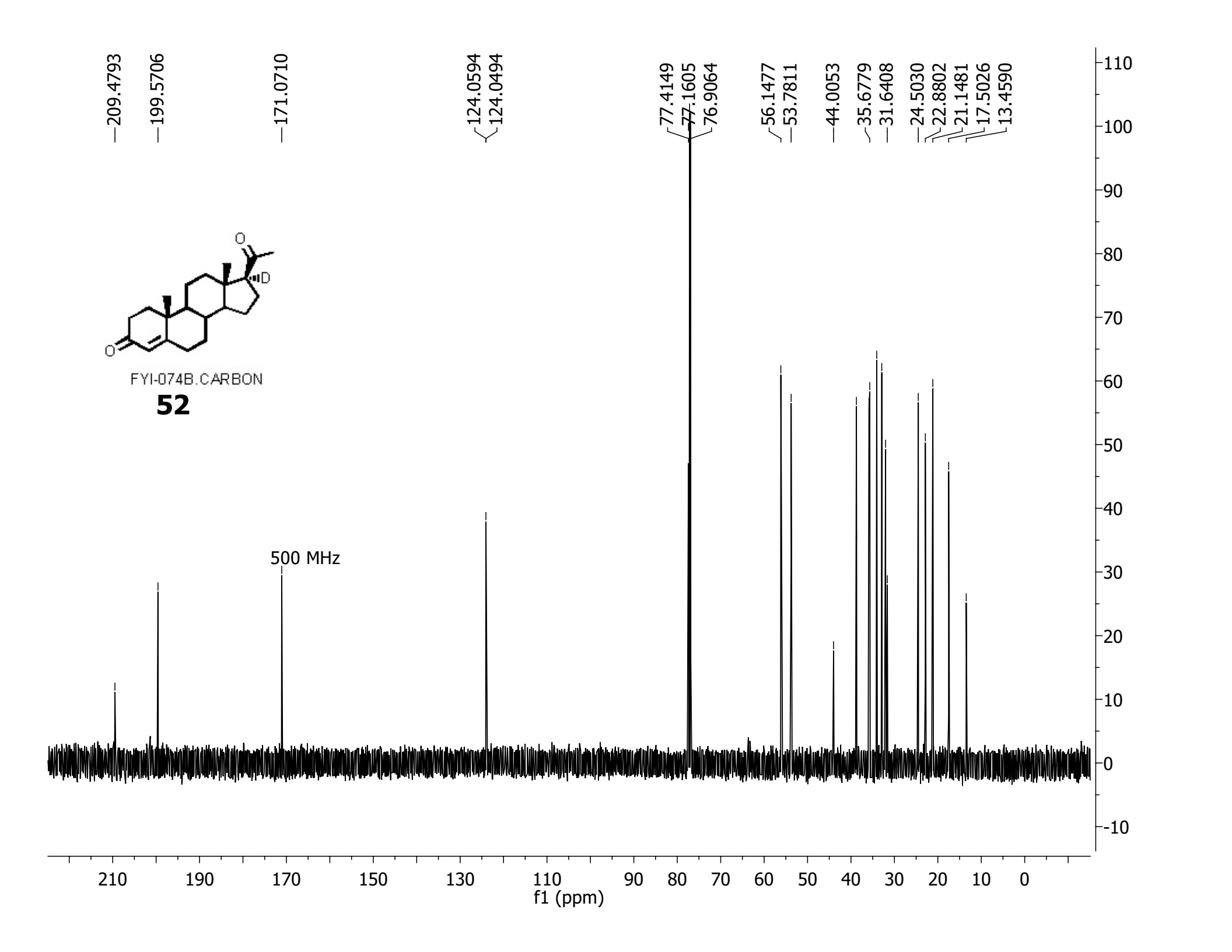

7.2597
5.3771
4.8357
4.8173
4.7949
4.6093
4.5959
3.1242
1.7144
1.6392
1.5141
1.2614
1.1646
1.0566
0.9172
0.6061
O
Br
AcO
FYIX-045
50
400 MHz
1.07
1.00
1.09
1.00
3.33
3.51
5.11
9.93
3.14
2.46
3.16
f1 (ppm)

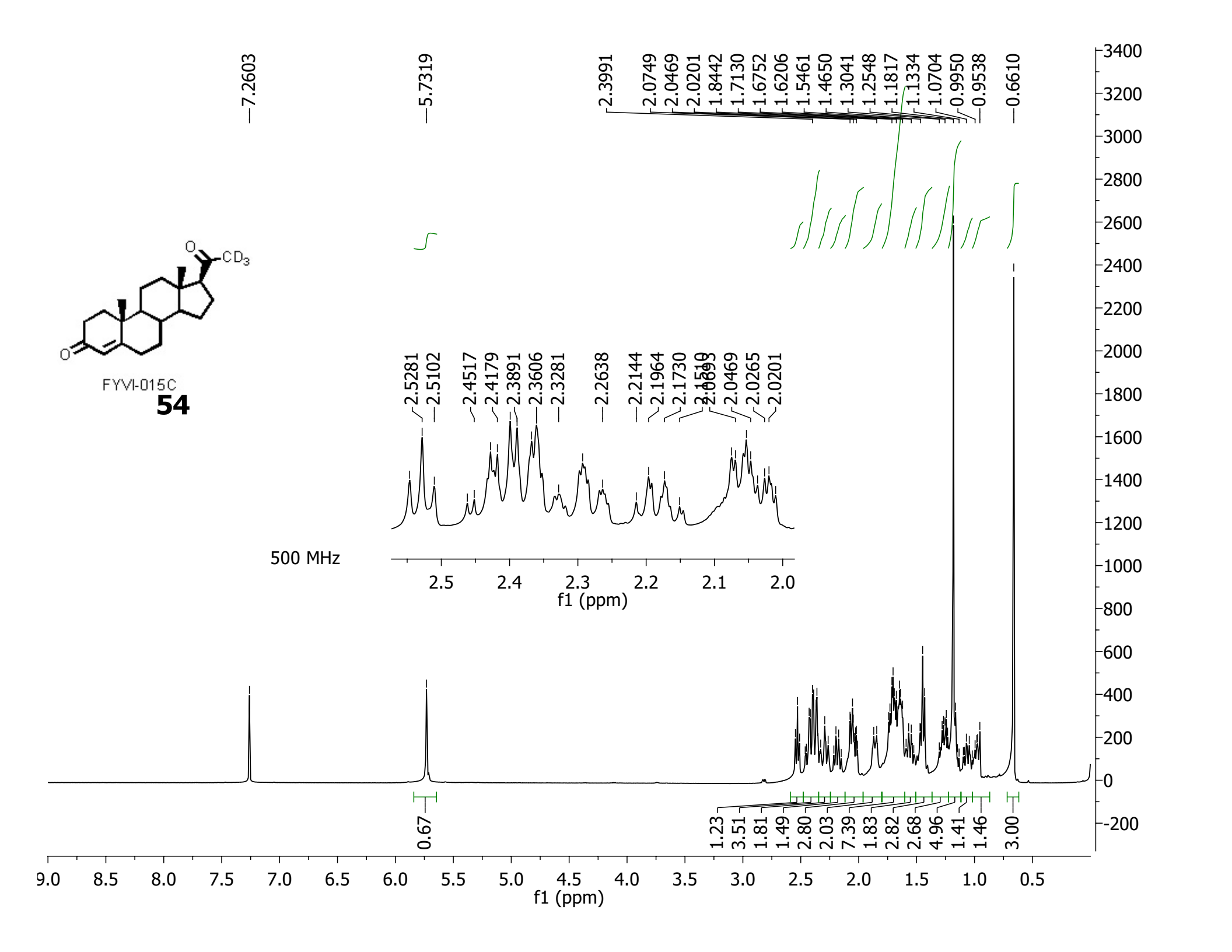

205.8281
170.4952
167.8108
139.6400
121.8921
77.3071
76.9893
76.6708
75.4294
73.6418
56.2980
54.5290
46.0662
38.4627
31.4032
27.6292
19.2452
13.5248
O
Br
AcO
FYIX-045
50
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

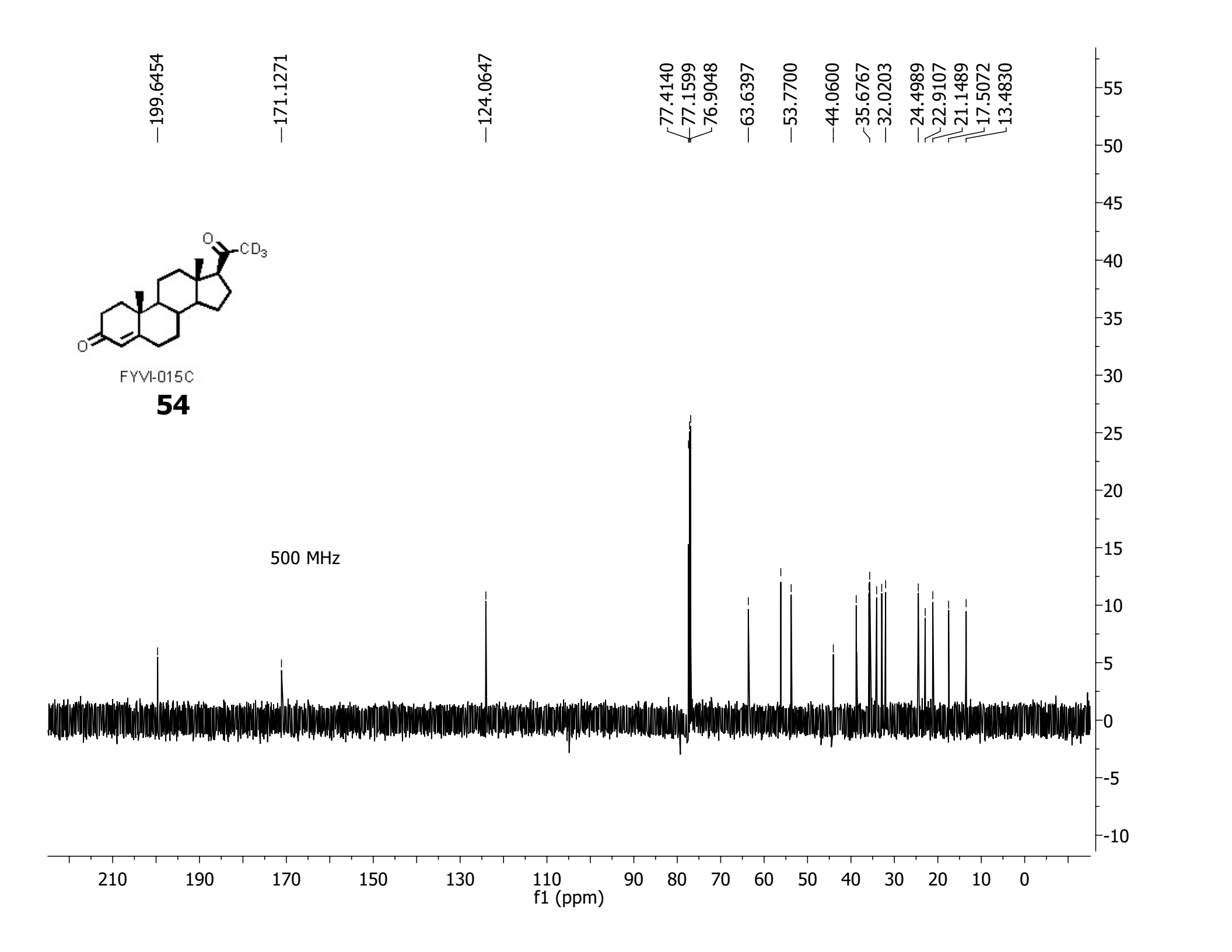

7.2600
5.3520
5.3467
5.3416
3.5449
3.5317
3.5225
3.5131
3.5000
3.4908
2.3834
2.1392
2.0284
1.8680
1.6692
1.5705
1.5020
1.4517
1.2473
1.1907
1.1235
1.0593
0.9588
0.6274
0.0640
O
D
HO
FYI-073B
51
500 MHz
1.26
1.00
1.48
1.26
1.30
3.55
3.32
3.14
14.90
6.05
5.75
3.69
30000
25000
20000
15000
10000
5000
0
-5000
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

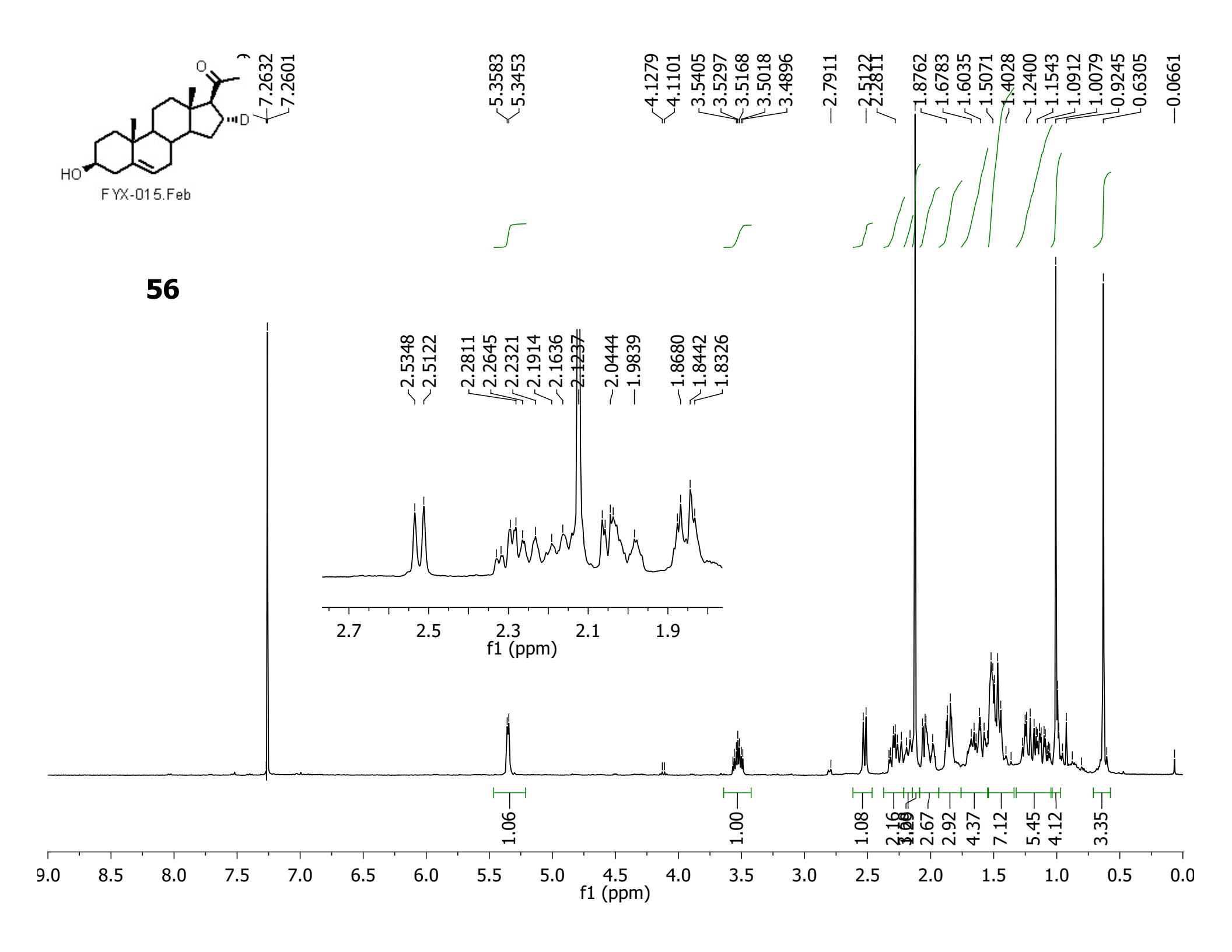

209.7682
140.9021
121.5369
121.5253
90.1907
77.1597
76.9053
71.8450
57.0594
50.1426
38.9451
32.0030
31.7031
24.6497
19.5336
13.3689
O
D
HO
FYI-073B CARBON2
51
500 MHz
f1 (ppm)

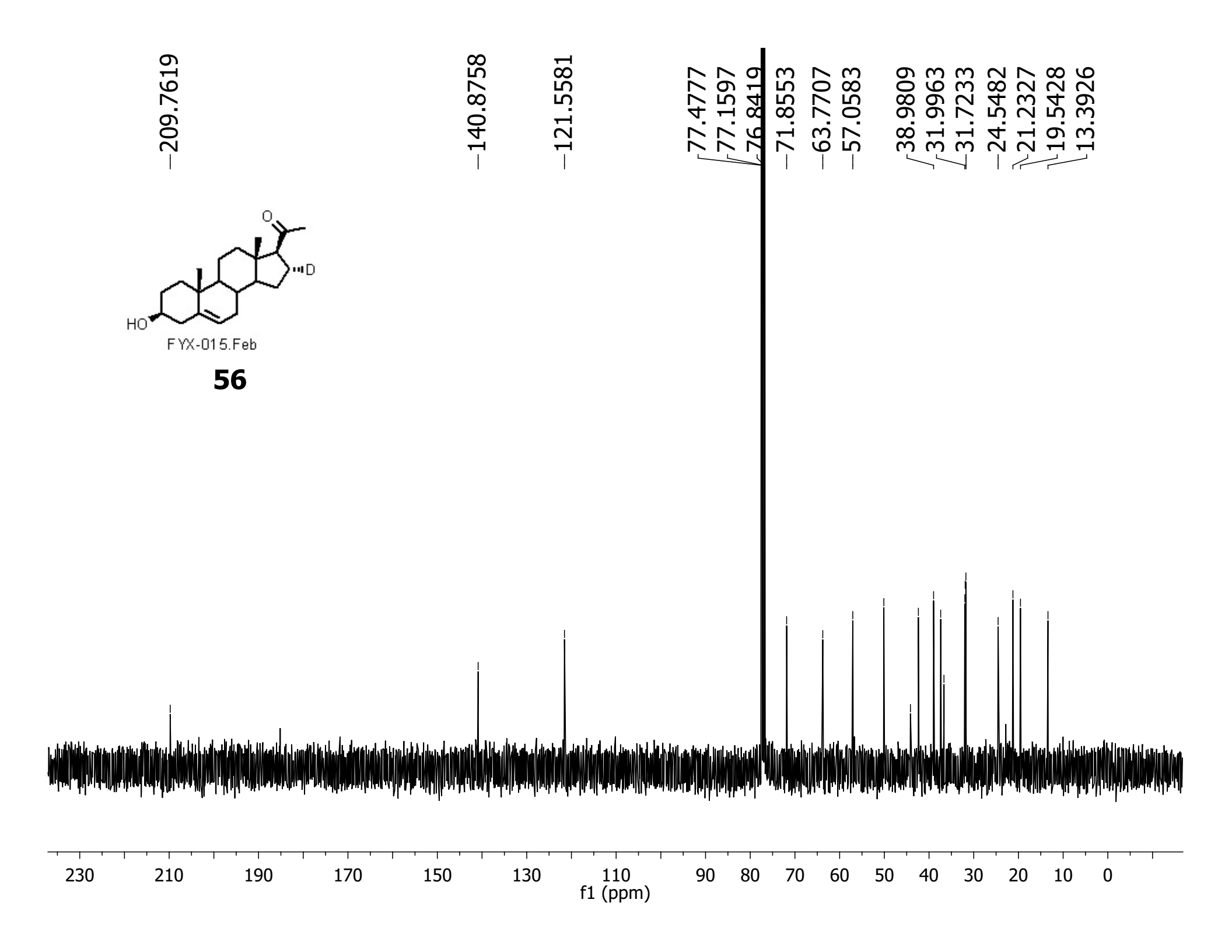

7.2605
5.7235
3.1195
2.4203
2.1668
2.0466
1.8389
1.6816
1.6146
1.4418
1.2394
1.0820
0.9985
0.6565
0.6206
O
O
D
FYI-074B
52
500 MHz
0.72
2.94
1.63
0.97
3.47
2.61
1.26
5.54
1.62
2.39
2.06
4.49
2.63
3.00
f1 (ppm)

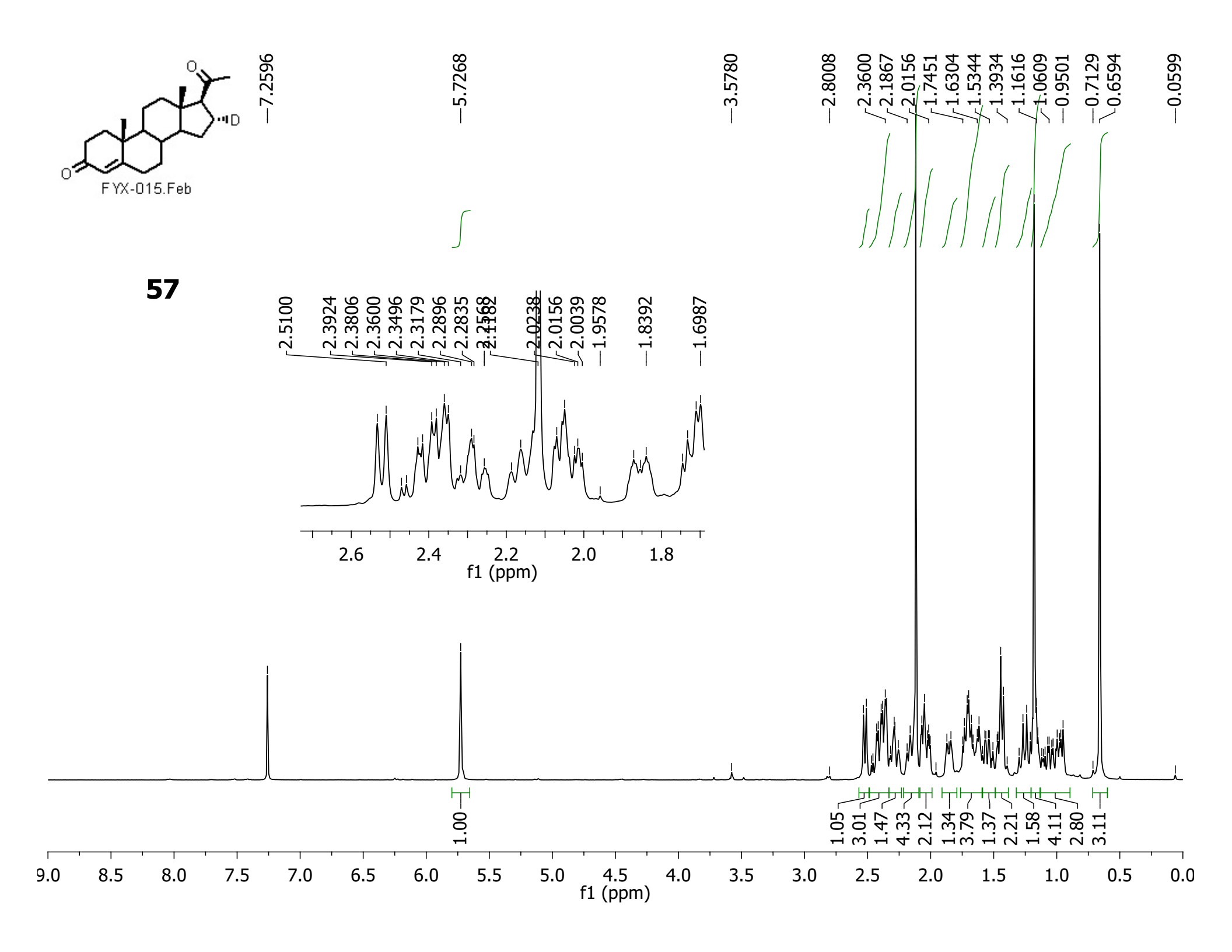

209.4793
199.5706
171.0710
124.0594
124.0494
77.4149
77.1605
76.9064
56.1477
53.7811
44.0053
35.6779
31.6408
24.5030
22.8802
21.1481
17.5026
13.4590
O
O
D
FYI-074B.CARBON
52
500 MHz
f1 (ppm)

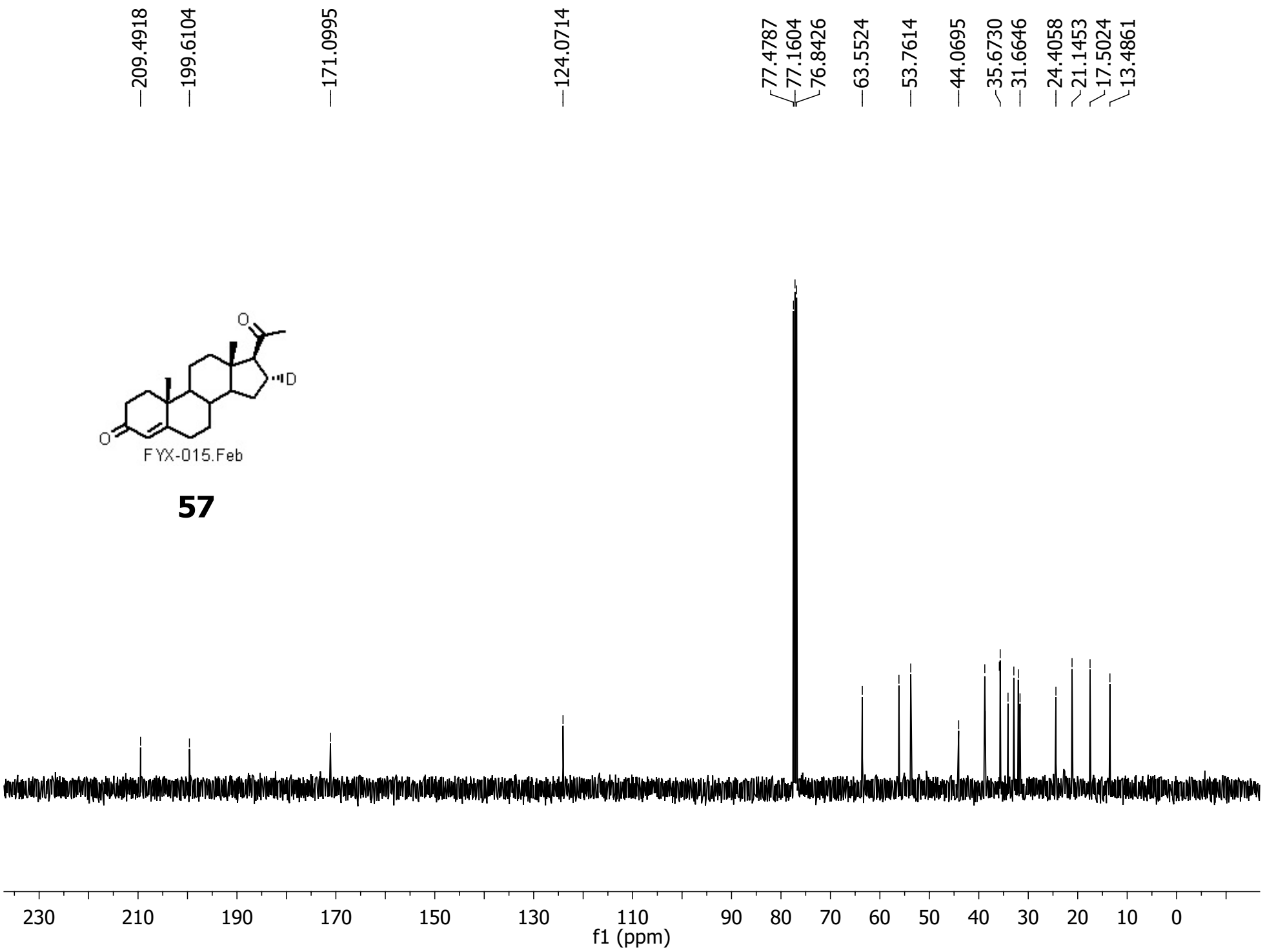

7.2603
5.7319
2.3991
2.0749
2.0469
2.0201
1.8442
1.7130
1.6752
1.6206
1.5461
1.4650
1.3041
1.2548
1.1817
1.1334
1.0704
0.9950
0.9538
0.6610
O
CD3
O
FYVI-015C
54
2.5281
2.5102
2.4517
2.4179
2.3891
2.3606
2.3281
2.2638
2.2144
2.1964
2.1730
2.0469
2.0265
2.0201
500 MHz
2.5
2.4
2.3
2.2
2.1
2.0
f1 (ppm)
0.67
1.23
3.51
1.81
1.49
2.80
2.03
7.39
1.83
2.82
2.68
4.96
1.41
1.46
3.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200


199.6454
171.1271
124.0647
77.4140
77.1599
76.9048
63.6397
53.7700
44.0600
35.6767
32.0203
24.4989
22.9107
21.1489
17.5072
13.4830
O
CD3
O
FYVI-015C
54
500 MHz
f1 (ppm)

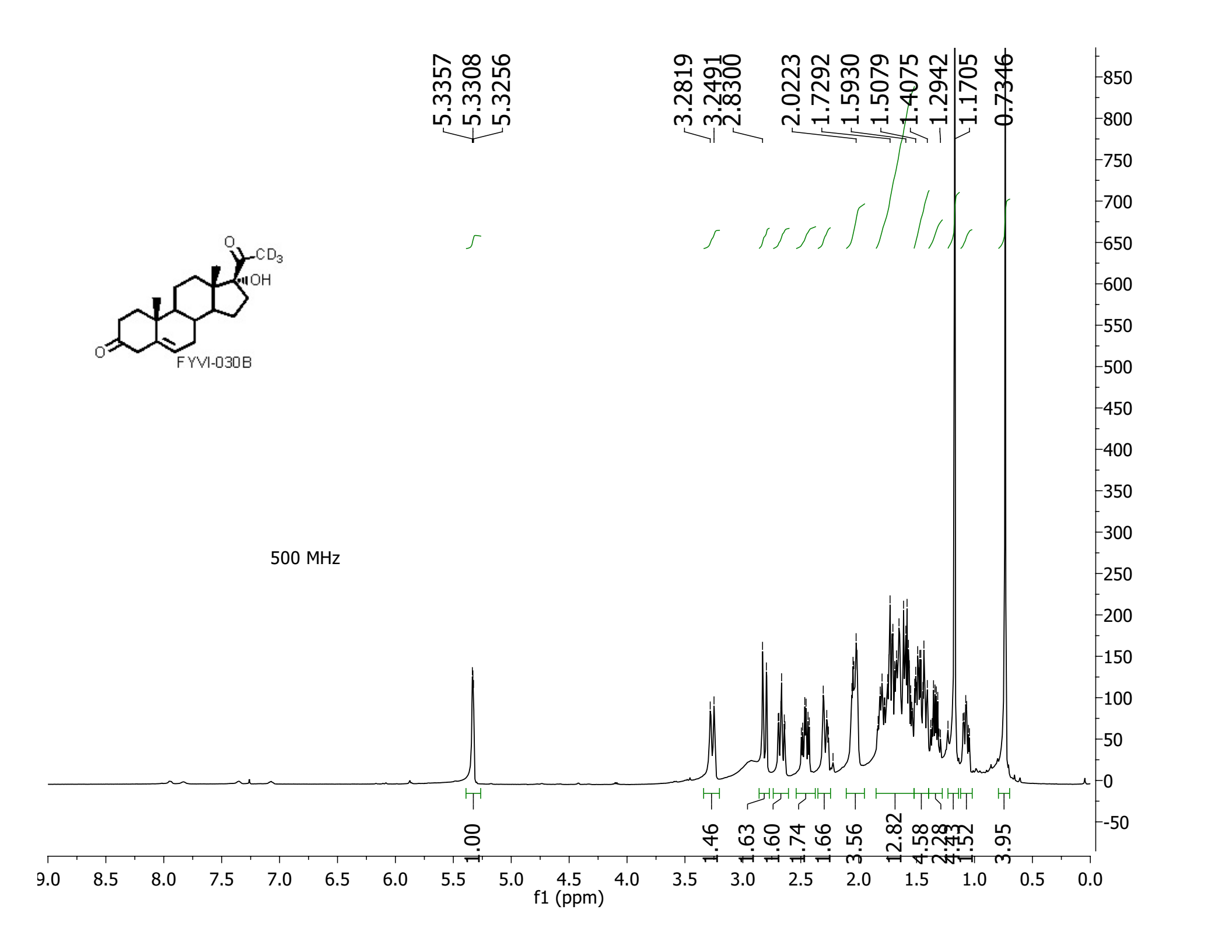

FYX-015.Feb
56
7.2632
7.2601
5.3583
5.3453
4.1279
4.1101
3.5405
3.5297
3.5168
3.5018
3.4896
2.7911
2.5122
2.2811
1.8762
1.6783
1.6035
1.5071
1.4028
1.2400
1.1543
1.0912
1.0079
0.9245
0.6305
0.0661
2.5348
2.5122
2.2811
2.2645
2.2321
2.1914
2.1636
2.1237
2.0444
1.9839
1.8680
1.8442
1.8326
2.7
2.5
2.3
2.1
1.9
f1 (ppm)
1.06
1.00
1.08
2.67
2.92
4.37
7.12
5.45
4.12
3.35
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

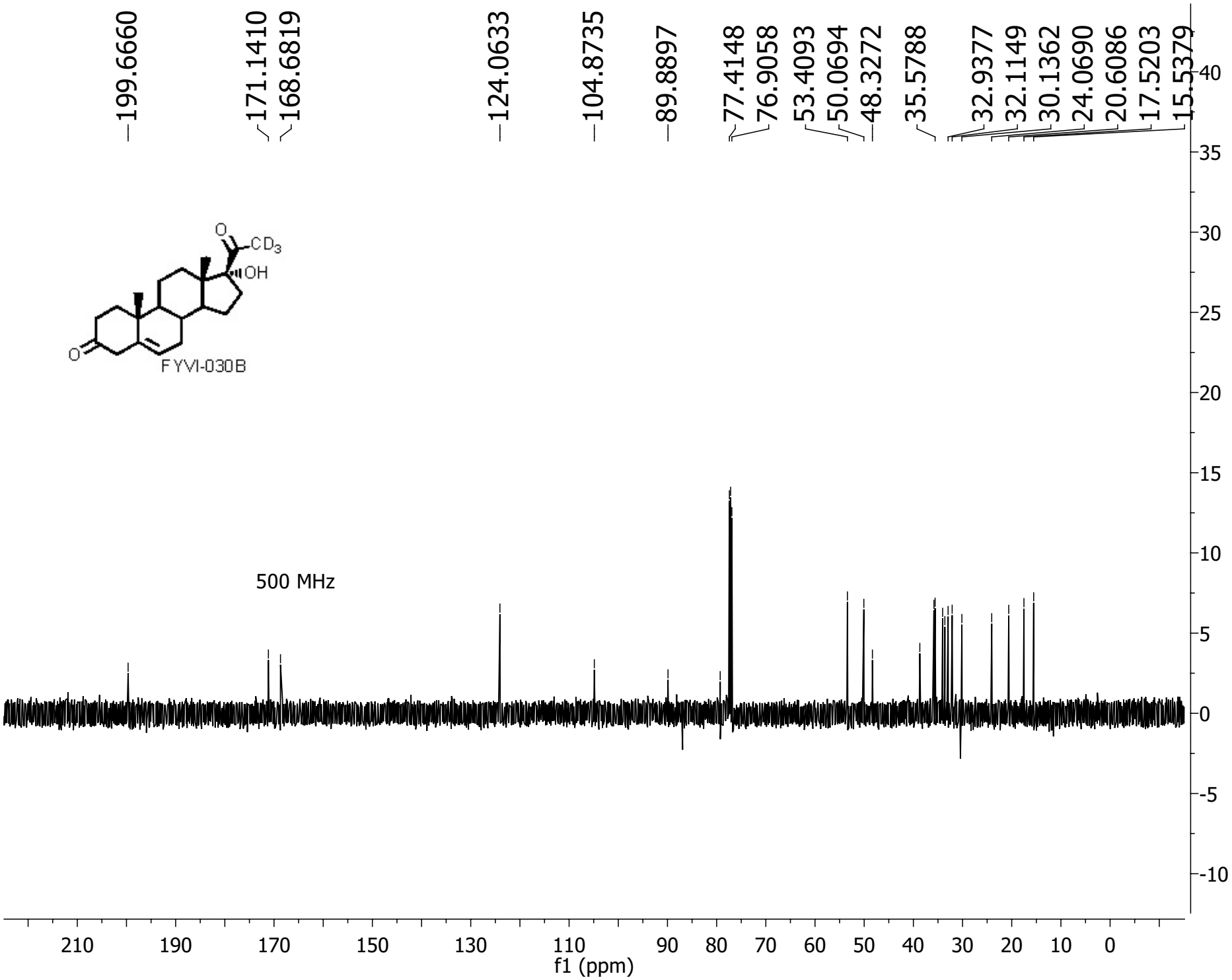

209.7619
140.8758
121.5581
77.4777
77.1597
76.8419
71.8553
63.7707
57.0583
38.9809
31.9963
31.7233
24.5482
21.2327
19.5428
13.3926
O
D
HO
FYX-015.Feb
56
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

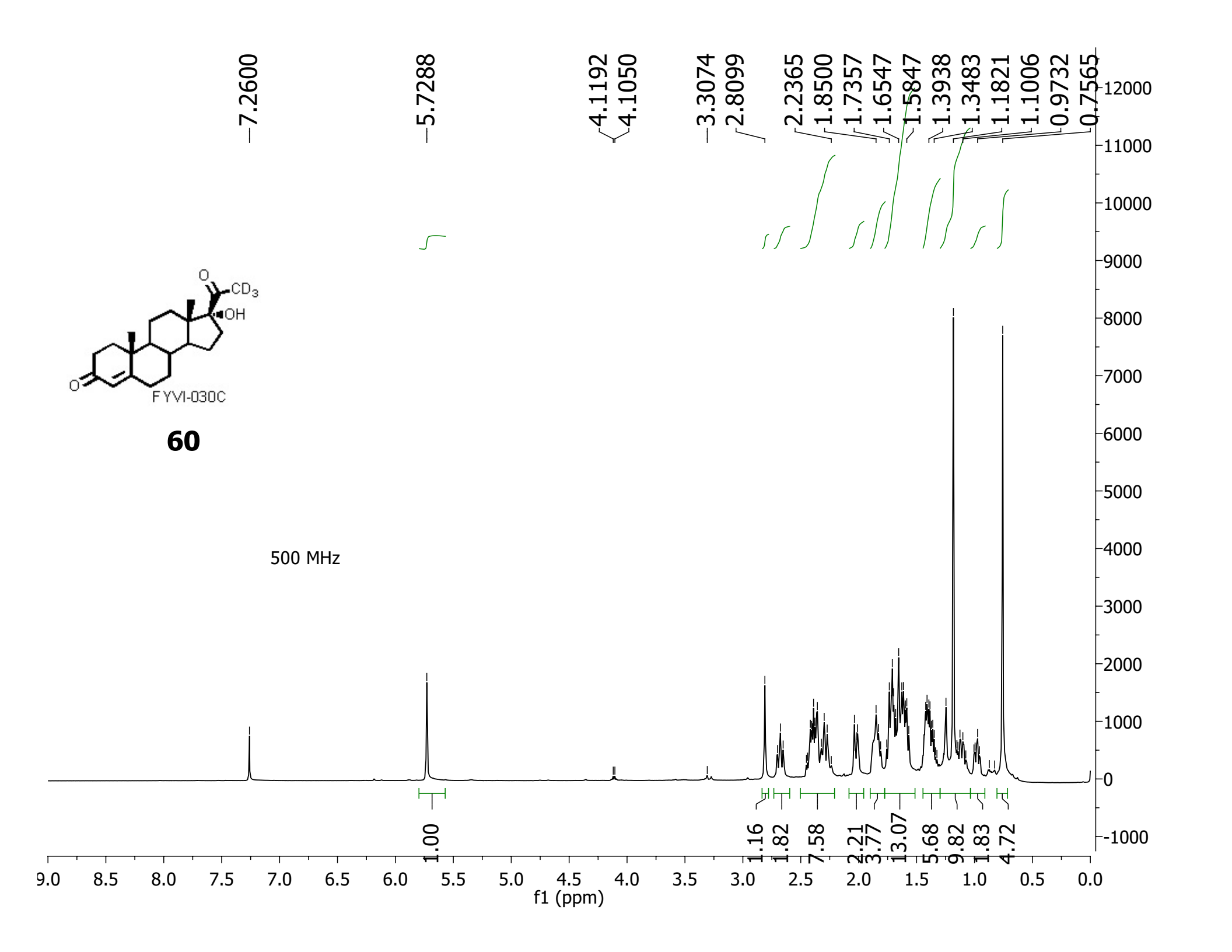

FYX-015.Feb
57
7.2596
5.7268
3.5780
2.8008
2.3600
2.1867
2.0156
1.7451
1.6304
1.5344
1.3934
1.1616
1.0609
0.9501
0.7129
0.6594
0.0599
2.5100
2.3924
2.3806
2.3600
2.3496
2.3179
2.2896
2.2835
2.0238
2.0156
2.0039
1.9578
1.8392
1.6987
f1 (ppm)
1.00
1.05
3.01
1.47
4.33
2.12
1.34
3.79
1.37
2.21
1.58
4.11
2.80
3.11
f1 (ppm)

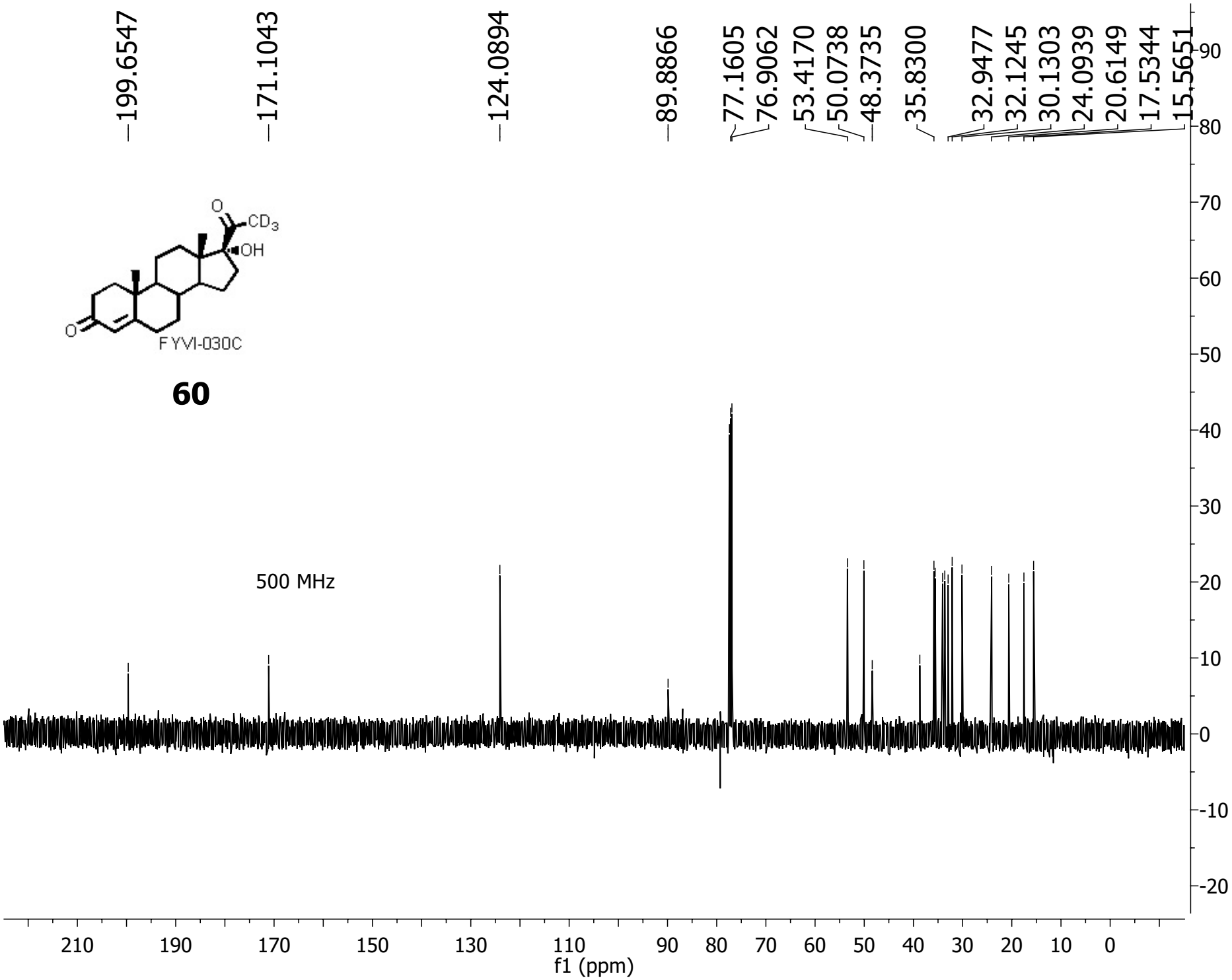

209.4918
199.6104
171.0995
124.0714
77.4787
77.1604
76.8426
63.5524
53.7614
44.0695
35.6730
31.6646
24.4058
21.1453
17.5024
13.4861
O
O
D
FYX-015.Feb
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

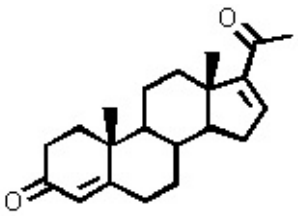
57


5.3357
5.3308
5.3256
3.2819
3.2491
2.8300
2.0223
1.7292
1.5930
1.5079
1.4075
1.2942
1.1705
0.7346
O
CD3
OH
O
FYVI-030B
500 MHz
1.00
1.46
1.63
1.60
1.74
1.66
3.56
12.82
4.58
2.28
4.43
1.52
3.95
f1 (ppm)


199.6660
171.1410
168.6819
124.0633
104.8735
89.8897
77.4148
76.9058
53.4093
50.0694
48.3272
35.5788
32.9377
32.1149
30.1362
24.0690
20.6086
17.5203
15.5379
O
CD3
OH
O
FYVI-030B
500 MHz
40
35
30
25
20
15
10
5
0
-5
-10
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


7.2600
5.7288
4.1192
4.1050
3.3074
2.8099
2.2365
1.8500
1.7357
1.6547
1.5847
1.3938
1.3483
1.1821
1.1006
0.9732
0.7565
O
CD3
OH
O
FYVI-030C
60
500 MHz
1.00
1.16
1.82
7.58
2.21
3.77
13.07
5.68
9.82
1.83
4.72
f1 (ppm)


199.6547
171.1043
124.0894
89.8866
77.1605
76.9062
53.4170
50.0738
48.3735
35.8300
32.9477
32.1245
30.1303
24.0939
20.6149
17.5344
15.5651
O
CD3
OH
O
FYVI-030C
60
500 MHz
f1 (ppm)

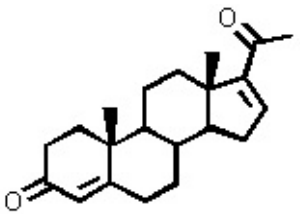


FYVII-044C 16.17dehydroprog

61

500 MHz

f1 (ppm)

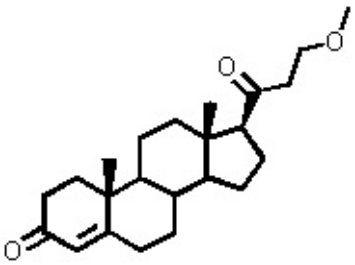


FYVII-044C 16.17dehydroprog

61

500 MHz

f1 (ppm)



11.05.2010.22OMe.prog

63

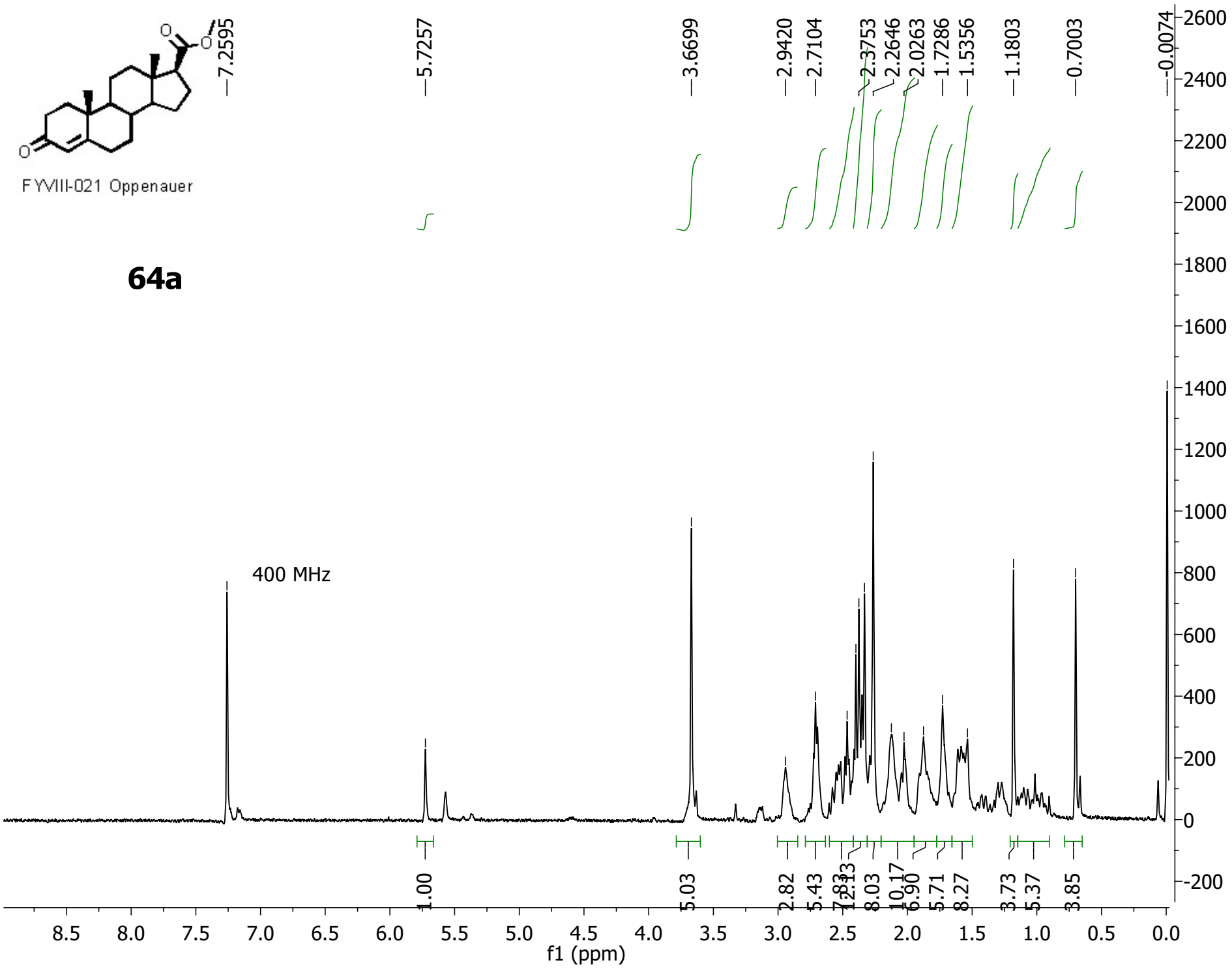
500 MHz

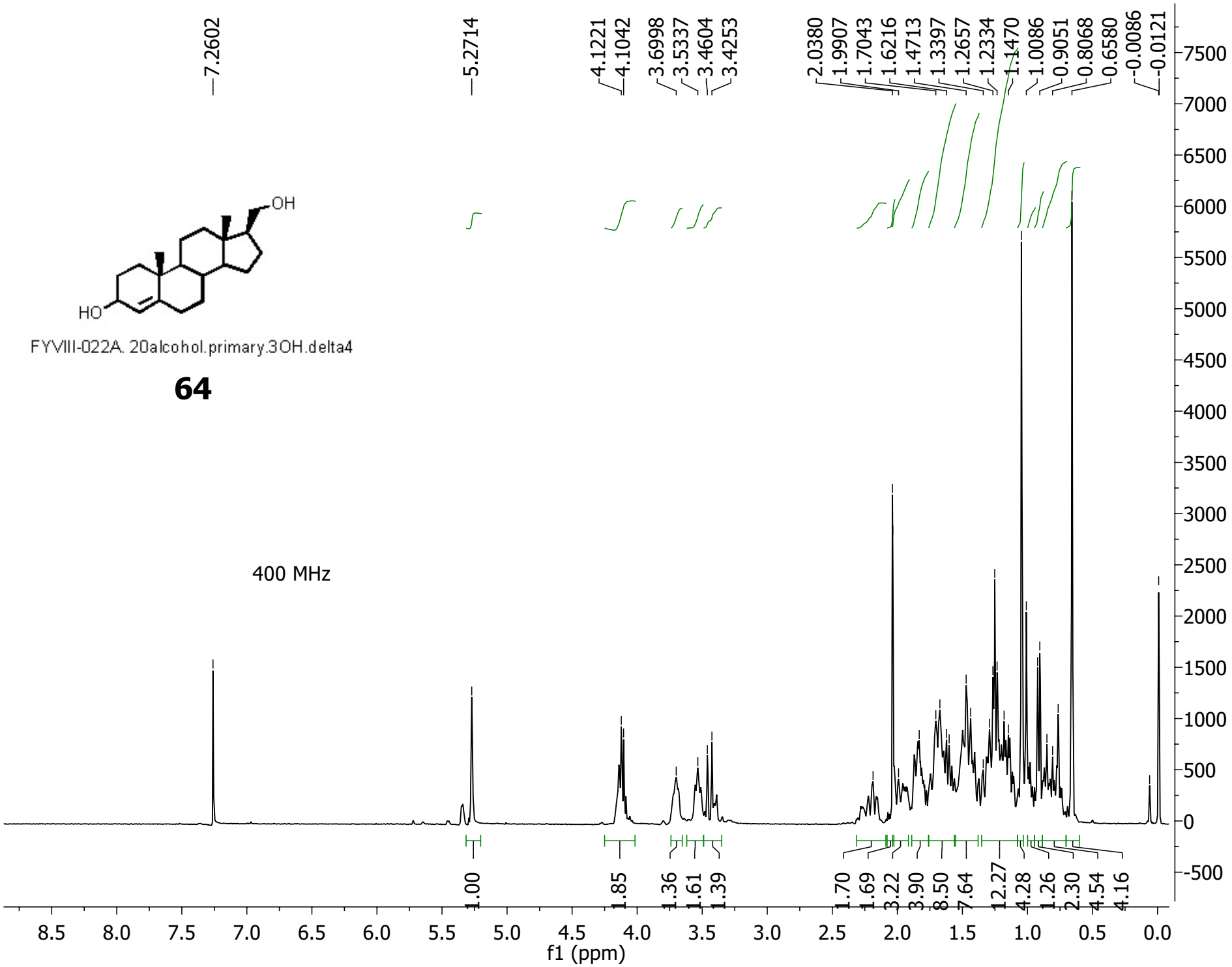
1.00 3.32 4.46 1.73 2.31 10.10 2.40 25.19 5.90 1.68 5.73

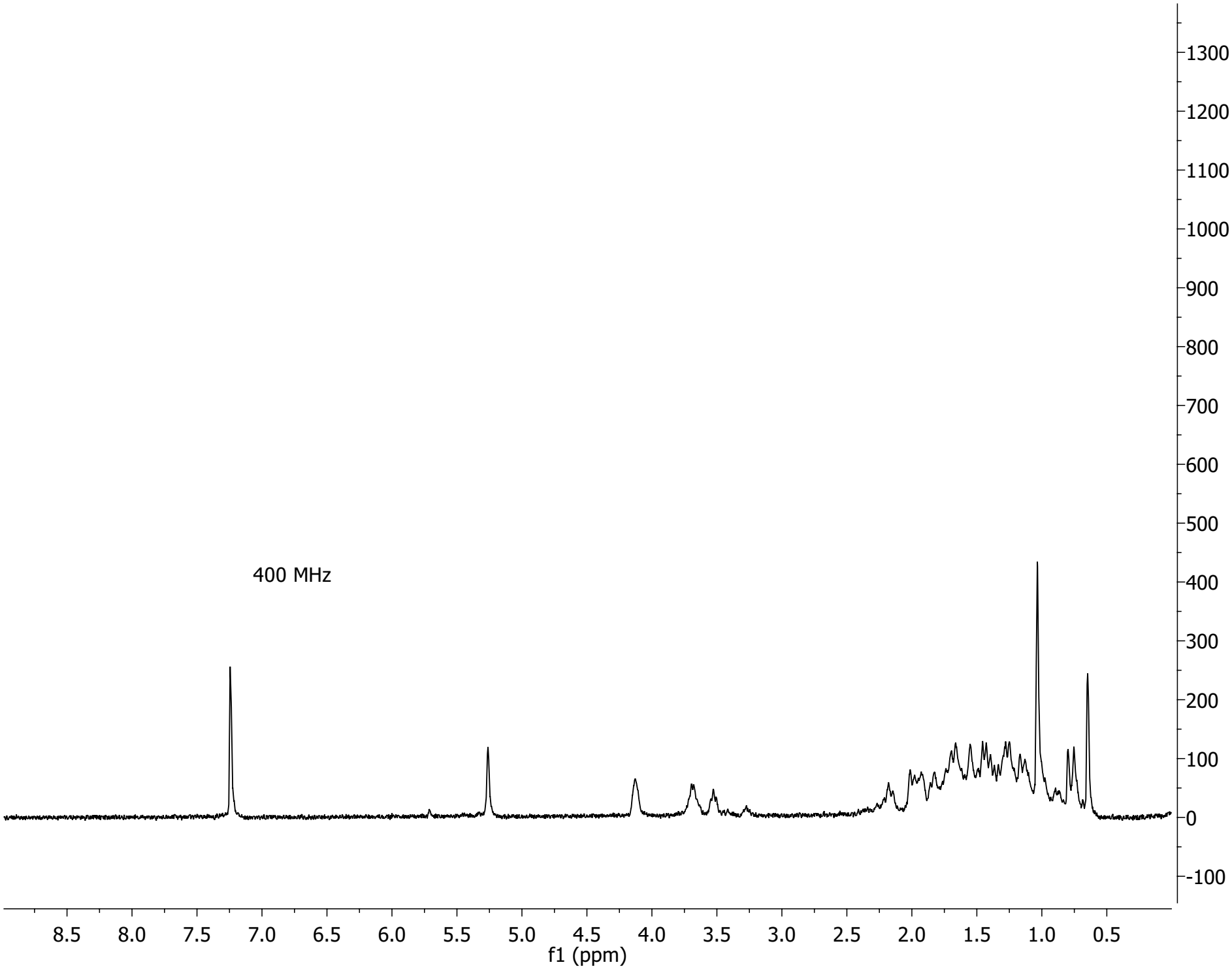
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

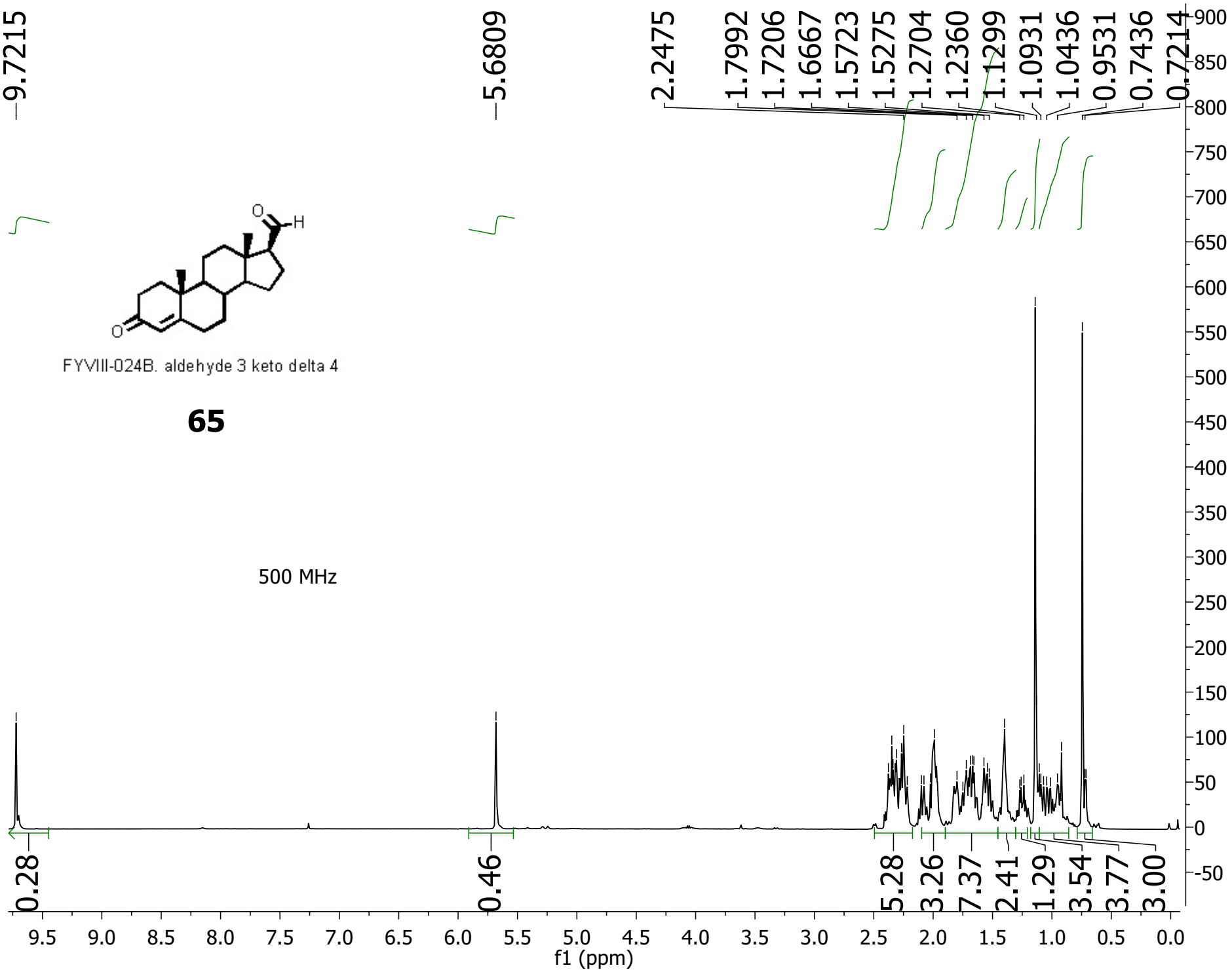
f1 (ppm)

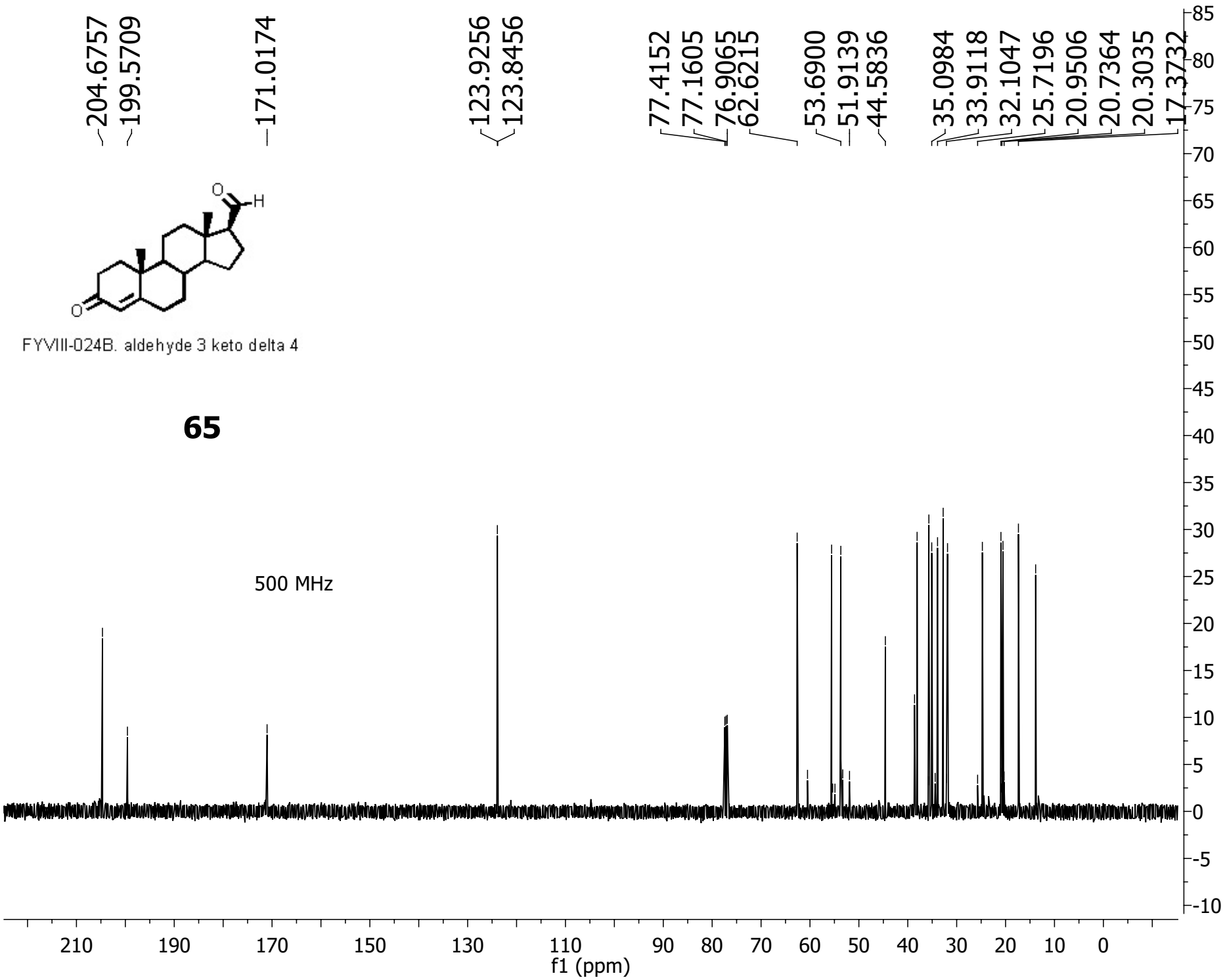
4500 4000 3500 3000 2500 2000 1500 1000 500 0

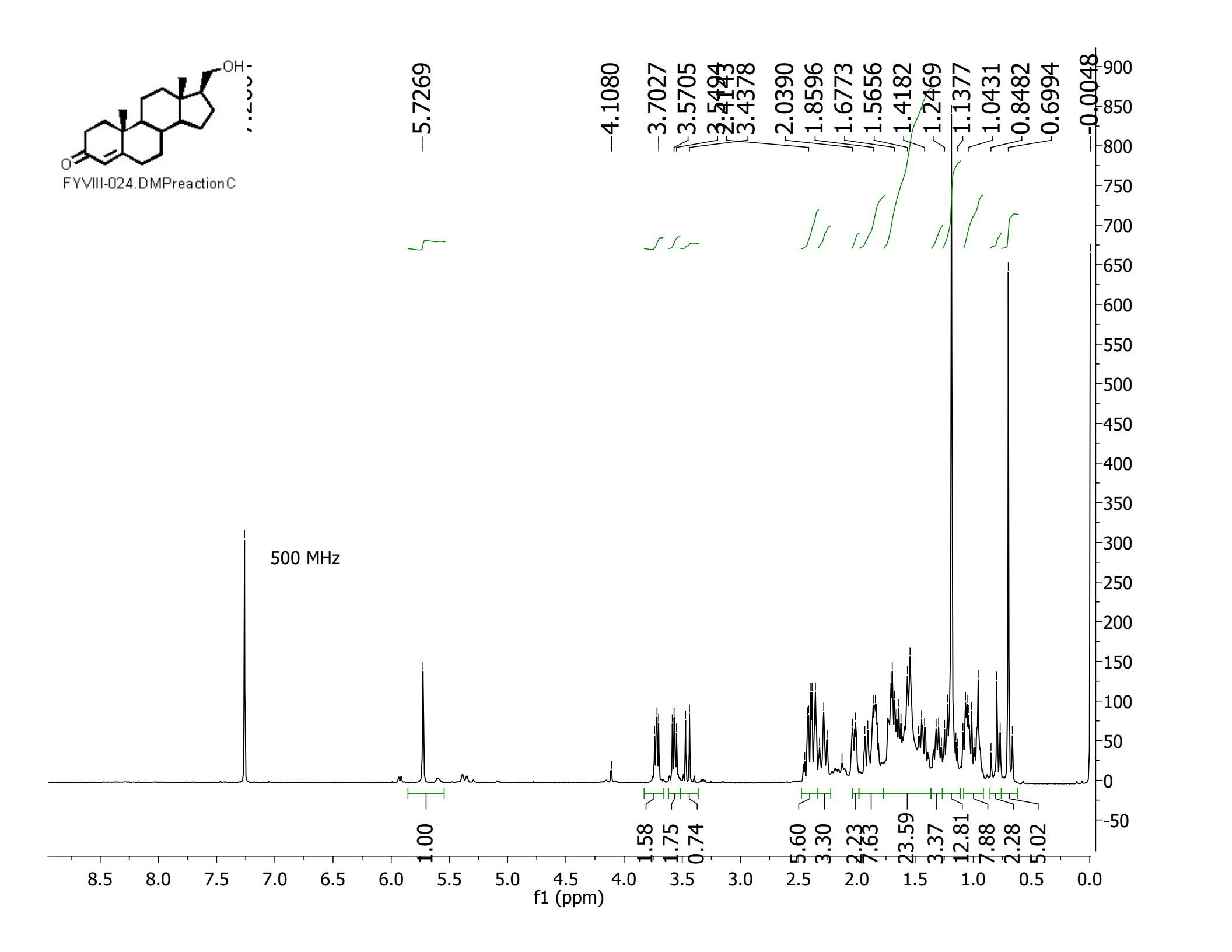

FYVIII-021 Oppenauer
64a
400 MHz
7.2595
5.7257
3.6699
2.9420
2.7104
2.3753
2.2646
2.0263
1.7286
1.5356
1.1803
0.7003
-0.0074
1.00
5.03
2.82
5.43
7.83
12.13
8.03
10.17
6.90
5.71
8.27
3.73
5.37
3.85
f1 (ppm)

