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Part I: Total Synthesis of Marine Natural Product Palmyramide A

Part II: Synthesis of New Hepatitis C Virus Translation Inhibitors

A Dissertation submitted in partial satisfaction of the requirements
for the degree Doctor of Philosophy

in

Chemistry

by

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2024

The Dissertation of Kevin Walsworth is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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2024

DEDICATION

This dissertation is dedicated to my wife, Kayla, and to my family. This journey would have been impossible without their love and support.

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LIST OF ABBREVIATIONS

Ac	Acetate
acac	Acetylacetonate
ACN	Acetonitrile
Alloc	allyl carbamate
aq	Aqueous
Bn	Benzyl
BrCN	Cyanogen bromide
cat	Catalytic
Cp	Cyclopentadiene
CSA	Camphorsulfonic Acid
Cy	Cyclohexyl
d.r.	Diastereomeric ratio
DBU	1,8-Diazabicylo[5,4,0]undec-7-ene
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIC	Diisopropylcarbodiimide
DIPEA	Diisopropylethylamine
DMAP	4-*N*,*N*-dimethylaminopyridine
DMF	Dimethylformamide
DMP	Dess-Martin periodinane
DMS	Dimethylsulfide
DMSO	Dimethylsulfoxide

DNA	Deoxyribonucleic acid
EDC	1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride
Et	Ethyl
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
FRET	Förster resonance energy transfer
HPLC	High performance liquid chromatography
HMDS	Hexamethyldisilizane
HMPA	Hexamethylphosphoramide
IRES	Internal ribosome entry site
imid	Imidazole
*i*Pr	Isopropyl
LAH	Lithium aluminum hydride
LDA	Lithium diisopropyl amide
mCPBA	*meta*-chloroperoxybenzoic acid
Me	Methyl
MOM	Methoxymethyl
MTBE	Methyl *tert*-butyl ether
PCC	Pyridinium chlorochromate
PDC	Pyridinium dichromate
Ph	Phenyl
RNA	Ribonucleic acid
sat	Saturated

TBABr	Tetrabutylammonium bromide
TBACl	Tetrabutylammonium chloride
TBAF	Tetrabutylammonium fluoride
TBAI	Tetrabutylammonium iodide
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
*t*Bu	*tert*-Butyl
TEA	Triethylamine
TES	Triethylsilyl
Tf	Trifluoromethanesulfonyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIPS	Triisopropylsilyl
TLC	Thin layer chromatography
TMEDA	N,N,N',N'-tetramethylethylaminediamine
TMS	Trimethylsilyl
T_3P	Propylphosphonic anhydride
TsOH	*para*-toluenesulfonic acid

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Schmit, D., Milewicz, U., Boerneke, M., Burley, S., **Walsworth, K**., Um, J., Hecht, D., Hermann, T., Bergdahl, BM. Syntheses and Binding Testing of N1-Alkylamino-Substituted 2-Aminobenzimidazole Analogues Targeting the Hepatitis C Virus Internal Ribosome Entry Site. *Aust. J. Chem* **2020**, 73, 212-221

Walsworth, K., Bender, A., Separovic, B., Bergdahl, BM., Metzger, R. The Conformations of Virginiamycin M 1 Diacetate, an Inhibitor of Guinea Pig Brain CCK-B Receptors, in Selected Solvents. *Aust. J. Chem* **2020**, 73, 230-235

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ABSTRACT OF THE DISSERTATION

Part I: Total Synthesis of the Marine Natural Product Palmyramide A
Part II: Synthesis of New Hepatitis C Translation inhibitors

by

Kevin James Walsworth

Doctor of Philosophy in Chemistry

University of California San Diego, 2024

San Diego State University, 2024

Professor B. Mikael Bergdahl, Chair
Professor Thomas Hermann, Co-Chair

The American Cancer Society estimates that there will be 97,000 new cases of colon cancer and 51,000 deaths in the United States in 2023Approximately 30% of these diagnoses are in patients under the age of 55. These numbers have been declining slightly in recent years as methods of detection are improving and people are making efforts to live healthier lifestyles. If

detected early, the 5 year survival rate is approximately 90%; however, if detected late, the survival rate decreases significantly (14% in stage IV disease). With people being diagnosed at earlier ages, and with only approximately 51% of people being up to date on the colorectal cancer screening, it is imperative that we have as many tools as possible at our disposal for treatment of the disease. While palmyramide A is a promising new drug candidate that may potentially help in the treatment of this disease, availability from natural sources is extremely low (471 g of dry bacteria used to isolate 6.1 mg palmyramide A). To aid in having enough of the compound available, we are proposing the first highly convergent total synthesis that will produce enough material for further testing. Part I of this dissertation describes the first total synthesis of palmyramide A, the biological evaluation of the natural product, and modification of the natural product to use as a probe that can determine its molecular mechanism of action.

The World Health organization estimates there are approximately 71 million people worldwide living with hepatitis C virus (HCV), with 15-30% of those developing advanced liver diseases such as cirrhosis or liver cancer. While current treatments can be effective, they are extremely cost prohibitive, limiting access for many people. Another issue is that most commercially available drugs target the same viral protein, non-structural protein 5B (NS5B), an RNA dependent RNA polymerase. Because HCV is a positive-sense RNA virus, its genome is translated and replicated with little proofreading, rendering it prone to mutations, and a mutation in NS5B could render these therapeutics ineffective. However, a small segment of the 5' non-coding RNA, known as the internal ribosome entry site subdomain IIa, is extremely highly conserved, and is an attractive target for therapeutics. Part II of this dissertation describes the design and synthesis of new classes of therapeutics targeting the HCV IRES.

PART I: TOTAL SYNTHESIS OF THE MARINE NATURAL PRODUCT PALMYRAMIDE A

CHAPTER 1: TOTAL SYNTHESIS OF THE MARINE NATURAL PRODUCT PALMYRAMIDE A

1.1 Introduction

Palmyramide A is a cyclic depsipeptide that has been shown to have potent cytotoxic effects against colon cancer cells, along with modest cytotoxicity against lung cancer cells.[1] Cyclic peptides and depsipeptides, especially those from marine sources, have long been studied for their therapeutic and anti-cancer effects.[2]

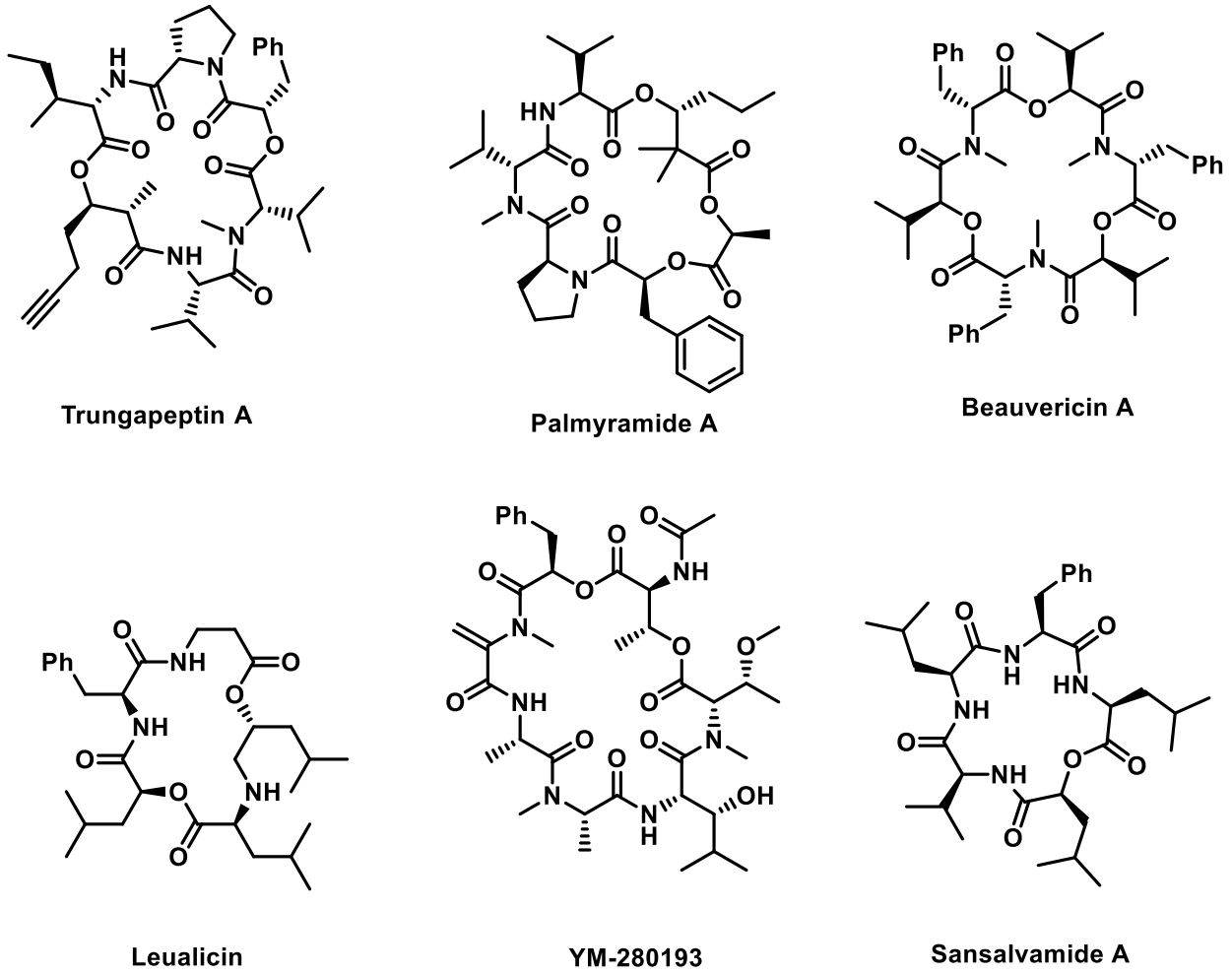


Figure 1.1: Representative cyclic depsipeptides.

1.2 Colon Cancer

The American Cancer Society estimates that there are approximately 107,000 new cases of colon cancer in the United States in 2023, with approximately 53,000 expected deaths. These numbers have been declining in recent years as treatments and screening have improved, and people have changed their lifestyle-related risk factors.[3] If detected early, the survival rate is relatively high (91% in stage I); however, the prognosis for more advanced disease is much worse (55% in state IIIC). Because of the prevalence and mortality of colon cancer, especially with people being diagnosed at much younger ages than in the past (estimated to increase by 124% by 2030)[4] and with new drug resistant strains of colon cancers, it is imperative that we have as many tools as possible at our disposal for the treatment of the disease. Palmyramide A is a promising new drug candidate for the treatment of colon cancer; however, availability from natural sources is extremely limited. Due to this low supply of the compound the mode of action of palmyramide, along with its molecular target, is not understood. The work presented in part I of this thesis is a novel and robust route for the synthesis of palmyramide A that could allow for the multigram synthesis of the compound along with the ability to easily synthesize analogues that can help to understand its mode of action and increase its activity.

1.2.1 Colon Cancer Treatments

Until the mid-1980's, most colorectal cancer patients underwent surgery alone. This led to high rates of pelvic failure, morbidity, and death.[5] Clinical trials performed in the 1980's and 1990's showed that surgery and postoperative chemotherapy and radiotherapy led to improved survival and lower pelvic failure rates, leading to their incorporation in patients with stage II and stage III disease.

The current standard of treatment for colon cancer depends on the stage of the disease. For stage 0 and stage I, typical treatment is just removal of the polyp, and as long as there are clean margins, no further treatment is needed. In stage II, the cancer has grown through the wall of the colon, potentially into nearby tissue. At this stage, surgery to remove the section of the colon containing the cancer, along with nearby lymph nodes, can be all the treatment that is needed; however, it may be recommended to undergo chemotherapy if the cancer is high risk.[6]

For stage III disease, the cancer has spread to the lymph nodes but not to other parts of the body. Surgery to remove the section of the colon with the cancer, removal of nearby lymph nodes, followed by chemotherapy is the standard of treatment. For chemotherapies, typically either FOLFOX (5-fluorouracil, leucovorin, and oxaliplatin) or CapeOx (capecitabine and oxaliplatin) are the most common.[7]

Stage IV colon cancers have spread outside of the colon to distant organs. The most common area for colon cancer to spread is the liver, though it can also spread to other places. Surgery is unlikely to cure these cancers in most cases. Surgery helps patients live longer if there are only a few small metastases, but typical treatments include chemotherapy to shrink the tumors before surgery. If the tumors shrink enough to be removed, then surgery can be performed. In stage IV disease, there are a variety of chemotherapy treatments that may be used depending on the extent of the cancer and the health of the patient. The chemical structures of a few of these treatments are shown in **Figure 1.2**.

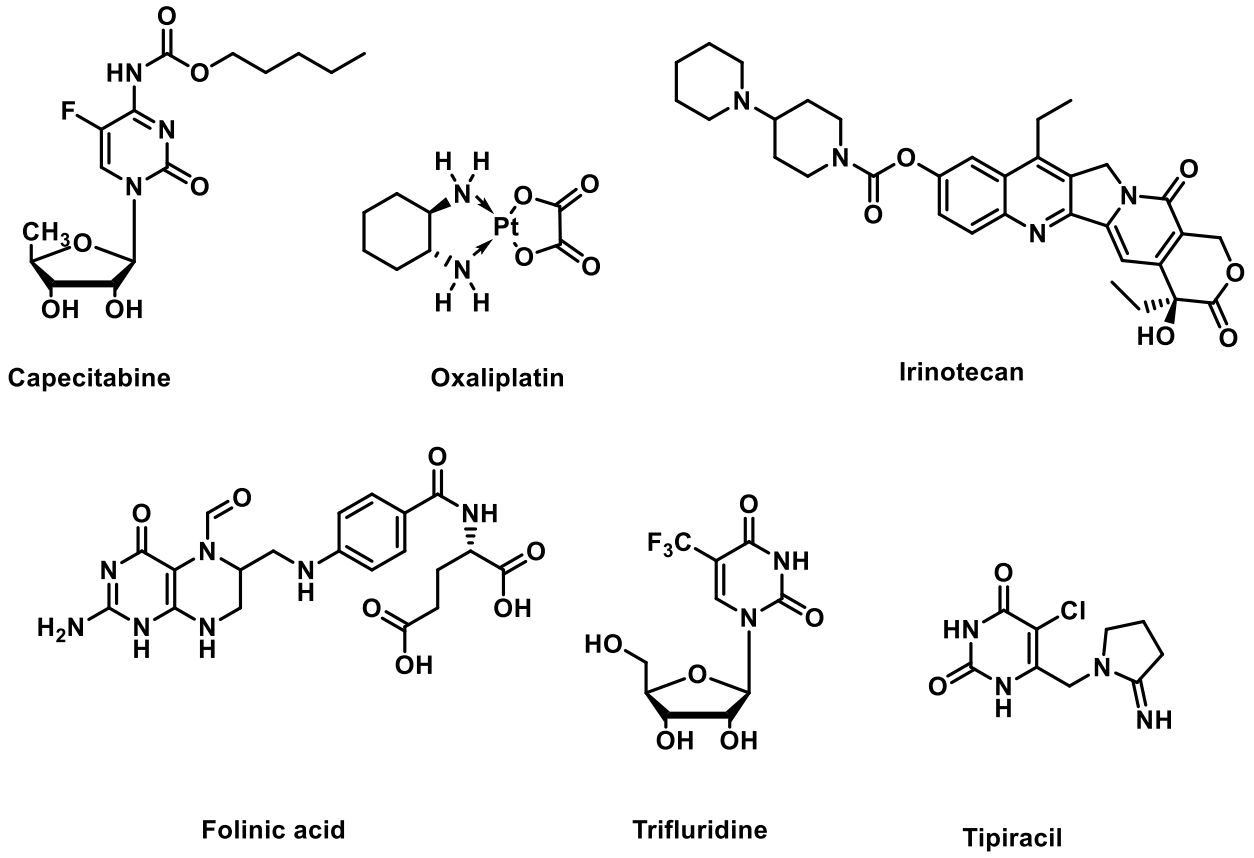


Figure 1.2: Representative chemotherapy agents used against colon cancer.

A major issue with most colon cancer treatments is the severe side effects of chemotherapies and the invasiveness of surgery. Also, the genetic makeup of colon cancers is varied and difficult to understand; therefore, while there a few targeted therapies (such as drugs that target VEGF or EGFR), options are limited. These limitations justify the need to learn as much as possible about colon cancer to find further treatments. The remainder of chapter 1 of this dissertation will discuss the first total synthesis of palmyramide A, a promising new compound that has shown activity against colon cancer cells, as well as the modification of the natural product to attempt to learn its mechanism of action.

1.3 Isolation and Structural Elucidation of Palmyramide A

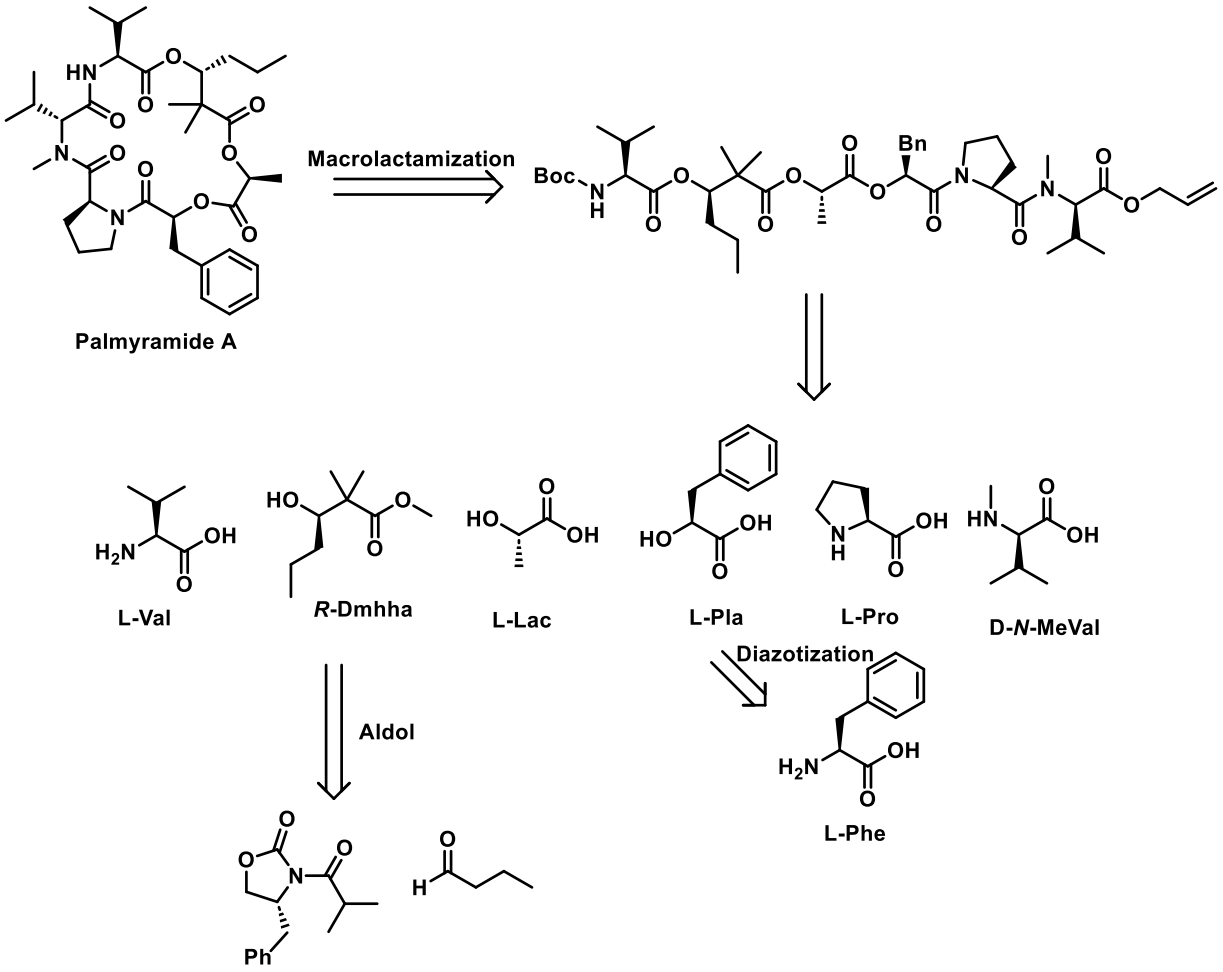
Palmyramide A was originally isolated from the cyanobacteria *L. majuscula* by the Gerwick group in 2010,[1] along with curacin D and malyngamide C, while performing bioassay guided fractionation of a consortium of marine cyanobacterium and red alga. The crude extract was subjected to vacuum chromatography and assayed for sodium channel blocking activity. The active fractions were further purified by reverse phase HPLC to yield palmyramide A as a colorless glassy solid. A total of 6.1 mg of palmyramide A was isolated from 417.1 g of the dry consortium.

The chemical formula was determined by high-resolution mass spectroscopy to be $C_{36}H_{53}N_3O_9$. Using NMR, they determined there were six ester/amide bonds, five aromatic protons, and an *N*-methyl group. Analysis of 2D-NMR led to the assignments of the amino acids valine, *N*-methyl valine, and proline, and the hydroxy acids lactic acid, 3-phenyllactic acid, and 2,2-dimethyl-3-hydroxyhexanoic acid (Dmhha). The sequence of the residues was accomplished by analysis of HMBC correlations. Absolute configuration of the amino acids was determined by HPLC analysis of the acids derivatized by Marfey's reagent. Absolute configuration of the hydroxy acids was determined by the Mosher ester method.

In their original publication, the Gerwick group assayed palmyramide A for cancer cell activities as well as sodium channel blocking. In the cancer cell activity assays, it was shown to have modest cytotoxic effects against H-460 human lung carcinoma cells, having an IC_{50} of 39.7 μM. In the sodium channel blocking assay, it showed an IC_{50} value of 17.2 μM. More recently, natural palmyramide A was assayed against HCT-116 human colon cancer cells and gave a more interesting IC_{50} value of 8.9 nM. Previous to the work described below, to our knowledge, no other studies of palmyramide's activity against normal human cells or determining its mode of action have been conducted.

1.4 Route to Palmyramide A

Palmyramide A is a cyclic depsipeptide consisting of three amino acids and three hydroxy acids. The retrosynthetic analysis is summarized in **Scheme 1.1**.



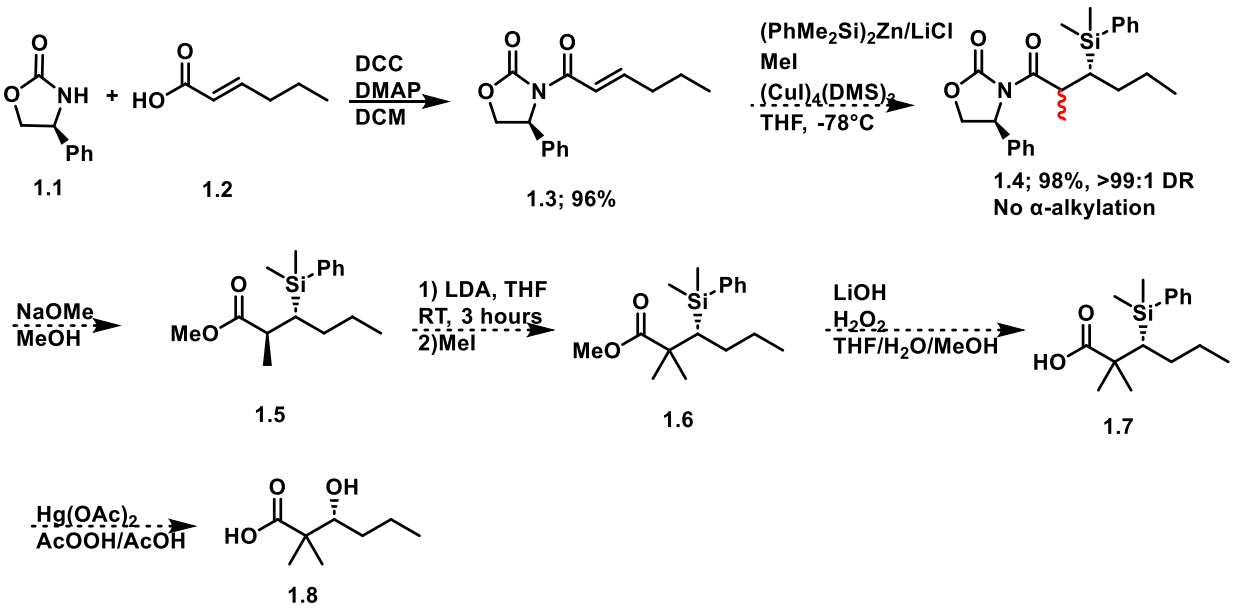
Scheme 1.1: Retrosynthetic analysis of palmyramide A.

Proline and valine are commercially available. D-*N*-Methylvaline is easily synthesized from *N*-boc-valine, sodium hydride, and iodomethane.[8] (l)-Phenyllactic acid (L-Pla) is easily synthesized by diazotization of phenylalanine.[9] *R*-Dimethylhydroxyhexanoic acid (DMHHA), however, proved to be more difficult to synthesize and work with, and two methods of its production are presented below.

The synthetic plan was to initially form two trimers, one with esters and one with peptides. Once these were complete, the next step would be to combine them into a linear intermediate, then after global deprotection use a powerful coupling reagent, at low concentration to prevent oligomerization, to form palmyramide A.

1.4.1 Initial Attempts to Synthesize DMHHA

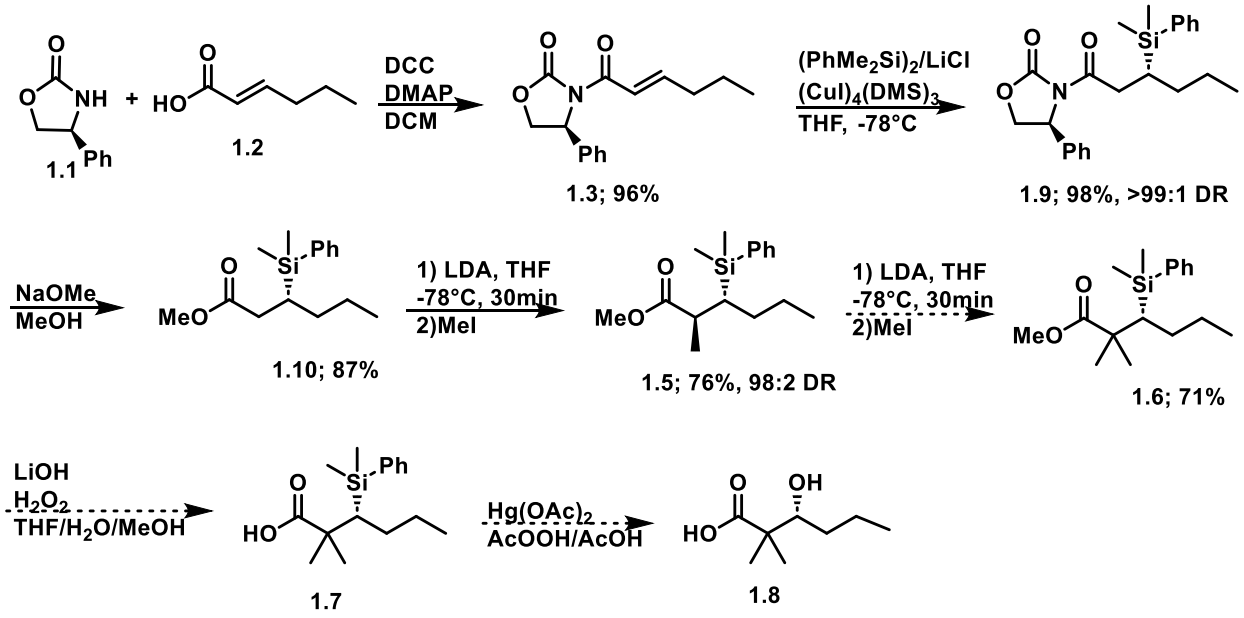
Initially, attempts were made to synthesize DMHHA using a $(CuI)_4(DMS)_3$ catalyzed conjugate addition of a silane to an α,β-unsaturated enone attached to a chiral auxiliary. This methodology was developed "in-house" and has shown promising results for being used in this synthesis. This synthetic plan is summarized in scheme 1.2.



Scheme 1.2: Initial attempts to synthesize DMHHA via a copper catalyzed conjugate addition followed by Fleming oxidation.

Coupling of **1.1** and **1.2** was successful employing the Steglich reaction conditions. Reaction of enone **1.3** with disilylzinc reagent $(PhMe_2Si)_2Zn$ in the presence of $(CuI)_4(DMS)_3$ and iodomethane, however, only gave 1,4 addition of the silane, but incorporation of the methyl group using MeI was not observed. Previous reactions using aldehydes as the electrophile to trap

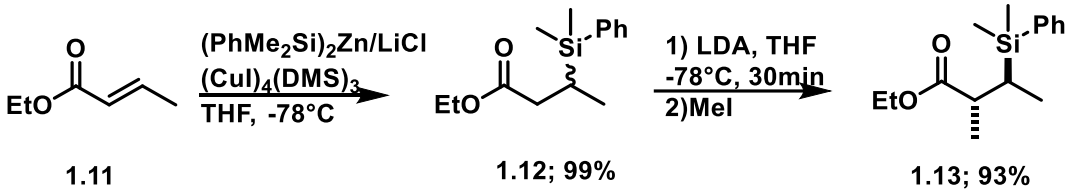
the enolate formed *in situ* worked out extremely well, so it was hypothesized that increasing the reactivity of the methyl group would be beneficial. Attempts to weaken the bond between carbon and iodine in iodomethane were made by addition of Ag_2O were unsuccessful. Attempts to increase reactivity by increasing the strength of the leaving group by exchanging iodomethane for methyl tosylate, dimethyl sulfate, and methyl triflate were also unsuccessful. After these reactions were unsuccessful, it was hypothesized that rather than the reactivity of the methyl group being too low, the problems might have arisen from steric hinderance of the enolate. Isolation of 1,4 product **1.9** and attempts to form the enolate with LDA and trap it with iodomethane were also unsuccessful; however, removal of the oxazolidinone with NaOMe followed by LDA mediated α-alkylation was successful, showing that having the bulky silane and the bulk from the oxazolidinone on either side of the enolate was the culprit. With the α-methylated intermediate now in hand, the route to DMHHA was updated and continued.



Scheme 1.3: Updated conjugate addition route to DMHHA.

The first α-methylation to produce **1.5** worked with little difficulty. However, subjecting **1.5** to the same conditions as **1.10** gave back only the monoalkylated starting material. A model

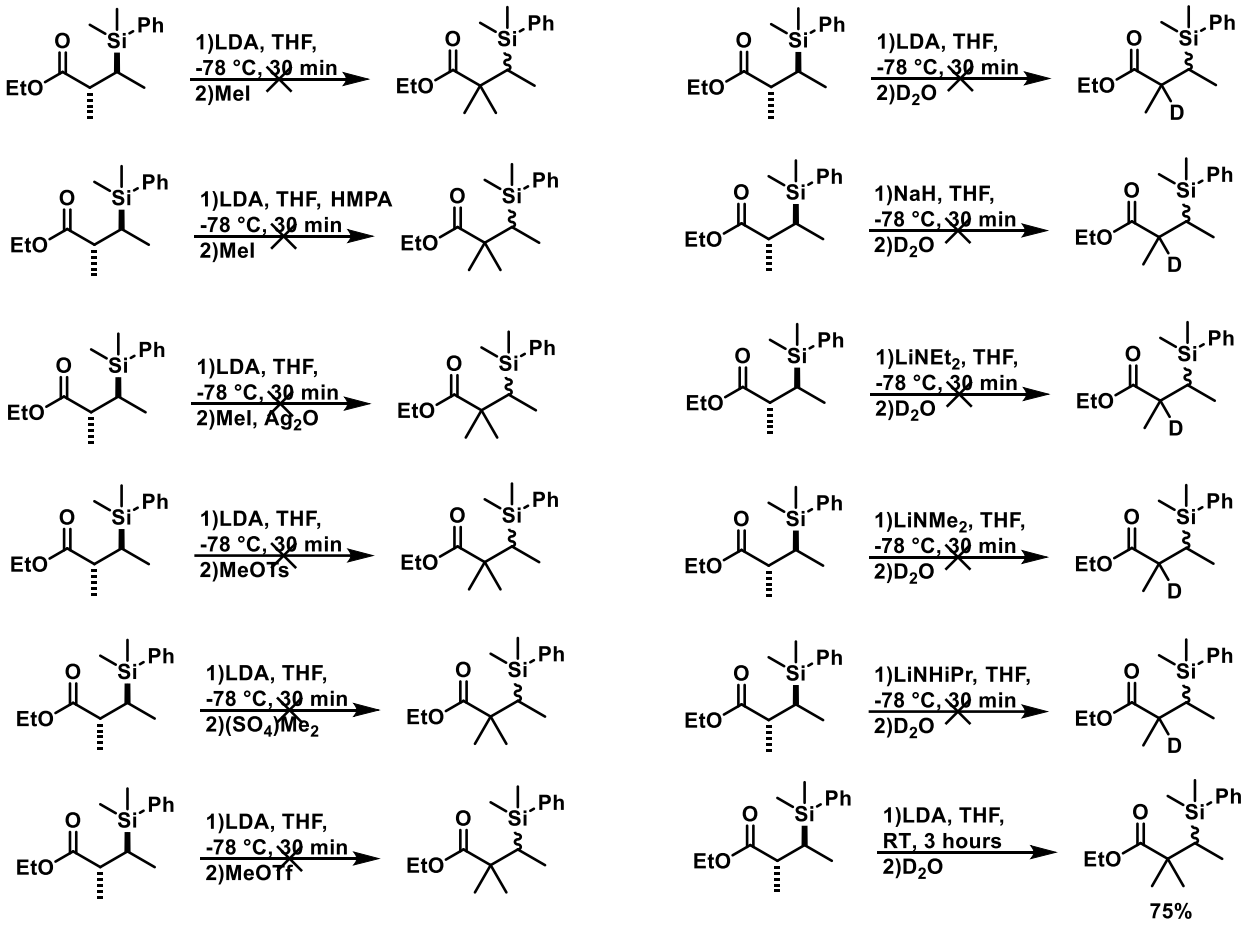
compound made with less expensive and more easily accessible starting material was used to test the second alkylation.



1.11 1.12; 99% 1.13; 93%

Scheme 1.4: Synthesis of model compound 3.13 from ethyl crotonate.

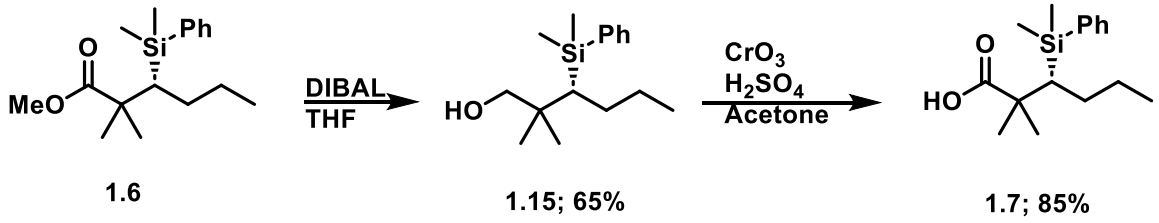
As expected, the copper catalyzed 1,4 silyl addition worked extremely well, giving the β-silane **1.12** in essentially quantitative yield. Monomethylation of **1.12** also worked well, giving **1.13** in 93%. First, attempts were made again to increase the reactivity of both the enolate and the electrophile. Electrophile activity was increased by either the addition of Ag_2O, or by replacing iodomethane with methyl tosylate, dimethyl sulfate, or methyl triflate; however, these attempts were unsuccessful. Attempts to increase the reactivity of the enolate was done by the addition of HPMA, but this also was ineffective. To assess if the enolate was even being formed, attempts were made to trap it with D_2O; however, that was also unsuccessful. With the theory that LDA was too bulky to deprotonate the α carbon, different bases were screened; however, NaH, lithium diethylamide, lithium dimethylamide, and lithium isopropylamide were all unsuccessful.



Scheme 1.5: Attempts at dimethylation and deuterium incorporation of compound **1.13**.

Next, temperature studies were performed, and while no deuterium incorporation was observed a -78 ℃ or -40 ℃, a small amount of deuterium was incorporated at 0 ℃ (5%), and even more at room temperature (20%) using LDA as the base and stirring for 30 minutes before quenching. Knowing that the enolate could be formed with LDA at room temperature, time studies were performed, and it was found that deuterium incorporation leveled out quenching after three hours (75%). With this knowledge in mind, attempting to trap the enolate after three hours at room temperature with iodomethane worked in similar fashion as deuterium trapping (68% isolated yield). Transitioning this methodology to **1.5** also worked in similar fashion, giving **1.6** in 71%. Fortunately, this methodology held up on scale, and there was no discernable difference using up to 1 g of **1.5**.

With **1.6** in hand, the next step would either be the saponification of the methyl ester, or the Fleming oxidation of the silane to the corresponding alcohol. Studies were performed to determine which step should be completed next; however, neither option was ideal. The first attempts were done on the Fleming oxidation, and while the reaction proceeded to completion by TLC, yield of the corresponding alcohol was low, probably due to the low molecular weight and corresponding volatility of the free alcohol. Saponification of the methyl ester was also unsuccessful; over 20 different combinations of solvents, bases, and acid catalysts were used to remove the ester, but no reaction was observed. Finally, the ester was converted into the carboxylic acid in 2 steps, first by reducing the ester to an alcohol using DIBAL, then oxidizing it back to the carboxylic acid with a Jones oxidation.

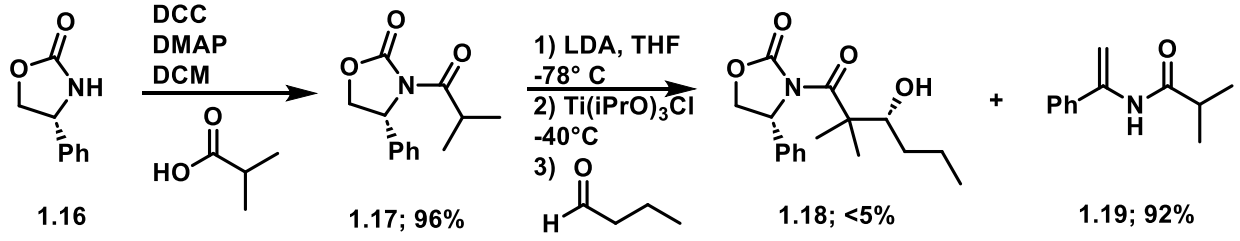


Scheme 1.6: DIBAL reduction and subsequent Fleming oxidation to form free acid **1.7**.

From here, attempts were made to esterify **1.7** with ethyl lactate; however, no matter which coupling reagents were used, no product was formed. With the idea that having the bulky silane in the β position along with the quaternary α was blocking nucleophilic attack at the carbonyl carbon, attempts to circumvent this were made. Fleming oxidation of **1.7** worked but was low yielding (typical reactions gave between 5 and 10%). Subsequent protections of the alcohol went smoothly, but there was still no reaction when attempting esterification. At this point, especially with the bottleneck in the Fleming oxidation, it became obvious that this route to DMHHA was not feasible, and a pivot was needed.

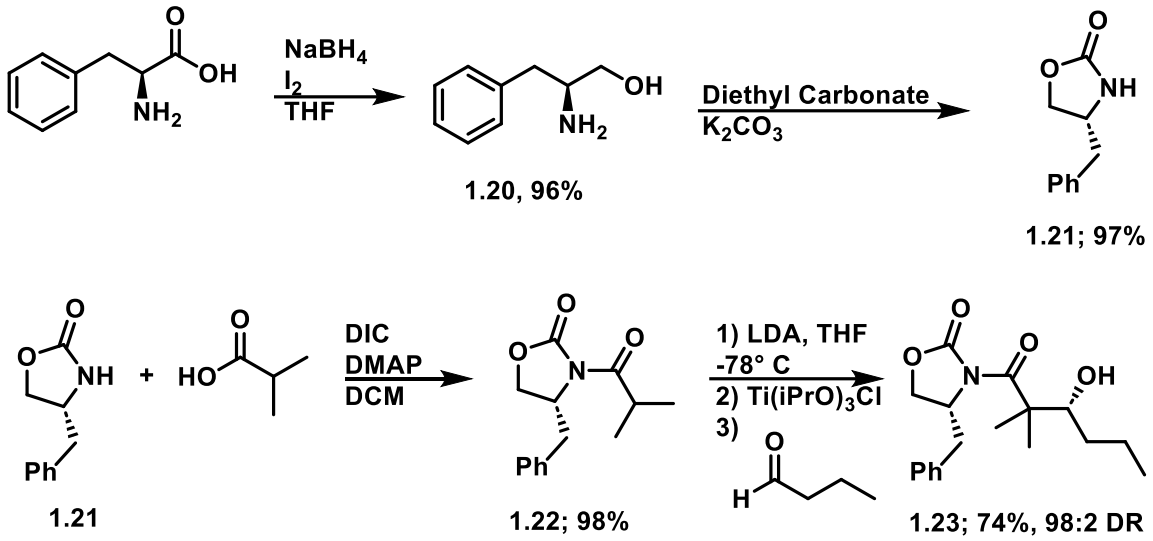
1.4.2 Aldol Route to DMHHA and Forming the Ester Half

Once it became clear the Fleming route was unfeasible to produce enough DMHHA to continue to palmyramide A, studies toward using different routes were conducted. The Gerwick group had published a paper on using a tertiary titanium-mediated aldol reaction and trapping various aldehydes.[10] Attempts to replicate this, however, were initially unsuccessful; the Gerwick group only reported 35% isolated yield, and even that was difficult to replicate. The aldol reaction gave a large amount of a crystalline white solid side product, and it was difficult to isolate **1.18** from it. A deeper look at the Gerwick study showed that they had difficulty with a decomposition product, and they got around it by using the superquat oxazolidinone.[11] However, that change was ineffective. It was also difficult to determine the identity of the side product, but eventually it was determined to be a decomposition product; LDA was deprotonating the benzyl proton of **1.17**, which decarboxylated the oxazolidinone and gave substituted styrene **1.19**.



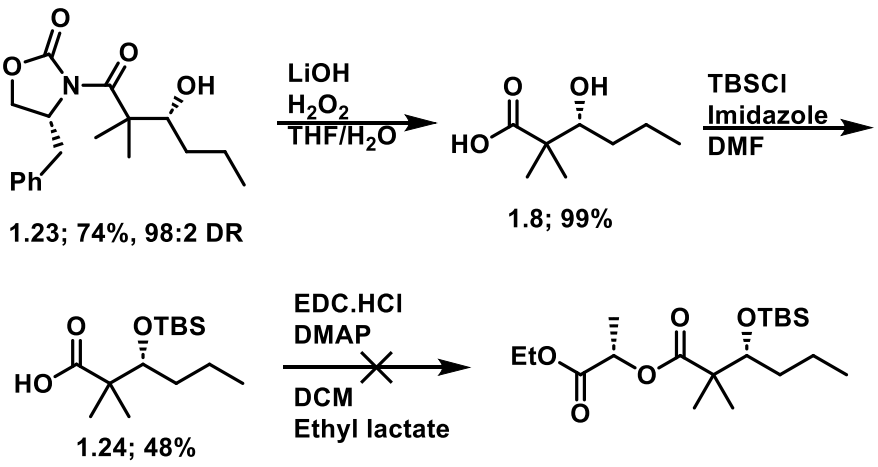
Scheme 1.7: Decomposition of phenyl oxazolidinone with LDA.

Once the identity of the side product had been confirmed, synthesis of the benzyl oxazolidinone was performed, and the problems circumvented. In fact, by simply keeping the reaction at -78 °C for the duration of the reaction rather than warming to -40 °C after the titanium addition significantly increased the yield. With a simple and efficient route that was scalable to at least 2 g, studies towards completing the ester half of the molecule could continue.



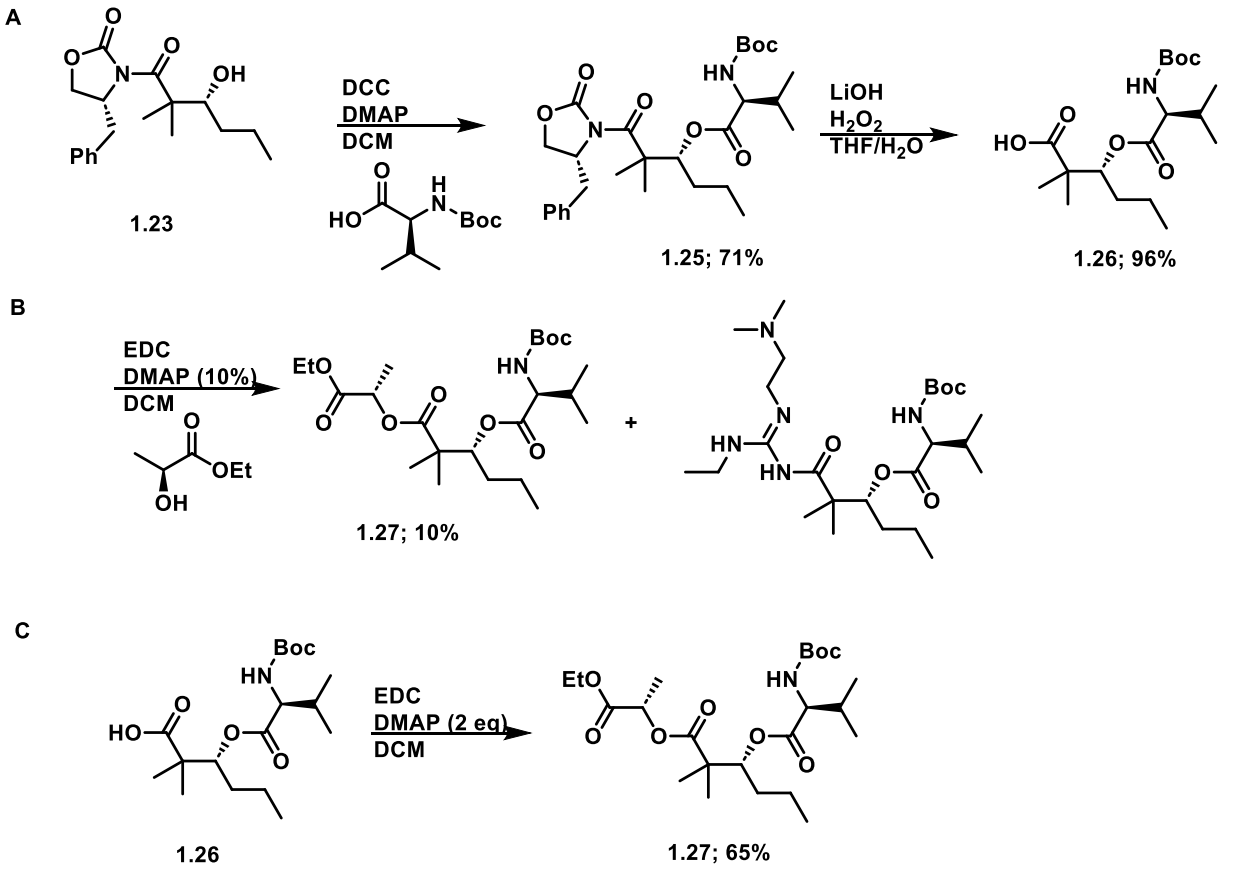
Scheme 1.8: Aldol route to **1.23**.

With ample quantities of **1.23** in hand, the next step was to determine the optimal order of forming the esters. With the theory that if valine was coupled to the alcohol portion of **1.23**, oxazolidinone removal conditions would also have an effect on the DMHHA-valine ester, attempts were made to protect the alcohol and remove the oxazolidinone first. Thus, different alcohol protecting groups, such as TBS, MOM, BOM, and the allylic ether, were screened to determine which would allow easy esterification of the carbonyl. However, problems arose when attempting to remove the oxazolidinone auxiliary. In the absence of an alcohol protecting group, the oxazolidinone comes off smoothly in 99% yield using lithium hydroxide and 30% aqueous hydrogen peroxide in 4:1 THF:water. Using the TBS and BOM protecting groups, there was no reaction. The allylic ether reacted with hydrogen peroxide. To circumvent this, the oxazolidinone was cleaved off prior the protection of the alcohol, and the protecting group of choice was TBS. The other protecting groups attempted either gave no reaction or reacted with both the alcohol and the carboxylic acid. However, although the TBS alcohol was able to be isolated, attempts to esterify the acid with ethyl lactate were unsuccessful.



Scheme 1.9: Attempts to synthesize the ester half with TBS protection.

To circumvent this, rather than removing the oxazolidinone immediately after the aldol reaction, compound **1.23** was first coupled to valine using EDC and DMAP in DCM. Surprisingly, removal of the oxazolidinone went smoothly with no effect on the DMHHA-Val ester. Esterification of the free acid portion of DMHHA with ethyl lactate, also with EDC and DMAP in DCM, was still sluggish initially due to the formation of a side product. The side product was determined to be the formation of a guanidine amide, known to form during slow reactions with carbodiimide-activated esters[12]. Fortunately, the problem was easily circumvented by increasing the equivalents of DMAP from 0.1 equivalents to 2 equivalents, raising the yield of the desired ester from 10% to 65%.

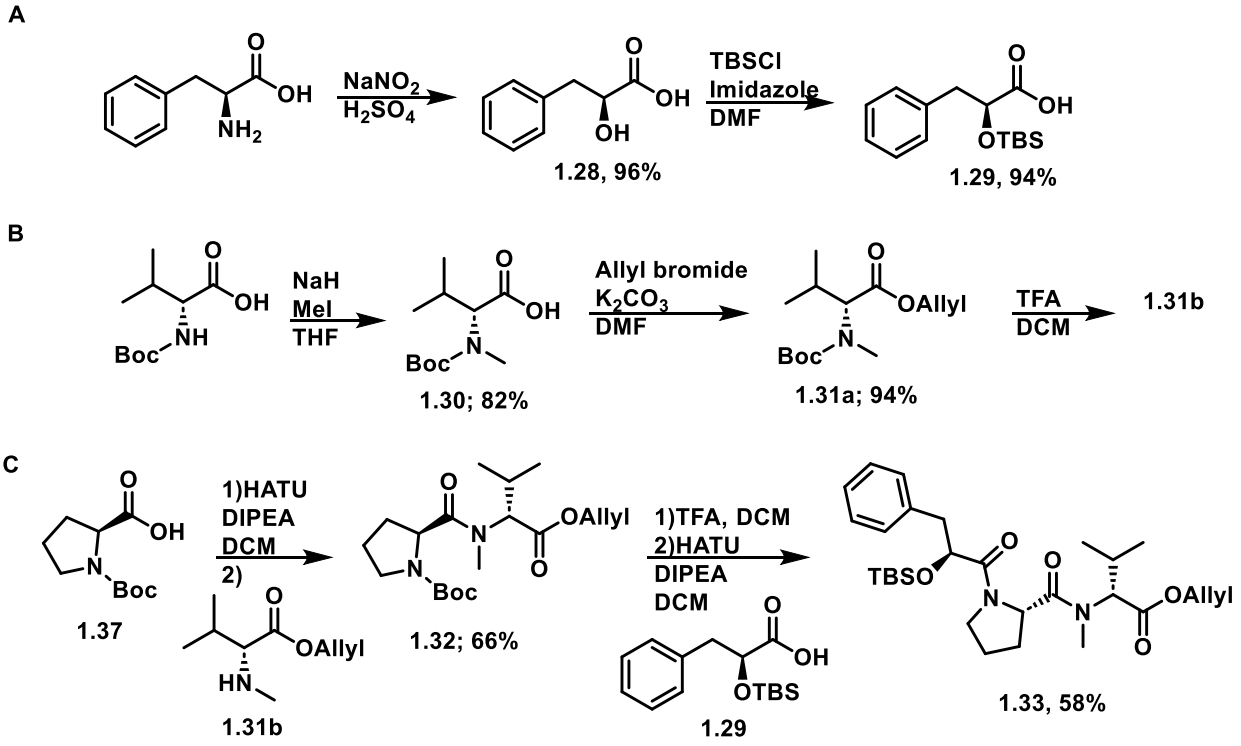


Scheme 1.10: A) Steglich coupling of aldol product **3.23** followed by oxazolidinone removal. B) Formation of guanidine amide side product from sluggish esterification. C) Final esterification with two equivalents of DMAP.

1.4.3 Synthesis of the Peptide Half

Synthesis of the peptide half of palmyramide A began with preparation of the amino acids. Amino acid **1.31a** was prepared by reacting *N*-boc-D-valine with sodium hydride and iodomethane in THF to give the *N*-methylated amino acid **1.30** in 82% yield. D-valine could also be *N*-methylated by reacting the Fmoc protected D-valine with paraformaldehyde in the presence of catalytic *p*-toluenesulfonic acid in refluxing toluene to give the corresponding 5-oxazilidinone, and then reducing it back to the amino acid using triethylsilane and TFA in DCM to give *N*-methylated D-valine in 94%. This method gave slightly better yields of the *N*-methylated valine; however, due to the simplicity of the NaH method, along with it being clean enough to not need purification, it was decided to be satisfactory to proceed with the slightly lower yield. The amino

acid was then *O*-allyl protected with allyl bromide and potassium carbonate in DMF in 94% yield. Boc-deprotection with TFA in DCM and subsequent peptide coupling to *N*-boc-proline gave dipeptide **1.32** in moderate 66% yield. L-phenyllactic acid (L-Pla, **1.28**) was prepared by diazotization of L-phenylalanine in excellent 96% yield with retention of stereochemistry. The newly formed alcohol was protected as the TBS-alcohol with imidazole in DMF in 94% yield. Following Boc-deprotection of dipeptide **1.32** using 1:1 TFA in DCM, **1.29** was then coupled with the dipeptide in moderate 58%. The route is summarized in **Scheme 1.11** below.

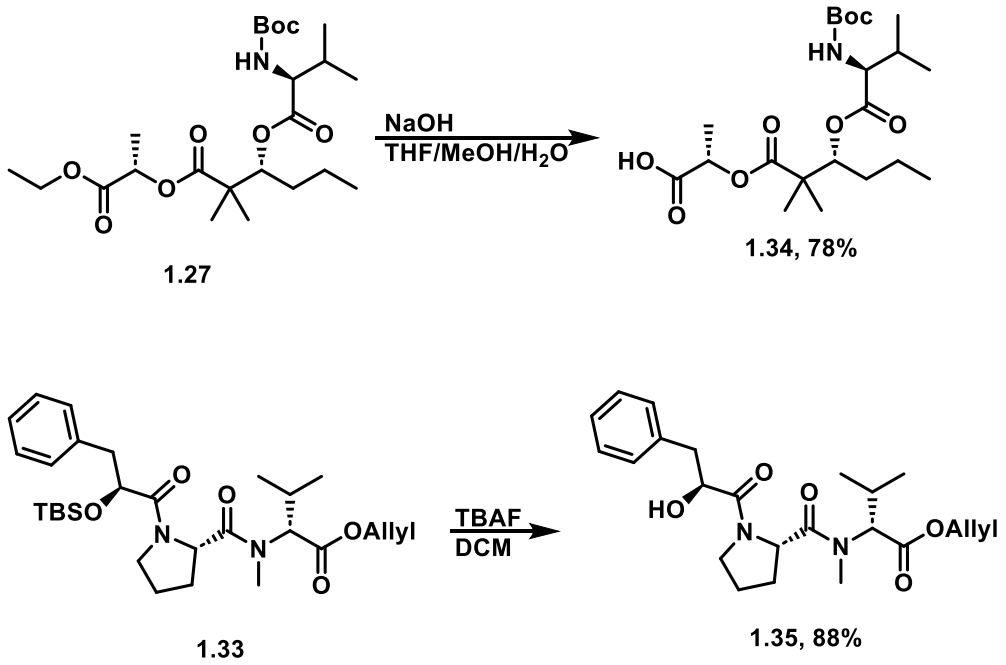


Scheme 1.11: A) Diazotation and subsequent TBS protection of phenylalanine. B) *N*-methylation and allyl protection of D-valine. C) Peptide couplings to form the peptide half **1.33** of palmyramide A.

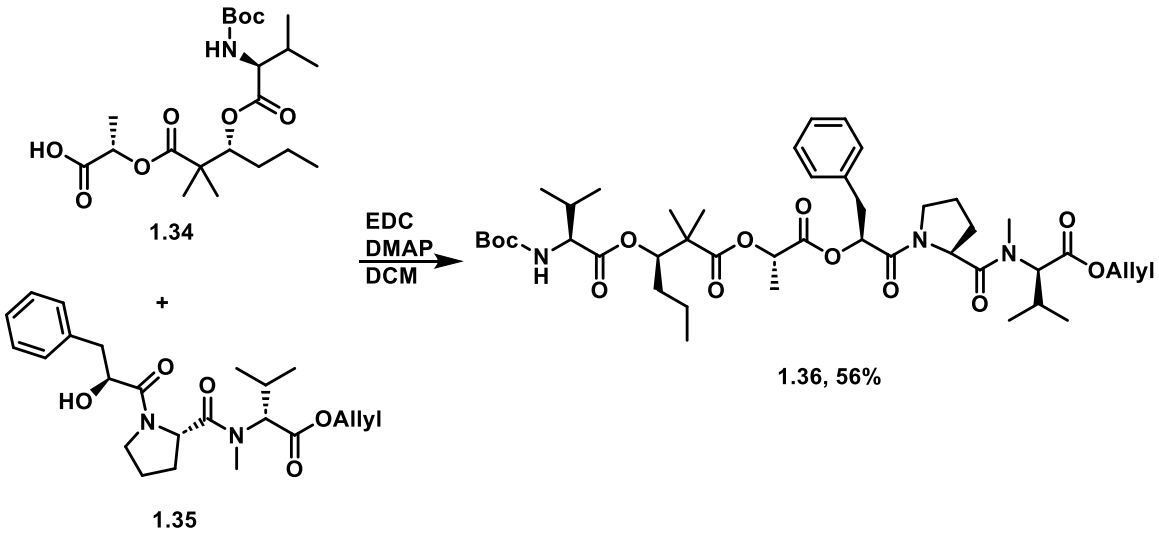
1.4.4 Forming the Linear Intermediate and Completion of Palmyramide A

With the ester and the peptide halves of palmyramide A completed, work towards coupling and cyclization could commence. The peptide half was easily deprotected with TBAF in THF to give free alcohol **1.35** in 88% yield, and the ester half was deprotected with NaOH in

methanol/THF/water to give free acid **1.34** in 78%. Peptide coupling of the two major fragments using EDC gave the protected linear intermediate in reasonable 56% yield.



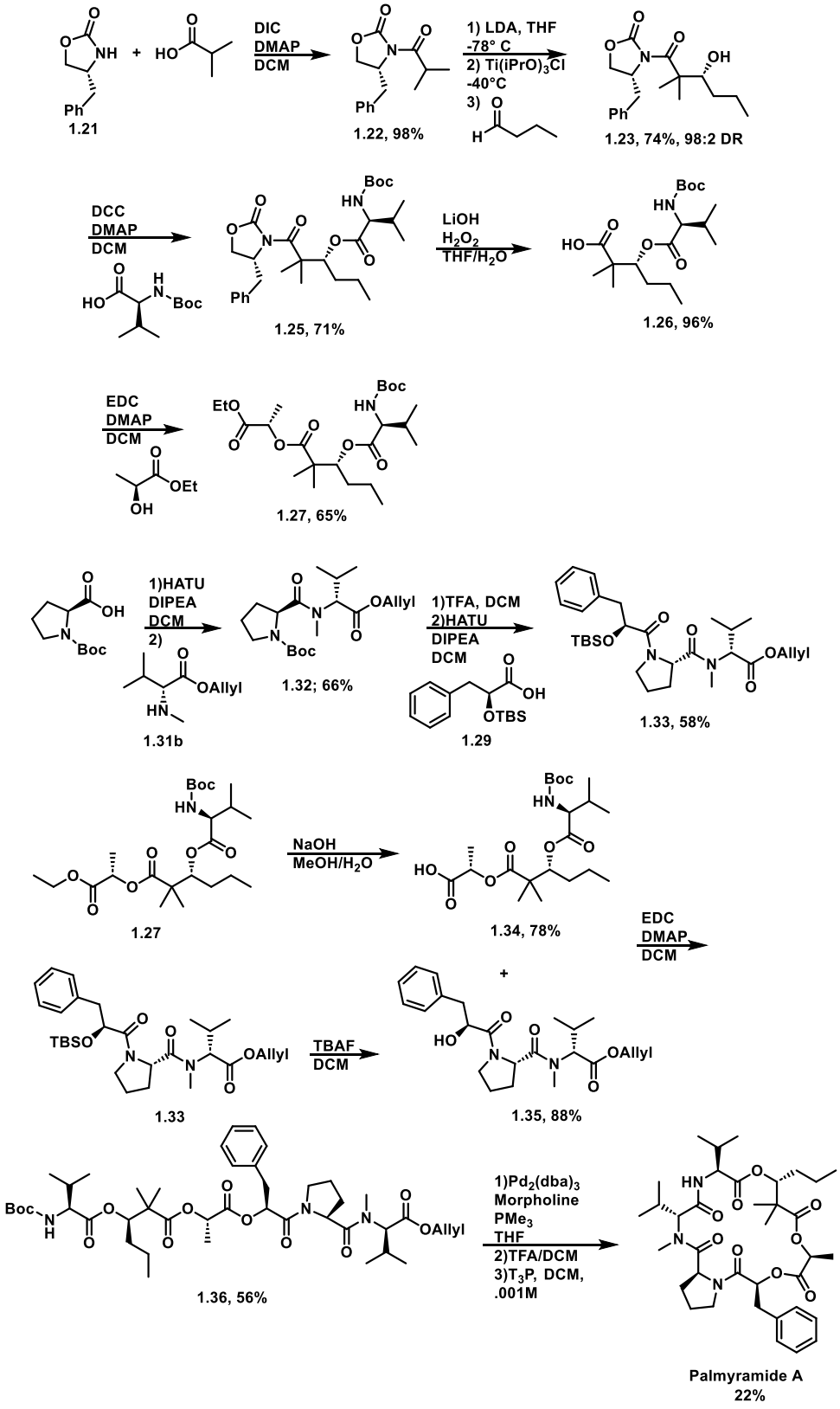
Scheme 1.12: Deprotection of the two palmyramide halves.



Scheme 1.13: Formation of the linear intermediate.

