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Application of Synthetic Organic and Medicinal Chemistry Toward Medical Advances in Cancer, Antibiotics, and Drug Delivery

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APPLICATION OF SYNTHETIC ORGANIC AND MEDICINAL CHEMISTRY TOWARD MEDICAL ADVANCES IN CANCER, ANTIBIOTICS, AND DRUG DELIVERY

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What we observe is not nature itself,
but nature exposed to our method of questioning.
— Werner Heisenberg

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LIST OF ABBREVIATIONS

DCM	Dichloromethane or Methylene Chloride
ND	Not Determined
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
Na_2SO_4	Sodium Sulfate
$MgSO_4$	Magnesium Sulfate
CTV	Cyclotriveratrylene
EtOAc	Ethyl Acetate
IPOAc	Isopropyl Acetate
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethylsulfoxide
DMAc	N,N-Dimethylacetamide
USP H_2O	United States Pharmacopeia Purified Water
H_2O	Water
NMR	Nuclear Magnetic Resonance
MS	Mass Spectroscopy
HRMS	High Resolution Mass Spectrometry
HPLC	High Performance Liquid Chromatography
FTIR	Fourier-transform infrared spectroscopy
UPLC	Ultra Performance Liquid Chromatography
UHPLC	Ultra High Performance Liquid Chromatography
Tau (τ)	Residence Time
μW	Microwave
MRI	Magnetic Resonance Imaging
Cu (II)	copper(II)
$CuSO_4$	Copper sulfate
K_2CO_3	Potassium Carbonate
NaOH	Sodium Hydroxide
MeI	Methyl Iodide or Iodomethane
n-BuLi	n-butyl lithium
SM	Starting Material
AcOH	Acetic Acid
$^{11}B_{10}H_{14}$	Decaborane(14) (typically 80% ^{11}B isotope and 20% ^{10}B isotope)
$^{10}B_{10}H_{14}$	Decaborane(14) (enriched ^{10}B isotope)
SDAP	N-succinyl-L-diamino-pimelic acid

DapE	dapE-encoded N-succinyl-L,L-diaminopimelic acid desuccinylase
MeOH	Methanol
EtOH	Ethanol
IPA	Isopropanol
Et_2O	Diethyl Ether
HCl	Hydrogen Chloride or Hydrochloric acid
HBr	Hydrogen Bromide or Hydrobromic acid
Hept	Heptanes or n-Heptane
Hex	Hexanes or n-Hexane
N_2	molecular nitrogen
Ar	molecular argon
H_2	molecular hydrogen
PhMe	Toluene
Tol	Toluene
ACN	Acetonitrile
MeCN	Acetonitrile
SNAr	nucleophilic aromatic substitution
SN2	bimolecular nucleophilic substitution
H2NNH2•H2O	Hydrazine Hydrate
TMS	Tetramethylsilane
TBDMSCl	*tert*-Butyldimethylsilyl chloride
TBDPSCl	*tert*-Butyldiphenylsilyl chloride
RT	Room Temperature (25 $\pm$ 5 ^{o}C)
LiHMDS	Lithium hexamethyldisilazide
PCC	Pyridinium chlorochromate
PDC	Pyridinium dichromate
MeI	Methyl iodide or iodomethane
Pd/C	Palladium on carbon
CDI	Carbonyldiimidazole
$SOCl_2$	Thionyl chloride
TFA	Trifluoroacetic acid
MSA	Methanesulfonic acid
H_2SO_4	Sulfuric acid
D_2O	Deuterium oxide
Ag_2O	Silver(I) oxide
$NaBH_4$	Sodium borohydride
NH_4Cl	Ammonium chloride
SiO_2	Silica gel
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
$KMnO_4$	Potassium permanganate
MnO_2	Manganese dioxide
NH_2OH-HCl	Hydroxylamine hydrochloride

NaN_3	Sodium azide
KI	Potassium iodide
TBAF	tetrabutylammonium fluoride
STAB	Sodium triacetoxyborohydride
THP	Tetrahydropyranyl
DHP	Dihydropyran
BNCT	Boron Neutron Capture Therapy
$PdCl_2$	Palladium(II) chloride
$CDCl_3$	Deuterated chloroform
DMSO-d_6	Deuterated dimethylsulfoxide
MeOH-d_4	Deuterated methanol
$NaHCO_3$	Sodium bicarbonate
$(COCl)_2$	Oxalyl chloride
Et_3N	Triethylamine
TEA	Trethylamine
UHP	Urea Hydrogen Peroxide
PA	Phthalic anhydride
mCPBA	meta-chloroperbenzoic acid
EDCI or EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
NMM	N-methylmorpoline

CHAPTER ONE

INTRODUCTION TO SUPRAMOLECULAR AND MEDICINAL CHEMISTRY

When referring to medicinal chemistry, one often does not typically think about supramolecular chemistry as a related field. However, medicinal chemists are often tasked to synthesize biologically active compounds with the objective of targeting a specific cell receptor, anti-body, or an enzyme. One should remember that these targets, such enzymes or receptors, are host-guest complexes. In a very real sense, these enzymes are supramolecular entities that function as hosts that have affinity to specific guests that encompass the correct electronic and structural properties to complement the macrocyclic host. In supramolecular chemistry, chemists build supramolecular complexes to study host-guest chemistry effects, attempting to mimic biological systems, drug delivery systems, molecular self-assembly techniques to construct lipophilic micelles or vesicles, and build macrocyclic tweezers to function as either artificial cell receptors or part of a cavity of an enzyme. It is these concepts of host-guest interactions that essentially tie medicinal and supramolecular chemistry together, and each field truly complements the other. Supramolecular chemistry is a field that will continue to grow, and researchers will continue to develop and

revolutionize macrocyclic systems that mimic and improve our understanding of biological systems, or to develop new methods to safely and efficiently deliver drugs minimizing undesired drug side-effects and efficiently eliciting the proper biological response or termination of cancer cells.

Supramolecular chemistry involves the formation of complex molecular entities that have the capacity to participate in specific molecular recognition of guest molecules. Supramolecular structures are often pursued due to their intrinsic ability to be chiral and to mimic biological systems and dynamics. A commonly employed scaffold in supramolecular chemistry is the trimeric crown-shaped molecule cyclotriveratrylene (CTV). CTV has been studied extensively for its capability of binding a number of smaller organic and organometallic guests within its bowl-shaped cleft and has been used as a building block enabling the construction of more complex cryptophanes. The goal of this research is the synthesis and characterization of novel chiral macrocyclic derivatives. These new cyclophanes should have greater versatility than the parent CTV hydrocarbon macrocycle including greatly enhanced water solubility, the ability to coordinate to metal ligands, the potential for use in drug delivery, and unique optical and liquid crystal properties. Enantiopurity assessment of a chiral macrocyclic lactam was addressed and accomplished using a chiral shift reagent (Chirabite-AR)—

itself a chiral macrocycle, which has been used for the enantiopurity determination of small molecules that can fit into its macrocyclic cleft, but not previously for other supramolecular scaffolds.

Globally, cancer has affected many people and families and there are many types of cancer. The challenge with cancer is to design active pharmaceutical compounds to selectively target cancer cells without affecting normal healthy cells. It is the ultimate goal for medicinal chemists to design such compounds that have high specificity for cancer cells as opposed to healthy cells. Normal treatments of cancer involve chemotherapy and radiation, however these methods are typically hard on the body because they affect normal cells. Often times, cancer patients with weak immune systems cannot tolerate radiation or chemotherapy because their immune system would severely be affected making them susceptible to infections, thus losing the battle against cancer. On the other hand, localized delivery of radiation is a targeted therapy that has been gaining much attention in the medical community. One of the methods of *in situ* generated of radiation is boron neutron capture therapy (BNCT). Coupling medicinal chemistry and BNCT is a powerful tool in delivering boron-based compounds to cancer cells, which in combination with a neutron source generate an internal source of localized radiation within the cancer cell and ultimately destroys cancer cells without affecting normal cells. Within this dissertation,

the objective of this anticancer work was to utilize a potent pharmacophore that has the potential to target multiple forms of cancer (brain, prostate, ovarian, breast, liver, colon, pancreatic, lung, head/neck, mouth, bone), and even rheumatoid arthritis which also involves upregulated matrix metalloprotease (MMP) enzymes. Toward accomplishing the generation of localized radiation within cancer cells, the essential pharmacophore (missile) was coupled with a carborane moiety which is the ultimate source (warhead) of the radiation once exposed to neutrons. Targeting cancer cells can be accomplished by identifying a cellular receptor or enzyme that is upregulated in cancer cells. In this dissertation, the host enzymes that are of interest are MMP enzymes since these enzymes are upregulated and are highly over-expressed in cancer cells. Establishing host-guest relationships of inhibitors bound to MMPs is of interest since it is these interactions that allow an active compound to be bound to an enzyme. Establishing bioavailability for oral dosing is not as important since the boron-containing drug will be administered i.v. Within this dissertation is described the synthesis of two novel MMP inhibitors that contain a carborane moiety. The key chemistry involved installation of the carborane group using Click chemistry. These Click carborane products were evaluated by *in vitro* MMP inhibition testing against MMP-1, MMP-2, MMP-9 and showed nanomolar potency against MMP-2 and MMP-9 and also sparing MMP-1.

Understanding host-guest interactions is an essential key for medicinal and supramolecular chemists. It is these interactions that allows researchers to evaluate how specific enzymes functions. From these lessons, the researchers generally prepare new substrate analogs to target a specific enzyme with the aim that these new analogs will bind more strongly than the native substrate. Based on this, molecular modeling also provides another powerful tool to assess whether designed guest molecules would serve as proper substrates for a given enzyme. Within this research, molecular modeling and docking illustrated key structural differences between methylated L,L-SDAP ligands inside the active site of succinyl-diaminopimelate desuccinylase (DapE) that allowed the focus to synthesize the best analog (monomethyl L,L-SDAP) for a novel ninhydrin-based assay developed within our research group. During route selection of the monomethyl substrate, an asymmetric synthesis was accomplished providing the alternate L,L-SDAP substrate in high enantiomeric purity.

CHAPTER TWO

FORMATION AND ENANTIOMERIC PURITY DETERMINATION OF BECKMANN-DERIVED CTV LACTAM WITH CHIRABITE-AR AND ATTEMPTED RESOLUTION

Section 1: Introduction

There are four characteristics[1] to be expected in cyclophanes[2] and supramolecular[3] scaffolds. The first is the electronic interaction between aromatic rings positioned in a "face-to-face" manner. Second, the substitution on one aromatic ring influences reactivity on adjacent rings within the cyclophane influencing intramolecular/intermolecular substitution reactions of the aromatic rings. Third, intramolecular charge-transfer complexes are possible. Lastly, the ring strain, steric strain, and transannular strain present in a macrocyclic molecule will dramatically impact the chemistry of the entire system.

Section 2: Introduction to Supramolecular Chemistry with Particular Emphasis on Cyclotriveratrylene (CTV)

Supramolecular chemistry involves the formation of complex molecular entities that have the capacity to participate in specific molecular recognition of guest molecules.[3] Cyclophanes are supramolecular structures comprised of aromatic units with bridging chains forming cage-like structures[2] and have

found applications in molecular recognition as synthetic receptors, as models for intercalation, as building blocks for organic catalysts, and in the preparation of crown ethers and cryptands.[1] Cyclophanes have been utilized as molecular scaffolds in the design of new pharmaceuticals[4-7]including use as cholesterol shuttles to modulate cholesterol metabolism,[8] as potent human choline kinase (ChoK) inhibitors,[9] and as inhibitors of HIV protease.[10] Recently, CTV analogs that have been constructed to be water-soluble motifs have been designed to function as drug delivery agents and successfully tested against HepG2 (human liver hepatocellular carcinoma) cell lines.[11] Supramolecular entities have received much attention such that they are being employed to improve therapeutics by utilizing the macrocycle's ability to target and host drugs as guests and provide subsequent delivery of the drug based on physiological indicators.[12]

The crown-shaped cyclophane cyclotriveratrylene (CTV, **1**, hexamethoxy tribenzocyclononene)[13] has been employed extensively as a scaffold in supramolecular chemistry. This [1.1.1]orthocyclophane is readily prepared from the trimerization of veratryl alcohol in acid and has been extensively studied for its capability of binding a variety of smaller organic and organometallic guests within its bow-shaped cleft.[14-16] Many clathrates of CTV and of CTV derivatives[17-20]have been structurally characterized including clathrates with anionic C_{70} dimers.[21] Thioether derivatives of CTV

have recently been employed to immobilize C_{60} onto gold surfaces,[22, 23] and water soluble CTV derivatives have been developed for biomedical applications including the biological delivery of fullerenes[24] and selective anion sensing.[25] Other clathrates of CTV have been structurally characterized, including DMSO and ethanol,[26] chlorinated organics,[18] xenon,[19] lanthanides,[20] organometallic complexes,[17] C_{60},[27] and anionic C_{70} dimers.[21] CTV has been employed as a supramolecular building block to construct more complex cage-like cryptophanes,[28] and as a crystal engineering tecton giving network structures of unusual topology.[29] CTV derivatives have also been studied for their mesomorphic properties[30] and are capable of forming liquid crystals by themselves[31] and as complexes with C60.[31] CTV is a natural product and was isolated from the bark of *Zanthoxylum conspersipunctatum* found in New Guinea.[32]

Section 3: Background Including Previously-Published Work on the Synthesis of Selected CTV Derivatives

Our work in supramolecular chemistry is based on the supramolecular scaffold cyclotriveratrylene (CTV, **1**), which has enjoyed much attention in the literature, but almost no focus on functionalization of the apex of the structure (see Figure 1). We have focused both on functionalization of the apex *via* oxidation reactions, as well as on a scaffold wherein the three apical methylenes are replaced by nitrogen atoms[33] ("N3-CTV").

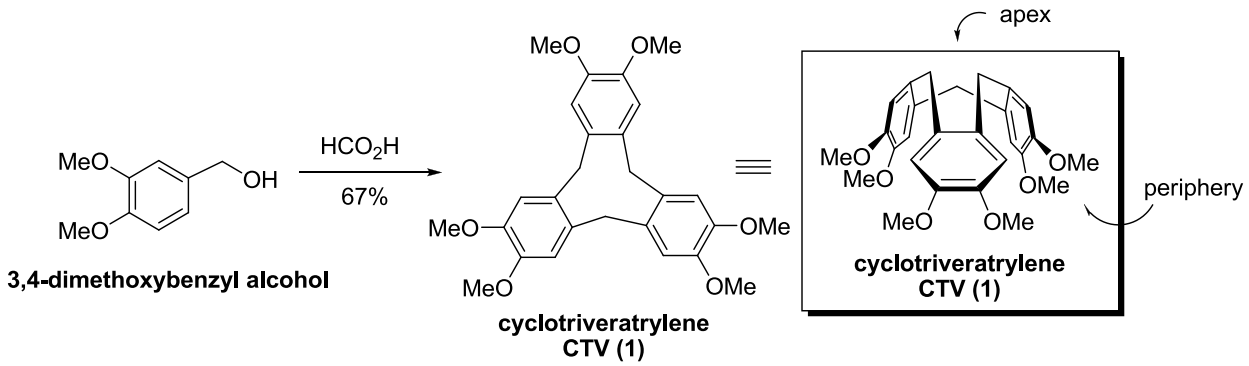
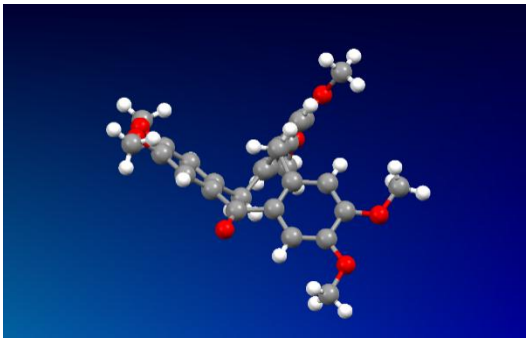
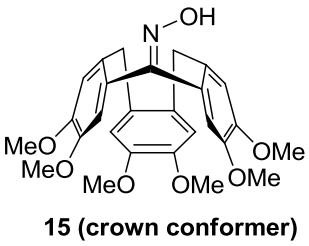


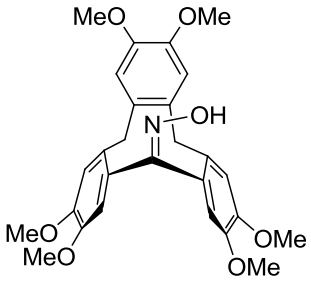
Figure 1. Classic Preparation of CTV[13] and designation of apical and peripheral regions

The bowl-shaped crown conformer is essential for host-guest chemistry with CTV and its derivatives. CTV itself exists almost exclusively in the crown conformer. Oxidation of CTV to the monoketone **2** afforded the crystalline product, which was shown by X-ray crystallography to crystallize in an enantiomerically pure crystal. Formation of the oxime of the ketone afforded two products, surprisingly, which we demonstrated were the crown (**15**) and saddle (**16**) conformers of the CTV oxime.[34] Given the importance of the kinetics and thermodynamics for host-guest chemistry, we pursued a study of the interconversion of the oxime conformers (see Figure 2), followed by proton NMR. The oxime saddle conformers of **16** are favored in $CDCl_3$ (K_{eq} = [saddle]/[crown] = 1.4), whereas the CTV oxime crown conformer **15** is favored in three more polar solvents studied (DMSO-d_6, acetonitrile-d_3, acetone-d_6). The $t_{1/2}$ of the saddle **16** was determined to be 2.45 ± 0.15 h in $CDCl_3$ at 25 °C, and 3.71 ± 0.07 h in DMSO-d_6.[35]





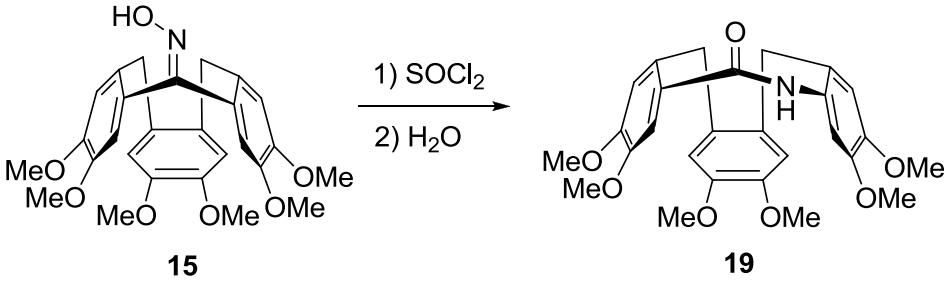
15 (crown conformer)



16 (saddle conformer)

Figure 2. X-Ray crystal structure of CTV monoketone **2**, and the two atropisomers of CTV oxime (**15** and **16**)

To expand the macrocycle from a 9-membered ring macrocycle to a 10-membered ring we employed the Beckmann rearrangement of the oxime **15**, which proceeded in high yield with thionyl chloride to generate the 10-membered lactam **19** (see Figure 3).[36]



15 19

Figure 3. Beckmann rearrangement of CTV-oxime to the ring-expanded lactam

We found that the 10-membered N-acetyl macrocyclic imide **21** crystallizes as a solid solution of racemic columnar assemblies comprised of alternating enantiomers in a stacked-cup array, each as a chloroform

monosolvate, but with a random distribution of the columns within the crystal leading to whole molecule disorder within the crystal, comprising a 2-D solid solution (see Figure 4).[37]

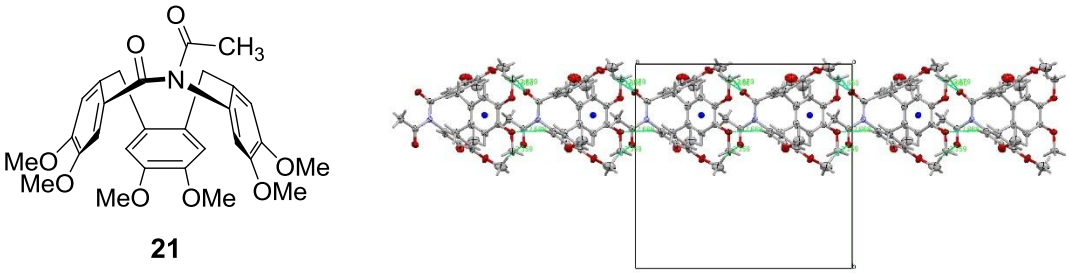


Figure 4. Columns of N-acetyl CTV imide **21** comprising the 2D solid solution observed in the X-ray crystal structure

Interestingly, under certain conditions for the Beckmann rearrangement for making the 10-membered lactam **19**, a helical pentacycle **20** was obtained, which arose *via* an unexpected tandem Beckmann-Electrophilic Addition cascade sequence (see Figure 5).[36]

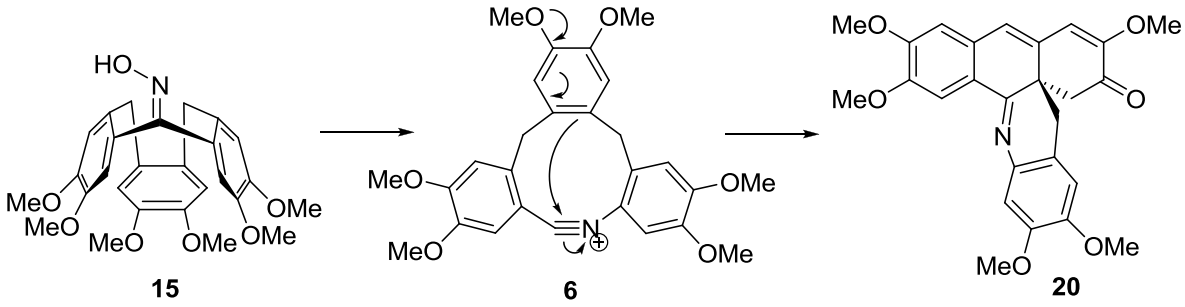


Figure 5. Observation of a Tandem-Beckmann/electrophilic aromatic addition cascade

The observation of the tandem-Beckmann cascade drew our attention to the possibility of a synthetically useful intermolecular metal-free tandem

Beckmann-electrophilic aromatic substitution cascade affording diaryl imines, ketones, amines and quinazolines, which was realized (Figure 6).[38]

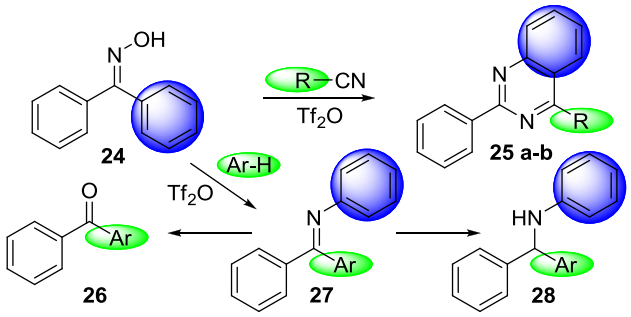


Figure 6. Intermolecular Tandem-Beckmann rearrangement

Oxidation of cyclotriveratrylene (CTV, **1**) to the corresponding diketone **3** and subsequent bromination resulted in an unexpected rearrangement to a highly functionalized 9-aryl-10-bromoanthracene derivative **11** and **12**. Electroluminescent 9,10-diaryl anthracenes have been shown to be promising host and hole-transporting materials in organic electroluminescence due to their high thermal stability, electrochemical reversibility, and wide band gap useful for organic light-emitting diodes (OLEDs), especially blue OLEDs, so we employed Suzuki couplings to synthesize a series of 9,10-diaryl compounds (**13a-c** and **14a-c**) that are structural analogues of anthracene derivatives used in the preparation of OLEDs but are more highly functionalized, including electron-donating methoxy groups in addition to substitution by a carboxylic acid moiety (see Figure 7).[39]

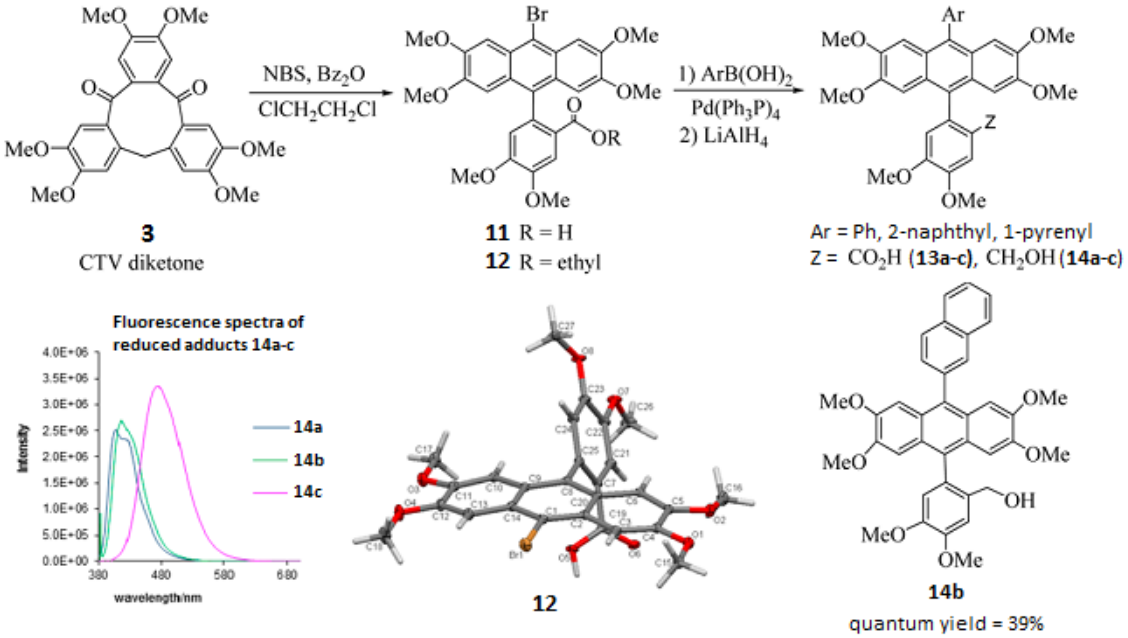


Figure 7. Electroluminescent 9,10-diaryl anthracenes (**13a-c**, **14a-c**) derived from diketone **3**

Furthering the apical functionalization of CTV **1**, we also explored the apical functionalization of the veratrylene tetramer, cyclotetraveratrylene (CTTV, **7**), obtaining the CTTV tetraketone **8** (see Figure 8). Curiously, the tetraketone **8** undergoes two distinct trans-annular cyclization reactions, depending upon the conditions, either to a bis-spiro lactone **9** reminiscent of the closed, lactone form of fluorescein or to **10**. When tetraketone **8** is heated with acid, the CTTV tetraketone **8** produces a highly symmetric diketone **10** in high yield (see Figure 8).[40]

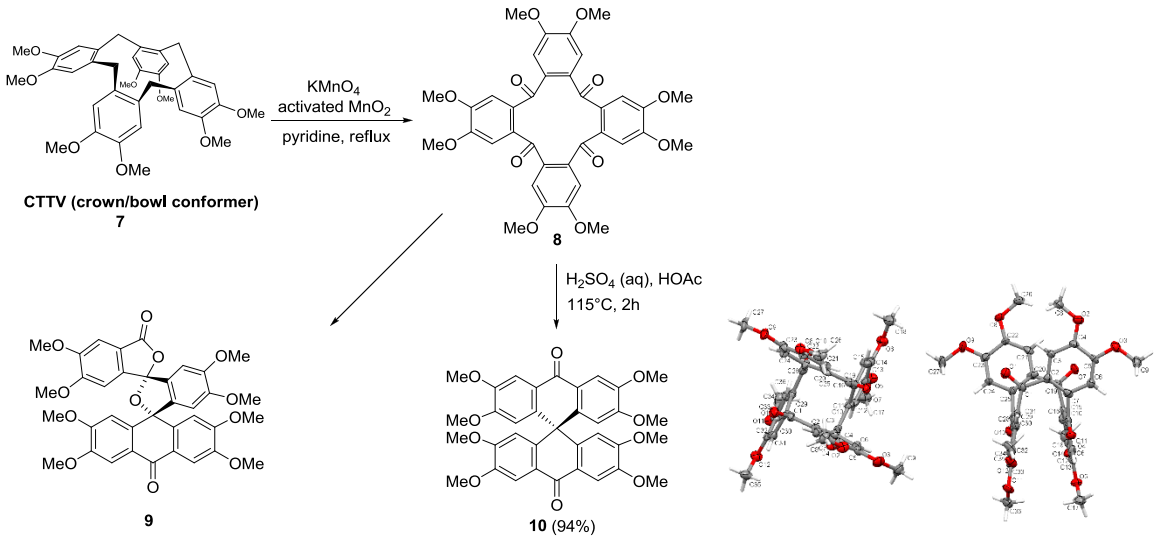


Figure 8. Preparation of CTTV tetraketone **8** and two different rearrangement products **9** and **10**. X-ray crystal structures of CTTV tetraketone **8**

Further, as a complementary avenue for apical functionalized analogs of CTV **1**, we targeted the tris-N derivative (**N3-CTV**), which conceptually is the combination of CTV and the well-known ligand TACN (triazacyclononane). The nitrogen atoms should provide both a handle for functionalization on the apex through alkylation, as well as the possibility of metal complexation (see Figure 9).[41]

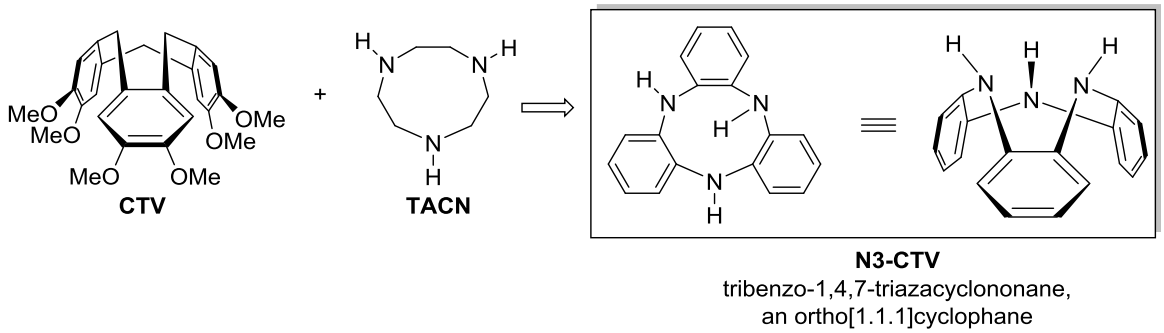


Figure 9. Design of "N3-CTV" as a conceptual combination of CTV and TACN

Section 4: Discussion of Current Work Including Improvement in the Synthesis of CTV Analogs

This section summarizes advances in the synthesis of cyclotriveratrylene (CTV) derivatives that have not already been published. These novel macrocyclic molecules have unique and intriguing properties including pseudorotation, rapid nitrogen umbrella inversions, elegant intramolecular transannular cyclizations, the ability to crystallize in random columnar assemblies, the ability to undergo ring expansions through both traditional and tandem Beckmann rearrangements, formation of helical like bonds, oxidative rearrangements, and the possibility of the electroluminescent properties which are enhanced by the highly functionalized moieties present on the molecules synthesized within this section. Cyclotriveratrylene (CTV, **1**) was the starting material for the synthesis of various CTV derivatives. The synthetic process improvements for CTV analogs of interest were monoketone **2**, oxime conformers **15** and **16**, lactam **19**, and menthyloxyacetic acid imide **23**.

One of the oxidized products of CTV, monoketone **2,** was previous synthesized utilizing either acidic conditions adopted by Cookson and Stevens[42] or slightly basic conditions. We found that using slightly alkaline conditions increased the rate of reaction, made isolation of the desired oxidized products easier, and eliminated the formation of spirolactone**5**. Within this work, the process of preparing monoketone was further improved using neutral conditions that employed ethyl acetate as the

solvent which avoided the use of pyridine. Furthermore, the process implemented a simple trituration step for purification of monoketone **2** rather than resorting to normal phase chromatography.

Another intermediate compound prepared in this work was the oxime of CTV which exists as the individual crown (**15**) and saddle (**16**) conformers that were previously isolated and characterized.[34] Previous isolation of CTV oximes consisted of concentration from pyridine, aqueous workup, and a tedious chromatography. Within the present work, the process was improved to isolate the CTV oximes as a mixture of atropisomers directly from the crude reaction mixture such that both saddle and crown oxime conformers were isolated together as a filterable solid.

The Beckmann rearrangement of CTV oximes to prepare racemic CTV lactam **19** has been modified to avoid the use of corrosive thionyl chloride, prevent the formation of helical pentacycle (**6**) and simplify for the purification process. Furthermore, previous methods required the use of pure crown CTV oxime conformer to achieve high yields of CTV lactam, however this work employs a newly-modified procedure where a mixture of oxime conforms can be used and still achieve high isolated yields of desired CTV lactam **19**.

CTV lactam **19** exists as a mixture of enantiomers (racemate) and these supramolecular enantiomers were observed spectroscopically *via* ^{1}H

NMR using commercially-available Chirabite-AR chiral shift reagent. Furthermore, the CTV lactam racemate (**19a** + **19b**) was derivatized using a chiral auxiliary, (-)-menthoxyacetyl chloride, generating an enriched diastereomeric imide **23** (87:13) confirmed by ^{1}H NMR and X-ray analysis. The use of Chirabite-AR was employed to monitor the chiral purity of CTV lactam after hydrolytic removal of the chiral auxiliary.

Section 5: Oxidations of Cyclotriveratrylene (CTV)

We are interested in the preparation of novel apex-functionalized derivatives of CTV and have reported the isolation and the kinetics and thermodynamics of the interconversion of the saddle and crown conformers of CTV oxime ,[34] and reported the discovery of a new tandem Beckmann-aromatic addition reaction sequence affording a helical pentacycle **20** from the CTV oxime **15**.[38, 46] We then turned our attention to the preparation of the elusive CTV triketone **4** due to the potential applications of this highly symmetric molecule as a new supramolecular host molecule as well as its potential utility as a synthetic intermediate.

Cookson and Stevens had reported the isolation of CTV monoketone **2** and CTV diketone **3** by the direct oxidation of CTV **1** with sodium dichromate in acetic acid under reflux.[42] Cookson and Stevens additionally reported[42] the isolation of another CTV derivative from this oxidation that they indicated was CTV triketone **4**. The correct structure was given shortly

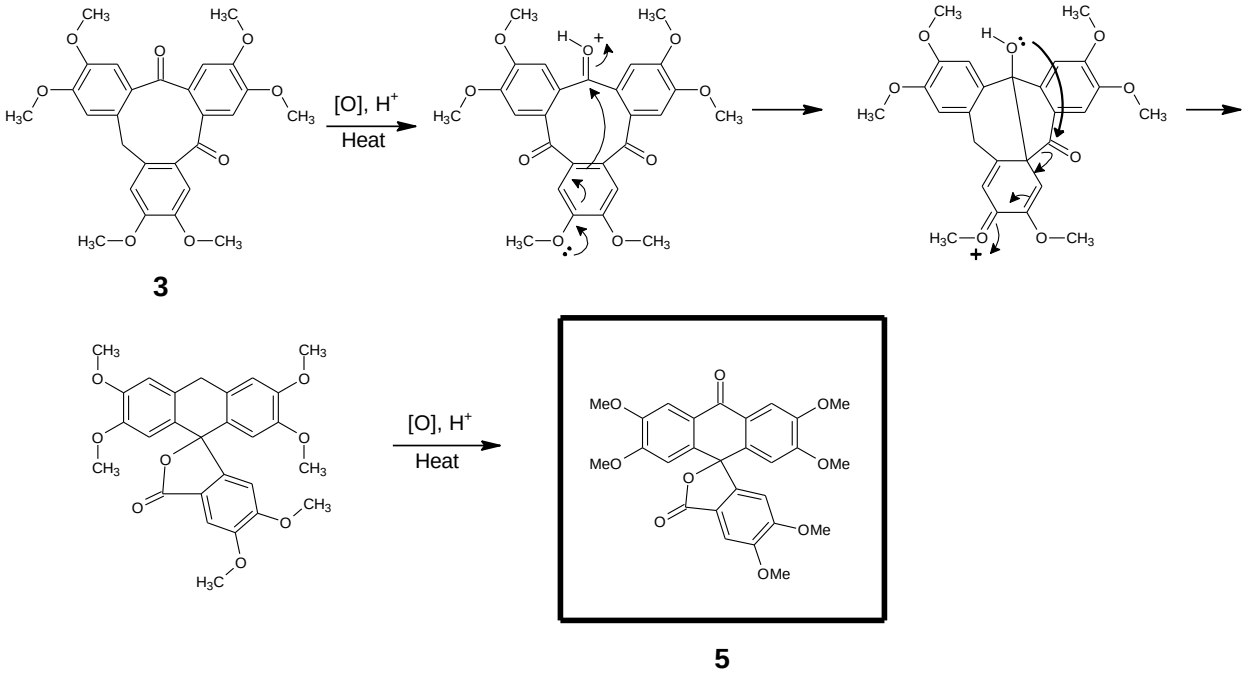
thereafter by Baldwin[43] as spirocyclic compound **5**,[44] an isomer of the CTV triketone thus giving rise to misleading combustion and mass spectrometry analyses. We believe that the triketone *may* be produced under the sodium dichromate oxidation conditions, but also that the spirocycle **5** results from rapid acid-catalyzed electrophilic addition and rearrangement of the intermediate diketone **3** or triketone **4**.

Furthermore, it is very well possible and seems more reasonable that CTV diketone **3** is the intermediate for the formation of spirocycle **5**. Below are two schemes which illustrate two different mechanisms for the formation of spirocycle **5**. Another rationale that diketone **3** may be the precursor to **5** is that after intramolecular transannular cyclization, the corresponding benzylic carbon is even more activated for oxidation to the ketone. CTV diketone **3** is difficult to oxidize to the triketone **4** due to the incorrect dihedral angles of the benzylic carbon making it an unactivated methylene, as discussed below.

From Schemes 1 and 2, with respect to the formation of spirolactone **5**, the diketone route (see Scheme 2) actually seems more reasonable than the triketone route, because when the aryl π-electrons of the diketone undergo intramolecular nucleophilic attack on the protonated carbonyl, the *ortho* methylene is electron donating (ortho activated) thus creating a more

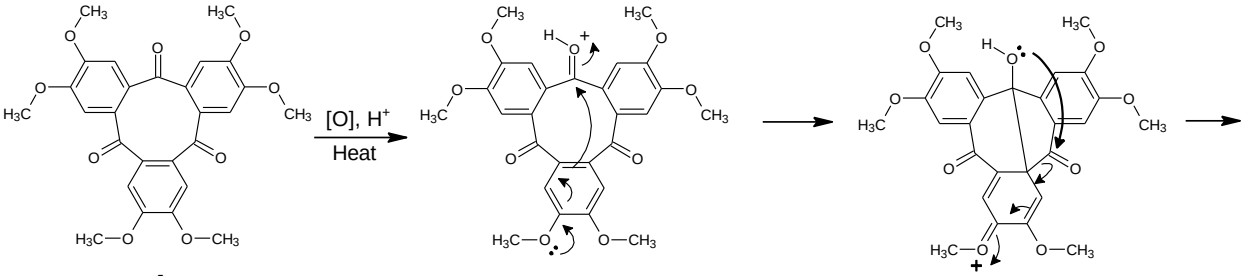
reactive nucleophilic, in contrast to the triketone which has an electron withdrawing group *ortho* to the aryl π-system.

Scheme 1. Mechanistic Hypothesis of the possible conversion of CTV Diketone **3** to Spirocycle **5**

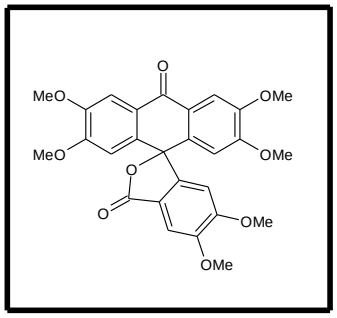


Spirolactone **5** was formed *via* a transannular electrophilic addition of a putative intermediate cyclotriveratrylene triketone **4** and is made up of both an anthrone and an isobenzofuranone ring that are connected *via* one C atom to form a spiro compound. The anthracene and isobenzofuranone ring systems of the spiro compound are both essentially planar and are perpendicular to each other, with a dihedral angle of 89.90° between them.

Scheme 2. Mechanistic Hypothesis of the Possible Conversion of CTVTriketone **4** to Spirocycle **5**



4

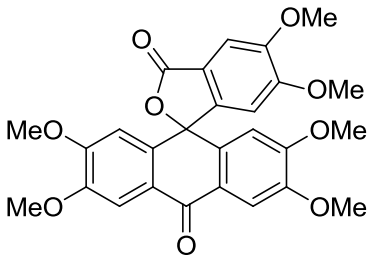


5

Cookson et al.[42] described the first synthesis of the spirolactone molecule **5**. Baldwin & Kelly[43] subsequently reported its correct identification as the spiro compound by UV and NMR methods.

Cookson et al.[42] reported the isolation of a CTV derivative from oxidation of CTV **1** with sodium dichromate in acetic acid that they thought was the CTV triketone **4**. The triketone **4**, however, has in fact never been isolated, and the compound isolated by Cookson and was shortly thereafter identified using UV–Vis and NMR-spectroscopic methods[43] to have a spiro structure (structure **5**), produced *via* acid-catalyzed electrophilic addition and rearrangement of the putative triketone under the acidic conditions.

Both this spirolactone **5** and the tandem Beckman/electrophilic addition products[36, 44, 45] are formed *via* transannular electrophilic addition to somehow-related cationic intermediates. Interestingly, the spiro derivative is a structural analogue of the cyclized lactone form of the exceedingly useful fluorescent spirolactone fluorescein. The spirolactone **5** contains a diaryl ketone rather than the diaryl ether of fluorescein.



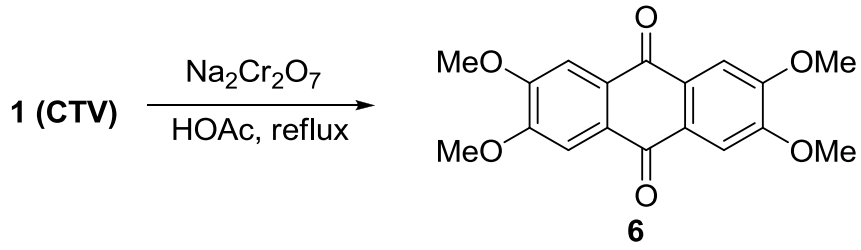
5

Figure 10.Structure of Spirolactone (Cmpd 5)

We initially suspected the presence of triketone **4** in the Cookson oxidation[42] method and isolated a small amount (1.5-3.5%) of high-melting yellow solid (mp 338-340ºC) which we thought had C_{3V} symmetry, thus displaying only two singlets in the ^{1}H NMR in a ratio of 1:3 at 7.68 and 4.07 ppm respectively, and only five signals total in the ^{13}C NMR corresponding to the carbonyl, the methoxy, and three aryl carbons. High resolution mass spectrometry demonstrated the presence of the appropriate ion for the trimeric triketone, although this must have been detection of a minor component in the material which was subsequently confirmed to be the

known dimeric 2,3,7,8-tetramethoxyanthraquinonone (structure **6**). The main component was certainly unexpected given the presumed integrity of the trimeric CTV system, but we hypothesize that it was formed *via* a reverse electrophilic aromatic substitution under the acidic conditions and expulsion of one of the three 3,4-dimethoxybenzyl moieties and re-closure to the dimeric anthraquinone **6** (see Scheme 3).

Scheme 3. Dimeric Anthraquinone **6** via Oxidation of CTV



6

Based on the fact that oxidation of CTV **1** itself, or oxidation of the mono/diketone analogs (**3/4)** under acidic conditions afforded the undesired spirolactone **5**, other means of carrying out the oxidation was investigated. Previously, alternative oxidative conditions of CTV **1** (Scheme 4) were developed using an excess of $KMnO_4$(36 eq) supported on activated MnO_2 (72 eq) in refluxing pyridine. After 3 h at reflux, the reaction was complete and isolation afforded a 35% yield of CTV monoketone **2** and 16% CTV diketone **3**. These basic conditions were convenient due to the fact that spirolactone **5** does not form and chromatography is much easier in terms of separating CTV monoketone **2** from diketone **3** after normal phase

chromatography. The isolated monoketone **2** and diketone **3** were analyzed *via* X-ray analysis (Figure 11). Interestingly, the monoketone **2** crystallizes as a single chiral conformer.[35] The X-ray crystal structure of the diketone **3** has not been published.

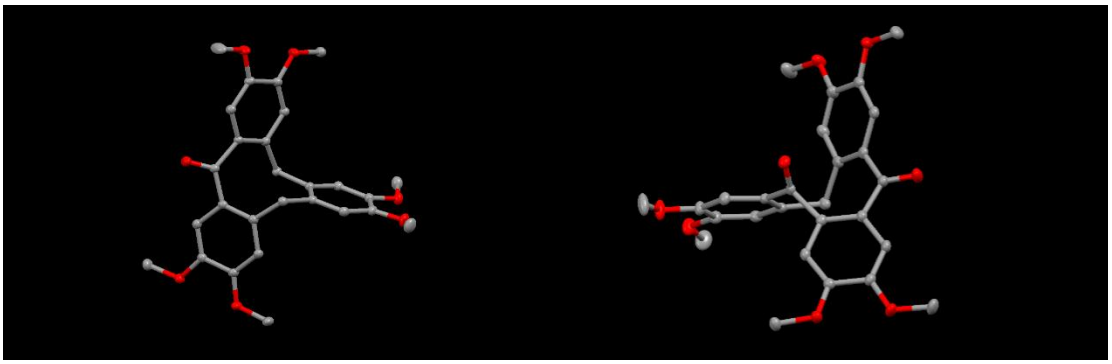
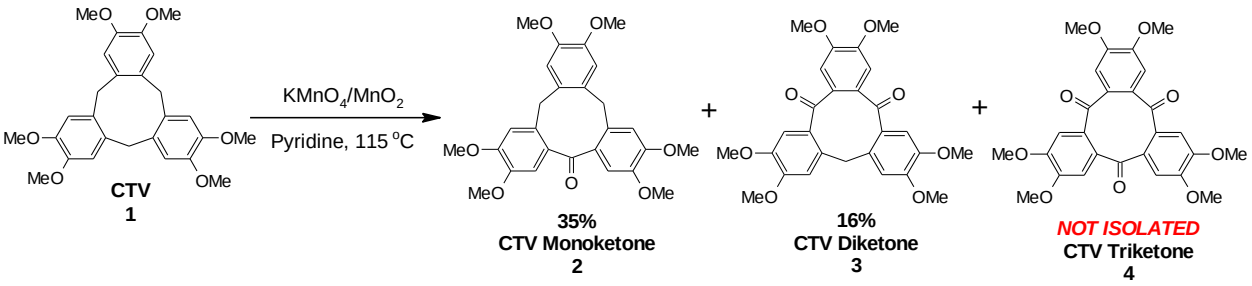


Figure 11.X-Ray structure of CTV monoketone (**2**) and CTV diketone (**3**). Hydrogens were omitted for clarity

Scheme 4. $KMnO_4$ supported on MnO_2 oxidation of CTV



One drawback of the $KMnO_4/MnO_2$ conditions is that when the reaction is deemed complete by HPLC and TLC analysis, the crude material after filtration is concentrated and the resulting mass balance is *always* only 50-60% of the theoretical amount. The low mass balance may be due to that

some of the starting material or desired products are decomposing by permanganate over-oxidation side reactions, or the desired products exist as manganese complex salts, perhaps even as a triketone manganese complex, and if true, then the lost mass may represent material that was successively oxidized to the triketone.

Unfortunately, CTV triketone **4** has not yet been isolated from these conditions. To investigate the robustness of the $KMnO_4/MnO_2$ conditions, CTV diketone **3** was treated with 36 eq $KMnO_4$ on activated MnO_2 in refluxing pyridine or quinoline to determine if CTV triketone **4** can be prepared, but only starting material **3** was observed even after seven days of heating. This result indicates that perhaps the dihedral angle of hydrogen abstraction on the methylene carbon of CTV diketone **3** is not ideal for proper radical stabilization. However, Synder et al.[46] is the only report to-date of successful preparation of a CTV triketone derivative, and in that report, the methoxy moieties were *ortho* to the benzylic carbon. Synder performed DFT calculations of their triketone derivative compared to CTV-derived triketone **4** (both in protonated form) and showed that the adjacent methoxy groups provided better stabilization of the protonated ketone thus discouraging unproductive intramolecular transannular cyclization. Based on his findings, it is likely that under basic conditions the mechanism may involve radical intermediates. If the process is operating under radical type pathway, then

the *ortho*-methoxy groups should also provide proper radical stabilization that generates significant twisting allowing the methylene and phenyl ring to be perpendicular and in turn this creating the correct dihedral angle for oxidation to occur.

Based on Synder's findings, the synthesis of triketone **4** was no longer pursued. However, during screening reaction conditions for triketone **4**, it was found that some of the results showed high conversion to monoketone/diketone and thus the preparation of CTV monoketone can be carried out efficiently in a more suitable and more green solvent, ethyl acetate. Details about this will be discussed below.

Section 6: Process Developments for Improving the Synthesis and Isolation of CTV Monoketone

Based on previous methods for the preparation of CTV monoketone, there was a need to develop a more suitable process for CTV monoketone **3**, since that the monoketone is key starting material in subsequent research described below, that did not involve the use of sodium dichromate or pyridine as the solvent. As mentioned above, during the search for preparing CTV triketone, many conditions were investigated which evaluated the effect of various solvents, temperatures, and ratio of potassium permanganate and manganese dioxide. Each experiment was monitored by TLC and/or HPLC analysis to determine the progress of oxidizing CTV **1** to CTV monoketone

2and diketone **3**. Table 1 summarizes the results from the solvent screening at specified temperatures.

Table 1. Solvent screening for CTV oxidation to monoketone at various temperatures

Experiment*	Solvent	Temp (°C)	TLC Analysis Monoketone 2 Present?	Comments
1	pyridine	100 °C	Yes	
2	pyridine	65 °C	Yes	
3	NMP	100 °C	No	Only SM.
4	NMP	65 °C	No	
5	MeOH	65 °C	No	Only SM.
6	MeOH	25 °C	No	Only SM.
7	t-BuOH	65 °C	No	Only SM.
8	sulfolane	100 °C	Yes	
9	sulfolane	65 °C	Yes	
10	MeCN	65 °C	Yes	
11	EtOAc	65 °C	Yes	
12	EtOAc	80 °C	Yes	
13	THF	65 °C	Yes (faint spot)	
14	chloroform	65 °C	Yes (faint spot)	
15	$MeNO_2$	100 °C	No	Only SM.
16	$MeNO_2$	65 °C	No	Only SM.
17	dioxane	65 °C	Yes (faint spot)	
18	dioxane	100 °C	Yes (faint spot)	

*All experiments used $KMnO_4/MnO_2$ (34 eq/52 eq) and 15 mL of solvent. Each experiment was heated for 18 h then analyzed by TLC analysis using EtOAc/DCM (20/80).

Based on the solvent screening and using TLC analysis to judge the presence of monoketone **2**, selected solvents that contained monoketone were further evaluated and analyzed by HPLC to quantitatively assess how much monoketone **2** and diketone **3** formed along with how much unreacted

starting material (CTV, **1**) remained. Table 2 illustrates the HPLC results from this study.

Table 2. Selected solvents studied for the oxidation of CTV and monitoring conversion to monoketone by HPLC analysis

Entry	Solvent	Temp (^{o}C)	Time	HPLC Analysis		
				%CTV 1	%MK 2	%DK 3
1	pyridine	65 ^{o}C	3 h	62	25	1
2	pyridine	65 ^{o}C	17 h	43	45	3
3	pyridine	100 ^{o}C	3 h	22	58	6
4	sulfolane	65 ^{o}C	3 h	72	15	3
5	sulfolane	65 ^{o}C	17 h	50	31	10
6	sulfolane	100 ^{o}C	10 h	30	56	7
7	sulfolane	100 ^{o}C	28 h	30	56	7
8	MeCN	65 ^{o}C	3 h	75	17	1
9	MeCN	65 ^{o}C	17 h	75	17	1
10	EtOAc	65 ^{o}C	3 h	74	19	1
11	EtOAc	65 ^{o}C	17 h	42	51	4
12	EtOAc	80 ^{o}C	18 h	16	70	9
13	EtOAc	80 ^{o}C	37 h	15	72	9

*All experiments used $KMnO_4/MnO_2$ (34 eq/52 eq) and 15 mL of solvent.

Based on the results in Table 2, performing the oxidation in ethyl acetate was superior compared to the other solvents employed in the study. The use of ethyl acetate also presents the opportunity to eliminate the use of pyridine which is toxic, has a strong disagreeable odor, and can cause impotence. Following these results, the next step was to optimize the amount of $KMnO_4/MnO_2$ in the oxidation of CTV in ethyl acetate. Table 3 illustrates the results of varying the amounts of $KMnO_4/MnO_2$ at specific temperatures.

Table 3. Optimization of $KMnO_4/MnO_2$ in the oxidation of CTV in ethyl acetate and subsequent scale up

Entry	CTV Qty.	$KMnO_4$	MnO_2	Temp (^oC)	Time	HPLC Analysis		
						%CTV 1	%MK 2	%DK 3
1	0.25 g	11 eq	20 eq	65 ^oC	18 h	9.9	76.4	13.7
2		11 eq	20 eq	80 ^oC		0.5	86.4	13.2
3		20 eq	20 eq	65 ^oC		9.3	74.3	16.4
4		20 eq	20 eq	80 ^oC		0	81.3	18.7
5		30 eq	20 eq	65 ^oC		9.6	75.9	14.5
6		30 eq	20 eq	80 ^oC		0	79.4	20.6
7		11 eq	20 eq	65 ^oC	37 h	2.4	83.9	13.6
8		11 eq	20 eq	80 ^oC		0.4	86.9	12.7
9		20 eq	20 eq	65 ^oC		1.1	80.9	18.0
10		20 eq	20 eq	80 ^oC		0	77.3	22.7
11		30 eq	20 eq	65 ^oC		1.2	78.7	20.1
12		30 eq	20 eq	80 ^oC		0	63.5	36.5
13	5.00 g	10 eq	20 eq	80 ^oC	2 h	41.3	56.6	2.1
					5 h	21.6	72.8	5.6
					19 h	0	91.7	8.3
14	0.25 g	7 eq	14 eq	80 ^oC	2 h	61.3	36.7	2.1
					5 h	43.4	52.3	4.3
					19 h	9.2	83.9	6.9
15	76.2 g	9 eq	18 eq	80 ^oC	18 h	0	92.0	8.0

Table 3 illustrates that performing the oxidation at 65 ^oC for 18 h did not achieve full consumption of CTV **1** even with higher loadings of $KMnO_4$. However, when the reactions were carried out at 80 ^oC for 18 h (entries 2, 4, 6), HPLC analysis revealed that CTV **1** was completely consumed and higher conversion of monoketone **2** and diketone **3** were observed. Increasing the reaction time did not show any benefit for enhancing the conversion to monoketone, however when increasing the amount of $KMnO_4$ to >11 eq,

diketone conversion was enhanced especially at 80 ^{o}C. Interestingly, entry 13 employed slightly lower loadings of oxidizing mixture (10 eq KMnO4/20 eq MnO_2) and after 19 h generated 91.7% of monoketone **2** and 8.3% diketone **3**. Scaling the oxidation (per entry 15) using 76 g of CTV and employing even lower loadings of $KMnO_4$ (9 eq)/MnO_2 (18 eq) in ethyl acetate at 80 ^{o}C generated the same conversions of monoketone/diketone as entry 13.

The next step was to identify purification conditions for monoketone **2** without resorting to column chromatography and subsequent recrystallization. Fourteen solvents were evaluated using crude monoketone (starting purity: 85% pure by HPLC that contained 11% diketone **3**) and the solvents that showed promise were acetonitrile, n-butanol, toluene, ethyl acetate, and xylenes. Ultimately, acetonitrile showed the best results for purifying CTV monoketone **2**. Recrystallization from acetonitrile provided 96% purity but with only 46% recovery of monoketone. Performing a trituration in acetonitrile (3 volumes) at 70 ^{o}C overnight (~16 h), cooling to room temperature, and isolating by filtration provided 98% pure monoketone with 70% recovery. Scaling the trituration of the crude monoketone (51.0 g) in acetonitrile (3 volumes) at 70 ^{o}C overnight provided the first crop of monoketone (32.5 g, 65% recovery, 98% pure by HPLC). The mother liquors were partially concentrated removing about half of the

solvent to generate a slurry which was filtered to yield a second crop of monoketone (15.8 g, 31% recovery, 95% pure). The overall recovery of monoketone was determined to be 96%.

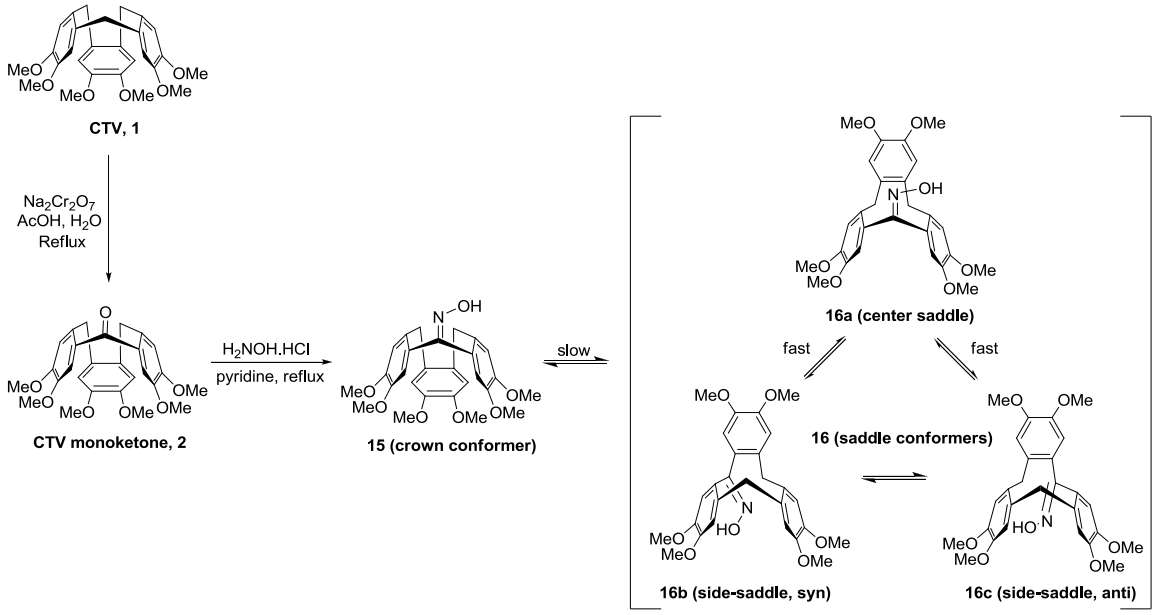
It is evident that the process for preparing monoketone has been substantially improved compared to previous reported methods. The benefits of the improved oxidation method include that the process does not use toxic and pungent smelling pyridine but rather the process uses a friendlier and less harmful solvent such as ethyl acetate, allows one to use considerably less $KMnO_4/MnO_2$, allows for higher conversion to monoketone, and lastly purification *via* a trituration in acetonitrile which proved to be very efficient and eliminated the need to employ a tedious and time-consuming column chromatography purification.

Section 7: Improved Synthesis for the Beckmann Rearrangement of cyclotriveratrylene (CTV) Oxime

The preparation of novel apex-functionalized derivatives of CTV (**1**) is of great interest in our research group. Toward this end, we have previously synthesized the oxime derivatives of CTV **15/16***via* the CTV monoketone (**2**) (see Scheme 5). Monoketone (**2**) was originally prepared utilizing a modification[47] of Steven's method[42] for oxidation of CTV with sodium dichromate in acetic acid under reflux, however this process was improved as described above using $KMnO_4/MnO_2$ in ethyl acetate and avoiding

chromatography. The conversion of **2** to the corresponding oximes **15**/**16** was quite sluggish and required an excess of hydroxylamine and overnight heating in pyridine to push the reaction to completion. The indolent nature of the monoketone in forming the oxime was presumably due to the aryl hydrogens ortho to the ketone hindering the approach of the nucleophilic hydroxylamine. The ortho hydrogen atoms are almost at contact distance, with 2.5 Å between their centers.[13]Furthermore, the conformational flexibility of monoketone may suggest that the pseudorotation also allows the phenyl rings to block the electrophilic carbonyl and prevent attack from the incoming hydroxylamine.

Scheme 5. Synthesis of Crown (**15**) and Saddle CTV-Oximes (**16**)



Our former procedure for preparation of CTV oximes **15**/**16** involved concentration of pyridine under reduced pressure, performing aqueous workup in ethyl acetate, and chromatographic purification over silica gel to isolate pure crown CTV oxime conformer **15** in 45-50% yield and pure saddle CTV oxime conformer **16** in 45-50% yield. However, since water and pyridine are miscible, we found that that CTV oximes **15**/**16** can be isolated as a filterable solid directly from diluting the partially concentrated reaction mixture with water and the isolated oxime product can be used in the Beckmann rearrangement. HPLC analysis of the filtered oxime solid indicated that the mixture was comprised of an 89:11 ratio (normalized) of crown to saddle conformers as illustrated in Figure 12. The 11.7 min component in the HPLC chromatogram is not the tandem product **20**, but rather an unidentified impurity that was not characterized.

We previously found that the Beckmann rearrangement performed on pure CTV oxime crown conformer **15** promoted with thionyl chloride in dilute solution at 0 ^{o}C proceeds in essentially quantitative yield (99%) to afford the 10-membered lactam **19**. Modified conditions afforded a helical pentacycle **20** derived from an unusual tandem Beckmann rearrangement and electrophilic aromatic addition followed by demethylation and tautomerization as illustrated in Scheme 6.[36]

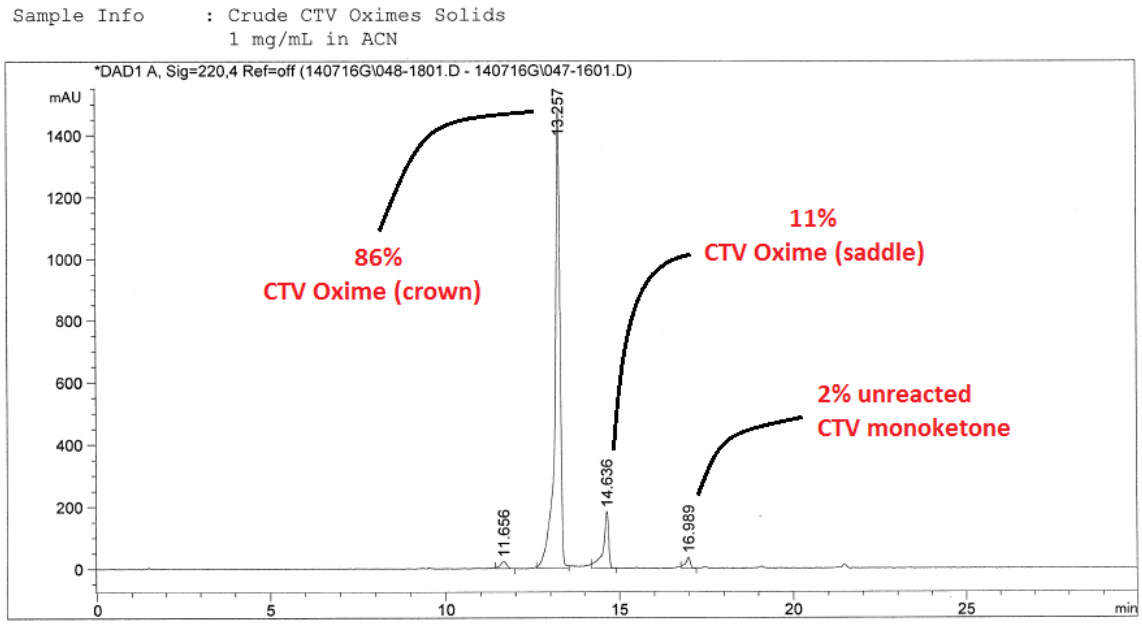
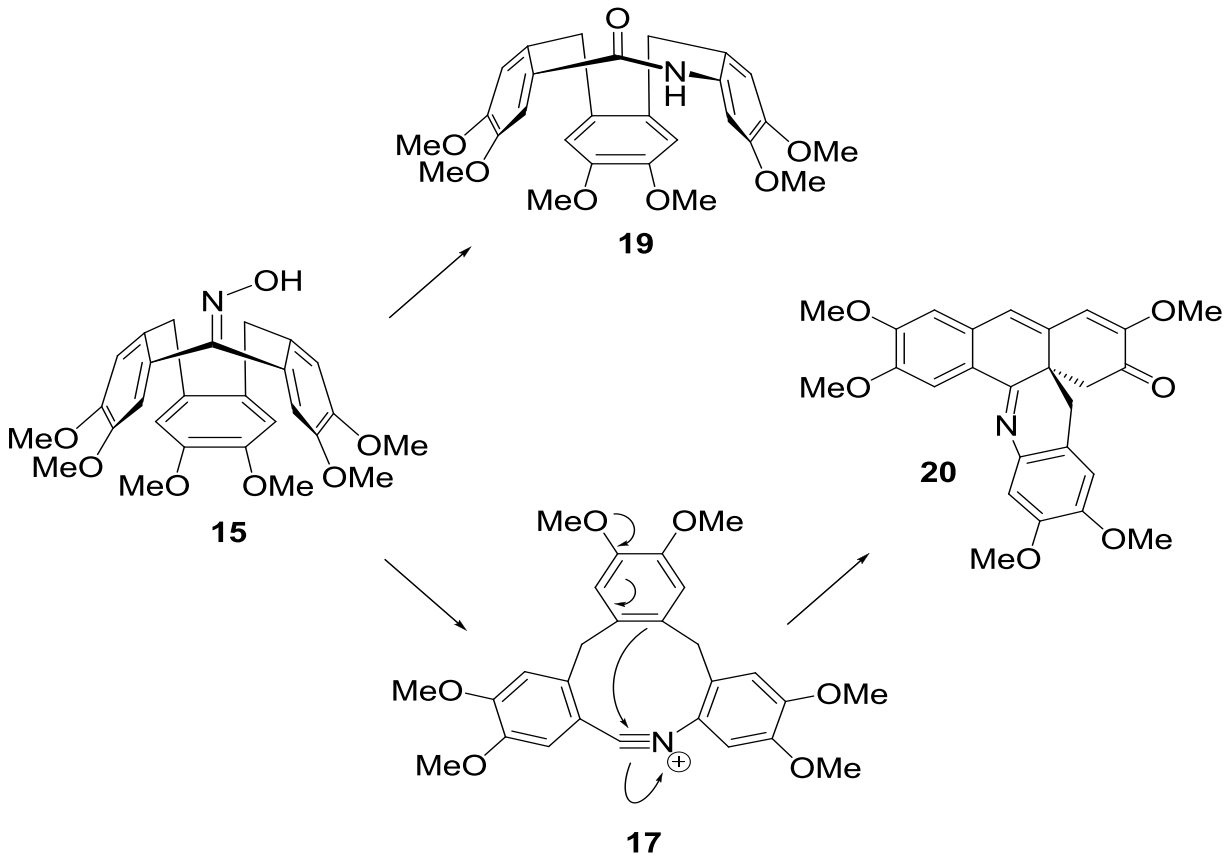


Figure 12. HPLC chromatogram of isolated CTV oximes **15**/**16** from pyridine/water

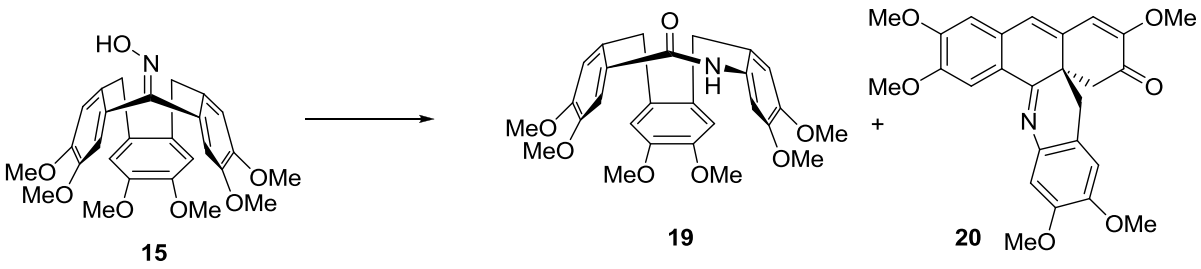
The ring-expanded lactam exists at room temperature exclusively as the crown conformer **15** based on the geminal coupling observed in the proton NMR [4.67 (1H, d, J = 15.0 Hz), 4.44 (1H, d, J = 15.3 Hz), 3.70 (1H, d, J = 15.0 Hz), 3.56 (1H, d, J = 15.3 Hz)], as pseudorotation of the flexible saddle conformation **16** is known to lead to magnetic equivalence of the geminal benzylic methylene protons. Furthermore, the N-acetyl CTV imide **21** showed that the 10-membered macrocycle exists in a crown conformation.[37] Interestingly, the lactam crown conformer **19** is a structurally chiral molecule, although lacking chiral tetrahedral carbon atoms (Schemes 6 and 7, structure **19**).

Scheme 6.Beckmann rearrangements of cyclotriveratrylene (CTV) oxime **15**



Based on our previous reported work, it is known that the Beckmann rearrangement of oxime **15** under certain conditions produces lactam **19** and helical pentacycle **20** resulting from a tandem Beckmann rearrangement and intramolecular electrophilic aromatic addition (see Scheme 7).

Scheme 7.Beckmann rearrangement of CTV oxime crown conformer to Beckmann lactam **19** and tandem Beckmann/electrophilic aromatic addition product **20**.



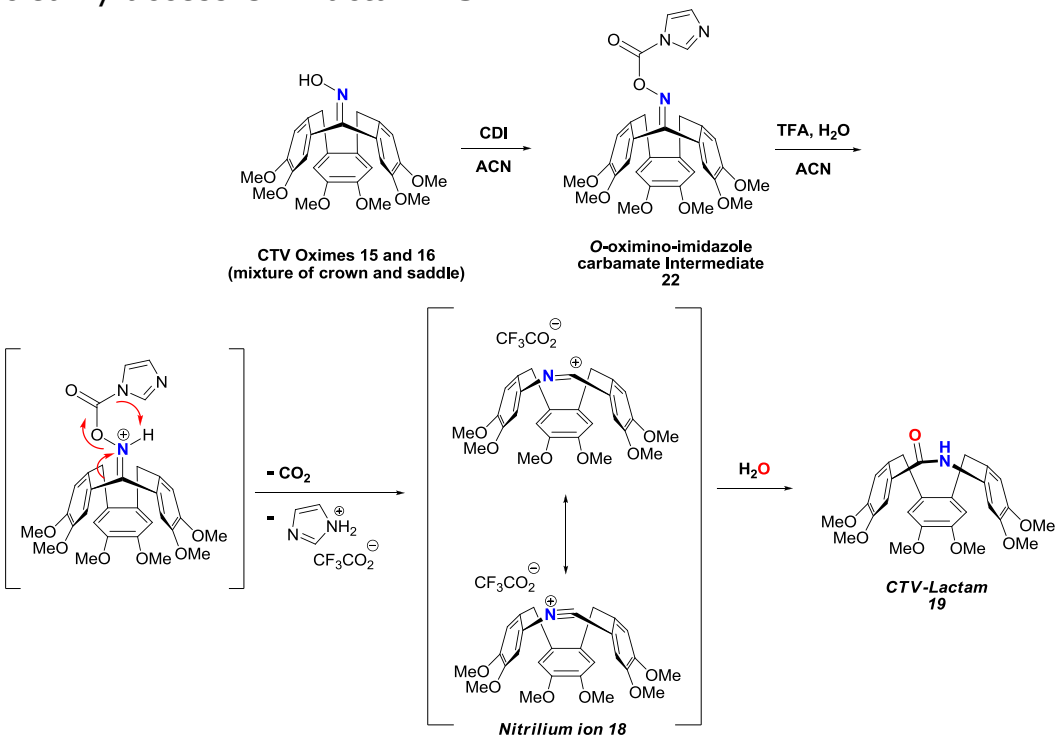
As reported previously, the product distribution in the Beckmann rearrangement was dependent upon the reaction conditions[36] when employing thionyl chloride in a diethyl ether/DCM mixture. Further efforts to develop conditions for preparing CTV lactam were evaluated in hope to minimize or eliminate the formation of the helical byproduct **20**. In addition, the previous procedure that used thionyl chloride in large excess required the use of pure crown form of CTV oxime **15**, plus it was preferred to eliminate the use of noxious thionyl chloride from the process.

Evaluation of other Beckmann reaction conditions were screened to determine if the formation of the tandem product **20** could be prevented. Thus, treatment of the oxime **15** with acetic anhydride in xylenes with microwave heating to 200–210 ^{o}C for 1.5 h under conditions employed by Savarin[40] to produce isoindoles from oximes gave only 26% of lactam **19** and none of the tandem product **20**. Heating the oxime **15** to 75 ^{o}C for 4 h with *p*-toluenesulfonyl chloride and DMAP in pyridine gave a 56% isolated

yield of lactam **19**, and none of tandem-derived product **20**. On the other hand, heating the oxime directly in polyphosphoric acid at 140 ^{o}C for 7 min gave pentacycle **20** in 22% yield with none of the Beckmann lactam **19**. Subjecting oxime **15** to iodine and triphenylphosphine in acetonitrile at 60 ^{o}C provided 14.1% conversion by HPLC to lactam **19**, and formation of **20** was not observed. Reaction of oxime **15** with catalytic ruthenium chloride hydrate in MeCN at 80 ^{o}C for 20 h resulted in only 17% conversion to CTV lactam **19** and unreacted starting material.

Ultimately, it was found that reacting CTV oxime **15** with carbonyldimidazole (CDI) generated a stable oximo-carbamate intermediate **22** which was subsequently treated with aqueous trifluoroacetic acid at 70^{o}C to generate clean conversion to CTV lactam **19** without formation of the tandem-derived product **20** as illustrated in Scheme 8. The 10-membered lactam **19** was isolated directly from the reaction mixture in 82-85% yield with 95% purity according to HPLC analysis.

Scheme 8. Modified Beckmann rearrangement using CDI/TFA/water to cleanly access CTV lactam **19**

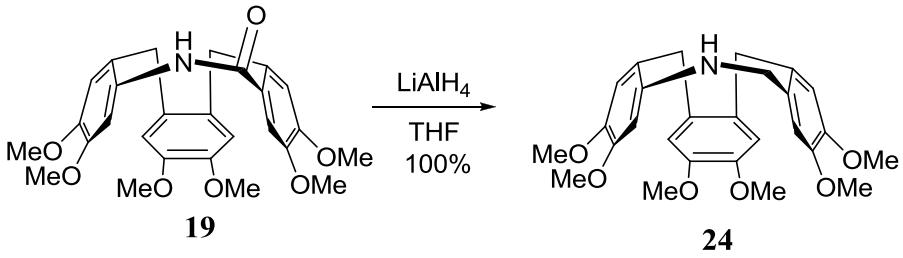


In conclusion, the Beckmann rearrangement process for preparing lactam **15** has been simplified and has been improved mainly by eliminating the use of thionyl chloride which required careful quenching of excess unreacted thionyl chloride followed by neutralization of hydrochloric acid waste streams. The improved procedure does not require the neutralization since the resulting waste stream is a mixture of imidazole and trifluoroacetic acid and the pH is within allowable disposal limits. Furthermore, the use of the improved method also allows one to use mixtures of oxime conformers **15** and **16** and easily access CTV-lactam **19** rather than using solely crown oxime **15** which was required when using thionyl chloride. Note also that the

oximes used for this work were isolated directly from the hydroxylamine reaction in pyridine by addition of water and the mixture was triturated, filtered, and dried to afford oximes as an isolatable solid which is another process improvement in its own right. Previous isolation of CTV oximes required extractive workup after removing pyridine and subsequent chromatography over silica gel which afforded foams or glass solids post concentration. Furthermore, the Beckmann rearrangement process can now be implemented into our research lab and allow undergraduates to easily carry out the preparation of racemic CTV lactam **19** using less hazardous conditions. With an improved procedure for CTV lactam, further studies were evaluated to determine if resolution of the chiral lactam enantiomers (**19a** and **19b**) could be achieved as mention in the next section.

Reduction of the crown shaped lactam **19** with lithium aluminum hydride in THF under reflux gave the corresponded racemic amine **24** in quantitative yield (see Scheme 9). The amine could potentially be applied as a linker to stationary phases or peptides which lead to the capability of allowing host-guest chemistry reactions to occur. In addition, amine **24** is envisioned to increase water solubility of the CTV-like scaffold allowing **24** to be a potential precursor for drug-delivery systems.

Scheme 9. Reduction of **19** with Lithium Aluminum Hydride to afford water soluble CTV derivative **24**



19 **24**

Section 8: Attempted Resolution and Observed Racemization of the Beckmann-Derived CTV-Lactam and the Use of Chirabite to Determine Optical Purity of the Supramolecular Scaffold

Chiral cyclophanes have applications in enantiodiscrimination processes including catalysis, recognition and sensing, determination of enantiomeric excess, and signaling chiral information of guests,[48, 49] as with chiral molecular tweezers that exhibit selective binding and chiral recognition of specific guests.[50] CTV and its [1.1.1]cyclophane congeners in their rigid crown conformation are unique bowl-shaped molecules have applications in sensors, self-organized materials, liquid crystals, and metallosupramolecular chemistry.[51] CTV and its cryptophane derivatives are of great interest in molecular recognition.[52] CTV derivatives are members of a larger family of inherently chiral concave molecules that have applications in chiral recognition and asymmetric synthesis.[49] A chiral CTV derivative bearing Kemp's triacid was shown to induce triple helix formation of collagen peptides.[53] Chiral CTV derivatives have been used for the dynamic covalent

synthesis of chiral nanocubes with potential utility as vessels for biomacromolecules,[54] and a similar dynamic thermodynamic resolution strategy was recently reported of racemic CTV units by remote stereogenic centers.[55] Helically chiral CTV units have been employed to construct enantiopure molecular cages,[56] along with a host of elegant CTV-derived coordination cages.[57] Some of the fascinating supramolecular structures of self-assembled cages derived from CTV-type scaffolds have recently been reviewed,[58] such as a racemic C3-symmetric bipyridyl-bearing CTV ligand with zinc shown to self-assemble into triply interlocked chiral catenanes within an overall chiral crystal.[59] CTV-based host compounds bearing three binaphthol moieties have been reported as chiral sensors with recognition of sugar derivatives.[60]

The crown form of the cyclotriveratrylene can undergo umbrella inversion which inverts chiral derivatives to their enantiomeric structure, as Collet demonstrated by observing the slow racemization of structurally chiral cyclotriveratrylene derivatives.[61] Resolution and NMR studies have been performed on the crown and saddle conformers of a CTV derivative toward chiral liquid crystals.[62] We have studied the kinetics and thermodynamics of the crown-saddle equilibria in CTV derivatives.[35] Indeed, while most work with CTV has focused on peripheral functionalization, we selected to focus on apical functionalization, enabling attachment of CTV “bowl-out” receptors on

surfaces.[63] We observed that CTV-derived oxime **15**[34] undergoes facile Beckman rearrangement to afford the macrocyclic lactam **19** which is chiral as the crown conformer and thus potentially resolvable.

The great interest in synthetic macrocyclic receptors includes applications in chiral analysis and separation,[64] and Ema has developed chiral selectors with multiple H-bonding sites in macrocyclic cavities[65-67] including the commercially available Chirabite-AR.

While the macrocycle of lactam **19** contains a larger 10-membered ring, lactam amide resonance[68] reduces flexibility through restricting rotation around the carbonyl C-N bond. We were interested if Chirabite-AR, which is designed for determining the e.e. of small molecules that can ideally be contained within its macrocycle, might be used to determine the e.e. of a larger supramolecular scaffold including the CTV-derived lactam **19**, and if lactam **19** might be resolvable *via* attachment of a chiral auxiliary through N-functionalization of the lactam as in derivative **21**.

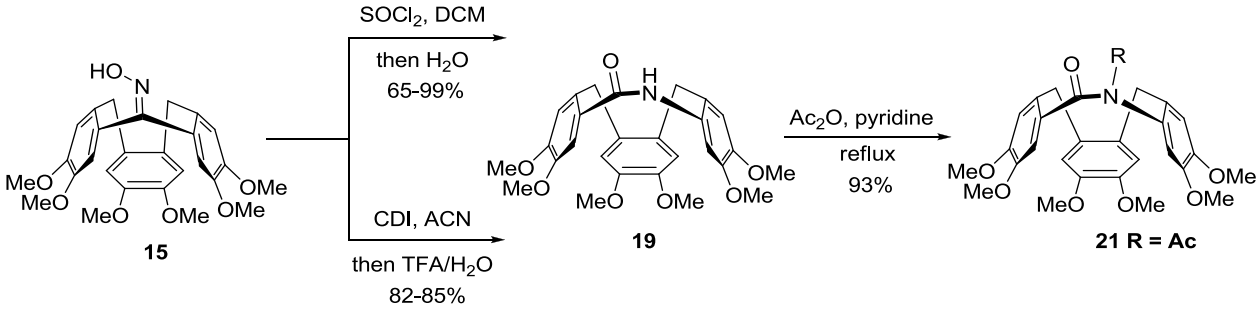
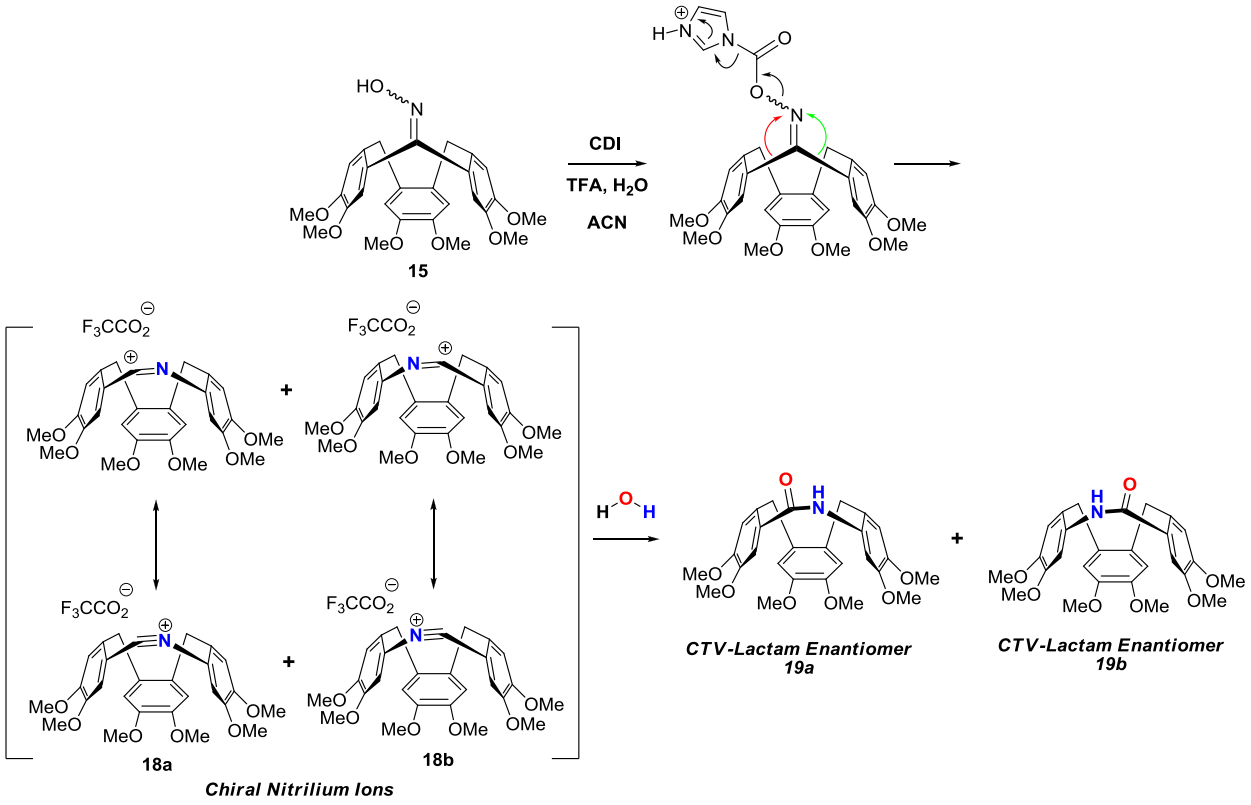


Figure 13. Synthesis of the 10-membered CTV-derived Lactam **19** and N-acetyl imide derivative **21**

Results and Discussion

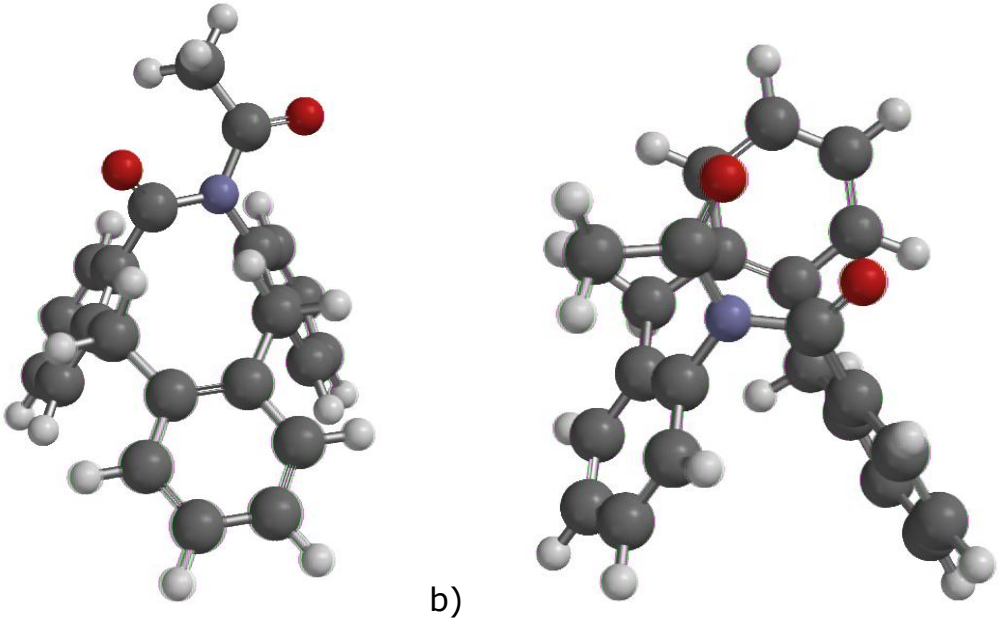
The Beckmann rearrangement of CTV-oxime generates CTV-lactam**19** as a racemic mixture of CTV-lactam enantiomers (**19a** and **19b**). As shown in Scheme 10, either of two phenyl rings can migrate (as denoted by either a red or green arrow) to form the chiral nitrilium ions (**18a-b**) which are trapped by water leading to a 50:50 mixture of enantiomers**19a-b** as a racemate.

Scheme 10. Formation and Mechanism for Racemic Cyclotriveratrylene (CTV) Lactam**19**



Assessment of the relative energies of the crown and saddle conformers of lactam **19**were assessed through calculations on model

structures lacking the six methoxy groups. Density functional theory (DFT) calculations (EDF2/6-31G*) were performed using Spartan '16 (Wavefunction, Inc., Irvine, CA). It was found that the crown conformer is 9.22 kcal/mol more stable than saddle conformer of lactam **19**. AM1 calculations that we reported earlier for the 9-membered ring CTV monoketone**2** revealed the saddle conformer as (3.15 to 5.23 kcal/mol, i.e. 13.2 to 21.9 kJ/mol) higher in energy than the more stable crown conformer.[35]



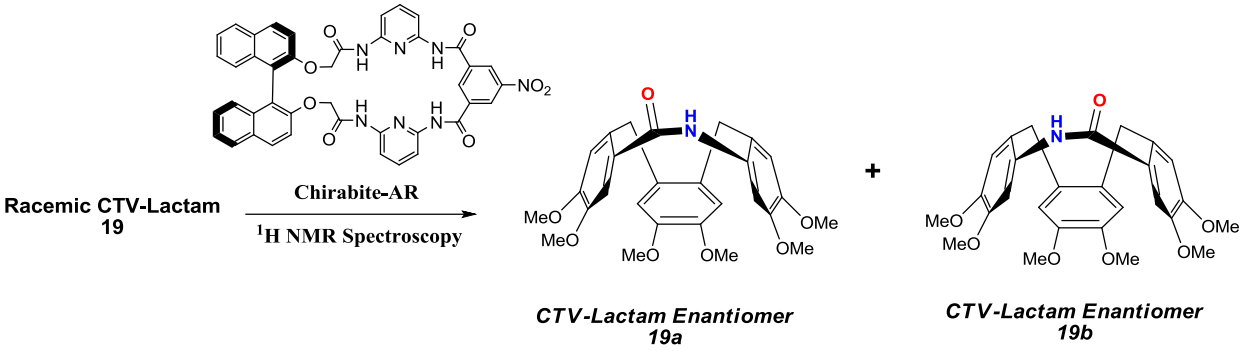
a) b)

Figure 14. DFT energy minimized structures for a) crown conformer, b) saddle conformer

Given that lactam **19** exists as a racemate in the crown form, attempting to observe the two enantiomeric lactam components was evaluated using ^{1}H NMR and Chirabite-AR as illustrated in Scheme

11.Chirabite-AR is a macrocyclic compound that structurally has a unique cavity where the hydrogen-bond donor and acceptor sites are well organized to facilitate the binding of a wide range of smaller compounds as guest molecules. Once the guest molecules interact with Chirabite-AR, the guest molecule will experience a strong anisotropic ring-current effect arising from the BINOL moiety, which is a chiral source, and which allows the chemical-shift nonequivalence between the two diastereomeric complexes to be distinguished.

Scheme 11. Resolution of racemic macrocyclic lactam **19** using macrocycle chiral shift reagent Chirabite-AR



The racemic macrocyclic lactam **19** was treated with increasing amounts Chirabite in $CDCl_3$ solution and examined by ^{1}H NMR spectroscopy, demonstrating that the individual enantiomers could be baseline separated in the NMR for ultimate optical purity determination. The ratio of Chirabite was increased from 0.01 equivalent to 0.3 equivalents, and separation was already evident with only 0.01 equivalent of Chirabite, but 0.05 equivalent

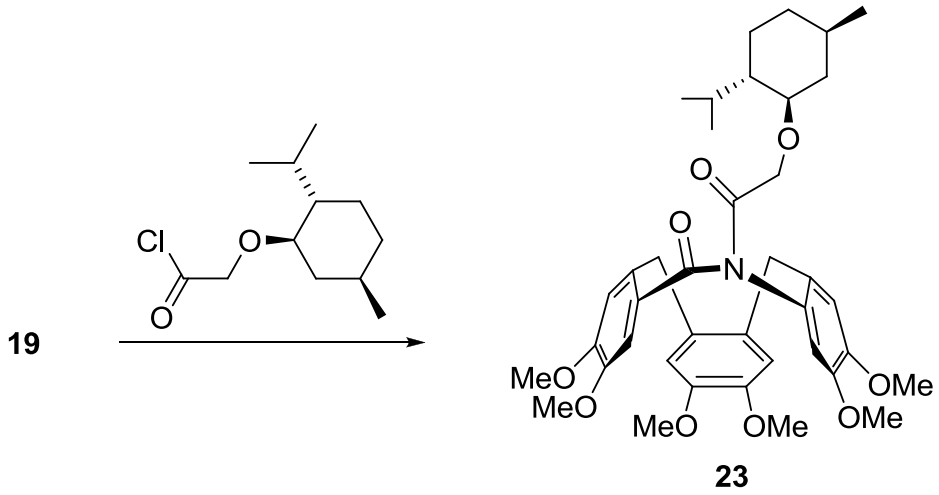
was optimal to provide baseline separation for the proton resonances in the aromatic region of the two enantiomers, while only 0.025 equivalent was sufficient for baseline separation of some methoxy resonances. Additional quantities of Chirabite (> 0.30 eq) were counterproductive, leading to large chemical shift displacements and compromising the ability to assign the peaks. We surmise that Chirabite is interacting most strongly with the lactam carbonyl oxygen, which bears a calculated Mulliken charge of -0.489 au which is the greatest point electron density on the molecule, as expected, and should H-bond accept strongly from an H-bond donor of Chirabite.

Toward resolution of the chiral lactam, analogs of the macrocyclic N-acetyl imide should be resolvable when utilizing chiral acyl substituent in place of the acetyl moiety of **3**, since resolving the macrocyclic lactam **2** into its enantiomers may provide a new and potentially useful chiral scaffold, although admittedly, the cavity of the macrocyclic CTV-lactam or macrocyclic N-acetyl imide is modest in size and the asymmetry of the lactam moiety is on the apical side of the molecule.

Several different chiral auxiliaries were explored for reaction with lactam **2**, including (1S)-(-)-camphanic chloride, (S)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride, diacetyl-L-tartaric anhydride and ketopinic acid chloride, which did not provide N-acylated adducts under a number of conditions explored, including heating in pyridine as solvent.

However, (-)-menthyloxyacetic acid chloride was successful in providing diastereomeric imide adducts in good yield using an excess of menthyloxyacetic acid chloride in pyridine at reflux providing a 69% yield of the desired imide **23** (see Scheme 12).

Scheme 12. Preparation of the diastereomeric menthyloxyacetic acid adduct **23**



23

After purification, the ^{1}H NMR revealed a mixture of diastereomers in an 87:13 ratio. Attempts to separate the diastereomers and improve the diastereomeric purity using recrystallization and achiral column chromatography techniques were unsuccessful. Recrystallization from DCM/hexane provided X-ray quality crystals. In fact, the crystal structure obtained through X-ray crystallography also confirmed the diastereomeric ratio through relative densities and the overall molecular structure including the absolute configuration of the chiral bowl-shaped CTV-imide **23**.

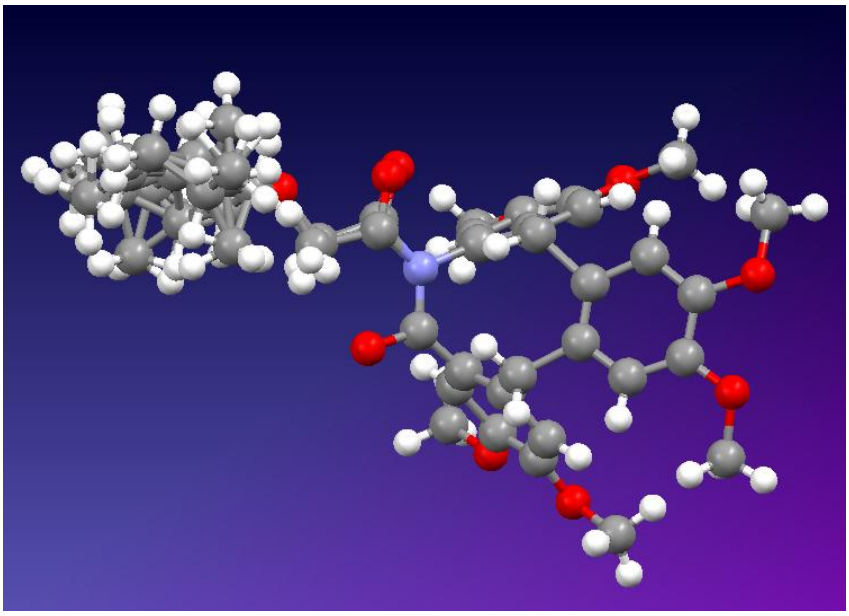


Figure 15. X-ray crystal structure of the menthyloxyacetic acid-CTV imide adduct **23**

Cleaving the chiral auxiliary of the 87:13 mix of diastereomers was accomplished using aqueous LiOH in THF. Optical rotation of the lactam proved to be unsuccessful to determine the rate of racemization from 87:13 to 50:50. We also used $Eu(fod)_3$, a chiral lanthanide shift reagent, to try to show the mix of enantiomers of the chiral CTV lactam in ^1H NMR , but, while the $Eu(fod)_3$ did complex with the lactam, the peaks were too broad to obtain any conclusions. An alternative chiral shift reagent, Chirabite AR, functioned significantly better giving better resolution and complexation with the chiral CTV lactam entities.

Shown below in Figures 16-20 are the overlap of ^{1}H NMR spectra showing resolution of racemic CTV-lactam into two enantiomeric components at different loading equivalents of Chirabite-AR reagent.

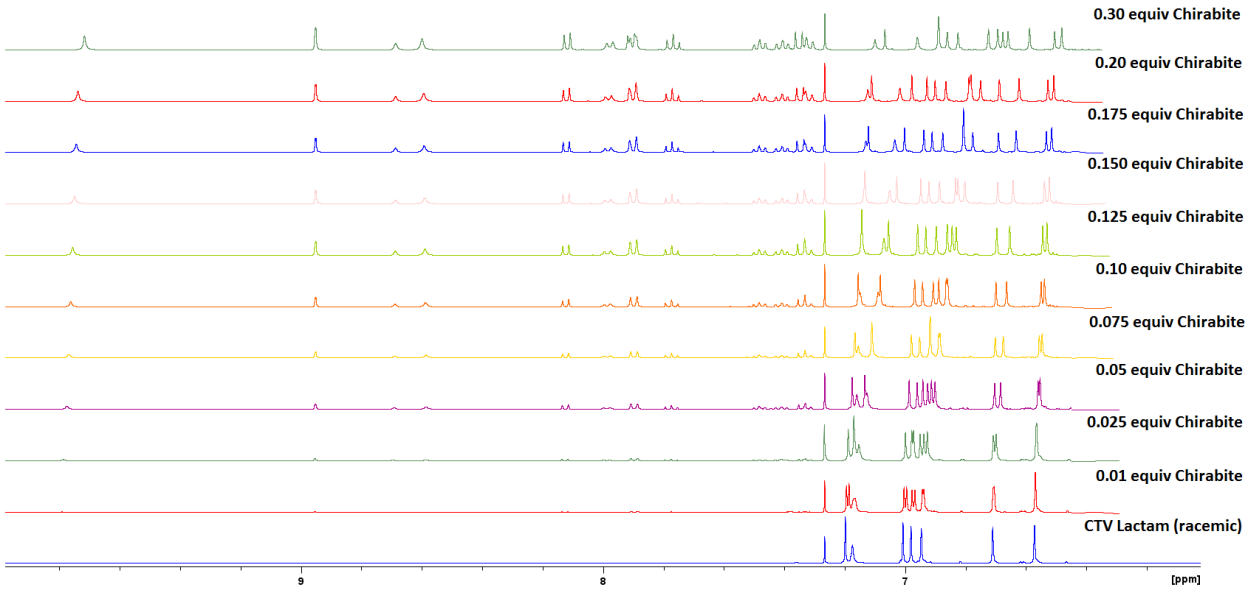


Figure 16. Overlap of the 6-10 ppm region for the ^{1}H NMR Spectrum of CTV-Lactam and different loadings of Chirabite

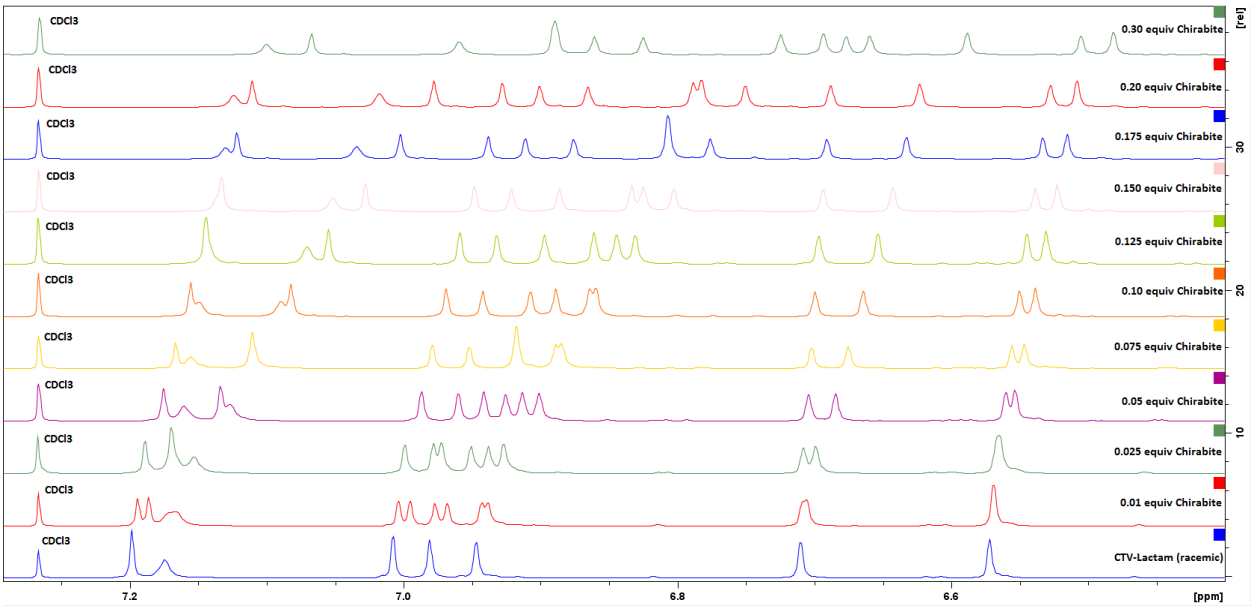


Figure 17. Overlap of the 6.5-7.3 ppm region for the ^{1}H NMR Spectrum of CTV-Lactam and different loadings of Chirabite

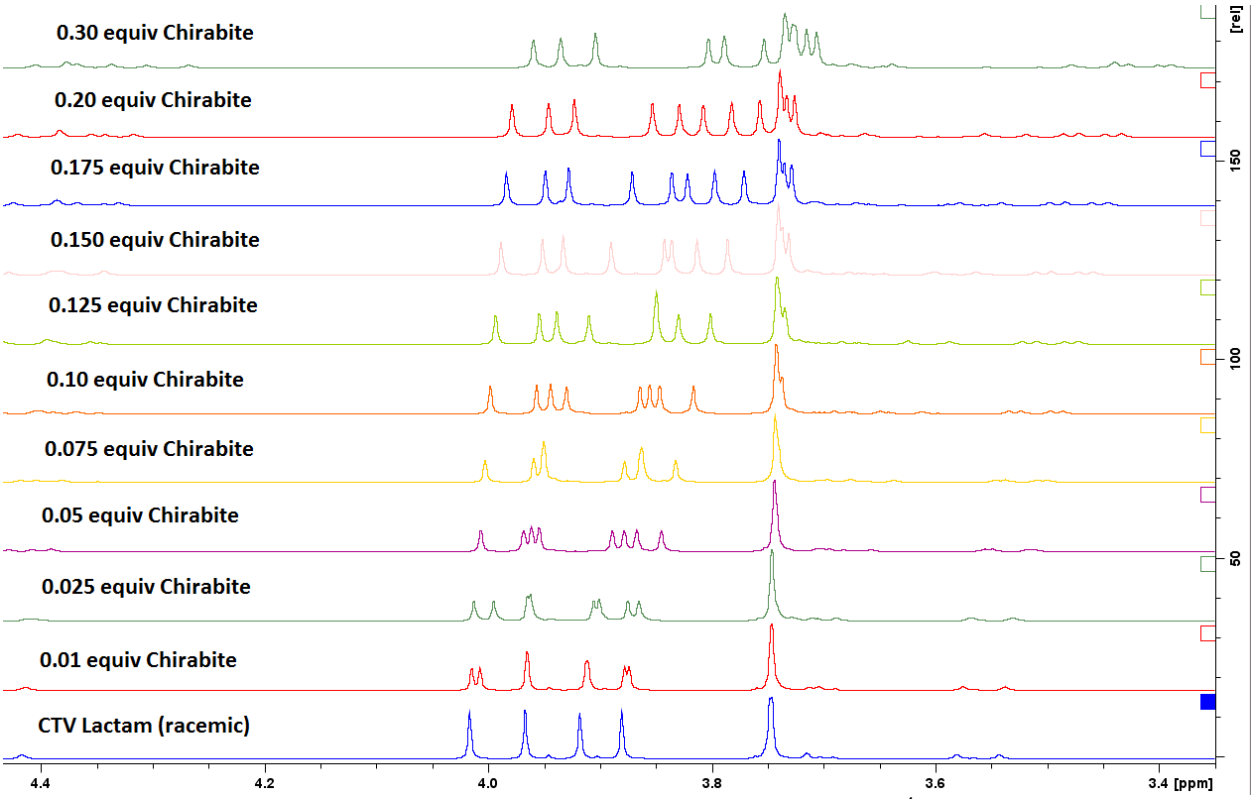


Figure 18. Overlap of the 3.35-4.05 ppm region for the ^{1}H NMR Spectrum of CTV-Lactam and different loading of Chirabite

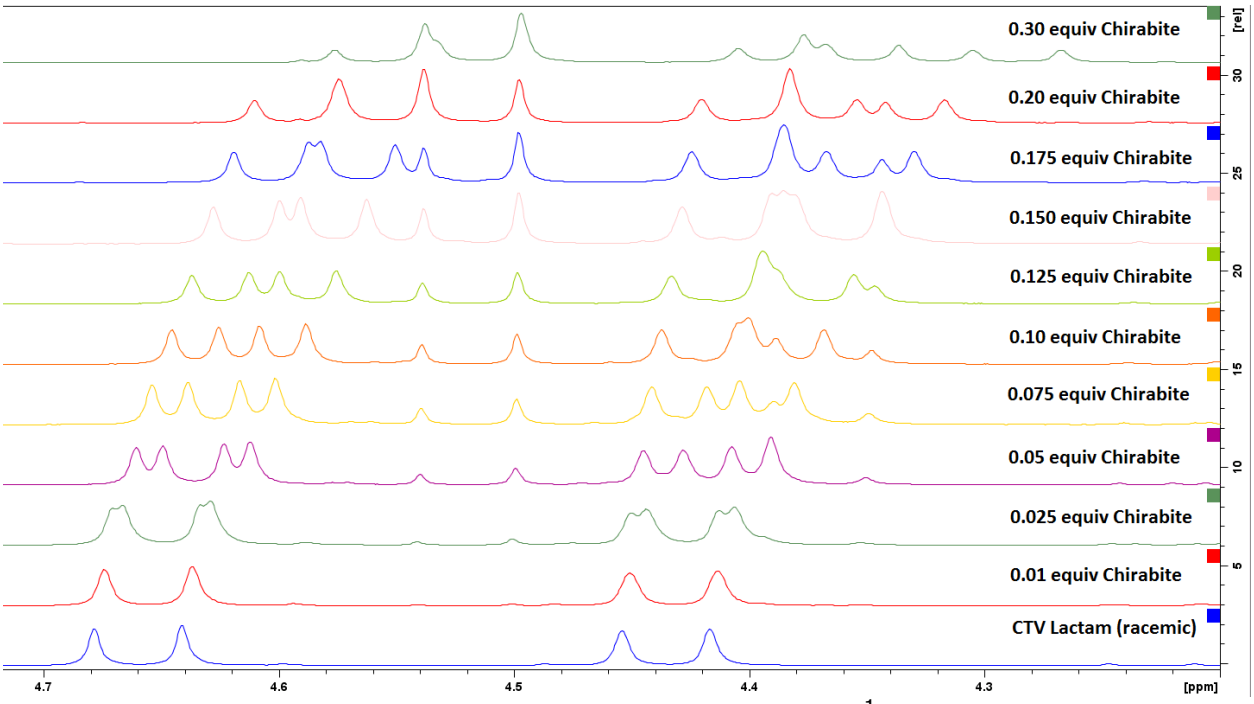


Figure 19. Overlap of the 4.2-4.7 ppm region for the ^{1}H NMR Spectrum of CTV-Lactam and different loading of Chirabite

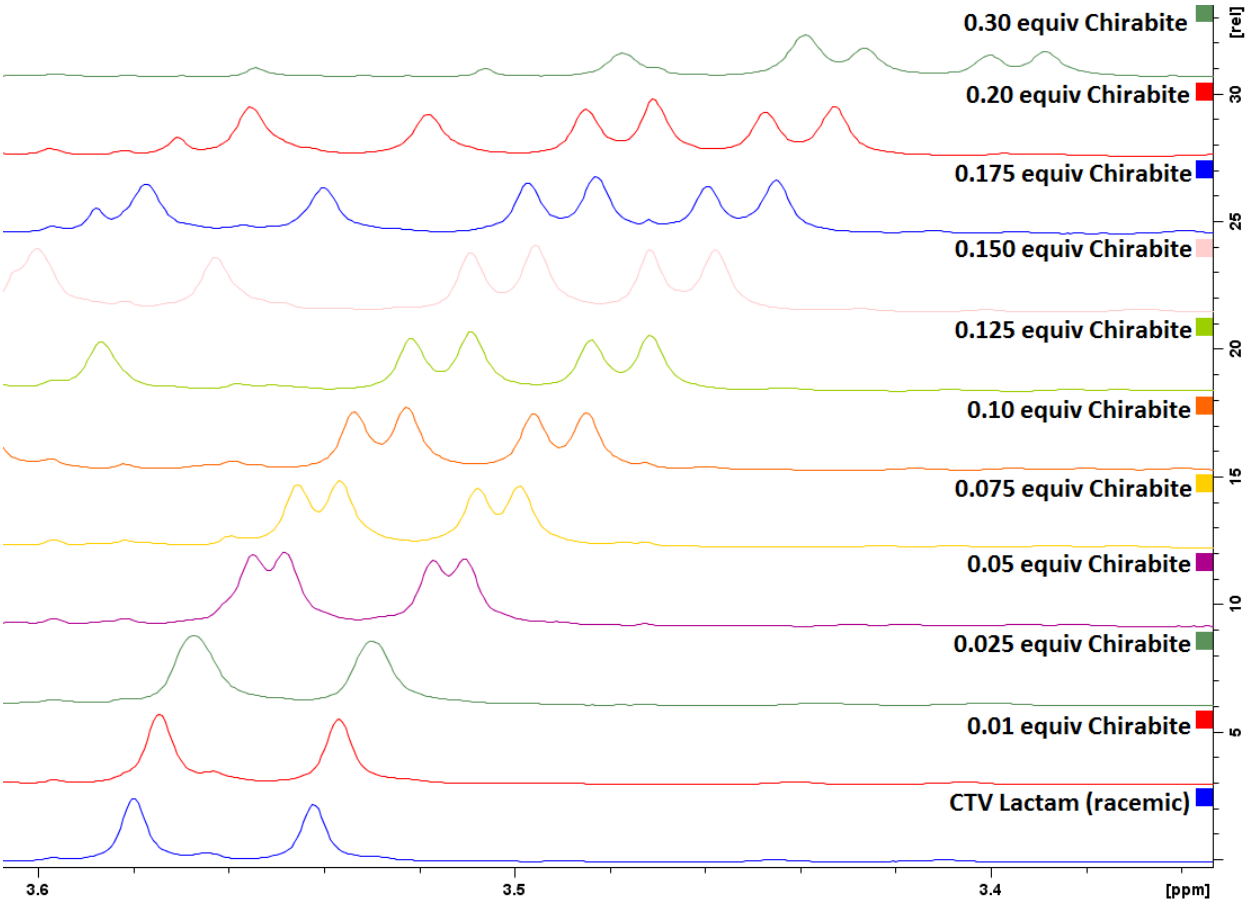


Figure 20. Overlap of the 3.2-3.6 ppm region for the ^{1}H NMR Spectrum of CTV-Lactam and different loading of Chirabite

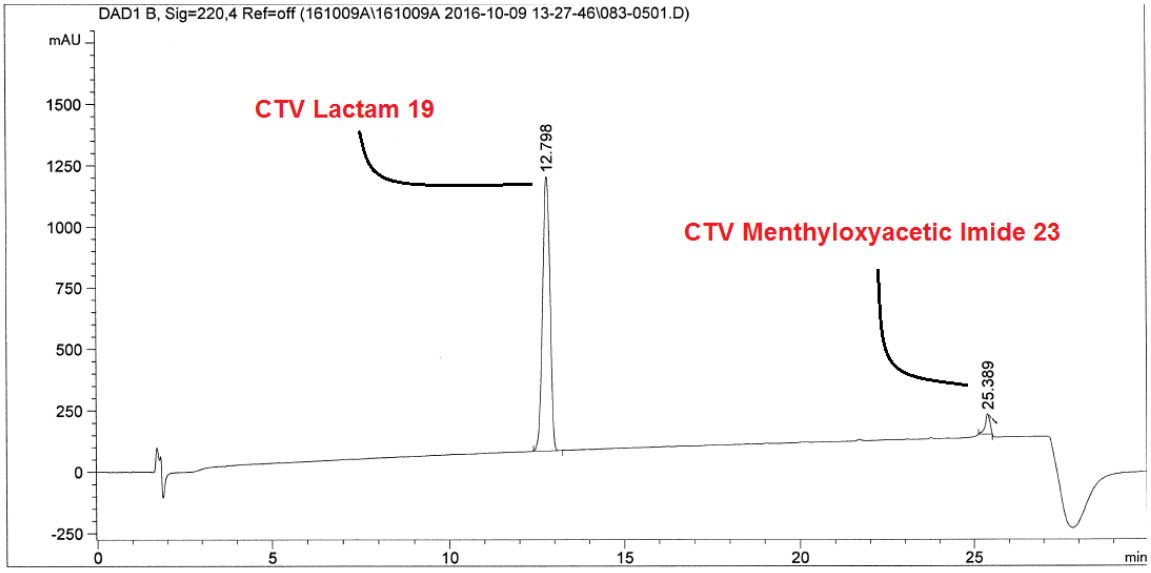
There are multiple regions of the ^{1}H NMR spectra that provide target peaks to track and integrate the enantiomeric CTV-lactam components. Within the aromatic region (6.5-7.3 ppm) shown in Figures 16 and 17, the six aromatic CH peaks all resolved fairly adequately where each of the six peaks resolved into 12 separate resonances when 0.125 equiv of Chirabite-AR was employed. It is even discernible that the lactam NH resonances can be differentiated when 0.30 equiv of Chirabite was utilized. Furthermore, the aromatic CH peak at 6.7 ppm was resolved quite beautifully giving near

baseline separation using very low loading of Chirabite-AR (0.05 equiv). In the methoxy region (3.7-4.05 ppm), nearly all the methoxy moieties from each enantiomeric lactam component were separated into nearly 12 separate peaks especially when 0.175-0.20 equivalents of Chirabite-AR was used as illustrated in Figure 18. Interestingly when focusing on the apex portion of CTV-lactam in Figure 19, the methylene C-H signals can be also distinguished into separate enantiomeric peaks when using 0.05 equivalents of Chirabite. Furthermore, the remaining methylene C-H peaks that resonate at 3.54 and 3.58 ppm also exhibited nearly baseline separation when 0.15-0.20 equivalents of Chirabite-AR was employed (see Figure 20). Furthermore, the NMR resonances for Chirabite-AR did not overlap with any of the peaks for CTV-lactam due to the fact that most of the Chirabite resonances occurred downfield of 7.27 ppm where there are no peaks relating to CTV-lactam **19a-b**.

Another potential analysis tool to determine chiral purity is the ability to do chiral separation by supercritical fluid chromatography (SFC) or chiral HPLC. Chiral screening on SFC and HPLC chiral stationary phases were evaluated but unfortunately there was not any success in resolving the enantiomers of CTV-lactam **19**. Based on the fact that ^{1}H NMR spectroscopy provided excellent results in distinguishing enantiomeric peaks of racemic CTV lactam **19**, the next objective was to determine if the enriched CTV

menthoxyacetic imide **23** can be hydrolyzed to release the chiral auxiliary (menthyloxyacetyl) and in parallel use Chirabite-AR to observe a 87:13 enantiomeric ratio of the resolved CTV lactam.

Before performing the hydrolysis, the time required for complete hydrolysis was determined by HPLC and it was confirmed that the hydrolysis reaction is completed (> 95% conversion) within 15 min and verified that the cleaving the chiral auxiliary group from **23** (retention time at 25.4 min) was accomplished giving CTV lactam **19** (retention time at 12.8 min). See below the HPLC chromatogram in Figure 21.



Area Percent Report

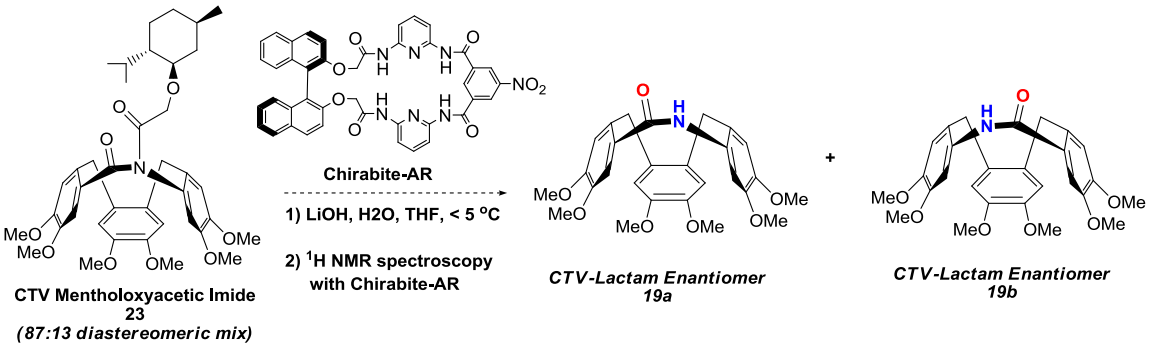
Signal 1: DAD1 B, Sig=220,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.798	BB	0.2369	1.65539e4	1116.71362	95.5610
2	25.389	MM	0.1544	768.95526	83.00005	4.4390

Figure 21 - HPLC Chromatogram illustrating the hydrolysis of Chiral Auxiliary Group on **23** with LiOH in aq THF

As illustrated in Scheme 13, the hydrolysis of chiral auxiliary was carried out on enriched CTV menthyloxyacetic imide **23** in aqueous THF using lithium hydroxide (3.5 eq) at < 5 ^{o} C for 15 min, then the reaction mixture was sampled, quickly concentrated, redissolved in $CDCl_3$ containing Chirabite-AR (0.30 equiv), and finally analyzed by ^{1}H NMR.

Scheme 13. Hydrolysis of the enriched diastereomeric menthyloxyacetic imide and subsequent enantiomeric purity assessment using Chirabite-AR



Shown below in Figure 22 and 23 is the data from ^{1}H NMR analysis post cleavage of the menthyloxyacetic group (shown in green), and the data showed that the CTV lactam product **19** had racemized to a 50:50 mixture based on the two sets of peaks (6.51/6.53 and 6.63/6.69 ppm) that integrated with the same area under the curve. In regards to Figure 23, it is evident that the set of peaks of interest to monitor enantiomeric purity are at 6.63/6.69 ppm and 6.51/6.53 ppm as indicated by the black circles. These black circles also illustrate which peaks corresponds to the singlet peaks in racemic CTV lactam as denoted in (A). These results suggest that once the chiral auxiliary is cleaved that the nitrogen umbrella inversion is

quite rapid furnishing a racemate. In more detail regarding Figure 23, there are two explanations of why the chemical shifts were different for (B) and (D). First explanation is there was an excess of hydroxide used in (B) that likely reduced the potency of Chirabite used (0.30 eq), therefore the actual amount of Chirabite used in the experiment is < 0.30 eq. Therefore, referring to Figure 17 where different loading equivalents of Chirabite was studied, the ^{1}H NMR spectrum profile that best fit to (B) was when 0.175 eq Chirabite was employed thus indicating that there was about 0.175 eq of Chirabite present in (B). The second explanation is that hydroxide is competing with CTV lactam and the lactam moieties of the Chirabite scaffold, since lactams are an H-bond acceptor, and hydroxide is an H-bond acceptor as well.

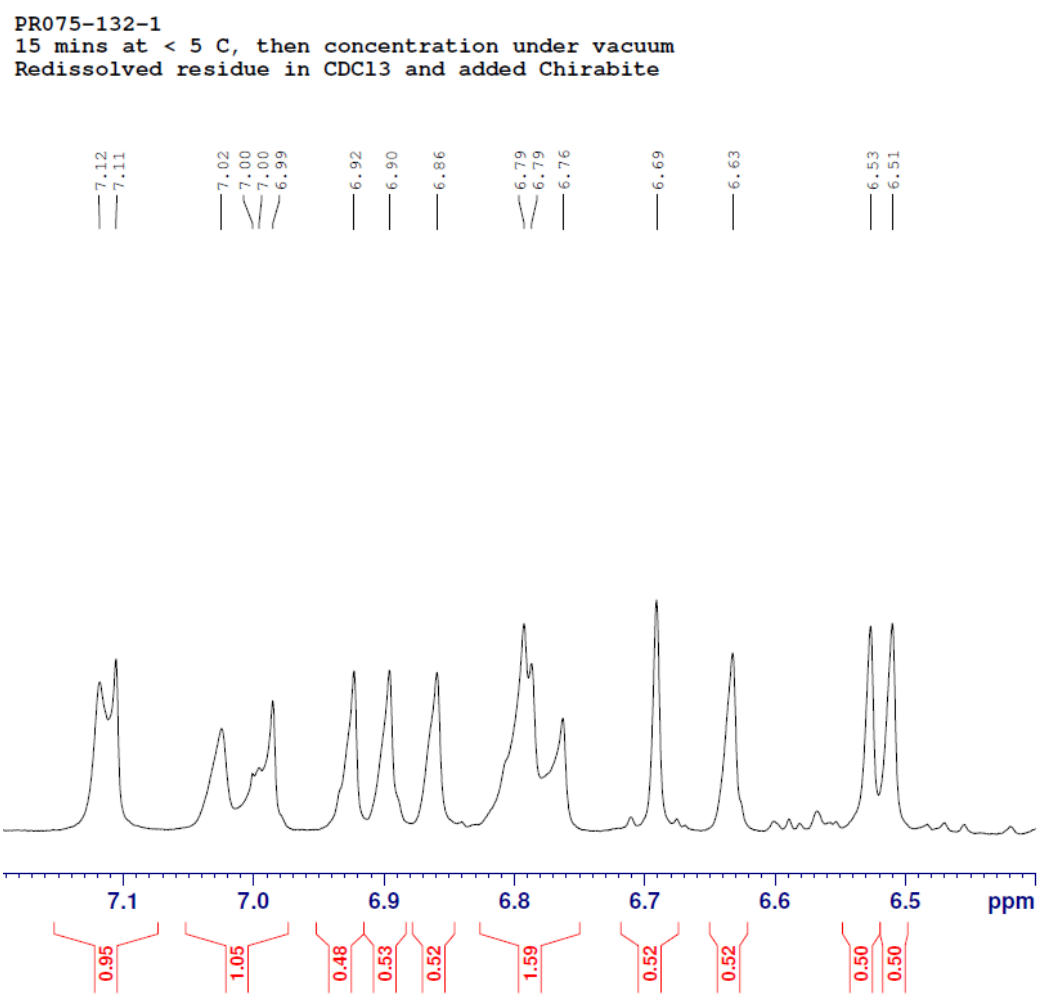
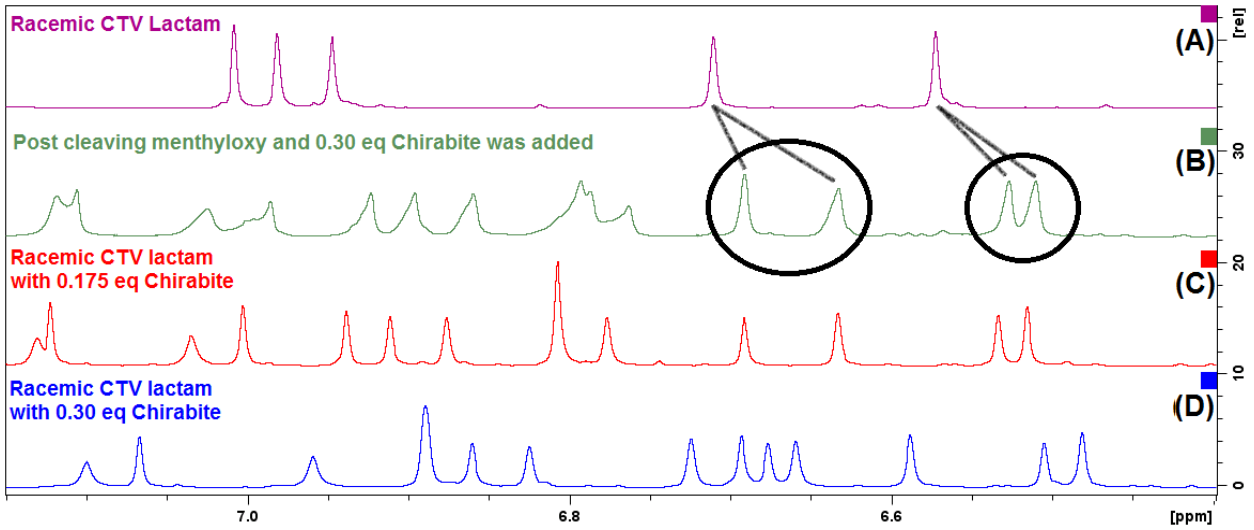


Figure 22.[1]H NMR spectrum of hydrolyzed **23** in the presence of Chirabite showing the integrated peaks that indicate racemization



Note. **A** (purple) shows racemic lactam without Chirabite showing singlets. **B** (green) shows the ^{1}H NMR spectrum of crude reaction mixture (post cleavage of chiral auxiliary group, menthyloxyacetate) containing Chirabite (0.30 eq) and excess hydroxide that shows a 1:1 ratio of singlets revealing sadly racemization of lactam. **C** (red) illustrates the enantiomeric separation of peaks for Racemic CTV Lactam in the presence of 0.175 eq Chirabite which the profile closely resembles **B** likely due to that excess hydroxide is reducing the potency of Chirabite. **D** (blue) illustrates the enantiomeric separation of peaks for racemic lactam using Chirabite (0.30 eq) affording two separated singlets at 6.51 and 6.53 ppm.

Figure 23. Post hydrolysis of **23** and using Chirabite-AR to determine enantiomeric purity of resolved lactam **19**

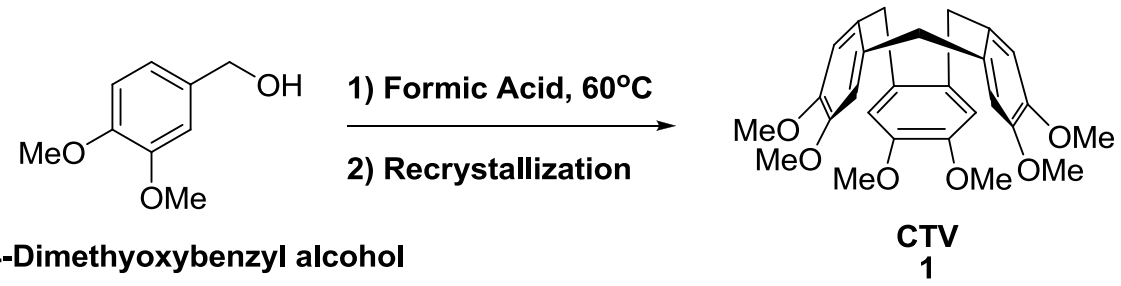
In addition to ^{1}H NMR spectroscopy, optical rotation was attempted to study if the enriched diastereomeric CTV mentholoxyacetic imide **23** could be cleaved such that the chiral purity of the resolved CTV lactam could be determined. However, the data generated provided inconclusive results since the byproduct of the reaction (mentholoxyacetic acid) is chiral. Attempts to run a standard sample of (-)-menthyloxyacetic acid in the same solvent medium did not provide satisfactory results and this analytical approach was no longer pursued.

Section 9: Conclusion

Within this work, the previously-reported processes to prepare and isolate specific CTV analogs (monoketone **2**, oxime **15/16**, lactam **19**) were improved, which allowed the sequential steps to be carried out more efficiently. We have shown that racemic CTV lactam **19** can be kinetically driven to an enriched diastereomer **23** by reaction of lactam racemate with (-)-menthyloxyacetyl chloride affording an 87:13 mixture of diastereomers that was confirmed by ^{1}H NMR and X-ray analysis. Furthermore, for the first time, we report that Chirabite-AR can be utilized employing substoichiometric amounts on a racemic macrocycle providing successful observation of the two macrocylic lactam enantiomers **19a-b** with nearly baseline separation and resolution. Basic hydrolysis of the 87:13 diastereomeric imide **23**, however, proceeded with rapid bowl inversion to yield only racemic lactam **19** as confirmed by ^{1}H NMR in the presence of Chirabite-AR.

Section 10: Experimental

Cyclotriveratrylene (CTV, 1, hexamethoxy-tribenzocyclononene)



3,4-Dimethyoxybenzyl alcohol

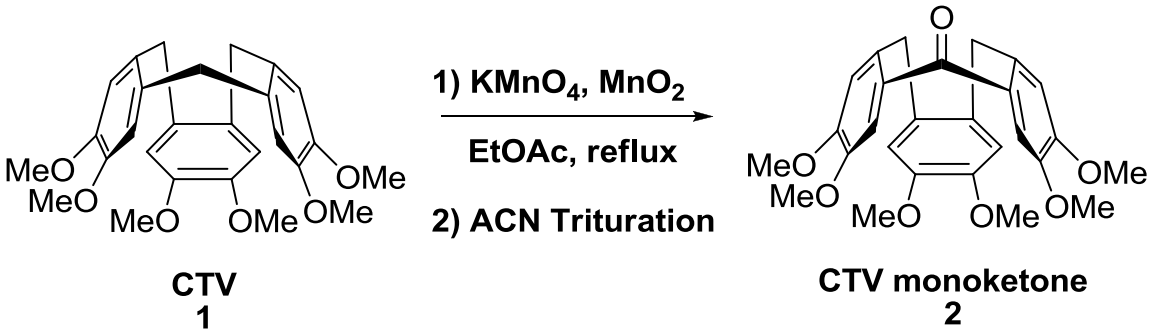
CTV
1

To a 5-liter glass reactor (3-necked), equipped with an overhead mechanical stirrer, J-Kem thermocouple, heating mantle, reflux condenser, and nitrogen inlet, was added 3,4-dimethoxybenzyl alcohol (201.7 g, 1.20 moles). The mixture was heated to 60°C followed by addition of formic acid (99%, 3 liters) using a dropping funnel over a period of 15 min which the temperature had decreased 40°C. The resulting mixture was a white slurry. The slurry was reheated to 60°C and held at 60°C for an additional 3 h to give a light blue slurry. The slurry was cooled to room temperature and USP purified water (2 liters) was added. The slurry was cooled to < 10°C and held at < 10°C overnight. The following day (17 h later), the cold slurry was filtered over a fritted funnel and the wetcake was washed with USP purified water (4 x 1 liter), pulled dry, and further dried *in vacuo* at 75°C to provide crude CTV (133.51 g, white solids).

Recrystallization of crude CTV

To a 5-liter glass reactor (3-necked), equipped with an overhead mechanical stirrer, J-Kem thermocouple, heating mantle, reflux condenser, and nitrogen inlet, was charged crude CTV (132.7 g) and toluene (2.9 L). The slurry was heated to reflux (102.8°C) to give a slight hazy solution. While at reflux, chloroform (100 mL) and methanol (50 mL) was added slowly over 10 min to provide a homogeneous pale yellow solution. The solution was allowed to gradually cool to room temperature. After 3.5 h later the temperature was 25°C. The white slurry was filtered over a fritted funnel and the wetcake was washed with ice cold toluene (2 x 100 mL), pulled dry, and further dried *in vacuo* at 40 °C to provide purified CTV **1** (82.57 g, 46.2% yield, white solids). HPLC (220 nm): 98.3%. The ^{1}H NMR data is in accordance with that reported in the literature.[69, 70]

Improved Procedure for the Synthesis of CTV monoketone (10,15-dihydro-2,3,7,8,12,13-hexamethoxy-5H Tribenzo[a,d,g]cyclononen-5-one, 2)



CTV
1

CTV monoketone
2

To a 5-liter glass reactor (3-necked), equipped with an overhead mechanical stirrer, J-Kem thermocouple, heating mantle, reflux condenser, and nitrogen inlet was charged in the following order: CTV **1** (76.22 g, 0.17 moles, 18 eq), ethyl acetate (2 L), activated manganese dioxide (264.7 g, 3.05 moles), ground potassium permanganate (241.1 g, 1.53 moles, 9 eq) and lastly ethyl acetate (0.29 L) as a rinse. The reaction was allowed to stir at room temperature to ensure that there was no exotherm. The mixture was then heated to reflux and stirred overnight. After 18 h, TLC and HPLC analysis indicated that the reaction was deemed complete. Note: the reaction solution was colorless (once solids settled) indicating that all KMnO4 had reacted. The reaction mixture was cooled to 50°C, filtered over a pad of Celite (70.6 g), and Celite Cake was rinsed with ethyl acetate (3 x 500 mL) and DCM (2 x 500 mL). The filtrate was concentrated under reduced pressure at 40-50°C to give crude monoketone **(**61.91 g, pale yellow solids).

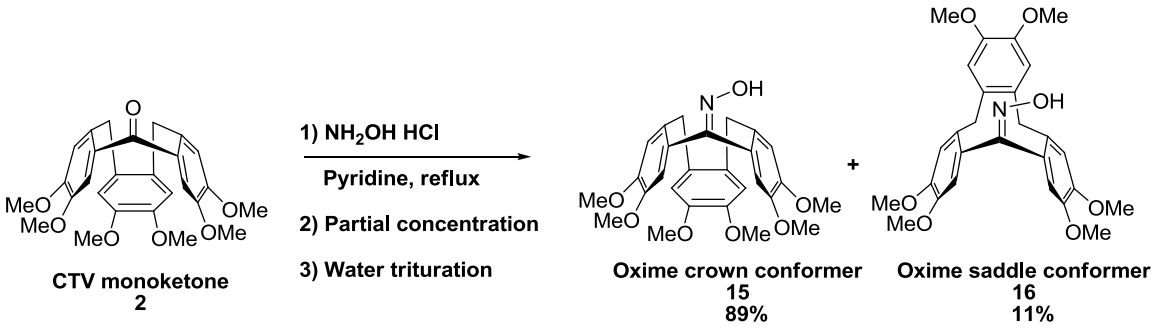
Purification of crude CTV Monoketone

Crude monoketone (51.00 g) was placed into a 250-mL glass reactor (2-necked), equipped with a large magnetic stir bar, J-Kem thermocouple, heating mantle, and reflux condenser. To the reactor was added acetonitrile (150 mL, HPLC grade). Heated mixture to 70 °C and allowed slurry to stir (250-300 rpm) at 70°C for 18 h. The slurry mixture was allowed to gradually cool to room temperature over 4 h, then continued to stir at room

temperature for approximately 3 h. The slurry was filtered over a fitted funnel, the wetcake was washed with acetonitrile (60 mL), pulled dry, and further dried *in vacuo* at 60°C overnight to give purified CTV monoketone **2** (32.54 g white solids, 65% yield).

The mother liquor was partially concentrated to remove about 75% of the solvent to provide a slurry. The slurry was filtered and the wetcake was washed with ethyl acetate/heptane (50/50, 10 mL) and dried to give a second crop (15.8 g, 31% yield, white solids) of purified CTV-monoketone **2**. Total recovery = 96%. Total yield: 74% yield (corrected). The ^{1}H NMR data is in accordance with that reported in the literature.[35]

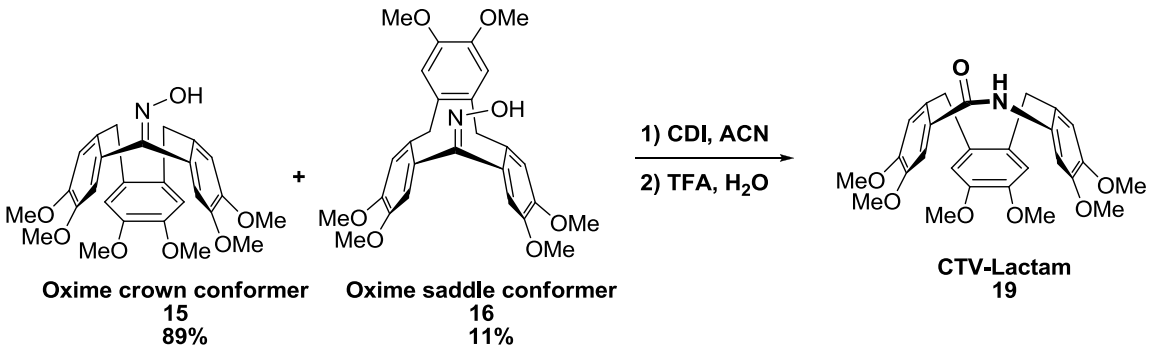
10,15-Dihydro-2,3,7,8,12,13-hexamethoxy-5H-tribenzo[a,d,g]cyclononen-5-oxime,CTV Oxime Crown (15) and CTV Oxime Saddle (16)



To a 500-mL glass reactor (3-necked), equipped with a magnetic stir bar, reflux condenser, J-Kem thermocouple, and nitrogen inlet, was charged CTV monoketone **2** (19.00 g, 40.91 mmol), hydroxylamine hydrochloride

(42.64 g, 613.6 mmol, 15.0 eq), and pyridine (190 mL). The mixture was heated to reflux (110 °C) under a nitrogen atmosphere. After 16.5 h, the heat was turned off and the reactor was sampled for TLC analysis (EtOAc/DCM, 20/80) and showed that the reaction was deemed complete. The reaction mixture was transferred to a 500 mL flask (1 neck) and partially concentrated at 60°C under reduced pressure to provide a crude residue. To the crude residue was added USP purified water (275 mL) and the mixture was allowed to triturate at room temperature for about 30 min. The slurry was filtered, and the wetcake was washed with USP purified water (2 x 50 mL), pulled dry, and further dried *in vacuo* at 50 °C overnight to provide a pure mixture of CTV-oximes (saddle/crown, 17.63 g, white solids, 90% yield). The filtrate was extracted with ethyl acetate (3 x 75 mL). The combined organic extracts were washed with 1N HCl (1 x 50 mL), brine (75 mL), dried with sodium sulfate, filtered, and concentrated under reduced pressure to provide an additional quantity of CTV-oximes (2.29 g). HPLC analysis showed a total purity of 96% for both oxime conformers which were a ratio of 89:11 (crown:saddle). The ^{1}H NMR data is in accordance with that reported in the literature.[35]

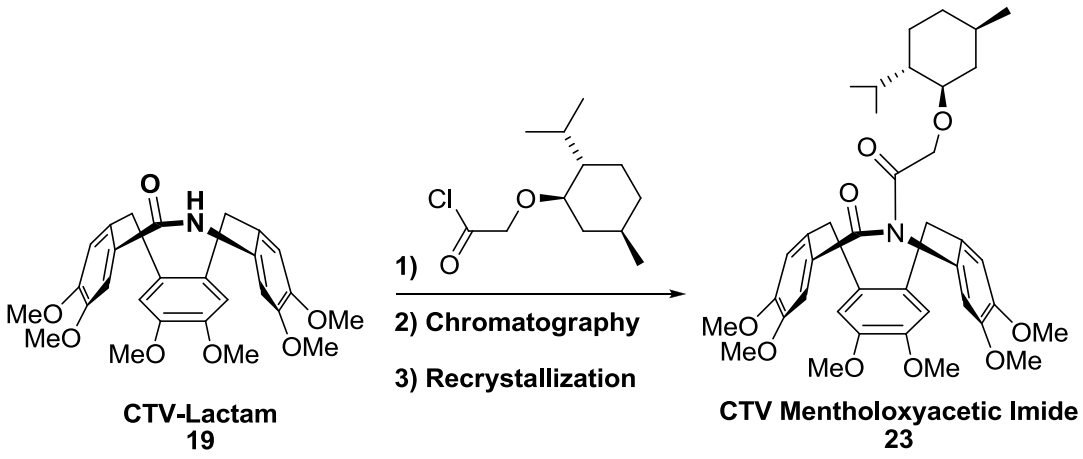
Improved Procedure for the Synthesis of CTV-lactam (19) (2,3,8,9,13,14-hexamethoxy-11,16-dihydrotribenzo[b,e,h]azecin-6(5H)-one)



Based on a similar procedure,[36] this process was modified and improved. To a 1-liter round bottom flask (3-necked), equipped with an overhead mechanical stirrer, nitrogen inlet, and J-Kem thermocouple, was added CTV oxime (10.00 g) and acetonitrile (200 mL). The mixture was allowed to stir at ambient temperature for 20 min, then carbonyldiimidazole (CDI, 7.18 g) was added. Additional acetonitrile (20 mL) was used to rinse contents off the reactor flask walls. After 1 hour at room temperature, TLC analysis (EtOAc/DCM, 80/20) revealed that the oxime starting material was completely consumed and a new spot appeared (oxime O-acyl imidazole intermediate **22**, R_f = 0.10). The reaction mixture was cooled to < 5^{o}C and de-ionized water (50 mL) was added followed by drop-wise addition of trifluoroacetic acid (TFA, 50 mL) over 20 min using a dropping funnel. After 20 h at room temperature, TLC analysis (EtOAc/DCM, 80/20) showed

complete conversion of acyl imidazole intermediate to CTV-lactam **19** (Rf = 0.18). To the reaction mixture was added de-ionized water (600 mL) drop-wise over 30 min providing an opaque pink slurry. The slurry was allowed to stir for 20 h and filtered. The wetcake was washed with water (50 mL), pulled dry, and further dried *in vacuo* at 50 ^{o}C to provide CTV-lactam (8.13 g, 82% yield). HPLC purity (220 nm): 95.2%. The ^{1}H NMR data is in accordance with that reported in the literature.[36]

5-[1,2,3,4-Tetrahydro-1-[[[(1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexyl]oxy]acetyl]-]acetyl-11,16-dihydro-2,3,8,9,13,14-hexamethoxy-tribenz[b,e,h]azecin-6(5H)-one (**23)**



CTV-Lactam 19

CTV Mentholoxyacetic Imide 23

Preparation Method 1:

To a solution of CTV lactam **19**(175 mg, 0.36 mmol) in pyridine (1.8 mL) was added (-)-menthyloxyacetic acid chloride (913 mg, 3.92 mmol) at room temperature and the solution was heated to reflux for 2 h. The mixture was then concentrated under reduced pressure, and the resulting

residue was diluted in dichloromethane and poured onto ice. The layers were separated, and the aqueous layer was extracted two additional times with dichloromethane. The combined organic layers were washed successively with 1N hydrochloric acid (2x), saturated aqueous sodium bicarbonate, distilled water, brine, dried over sodium sulfate and concentrated to afford the crude product as a solid (999 mg). The mixture was passed through neutral alumina (17 g) eluting with ethyl acetate/dichloromethane (20/80) to remove excess menthyloxyacetic acid, followed by elution of the cyclotriveratrylene lactam starting material that was recovered (40 mg, 25% recovered). The remaining material (477 mg) eluted from the alumina was chromatographed on silica gel (24 g) eluting with a gradient from pure dichloromethane to ethyl acetate/dichloromethane (50/50) to afford the desired imide **23** (167 mg, 69%) as a pale yellow solid. The product was crystallized using DCM and hexane yielding an 87:13 mixture of diastereomers: mp 200-203 ^{o}C; $[\alpha]_D$ = -218 ^{o} (c = 2.4 g/100 mL); **IR** 2998.6, 2953.5, 2927.6, 2868.3, 1723.0, 1696.3, 1608.0 , 1517.9, 1463.1 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 6.87 (bs, 2H), 6.71 (bs, 0.12H), 6.69 (bs, 0.11H), 6.68 (bs, 0.11H), 6.67 (bs, 0.13H), 6.60 (bs, 0.22H), 6.59-6.52 (complex, 3.2H), 6.47 (bs, 0.13H), 6.46 (bs, 0.13H), 6.39 (bs, 0.45H), 6.38 (bs, 0.55H), 5.07 (bs, 0.19H), 5.03 (bs, 0.26H), 4.98-4.84 (m, 0.87H), 4.80 (bs, 0.27H), 4.76 (0.20H), 4.47 (bs, 0.03H), 4.42 (bs,

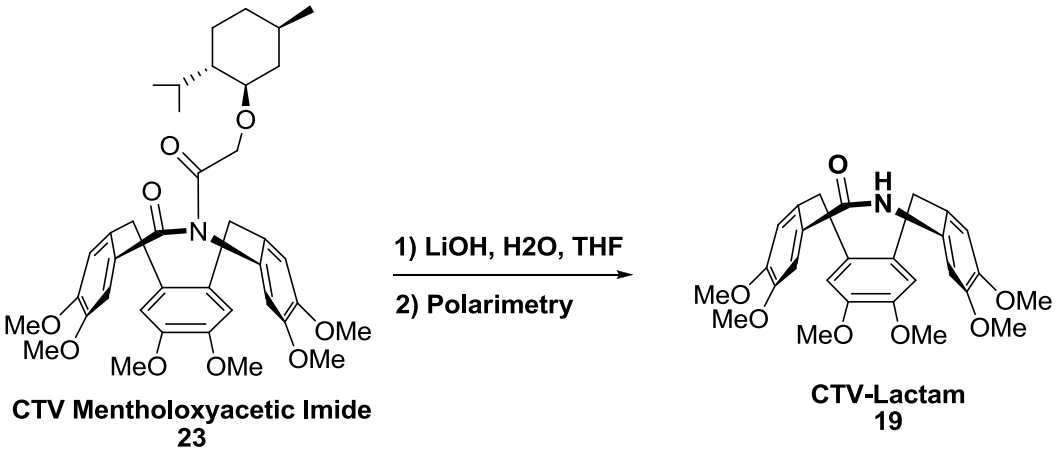
0.04H), 4.37 (bs, 0.04H), 4.32 (bs, 0.31H), 4.29 (bs, 0.28H), 4.26 (bs, 0.24H), 4.23 (0.26H), 4.16 (bs, 0.04H), 4.13-4.11 (complex, 0.27H), 4.10-4.05 (complex, 0.45H), 4.04-4.02 (complex, 0.52H), 4.01-3.97 (complex, 0.43H), 3.95-3.91 (complex, 6.2H), 3.90 (bd, 1H), 3.87 (bd, 1H), 3.83 (bs, 1H), 3.81 (bs, 0.43H), 3.80-3.78 (complex, 1.2H), 3.74 (bd, 3.2H), 3.73 (bs, 3.2H), 3.71-3.60 (complex, 6.4H), 3.62-3.58 (complex, 1H), 3.55 (bd, 1H), 3.49 (bs, 0.30H), 3.45 (bd, 0.54H), 3.42 (bs, 0.28H), 3.33-3.27 (td, 1.1H), 3.20-3.07 (complex, 0.32H), 2.41-2.24 (complex, 1.35H), 2.25-2.19 (complex, 1.23H), 2.12-2.00 (complex, 0.55H), 1.68-1.58 (complex, 5.4H), 1.46-1.18 (complex, 8H), 1.08-0.71 (complex, 22.2H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ 16.1, 16.2, 16.3, 20.9, 21.0, 21.1, 21.9, 22.1, 22.3, 23.2, 23.3, 25.4, 25.5, 25.6, 29.6, 31.5, 33.8, 33.9, 34.2, 34.4, 34.5, 35.7, 35.8, 40.0, 40.2, 48.2, 48.3, 55.5, 55.6, 55.7, 55.8, 55.9, 56.0, 56.1, 56.2, 70.9, 71.2, 76.6, 77.0, 77.2, 77.4, 80.1, 80.5, 80.6, 109.2, 109.4, 111.2, 111.3, 111.5, 111.6, 111.7, 111.9, 112.1, 112.2, 112.5, 113.6, 114.2, 114.3, 115.4, 127.5, 128.1, 128.8, 129.9, 130.0, 130.5, 130.8, 131.0, 131.1, 131.3, 146.8, 147.4, 147.7, 147.8, 148.3, 148.8, 149.6, 173.4, 174.4 **MS**: Calculated for $C_{39}H_{50}NO_9^+$ [M+H]: m/z 676.3, found 676.3.

Preparation Method 2:

To a solution of CTV lactam **19** (400 mg, 0.83 mmol) in pyridine (4.1 mL) was added commercially available (-)-menthyloxyacetic acid chloride

(0.75 mL, 0.78 g, 3.35 mmol) at room temperature and the solution was heated to 80 ^{o}C for 2 h. The mixture was then concentrated under reduced pressure to give a crude residue. The residue was diluted in ethyl acetate (30 mL), washed with 1N HCl (20 mL), 5% aqueous $NaHCO_3$ (2 x 20 mL), saturated brine solution (20 mL), dried with sodium sulfate, filtered, and concentrated under reduced pressure to give crude product (1.20 g) as a dark yellow glass solid. The crude product was dissolved in DCM (20 mL)/triethylamine (0.5 mL) and silica gel (2.1 g, 60-200 micron) was added and the resulting mixture was concentrated to dryness to provide crude product adsorbed onto silica gel which was loaded into a solid loading cartridge (60 g). Purification was accomplished on a Teledyne Isco Rf200 unit using a 40 g RediSep Rf Gold silica gel cartridge column and an ethyl acetate/n-heptane gradient containing 0.5% triethylamine to afford the desired imide **23** (205 mg, 36% yield). The product was recrystallized using DCM/heptane or ethyl acetate affording an 87:13 ratio of diastereomers.

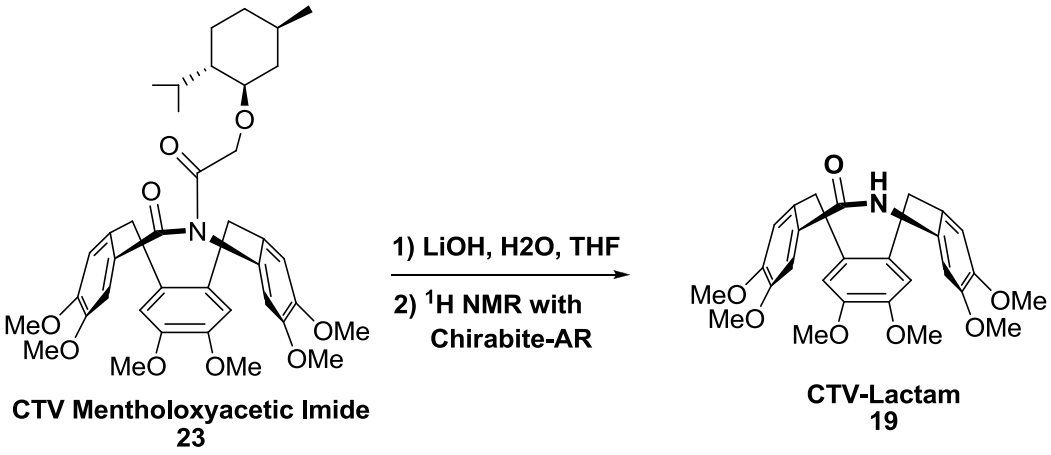
Hydrolysis of Imide 23 and the use of Optical Rotation to determine resolution of CTV-Lactam:



CTV Lactam 19 from hydrolysis of the imide23

To enriched CTV-menthyloxyacetic imide **23** (24 mg, 0.037 mmol), at 0 °C in THF/water (3:1, v/v, 0.75 mL), was added lithium hydroxide (3 mg, 0.911 mmol) and stirred for 15 min. The mixture was extracted with dichloromethane (3 x 20 mL). The combined organic layers were washed with distilled water (10 mL), brine (10 mL), filtered over sodium sulfate, and concentrated under reduced pressure to afford a solid (24 mg). The product was filtered through alumina eluting with ethyl acetate/dichloromethane (20/80) to isolate CTV lactam **19** (17 mg); $[\alpha]_D = 0^o$ (3.4g/100mL).

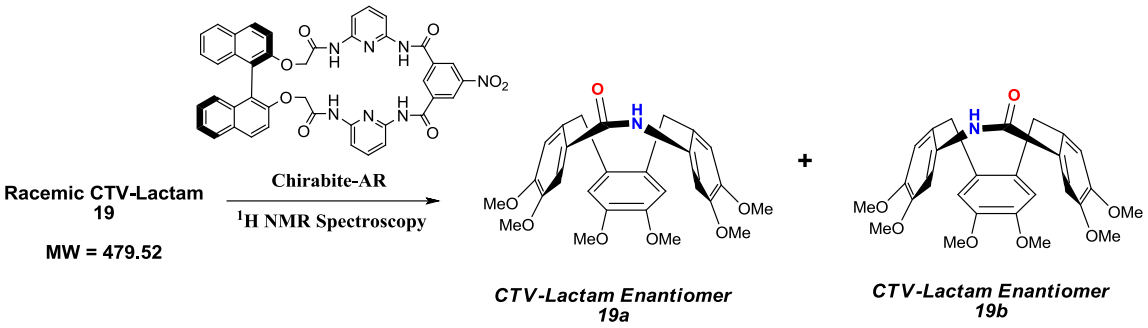
Hydrolysis of Imide and the use of Chirabite-AR to determine resolution of CTV-Lactam:



CTV Lactam 19 from hydrolysis of the imide23

To enriched CTV-menthyloxyacetic imide **23** (13.2 mg, 0.019 mmoles) was dissolved in THF (0.75 mL) and then cooled to 0-5 °C using an ice-water bath. A solution of 0.24 M lithium hydroxide monohydrate (0.275 mL, 0.068 mmol, 3.5 eq) was added. The mixture was allowed to stir at 0-5 °C for 15 min, then the reaction mixture was transferred to a 20 mL scintillation vial and concentrated under high vacuum for 5 min. The residue was dissolved in $CDCl_3$ (0.75 mL) followed by addition of 0.10 M Chirabite-AR solution in $CDCl_3$ (60 µL, 0.006 mmol, 0.3 equiv.) and this mixture was placed in a NMR tube. Two min later, NMR analysis started. ^{1}H NMR analysis showed that the resolution was lost and the mixture was a racemate based on that the two peaks at 6.51 and 6.53 ppm were 1:1 by integration and peak height.

Chirabite-AR Study with Racemic CTV-Lactam



To racemic CTV-lactam **19** (20 mg, 0.042 mmoles) was added $CDCl_3$ (1.0 mL) to provide a solution and this solution was transferred to a 400 MHz tube. A solution of 0.10 M Chirabite-AR in $CDCl_3$ was prepared by mixing Chirabite-AR (38 mg, 0.05 mmoles) in $CDCl_3$ (0.5 mL).

Below in Table 4 denotes the following equivalents of Chirabite-AR solution added to the NMR tube. The equivalents of Chirabite-AR that were evaluated were 0.01, 0.025, 0.050, 0.075, 0.10, 0.125, 0.150, 0.175, 0.20, and 0.30 equiv. After addition of the specified amount of Chirabite solution into the NMR sample containing racemic CTV-lactam, the sample was placed into the NMR spectrometer, analyzed, then proceeded to the next charge of Chirabite solution.

Table 4. Loading of Chirabite-AR Solution

Loading of Chirabite-AR Solution (0.10 M in $CDCl_3$)	MW	Loading equiv.	Total Volume *u*L	Vol to be added portion-wise to NMR Sample	mmole
Chirabite-AR Solution in $CDCl_3$	759.72	0.010	4.2	4.2	0.0004
Chirabite-AR Solution in $CDCl_3$	759.72	0.025	10.4	6.3	0.0010
Chirabite-AR Solution in $CDCl_3$	759.72	0.050	20.9	10.4	0.0021
Chirabite-AR Solution in $CDCl_3$	759.72	0.075	31.3	10.4	0.0031
Chirabite-AR Solution in $CDCl_3$	759.72	0.100	41.7	10.4	0.0042
Chirabite-AR Solution in $CDCl_3$	759.72	0.125	52.1	10.4	0.0052
Chirabite-AR Solution in $CDCl_3$	759.72	0.150	62.6	10.4	0.0063
Chirabite-AR Solution in $CDCl_3$	759.72	0.175	73.0	10.4	0.0073
Chirabite-AR Solution in $CDCl_3$	759.72	0.200	83.4	10.4	0.0083
Chirabite-AR Solution in $CDCl_3$	759.72	0.300	125.1	41.7	0.0125

CHAPTER THREE

CARBORANE HYDROXAMATE MMP INHIBITORS FOR TREATMENT OF CANCER AND RHEUMATOID ARTHRITIS EMPLOYING BNCT

Section 1: Introduction

Antimicrobial therapy has saved millions of lives over the past 80 years, yet our arsenal of effective antibiotics is increasingly diminished by the alarming rise of bacteria that are resistant to all currently available antibiotics.[1] Over two million people annually in the United States acquire infections that are resistant to antibiotics, and at least 23,000 people die as a result, according to are port issued by the Centers for Disease Control and Prevention.[2] In the U.S., antibiotic resistance adds $20 billion in additional direct health care costs, with lost productivity as high as an additional $35 billion annually.[2]

There is an urgent need for antibacterial agents with new cellular mechanisms of action, yet the pharmaceutical industry shows little interest in antibiotic R&D due to the high cost of development and the typically short-term treatment regimen of antibiotics which is considered to be less profitable than the treatment of chronic diseases.[3]

The sharp increase in mortality and morbidity due to rising bacterial infections caused by antibiotic-resistant bacteria[4] underlines the need to discover and identify previously-unexplored enzymes as novel antibiotic targets with the goal of developing new molecular leads. For example, invasive methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious and growing health problem.[5] Several newly discovered strains of MRSA show antibiotic resistance even to vancomycin, which has been considered for decades as the last line of defense for the treatment of systemic infections.[6]

An attractive bacterial target is the *dapE*-encoded N-succinyl-L,L-diaminopimelic acid desuccinylase (DapE, E.C. 3.5.1.18),[7] which is a member of the lysine biosynthetic pathway in bacteria (see Figure 24) that provides lysine and *meso*-diaminopimelate (m-DAP),[8] both of which are essential for peptidoglycan cell-wall synthesis.

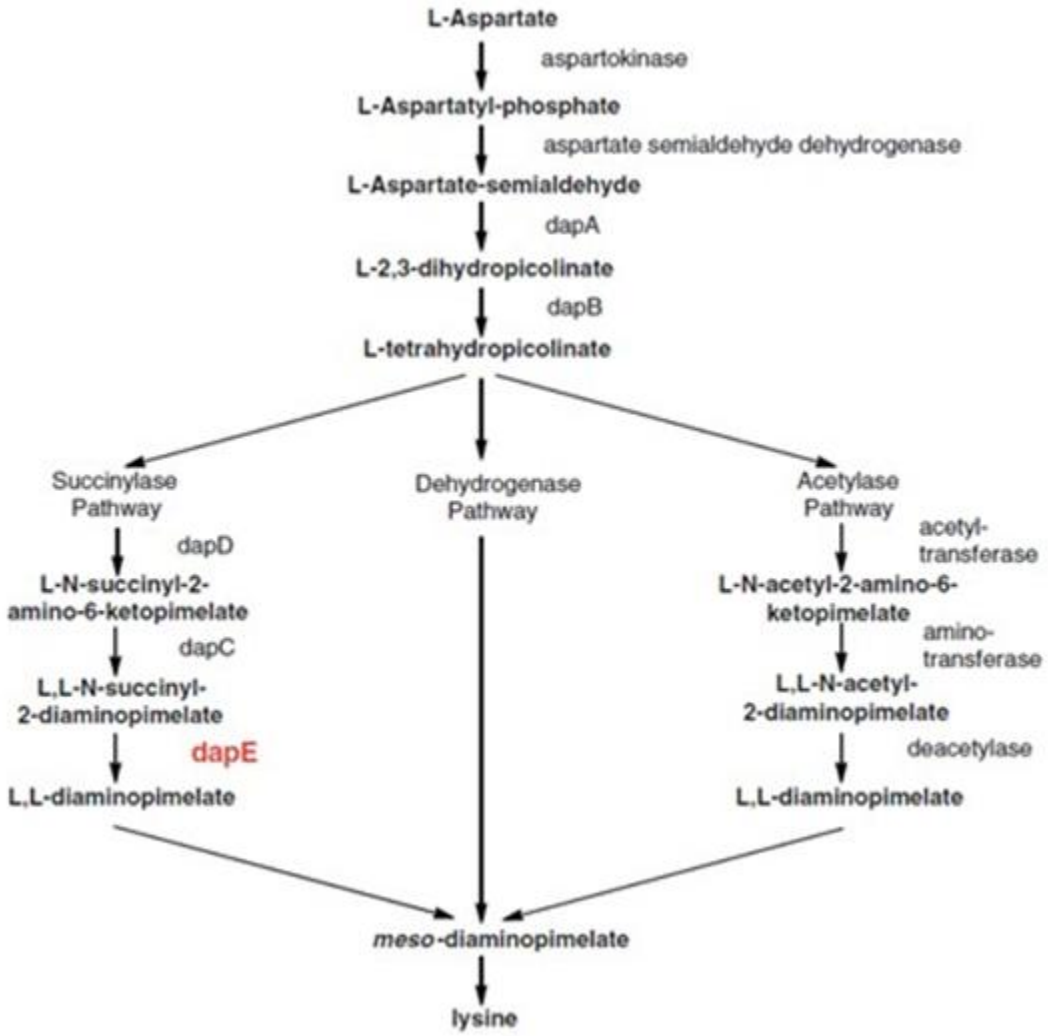
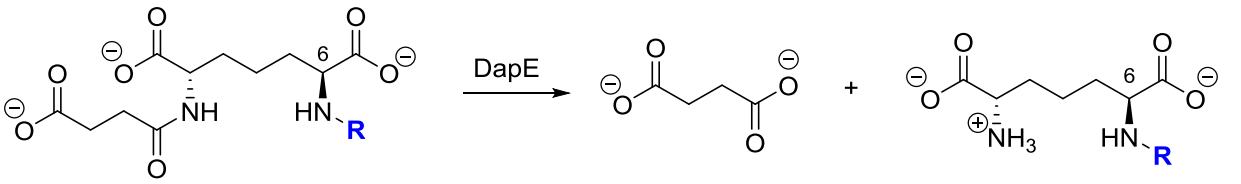


Figure 24. Biosynthetic pathways producing mDAP and lysine in bacteria

As illustrated in Figure 25, DapE catalyzes the hydrolysis of N-succinyl-L,L-diaminopimelic acid (L,L-SDAP) to succinate and L,L-diaminopimelic acid (L,L-DAP), and is ultimately responsible for synthesizing lysine for bacterial peptide synthesis, and for providing both mDAP and lysine for peptidoglycan cell wall formation in both Gram-negative and most Gram-positive bacteria.[8] Deletion of the DapE gene is lethal to *Helicobacter pylori* and *Mycobacterium*

smegmatis, demonstrating the indispensable role of this bacterial enzyme in pathogens.[9,10] Furthermore, lack of a similar pathway in humans suggests that inhibition of DapE may be selectively toxic to bacteria but not to human hosts, eliminating the concern of mechanism-based toxicity and making DapE a very promising target for the development of antibiotics with a new mechanism of action.[7]



1a R = H L,L-SDAP
1b R = CH_3 N^6-Me-L,L-SDAP
1c R = Ac N^6-Ac-SDAP

3a R = H L,L-DAP
3b R = CH_3 N^6-Me-L,L-DAP
3c R = Ac N^6-Ac-L,L-DAP

Note. L,L-SDAP (**1a**) and analogs N^6-methyl SDAP (**1b**), and N^6-acetyl-SDAP (**1c**) with formation of hydrolysis products succinate (**2**) and L,L-diaminopimelic acid derivatives (**3a-c**). Hydrolysis was not observed for N-acetyl analog **1c**

Figure 25. Hydrolysis of L,L-SDAP and analogs by *Hi*DapE

Previously, it was shown that DapE isozymes exhibit >60% of their maximal activity towards L,L-SDAP when only one Zn(II) ion is present in its active site,[11] with the tighter binding site confirmed by high-resolution X-ray crystallography. These structures obtained from X-ray crystallography have enabled further refinement of a mechanistic hypothesis of amide bond cleavage by DapE enzymes, which have facilitated inhibitor identification.[12]A small, focused screen of compounds containing zinc-binding groups

identified the thiol-containing ACE inhibitor captopril as a low micromolar competitive inhibitor (IC_{50} = 3.3 μM) of the DapE from *Haemophilus influenza* (*Hi*DapE) along with several other small molecule inhibitors including 3-mercaptobenzoic acid (IC_{50} = 35 μM), phenylboronic acid (IC_{50} = 107 μM), and 2-thiopheneboronic acid (IC_{50} = 92 μM).[13] A high-resolution (1.8 Å) X-ray crystal structure of captopril bound to the DapE from *Neisseria meningitidis* (*Nm*DapE) revealed a thiolate-bridged dinuclear Zn(II) active site providing a model for *in silico* approaches to identifying potential inhibitors of DapEs.[14]

The most widely used DapE assay monitors amide bond cleavage of L,L-SDAP spectrophotometrically at 225 nm.[13] While this assay is simple and reliable for simple molecules, it cannot be used to test potential inhibitors that absorb strongly in the UV region, precluding its use in testing many of the preferred medicinal chemistry leads and analogs. While several assays have been developed for evaluating inhibitors of DapEs, these assays are technically troublesome and/or difficult to reproduce.[15] For example, Gelb *et al*.[16] exploited the fact that L,L-DAP reacts somewhat faster with ninhydrin than L,L-SDAP; however, this assay suffers from poor reproducibility. Two alternative assays were also reported, the first of which employed ^{14}C-labeled L,L-SDAP while the second was a coupled assay that utilizes porcine succinate thiokinase and inositol triphosphate to convert

liberated succinate to succinyl-CoA (CoA = coenzyme A) and inositol diphosphate. The inositol diphosphate was then detected by its reaction with phosphoenolpyruvate to yield liberated pyruvate, itself being detected spectrophotometrically using lactate dehydrogenase. The ^{14}C-labeled substrate assay requires working with radioactivity, necessitating extra safety protocols and waste disposal issues, while the coupled assay is cumbersome and expensive while also being technically difficult and therefore challenging to reproduce. Moreover, none of these assays is amenable to high-throughput screening studies, a necessity for the discovery of new lead compounds with antibacterial properties.

Recognizing the ease and reliability of ninhydrin-based assays to detect primary amino groups, such as that formed upon the hydrolysis of L,L-SDAP, and the importance of the N^6-amino group of L,L-SDAP,[17] we synthesized L,L-SDAP derivatives with partially blocked free amino groups to prevent ninhydrin side reactions. N^6-Acetylated, N^6-methylated, and N^6,N^6-dimethyl derivatives of L,L-SDAP were investigated and prioritized using molecular docking and modeling. Based on these data, a new enzymatic assay for *Hi*DapE is described using N^6-methylated L,L-SDAP, which we have shown is simple, robust, and amenable to high-throughput screening. These data open the door to medicinal chemistry efforts toward the discovery of *Hi*DapE inhibitors that can function as a new class of antibiotics.

Section 2: Existing Synthetic Preparations of 2,6-Diaminopimelic Acid (DAP)

The 2,6-diaminopimelic acid (DAP) analogs are relatively difficult to synthesize by conventional routes, due to the fact that the spacing of the groups on synthetic intermediates tends to favor intramolecular ring closure to form a piperidine system. For instance, one can envision the possible synthesis of 2,6-diaminopimelic acid from either 2,6-dibromopimelic acid or 2,6-dibromopimelate ester in the presence of ammonia or some other amine, however once one molecule of ammonia attacks one of the carbons bearing the bromo group, the resulting primary amine has the opportunity to undergo an intramolecular ring closure attacking the other carbon bearing the bromo substituent which generates a 2,6-disubstituted piperidine derivative rather than the seven-carbon linear chain structure that bears the equally spaced amino groups.[18-23]

In 1964, a patent was awarded to Dow Chemical[24] for a synthesis of racemic DAP starting with either glutaraldehyde or 2,6-dihydroxypimelonitrile to furnish trimethylene-1,3-bis(5-hydratoin). The process of forming the bis-hydantoin starting from glutaraldehyde requires the use of sodium sulfite (2.1 eq) and sodium cyanide (2.04 eq) in water which, after extractions with diethyl ether, provides 2,6-dihydroxypimelonitrile in 90% yield, which is then converted to a bis-

hydantoin using a pressure bomb reactor at 85 ^{o}C in the presence of ammonium carbonate and 14N ammonium hydroxide. Subsequent reaction of the intermediate hydantoin under high temperature acidic or alkaline hydrolytic conditions (48% HBr or 50% NaOH at 175-185 ^{o}C in a stainless steel bomb) hydrolyzed the hydantoin intermediate to a mixture of diastereomers of 2,6-diaminopimelic acid which then required purification on ion-exchange resin and recrystallization from ethanol/water to access the DAP as the bis-ammonium salt in 37% yield. This procedure is most likely the commercial route for preparing racemic DAP; however, this route in an academic setting is not ideal due to the use of cyanide, pressure bombs, and elevated temperature of volatile mixtures (acidic/alkaline) which are serious safety concerns leaving many researchers little choice except to purchase synthetic DAP. However, it still raises concerns for individual working in manufacturing who are assigned to carry out this process.

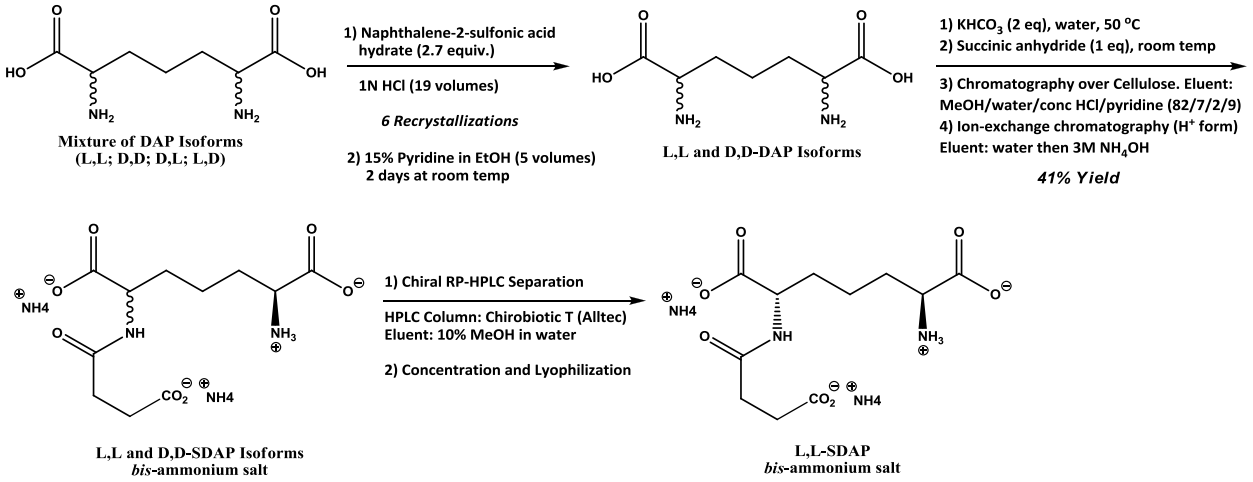
Developing a method to synthesize sufficient quantities of DAP and SDAP materials for the ninhydrin-based assay developed by Dr. Tahirah Heath at Loyola University Chicago was a challenging process that would have required the use of synthetic processes that were generally low yielding and inefficient if only making use of the known methodology. Alternatively, L,L-DAP can be purchased at significantly higher costs and associated lead times. We needed the synthetic flexibility to produce specific derivatives of

L,L-DAP and L,L-SDAP that were not possible from the commercial material. Thus, there was an urgent need to develop a safer, scalable and more flexible process for the asymmetric synthesis of DAP, SDAP, and related analogues.

Section 3: SDAP Route Comparisons and Improvements

Previous synthetic routes of L,L-N-succinyl-diaminopimelic acid (L,L-SDAP) were generally tedious and non-stereoselective involving multiple purifications that ultimately made the synthesis a very low throughput process. Typically, one needed to start with D,L-diaminopimelic acid (DAP) isoforms and perform multiple recrystallizations using naphthalene- β -sulfonic acid[25]to produce a 1:1 mixture of L,L and D,D-DAP isoforms. The general approach for preparation of diastereomerically pure L,L-SDAP consisted of a laborious and time consuming process as illustrated in Scheme 14 below.

Scheme 14. General Preparation of L,L-SDAP



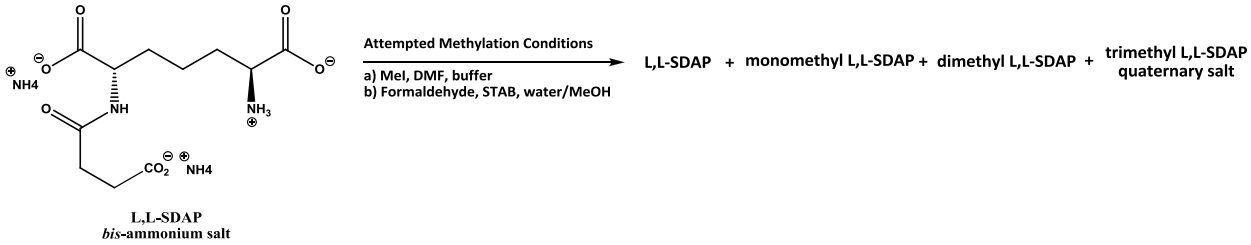
This process of preparing L,L-SDAP required separation of D,D and L,L-isoforms from the D,L/L,D isoforms through a classical recrystallization method using naphthalene-β-sulfonic acid[25,26] to form sparingly-soluble ammonium salts in 1N HCl. To ensure that the D,L/L,D-DAP isoforms are removed, in excess of six recrystallizations from 1N HCl were required, with no indication in the literature of percent recovery after each recrystallization.[26] The subsequent step required conversion to the free base of the resolved D,D/L,L-isoforms using 15% pyridine in ethanol for 2 days at room temperature releasing naphthalene-β-sulfonic acid. Installation of the succinyl group on D,D/L,L-DAP then required the method of Lin *et al*[15] which involved treatment of D,D/L,L-DAP with potassium bicarbonate (2.2 eq) in water in the presence of succinic anhydride (1.01 equiv) and, after overnight at room temperature, the reaction mixture was adjusted to pH 2 with dilute

hydrochloric acid, concentrated, and loaded onto a purification column packed with microcrystalline cellulose that was eluted with a quaternary solvent system (methanol/water/concentrated HCl/pyridine). After purification over cellulose, the corresponding fractions were analyzed using TLC on cellulose plates, and pure D,D/L,L-SDAP fractions were then pooled and concentrated. The resulting concentrate was further purified by ion-exchange chromatography using H^+ form resin to eliminate residual succinate as well as pyridinum hydrochloride. After ion-exchange purification, a yield of 41% was reported which provided 1.1 g of DL-SDAP ammonium salt. The ion-exchange purification required about 1 liter of water and 3M ammonium hydroxide to flush the product from the resin. After this step, the final step still required reverse-phase chiral HPLC on an expensive chiral HPLC column (Chirobiotic T column, Alltec) to separate the D,D and L,L-SDAP isomers. It is evident that this process for achieving diastereomerically pure L,L-SDAP is highly inefficient and exhaustive due to issues involving multiple recrystallizations of nosylate salts (naphthalene-sulfonic/DAP salts), purification over cellulose, low-throughput purification over ion-exchange resin, and finally extremely low throughput and a time-consuming separation to isolate the L,L-isoform followed by concentration of aqueous streams to isolate desired L,L-SDAP isoform *via* chiral reverse phase HPLC.

Section 4: Development of Routes to Access L,L-DAP, L,L-SDAP, N^6-Monomethyl-SDAP, and N^6,N^6-Dimethyl-SDAP as Potential DapE Substrates

Previous attempts in our lab to prepare monomethyl and dimethyl-SDAP involved the direct methylation of racemic SDAP or L,L-SDAP, which produced an inseparable mixture of unreacted starting material, monomethyl, dimethyl, and quaternary ammonium salt as illustrated below in Scheme 15. Depending on the conditions, methylation of the carboxyl moieties was also of concern since formation of methyl ester(s) are possible. Reductive amination of racemic SDAP was difficult since isolation of the monomethyl or dimethyl products was troublesome due to high water solubility of the products, combined with the need for isolation from inorganic salts. Based on these synthetic difficulties for preparing monomethyl SDAP, alternative routes were outlined to develop a scalable process.

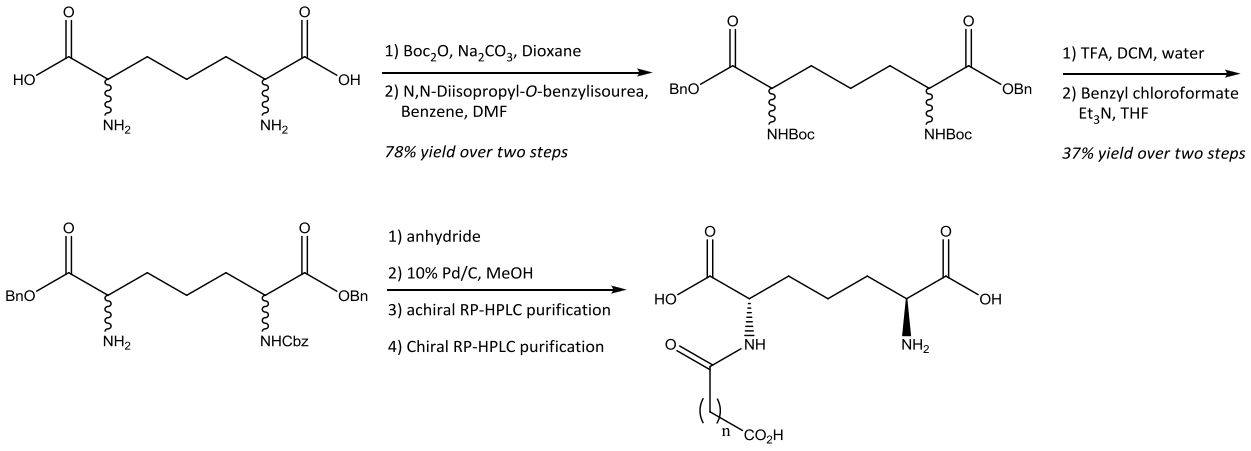
Scheme 15. Attempts to form Monomethyl and Dimethyl SDAP



During literature searching, it was observed that many syntheses focused on the synthetic preparation of diaminopimelic acid (DAP) or acylated derivatives.[27] Hlavácek demonstrated that racemic DAP (*R,S*)-2,6-diaminopimelic acid) can be selectively protected which required the free amino group to be protected with a Boc group and the carboxyl groups protected as benzyl esters using *N*,*N´*-diisopropyl-*O*-benzylisourea (Scheme 16). This fully protected material is much more soluble in many organic solvents compared to its parent, unprotected structure (diaminopimelic acid) which has poor organic solubility and requires polar solvents (such as water, methanol, DMF, or DMSO) to ensure material is dissolved and allow chemical transformations to occur more readily in a homogeneous manner. The Boc moieties were removed under acidic conditions followed by mono-protection of one of the free amino groups using Cbz-Cl giving a 37% yield over the two steps of Boc deprotection and carbamate formation after extensive purification. At this point, the mono-acylated free amino component was then acylated with an anhydride, hydrogenated to cleave the benzyl groups, and then purified by achiral reverse-phase HPLC followed by chiral reverse phase HPLC to afford limited quantities of pure L,L-SDAP with high diastereomeric purity. This method offered some advantages, however the use of racemic DAP did not seem appealing since reverse-phase chromatography is typically a low-throughput method, and higher quantities

were required for the ninhydrin assay. Furthermore, the mono-protection step using Cbz-Cl is low yielding, and based on the expense of purchasing DAP, this route would not be useful in preparing sufficient quantities of monomethylated SDAP.