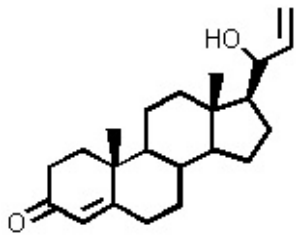

7.2602
5.2714
4.1221
4.1042
3.6998
3.5337
3.4604
3.4253
2.0380
1.9907
1.7043
1.6216
1.4713
1.3397
1.2657
1.2334
1.1470
1.0086
0.9051
0.8068
0.6580
-0.0086
-0.0121
OH
HO
FYVIII-022A. 20alcohol.primary.3OH.delta4
64
400 MHz
1.00
1.85
1.36
1.61
1.39
1.70
1.69
3.22
3.90
8.50
7.64
12.27
4.28
1.26
2.30
4.54
4.16
f1 (ppm)


400 MHz
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


9.7215
5.6809
2.2475
1.7992
1.7206
1.6667
1.5723
1.5275
1.2704
1.2360
1.1299
1.0931
1.0436
0.9531
0.7436
0.7214
O
H
O
FYVIII-024B. aldehyde 3 keto delta 4
65
500 MHz
0.28
0.46
5.28
3.26
7.37
2.41
1.29
3.54
3.77
3.00
f1 (ppm)


204.6757
199.5709
171.0174
123.9256
123.8456
77.4152
77.1605
76.9065
62.6215
53.6900
51.9139
44.5836
35.0984
33.9118
32.1047
25.7196
20.9506
20.7364
20.3035
17.3732
O
H
O
FYVIII-024B. aldehyde 3 keto delta 4
65
500 MHz
f1 (ppm)


OH
O
FYVIII-024.DMPreactionC
5.7269
4.1080
3.7027
3.5705
3.5494
3.4143
3.4378
2.0390
1.8596
1.6773
1.5656
1.4182
1.2469
1.1377
1.0431
0.8482
0.6994
-0.0048
500 MHz
1.00
1.58
1.75
0.74
5.60
3.30
2.23
7.63
23.59
3.37
12.81
7.88
2.28
5.02
f1 (ppm)

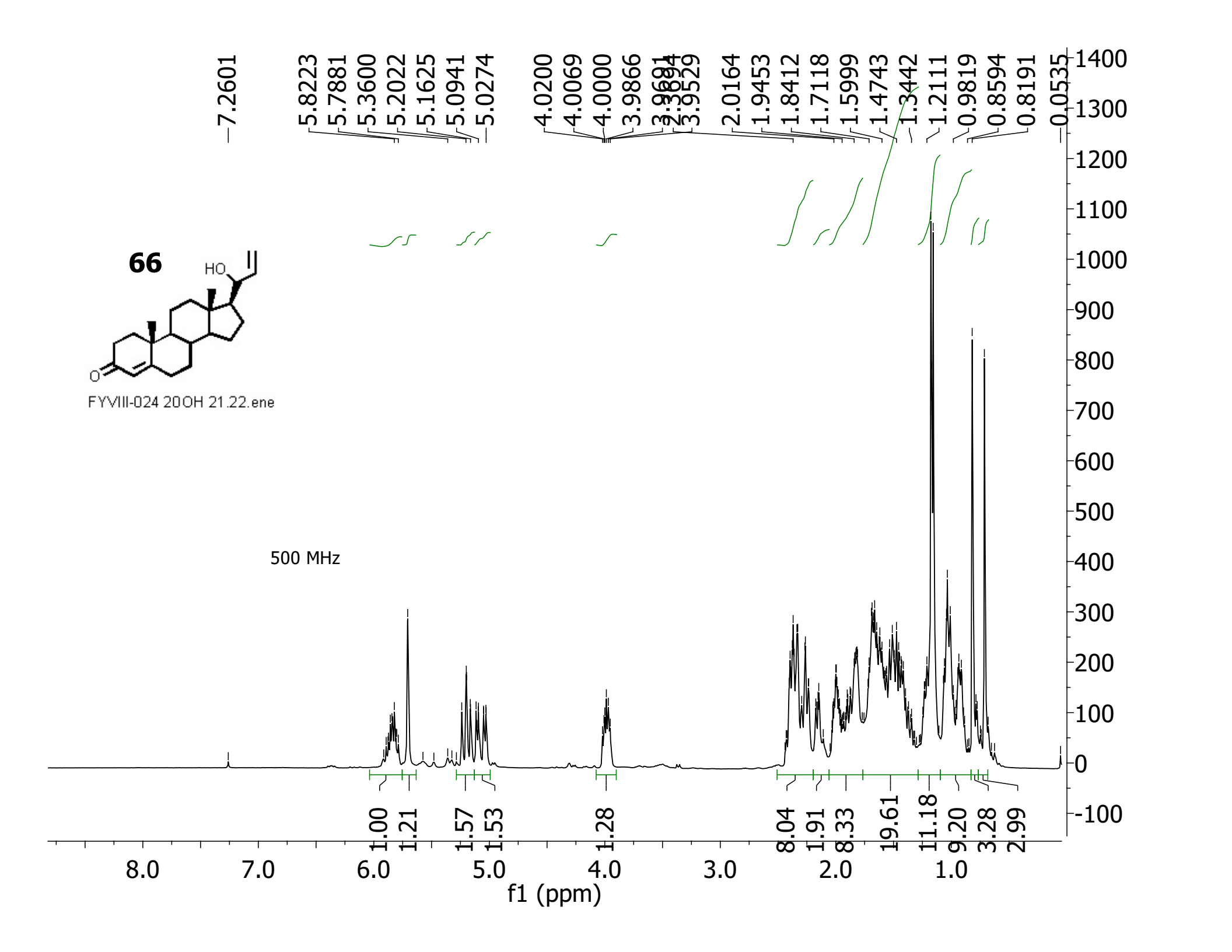


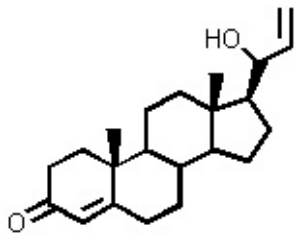
FYVIII-024 20OH 21.22.ene

66

400 MHz

f1 (ppm)


66
HO
O
FYVIII-024 20OH 21.22.ene
500 MHz
7.2601
5.8223
5.7881
5.3600
5.2022
5.1625
5.0941
5.0274
4.0200
4.0069
4.0000
3.9866
3.9681
3.9529
2.0164
1.9453
1.8412
1.7118
1.5999
1.4743
1.3442
1.2111
0.9819
0.8594
0.8191
0.0535
1.00
1.21
1.57
1.53
1.28
8.04
1.91
8.33
19.61
11.18
9.20
3.28
2.99
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
f1 (ppm)
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100

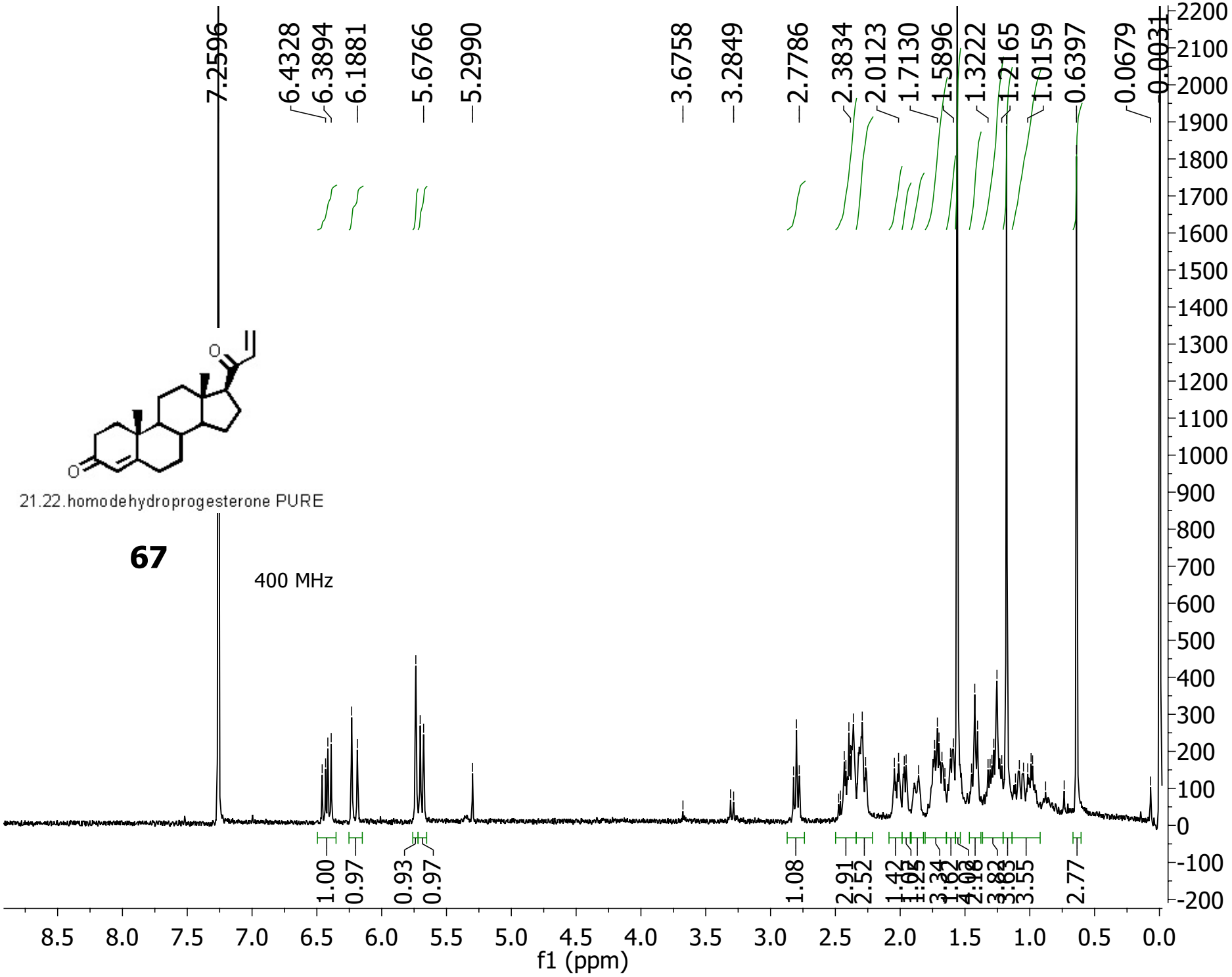


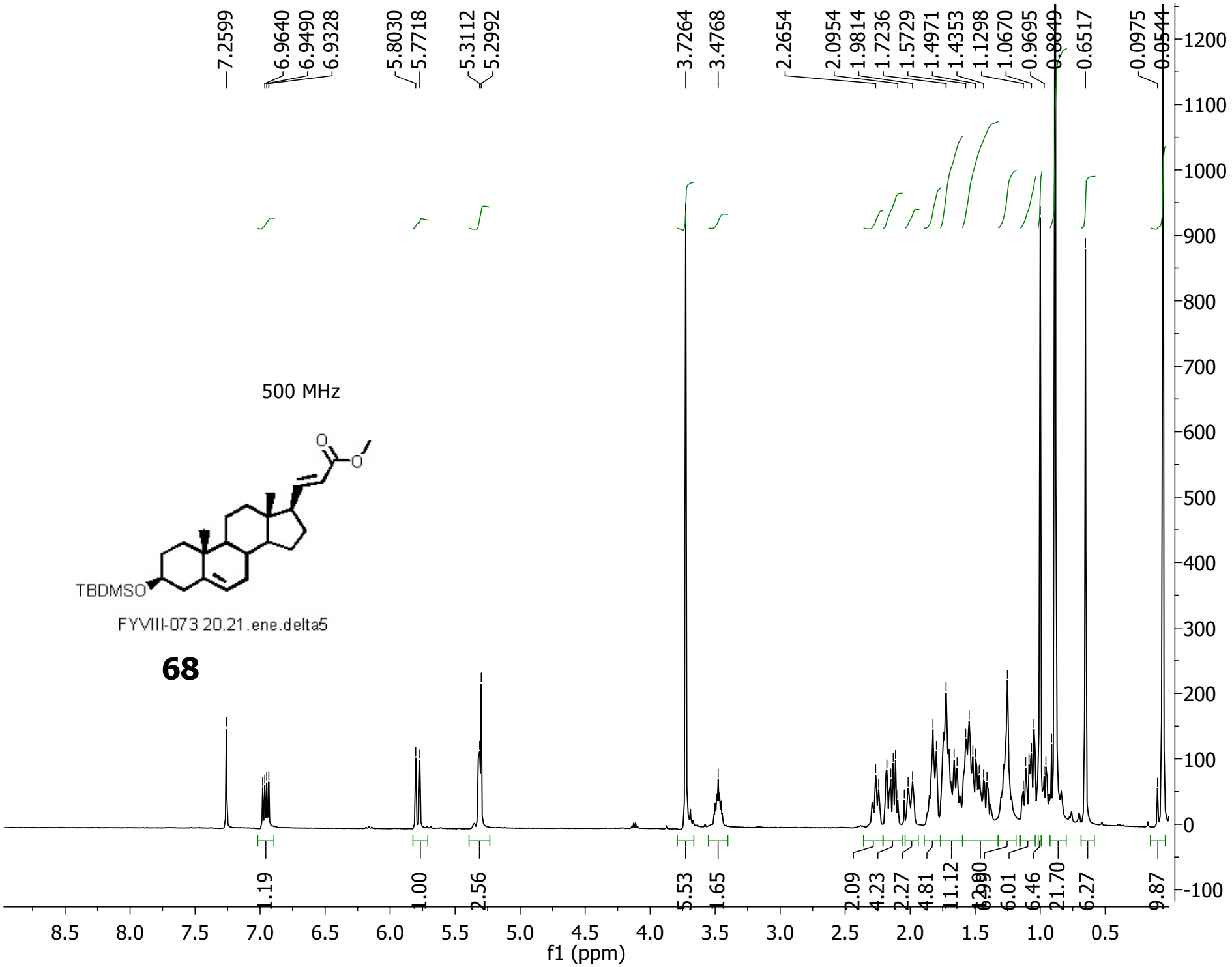
FYVIII-024 20OH 21.22.ene

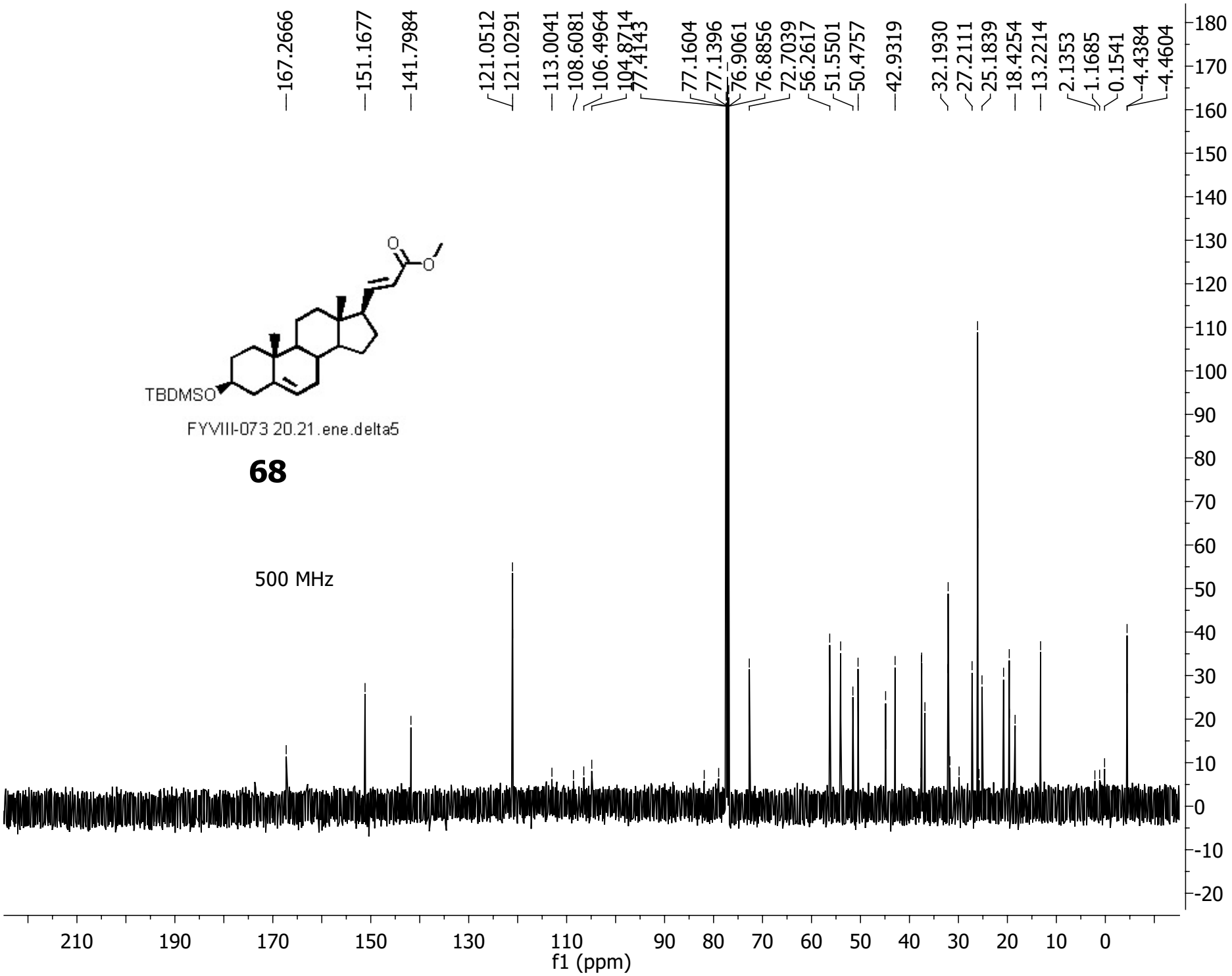
66

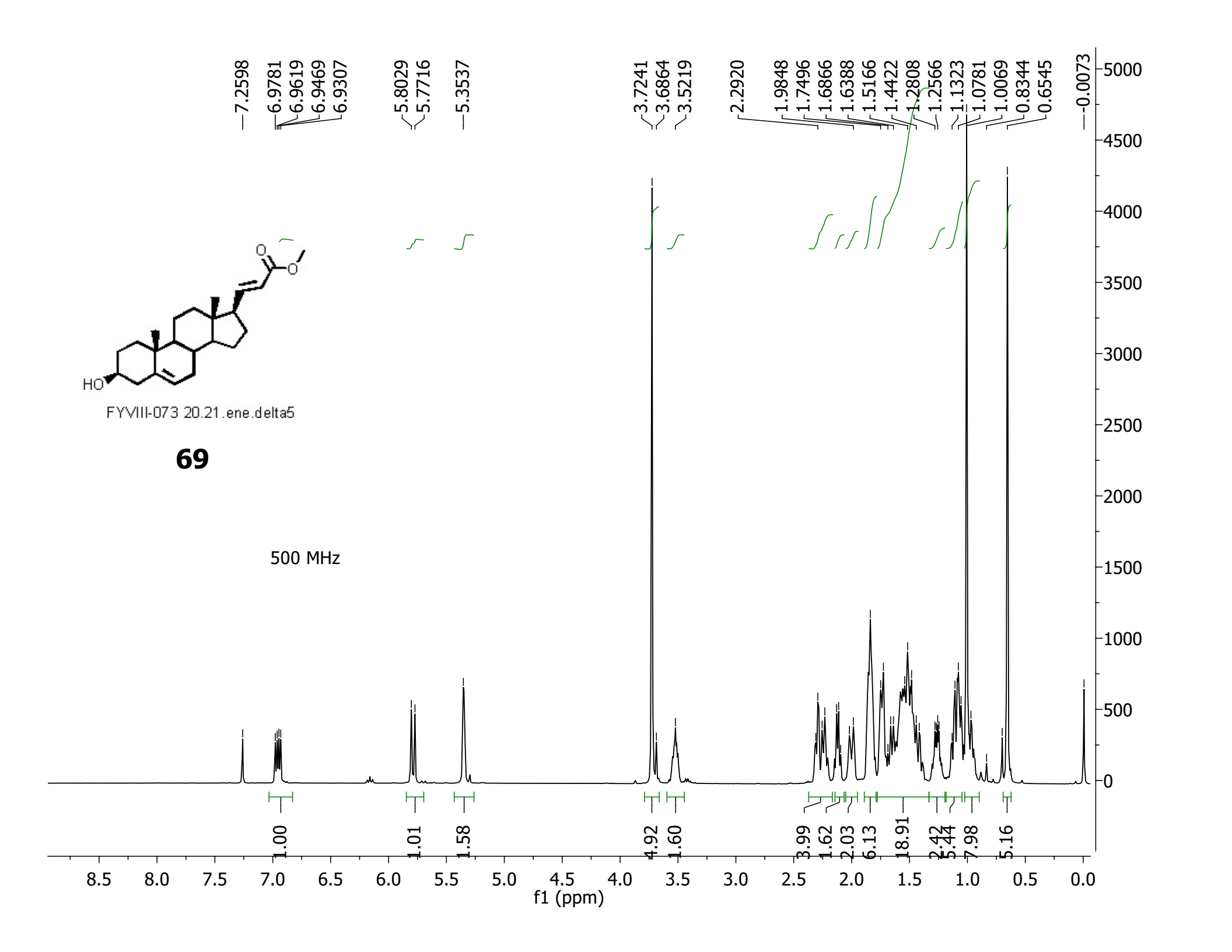
500 MHz

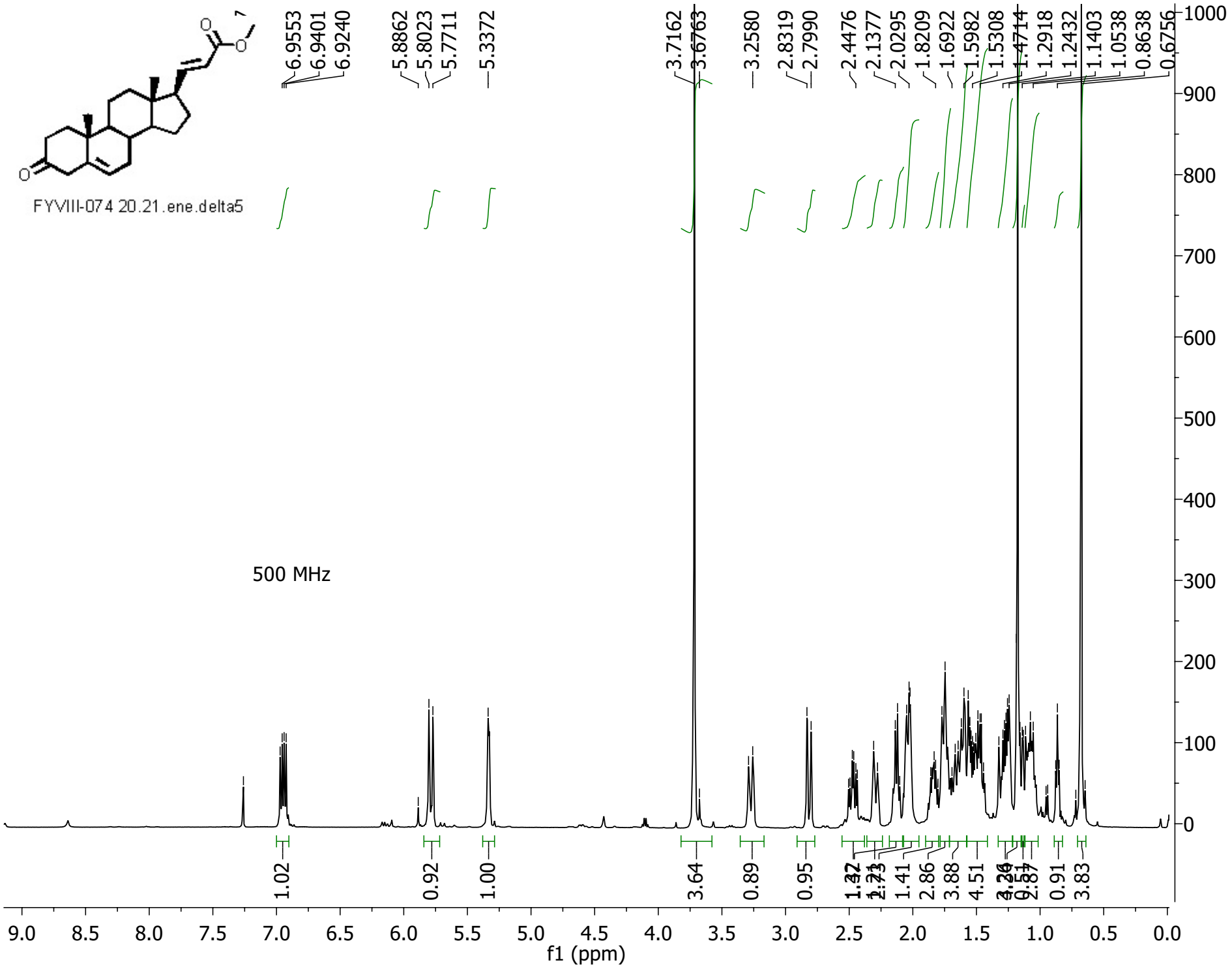
f1 (ppm)

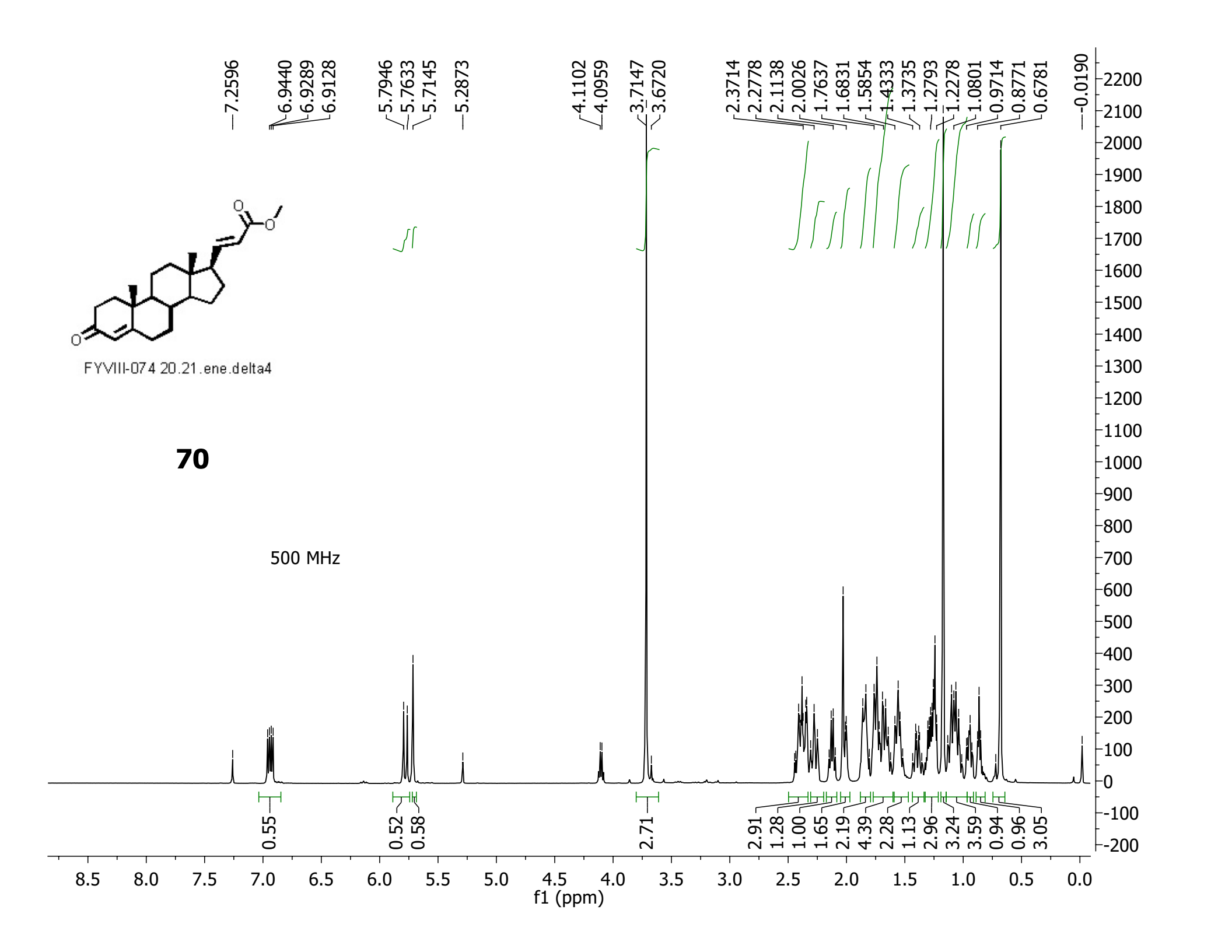

7.2596
6.4328
6.3894
6.1881
5.6766
5.2990
3.6758
3.2849
2.7786
2.3834
2.0123
1.7130
1.5896
1.3222
1.2165
1.0159
0.6397
0.0679
0.0031
21.22.homodehydroprogesterone PURE
67
400 MHz
1.00
0.97
0.93
0.97
1.08
2.91
2.52
2.77
f1 (ppm)

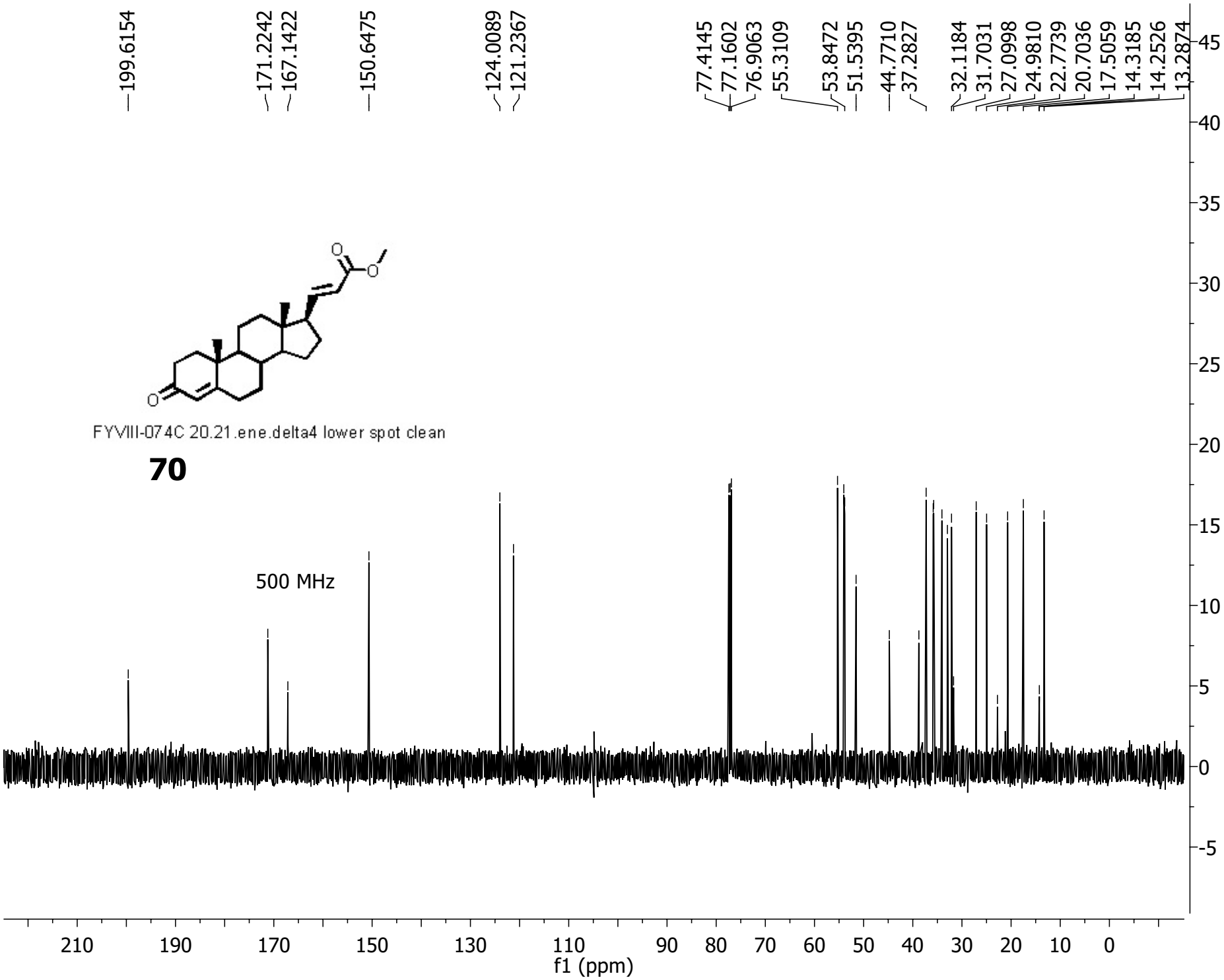

7.2599
6.9640
6.9490
6.9328
5.8030
5.7718
5.3112
5.2992
3.7264
3.4768
2.2654
2.0954
1.9814
1.7236
1.5729
1.4971
1.4353
1.1298
1.0670
0.9695
0.8849
0.6517
0.0975
0.0544
500 MHz
TBDMSO
FYVIII-073 20.21.ene.delta5
68
1.19
1.00
2.56
5.53
1.65
2.09
4.23
2.27
4.81
11.12
6.01
6.46
21.70
6.27
9.87
f1 (ppm)

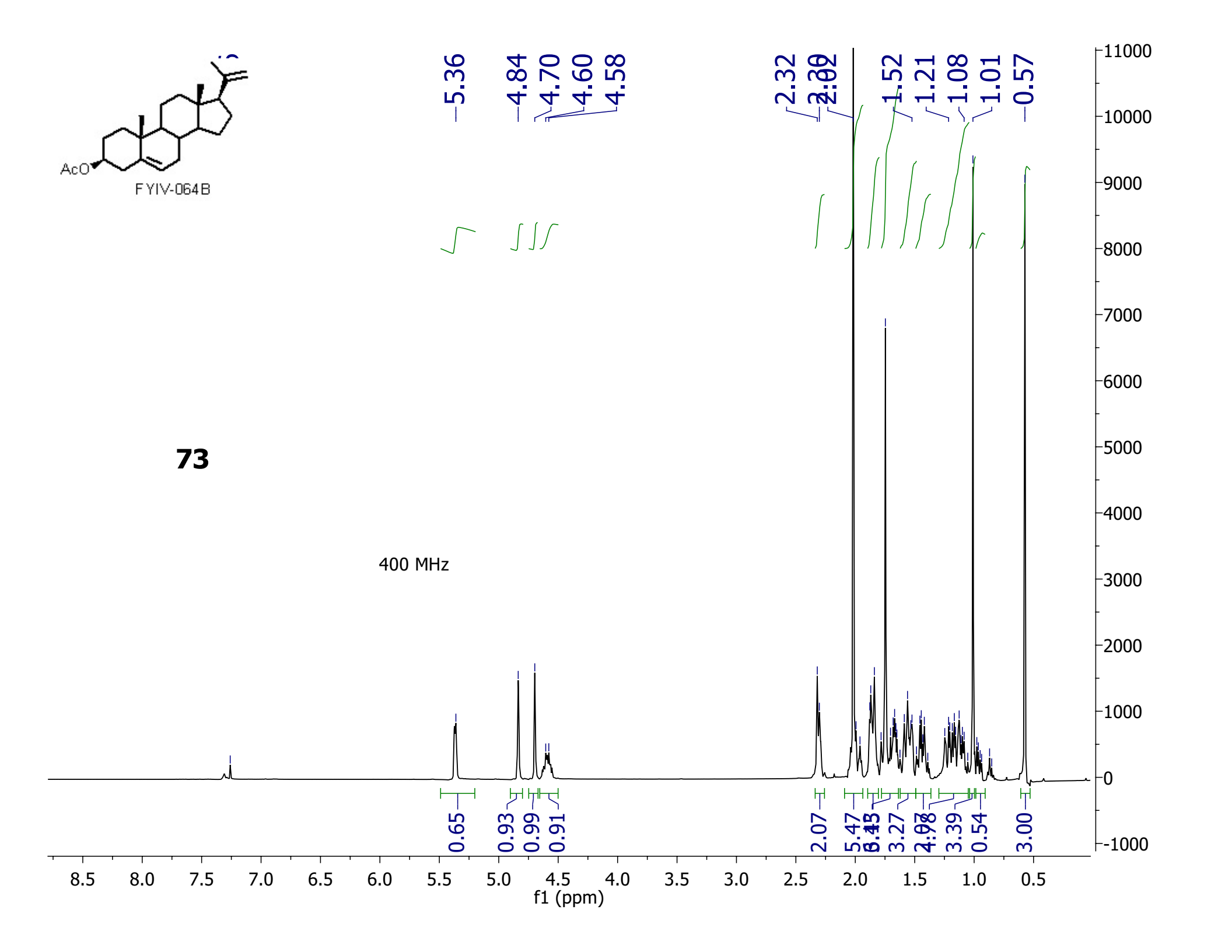

167.2666
151.1677
141.7984
121.0512
121.0291
113.0041
108.6081
106.4964
104.8714
77.4143
77.1604
77.1396
76.9061
76.8856
72.7039
56.2617
51.5501
50.4757
42.9319
32.1930
27.2111
25.1839
18.4254
13.2214
2.1353
1.1685
0.1541
-4.4384
-4.4604
TBDMSO
FYVIII-073 20.21.ene.delta5
68
500 MHz
f1 (ppm)

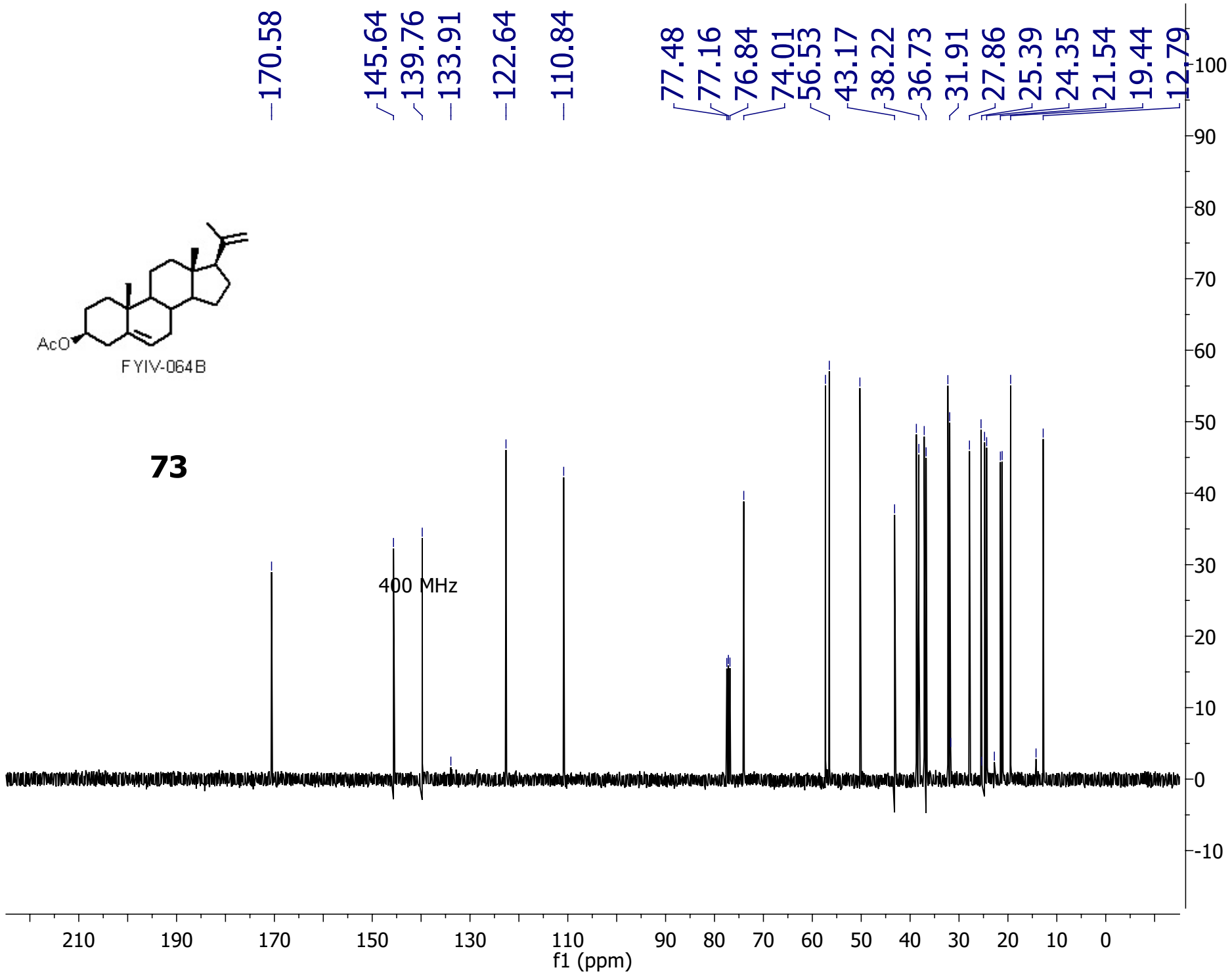

7.2598
6.9781
6.9619
6.9469
6.9307
5.8029
5.7716
5.3537
3.7241
3.6864
3.5219
2.2920
1.9848
1.7496
1.6866
1.6388
1.5166
1.4422
1.2808
1.2566
1.1323
1.0781
1.0069
0.8344
0.6545
-0.0073
HO
O
O
FYVIII-073 20.21.ene.delta5
69
500 MHz
1.00
1.01
1.58
4.92
1.60
3.99
1.62
2.03
6.13
18.91
2.42
5.44
7.98
5.16
f1 (ppm)

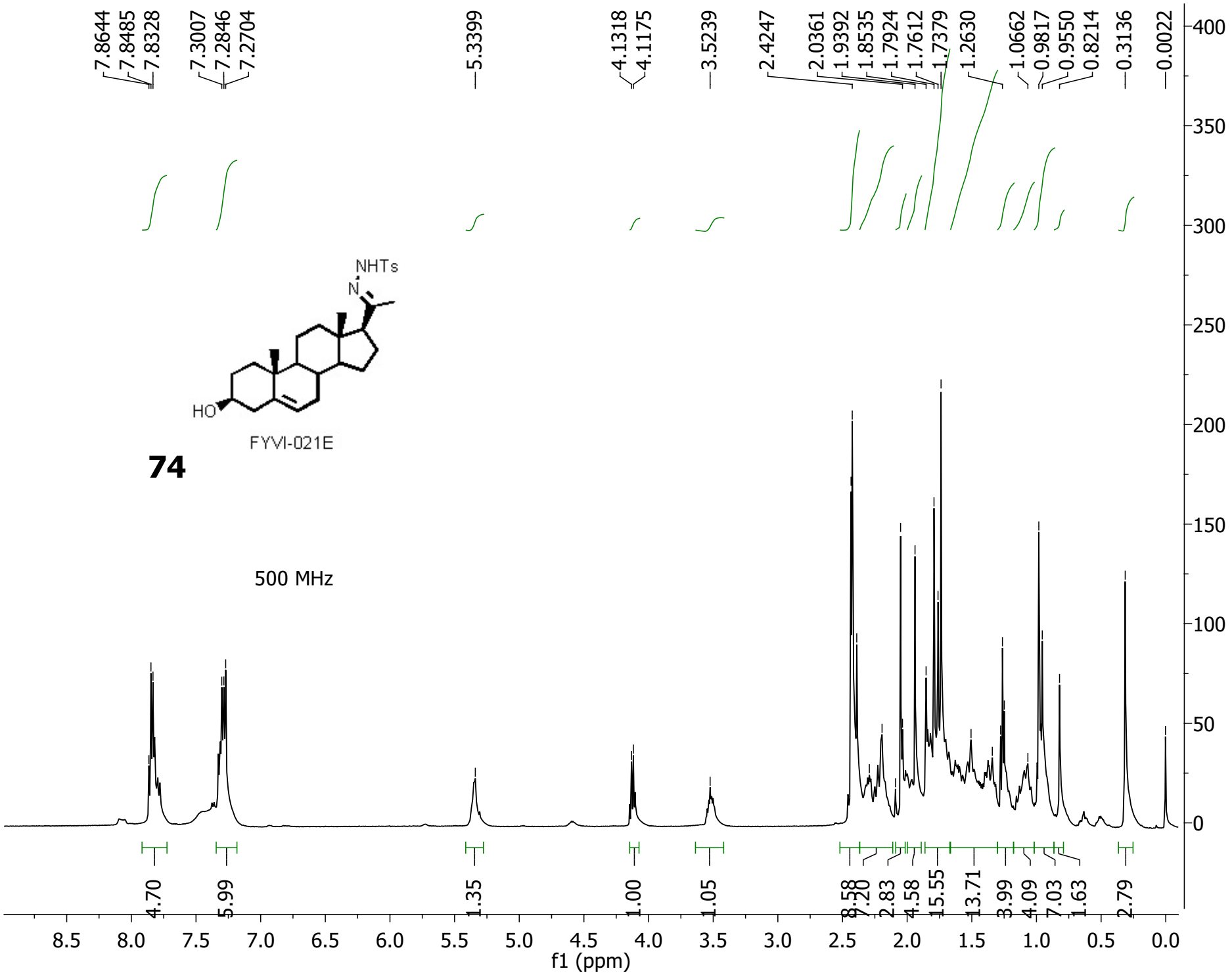

FYVIII-074 20.21.ene.delta5
6.9553
6.9401
6.9240
5.8862
5.8023
5.7711
5.3372
3.7162
3.6763
3.2580
2.8319
2.7990
2.4476
2.1377
2.0295
1.8209
1.6922
1.5982
1.5308
1.4714
1.2918
1.2432
1.1403
1.0538
0.8638
0.6756
500 MHz
1.02
0.92
1.00
3.64
0.89
0.95
1.41
2.86
3.88
4.51
0.91
3.83
f1 (ppm)

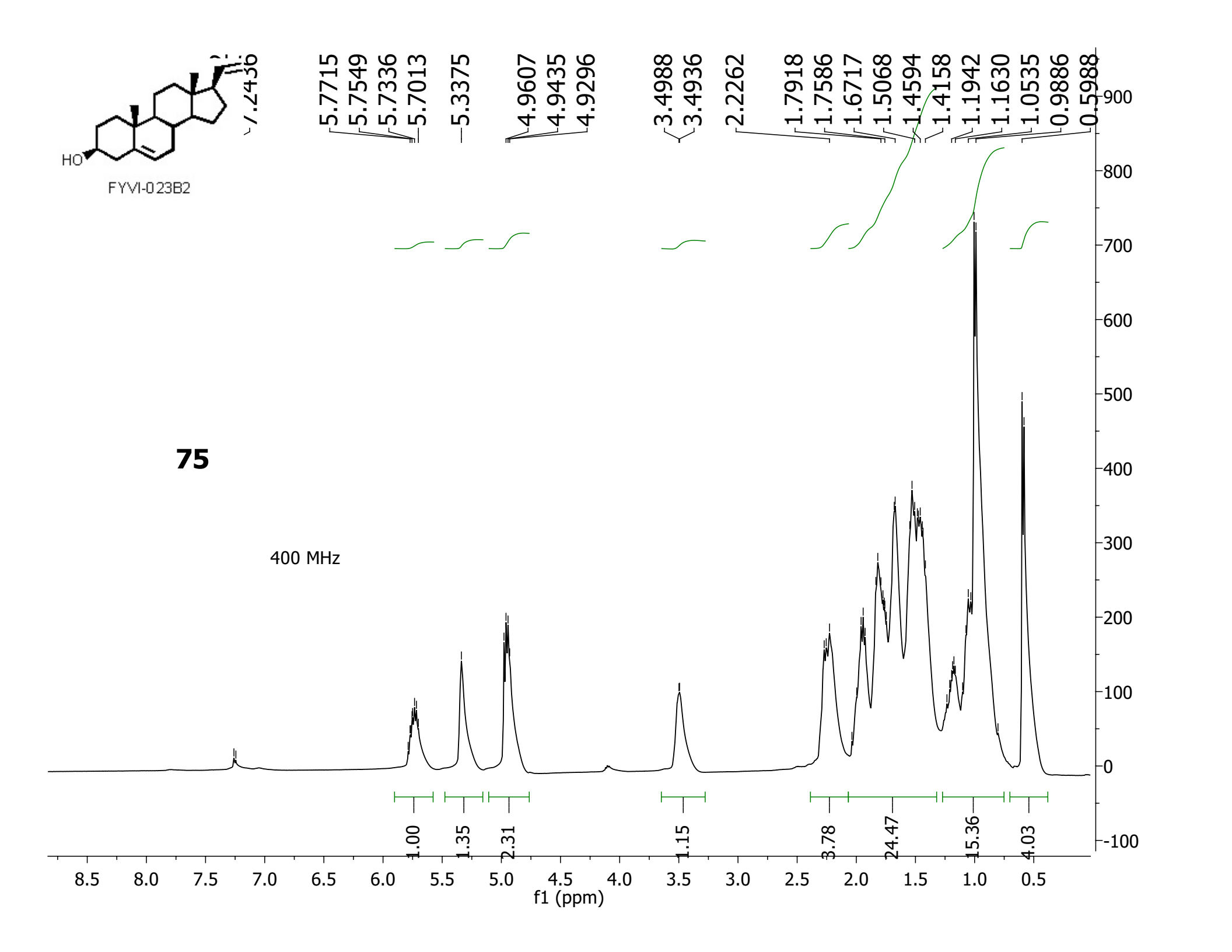

7.2596
6.9440
6.9289
6.9128
5.7946
5.7633
5.7145
5.2873
4.1102
4.0959
3.7147
3.6720
2.3714
2.2778
2.1138
2.0026
1.7637
1.6831
1.5854
1.4333
1.3735
1.2793
1.2278
1.0801
0.9714
0.8771
0.6781
-0.0190
FYVIII-074 20.21.ene.delta4
70
500 MHz
0.55
0.52
0.58
2.71
2.91
1.28
1.00
1.65
2.19
4.39
2.28
1.13
2.96
3.24
3.59
0.94
0.96
3.05
f1 (ppm)

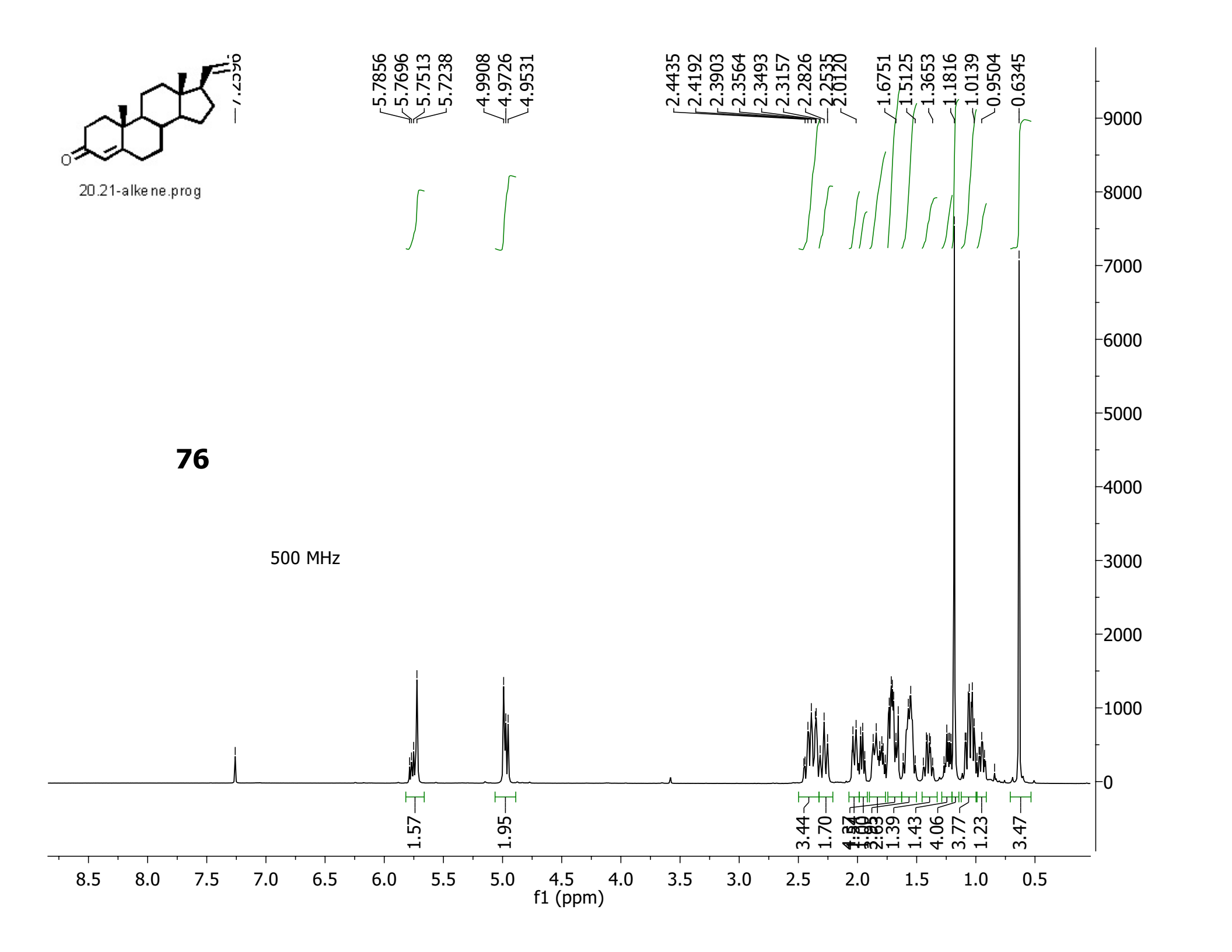

199.6154
171.2242
167.1422
150.6475
124.0089
121.2367
77.4145
77.1602
76.9063
55.3109
53.8472
51.5395
44.7710
37.2827
32.1184
31.7031
27.0998
24.9810
22.7739
20.7036
17.5059
14.3185
14.2526
13.2874
FYVIII-074C 20.21.ene.delta4 lower spot clean
70
500 MHz
f1 (ppm)

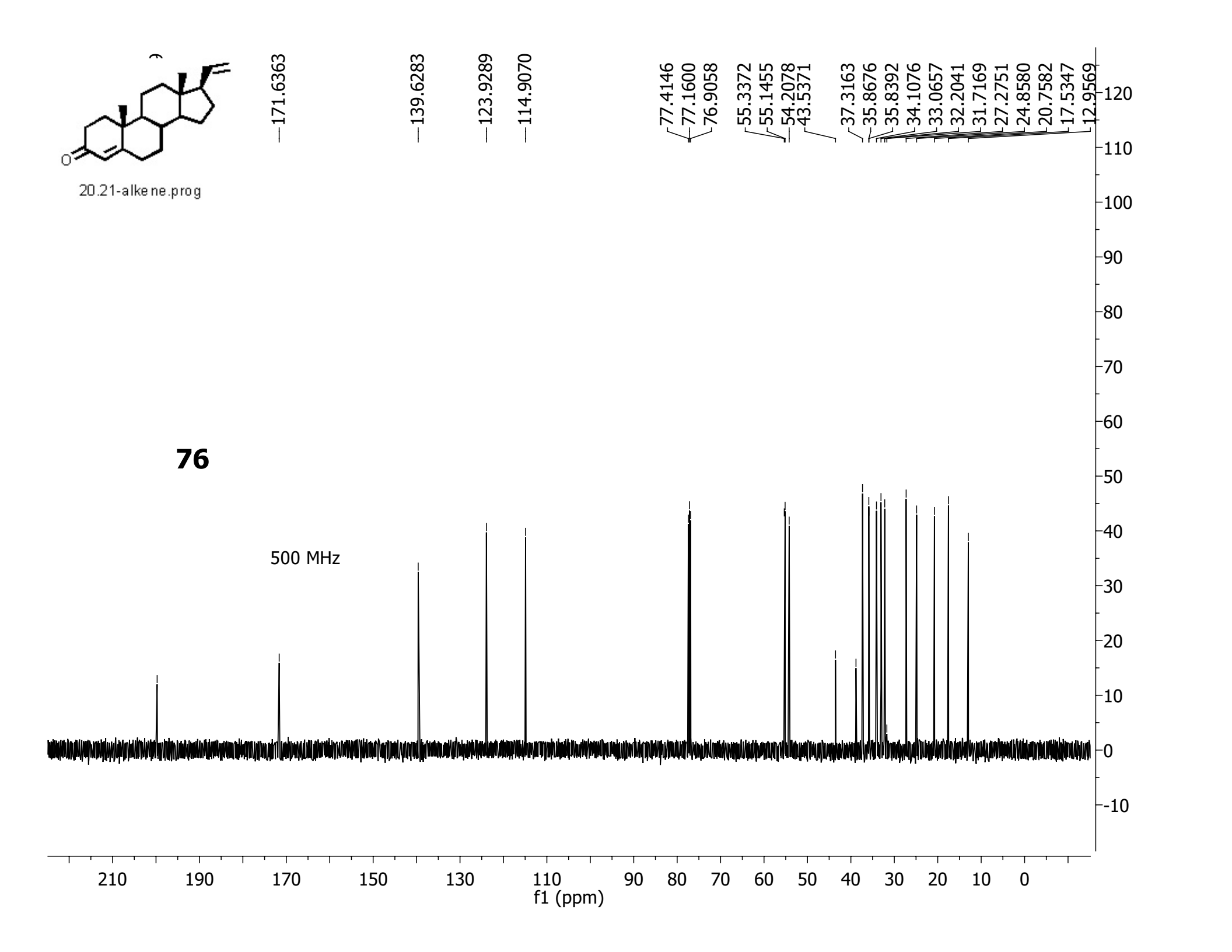

AcO
FYIV-064B
73
400 MHz
5.36
4.84
4.70
4.60
4.58
2.32
1.52
1.21
1.08
1.01
0.57
0.65
0.93
0.99
0.91
2.07
3.27
3.39
0.54
3.00
f1 (ppm)

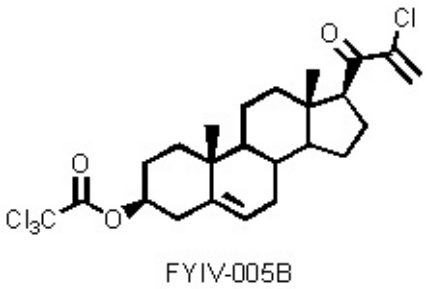

170.58
145.64
139.76
133.91
122.64
110.84
77.48
77.16
76.84
74.01
56.53
43.17
38.22
36.73
31.91
27.86
25.39
24.35
21.54
19.44
12.79
AcO
FYIV-064B
73
400 MHz
f1 (ppm)


7.8644
7.8485
7.8328
7.3007
7.2846
7.2704
5.3399
4.1318
4.1175
3.5239
2.4247
2.0361
1.9392
1.8535
1.7924
1.7612
1.7379
1.2630
1.0662
0.9817
0.9550
0.8214
0.3136
0.0022
NHTs
N
HO
FYVI-021E
74
500 MHz
4.70
5.99
1.35
1.00
1.05
8.58
9.20
2.83
4.58
15.55
13.71
3.99
4.09
7.03
1.63
2.79
f1 (ppm)


HO
FYVI-023B2
7.2436
5.7715
5.7549
5.7336
5.7013
5.3375
4.9607
4.9435
4.9296
3.4988
3.4936
2.2262
1.7918
1.7586
1.6717
1.5068
1.4594
1.4158
1.1942
1.1630
1.0535
0.9886
0.5988
75
400 MHz
1.00
1.35
2.31
1.15
3.78
24.47
15.36
4.03
f1 (ppm)


20.21-alkene.prog
76
500 MHz
5.7856
5.7696
5.7513
5.7238
4.9908
4.9726
4.9531
2.4435
2.4192
2.3903
2.3564
2.3493
2.3157
2.2826
2.2535
2.0120
1.6751
1.5125
1.3653
1.1816
1.0139
0.9504
0.6345
1.57
1.95
3.44
1.70
1.39
1.43
4.06
3.77
1.23
3.47
f1 (ppm)

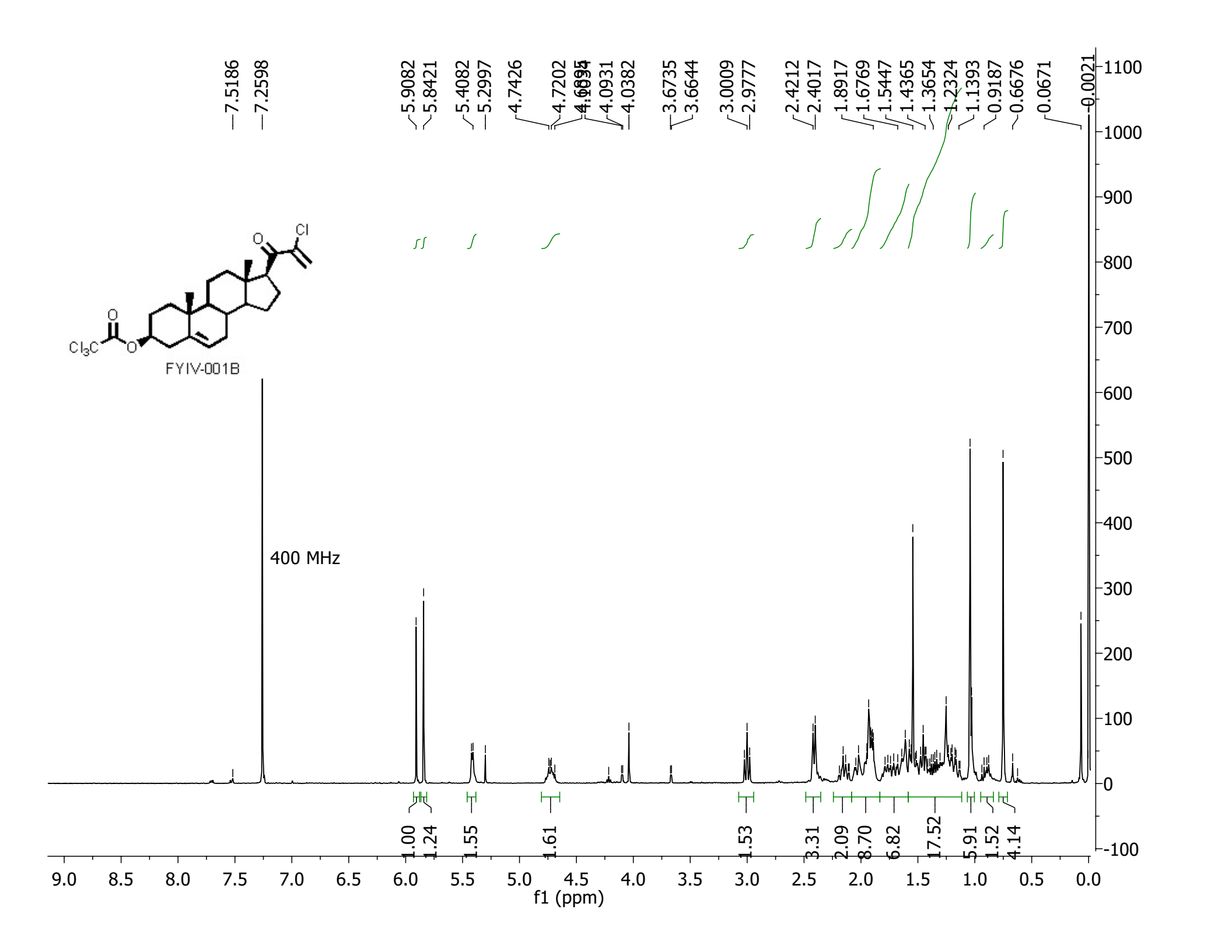

171.6363
139.6283
123.9289
114.9070
77.4146
77.1600
76.9058
55.3372
55.1455
54.2078
43.5371
37.3163
35.8676
35.8392
34.1076
33.0657
32.2041
31.7169
27.2751
24.8580
20.7582
17.5347
12.9569
20.21-alkene.prog
76
500 MHz
f1 (ppm)

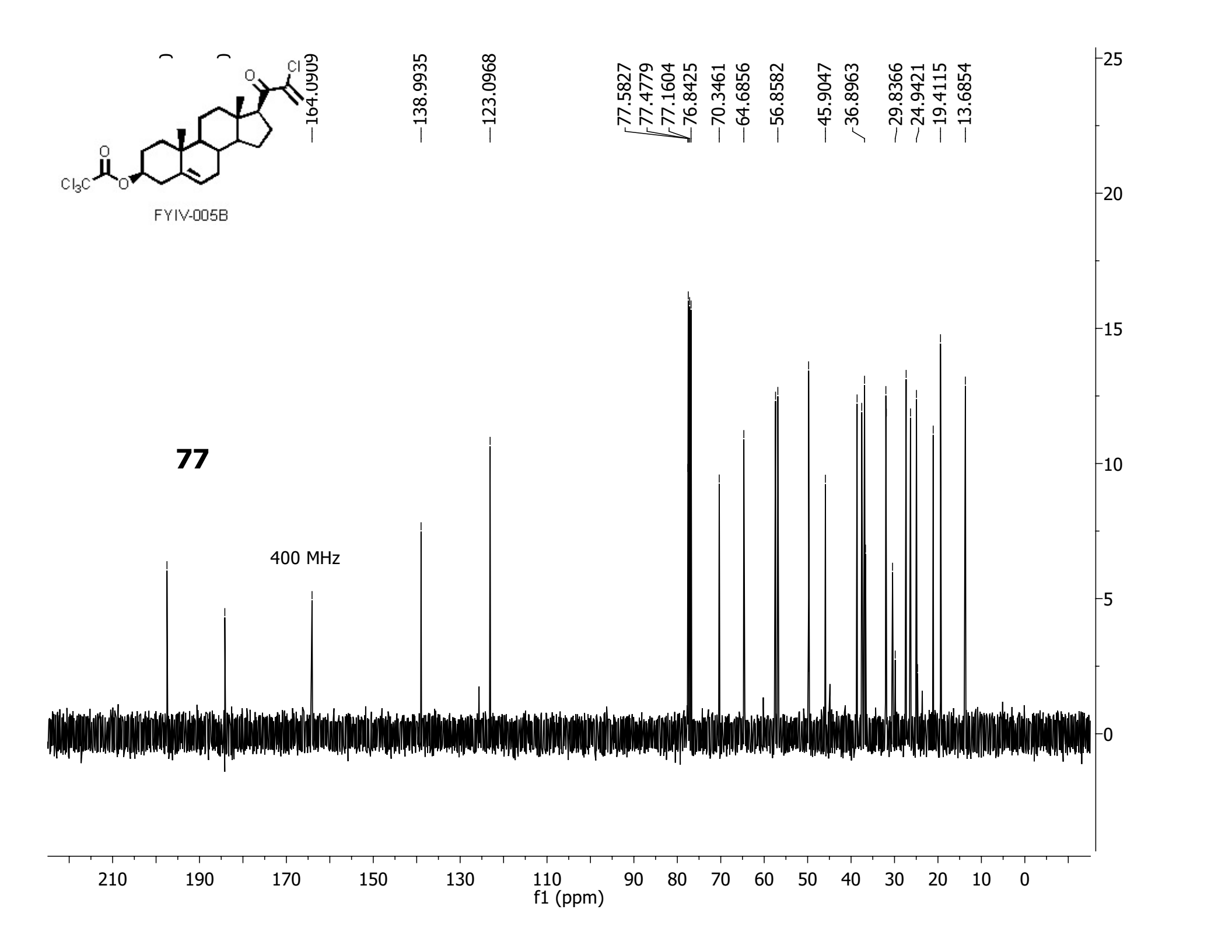


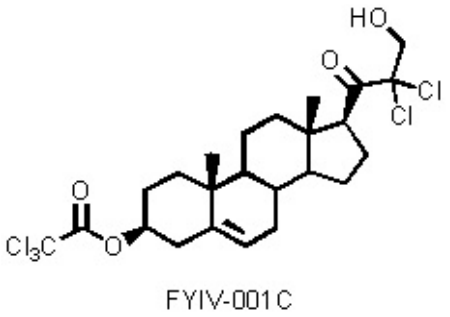
FYIV-005B

77

400 MHz


7.5186
7.2598
5.9082
5.8421
5.4082
5.2997
4.7426
4.7202
4.0931
4.0382
3.6735
3.6644
3.0009
2.9777
2.4212
2.4017
1.8917
1.6769
1.5447
1.4365
1.3654
1.2324
1.1393
0.9187
0.6676
0.0671
-0.0021
FYIV-001B
400 MHz
1.00
1.24
1.55
1.61
1.53
3.31
2.09
8.70
6.82
17.52
5.91
1.52
4.14
f1 (ppm)