With the linear intermediate in hand, attempts at deprotecting and cyclizing palmyramide A could commence. Boc deprotection of the N-terminus went smoothly; however, deprotection

of the C terminus was more challenging. Tetrakis(triphenylphosphine)palladium(0) removed the allyl ester, but there was significant contamination by triphenylphosphine that could not be removed by flash chromatography, hplc, or by chelating with zinc chloride in ethanol.[13] Cyclization was still attempted with the impurity present to determine if the phosphine could be removed in the next step. Cyclizing in dilute DCM with T3P as the coupling reagent over 48 hours was successful; however, triphenylphosphine oxide remained in the sample even after flash chromatography and HPLC. Weix and coworkers reported that triphenylphosphine oxide can be removed from samples by precipitation from ethanol with $ZnCl_2$;[13] however, this was also unsuccessful in the palmyramide A sample. To circumvent using Tetrakis(triphenylphosphine)palladium(0), using different sources of palladium (0) and different ligands were explored. Literature has shown that dppe can be used in combination with a palladium (II) source to remove allyl ethers and amines;[14] however, attempts to remove the allyl ester with palladium (II) acetate and tppe were unsuccessful. To ensure that the palladium (II) not being reduced wasn't the problem, tris(dibenzylideneacetone)dipalladium(0) was used instead as the palladium source, but this also only gave back starting material. The next attempt at deallylation was to use a phosphine that could more easily be removed than triphenylphosphine. Trimethylphosphine was attempted with the thought that it could be easily removed under vacuum due to its low boiling point, and this was successful. Finally, with the clean deprotected linear intermediate in hand, cyclization was again attempted at 0.001 M in DCM with T_3P as the activating agent and the reaction gave palmyramide A in 22% yield.



Scheme 1.14: Finalized route to palmyramide A.

The final route to palmyramide A shown in scheme **1.14** consists of 11 steps with an overall yield of 1% due to the low yielding final step. Disscounting the final cyclization step, the overall yield was 5%, thus with optimization the overall yield may increase. Comparison of the spectra of synthetic palmyramide and natural palmyramide confirmed within reasonable doubt that they are the same. Analysis of ^{13}C NMR was particularly close, with the synthetic compound having a maximum difference of 0.3 ppm (carbon 4 of DMHHA). Proton NMR is also very similar, with some small differences in J-values most likely due to concentration and temperature differences when the experiment was collected.[15] Comparisons of two-dimensional spectra of the synthetic and natural compounds also confirmed the structures were the same.

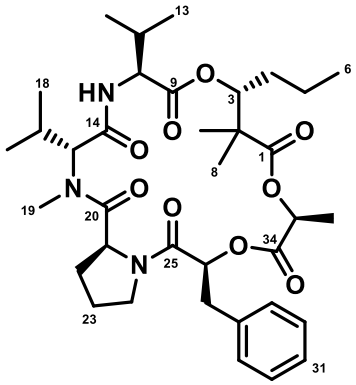


Figure 1.3: A) Table of chemical shifts of for ^{1}NMR of palmyramide A. The columns in blue contain the data from the synthetic product. B) ^{13}C NMR of palmyramide A, with a column showing the difference in chemical shifts between the synthetic product and the naturally occurring compound.

^{1}H NMR							
Residue	Position	1H δ Exp	Mult, *J* -values	1H δ Literature	Mult, *J* -values	Difference	abs diff
Dmhha	1					0	0
	2					0	0
	3	5.62	dd, 2.0, 9.6	5.57	dd, 9.6, 1.8	0.05	0.05
	4a	1.55	m	1.43	m	0.12	0.12
	4b	1.59	m	1.52	m	0.07	0.07
	5	1.34	m	1.36	m	-0.02	0.02
	6	0.88	t, 6.8	0.92	t, 7.2	-0.04	0.04
	7	1.25	s	1.21	s	0.04	0.04
	8	1.2	s	1.16	s	0.04	0.04
Val	9					0	0
	10	4.5	dd, 3.1, 5.4	4.46	dd, 5.4, 3.0	0.04	0.04
	11	2.42	td, 3.0, 7.1, 7.1	2.38	dqq, 3.0, 7.2, 7.2	0.04	0.04
	12	0.93	d. 7.0	0.89	d, 7.2	0.04	0.04
	13	1.02	d, 7.1	0.98	d, 7.2	0.04	0.04
	NH	6.58	d, 5.3	6.55	d, 5.4	0.03	0.03
N-MeVal	14					0	0
	15	4.46	d, 11.2	4.42	d, 10.8	0.04	0.04
	16	2.17	m	2.13	m	0.04	0.04
	17	0.75	d, 6.6	0.7	d, 6.6	0.05	0.05
	18	0.98	d, 6.6	0.94	d, 6.6	0.04	0.04
	19 (N-CH3)	2.89	s	2.85	s	0.04	0.04
Pro	20					0	0
	21	3.28	dd, 2.9, 8.5	3.22	dd, 7.8, 3.0	0.06	0.06
	22	1.55	m	1.5	m	0.05	0.05
	23a	1.6	m	1.56	m	0.04	0.04
	23b	1.71	m	1.68	m	0.03	0.03
	24a	3.46	dt, 7.1, 11.5	3.42	ddd, 12.0, 6.6, 6.6	0.04	0.04
	24b	3.61	ddd, 4.2, 7.8, 12.0	3.57	ddd, 12.0, 8.4, 4.8	0.04	0.04
Pla	25					0	0
	26	5.07	dd, 4.7, 11.2	5.02	dd, 12.0, 4.8	0.05	0.05
	27a	2.99	dd, 4.7, 12.4	2.95	dd, 12.0, 4.8	0.04	0.04
	27b	3.17	t, 11.8	3.13	dd, 12.0, 4.8	0.04	0.04
	28					0	0
	29/33	7.27	m	7.23	m	0.04	0.04
	30/32	7.31	m	7.28	m	0.03	0.03
	31	7.27	m	7.25	m	0.02	0.02
Lac	34					0	0
	35	4.85	q, 7.1	4.81	q, 6.6	0.04	0.04
	36	1.51	d, 7.2	1.47	d, 6.6	0.04	0.04

Figure 1.3 cont.

^{13}C NMR					
Residue	Position	Published δ	Assignment	Experimental δ	Difference
DMHHA	1	174.9	qC	175	-0.1
	2	46.4	qC	46.5	-0.1
	3	77.2	CH	77.4	-0.2
	4a	31.6	CH2	31.9	-0.3
	4b				
	5	19.6	CH2	19.7	-0.1
	6	13.9	CH3	14	-0.1
	7	16.9	CH3	17	-0.1
	8	24.3	CH3	24.4	-0.1
L-Valine	9	170.1	qC	170.2	-0.1
	10	58.3	CH	58.5	-0.2
	11	30.5	CH	30.6	-0.1
	12	17.8	CH3	17.9	-0.1
	13	18.3	CH3	18.5	-0.2
	NH				
D-N-MeVal	14	169.7	qC	169.8	-0.1
	15	61.7	CH	61.9	-0.2
	16	27.3	CH	27.4	-0.1
	17	18.8	CH3	18.9	-0.1
	18	19.3	CH3	19.4	-0.1
	19 N-CH3	30.5	CH3	30.6	-0.1
Pro	20	171.2	qC	171.4	-0.2
	21	56.7	CH	56.8	-0.1
	22	30.7	CH2	30.9	-0.2
	23a	22	CH2	22.1	-0.1
	23b				
	24a	46.7	CH2	46.8	-0.1
	24b				
Pla	25	167.2	qC	167.2	0
	26	70.9	CH	71	-0.1
	27a	39.2	CH2	39.3	-0.1
	27b				0
	28	134.9	qC	135.1	-0.2
	29/33	129.7	CH	129.8	-0.1
	30/32	128.6	CH	128.7	-0.1
	31	127.4	CH	127.4	0
Lac	34	168.3	qC	168.4	-0.1
	35	69	CH	69.1	-0.1
	36	16.7	CH3	16.8	-0.1

Figure 1.3 cont.

A sample of palmyramide A was provided to the Gerwick group to test its anti-cancer properties against human lung carcinoma cell line H-460. Synthetic palmyramide A had an IC_{50} of 54 μM, which is similar to the published value of 40 μM for the isolated natural product.

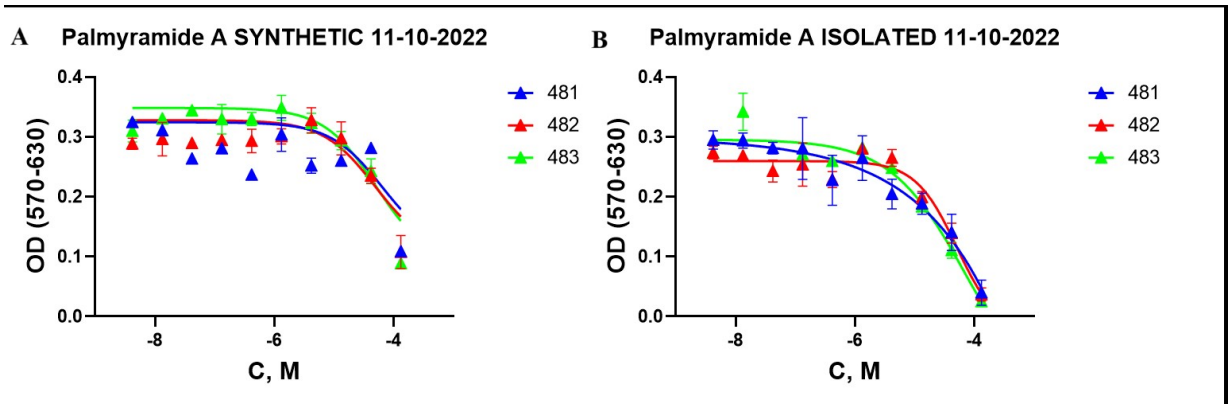


Figure 1.4: Comparison of activities of synthetic palmyramide A (A) and naturally occuring palmyramide A (B) against H-460 lung cancer cells. Naturally occuring palmyramide A had an IC_{50} of 40 μM, and synthetic palmyramide A had an IC_{50} of 54 μM.

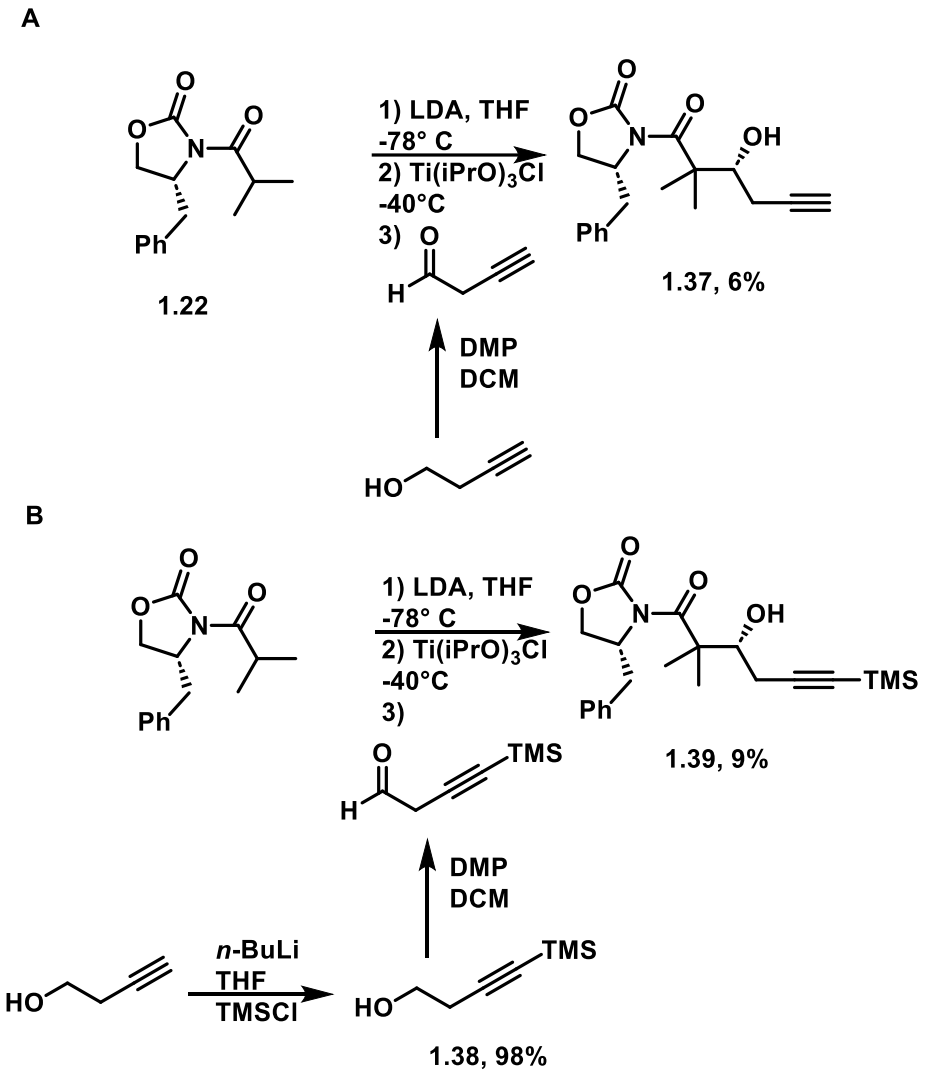
However, when tested against colon cancer, synthetic palmyramide A had no activity, while the naturally occurring compound had good activity (8.9 nM). At this point, it is still unclear what is causing this discrepancy, though there are many factors that could come into play. For example, even though the structural connectivity is the same, there could be differences in the conformations; the natural compound may have been put together in a different order than the synthetic compound, and the natural compound was not synthesized in organic solvents using synthetic coupling agents. Another possibility is that the natural compound may have a small quantity of an unknown impurity that has excellent activity against colon cancer.[16]

1.5 Synthesis of Tagged Palmyramide A

With the knowledge that synthetic palmyramide A behaves similarly in biological assays as the naturally occurring compound, the next step was to determine its biological target. There are many methods to determine this[17]. The method that was chosen was to modify part of the compound by adding on an alkyne, which could be used as a handle to attach a tag such as biotin

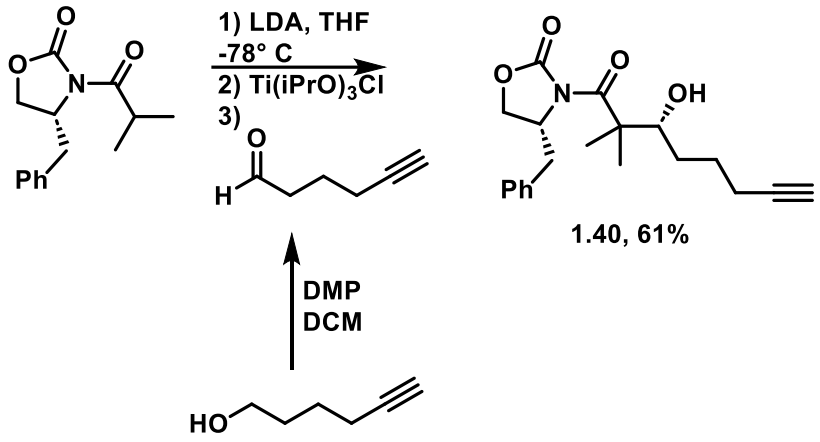
and do a traditional pull-down study to find out where palmyramide A is binding in the active site.[18] DMHHA, being an aliphatic chain which was theorized to stick outside of the binding pocket, was chosen as the site of modification.

To keep the modified DMHHA structure as close as possible to the unaltered version, butyraldehyde was originally substituted with but-3-ynal, which was formed by oxidation of but-3-yn-1-ol with DMP in DCM. This oxidation went smoothly; however, the aldol reaction with 1.22 under the same conditions as previously used for butyraldehyde gave back mostly starting material and very little product. This low yield was probably due to the resonance stabilization of the enolate formed from the but-3-ynal. Thus the α-proton was easily deprotonated by the enolate instead of the anticipated aldol reaction. Once the enolate deprotonates the aldehyde rather than reaction with the carbonyl carbon, the desired reaction is unable to proceed. Protecting the alkyne with a TMS group unfortunately did not significantly improve the aldol yield.



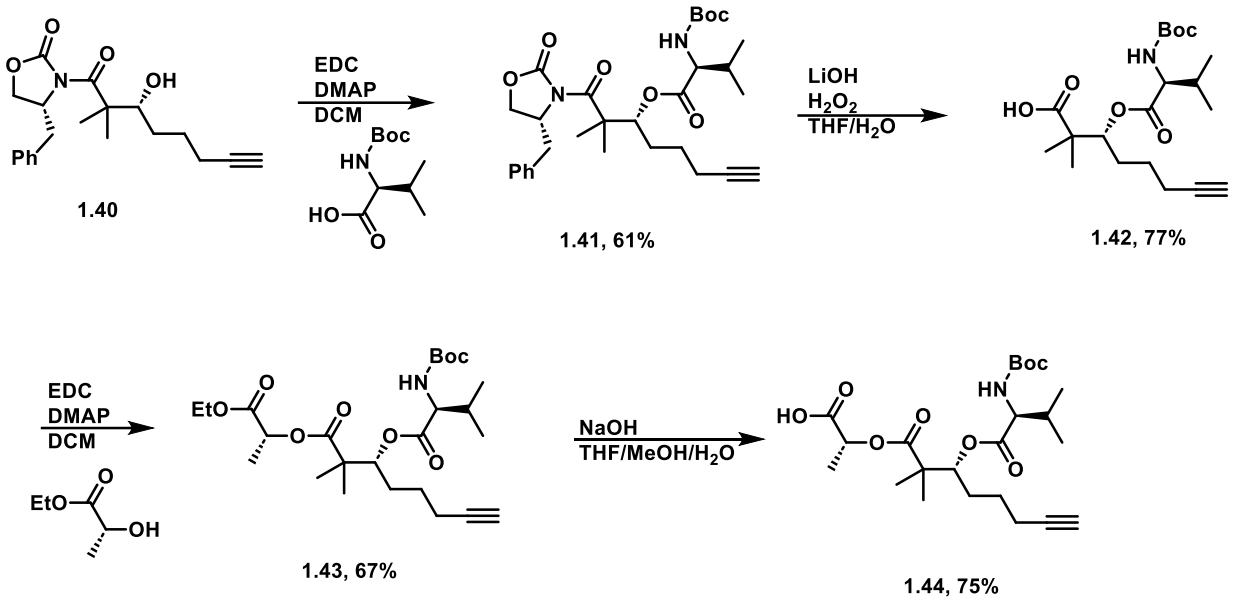
Scheme 1.15: Synthetic scheme towards alkyne labeled DMHHA with butynal (A) and with TMS protected butynal (B).

To avoid this, 3-butyn-1-ol was switched out to 5-hexyn-1-ol with the idea that lengthening hydrocarbon chain would not be detrimental to the biological activity of palmyramide A, and it might even be helpful for the pull-down study by adding distance between the active site, the triazole ring formed by the click reaction, and the biotin that was added by the click reaction.



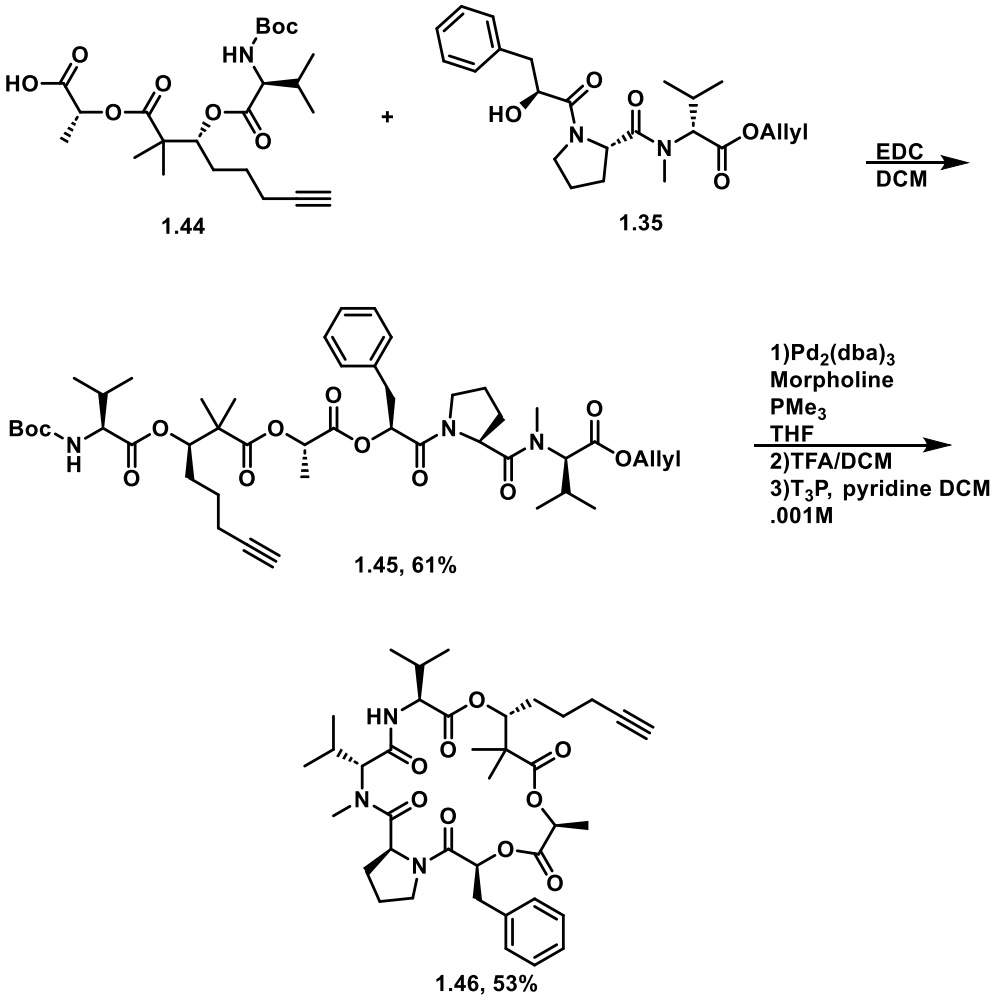
Scheme 1.16: Aldol reaction with oxidized hex-5-yn-1-ol.

Switching to 5-hexynol made little difference on the DMP oxidation, but had a large effect on the aldol reaction, improving the yield significantly to 61%. Once the aldol reaction was optimized, the rest of the synthesis went as predicted. Steglich esterification of the secondary alcohol on **1.40** with Boc-L-valine gave ester **1.41** in moderate 61%. Cleavage of the oxazolidinone with lithium hydroxide and 30% hydrogen peroxide proceeded smoothly, even in the presence of the alkyne, to give free acid **1.42** in 77%. Steglich conditions were used again to couple free acid **1.42** to ethyl lactate to give the ester **1.43** in 67% yield. Selective saponification of the ethyl ester of **1.43** with sodium hydroxide in THF and water gave free acid **1.44** in 75%.



Scheme 1.17: Synthesis of the alkyne tagged ester half of palmyramide A.

Following the synthesis of unmodified palmyramide A, Steglich conditions again were used to give linear intermediate **3.45** in moderate 61% yield. Allyl deprotection using $Pd_2(dba)_3$, trimethylphosphine, and morpholine in THF went smoothly. Boc deprotection with TFA in DCM followed worked in a similar manner to the formation of palmyramide A. Finally, cyclization of the deprotected linear intermediate with T3P in the presence of pyridine in DCM was slower than cyclization of unmodified palmyramide A (**3.46**), but also gave a slightly better 53% yield.



Scheme 1.18: Formation of the alkyne tagged linear intermediate and completion of tagged palmyramide A.

The modified palmyramide A was supplied to the Gerwick lab for click chemistry and the subsequent pull-down study.

1.6 Conclusions

Palmyramide A was synthesized in 11 synthetic steps with a 1% overall yield, though the final yield can be increased with optimization of the final ring closing step. The original synthesis attempted to use a novel 1,4 addition of a silane catalyzed by $(CuI)_4(DMS)_3$ to 2-hexenoic acid with an oxazolidinone to drive stereochemistry. Attempts to trap iodomethane by the in-situ generated enolate failed, probably due to the steric bulk from the aforementioned oxazolidinone and the newly installed silane. Due to the unforeseen length of this synthesis

brought upon by the difficulty outlined above, as well as the low yield of the Fleming oxidation, a new route was developed using a non-Evans aldol reaction. The non-Evans aldol, using the unusual $Ti(iPrO)_3Cl$ as the Lewis acid, gave the *trans* product in very good 74% yield with 98:2 DR. The linear intermediate was constructed by first making two trimers, the ester half and the peptide half. The ester half was formed using Steglich conditions (EDC, DMAP, DCM), and the peptide half was formed using typical HATU coupling conditions (HATU, DIPEA, DCM). The linear intermediate was formed again using Steglich conditions. Deprotection of the allyl protecting group went smoothly with $Pd(PPh_3)_4$; however, PPh_3 proved difficult to remove from the product. $Pd(PPh_3)_4$ was replaced with $Pd_2(dba)_3$ and PMe_3, and the reaction again proceeded smoothly. This time, fortunately, PMe_3 was easily removed under vacuum. Boc deprotection and subsequent cyclization, dilute in DCM using T_3P, yielded palmyramide A.

The synthetic compound's structure was confirmed by 1D and 2D ^{1}H- and ^{13}C-NMR, as well as by mass spectrometry. The compound was subjected to biological evaluation and showed similar activity to the natural product against HC-460 lung cancer cells, although the synthetic compound showed no activity against colon cancer, with the natural product showing modest activity. A synthetic analogue of palmyramide A was also synthesized to determine its molecular mechanism of action.

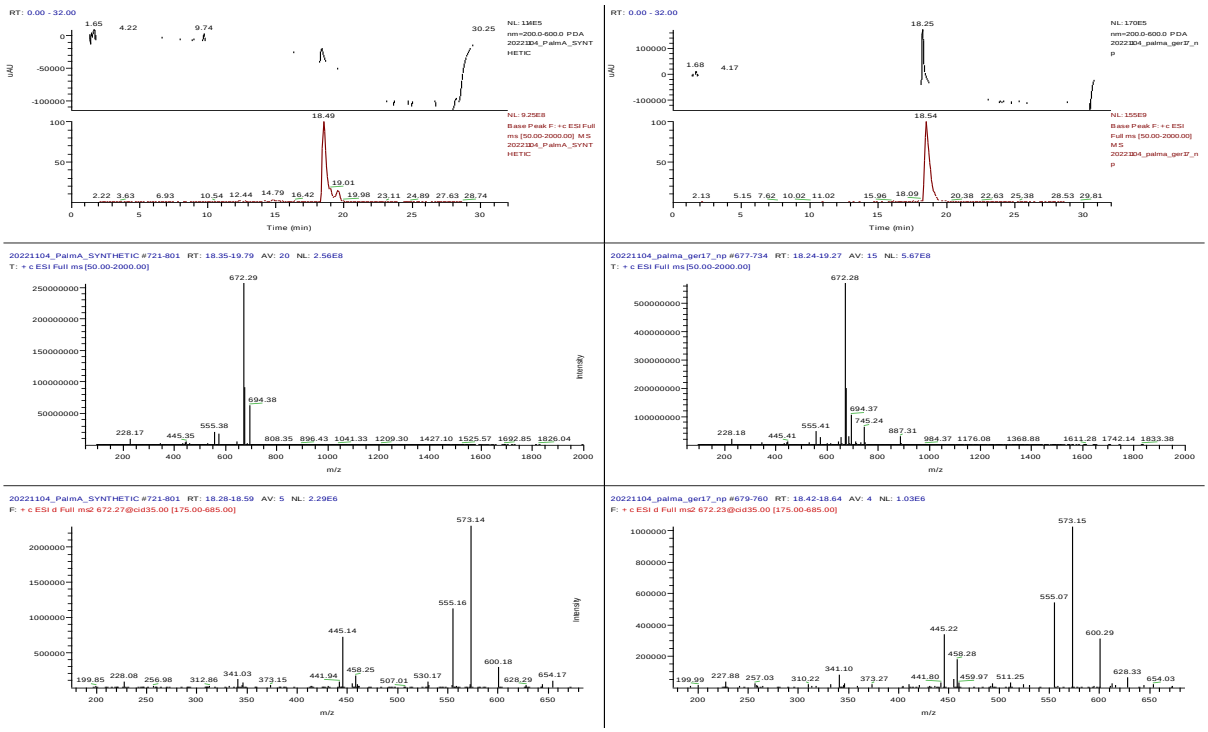


Figure 1.5: Comparison of synthetic palmyramide A (left) vs isolated palmyramide A (right). The top compares the HPLC trace, showing both compounds have the same retention time. The middle shows the mass spectrum, with the synthetic and natural compounds having the same molecular ion peak. The bottom shows that the synthetic and natural compounds have identical fragmentation patterns.

The synthetic analogue with an alkyne at the end of the DMHHA fragment was synthesized in much the same way as the natural product. Using the interesting non-Evans aldol reaction, but with hexynal replacing butanal, proceeded smoothly. Forming the modified ester half using Steglich conditions also worked as before. Construction of the modified linear intermediate, as well as deprotection with $Pd_2(dba)_3$ and PMe_3, worked in the same manner as with the natural product as well. Finally, cyclization to form the modified natural product was done dilute in DCM with T_3P as the activating agent and yielded the alkyne modified natural product.

Future work for palmyramide A would be to use click chemistry to attach a handle to the synthetic analogue described in chapter 3.4.4 to determine its molecular target in lung cancer.

There is a possibility that it acts in a novel fashion, and by learning how it works we can develop targeted analogues that have better activity and less systemic toxicity. Even if it does not act upon a novel target, knowing its activity can help in further development of future analogues.

Chapter 1 contained information being prepared for submission for publication. Walsworth, Kevin; Bergdahl, B. Mikael. The dissertation author is the principal investigator and author of this material.

PART II: SYNTHESIS OF NEW HEPATITIS C VIRUS TRANSLATION INHIBITORS

CHAPTER 2: INTRODUCTION

2.1 Introduction

The World Health Organization estimates that there are approximately 58 million people worldwide with a chronic hepatitis C virus (HCV) infection, with an estimated 1.5 million new infections per year.[19] HCV can cause both acute and chronic infections, with symptoms ranging from mild to severe lifelong illness, including hepatocellular carcinoma and liver cirrhosis. In contrast to hepatitis A virus (HAV), which is transmitted orally, and hepatitis B virus (HBV), which is transmitted through transmission of bodily fluids, HCV is primarily transmitted through blood contact.

Common sources of contamination are the use of contaminated needles associated with intravenous drug use or improper procedures by healthcare providers, blood transfusions with infected blood, or transmission from mother to fetus *in utero*.[20,21]

Until recently, HCV treatments have been limited, and suffered from low efficacy and adverse side-effects. Recently, many pharmaceutical companies have released improved drugs and therapies to treat HCV.[20] These drugs target the proteins coded by HCV and disrupt the virus's ability to replicate its genome. In 2005, researchers at Ionis Pharmaceuticals discovered a novel target, located in the 5' untranslated region of the virus's genome, that could be targeted by small molecules to disrupt translation.[22] The novel target, known as the internal ribosome entry site (IRES), is a highly conserved region of RNA across all genotypes or HCV, making it an attractive therapeutic target.[23]

2.2 Small Molecules Targeting RNA

As our understanding of the role RNA plays in many biological processes has increased, it has become of more interest as a target for small and large molecule drugs.[24] Although RNA

is typically considered for its role as messenger RNA (mRNA), it has a multitude of other biological functions. For example, non-coding RNA can fold into complex three-dimensional structures that can interact with small molecules or macromolecules, such as ribosomal RNA interacting with transfer RNA in translation. The interactions of these structures not directly involved in protein translation is of particular interest, as the biological effects can be extremely diverse.[25]

The discovery of the natural product streptomycin in 1944 was the first evidence that RNA could be a therapeutic target.[26] Streptomycin, like most therapeutics that target RNA, targets ribosomal RNA (16S bacterial ribosomal RNA specifically for streptomycin). However, the true power of RNA as a target was the discovery of how the three-dimensional structure of RNA can have a diverse array of biological effects. Examples of these include the tat-TAR protein-RNA complex in HIV,[27] bacterial riboswitches,[28] and the HCV IRES.

RNA is typically considered difficult to target with small molecules.[29] The pockets that are targeted are hydrophilic, which is in contrast to hydrophobic protein pockets that are more commonly targeted for therapeutics.[30] This can cause problems with lead compounds not being specific enough, with ligand promiscuity being a common and difficult problem to overcome. Another problem with targeting RNA is how quickly the RNA can mutate, making drug resistance a major issue.[31] Because of this, current approaches target highly conserved and structured segments of RNA. The HCV IRES fits this description, along with playing an important role in the life cycle of the virus.

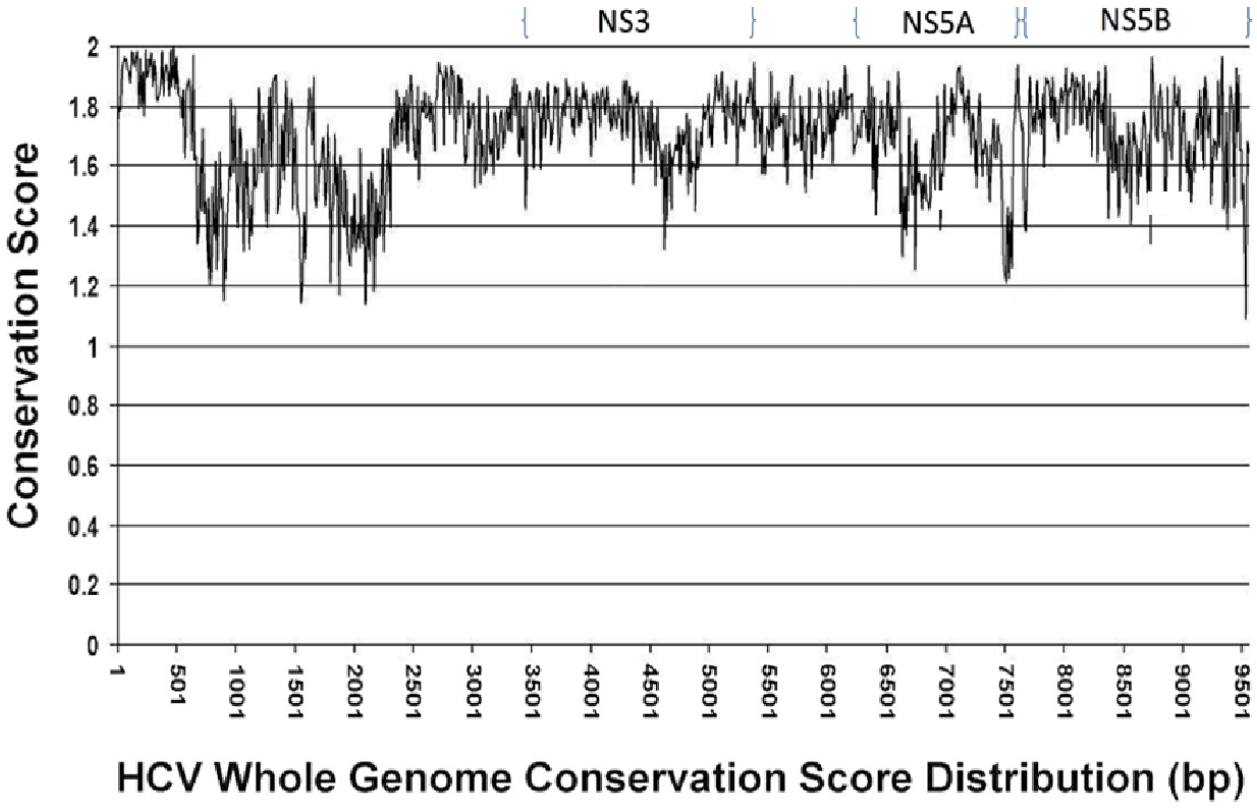


Figure 2.1: Conservation Score of HCV genome. Figure modified from ref.[32]

As seen in **figure 2.1** above from a study performed by Merck,[32] the genetic material that codes for HCV's proteins, including NS5B targeted by most current therapeutics, have segments that are not well conserved. The HCV IRES, located in bases 39 to 371, however, is very well conserved amongst all genotypes of HCV. Presuming there is an evolutionary reason for this high level of conservation, the HCV IRES may be a target of interest.

2.3 Hepatitis C Virus

HCV is single stranded positive sense RNA virus in the flavivirus family with a genome that acts as messenger RNA, being translated into viral proteins with little proofreading. The genome contains structured untranslated regions with high conservation amongst the six genotypes of HCV that allow the virus to bypass most of the host cell initiation factors required

in eukaryotic translation. HCV, along with other flaviviruses, contain a highly structured and conserved IRES in the 5' untranslated region.

Despite there being multiple treatments, including direct acting antiviral agents with up to a 95% cure rate, there are still approximately 71 million people worldwide with chronic HCV infections, and approximately 1.5 million new people infected every year and approximately 400,000 people die per year from complications, typically from hepatocellular carcinoma or liver cirrhosis.

2.4 HCV Treatments

In 1989, non-A and non-B hepatitis was officially named hepatitis C.[33] Interferon-α (IFN-α) was used in chronic hepatitis patients starting in 1986, and its use was continued when HCV was identified with some success (FDA approved in 1991). Patients showed marked immediate lowering of HCV content in blood serum; however, the virus showed resistance after only 6 months, with only a 6-12% efficacy rate at that time. In the late 90's it was discovered that IFN-α in combination with ribavirin increased the response rate up to 40%.[20]

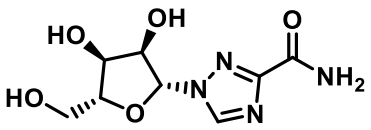


Figure 2.2: Chemical structure of ribavirin.

In 2000, it was discovered that adding a large polyethylene glycol (PEG) unit to IFN-α increased its efficacy up to 56% while also having better pharmacokinetic profile and longer half-life. For these reasons, along with the lack of a vaccine against HCV and that there were no other good options, PEG-IFN-α became the gold standard of HCV. However, PEG-IFN-α has many suboptimal side-effects against both body and mind, including ulcers, sores on the mouth, lack of appetite, tiredness, and depression.[34]

2.4.1 Boceprevir and Telaprevir

In 2011, the FDA approved the use of two Direct Acting Antivirals (DAA's) for use in combination with PEG-IFN-α called boceprevir (trade name Victrelis) and telaprevir (trade name Incivek). These DAA's target viral proteins and proteases.[35]

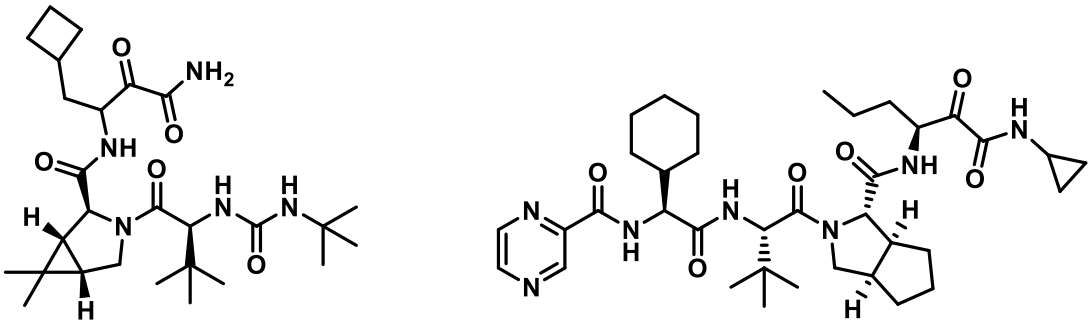


Figure 2.3: Chemical structure of boceprevir and telaprevir.

Boceprevir was originally developed by Schering-Plough prior being purchased by Merck in 2009. FDA approved in 2011 for genotype 1 of HCV, it non-covalently binds to HCV non-structural protein 3 (NS3), a serine protease. In combination with PEG-IFN-α and ribavirin it had an efficacy rate of 73% in genotype 1 of HCV. It was discontinued in 2015 due to superior activity of newer antivirals on the market.[36]

Telaprevir was developed in a collaborative effort by Johnson & Johnson and Vertex. Another drug specifically developed for genotype 1, telaprevir is a non-covalent inhibitor of NS3 serine protease and was FDA approved in 2011.[35] Again, it was most effective when used in triplicate with ribavirin and PEG-IFN-α, and had an efficacy rate of up to 75% when taken 3 times daily.[36]

2.4.2 Simeprevir

Simeprevir, sold under the brand name Olysio, was specific for HCV genotype 1. It is a DAA developed by Medivir AB and Janssen Pharmaceuticals, received FDA approval in 2013

for patients with compensated liver disease, though it was also commonly used off-label for genotype 4. It was also used in conjunction with ribavirin and PEG-IFN-α, and it is an inhibitor of the HCV NS3 serine protease. It showed the best efficacy rates of any DAA's at the time, with rates nearing 90% after 12 weeks.[37]

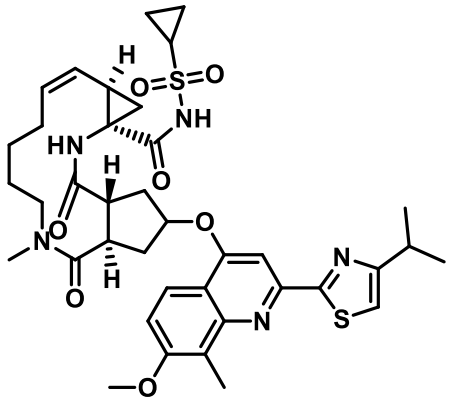


Figure 2.4: Chemical structure of simeprevir.

2.4.3 Sofosbuvir

Sofosbuvir, sold under the trade name Sovaldi, is a different DAA developed by Gilead, which is the current gold standard treatment for HCV. It was approved for use in all genotypes of HCV in 2013 and is on the World Health Organization list of essential medicines. It is a nucleoside analogue that inhibits HCV non-structural protein 5b (NS5b), an RNA-dependent RNA polymerase. In combination with velpatasvir (also developed by Gilead), it is recommended for all six genotypes and has an efficacy rate of higher than 90%, and in many cases, it is 100%. It should be noted that while it has less severe side effects than its predecessors, it may reactivate hepatitis B in patients that had been previously infected.[38]

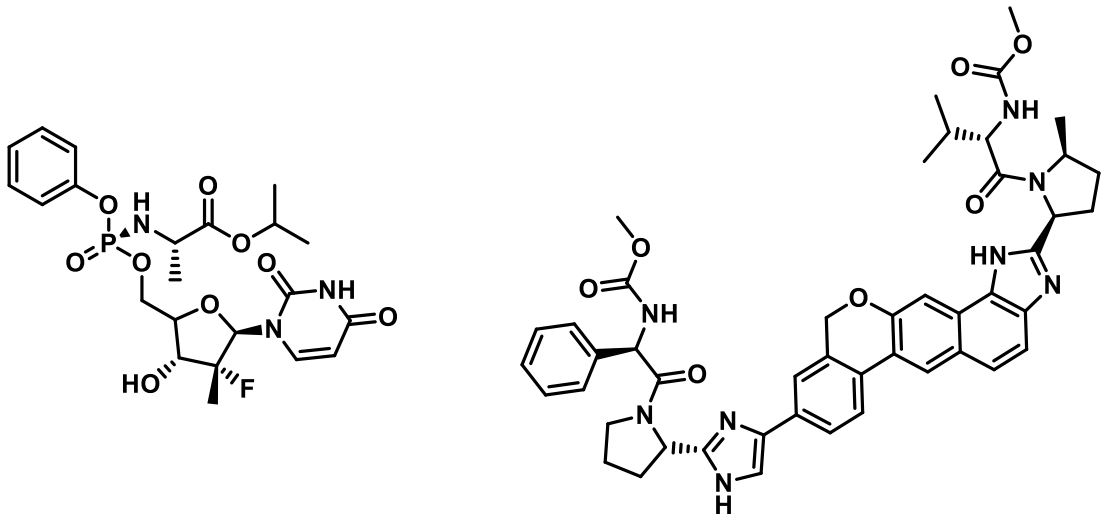


Figure 2.5: Chemical structures of sofosbuvir and velpatasvir.

CHAPTER 3: PROBING THE HCV IRES

3.1 HCV Internal Ribosome Entry Site

HCV is a member of the *Flaviviridae* family of viruses. The *Flaviviridae* family is broken up into three genera: flavivirus, pestivirus, and hepacivirus, with HCV and its six genotypes being a member of the hepacivirus genera. Other members of the hepacivirus genera include tamarin virus and GB virus B.

Members of the *Flaviviridae* family share many basic virological and structural characteristics. They are enveloped in a lipid bilayer in which two or more envelope proteins are anchored. Inside the envelope is a nucleocapsid which contains the RNA genome.[39]

As a member of the *Flaviviridae* family, HCV is a single stranded, positive-sense RNA virus whose genome serves directly as viral messenger RNA. The viral genome, approximately 9.6 kilobases in length, contains structured untranslated regions at both the 5' and 3' ends that surround the translated region. The translated region of the genome codes for one long polyprotein that is co- and post-translationally processed into mature gene products.

The 5' untranslated region of the HCV genome contains a highly structured and conserved IRES which allows the virus to translate its genome into proteins and to replicate its genome while bypassing many of the initiation factors required in cap-dependent eukaryotic translation. Cap independent viral translation offers several benefits, including escaping antiviral countermeasures of the cell and that the ends of the RNA genome do not need to serve functions in translation control.[40] Because of the importance of the IRES in viral translation, as well as its high conservation among all genotypes of HCV, it has high potential as a therapeutic target.[41]

Domain IIa of the HCV IRES is of particular interest. This region contains an RNA switch mechanism, with an internal loop that is flexible and undergoes a ligand dependent

conformational change between the ligand-free 90° L-shaped conformation and the ligand bound 180° straight conformation.[42] If this process is inhibited, the viral RNA is prevented from undergoing IRES driven translation initiation. Interestingly, according to a study of over 1000 clinical isolates of HCV from all six genotypes this region is particularly well conserved (**Figure 3.2**), with only two bases being lower than 84% conserved.

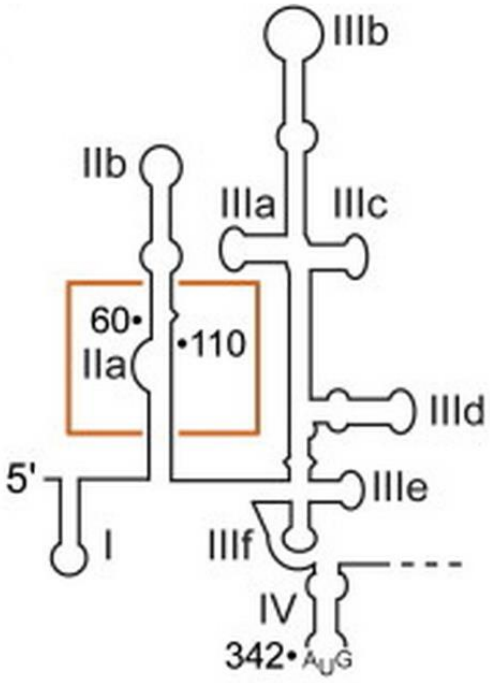


Figure 3.1: Secondary structure of the HCV IRES with stem-loop IIa marked in orange. Figure modified from ref.[43]

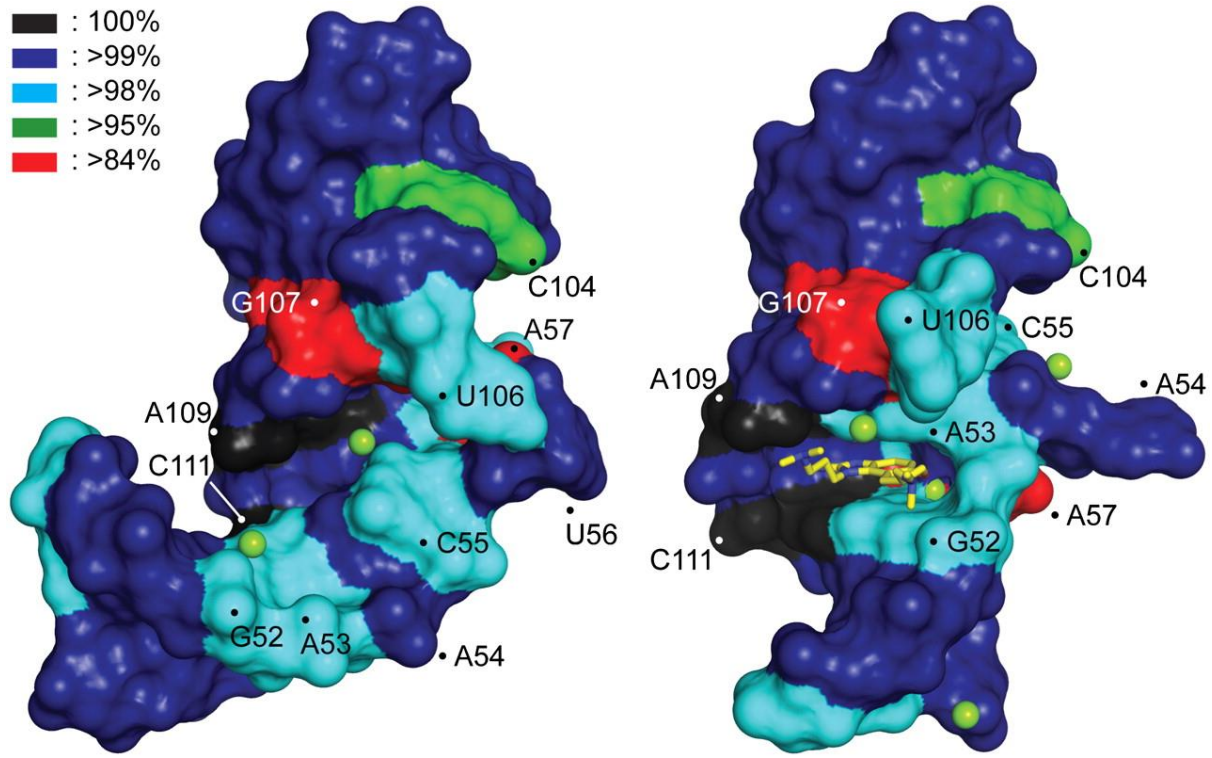
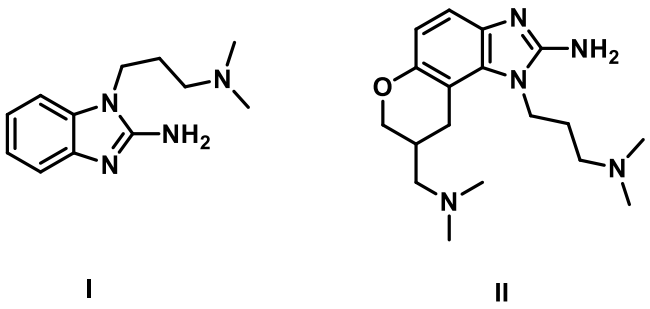


Figure 3.2: Conservation heat map of HCV IRES Domain IIa. Figure modified from ref.[44]

3.2 Benzimidazoles and FRET Development

In 2005, Seth and coworkers at Ionis Pharmaceuticals conducted a high-throughput mass spectrometry-based study to identify potential HCV translation inhibitors.[22] From their screening library, they discovered benzimidazole I had modest affinity and selectivity. Further derivatization gave them benzimidazole II, which was measured to have a K_D of 0.72 μM, a 140-fold increase in binding affinity relative to the initial hit.[22] While this hit was not strong enough for Ionis to move it down the drug discovery pipeline, it was potentially strong enough that a co-crystal structure of the RNA with the ligand bound could be found, which could elucidate the interactions between the ligand and RNA.



I II

Figure 3.3: Structures of Ionis benzimidazoles.

In 2013, the Hermann group published a crystal structure of the subdomain IIa RNA as a standalone unit and a co-crystal structure of the RNA with the benzimidazole II.[45] These crystal structures confirmed the conformational change from the 90° L-shaped conformation with no ligand bound and the 180° straight conformation with the ligand present. Based on this information, a FRET based assay was designed to test for compounds binding to this RNA switch. Fluorophores, specifically Cy3 and Cy5, were added by the Hermann group on the 5' end of both constructs taken from a portion of the RNA switch. With no ligand bound, the RNA in the L-shaped configuration has both fluorophores in the Förster radius, and FRET transfer between the dyes is observed. When the ligand is bound, the RNA takes the straight configuration the dyes are outside of the Förster radius, and the signal is quenched. Therefore, in this assay, the FRET signal will decrease in response to increasing concentration of a potential inhibitor, allowing the determination of the structure activity relationship between ligands and the binding pocket. Published in 2020,[46] our group attempted to further optimize benzimidazole II; however, our attempts were unsuccessful, and benzimidazole II still gave the best activity.

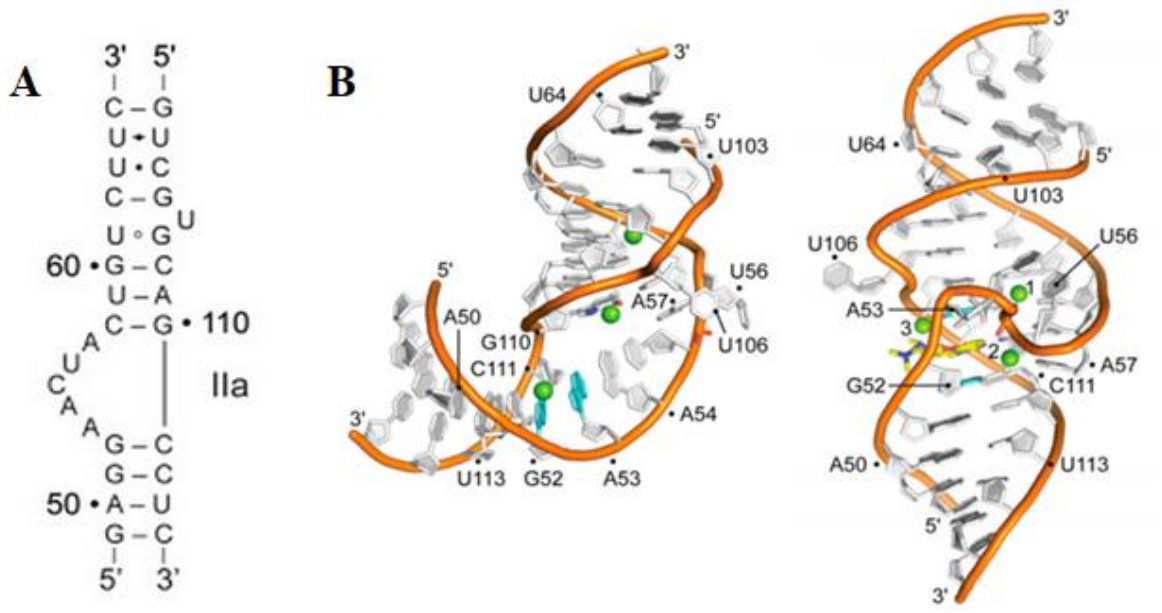


Figure 3.4: A) Secondary structure of domain IIa. B) Crystal structure of domain IIa in the non-ligand bound L shape conformation (left) and the ligand bound straight conformation (right). Figure adapted from ref.[45]

3.3 Improving Shape Complementarity

Being able to visualize the binding pocket, the native ligand in the 40S ribosome was determined to be guanine. Overlaying the guanine and Ionis benzimidazole **II**, it was possible to align the crucial hydrogen bonding interactions. This allowed us to determine available space in the back of the binding pocket not being accessed by the benzimidazole ligand. Moreover, it allowed the Hermann group to explore space-filling interactions in the RNA pocket not previously explored by the benzimidazoles.

In 2016, the Hermann group published an article probing the RNA pocket.[42] Guanine (**III**), the determined native ligand, was found to have an EC_{50} of 1 mM, which is significantly weaker activity than the original benzimidazole **II**. When the imidazole was replaced with a phenyl ring, such as for 2-aminoquinazoline (**IV**), an improved activity was observed, approximately two-fold over guanine **III**, which might be due to increased pi-stacking. To test this hypothesis, a non-planar compound was also tested to determine if they could access the

empty space in the back of the binding pocket. They replaced the carbonyl with a dimethyl moiety (**V**) and a spirocyclopropyl substituent (**VI**). When subjected to the FRET assay, **V** gave a similar EC_{50} to compound **IV**; however, spirocyclopropyl compound **VI** gave greater than a four-fold increase in activity at 100 μM. The difference in activity is likely due to the availability of space for the groups to fill; the spirocyclic compound constrains the bond angles to 60°, while the dimethyl compound is unrestrained, with bond angles of around 109° and will therefore demand more space in the binding pocket. As a control, the Hermann group also synthesized compound **VII** with the purpose of replacing the space-filling groups with just two hydrogens. This compound had no activity in the FRET assay.

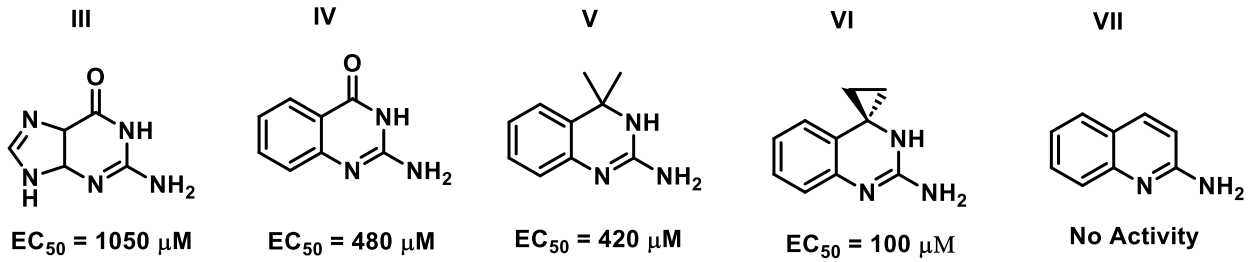


Figure 3.5: Compounds tested by the Hermann group through the FRET assay.

With this data in hand, the Hermann group next attempted to derivatize compound **VI**. Thus, benzothiadiazene **VIII** was synthesized in an attempt to make the compound more drug-like as well as believing that the sulfonylamide substituent, being space-filling but polar, would be able to improve the binding affinity. Benzothiadiazene moieties are also found in many FDA approved pharmaceuticals. This compound showed an excellent activity improvement, with an EC_{50} of 47 μM. Further derivatization by adding the dimethylpropyl chain (**IX**) to make the compound structurally similar to benzimidazole **II** and to potentially improve the hydrogen bonding interactions outside of the binding pocket, an improved activity of another four-fold was observed (EC_{50} ~ 11 μM).[47] The benzothiadiazene scaffold **VIII** and **IX** have not been as

thoroughly explored as the benzimidazole scaffold **II**. Thus, the discussion of the following compounds are an exploration of derivatives of benzothiadiazene **VIII**.

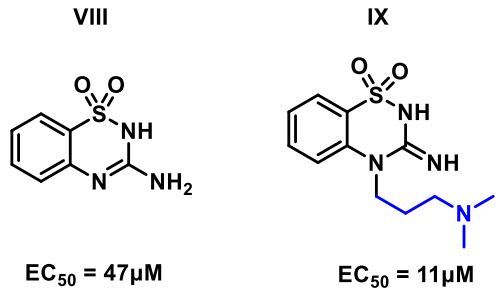
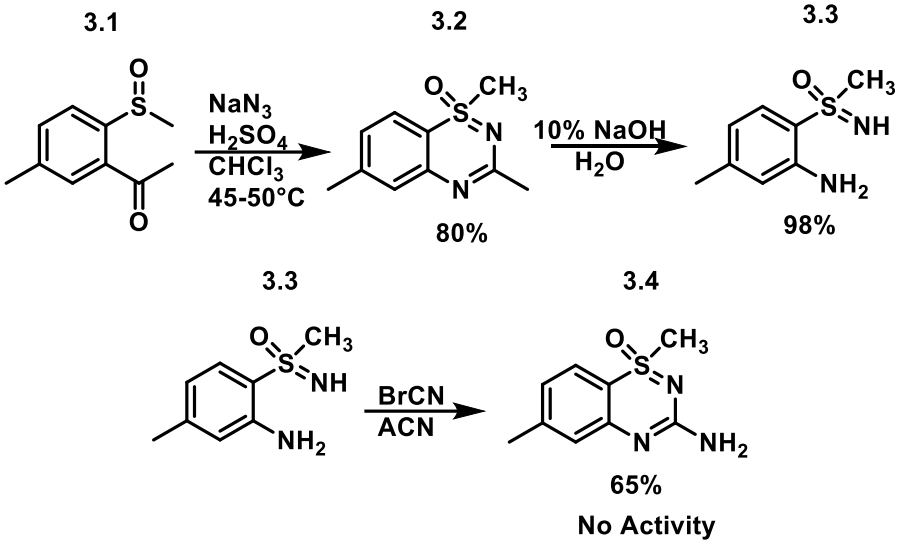


Figure 3.6: Benzothiadiazenes designed from information gleaned from preveious assays.

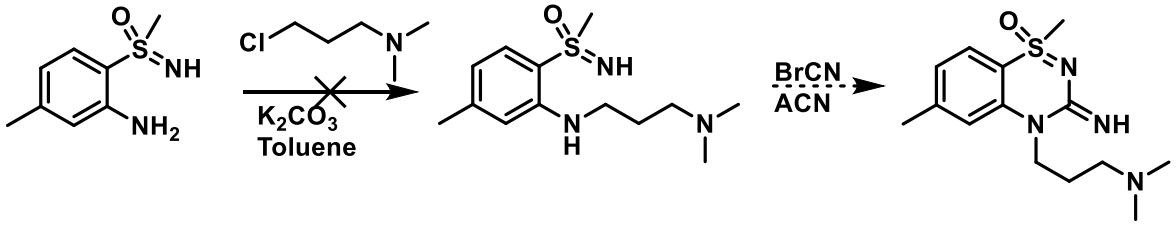
3.4 Methylsulfoximines

To further probe the binding pocket, a variety of methylsulfoximines, which contain both a non-polar space filling methyl group and a polar sulfoxide, were synthesized. This scaffold is interesting for a few reasons; first, the sulfur is chiral, and if there was activity it would have been interesting to determine a potential stereochemical bias in the binding pocket. Second, it contains both a non-polar space-filling methyl group, as well as a polar hydrogen-bond accepting polar group, which would be interesting to determine if the combined pair had any effect. Using a modified literature procedure,[48] commercially available **3.1** was reacted with sodium azide and sulfuric acid in chloroform to do a one-pot Schmitt reaction and sulfoximine formation to give **3.2** in good yield. Compound **3.2** was then hydrolyzed by refluxing in 10% sodium hydroxide in water to give the free aniline and free sulfoximine **3.3** in excellent 98% yield. The free nitrogen compound **3.3** was then cyclized with cyanogen bromide in ACN to form cyclic guanidine derivative **3.4** in moderate 65% yield as the hydrobromide salt. This compound was subjected to the FRET assay, and surprisingly showed no activity, presumably due to the long sulfur-carbon bond taking up space in the binding pocket.



Scheme 3.1: Synthetic route to methylsulfoximine **3.4**.