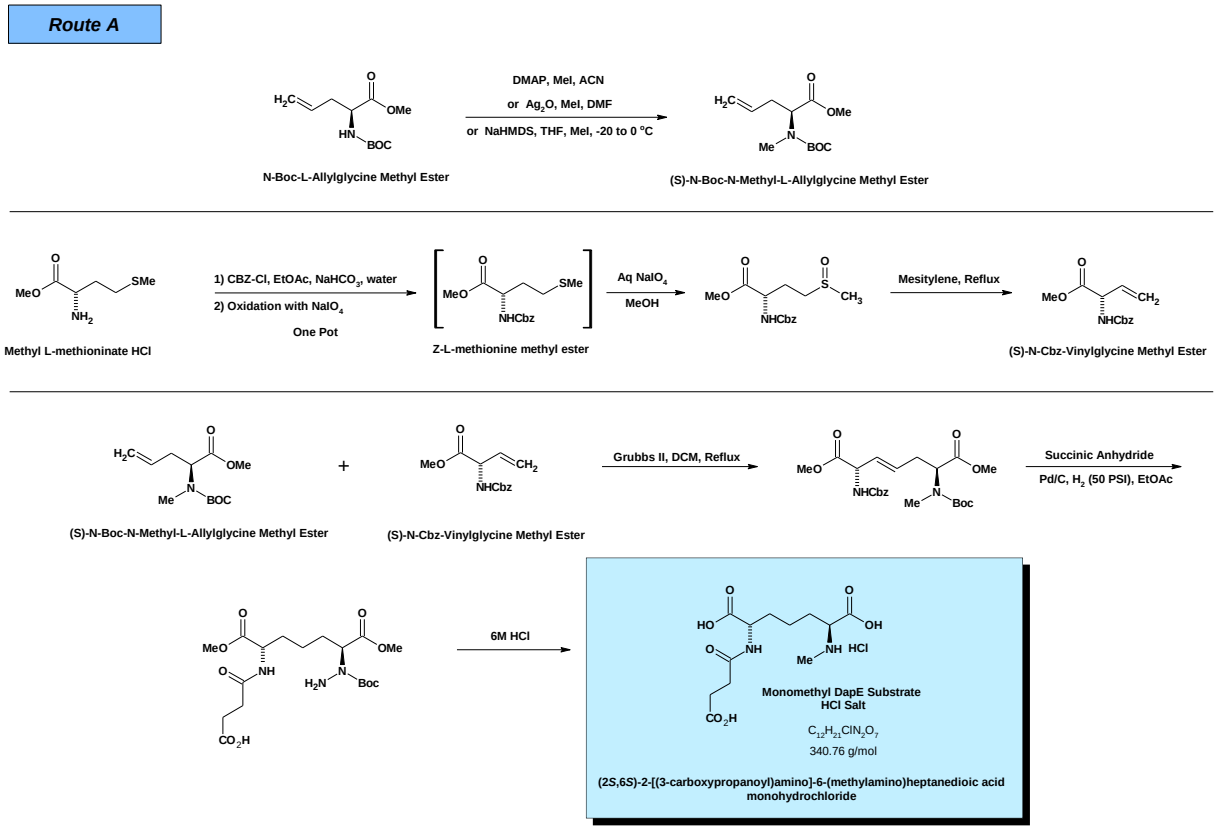
Scheme 16. Hlavácek synthesis of SDAP and its analogs



One of the methods of producing orthogonally-protected S,S-DAP was accomplished *via* olefin cross-metathesis which was reported by Blicher.[28] Drawbacks of this method included a challenging selective hydrogenation of the alkene in the presence of the Cbz groups, and also the need to minimize the self-crossed metathesis byproducts which depends on the generation of Grubb's catalysts used. In relation to Blicher's route, a similar synthesis reported by Takahata[29] which demonstrated that protected *meso*-DAP can be prepared by cross metathesis of the Garner aldehyde-derived vinyl glycine with protected allyl glycine in the presence of Grubb's second-generation catalyst. Based on this Blicher's method, the proposed route

shown below in Scheme 17 (Route A) illustrates how Blicher's method could be employed to prepare monomethylated SDAP. The proposed route was modified such that the Cbz group could be exchanged for a Boc group. This would simplify the hydrogenation because the alkene and Cbz groups could be selectively reduced in the presence of succinic anhydride to generate the succinate amide. However, in order to perform the cross metathesis reaction, the requisite starting materials would need to be prepared, specifically the *N*-methylated Boc protected vinyl glycine and *N*-Cbz vinyl glycine. It was decided not to pursue this route since formation of self-cross metathesis products is a significant drawback that would give rise to a 30-40% yield loss of desired product as reported by Blicher[28] and Takahata.[29]

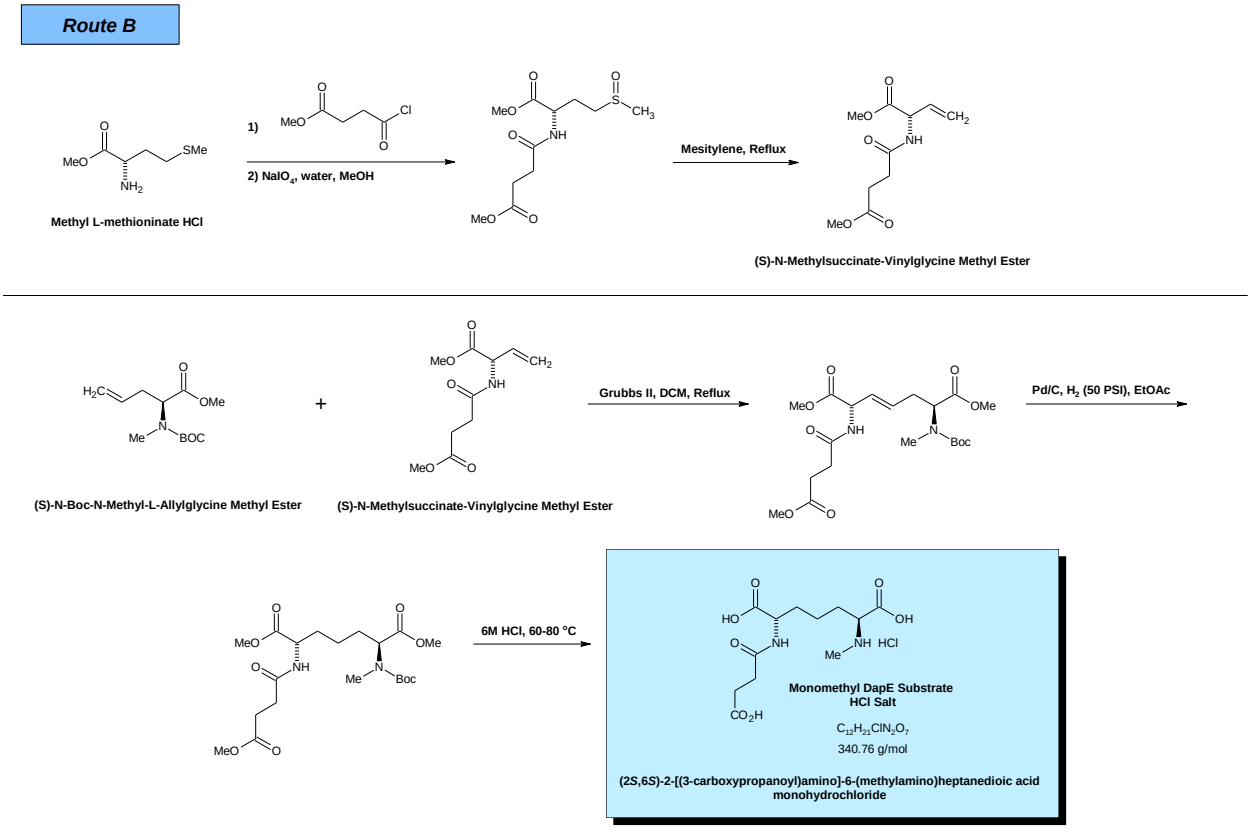
Scheme 17. Proposed Route A for the Preparation of Monomethyl L,L-SDAP



Another interesting approach to preparing diastereomerically pure monomethyl SDAP is illustrated in Scheme 18 (Route B). The synthesis starts with the commercially-available and inexpensive starting material, methyl L-methioninate hydrochloride, which reacts with methyl succinyl chloride to form the succinate amide methyl ester. The subsequent step involves the oxidation of the sulfide using sodium periodate to form the sulfoxide component. Under high temperatures and/or high temperature/high pressure conditions either in a microwave reactor or continuous flow, the sulfoxide is eliminated to afford the corresponding

alkene. The generated alkene after elimination of the sulfoxide would couple with the N-methyl-N-Boc allyl glycine in a cross-metathesis reaction using a Grubb's II generation catalyst. Upon formation of the desired alkene, this product would undergo hydrogenation to reduce the alkene to the saturated form, then global deprotection of the trimethyl ester to the corresponding carboxylic acid and removal of the Boc group to afford the monomethyl SDAP ammonium salt. This proposed route was conceptually attractive, however the generation of the alkene *via* sulfoxide elimination might be a problematic step since these reactions require high temperatures and it is not known whether the succinate amide would epimerize through alkene isomerization of the double bond, which would prefer to be conjugated to the carbonyl ester system with generation of an enamide. Based on these concerns, this route was not pursued.

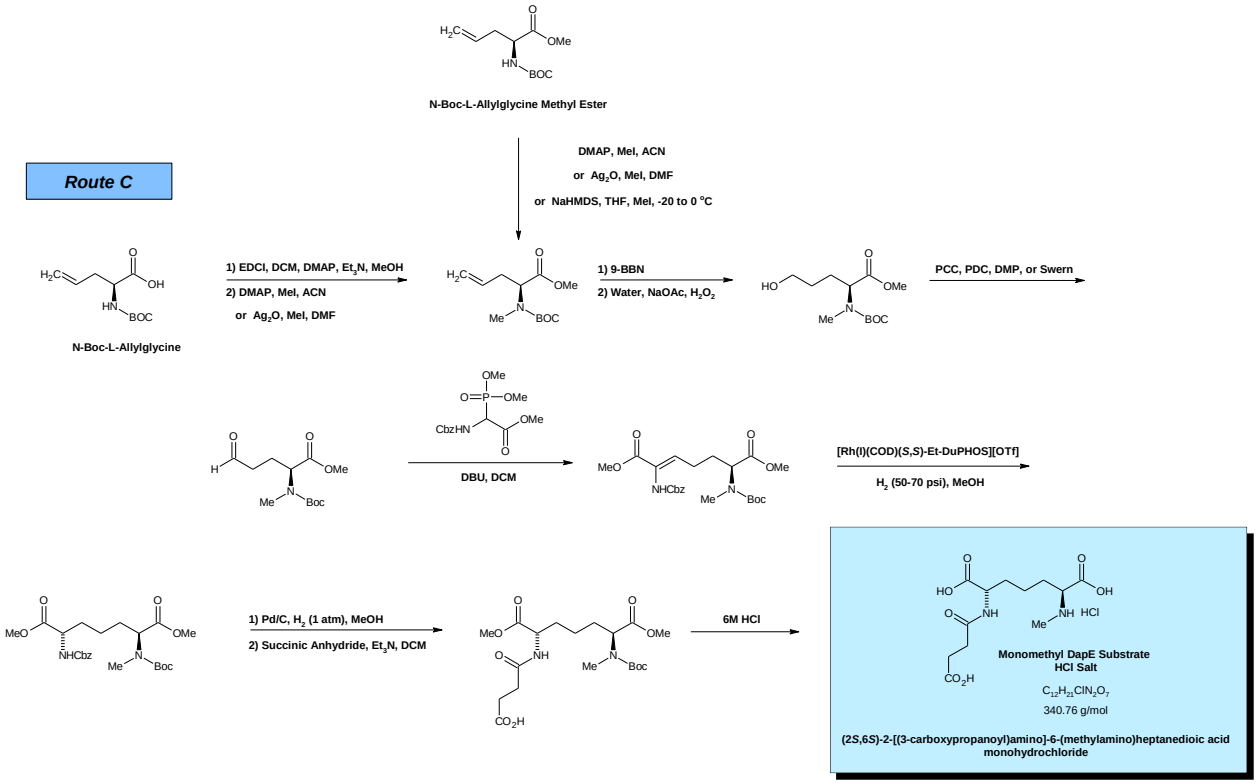
Scheme 18. Proposed Route B for the Preparation of Monomethyl L,L-SDAP



The next proposed route illustrated in Scheme 19 (Route C) for preparation of diastereomerically pure monomethyl SDAP was designed around the preparation of an aldehyde through the use of commercially-available *N*-Boc-L-allylglycine. This route is very similar to the route ultimately selected for preparation of monomethyl SDAP (refer to Route D), however this route primarily is designed around an alternative preparation of the aldehyde. The first step requires the formation of the methyl ester followed by installation of the methyl group onto the *N*-Boc moiety. Next, the hydration of the allyl group then proceeds *via* hydroboration followed by

oxidation of the primary alcohol to aldehyde using either PCC/PDC or Swern conditions. This route relies on the fact that aldehydes and phosphonoglycine esters can couple efficiently to form enamides through the use of the Horner-Emmons-Wadsworth olefination reaction. The subsequent step involving hydrogenation of the Cbz group followed by succinylation yields the succinate amide. Lastly, aqueous acid to perform global deprotection *via* hydroylsis is expected to furnish the monomethyl-SDAP salt, however the hydrolysis of the trimethyl ester may require lower temperature/lower acid concentration to prevent hydrolysis of the amide group. This route does seem promising for the preparation of monomethyl SDAP, however there are a few concerns regarding the initial two steps such as installation of methyl group onto the Boc-amide group. Installation of the methyl group using silver(I) oxide could be problematic since silver(I) species interact with olefins which could complicate the desired methylation of Boc-amide. Alternatively, methylation under alkaline conditions using DMAP/MeI or NaTMDS/MeI could be employed but there is the risk that alkene isomerization will occur, which would affect the hydroboration reaction leading to incorrect functionalization of the hydroxyl moiety and thus leading to formation of ketone rather than desired aldehyde.

Scheme 19. Proposed Route C for the Preparation of Monomethyl L,L-SDAP

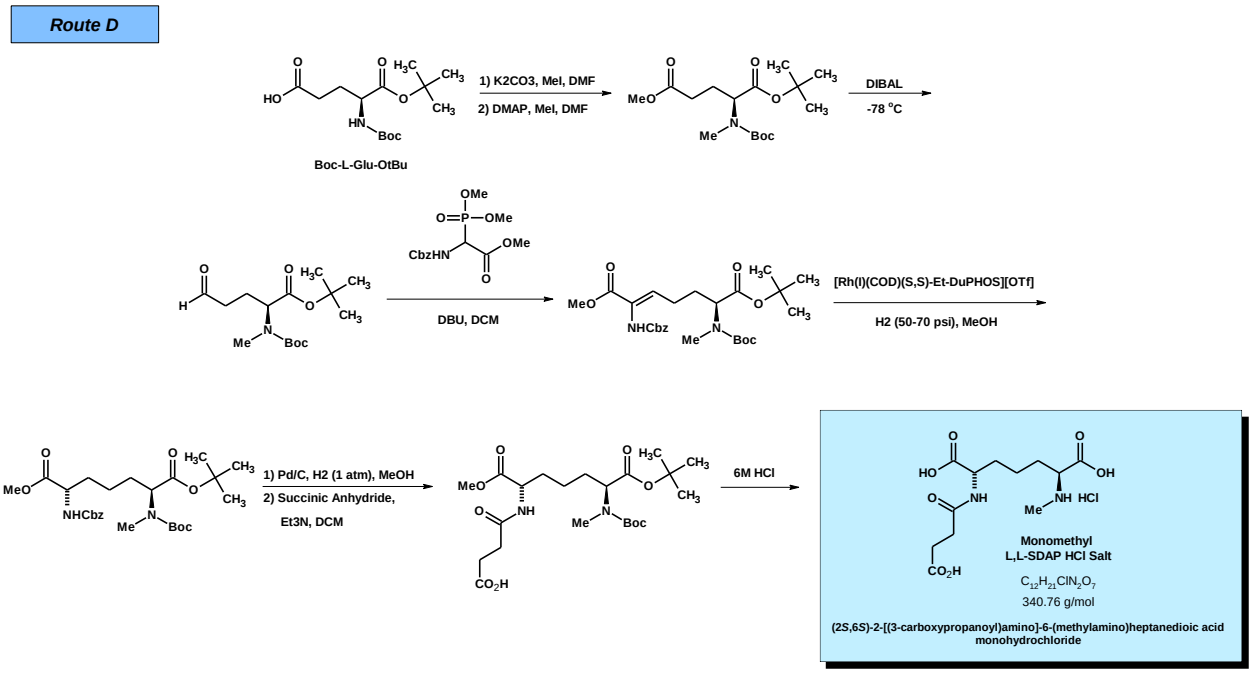


A synthesis of diaminosuberic acid (DAS) was developed by Nájera et al[30]using a palladium-catalyzed allylic double substitution which could be applied to the synthesis of DAP. This approach generates a 1:1 mixture of diastereomers and thus is not appropriate for the preparation of diastereomerically pure diaminopimelic acid (DAP). Other known preparations of diaminopimelic acid (DAP) have been cited, however these preparations generally give a mixture of diastereomers.[31-33]

An orthogonally synthetic method for producing a protected *meso*-DAP derivative was reported by Fukase.[34] Fukase's synthetic methodology focused on the use of a Kocienski-modified Julia olefination that effectively

coupled a homologated-serine based Garner aldehyde, itself derived from Boc-protected D-serine[35] and a modified Julia sulfone (also derived from Cbz protected D-serine)[36] to yield a key seven-carbon backbone intermediate that leads to the formation of a protected *meso*-DAP derivative after 5 additional chemical steps. Thus, Fukase reported an elegant synthesis in which no epimerization was observed, however this route is not amenable toward the synthesis of monomethyl L,L-SDAP since the homologated Garner aldehyde starting material (L or D-serine) requires 7 chemical steps[35, 37] to access the homologated Boc-protected Garner aldehyde and attempting to install a methyl group during the first three steps route will prohibit the formation of Garner aldehyde. Thus, the only means to form the N-methyl would be at a time before reducing the methyl ester to the aldehyde/alcohol. The installation of a methyl group for the sulfone precursor is also not attractive due the requirement of a multi-step approach to furnish a monomethyl starting material. Granted that Fukase's method proved successful for his objectives, the synthesis is not practical for preparation of monomethyl L,L-SDAP at larger scale due to the high cost and time-demanding multi-step synthesis to furnish a monomethyl adduct.

Scheme 20. Proposed and Selected Route D for the Preparation of Monomethyl L,L-SDAP

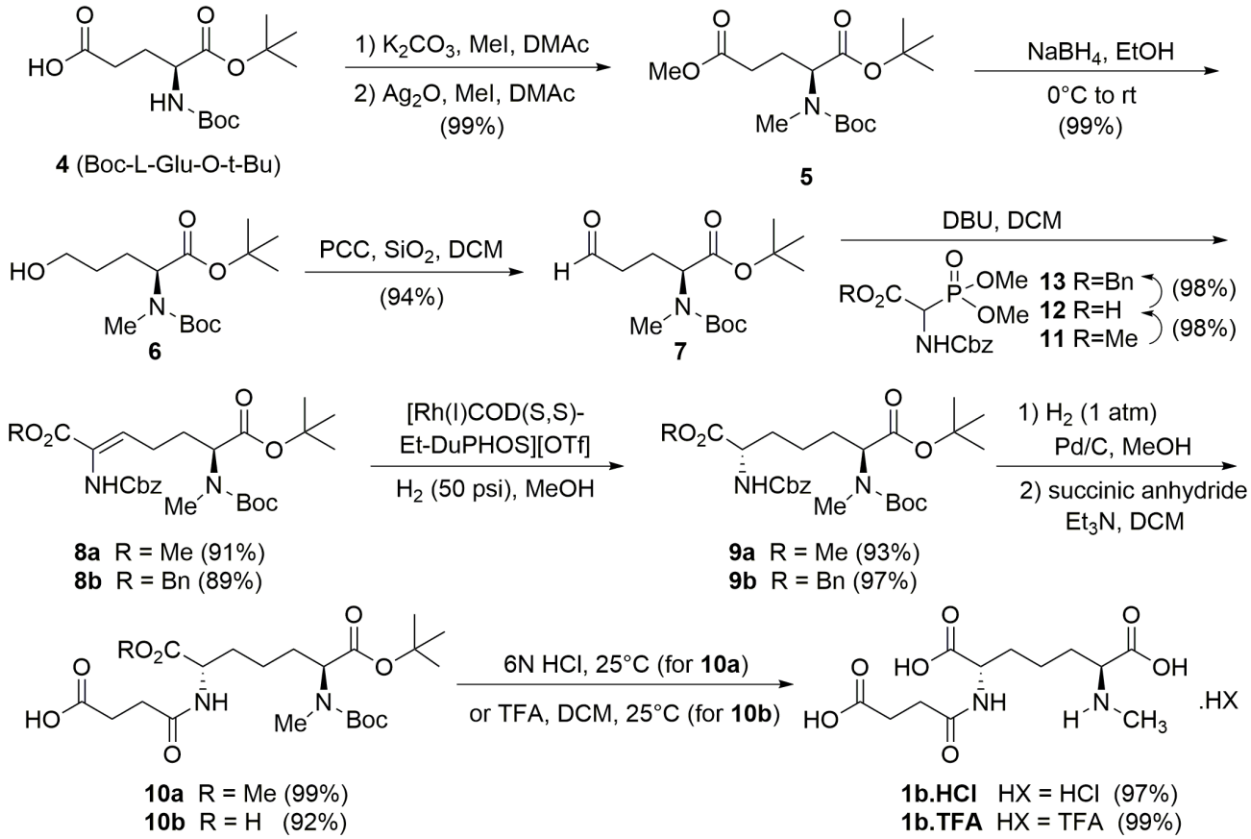


Proposed route D (see Scheme 20) was partly based on the previous work by Hruby[38] which demonstrates that DAP can be enantiomerically synthesized through asymmetric hydrogenation of an enamide precursor. In this route, the starting material, Boc-L-Glu-OtBu, was selected due to its commercially availability and low cost. The methyl ester could have been purchased, however this was not as commercially available, and the cost was higher compared to the free acid. The preparation of the methyl ester can be accomplished by a variety of methods, however the simplest approach was using methyl iodide or dimethyl sulfate. Methyl iodide and potassium carbonate in DMF was selected for the formation of the methyl

ester for the work described herein. The next step to install a methyl group on the *N*-Boc moiety was planned to be carried out in the same pot to avoid aqueous workup of the methyl ester. Following Hruby's method of reducing the methyl ester to the aldehyde using DIBAL at cryogenic conditions, it was found that this method was not applicable for the *N*-methyl-Boc substrate due to the formation of mixtures, thus alternative methods for a high-yielding reduction/reoxidation protocol were investigated. The next step incorporated the Horner-Emmons-Wadsworth olefination by treating the prepared aldehyde with Cbz-α-phosphonoglycine ester and DBU. The Horner-Emmons reaction using Cbz-α-phosphonoglycine dimethyl ester free carboxylic acid was also attempted but this approach was unsuccessful with no conversion of starting materials observed. The asymmetric reduction seemed very promising for the monomethyl-SDAP since it was determined that Rh-DUPHOS is an effective chiral catalyst to access the enantiomerically pure L,L-DAP scaffold. The subsequent step requires removal of the Cbz group using hydrogenation followed by succinylation using succinic anhydride and triethylamine in DCM. Lastly, based on literature precedent, the concurrent hydrolysis of the methyl ester, Boc group, and *t*-butyl ester can be accomplished using aqueous hydrochloric acid or aqueous hydrobromic acid, which Hruby employed for hydrolysis of the methyl ester and Cbz groups to access diaminopimelic acid (DAP).

Executing the proposed route for preparation of monomethyl L,L-SDAP followed a similar approach of Hruby[38] but the route was modified and significantly improved as needed for subsequent scale-up for preparation of intermediates, finally yielding enantiomerically pure monomethyl L,L-SDAP. Scheme 21 depicts the modified synthetic routes utilized for preparation of monomethyl L,L-SDAP denoted as **1b.HCl** and **1b.TFA** for the hydrochloride and trifluoroacetate salts, respectively.

Scheme 21. Asymmetric synthesis of N^6-Methyl-L,L-SDAP **1b**. Synthetic route for preparation of monomethyl substrate analog as the hydrochloride salt (**1b.HCl**) via the methyl ester or the trifluoroacetate salt (**1b.TFA**) via the benzyl ester



N^2-Succinyl-N^6-methyl-L,L-DAP **1b** was prepared enantioselectively with the key olefination and asymmetric hydrogenation steps[38] modeling the synthesis of diaminopimelic acid, which lacks the N^6-methyl as well as the succinate. In addition, the present route avoids any use of ion exchange chromatography that was used in previous smaller-scale work.[38] The first step involves the one-pot methylation of the BOC-L-glutamic acid t-butyl ester **4** with potassium carbonate and methyl iodide, in the presence of silver oxide, to afford the N-methylated ester **5**. Reduction of the methyl ester with sodium borohydride afforded the primary alcohol **6**, which was oxidized to the aldehyde **7** with PCC or PDC, or under Swern conditions. The oxidation using PCC or PDC was more convenient than Swern conditions which typically formed an impurity (believed to be a mixed thioacetal) that was difficult to remove by normal-phase chromatography and resulted in lower yields of the desired aldehyde. Furthermore, the use of PDC appeared to be more facile and typically produced less baseline material as determined by TLC analysis. Attempts to access the aldehyde directly from the methyl ester using DIBAL were unsuccessful and typically gave mixtures of starting material, aldehyde, and alcohol. Oxidation of alcohol to aldehyde with activated manganese dioxide was also not successful. Horner-Wadsworth-Emmons olefination with Cbz-α-phosphonoglycine ester (Cbz = carboxybenzyl) gave the enamide in excellent yield. Alternatively, the

Horner-Emmons reaction using Cbz-α-phosphonoglycine dimethyl ester free carboxylic acid was attempted but this approach was unsuccessful with no conversion of starting materials. The enamide product was enantioselectively hydrogenated in the presence of catalytic amounts of 1,2-bis[(2*S*,5*S*)-2,5-diethylphospholano]benzene(1,5-cyclooctadiene)rhodium(I) trifluoromethanesulfonate affording the L,L-Cbz-protected amino acid **9a** in 93% yield. This asymmetric hydrogenation and catalyst selection follows previous work that established an asymmetric synthesis of diaminopimelic acid.[38] The asymmetric hydrogenation of the enamide described within this work was accomplished using lower hydrogen pressure (50 psi) compared to Hruby's method which employed 70 psi of hydrogen using a Parr Shaker. Furthermore, the asymmetric hydrogenation process described within this work was executed safely using lower hydrogen pressure and the process was carried out in a more controlled manner where the pressure vessel was equipped with mechanical agitation and better thermal control throughout the duration of the reaction. Removal of the Cbz protecting group by hydrogenolysis followed by reaction with succinic anhydride afforded the succinamide derivative in 99% yield, which was subjected to hydrolysis with aqueous HCl to afford N^6-methyl L,L-SDAP as the hydrochloride salt (**1a.HCl**) in 97% yield. The hydrochloride salt was very hygroscopic, and

eventually the material became an un-weighable solid and the quality of the product declined presumably due to desuccinylation.

In more detail, the overall route to monomethyl SDAP was modified and improved based on Hruby's method, since that installation of the methyl group on the N-Boc moiety was required. Hruby's method of accessing the aldehyde strictly depended on the bis-Boc-amide which under reductive conditions using DIBAL gave primarily the aldehyde. It was found that once the methyl ester-*N*-methyl-Boc **5** was prepared, the reduction of methyl ester to aldehyde using DIBAL was problematic producing a mixture of aldehyde, alcohol, and unreacted starting material methyl ester which could be oxidized to primarily aldehyde using PDC/PCC but made isolation of aldehyde very difficult. Furthermore, carrying forward the methyl ester starting material as an impurity would lead to its hydrolysis if carried to the final step, which would create problems downstream during the synthetic sequence. Therefore, conditions for accessing the aldehyde were modified and then accomplished by reducing the methyl ester cleanly to the alcohol using sodium borohydride followed by an efficient oxidation to aldehyde using either PDC/PCC or Swern conditions. Furthermore, the use of sodium borohydride left the *tert*-butyl ester unaffected due to the bulky nature of the *t*-butyl group.

The use of hydrobromic acid in acetic acid, based on literature method,[38] to hydrolyze the methyl ester and Cbz group was not amenable for the hydrolysis of monomethyl SDAP due to the fact that the succinyl group was also hydrolyzed affording monomethyl-diaminopimpelic acid as an impurity which proved to be an inhibitor in the ninhydrin assay. Given that the combination of acid and heat does increase the risk of epimerization of the amino substituent(s), two alternative methods were developed to control epimerization and prevent hydrolysis of the succinyl group. The two methods evaluated were the use of 6M HCl at ambient temperature to control the epimerization but this method also eventually produced small amounts of desuccinylated by-product giving a primary amine. The second method is based on the benzyl ester analog (discussed below) that consisted of non-aqueous conditions using trifluoroacetic acid to cleave both the t-butyl and Boc groups that generated the mono-methyl SDAP analog cleanly without any desuccinylated impurity and with better control in avoiding epimerization.

The Horner-Emmons-Wadsworth monomethyl product did show evidence of *cis-trans* alkene isomers based on ^{1}H NMR analysis, although the ultimate enantioselectivity was not compromised. During the asymmetric hydrogenation of the monomethyl enamide intermediate, ^{1}H NMR was employed to monitor the progress of the reaction. Isomerization of the

alkene was not observed, and both *E*/*Z* enamides were hydrogenated to achieve high e.e. as described and supported by Burk's study.[39, 40]

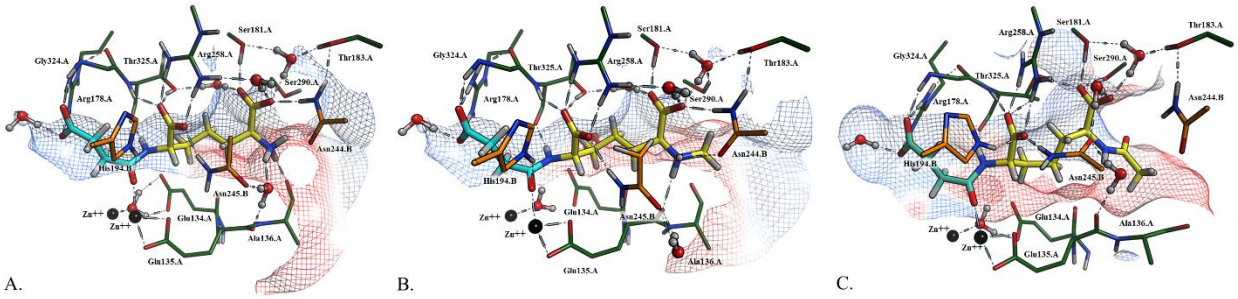
The use of the benzyl ester route proved to be more amenable to large-scale reactions as the benzyl moiety can easily be deprotected after the asymmetric hydrogenation. Conversely, the intrinsic substrate selectivity for asymmetric hydrogenation of the methyl ester was actually better than for the benzyl ester enamide scaffold, as determined by HPLC analysis; the methyl ester chiral purity was 97.4% e.e. whereas 92.0% e.e. was achieved for the benzyl ester. The benzyl ester still remained the most attractive procedure since hydrolysis of the methyl ester is often accompanied by desuccinylation generating a primary amino moiety which dramatically affects the ninhydrin assay, therefore the benzyl ester enamide route proved to be superior in generating cleaner, mono-methyl SDAP, combined with the fact that a final crystallization in the benzyl ester route afforded material over very high e.e., as detailed below.

A second route was also developed that was an improvement, based on yield, over the initial methyl ester route by utilizing a benzyl ester. In this synthetic scheme, the Wadsworth-Emmons reaction of the aldehyde **7** with benzyl 2-{[(benzyloxy)carbonyl]amino}-2-(dimethoxyphosphoryl) acetate (R = Bn) afforded enamide **8b**, which was enantioselectively hydrogenated in the presence of catalytic 1,2-bis[(2*S*,5*S*)-2,5-

diethylphospholano]benzene(1,5-cyclooctadiene)rhodium(I) trifluoromethanesulfonate to afford the L,L-Cbz-protected amino acid benzyl ester **9b** in 97% yield. Hydrogenolytic removal of both Cbz and benzyl groups followed by reaction with succinic anhydride gave the succinate amide (92-97% for **10b)**. Removal of the *tert*-butyl ester with trifluoroacetic acid in methylene chloride gave the trifluoroacetate salt of **1b** in nearly quantitative yield. Overall, the benzyl ester route proved to be more amenable for large scale reactions, although the intrinsic substrate diastereoselectivity for asymmetric reduction of the methyl ester yielded material of 97.4% e.e. for methyl ester **9a**, compared to 92.0% e.e. for the benzyl ester **9b**. Nevertheless, crystallization at a later stage ultimately afforded the final product **1b** in >99% e.e. from the benzyl ester, and the methyl ester route was often accompanied by desuccinylation generating a primary amino-containing impurity which would negatively affect the ninhydrin assay, therefore the benzyl ester route was the superior route. Furthermore, the benzyl ester route provides the TFA salt which was not observed to be hygroscopic, in contrast to the hydrochloride salt from the methyl ester route which is very hygroscopic and converts to a sticky and un-weighable solid over time in storage.

Based on molecular modeling data performed by Dr. Cory Reidl and Estefany Guzman, N^6-methyl-L,L-SDAP #**1b** is predicted to retain an overall

similar binding pose to L,L-SDAP #**1a**, making it a likely substrate analog while N^6-acetyl-L,L-SDAP #**1c** suffers significant binding alterations due to the presence of the acetyl group, in addition to loss of the positive charge on the nitrogen. Therefore, N^2-succinyl-N^6-methyl-L,L-DAP #**1b** was prepared enantioselectively, as illustrated in Figure 26.



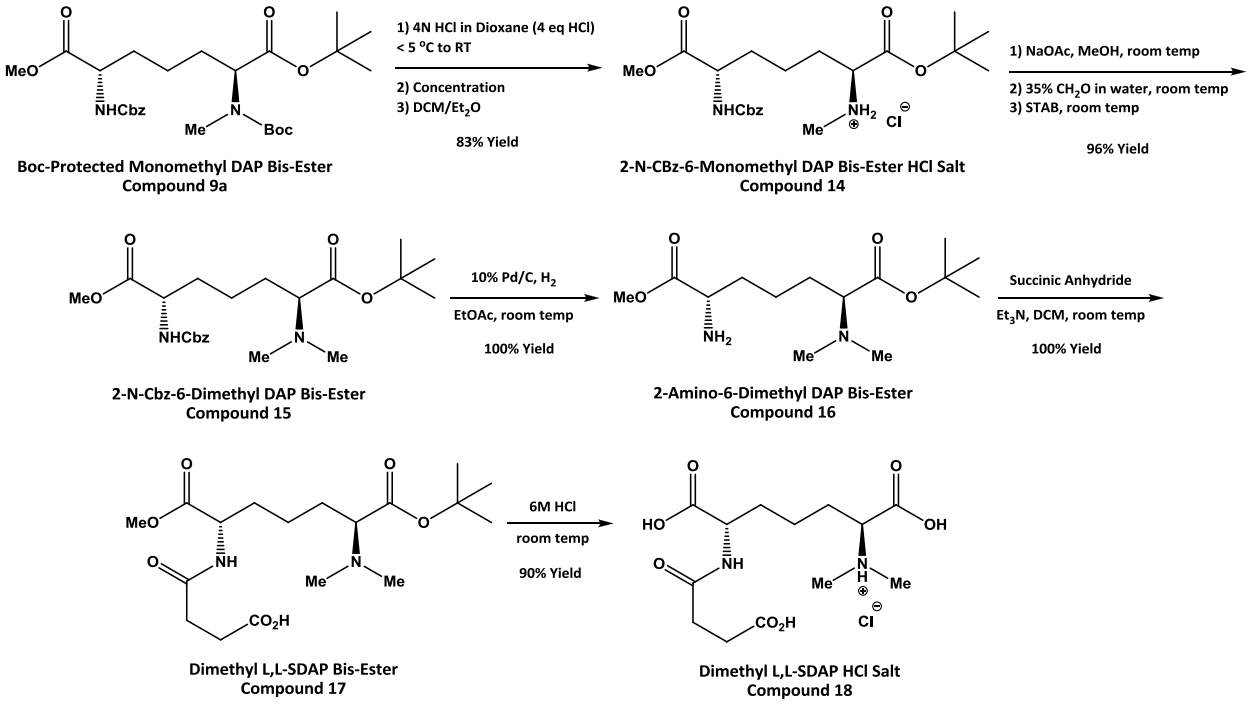
Note. The diaminopimelate moiety is depicted in yellow and the succinate in turquoise. A) Native substrate L,L-SDAP, B) N^6-methyl-L,L-SDAP, and C) N^6-acetyl-L,L-SDAP. The catalytic domain of Chain A is depicted in green, whereas the dimerization domain of Chain B is shown in orange.

Figure 26. Minimized substrate/analogs docked and modeled in the HiDapE active site

Section 5: Novel Preparation of N^6,N^6-Dimethyl-L,L-SDAP

Based on the developed route for asymmetric preparation of monomethyl L,L-SDAP, the synthesis of N^6,N^6-dimethyl-L,L-SDAP was executed starting with a protected DAP intermediate **9a** as illustrated in Scheme 22 below.

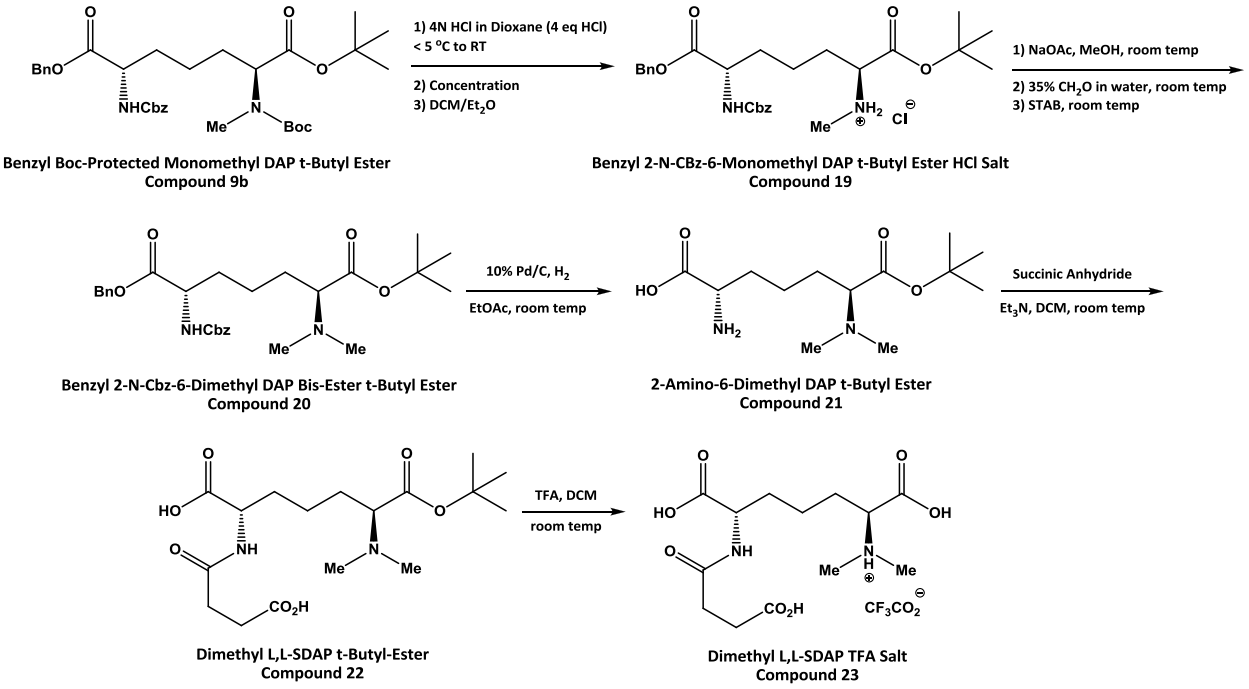
Scheme 22. Preparation of N^6,N^6-dimethyl-L,L-SDAP



The synthesis of dimethyl L,L-SDAP begins with deprotection of the Boc moiety from **9a** using 4N HCl in dioxane which cleanly removes the Boc group. After overnight reaction at room temperature and subsequent concentration of the reaction mixture, the crude residue was dissolved in DCM and the hydrochloride salt was precipitated by addition of diethyl ether affording an 83% yield of **14**. HPLC analysis of the resulting hydrochloride salt **14** showed a 96% purity for the desired product plus 4% of a component where both Boc and *t*-butyl groups were cleaved. Subjecting the monomethyl hydrochloride **14** to reductive amination provided the dimethyl-DAP intermediate **15** in 96% yield after extractive workup which provided

100% purity by HPLC, and MS analysis confirmed the correct molecular ion for the desired dimethyl-DAP bis-ester **15**. Subsequent hydrogenation of **15** (N^6,N^6-dimethyl-DAP bis-ester) provided the free amine **16** in quantitative yield followed by succinylation which yielded the protected N^6,N^6-dimethyl-L,L-SDAP analog **17** in quantitative yield. Finally, hydrolysis of protected dimethyl L,L-SDAP analog **17** in 6M HCl provided N^6,N^6-dimethyl-L,L-SDAP HCl salt **18** in 90% yield. This chemistry was developed during process development of monomethyl L,L-SDAP and it was also found that the dimethyl L,L-SDAP HCl salt was hygroscopic as well. However, the benzyl ester route was not applied in the synthesis of dimethyl L,L-SDAP TFA salt. In the future, it is recommended that the synthesis be executed using the route proposed illustrated below in Scheme 23.

Scheme 23. Proposed Route to Improve the Synthesis for Dimethyl L,L-SDAP



Section 6: Conclusion

Overall, the described novel synthesis of the N^6-monomethyl-L,L-SDAP DapE substrate proved to be a scalable process, which will allow our group and the scientific community at large to easily synthesize large quantities of this new DapE substrate. Furthermore, this synthesis will enable other research groups to use our newly-developed ninhydrin assay and easily access the monomethyl substrate through synthesis or purchase from a commercial supplier such as Regis Technologies. Process improvements which have been achieved with this new synthesis of monomethyl L,L-SDAP include higher atom economy due to the asymmetric hydrogenation, and

that the process does not require the use of expensive and time-consuming reverse-phase chromatographic separations or ion-exchange resin purifications, and lastly this process employs very low loadings of normal phase silica gel which increases throughput and reduces cost, and finally, that organic solvents used in the purification can be easily removed in contrast to aqueous media.

In summary, N^6-methyl-L,L-SDAP **1b** was predicted to be a potential substrate for *Hi*DapE whereas N^6,N^6-dimethyl-L,L-SDAP was predicted to suffer significant binding alterations due to the presence of the second methyl group based on molecular modeling. Two synthetic routes to DapE substrate **1b** were developed enabling the synthesis of **1b** in nearly quantitative yield on a multi-gram scale. Kinetic studies proved that substrate **1b** can be hydrolyzed by *Hi*DapE at comparable rates to the endogenous substrate, L,L-SDAP, establishing **1b** as a viable substrate for DapE enzymes and for our new ninhydrin-based assay. Since the amine-containing product of the hydrolysis of **1b** contains only one primary amine, conditions that selectively modify the primary amine *versus* the secondary amine with ninhydrin were developed, resulting in a new spectrophotometric assay for DapE enzymes that is inexpensive, robust and reproducible. The newly discovered DapE substrate,**1b**, coupled with ninhydrin development, is superior to the standard UV-based (225 nm) assay which uses L,L-SDAP as

the substrate, as it is alleviates interference from potential inhibitor absorption at 225 nm, a common absorption region for aryl containing compounds that are of interest to medicinal chemists. The feasibility of the new **1b**-ninhydrin-coupled DapE assay was established by accurately determining IC_{50} values of known *Hi*DapE inhibitors. In summary, this new ninhydrin-based assay provides a method for the discovery of lead compounds for DapE enzymes and for driving medicinal chemistry structure-activity relationships (SAR) and thus provide a critical tool for the discovery of a new class of antibiotics that target DapE enzymes.

Section 7: Experimental

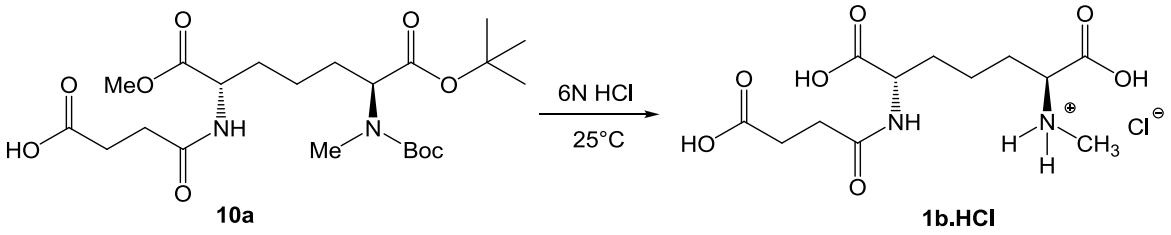
Materials and Methods

All solvents were distilled prior to use and all reagents were used without further purification unless otherwise noted. All synthetic reactions were conducted under a nitrogen or argon atmosphere. The term reactor refers to a large round-bottom flask. Silica gel 60 Å, 40−75 μm (200 × 400 mesh) or 60-200 μm (Silicycle), was used for column chromatography. Aluminum-backed silica gel 200 μm plates were used for TLC (thin-layer chromatography). ^{1}H NMR spectra were obtained using a 300, 400, or 500 MHz spectrometer with trimethylsilane (TMS) as the internal standard. ^{13}C NMR spectra were obtained using a 75, 100, or 125 MHz spectrometer. The purity of all compounds was determined to be ≥95%, unless otherwise

noted, by high performance liquid chromatography (HPLC) employing a mobile phase A = 0.1% TFA in water and a mobile phase B = 0.1% TFA in acetonitrile with a gradient of 60% B increasing to 95% over 10 min, holding at 95% B for 5 min, then returning to 60% B and holding for 5 min. Chiral purities were determined using Chiral HPLC on various chiral HPLC columns using varying chromatographic conditions, therefore for any procedure that reports chiral purity will describe the chiral HPLC method used. HRMS spectra were measured on a TOF instrument by electrospray ionization (ESI). HRMS spectra were collected using a Waters Acquity I class UPLC and Xevo G2-XS QTof mass spectrometer with Waters Acquity BEH C18 column (1.7 µm, 2.1x50 mm). Mobile phase A was 0.05% formic acid in water and mobile phase B was 0.05% formic acid in acetonitrile. A gradient of 5% to 90% B over 5 min was applied.

Organic Synthesis

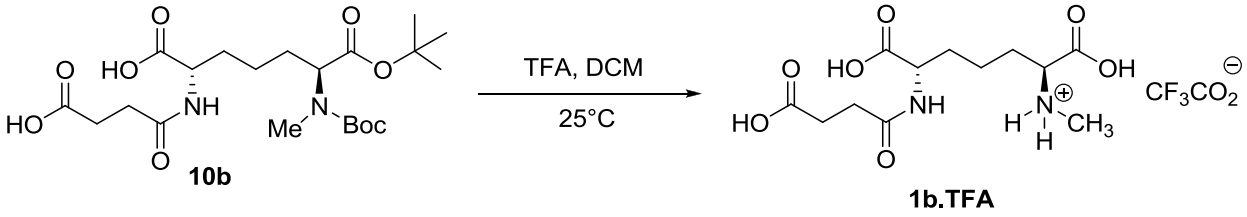
(2*S*,6*S*)-2-(3-Carboxypropanamido)-6-(methylamino)heptanedioic acid hydrochloride (1b.HCl)



10a **1b.HCl**

To a 100 mL reactor was added **10a** (1.50 g, 3.16 mmol) and a freshly prepared 6M HCl solution (30 mL). The reaction mixture was allowed to stir at 25°C for 5 h and concentrated under reduced pressure (0.1-10 mbar) at 25°C on a rotary evaporator and triturated in acetonitrile overnight at room temperature. The resulting slurry was filtered and dried under reduced pressure overnight to provide **1b** as the hydrochloride salt (**1b.HCl**, 1.04 g, 97%, deliquescent solid) as a white foam.**^{1}H NMR** (400 MHz, D_2O): δ 4.35 (q, 1 H, *J* = 9.0, 5.0 Hz), 3.95 (t, 1 H, *J* = 6.2 Hz), 2,73 (s, 3 H), 2.64 (m, 2 H), 2.58 (m, 2 H), 2.03-1.88 (m, 3 H), 1.81-1.72 (m, 1 H), 1.58-1.46 (m, 1 H), 1.43-1.36 (m, 1 H). **HRMS (IT-Tof)**: m/z calcd for $C_{12}H_{21}N_2O_7^+[M+H]^+$:305.1349, found 305.1309.

(2S,6S)-2-(3-Carboxylatopropanamido)-6-(methylamino)heptanedioate, 2,2,2-trifluoroacetate salt (1b.TFA)



To a solution of succinyl t-butyl ester **10b** (0.608 g, 1.32 mmol) in DCM (5.75 mL) was added TFA/DCM solution (30/70, 15 mL) under argon. The mixture was stirred at room temperature for 4 hours and concentrated under reduced pressure. The resulting residue was dissolved in dry methanol (1.0 mL) and slowly added to diethyl ether (50 mL) with vigorous stirring to

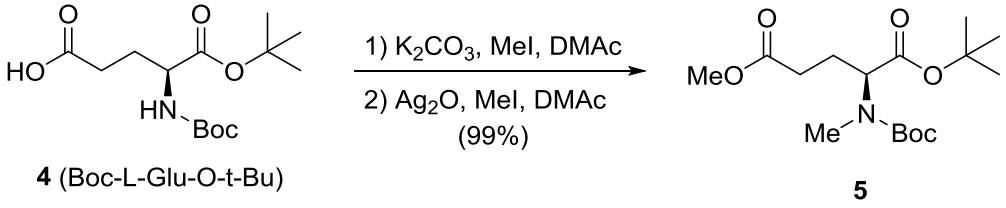
precipitate TFA salt. The slurry was filtered and the wetcake was rinsed with diethyl ether (2.0 mL), hexane (10 mL), and dried *in vacuo* at 35°C overnight to provide the mono-methyl SDAP TFA salt (550 mg, 99.6% yield) as a white solid. **^{1}H NMR** (400 MHz, D_2O): δ 4.36 (dd, 1H, *J* = 9.2, 5.2 Hz), 3.76 (t, 1H, *J* = 6.4 Hz), 2.72 (s, 3H), 2.69-2.66 (m, 2H), 2.66-2.59 (m, 2H), 1.98-1.92 (m, 3H), 1.80-1.77 (m, 1H), 1.52-1.50 (m, 1H), 1.44-1.42 (m, 1H). **^{13}C NMR** (100 MHz, D_2O): δ 176.9, 175.6, 174.9, 172.3, 62.1, 52.3, 31.5, 30.0, 29.9, 29.0, 28.2, 20.5. **HRMS** (ESI-ToF): m/z calcd for $C_{12}H_{21}N_2O_7^+[M+H]^+$: 305.1349, found: 305.1365. **Achiral HPLC purity**: 100%. **Chiral HPLC purity**: 100% (100% e.e.). Chiral HPLC was performed using either two methods. Chiral HPLC method 1: a RegisPack column (250x4.6 mm, 5 micron) eluting with an isocratic gradient of hexane/IPA (90/10) + 0.1%TFA at 1.5 mL/min and monitoring at 210 nm. Chiral HPLC method 2: Chirosil SCA(-) column (150 mm x 4.6 mm, 5 micron) with isocratic elution employing 84% MeOH in water containing 5 mM $HClO_4$ with an elution time of 15 minutes with column temp at 25°C and a flow rate of 0.75 mL/min monitoring at a wavelength of 210 nm.

Scale-up of the TFA Salt:

To a solution of succinyl *t*-butyl ester **10b** (6.08 g, 14.8 mmol) in DCM (60 mL) was added a TFA/DCM solution (30/70, 135 mL) under nitrogen. The mixture was stirred at room temperature for 6 h and concentrated on a

rotary evaporator at 35-40°C. The resulting residue was dissolved in anhydrous methanol (6.0 mL) and slowly added to anhydrous diethyl ether (450 mL, HPLC grade, inhibitor-free) with vigorous stirring to precipitate the TFA salt. The slurry was allowed to stir overnight at room temperature. The slurry was filtered, and the wet cake was rinsed with anhydrous diethyl ether (50 mL), n-hexane (100 mL), and dried *in vacuo* at room temperature overnight to provide **1b** as the mono-methyl L,L-SDAP TFA salt (L,L-succinyl diaminopimelate trifluoroacetate; 6.06 g, 98% yield) as a white solid.

1-(*tert*-Butyl) 5-methyl (*tert*-butoxycarbonyl)-L-glutamate (5)

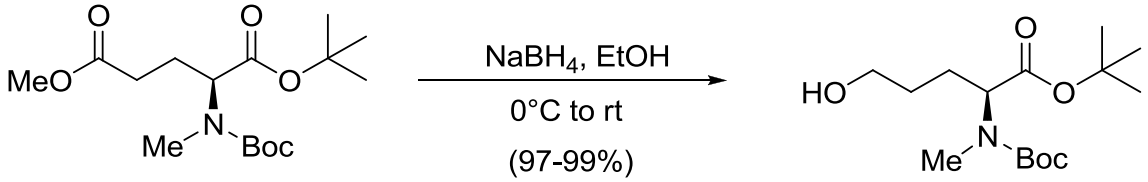


4 (Boc-L-Glu-O-t-Bu) **5**

According to the general and herein improved method of Glinka,[41] to a 2 L reactor was added Boc-L-Glu-O*t*Bu (**4**) (95.65 g, 315.3 mmol), milled K_2CO_3 (69.72 g, 504.5 mmol), and anhydrous DMAC (480 mL). To the reaction mixture was added methyl iodide (49.3 g, 21.2 mL, 112 mmol). The reaction was agitated with mechanical stirring (300 rpm) at 25°C for 18 h which ^{1}H NMR and TLC analysis confirmed that the reaction had gone to completion. To the reaction mixture was added methyl iodide (228 g, 100 mL, 1.61 mol) followed by Ag_2O (110.0 g, 474.7 mmol). The resulting reaction mixture was stirred at 400 rpm with a water bath (15°C) in place to

control the exotherm and allowed to further stir overnight (12 hours) and gradually warm to room temperature. ^{1}H NMR analysis confirmed that the reaction had gone to completion. The reaction mixture was then diluted with EtOAc (750 mL) and treated with Celite (43 g), stirred for 15 min, filtered over a fritted funnel (fine porosity) containing a pad of Celite. The filter was washed with EtOAc (500 mL). The filtrate was concentrated under reduced pressure at 40^{o}C to remove EtOAc and DMAC to provide crude residue oil (168.0 g). The crude oil was diluted with EtOAc (500 mL) and heptane (400 mL) and subsequently washed with 50 wt% sodium thiosulfate pentahydrate solution (2 x 500 mL), USP purified water (3 x 500 mL), brine (500 mL), dried with sodium sulfate (40 g)/silica gel (20 g), filtered, and concentrated to provide title compound **5** (101.6 g, 97%) as a colorless oil. **HPLC purity:** 97.7%.**^{1}H NMR**(400 MHz, $CDCl_3$) δ 4.59 (m, amide rotamer, 0.50 H), 4.37 (m, amide rotamer, 0.50 H), 2.77 (d, mixture of amide rotamers, 3H), 2.38-2.20 (m, 3H), 2.00 (m, 1H), 1.45 (s, 18H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ 173.4, 173.3, 170.4, 170.3, 156.3, 155.8, 81.6, 81.5, 80.3, 80.0, 59.5, 58.4, 51.7, 31.3, 31.2, 30.9, 30.5, 28.4, 28.1, 24.3, 24.1. **HRMS (ESI-ToF)**: m/z calcd for $C_{16}H_{29}NNaO_6^+$ $[M+Na]^+$: 354.1887, found: 354.1920. HRMS also found $[M+Li]^+$: 338.2166 and $[M+K]^+$: 370.1658.

***tert*-Butyl-(S)-2-((tert-butoxycarbonyl)(methyl)amino)-5-hydroxypentanoate (6)**

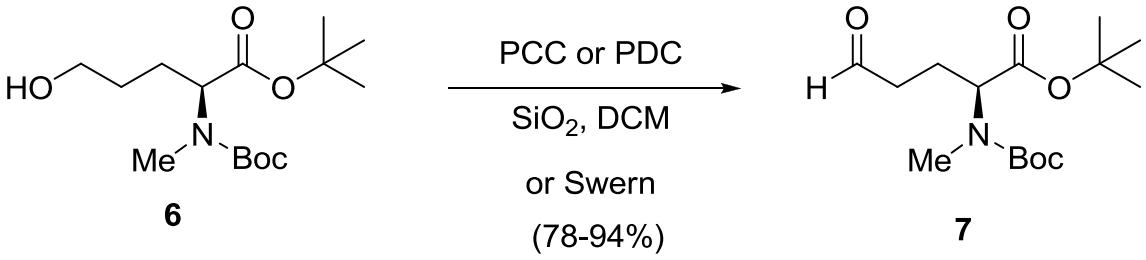


5 **6**

To a 3 L 3-necked reactor was added methyl ester **5** (100.0 g, 301.7 mmol) and ethanol (HPLC grade, 1500 mL) and the mixture was cooled to < 5°C in an ice water bath. Sodium borohydride (57.6 g, 1520 mmol) was added in 5 portions over 15 minutes. The reaction was allowed to stir at < 5°C and subsequently allowed to gradually warm to room temperature overnight while the cooling bath remained in place. After 20 hours, TLC analysis showed complete consumption of methyl ester starting material. To a 12 L reactor containing a cold solution of 50% aqueous ammonium chloride solution (3000 mL, < 5°C) was slowly added the reaction mixture over a period of 15 min such that the temperature remained < 15°C. The mixture was allowed to stir at 15-20°C for 30 min, then EtOAc (1000 mL) was added. The organic layer was separated, and the aqueous layer was further extracted with EtOAc (3 x 1000 mL). The combined organic layers were washed with brine (300 mL), dried with sodium sulfate, filtered, and concentrated to a colorless oil (90.77 g). The crude oil was dissolved in

EtOAc/heptane (20/80, 100 mL), which was purified through a silica gel plug (1000 g) and the plug was flushed with EtOAc/heptane (20/80, 2 L) and EtOAc/heptane (40/60, 6 L) to afford the title alcohol **6** (88.60 g, 97%) as a colorless oil. **^{1}H NMR**: (400 MHz, $CDCl_3$): δ 4.65 (m, amide rotamer, 0.50 H), 4.35 (m, amide rotamer, 0.50 H), 3.68 (t, 2H, *J* = 6.4 Hz), 2.78 (m, mixture of amide rotamers, 3H), 2.04-1.71 (m, 3H), 1.69-1.53 (m, 2H), 1.45 (s, 18H). **^{13}C NMR**(100 MHz, $CDCl_3$): δ 171.1, 171.0, 156.6, 155.9, 81.4, 81.4, 80.2, 80.0, 62.2, 62.1, 59.7, 58.4, 30.6, 29.4, 29.3, 28.5, 28.1, 25.5, 25.3. **MS:** M+1 (304 m/z), M+23 (326 m/z), M+39 (342 m/z), and 2M+23 (629 m/z). **HRMS (ESI-ToF)**: m/z calcd for $C_{15}H_{29}NNaO_5^+[M+Na]^+$: 326.1938, found: 326.1969.

***tert*-Butyl (S)-2-((tert-butoxycarbonyl)(methyl)amino)-5-oxopentanoate (7)**



6 **7**

To a 250 mL reactor containing alcohol **6** (6.09 g, 20.1 mmol) was added DCM (90 mL) followed sequentially by PCC (pyridinium chlorochromate, 6.50 g, 44.1 mmol) and silica gel (60-200 micron, 7.00 g) with DCM (18 mL) as a rinse. The mixture was allowed to stir at room

temperature for 1 hour after which time TLC showed complete consumption of starting material. The reaction mixture was filtered through a plug of silica gel (60-200 micron, 90.00 g) packed in heptane, and product was eluted through plug with a gradient of DCM (350 mL), 20% EA/heptane (500 mL), 40% EA/heptane (500 mL), and 100% EA (250 mL) to afford aldehyde **7** (5.67 g, 94%) as a colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.78 (d, 1H, amide rotamer), 4.59 (m, amide rotamer, 0.50 H), 4.34 (m, amide rotamer, 0.50 H), 2.77 (d, mixture of amide rotamers, 3H), 2.51-2.40 (m, 2H), 2.28-2.22 (m, 1H), 1.98 (m, 1H), 1.46 (s, 18H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ 201.4, 201.1, 177.8, 170.3, 170.2, 156.5, 155.9, 81.8, 80.6, 80.3, 59.4, 59.4, 58.6, 58.4, 40.8, 40.4, 31.4, 31.3, 30.8, 30.4, 28.5, 28.1, 24.1, 24.0, 21.7, 21.5. **HRMS (ESI-ToF)**: m/z calcd for $C_{15}H_{27}NNaO_5^+$ $[M+Na]^+$: 324.1781, found: 324.1816. HRMS also detected $[M+Li]^+$: 308.2084, $[M+K]^+$: 340.1542 and $[M+Cr]^+$: 354.4117.

Scale-up using alternative oxidation conditions using Pyridinium Dichromate (PDC)

To a 3-L 3-necked reactor containing alcohol **6** (62.5 g, 206.01 mmol) was added DCM (1.6 L) and cooled to < 10^{o}C. To the reaction mixture was added PDC (116.2 g, 309.0 mmol) and silica gel (60-200 micron, 100 g). The mixture was allowed to stir overnight (16 hr) and allowed to gradually warm to room temperature while the cooling bath was in place. The reaction

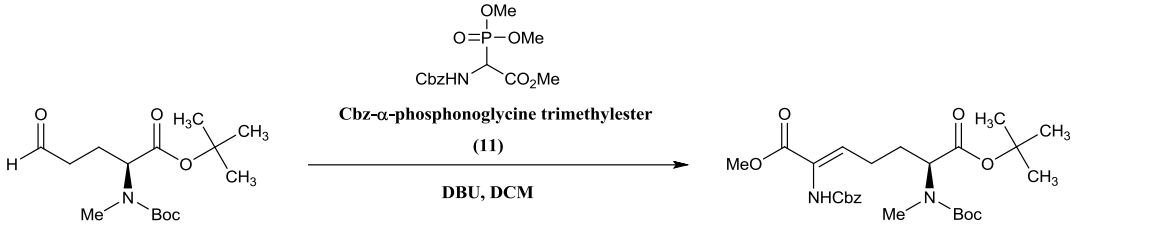
mixture was then partially concentrated to remove approximately 1 L of DCM. The reaction mixture was loaded onto a silica plug (940 g, 60-200 micron) packed in EtOAc/heptane (10/90) followed by flushing the plug with EtOAc/heptane (20/80, 2.5 L), EtOAc/heptane (30/70, 2.5 L), and EtOAc/heptane (40/60, 2.5 L) to furnish aldehyde **7** (57.43 g, 92.5%) as a colorless oil.

Scale-up using alternative Swern oxidation conditions

To a 1-L 3-necked reactor was equipped with a mechanical stirrer, J-Kem thermocouple, dropping funnel, nitrogen inlet, and a dry-ice Dewar bath. The reactor was placed under a nitrogen atmosphere and was charged with anhydrous DCM (170 mL) and anhydrous DMSO (13.2 mL, 14.5 g, 186 mmol). This mixture was cooled to -78 ^{o}C (dry ice/acetone). To the cryogenic solution (stir rate: 200 rpm) was slowly added oxalyl chloride (8.00 mL, 11.8 g, 93.3 mmol) using a syringe over a period of 6 minutes such that the temperature was maintained $\leq$ -65^{o}C. The resulting mixture was allowed to stir at -78^{o}C for an additional 15 minutes, then a solution of alcohol **6** (14.00 g, 46.15 mmol) in anhydrous DCM (100 mL) was added over 15 minutes. An additional portion of anhydrous DCM (20 mL) was used to quantitatively transfer all alcohol to the reactor. The reaction continued to stir at -78 ^{o}C for an additional 45 minutes. The rate of stirring was increased to 550 rpm, then triethylamine (57 mL, 41.61 g, 411.2 mmol) was slowly

charged to the reactor over 15 minutes such that the temperature was maintained $\leq$ -65 ^{o}C. The reaction mixture was allowed to stir at -78 ^{o}C for 30 minutes, then allowed to warm to room temperature, stirred an additional 30 minutes at ambient temperature, and quenched with 25 wt% ammonium chloride solution in USP purified water (400 mL). The pH of the mixture was adjusted to 7 using 2M HCl, then the organic layer was separated. The aqueous stream was extracted with DCM (2 x 100 mL). The combined organic layers were successfully washed with USP purified water (300 mL), brine (300 mL), dried with sodium sulfate, and concentrated under reduced pressure to provide crude aldehyde (14.93 g) as a golden yellow oil. The crude aldehyde was purified using a glass gravity column using silica gel (400 g, 60-200 micron) and the desired product was eluted with an EtOAc/Heptane gradient (0/100 to 30/70) to furnish purified aldehyde (10.8 g, 77.6% isolated yield, pale yellow oil).

(S,Z)-7-*tert*-Butyl 1-methyl 2-(benzyloxycarbonylamino)-6-(*tert*-butoxycarbonyl(methyl)amino)hept-2-enedioate (8a)



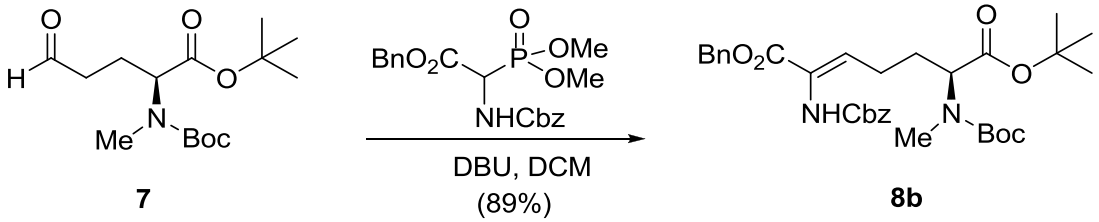
(*S*)-*tert*-butyl 2-(*tert*-butoxycarbonyl(methyl)amino)-5-oxopentanoate (7)

(*S*,*Z*)-7-*tert*-butyl 1-methyl 2-(benzyloxycarbonylamino)-6-(*tert*-butoxycarbonyl(methyl)amino)hept-2-enedioate (8a)

In a 250 mL round-bottom reactor was placed, under an argon atmosphere, (±)-Cbz-α-phosphonoglycine trimethyl ester **11** (3.96 g, 11.92 mmol) and DCM (30 mL), then DBU (1.71 g, 11.21 mmol) was added followed by a DCM rinse (7 mL). The mixture was allowed to stir at room temperature for 45 min and subsequently added to a solution of aldehyde (3.00 g, 9.93 mmol) in DCM (40 mL) over 2 minutes to give a pale-yellow solution. The reaction was allowed to stir for 2 hours and then concentrated under reduced pressure at 40-45 ^{o}C. The residue was dissolved into isopropyl acetate (100 mL) and was washed with 6% citric acid (3 x 100 mL), USP purified water (75 mL), brine (75 mL), dried with sodium sulfate for 30 min, filtered, and concentrated to give a crude oil. The crude oil (dissolved in EtOAc/heptane, 20/80) was purified over a glass gravity column (10 in x 2.75 in) packed with silica gel (Silicycle, 60-200 micron, 125 g) in heptane. The column was eluted with EtOAc/heptane gradient (10/90 to 40/60). Appropriate fractions containing pure product were combined and concentrated to afford the enamide **8a** (4.55 g, 90% yield, viscous colorless oil). **HPLC purity**: 96.4%. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.36-7.31 (m, 5 H), 6.76 (bs, amide rotamer, 0.70 H), 6.59 (m, 1 H), 6.28 (bs, 0.30 H), 5.12 (m, 2 H), 4.55 (m, 0.60 H), 4.30 (m, 0.40 H), 3.76 (bs, 3 H), 2.77-2.71 (s, 3 H, amide rotamers), 2.23 (m, 2 H), 2.01 (m, 1 H), 1.86 (m, 1 H), 1.45 (bs, 18 H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ 170.4, 165.1, 164.9, 156.9,

155.8, 154.6, 154.0, 136.4, 136.2, 135.9, 128.7, 128.6, 128.5, 128.3, 128.2, 128.1, 81.6, 80.4, 80.3, 67.5, 67.2, 59.5, 58.0, 52.4, 52.3, 30.7, 30.6, 28.3, 28.0, 27.6, 26.6, 25.2, 24.8. **HRMS (ESI-ToF)**: m/z calcd for $C_{26}H_{38}N_2NaO_8$ $[M+Na]^+$: 529.2520, found: 529.2553. HRMS also detected $[M+K]^+$: 545.2288.

1-Benzyl 7-(*tert*-butyl) (S,Z)-2-(((benzyloxy)carbonyl)amino)-6-((*tert*-butoxycarbonyl)(methyl)amino)hept-2-enedioate (8b)

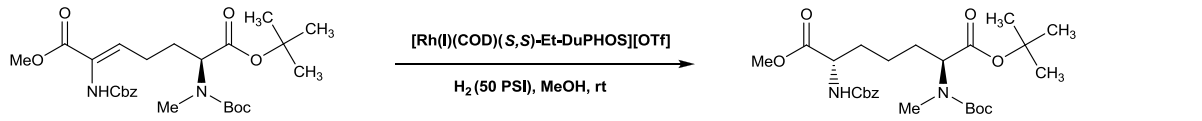


7 **8b**

To a 500 mL reactor was added benzyl Z-phosphinoglycine dimethyl ester **13** (22.00 g, 54.00 mmol) and DCM (100 mL). At ambient temperature, DBU (7.88 g, 51.65 mmol) was added to the mixture under argon. After 30 minutes, a solution of aldehyde **7** (14.15 g, 84% pure based on ^{1}H NMR, 39.44 mmol) in DCM (150 mL) was slowly added *via* dropping funnel over 10 min. The reaction mixture was allowed to stir at room temperature for 21 h and subsequently was concentrated, diluted with ethyl acetate (350 mL), and washed with USP purified water (175 mL). The aqueous stream was extracted with ethyl acetate (2 x 85 mL). The combined organic streams were successively washed with 6% citric acid (110 mL), saturated sodium bicarbonate (110 mL), brine (110 mL), dried

with sodium sulfate and concentrated to provide the crude enamide product (30.67 g) as a yellow oil. The crude product dissolved in diethyl ether (350 mL) and silica gel (48 g, 60-200 micron) were combined and concentrated to dryness to afford a dry-loaded material that was placed in a 750 g cartridge coupled to another cartridge containing silica gel (615 g, 60-200 micron) equilibrated with heptane. Purification was performed on a Teledyne Isco Rf Flash chromatography unit eluting with a step-gradient of 100% heptane (2 CV), 2% IPA in heptane (5 CV), and 5% IPA in heptane (4.5 CV) at 200 mL/min providing pure enamide Horner-Emmons product **8b** (21.40 g, 93%) as a colorless viscous oil. **HPLC purity:** 98.4%. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.34 (bm, 10H), 6.77 (bs, amide rotamer, 0.60H), 6.62 (m, 1H), 6.29 (bs, amide rotamer, 0.40H), 5.19 (s, 2H), 5.11 (d, amide rotamer, 2H), 4.55 (m, amide rotamer, 0.60 H), 4.29 (m, amide rotamer, 0.40 H), 2.73 (d, mixture of amide rotamer, 3H), 2.24 (m, 2H), 2.02 (m, 1H), 1.98 (m, 1H), 1.85 (m, 1H), 1.43 (s, 18H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ 170.5, 164.5, 164.4, 156.9, 155.8, 154.6, 154.1, 136.7, 136.2, 136.0, 135.7, 135.5, 128.6, 128.6, 128.5, 128.3, 128.2, 81.7, 80.4, 80.4, 67.5, 67.4, 67.3, 67.2, 59.6, 58.2, 30.7, 28.4, 28.1. **HRMS (ESI-ToF):** m/z calcd for $C_{32}H_{42}N_2NaO_8^+[M+Na]^+$:605.2833, found: 605.2928. HRMS also detected $[M+K]^+$:621.2646.

(2S,6S)-7-*tert*-Butyl 1-methyl 2-(benzyloxycarbonylamino)-6-(*tert* butoxycarbonyl(methyl)amino)heptanedioate (9a)



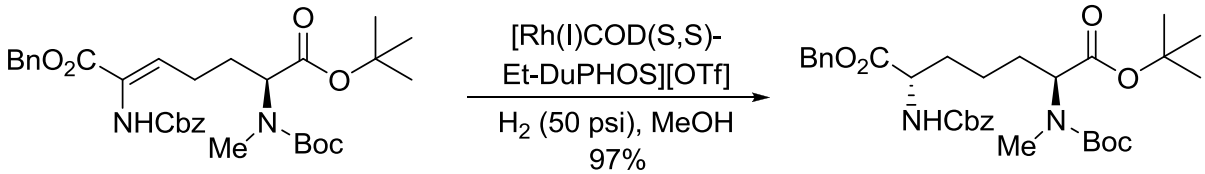
(*S*,*Z*)-7-*tert*-butyl 1-methyl 2-(benzyloxycarbonylamino)-6-(*tert*-butoxycarbonyl(methyl)amino)hept-2-enedioate (8a)

(2*S*,6*S*)-7-*tert*-butyl 1-methyl 2-(benzyloxycarbonylamino)-6-(*tert*-butoxycarbonyl(methyl)amino)heptanedioate (9a)

To a Mettler Toledo RC1e system equipped with a MP06-HC 1.2-liter pressure glass reactor, mechanical stirrer, and an external temperature control unit under nitrogen was added a solution of enamide **8a** (4.00 g, 7.90 mmol) in HPLC-grade methanol (110 mL, degassed with argon). A solution of Rh(I)(COD)(*S,S*)-Et-DuPHOS catalyst (86 mg, 0.12 mmol) in degassed methanol (HPLC grade, 10 mL) was added and the reactor was pressurized with nitrogen (3x) and hydrogen (3x), then placed under 50 psi of hydrogen. The mixture was agitated with mechanical stirring (500 rpm) at 25°C for 25 h after which ^{1}H NMR indicated complete consumption of the alkene. The reaction mixture was purged with nitrogen three times and then concentrated under reduced pressure. The crude residue (4.04 g) was dissolved in ethyl acetate/heptane (50/50, 8 mL) and passed through a silica gel plug (20.5 g) eluting with ethyl acetate/heptane (50/50, 200 mL) to afford Cbz-protected amino acid derivative **9a** (3.74g, 93% yield) as a colorless viscous syrup. **HPLC purity (achiral)**: 100% AUC (area under the

curve). **Chiral HPLC**: e.e. = 97.4%. Chiral HPLC was performed using a Chiralpak AD-H column (250x4.6 mm, 5 micron) eluting with a gradient of hexane/EtOH (90/10 to 30/70) over 20 min at 1.0 mL/min and monitoring at 210 nm. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.36-7.30 (m, 5 H), 5.30-5.10 (s, 3 H, amide rotamers), 4.60 (m, 0.55 H), 4.34 (m, 1.45 H, amide rotamer), 3.74-3.68 (s, 3 H, amide rotamers), 2,76-2.71 (s, 3 H, amide rotamers), 1.83 (m, 2 H), 1.74 (m, 2 H), 1.44-1.26 (m, 20 H). **^{13}C NMR** (100 MHz, CDCl3): δ 172.9, 172.8, 170.9, 170.7, 156.5, 156.0, 155.8, 136.2, 128.5, 128.1, 81.4, 81.3, 80.1, 79.9, 67.0, 59.4, 57.9, 53.7, 53.6, 52.4, 32.1, 31.9, 30.5, 28.4, 28.0, 21.8. **HRMS (ESI-ToF):** m/z calcd for $C_{26}H_{40}N_2NaO_8^+$ $[M+Na]^+$: 531.2677, found: 531.2726.

1-Benzyl-7-(*tert*-butyl) (2S,6S)-2-(((benzyloxy)carbonyl)amino)-6-((*tert*-butoxycarbonyl)(methyl)amino)heptanedioate (9b)



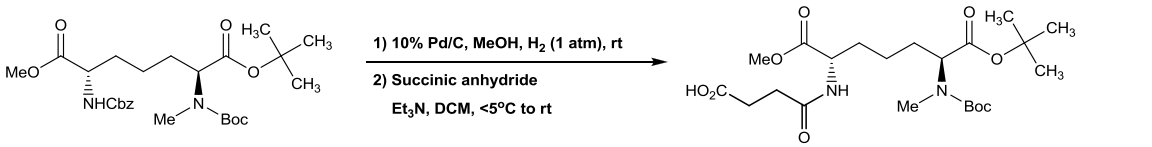
8b **9b**

To a Mettler Toledo RC1e system equipped with a MP06-HC 1.2-liter pressure glass reactor, mechanical stirrer, and an external temperature control unit under nitrogen was added a solution of enamide **8b** (19.9 g, 34.1mmol) in HPLC grade methanol (110 mL, degassed with argon). This

mixture was sparged with argon for an additional 30 min. A solution of Rh(I)(COD)(*S,S*)-Et-DuPHOS catalyst (378 mg, 0.52 mmol) in degassed methanol (HPLC grade, 50 mL) was added and the reactor was pressurized sequentially with nitrogen (3x), hydrogen (3x), and finally placed under 50 psi of hydrogen. The mixture was agitated with mechanical stirring (500 rpm) at room temperature for 21 hours after which ^{1}H NMR indicated complete consumption of the alkene. The reaction mixture purged with nitrogen three times, then concentrated under reduced pressure. The crude residue (19.7 g) was dissolved in ethyl acetate/heptane (50/50, 45 mL) and passed through a silica gel plug (106 g) using ethyl acetate/heptane (50/50, 600 mL) to afford Cbz-protected amino acid derivative **9b** (19.3 g, 97% yield) as a colorless viscous oil. **HPLC analysis (achiral):** 95.1%. **Chiral HPLC:** e.e. = 92%. Chiral HPLC was performed using a RegisPack column (250x4.6 mm, 5 micron) eluting with an isocratic gradient for 15 minutes consisting of hexane/IPA (70/30) + 0.1%DEA at 1.5 mL/min and monitoring at 220 nm. **^{1}H NMR**(400 MHz, $CDCl_3$): δ 7.35 (m, 10 H), 5.28 (t, 0.89 H, *J* = 8.0 Hz, amide rotamer), 5.17-5.10 (m, amide rotamer, 4.10 H), 4.56 (m, 0.53 H, amide rotamer), 4.41 (m, 0.90 H, amide rotamer), 4.23 (m, 0.58 H, amide rotamer), 2.71 (d, mixture of amide rotamers, 3H), 1.89-1.59 (m, 4 H), 1.50-1.25 (m, 20 H**). ^{13}C NMR** (100 MHz, $CDCl_3$): δ 172.4, 172.3, 170.9, 170.8, 156.6, 156.1, 156.0, 155.9, 136.3, 135.4, 135.3, 128.7,

128.6, 128.5, 128.4, 128.3, 128.2, 81.5, 81.4, 80.2, 80.0, 67.3, 67.3, 67.1, 59.6, 58.1, 54.0, 53.8, 32.2, 32.0, 30.6, 28.6, 28.5, 28.1, 22.02. **HRMS (ESI-ToF):** m/z calcd for $C_{32}H_{44}N_2NaO_8^+$ $[M+Na]^+$: 607.2990, found: 607.3088. HRMS also detected $[M+K]^+$:623.2815.

4-((2S,6S)-7-*tert*-Butoxy-6-(tert-butoxycarbonyl(methyl)amino)-1-methoxy-1,7-dioxoheptan-2-ylamino)-4-oxobutanoic acid (10a)



(2*S*,6*S*)-7-*tert*-butyl 1-methyl 2-(benzyloxycarbonylamino)-6-(*tert*-butoxycarbonyl(methyl)amino)heptanedioate (9a)

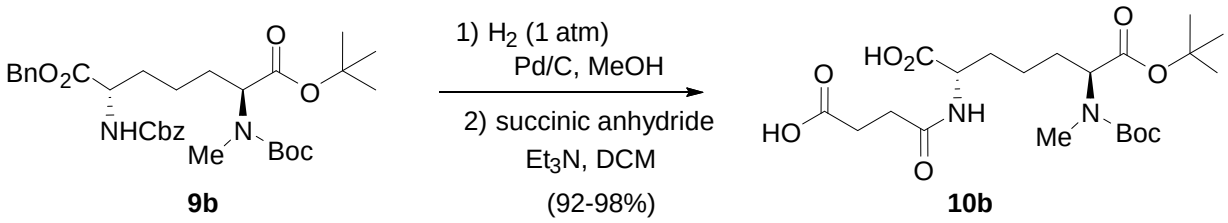
4-((2*S*,6*S*)-7-*tert*-butoxy-6-(*tert*-butoxycarbonyl(methyl)amino)-1-methoxy-1,7-dioxoheptan-2-ylamino)-4-oxobutanoic acid (10a)

To a 250 mL reactor under argon was added a solution of **9a** (3.15 g, 6.19 mmol) in HPLC grade methanol (85 mL). The mixture was sparged with argon for 10 min followed by addition of 10% Pd/C (320 mg). The reactor was flushed with argon for 5 min, hydrogen for 5 min, then placed under balloon pressure of hydrogen and allowed to stir overnight (16 h) at room temperature, when TLC analysis (EtOAc/DCM/MeOH, 20/75/5) indicated complete consumption of starting material. The mixture was sparged with argon, filtered over a pad of Celite®, rinsed with methanol (50 mL) and the resulting filtrate was concentrated and chased with toluene to provide the free deprotected amino methyl ester (2.34 g, 100%) as a colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.60 (m, 0.50 H, amide rotamer), 4.32 (m, 0.50 H, amide rotamer), 3.72 (s, 3H), 3.47 (m, 1H), 2.77 (s, 3H, amide rotamer),

1.90-1.60 (m, 6H), 1.47 (m, 20H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ 176.5, 171.2, 171.0, 156.5, 156.0, 81.4, 81.3, 80.2, 79.9, 59.6, 58.3, 54.4, 54.3, 52.1, 34.4, 34.4, 30.7, 28.8, 28.5, 28.1, 22.4. **HRMS (ESI-ToF):** m/z calcd for $[M+H]^+$ for $C_{18}H_{35}N_2O_6^+$: 375.2490, found: 375.2514.

To a 250 mL reactor was added Cbz-deprotected amine (2.25 g, 6.01 mmol), succinic anhydride (0.66 g, 6.60 mmol), and DCM (50 mL). The mixture was cooled to < 5°C and triethylamine (0.92 mL, 6.60 mmol) was added dropwise over 2 min. The cooling bath was removed, and the reaction was allowed to stir overnight at room temperature. After 24 hours, the reaction mixture was diluted with DCM (50 mL), washed sequentially with 6% citric acid (2 x 50 mL), brine (25 mL), dried with sodium sulfate, filtered, and concentrated under reduced pressure and chased with DCM and heptane to furnish **10a** (2.90 g, 99%) as a colorless viscous syrup, which was used without further purification.**^{1}H NMR** (400 MHz, $CDCl_3$): δ 6.43 (d, 0.50H, *J* = 8.0 Hz, amide rotamer), 6.32 (d, 0.50H, *J* = 8.0 Hz, amide rotamer), 4.69-4.57 (m, 1.5H, amide rotamer), 4.36-4.32 (m, 0.50H, amide rotamer), 3.75 (d, 3H, mixture of amide rotamers), 2.83-2.72 (m, 4H), 2.65-2.42 (m, 3H), 1.88-1.70 (m, 5H), 1.46-1.44 (m, 19H). **HRMS (ESI-ToF):** m/z calcd for $C_{22}H_{38}N_2NaO_9^+$ $[M+Na]^+$: 497.2470, found: 497.2493.

4-(((2S,6S)-1-(Benzyloxy)-7-(*tert*-butoxy)-6-((*tert*-butoxycarbonyl)(methyl)amino)-1,7-dioxoheptan-2-yl)amino)-4-oxobutanoic acid (10b)



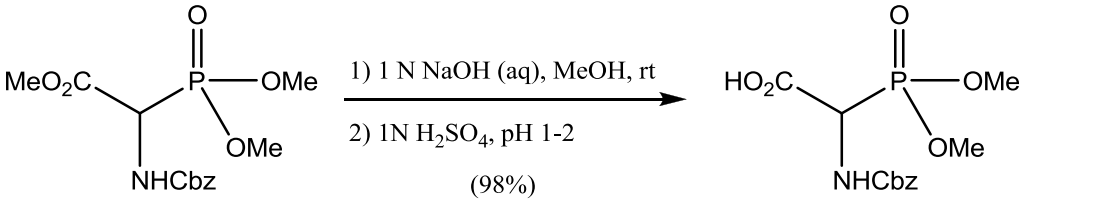
9b **10b**

To a 500 mL reactor was added Cbz-protected amino acid **9b** (10.65 g, 18.2 mmol) and methanol (275 mL). The mixture was sparged with nitrogen for 10 min followed by addition of 10% Pd/C (1.04 g). The reactor was flushed with nitrogen for 5 min, hydrogen for 5 min, then placed under balloon pressure of hydrogen for 6 h. The mixture was treated with Celite® (5.0 grams) and sparged with nitrogen for 10 min, filtered over a pad of Celite®, and rinsed with methanol (200 mL). The resulting filtrate was concentrated to provide the free **amino acid intermediate 10b** (6.39 g, 97% yield) as an off-white solid. **HPLC purity:** 100%. UPLC/MS analysis of the free amino acid: $[M+H]^+$ = 361.3. **HRMS (ESI-ToF):** m/z calcd $[M+H]^+$ for $C_{17}H_{33}N_2O_6^+$: 361.2333, found: 361.2377. HRMS also detected $[M+Na]^+$: 383.2194 and $[M+K]^+$: 399.1936. The crude **free amino acid intermediate10b** was carried forward without any further purification.

To a 500 mL reactor was added the **free amino acid intermediate 10b** (6.02 g, 16.7 mmol), succinic anhydride (1.73 g, 17.3 mmol), and DCM (175 mL). The mixture cooled to < 5°C and triethylamine (3.66 g, 36.17 mmol) was added dropwise over 3 min. The cooling bath was removed, and the reaction was allowed to stir for 2 h at room temperature. HPLC/UPLC/TLC analysis showed that the reaction was deemed complete. The reaction mixture was concentrated, diluted with EtOAc (300 mL), washed sequentially with 0.10 N HCl (390 mL), USP purified water, (250 mL), brine (200 mL), dried with sodium sulfate, filtered, and concentrated to provide crude **10b** (7.40 g). The crude product **10b** was dissolved in EtOAc (25 mL) and was purified by passing through a silica gel plug (52 g, 60-200 micron, equilibrated with EtOAc/THF then EtOAc). The silica plug was flushed with EtOAc (100 mL) and EtOAc/THF (1:1, 250 mL). Appropriate fractions were combined, concentrated, and chased with DCM followed by anhydrous 1,4-dioxane affording pure succinamide adduct **10b** (7.10 g, 92% yield). **HPLC purity:** 98.6% AUC**. Chiral HPLC:** e.e. = 100%. Chiral HPLC was performed using a RegisPack column (250x4.6 mm, 5 micron) eluting with an isocratic gradient for 15 minutes consisting of hexane/IPA (90/10) + 0.1%TFA at 1.5 mL/min and monitoring at 210 nm. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 10.74 (bs, 2H), 7.10 (d, 1H, *J* = 8.0 Hz), 4.65-4.51 (m, amide rotamer, 1.5H), 4.29-4.27 (m, amide rotamer, 0.5H), 2.78-2.49 (m, mixture

of amide rotamers, 7H), 1.87-1.60 (m, 4H), 1.45 (d, 18H), 1.38-1.16 (m, 2H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ 176.5, 176.5, 175.5, 175.4, 173.1, 172.9, 170.8, 157.1, 156.8, 81.7, 81.0, 80.8, 67.1, 59.9, 58.4, 53.5, 52.5, 52.1, 31.3, 30.7, 30.6, 30.4, 29.7, 28.5, 28.3, 28.1, 22.3, 21.7. **HRMS (ESI-ToF):** m/z calcd for $C_{21}H_{36}N_2NaO_9$ $[M+Na]^+$: 483.2313, found: 483.2368. HRMS also detected $[M+K]^+$: 499.2093.

(±)-Cbz-α-phosphonoglycine dimethyl ester (12)



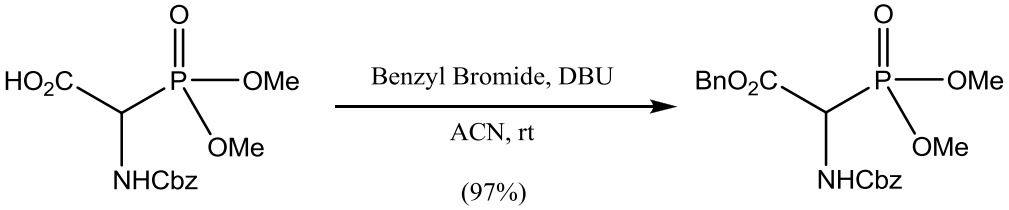
(±)-Cbz-α-phosphonoglycine trimethyl ester (11)

(±)-Cbz-α-phosphonoglycine dimethyl ester (12)

According to a reported patent procedure,[42] to a 1 L 3-neck reactor equipped with a mechanical stirrer, J-Kem thermocouple, nitrogen inlet, and water bath (20-25°C), were added (±)-Cbz-α-phosphonoglycine trimethyl ester **11** (33.13 g, 100.0 mmol), MeOH (180 mL), and USP purified water (20 mL). To the slightly hazy mixture was added a solution of 1N aq NaOH (102 mL, 102.0 mmol) *via* dropping funnel over a period of 1 hr to give a homogeneous mixture. The resulting mixture was allowed to stir for an additional 1 hr at room temperature and subsequently concentrated under reduced pressure at 25-30°C to remove methanol. The aqueous residue was polish filtered through filter paper and pH adjusted to pH 1.5 with 1N

H_2SO_4solution (100 mL) to provide a white murky solution. The mixture was extracted with EtOAc (4 x 150 mL) and the combined organic streams were washed with brine (150 mL), dried with magnesium sulfate, and concentrated under reduced pressure to afford (±)-Cbz-α-phosphonoglycine dimethyl ester **12** (30.98 g, 98%) as a viscous oil that solidifies to a white solid upon standing. **HPLC purity:** 97.4%. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.90 (bs, 2H), 7.36-7.29 (m, 5H), 5.96 (d, 1H, *J* = 12.0 Hz), 5.16 (d, 1H, *J* = 12.0 Hz), 5.11 (d, 1H, *J* = 12.0 Hz), 5.00 (dd, 1H, *J* = 24.0, 8.0 Hz), 3.84 (d, 3H, *J* = 8.0 Hz), 3.79 (d, 3H, *J* = 8.0 Hz). **HRMS (ESI-ToF):** m/z calcd for $C_{12}H_{17}NO_7P^+$ $[M+H]^+$: 318.0737, found: 318.0782. HRMS also detected $[M+Na]^+$: 340.0599 and $[M+K]^+$: 356.0319.

Benzyl 2-(benzyloxycarbonylamino)-2-(dimethoxyphosphoryl)acetate (13)



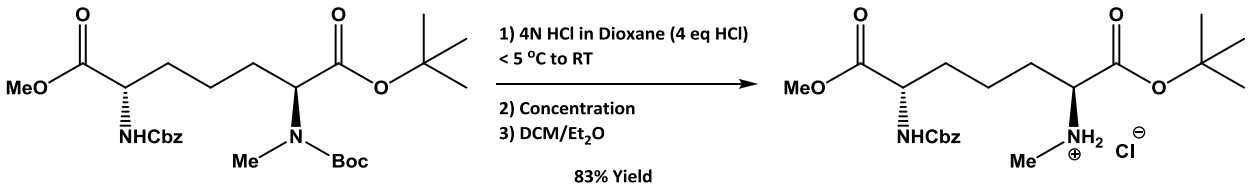
(±)-Cbz-α-phosphonoglycine dimethyl ester (12)

benzyl 2-(benzyloxycarbonylamino)-2-(dimethoxyphosphoryl)acetate (13)

According to the general and herein improved procedure of Nakanishi,[43] a 500 mL 3-neck reactor was charged with (±)-Cbz-α-phosphonoglycine dimethyl ester **12** (30.77 g, 97.00 mmol), ACN (80 mL), and DBU (15.2 g, 21.0 mL, 99.9 mmol) to provide a clear solution. To the

reaction mixture was added benzyl bromide (17.4 g, 102 mmol) dropwise over 5 min which a cooling bath (ambient temperature) was used to control the exotherm. After 2 hours at room temperature, the reaction mixture was concentrated under reduced pressure and diluted with EtOAc (350 mL). The organic mixture was washed with USP purified water (130 mL), 3% citric acid solution (1 x 110 mL), saturated $NaHCO_3$ solution (100 mL), brine (100 mL), dried with sodium sulfate, and concentrated under reduced pressure to give crude product (43.5 g). The crude product was dissolved in EtOAc/heptane (1:1, 90 mL) at 45^{o}C, seeded, and diluted with heptane (350 mL) and the resulting slurry was allowed to stir an additional 1 hr at ambient temperature, and filtered. The filter cake was washed with heptane (50 mL) and pulled dry to give benzyl 2-(benzyloxycarbonylamino)-2-(dimethoxyphosphoryl)acetate **13** (38.7 g, 98%) as a white solid. **HPLC purity:** 95.9% AUC. **^{1}H NMR:** (400 MHz, $CDCl_3$): δ 7.35 (m, 10H), 5.62 (d, 1H, *J* = 12.0 Hz), 5.29 (d, 1H, *J* =12.0 Hz), 5.22 (d, 1H, *J* = 12.0 Hz), 5.14 (dd, 2H, *J* = 12.0, 4.0 Hz), 4.96 (dd, 24.0, 12.0 Hz), 3.72 (d, 6H. *J* = 12.0 Hz). **HRMS (ESI-ToF):** m/z calcd for $C_{19}H_{23}NO_7P^+$ $[M+H]^+$:408.1207, found: 408.1255. HRMS also detected $[M+Na]^+$: 430.1061 and $[M+K]^+$: 446.0810.

(2S,6S)-6-(Benzyloxycarbonylamino)-1-*tert*-butoxy-7-methoxy-N-methyl-1,7-dioxoheptan-2-aminium chloride (14)

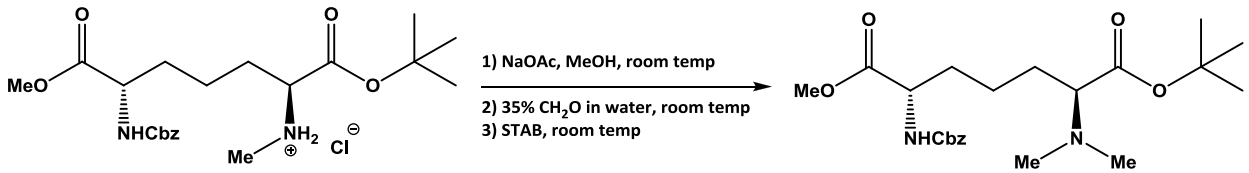


Boc-Protected Monomethyl DAP Bis-Ester
Compound 9a

2-N-CBz-6-Monomethyl DAP Bis-Ester HCl Salt
Compound 14

To a 20 mL scintillation vial was added Boc-protected monomethyl DAP Bis-ester **9a** (400 mg, 0.80 mmol), magnetic stirring bar, and anhydrous 1,4-dioxane (4 mL). The mixture was purged with argon and then cool to 0-5 ^{o}C, then a solution of 4N HCl in 1,4-dioxane (0.8 mL, 3.2 mmol) was added. The thick slurry was allowed to stir at 0-5 ^{o}C for 20 minutes then allowed to stir overnight at room temperature. After 26 hours at ambient temperature, the reaction mixture was deemed complete by HPLC analysis. The reaction mixture was concentrated under nitrogen, diluted with DCM (3 mL), and diethyl ether (4 mL) was added to precipitate the desired product. The slurry was allowed to stir overnight at room temperature. The following day, the slurry was filtered and the filter cake was washed with diethyl ether (2 x 4 mL), pulled dry, and further dried *in vacuo* at room temperature to provide the monomethyl hydrochloride product **14** (290 mg, 83% yield) as a white solid and taken forward without further purification. **HPLC purity**: 96% AUC. **MS (ESI)**: m/z calcd for $C_{21}H_{33}N_2O_6^+$ $[M+H]^+$: 409.5, found: 409.5.

(2S,6S)-7-*tert*-Butyl 1-methyl 2-(benzyloxycarbonylamino)-6-(dimethylamino)heptanedioate (15)



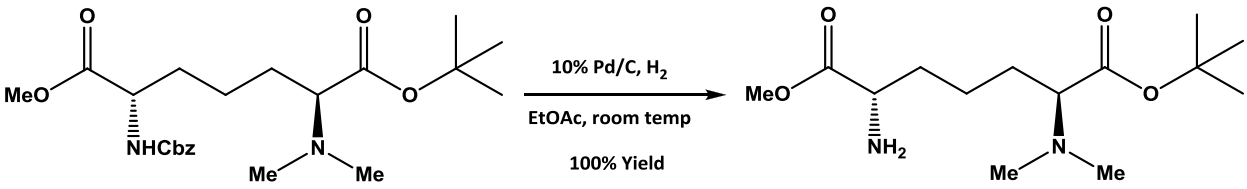
2-N-CBz-6-Monomethyl DAP Bis-Ester HCl Salt
Compound 14

2-N-Cbz-6-Dimethyl DAP Bis-Ester
Compound 15

To a 10 mL vial was added monomethyl HCl salt **14** (150 mg, 0.34 mmol), magnetic stirring bar, sodium acetate (29 mg, 0.35 mmol), and methanol (HPLC grade, 1.0 mL). The mixture was allowed to stir for 5 minutes at room temperature, then a solution of 35% formaldehyde in water (40 µL, 0.50 mmol, stabilized with methanol) was added. The mixture was allowed to stir 15 minutes at room temperature, then sodium triacetoxyborohydride (STAB, 143 mg, 0.67 mmol) was added in 5 portions. The reaction mixture was allowed to stir overnight at room temperature. After 17 hours, the reaction was sampled for HPLC analysis which indicated that the reaction was complete. The reaction mixture was added to ethyl acetate (10 mL) and to this mixture was added USP purified water (6 mL). The mixture was allowed to stir for 5 minutes, then a saturated aqueous sodium bicarbonate solution (2 mL) was added to adjust the pH of the aqueous phase to pH 8-9. The organic layer was separated and the aqueous phase was extracted with ethyl acetate (3 x 4 mL). The combined organic layers were washed with brine, dried with sodium sulfate, and concentrated

under reduced pressure to afford dimethyl DAP bis-ester product **15** (136 mg, 96% yield) as a colorless oil and taken forward without further purification. **HPLC purity**: 100% AUC. **MS (ESI)**: m/z calcd for $C_{22}H_{35}N_2O_6^+$ $[M+H]^+$: 423.5, found: 423.5.

(2S,6S)-7-*tert*-Butyl 1-methyl 2-amino-6 (dimethylamino)heptanedioate (16)



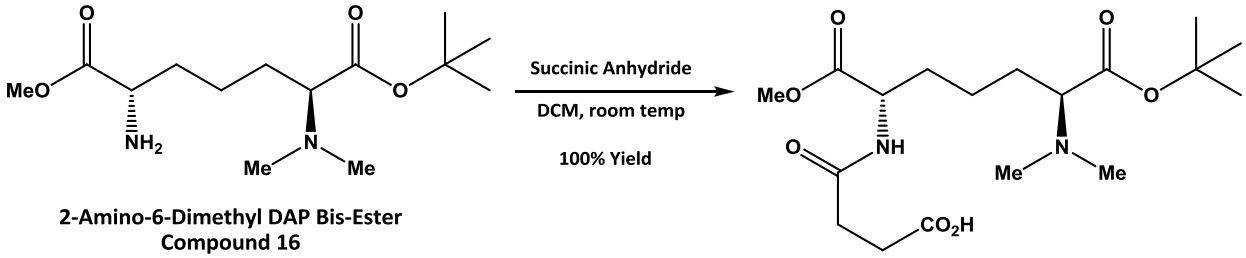
2-N-Cbz-6-Dimethyl DAP Bis-Ester Compound 15

2-Amino-6-Dimethyl DAP Bis-Ester Compound 16

To a 10 mL vial were added dimethyl DAP bis-ester product **15** (117 mg, 0.26 mmol), a magnetic stirring bar, and ethyl acetate (3 mL). The solution was sparged with argon for 5 minutes at room temperature then 10% Pd/C (20 mg) was added. The mixture was sparged for an additional 2 minutes with argon, then sparged with hydrogen for 5 minutes, and finally placed the reaction mixture under hydrogen atmosphere (~1 atm, balloon pressure). After 5 hours at room temperature, TLC analysis showed complete consumption of Cbz-protected starting material (Rf = 0.35 in EtOAc/DCM/MeOH, 20/75/5). The reaction mixture was filtered over a pad of Celite®, the filter cake was washed with ethyl acetate (2 x 5 mL), and the corresponding filtrate was concentrated under reduced pressure to provide

the free amine **16** (76 mg, 100% yield) as a colorless viscous oil. The free amine product was taken forward without any further purification.

4-((2S,6S)-7-*tert*-Butoxy-6-(dimethylamino)-1-methoxy-1,7-dioxoheptan-2-ylamino)-4-oxobutanoic acid (17)

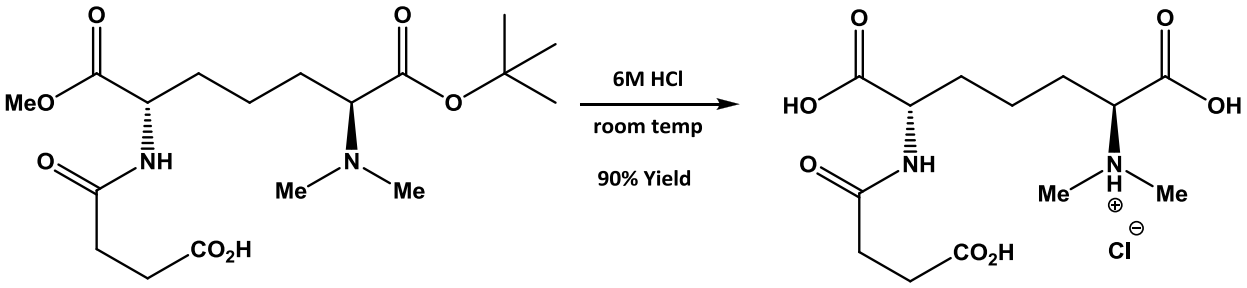


2-Amino-6-Dimethyl DAP Bis-Ester
Compound 16

Dimethyl L,L-SDAP Bis-Ester
Compound 17

To a 10 mL vial containing free amine **16** (76 mg, 0.26 mmol) was added DCM (2 mL) and a magnetic stirring bar. To the reaction mixture was added succinic anhydride (26 mg, 0.26 mmol) and DCM (1 mL) as a rinse. The resulting reaction mixture was allowed to stir overnight at ambient temperature. After 16 hours, the reaction mixture was analyzed by TLC analysis (EtOAc/$CHCl_3$/MeOH/H_2O/AcOH, 50/24/18/5.5/2.5, ninhydrin stain) which revealed that the free amine (Rf = 0.07, red spot) was entirely consumed and a new spot appeared (desired succinate amide, Rf = 0.28, yellowish-peach spot). The reaction mixture was concentrated under reduced pressure to provide protected dimethyl L,L-SDAP **17** (103 mg, 100% yield) as a colorless glass film and was carried forward without any further purification.

(1S,5S)-1,5-Dicarboxy-5-(3-carboxypropanamido)-N,N-dimethylpentan-1-aminium chloride (18)



Dimethyl L,L-SDAP Bis-Ester Compound 17

Dimethyl L,L-SDAP HCl Salt Compound 18

To a 10 mL vial containing protected dimethyl L,L-SDAP **17** (102 mg, 0.26 mmol) was added a magnetic stirring bar, and 6M HCl (2 mL). Purged with nitrogen and the reaction mixture was allowed to stir at room temperature. After 7 hours at room temperature, ^{1}H NMR analysis revealed that the reaction mixture contained 96% desired product and 4% unreacted starting material. The reaction mixture was concentrated under reduced pressure to provide dimethyl L,L-SDAP HCl salt **18** as a white foam (82 mg, 90% yield). **^{1}H NMR**: (400 MHz, $CDCl_3$): δ 4.41 (dd, 1H, *J* = 8.0, 4.0 Hz), 3.87 (dd, 1H, *J* = 12.0, 8.0 Hz), 2.95 (bs, 3H), 2.92 (bs, 3H), 2.71-2.60 (m, 4H), 2.05-1.93 (m, 3H), 1.87-1.76 (m, 1H), 1.62-1.41 (m, 2H).

CHAPTER FOUR

PROCESS-SCALE ASYMMETRIC SYNTHESIS OF L,L-DAP, N^6-MONOMETHYL-L,L-SDAP, AND N^6,N^6-DIMETHYL-L,L-SDAP ENABLING A NEW DAPE INHIBITION ASSAY

Carboranes are boron cage molecules that have found use in the treatment of diseases including various cancers and rheumatoid arthritis, most notably through boron neutron capture therapy (BNCT). Carboranes are also distinguished with a rich and diverse synthetic chemistry.[1] BNCT is a useful binary cancer treatment, in which a drug containing ^{10}B atoms is selectively transported into tumor cells and then irradiated with thermal neutrons. Thereupon, a ^{10}B nucleus absorbs a neutron to form an excited^{11}B nucleus that undergoes decay *via* fission, emitting an α-particle ($^4He^{2+}$) as well as a $^7Li^{3+}$ ion, both with high kinetic energy. These highly charged particles damage the surrounding tissue, but they have a range of only about one cell diameter (5-9 μm), which limits the radiation damage to the cell in which they arise, thus avoiding damage to the surrounding tissue.[2]

As noted recently,[3]BNCT is a very promising treatment for cancers given its exquisitely ability to target cancer cells, given that it relies on the induced fission of boron within tumor cells. Ideally, a BNCT agent should

concentrate in tumor cells while avoiding healthy cells. The U.S. FDA-approved BNCT drugs boronophenylalanine (BPA), sodium borocaptate and sodium decahydrodecaborate and sodium decahydrodecaborate, which were developed in the 1950's and 1960's are neither tumor-specific nor do they accumulate with in tumor cells.[3] Thus, there is an urgent need for boron-containing therapeutics that both contain a higher density of boron atoms, i.*e*. which have a high neutron-capture cross section, and which are selective for tumor cells. Similarly, agents are needed for boron neutron capture synovectomy for the treatment of severe cases of rheumatoid arthritis, which works through the same principle and mechanism as BNCT for cancer.[1]

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that are involved in the remodeling and degradation of all components of the extracellular matrix (ECM).[4] MMP enzymes play a key role in normal development, morphogenesis, bone remodeling, wound healing, and angiogenesis, yet inappropriately high MMP activity has been implicated in a number of disease states including tumor growth and metastasis and in the degradation of articular cartilage in arthritis.[5] Thus, MMP enzymes are over expressed in both tumors and in arthritic cartilage. In order to halt disease progression resulting from exaggerated matrix remodeling mediated by MMPs, MMP inhibitors (MMPi's) have been

extensively explored with the goal of discovering and developing potent, selective, and druggable MMP inhibitors.[6-8]

MMPs are known to be over expressed in tumors and also in articular cartilage in patients suffering from rheumatoid and osteoarthritis. Interestingly, MMP inhibitors have been used in the imaging of cancer cells by binding tightly and specifically to MMP receptors.[9] The goal of this research is to discover orally bioavailable MMP inhibitors that bind with high potency and selectivity to MMP enzymes, for it is these enzymes that are over expressed in tumors and in arthritic tissues enabling the delivery of a high density of boron atoms to tumor and/or arthritic tissue, thus with a high neutron-capture cross section, to enable binary treatment of tumors utilizing BNCT as well as the treatment of RA with neutron therapy. In addition, it is appropriate to target potent inhibitors of a several MMP enzymes specifically, including the collagenase MMP-13 and the gelatinases MMP-2 and MMP-9, but with much lower potencies at MMP-1 and MMP-7 enzymes, enabling these inhibitors to inhibit angiogenesis, invasion, and metastasis of tumors as well as inhibiting the MMP-induced destruction of articular cartilage.

The presence of fluorine (^{19}F) atoms in several derivatives among the presently-described ligands enables the use of magnetic resonance spectroscopy for the detection and localization of fluorinated drug in tumors,

which will be very valuable in demonstrating the localization of tissues to be irradiated with neutrons in therapy.[10]

Section 1: Synthesis of BNCT Analogs and Discussion

Carborane-containing MMP inhibitors to be applied in BNCT have been designed and synthesized. A multitude of synthetic targets were explored intellectually, and some were carried out experimentally. The synthesis of carborane derived analogs were constructed by utilizing the alkyne moiety of the THP-protected hydroxamate (**Cmpd9**). The nature and reactivity of alkynes, both internal and terminal, enables the use of many different types of chemistry to covalently bind carboranyl clusters. In this work, the chemistry focuses on the use of *ortho*-*closo*-carboranes. The chemistries that were evaluated focused around the reactivity of alkynes with specific reactants carrying the carboranyl cluster. More specifically, the chemistries evaluated include the reaction of alkynes with an activated decaborane complex ($B_{10}H_{12}$*2MeCN) forming ortho-closo-carboranes, reaction of alkynes and azides either *via* thermal Huisgen 1,3-dipolar cycloaddition or copper-mediated azide-alkyne cycloaddition (CuAAC), or the ruthenium-catalyzed azide alkyne cycloaddition (RuAAC) affording triazole compounds, as well as reaction of alkynes with thiols affording alkenyl sulfides.

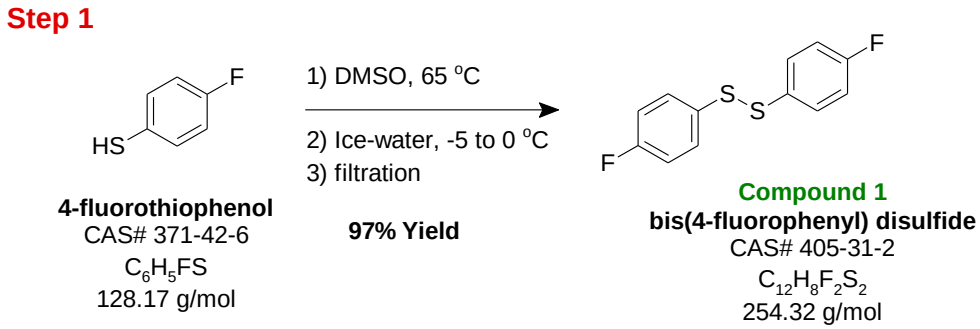
The chemistry begins with the preparation of THP-MMP-18 (**Cmpd 9**). The synthetic route was based on the literature preparation of THP-MMP-

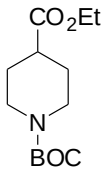
18[7,8] and the route was improved during process development. In order to prepare carboranyl derivatives using the THP-MMP-18 scaffold, sufficient quantities of the MMP-18 starting material was synthesized. The alkyne was selected based on the fact that decaborane can react to produce carboranyl derivatives, but the alkyne can also be used in Click chemistry to produce triazolecarboranyl derivatives.

Section 2: Preparation and Process Development of THP-MMP-18 (Cmpd 9)

Preparation of the THP-MMP-18 (**Cmpd 9**) was accomplished using a modified procedure based on Becker *et al*.[7,8] The improved preparation of THP-MMP-18 is illustrated in Scheme 24.

Scheme 24. Improved Synthetic Route to THP-MMP-18 (Cmpd 9)





Compound 2
Ethyl N-Boc-piperidine-4-carboxylate
CAS# 142851-03-4
257.33 g/mol

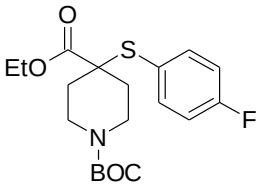
Step 2

1) LDA then

Compound 1

2) Silica plug

86% Yield



Compound 3
$C_{19}H_{26}FNO_4S$
383.48 g/mol

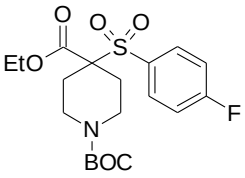
Step 3

1) UHP, Phthalic Anhydride
EtOAc, room temp

2) Aq Sodium Sulfite

3) Adjust pH to 8

99% Yield



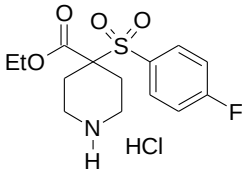
Compound 4
$C_{19}H_{26}FNO_6$
415.48 g/mol

Step 4

HCl (g)

IPOAc

100% Yield



Compound 5
$C_{14}H_{18}FNO_4S$
351.82 g/mol

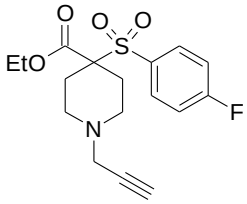
Step 5

1) propargyl bromide

K_2CO_3, DMF, rt

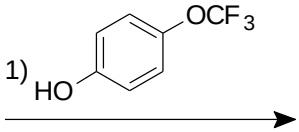
2) water

94% Yield



Compound 6
$C_{17}H_{20}FNO_4S$
353.41 g/mol

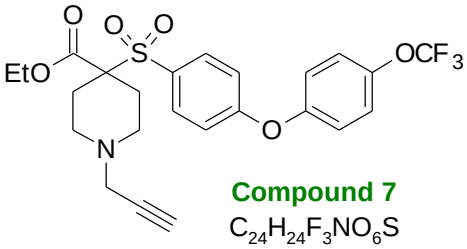
Step 6



90°C, DMF

2) Aqueous workup

99% Yield



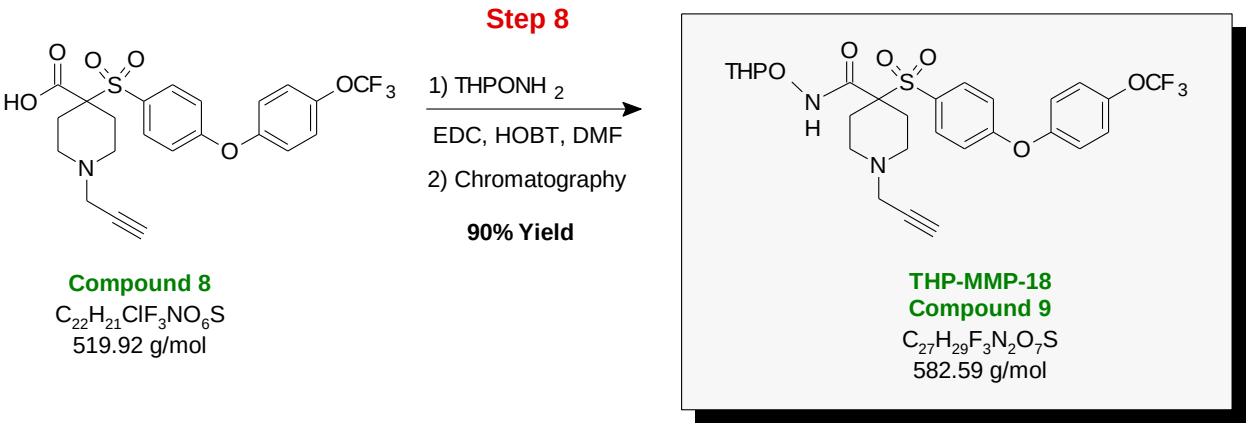
Compound 7
$C_{24}H_{24}F_3NO_6S$
511.51 g/mol

Step 7

1) NaOH, 60 °C

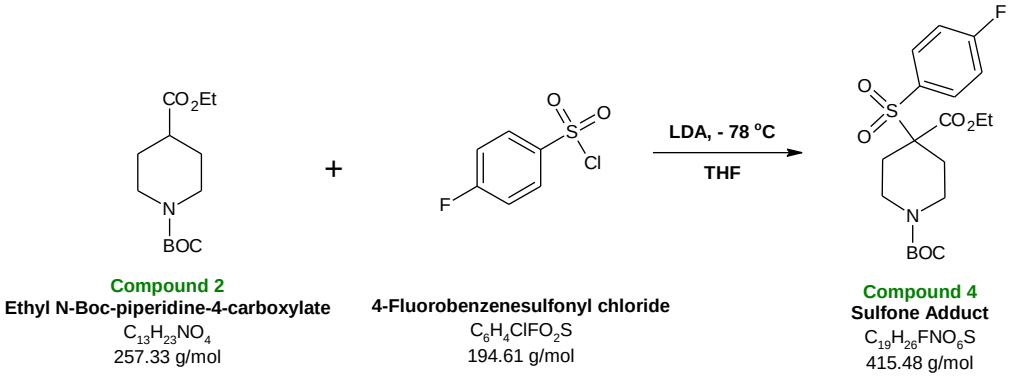
2) 2M HCl

98% Yield



Step 1 involved the formation of the disulfide (**Cmpd 1**) and the isolation procedure was improved such that the yield increased dramatically if the product slurry was chilled prior to isolation. Step 2 required the formation of the enolate anion **2** which was subsequently reacted with the disulfide **1** to produce the corresponding sulfide adduct (**Cmpd 3**). Step 3 involved oxidation of the sulfide **3** to the sulfone (**Cmpd 4**) using recently-developed novel oxidative conditions.[11] During the development of Step 2, it was determined that the sulfone (**Cmpd 4**) can accessed directly by reaction of enolate **2** with 4-fluorophenyl sulfonyl chloride as shown below (Scheme 25). The reaction proved to be successful in cleanly producing the desired product in 80% yield. For future preparation, this route can be used as an alternative route to access the sulfone directly from **2** either in batch or potentially in continuous flow mode.

Scheme 25. Enolate and Sulfonyl chloride to produce Sulfone Adduct **4**



Compound 2
Ethyl N-Boc-piperidine-4-carboxylate
$C_{13}H_{23}NO_4$
257.33 g/mol

4-Fluorobenzenesulfonyl chloride
$C_6H_4ClFO_2S$
194.61 g/mol

Compound 4
Sulfone Adduct
$C_{19}H_{26}FNO_6S$
415.48 g/mol

Step 4 entailed removal of the BOC moiety using hydrogen chloride gas in isopropyl acetate to access the ammonium hydrochloride salt (**5**). Step 5 involved installation of the propargyl group *via* N-alkylation using propargyl bromide and potassium carbonate to give exclusively the tertiary amine product (**6**). Step 6 involved the preparation of the diaryl ether (**7**) using SNAr conditions. Step 7 involved alkaline hydrolysis of the ethyl ester to the carboxylic acid (**8**) which required overnight heating to achieve >90% conversion by HPLC. This ester is highly hindered, and it was surprising that this reaction did not go to completion. This type of reaction would be highly amendable for flow chemistry at high temperatures. The final step, Step 8 is a peptide coupling reaction to form the protected hydroxamate material (**9**) which also required 2-3 days to fully consume the starting material.

As shown in Scheme 24, the synthesis commences with the reaction of inexpensive and commercially available ethyl N-Boc-piperidine-4-carboxylate (**2**) with Lithium Diisopropylamide (LDA) under cryogenic conditions to

produce the enolate anion that subsequently reacts with disulfide (**1**) to produce the sulfide adduct (**3**). The preparation of disulfide (**1**) was easily achieved by the reaction of 4-fluorothiophenol in DMSO. It is noteworthy that the process and yield of the disulfide (**1**) was drastically improved by isolating the disuflide at 0-5 ^{o}C providing crystalline material as a low melting solid. The corresponding sulfide adduct (**2**) was originally oxidized to the sulfone (**3**) using MCPBA and required chromatographic purification.[7,8] However, the process for oxidation of the sulfide to the sulfone was significantly improved with the use of the newly-developed, safe and efficient, scalable, and cost-efficient urea hydrogen peroxide (UHP)/phthalic anhydride system[11] in ethyl acetate, which allowed this oxidation to be easily carried out on 180 g scale and enabled facile isolation of highly pure sulfone *via* an extractive workup eliminating the need for chromatography. The corresponding *N*-Boc sulfone (**4**) was reacted with hydrogen chloride gas in isopropryl acetate to cleave the Boc protecting group producing the piperidine as the hydrochloride salt (**5**), which is then alkylated with propargyl bromide and potassium carbonate in DMF to cleanly produce the propargylamine adduct (**6**) in high yield. SNAr reaction of propargylamine (**6**) with 4-(trifluoromethoxy)phenol and potassium carbonate in DMF cleanly produced the diphenyl ether adduct (**7**) in high yield. Alkaline hydrolysis of diphenyl ether (**7**) in ethanol required an excess (10 equiv. of NaOH) of

hydroxide and overnight heating to achieve at least 90% conversion of ester to carboxylic acid (**8**). Lastly, the reaction of carboxylic acid (**8**) with O-(tetrahydro-2H-pyran-2-yl)hydroxylamine using EDC, HOBt and DIPEA in DMF afforded pure protected hydroxamate (**9**) after purification *via* normal phase chromatography.

Section 3: Borane-Alkyne Reactions and Synthesis of the 1,2-C_2B_{10}Carborane Cluster

The reaction involving alkynes and decaborane have been a useful method of covalently attaching boron clusters to carrier molecules[12] for a wide range of chemical fields including electronics, nanomaterials, and medicine. There are a variety of methods of carrying out reactions of alkynes and decaborane to prepare *ortho-closo*-carboranes. The classical method requires the formation of an activated decaborane complex, $B_{10}H_{12}(MeCN)_2$, which can be prepared *in situ* or isolated and can be stored at ambient temperature for some time, however the isolated yield of activated decaborane complex is undesirably low with yields of only 20-40% isolated yield. $B_{10}H_{12}(MeCN)_2$ is typically prepared in toluene and acetonitrile (typically in excess) and the generated $B_{10}H_{12}(MeCN)_2$ complex can be isolated as a solid with a moderate shelf-life when stored under an inert atmosphere, or the $B_{10}H_{12}(MeCN)_2$ complex can be prepared *in situ* for direct carborane synthesis. Preparation of the $B_{10}H_{12}(MeCN)_2$ complex is usually

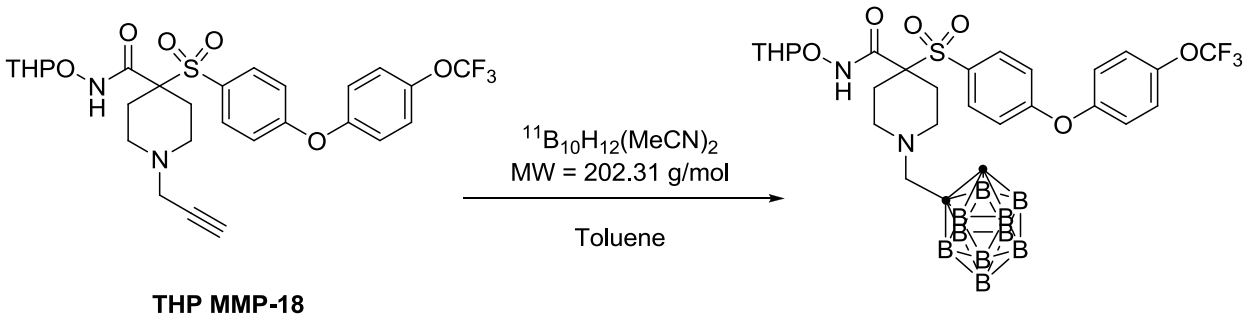
performed at 80-120^{o}C for at least 1 hour. Over the course of the reaction hydrogen gas is generated as a byproduct and 2 molar equivalents of acetonitrile as a Lewis base replaces the liberated hydrogen. Subsequent reaction of $B_{10}H_{12}(MeCN)_2$ and an alkyne at 80-120 ^{o}C affords the *closo*-carboranyl derivative, usually requiring overnight heating to fully consume the alkyne starting material. However, the reaction can be accelerated when a catalytic amount of silver nitrate is added reducing the reaction, reducing the reaction time to 1-4 hours.[13] In addition, direct reaction of alkynes with decaborane can be carried out efficiently using ionic liquids as the solvent/activator, and this process does not require the use of a Lewis base to form a $B_{10}H_{12}$(Lewis base)$_2$ complex. The use of an ionic liquid allows complete conversion in much shorter timeframe at 100-120^{o}C, which makes this process amendable for flow chemistry since it is a fast reaction, and the toxic decaborane can be more safely handled.

It was projected that the reaction of THP-MMP-18 and $B_{10}H_{12}(MeCN)_2$ would serve as a convenient pathway to prepare and isolate carboranyl targets for BNCT. However, we eventually realized that the reaction was not as straightforward as one might perceive. A significant amount of time was spent on scoping process conditions in attempt to form the desired carboranyl product (Cmpd 24) as illustrated in Scheme 26. Conditions that were evaluated included temperature, solvent, prior preparation of the B-

$_{10}H_{12}(MeCN)_2$ complex, *in situ* generation of the $B_{10}H_{12}(MeCN)_2$ complex, stoichiometry of $B_{10}H_{12}(MeCN)_2$, and catalytic addition of silver nitrate. Table 4 summarizes the results from the reaction of THP-MMP-18 with decaborane in attempt to form the *closo*-carborane. Pilot reactions performed were monitored by HPLC and TLC analysis.

The results summarized in Table 5 showed that the reactions performed generated a multitude of products which were not identified or characterized. It was postulated that the hydroxamate moiety was undergoing chelation with decaborane or the corresponding decaborane activated complex.

Scheme 26. Reaction of THP-MMP 18 with decaborane activated complex to form Compound 24



THP MMP-18
Compound 9
Chemical Formula: $C_{27}H_{29}F_3N_2O_7S$
Exact Mass: 582.16
Molecular Weight: 582.59

(^{11}B)-THP MMP-18 *closo*-carborane
Compound 24
Chemical Formula: $C_{27}H_{39}B_{10}F_3N_2O_7S$
Molecular Weight: 700.78

Note: All B atoms are actually BH
all boron atoms are predominantly
11 isotope (80% and 20% 10 isotope).

Table 5. Reaction Conditions for the coupling of Decaborane with THP-MMP-18 (Cmpd 9)

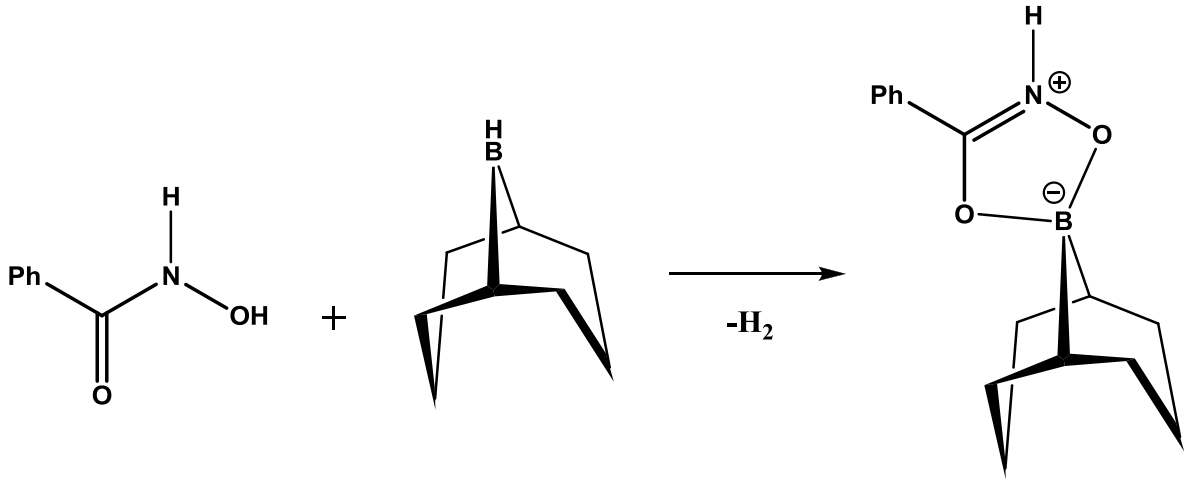
Pilot	Reactions Conditions	Temp	Time	HPLC/TLC Result
Pilot 1 PR075-113	$B_{10}H_{14}$ (1.2 equiv) ACN (100 equiv) Toluene THP-MMP-18 (1 equiv)	110°C	21 h	Highly complex mixture
Pilot 2 PR075-133	$B_{10}H_{14}$ (1.2 equiv) ACN (100 equiv) Toluene THP-MMP-18 (1 equiv)	50°C	13 h	Highly complex mixture
Pilot 3 PR075-148	$B_{10}H_{14}$ (2 equiv) ACN (100 equiv) Toluene THP-MMP-18 (1 equiv)	40°C	21 h 37 h 11 days	Highly complex mixture Highly complex mixture Highly complex mixture
		110°C	7 h 22 h 2.5 days	Highly complex mixture Highly complex mixture Highly complex mixture
Pilot 4 PR075-149	$B_{10}H_{14}$ (3 equiv) Toluene ACN (100 equiv) THP-MMP-18 (1 equiv)	40°C	21 h 37 h 11 days	Highly complex mixture Highly complex mixture Highly complex mixture
		110°C	7 h 22 h 2.5 days	Highly complex mixture Highly complex mixture Highly complex mixture
Pilot 5 PR075-150	$B_{10}H_{14}$ (3 equiv) Toluene ACN (100 equiv) THP-MMP-18 (1 equiv) $AgNO_3$ (0.10 equiv)	25°C	21 h 32 h 11 days	Highly complex mixture Highly complex mixture Highly complex mixture
		110°C	2 h	Highly complex mixture
Pilot 6 PR075-156	$B_{10}H_{14}$ (0.5 equiv) ACN (100 equiv) THP-MMP-18 (1 equiv)	40°C	15 h	Highly complex mixture
		110°C	7 h 2.5 days	Highly complex mixture
Pilot 7 PR075-157	$B_{10}H_{14}$ (0.5 equiv) Toluene ACN (100 equiv) THP-MMP-18 (1 equiv) $AgNO_3$ (0.10 equiv)	40°C	15 h	Highly complex mixture
		110°C	2 h	Highly complex mixture

Pilot	Reactions Conditions	Temp	Time	HPLC/TLC Result
Pilot 8 PR075-164	$B_{10}H_{12}(MeCN)_2$ (0.5 equiv) Toluene THP-MMP-18 (1 equiv)	110°C	2 h 16 h 2 days	Highly complex mixture
Pilot 9 PR075-165	$B_{10}H_{12}(MeCN)_2$ (1.2 equiv) Toluene THP-MMP-18 (1 equiv)	110°C	2 h 16 h 2 days	Highly complex mixture
Pilot 10 PR075-166	$B_{10}H_{12}(MeCN)_2$ (1.2 equiv) Toluene THP-MMP-18 (1 equiv)	110°C	2 h 16 h 2 days	Highly complex mixture

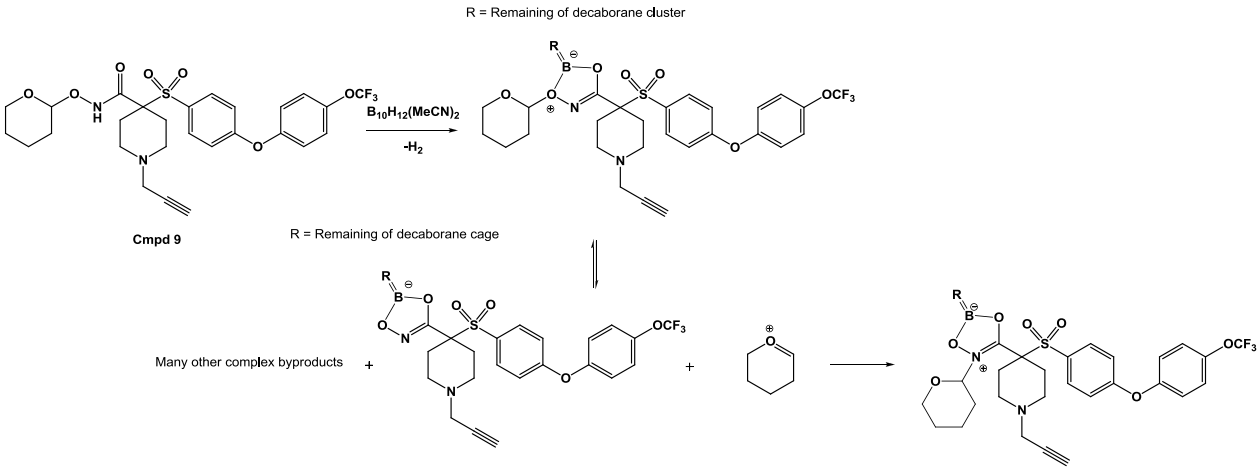
According to Kliegel et al,[14] reaction of 9-BBN with a THP protected hydroxymate can generate boron chelates[14] (illustrated in Scheme 27) and based on this precedent, it was suspected that this chelation behavior was a contributing factor to the complex reaction of THP-MMP-18 with decaborane or $B_{10}H_{12}(MeCN)_2$. Kliegel et al[14] showed that hydroxamic acids chelate 9-BBN, but he also demonstrated that O-THP protected hydroxamates can chelate to Lewis acidic boron compounds such as 9-BBN, BF_3, and diphenylborinic anhydride. Based on the work by Kliegel et al[14] showing that O-THP protected hydroxamates chelate to boron containing compounds, it was hypothesized that the reaction of THP-MMP-18 with decaborane or the decarborane activated complex resulted in cleavage of the THP group as illustrated in Scheme 28. Further evidence for the deprotection of THP ethers were reported by Yung[15] who employed the use of catalytic amounts of decaborane in various solvents (MeOH, EtOH, MeOH/THF) to cleave THP

ethers. Gigg et al. also reported that boric acid at 90°C cleaves THP ethers.[16] Furthermore, the use of borane in THF cleaves tetrahydropyranyl ethers at room temperature according to Cossy et al.[17]

Scheme 27. 9-BBN Reaction with Free Hydroxamic Acid (based on ChemischeBerichte, 116(7), pg 2616, 1983)



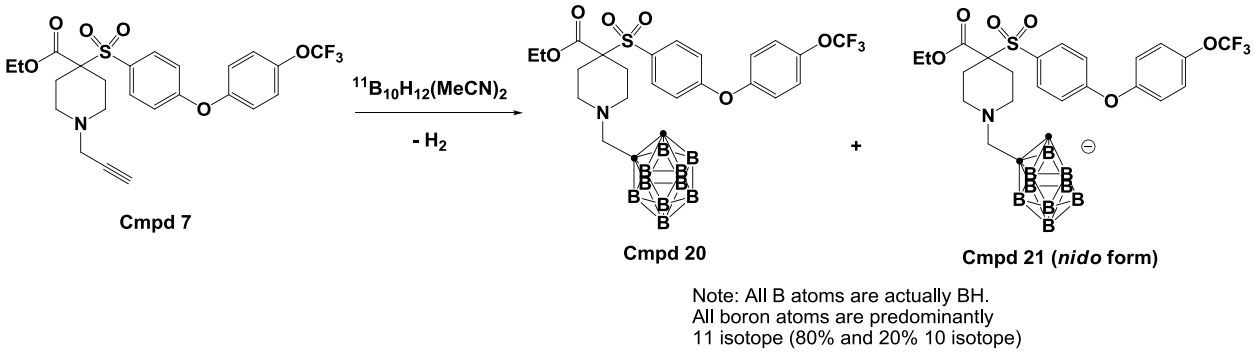
Scheme 28. Proposed undesired reaction of THP-MMP-18 (Cmpd 9) with decaborane/decaborane activated complex



Given that hydroboration of decaborane and THP-MMP-18 (Cmpd 9) proved undesirable, an alternative approach was considered. The alternative approach, illustrated in Scheme 29, involved hydroboration of ethyl ester (**7**) with $B_{10}H_{12}(MeCN)_2$ which proved successful in generating the *ortho-closo*-carborane ethyl ester adduct **20**. In addition to the carboranyl ethyl ester (**20**), a side-product was formed and identified to be the *nido* ethyl ester form (**23**). *Nido* formation is typically a result of deboronation of the carborane cluster under alkaline conditions (> pH 9) either with alkali hydroxide or organic amines. Given that ethyl ester carborane (**22**) does contain an organic amine moiety from the piperidine scaffold, the formation of the *nido* complex is likely due to the presence of the piperidine scaffold. It has been shown that treatment of boron clusters in toluene and triethylamine at reflux generate the *nido* form.[18] Additionally, Viñas *et al*. showed that using piperidine in ethanol generated *nido* carboranes.[19]

Since electron donors (such as MeCN, SEt_2, or Et_3N) coordinate to decaborane to form decaborane activated complexes, it is quite possible that $B_{10}H_{12}(MeCN)_2$ underwent an exchange with the piperidine nitrogen to eject one equivalent of acetonitrile. This swapping reaction could also explain why the HPLC/TLC analysis resulted in a significantly complex mixture of byproducts or why lower isolated yields for ethyl ester carboranyl adduct were achieved.

Scheme 29. MMP-18 Ethyl Ester (Cmpd 7) Reaction with Activated Decaborane



The reaction of MMP-18 ethyl ester (Cmpd 7) with activated decaborane was monitored by HPLC analysis. After 19 h at 80-85 ^{o}C, the reaction completely consumed starting material and resulted in a 70% conversion to the desired carborane (Cmpd 20) along with 16% of a major impurity which was identified and assigned as the *nido* ethyl ester species (Cmpd 21) based on HRMS analysis. Purification by flash chromatography afforded the desired MMP-18 carboranyl ethyl ester (Cmpd 20) in 35% yield and >98% purity. It is still undetermined why the isolated yield is lower than expected since the conversion was 70% by HPLC. Based on the observation that ethyl ester ***7*** coupled effectively to decaborane, the interaction of the alkyne moiety of Cmpd 9 with decaborane should proceed as expected, and shows that Lewis acidic decaborane was probably chelating to the hydroxamate moiety.

With experiment PR074-052 (see Scheme 30 below), direct access to MMP-18 carboranyl acid (Cmpd 22) was explored by using an MMP-18 carboxylic acid (Cmpd 8) and activated decaborane which led to essentially complete consumption of MMP-18 carboxylic acid (Cmpd 8) after 17 h at 80°C. The resultant reaction mixture contained only 18% of the desired carboranyl acid **22**. The HPLC chromatogram for this reaction of MMP-18 carboxylic acid and activated decaborane is shown in Figure 27.

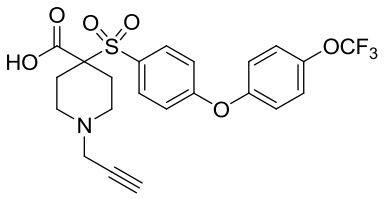
Scheme 30. Reaction of MMP-Carboxylic Acid (Cmpd 8) with activated decaborane

PR074-052

Purpose:
Determine if hydroboration reaction of MMP-18 CO2H (Cmpd 8) and $B_{10}H_{12}(MeCN)_2$ can be accomplished.

$B_{10}H_{12}(MeCN)_2$ is prepared by heating $B_{10}H_{14}$ in Toluene and acetonitrile.

$B_{10}H_{12}(MeCN)_2$ Preparation Time = 1 hr at 100 C

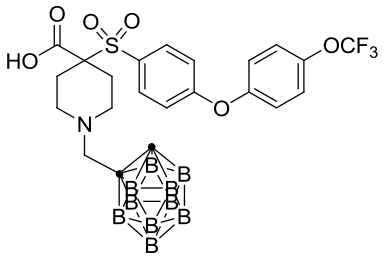


MMP-18 CO_2H
Cmpd 8
Chemical Formula: $C_{22}H_{20}F_3NO_6S$
Molecular Weight: 483.46

1) $^{11}B_{10}H_{14}$, Toluene, ACN

(100 °C, 1 hr)

2) Add MMP-18 CO2H
80-85 °C



***closo*-carborane MMP-18 CO_2H**
Cmpd 22
Chemical Formula: $C_{22}H_{30}B_{10}F_3NO_6S$
Molecular Weight: 601.65
Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope)

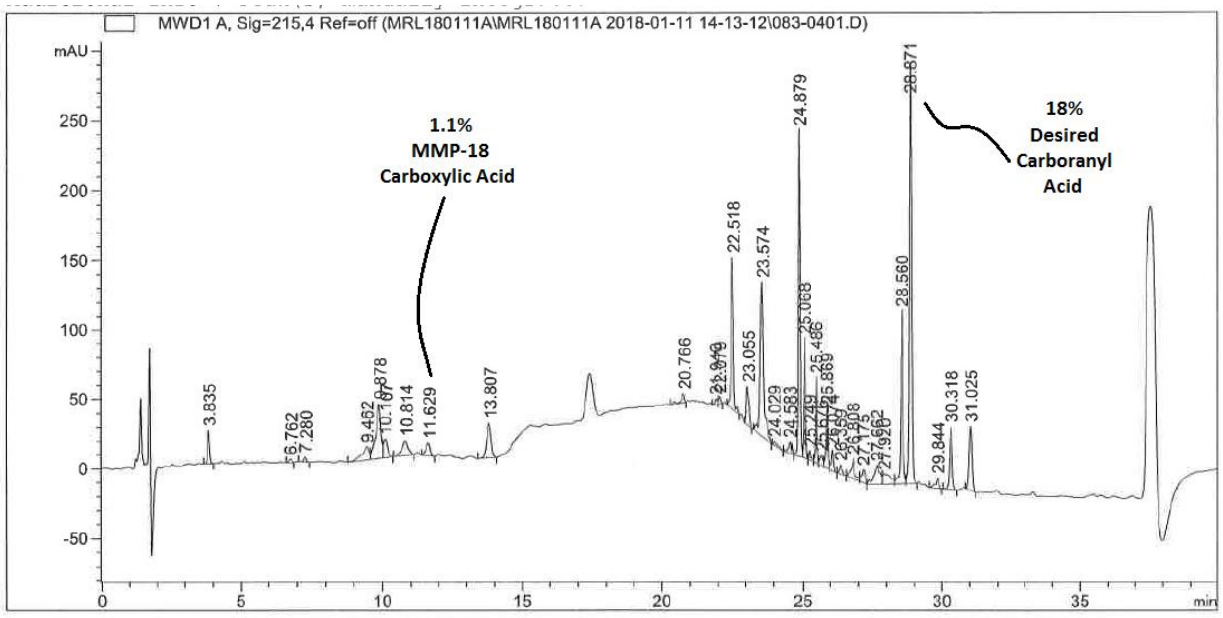


Figure 27. Reaction of MMP-18 carboxylic acid and activated decaborane

The next step was to determine if the ethyl ester carborane (Cmpd 20) can be hydrolyzed to its corresponding carboxylic acid derivative (Cmpd 22). The first attempt to hydrolyze the ethyl ester using sodium hydroxide in aqueous ethanol at room temperature proved unsuccessful since deboronation occurred much faster than hydrolysis of the hindered ester group leading to the *nido* form as the major component. See below in Figure 28, Figure 29, and Table 6 that show two HPLC chromatograms and tabulated data which illustrate that deboronation occurred faster than ester hydrolysis and that a significant amount of ethyl ester starting material remained unreacted even after 210 h at room temperature. Heating this alkaline mixture was not explored since deboronation is likely to be enhanced leading to an even higher population of *nido* ester or *nido* acid.

Note that the *nido* form is of interest in the future for preparing water soluble MMP carboranyl inhibitors, however the initial goal of this project is to prepare the analogs that encompass the *closo*-carborane cage.

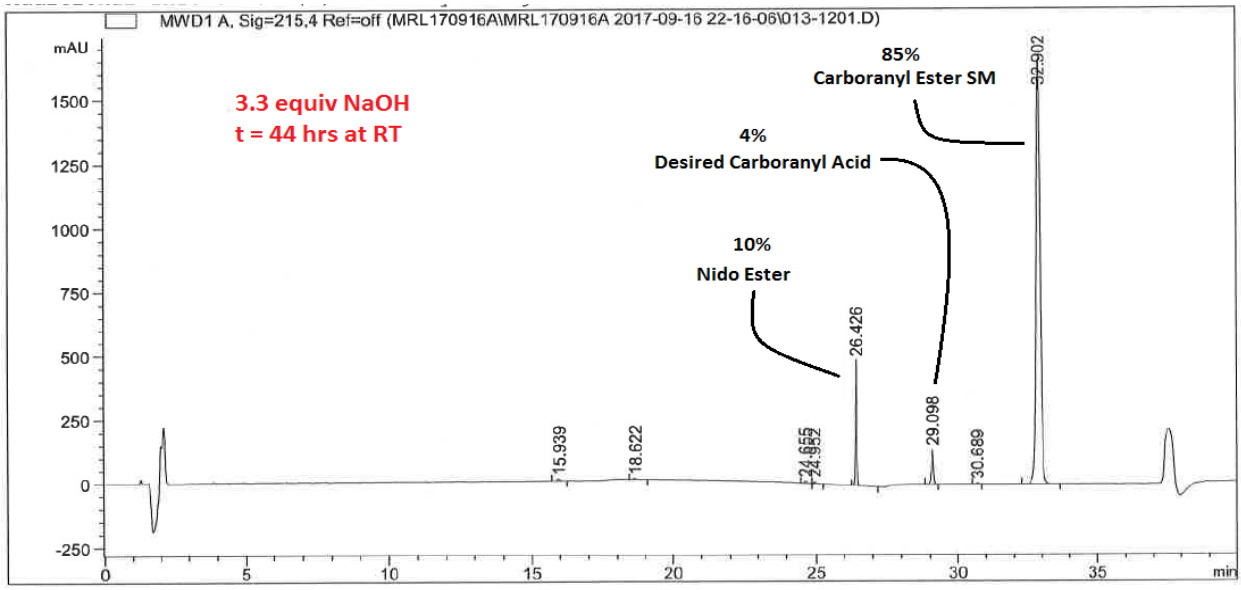


Figure 28. Alkaline Hydrolysis of Ethyl Ester Carborane at 44 hrs at RT

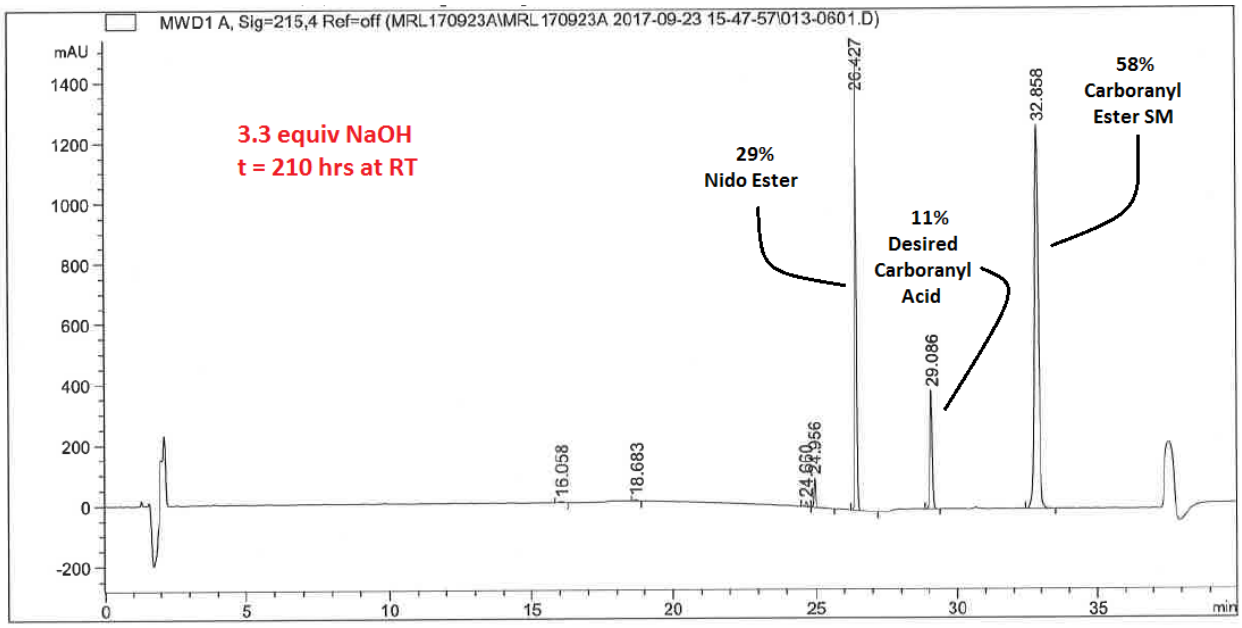


Figure 29. Alkaline Hydrolysis of Ethyl Ester Carborane at 210 hrs at RT

Table 6. Alkaline Hydrolysis and Deboronation of Ethyl Ester Carborane over 210 hours at RT

Time at RT	HPLC Carboranyl Ester Cmpd 20 (%AUC)	HPLC Desired Carboranyl Acid Cmpd 22 (%AUC)	HPLC Nido Ester Cmpd 21 (26.4 mins) (%AUC)
44 h	85	4	10
84 h	82	5	12
108 h	73	7	18
162 h	64	9	22
210 h	58	11	29

The steric hindrance of MMP-18 Ethyl Ester contributed to the very slow hydrolysis using sodium hydroxide in aqueous ethanol at 60^{o}C overnight. This demonstrates that the ester would prove to be very difficult to completely convert to the carboxylic acid.

As it appeared that alkaline hydrolysis of carboranyl ester (Cmpd 20) is not a viable pathway to access the carboxylic acid carborane, since deboronation and *nido* formation would predominate, conditions employing acidic hydrolysis were pursued. Below in Table 7 is summarized the process development toward hydrolysis of the hindered ethyl ester using acidic mixtures employing conventional thermal heating in batch mode.

Table 7. Screening Results for Hydrolysis Hindered MMP-18 Carboranyl Ethyl Ester

Reaction Conditions				HPLC (%AUC)						Comments
Experiment	Solvent Medium	Temp	Time	Ester Cmpd 20	Acid Cmpd 22	*Nido* Ester Cmpd 21	*Nido* Ester Isomer	*Nido* Acid	*Arachno* Acid	
Pilot 1 PR075-147-A	33% HBr in aq AcOH sealed vial	100 °C	27 h	61	30	2	2	0	0	
			48 h	56	33	3	2	0	0	
			80 h	42	37	3	2	1	0	recharged with additional water and 33% HBr
Pilot 2 PR075-147-B	6M HCl (aq) in AcOH sealed vial	100 °C	15 h	76	15	2	0	3	0	
			49 h	76	16	1	0	3	0	
			71 h	66	21	2	0	3	0	recharged with additional water and 33% HBr
Pilot 3 PR075-153	6M HCl (aq) in AcOH sealed vial	100 °C	2 days	52	15	13	0	0.6	0	
			3 days	38	13	21	0	8	0	
			7 days	20	22	4	0	15	16	
Pilot 4 PR075-168	70% H2SO4 in AcOH sealed vial	100 °C	6 h	92	8	0	0	0	0	
			1 day	69	31	0	0	0	0	
			3 days	44	56	0	0	0	0	
			4 days	31	68	0	0	0	0	
			5 days	21	79	0	0	0	0	
Pilot 5 PR075-169	50% MsOH in AcOH sealed vial	100 °C	6 h	96	4	0	0	0	0	
			1 day	85	15	0	0	0	0	
			3 days	61	39	0	0	0	0	
			4 days	51	48	0	0	0	0	
			5 days	45	55	0	0	0	0	
Pilot 6 PR075-170	33% HBr in aq AcOH	100 °C	6 h	50	18	10	11	0	1	
			1 day	18	47	9	12	0	1	
			2 days	4	61	10	12	0	2	

Reaction Conditions				HPLC (%AUC)						Comments
Experiment	Solvent Medium	Temp	Time	Ester Cmpd 20	Acid Cmpd 22	*Nido* Ester Cmpd 21	*Nido* Ester Isomer	*Nido* Acid	*Arachno* Acid	
	sealed vial		3 days	0	43	18	15	0	2	

As seen in Table 7, acids that were screened included hydrochloric acid, hydrobromic acid, sulfuric acid, and methanesulfonic acid. Screening was limited to 100 ^{o}C, limited by the boiling point of water and given the azeotropic nature of aqueous acetic acid mixtures. The addition of acetic acid was necessary to ensure that the mixtures remained homogeneous. During screening of these acids in batch mode at 100 ^{o}C (standard thermal heating), the acids screened generated the desired carboxylic acid as well as the *nido* carborane ethyl ester and carboxylic acid forms. However, the formation of the *nido* ethyl ester and carboxylic acid forms depended on the acid used. For instance, hydrochloric acid generated larger amounts of *nido* carboxylic acid form, whereas sulfuric acid generated lower levels of *nido* carboxylic acid. Interestingly, methanesulfonic acid also generated larger amounts of *nido* carboxylic acid form compared to sulfuric acid. Solubility of the carborane ethyl ester in mineral acids and methanesulfonic acid was relatively poor and required the addition of acetic acid to ensure complete dissolution. Given that the batch reaction was excessively slow, the use of

microwave heating at elevated temperature was evaluated. During the evaluation using microwave heating, only sulfuric acid/acetic acid conditions were employed since these conditions appeared to form less of the *nido* ethyl ester or carboxylic acid form(s). Note that the *nido* carboxylic acid form is a degradation product from deboronation of the desired carboranyl carboxylic acid. Table 8 summarizes the HPLC results of the hydrolysis of carboranyl ethyl ester (Cmpd 20) under microwave irradiation.

Table 8. Microwave Irradiation of Carboranyl Ethyl Ester **20** using sulfuric acid/acetic acid

Sample	H_2SO_4		AcOH (volumes)	Temp (°C)	τ (mins)	Carboranyl Ethyl Ester (Cmpd 20) 32.3 min (%AUC)	29.3 min Impurity A (%AUC)	*DESIRED* Carboranyl Acid Product (Cmpd 22) 28.8 min (%AUC)	26.3 min Impurity B (*nido*) Ester (%AUC)	25.7 min Impurity C (*nido*) Acid (%AUC)	16.1 min Impurity D (*arachno*) Ester (%AUC)	9.7-9.9 min Impurity E (*arachno*) Acid (%AUC)
	%wt	volumes										
PR075-173	70	325	650	200	5	0	2	28	8	1	0	0
PR075-174	70	325	650	175	10	0	17	50	6	20	0	1
PR075-175	70	325	650	135	10	91	0	9	0	0	0	0
				160	+20	28	3	66	0	1	0	1
PR075-176	70	325	650	150	10	50	1	46	0	2	0	0
					20	28	2	65	0	3	0	1
					30	13	3	75	0	6	0	1
PR074-069-A	70	77	154	150	30	46	0	51	0	1	0	0
PR074-069-B	50	250	500	150	30	69	0	29	0	0	0	0
PR074-069-C	70	250	500	150	30	48	0	49	0	1	0	0
PR074-069-D	70	250	500	150	45	39	0	58	0	1	0	0
PR074-069-E	70	250	500	150	60	33	0	63	0	1	0	0

PR074-069-L	70	125	250	150	60	33	0	64	0	1	0	0
PR074-069-F	90	250	500	150	60	6	4	83	0	4	0	0
PR074-069-G	90	125	500	150	60	33	1	63	0	1	0	0
PR074-069-H	90	125	375	150	60	21	1	75	0	1	0	0
PR074-069-I	90	125	250	150	60	1	9	78	0	8	0	0
PR074-069-K	90	125	250	150	50	5	5	82	0	5	0	0
PR074-069-J	90	125	250	140	60	28	1	68	0	1	0	0
PR074-069-M	0	0	250 (AcOH) 125 (H_2O)	140-145	10	27	0	0	70	0	3	0
PR074-069-N	0	0	375 (AcOH) 250 (H_2O)	150-170	10	0	0	0	67	0	23	0

Section 4: Discussion about Results

Initial screening under microwave irradiation at 200^{o}C resulted in rapid consumption of the carboranyl ethyl ester, however severe degradation occurred resulting in only 28% desired product with many unknown byproducts accompanying an undesirable stench. Decreasing the temperature to 175^{o}C resulted in consumption of starting material and produced 50% desired product along with associated *nido* byproducts. Operating at 135^{o}C for 10 minutes resulted in 91% unreacted starting material and 9% desired product, and subjecting the same reaction mixture to 160^{o}C for an additional 20 minutes generated 66% desired carboxylic acid product, 28% unreacted starting material, and trace amounts of *nido*

species. Based on those observations, a convenient procedure is warranted for scaling the hydrolysis of carboranyl ethyl ester using continuous flow, the maximum operating temperature is restricted to 150 ^{o}C with the current flow chemistry configuration due to that sulfuric acid is employed and the thermal limitation of the PFA plug flow reactor is typically restricted to 150 ^{o}C for safety and durability considerations. The next phase was to evaluate microwave irradiation at 150 ^{o}C and to optimize the residence time (Tau or τ), minimize the formation of *nido* species, and evaluate the solvent volumes and concentration of sulfuric acid. It was found that performing the hydrolysis for 60 minutes using 90% sulfuric acid (125 volumes) and acetic acid (250 volumes) gave the best conversion to the desired carboranyl acid product (~80%), with near consumption of starting material, and <10% of *nido* impurities. Interestingly, when carrying out microwave heating using solely aqueous acetic acid (experiment PR074-069-M and -N), hydrolysis of the ethyl ester was not affected, however the only products formed were mono and di-deboronated compounds that were identified by HRMS to be *nido*-ester and *arachno*-ester adducts.

To confirm that the desired carboranyl acid was forming, one of the pilot reactions (PR074-069-C) was analyzed by HPLC followed by UPLC/MS. Before analysis, the pilot reaction (PR074-069) was concentrated to remove acetic acid under reduced pressure followed by adjusting the pH of the

sulfuric acid residue to pH 5-6 (by litmus paper) using saturated aqueous sodium bicarbonate solution and subsequently extracting the organic material with dichloromethane. Analysis of the organic layer by HPLC showed that the ratio of starting material carboranyl ester and desired carboranyl acid remained the same (see below). Furthermore, UPLC/MS of this mixture showed the correct molecular ion $[M+H]^+$ = 602 m/z for the carboranyl acid, and this result confirmed that the carboranyl acid is forming under acid hydrolysis. The $[M+H]^+$ = 631 m/z is unreacted carboranyl ethyl ester starting material. The UPLC/MS data is depicted in Figure 30 below.

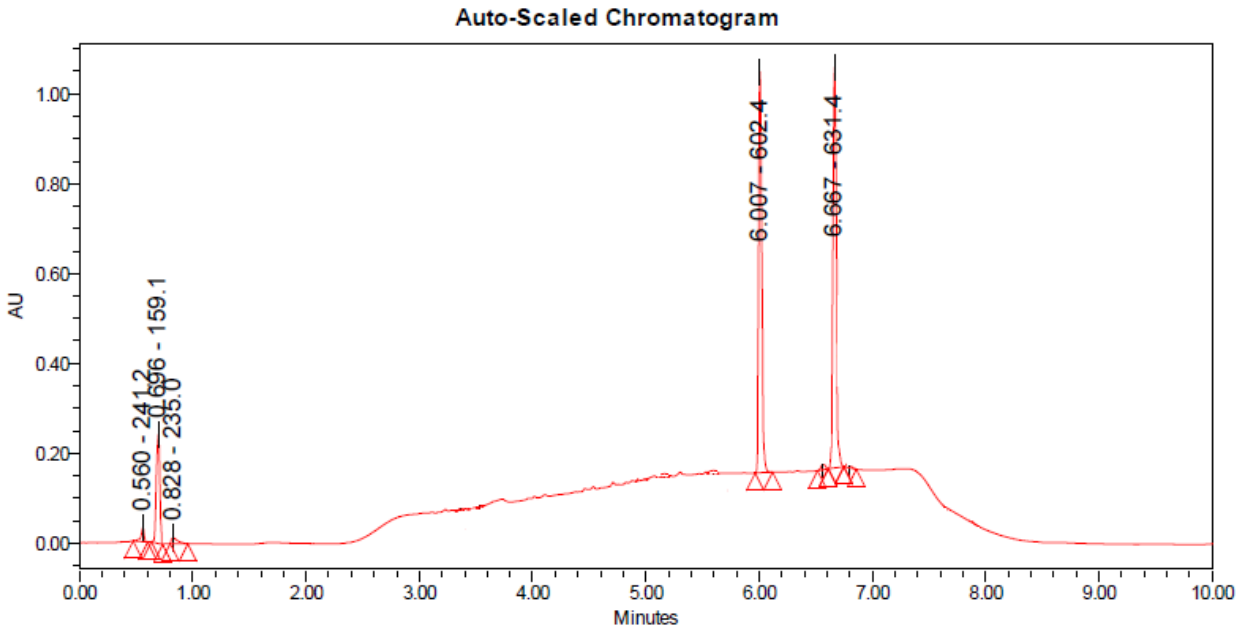


Figure 30. UPLC/MS - Carboranyl MMP-18 Carboxylic Acid (Compound 22)

Based on the microwave irradiation experiments, the next step was to evaluate the hydrolysis of carboranyl ethyl ester under continuous flow mode

using conditions developed with microwave batch irradiation. Under continuous flow mode at 150 ^{o}C for 60 minutes residence time, the formation of *nido* ester was significantly lower than microwave heating, however hydrolysis of the ethyl ester was slightly slower than expected. It was postulated that the heat transfer property under microwave heating is significantly higher than flow mode since glass has better heat transfer properties than PFA (Perfluoroalkoxy) tubing which is widely used in continuous mode application especially with corrosive or oxidizing agents. Furthermore, the higher levels of formation of the *nido* form under microwave heating is likely due to that the concentration of water (within the reaction medium) decreased since some of the water within the reaction was in the headspace of the microwave vessel as the vapor state. The HPLC chromatogram for a residence time of 60 minutes is depicted in Figure 31.

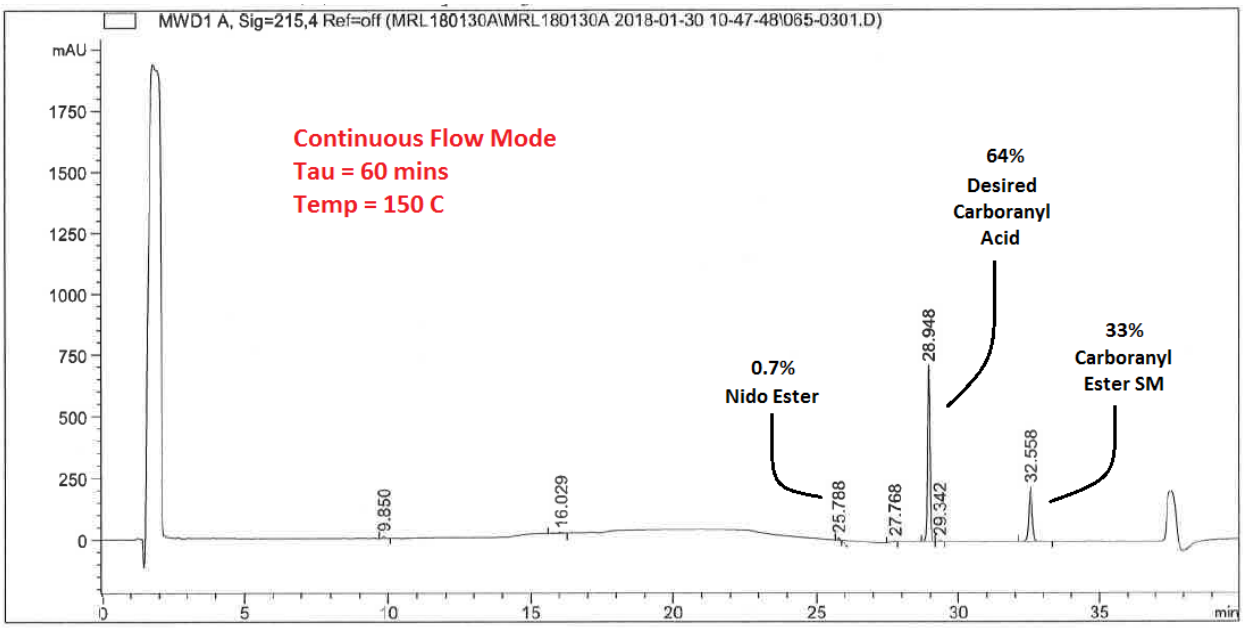


Figure 31. Hydrolysis of Ethyl Ester Carborane under Continuous Flow at 150 ^{o}C with τ = 60 mins

Given that the conversion was ~10-15% lower than conditions developed using microwave heating, the residence time was increased to 75 minutes at 150 ^{o}C which provided the same conversion observed with microwave irradiation. The HPLC chromatogram for the hydrolysis at 150 ^{o}C for 75 minutes under continuous flow mode is illustrated in Figure 32.

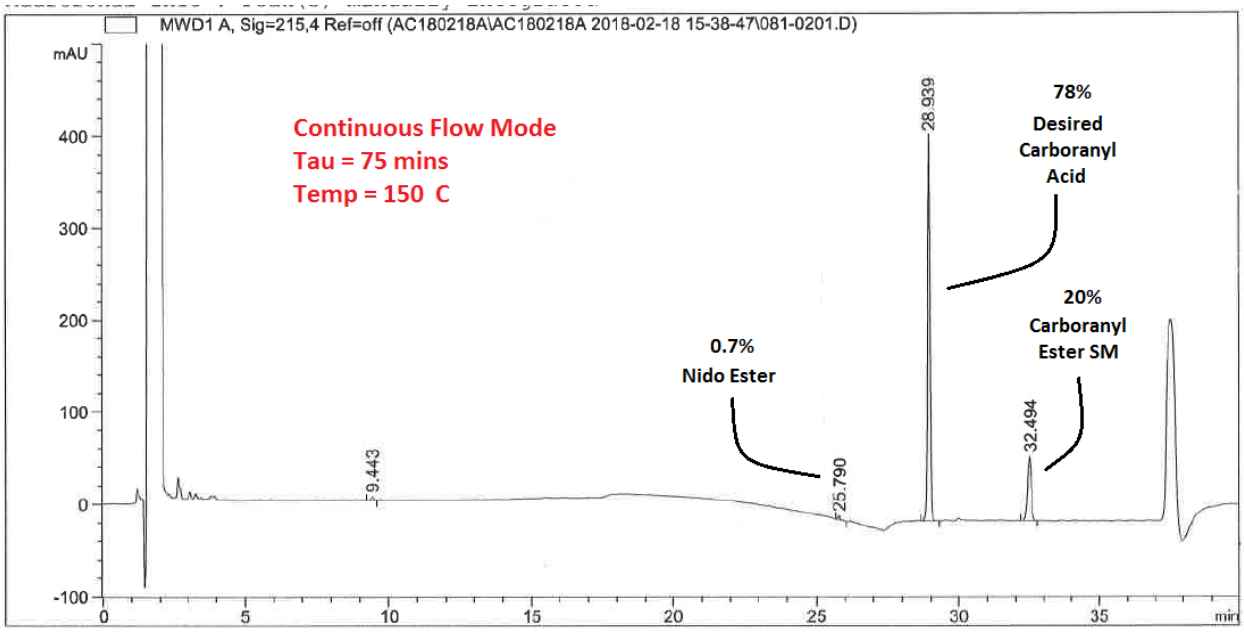


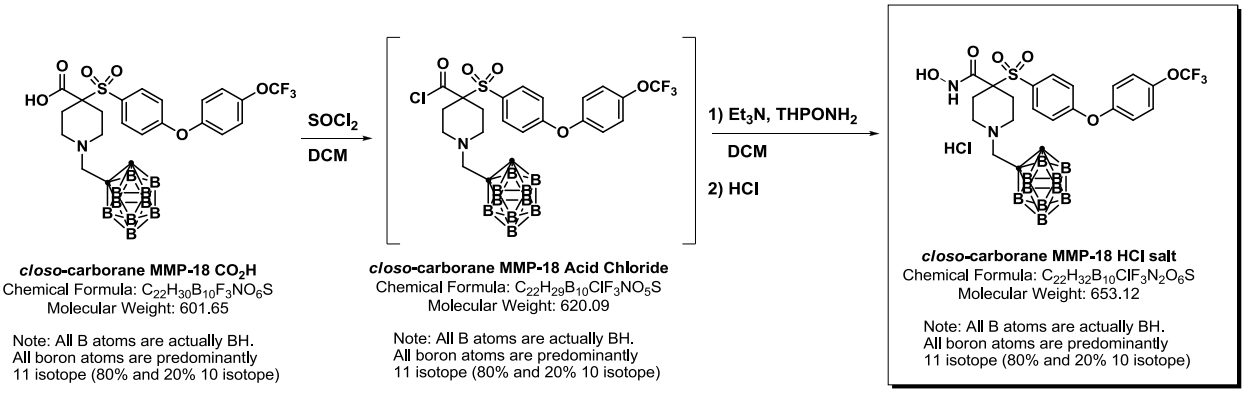
Figure 32. Hydrolysis of Ethyl Ester Carborane under Continuous Flow at 150 ^{o}C with τ = 75 mins

The use of continuous flow with a residence time of 75 minutes achieved 78-80% conversion to product that was substantially cleaner and contained 18-20% unreacted starting materiel ethyl ester which can be easily recovered by normal-phase chromatography and subsequently recycled for continuous flow processing.

With the desired carboranyl acid (Cmpd 22) in hand, the rest of the synthesis focused on preparation of the acid chloride, subsequently forming the protected hydroxamate using $THPONH_2$ and lastly cleaving the THP group with acidic reagent to isolate the carboranyl MMP-18 salt. The scheme for this synthetic pathway is illustrated in Scheme 31.

Formation of acid chloride and subsequent hydroxamate formation has not been pursued. Current efforts on this front will be addressed in due time.

Scheme 31. Synthetic Pathway to access Carboranyl MMP-18 salt

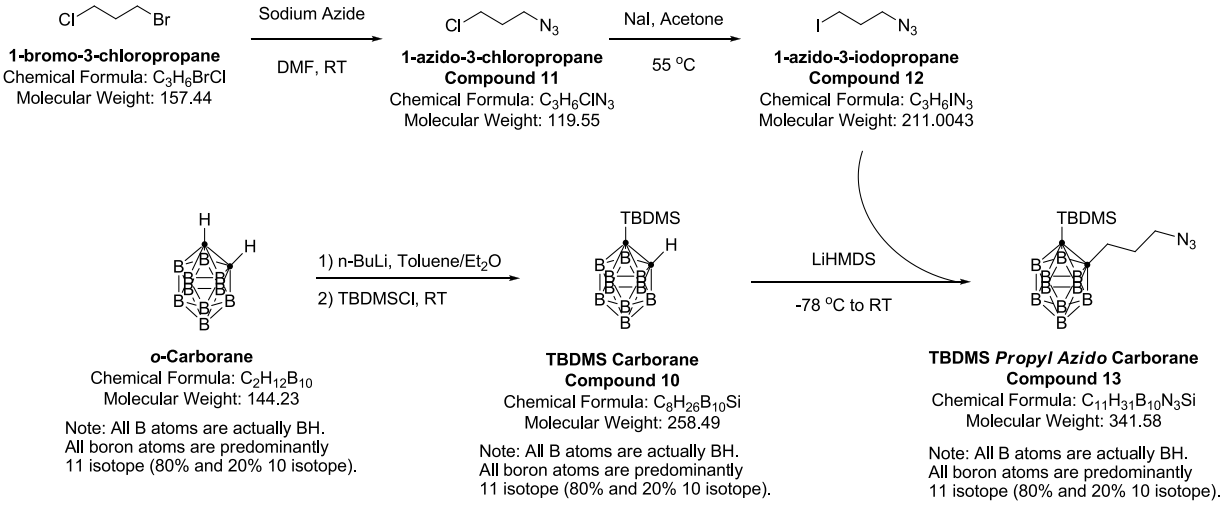


Section 5: Utilizing Click Chemistry to Attach Boron Cluster to THP-MMP-18

The scope of the next phase of this project revolved around the use of Click chemistry to attach boron clusters to the alkyne moiety of THP-MMP-18 (Cmpd 9). There are a number of methods of employing Click chemistry to couple azides to alkynes either thermally or *via* copper or ruthenium catalysis (CuAAC or RuAAC, respectively). Since we are targeting matrix metalloproteinases (MMPs), the use of copper has the potential to cause issues downstream since MMPs have a natural affinity to divalent metals (zinc, cobalt, copper). Based on this, the thermal Click reaction (without metal catalysis) was used to prepare both the 1,4-disubstituted and 1,5-disubstituted Click products.

Synthesis of both the 1,4-disubstituted and 1,5-disubstituted Click products was accomplished by preparation of protected carboranyl propyl azide and subsequent Click reaction with THP-MMP-18 in toluene at elevated temperatures (120-140^{o}C). The synthetic scheme for preparation of carboranyl propyl azide is shown in Scheme 32.

Scheme 32. Preparation of TBDMS Propyl Azido Carborane (**13**)



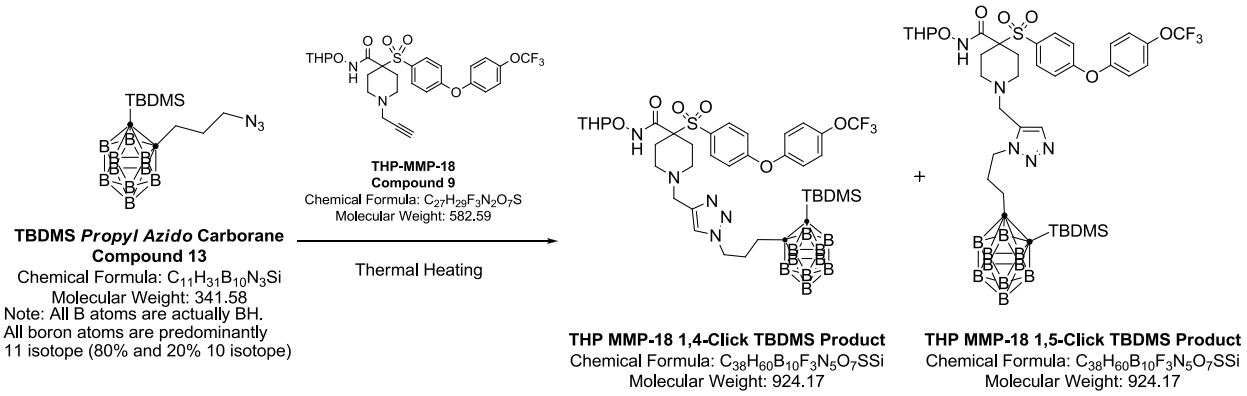
Formation of 1-azido-iodopropane was accomplished based on literature precedent (reference: ACIEE, 56(26), 7420-7424; 2017) which entailed SN_2 reaction of sodium azide and 1-bromo-3-chloropropane in DMF affording a 95% yield of 1-azido-3-chloropropane that contained ~15% of 1-azido-3-bromopropane and was taken forward without further purification. The crude 1-azido-3-halopropane was subjected to Finkelstein conditions using sodium iodide in warm acetone that generated pure 1-azido-3-iodopropane in 81% isolated yield after silica plug purification. The next

reaction involved preparation of the TBDMS-protected carborane derivative (**13**) which involved deprotonation of the *o*-carborane with *n*-butyllithium (*n*-BuLi) generating the carborane anion which subsequently attacks TBDMSCl affording highly pure TBDMS carborane (**10**) in 94% isolated yield post silica plug purification. The last step to prepare the TBDMS propyl azido carborane (**13**) uses novel anion generating conditions not reported in literature for this particular reaction. The deprotonation was accomplished using lithium hexamethyldisilazide (LiHMDS) to generate the carboranyl anion that sequentially attacks 1-azido-3-iodo-propane to furnish pure TBDMS propyl azido carborane in 98% isolated yield post silica plug purification.

The thermal heating of an azide and an alkyne without copper or ruthenium agents generates a mixture of 1,4-disubstituted and 1,5-disubstituted triazole products. Executing the Click chemistry on TBDMS propyl azido carborane (**13**) and THP-MMP-18 did confirm that a mixture of two products were formed as determined by TLC and HPLC analysis. In order to determine which Click product by TLC and HPLC correlated to the 1,4-disubstituted triazole and 1,5-disubstituted triazole products, two separate reactions were carried out using copper to facilitate the selective formation of the 1,4-disubstiuted Click product and ruthenium catalysis to promote selective formation of the 1,5-disubstituted Click product. Scheme 33 depicts the thermal click reaction and Figure 35 also shows the HPLC profile of a

thermal Click reaction. Furthermore, Figures 33 and 34 provide the mechanism of how each regioisomer is formed.

Scheme 33. Thermal click reaction between alkyne (**9**) and azide (**13**)



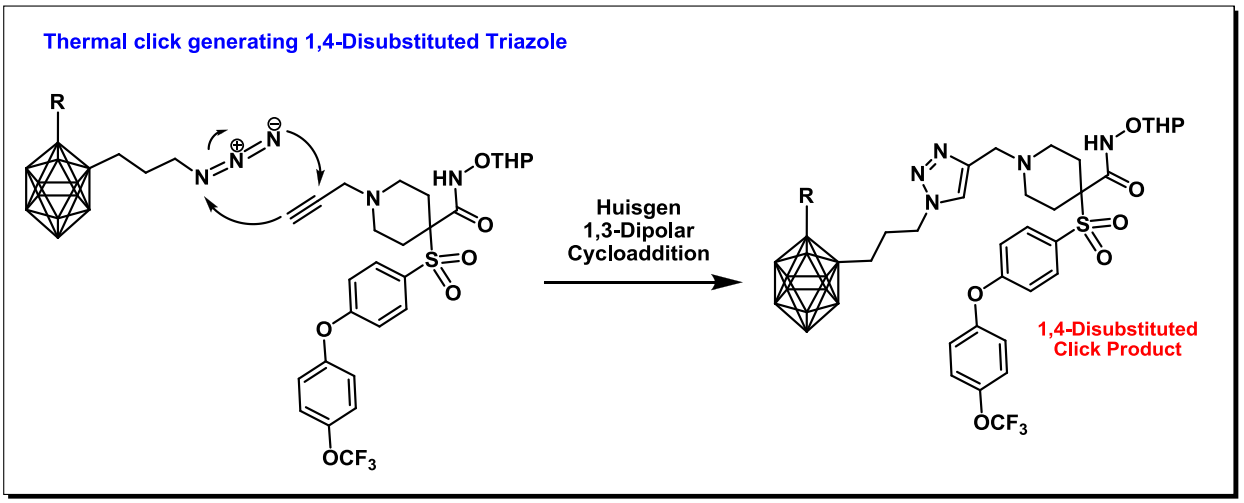


Figure 33. Mechanism for 1,4-Click Product

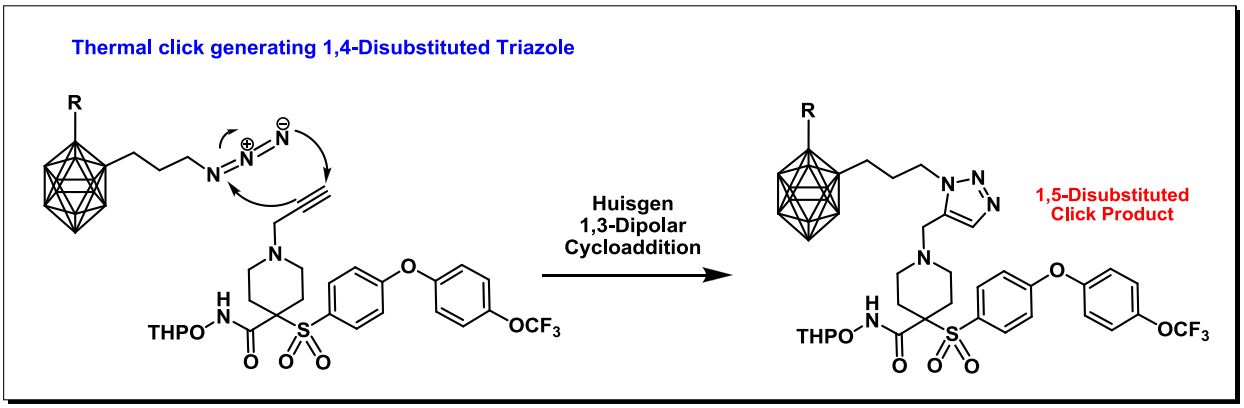


Figure 34. Mechanism for 1,5-Click Product

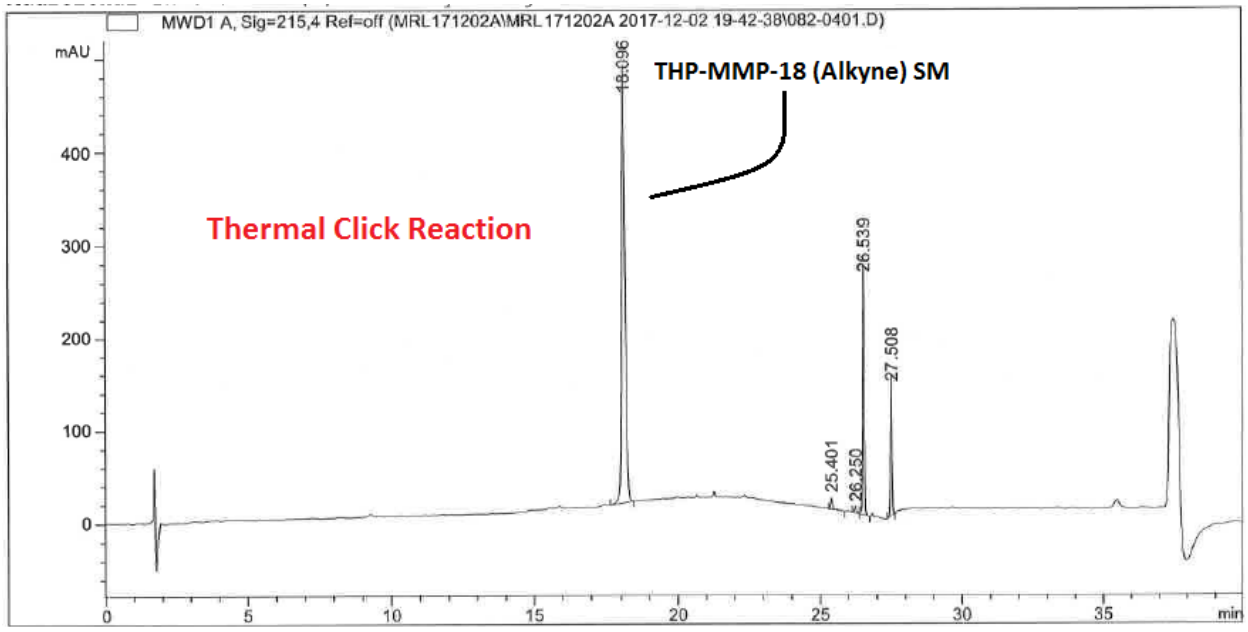
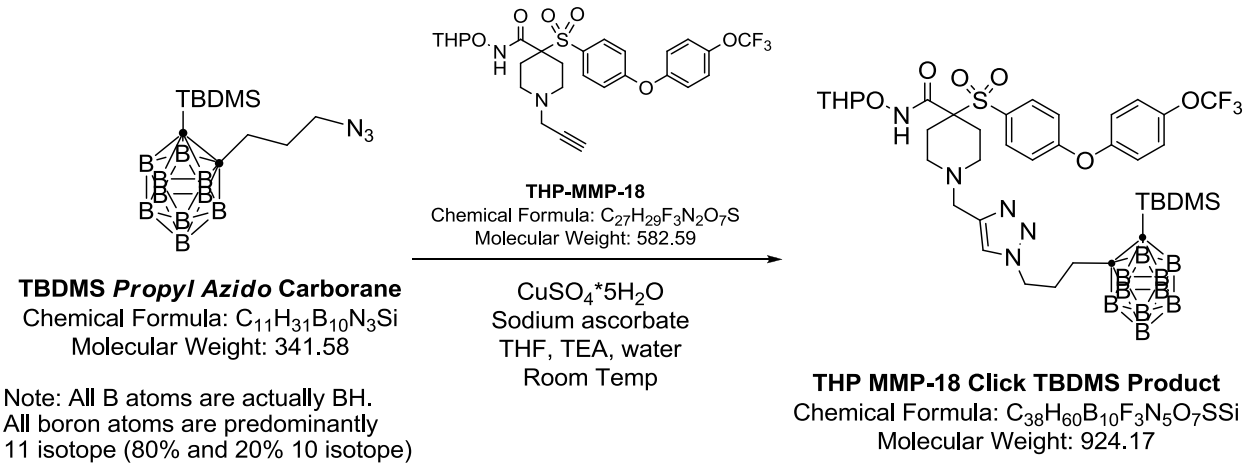


Figure 35. HPLC chromatogram of the thermal click reaction between Alkyne (**9**) and Azide (**13**)

Using stoichiometric copper sulfate and excess sodium ascorbate in aqueous THF at room temperature, the CuAAC reaction (Scheme 34) produced solely the 26.5 min component by HPLC (Figure 36) and confirmed that this component was the 1,4-disubstituted triazole. Figure 37 depicts the

mechanism for the formation of the 1,4-regioisomer under CuAAC conditions.