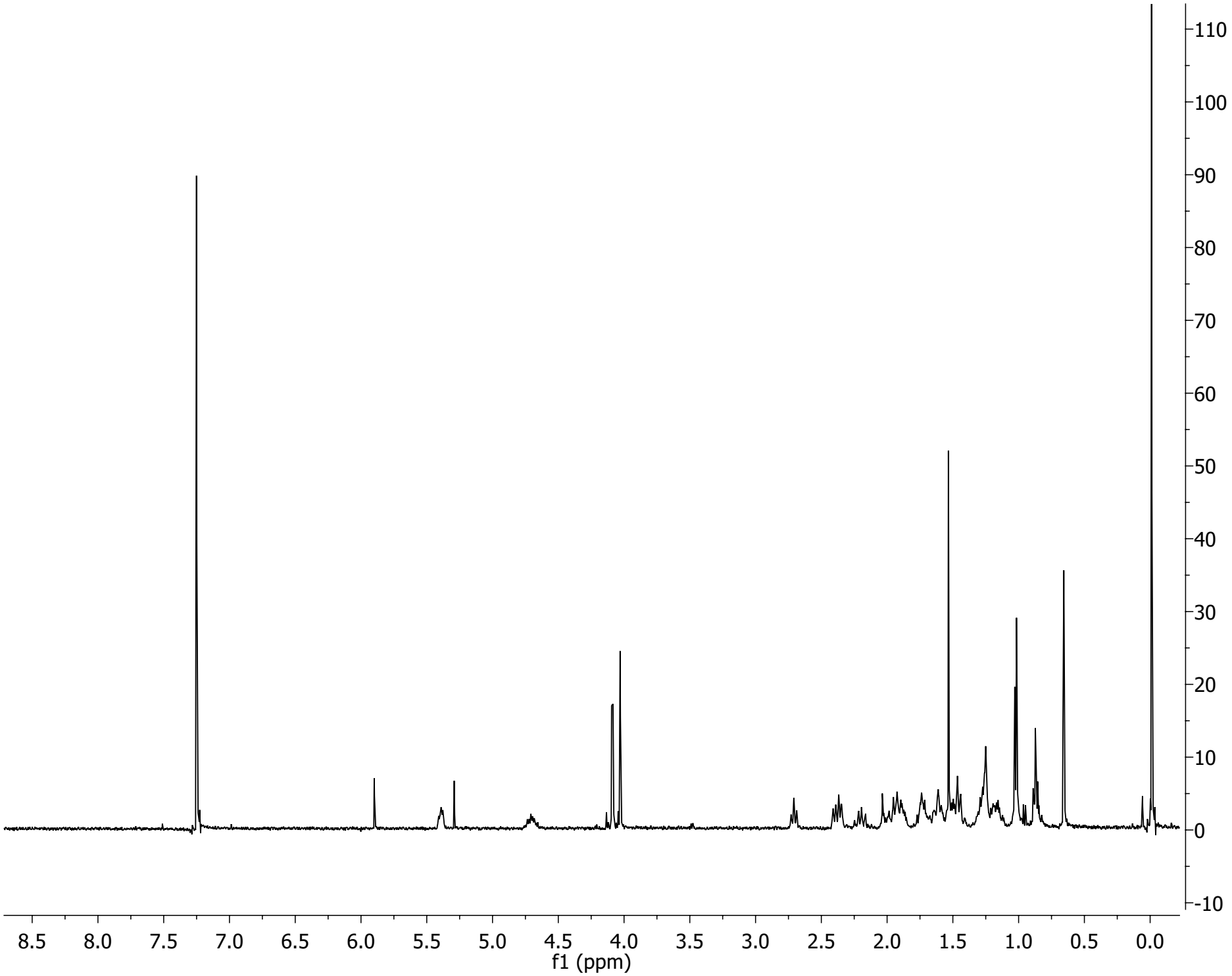

FYIV-005B
164.0909
138.9935
123.0968
77.5827
77.4779
77.1604
76.8425
70.3461
64.6856
56.8582
45.9047
36.8963
29.8366
24.9421
19.4115
13.6854
77
400 MHz
f1 (ppm)

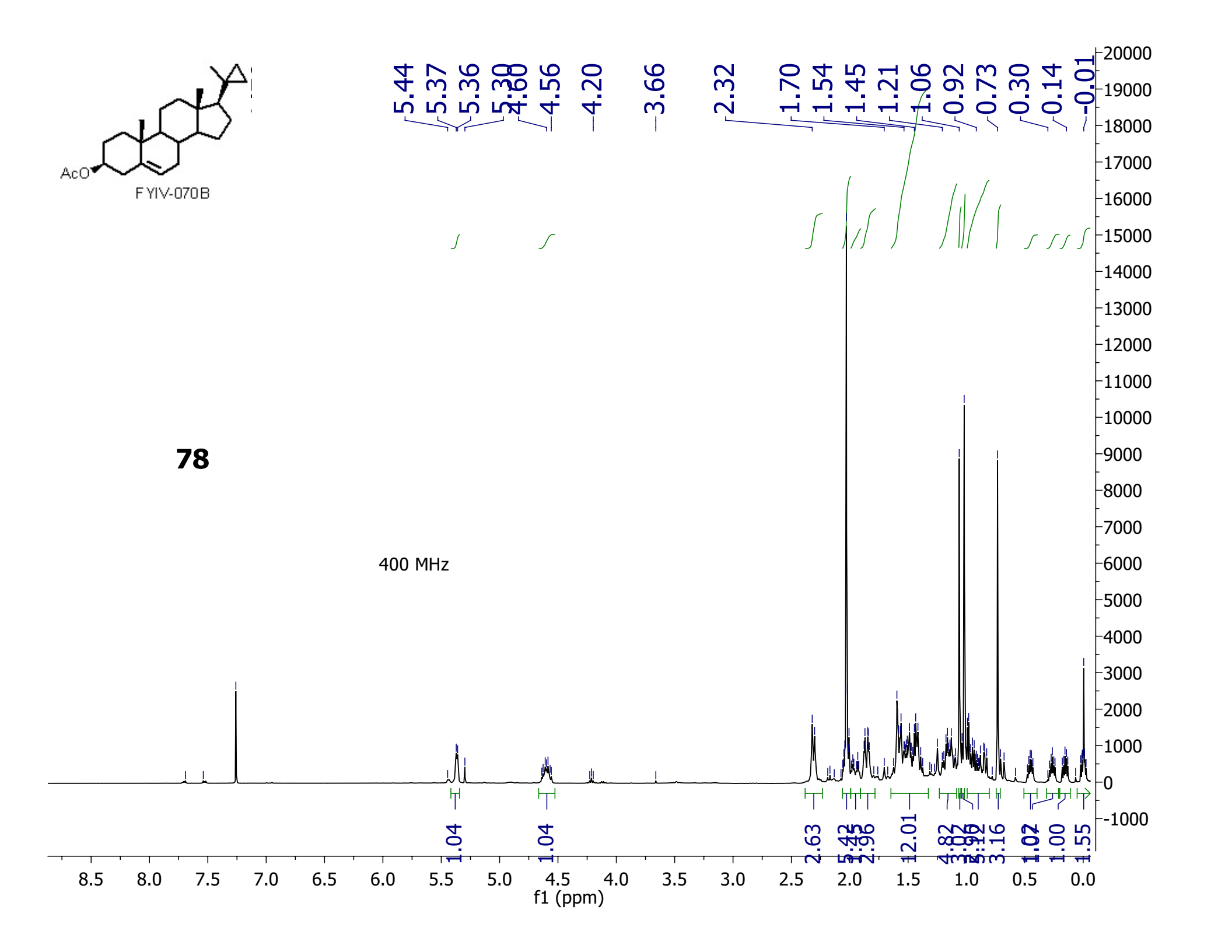


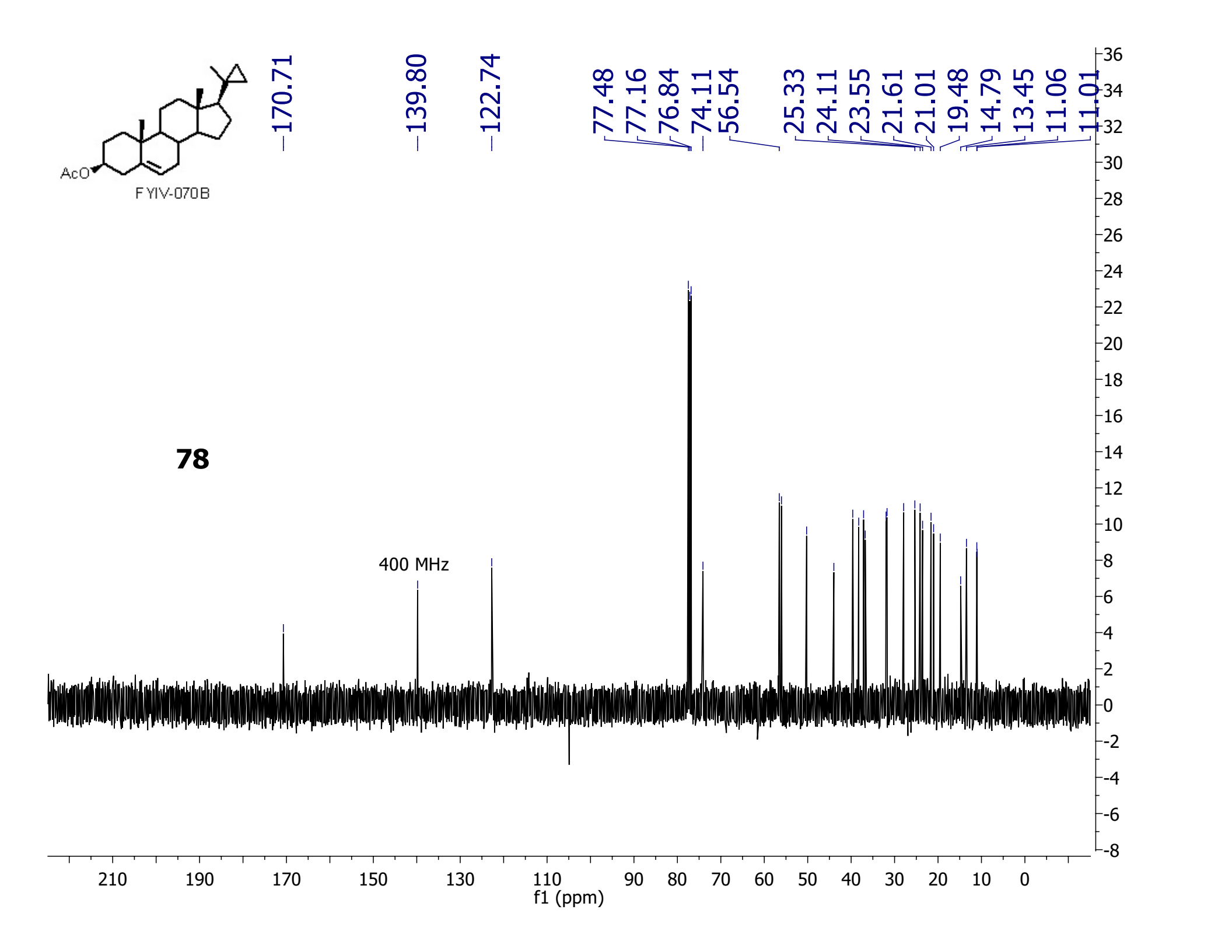
FYIV-001C

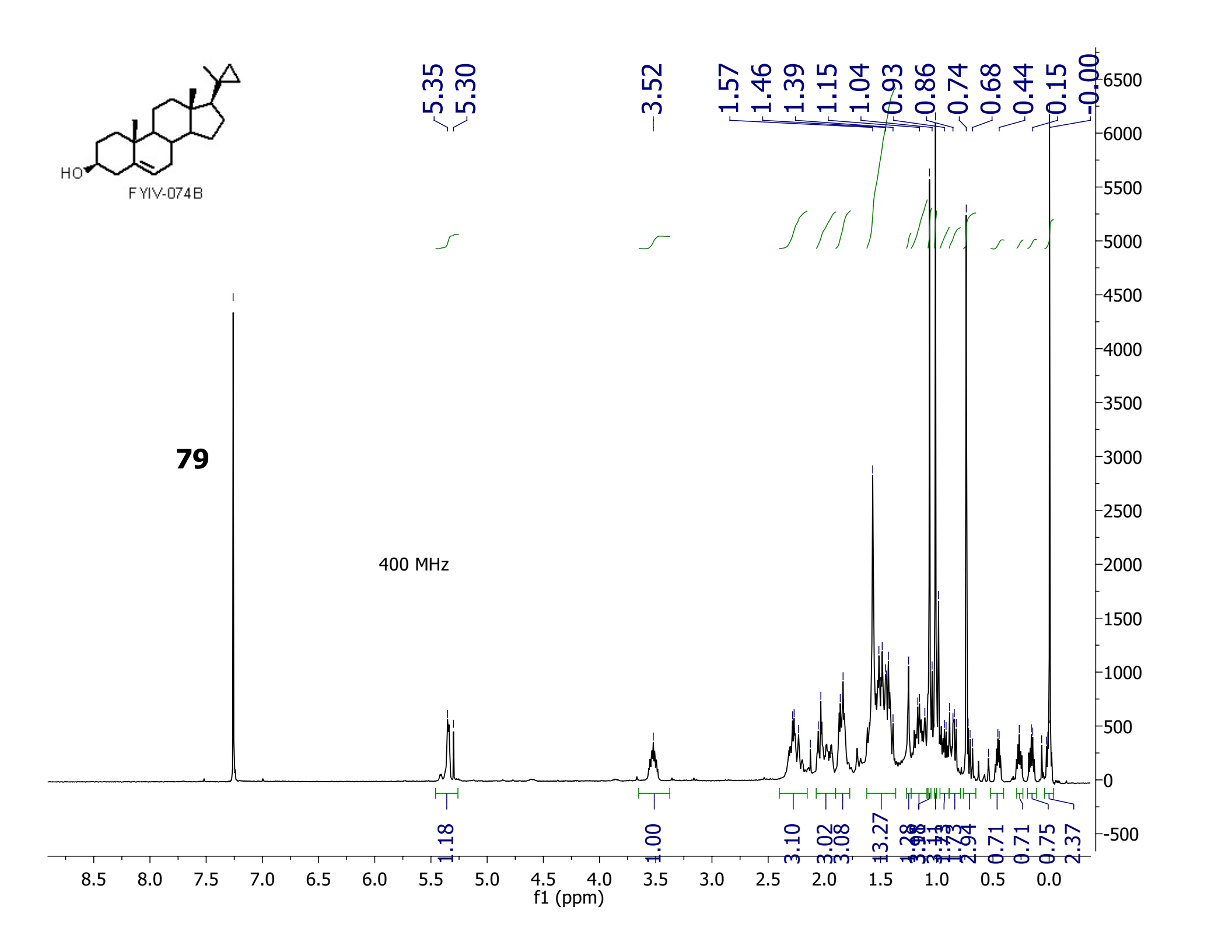
400 MHz

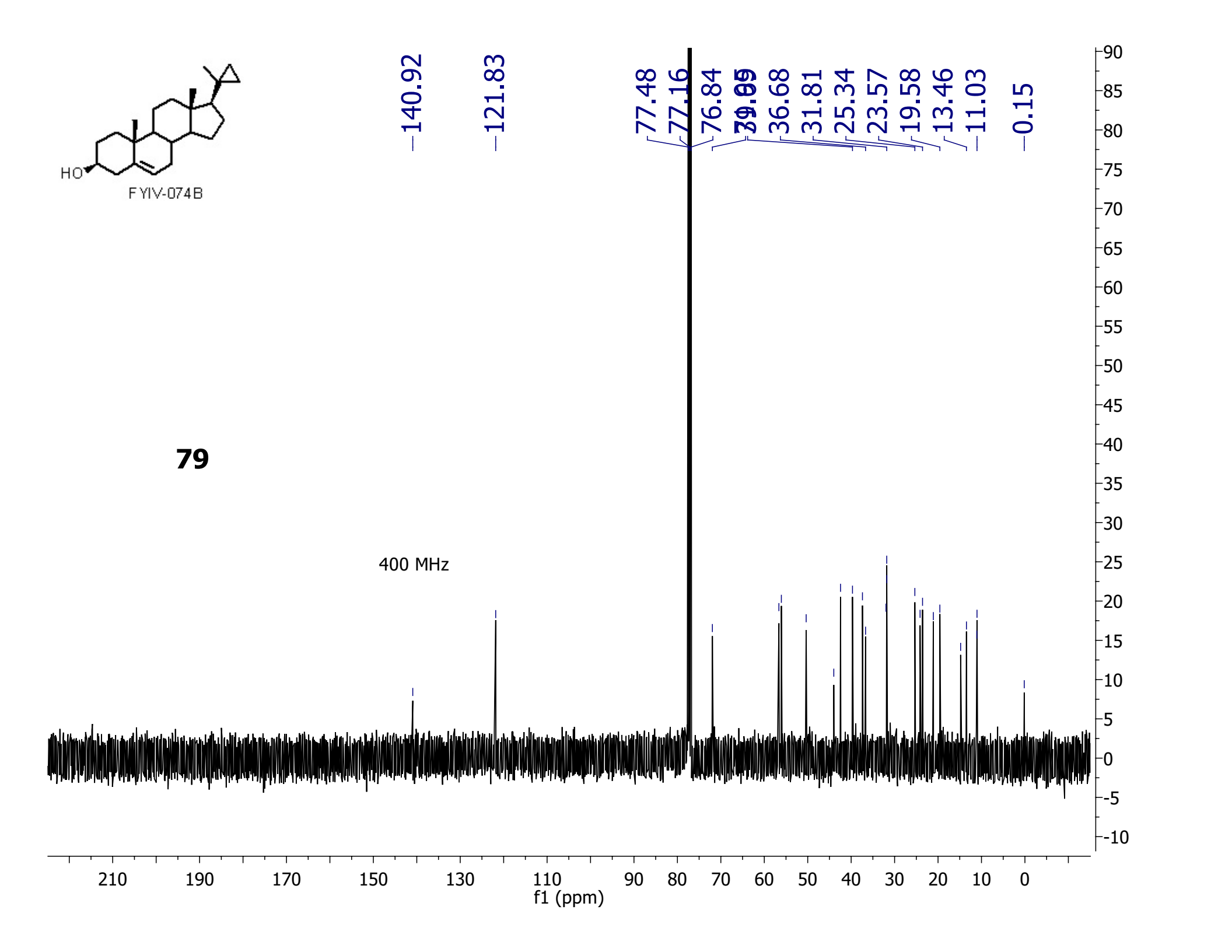
f1 (ppm)

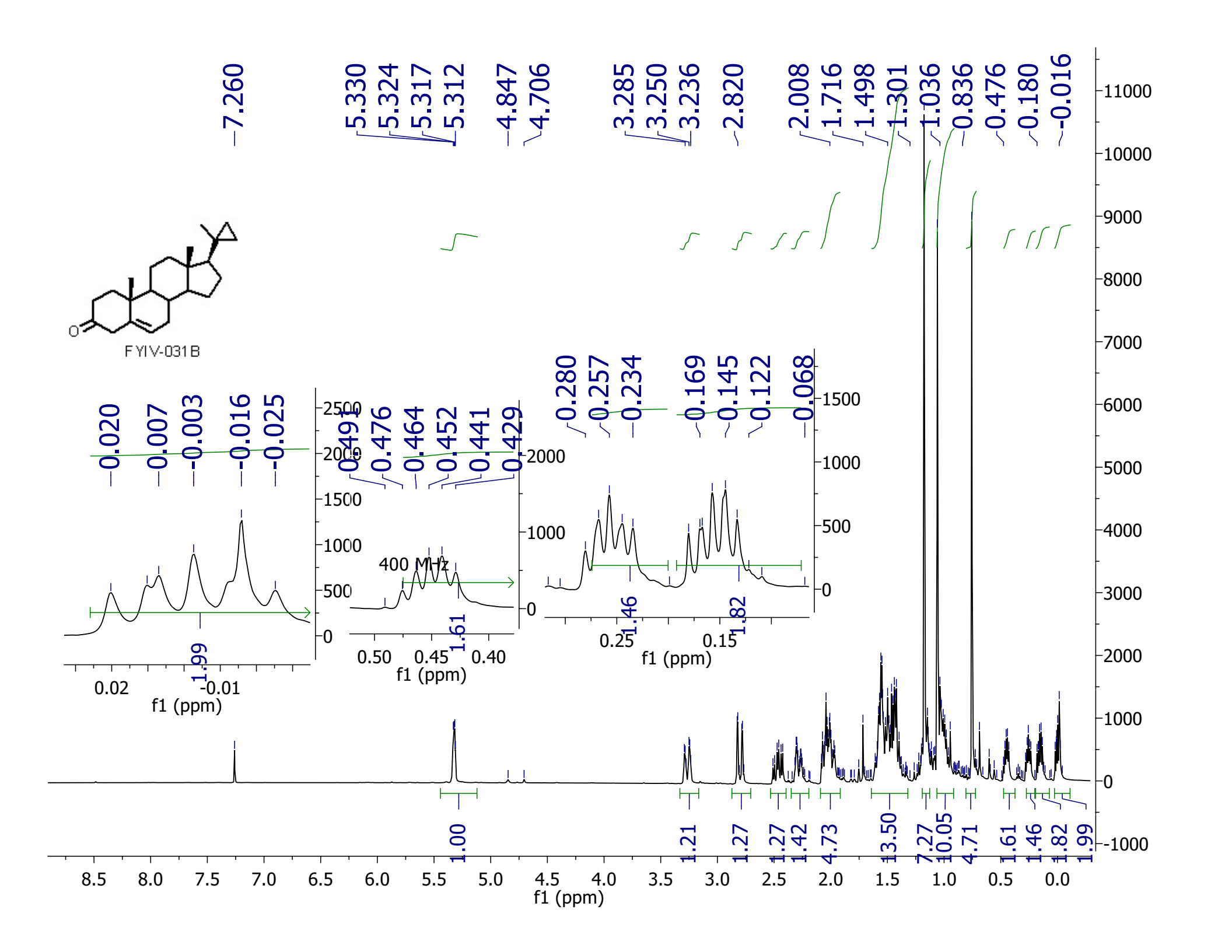

110
100
90
80
70
60
50
40
30
20
10
0
-10
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

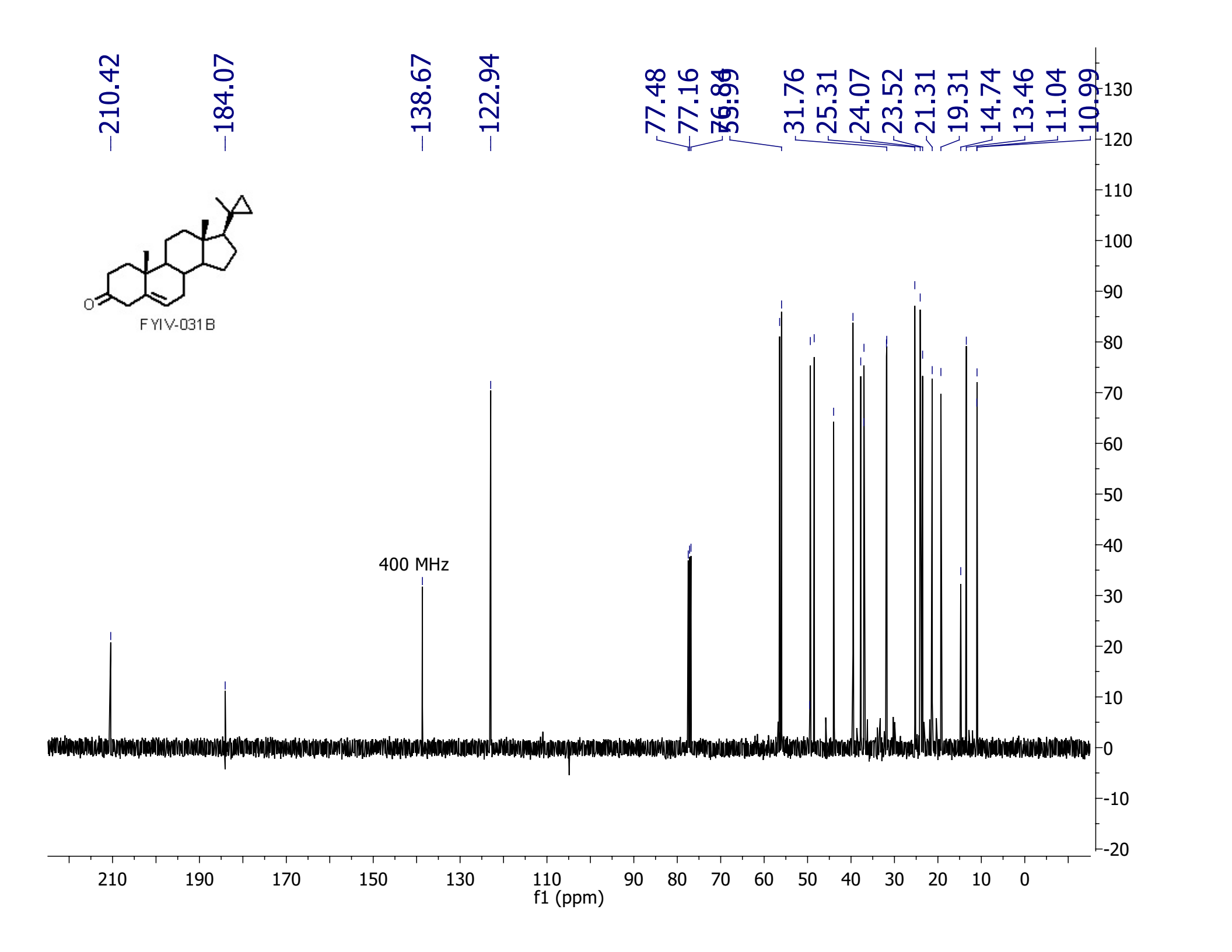

AcO
FYIV-070B
78
400 MHz
5.44
5.37
5.36
5.30
4.60
4.56
4.20
3.66
2.32
1.70
1.54
1.45
1.21
1.06
0.92
0.73
0.30
0.14
-0.01
1.04
1.04
2.63
12.01
3.16
1.00
1.55
f1 (ppm)

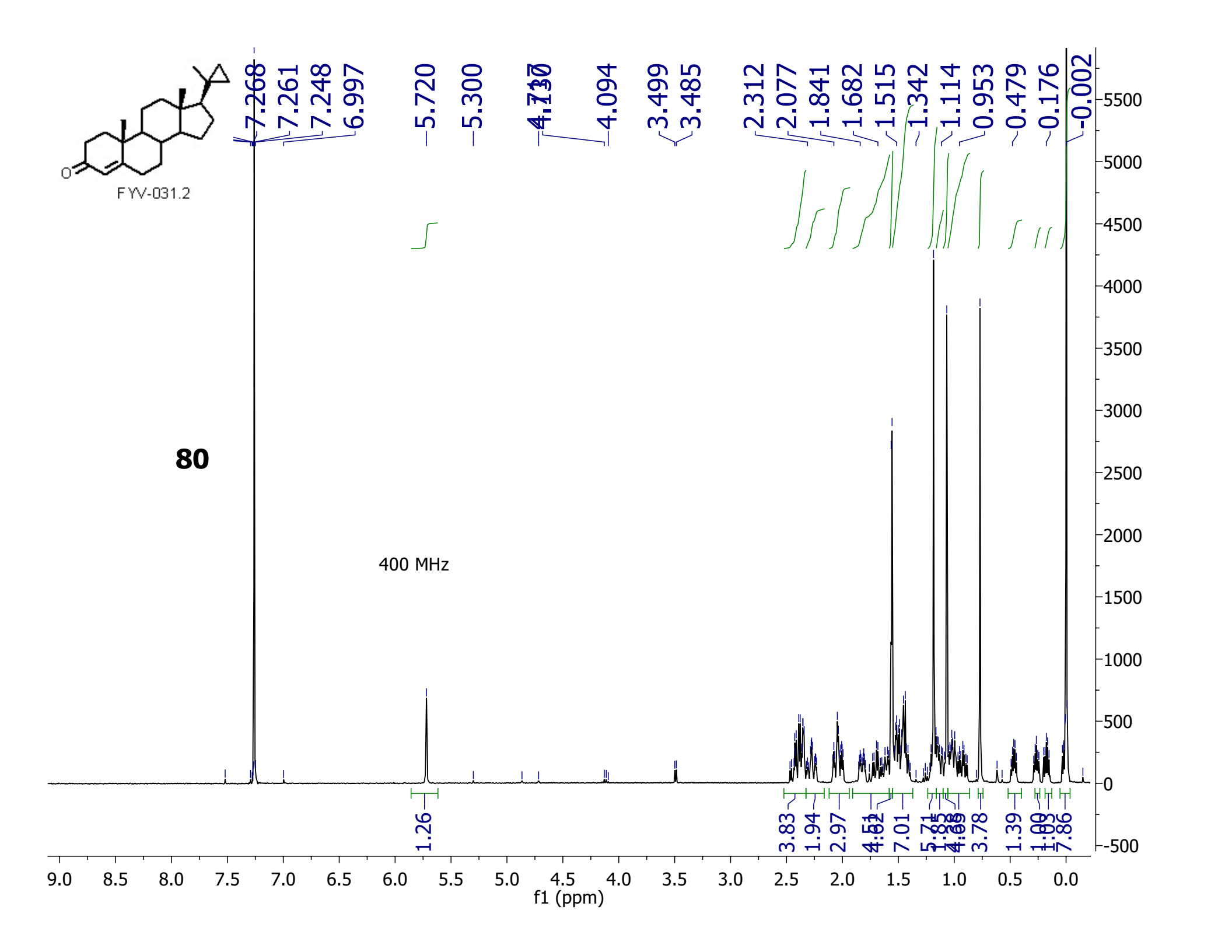

AcO
FYIV-070B
170.71
139.80
122.74
77.48
77.16
76.84
74.11
56.54
25.33
24.11
23.55
21.61
21.01
19.48
14.79
13.45
11.06
11.01
78
400 MHz
f1 (ppm)

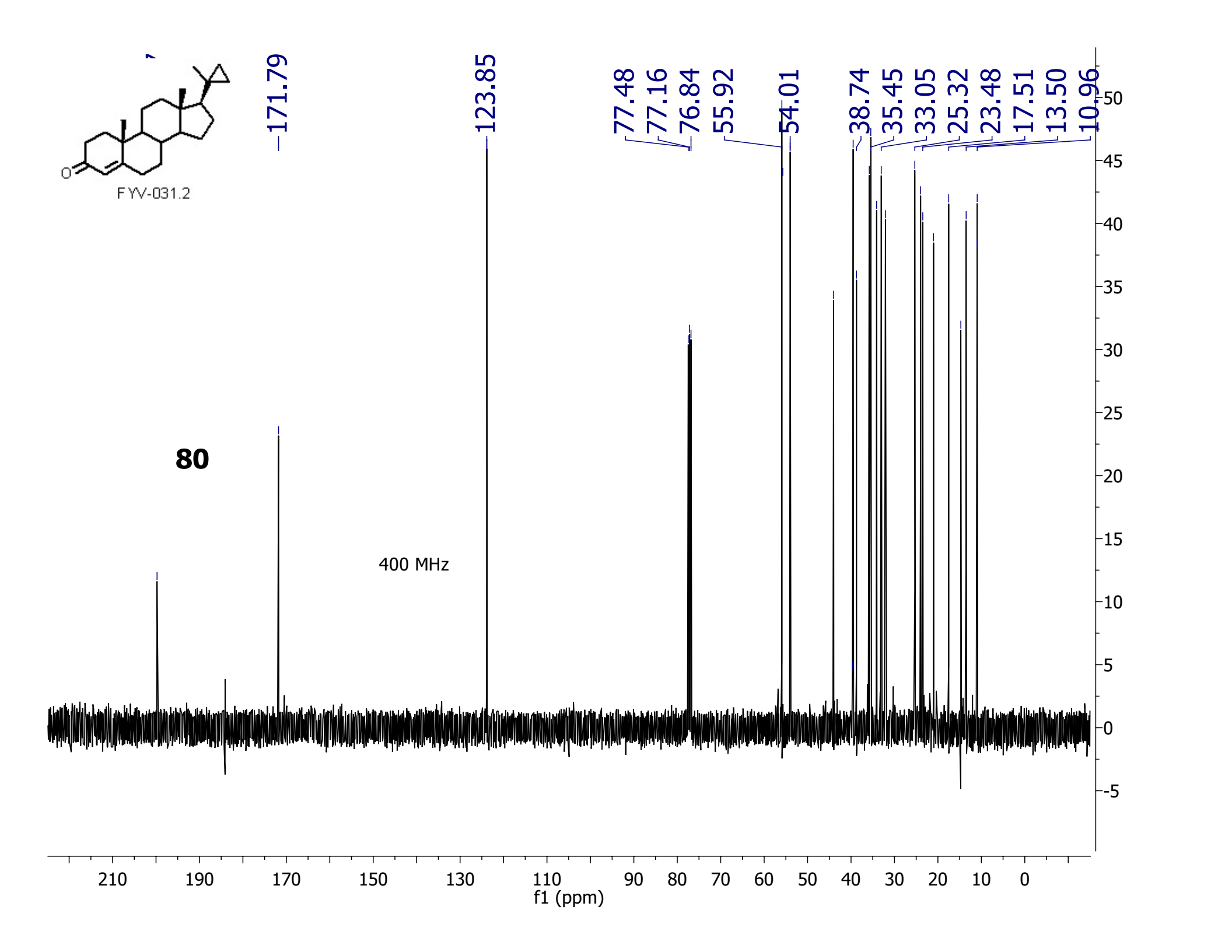

HO
FYIV-074B
79
400 MHz
5.35
5.30
3.52
1.57
1.46
1.39
1.15
1.04
0.93
0.86
0.74
0.68
0.44
0.15
-0.00
1.18
1.00
3.10
3.02
3.08
13.27
2.94
0.71
0.71
0.75
2.37
f1 (ppm)

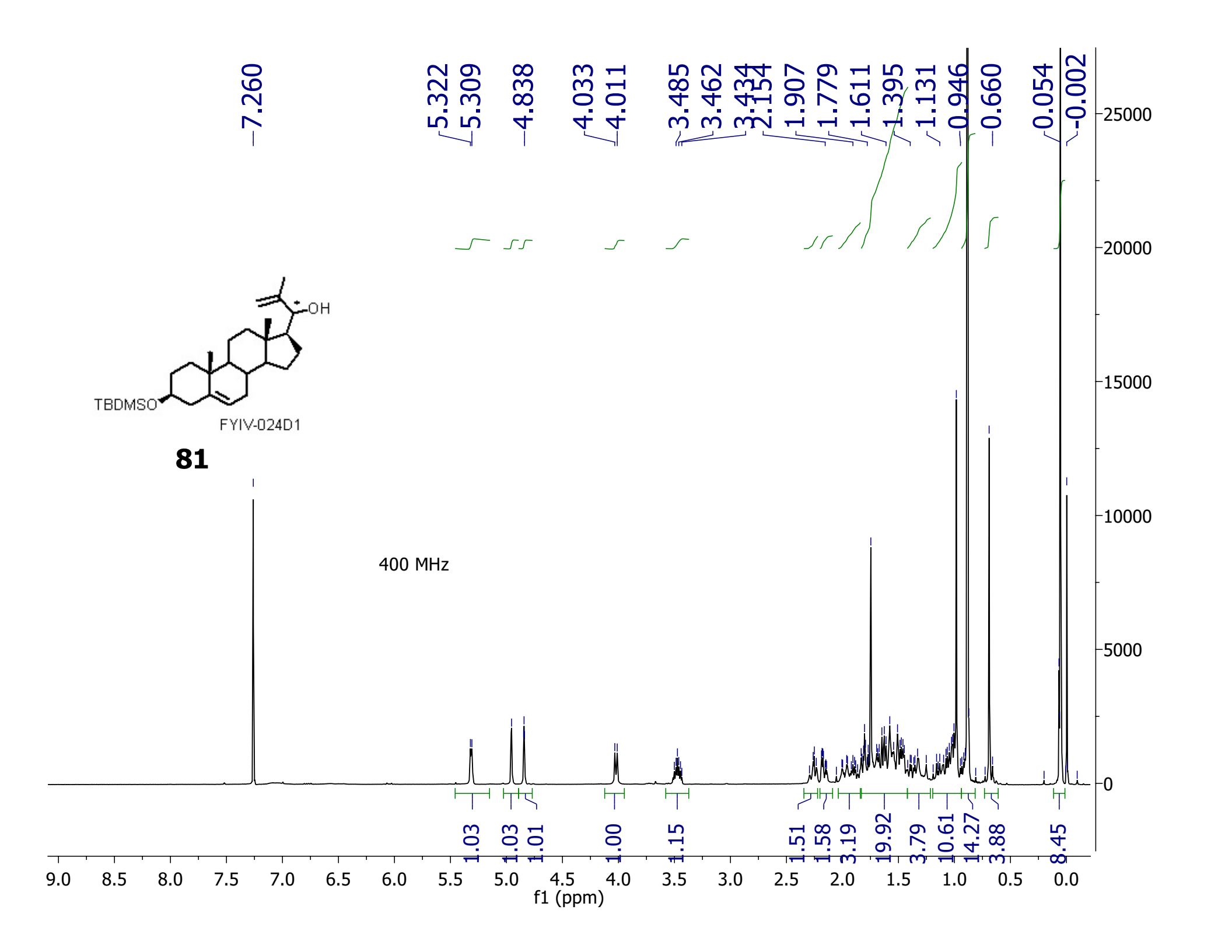

HO
FYIV-074B
140.92
121.83
77.48
77.16
76.84
36.68
31.81
25.34
23.57
19.58
13.46
11.03
0.15
79
400 MHz
f1 (ppm)

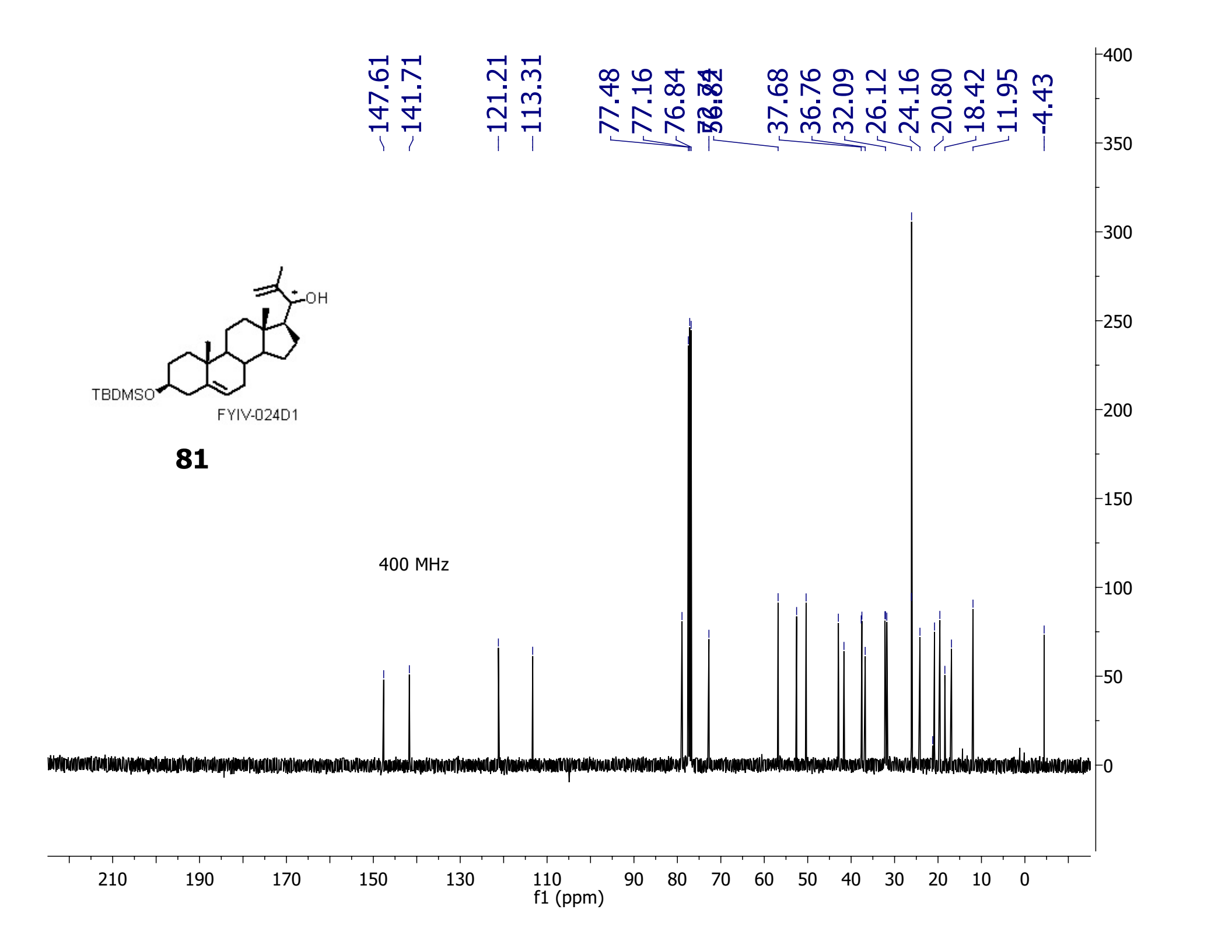

7.260
5.330
5.324
5.317
5.312
4.847
4.706
3.285
3.250
3.236
2.820
2.008
1.716
1.498
1.301
1.036
0.836
0.476
0.180
-0.016
FYIV-031B
-0.020
-0.007
-0.003
-0.016
-0.025
0.491
0.476
0.464
0.452
0.441
0.429
0.280
0.257
0.234
0.169
0.145
0.122
0.068
400 MHz
f1 (ppm)
1.00
1.21
1.27
1.27
1.42
4.73
13.50
7.27
10.05
4.71
1.61
1.46
1.82
1.99

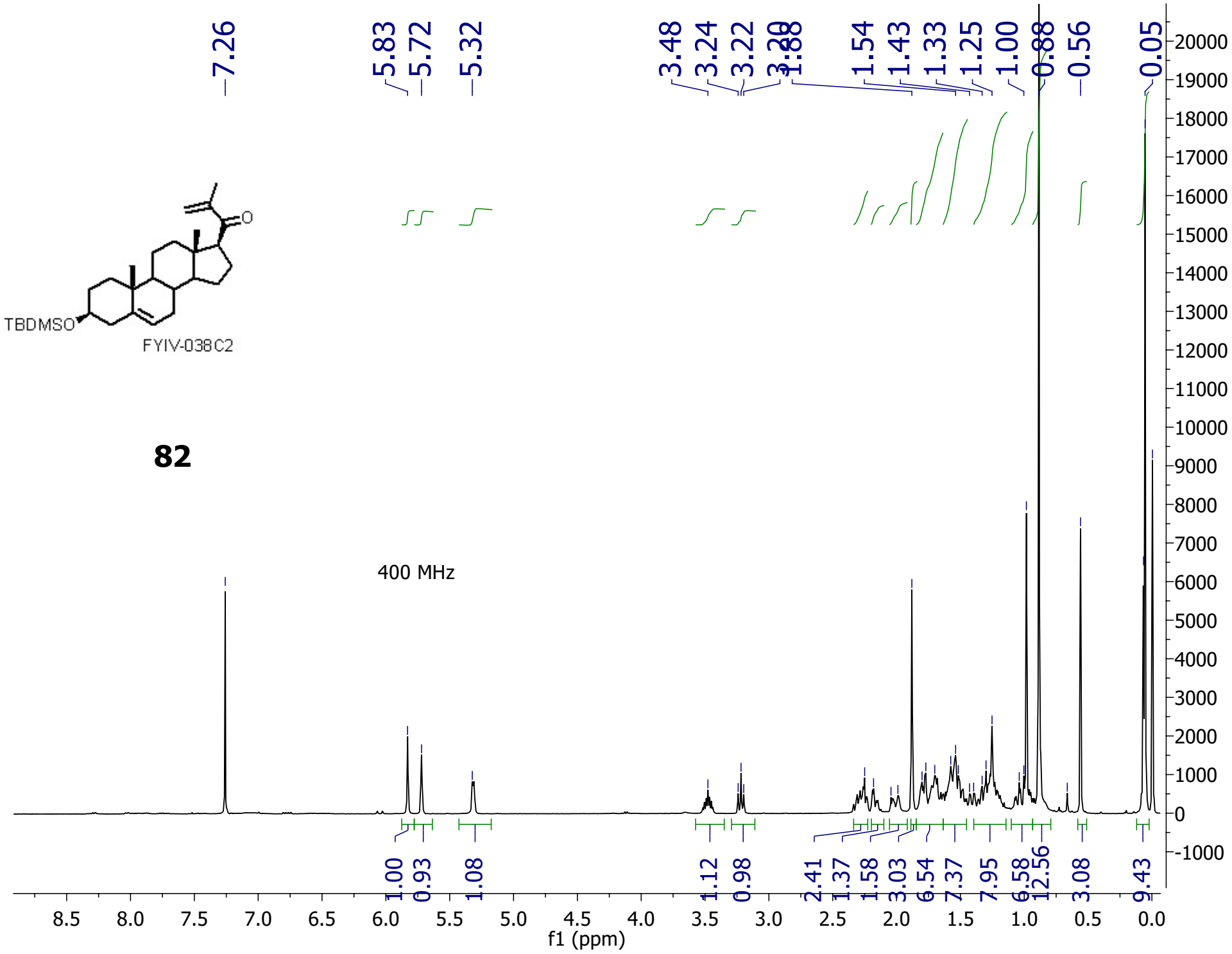

210.42
184.07
138.67
122.94
77.48
77.16
76.84
55.99
31.76
25.31
24.07
23.52
21.31
19.31
14.74
13.46
11.04
10.99
O
FYIV-031B
400 MHz
f1 (ppm)

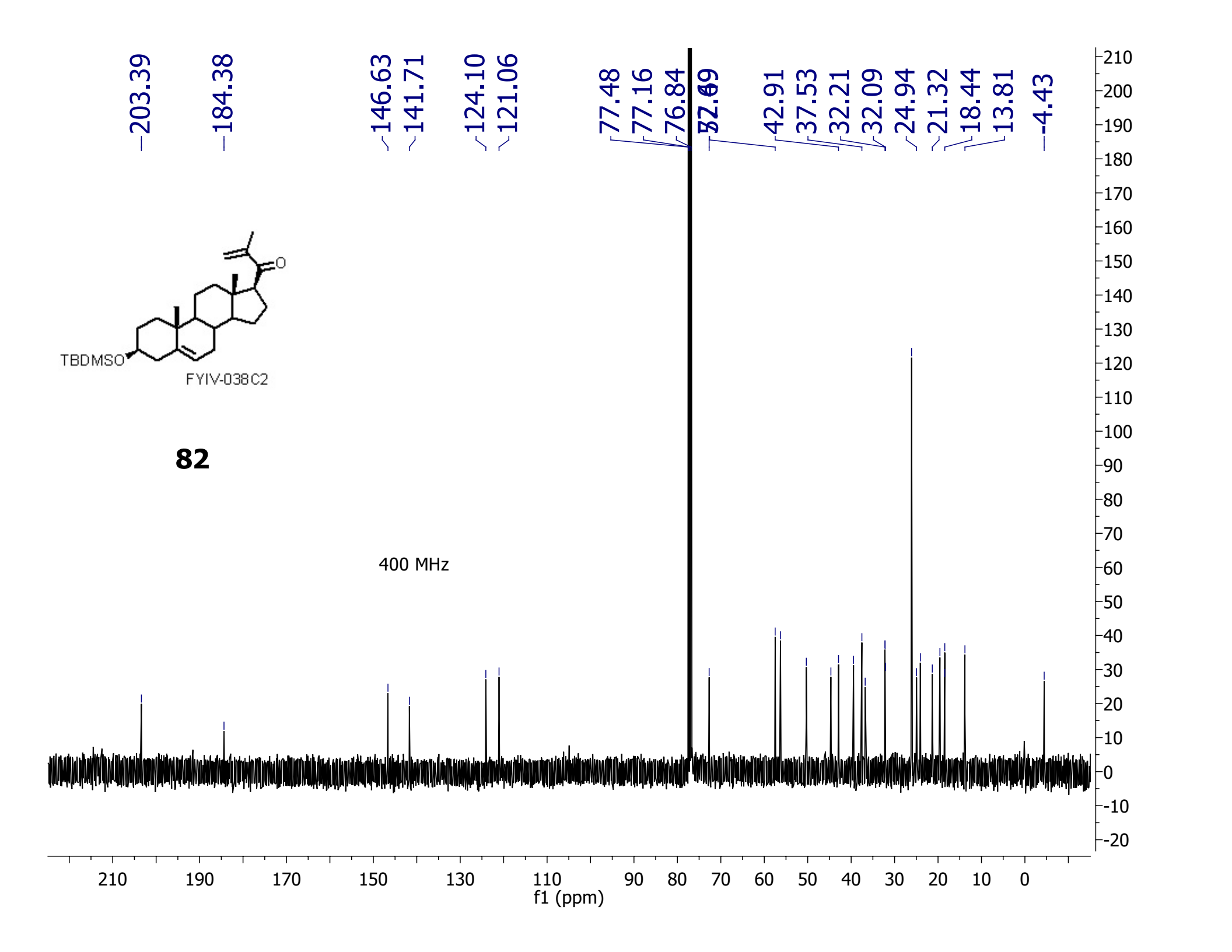

FYV-031.2
80
400 MHz
7.268
7.261
7.248
6.997
5.720
5.300
4.094
3.499
3.485
2.312
2.077
1.841
1.682
1.515
1.342
1.114
0.953
0.479
0.176
-0.002
1.26
3.83
1.94
2.97
7.01
3.78
1.39
7.86
f1 (ppm)

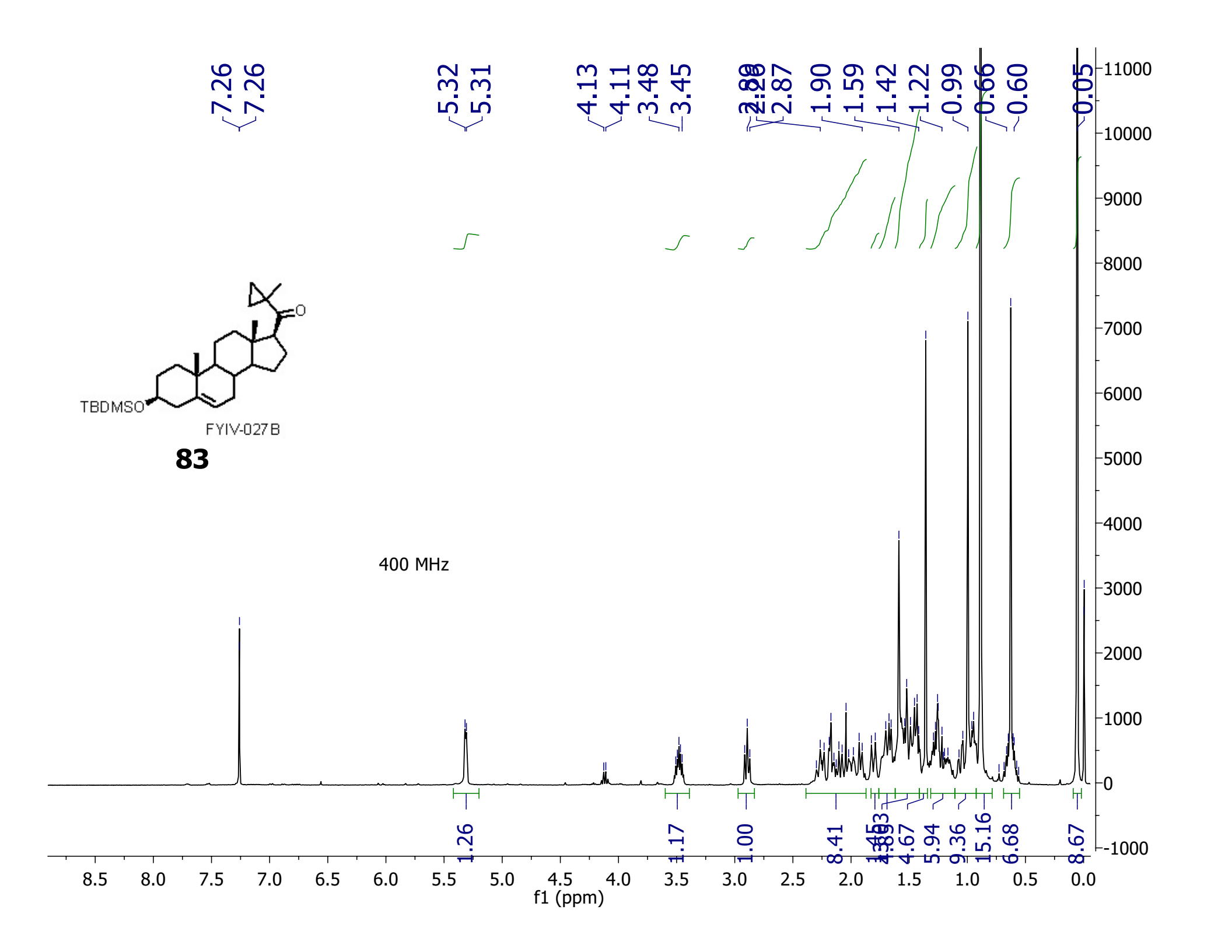

FYV-031.2
171.79
123.85
77.48
77.16
76.84
55.92
54.01
38.74
35.45
33.05
25.32
23.48
17.51
13.50
10.96
80
400 MHz
f1 (ppm)

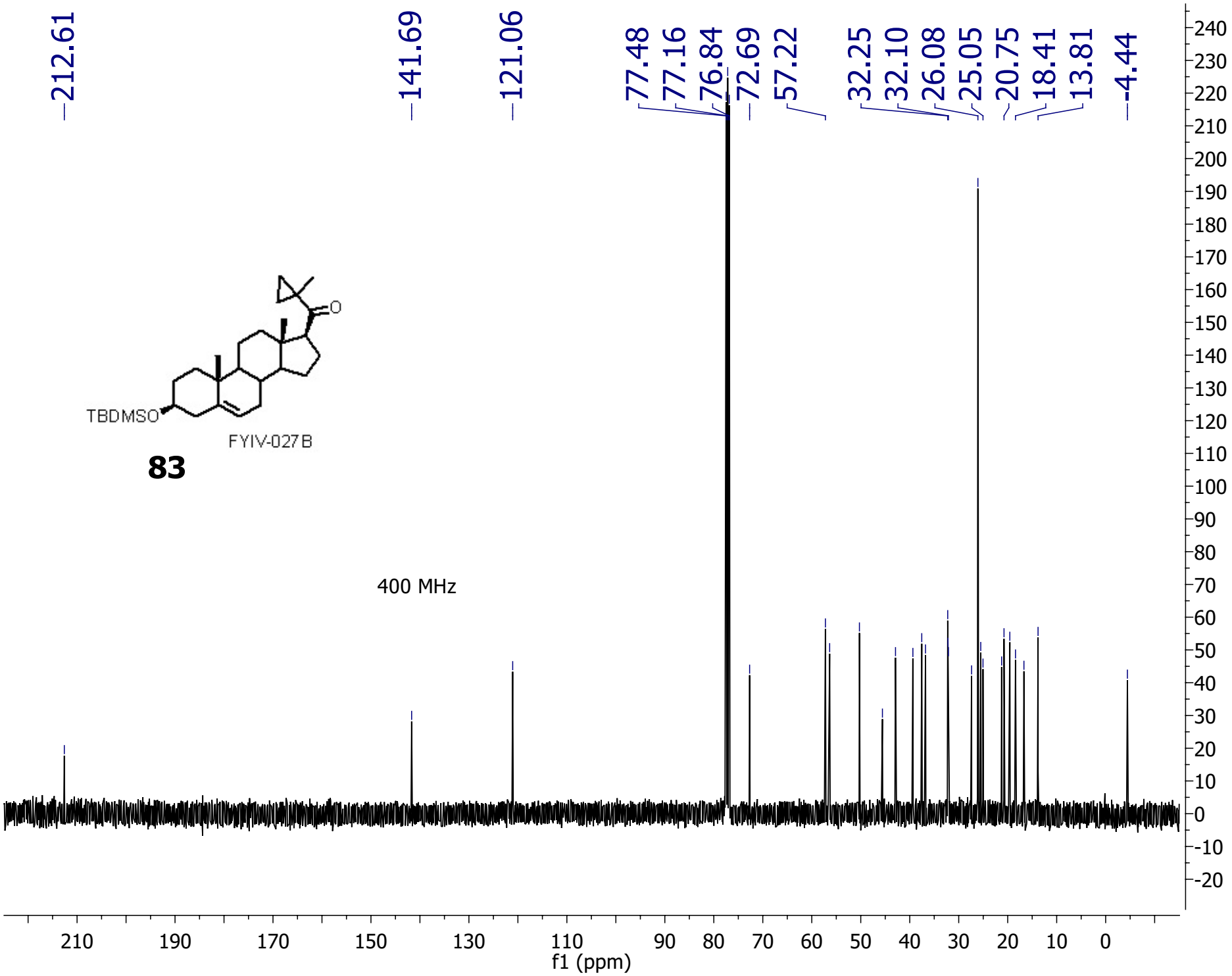

7.260
5.322
5.309
4.838
4.033
4.011
3.485
3.462
3.434
2.154
1.907
1.779
1.611
1.395
1.131
0.946
0.660
0.054
-0.002
OH
TBDMSO
FYIV-024D1
81
400 MHz
1.03
1.03
1.01
1.00
1.15
1.51
1.58
3.19
19.92
3.79
10.61
14.27
3.88
8.45
f1 (ppm)

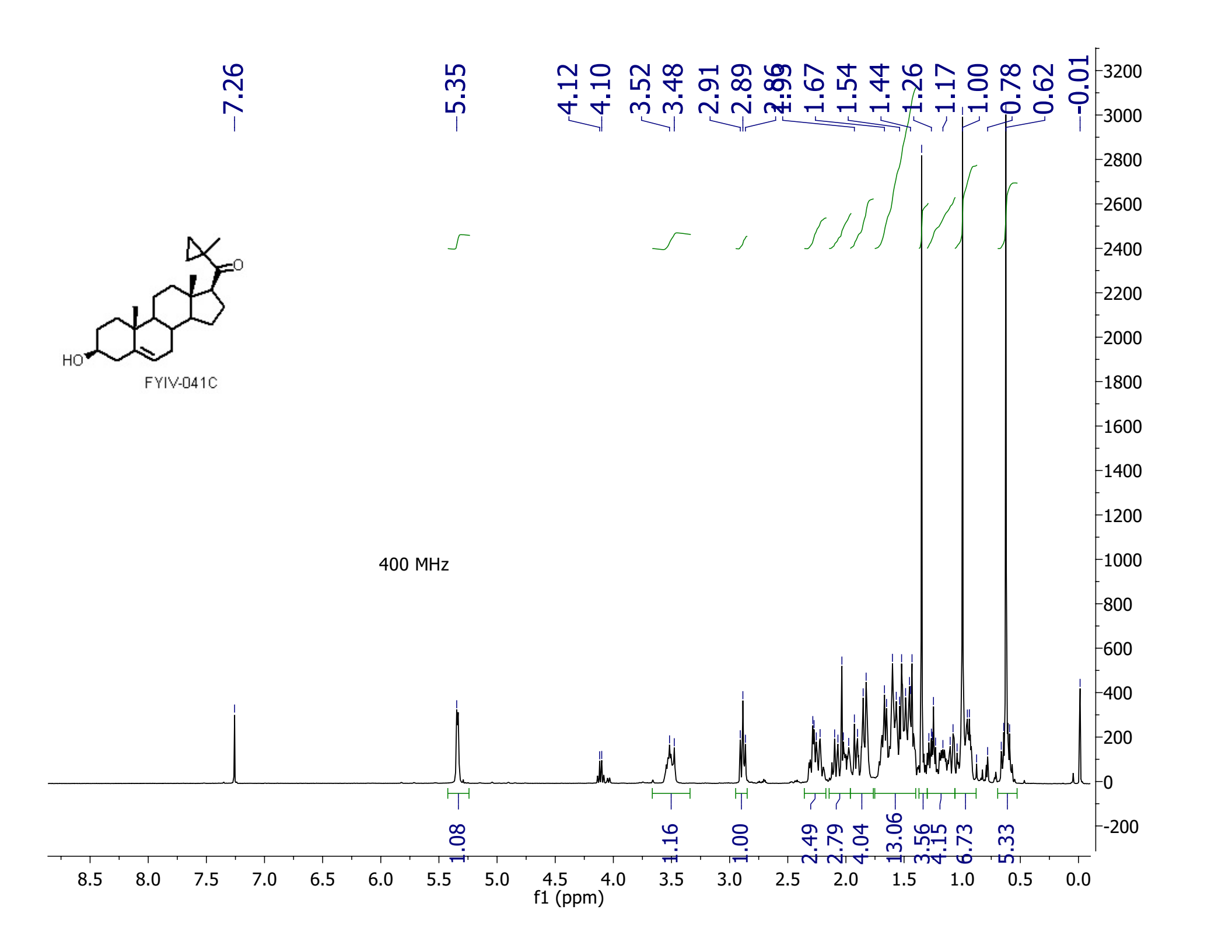

147.61
141.71
121.21
113.31
77.48
77.16
76.84
37.68
36.76
32.09
26.12
24.16
20.80
18.42
11.95
-4.43
OH
TBDMSO
FYIV-024D1
81
400 MHz
f1 (ppm)

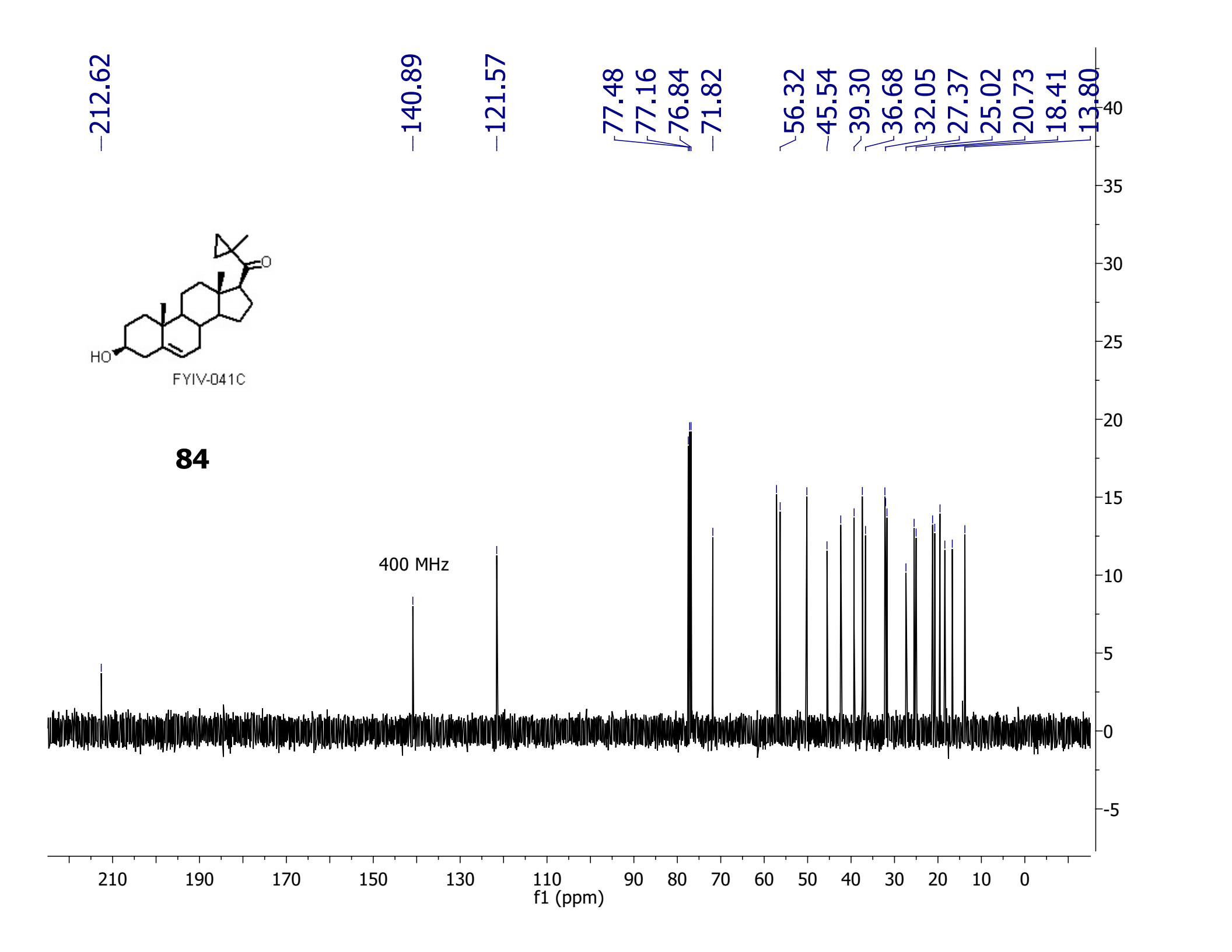

7.26
5.83
5.72
5.32
3.48
3.24
3.22
1.54
1.43
1.33
1.25
1.00
0.88
0.56
0.05
TBDMSO
O
FYIV-038C2
82
400 MHz
1.00
0.93
1.08
1.12
0.98
2.41
1.37
1.58
3.03
6.54
7.37
7.95
6.58
12.56
3.08
9.43
f1 (ppm)

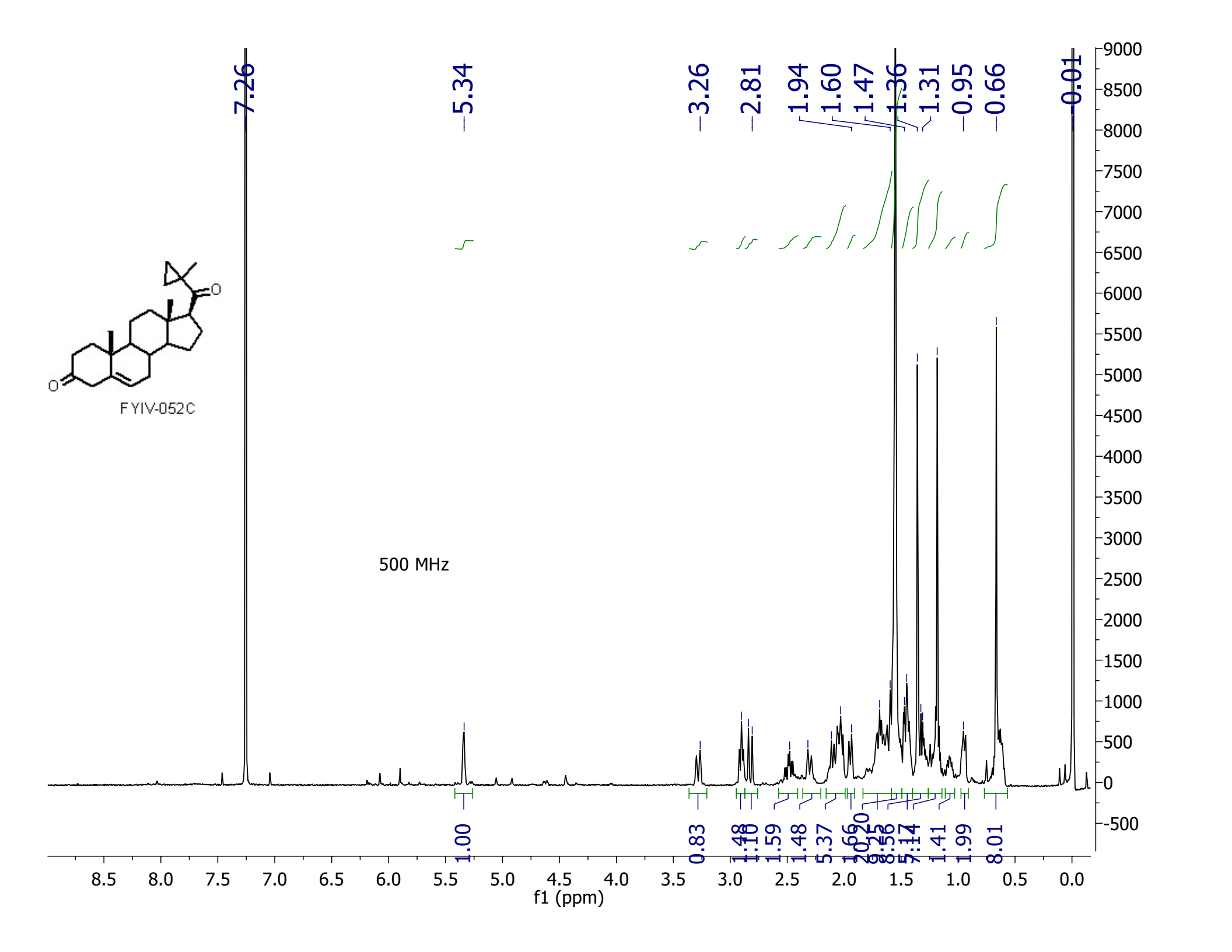

203.39
184.38
146.63
141.71
124.10
121.06
77.48
77.16
76.84
52.49
42.91
37.53
32.21
32.09
24.94
21.32
18.44
13.81
-4.43
TBDMSO
O
FYIV-038C2
82
400 MHz
f1 (ppm)