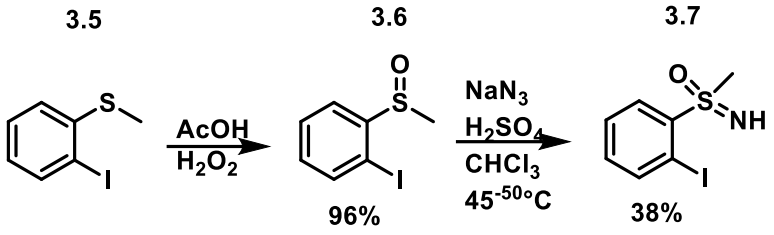
To further verify, and to determine if sulfoximines are at all viable ligands, the scaffold was modified to incorporate the dimethylaminopropyl, which had previously been shown to activity four-fold in previously synthesized compounds. The first attempt at adding the arm was done in an S_N2 fashion, with precedence from the benzothiadiazine synthesis, using the hydrochloride salt of 3-chloro-N,N-dimethylpropan-1-amine and potassium carbonate in refluxing toluene. However, only starting material was recovered, presumably due to the strongly electron withdrawing nature of the sulfoximine ortho to the aniline.



Scheme 3.2: First attempts at aniline derivitization.

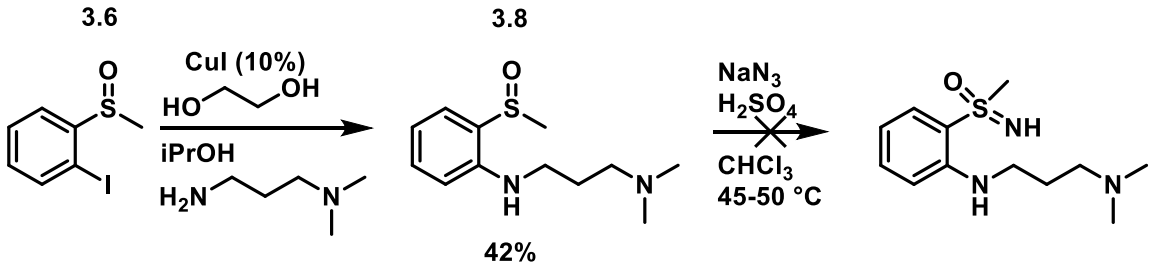
Having the electron poor aniline also ruled out reductive amination. Due to these constraints, a different synthetic route was employed, using an Ullman amination to install the dimethylaminopropyl arm. Commercially available thioanisole **3.5** was oxidized with acetic

peracid to give sulfoxide **3.6** in excellent 96% yield.[49] In situ made hydrazoic acid in chloroform was used to convert the sulfoxide to the corresponding sulfoximine **3.7**.



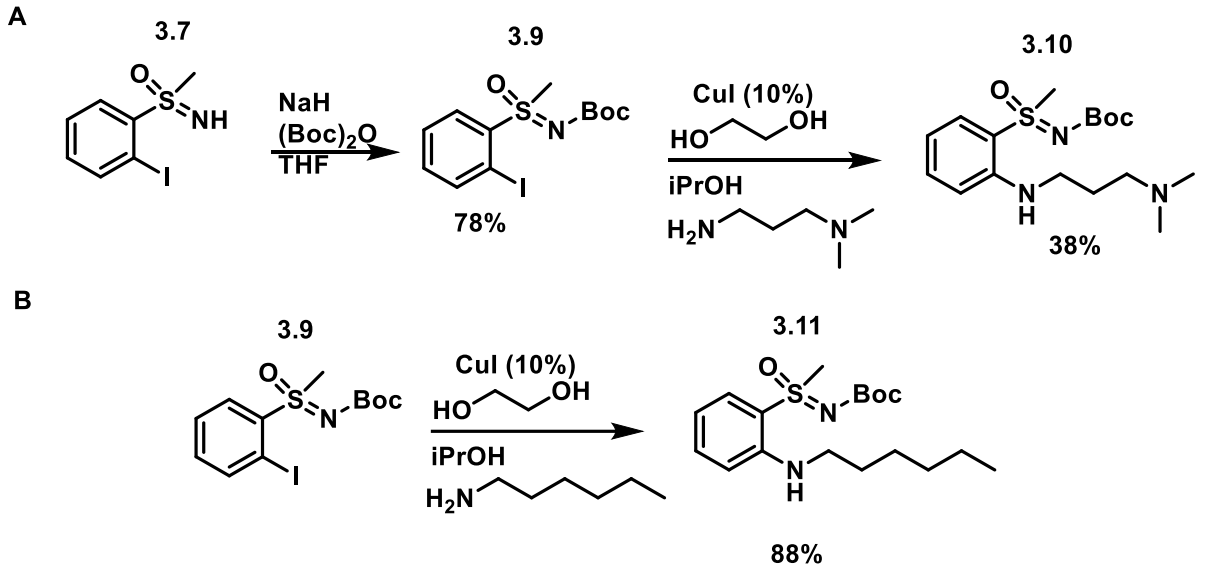
Scheme 3.3: Oxidation and sulfoximine formation.

With the sulfoximine in hand, the Ullman amination was next attempted; however, the nitrogen of the sulfoximine seems to also heavily coordinate to copper(I), resulting in only polymerization.[50] The Ullman amination of sulfoxide **3.6** provided the substituted aniline **3.8** in moderate 42% yield. Attempts to convert the sulfoxide (**3.8**) to the sulfoximine failed due to decomposition in the presence of sulfuric acid.



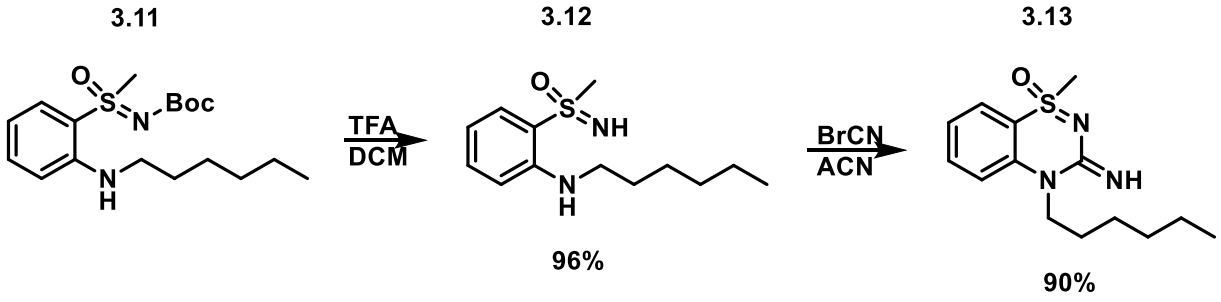
Scheme 3.4: Ullman amination and sulfoximine formation attempts.

The next logical step was to protect the sulfoximine nitrogen to prevent oligomer formation. Sulfoximine **3.7** was reacted with sodium hydride and boc anhydride in anhydrous THF to give the protected sulfoximine **3.9** in 78% yield.[51] Sulfoximine was then subjected to the Ullman conditions outlined previously, and the reaction gave **3.10** in 38% yield. As a test to ensure that there was no procedural error, N^1,N^1-dimethylpropane-1,3-diamine was replaced with hexylamine, and the coupling proceeded to give **3.11** in an excellent 88%, which shows that the tertiary amine can also coordinate with copper(I), giving the lower yield.



Scheme 3.5: A) Boc protection and subsequent Ullman amination. B) Ullman amination using hexylamine.

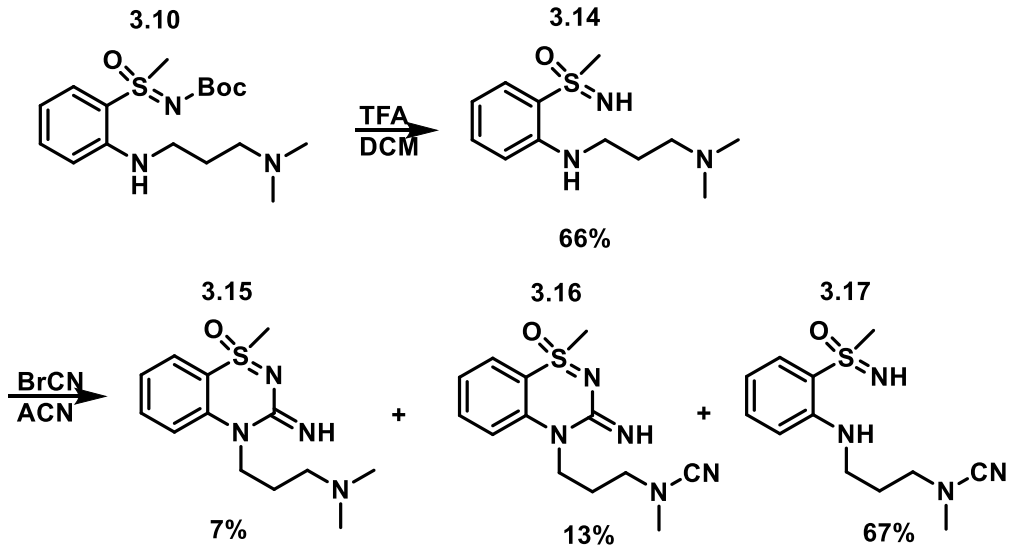
Due to the low yielding Ullman reaction with the desired dimethylamine, the rest of the synthesis was continued with the C6-alkyl moiety present in order to evaluate the viability of the route. Moreover, this compound would be a negative control, containing the correct tautomer of the final cyclized product but with a hydrophobic arm that should not interact with the phosphate backbone of the IRES. Boc-deprotection of **3.11** with TFA in DCM went smoothly, giving the free sulfoximine **3.12** in an excellent 96%. Subsequent cyclization then proceeded smoothly, providing the final cyclized product **3.13** in 90% yield.



Scheme 3.6: Boc deprotection and cyclization of hexane derivative.

The dimethylaminopropyl compound synthesis went smoothly, albeit with lower yields than the compound with the hexyl chain, presumably due to the dimethylamino moiety. Boc-

deprotection of **3.10** gave the free sulfoximine **3.14** in moderate 66%. Cyclization with cyanogen bromide in acetonitrile gave a mixture of products (Scheme 2.7). The main product was the Von Braun product **3.17**, converting one of the methyl groups on the chain to a cyanamide, in 67%. A minor product was the cyclization of the Von Braun product (**3.16**) in 13%. The desired product **3.15** was isolated in 7%, which was enough compound needed for the FRET assay.



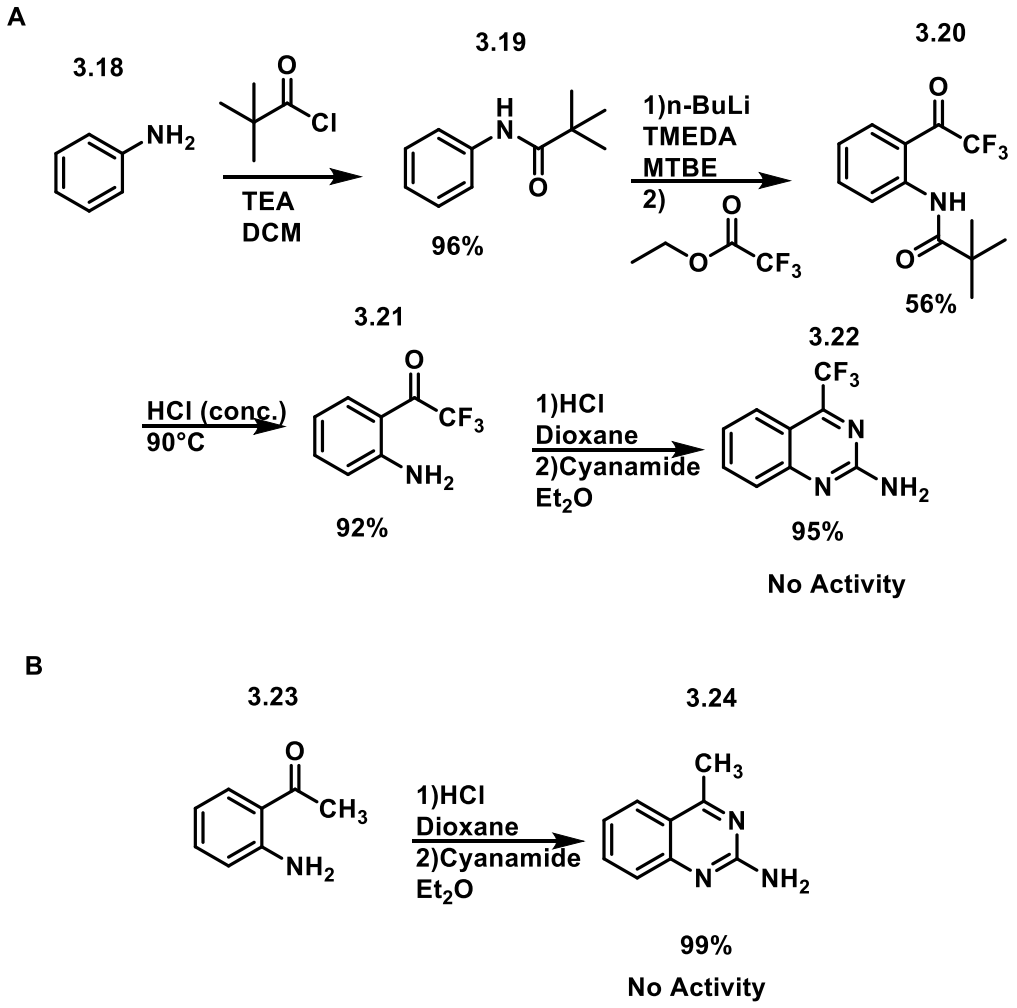
Scheme 3.7: Boc deprotection and BrCN cyclization.

Compounds **3.13**, **3.15**, **3.16**, and **3.17** unfortunately did not show activity in the FRET assay.

3.5 Trifluoromethylated Compound

In an attempt to not only derivatize and optimize the quinazolinone to have similar dipolar properties, but also to take advantage of the empty space in the IRES binding pocket, a derivative containing a trifluoromethyl group (**3.22**) in place of the carbonyl was synthesized, along with a control compound containing a CH_3 Moiety (**3.24**). To synthesize the trifluoromethyl derivative, aniline was first reacted with pivaloyl chloride in the presence of TEA in DCM to give compound **3.19** in 96% yield. Trifluoroacetophenone derivative **3.20** was then formed by a Snieckus style ortho-lithiation with *n*-butyllithium and TMEDA in MTBE and

trapping the lithiated intermediate with ethyl trifluoroacetate in modest 56% yield.[52] The aniline was next formed via deprotection of the Boc-group by heating **3.20** in concentrated $HCl_{(aq)}$, giving **3.21** in 92%. The cyclized product was formed by reaction with cyanamide in ether to give **3.22** in 95%.[53] For control compound **3.22** with a methyl group instead of the trifluoromethyl moiety, acetophenone **3.23** was cyclized with cyanamide in ether to give **3.24** in excellent 99% yield. Compounds **3.22** and **3.24** did not display activity in the FRET assay.

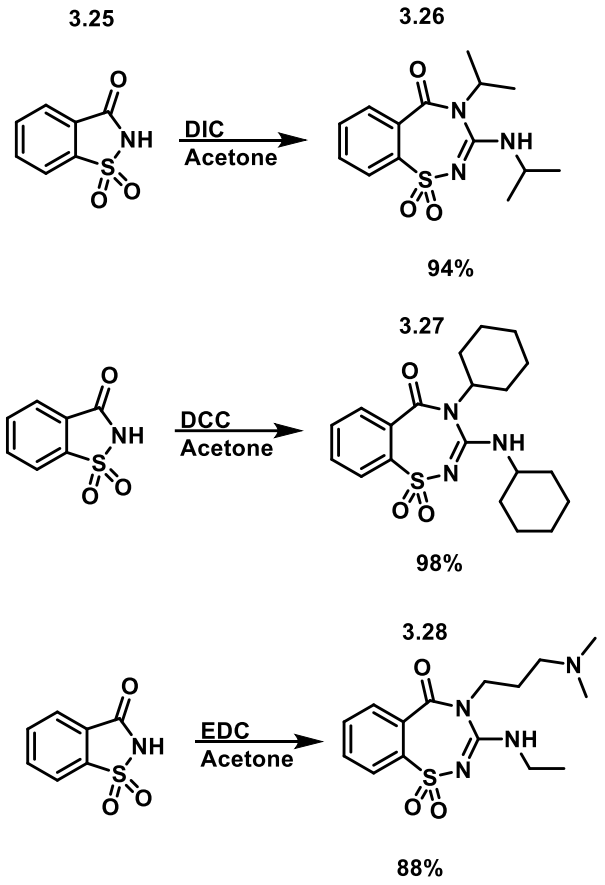


Scheme 3.8: A) Route to trifluoromethylated derivative. B) Route to methyl control compound.

3.6 Benzothiadiazapenes

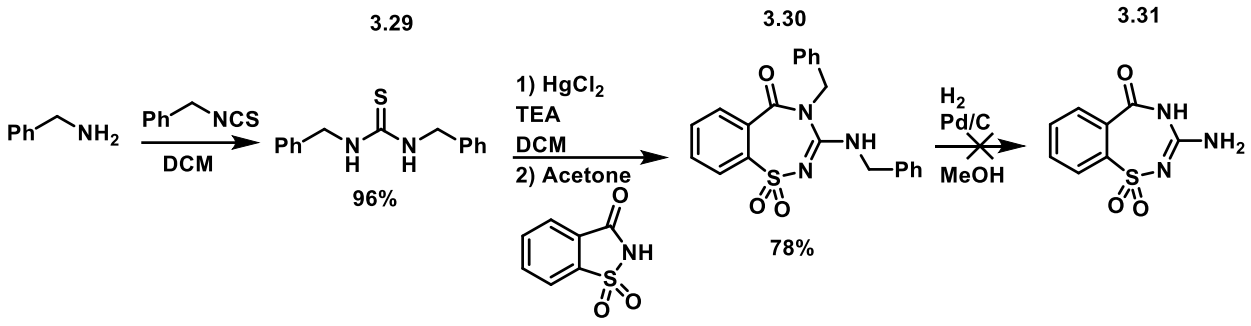
The Ionis benzimidazole **II** had a five-membered imidazole ring that fits nicely into the IRES binding pocket. Expanding the size to a six-membered ring, as in the quinazolinone compound **IV** and the native guanine ligand **III**, had fortuitous effects on the activity in the FRET assay. To evaluate whether further expansion to a seven-membered ring would also be beneficial, a benzothiadiazapine structure was theorized, but the synthesis of the compound proved troublesome. The synthetic plan was to use an interesting ring expansion of saccharine (**3.25**) using a carbodiimide,[54] then deprotect the carbodiimide to free the nitrogens. A non-commercially available carbodiimide had to be used for ease of deprotection, and those can be made easily by desulfurating the corresponding thiourea with mercury (II) chloride.

The first strategy was to use Boc as the protecting group since its removal is quite simple and robust. Forming diboccarbodiimide from dibocthiourea was simple enough, however the corresponding cyclization gave back only starting material. To ensure there was not a procedural error, ring expansions were done with commercially available DCC, DIC, and EDC. All these reactions gave the expansion products in good yield. Thus, the problem was the choice of carbodiimide.



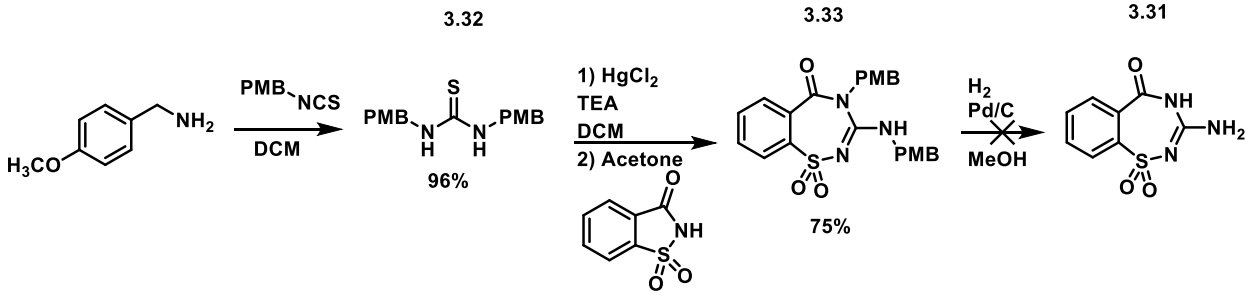
Scheme 3.9: Test reactions of **3.25** with commercially available carbodiimides.

To alleviate the problem with the expansion, dibenzylcarbodiimide was used in its place of diboccarbodiimide. Adding benzylamine to benzylisothiocyanate gave dibenzylthiourea **3.29** in excellent 98% yield. Desulfurization and subsequent ring expansion proceeded to give **3.30** in good 78% yield. However, attempts at hydrogenation were unsuccessful; neither standard 10% palladium on carbon under a hydrogen atmosphere nor platinum (IV) oxide under a hydrogen atmosphere in methanol, ethanol, or THF, nor did boron tribromide in DCM.



Scheme 3.10: Route to benzothiadiazopene through benzyl thiourea.

In an attempt to alleviate this challenge, the more easily reduced PMB group was used in place of the benzyl group. PMB-isothiocyanate was reacted with PMB amine in DCM to give compound **3.32** in excellent 96% yield. The ring opening reaction of saccharine to form benzothiadiazapine **3.33** proceeded in good 78% yield. However, there were still challenges with the deprotection reaction. Hydrogenation again failed, both with 10% palladium on carbon and with titanium (IV) oxide. Boron tribromide in DCM was also unsuccessful. Other protecting groups were also attempted, but unfortunately the ring expansion reaction was unsuccessful with both dialloccarbodiimide and diallylcarbodiimide.



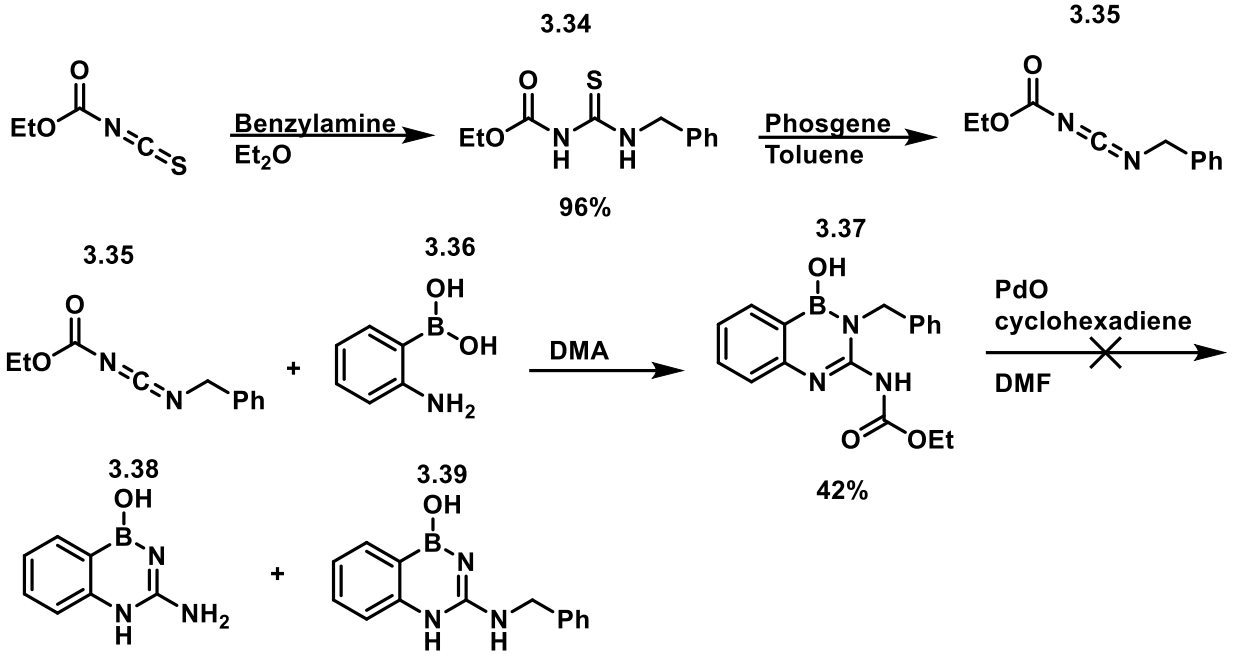
Scheme 3.11: Route to benzothiadiazopene through PMB urea.

With attempts at deprotection failing, the protected compounds were subjected to the FRET assay to determine if further attempts were worthwhile. The dicyclohexyl, dibenzyl, and di-PMB derivatives had no activity. The diisopropyl derivative gave an EC_{50} of 100 μM, and the derivative from EDC gave an EC_{50} of 160 μM.

3.7 Boronamide

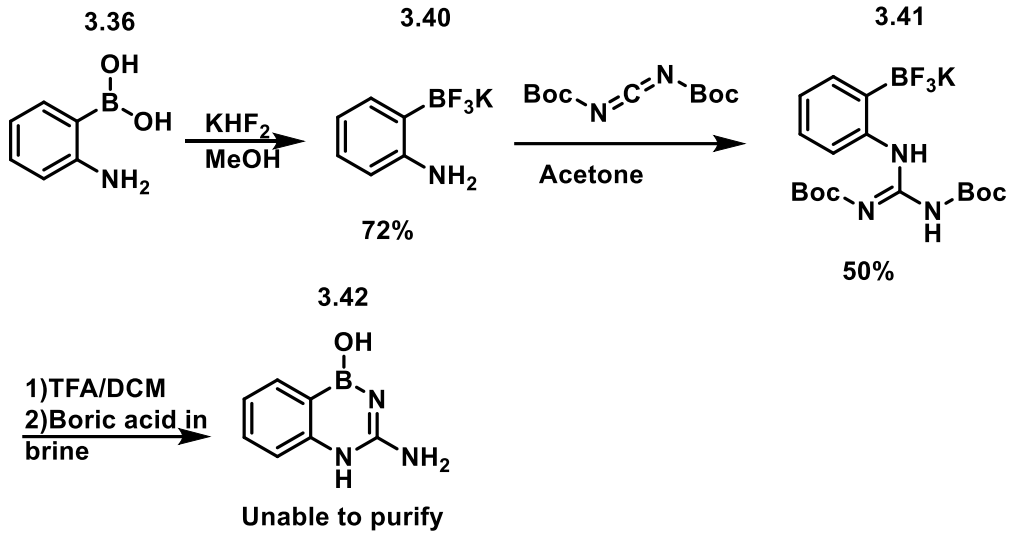
Boronic acids are becoming a more common moiety in medicinal chemistry, with a few recently FDA approved drugs containing the functional group.[55] While those drugs use it as a prodrug, it would be interesting to probe the IRES binding pocket with a boronic acid, especially considering that in aqueous environments the boronic acid will be hydrated, putting two hydrogen bond donors and acceptors in a shape that could also fill the binding pocket. There is literature precedence for its synthesis,[56] and attempts were made to replicate their methodology, though the authors note that **2.36** is only a side product in their scheme. With that in mind, the methodology was pushed forward with hopes that enough material would be isolated for the FRET assay.

In a similar manner to the benzothiadiazapenes, the method employed by Robinson *et al*[56] involved trapping a carbodiimide (originally formed by desulfurization of a thiourea) with an aniline, and the corresponding guanidine would spontaneously cyclize, with nitrogen donating electrons to the empty P orbital of boron. The formation of thiourea **3.32** went smoothly, reacting *O*-ethyl carbonisothiocyanatidate with benzylamine in ether to give **3.34** in 96% yield. Desulfurization with a solution of 15% phosgene in toluene to give carbodiimide **3.35** also went smoothly, and the corresponding carbodiimide was reacted with boronic acid **3.36** to give protected compound **3.37** in 42%. However, as predicted in the manuscript, only trace amounts of desired boronamide **3.38** was formed upon hydrogenation, with the major product being compound **3.39**. Although the desired product was detected using mass spectroscopy, attempts at isolation failed. Changing hydrogen donor sources and solvents unfortunately had no effect.



Scheme 3.12: First attempt at boronamide synthesis.

It became apparent that a different synthetic strategy needed to be used to produce an isolable amount of **3.38**. The new strategy, as outlined in **Scheme 3.13**, used potassium bifluoride in methanol to protect the boron as the potassium trifluoroborate salt, giving **3.40** in 72% yield. Trapping diboccarbodiimide with the free aniline in acetone gave protected guanidine **3.39** in a modest 50%. Boc deprotection by TFA in DCM, followed by deprotection of the trifluoroborate with boric acid in brine gave the desired product by mass spectroscopy; however, isolation of **3.42** was again challenging. The compound decomposed on silica, and attempts at recrystallization failed. It could successfully be converted to the methyl boronic ester by dissolving in methanol and removing the solvent in vacuo, but this too decomposed on silica, and crystallization attempts were again unsuccessful.



Scheme 3.13: Boronamide synthesis via trifluoroborate.

Both the free boronic acid and the dimethoxy adduct significantly decomposed in air if left for more than an hour and would decompose in the freezer under argon in less than a week. Due to the low stability of the compound, we decided to move away from boronamides.

3.8 Conclusions

In part II of this thesis, a variety of compounds have been synthesized and tested to explore the viability of a highly concerted region of the HCV IRES as a drug target. This region of RNA, domain IIa, acts as conformational switch, and is involved in HCV RNA translation. This switch provides a unique target for small molecules within the native ligand binding pocket. Other viruses, namely the viruses in the *Flaviviridae* family of viruses such as dengue virus and tamarin virus, also contain an IRES with a similar switch mechanism that could potentially be manipulated in a similar manner. The work described in part II of this dissertation focused on using structure activity relationships gleaned from a crystal structure of domain IIa bound to a ligand to design new ligands to inhibit HCV translation. These compounds were relatively simple to synthesize (as few as three steps) and had activities in the same order of magnitude as the Ionis benzimidazoles, which were much more synthetically challenging.[22,45] This was

accomplished by synthesizing compounds that take advantage of the empty space in the HCV IRES domain IIa in a similar manner as to the spirocyclopropyl compound (compound **VI**), exploring compounds that fill the empty space in the binding pocket by expanding to a seven-membered ring, with compounds that have differing degrees of polar and non-polar constituents that fit into the binding pocket. The compounds with the best affinity were the benzothiadiazine derivatives synthesized by my colleague in the Hermann group[47]. Other non-planar compounds with varying degrees of polarity and differing size were explored with mixed results. The benzothiadiazapene compounds showed weaker affinity; however, they still contained their bulky protecting groups. It will be worthwhile to continue down that path and attempting to find a way to form the free benzothiadiazapene.

These compounds, as mentioned previously, are simple and straightforward to synthesize, over as few as three steps, and therefore are a nice starting point for future studies attempting to improve our understanding of how to inhibit the HCV IRES. Next steps in the project would be to synthesize derivatives of the benzothiadiazapene compounds (**VIII** and **IX**). For example, addition of the chroman ring to make it more similar to the Ionis benzimidazole (compound **II**) would be interesting to see if the activity could be further increased would be an obvious starting point to optimize these ligands.

Chapter 3 contains information being prepared for submission for publication. Walsworth, Kevin; Frauman, Walter; Bergdahl, B. Mikael; Hermann, Thomas. The dissertation author was the principal investigator and author of this material.

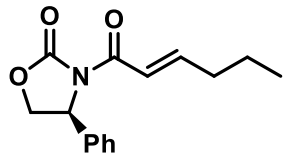
APPENDIX

A1 Instrumentation and Chemicals

General: All air and water sensitive reactions were conducted under an argon atmosphere in an oven-dried flask equipped with a rubber septum. Reaction yields were determined based on purified material (>96% by ^{1}H spectroscopy) unless otherwise specified. NMR spectra were recorded on a Bruker 400-MHz, Varian 400, 500, or 600-MHz (^{1}H NMR) and 150 MHz (^{13}C NMR) using tetramethylsilane as an internal standard ($\delta = 0.0$ ppm). Coupling for ^{1}H spectra are abbreviated as follows: s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), p (pentet), h (hextet), sept (septet), m (multiplet). In the cases of combined multiplicities, the multiplicity with the higher J value is listed first. *Partially hidden* couplings refer to signals that overlapped but were definable. The peaks for ^{13}C NMR are reported in ppm excluding solvent and tetramethylsilane. Flash chromatography was conducted either by hand or using a Biotage Isolera One instrument with self-packed Biotage SNAP columns filled with silica gel (Sorbent Technologies, 60 Å, 230-400 mesh).

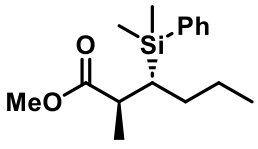
Materials: THF and Et_2O were distilled under argon from sodium-benzophenone. DCM was distilled under argon from calcium hydride. All other air and water sensitive reagents were used as purchased from the manufacturer (Sigma Aldrich) through sure-seal septa unless otherwise specified. Solvents used for extractions and purifications were technical grade and used as purchased from the manufacturer (Fisher Scientific). All other chemicals were used as purchased from the manufacturer unless otherwise specified.

A2 Experimental Procedures and Compound Characterization for Part I: Total Synthesis of the Marine Natural Product Palmyramide A



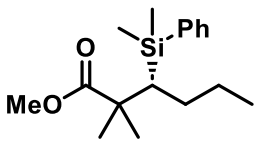
1.3

(S,E)-3-(hex-2-enoyl)-4-phenyloxazolidin-2-one: A solution of oxazolidinone **1.1** (1 g, 6.12 mmol, 1 eq), DMAP (119 mg, .974 mmol, 0.16 eq), and *trans*-2-hexenoic acid (**1.2**) (908 mg, 7.97 mmol, 1.3 eq) in 10 mL distilled DCM was cooled to 0 °C. DCC (1.25 mL, 7.97 mmol, 1.3 eq) was added slowly and the mixture was stirred for 2 hours while coming to room temperature. The mixture was filtered through Celite and washed with DCM. The organics were washed with saturated aqueous $NaHCO_3$ (1 x 10 mL), brine (1 x 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica (30% EtOAc/hexanes, isocratic) to give product **1.3** as a white crystalline solid (1.527 g, 96%). **TLC**: (30% EtOAc/hexanes) R_f = 0.6. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.41 – 7.36 (m, Ar*H*, 2H), 7.33 (td, Ar*H*, *J* = 7.1, 1.4 Hz, 1H) 7.26 (tt, C*H*$^{\alpha}$=CH$^{\beta}$, *J* = 6.6, 1.4 Hz), 7.09 (dt, CH$^{\alpha}$=C*H*$^{\beta}$, *J* = 15.3, 6.9 Hz, 1H), 5.49 (dd, O-C*H*$_2$, *J* = 8.7, 3.9 Hz, 1H), 4.70 (t, Ar-C*H*, *J* = 8.8 Hz, 1H), 4.28 (dd, O-C*H*$_2$ *J* = 8.9, 3.9 Hz, 1H), 2.23 (qd, CH_3-CH_2-C*H*$_2$-CH$^{\beta}$, *J* = 7.1, 1.5 Hz, 2H), 1.50 (h, CH_3-C*H*$_2$-CH_2 *J* = 7.4 Hz, 2H), 0.93 (t, C*H*$_3$-CH_2, *J* = 7.4 Hz, 3H). **^{13}C NMR** (125.6 MHz, $CDCl_3$) δ 164.67, 152.06, 141.55, 139.14, 129.17, 128.65, 125.95, 120.29, 69.93, 57.76, 34.69, 21.32, 13.69.



1.5

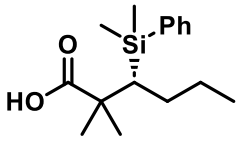
Methyl (2S,3R)-3-(dimethyl(phenyl)silyl)-2-methylhexanoate: To a solution of freshly distilled diisopropylamine (0.32 mL, 2.27 mmol, 1.2 eq) in 9 mL anhydrous THF in a round bottom flask under Ar(g) at -78 °C was added 2.5 M *n*-butyllithium (0.8 mL, 2.08 mmol, 1.1 eq) dropwise. The mixture was stirred 5 minutes at -78 °C, warmed to 0 °C for 20 minutes, then cooled back to -78 °C. A solution of substrate **1.10** (501 mg, 1.89 mmol, 1 eq) was dissolved in 18 mL anhydrous THF was added to the reaction flask and stirred 30 minutes. The reaction turned a light pink. A solution of iodomethane (0.14 mL, 2.27 mmol, 1.2 eq) in 9 mL anhydrous THF was added to the reaction, which turned a pale yellow, and was allowed to stir for 3 hours, monitoring by TLC. After completion, the reaction was quenched with saturated $NH_4Cl_{(aq)}$, extracted with EtOAc (3 x 20 mL), the combined organics were washed with brine (1 x 20 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude mixture was purified by flash chromatography on silica gel (gradient, 10 to 40% EtOAc/hexanes) to yield **1.5** as a clear colorless oil (400 mg, 76%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.55 – 7.49 (m, Ar*H*, 2H), 7.38 – 7.32 (m, Ar*H*, 3H), 3.57 (s, C*H*$_3$-O, 3H), 2.59 (qd, O_2C-C*H*, *J* = 7.1, 3.6 Hz, 1H), 1.52 – 1.47 (m, Si-C*H*, 1H), 1.45 – 1.33 (m, CH_3-CH_2-C*H*$_2$, 2H), 1.30 – 1.13 (m, CH_3C*H*$_2$, 2H), 1.05 (d, C*H*$_3$-CH, *J* = 7.1 Hz, 3H), 0.79 (t, C*H*$_3$-CH_2, *J* = 7.3 Hz, 3H), 0.32 (s, (C*H*$_3$)$_2$-Si 6H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 177.61, 138.78, 133.87, 128.88, 127.73, 51.39, 39.12, 28.91, 28.48, 22.83, 14.29, 13.90, -2.96, -3.08.



1.6

Methyl (R)-3-(dimethyl(phenyl)silyl)-2,2-dimethylhexanoate: To a solution of freshly distilled diisopropylamine (0.52 mL, 3.70 mmol, 1.5 eq) in 10 mL anhydrous THF under Ar(g) at – 78 °C

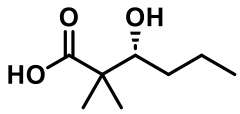
was added 2.5 M *n*-butyllithium (1.4 mL, 3.46 mmol, 1.4 eq) dropwise. This mixture was stirred at -78 °C for 5 minutes before being warmed to 0 °C for 20 minutes. A solution of substrate **1.5** (687 mg, 2.47 mmol, 1 eq) in 20 mL anhydrous THF was added to the reaction, warmed to room temperature, and stirred for 3 hours. A solution of iodomethane (0.23 mL, 3.70 mmol, 1.5 eq) in 10 mL anhydrous THF was added to the reaction and allowed to stir 12 hours, monitoring by TLC. Upon completion, the reaction was quenched with saturated $NH_4Cl_{(aq)}$, extracted with EtOAc (3 x 25 mL), the combined organics were washed with brine (1 x 25 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica (gradient, 10 to 40% EtOAc/hexanes) to yield **1.6** as a clear colorless oil (512 mg, 71%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.52 (m, 2H), 7.33 (m, 3H), 3.54 (s, 3H), 1.43 (dd, *J* = 6.5, 4.2 Hz, 1H), 1.35 (m, 4H), 1.16 (s, 3H), 1.13 (s, 3H), 0.72 (t, *J* = 7.3 Hz, 3H), 0.32 (s, 3H), 0.29 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 179.15, 140.13, 133.94, 128.64, 127.57, 51.49, 45.39, 34.30, 29.71, 25.81, 25.83, 24.55, 14.41, -1.10, -2.31.



1.7

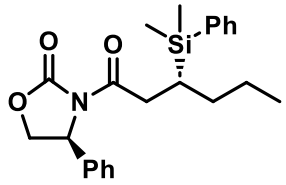
(R)-3-(dimethyl(phenyl)silyl)-2,2-dimethylhexanoic acid: To a solution of substrate **1.15** (212 mg, 0.856 mmol, 1 eq) in 5 mL acetone at 0 °C was added a solution of chromium trioxide in acetone dropwise until the orange color persisted for 30 minutes. The reaction was then quenched with isopropanol until it turned blue. The suspension was filtered through celite, washed with EtOAc, and concentrated under reduced pressure to yield **1.7** as a yellow oil (220 mg, 98%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.54 (dt, Ar*H*, *J* = 7.0, 3.1 Hz, 2H), 7.35 – 7.30 (m, Ar*H*, 3H), 1.43 (q, CH_3-CH_2-CH_2-CH, *J* = 5.8 Hz, 2H), 1.37 (td, Si-CH, *J* = 8.9, 8.0, 4.3 Hz,

1H), 1.20 (s, (C*H*$_3$)$_2$ 3H), 1.16 (s, (C*H*$_3$)$_2$ 3H), 1.12 – 1.03 (m, CH$_3$-C*H*$_2$, 2H), 0.72 (t, C*H*$_3$-CH$_2$, *J* = 7.2 Hz, 3H), 0.35 (s, Si-C*H*$_3$, 3H), 0.33 (s, Si-CH$_3$, 3H).



1.8

(R)-3-hydroxy-2,2-dimethylhexanoic acid: Substrate **1.23** (184 mg, .577 mmol, 1 eq) was dissolved in 5 mL of a 4:1 mixture of THF and deionized water. This solution was cooled to 0 °C before lithium hydroxide (39 mg, 0.923 mmol, 1.6 eq) was added. 30% hydrogen peroxide in water (0.21 mL, 2.08 mmol, 3.6 eq) was added dropwise and the reaction was stirred for 1 hour. The reaction was quenched with 306 mg sodium sulfite then the volatiles were removed under reduced pressure. The resulting suspension was diluted with water and extracted with DCM (2 x 20 mL). The pH of the resulting aqueous solution was adjusted to 2 with 1 M HCl, then extracted with Et$_2$O (3 x 20 mL), the combined ethereal organics were combined and dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure to yield the product **1.8** as a white solid (91 mg, 99%). **^{1}H NMR** (400 MHz, CDCl$_3$) δ 3.66 (dd, *J* = 10.1, 2.2, 1H), 1.69 – 1.56 (m, 1H), 1.53 – 1.42 (m, 1H), 1.42 – 1.29 (m, 4H), 1.25 (s, 3H), 1.20 (s, 3H), 0.95 (t, *J* = 7.1 Hz, 3H). **HRMS** (ESI) Calcd for [C$_8$H$_{15}$O]$^-$ 159.1021, found 159.1022.

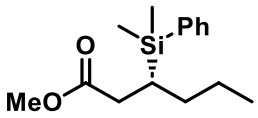


1.9

(S)-3-((R)-3-(dimethyl(phenyl)silyl)hexanoyl)-4-phenyloxazolidin-2-one: **Silyl Lithium Prep**: To a round bottom flask under Ar(g) was added Li^0 (645 mg, 93 mmol, 15 eq) and 20 mL anhydrous THF. This suspension was cooled to 0 °C then phenyldimethylsilyl chloride (3.1 mL, 18.6

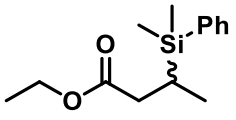
mmol, 3 eq) was added dropwise over 10 minutes. This mixture was stirred 4 hours at 0 °C, turning a deep, dark red. The septum was replaced with a fresh one and the flask was stored in the freezer until use.

Reaction: To a round bottom flask under Ar(g) was added 26 mL anhydrous THF and a 0.7M solution of $ZnCl_2$ in THF (13 mL, 9.31 mmol, 1.5 eq). This solution was cooled to -78 °C, then the silyl lithium solution was added, followed by a 11mL rinse of the silyl lithium flask with anhydrous THF, and was stirred for 30 minutes. To another round bottom flask under Ar(g) was added $(CuI)_4(DMS)_3$ (294 mg, 0.31 mmol, 0.05 eq) and 63 mL anhydrous THF. This suspension was cooled to -78 °C, then the silyl zinc solution was added via cannula and stirred 5 minutes. The substrate **1.3** (1.795 g, 6.20 mmol, 1 eq) was dissolved in 29 mL anhydrous THF, added to the reaction flask, and stirred 4 hours at -78 °C. The deep red reaction was then quenched with a pH 10 NH_4Cl/NH_3 solution and allowed to equilibrate overnight, coming to room temperature. The blue biphasic solution was extracted with EtOAc (3 x 75 mL), the combined organics were washed with brine (1 x 100 mL), dried over anhydrous Na_2SO_4, and the solvent removed under reduced pressure. The crude mixture was purified by flash chromatography on silica (gradient, 0 to 40% EtOAc/hexanes) to yield **1.9** as a clear colorless oil (2.422g, 99%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.54 (dd, Ar*H*, *J* = 7.3, 2.0 Hz, 1H), 7.49 – 7.45 (m, Ar*H*, 2H), 7.39 – 7.28 (m, Ar*H*, 7H), 5.28 (dd, O-C*H*$_2$-CH, *J* = 8.7, 3.7 Hz, 1H), 4.60 (t, Ar-C*H*, *J* = 8.8 Hz, 1H), 4.23 (dd, O-C*H*$_2$-CH, *J* = 8.9, 3.7 Hz, 1H), 2.92 (dd, O=C-C*H*$_2$, *J* = 16.9, 5.9 Hz, 1H), 2.87 (dd, O=C-C*H*$_2$, *J* = 16.9, 8.1 Hz, 1H), 1.41 – 1.28 (m, R_3Si-C*H*, 1H), 1.22 – 1.07 (m, CH_3-C*H*$_2$-C*H*$_2$, partially hidden, 4H), 0.73 (t, C*H*$_3$-CH_2, *J* = 6.9 Hz, 3H), 0.33 (s, Si-C*H*$_3$, 3H), 0.23 (s, Si-C*H*$_3$ 3H). **^{13}C NMR** (125.7 MHz, $CDCl_3$) δ 172.29, 138.27, 137.38, 133.09, 132.13, 128.38, 128.22, 128.02, 127.77, 126.81, 125.19, 69.00, 56.76, 35.62, 31.71, 21.28, 19.63, 13.37, -4.88, -5.06.



1.10

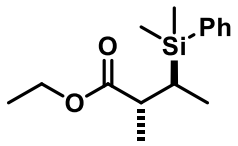
Methyl (R)-3-(dimethyl(phenyl)silyl)hexanoate: To a round bottom flask under Ar(g) containing 73 mL anhydrous methanol at 0 °C was added freshly cut Na^0 (171 mg, 7.44 mmol, 1.2 eq). This suspension was stirred until all Na had reacted (approximately 45 minutes). Substrate **1.9** was dissolved in 10 mL anhydrous methanol and added to the sodium methoxide solution. The reaction was allowed to warm to room temperature and monitored by TLC (20% EtOAc/hexanes, product R_f = 0.4) until the consumption of the starting material (approximately 2 hours). Upon completion, the reaction was quenched with a saturated solution of $NH_4Cl_{(aq)}$ and then extracted with EtOAc (3 x 50 mL), the combined organics were washed with brine (1 x 50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude material was purified by flash chromatography on silica (gradient, 0 to 30% EtOAc/hexanes) to yield **1.10** as a clear colorless oil (1.163 g, 71 %). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.50 (m, Ar*H*, 2H), 7.35 (m, Ar*H*, 3H), 3.57 (s, C*H*$_3$-O, 3H), 2.33 (dd, O_2C-C*H*$_2$-CH, *J* = 15.6, 5.4 Hz, 1H), 2.22 (dd, O_2C-C*H*$_2$-CH, *J* = 15.6, 8.2 Hz, 1H), 1.50 – 1.39 (m, CH_3-CH_2-C*H*$_2$, 2H), 1.38 – 1.28 (m, Si-C*H*, 1H), 1.28 – 1.15 (m, CH_3-C*H*$_2$, 2H), 0.83 (t, C*H*$_3$-CH_2, *J* = 7.0 Hz, 3H), 0.29 (s, Si-C*H*$_3$, 3H), 0.28 (s, Si-C*H*$_3$, 3H). **^{13}C NMR** (125.7 MHz, $CDCl_3$) δ 189.87, 178.84, 142.08, 138.03, 133.09, 131,84, 38.97, 36.72, 26.05, 25.90, 18.34, -0.31.



1.12

ethyl 3-(dimethyl(phenyl)silyl)butanoate: **Silyl lithium prep**: To a suspension of Li^0 (497 mg, 71.55 mmol, 15 eq) in 10 mL anhydrous THF at 0 °C under Ar(g) was added

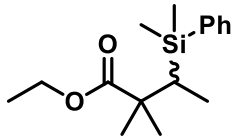
phenyldimethylchlorosilane (2.3 mL, 14.31 mmol, 3 eq) dropwise. The reaction was stirred 4 hours and turned a deep, dark red. A fresh septum was placed on the flask and the silyl lithium was stored in the freezer until use. **Reaction**: To a round bottom flask containing 20 mL anhydrous THF under Ar(g) was added a 0.7 M solution of $ZnCl_2$ in THF (10.22 mL, 7.155 mmol, 1.5 eq). This solution was cooled to -78 °C, then the silyl lithium was added via cannula. The silyl lithium flask was rinsed with an additional 10 mL anhydrous THF, and the reaction was stirred 20 minutes. This solution was added via cannula to another round bottom flask under Ar(g) at -78 °C containing a suspension of $(CuI)_4(DMS)_3$ (226 mg, .239 mmol, 0.05 eq) in 48 mL anhydrous THF. After 5 minutes, a solution of freshly distilled ethyl crotonate (0.5 mL, 4.77 mmol, 1 eq) in anhydrous THF was added to the reaction and stirred 4 hours, monitoring by TLC (5% EtOAc/hexanes). Once complete, the reaction was quenched with an aqueous pH 10 buffer of NH_3/NH_4Cl and allowed to equilibrate at room temperature over 12 hours. The now blue biphasic solution was extracted with EtOAc (3 x 50 mL), the combined organics were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica (isocratic, 5% EtOAc/hexanes) to give product **1.12** as a clear colorless oil (1.057g, 99%). **^{1}H NMR** (500MHz, $CDCl_3$) δ 7.54 – 7.45 (m, 2H), 7.41 – 7.31 (m, 3H), 4.18 – 4.02 (q, *J* = 7.2 Hz, 2H), 2.37 (ddd, *J* = 15.0, 4.1, 1.7 Hz, 1H), 1.49 – 1.38 (m, 1H), 1.24 (t, *J* = 7.1 Hz, 3H), 0.97 (dd, *J* = 7.5, 1.7 Hz, 3H), 0.28 (s, 6H).



1.13

ethyl 3-(dimethyl(phenyl)silyl)-2-methylbutanoate: To a solution of diisopropylamine (0.71 mL, 5.05 mmol, 1.2 eq) in 20 mL amhydrous THF at -78 °C under Ar(g) was added 2.5 M *n*-

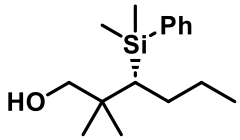
butyllithium in hexane (1.84 mL, 4.63 mmol, 1.1 eq). This solution was warmed to 0 °C for 20 minutes, then cooled back to -78 °C. A solution of substrate **1.12** (1.057 g, 4.21 mmol, 1 eq) in 40 mL anhydrous THF was added to the reaction and allowed to stir 30 minutes. To this reaction mixture was added a solution of iodomethane (0.3 mL, 5.05 mmol, 1.2 eq) in 20 mL anhydrous THF, and this solution was stirred for 3 hours. The reaction was quenched with saturated NH_4Cl, then extracted with EtOAc (3 x 40 mL), the combined organics washed with brine (1 x 40 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (isocratic, 15% EtOAc/hexanes) to give product **1.13** as a clear colorless oil (1.040g, 93%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.52 (dd, Ar*H*, *J* = 6.5, 3.1 Hz, 2H), 7.39 – 7.31 (m, Ar*H*, 3H), 4.03 (q, CH_3-C*H*$_2$-O, *J* = 7.1 Hz, 2H), 2.56 (qd, O_2C-C*H*, *J* = 7.1, 4.7 Hz, 1H), 1.50 (qd, CH_3-C*H*, *J* = 7.6, 4.7 Hz, 1H), 1.21 (t, C*H*$_3$-CH_2-O, *J* = 7.1 Hz, 3H), 1.04 (d, C*H*$_3$-CH-CO_2, *J* = 7.1 Hz, 3H), 0.94 (d, C*H*$_3$-CH-Si, *J* = 7.6 Hz, 3H), 0.32 (s, C*H*$_3$-Si, 3H), 0.30 (s, CH_3-Si, 3H). **^{13}C NMR** (127 MHz, $CDCl_3$) δ 178.49, 139.93, 135.70, 130.75, 129.51, 61.87, 41.85, 24.32, 16.02, 15.45, 12.03, -2.09, -2.15.



1.14

ethyl 3-(dimethyl(phenyl)silyl)-2,2-dimethylbutanoate: To a solution of diisopropylamine (0.1 mL, 0.748 mmol, 1.5 eq) in 2 mL anhydrous THF under Ar(g) at -78 °C was added 2.5 M *n*-butyllithium in hexane (0.28 mL, 0.694 mmol, 1.4 eq). This solution was warmed to 0 °C for 20 minutes, then a solution of substrate **1.13** (132 mg, 0.499 mmol, 1 eq) in 4 mL anhydrous THF was added. The reaction was warmed to room temperature and stirred for 3 hours, then a solution of iodomethane (46 μL, 0.748 mmol, 1.5 eq) was added and stirred at room temperature,

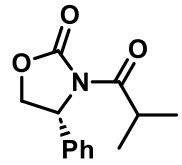
monitored by TLC (15% EtOAc/hexanes). Once complete (approximately 20 hours), the reaction was quenched with saturated $NH_4Cl_{(aq)}$, then extracted with EtOAc (3 x 10 mL), the combined organics were washed with brine (1 x 10 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (isocratic, 15% EtOAc/hexanes) to yield product **1.14** as a clear yellow oil (92 mg, 66%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.56 – 7.48 (m, Ar*H*, 2H), 7.38 – 7.30 (m, Ar*H*, 3H), 4.03 (dtdd, CH_3-C*H*$_2$-O, *J* = 23.5, 10.8, 7.2, 3.7 Hz, 2H), 1.25 – 1.19 (m, C*H*$_3$-CH_2-O, 3H), 1.12 (d, $(CH_3)_2$, 1.09 (d, $(CH_3)_2$, *J* = 7.1 Hz, 3H) *J* = 4.4 Hz, 3H), 0.97 (d, CH_3-C*H*-Si *J* = 7.5 Hz, 1H), 0.93 (d, C*H*$_3$-CH, *J* = 7.6 Hz, 3H), 0.31 (s, C*H*$_3$-Si 3H), 0.30 (s, C*H*$_3$-Si, 3H) **^{13}C NMR** (127 MHz, $CDCl_3$) δ 178.51, 139.53, 133.97, 128.74, 127.62, 60.18, 28.41, 24.73, 24.35, 17.35, 14.14, 11.34, -3.00, -3.60.



1.15

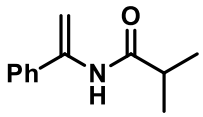
(R)-3-(dimethyl(phenyl)silyl)-2,2-dimethylhexan-1-ol: To a solution of substrate **1.6** (742 mg, 2.54 mmol, 1 eq) in 23 mL anhydrous THF under Ar(g) at 0 °C was added 1M DIBAL in toluene (5.1 mL, 5.10 mmol, 2 eq). This solution was stirred for 30 minutes at 0 °C then was warmed to room temperature and allowed to stir 12 hours. The reaction was quenched with a saturated solution of Rochelle salt, then extracted with EtOAc (3 x 15 mL), the combined organics dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (gradient, 5 to 25% EtOAc/hexanes) to yield **1.15** as a clear yellow oil (436 mg, 65%). **^{1}H NMR** (400 MHz) δ 7.57 – 7.53 (m, Ar*H*, 2H), 7.34 (t, Ar*H*, *J* = 3.2 Hz, 3H), 3.27 (d, HO-C*H*$_2$, *J* = 10.9 Hz, 1H), 3.19 (d, HO-C*H*$_2$, *J* = 10.9 Hz, 1H),

1.40 – 1.32 (m, CH_3-CH_2-CH_2, 2H), 1.26 (ddt, Si-C*H*, *J* = 14.0, 9.7, 7.0 Hz, 1H), 1.10 (ddt, CH_3-CH_2, *J* = 13.6, 9.6, 7.0 Hz, 2H), 0.87 (s, (CH_3)$_2$, 3H), 0.85 (s, (CH_3)$_2$, 3H), 0.77 (t, CH_3-CH_2, *J* = 7.2 Hz, 3H), 0.40 (s, Si-CH_3, 3H), 0.32 (s, Si-CH_3, 3H).



1.17

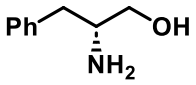
(R)-3-isobutyryl-4-phenyloxazolidin-2-one: To a solution of phenyl oxazolidinone (1.000 g, 6.12 mmol, 1 eq), isobutyric acid (0.74 mL, 7.97 mmol, 1.3 eq), and DMAP (97 mg, 0.797 mmol, 0.13 eq) in 10 mL DCM at 0 °C was added DIC (1.2 mL, 7.97 mmol, 1.3 eq). This solution was warmed to room temperature and stirred overnight. The next day, the reaction was filtered through celite, washed with saturated $NaHCO_{3(aq)}$ (1 x 10 mL), brine (1 x 10 mL), and concentrated under reduced pressure. The crude solid was purified by flash chromatography on silica gel (gradient, 10 to 25% EtOAc/hexanes) to give **1.17** as a crystalline white solid (1.369 g, 96%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.41 – 7.32 (m, 2H), 7.31 – 7.27 (m, 3H), 5.43 (dd, *J* = 8.8, 3.9 Hz, 1H), 4.69 (t, *J* = 8.8 Hz, 1H), 4.26 (dd, *J* = 8.9, 3.9 Hz, 1H), 3.79 (hept, *J* = 6.6 Hz, 1H), 1.14 (dd, *J* = 6.8, 4.6 Hz, 7H).



1.19

N-(1-phenylvinyl)isobutyramide: To a solution of diisopropylamine (0.53 mL, 3.78 mmol, 2.1 eq) in 18 mL anhydrous THF at -78 °C was slowly added 2.5 M *n*-butyllithium in hexane (1.44 mL, 3.60 mmol, 2 eq). The solution was stirred for 15 minutes. Meanwhile, substrate **1.17** (420 mg, 1.80 mmol, 1 eq) was dissolved in 1.8 mL anhydrous THF. This substrate solution was

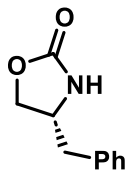
added to the reaaction via syringe slowly, then stirred 45 minutes. The reaction was quenched with saturated $NH_4Cl_{(aq)}$. EtOAc was added, then the layers were separated. The aqueous layer was extracted with EtOAc (3 x 15 mL), the combined organics were washed with brine (1 x 15 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude oil was purified by flash chromatography on silica gel (gradient, 10 to 25% EtOAc/hexanes) to give **1.19** as a white crystalline solid (230 mg, 67%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.49 – 7.32 (m, 5H), 6.75 (s, N*H*, 1H), 5.93 (s, 1H), 5.08 (s, 1H), 2.48 (p, *J* = 6.9 Hz, 1H), 1.25 (d, *J* = 6.9 Hz, 6H).



1.20

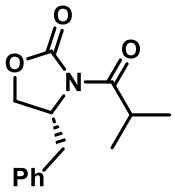
(R)-2-amino-3-phenylpropan-1-ol: To a solution of *D*-phenylalanine (20.000 g, 121.07 mmol, 1 eq) in 300 mL anhydrous THF in a two-neck round bottom flask at 0 °C under Ar(g) was added sodium borohydride (10.992 g, 290.57 mmol, 2.4 eq) slowly over 10 minutes. Once the addition was complete, a solution of I_2 (30.729 g, 121.07 mmol, 1 eq) in 80 mL anhydrous THF was added dropwise via addition funnel over 1 hour. Once the addition was complete, the reaction flask was equipped with a condenser and the reaction was heated to reflux. After 12 hours, the reaction was cooled to room temperature, 100 mL methanol was added, and the mixture was stirred an additional 30 minutes. The reaction was concentrated under reduced pressure, then the residue was taken up in 300 mL 20% aqueous KOH and stirred 4 hours. The solution was extracted with DCM (3 x 70 mL), the combined organics dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give the product **1.20** as a white crystalline solid (17.574 g, 96%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.40 – 7.28 (m, Ar*H*, 2H), 7.25 – 7.17 (m, Ar*H*, 3H), 3.66 – 3.61 (dd, HO-CH_2, *J* = 10.5, 4.0, 1H), 3.38 (dd, HO-CH_2, *J* = 10.6, 7.2 Hz, 1H), 3.12

(dddd, H_2H-C*H*, *J* = 9.0, 7.3, 5.3, 4.0 Hz, 1H), 2.80 (dd, Ar-CH_2, *J* = 13.5, 5.2 Hz, 1H), 2.53 (dd, Ar-CH_2, *J* = 13.5, 8.5 Hz, 1H).



1.21

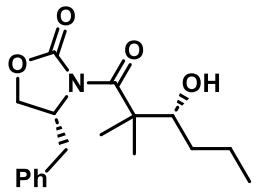
(R)-4-benzyloxazolidin-2-one: To a solution of phenylalinol (**1.20**) (9.071 g, 59.99 mmol, 1 eq) in diethylcarbonate (18.9 mL, 155.97 mmol, 2.6 eq) was added K_2CO_3 (829 mg, 5.999 mmol, 0.1 eq). This suspension was heated to 110 °C and stirred overnight. The reaction was then cooled to room temperature and diluted with DCM. The organic was washed with water (3 x 25 mL), brine (2 x 25 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude solid was purified by recrystallization from 2:1 EtOAc/hexanes to give oxazolidinone **1.21** as a white crystalline solid (7.689 g, 72%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.38 – 7.27 (m, 3H), 7.21 – 7.16 (m, 2H), 4.90 (bs, 1H), 4.49 (t, *J* = 8.3 Hz, 1H), 4.16 (dd, *J* = 8.6, 5.5 Hz, 1H), 4.13 – 4.04 (m, 1H), 2.87 (qd, *J* = 13.6, 7.0 Hz, 2H).