Scheme 34. CuAAC Reaction of THP-MMP-18 Alkyne and Carboranyl Azide



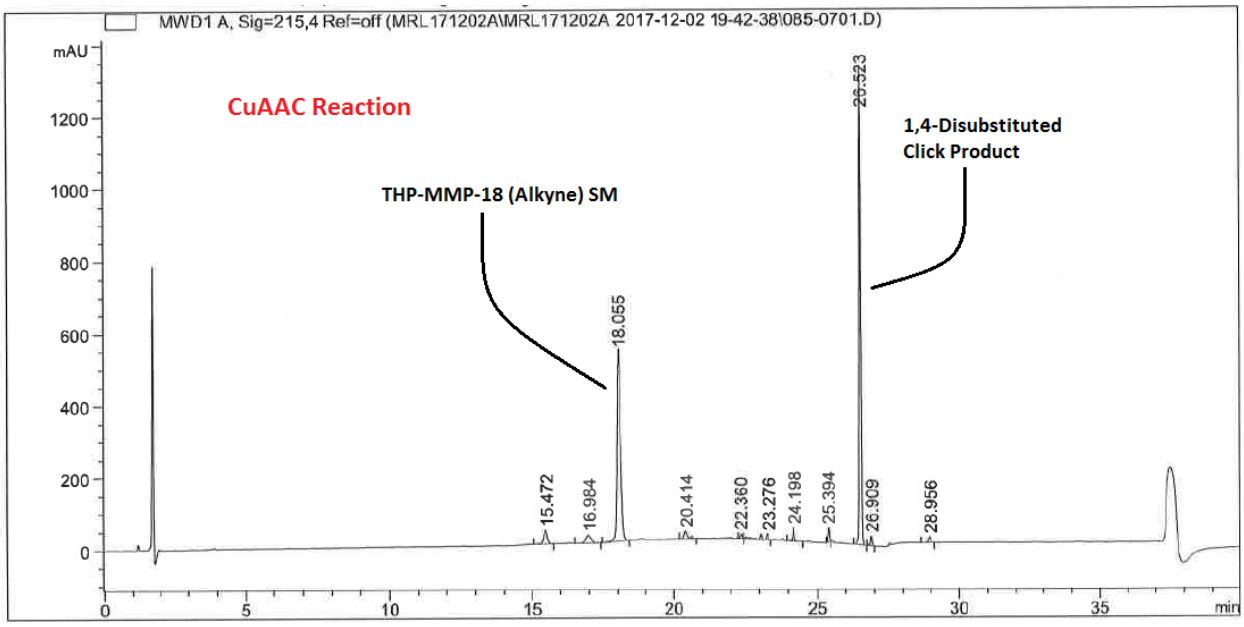


Figure 36. CuAAC Reaction of THP-MMP-18 Alkyne and Carboranyl Azide

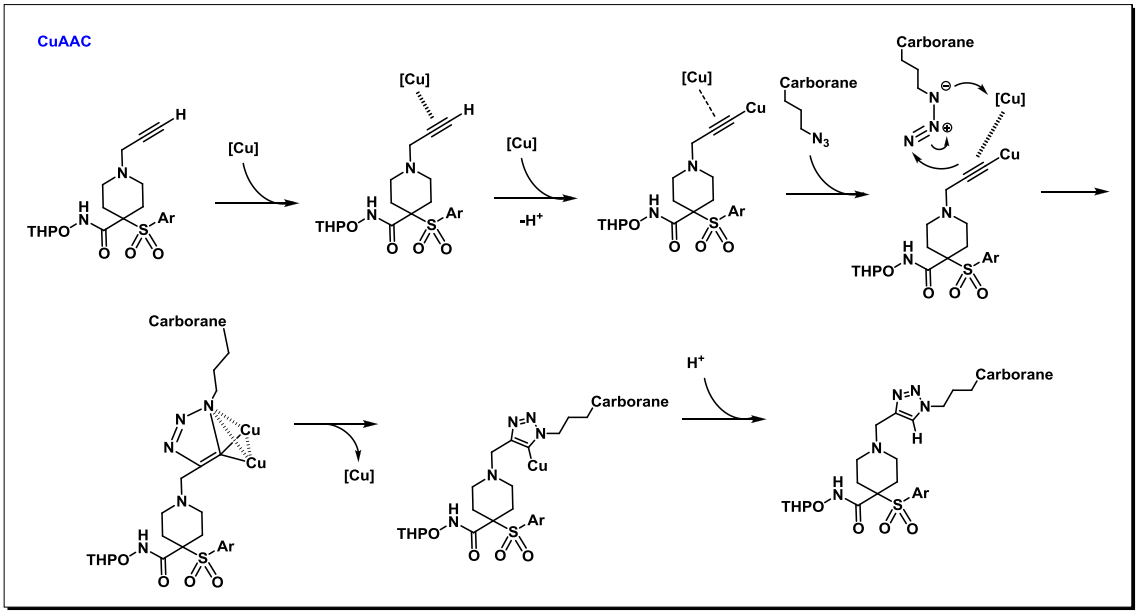
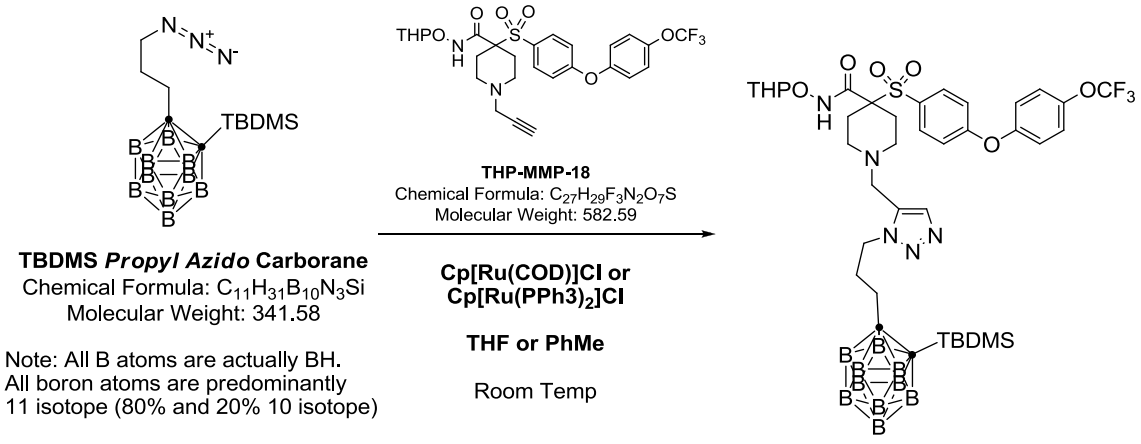


Figure 37. CuAAC mechanism for the formation of the 1,4-disubstituted triazole

The RuAAC reaction (Scheme 35) generated the 27.5 minute component by HPLC (Figure 38) and verified that this component was the 1,5-disubstituted triazole. The RuAAC affords predominately the 1,5-click product, but trace amounts (<2% AUC) of the 1,4-click product are produced when performing the reaction at ambient temperature. The mechanism for the formation of the 1,5-disubstituted triazole click product is shown in Figure 39.

Scheme 35:RuAAC Reaction of THP-MMP-18 Alkyne and Carboranyl Azide



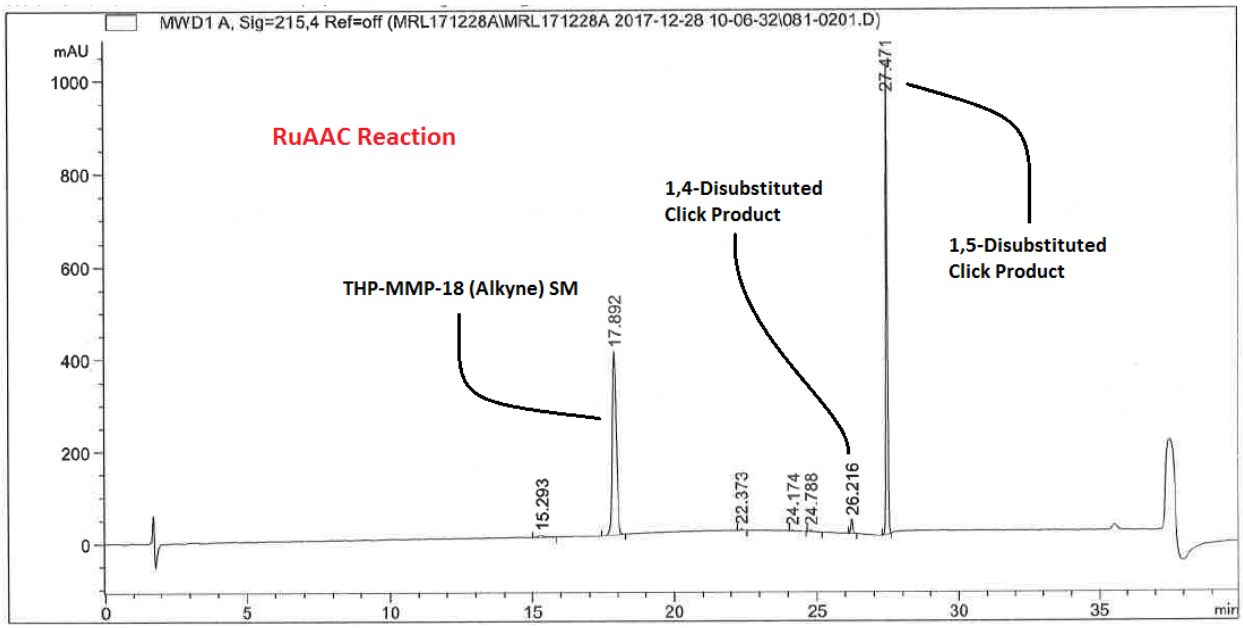


Figure 38.RuAAC Reaction of THP-MMP-18 Alkyne and Carboranyl Azide

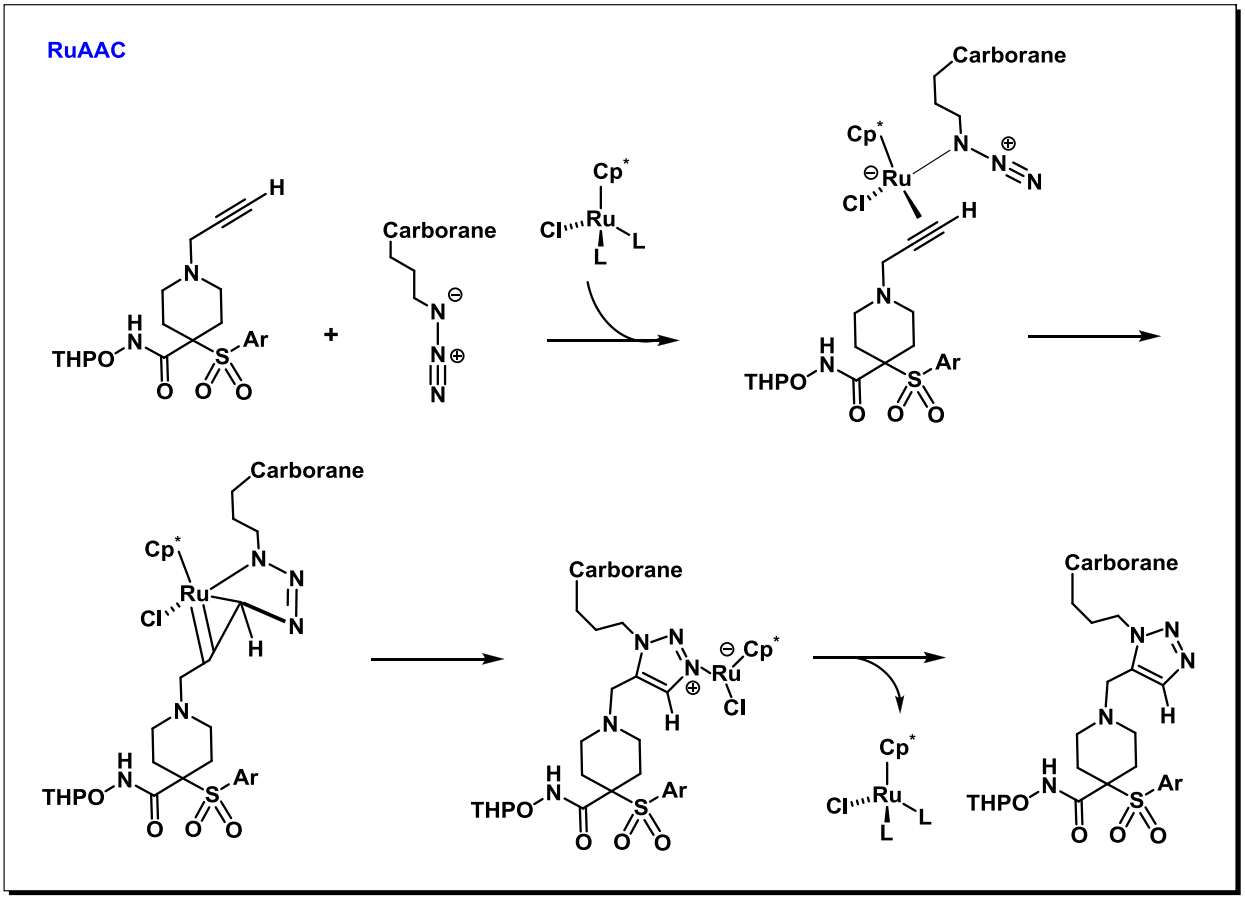


Figure 39.RuAAC mechanism for the formation of the 1,5-disubstituted triazole

To evaluate the RuAAC chemistry, two ruthenium catalysts were screened in toluene or tetrahydrofuran to determine which catalyst/solvent system was most effective in generating the 1,5-disubstituted triazole. The results of this screening are summarized in Table 9. It is apparent that the chloro(pentamethylcyclopentadienyl)(cyclooctadiene)ruthenium(II) (Cp*Ru(COD)Cl) catalyst was superior compared to pentamethylcyclopentadienylbis-(triphenylphosphine)ruthenium(II) chloride (Cp*Ru(PPh3)2Cl) catalyst, and performing the reaction in a polar solvent

(THF) generated higher conversion to the 1,5-click product compared to running the reaction in non-polar media such as toluene. Granted that the reaction could have been pushed to completion by adding in more catalyst, but this was not explored. It is expected that ruthenium catalysis may serve useful for future selective preparation of carboranyl Click products and even trace levels of ruthenium should not interfere with binding to MMPs or coordinate with the hydroxamate ligand of the MMP-18 scaffold.

Table 9. RuAAC Reaction screening catalyst type and solvent

Pilot Rxn	Catalyst‡	Solvent	Time (Temp)	Alkyne SM %AUC	1,5-Click Product %AUC
Pilot 1 PR074-035-A1	Cp*Ru(COD)Cl	THF	40 h (RT) 65 h (50°C)	48 34	49 54
Pilot 2 PR074-035-A2	$Cp^*Ru(PPh_3)_2Cl$	Toluene	40 h (RT) 65 h (50°C)	88 73	7 20
Pilot 3 PR074-035-B1	Cp*Ru(COD)Cl	THF	40 h (RT) 65 h (50°C)	76 69	22 27
Pilot 4 PR074-035-B2	$Cp^*Ru(PPh_3)_2Cl$	Toluene	40 h (RT) 65 h (50°C)	86 -----	7 -----

‡Catalyst loading was 0.025 equivalents.

The next phase was to optimize the thermal click reaction in toluene as the solvent. The goal of this phase was to determine the optimum temperature that provided good conversion to Click products and to minimize the formation of impurities such as *nido* species. Formation of *nido* species was expected since the starting material alkyne (Cmpd 9) does

contain an internal tertiary organic piperidine base and the resulting Click products are alkaline as well, although not as basic as the piperidine nitrogen, and with the combination of heat and basicity leads to formation of *nido* complexes. The temperatures screened for the thermal click reaction were 100 ^{o}C, 120 ^{o}C, and 140 ^{o}C and the HPLC results from the evaluated temperatures are illustrated in Table 9. It is evident that the Click reaction at 100 ^{o}C is significantly slower compared to the reactions at 120 ^{o}C and 140 ^{o}C. The reaction at 120 ^{o}C progressed to 62% conversion (sum of both Click regioisomers) after 42 h whereas the reaction at 140 ^{o}C for 19 h performed slightly better with a total conversion of 69% (sum of both Click regioisomers) and contained less starting material alkyne (22%). The formation of impurities (*nido*) was lower at 120 ^{o}C even after 42 h and slightly elevated at 140 ^{o}C after 19 h, however heating the mixture for longer periods of time at 140 ^{o}C does consume more starting material alkyne, but at the expense of forming significantly higher amount of *nido*-like impurities which makes purification difficult downstream.

Table 10. Temperature Evaluation for the Thermal Click Reaction

Pilot Rxn	Temp	Time	Alkyne SM %AUC	1,4-Click Product %AUC	1,5-Click Product %AUC	Impurities %AUC
Pilot 1 PR074-023	100 °C	2.5 days 5 days 12 days 15 days	79 70 29 24	12 18 29 29	7 10 19 20	2 2 23 27
Pilot 2 PR074-036-A	120 °C▲	19 h 42 h	56 34	26 38	16 24	2 4
Pilot 3 PR074-036-B	140 °C▲	19 h 42 h	22 9	42 32	27 23	9 36

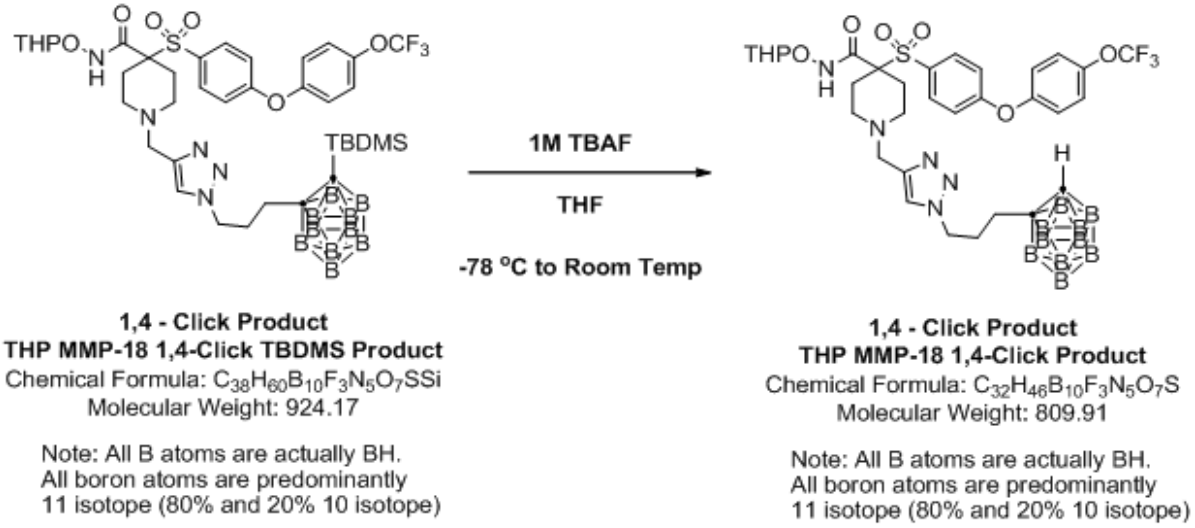
▲ - Reaction was performed in a pressure vial.

Based on the development of the thermal click chemistry work, scaling this procedure was attempted in a microwave vessel, however the poor microwave absorption of toluene prohibited the reaction from proceeding smoothly. A safer and more efficient method that would allow the preparation of Click products would be to perform the Click reaction in continuous flow which will allow the reaction to be rapidly heated to target temperature, and also to safely handle organo-azides especially at elevated temperatures. Continuous flow was not evaluated at this time, therefore the Click regioisomers was executed on a gram scale using batch-type pressure vessels at 120°C for 58 h. HPLC analysis of the reaction after 58 h showed ~65% conversion (sum of Click regioisomers) and indicated that there was

~20% unreacted alkyne. The reaction was deemed complete at this point to avoid degradation of product and introduction of *nido*-based impurities that are difficult to remove. Furthermore, HPLC analysis confirmed that the thermal Huisgen 1,3-dipolar cycloaddition reaction gives 1,4-Click and 1,5-Click products with a ratio of 1.6 to 1, respectively, which agrees with Sharpless' study[20] the component ratio of the click regioisiomers.

To access the final targets intended for assaying MMP inhibition and subsequent biodistribution studies, the protected carboranyl Click products (1,4- and 1,5-disubstituted) require deprotection of both the TBDMS and THP groups. Cleavage of the TBDMS group was executed using 1M tetrabutylammonium fluoride (TBAF) in THF which is shown in Scheme 36 for the 1,4-Click regioisomer and in Scheme 37 for the 1,5-Click regioisomer. The reaction conditions require cooling the mixture to cryogenic conditions prior to charging 1M TBAF to control the exotherm and also to prevent deboronation. Once the TBAF was added, the reaction was allowed to warm temperature and the reaction was complete within 30 minutes at ambient temperature.

Scheme 36. Cleaving the TBDMS group of the 1,4-Click regioisomer



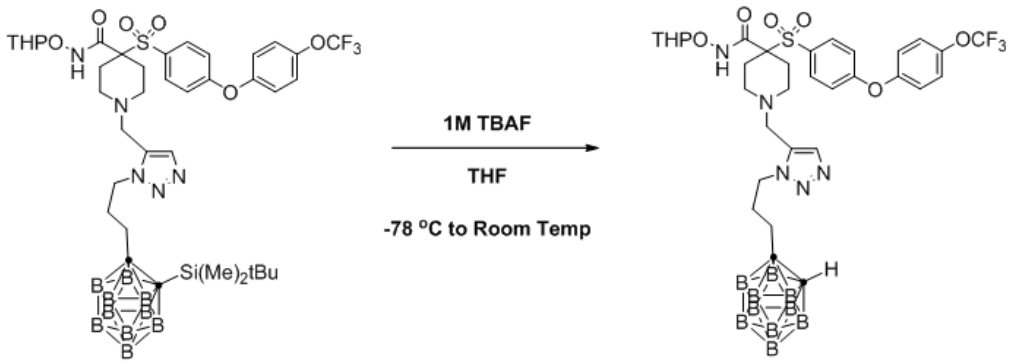
1,4 - Click Product
THP MMP-18 1,4-Click TBDMS Product
Chemical Formula: $C_{38}H_{60}B_{10}F_3N_5O_7SSi$
Molecular Weight: 924.17

Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope)

1,4 - Click Product
THP MMP-18 1,4-Click Product
Chemical Formula: $C_{32}H_{46}B_{10}F_3N_5O_7S$
Molecular Weight: 809.91

Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope)

Scheme 37. Cleaving the TBDMS group of the 1,5-Click regioisomer



THP MMP-18 1,5-Disubstituted Click TBDMS Product
Chemical Formula: $C_{38}H_{60}B_{10}F_3N_5O_7SSi$
Molecular Weight: 924.17

Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope)

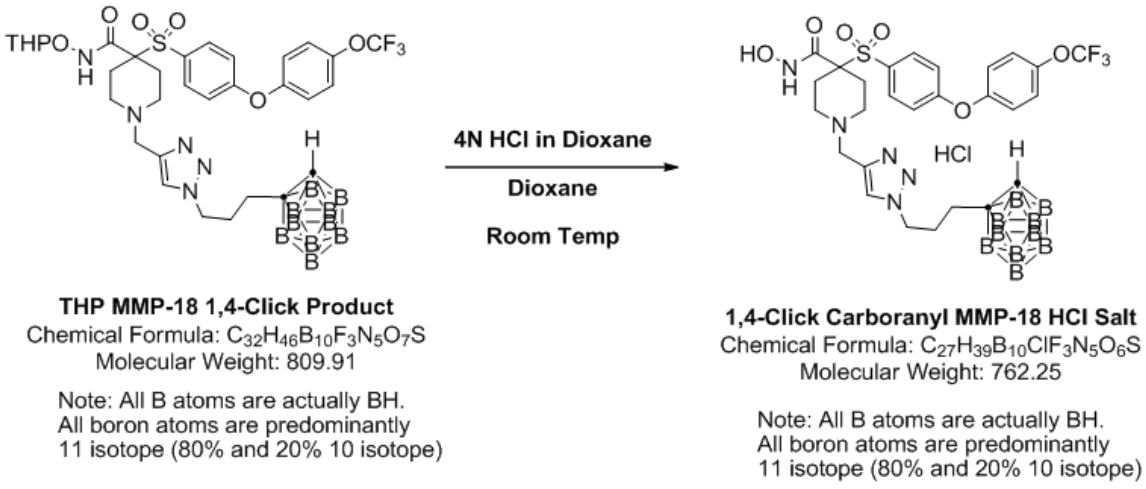
1,5 - Click Product
THP MMP-18 1,5-Click Product
Chemical Formula: $C_{32}H_{46}B_{10}F_3N_5O_7S$
Molecular Weight: 809.91

Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope)

The final stage of chemistry required the deprotection of the THP protecting group which was executed using 4N HCl in 1,4-dioxane as depicted in Schemes 38 and 39. The reaction was performed at room temperature and monitored by HPLC to ensure complete deprotection of THP

group, since TLC would lead to false interpretations of reaction completion since the starting material would simply form a hydrochloride salt and remain at the baseline along with desired product. Once the reaction was complete, the reaction mixture was concentrated, redissolved in dichloromethane, and subsequently precipitated using diethyl ether.

Scheme 38. Cleaving the THP group of the 1,4-Click regioisomer



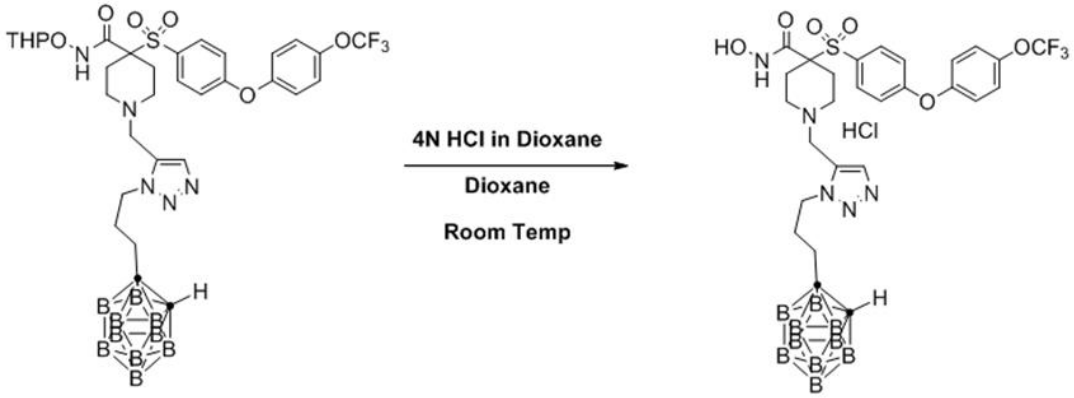
THP MMP-18 1,4-Click Product
Chemical Formula: $C_{32}H_{46}B_{10}F_3N_5O_7S$
Molecular Weight: 809.91

Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope)

1,4-Click Carboranyl MMP-18 HCl Salt
Chemical Formula: $C_{27}H_{39}B_{10}ClF_3N_5O_6S$
Molecular Weight: 762.25

Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope)

Scheme 39. Cleaving the THP group of the 1,5-Click regioisomer



1,5 - Click Product
THP MMP-18 Click Product
Chemical Formula: $C_{32}H_{46}B_{10}F_3N_5O_7S$
Molecular Weight: 809.91

Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope)

1,5-Click Carboranyl MMP-18 HCl Salt
Chemical Formula: $C_{27}H_{39}B_{10}ClF_3N_5O_6S$
Molecular Weight: 762.25

Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope)

Section 6: Conclusion and MMP Inhibition Data of the Click Products

The two Click products (1,4-Click and 1,5-Click) that were synthesized as hydrochloride salts were tested against three MMP enzymes (MMP-1, MMP-2, and MMP-9). In parallel to testing the Click products, Compound 9 was deprotected to produce the free hydroxamate as the hydrochloride salt and this sample was designed to be a control since it does not have a carborane cluster attached to it. The inhibition results shown in Table 10 illustrate that the 1,4-Click product and 1,5-Click product remained highly potent against MMP-2 and MMP-9. It is worth noting that these Click products showed insignificant binding to MMP-1 which is a positive result since reduced binding of MMP-1 will avoid the musculoskeletal syndrome (MSS) side effects if administered to humans. The control sample

(Compound 9 deprotected) showed sub-nanomolar potency for MMP-2 and MMP-9 (0.24 and 0.3 nM) as well but did not spare MMP-1 (860 nM). It is interesting that the 1,5-Click product was more potent for MMP-2 and MMP-9 compared to 1,4-Click product. A possible explaination of why the 1,5-Click product was more potent is that while being bound inside MMP-2 or MMP-9, the functionality extending from the piperidine nitrogen is essentially sticking out in solvent more than the 1,4-Click product. Furthermore, the regiochemistry is different between the 1,4 and 1,5-Click product and it could be that the carboranyl moiety of the 1,4-Click product is intramolecularly interacting with the hydroxamic acid through desirable lewis acid and lewis base chemistry since carboranes are lewis acidic and hydroxamic acids are lewis basic/chelators.

The results for the Click products positively indicate that the installation of the carborane moiety does not affect the host-guest interaction of MMP-2 and MMP-9 with 1,4 and 1,5-Click carboranyl substrates. In fact, it is likely that the carborane moiety is outside in solvent whereas the remaining of the substrate is acting as the pharmacophore and is bound tightly inside the active site of either MMP-2 and MMP-9.

In conclusion, this research has demonstrated that the two Click derived analogs can target MMP enzymes at nanomolar potency making BNCT a more viable route fighting and killing cancer cells.

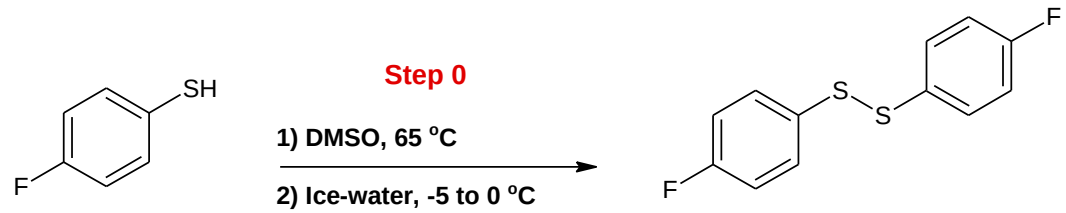
Table 11.MMP Inhibition of Click Products vs. MMP-1, MMP-2, and MMP-9

Compounds	IC_{50} (μM) or % Inhibition		
	MMP-1	MMP-2	MMP-9
PRO74-092-1[2, 3] Compound 9 deprotected	0.86	0.00024	0.0003
PRO74-062-5 1,4-Click Product	>10μM, *NI at 10μM	0.037 >270x sel	0.046 >217x sel
PRO74-088-2 1,5-Click Product	>10μM, 20% at 10μM	0.0098 1020x sel	0.013 769x sel
NNGH[1]	0.12	0.0027	0.0051

[1]NNGH (BML-205) is N-Isobutyl-N-(4-methoxyphenylsulfonyl)glycyl hydroxamic acid standard MMP inhibitor.
[2]PRO74-092-1 is compound **9** deprotected.
[3]Literature MMPi for **9** deprotected [Becker, J. Med. Chem. 2010, 53, 6653-6680]: MMP-1 IC_{50} = 2.6 μM, MMP-2 IC_{50} = <0.0001 μM, MMP-9 IC_{50} = 0.0001 μM.

Section 7: Experimental

Preparation of Bis(4-Florophenyl) disulfide (Compound 1)



4-Fluorothiophenol
C_6H_5FS
128.17 g/mol

Compound 1
Bis(4-Fluorophenyl) Disulfide
$C_{12}H_8F_2S_2$
254.32 g/mol

Procedure

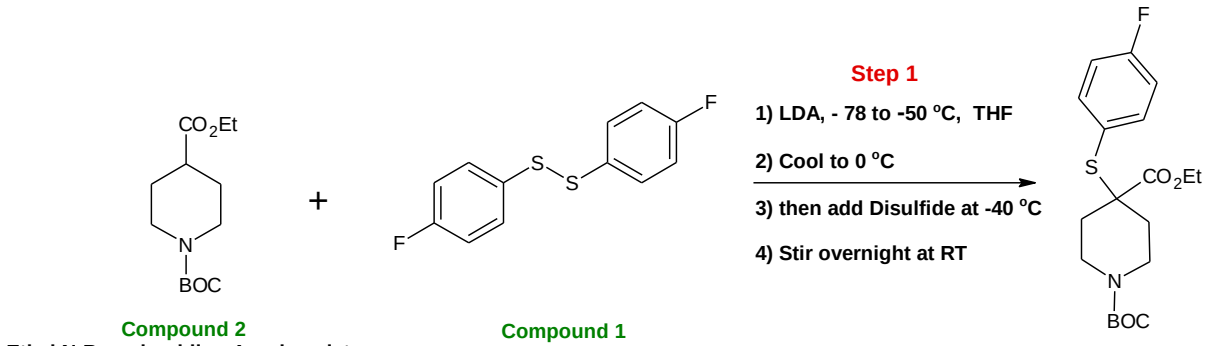
The reaction consisted of heating a mixture of 4-fluorothiophenol (215.25 g, 1.68 moles) and DMSO (1.4 liters) at 65 °C for 8 hours. The

mixture was cooled to 15 °C and this mixture was added (*via* vacuum transfer using a PTFE transfer line) to a second reactor equipped with an overhead mechanical stirrer containing a mixture of ice/USP purified water (5 Kg) to afford a white slurry which was stirred overnight at 0-5 °C. The slurry was filtered, and the wetcake was washed with cold USP water (1.5 L). Cmpd 1 was pulled dry overnight under a stream of nitrogen to provide white crystalline solids (Lot PR075-028-2, 207.4 g, low melting solid, 97.1% yield, HPLC purity: 100% AUC, ^{1}H NMR and ^{13}C NMR analysis: conforms to structure). Stored in the fridge under nitrogen.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.44 (m, 4H), 7.00 (m, 4H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 163.96, 161.49, 132.32, 132.29, 131.43, 131.34, 116.51, 116.29, 77.47, 77.16, 76.84.

Preparation of 1-*tert*-butyl 4-ethyl 4-(4-fluorophenylthio)piperidine-1,4-dicarboxylate (Compound 3)



Compound 2
Ethyl N-Boc-piperidine-4-carboxylate
$C_{13}H_{23}NO_4$
257.33 g/mol

Compound 1
Bis(4-Fluorophenyl) Disulfide
$C_{12}H_8F_2S_2$
254.32 g/mol

Compound 3
Sulfide Adduct
$C_{19}H_{26}FNO_4S$
383.48 g/mol

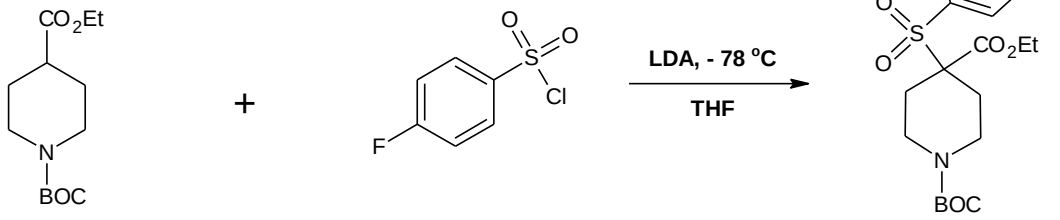
Procedure

A solution of Compound 2 (150.00 g, 0.58 moles) in anhydrous THF (1.8 liters) was cooled to -78 ^{o}C (dry ice/acetone). To this mixture was slowly added a solution of LDA (2.0 M in THF/Ethylbenzene, 350 mL, 0.70 moles) over 22 minutes maintaining the temperature $\leq$ -70 ^{o}C. The resulting mixture was allowed to warm to 0 ^{o}C, then cooled to -40 ^{o}C, and subsequently added a solution of Compound 1 (148.75 g, 0.583 mole) in anhydrous THF (600 mL) *via* addition funnel over 30 minutes. The mixture was allowed to warm to ambient temperature overnight. The following day, the reaction was deemed completed by HPLC analysis. The reaction mixture was quenched with a solution of acetic acid (7.7 g) in USP water (200 mL), concentrated under reduced pressure at 35-40 ^{o}C. The aqueous residue was extracted with ethyl acetate (3 x 450 mL) and the combined organic layers were successively washed with USP water (300 mL), and brine (500 mL). After drying with sodium sulfate, the solution was concentrated under reduced pressure to provide crude sulfide adduct (232.4 g). A silica plug (1.2 Kg, 60-200 micron, 5:1 ratio) purification of crude sulfide using ethyl acetate/heptane provided Compound 3 as a light yellow oil (Lot PR075-036-4, 192.26 g, 86.0% yield, HPLC purity: 99% AUC, ^{1}H NMR and ^{13}C NMR analysis: conforms to structure).

^{1}H NMR ($CDCl_3$, 400 MHz): δ =7.41 (m, 2H), 7.02 (m, 2H), 4.13 (q, 2H, *J* = 14.4 Hz), 3.80 (bd, 2H), 3.09 (m, 2H), 2.09 (m, 2H), 1.76 (m, 2H), 2.12-2.06 (m, 2H), 1.75 (m, 2H), 1.45 (s, 9H), 1.22 (t, 3H, *J* = 7.2 Hz).

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.72, 165.16, 162.67, 154.71, 139.17, 139.08, 125.16, 125.13, 116.12, 115.90, 79.81, 77.48, 77.16, 76.84, 61.33, 53.79, 33.17, 28.48, 14.16.

Preparation of 1-*tert*-butyl 4-ethyl 4-(4-fluorophenylsulfonyl)piperidine-1,4-dicarboxylate (Compound 4) - Alternative Route



Compound 2
Ethyl N-Boc-piperidine-4-carboxylate
$C_{13}H_{23}NO_4$
257.33 g/mol

4-Fluorobenzenesulfonyl chloride
$C_6H_4ClFO_2S$
194.61 g/mol

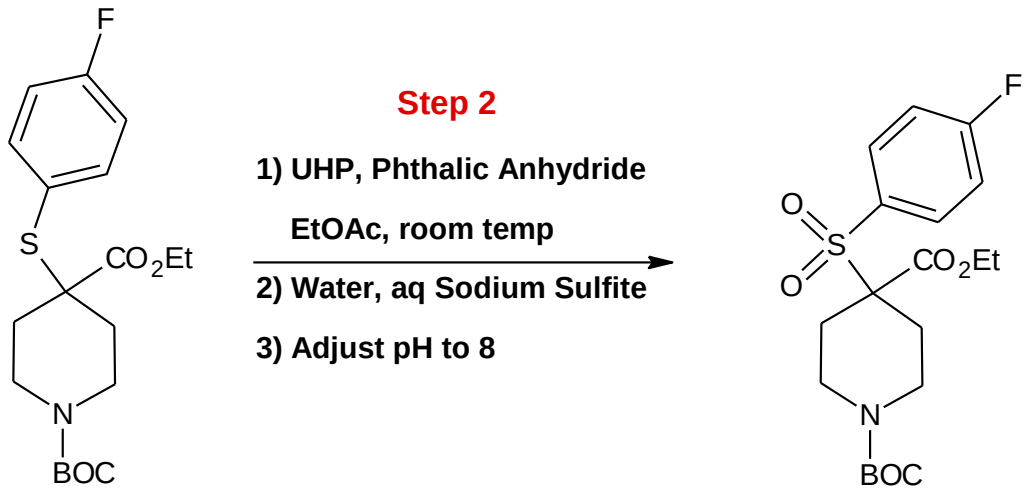
Compound 4
Sulfone Adduct
$C_{19}H_{26}FNO_6S$
415.48 g/mol

Procedure

A pilot reaction was performed to determine if the sulfone adduct (Compound 4) can be easily accessible through anion generation of piperidine scaffold via LDA followed by addition of aryl sulfonyl chloride. The reaction proved to be successful cleanly producing 80% conversion to desired product. No workup was performed on this reaction as it was solely

for information only. For future preparation, this route can be used to directly access sulfone.

Preparation of 1-*tert*-butyl 4-ethyl 4-(4-fluorophenylsulfonyl)piperidine-1,4-dicarboxylate (Compound 4)



Compound 3
Sulfide Adduct
$C_{19}H_{26}FNO_4S$
383.48 g/mol

Compound 4
Sulfone Adduct
$C_{19}H_{26}FNO_6S$
415.48 g/mol

Procedure

To a 5 L glass reactor (3-necked), equipped with an overhead mechanical stirrer, J-Kem thermocouple, cooling bath (water bath at 15-20 ^{o}C) , nitrogen inlet, and an additional funnel, was charged with ethyl acetate (1.5 L), urea hydrogen peroxide (UHP, 132.61 g, 1.41 moles), and Compound 3 (180.00 g, 0.469 moles). Solid phthalic anhydride (209.16 g, 1.41 moles) was added over 5 minutes and ethyl acetate (700 mL) was used as a rinse. The resulting mixture was allowed to stir overnight while the

cooling bath (15-20 ^{o}C) remained in place. After 18 hours, HPLC analysis showed complete conversion to sulfone. The reaction slurry was filtered (to remove urea and phthalic acid) and the filtrate was washed with a solution of aqueous 10% sodium sulfite (1.27 liters). The organic layer was digested with aqueous 10% sodium carbonate (800 mL), washed with brine, dried with sodium sulfate, concentrated and finally chased with heptane to provide Compound 4 (Lot PR075-042-2, off-white solid, 193.61 g, 99.3% yield, HPLC purity: 100% AUC, ^{1}H NMR and ^{13}C NMR analysis: conforms to structure).

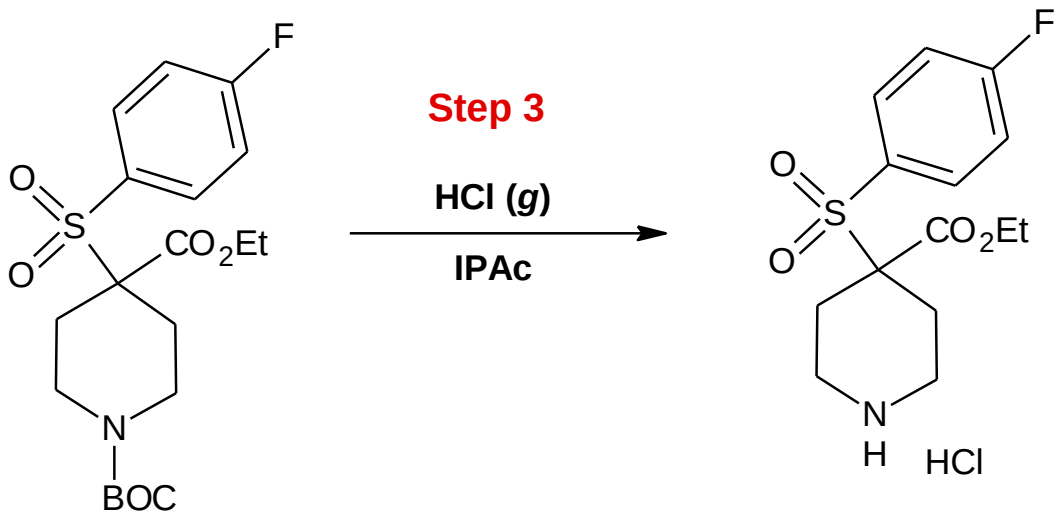
^{1}H NMR ($CDCl_3$, 400 MHz): δ =7.81 (m, 2H), 7.25 (m, 2H), 4.20 (m, 4H), 2.63 (vbs, 2H), 2.30 (bs, 2H), 2.04 (td, 2H, *J* = 12.0, 4.0 Hz), 1.45 (s, 9H), 1.25 (t, 3H, *J* = 8.0 Hz)

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 167.66, 166.7, 165.10, 154.40, 133.26, 133.16, 131.11, 131.08, 116.41, 116.18, 80.26, 77.48, 77.16, 76.84, 72.58, 62.77, 28.41, 27.80, 13.99, 0.05

HRMS (ESI-ToF): m/z calcd for $C_{19}H_{26}FNNaO_6S^+$ $[M+Na]^+$: 438.1363, found 438.1361.

m.p. 89-97 ^{o}C.

Preparation of ethyl 4-(4-fluorophenylsulfonyl)piperidine-4-carboxylate hydrochloride (Compound 5)



Compound 4
Sulfone Adduct
$C_{19}H_{26}FNO_6S$
415.48 g/mol

Compound 5
$C_{14}H_{19}ClFNO_4S$
351.82 g/mol

Procedure

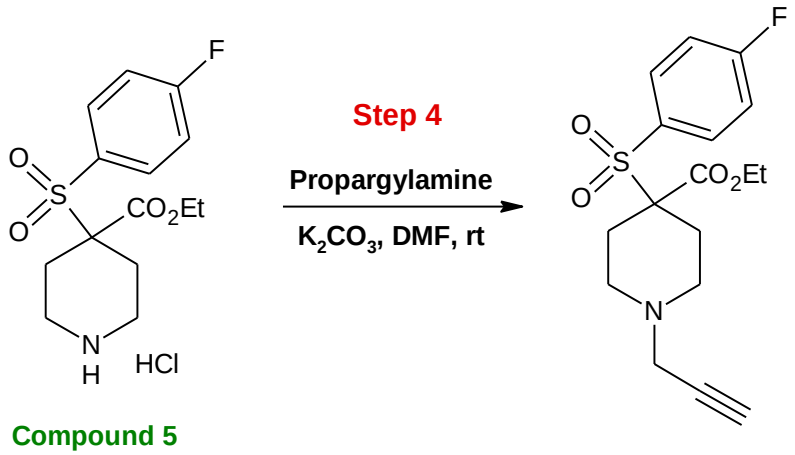
The reaction consisted of sparging HCl gas (10.00 g) into a solution of Compound 4 (10.25 g, 24.7 mmoles) in isopropylacetate (IPAc, 120 mL) and the resultant mixture was allowed to stir overnight at room temperature. HPLC analysis confirmed that the reaction was complete and then the mixture was concentrated under reduced pressure to afford Compound 5 as a white solid (8.72 g, 100% yield, HPLC purity: 100% AUC, ^{1}H NMR and ^{13}C NMR analysis: conforms to structure.

^{1}H NMR (DMSO-d_6, 400 MHz): δ =9.65 (bs, 1H), 9.42 (bs, 1H), 7.89 (m, 2H), 7.58 (t, 2H, *J* = 8.8 Hz), 4.13 (q, 2H, *J* = 14.4 Hz), 3.41 (d, 2H *J* = 13.2 Hz), 2.76 (bs, 2H), 2.32 (m, 4H), 1.10 (t, 3H, *J* = 7.2 Hz)

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.74, 167.94, 165.97, 165.36, 133.46, 133.36, 130.33, 130.30, 116.79, 116.56, 77.47, 77.15, 76.84, 70.05, 67.69, 63.58, 41.32, 25.12, 21.88, 21.50, 13.92, 0.05

HRMS (ESI-ToF): m/z calcd for $C_{14}H_{19}FNO_4S^+$ $[M+H]^+$: 316.1019, found 316.1018.

Preparation of ethyl 4-(4-fluorophenylsulfonyl)-1-(prop-2-ynyl)piperidine-4-carboxylate (Compound 6)



Compound 5
Sulfone Adduct HCl Salt
$C_{14}H_{19}ClFNO_4S$
351.82 g/mol

Compound 6
Propargylamine Adduct
$C_{17}H_{20}FNO_4S$
353.41 g/mol

Procedure

The reaction was carried out in a 2-liter reactor which involved charging Compound 5 (30.00 g, 85.27 mmoles), anhydrous DMF (600 mL),

and potassium carbonate (23.8 g, 172.20 mmoles) and the resulting mixture was allowed to stir (mechanical stirring) for 10 minutes. Propargylamine (97% purity, 10.35 g, 87.00 mmoles) was added and the reaction was allowed to stir for 23 hours at room temperature in which HPLC analysis revealed 11.75% AUC of unreacted Compound 5 and 87.3%AUC of Compound 6. The reaction was charged with an additional portion of propargylamine (1.58 g, 1.00 mL, 8.41 mmoles) and the reaction was allowed to stir for an additional 1 hour at room temperature in which HPLC showed that the reaction was deemed complete (2.3% AUC of unreacted Compound 5 was noted and 96.3% AUC of Compound 6).

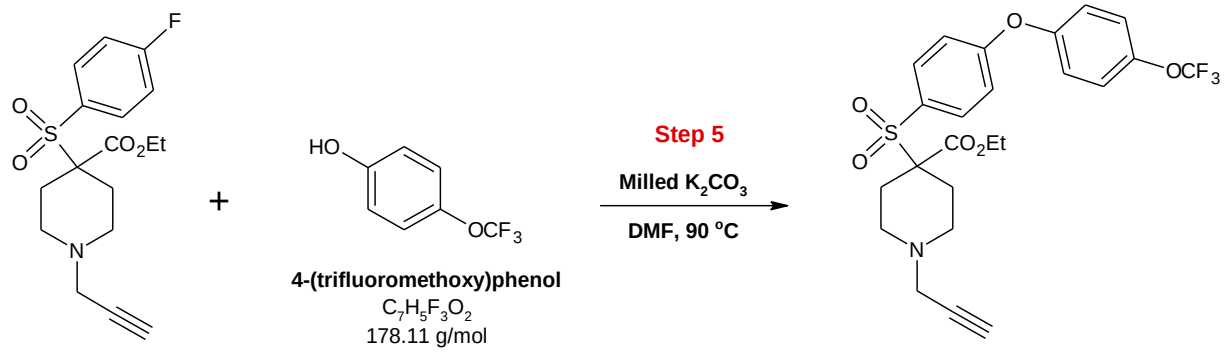
The reaction mixture was transferred to a 5-liter reactor and diluted with ice-cold USP purified water (2.5 liters) to initiate crystallization. The resulting slurry was stirred overnight at 0-5 ^{o}C *via* mechanical stirring, filtered, washed with USP water (150 mL), and pulled dry under a stream of nitrogen to provide Compound 6 (28.44 g, 94.4% yield, Lot PR075-053-3, light beige solids, HPLC analysis: 99.81% AUC, ^{1}H NMR and ^{13}C NMR analysis: conforms to desired product).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.82 (m, 2H), 7.23 (m, 2H), 4.21 (q, 2H, *J* = 12.0 Hz), 3.26 (d, 2H, *J* = 4.0 Hz), 2.90 (m, 2H), 2.39 (m, 2H), 2.20 (m, 5H), 1.25 (t, 3H, *J* = 12.0 Hz)

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 167.5, 166.8, 165.0, 133.2, 133.1, 131.4, 116.3, 116.1, 78.2, 77.4, 77.1, 76.8, 73.5, 71.9, 62.5, 49.1, 46.6, 49.1, 46.6, 28.1, 14.0

HRMS (ESI-ToF): m/z calcd for $C_{17}H_{21}FNO_4S^+$ $[M+H]^+$: 354.1175, found 354.1176.

Preparation of ethyl 1-(prop-2-ynyl)-4-(4-(4-(trifluoromethoxy)phenoxy)phenylsulfonyl)piperidine-4-carboxylate (Compound 7)



Compound 6
Propargylamine Adduct
$C_{17}H_{20}FNO_4S$
353.41 g/mol

Compound 7
Diaryl Ether Adduct
$C_{24}H_{24}F_3NO_6S$
511.51 g/mol

Procedure

To a 500-mL reactor equipped with an overhead mechanical stirrer, J-Kem thermocouple, heating mantle, nitrogen inlet, and an additional funnel, was charged with Compound 6 (24.86 g, 70.34 mmoles), anhydrous DMF (125 mL), milled potassium carbonate (19.54 g, 141.38 mmoles), and 4-(trifluoromethoxy)phenol (25.10 g, 140.69 mmoles). The reaction was

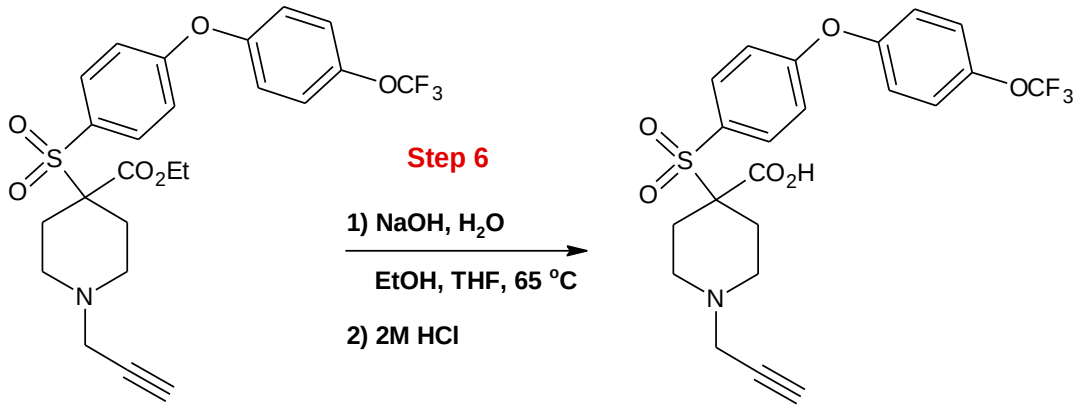
heated at 90 ^{o}C for 19 hours which HPLC analysis showed that the reaction was deemed complete. The reaction mixture was cooled to room temperature and DMF was removed on a rotary evaporator under reduced pressure at 40-60 ^{o}C. The residue was diluted with MTBE/Toluene (1:1, 300 mL) and washed with 1M NaOH (2 x 250 mL), USP purified water (250 mL), brine (250 mL), dried with sodium sulfate, filtered, and concentrated to furnish Compound 7 (36.06 g, 100% yield, Lot PR075-058-4, yellow oil, HPLC analysis: 98.03% AUC, ^{1}H NMR and ^{13}C NMR analysis: conforms to desired product).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.76 (m, 2H), 7.27 (m, 2H), 7.10 (m, 2H), 7.06 (m, 2H), 4.23 (m, 2H), 3.25 (s, 2H), 2.90 (bs, 2H), 2.40 (bd, 2H), 2.19 (m, 5H), 1.27 (m, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 167.0, 162.5, 153.3, 146.1, 132.8, 129.6, 123.0, 121.6, 117.3, 78.3, 77.4, 77.1, 76.7, 73.4, 72.0, 62.4, 49.3, 46.7, 28.3, 14.0

HRMS (ESI-ToF): m/z calcd for $C_{24}H_{25}F_3NO_6S^+$ $[M+H]^+$: 512.1355, found 512.1353.

Preparation of ethyl 1-(prop-2-ynyl)-4-(4-(4-(trifluoromethoxy)phenoxy)phenylsulfonyl)piperidine-4-carboxylate (Compound 8)



Compound 7
Diaryl Ether Adduct
$C_{24}H_{24}F_3NO_6S$
511.51 g/mol

Compound 8
Diaryl Ether Free Acid
$C_{22}H_{20}F_3NO_6S$
483.46 g/mol

Procedure

To a 2-Liter reactor equipped with an overhead mechanical stirrer, J-Kem thermocouple, heating mantle, reflux condenser with nitrogen inlet, and an additional funnel, was charged with a solution of Compound 7 (28.50 g, 55.72 mmoles) in EtOH/THF (1:1, 850 mL) and a solution of NaOH (22.30 g, 557.17 mmoles) in USP water (425 mL). The reaction mixture was heated to 65 °C and allowed to stir for 18 hours which HPLC analysis showed complete consumption of Compound 7. The mixture was allowed to cool to ambient temperature, diluted with USP water (300 mL), and concentrated at 45-50 °C on a rotary evaporator to remove EtOH and THF. The aqueous residue

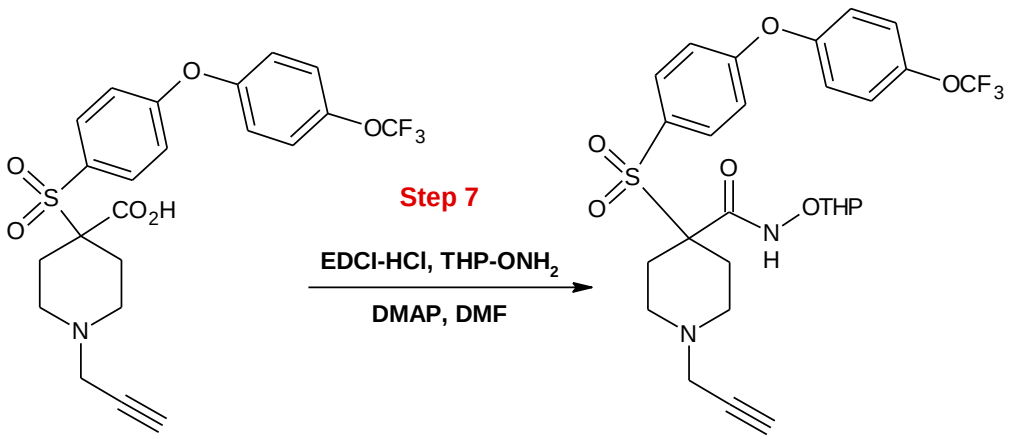
was diluted with USP water (600 mL) and the pH was adjusted to pH 2.25 using 2M HCl (282 mL) providing a thick white slurry. The slurry was filtered, washed with USP water (750 mL), pulled dry, and dried further in a vacuum oven at 40 ^{o}C to provide Compound 8 (26.30 g, 97.6% yield, Lot PR075-063-2, white solid, HPLC analysis: 94.4% AUC, ^{1}H NMR and ^{13}C NMR analysis: conforms to desired product).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.78 (m, 2H), 7.49 (d, 2H, *J* = 8.0 Hz), 7.31 (m, 2H), 7.19 (m, 2H), 3.31 (s, 2H), 3.17 (s, 1H), 2.85 (d, 2H, *J* = 12.0 Hz), 2.19 (d, 2H, *J* = 12.0 Hz), 2.11 (t, 2H, *J* = 12.0 Hz), 1.92 (td, 2H, *J* = 12.0, 4.0 Hz)

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 167.5, 161.7, 153.3, 145.03, 145.01, 132.8, 129.2, 123.32, 122.0, 117.4, 78.5, 76.3, 71.0, 48.4, 45.7, 27.5

HRMS (ESI-ToF): m/z calcd for $C_{22}H_{21}F_3NO_6S^+$ $[M+H]^+$: 484.1042, found 484.1042.

Preparation of 1-(prop-2-ynyl)-N-(tetrahydro-2H-pyran-2-yloxy)-4-(4-(4-(trifluoromethoxy)phenoxy)phenylsulfonyl)piperidine-4-carboxamide (Compound 9)



Compound 8
Diaryl Ether Free Acid
$C_{22}H_{20}F_3NO_6S$
483.46 g/mol

Compound 9
MMP-18 THP Free Base
$C_{27}H_{29}F_3N_2O_7S$
582.59 g/mol

Procedure

To a 500-mL reactor, equipped with a large magnetic stirring bar, J-Kem thermocouple, cooling dewer, and nitrogen inlet, was charged with Compound 8 (20.00 g, 41.37 mmoles) and anhydrous DMF (180 mL). The mixture was allowed to stir for 10 minutes at ambient temperature then cooled the mixture to < 5 ^{o}C. To the cooled mixture was sequentially added O-(Tetrahydro-2H-pyran-2-yl)hydroxylamine (7.50 g, 64.02 mmoles), EDCI-HCl (10.00 g, 81.85 mmoles), and DMAP (8.00 g, 65.36 mmoles). The mixture was allowed to stir overnight and gradually warmed to room temperature while the reactor remained in the cooling bath. After 17 hours,

HPLC analysis revealed 16.1% of unreacted Compound 8 free acid. The cooling bath was removed and the reaction mixture was allowed to stir an additional 33 hours at room temperature whereby HPLC analysis confirmed that the reaction was deemed complete. The reaction was quenched with USP water (5 mL) and concentrated at 30 ^{o}C on a rotovap evaporator to remove DMF. The residue was diluted with ethyl acetate (350 mL) and subsequently washed with saturated aqueous sodium bicarbonate solution (200 mL), brine (200 mL), dried with magnesium sulfate, filtered, and concentrated under reduced pressure to provide crude Compound 9 (34.31 g).

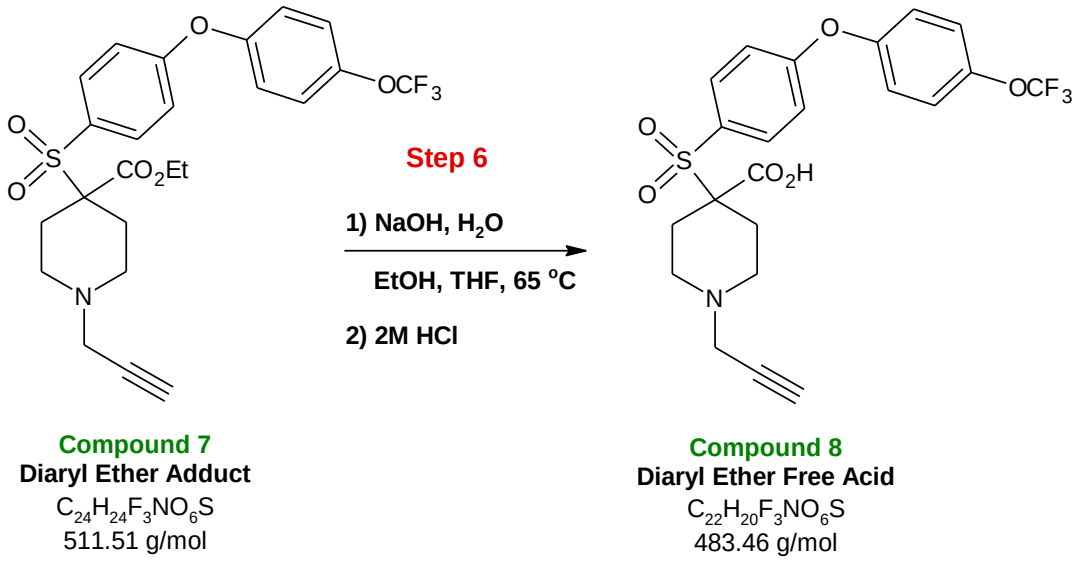
Crude Compound 9 (34.31 g) was purified by silica gel chromatography using a glass gravity column (24 in x 3 in) and silica gel (680 g, 60-200 micron, Silicycle, equilibrated with 30% EtOAc in heptane). Crude Compound 9 was dissolved in ethyl acetate (20 mL) then diluted with heptane (20 mL) and subsequently loaded onto the silica bed. The column was eluted with an EtOAc/Heptane gradient (30% to 70%). Appropriate fractions were combined and concentrated to provide Compound 9 (21.78 g, 90.4% yield, Lot PR075-070-9, white solid, HPLC analysis: 97% AUC, ^{1}H NMR and ^{13}C NMR analysis: conforms to desired product, Mass Spec analysis: $[M+H]^{+}$ = 583.46 m/z, FTIR: conforms to desired product).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 9.40 (s, 1H), 7.80 (dd, 2H, *J* = 6.9, 2.0 Hz), 7.27 (m, 2H), 7.12 (dd, 2H, *J* = 6.8, 2.3 Hz), 7.05 (dd, 2H, *J* = 6.9, 2.0 Hz), 5.00 (t, 1H, *J* = 2.8 Hz), 4.00 (td, 1H, *J* = 11.2, 2.4 Hz), 3.69 (m, 1H), 3.23 (d, 2H, *J* = 2.4 Hz), 2.92 (m, 2H), 2.35-2.30 (m, 3H), 2.25-2.20 (m, 4H), 1.88-1.76 (m, 3H), 1.68-1.58 (m, 3H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 164.0, 162.8, 153.0, 146.1, 146.0, 132.6, 128.0, 123.0, 121.8, 121.7, 119.2, 117.4, 102.0, 78.6, 73.4, 70.3, 62.3, 49.0, 48.9, 46.6, 28.6, 28.4, 27.8, 25.0, 18.3.

HRMS (ESI-ToF): m/z calcd for $C_{27}H_{30}F_3N_2O_7S^+$ $[M+H]^+$: 583.1726, found 583.1723.

Preparation of ethyl 1-(prop-2-ynyl)-4-(4-(4-(trifluoromethoxy)phenoxy)phenylsulfonyl)piperidine-4-carboxylate (Compound 8)



Compound 7
Diaryl Ether Adduct
$C_{24}H_{24}F_3NO_6S$
511.51 g/mol

Compound 8
Diaryl Ether Free Acid
$C_{22}H_{20}F_3NO_6S$
483.46 g/mol

Procedure

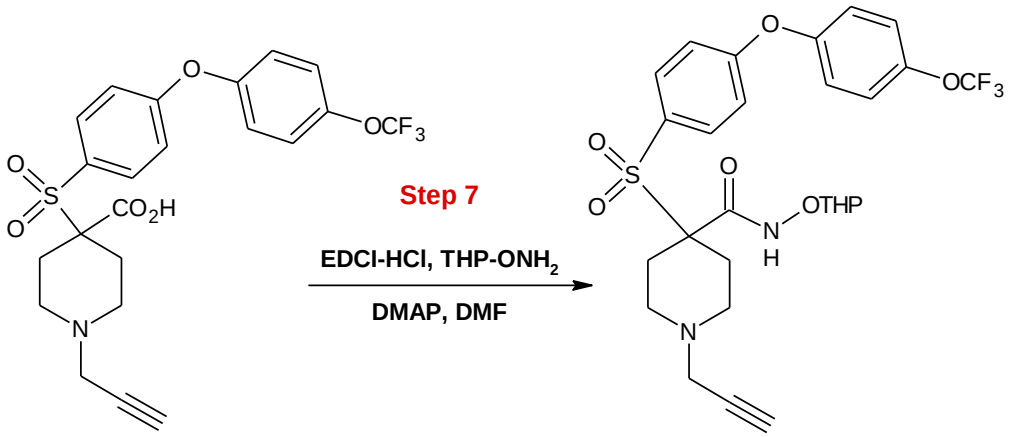
To a 2-Liter reactor equipped with an overhead mechanical stirrer, J-Kem thermocouple, heating mantle, reflux condenser with nitrogen inlet, and an additional funnel, was charged with a solution of Compound 7 (28.50 g, 55.72 mmoles) in EtOH/THF (1:1, 850 mL) and a solution of NaOH (22.30 g, 557.17 mmoles) in USP water (425 mL). The reaction mixture was heated to 65 ^{o}C and allowed to stir for 18 hours which HPLC analysis showed complete consumption of Compound 7. The mixture was allowed to cool to ambient temperature, diluted with USP water (300 mL), and concentrated at 45-50 ^{o}C on a rotary evaporator to remove EtOH and THF. The aqueous residue was diluted with USP water (600 mL) and the pH was adjusted to pH 2.25 using 2M HCl (282 mL) providing a thick white slurry. The slurry was filtered, washed with USP water (750 mL), pulled dry, and dried further in a vacuum oven at 40 ^{o}C to provide Compound 8 (26.30 g, 97.6% yield, Lot PR075-063-2, white solid, HPLC analysis: 94.4% AUC, ^{1}H NMR and ^{13}C NMR analysis: conforms to desired product).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.78 (m, 2H), 7.49 (d, 2H, *J* = 8.0 Hz), 7.31 (m, 2H), 7.19 (m, 2H), 3.31 (s, 2H), 3.17 (s, 1H), 2.85 (d, 2H, *J* = 12.0 Hz), 2.19 (d, 2H, *J* = 12.0 Hz), 2.11 (t, 2H, *J* = 12.0 Hz), 1.92 (td, 2H, *J* = 12.0, 4.0 Hz)

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 167.5, 161.7, 153.3, 145.03, 145.01, 132.8, 129.2, 123.32, 122.0, 117.4, 78.5, 76.3, 71.0, 48.4, 45.7, 27.5

HRMS (ESI-ToF): m/z calcd for $C_{22}H_{21}F_3NO_6S^+$ $[M+H]^+$: 484.1042, found 484.1042.

Preparation of 1-(prop-2-ynyl)-N-(tetrahydro-2H-pyran-2-yloxy)-4-(4-(4-(trifluoromethoxy)phenoxy)phenylsulfonyl)piperidine-4-carboxamide (Compound 9)



Compound 8
Diaryl Ether Free Acid
$C_{22}H_{20}F_3NO_6S$
483.46 g/mol

Compound 9
MMP-18 THP Free Base
$C_{27}H_{29}F_3N_2O_7S$
582.59 g/mol

Procedure

To a 500-mL reactor, equipped with a large magnetic stirring bar, J-Kem thermocouple, cooling dewer, and nitrogen inlet, was charged with Compound 8 (20.00 g, 41.37 mmoles) and anhydrous DMF (180 mL). The mixture was allowed to stir for 10 minutes at ambient temperature then cooled the mixture to < 5 ^{o}C. To the cooled mixture was sequentially added

O-(Tetrahydro-2H-pyran-2-yl)hydroxylamine (7.50 g, 64.02 mmoles), EDCI-HCl (10.00 g, 81.85 mmoles), and DMAP (8.00 g, 65.36 mmoles). The mixture was allowed to stir overnight and gradually warmed to room temperature while the reactor remained in the cooling bath. After 17 hours, HPLC analysis revealed 16.1% of unreacted Compound 8 free acid. The cooling bath was removed and the reaction mixture was allowed to stir an additional 33 hours at room temperature whereby HPLC analysis confirmed that the reaction was deemed complete. The reaction was quenched with USP water (5 mL) and concentrated at 30 ^{o}C on a rotovap evaporator to remove DMF. The residue was diluted with ethyl acetate (350 mL) and subsequently washed with saturated aqueous sodium bicarbonate solution (200 mL), brine (200 mL), dried with magnesium sulfate, filtered, and concentrated under reduced pressure to provide crude Compound 9 (34.31 g).

Crude Compound 9 (34.31 g) was purified by silica gel chromatography using a glass gravity column (24 in x 3 in) and silica gel (680 g, 60-200 micron, Silicycle, equilibrated with 30% EtOAc in heptane). Crude Compound 9 was dissolved in ethyl acetate (20 mL) then diluted with heptane (20 mL) and subsequently loaded onto the silica bed. The column was eluted with an EtOAc/Heptane gradient (30% to 70%). Appropriate fractions were combined and concentrated to provide Compound 9 (21.78 g,

90.4% yield, Lot PR075-070-9, white solid, HPLC analysis: 97% AUC, ^{1}H NMR and ^{13}C NMR analysis: conforms to desired product, Mass Spec analysis: $[M+H]^+$ = 583.46 m/z, FTIR: conforms to desired product).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 9.40 (s, 1H), 7.80 (dd, 2H, *J* = 6.9, 2.0 Hz), 7.27 (m, 2H), 7.12 (dd, 2H, *J* = 6.8, 2.3 Hz), 7.05 (dd, 2H, *J* = 6.9, 2.0 Hz), 5.00 (t, 1H, *J* = 2.8 Hz), 4.00 (td, 1H, *J* = 11.2, 2.4 Hz), 3.69 (m, 1H), 3.23 (d, 2H, *J* = 2.4 Hz), 2.92 (m, 2H), 2.35-2.30 (m, 3H), 2.25-2.20 (m, 4H), 1.88-1.76 (m, 3H), 1.68-1.58 (m, 3H).

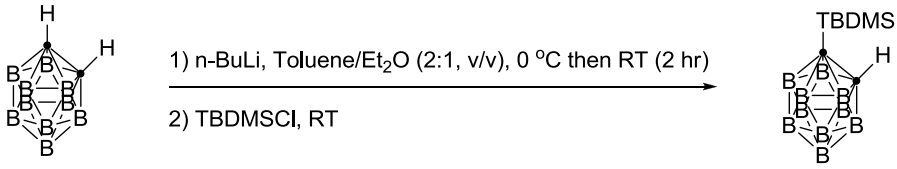
^{13}C NMR ($CDCl_3$, 100 MHz): δ = 164.0, 162.8, 153.0, 146.1, 146.0, 132.6, 128.0, 123.0, 121.8, 121.7, 119.2, 117.4, 102.0, 78.6, 73.4, 70.3, 62.3, 49.0, 48.9, 46.6, 28.6, 28.4, 27.8, 25.0, 18.3.

HRMS (ESI-ToF): m/z calcd for $C_{27}H_{30}F_3N_2O_7S^+$ $[M+H]^+$: 583.1726, found 583.1723.

Preparation of TBDMS Carborane (Compound 10)

PR075-190

Purpose: Scale the preparation of TBDMS functionalized o-carborane using reference J. Med. Chem., 2011, 54, 2368 and PR075-185.



***o*-Carborane**
Chemical Formula: $C_2H_{12}B_{10}$
Molecular Weight: 144.23

Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope).

TBDMS Carborane
Chemical Formula: $C_8H_{26}B_{10}Si$
Molecular Weight: 258.49

Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope).

EXPECTED CHARGES

Reagent	MW	Loading eq	amount g	amount mL	mmole
o-Carborane	144.23	1.00	**3.00**	----------	20.80
Toluene (anhydrous)	----------	----------	----------	**12.00**	----------
Diethyl Ether (anhydrous with BHT)	----------	----------	----------	**6.00**	----------
nBuLi (1.66 M in hexanes)	----------	1.05	----------	**13.16**	21.84
TBDMSCl	150.72	1.10	**3.45**	----------	22.88
USP Water	----------	----------	----------	**45.00**	----------
Diethyl Ether (3 extractions each)	----------	----------	----------	**30.00**	----------

Theoretical Yield of Product (in grams)	**5.38**

Procedure

To a 100 mL RBF was added 3.00 g of o-carborane, 12 mL anhydrous Toluene, and 6 mL anhydrous Et2O. Stirred at room temperature until completely dissolved then cooled to < 5°C. Added nBuLi solution (1.66M, 13.2 mL) over about 5 minutes to give a murky turbid white mixture. Removed cooling bath after 5 minutes and allowed to store at room temperature. After 2.5 hours, solid TBDMSCl (3.47 g) was added at room temperature as one portion which addition was endothermic. Reaction is a murky while solution. After 22.5 hours, the reaction slurry was analyzed by TLC (80% Hexane and 20% Et2O) and showed a trace amount of starting material and the reaction was deemed complete. The reaction mixture was quenched reaction with 30 mL of USP purified H2O, then extracted with Et2O (3x30 mL). The combined organic layers were dried with MgSO4, filtered and concentrated to give crude product 7.07g (pale yellow oil, lot PR075-190-2). The crude oil was purified over silica gel as follows. To a glass gravity fitted column (12x2in) was added sand, n-hexane, a slurry of 140 g of silica gel (60-200 micron) in n-hexane, further packed under a positive pressure of nitrogen, and lastly placed a layer of sand on top of the silica bed. Loaded neat crude product (7.07 g) onto glass gravity column and used n-hexane as a rinse. The column was eluted with n-hexane (250 mL) and 10% Et2O/hexane (825 mL). Collected 10 fractions (each approximately 75 mL).

Analyzed fractions using TLC. Appropriate fractions were combined and concentrated on rotovap at 25-30°C to give 5.05 g of Compound 10 as white crystalline solids (Lot PR075-190-3, 93.9% yield). *Literature reference that this procedure was followed: J. Med. Chem. 2011, 54, pg 2368.*

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 3.44 (bs,1H), 2.87-1.54 (m, 10H), 1.02 (s, 9H), 0.23 (s, 6H).

^{11}B NMR (Decoupled, 100 MHz): δ = 0.34, -1.76, -7.02, -10.73, -12.31, -13.26

^{11}B NMR (Coupled, 100 MHz): δ = 1.01, -0.94, -2.57, -6.29, -7.87, -9.99, -11.62, -12.41, -13.2, -14.26

Preparation of 1-azido-3-chloropropane (Compound 11)

Safety Note on Handling Azido Compounds (refer to references 1, 2, 3 below)

Handling Sodium Azide
Sodium azide is extremely toxic (LC_{50} Inhalation = 37 mg/m^3 for rats, LD_{50} Dermal = 20mg/kg for rabbits) and very soluble in water (>30 g/100 mL at 0 °C). Sodium azide can be easily absorbed dermally and consequently must be handled with appropriate personal protection equipment (PPE).
Sodium azide decomposes above 275 °C, generating highly reactive sodium metal. Sodium azide is not compatible with any acid as it spontaneously forms highly explosive hydrazoic acid on contact, even in dilute solution.

Low Molecular Weight Organic Azides
Low molecular weight organic azides are potentially explosive substances that can decompose with a slight input of external energy (heat, friction, pressure etc). Although there has not been any documented explosions on this end of work, any organic azides where the weight attributed to the azido

group exceeds 25% of the molecular weight should be handled with significant caution. It is recommended that a blast shield be used during synthesis and avoid of very large-scale reactions when dealing with these substances.

1. Sigma-Aldrich, *Sodium Azide*; MSDS No. 13412 [Online]; Auckland, NZ, **Nov 05, 2012**
http://www.sigmaaldrich.com/catalog/product/sial/13412 (accessed March 29, 2017).

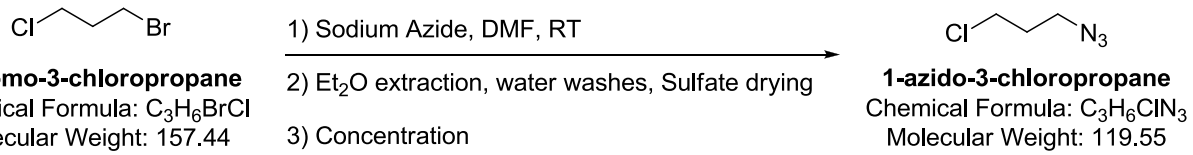
2. T. Archibald in *Managing Hazardous Reactions and Compounds in Process Chemistry*, *Vol*. *1181*
(Eds.: J. A. Pesti, A. F. Abdel-Magid), American Chemical Society: Washington, DC, **2014**; pp. 87-
109.

3. T. Keicher, S. Löbbecke in *Organic Azides: Syntheses and Applications*; (Eds.: S. Bräse, K. Banert),Wiley: Chichester, U.K., **2010**; pp 3.

PR075-191

Purpose:

To scale the preparation of azido-propyl chloride according to ACIEE, 56(26), 7420-7424; 2017



1-bromo-3-chloropropane
Chemical Formula: C_3H_6BrCl
Molecular Weight: 157.44

1-azido-3-chloropropane
Chemical Formula: $C_3H_6ClN_3$
Molecular Weight: 119.55

Procedure

To a 250 mL round bottomed flask was added 100 mL of anhydrous DMF and 10.02 g of 1-bromo-3-chloropropane and then 4.2 g of sodium azide. The reaction was placed in an ambient water bath and stirred overnight (16 hrs) at room temperature. The reaction mixture was diluted with 50 mL of Et2O and 50 mL USP purified water. Stirred 2-3 minutes then separated the organic layer (top). Extracted the bottom aqueous layer with

Et2O (2x 60 mL). The combined organic layers were washed with USP purified water (3x 50 mL), dried with Na2SO4, filtered and concentrated at 25-30°C under reduced pressure to give Compound 11 as a colorless oil, lot PR075-191-1 (7.21g), 95% crude yield which was taken forward without any further purification. This gives a mixture of 1-azido-3-chloropropane as the major product (81%) and does contain ~19% of 1-azido-3-bromopropane. The next step involves iodide formation which under Finkelstein conditions will convert chloro/bromo to iodo as the sole product. Precedent: Angewandte Chemie International Edition, 56(26), 7420-7424; 2017

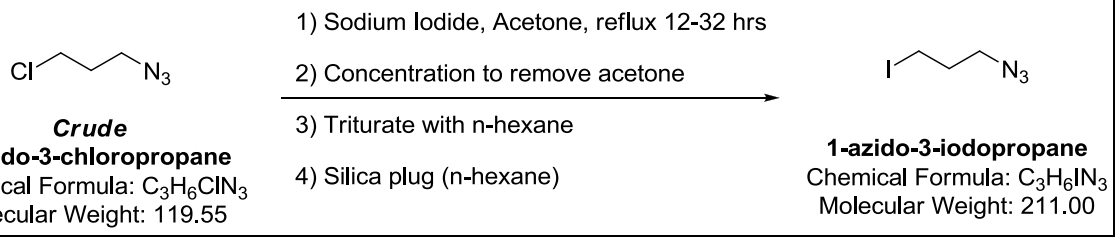
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 3.64 (t, 2H, *J* = 8.0 Hz), 3.51 (t, 3H, *J* = 8.0 Hz), 2.02 (p, 2H, *J* = 12.0, 4.0 Hz)

Procedure for 1-azido-3-iodopropane (Compound 12)

PR074-018

Purpose:

Scale up the preparation of azido-propyl iodide following the improved process from PR074-013 which was derived from process described in precedent ACIEE, 56(26), 7420-7424; 2017.



Crude
1-azido-3-chloropropane
Chemical Formula: $C_3H_6ClN_3$
Molecular Weight: 119.55

1-azido-3-iodopropane
Chemical Formula: $C_3H_6IN_3$
Molecular Weight: 211.00

EXPECTED CHARGES						
Reagent	MW	Density	Loading eq	amount g	amount mL	mmole
Crude 1-azido-3-chloropropane	119.55	----------	1.00	**7.50**	----------	62.74
Acetone	----------	----------	----------	----------	**187.50**	----------
Sodium Iodide	149.89	----------	2.00	**18.81**	----------	125.47
Silica Gel (60-200 micron)	----------	----------	----------	----------	**75.00**	----------

Theoretical Yield of Product 12 (in grams)	**13.24**

Procedure for 1-azido-3-iodopropane (Compound 12)

To a 1000 mL round bottomed flask (3-necked) was added 19.35 g of NaI, 7.5 g of crude 1-azido-3-chloropropane, and 190 mL of acetone. Purged with N2 and heated to 52ºC. Covered with foil. After 40 hours, the reaction

was allowed reaction to cool to room temperature. The reaction mixture (yellow slurry) was filtered over a pad of Celite, washed funnel and flask with acetone (~100mL), then concentrated the yellow filtrate on a rotavap at 25-30ºC to remove acetone. After concentration, an orange-yellow residue was obtained (oily solids, 26.2 g). Added 50 mL hexane to oil/solid residue (yellowish-orange) which changed the color to a greenish solid. Stirred the slurry overnight at room temperature then passed over a short silica plug(65g) packed in hexane. Flushed with n-Hexane to collect fractions (each 50-70 mL). Appropriate fractions were combined and concentrated at 25ºC to give 10.77 g of Compound 12 as a colorless oil (81.3% yield, Lot PR074-018-3). Precedent: Angewandte Chemie International Edition, 56(26), 7420-7424; 2017

^{1}H NMR ($CDCl_3$, 400 MHz): δ= 3.44 (t, 2H), 3.25 (t, 2H), 2.04 (p, 2H)

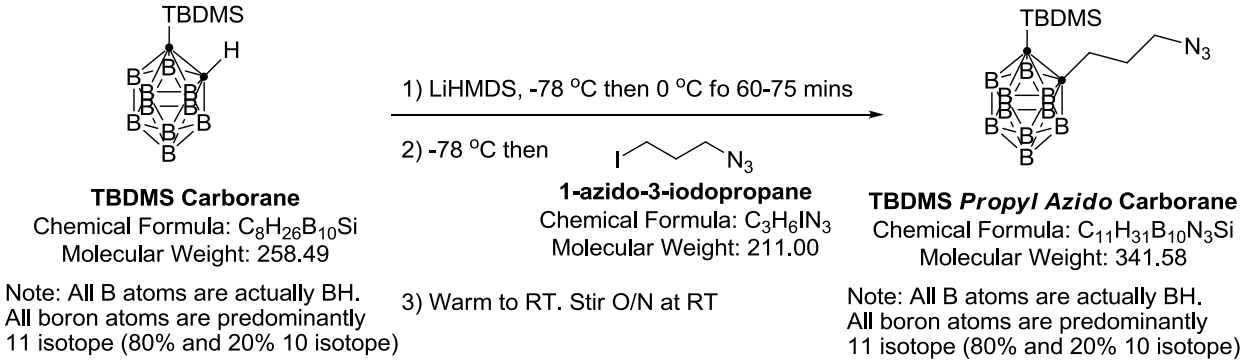
Preparation of TBDMS Propyl Azido Carborane (Compound 13)

PR074-027

Purpose:

Scale the preparation of TBDMS Propyl Azido Carborane using 2 grams of TBDMS Carborane and LiHMDS as the base and THF as the reaction medium.

Precedents: PR074-016 and ACIEE, 56(26), 7420-7424; 2017
Note: the ACIEE precedent does not respresent the exact chemistry below, but it shows that an enolate formed by LiHMDS can react with iodo-alkyl-azide.



TBDMS Carborane
Chemical Formula: $C_8H_{26}B_{10}Si$
Molecular Weight: 258.49

Note: All B atoms are actually BH. All boron atoms are predominantly 11 isotope (80% and 20% 10 isotope)

1-azido-3-iodopropane
Chemical Formula: $C_3H_6IN_3$
Molecular Weight: 211.00

TBDMS *Propyl Azido* Carborane
Chemical Formula: $C_{11}H_{31}B_{10}N_3Si$
Molecular Weight: 341.58

Note: All B atoms are actually BH. All boron atoms are predominantly 11 isotope (80% and 20% 10 isotope)

EXPECTED CHARGES

Reagent	MW	Density	Loading eq	amount g	amount mL	mmole
TBDMS Carborane	258.49	----------	1.00	**2.00**	----------	7.74
THF (anhydrous) for Carborane	----------	----------	----------	----------	**10.00**	----------
LiHMDS (1M in THF)	----------	----------	1.25	----------	**9.67**	9.67
THF (anhydrous) for LiHMDS	----------	----------	----------	----------	**20.00**	----------
1-azido-3-iodopropane	211.00	----------	1.30	**2.12**	----------	10.06
THF (anhydrous)	----------	----------	----------	----------	**12.00**	----------

Theoretical Yield of Product 13 (in grams)	**2.64**

Procedure

To a dry 100 mL round bottomed flask under a nitrogen atmosphere was added anhydrous THF (18 mL) and 1M LiHMDS (9.7 mL). The mixture was cooled to -78 ^{o}C. A solution of TBDMS Carborane (2.00 g) in anhydrous THF (10 mL) was added to the cryogenic mixture *via* syringe over 5 mins such that the temperature was maintained $\leq$ -65 ^{o}C. The reaction mixture was allowed to stir an additional 5 minutes at -78 ^{o}C then allowed to warm to 0ºC, stirred an additional 1.25 hrs at 0 ^{o}C, and cooled to -78 ^{o}C. A solution of 1-azido-3-iodopropane (2.15 g) in anhydrous THF (12 mL) was added over 3 minutes at -78 ^{o}C. The reaction was allowed reaction to stir at -78 ^{o}C for 10 minutes then allowed to warm to room temperature and stirred an additional 1.25 hours at ambient temperature. The reaction was cooled to 0ºC, quenched with USP purified water (5 mL), concentrated under reduced pressure, extracted with diethyl ether (2x 20mL). The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduce pressure to give a crude yellow oil (3.13 g). The crude oil (3.13 g) was dissolved in DCM/n-hexane (3.5 mL, 25/75, v/v) and passed through a large silica plug (40g) packed in DCM/n-hexane (25/75, v/v). The silica plug was flushed with DCM/n-hexane (200 mL, 25/75, v/v) to collect 8 fractions (each about 10-15 mL). Fractions 2-6 were combined and concentrated to

give 2.58 g of Compound 13 (97.7% yield, white solid). Stored in fridge (5-10ºC). This is a novel compound.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 3.32 (t, 2H), 3.15-1.5 (m, 14H), 1.07 (s, 9H), 0.34 (s, 6H)

^{11}B NMR (Decoupled, 100 MHz): δ = 0.29, -3.76, -7.29, -10.18

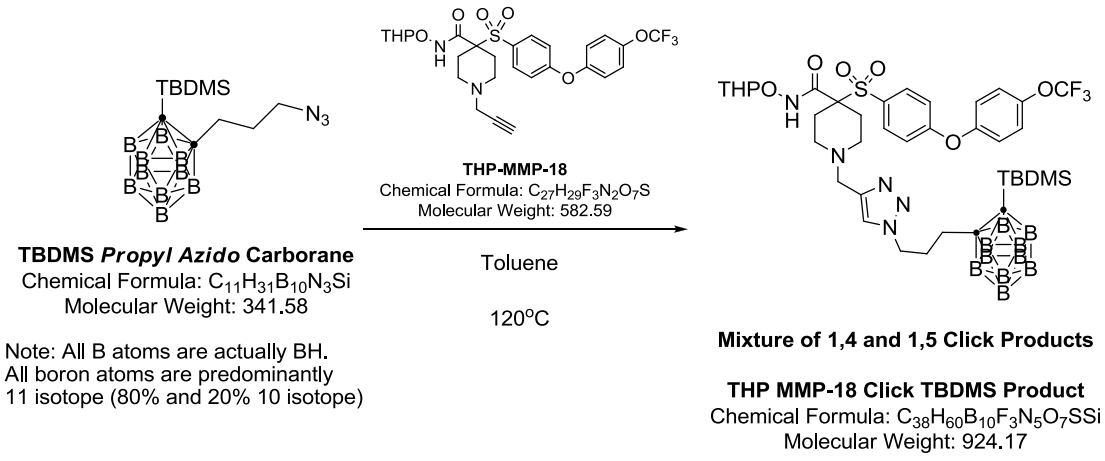
^{11}B NMR (Coupled, 100 MHz): δ = 0.99, -0.56, -3.13, -4.62, -6.57, -8.16, -9.48, -11.24

Preparation of 1,4 and 1,5-Disubstituted Click Products *via* Thermal Click Reaction(Compound 14 and Compound 17)

PR074-070

Purpose:

Perform Click reaction using TBDMS-Propyl-Azide (1.00 g) and THP-MMP-18 alkyne. Reaction will be done using thermal heating at 120 C.



TBDMS *Propyl Azido* Carborane
Chemical Formula: $C_{11}H_{31}B_{10}N_3Si$
Molecular Weight: 341.58

Note: All B atoms are actually BH.
All boron atoms are predominantly 11 isotope (80% and 20% 10 isotope)

Mixture of 1,4 and 1,5 Click Products

THP MMP-18 Click TBDMS Product
Chemical Formula: $C_{38}H_{60}B_{10}F_3N_5O_7SSi$
Molecular Weight: 924.17

EXPECTED CHARGES					
Reagent	MW	Loading eq	amount g	amount mL	mmole
TBDMS Propyl Azido Carborane	341.58	1.00	1.000	----------	2.928
THP-MMP-18	585.59	0.90	1.543	----------	2.635
Toluene (anhydrous)	----------	----------	----------	50.00	----------

Theoretical Yield of TBDMS Product	**2.44 g**
Corrected Theo based on recovered SM, see below in procedure	**1.87 g**

Procedure for the Thermal Click Products (Compounds 14 and 17):

To a 100 mL round bottomed flask was added carboranyl azide (1.00 g), THP-MMP-18 alkyne (1.54 g) and anhydrous toluene (50 mL). The mixture was stirred for 10 minutes at ambient temperature and the mixture was equally portioned into 6 different pressure vials (ChemGlass, 40 mL) where each vial contained 8.3-8.5 mL of mixture. Each mixture was purged with argon, placed on a heating block at 120 ^{o}C, and allowed to stir at 120 ^{o}C for 58 hours. HPLC analysis of each reaction vial indicated that there was ~20% unreacted alkyne and the reaction was deemed complete at this point to avoid degradation of product and introduction of impurities that are difficult to remove downstream. Furthermore, HPLC analysis confirmed that the thermal Huisgen 1,3-Dipolar Cycloaddition reaction gives 1,4-Click and 1,5-Click products with a ratio of 1.6 to 1, respectively, which agrees with Sharpless' study (Sharpless, *Angew. Chem. Int. Ed.*, **2002**, *41*, 2596-2599) of determining the component ratio of the regioisiomers.

All six reactions (at room temperature) were combined and concentrated under reduced pressure to provide a crude oil (2.76 g). The crude oil was dissolved in DCM (30 mL), treated with silica gel (6.9 g, 60-200 micron, Silicylcle), and concentrated to dryness to give dry-loaded material on silica which was placed and packed in a 65 g solid loading cartridge. An 80 g RediSep Rf Gold silica gel column cartridge was

equilibrated with ethyl acetate/n-hexane (40/60, 2CV), 100% ethyl acetate (1CV), and ethyl acetate/n-hexane (40/60, 2CV). The purification was accomplished using a ethyl acetate/n-hexane step-gradient from ethyl acetate/n-hexane (40/60) to 100% ethyl acetate. The elution of components were in the following order: starting material, 1,5-Click product (Compound 17), and lastly 1,4-click product (Compound 14). Appropriate fractions were combined and concentrated to isolate recovered starting material (THP-MMP-18/Compound 9, 0.36 g, colorless oil, TLC Rf = 0.51 using 100% EtOAc), 1,5-click product (Compound 17, colorless glass solid, 0.60 g, 32.1% yield, *corrected yield for recovered SM*, TLC Rf = 0.35 using 100% EtOAc), and 1,4-click product (Compound 14, white solid, 0.90 g, 48.1% yield, *corrected yield for recovered SM*, TLC Rf = 0.15 using 100% EtOAc).

Analytical data for 1,4-Click (Compound 14) and 1,5-Click (Compound 17) Products:

- (1,4-Click) - Compound 14

HPLC purity: 96% AUC (retention time: 26.5 mins).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 9.38 (s, 1H), 7.80 (m ,2H), 7.41 (s, 1H), 7.28 (d, 2H), 7.11 (d, 2H), 7.04 (d, 2H), 4.99 (s, 1H), 4.33 (t, 2H, *J* = 6.4 Hz), 3.99 (bt, 1H, *J* = 10.3 Hz), 3.68 (bd, 1H), 3.59 (s, 2H), 2.93 (bs, 2H), 2.40-1.20 (m, 26H), 0.98 (s, 9H), 0.23 (s, 6H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ =164.1, 162.9, 152.9, 146.1, 145.3, 132.5,

128.5, 123.0, 122.3, 121.8, 117.4, 102.1, 79.8, 77.4, 77.2, 77.0, 76.7, 76.2, 70.5, 62.4, 52.9, 49.8, 49.8, 49.2, 34.9, 30.7, 28.5, 28.3, 27.8, 27.4, 24.9, 20.3, 18.24, -2.60.

^{11}B NMR (Decoupled, 100 MHz): δ = 0.27, -3.86, -7.47, -10.51

^{11}B NMR (Coupled, 100 MHz): δ = 1.09, -0.59, -3.17, -4.34, -6.64, -8.41, -9.41, -10.81

HRMS (ESI-ToF): m/z calcd for $C_{38}H_{61}B_{10}F_3N_5O_7SSi^+$ $[M+H]^+$: 926.4944, found 926.5007.

FTIR: 2941.1, 2863.3, 2575.8, 1686.5, 1587, 1501.4, 1488.6, 1316.7, 1244.2, 1220.2, 1185.8, 1149.4, 1133.2, 1086.5, 1038.0, 944.4, 896.8, 858.2, 838.3, 752.8, 731.2, 677.0 cm^{-1}.

- (1,5-Click) - Compound 17

HPLC purity: 95% AUC (retention time: 27.4 mins)

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 9.43 (s, 1H), 7.8 (d, 2H), 7.49 (s, 1H), ? (?, 3H), 7.1 (d,2H), 7.05 (d,2H), 5.01 (bs,1H), 4.39 (t, 2H), 3.99 (bt,1H), 3.7 (d, 1H), 3.49 (s, 2H), 2.8 (?, 2H), 1.58-2.30 (c, 24H), 1.25 (s, 1H), 1.00 (s, 9H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 164.16, 162.91, 152.91, 1461, 134.74, 132.63, 132.42, 127.82, 123.03, 121.80, 121.71, 117.50, 101.87, 80.13, 77.35, 77.23, 77.03, 76.71, 76.35, 70.15, 62.30, 50.20, 49.93, 49.74,

47.42, 35.04, 30.11, 28.30, 28.10, 27.77, 27.43, 24.92, 20.32, 18.12, -2.59.

^{11}B NMR (Decoupled, 100 MHz): δ = 0.37, -3.85, -7.43, -10.47

^{11}B NMR (Coupled, 100 MHz): δ = 0.94, -0.47, -4.55, -6.70, -8.43, -10.83

HRMS (ESI-ToF): m/z calcd for $C_{38}H_{61}B_{10}F_3N_5O_7SSi^+$ $[M+H]^+$: 926.4944, found 926.4999.

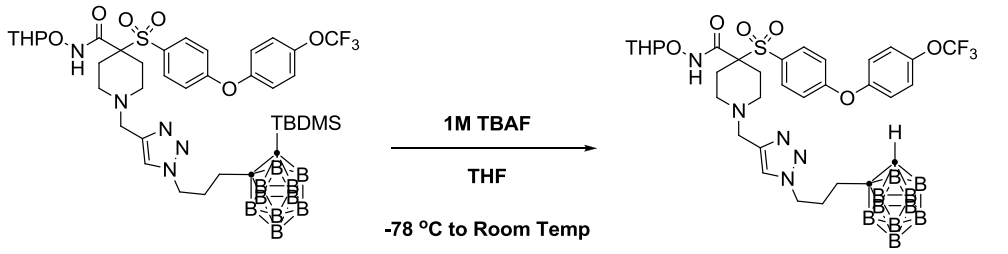
FTIR: 2939.8, 2869.6, 2579.3, 1685.9, 1586.8, 15014, 1488.7, 1319.0, 1295.5, 1220.0, 1243.6, 1185.7, 1149.9, 1134.3, 1086.8, 1037.2, 957.7, 944.6, 896.9, 873.3, 858.7, 838.3, 818.4, 732.1, 677.3 cm^{-1}

Preparation of the THP MMP-18 1,4-Click Product (Compound 15)

PR074-055

Purpose:

Deprotect 1,4-Click TBDMS protected product using 1 M TBAF solution in THF.



1,4 - Click Product
THP MMP-18 Click TBDMS Product
Chemical Formula: $C_{38}H_{60}B_{10}F_3N_5O_7SSi$
Molecular Weight: 924.17

Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope)

1,4 - Click Product
THP MMP-18 1,4-Click Product
Chemical Formula: $C_{32}H_{46}B_{10}F_3N_5O_7S$
Molecular Weight: 809.91

Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope)

EXPECTED CHARGES					
Reagent	MW	Loading eq	amount g	amount mL	mmole
1,4-Click Product TBDMS-THP Protected	924.17	1.00	**0.128**	----------	0.139
1M TBAF in THF	----------	1.20	----------	**0.17**	0.166
Anhydrous THF	----------	----------	----------	**1.28**	----------

Theoretical Yield of Product 15 (in g)	**0.112**

Procedure

To 1,4-click TBDMS protected product (Compound 14, 128 mg) was added anhydrous THF (1.25 mL) and the resulting mixture was cooled solution to –78ºC. To the cryogenic mixture was added a solution of 1M

TBAF in THF (0.17 mL) over approximately 30 seconds. After 5 minutes, the cooling bath was removed and then the reaction was permitted to warm to room temperature. After 30 minutes at room temperature, TLC analysis (100% EtOAc) showed complete consumption of starting material. The reaction mixture was concentrated to a crude oil residue which was dissolved in ethyl acetate (2 mL) and washed with water (1 mL, pH 7-7.5). The aqueous phase was extracted ethyl acetate (1 mL). The combined organic layers were washed with water (pH 7-7.5, 1 mL), dried with sodium sulfate, filtered, and concentrated under reduced pressure to give crude product (108 mg , lot PR074-055-2). The crude oil (108 mg) was dissolved in ethyl acetate (0.5 mL) and passed through a silica plug (0.27 g) in ethyl acetate. The plug was flushed with ethyl acetate. Appropriate fractions were combined and concentrated to afford desired 1,4-click THP protected product (Compound 15, 88 mg, 78.6% yield, colorless oil that solidifies upon standing) which was taken forward without any further purification since it contains residual *tert*-butyldimethylsilyl fluoride (which will be removed downstream).

HPLC purity: 92.3% AUC (retention time: 24.1 mins)

HRMS (ESI-ToF): m/z calcd for $C_{32}H_{47}B_{10}F_3N_5O_7S^+$ $[M+H]^+$: 812.4079, found 812.4131.

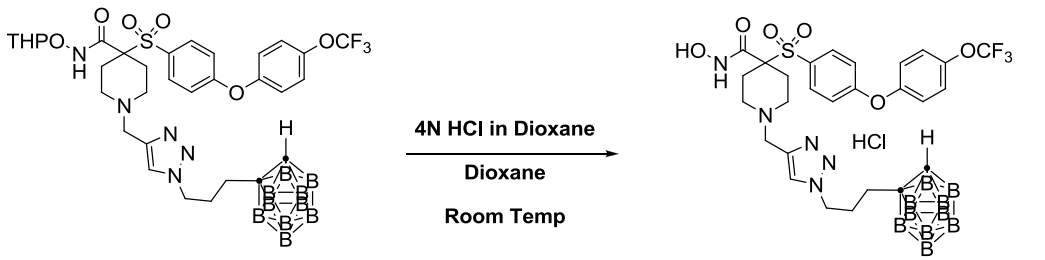
FTIR: 2946.6, 2850.4, 2586.7, 1682.0, 1587.0, 1501.5, 1488.4, 1461.3, 1293.7, 1219.9, 1244.5, 1185.9, 1132.6, 1149.0, 1086.1, 1037.5, 1021.5, 956.2, 944.2, 896.0, 873.1, 835.1, 816.0, 754.5, 722.7, 678.9, 645.0 cm^{-1}

Preparation of 1,4-Click Carboranyl MMP-18 HCl Salt (Compound 16)

PR074-062

Purpose:

Deprotect 1,4-Click THP protected product using 4N HCl in dioxane.



THP MMP-18 Click Product
Chemical Formula: $C_{32}H_{46}B_{10}F_3N_5O_7S$
Molecular Weight: 809.91

Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope)

1,4-Click Carboranyl MMP-18 HCl Salt
Chemical Formula: $C_{27}H_{39}B_{10}ClF_3N_5O_6S$
Molecular Weight: 762.25

Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope)

EXPECTED CHARGES					
Reagent	MW	Loading eq	amount g	amount mL	mmole
THP Protected 1,4-Click Product	809.91	1.00	**0.088**	----------	0.109
Dioxane (anhydrous)	----------	----------	----------	**0.88**	----------
4N HCl in Dioxane	----------	5.00	----------	**0.14**	0.543

Theoretical Yield of Product 16 (in g)	**0.083**

Procedure

To a 10 mL vial containing THP protected 1,4-Click product (Compound 15, 88 mg) under nitrogen atmosphere was added anhydrous dioxane (0.9 mL) and allowed mixture to stir until complete dissolution was achieved. To the solution was added 4N HCl in dioxane (0.14 mL) and the reaction was allowed to stir for 2 hours at room temperature where HPLC analysis revealed that the reaction was deemed complete. The reaction mixture was concentrated under reduced pressure at 30 $\pm$ 5 ^{o}C to give a crude oil. The crude oil was dissolved in dichloromethane (1 mL) and diethyl ether (3 mL) was added to generate a white slurry. The slurry was allowed to stir at ambient temperature for 1.5 hours, filtered, and the filter cake was washed with diethyl ether (2 mL) and n-heptane (5 mL), pulled dry under nitrogen, and further dried *in vacuo* at room temperature to provide the title 1,4-Click Carboanyl MMP-18 HCl salt (Compound 16, 60 mg, white solids, 72.2% yield, Lot PR074-062-5).

HPLC purity: 95.1% AUC (retention time: 22.7 mins)

^{11}B NMR (Decoupled, 100 MHz): δ = 18.56, -2.94, -6.09, -9.67, -12.20, -13.17

^{11}B NMR (Coupled, 100 MHz): δ = 18.56, -2.15, -3.64, -5.21, -6.32, -8.91, -11.08, -12.95, -13.86

HRMS (ESI-ToF): m/z calcd for $C_{27}H_{39}B_{10}F_3N_5O_6S^+$ $[M+H]^+$: 728.3504, found 728.3540.

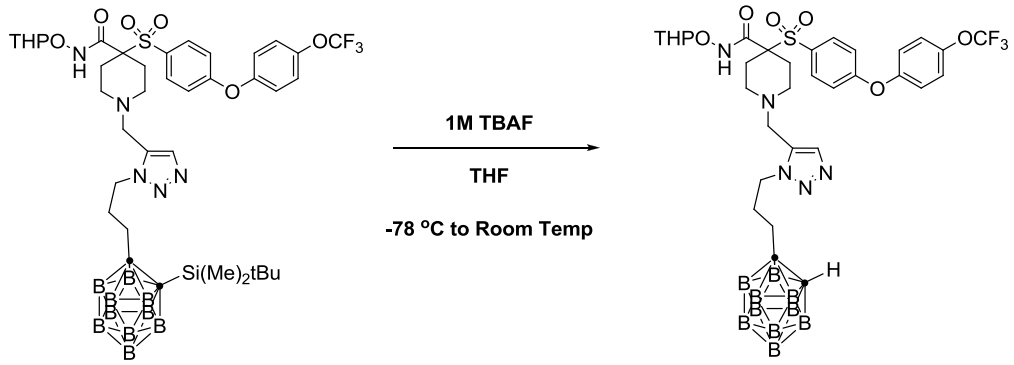
X-Ray crystallography: PENDING

Preparation of the THP MMP-18 1,5-Click Product (Compound 18)

PR074-080

Purpose:

Deprotect 1,5-Click TBDMS protected product using 1 M TBAF solution in THF.



THP MMP-18 1,5-Disubstituted Click TBDMS Product
Chemical Formula: $C_{38}H_{60}B_{10}F_3N_5O_7SSi$
Molecular Weight: 924.17

Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope)

1,5 - Click Product
THP MMP-18 Click Product
Chemical Formula: $C_{32}H_{46}B_{10}F_3N_5O_7S$
Molecular Weight: 809.91

Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope)

EXPECTED CHARGES					
Reagent	MW	Loading eq	amount g	amount mL	mmole
1,5-Click Product TBDMS-THP Protected	924.17	1.00	**0.071**	----------	0.077
1M TBAF in THF	----------	1.20	----------	**0.092**	0.092
Anhydrous THF	----------	----------	----------	**0.71**	----------

Theoretical Yield of Product 18 (in g)	**0.062**

Procedure

To 1,5-click TBDMS protected product (Compound 17, 71 mg) was added anhydrous THF (0.9 mL) and the resulting mixture was cooled solution to −78ºC. To the cryogenic mixture was added a solution of 1M TBAF in THF (90 µL). After 5 minutes, the cooling bath was removed and then the reaction was allowed to warm to room temperature. After 75 minutes at room temperature, TLC analysis (100% EtOAc) showed complete consumption of starting material. The reaction mixture was concentrated to a crude oil residue which was dissolved in ethyl acetate (2 mL) and washed with water (1 mL, pH 7-7.5). The aqueous phase was extracted ethyl acetate (2 mL). The combined organic layers were washed with water (pH 7-7.5, 1 mL), dried with sodium sulfate, filtered, and concentrated under reduced pressure to give crude desired product Compound 18 (82 mg , lot PR074-080-2, theoretically 62 mg present) which was taken forward without any further purification since it contains residual *tert*-butyldimethylsilyl fluoride (which will be removed downstream).

HPLC purity: 95.2% AUC (retention time: 26.4 mins)

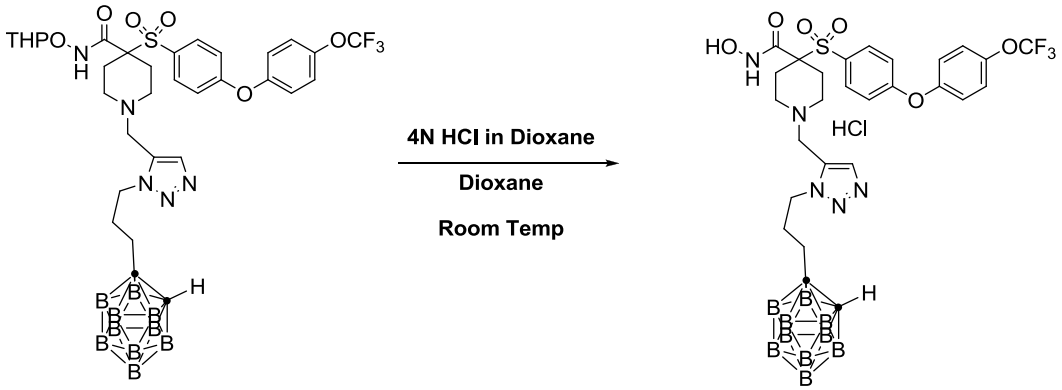
HRMS (ESI-ToF): m/z calcd for $C_{32}H_{47}B_{10}F_3N_5O_7S^+$ $[M+H]^+$: 812.4079, found 812.4139.

Preparation of 1,5-Click Carboranyl MMP-18 HCl Salt (Compound 19)

PR074-088

Purpose:

Deprotect 1,5-Click THP protected product using 4N HCl in dioxane.



1,5 - Click Product
THP MMP-18 Click Product
Chemical Formula: $C_{32}H_{46}B_{10}F_3N_5O_7S$
Molecular Weight: 809.91

Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope)

1,5-Click Carboranyl MMP-18 HCl Salt
Chemical Formula: $C_{27}H_{39}B_{10}ClF_3N_5O_6S$
Molecular Weight: 762.25

Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope)

EXPECTED CHARGES					
Reagent	MW	Loading eq	amount g	amount mL	mmole
THP Protected 1,5-Click Product (crude, contains residual TBDMS Fluoride)	809.91	1.00	**0.082**	----------	0.101
Dioxane (anhydrous)	----------	----------	----------	**0.82**	----------
4N HCl in Dioxane	----------	5.00	----------	**0.13**	0.506

Theoretical Yield of Product 19 (in g)	**0.077**
Corrected Theo yield based on 62 mg SM since TBDMSF is present (in g)	**0.0584**

Procedure

To a 10 mL vial containing crude THP protected 1,5-Click product (Compound 18, 82 mg) under nitrogen atmosphere was added anhydrous dioxane (0.9 mL) and allowed mixture to stir until complete dissolution was achieved. To the solution was added 4N HCl in dioxane (0.14 mL) and the reaction was allowed to stir for 2.5 hours at room temperature where HPLC analysis revealed that the reaction was deemed complete. The reaction mixture was concentrated under reduced pressure at 30 $\pm$ 5 ^{o}C to give a crude oil. The crude oil was dissolved in dichloromethane (0.5 mL) and diethyl ether (3 mL) was slowly added to generate a white slurry. The slurry was allowed to stir at ambient temperature for 20 mins, filtered, and the filter cake was washed with diethyl ether (2.5 mL) and n-hexane (5 mL), pulled dry under nitrogen, and further dried *in vacuo* at room temperature to provide the title 1,5-Click Carboanyl MMP-18 HCl salt (Compound 19, 58 mg, white solids, 99% yield, Lot PR074-088-2).

HPLC purity: 92.2% AUC (retention time: 23.2 mins)

^{11}B NMR (Decoupled, 100 MHz): δ = 18.55, -2.90, -6.26, -9.74, -12.24, -13.14

^{11}B NMR (Coupled, 100 MHz): δ = 18.58, -2.13, -3.71, -8.99, -11.11, -12.88

HRMS (ESI-ToF): m/z calcd for $C_{27}H_{39}B_{10}F_3N_5O_6S^+$ $[M+H]^+$: 728.3504, found 728.3538.

X-Ray crystallography: PENDING

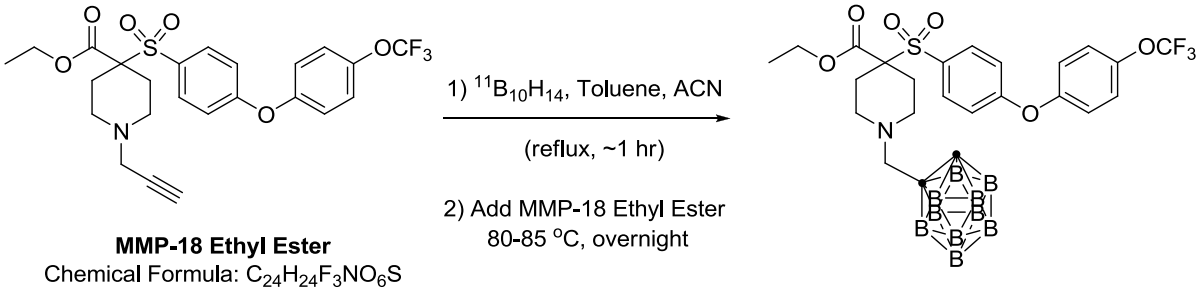
Preparation of MMP-18 Carboranyl Ethyl Ester (Compound 20)

PR074-057

Purpose:
1) Scale the hydroboration reaction of MMP-18 Ethyl Ester (Cmpd 7) and $B_{10}H_{12}(MeCN)_2$.
Reference batches: PR075-142 and PR074-050 (Pilot A). Mixture will be refluxed overnight based on that heating overnight at reflux consumes starting material alkyne and 60% conversion to product is typically achieved based on HPLC analysis.

$B_{10}H_{12}(MeCN)_2$ is prepared by heating $B_{10}H_{14}$ in Toluene and acetonitrile.

$B_{10}H_{12}(MeCN)_2$ Preparation Time = 1 hr at 100 C



MMP-18 Ethyl Ester
Chemical Formula: $C_{24}H_{24}F_3NO_6S$
Molecular Weight: 511.51

***closo*-carborane MMP-18 Ethyl Ester**
Chemical Formula: $C_{24}H_{34}B_{10}F_3NO_6S$
Molecular Weight: 629.70

Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope)

EXPECTED CHARGES					
Reagent	MW	Loading eq	amount g	amount mL	mmole
Decaborane, $^{11}B_{10}H_{14}$	122.22	1.20	**0.860**	NA	7.04
Acetonitrile (anhydrous)	41.05	100.00	24.08	**30.63**	586.50
Toluene (anhydrous) for $^{11}B_{10}H_{14}$	--------	--------	--------	**45.00**	--------
Toluene (anhydrous) for MMP-18 Ester	--------	--------	--------	**15.00**	--------

MMP-18 Ethyl Ester (Cmpd 7)	511.51	1.00	**3.00**	NA	5.86

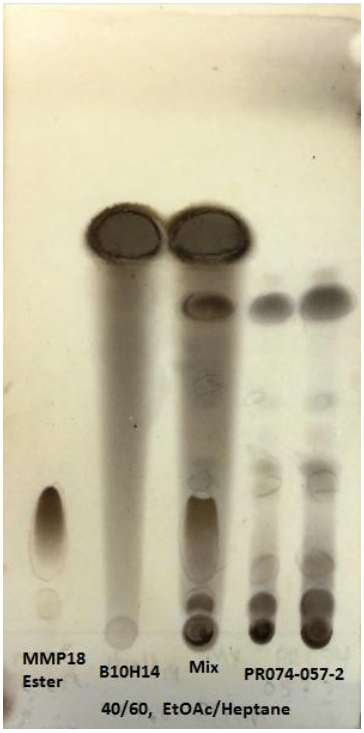
Theoretical Yield of Product 20 **(in grams)**	**3.69**

Procedure

A 250 mL round bottomed flask (1-necked) was equipped with a Claisen adapter, reflux condenser with nitrogen inlet, magnetic stirring bar, and a J-Kem thermocouple. To the reactor under nitrogen was added white decaborane (0.90 g), anhydrous toluene (45 mL), and anhydrous ACN (31 mL). The mixture was purged with nitrogen and the reaction flask was covered with foil. Heated the mixture to reflux (temp 82ºC) for 80 minutes followed by cooling the mixture to room temperature. To the reaction mixture (light yellow solution) at ambient temperature was added a solution of MMP-18 Ethyl Ester (Compound 7, 3.13 g) in anhydrous toluene (10 mL) and additional anhydrous toluene (5 mL) was used to quantitatively transfer all ester to the reactor. The reactor was purged with nitrogen and heated to reflux. After 19 hours, the reaction was a dark yellowish-orange solution and the reaction was sampled for HPLC analysis (8 drops of reaction mixture was concentrated and dissolved in 4 mL of ACN). HPLC analysis indicated that the starting material alkynyl ester (18.4 mins) was completely consumed. The reaction mixture was cooled to room temperature and was concentrated under reduced pressure at 40-50ºC to give crude Compound 20 as an orange

foam (4.29 g, Lot PR074-057-2). Crude product (lot PR074-057-2) was analyzed by HPLC and TLC*.

TLC Analysis of PR074-057-2 (crude, EtOAc/hept 40/60). TLC analysis was done using a glass backed TLC plate visualizing with UV and 0.4% PdCl2 in 3M HCl:



Purification of PR074-057-2 *via* flash chromatography:

Crude product (PR074-057-2, 4.29 g) was dissolved in DCM and transferred to a 250 mL round bottomed flask. To the flask was added silica gel (10.0 g, 60-200 micron, Silicycle) and this mixture was concentrated to dryness on rotovap at 25-30ºC to provide dry-loaded crude product. The dry-loaded crude product was loaded into a 65 g solid loading cartridge. An 80 g Redisep Rf Gold silica gel cartridge column was equilibrated with n-

hexane (165 mL), ethyl acetate/n-hexane (50/50, 195 mL), and n-hexane (275 mL). Purification on an Isco Rf unit was accomplished using an ethyl acetate/n-hexane gradient (0/100 to 100/0) over 16.5 column volumes. Collected fractions were analyzed by HPLC (refer to the table below for information regarding purity of the fraction analyzed.

Table: HPLC Analysis of Fractions from Flash Chrom. Purification

Fraction	% AUC Product (32.4 min)	% AUC 26.4 min Impurity	Comments
1	0	0	Toluene (100% AUC, 17.4 mins)
3	100 (97 mAu)	0	N/A
5	100 (237 mAu)	0	N/A
7	100 (180 mAu)	0	N/A
9	97.4 (111 mAu)	0	2.6% (28.7 mins)
11	100 (72 mAu)	0	N/A
13	100 (47 mAu)	0	N/A
15	90.6 (32 mAu)	0	9.4% (30.3 mins)
17	78.9 (25 mAu)	0	9.4% (30.3 mins), 14.1% (30.9 mins)
19	75.5 (23 mAu)	0	24.5% (22.5 mins)
21	38.1 (12 mAu)	0	61.9% (25.2 mins)
23	30.7 (9 mAu)	0	47.8% (25.2 mins), 21.5% (28.2 mins)
25	10.9 (7 mAu)	0	13.5% (24.6 mins), 14.0% (25.2 mins), 61.5% (28.2 mins)
27	8.8 (8 mAu)	0	17.5% (24.6 mins), 15.5% (25.2 mins), 33.5% (26.9 mins), 24.6% (28.2 mins)
29	0	90.4 (816	Many small impurities present

Fraction	% AUC Product (32.4 min)	% AUC 26.4 min Impurity	Comments
		mAu)	
31	0	73.6 (212 mAu)	Other small impurities present
33	0	28.5 (23 mAu)	Many small impurities present
35	0	29.5 (6 mAu)	70.5% (27.5 mins)
37	0	trace	N/A
40	0	trace	N/A

Fractions 3-14 were combined and concentrated under reduced pressure to give desired carboranyl ethyl ester product (Compound 20, 1.30 g, 33.8% yield, white foam solid, Lot PR074-057-3).

HPLC purity: 98.9% AUC (retention time: 32.4 mins)

^{1}H NMR ($CDCl_3$, 400 MHz): □ = 7.73 (m, 2H), 7.27 (m, 2H), 7.13-7.04 (m, 4H), 4.18 (q, 2H, *J* = 8.0 Hz), 3.91 (s, 1H), 3.10-1.50 (complex, 20H), 1.24 (t, 3H, *J* = 8.0 Hz)

^{13}C NMR ($CDCl_3$, 100 MHz): □ = 166.87, 162.67, 153.06, 146.07, 146.06, 132.62, 128.77, 123.05, 121.71, 121.66, 119.15, 117.17, 77.34, 77.02, 76.71, 74.45, 71.41, 62.62, 58.16, 51.96, 13.95

DEPT135: 132.62, 123.06, 121.67, 117.18, 62.63, 58.17, 51.95, 13.96

C13APT: 166.87, 162.67, 153.06, 146.06, 132.62, 128.78, 123.06, 121.67, 117.18, 77.35, 77.03, 76.71, 74.45, 71.41, 62.62, 58.17, 51.96, 13.96

^{11}B NMR (Decoupled, 100 MHz): δ = -2.82, -5.21, -8.98, -11.69, -12.89

^{11}B NMR (Coupled, 100 MHz): δ = -2.10, -3.73, -5.97, -8.20, -9.83, -12.35

HRMS (ESI-ToF): m/z calcd for $C_{24}H_{35}B_{10}F_3NO_6S^+$ $[M+H]^+$: 632.3068, found 632.3112.

Fractions 28-32 were pooled and concentrated to give 0.80 g of Compound 21 as a yellow foam solid (Lot PR074-057-5) which is assigned to be the *nido*-form of carboranyl ethyl ester product.

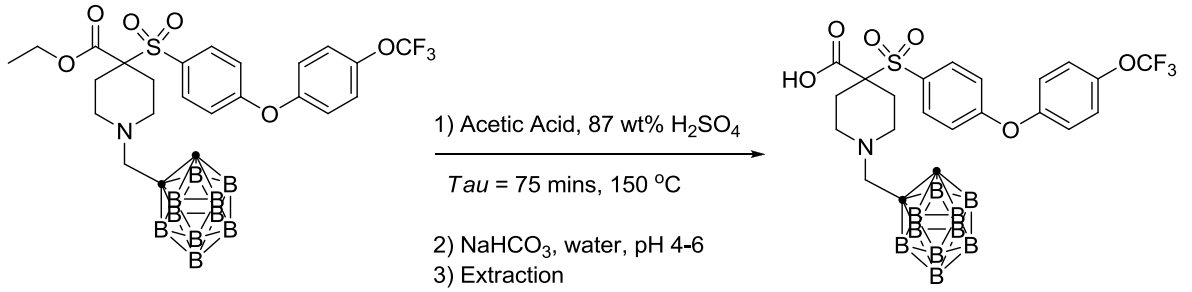
HPLC purity: 68.7% AUC (retention time: 26.4 mins)

HRMS (ESI-ToF): m/z calcd for $C_{24}H_{34}B_9F_3NO_6S^+$ $[M+H]^+$: 620.2896, found 620.3022.

Hydrolysis of MMP-18 Carboranyl Ethyl Ester *via* Continuous Flow Mode (Compound 22)

PR074-085

Purpose:
Hydrolyze the ethyl ester moiety to the carboxylic acid under continuous flow using 87.4 wt% sulfuric acid in acetic acid at 150 °C.



***closo*-carborane MMP-18 CO_2Et**
Chemical Formula: $C_{24}H_{34}B_{10}F_3NO_6S$
Molecular Weight: 629.70

Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope)

***closo*-carborane MMP-18 CO_2H**
Chemical Formula: $C_{22}H_{30}B_{10}F_3NO_6S$
Molecular Weight: 601.65

Note: All B atoms are actually BH.
All boron atoms are predominantly
11 isotope (80% and 20% 10 isotope)

EXPECTED CHARGES					
Reagent	MW	Loading eq	amount g	amount mL	mmole
MMP-18 Carboranyl Ethyl Ester, Cmpd 20	629.70	1.00	**0.600**	NA	0.96
Acetic Acid	41.05	100.00	157.50	**150.00**	--------
87.4 wt% H2SO4	--------	--------	--------	**75.00**	--------

Theoretical Yield of Product 21 (in grams)	**0.575**

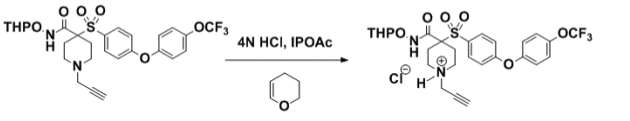
Procedure

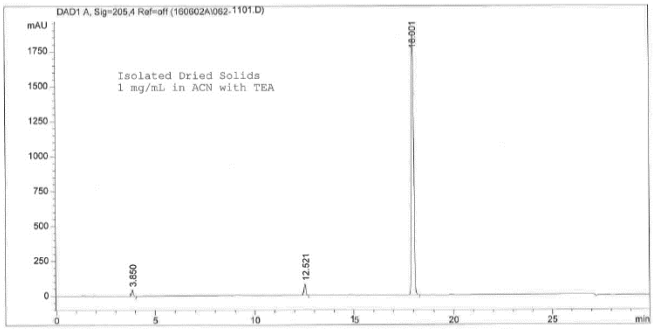
MMP-18 Carboranyl Ethyl Ester (Compound 20, 0.60 g) was dissolved in acetic acid (150 mL) and allowed to stir at room temperature until complete dissolution was achieved. The solution of ester was diluted with 87% sulfuric acid (75 mL), stirred for 10 minutes at room temperature, then processed through a Vapourtec plug flow reactor (10 mL) at 150 ^{o}C with a flow rate of 0.133 mL/min providing a residence time of 75 minutes. Once all the starting material feed was processed, the flow reactor was flushed with glacial acetic acid at 150 ^{o}C at 2 mL/min for 30 minutes. The collected crude product was concentrated at 60 ^{o}C under reduced pressure to remove acetic acid. The resulting sulfuric acid residue was diluted with DCM (300 mL) and then placed in a cooling bath (10 ^{o}C). The mixture was carefully diluted with saturated sodium bicarbonate solution (300 mL) followed by slow addition of

solid sodium bicarbonate (210 g) to achieved a pH of 3.5. The mixture was further diluted with 300 mL of USP purified water and then extracted with DCM (2 x 200 mL). The combined organic layers were dried with sodium sulfate, filtered, and concentrated to give 0.52 g of crude Compound 22.

Formation of THP protected Hydroxamate hydrochloride salt:

HCl salt formation of THP-protected MMP-18 (Compound 9) *via* addition of 4N HCl in Dioxane into a solution THP-protected MMP-18 in isopropyl acetate was unsuccessful and only generated free MMP-18 hydroxamic acid and some minor impurities (refer to PR075-074). However, treating a solution of THP-protected MMP-18 (Compound 9, 150 mg) in isopropyl acetate (1.5 mL) and 3,4-Dihydro-2H-pyran (1.0 mL) with 4N HCl in dioxane (0.25 mL) provided THP-protected MMP-18 HCl salt (159 mg, 97.5% yield, HPLC purity: 95% THP HCl salt, and 3% Free Hydroxamic acid). Refer to PR075-075 for this interesting work where THP group remained stable!





Area Percent Report

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Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

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Salt formation of THP-MMP-18 (Compound 9) was also successful by treating a solution of THP-MMP-18 in ethyl acetate with a solution of oxalic acid dihydrate (1.1 eq) in EtOH/EtOAc. Refer to PR075-075 for this work regarding oxalate salt formation!

Section 8: Acknowledgements

It is with utmost pleasure to acknowledge Dr. Marta Wenzler for her contributions for attaining HRMS data for all BNCT compounds and intermediate, and we are extremely grateful. Additionally, it is with great pleasure to acknowledge Andre Colorina for his significant contributions in assisting with synthesis of BNCT compounds. Furthermore, many thank to Dr. Narayan Hosmane for performing ^{11}B NMR analyses on carborane compounds.

APPENDIX A

SUPPLEMENTAL DATA FOR CHAPTER TWO

HPLC for Racemic CTV-Lactam (19)

Data File C:\HPCHEM\1\DATA\150316B\003-0301.D Sample Name: PT1-11-3

PT1-11-3

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Sample Name     : PT1-11-3                       Location : Vial 3
Acq. Operator   : Jake                              Inj :   1
Acq. Instrument : Instrument 1                  Inj Volume : 5 µl
Method          : C:\HPCHEM\1\METHODS\CTVTK.M
Last changed    : 1/26/2015 2:10:18 PM by Jake
Zorbax SB-CN 4.6MM ID X 15CM
HPLC Method for CTV Triketone Research
B= 0.1% Formic Acid in ACN
C = DI Water
1.0 mL/min, 220 nm
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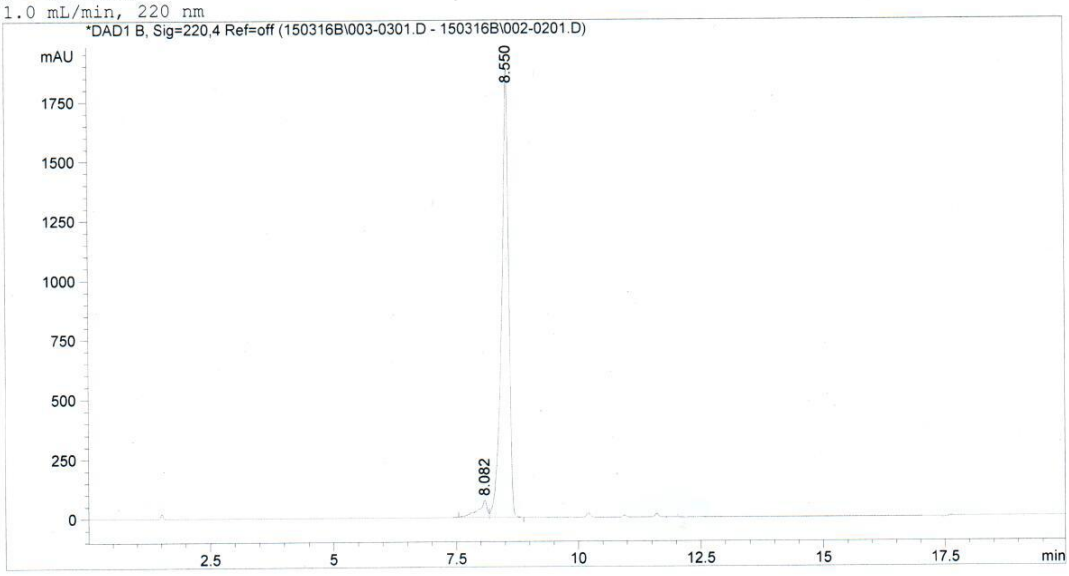

Area Percent Report

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Use Multiplier & Dilution Factor with ISTDs


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 Signal has been modified after loading from rawdata file!

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   2   8.550 VB    0.1497 1.87809e4  1860.92346  95.2343

Totals :                  1.97207e4  1933.91434

 Results obtained with enhanced integrator!
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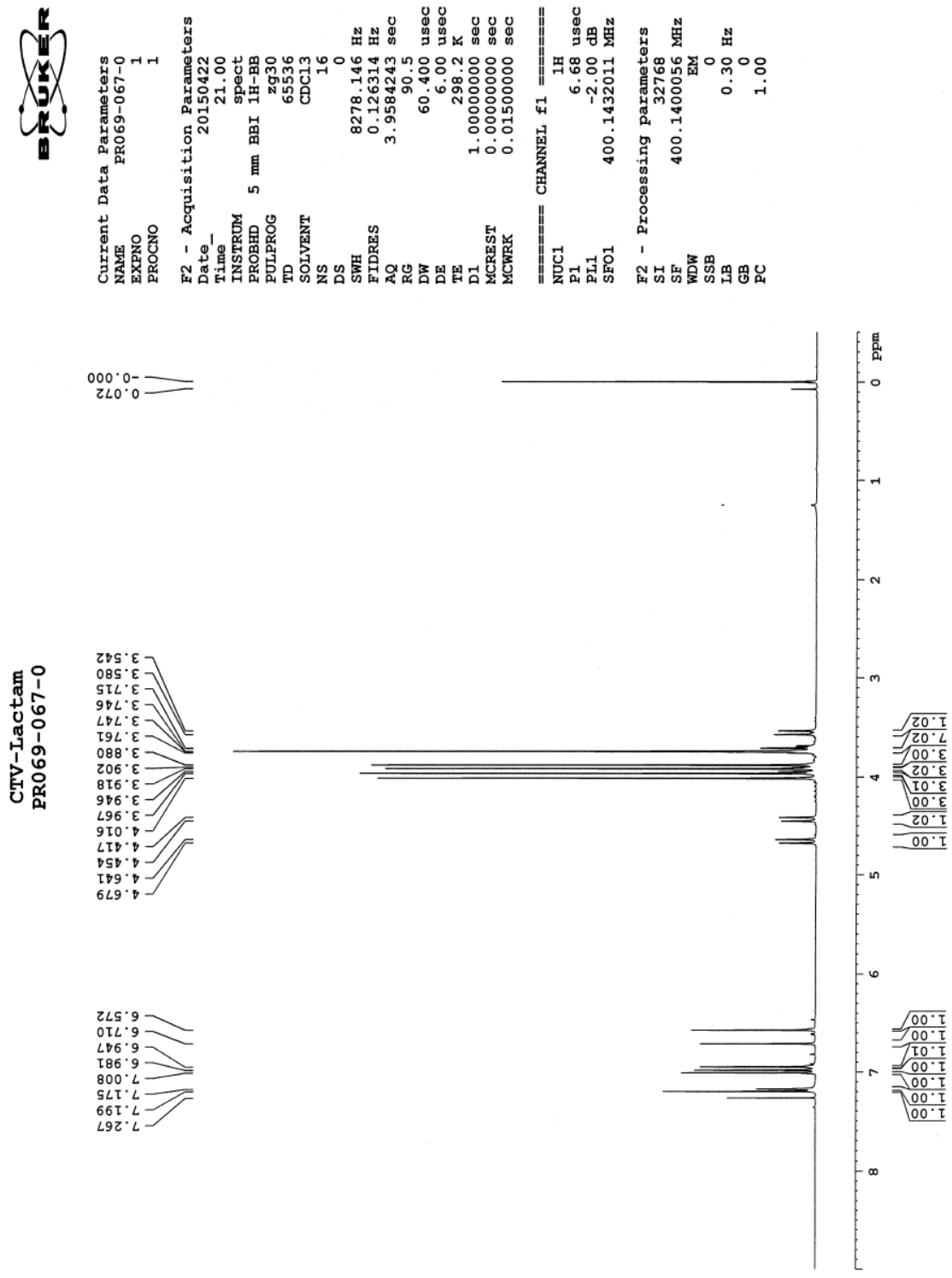
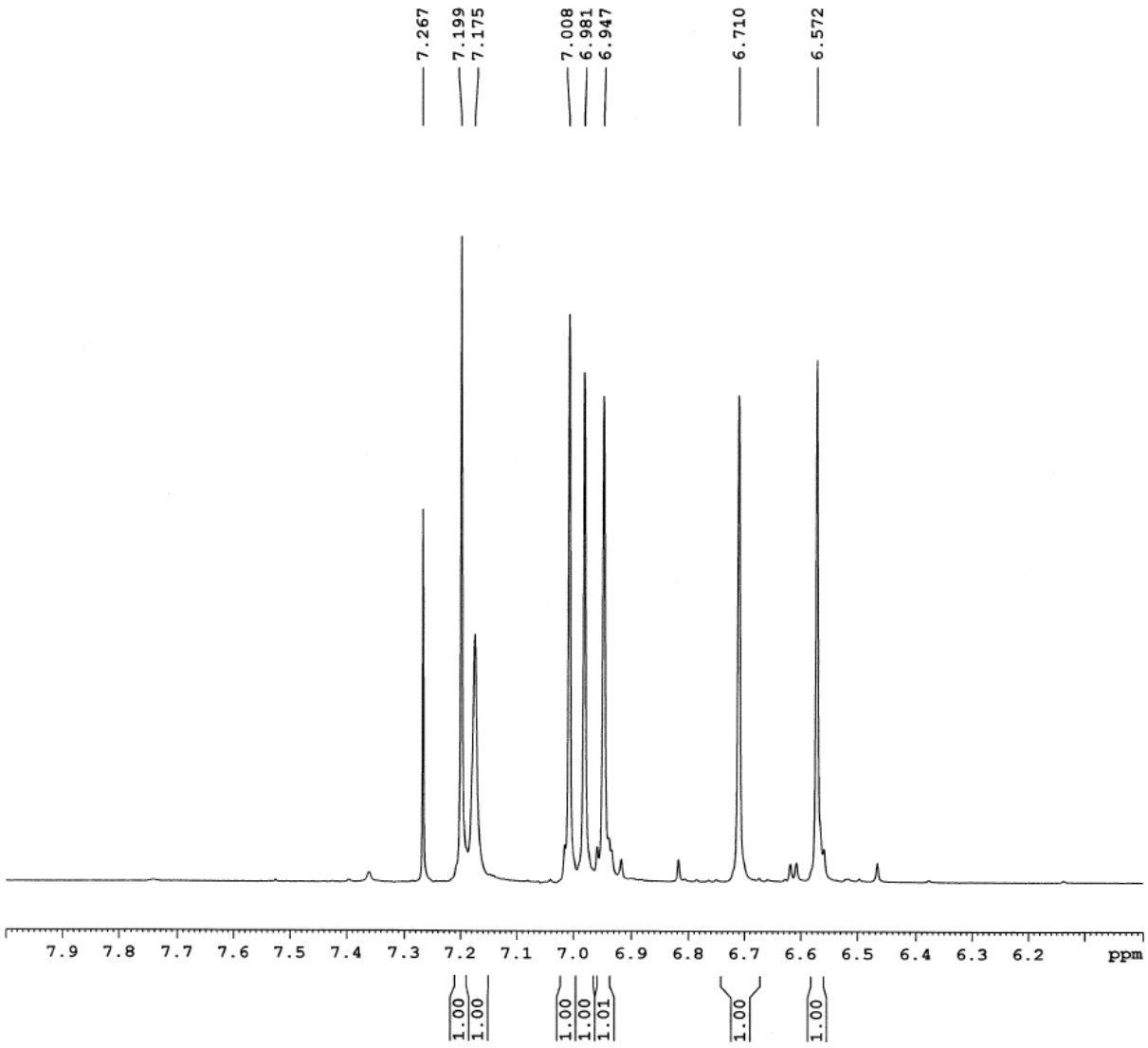
*** End of Report ***

[1]H NMR Spectra for Racemic CTV-Lactam (19)



CTV-Lactam
PR069-067-0



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PROCNO	1

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FIDRES	0.126314 Hz
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RG	90.5
DW	60.400 usec
DE	6.00 usec
TE	298.2 K
D1	1.00000000 sec
MCREST	0.00000000 sec
MCWRK	0.01500000 sec

======== CHANNEL f1 ========

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PL1	-2.00 dB
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F2 - Processing parameters

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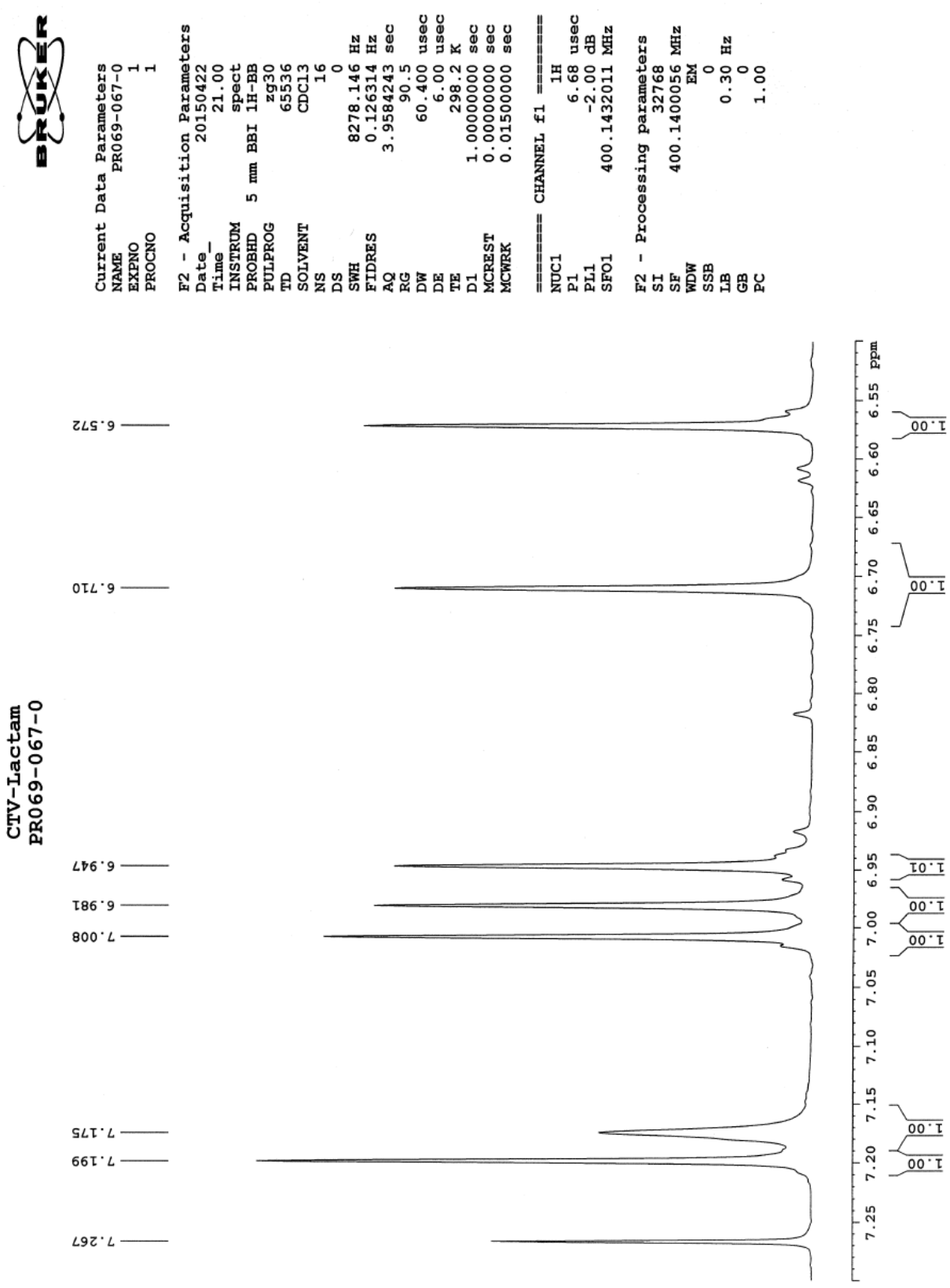
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PR069-067-0
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FIDRES 0.126314 Hz
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DW 60.400 usec
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TE 298.2 K
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MCREST 0.00000000 sec
MCWRK 0.01500000 sec
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PL1 -2.00 dB
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F2 - Processing parameters
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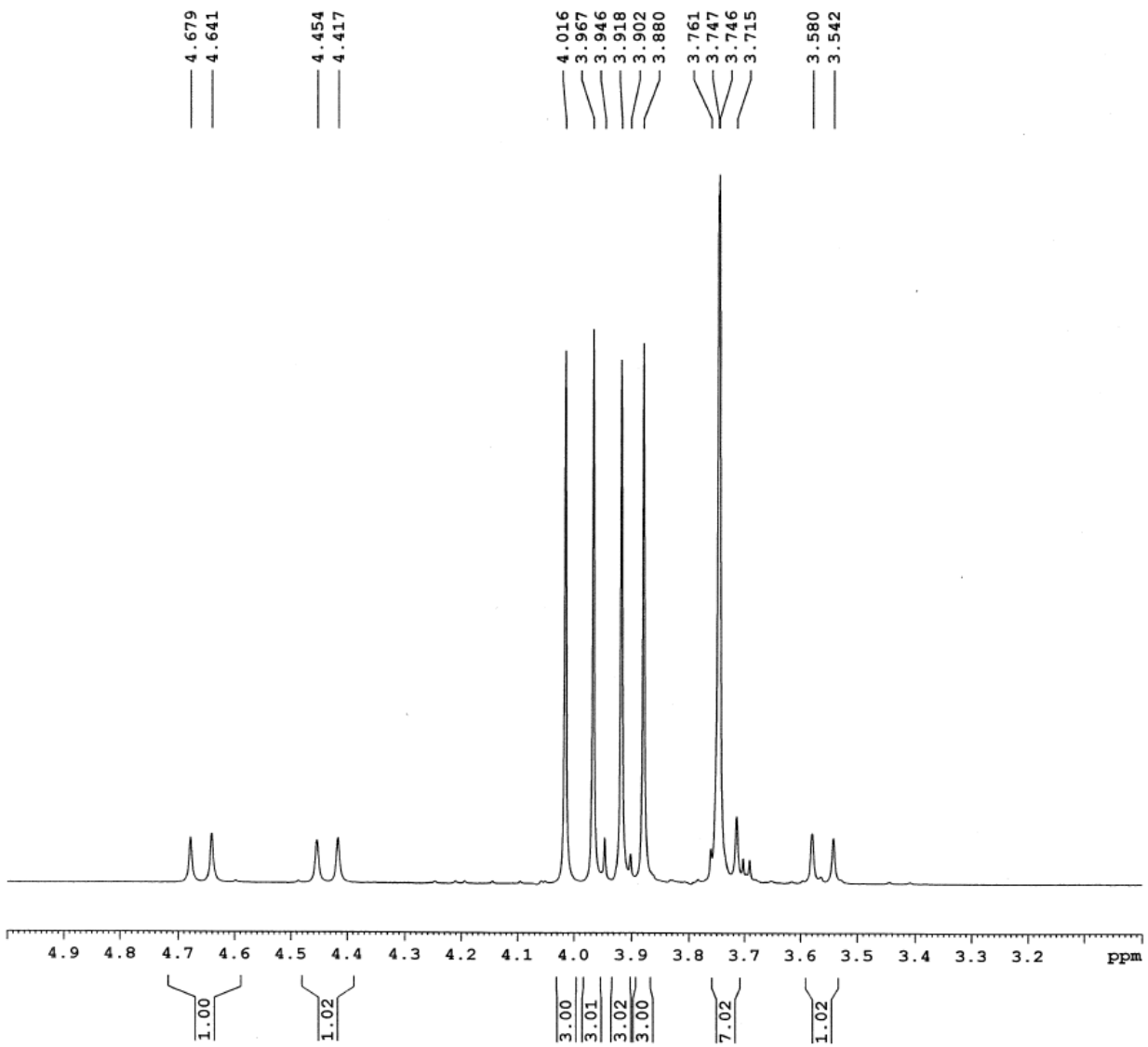
ppm

CTV-Lactam
PR069-067-0

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PROCNO                  1

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MCWRK         0.01500000 sec

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PL1                -2.00 dB
SFO1          400.1432011 MHz

F2 - Processing parameters
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SSB                     0
LB                   0.30 Hz
GB                      0
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Achiral HPLC of Recrystallized CTV-Menthyloxyacetic acid Imide (23)

Trial Recry from DCM/Hept
Crystal Solid, 1.0 mg/mL in ACN

```
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Acq. Instrument : Instrument 1                  Inj Volume : 5 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 2 µl
Method          : C:\HPCHEM\1\METHODS\LACTAM.M
Last changed    : 3/15/2015 5:08:43 PM by MRL
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C = DI Water
1.0 mL/min, 220 nm
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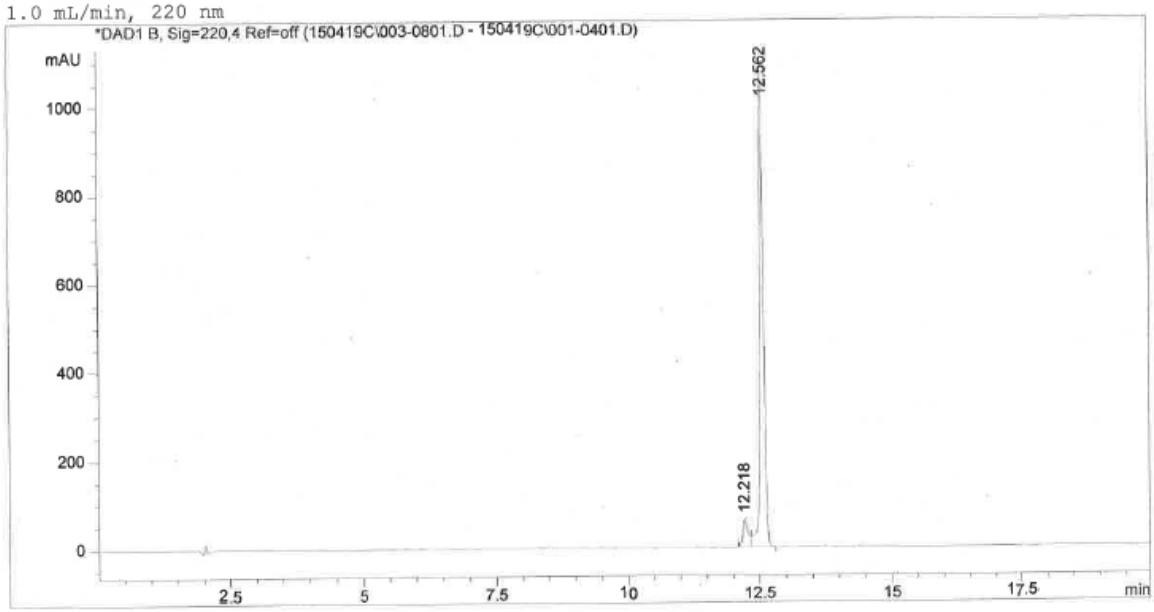


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Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


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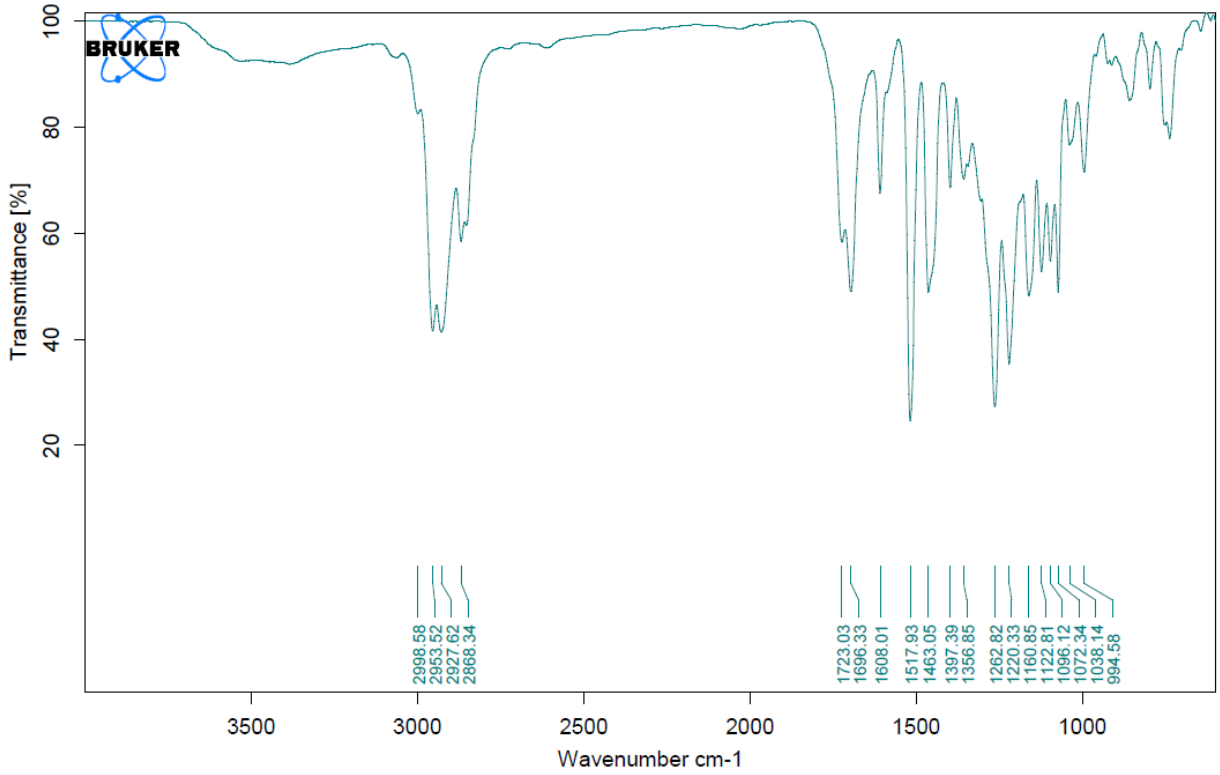
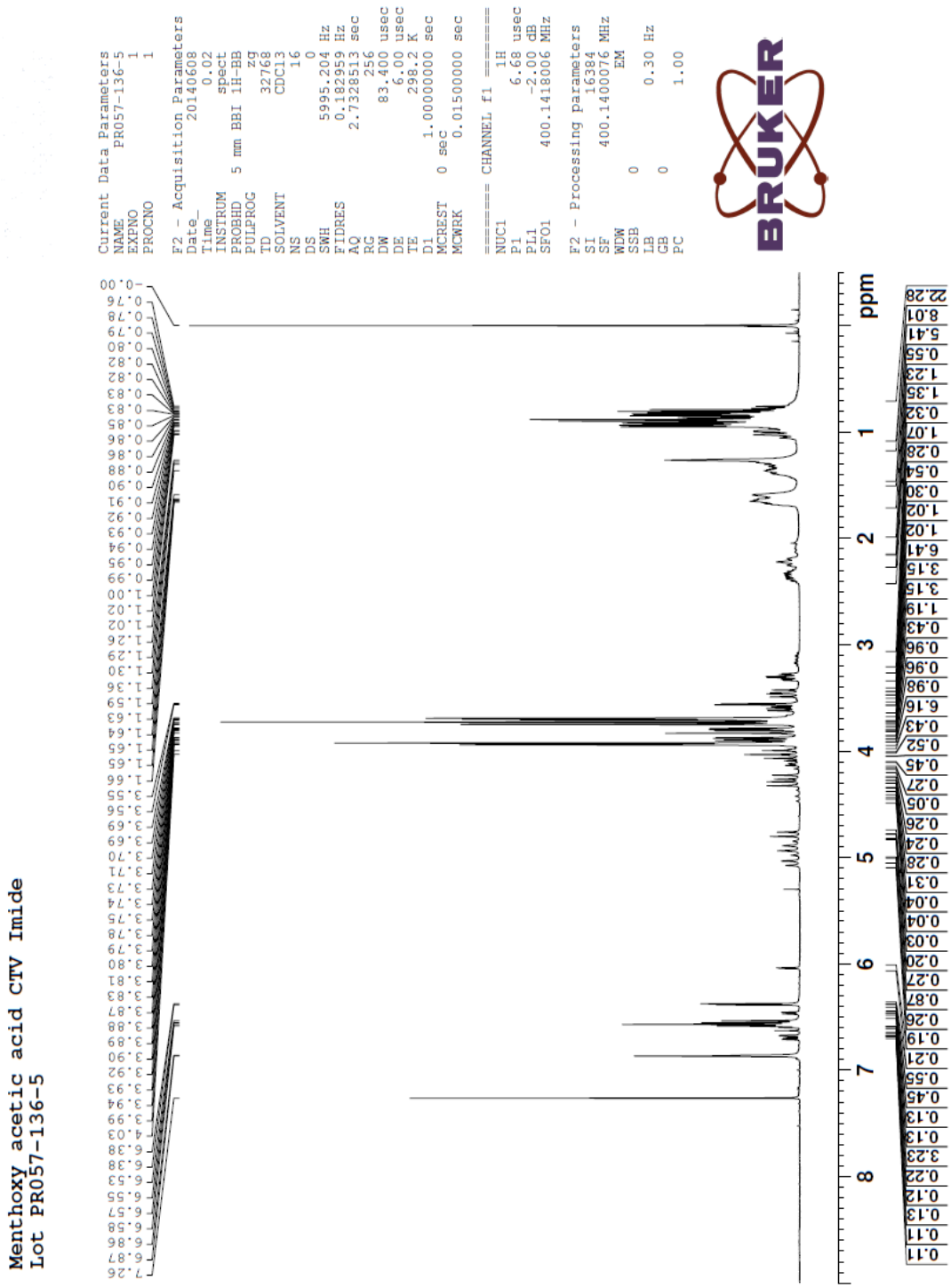
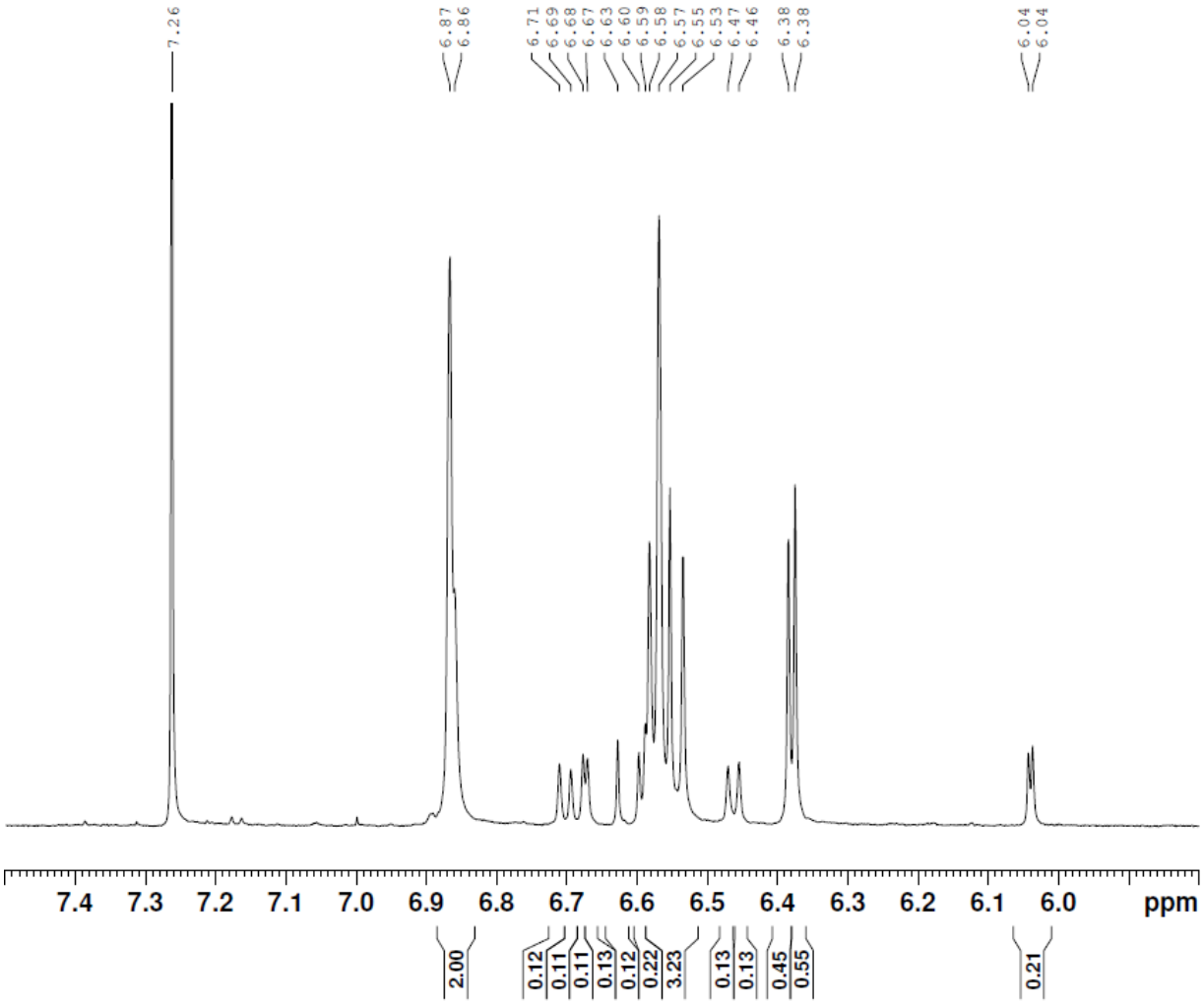
IR of CTV lactam menthyloxyacetic acid imide (23)



^{1}H NMR Spectra for Different Lots of CTV lactam menthyloxyacetic acid imide (23)



Menthoxy acetic acid CTV Imide
Lot PR057-136-5

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PROCNO                 1

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PULPROG               zg
TD                 32768
SOLVENT            CDCl3
NS                    16
DS                     0
SWH             5995.204 Hz
FIDRES          0.182959 Hz
AQ             2.7328513 sec
RG                   256
DW                83.400 usec
DE                 6.00 usec
TE                298.2 K
D1           1.00000000 sec
MCREST   0 sec
MCWRK        0.01500000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 6.68 usec
PL1               -2.00 dB
SFO1         400.1418006 MHz

F2 - Processing parameters
SI                 16384
SF           400.1400076 MHz
WDW                   EM
SSB      0
LB                 0.30 Hz
GB       0
PC                 1.00
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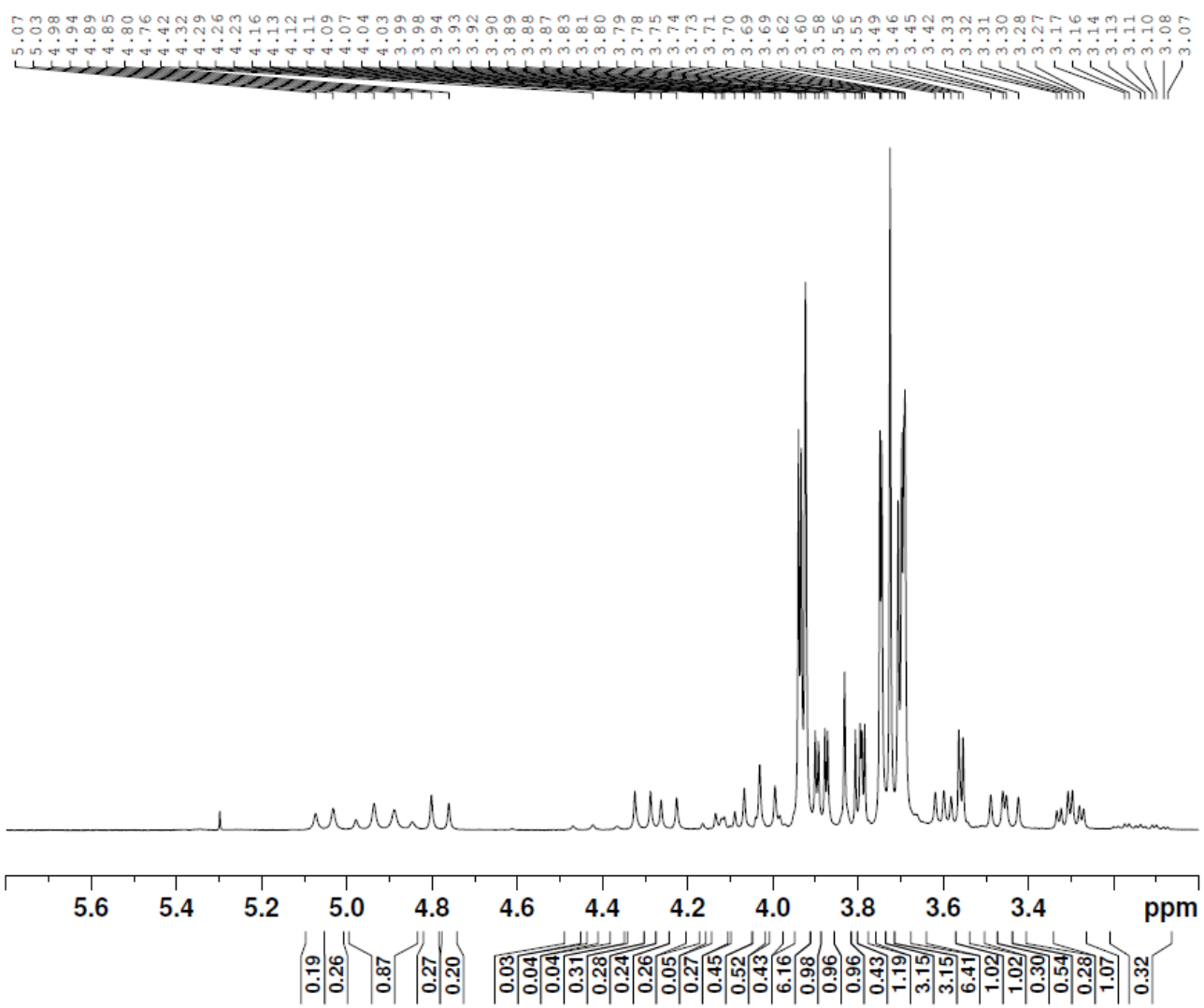
Menthoxy acetic acid CTV Imide
Lot PR057-136-5

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Current Data Parameters
NAME         PR057-136-5
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
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Time                0.02
INSTRUM            spect
PROBHD   5 mm BBI 1H-BB
PULPROG               zg
TD                 32768
SOLVENT            CDC13
NS                    16
DS                     0
SWH             5995.204 Hz
FIDRES          0.182959 Hz
AQ             2.7328513 sec
RG                   256
DW                83.400 usec
DE                 6.00 usec
TE                298.2 K
D1           1.00000000 sec
MCREST   0 sec
MCWRK        0.01500000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 6.68 usec
PL1               -2.00 dB
SFO1        400.1418006 MHz

F2 - Processing parameters
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SF          400.1400076 MHz
WDW                   EM
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LB                 0.30 Hz
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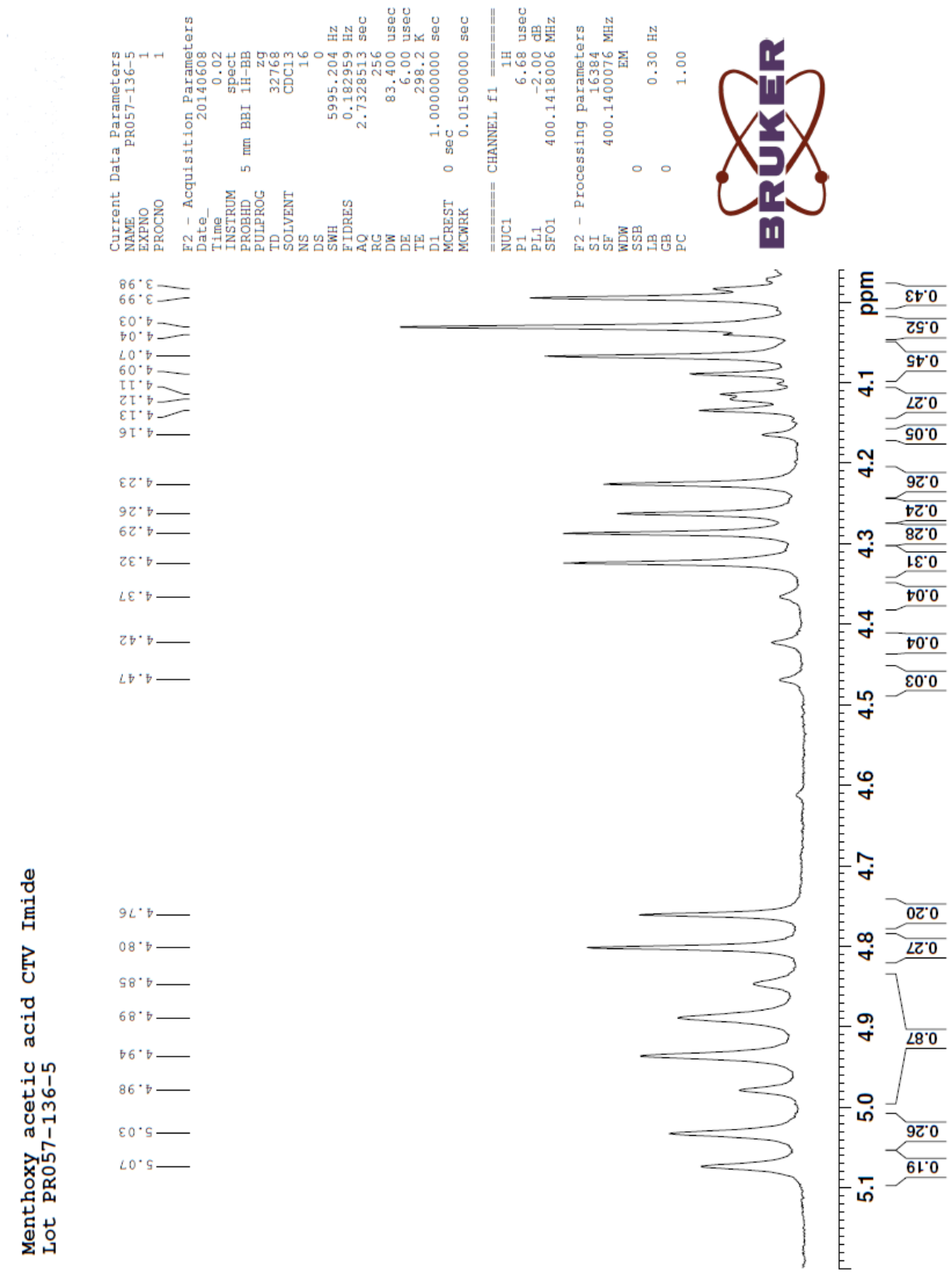
Menthoxy acetic acid CTV Imide
Lot PR057-136-5
Current Data Parameters
NAME PR057-136-5
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140608
Time 0.02
INSTRUM spect
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PULPROG zg
TD 32768
SOLVENT CDC13
NS 16
DS 0
SWH 5995.204 Hz
FIDRES 0.182959 Hz
AQ 2.7328513 sec
RG 256
DW 83.400 usec
DE 6.00 usec
TE 298.2 K
D1 1.00000000 sec
MCREST 0 sec 0.01500000 sec
MCWRK
======== CHANNEL f1 ========
NUC1 1H
P1 6.68 usec
PL1 -2.00 dB
SFO1 400.1418006 MHz
F2 - Processing parameters
SI 16384
SF 400.1400076 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
BRUKER
3.98
3.99
4.03
4.04
4.07
4.09
4.11
4.12
4.13
4.16
4.23
4.26
4.29
4.32
4.37
4.42
4.47
4.76
4.80
4.85
4.89
4.94
4.98
5.03
5.07
ppm
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5.0
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4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
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0.26
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0.27
0.20
0.03
0.04
0.04
0.31
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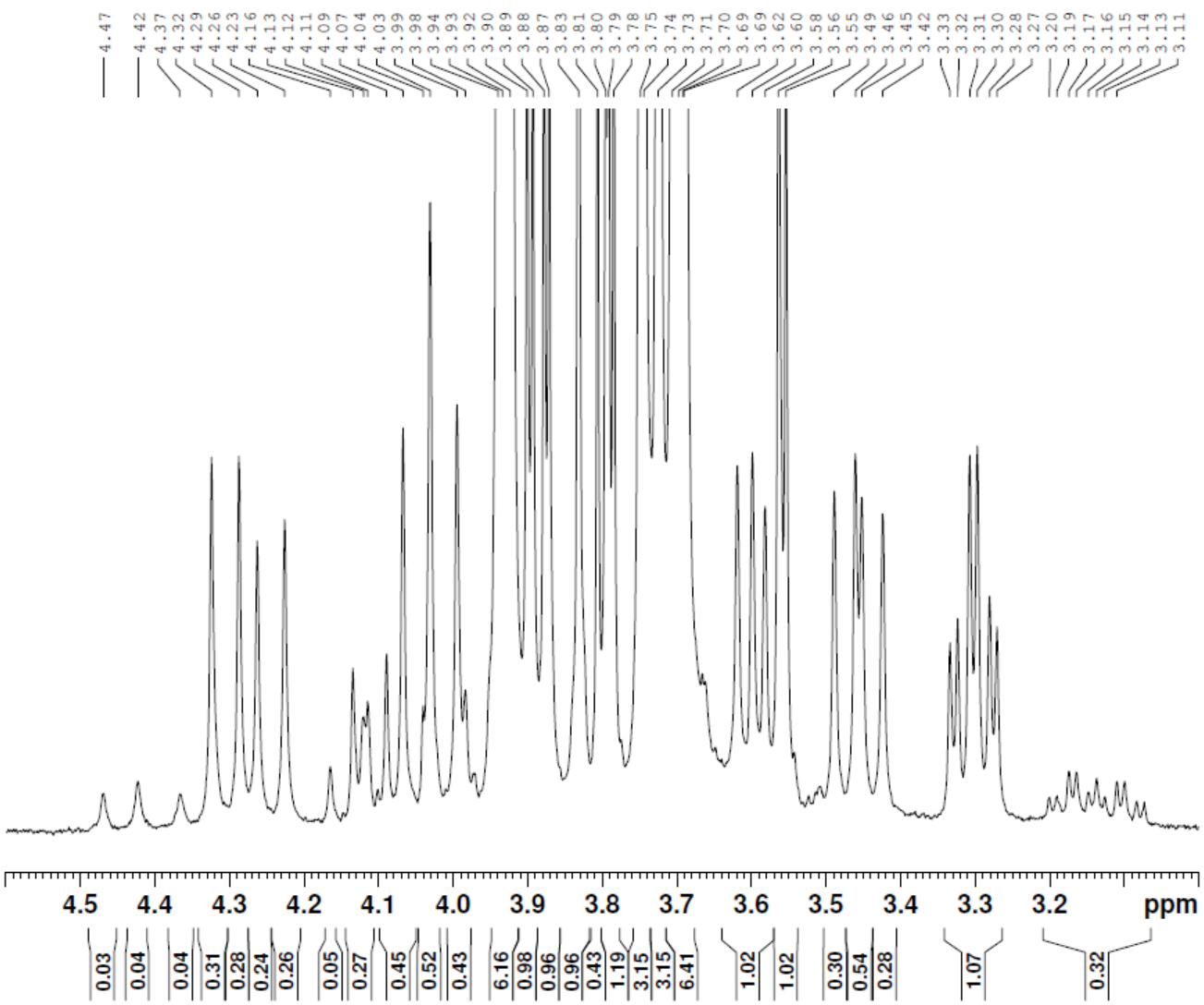
0.05
0.27
0.45
0.52
0.43

Menthoxy acetic acid CTV Imide
Lot PR057-136-5



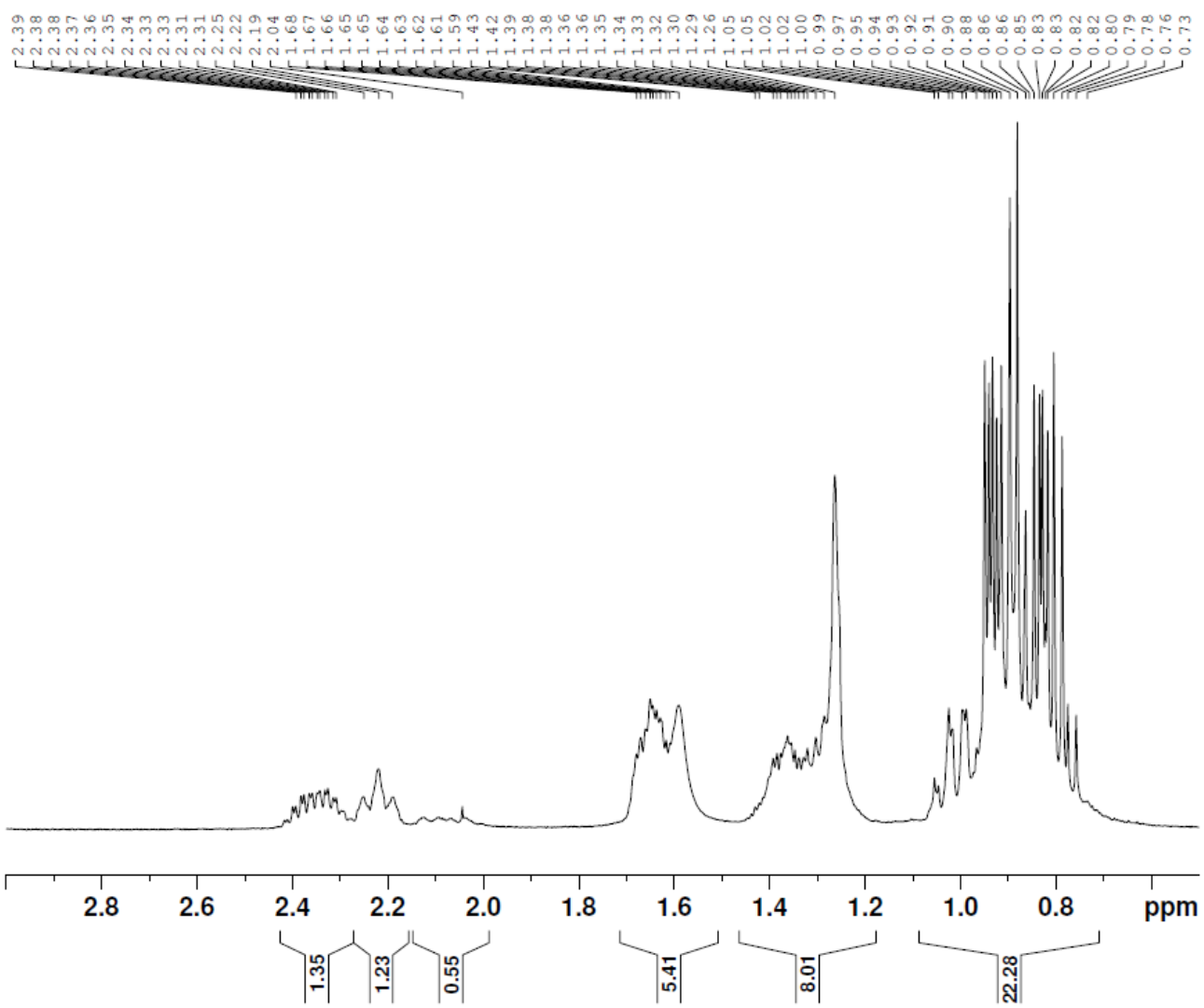

```
Current Data Parameters
NAME         PR057-136-5
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20140608
Time                0.02
INSTRUM            spect
PROBHD   5 mm BBI 1H-BB
PULPROG               zg
TD                 32768
SOLVENT            CDCl3
NS                    16
DS                     0
SWH             5995.204 Hz
FIDRES          0.182959 Hz
AQ             2.7328513 sec
RG                   256
DW                83.400 usec
DE                 6.00 usec
TE                298.2 K
D1           1.00000000 sec
MCREST   0 sec
MCWRK         0.01500000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 6.68 usec
PL1               -2.00 dB
SFO1         400.1418006 MHz

F2 - Processing parameters
SI                 16384
SF          400.1400076 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

Menthoxy acetic acid CTV Imide
Lot PR057-136-5



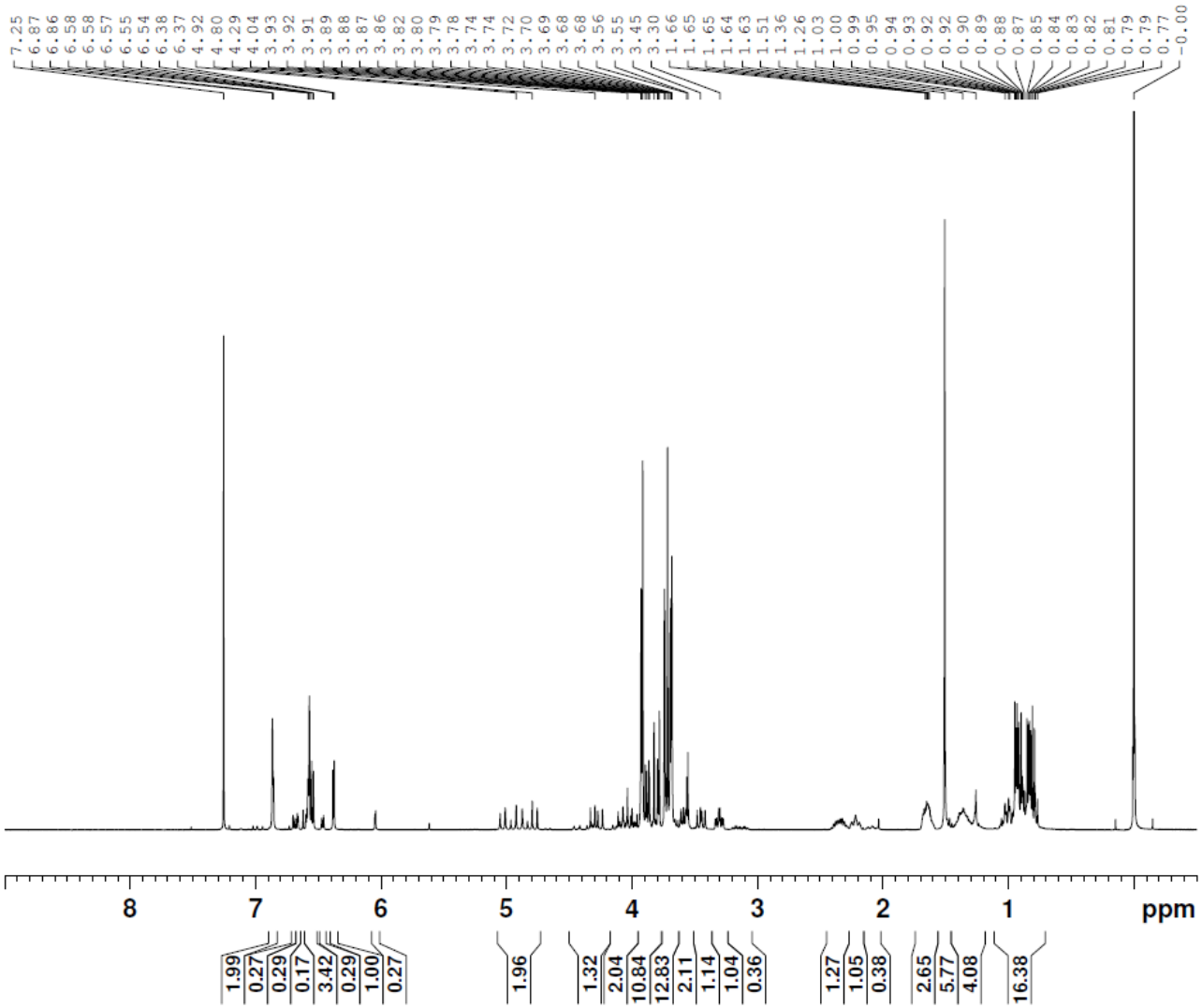

```
Current Data Parameters
NAME         PR057-136-5
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20140608
Time                0.02
INSTRUM            spect
PROBHD   5 mm BBI 1H-BB
PULPROG               zg
TD                 32768
SOLVENT            CDCl3
NS                    16
DS                     0
SWH             5995.204 Hz
FIDRES          0.182959 Hz
AQ              2.7328513 sec
RG                   256
DW                83.400 usec
DE                 6.00 usec
TE                298.2 K
D1             1.00000000 sec
MCREST    0 sec
MCWRK          0.01500000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 6.68 usec
PL1               -2.00 dB
SFO1         400.1418006 MHz

F2 - Processing parameters
SI                 16384
SF           400.1400076 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

PR069-062-B1
Enriched CTV-Menthoxyacetyl Imide

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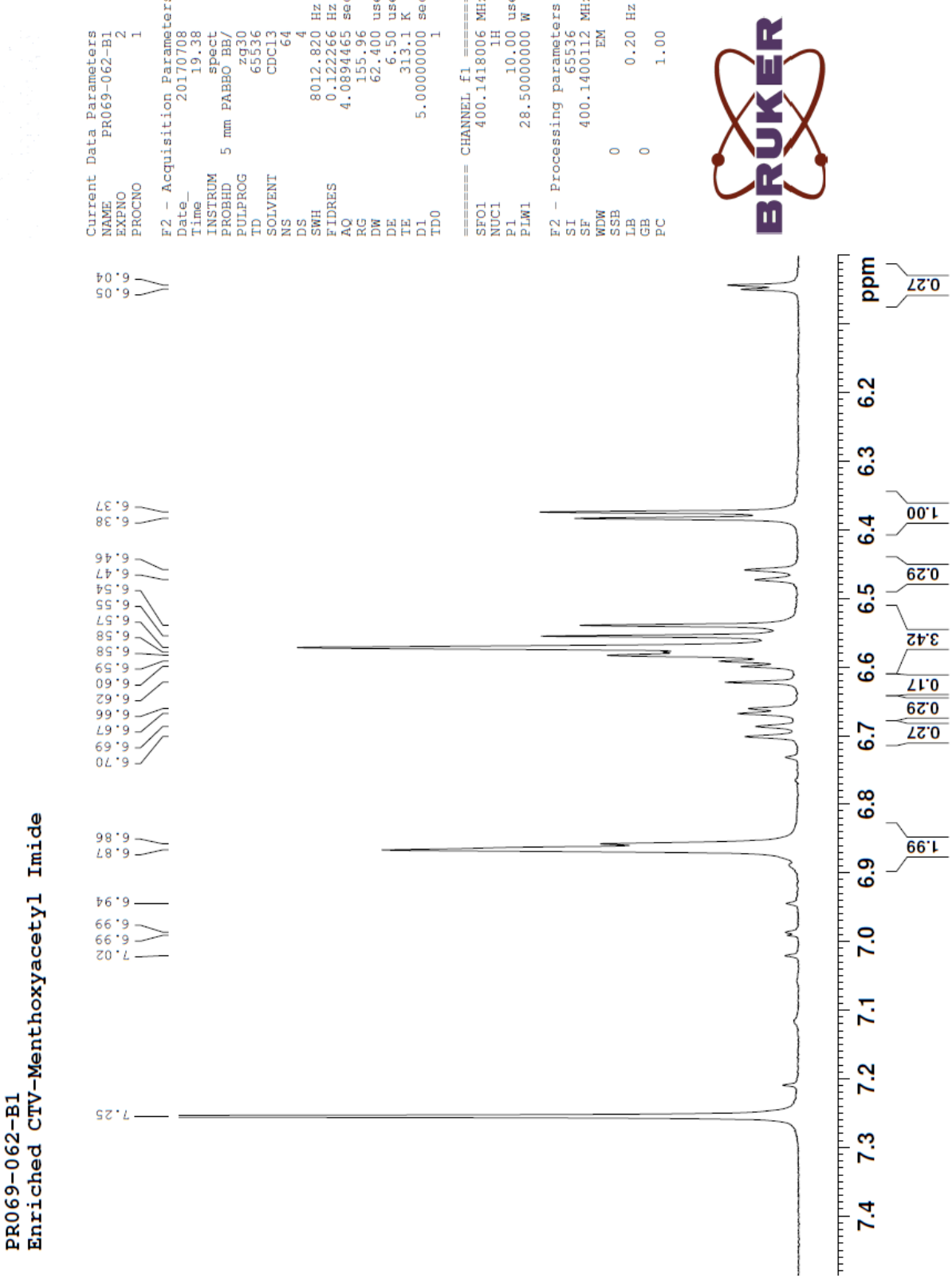
Current Data Parameters
NAME        PR069-062-B1
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20170708
Time               19.38
INSTRUM            spect
PROBHD   5 mm PABBO BB/
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    64
DS                     4
SWH             8012.820 Hz
FIDRES          0.122266 Hz
AQ              4.0894465 sec
RG                155.96
DW                62.400 usec
DE                 6.50 usec
TE                313.1 K
D1             5.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          400.1418006 MHz
NUC1                  1H
P1                 10.00 usec
PLW1          28.50000000 W

F2 - Processing parameters
SI                 65536
SF            400.1400112 MHz
WDW                   EM
SSB       0
LB                 0.20 Hz
GB        0
PC                 1.00
```