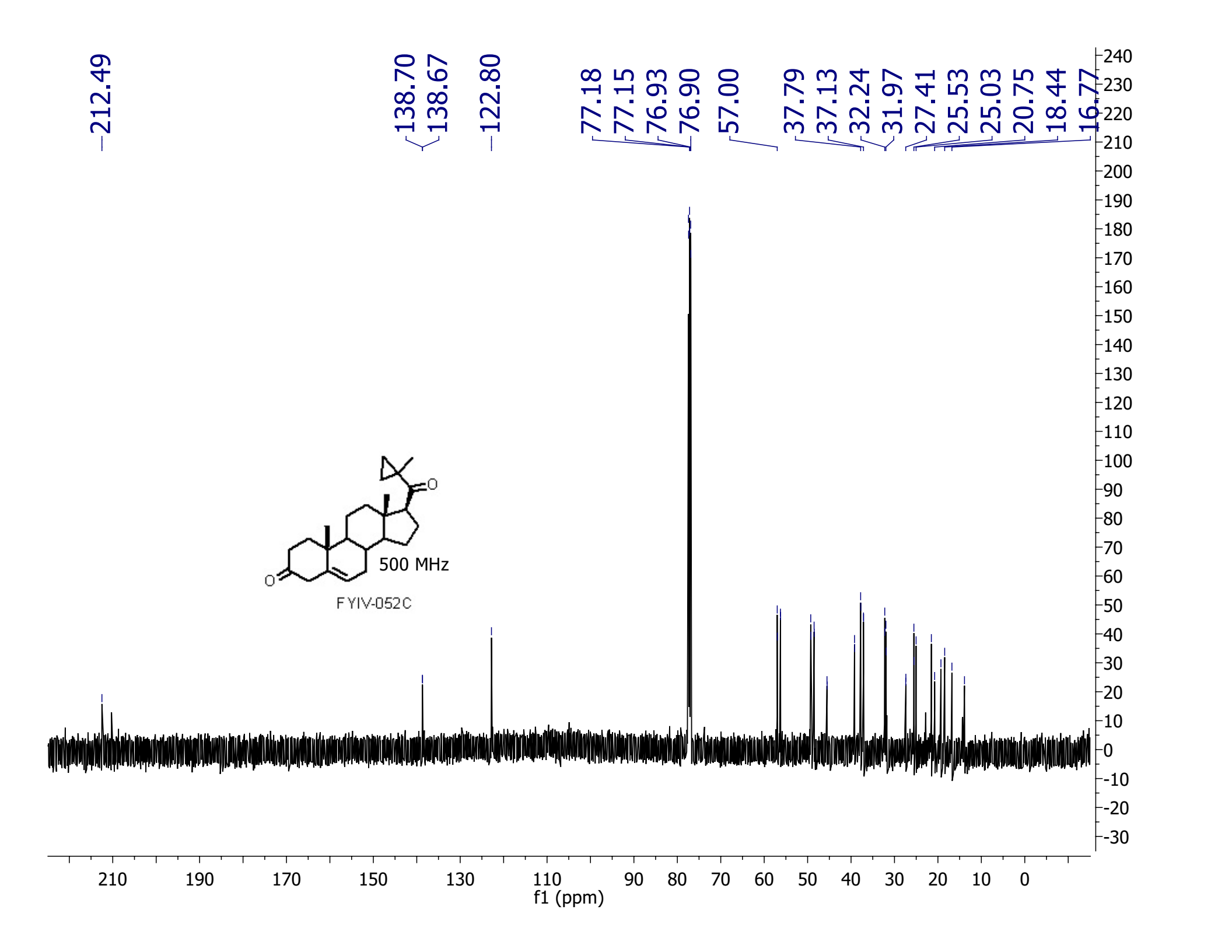

7.26
7.26
5.32
5.31
4.13
4.11
3.48
3.45
2.87
1.90
1.59
1.42
1.22
0.99
0.66
0.60
0.05
TBDMSO
O
FYIV-027B
83
400 MHz
1.26
1.17
1.00
8.41
4.67
5.94
9.36
15.16
6.68
8.67
f1 (ppm)

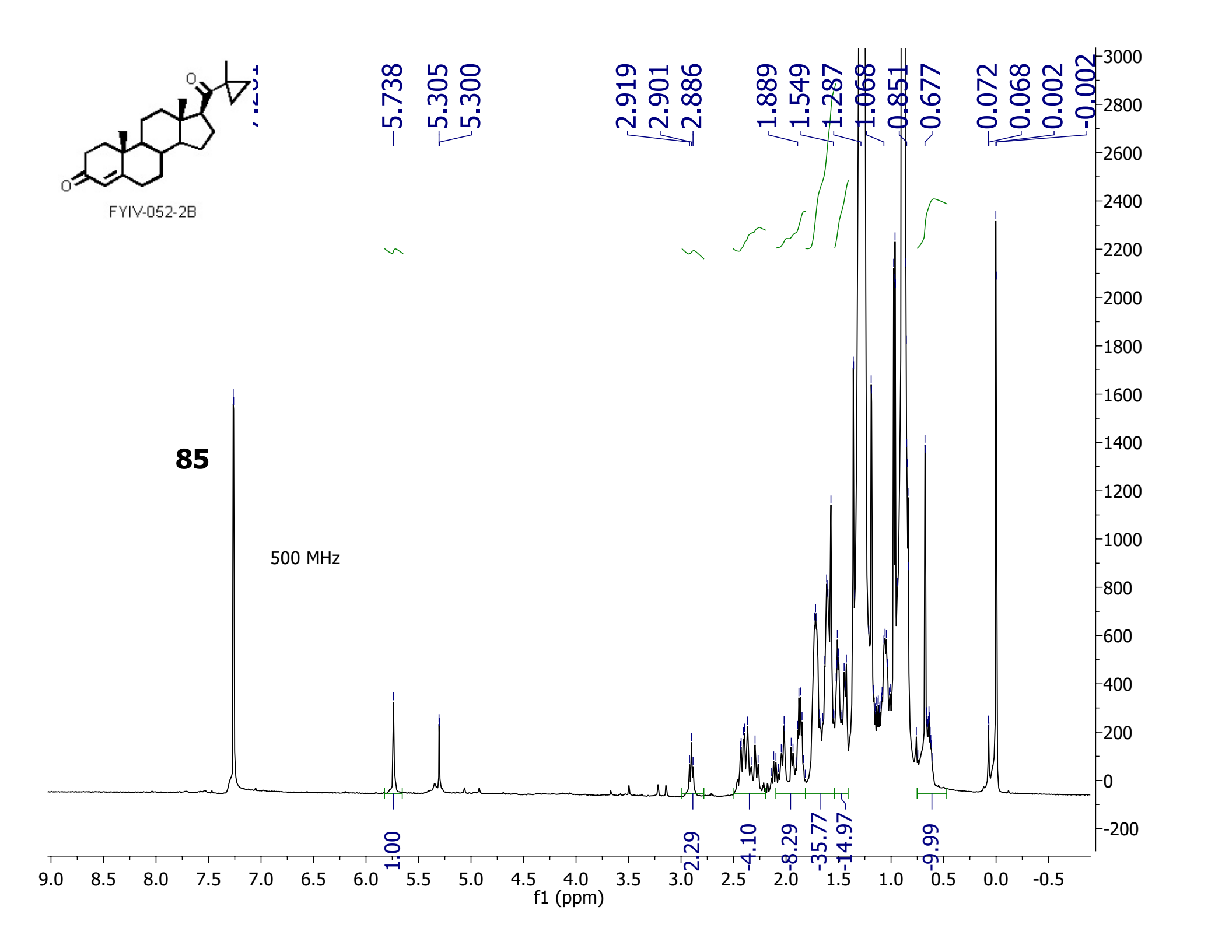

212.61
141.69
121.06
77.48
77.16
76.84
72.69
57.22
32.25
32.10
26.08
25.05
20.75
18.41
13.81
-4.44
TBDMSO
O
FYIV-027B
83
400 MHz
f1 (ppm)

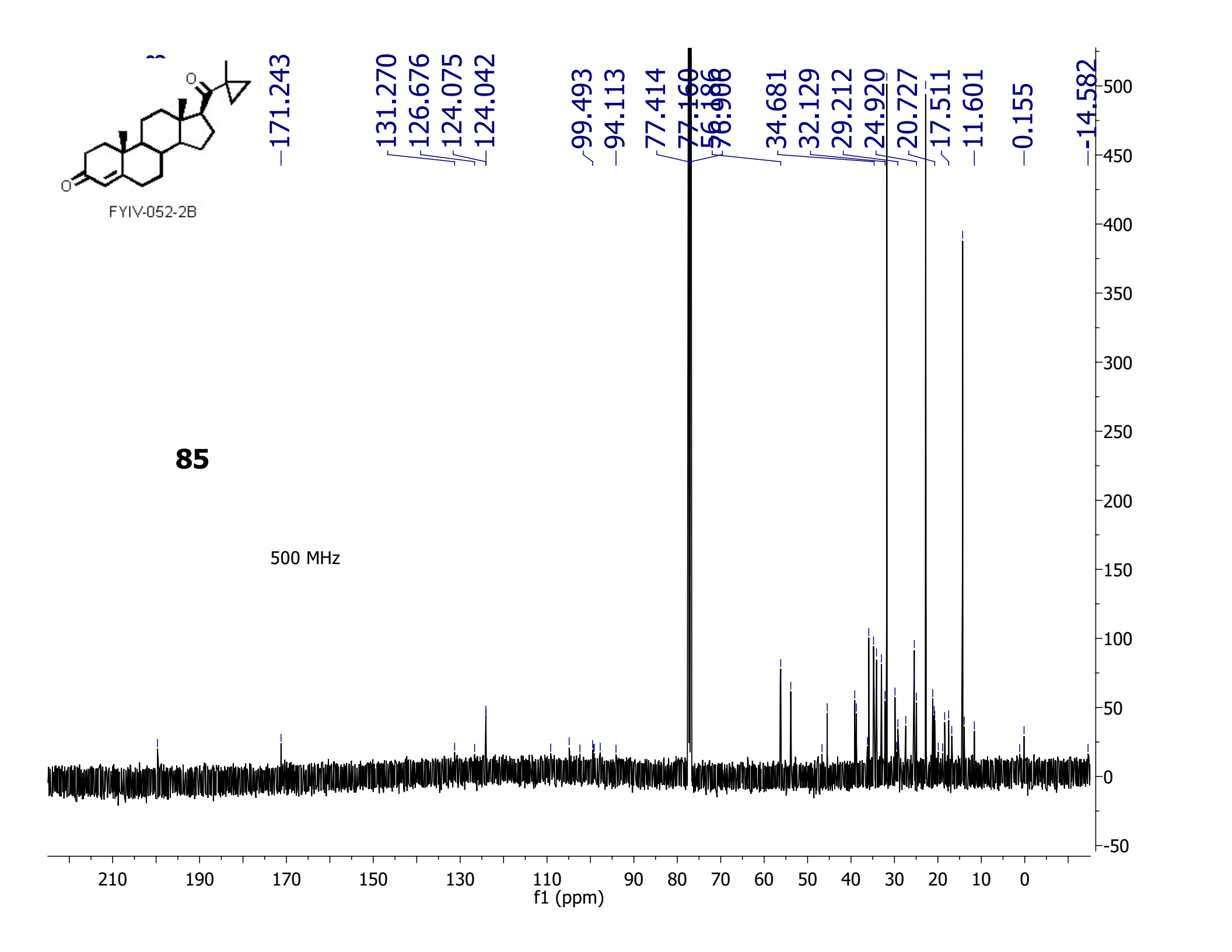

7.26
5.35
4.12
4.10
3.52
3.48
2.91
2.89
2.86
1.67
1.54
1.44
1.26
1.17
1.00
0.78
0.62
-0.01
HO
O
FYIV-041C
400 MHz
1.08
1.16
1.00
2.49
2.79
4.04
13.06
3.56
4.15
6.73
5.33
f1 (ppm)

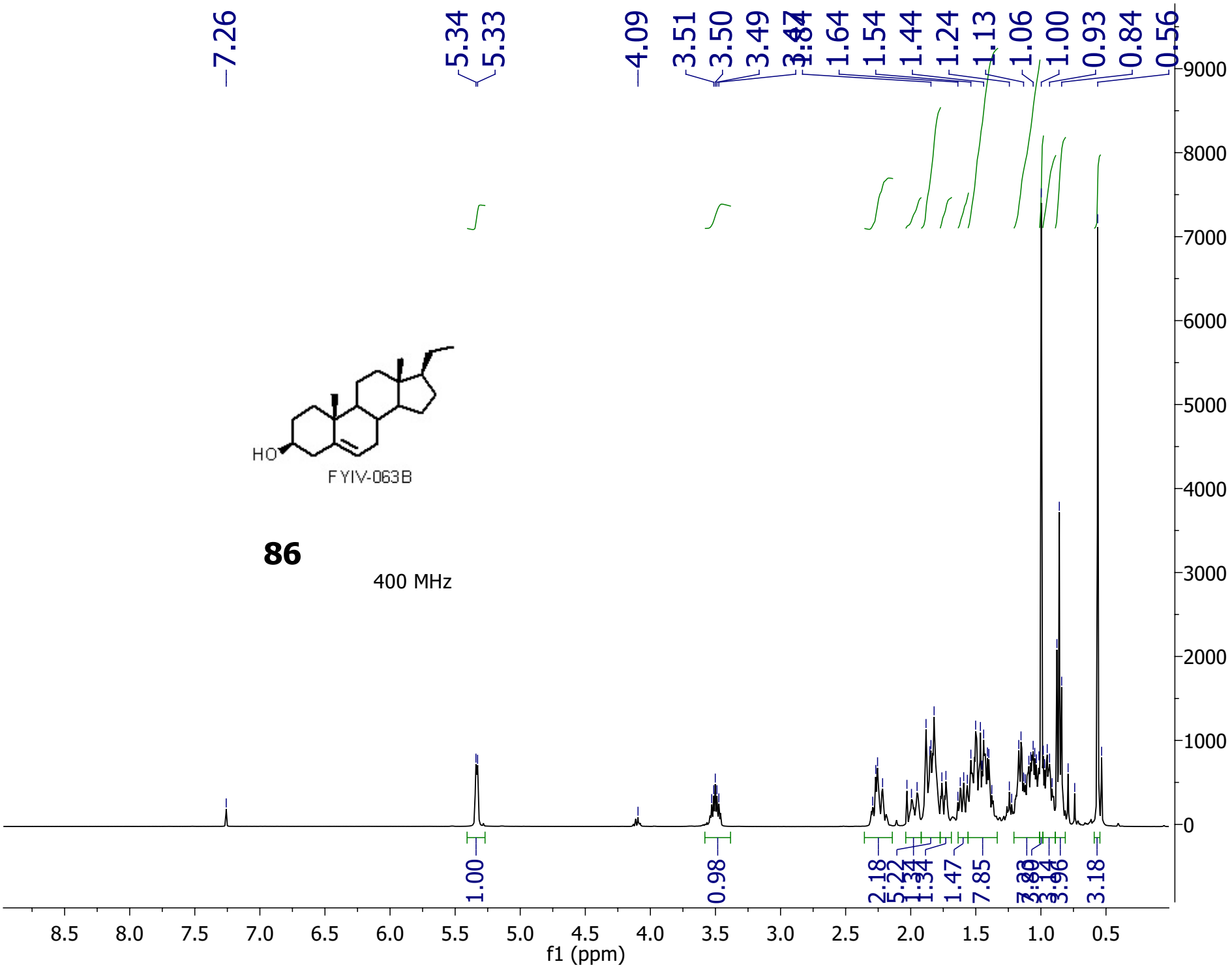

212.62
140.89
121.57
77.48
77.16
76.84
71.82
56.32
45.54
39.30
36.68
32.05
27.37
25.02
20.73
18.41
13.80
HO
O
FYIV-041C
84
400 MHz
f1 (ppm)

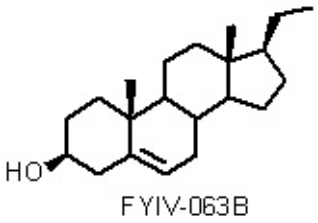

7.26
5.34
3.26
2.81
1.94
1.60
1.47
1.36
1.31
0.95
0.66
0.01
FYIV-052C
500 MHz
f1 (ppm)


212.49
138.70
138.67
122.80
77.18
77.15
76.93
76.90
57.00
37.79
37.13
32.24
31.97
27.41
25.53
25.03
20.75
18.44
16.77
O
O
500 MHz
FYIV-052C
f1 (ppm)


FYIV-052-2B
85
500 MHz
5.738
5.305
5.300
2.919
2.901
2.886
1.889
1.549
1.287
1.068
0.851
0.677
0.072
0.068
0.002
-0.002
1.00
2.29
4.10
8.29
35.77
14.97
9.99
f1 (ppm)

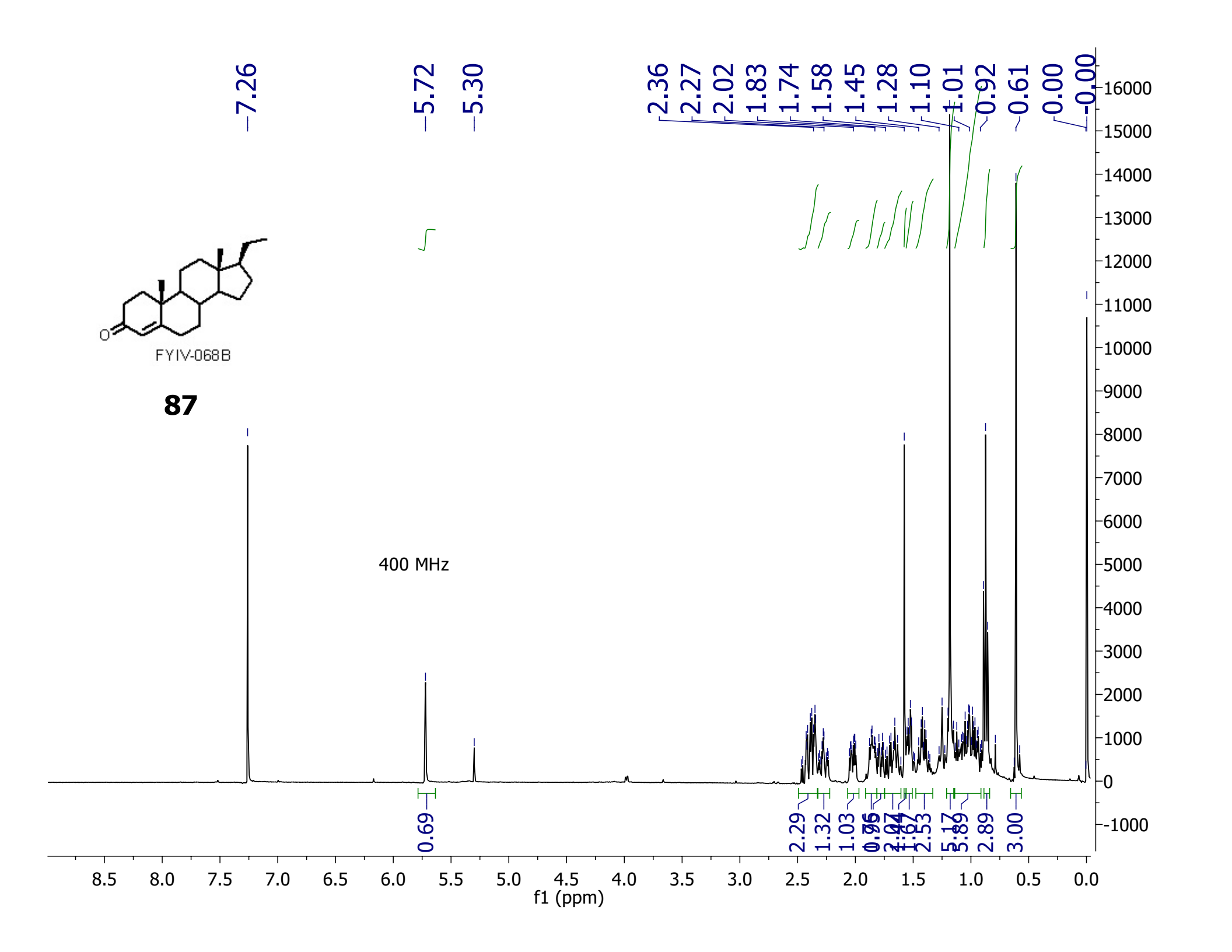

FYIV-052-2B
171.243
131.270
126.676
124.075
124.042
99.493
94.113
77.414
77.160
56.186
34.681
32.129
29.212
24.920
20.727
17.511
11.601
0.155
-14.582
85
500 MHz
f1 (ppm)

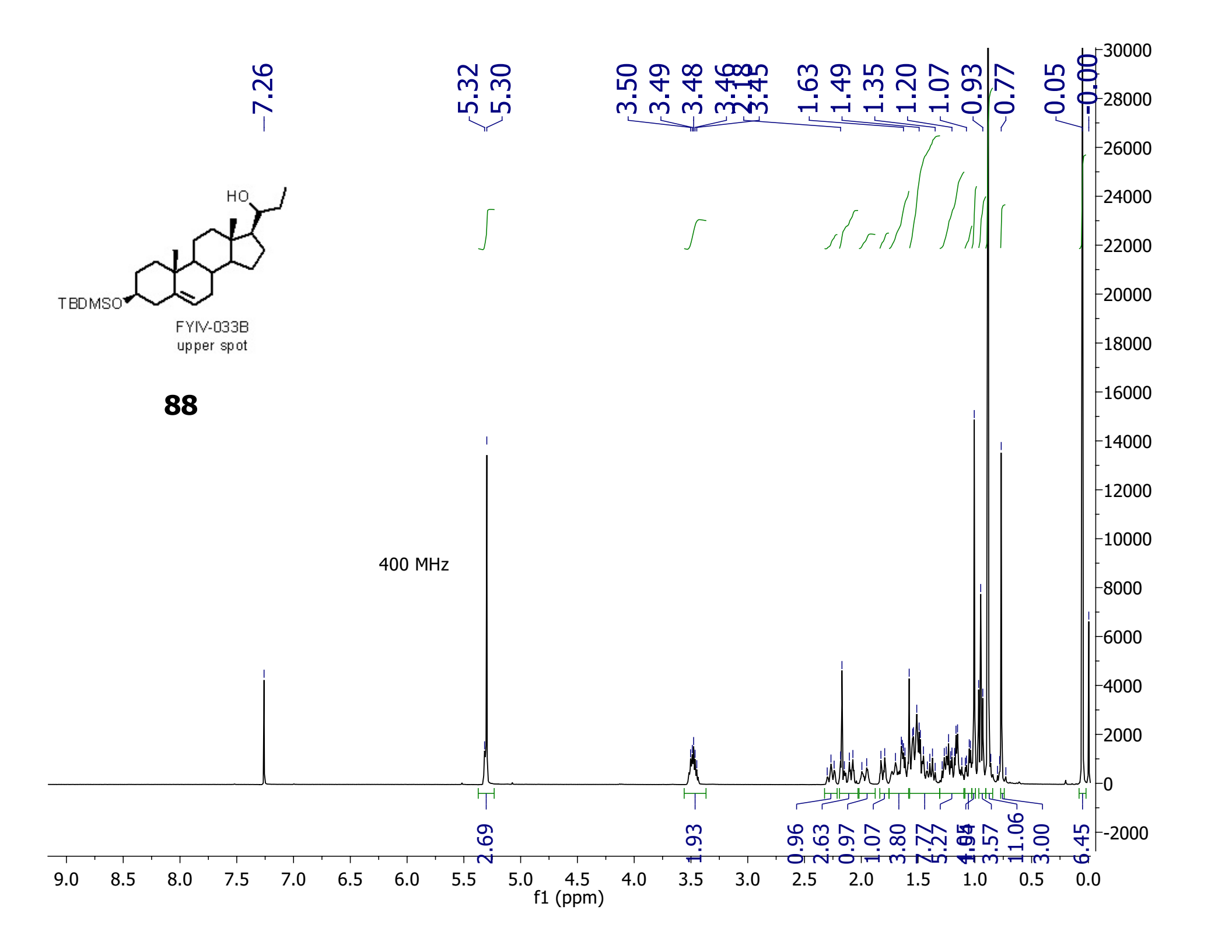

7.26
5.34
5.33
4.09
3.51
3.50
3.49
3.47
3.44
1.64
1.54
1.44
1.24
1.13
1.06
1.00
0.93
0.84
0.56
HO
FYIV-063B
86
400 MHz
1.00
0.98
2.18
5.22
1.34
1.47
7.85
3.18
f1 (ppm)

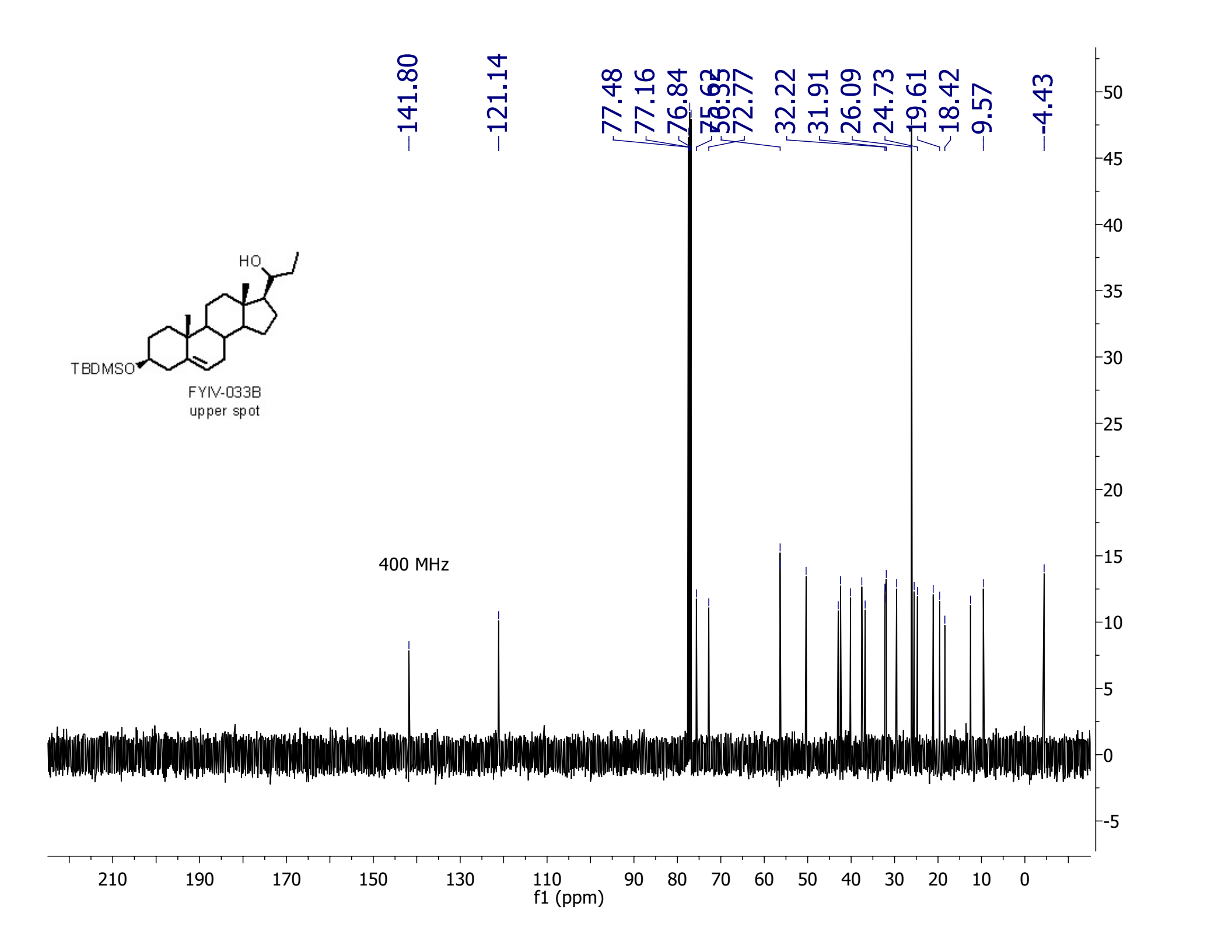

HO
FYIV-063B

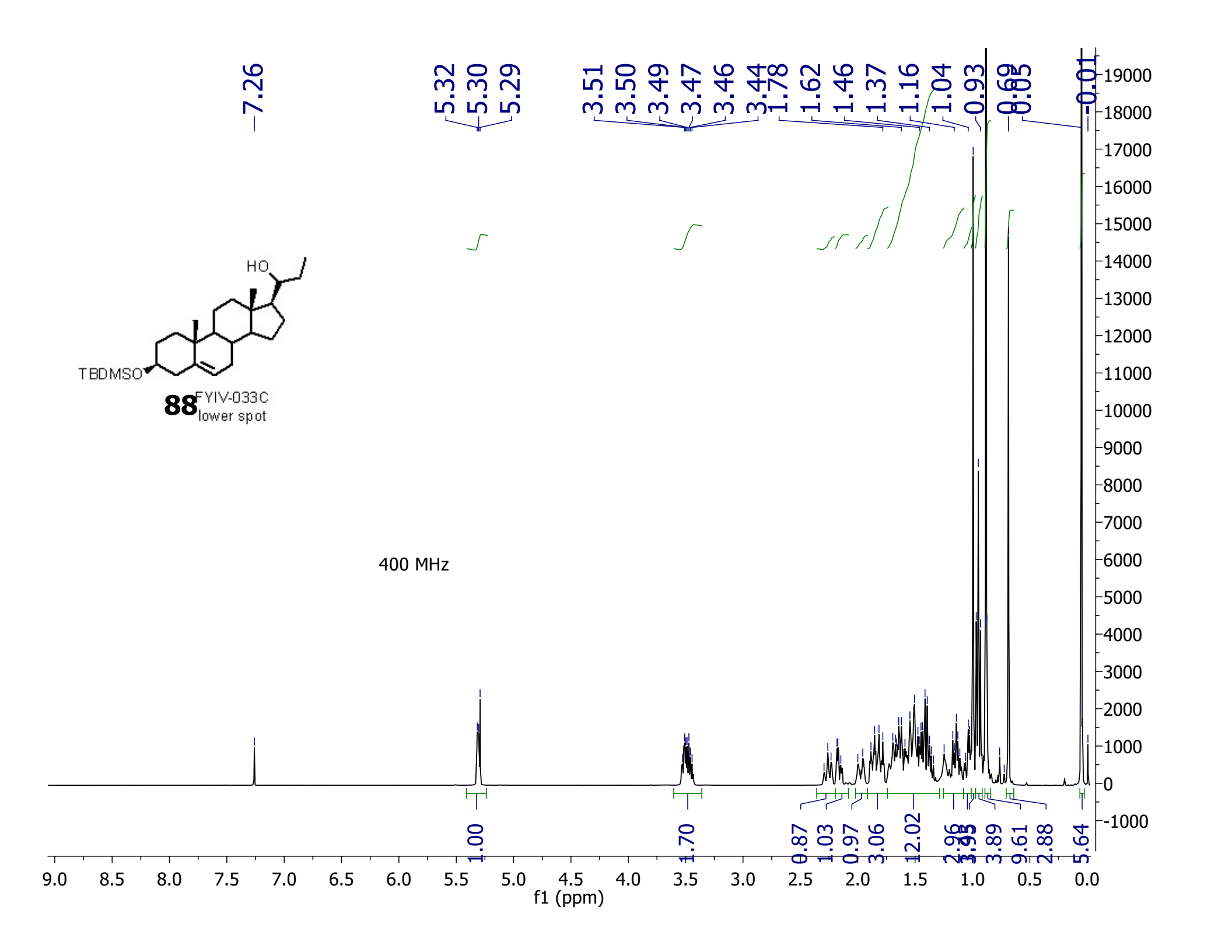
86

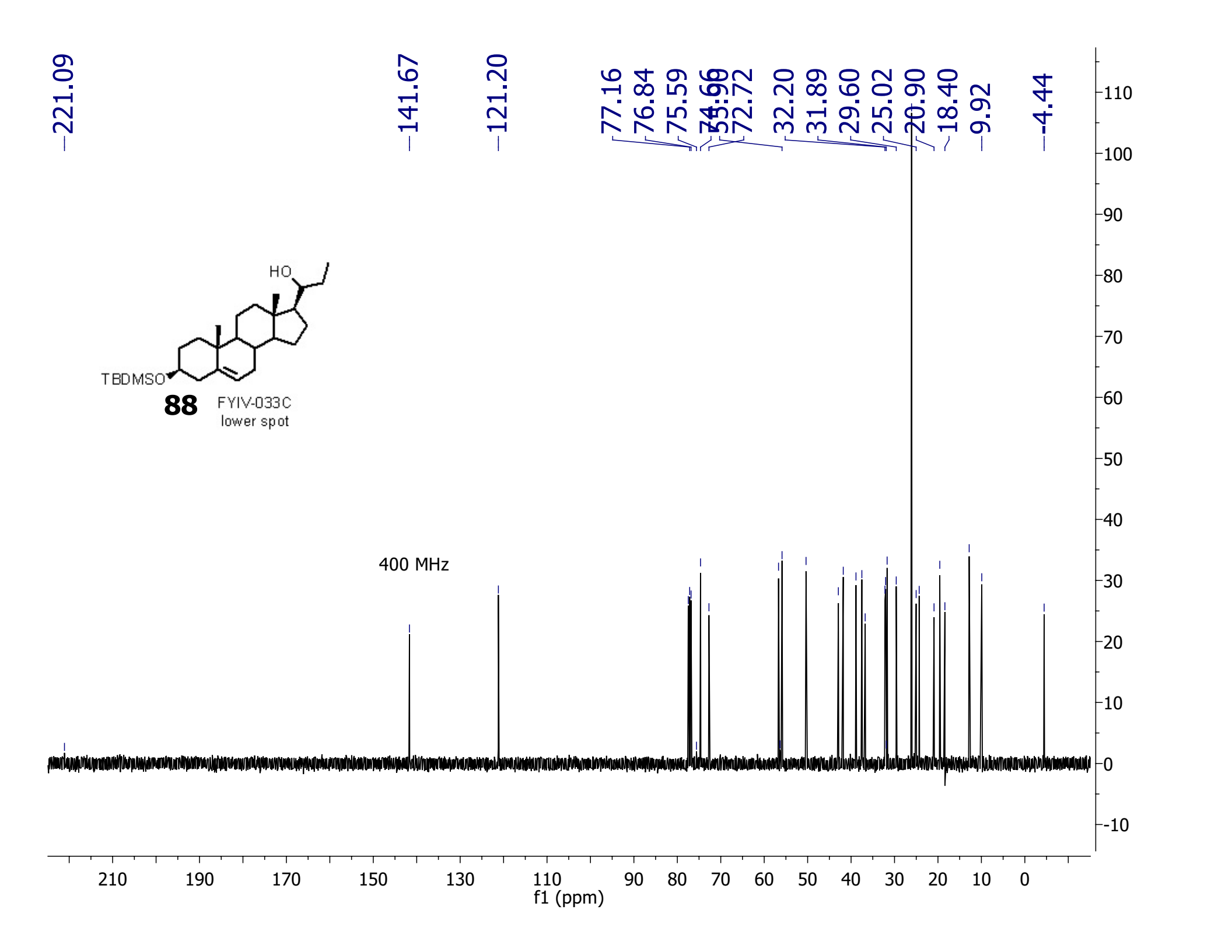
140.92
121.79
77.48
77.16
76.84
71.83
56.33
53.06
50.67
37.43
32.12
31.73
24.75
21.15
19.54
13.48
400 MHz
f1 (ppm)

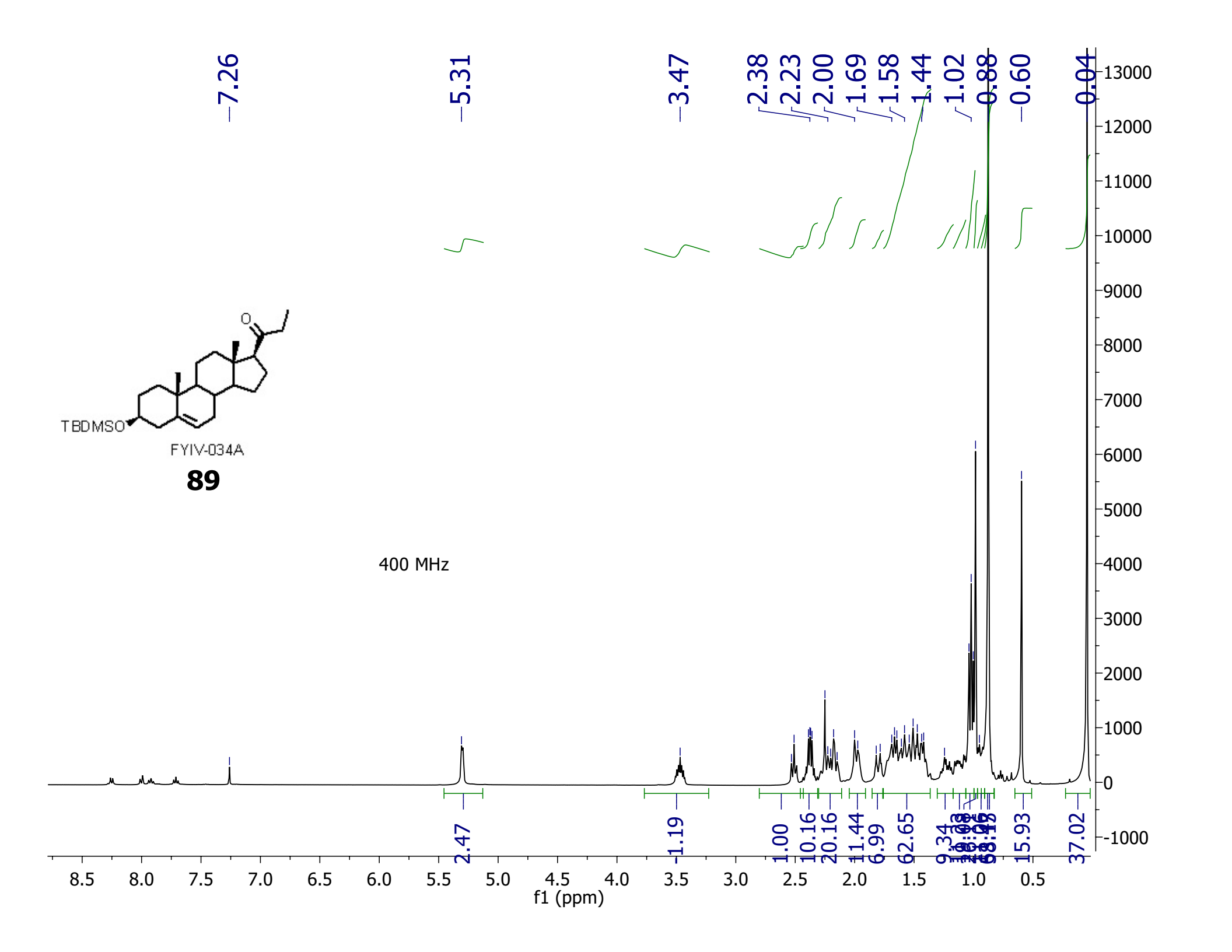

7.26
5.72
5.30
2.36
2.27
2.02
1.83
1.74
1.58
1.45
1.28
1.10
1.01
0.92
0.61
0.00
-0.00
FYIV-068B
87
400 MHz
0.69
2.29
1.32
1.03
2.53
5.17
5.89
2.89
3.00
f1 (ppm)

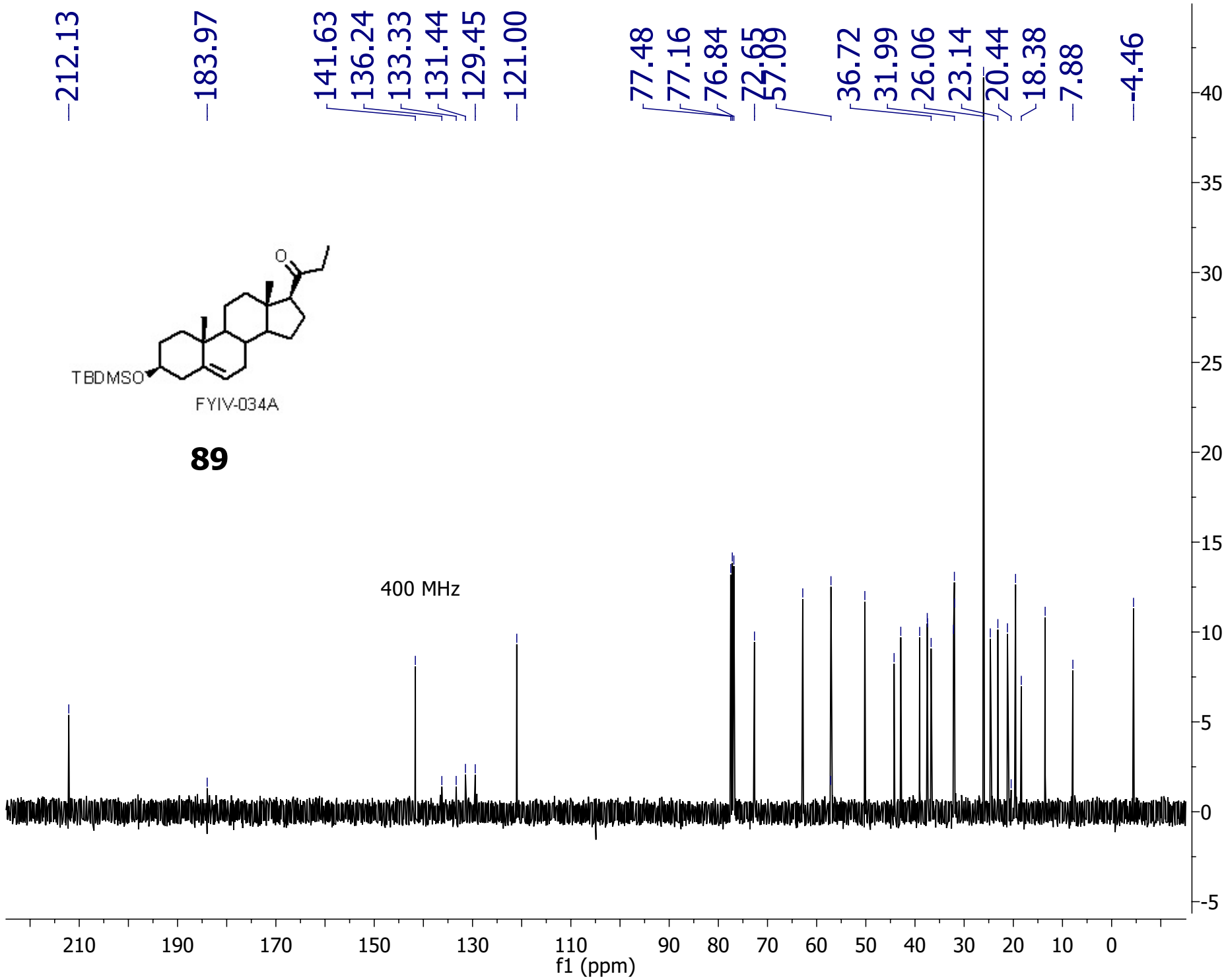

7.26
5.32
5.30
3.50
3.49
3.48
3.46
3.45
1.63
1.49
1.35
1.20
1.07
0.93
0.77
0.05
0.00
HO
TBDMSO
FYIV-033B
upper spot
88
400 MHz
f1 (ppm)

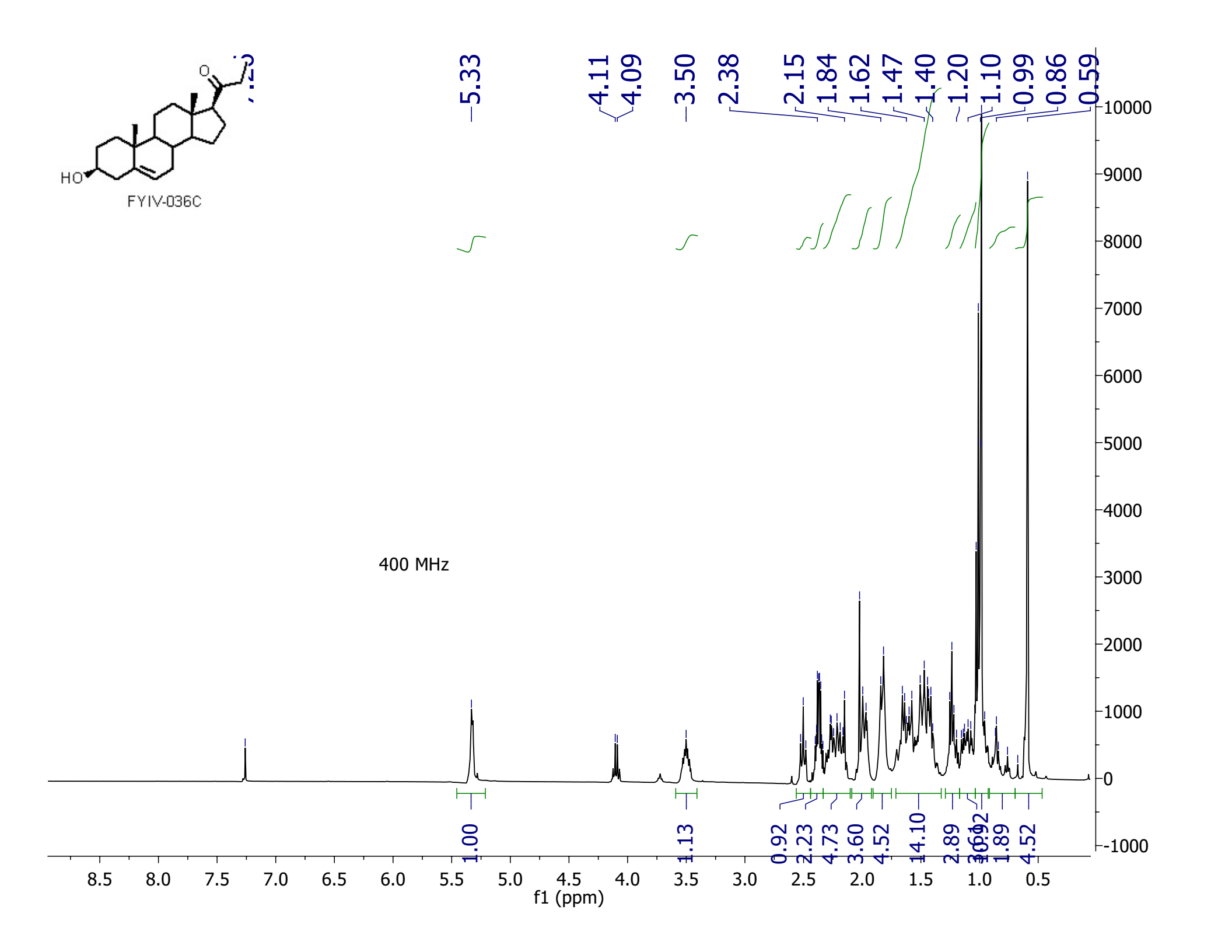

141.80
121.14
77.48
77.16
76.84
72.77
32.22
31.91
26.09
24.73
19.61
18.42
9.57
-4.43
HO
TBDMSO
FYIV-033B
upper spot
400 MHz
f1 (ppm)

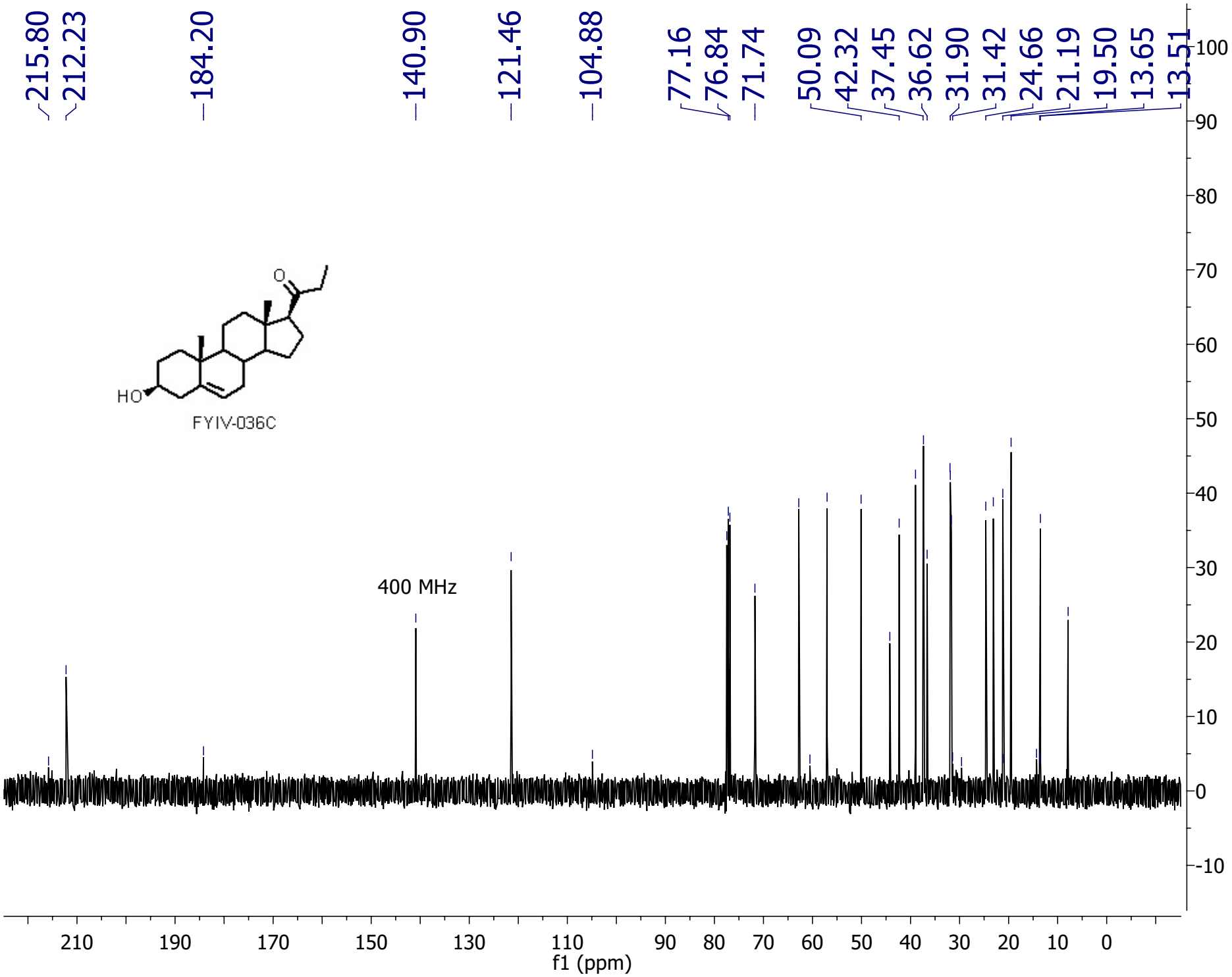

7.26
5.32
5.30
5.29
3.51
3.50
3.49
3.47
3.46
3.44
1.78
1.62
1.46
1.37
1.16
1.04
0.93
0.69
0.05
-0.01
HO
TBDMSO
88
FYIV-033C
lower spot
400 MHz
1.00
1.70
0.87
1.03
0.97
3.06
12.02
2.96
3.89
9.61
2.88
5.64
f1 (ppm)

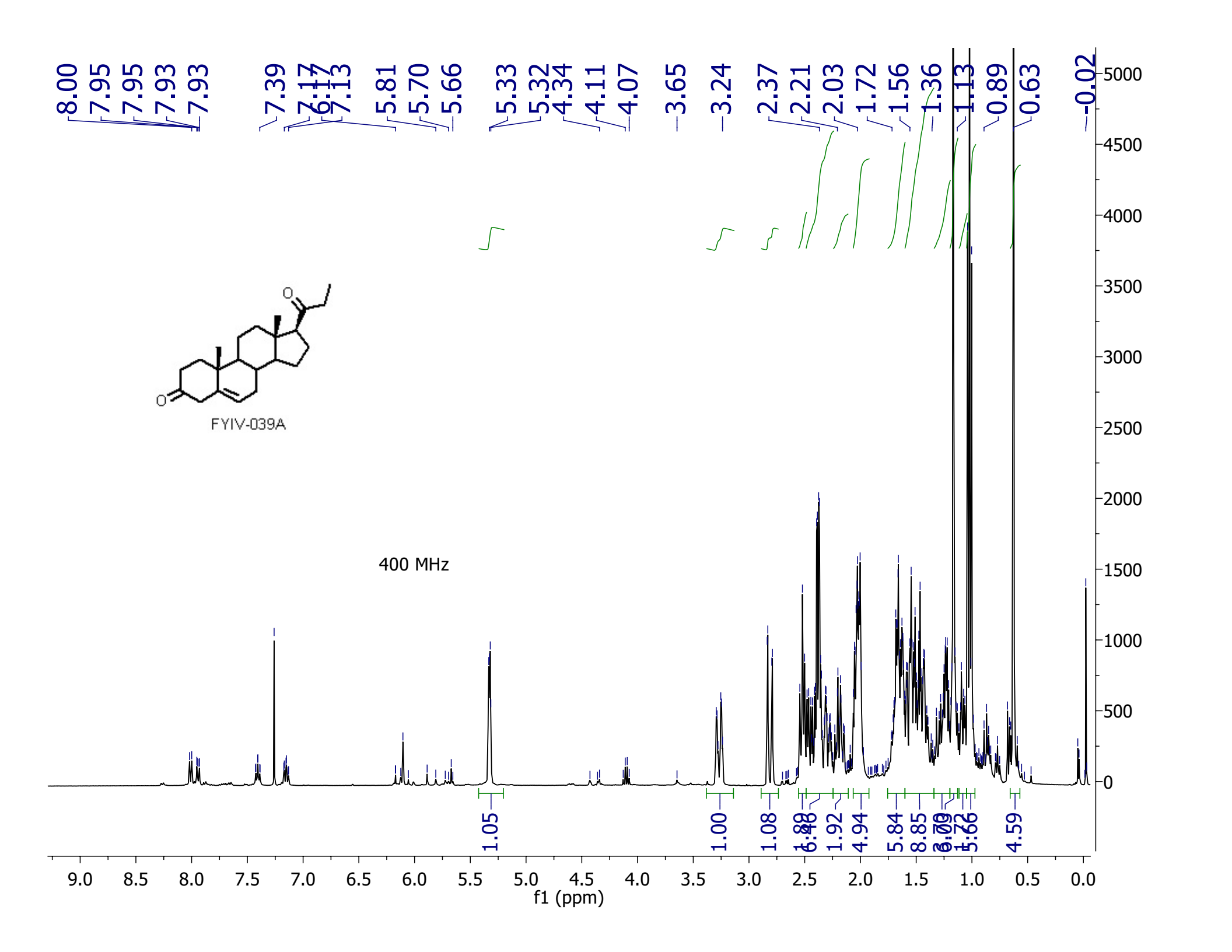

221.09
141.67
121.20
77.16
76.84
75.59
74.66
55.90
72.72
32.20
31.89
29.60
25.02
20.90
18.40
9.92
-4.44
HO
TBDMSO
88
FYIV-033C
lower spot
400 MHz
f1 (ppm)

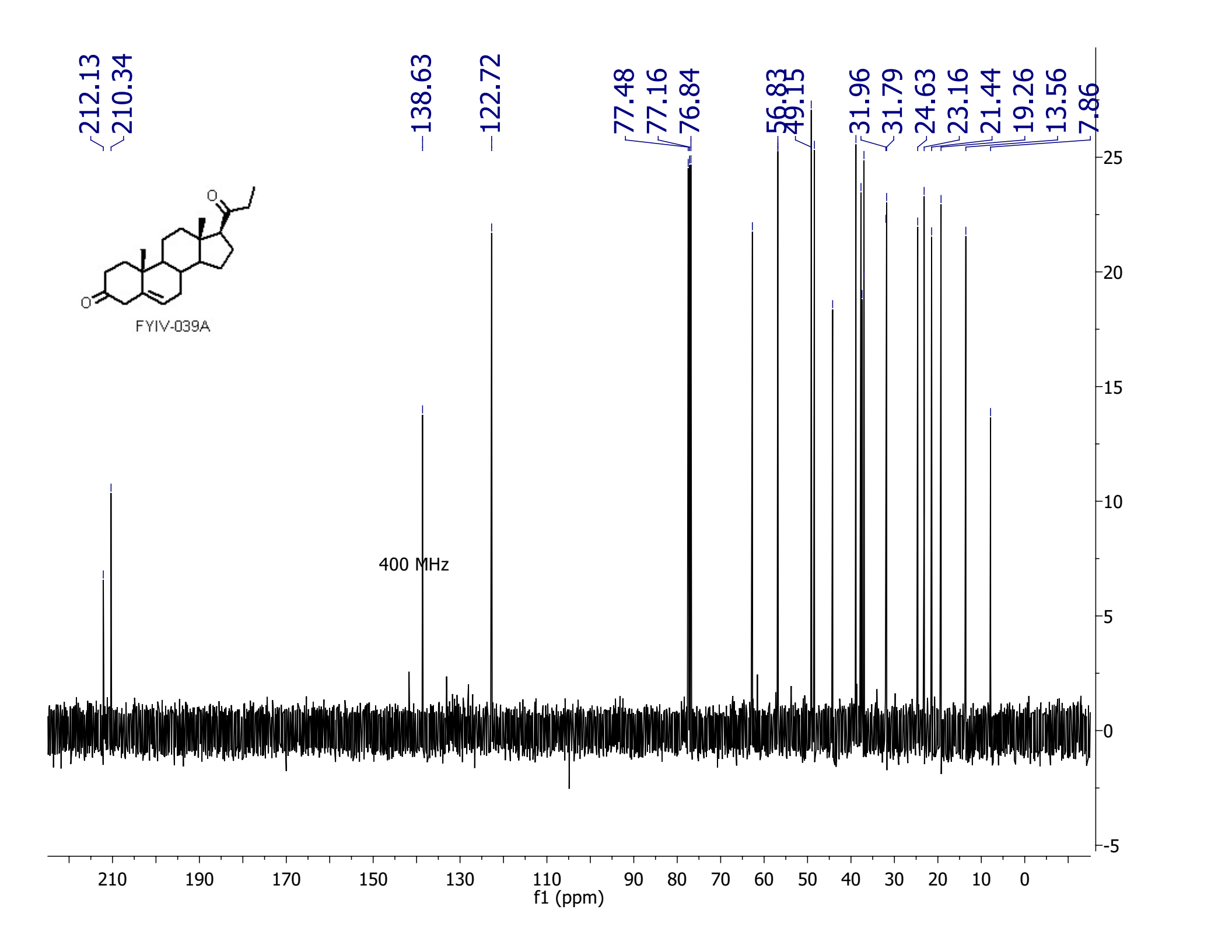

7.26
5.31
3.47
2.38
2.23
2.00
1.69
1.58
1.44
1.02
0.88
0.60
0.04
O
TBDMSO
FYIV-034A
89
400 MHz
2.47
1.19
1.00
10.16
20.16
11.44
6.99
62.65
9.34
15.93
37.02
f1 (ppm)

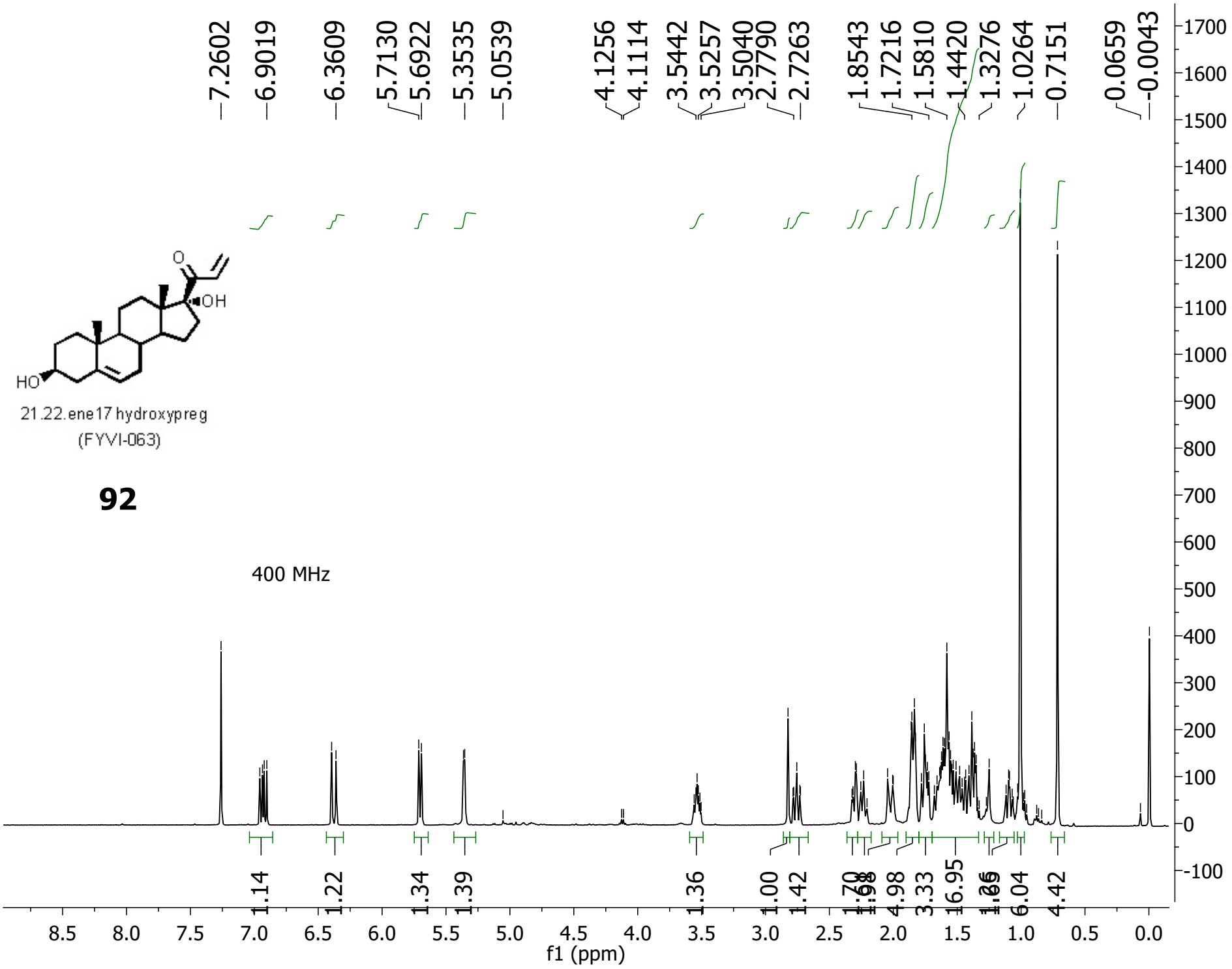

212.13
183.97
141.63
136.24
133.33
131.44
129.45
121.00
77.48
77.16
76.84
72.65
57.09
36.72
31.99
26.06
23.14
20.44
18.38
7.88
-4.46
O
TBDMSO
FYIV-034A
89
400 MHz
40
35
30
25
20
15
10
5
0
-5
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

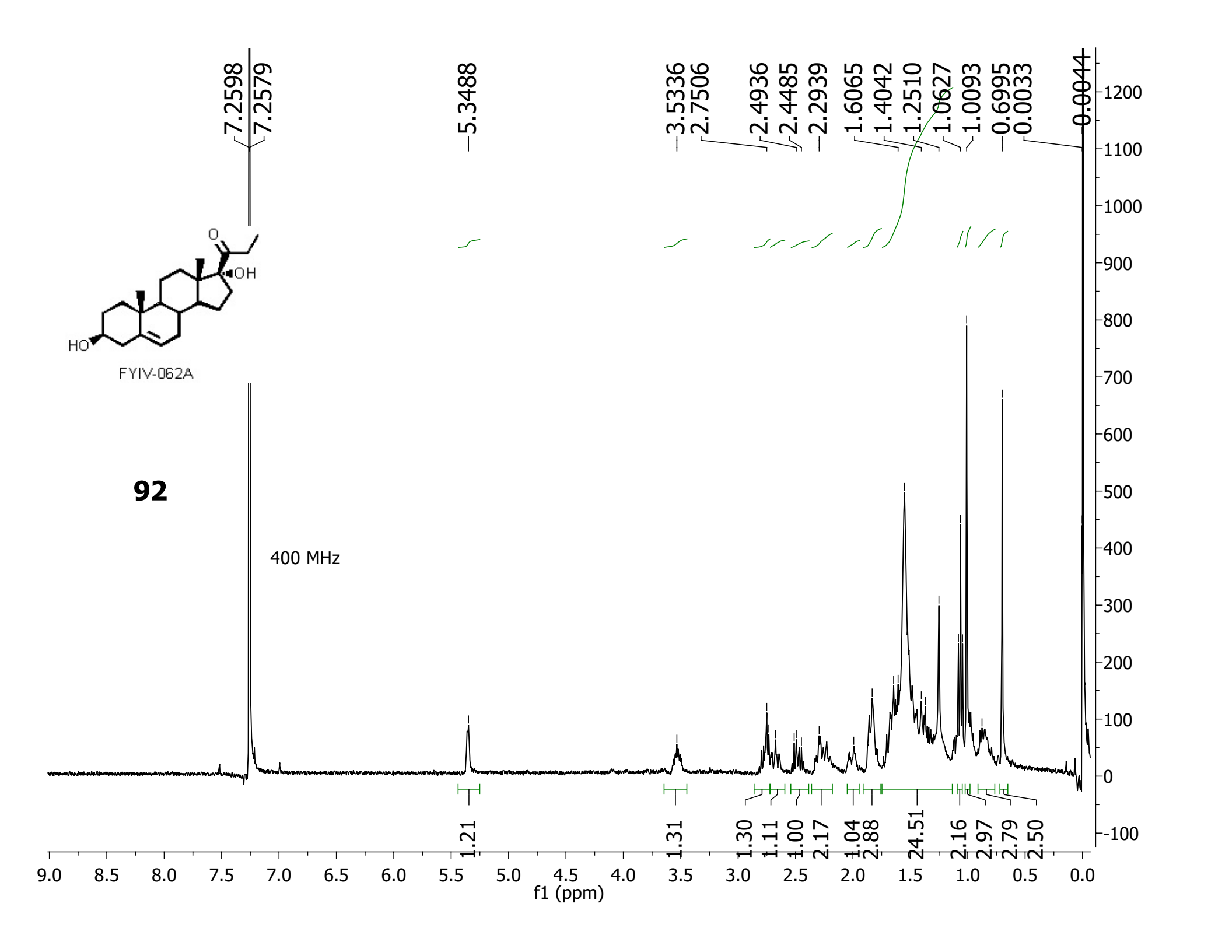

HO
O
FYIV-036C
5.33
4.11
4.09
3.50
2.38
2.15
1.84
1.62
1.47
1.40
1.20
1.10
0.99
0.86
0.59
400 MHz
1.00
1.13
0.92
2.23
4.73
3.60
4.52
14.10
2.89
1.89
4.52
f1 (ppm)