1.22

(R)-4-benzyl-3-isobutyryloxazolidin-2-one: To a solution of (R)-4-benzyloxazolidin-2-one **(1.21**) (2.000 g, 11.29 mmol, 1 eq), isobutyric acid (1.36 mL, 14.77 mmol, 1.3 eq), and DMAP (127 mg, 1.129 mmol, 0.1 eq) in 18.4 mL distilled DCM at 0 °C under Ar(g) was added DIC (2.3 mL, 14.67 mmol, 1.3 eq) dropwise. This reaction was warmed to room temperature and allowed to stir 2 hours, monitoring by TLC (25% EtOAc/hexanes). When complete, the reaction was

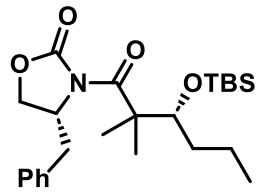
filtered through celite, rinsed with DCM, and washed with saturated $NaHCO_{3(aq)}$ (1 x 20 mL), brine (1 x 20 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (gradient, 10 to 25% EtOAc/hexanes) to yield **1.22** as a white crystalline solid (2.593g, 93%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.36 – 7.28 (m, Ar*H*, 3H), 7.24 – 7.19 (m, Ar*H*, 2H), 4.68 (ddt, *J* = 10.5, 6.7, 3.2 Hz, 1H), 3.77 (hept, *J* = 6.8 Hz, 1H), 3.27 (dd, *J* = 13.3, 3.3 Hz, 1H), 2.77 (dd, *J* = 13.3, 9.6 Hz, 1H), 1.22 (dd, *J* = 18.9, 6.8 Hz, 6H).



1.23

(*R)-4-benzyl-3-((R)-3-hydroxy-2,2-dimethylhexanoyl)oxazolidin-2-one*: To a round bottom flask under Ar(g) cooled to -78 °C containing diisopropylamine (1.1 mL, 7.76 mmol, 1.6 eq) was added 2.5M *n*-butyllithium in hexane (2.9 mL, 7.76 mmol, 1.5 eq) dropwise. The reaction stirred 5 minutes, giving a white precipitate, before the solvent being removed via vacuum while coming to room temperature. Once the solvent was removed, the white solid was cooled back to -78 °C and dissolved in 57 mL anhydrous THF. Substrate **1.22** (1.2 g, 4.85 mmol, 1 eq) was dissolved in 57 mL anhydrous THF, added to the reaction flask via cannula, and stirred 20 minutes. Chlorotriisopropoxytitanium(IV) chloride (3.115 g, 19.4 mmol, 4 eq) was dissolved in 19 mL anhydrous THF, added to the reaction flask, and stirred1 hour. Butanal (1.3 mL, 14.6 mmol, 4 eq) was dissolved in 19 mL anhydrous THF, and stirred an additional 3 hours, monitoring by TLC. When complete, the reaction was quenched with saturated $NH_4Cl_{(aq)}$, extracted with EtOAc (3 x 60 mL), the combined organics were washed with brine (1 x 60 mL),

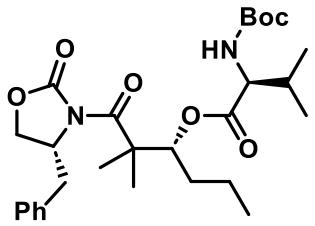
dried over Na_2SO_4, and concentrated under reduced pressure. The crude material was purified by flash chromatography on silica gel (gradient, 5 to 25% EtOAc/hexanes) to yield product **1.23** as a clear colorless oil (1.368 g, 88%). **^{1}H NMR** δ (400 MHz, $CDCl_3$) δ 7.37 – 7.28 (m, 2H), 7.24 – 7.14 (m, 3H), 5.12 (m, 1H), 4.24 – 4.15 (m, 1H), 4.09 (dt, *J* = 11.7, 8.1 Hz, 1H), 3.90 (dt, *J* = 12.0, 3.5, 1H), 3.32 – 3.21 (m, 1H), 3.05 (dd, *J* = 13.8, 6.2 Hz, 1H), 2.35 (dd, *J* = 17.0, 6.6, 1H), 1.38 (d, *J* = 21.0 Hz, 1H), 1.31 – 1.21 (m, 3H), 1.12 (s, 3H), 0.89 – 0.84 (m, 6H). **HRMS** (ESI) Calcd for $[C_{18}H_{25}NO_4Na]^+$ 342.1684, found 342.1706.



1.24

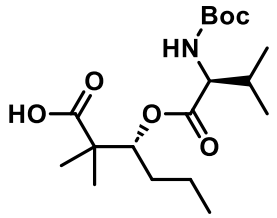
(R)-4-benzyl-3-((R)-3-((tert-butyldimethylsilyl)oxy)-2,2-dimethylhexanoyl)oxazolidin-2-one: To a solution of substrate **1.23** (101 mg, 0.316 mmol, 1 eq) and imidazole (52 mg, 0.758 mmol, 2.4 eq) in 2.5 mL DCM at 0 °C under Ar(g) was added TBSCl (56 mg, 379 mmol, 1.2 eq) slowly. The reaction was warmed to room temperature and monitored by TLC (15% EtOAc/hexanes). Once complete (approximately 2 hours) the reaction was quenched with saturated $NaHCO_3$, extracted with DCM (3 x 10 mL), the combined organics washed with brine (1 x 10 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica (gradient, 10-25% EtOAc/hexanes) to give product **1.24** as a clear colorless oil (49 mg, 36%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.32 – 7.19 (m, Ar*H*, 5H), 5.23 – 5.13 (m, C*H*-CH_2-Ar, 1H), 4.22 – 4.13 (m, C*H*$_2$-CH-Bn, 1H, partially hidden), 3.83 (bm, C*H*-OTBS, 1H) 3.88 (dd, C*H*$_2$-CH-Bn, *J* = 10.1, 5.8 Hz, 1H), 3.25 (dd, C*H*$_2$-Ar, *J* = 13.6, 10.9 Hz, 1H), 3.05 (dd, C*H*$_2$-Ar, *J* = 13.6, 6.0 Hz, 1H), 1.69 – 1.57 (m, CH_3-CH_2-C*H*$_2$, 2H), 1.42 – 1.29 (m, CH_3-C*H*$_2$, 2H), 1.14 (s, CH_3-CR_3, 3H) 0.92 (s, $(CH_3)_3$-C-Si, 9H, partially hidden),

0.90 (t, C*H*$_3$-CH$_2$, *J* = 7.0, 3H, partially hidden), 0.84 (s, CH$_3$-CR$_3$, 3H), 0.10 (s, CH$_3$-Si, 3H), 0.10 (s, CH$_3$-Si, 3H). **HRMS** (ESI) Calcd for $[C_{24}H_{39}NO_4SiH]^+$ 434.2726, found 434.2751.



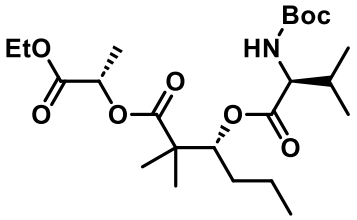
1.25

(R)-1-((R)-4-benzyl-2-oxooxazolidin-3-yl)-2,2-dimethyl-1-oxohexan-3-yl (tert-butoxycarbonyl)-L-valinate: To a solution of substrate **1.23** (689 mg, 2.16 mmol, 1 eq), DMAP (31 mg, .280 mmol, .16 eq) and *N*-Boc-*L*-valine (608 mg, 2.80 mmol, 1.3 eq) in 3.5 mL DCM under Ar(g) at 0 °C was added DIC (0.55 mL, 2.8 mmol, 1.3 eq) dropwise. White solids formed after 30 seconds of stirring, and the reaction was allowed to stir 2 hours, monitoring by TLC (25% EtOAc/hexanes). Once complete, the suspension was filtered through celite and rinsed with additional DCM. The filtrate was washed with saturated $NaHCO_{3(aq)}$ (1 x 10 mL), brine (1 x 10 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (gradient, 5 to 25% EtOAc/hexanes) to yield **1.25** as a white solid (910 mg, 82%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ **^{13}C NMR** (126 MHz, $CDCl_3$) rotameric mixture, minor rotamer in parenthesis, δ 175.74, 172.14, 155.41, 152.57, 136.23, 129.49, 128.83, 127.05, 79.45, 75.55, 58.38, 58.13, (37.34), 37.13, (31.44), 31.31, 31.16, 31.07, 28.31, 28.25, 28.08, (27.79), 20.58, (20.53), 20.46, (19.47), 19.31, (17.48), 17.07, 13.74. **HRMS** (ESI) Calcd for $[C_{28}H_{42}N_2O_7Na]^+$ 541.2892, found 541.2915.



1.26

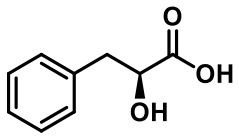
(R)-3-(((tert-butoxycarbonyl)-L-valyl)oxy)-2,2-dimethylhexanoic acid: Substrate **1.25** (2.903 g, 5.619 mmol, 1 eq) was dissolved in 50 mL of a 4:1 solution of THF:water and cooled to 0 °C. LiOH (377 mg, 8.990 mmol, 1.6 eq) was added followed by the dropwise addition of 30% hydrogen peroxide in water (2.1 mL, 20.23 mmol, 3.6 eq). This mixture was stirred for 1 hour, then was quenched by the addition of 2.980 g sodium sulfite. The volatiles were removed under reduced pressure, then the remaining suspension was diluted with deionized water and extracted with DCM (2 x 40 mL). The aqueous layer was acidified to pH 2 with 1M HCl, then extracted with Et_2O (3 x 50 mL), the combined organics were washed with brine (1 x 50 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure to yield **1.26** as a sticky white solid (1.939 g, 96%). **^{1}H NMR** (400 MHz, $CDCl_3$) rotameric mixture δ 5.27 (dt, *J* = 10.1, 1.7 MHz, 1H), 4.96 (t, *J* = 9.7 Hz, 1H), 4.24 and 4.17 (2 dd, *J* = 9.2, 4.7 Hz, 1H, rotamer), 2.24 – 2.11 (m, 1H), 1.63 – 1.48 (m, 1H), 1.46 – 1.42 (m, 12H), 1.32 (dt, *J* = 13.5, 6.7 Hz, 1H), 1.24 – 1.17 (m, 6H), 1.03 – 0.96 (m, 3H), 0.95 – 0.85 (m, 6H).



1.27

(S)-1-ethoxy-1-oxopropan-2-yl-(R)-3-(((tert-butoxycarbonyl)-L-valyl)oxy)-2,2-dimethylhexanoate: To a solution of substrate **1.26** (584 mg, 1.62 mmol, 1 eq), ethyl lactate (0.37

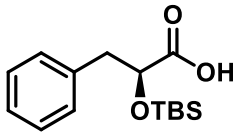
mL, 3.24 mmol, 2 eq), and DMAP (397 mg, 3.24 mmol, 2 eq) in 2.7 mL DCM at 0 °C under Ar(g) was added EDC (311 mg, 1.62 mmol, 1 eq) slowly. The reaction was stirred and monitored by TLC (15% EtOAc/hexanes). Once complete (approximately 2 hours) the reaction was diluted with additional DCM and deionized water. The layers were separated, and the organic layer was washed with deionized water (1 x 10 mL), saturated $NaHCO_{3(aq)}$ (1 x 10 mL), brine (1 x 10 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified via flash chromatography on silica gel (gradient, 5 to 25% EtOAc/hexanes) to yield the product **1.27** as a clear colorless oil (455 mg, 65%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.30 (dd, *J* = 10.1, 2.1 Hz, 1H), 5.10 – 5.00 (m, 2H), 4.22 (dq, *J* = 22.8, 7.2 Hz, 5H), 2.79 (d, *J* = 5.1 Hz, 1H), 2.25 – 2.10 (m, 1H), 1.65 – 1.54 (m, 2H), 1.49 (d, *J* = 7.1 Hz, 3H), 1.44 (s, 9H), 1.42 (d, *J* = 7.2 Hz, 3H), 1.33 – 1.24 (m, 6H), 1.23 (s, 3H), 1.21 (s, 3H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.93 – 0.87 (m, 6H).



1.28

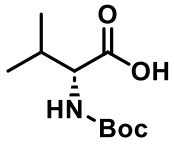
(S)-2-hydroxy-3-phenylpropanoic acid: To a 2-neck round bottom flask containing L-phenylalanine (10.00 g, 60.5 mmol, 1 eq) was added 2 M H_2SO_4 (70 mL, 139 mmol, 2.3 eq). This solution was cooled to 0 °C, then a 30% solution of $NaNO_2$ in water (55.6 mL, 242 mmol, 4 eq) was added dropwise through an addition funnel over 1 hour, using an air hose to blow away the brown gas formed. Once the addition was complete, the reaction was allowed to stir 18 hours, coming to room temperature. Once complete, solid NaCl was added until the solution was saturated. The suspension was then extracted with EtOAc (3 x 100 mL), the combined organics were washed with brine (1 x 100 mL), dried over Na_2SO_4, and concentrated under reduced

pressure to yield the product **1.28** as a white fluffy solid (7.255 g, 72%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 12.44 (s, 1H), 7.38 – 7.08 (m, 5H), 5.26 (s, 1H), 4.13 (dd, *J* = 8.4, 4.5 Hz, 1H), 3.31 (s, 1H), 2.94 (dd, *J* = 13.7, 4.5 Hz, 1H), 2.76 (dd, *J* = 13.7, 8.3 Hz, 1H), 2.48 (p, *J* = 1.9 Hz, 2H).



1.29

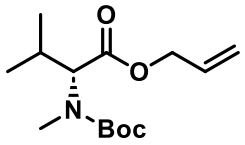
(S)-2-((tert-butyldimethylsilyl)oxy)-3-phenylpropanoic acid: To a solution of phenyllactic acid (1.000 g, 6.02 mmol, 1 eq) and imidazole (1.722 g, 25.3 mmol, 4.2 eq) in 22 mL DMF at 0 °C under Ar(g) was added TBSCl slowly. This reaction was stirred, monitoring by TLC (30% EtOAc/hexanes). Once complete (approximately 4 hours) the reaction was diluted with EtOAc and washed with 10% citric $acid_{(aq)}$ (1 x 20 mL), saturated $NaHCO_3$ (1 x 20 mL), brine (1 x 20 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica (gradient, 15 to 40% EtOAc/hexanes) to yield product **1.29** as a white crystalline solid (1.586 g, 94%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.31 – 7.16 (m, 5H), 4.28 (dd, *J* = 8.5, 3.9 Hz, 1H), 3.06 (dd, *J* = 13.4, 4.0 Hz, 1H), 2.87 (dd, *J* = 13.4, 8.6 Hz, 1H), 0.80 (s, 9H), -0.09 (s, 3H), -0.21 (s, 3H).



1.30

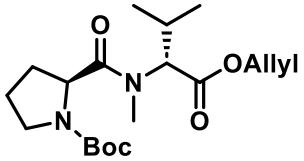
(tert-butoxycarbonyl)-D-valine: To a solution of D-Valine (5.000 g, 42.68 mmol, 1 eq) in 170 mL 0.63 M $NaOH_{(aq)}$ and 100 mL *t*-butyl alcohol was added di-*tert*-butyl dicarbonate (11.178 g, 51.12 mmol, 1.2 eq). This solution was stirred for 12 hours, then the pH was adjusted to 2 with 1 M HCl. The resulting solution was extracted with EtOAc (3 x 100 mL), the conbined organics

dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by recrystallization from Et_2O to give product **1.30** as a white crystalline solid (9.014 g, 97%). **^{1}H NMR** (400 MHz, $CDCl_3$) rotameric mixture δ 5.00 and 4.25 (d, N*H*, *J* = 7.9, 1H, rotamer), 2.31 – 2.14 (m, $(CH_3)_2$-C*H*, 1H), 1.45 (s, $(CH_3)_3$-O, 9H), 1.01 (d, $(CH_3)_2$-CH, *J* = 6.9 Hz, 3H), 0.94 (d, $(CH_3)_2$-CH, *J* = 6.9 Hz, 3H).



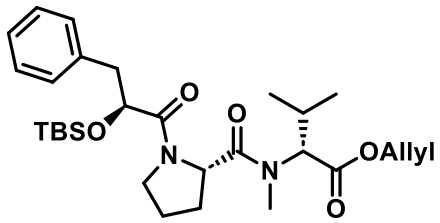
1.31a

Allyl N-(tert-butoxycarbonyl)-N-methyl-D-valinate: To a solution of amino acid X (1.812 g, 7.83 mmol, 1 eq) and K_2CO_3 (1.624 g, 11.8 mmol, 1.5 eq) in 20 mL anhydrous DMF was added allyl bromide (0.74 mL, 8.61 mmol, 1.1 eq). The reaction was stirred at room temperature overnight. The next day, the reaction was diluted with distilled H_2O, then was extracted with EtOAc (3 x 30 mL). The combined organics were washed with distilled water (2 x 30 mL), 10% $LiCl_{(aq)}$ (1 x 30 mL), brine (1 x 30 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give allyl ester **1.31a** as a clear colorless oil (1.950 g, 92%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.92 (ddt, *J* = 16.5, 10.4, 5.8 Hz, 1H), 5.40 – 5.21 (m, 2H), 5.02 (d, *J* = 9.2 Hz, 1H), 4.64 (ddt, *J* = 7.3, 5.8, 1.4 Hz, 2H), 4.25 (dd, *J* = 9.3, 4.7 Hz, 1H), 2.95 (s, *J* = 6.7 Hz, 3H), 2.22 – 2.08 (m, 1H), 1.45 (s, 9H), 0.97 (d, *J* = 6.9 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 3H).



1.32

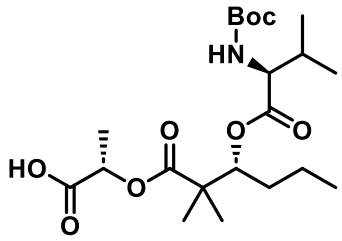
tert-butyl (S)-2-(((R)-1-(allyloxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)pyrrolidine-1-carboxylate: Substrate **1.31a** (730 mg, 1.85 mmol, 1 eq) was dissolved in 7 mL of a 1:1 mixture of acetonitrile and diethylamine. The reaction was stirred one hour, concentrated under reduced pressure, then re-dissolved in 2 mL DCM. To a solution of *N*-boc-L-Proline (519 mg, 2.41 mmol, 1.3 eq) and HATU (916 mg, 2.41 mmol, 1.3 eq) in 19 mL DCM under Ar(g) was added DIPEA (0.4 mL, 2.41 mmol, 1.3 eq) slowly. After stirring for 20 minutes, the solution of deprotected X was added to the reaction and stirred 4 hours. The reaction was diluted with more DCM then washed subsequently with $NaHCO_3$ (2 x 20 mL), brine (1 x 20 mL), dried over anhydrous Na_2SO_4, then concentrated under reduced pressure. The crude product was purified by flash chromatography on silica (gradient, 10 to 50% EtOAc/hexanes) to give product **1.32** as a white solid (480 mg, 70%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.90 (dd, *J* = 16.9, 10.6 Hz, 1H), 5.39 – 5.16 (m, 2H), 4.92 (d, *J* = 10.5 Hz, 1H), 4.76 – 4.47 (m, 3H), 3.69 – 3.33 (m, 1H), 3.08 (d, *J* = 21.5 Hz, *rotamers*, N-*CH_3*, 3H), 2.24 (s, 2H), 1.84 (s, 2H), 1.57 (s, 9H), 1.00 (d, *J* = 6.6 Hz, 3H), 0.86 (d, *J* = 6.7 Hz, 3H)



1.33

allyl N-(((S)-2-((tert-butyldimethylsilyl)oxy)-3-phenylpropanoyl)-L-prolyl)-N-methyl-D-valinate: Dipeptide **1.32** (355 mg, 0.964mmol, 1 eq) was dissolved in 12 mL of a 1:1 mixture of DCM and TFA. This reaction was stirred for 2 hours then concentrated under reduced pressure. To a solution of **1.29** (351 mg, 1.25 mmol, 1.3 eq) and 1-hydroxy-7-azabenzotriazole (171 mg, 1.25 mmol, 1.3 eq) in 10 mL DCM under Ar(g) was added EDC (240 mg, 1.25 mmol, 1.3 eq) slowly

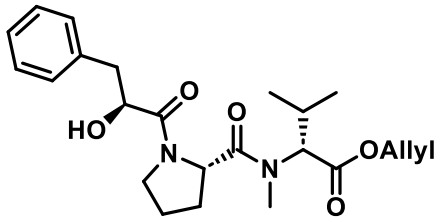
and stirred 30 minutes. The deprotected dipeptide was dissolved in 2 mL DCM, added to the reaction flask, then stirred 4 hours. Once complete, the reaction was diluted with DCM and washed with saturated $NaHCO_3$ (2 x 10 mL), brine (1 x 20 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The reaction was purified by flash chromatography on silica (gradient, 35 to 80% EtOAc/hexanes) to give tripeptide **1.33** as a clear colorless oil (296 mg, 58%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.36 – 7.14 (m, 5H), 5.97 – 5.83 (m, 1H), 5.06 – 4.85 (m, 1H), 4.72 – 4.57 (m, 2H), 4.51 – 4.41 (m, 1H), 3.93 – 3.56 (m, 1H), 3.19 – 2.86 (m, 4H, rotamers, N-C*H*$_3$), 2.40 – 2.16 (m, 2H), 1.97 – 1.71 (m, 2H) 1.09 (dd, *J* = 12.7, 6.8 Hz, 1H), 1.05 – 0.97 (m, 3H), 0.87 (s, 9H), 0.78 (s, 6H), -0.04 – -0.11 (m, 3H, rotamer), -0.15 – -0.24 (m, 3H, rotamer). **HRMS** (ESI) Calcd for $[C_{23}H_{32}N_2O_5H]^+$ 417.2389, found 417.2378.



1.34

(6S,9R,13S)-6-isopropyl-2,2,10,10,13-pentamethyl-4,7,11-trioxo-9-propyl-3,8,12-trioxa-5-azatetradecan-14-oic acid: Substrate **1.27** (455 mg, 1.06 mmol, 1 eq) was dissolved in 0.12 mL THF, then 4 M NaOH (0.72 mL, 3.17 mmol, 3 eq) was added. The reaction was stirred 4 hours, then diluted with water. The pH of the solution was adjusted to 2 with 1 M HCl, then was extracted with DCM (3 x 10 mL), the combined organics were washed with brine (1 x 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure to yield the product **1.34** as a clear colorless oil (333 mg, 78%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.11 (q, *J* = 7.0 Hz, 1H), 4.15 (dd, *J* = 9.8, 3.7 Hz, 1H), 3.79 – 3.72 (m, 1H), 2.34 – 2.09 (m, 3H), 1.90 – 1.82 (m, 1H), 1.58 – 1.43

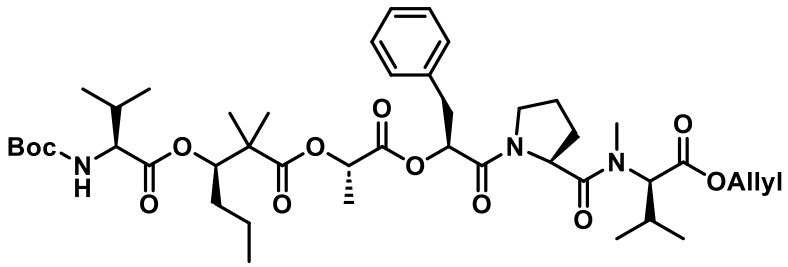
(m, 4H), 1.45 (s, 9H, *partially hidden*), 1.28 – 1.17 (m, 8H), 1.00 (t, *J* = 7.2 Hz, 3H), 0.96 – 0.82 (m, 8H).



1.35

allyl N-(((S)-2-hydroxy-3-phenylpropanoyl)-L-prolyl)-N-methyl-D-valinate: To a solution of tripeptide **1.33** (170 mg, 0.32 mmol, 1 eq) in 3.2 mL anhydrous THF under Ar(g) was added 1 M TBAF in THF (0.35 mL, 0.35 mmol, 1.1 eq) and was stirred 2 hours. The solution was then diluted with DCM and quenched with water. The organic layer was washed with brine (1 x 5 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (gradient, 40 to 80% EtOAc in hexanes) to give the free alcohol **1.35** as a clear yellow oil (117 mg, 88%). **^{1}H NMR** (500 MHz, $CDCl_3$) rotameric mixture δ 7.33 – 7.19 (m, Ar*H*, 5H), 5.91 (ddt, CH_2=C*H*-CH_2, *J* = 16.3, 11.0, 5.5 Hz, 1H), 5.34 (d, C*H*$_2$=CH, *J* = 17.1 Hz, 1H), 5.29 and 5.23 (*J* = 10.3 Hz, 1H, rotamer), 5.07 – 4.93 (m, CH_2=CH-C*H*$_2$-O, 1H), 4.71 and 4.64 (d, C*H*-N-CH_3, 1H, rotamer, partially hidden), 4.64 (t, HO-C*H*, *J* = 5.8, Hz, 1H, partially hidden), 4.50 – 4.40 (m, O_2C-C*H* (proline), 1H), 3.65 – 3.57 (m, N-C*H*$_2$ (proline), 1H), 3.46 (d, N-CH_2 (proline), *J* = 7.9 Hz, 1H), 3.15 and 2.93 (s, N-C*H*$_3$, 3H, rotamer), 3.09 (dd, Ar-C*H*$_2$, *J* = 14.4, 3.5, 1H), 2.85 (dd, Ar-C*H*$_2$, *J* = 14.6, 8.1 Hz, 1H), 2.32 (dq, C*H*$_2$-CH-N (proline) *J* = 11.0, 6.3 Hz, 1H), 2.27 – 2.11 (m, C*H*$_2$-CH-N (proline), 1H, partially hidden), 2.27 – 2.11 (m, CH_2-C*H*$_2$-N (proline), 1H, partially hidden), 2.03 – 1.88 (m, CH_2-C*H*$_2$-N (proline), 1H), 1.06 and 1.03 (d, (C*H*$_3$)$_2$-CH, *J* = 6.7 Hz, 3H, rotamer), 0.88 and 0.80 (d, (C*H*$_3$)-CH, *J* = 6.7 Hz, 3H, rotamer). **^{13}C NMR** (126 MHz, $CDCl_3$) rotameric mixture, minor

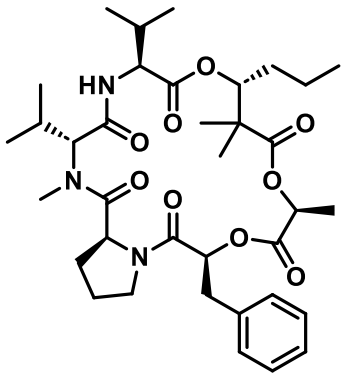
rotamer in parenthases δ (172.21) and 172.13, (172.10) and 172.02, 170.25, 137.22, 131.33, 129.63, 128.30, 126.46, 118.40, (70.66) and 70.37, 65.86, 65.44, 62.88, 57.04, (46.83) and 46.69, (40.91) and 40.73, (28.83) and 28.42, (25.02) and 24.92, 20.04, 18.78. **HRMS** (ESI) Calcd for $[C_{29}H_{46}N_2O_5SiH]^+$ 531.3254, found 531.3284.



1.36

(S)-1-(((S)-1-((S)-2-(((R)-1-(allyloxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)pyrrolidin-1-yl)-1-oxo-3-phenylpropan-2-yl)oxy)-1-oxopropan-2-yl-(R)-3-(((tert-butoxycarbonyl)-L-valyl)oxy)-2,2-dimethylhexanoate: To a solution of tripeptide **1.35** (81 mg, 0.194 mmol, 1 eq), triester **1.34** (84 mg, 0.194 mmol, 1 eq), and DMAP (24 mg, 0.194 mmol, 1 eq) in 0.3 mL DCM under Ar(g) was added EDC (81 mg, 0.194 mmol, 1 eq). The reaction was stirred, monitored by TLC (5% methanol/DCM). Once complete (approximately 12 hours) the reaction was diluted with DCM then washed with saturated $NaHCO_3$ (1 x 5 mL), brine (1 x 5 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica (gradient, 1 to 5% methanol/DCM) to give product **1.36** as a clear colorless oil (90 mg, 56%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.38 – 7.17 (m, 5H), 5.92 (tt, *J* = 11.3, 5.7 Hz, 1H), 5.40 – 5.17 (m, 4H), 5.13 – 4.88 (m, 2H), 4.77 – 4.55 (m, 3H), 4.29 – 4.05 (m, 1H), 3.90 – 3.39 (m, 2H), 3.29 – 3.05 (m, 3H), 2.97 – 2.76 (m, 1H), 2.16 (s, 3H), 2.07 – 1.76 (m, 1H), 1.50 (dd, *J* = 6.9, 3.1 Hz, 2H), 1.48 – 1.37 (m, 9H), 1.29 – 1.14 (m, 3H), 1.10 – 0.95 (m, 6H), 0.94 – 0.76 (m, 6H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 172.14, 170.29, 168.74, 167.97, 167.07, 166.04, 162.76, 136.26, 131.93, 131.36, 129.69, 129.35, 129.32, 128.55, 128.51, 128.29,

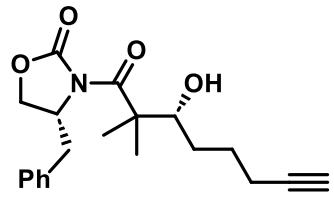
126.85, 118.42, 79.64, 68.45, 65.43, 56.88, 53.42, 46.83, 46.71, 36.77, 32.92, 32.11, 31.36, 30.82, 29.15, 28.69, 28.32, 27.80, 24.70, 22.99, 19.98, 19.41, 19.34, 18.73, 17.42, 16.75, 13.76. **HRMS** (ESI) Calcd for $[C_{44}H_{67}N_3O_{12}H]^+$ 830.4803, found 830.4818.



Palmyramide A

Palmyramide A: To a solution of linear intermediate **1.36** (85 mg, 0.1024 mmol, 1 eq) and $Pd_2(dba)_3$ (5 mg, 0.00512 mmol, .05 eq) in 0.8 mL THF under Ar(g) was added trimethylphosphine (5 µL, .0169 mmol, 0.35 eq). This solution was cooled to 0 °C, then morpholine (90 µL, 1.024 mmol, 10 eq) was added slowly. The reaction was warmed to room temperature, then stirred while monitoring by TLC (5% MeOH/DCM). After completion (approximately 2 hours), the volatiles were removed under reduced pressure. The crude oil was dissolved in DCM, then washed with 1 M HCl (1 x 10 mL), water (1 x 10 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give the free acid as a clear yellow oil that was used without further purification. The oil was taken up in 1 mL each of DCM and TFA and stirred one hour. Once compete, the volatiles were removed in vacuo to give the TFA salt of the deprotected linear intermediate as a yellow oil that was dried in a vacuum desiccator and used without further purification. The oil dissolved in 102 mL DCM under Ar and pyridine (30 µL, 0.307 mmol, 3 eq) was added, followed by T3P (0.12 mL, 0.205 mmol, 2 eq). The reaction was stirred at room temperature for 120 hours. Once complete, the solution

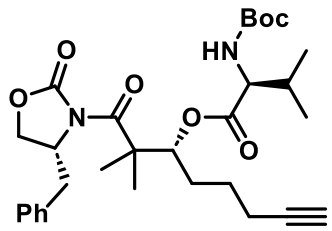
was washed with saturated aqueous $NaHCO_3$ (1 x 20 mL), water (1 x 20 mL), brine (1 x 20 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica (gradient, 1 to 10% MeOH/DCM) to give palmyramide A as a clear colorless oil (8.6 mg, 13%). **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.37 – 7.29 (m, 5H), 6.58 (d, *J* = 5.3 Hz, 1H), 5.62 (dd, *J* = 9.6, 2.0 Hz, 1H), 5.07 (dd, *J* = 11.2, 4.7 Hz, 1H), 4.85 (q, *J* = 7.1 Hz, 1H), 4.50 (dd, *J* = 5.4, 3.1 Hz, 1H), 4.46 (d, *J* = 11.2 Hz, 1H), 3.61 (ddd, *J* = 12.0, 7.8, 4.2 Hz, 1H), 3.46 (dt, *J* = 11.5, 7.1 Hz, 1H), 3.28 (dd, *J* = 8.5, 2.9 Hz, 1H), 3.17 (t, *J* = 11.8 Hz, 1H), 2.99 (dd, *J* = 12.4, 4.6 Hz, 1H), 2.89 (s, 3H), 2.42 (td, *J* = 7.1, 3.0 Hz, 1H), 2.17 (dt, *J* = 12.6, 6.4 Hz, 2H), 1.71 (m, 1H), 1.6 (m, 1H), 1.55 (m, 2H), 1.52 (m, 1H), 1.51 (d, *J* = 7.2 Hz, 3H), 1.43 (m, 1H), 1.38 (m, 2H), 1.25 (s, 3H), 1.20 (s, 3H), 1.02 (d, *J* = 7.1 Hz, 3H), 0.98 (d, *J* = 6.6 Hz, 3H), 0.93 (d, *J* = 7.0 Hz, 3H), 0.88 (t, *J* = 6.8 Hz, 3H), 0.75 (d, *J* = 6.6 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 174.9, 171.2, 170.1, 169.7, 168.3, 167.2, 134.9, 129.7, 128.6, 127.4, 77.2, 70.9, 69.0, 61.7, 58.3, 56.7, 46.7, 46.4, 39.2, 31.6, 30.7, 30.5, 30.5, 27.3, 24.3, 22.0, 19.6, 19.3, 18.8, 18.3, 17.8, 16.9, 16.7, 13.9. **HRMS** (ESI) Calcd for $[C_{36}H_{53}N_3O_9H]^+$ 672.3860, found 672.4051.



1.40

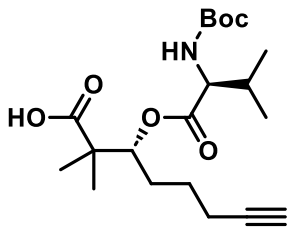
(R)-4-benzyl-3-((R)-3-hydroxy-2,2-dimethyloct-7-ynoyl)oxazolidin-2-one: To a round bottom flask containing diisopropylamine (0.91 mL, 6.47 mmol, 1.6 eq) under Ar(g) at -78 °C was added a 2.5 M solution of *n*-butyllithium in hexane (2.4 mL, 6.07 mmol, 1.5 eq). This was stirred 5 minutes at -78 °C then slowly warmed to room temperature. Once at room temperature,

the solution was placed under vacuum until all volatiles were removed, leaving a white solid. The flask was then re-flushed with Ar(g) before the solids were dissolved in 48 mL anhydrous THF and cooled to -78 °C. A solution of substituted oxazolidinone **1.22** (1.000 g, 4.04 mmol, 1 eq) dissolved in 48 mL anhydrous THF was added slowly and allowed to stir 30 minutes. A solution of chlorotriisopropoxy titanium (IV) (4.215 g, 16.2 mmol, 4 eq) dissolved in 16 mL anhydrous THF was added to the reaction and allowed to stir 45 minutes. A solution of hex-5-ynal (1.166 g, 12.1 mmol, 3 eq) dissolved in 16 mL anhydrous THF was added to the reaction flask and stirred 3 hours. Once complete, the reaction was quenched with saturated aqueous NH_4Cl and allowed to come to room temperature. The biphasic mixture was extracted with EtOAc (3 x 30 mL), the combined organics were washed with brine (1 x 25 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatograph on silica (gradient, 10 to 25% EtOAc/hexanes) to give aldol product **1.40** as a clear colorless oil (405 mg, 29%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.37 – 7.21 (m, 5H), 4.70 (ddt, *J* = 10.0, 7.2, 3.0 Hz, 1H), 4.26 – 4.10 (m, 3H), 3.69 (t, *J* = 6.2 Hz, 1H), 3.27 (dd, *J* = 13.3, 3.3 Hz, 1H), 2.77 (dd, *J* = 13.3, 9.8 Hz, 1H), 2.27 (td, *J* = 6.8, 2.7 Hz, 2H), 1.95 (t, *J* = 2.6 Hz, 1H), 1.92 – 1.77 (m, 1H), 1.73 – 1.55 (m, 2H), 1.55 – 1.44 (m, 1H, partially hidden), 1.42 (s, 3H), 1.37 (s, 3H) **^{13}C NMR** (126 MHz, $CDCl_3$) δ 178.18, 152.26, 135.48, 129.42, 128.94, 127.34, 84.30, 75.34, 68.56, 66.33, 57.76, 50.12, 37.82, 30.14, 25.61, 20.34, 18.48, 18.23. **HRMS** (ESI) calcd for $[C_{20}H_{25}NO_4Na]^+$ 366.1681, found 366.1691.



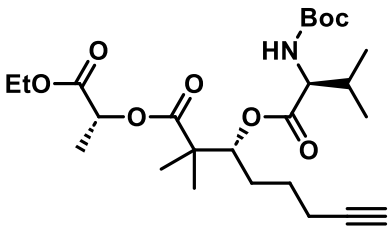
1.41

(R)-1-((R)-4-benzyl-2-oxooxazolidin-3-yl)-2,2-dimethyl-1-oxooct-7-yn-3-yl (tert-butoxycarbonyl)-L-valinate: To a solution of substituted oxazolidinone **1.40** (405 mg, 1.18 mmol, 1 eq) in 1.9 mL DCM under Ar(g) was added DMAP (17 mg, 0.153 mmol, 0.13 eq) and *N*-Boc-L-valine (332 mg, 1.53 mmol, 1.3 eq). This solution was cooled to 0 °C, then EDC (294 mg, 1.53 mmol, 1.3 eq) was added. The reaction was stirred 5 minutes at 0 °C, then warmed to room temperature for 2 hours. Once the starting material was consumed by TLC (15% EtOAc/hexanes), the reaction was diluted with DCM then washed with saturated aqueous $NaHCO_3$ (1 x 5 mL), brine (1 x 10 mL), the organic layer was dried over anhydrous Na_2SO_4, then concentrated under reduced pressure. The crude product was purified by flash chromatography on silica (gradient, 5 to 25% EtOAc/hexanes) to give product **1.41** as a clear, light yellow oil (310 mg, 48%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.34 – 7.18 (m, 5H), 7.15 (d, *J* = 7.7 Hz, 1H), 6.16 (dd, *J* = 9.5, 2.7 Hz, 1H), 4.96 (d, *J* = 9.2 Hz, 1H), 4.58 (td, *J* = 7.0, 3.1 Hz, 1H), 4.21 (dd, *J* = 9.2, 4.2 Hz, 1H), 4.15 – 4.09 (m, 2H), 3.23 (dd, *J* = 13.1, 3.4 Hz, 1H), 2.59 (d, *J* = 12.0 Hz, 1H), 2.23 (t, *J* = 7.1 Hz, 3H), 2.15 (q, *J* = 8.5, 6.9 Hz, 2H), 2.02 (d, *J* = 7.1, 1H) 1.95 – 1.89 (m, 1H), 1.77 – 1.65 (m, 2H), 1.34 – 1.31 (m, 2H) 1.43 (d, *J* = 8.2 Hz, 2H) 1.26 (s, 9H) 0.95 (d, *J* = 6.7 Hz, 3H) 0.86 (d, *J* = 6.8 Hz). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 175.64, 172.17, 155.44, 152.58, 136.14, 129.47, 128.87, 127.12, 83.74, 75.41, 68.77, 66.38, 58.50, 58.05, 49.54, 37.31, 31.16, 28.59, 28.35, 28.15, 25.08, 20.56, 20.31, 19.32, 18.02, 17.25.



1.42

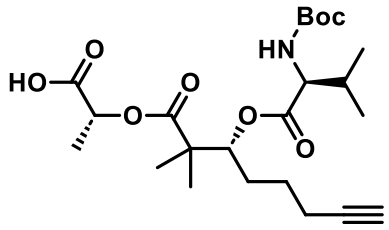
(R)-3-(((tert-butoxycarbonyl)-L-valyl)oxy)-2,2-dimethyloct-7-ynoic acid: Substrate **1.41** (310 mg, 0.571 mmol, 1 eq) was dissolved in 4.8 mL of a 4:1 mixture of THF and water. The solution was cooled to 0 °C, then 30% H_2O_2 in water (0.21 mL, 2.06 mmol, 3.6 eq) was added, followed by LiOH (38 mg, 0.914 mmol, 1.3 eq). The reaction was stirred 2 hours at 0 °C. Once complete, the reaction was quenched by the addition of 298 mg Na_2SO_3, then the volatiles were removed in vacuo. The remaining suspension was diluted with water, then extracted with DCM (2 x 10 mL). The pH of the aqueous layer was adjusted to 2 with 1 M HCl, then was extracted by Et_2O (3 x 10 mL). The combined organics were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give free acid **1.42** as a clear colorless oil (167 mg, 76%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.26 (d, *J* = 10.0 Hz, 1H), 4.96 (d, *J* = 9.4 Hz, 1H), 4.18 (dd, *J* = 9.3, 4.7 Hz, 1H), 2.23 (td, *J* = 6.9, 2.6 Hz, 3H), 2.19 – 2.11 (m, 1H), 1.97 – 1.91 (m, 1H), 1.76 – 1.63 (m, 2H), 1.54 (h, *J* = 6.9 Hz, 2H), 1.44 (s, 12H), 1.22 (s, 3H, partially hidden) 0.99 (d, *J* = 6.8 Hz, 3H), 0.91 (d, *J* = 6.9 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 178.83, 172.15, 155.90, 83.54, 77.68, 76.29, 68.91, 58.96, 46.57, 29.37, 28.34, 25.02, 21.64, 19.35, 17.99, 17.56. **HRMS** (ESI) calcd for $[C_{20}H_{33}NO_6Na]^+$ 406.2206, found 406.2198.



1.43

(R)-1-ethoxy-1-oxopropan-2-yl (R)-3-(((tert-butoxycarbonyl)-L-valyl)oxy)-2,2-dimethyloct-7-ynoate: To a solution of free acid **1.42** (167 mg, 0.435 mmol, 1 eq) in 0.8 mL DCM was added ethyl lactate (0.1 mL, 0.871 mmol, 2 eq) and DMAP (106 mg, 0.871 mmol, 2 eq). The solution was cooled to 0 °C, then EDC (86 mg, 0.435 mmol, 1 eq) was added. The reaction was stirred 5

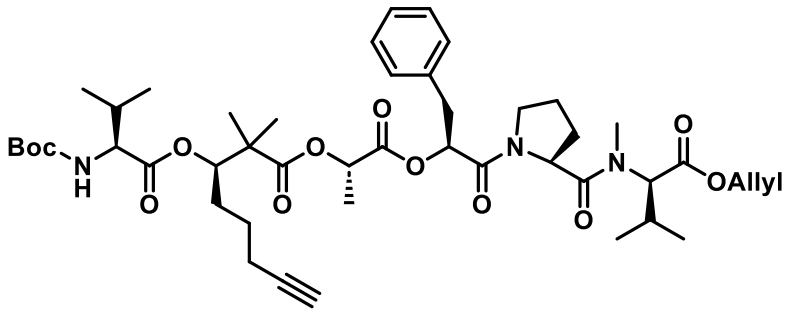
minutes at 0 °C, then was warmed to room temperature and stirred 16 hours. Once complete, the reaction was diluted with DCM, washed with saturated $NaHCO_3$ (1 x 10 mL), brine (1 x 10 mL), dried over anhydrous $NaSO_4$, and concentrated under reduced pressure. The crude product was purified by flash chromatograph on silica (gradient, 5 to 20% EtOAc/hexanes) to give diester **1.43** as a clear yellow oil (140 mg, 67%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.33 – 5.23 (m, 1H), 5.12 – 4.97 (m, 1H), 4.19 (q, *J* = 7.1 Hz, 2H), 2.78 (s, 1H), 2.21 (td, *J* = 6.8, 2.9 Hz, 2H), 1.95 – 1.89 (m, 1H), 1.67 (p, *J* = 7.5 Hz, 2H), 1.63 – 1.53 (m, 3H), 1.50 (d, *J* = 7.0 Hz, 3H), 1.44 (s, 9H), 1.43 – 1.40 (m, 4H), 1.33 – 1.28 (m, 5H), 1.27 – 1.20 (m, 6H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 180.46, 175.72, 172.16, 170.47, 83.66, 76.01, 68.97, 68.79, 66.78, 61.67, 46.64, 30.91, 29.74, 29.55, 28.34, 25.13, 22.68, 20.43, 19.36, 17.97, 17.52, 16.77, 14.10. **HRMS** (ESI) calcd for $[C_{25}H_{41}NO_8Na]^+$ 506.2730, found 506.2723.



1.44

(6S,9R,13R)-6-isopropyl-2,2,10,10,13-pentamethyl-4,7,11-trioxo-9-(pent-4-yn-1-yl)-3,8,12-trioxa-5-azatetradecan-14-oic acid: To a solution of triester **1.43** (140 mg, 0.290 mmol, 1 eq) in 0.25 mL of a 5:1 mixture of THF:water was added a 4 M solution of NaoH in water (0.22 mL, 0.870 mmol, 3 eq). This reaction was stirred at room temperature for 12 hours. Once complete, the reaction was diluted with water, then the pH was adjusted to 1 with 1 M HCl. The solution was extracted with DCM (3 x 10 mL), the combined organics were washed with brine (1 x 10 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give free acid **1.44** as a clear yellow oil (78 mg, 60%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.15 – 4.94 (m, 2H),

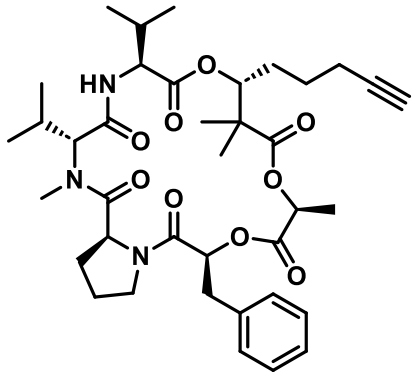
4.24 and 4.18 (td, *J* = 9.7, 8.7, 4.3 Hz, rotameric mixure, 1H), 3.68 (dd, *J* = 10.6, 1.9 Hz, 1H), 2.29 – 2.18 (m, 3H), 1.96 and 1.94 (t, *J* = 2.7 Hz, rotameric mixture, 1H), 1.52 (d, *J* = 7.1 Hz, 3H), 1.45 (d, *J* = 3.7 Hz, 9H), 1.25 (d, *J* = 4.5 Hz, 6H), 1.24 – 1.20 (m, 6H), 0.99 (d, *J* = 6.8 Hz, 6H), 0.96 – 0.86 (m, 6H).



1.45

(S)-1-(((S)-1-((S)-2-(((R)-1-(allyloxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)pyrrolidin-1-yl)-1-oxo-3-phenylpropan-2-yl)oxy)-1-oxopropan-2-yl (R)-3-(((tert-butoxycarbonyl)-L-valyl)oxy)-2,2-dimethyloct-7-ynoate: To a solution of peptide **1.35** (71 mg, 0.170 mmol, 1 eq) and ester **1.44** (78 mg, 0.170 mmol, 1 eq) in 0.3 mL DCM under Ar(g) was added DMAP (21 mg, 0.170 mml, 1 eq). This solution was cooled to 0 °C, then EDC (33 mg, 0.170 mmol, 1 eq) was added. The reaction was stirred at 0 °C for 5 minutes, then warmed to room temperature and stirred for 16 hours. Once complete, the reaction was diluted with DCM then washed with saturated $NaHCO_3$ (1 x 10 mL), water (1 x 10 mL), brine (1 x 10 mL), dried over anhydrous Na_2SO_4, then concentrated under reduced pressure. The crude product was purified by flash chromatography on silica (gradient, 1 to 10% MeOH/DCM) to give protected linear intermediate **1.45** as a clear yellow oil (89 mg, 61%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.33 – 7.22 (m, 5H), 5.92 (ddt, *J* = 20.6, 10.1, 5.1 Hz, 1H), 5.39 – 5.16 (m, 4H), 5.12 – 4.91 (m, 1H), 4.70 (d, *J* = 10.5 Hz, 1H), 4.63 (t, *J* = 7.0 Hz, 2H), 4.20 (d, *J* = 11.4 Hz, 1H), 4.11 (d, *J* = 10.6 Hz, 1H), 3.83 – 3.69 (m, 1H), 3.61 – 3.47 (m, 1H), 3.25 – 3.08 (m, 2H), 2.90 and 2.80 (s, rotameric mixture, 3H),

2.40 – 2.07 (m, 4H), 1.94 and 1.90 (t, *J* = 2.8 Hz, 1H), 1.72 – 1.55 (m, 6H), 1.52 (d, *J* = 7.3 Hz, 2H), 1.44 and 1.43 (s, rotameric mixture, 9H), 1.27 – 1.22 (m, 2H), 1.19 (d, *J* = 9.0 Hz, 3H), 1.03 – 0.96 (m, 6H), 0.95 – 0.84 (m, 6H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 176.59, 172.20, 170.60, 169.78, 168.95, 159.14, 129.36, 129.33, 128.53, 126.86, 119.92, 118.42, 87.90, 83.67, 82.54, 77.94, 75.02, 68.80, 65.86, 65.79, 65.44, 56.90, 38.63, 36.80, 30.86, 30.81, 29.78, 28.34, 28.00, 27.82, 25.14, 24.73, 22.88, 19.99, 19.73, 19.36, 18.75, 17.95, 17.50, 16.76, 17.72. **HRMS** (ESI) calcd for $[C_{46}H_{67}N_3O_{12}Na]^+$ 876.4622, found 876.4620.

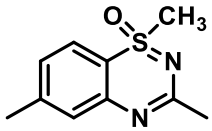


1.46

(3R,6S,9R,13S,16S,21aS)-16-benzyl-3,6-diisopropyl-2,10,10,13-tetramethyl-9-(pent-4-yn-1-yl)octahydro-1H,9H-pyrrolo[1,2-g][1,4,16]trioxa[7,10,13]triazacyclononadecine-1,4,7,11,14,17(10H,13H,16H)-hexaone: To a solution of linear intermediate **1.45** (89 mg, 0.104 mmol, 1 eq) and $Pd_2(DBA)_3$ (5 mg, 0.00521 mmol, 0.05 eq) in 0.8 mL anhydrous THF under argon was added trimethylphosphine (3 μL, 0.0364 mmol, 0.35 eq) and morpholine (0.09 mL, 1.04 mmol, 10 eq). This reaction was allowed to stir at room temperature for 12 hours. Once complete, the volatiles were removed in vacuo and the residue taken up in DCM. The solution was washed with 2 M HCl (2 x 10 mL), water (1 x 10 mL), brine (1 x 10 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give the free acid which was used without further purification. The crude product was taken up in 1 mL each of DCM and

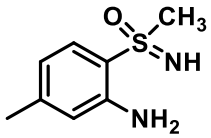
TFA and stirred for 2 hours. Once complete, the solution was concentrated in vacuo to give the TFA salt of the free amine which was dried in a vacuum desiccator and used without further purification. The crude product was dissolved in 104 mL distilled DCM under argon. Pyridine (0.03 mL, 0.312 mmol, 3 eq) was added, followed by the addition of a 50% solution of T_3P in ethyl acetate (0.12 mL, 0.208 mmol, 2 eq). The reaction was stirred for 120 hours at room temperature. Once the cyclization was complete, it was washed with saturated $NaHCO_3$ (1 x 20 mL), water (1 x 20 mL), brine (1 x 20 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica (gradient, 1 to 10% MeOH/DCM) to give cyclized product **1.46** as a clear yellow oil (38 mg, 53%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.40 – 7.16 (m, 5H), 6.60 (d, *J* = 5.3 Hz, 1H), 5.31 – 5.19 (m, 1H), 5.06 (dd, *J* = 11.0, 4.8 Hz, 1H), 4.85 (p, *J* = 6.3 Hz, 1H), 4.50 (dd, *J* = 5.3, 3.1 Hz, 1H), 4.46 (d, *J* = 11.1 Hz, 1H), 4.30 (dq, *J* = 11.9, 4.1 Hz, 1H), 4.22 – 4.07 (m, 2H), 3.76 – 3.56 (m, 2H), 3.53 – 3.38 (m, 1H), 3.30 (dd, *J* = 7.3, 3.6 Hz, 1H), 3.17 (t, *J* = 11.8 Hz, 1H), 2.99 (dd, *J* = 12.5, 4.7 Hz, 1H), 2.09 and 2.81 (s, rotameric mixture, 3H), 2.33 (dt, *J* = 11.7, 7.4 Hz, 2H), 2.11 – 2.06 (m, 3H), 2.04 (s, 2H), 1.62 (q, *J* = 7.0, 6.2 Hz, 3H), 1.55 – 1.45 (m, 4H), 1.35 – 1.20 (m, 9H), 1.02 (d, *J* = 7.0 Hz, 1H), 0.99 (d, *J* = 6.6 Hz, 1H), 0.93 (d, *J* = 7.0 Hz, 1H), 0.88 (t, *J* = 6.7 Hz, 3H), 0.75 (d, *J* = 6.6 Hz, 1H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 176.47, 173.81, 173.22, 170.85, 170.40, 169.76, 135.06, 129.76, 128.63, 127.39, 83.34, 74.42, 69.15, 68.28, 65.24, 65.01, 62.30, 61.97, 58.44, 56.79, 46.82, 46.55, 34.11, 31.70, 29.46, 29.16, 35.24, 24.82, 22.53, 21.22, 20.61, 18.53, 18.03, 16.99, 16.75, 13.99. **HRMS** (ESI) calcd for $[C_{38}H_{53}N_3O_9Na]^+$ 718.3679, found 718.3684.

A3 Experimental Procedures and Compound Characterization for Part II: Synthesis of New Hepatitis C Virus Translation Inhibitors



3.2

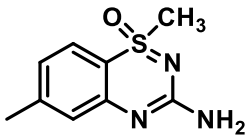
1,3,6-trimethyl-1λ⁴-benzo[e][1,2,4]thiadiazine 1-oxide: To a mixture of substrate **3.1** (1.000 g, 5.095 mmol, 1 eq) and concentrated sulfuric acid (5 mL, 93.75 mmol, 18.4 eq) in 50 mL distilled chloroform was added sodium azide (729 mg, 11.21 mmol, 2.2 eq) in 4 portions every 30 minutes. Once the additions were complete, the reaction was heated to 45 °C (NOTE: The reaction must not be allowed to reflux to prevent the formation of triazidomethane) and stirred 18 hours. The reaction was then cooled to room temperature and diluted with crushed ice. The now red biphasic solution was neutralized with Na_2CO_3, extracted with DCM (3 x 25 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by recrystallization from boiling acetone to give the product **3.2** as a white crystalline solid (848 mg, 80%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.66 (d, Ar*H*, *J* = 8.1 Hz, 1H), 7.32 (s, Ar*H*, 1H), 7.22 (d, Ar*H*, *J* = 8.3 Hz, 1H), 3.45 (s, Ar-C*H*$_3$, 3H), 2.45 (s, S-C*H*$_3$, 3H), 2.41 (s, N=C-C*H*$_3$, 3H).



3.3

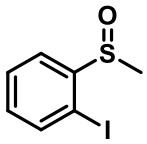
(2-amino-4-methylphenyl)(imino)(methyl)-λ⁶-sulfanone: Substrate **3.2** (639 mg, 3.07 mmol, 1 eq) was dissolved in 27 mL 5% aqueous NaOH and heated to reflux for 4 hours. The reaction was then cooled to room temperature, extracted with DCM (3 x 15 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give product **3.3** as a clear colorless oil that crystallized upon storage in the freezer (444 mg, 79%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.09 (d,

Ar*H*, *J* = 7.9 Hz, 1H), 6.55 (d, Ar*H*, *J* = 7.9 Hz, 1H), 6.50 (s, Ar*H*, 1H), 2.90 (s, S-C*H*$_3$, 3H), 2.26 (s, Ar-C*H*$_3$, 3H).



3.4

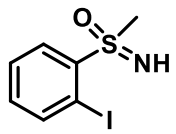
3-amino-1,6-dimethyl-1λ^4-benzo[e][1,2,4]thiadiazine 1-oxide: Substrate **3.3** (240 mg, 1.30 mmol, 1 eq) was added to a round bottom flask containing a 0.5744 M cyanogen bromide solution in ACN (2.7 mL, 1.56 mmol, 1.2 eq). This solution was heated to reflux and allowed to react 12 hours. Once complete, Et_2O was added, and a white powder crashed out of solution. This powder was filtered, rinsed with additional Et_2O, then recrystallized from boiling isopropanol to give product **3.4** as a white crystalline solid (177 mg, 65%). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.13 (d, Ar*H*, *J* = 8.2 Hz, 1H), 7.34 (d, Ar*H*, *J* = 8.1 Hz, 1H), 7.15 (s, Ar*H*, 1H), 3.94 (s, S-C*H*$_3$, 3H), 2.43 (s, Ar-C*H*$_3$, 3H).



3.6

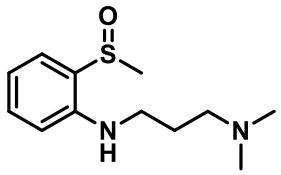
1-iodo-2-(methylsulfinyl)benzene: To a solution of *o*-iodothioanisole (3 mL, 21.4 mmol, 1 eq) in glacial acetic acid (65 mL, 1.17 mmol, 55 eq) was added a 30% solution of hydrogen peroxide (24 mL, 214 mmol, 10 eq) slowly. The reaction turned cloudy and was stirred until it turned clear (approximately 30 minutes). Once complete, the reaction was diluted with water and EtOAc then neutralized with solid Na_2CO_3. Once neutralized, the solution was extracted with EtOAc (3 x 100 mL), the combined organics were washed with saturated aqueous Na_2CO_3 (1 x 100 mL), brine (1 x 100 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced

pressure to give the oxidized product **3.6** as a clear yellow oil (5.122 g, 90%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.94 (dd, Ar*H*, *J* = 7.8, 1.6 Hz, 1H), 7.84 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.64 (td, Ar*H*, *J* = 7.6, 1.1 Hz, 1H), 7.25 (td, Ar*H*, *J* = 7.5, 1.7 Hz, 1H), 2.81 (s, S-CH_3, 1H).



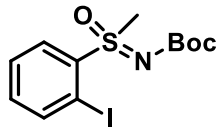
3.7

imino(2-iodophenyl)(methyl)-λ^6-sulfanone: To a solution of substrate **3.6** (0.31 mL, 2.25 mmol, 1 eq) and concentrated sulfuric acid (2 mL, 37.52 mmol, 16.68 eq) in 20 mL distilled chloroform was added sodium azide (440 mg, 6.76 mmol, 3 eq) in 4 portions at 30 minute intervals. After the addition was complete, the reaction was heated to 45 °C (NOTE: Do not let the reaction reflux to prevent formation of triazidomethane) and stirred 12 hours. Once complete, the reaction was cooled to room temperature and diluted with crushed ice. Once the ice melted, the now orange biphasic mixture was neutralized with solid Na_2CO_3, extracted with DCM (3 x 15 mL), the combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica (isocratic, 50% EtOAc/hexanes) to give the product **3.7** as a dark brown oil (239 mg, 38%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.31 (dd, Ar*H*, *J* = 7.9, 1.7 Hz, 1H), 8.13 (dd, Ar*H*, *J* = 7.8, 1.2 Hz, 1H), 7.55 (td, Ar*H J* = 7.9, 1.2 Hz, 1H), 7.23 (td, Ar*H*, *J* = 7.6, 1.7 Hz, 1H), 3.29 (s, S-C*H*$_3$, 3H).



3.8

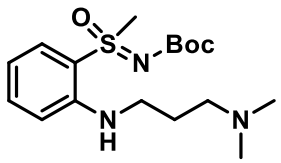
N^1,N^1-dimethyl-N^3-(2-(methylsulfinyl)phenyl)propane-1,3-diamine: To a suspension of substrate **3.6** (237 mg, 0.891 mmol, 1 eq), copper (I) iodide (17 mg, 0.0891 mmol, 0.1 eq), and potassium phosphate tribasic (378 mg, 1.78 mmol, 2 eq) in 1 mL isopropanol under Ar(g) was added ethylene glycol (0.10 mL, 1.782 eq, 2 eq) and amine X (0.22 mL, 1.78 mmol, 2 eq). The blue suspension was then heated to 80 °C and was stirred15 hours. Once complete, the reaction was cooled to room temperature and diluted with water and Et_2O. The mixture was extracted with Et_2O (3 x 10 mL), the combined organics were washed with brine (1 x 10 mL), dried over anhydrous Na_2SO_4, and concentrated under reduce pressure. The crude product was purified by flash chromatography on silica (isocratic, 10% methanol/1% NH_4OH/DCM) to give the product **3.8** as a dark yellow oil (82 mg, 38%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.33 – 7.27 (m, Ar*H*, 2H), 6.74 – 6.68 (m, Ar*H*, 2H), 6.60 (bs, N*H*, 1H), 3.27 – 3.17 (m, NH-C*H*$_2$, 2H), 2.88 (s, S-C*H*$_3$, 3H), 2.43 (t, $(CH_3)_2$-N-C*H*$_2$, *J* = 6.8 Hz, 2H), 2.26 (s, $(CH_3)_2$-N, 6H), 1.84 (pd, CH_2-C*H*$_2$-CH_2, *J* = 6.9, 4.7 Hz, 2H).



3.9

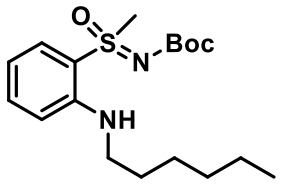
tert-butyl ((2-iodophenyl)(methyl)(oxo)-l6-sulfaneylidene)carbamate: To a solution of substrate **3.7** (1.005 g, 3.56 mmol, 1 eq) in 15 mL anhydrous THF was added a 60% dispersion of NaH in mineral oil (214 mg, 5.34 mmol, 1.5 eq). The mixture bubbled and was allowed to stir 20 minutes, then di-*t*-butyl dicarbonate (1.552 g, 7.11 mmol, 2 eq) was added, and the reaction was stirred for 16 hours. Once complete, the reaction was quenched with saturated aqueous NH_4Cl. The solution was then extracted with DCM (4 x 10 mL), the combined organics were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by

flash chromatography on silica (isocratic, 50% EtOAc/hexanes) to give the boc protected product **3.9** as a clear brown oil (1.028 g, 76%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.31 (dd, Ar*H*, *J* = 8.0, 1.6 Hz, 1H), 8.14 (dd, Ar*H*, *J* = 7.8, 1.2 Hz, 1H), 7.62 (td, Ar*H*, *J* = 7.4, 1.2 Hz, 1H), 7.29 (td, Ar*H*, *J* = 7.4, 1.6 Hz, 1H), 3.36 (s, S-C*H*$_3$, 3H), 1.37 (s, (C*H*$_3$)$_3$-C-O, 9H).