PR069-062-B1
Enriched CTV-Menthoxyacetyl Imide
Current Data Parameters
NAME PR069-062-B1
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170708
Time_ 19.38
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDC13
NS 64
DS 4
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 155.96
DW 62.400 usec
DE 6.50 usec
TE 313.1 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1418006 MHz
NUC1 1H
P1 10.00 usec
PLW1 28.50000000 W
F2 - Processing parameters
SI 65536
SF 400.1400112 MHz
WDW EM
SSB 0
LB 0.20 Hz
GB 0
PC 1.00
BRUKER
7.25
7.02
6.99
6.99
6.94
6.87
6.86
6.70
6.69
6.67
6.66
6.62
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6.58
6.57
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6.46
6.38
6.37
6.05
6.04
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7.1
7.0
6.9
6.8
6.7
6.6
6.5
6.4
6.3
6.2
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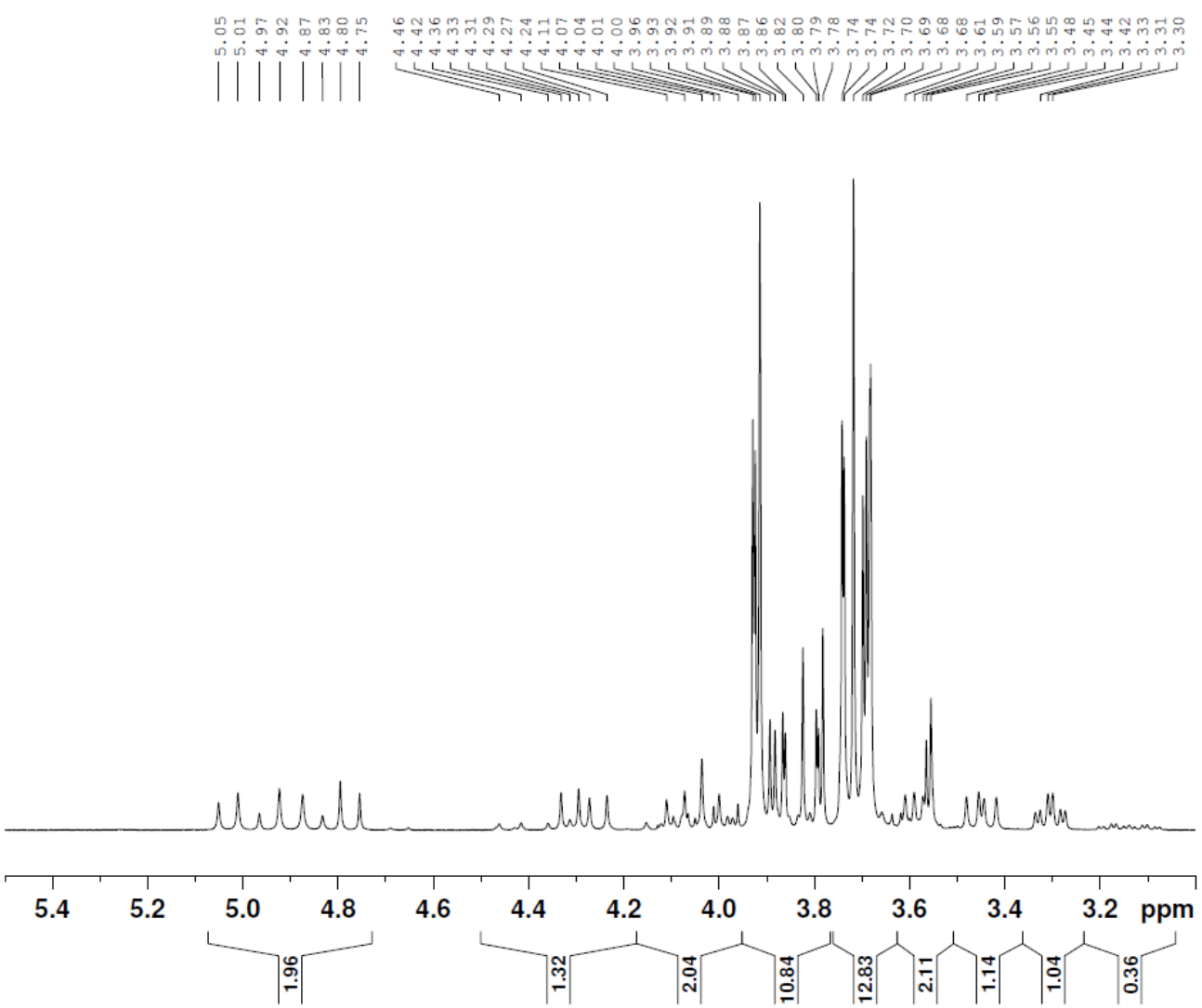
0.17
0.29
0.27
1.99

PR069-062-B1
Enriched CTV-Menthoxyacetyl Imide



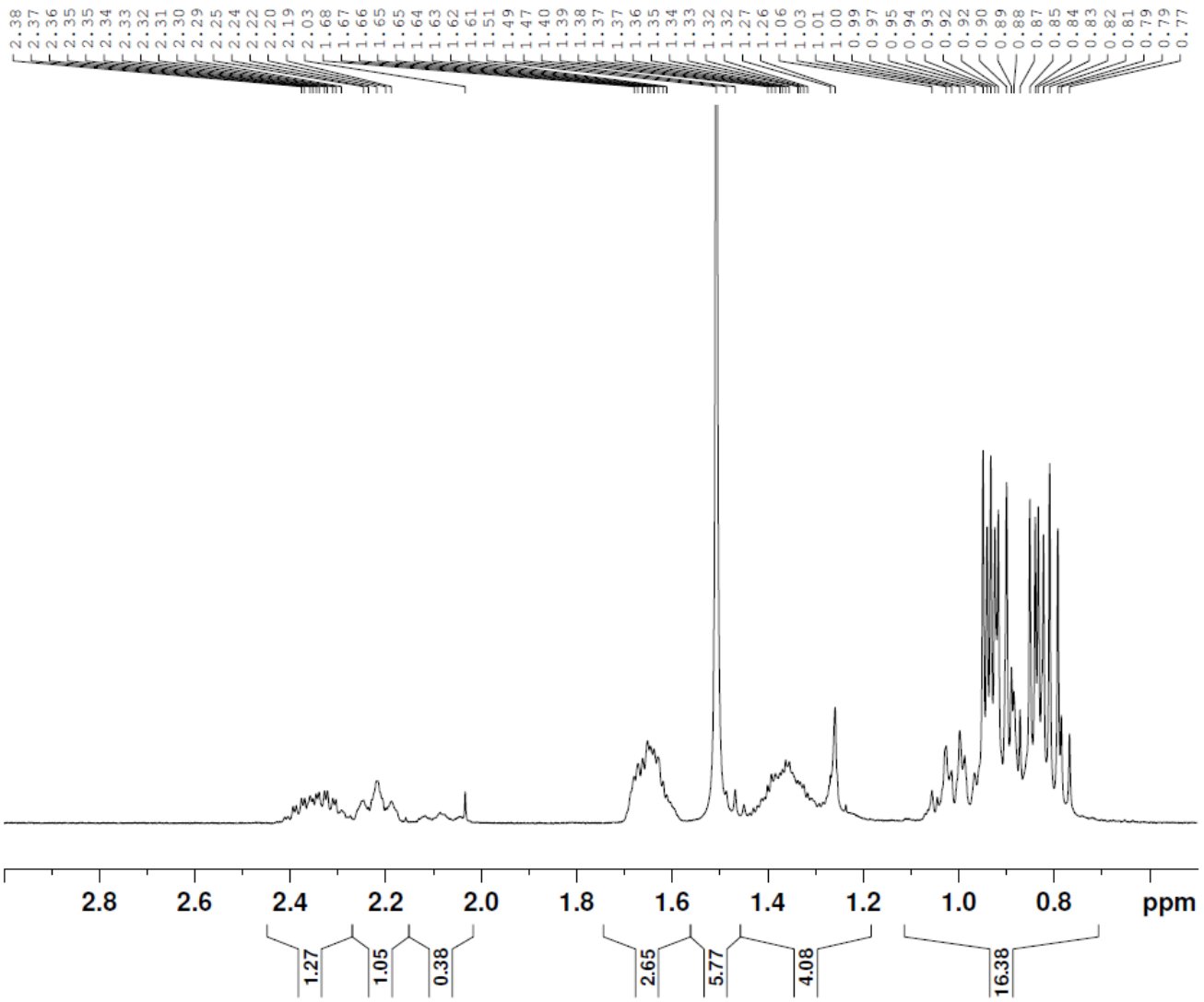

```
Current Data Parameters
NAME        PR069-062-B1
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20170708
Time               19.38
INSTRUM            spect
PROBHD   5 mm PABBO BB/
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    64
DS                     4
SWH             8012.820 Hz
FIDRES          0.122266 Hz
AQ              4.0894465 sec
RG                155.96
DW                62.400 usec
DE                 6.50 usec
TE                313.1 K
D1             5.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.1418006 MHz
NUC1                   1H
P1                 10.00 usec
PLW1         28.50000000 W

F2 - Processing parameters
SI                 65536
SF           400.1400112 MHz
WDW                   EM
SSB       0
LB                 0.20 Hz
GB        0
PC                 1.00
```

PR069-062-B1
Enriched CTV-Menthoxyacetyl Imide



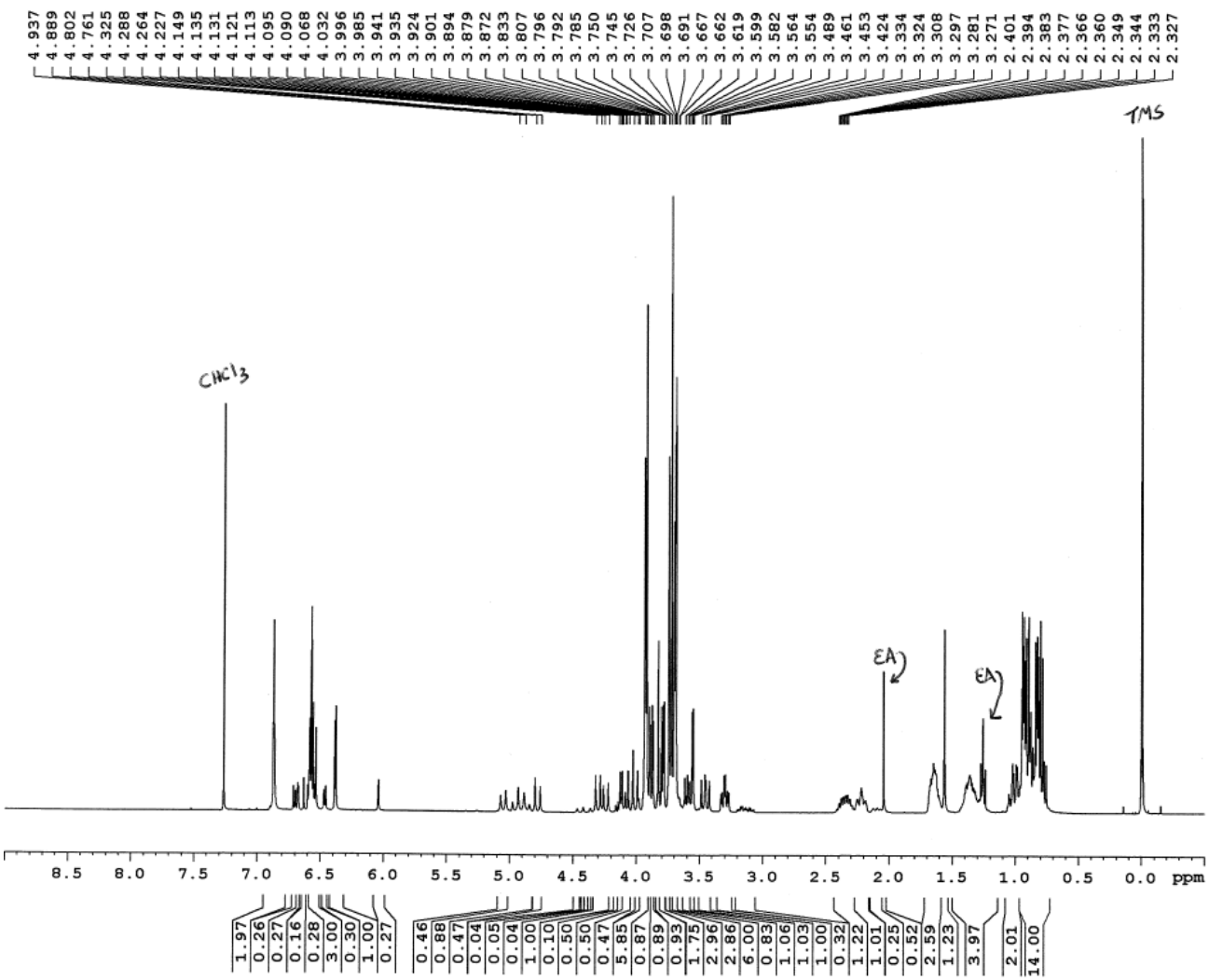

```
Current Data Parameters
NAME        PR069-062-B1
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20170708
Time              19.38
INSTRUM           spect
PROBHD   5 mm PABBO BB/
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                   64
DS                    4
SWH            8012.820 Hz
FIDRES        0.122266 Hz
AQ            4.0894465 sec
RG               155.96
DW               62.400 usec
DE                6.50 usec
TE                313.1 K
D1           5.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         400.1418006 MHz
NUC1                 1H
P1                10.00 usec
PLW1         28.50000000 W

F2 - Processing parameters
SI                65536
SF           400.1400112 MHz
WDW                  EM
SSB       0
LB                0.20 Hz
GB        0
PC                1.00
```

Menthoxy acetic acid CTV Imide
Lot PR057-140-5
*Recrystallized from EA/Hept
10 mg in 0.75 mL CDCl3



BRUKER

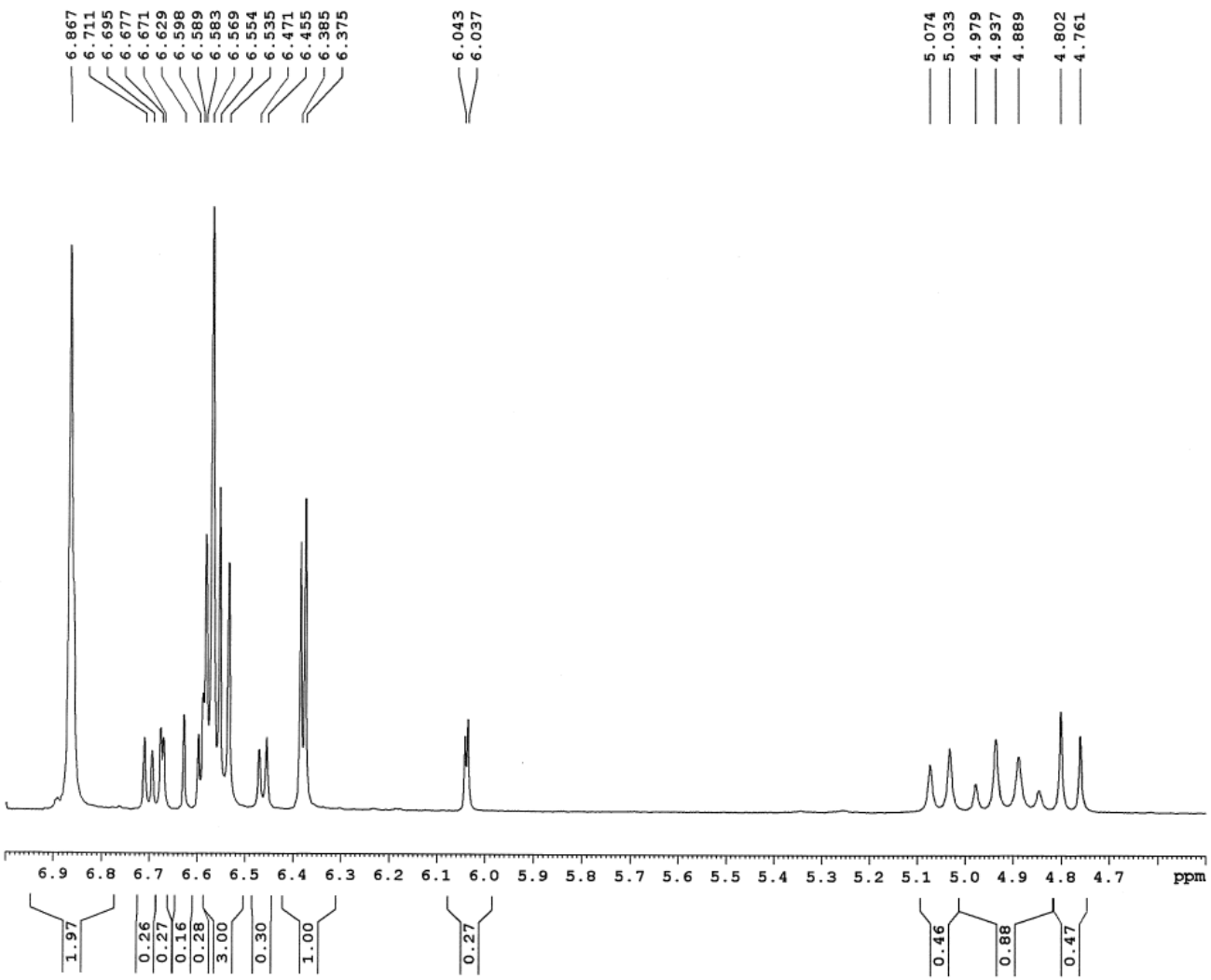
```
Current Data Parameters
NAME                  PR057-140-5
EXPNO                           1
PROCNO                          1

F2 - Acquisition Parameters
Date_                    20140608
Time                         0.36
INSTRUM                     spect
PROBHD    5 mm BBI 1H-BB
PULPROG                        zg
TD                          32768
SOLVENT                     CDCl3
NS                             64
DS                              0
SWH                      5995.204 Hz
FIDRES                   0.182959 Hz
AQ                       2.7329011 sec
RG                            256
DW                          83.400 usec
DE                           6.00 usec
TE                          298.2 K
D1                    1.00000000 sec
MCREST                0.00000000 sec
MCWRK                 0.01500000 sec

======== CHANNEL f1 ========
NUC1                           1H
P1                           6.68 usec
PL1                         -2.00 dB
SFO1                400.1418006 MHz

F2 - Processing parameters
SI                         16384
SF                  400.1400076 MHz
WDW                           EM
SSB                            0
LB                          0.30 Hz
GB                             0
PC                          1.00
```

Menthoxy acetic acid CTV Imide
Lot PR057-140-5
*Recrystallized from EA/Hept
10 mg in 0.75 mL CDCl3



BRUKER

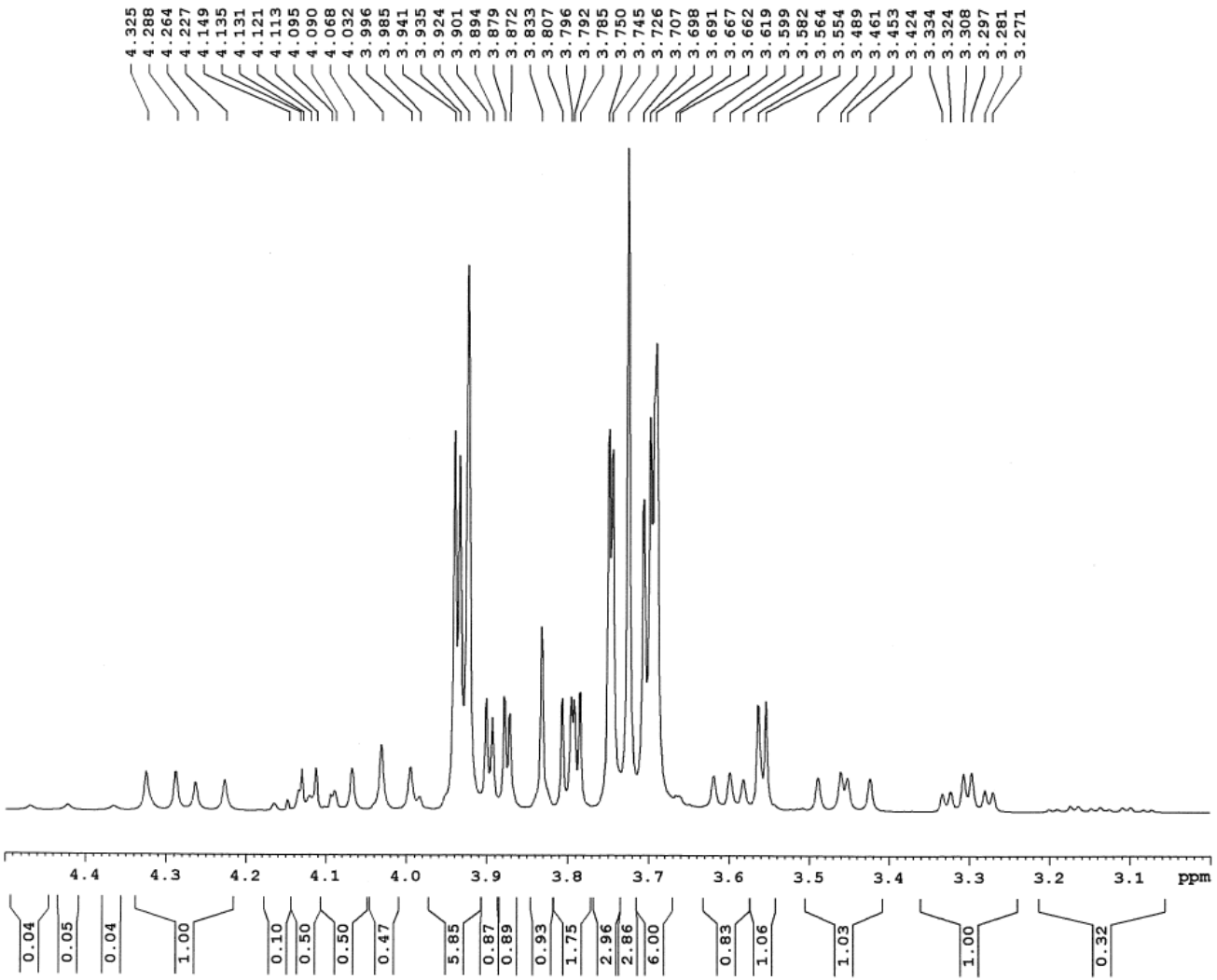
Current Data Parameters
NAME PR057-140-5
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20140608
Time 0.36
INSTRUM spect
PROBHD 5 mm BBI 1H-BB
PULPROG zg
TD 32768
SOLVENT CDCl3
NS 64
DS 0
SWH 5995.204 Hz
FIDRES 0.182959 Hz
AQ 2.7329011 sec
RG 256
DW 83.400 usec
DE 6.00 usec
TE 298.2 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 6.68 usec
PL1 -2.00 dB
SFO1 400.1418006 MHz

F2 - Processing parameters
SI 16384
SF 400.1400076 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

Menthoxy acetic acid CTV Imide
Lot PR057-140-5
*Recrystallized from EA/Hept
10 mg in 0.75 mL CDCl3



BRUKER

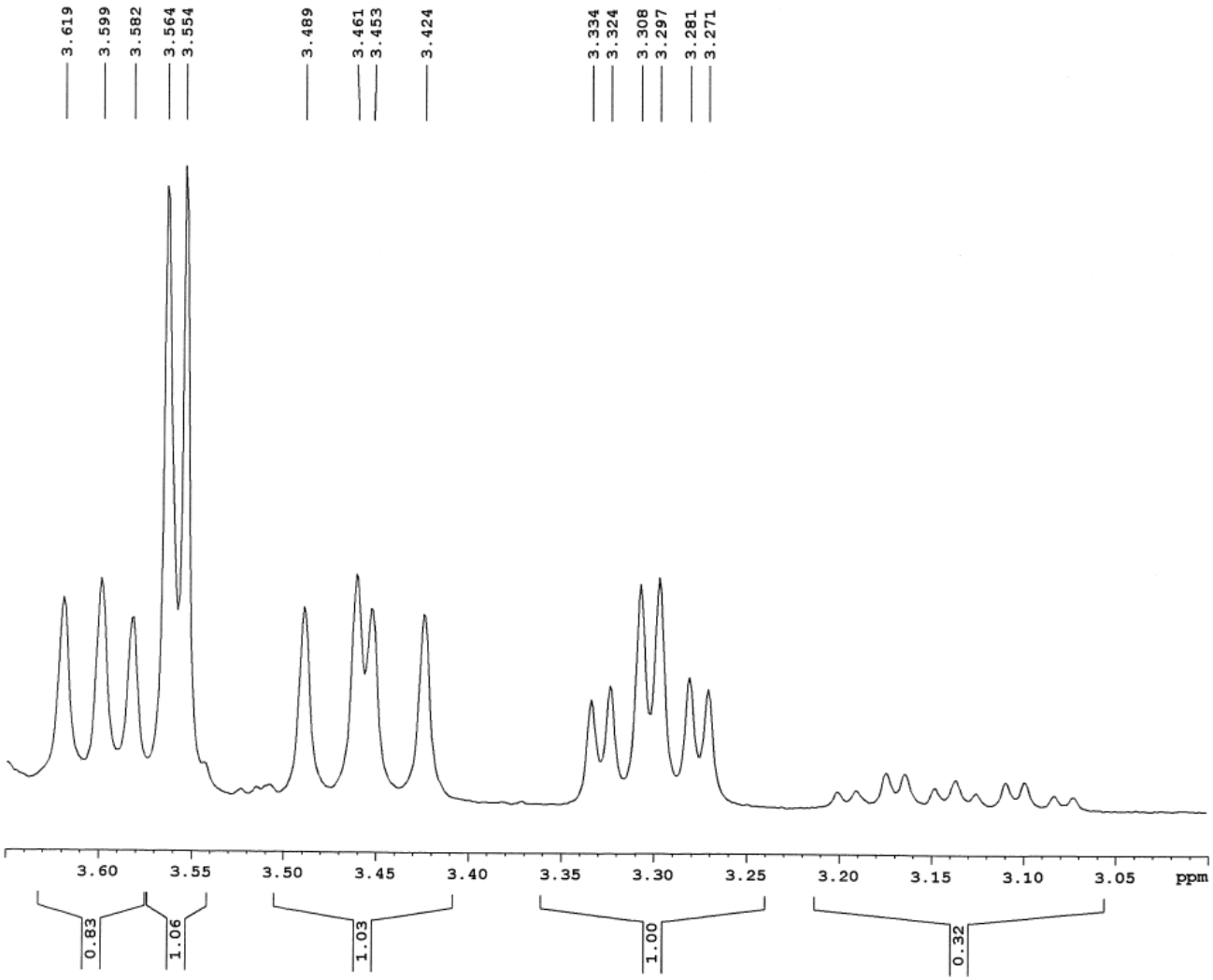
Current Data Parameters
NAME PR057-140-5
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20140608
Time 0.36
INSTRUM spect
PROBHD 5 mm BBI 1H-BB
PULPROG zg
TD 32768
SOLVENT CDCl3
NS 64
DS 0
SWH 5995.204 Hz
FIDRES 0.182959 Hz
AQ 2.7329011 sec
RG 256
DW 83.400 usec
DE 6.00 usec
TE 298.2 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 6.68 usec
PL1 -2.00 dB
SFO1 400.1418006 MHz

F2 - Processing parameters
SI 16384
SF 400.1400076 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

Menthoxy acetic acid CTV Imide
Lot PR057-140-5
*Recrystallized from EA/Hept
10 mg in 0.75 mL CDCl3



BRUKER

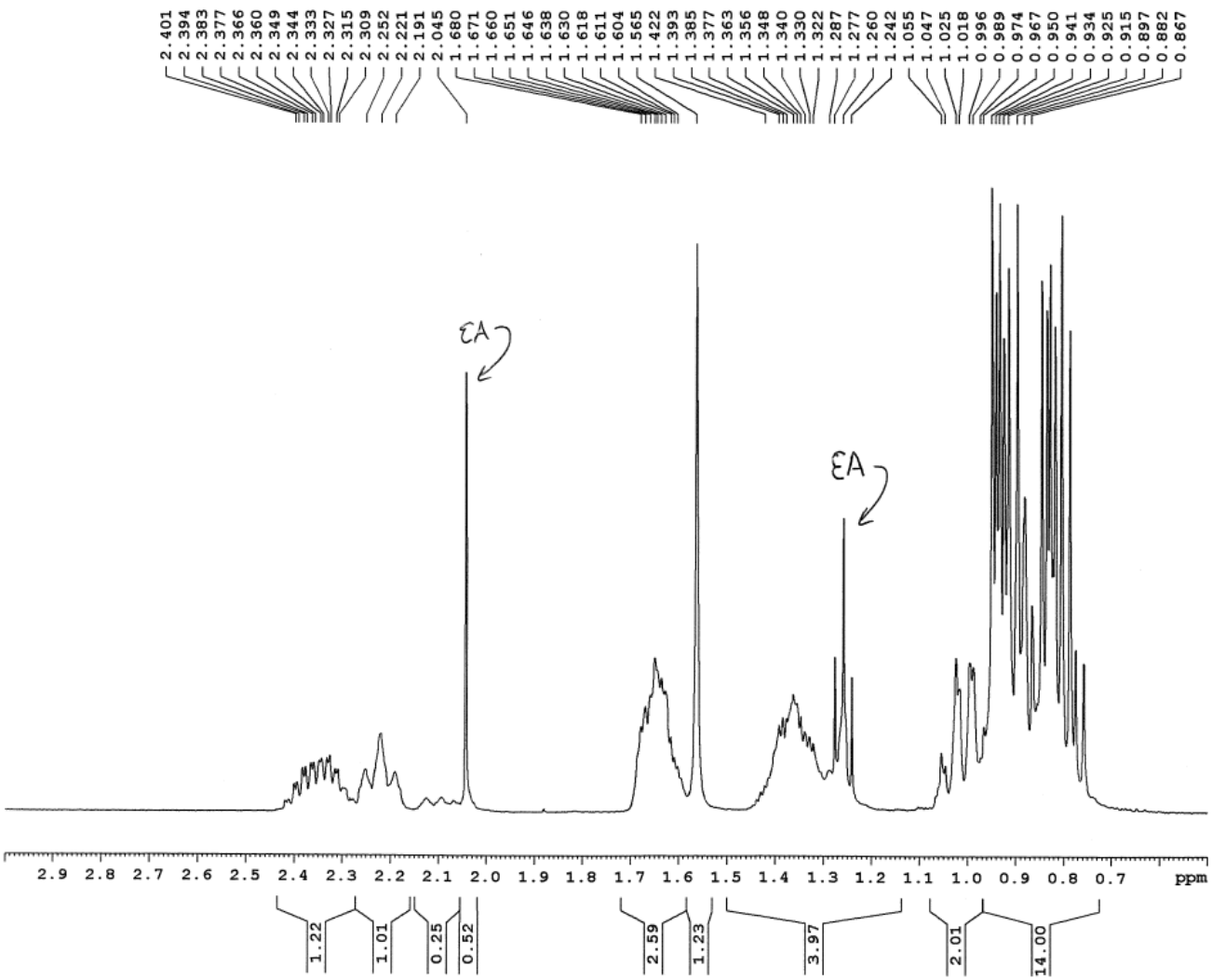
```
Current Data Parameters
NAME                  PR057-140-5
EXPNO                           1
PROCNO                          1

F2 - Acquisition Parameters
Date_                    20140608
Time                         0.36
INSTRUM                     spect
PROBHD          5 mm BBI 1H-BB
PULPROG                       zg
TD                         32768
SOLVENT                    CDCl3
NS                            64
DS                             0
SWH                     5995.204 Hz
FIDRES                  0.182959 Hz
AQ                      2.7329011 sec
RG                           256
DW                        83.400 usec
DE                         6.00 usec
TE                        298.2 K
D1                    1.00000000 sec
MCREST                0.00000000 sec
MCWRK                 0.01500000 sec

======== CHANNEL f1 ========
NUC1                          1H
P1                         6.68 usec
PL1                       -2.00 dB
SFO1                400.1418006 MHz

F2 - Processing parameters
SI                         16384
SF                  400.1400076 MHz
WDW                           EM
SSB                            0
LB                          0.30 Hz
GB                             0
PC                          1.00
```

Menthoxy acetic acid CTV Imide
Lot PR057-140-5
*Recrystallized from EA/Hept
10 mg in 0.75 mL CDCl3



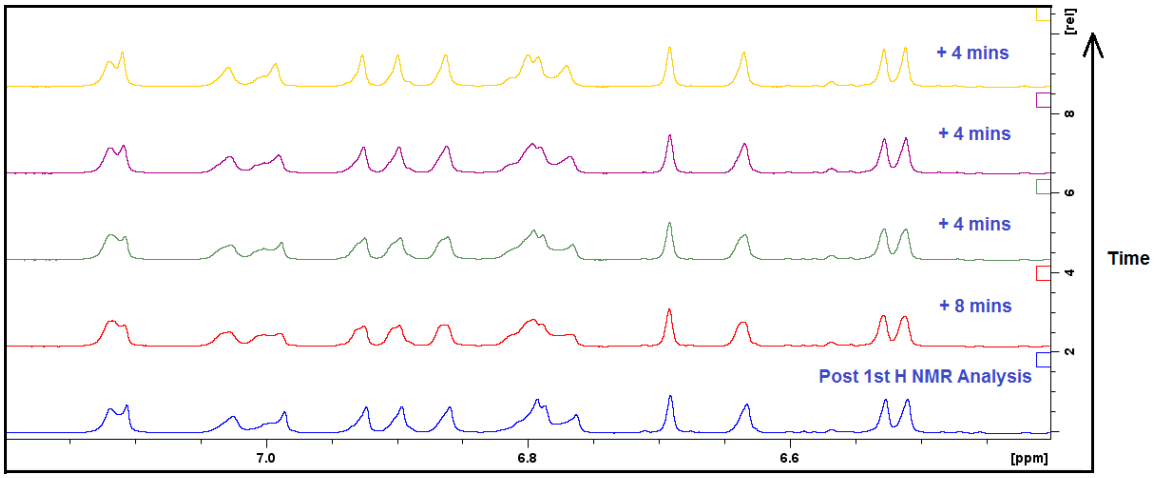
Current Data Parameters
NAME PR057-140-5
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20140608
Time 0.36
INSTRUM spect
PROBHD 5 mm BBI 1H-BB
PULPROG zg
TD 32768
SOLVENT CDCl3
NS 64
DS 0
SWH 5995.204 Hz
FIDRES 0.182959 Hz
AQ 2.7329011 sec
RG 256
DW 83.400 usec
DE 6.00 usec
TE 298.2 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 6.68 usec
PL1 -2.00 dB
SFO1 400.1418006 MHz

F2 - Processing parameters
SI 16384
SF 400.1400076 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

Overlap of ^{1}H NMR spectra of hydrolyzed 23 to 19 and analyzing the same NMR sample over 20 min shown below.

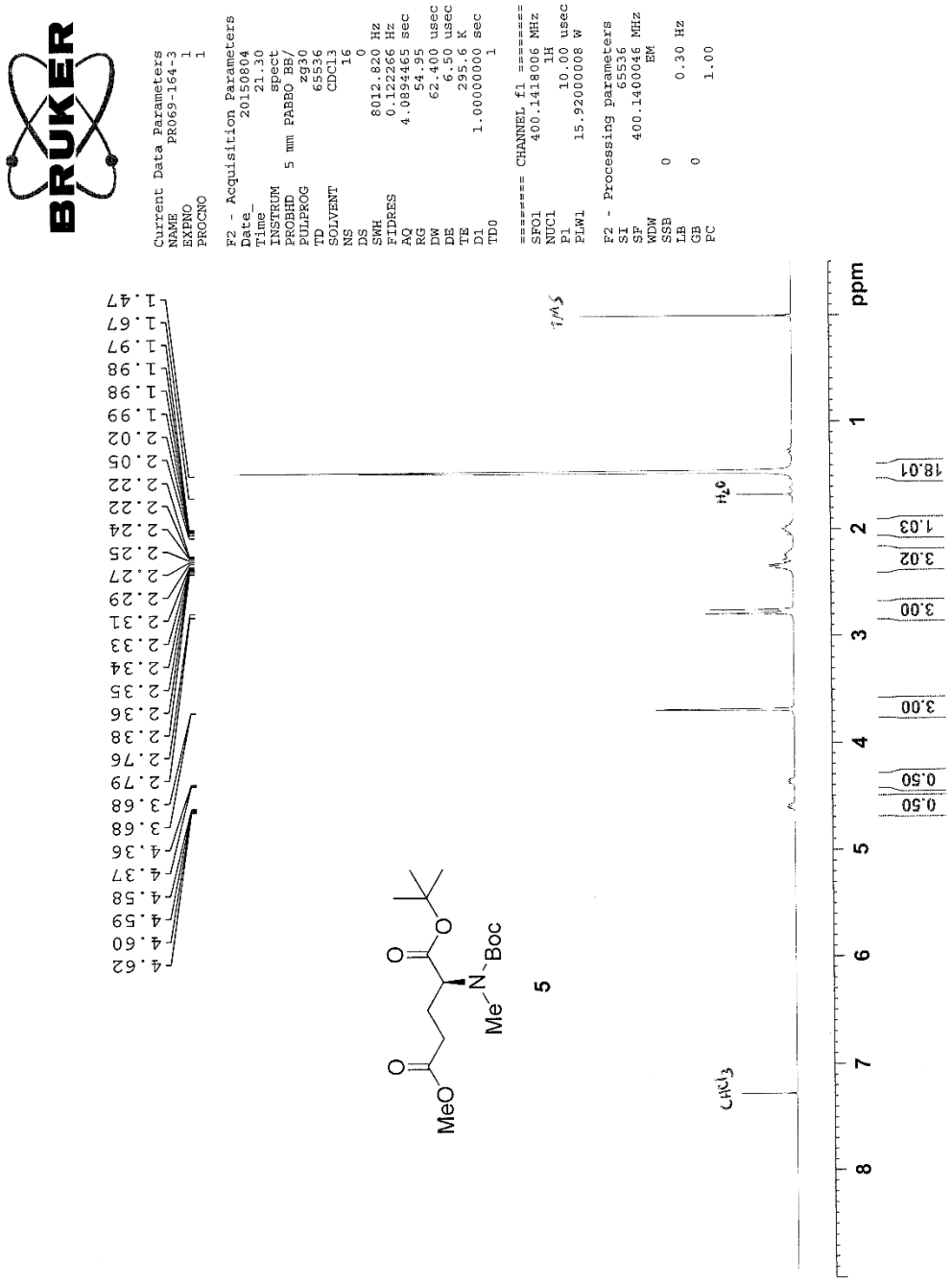


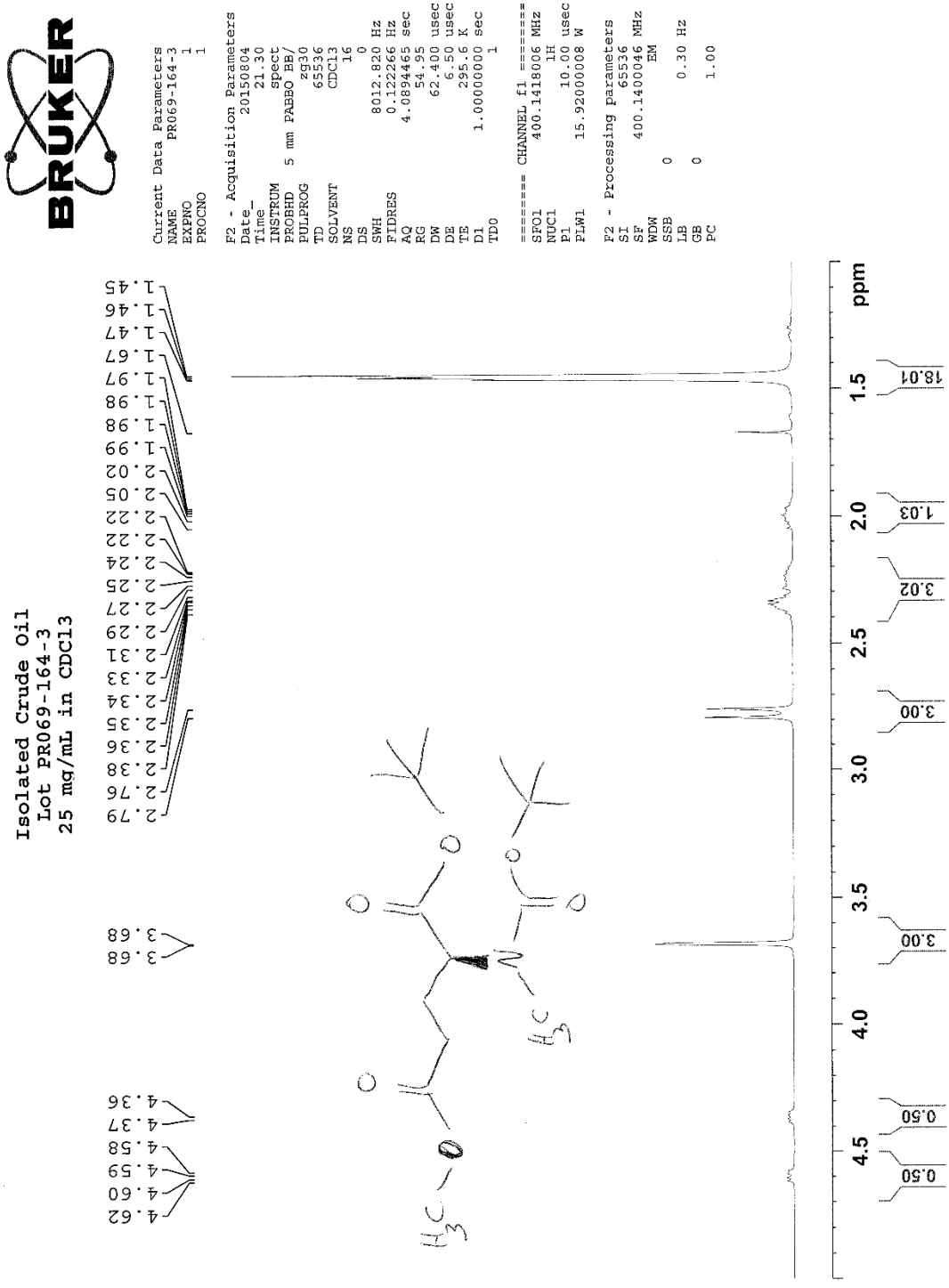
When the chiral auxiliary group was cleaved, concentrated, dissolved in CDCl3 and added Chirabite (0.30 eq), the sample was analyzed by ^{1}H NMR analysis over time to determine if there was any change. The figure above clearly shows that there wasn't any change and this result further proves that racemization occurs fast.

APPENDIX B

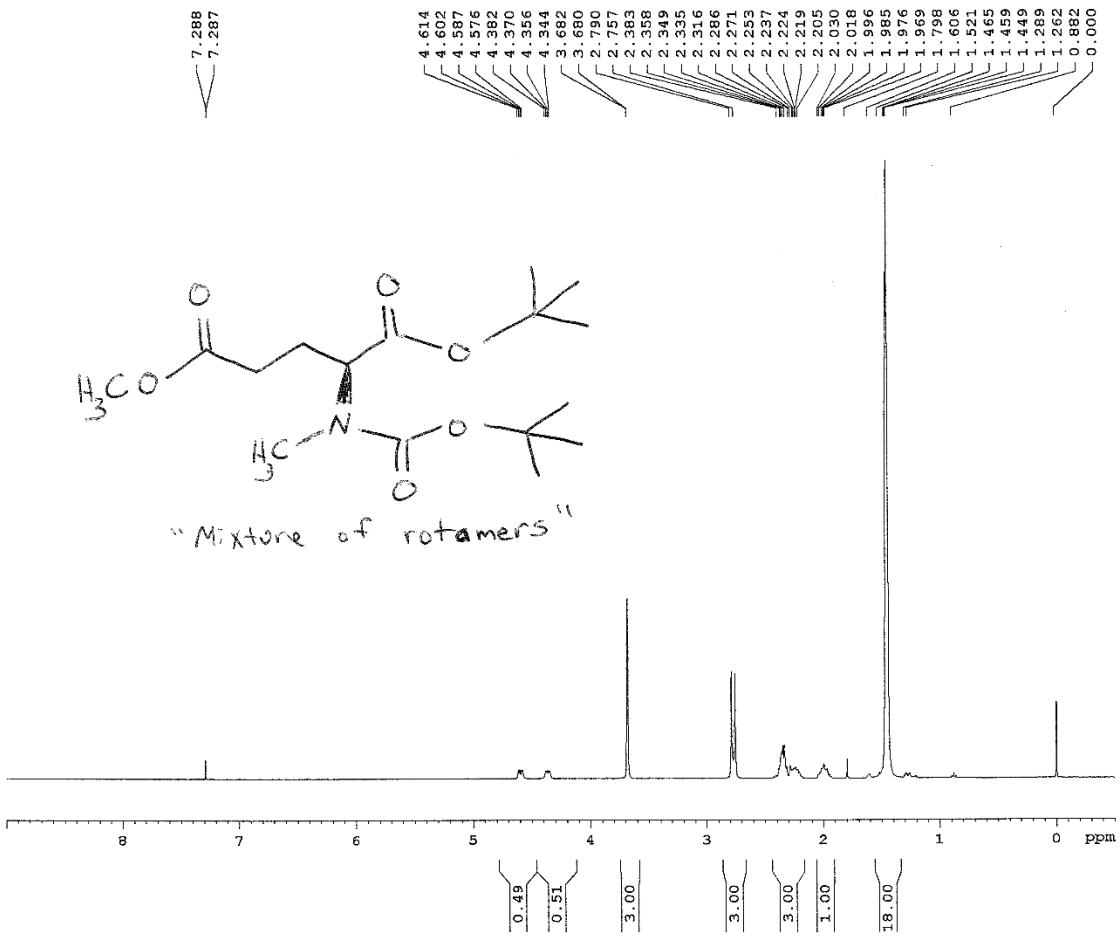
SUPPLEMENTAL DATA FOR CHAPTER THREE

^{1}H NMR - Compound 5




Isolated Crude Oil
Lot PR069-164-3
25 mg/mL in CDCl3
Current Data Parameters
NAME PR069-164-3
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20150804
Time 21.30
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 0
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 54.95
DW 62.400 usec
DE 6.50 usec
TE 295.6 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1418006 MHz
NUC1 1H
P1 10.00 usec
PLW1 15.92000008 W
F2 - Processing parameters
SI 65536
SF 400.1400046 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
1.45
1.46
1.47
1.67
1.97
1.98
1.98
1.99
2.02
2.05
2.22
2.22
2.24
2.25
2.27
2.29
2.31
2.33
2.34
2.35
2.36
2.38
2.76
2.79
3.68
3.68
4.36
4.37
4.58
4.59
4.60
4.62
ppm
4.5
4.0
3.5
3.0
2.5
2.0
1.5
0.50
0.50
3.00
3.00
3.02
1.03
18.01

N,N'-Methyl-Boc-L-Glu(OMe)-OtBu
Isolated Crude Oil
PR069-112-3
50 mg/mL in CDCl3



Current Data Parameters

NAME	PR069-112-3
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

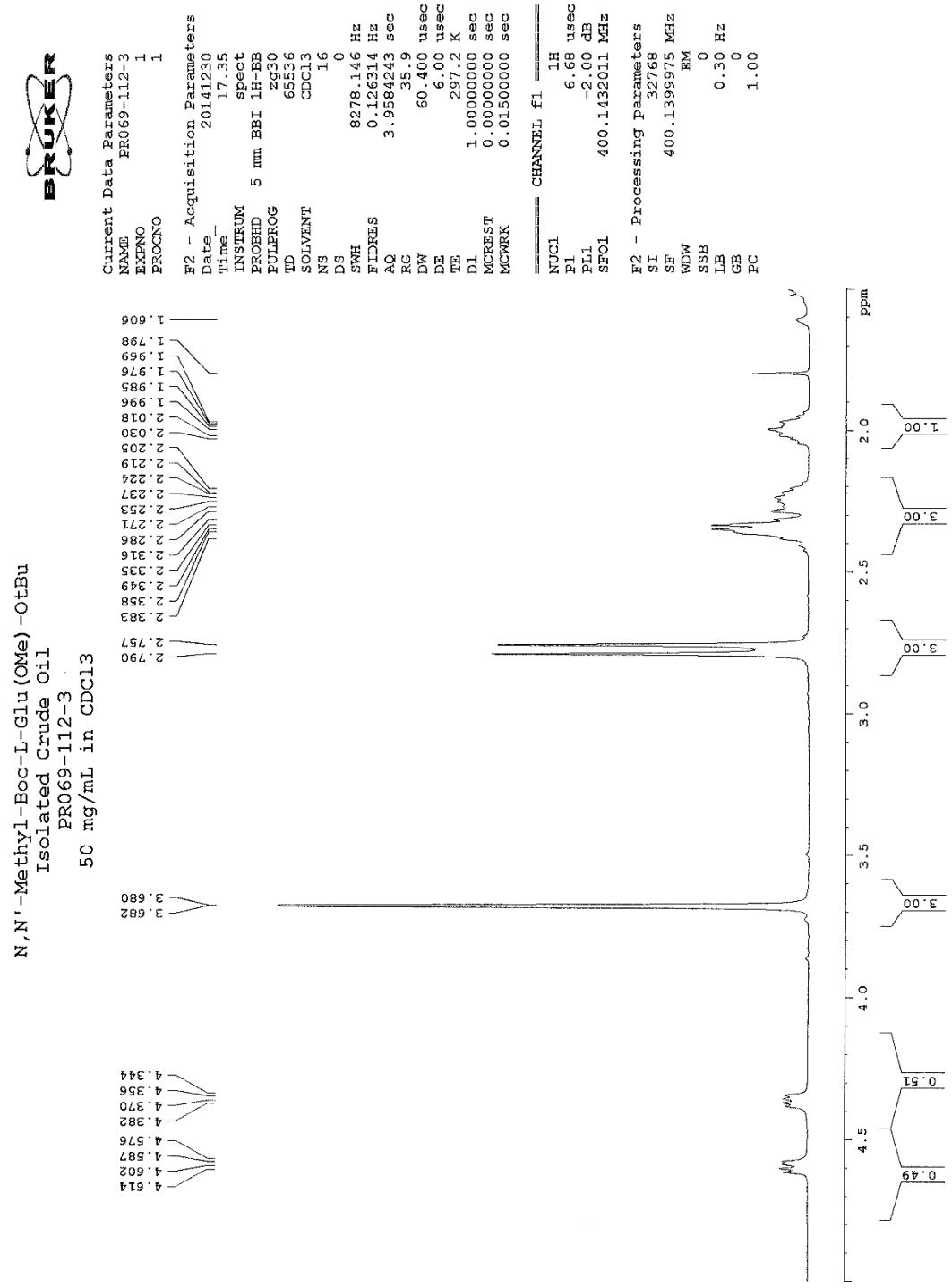
Date	20141230
Time	17.35
INSTRUM	spect
PROBHD	5 mm BBI 1H-BB
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	0
SWH	8278.146 Hz
FIDRES	0.126314 Hz
AQ	3.9584243 sec
RG	35.9
DW	60.400 usec
DE	6.00 usec
TE	297.2 K
D1	1.00000000 sec
MCREST	0.00000000 sec
MCWRK	0.01500000 sec

======== CHANNEL f1 ========

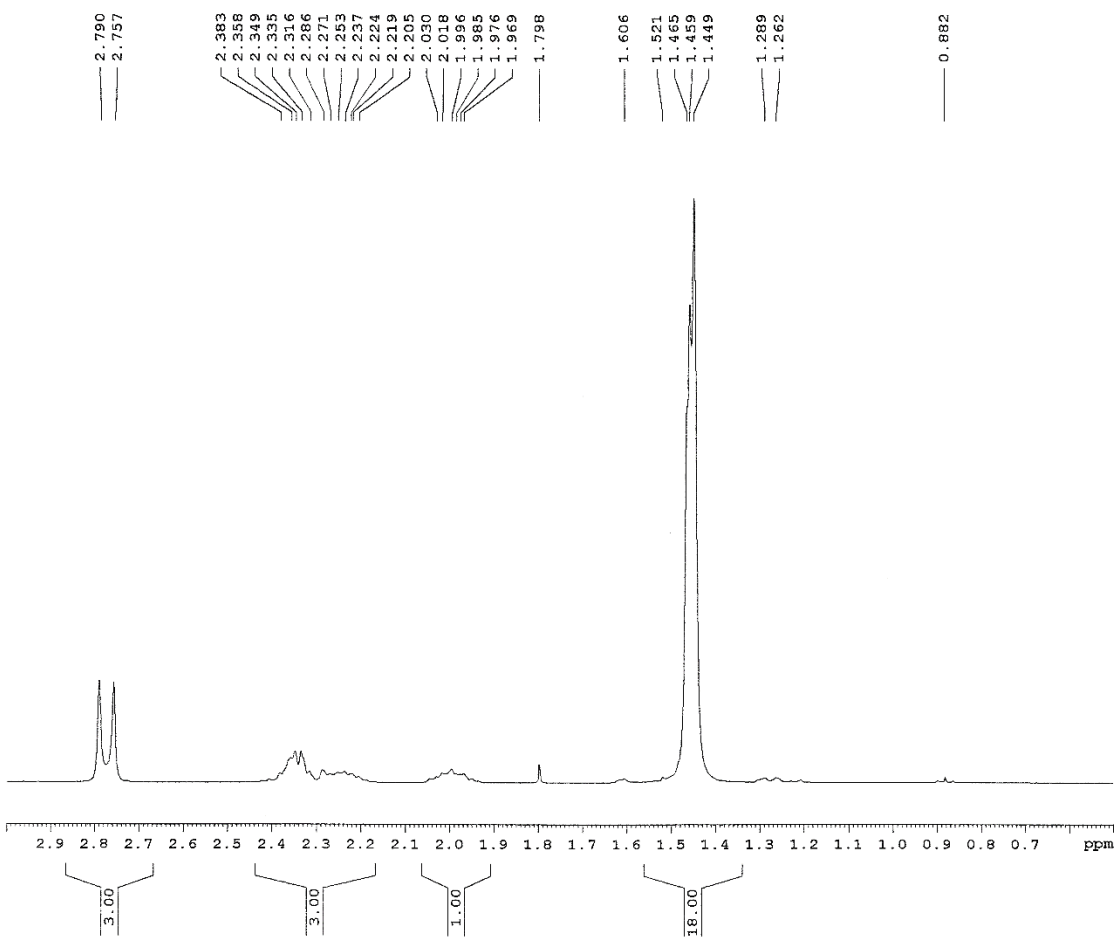
NUC1	1H
P1	6.68 usec
PL1	-2.00 dB
SFO1	400.1432011 MHz

F2 - Processing parameters

SI	32768
SF	400.1399975 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00


N,N'-Methyl-Boc-L-Glu(OMe)-OtBu
Isolated Crude Oil
PR069-112-3
50 mg/mL in CDCl3
Current Data Parameters
NAME PR069-112-3
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20141230
Time_ 17.35
INSTRUM spect
PROBHD 5 mm BBI 1H-BB
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 0
SWH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9584243 sec
RG 35.9
DW 60.400 usec
DE 6.00 usec
TE 297.2 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
CHANNEL f1
NUC1 1H
P1 6.68 usec
PL1 -2.00 dB
SFO1 400.1432011 MHz
F2 - Processing parameters
SI 32768
SF 400.1399975 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
1.606
1.798
1.969
1.976
1.985
1.996
2.018
2.030
2.205
2.219
2.224
2.237
2.253
2.271
2.286
2.316
2.335
2.349
2.358
2.383
2.757
2.790
3.680
3.682
4.344
4.356
4.370
4.382
4.576
4.587
4.602
4.614
ppm
4.5
4.0
3.5
3.0
2.5
2.0
0.49
0.51
3.00
3.00
3.00
3.00
1.00

N,N'-Methyl-Boc-L-Glu(OMe)-OtBu
Isolated Crude Oil
PR069-112-3
50 mg/mL in CDCl3



Current Data Parameters

NAME	PR069-112-3
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20141230
Time	17.35
INSTRUM	spect
PROBHD	5 mm BBI 1H-BB
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	0
SWH	8278.146 Hz
FIDRES	0.126314 Hz
AQ	3.9584243 sec
RG	35.9
DW	60.400 usec
DE	6.00 usec
TE	297.2 K
D1	1.00000000 sec
MCREST	0.00000000 sec
MCWRK	0.01500000 sec

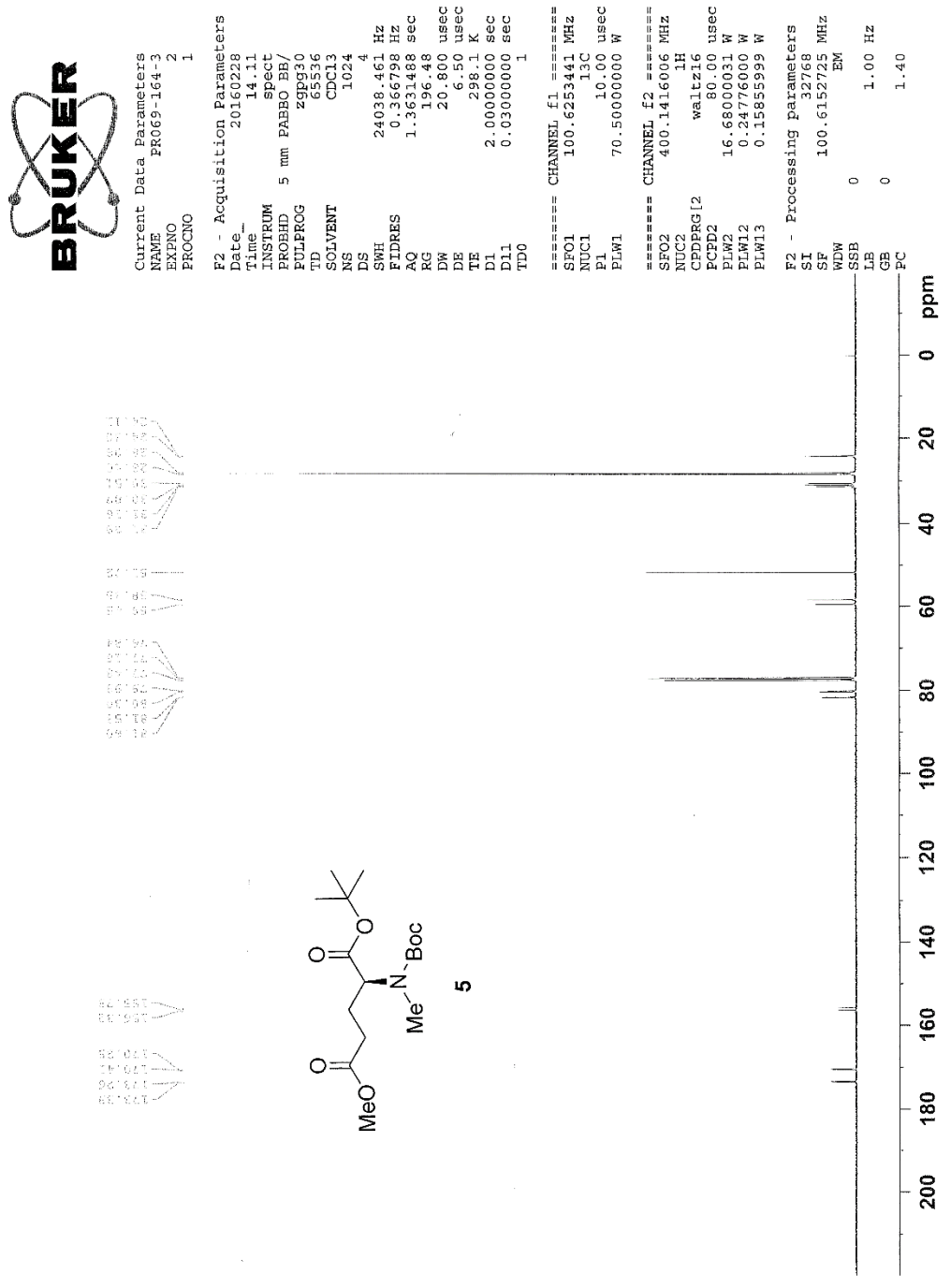
======== CHANNEL f1 ========

NUC1	1H
P1	6.68 usec
PL1	-2.00 dB
SFO1	400.1432011 MHz

F2 - Processing parameters

SI	32768
SF	400.1399975 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

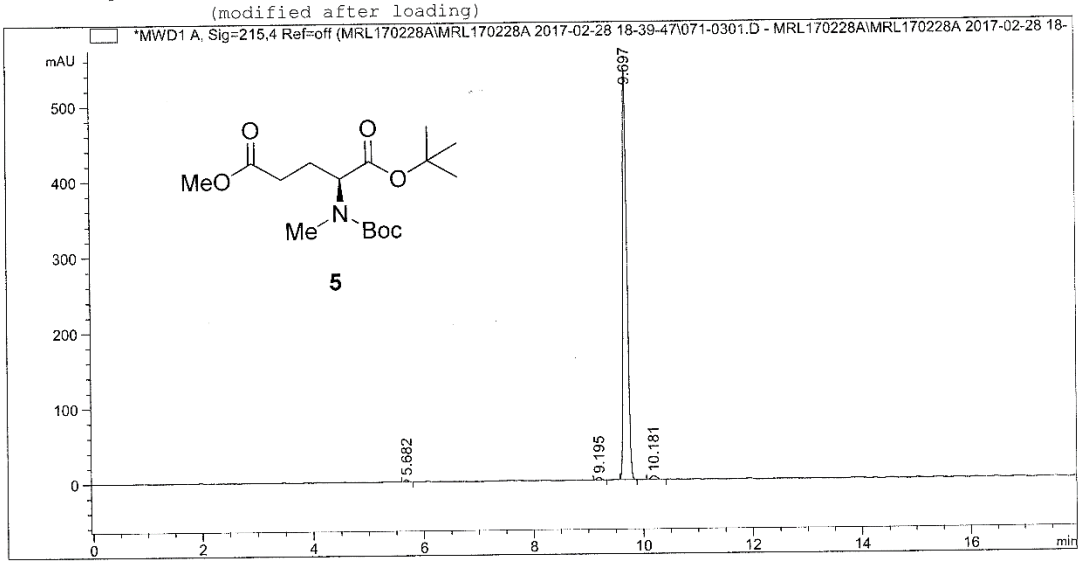
^{13}C NMR - Compound 5



Achiral HPLC - Compound 5

Data File C:\CHEM32\1\DATA\MRL170228A\MRL170228A 2017-02-28 18-39-47\071-0301.D
Sample Name: PR069-122-3

Acq. Operator	: MRL	Seq. Line	: 3
Acq. Instrument	: Instrument 1	Location	: Vial 71
Injection Date	: 02/28/17 7:24:45 PM	Inj	: 1
		Inj Volume	: 3.0 µl
Acq. Method	: C:\CHEM32\1\DATA\MRL170228A\MRL170228A 2017-02-28 18-39-47\SDAP3.M		
Last changed	: 02/28/17 6:37:32 PM by MRL		
Analysis Method	: C:\CHEM32\1\METHODS\SDAP3.M		
Last changed	: 03/01/17 10:16:40 AM by MRL (modified after loading)		



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: MWD1 A, Sig=215,4 Ref=off
Signal has been modified after loading from rawdata file!

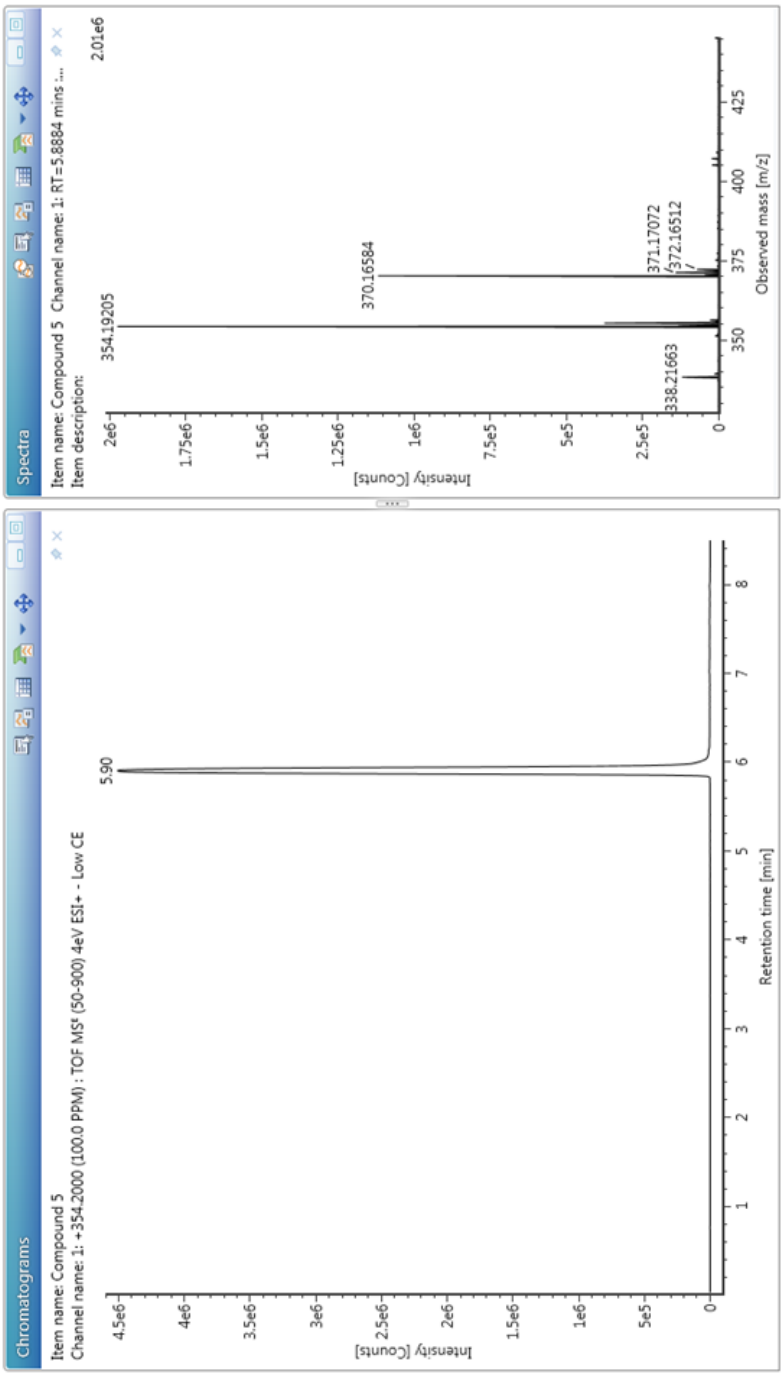
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.682	BB	0.0685	13.54313	3.13141	0.4709
2	9.195	BB	0.0767	18.68857	3.84549	0.6498
3	9.697	BB	0.0802	2808.89868	544.60742	97.6665
4	10.181	BB	0.1206	34.87980	4.69699	1.2128
Totals :				2876.01017	556.28132	

HRMS - Compound 5

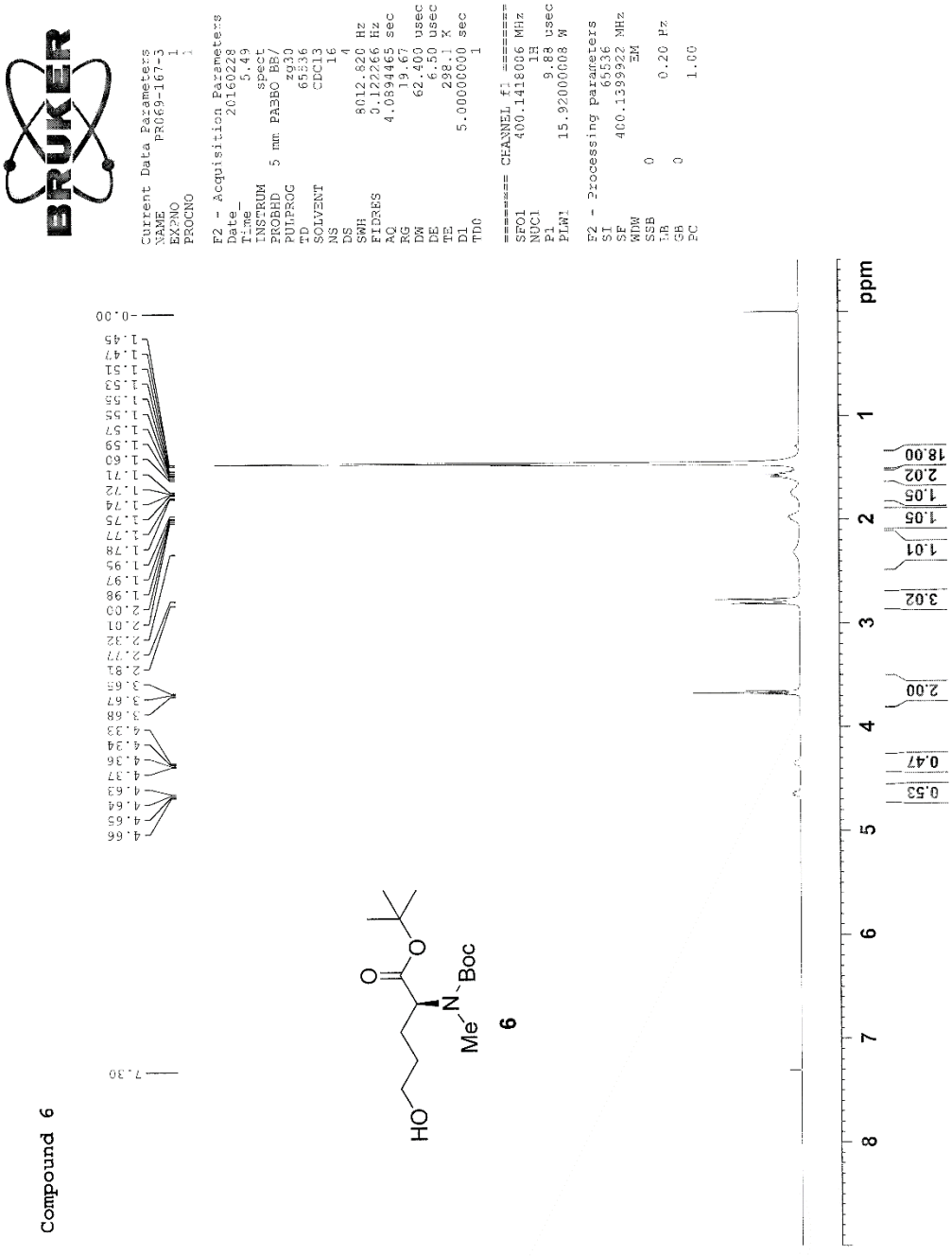
[Compound 5 +Na]$^+$

Calculated $[M+Na]^+$ for $C_{16}H_{29}NNaO_6^+$: 354.1887

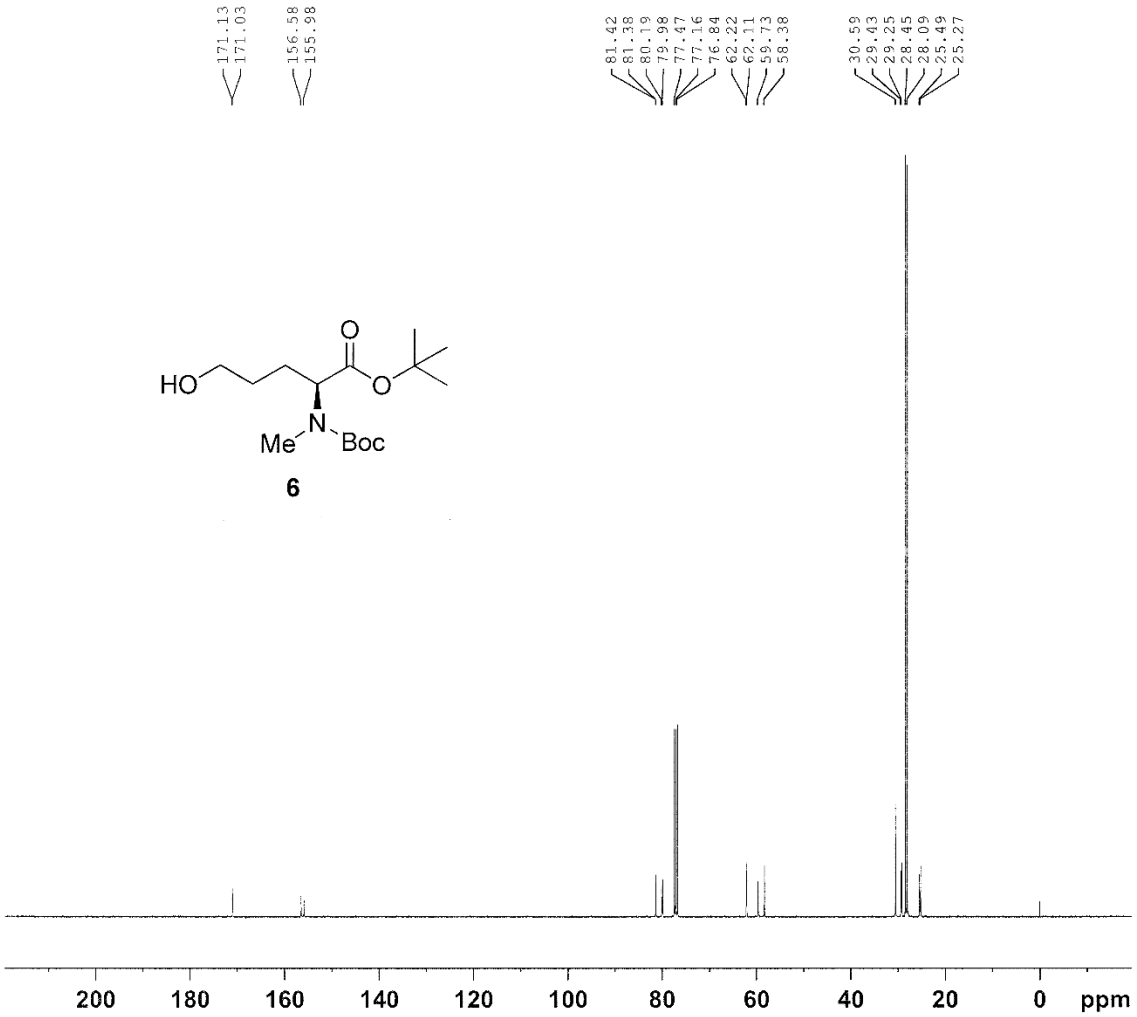
Found: 354.1920



^{1}H NMR - Compound 6



Compound 6




```
Current Data Parameters
NAME          PR069-167-3
EXPNO                   2
PROCNO                  1

F2 - Acquisition Parameters
Date_            20160228
Time                6.49
INSTRUM            spect
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ            1.3631488 sec
RG               196.48
DW               20.800 usec
DE                6.50 usec
TE                298.1 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1        100.6253441 MHz
NUC1               13C
P1               10.00 usec
PLW1        70.50000000 W

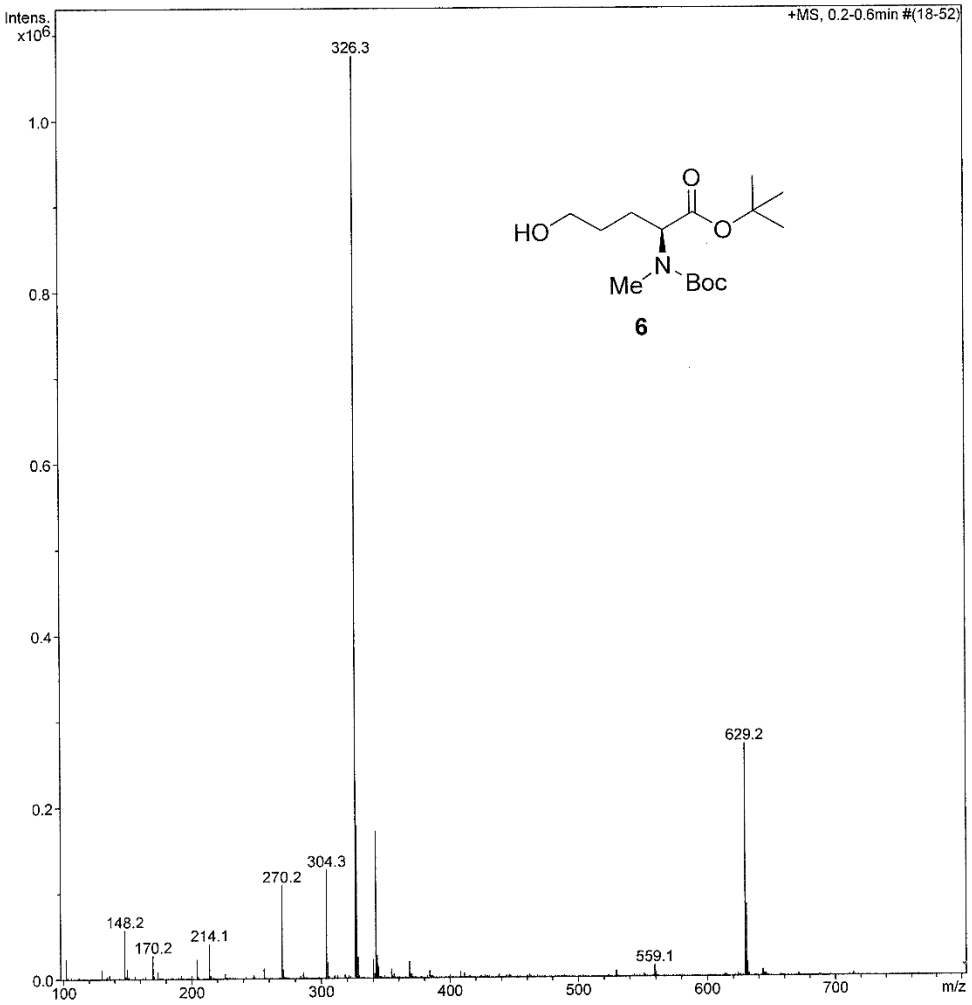
======== CHANNEL f2 ========
SFO2        400.1416006 MHz
NUC2               1H
CPDPRG[2        waltz16
PCPD2            80.00 usec
PLW2        16.68000031 W
PLW12        0.24776000 W
PLW13        0.15855999 W

F2 - Processing parameters
SI                32768
SF          100.6152792 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

MS - Compound 6

Display Report - Selected Window Selected Analysis

Analysis Name: 00000001.d **Instrument:** LC-MSD-Trap **Print Date:** 1/7/2015 4:12:32 AM
Method: ProcessResearch.m **Operator:** regis **Acq. Date:** 1/7/2015 4:06:07 AM
Sample Name: PR069-119-3
Analysis Info: Crude Oil

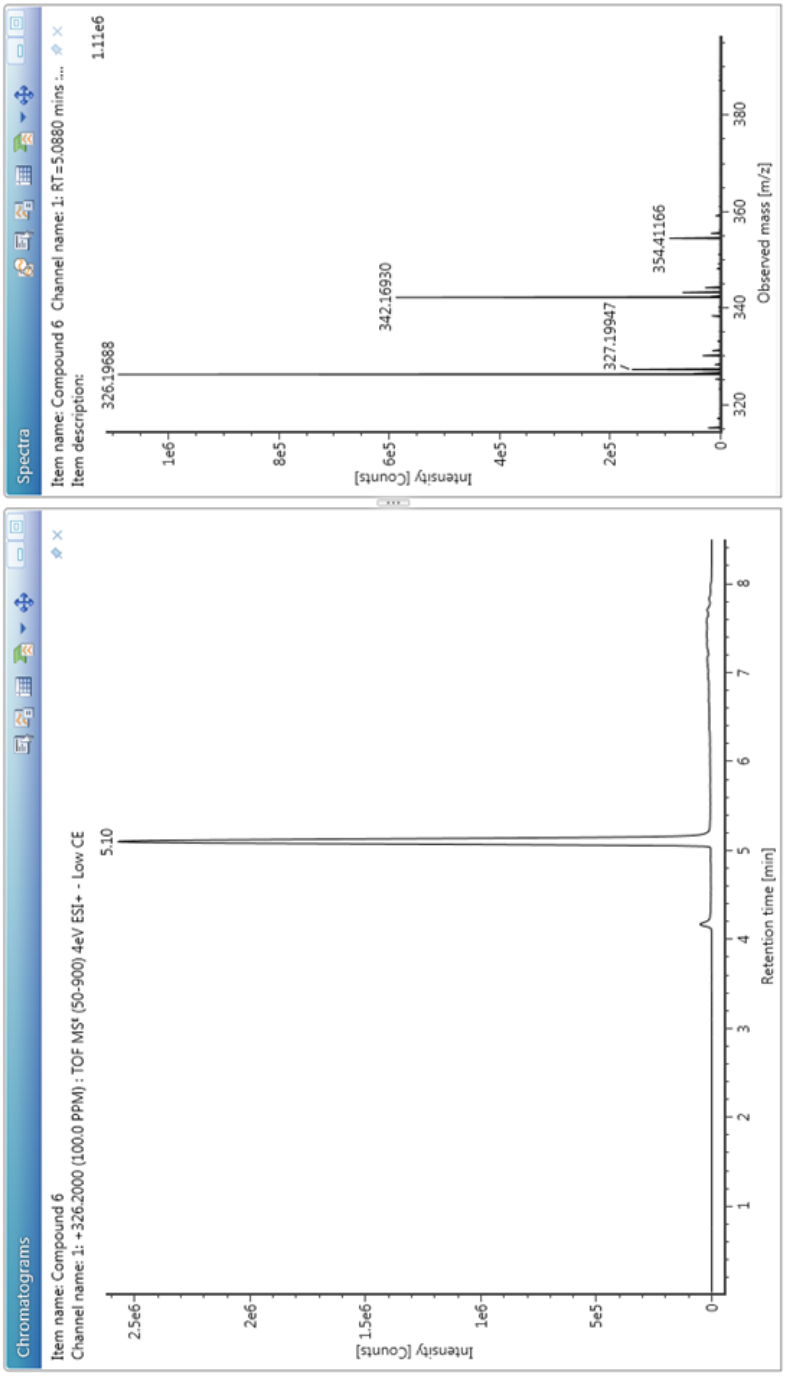


HRMS - Compound 6

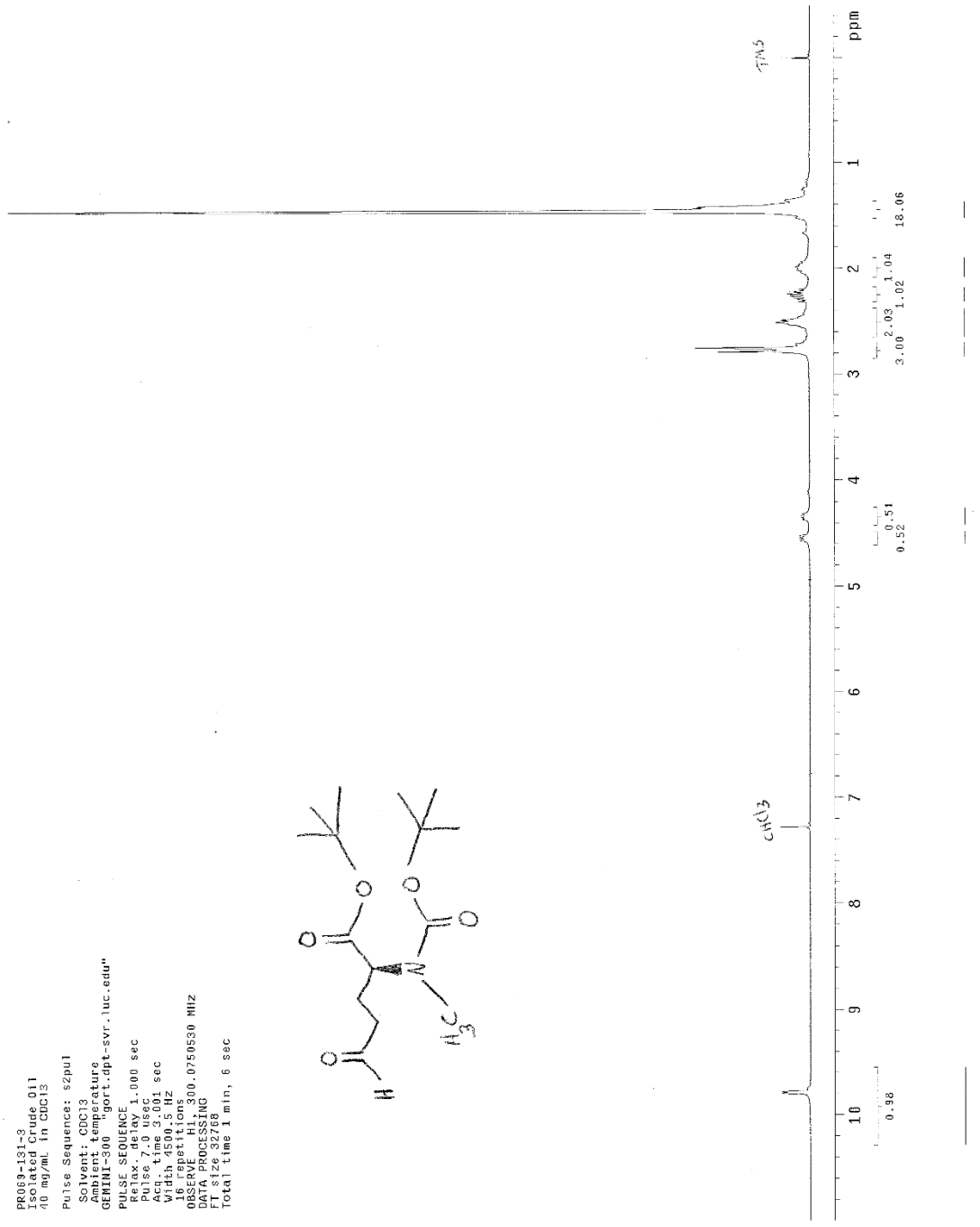
[Compound 6 +Na]$^+$

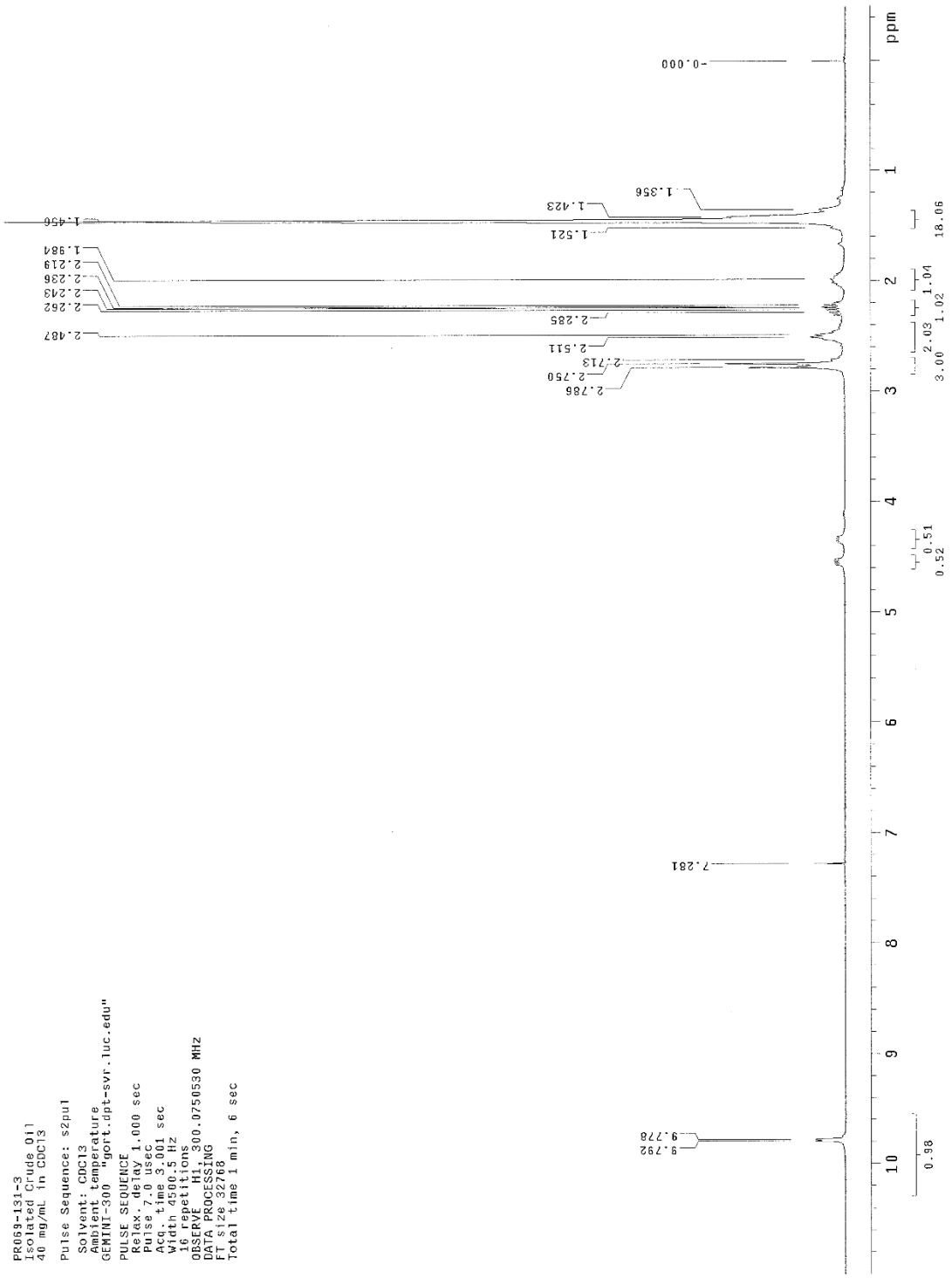
Calculated $[M+Na]^+$ for $C_{15}H_{29}NNaO_5^+$: 326.1938

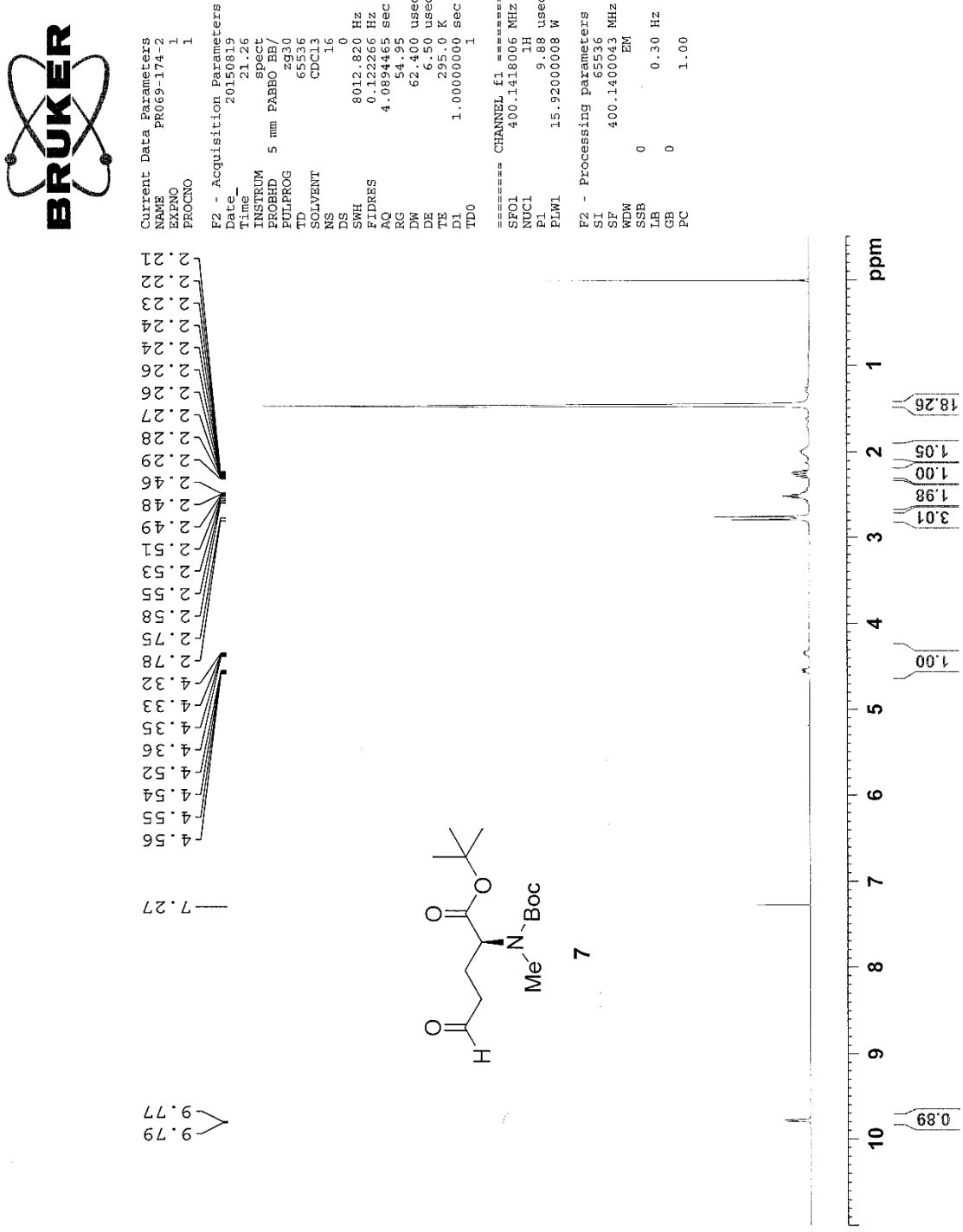
Found: 326.1969

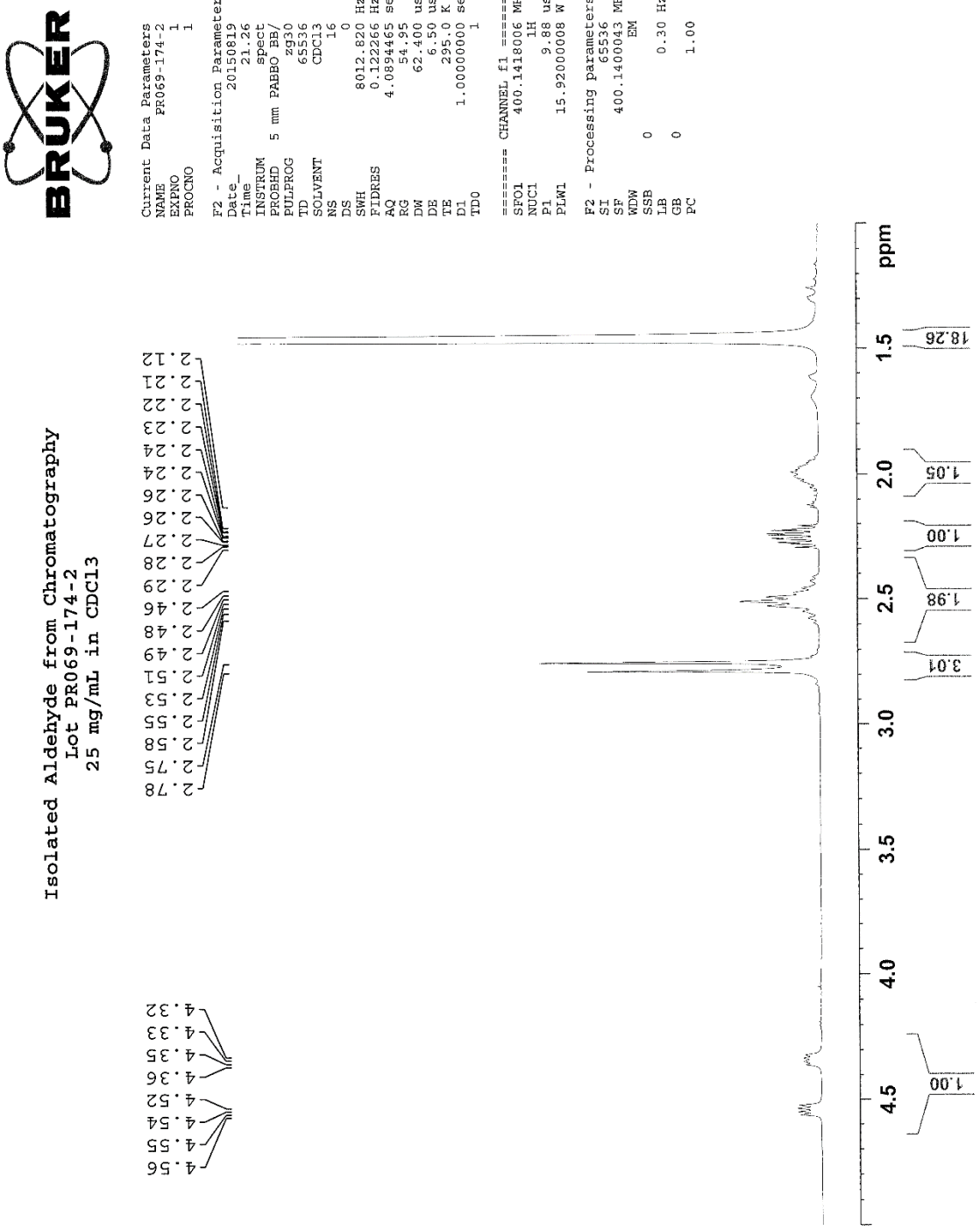


^{1}H NMR - Compound 7

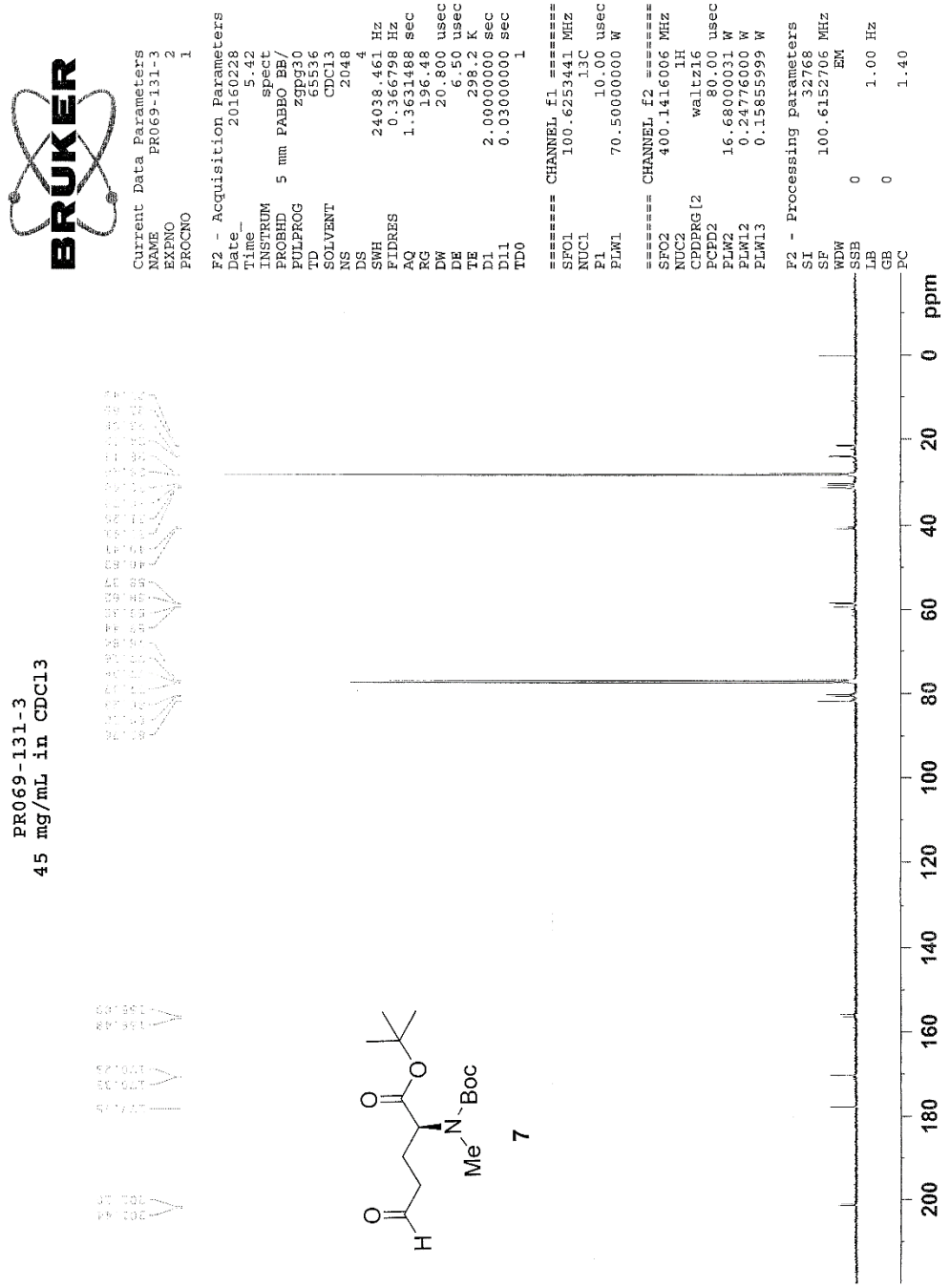


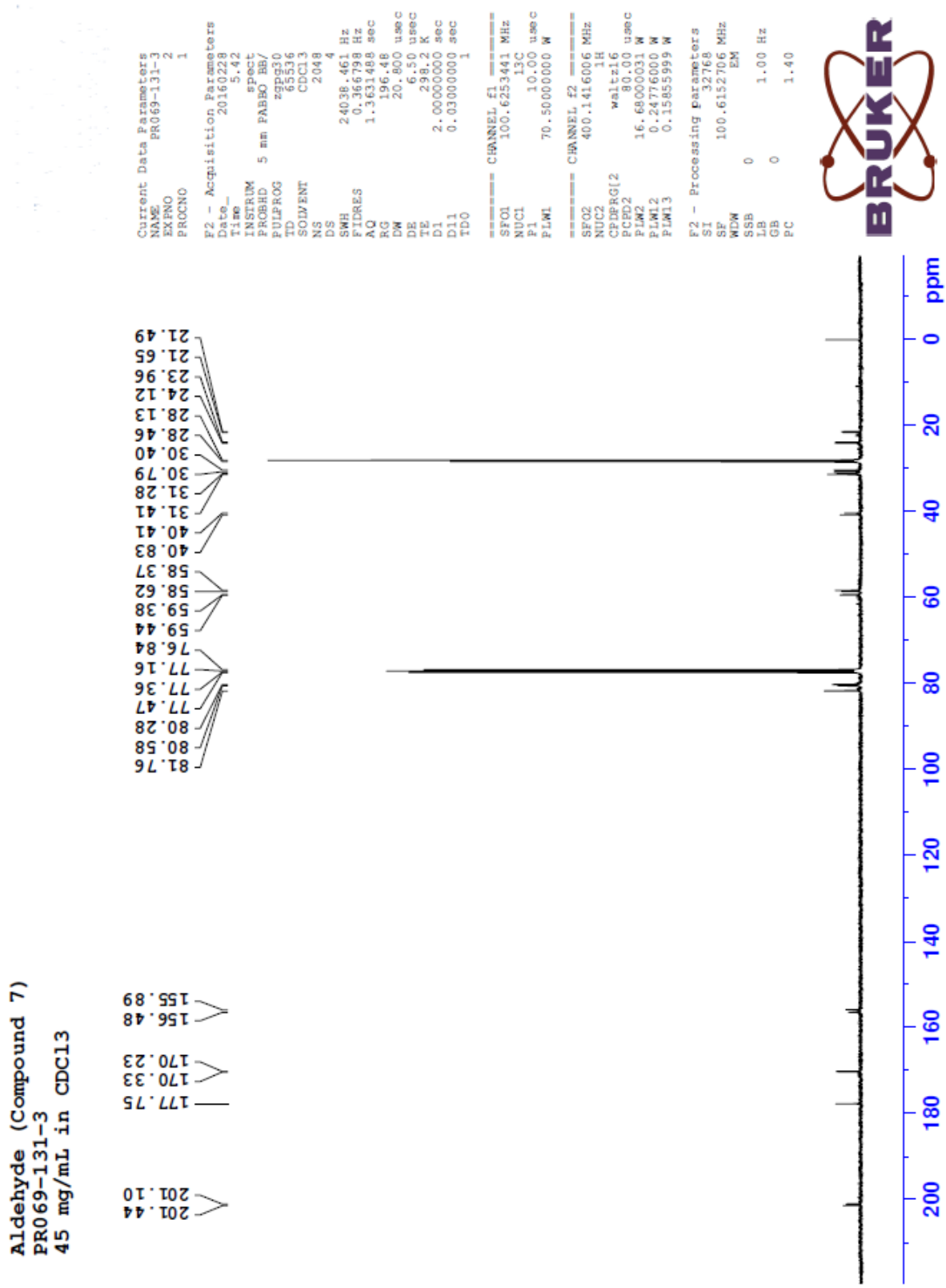

PR069-131-3
Isolated Crude Oil
40 mg/mL in CDCl3
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 7.0 usec
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750530 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
9.792
9.778
7.281
2.487
2.262
2.243
2.236
2.219
1.984
1.456
2.785
2.511
2.713
2.750
2.786
1.521
1.423
1.356
0.000
ppm
0.98
0.51
0.52
2.03
1.04
3.00
1.02
18.06


Current Data Parameters
NAME PR069-174-2
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20150819
Time 21.26
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 0
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 54.95
DW 62.400 usec
DE 6.50 usec
TE 295.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1418006 MHz
NUC1 1H
P1 9.88 usec
PLW1 15.92000008 W
F2 - Processing parameters
SI 65536
SF 400.1400043 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
2.21
2.22
2.23
2.24
2.24
2.26
2.26
2.27
2.28
2.29
2.46
2.48
2.49
2.51
2.53
2.55
2.58
2.75
2.78
4.32
4.33
4.35
4.36
4.52
4.54
4.55
4.56
7.27
9.77
9.79
ppm
18.26
1.05
1.00
1.98
3.01
1.00
0.89
Me
N
Boc
O
O
O
H
7

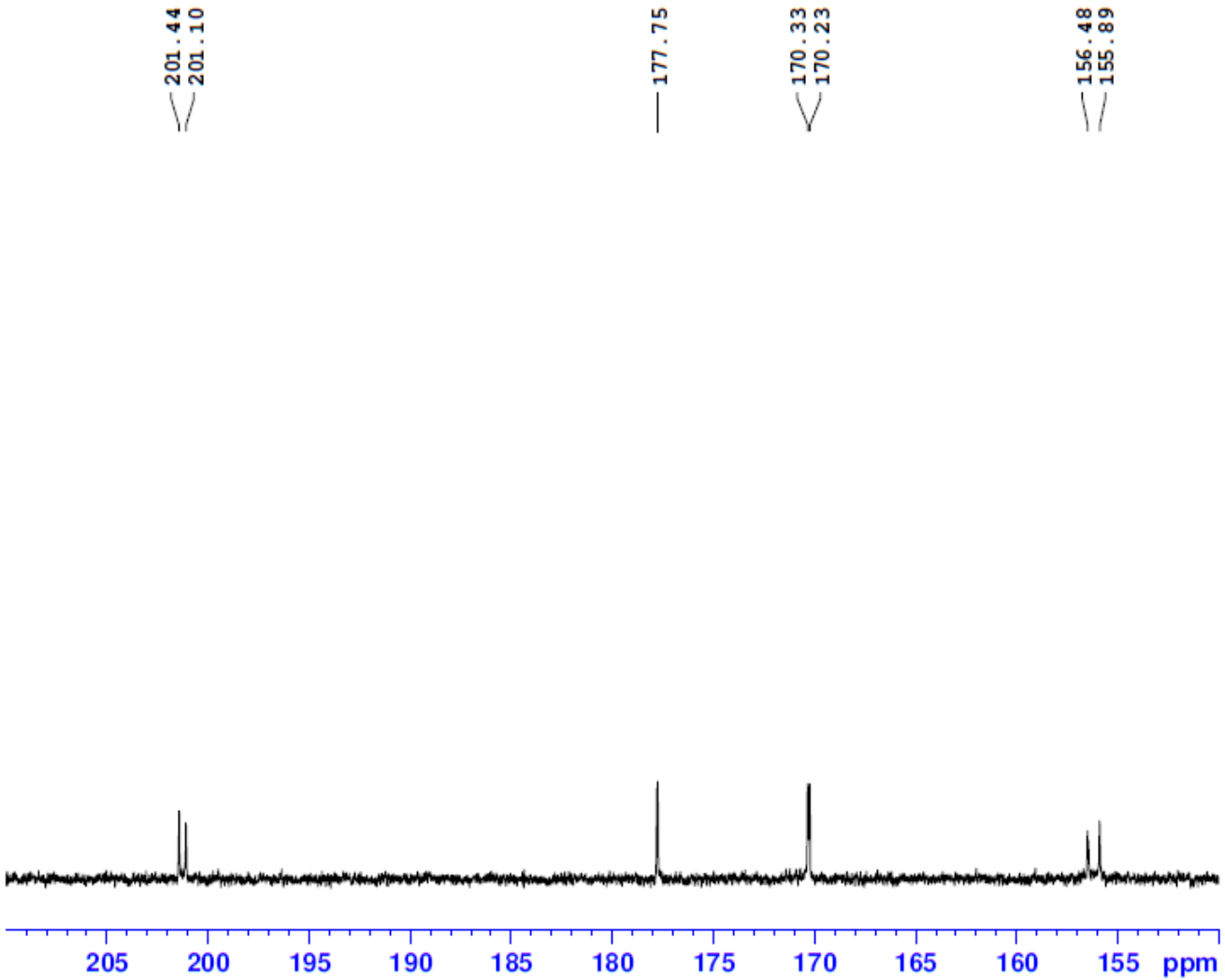

Isolated Aldehyde from Chromatography
Lot PR069-174-2
25 mg/mL in CDCl3
Current Data Parameters
NAME PR069-174-2
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20150819
Time_ 21.26
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 0
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 54.95
DW 62.400 usec
DE 6.50 usec
TE 295.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1418006 MHz
NUC1 1H
P1 9.88 usec
PLW1 15.92000008 W
F2 - Processing parameters
SI 65536
SF 400.1400043 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
2.12
2.21
2.22
2.23
2.24
2.24
2.26
2.26
2.27
2.28
2.29
2.46
2.48
2.49
2.51
2.53
2.55
2.58
2.75
2.78
4.32
4.33
4.35
4.36
4.52
4.54
4.55
4.56
ppm
18.26
1.05
1.00
1.98
3.01
1.00

^{13}C NMR - Compound 7




Aldehyde (Compound 7)
PR069-131-3
45 mg/mL in CDCl3
201.44
201.10
177.75
170.33
170.23
156.48
155.89
81.76
80.58
80.28
77.47
77.36
77.16
76.84
59.44
59.38
58.62
58.37
40.83
40.41
31.41
31.28
30.79
30.40
28.46
28.13
24.12
23.96
21.65
21.49
Current Data Parameters
NAME PR069-131-3
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160228
Time 5.42
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 2048
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 196.48
DW 20.800 usec
DE 6.50 usec
TE 298.2 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6253441 MHz
NUC1 13C
P1 10.00 usec
PLW1 70.50000000 W
======== CHANNEL f2 ========
SFO2 400.1416006 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 16.68000031 W
PLW12 0.24776000 W
PLW13 0.15855999 W
F2 - Processing parameters
SI 32768
SF 100.6152706 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
BRUKER
200
180
160
140
120
100
80
60
40
20
0
ppm

Aldehyde (Compound 7)
PR069-131-3
45 mg/mL in CDC13




```
Current Data Parameters
NAME          PR069-131-3
EXPNO                   2
PROCNO                  1

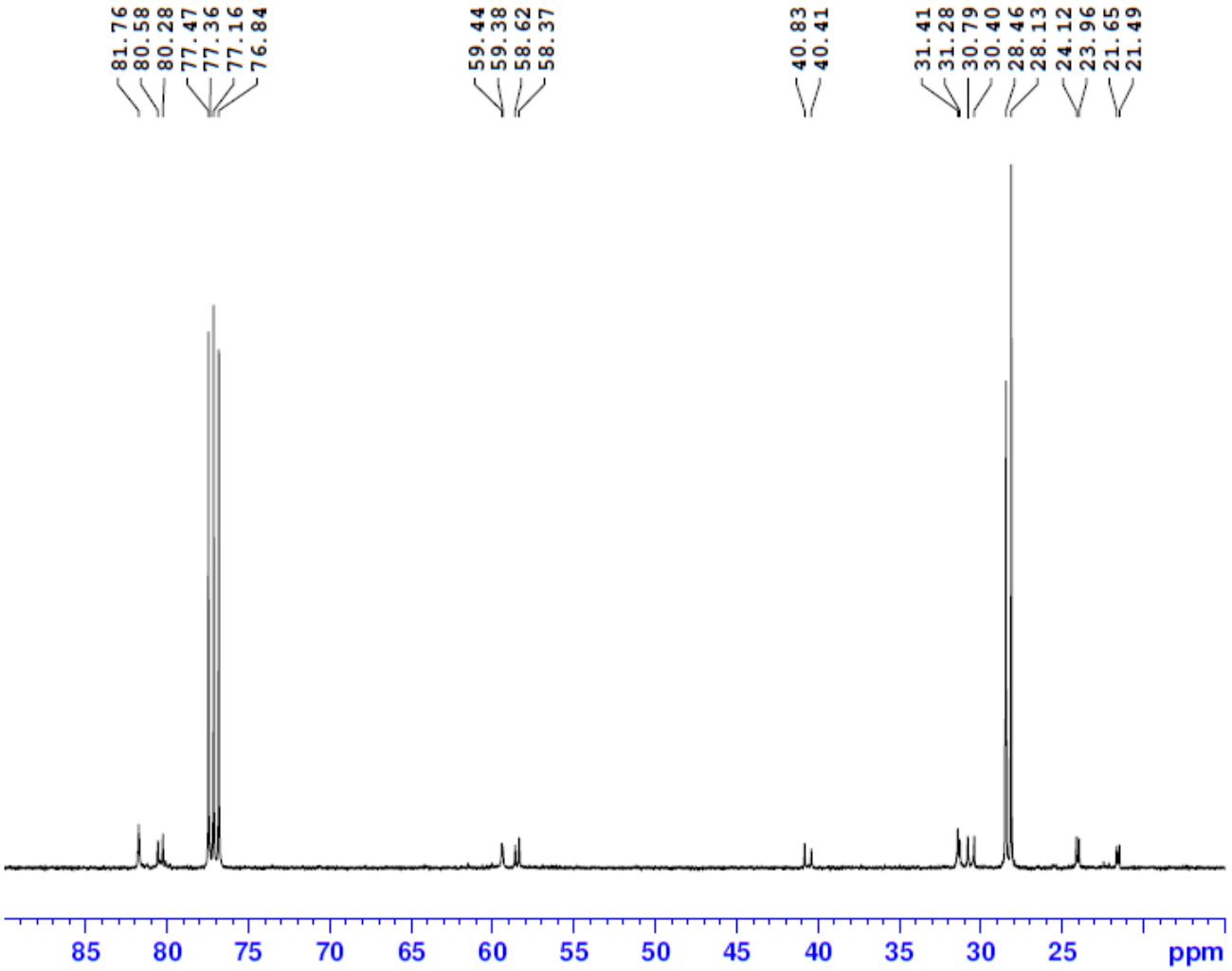
F2 - Acquisition Parameters
Date_          20160228
Time               5.42
INSTRUM           spect
PROBHD   5 mm PABBO BB/
PULPROG         zgpg30
TD               65536
SOLVENT           CDC13
NS                 2048
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ             1.3631488 sec
RG                196.48
DW                20.800 usec
DE                 6.50 usec
TE                298.2 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

======= CHANNEL f1 =======
SFO1        100.6253441 MHz
NUC1                13C
P1                10.00 usec
PLW1         70.50000000 W

======= CHANNEL f2 =======
SFO2        400.1416006 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2         16.68000031 W
PLW12         0.24776000 W
PLW13         0.15855999 W

F2 - Processing parameters
SI                 32768
SF          100.6152706 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

Aldehyde (Compound 7)
PR069-131-3
45 mg/mL in CDCl3




```
Current Data Parameters
NAME          PR069-131-3
EXPNO                   2
PROCNO                  1

F2 - Acquisition Parameters
Date_            20160228
Time                5.42
INSTRUM            spect
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 2048
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ             1.3631488 sec
RG                196.48
DW                20.800 usec
DE                 6.50 usec
TE                298.2 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======= CHANNEL f1 =======
SFO1         100.6253441 MHz
NUC1                13C
P1                10.00 usec
PLW1         70.50000000 W

======= CHANNEL f2 =======
SFO2         400.1416006 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2         16.68000031 W
PLW12         0.24776000 W
PLW13         0.15855999 W

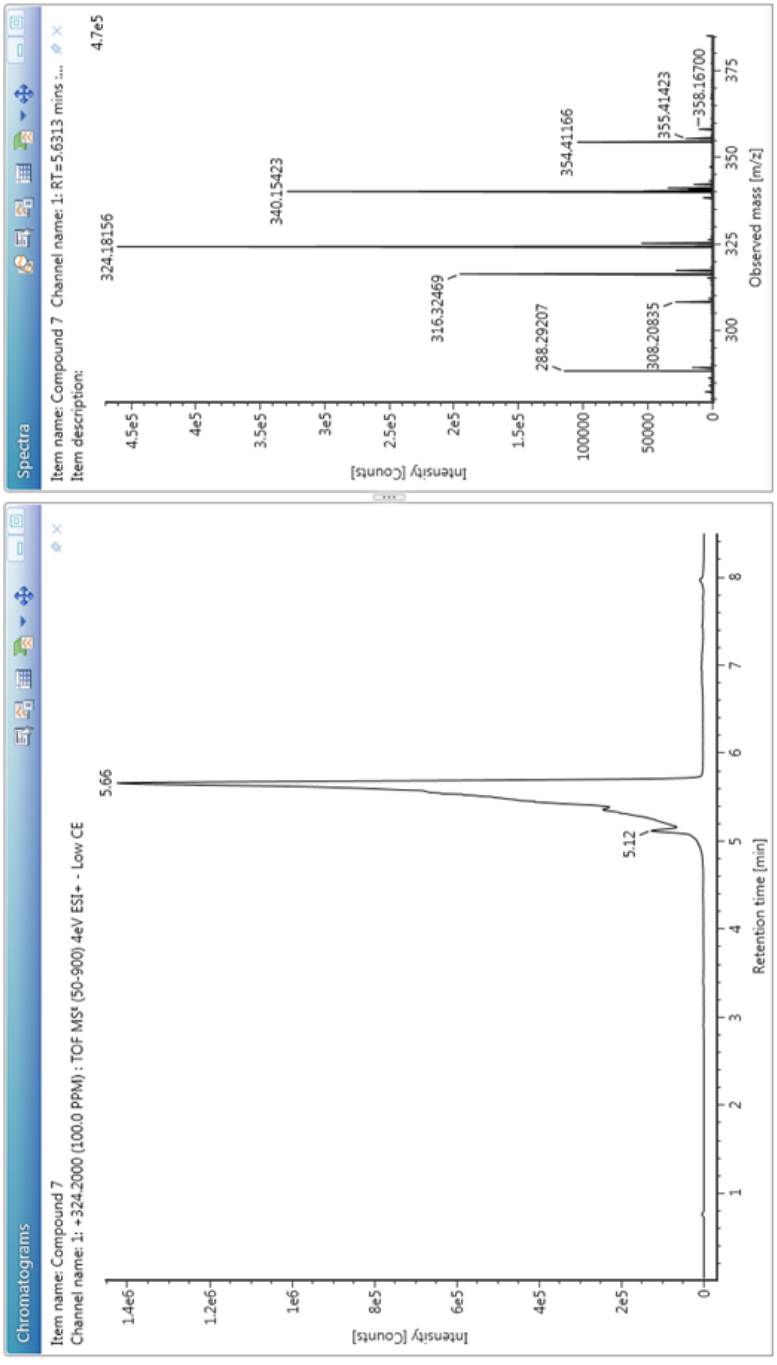
F2 - Processing parameters
SI                 32768
SF           100.6152706 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

HRMS - Compound 7

$[Compound\ 7 + Na]^+$

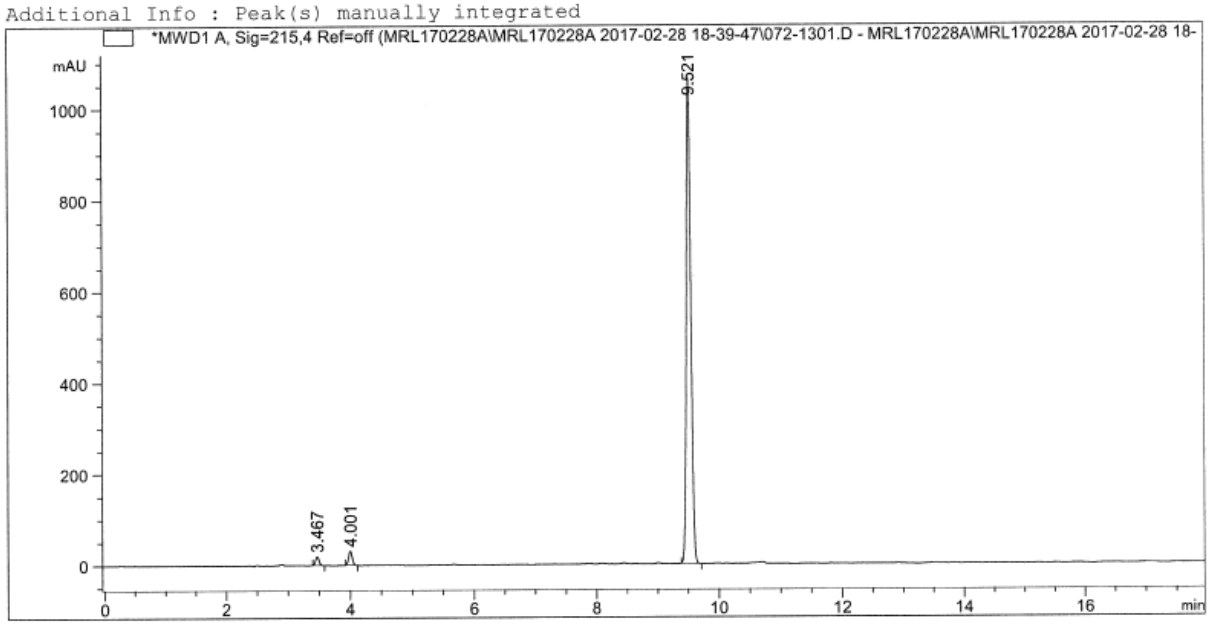
Calculated $[M+Na]^+$ for $C_{15}H_{27}NNaO_5^+$: 324.1781

Found: 324.1816



Achiral HPLC - Compound 8a

```
=====================================================================
Acq. Operator   : MRL                          Seq. Line :  13
Acq. Instrument : Instrument 1                  Location : Vial 72
Injection Date  : 02/28/17  11:01:34 PM              Inj :   1
                                               Inj Volume : 3.0 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 1.0 µl
Acq. Method     : C:\CHEM32\1\DATA\MRL170228A\MRL170228A 2017-02-28 18-39-47\SDAP3.M
Last changed    : 02/28/17  6:37:32 PM by MRL
Analysis Method : C:\CHEM32\1\METHODS\SDAP3.M
Last changed    : 03/01/17  10:16:40 AM by MRL
                  (modified after loading)
Additional Info : Peak(s) manually integrated
```



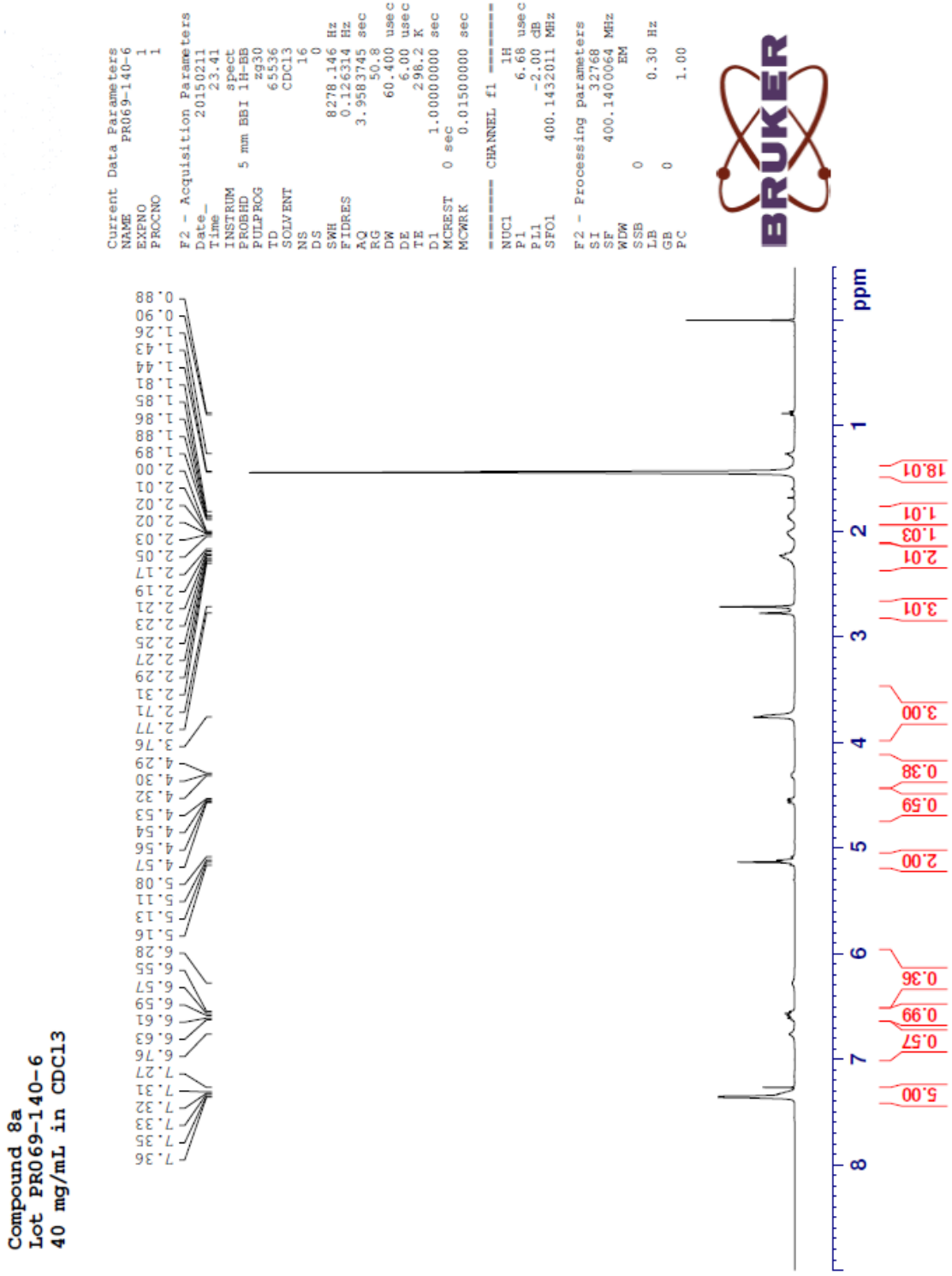

```
=====================================================================
                        Area Percent Report
=====================================================================

Signal 1: MWD1 A, Sig=215,4 Ref=off
 Signal has been modified after loading from rawdata file!

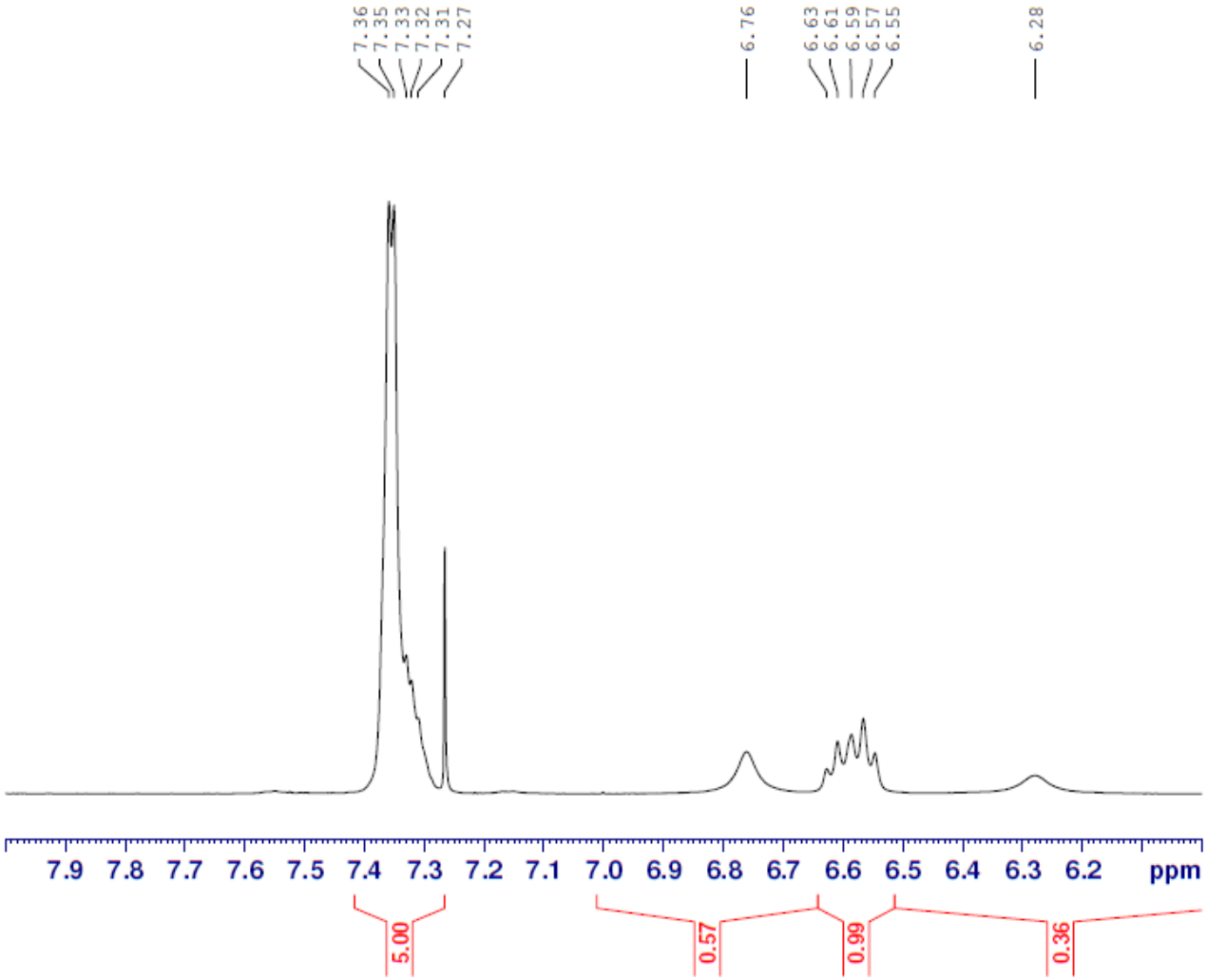
Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.467 BB    0.0602   76.39406   19.28664   1.3561
   2   4.001 BB    0.0629  126.81283   31.52904   2.2512
   3   9.521 BB    0.0794 5429.95850 1065.78503  96.3927

Totals :                   5633.16538 1116.60071
=====================================================================
```

^{1}H NMR - Compound 8a



Compound 8a
Lot PR069-140-6
40 mg/mL in CDCl3

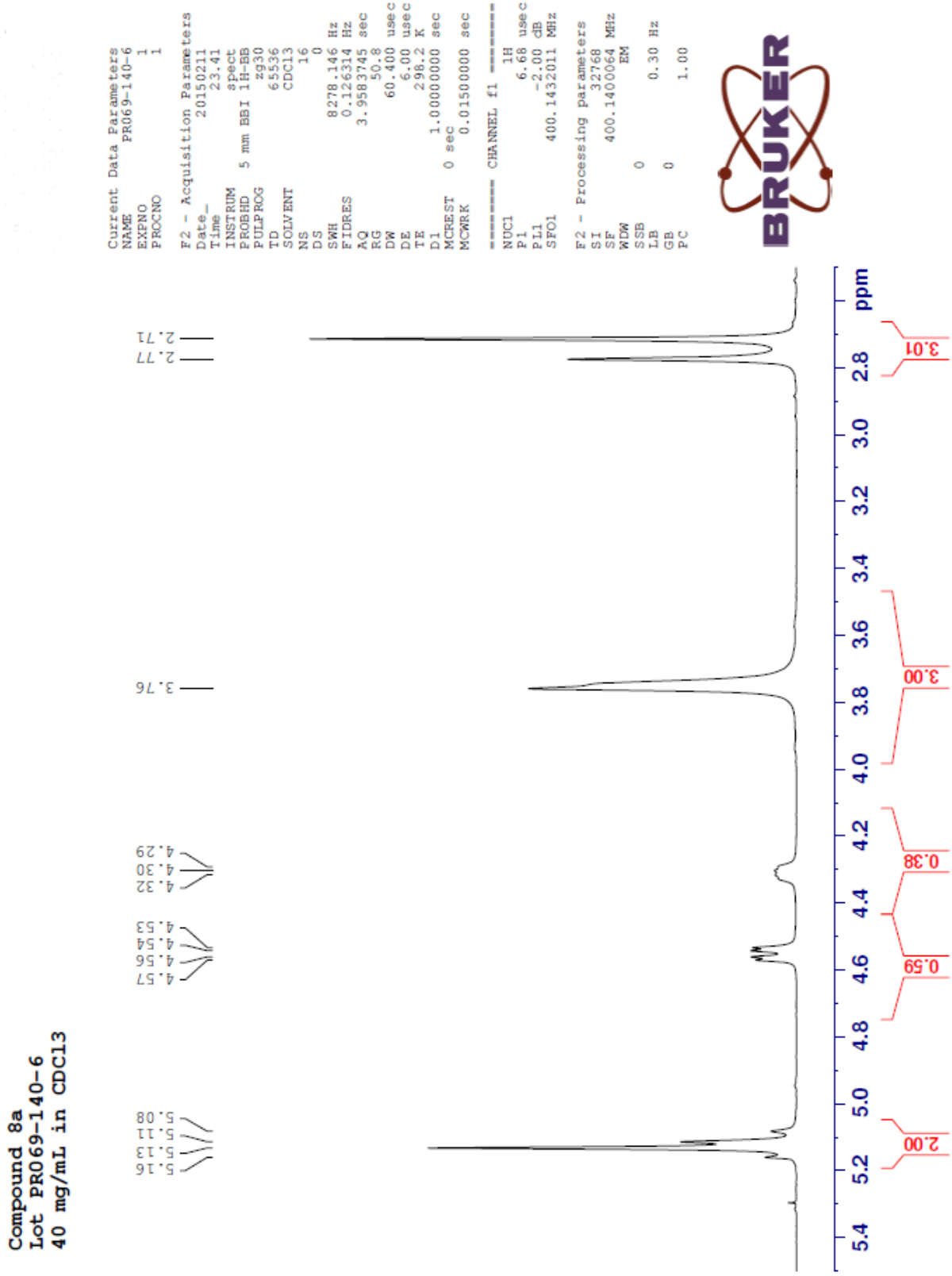



```
Current Data Parameters
NAME          PR069-140-6
EXPNO                   1
PROCNO                  1

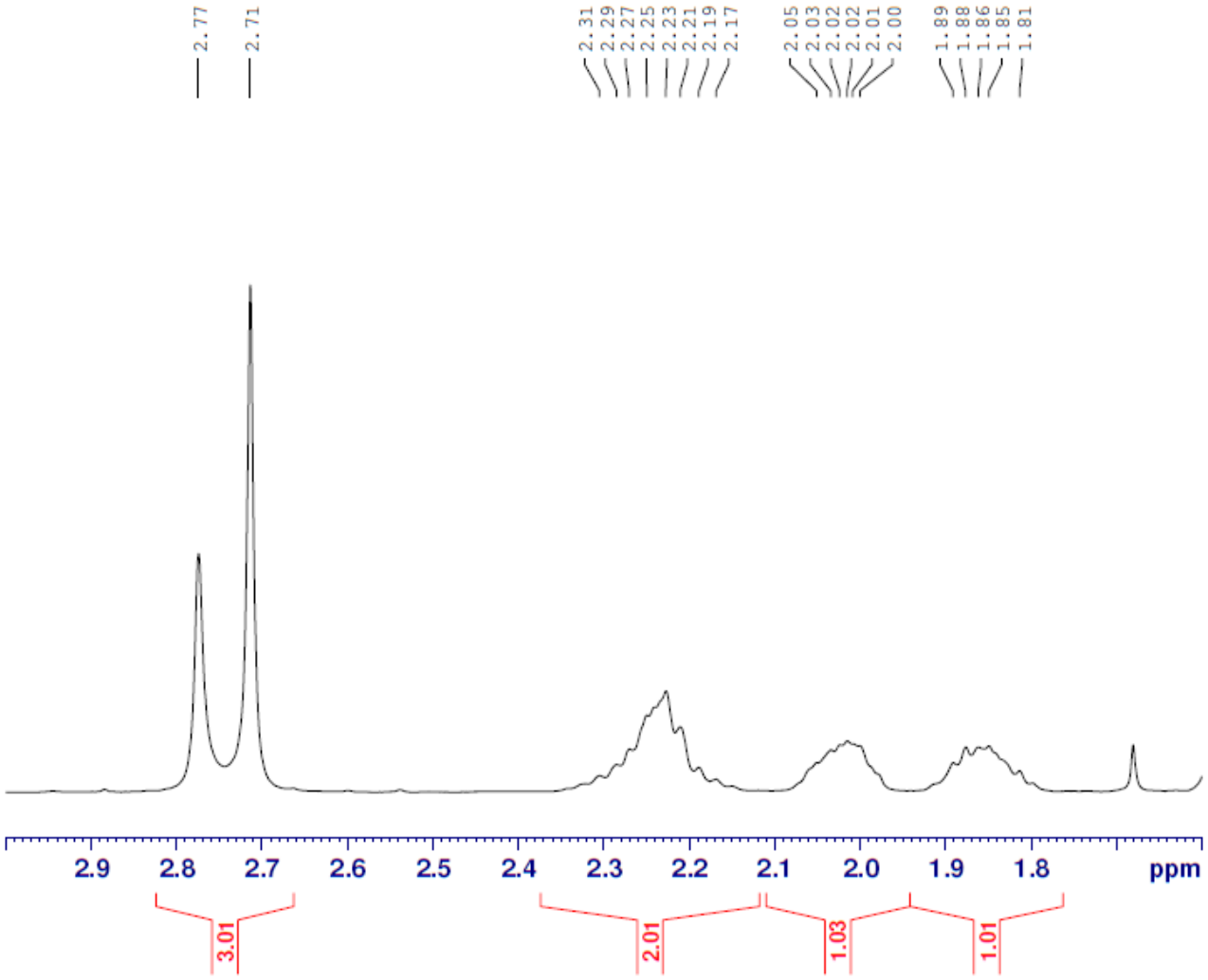
F2 - Acquisition Parameters
Date_            20150211
Time                23.41
INSTRUM             spect
PROBHD   5 mm BBI 1H-BB
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     0
SWH             8278.146 Hz
FIDRES          0.126314 Hz
AQ             3.9583745 sec
RG                  50.8
DW                60.400 usec
DE                 6.00 usec
TE                298.2 K
D1           1.00000000 sec
MCREST   0 sec
MCWRK        0.01500000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                   6.68 usec
PL1                 -2.00 dB
SFO1         400.1432011 MHz

F2 - Processing parameters
SI                 32768
SF           400.1400064 MHz
WDW                   EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```


Compound 8a
Lot PR069-140-6
40 mg/mL in CDCl3
Current Data Parameters
NAME PR069-140-6
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20150211
Time 23.41
INSTRUM spect
PROBHD 5 mm BBI 1H-BB
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 0
SWH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9583745 sec
RG 50.8
DW 60.400 usec
DE 6.00 usec
TE 298.2 K
D1 1.00000000 sec
MCREST 0 sec 0.01500000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 6.68 usec
PL1 -2.00 dB
SFO1 400.1432011 MHz
F2 - Processing parameters
SI 32768
SF 400.1400064 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
BRUKER
2.71
2.77
3.76
4.29
4.30
4.32
4.53
4.54
4.56
4.57
5.08
5.11
5.13
5.16
ppm
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
3.01
3.00
0.38
0.59
2.00

Compound 8a
Lot PR069-140-6
40 mg/mL in CDCl3



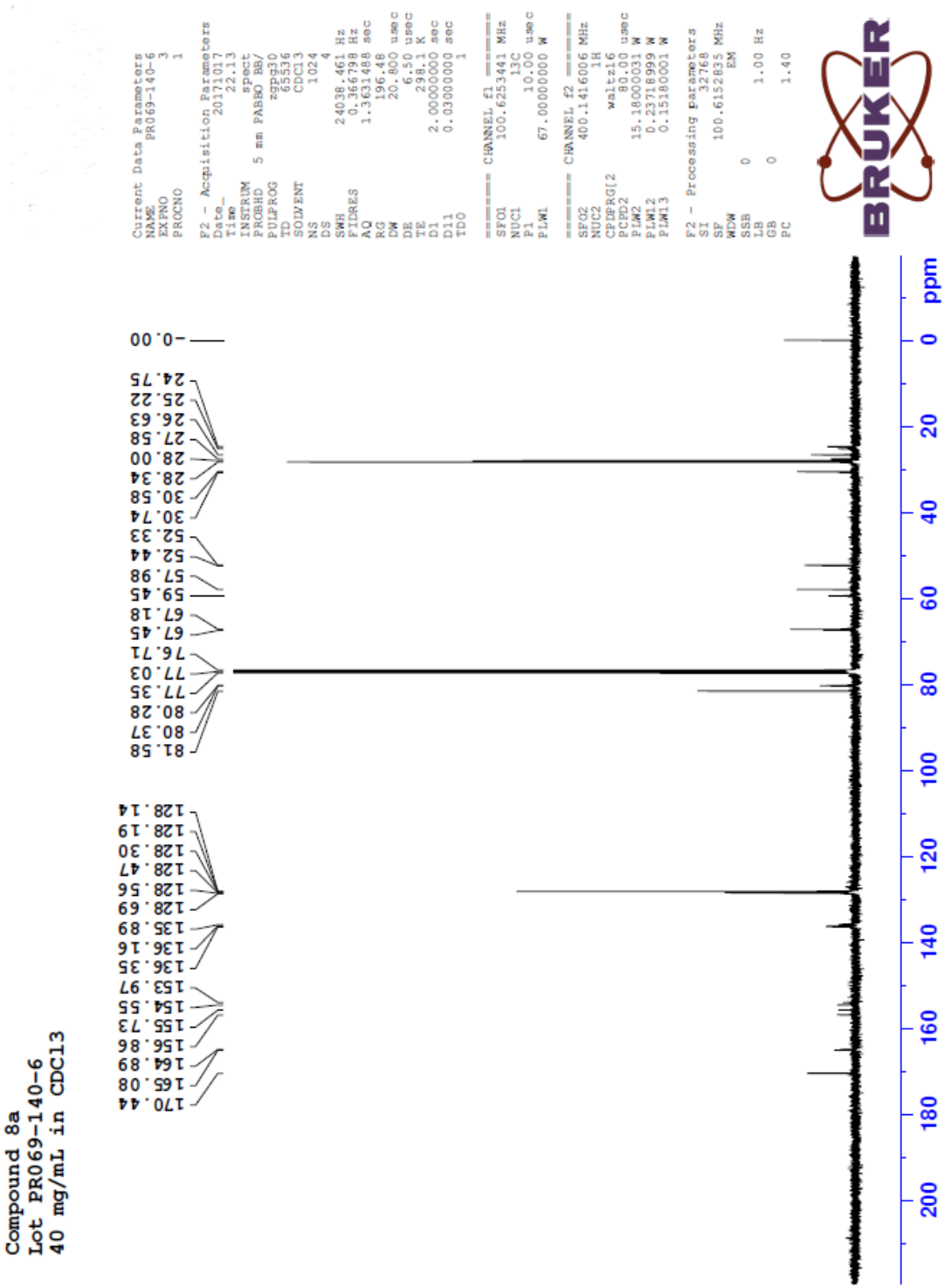

```
Current Data Parameters
NAME         PR069-140-6
EXPNO                  1
PROCNO                 1

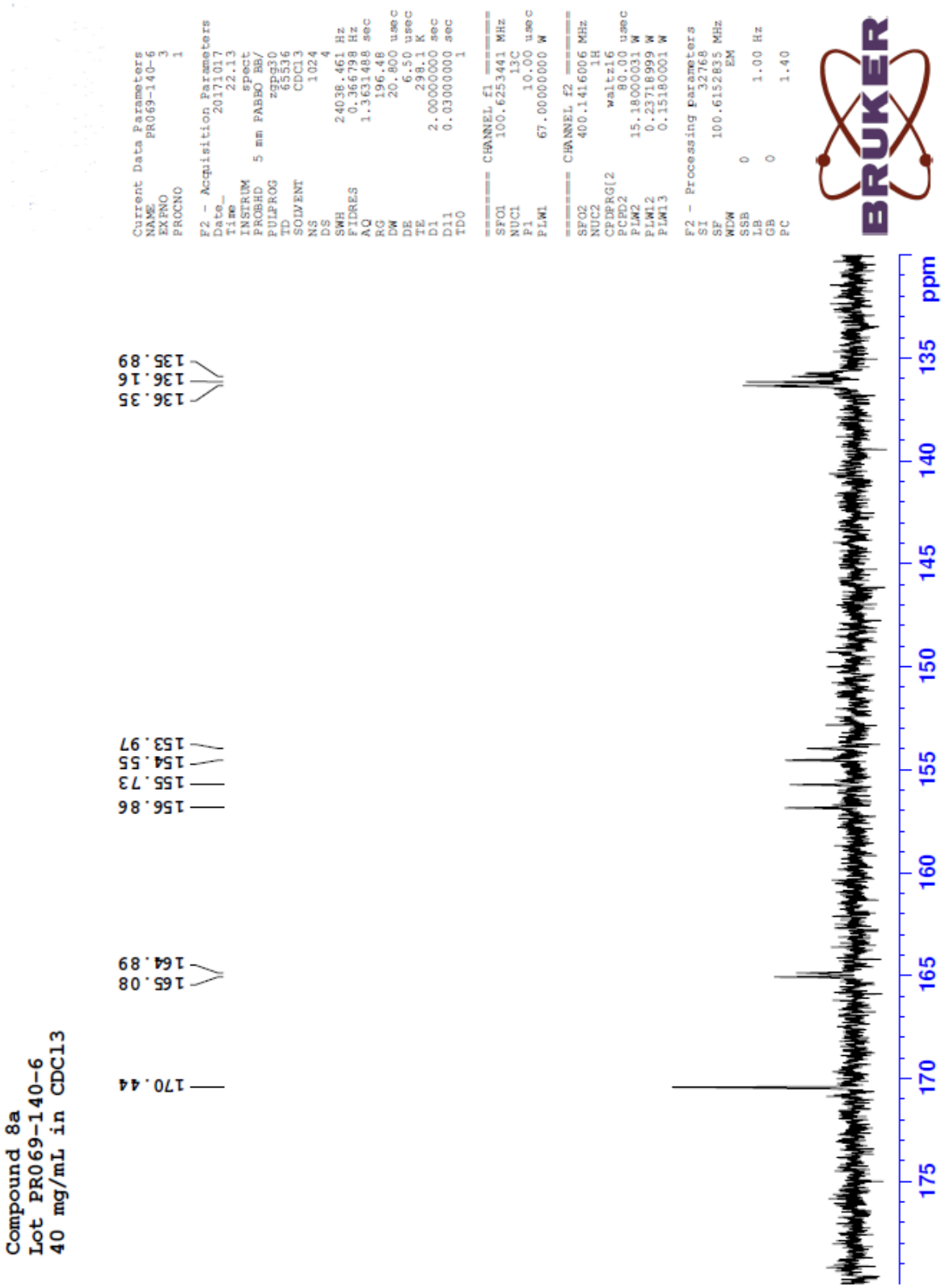
F2 - Acquisition Parameters
Date_           20150211
Time               23.41
INSTRUM            spect
PROBHD   5 mm BBI 1H-BB
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     0
SWH             8278.146 Hz
FIDRES          0.126314 Hz
AQ              3.9583745 sec
RG                  50.8
DW                60.400 usec
DE                 6.00 usec
TE                298.2 K
D1           1.00000000 sec
MCREST   0 sec
MCWRK         0.01500000 sec

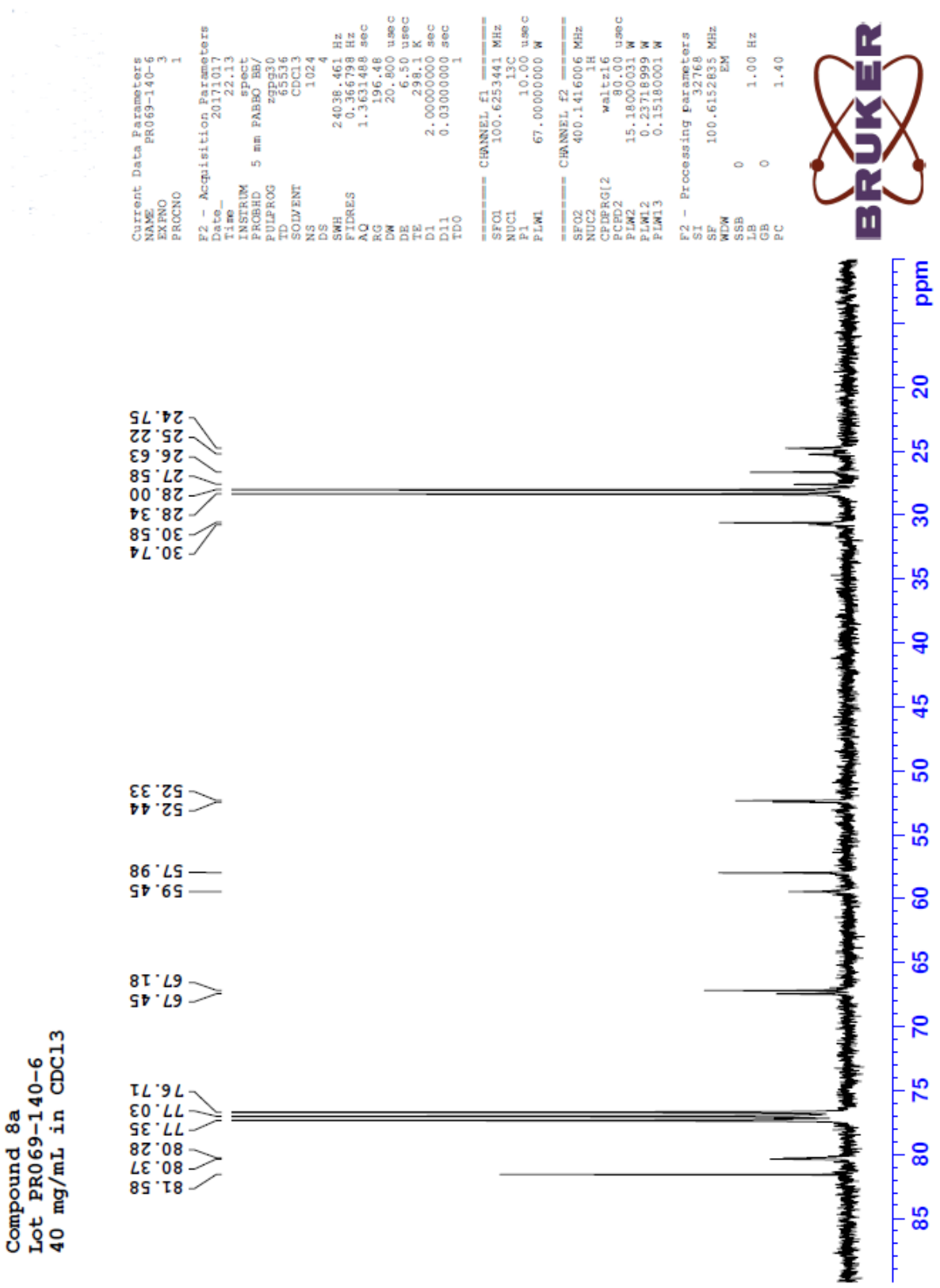
======== CHANNEL f1 ========
NUC1                  1H
P1                 6.68 usec
PL1               -2.00 dB
SFO1         400.1432011 MHz

F2 - Processing parameters
SI                 32768
SF           400.1400064 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

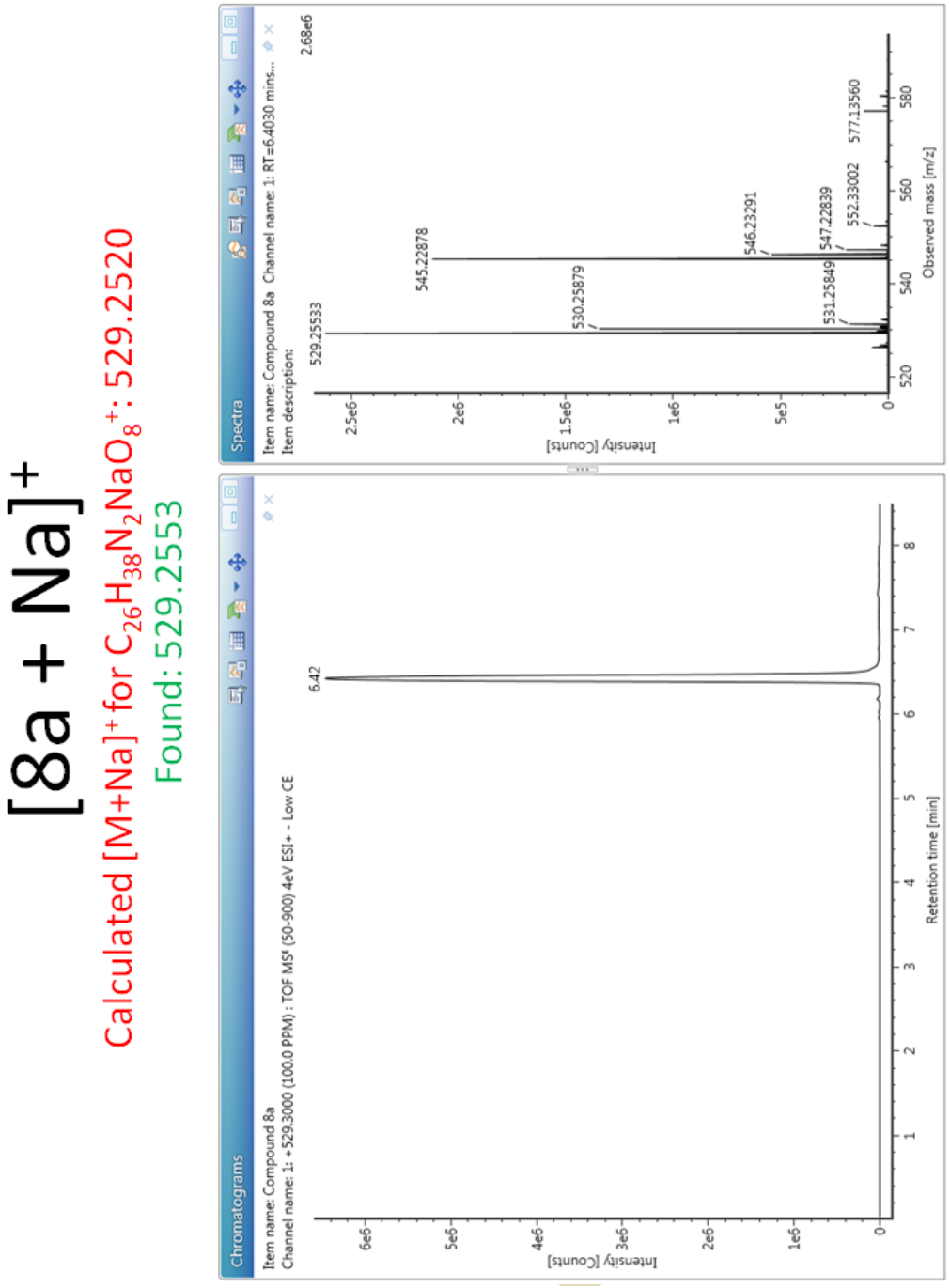
^{13}C NMR - Compound 8a




Compound 8a
Lot PR069-140-6
40 mg/mL in CDCl3
170.44
165.08
164.89
156.86
155.73
154.55
153.97
136.35
136.16
135.89
Current Data Parameters
NAME PR069-140-6
EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
Date_ 20171017
Time 22.13
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 1024
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 196.48
DW 20.800 usec
DE 6.50 usec
TE 298.1 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
CHANNEL f1
SFO1 100.6253441 MHz
NUC1 13C
P1 10.00 usec
PLW1 67.00000000 W
CHANNEL f2
SFO2 400.1416006 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 15.18000031 W
PLW12 0.23718999 W
PLW13 0.15180001 W
F2 - Processing parameters
SI 32768
SF 100.6152835 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
BRUKER
175
170
165
160
155
150
145
140
135
ppm

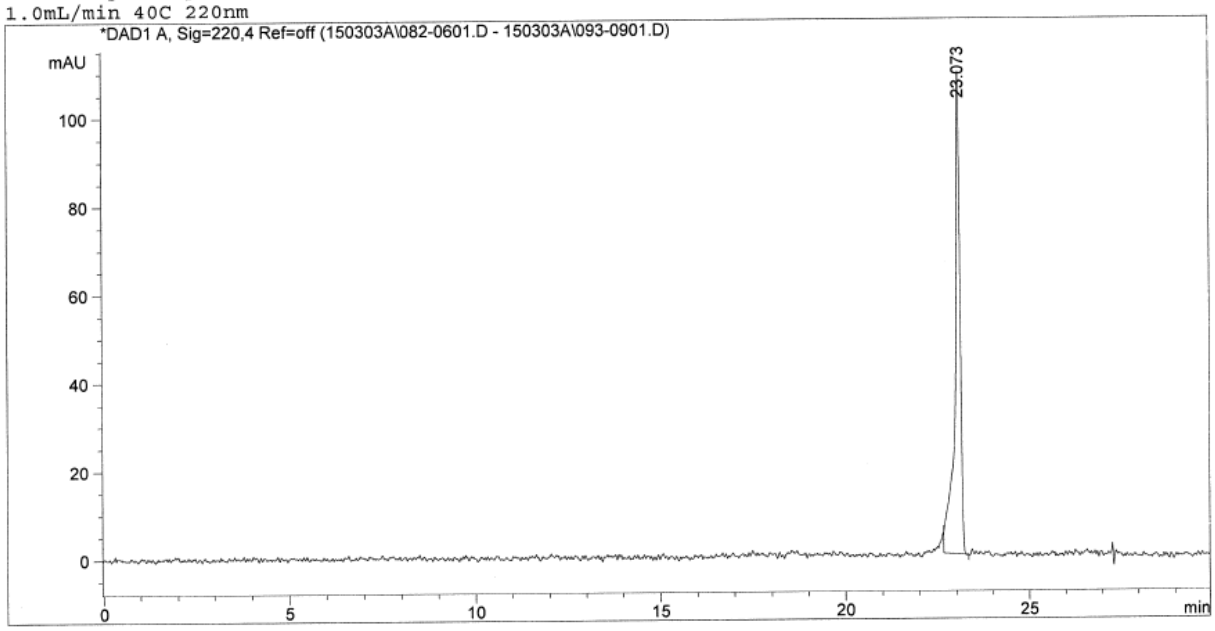

Compound 8a
Lot PR069-140-6
40 mg/mL in CDC13
Current Data Parameters
NAME PR069-140-6
EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
Date_ 20171017
Time 22.13
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT CDC13
NS 1024
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 196.48
DW 20.800 usec
DE 6.50 usec
TE 298.1 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6253441 MHz
NUC1 13C
P1 10.00 usec
PLW1 67.00000000 W
======== CHANNEL f2 ========
SFO2 400.1416006 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 15.18000031 W
PLW12 0.23718999 W
PLW13 0.15180001 W
F2 - Processing parameters
SI 32768
SF 100.6152835 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
BRUKER
24.75
25.22
26.63
27.58
28.00
28.34
30.58
30.74
52.33
52.44
57.98
59.45
67.18
67.45
76.71
77.03
77.35
80.28
80.37
81.58
ppm

HRMS - Compound 8a



Achiral HPLC - Compound 9a

```
======================================================================
Injection Date  : 3/3/2015 11:38:19 PM          Seq. Line :   6
Sample Name     : PR069-122-3                    Location : Vial 82
Acq. Operator   : MRL                               Inj :   1
Acq. Instrument : Instrument 1                 Inj Volume : 5 µl
Method          : C:\HPCHEM\1\METHODS\
Last changed    : 8/18/2014 5:35:20 PM by MRL
Generic HPLC Method for Project - Reaction Monitoring and Purity Assay
HPLC 31
C=10mM K3PO4 pH 3 in water + 5% ACN
D=ACN
Waters Symmetry Shield RP-18 150mmx4.6mm 5 microns
1.0mL/min 40C 220nm
```




```
======================================================================
                        Area Percent Report
======================================================================

Signal 1: DAD1 A, Sig=220,4 Ref=off
 Signal has been modified after loading from rawdata file!