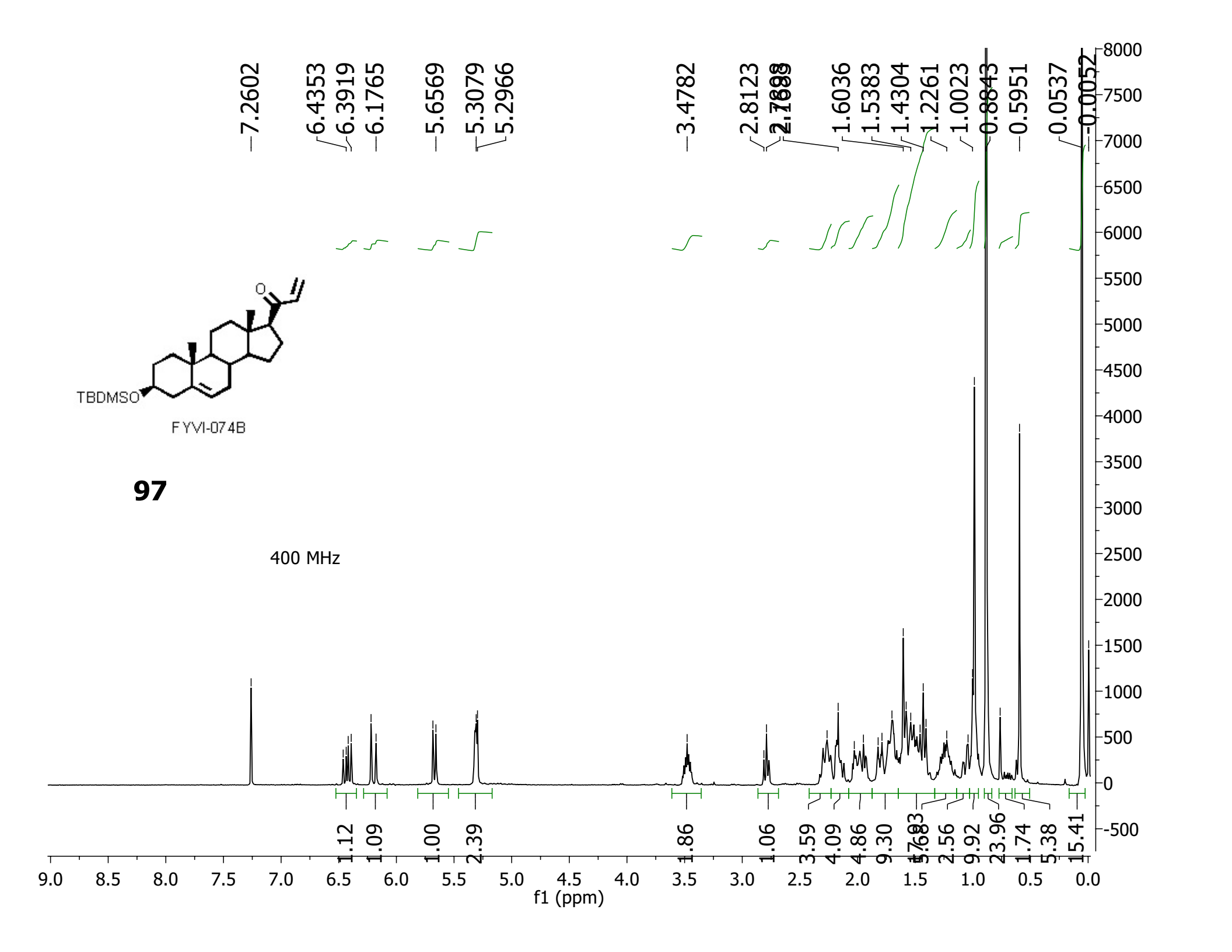

215.80
212.23
184.20
140.90
121.46
104.88
77.16
76.84
71.74
50.09
42.32
37.45
36.62
31.90
31.42
24.66
21.19
19.50
13.65
13.51
O
HO
FYIV-036C
400 MHz
f1 (ppm)

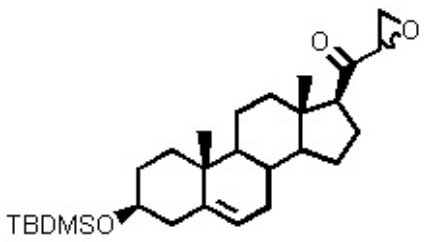

8.00
7.95
7.95
7.93
7.93
7.39
7.17
7.13
5.81
5.70
5.66
5.33
5.32
4.34
4.11
4.07
3.65
3.24
2.37
2.21
2.03
1.72
1.56
1.36
1.13
0.89
0.63
-0.02
O
O
FYIV-039A
400 MHz
1.05
1.00
1.08
1.92
4.94
5.84
8.85
4.59
f1 (ppm)


212.13
210.34
138.63
122.72
77.48
77.16
76.84
56.83
49.15
31.96
31.79
24.63
23.16
21.44
19.26
13.56
7.86
O
O
FYIV-039A
400 MHz
25
20
15
10
5
0
-5
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


7.2602
6.9019
6.3609
5.7130
5.6922
5.3535
5.0539
4.1256
4.1114
3.5442
3.5257
3.5040
2.7790
2.7263
1.8543
1.7216
1.5810
1.4420
1.3276
1.0264
0.7151
0.0659
-0.0043
O
OH
HO
21.22.ene17 hydroxypreg
(FYVI-063)
92
400 MHz
1.14
1.22
1.34
1.39
1.36
1.00
1.42
4.98
3.33
16.95
6.04
4.42
f1 (ppm)


7.2598
7.2579
5.3488
3.5336
2.7506
2.4936
2.4485
2.2939
1.6065
1.4042
1.2510
1.0627
1.0093
0.6995
0.0033
0.0044
O
OH
HO
FYIV-062A
92
400 MHz
1.21
1.31
1.30
1.11
1.00
2.17
1.04
2.88
24.51
2.16
2.97
2.79
2.50
f1 (ppm)


7.2602
6.4353
6.3919
6.1765
5.6569
5.3079
5.2966
3.4782
2.8123
1.6036
1.5383
1.4304
1.2261
1.0023
0.8843
0.5951
0.0537
-0.0052
TBDMSO
O
FYVI-074B
97
400 MHz
1.12
1.09
1.00
2.39
1.86
1.06
3.59
4.09
4.86
9.30
2.56
9.92
23.96
1.74
5.38
15.41
f1 (ppm)



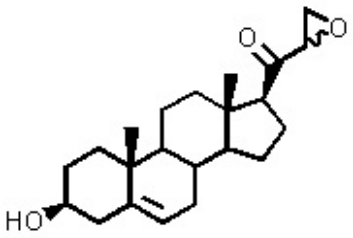
FYVI-074B.second.rxn epoxidation
(epimeric mixture at C20)

400 MHz

8000
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500

8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

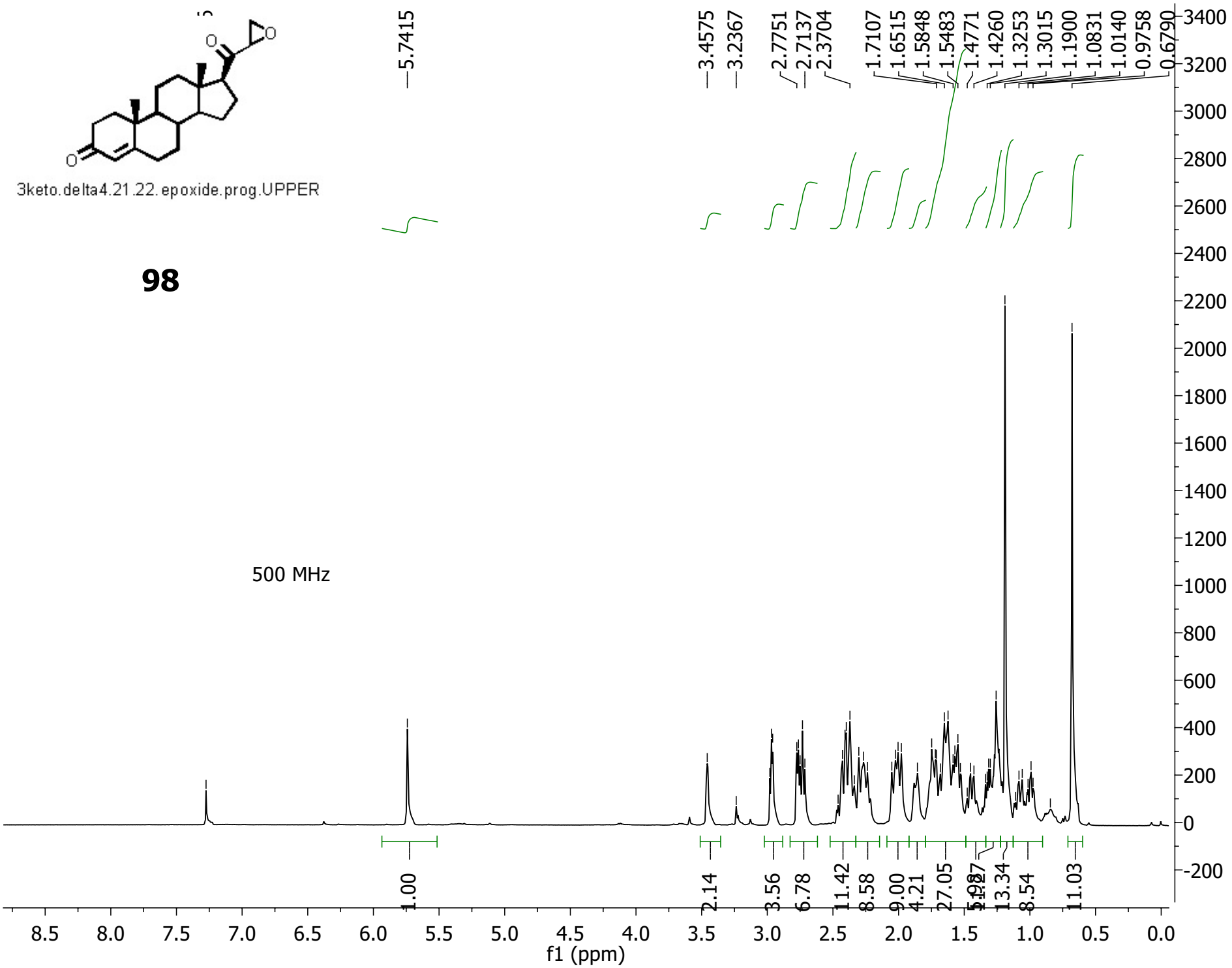
f1 (ppm)

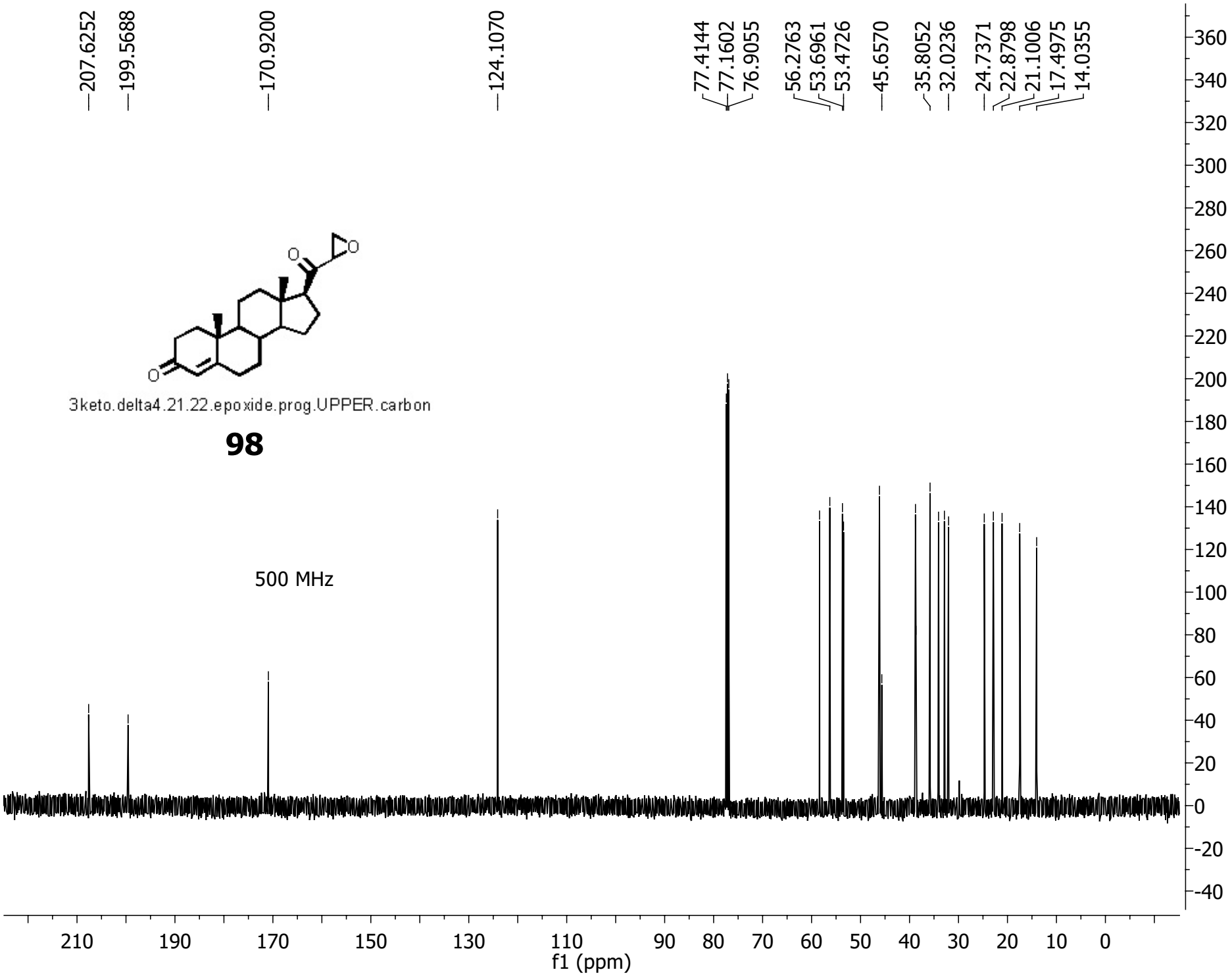


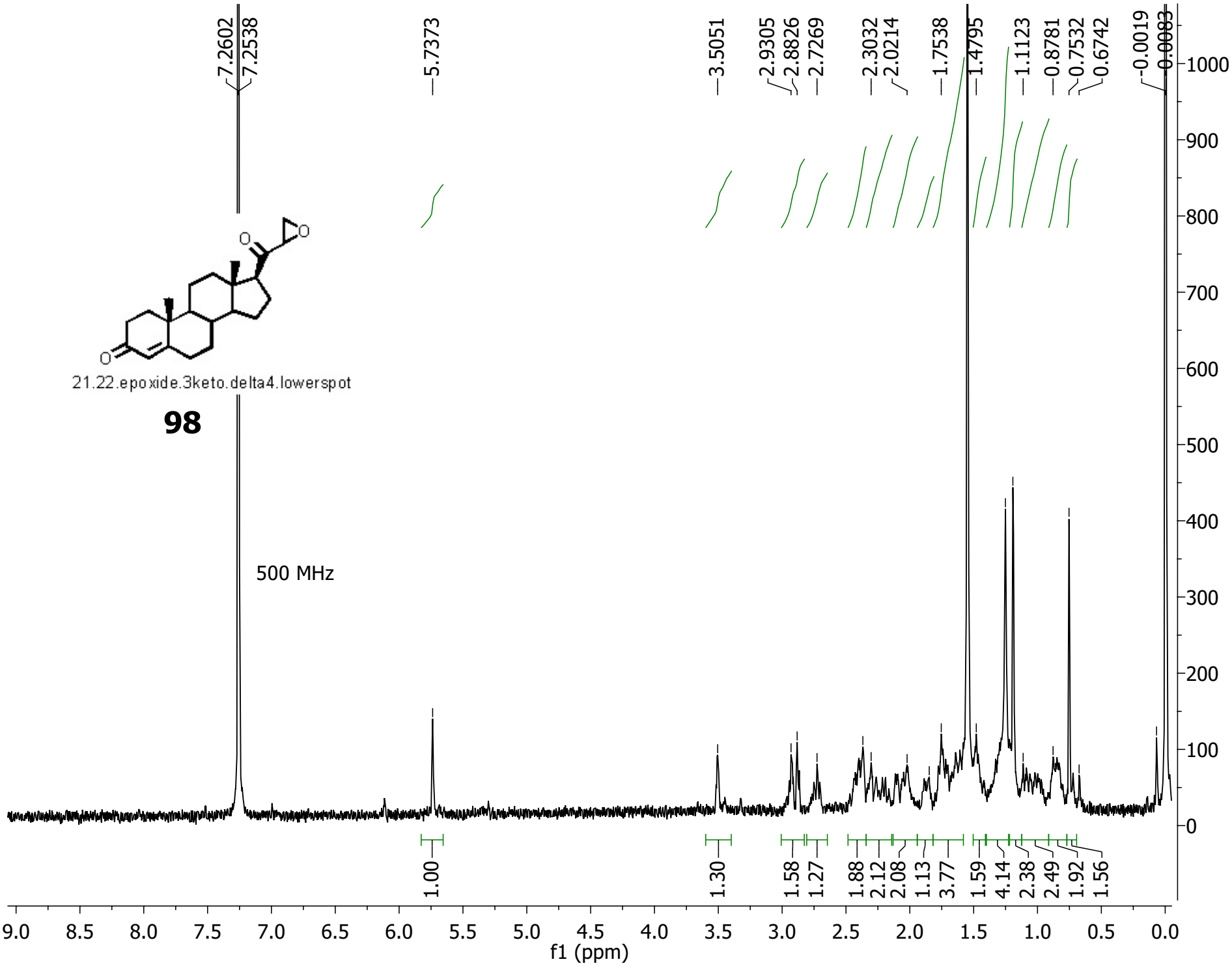
FYVI-075B

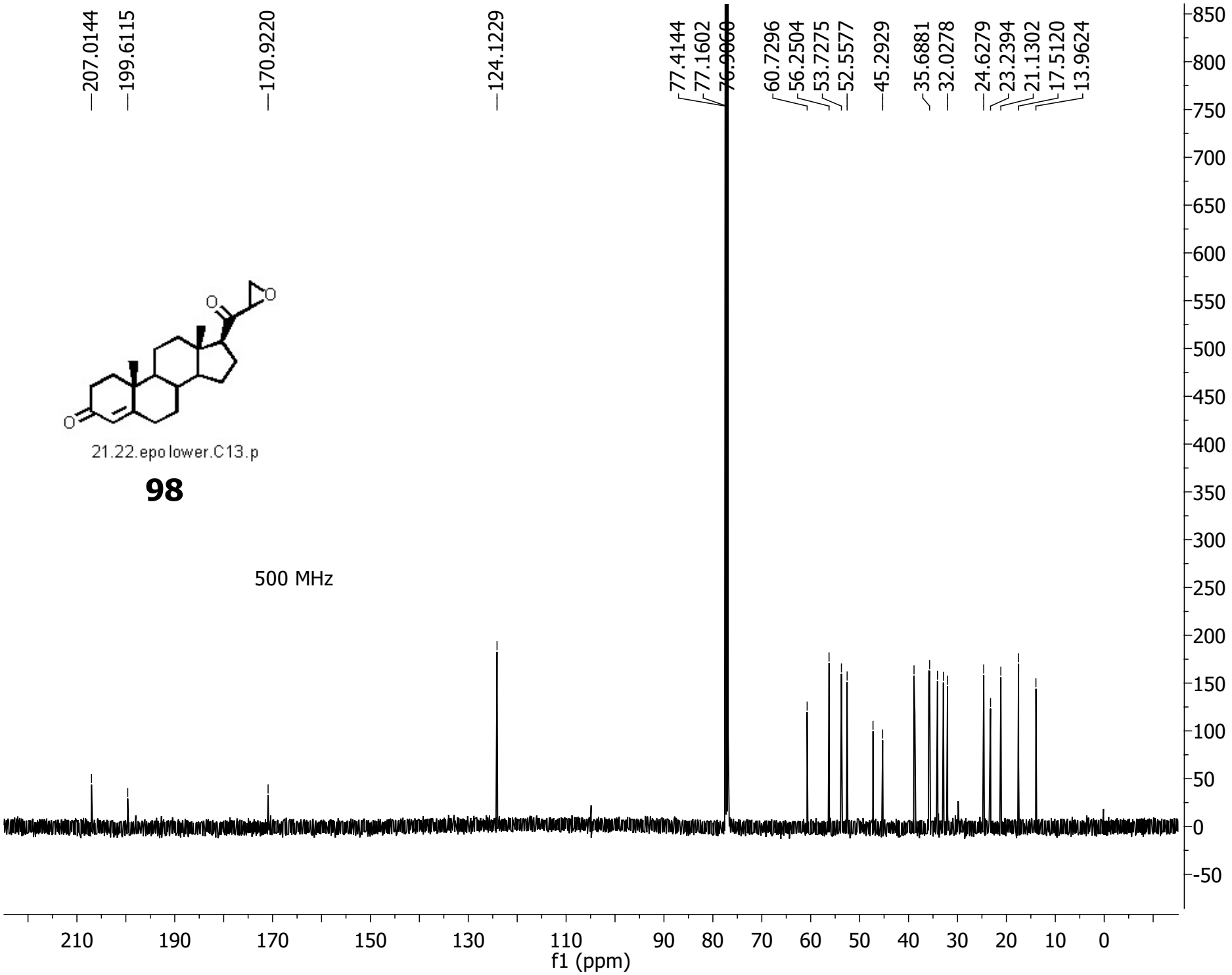
400 MHz

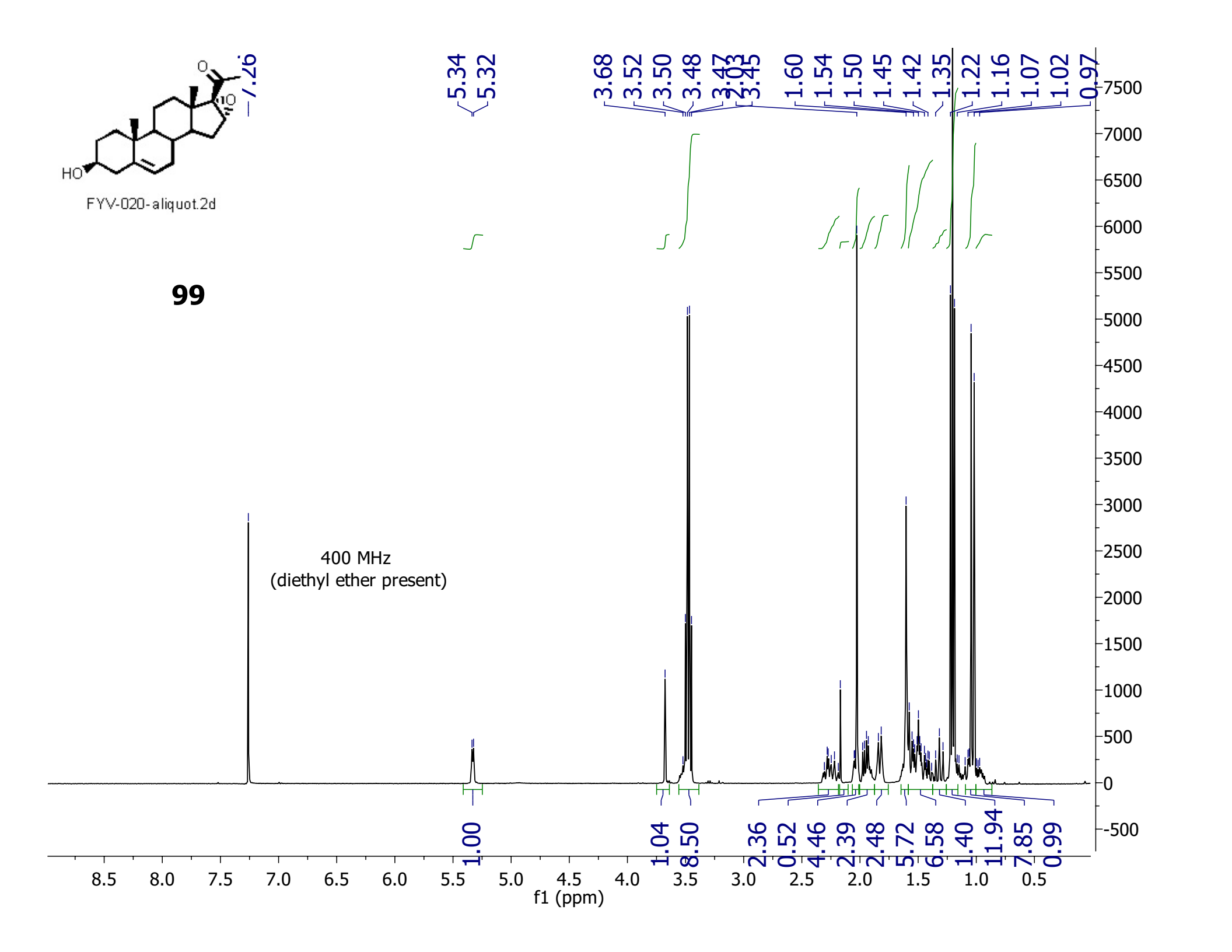
f1 (ppm)

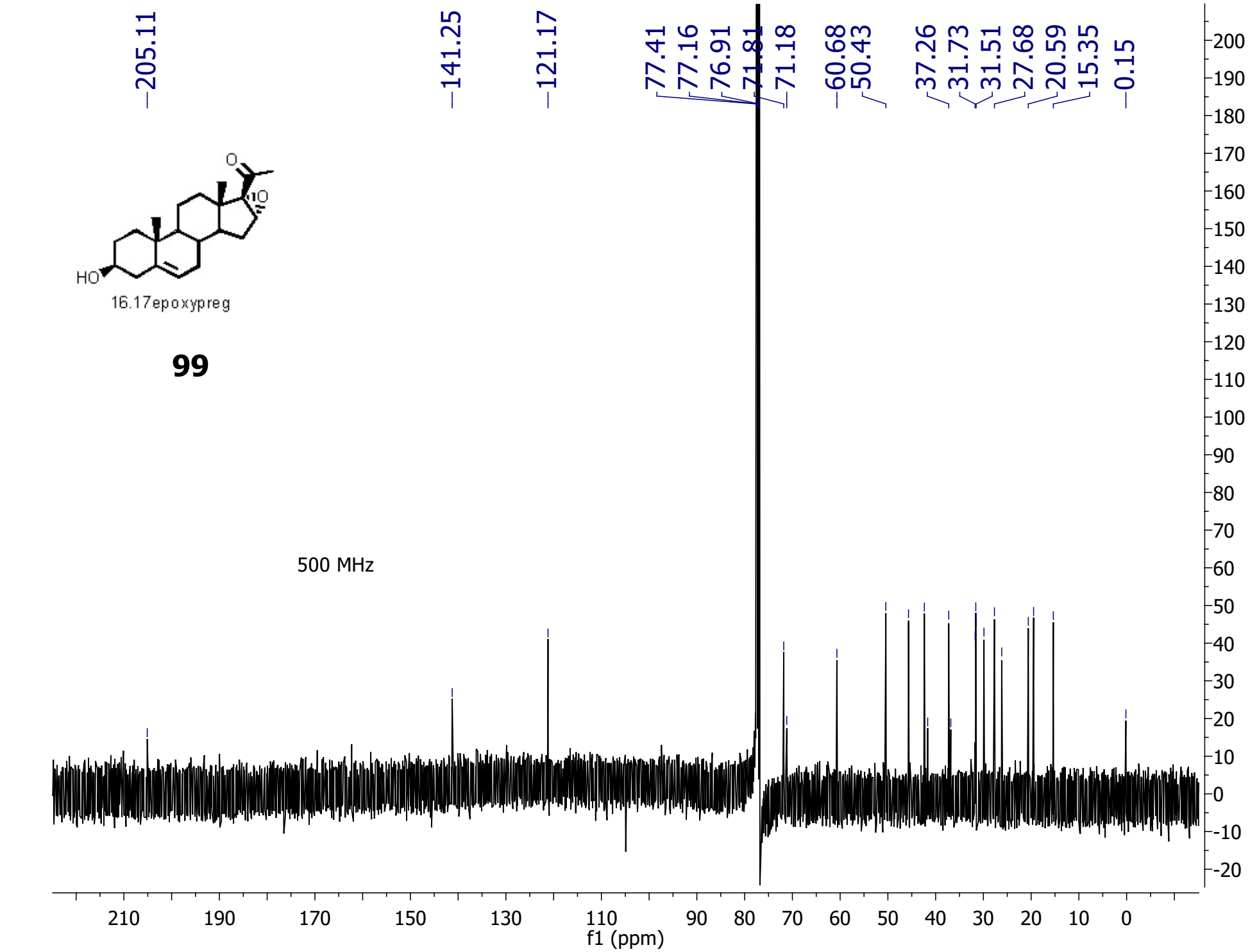

3keto.delta4.21.22.epoxide.prog.UPPER
98
500 MHz
5.7415
3.4575
3.2367
2.7751
2.7137
2.3704
1.7107
1.6515
1.5848
1.5483
1.4771
1.4260
1.3253
1.3015
1.1900
1.0831
1.0140
0.9758
0.6790
1.00
2.14
3.56
6.78
11.42
8.58
9.00
4.21
27.05
13.34
8.54
11.03
f1 (ppm)

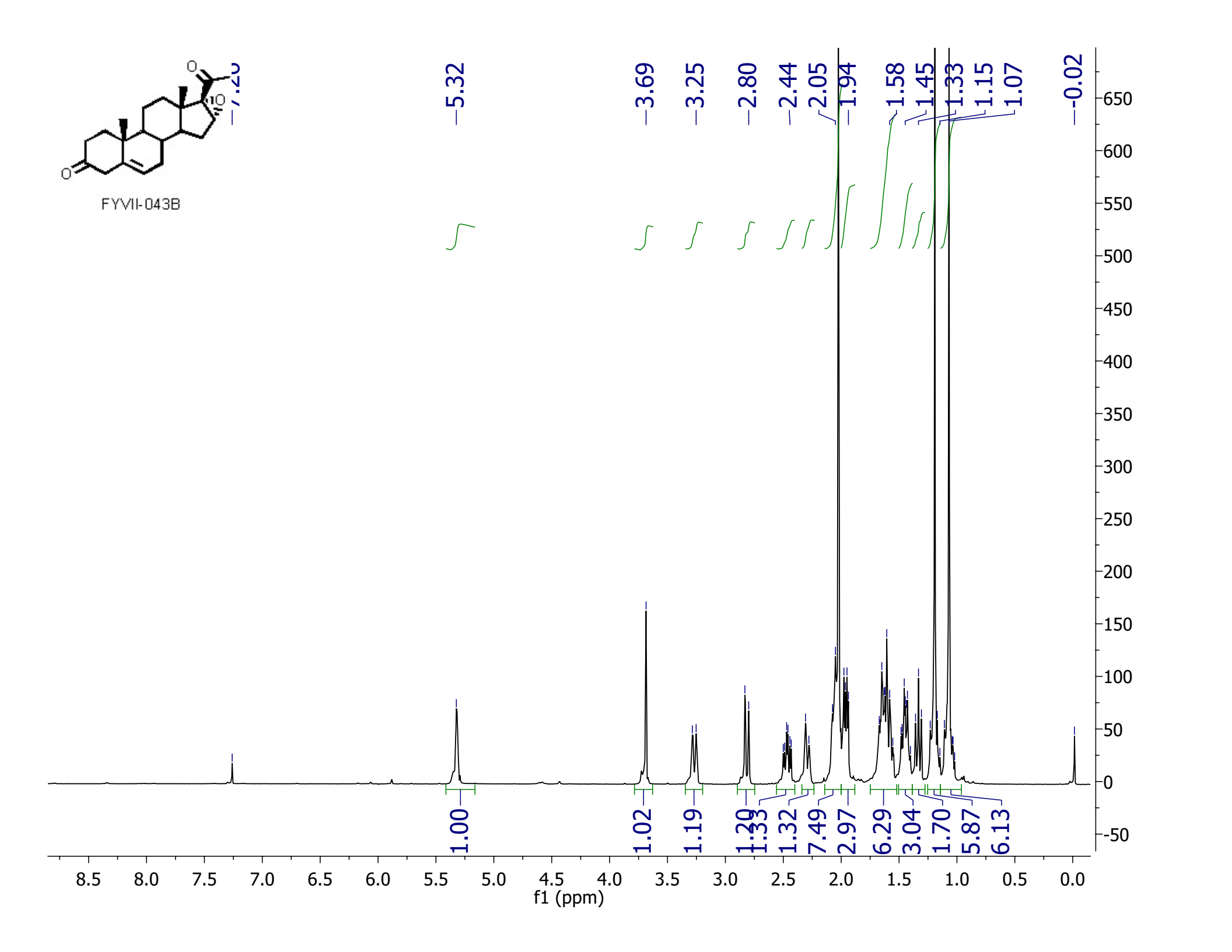

207.6252
199.5688
170.9200
124.1070
77.4144
77.1602
76.9055
56.2763
53.6961
53.4726
45.6570
35.8052
32.0236
24.7371
22.8798
21.1006
17.4975
14.0355
3keto.delta4.21.22.epoxide.prog.UPPER.carbon
98
500 MHz
f1 (ppm)

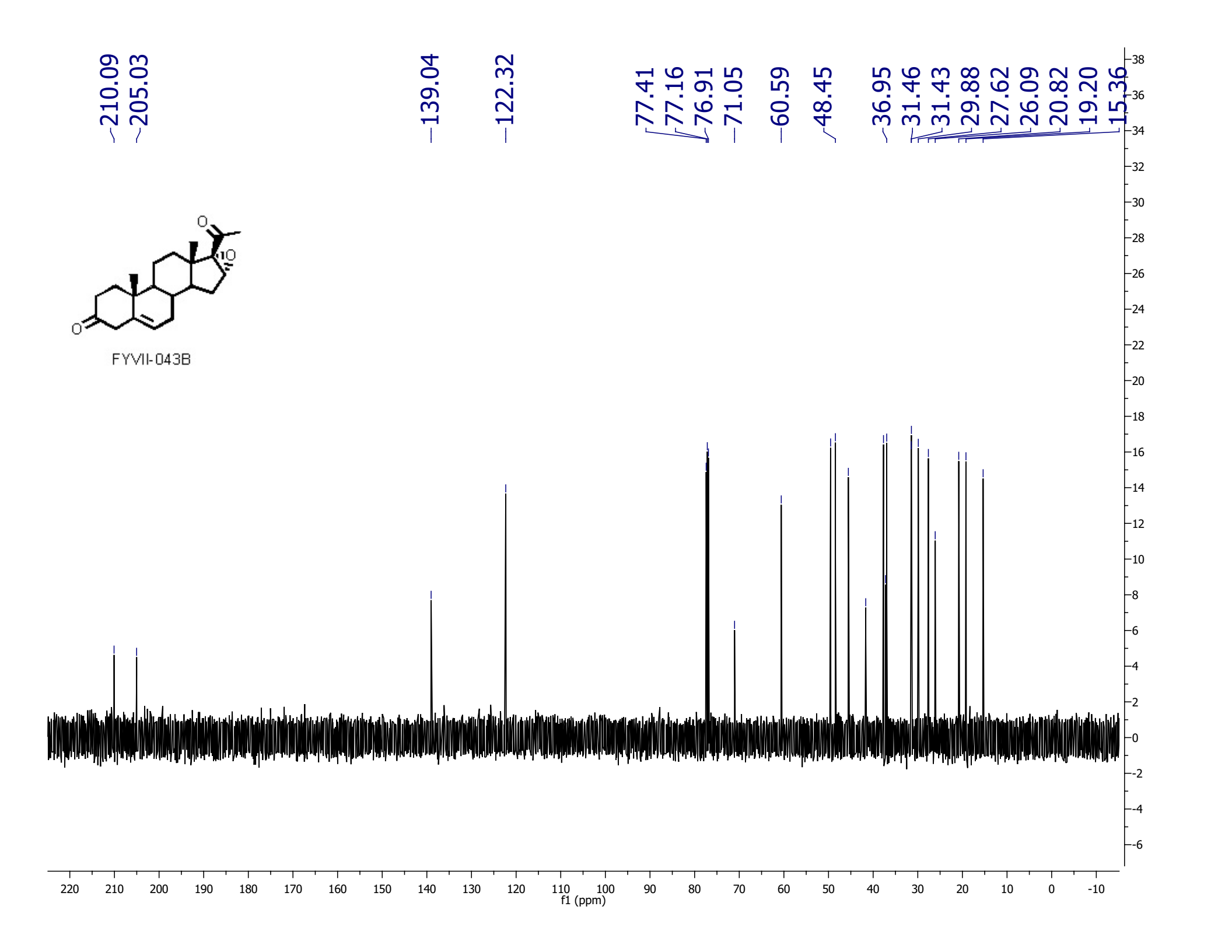

7.2602
7.2538
5.7373
3.5051
2.9305
2.8826
2.7269
2.3032
2.0214
1.7538
1.4795
1.1123
0.8781
0.7532
0.6742
0.0019
0.0083
21.22.epoxide.3keto.delta4.lowerspot
98
500 MHz
1.00
1.30
1.58
1.27
1.88
2.12
2.08
1.13
3.77
1.59
4.14
2.38
2.49
1.92
1.56
f1 (ppm)

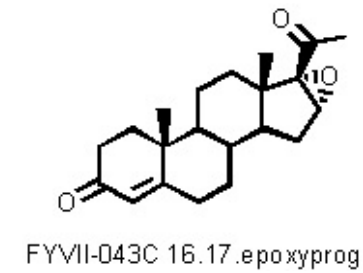

207.0144
199.6115
170.9220
124.1229
77.4144
77.1602
76.9060
60.7296
56.2504
53.7275
52.5577
45.2929
35.6881
32.0278
24.6279
23.2394
21.1302
17.5120
13.9624
21.22.epo lower.C13.p
98
500 MHz
f1 (ppm)


FYV-020-aliquot.2d
99
400 MHz
(diethyl ether present)
f1 (ppm)


205.11
141.25
121.17
77.41
77.16
76.91
71.81
71.18
60.68
50.43
37.26
31.73
31.51
27.68
20.59
15.35
0.15
HO
O
O
16.17epoxypreg
99
500 MHz
f1 (ppm)


FYVII-043B
5.32
3.69
3.25
2.80
2.44
2.05
1.94
1.58
1.45
1.33
1.15
1.07
-0.02
1.00
1.02
1.19
1.20
1.33
1.32
7.49
2.97
6.29
3.04
1.70
5.87
6.13
f1 (ppm)


210.09
205.03
139.04
122.32
77.41
77.16
76.91
71.05
60.59
48.45
36.95
31.46
31.43
29.88
27.62
26.09
20.82
19.20
15.36
FYVII-043B
f1 (ppm)



FYVII-043C 16.17.epoxyprog

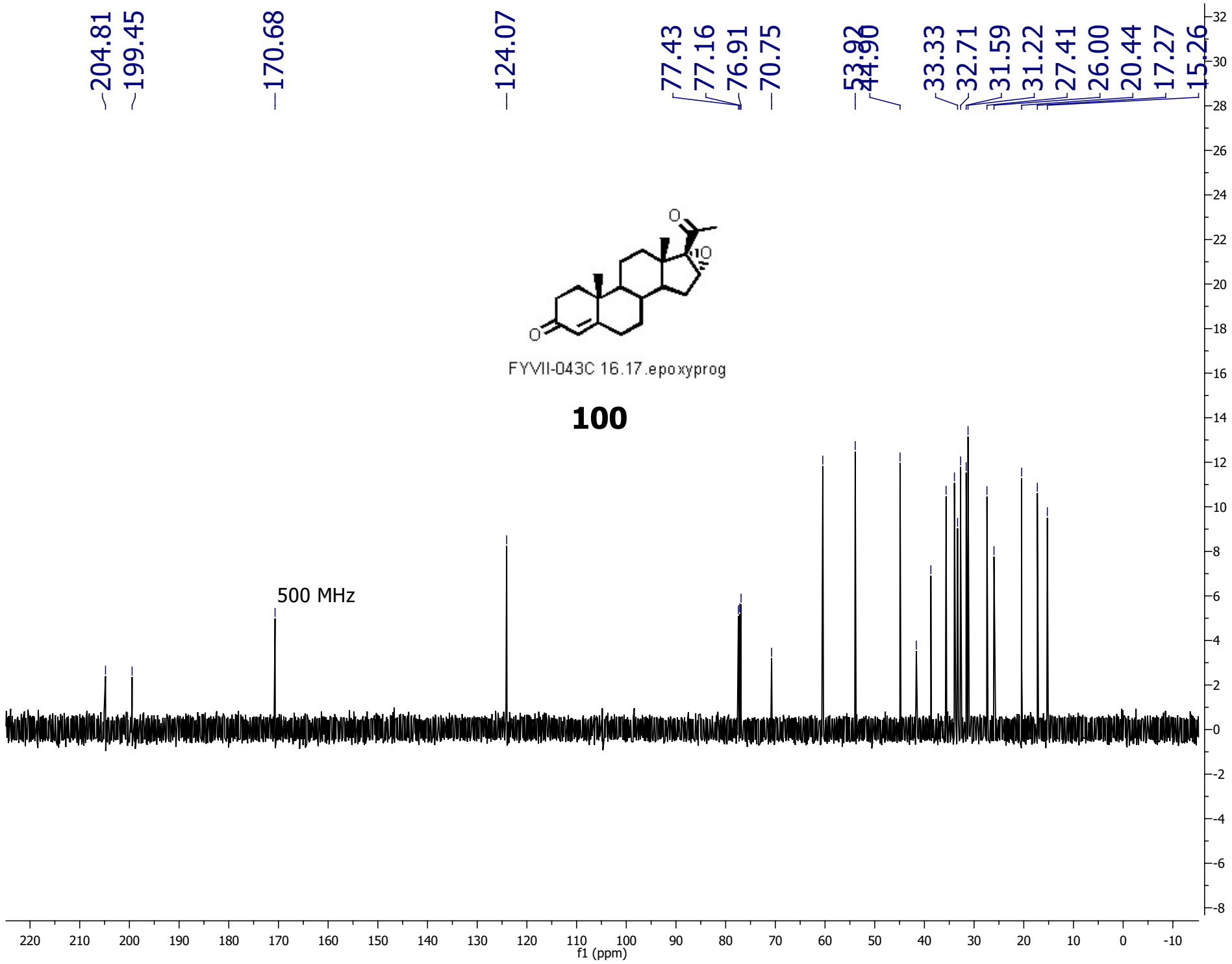
100

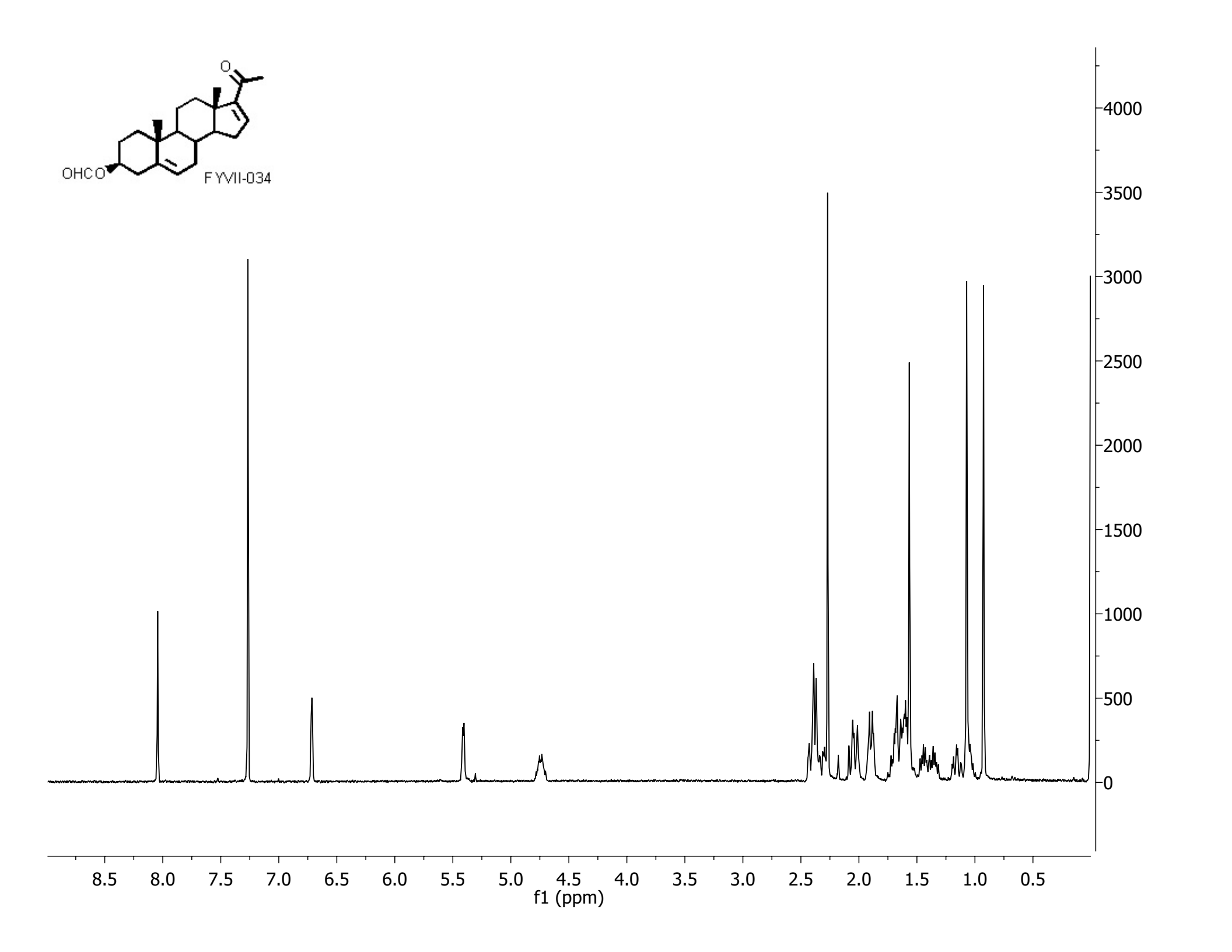
500 MHz

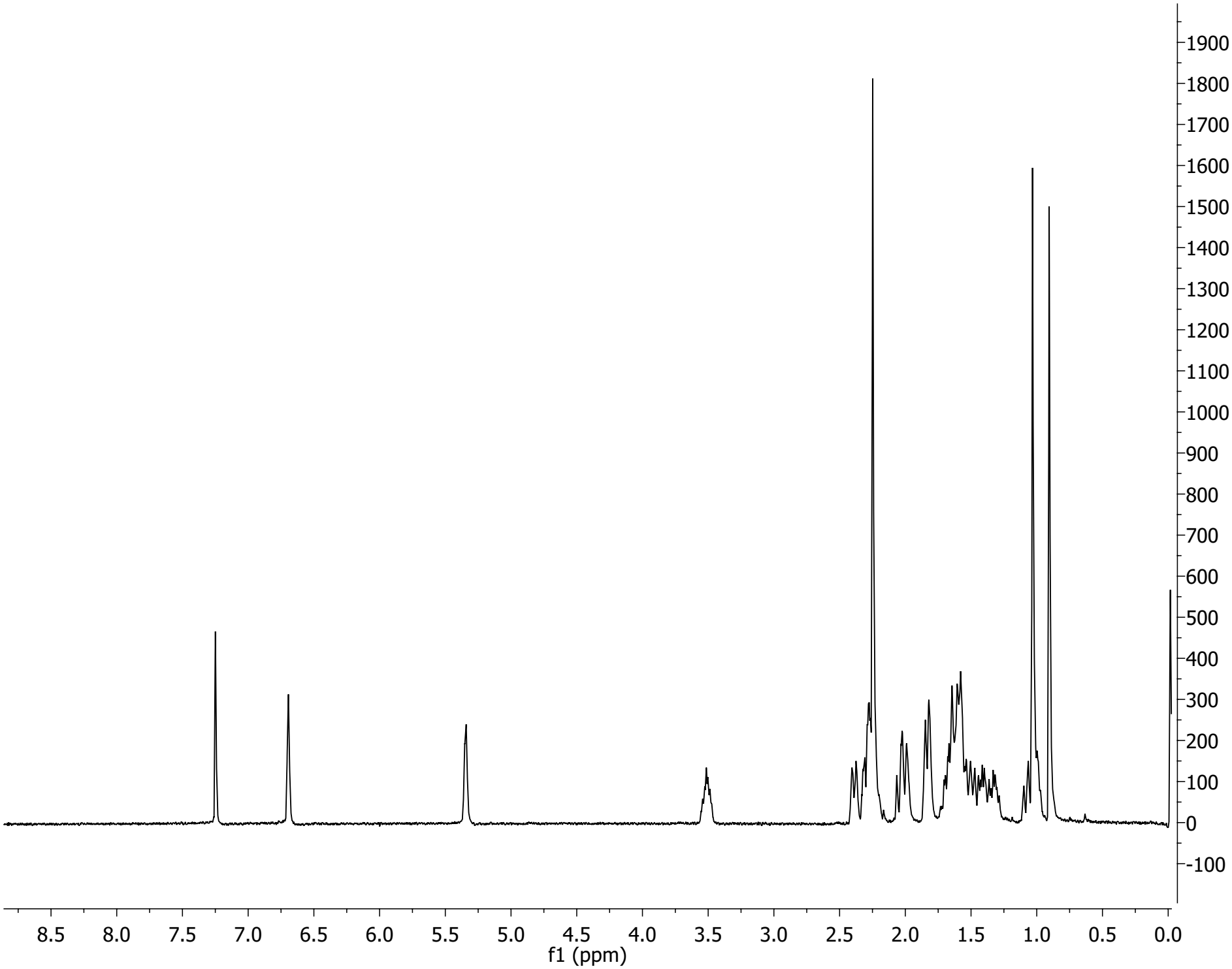
5.6825
5.6737
3.6650
1.9633
1.9251
1.6734
1.6168
1.4536
1.3874
1.3369
1.1565
1.1163
1.0318
0.9121
-0.0396

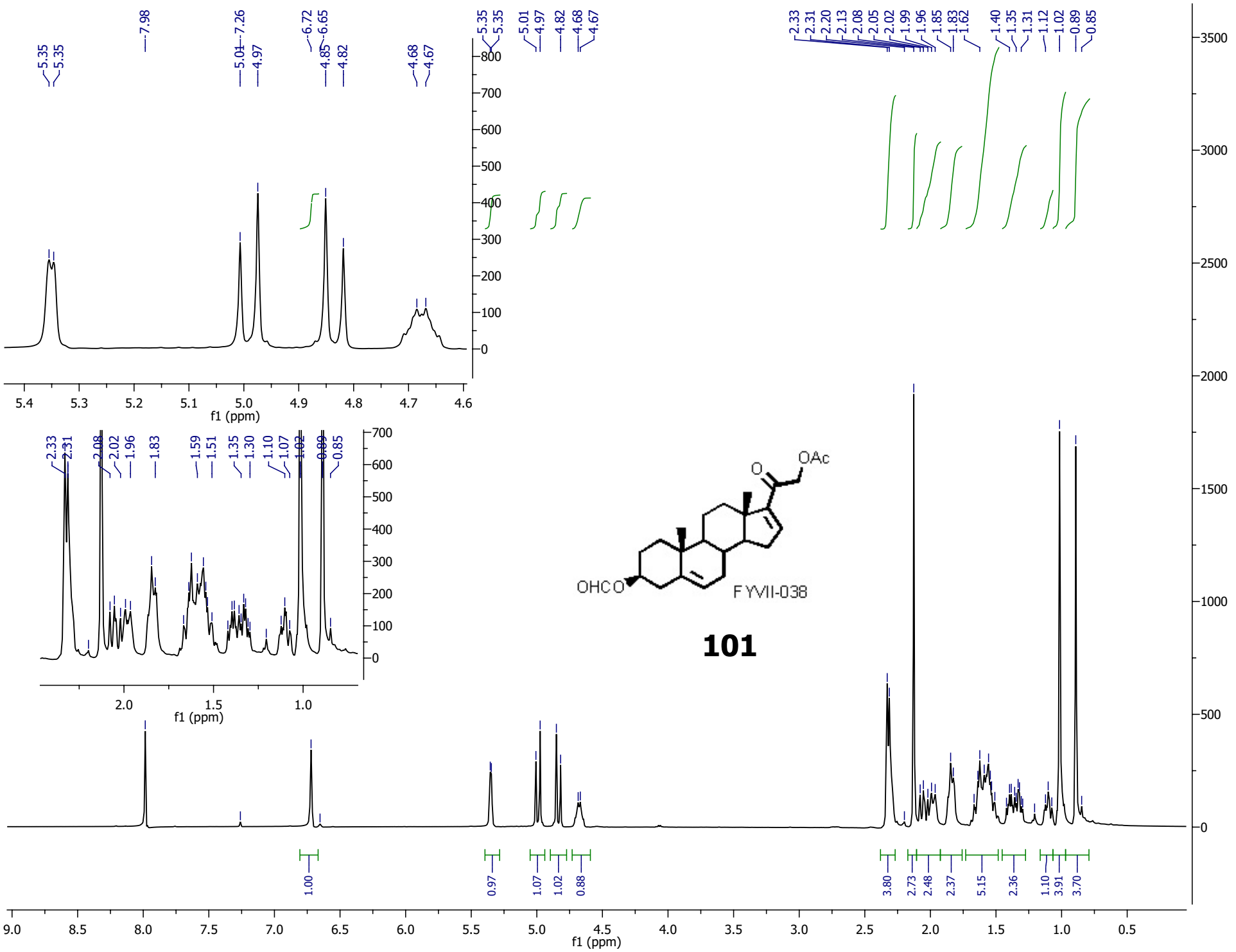
1.00
0.96
5.66
8.31
1.38
4.30
4.29
5.29
5.58
1.41

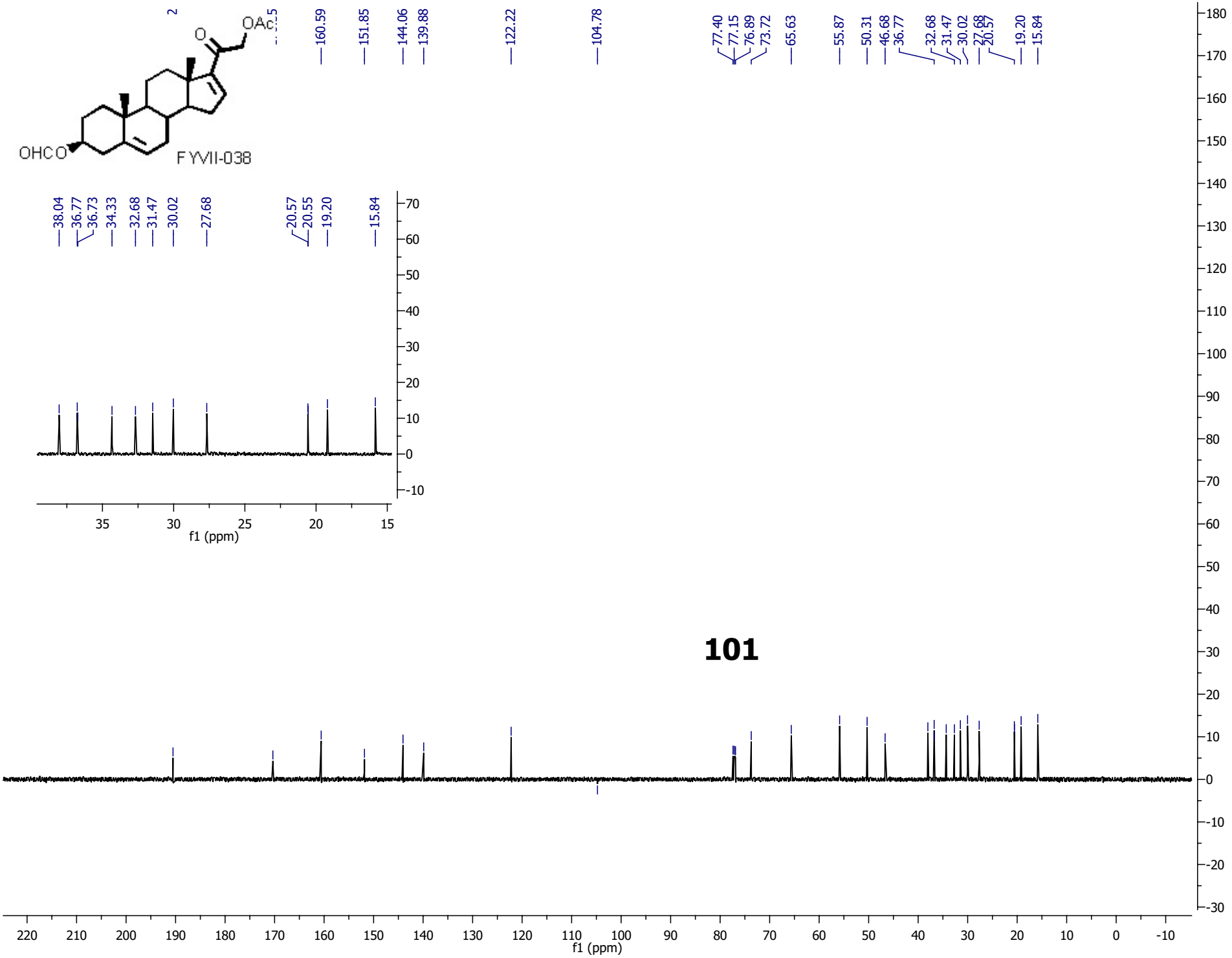
f1 (ppm)

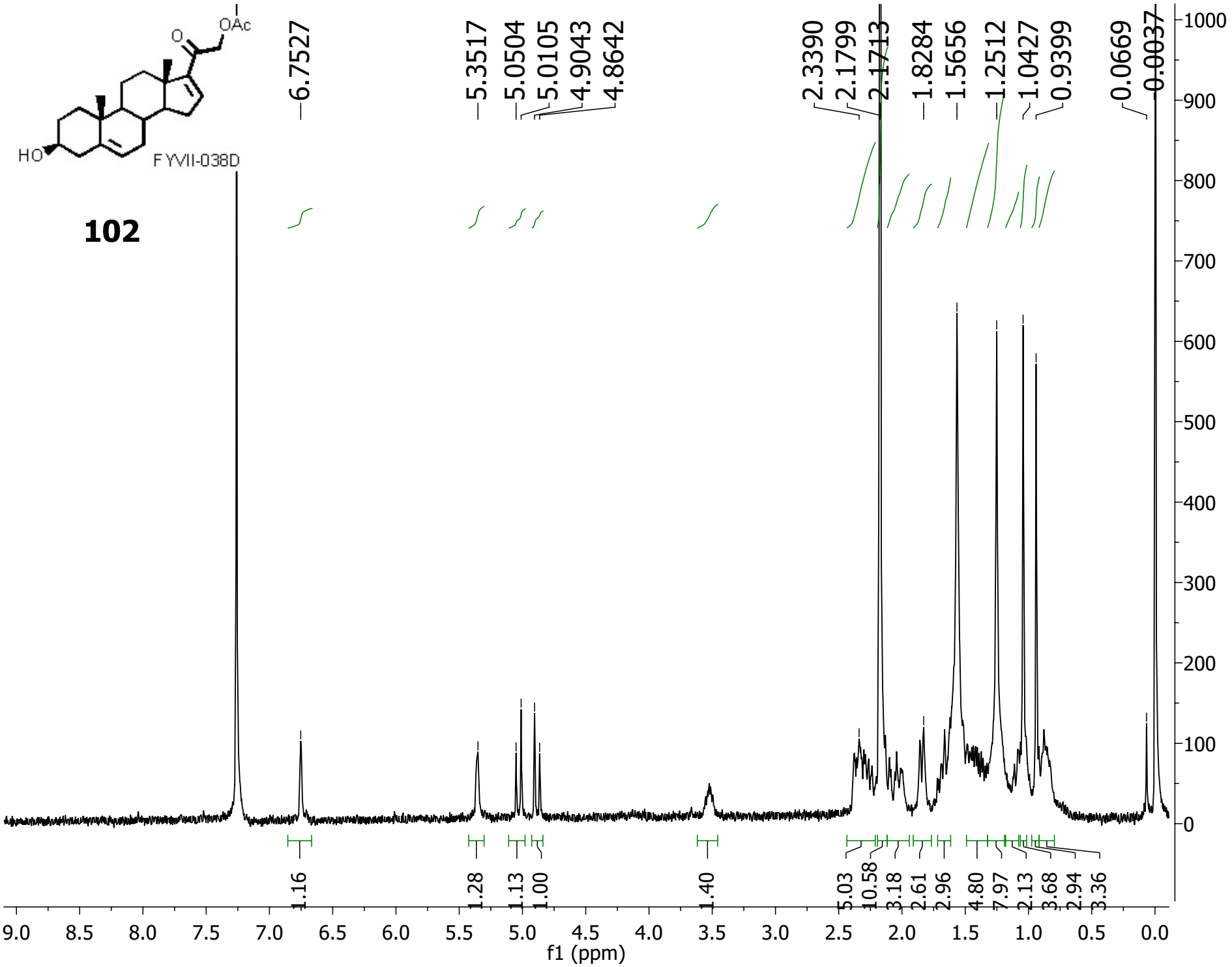

204.81
199.45
170.68
124.07
77.43
77.16
76.91
70.75
53.92
44.90
33.33
32.71
31.59
31.22
27.41
26.00
20.44
17.27
15.26
FYVII-043C 16.17.epoxyprog
100
500 MHz
f1 (ppm)

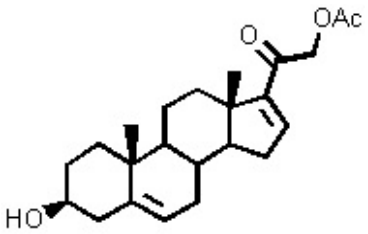

O
OHCO
FYVII-034
4000
3500
3000
2500
2000
1500
1000
500
0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


OAc
O
OHC O
FYVII-038
101
f1 (ppm)


OAc
O
OHC O
FYVII-038
160.59
151.85
144.06
139.88
122.22
104.78
77.40
77.15
76.89
73.72
65.63
55.87
50.31
46.68
36.77
32.68
31.47
30.02
27.68
20.57
19.20
15.84
38.04
36.77
36.73
34.33
32.68
31.47
30.02
27.68
20.57
20.55
19.20
15.84
f1 (ppm)
101
f1 (ppm)


OAc
O
HO
FYVII-038D
102
6.7527
5.3517
5.0504
5.0105
4.9043
4.8642
2.3390
2.1799
2.1713
1.8284
1.5656
1.2512
1.0427
0.9399
0.0669
0.0037
1.16
1.28
1.13
1.00
1.40
5.03
10.58
3.18
2.61
2.96
4.80
7.97
2.13
3.68
2.94
3.36
f1 (ppm)



FYVII-038B 3 OH

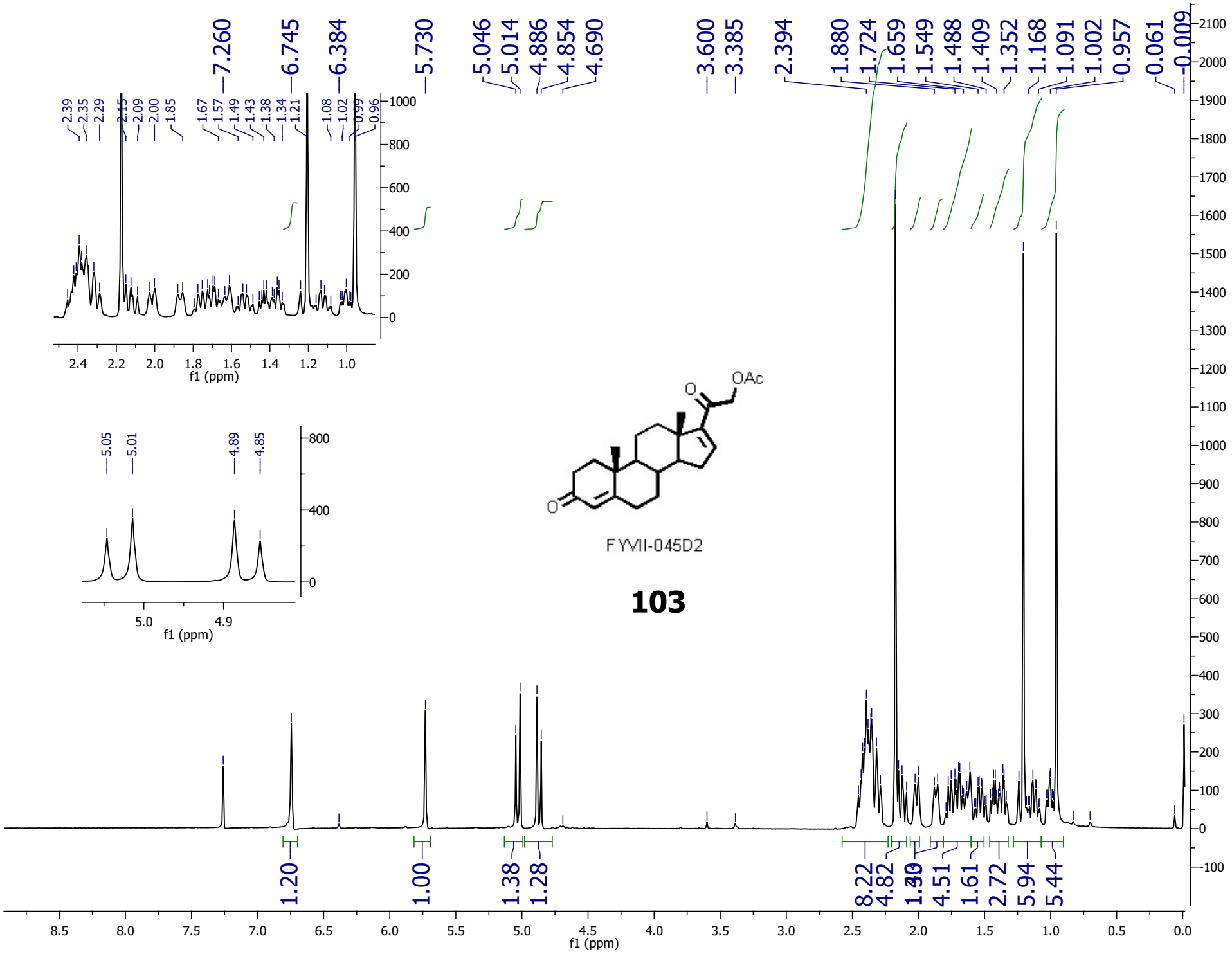
102

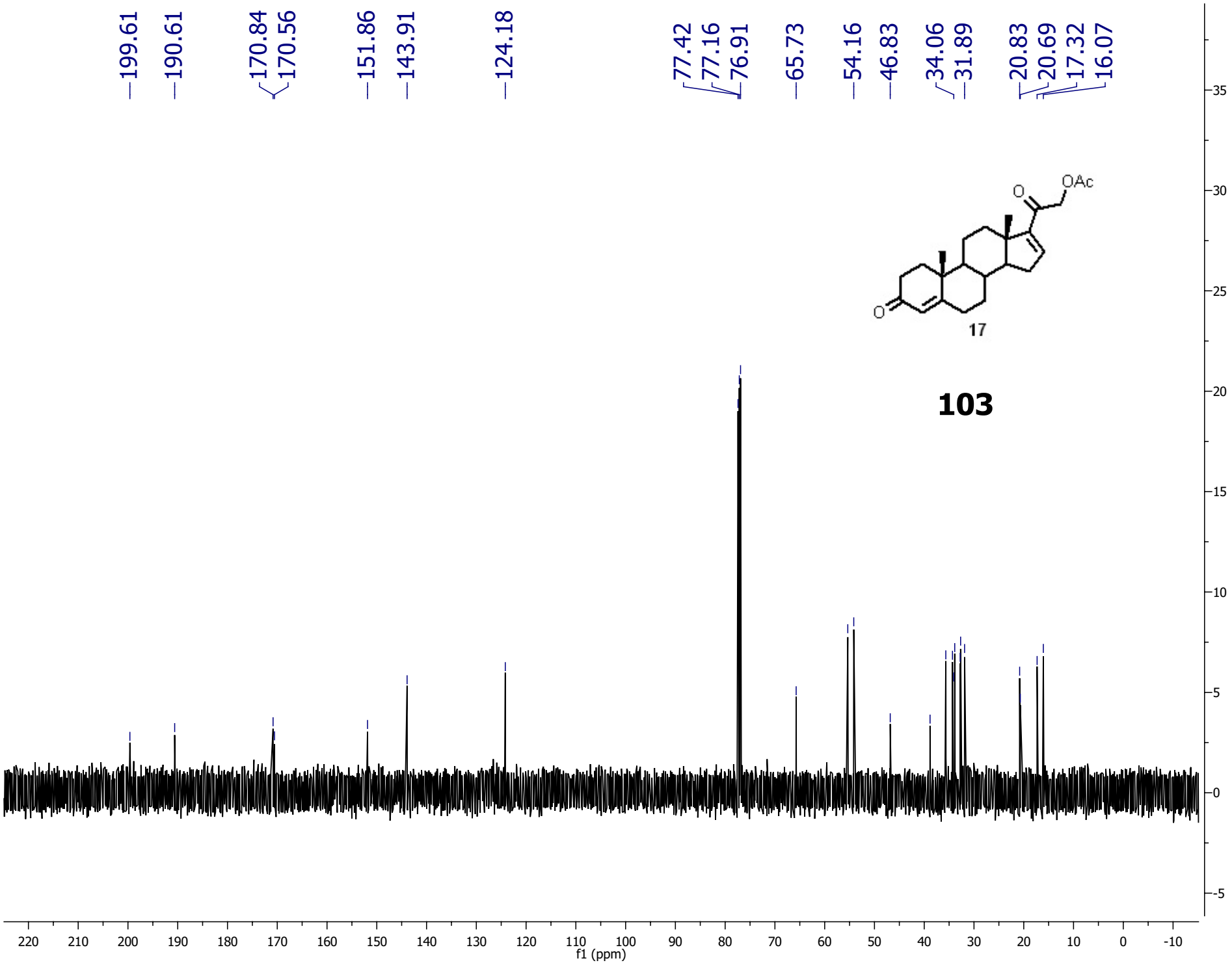
400 MHz

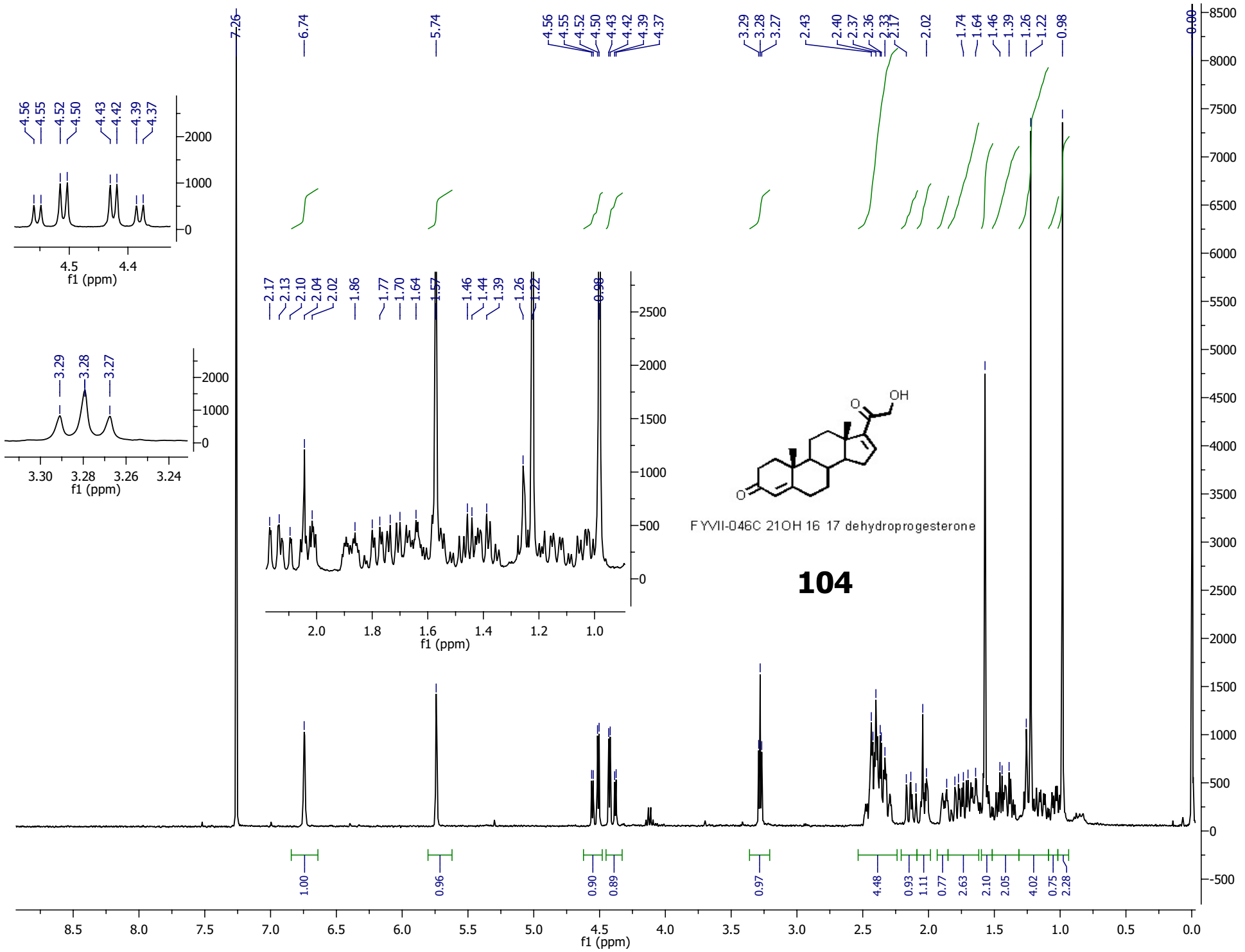
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500