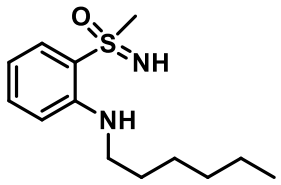
3.10

Tert-butyl((2-((3-(dimethylamino)propyl)amino)phenyl)(methyl)(oxo)-λ^6 sulfaneylidene)carbamate: To a suspension of substrate **3.9** (377 mg, 0.989 mmol, 1 eq), copper (I) iodide (19 mg, 0.0989 mg, 0.1 eq), and potassium phosphate tribasic (418 mg, 1.97 mmol, 2 eq) in 1 mL isopropanol under Ar(g) was added ethylene glycol (0.11 mL, 1.97 mmol, 2 eq) and amine X (0.125 mL, 1.97 mmol, 2 eq). The blue suspension was then heated to 90 °C and stirred 36 hours. Once complete, the reaction was diluted with water and DCM. The mixture was extracted with DCM (5 x 10 mL, until organic layer was colorless), the combined organics were washed with brine (1 x 10 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica (isocratic, 10% methanol/1% NH_4OH/DCM) to give product **3.10** as a clear brown oil (134 mg, 38%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.78 (dd, Ar*H*, *J* = 8.3, 1.6 Hz, 1H), 7.45 (td, Ar*H*, *J* = 8.5, 7.2, 1.6 Hz, 1H), 6.82 – 6.77 (m, Ar*H*, 2H), 6.35 (t, N*H*, *J* = 5.3 Hz, 1H), 3.26 (s, S-C*H*$_3$, 3H), 3.25 – 3.22 (m, NH-C*H*$_2$, 2H), 2.45 (tt, $(CH_3)_2$-N-C*H*$_2$, *J* = 8.7, 4.5 Hz, 2H), 2.29 (s, (C*H*$_3$)$_2$-N, 6H), 1.87 (p, CH_2-C*H*$_2$-CH_2, *J* = 6.9 Hz, 2H), 1.40 (s, (C*H*$_3$)$_3$-C-O, 9H).



3.11

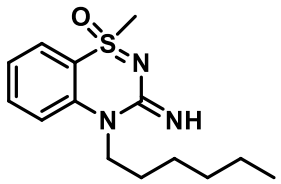
Tert-butyl ((2-(hexylamino)phenyl)(methyl)(oxo)-λ^6-sulfaneylidene)carbamate: To a suspension of substrate **3.9** (100 mg, 0.262 mmol, 1 eq), copper (I) iodide (5 mg, 0.0262 mmol, 0.1 eq), and potassium phosphate tribasic (111 mg, 0.524 mmol, 2 eq) in 3 mL anhydrous isopropanol under Ar(g) was added ethylene glycol (30 μL, 0.524 mmol, 2 eq) and hexylamine (0.15 mL, 1.05 mmol, 4 eq). This suspension was heated to 80 °C and stirred 24 hours. Once complete, the reaction was diluted with water and extracted with DCM (4 x 10 mL, until organic layer was clear), the combined organic layers were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica (isocratic, 30% EtOAc/hexanes) to give product **3.11** as a clear yellow oil (81 mg, 88%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.62 – 7.59 (m, 1H), 7.42 – 7.38 (m, 1H), 7.37 – 7.27 (m, 3H), 3.10 (s, 3H), 2.22 (t, *J* = 6.6 Hz, 2H), 1.56 (s, 9H), 1.34 (s, 2H), 1.24 – 1.08 (m, 4H), 0.78 (t, *J* = 6.9 Hz, 3H).



3.12

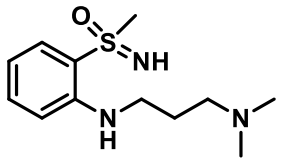
(2-(hexylamino)phenyl)(imino)(methyl)-λ^6-sulfanone: Substrate **3.11** (188 mg, 0.530 mmol, 1 eq) was dissolved in 1.5 mL DCM, then 0.5 mL TFA was added. The reaction was stirred 3 hours. Once complete, the reaction was diluted with DCM and water, then neutralized with solid Na_2CO_3. Once neutralized, the mixture was extracted with DCM (3 x 10 mL), the combined organics were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give

product **3.12** as a clear yellow oil (130 mg, 96%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.82 (dd, Ar*H*, *J* = 8.1, 1.7 Hz, 1H), 7.42 (td, Ar*H*, *J* = 7.2, 1.7 Hz, 1H), 6.82 (d, Ar-N*H*, *J* = 4.8 Hz, 1H), 6.76 – 6.72 (m, Ar*H*, 2H), 3.16 (tdd, N-CH_2, *J* = 7.0, 5.0, 1.9 Hz, 2H), 3.10 (s, S-CH_3, 3H), 2.76 (s, S=N*H*, 1H), 1.69 (p, N-CH_2-CH_2, *J* = 7.2 Hz, 2H), 1.48 – 1.40 (m, N-CH_2-CH_2-CH_2, 2H), 1.38 – 1.30 (m, CH_3-CH_2-CH_2, 4H), 0.94 – 0.88 (m, CH_3-CH_2, 3H). **^{13}C NMR** (125 Hz, $CDCl_3$) δ 147.25, 134.88, 129.99, 122.96, 115.57, 111.95, 43.20, 42.91, 31.51, 28.98, 26.91, 22.58, 14.01.



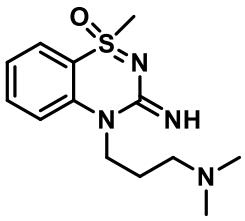
3.13

4-hexyl-3-imino-1-methyl-3,4-dihydro-1λ^4-benzo[e][1,2,4]thiadiazine 1-oxide: Substrate **3.12** (248 mg, 0.975 mmol, 1 eq) was dissolved in 1.8 mL anhydrous ACN under Ar(g) before a 1 M solution of cyanogen bromide in ACN was added (1.16 mL, 1.16 mmol, 1.2 eq). The reaction was heated to 80 °C and stirred 18 hours. Once complete, Et_2O was added and a white solid formed. The solid was filtered, washed with more Et_2O, the dissolved in 1 M aqueous NaOH. The solution was extracted with DCM (3 x 10 mL), the combined organics were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give the product **3.13** as a clear yellow oil (244 mg, 90%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.67 (dd, Ar*H*, *J* = 7.8, 1.6 Hz, 1H), 7.60 (ddd, Ar*H*, *J* = 8.8, 7.3, 1.6 Hz, 1H), 7.11 – 7.05 (m, Ar*H*, 2H), 4.25 – 4.02 (bm, N-CH_2, 2H), 3.32 (s, S-CH_3, 3H), 1.73 (p, N-CH_2-CH_2, *J* = 8.2 Hz, 2H), 1.42 (p, N-CH_2-CH_2-CH_2, *J* = 7.2 Hz, 2H), 1.39 – 1.29 (m, CH_3-CH_2-CH_2, 4H), 0.90 (t, CH_3-CH_2, *J* = 7.1 Hz, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$) δ 151.38, 141.53, 135.59, 125.08, 120.08, 115.67, 115.27, 45.32, 31.62, 26.54, 26.25, 22.68, 14.02.



3.14

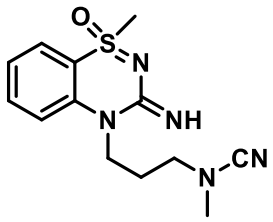
(2-((3-(dimethylamino)propyl)amino)phenyl)(imino)(methyl)-λ^6-sulfanone: To a solution of substrate **3.10** (320 mg, 0.937 mmol, 1 eq) in 1.5 mL DCM was added 0.5 mL TFA. The reaction was stirred 5 hours, then diluted with water and neutralized with solid $NaHCO_3$. Once neutral, the mixture was extracted with DCM (4 x 10 mL), the combined organics were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give the product **3.14** as a clear brown oil (150 mg, 66%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.82 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.44 – 7.38 (m, 1H), 6.93 (s, 1H), 6.79 – 6.72 (m, 2H), 3.26 (dt, *J* = 11.4, 6.3 Hz, 2H), 3.10 (s, 3H), 2.49 (t, *J* = 6.9 Hz, 2H), 2.31 (s, 6H), 1.92 – 1.85 (m, 2H).



3.15

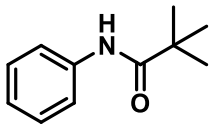
4-(3-(dimethylamino)propyl)-3-imino-1-methyl-3,4-dihydro-1λ^4-benzo[e][1,2,4]thiadiazine-1-oxide: To a solution of substrate **3.14** (150 mg, 0.587 mmol, 1 eq) in 2.6 mL anhydrous ACN under Ar(g) was added a 0.5 M solution of cyanogen bromide in ACN (1.4 mL, 0.705 mmol, 1.2 eq). The reaction was stirred for 16 hours. Once starting material was consumed by TLC (10% methanol/1% NH_4Cl/DCM), Et_2O was added, and a white solid crashed out of solution. This solid was filtered then dissolved in 1 M aqueous NaOH, then extracted with DCM (3 x 10 mL), the combined organics were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica (isocratic, 10%

methanol/1% NH_4OH/DCM) to give the product **3.15** as a clear yellow oil (10 mg, 6%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.84 (dd, Ar*H*, *J* = 7.9, 1.6 Hz, 1H), 7.70 (td, Ar*H*, *J* = 7.3, 1.6 Hz, 1H), 7.38 (d, Ar*H*, *J* = 8.7 Hz, 1H), 7.25 (t, Ar*H*, *J* = 7.6 Hz, 1H), 5.46 (bs, N*H*, 1H), 4.34 – 4.14 (m, N-CH_2, 2H), 3.56 (s, S-CH_3, 3H), 2.54 – 2.42 (m, $(CH_3)_2$-N-CH_2, 2H), 2.31 (s, $(CH_3)_2$-N, 6H), 2.08 – 1.94 (m, CH_2-CH_2-CH_2, *J* = 6.7 Hz, 2H). **^{13}C NMR** (125 MHz, $CDCl_3$) δ 152.3, 140.4, 136.3, 125.6, 122.4, 116.1, 115.6, 55.8, 45.8, 45.0, 44.5, 24.8.



3.16

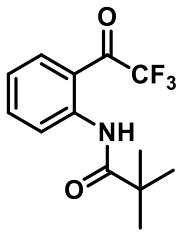
N-(3-(3-imino-1-methyl-1-oxido-1λ^4-benzo[e][1,2,4]thiadiazin-4(3H)-yl)propyl)-N-methylcyanamide: Using the same procedure as outline for **3.15** also yielded **3.16** as a clear yellow oil (22 mg, 13%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.84 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.78 (ddd, *J* = 8.8, 7.4, 1.6 Hz, 1H), 7.50 (d, *J* = 8.7 Hz, 1H), 7.40 – 7.36 (m, 1H), 4.31 – 4.07 (m, 2H), 3.58 (s, 3H), 2.43 (t, *J* = 6.7 Hz, 2H), 2.29 (s, 3H), 1.99 – 1.83 (m, 2H).



3.19

N-phenylpivalamide: To a solution of aniline (5.00 mL, 54.76 mmol, 1 eq) and triethylamine (8.4 mL, 60.24 mmol, 1.1 eq) in 165 mL distilled DCM at 0 °C under Ar(g) was added pivaloyl chloride (7.4 mL, 60.24 mmol, 1.1 eq) slowly. The reaction was warmed to room temperature and stirred 12 hours. Once complete, the reaction was quenched by slow addition of 1 M aqueous HCl. The biphasic mixture was extracted with EtOAc (3 x 75 mL), washed with brine (2

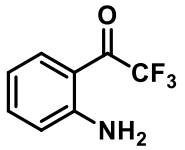
x 75 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude solid was purified by recrystallization from DCM/hexanes to give the product **3.19** as a white crystalline solid (9.263 g, 95%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.53 (dd, Ar*H*, *J* = 8.6, 1.1 Hz, 2H), 7.32 (td, Ar*H*, *J* = 10.0, 9.4, 1.8 Hz, 2H), 7.10 (tt, Ar*H*, *J* = 7.4, 1.0 Hz, 1H), 1.32 (s, C*H*$_3$-C-CO, 9H). **^{13}C NMR** (125 MHz, $CDCl_3$) δ 176.53, 138.01, 128.96, 124.19, 119.93, 39.61, 27.65.



3.20

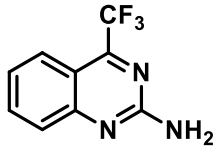
N-(2-(2,2,2-trifluoroacetyl)phenyl)pivalamide: To a solution of substrate **3.19** (3.000 g, 16.93 mmol, 1 eq) and distilled TMEDA (2.54 mL, 16.93 mmol, 1 eq) in 33 mL anhydrous methyl *tert*-butylmethyl ether at -78 °C under Ar(g) was added 2.5 M *n*-butyllithium in hexane (20.3 mL, 50.79 mmol, 3 eq) slowly. Once the addition was complete the reaction was warmed to 0 °C and stirred 3 hours. Once lithiation was complete, the reaction was cooled back to -78 °C and ethyl trifluoroacetate (8.4 mL, 59.26 mmol, 3.5 eq) was added. The reaction was stirred 30 minutes at -78 °C, then warmed to 0 °C and stirred another 30 minutes. Once complete, the reaction was quenched with saturated aqueous NH_4Cl, extracted with EtOAc (3 x 50 mL), the combined organics were washed with brine (1 x 50 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica (gradient, 5 to 20% EtOAc/hexanes) to give the product **3.20** as a bright yellow oil (2.53 g, 55%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 11.25 (bs, N*H*, 1H), 8.90 (d, Ar*H*, *J* = 8.5 Hz, 1H), 7.99 (d, Ar*H*, *J* = 8.3 Hz, 1H), 7.70 (dd, Ar*H*, *J* = 8.8, 7.2 Hz, 1H), 7.19 (td, Ar*H*, *J* = 7.8, 7.1, 1.2 Hz,

1H), 1.37 (s, (CH_3)$_3$-C J = 1.3 Hz, 9H). **^{13}C NMR** (125 MHz, $CDCl_3$) δ 182.97 (q, CF_3, $J_{C\text{-}F}$ = 34.2 Hz), 178.44, 144.03, 137.78, 131.90, 131.84, 122.48, 121.22, 117.78, 115.38, 40.64, 27.54.



3.21

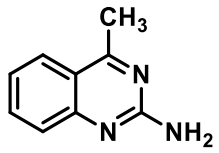
1-(2-aminophenyl)-2,2,2-trifluoroethan-1-one: Susbstrate **3.20** (265 mg, 0.973 mmol, 1 eq) was dissolved in 1.2 mL concentrated HCl, then heated to 90 °C for 6 hours. The reaction was then cooled to room temperature, diluted with water, and quenched with saturated aqueous Na_2CO_3. The now basic solution was extracted with EtOAc (3 x 20 mL), the combined organics were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the free aniline **3.21** as a bright yellow oil (169 mg, 92%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.76 (d, Ar*H*, J = 8.5 Hz, 1H), 7.38 (d, Ar*H*, J = 7.6 Hz, 1H), 6.76 – 6.67 (m, Ar*H*, 2H), 6.43 (bs, NH_2, 2H).



3.22

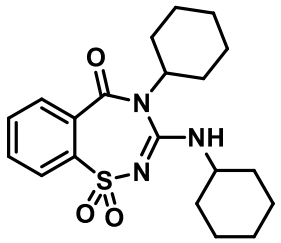
4-methylquinazolin-2-amine: Compound **3.22** (169 mg, 0.894 mmol, 1 eq) was dissolved in 4.6 mL anhydrous Et_2O before 4 M HCl in dioxane (0.54 mL, 2.14 mmol, 2.4 eq) was added. The mixture was stirred 10 minutes at room temperature then concentrated under reduced pressure. In a separate flask, cyanamide (225 mg, 5.36 mmol, 6 eq) was dissolved in 5.4 mL anhydrous Et_2O. The cyanamide solution was added to the protonated substrate and stirred 10 minutes before being concentrated under reduced pressure. The neat mixture was heated to 50 °C for 1 hour. The reaction was then cooled to room temperature, diluted with DCM, then washed with 10% NaOH (1 x 10 mL), brine (1 x 10 mL), dried over anhydrous Na_2SO_4, and concentrated

under reduced pressure to give compound **3.22** as a yellow solid (45 mg, 24%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8.6 Hz, 1H), 7.78 (t, *J* = 7.9 Hz, 1H), 7.68 (d, *J* = 8.6 Hz, 1H), 7.37 (t, *J* = 7.9 Hz, 1H), 5.39 (s, 2H). **^{13}C NMR** (125 MHz, $CDCl_3$) δ 155.58, 154.23, 135.11, 126.423, 124.65, 124.39, 124.32, 121.00 (q, *J* = 277.6 Hz, *C*F_3), 115.40. **^{19}F NMR** (376 MHz, $CDCl_3$) 65.08.



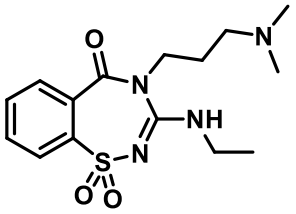
3.24

4-methylquinazolin-2-amine: 4 M HCl in dioxane was prepared by stirring acetyl chloride (1.4 mL, 19.74 mmol, 2.4 eq) and anhydrous methanol (0.8 mL, 19.74 mmol, 2.4 eq) in 5 mL anhydrous dioxane under Ar(g) for 10 minutes. *O*-aminoacetophenone (1 mL, 8.227 mmol, 1 eq) was dissolved in 42 mL anhydrous Et_2O, then the 4 M HCl in dioxane was added and stirred 10 minutes. The solution was concentrated under reduced pressure, then a solution of cyanamide (2.075 g, 49.36 mmol, 6 eq) in 25 mL Et_2O was added and stirred 10 more minutes. The mixture was concentrated under reduced pressure, then was heated to 50 °C for 1 hour. The reaction was then cooled to room temperature, diluted with DCM, then washed with 10% aqueous NaOH (1 x 30 mL), brine (1 x 30 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica (gradient, 5 to 25% EtOAc/hexanes) to give the product **3.24** as a white solid (1.211 g, 92%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.89 (dd, Ar*H*, *J* = 8.2, 1.5 Hz, 1H), 7.68 (td, Ar*H*, *J* = 8.3, 6.9, 1.4 Hz, 1H), 7.57 (d, Ar*H*, *J* = 8.4 Hz, 1H), 7.28 (td, Ar*H*, *J* = 8.2, 7.1, 1.0 Hz, 1H, partially hidden), 5.08 (bs, N*H*$_2$, 2H), 2.80 (s, Ar-CH_3, 3H).



3.27

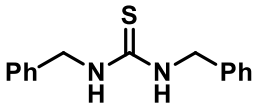
4-cyclohexyl-3-(cyclohexylamino)benzo[f][1,2,4]thiadiazepin-5(4H)-one 1,1-dioxide: A solution of saccharine (100 mg, 0.545.mmol, 1 eq) and DCC (0.09 mL, 0.545 mmol, 1 eq) in 5.5 mL anhydrous acetone under Ar(g) was heated to reflux for 12 hours. Once complete, the reaction cooled to room temperature and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica (gradient, 30 to 50% EtOAc/hexanes) to give product **3.27** as a white solid (211 mg, 99%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.93 (dd, *J* = *J* = 5.6, 3.4 Hz, 1H), 7.89 (t, *J* = 5.6, 3.4, 1H) 7.61 (dd, *J* = 5.6, 3.4 Hz, 2H), 5.24 – 5.15 (m, 1H), 4.56 – 4.44 (m, 1H), 3.92 – 3.78 (m, 1H), 3.55 – 3.42 (m, 1H), 2.38 – 2.21 (m, 2H), 2.02 (s, 1H), 1.97 – 1.87 (m, 3H), 1.77 (d, *J* = 13.5 Hz, 1H), 1.75 – 1.66 (m, 2H), 1.37 (t, *J* = 12.8 Hz, 2H), 1.28 – 1.04 (m, 8H).



3.28

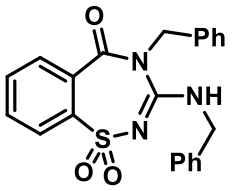
4-(3-(dimethylamino)propyl)-3-(ethylamino)benzo[f][1,2,4]thiadiazepin-5(4H)-one 1,1-dioxide: A solution of saccharine (1.000 g, 5.50 mmol, 1 eq) and EDC (1.047 g, 5.50 mmol, 1 eq) in 50 mL anhydrous acetone was heated to reflux and allowed to stir overnight. The next day, the reaction was cooled to room temperature and concentrated under reduced pressure. The crude

product was purified by flash chromatography on silica (gradient, 1 to 6% MeOH/DCM) to give **3.28** as a white crystalline solid (1.625 g, 88%). **^{1}H NMR** (400 MHz, acetone-*d6*) δ 7.77 – 7.72 (m, 2H), 7.70 – 7.62 (m, 2H), 3.94 (bs, 1H), 3.24 (d, *J* = 8.6 Hz, 2H), 2.42 (d, *J* = 8.2 Hz, 2H), 2.25 (s, 6H), 2.01 (p, *J* = 2.5 Hz, 6H), 1.13 (t, *J* = 7.2 Hz, 3H).



3.29

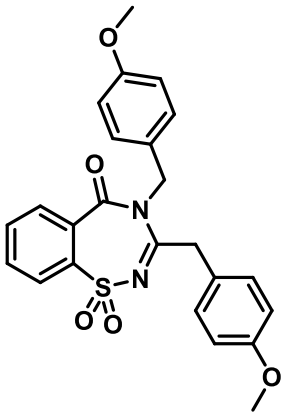
1,3-dibenzylthiourea: To a solution of benzylamine (0.55 mL, 5 mmol, 1 eq) in 10 mL distilled DCM under Ar(g) was added benzylisothiocyanate (0.66mL, 5 mmol, 1 eq) slowly. The reaction was allowed to stir overnight. Once complete, a white solid formed. This solid was filtered and rinsed with DCM. The crude product was purified by recrystallization from Et_2O/petroleum ether to give the product **3.29** as a white crystalline solid (1.225 g, 96%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.32 (dd, *J* = 7.9, 7.3 Hz, 6H), 7.27 – 7.21 (m, 4H, partially hidden), 6.02 (bs, 2H), 4.63 (s, 4H).



3.30

4-benzyl-3-(benzylamino)benzo[f][1,2,4]thiadiazepin-5(4H)-one 1,1-dioxide: Carbodiimide prep: To a solution of dibenzylthiourea (200 mg, 0.780 mmol, 1 eq) and TEA (0.45 mL3.25 mmol, 4.17 eq) in DCM at 0 °C was added $HgCl_2$ (229 mg, 0.842 mmol, 1.08 eq). This reaction was stirred for 5 minutes before a yellow precipitate formed. The reaction was then warmed to room temperature and stirred for a further 20 minutes. The suspension was then filtered through celite, rinsed with DCM, then concentrated under reduced pressure to give dibenzylcarbodiimide

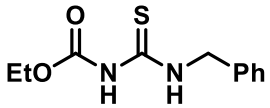
as a yellow solid that was used without further purification. Reaction: The previously prepared carbodiimide was taken up in 10 mL dry acetone and saccharine (143 mg, 0.780 mmol, 1 eq) was added. The solution was heated to reflux and allowed to stir overnight. The next day, the reaction was cooled to room temperature and concentrated under reduced pressure. The residue was taken up in DCM and washed with H_2O (2 x 10 mL), brine (2 x 10 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica (isocratic, 2% MeOH in DCM) to give **3.30** as a white solid (245 mg, 78%). **^{1}H NMR** (500 MHz, Methanol-*d*6) δ 7.94 (dd, *J* = 5.8, 3.2 Hz, 1H), 7.87 (dd, *J* = 5.9, 3.2 Hz, 1H), 7.75 (dd, *J* = 5.8, 3.3 Hz, 2H), 7.46 – 7.41 (m, 2H), 7.35 – 7.26 (m, 4H), 7.23 – 7.13 (m, 4H), 6.93 (d, *J* = 6.2 Hz, 2H), 4.54 (s, 2H), 4.26 (s, 2H).



3.33

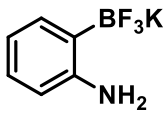
3,4-bis(4-methoxybenzyl)benzo[f][1,2,4]thiadiazepin-5(4H)-one 1,1-dioxide: Carbodiimide prep: Di-*p*-methoxybenzylthiourea (600 mg, 2.34 mmol, 1 eq) and TEA (1.36 mL, 9.76 mmol, 4.17 eq) were dissolved in 20 mL DCM. This solution was cooled to 0 °C before $HgCl_2$ (687 mg, 2.53 mmol, 1.08 eq) was added. The reaction stirred for 5 minutes at 0 °C, during which yellow solids began to precipitate, and was warmed to room temperature for a further 20 minutes. The reaction was then filtered through Celite, rinsed with DCM, and concentrated under reduced pressure to give di-*p*-methoxybenzylthiourea as a yellow solid that was used without further

purification. Reaction: The previously synthesized carbodiimide (1 eq) was taken up in 24 mL anhydrous acetone. Saccharine was then added and the solution was heated to reflux overnight. The next day, the reaction was cooled to room temperature and concentrated under reduced pressure. The residue was taken up in DCM and washed with H_2O (2 x 15 mL), brine (1 x 15 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (gradient, 0 to 5% MeOH/DCM) to give benzothiadiazapene **3.33** as a white solid (721 mg, 76%). **^{1}H NMR** (500 MHz, MeOH-*d6*) δ 8.06 – 7.97 (m, 2H), 7.73 – 7.65 (m, 2H), 7.19 (d, *J* = 8.3 Hz, 2H), 7.00 (d, *J* = 8.2 Hz, 2H), 6.82 (dd, *J* = 8.7, 3.5 Hz, 4H), 5.45 (bs, 1H), 4.30 (s, 2H), 3.81 (s, 3H), 3.79 (s, 3H).



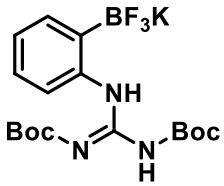
3.34

1-benzyl-3-ethylcarbamoylthiourea: Benzylamine (0.83 mL, 7.62 mmol, 1 eq) was dissolved in 30 mL Et_2O at 0 °C. *O*-ethyl carbonisothiocyanatidate was added dropwise, then the reaction was allowed to come to room temperature and stir an additional 30, during which white solids precipitated. The solids were filtered and rinsed with hexane. The crude solids were recrystallized from Et_2/hexane to give **3.32** as a white crystalline solid (1.136 g, 63%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.95 (s, 1H), 8.05 (s, 1H), 7.44 – 7.27 (m, 5H), 4.86 (d, *J* = 5.4 Hz, 2H), 4.21 (q, *J* = 7.1 Hz, 2H), 1.30 (t, *J* = 7.1 Hz, 3H).



3.38

2-(trifluoro-λ^4-boraneyl)aniline, potassium salt: *O*-aminophenylboronic acid (200 mg, 1.46 mmol, 1 eq) was dissolved in 7.4 mL MeOH and cooled to 0 °C. 5 M KHF_2 (2.18 mL, 5.48 mmol, 7.5 eq) was added slowly and stirred for an additional 10 minutes. The reaction was warmed to room temperature for an additional 20 minutes before being concentrated under reduced pressure. The residue was dissolved in ACN and 2 g K_2CO_3 was added and stirred 4 hours. This suspension was concentrated under reduced pressure, then the solids were rinsed and filtered with acetone. Et_2O was added to the acetone solution, and white solids precipitated out. These solids were filtered and rinsed with additional Et_2O to give trifluoroborate salt **3.38** as a crystalline white solid (208 mg, 72%). **^{1}H NMR** (500 MHz, *DMSO-d6*) δ 7.08 (d, *J* = 7.0 Hz, 1H), 6.76 (td, *J* = 7.5, 1.8 Hz, 1H), 6.35 (t, *J* = 7.1 Hz, 2H), 4.44 (bs, 2H)



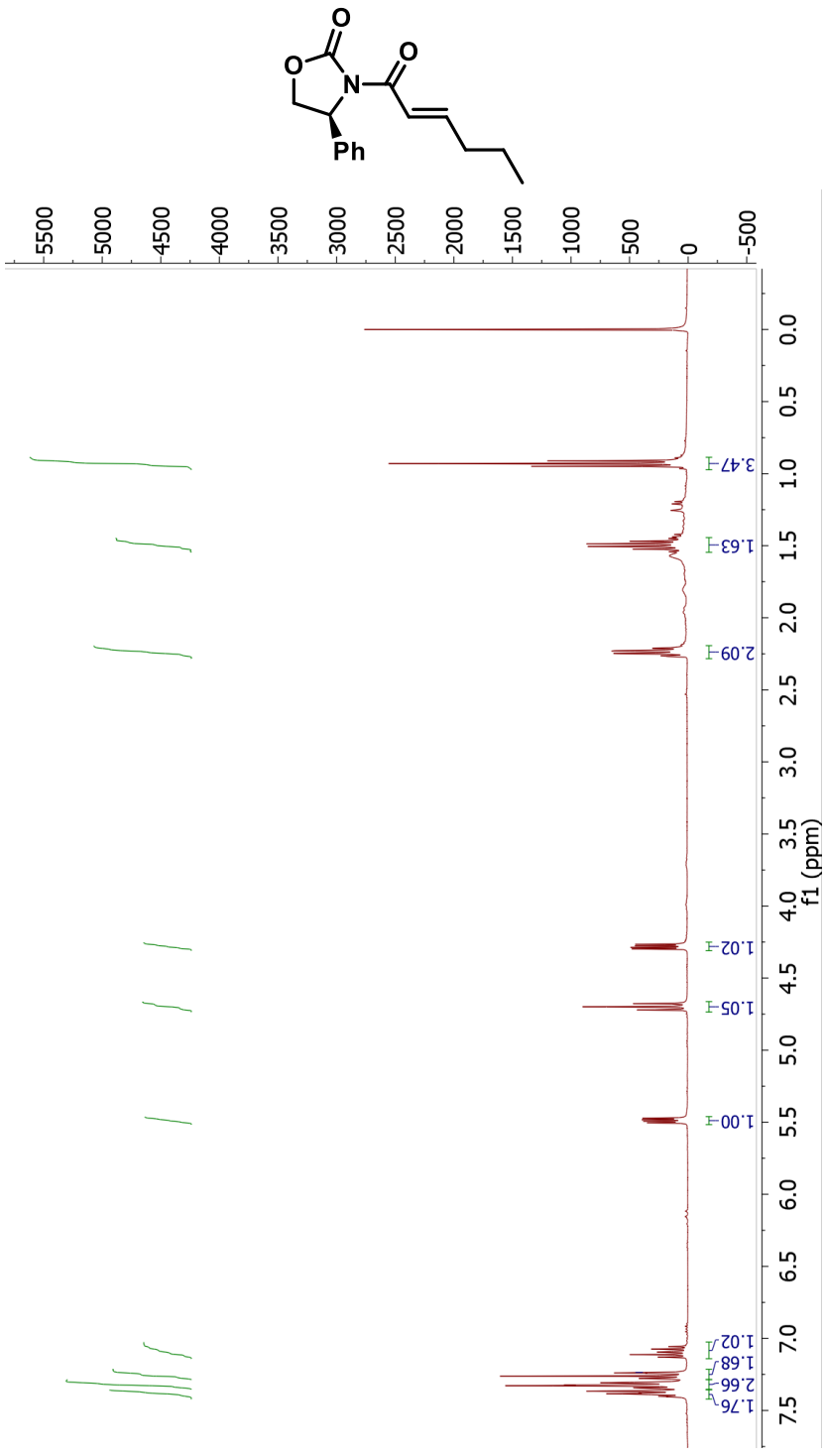
3.39

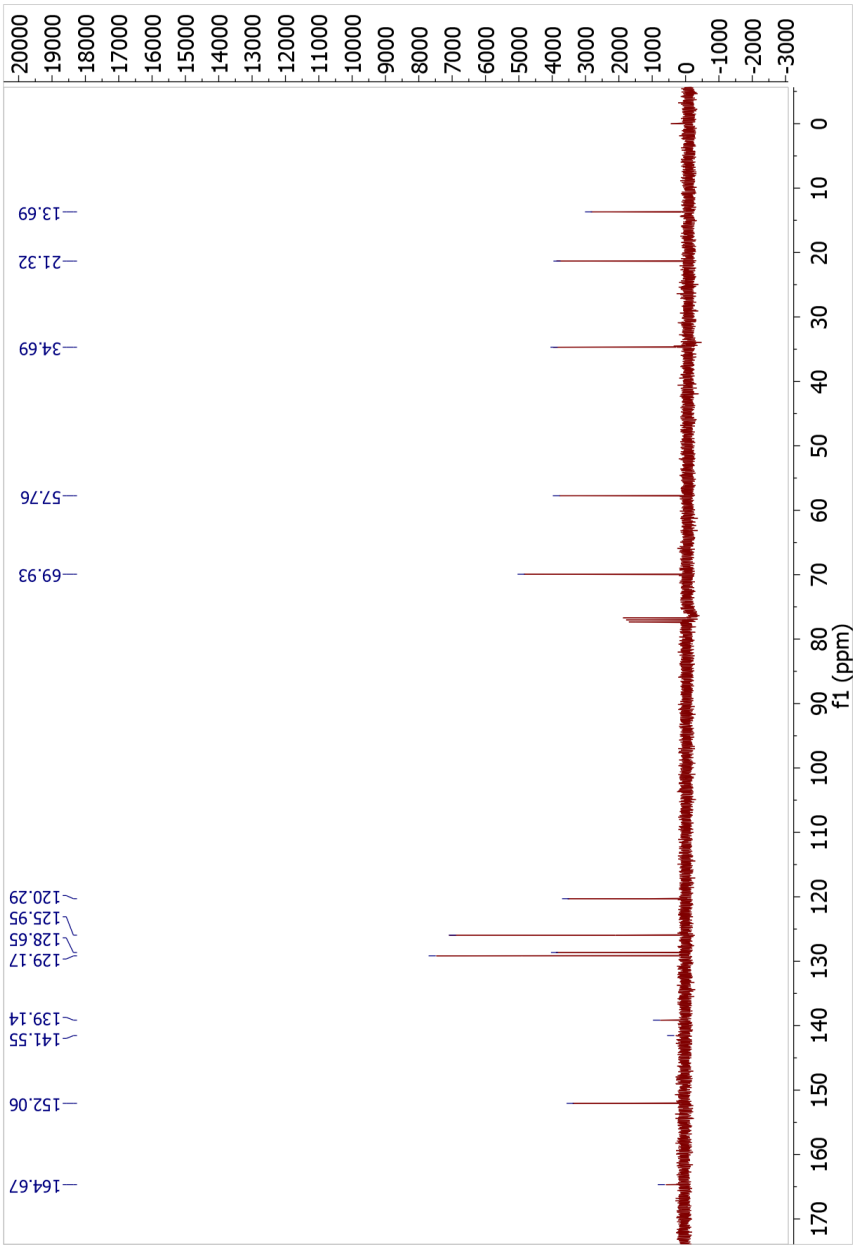
1,2-diboc-3-(2-(trifluoro-l4-boraneyl)phenyl)guanidine, potassium salt: Carbodiimide prep: dibocthiourea (153 mg, 0.553 mmol, 1 eq) was dissolved in 4 mL DCM at 0 °C. TEA (0.4 mL, 2.71 mmol, 5 eq) was added, then $HgCl_2$ (162 mg, .597 mmol, 1.02 eq). The solution turned yellow after 5 minutes, then was warmed to room temperature and stirred an additional 20 minutes. The suspension was filtered through Celite, rinsed with DCM, and concentrated under reduced pressure to give the crude carbodiimide as a yellow oil that was used without further purification. Reaction: The carbodiimide synthesized previously (1.67 eq) was dissolved in 4 mL dry acetone, then compound **3.38** was added. This solution was stirred overnight at room temperature. The next day, the solution was concentrated under reduced pressure. The crude

material was crystallized from 2:1 Et_2O:pentane to give compound **3.39** as a white crystalline solid (97 mg, 66%). **^{1}H NMR** (500 MHz, *DMSO-d6*) δ 7.08 and 6.53 ((d, *J* = 6.9 Hz, 1H, rotamers), 6.98 (t, *J* = 7.7 Hz, 2H), 6.53 (d, *J* = 7.8 Hz, 2H), 6.53 (d, *J* = 7.8 Hz, 2H), 6.46 and 6.38 (t, *J* = 7.3 Hz, 1H, rotamers), 1.43 (s, 18H).

A4 Spectra

^{1}H and ^{13}C NMR for Compound 1.3

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O
N
O
Ph
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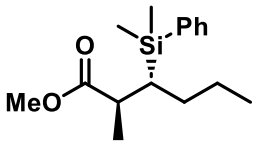
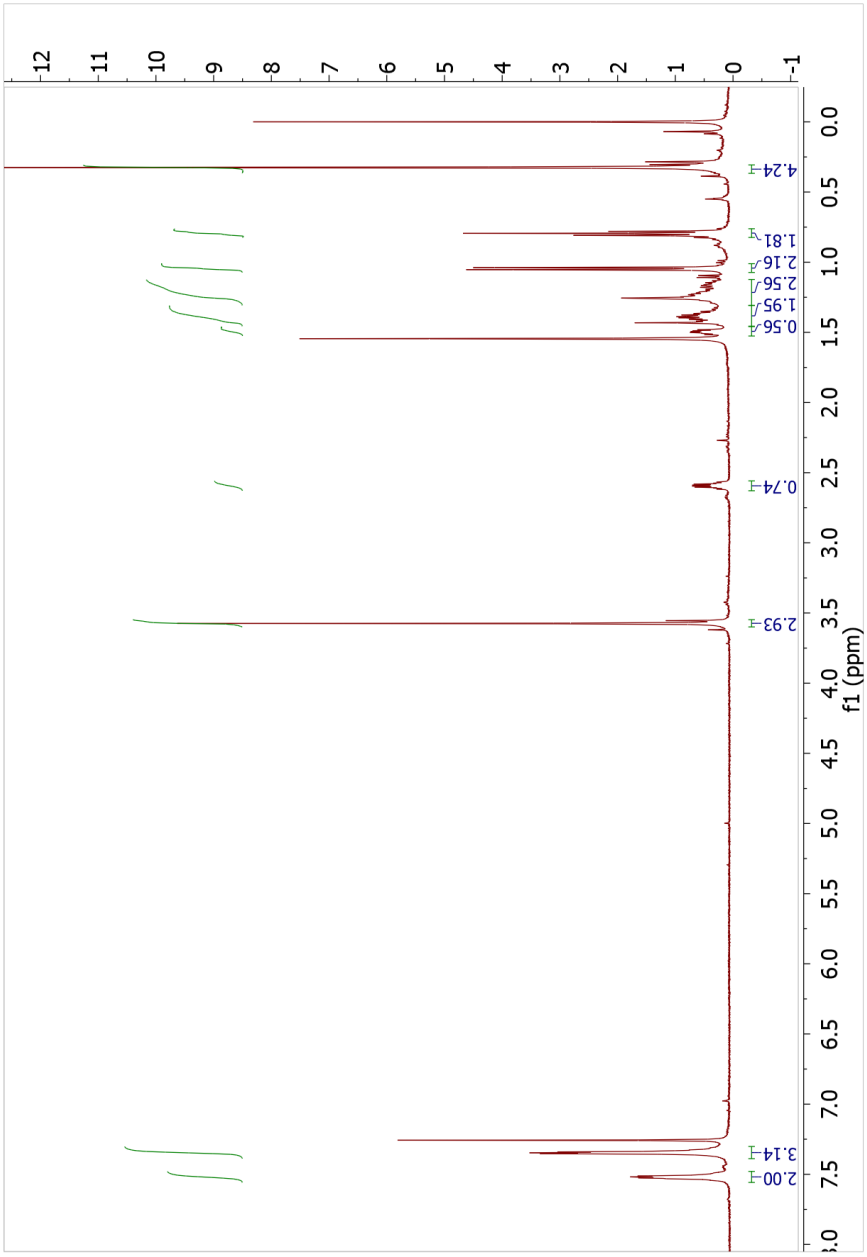
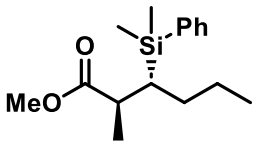
21.32
34.69
57.76
69.93
120.29
125.95
128.65
129.17
139.14
141.55
152.06
164.67
f1 (ppm)

^{1}H and ^{13}C NMR for compound 1.5



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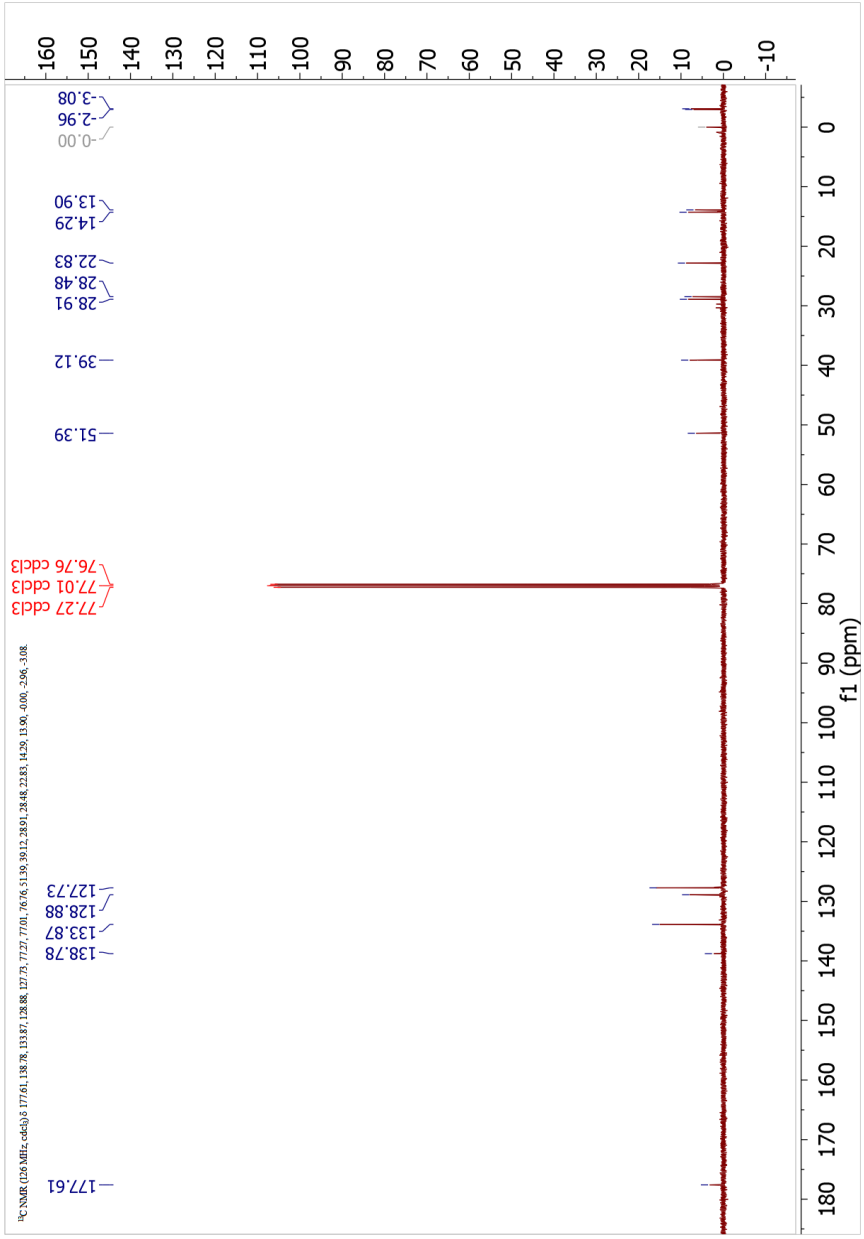
Si
Ph
MeO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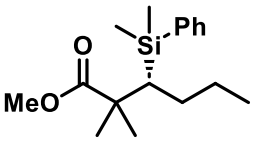
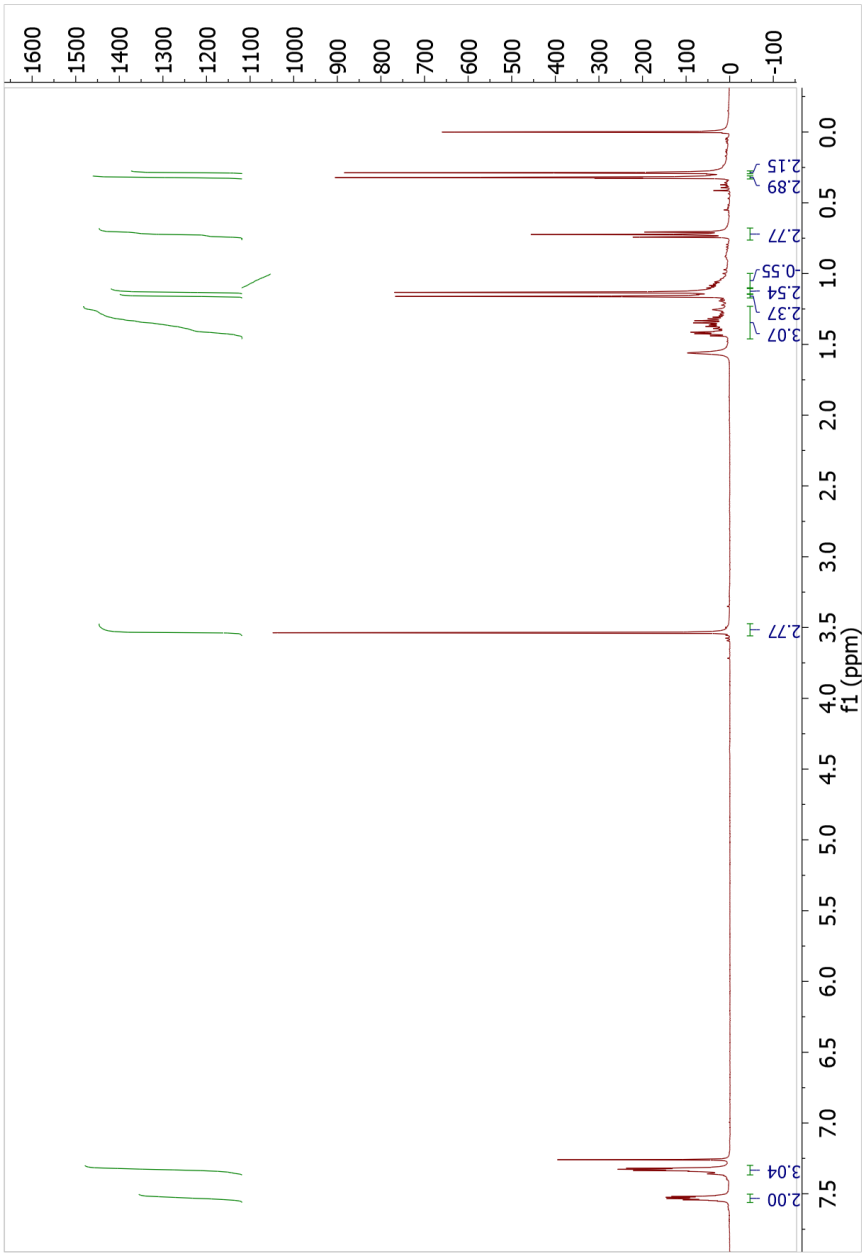
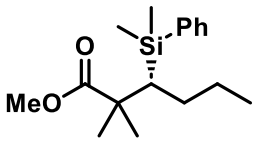
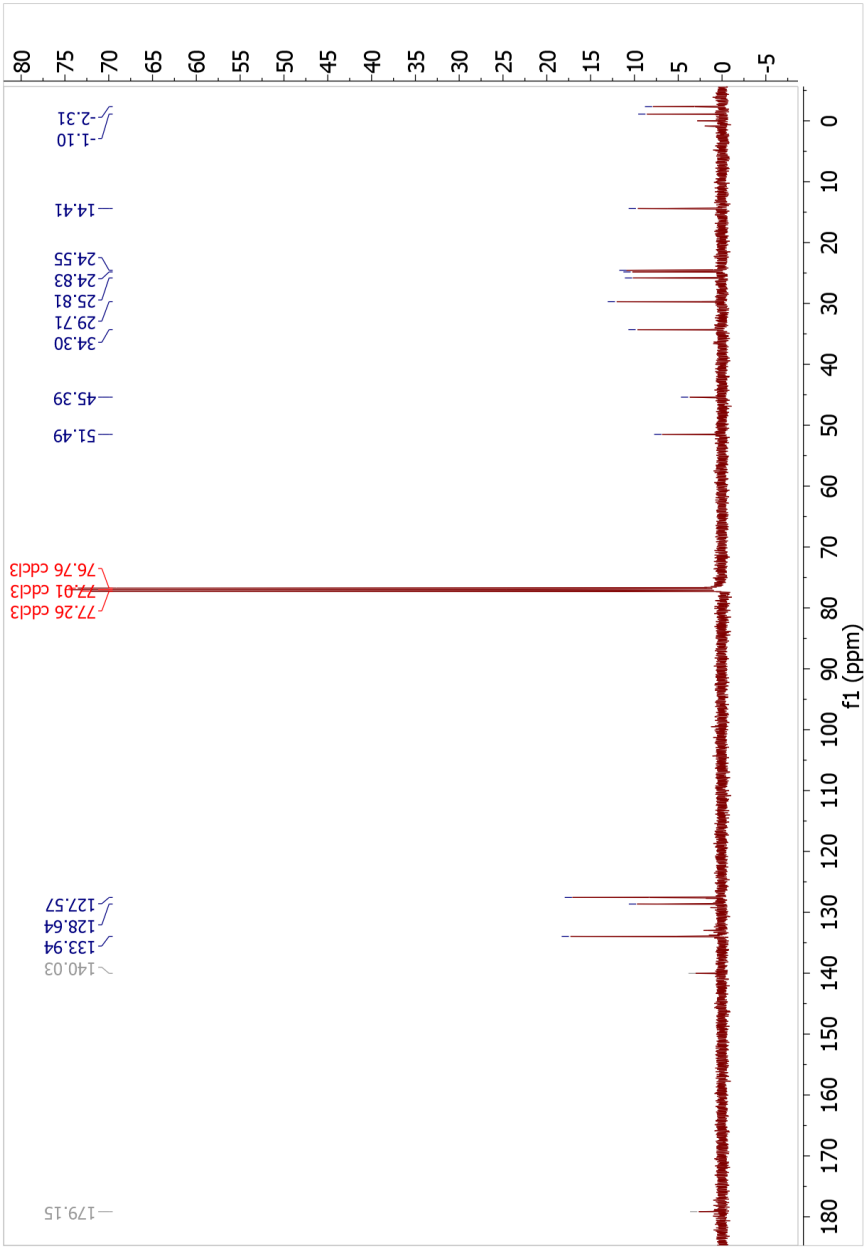
28.48
28.91
39.12
51.39
76.76 cdcl3
77.01 cdcl3
77.27 cdcl3
127.73
128.88
133.87
138.78
177.61
f1 (ppm)

^{1}H and ^{13}C NMR for Compound 1.6






O
Si
Ph
MeO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76.76 cdcl3
77.01 cdcl3
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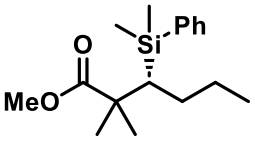
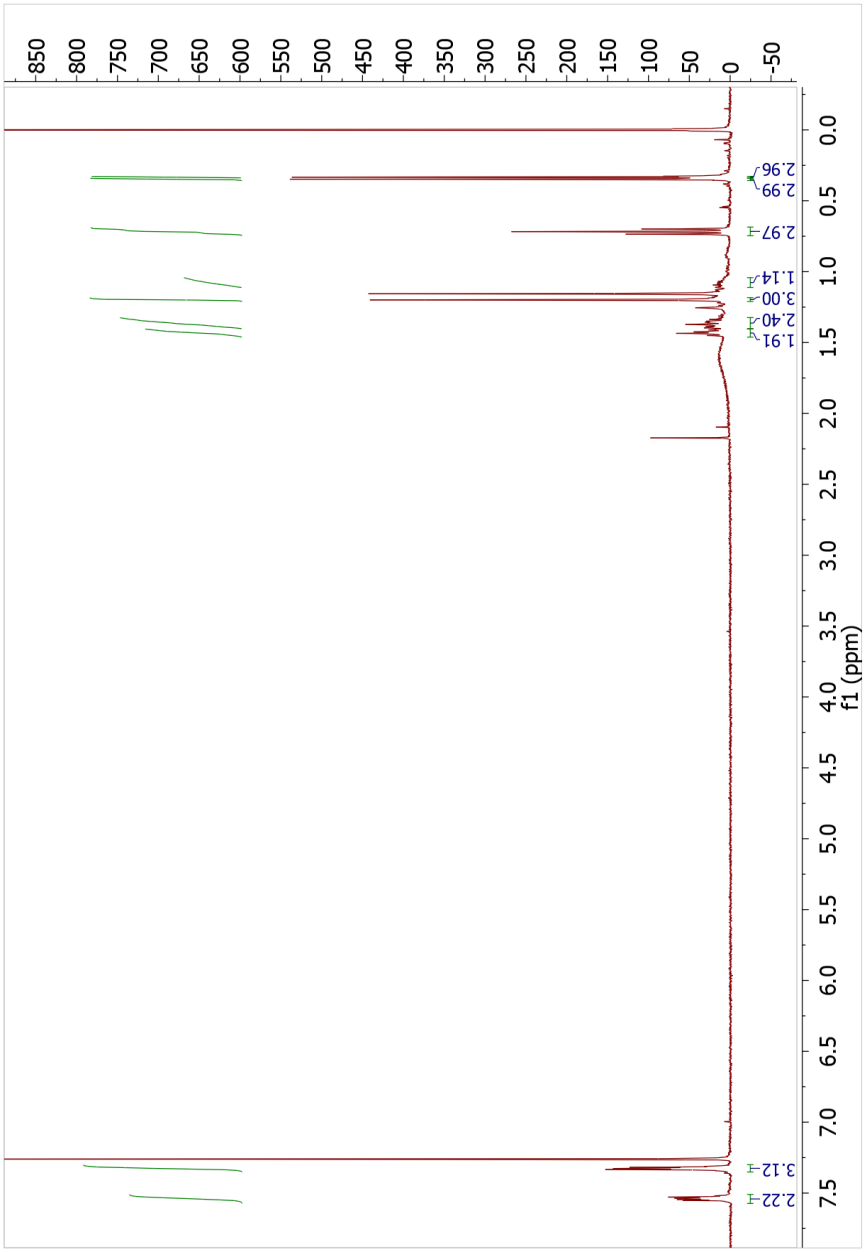
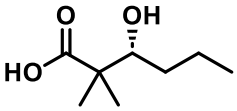
127.57
128.64
133.94
140.03
179.15
f1 (ppm)

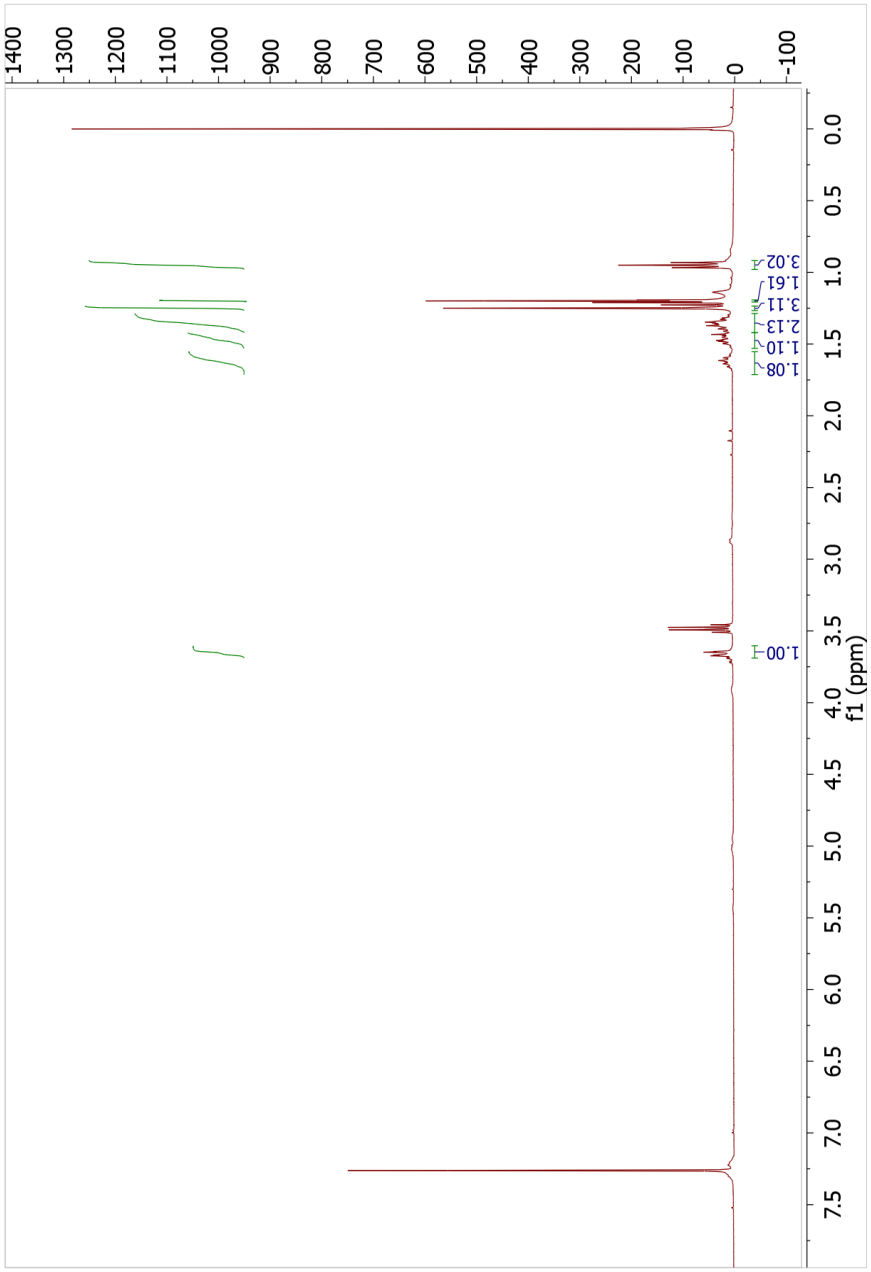
^{1}H NMR for Compound 1.7

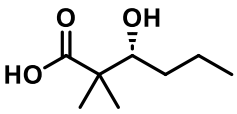


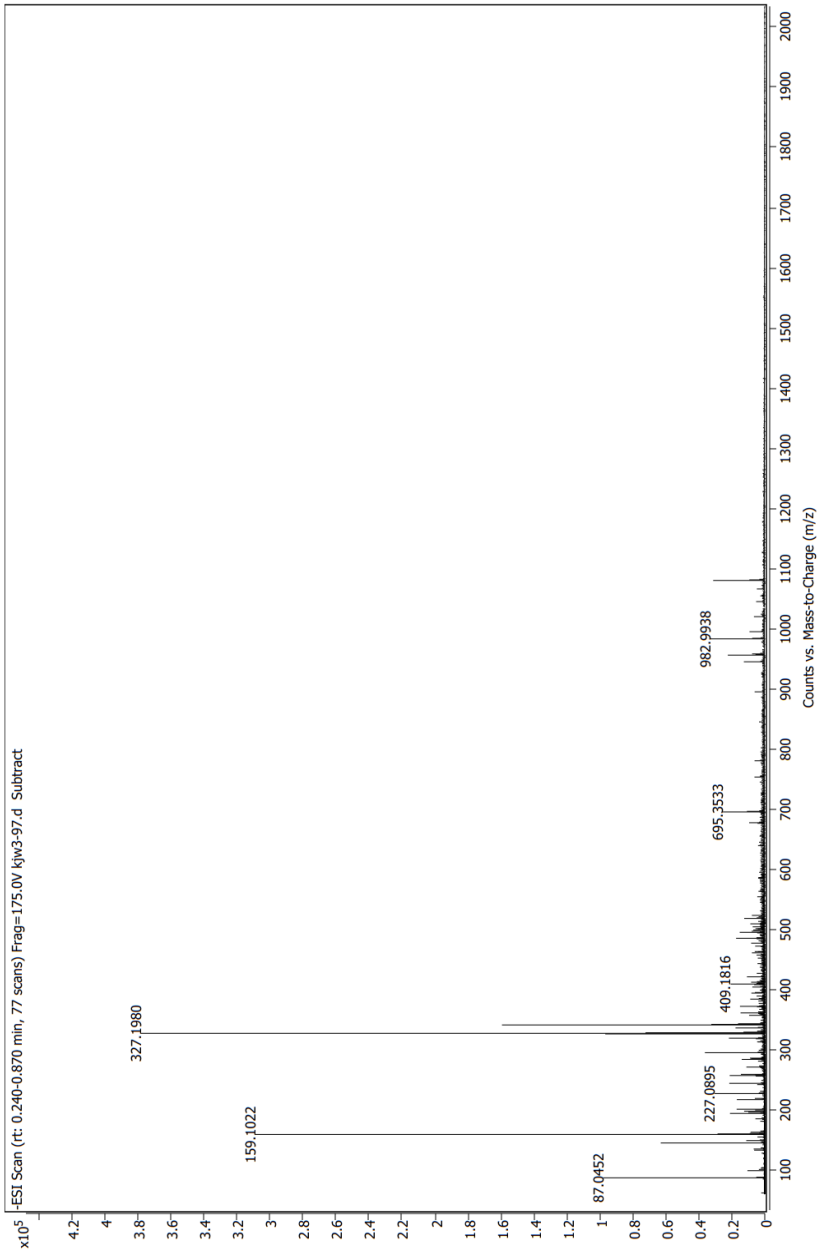


^{1}H NMR and HRMS for Compound 1.8

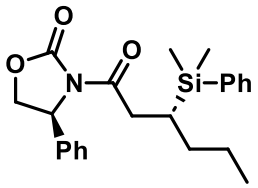


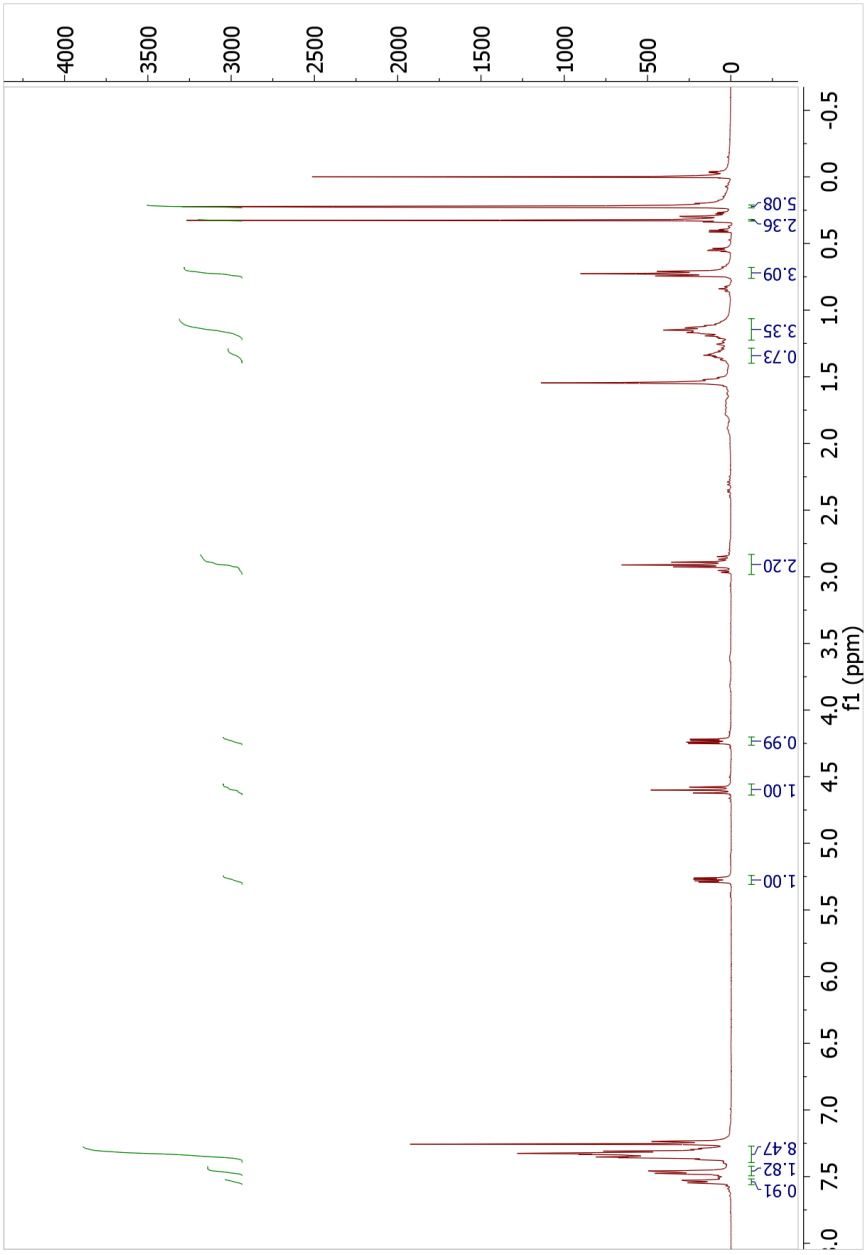


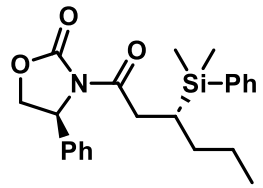

O
OH
HO

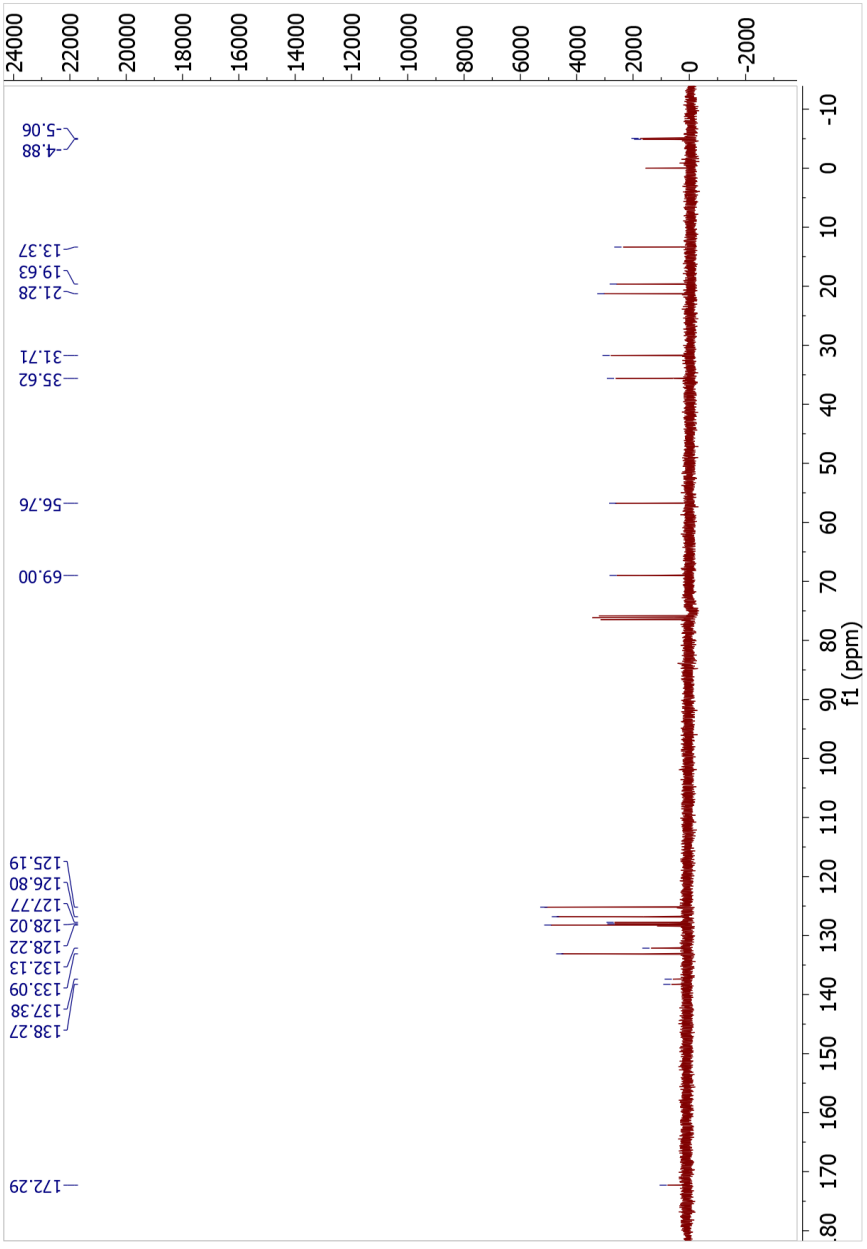

-ESI Scan (rt: 0.240-0.870 min, 77 scans) Frag=175.0V kjw3-97.d Subtract
x10^5
Counts vs. Mass-to-Charge (m/z)
87.0452
159.1022
227.0895
327.1980
409.1816
695.3533
982.9938