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]    [mAU*s]     [mAU]      %
----|-------|----|-------|----------|----------|--------|
   1  23.073 BB    0.1492 1096.58057  109.18481 100.0000

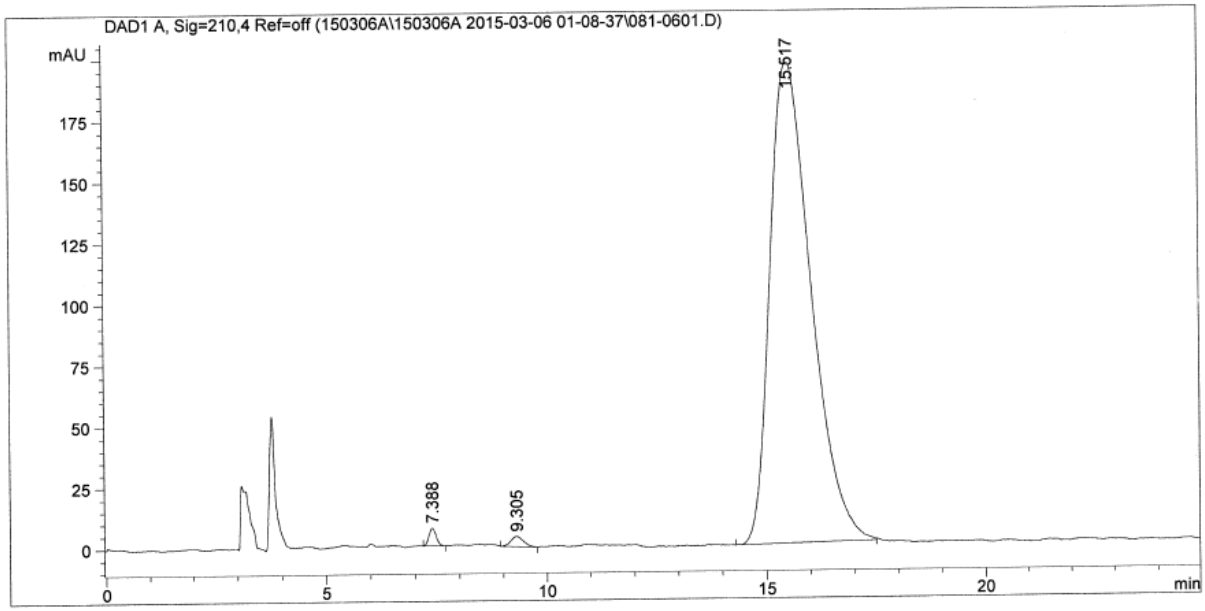
Totals :                  1096.58057  109.18481

======================================================================
```

Chiral HPLC – Compound 9a

```
======================================================================
Acq. Operator   : MRL                              Seq. Line :   6
Acq. Instrument : HPLC-30                           Location : Vial 81
Injection Date  : 3/6/2015 3:24:40 AM                    Inj :   1
                                                  Inj Volume : 5 µl
Acq. Method     : C:\HPCHEM\1\DATA\150306A\150306A 2015-03-06 01-08-37\SDAP-CHIRAL4.M
Last changed    : 3/5/2015 1:45:43 AM by MRL
Analysis Method : C:\HPCHEM\1\METHODS\SDAP-CHIRAL4.M
Last changed    : 3/6/2015 1:58:20 AM by MRL
                  (modified after loading)
Method Info     : Chiral HPLC Method to Assess Chiral Purity of SDAP Related Compounds
                  Column:  Chiralpak AD-H; 25cm x 4.6mm, 5um
                  A = n-Hexane
                  B = Ethanol
                  ELSD: 45°C, 1.8L/min gas flow
                  HPLC: 210-340 nm, 1.0 mL/min, Column Temp = 30 C

Sample Info     : Asymmetric Reduced Horner Emmons Product (2S,6S)
                  3.0 mg/mL in EtOH
```



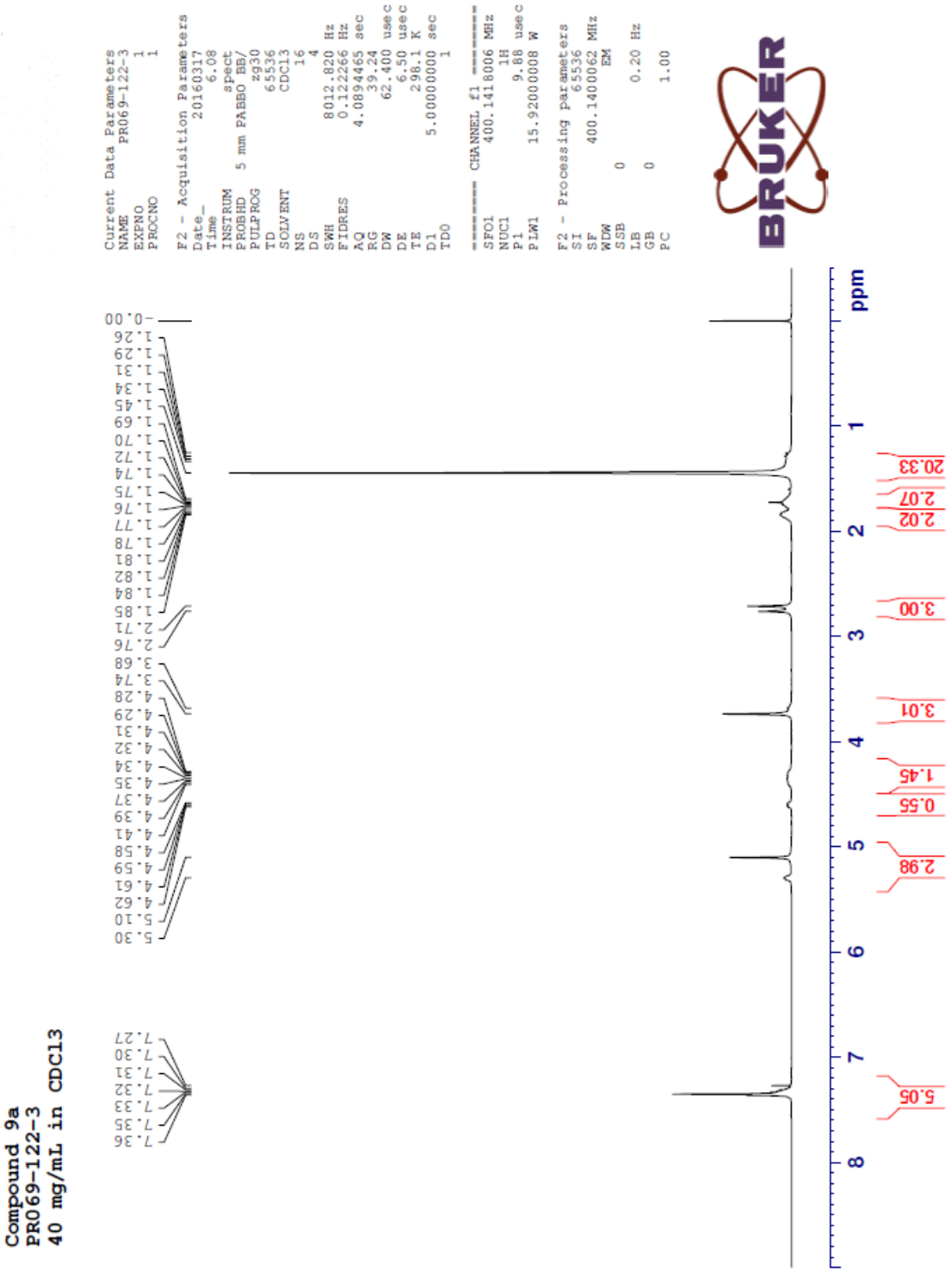

```
======================================================================
                        Area Percent Report
======================================================================
Signal 1: DAD1 A, Sig=210,4 Ref=off

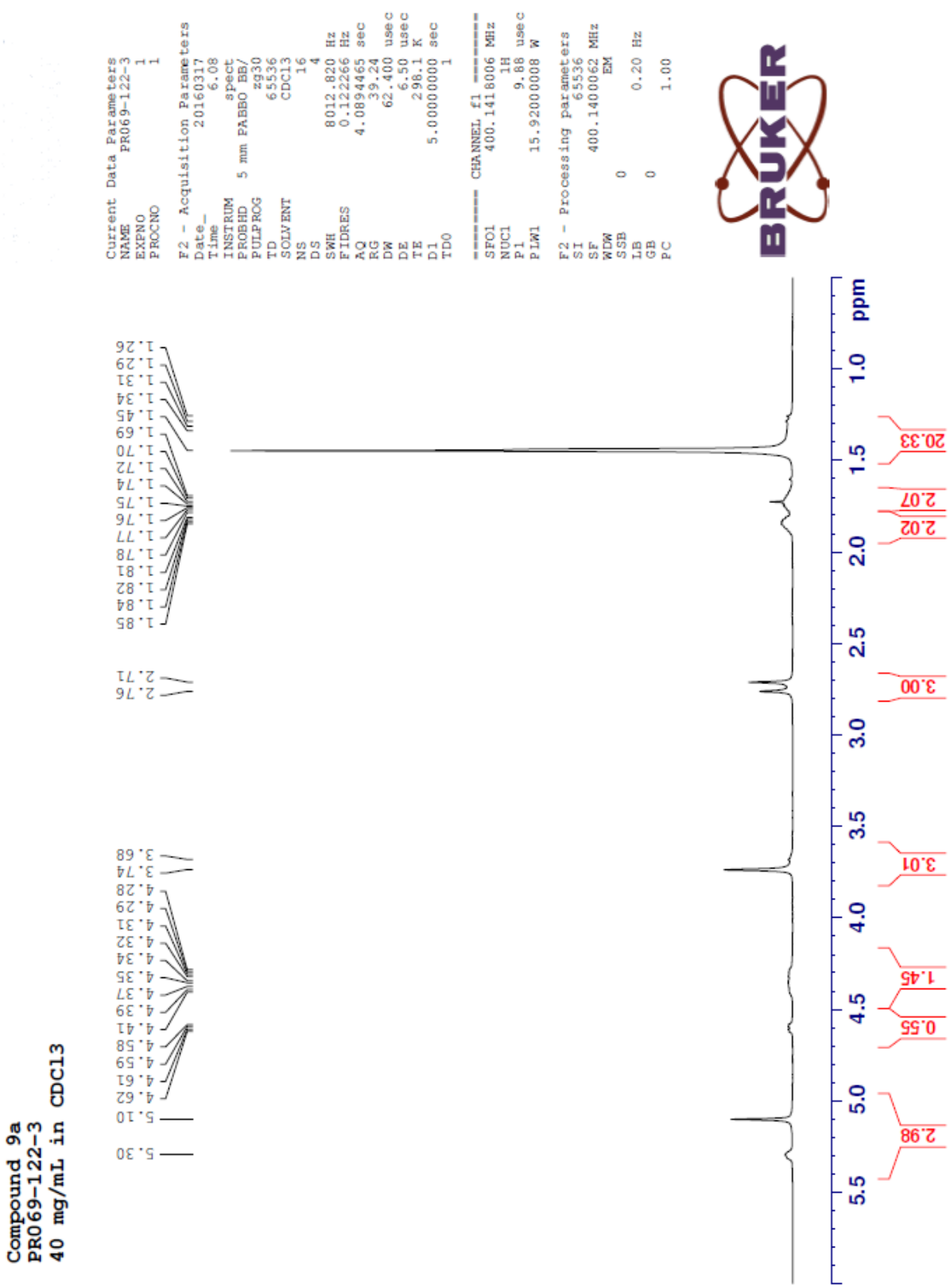
Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   7.388 BB    0.1623   79.51912    7.23195   0.6138
   2   9.305 BV    0.2455   84.48681    4.22113   0.6521
   3  15.517 BB    0.8422 1.27920e4   196.84250  98.7341

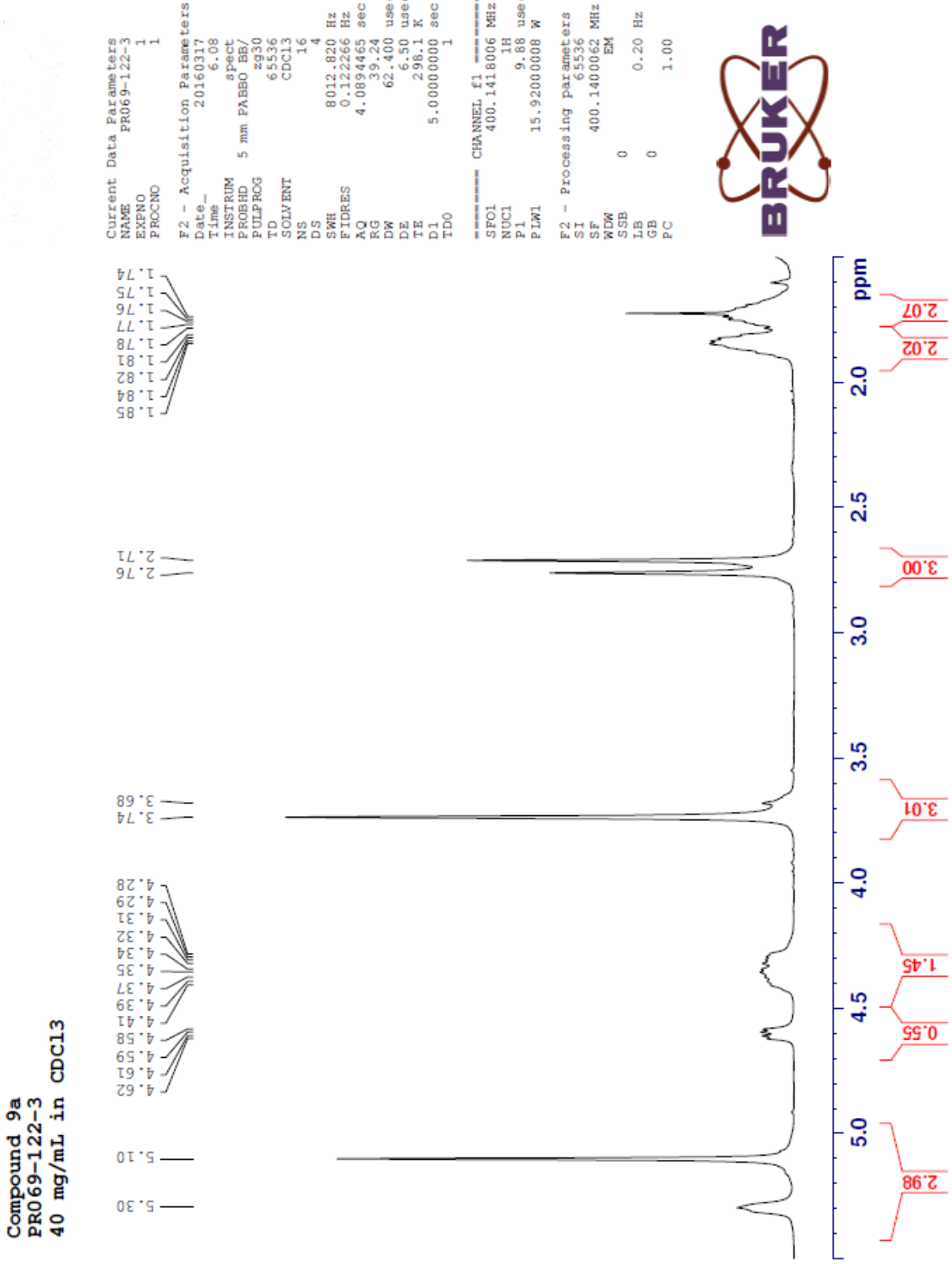
Totals :                  1.29560e4   208.29558

======================================================================
```

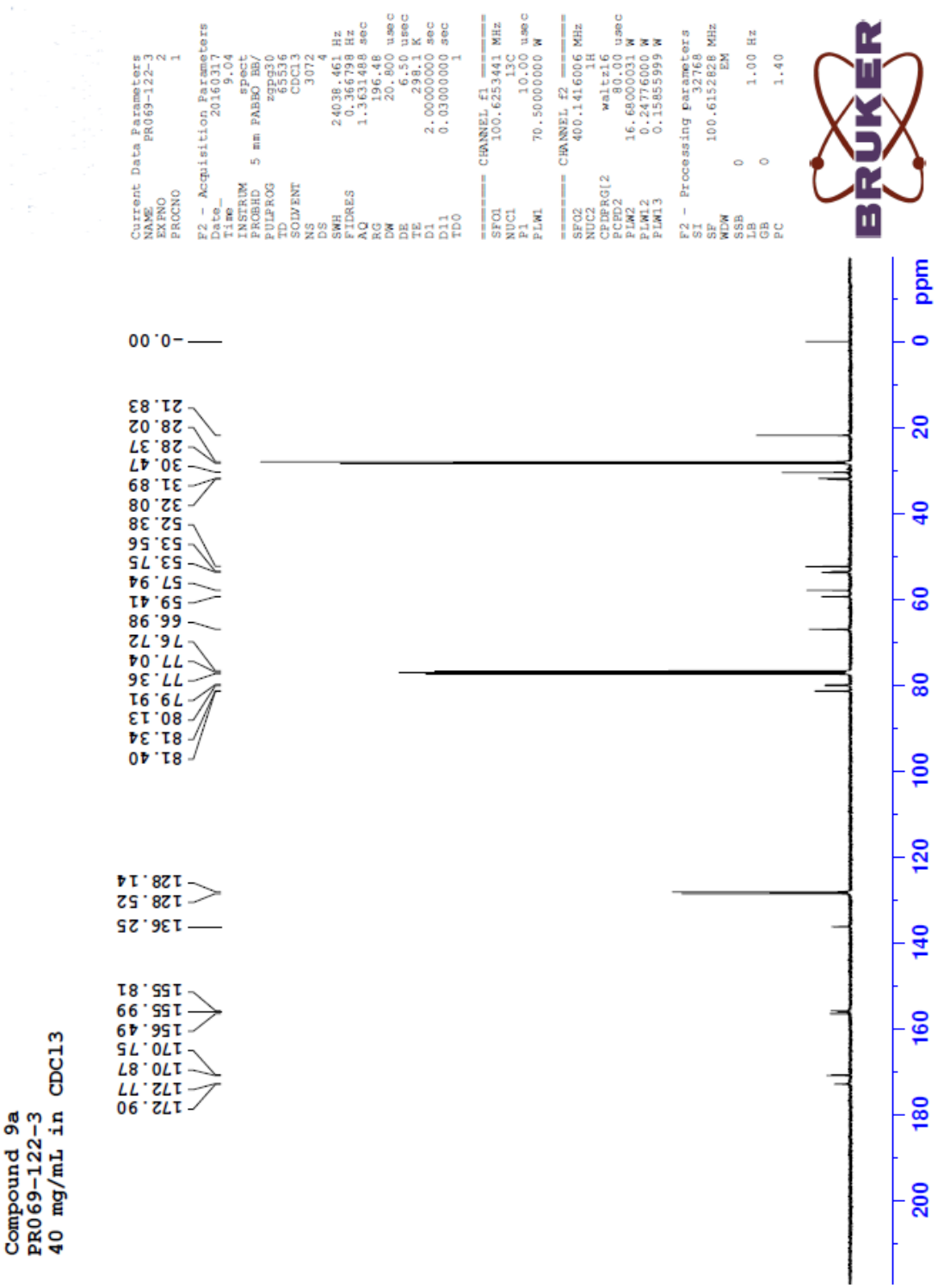
^{1}H NMR - Compound 9a

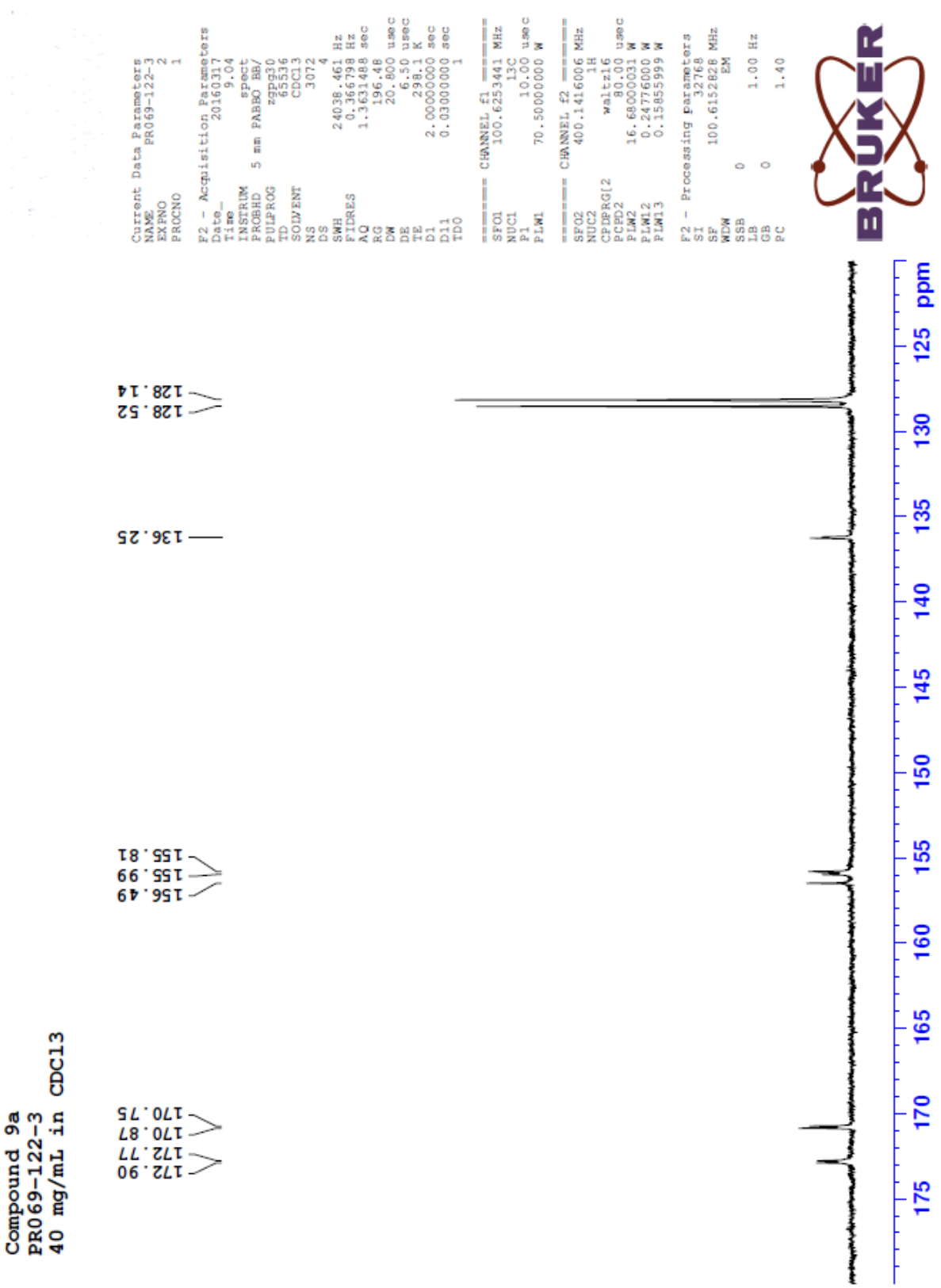


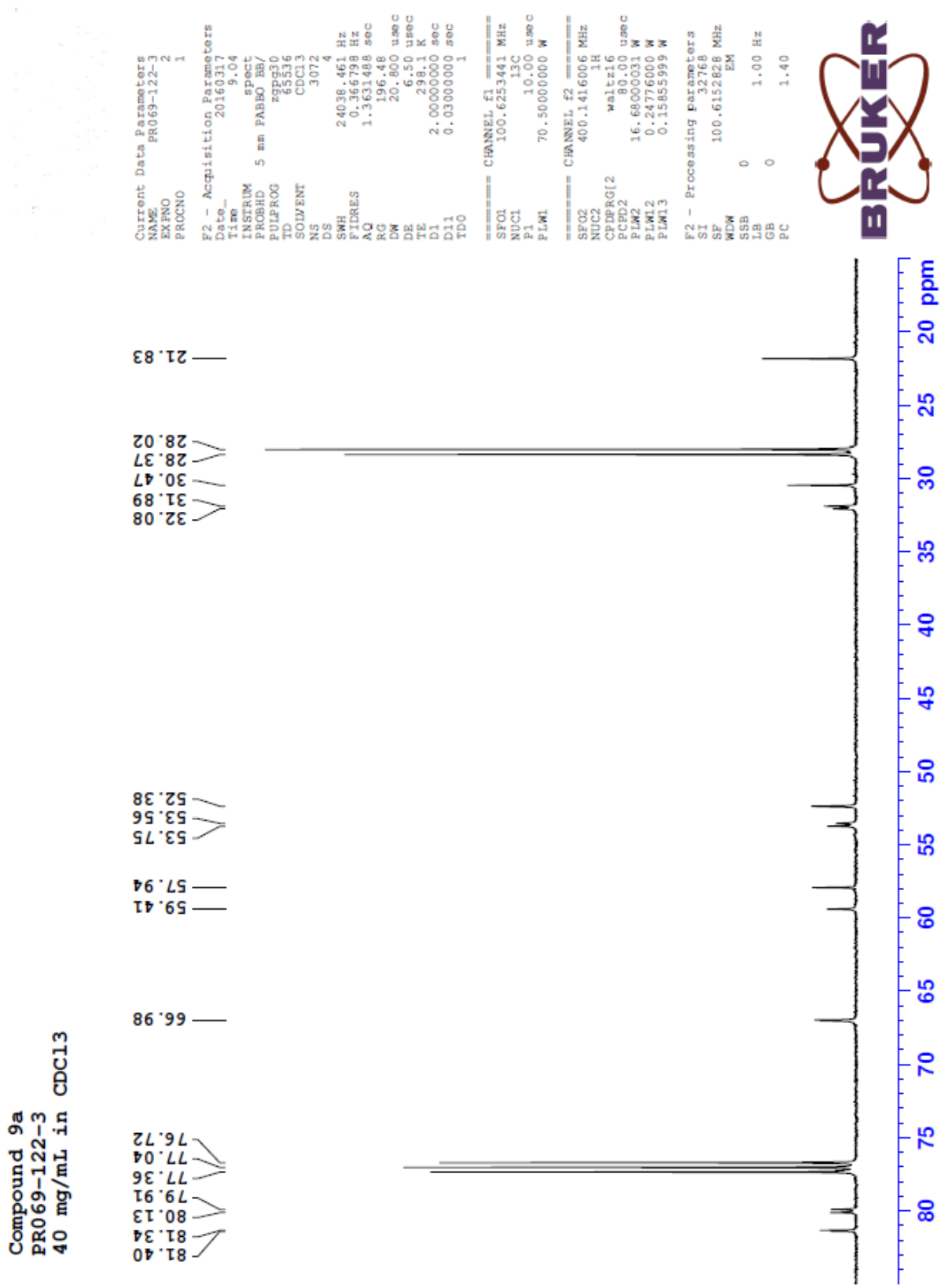

Compound 9a
PR069-122-3
40 mg/mL in CDC13
Current Data Parameters
NAME PR069-122-3
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160317
Time 6.08
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDC13
NS 16
DS 4
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 39.24
DW 62.400 usec
DE 6.50 usec
TE 298.1 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1418006 MHz
NUC1 1H
P1 9.88 usec
PLW1 15.92000008 W
F2 - Processing parameters
SI 65536
SF 400.1400062 MHz
WDW EM
SSB 0
LB 0.20 Hz
GB 0
PC 1.00
BRUKER
5.30
5.10
4.62
4.61
4.59
4.58
4.41
4.39
4.37
4.35
4.34
4.32
4.31
4.29
4.28
3.74
3.68
2.76
2.71
1.85
1.84
1.82
1.81
1.78
1.77
1.76
1.75
1.74
1.72
1.70
1.69
1.45
1.34
1.31
1.29
1.26
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
2.98
0.55
1.45
3.01
3.00
2.02
2.07
20.33


Compound 9a
PR069-122-3
40 mg/mL in CDCl3
Current Data Parameters
NAME PR069-122-3
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160317
Time 6.08
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 39.24
DW 62.400 usec
DE 6.50 usec
TE 298.1 K
D1 5.00000000 sec
TD0 1
CHANNEL f1
SFO1 400.1416006 MHz
NUC1 1H
P1 9.88 usec
PLW1 15.92000008 W
F2 - Processing parameters
SI 65536
SF 400.1400062 MHz
WDW EM
SSB 0
LB 0.20 Hz
GB 0
PC 1.00
BRUKER
ppm
5.0
4.5
4.0
3.5
3.0
2.5
2.0
2.98
0.55
1.45
3.01
3.00
2.02
2.07

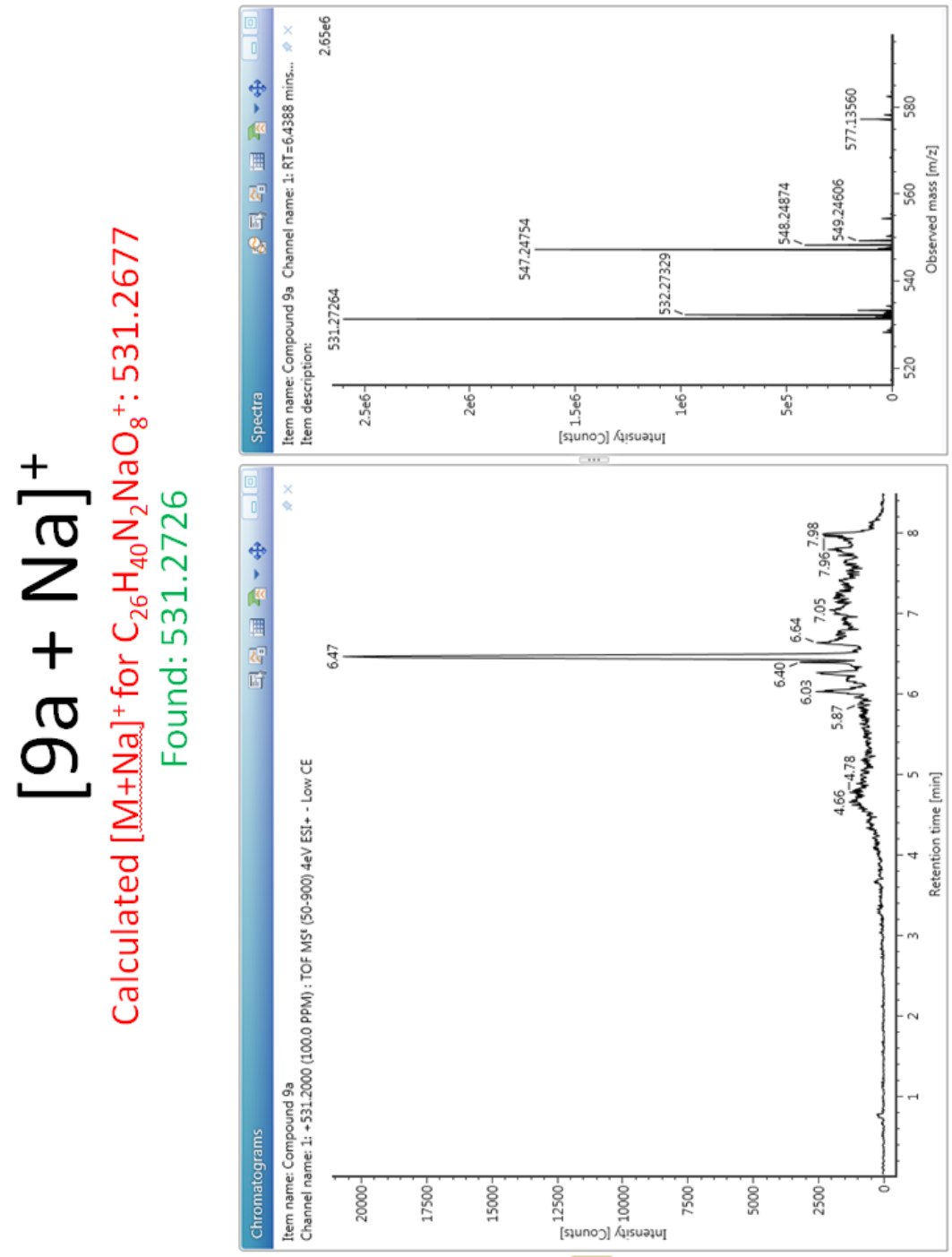
^{13}C NMR - Compound 9a



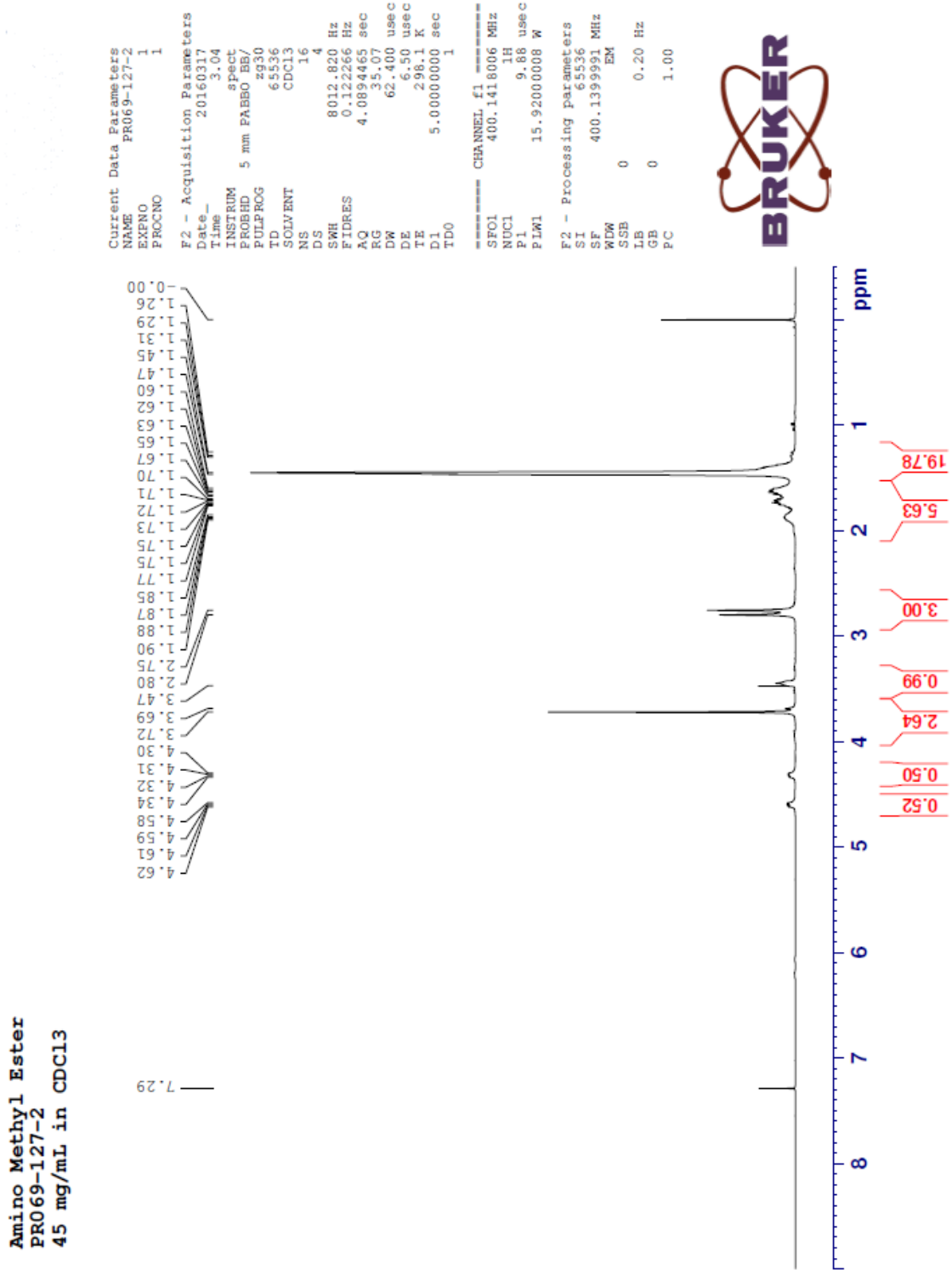

Compound 9a
PR069-122-3
40 mg/mL in CDCl3
172.90
172.77
170.87
170.75
156.49
155.99
155.81
136.25
128.52
128.14
175
170
165
160
155
150
145
140
135
130
125 ppm
Current Data Parameters
NAME PR069-122-3
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160317
Time 9.04
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 3072
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 196.48
DW 20.800 usec
DE 6.50 usec
TE 298.1 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6253441 MHz
NUC1 13C
P1 10.00 usec
PLW1 70.50000000 W
======== CHANNEL f2 ========
SFO2 400.1416006 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 16.68000031 W
PLW12 0.24776000 W
PLW13 0.15855999 W
F2 - Processing parameters
SI 32768
SF 100.6152828 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
BRUKER


Compound 9a
PR069-122-3
40 mg/mL in CDCl3
81.40
81.34
80.13
79.91
77.36
77.04
76.72
66.98
59.41
57.94
53.75
53.56
52.38
32.08
31.89
30.47
28.37
28.02
21.83
Current Data Parameters
NAME PR069-122-3
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160317
Time 9.04
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 3072
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 196.48
DW 20.800 usec
DE 6.50 usec
TE 298.1 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6253441 MHz
NUC1 13C
P1 10.00 usec
PLW1 70.50000000 W
======== CHANNEL f2 ========
SFO2 400.1416006 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 16.68000031 W
PLW12 0.24776000 W
PLW13 0.15855999 W
F2 - Processing parameters
SI 32768
SF 100.6152828 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
BRUKER
80 75 70 65 60 55 50 45 40 35 30 25 20 ppm

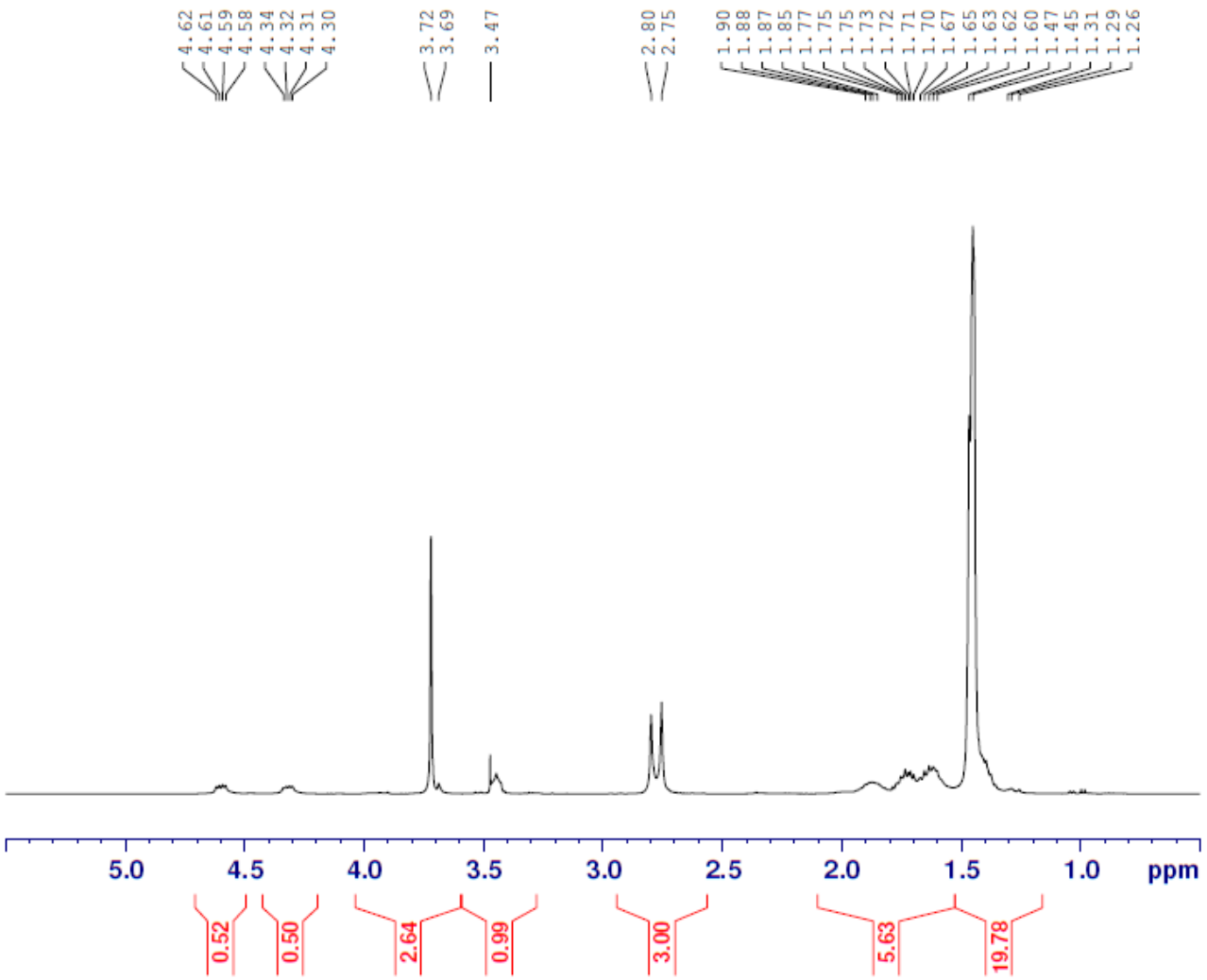
HRMS - Compound 9a



^{1}H NMR - Amino Methyl Ester



Amino Methyl Ester
PR069-127-2
45 mg/mL in CDCl3

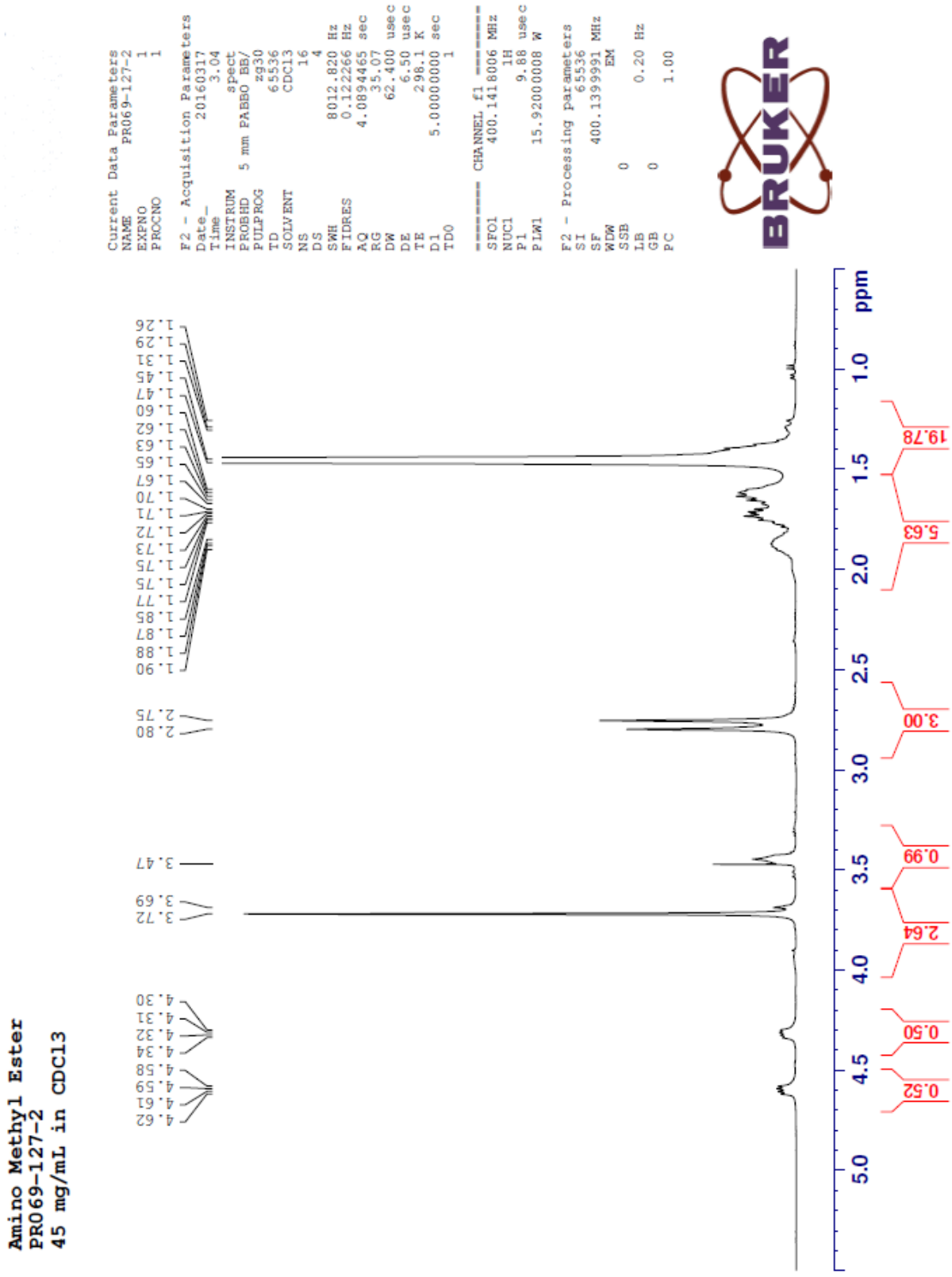



```
Current Data Parameters
NAME          PR069-127-2
EXPNO                   1
PROCNO                  1

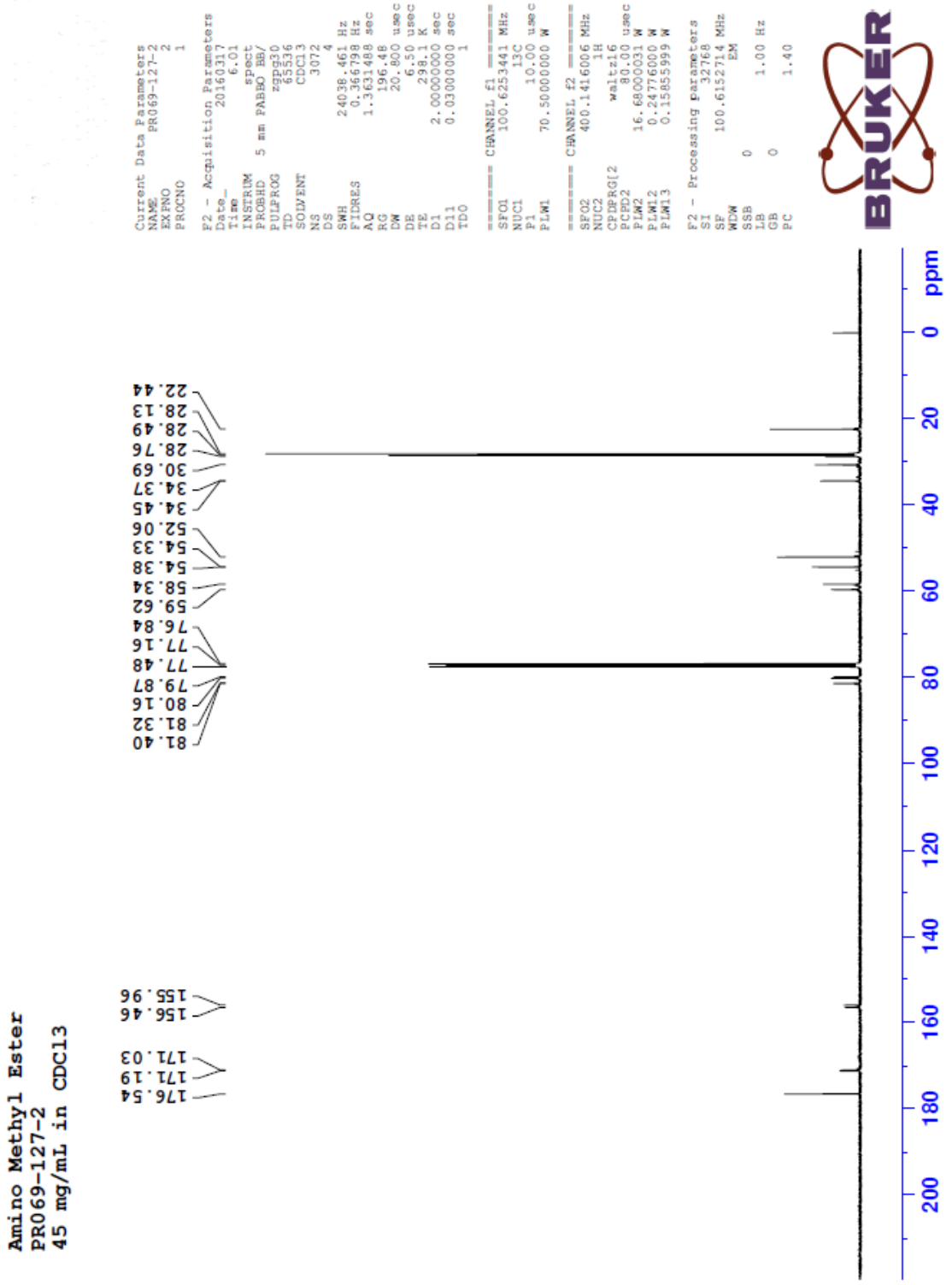
F2 - Acquisition Parameters
Date_            20160317
Time                 3.04
INSTRUM             spect
PROBHD    5 mm PABBO BB/
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                     16
DS                      4
SWH              8012.820 Hz
FIDRES           0.122266 Hz
AQ               4.0894465 sec
RG                  35.07
DW                62.400 usec
DE                 6.50 usec
TE                298.1 K
D1             5.00000000 sec
TD0                     1

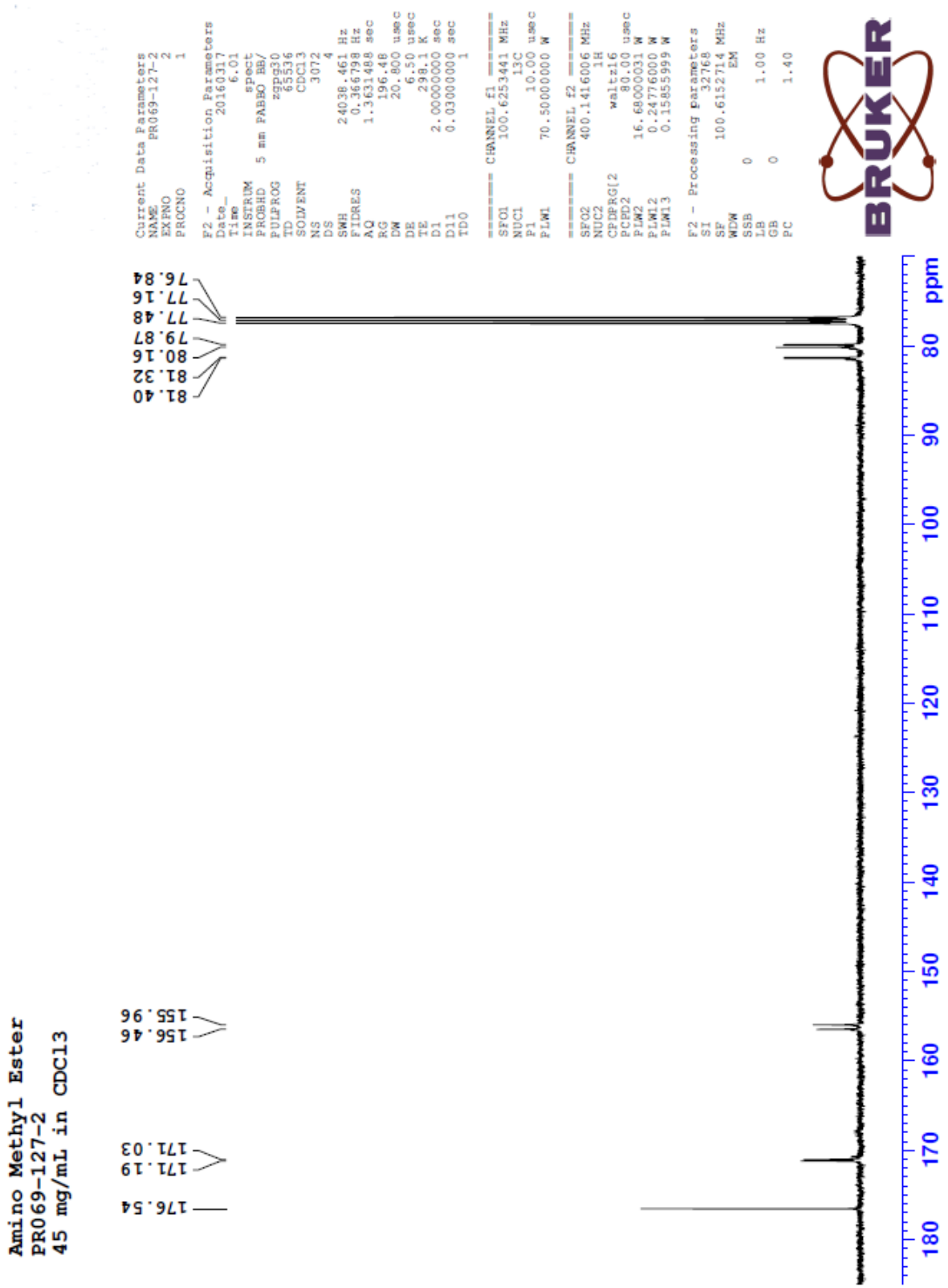
======== CHANNEL f1 ========
SFO1          400.1418006 MHz
NUC1                   1H
P1                  9.88 usec
PLW1         15.92000008 W

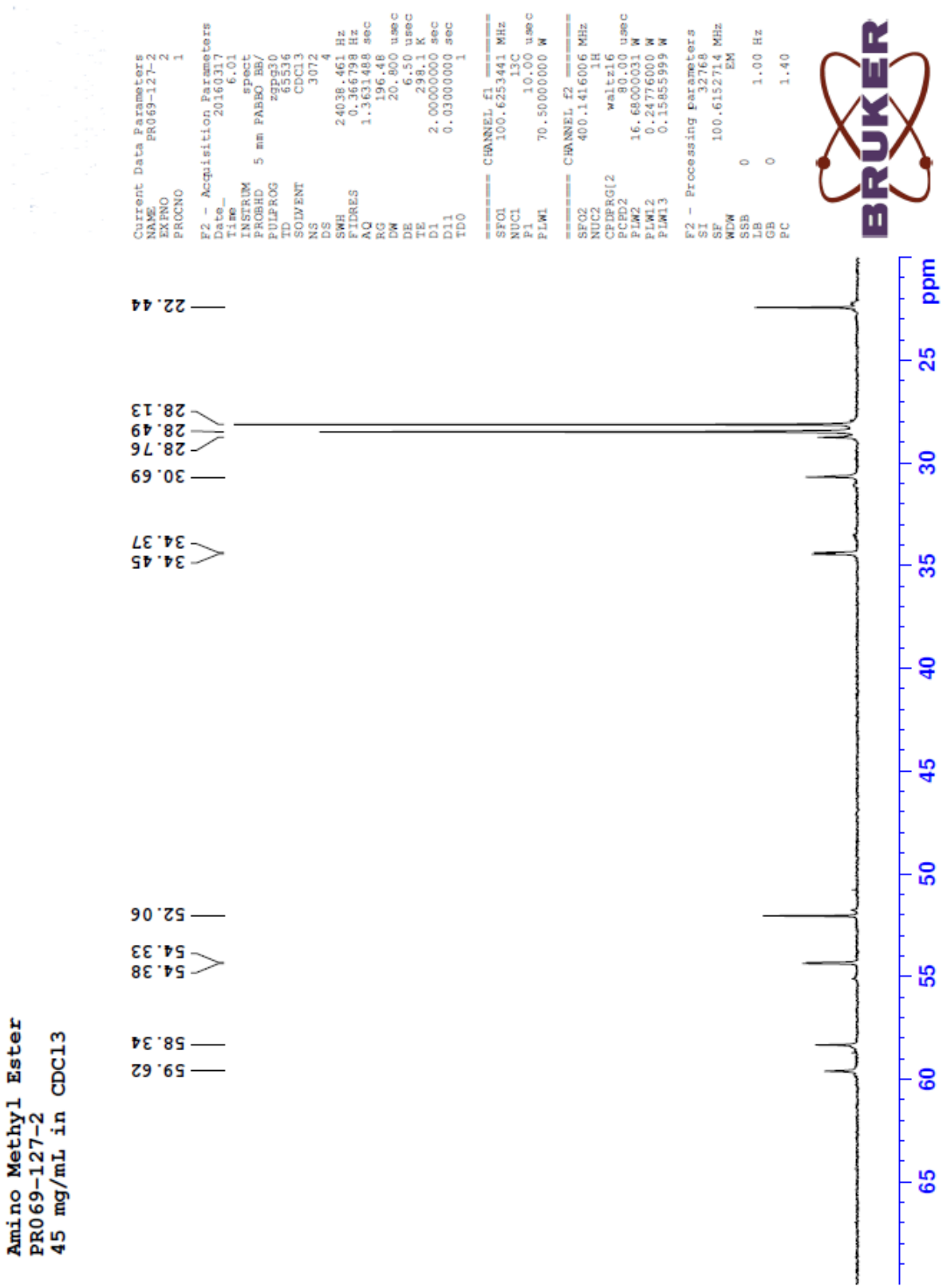
F2 - Processing parameters
SI                  65536
SF            400.1399991 MHz
WDW                    EM
SSB       0
LB                  0.20 Hz
GB        0
PC                  1.00
```


Amino Methyl Ester
PR069-127-2
45 mg/mL in CDCl3
Current Data Parameters
NAME PR069-127-2
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160317
Time 3.04
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 35.07
DW 62.400 usec
DE 6.50 usec
TE 298.1 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1418006 MHz
NUC1 1H
P1 9.88 usec
PLW1 15.92000008 W
F2 - Processing parameters
SI 65536
SF 400.1399991 MHz
WDW EM
SSB 0
LB 0.20 Hz
GB 0
PC 1.00
BRUKER
1.26
1.29
1.31
1.45
1.47
1.60
1.62
1.63
1.65
1.67
1.70
1.71
1.72
1.73
1.75
1.75
1.77
1.85
1.87
1.88
1.90
2.75
2.80
3.47
3.69
3.72
4.30
4.31
4.32
4.34
4.58
4.59
4.61
4.62
19.78
5.63
3.00
0.99
2.64
0.50
0.52
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

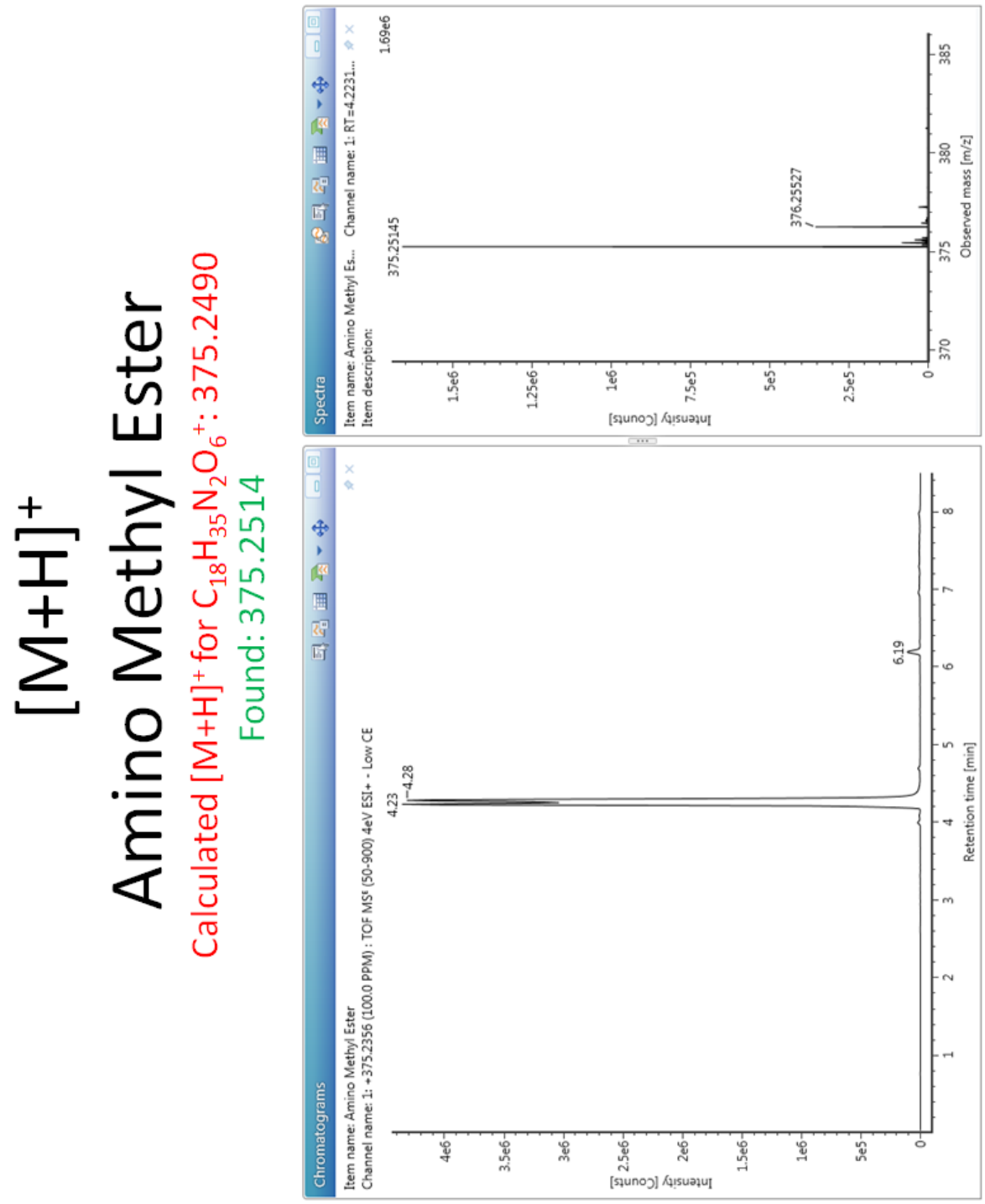
^{13}C NMR - Amino Methyl Ester



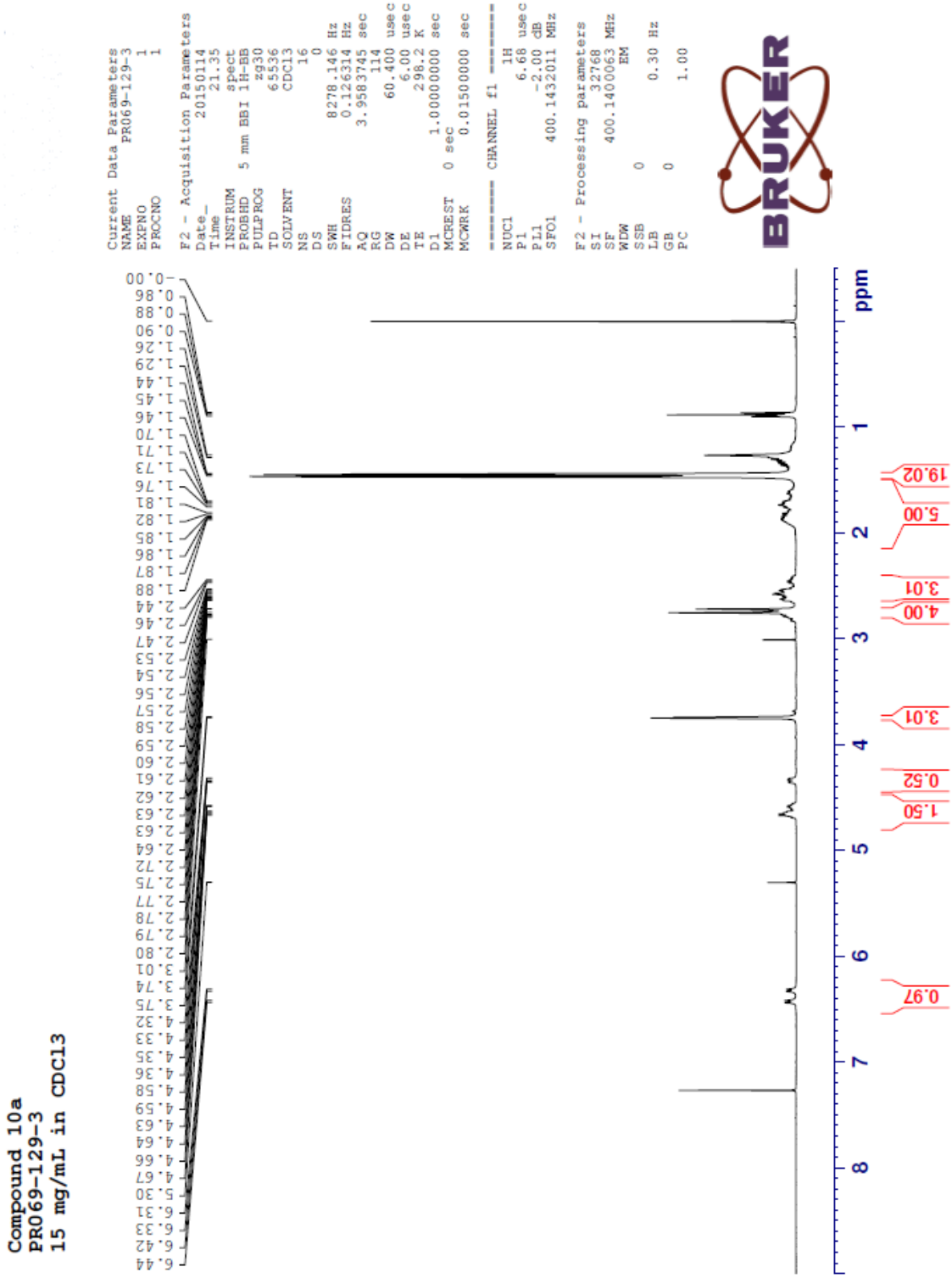

Amino Methyl Ester
PR069-127-2
45 mg/mL in CDCl3
176.54
171.19
171.03
156.46
155.96
81.40
81.32
80.16
79.87
77.48
77.16
76.84
Current Data Parameters
NAME PR069-127-2
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160317
Time 6.01
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 3072
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 196.48
DW 20.800 usec
DE 6.50 usec
TE 298.1 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6253441 MHz
NUC1 13C
P1 10.00 usec
PLW1 70.50000000 W
======== CHANNEL f2 ========
SFO2 400.1416006 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 16.68000031 W
PLW12 0.24776000 W
PLW13 0.15855999 W
F2 - Processing parameters
SI 32768
SF 100.6152714 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
BRUKER
180 170 160 150 140 130 120 110 100 90 80 ppm

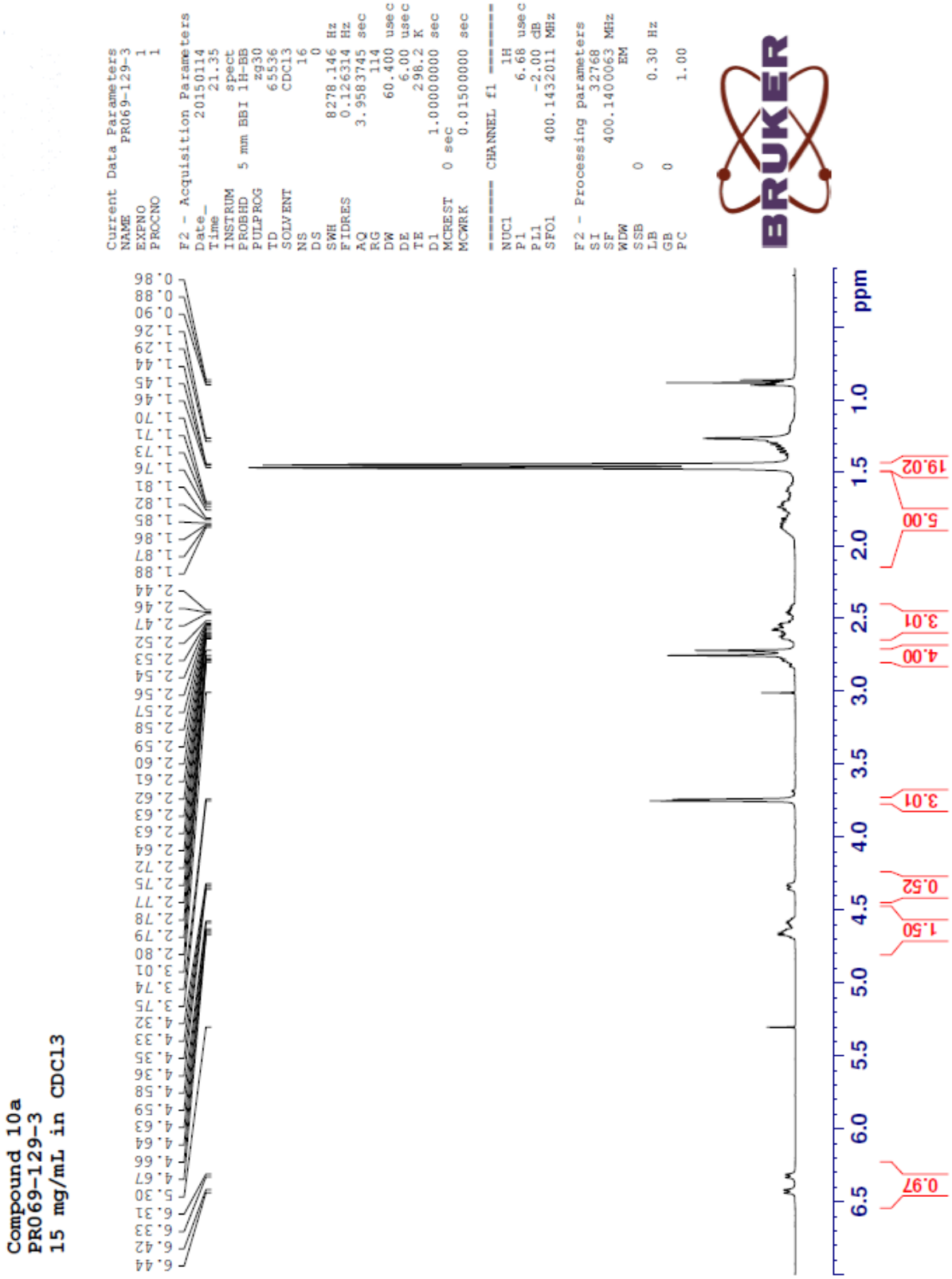

Amino Methyl Ester
PR069-127-2
45 mg/mL in CDCl3
22.44
28.13
28.49
28.76
30.69
34.37
34.45
52.06
54.33
54.38
58.34
59.62
ppm
Current Data Parameters
NAME PR069-127-2
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160317
Time 6.01
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 3072
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 196.48
DW 20.800 usec
DE 6.50 usec
TE 298.1 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6253441 MHz
NUC1 13C
P1 10.00 usec
PLW1 70.50000000 W
======== CHANNEL f2 ========
SFO2 400.1416006 MHz
NUC2 1H
CPDPRG2 waltz16
PCPD2 80.00 usec
PLW2 16.68000031 W
PLW12 0.24776000 W
PLW13 0.15855999 W
F2 - Processing parameters
SI 32768
SF 100.6152714 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
BRUKER

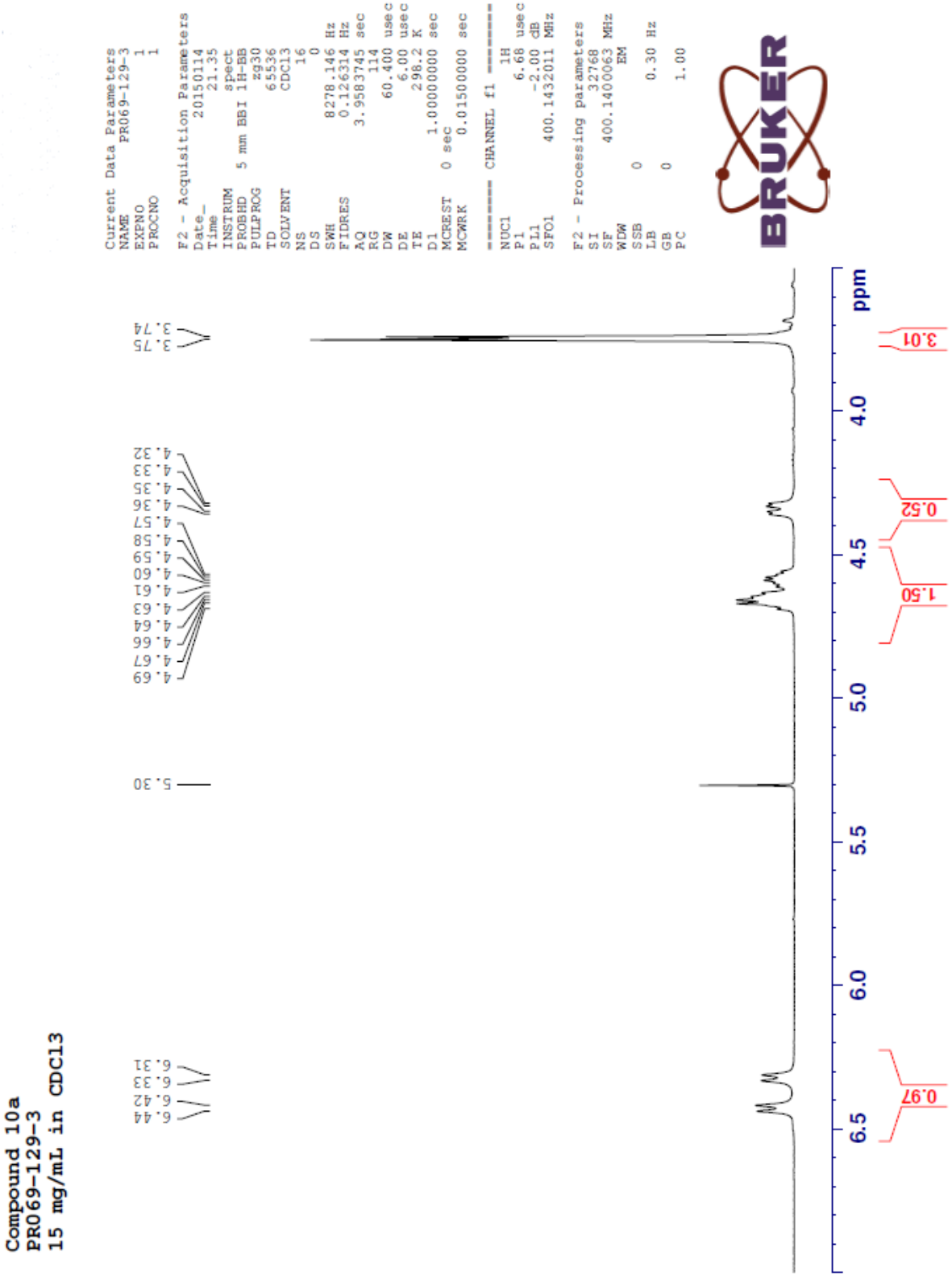
HRMS - Amino Methyl Ester

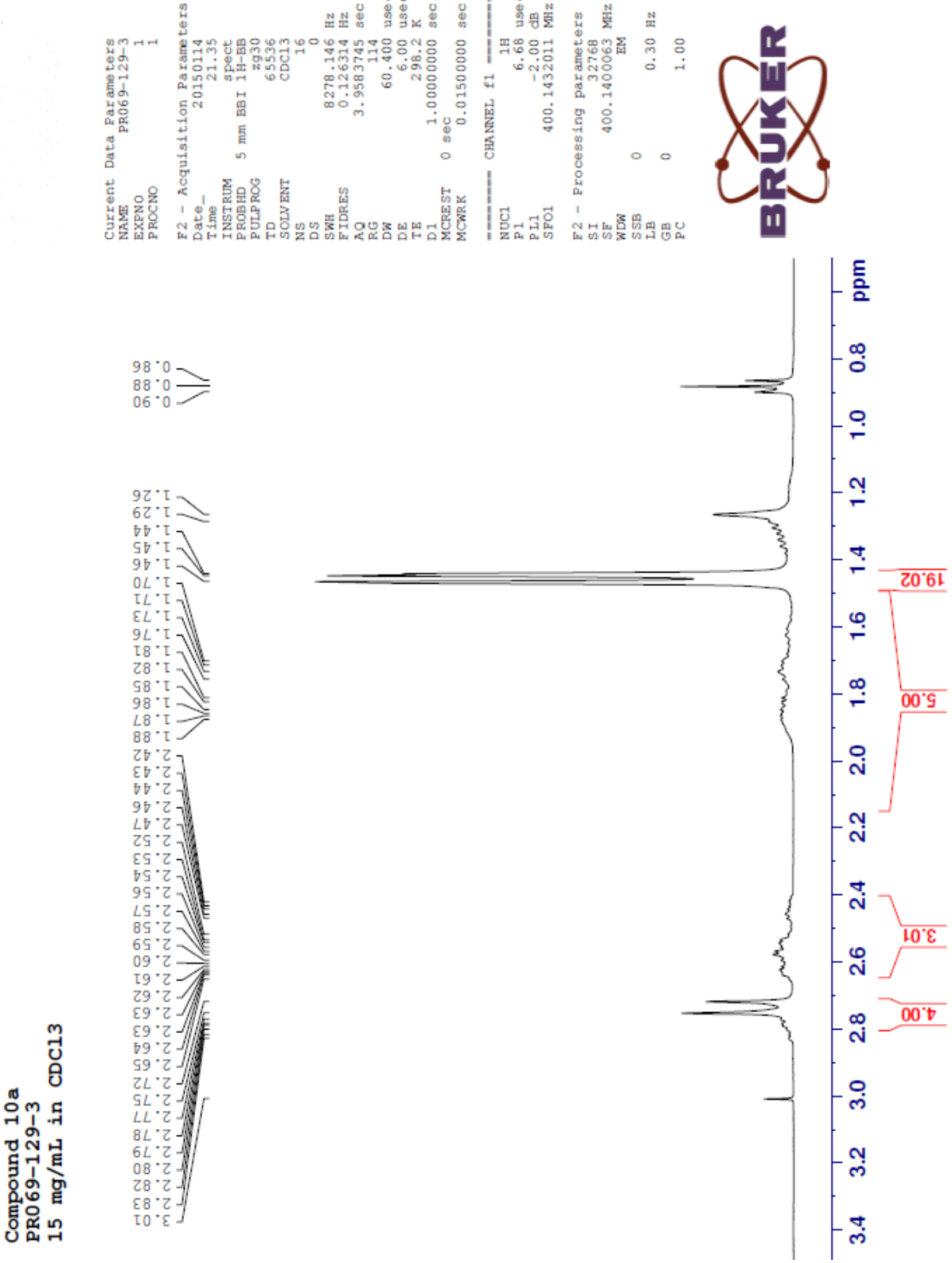


^{1}H NMR - Compound 10a

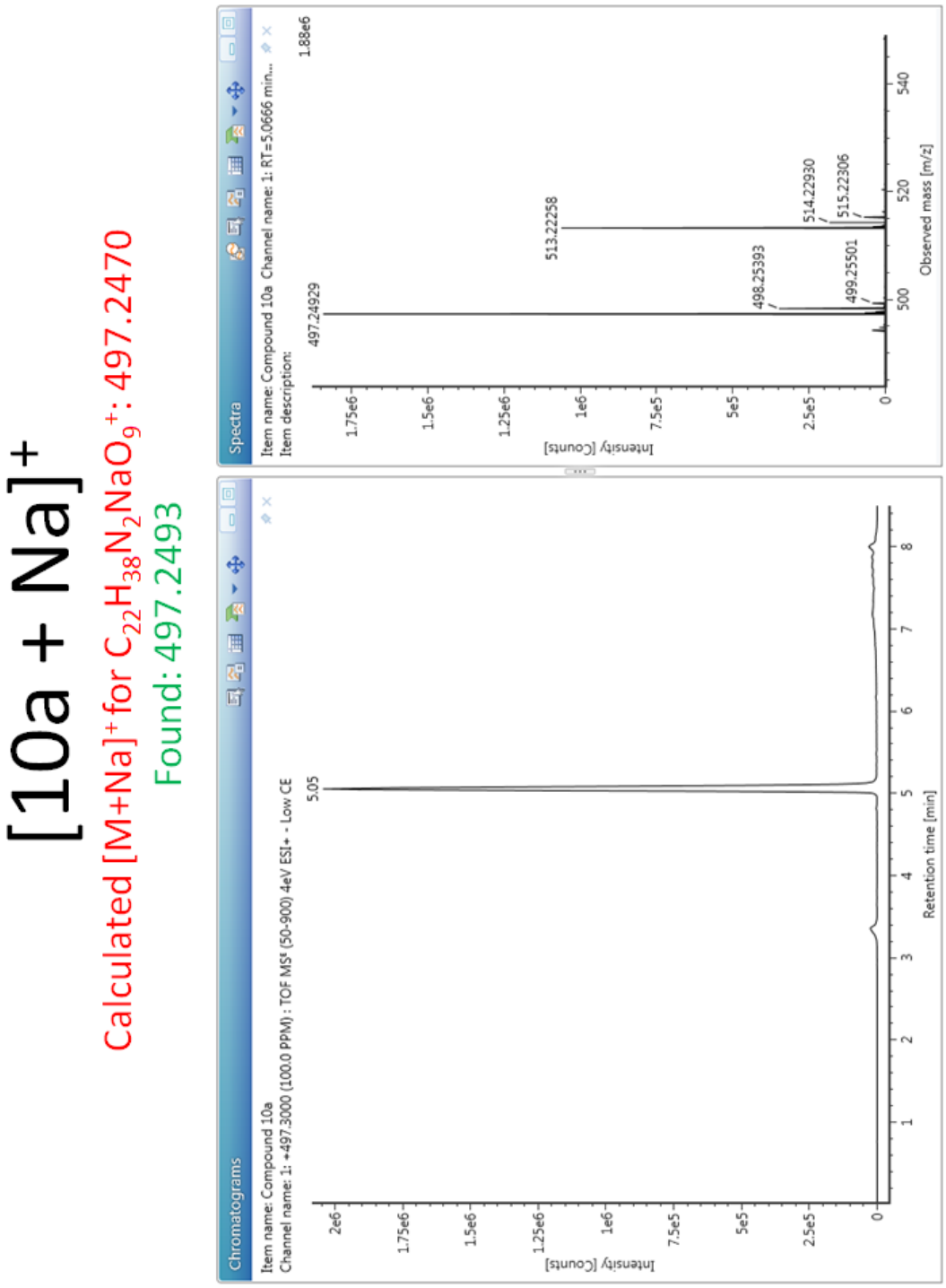



Compound 10a
PR069-129-3
15 mg/mL in CDCl3
Current Data Parameters
NAME PR069-129-3
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20150114
Time 21.35
INSTRUM spect
PROBHD 5 mm BBI 1H-BB
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 0
SWH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9583745 sec
RG 114
DW 60.400 usec
DE 6.00 usec
TE 298.2 K
D1 1.00000000 sec
MCREST 0 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 6.68 usec
PL1 -2.00 dB
SFO1 400.1432011 MHz
F2 - Processing parameters
SI 32768
SF 400.1400063 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
BRUKER
ppm
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.97
1.50
0.52
3.01
4.00
3.01
5.00
19.02

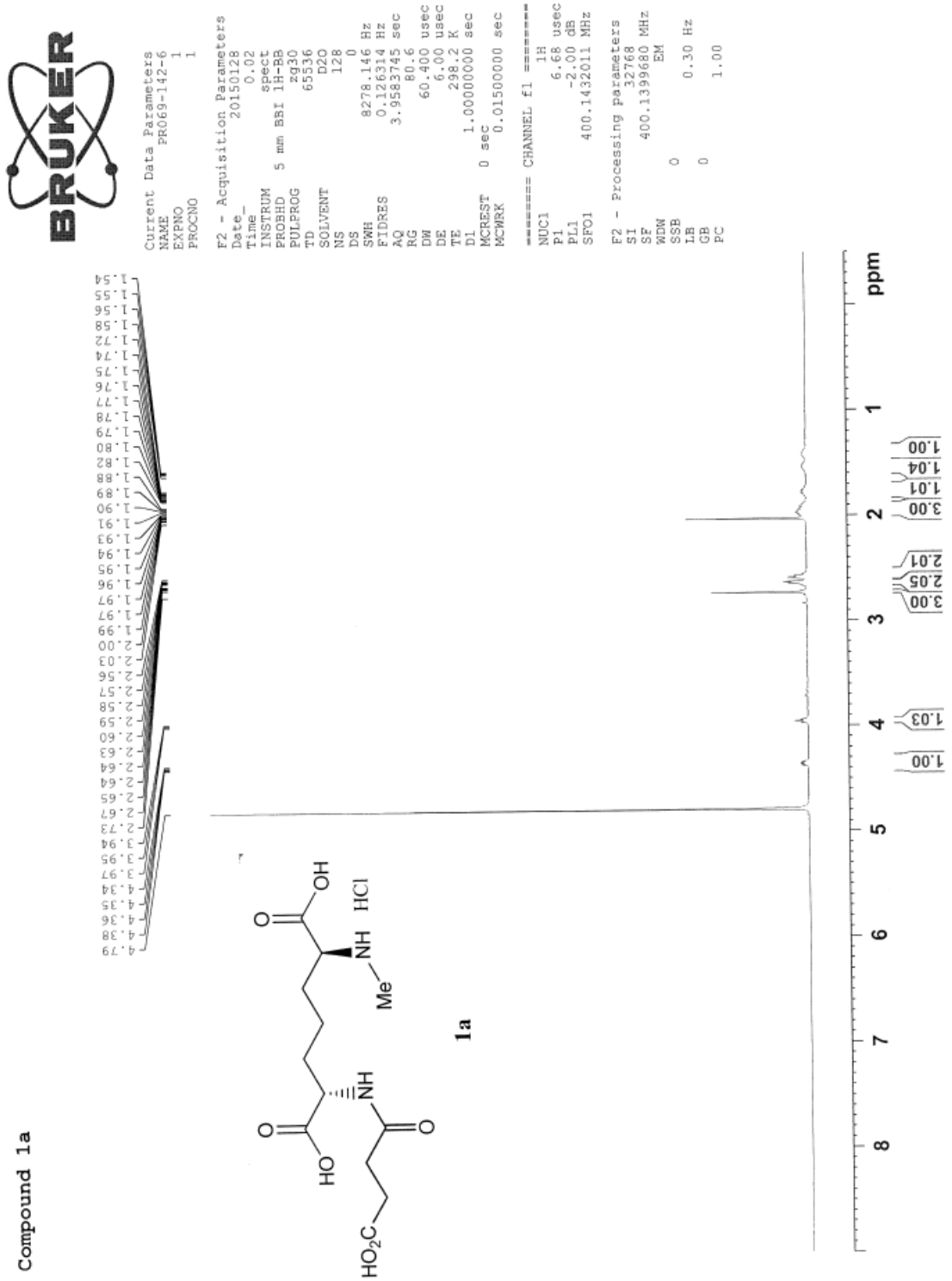

Compound 10a
PR069-129-3
15 mg/mL in CDCl3
Current Data Parameters
NAME PR069-129-3
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20150114
Time 21.35
INSTRUM spect
PROBHD 5 mm BBI 1H-BB
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 0
SWH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9583745 sec
RG 114
DW 60.400 usec
DE 6.00 usec
TE 298.2 K
D1 1.00000000 sec
MCREST 0 sec 0.01500000 sec
MCWRK 0.01500000 sec
CHANNEL f1
NUC1 1H
P1 6.68 usec
PL1 -2.00 dB
SFO1 400.1432011 MHz
F2 - Processing parameters
SI 32768
SF 400.1400063 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
BRUKER
6.44
6.42
6.33
6.31
5.30
4.69
4.67
4.66
4.64
4.63
4.61
4.60
4.59
4.58
4.57
4.36
4.35
4.33
4.32
3.75
3.74
6.5
6.0
5.5
5.0
4.5
4.0
ppm
0.97
1.50
0.52
3.01


Compound 10a
PR069-129-3
15 mg/mL in CDCl3
Current Data Parameters
NAME PR069-129-3
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20150114
Time_ 21.35
INSTRUM spect
PROBHD 5 mm BBI 1H-BB
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 0
SWH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9583745 sec
RG 114
DW 60.400 usec
DE 6.00 usec
TE 298.2 K
D1 1.00000000 sec
MCREST 0 sec 0.01500000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 6.68 usec
PL1 -2.00 dB
SFO1 400.1432011 MHz
F2 - Processing parameters
SI 32768
SF 400.1400063 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
BRUKER
0.86
0.88
0.90
1.26
1.29
1.44
1.45
1.46
1.70
1.71
1.73
1.76
1.81
1.82
1.85
1.86
1.87
1.88
2.42
2.43
2.44
2.46
2.47
2.52
2.53
2.54
2.56
2.57
2.58
2.59
2.60
2.61
2.62
2.63
2.63
2.64
2.65
2.72
2.75
2.77
2.78
2.79
2.80
2.82
2.83
3.01
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
ppm
4.00
3.01
5.00
19.02

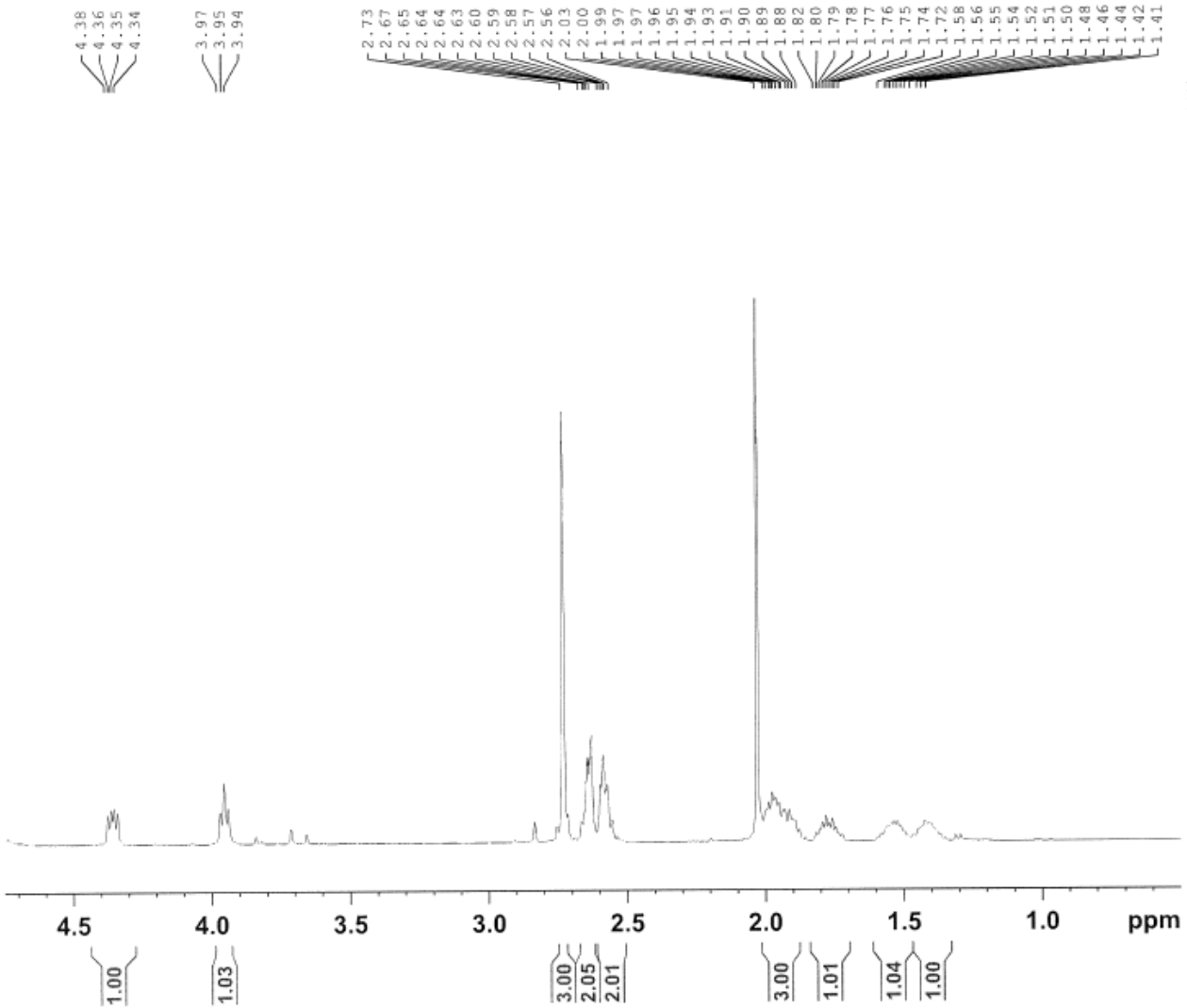
HRMS - Compound 10a



^{1}H NMR - Compound 1a.HCl



Compound 1a



BRUKER

Current Data Parameters
NAME PR069-142-6
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20150128
Time 0.02
INSTRUM spect
PROBHD 5 mm BBI 1H-BB
PULPROG zg30
TD 65536
SOLVENT D2O
NS 128
DS 0
SWH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9583745 sec
RG 80.6
DW 60.400 usec
DE 6.00 usec
TE 298.2 K
D1 1.00000000 sec
MCREST 0 sec
MCWRK 0.01500000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 6.68 usec
PL1 -2.00 dB
SFO1 400.1432011 MHz

F2 - Processing parameters
SI 32768
SF 400.1399680 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

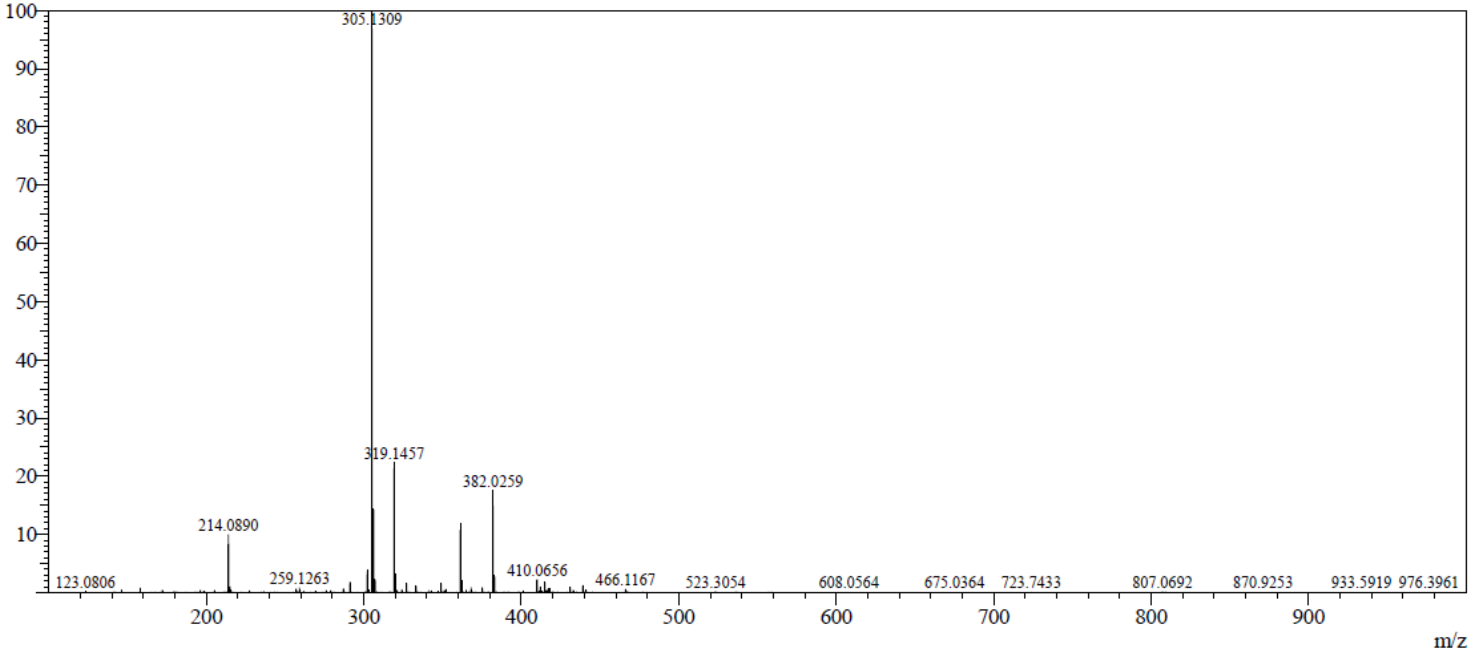
Shimadzu IT-TOF Accurate Mass Report

Monomethyl L,L-SDAP HCl Salt (Compound 1a)
Lot PR069-142-6

Mass Spectrum

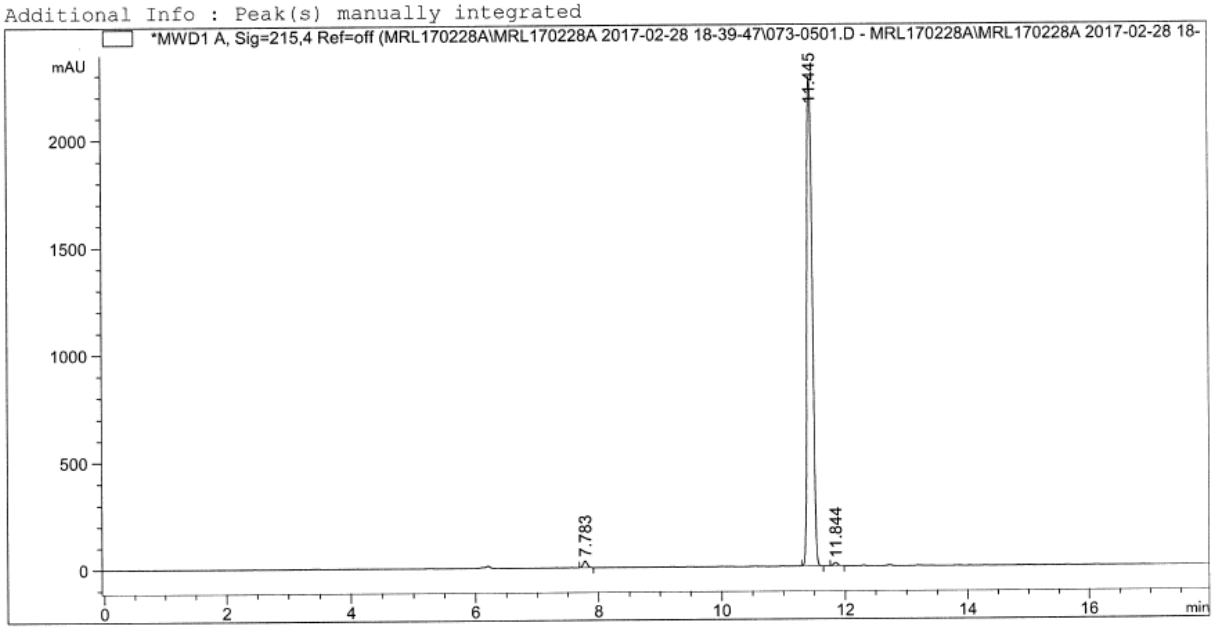
MS Spectrum Graph

#:1 Ret.Time:Averaged 0.143-0.665(Scan#:19-85)
BG Mode:None
Mass Peaks:850 Base Peak:305.13(7501548) MS Stage:MS Polarity:Pos Segment1 - Event1 Precursor:----- Cutoff: Ionization Mode:ESI



Achiral HPLC - Compound 8b

```
==================================================================
Acq. Operator   : MRL                          Seq. Line :   5
Acq. Instrument : Instrument 1                  Location : Vial 73
Injection Date  : 02/28/17  8:08:02 PM              Inj :   1
                                               Inj Volume : 3.0 µl
Acq. Method     : C:\CHEM32\1\DATA\MRL170228A\MRL170228A 2017-02-28 18-39-47\SDAP3.M
Last changed    : 02/28/17  6:37:32 PM by MRL
Analysis Method : C:\CHEM32\1\METHODS\SDAP3.M
Last changed    : 03/01/17  10:16:40 AM by MRL
                  (modified after loading)
Additional Info : Peak(s) manually integrated
```




```
==================================================================
                        Area Percent Report
==================================================================

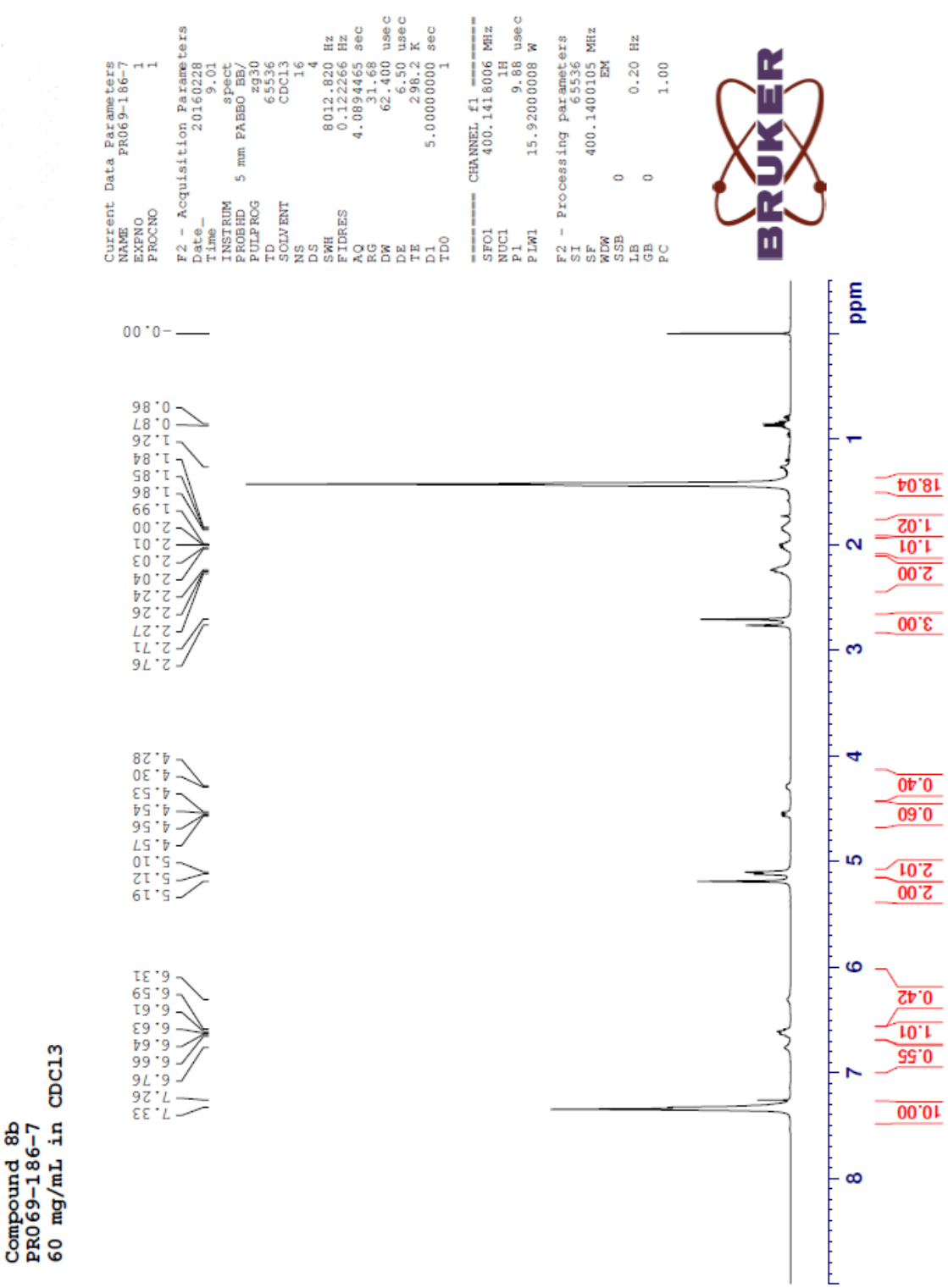
Signal 1: MWD1 A, Sig=215,4 Ref=off
 Signal has been modified after loading from rawdata file!

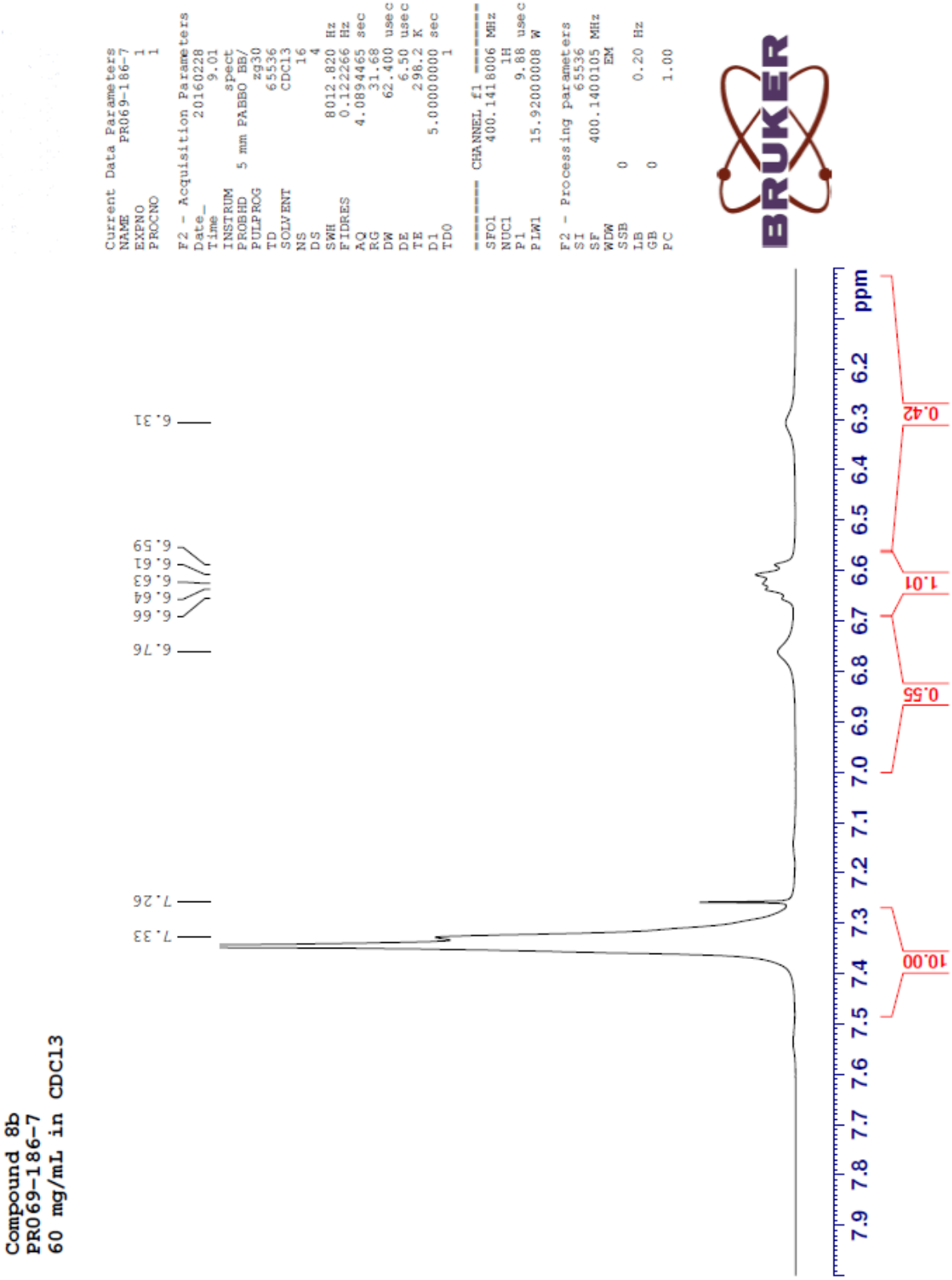
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   7.783 BB    0.0726  143.68234   30.69515   1.0318
   2  11.445 BB    0.0980 1.36981e4  2282.33813  98.3703
   3  11.844 BB    0.0763   83.24905   16.66619   0.5978

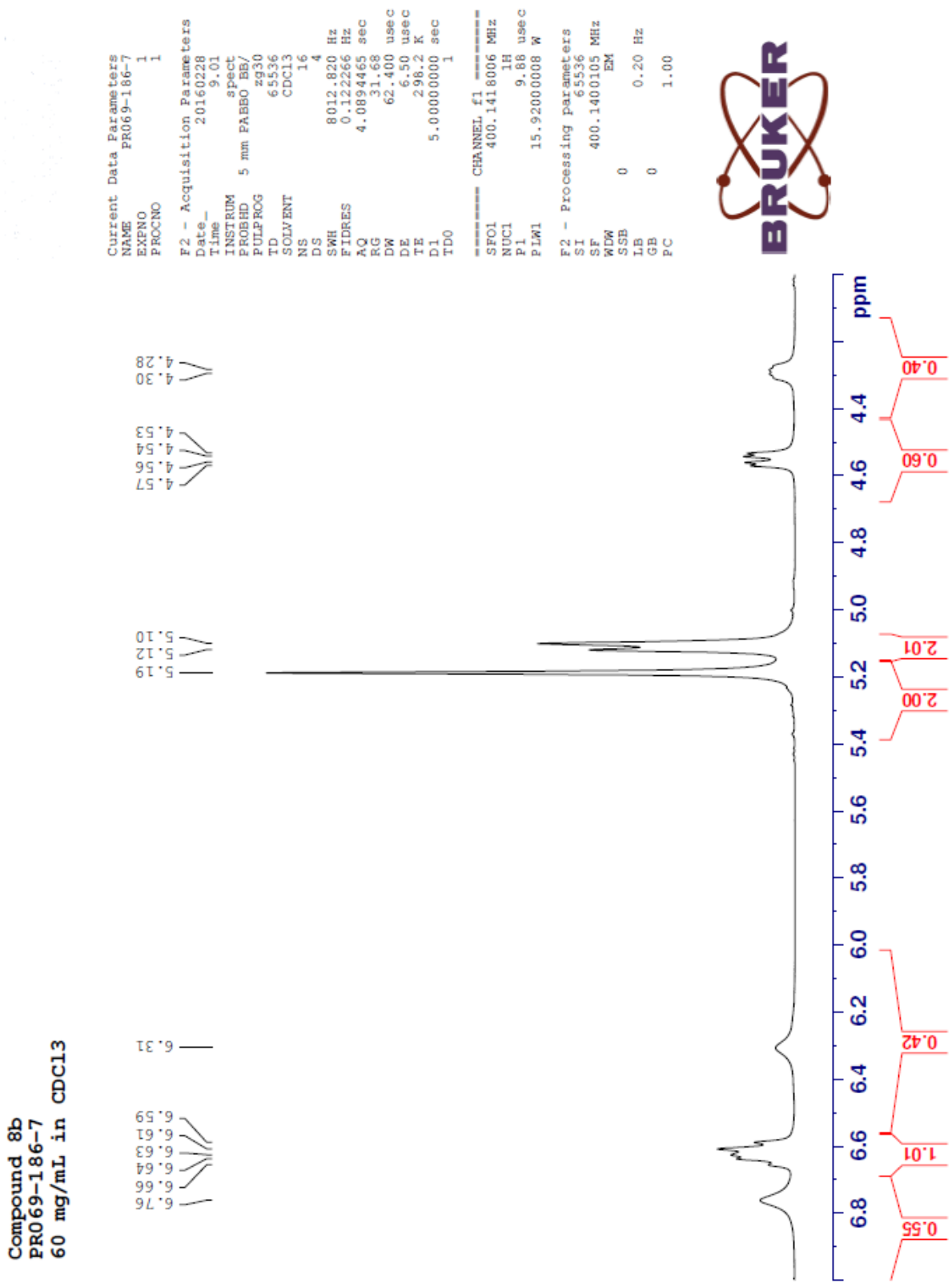
Totals :                  1.39251e4  2329.69947

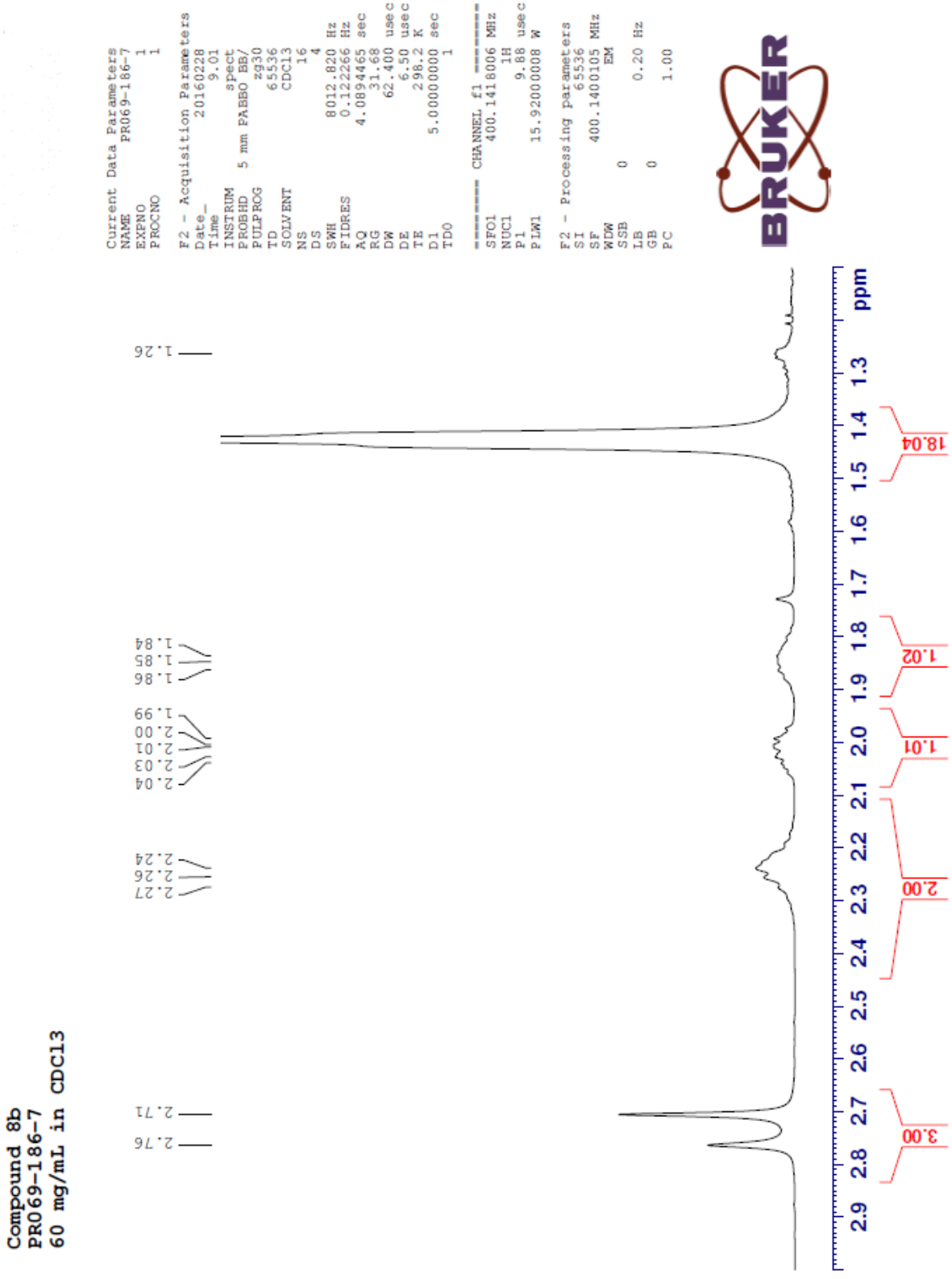
==================================================================
```

^{1}H NMR - Compound 8b

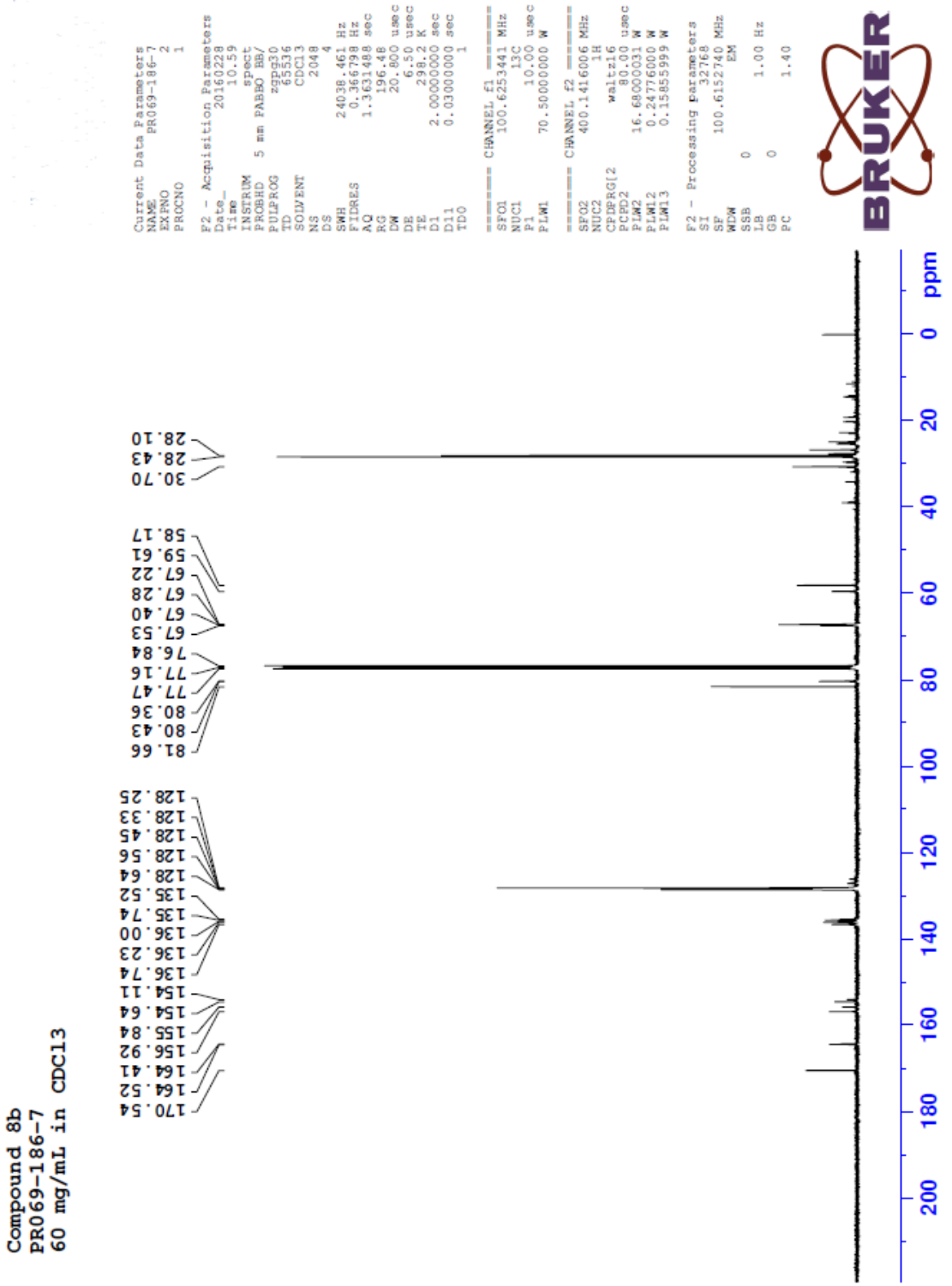



Compound 8b
PR069-186-7
60 mg/mL in CDCl3
Current Data Parameters
NAME PR069-186-7
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160228
Time_ 9.01
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 31.68
DW 62.400 usec
DE 6.50 usec
TE 298.2 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1418006 MHz
NUC1 1H
P1 9.88 usec
PW1 15.92000008 W
F2 - Processing parameters
SI 65536
SF 400.1400105 MHz
WDW EM
SSB 0
LB 0.20 Hz
GB 0
PC 1.00
BRUKER
6.31
6.59
6.61
6.63
6.64
6.66
6.76
7.26
7.33
7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9 6.8 6.7 6.6 6.5 6.4 6.3 6.2 ppm
10.00
0.55
1.01
0.42

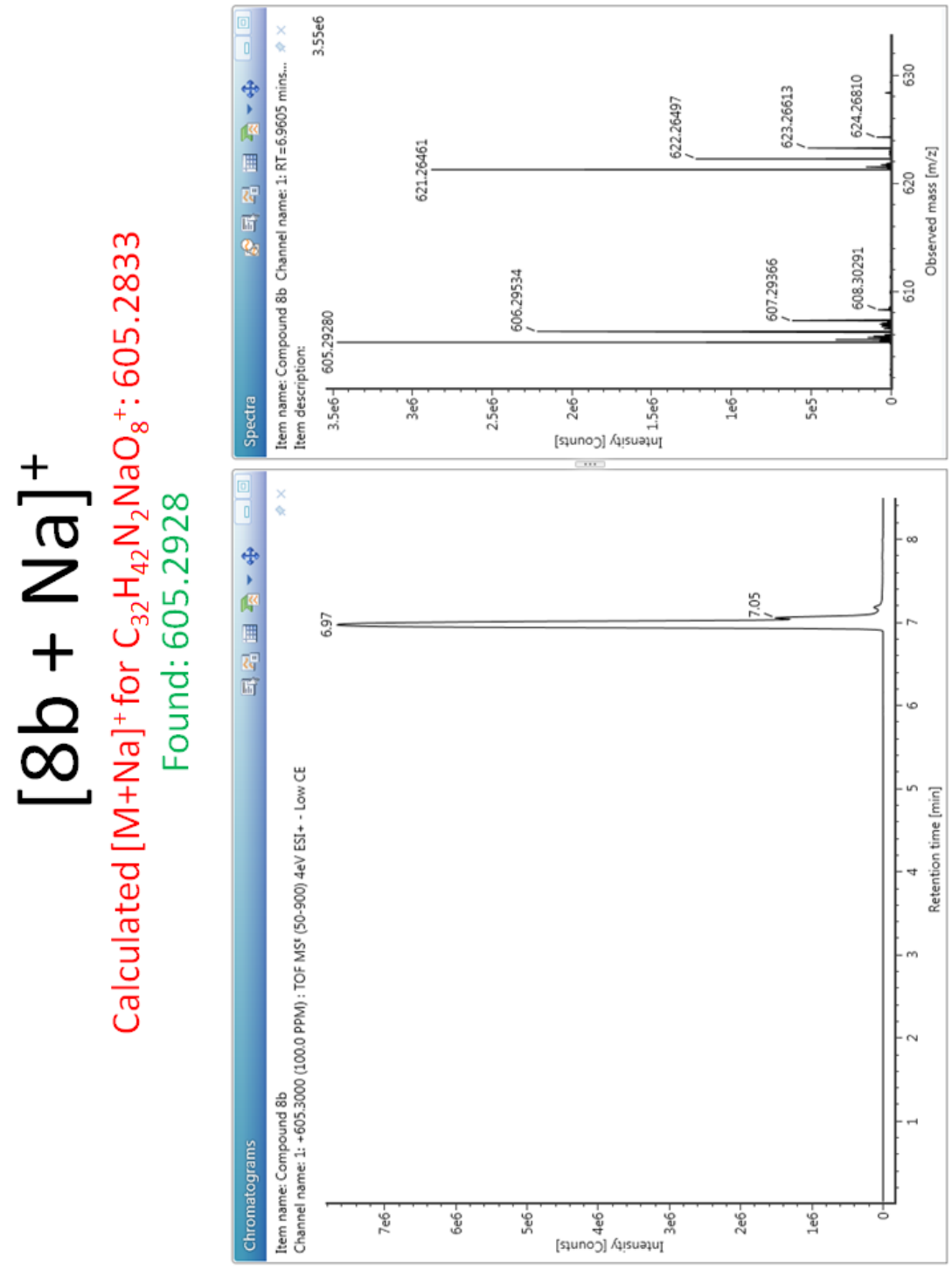

Compound 8b
PR069-186-7
60 mg/mL in CDCl3
Current Data Parameters
NAME PR069-186-7
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160228
Time 9.01
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 31.68
DW 62.400 usec
DE 6.50 usec
TE 298.2 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1418006 MHz
NUC1 1H
P1 9.88 usec
PLW1 15.92000008 W
F2 - Processing parameters
SI 65536
SF 400.1400105 MHz
WDW EM
SSB 0
LB 0.20 Hz
GB 0
PC 1.00
BRUKER
6.76
6.66
6.64
6.63
6.61
6.59
6.31
5.19
5.12
5.10
4.57
4.56
4.54
4.53
4.30
4.28
0.55
1.01
0.42
2.00
2.01
0.60
0.40
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
ppm


Compound 8b
PR069-186-7
60 mg/mL in CDCl3
Current Data Parameters
NAME PR069-186-7
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160228
Time 9.01
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 31.68
DW 62.400 usec
DE 6.50 usec
TE 298.2 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1418006 MHz
NUC1 1H
P1 9.88 usec
PLW1 15.92000008 W
F2 - Processing parameters
SI 65536
SF 400.1400105 MHz
WDW EM
SSB 0
LB 0.20 Hz
GB 0
PC 1.00
BRUKER
2.76
2.71
2.27
2.26
2.24
2.04
2.03
2.01
2.00
1.99
1.86
1.85
1.84
1.26
2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 ppm
3.00
2.00
1.01
1.02
18.04

^{13}C NMR - Compound 8b



HRMS - Compound 8b



Achiral HPLC - Compound 9b

```
================================================================================
Acq. Operator    : MRL                                  Seq. Line :  10
Acq. Instrument  : Instrument 1                          Location : Vial 75
Injection Date   : 02/28/17  9:56:43 PM                      Inj :   1
                                                      Inj Volume : 3.0 µl

Acq. Method      : C:\CHEM32\1\DATA\MRL170228A\MRL170228A 2017-02-28 18-39-47\SDAP3.M
Last changed     : 02/28/17  6:37:32 PM by MRL
Analysis Method  : C:\CHEM32\1\METHODS\SDAP3.M
Last changed     : 03/01/17  10:16:40 AM by MRL
                   (modified after loading)
Additional Info  : Peak(s) manually integrated
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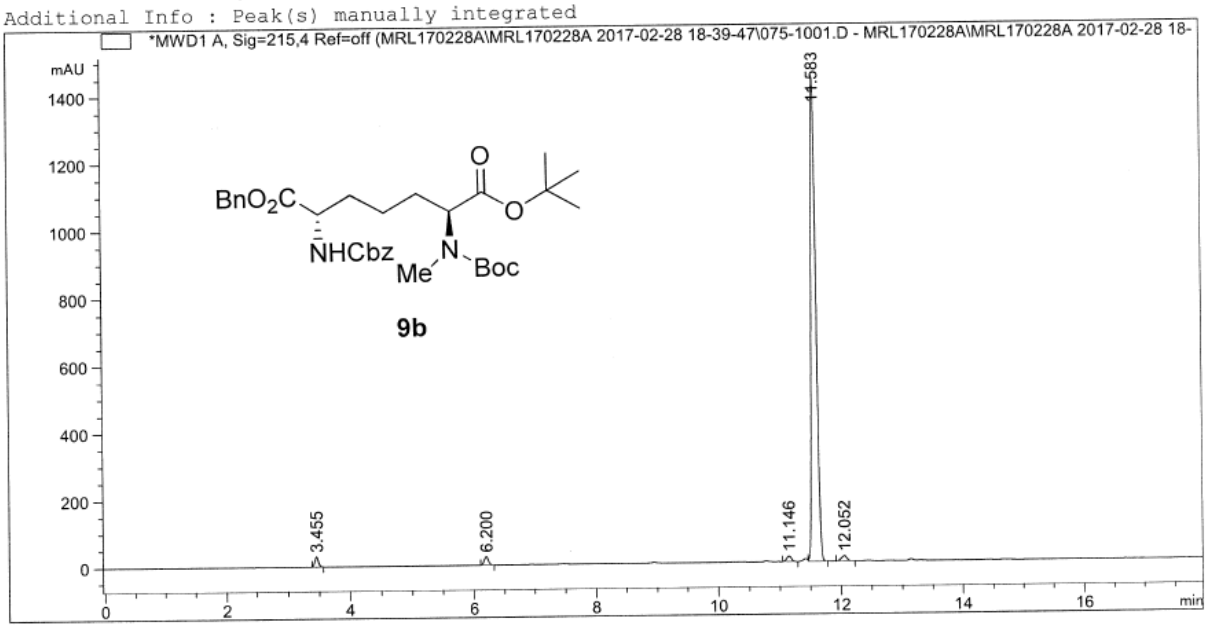



```
================================================================================
                            Area Percent Report
================================================================================

Signal 1: MWD1 A, Sig=215,4 Ref=off
 Signal has been modified after loading from rawdata file!

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.455 BB    0.0584  115.78800   30.40140   1.3504
   2   6.200 BB    0.0721  118.88693   25.66010   1.3865
   3  11.146 BB    0.0810   87.09289   16.66339   1.0157
   4  11.583 VB    0.0918 8150.43506 1444.79565  95.0551
   5  12.052 BB    0.0929  102.23224   16.36980   1.1923

Totals :                  8574.43512 1533.89035

================================================================================
```

Chiral HPLC - Compound 9b

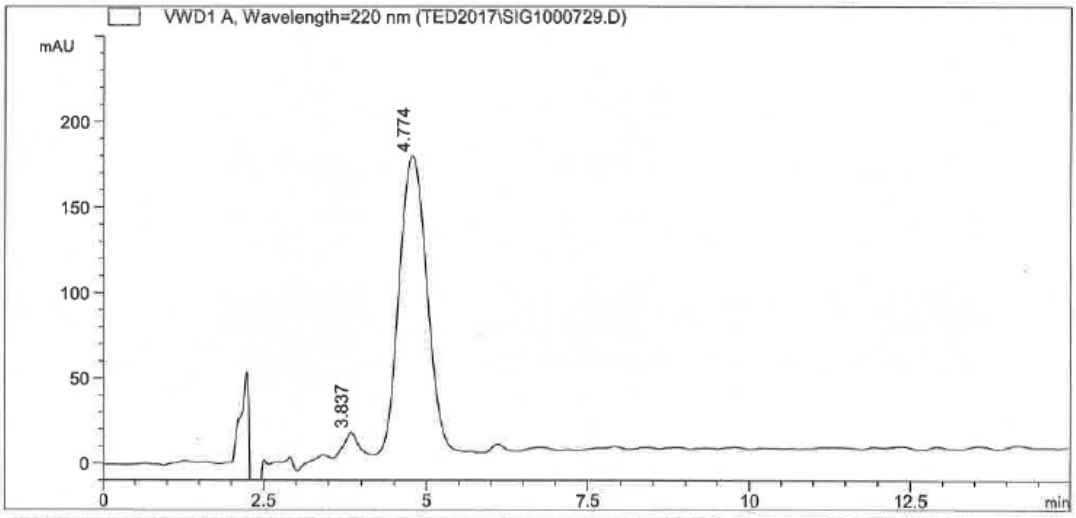
REGIS® TECHNOLOGIES, INC.
8210 Austin Avenue
Morton Grove, IL 60053

E-mail: teds@registech.com
Phone: (847) 583-7661
www.registech.com

LC Chiral Screening Data Report

C:\CHEM32\1\DATA\TED2017\SIG1000729.D

```
Column Name    :  RegisPack
Catalog #      :  783104
Column Size    :  250 mm x 4.6 mm
Particle Size  :  5 micron
Sample Name    :  PR075-001-3
Mobile Phase   :  Hexane/IPA (70/30) + 0.1% DEA
Flow Rate      :  1.5  mL/min
Injection Vol  :  10.0 uL
Pressure       :  82.5     bar
```



Peak #	RT [min]	Width [min]	Area	Area %
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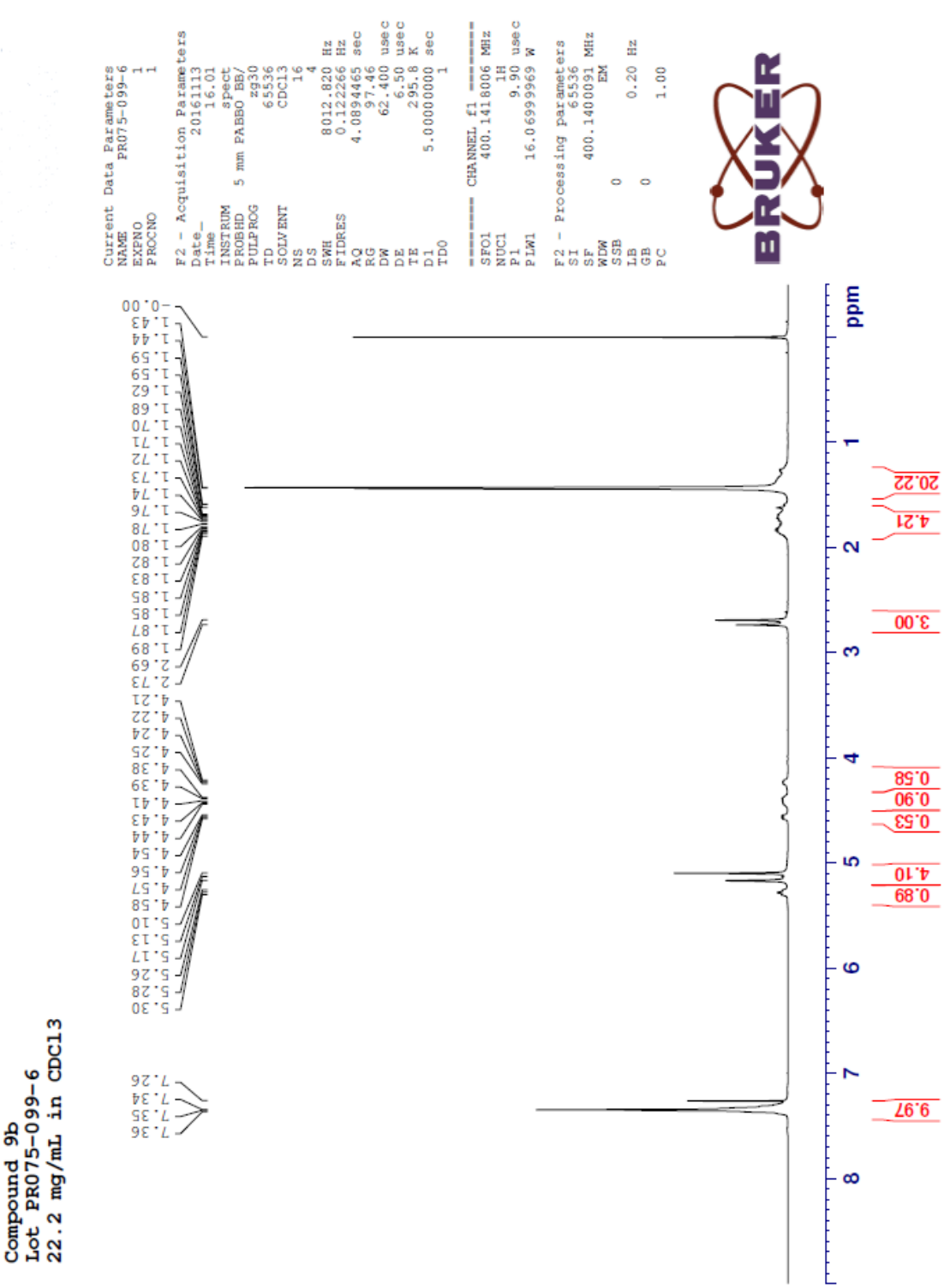
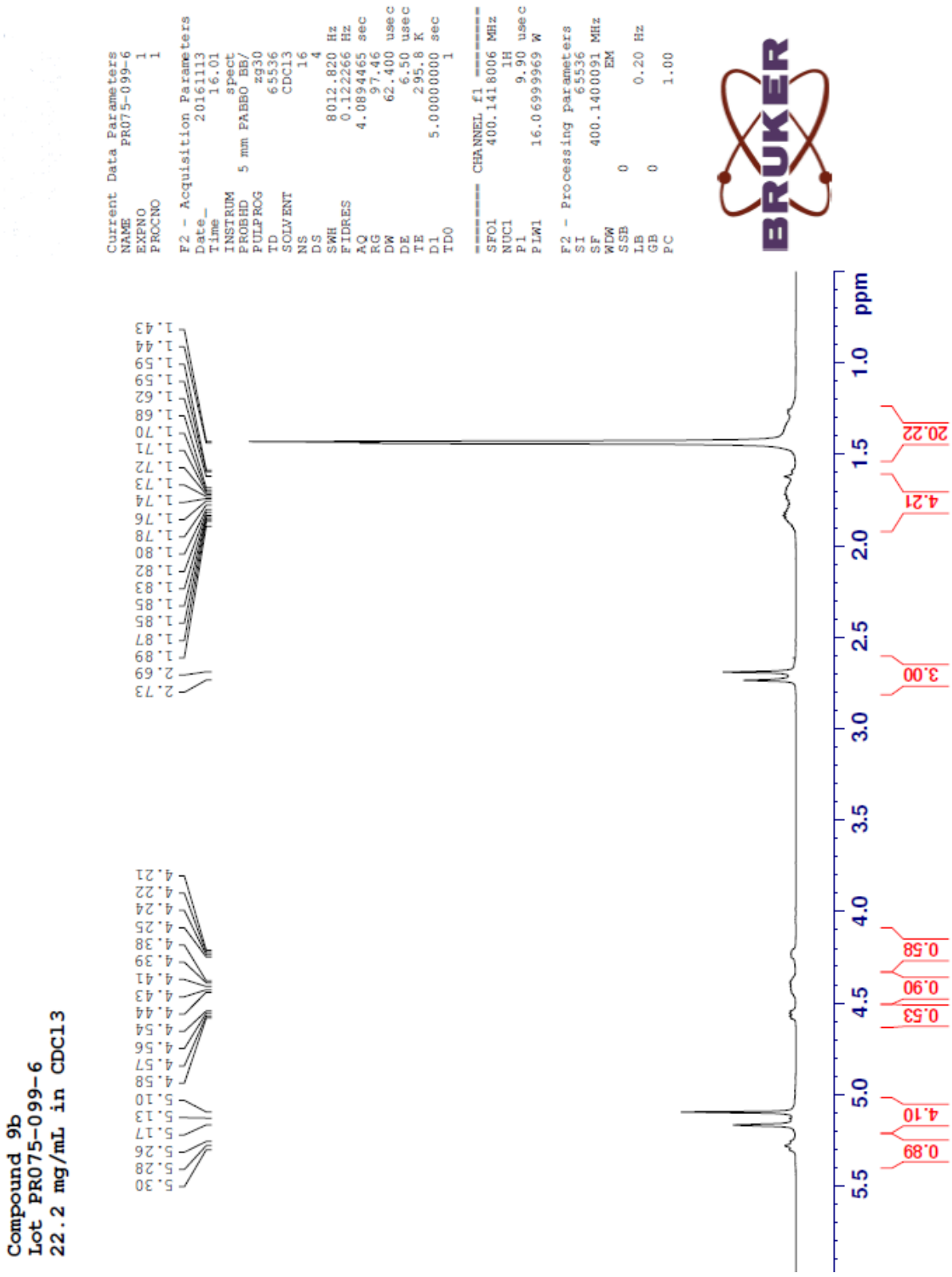
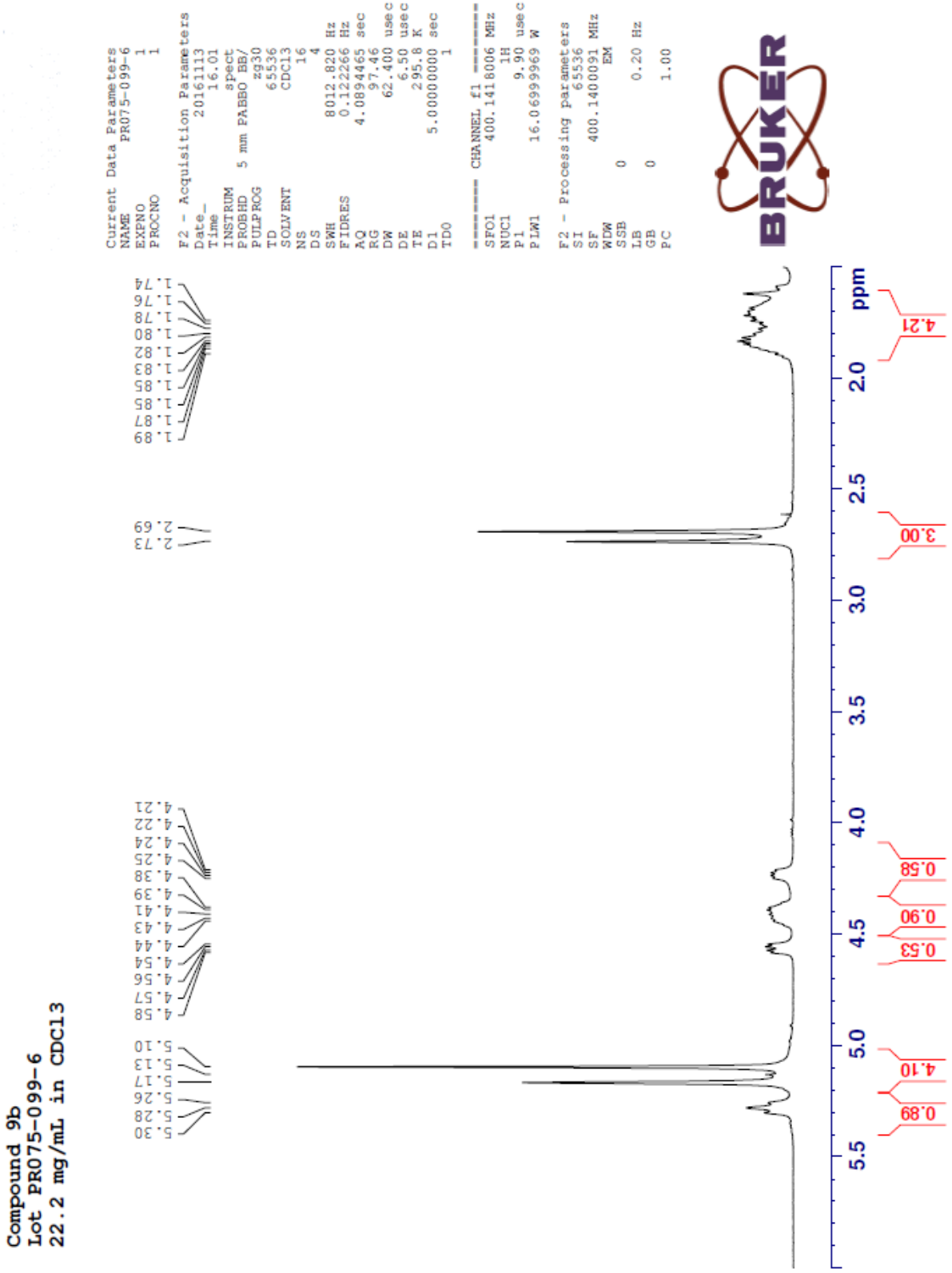
2	4.77	0.488	5072.15	96.05

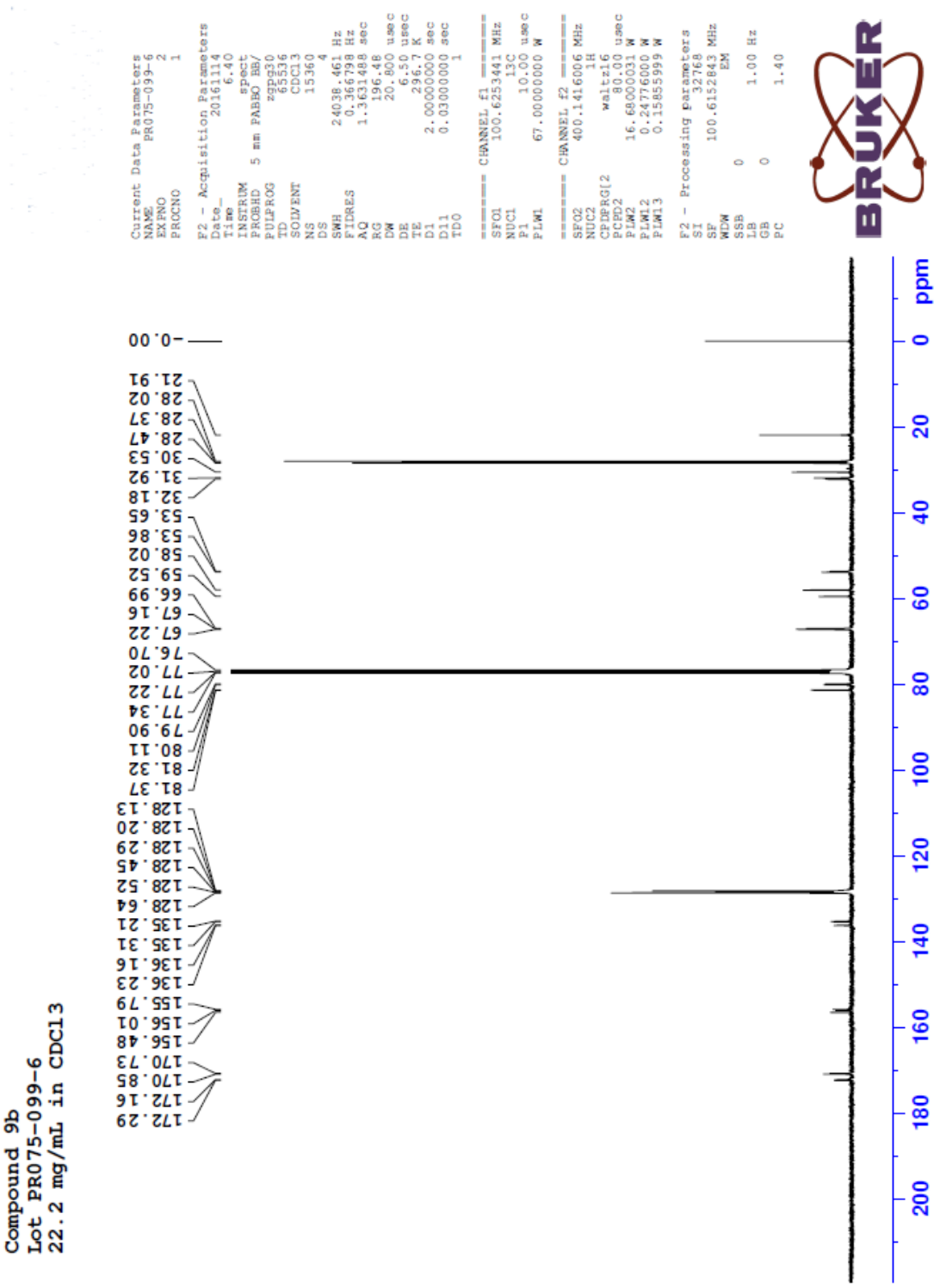
^{1}H NMR - Compound 9b



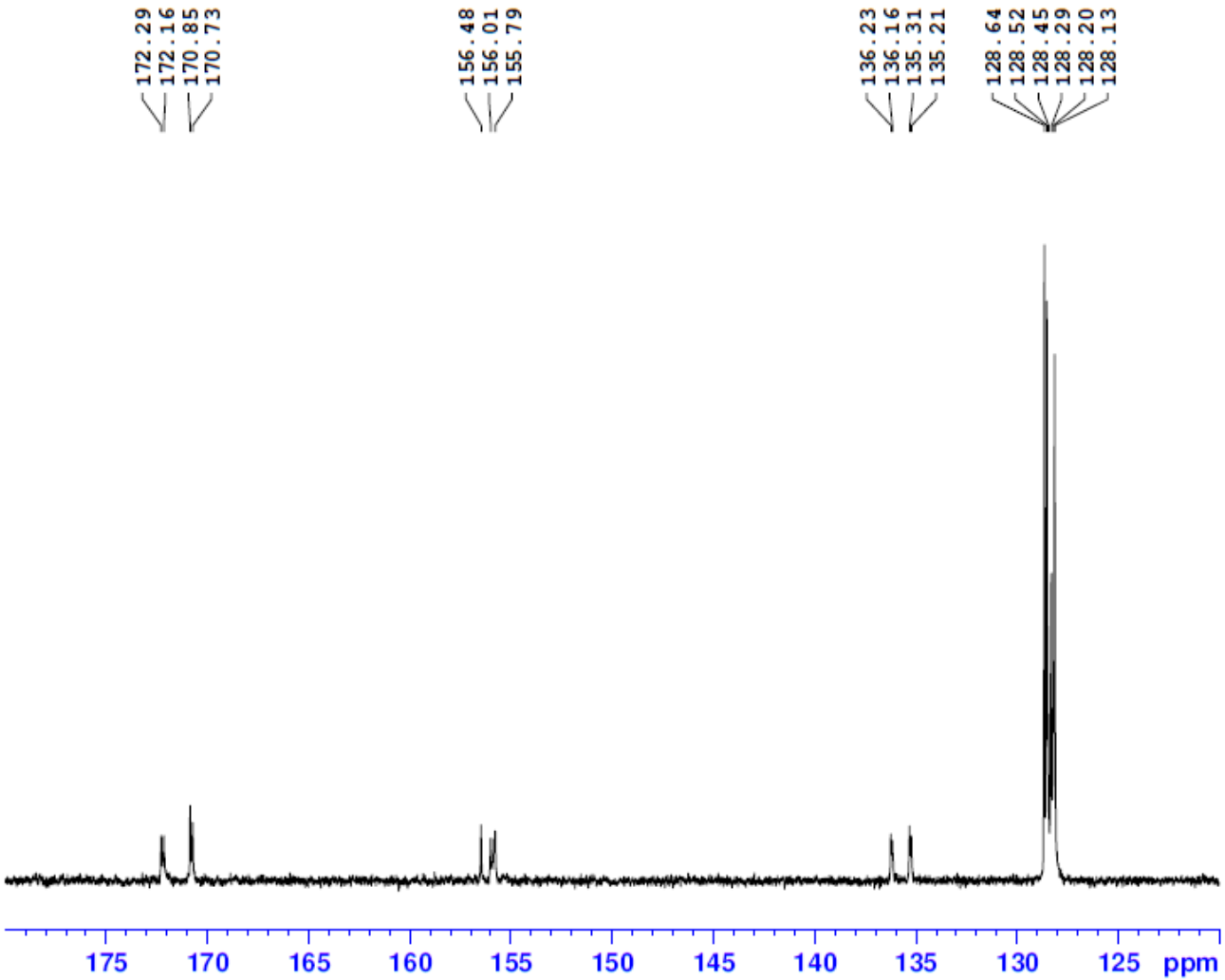

Compound 9b
Lot PR075-099-6
22.2 mg/mL in CDCl3
Current Data Parameters
NAME PR075-099-6
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20161113
Time 16.01
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 97.46
DW 62.400 usec
DE 6.50 usec
TE 295.8 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1418006 MHz
NUC1 1H
P1 9.90 usec
PLW1 16.06999969 W
F2 - Processing parameters
SI 65536
SF 400.1400091 MHz
WDW EM
SSB 0
LB 0.20 Hz
GB 0
PC 1.00
BRUKER
ppm
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.89
4.10
0.53
0.90
0.58
3.00
4.21
20.22


Compound 9b
Lot PR075-099-6
22.2 mg/mL in CDCl3
Current Data Parameters
NAME PR075-099-6
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20161113
Time_ 16.01
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 97.46
DW 62.400 usec
DE 6.50 usec
TE 295.8 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1418006 MHz
NUC1 1H
P1 9.90 usec
PLW1 16.06999969 W
F2 - Processing parameters
SI 65536
SF 400.1400091 MHz
WDW EM
SSB 0
LB 0.20 Hz
GB 0
PC 1.00
BRUKER
1.74
1.76
1.78
1.80
1.82
1.83
1.85
1.85
1.87
1.89
2.69
2.73
4.21
4.22
4.24
4.25
4.38
4.39
4.41
4.43
4.44
4.54
4.56
4.57
4.58
5.10
5.13
5.17
5.26
5.28
5.30
ppm
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
0.89
4.10
0.53
0.90
0.58
3.00
4.21

^{13}C NMR - Compound 9b



Compound 9b
Lot PR075-099-6
22.2 mg/mL in CDCl3




```
Current Data Parameters
NAME          PR075-099-6
EXPNO                   2
PROCNO                  1

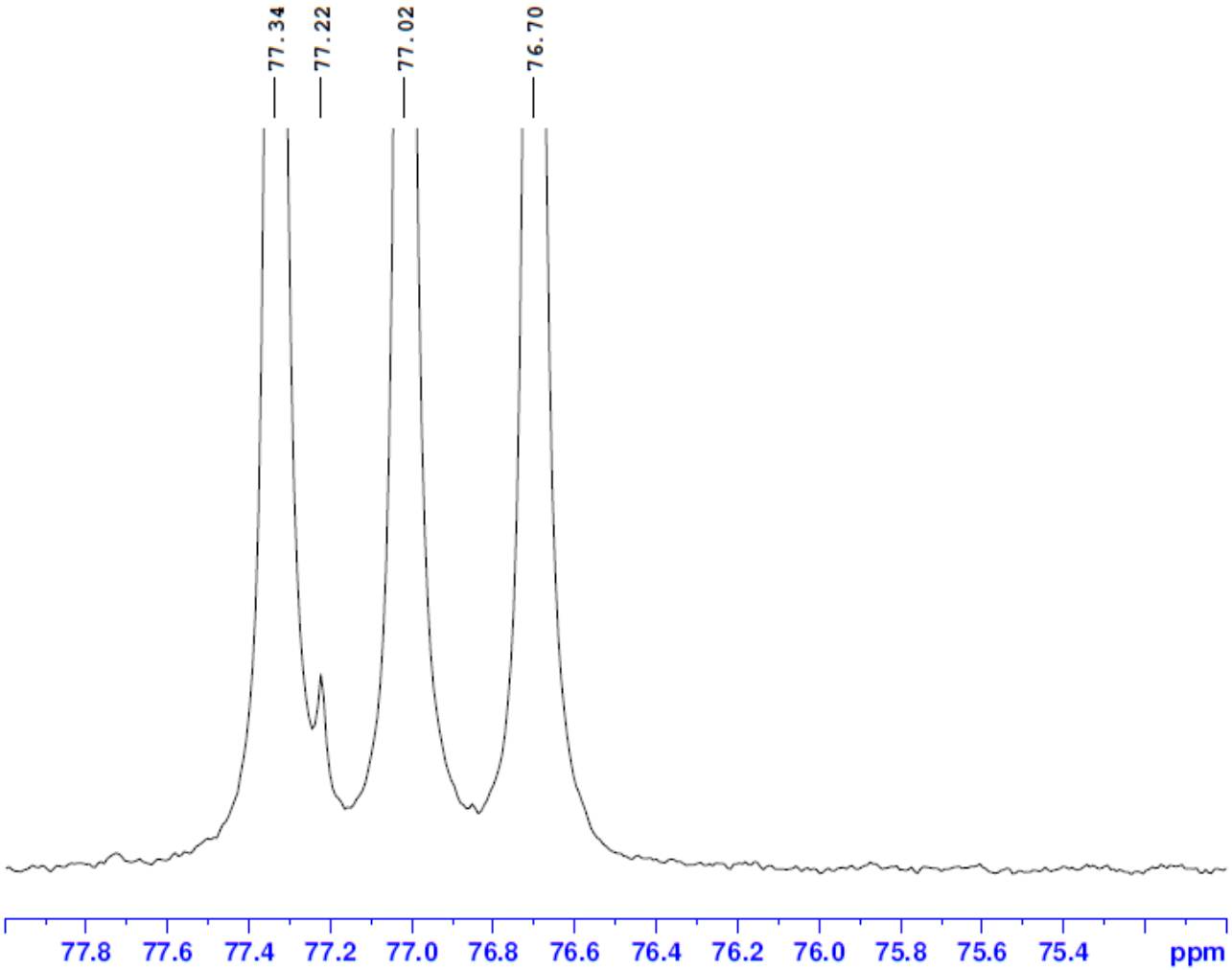
F2 - Acquisition Parameters
Date_          20161114
Time               6.40
INSTRUM           spect
PROBHD   5 mm PABBO BB/
PULPROG         zgpg30
TD               65536
SOLVENT          CDCl3
NS               15360
DS                   4
SWH          24038.461 Hz
FIDRES        0.366798 Hz
AQ            1.3631488 sec
RG               196.48
DW              20.800 usec
DE               6.50 usec
TE              296.7 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                  1

======== CHANNEL f1 =======
SFO1        100.6253441 MHz
NUC1               13C
P1              10.00 usec
PLW1        67.00000000 W

======== CHANNEL f2 =======
SFO2        400.1416006 MHz
NUC2               1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2        16.68000031 W
PLW12        0.24776000 W
PLW13        0.15855999 W

F2 - Processing parameters
SI                32768
SF          100.6152843 MHz
WDW                EM
SSB       0
LB              1.00 Hz
GB        0
PC              1.40
```

Compound 9b
Lot PR075-099-6
22.2 mg/mL in CDCl3



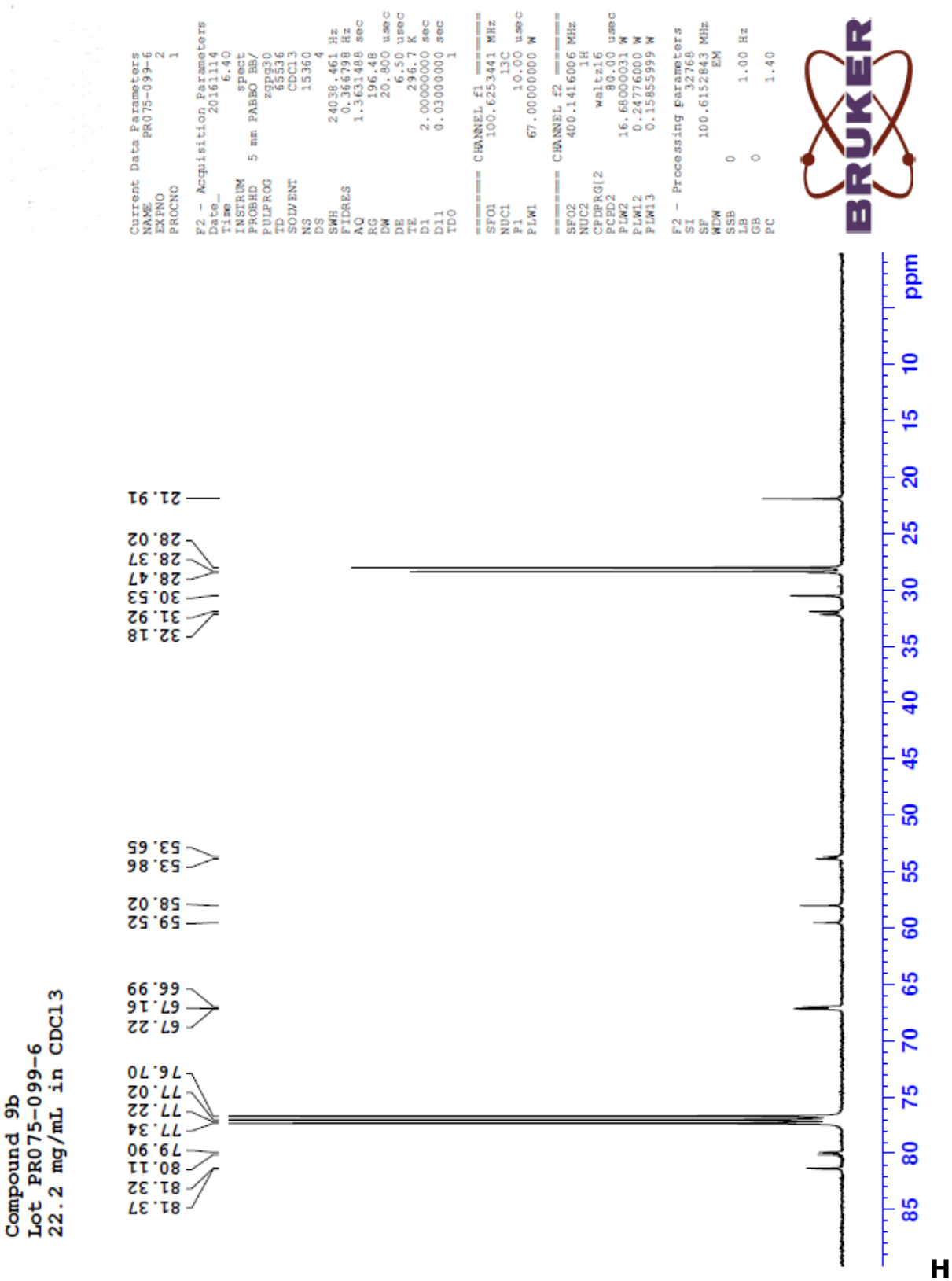

```
Current Data Parameters
NAME          PR075-099-6
EXPNO                   2
PROCNO                  1

F2 - Acquisition Parameters
Date_          20161114
Time               6.40
INSTRUM           spect
PROBHD   5 mm PABBO BB/
PULPROG         zgpg30
TD               65536
SOLVENT          CDCl3
NS               15360
DS                   4
SWH          24038.461 Hz
FIDRES        0.366798 Hz
AQ            1.3631488 sec
RG               196.48
DW               20.800 usec
DE                6.50 usec
TE               296.7 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                  1

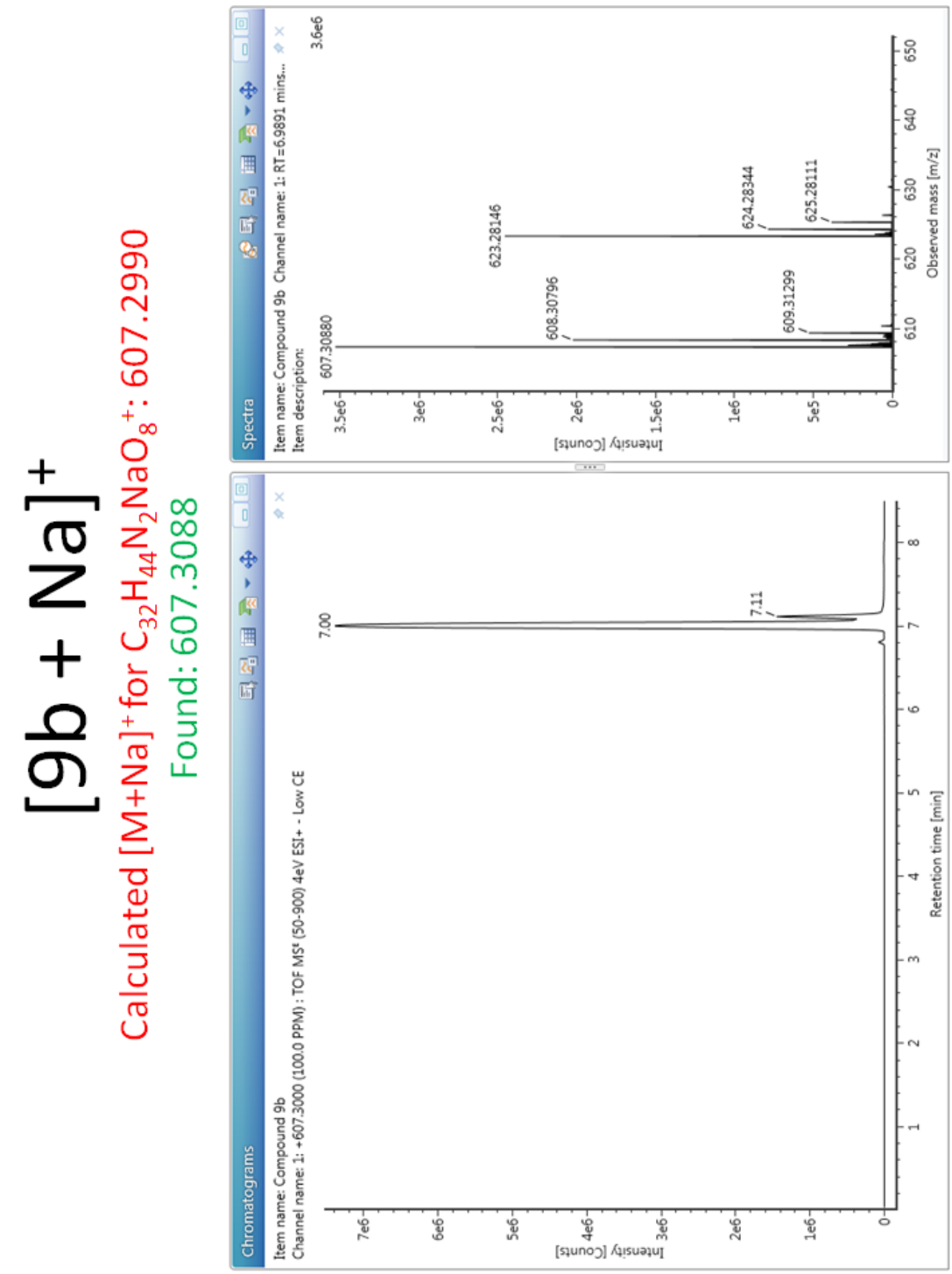
======== CHANNEL f1 ========
SFO1        100.6253441 MHz
NUC1                13C
P1                10.00 usec
PLW1         67.00000000 W

======== CHANNEL f2 ========
SFO2        400.1416006 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2         16.68000031 W
PLW12         0.24776000 W
PLW13         0.15855999 W

F2 - Processing parameters
SI                 32768
SF          100.6152843 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```


Compound 9b
Lot PR075-099-6
22.2 mg/mL in CDCl3
Current Data Parameters
NAME PR075-099-6
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20161114
Time 6.40
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 1536
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 196.48
DW 20.800 usec
DE 6.50 usec
TE 296.7 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6253441 MHz
NUC1 13C
P1 10.00 usec
PLW1 67.00000000 W
======== CHANNEL f2 ========
SFO2 400.1416006 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 16.68000031 W
PLW12 0.24776000 W
PLW13 0.15855999 W
F2 - Processing parameters
SI 32768
SF 100.6152843 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
BRUKER
21.91
28.02
28.37
28.47
30.53
31.92
32.18
53.65
53.86
58.02
59.52
66.99
67.16
67.22
76.70
77.02
77.22
77.34
79.90
80.11
81.32
81.37
ppm
H

MS - Compound 9b



UPLC/MS - Amino Acid Intermediate 10b

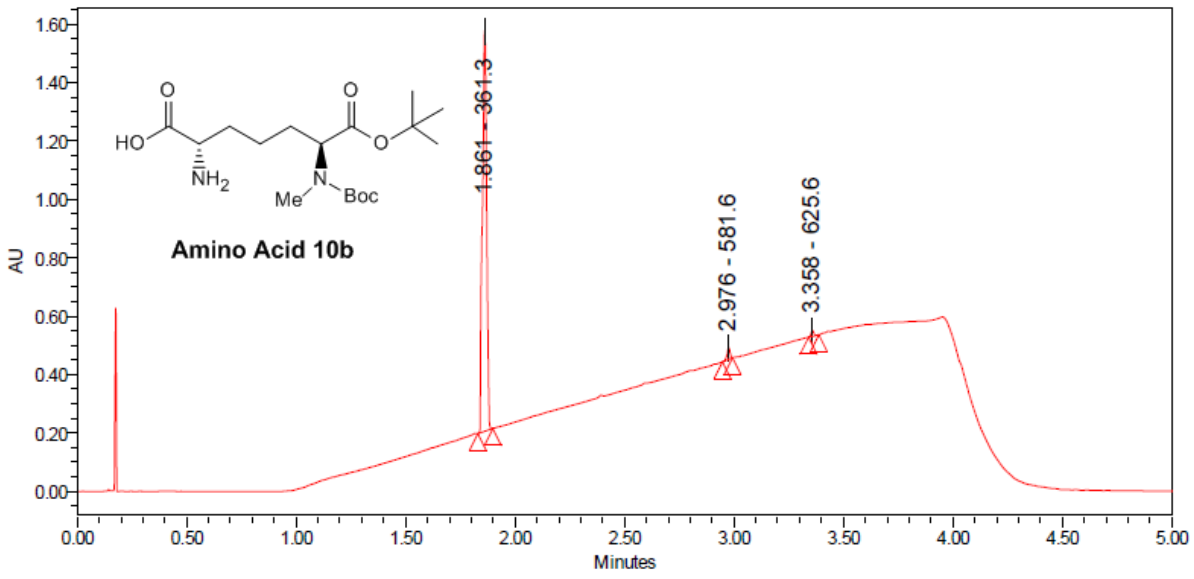
Empower 3 SOFTWARE

QDA Report

SAMPLE INFORMATION

Sample Name:	PR075-104-1	Acquired By:	Marlon
Sample Type:	Unknown	Sample Set Name	20161103
Vial:	1:F,3	Acq. Method Set:	Gradient AB Positive
Injection #:	1	Processing Method	Process 0729
Injection Volume:	5.00 ul	Channel Name:	PDA Max Plot 190.0 - 800.0
Run Time:	5.0 Minutes	Proc. Chnl. Descr.:	PDA Spectrum PDA MaxPlot
Date Acquired:	11/03/16 11:59:25 AM CDT		
Date Processed:	11/03/16 12:04:27 PM CDT		

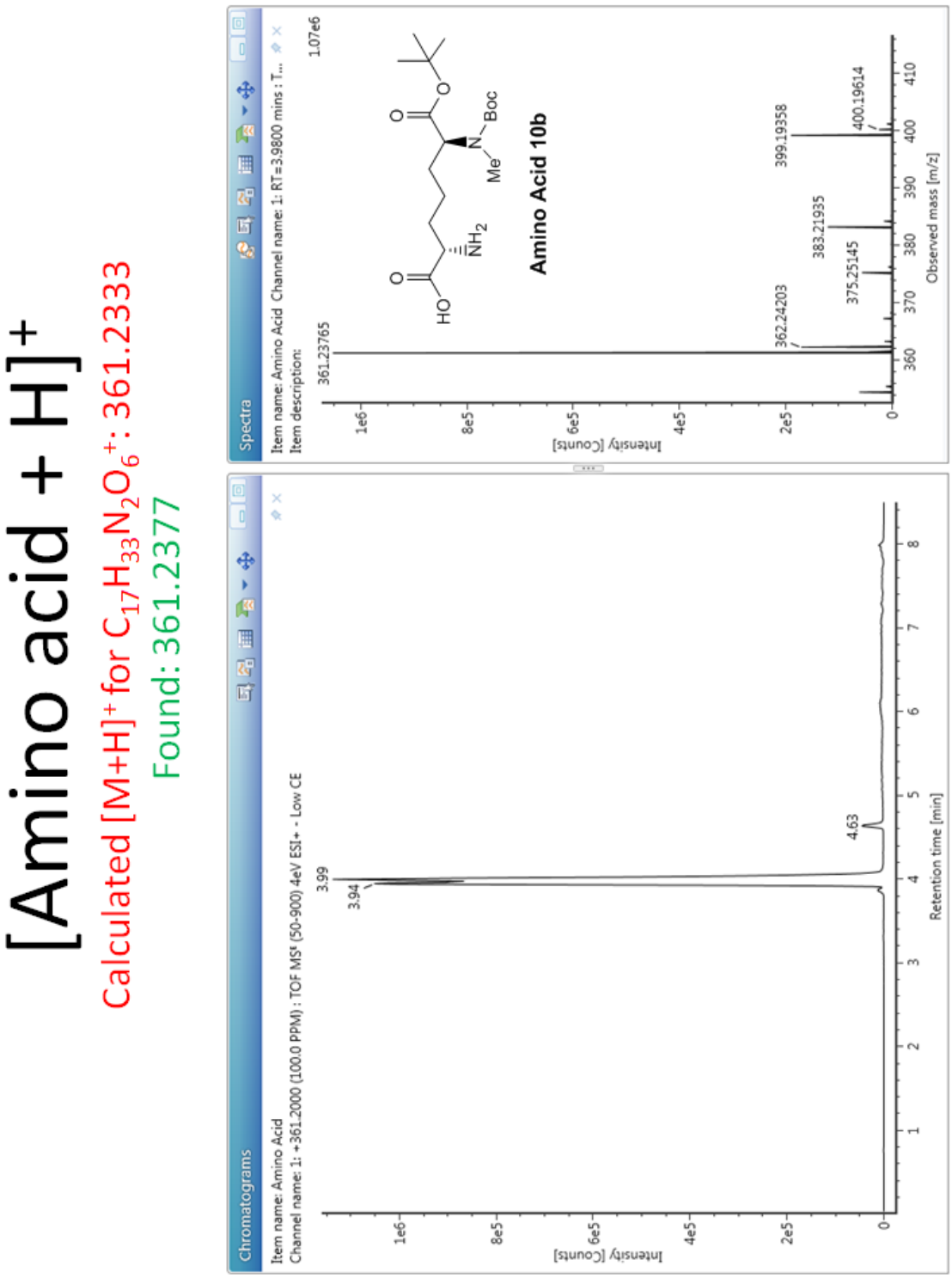
Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	% Area	Height
1		1.861	1852192	97.15	1372625
2		2.976	37713	1.98	39398
3		3.358	16630	0.87	20675

HRMS - Amino Acid Intermediate 10b



Chiral HPLC - Compound 10b

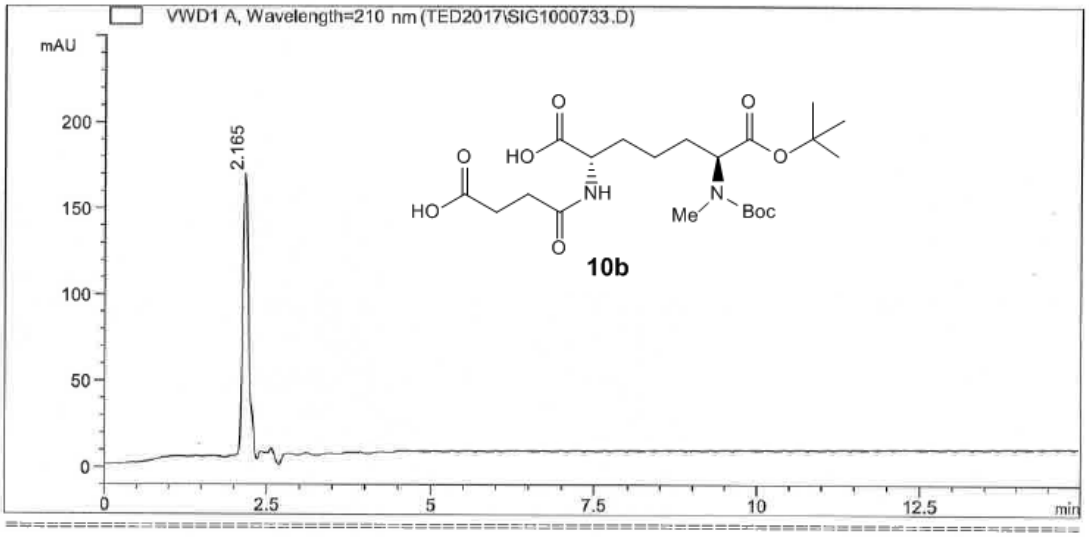
REGIS® TECHNOLOGIES, INC.
8210 Austin Avenue
Morton Grove, IL 60053

E-mail: teds@registech.com
Phone: (847) 583-7661
www.registech.com

LC Chiral Screening Data Report

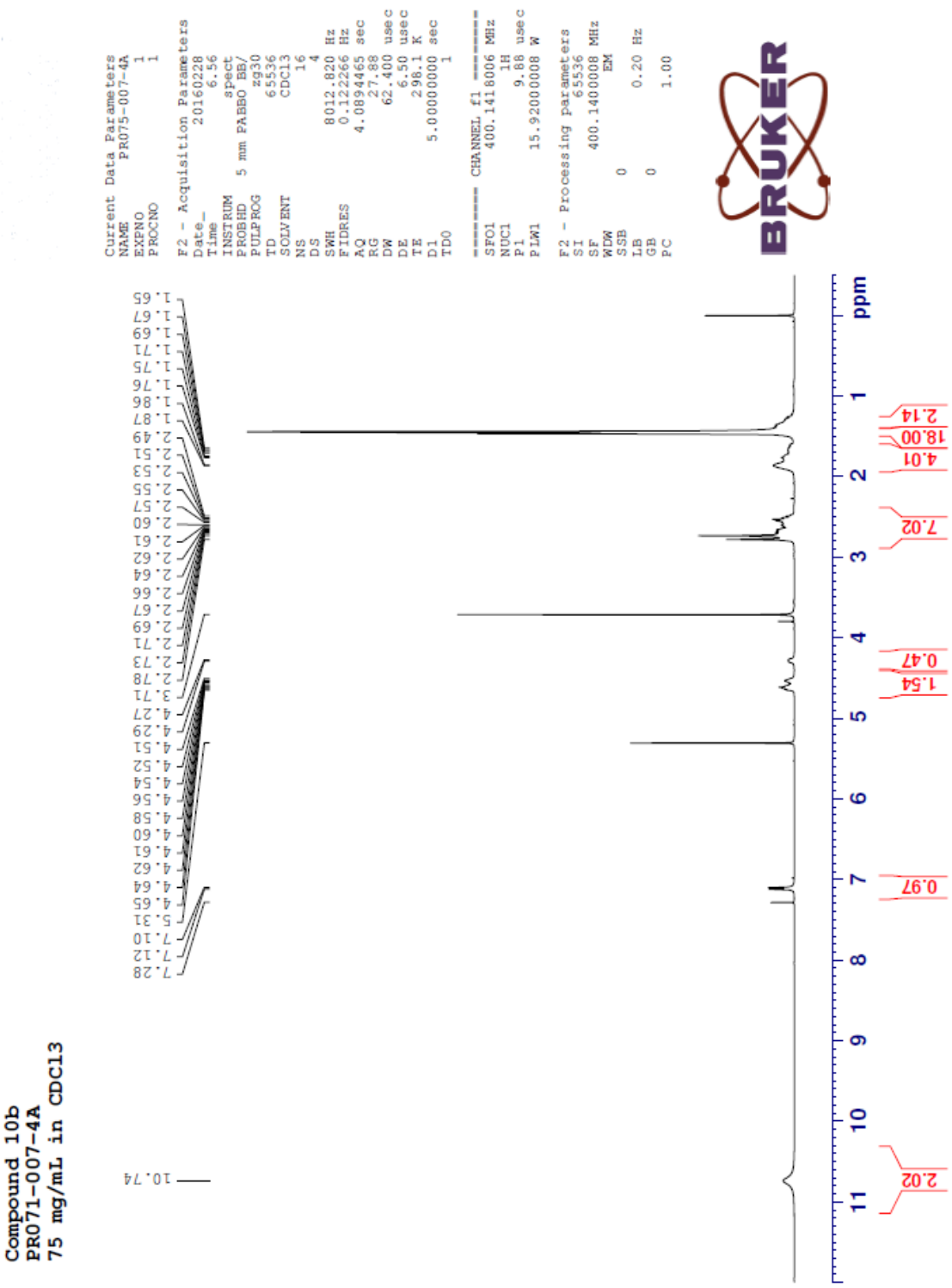
C:\CHEM32\1\DATA\TED2017\SIG1000733.D

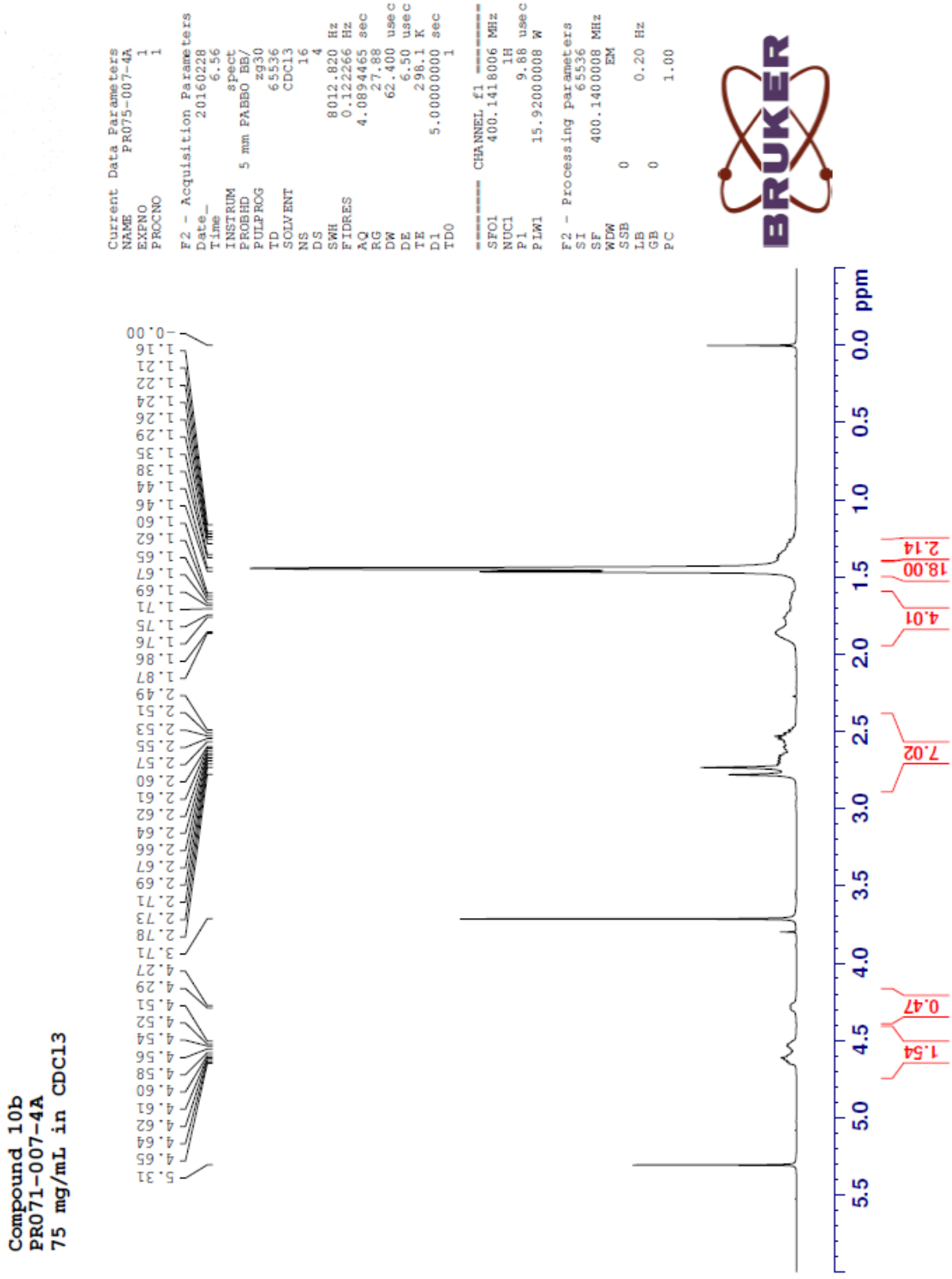
Column Name	:	RegisPack
Catalog #	:	783104
Column Size	:	250 mm x 4.6 mm
Particle Size	:	5 micron
Sample Name	:	PR075-007-4A
Mobile Phase	:	Hexane/IPA (90/10) + 0.1% TFA
Flow Rate	:	1.5 mL/min
Injection Vol	:	10.0 uL
Pressure	:	58.8 bar



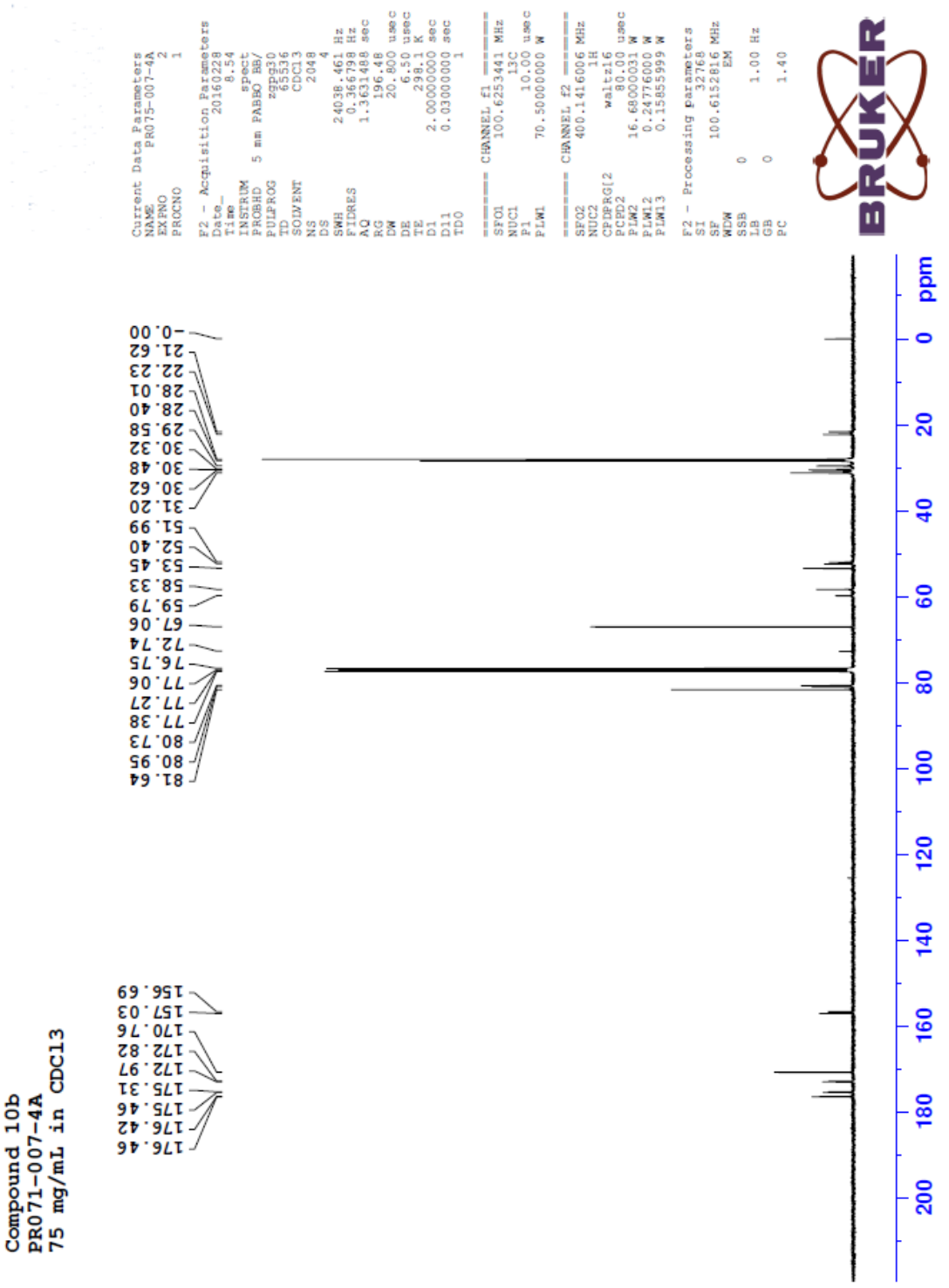
Peak #	RT [min]	Width [min]	Area	Area %
1	2.16	0.109	1196.47	100.00

^{1}H NMR - Compound 10b

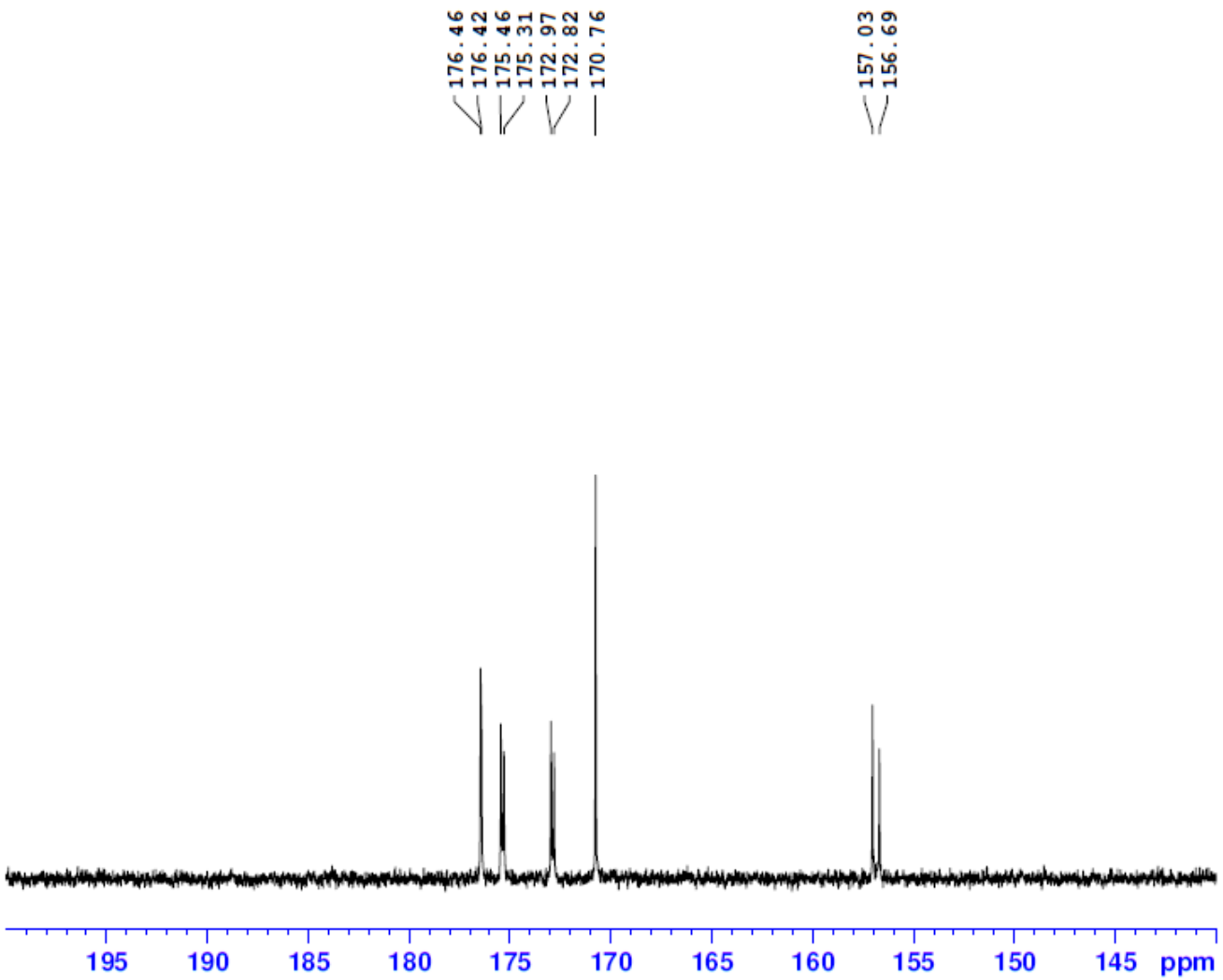



Compound 10b
PR071-007-4A
75 mg/mL in CDCl3
Current Data Parameters
NAME PR075-007-4A
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160228
Time 6.56
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 27.88
DW 62.400 usec
DE 6.50 usec
TE 296.1 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1418006 MHz
NUC1 1H
P1 9.88 usec
PLW1 15.92000008 W
F2 - Processing parameters
SI 65536
SF 400.1400008 MHz
WDW EM
SSB 0
LB 0.20 Hz
GB 0
PC 1.00
BRUKER
ppm
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
2.14
18.00
4.01
7.02
0.47
1.54

^{13}C NMR - Compound 10b



Compound 10b
PR071-007-4A
75 mg/mL in CDCl3



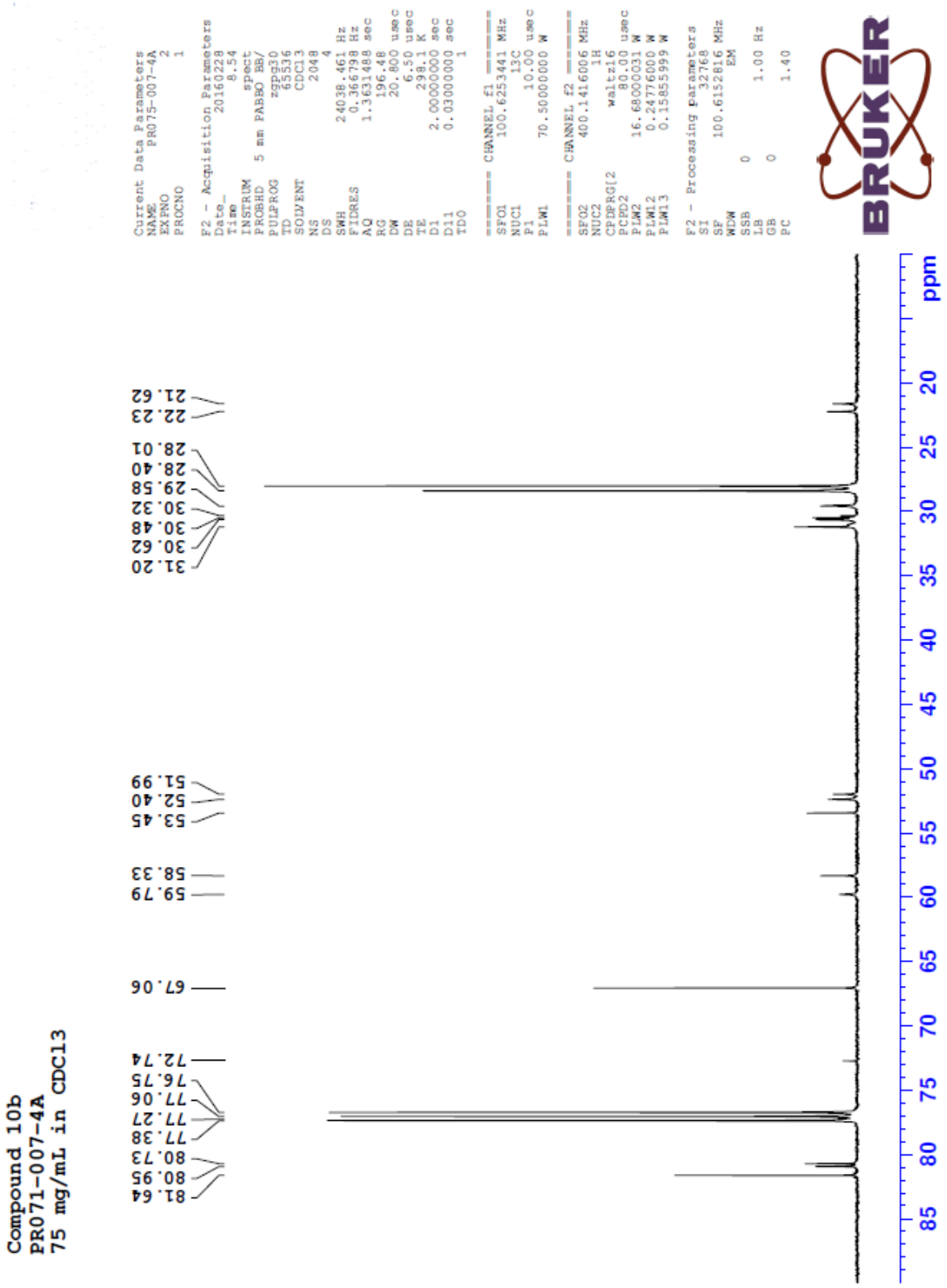

```
Current Data Parameters
NAME         PR075-007-4A
EXPNO                   2
PROCNO                  1

F2 - Acquisition Parameters
Date_           20160228
Time                8.54
INSTRUM            spect
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 2048
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ            1.3631488 sec
RG               196.48
DW               20.800 usec
DE                6.50 usec
TE                298.1 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

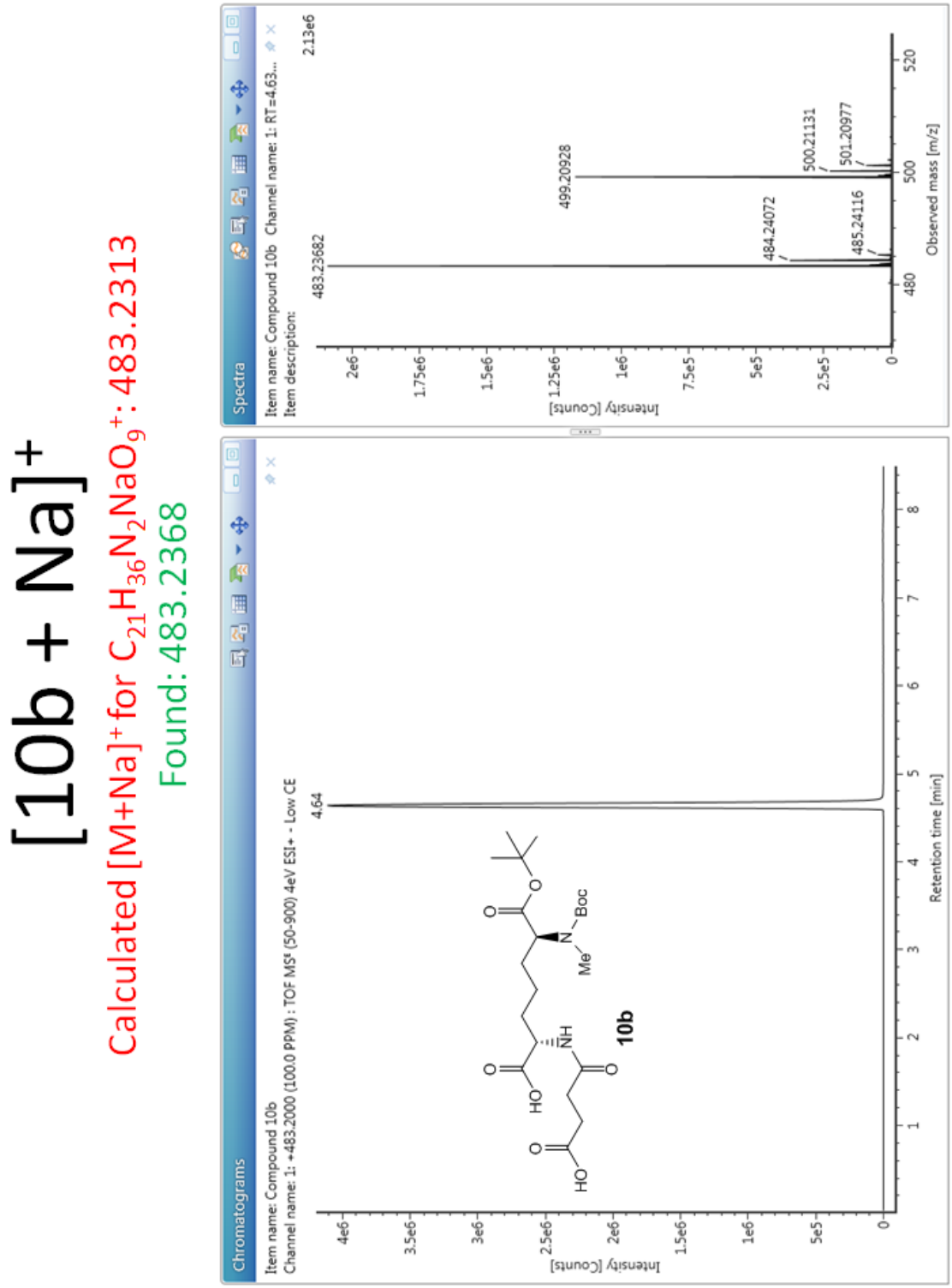
======== CHANNEL f1 =======
SFO1        100.6253441 MHz
NUC1                13C
P1                10.00 usec
PLW1         70.50000000 W

======== CHANNEL f2 =======
SFO2        400.1416006 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2         16.68000031 W
PLW12         0.24776000 W
PLW13         0.15855999 W

F2 - Processing parameters
SI                 32768
SF          100.6152816 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```


Compound 10b
PR071-007-4A
75 mg/mL in CDC13
Current Data Parameters
NAME PR075-007-4A
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160228
Time 8.54
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT CDC13
NS 2048
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 196.48
DW 20.800 usec
DE 6.50 usec
TE 298.1 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
CHANNEL f1
SFO1 100.6253441 MHz
NUC1 13C
P1 10.00 usec
PLW1 70.50000000 W
CHANNEL f2
SFO2 400.1416006 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 16.68000031 W
PLW12 0.24776000 W
PLW13 0.15855999 W
F2 - Processing parameters
SI 32768
SF 100.6152816 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
BRUKER
21.62
22.23
28.01
28.40
29.58
30.32
30.48
30.62
31.20
51.99
52.40
53.45
58.33
59.79
67.06
72.74
76.75
77.06
77.27
77.38
80.73
80.95
81.64
ppm
85
80
75
70
65
60
55
50
45
40
35
30
25
20