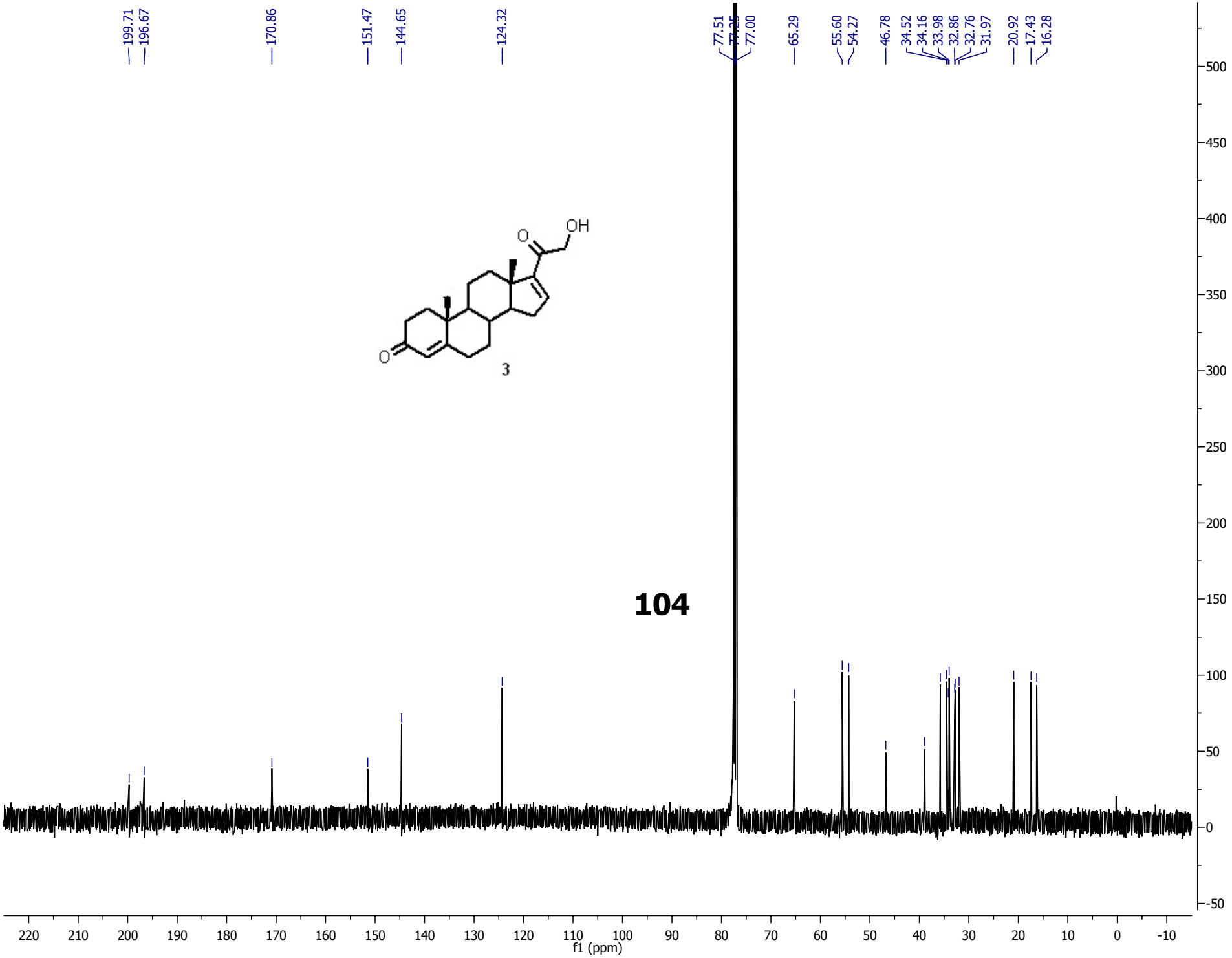
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

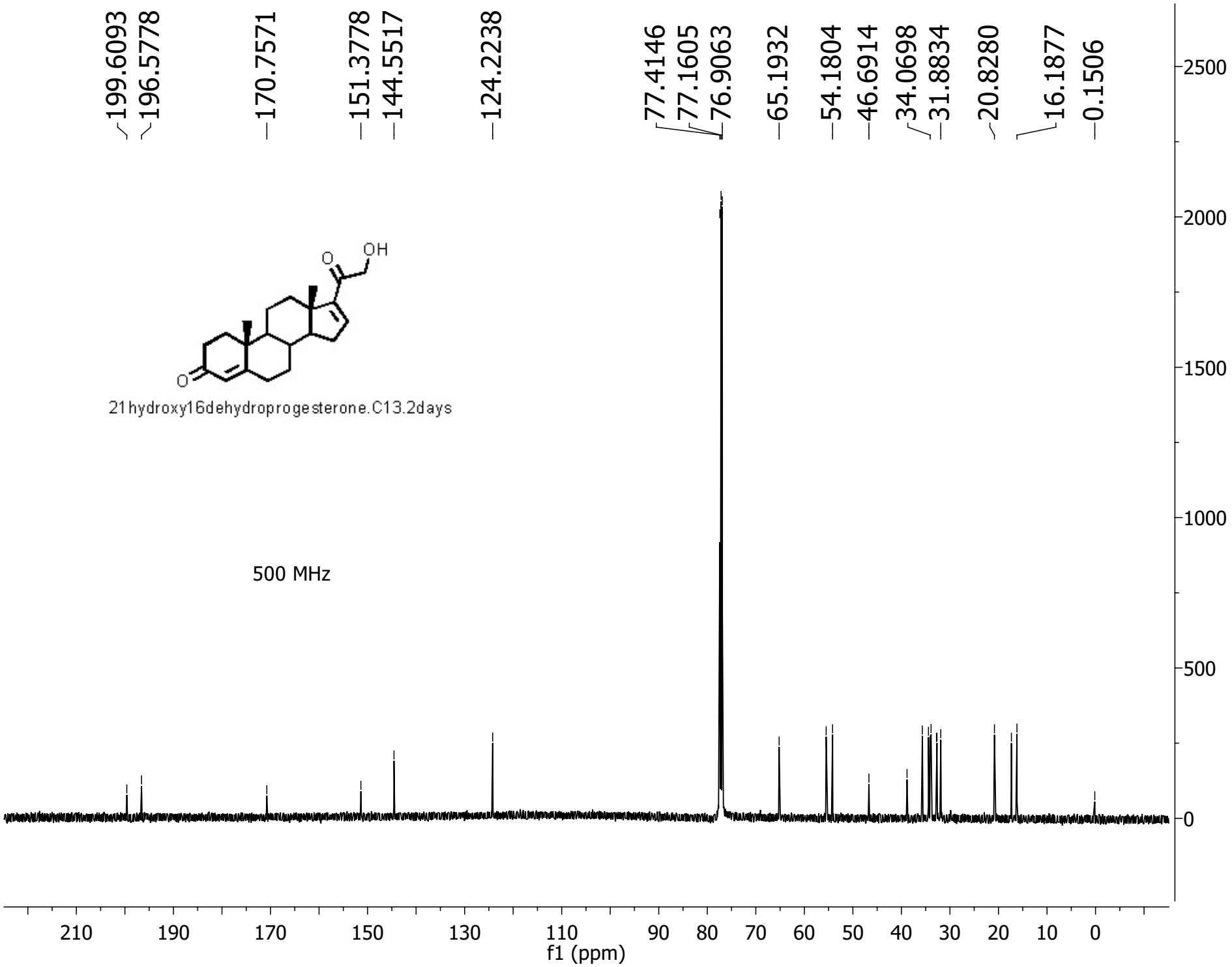
f1 (ppm)

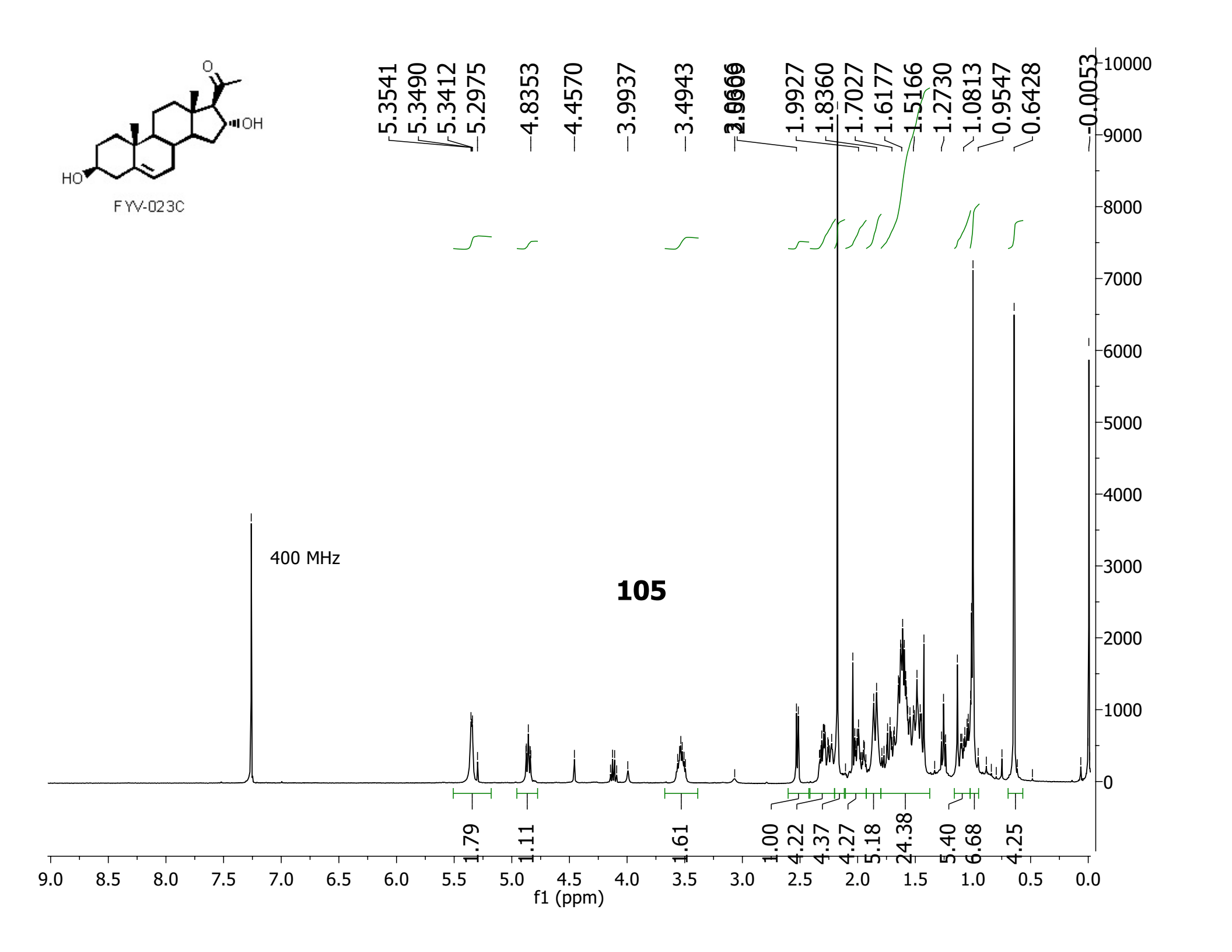

7.260
6.745
6.384
5.730
5.046
5.014
4.886
4.854
4.690
3.600
3.385
2.394
1.880
1.724
1.659
1.549
1.488
1.409
1.352
1.168
1.091
1.002
0.957
0.061
-0.009
OAc
O
O
FYVII-045D2
103
f1 (ppm)
1.20
1.00
1.38
1.28
8.22
4.82
4.51
1.61
2.72
5.94
5.44

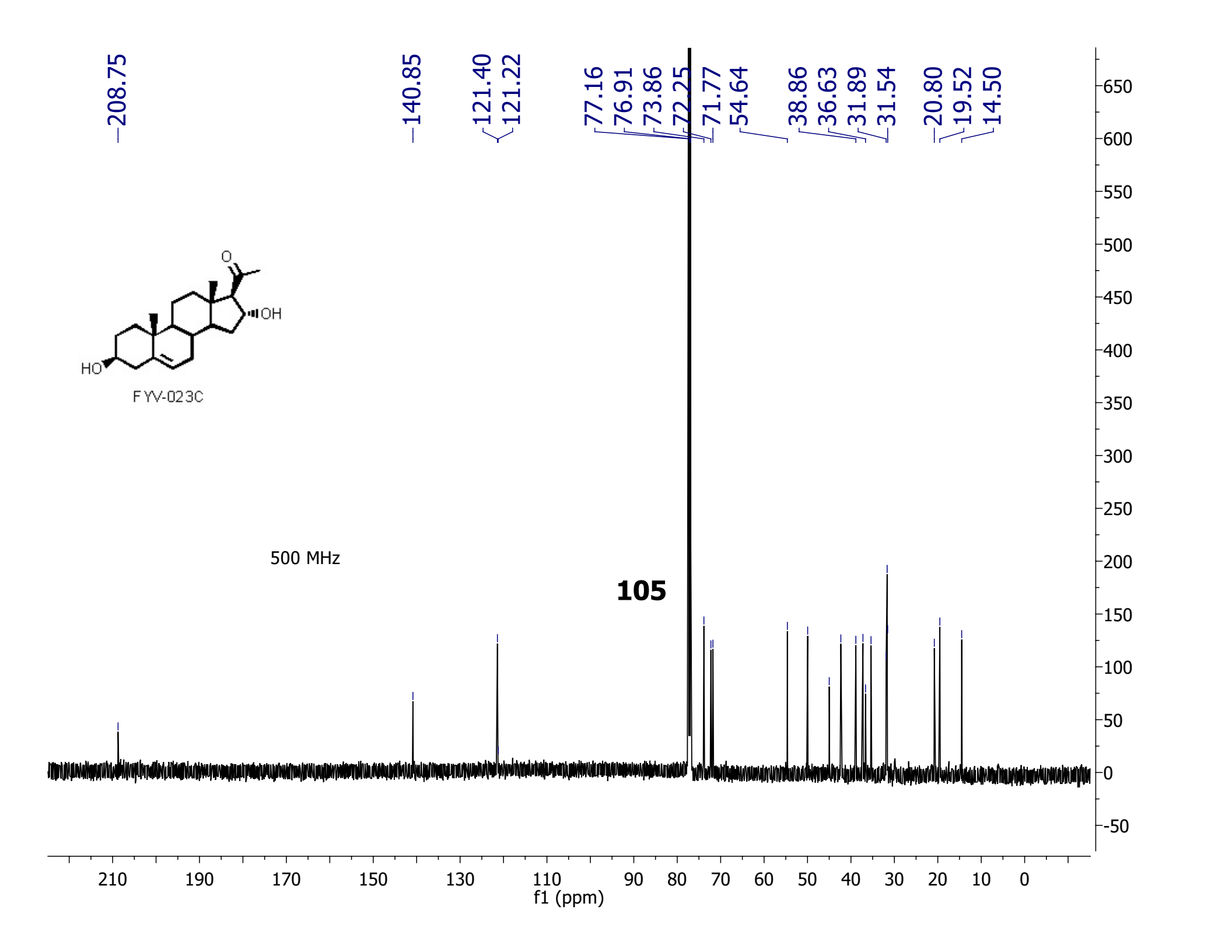

199.61
190.61
170.84
170.56
151.86
143.91
124.18
77.42
77.16
76.91
65.73
54.16
46.83
34.06
31.89
20.83
20.69
17.32
16.07
OAc
O
O
17
103
f1 (ppm)

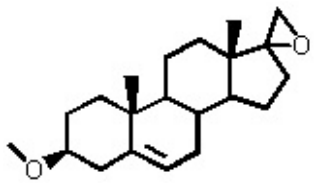

FYVII-046C 21OH 16 17 dehydroprogesterone
104
f1 (ppm)


199.71
196.67
170.86
151.47
144.65
124.32
77.51
77.25
77.00
65.29
55.60
54.27
46.78
34.52
34.16
33.98
32.86
32.76
31.97
20.92
17.43
16.28
OH
O
O
3
104
f1 (ppm)


199.6093
196.5778
170.7571
151.3778
144.5517
124.2238
77.4146
77.1605
76.9063
65.1932
54.1804
46.6914
34.0698
31.8834
20.8280
16.1877
0.1506
OH
O
O
21hydroxy16dehydroprogesterone.C13.2days
500 MHz
2500
2000
1500
1000
500
0
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

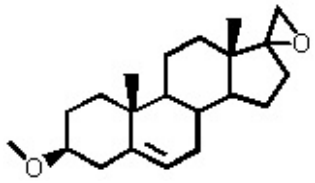

FYV-023C
400 MHz
105
f1 (ppm)


208.75
140.85
121.40
121.22
77.16
76.91
73.86
72.25
71.77
54.64
38.86
36.63
31.89
31.54
20.80
19.52
14.50
O
OH
HO
FYV-023C
500 MHz
105
650
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



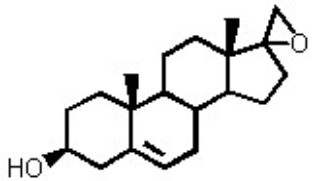
400 MHz

f1 (ppm)



400 MHz

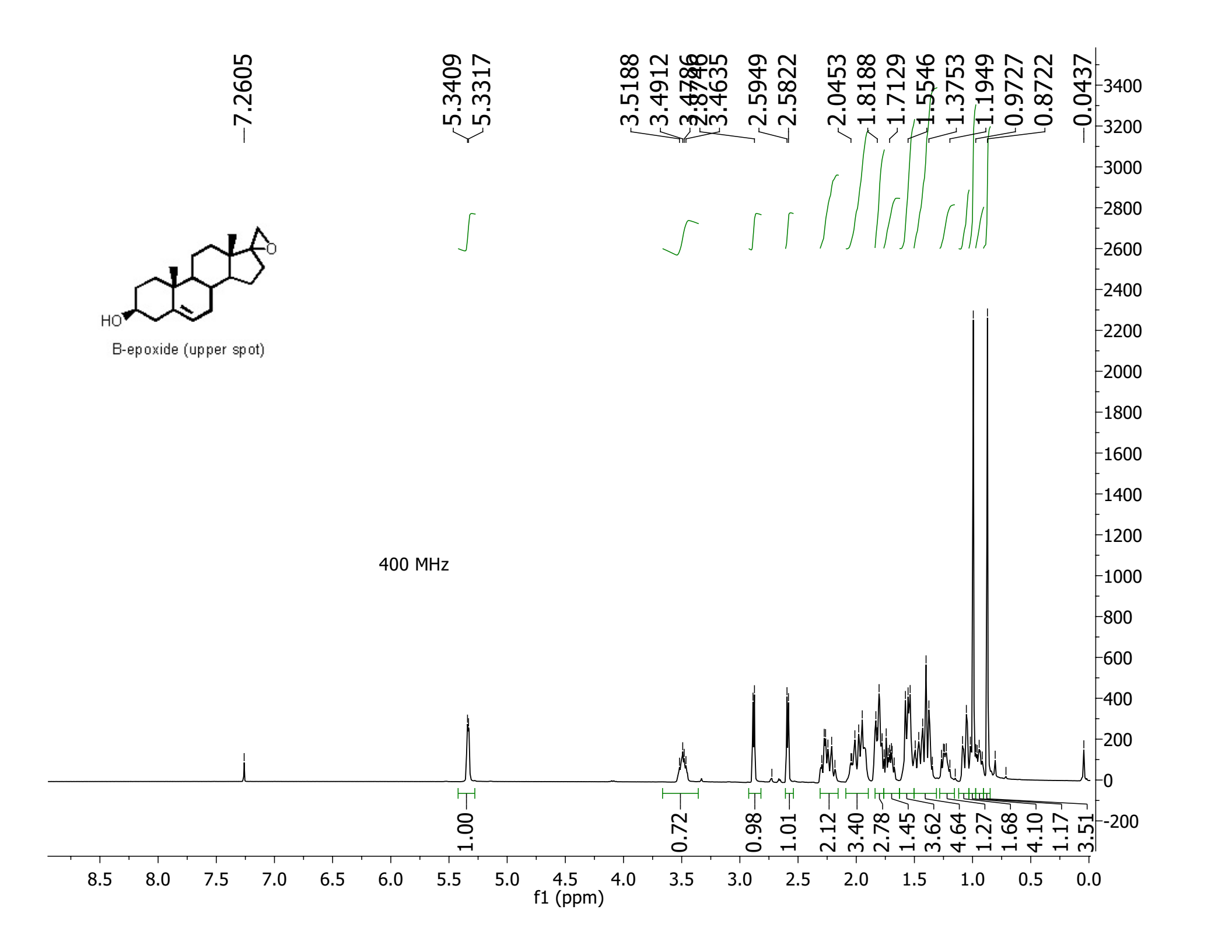
f1 (ppm)

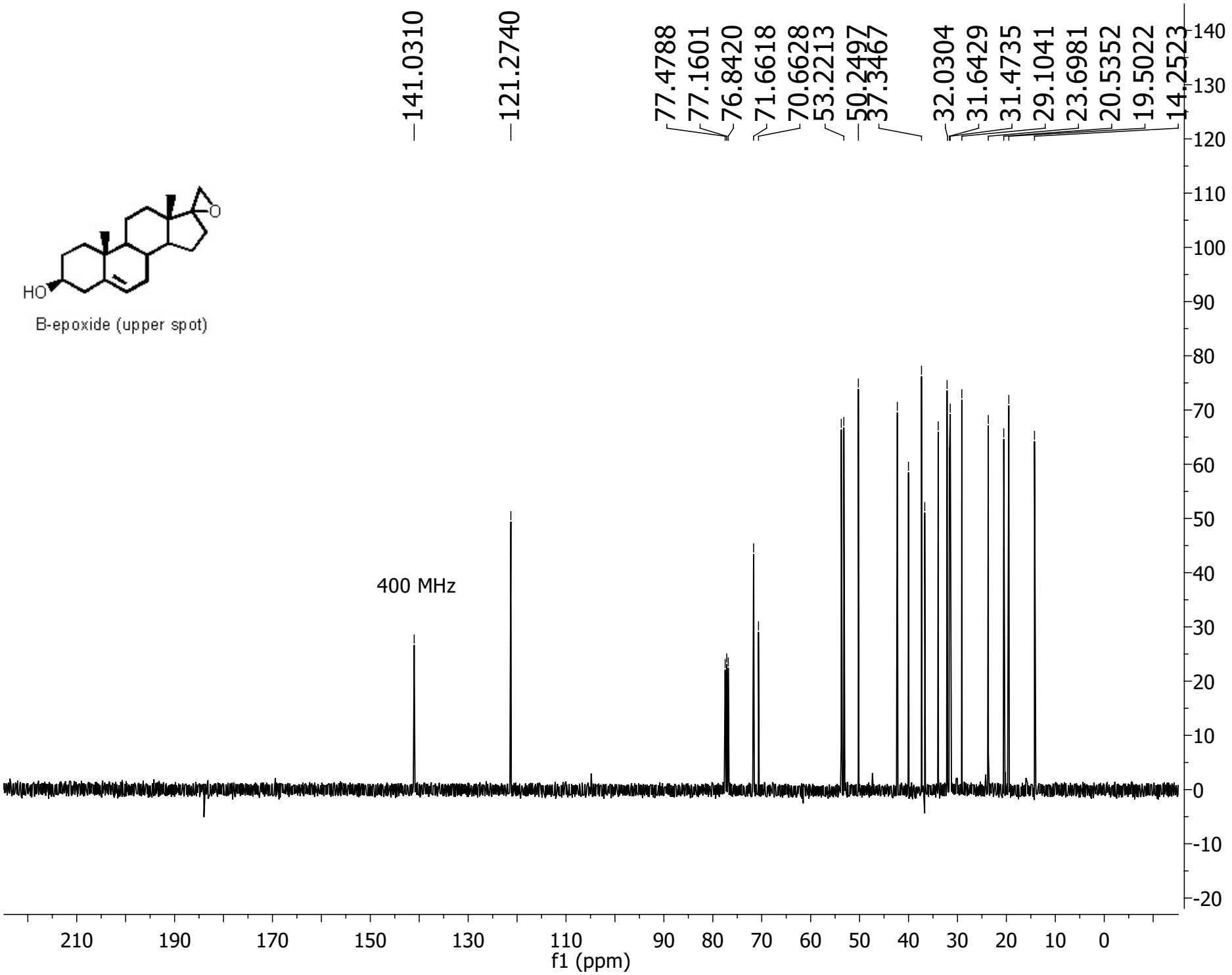


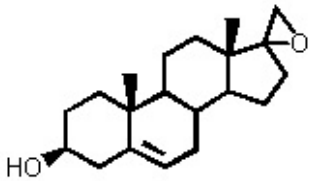
FYV-050B

400 MHz

f1 (ppm)


7.2605
5.3409
5.3317
3.5188
3.4912
3.4786
3.4635
2.5949
2.5822
2.0453
1.8188
1.7129
1.5546
1.3753
1.1949
0.9727
0.8722
0.0437
HO
O
B-epoxide (upper spot)
400 MHz
1.00
0.72
0.98
1.01
2.12
3.40
2.78
1.45
3.62
4.64
1.27
1.68
4.10
1.17
3.51
f1 (ppm)

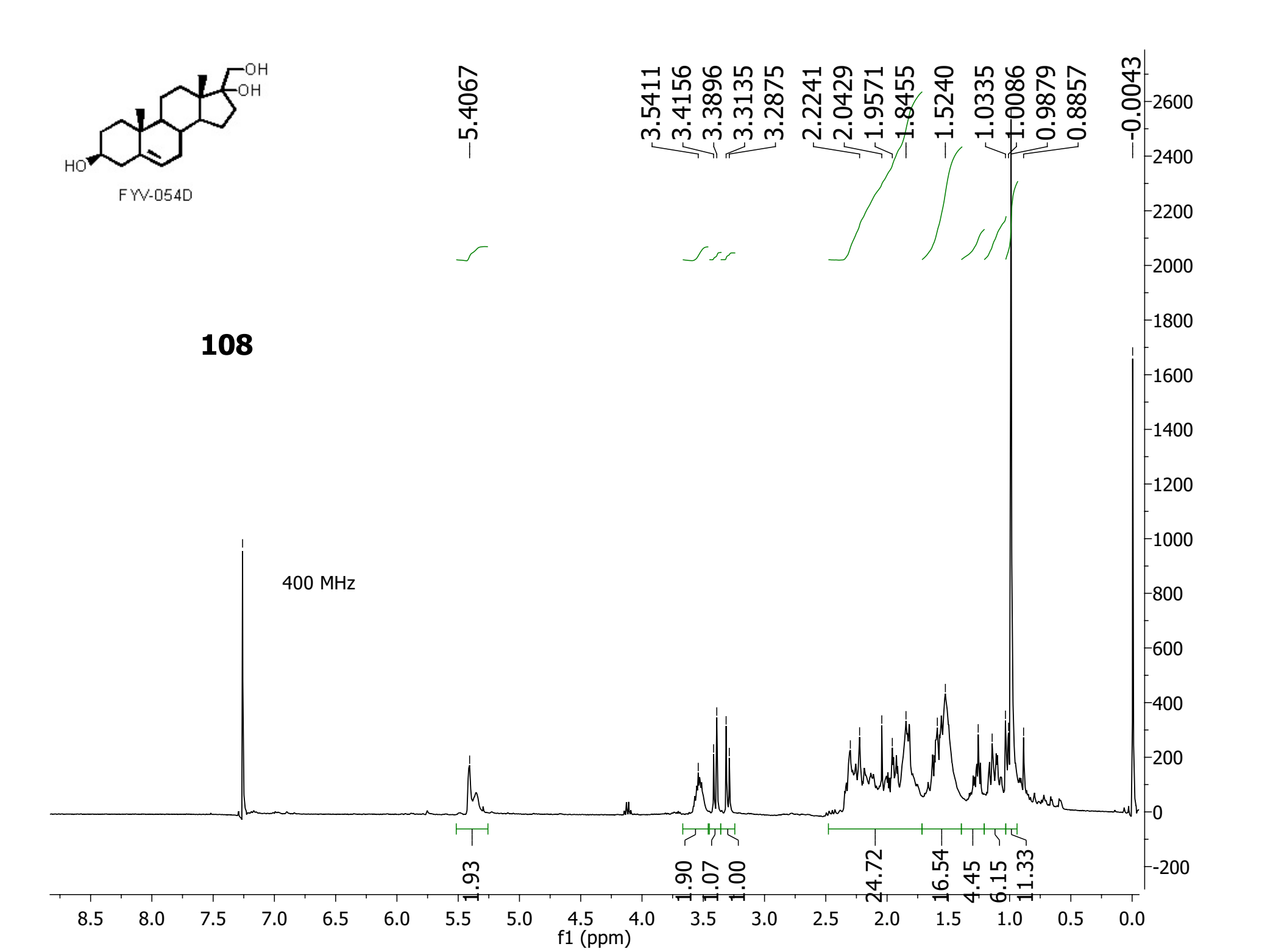

141.0310
121.2740
77.4788
77.1601
76.8420
71.6618
70.6628
53.2213
50.2497
37.3467
32.0304
31.6429
31.4735
29.1041
23.6981
20.5352
19.5022
14.2523
HO
O
B-epoxide (upper spot)
400 MHz
f1 (ppm)

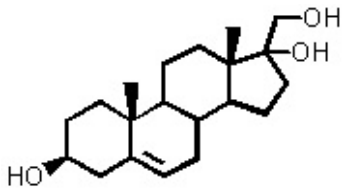


C-epoxide (lower spot, minor product)

107

400 MHz

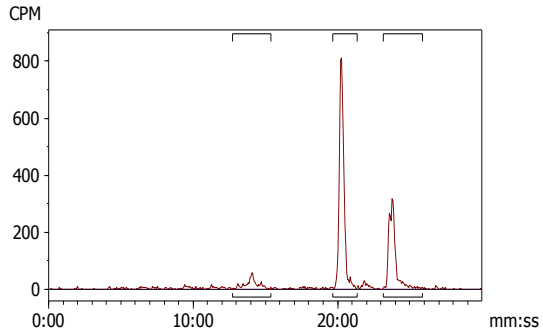

OH
OH
HO
FYV-054D
108
400 MHz
5.4067
3.5411
3.4156
3.3896
3.3135
3.2875
2.2241
2.0429
1.9571
1.8455
1.5240
1.0335
1.0086
0.9879
0.8857
-0.0043
1.93
1.90
1.07
1.00
24.72
16.54
4.45
6.15
11.33
f1 (ppm)



FYV-054H2SO4 triol

400 MHz

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	12:42	15:22	14:06	2573	8.28	7.26
Region 2	19:41	21:22	20:14	18307	58.94	51.65
Region 3	23:10	25:54	23:47	10181	32.78	28.72
3 Peaks				31061	100.00	87.64

Total Area: 35443 CPM
Average Background: 0 CPM

FYX-007. 17Dpreg (40 uM) with [^{3}H]preg and C17WT then cholesterol oxidase.E.CMethod:Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, January 27, 2012 9:32:58 PM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Monday, February 06, 2012 3:00:59 PM

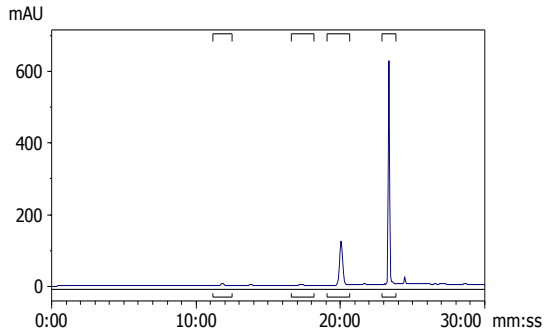
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 13
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:11	12:31	11:48	92	1.54	0.82
Region 2	16:37	18:11	17:15	70	1.17	0.63
Region 3	19:03	20:40	20:00	1852	30.87	16.51
Region 4	22:54	23:51	23:19	3983	66.41	35.52
4 Peaks				5998	100.00	53.49

Total Area: 11212 mAU
Average Background: N/A mAU

FYX-007. 17Dpreg (40 uM) with [^{3}H]preg and C17WT then cholesterol oxidase.E.CMethod:Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, January 27, 2012 9:32:58 PM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Monday, February 06, 2012 3:00:59 PM

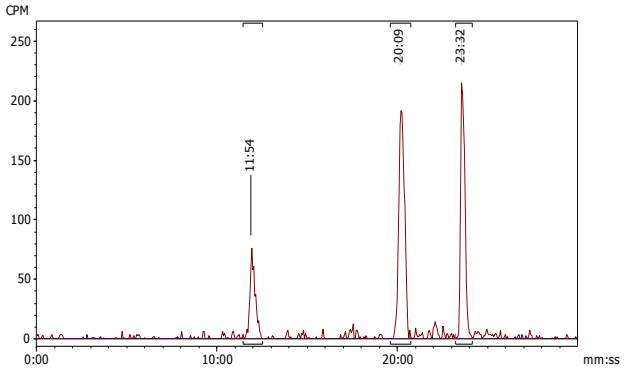
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 13
Injection Volume: 5 µL

Comments:

Chromatogram: ^{14}C



Regions: ^{14}C Detector: PMT

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:28	12:32	11:54	1488	15.63	12.79
Region 2	19:38	20:44	20:09	4402	46.24	37.85
Region 3	23:14	24:08	23:32	3629	38.12	31.20
3 Peaks				9518	100.00	81.84

Total Area: 11630 CPM
Average Background: 0 CPM

C17WT 17-D-prog with [^{14}C]-prog C (VIAL 22)Method: Halogens_Test1_FOR.^{14}C.detection

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, January 27, 2012 7:13:54 PM
Method by: Super User on Thursday, January 26, 2012 7:45:12 PM
Evaluation by: Super User on Saturday, January 28, 2012 8:34:50 PM

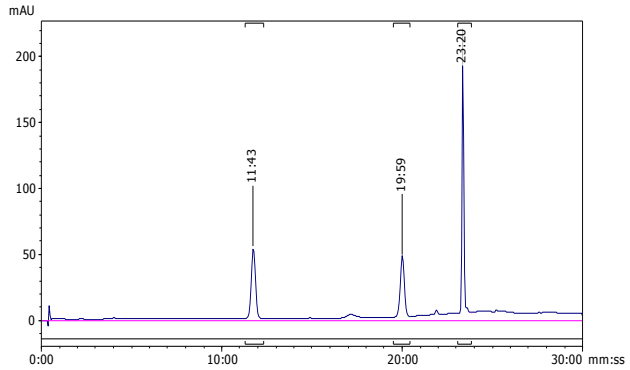
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{14}C	20-1000	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 22
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:18	12:18	11:43	864	30.07	10.87
Region 2	19:32	20:25	19:59	724	25.20	9.11
Region 3	23:06	23:49	23:20	1285	44.73	16.16
3 Peaks				2872	100.00	36.14

Total Area: 7948 mAU
Average Background: N/A mAU

C17WT 17-D-prog with [^{14}C]-prog C (VIAL 22)Method: Halogens Test1 FOR.^{14}C.detection

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, January 27, 2012 7:13:54 PM
Method by: Super User on Thursday, January 26, 2012 7:45:12 PM
Evaluation by: Super User on Saturday, January 28, 2012 8:34:50 PM

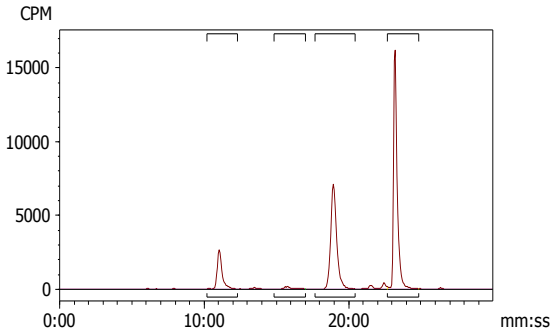
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{14}C	20-1000	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 22
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:10	12:18	11:01	64757	11.29	10.63
Region 2	14:52	17:00	15:42	9197	1.60	1.51
Region 3	17:41	20:27	18:55	209338	36.49	34.36
Region 4	22:42	24:52	23:10	290410	50.62	47.66
4 Peaks				573701	100.00	94.16

Total Area: 609283 CPM
Average Background: 0 CPM

C17WT.3uL.17Dprog.40uM.[^{3}H].prog.30uL (10 x hot).V61.C17.AMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Saturday, February 04, 2012 6:53:30 PM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Monday, February 06, 2012 10:36:13 AM

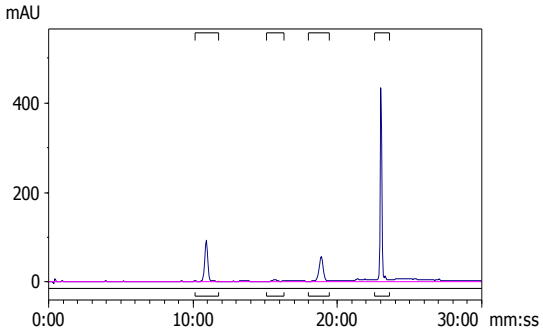
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 61
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:10	11:45	10:53	1306	22.22	11.96
Region 2	15:07	16:18	15:38	64	1.08	0.58
Region 3	18:01	19:26	18:50	1097	18.66	10.04
Region 4	22:35	23:37	22:59	3412	58.04	31.24
4 Peaks				5879	100.00	53.83

Total Area: 10921 mAU
Average Background: N/A mAU

C17WT.3uL.17Dprog.40uM.[^{3}H].prog.30uL (10 x hot).V61.C17.AMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Saturday, February 04, 2012 6:53:30 PM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Monday, February 06, 2012 10:36:13 AM

Run Length: 30m
Dwell: 1s

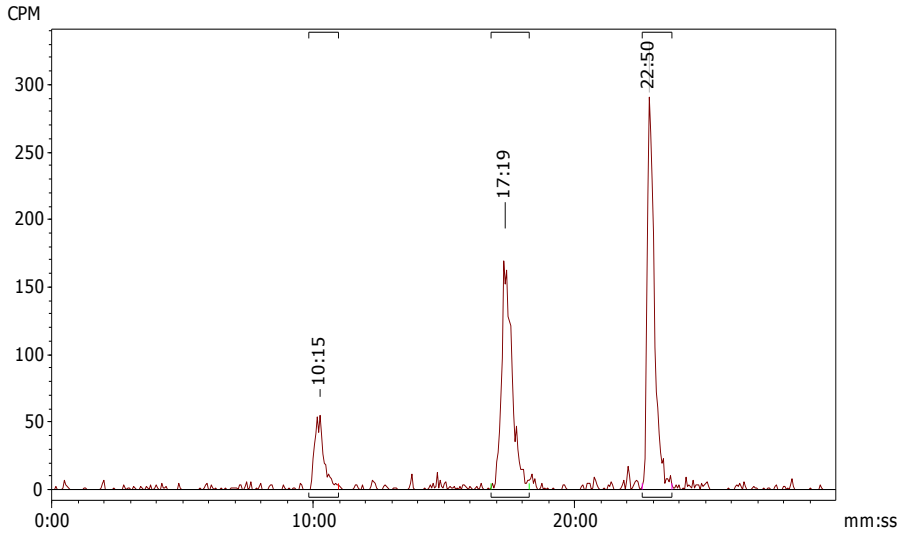
Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 61
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	9:49	10:59	10:15	1349	11.55	9.82
Region 2	16:47	18:15	17:19	4766	40.81	34.70
Region 3	22:35	23:42	22:50	5563	47.64	40.50
3 Peaks				11678	100.00	85.01

Total Area: 13738 CPM
Average Background: 0 CPM

FYX-001 C17WT prog.and.hot.prog.1H.AMethod: Halogens_Test1

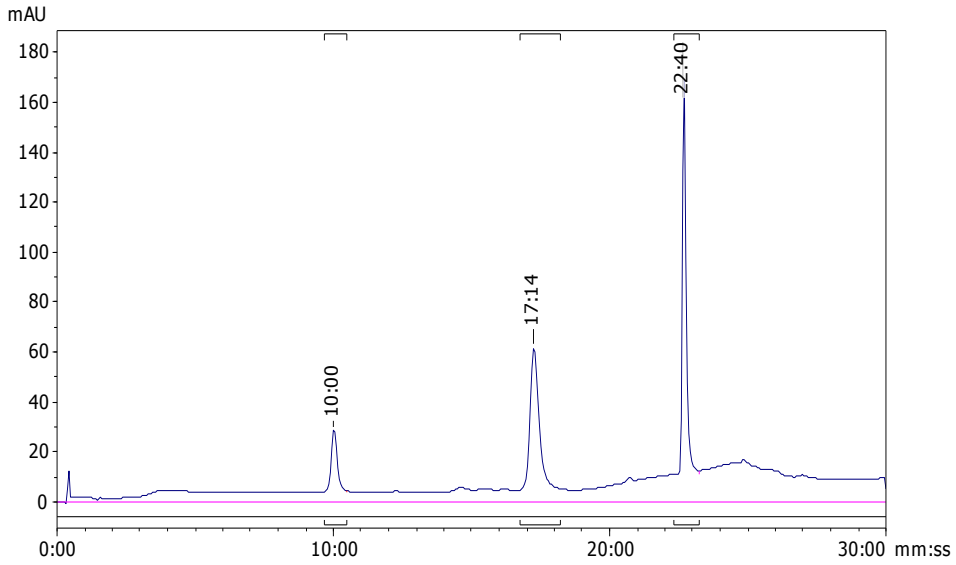
Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, January 17, 2012 9:12:23 PM
Method by: Super User on Monday, December 05, 2011 2:07:44 PM
Evaluation by: Super User on Tuesday, January 17, 2012 9:45:46 PM

Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 5
Injection Volume: 5 µL

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:41	10:30	10:00	371	12.07	2.59
Region 2	16:46	18:14	17:14	1280	41.60	8.92
Region 3	22:21	23:16	22:40	1425	46.32	9.93
3 Peaks				3076	100.00	21.44

Total Area: 14347 mAU
Average Background: N/A mAU

FYX-001 C17WT prog.and.hot.prog.1H.AMethod: Halogens_Test1

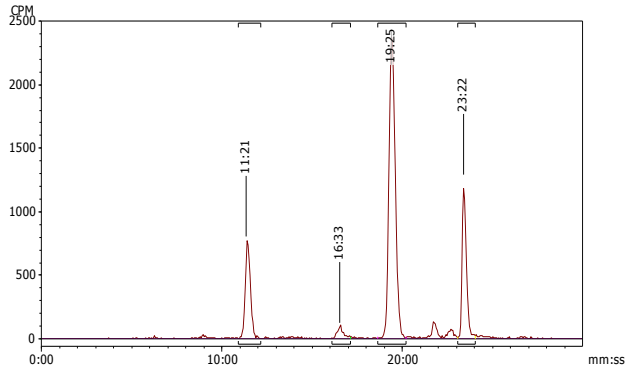
Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, January 17, 2012 9:12:23 PM
Method by: Super User on Monday, December 05, 2011 2:07:44 PM
Evaluation by: Super User on Tuesday, January 17, 2012 9:45:46 PM
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 5
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:55	12:08	11:21	15504	16.08	14.42
Region 2	16:06	17:08	16:33	2467	2.56	2.29
Region 3	18:39	20:11	19:25	58584	60.75	54.49
Region 4	23:05	24:03	23:22	19886	20.62	18.50
4 Peaks				96442	100.00	89.70

Total Area: 107520 CPM
Average Background: 0 CPM

FYX-011.3uL.C17WT.40uM.21.21.21.D3.Prog.hot.prog.vial.71.C17.AMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Saturday, January 28, 2012 12:02:27 PM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Saturday, January 28, 2012 5:04:31 PM

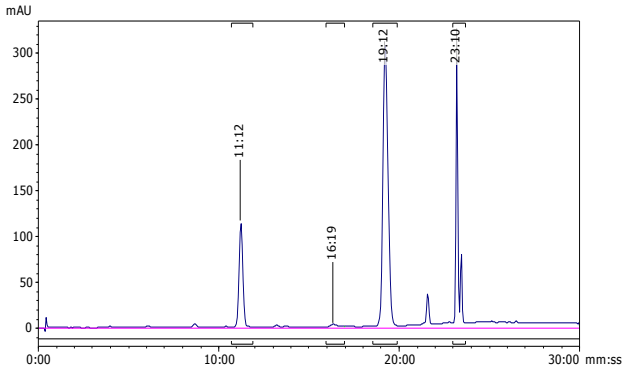
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 71
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:42	11:54	11:12	1637	16.31	10.46
Region 2	15:58	16:57	16:19	50	0.50	0.32
Region 3	18:32	19:55	19:12	5988	59.66	38.25
Region 4	23:00	23:40	23:10	2361	23.53	15.09
4 Peaks				10036	100.00	64.11

Total Area: 15654 mAU
Average Background: N/A mAU

FYX-011.3uL.C17WT.40uM.21.21.21.D3.Prog.hot.prog.vial.71.C17.AMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Saturday, January 28, 2012 12:02:27 PM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Saturday, January 28, 2012 5:04:31 PM

Run Length: 30m
Dwell: 1s

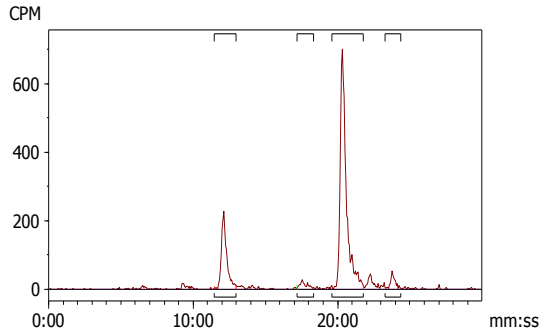
Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %

Off

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 71
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:26	12:59	12:03	5770	20.32	17.86
Region 2	17:13	18:20	17:28	826	2.91	2.55
Region 3	19:38	21:48	20:17	20635	72.69	63.86
Region 4	23:18	24:23	23:45	1157	4.08	3.58
4 Peaks				28387	100.00	87.85

Total Area: 32314 CPM
Average Background: 0 CPM

FYX-007.17Dprog.[^{3}H]prog.C17WT.1BMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, January 26, 2012 1:32:16 AM
Method by: Super User on Monday, December 05, 2011 2:07:44 PM
Evaluation by: Super User on Monday, February 06, 2012 1:56:39 PM

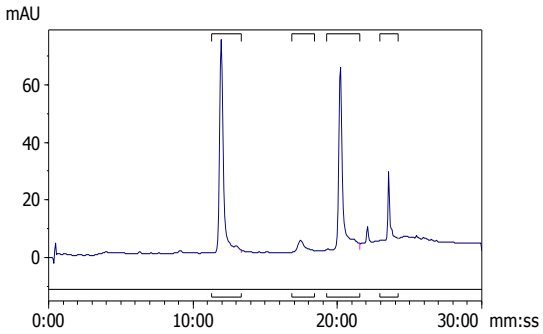
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 6
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:18	13:21	11:55	1363	48.77	16.42
Region 2	16:52	18:25	17:24	123	4.41	1.48
Region 3	19:16	21:33	20:10	1097	39.23	13.21
Region 4	22:55	24:11	23:31	212	7.59	2.56
4 Peaks				2795	100.00	33.67

Total Area: 8302 mAU
Average Background: N/A mAU

FYX-007.17Dprog.[^{3}H]prog.C17WT.1BMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, January 26, 2012 1:32:16 AM
Method by: Super User on Monday, December 05, 2011 2:07:44 PM
Evaluation by: Super User on Monday, February 06, 2012 1:56:39 PM

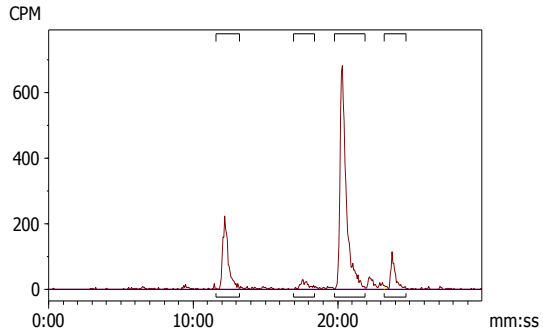
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 6
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:36	13:13	12:09	5918	19.79	17.61
Region 2	16:57	18:23	17:31	1066	3.56	3.17
Region 3	19:47	21:53	20:16	20472	68.45	60.93
Region 4	23:15	24:45	23:45	2453	8.20	7.30
4 Peaks				29909	100.00	89.01

Total Area: 33600 CPM
Average Background: 0 CPM

FYX-007.16Dprog.[^{3}H]prog.C17WT.2CMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, January 26, 2012 3:51:25 AM
Method by: Super User on Monday, December 05, 2011 2:07:44 PM
Evaluation by: Super User on Monday, February 06, 2012 1:54:51 PM

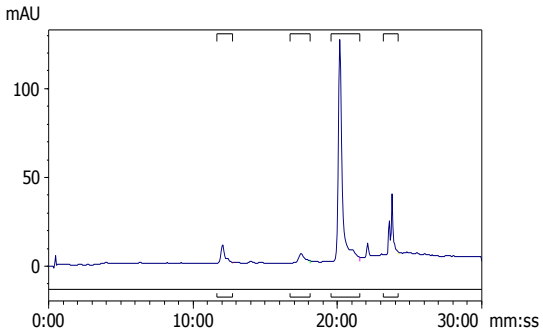
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 10
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:39	12:44	12:01	196	6.45	2.24
Region 2	16:42	18:08	17:27	120	3.96	1.38
Region 3	19:34	21:34	20:08	2286	75.33	26.17
Region 4	23:11	24:14	23:45	433	14.26	4.95
4 Peaks				3034	100.00	34.74

Total Area: 8734 mAU
Average Background: N/A mAU

FYX-007.16Dprog.[^{3}H]prog.C17WT.2CMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, January 26, 2012 3:51:25 AM
Method by: Super User on Monday, December 05, 2011 2:07:44 PM
Evaluation by: Super User on Monday, February 06, 2012 1:54:51 PM

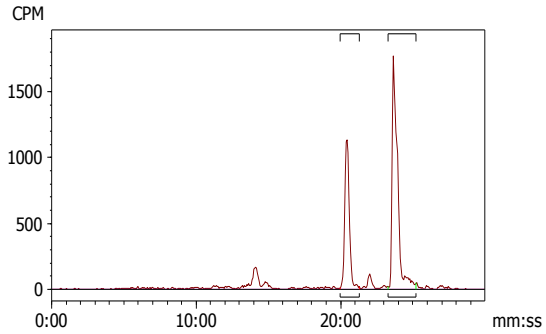
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 10
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	19:57	21:17	20:23	25320	35.22	27.40
Region 2	23:18	25:13	23:38	46565	64.78	50.39
2 Peaks				71885	100.00	77.79

Total Area: 92410 CPM
Average Background: 0 CPM

FYX-007. 17Dpreg (40 uM) with [^{3}H]preg and C17A105L then cholesterol oxidas.EM.CMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, January 27, 2012 11:17:14 PM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Monday, February 06, 2012 2:45:35 PM

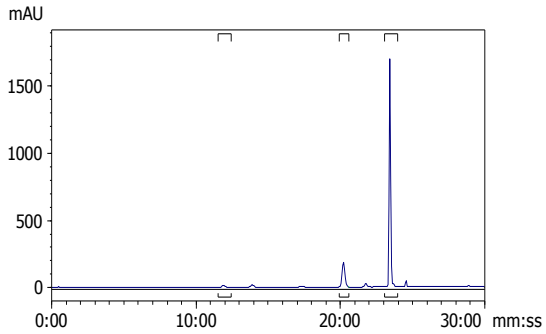
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 16
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:31	12:27	11:52	270	1.74	1.20
Region 2	19:54	20:36	20:10	2563	16.56	11.44
Region 3	23:02	23:57	23:23	12646	81.70	56.45
3 Peaks				15478	100.00	69.09

Total Area: 22403 mAU
Average Background: N/A mAU

FYX-007. 17Dpreg (40 uM) with [^{3}H]preg and C17A105L then cholesterol oxidas.EM.CMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, January 27, 2012 11:17:14 PM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Monday, February 06, 2012 2:45:35 PM

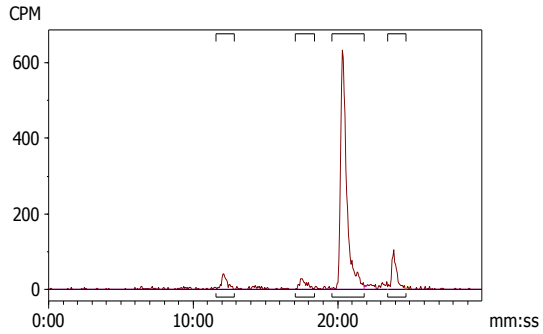
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 16
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:36	12:51	12:07	1152	4.97	4.41
Region 2	17:04	18:23	17:25	979	4.22	3.75
Region 3	19:38	21:51	20:20	18634	80.37	71.29
Region 4	23:28	24:45	23:53	2419	10.43	9.26
4 Peaks				23184	100.00	88.71

Total Area: 26136 CPM
Average Background: 0 CPM

FYX-007.21.21.21.D3prog.[^{3}H]prog.C17A105L.5BMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, January 26, 2012 6:45:21 AM
Method by: Super User on Monday, December 05, 2011 2:07:44 PM
Evaluation by: Super User on Monday, February 06, 2012 2:10:07 PM

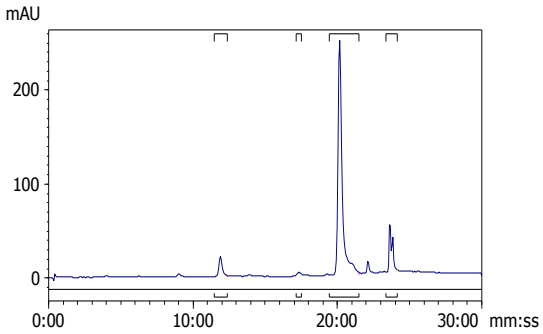
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 18
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:27	12:22	11:51	320	5.27	2.65
Region 2	17:09	17:32	17:17	24	0.39	0.20
Region 3	19:25	21:29	20:07	4981	81.94	41.24
Region 4	23:20	24:10	23:35	754	12.40	6.24
4 Peaks				6079	100.00	50.33

Total Area: 12078 mAU
Average Background: N/A mAU

FYX-007.21.21.21.D3prog.[^{3}H]prog.C17A105L.5BMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, January 26, 2012 6:45:21 AM
Method by: Super User on Monday, December 05, 2011 2:07:44 PM
Evaluation by: Super User on Monday, February 06, 2012 2:10:07 PM

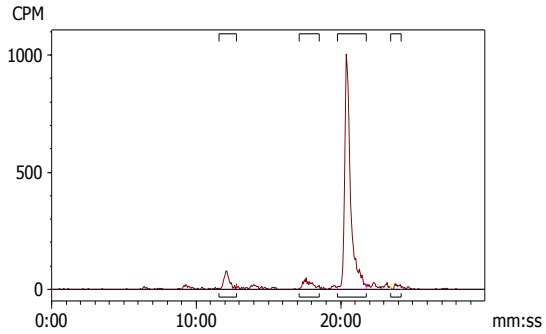
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 18
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:35	12:46	12:04	2131	6.53	5.66
Region 2	17:09	18:31	17:34	1838	5.63	4.89
Region 3	19:46	21:48	20:22	28118	86.11	74.73
Region 4	23:27	24:12	23:51	566	1.73	1.51
4 Peaks				32654	100.00	86.78

Total Area: 37627 CPM
Average Background: 0 CPM

FYX-007.17Dprog.[^{3}H]prog.C17A105L.3CMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, January 26, 2012 4:26:13 AM
Method by: Super User on Monday, December 05, 2011 2:07:44 PM
Evaluation by: Super User on Monday, February 06, 2012 2:09:21 PM

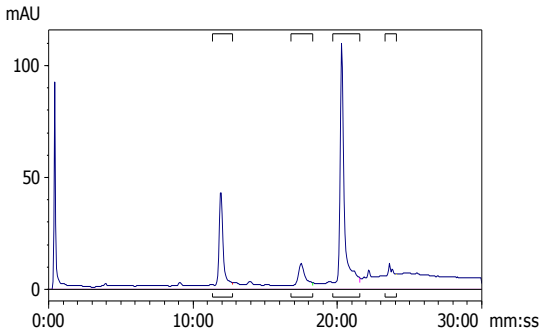
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 13
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:22	12:44	11:53	726	24.61	7.74
Region 2	16:46	18:18	17:27	250	8.46	2.66
Region 3	19:41	21:33	20:15	1912	64.82	20.39
Region 4	23:17	24:04	23:34	63	2.12	0.67
4 Peaks				2951	100.00	31.47

Total Area: 9377 mAU
Average Background: N/A mAU

FYX-007.17Dprog.[^{3}H]prog.C17A105L.3CMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, January 26, 2012 4:26:13 AM
Method by: Super User on Monday, December 05, 2011 2:07:44 PM
Evaluation by: Super User on Monday, February 06, 2012 2:09:21 PM

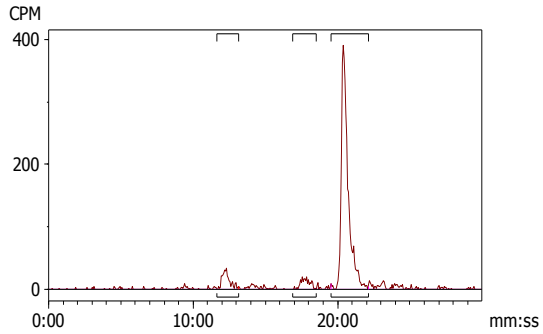
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 13
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:39	13:10	12:07	1166	7.94	6.92
Region 2	16:52	18:31	17:32	821	5.59	4.87
Region 3	19:31	22:07	20:22	12706	86.48	75.35
3 Peaks				14693	100.00	87.13

Total Area: 16862 CPM
Average Background: 0 CPM

FYX-007.16Dprog.[^{3}H]prog.C17A105L.4AMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, January 25, 2012 10:38:22 PM
Method by: Super User on Monday, December 05, 2011 2:07:44 PM
Evaluation by: Super User on Monday, February 06, 2012 2:08:46 PM

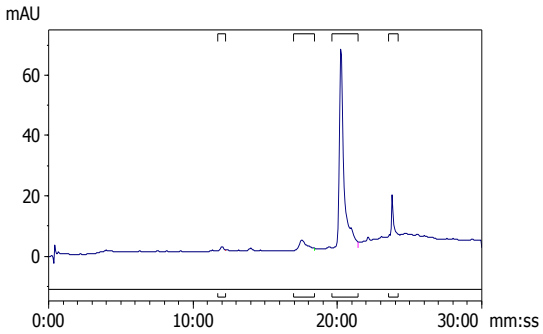
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 14
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:42	12:15	11:58	18	1.16	0.26
Region 2	16:57	18:25	17:30	106	6.61	1.51
Region 3	19:36	21:24	20:13	1362	85.22	19.42
Region 4	23:32	24:13	23:45	112	7.01	1.60
4 Peaks				1598	100.00	22.79

Total Area: 7011 mAU
Average Background: N/A mAU

FYX-007.16Dprog.[^{3}H]prog.C17A105L.4AMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, January 25, 2012 10:38:22 PM
Method by: Super User on Monday, December 05, 2011 2:07:44 PM
Evaluation by: Super User on Monday, February 06, 2012 2:08:46 PM

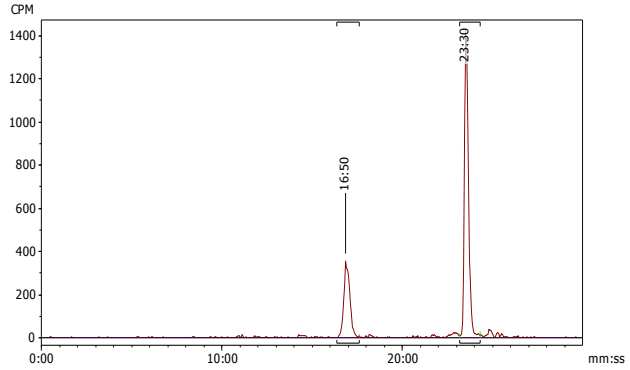
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 14
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	16:22	17:36	16:50	8270	26.89	23.57
Region 2	23:10	24:20	23:30	22488	73.11	64.10
2 Peaks				30758	100.00	87.67

Total Area: 35083 CPM
Average Background: 0 CPM

FYX-011.20uL.C21WT.20uM.Prog.hot.prog.vial.41.1AMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Saturday, January 28, 2012 2:11:07 AM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Saturday, January 28, 2012 4:10:58 PM

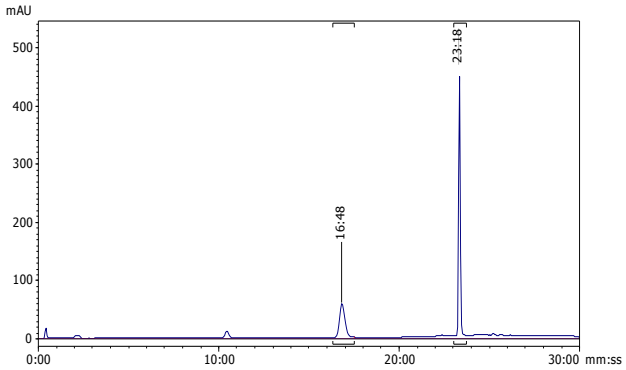
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 41
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	16:18	17:30	16:48	1209	29.03	12.73
Region 2	23:02	23:43	23:18	2955	70.97	31.11
2 Peaks				4164	100.00	43.84

Total Area: 9500 mAU
Average Background: N/A mAU

FYX-011.20uL.C21WT.20uM.Prog.hot.prog.vial.41.1AMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Saturday, January 28, 2012 2:11:07 AM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Saturday, January 28, 2012 4:10:58 PM

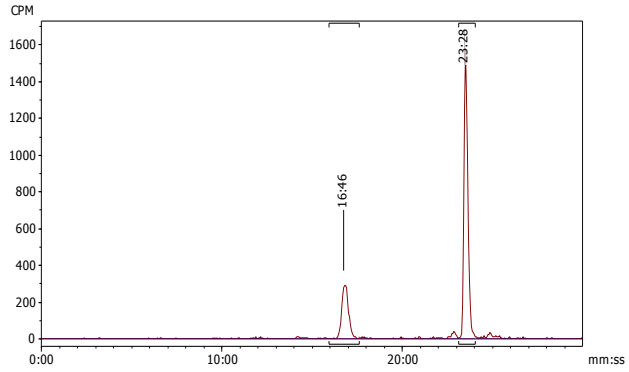
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 41
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	15:55	17:38	16:46	7805	24.87	22.12
Region 2	23:06	24:02	23:28	23582	75.13	66.83
2 Peaks				31387	100.00	88.94

Total Area: 35290 CPM
Average Background: 0 CPM

FYX-011.20uL.C21WT.20uM.21.21.21D3.Prog.hot.prog.vial.44.2A Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Saturday, January 28, 2012 3:55:26 AM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Saturday, January 28, 2012 4:25:28 PM

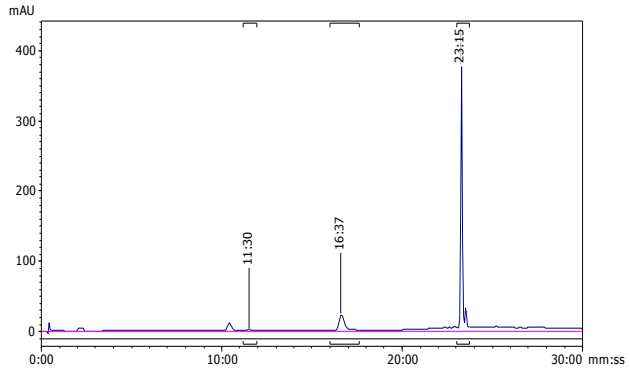
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 44
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:10	11:58	11:30	26	0.83	0.31
Region 2	16:00	17:36	16:37	470	15.12	5.62
Region 3	23:03	23:44	23:15	2611	84.05	31.24
3 Peaks				3107	100.00	37.17