^{1}H and ^{13}C NMR for Compound 1.9

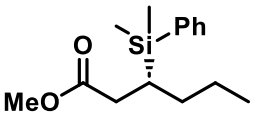


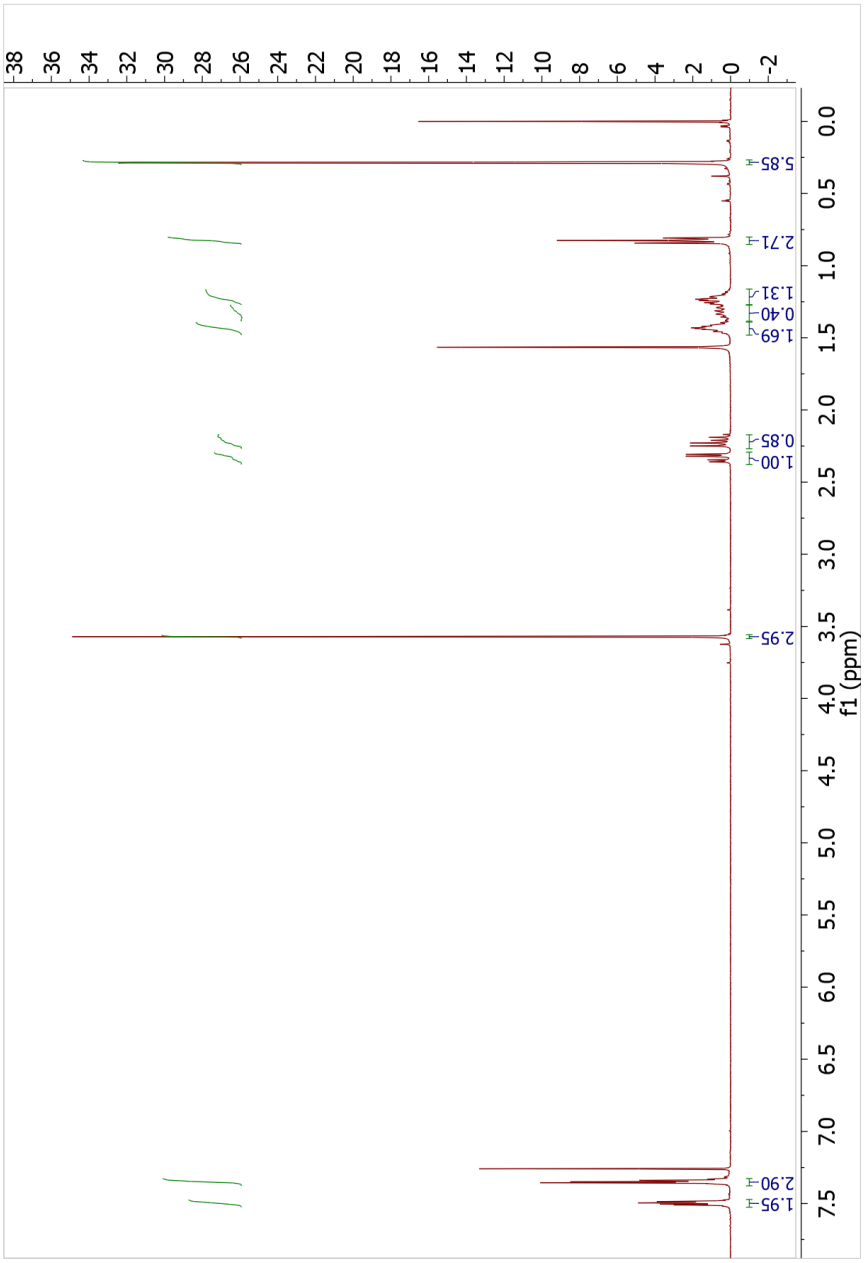


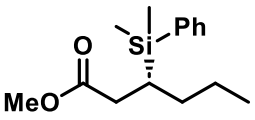

O
O
N
O
Si–Ph
Ph

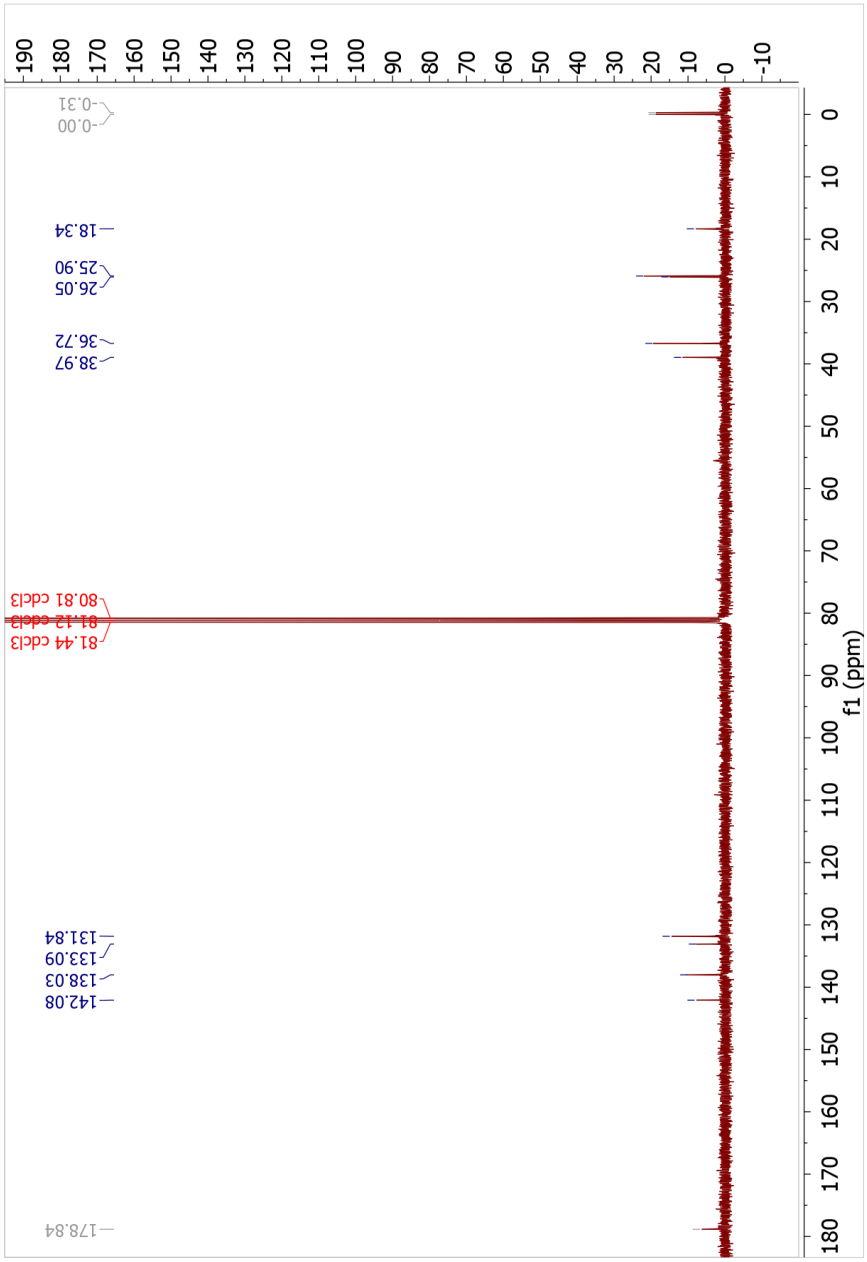

-4.88
-5.06
13.37
19.63
21.28
31.71
35.62
56.76
69.00
125.19
126.80
127.77
128.02
128.22
132.13
133.09
137.38
138.27
172.29
f1 (ppm)

^{1}H and ^{13}C NMR for 1.10

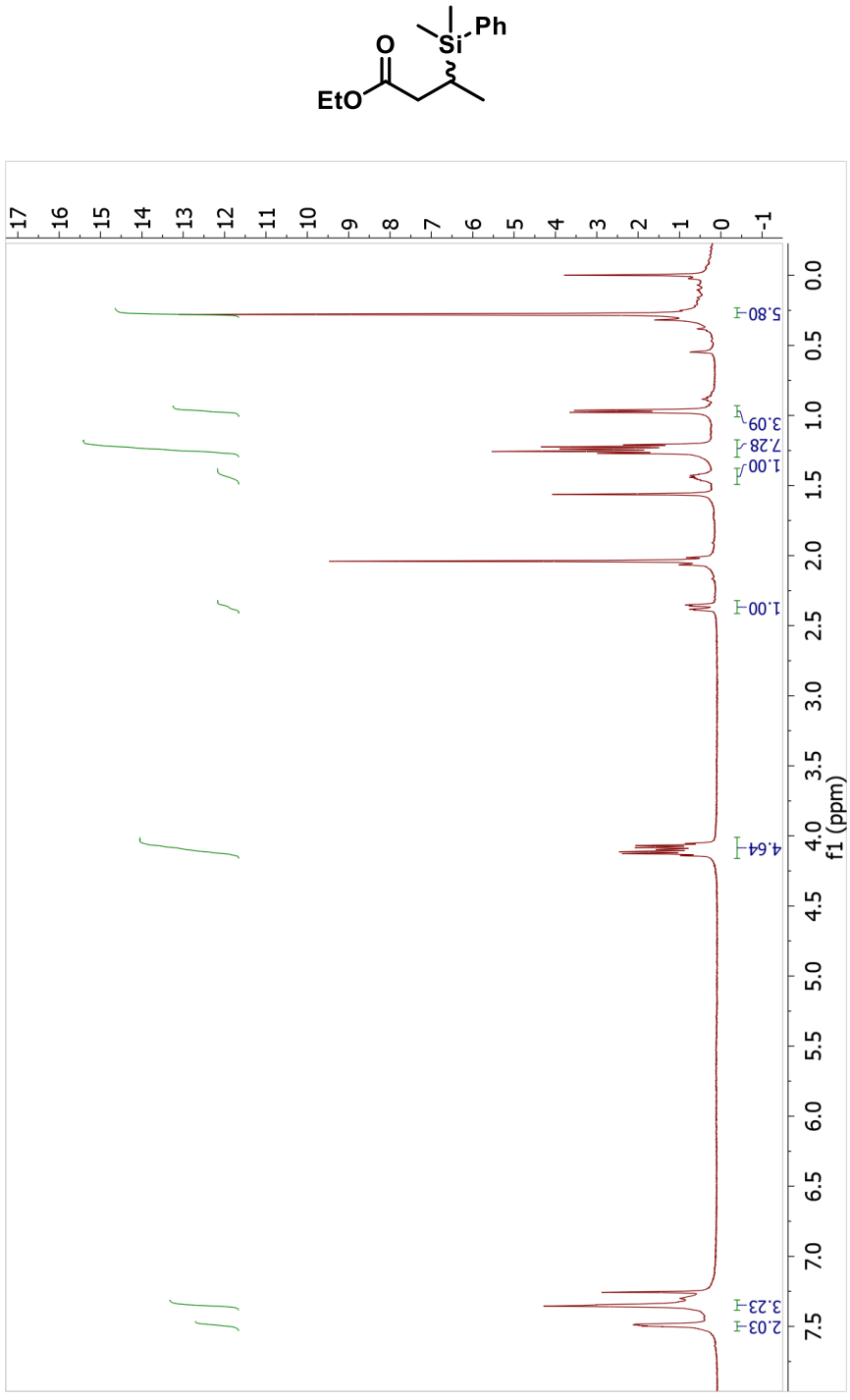




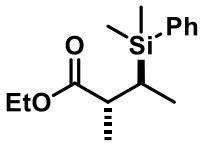

O
Si
Ph
MeO

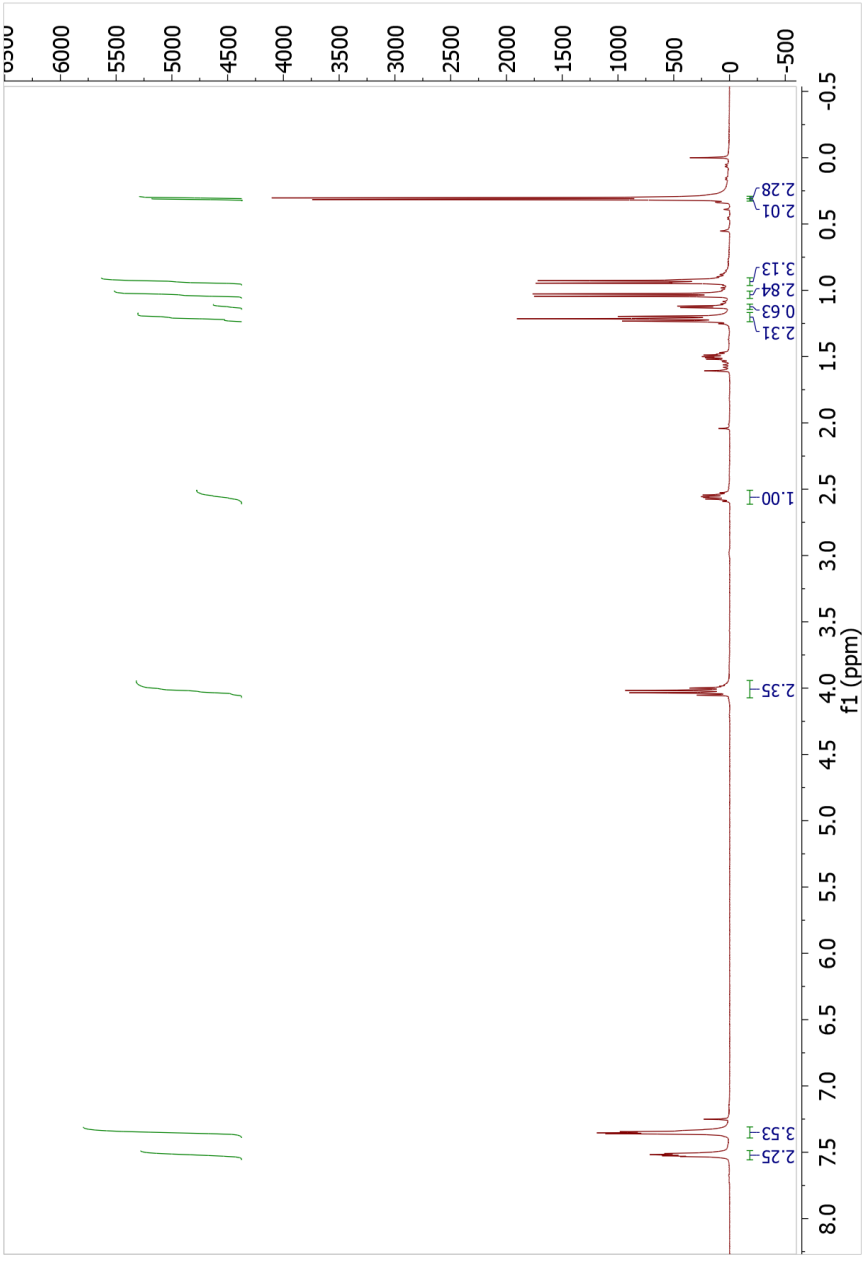

-0.31
-0.00
18.34
25.90
26.05
36.72
38.97
80.81 cdcl3
81.12 cdcl3
81.44 cdcl3
131.84
133.09
138.03
142.08
178.84
f1 (ppm)

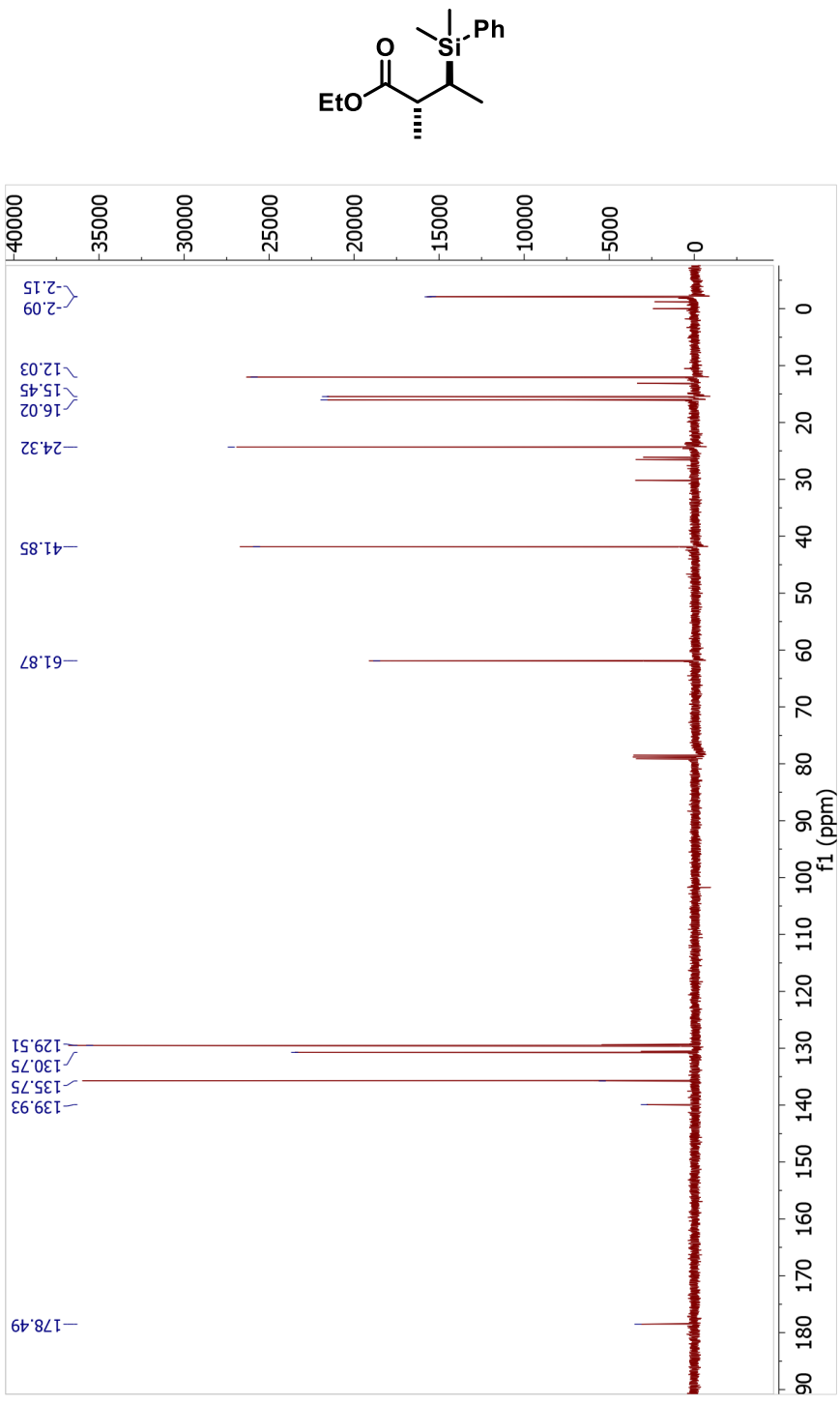
^{1}H NMR for Compound 1.12



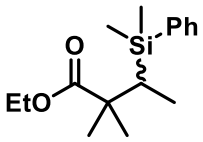
^{1}H and ^{13}C NMR for Compound 1.13

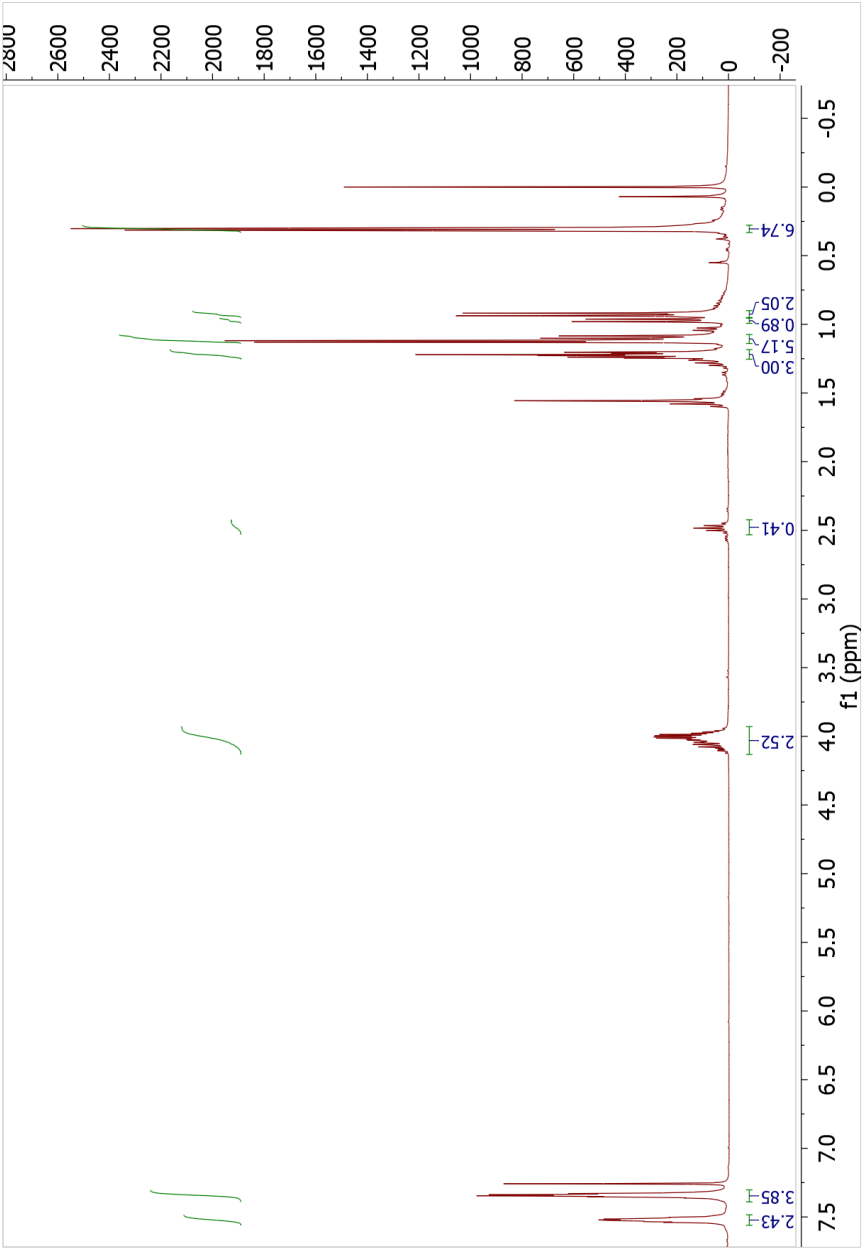


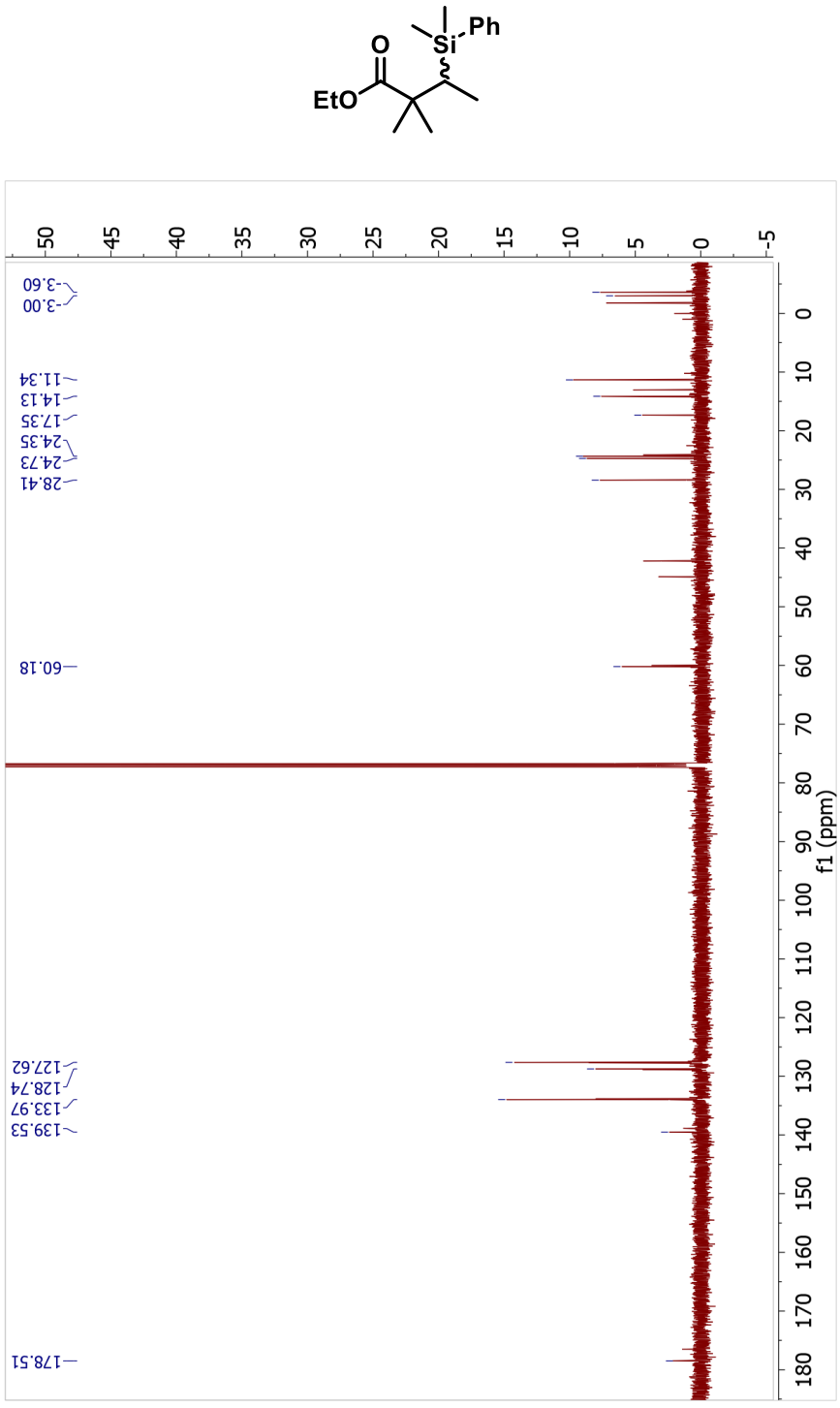



Ph
Si
O
EtO
-2.15
-2.09
12.03
15.45
16.02
24.32
41.85
61.87
129.51
130.75
135.75
139.93
178.49
f1 (ppm)

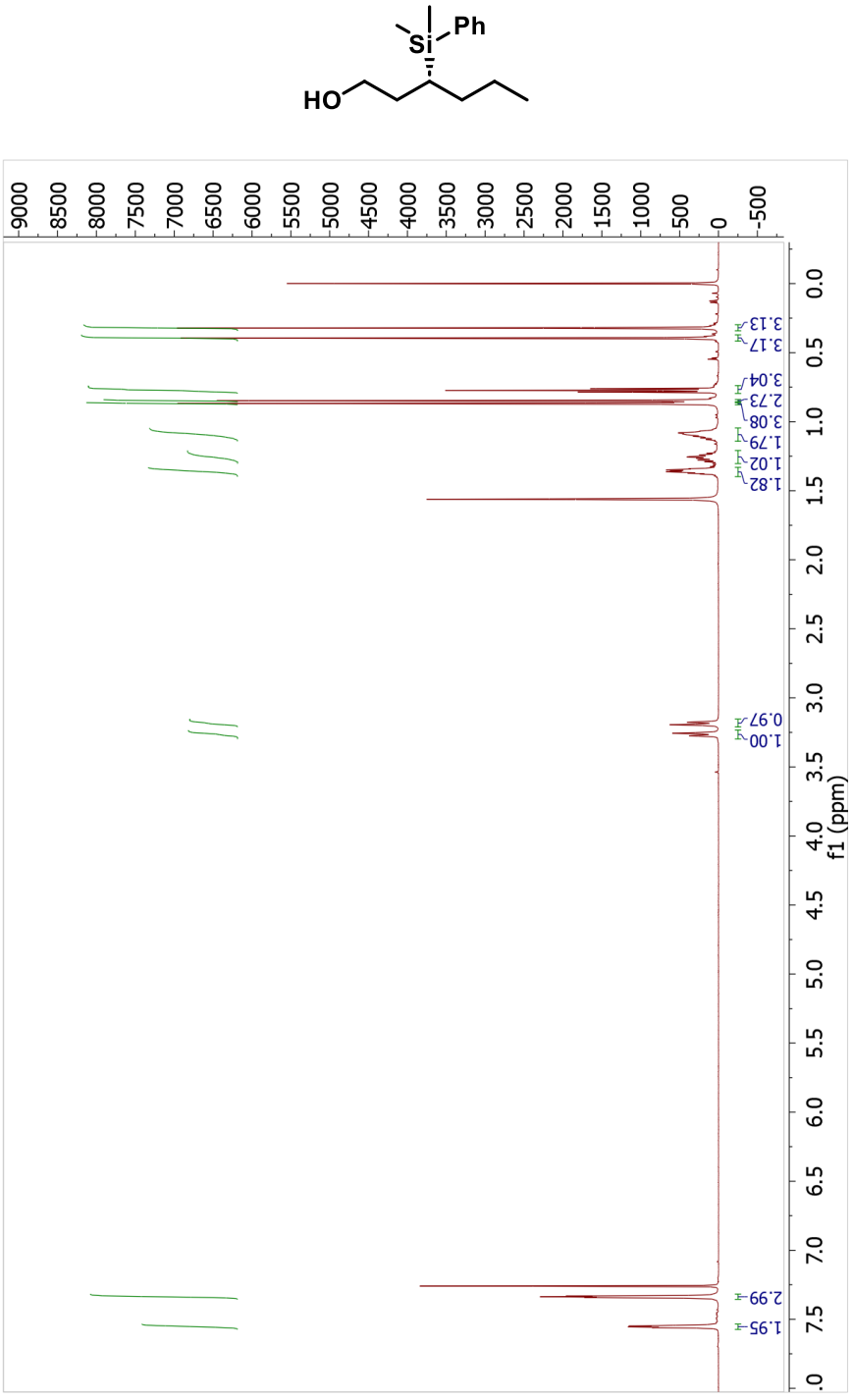
^{1}H and ^{13}C NMR for Compound 1.14



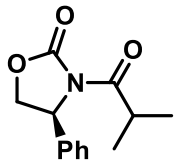


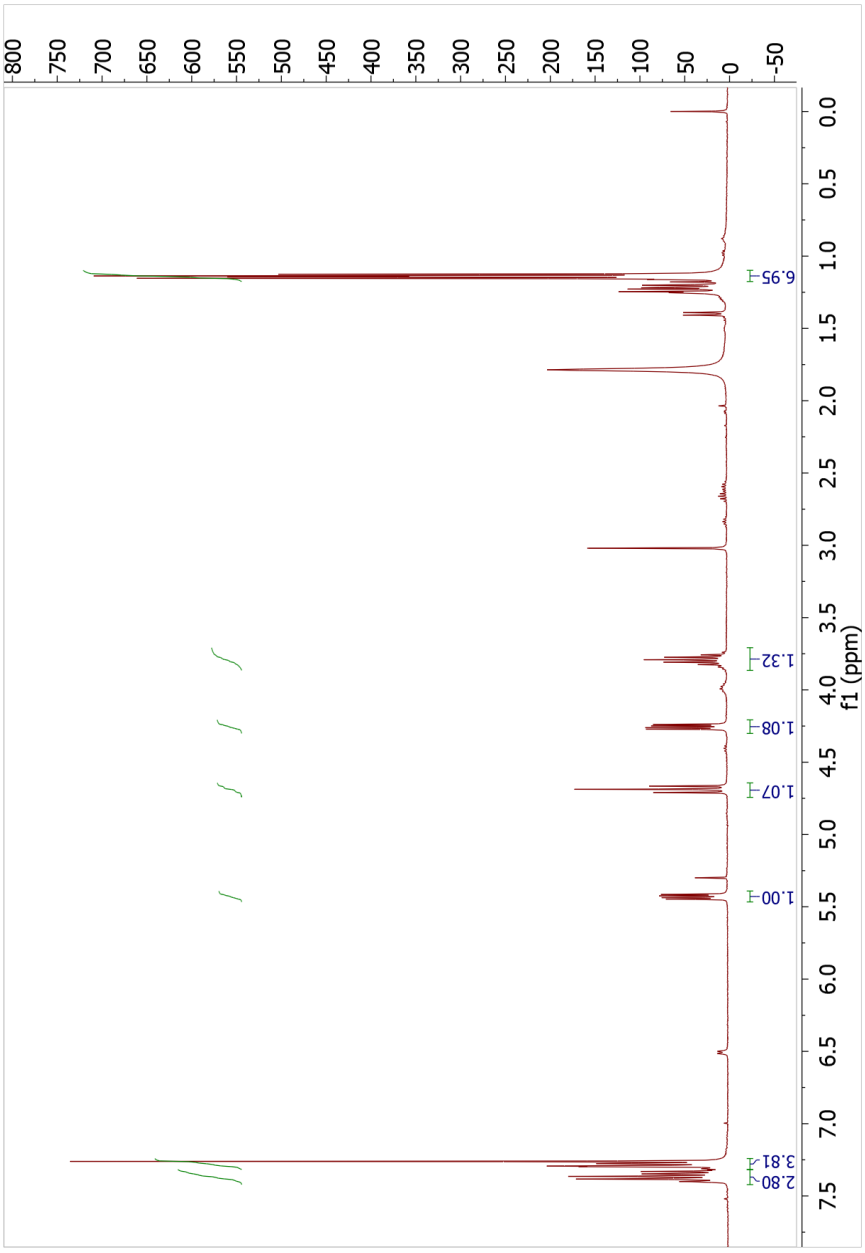

Ph
Si
O
EtO
-3.60
-3.00
11.34
14.13
17.35
24.35
24.73
28.41
60.18
127.62
128.74
133.97
139.53
178.51
f1 (ppm)

^{1}H NMR for Compound 1.15

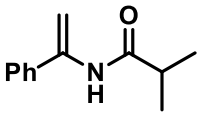


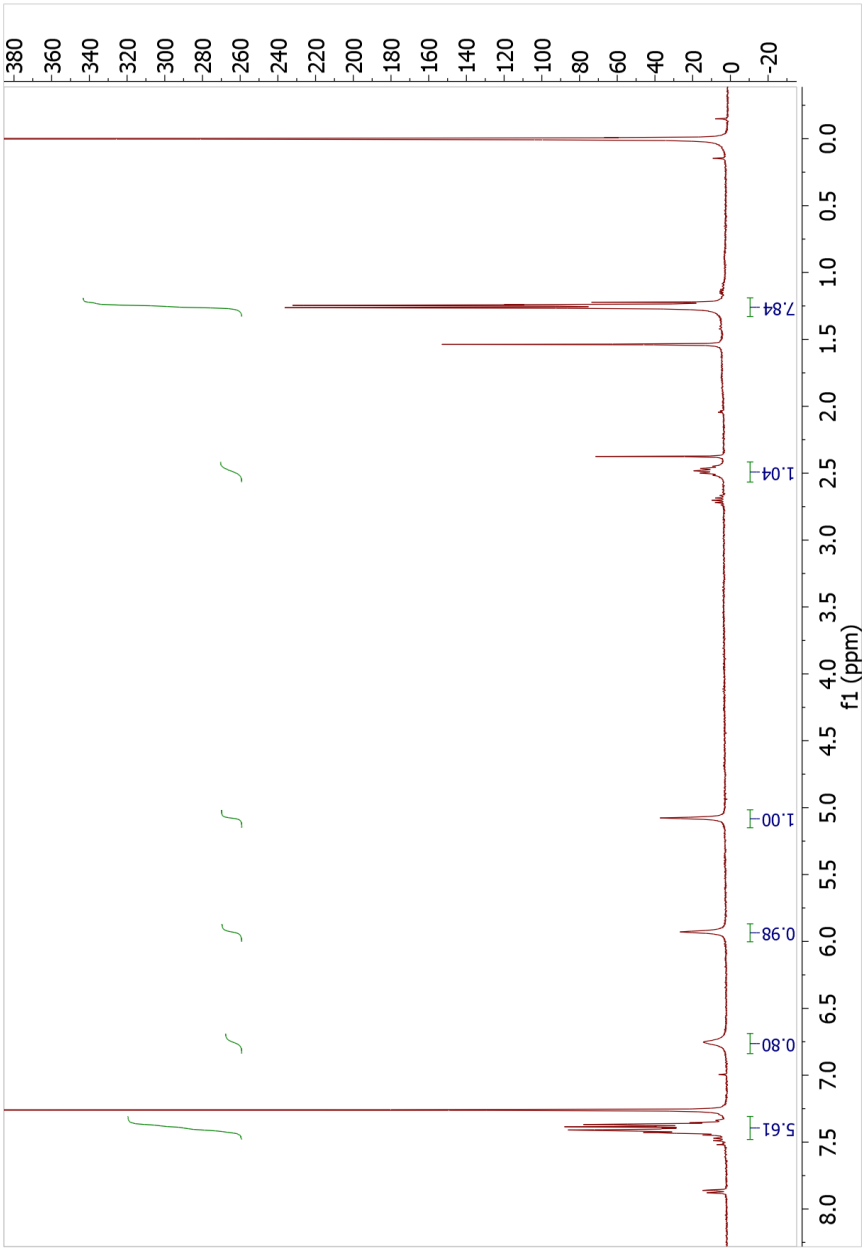
^{1}H NMR for Compound 1.17



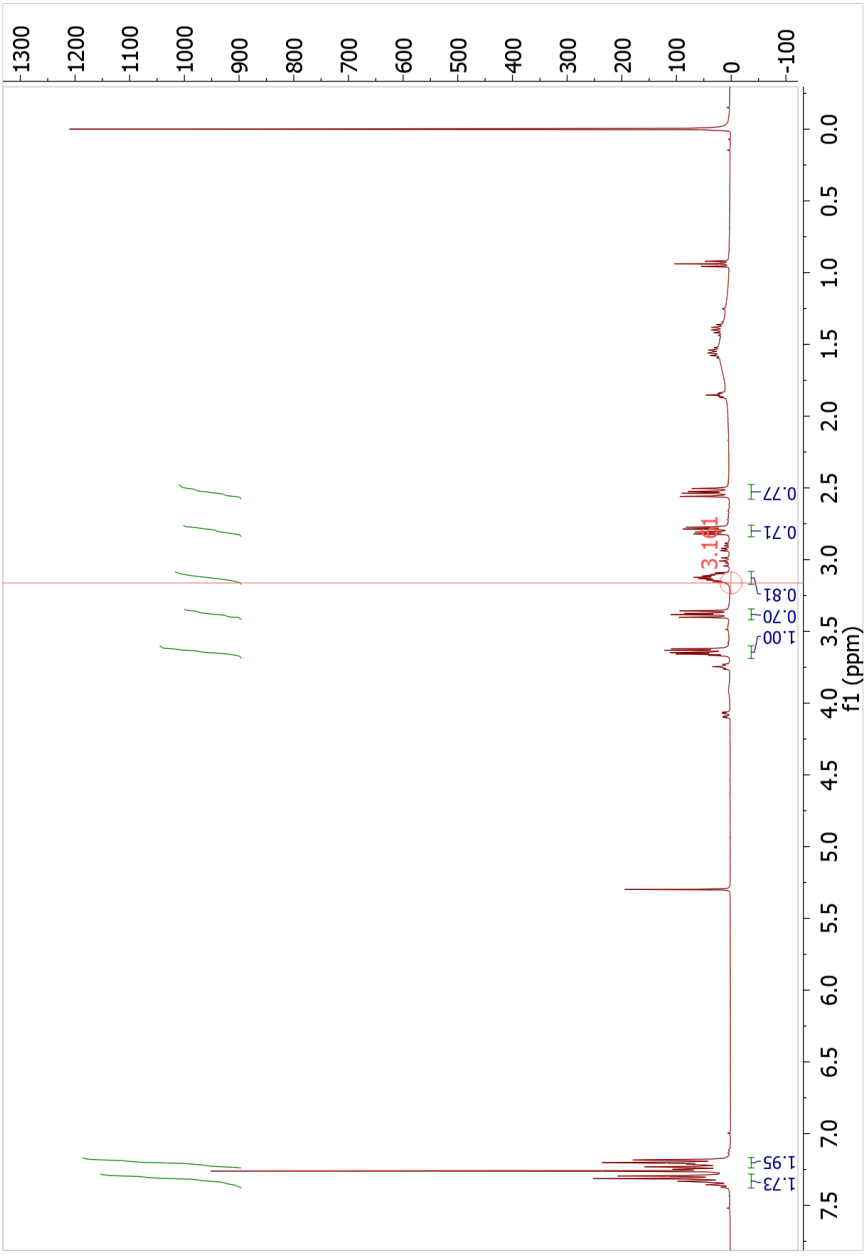


^{1}H NMR for Compound 1.19

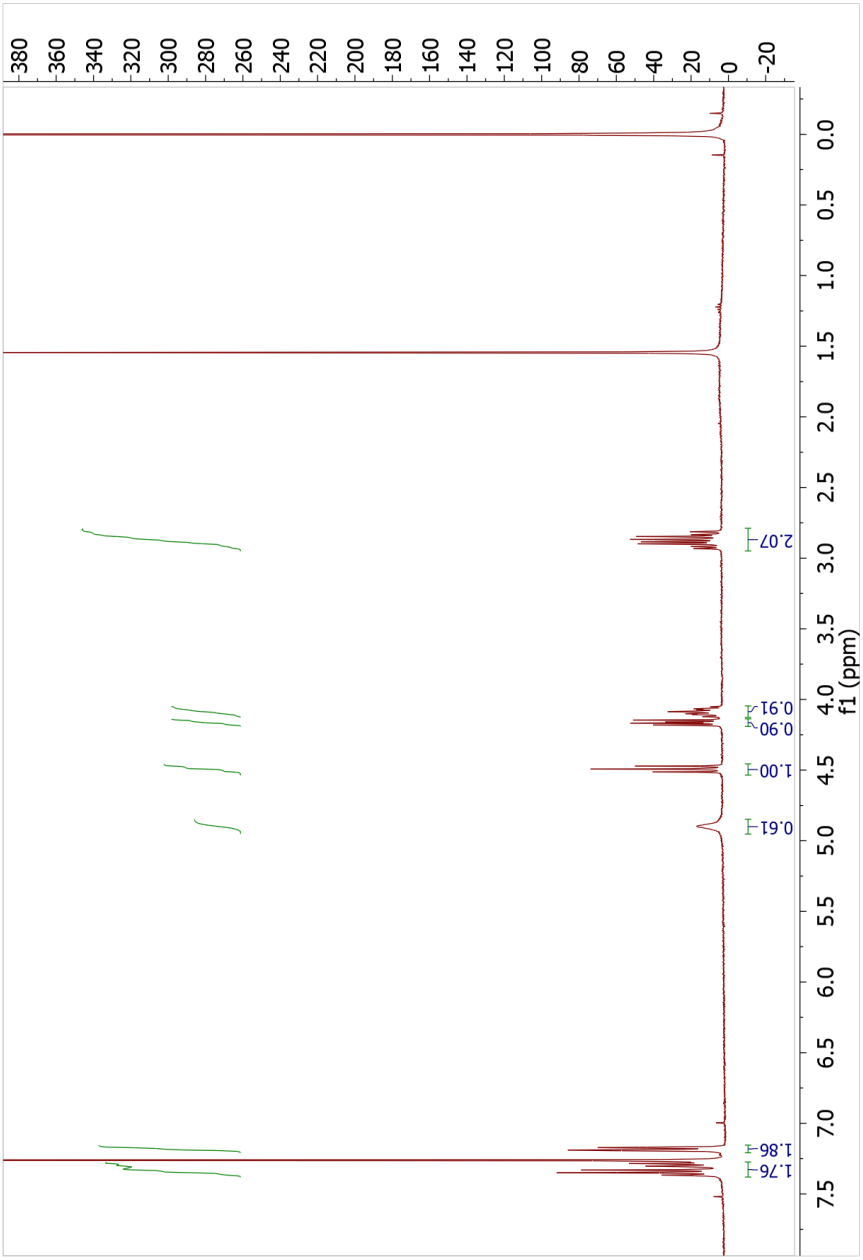




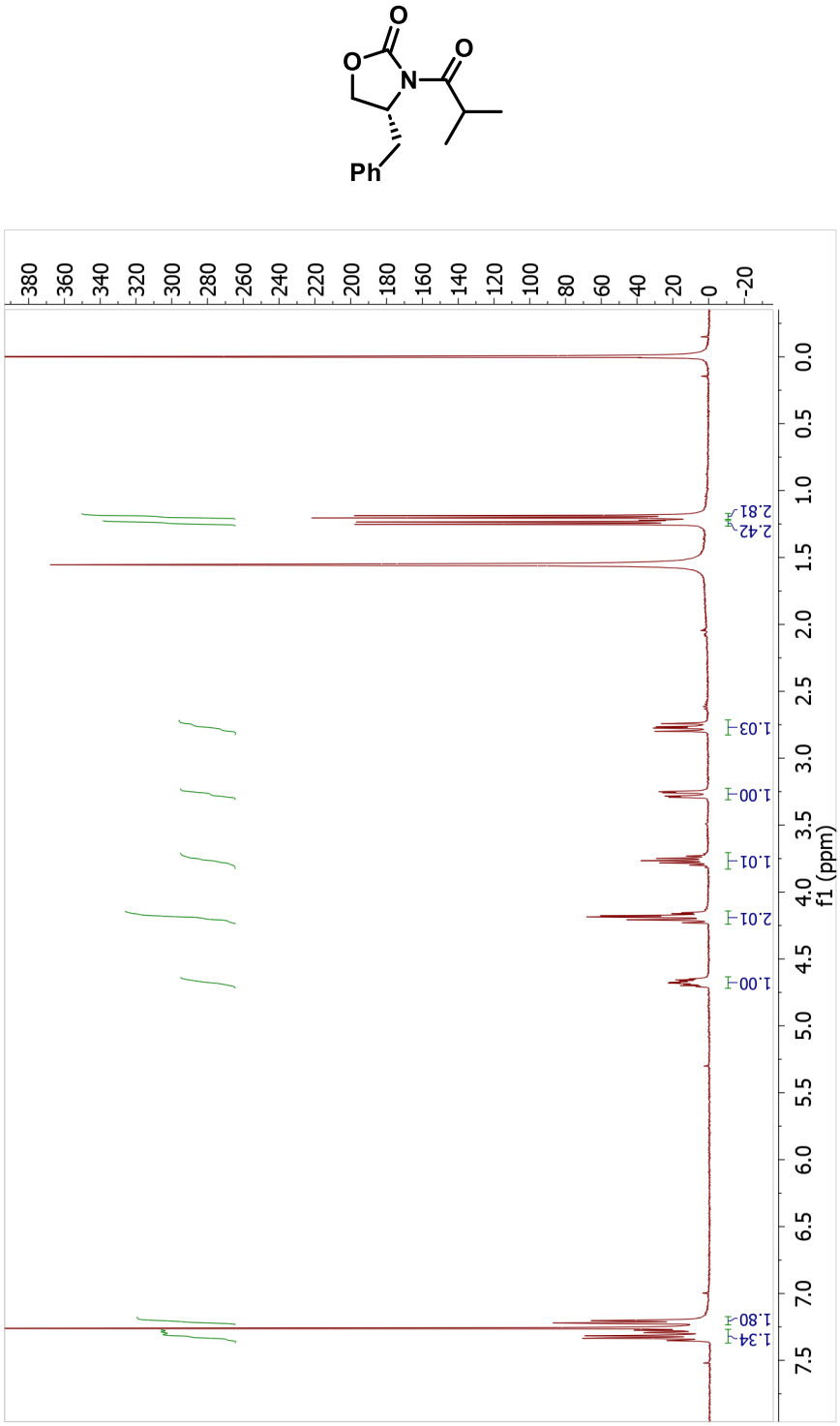
^{1}H NMR for Compound 1.20



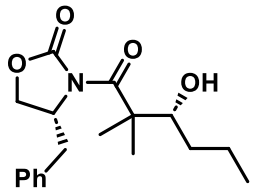
^{1}H NMR for Compound 1.21

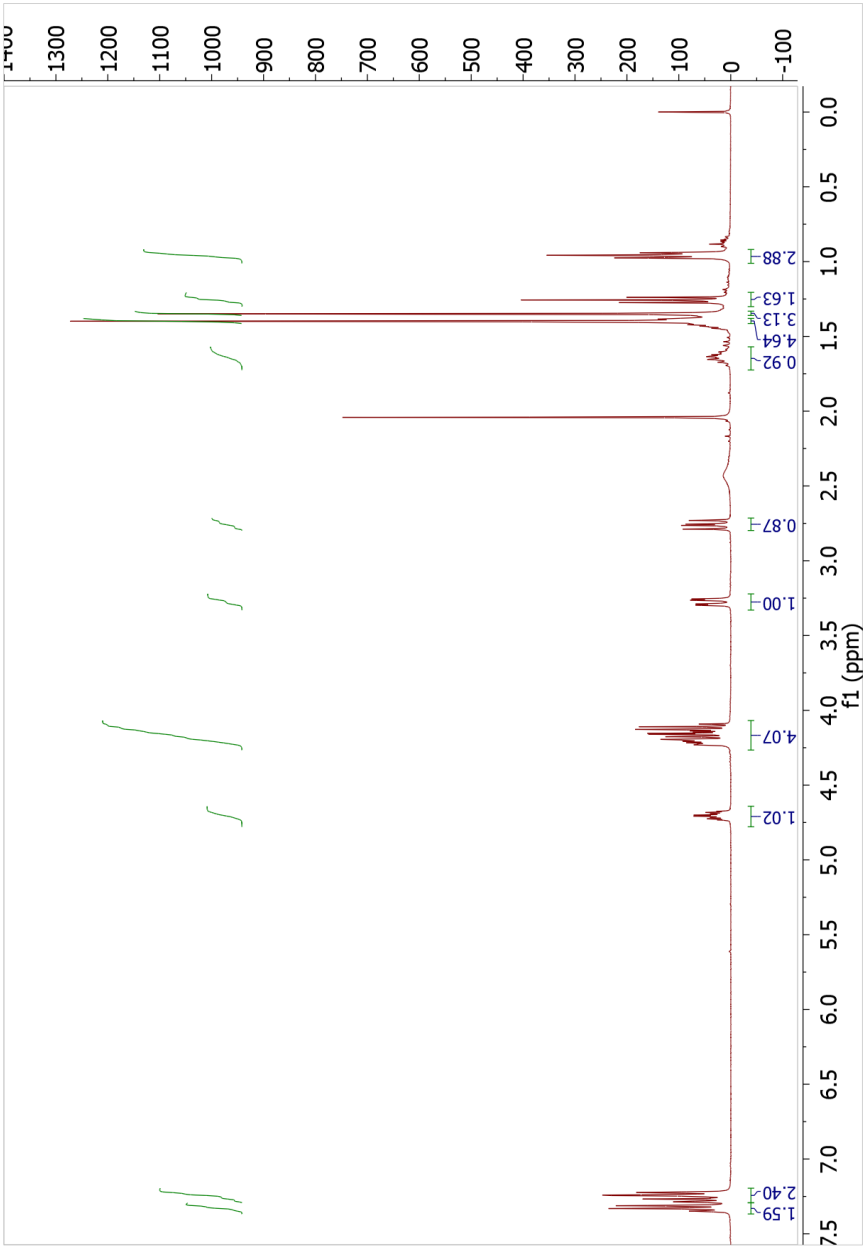


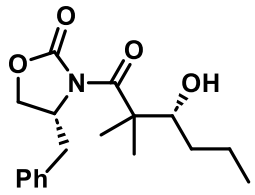
^{1}H NMR for Compound 1.22

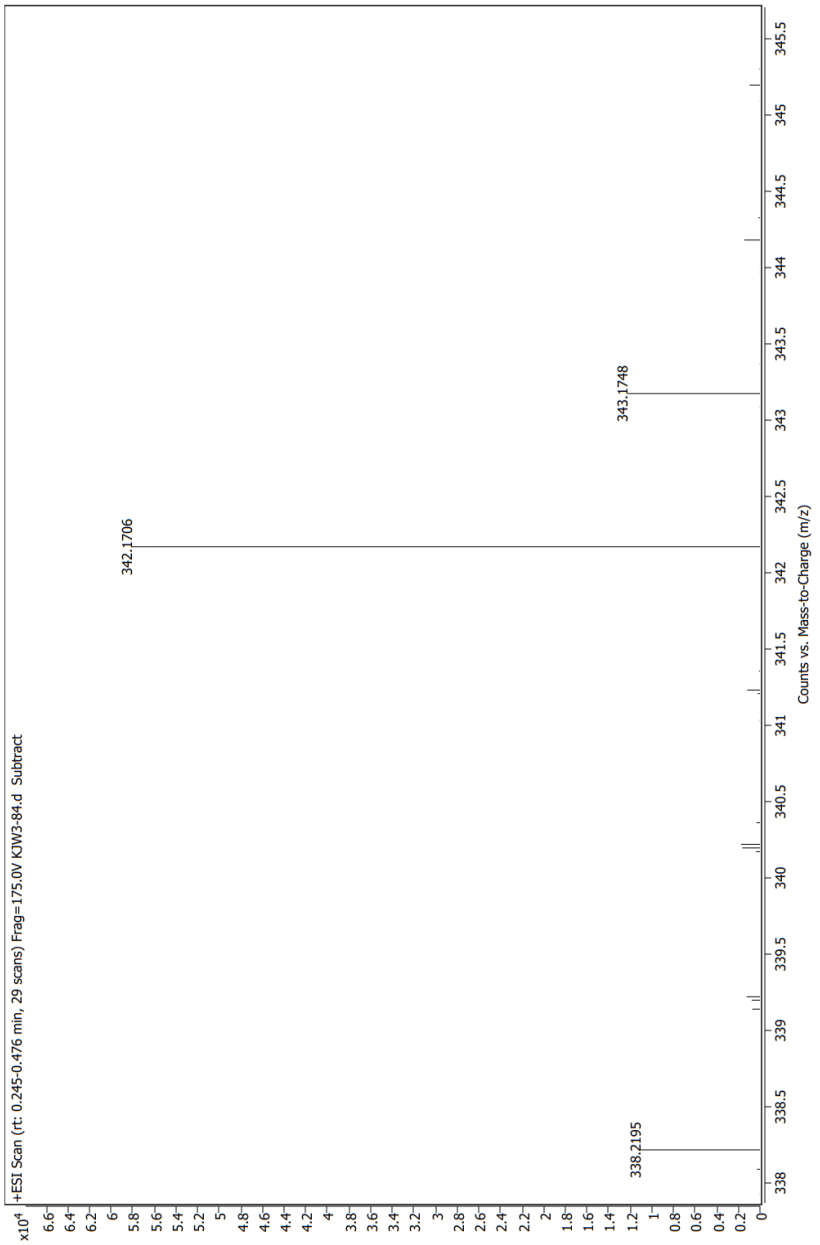


^{1}H NMR and HRMS for Compound 1.23

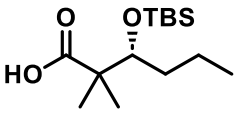


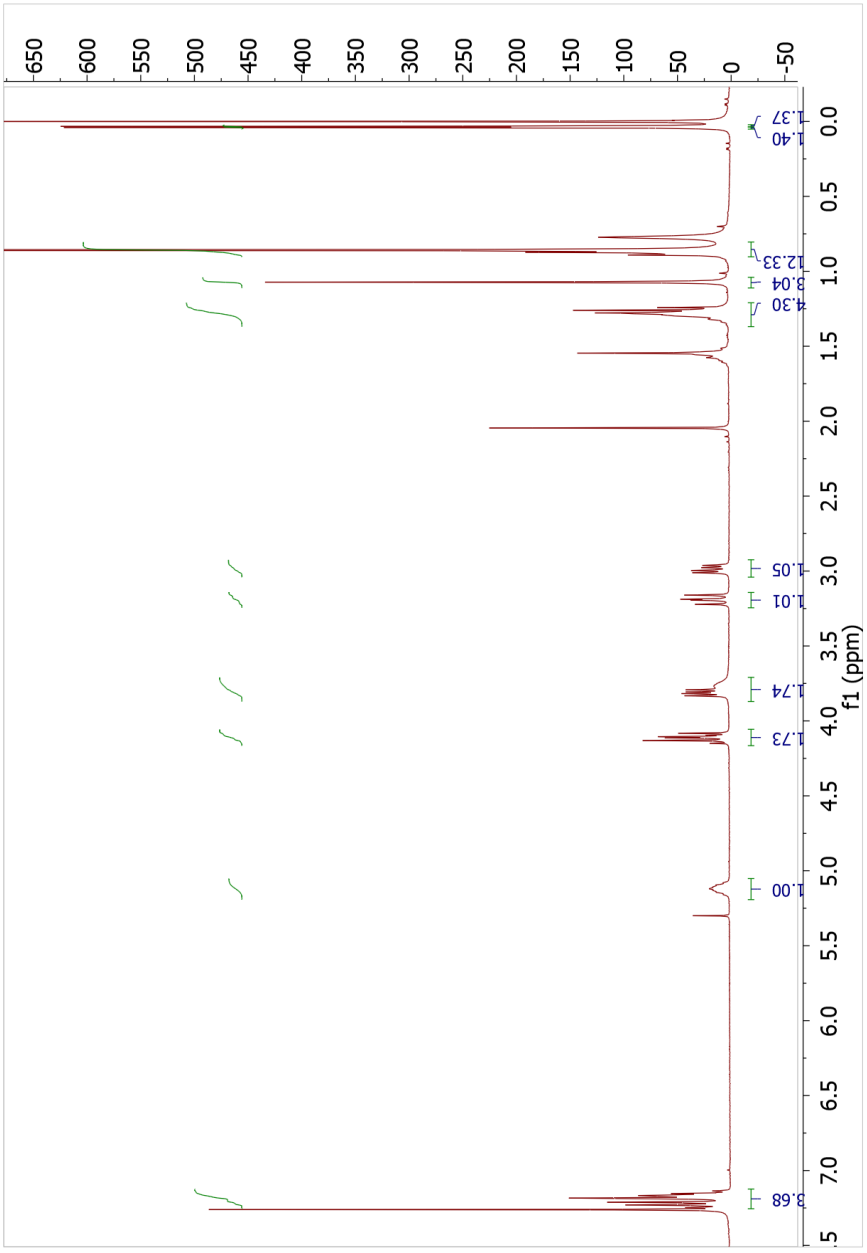


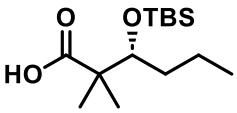

O
O
N
O
OH
Ph

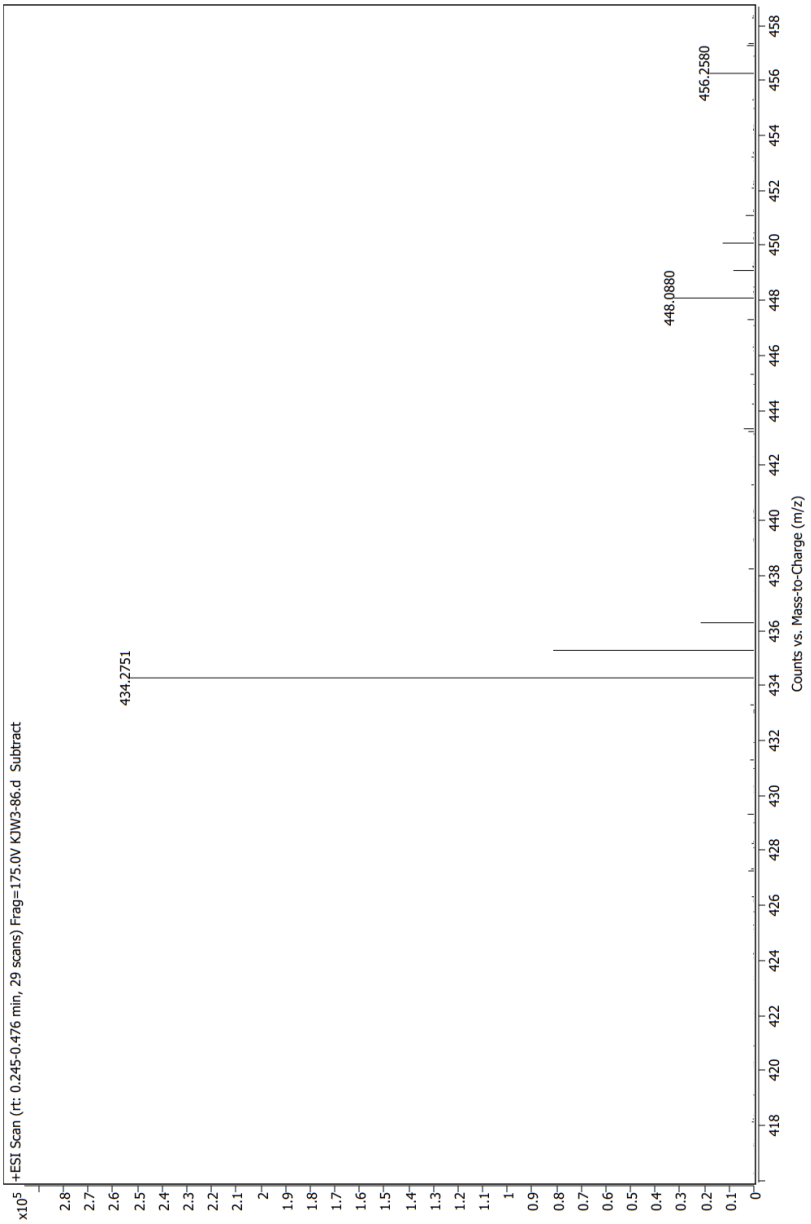

+ESI Scan (rt: 0.245-0.476 min, 29 scans) Frag=175.0V KJW3-84.d Subtract
x10^4
6.6
6.4
6.2
6
5.8
5.6
5.4
5.2
5
4.8
4.6
4.4
4.2
4
3.8
3.6
3.4
3.2
3
2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
338.2195
342.1706
343.1748
338
338.5
339
339.5
340
340.5
341
341.5
342
342.5
343
343.5
344
344.5
345
345.5
Counts vs. Mass-to-Charge (m/z)