HRMS - Compound 10b

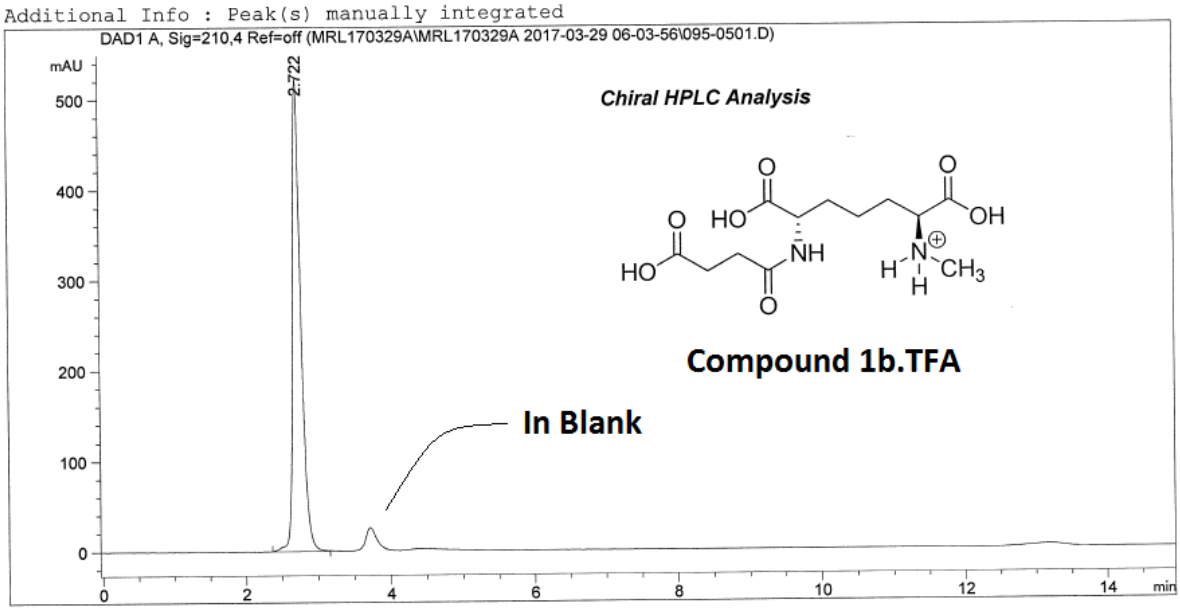


Chiral HPLC - Compound 1b.TFA on a Chirosil SCA(-) column (150 mm x 4.6 mm, 5 micron)

```
==========================================================================
Acq. Operator   : MRL                                Seq. Line :   5
Acq. Instrument : HPLC-31                             Location : Vial 95
Injection Date  : 03/29/17  7:13:03 AM                    Inj :   1
                                                   Inj Volume : 5.0 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 2.5 µl
Acq. Method     : C:\CHEM32\2\DATA\MRL170329A\MRL170329A 2017-03-29 06-03-56\SDAP-CHIRAL8.M
Last changed    : 03/29/17  6:02:45 AM by MW
Analysis Method : C:\CHEM32\2\METHODS\SDAP-CHIRAL8.M
Last changed    : 03/29/17  6:02:45 AM by MW
Method Info     : HPLC 31
                  C=84% MeOH in water, 5 mM HClO4
                  (-), 150mmx4.6mm 5 microns
                  0.75 mL/min 25C 210, 220 nm

Sample Info     : PR075-110-7,   5 mg/mL

Additional Info : Peak(s) manually integrated
```




```
==========================================================================
                          Area Percent Report
==========================================================================

Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   2.722 BB    0.1190 4170.30273  523.04901 100.0000

Totals :                  4170.30273  523.04901

==========================================================================
                          *** End of Report ***
```

Chiral HPLC - Compound 1b.TFA on a RegisPack column (250x4.6 mm, 5 micron)

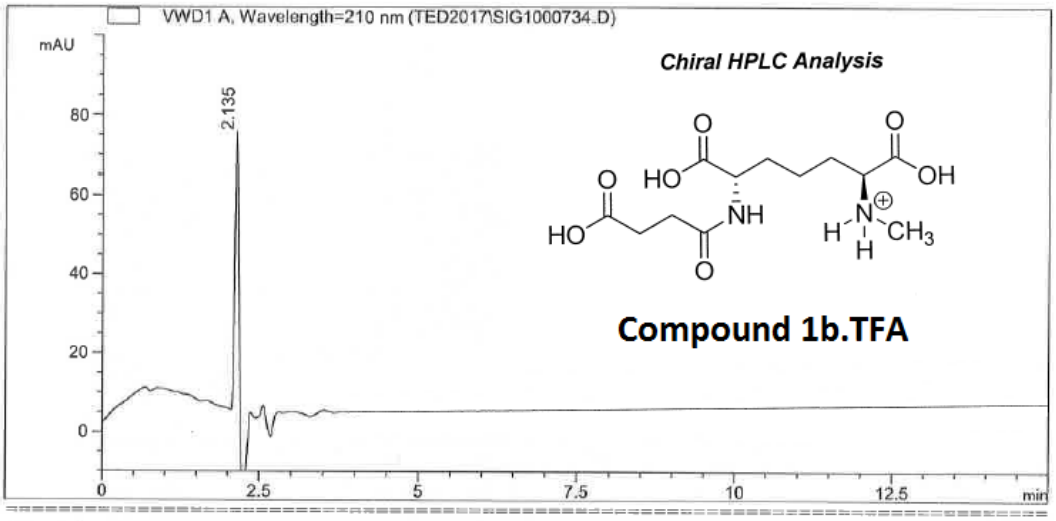
REGIS® TECHNOLOGIES, INC.
8210 Austin Avenue
Morton Grove, IL 60053

E-mail: teds@registech.com
Phone: (847) 583-7661
www.registech.com

LC Chiral Screening Data Report

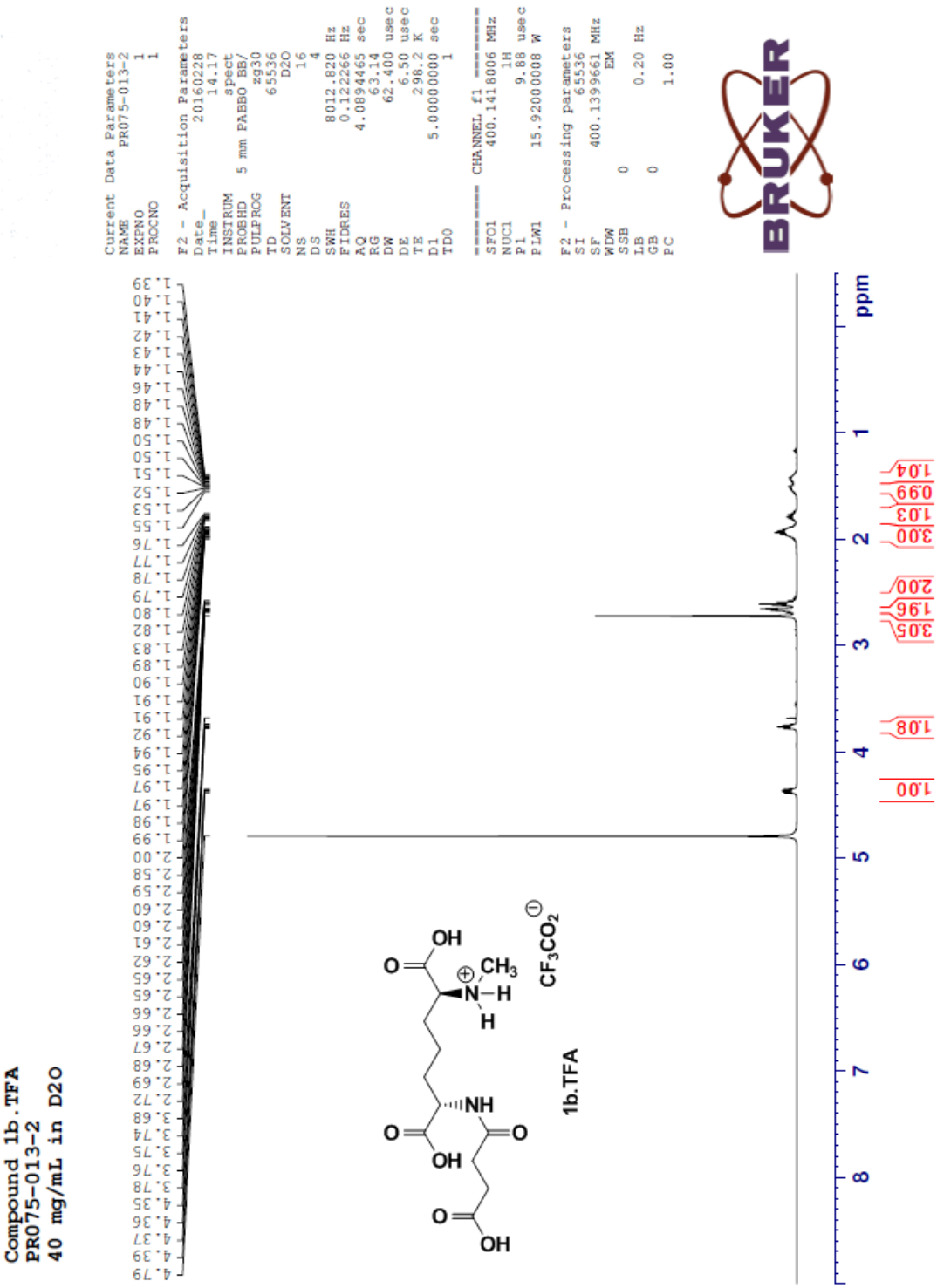
C:\CHEM32\1\DATA\TED2017\SIG1000734.D

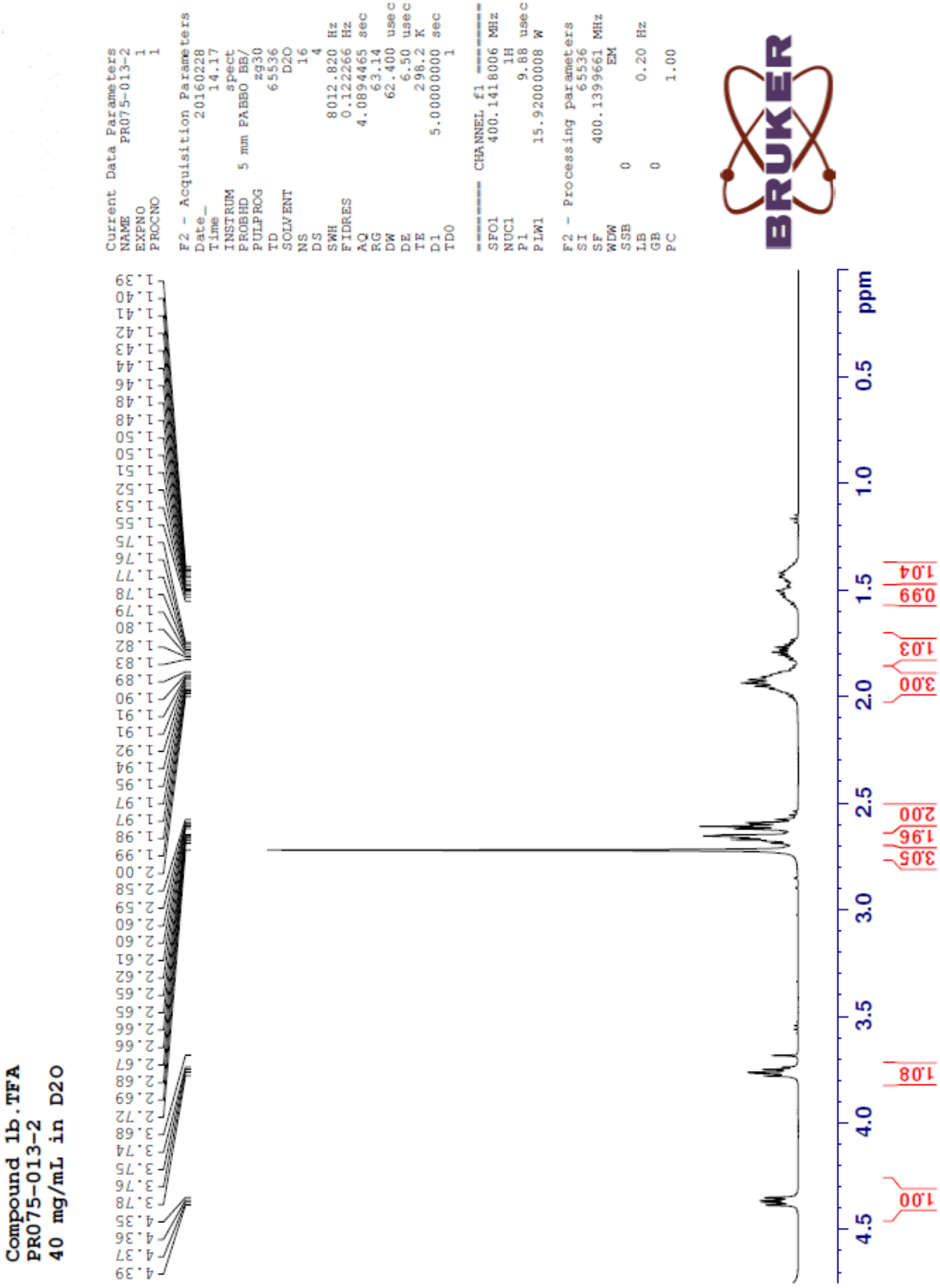
Column Name	:	RegisPack
Catalog #	:	783104
Column Size	:	250 mm x 4.6 mm
Particle Size	:	5 micron
Sample Name	:	PR075-110-7
Mobile Phase	:	Hexane/IPA (90/10) + 0.1% TFA
Flow Rate	:	1.5 mL/min
Injection Vol	:	10.0 uL
Pressure	:	58.5 bar



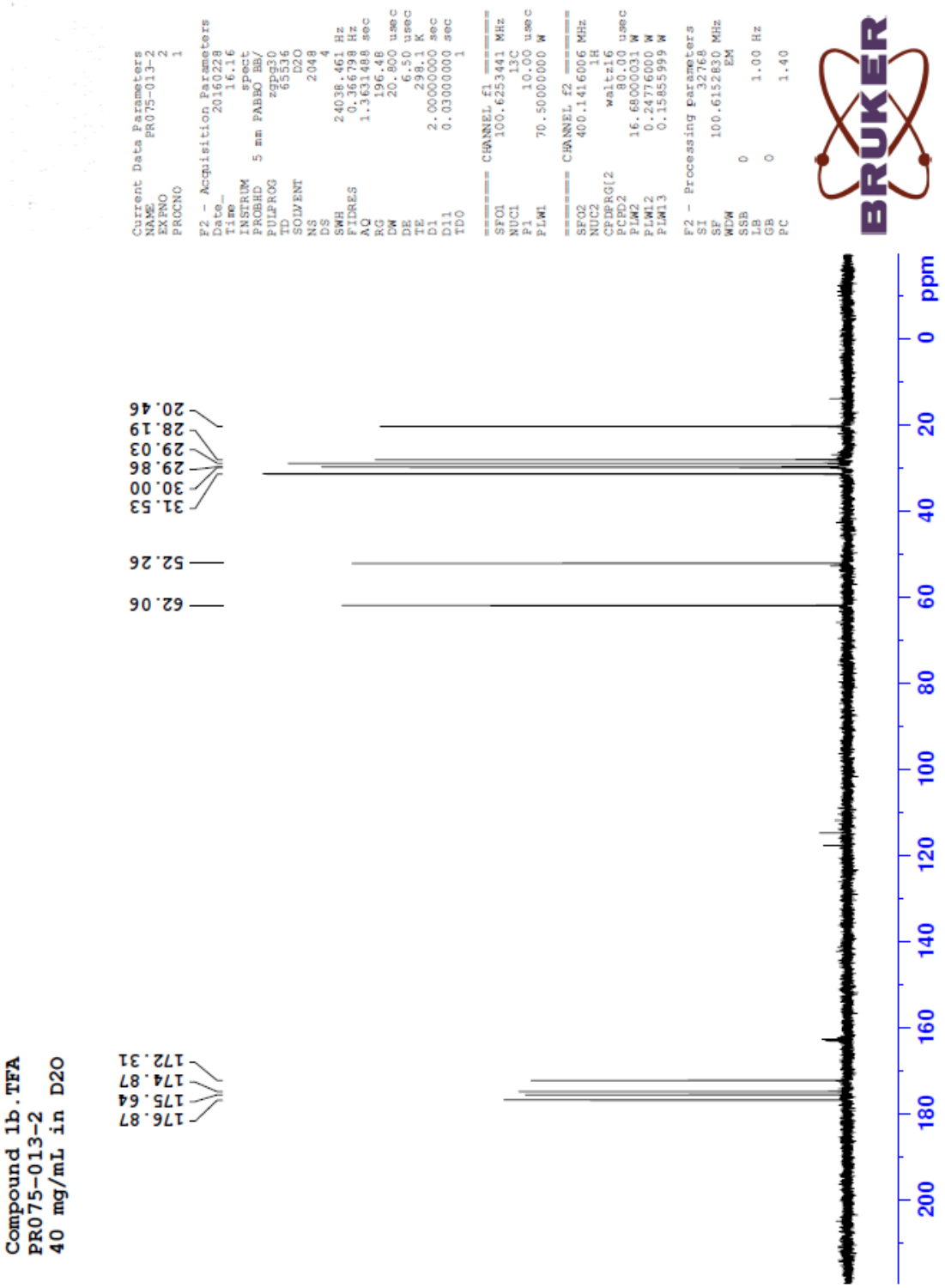
Peak #	RT [min]	Width [min]	Area	Area %
1	2.13	0.088	520.32	100.00

^{1}H NMR - Compound 1b.TFA

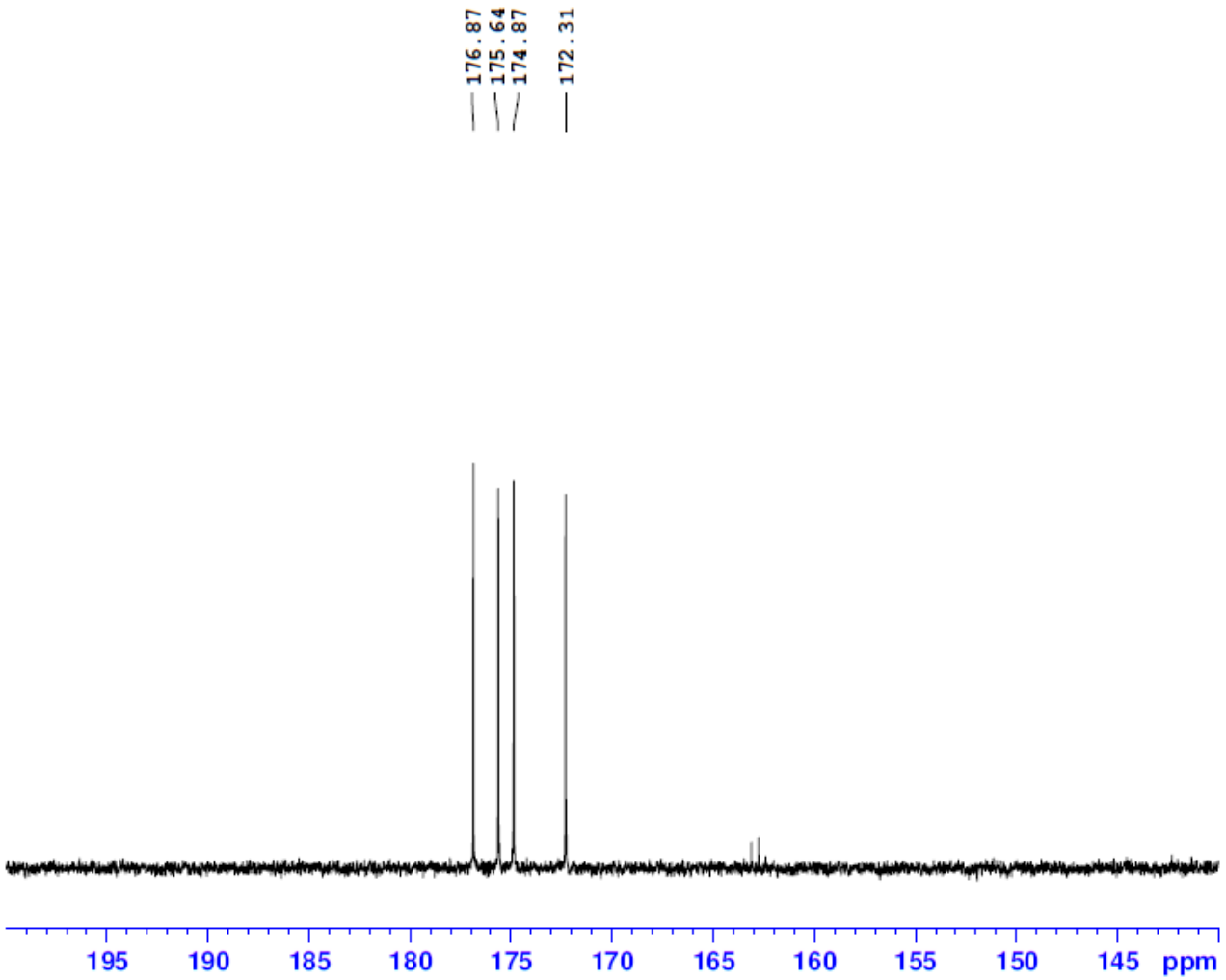



Compound 1b.TFA
PR075-013-2
40 mg/mL in D2O
Current Data Parameters
NAME PR075-013-2
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160228
Time 14.17
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT D2O
NS 16
DS 4
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 63.14
DW 62.400 usec
DE 6.50 usec
TE 298.2 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1418006 MHz
NUC1 1H
P1 9.88 usec
PLW1 15.92000008 W
F2 - Processing parameters
SI 65536
SF 400.1399661 MHz
WDW EM
SSB 0
LB 0.20 Hz
GB 0
PC 1.00
BRUKER
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
1.08
3.05
1.96
2.00
3.00
1.03
0.99
1.04

^{13}C NMR - Compound 1b.TFA



Compound 1b.TFA
PR075-013-2
40 mg/mL in D2O



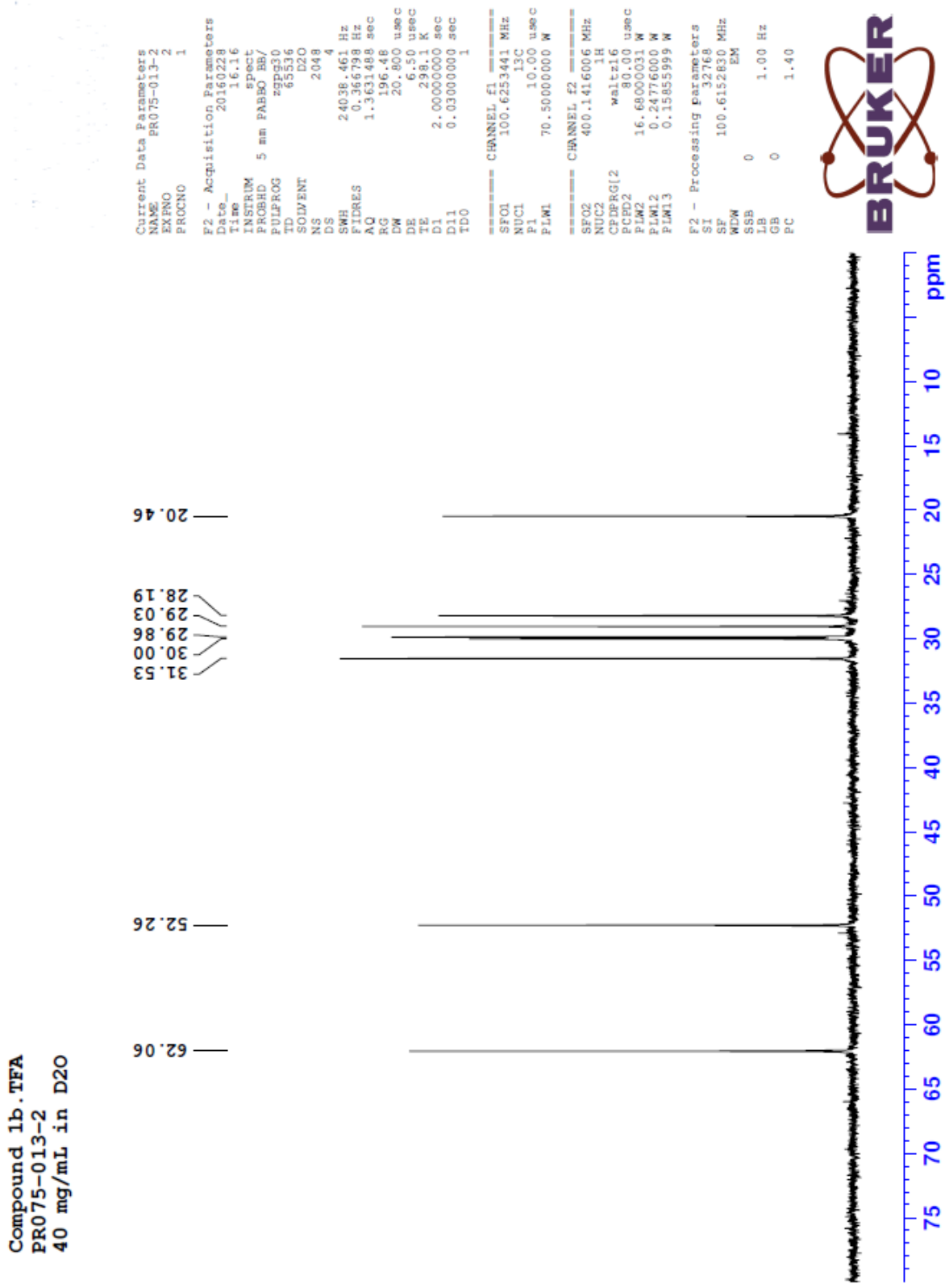

```
Current Data Parameters
NAME          PR075-013-2
EXPNO                   2
PROCNO                  1

F2 - Acquisition Parameters
Date_            20160228
Time                16.16
INSTRUM             spect
PROBHD   5 mm PABBO BB/
PULPROG           zgpg30
TD                 65536
SOLVENT              D2O
NS                  2048
DS                     4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ             1.3631488 sec
RG                196.48
DW                20.800 usec
DE                 6.50 usec
TE                298.1 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1

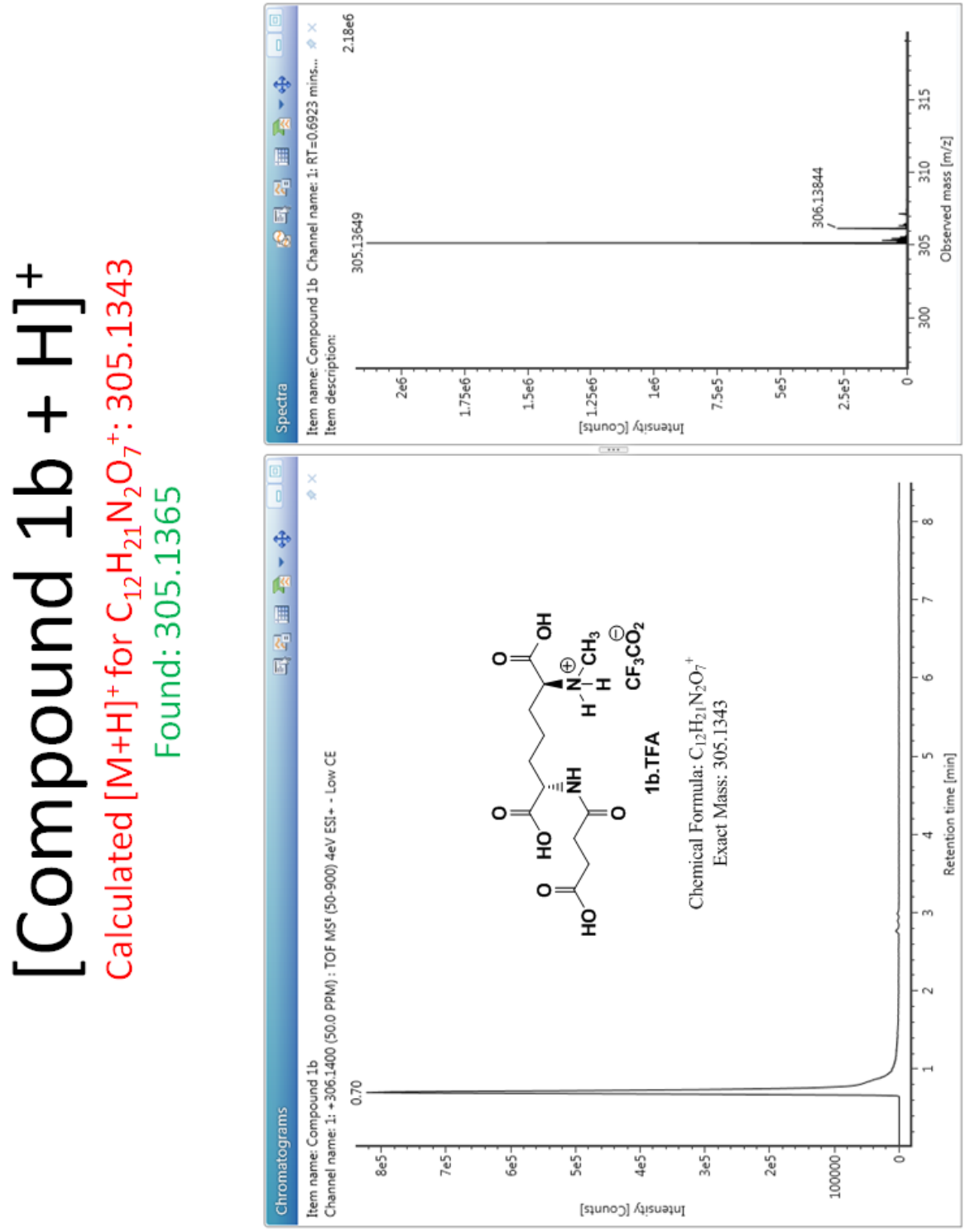
======= CHANNEL f1 =======
SFO1         100.6253441 MHz
NUC1                 13C
P1                 10.00 usec
PLW1         70.50000000 W

======= CHANNEL f2 =======
SFO2         400.1416006 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2              80.00 usec
PLW2         16.68000031 W
PLW12         0.24776000 W
PLW13         0.15855999 W

F2 - Processing parameters
SI                  32768
SF           100.6152830 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```


Compound 1b.TFA
PR075-013-2
40 mg/mL in D2O
Current Data Parameters
NAME PR075-013-2
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160228
Time 16.16
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 4
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 196.48
DW 20.800 usec
DE 6.50 usec
TE 298.1 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6253441 MHz
NUC1 13C
P1 10.00 usec
PLW1 70.50000000 W
======== CHANNEL f2 ========
SFO2 400.1416006 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 16.68000031 W
PLW12 0.24776000 W
PLW13 0.15855999 W
F2 - Processing parameters
SI 32768
SF 100.6152630 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
BRUKER
20.46
28.19
29.03
29.86
30.00
31.53
52.26
62.06
75 70 65 60 55 50 45 40 35 30 25 20 15 10 ppm

HRMS - Compound 1b.TFA

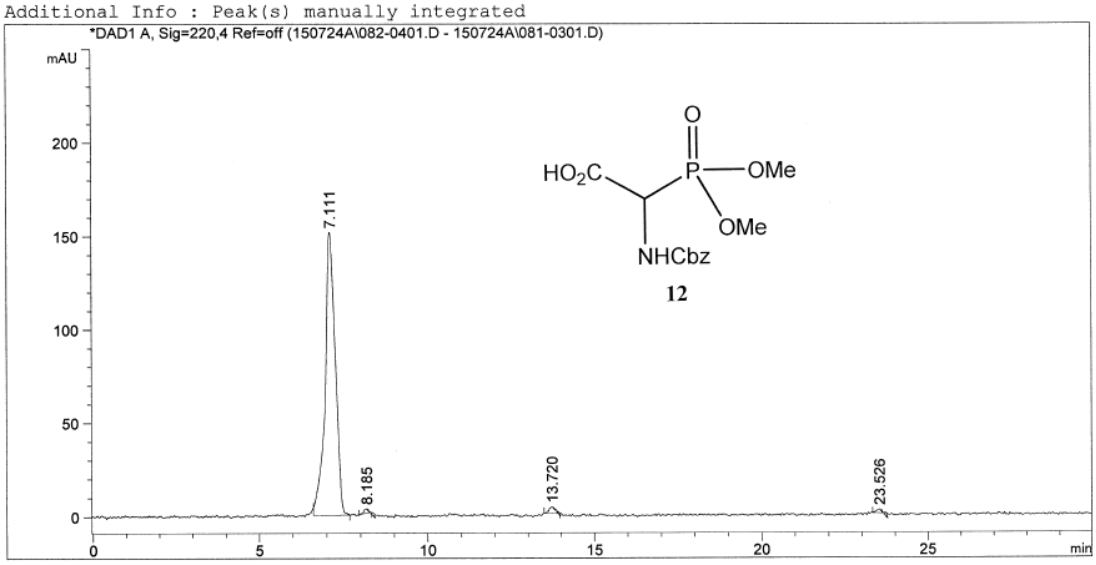


HPLC (achiral) - Compound 12

```
======================================================================
Acq. Operator   : IL                              Seq. Line :   4
Acq. Instrument : Instrument 1                     Location : Vial 82
Injection Date  : 07/24/15  8:25:49 PM                Inj :   1
                                                  Inj Volume : 5.000 µl
Acq. Method     : C:\HPCHEM\1\METHODS\SDAP.M
Last changed    : 01/01/15  5:49:26 PM by MRL
Analysis Method : C:\CHEM32\2\METHODS\SDAP.M
Last changed    : 03/25/17  3:07:53 PM by MW
                  (modified after loading)
Method Info     : Generic HPLC Method for SDAP Project - Reaction Monitoring and Purity
                  Assay
                  HPLC 31
                  C=10mM K3PO4 pH 3 in water + 5% ACN
                  D=ACN
                  Waters Symmetry Shield RP-18 150mmx4.6mm 5 microns
                  1.0mL/min 40C 220, 254, 275 nm

Sample Info     : Crude Saponified Z-Phosphoglycine diethyl ester
                  PR069-158-2
                  2 mg/mL in ACN

Additional Info : Peak(s) manually integrated
```




```
======================================================================
                        Area Percent Report
======================================================================

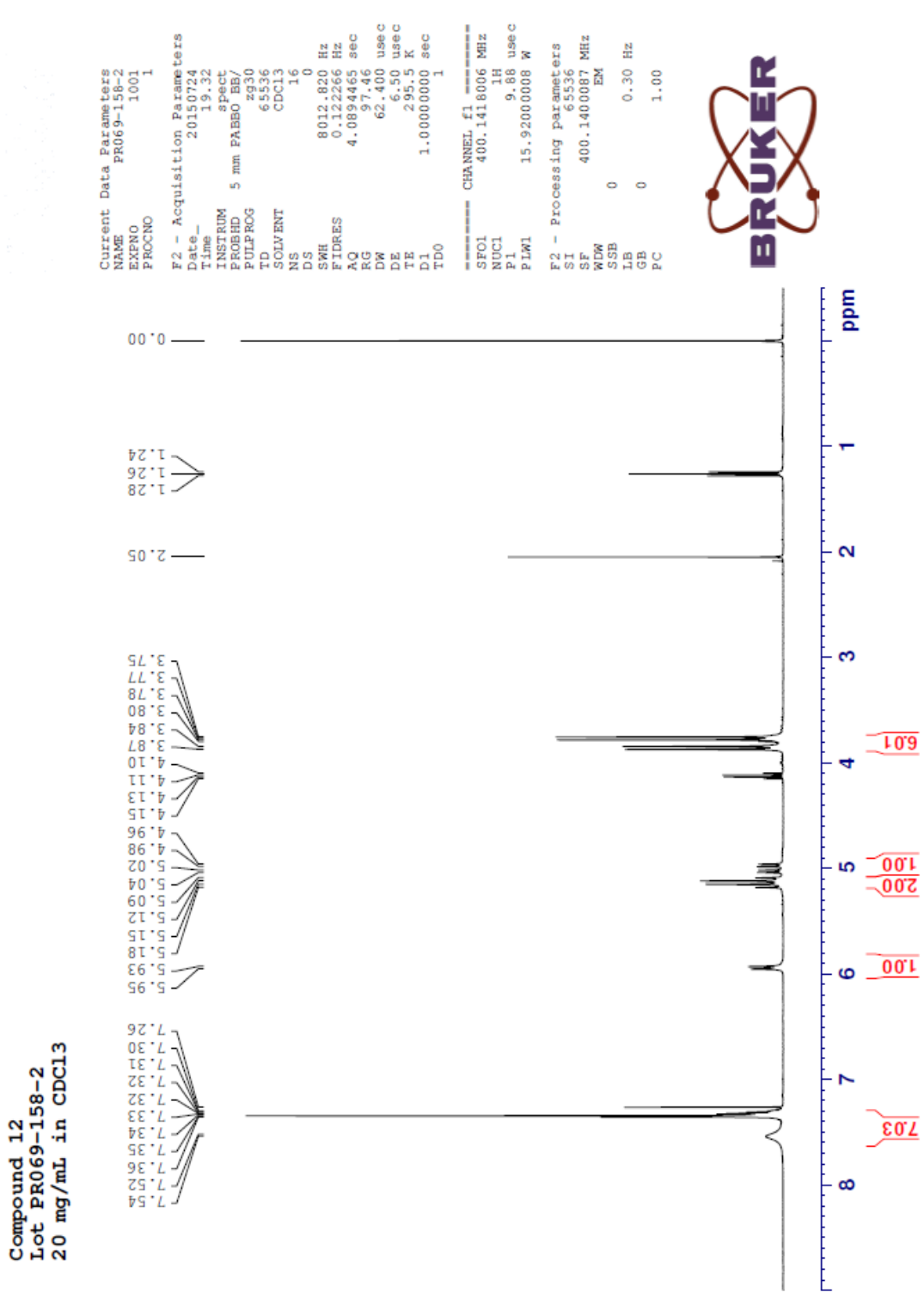
Signal 1: DAD1 A, Sig=220,4 Ref=off
 Signal has been modified after loading from rawdata file!

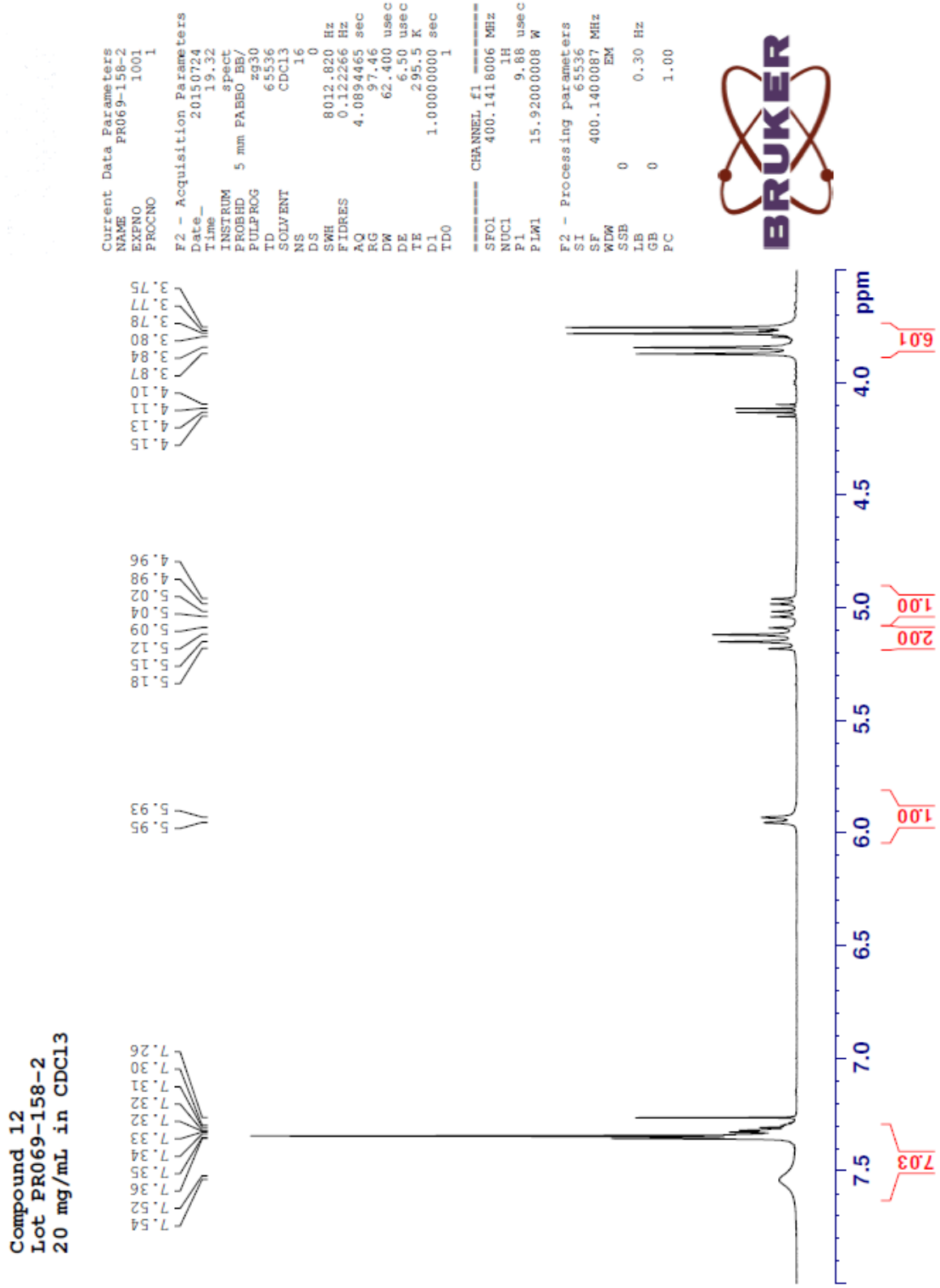
Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   7.111 BB    0.3001 3167.10864  151.39572  97.3588
   2   8.185 MM    0.1609   22.54046    2.33443   0.6929
   3  13.720 MM    0.2047   40.95317    3.33483   1.2589
   4  23.526 MM    0.1817   22.42507    2.05666   0.6894

Totals :                  3253.02735  159.12164

======================================================================
```

^{1}H NMR - Compound 12



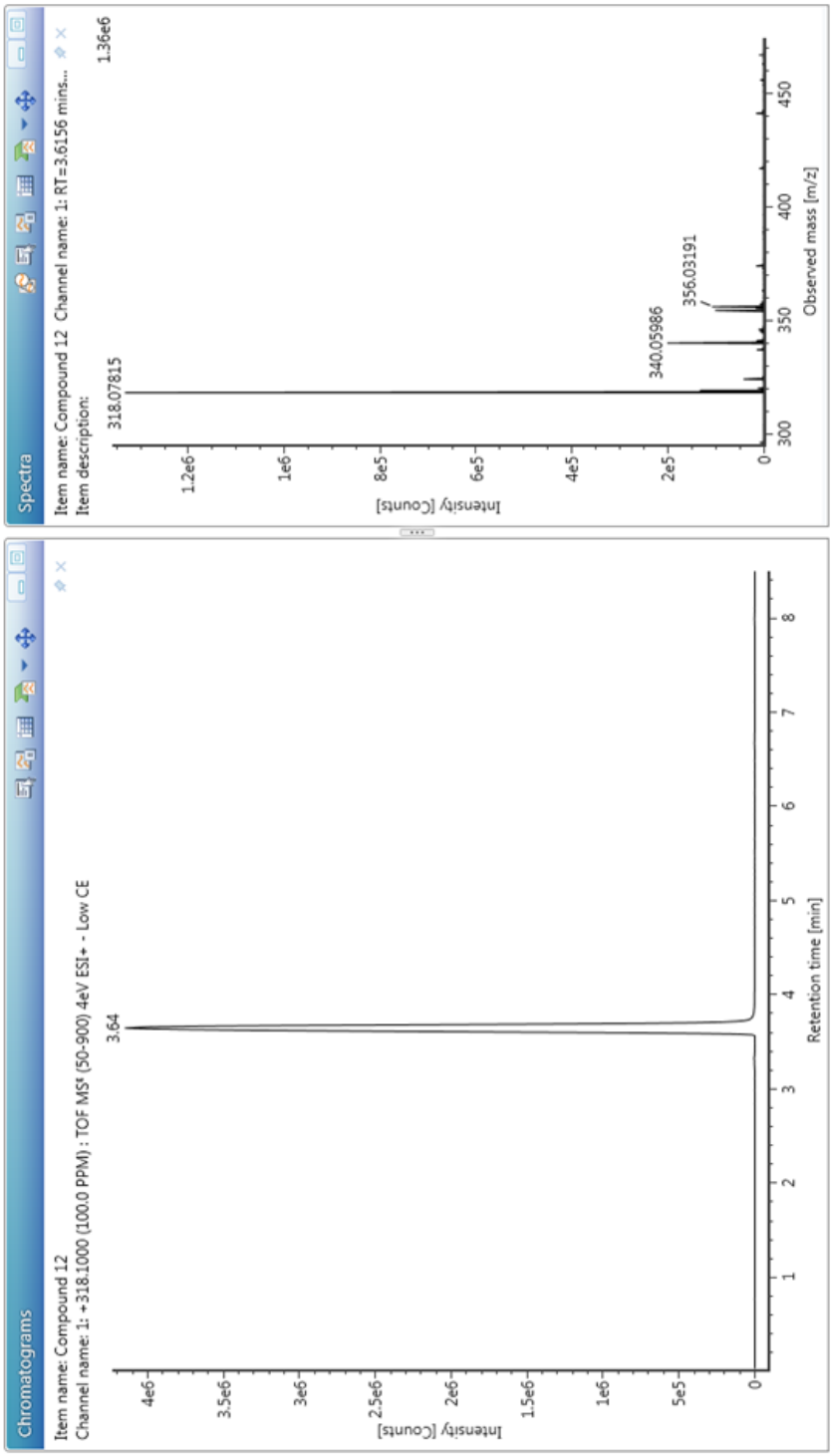

Compound 12
Lot PR069-158-2
20 mg/mL in CDCl3
Current Data Parameters
NAME PR069-158-2
EXPNO 1001
PROCNO 1
F2 - Acquisition Parameters
Date_ 20150724
Time 19.32
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 0
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 97.46
DW 62.400 usec
DE 6.50 usec
TE 295.5 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1418006 MHz
NUC1 1H
P1 9.88 usec
PLW1 15.92000008 W
F2 - Processing parameters
SI 65536
SF 400.1400087 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
BRUKER
7.54
7.52
7.36
7.35
7.34
7.33
7.32
7.32
7.31
7.30
7.26
5.95
5.93
5.18
5.15
5.12
5.09
5.04
5.02
4.98
4.96
4.15
4.13
4.11
4.10
3.87
3.84
3.80
3.78
3.77
3.75
7.0
6.5
6.0
5.5
5.0
4.5
4.0
ppm
7.03
1.00
1.00
2.00
1.00
6.01

HRMS - Compound 12

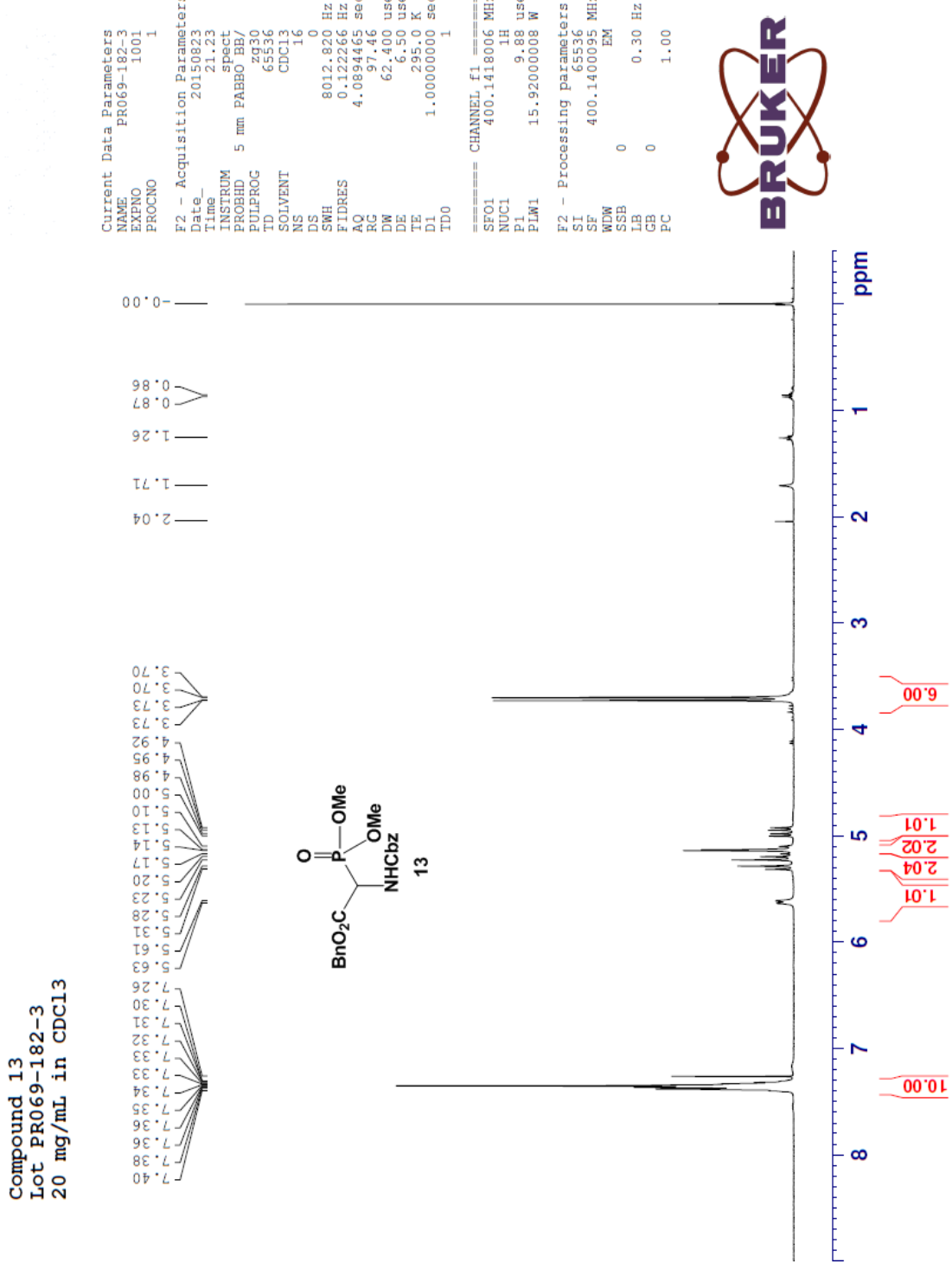
$[12 + H]^+$

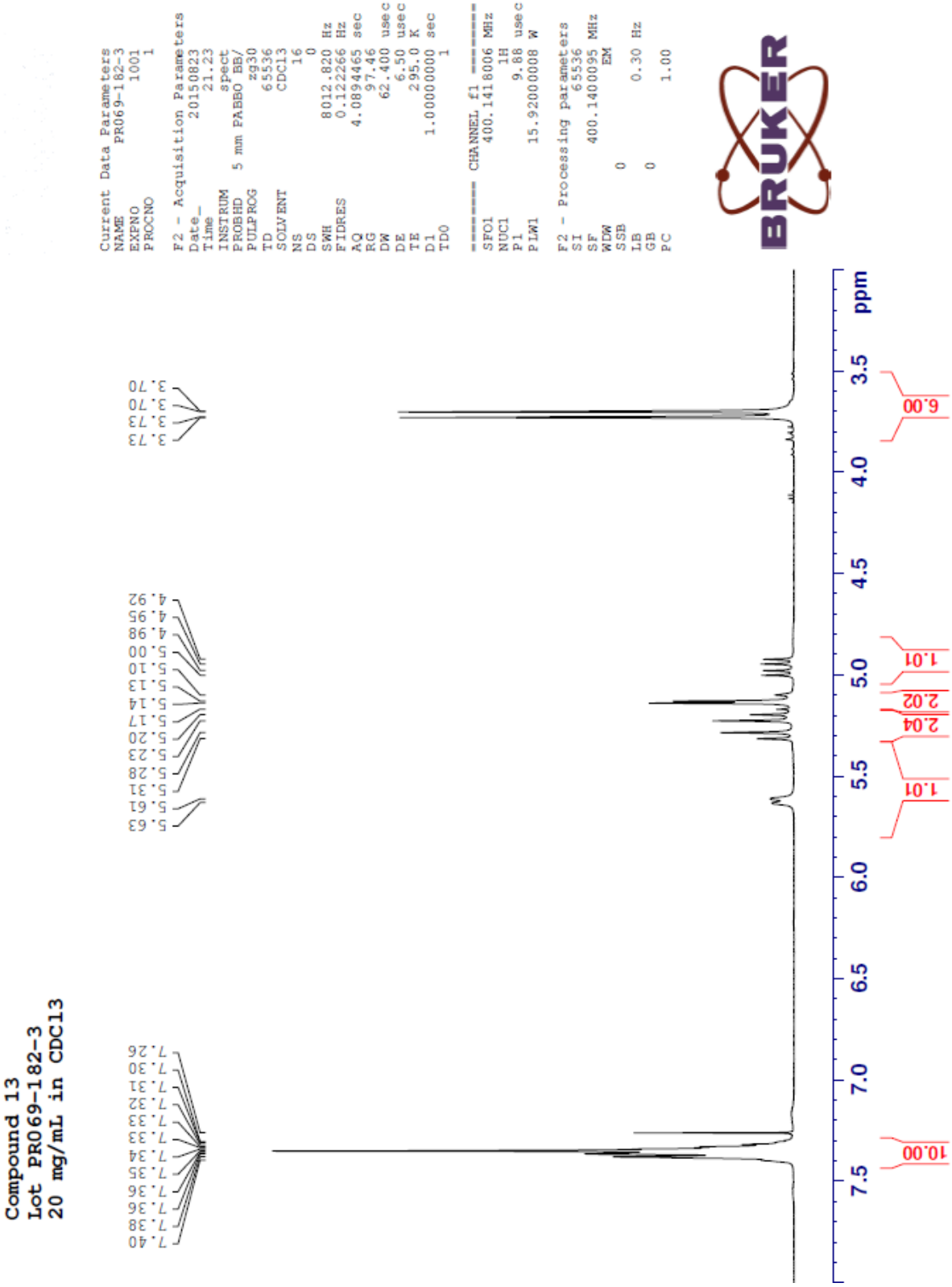
Calculated $[M+H]^+$ for $C_{12}H_{17}NO_7P^+$: 318.0737

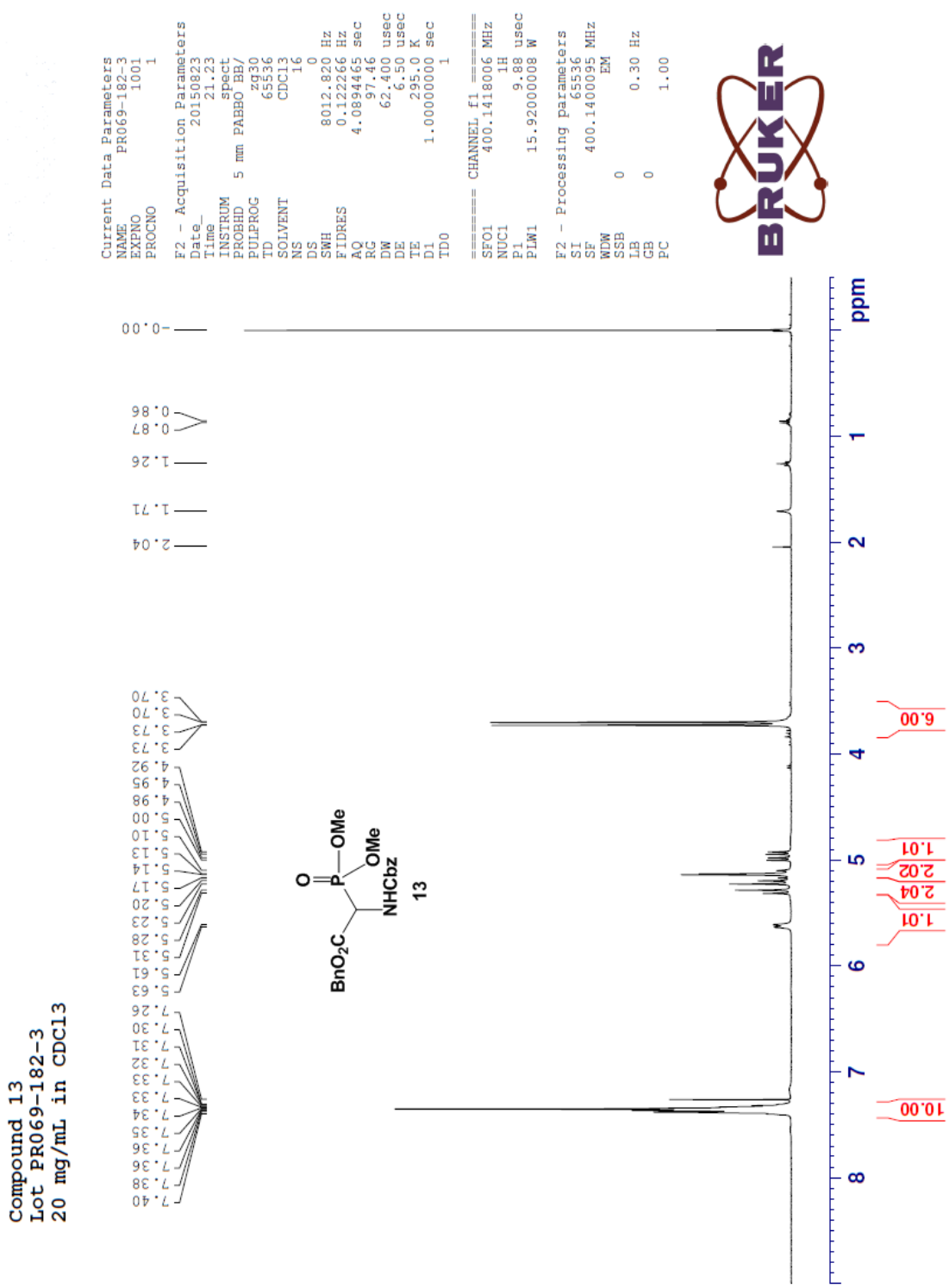
Found: 318.0782



^{1}H NMR - Compound 13




Compound 13
Lot PR069-182-3
20 mg/mL in CDC13
Current Data Parameters
NAME PR069-182-3
EXPNO 1001
PROCNO 1
F2 - Acquisition Parameters
Date_ 20150823
Time 21.23
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDC13
NS 16
DS 0
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 97.46
DW 62.400 usec
DE 6.50 usec
TE 295.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1418006 MHz
NUC1 1H
P1 9.88 usec
PLW1 15.92000008 W
F2 - Processing parameters
SI 65536
SF 400.1400095 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
BRUKER
7.40
7.38
7.36
7.36
7.35
7.34
7.33
7.33
7.32
7.31
7.30
7.26
5.63
5.61
5.31
5.28
5.23
5.20
5.17
5.14
5.13
5.10
5.00
4.98
4.95
4.92
3.73
3.73
3.70
3.70
10.00
1.01
2.04
2.02
1.01
6.00
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
ppm

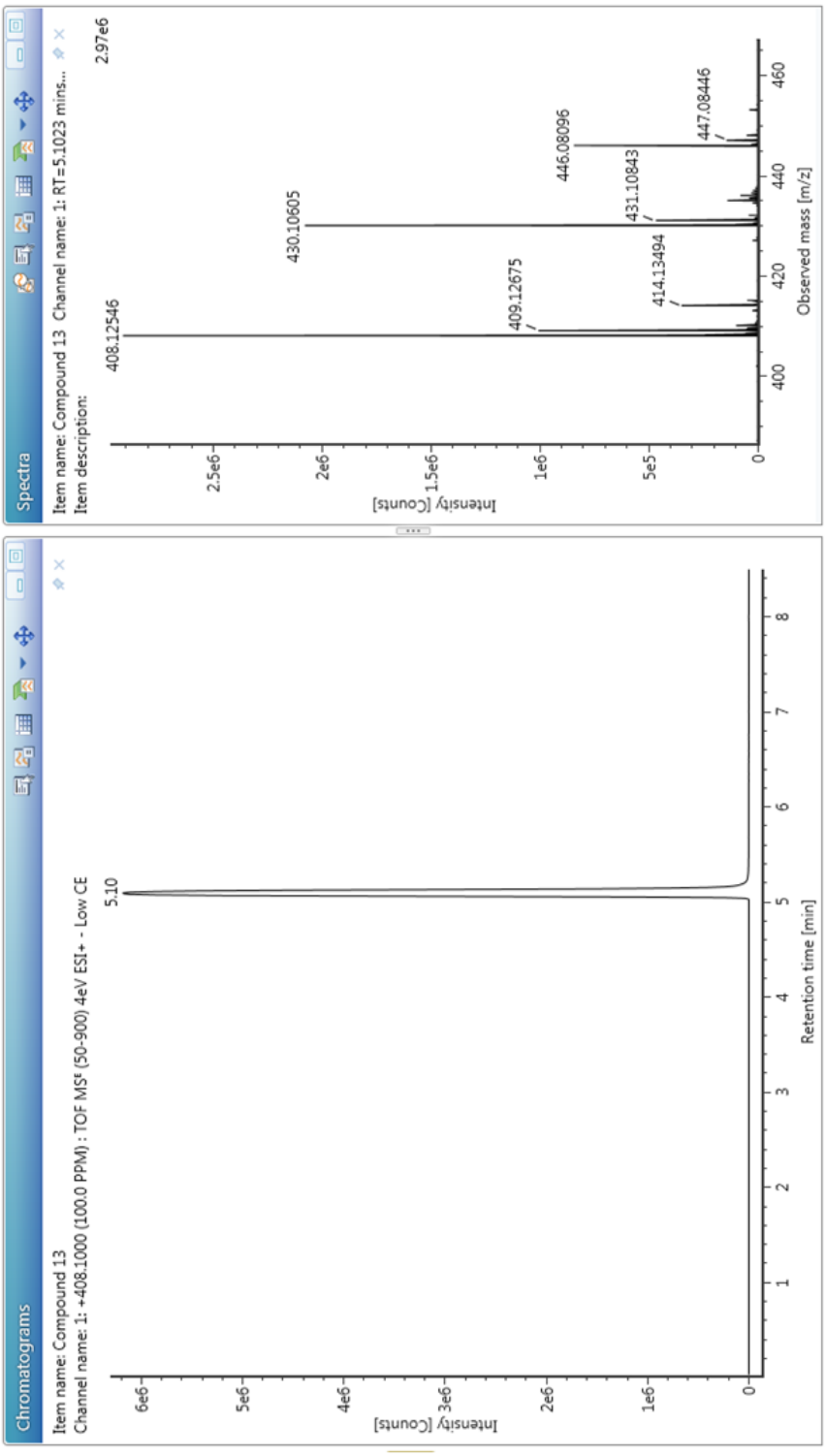

Compound 13
Lot PR069-182-3
20 mg/mL in CDCl3
Current Data Parameters
NAME PR069-182-3
EXPNO 1001
PROCNO 1
F2 - Acquisition Parameters
Date_ 20150823
Time 21.23
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 0
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 97.46
DW 62.400 usec
DE 6.50 usec
TE 295.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1418006 MHz
NUC1 1H
P1 9.88 usec
PLW1 15.92000008 W
F2 - Processing parameters
SI 65536
SF 400.1400095 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
BRUKER
O
P
OMe
OMe
NHCbz
BnO2C
13
ppm
6.00
1.01
2.04
2.02
1.01
10.00

HRMS - Compound 13

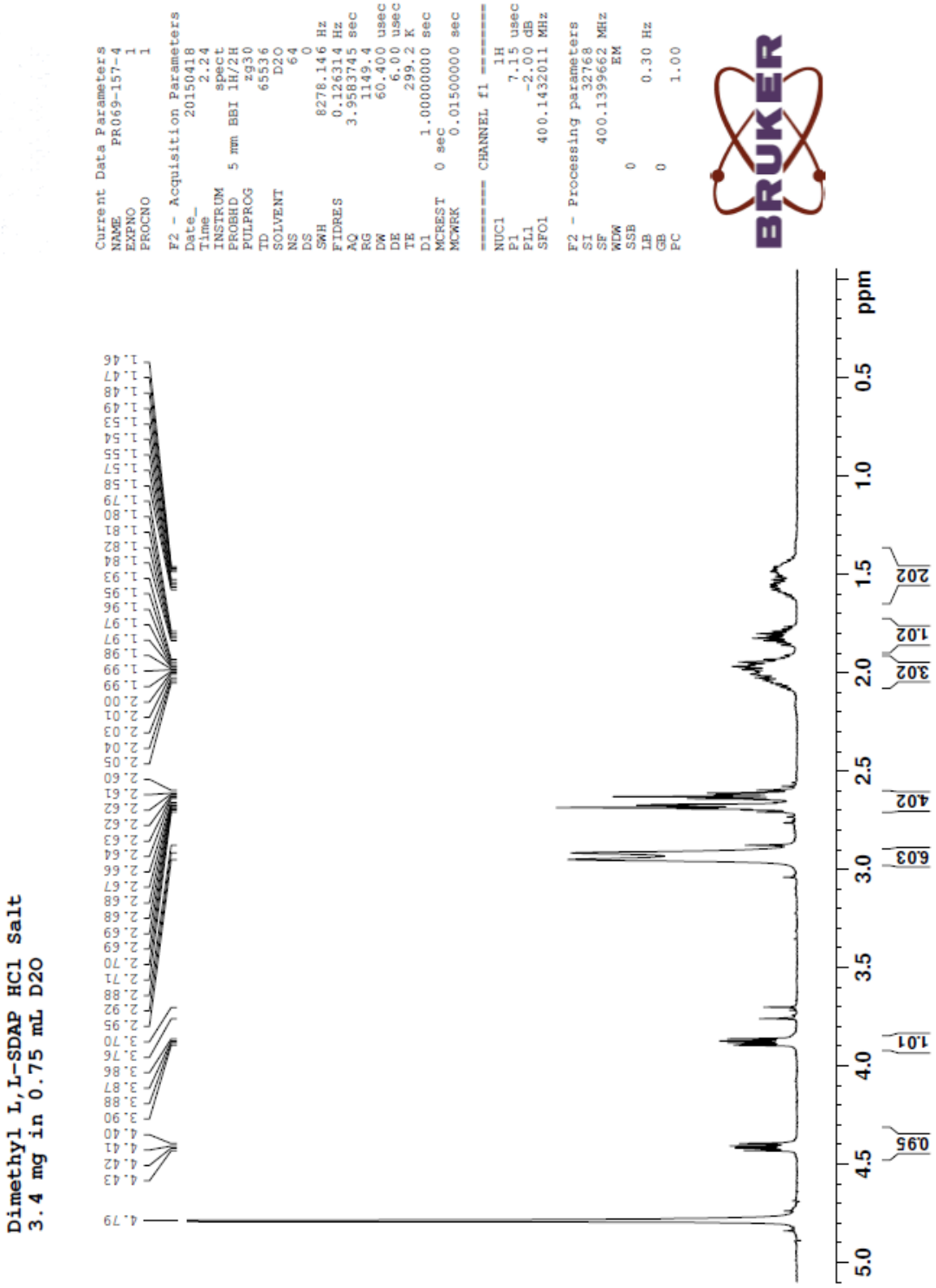
$[13 + H]^+$

Calculated $[M+H]^+$ for $C_{19}H_{23}NO_7P^+$: 408.1207

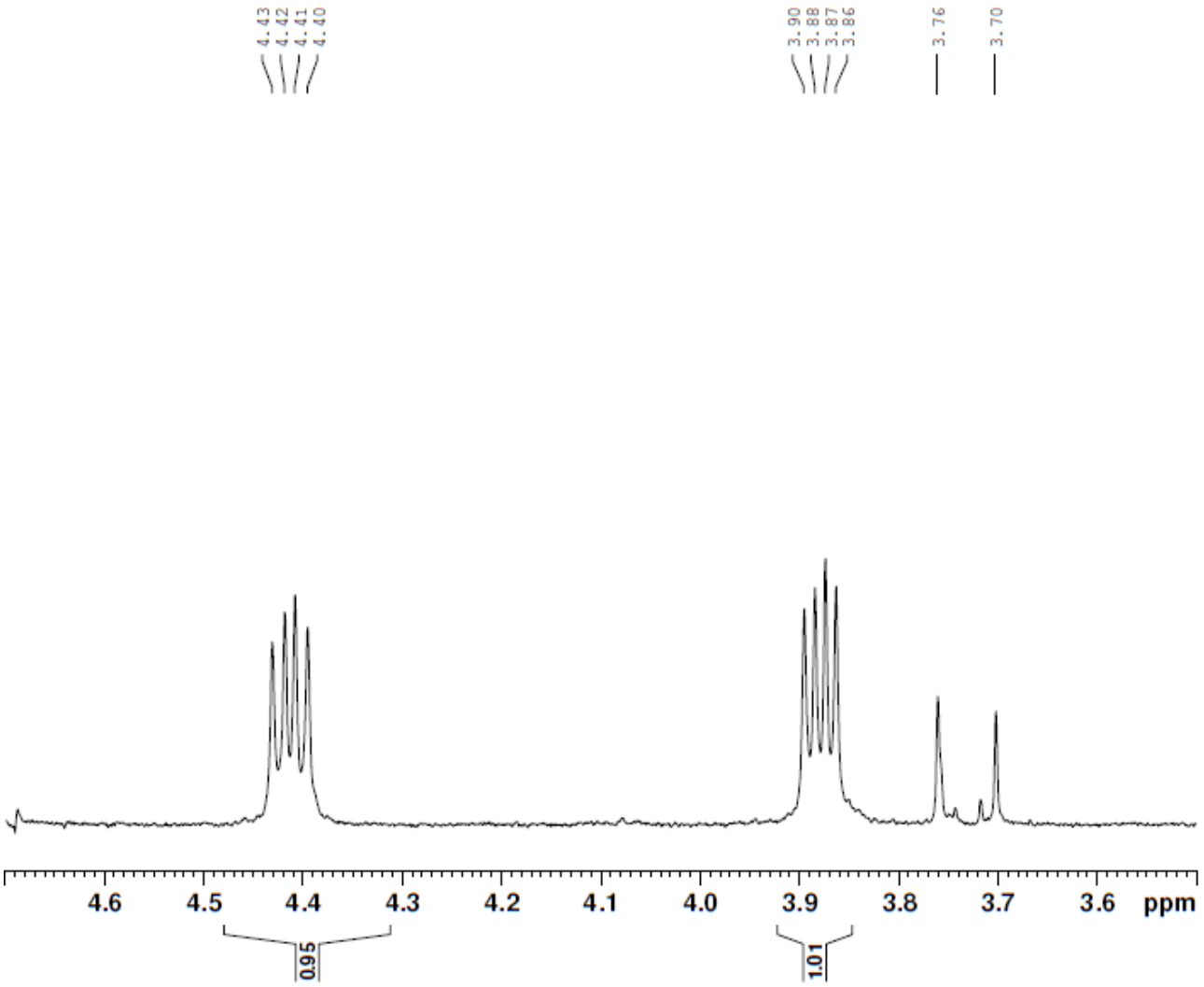
Found: 408.1255



^{1}H NMR - Compound 18



Dimethyl L,L-SDAP HCl Salt
3.4 mg in 0.75 mL D2O



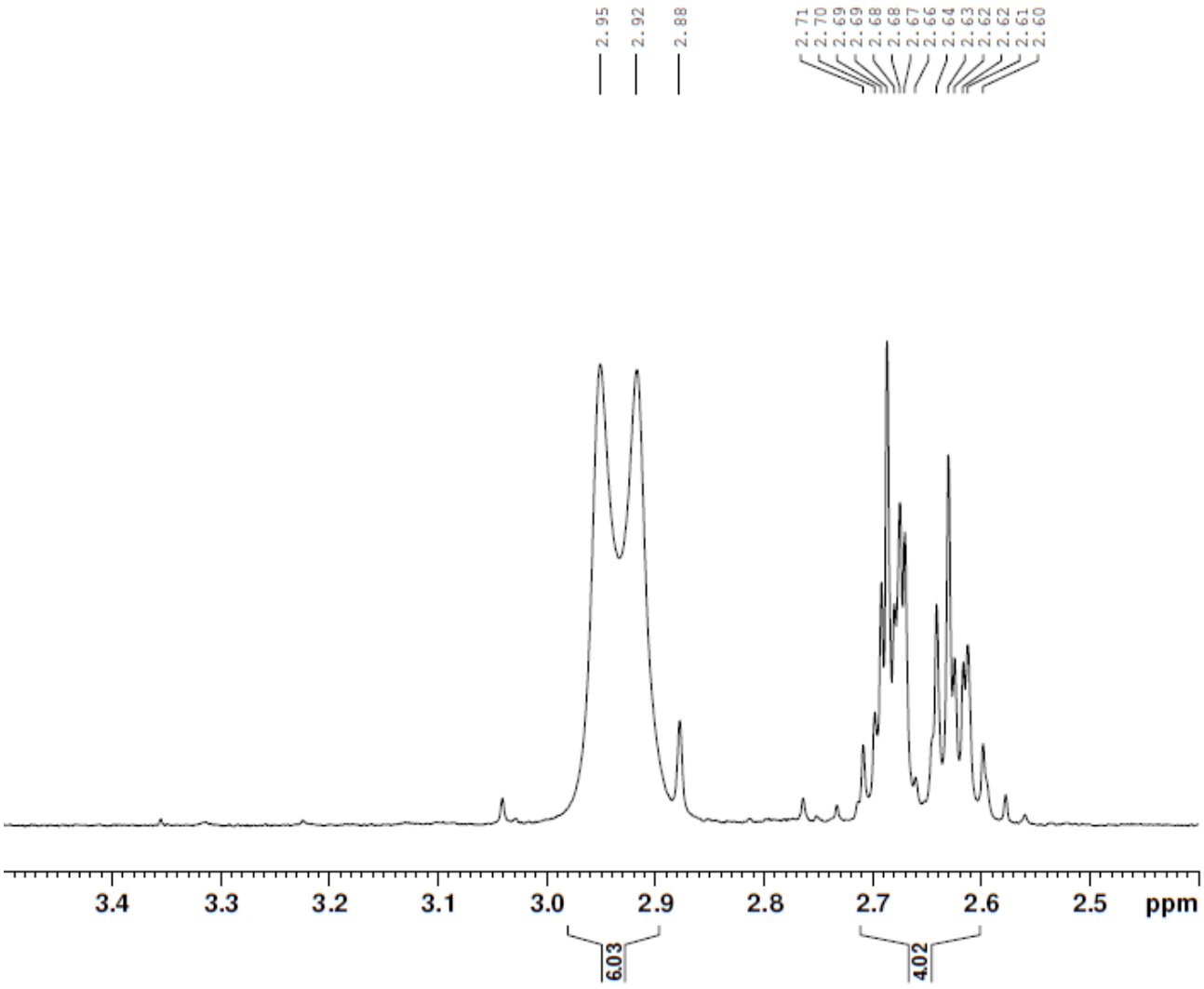

```
Current Data Parameters
NAME         PR069-157-4
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150418
Time                2.24
INSTRUM            spect
PROBHD   5 mm BBI 1H/2H
PULPROG             zg30
TD                 65536
SOLVENT              D2O
NS                    64
DS                     0
SWH            8278.146 Hz
FIDRES        0.126314 Hz
AQ            3.9583745 sec
RG               1149.4
DW              60.400 usec
DE               6.00 usec
TE              299.2 K
D1           1.00000000 sec
MCREST   0 sec
MCWRK        0.01500000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 7.15 usec
PL1               -2.00 dB
SFO1        400.1432011 MHz

F2 - Processing parameters
SI                 32768
SF          400.1399662 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

Dimethyl L,L-SDAP HCl Salt
3.4 mg in 0.75 mL D2O



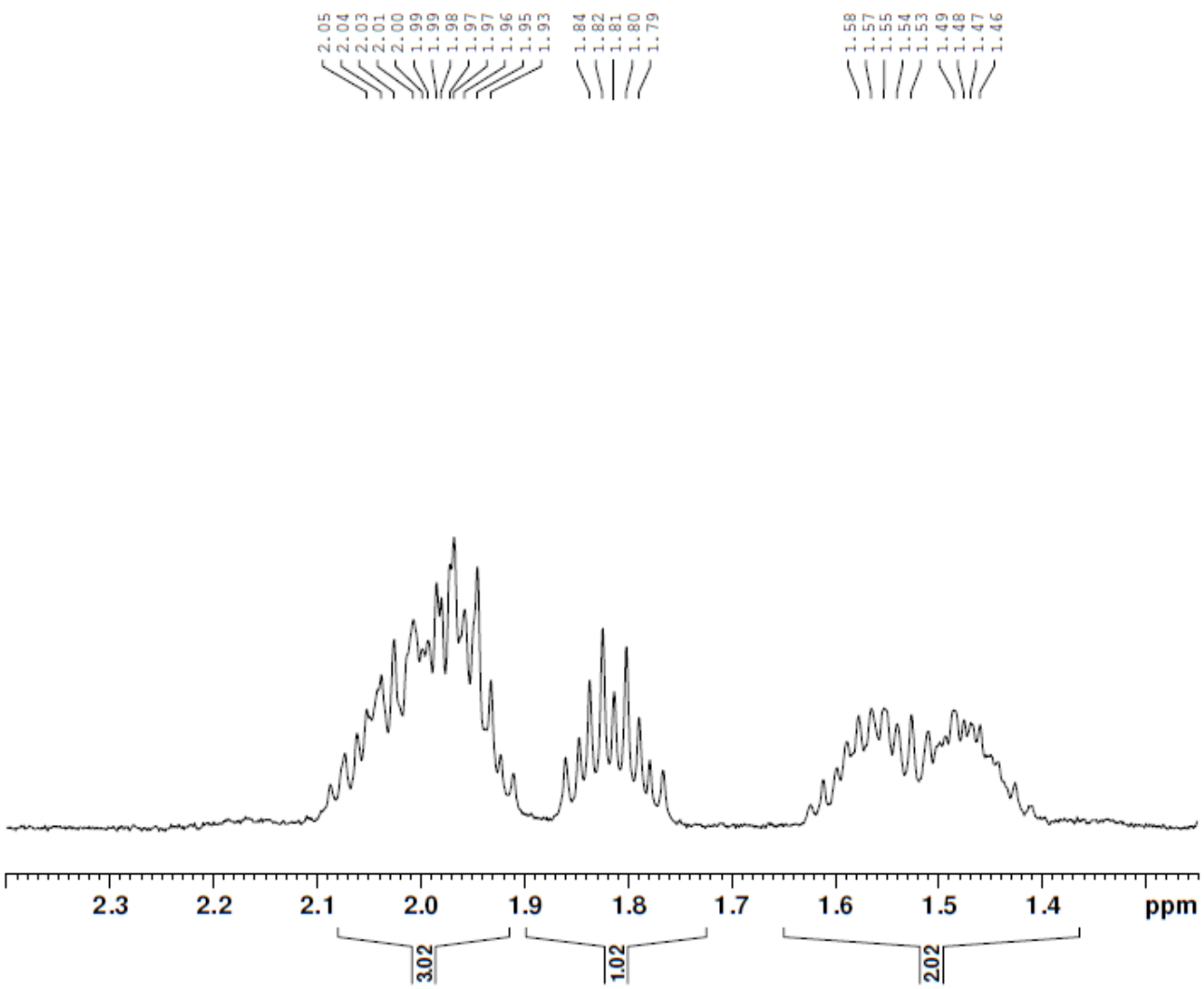

```
Current Data Parameters
NAME         PR069-157-4
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150418
Time                2.24
INSTRUM            spect
PROBHD   5 mm BBI 1H/2H
PULPROG             zg30
TD                 65536
SOLVENT              D2O
NS                    64
DS                     0
SWH             8278.146 Hz
FIDRES          0.126314 Hz
AQ              3.9583745 sec
RG                 1149.4
DW                60.400 usec
DE                 6.00 usec
TE                299.2 K
D1             1.00000000 sec
MCREST   0 sec
MCWRK          0.01500000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 7.15 usec
PL1               -2.00 dB
SFO1         400.1432011 MHz

F2 - Processing parameters
SI                 32768
SF           400.1399662 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

Dimethyl L,L-SDAP HCl Salt
3.4 mg in 0.75 mL D2O




```
Current Data Parameters
NAME         PR069-157-4
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150418
Time                2.24
INSTRUM            spect
PROBHD   5 mm BBI 1H/2H
PULPROG             zg30
TD                 65536
SOLVENT              D2O
NS                    64
DS                     0
SWH            8278.146 Hz
FIDRES        0.126314 Hz
AQ            3.9583745 sec
RG               1149.4
DW                60.400 usec
DE                 6.00 usec
TE               299.2 K
D1           1.00000000 sec
MCREST   0 sec
MCWRK        0.01500000 sec

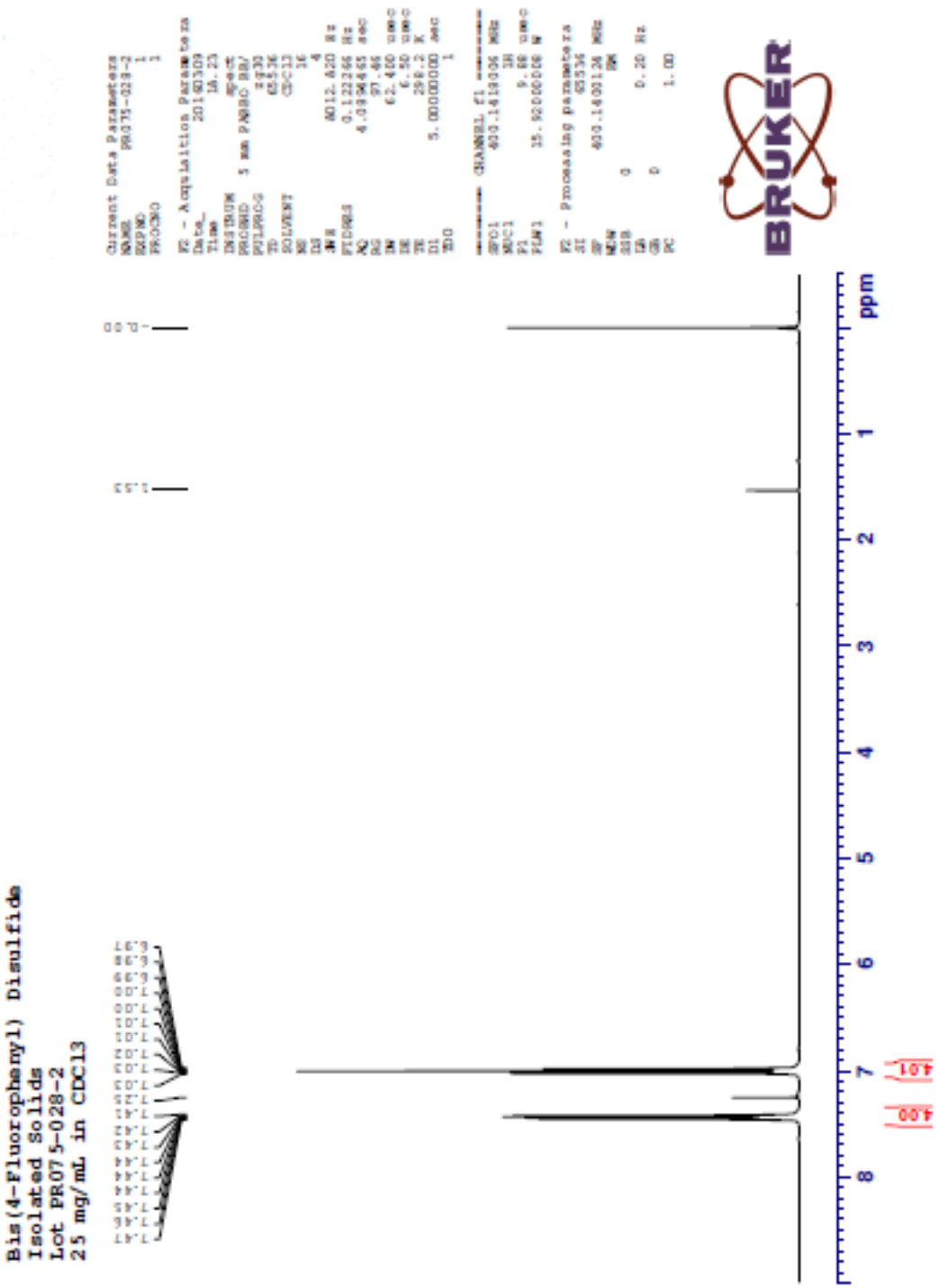
======== CHANNEL f1 ========
NUC1                  1H
P1                 7.15 usec
PL1               -2.00 dB
SFO1        400.1432011 MHz

F2 - Processing parameters
SI                 32768
SF          400.1399662 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

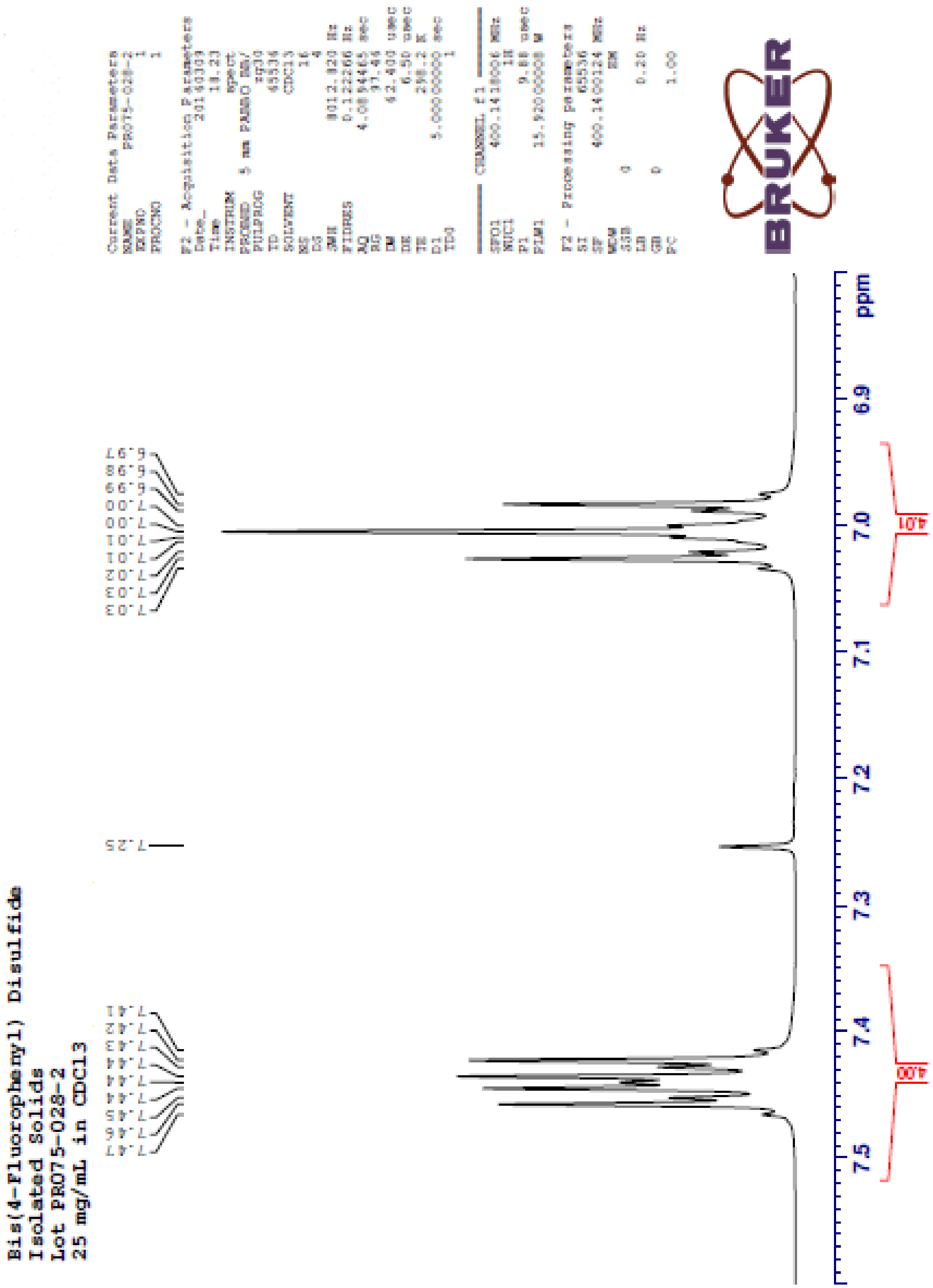
APPENDIX C

SUPPLEMENTAL DATA FOR CHAPTER FOUR

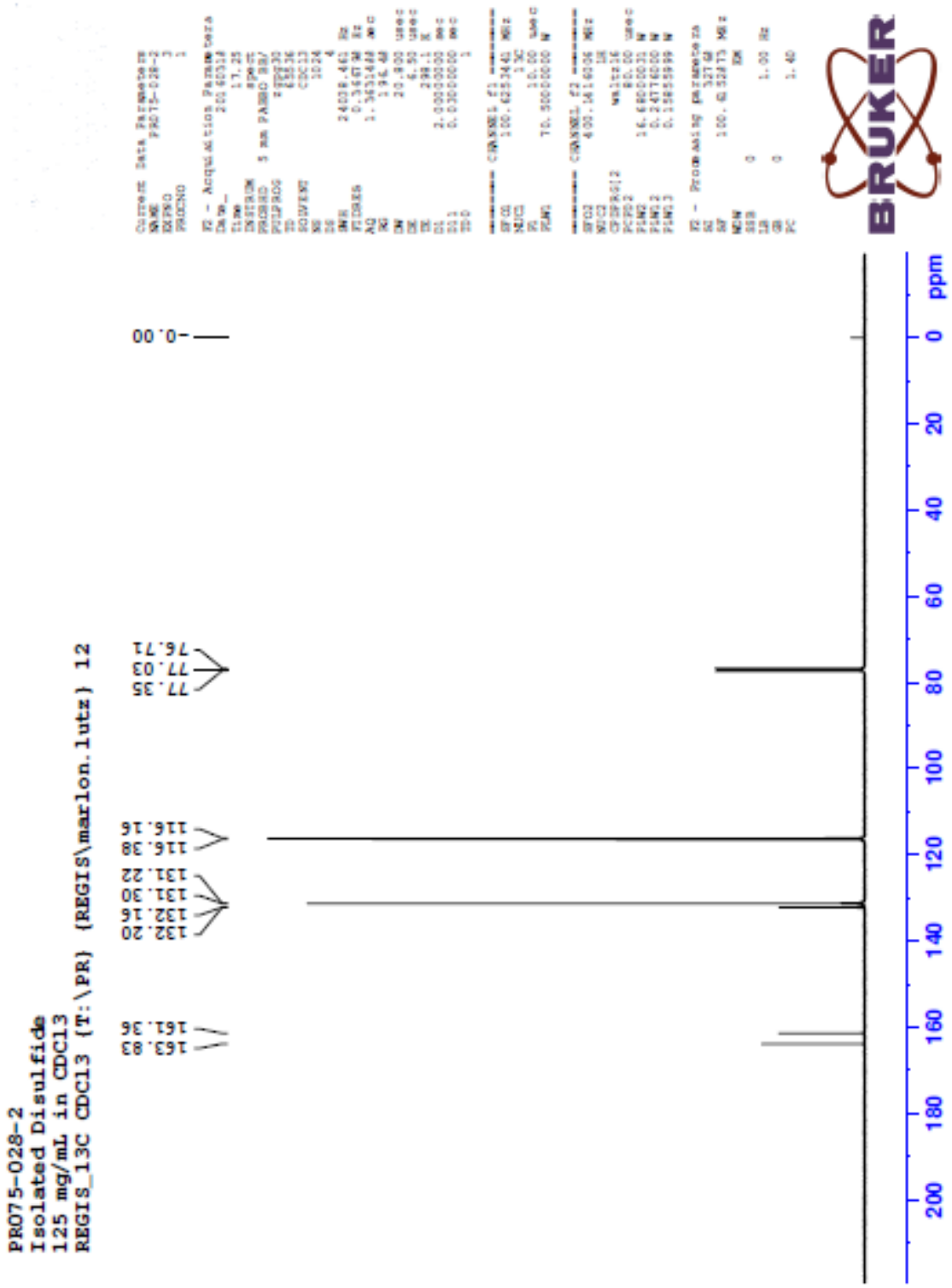
H NMR - Bis(4-Fluorophenyl) Disulfide (Compound 1)



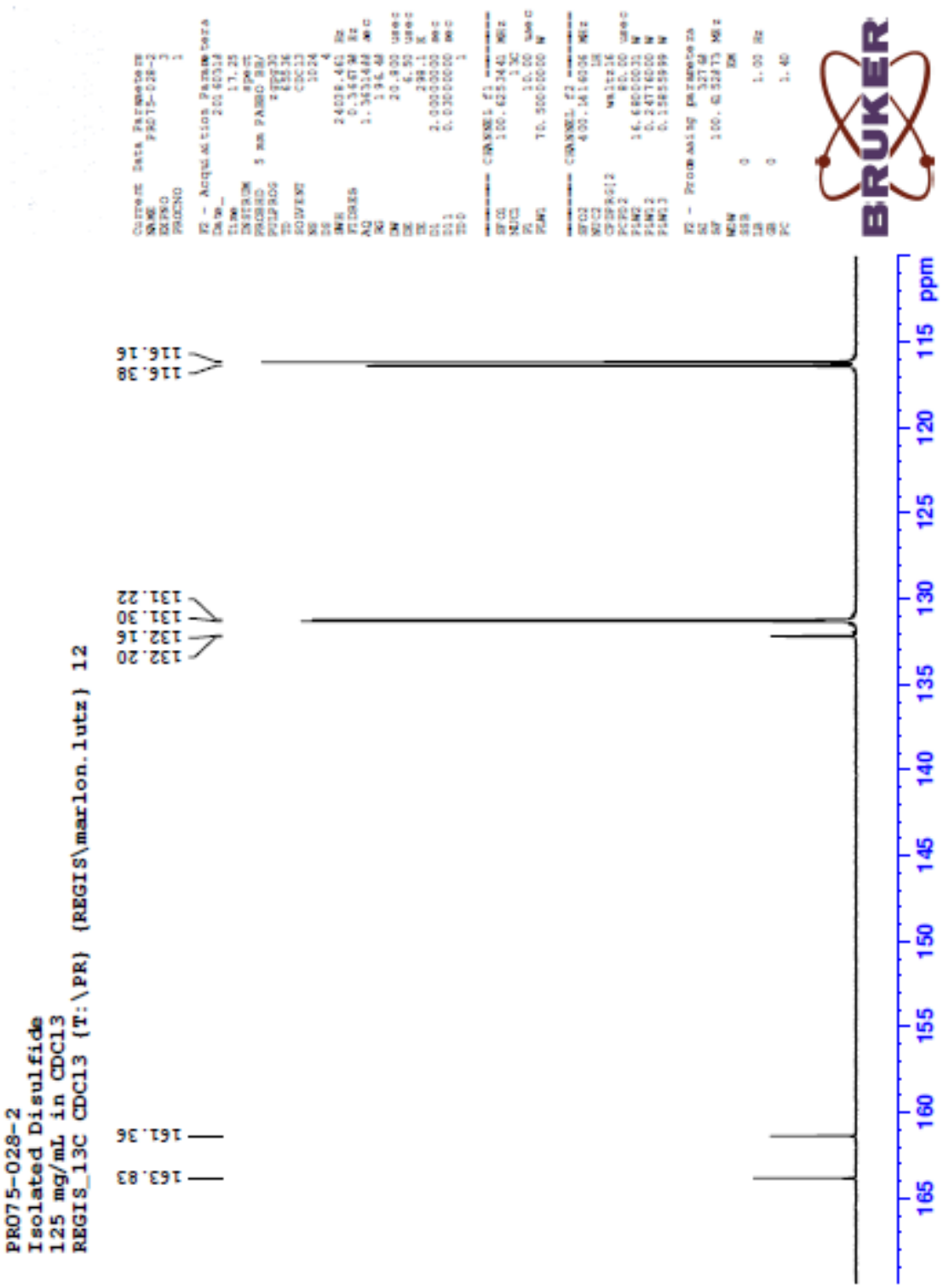
^{1}H NMR - Bis(4-Fluorophenyl) Disulfide (Compound 1)



^{13}C NMR - Bis(4-Fluorophenyl) Disulfide (Compound 1)



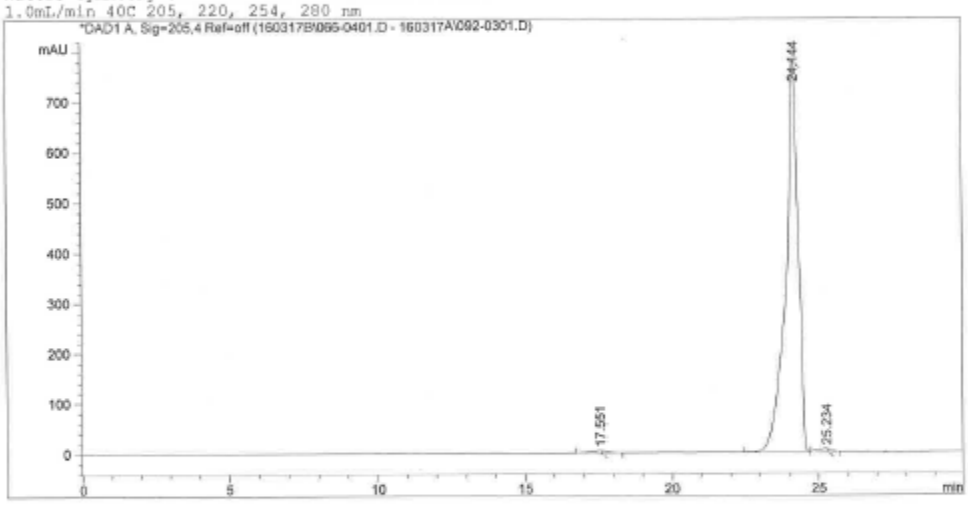
^{13}C NMR - Bis(4-Fluorophenyl) Disulfide (Compound 1)



HPLC - Isolated Sulfide Adduct (Compound 3)

Isolated Sulide Adduct
PR075-036-4
2.7 mg/mL in ACN

Injection Date : 3/17/2016 9:05:33 PM Seq. Line : 4
Sample Name : PR075-036-4 Location : Vial 66
Acq. Operator : MRL Inj : 1
Acq. Instrument : Instrument 1 Inj Volume : 5 µl
Method : C:\HPCHEM\1\METHODS\LUC-MMP.M
Last changed : 2/26/2016 10:22:15 PM by MRL
Generic HPLC Method for Loyola MMP Project - Rxn Monitoring and Purity Assay
HPLC 31
C=10mM K3PO4 pH 3 in water + 5% ACN
D=ACN
Waters Symmetry Shield RP-18 150mmx4.6mm 5 microns
1.0mL/min 40C 205, 220, 254, 280 nm



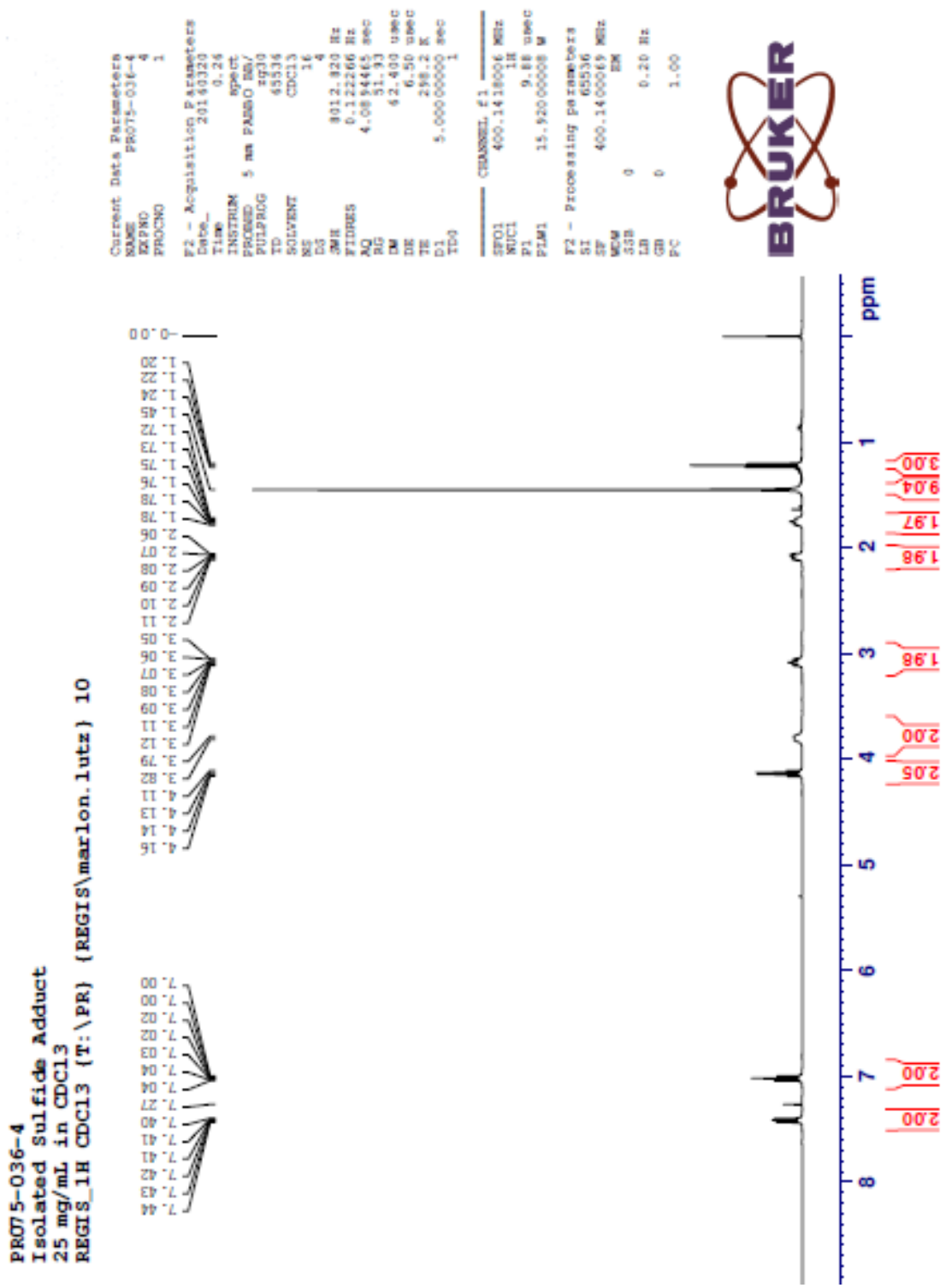
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

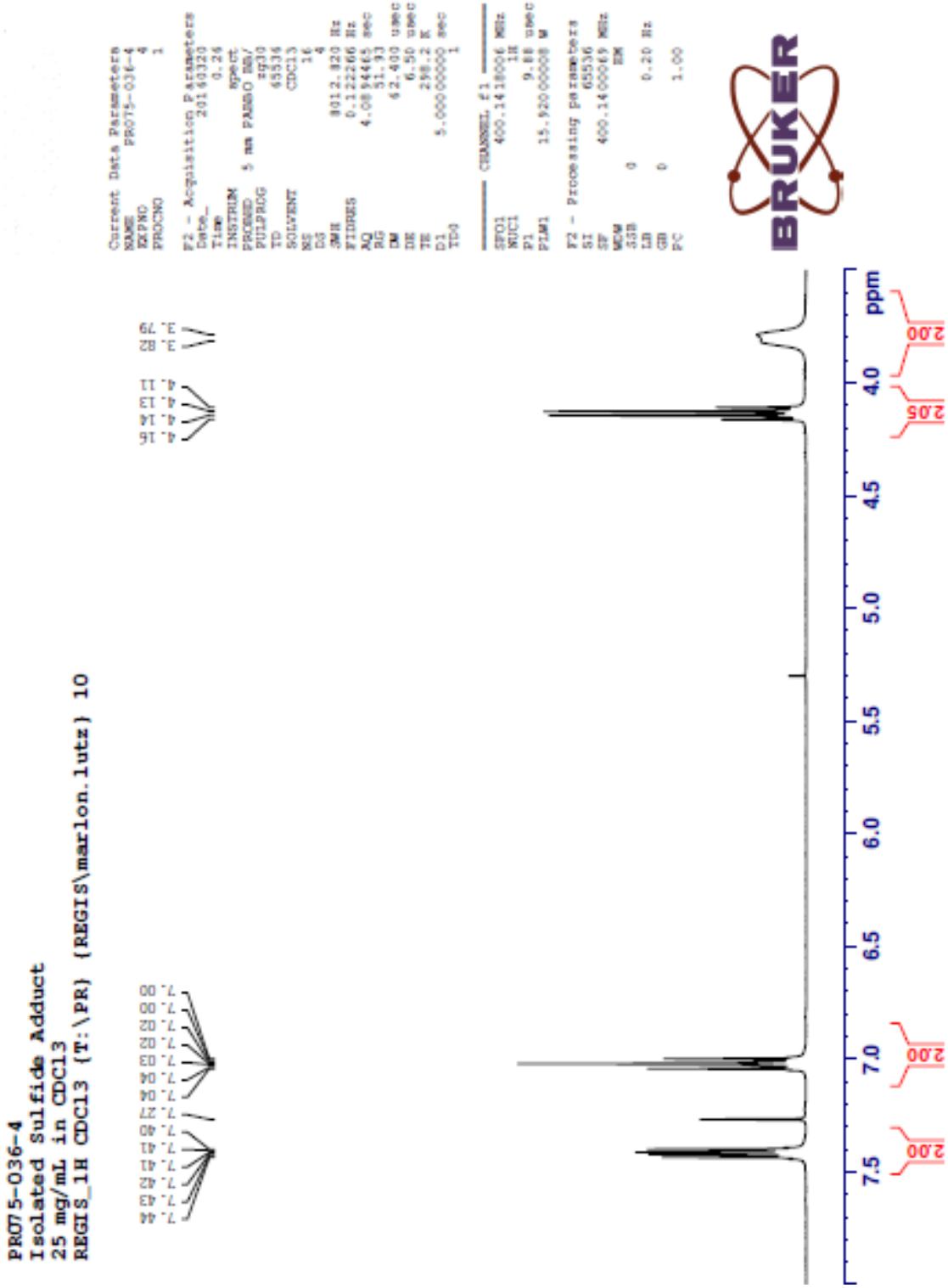
Signal 1: DAD1 A, Sig=205,4 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.551	MM	0.5563	137.34030	4.11496	0.5869
2	24.144	MM	0.4932	2.31125e4	781.07788	98.7706
3	25.234	MM	0.4237	150.34215	5.91411	0.6425
Totals :				2.34002e4	791.10695	

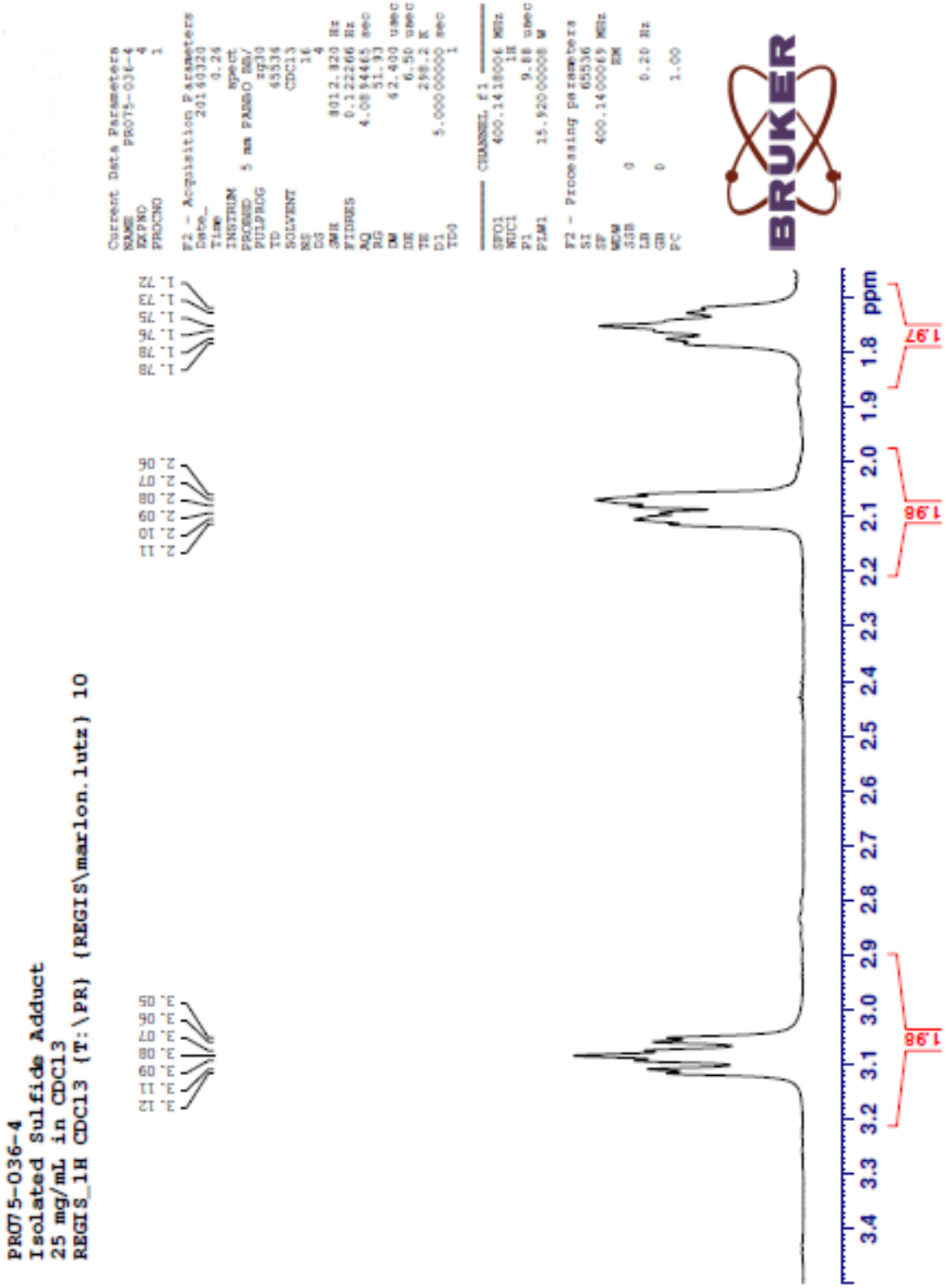
^{1}H NMR - Isolated Sulfide Adduct (Compound 3)



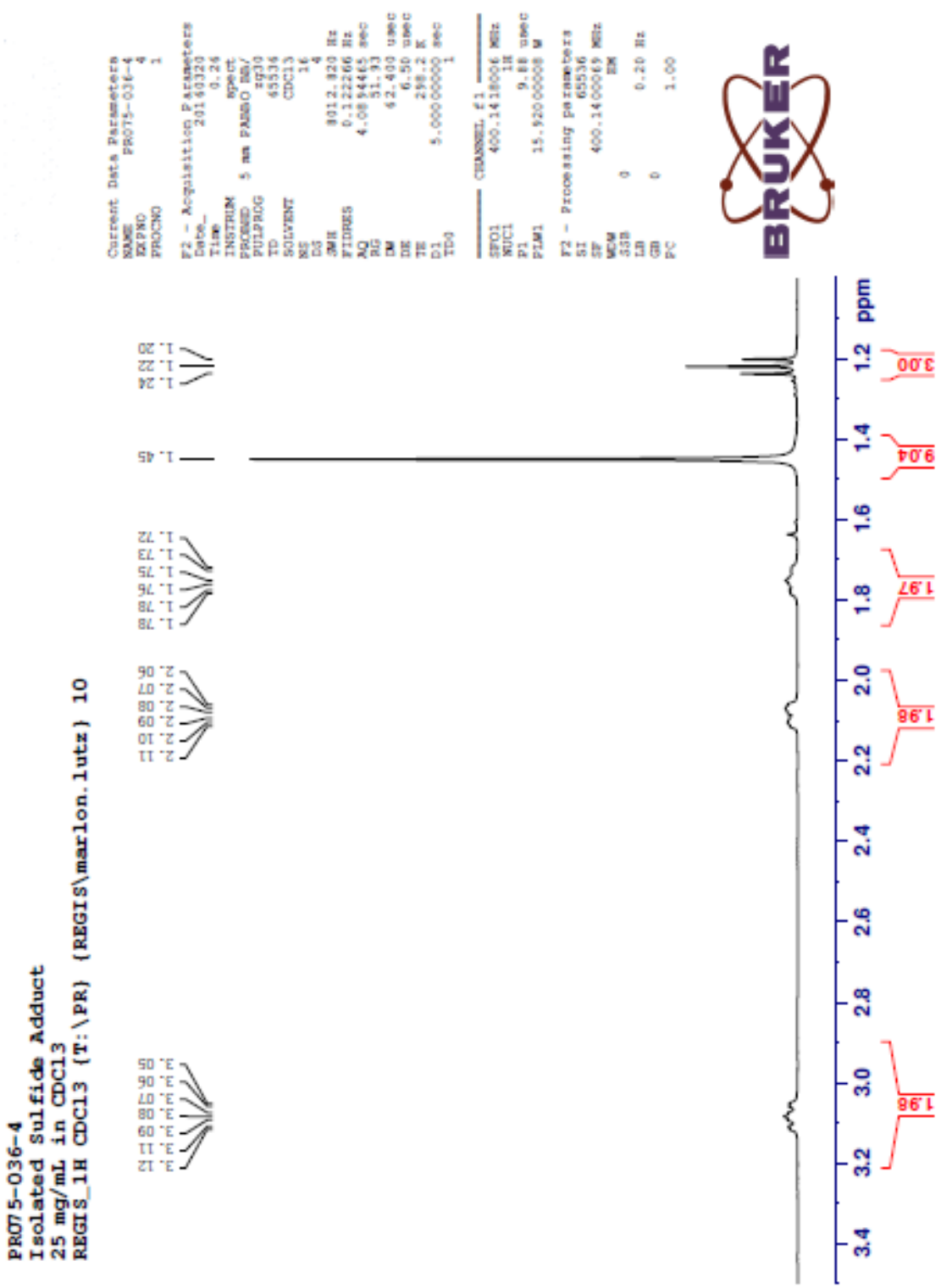
^{1}H NMR - Isolated Sulfide Adduct (Compound 3)



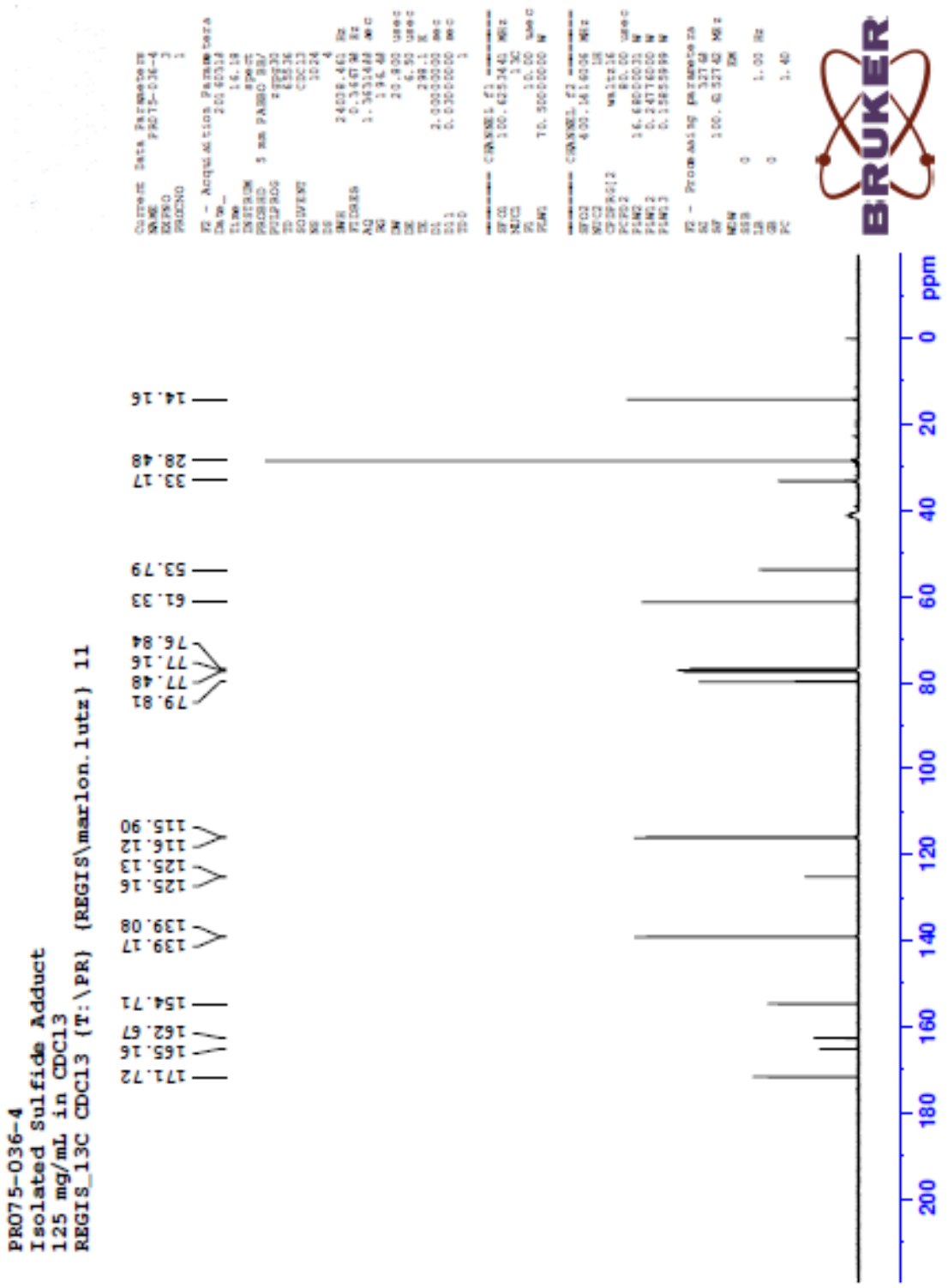
^{1}H NMR - Isolated Sulfide Adduct (Compound 3)



^{1}H NMR - Isolated Sulfide Adduct (Compound 3)



^{13}C NMR - Isolated Sulfide Adduct (Compound 3)



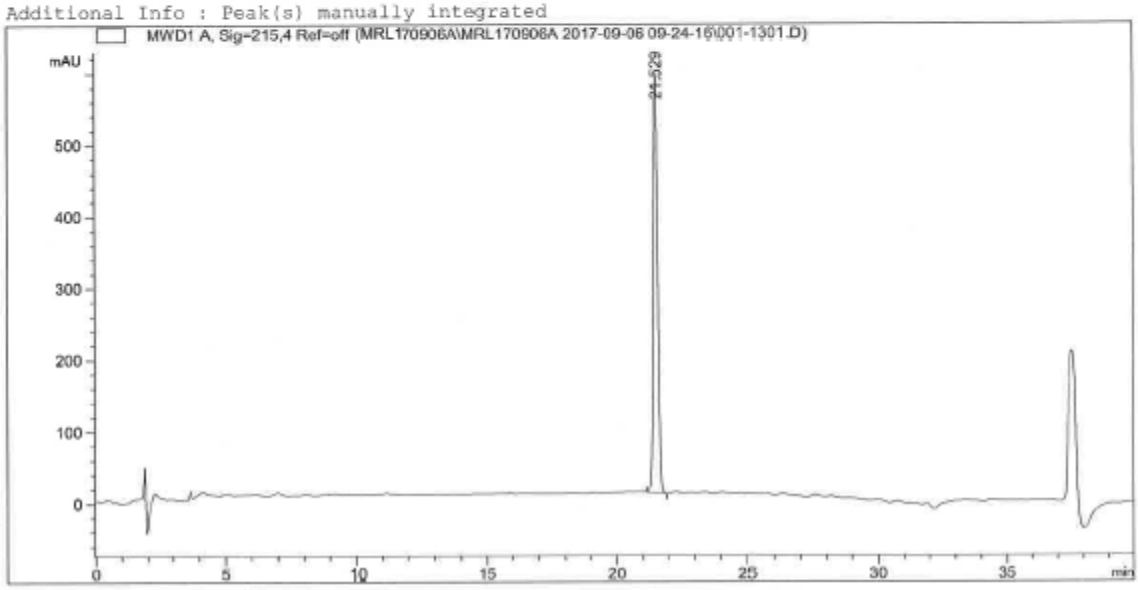
HPLC - Sulfone Adduct (Compound 4)

Sample Name: PR075-042-2

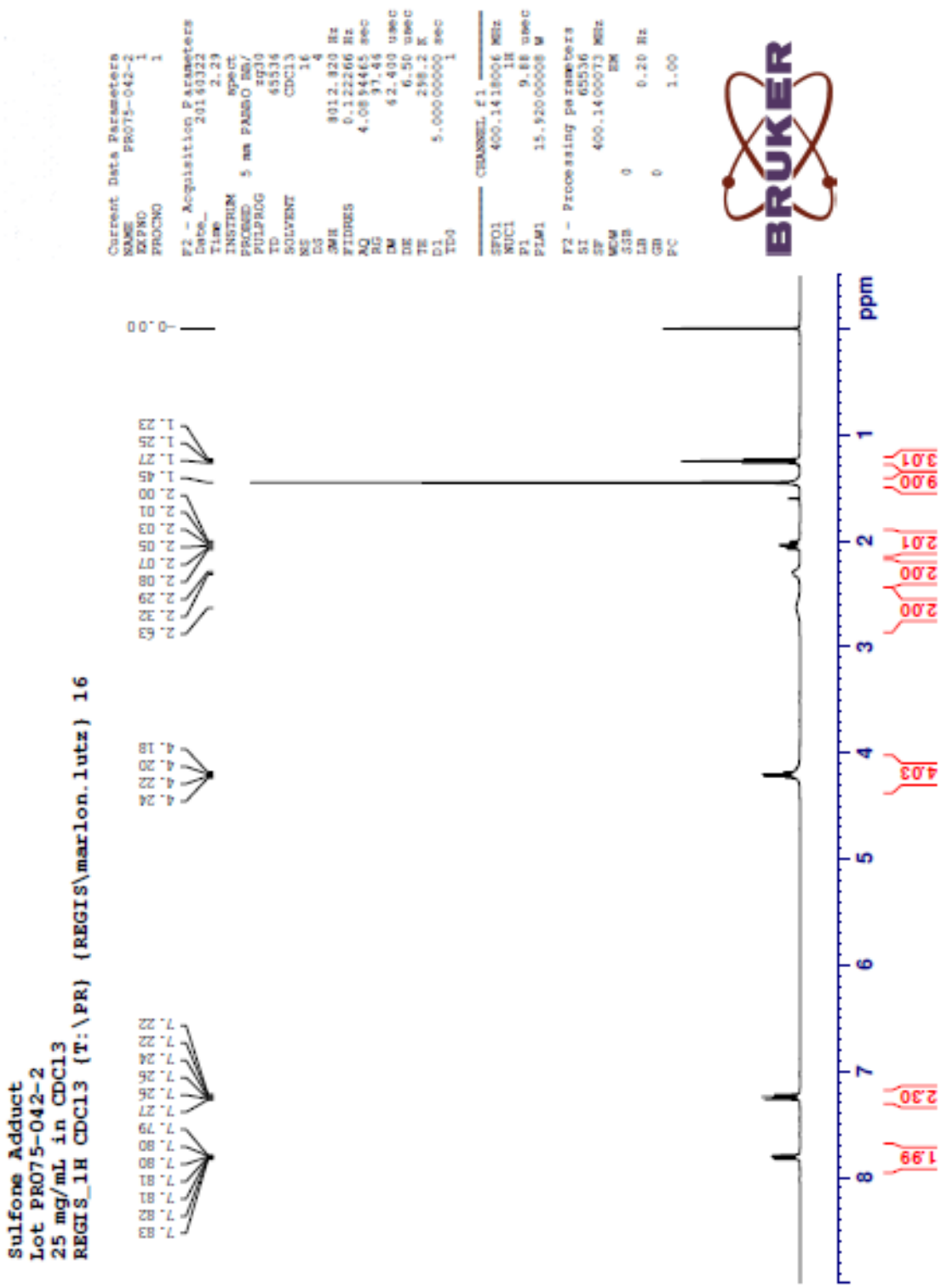
```
Acq. Operator   : MRL                                  Seq. Line :  13
Acq. Instrument : Instrument 1                        Location : Vial 1
Injection Date  : 09/06/17  5:41:02 PM                     Inj :   1
                                                     Inj Volume : 5.0 µl
Acq. Method     : C:\CHEM32\1\DATA\MRL170906A\MRL170906A 2017-09-06 09-24-16\BNCT-LONG1.M
Last changed    : 08/23/17  11:55:00 AM by MRL
Analysis Method : C:\CHEM32\1\METHODS\BNCT-LONG1.M
Last changed    : 08/23/17  11:55:00 AM by MRL
Method Info     : HPLC Method for Monitoring and Assaying BNCT products and reactions
                  HPLC 12 (Lab 2)
                  A = 0.10% TFA in water
                  B = 0.10% TFA in ACN
                  Waters Sunfire C8, 4.6x150 mm, 3.5 micron
                  Flow: 1.0 mL/min, Column Temp: 40 C

Sample Info     : Boc-Sulfone-Ethyl Ester
                  Lot PR075-042-2
                  1 mg/mL in ACN

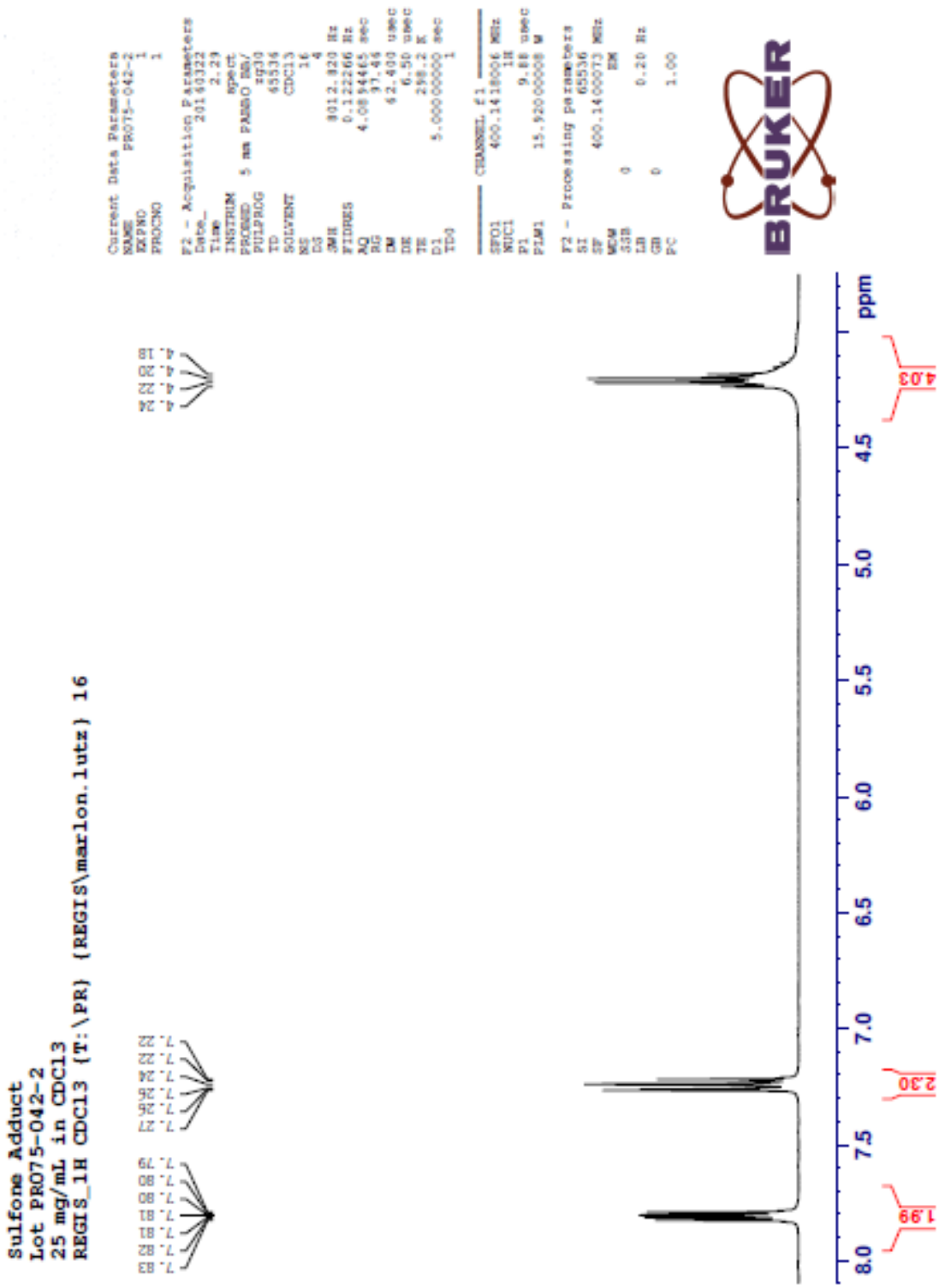
Additional Info : Peak(s) manually integrated
```



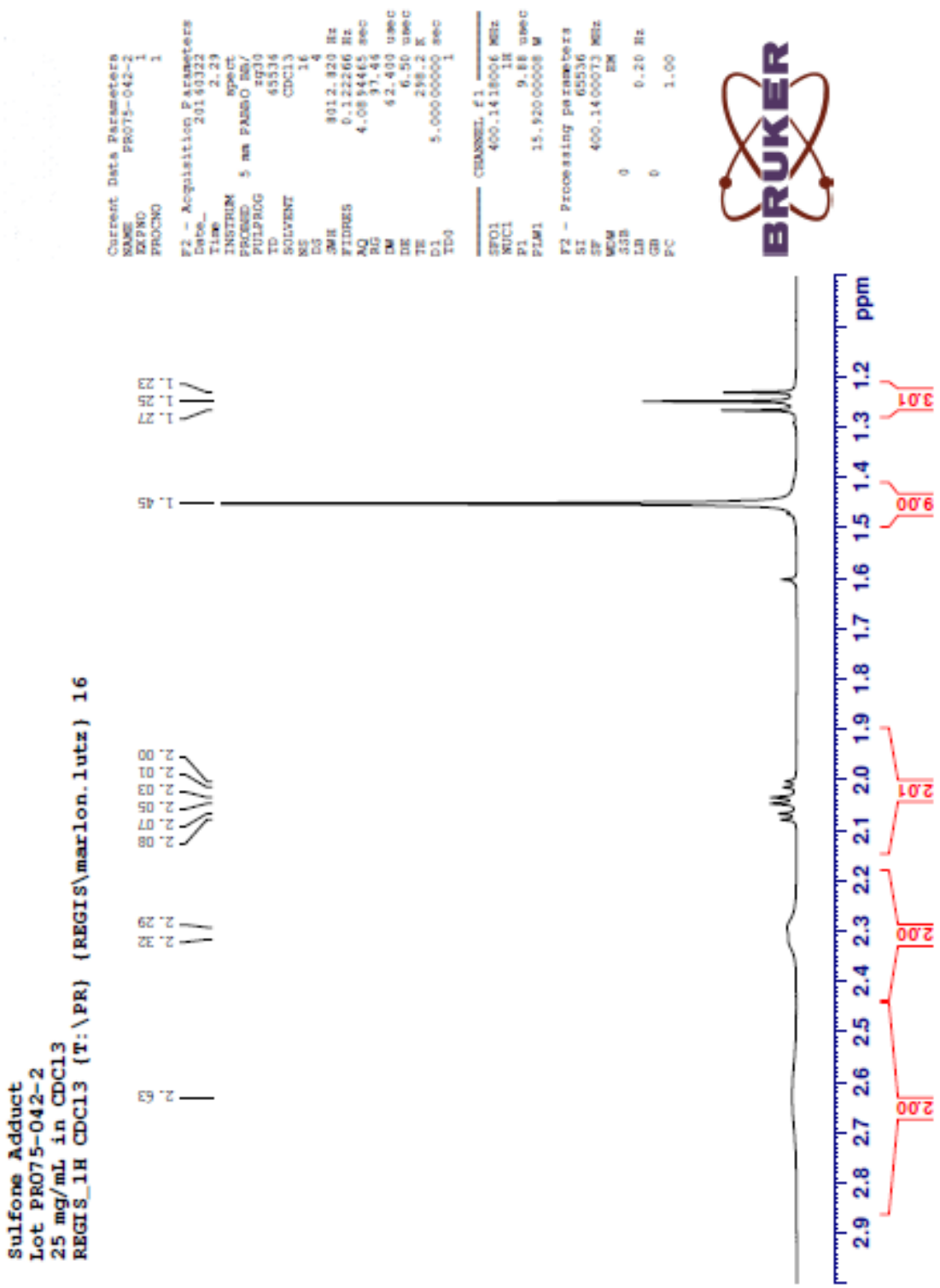
^{1}H NMR - Sulfone Adduct (Compound 4)



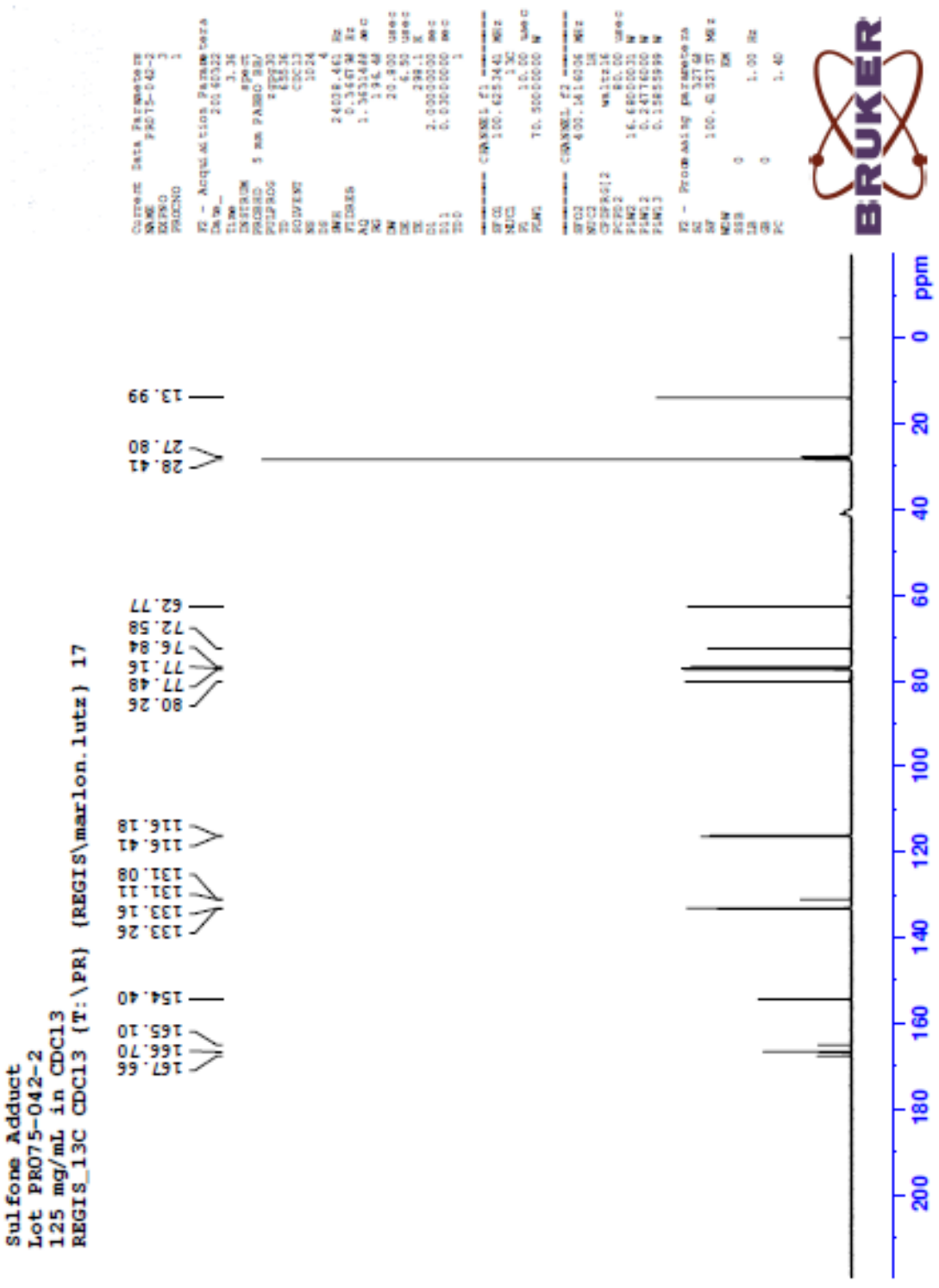
^{1}H NMR - Sulfone Adduct (Compound 4)



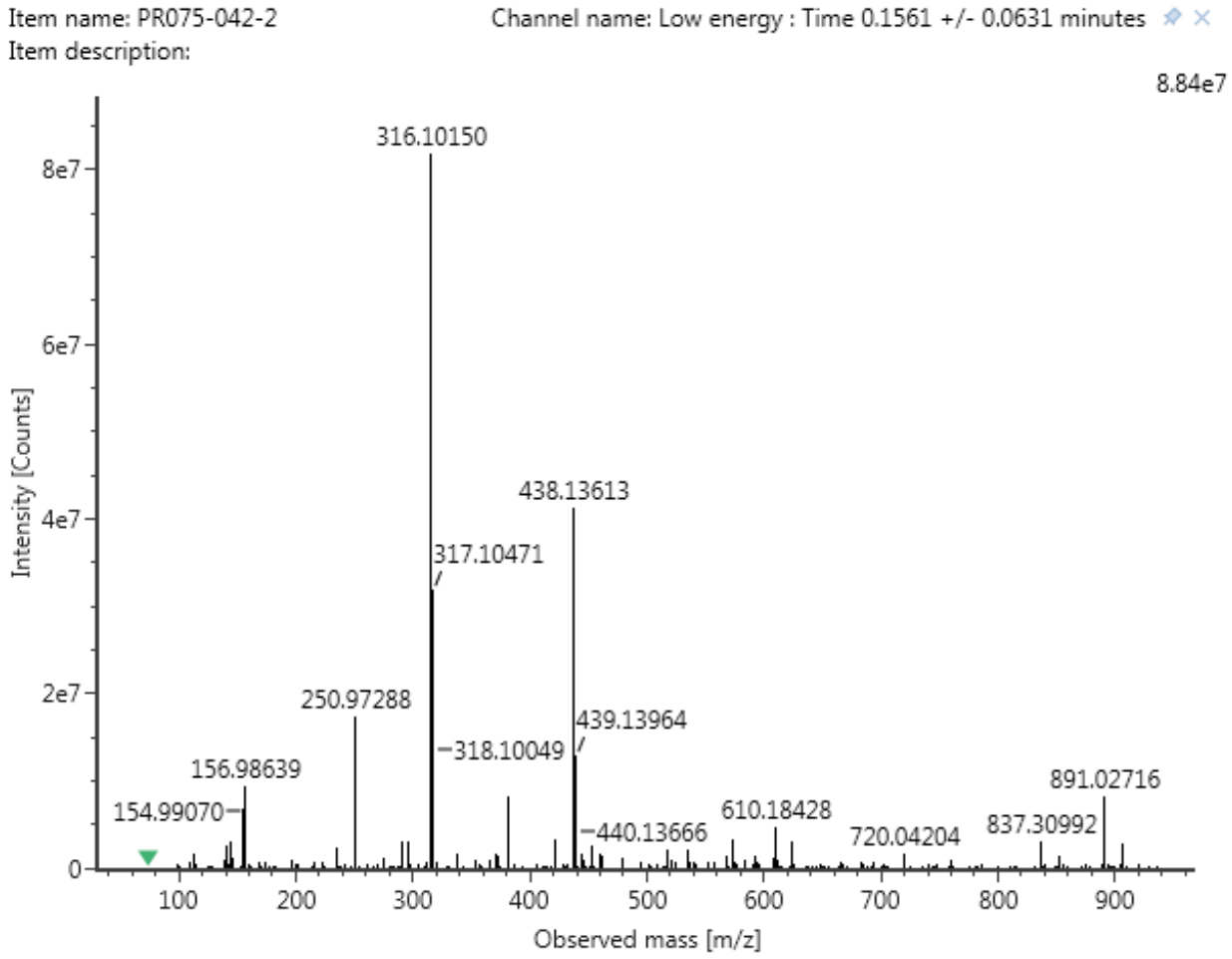
^{1}H NMR - Sulfone Adduct (Compound 4)



^{13}C NMR - Sulfone Adduct (Compound 4)



HRMS - Sulfone Adduct (Compound 4)



HPLC - Sulfone Adduct HCl Salt (Compound 5)

```
Sulfone Adduct HCl Salt
PR075-044-2,  4 mg/mL in ACN

================================================================================
Injection Date  : 4/5/2016 10:52:29 PM          Seq. Line :   8
Sample Name     : PR075-044-2                    Location : Vial 84
Acq. Operator   : MRL                               Inj :   1
Acq. Instrument : Instrument 1                 Inj Volume : 5 µl
Method          : C:\HPCHEM\1\METHODS\LOC-MMP.M
Last changed    : 2/26/2016 10:22:15 PM by MRL
Generic HPLC Method for Loyola MMP Project - Rxn Monitoring and Purity Assay
HPLC 31
C=10mM K3PO4 pH 3 in water + 5% ACN
D=ACN
Waters Symmetry Shield RP-18 150mmx4.6mm 5 microns
1.0mL/min 40C 205, 220, 254, 280 nm
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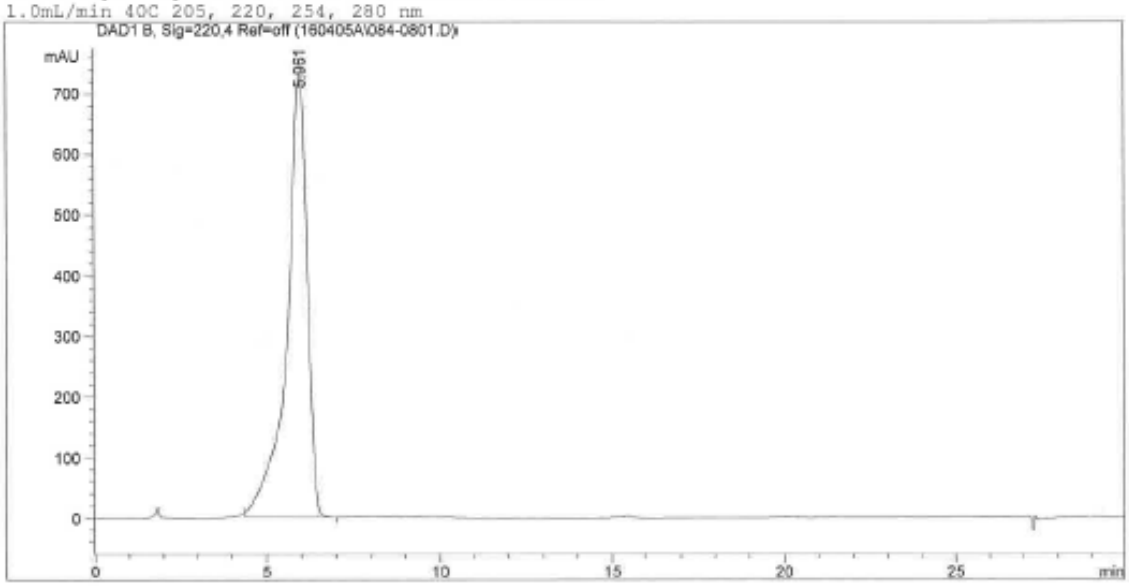


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                              Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 B, Sig=220,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
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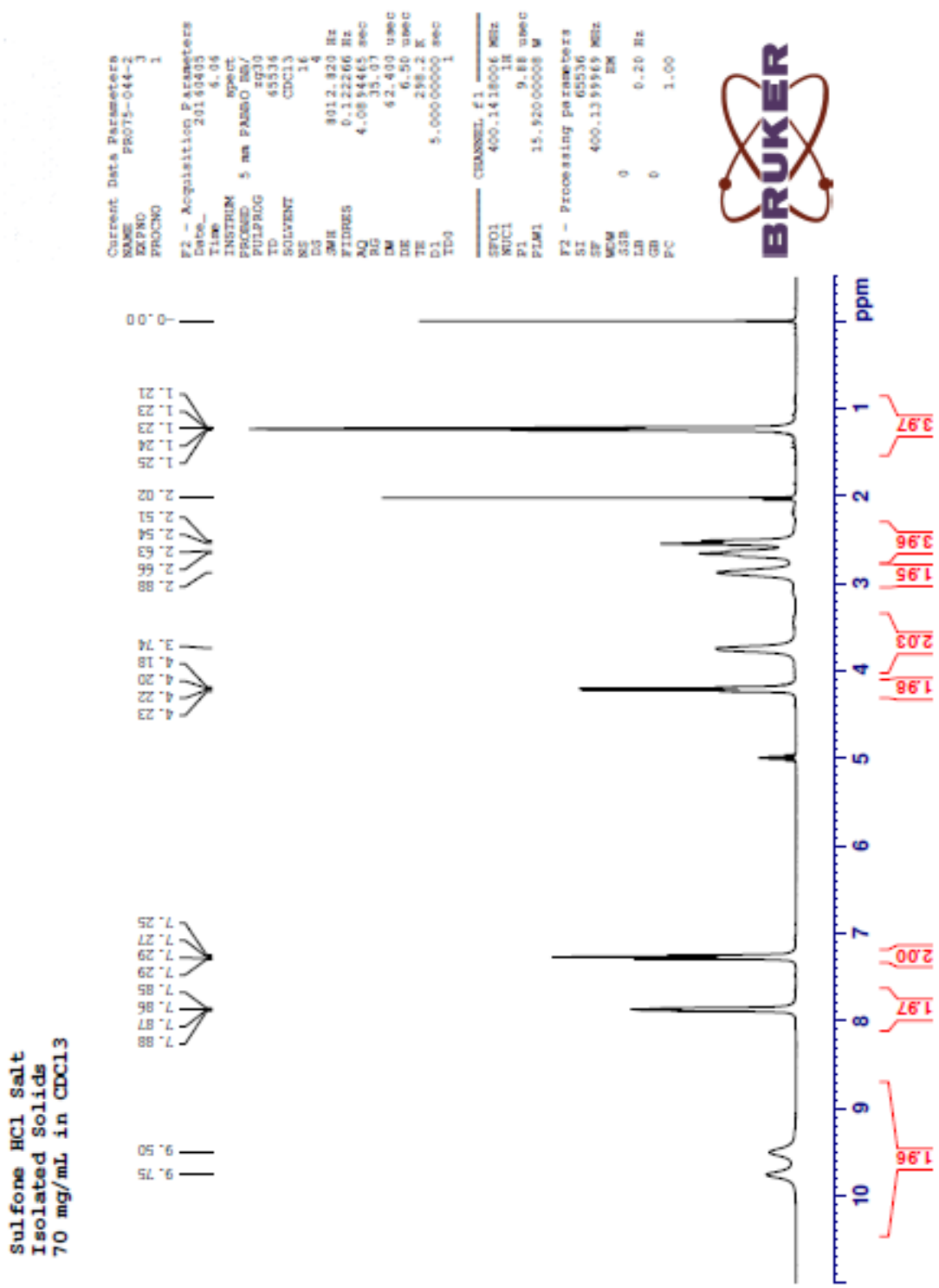
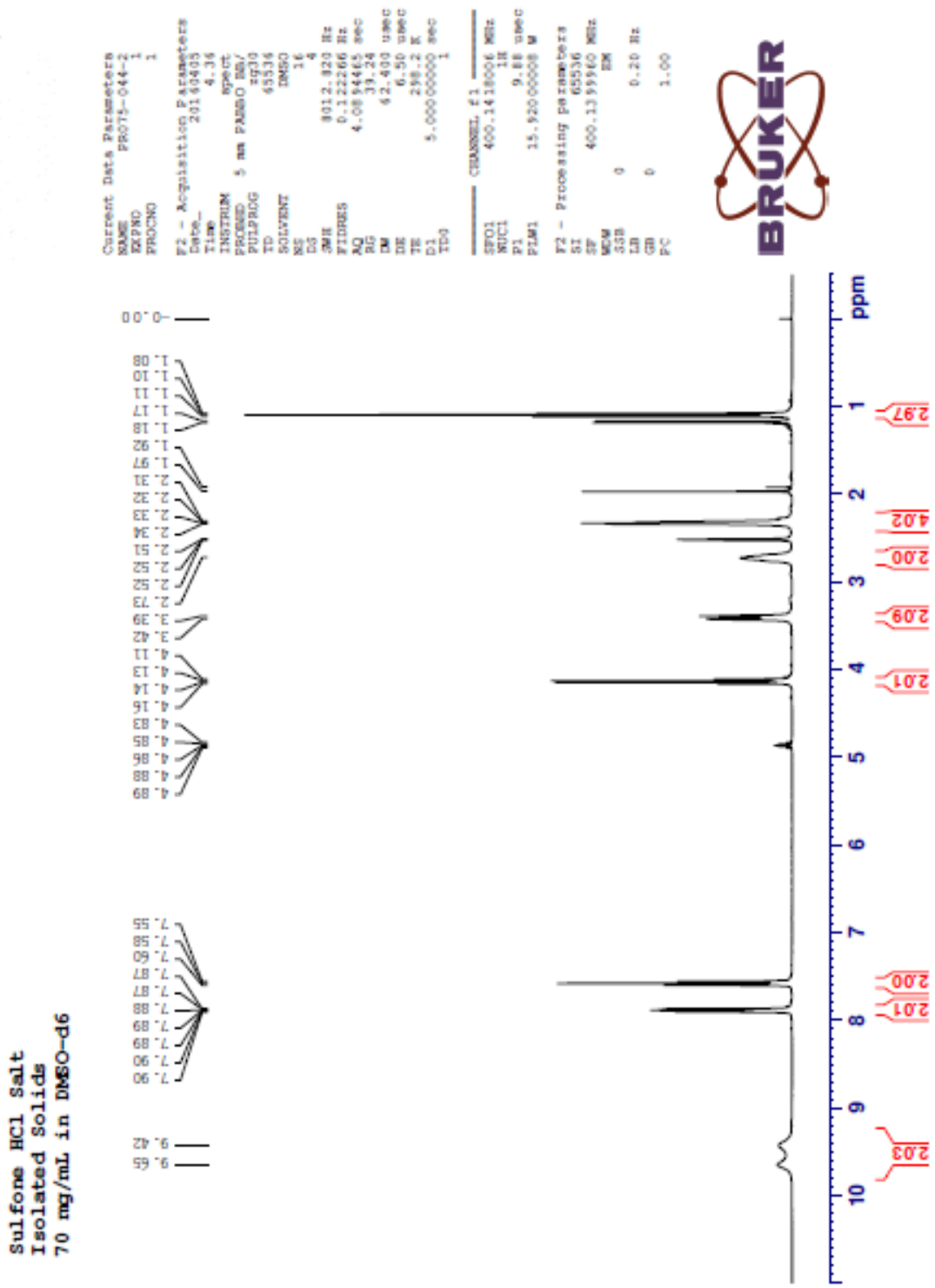
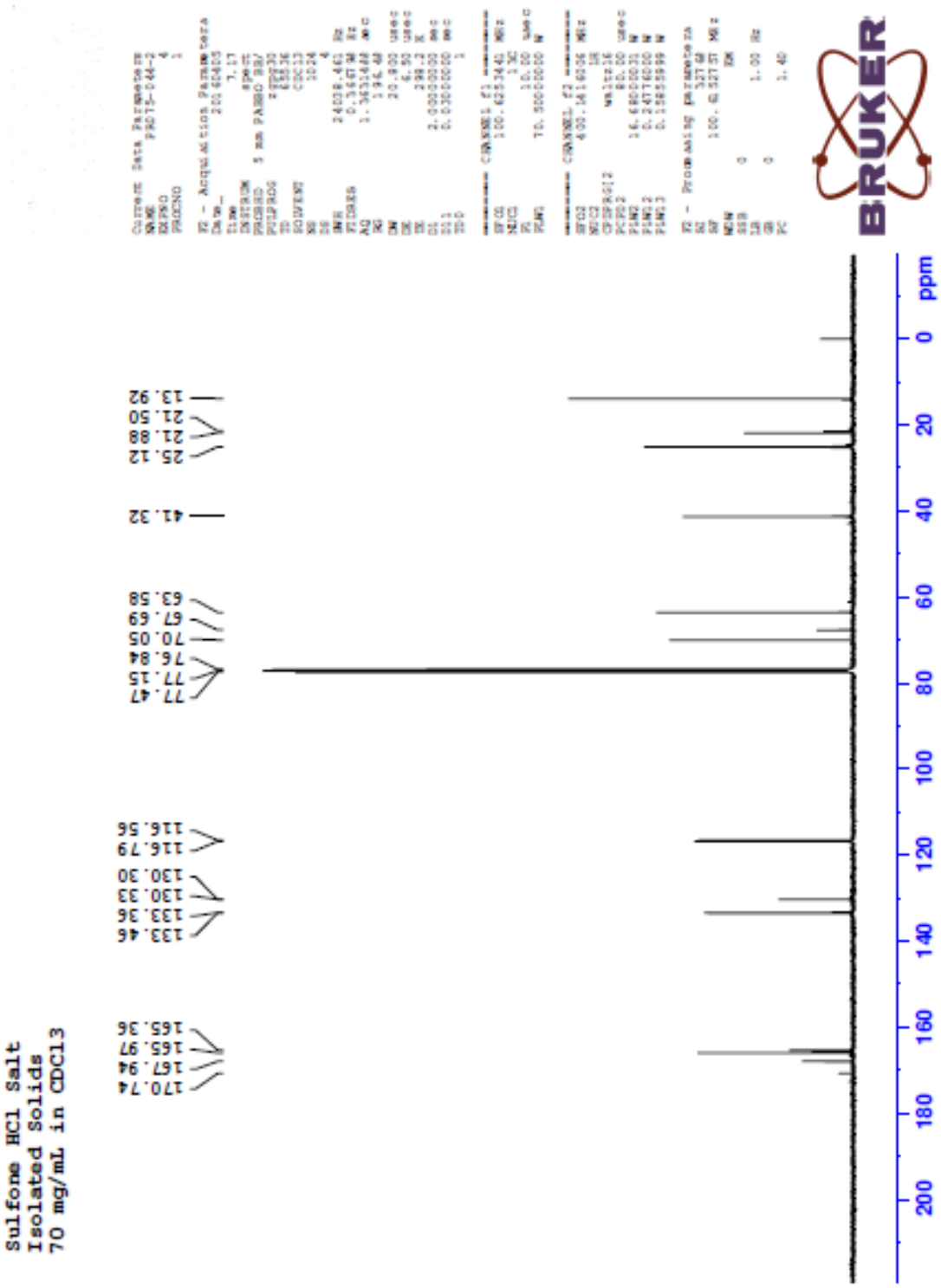
^{1}H NMR - Sulfone Adduct HCl Salt (Compound 5)



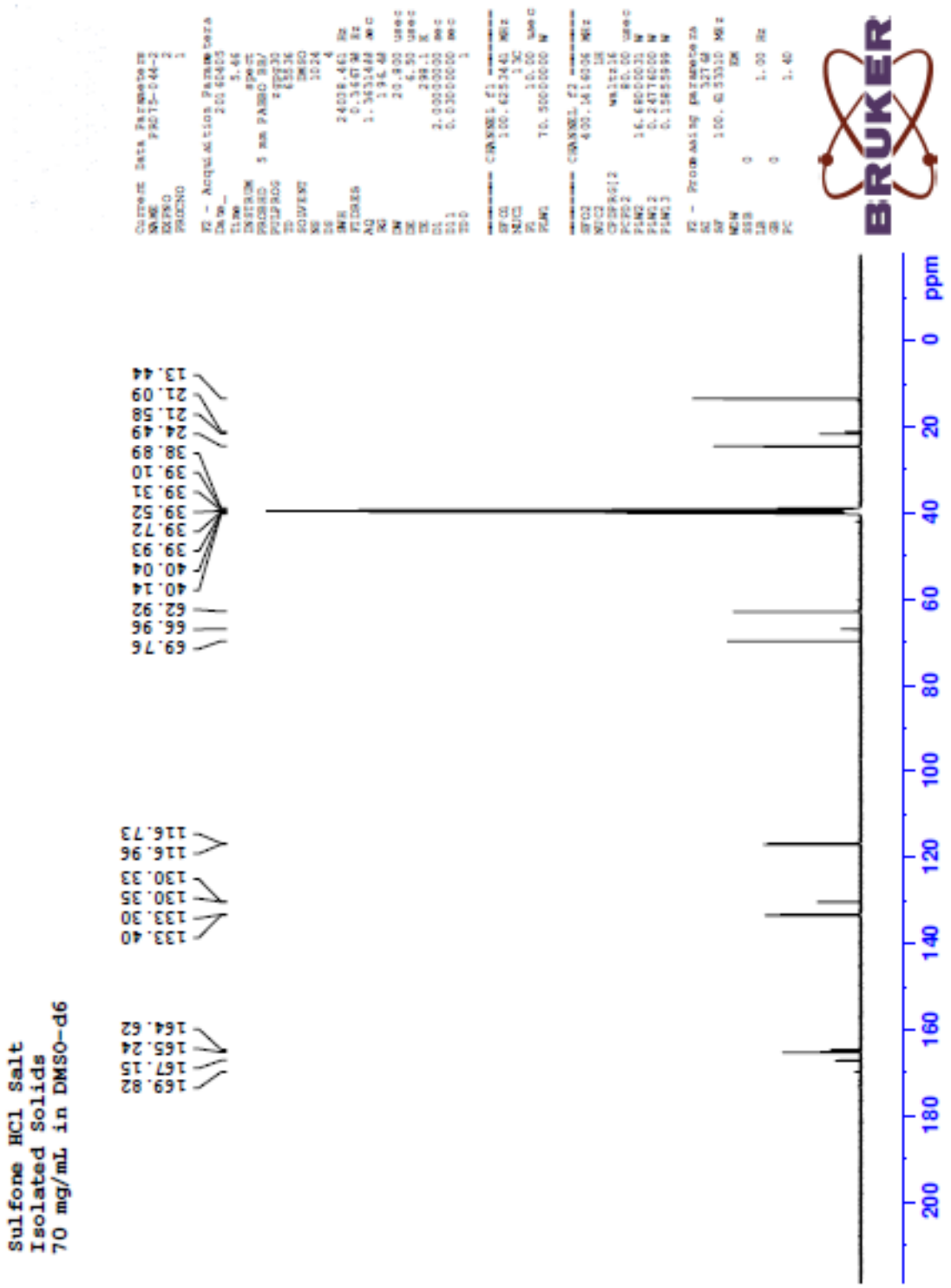
^{1}H NMR - Sulfone Adduct HCl Salt (Compound 5)



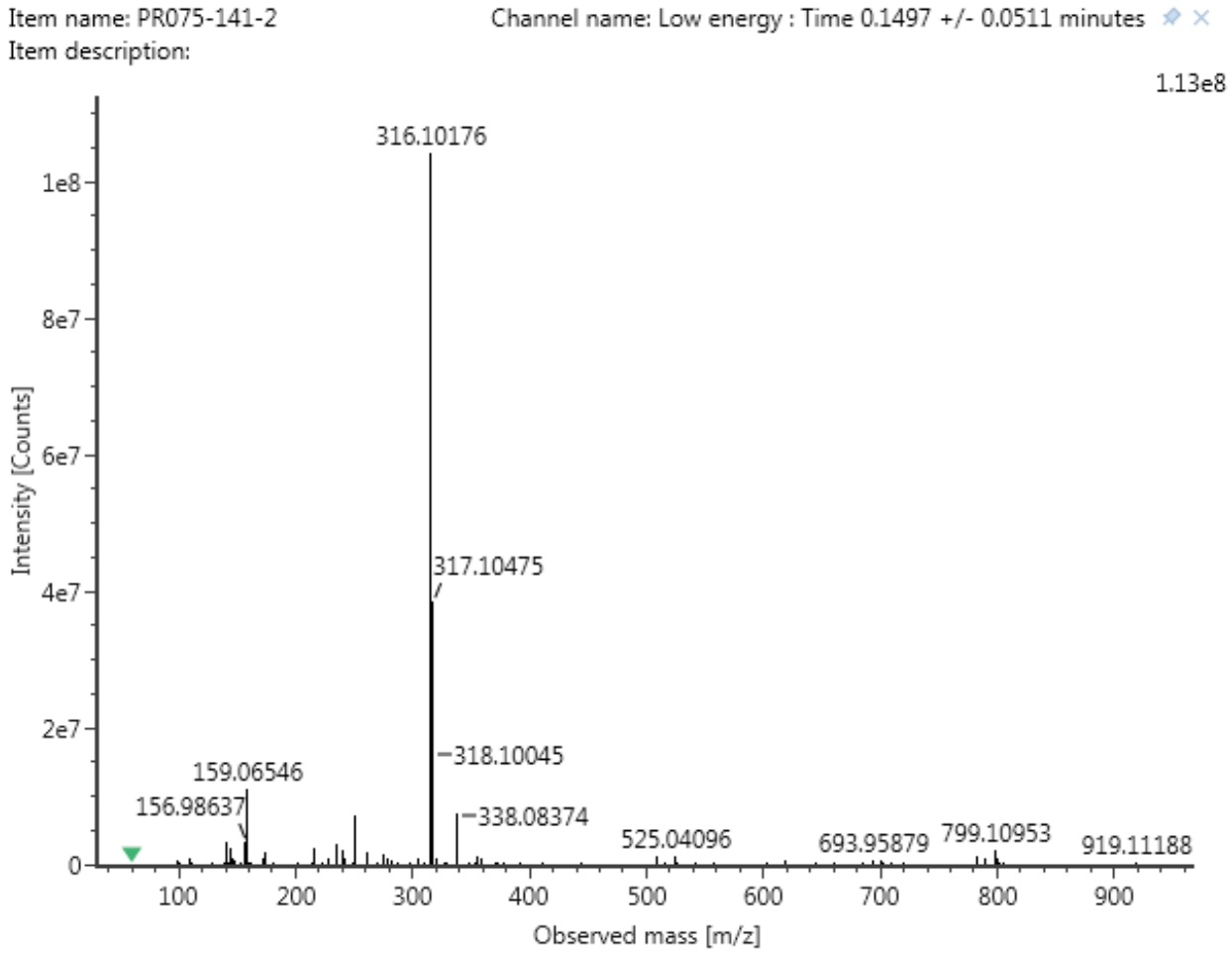
^{13}C NMR - Sulfone Adduct HCl Salt (Compound 5)



^{13}C NMR - Sulfone Adduct HCl Salt (Compound 5)



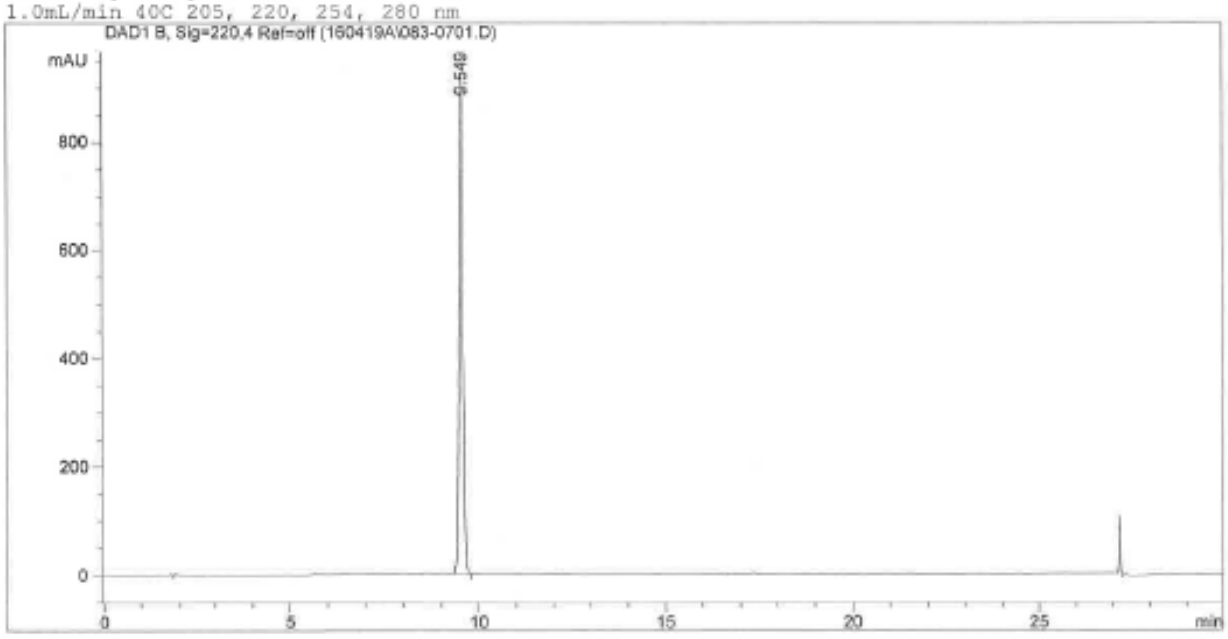
HRMS - Sulfone Adduct HCl Salt (Compound 5)



HPLC - Propargylamine Adduct (Compound 6)

```
Isolated Propargylamine Adduct
1.0 mg/mL in ACN/water (1:1)

=====================================================================
Injection Date  : 4/19/2016 10:46:08 PM          Seq. Line :   7
Sample Name     : PR075-053-3                     Location : Vial 83
Acq. Operator   : MRL                                 Inj :   1
Acq. Instrument : Instrument 1
Method          : C:\HPCHEM\1\METHODS\LUC-MMP.M
Last changed    : 2/26/2016 10:22:15 PM by MRL
Generic HPLC Method for Loyola MMP Project - Rxn Monitoring and Purity Assay
HPLC 31
C=10mM K3PO4 pH 3 in water + 5% ACN
D=ACN
Waters Symmetry Shield RP-18 150mmx4.6mm 5 microns
1.0mL/min 40C 205, 220, 254, 280 nm
```

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                        Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 B, Sig=220,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
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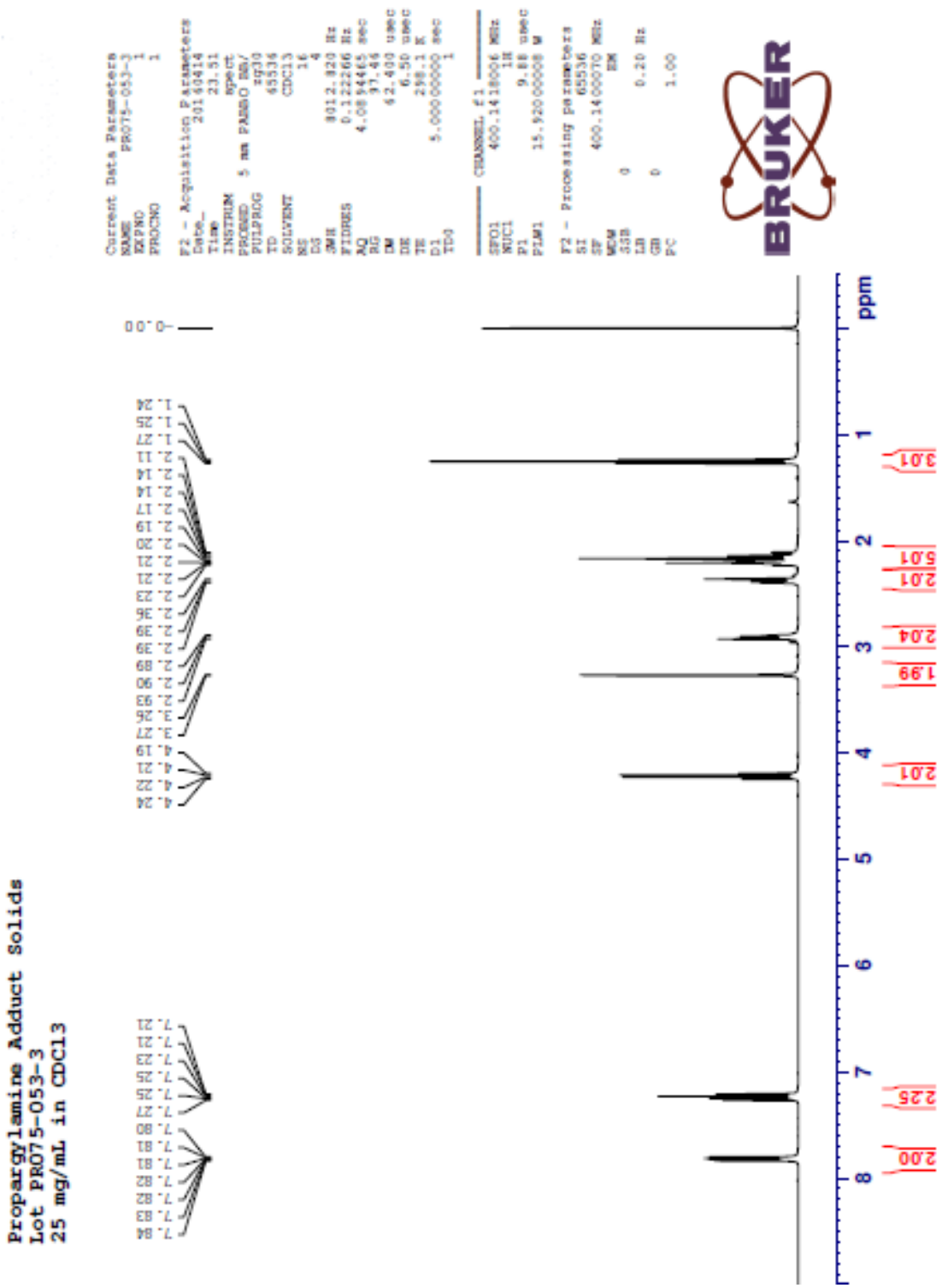
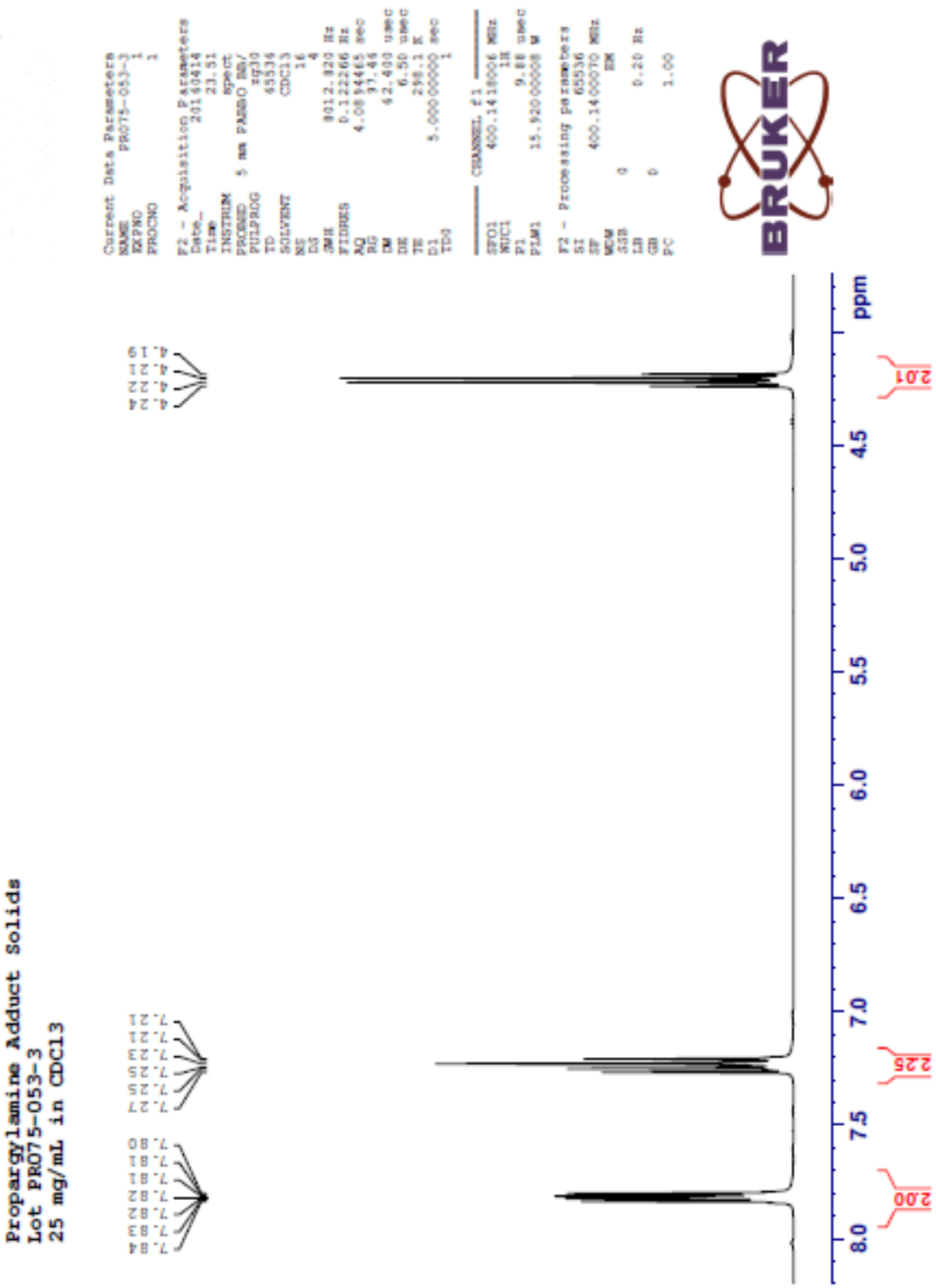
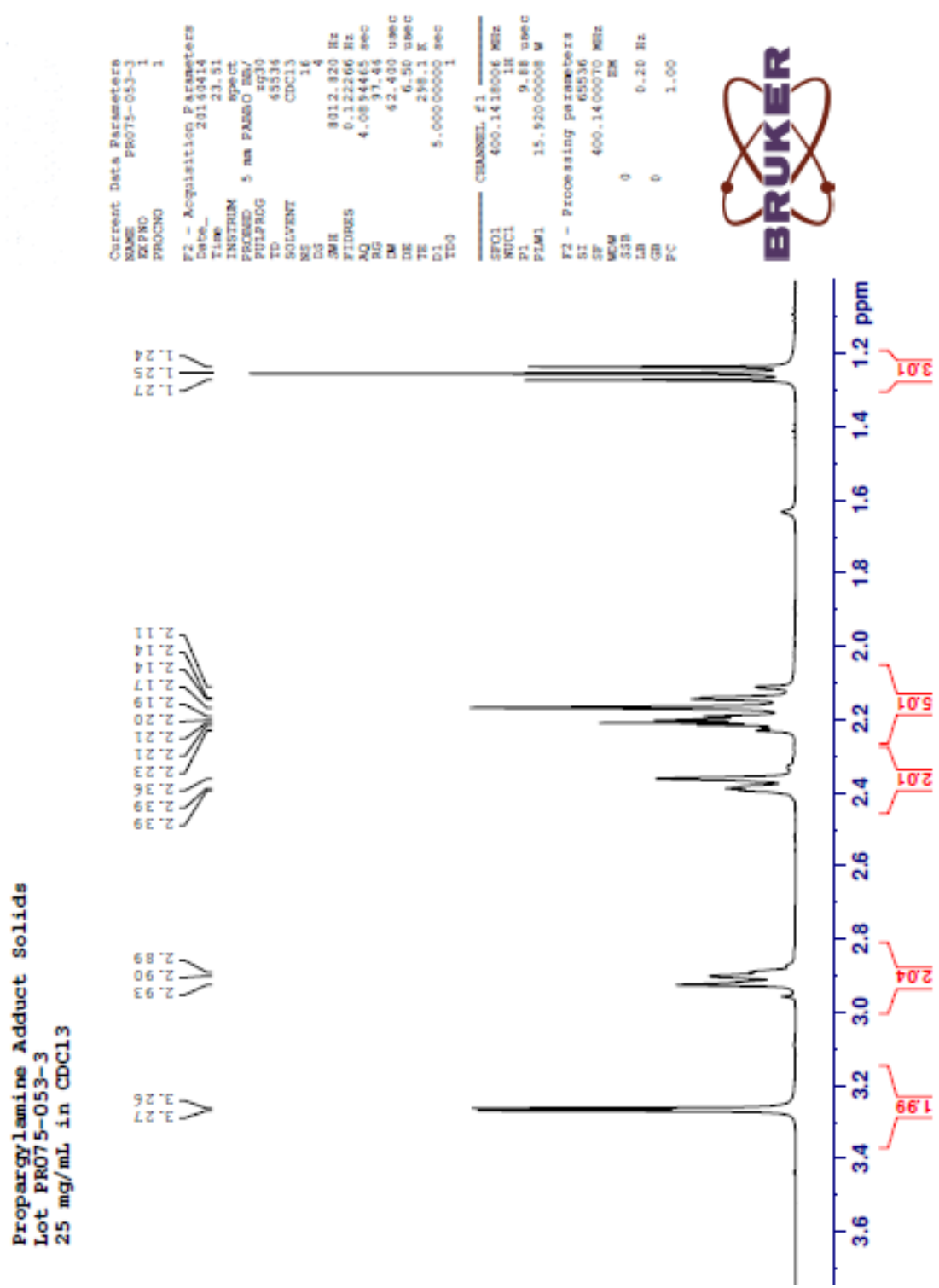
^{1}H NMR - Propargylamine Adduct (Compound 6)



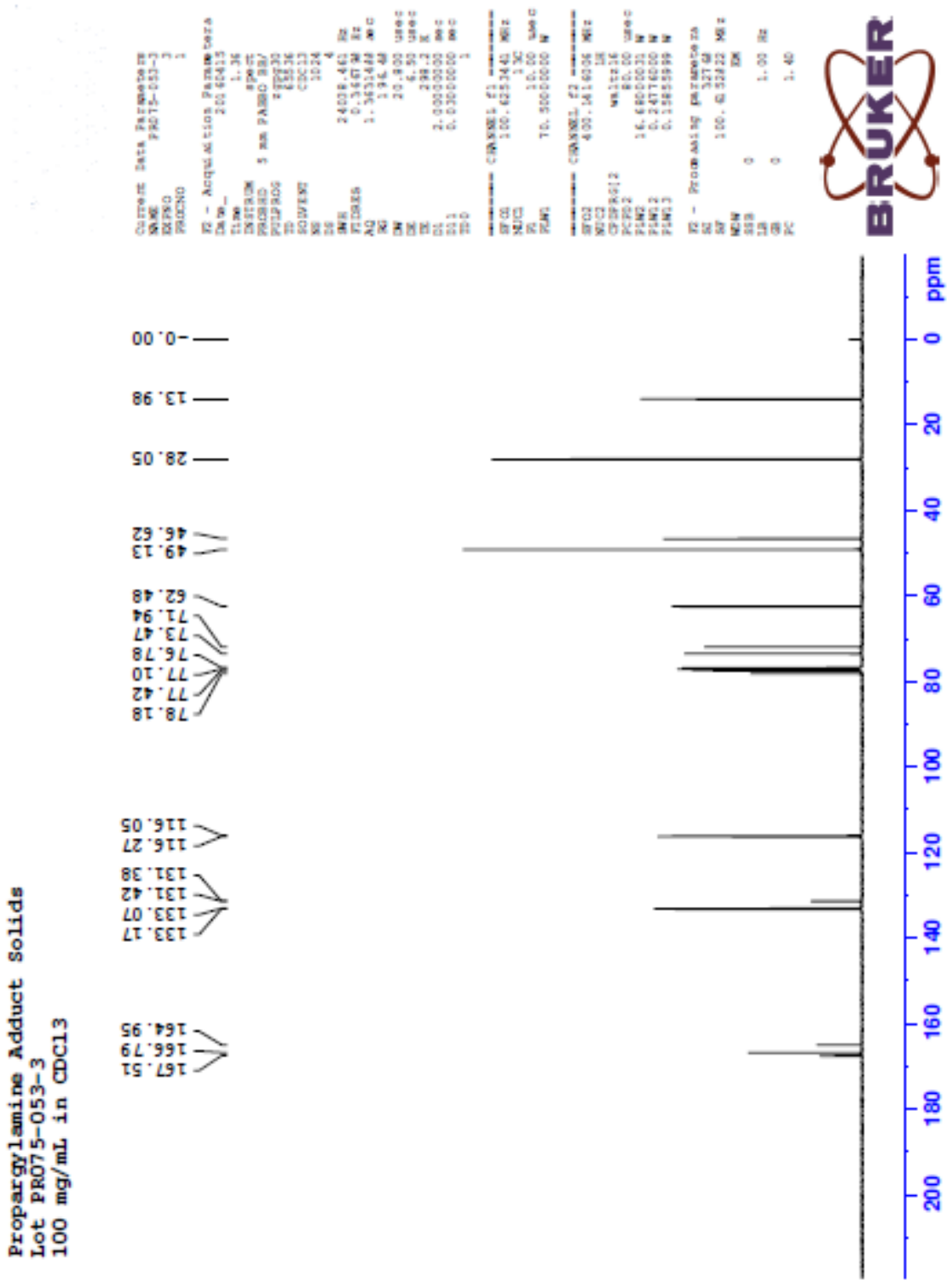
^{1}H NMR - Propargylamine Adduct (Compound 6)



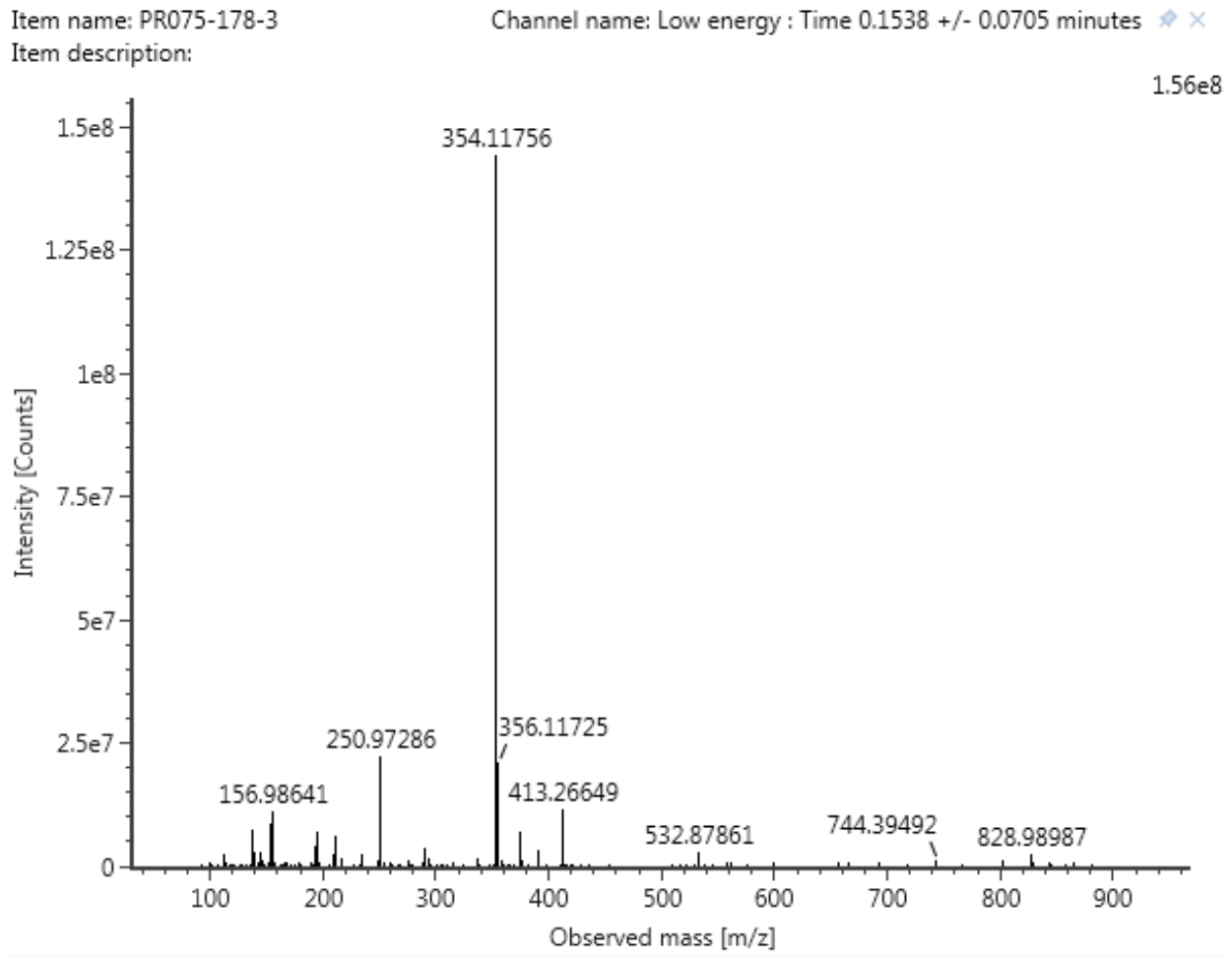
^{1}H NMR - Propargylamine Adduct (Compound 6)



^{13}C NMR - Propargylamine Adduct (Compound 6)



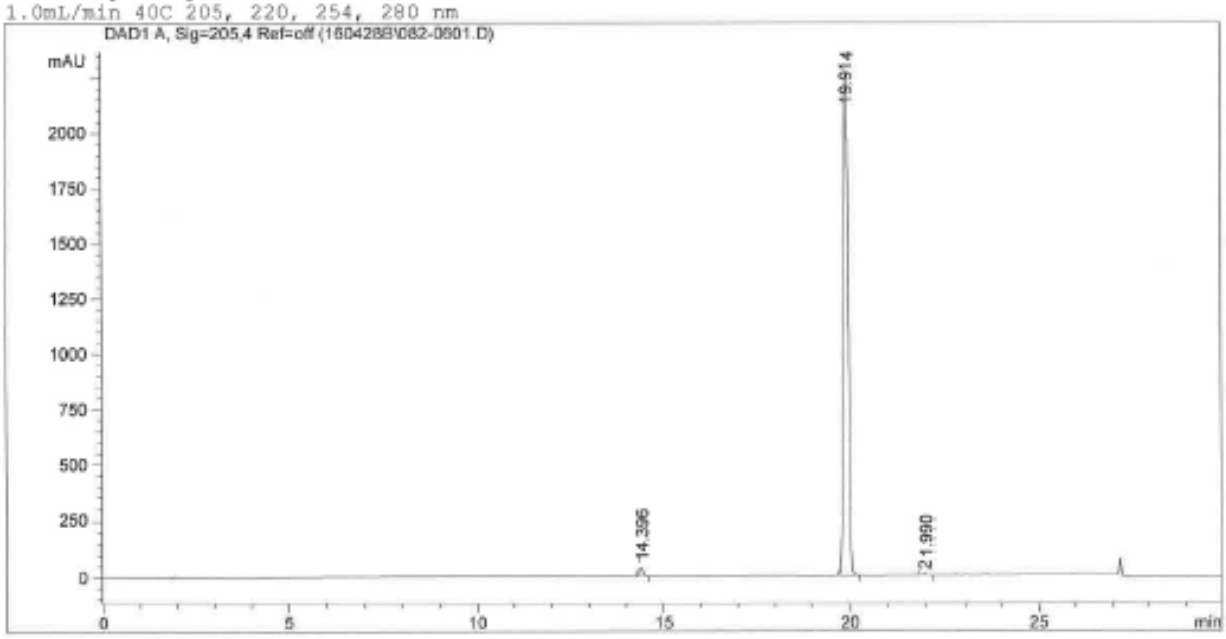
HRMS - Propargylamine Adduct (Compound 6)



HPLC - Diaryl Ether Adduct (Compound 7)