Total Area: 8359 mAU
Average Background: N/A mAU

FYX-011.20uL.C21WT.20uM.21.21.21D3.Prog.hot.prog.vial.44.2AMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Saturday, January 28, 2012 3:55:26 AM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Saturday, January 28, 2012 4:25:28 PM

Run Length: 30m
Dwell: 1s

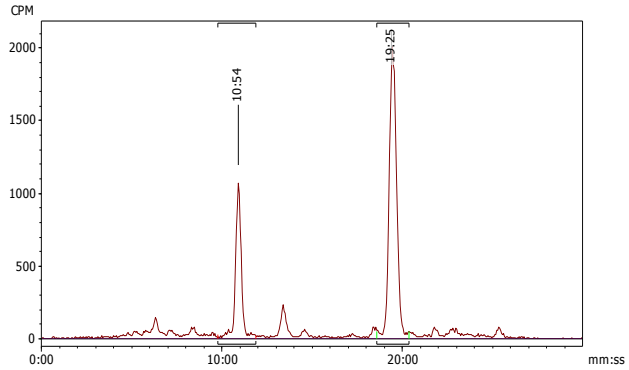
Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %

Off

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 44
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	9:48	11:52	10:54	24163	29.52	20.08
Region 2	18:34	20:23	19:25	57677	70.48	47.93
2 Peaks				81840	100.00	68.00

Total Area: 120346 CPM
Average Background: 0 CPM

FYX-011.20uL.C21WT.20uM.21.21.21.D3.17OH.Prog.hot.17OHprog.vial.53.5AMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Saturday, January 28, 2012 9:08:29 AM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Saturday, January 28, 2012 5:24:31 PM

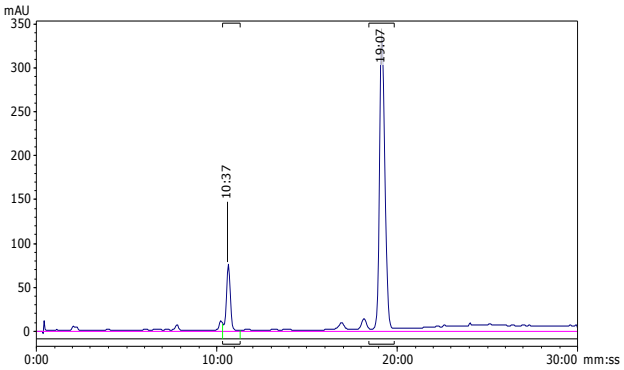
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 53
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:18	11:18	10:37	1190	14.86	8.42
Region 2	18:26	19:51	19:07	6818	85.14	48.24
2 Peaks				8008	100.00	56.65

Total Area: 14135 mAU
Average Background: N/A mAU

FYX-011.20uL.C21WT.20uM.21.21.21.D3.17OH.Prog.hot.17OHprog.vial.53.5AMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Saturday, January 28, 2012 9:08:29 AM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Saturday, January 28, 2012 5:24:31 PM

Run Length: 30m
Dwell: 1s

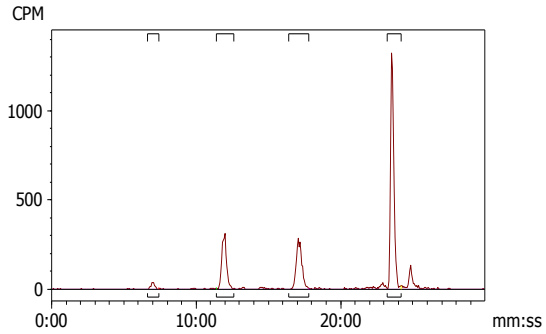
Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %

Off

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 53
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	6:37	7:26	6:53	734	2.00	1.71
Region 2	11:24	12:37	11:57	7157	19.52	16.65
Region 3	16:25	17:47	17:03	7622	20.79	17.74
Region 4	23:12	24:10	23:30	21158	57.70	49.23
4 Peaks				36672	100.00	85.32

Total Area: 42979 CPM
Average Background: 0 CPM

FYX-009 vial 9 C21V359A 21.21.21.D3.prog with [^{3}H]-prog.(vial 9).3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, January 27, 2012 6:04:19 PM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Monday, February 06, 2012 1:33:37 PM

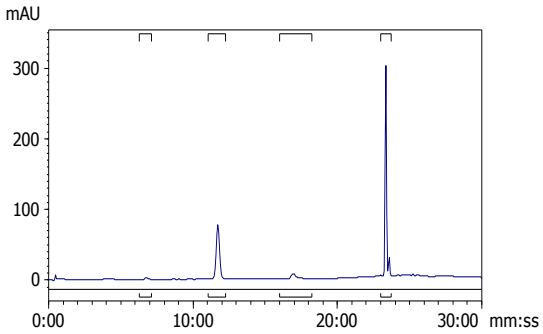
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 9
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	6:16	7:08	6:47	33	0.90	0.38
Region 2	11:03	12:16	11:41	1261	34.90	14.70
Region 3	15:59	18:12	16:54	211	5.84	2.46
Region 4	23:00	23:44	23:19	2108	58.36	24.58
4 Peaks				3613	100.00	42.13

Total Area: 8577 mAU
Average Background: N/A mAU

FYX-009 vial 9 C21V359A 21.21.21.D3.prog with [^{3}H]-prog.(vial 9).3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, January 27, 2012 6:04:19 PM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Monday, February 06, 2012 1:33:37 PM

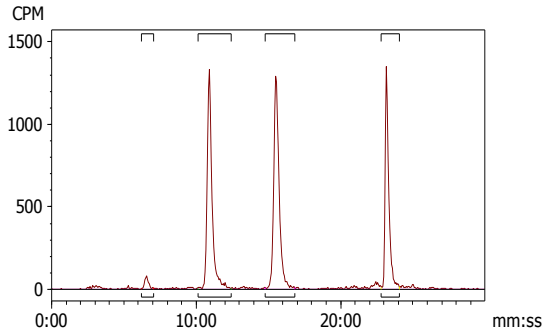
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 9
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	6:14	7:05	6:31	1579	1.76	1.61
Region 2	10:09	12:24	10:52	30682	34.23	31.22
Region 3	14:48	16:49	15:28	33667	37.56	34.26
Region 4	22:48	24:05	23:08	23712	26.45	24.13
4 Peaks				89640	100.00	91.22

Total Area: 98270 CPM
Average Background: 0 CPM

C21WT.3uL.21.d3.16D.prog.20uM.[^{3}H].prog.6.7uL.Vial.71.3C ACTUALLY V359AMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Sunday, February 05, 2012 12:41:27 AM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Monday, February 06, 2012 10:32:05 AM

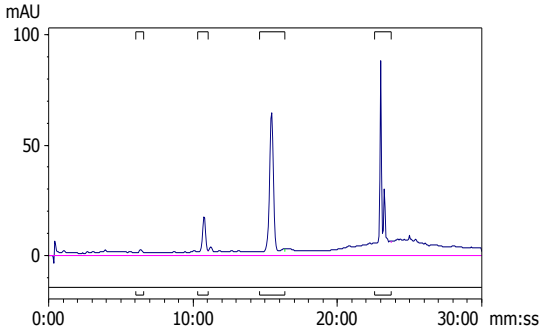
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 71
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	6:03	6:36	6:20	14	0.67	0.19
Region 2	10:18	11:02	10:44	223	10.53	3.00
Region 3	14:37	16:21	15:24	1124	53.19	15.15
Region 4	22:33	23:43	22:58	753	35.61	10.14
4 Peaks				2114	100.00	28.47

Total Area: 7422 mAU
Average Background: N/A mAU

C21WT.3uL.21.d3.16D.prog.20uM.[^{3}H].prog.6.7uL.Vial.71.3C ACTUALLY V359AMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Sunday, February 05, 2012 12:41:27 AM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Monday, February 06, 2012 10:32:05 AM

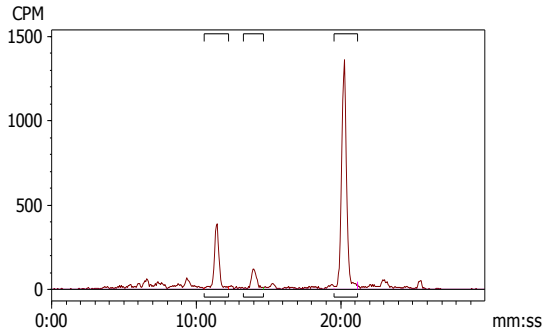
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 71
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:34	12:13	11:23	8870	19.77	13.77
Region 2	13:16	14:41	13:54	3403	7.59	5.28
Region 3	19:33	21:09	20:14	32592	72.64	50.60
3 Peaks				44866	100.00	69.65

Total Area: 64416 CPM
Average Background: 0 CPM

FYX-009 vial 10 C21V359A 21.21.21.D3.17OHprog with [^{3}H]-17OHprog.(vial 10).4Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, January 27, 2012 6:39:05 PM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Monday, February 06, 2012 1:38:42 PM

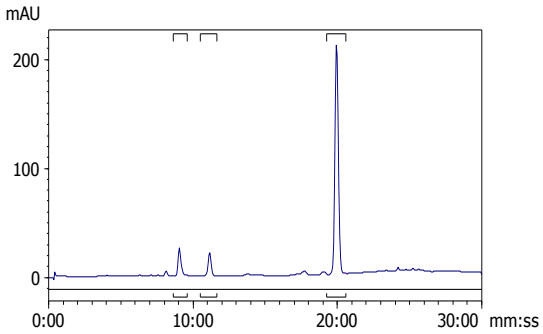
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 10
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	8:38	9:36	9:01	365	8.82	3.83
Region 2	10:31	11:38	11:07	305	7.37	3.20
Region 3	19:14	20:34	19:54	3475	83.81	36.39
3 Peaks				4146	100.00	43.41

Total Area: 9549 mAU
Average Background: N/A mAU

FYX-009 vial 10 C21V359A 21.21.21.D3.17OHprog with [^{3}H]-17OHprog.(vial 10).4Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, January 27, 2012 6:39:05 PM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Monday, February 06, 2012 1:38:42 PM

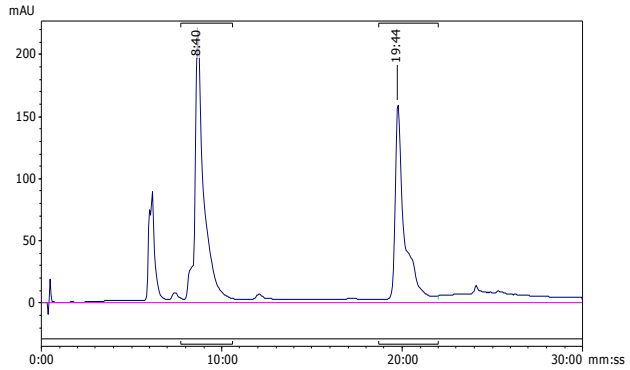
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 10
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	7:43	10:37	8:40	7105	57.92	33.81
Region 2	18:41	21:59	19:44	5163	42.08	24.56
2 Peaks				12269	100.00	58.37

Total Area: 21019 mAU
Average Background: N/A mAU

16.17.dihydroxyprog std with 17OHprog std vial 91Method: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, January 27, 2012 10:35:13 AM
Method by: Super User on Friday, January 27, 2012 9:48:45 AM
Evaluation by: Super User on Saturday, January 28, 2012 5:28:55 PM

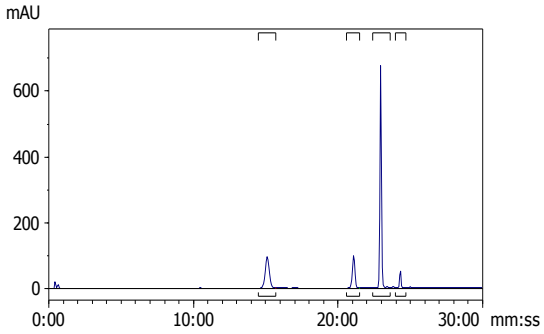
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
Off			
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 2.00 mL/min
Residence Time: 12.5s
Vial No: 91
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	14:28	15:41	15:04	1877	21.69	17.53
Region 2	20:35	21:31	21:03	1228	14.19	11.47
Region 3	22:25	23:36	22:55	5199	60.09	48.57
Region 4	23:59	24:41	24:16	348	4.03	3.26
4 Peaks				8652	100.00	80.83

Total Area: 10705 mAU
Average Background: N/A mAU

Standards.16.dehydroprog.16.dehydropreg.21OH.16dehydroprog.16epoxyprog.vial41Method: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, February 23, 2012 7:41:18 AM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Saturday, February 25, 2012 11:43:27 AM

Run Length: 30m
Dwell: 1s

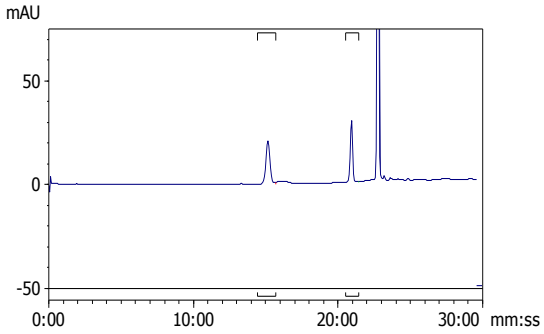
Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 41
Injection Volume: 3 µL

Comments:

Chromatogram: DA-B@254nm FYX-027 16-dehydroprog 21.22.eneprog5 Run 2



Regions: DA-B@254nm FYX-027 16-dehydroprog 21.22.eneprog5 Run 2 Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	14:24	15:41	15:07	382	53.80	7.95
Region 2	20:30	21:25	20:53	328	46.20	6.83
2 Peaks				710	100.00	14.78

Total Area: 4803 mAU
Average Background: N/A mAU

FYX-027.C17WT.6uL.16.dehydroprog(6 mM).10uL.vial45.1AMethod: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Saturday, February 18, 2012 2:44:43 PM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Sunday, February 19, 2012 3:13:26 PM

Run Length: 30m
Dwell: 1s

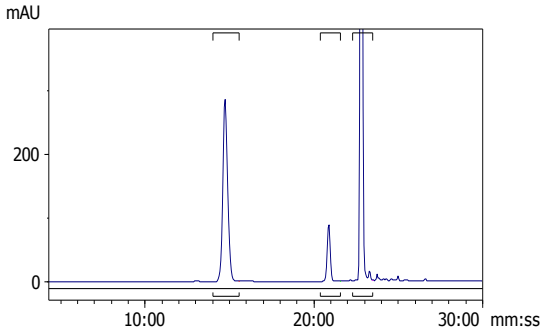
Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 45
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	14:02	15:33	14:42	5790	24.24	22.13
Region 2	20:22	21:35	20:51	1110	4.65	4.24
Region 3	22:17	23:29	22:46	16991	71.12	64.93
3 Peaks				23891	100.00	91.29

Total Area: 26169 mAU
Average Background: N/A mAU

FYX-027.C17A105L.6uL.16.dehydroprog(6 mM).10uL.vial49.2BMethod: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Saturday, February 18, 2012 6:48:37 PM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Saturday, February 25, 2012 10:52:03 AM

Run Length: 30m
Dwell: 1s

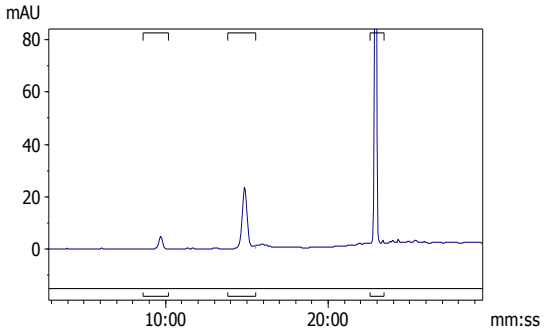
Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 49
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	8:39	10:12	9:41	73	3.27	1.74
Region 2	13:48	15:34	14:50	454	20.44	10.89
Region 3	22:35	23:24	22:53	1696	76.30	40.64
3 Peaks				2223	100.00	53.27

Total Area: 4172 mAU
Average Background: N/A mAU

FYX-028 C21WT 10uL. 16-dehydroprog 5uM.vial54.3Method: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Sunday, February 19, 2012 7:09:19 PM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Saturday, February 25, 2012 11:09:31 AM

Run Length: 30m
Dwell: 1s

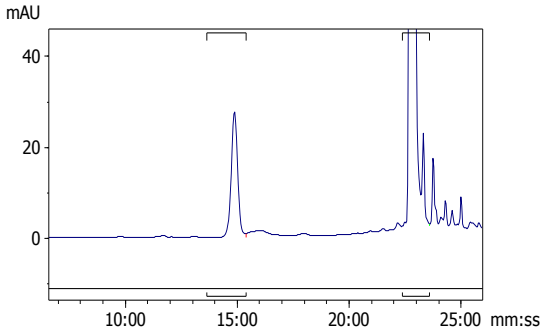
Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 54
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	13:38	15:24	14:52	510	1.93	1.78
Region 2	22:23	23:36	22:45	25848	98.07	90.42
2 Peaks				26358	100.00	92.20

Total Area: 28588 mAU
Average Background: N/A mAU

FYX-027.C21V359A.10uL.16.dehydroprog(6 mM).10uL.vial54.4AMethod: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Saturday, February 18, 2012 9:42:56 PM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Saturday, February 25, 2012 11:12:10 AM

Run Length: 30m
Dwell: 1s

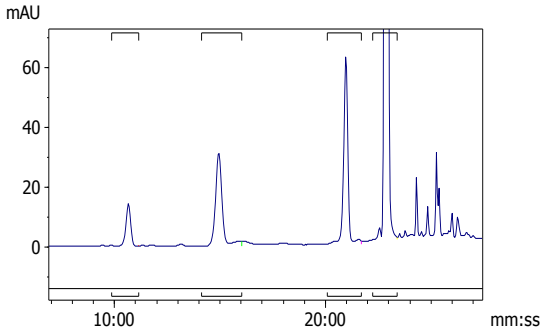
Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 54
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:52	11:09	10:39	210	1.72	1.41
Region 2	14:07	16:02	14:55	597	4.89	3.99
Region 3	20:04	21:41	20:57	827	6.78	5.53
Region 4	22:13	23:24	22:51	10569	86.60	70.67
4 Peaks				12204	100.00	81.60

Total Area: 14957 mAU
Average Background: N/A mAU

FYX-027 16.dehdropreg(1mM).40uL.C17WTyeast.6uL.V38.12C Method: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, February 23, 2012 5:56:43 AM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Thursday, February 23, 2012 6:15:39 PM

Run Length: 30m
Dwell: 1s

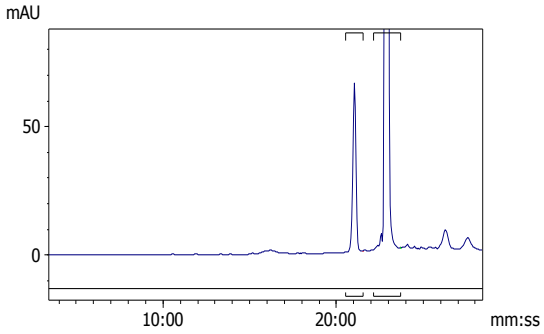
Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 38
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	20:32	21:31	21:01	817	4.36	3.91
Region 2	22:07	23:42	22:50	17918	95.64	85.82
2 Peaks				18735	100.00	89.73

Total Area: 20878 mAU
Average Background: N/A mAU

FYX-027 16.dehdropreg(1mM).40uL.C17purified.6uL.V35.B3CMethod: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, February 23, 2012 4:12:09 AM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Thursday, February 23, 2012 6:02:02 PM

Run Length: 30m
Dwell: 1s

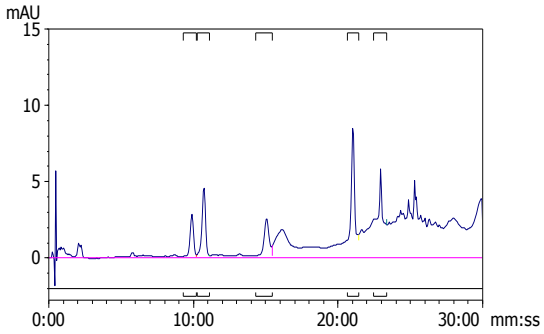
Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 35
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:17	10:11	9:51	43	15.42	2.07
Region 2	10:14	11:08	10:41	74	26.42	3.55
Region 3	14:19	15:28	15:01	36	12.90	1.73
Region 4	20:37	21:24	21:00	96	34.60	4.65
Region 5	22:29	23:20	22:55	30	10.66	1.43
5 Peaks				278	100.00	13.43

Total Area: 2071 mAU
Average Background: N/A mAU

FYX-028 16.dehdropreg(1mM).1uL.C17WTyeast.6uL.V39.5Method: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, February 23, 2012 6:31:35 AM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Saturday, February 25, 2012 11:41:06 AM

Run Length: 30m
Dwell: 1s

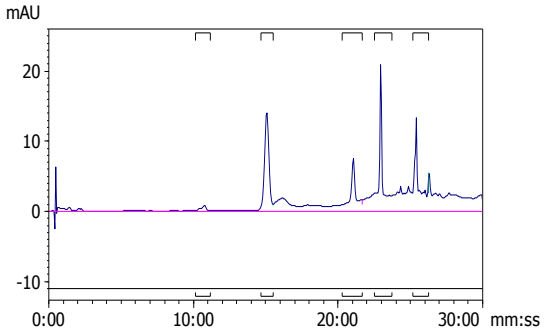
Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 39
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:10	11:10	10:43	16	3.16	0.68
Region 2	14:39	15:32	15:02	261	51.47	11.13
Region 3	20:18	21:40	21:01	83	16.26	3.51
Region 4	22:31	23:44	22:55	146	28.72	6.21
Region 5	25:09	26:15	25:22	2	0.39	0.08
5 Peaks				508	100.00	21.62

Total Area: 2350 mAU
Average Background: N/A mAU

FYX-028 16.dehdropreg(1mM).1uL.C17A105Lyeast.6uL.V40.6Method: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, February 23, 2012 7:06:26 AM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Saturday, February 25, 2012 11:40:01 AM

Run Length: 30m
Dwell: 1s

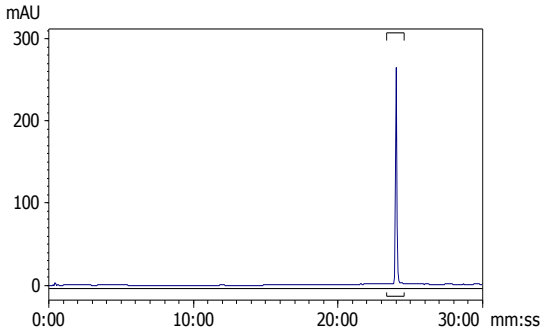
Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 40
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	23:21	24:35	23:59	1688	100.00	61.15
1 Peak				1688	100.00	61.15

Total Area: 2760 mAU
Average Background: N/A mAU

21.22.homodehydroprogesterone.std.10uL.3mM.in.60uLMeOHMethod: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, February 22, 2012 6:45:08 PM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Wednesday, February 22, 2012 7:18:02 PM

Run Length: 30m
Dwell: 1s

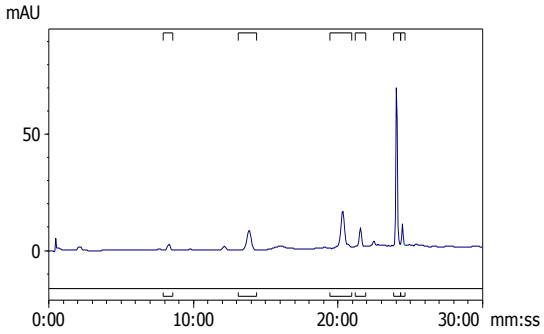
Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 31
Injection Volume: 3 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	7:55	8:33	8:16	36	3.08	1.27
Region 2	13:05	14:22	13:49	182	15.65	6.47
Region 3	19:26	20:55	20:17	305	26.21	10.83
Region 4	21:12	21:54	21:31	87	7.44	3.07
Region 5	23:50	24:19	24:01	502	43.17	17.84
Region 6	24:19	24:38	24:25	52	4.44	1.83
6 Peaks				1163	100.00	41.31

Total Area: 2816 mAU
Average Background: N/A mAU

FYX-029.21.22.ene.prog.(3mM).5.uL.C17A1.10uL.v21.1 Method: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, February 22, 2012 7:20:01 PM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Saturday, February 25, 2012 10:55:55 AM

Run Length: 30m
Dwell: 1s

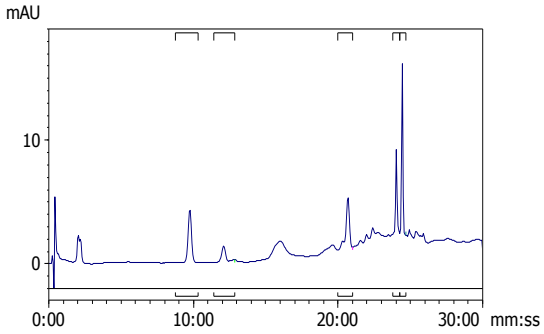
Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 21
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	8:44	10:19	9:43	70	24.63	3.57
Region 2	11:26	12:53	12:03	21	7.23	1.05
Region 3	20:00	21:02	20:39	71	24.88	3.60
Region 4	23:48	24:17	24:00	43	15.12	2.19
Region 5	24:17	24:41	24:24	80	28.13	4.07
5 Peaks				284	100.00	14.48

Total Area: 1963 mAU
Average Background: N/A mAU

FYX-029.21.22.ene.prog.(3mM).5.uL.C21A2.10uL.v27.3Method: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, February 22, 2012 8:29:40 PM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Saturday, February 25, 2012 10:57:22 AM

Run Length: 30m
Dwell: 1s

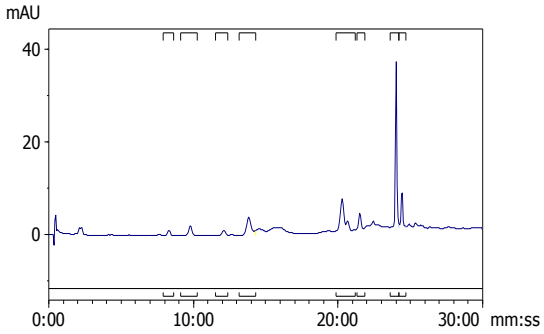
Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 27
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	7:53	8:37	8:16	15	2.67	0.90
Region 2	9:07	10:16	9:45	30	5.15	1.73
Region 3	11:32	12:23	12:04	20	3.48	1.17
Region 4	13:11	14:17	13:48	59	10.35	3.48
Region 5	19:50	21:11	20:15	137	23.85	8.02
Region 6	21:20	21:52	21:28	25	4.43	1.49
Region 7	23:35	24:13	23:59	231	40.26	13.54
Region 8	24:13	24:42	24:23	56	9.81	3.30
8 Peaks				573	100.00	33.63

Total Area: 1705 mAU
Average Background: N/A mAU

FYX-029.1+3.21.22.ene.prog.(3mM).5.uL.C21A2.C17WTmix.v21.mix.v27.3Method: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, February 22, 2012 9:48:50 PM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Saturday, February 25, 2012 11:01:25 AM

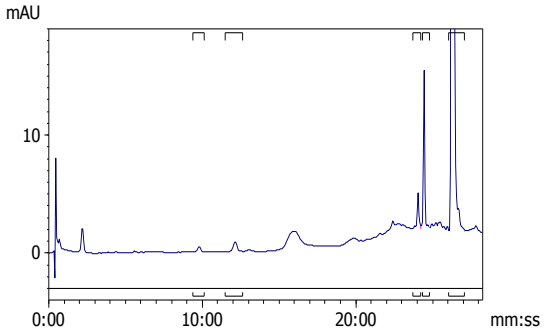
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
Off			
Off			

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 21
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:22	10:08	9:47	7	0.36	0.20
Region 2	11:30	12:37	12:06	15	0.75	0.42
Region 3	23:43	24:13	24:01	20	1.00	0.55
Region 4	24:19	24:48	24:25	72	3.67	2.04
Region 5	26:02	27:06	26:15	1849	94.22	52.27
5 Peaks				1963	100.00	55.47

Total Area: 3538 mAU
Average Background: N/A mAU

FYX-029.21.22.ene.prog.(3mM).5.uL.abiraterone(2mM).10uL.C17A1.10uL.v23.1AMethod: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, February 22, 2012 9:04:30 PM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Saturday, February 25, 2012 10:58:43 AM

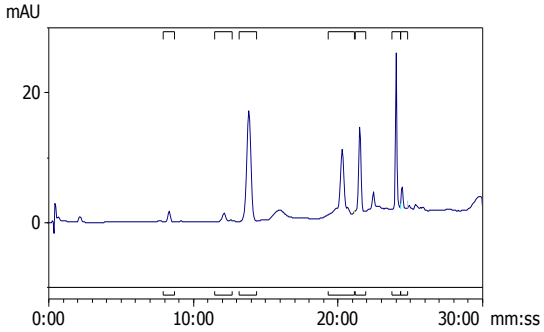
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
Off			
Off			

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 23
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	7:56	8:41	8:17	20	2.29	0.76
Region 2	11:28	12:39	12:05	19	2.19	0.72
Region 3	13:09	14:22	13:48	352	40.34	13.35
Region 4	19:19	21:07	20:15	182	20.89	6.91
Region 5	21:10	21:53	21:28	147	16.91	5.60
Region 6	23:43	24:19	23:59	143	16.44	5.44
Region 7	24:19	24:48	24:23	8	0.95	0.31
7 Peaks				872	100.00	33.09

Total Area: 2634 mAU
Average Background: N/A mAU

FYX-029.21.22.ene.prog.(3mM).5.uL.C17A105L.10uL.v24.2Method: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Wednesday, February 22, 2012 10:23:39 PM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Saturday, February 25, 2012 11:02:26 AM

Run Length: 30m
Dwell: 1s

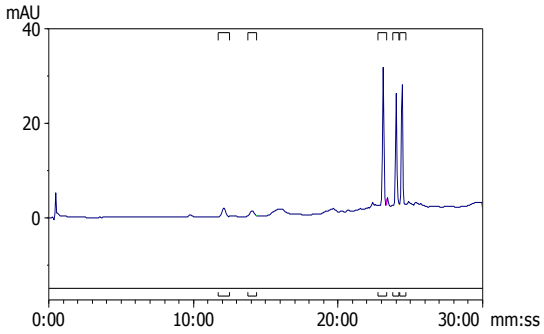
Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 24
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:42	12:28	12:04	31	5.60	1.24
Region 2	13:46	14:22	14:01	18	3.34	0.74
Region 3	22:46	23:22	23:05	182	33.09	7.32
Region 4	23:48	24:13	23:59	145	26.33	5.83
Region 5	24:16	24:41	24:23	174	31.64	7.00
5 Peaks				551	100.00	22.13

Total Area: 2490 mAU
Average Background: N/A mAU

FYX-029.21.22.ene.prog.(3mM).5.uL.C21V359A.10uL.v29.4Method: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, February 23, 2012 12:43:02 AM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Saturday, February 25, 2012 11:05:04 AM

Run Length: 30m
Dwell: 1s

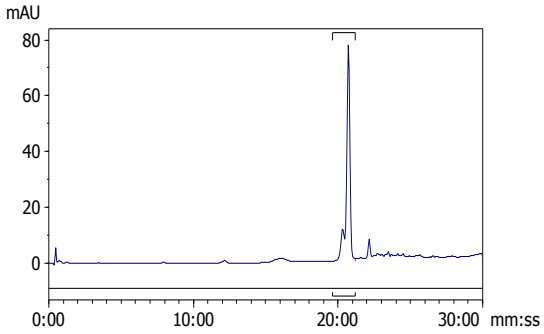
Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 29
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	19:38	21:11	20:41	1294	100.00	41.13
1 Peak				1294	100.00	41.13

Total Area: 3147 mAU
Average Background: N/A mAU

21.22.epoxyprog.epimeric mixture.std.v32Method: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, February 23, 2012 1:52:46 AM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Saturday, February 25, 2012 11:06:30 AM

Run Length: 30m
Dwell: 1s

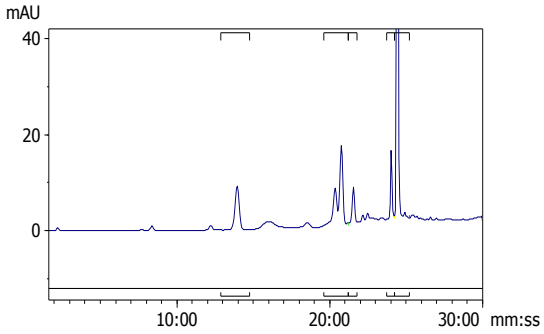
Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 32
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	12:52	14:44	13:55	172	7.76	4.20
Region 2	19:37	21:13	20:44	348	15.67	8.47
Region 3	21:14	21:46	21:31	75	3.38	1.83
Region 4	23:43	24:15	24:00	94	4.26	2.30
Region 5	24:15	25:11	24:23	1530	68.93	37.27
5 Peaks				2220	100.00	54.07

Total Area: 4106 mAU
Average Background: N/A mAU

FYX-029.21.22.ene.prog.vial24.(2)C17A105L.mixed.with.21homome.prog.21.22.epoxyprMethod: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, February 23, 2012 2:27:38 AM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Saturday, February 25, 2012 11:08:00 AM

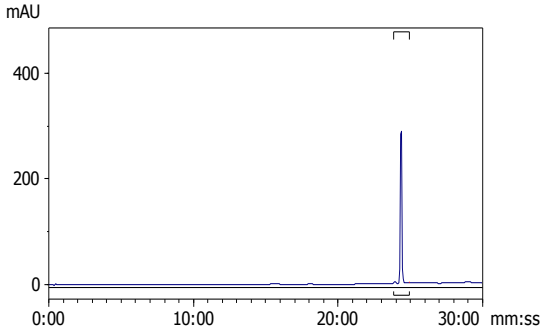
Run Length: 30m
Dwell: 1s

Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 24
Injection Volume: 5 µL

Comments:
Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	23:52	24:56	24:19	2139	100.00	46.59
1 Peak				2139	100.00	46.59

Total Area: 4591 mAU
Average Background: N/A mAU

21-homomethylprog 1 uL in 27 uL MeOHMethod: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Sunday, February 19, 2012 5:24:45 PM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Saturday, February 25, 2012 11:23:04 AM

Run Length: 30m
Dwell: 1s

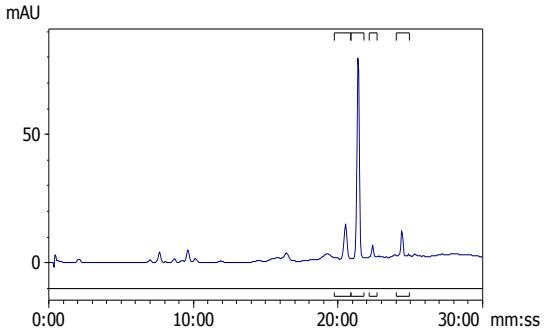
Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 51
Injection Volume: 1 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	19:43	20:52	20:29	192	15.77	5.17
Region 2	20:53	21:48	21:21	894	73.41	24.06
Region 3	22:09	22:41	22:21	36	2.92	0.96
Region 4	24:03	24:54	24:21	96	7.89	2.59
4 Peaks				1218	100.00	32.78

Total Area: 3717 mAU
Average Background: N/A mAU

FYX-028 C17WT 6uL. 21homomethylprog 5uM.vial56.7Method: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Sunday, February 19, 2012 5:59:37 PM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Saturday, February 25, 2012 11:15:41 AM

Run Length: 30m
Dwell: 1s

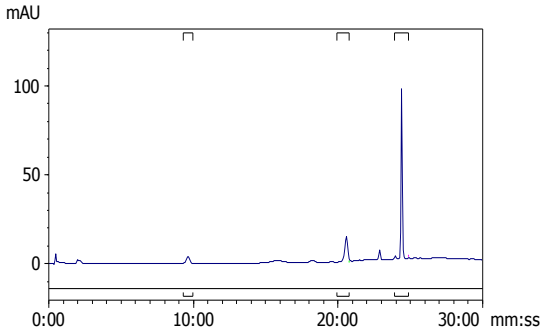
Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 56
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:17	9:58	9:36	57	7.61	1.91
Region 2	19:56	20:47	20:32	189	25.41	6.37
Region 3	23:56	24:52	24:22	500	66.99	16.81
3 Peaks				746	100.00	25.09

Total Area: 2972 mAU
Average Background: N/A mAU

FYX-028 C21WT 10uL. 21homomethylprog 5uM.vial58.9Method: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Sunday, February 19, 2012 6:34:28 PM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Saturday, February 25, 2012 11:16:39 AM

Run Length: 30m
Dwell: 1s

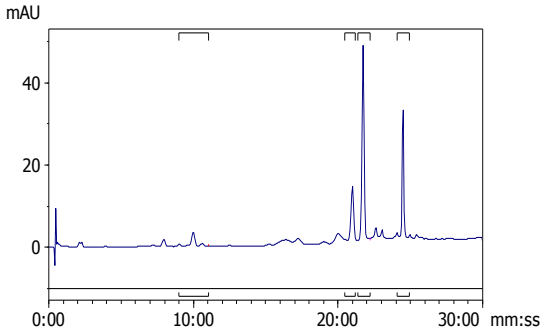
Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 58
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:00	11:01	9:57	44	4.85	1.47
Region 2	20:29	21:13	20:57	162	18.00	5.45
Region 3	21:21	22:12	21:42	483	53.71	16.25
Region 4	24:05	24:56	24:27	211	23.43	7.09
4 Peaks				900	100.00	30.26

Total Area: 2974 mAU
Average Background: N/A mAU

FYX-028 C17WTmixC21WT 21homomethylprog5uM.vial56.7+9Method: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Sunday, February 19, 2012 11:13:21 PM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Saturday, February 25, 2012 11:20:01 AM

Run Length: 30m
Dwell: 1s

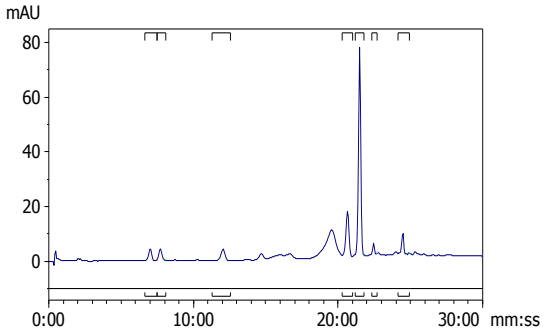
Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 56
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	6:40	7:29	6:59	47	3.56	1.17
Region 2	7:29	8:06	7:41	51	3.84	1.27
Region 3	11:18	12:33	12:01	75	5.64	1.86
Region 4	20:16	21:02	20:37	236	17.85	5.88
Region 5	21:10	21:48	21:28	835	63.08	20.79
Region 6	22:20	22:41	22:26	21	1.57	0.52
Region 7	24:07	24:54	24:27	59	4.46	1.47
7 Peaks				1324	100.00	32.96

Total Area: 4018 mAU
Average Background: N/A mAU

FYX-028 C17A105L 6uL. 21homomethylprog 5uM.vial57.8Method: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Sunday, February 19, 2012 9:28:45 PM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Saturday, February 25, 2012 11:18:57 AM

Run Length: 30m
Dwell: 1s

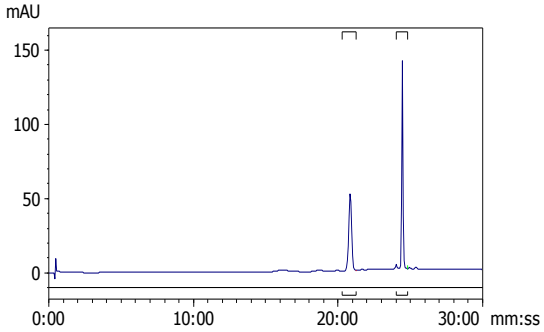
Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 57
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	20:16	21:16	20:49	720	49.95	20.96
Region 2	24:01	24:48	24:24	721	50.05	21.00
2 Peaks				1441	100.00	41.96

Total Area: 3435 mAU
Average Background: N/A mAU

FYX-028 C21V359A 10uL. 21homomethylprog 5uM.vial59.10Method: Halogens_Test1_NO_RADIOACTIVITY

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Sunday, February 19, 2012 10:03:36 PM
Method by: Super User on Friday, January 27, 2012 11:42:43 AM
Evaluation by: Super User on Saturday, February 25, 2012 11:21:04 AM

Run Length: 30m
Dwell: 1s

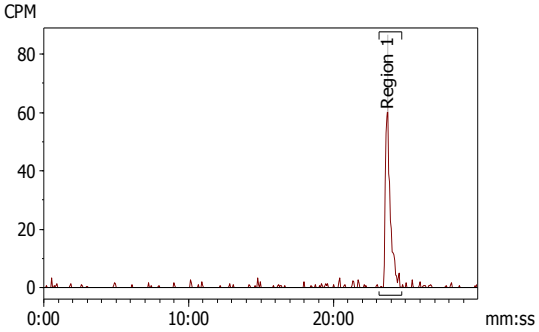
Channel Limits Efficiency Spill

Off
Off

Cell Volume: 500 µL
Cell Type: Solid
Eluate Flow: 0.40 mL/min
Scint Flow: N/A
Residence Time: 1m15s
Vial No: 59
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	23:10	24:45	23:45	1450	100.00	80.32
1 Peak				1450	100.00	80.32

Total Area: 1805 CPM
Average Background: 0 CPM

FYX-016 17T-preg.20 uM. COtreatment.30C.1h.V38.6B.IMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, February 14, 2012 2:17:49 AM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Saturday, February 25, 2012 11:32:46 AM

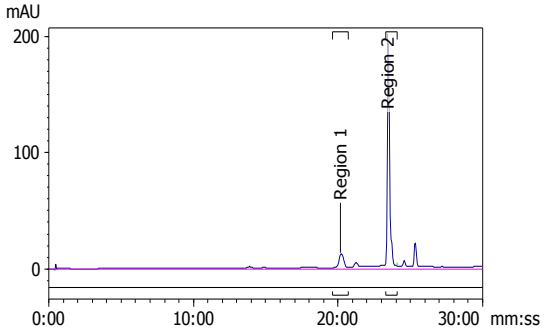
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 38
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	19:36	20:43	20:11	258	11.49	5.83
Region 2	23:17	24:06	23:26	1989	88.51	44.88
2 Peaks				2247	100.00	50.71

Total Area: 4431 mAU
Average Background: N/A mAU

FYX-016 17T-preg.20 uM. COtreatment.30C.1h.V38.6B.IMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, February 14, 2012 2:17:49 AM
Method by: Super User on Thursday, January 26, 2012 3:29:25 PM
Evaluation by: Super User on Saturday, February 25, 2012 11:32:46 AM

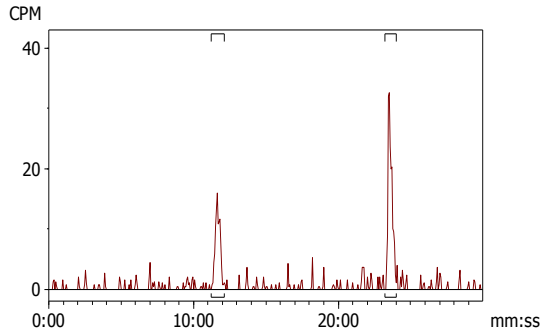
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 38
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:13	12:06	11:36	355	36.51	21.55
Region 2	23:14	24:01	23:25	618	63.49	37.48
2 Peaks				973	100.00	59.03

Total Area: 1648 CPM
Average Background: 0 CPM

FYX-028.C17WT 10uL.17Tpreg(3mM)10uL.CO.V21.1AMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, February 23, 2012 7:34:59 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Friday, February 24, 2012 10:40:46 AM

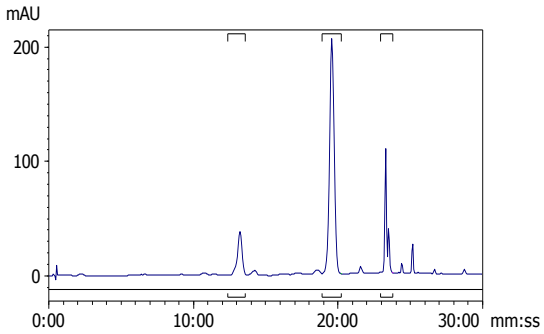
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 21
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	12:23	13:36	13:11	830	12.63	8.92
Region 2	18:55	20:14	19:32	4570	69.57	49.12
Region 3	22:57	23:47	23:15	1169	17.80	12.57
3 Peaks				6569	100.00	70.60

Total Area: 9304 mAU
Average Background: N/A mAU

FYX-028.C17WT 10uL.17Tpreg(3mM)10uL.CO.V21.1AMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, February 23, 2012 7:34:59 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Friday, February 24, 2012 10:40:46 AM

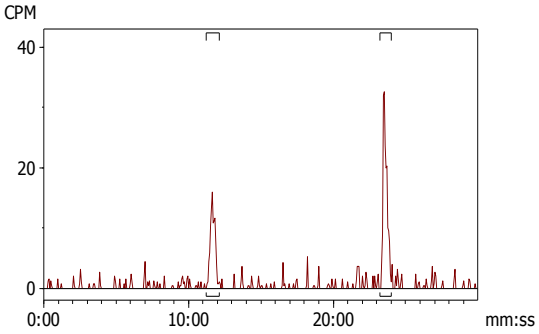
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 21
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:13	12:06	11:36	355	36.51	21.55
Region 2	23:14	24:01	23:25	618	63.49	37.48
2 Peaks				973	100.00	59.03

Total Area: 1648 CPM
Average Background: 0 CPM

FYX-028.C17WT 10uL.17Tpreg(3mM)10uL.CO.V21.1AMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, February 23, 2012 7:34:59 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Friday, February 24, 2012 10:40:46 AM

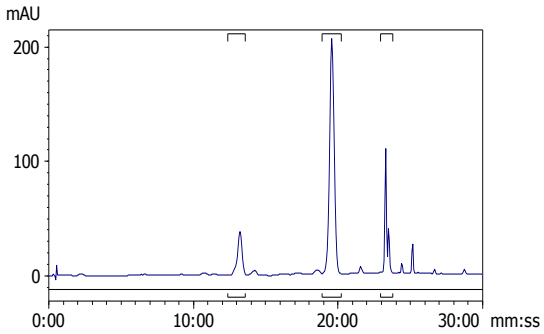
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 21
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	12:23	13:36	13:11	830	12.63	8.92
Region 2	18:55	20:14	19:32	4570	69.57	49.12
Region 3	22:57	23:47	23:15	1169	17.80	12.57
3 Peaks				6569	100.00	70.60

Total Area: 9304 mAU
Average Background: N/A mAU

FYX-028.C17WT 10uL.17Tpreg(3mM)10uL.CO.V21.1AMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, February 23, 2012 7:34:59 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Friday, February 24, 2012 10:40:46 AM

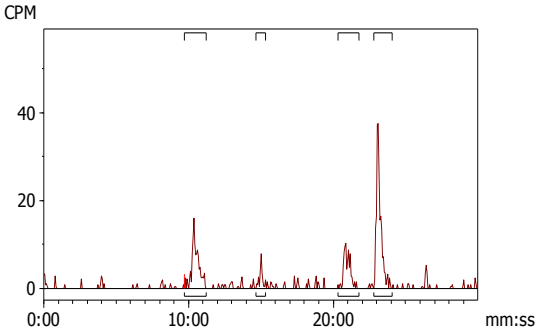
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 21
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	9:43	11:14	10:21	413	26.71	20.84
Region 2	14:40	15:21	14:58	93	6.00	4.68
Region 3	20:21	21:48	20:49	282	18.22	14.22
Region 4	22:50	24:03	23:05	758	49.07	38.29
4 Peaks				1546	100.00	78.03

Total Area: 1981 CPM
Average Background: 0 CPM

FYX-028.C17A105L 10uL.17Tpreg(3mM)10uL.CO.V25.2BMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, February 23, 2012 11:51:49 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Friday, February 24, 2012 10:47:05 AM

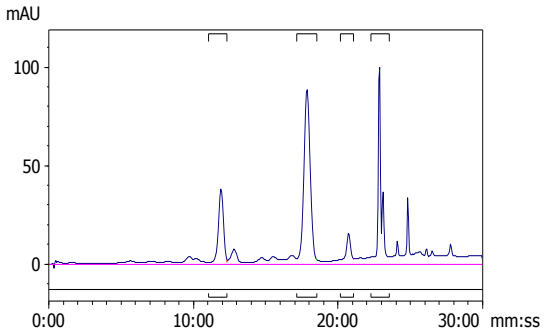
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 25
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:02	12:20	11:52	853	17.41	9.20
Region 2	17:07	18:32	17:49	2564	52.31	27.63
Region 3	20:11	21:05	20:42	229	4.68	2.47
Region 4	22:18	23:31	22:49	1255	25.60	13.52
4 Peaks				4902	100.00	52.82

Total Area: 9280 mAU
Average Background: N/A mAU

FYX-028.C17A105L 10uL.17Tpreg(3mM)10uL.CO.V25.2BMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, February 23, 2012 11:51:49 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Friday, February 24, 2012 10:47:05 AM

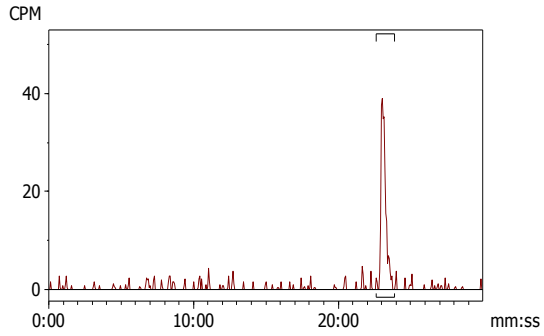
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 25
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	22:39	23:54	23:05	886	100.00	60.75
1 Peak				886	100.00	60.75

Total Area: 1459 CPM
Average Background: 0 CPM

FYX-028.C17purified 10uL.17Tpreg(3mM)10uL.CO.V28.3CMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, February 24, 2012 1:33:22 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Friday, February 24, 2012 10:50:46 AM

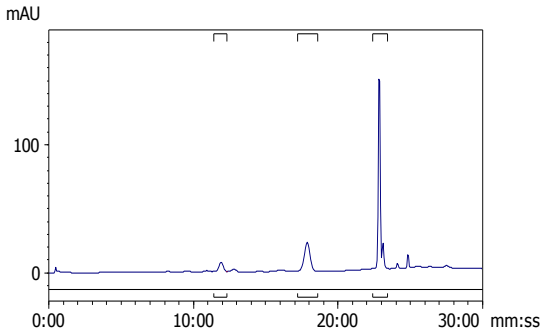
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 28
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:25	12:18	11:53	152	6.35	2.75
Region 2	17:11	18:36	17:50	629	26.28	11.38
Region 3	22:24	23:25	22:49	1613	67.37	29.18
3 Peaks				2394	100.00	43.31

Total Area: 5527 mAU
Average Background: N/A mAU

FYX-028.C17purified 10uL.17Tpreg(3mM)10uL.CO.V28.3CMethod: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, February 24, 2012 1:33:22 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Friday, February 24, 2012 10:50:46 AM

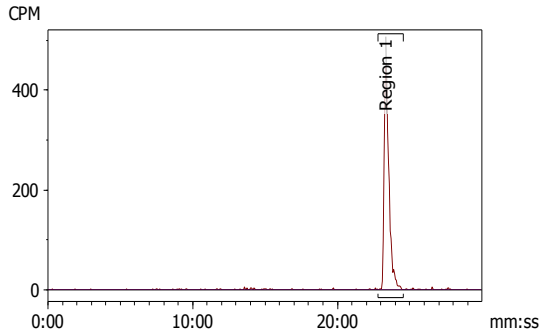
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 28
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	22:49	24:32	23:21	10579	100.00	94.40
1 Peak				10579	100.00	94.40

Total Area: 11206 CPM
Average Background: 0 CPM

FYX-038 Std.17Tpreg(80uM).Cholesterol oxidase.Control2.C2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Saturday, March 10, 2012 11:35:51 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 7:42:35 PM

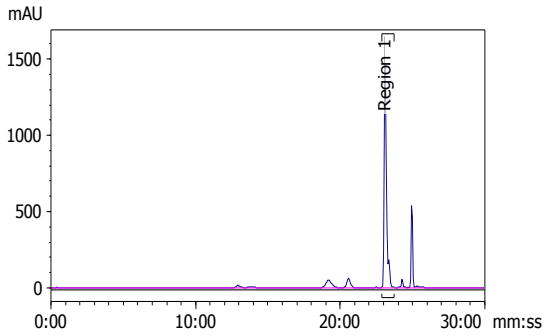
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 39
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	22:52	23:43	23:04	16127	100.00	61.56
1 Peak				16127	100.00	61.56

Total Area: 26199 mAU
Average Background: N/A mAU

FYX-038 Std.17Tpreg(80uM).Cholesterol oxidase.Control2.C2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Saturday, March 10, 2012 11:35:51 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 7:42:35 PM

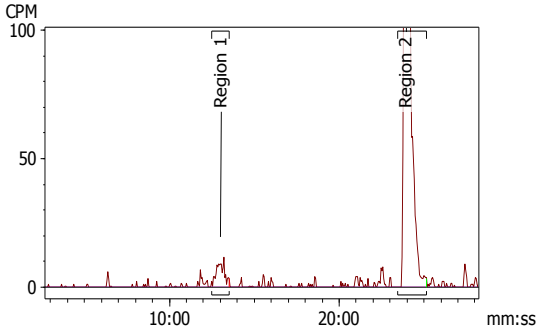
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 39
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	12:28	13:32	13:01	352	4.76	4.21
Region 2	23:28	25:09	23:55	7046	95.24	84.30
2 Peaks				7398	100.00	88.51

Total Area: 8358 CPM
Average Background: 0 CPM

FYX-038 C17WT(10uL).17Tpreg(80uM).RT(22C).TA1 Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 9:02:40 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:49:15 PM

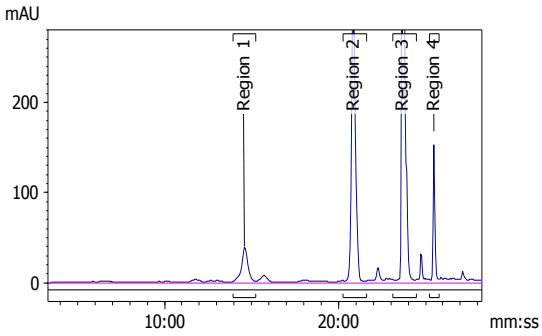
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 85
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	13:56	15:14	14:35	913	5.16	4.22
Region 2	20:16	21:36	20:49	5374	30.36	24.80
Region 3	23:06	24:28	23:39	10429	58.92	48.13
Region 4	25:14	25:46	25:27	984	5.56	4.54
4 Peaks				17701	100.00	81.68

Total Area: 21671 mAU
Average Background: N/A mAU

FYX-038 C17WT(10uL).17Tpreg(80uM).RT(22C).TA1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 9:02:40 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:49:15 PM

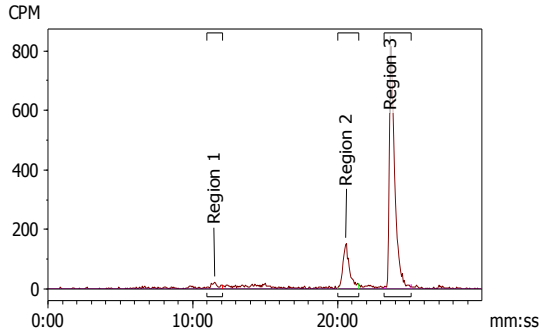
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 85
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:59	12:04	11:30	781	2.93	2.35
Region 2	20:00	21:29	20:35	4899	18.41	14.74
Region 3	23:15	25:05	23:40	20925	78.65	62.94
3 Peaks				26605	100.00	80.03

Total Area: 33245 CPM
Average Background: 0 CPM

FYX-038 C17WT(10uL).17Tpreg(80uM).[^{3}H]preg(6uL).RT(22C).THA1 Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 9:37:30 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:51:43 PM

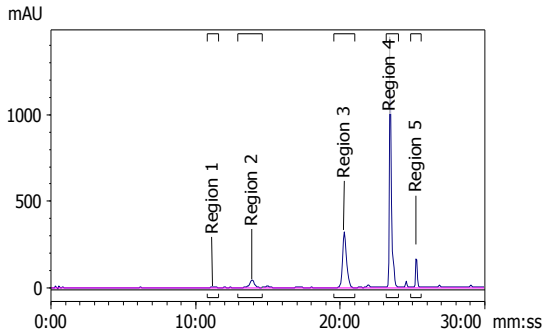
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 86
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:49	11:34	11:10	74	0.35	0.29
Region 2	12:56	14:36	13:52	996	4.73	3.93
Region 3	19:35	21:02	20:14	6098	29.00	24.10
Region 4	23:09	24:02	23:25	12488	59.39	49.34
Region 5	24:52	25:35	25:13	1371	6.52	5.42
5 Peaks				21026	100.00	83.08

Total Area: 25308 mAU
Average Background: N/A mAU

FYX-038 C17WT(10uL).17Tpreg(80uM).[^{3}H]preg(6uL).RT(22C).THA1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 9:37:30 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:51:43 PM

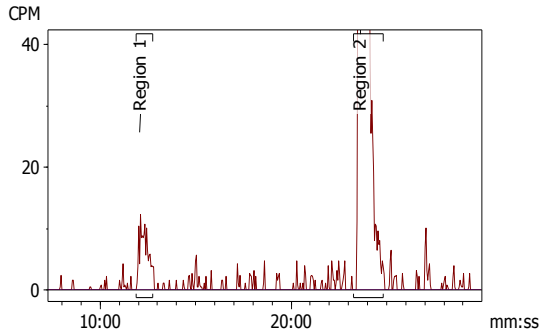
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 86
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:53	12:45	12:05	346	4.80	4.27
Region 2	23:16	24:48	23:37	6861	95.20	84.84
2 Peaks				7206	100.00	89.12

Total Area: 8086 CPM
Average Background: 0 CPM

FYX-038 C17WT(10uL).17Tpreg(80uM).17Dpreg(7uM).RT(22C).TDA1 Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 10:12:22 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:54:06 PM

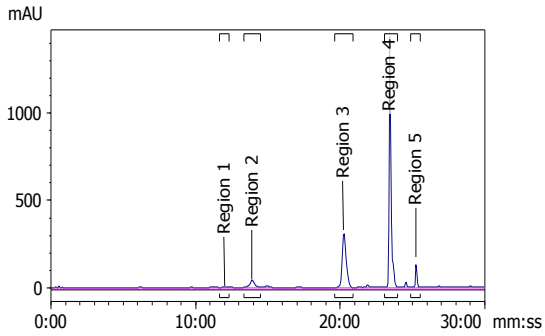
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 87
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:40	12:19	12:00	33	0.16	0.13
Region 2	13:21	14:29	13:53	872	4.27	3.47
Region 3	19:36	20:54	20:13	6078	29.74	24.17
Region 4	23:03	23:59	23:24	12516	61.25	49.77
Region 5	24:52	25:31	25:13	936	4.58	3.72
5 Peaks				20436	100.00	81.25

Total Area: 25150 mAU
Average Background: N/A mAU

FYX-038 C17WT(10uL).17Tpreg(80uM).17Dpreg(7uM).RT(22C).TDA1 Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 10:12:22 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:54:06 PM

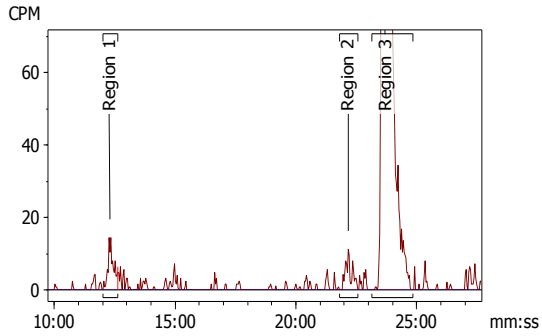
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 87
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	12:01	12:38	12:17	224	3.10	2.75
Region 2	21:48	22:35	22:10	186	2.57	2.28
Region 3	23:08	24:50	23:42	6819	94.33	83.80
3 Peaks				7229	100.00	88.83

Total Area: 8138 CPM
Average Background: 0 CPM

FYX-038 C17WT(10uL).17Tpreg(80uM).RT(22C).TA2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 10:47:14 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:55:51 PM

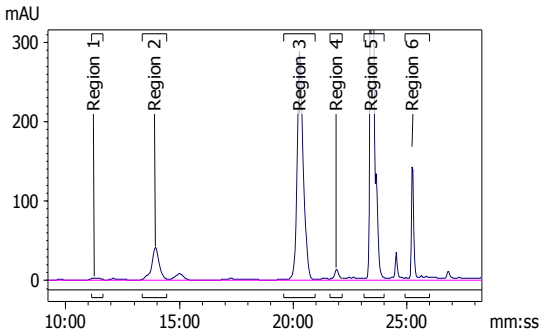
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 88
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:08	11:39	11:14	25	0.14	0.12
Region 2	13:22	14:26	13:56	872	4.99	4.12
Region 3	19:36	20:58	20:17	5240	29.98	24.76
Region 4	21:36	22:10	21:54	134	0.77	0.63
Region 5	23:07	24:00	23:26	10290	58.87	48.62
Region 6	24:56	25:59	25:14	919	5.26	4.34
6 Peaks				17480	100.00	82.59

Total Area: 21164 mAU
Average Background: N/A mAU

FYX-038 C17WT(10uL).17Tpreg(80uM).RT(22C).TA2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 10:47:14 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:55:51 PM

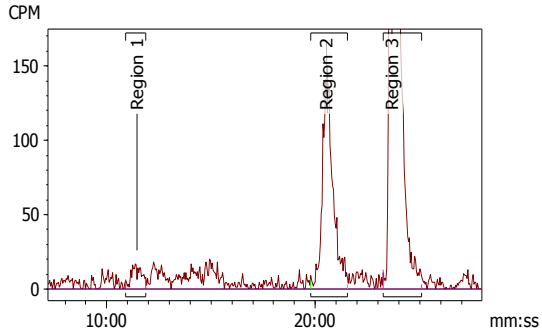
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 88
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:55	11:52	11:28	573	2.27	1.84
Region 2	19:48	21:33	20:33	5072	20.10	16.31
Region 3	23:15	25:05	23:41	19590	77.63	62.98
3 Peaks				25235	100.00	81.13

Total Area: 31104 CPM
Average Background: 0 CPM

FYX-038 C17WT(10uL).17Tpreg(80uM).[^{3}H]preg(6uL).RT(22C).THA2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 11:22:04 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:57:35 PM

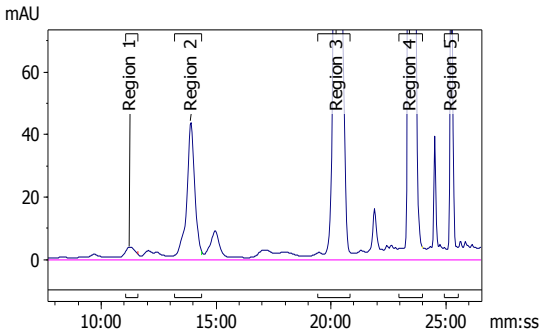
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 89
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:03	11:36	11:14	35	0.19	0.15
Region 2	13:12	14:22	13:53	945	5.09	4.12
Region 3	19:26	20:49	20:15	5716	30.76	24.91
Region 4	22:58	23:58	23:25	10952	58.94	47.74
Region 5	24:56	25:33	25:13	935	5.03	4.07
5 Peaks				18584	100.00	81.00

Total Area: 22943 mAU
Average Background: N/A mAU

FYX-038 C17WT(10uL).17Tpreg(80uM).[^{3}H]preg(6uL).RT(22C).THA2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 11:22:04 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:57:35 PM

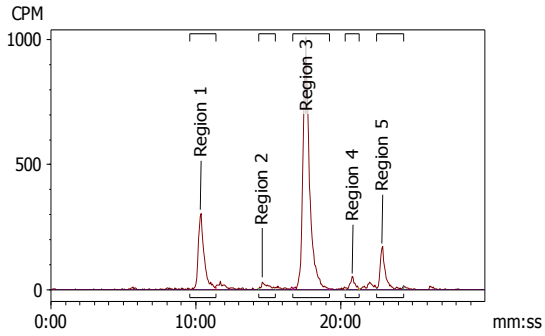
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 89
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	9:36	11:23	10:20	8566	18.56	16.79
Region 2	14:21	15:30	14:35	1062	2.30	2.08
Region 3	16:42	19:13	17:37	30986	67.14	60.74
Region 4	20:20	21:17	20:49	1149	2.49	2.25
Region 5	22:31	24:23	22:53	4390	9.51	8.61
5 Peaks				46154	100.00	90.47

Total Area: 51014 CPM
Average Background: 0 CPM

FYX-037 17Dprog(20uM).C17WT.5uL.RT(22 C).1A1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 3:00:07 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 9:15:15 PM

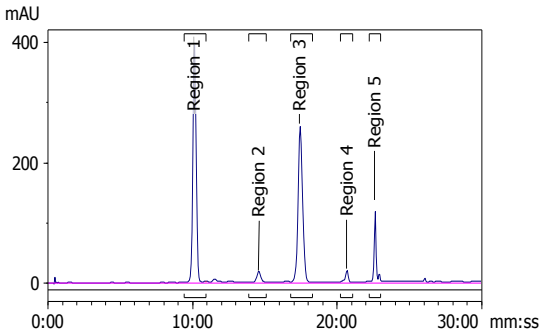
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 41
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:24	10:55	10:05	6217	46.28	36.60
Region 2	13:53	15:04	14:33	325	2.42	1.92
Region 3	16:47	18:18	17:24	5568	41.45	32.78
Region 4	20:13	21:03	20:39	252	1.87	1.48
Region 5	22:12	23:01	22:35	1071	7.97	6.30
5 Peaks				13433	100.00	79.08

Total Area: 16987 mAU
Average Background: N/A mAU

FYX-037 17Dprog(20uM).C17WT.5uL.RT(22 C).1A1 Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 3:00:07 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 9:15:15 PM

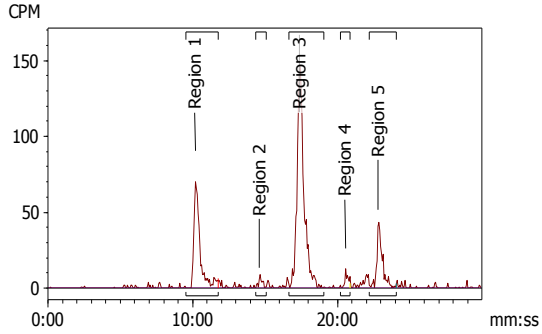
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 41
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	9:33	11:45	10:10	2048	24.55	22.16
Region 2	14:21	15:06	14:37	144	1.73	1.56
Region 3	16:39	19:03	17:23	4813	57.69	52.08
Region 4	20:13	20:52	20:33	189	2.26	2.04
Region 5	22:11	24:06	22:49	1149	13.77	12.43
5 Peaks				8342	100.00	90.27

Total Area: 9242 CPM
Average Background: 0 CPM

FYX-037 17Dprog(20uM).C17WT.5uL.RT(22 C).1A2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 3:34:59 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 9:16:34 PM

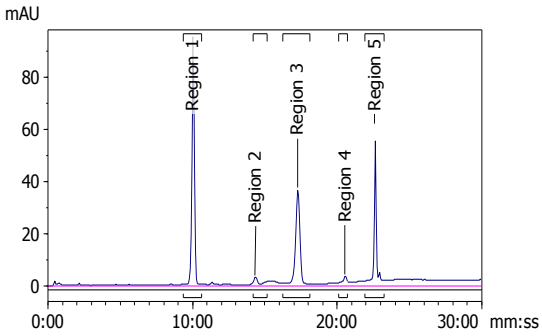
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 42
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:22	10:38	10:01	1096	47.90	26.61
Region 2	14:10	15:09	14:19	-5	-0.21	-0.12
Region 3	16:16	18:08	17:15	710	31.04	17.24
Region 4	20:06	20:41	20:32	26	1.16	0.64
Region 5	21:53	23:16	22:36	460	20.11	11.17
5 Peaks				2287	100.00	55.55

Total Area: 4118 mAU
Average Background: N/A mAU

FYX-037 17Dprog(20uM).C17WT.5uL.RT(22 C).1A2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 3:34:59 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 9:16:34 PM