^{1}H NMR and HRMS for Compound 1.24

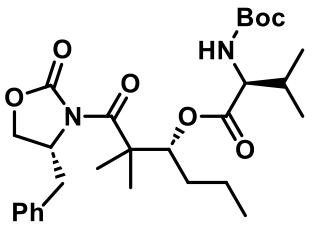


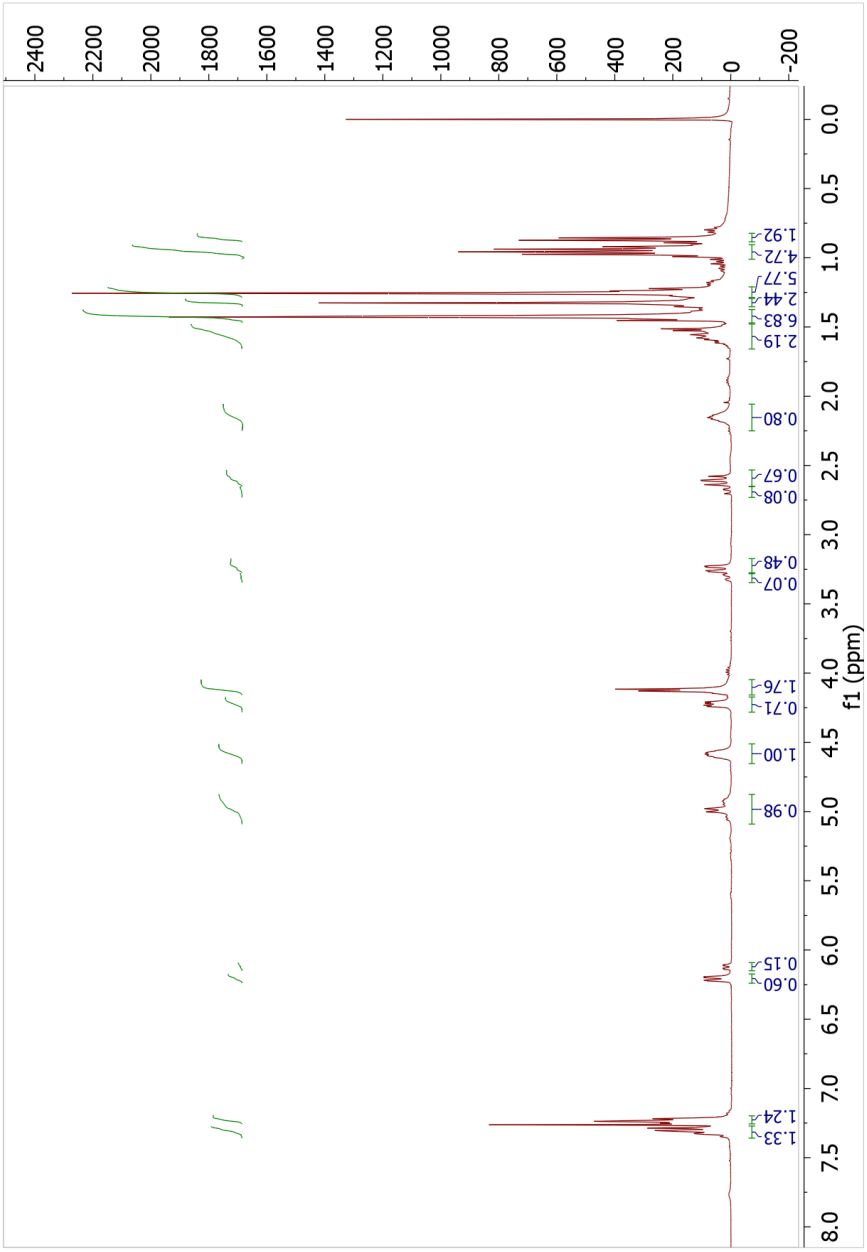


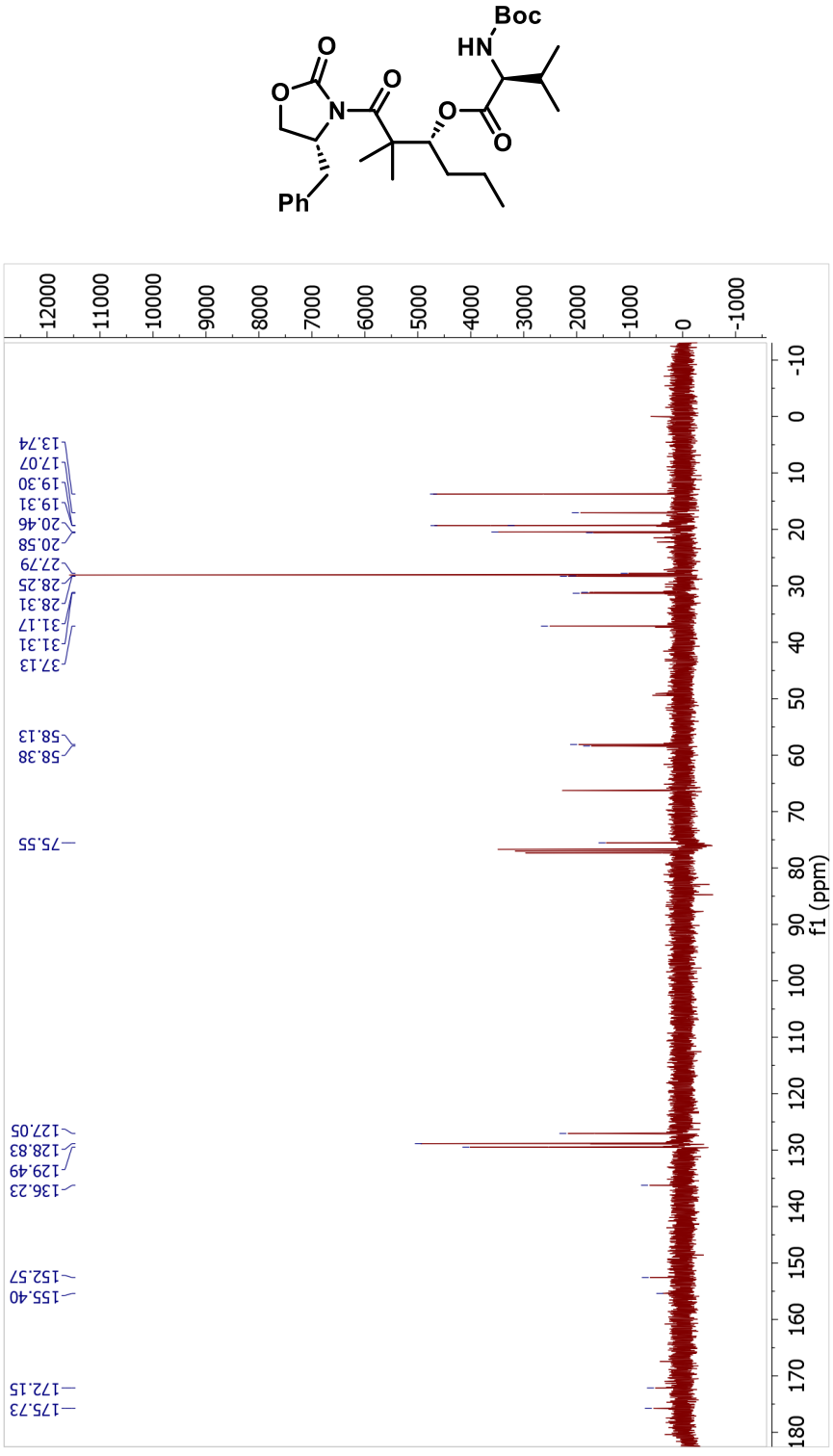

O
OTBS
HO

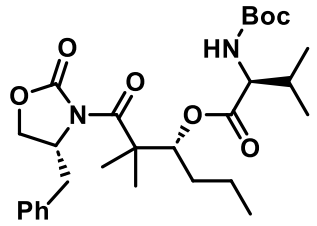

+ESI Scan (rt: 0.245-0.476 min, 29 scans) Frag=175.0V KJW3-86.d Subtract
x10^5
434.2751
448.0880
456.2580
Counts vs. Mass-to-Charge (m/z)

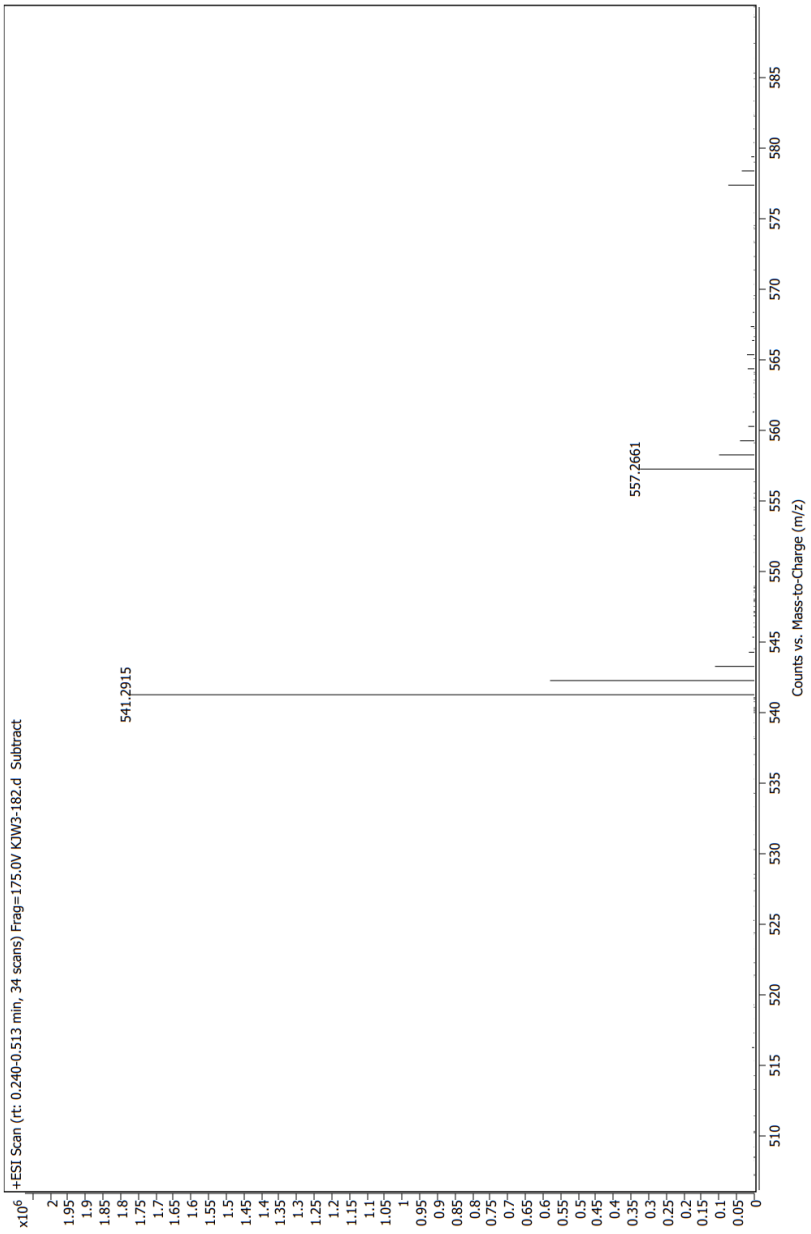
^{1}H and ^{13}C NMR and HRMS for Compound 1.25



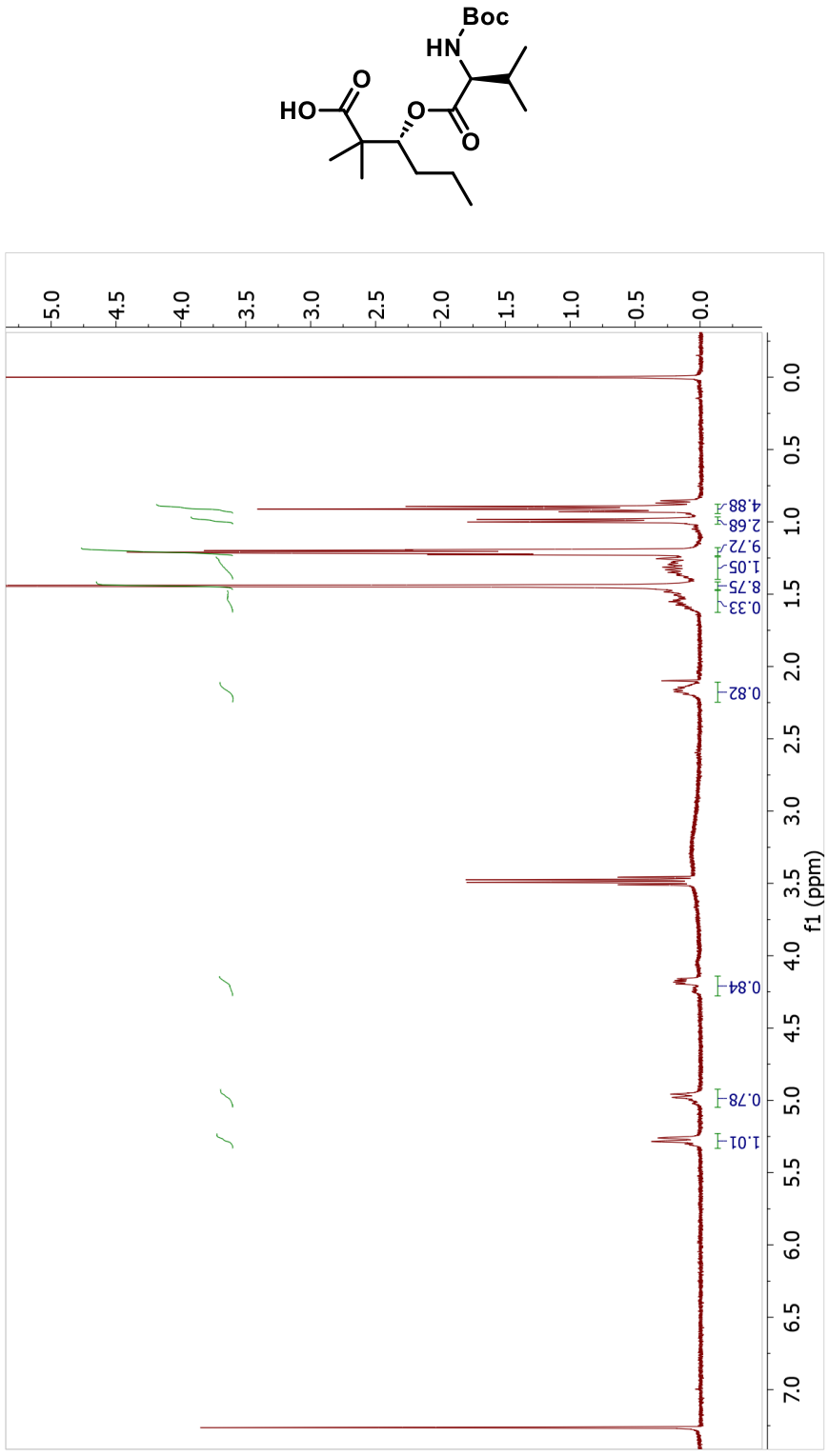



Boc
HN
O
O
O
N
O
O
O
Ph
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
-10
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
f1 (ppm)
13.74
17.07
19.30
19.31
20.46
20.58
27.79
28.25
28.31
31.17
31.31
37.13
58.13
58.38
75.55
127.05
128.83
129.49
136.23
152.57
155.40
172.15
175.73

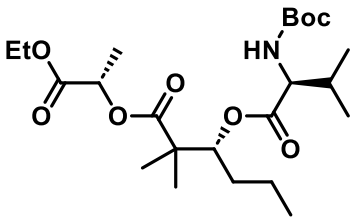

Boc
HN
O
O
O
N
O
O
Ph

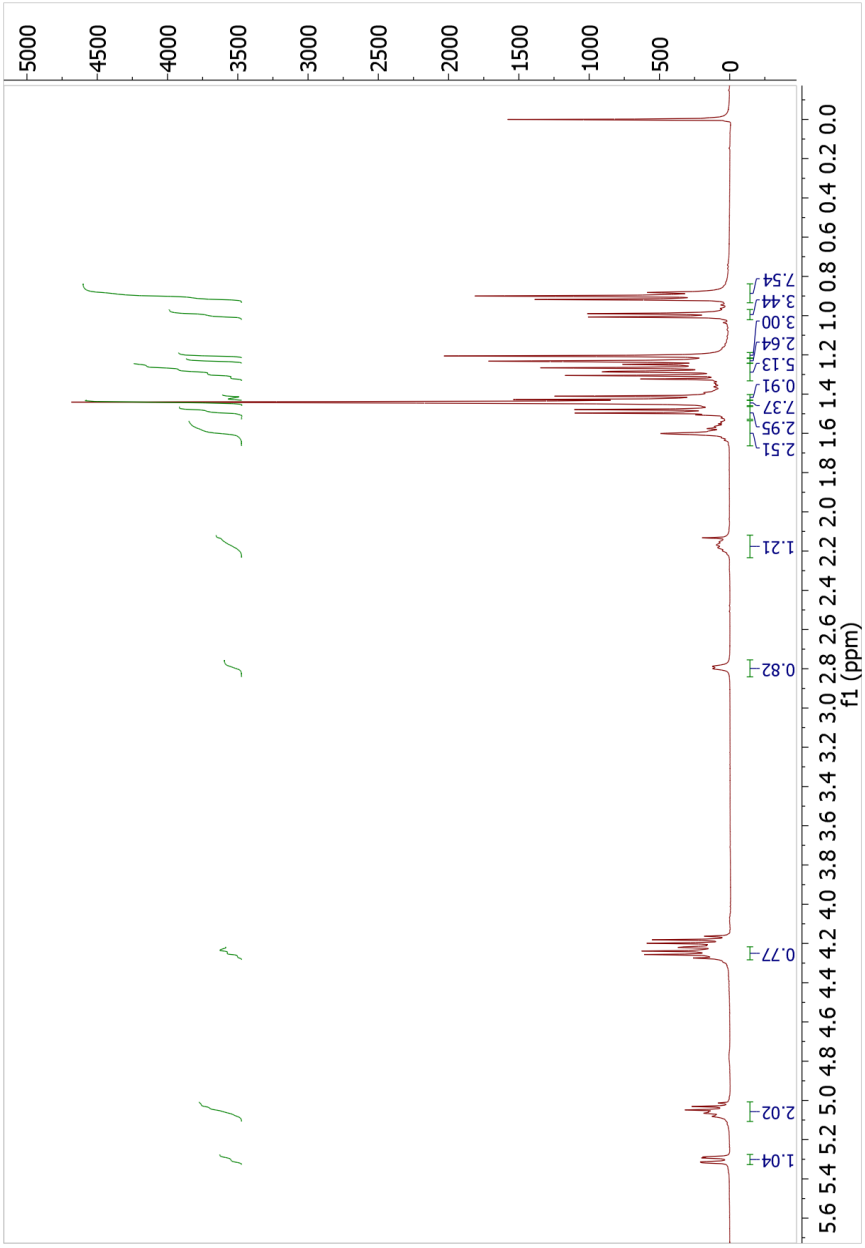

+ESI Scan (rt: 0.240-0.513 min, 34 scans) Frag=175.0V KW3-182.d Subtract
x10^6
541.2915
557.2661
Counts vs. Mass-to-Charge (m/z)

^{1}H NMR of Compound 1.26

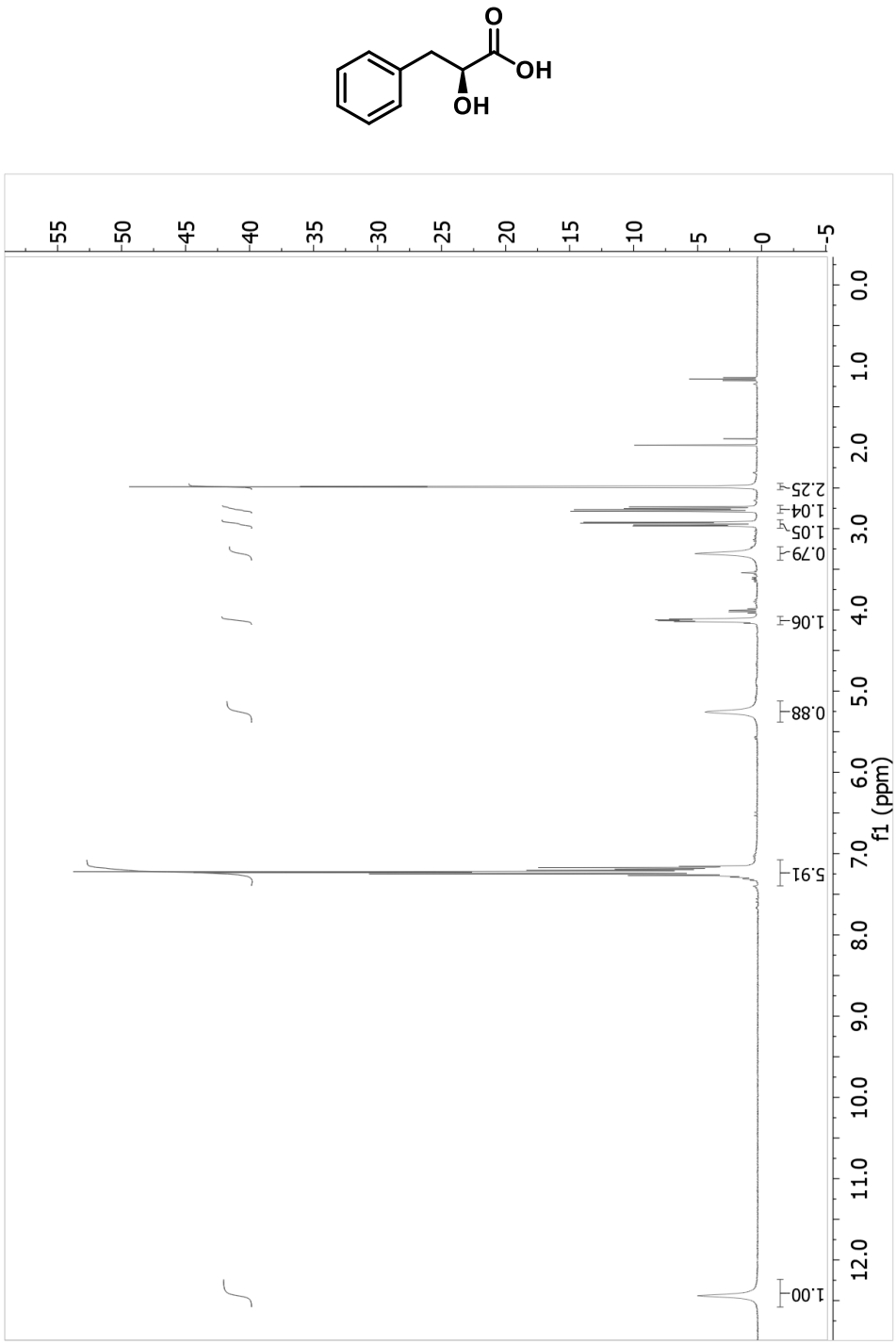


^{1}H NMR of Compound 1.27

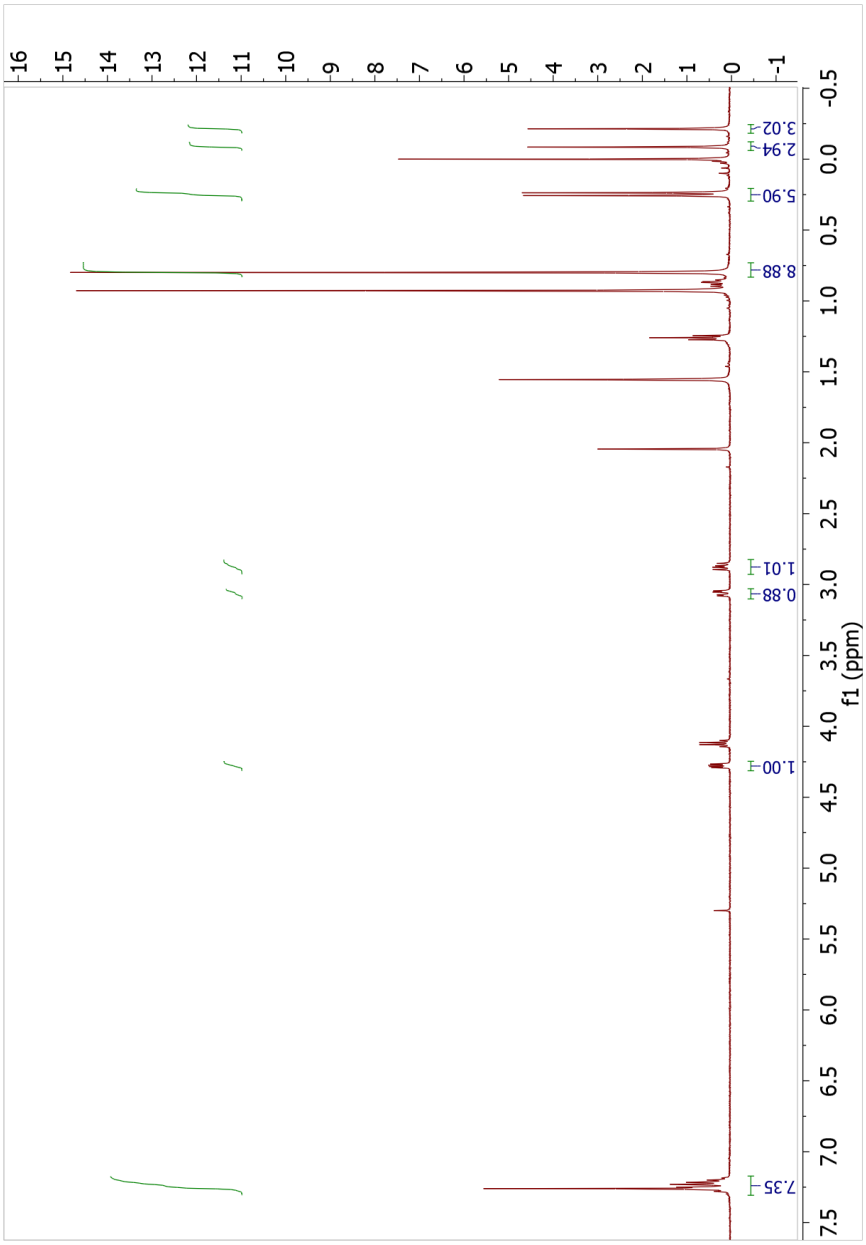




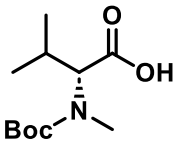
^{1}H NMR of Compound 1.28

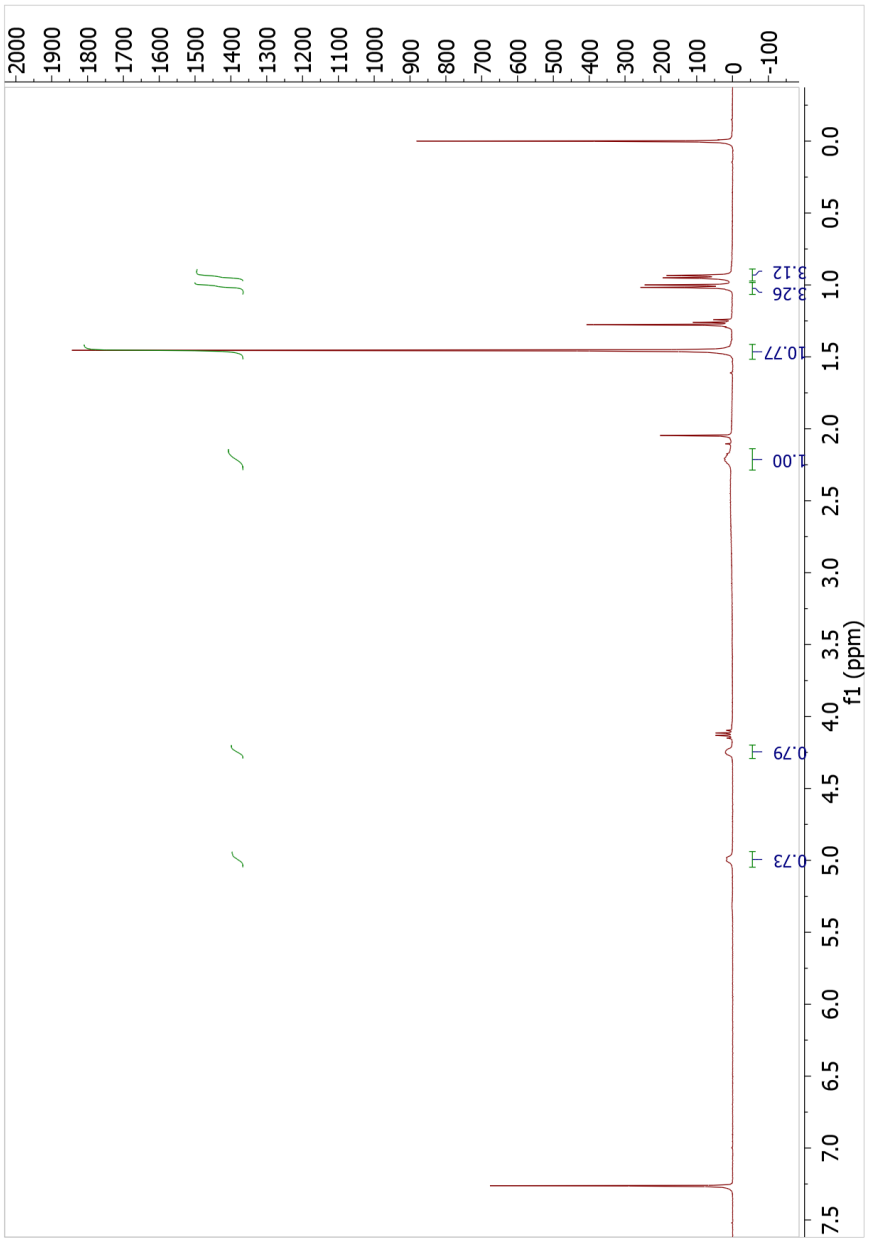


^{1}H NMR of Compound 1.29

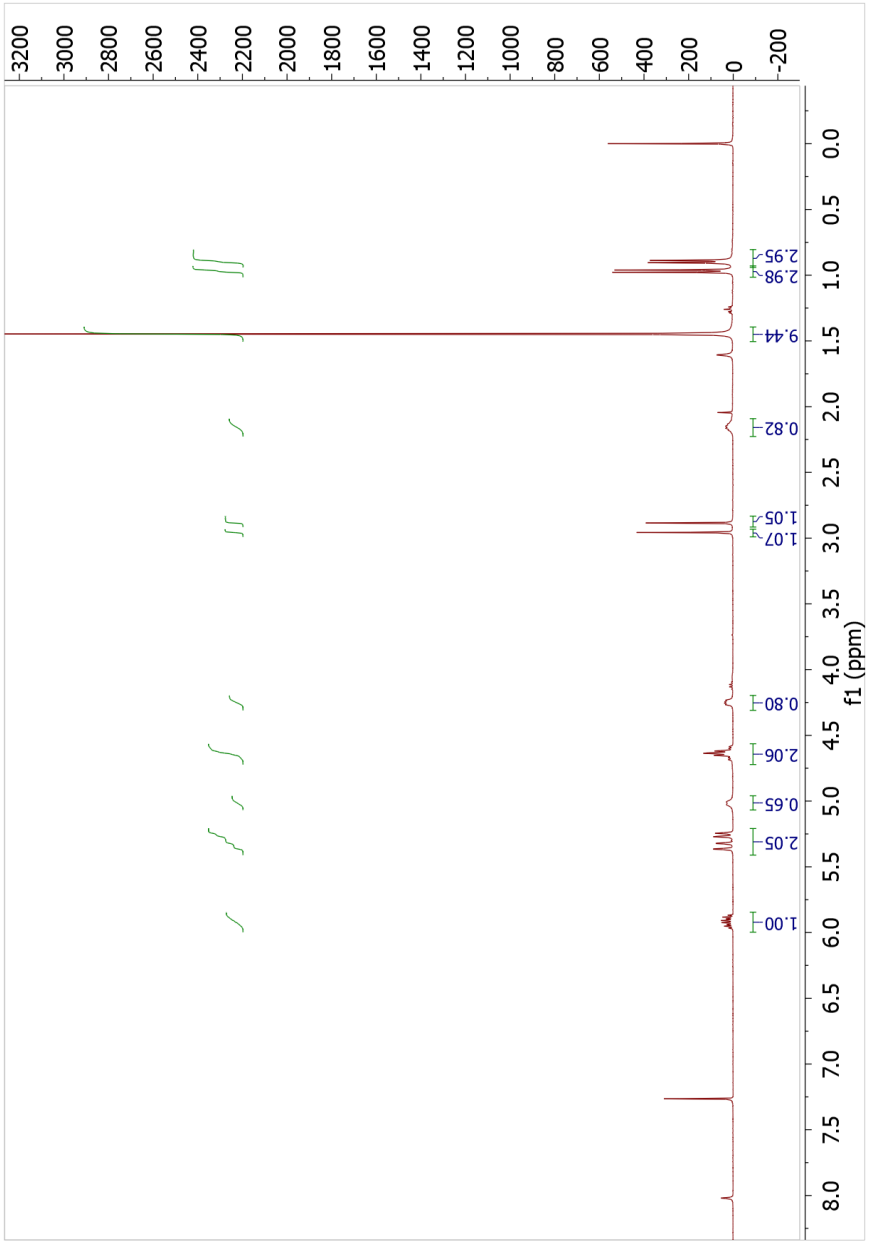


^{1}H NMR of Compound 1.30

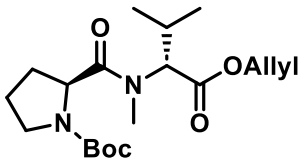


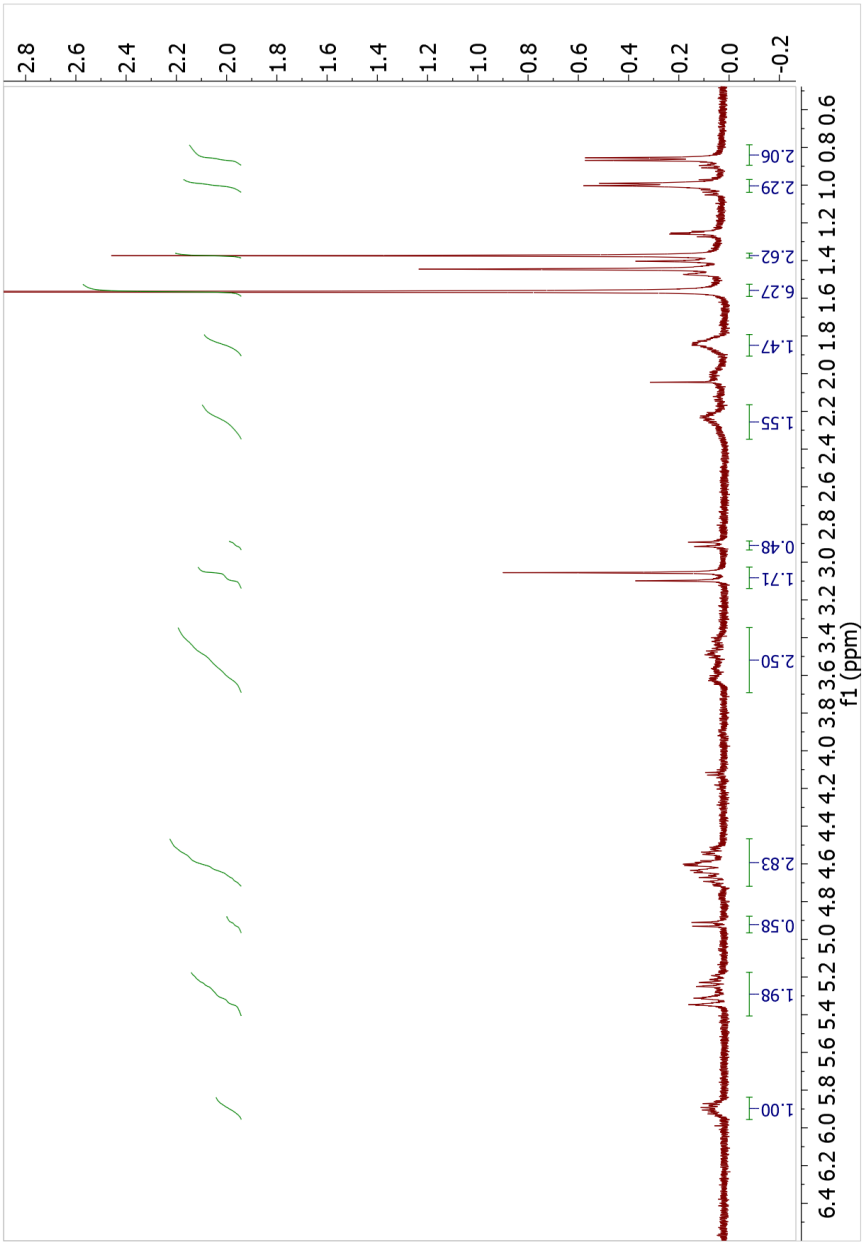


^{1}H NMR of Compound 1.31a

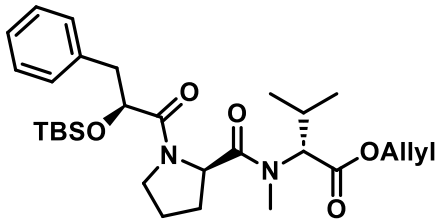


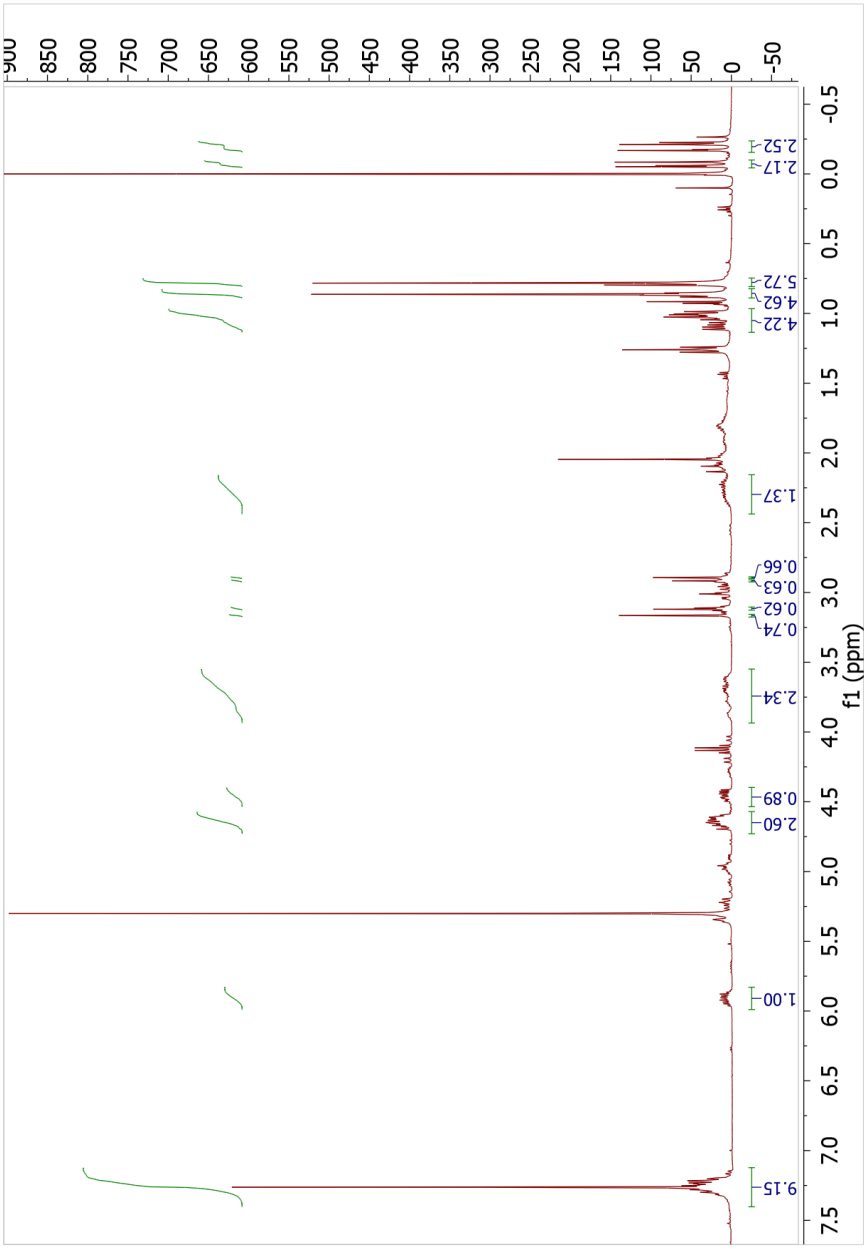
^{1}H NMR of Compound 1.32

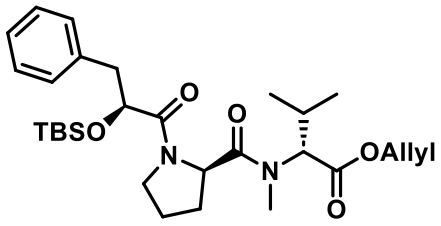


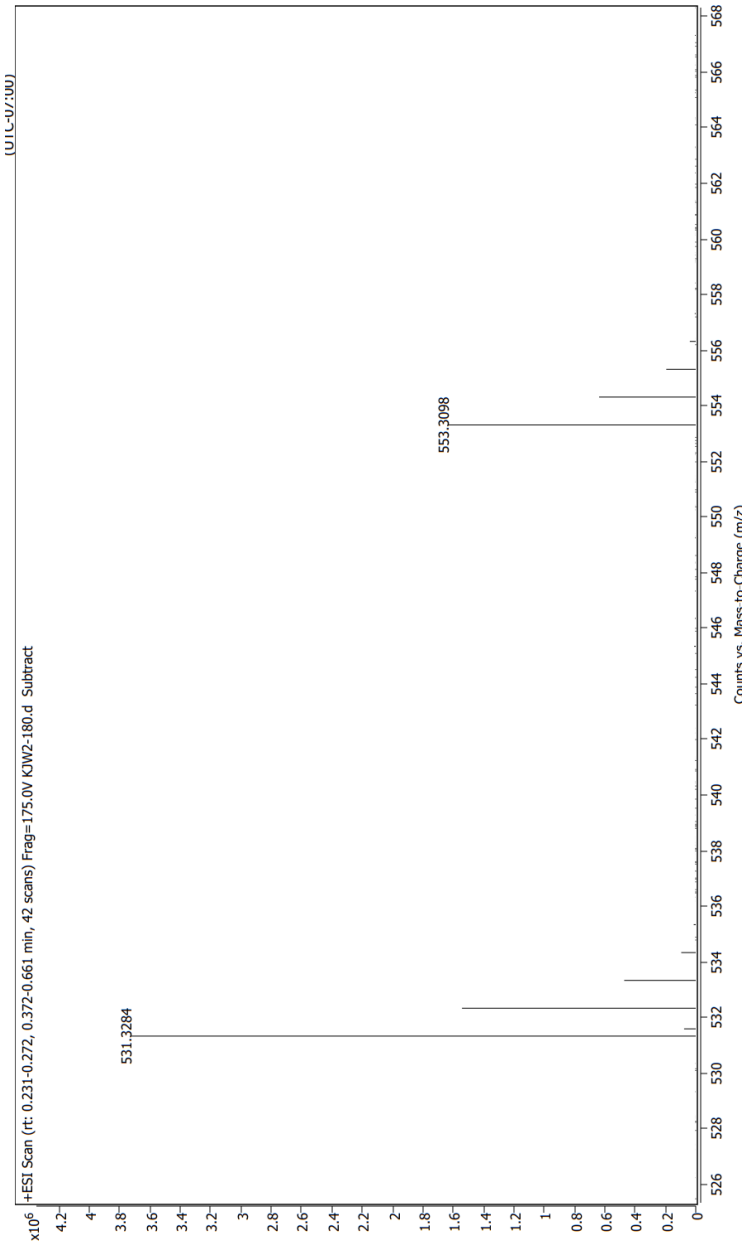


^{1}H NMR and HRMS of Compound 1.33

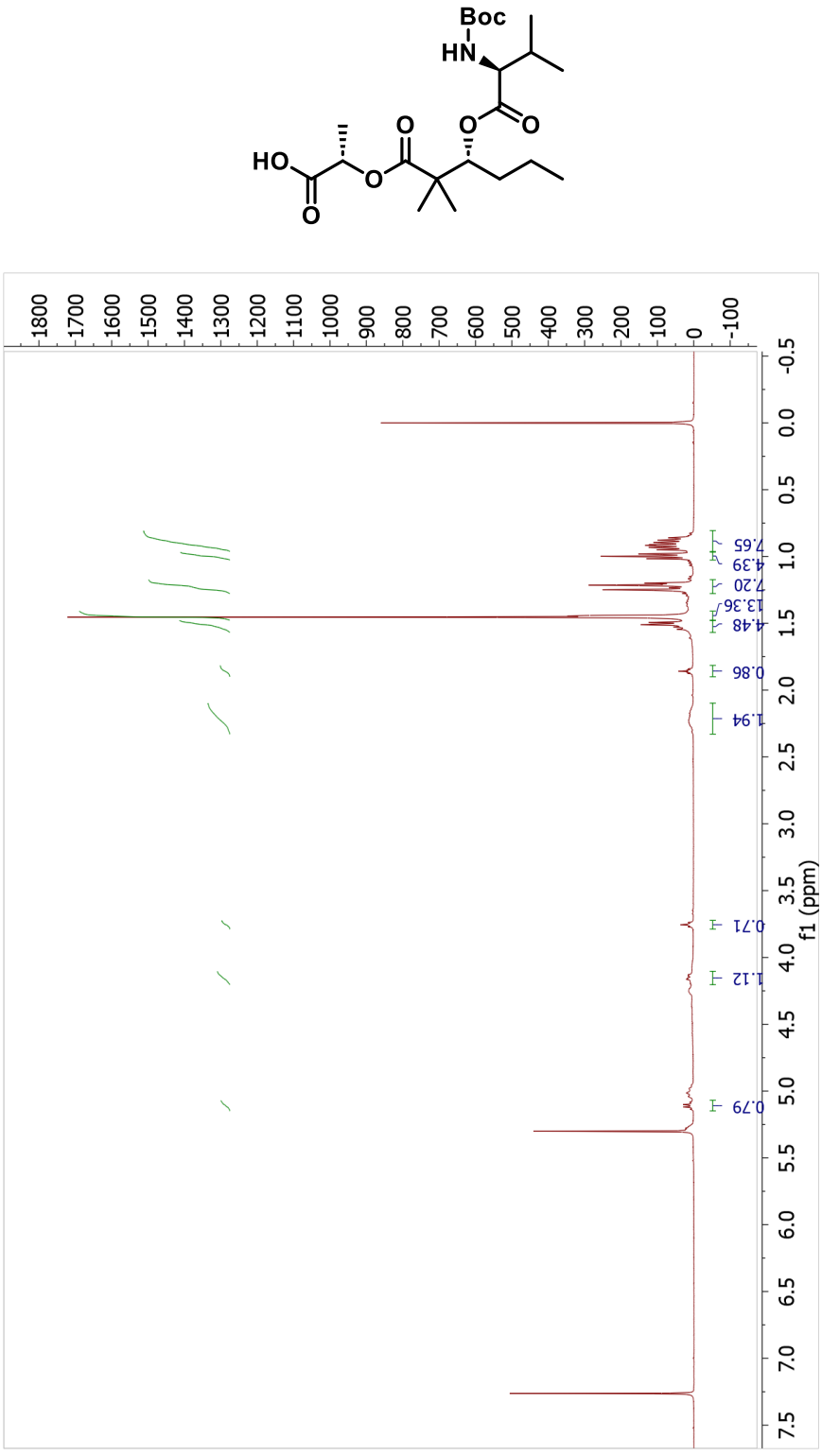




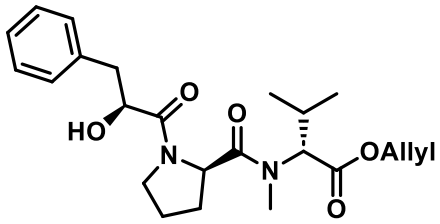

TBSO
O
N
O
N
OAllyl
O

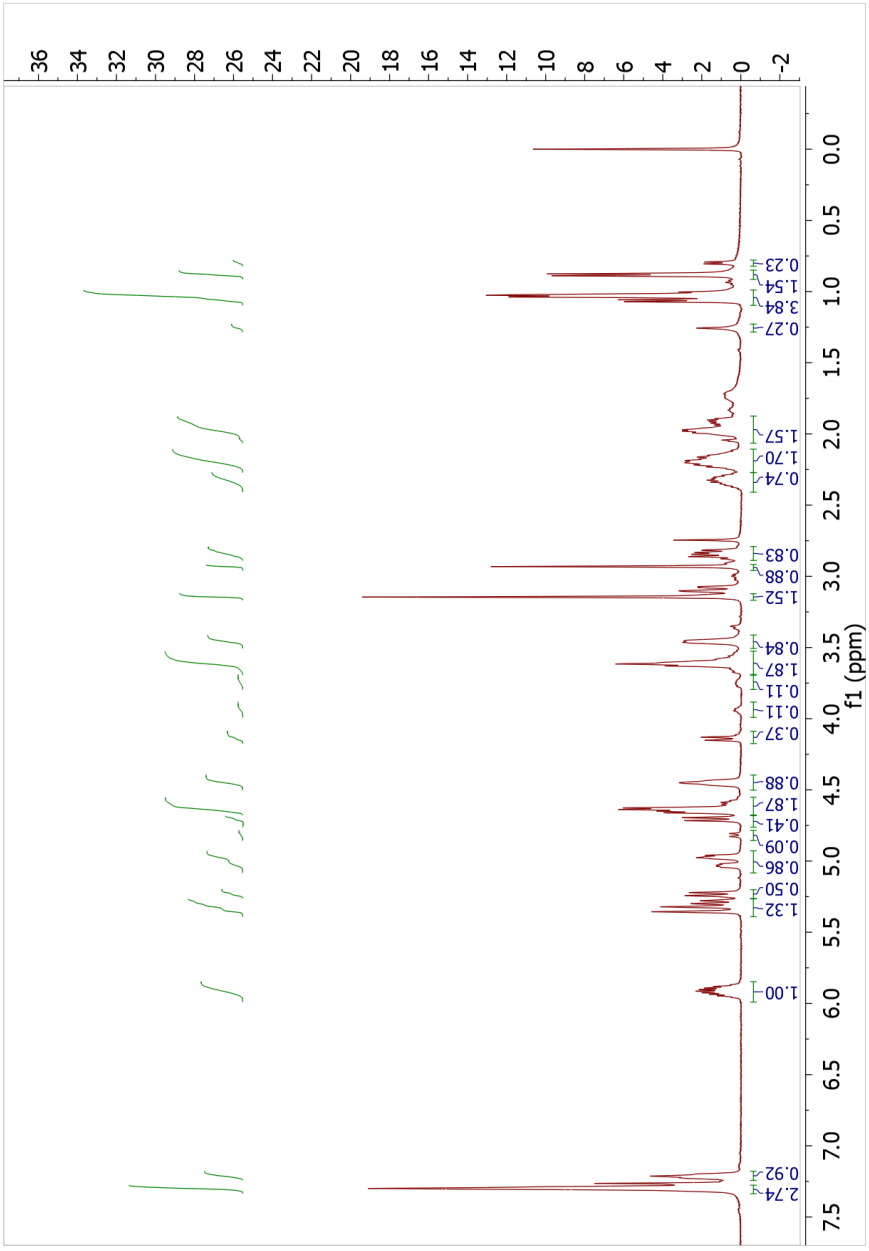

+ESI Scan (rt: 0.231-0.272, 0.372-0.661 min, 42 scans) Frag=175.0V KIW2-180.d Subtract
x10^6
531.3284
553.3098
Counts vs. Mass-to-Charge (m/z)

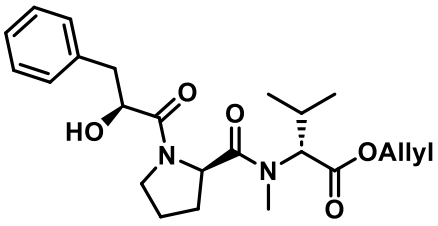
^{1}H NMR of Compound 1.34

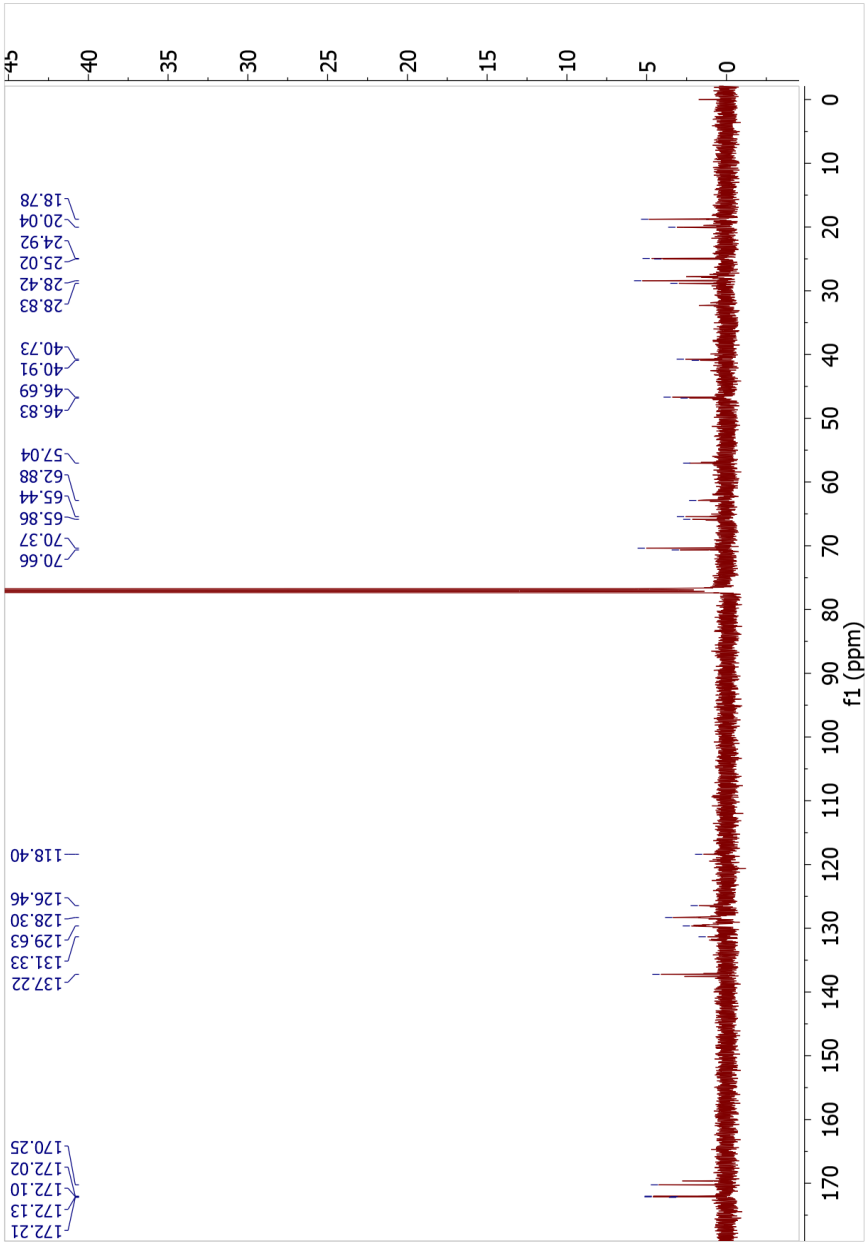


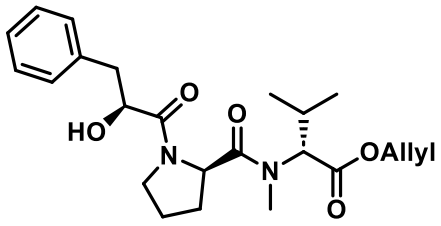
^{1}H and ^{13}C NMR and HRMS of Compound 1.35