```
Diaryl Ether
PR075-058-4, 1 mg/mL in ACN/water (1:1)

================================================================================
Injection Date  : 4/28/2016 9:05:01 PM          Seq. Line :   6
Sample Name     : PR075-058-4                    Location : Vial 82
Acq. Operator   : MRL                               Inj :   1
Acq. Instrument : Instrument 1                  Inj Volume : 5 µl
Method          : C:\HPCHEM\1\METHODS\LUC-MMP.M
Last changed    : 2/26/2016 10:22:15 PM by MRL
Generic HPLC Method for Loyola MMP Project - Rxn Monitoring and Purity Assay
HPLC 31
C=10mM K3PO4 pH 3 in water + 5% ACN
D=ACN
Waters Symmetry Shield RP-18 150mmx4.6mm 5 microns
1.0mL/min 40C 205, 220, 254, 280 nm
```

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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=205,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
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   1  14.396 BB    0.1066  260.65900   38.69700   1.3512
   2  19.914 BB    0.1345 1.89888e4  2248.54468  98.4360
   3  21.990 BB    0.1228   41.04656    5.27531   0.2128
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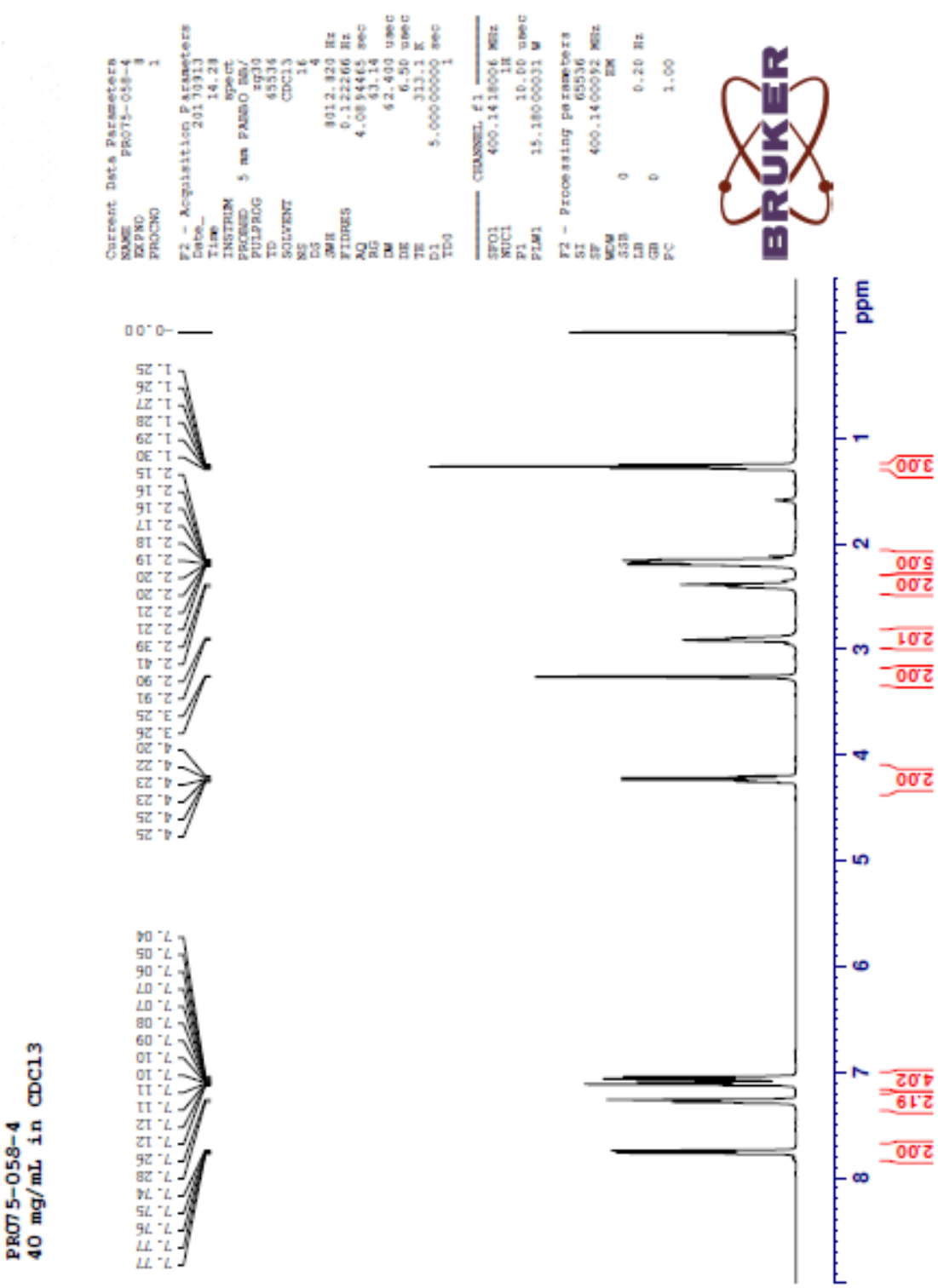
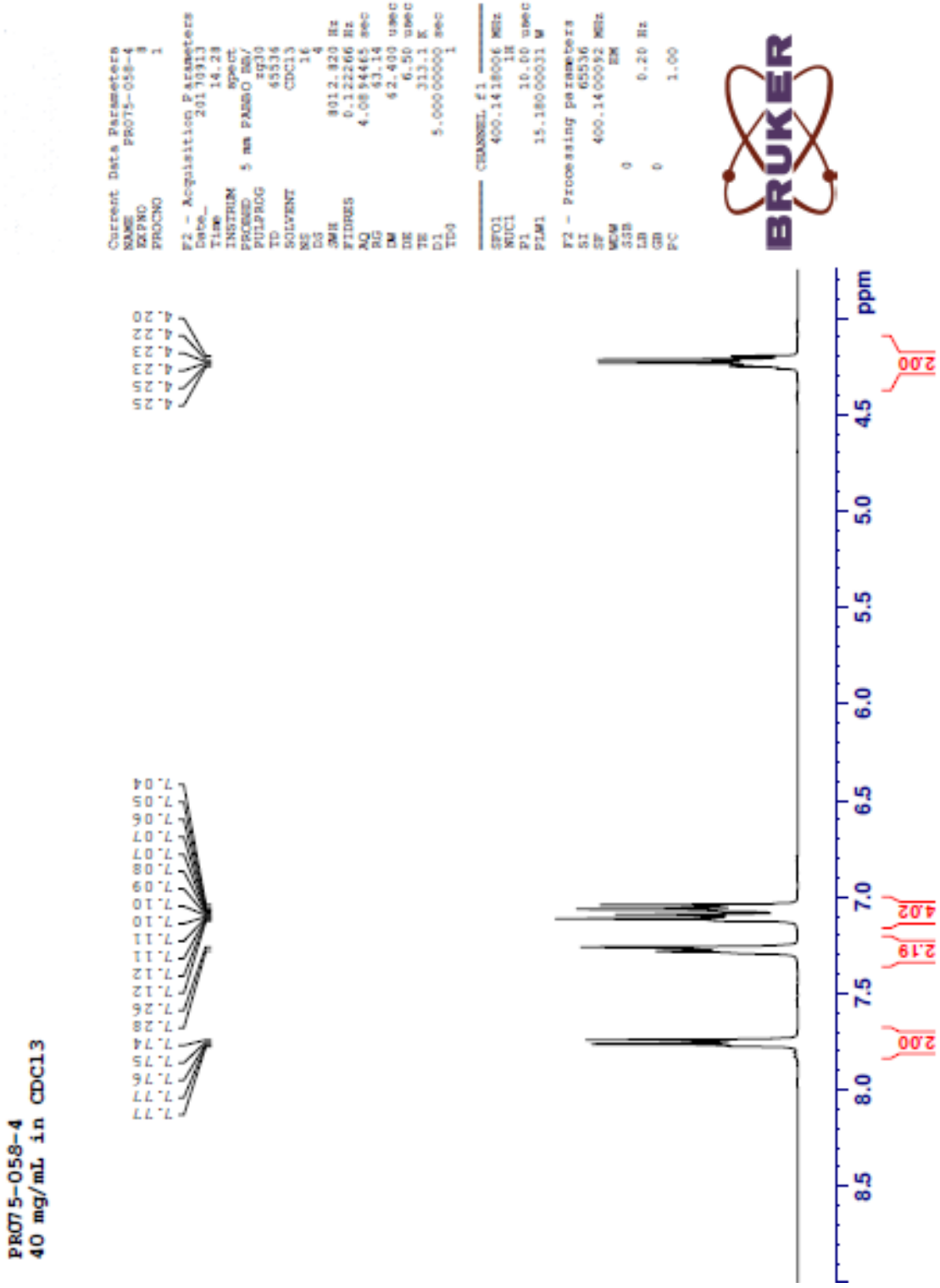
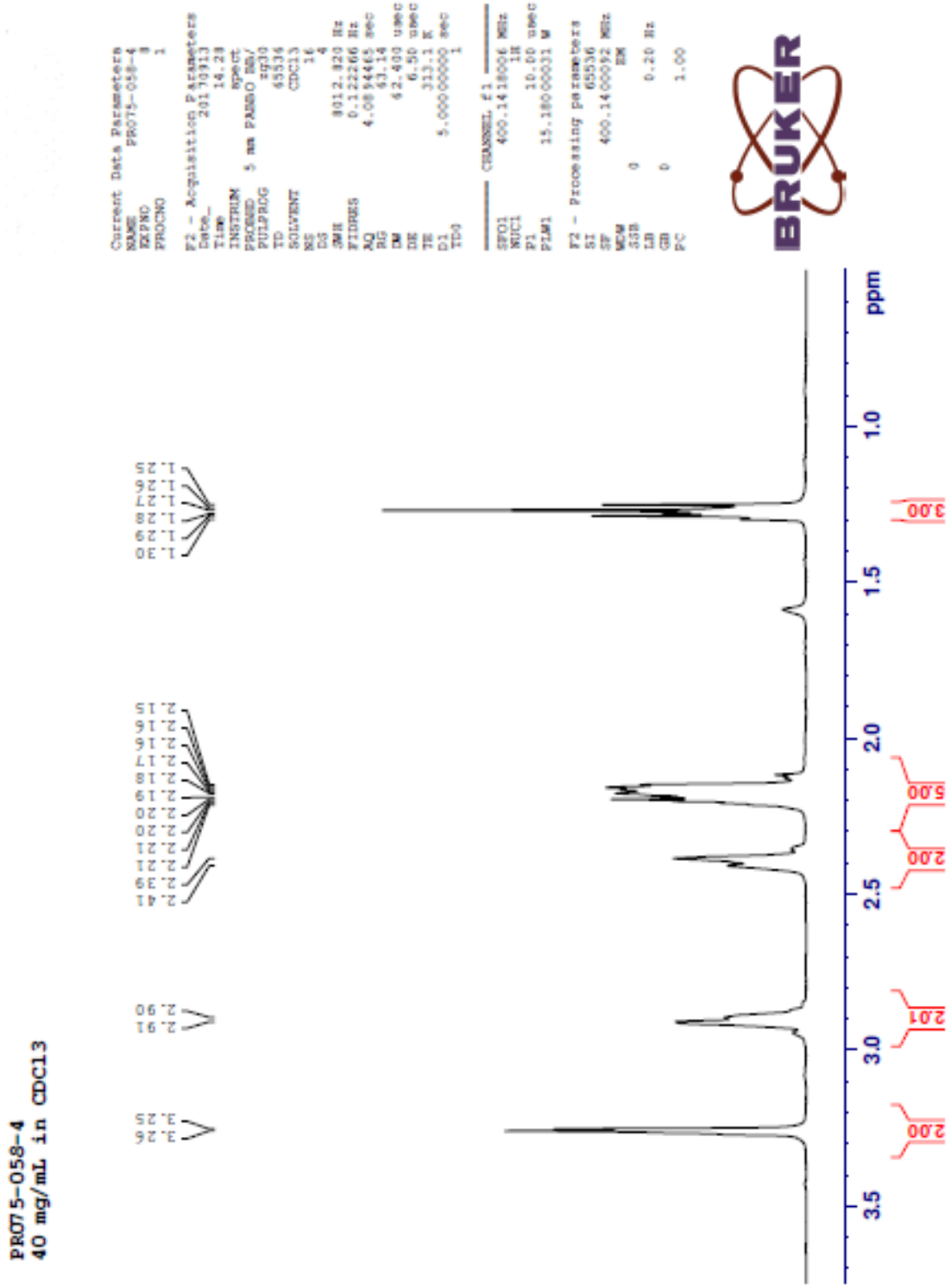
^{1}H NMR - Diaryl Ether Adduct (Compound 7)



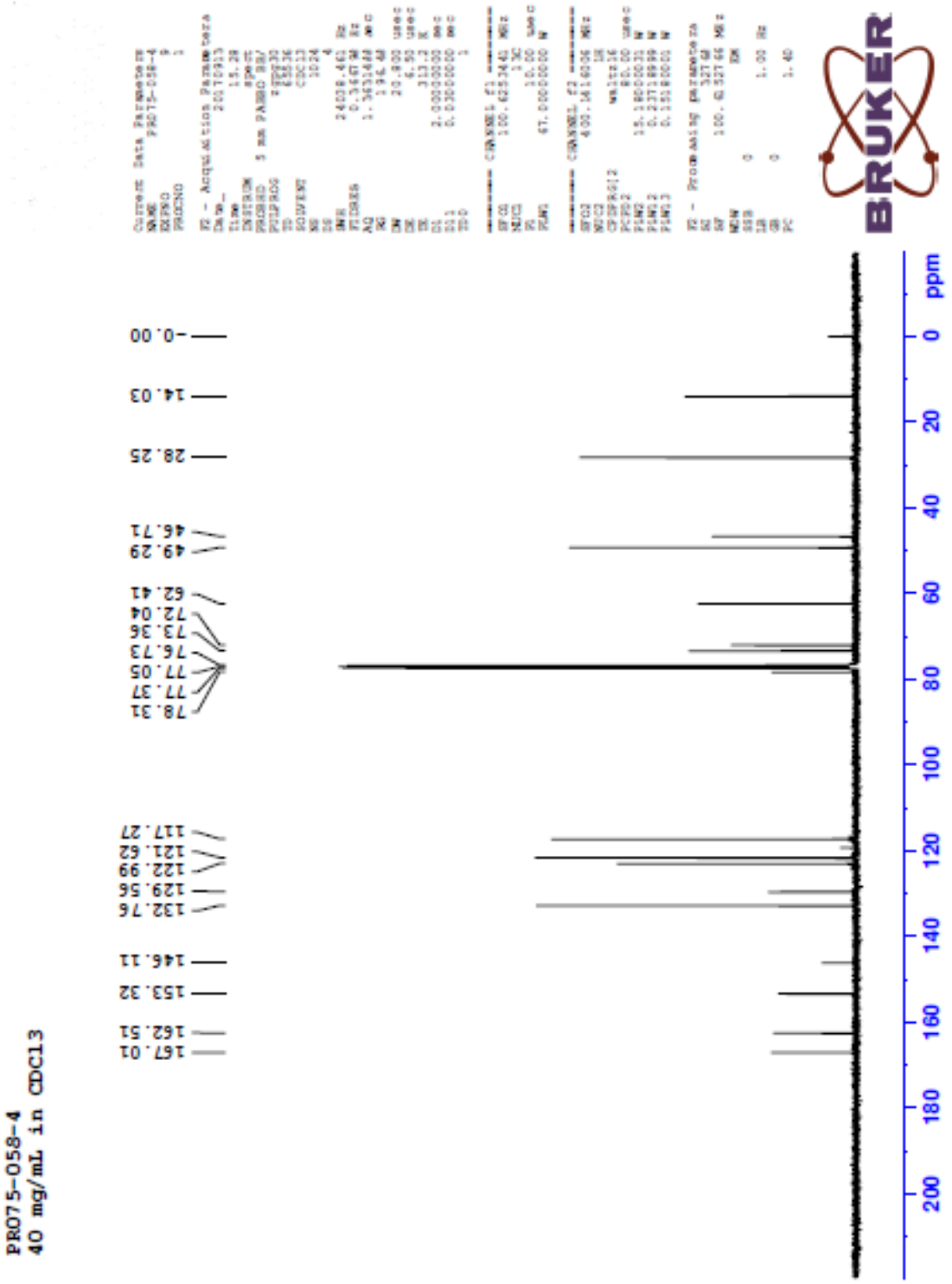
^{1}H NMR - Diaryl Ether Adduct (Compound 7)



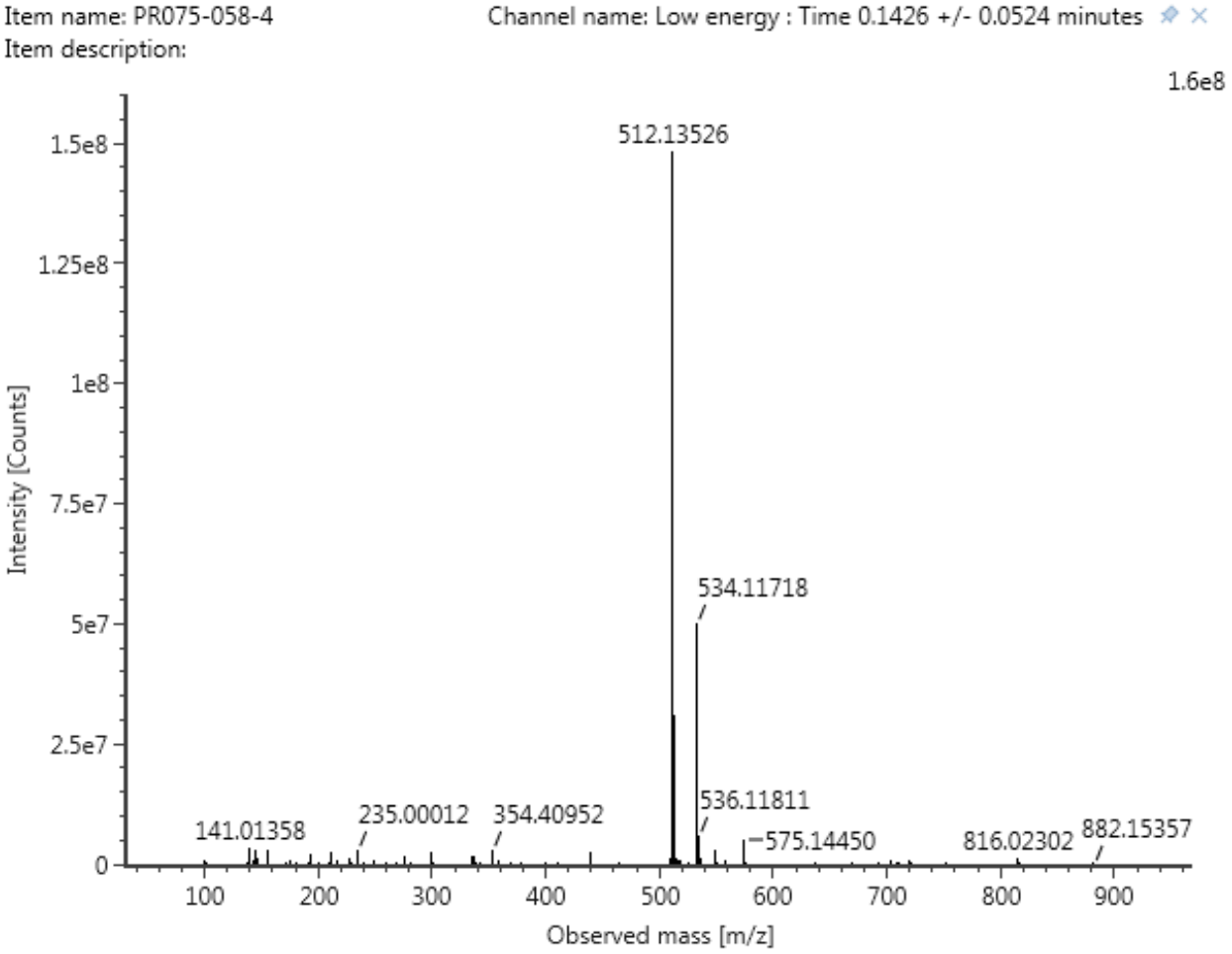
^{1}H NMR - Diaryl Ether Adduct (Compound 7)



^{13}C NMR - Diaryl Ether Adduct (Compound 7)



HRMS - Diaryl Ether Adduct (Compound 7)



HPLC - MMP-18 Carboxylic Acid (Compound 8)

```
Diaryl Ether Free Acid
Isolated Crude Solids
PR075-063-2, 1 mg/mL

================================================================================
Injection Date  : 5/20/2016 9:35:31 PM          Seq. Line :  11
Sample Name     : PR075-063-2                    Location : Vial 83
Acq. Operator   : MRL                               Inj :  1
Acq. Instrument : Instrument 1                 Inj Volume : 5 µl
Method          : C:\HPCHEM\1\METHODS\LUC-MMP.M
Last changed    : 2/26/2016 10:22:15 PM by MRL
Generic HPLC Method for Coco-Cola Project - Reaction Monitoring and Purity Assay
HPLC 31
C=10mM K3PO4 pH 3 in water + 5% ACN
D=ACN
Waters Symmetry Shield RP-18 150mmx4.6mm 5 microns
1.0mL/min 40C 205, 220, 254, 280, 390 nm
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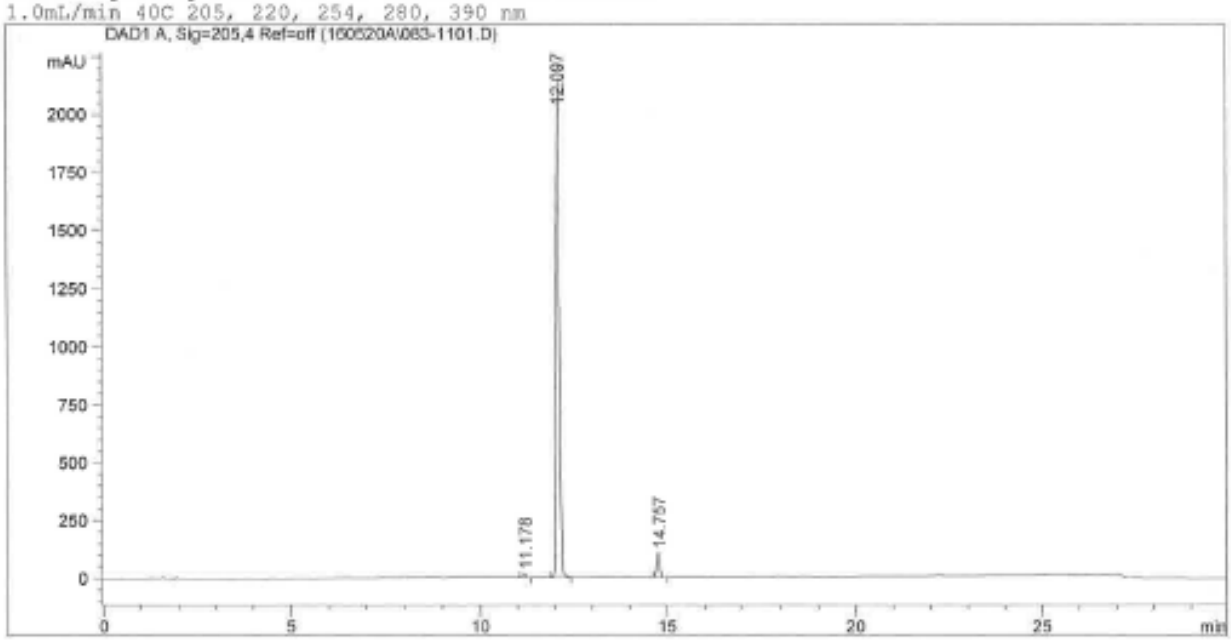


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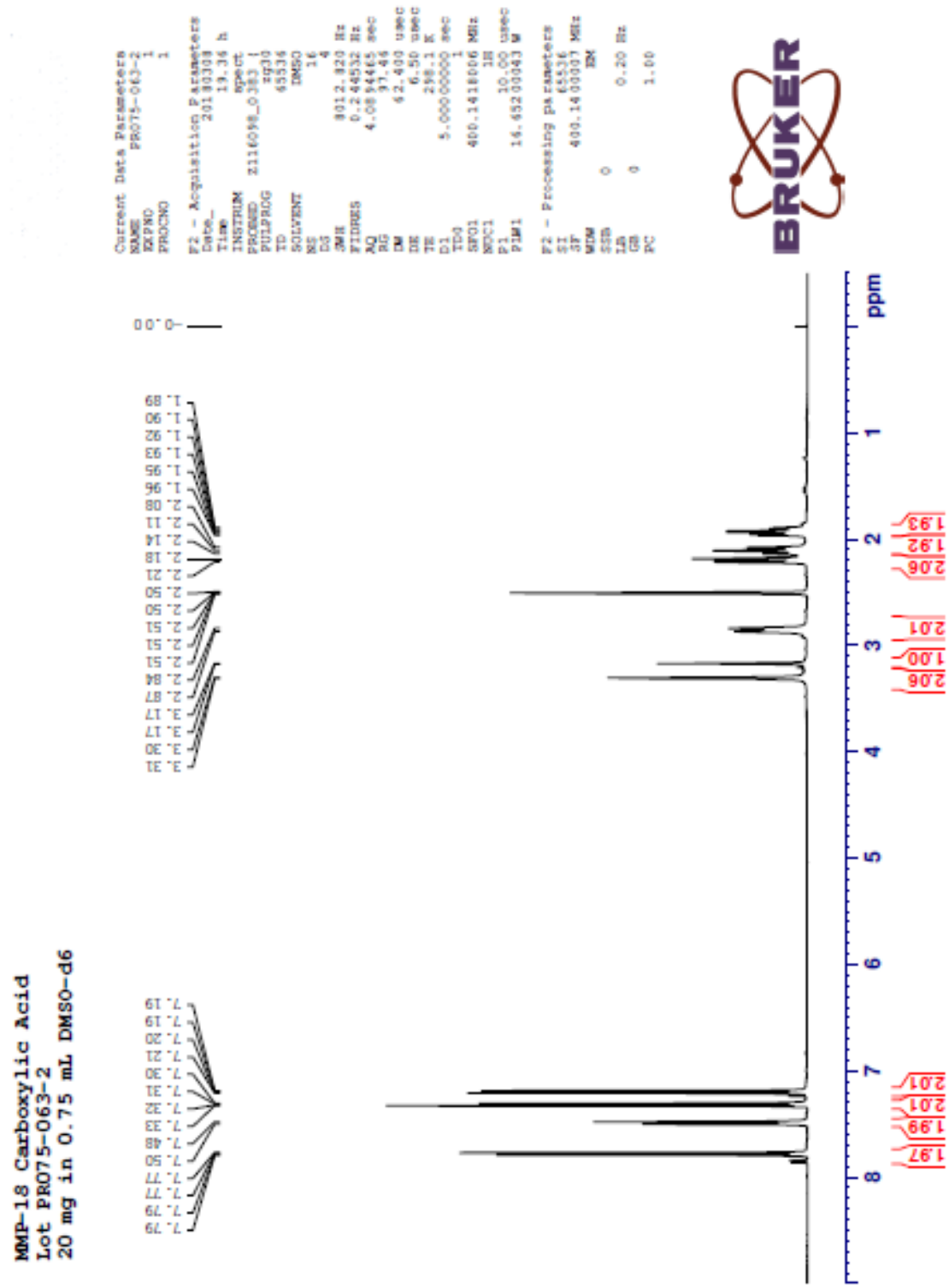
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

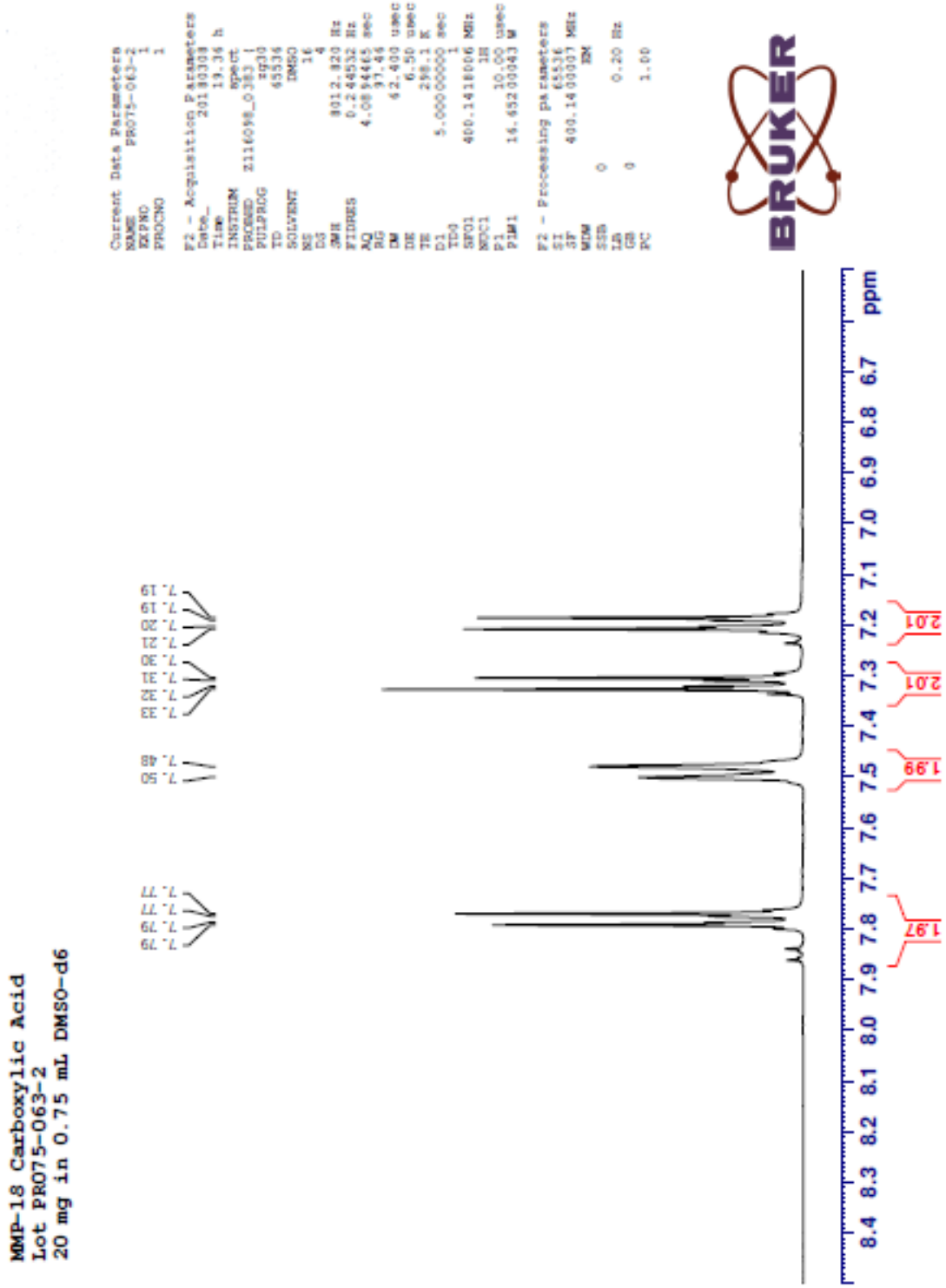

Signal 1: DAD1 A, Sig=205,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  11.178 BB    0.0858   85.31812   16.10917   0.6433
   2  12.097 VB    0.0923 1.25220e4  2140.29785  94.4110
   3  14.757 BB    0.1007  655.96240  105.19547   4.9457
```

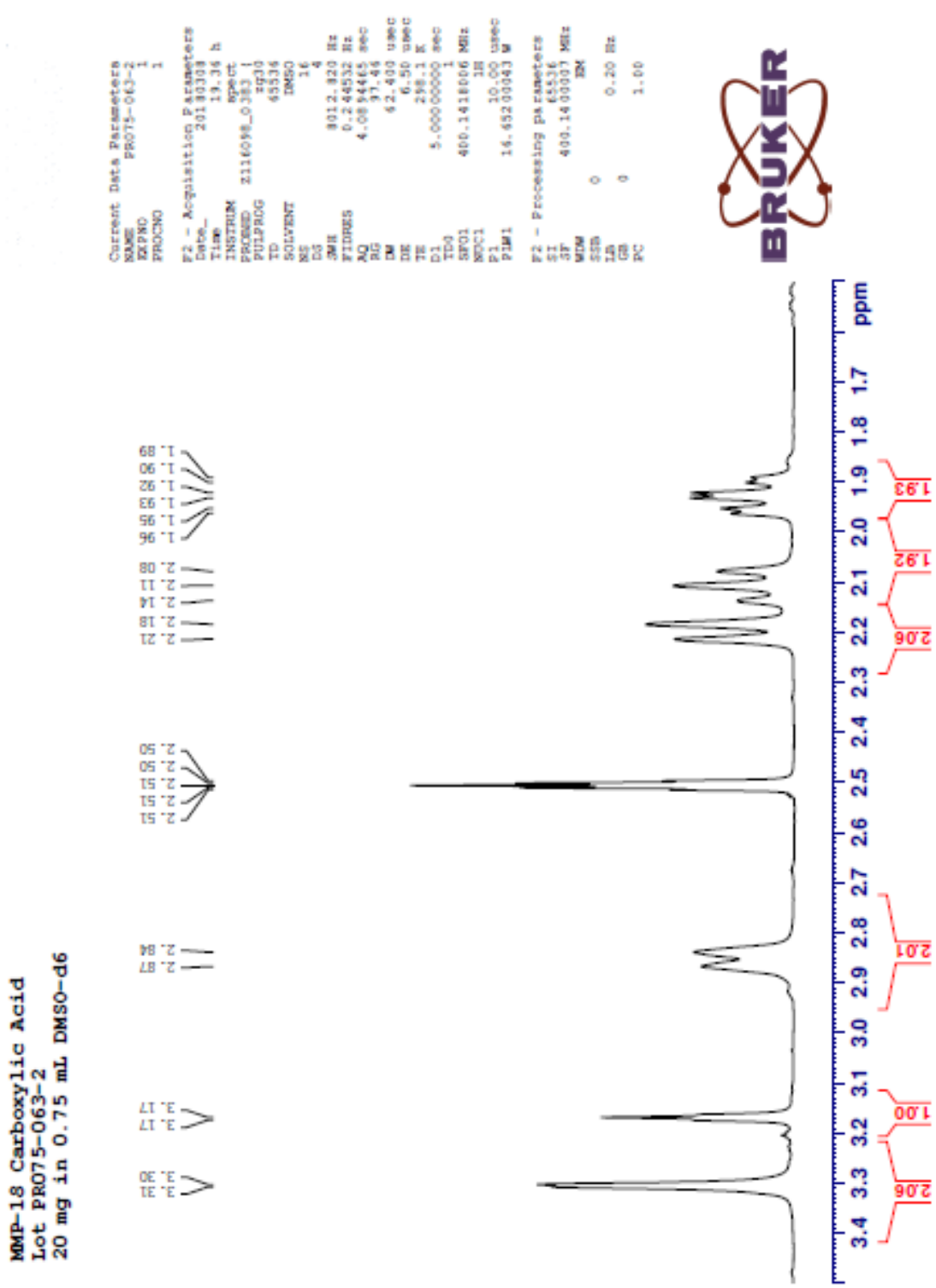
^{1}H NMR - MMP-18 Carboxylic Acid (Compound 8)



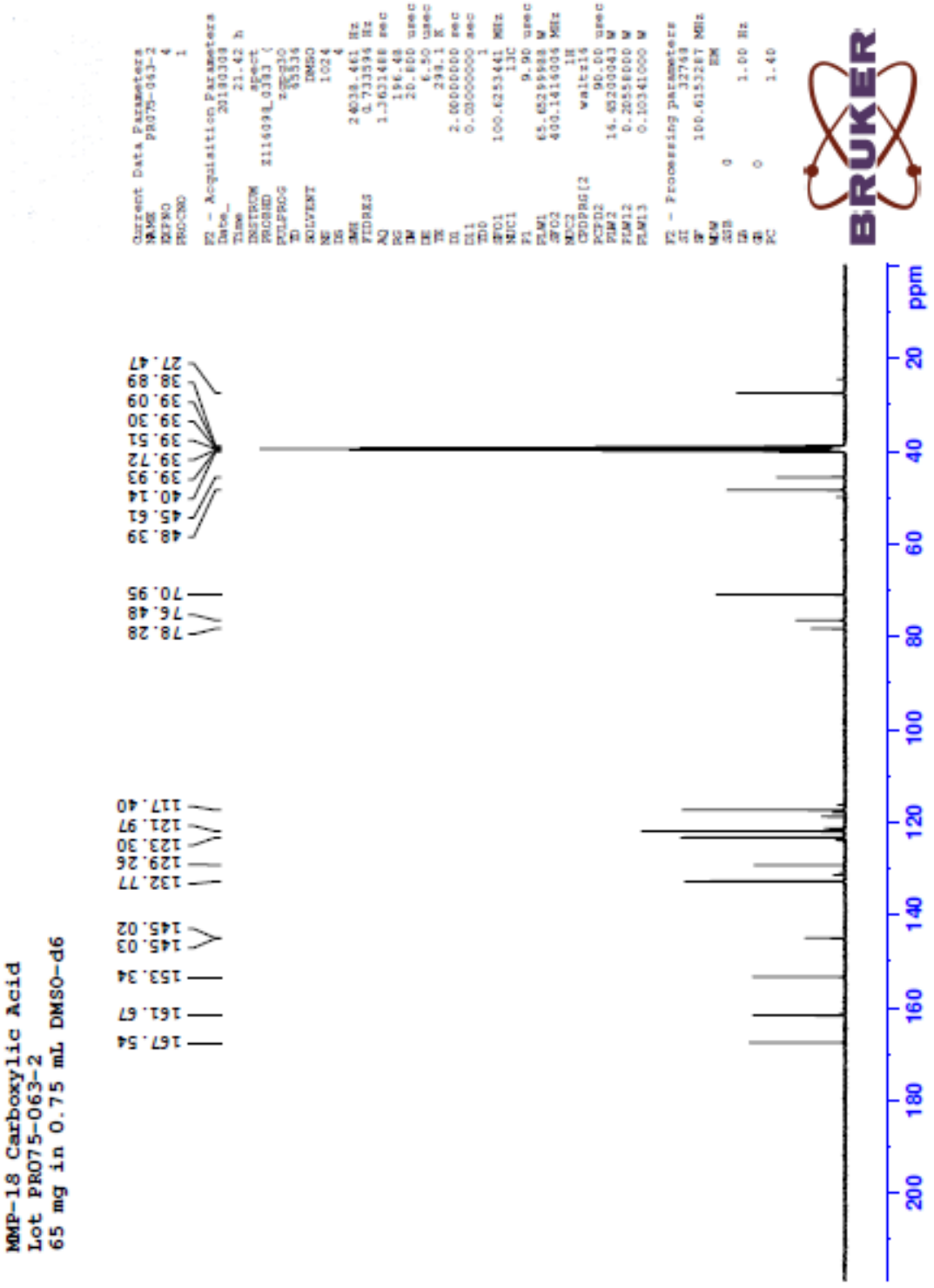
^{1}H NMR - MMP-18 Carboxylic Acid (Compound 8)



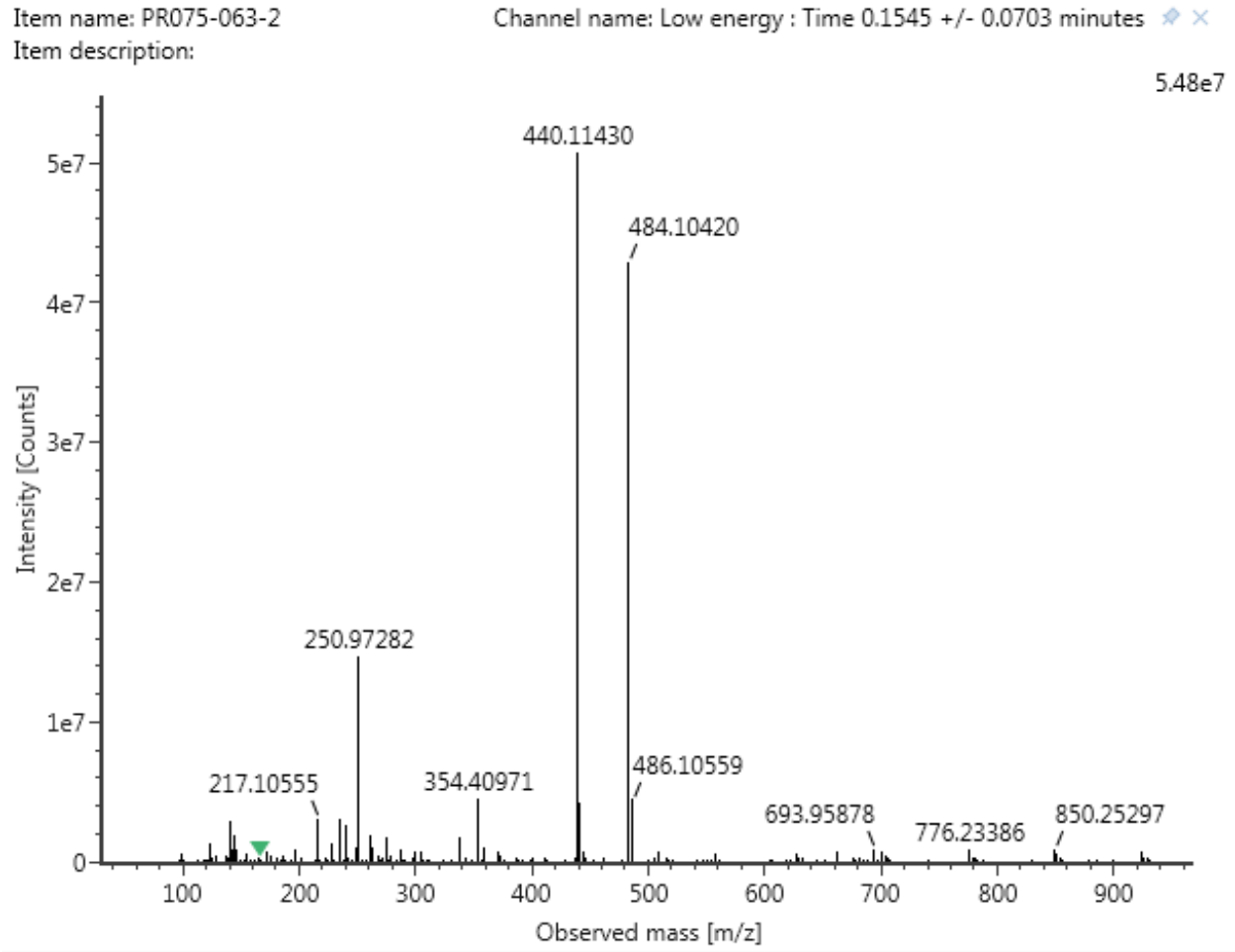
^{1}H NMR - MMP-18 Carboxylic Acid (Compound 8)



^{13}C NMR - MMP-18 Carboxylic Acid (Compound 8)



HRMS - MMP-18 Carboxylic Acid (Compound 8)



HPLC - THP-MMP-18 (Compound 9)

```
==========================================================================
Acq. Operator   : MRL                              Seq. Line :   5
Acq. Instrument : Instrument 1                      Location : Vial 24
Injection Date  : 09/19/17  11:20:59 AM                 Inj :   1
                                                  Inj Volume : 5.0 µl
Acq. Method     : C:\CHEM32\1\DATA\MRL170919A\MRL170919A 2017-09-19 08-32-20\BNCT-LONG2.M
Last changed    : 09/09/17  10:45:15 AM by MRL
Analysis Method : C:\CHEM32\1\METHODS\BNCT-LONG2.M
Last changed    : 09/09/17  10:45:15 AM by MRL
Method Info     : HPLC Method for Monitoring and Assaying BNCT products and reactions
                  HPLC 12 (Lab 2)
                  A = 0.10% TFA in water
                  B = 0.10% TFA in ACN
                  Waters Sunfire C8, 4.6x150 mm, 3.5 micron
                  Flow: 1.0 mL/min, Column Temp: 40 C

Sample Info     : THP-MMP-18
                  PR075-117-1

Additional Info : Peak(s) manually integrated
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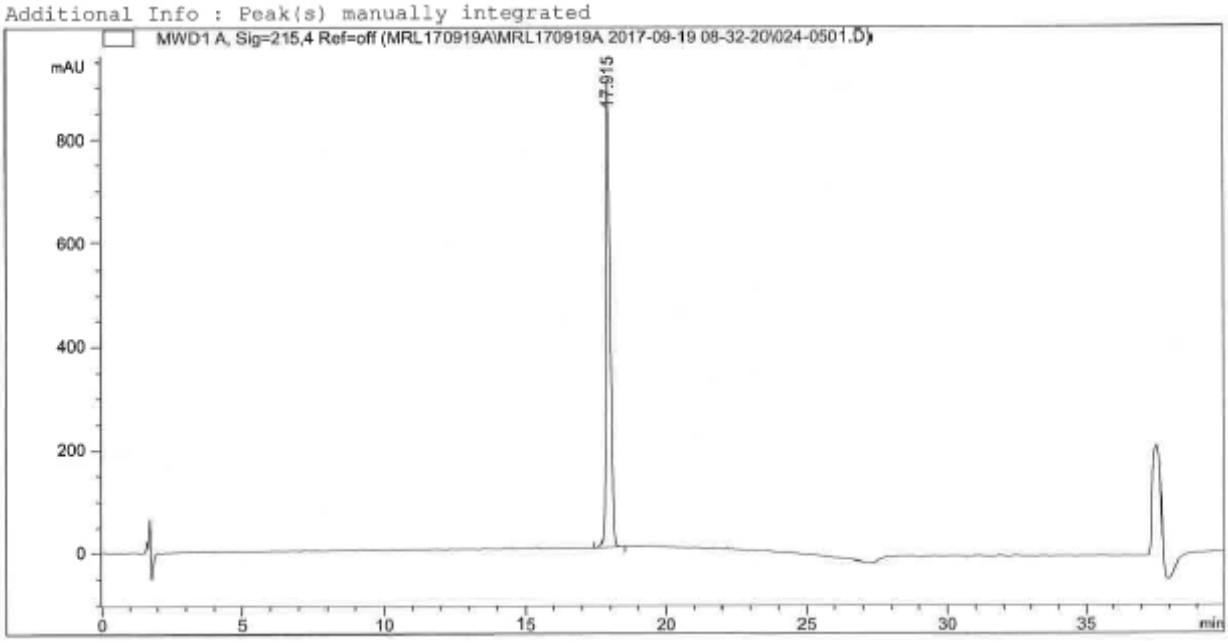
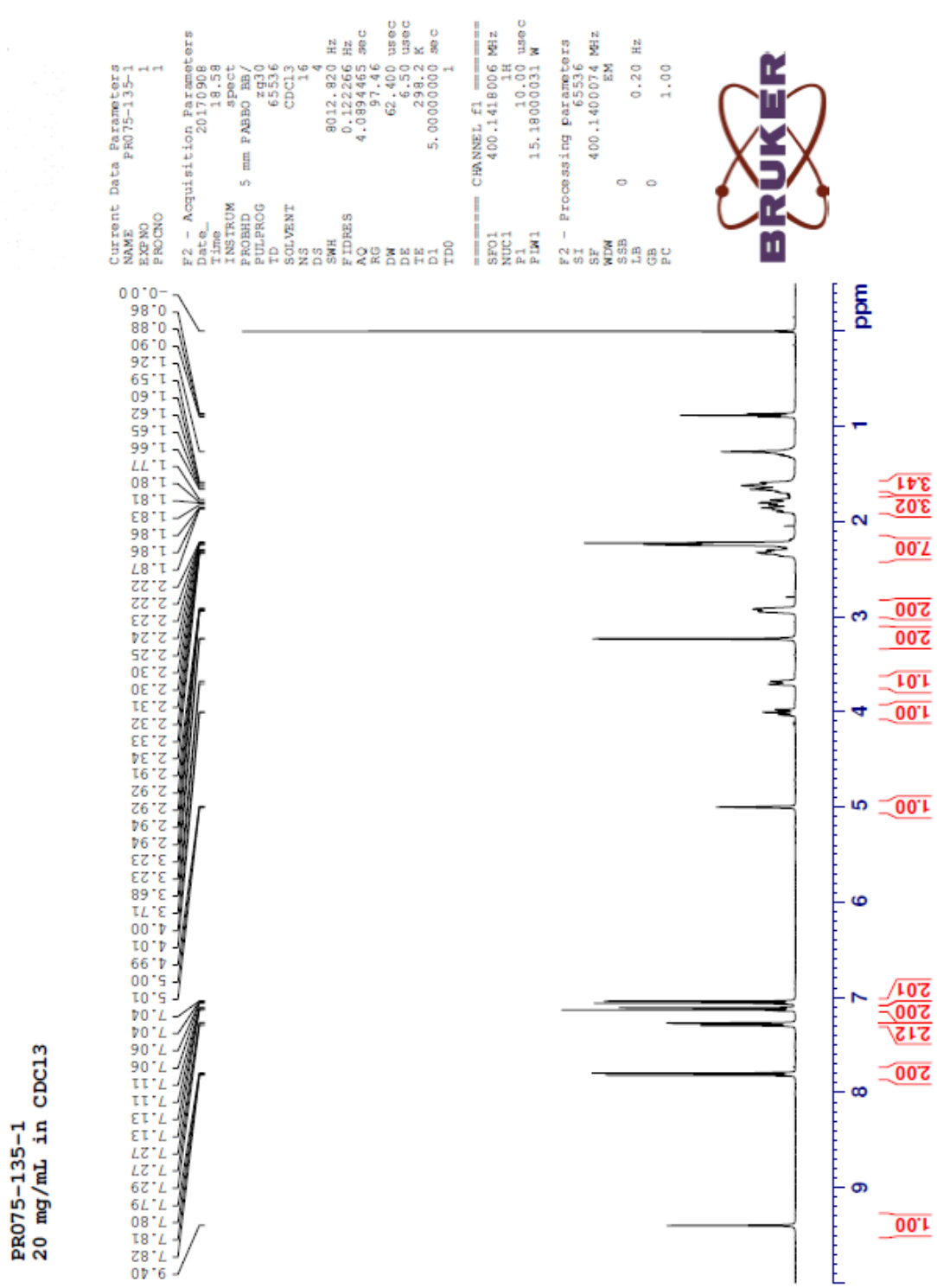
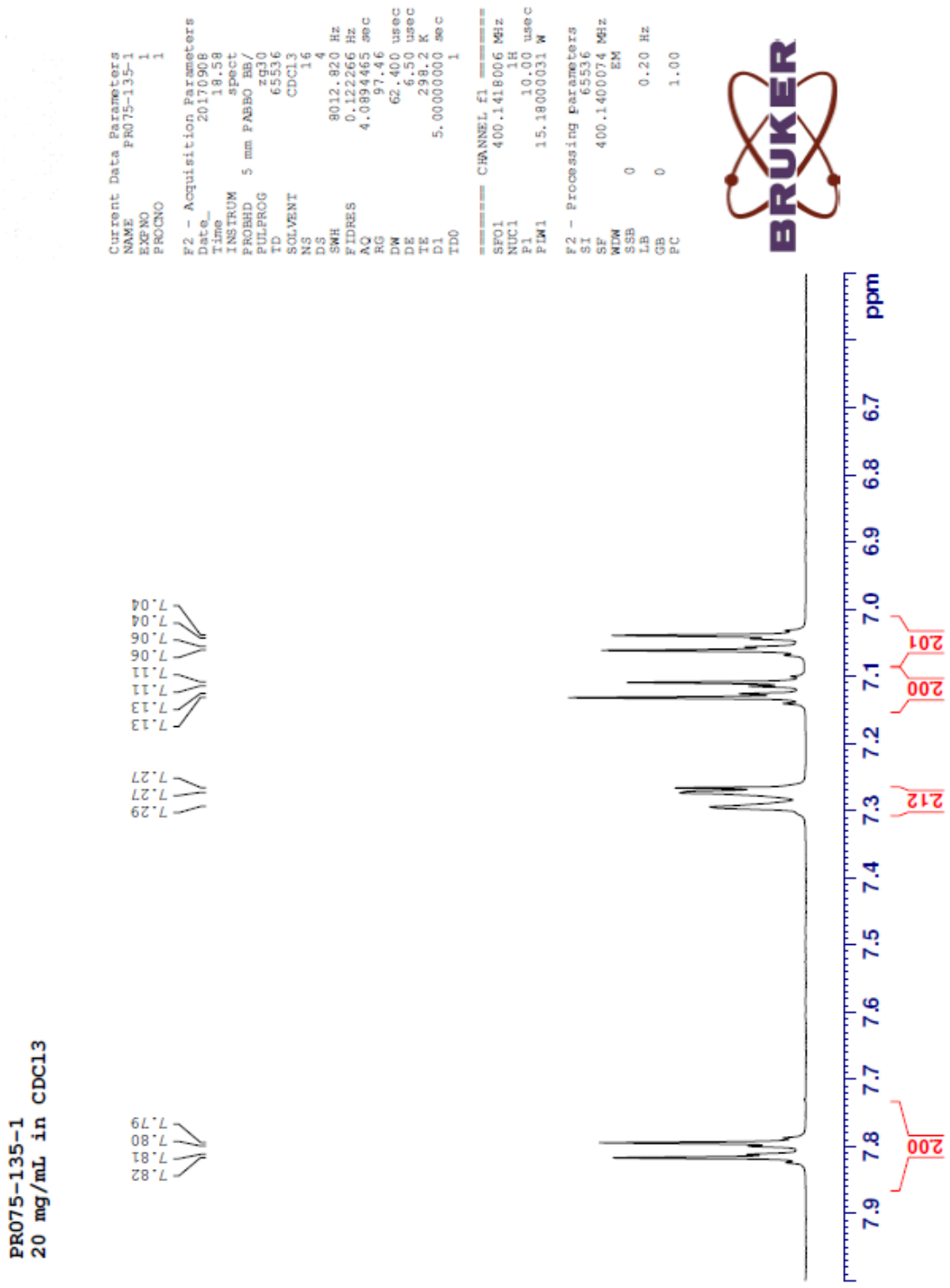


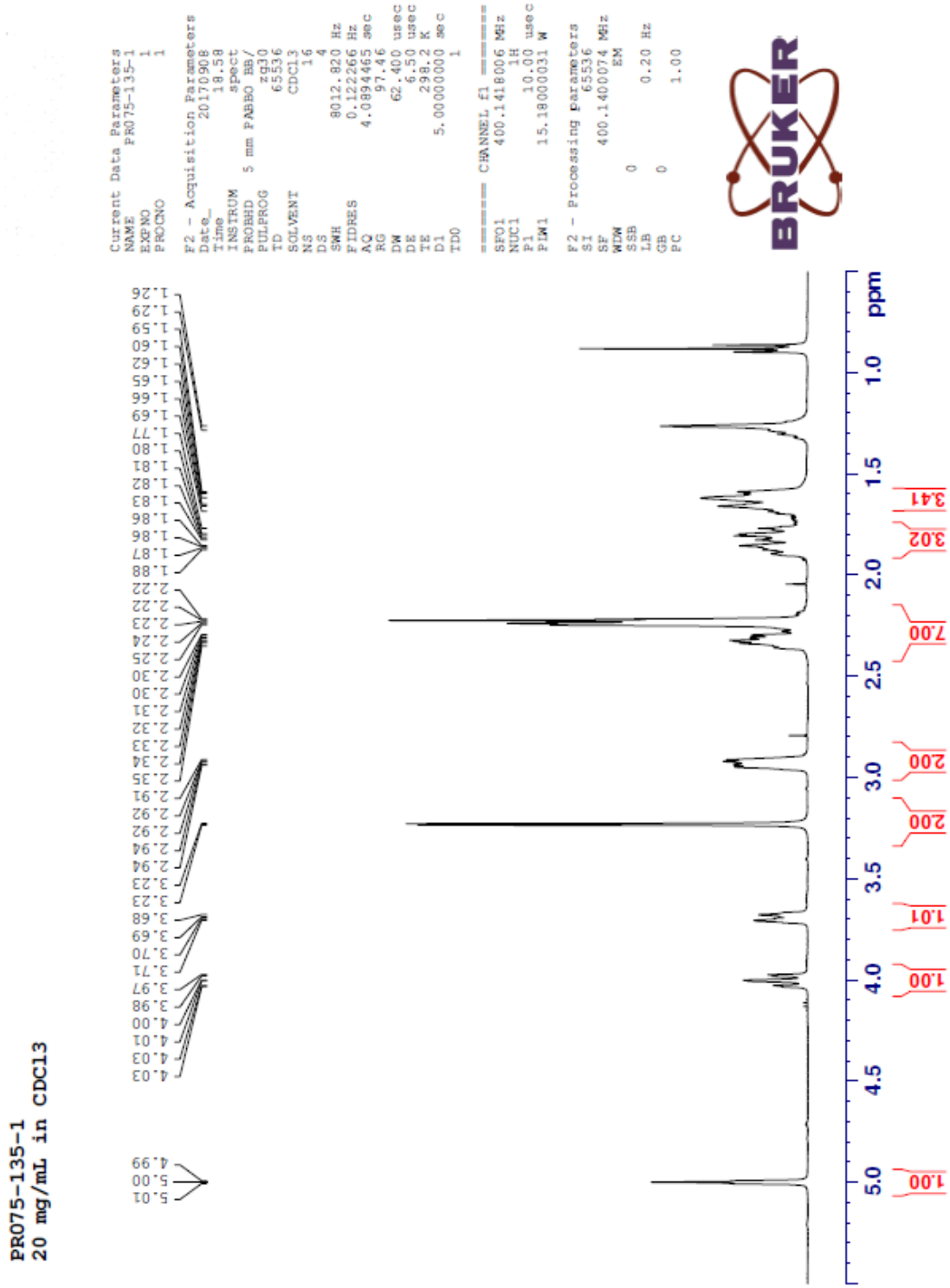
^{1}H NMR - THP-MMP-18 (Compound 9)



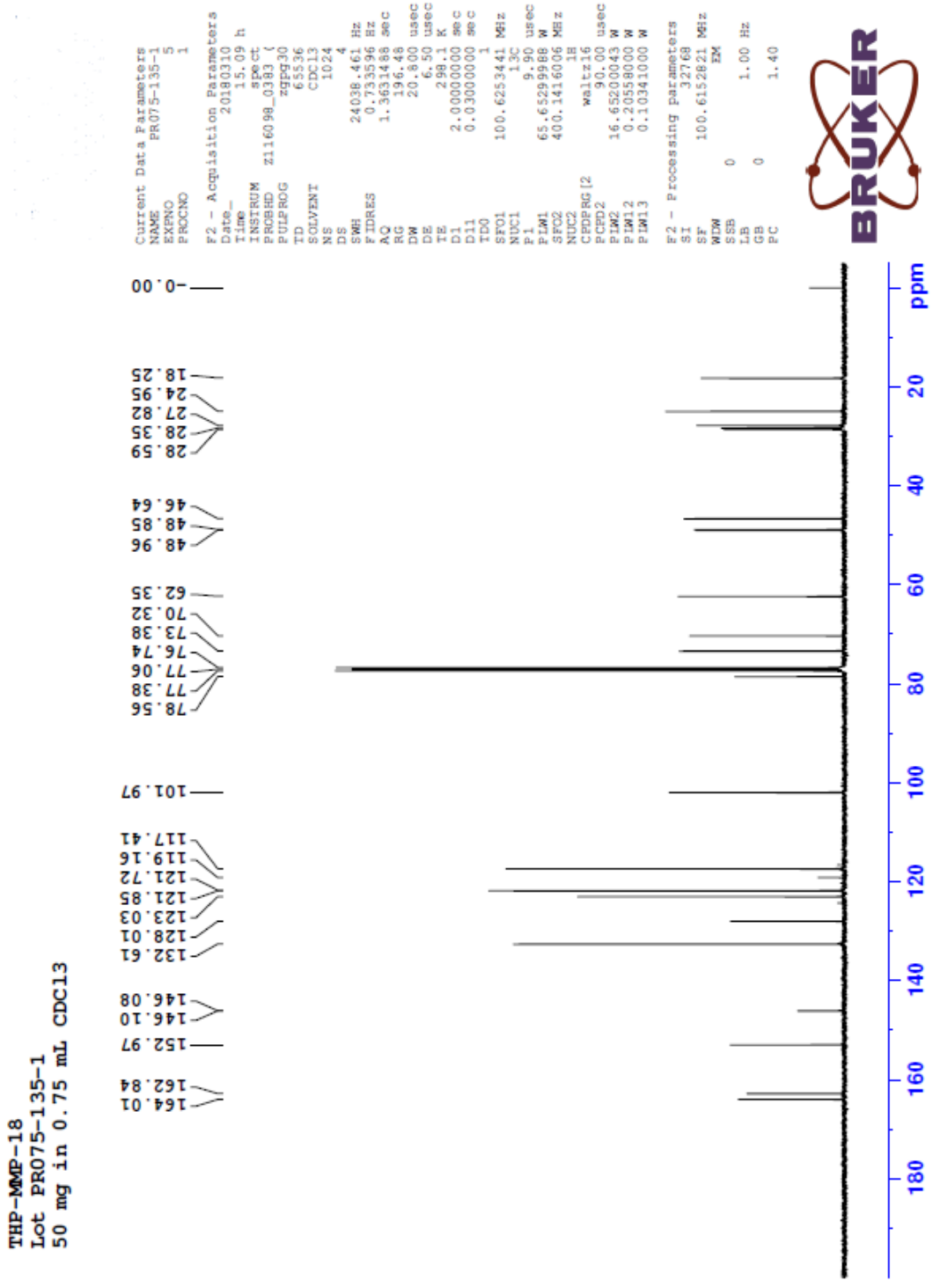
^{1}H NMR - THP-MMP-18 (Compound 9)



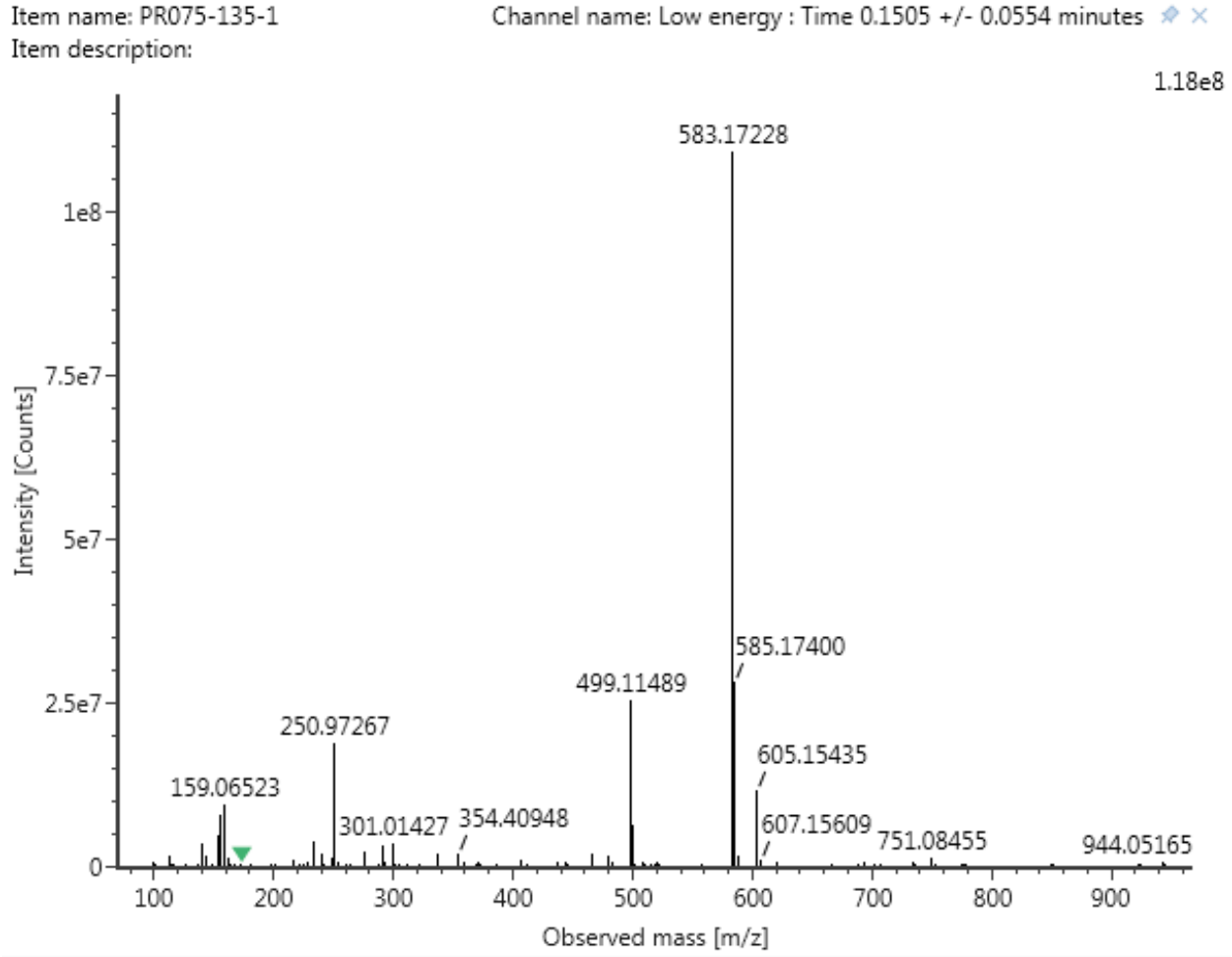
^{1}H NMR - THP-MMP-18 (Compound 9)



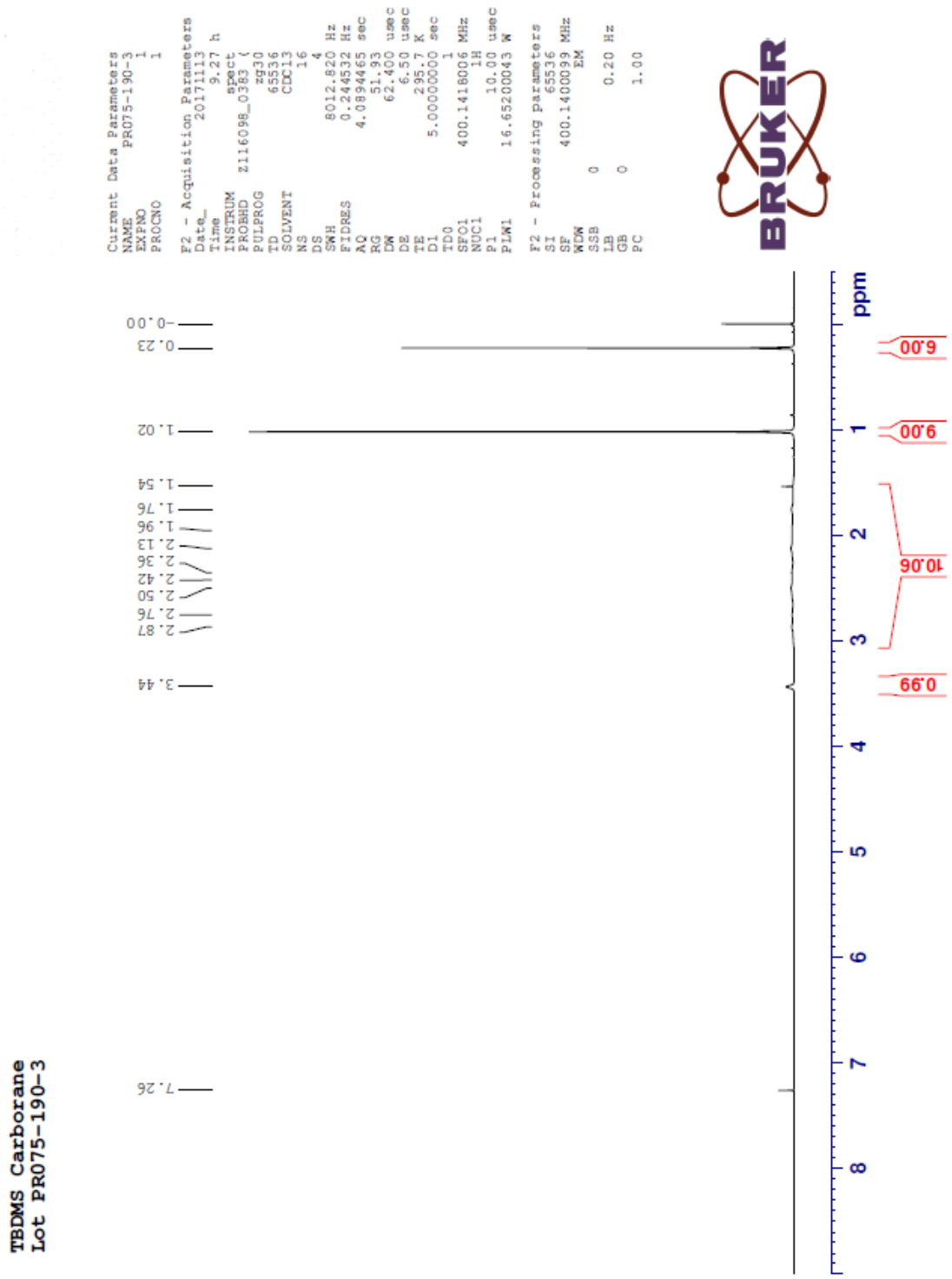
^{13}C NMR - THP-MMP-18 (Compound 9)



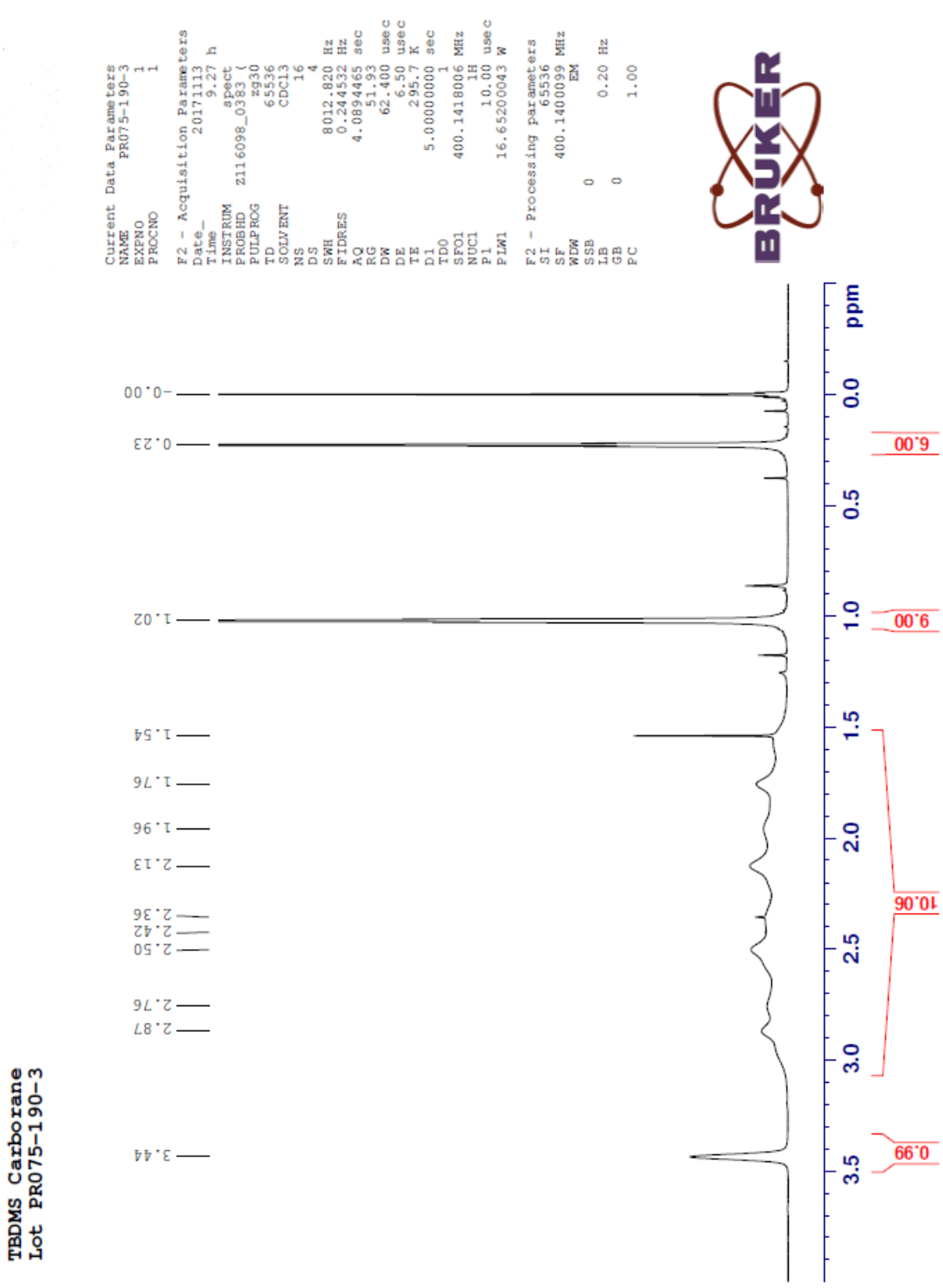
HRMS - THP-MMP-18 (Compound 9)



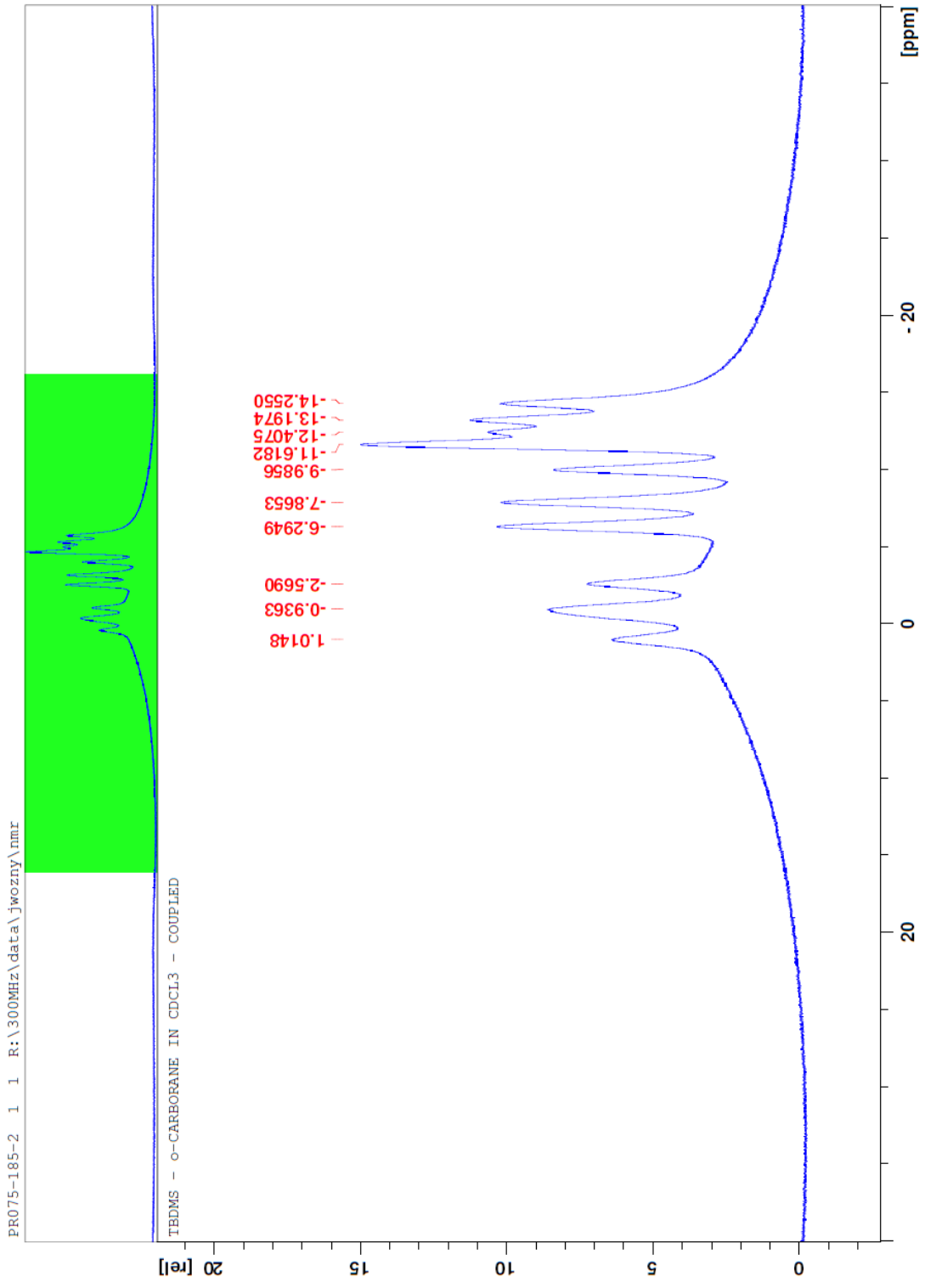
^{1}H NMR - TBDMS Carborane (Compound 10)



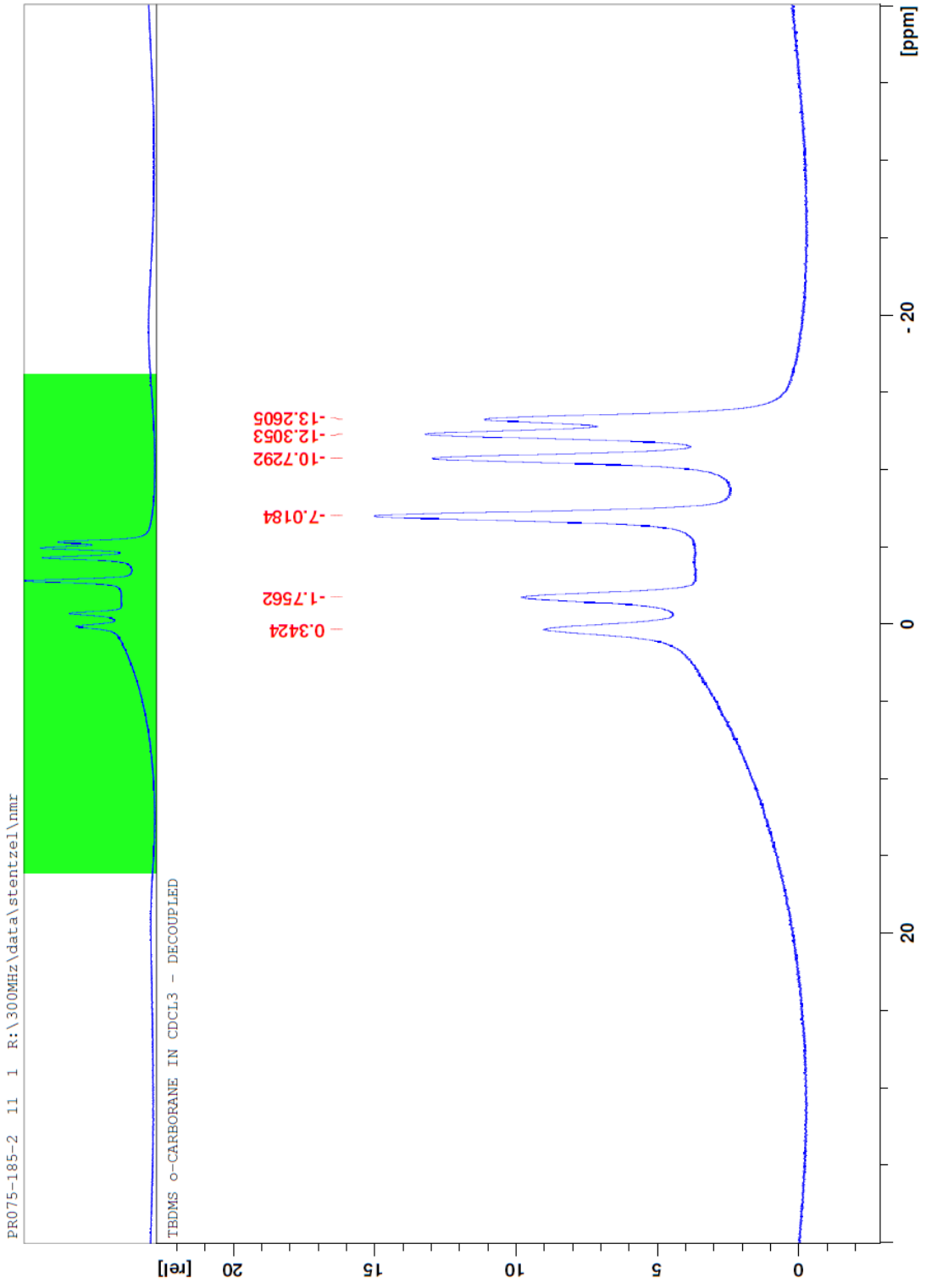
^{1}H NMR - TBDMS Carborane (Compound 10)



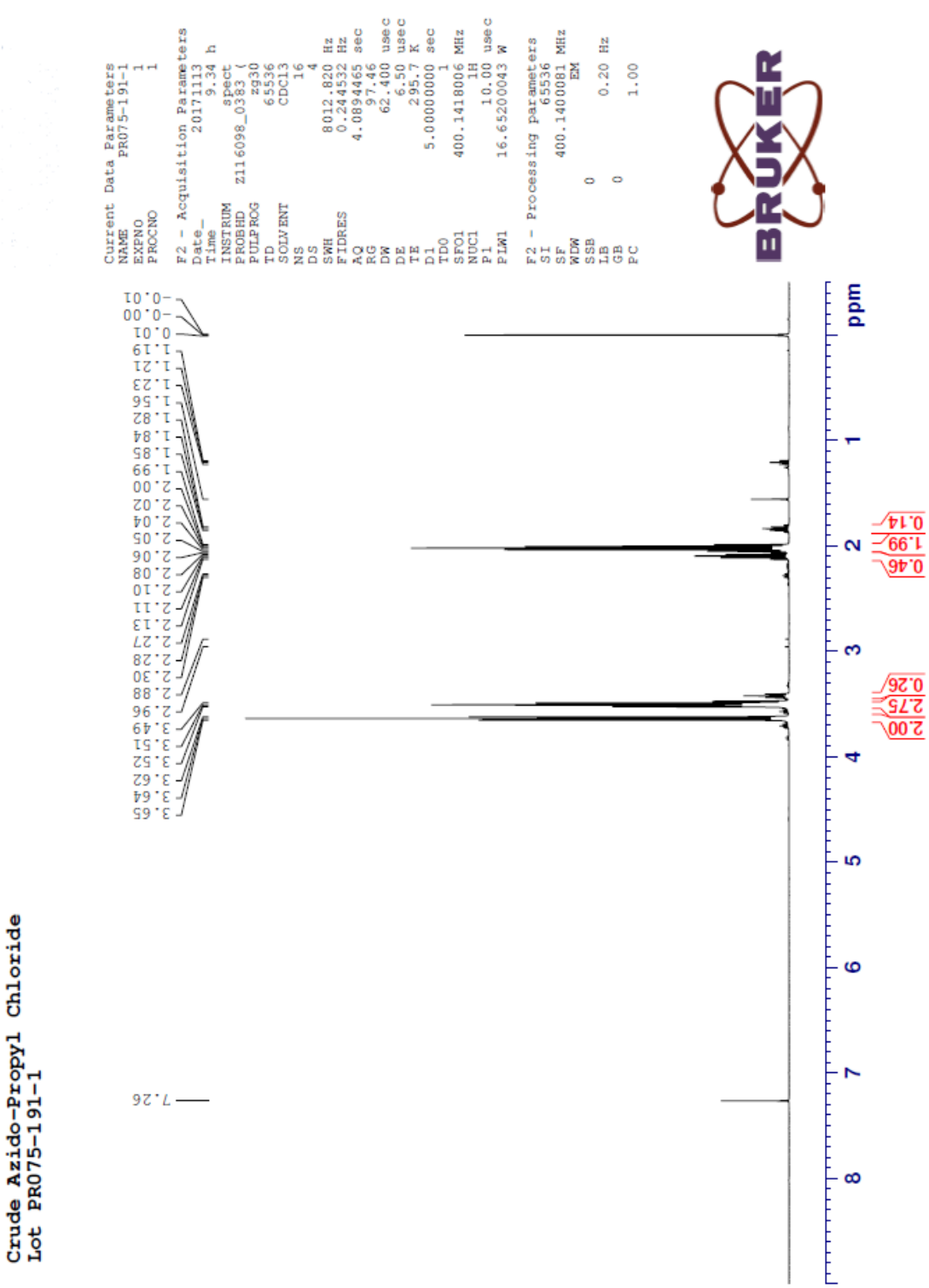
^{11}B NMR (Coupled) - TBDMS Carborane (Compound 10)



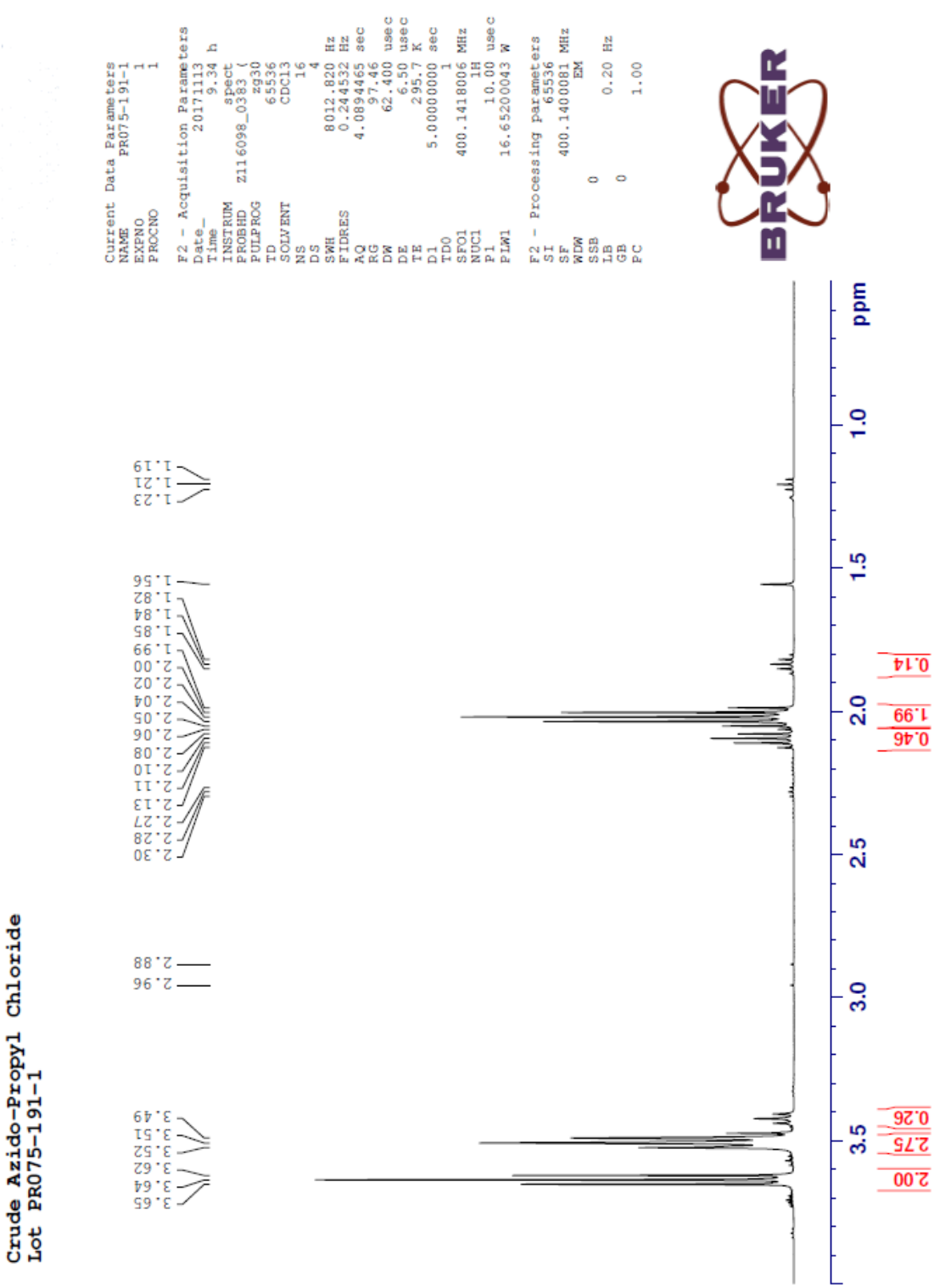
^{11}B NMR (Decoupled) - TBDMS Carborane (Compound 10)



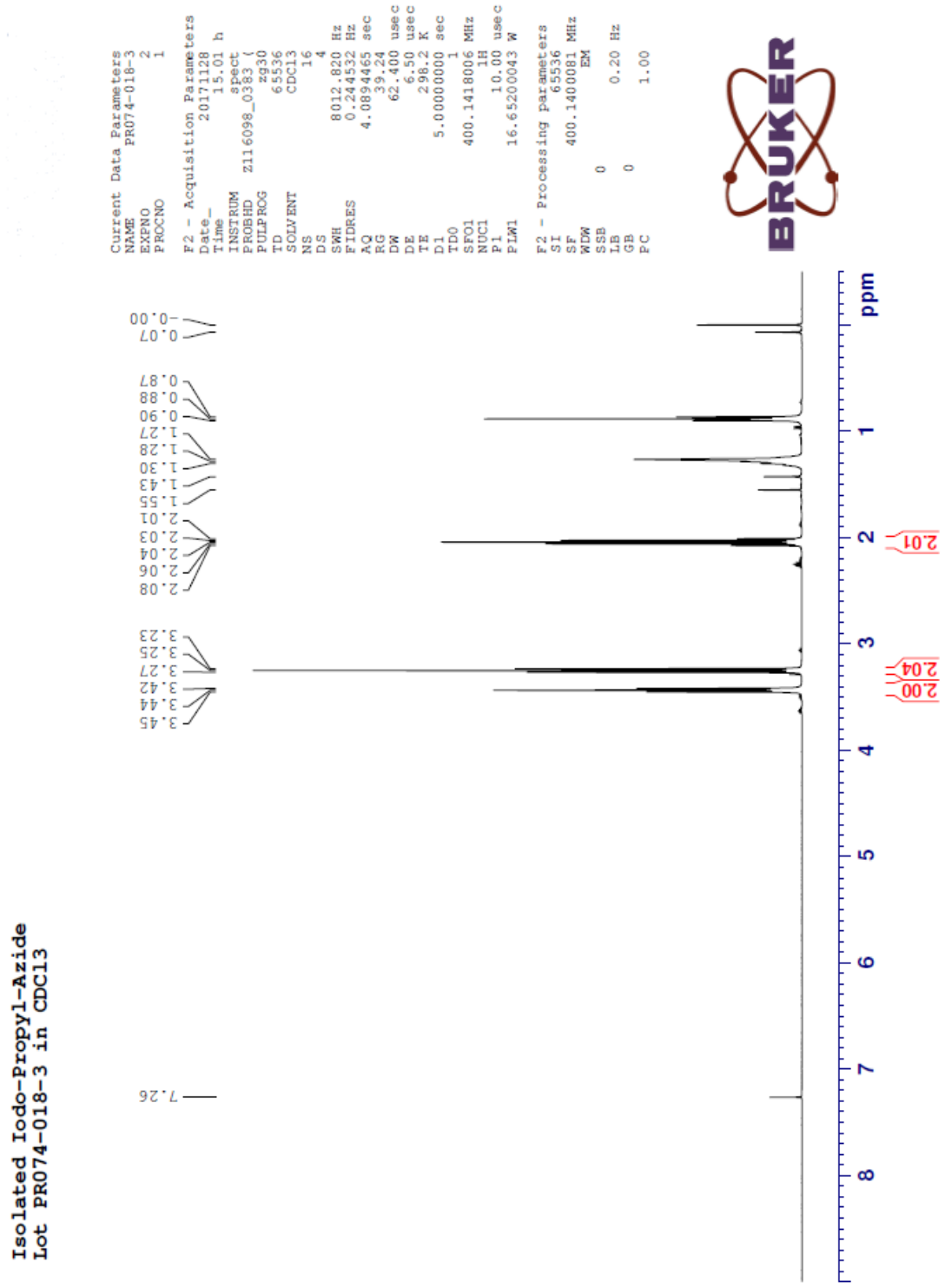
^{1}H NMR - Crude Chloro Propyl Azide (Compound 11)



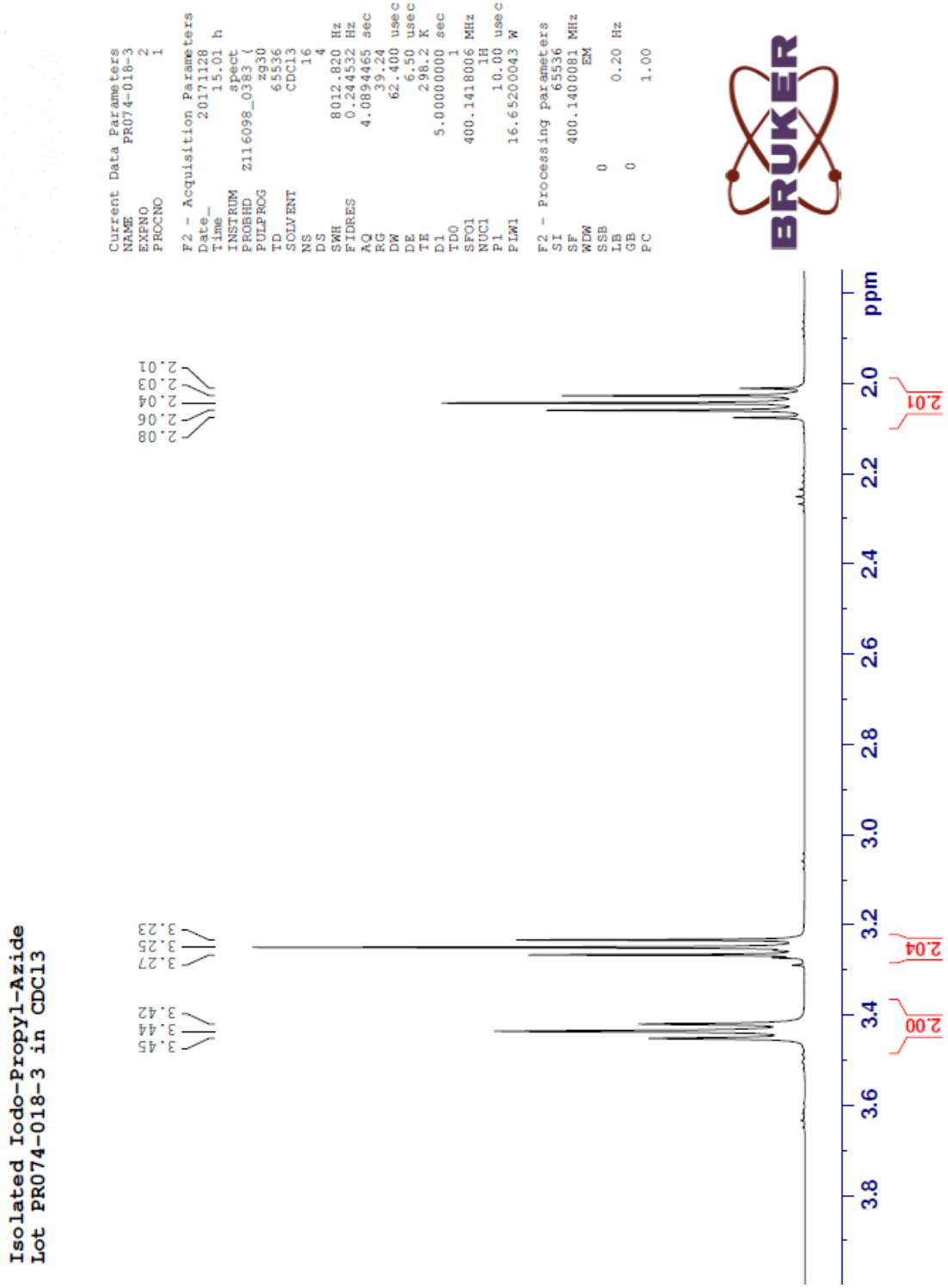
^{1}H NMR - Crude Chloro Propyl Azide (Compound 11)



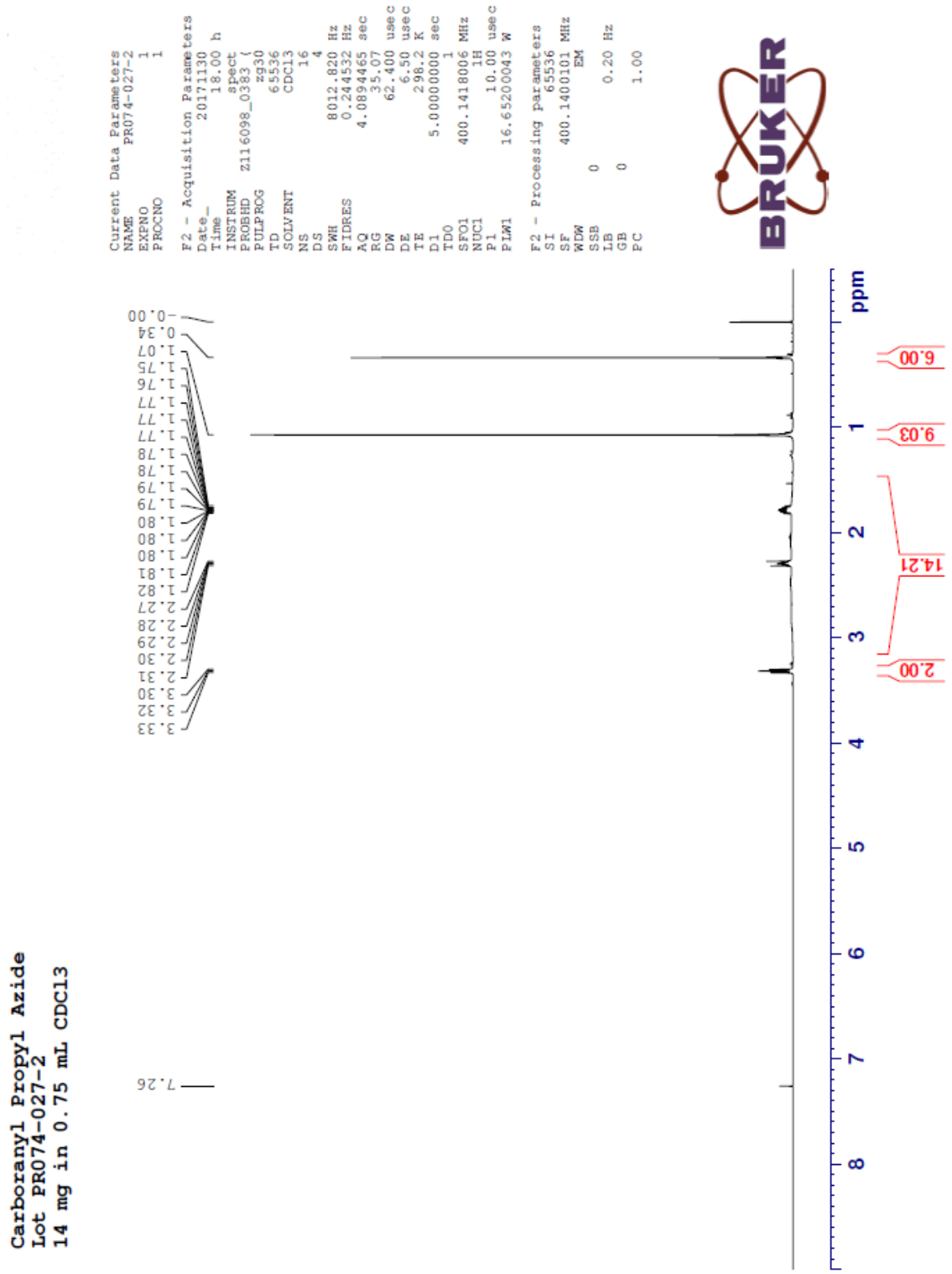
^{1}H NMR - Crude Chloro Propyl Azide (Compound 11)



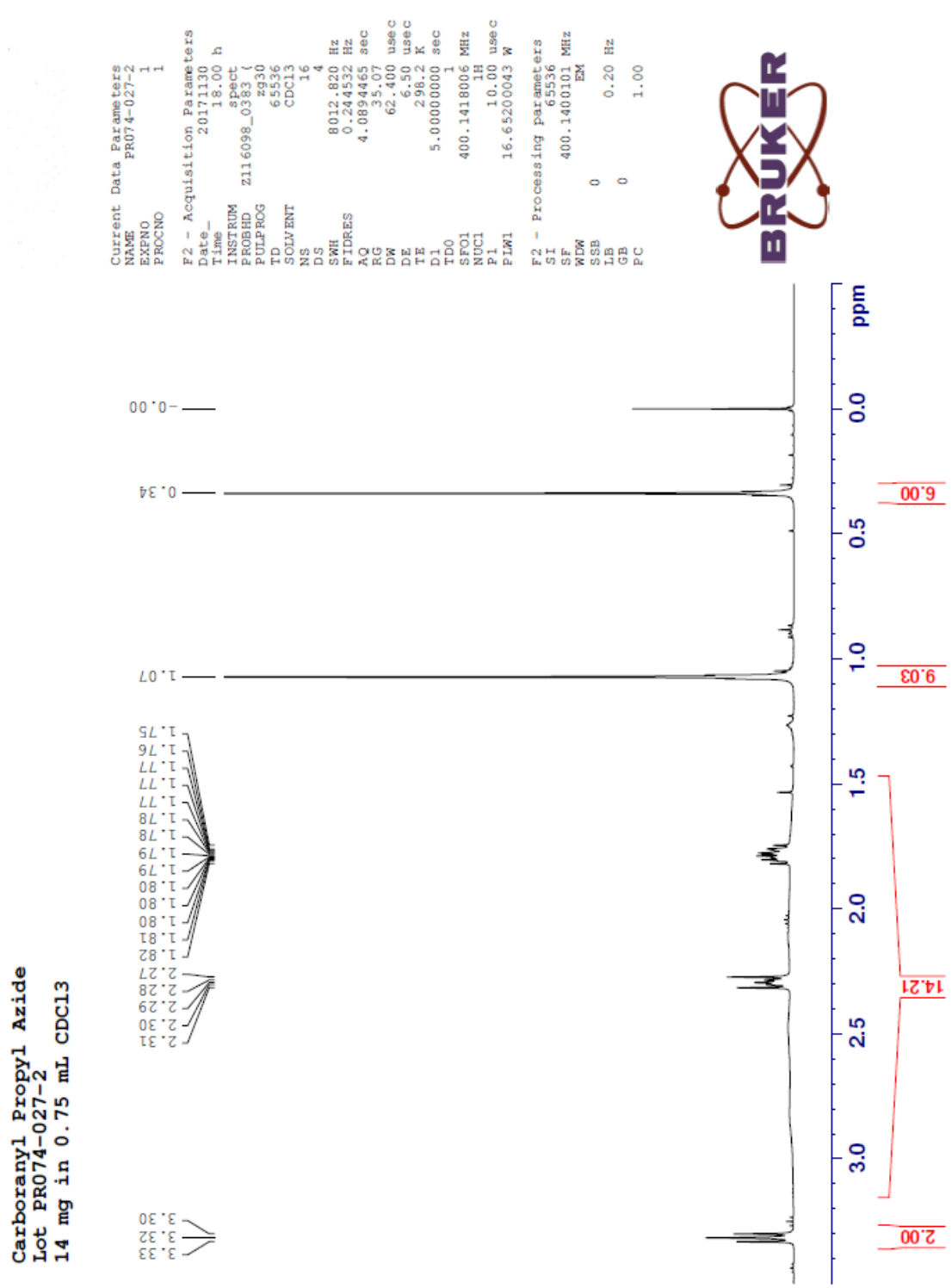
^{1}H NMR - Iodo Propyl Azide (Compound 12)



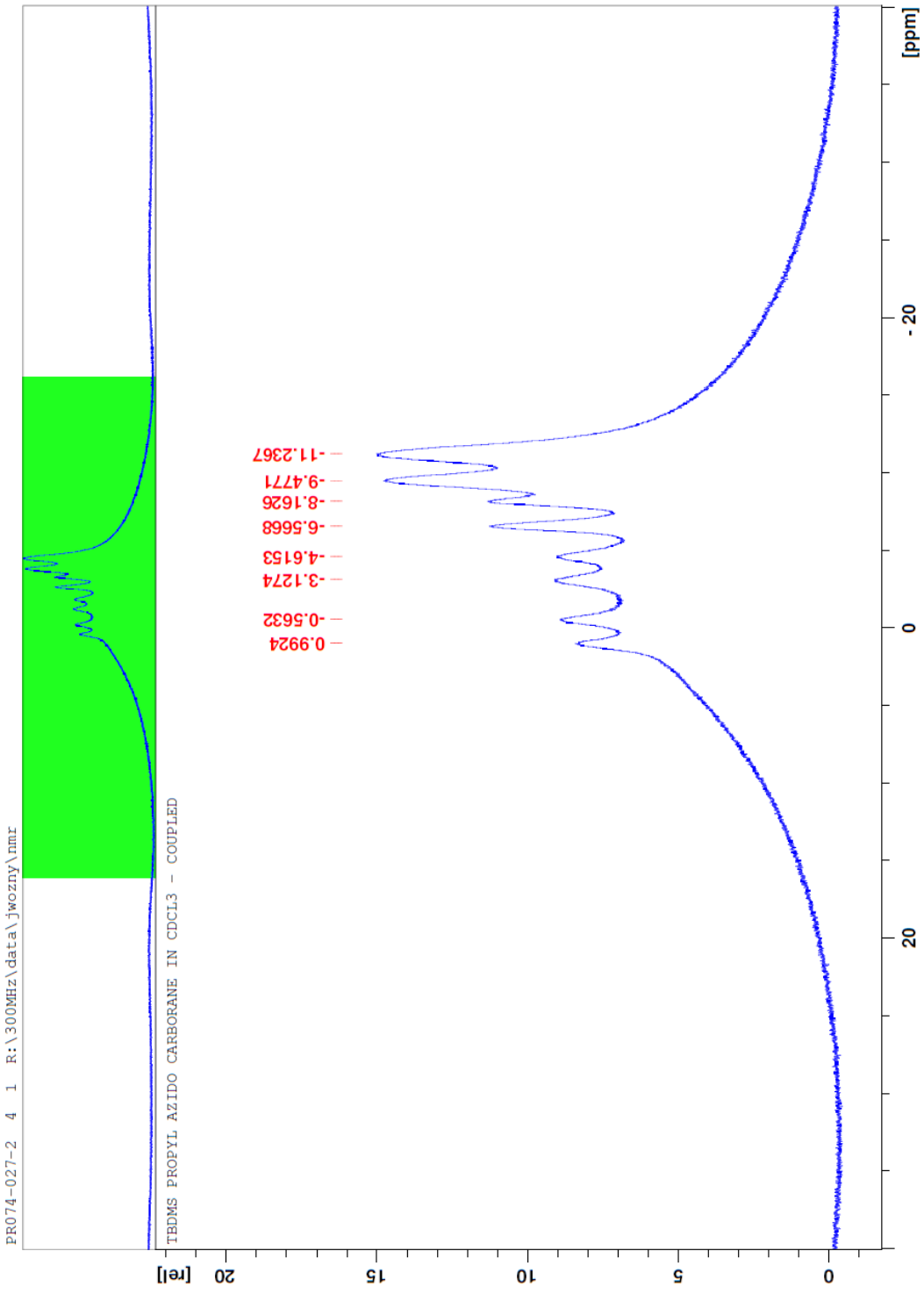
^{1}H NMR - Carboranyl Propyl Azide (Compound 13)



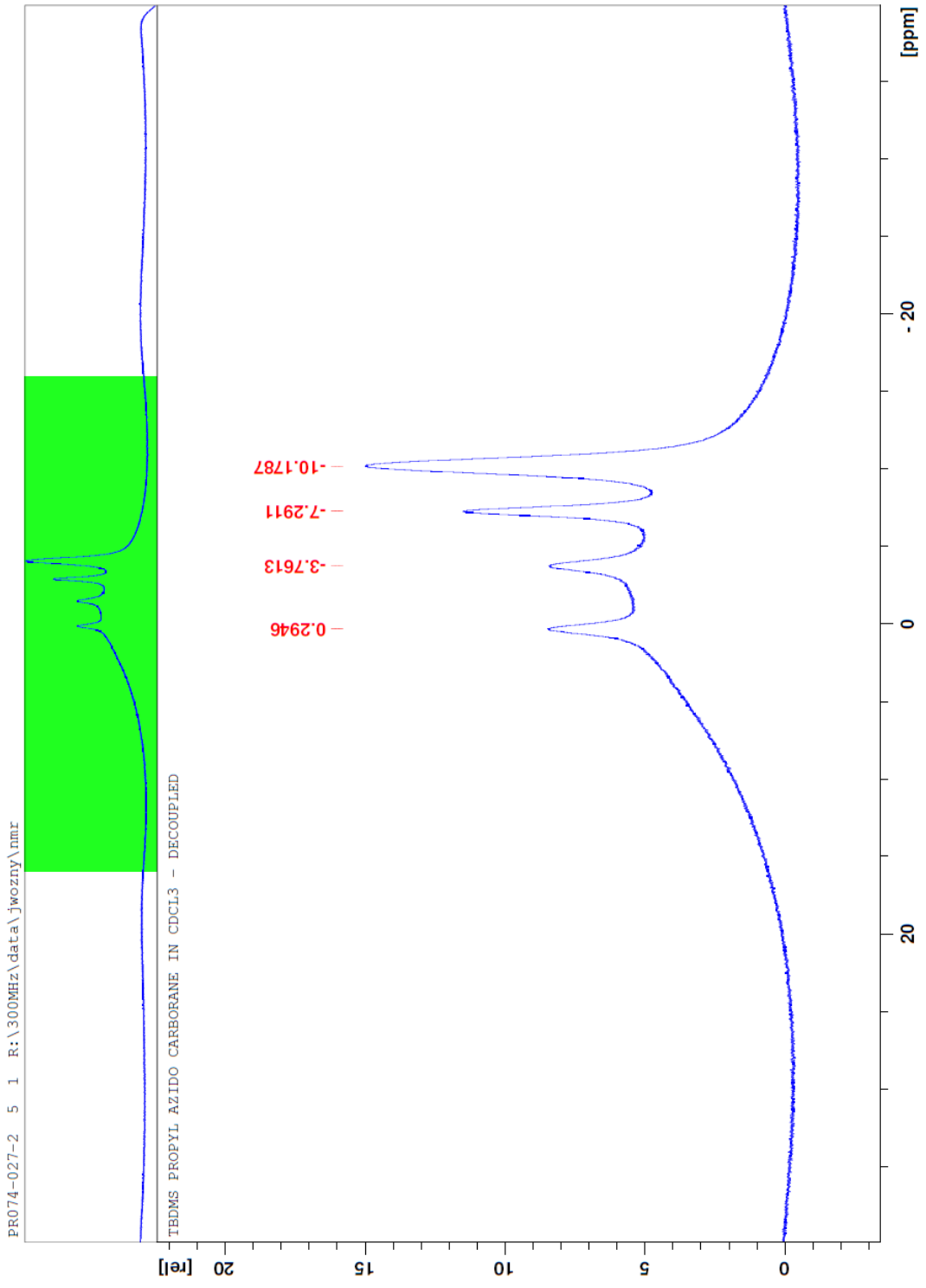
^{1}H NMR - Carboranyl Propyl Azide (Compound 13)



^{11}B NMR (Coupled) - Carboranyl Propyl Azide (Compound 13)



^{11}B NMR (Decoupled) - Carboranyl Propyl Azide (Compound 13)



HPLC - 1,4-Disubstituted Click Product (TBDMS and THP Protected) (Compound 14)

```
Acq. Operator    : MRL                              Seq. Line :   3
Acq. Instrument  : Instrument 1                      Location : Vial 84
Injection Date   : 02/27/18  8:02:41 PM                 Inj :   1
                                                   Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\1\DATA\AC180227A\AC180227A 2018-02-27 18-37-43\BNCT-LONG2.M
Last changed     : 10/18/17  2:57:54 PM by MRL
Analysis Method  : C:\CHEM32\1\METHODS\BNCT-LONG2.M
Last changed     : 02/28/18  8:01:59 AM by MRL
                   (modified after loading)
Method Info      : HPLC Method for Monitoring and Assaying BNCT products and reactions
                   HPLC 12 (Lab 2)
                   A = 0.10% TFA in water
                   B = 0.10% TFA in ACN
                   Waters Sunfire C8, 4.6x150 mm, 3.5 micron
                   Flow: 1.0 mL/min, Column Temp: 40 C

Sample Info      : 1,4 Click Product
                   Lot# PR074-070-3
                   Conc. 1 mg/mL in ACN

Additional Info : Peak(s) manually integrated
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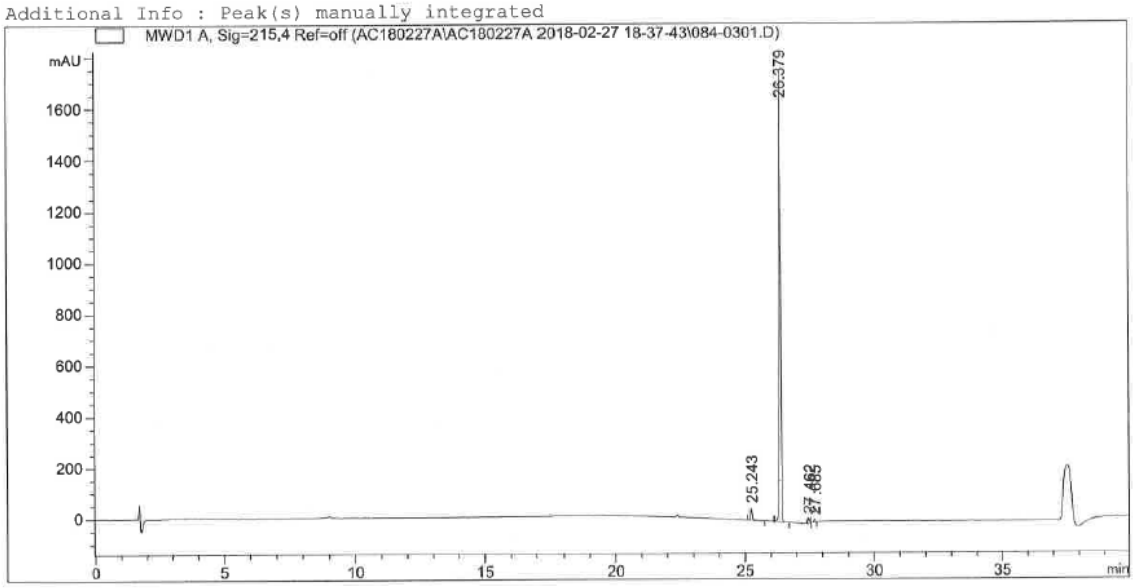
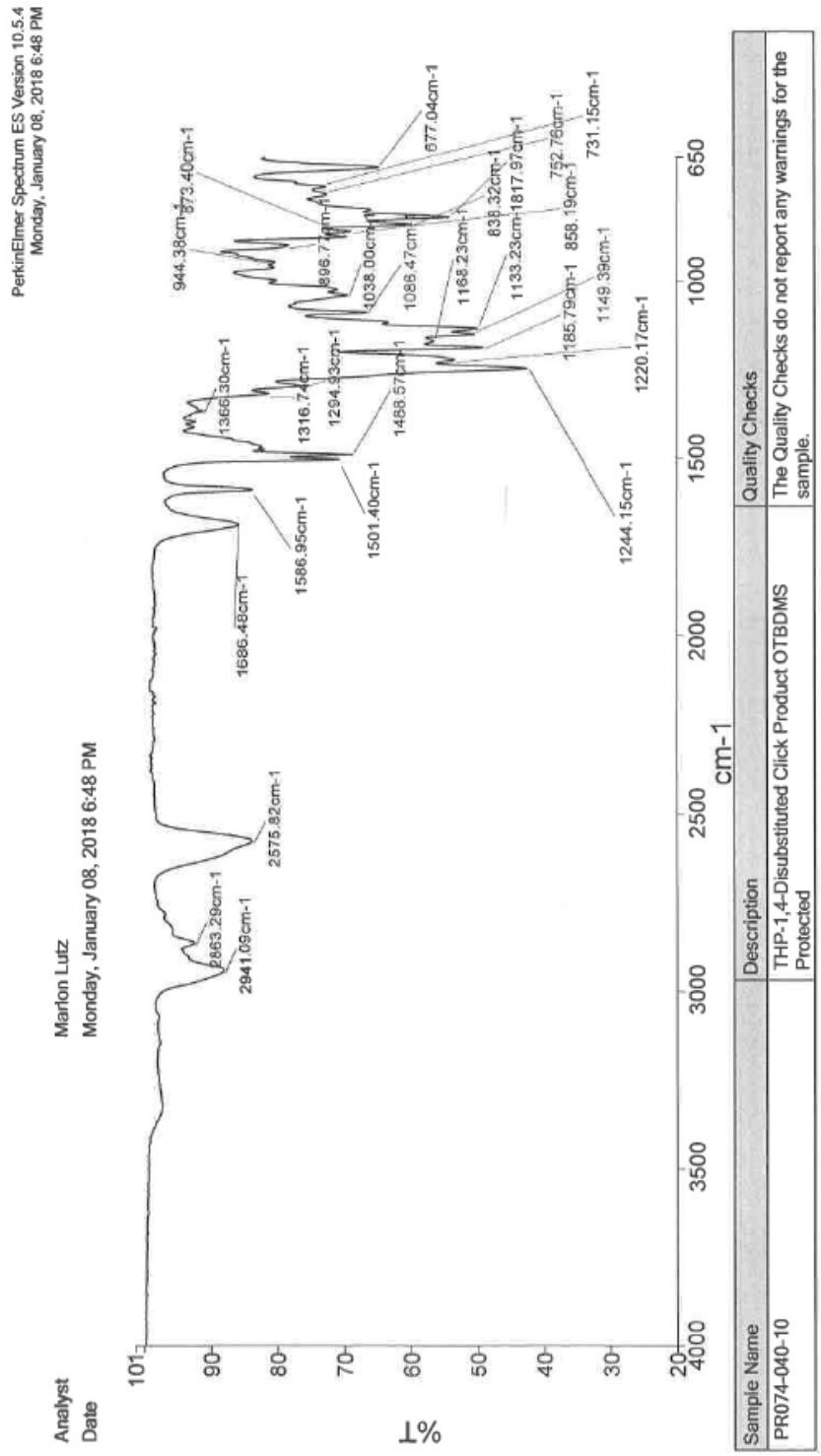


Area Percent Report

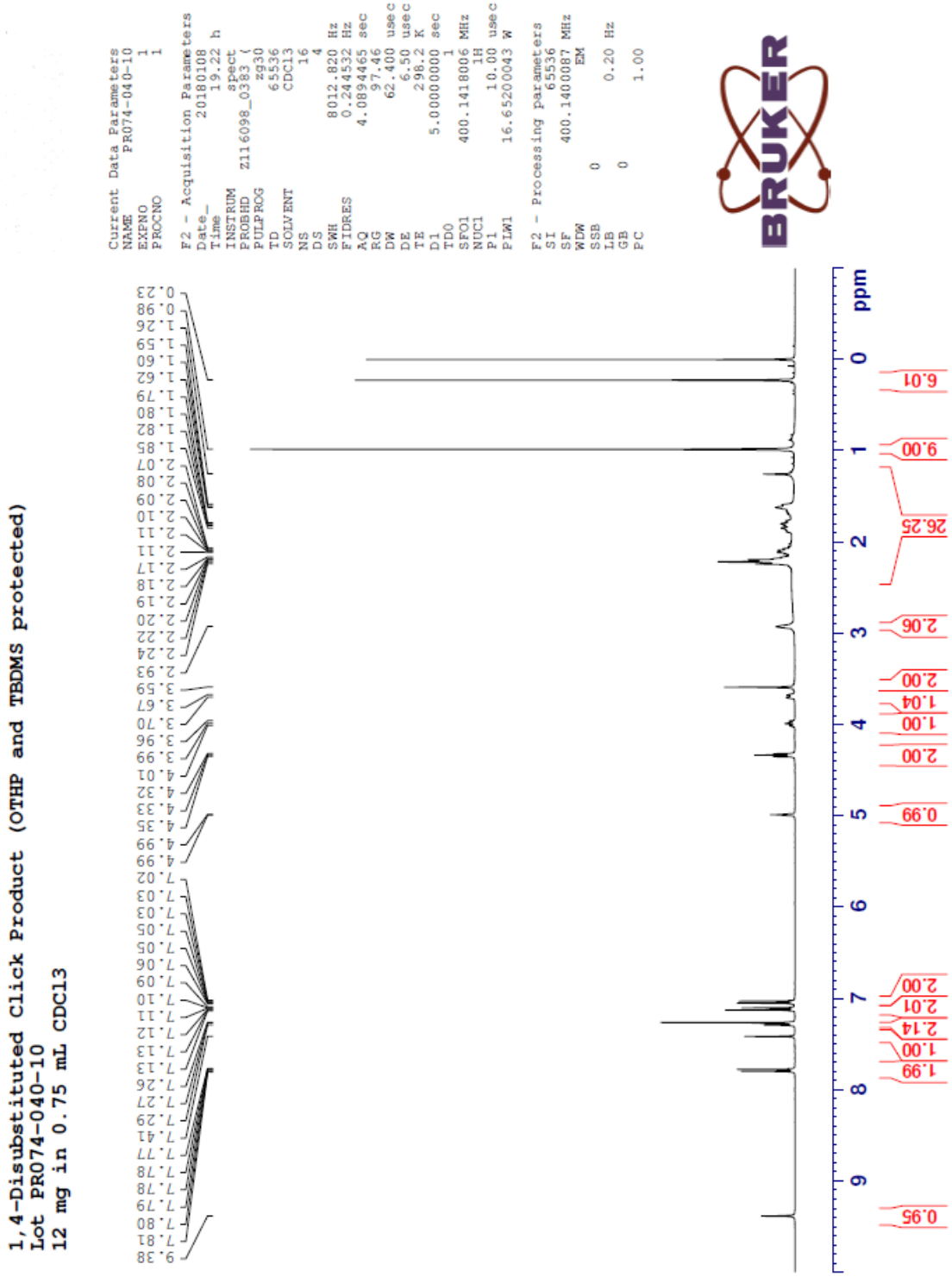
Signal 1: MWD1 A, Sig=215,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	25.243	BV	0.0753	229.43767	45.15441	3.2020
2	26.379	VB	0.0618	6800.98584	1730.79297	94.9142
3	27.462	BB	0.0594	80.61908	21.68694	1.1251
4	27.685	BB	0.0630	54.36395	13.49392	0.7587
Totals :				7165.40654	1811.12824	

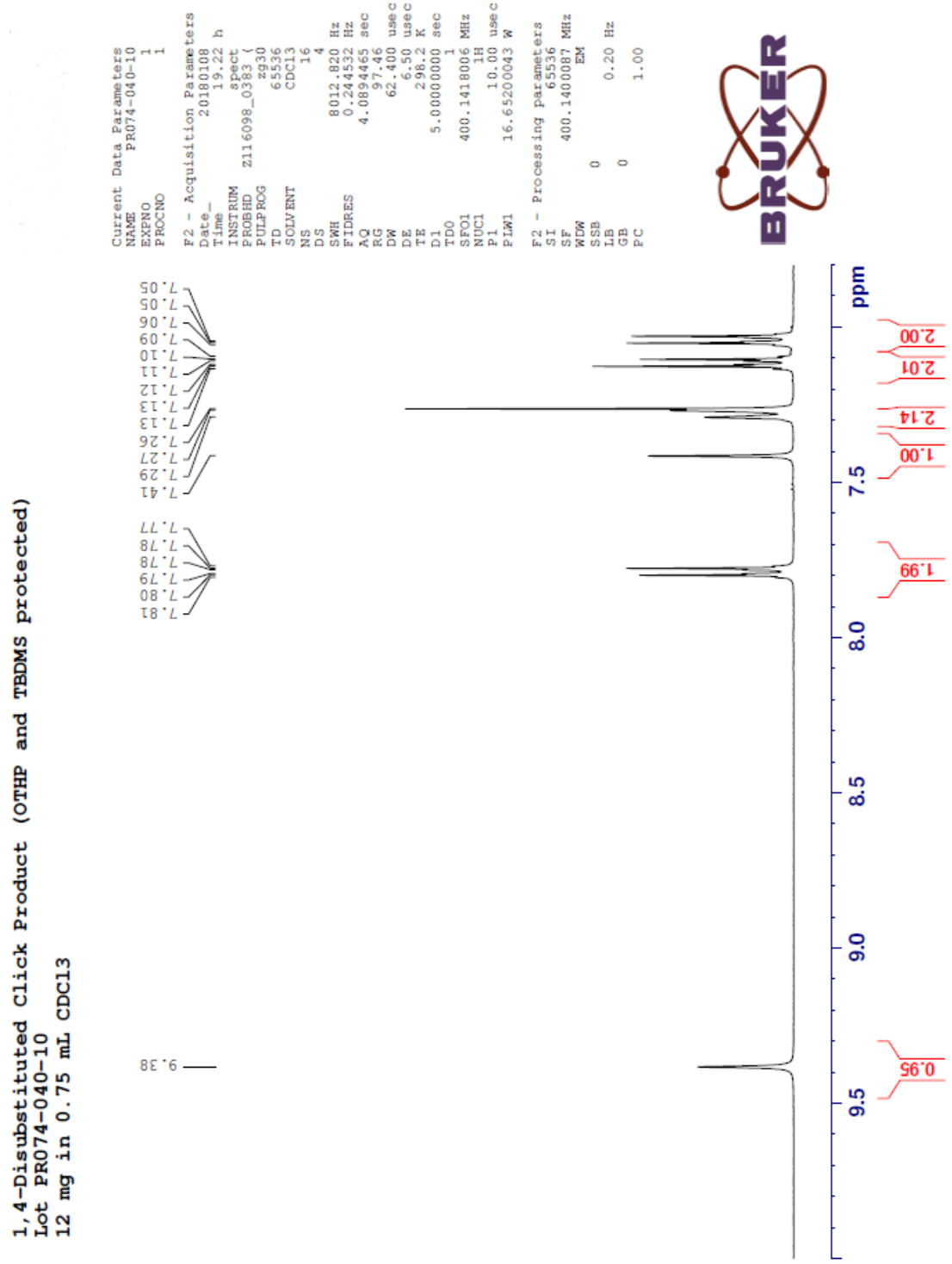
FTIR - 1,4-Disubstituted Click Product (TBDMS and THP Protected) (Compound 14)



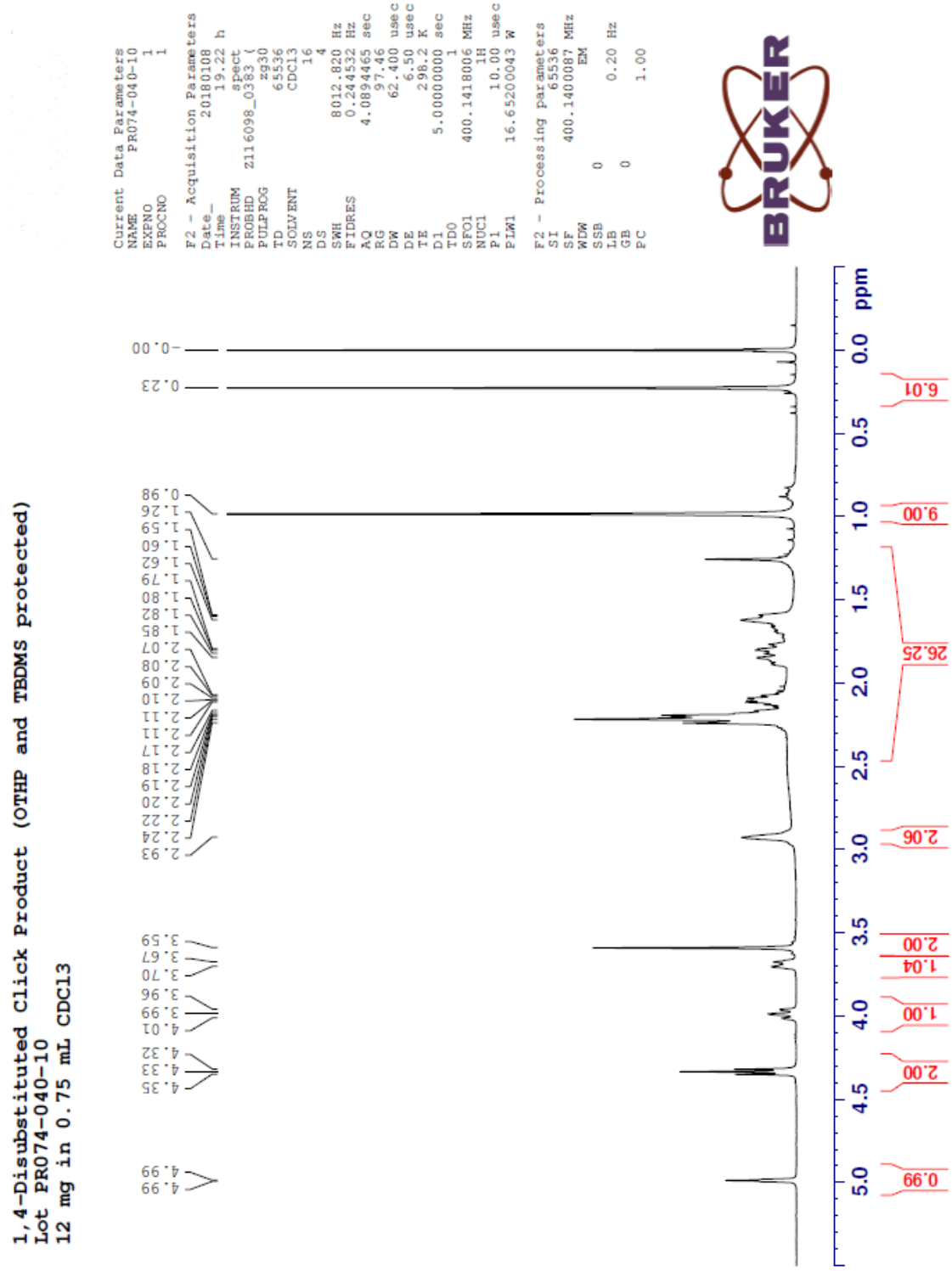
^{1}H NMR - 1,4-Disubstituted Click Product (TBDMS and THP Protected) (Compound 14)



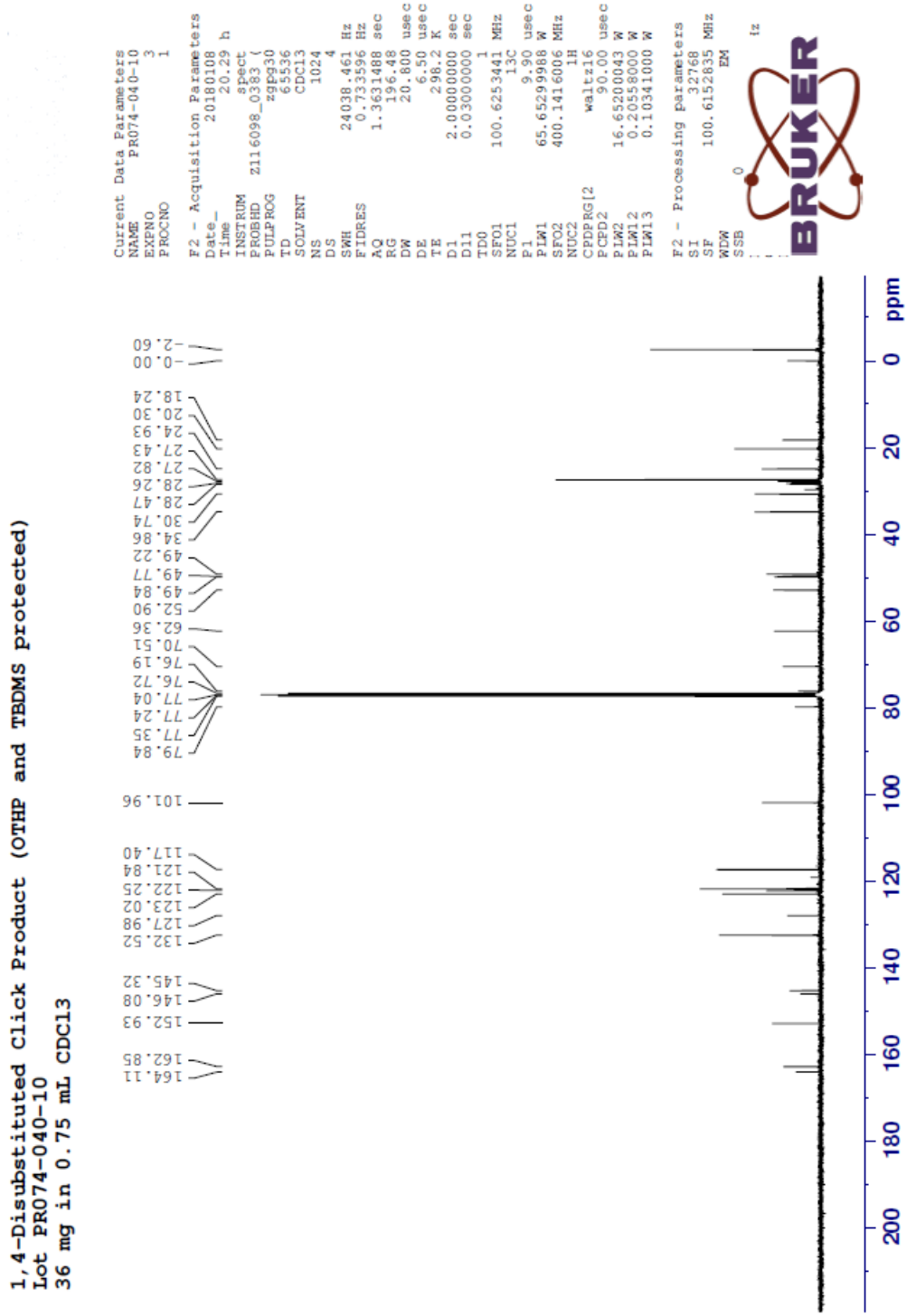
^{1}H NMR - 1,4-Disubstituted Click Product (TBDMS and THP Protected) (Compound 14)



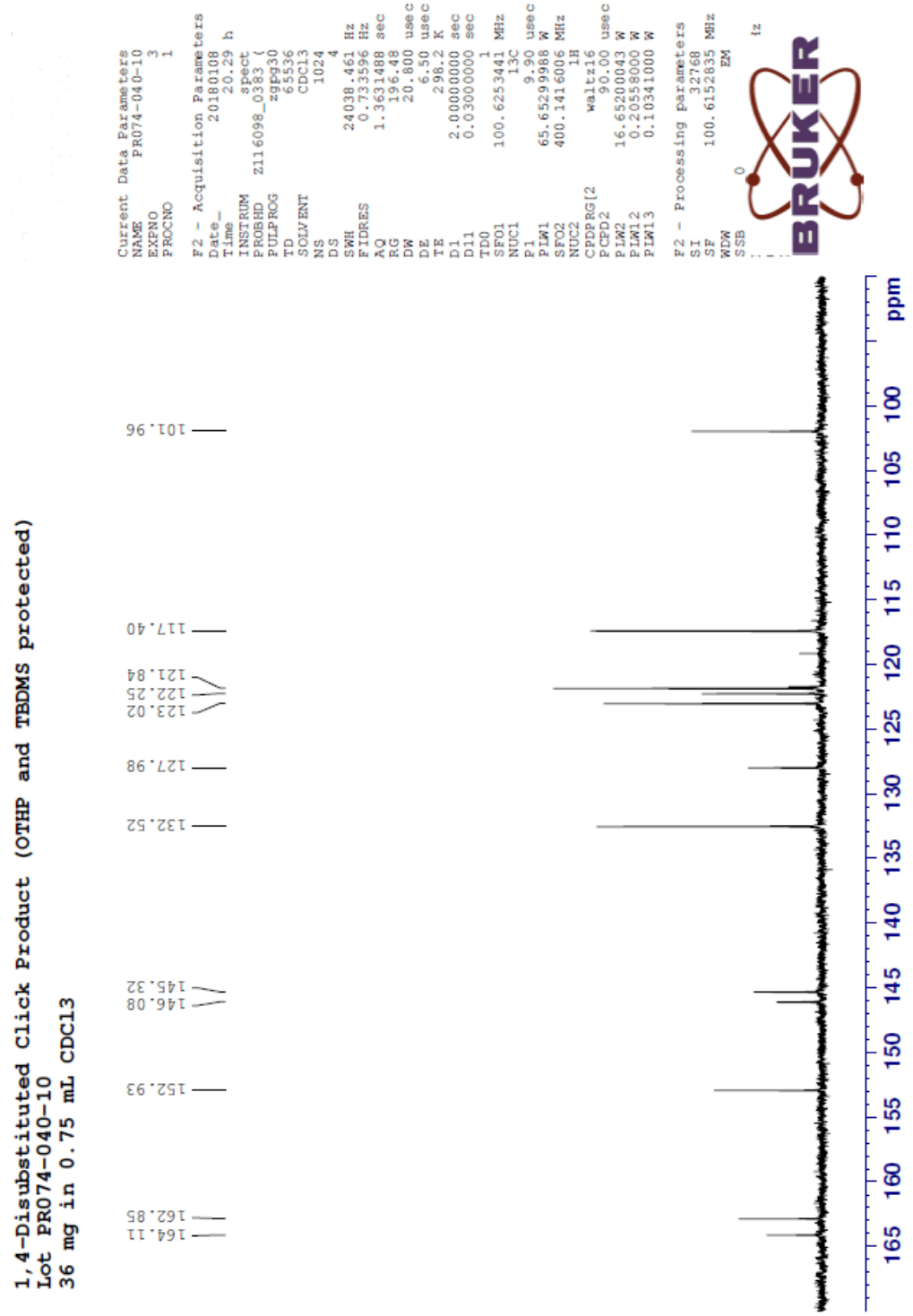
^{1}H NMR - 1,4-Disubstituted Click Product (TBDMS and THP Protected) (Compound 14)



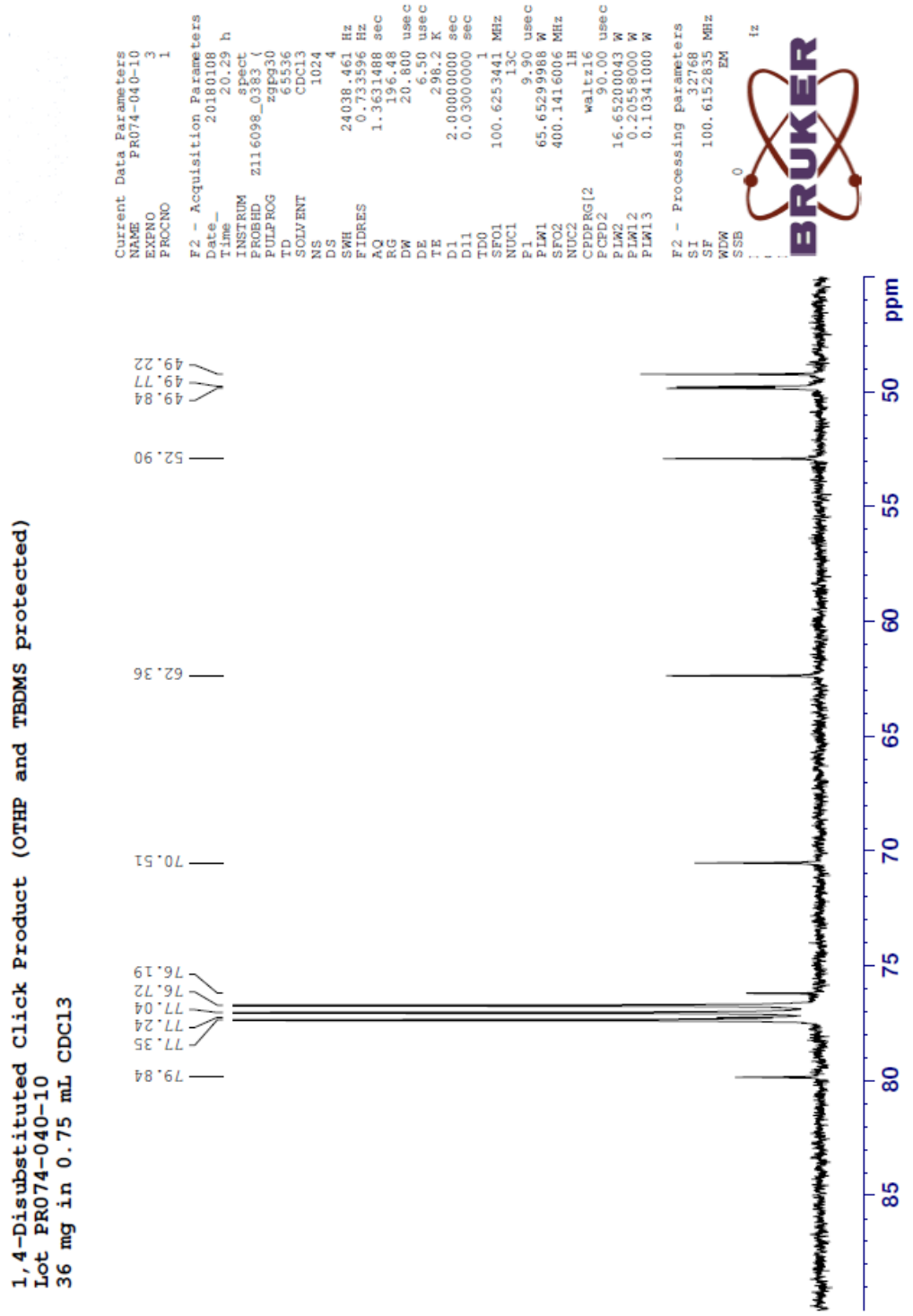
^{13}C NMR - 1,4-Disubstituted Click Product (TBDMS and THP Protected) (Compound 14)



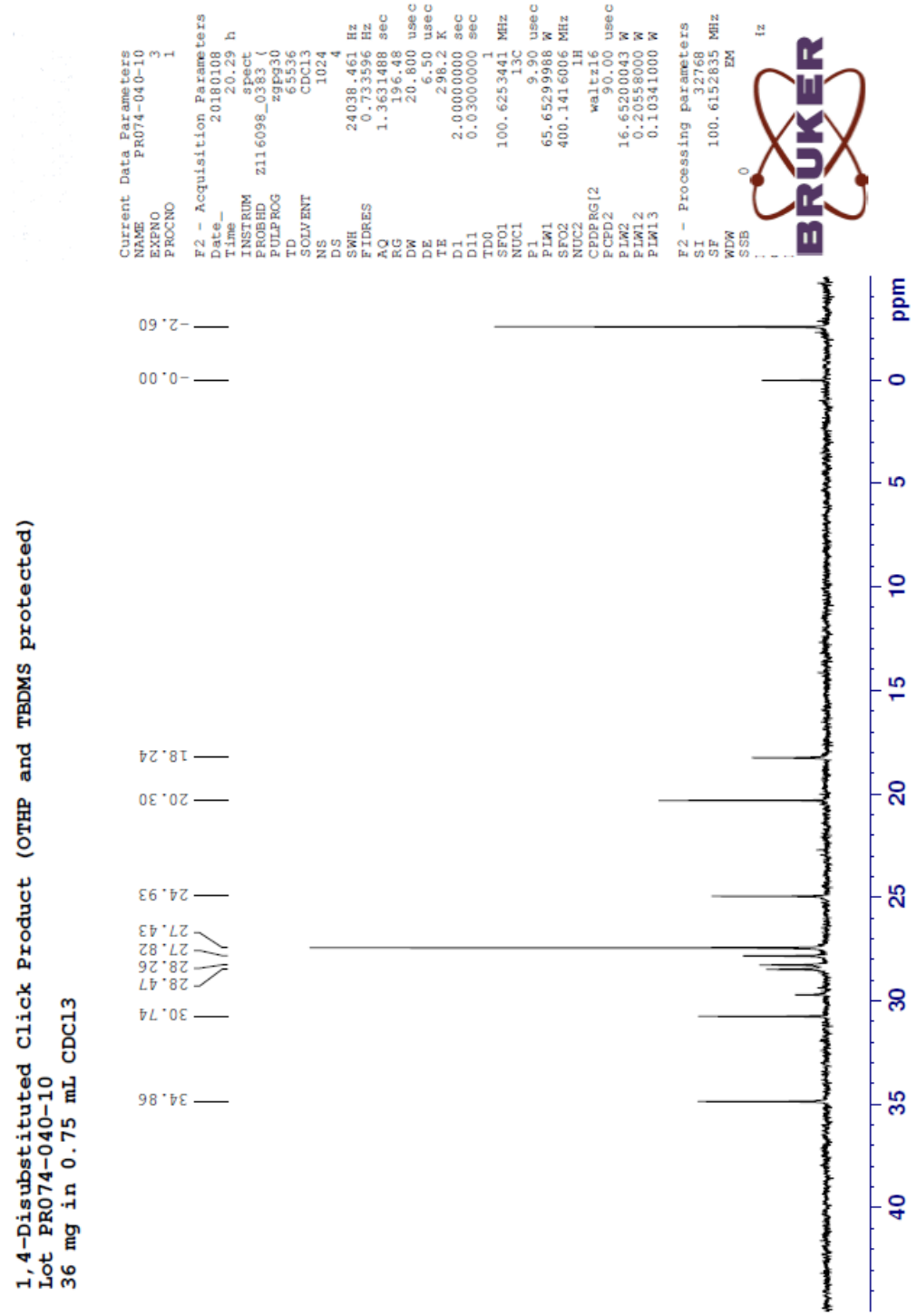
^{13}C NMR - 1,4-Disubstituted Click Product (TBDMS and THP Protected) (Compound 14)



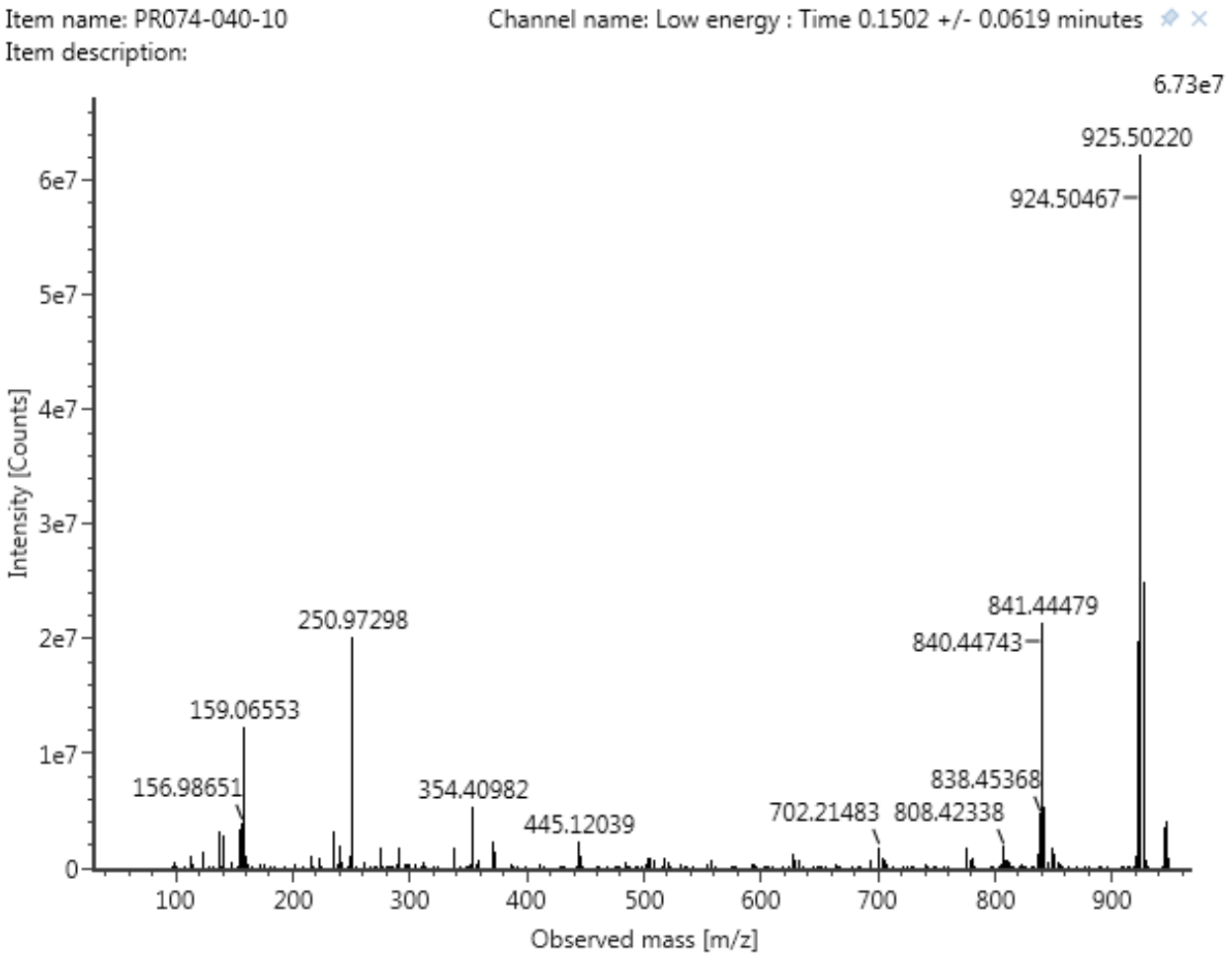
^{13}C NMR - 1,4-Disubstituted Click Product (TBDMS and THP Protected) (Compound 14)



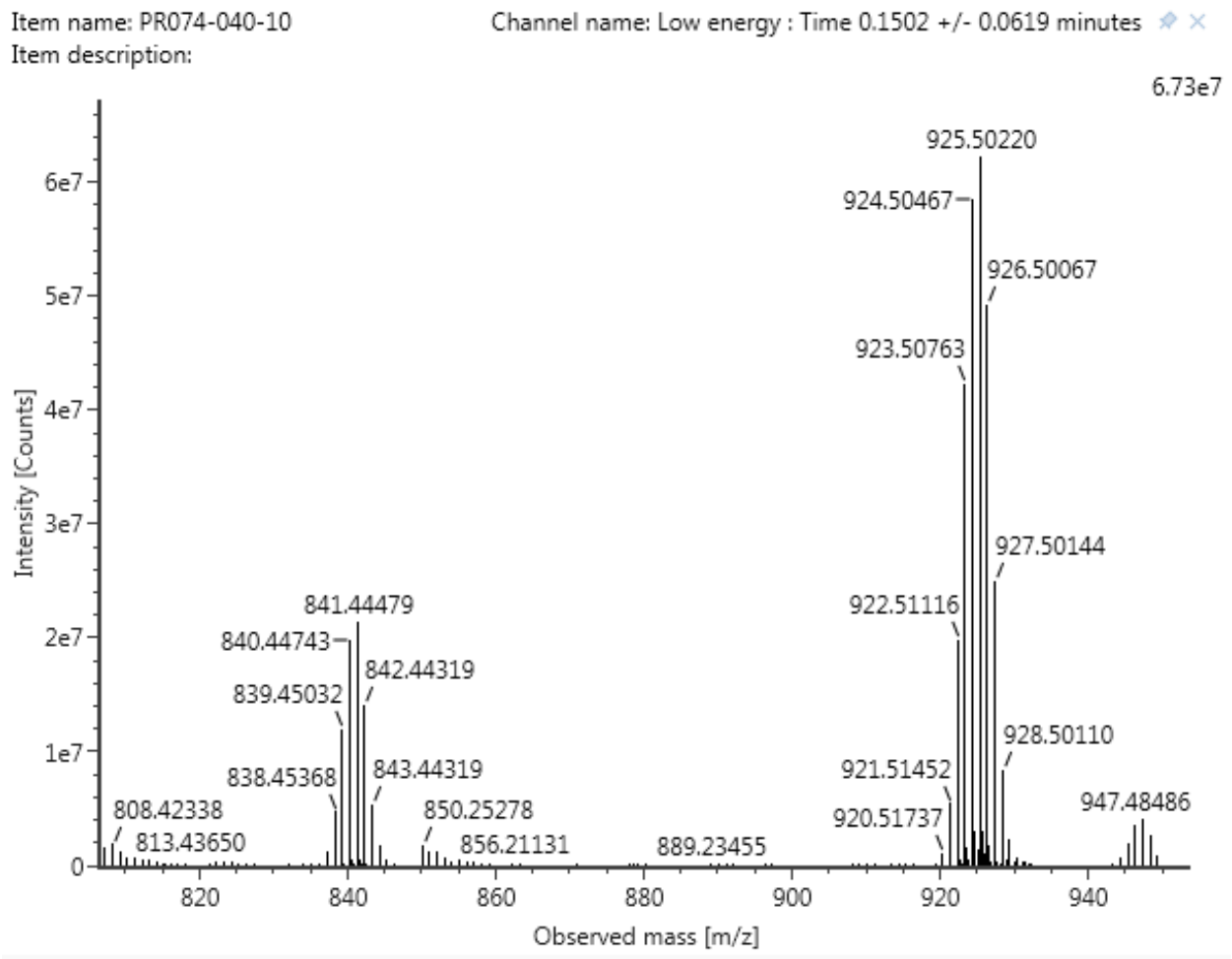
^{13}C NMR - 1,4-Disubstituted Click Product (TBDMS and THP Protected) (Compound 14)



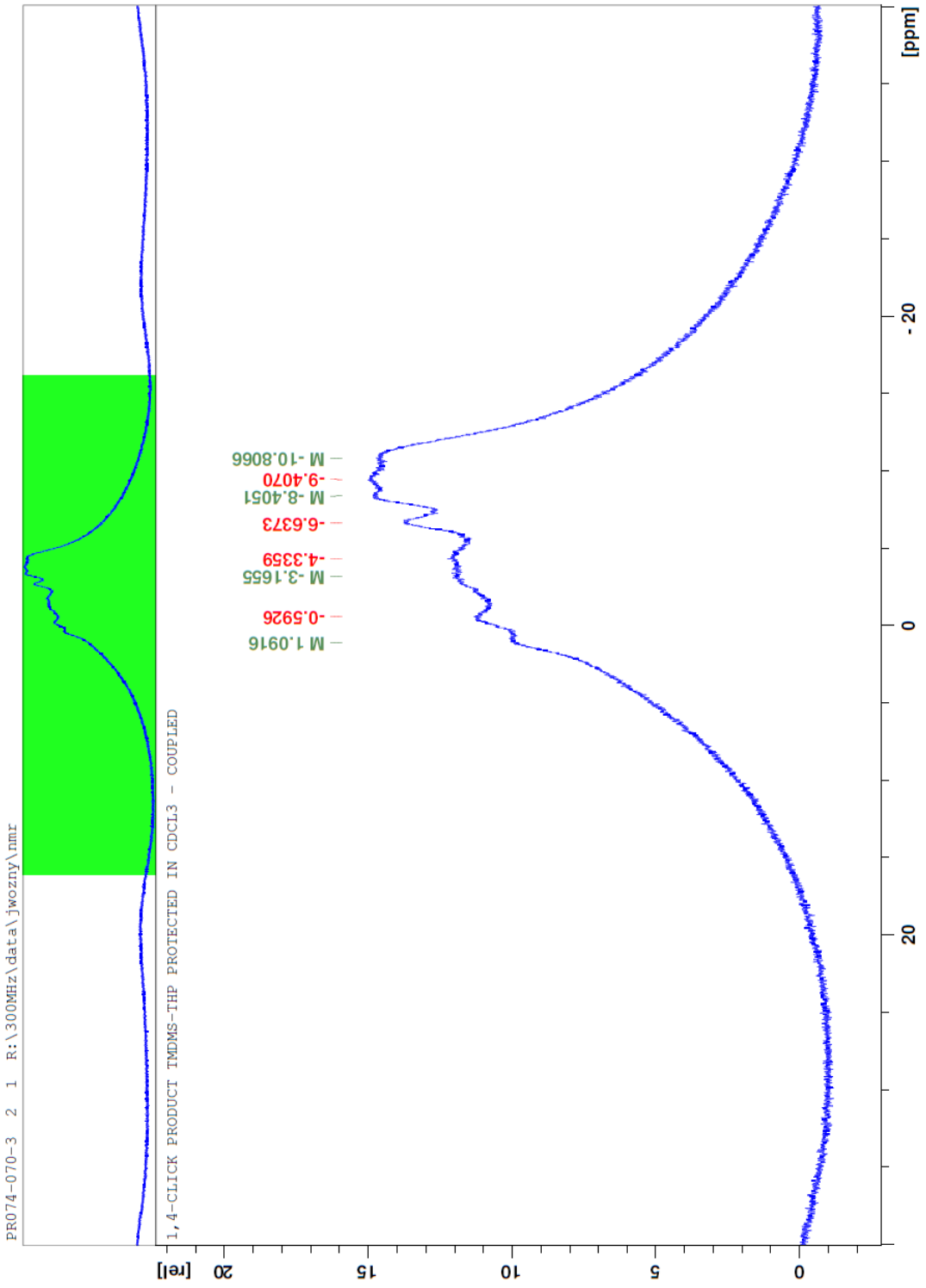
HRMS - 1,4-Disubstituted Click Product (TBDMS and THP Protected) (Compound 14)



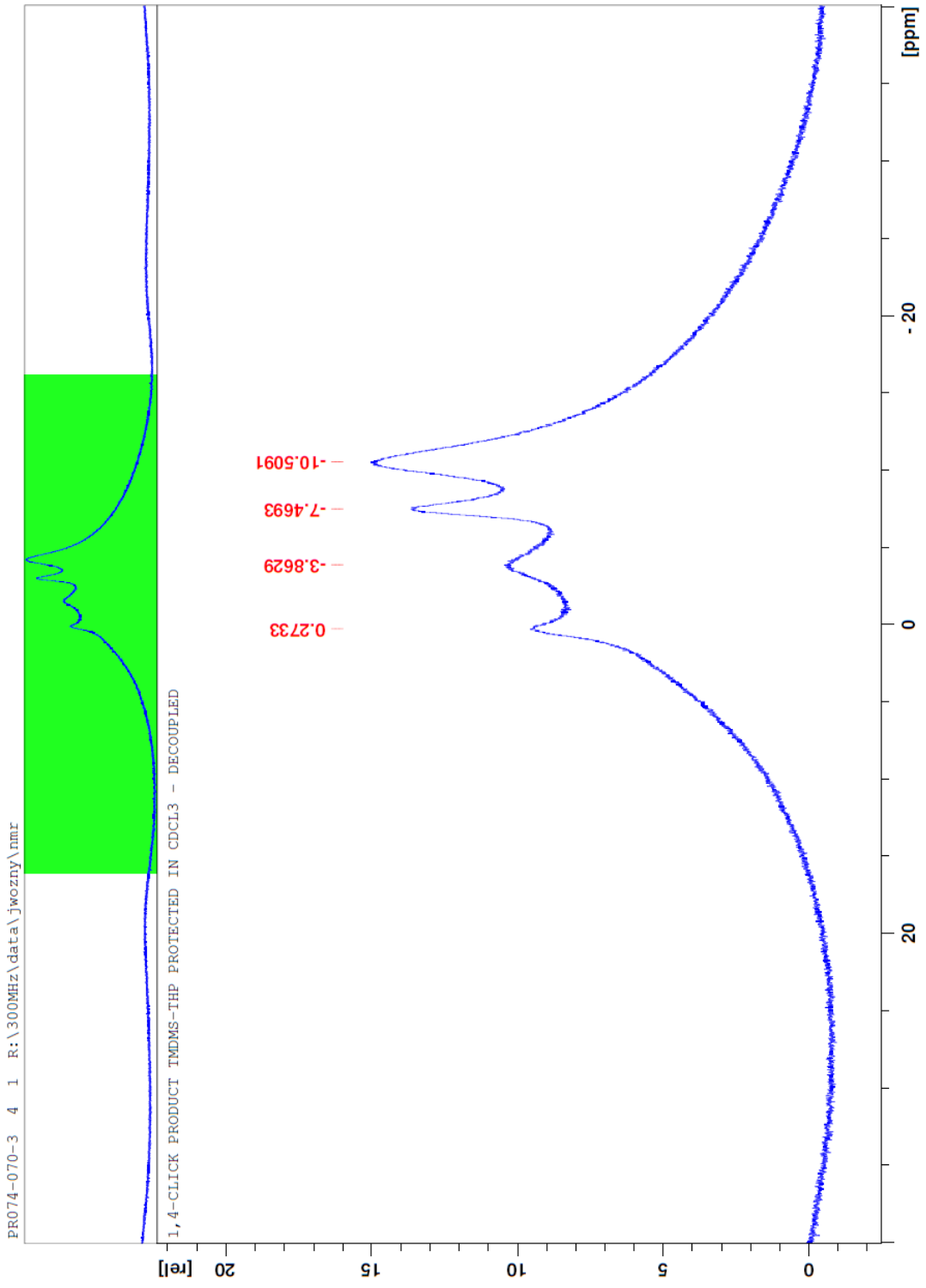
HRMS - 1,4-Disubstituted Click Product (TBDMS and THP Protected) (Compound 14)



^{11}B NMR (Coupled) - 1,4-Disubstituted Click Product (TBDMS/THP Protected) (Cmpd 14)



^{11}B NMR (Decoupled) - 1,4-Disubstituted Click Product (TBDMS/THP Protected) (Cmpd 14)



HPLC - THP MMP-18 1,4-Click Product (Compound 15)

```
Acq. Operator   : MRL                                Seq. Line :  10
Acq. Instrument : Instrument 1                        Location : Vial 82
Injection Date  : 01/15/18  2:13:15 AM                    Inj :   1
                                                    Inj Volume : 5.0 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 2.5 µl
Acq. Method     : C:\CHEM32\1\DATA\MRL180114A\MRL180114A 2018-01-14 19-56-32\BNCT-LONG2.M
Last changed    : 10/18/17  2:57:54 PM by MRL
Analysis Method : C:\CHEM32\1\METHODS\BNCT-LONG2.M
Last changed    : 01/15/18  12:25:15 PM by MRL
                  (modified after loading)
Method Info     : HPLC Method for Monitoring and Assaying BNCT products and reactions
                  HPLC 12 (Lab 2)
                  A = 0.10% TFA in water
                  B = 0.10% TFA in ACN
                  Waters Sunfire C8, 4.6x150 mm, 3.5 micron
                  Flow: 1.0 mL/min, Column Temp: 40 C

Sample Info     : PR074-055-3
                  Purified product

Additional Info : Peak(s) manually integrated
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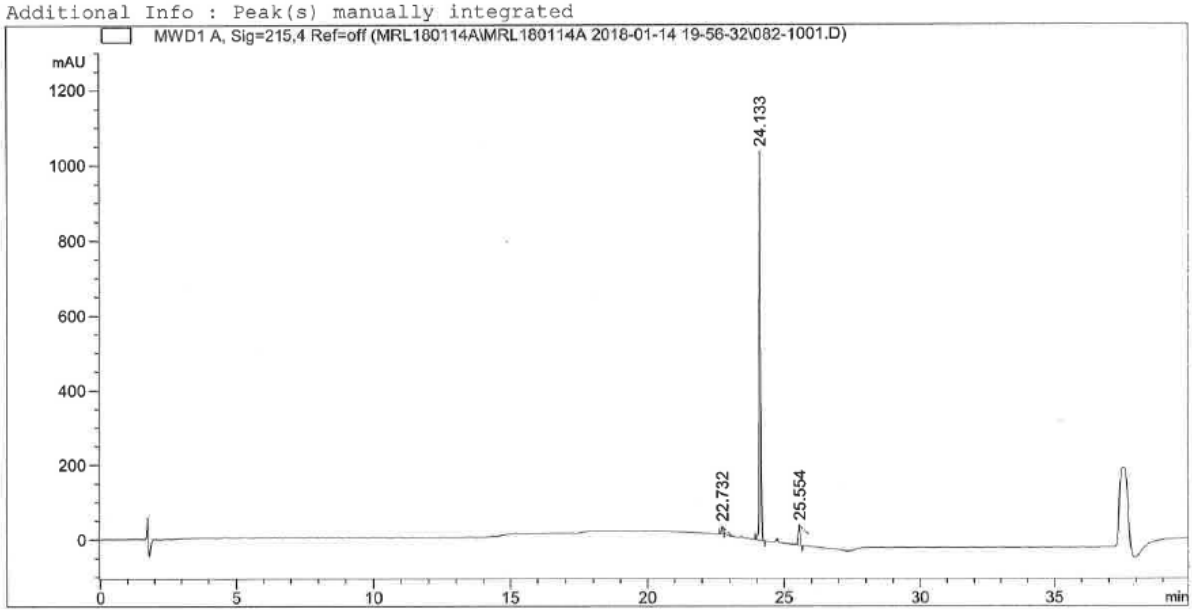
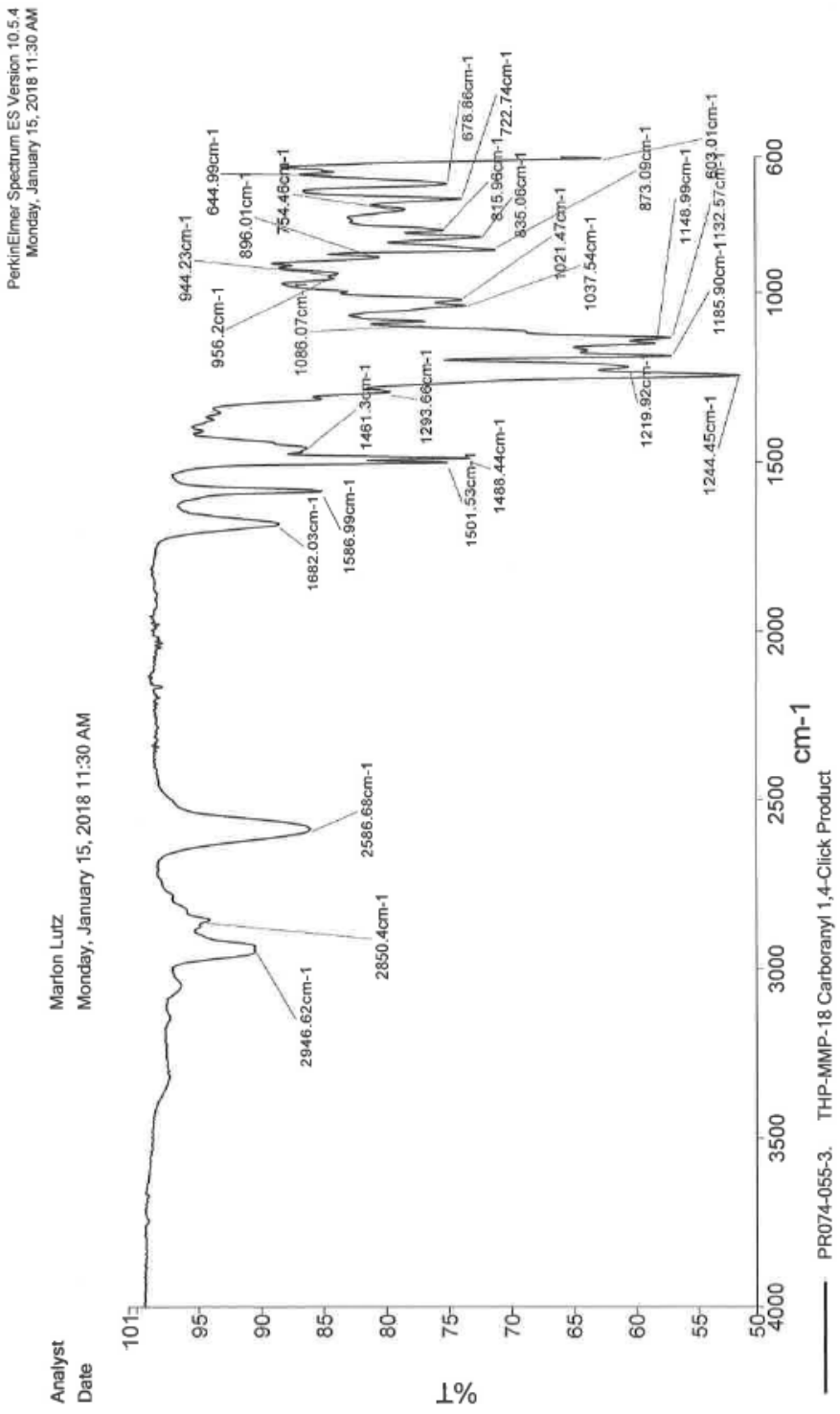


Area Percent Report

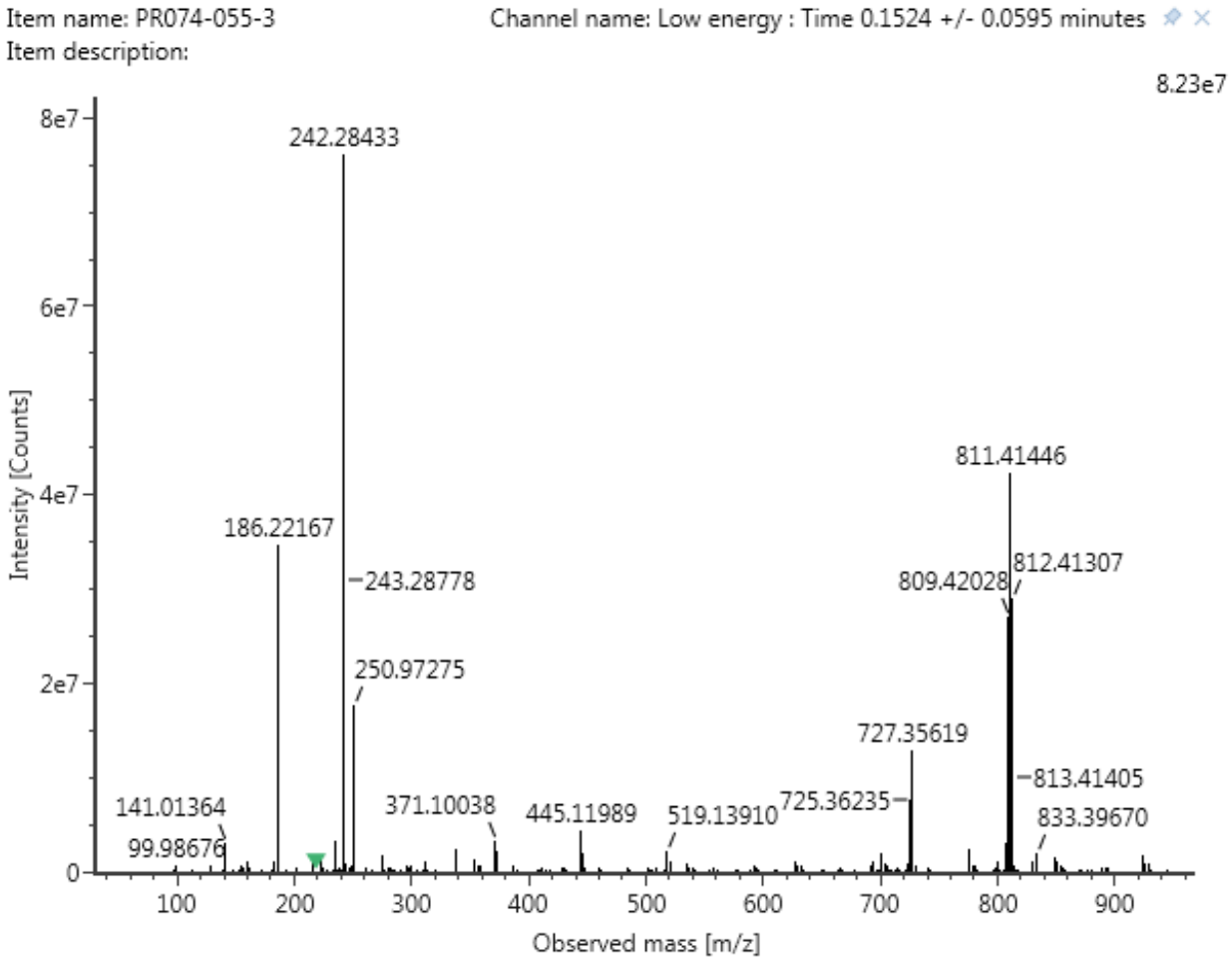
Signal 1: MWD1 A, Sig=215,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	22.732	MF	0.0805	113.96170	23.60163	2.6791
2	24.133	BB	0.0602	3927.70483	1037.55432	92.3360
3	25.554	FM	0.0644	212.04193	54.87874	4.9849
Totals :				4253.70847	1116.03470	

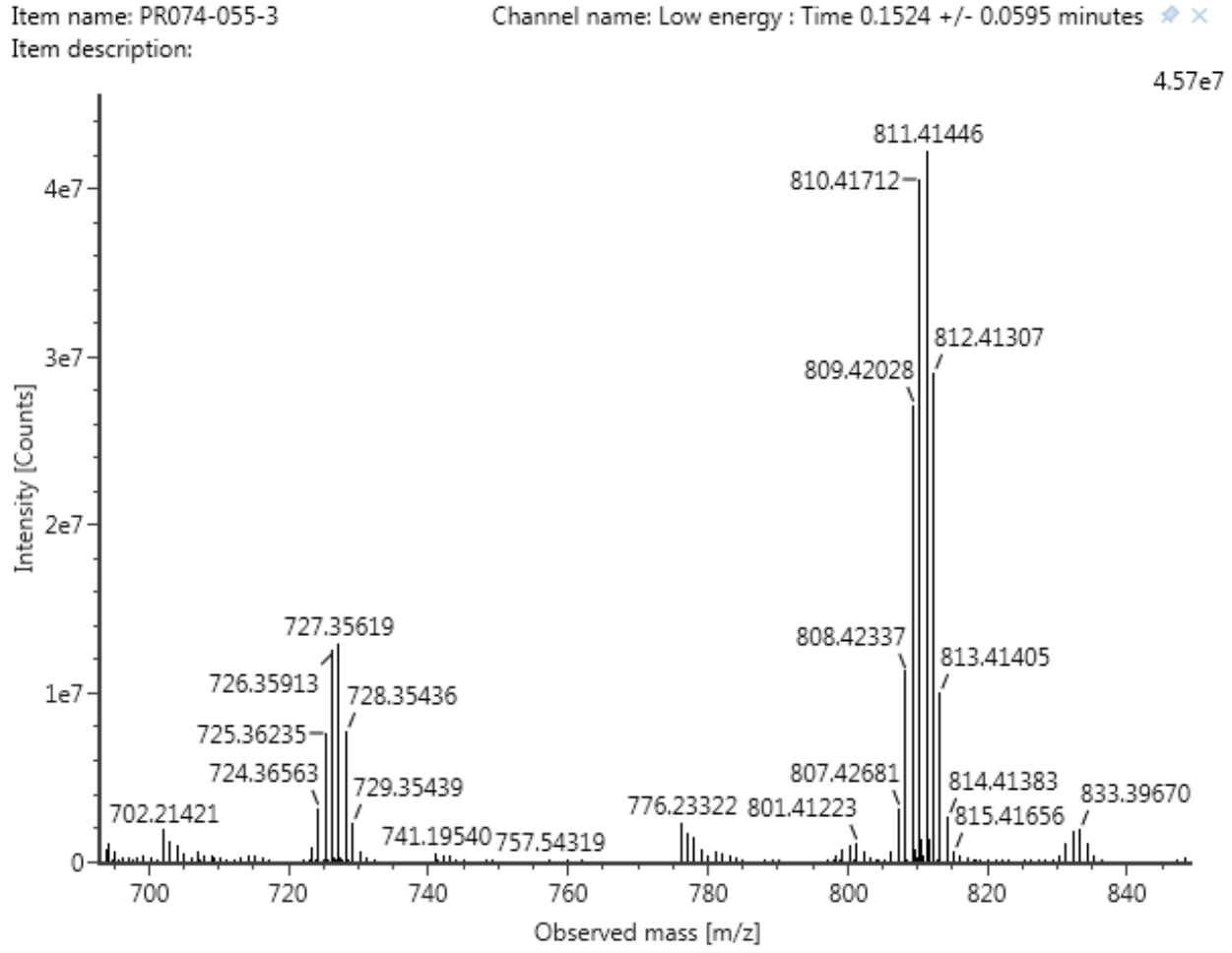
FTIR - THP MMP-18 1,4-Click Product (Compound 15)



HRMS - THP MMP-18 1,4-Click Product (Compound 15)



HRMS - THP MMP-18 1,4-Click Product (Compound 15)