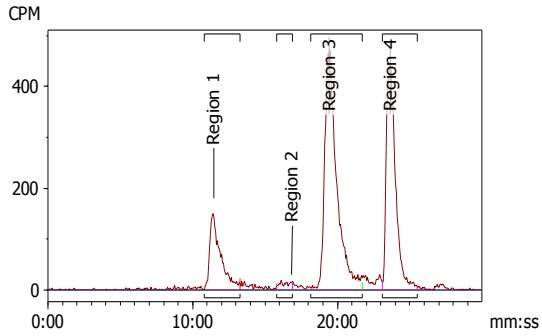
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 42
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:48	13:16	11:26	7907	14.17	13.04
Region 2	15:50	16:55	16:50	826	1.48	1.36
Region 3	18:09	21:42	19:26	27318	48.95	45.05
Region 4	23:08	25:32	23:40	19760	35.41	32.58
4 Peaks				55811	100.00	92.03

Total Area: 60646 CPM
Average Background: 0 CPM

FYX-037 17D.prog(20uM).C17WT.10uL.(26 C).1B1 Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 2:41:21 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:29:29 PM

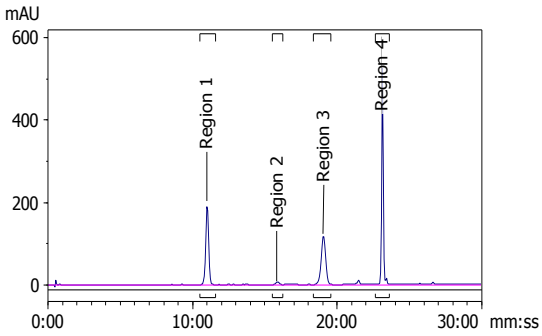
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 68
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:30	11:36	10:58	2704	28.88	23.20
Region 2	15:29	16:14	15:50	116	1.23	0.99
Region 3	18:21	19:34	19:01	2555	27.29	21.92
Region 4	22:37	23:37	23:05	3988	42.60	34.22
4 Peaks				9362	100.00	80.34

Total Area: 11654 mAU
Average Background: N/A mAU

FYX-037 17D.prog(20uM).C17WT.10uL.(26 C).1B1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 2:41:21 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:29:29 PM

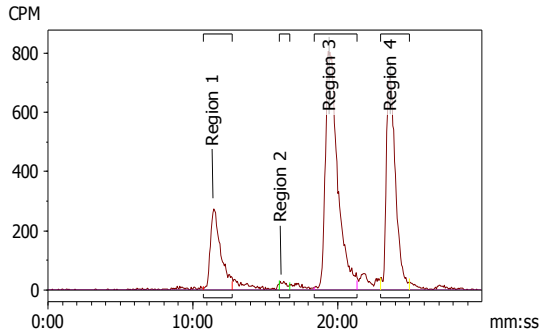
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 68
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:46	12:44	11:24	13766	14.53	13.05
Region 2	15:59	16:44	16:06	966	1.02	0.92
Region 3	18:24	21:23	19:26	47936	50.61	45.43
Region 4	22:59	25:00	23:39	32045	33.83	30.37
4 Peaks				94714	100.00	89.76

Total Area: 105514 CPM
Average Background: 0 CPM

FYX-037 17D.prog(20uM).C17WT.10uL.(26 C).1B2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 3:16:13 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:32:44 PM

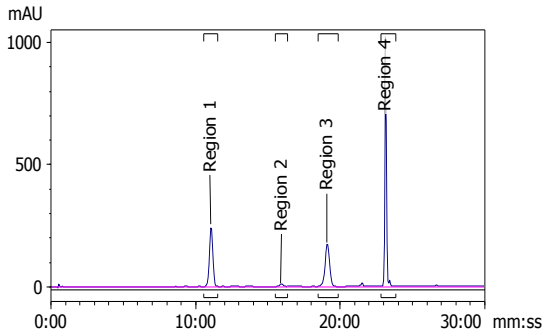
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 69
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:35	11:31	11:02	4021	27.24	23.13
Region 2	15:31	16:20	15:54	175	1.18	1.00
Region 3	18:27	19:52	19:04	3762	25.49	21.64
Region 4	22:48	23:52	23:06	6801	46.08	39.12
4 Peaks				14758	100.00	84.89

Total Area: 17385 mAU
Average Background: N/A mAU

FYX-037 17D.prog(20uM).C17WT.10uL.(26 C).1B2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 3:16:13 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:32:44 PM

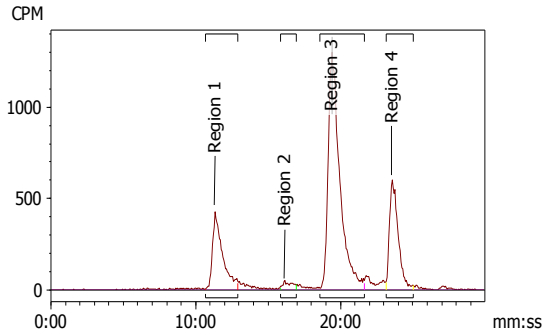
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 69
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:42	12:55	11:18	20758	17.14	15.54
Region 2	15:51	16:57	16:08	2182	1.80	1.63
Region 3	18:34	21:40	19:24	72880	60.18	54.57
Region 4	23:10	25:02	23:32	25283	20.88	18.93
4 Peaks				121104	100.00	90.67

Total Area: 133558 CPM
Average Background: 0 CPM

FYX-037 17D.prog(20uM).C17WT.10uL.(26 C).1B3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 3:51:06 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:38:34 PM

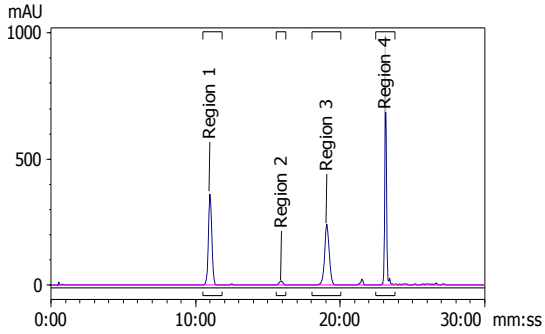
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 70
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:29	11:49	10:57	5791	32.34	27.79
Region 2	15:35	16:16	15:53	244	1.36	1.17
Region 3	18:02	20:01	19:02	5221	29.15	25.06
Region 4	22:29	23:48	23:06	6653	37.15	31.93
4 Peaks				17909	100.00	85.95

Total Area: 20835 mAU
Average Background: N/A mAU

FYX-037 17D.prog(20uM).C17WT.10uL.(26 C).1B3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 3:51:06 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:38:34 PM

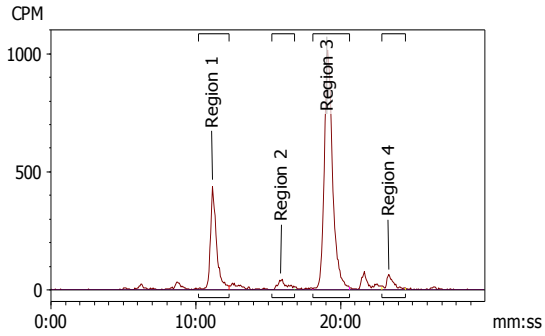
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 70
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:13	12:19	11:08	12621	22.05	19.36
Region 2	15:16	16:50	15:52	1862	3.25	2.86
Region 3	18:06	20:38	19:05	40461	70.69	62.06
Region 4	22:51	24:28	23:20	2291	4.00	3.51
4 Peaks				57235	100.00	87.79

Total Area: 65194 CPM
Average Background: 0 CPM

FYX-037 C17WT 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.30C.1C1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Sunday, March 11, 2012 12:22:04 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:20:48 PM

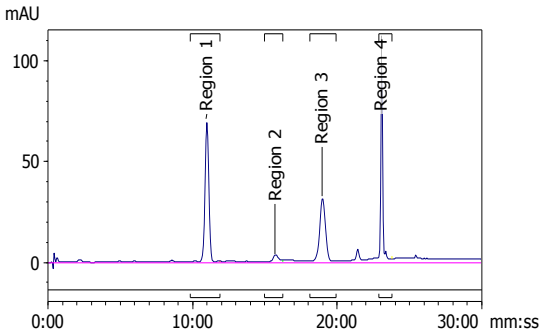
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 41
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:51	11:52	10:57	1248	41.97	27.25
Region 2	15:00	16:13	15:42	64	2.15	1.39
Region 3	18:08	19:54	18:57	853	28.70	18.64
Region 4	22:52	23:46	23:03	808	27.19	17.65
4 Peaks				2973	100.00	64.94

Total Area: 4578 mAU
Average Background: N/A mAU

FYX-037 C17WT 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.30C.1C1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Sunday, March 11, 2012 12:22:04 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:20:48 PM

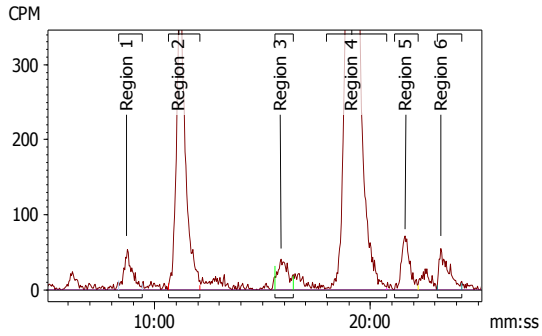
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 41
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	8:19	9:26	8:42	1379	2.50	2.28
Region 2	10:37	12:06	11:07	11389	20.65	18.84
Region 3	15:34	16:25	15:51	1357	2.46	2.24
Region 4	17:57	20:46	19:07	37475	67.94	61.99
Region 5	21:06	22:13	21:37	1926	3.49	3.19
Region 6	23:06	24:13	23:16	1635	2.96	2.70
6 Peaks				55162	100.00	91.25

Total Area: 60451 CPM
Average Background: 0 CPM

FYX-037 C17WT 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.30C.1C2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Sunday, March 11, 2012 12:56:56 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:23:07 PM

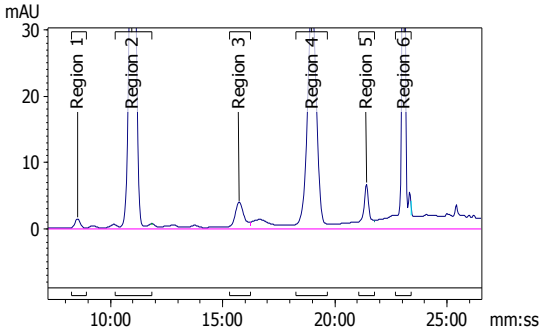
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 42
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	8:15	8:55	8:32	21	0.65	0.44
Region 2	10:13	11:50	10:58	1351	42.16	28.67
Region 3	15:19	16:15	15:43	73	2.27	1.55
Region 4	18:16	19:40	18:58	911	28.42	19.32
Region 5	21:03	21:46	21:24	68	2.11	1.44
Region 6	22:41	23:23	23:03	782	24.39	16.58
6 Peaks				3206	100.00	67.99

Total Area: 4715 mAU
Average Background: N/A mAU

FYX-037 C17WT 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.30C.1C2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Sunday, March 11, 2012 12:56:56 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:23:07 PM

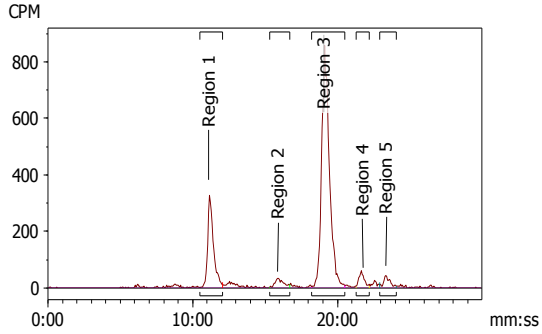
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 42
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:29	12:04	11:07	9120	20.11	18.19
Region 2	15:18	16:41	15:52	1478	3.26	2.95
Region 3	18:12	20:32	19:05	31728	69.97	63.27
Region 4	21:16	22:11	21:45	1574	3.47	3.14
Region 5	22:55	24:03	23:21	1443	3.18	2.88
5 Peaks				45344	100.00	90.42

Total Area: 50147 CPM
Average Background: 0 CPM

FYX-037 C17WT 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.30C.1C3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Sunday, March 11, 2012 1:31:47 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:24:29 PM

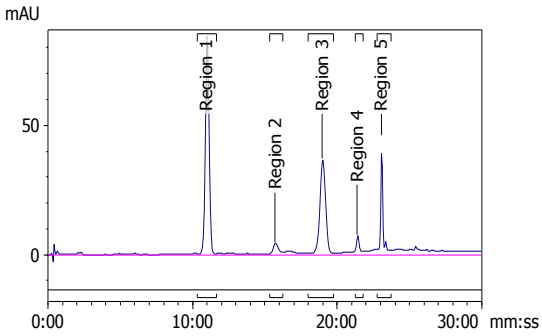
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 43
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:18	11:38	10:58	1507	51.05	34.24
Region 2	15:20	16:14	15:43	81	2.76	1.85
Region 3	18:00	19:44	18:58	972	32.92	22.08
Region 4	21:15	21:49	21:23	47	1.59	1.07
Region 5	22:45	23:42	23:03	345	11.68	7.83
5 Peaks				2952	100.00	67.07

Total Area: 4401 mAU
Average Background: N/A mAU

FYX-037 C17WT 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.30C.1C3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Sunday, March 11, 2012 1:31:47 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:24:29 PM

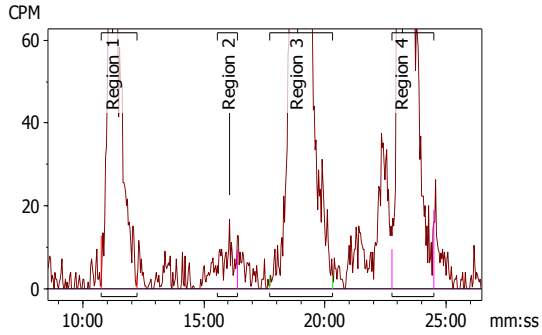
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 43
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:46	12:13	11:15	4074	9.90	9.03
Region 2	15:34	16:23	16:03	358	0.87	0.79
Region 3	17:43	20:19	18:51	14054	34.17	31.17
Region 4	22:46	24:30	23:10	22650	55.06	50.23
4 Peaks				41136	100.00	91.22

Total Area: 45094 CPM
Average Background: 0 CPM

FYX-037 C17WT 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.55C.1D1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 12, 2012 1:39:52 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:33:31 PM

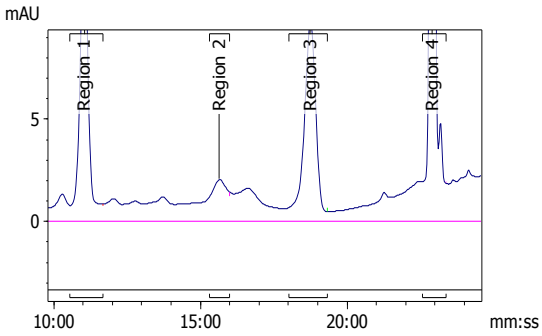
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 47
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:33	11:40	11:02	226	11.69	5.91
Region 2	15:19	16:00	15:39	16	0.82	0.41
Region 3	18:01	19:20	18:45	232	12.02	6.08
Region 4	22:35	23:22	22:55	1458	75.47	38.14
4 Peaks				1932	100.00	50.54

Total Area: 3824 mAU
Average Background: N/A mAU

FYX-037 C17WT 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.55C.1D1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 12, 2012 1:39:52 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:33:31 PM

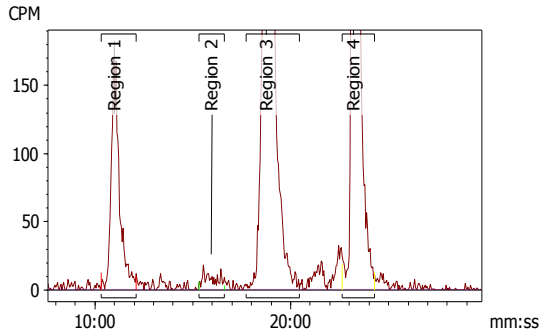
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 47
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:20	12:07	11:00	4976	11.28	10.43
Region 2	15:18	16:37	15:57	637	1.44	1.34
Region 3	17:43	20:26	18:47	18378	41.64	38.53
Region 4	22:39	24:18	23:14	20141	45.64	42.23
4 Peaks				44131	100.00	92.53

Total Area: 47693 CPM
Average Background: 0 CPM

FYX-037 C17WT 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.55C.1D2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 12, 2012 2:14:45 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:35:33 PM

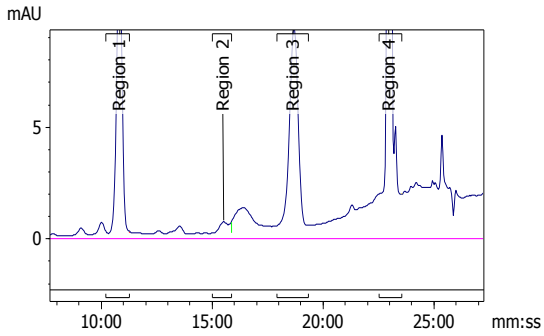
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 48
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:13	11:17	10:48	218	11.07	6.06
Region 2	15:00	15:52	15:30	3	0.14	0.08
Region 3	17:55	19:21	18:40	233	11.82	6.48
Region 4	22:32	23:33	22:58	1517	76.97	42.16
4 Peaks				1971	100.00	54.78

Total Area: 3599 mAU
Average Background: N/A mAU

FYX-037 C17WT 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.55C.1D2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 12, 2012 2:14:45 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:35:33 PM

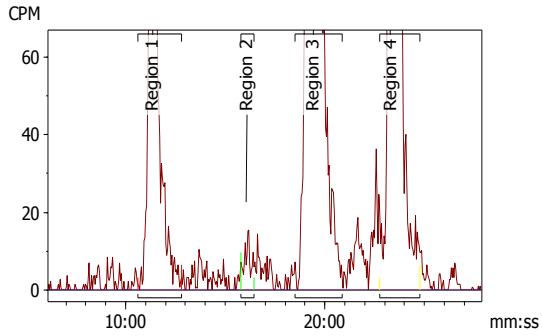
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 48
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:36	12:48	11:19	4829	12.57	11.59
Region 2	15:46	16:26	16:04	317	0.82	0.76
Region 3	18:31	20:52	19:24	15792	41.10	37.90
Region 4	22:46	24:46	23:18	17488	45.51	41.97
4 Peaks				38426	100.00	92.22

Total Area: 41667 CPM
Average Background: 0 CPM

FYX-037 C17WT 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.55C.1D3 Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 12, 2012 2:49:37 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:37:38 PM

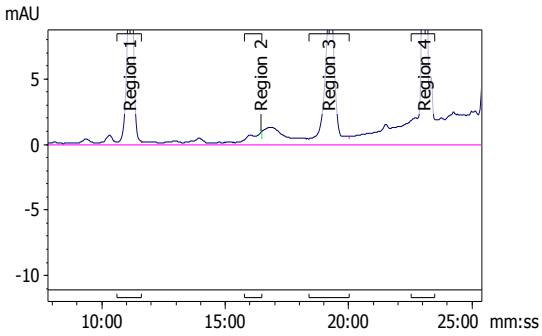
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 49
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:37	11:37	11:09	245	12.30	6.93
Region 2	15:47	16:29	16:28	0	0.01	0.01
Region 3	18:23	20:01	19:14	250	12.56	7.08
Region 4	22:31	23:30	23:04	1494	75.13	42.34
4 Peaks				1989	100.00	56.35

Total Area: 3529 mAU
Average Background: N/A mAU

FYX-037 C17WT 5 uL. 17Dprog (3 mM.9.3uL)20uM.[^{3}H]-prog.55C.1D3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 12, 2012 2:49:37 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 6:37:38 PM

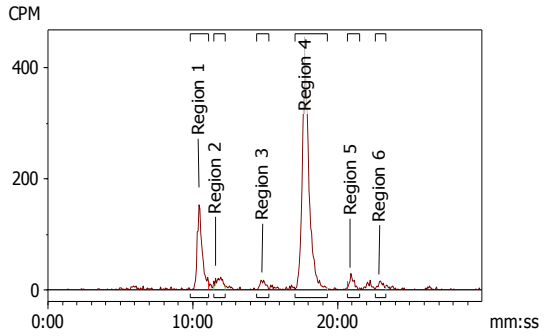
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 49
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	9:49	11:07	10:25	3702	19.05	17.15
Region 2	11:26	12:16	11:34	762	3.92	3.53
Region 3	14:24	15:17	14:47	470	2.42	2.18
Region 4	17:03	19:18	17:46	13546	69.68	62.74
Region 5	20:41	21:32	20:54	573	2.95	2.65
Region 6	22:37	23:21	22:52	387	1.99	1.79
6 Peaks				19440	100.00	90.04

Total Area: 21590 CPM
Average Background: 0 CPM

FYX-037 21.21.21.D3prog(20uM).C17WT.5uL.RT(22 C).2A1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 4:44:42 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:06:07 PM

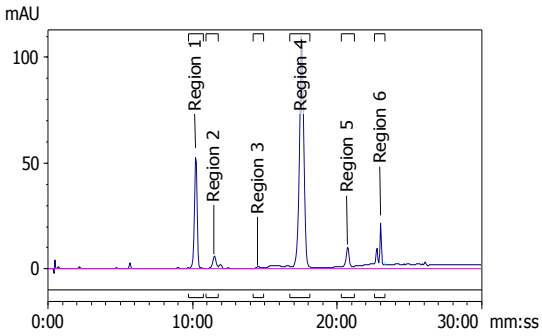
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 44
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:42	10:45	10:11	679	20.64	13.65
Region 2	10:56	11:47	11:28	69	2.10	1.39
Region 3	14:11	14:55	14:31	11	0.33	0.22
Region 4	16:44	18:08	17:30	2232	67.88	44.89
Region 5	20:16	21:10	20:42	124	3.78	2.50
Region 6	22:36	23:19	22:59	173	5.27	3.49
6 Peaks				3288	100.00	66.13

Total Area: 4972 mAU
Average Background: N/A mAU

FYX-037 21.21.21.D3prog(20uM).C17WT.5uL.RT(22 C).2A1 Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 4:44:42 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:06:07 PM

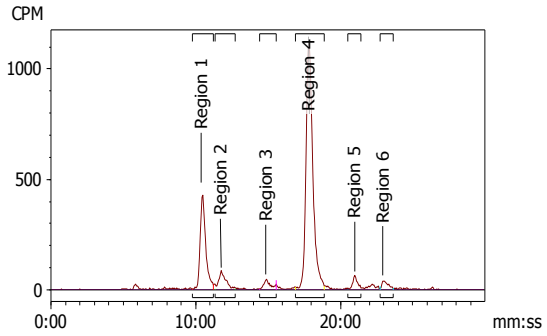
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 44
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	9:45	11:14	10:24	11693	20.84	19.07
Region 2	11:22	12:43	11:45	2973	5.30	4.85
Region 3	14:26	15:33	14:51	1565	2.79	2.55
Region 4	16:52	18:54	17:49	37130	66.17	60.55
Region 5	20:32	21:24	20:59	1488	2.65	2.43
Region 6	22:43	23:39	22:56	1264	2.25	2.06
6 Peaks				56112	100.00	91.50

Total Area: 61322 CPM
Average Background: 0 CPM

FYX-037 21.21.21.D3prog(20uM).C17WT.5uL.RT(22 C).2A2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 5:19:33 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:08:27 PM

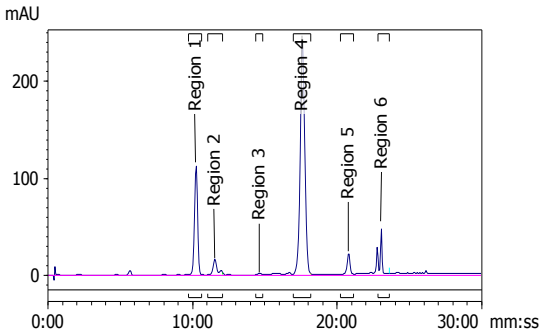
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 45
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:42	10:36	10:12	1666	21.80	16.44
Region 2	11:04	12:03	11:30	246	3.21	2.42
Region 3	14:22	14:51	14:36	21	0.27	0.21
Region 4	16:57	18:09	17:33	5239	68.55	51.68
Region 5	20:15	21:06	20:45	288	3.77	2.84
Region 6	22:49	23:37	23:01	184	2.40	1.81
6 Peaks				7643	100.00	75.40

Total Area: 10137 mAU
Average Background: N/A mAU

FYX-037 21.21.21.D3prog(20uM).C17WT.5uL.RT(22 C).2A2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 5:19:33 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:08:27 PM

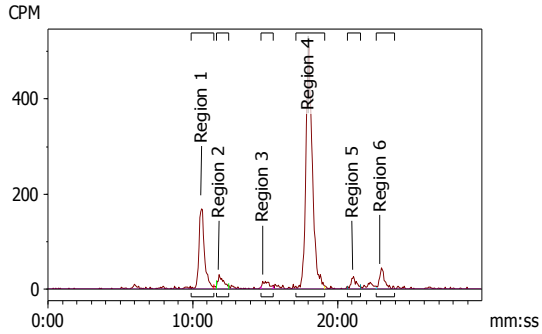
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 45
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	9:55	11:26	10:34	4506	19.27	17.56
Region 2	11:39	12:29	11:47	806	3.45	3.14
Region 3	14:43	15:33	14:49	534	2.29	2.08
Region 4	17:09	19:08	17:57	15786	67.51	61.52
Region 5	20:42	21:36	21:02	646	2.76	2.52
Region 6	22:40	23:56	22:57	1104	4.72	4.30
6 Peaks				23382	100.00	91.13

Total Area: 25658 CPM
Average Background: 0 CPM

FYX-037 21.21.21.D3prog(20uM).C17WT.5uL.RT(22 C).2A3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 5:54:25 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:15:50 PM

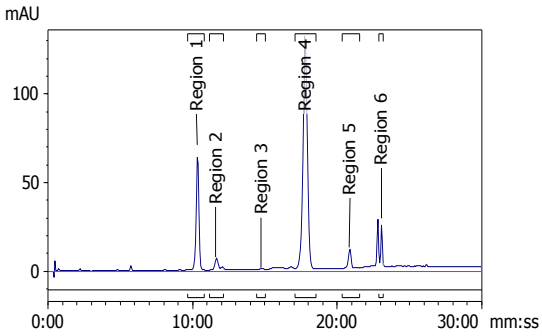
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 46
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:41	10:47	10:19	851	21.55	12.74
Region 2	11:11	12:07	11:37	83	2.11	1.25
Region 3	14:24	15:03	14:45	13	0.33	0.19
Region 4	17:05	18:32	17:45	2728	69.06	40.84
Region 5	20:20	21:33	20:50	133	3.37	1.99
Region 6	22:53	23:12	23:02	142	3.59	2.12
6 Peaks				3950	100.00	59.13

Total Area: 6679 mAU
Average Background: N/A mAU

FYX-037 21.21.21.D3prog(20uM).C17WT.5uL.RT(22 C).2A3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 5:54:25 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:15:50 PM

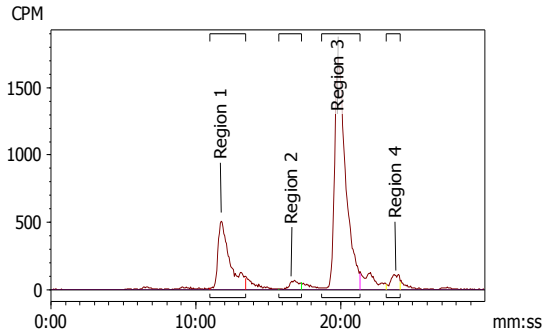
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 46
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:00	13:29	11:45	29469	22.63	19.46
Region 2	15:44	17:20	16:37	3370	2.59	2.23
Region 3	18:41	21:23	19:50	92646	71.14	61.18
Region 4	23:10	24:08	23:49	4739	3.64	3.13
4 Peaks				130224	100.00	86.00

Total Area: 151430 CPM
Average Background: 0 CPM

FYX-037 21.21.21.D3.prog(20uM).C17WT.10uL.(26 C).2B1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 4:26:00 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:40:59 PM

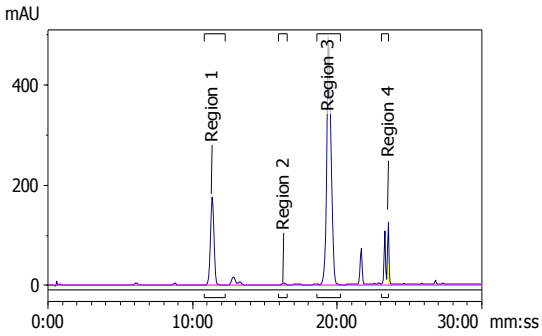
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 71
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:47	12:15	11:19	2989	20.91	15.94
Region 2	15:55	16:31	16:16	53	0.37	0.28
Region 3	18:35	20:12	19:22	10309	72.13	54.96
Region 4	23:02	23:34	23:30	942	6.59	5.02
4 Peaks				14293	100.00	76.20

Total Area: 18757 mAU
Average Background: N/A mAU

FYX-037 21.21.21.D3.prog(20uM).C17WT.10uL.(26 C).2B1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 4:26:00 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:40:59 PM

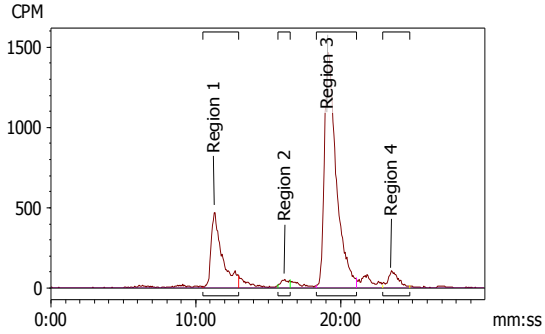
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 71
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:31	12:57	11:16	25040	21.61	19.14
Region 2	15:40	16:31	16:07	2067	1.78	1.58
Region 3	18:22	21:07	19:07	83379	71.95	63.72
Region 4	22:55	24:47	23:29	5402	4.66	4.13
4 Peaks				115888	100.00	88.56

Total Area: 130851 CPM
Average Background: 0 CPM

FYX-037 21.21.21.D3.prog(20uM).C17WT.10uL.(26 C).2B2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 5:00:53 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:43:13 PM

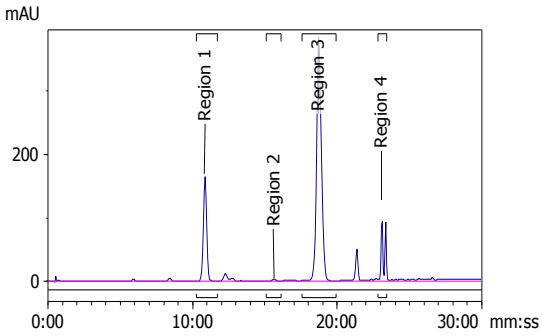
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 72
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:14	11:43	10:49	2616	20.49	16.24
Region 2	15:07	16:07	15:37	54	0.43	0.34
Region 3	17:33	19:54	18:41	8789	68.84	54.58
Region 4	22:48	23:27	23:03	1308	10.25	8.12
4 Peaks				12767	100.00	79.29

Total Area: 16102 mAU
Average Background: N/A mAU

FYX-037 21.21.21.D3.prog(20uM).C17WT.10uL.(26 C).2B2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 5:00:53 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:43:13 PM

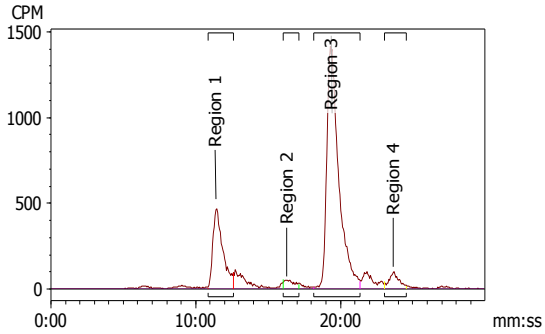
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 72
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:50	12:36	11:23	22803	20.50	17.65
Region 2	16:03	17:07	16:19	2666	2.40	2.06
Region 3	18:11	21:23	19:23	81011	72.82	62.70
Region 4	23:01	24:35	23:39	4765	4.28	3.69
4 Peaks				111245	100.00	86.10

Total Area: 129206 CPM
Average Background: 0 CPM

FYX-037 21.21.21.D3.prog(20uM).C17WT.10uL.(26 C).2B3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 5:35:43 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:45:13 PM

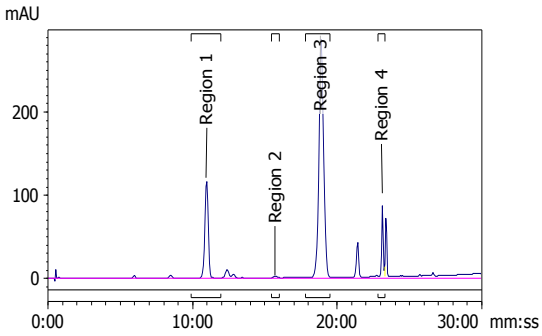
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 73
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:55	11:56	10:56	1911	21.63	14.83
Region 2	15:26	16:01	15:42	35	0.39	0.27
Region 3	17:50	19:30	18:51	6432	72.80	49.92
Region 4	22:48	23:17	23:06	458	5.18	3.55
4 Peaks				8835	100.00	68.57

Total Area: 12885 mAU
Average Background: N/A mAU

FYX-037 21.21.21.D3.prog(20uM).C17WT.10uL.(26 C).2B3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 5:35:43 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:45:13 PM

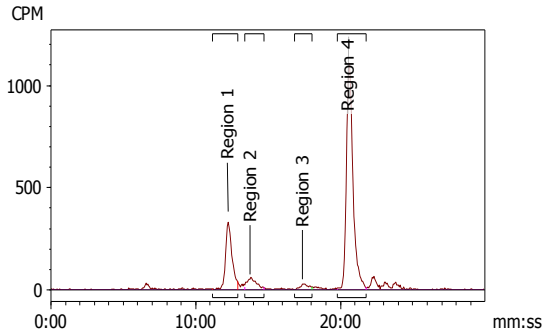
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 73
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:11	12:55	12:13	10410	20.92	17.92
Region 2	13:22	14:43	13:46	2778	5.58	4.78
Region 3	16:51	18:04	17:24	1226	2.46	2.11
Region 4	19:48	21:45	20:33	35341	71.03	60.84
4 Peaks				49754	100.00	85.65

Total Area: 58086 CPM
Average Background: 0 CPM

FYX-037 C17A1WT(5uL).21.21.21.D3prog(20 uM).30C.2C1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 12:05:03 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 3:25:15 PM

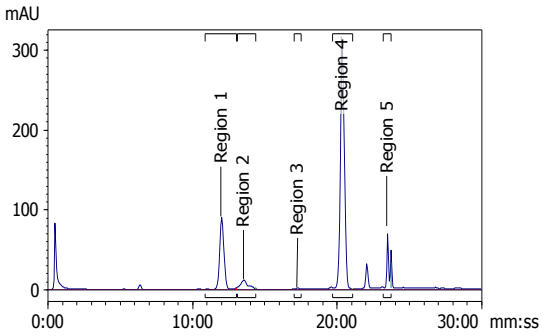
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 34
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:52	13:01	11:58	1738	21.41	14.22
Region 2	13:07	14:23	13:31	284	3.50	2.32
Region 3	17:01	17:29	17:13	12	0.15	0.10
Region 4	19:41	21:05	20:18	5841	71.98	47.79
Region 5	23:09	23:45	23:27	240	2.95	1.96
5 Peaks				8115	100.00	66.40

Total Area: 12222 mAU
Average Background: N/A mAU

FYX-037 C17A1WT(5uL).21.21.21.D3prog(20 uM).30C.2C1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 12:05:03 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 3:25:15 PM

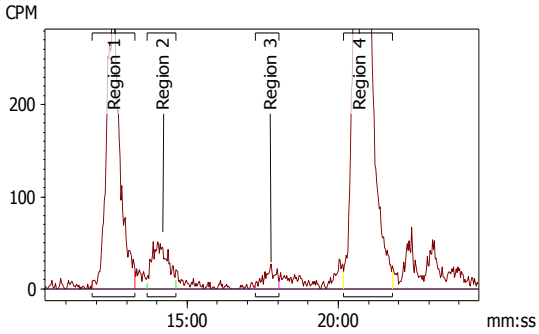
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 34
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:52	13:17	12:36	9504	22.12	18.50
Region 2	13:40	14:37	14:12	1827	4.25	3.56
Region 3	17:15	18:03	17:46	643	1.50	1.25
Region 4	20:11	21:48	20:43	30982	72.12	60.32
4 Peaks				42957	100.00	83.63

Total Area: 51363 CPM
Average Background: 0 CPM

FYX-037 C17A1WT(5uL).21.21.21.D3prog(20 uM).30C.2C2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 12:39:53 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 3:27:42 PM

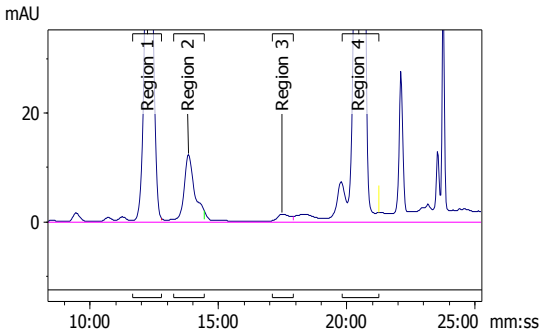
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 35
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:40	12:47	12:15	1782	24.34	17.83
Region 2	13:15	14:27	13:49	266	3.63	2.66
Region 3	17:06	17:54	17:29	18	0.24	0.18
Region 4	19:50	21:15	20:28	5257	71.79	52.60
4 Peaks				7323	100.00	73.26

Total Area: 9996 mAU
Average Background: N/A mAU

FYX-037 C17A1WT(5uL).21.21.21.D3prog(20 uM).30C.2C2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 12:39:53 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 3:27:42 PM

Run Length: 30m
Dwell: 1s

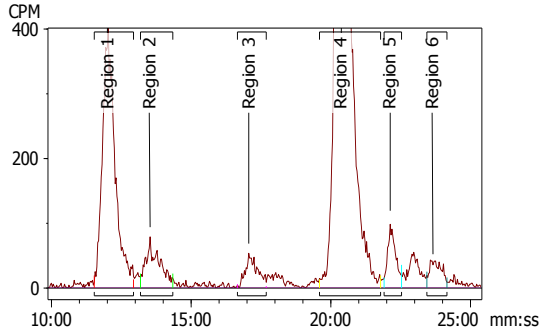
Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %

Off

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 35
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:33	12:57	12:02	12595	19.42	17.53
Region 2	13:12	14:21	13:32	2554	3.94	3.56
Region 3	16:39	17:42	17:04	1565	2.41	2.18
Region 4	19:35	21:46	20:22	44893	69.22	62.50
Region 5	21:55	22:31	22:07	1917	2.96	2.67
Region 6	23:26	24:09	23:39	1328	2.05	1.85
6 Peaks				64851	100.00	90.28

Total Area: 71830 CPM
Average Background: 0 CPM

FYX-037 C17A1WT(5uL).21.21.21.D3prog(20 uM).30C.2C3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 1:14:45 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 3:30:27 PM

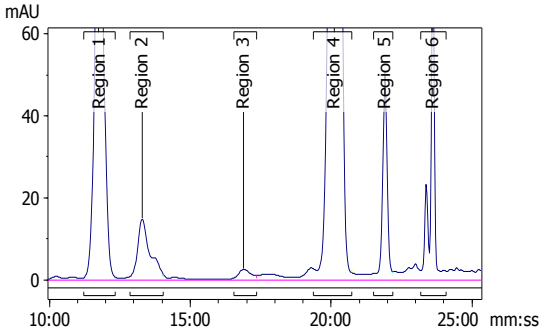
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 36
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:13	12:21	11:45	2360	19.67	16.58
Region 2	12:52	14:02	13:17	374	3.11	2.63
Region 3	16:33	17:22	16:53	41	0.34	0.29
Region 4	19:23	20:44	20:05	8071	67.29	56.71
Region 5	21:30	22:11	21:54	505	4.21	3.55
Region 6	23:10	24:05	23:36	644	5.37	4.53
6 Peaks				11995	100.00	84.29

Total Area: 14231 mAU
Average Background: N/A mAU

FYX-037 C17A1WT(5uL).21.21.21.D3prog(20 uM).30C.2C3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 1:14:45 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 3:30:27 PM

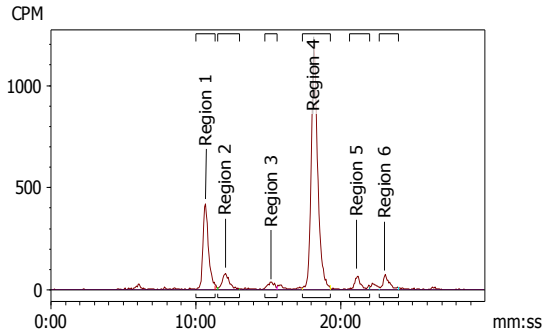
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 36
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:00	11:21	10:41	11290	19.75	18.03
Region 2	11:32	13:03	12:03	2838	4.97	4.53
Region 3	14:46	15:38	15:13	1270	2.22	2.03
Region 4	17:21	19:20	18:09	37331	65.31	59.64
Region 5	20:37	22:00	21:07	2106	3.68	3.36
Region 6	22:40	23:59	23:03	2323	4.06	3.71
6 Peaks				57158	100.00	91.31

Total Area: 62598 CPM
Average Background: 0 CPM

FYX-037 16Dprog(20uM).C17WT.5uL.RT(22 C).3A1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 6:29:18 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:19:15 PM

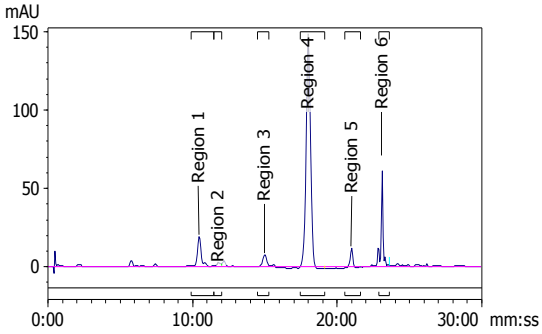
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 47
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:53	11:29	10:26	291	7.17	5.95
Region 2	11:29	11:59	11:45	19	0.48	0.40
Region 3	14:31	15:15	14:58	127	3.12	2.59
Region 4	17:27	19:07	17:58	3136	77.18	64.03
Region 5	20:32	21:36	20:58	143	3.51	2.91
Region 6	22:52	23:37	23:04	347	8.54	7.09
6 Peaks				4064	100.00	82.97

Total Area: 4898 mAU
Average Background: N/A mAU

FYX-037 16Dprog(20uM).C17WT.5uL.RT(22 C).3A1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 6:29:18 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:19:15 PM

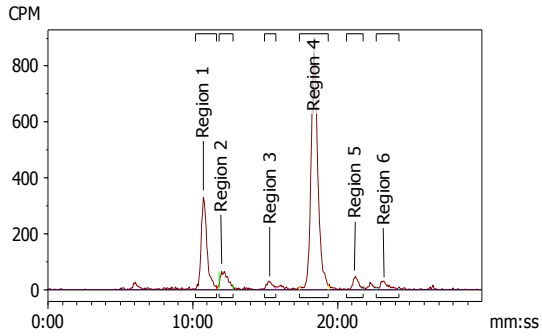
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 47
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:10	11:38	10:43	9242	21.27	19.21
Region 2	11:51	12:48	11:59	2330	5.36	4.84
Region 3	14:58	15:45	15:21	931	2.14	1.94
Region 4	17:23	19:23	18:23	28304	65.13	58.82
Region 5	20:37	21:46	21:11	1331	3.06	2.77
Region 6	22:40	24:16	23:12	1318	3.03	2.74
6 Peaks				43456	100.00	90.31

Total Area: 48118 CPM
Average Background: 0 CPM

FYX-037 16Dprog(20uM).C17WT.5uL.RT(22 C).3A2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 7:04:10 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:22:22 PM

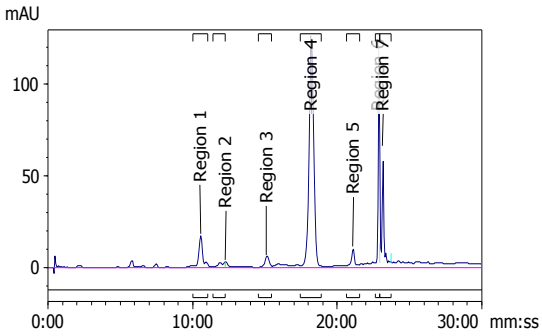
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 48
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:00	11:04	10:32	262	6.29	3.92
Region 2	11:25	12:17	12:16	-8	-0.20	-0.12
Region 3	14:34	15:26	15:07	103	2.46	1.53
Region 4	17:26	18:54	18:11	2737	65.78	40.95
Region 5	20:40	21:32	21:04	107	2.56	1.60
Region 6	22:39	22:58	22:51	656	15.76	9.81
Region 7	22:58	23:45	23:08	305	7.34	4.57
7 Peaks				4161	100.00	62.25

Total Area: 6685 mAU
Average Background: N/A mAU

FYX-037 16Dprog(20uM).C17WT.5uL.RT(22 C).3A2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 7:04:10 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:22:22 PM

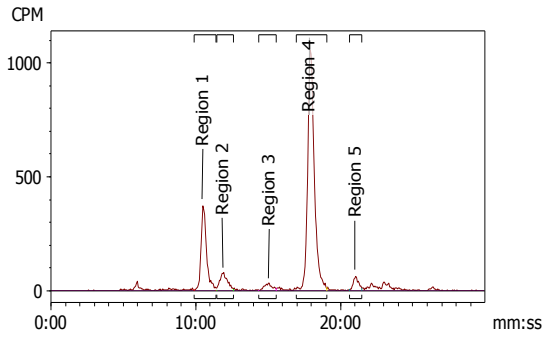
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 48
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	9:55	11:23	10:27	9917	19.98	17.52
Region 2	11:27	12:38	11:53	2947	5.94	5.21
Region 3	14:21	15:35	15:02	1283	2.58	2.27
Region 4	16:56	19:03	17:51	33782	68.05	59.69
Region 5	20:39	21:29	21:00	1712	3.45	3.02
5 Peaks				49642	100.00	87.71

Total Area: 56595 CPM
Average Background: 0 CPM

FYX-037 16Dprog(20uM).C17WT.5uL.RT(22 C).3A3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 7:39:03 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:28:18 PM

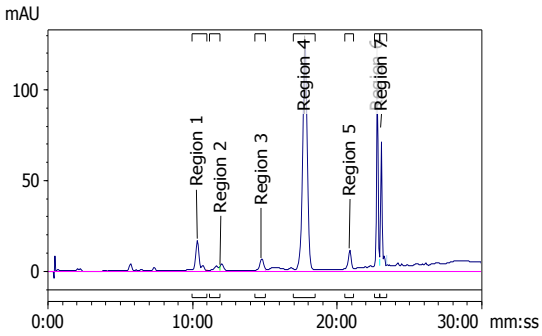
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 49
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	9:58	10:59	10:18	277	6.34	3.67
Region 2	11:11	11:55	11:54	-2	-0.05	-0.03
Region 3	14:17	15:02	14:45	106	2.42	1.40
Region 4	16:59	18:28	17:44	2720	62.23	35.98
Region 5	20:30	21:08	20:50	129	2.95	1.71
Region 6	22:34	22:55	22:44	748	17.11	9.89
Region 7	22:55	23:24	23:01	394	9.00	5.20
7 Peaks				4372	100.00	57.82

Total Area: 7561 mAU
Average Background: N/A mAU

FYX-037 16Dprog(20uM).C17WT.5uL.RT(22 C).3A3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Monday, March 05, 2012 7:39:03 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Saturday, March 17, 2012 11:28:18 PM

Run Length: 30m
Dwell: 1s

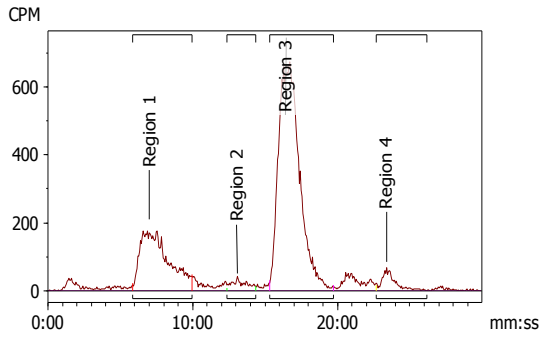
Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %

Off

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 49
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	5:51	9:56	7:01	24525	24.64	21.87
Region 2	12:22	14:21	13:05	2528	2.54	2.25
Region 3	15:21	19:43	16:28	68560	68.87	61.15
Region 4	22:40	26:11	23:23	3933	3.95	3.51
4 Peaks				99546	100.00	88.79

Total Area: 112118 CPM
Average Background: 0 CPM

FYX-037 16D.prog(20uM).C17WT.10uL.(26 C).3B1 Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 6:11:16 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:46:47 PM

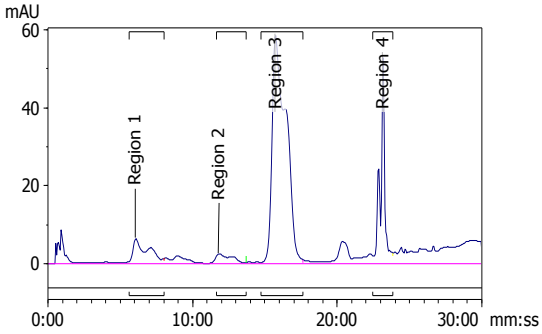
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 74
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	5:37	8:02	6:02	309	6.13	3.75
Region 2	11:38	13:41	11:48	29	0.58	0.35
Region 3	14:43	17:38	15:42	3815	75.62	46.24
Region 4	22:27	23:52	23:08	891	17.67	10.81
4 Peaks				5045	100.00	61.15

Total Area: 8250 mAU
Average Background: N/A mAU

FYX-037 16D.prog(20uM).C17WT.10uL.(26 C).3B1Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 6:11:16 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:46:47 PM

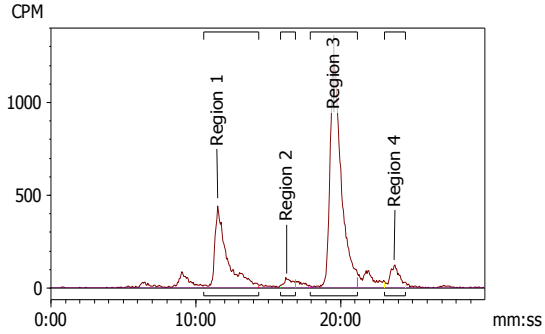
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 74
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:33	14:21	11:31	25830	25.77	22.06
Region 2	15:51	16:54	16:17	2182	2.18	1.86
Region 3	17:54	21:11	19:32	66717	66.55	56.99
Region 4	23:03	24:29	23:44	5514	5.50	4.71
4 Peaks				100243	100.00	85.63

Total Area: 117069 CPM
Average Background: 0 CPM

FYX-037 16D.prog(20uM).C17WT.10uL.(26 C).3B2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 6:46:10 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:48:24 PM

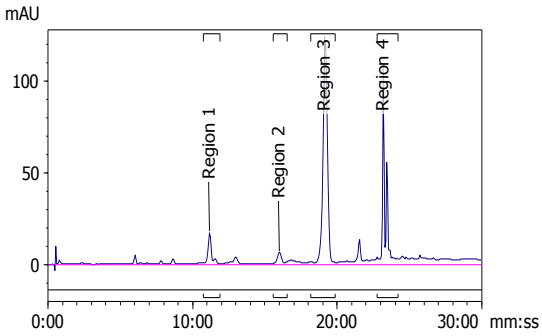
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 75
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:43	11:52	11:09	265	6.71	4.05
Region 2	15:35	16:33	15:58	93	2.35	1.42
Region 3	18:11	19:52	19:08	2507	63.56	38.39
Region 4	22:46	24:13	23:09	1080	27.38	16.53
4 Peaks				3945	100.00	60.39

Total Area: 6532 mAU
Average Background: N/A mAU

FYX-037 16D.prog(20uM).C17WT.10uL.(26 C).3B2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 6:46:10 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:48:24 PM

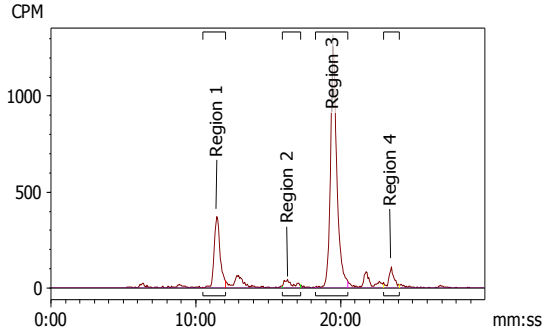
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 75
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	10:29	12:05	11:25	10771	19.83	16.54
Region 2	15:59	17:16	16:20	1840	3.39	2.83
Region 3	18:15	20:31	19:28	38986	71.77	59.86
Region 4	22:59	24:05	23:27	2723	5.01	4.18
4 Peaks				54320	100.00	83.40

Total Area: 65130 CPM
Average Background: 0 CPM

FYX-037 16D.prog(20uM).C17WT.10uL.(26 C).3B3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 7:21:03 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:50:13 PM

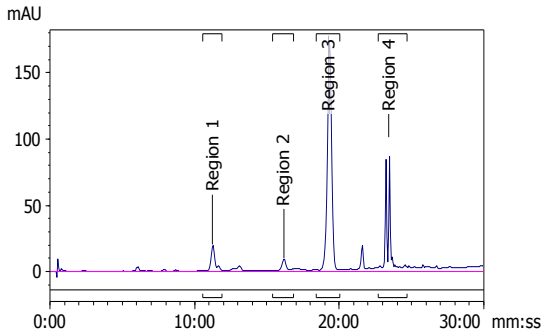
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 76
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	10:35	11:54	11:14	365	7.07	4.51
Region 2	15:25	16:50	16:09	132	2.55	1.63
Region 3	18:25	20:01	19:17	3507	67.94	43.34
Region 4	22:42	24:40	23:27	1158	22.43	14.31
4 Peaks				5162	100.00	63.79

Total Area: 8092 mAU
Average Background: N/A mAU

FYX-037 16D.prog(20uM).C17WT.10uL.(26 C).3B3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Tuesday, March 06, 2012 7:21:03 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 12:50:13 PM

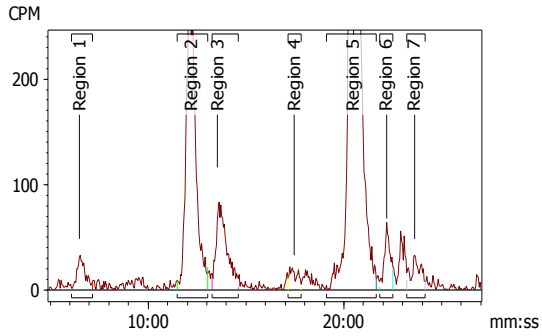
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 76
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	6:04	7:09	6:30	960	2.03	1.83
Region 2	11:29	13:03	12:12	9712	20.49	18.50
Region 3	13:17	14:36	13:33	3155	6.66	6.01
Region 4	17:10	17:49	17:28	573	1.21	1.09
Region 5	19:06	21:38	20:27	30634	64.63	58.35
Region 6	21:49	22:30	22:10	1283	2.71	2.44
Region 7	23:13	24:09	23:36	1085	2.29	2.07
7 Peaks				47402	100.00	90.30

Total Area: 52496 CPM
Average Background: 0 CPM

FYX-037 C17A1WT(5uL).16Dprog(20 uM).30C.3C1 Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 1:49:37 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:05:40 PM

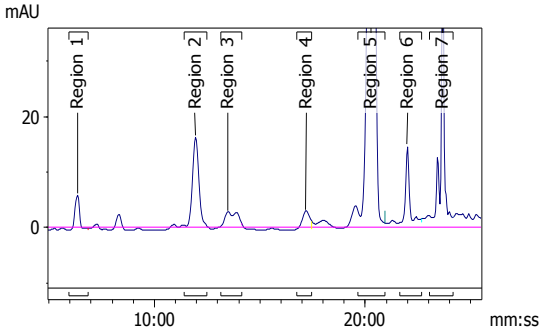
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 37
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	5:57	6:51	6:21	89	1.86	1.51
Region 2	11:25	12:29	11:56	326	6.84	5.55
Region 3	13:10	14:08	13:28	86	1.80	1.46
Region 4	16:45	17:28	17:11	51	1.06	0.86
Region 5	19:38	20:57	20:15	3602	75.53	61.29
Region 6	21:38	22:41	21:59	140	2.93	2.38
Region 7	23:02	24:11	23:39	476	9.98	8.10
7 Peaks				4770	100.00	81.14

Total Area: 5878 mAU
Average Background: N/A mAU

FYX-037 C17A1WT(5uL).16Dprog(20 uM).30C.3C1 Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 1:49:37 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:05:40 PM

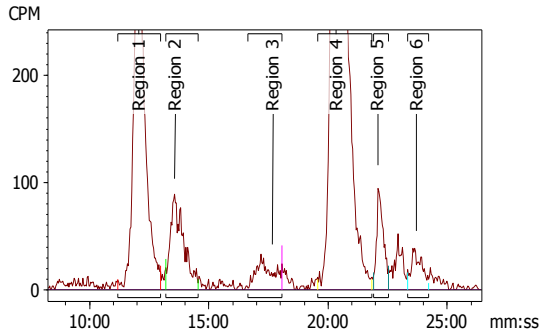
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 37
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:11	12:59	12:05	11235	19.19	17.38
Region 2	13:11	14:32	13:34	3398	5.80	5.26
Region 3	16:38	18:04	17:40	1434	2.45	2.22
Region 4	19:34	21:51	20:22	39443	67.36	61.03
Region 5	21:54	22:32	22:05	1885	3.22	2.92
Region 6	23:21	24:13	23:43	1162	1.98	1.80
6 Peaks				58557	100.00	90.60

Total Area: 64634 CPM
Average Background: 0 CPM

FYX-037 C17A1WT(5uL).16Dprog(20 uM).30C.3C2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 2:24:28 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:11:53 PM

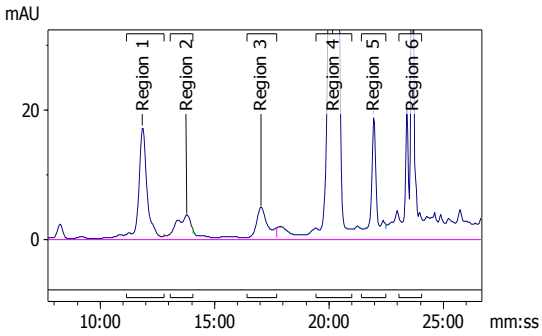
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 38
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:10	12:48	11:51	369	6.93	4.75
Region 2	13:04	14:02	13:47	79	1.48	1.01
Region 3	16:24	17:44	17:01	94	1.76	1.21
Region 4	19:26	21:00	20:10	3961	74.37	51.02
Region 5	21:24	22:30	21:57	185	3.48	2.38
Region 6	23:03	24:02	23:38	638	11.98	8.22
6 Peaks				5325	100.00	68.61

Total Area: 7762 mAU
Average Background: N/A mAU

FYX-037 C17A1WT(5uL).16Dprog(20 uM).30C.3C2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 2:24:28 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:11:53 PM

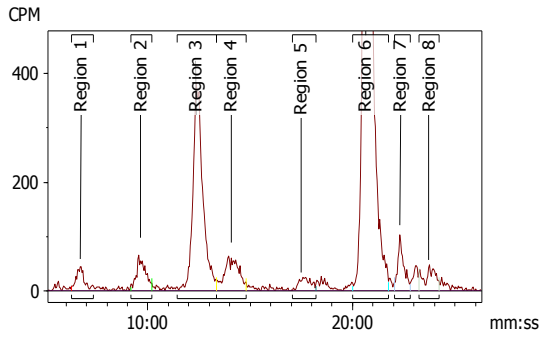
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 38
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	6:15	7:21	6:42	1264	1.91	1.77
Region 2	9:11	10:12	9:39	1981	3.00	2.77
Region 3	11:26	13:20	12:20	12771	19.34	17.84
Region 4	13:20	14:48	14:04	3117	4.72	4.35
Region 5	17:04	18:13	17:29	1053	1.59	1.47
Region 6	19:59	21:46	20:38	42179	63.87	58.91
Region 7	22:02	22:50	22:19	2054	3.11	2.87
Region 8	23:16	24:13	23:44	1616	2.45	2.26
8 Peaks				66035	100.00	92.23

Total Area: 71597 CPM
Average Background: 0 CPM

FYX-037 C17A1WT(5uL).16Dprog(20 uM).30C.3C3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 2:59:19 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:14:17 PM

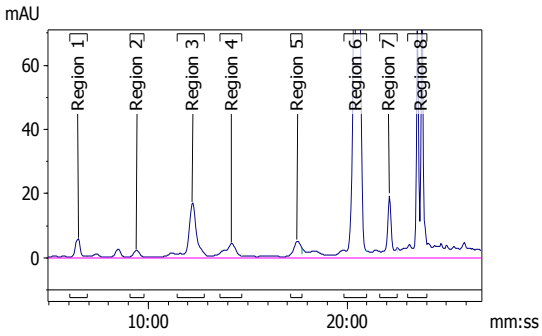
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 39
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	6:03	6:56	6:28	86	1.47	1.01
Region 2	9:04	9:46	9:24	39	0.68	0.46
Region 3	11:27	12:50	12:12	361	6.21	4.26
Region 4	13:35	14:42	14:11	95	1.63	1.12
Region 5	17:10	17:43	17:29	51	0.87	0.60
Region 6	19:51	20:59	20:27	3990	68.54	47.07
Region 7	21:39	22:31	22:07	161	2.77	1.90
Region 8	23:04	24:02	23:45	1039	17.84	12.25
8 Peaks				5821	100.00	68.67

Total Area: 8477 mAU
Average Background: N/A mAU

FYX-037 C17A1WT(5uL).16Dprog(20 uM).30C.3C3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Thursday, March 08, 2012 2:59:19 PM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 4:14:17 PM

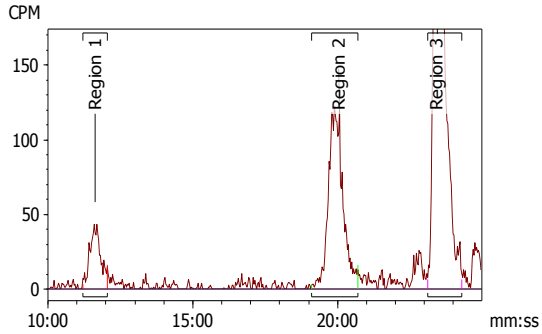
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 39
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:13	12:03	11:38	1126	6.48	5.37
Region 2	19:07	20:43	20:03	4250	24.44	20.28
Region 3	23:07	24:17	23:25	12010	69.08	57.31
3 Peaks				17386	100.00	82.96

Total Area: 20957 CPM
Average Background: 0 CPM

FYX-037 C17WT(5uL).16Dprog(20 uM).55C.3D1 Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 7:49:58 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:22:03 PM

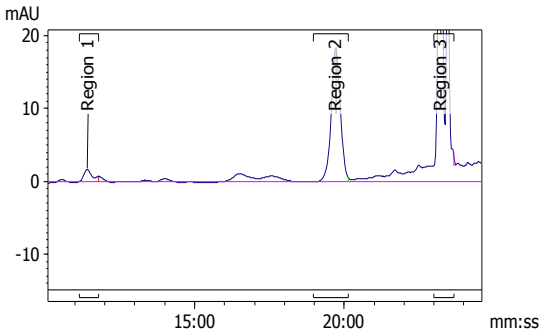
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 64
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:09	11:47	11:25	19	1.08	0.61
Region 2	18:58	20:09	19:43	373	21.74	12.26
Region 3	22:59	23:40	23:12	1325	77.18	43.52
3 Peaks				1717	100.00	56.38

Total Area: 3044 mAU
Average Background: N/A mAU

FYX-037 C17WT(5uL).16Dprog(20 uM).55C.3D1 Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 7:49:58 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:22:03 PM

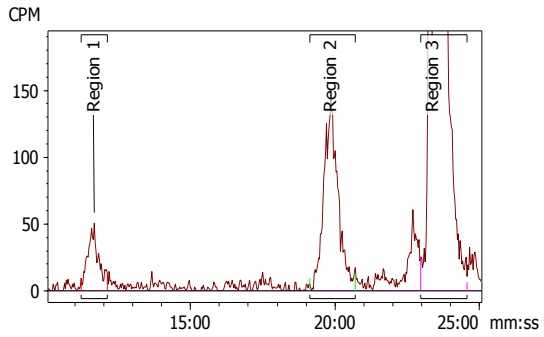
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 64
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:12	12:08	11:40	1296	2.59	2.36
Region 2	19:08	20:43	19:51	4880	9.77	8.89
Region 3	22:58	24:35	23:23	43773	87.64	79.74
3 Peaks				49949	100.00	90.99

Total Area: 54896 CPM
Average Background: 0 CPM

FYX-037 C17WT(5uL).16Dprog(20 uM).55C.3D2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 8:24:52 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:23:54 PM

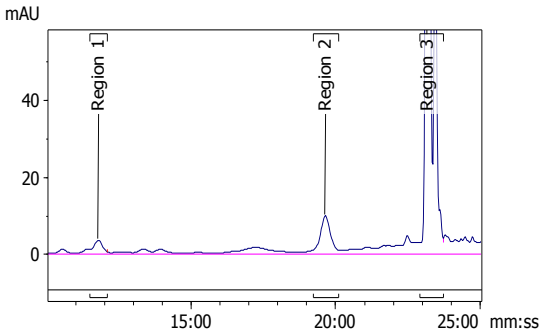
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 65
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:30	12:06	11:46	44	0.93	0.61
Region 2	19:14	20:07	19:38	190	4.00	2.60
Region 3	22:56	23:45	23:11	4531	95.07	61.97
3 Peaks				4766	100.00	65.18

Total Area: 7312 mAU
Average Background: N/A mAU

FYX-037 C17WT(5uL).16Dprog(20 uM).55C.3D2Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 8:24:52 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:23:54 PM

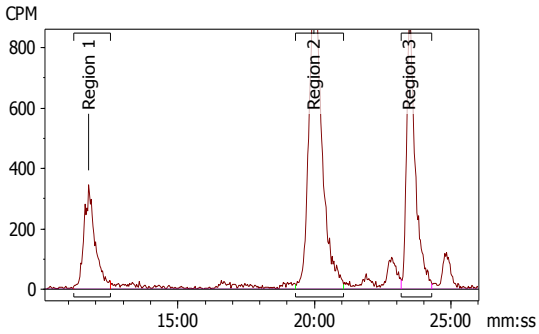
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 65
Injection Volume: 5 µL

Comments:

Chromatogram: ^{3}H



Regions: ^{3}H Detector: ß-RAM

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)
Region 1	11:12	12:31	11:44	9635	15.33	12.64
Region 2	19:18	21:03	20:00	33376	53.11	43.77
Region 3	23:11	24:18	23:29	19830	31.56	26.01
3 Peaks				62842	100.00	82.42

Total Area: 76246 CPM
Average Background: 0 CPM

FYX-037 C17WT(5uL).16Dprog(20 uM).55C.3D3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 8:59:43 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:25:46 PM

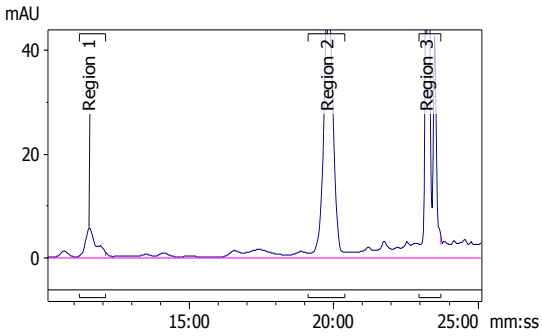
Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 66
Injection Volume: 5 µL

Comments:

Chromatogram: DA-B@254nm



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU)	%ROI (%)	%Total (%)
Region 1	11:12	12:06	11:32	109	3.92	2.09
Region 2	19:07	20:23	19:48	1100	39.65	21.08
Region 3	22:57	23:43	23:14	1565	56.43	30.00
3 Peaks				2773	100.00	53.18

Total Area: 5216 mAU
Average Background: N/A mAU

FYX-037 C17WT(5uL).16Dprog(20 uM).55C.3D3Method: Halogens_Test1

Instrument: ß-RAM Serial no 1101278
Measured by: Super User on Friday, March 09, 2012 8:59:43 AM
Method by: Super User on Thursday, February 23, 2012 10:16:56 AM
Evaluation by: Super User on Sunday, March 18, 2012 5:25:46 PM

Run Length: 30m
Dwell: 1s

Channel	Limits	Efficiency	Spill
^{3}H	0-200	100.00 %	0.00 %
Off			

Cell Volume: 500 µL
Cell Type: Liquid
Eluate Flow: 0.40 mL/min
Scint Flow: 1.20 mL/min
Residence Time: 18.8s
Vial No: 66
Injection Volume: 5 µL

Comments:

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