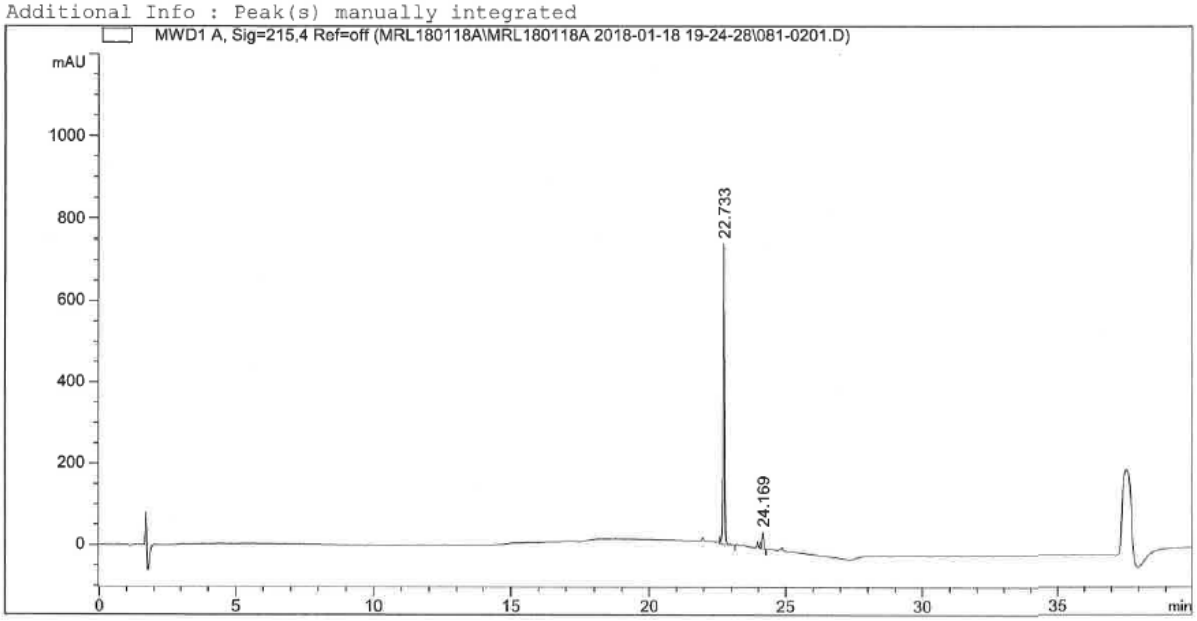


HPLC - 1,4-Click Carboranyl MMP-18 HCl Salt (Compound 16)

```
Acq. Operator   : MRL                                Seq. Line :   2
Acq. Instrument : Instrument 1                        Location : Vial 81
Injection Date  : 01/18/18  8:07:58 PM                    Inj :   1
                                                  Inj Volume : 5.0 µl
Acq. Method     : C:\CHEM32\1\DATA\MRL180118A\MRL180118A 2018-01-18 19-24-28\BNCT-LONG2.M
Last changed    : 10/18/17  2:57:54 PM by MRL
Analysis Method : C:\CHEM32\1\METHODS\BNCT-LONG2.M
Last changed    : 01/19/18  9:17:03 AM by MRL
                  (modified after loading)
Method Info     : HPLC Method for Monitoring and Assaying BNCT products and reactions
                  HPLC 12 (Lab 2)
                  A = 0.10% TFA in water
                  B = 0.10% TFA in ACN
                  Waters Sunfire C8, 4.6x150 mm, 3.5 micron
                  Flow: 1.0 mL/min, Column Temp: 40 C

Sample Info     : Isolated 1,4-Click MMP-18 Carboranyl Product
                  PR074-062-5

Additional Info : Peak(s) manually integrated
```

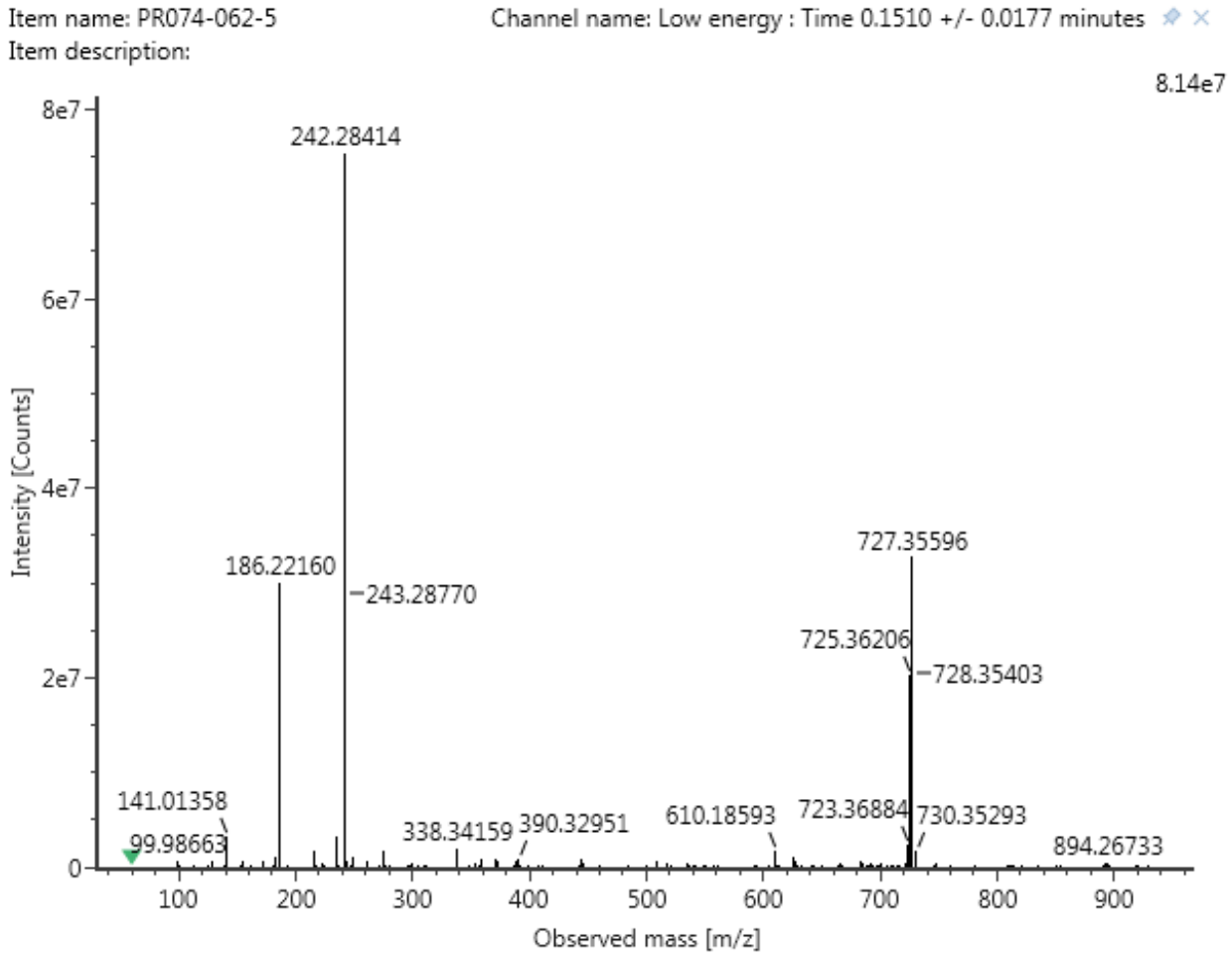


Area Percent Report

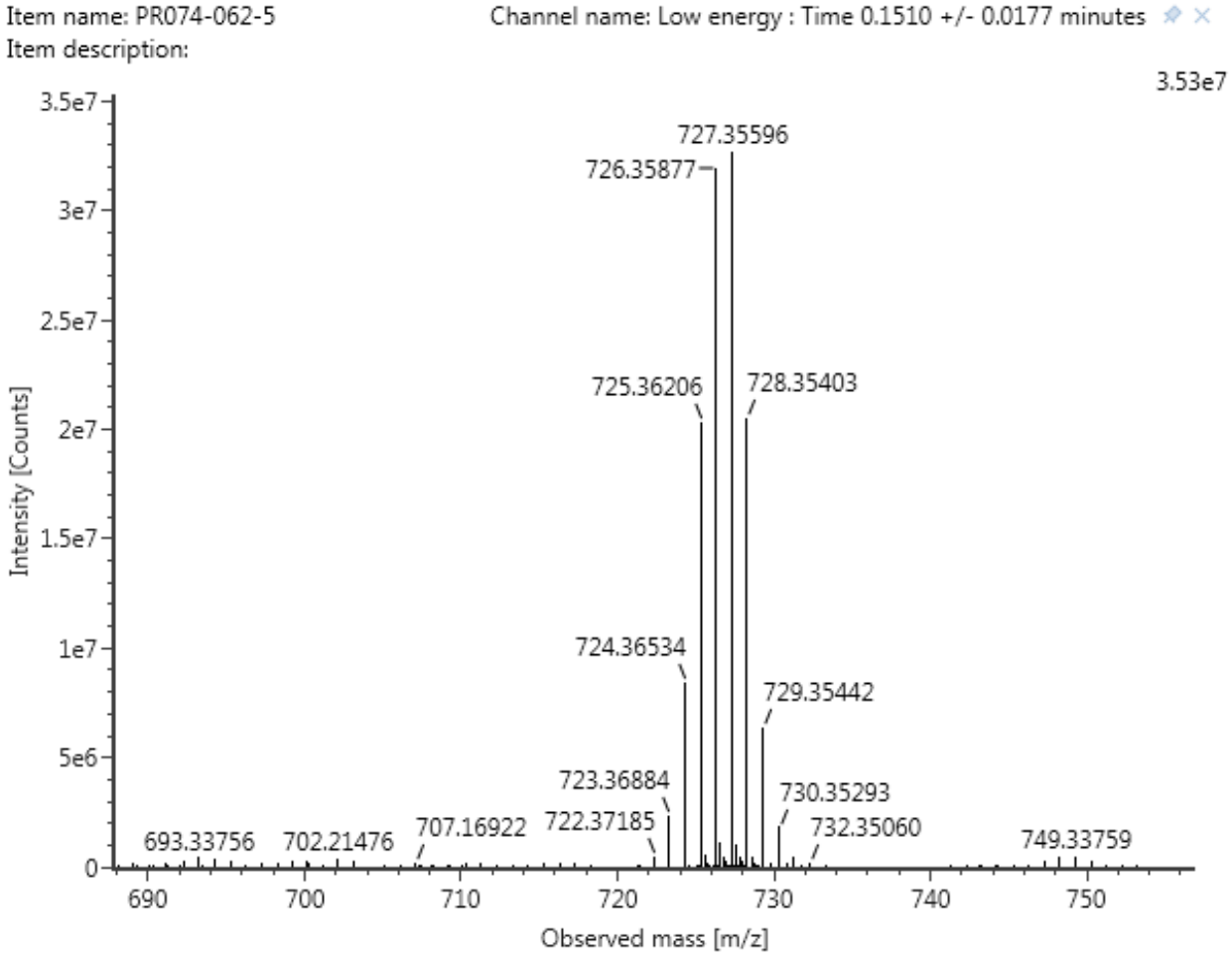
Signal 1: MWD1 A, Sig=215,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	22.733	VV	0.0612	2849.45630	734.94965	95.1416
2	24.169	VB	0.0595	145.50888	39.06241	4.8584
Totals :				2994.96518	774.01206	

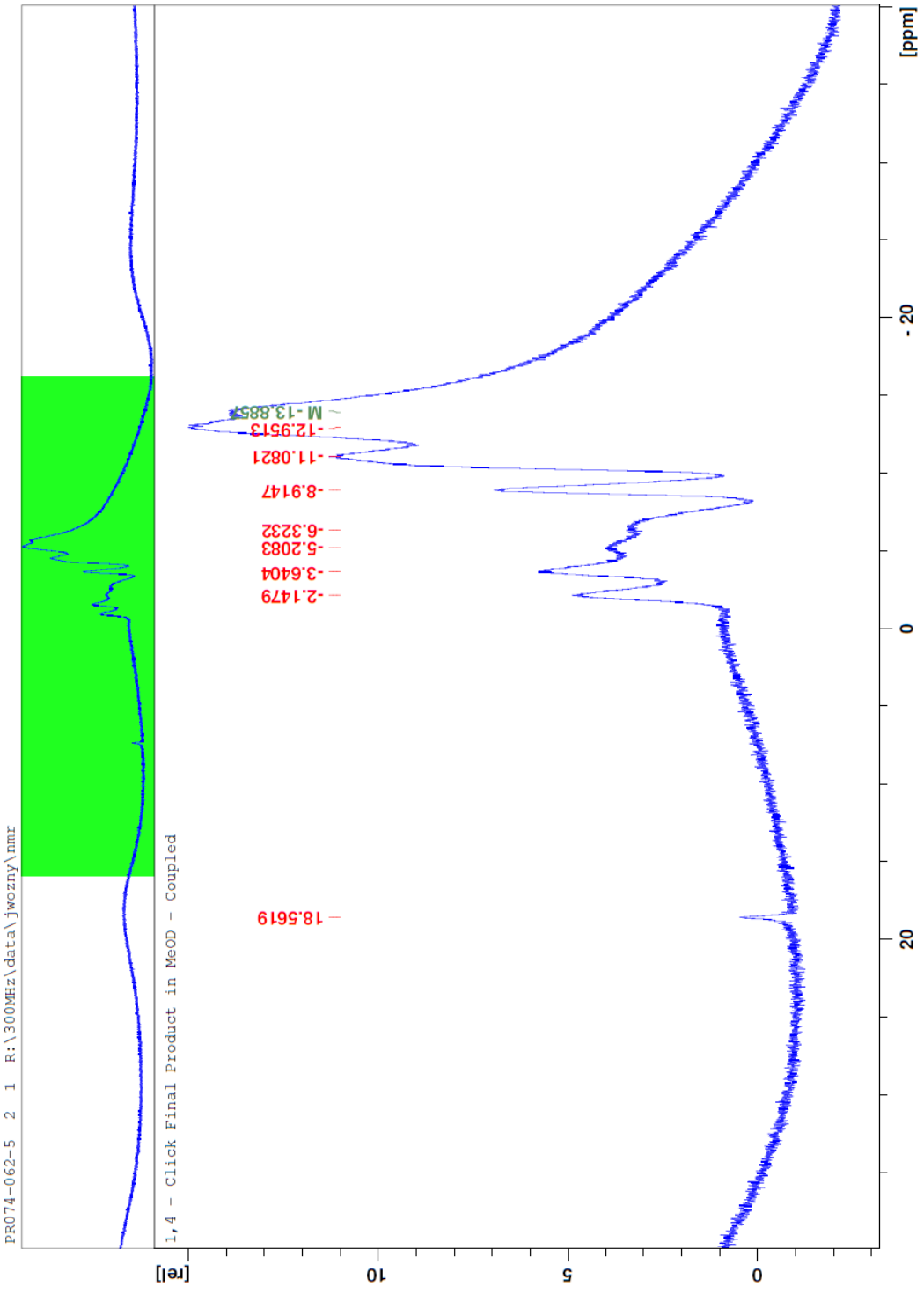
HRMS - 1,4-Click Carboranyl MMP-18 HCl Salt (Compound 16)



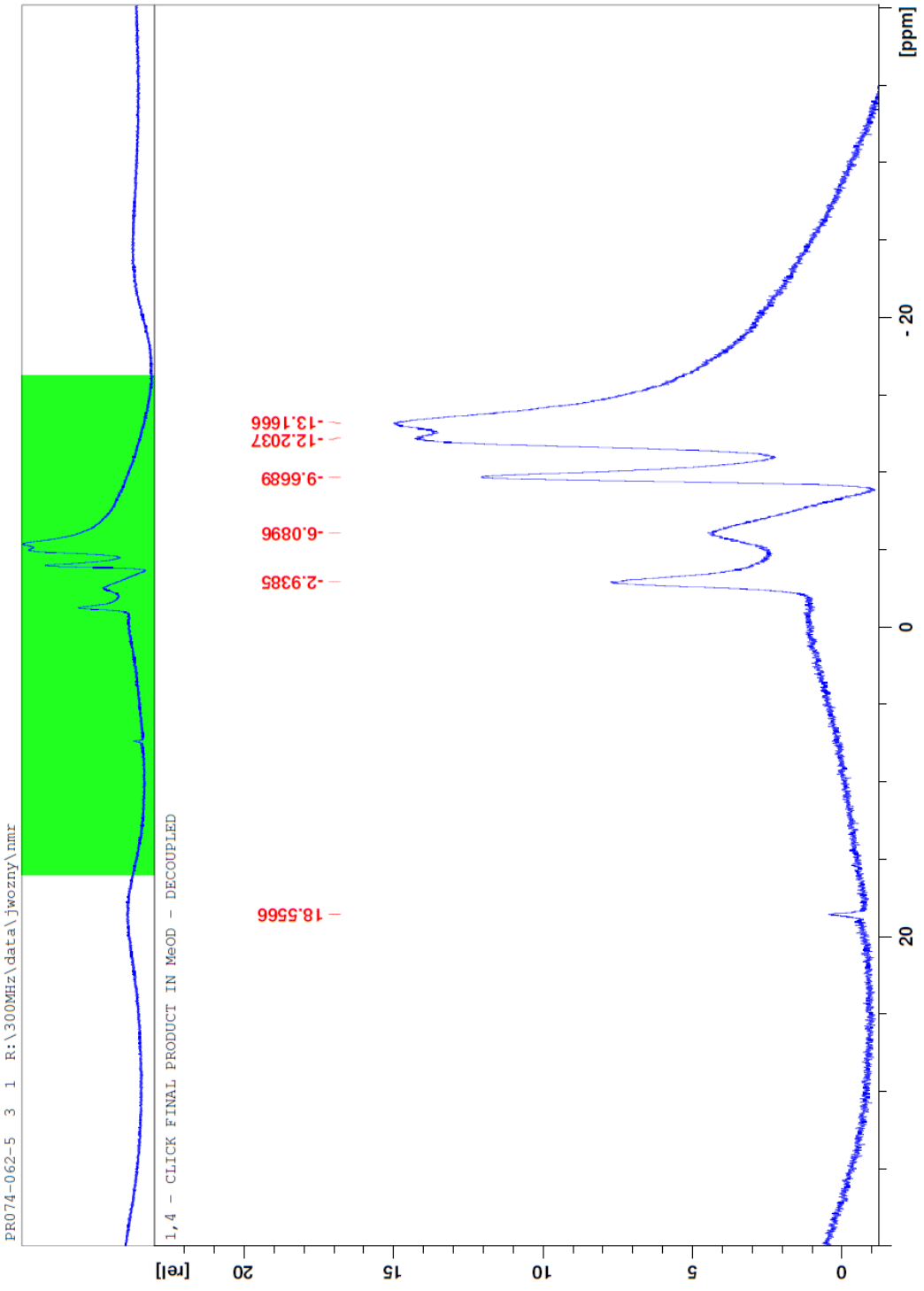
HRMS - 1,4-Click Carboranyl MMP-18 HCl Salt (Compound 16)



^{11}B NMR (Coupled) - 1,4-Click Carboranyl MMP-18 HCl Salt (Compound 16)



^{11}B NMR (Decoupled) - 1,4-Click Carboranyl MMP-18 HCl Salt (Compound 16)

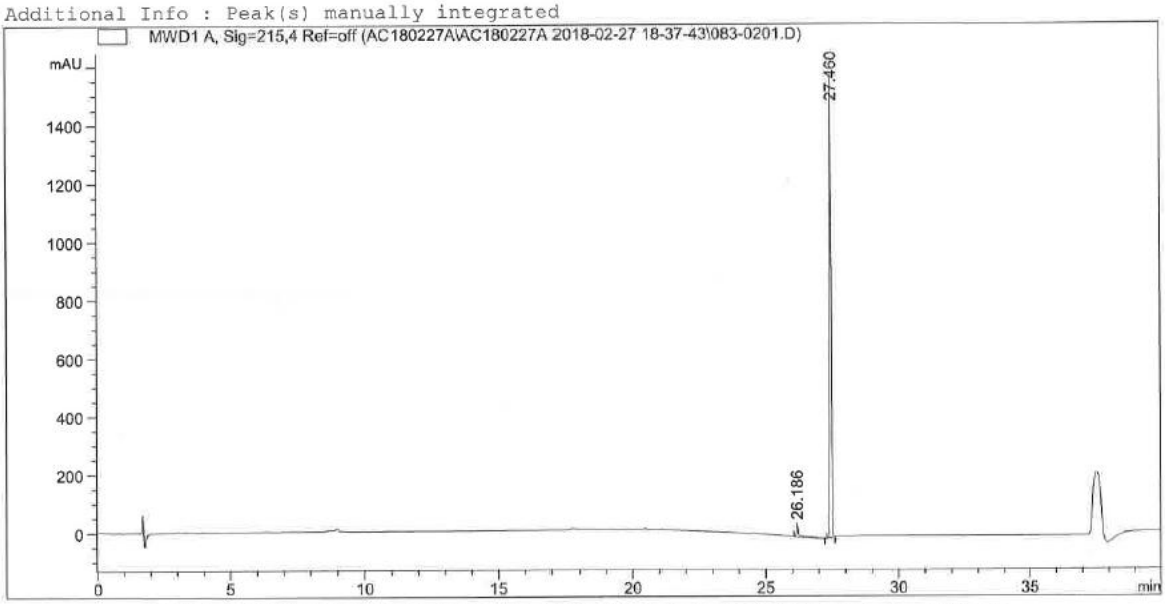


HPLC - 1,5-Disubstituted Click Product (TBDMS and THP Protected) (Compound 17)

```
Acq. Operator    : MRL                                  Seq. Line :   2
Acq. Instrument  : Instrument 1                          Location : Vial 83
Injection Date   : 02/27/18  7:20:59 PM                      Inj :   1
                                                      Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\1\DATA\AC180227A\AC180227A 2018-02-27 18-37-43\BNCT-LONG2.M
Last changed     : 10/18/17  2:57:54 PM by MRL
Analysis Method  : C:\CHEM32\1\METHODS\BNCT-LONG2.M
Last changed     : 02/28/18  8:01:59 AM by MRL
                   (modified after loading)
Method Info      : HPLC Method for Monitoring and Assaying BNCT products and reactions
                   HPLC 12 (Lab 2)
                   A = 0.10% TFA in water
                   B = 0.10% TFA in ACN
                   Waters Sunfire C8, 4.6x150 mm, 3.5 micron
                   Flow: 1.0 mL/min, Column Temp: 40 C

Sample Info      : 1,5 Click Product
                   Lot# PR074-070-2
                   Conc. 1 mg/mL in ACN

Additional Info : Peak(s) manually integrated
```



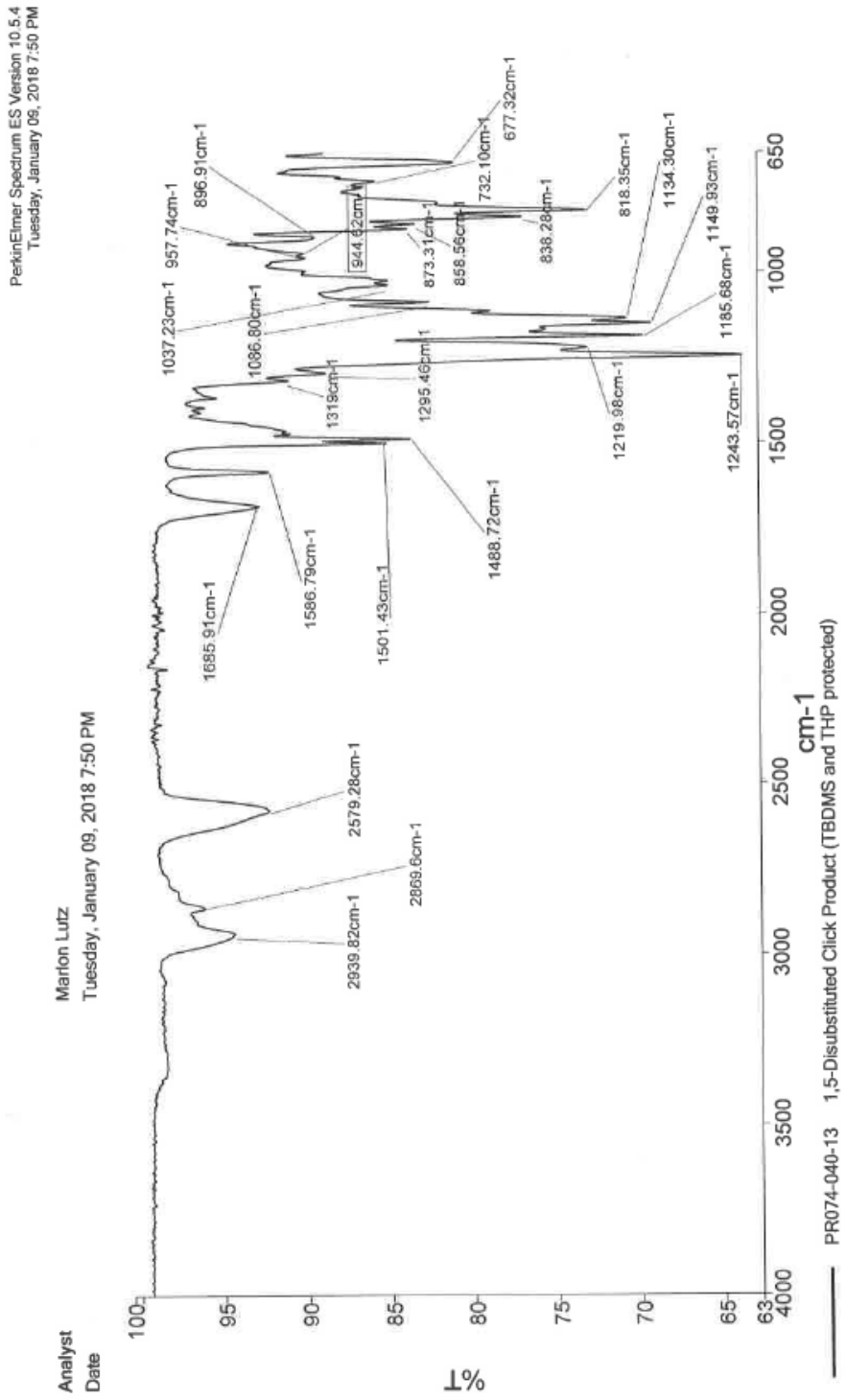

```
                          Area Percent Report

Signal 1: MWD1 A, Sig=215,4 Ref=off

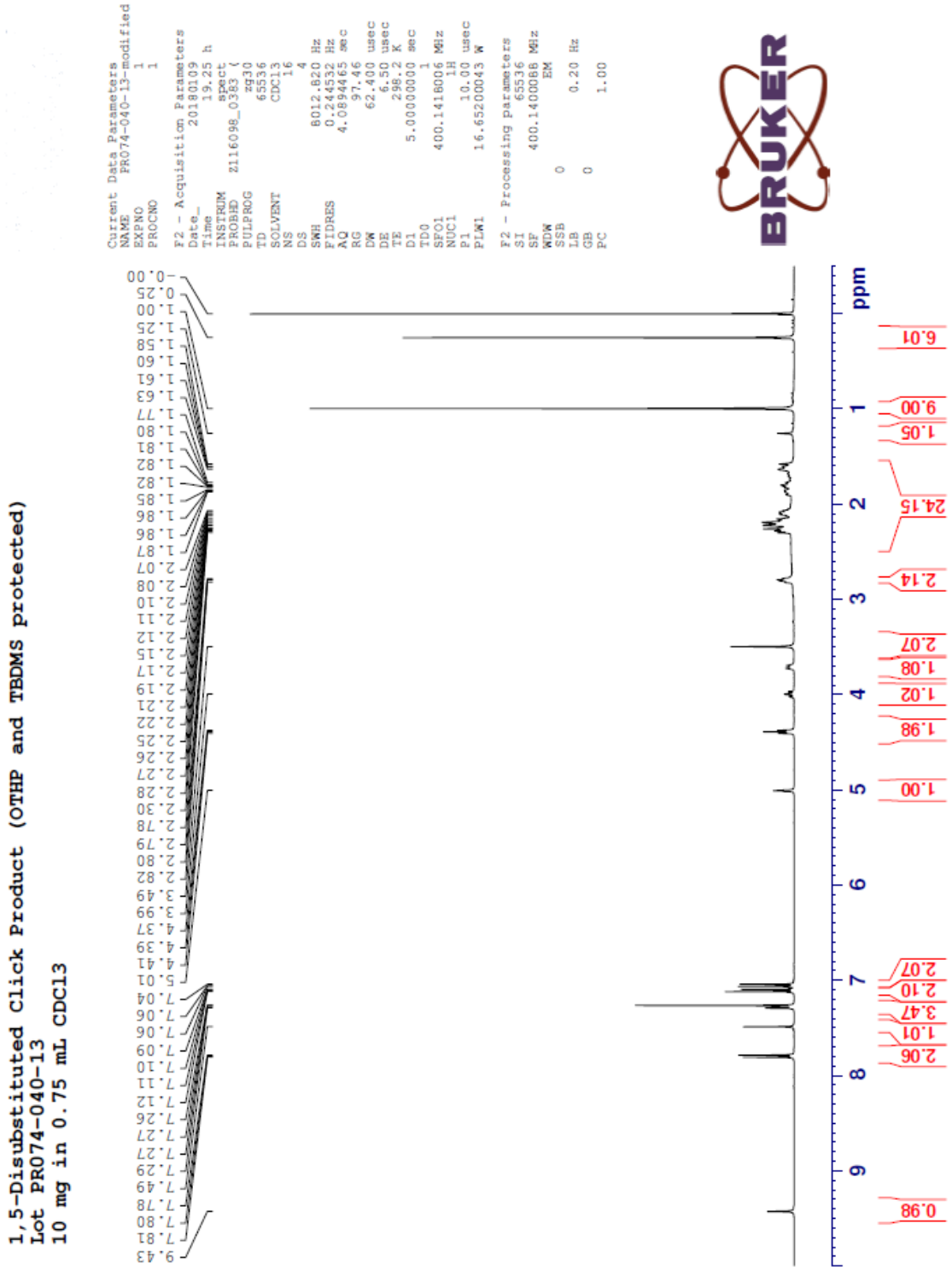
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  26.186 VB    0.1067  369.88660   47.59706   5.6269
   2  27.460 BB    0.0621 6203.61084 1568.41614  94.3731

Totals :                  6573.49744 1616.01320
```

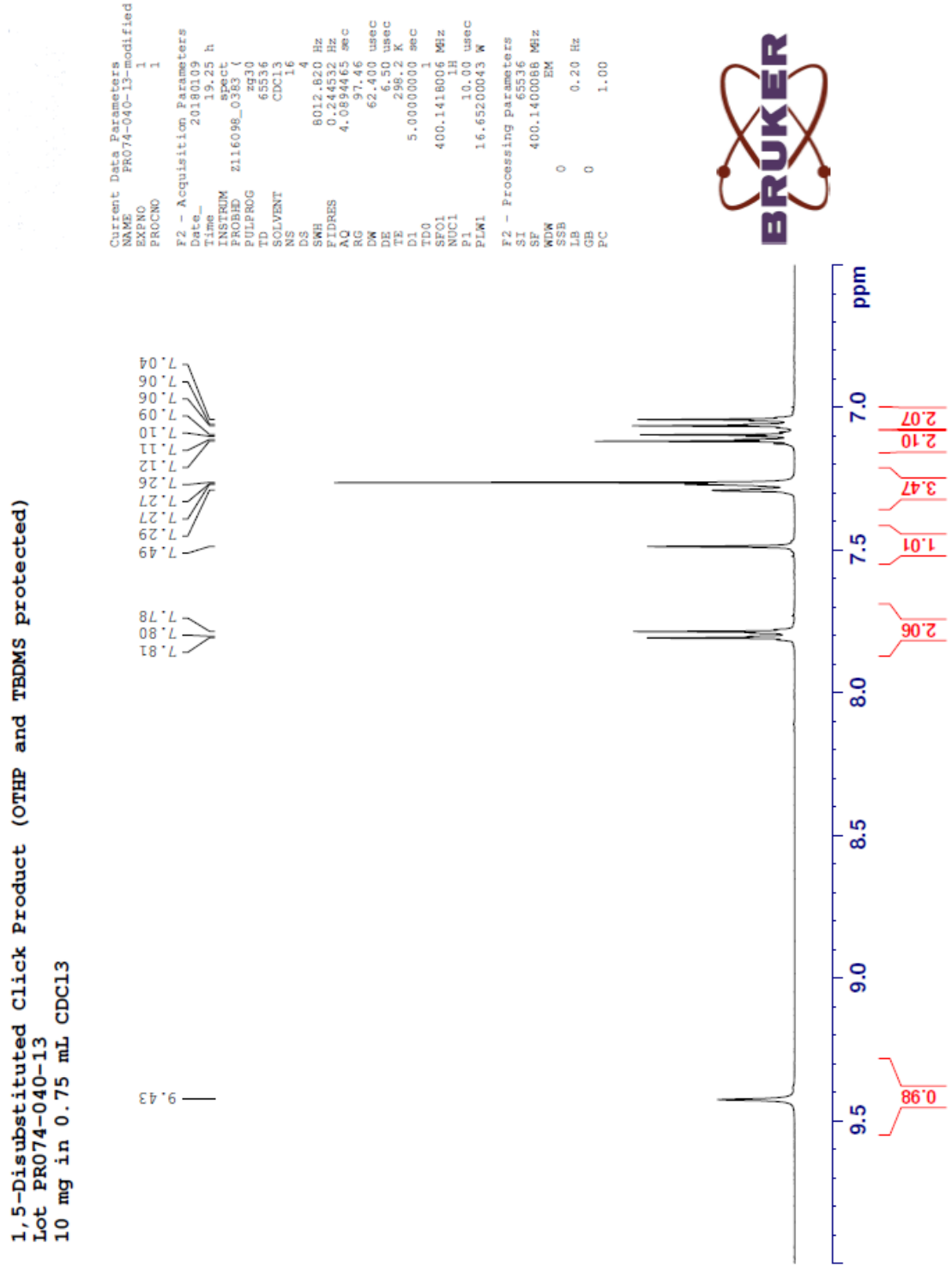
FTIR - 1,5-Disubstituted Click Product (TBDMS and THP Protected) (Compound 17)



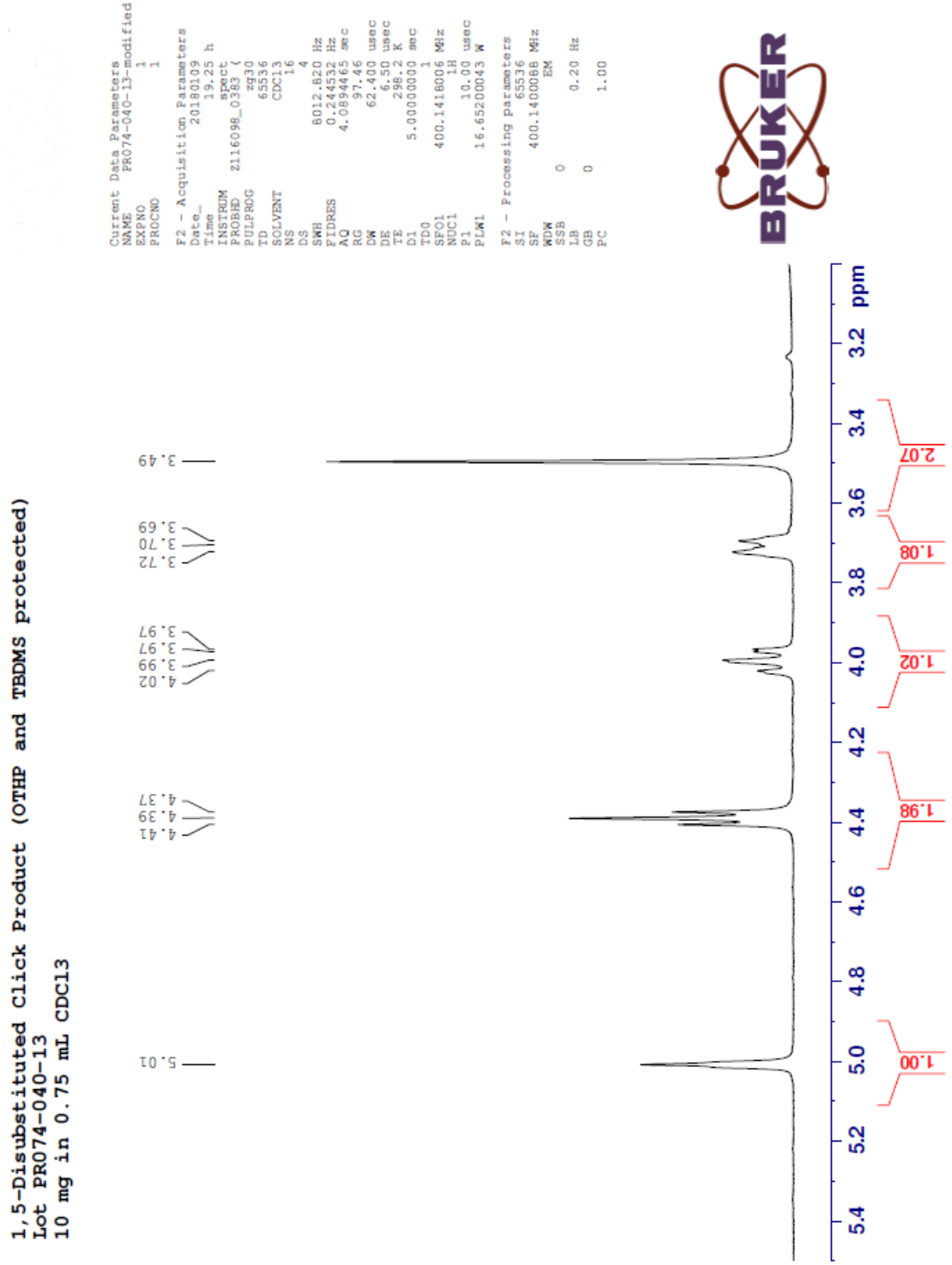
^{1}H NMR - 1,5-Disubstituted Click Product (TBDMS and THP Protected) (Compound 17)



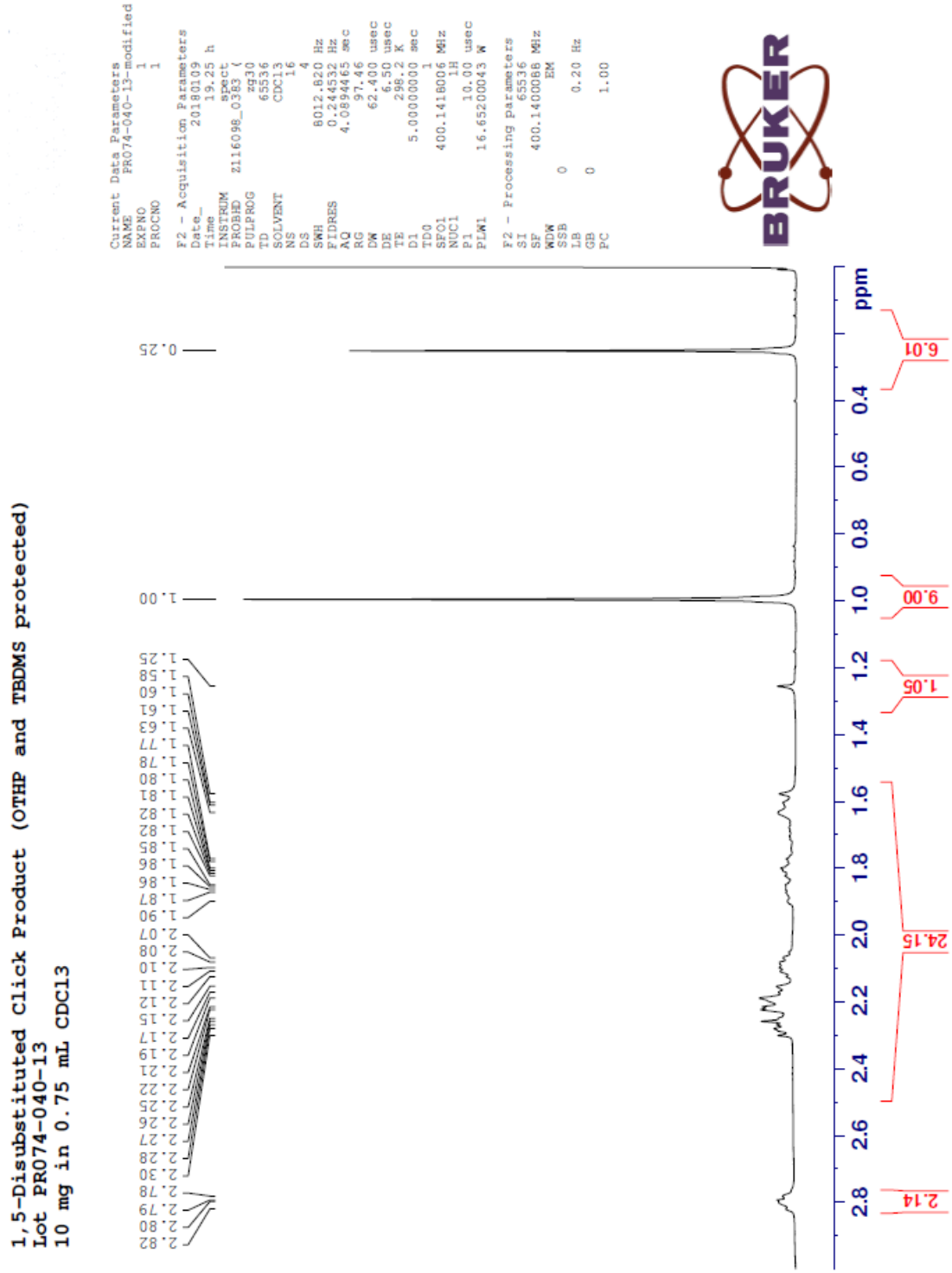
^{1}H NMR - 1,5-Disubstituted Click Product (TBDMS and THP Protected) (Compound 17)



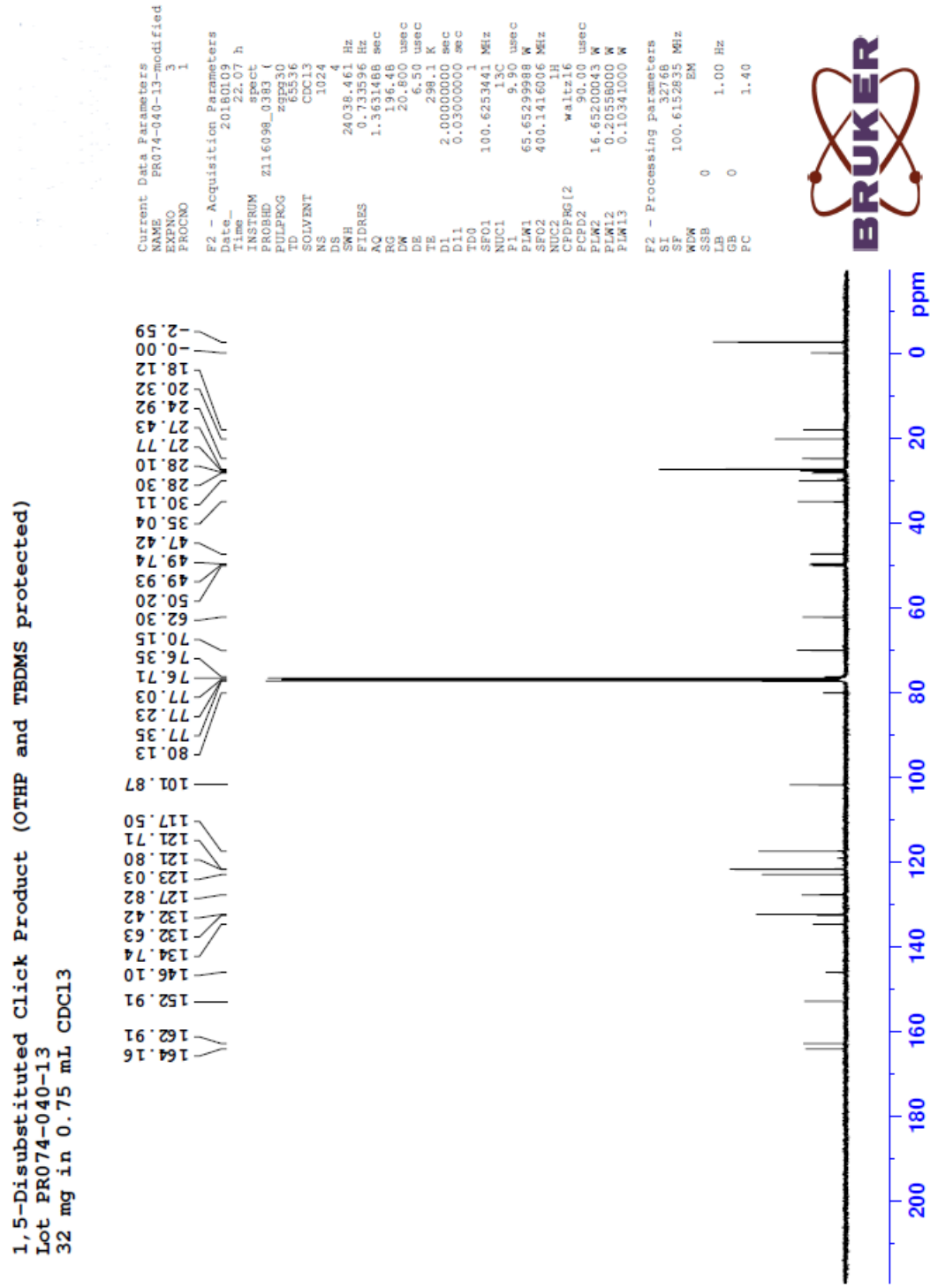
^{1}H NMR - 1,5-Disubstituted Click Product (TBDMS and THP Protected) (Compound 17)



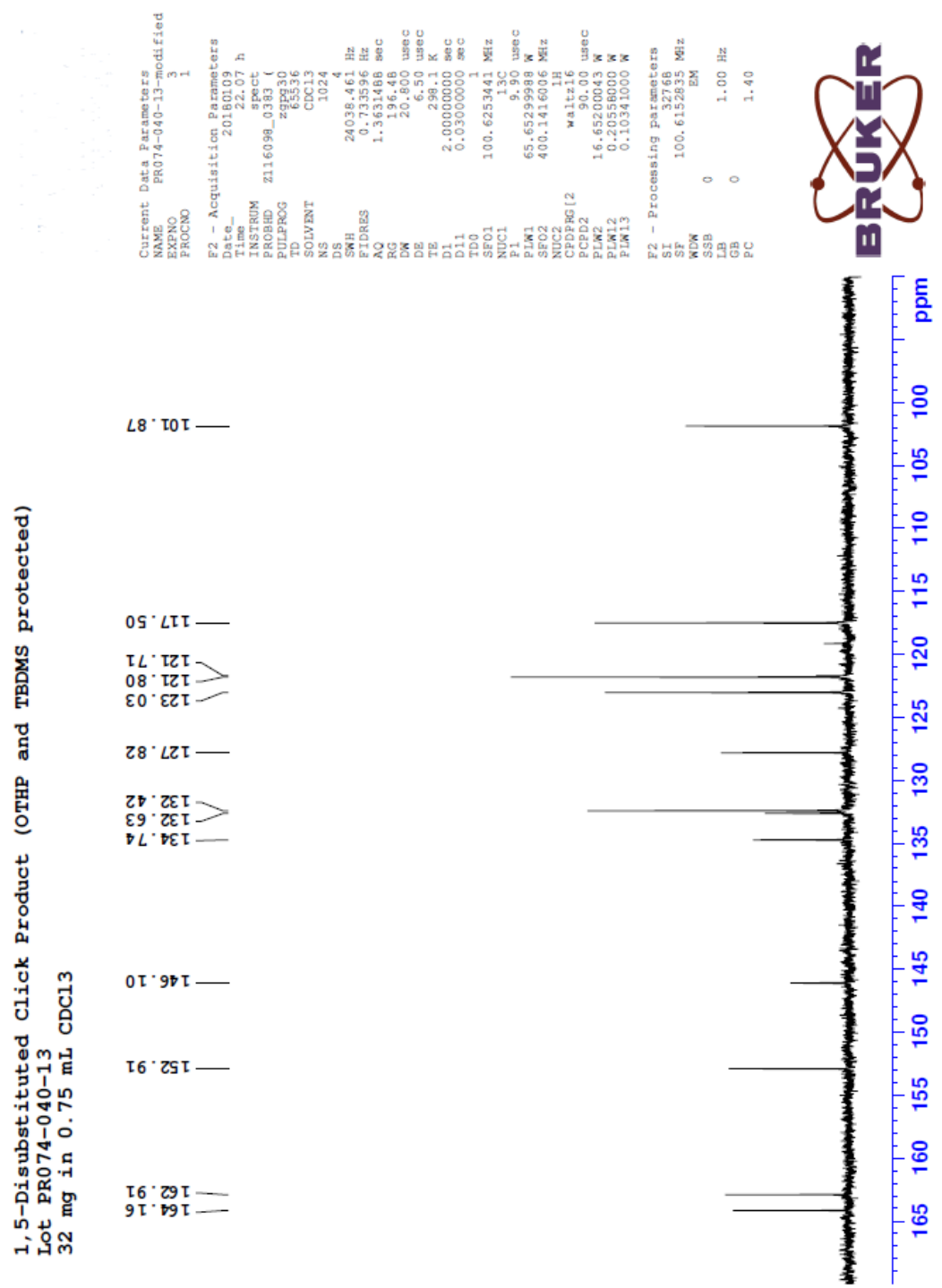
^{1}H NMR - 1,5-Disubstituted Click Product (TBDMS and THP Protected) (Compound 17)



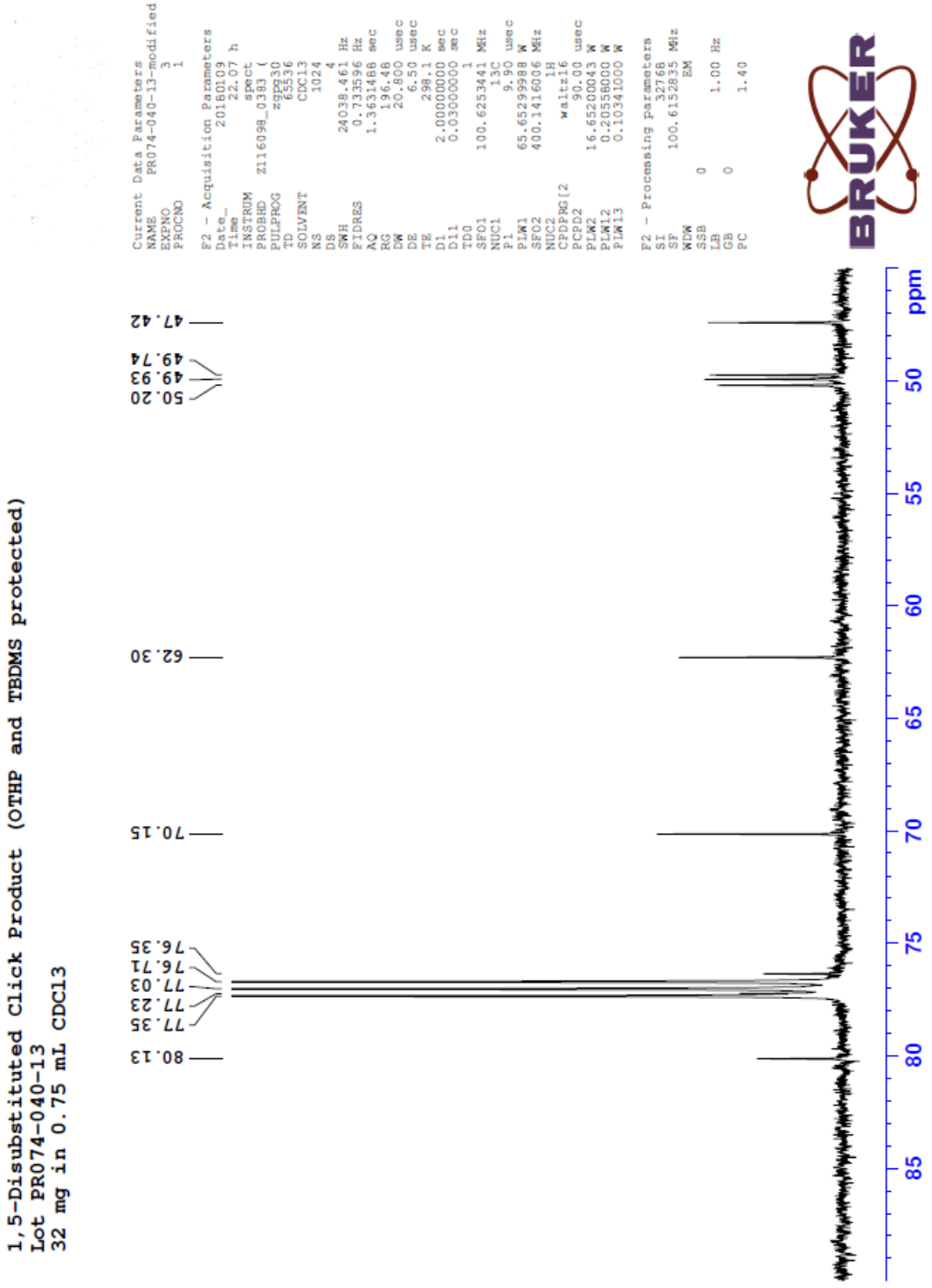
^{13}C NMR - 1,5-Disubstituted Click Product (TBDMS and THP Protected) (Compound 17)



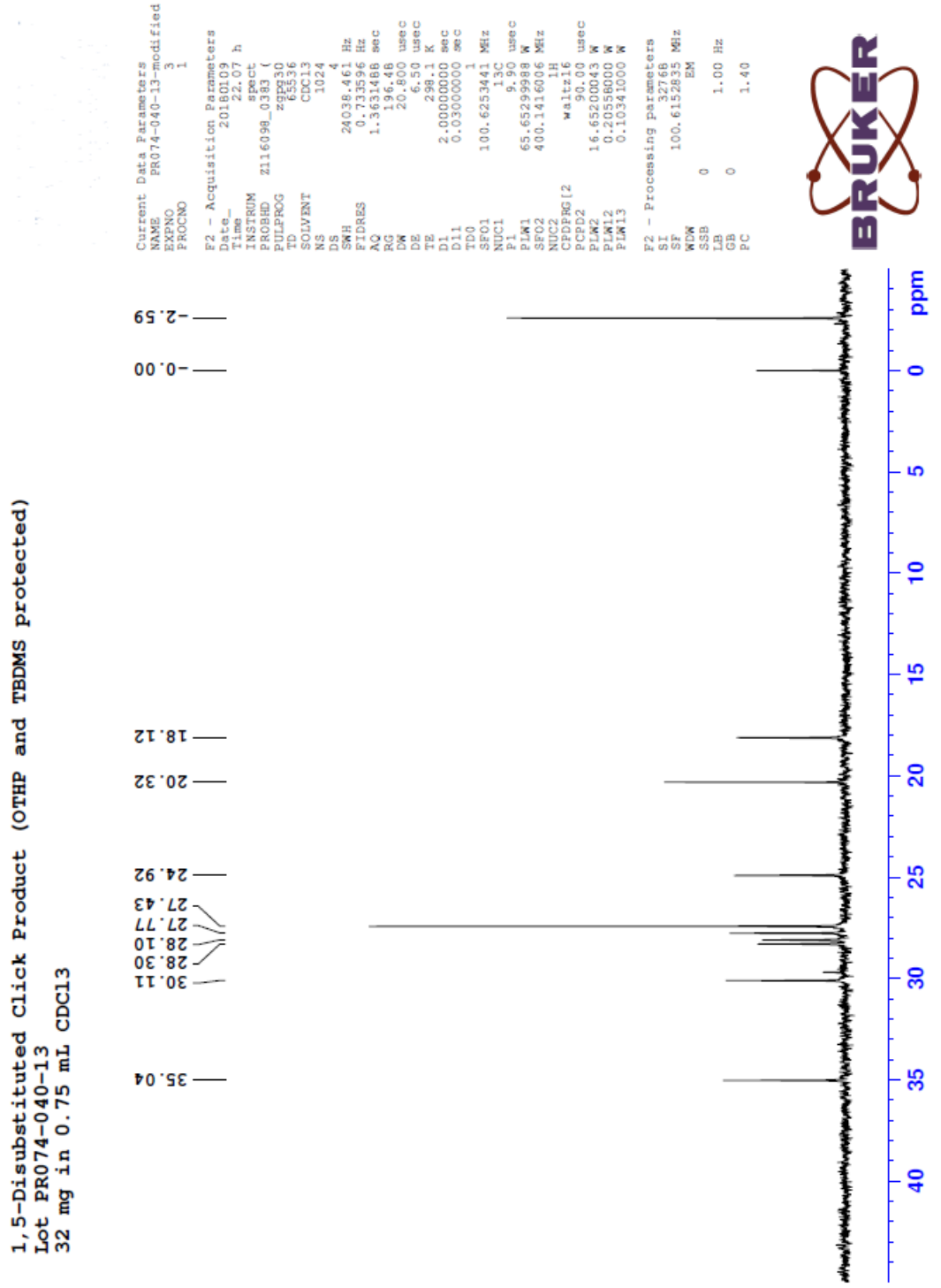
^{13}C NMR - 1,5-Disubstituted Click Product (TBDMS and THP Protected) (Compound 17)



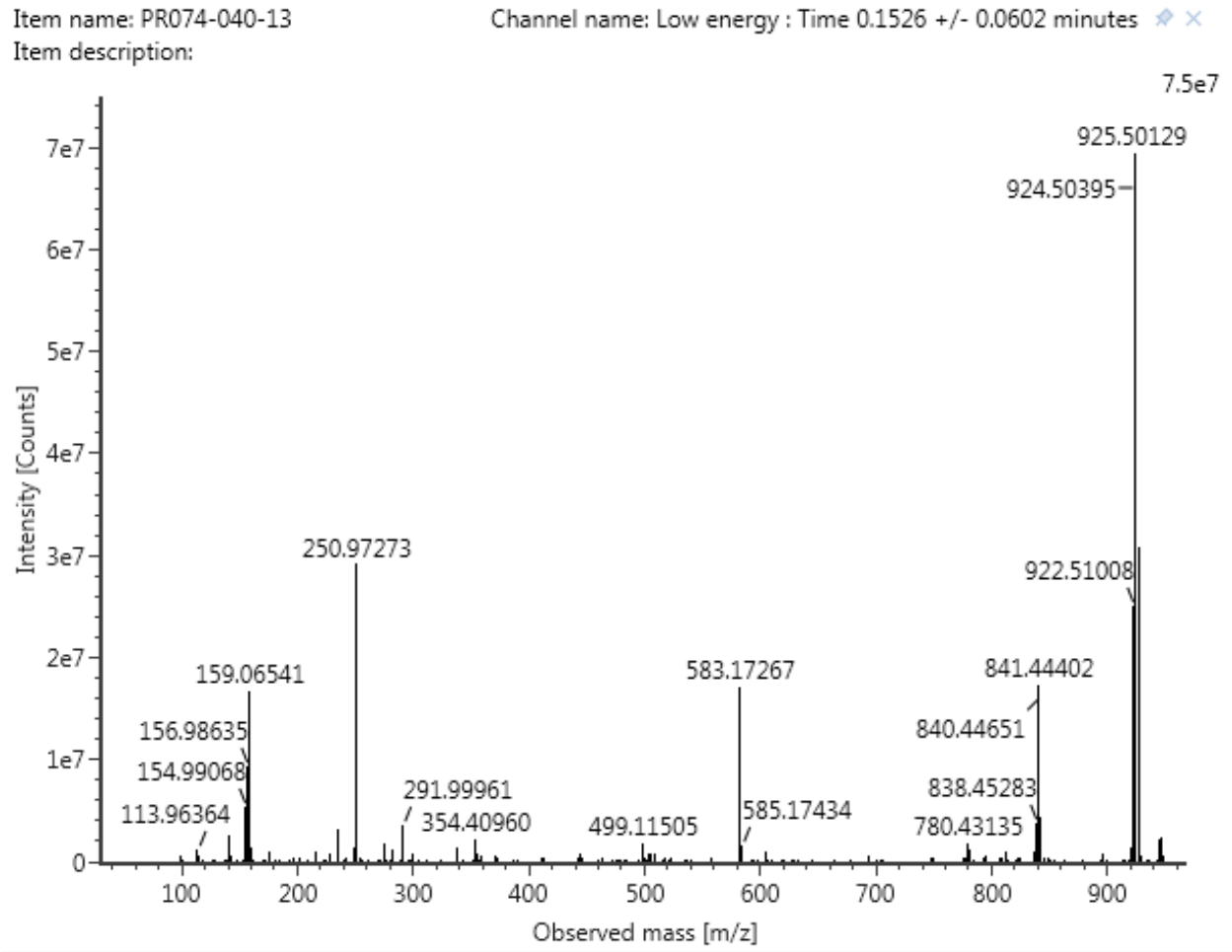
^{13}C NMR - 1,5-Disubstituted Click Product (TBDMS and THP Protected) (Compound 17)



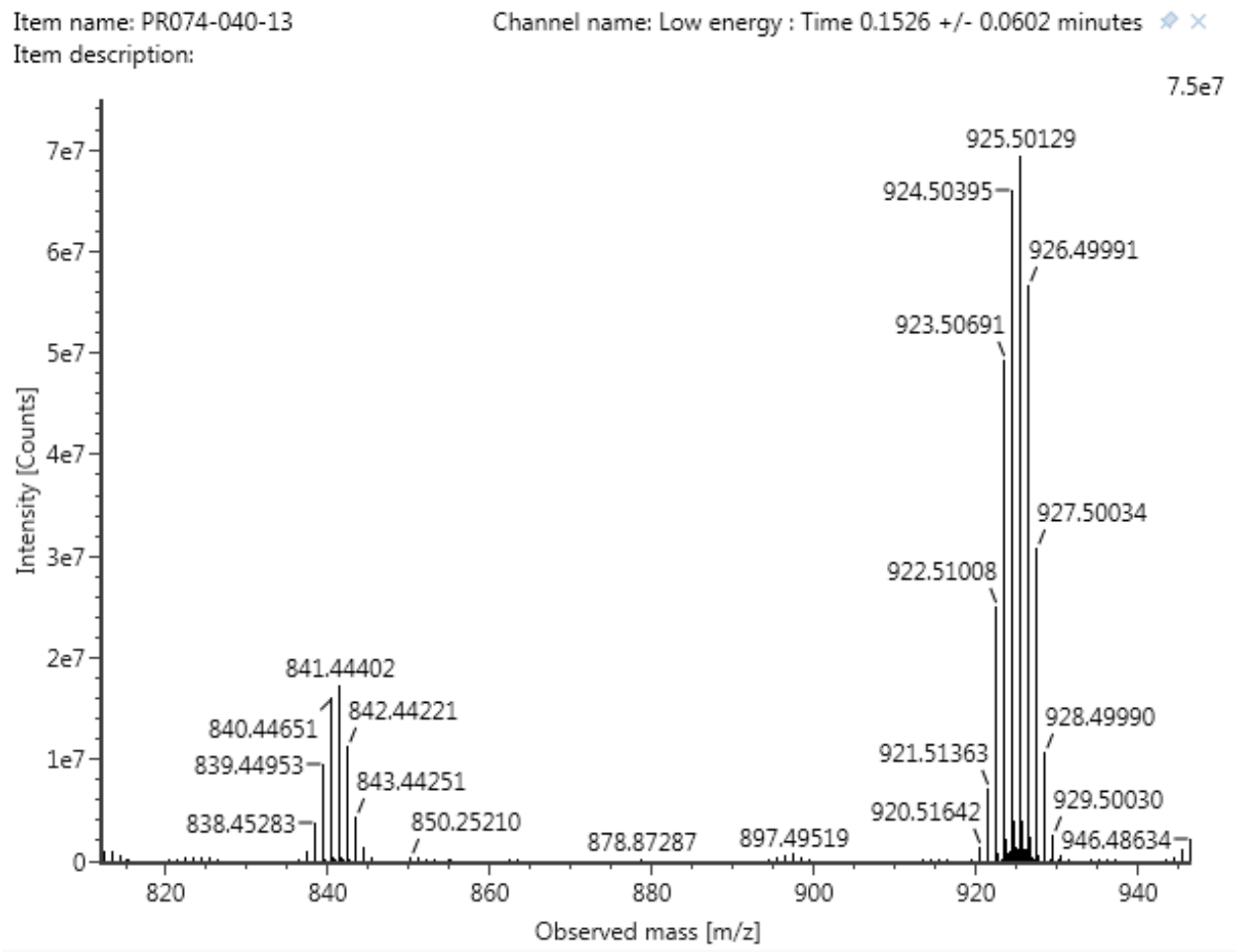
^{13}C NMR - 1,5-Disubstituted Click Product (TBDMS and THP Protected) (Compound 17)



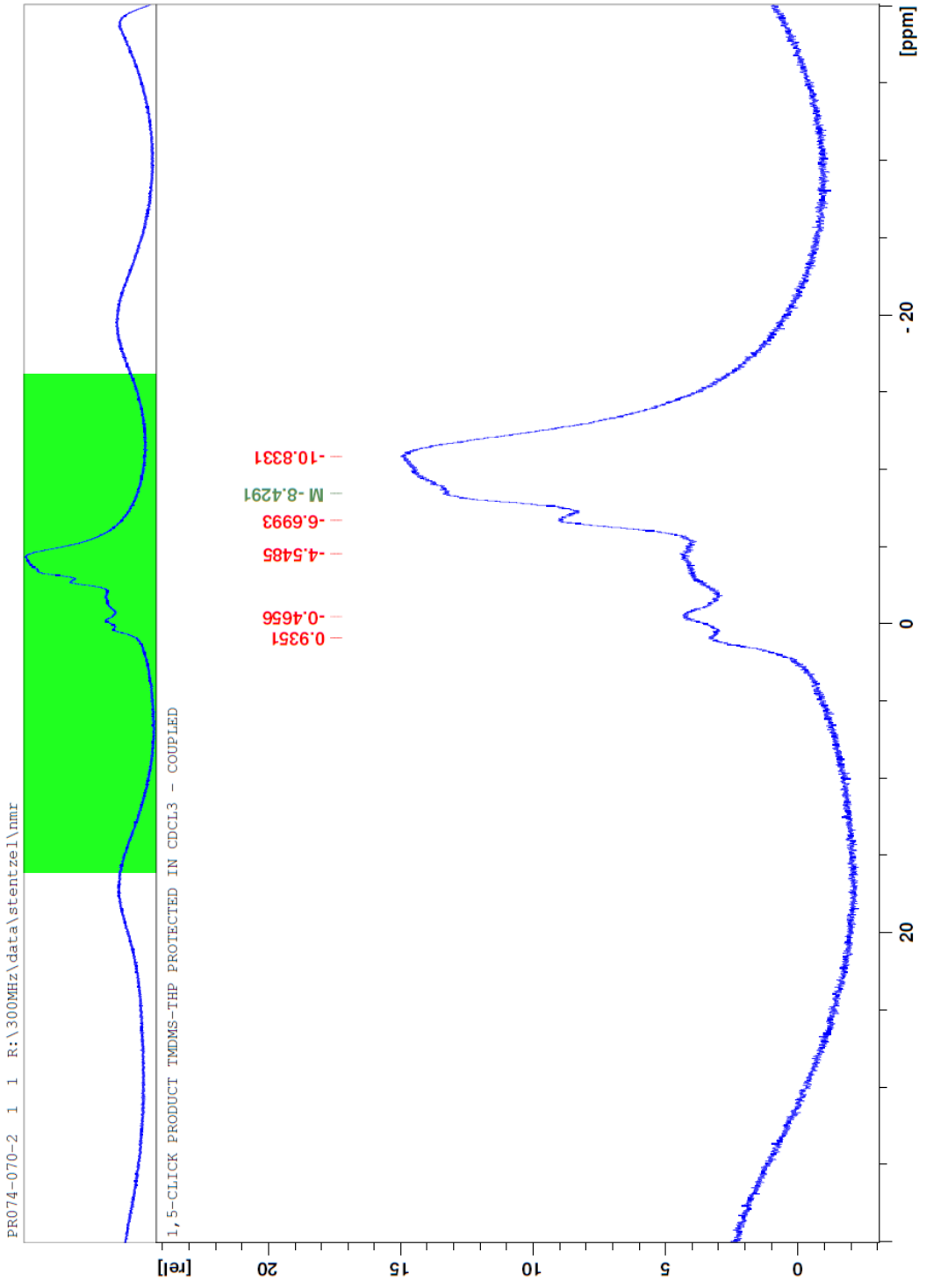
HRMS - 1,5-Disubstituted Click Product (TBDMS and THP Protected) (Compound 17)



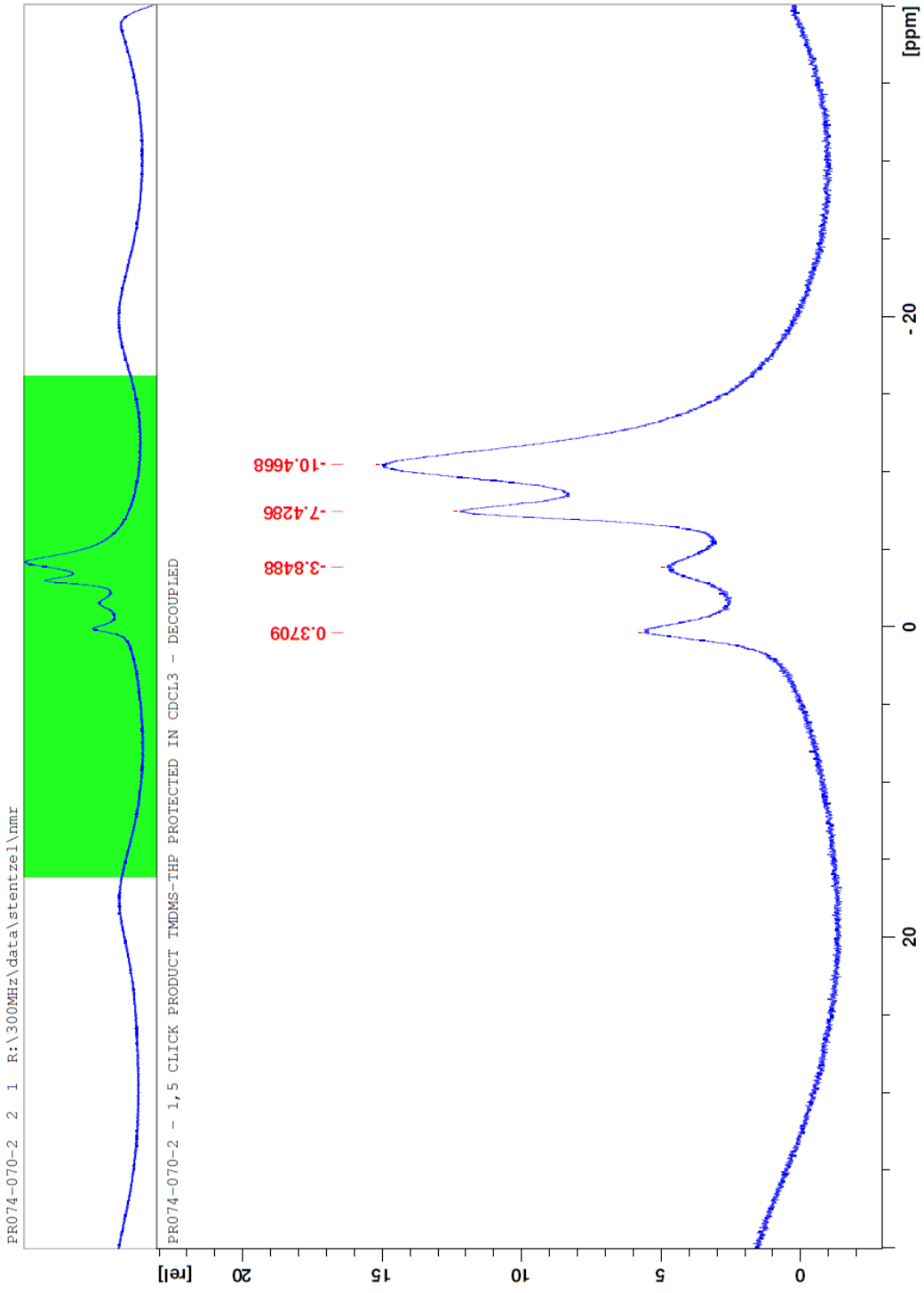
HRMS - 1,5-Disubstituted Click Product (TBDMS and THP Protected) (Compound 17)



^{11}B NMR (Coupled) - 1,5-Disubstituted Click Product (TBDMS/THP Protected) (Compound 17)



^{11}B NMR (Decoupled) - 1,5-Disubstituted Click Product (TBDMS/THP Protected) (Cmpd 17)

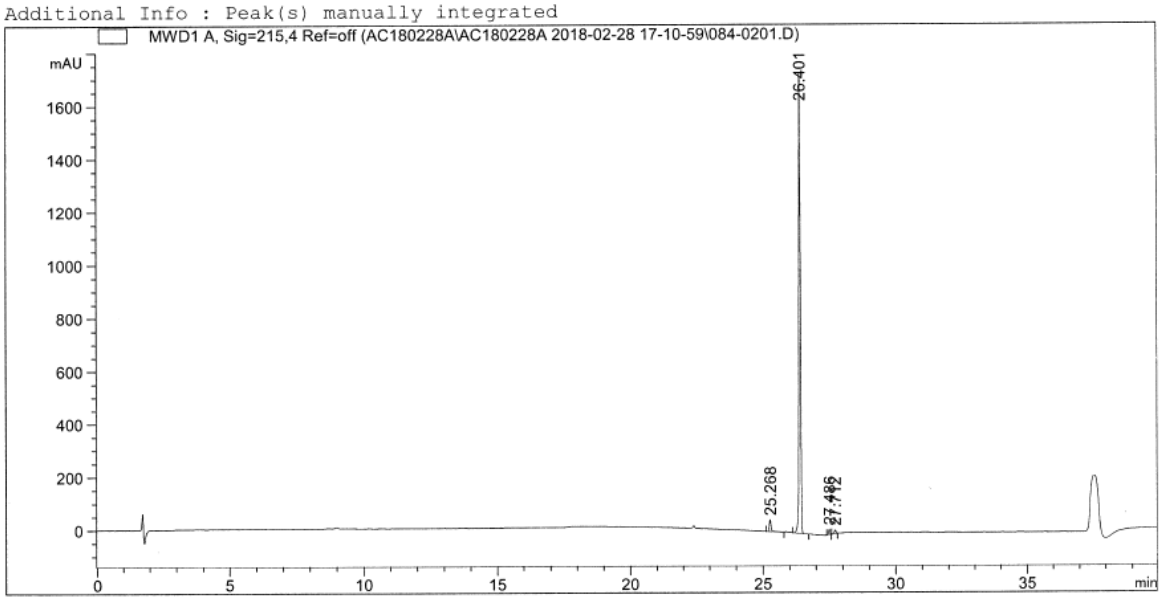


HPLC - THP MMP-18 1,5-Click Product (Compound 18)

```
======================================================================
Acq. Operator   : MRL                          Seq. Line :   2
Acq. Instrument : Instrument 1                  Location : Vial 84
Injection Date  : 02/28/18  5:54:19 PM               Inj :   1
                                               Inj Volume : 5.0 µl
Acq. Method     : C:\CHEM32\1\DATA\AC180228A\AC180228A 2018-02-28 17-10-59\BNCT-LONG2.M
Last changed    : 10/18/17  2:57:54 PM by MRL
Analysis Method : C:\CHEM32\1\METHODS\BNCT-LONG2.M
Last changed    : 03/03/18  4:51:44 PM by MRL
                  (modified after loading)
Method Info     : HPLC Method for Monitoring and Assaying BNCT products and reactions
                  HPLC 12 (Lab 2)
                  A = 0.10% TFA in water
                  B = 0.10% TFA in ACN
                  Waters Sunfire C8, 4.6x150 mm, 3.5 micron
                  Flow: 1.0 mL/min, Column Temp: 40 C

Sample Info     : 1,5 Click Product post deprotection with TBAF and aq workup
                  Lot# PR074-080-2
                  Conc. 1 mg/mL in ACN

Additional Info : Peak(s) manually integrated
```



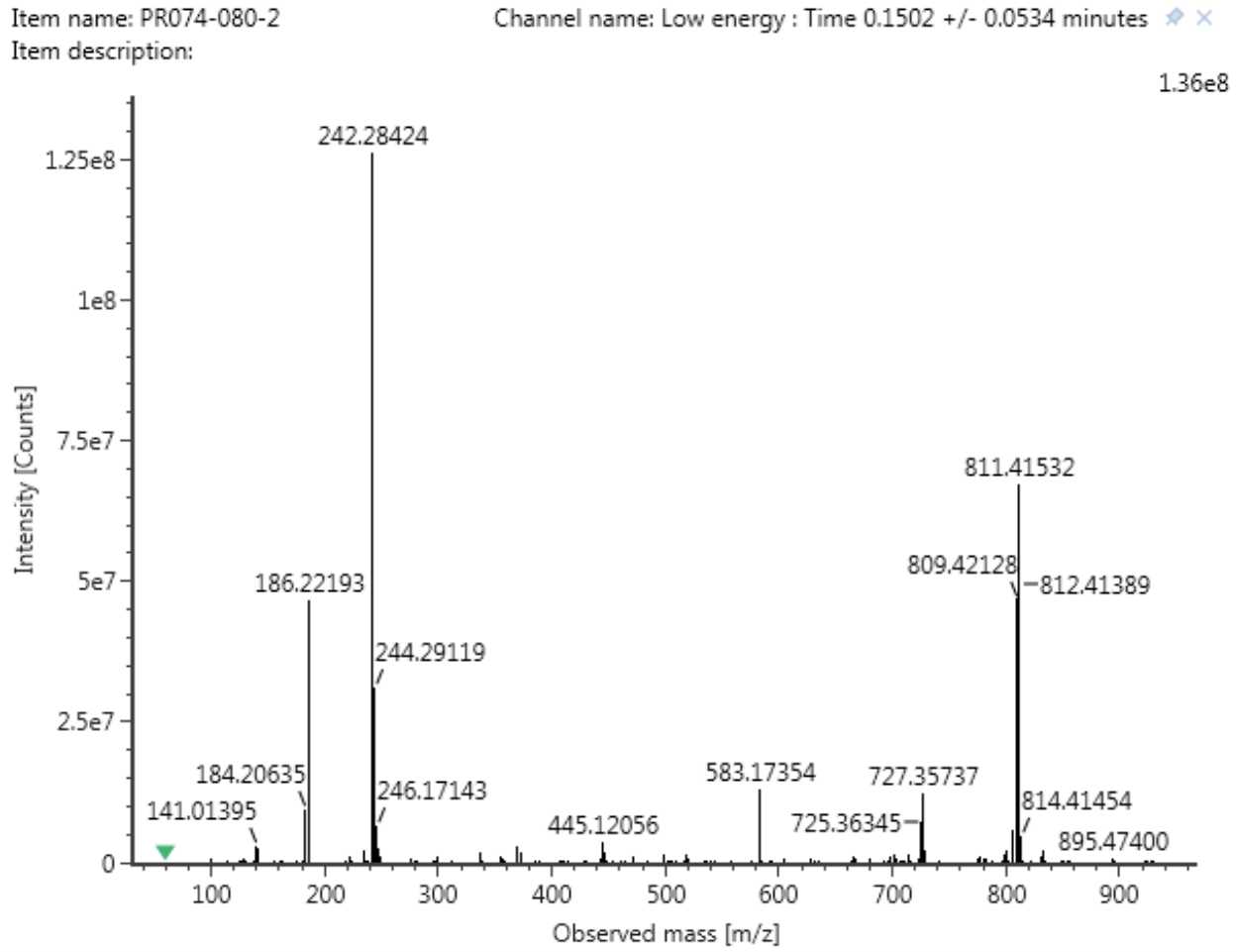

```
======================================================================
                        Area Percent Report
======================================================================

Signal 1: MWD1 A, Sig=215,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  25.268 BV    0.0697  205.69513   44.66614   2.8740
   2  26.401 BB    0.0620 6814.97900 1727.22119  95.2192
   3  27.486 BB    0.0595   78.36858   20.99575   1.0950
   4  27.712 BB    0.0703   58.10604   13.47774   0.8119

Totals :                  7157.14875 1806.36082
======================================================================
```

HRMS - THP MMP-18 1,5-Click Product (Compound 18)

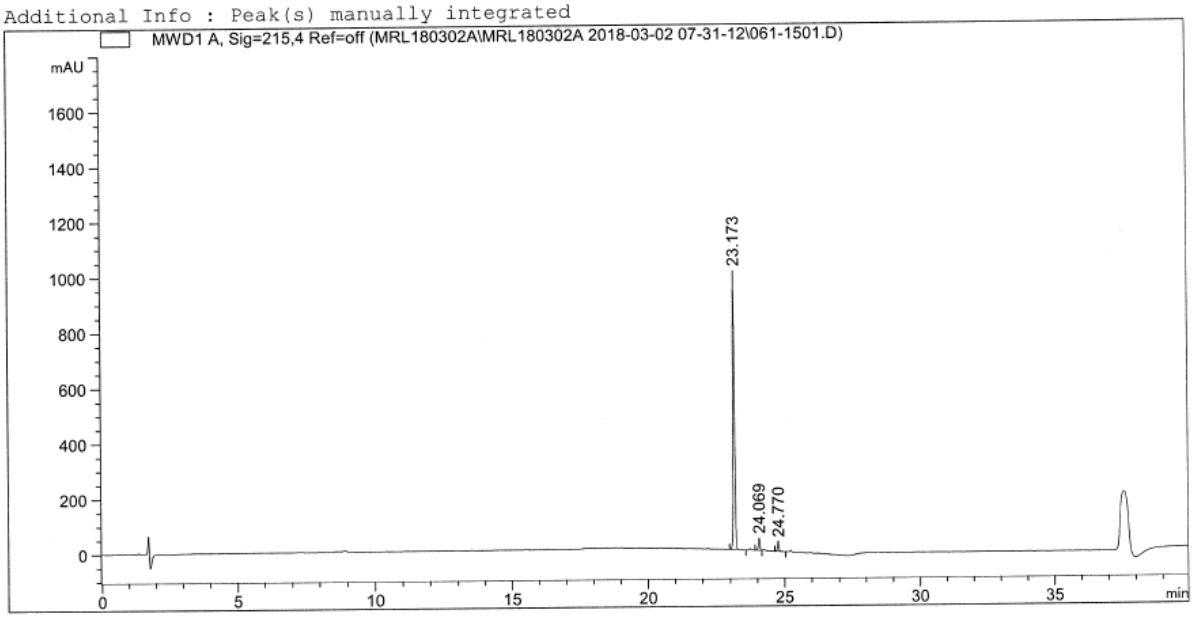


HPLC - 1,5-Click Carboranyl MMP-18 HCl Salt (Compound 19)

```
======================================================================
Acq. Operator   : MRL                          Seq. Line :  15
Acq. Instrument : Instrument 1                  Location : Vial 61
Injection Date  : 03/02/18  4:15:14 PM              Inj :   1
                                               Inj Volume : 5.0 µl
Acq. Method     : C:\CHEM32\1\DATA\MRL180302A\MRL180302A 2018-03-02 07-31-12\BNCT-LONG2.M
Last changed    : 10/18/17  2:57:54 PM by MRL
Analysis Method : C:\CHEM32\1\METHODS\BNCT-LONG2.M
Last changed    : 03/03/18  4:51:44 PM by MRL
                  (modified after loading)
Method Info     : HPLC Method for Monitoring and Assaying BNCT products and reactions
                  HPLC 12 (Lab 2)
                  A = 0.10% TFA in water
                  B = 0.10% TFA in ACN
                  Waters Sunfire C8, 4.6x150 mm, 3.5 micron
                  Flow: 1.0 mL/min, Column Temp: 40 C

Sample Info     : 1,5-Click MMP-18 Carboranyl HCl Salt
                  Lot PR074-088-2
                  1 mg/mL in ACN

Additional Info : Peak(s) manually integrated
```

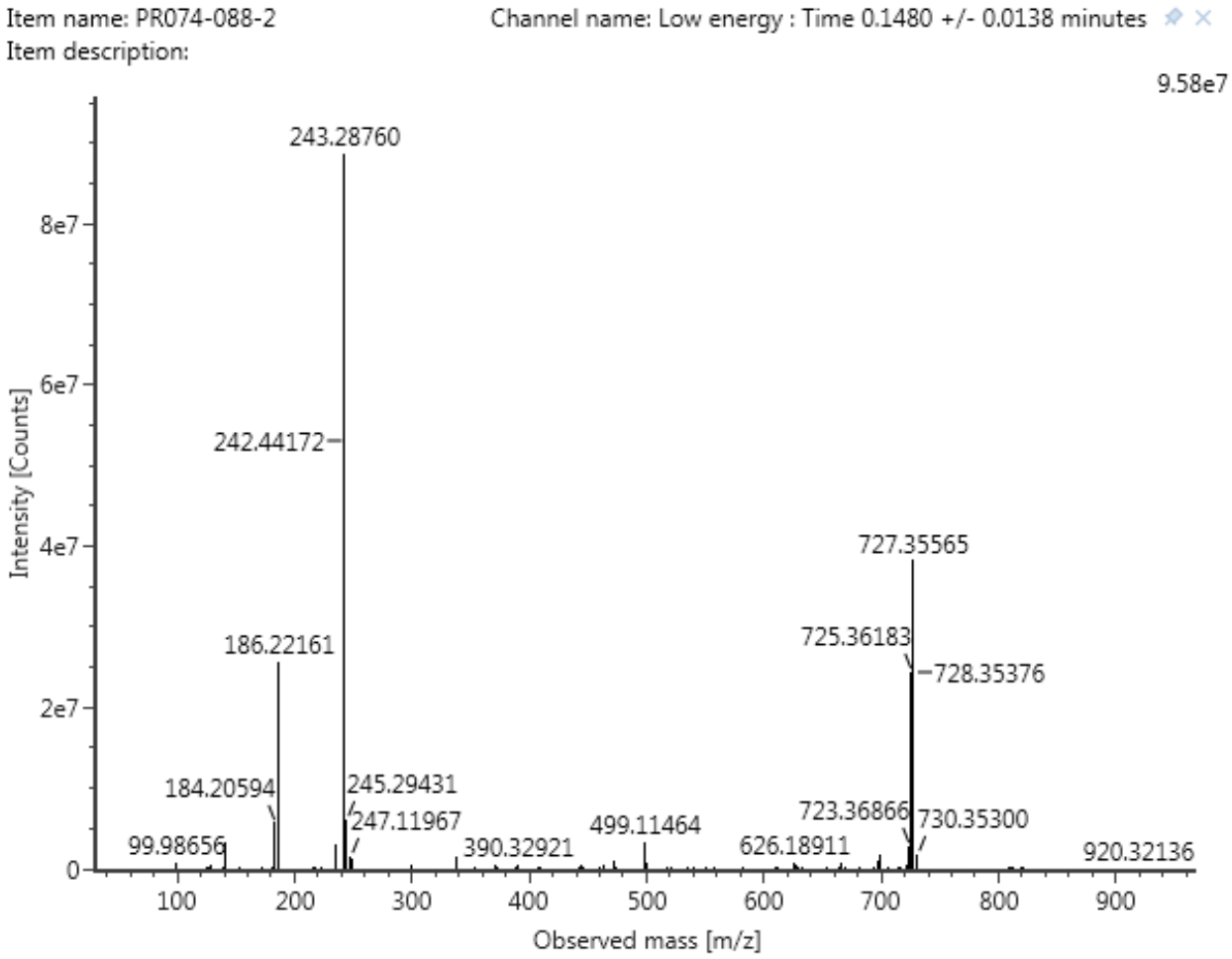



```
======================================================================
                        Area Percent Report
======================================================================
Signal 1: MWD1 A, Sig=215,4 Ref=off

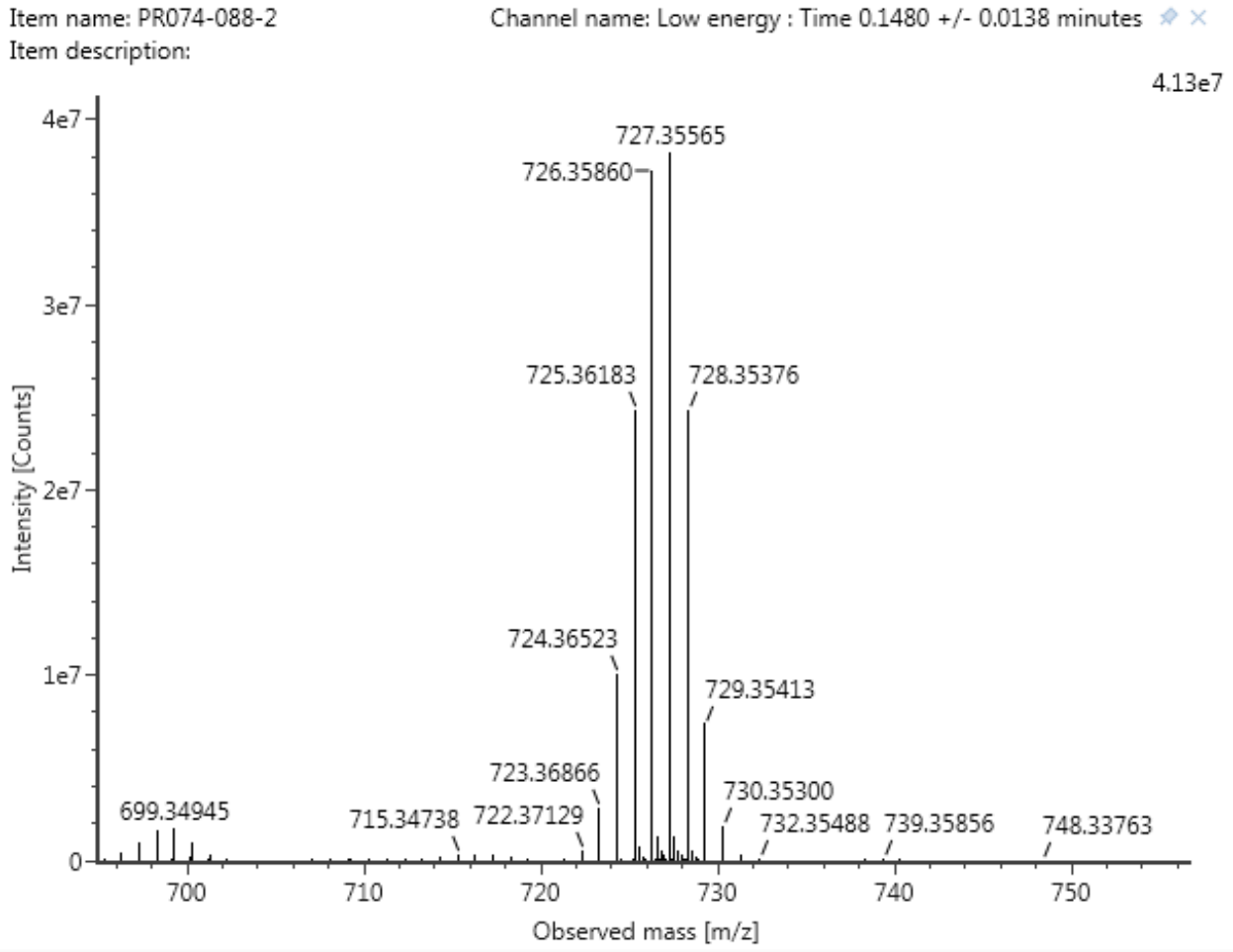
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  23.173 BB    0.0657 3943.21484 1007.29846  92.1925
   2  24.069 BV    0.0654  190.88611   45.06153   4.4629
   3  24.770 BB    0.0612  143.05429   36.93990   3.3446

Totals :                  4277.15524 1089.29989
======================================================================
```

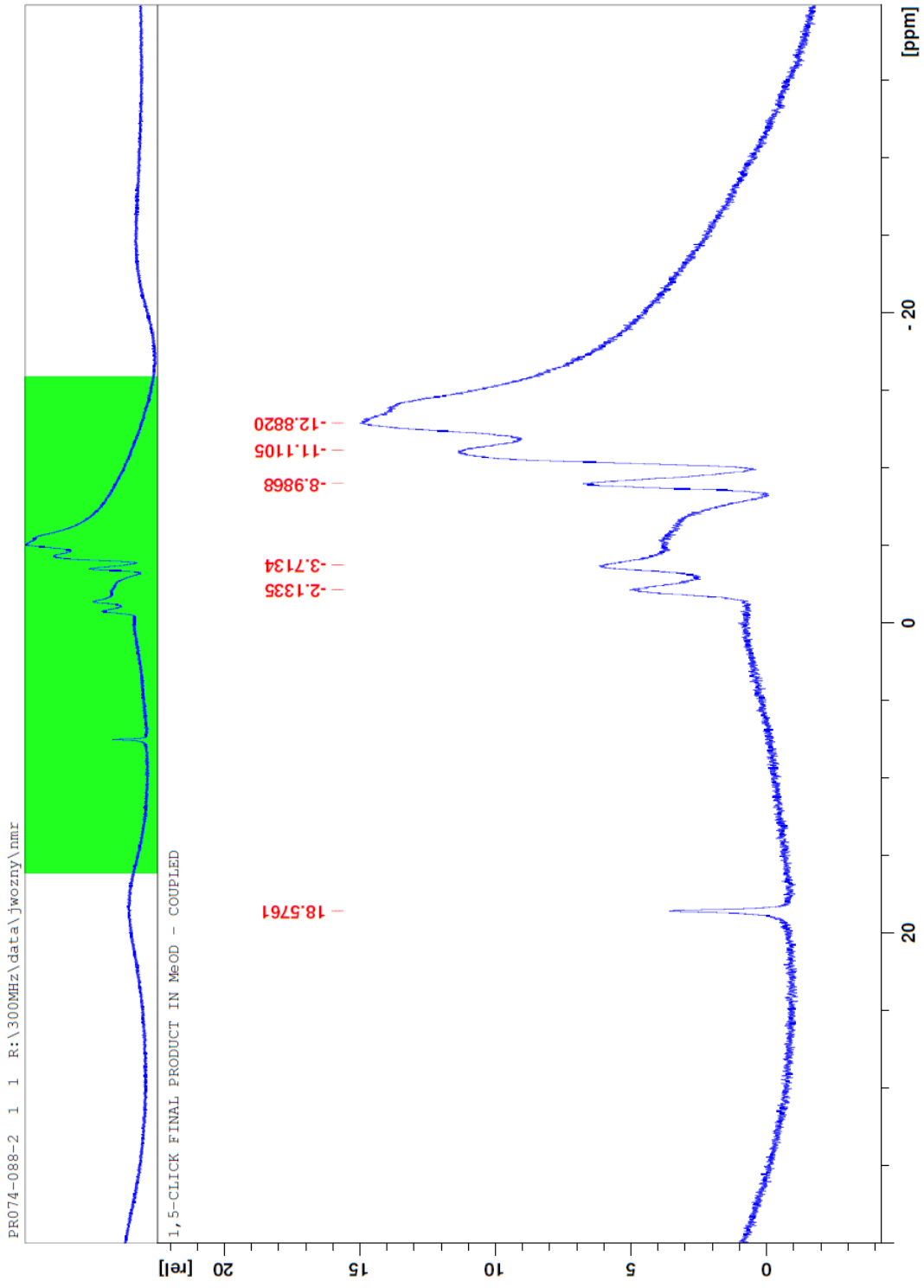
HRMS - 1,5-Click Carboranyl MMP-18 HCl Salt (Compound 19)



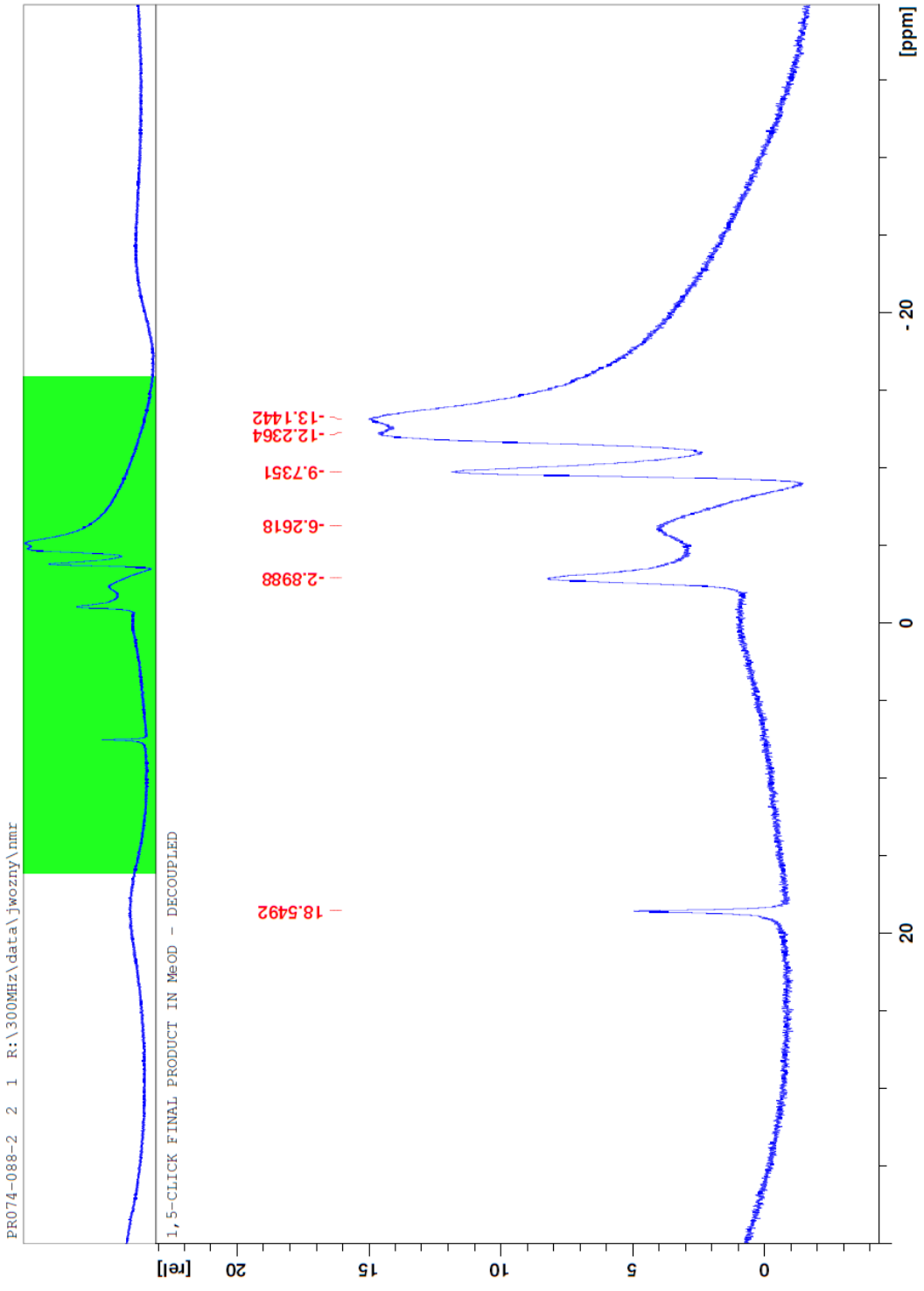
HRMS - 1,5-Click Carboranyl MMP-18 HCl Salt (Compound 19)



^{11}B NMR (Coupled) - 1,5-Click Carboranyl MMP-18 HCl Salt (Compound 19)



^{11}B NMR (Decoupled) - 1,5-Click Carboranyl MMP-18 HCl Salt (Compound 19)

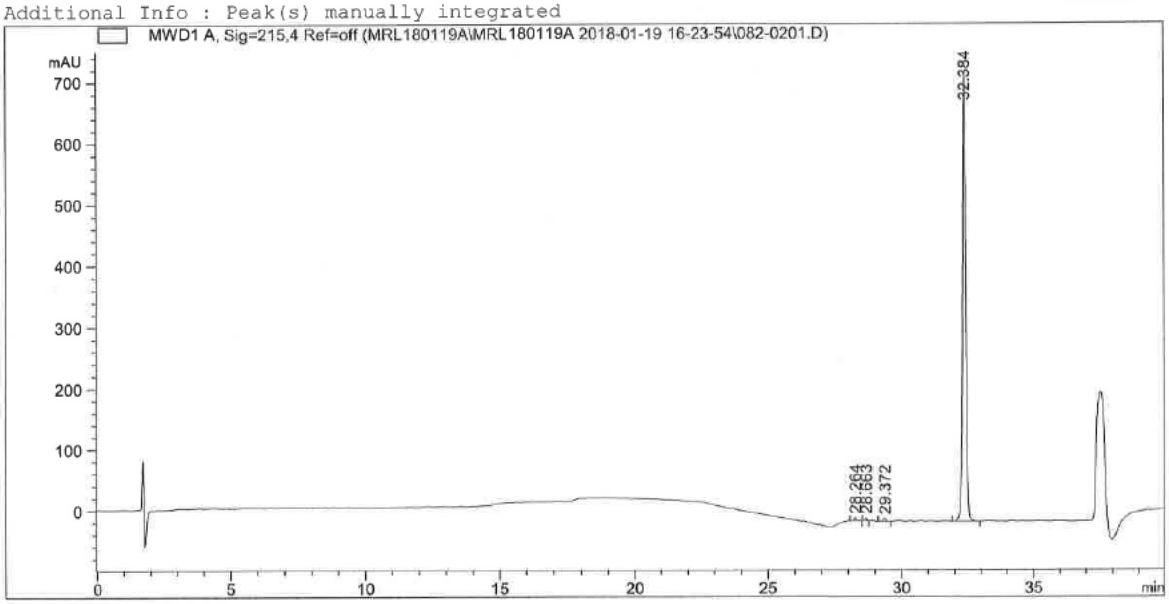


HPLC - Carboranyl MMP-18 Ethyl Ester (Compound 20)

```
Acq. Operator    : MRL                                  Seq. Line :   2
Acq. Instrument  : Instrument 1                          Location : Vial 82
Injection Date   : 01/19/18  5:07:34 PM                      Inj :   1
                                                       Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\1\DATA\MRL180119A\MRL180119A 2018-01-19 16-23-54\BNCT-LONG2.M
Last changed     : 10/18/17  2:57:54 PM by MRL
Analysis Method  : C:\CHEM32\1\METHODS\BNCT-LONG2.M
Last changed     : 01/19/18  9:19:02 AM by MRL
                   (modified after loading)
Method Info      : HPLC Method for Monitoring and Assaying BNCT products and reactions
                   HPLC 12 (Lab 2)
                   A = 0.10% TFA in water
                   B = 0.10% TFA in ACN
                   Waters Sunfire C8, 4.6x150 mm, 3.5 micron
                   Flow: 1.0 mL/min, Column Temp: 40 C

Sample Info      : Isolated Carboranyl MMP-18 Ethyl Ester
                   PR074-057-3, 1 mg/mL in ACN

Additional Info : Peak(s) manually integrated
```

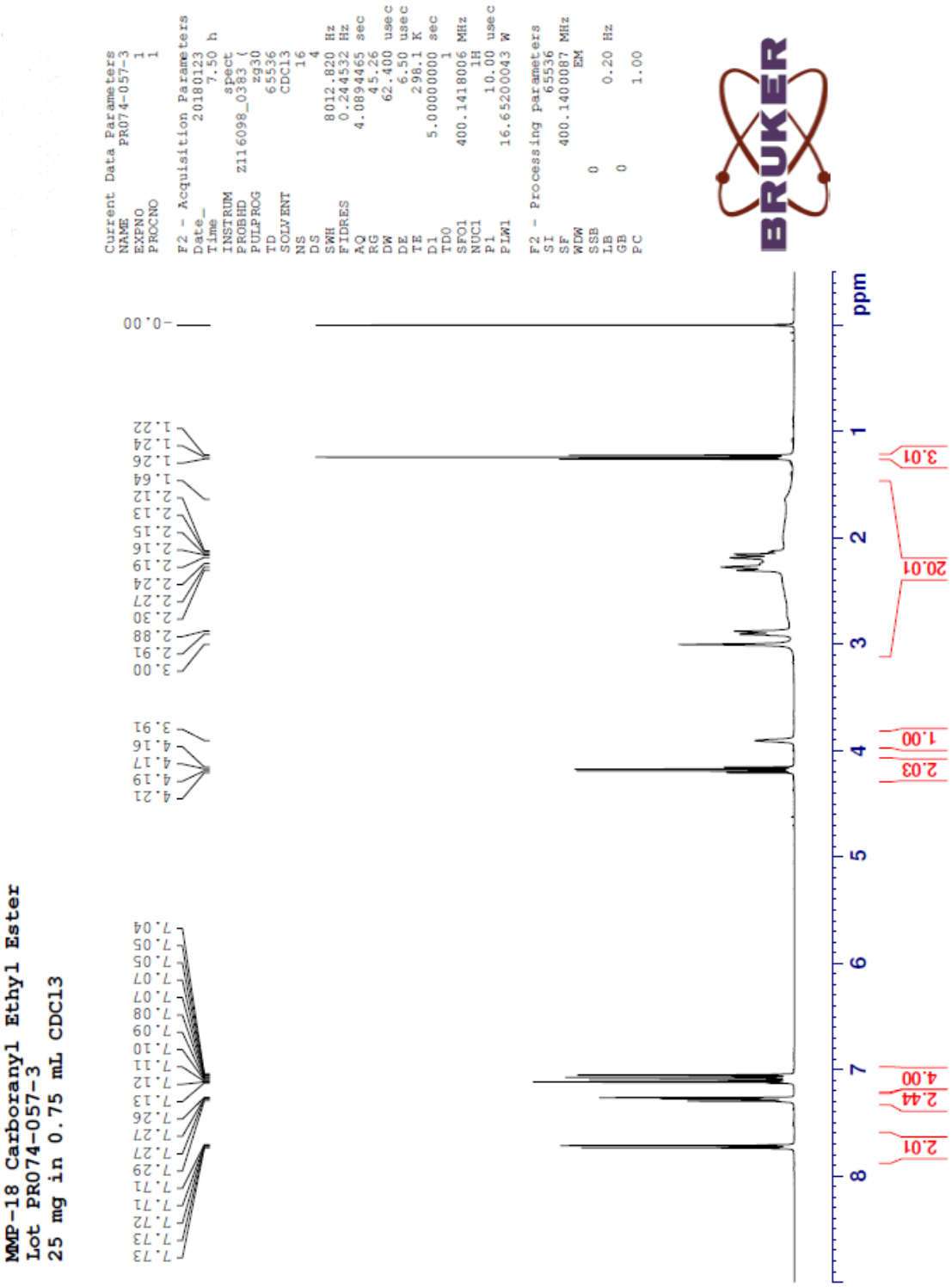


Area Percent Report

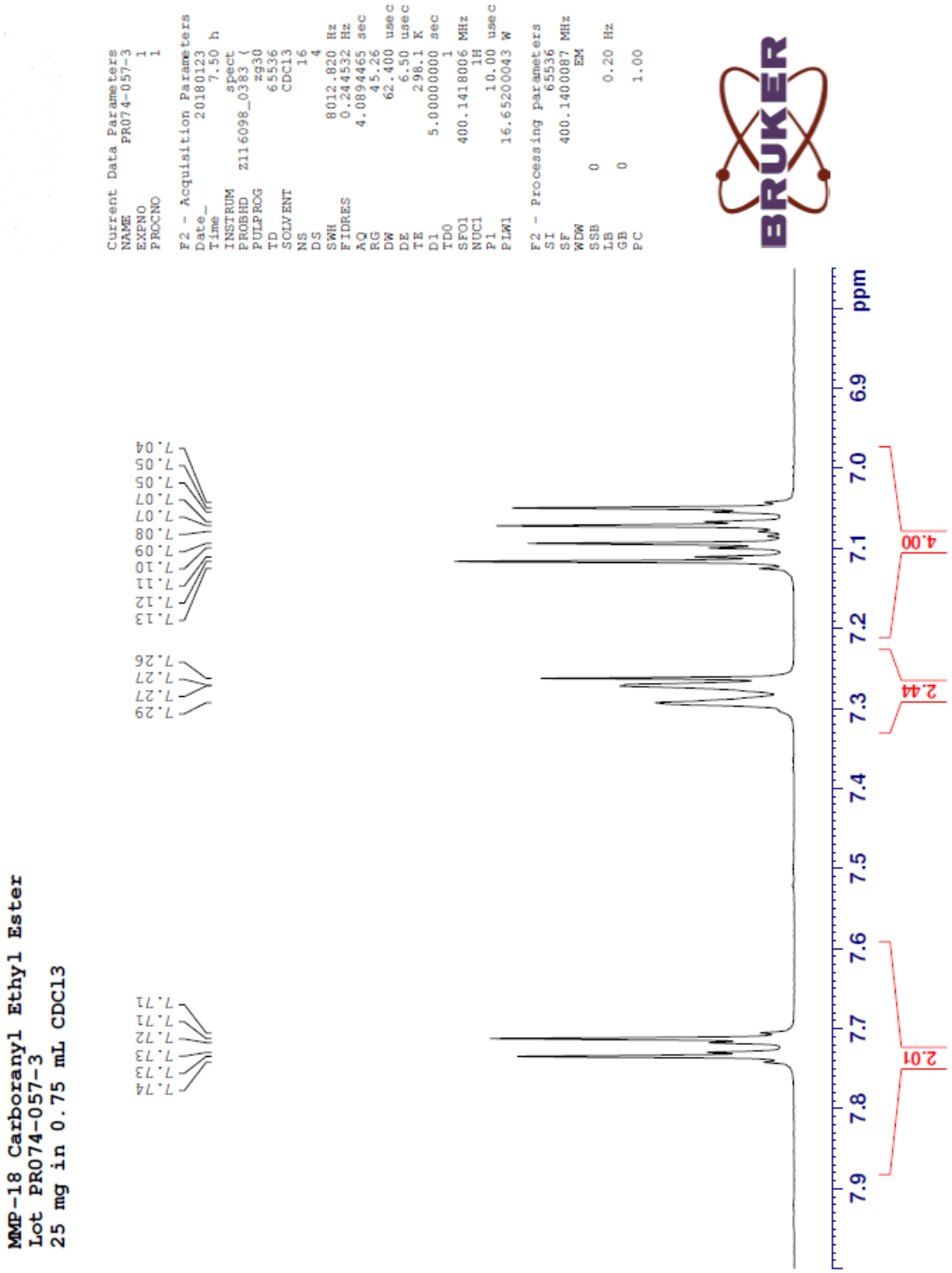
Signal 1: MWD1 A, Sig=215,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	28.264	BB	0.0889	31.37245	5.30928	0.4801
2	28.663	BB	0.0792	23.56598	4.64365	0.3607
3	29.372	BB	0.0963	31.66947	4.83967	0.4847
4	32.384	BB	0.1351	6447.33887	729.33942	98.6745
Totals :				6533.94678	744.13202	

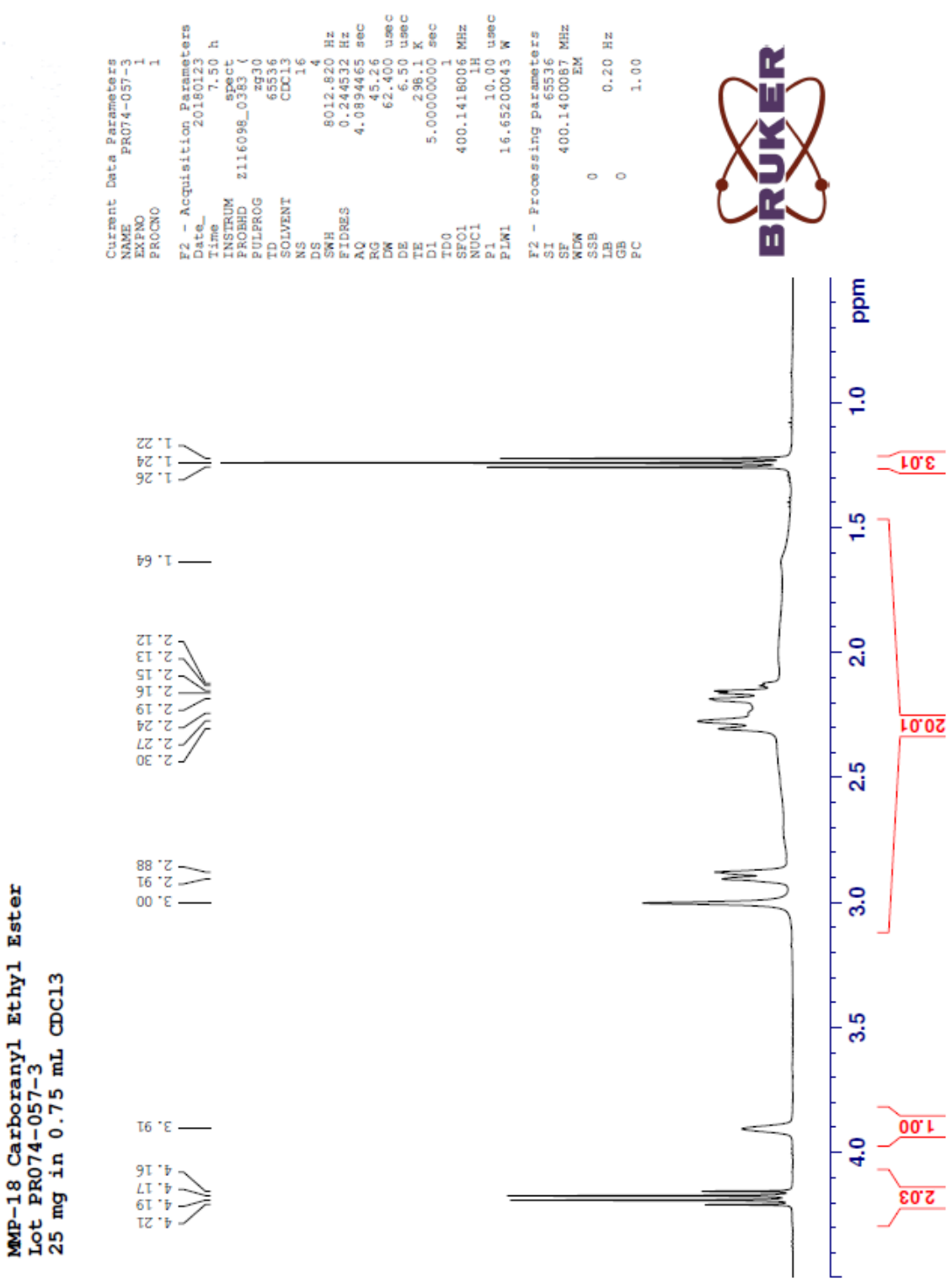
^{1}H NMR - Carboranyl MMP-18 Ethyl Ester (Compound 20)



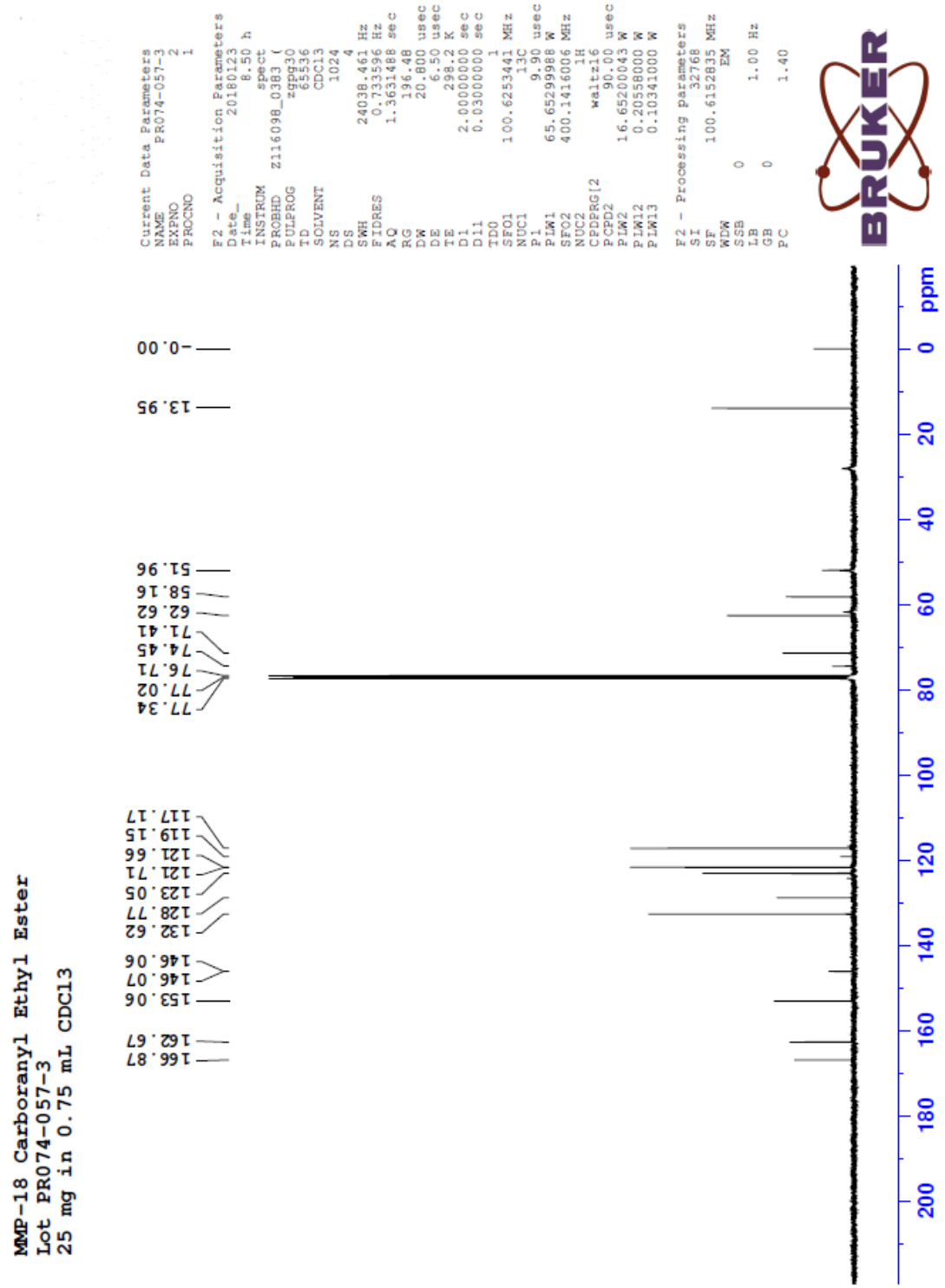
^{1}H NMR - Carboranyl MMP-18 Ethyl Ester (Compound 20)



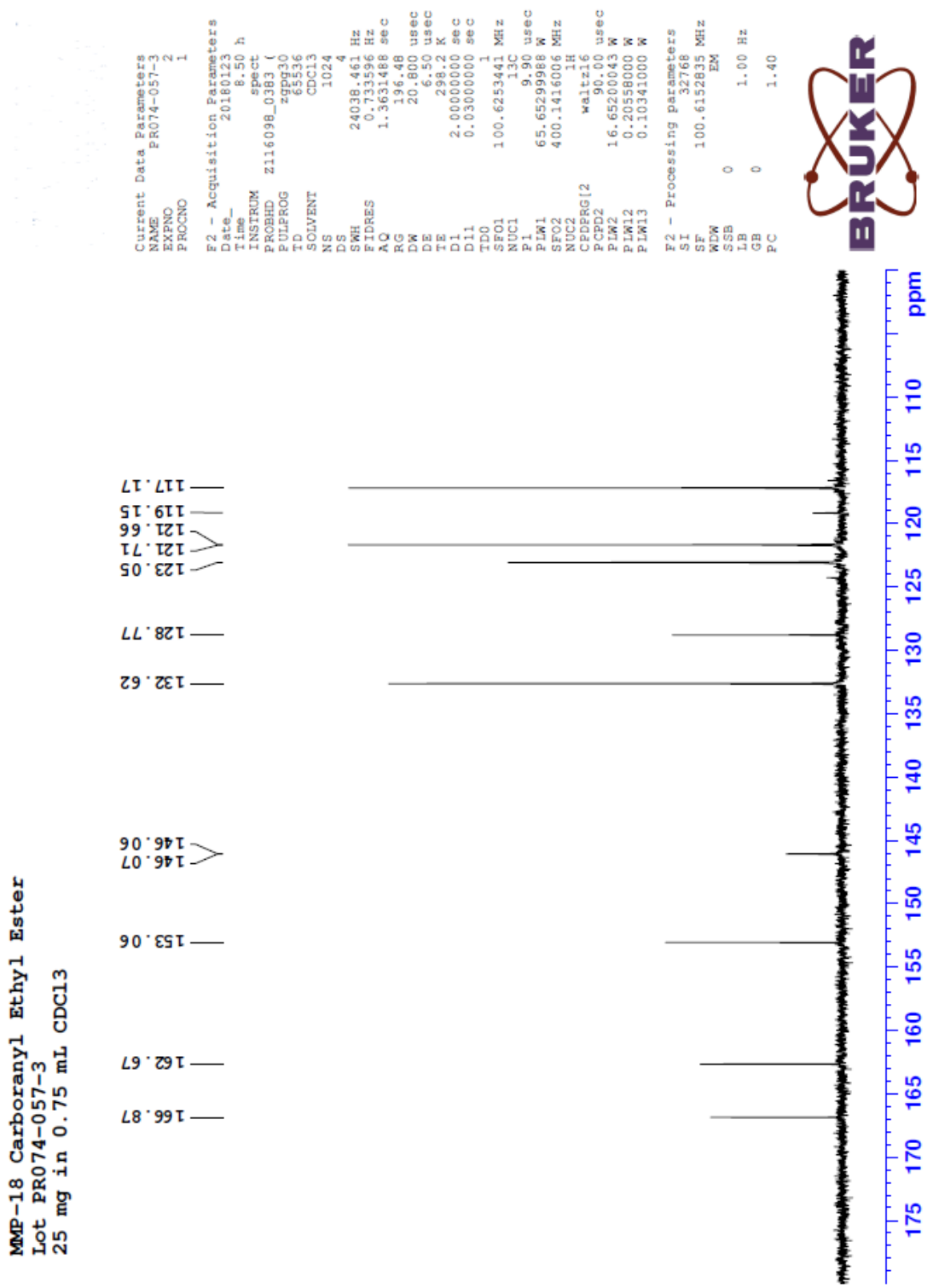
^{1}H NMR - Carboranyl MMP-18 Ethyl Ester (Compound 20)



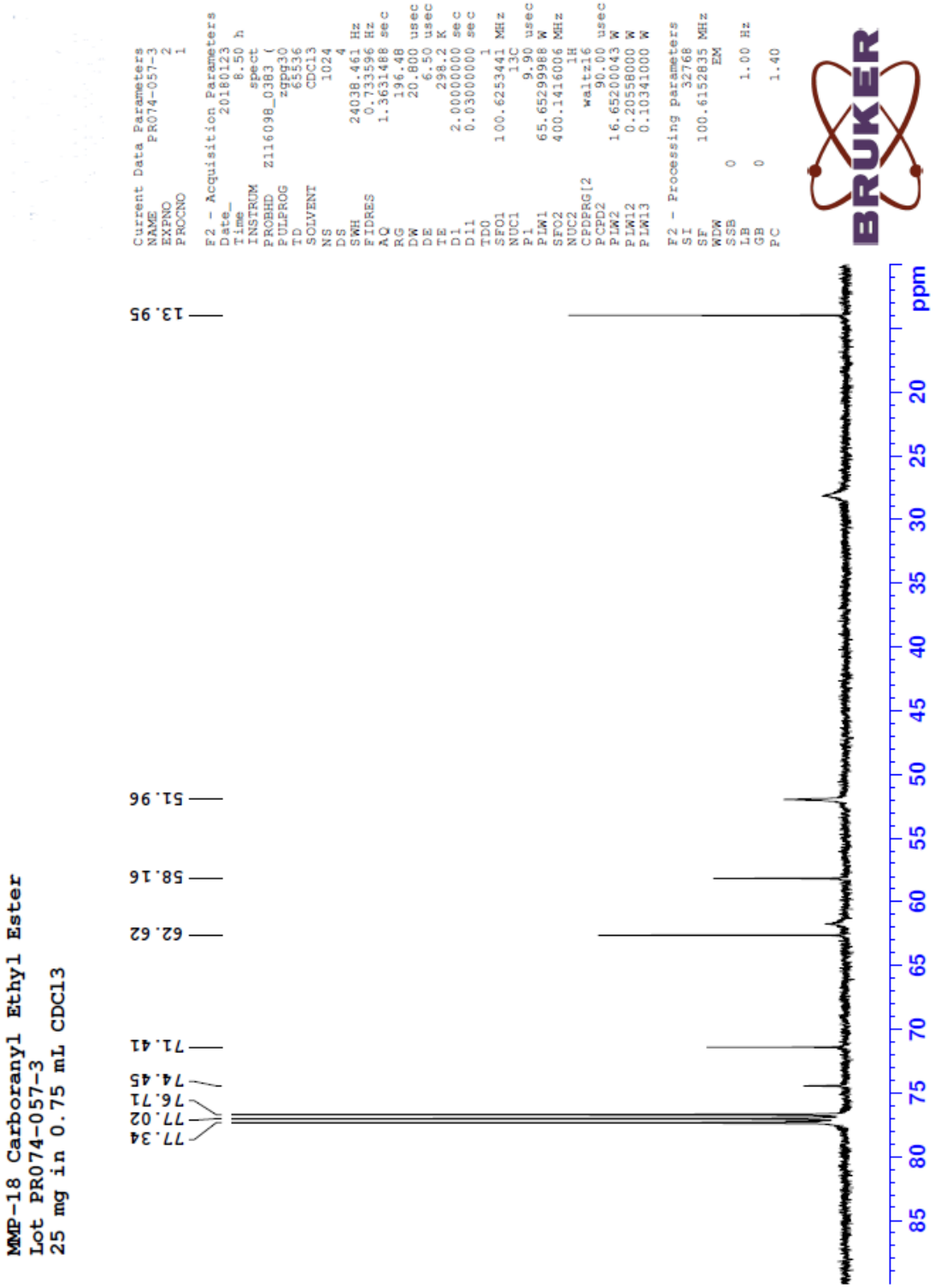
^{13}C NMR - Carboranyl MMP-18 Ethyl Ester (Compound 20)



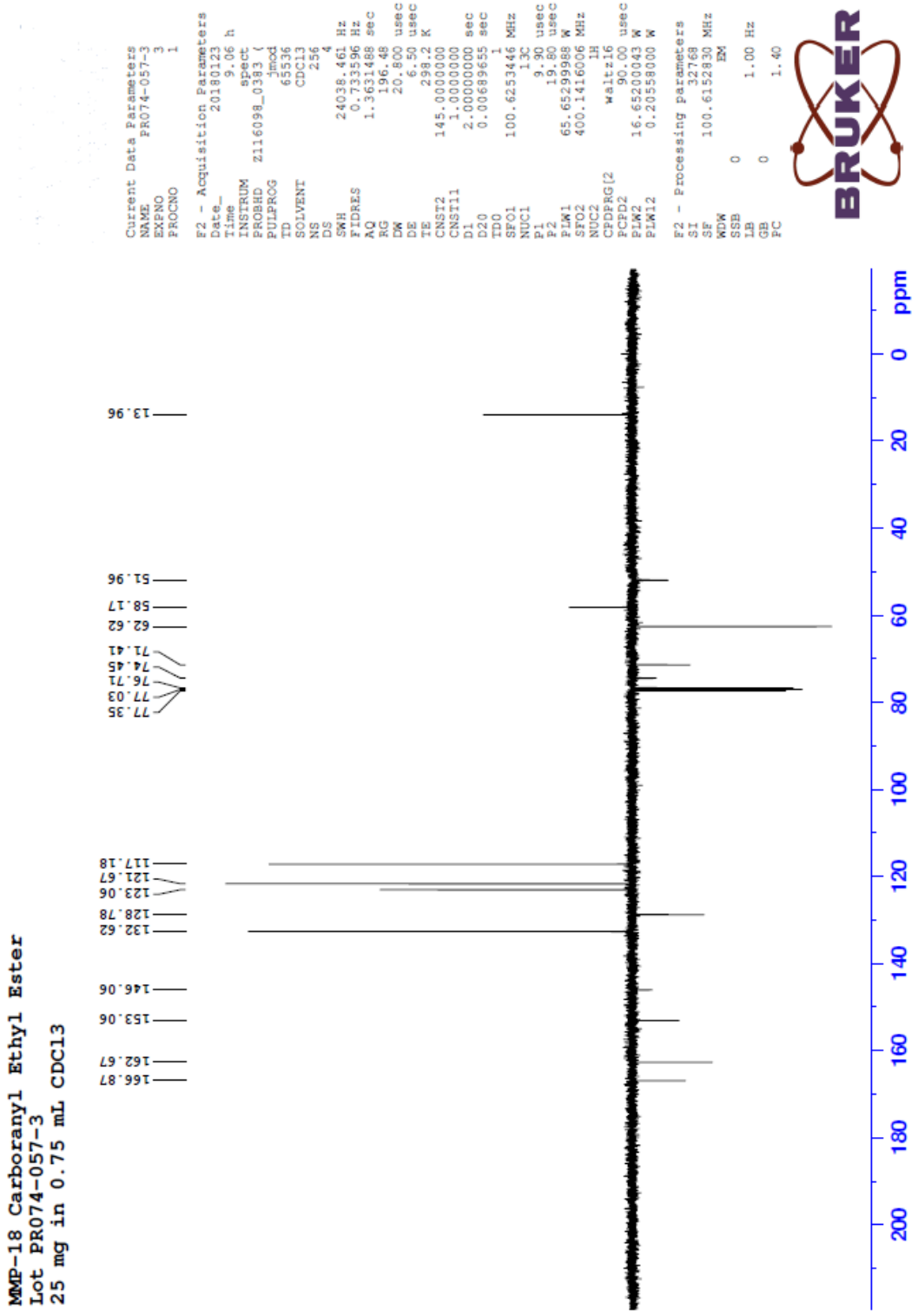
^{13}C NMR - Carboranyl MMP-18 Ethyl Ester (Compound 20)



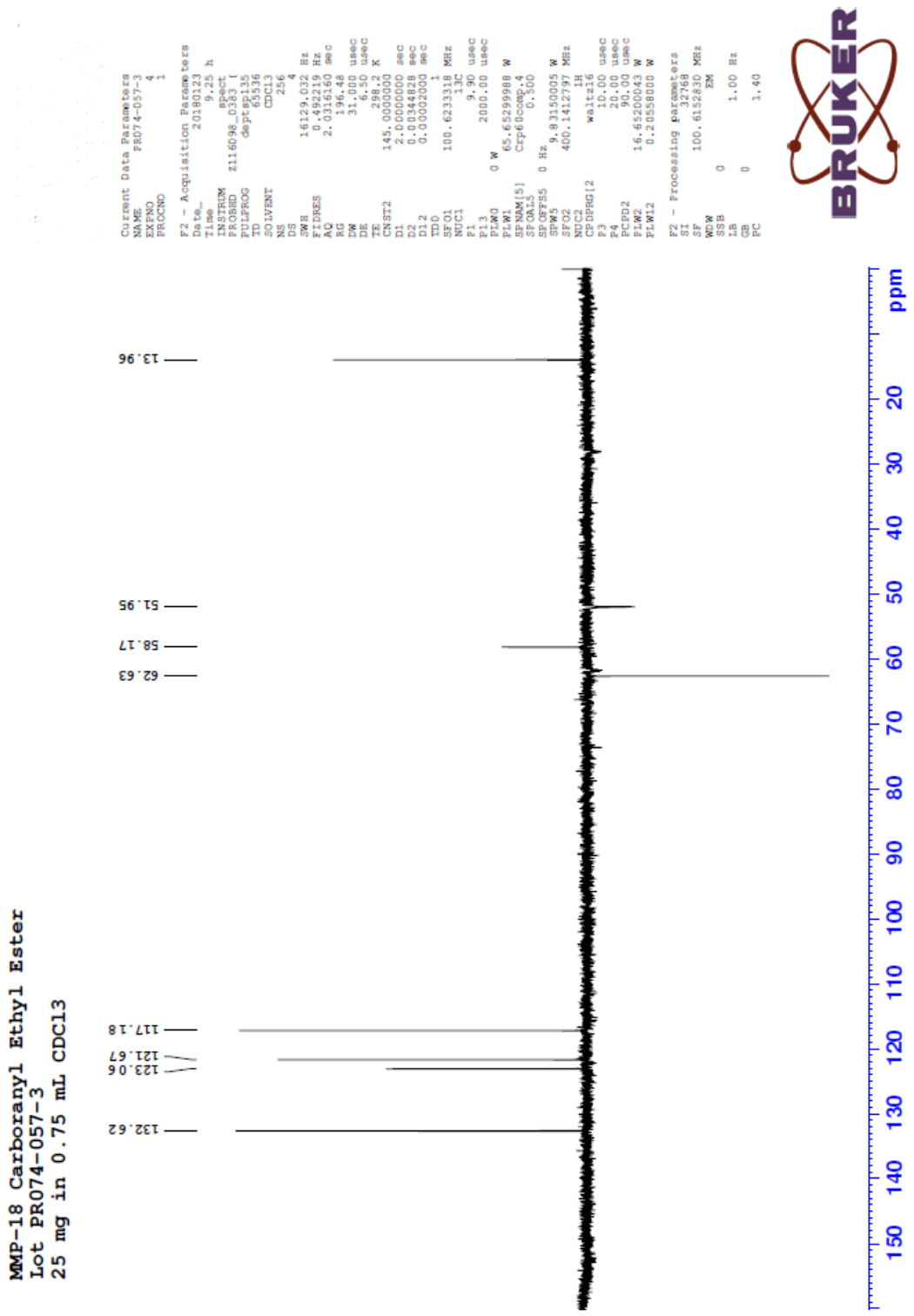
^{13}C NMR - Carboranyl MMP-18 Ethyl Ester (Compound 20)



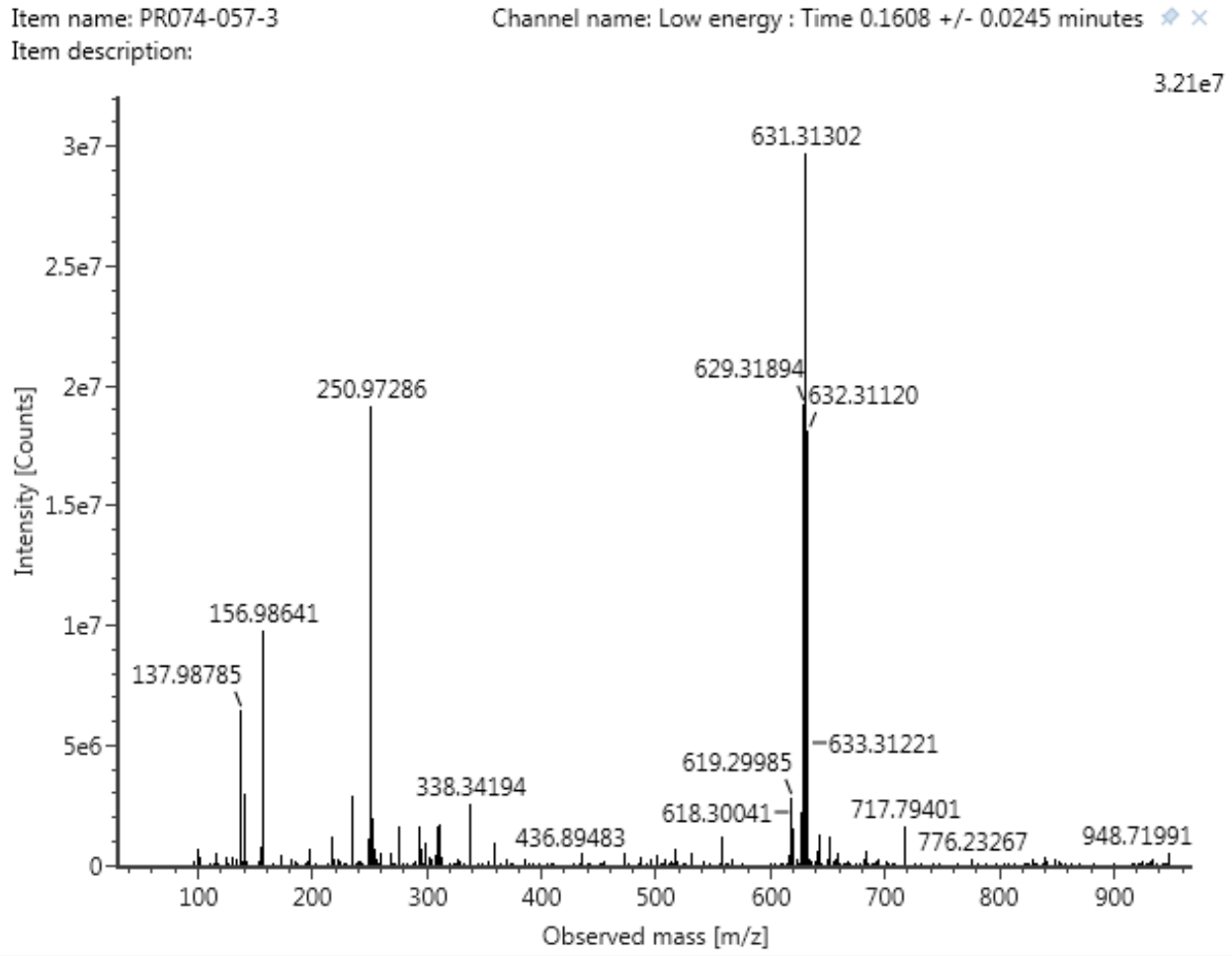
^{13}C APT NMR - Carboranyl MMP-18 Ethyl Ester (Compound 20)



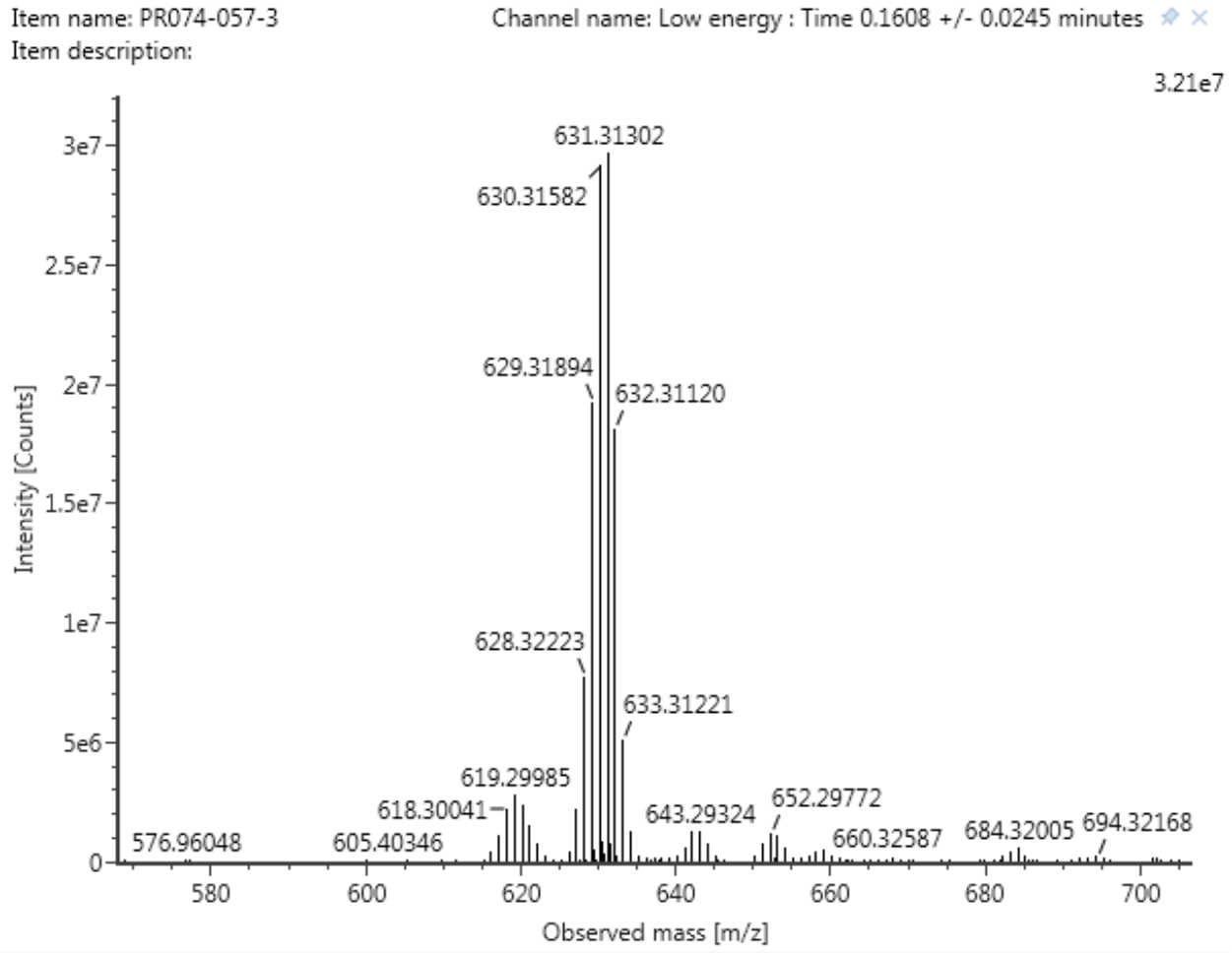
^{13}C DEPT135 NMR - Carboranyl MMP-18 Ethyl Ester (Compound 20)



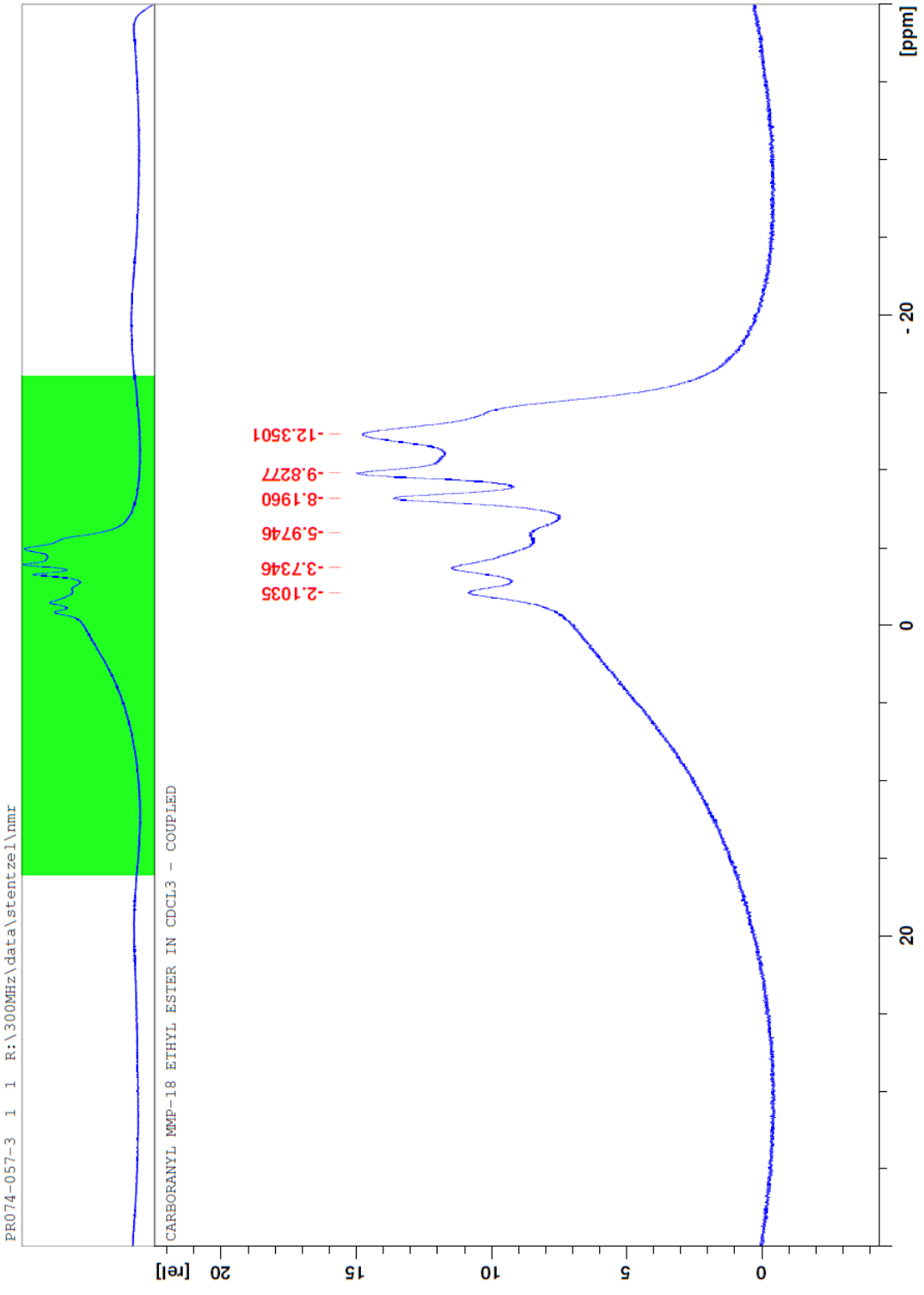
HRMS - Carboranyl MMP-18 Ethyl Ester (Compound 20)



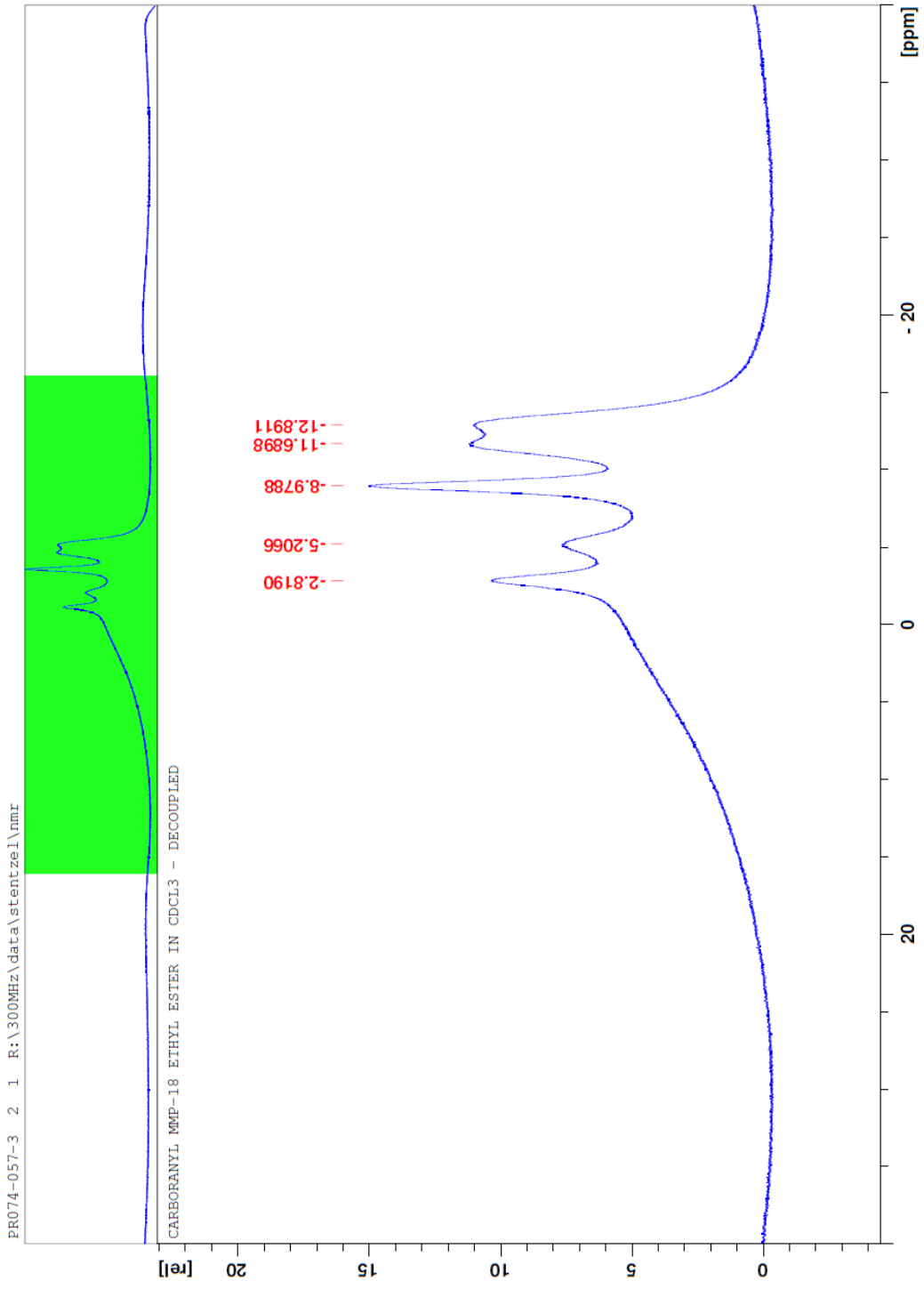
HRMS - Carboranyl MMP-18 Ethyl Ester (Compound 20)



^{11}B NMR (Coupled) - Carboranyl MMP-18 Ethyl Ester (Compound 20)



^{11}B NMR (Decoupled) - Carboranyl MMP-18 Ethyl Ester (Compound 20)



HPLC - *Nido* Carboranyl MMP-18 Ethyl Ester from B10H14 Reaction (Compound 21)

```
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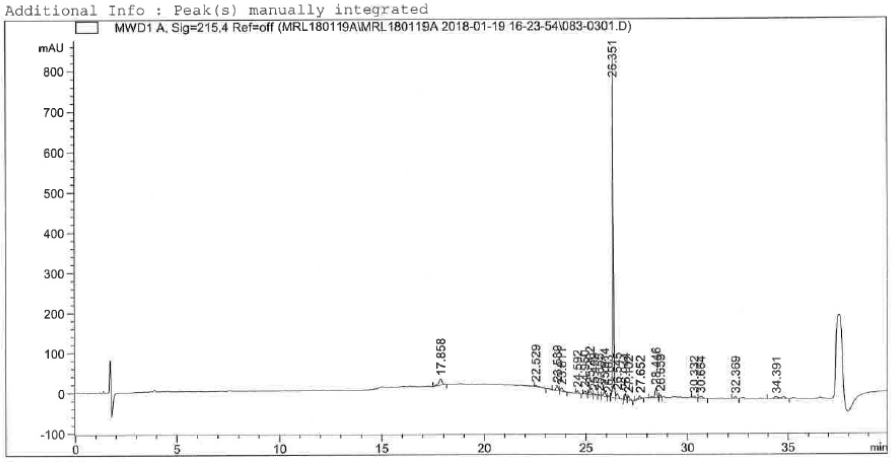
Acq. Instrument : Instrument 1                     Location : Vial 83
Injection Date  : 01/19/18  5:49:15 PM                Inj :   1
                                                  Inj Volume : 5.0 µl

Acq. Method     : C:\CHEM32\1\DATA\MRL180119A\MRL180119A 2018-01-19 16-23-54\BNCT-LONG2.M
Last changed    : 10/18/17  2:57:54 PM by MRL
Analysis Method : C:\CHEM32\1\METHODS\BNCT-LONG2.M
Last changed    : 01/19/18  9:19:02 AM by MRL
                  (modified after loading)
Method Info     : HPLC Method for Monitoring and Assaying BNCT products and reactions
                  HPLC 12 (Lab 2)
                  A = 0.10% TFA in water
                  B = 0.10% TFA in ACN
                  Waters Sunfire C8, 4.6x150 mm, 3.5 micron
                  Flow: 1.0 mL/min, Column Temp: 40 C

Sample Info     : Isolated 26.4 min impurity
                  PR074-057-5, 1 mg/mL in ACN

Additional Info : Peak(s) manually integrated
```

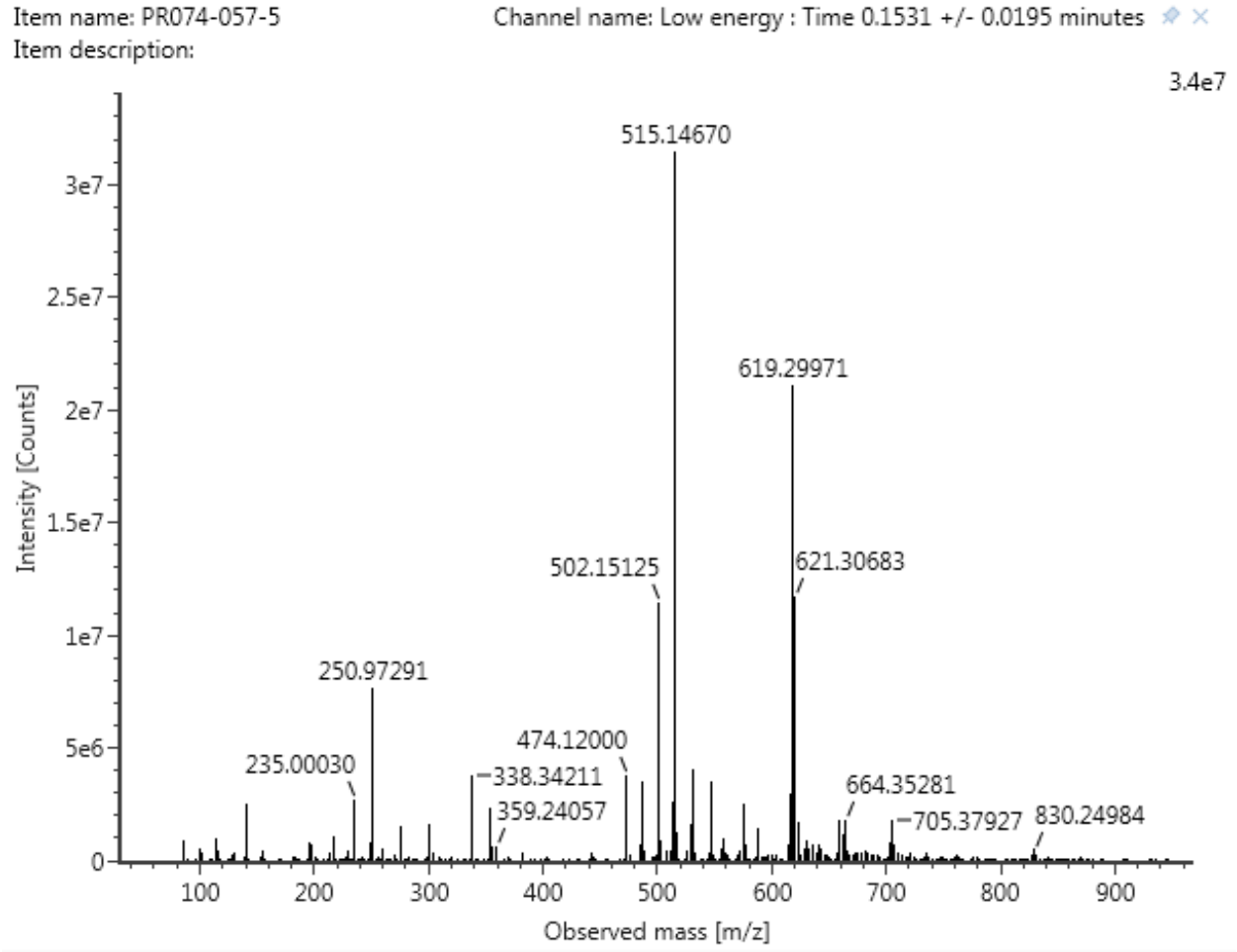


Area Percent Report

Signal 1: MWD1 A, Sig=215,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.858	BB	0.1763	194.04961	16.13411	4.0307
2	22.529	BB	0.1569	38.41038	3.18155	0.7978
3	23.589	BV	0.1269	97.47675	11.98632	2.0248
4	23.811	VB	0.1179	63.87245	8.29248	1.3267
5	24.592	BB	0.0786	33.65154	6.27060	0.6990
6	24.950	BV	0.0946	55.38588	8.66052	1.1505
7	25.202	VV	0.0791	103.04434	19.04369	2.1404
8	25.409	VB	0.0889	28.40926	4.31031	0.5901
9	25.655	BV	0.0574	13.35903	3.76682	0.2775
10	25.914	VB	0.0843	117.50207	20.06637	2.4407
11	26.103	BB	0.0845	19.37152	3.11699	0.4024
12	26.351	BV	0.0622	3307.39893	835.69855	68.7003
13	26.545	VB	0.0972	92.74319	12.69990	1.9264
14	26.944	BV	0.0645	70.68168	15.71924	1.4682
15	27.102	VB	0.0974	72.03439	10.86010	1.4963
16	27.652	BB	0.1215	72.68391	8.35457	1.5098
17	28.446	BV	0.0882	149.31303	24.11763	3.1015
18	28.659	VB	0.0774	34.62709	7.03583	0.7193
19	30.332	BB	0.1277	28.48396	3.47066	0.5917
20	30.664	BB	0.1814	48.23397	4.21900	1.0019
21	32.369	BB	0.1325	42.04502	5.08472	0.8733
22	34.391	BB	0.3670	131.46704	4.74818	2.7308
Totals :				4814.24504	1036.83814	

HRMS (Positive Mode) - *Nido* Carboranyl MMP-18 Ethyl Ester from B10H14 Reaction (Compound 21)

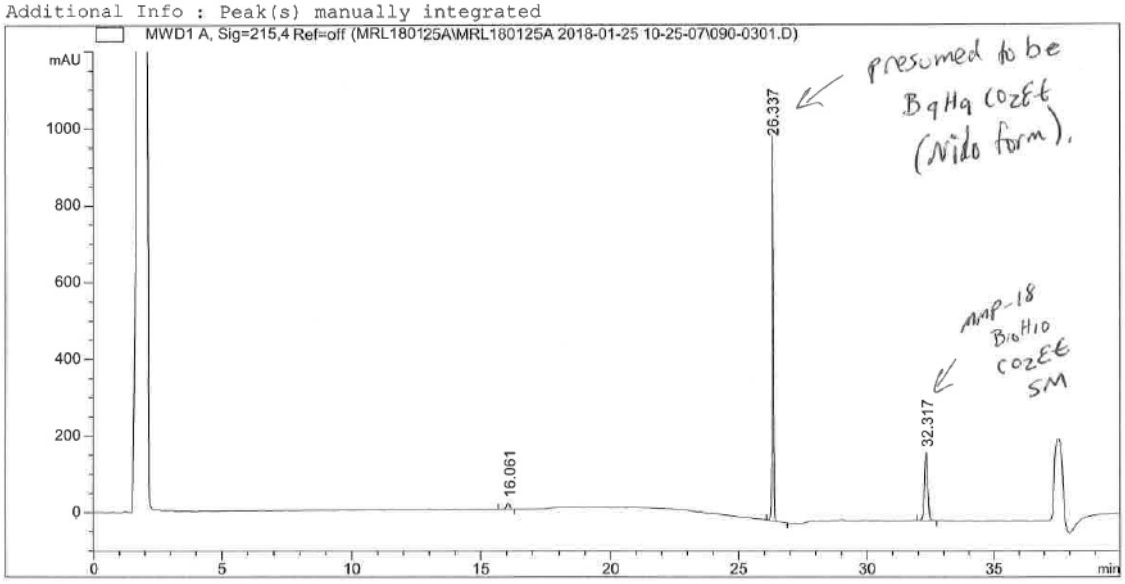


HPLC - *Nido* Carboranyl MMP-18 Ethyl Ester from microwave heating (Compound 21)

```
Acq. Operator    : MRL                              Seq. Line :   3
Acq. Instrument  : Instrument 1                      Location : Vial 90
Injection Date   : 01/25/18  3:28:15 PM                  Inj :   1
                                                   Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\1\DATA\MRL180125A\MRL180125A 2018-01-25 10-25-07\BNCT-LONG2.M
Last changed     : 10/18/17  2:57:54 PM by MRL
Analysis Method  : C:\CHEM32\1\METHODS\BNCT-LONG2.M
Last changed     : 01/25/18  4:28:27 PM by MRL
                   (modified after loading)
Method Info      : HPLC Method for Monitoring and Assaying BNCT products and reactions
                   HPLC 12 (Lab 2)
                   A = 0.10% TFA in water
                   B = 0.10% TFA in ACN
                   Waters Sunfire C8, 4.6x150 mm, 3.5 micron
                   Flow: 1.0 mL/min, Column Temp: 40 C

Sample Info      : Hydrolysis Carboranyl MMP-18 Ethyl Ester
                   2.0 mg SM, AcOH (0.50 mL),USP water (0.25 mL)
                   t = 10 mins at 140-145 C (microwave)....reaction didnt reach target temp:
                   200 C
                   sample prep: 4 drops rxn into 15 drops acetic acid

Additional Info : Peak(s) manually integrated
```

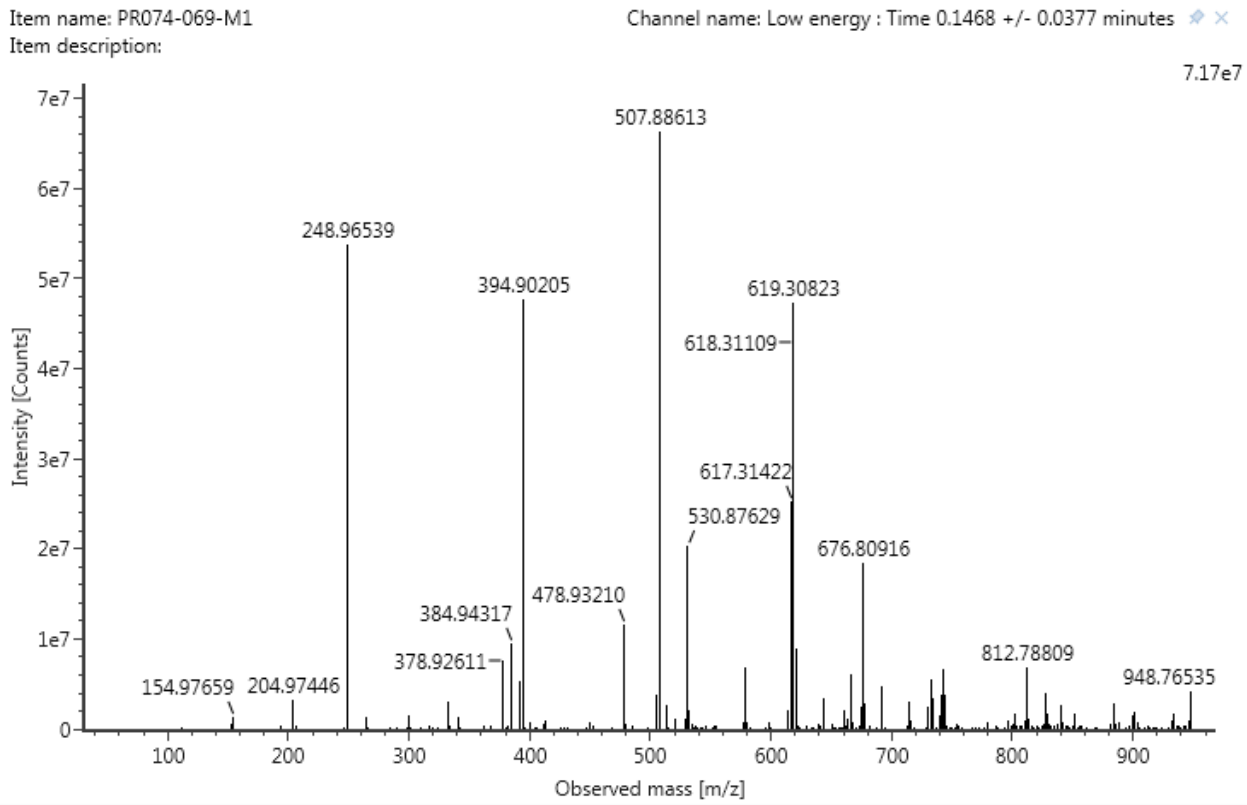


Area Percent Report

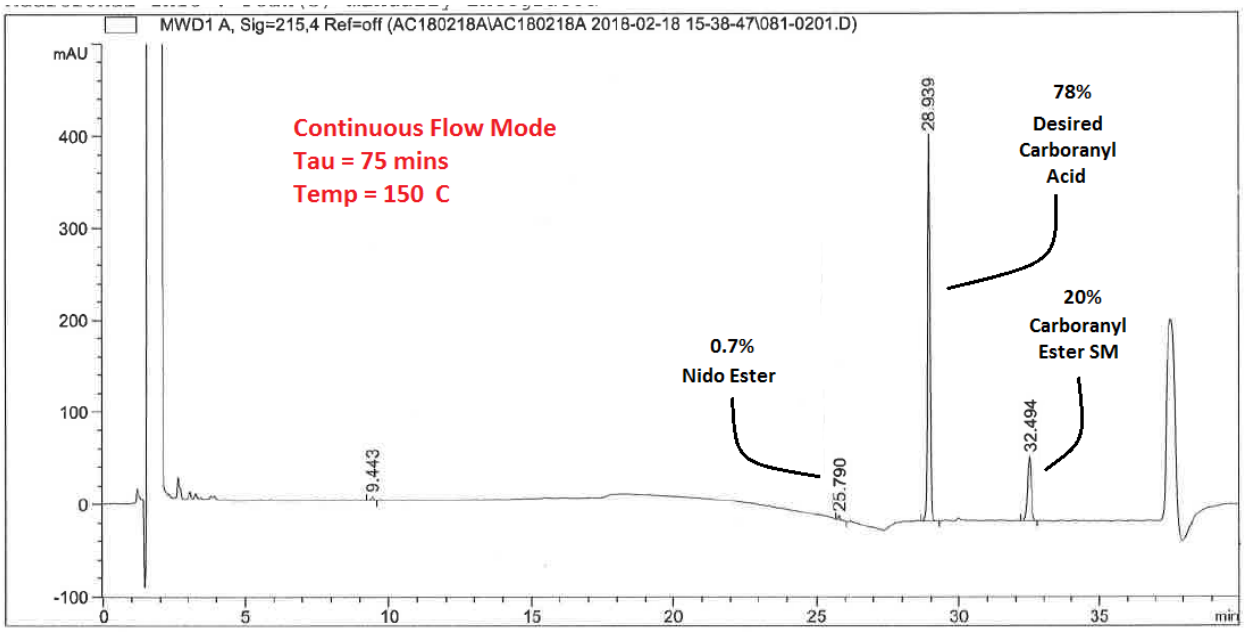
Signal 1: MWD1 A, Sig=215,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	16.061	BB	0.1369	147.49146	16.39601	2.6312
2	26.337	BB	0.0623	3928.60938	990.10999	70.0855
3	32.317	BB	0.1331	1529.35376	176.56261	27.2833
Totals :				5605.45459	1183.06861	

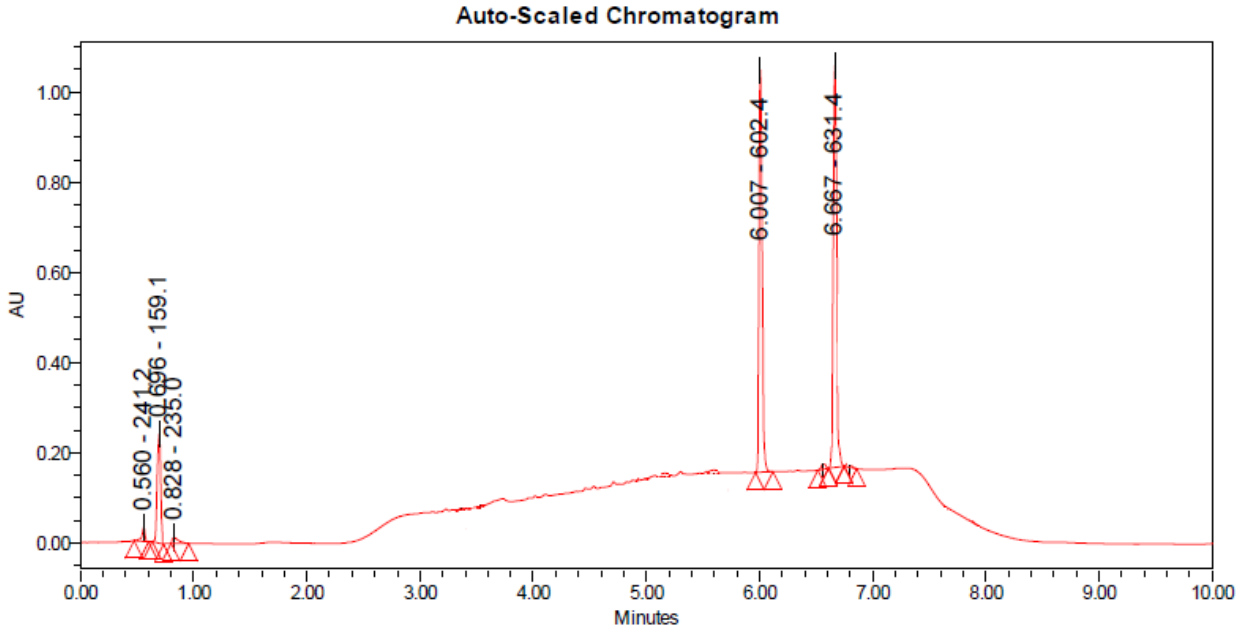
HRMS (Negative Mode) - *Nido* Carboranyl MMP-18 Ethyl Ester from microwave heating (Compound 21)



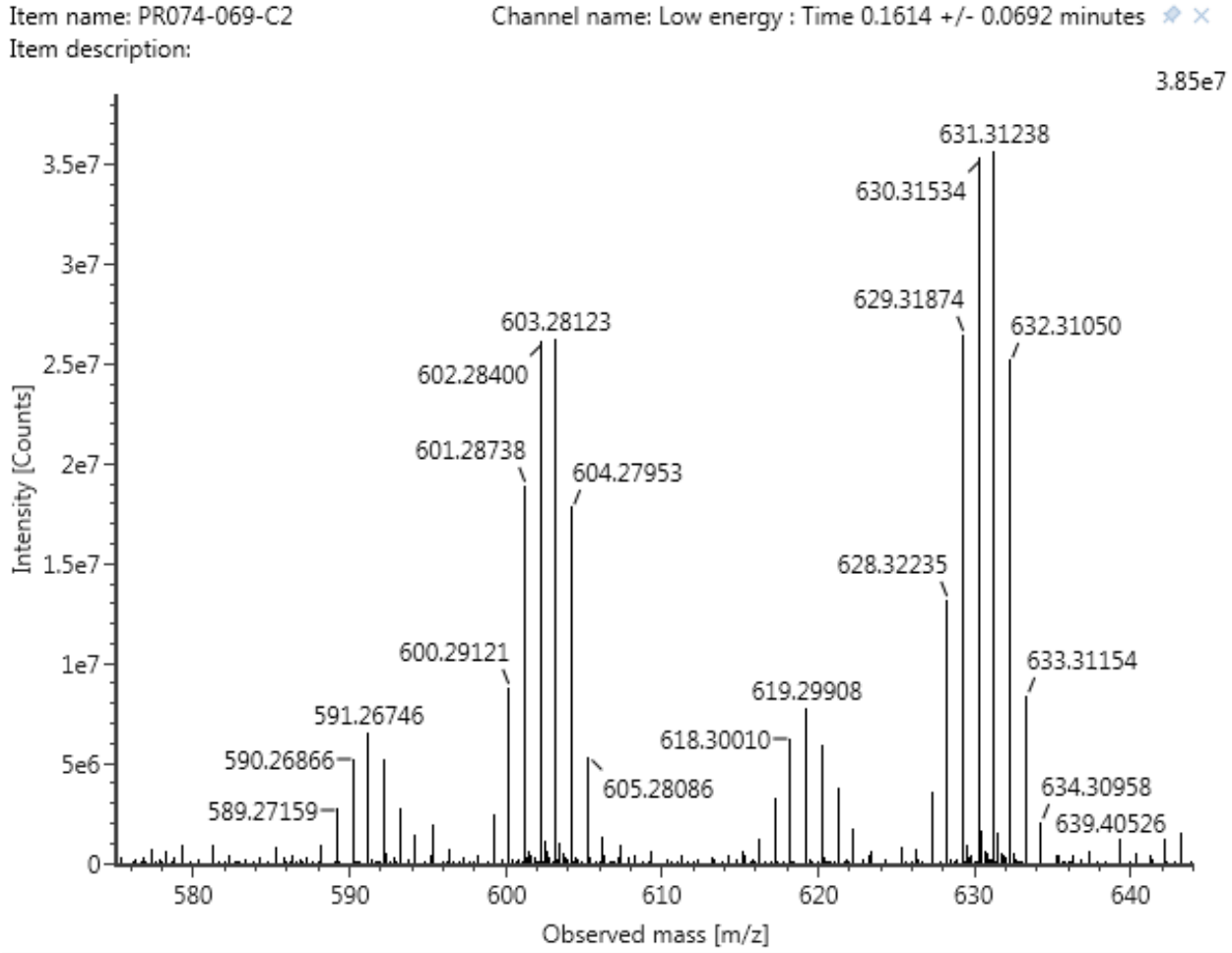
HPLC - Carboranyl MMP-18 Carboxylic Acid (Compound 22)



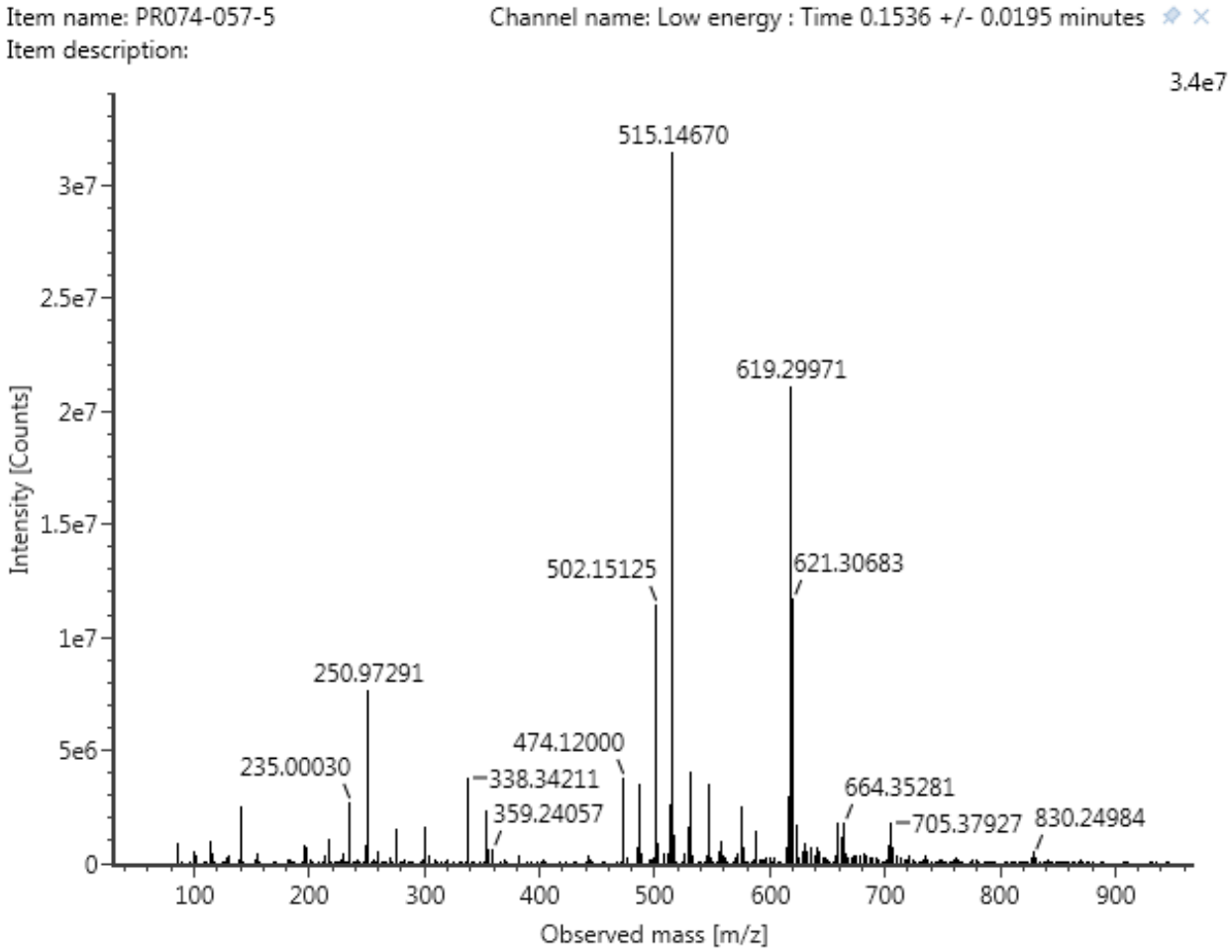
UPLC/MS - Carboranyl MMP-18 Carboxylic Acid (Compound 22)



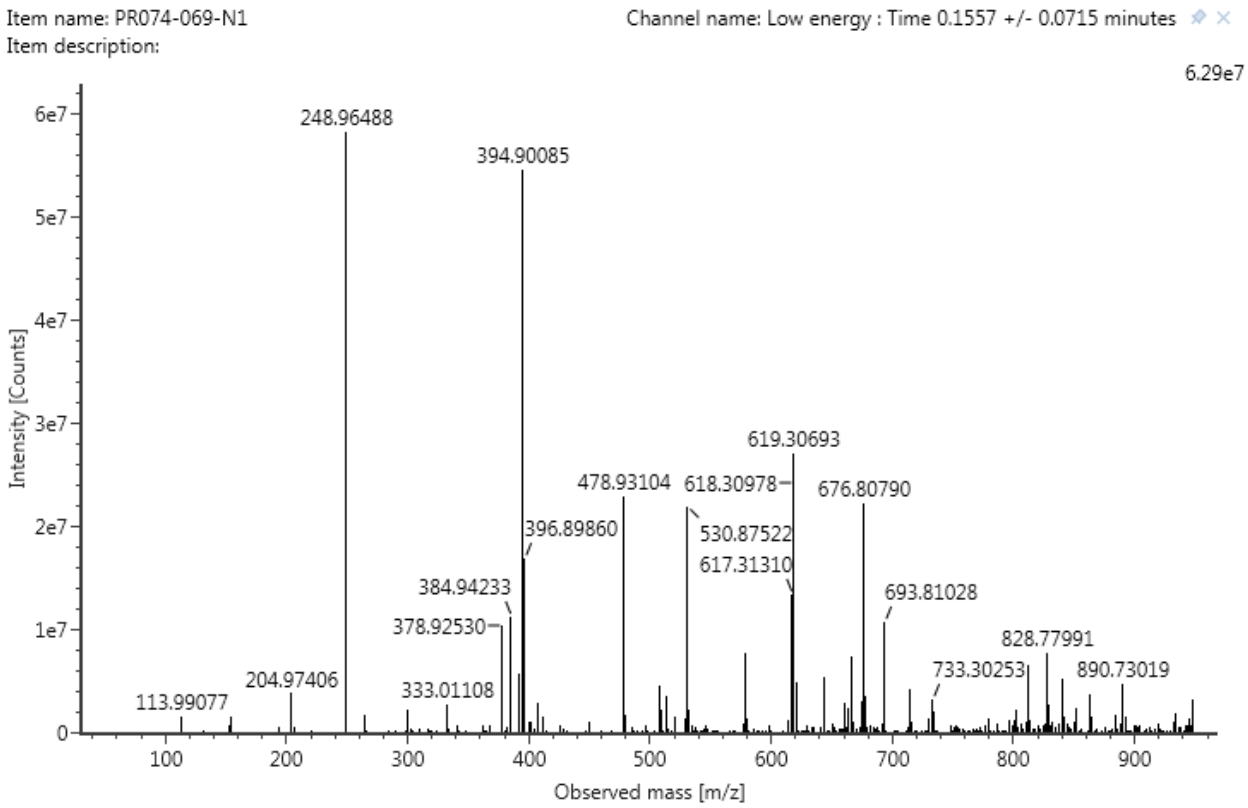
HRMS - Carboranyl MMP-18 Carboxylic Acid (Compound 22)



HRMS (Negative Mode) - *Nido* Carboranyl MMP-18 Ethyl Ester (Compound 21)



HRMS - Arachno Carboranyl MMP-18 Ethyl Ester (Compound 24)



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VITA

Marlon Ray Lutz Jr. is the eldest out of four siblings and also is the son of Catherine Lutz. He was born March 30, 1983 in Honolulu, Hawaii. When he was younger, he lived in many places in the United States. The majority of his lifetime was spent in Maine for a total of 17 years.

After graduating high school 4th in his class in 2001, Marlon went to the University of Maine at Machias (UMM) on a full scholarship for Marine Biology. After a year, he determined that Marine Sciences was not his passion, but rather chemistry. Over the years at UMM, he was inspired by Dr. Shallee Page who taught organic chemistry and later took all the chemistry courses that were offered at UMM. In the summer of 2005, he was awarded a REU-NSF internship at Loyola University Chicago (LUC) where he spent the summer researching the synthesis of cyclobutanones *via* intramolecular [2+2] cycloadditions as protease inhibitors with Dr. Daniel Becker. Given the academic and research opportunities at Loyola University Chicago, Marlon transferred to LUC in the Fall of 2005 to continue organic chemistry research with Dr. Daniel Becker, serving as a teaching assistant in

organic chemistry labs, and achieving a Bachelor of Science degree in chemistry in 2007.

Immediately after graduating, Marlon began his Master studies in chemistry with Dr. Daniel Becker and also had the opportunity to work as an intern at a Regis Technologies located in Morton Grove, IL and owned by the Glunz family. After the internship, Marlon was immediately hired as a full-time Regis Technologies employee where he is currently still working there as a Process Organic Chemist. During his two year graduate career, Marlon synthesized many cyclotriveratrylene (CTV) derivatives, few other larger macrocycles, published many papers, and had the opportunity to mentor three undergraduate students with his research. Marlon achieved his M.S. degree in chemistry in May 2009. In the Fall of 2012, Marlon decided to pursue further education and achieve his doctoral degree in Chemistry and Medicinal Chemistry. During this time, Marlon worked full-time at Regis Technologies while being a dedicated family man raising a family of 4 with his wife. Marlon made significant contributions to his doctoral research projects that encompassed novel targets for killing cancer cells more efficiently and safely and developing a novel and very efficient synthesis of a DapE substrate that allowed to target an antibacterial target (DapE) and research methods to overcome bacterial resistance. Furthermore, Marlon

evaluated and discovered a class of supramolecular host-guest interactions that has not been reported and this discovery will warrant many opportunities for other research groups to apply his findings with their chiral macrocycles. Over the years and during his professional career, Marlon gained extensive experience with continuous flow chemistry that allowed him to use flow chemistry as a tool to develop processes that were more efficient and safer. With this experience, Marlon equipped the Becker research group with equipment so that they could carry flow chemistry for troublesome or low yielding batch-type reactions.

Marlon Lutz achieved his Ph.D. in August 2018 and plans on continuing research for treating or curing cancer and other diseases. Furthermore, Marlon will continue to push the utilization of continuous flow chemistry since it is the future for the chemical industry but also for academia. Lastly, Marlon plans on teaching one day at the college level where he plans to perform his own research and will use his educational and industrial experience to inspire and teach future generation chemists.