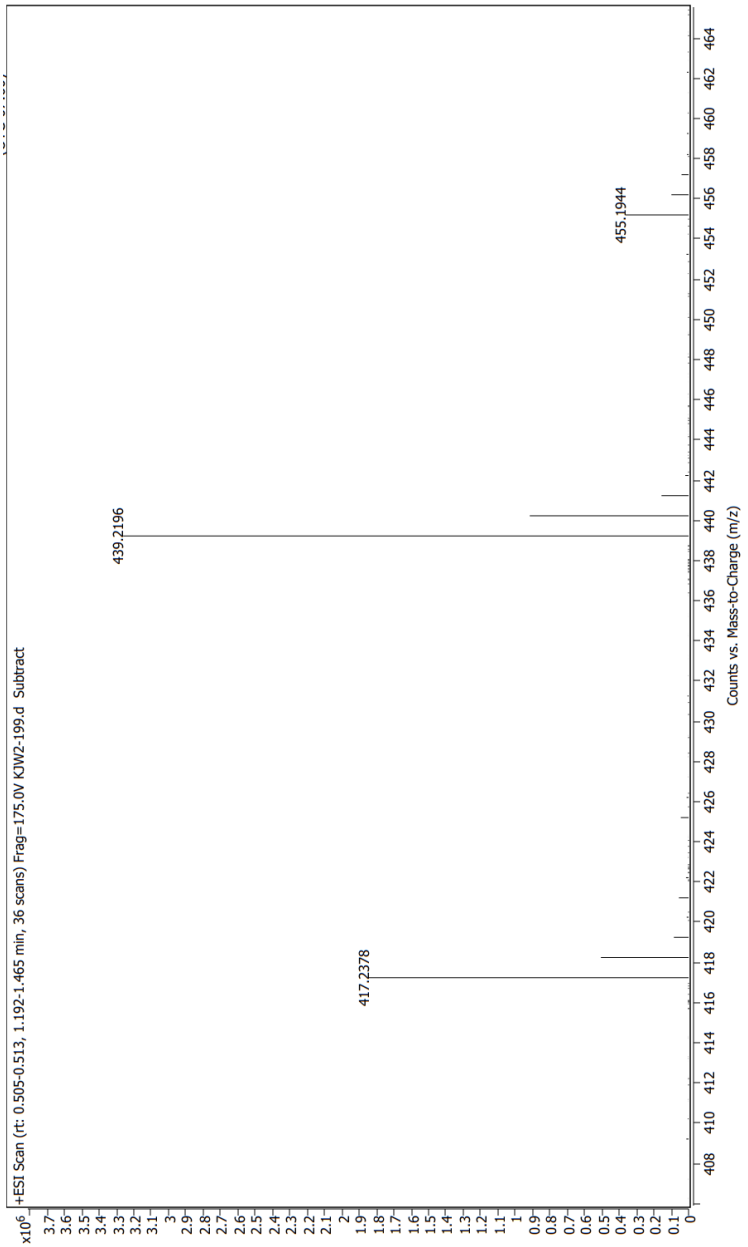




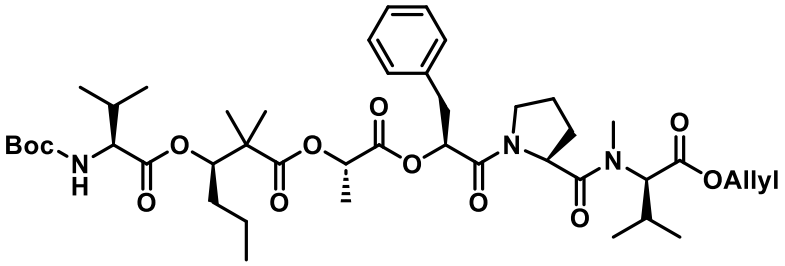

HO
O
N
O
N
OAllyl
O

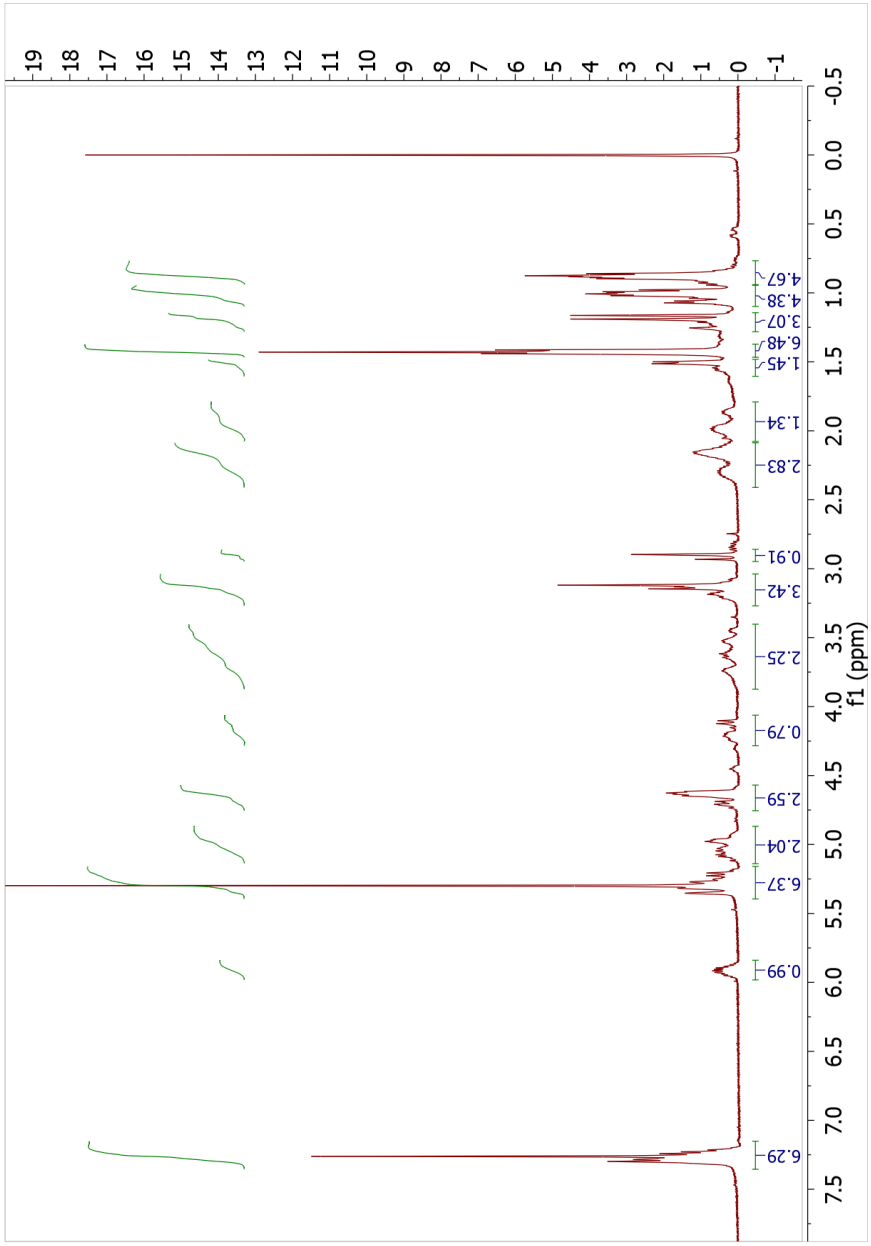

172.21
172.13
172.10
172.02
170.25
137.22
131.33
129.63
128.30
126.46
118.40
70.66
70.37
65.86
65.44
62.88
57.04
46.83
46.69
40.91
40.73
28.83
28.42
25.02
24.92
20.04
18.78
f1 (ppm)

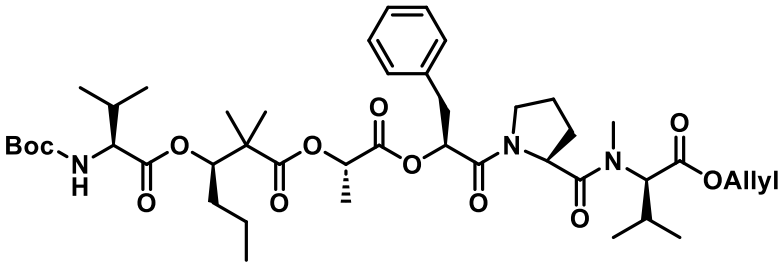

HO
O
N
O
N
OAllyl
O

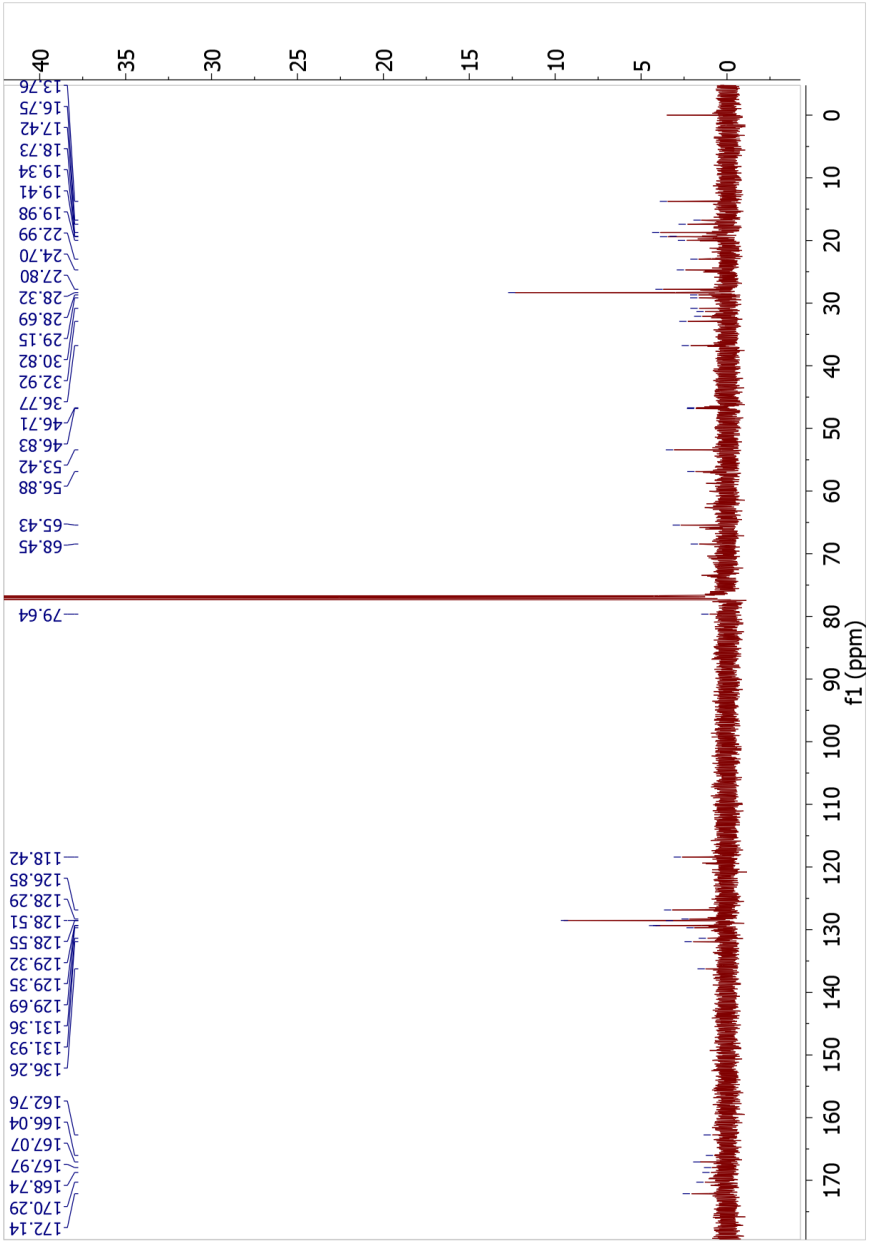

+ESI Scan (rt: 0.505-0.513, 1.192-1.465 min, 36 scans) Frag=175.0V KJW2-199.d Subtract
x10^6
439.2196
417.2378
455.1944
Counts vs. Mass-to-Charge (m/z)

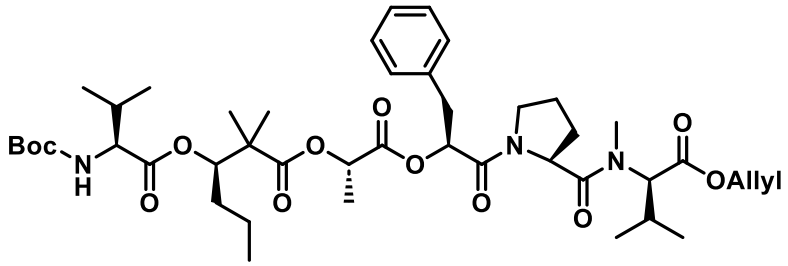
^{1}H and ^{13}C NMR and HRMS of Compound 1.36

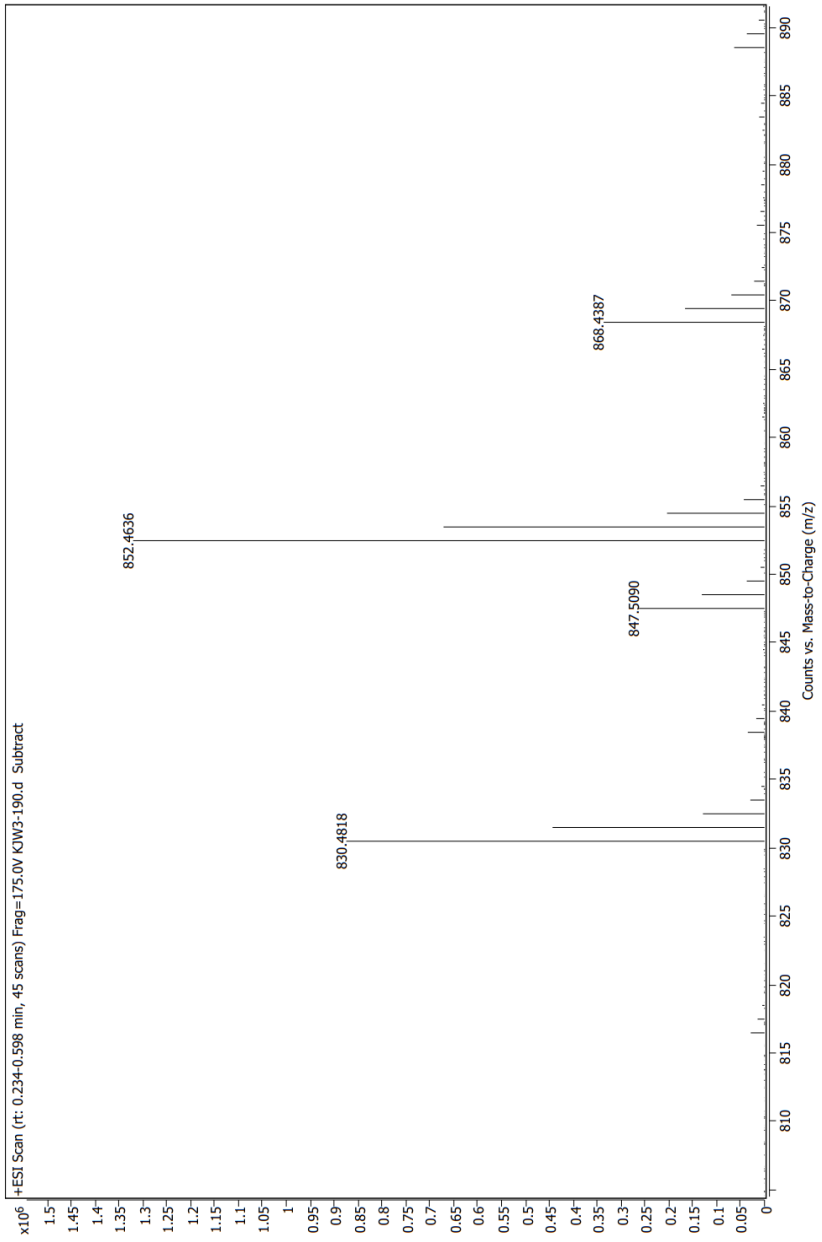




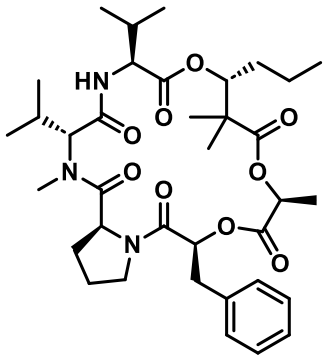

Boc
N
H
O
O
O
O
O
O
O
N
N
O
O
OAllyl

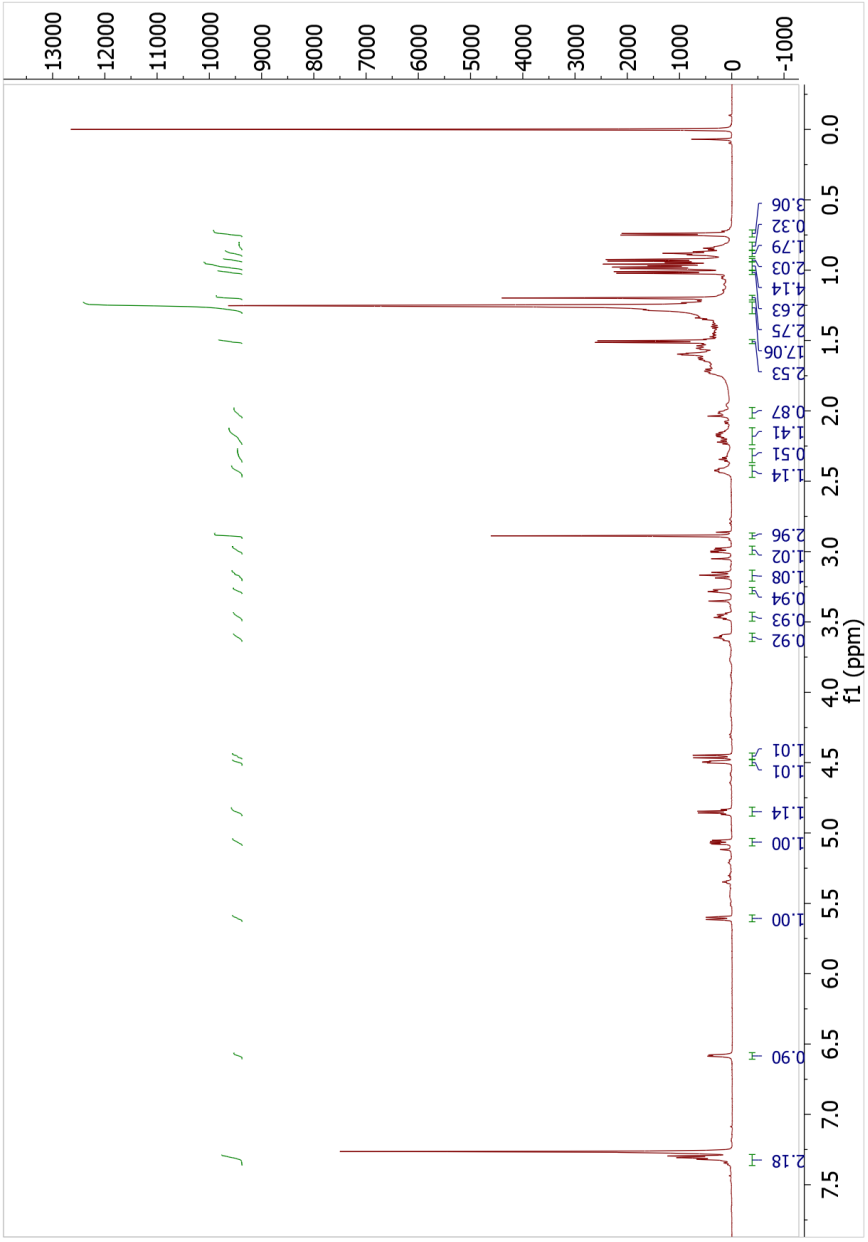

172.14
170.29
168.74
167.97
167.07
166.04
162.76
136.26
131.93
131.36
129.69
129.35
129.32
128.55
128.51
128.29
126.85
118.42
79.64
68.45
65.43
56.88
53.42
46.83
46.71
36.77
32.92
30.82
29.15
28.69
28.32
27.80
24.70
22.99
19.98
19.41
19.34
18.73
17.42
16.75
13.76
f1 (ppm)

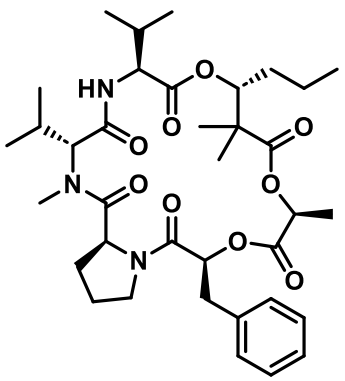

Boc
N
H
O
O
O
O
O
O
O
N
N
O
O
OAllyl

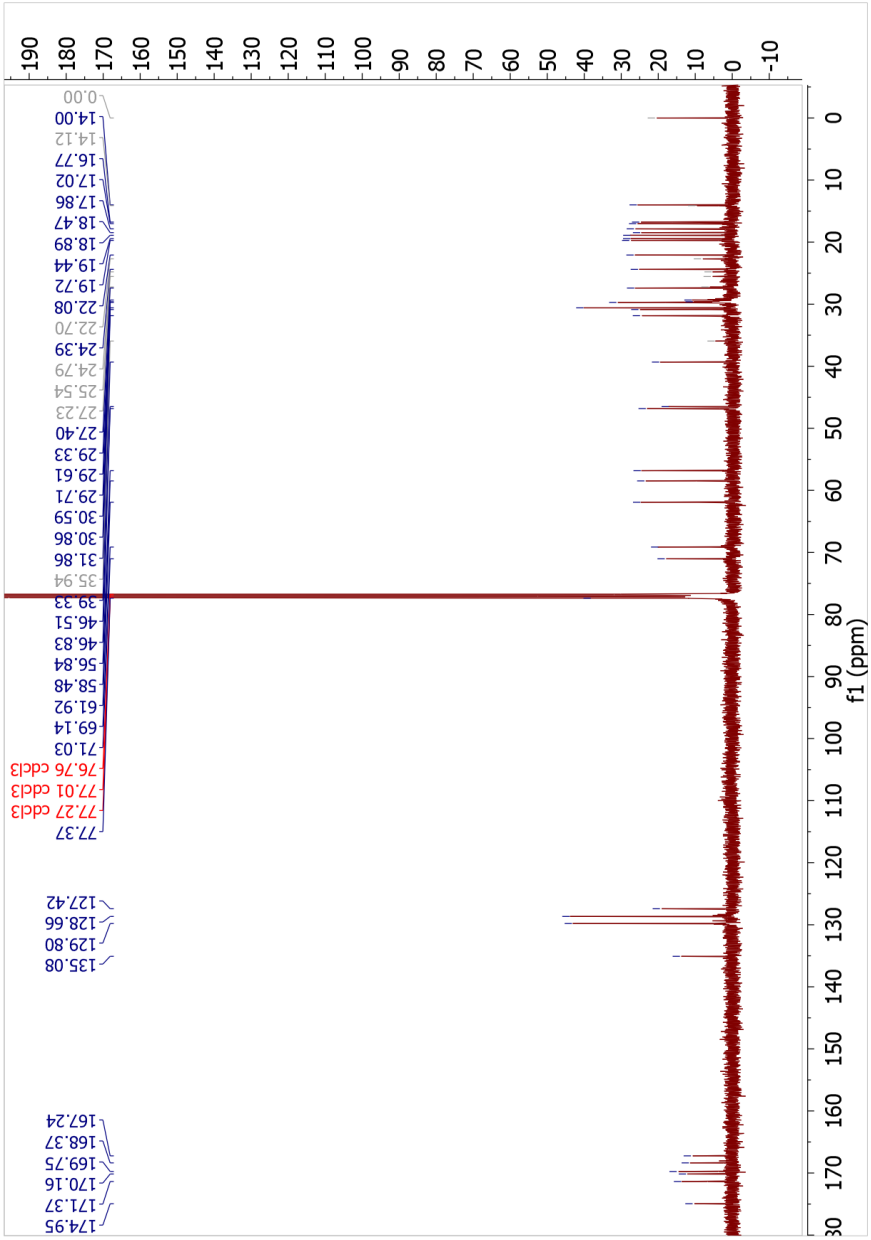

+ESI Scan (rt: 0.234-0.598 min, 45 scans) Frag=175.0V KJW3-190.d Subtract
x10^6
Counts vs. Mass-to-Charge (m/z)
868.4387
852.4636
847.5090
830.4818

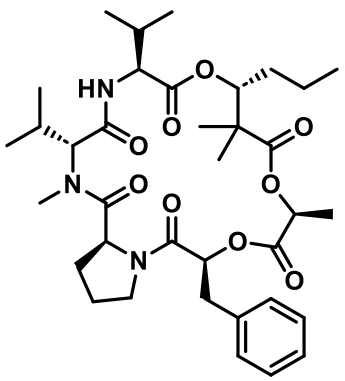
^{1}H, ^{13}C, COSY, HMBC, HSQC, and HRMS of Palmyramide A

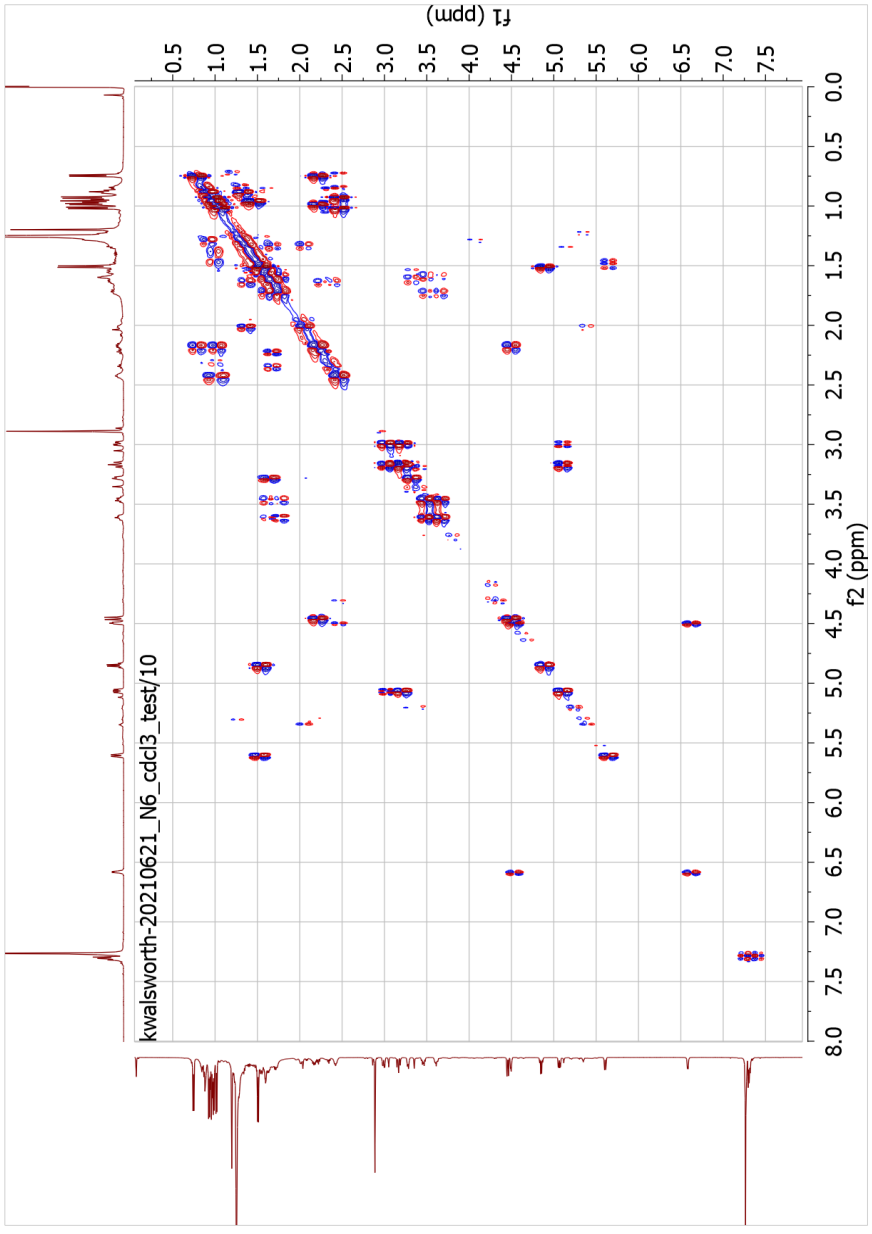


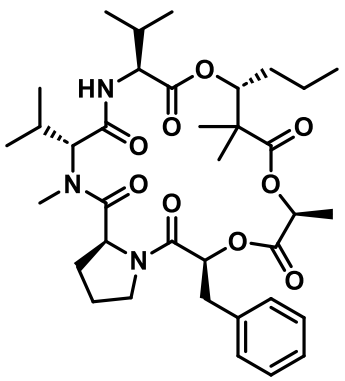


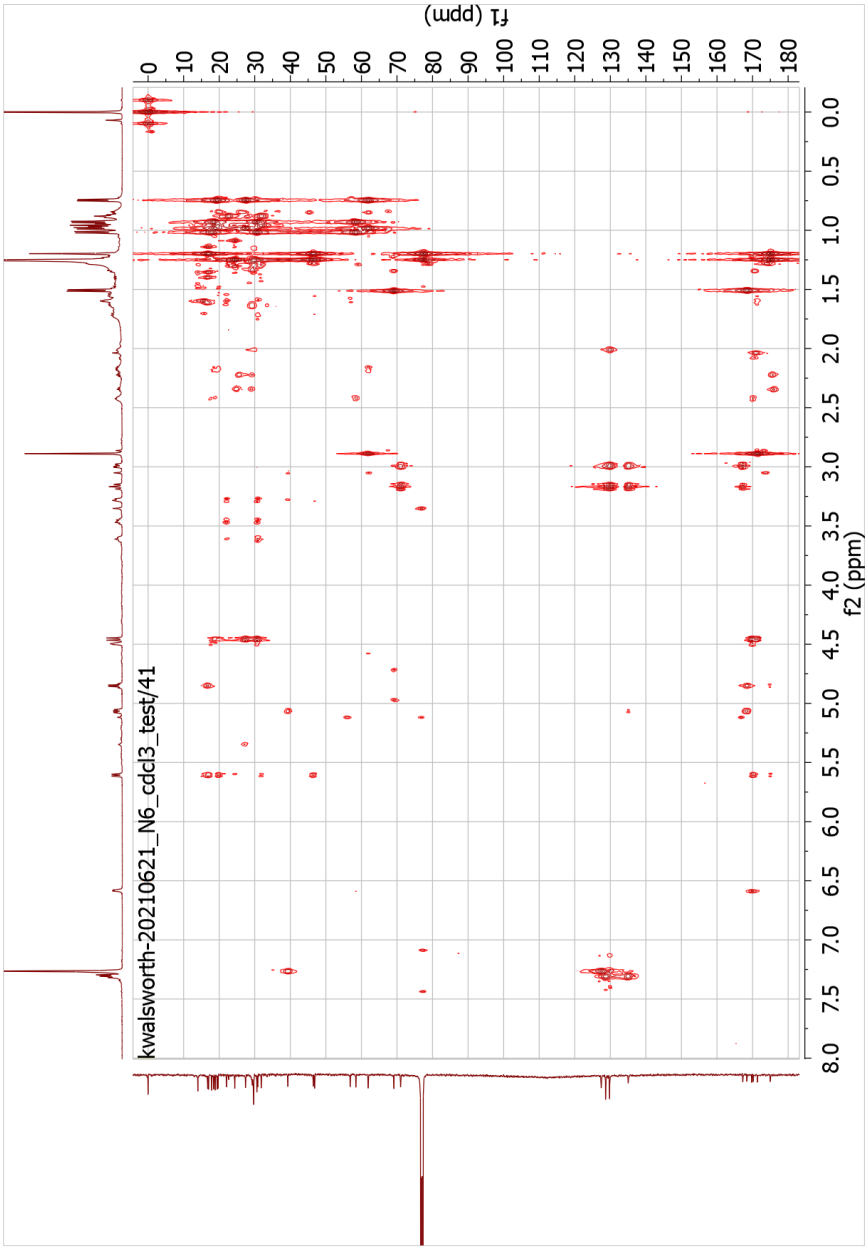

HN
O
O
O
O
O
O
O
O
O
O
N
N

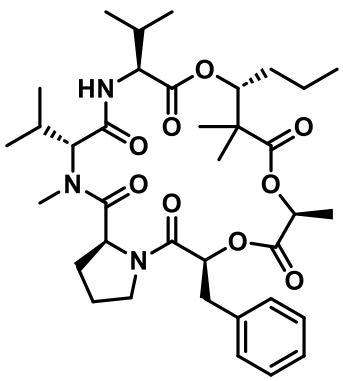

f1 (ppm)
174.95
171.37
170.16
169.75
168.37
167.24
135.08
129.80
128.66
127.42
77.37
77.27 cdcl3
77.01 cdcl3
76.76 cdcl3
71.03
69.14
61.92
58.48
56.84
46.83
46.51
39.33
35.94
31.86
30.86
30.59
29.71
29.61
29.33
27.40
27.23
25.54
24.79
24.39
22.70
22.08
19.72
19.44
18.89
18.47
17.86
17.02
16.77
14.12
14.00
0.00

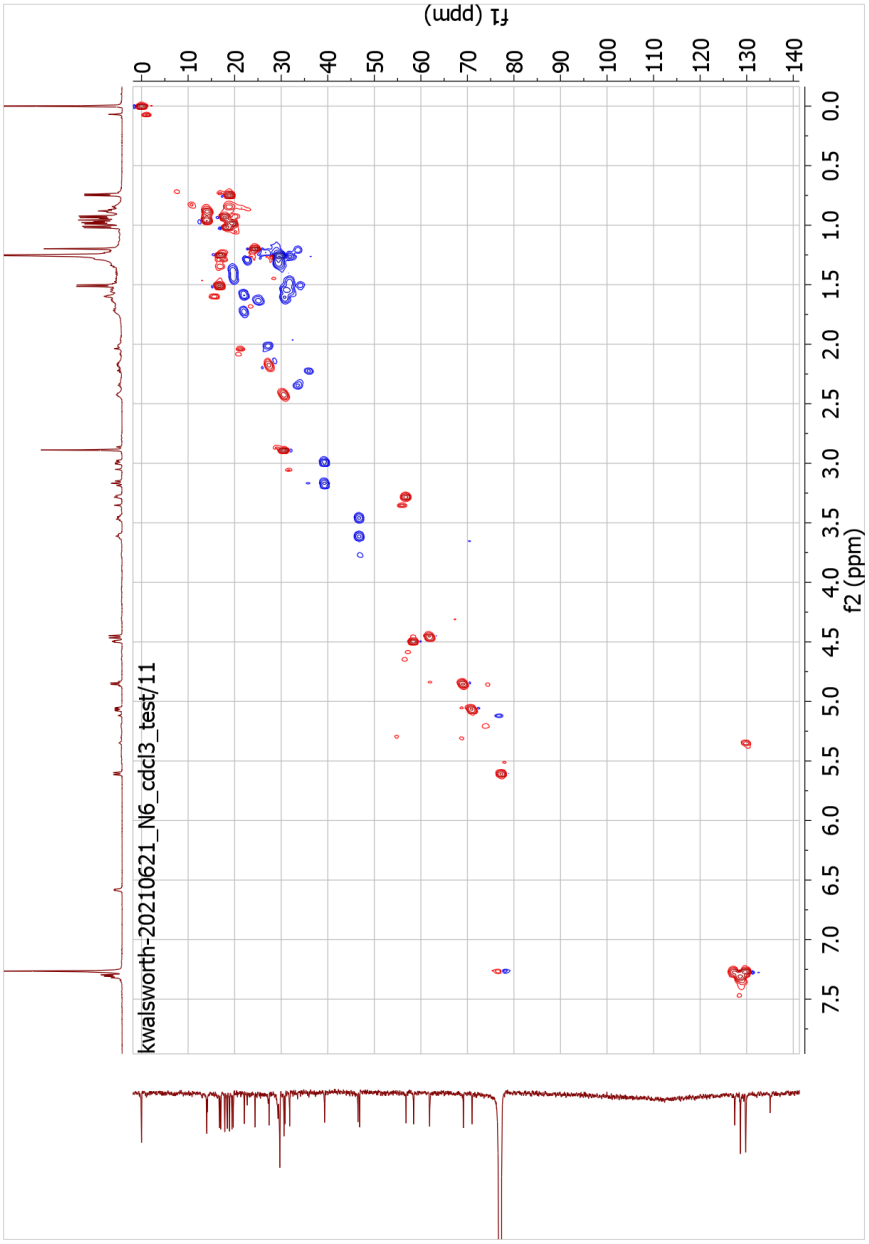

HN
O
O
O
O
O
O
O
O
O
O
N
N

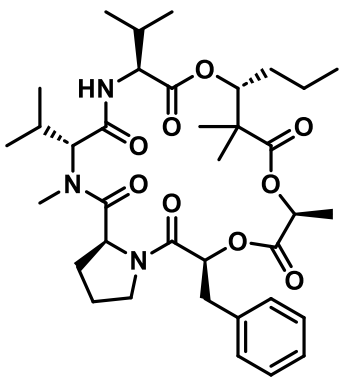

f1 (ppm)
f2 (ppm)
kwalsworth-20210621_N6_cdcl3_test/10

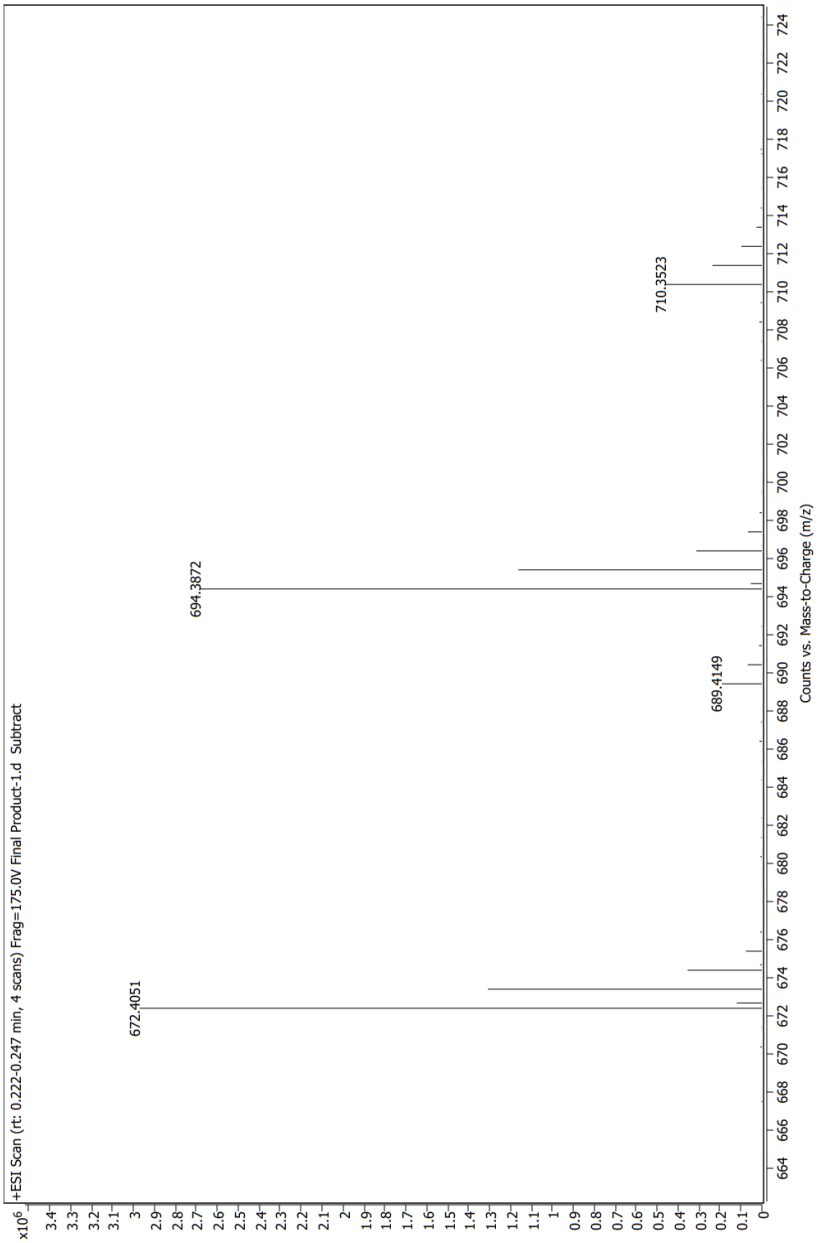

HN
O
O
O
O
O
O
O
O
O
O
N
N


f1 (ppm)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
f2 (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
6.0
6.5
7.0
7.5
8.0
kwalsworth-20210621_N6_cdcl3_test/41

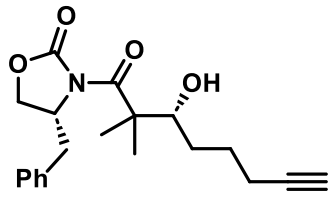

HN
O
O
O
O
O
O
O
O
O
O
N
N

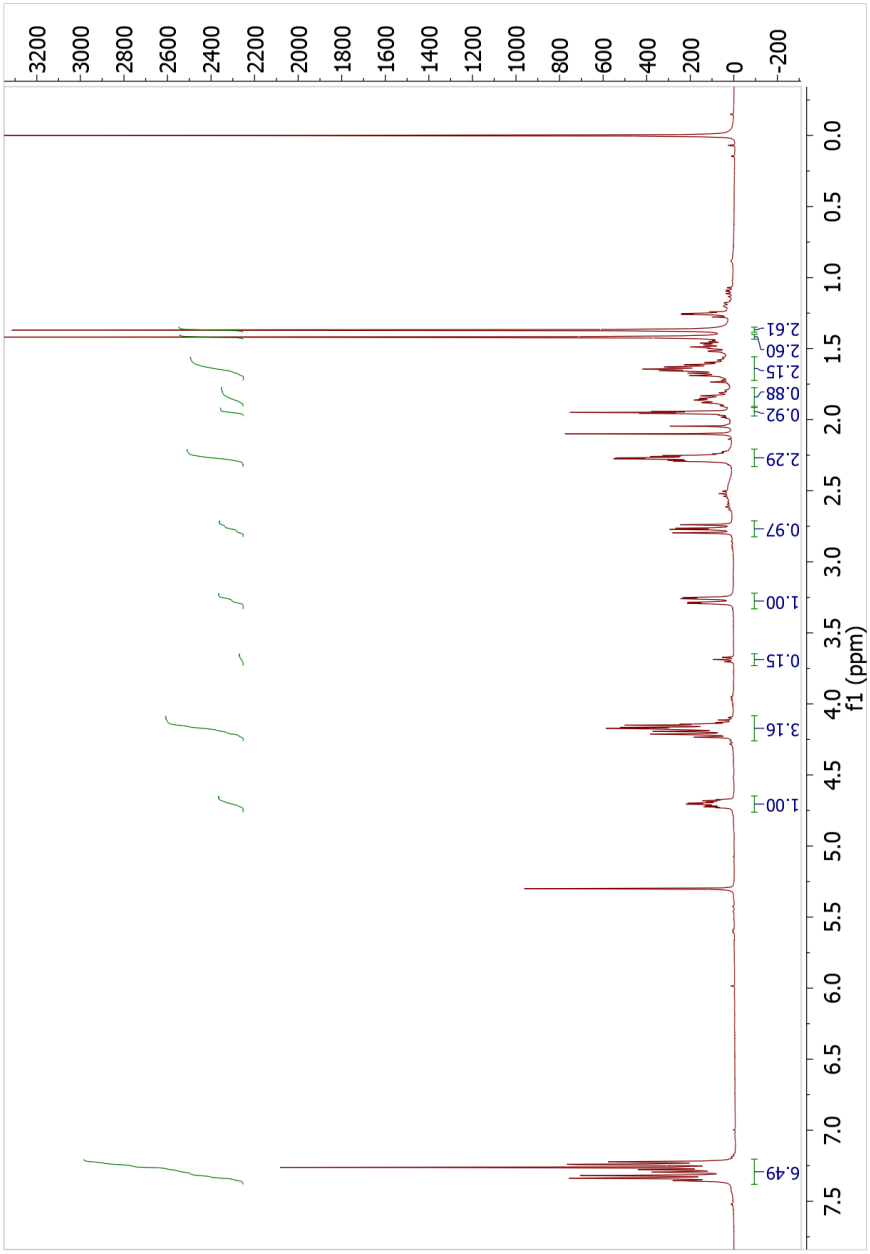

f1 (ppm)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
f2 (ppm)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
kwalsworth-20210621_N6_cdcl3_test/11

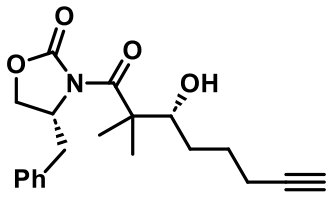

HN
O
O
O
O
O
O
N
O
O
N
O
O
O

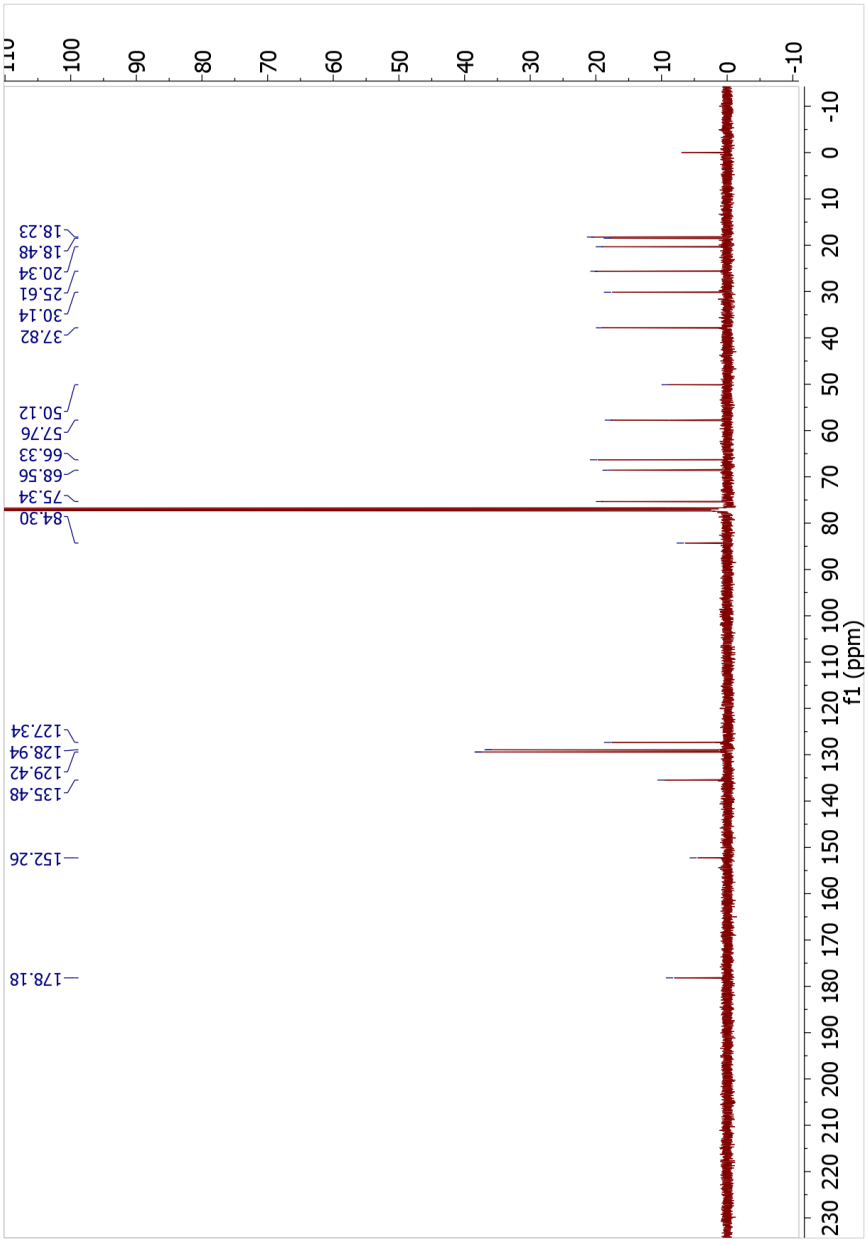

+ESI Scan (rt: 0.222-0.247 min, 4 scans) Frag=175.0V Final Product-1.d Subtract
x10^6
672.4051
689.4149
694.3872
710.3523
Counts vs. Mass-to-Charge (m/z)

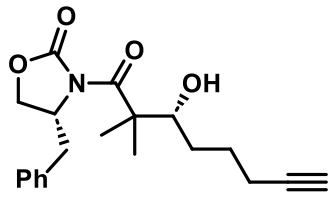
^{1}H and ^{13}C NMR and HRMS for Compound 1.40

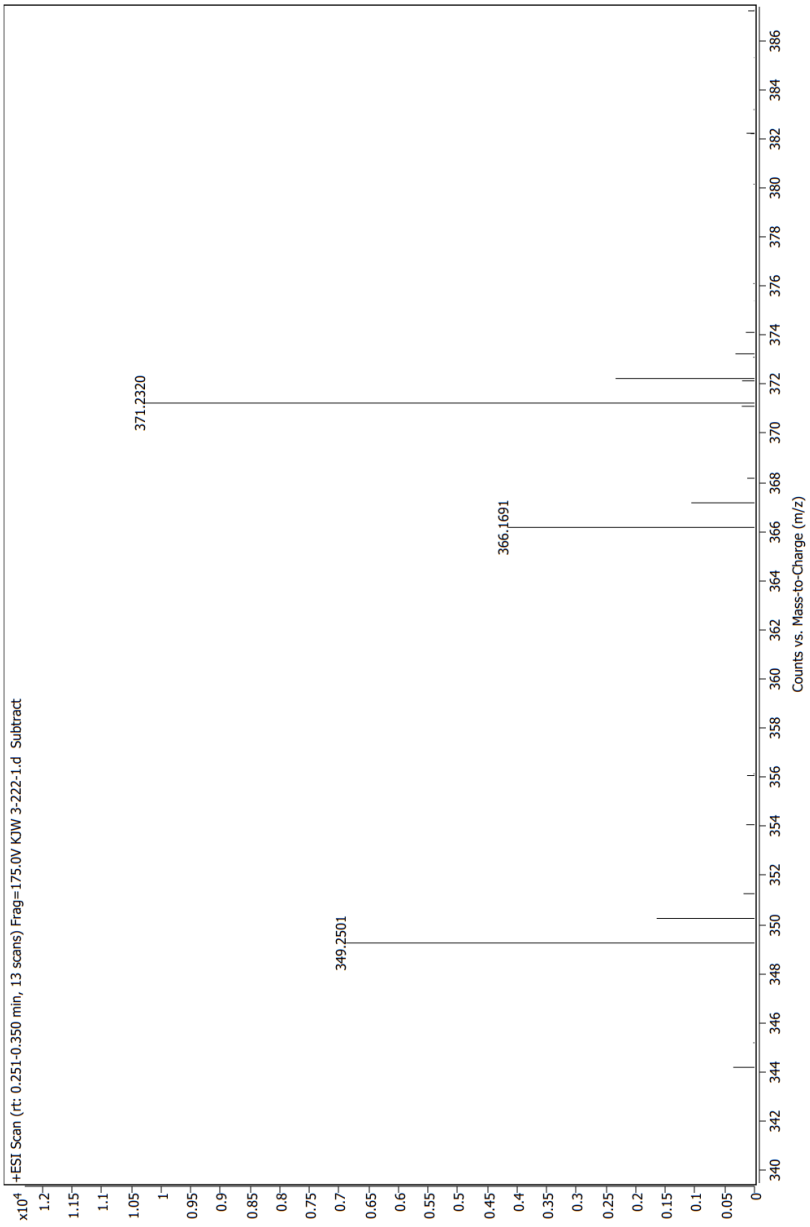




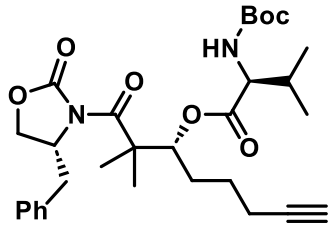

O
O
O
N
OH
Ph

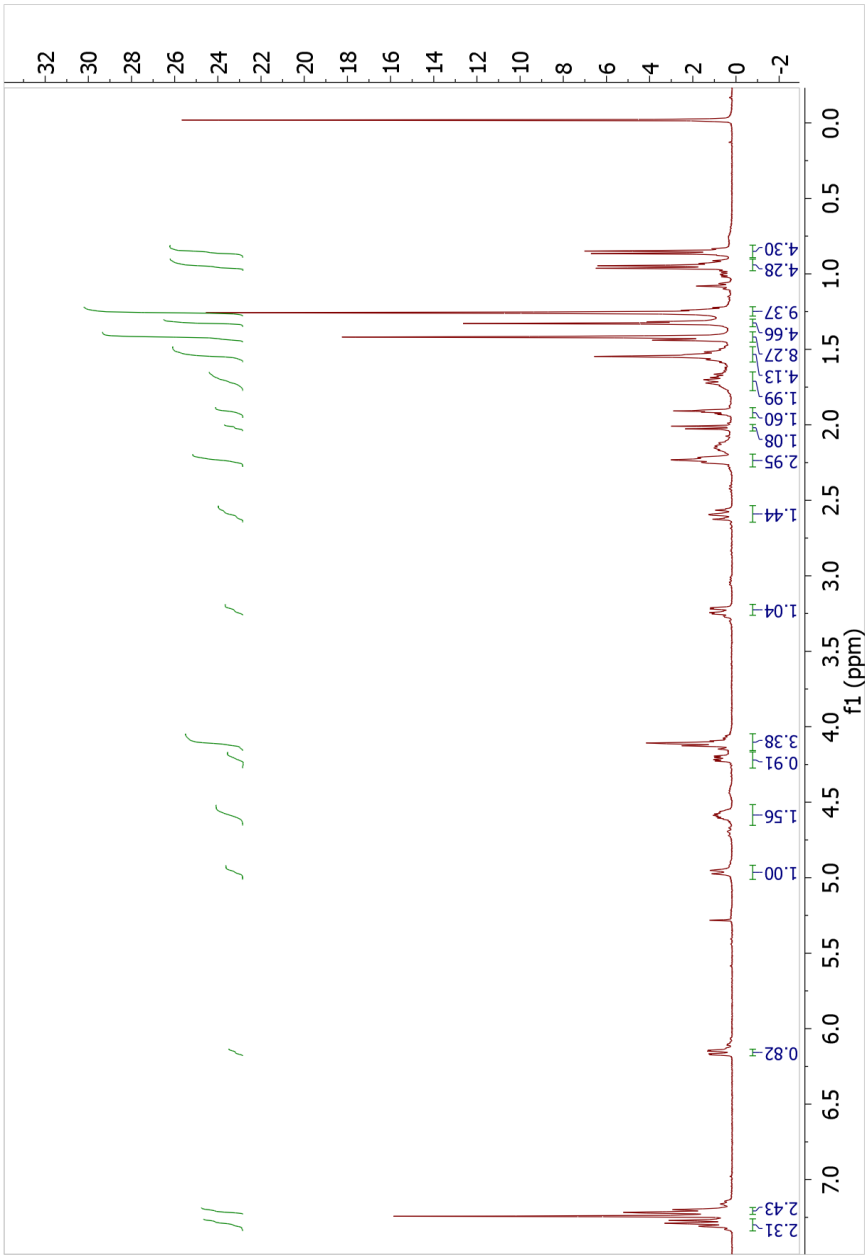

18.23
18.48
20.34
25.61
30.14
37.82
50.12
57.76
66.33
68.56
75.34
84.30
127.34
128.94
129.42
135.48
152.26
178.18
f1 (ppm)

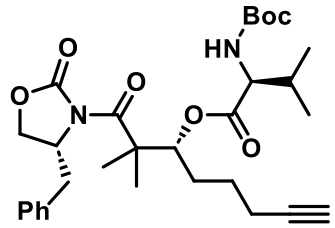

O
O
O
N
OH
Ph

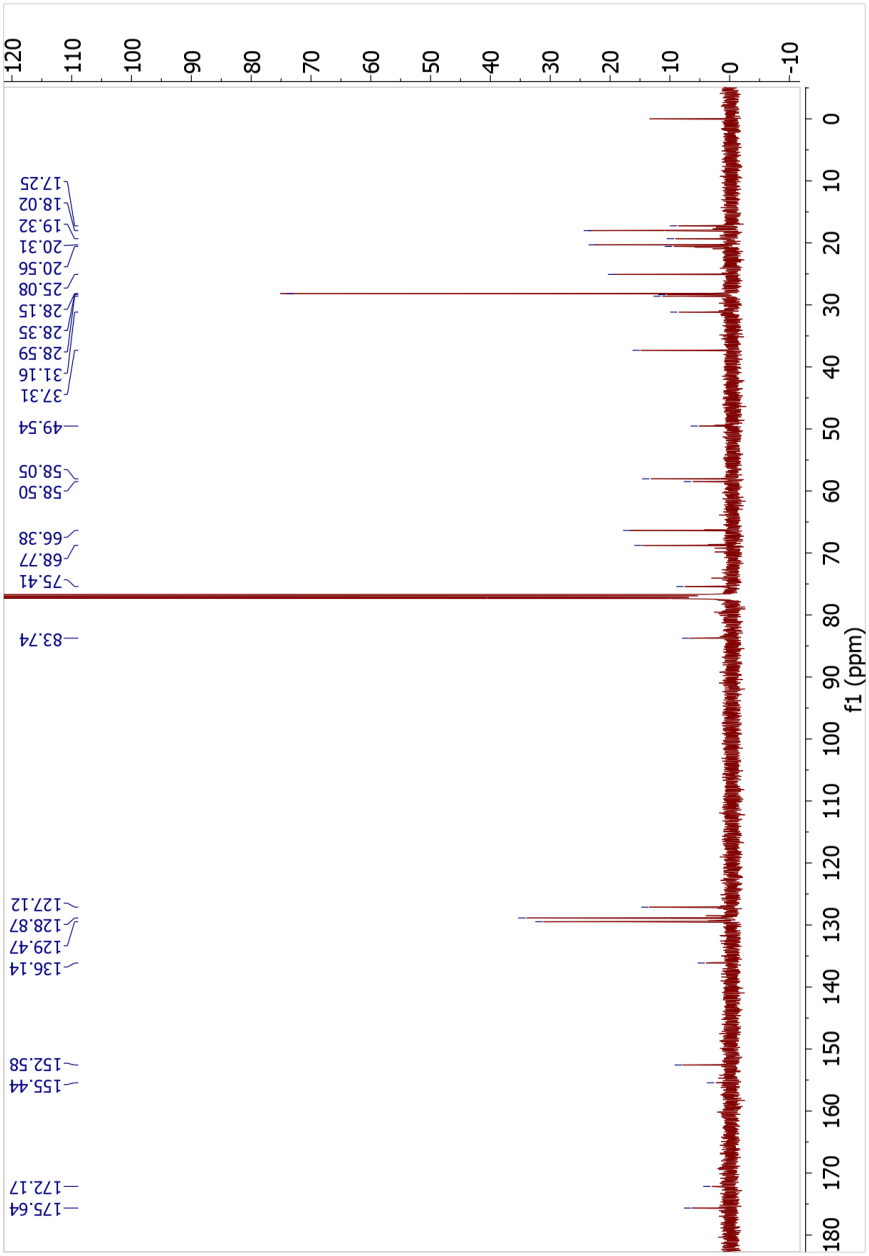

+ESI Scan (rt: 0.251-0.350 min, 13 scans) Frag=175.0V KJW 3-222-1.d Subtract
x10^4
1.2
1.15
1.1
1.05
1
0.95
0.9
0.85
0.8
0.75
0.7
0.65
0.6
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
340 342 344 346 348 350 352 354 356 358 360 362 364 366 368 370 372 374 376 378 380 382 384 386
Counts vs. Mass-to-Charge (m/z)
371.2320
366.1691
349.2501

^{1}H and ^{13}C NMR of Compound 1.41

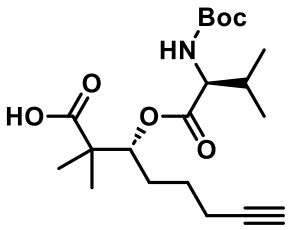


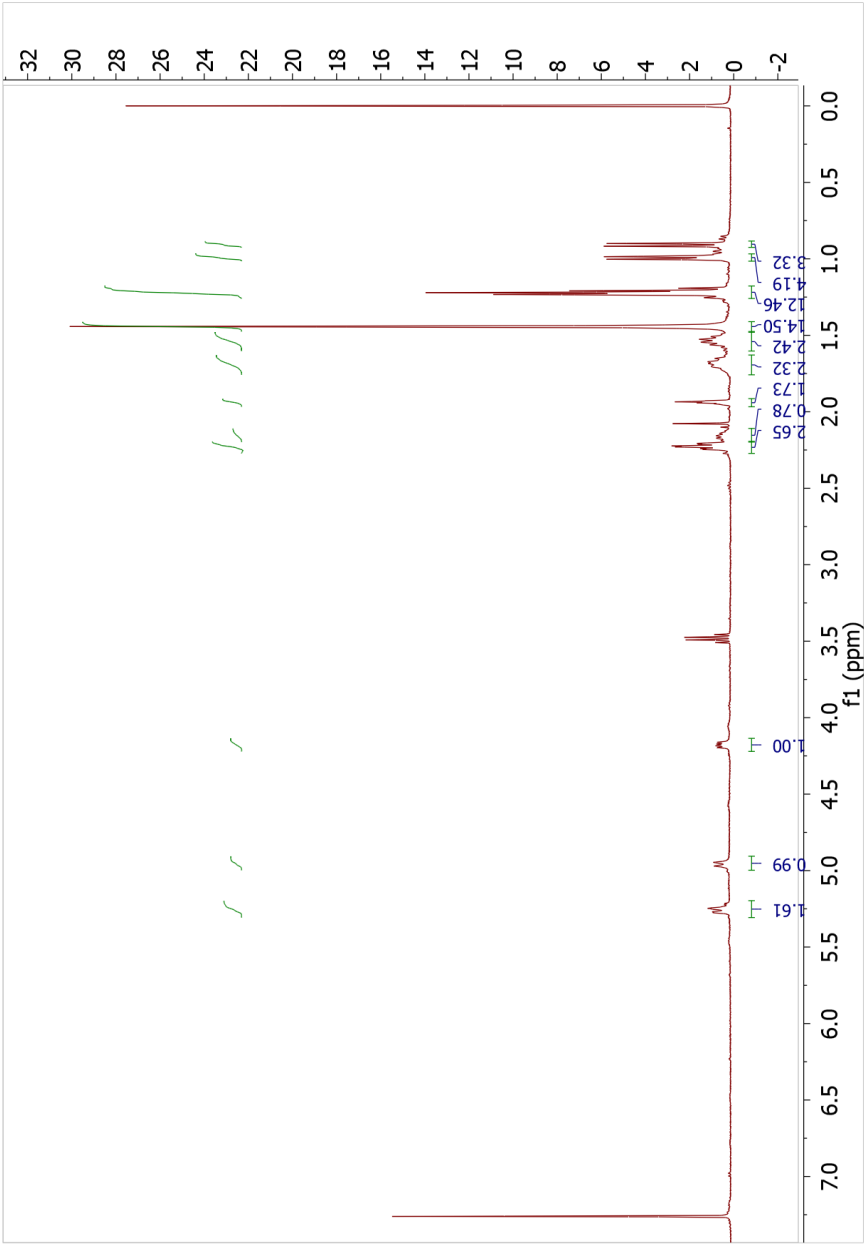


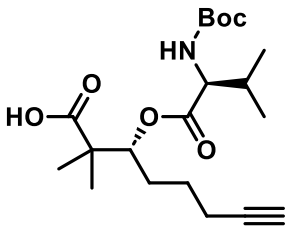

Boc
HN
O
O
O
N
O
O
O
Ph

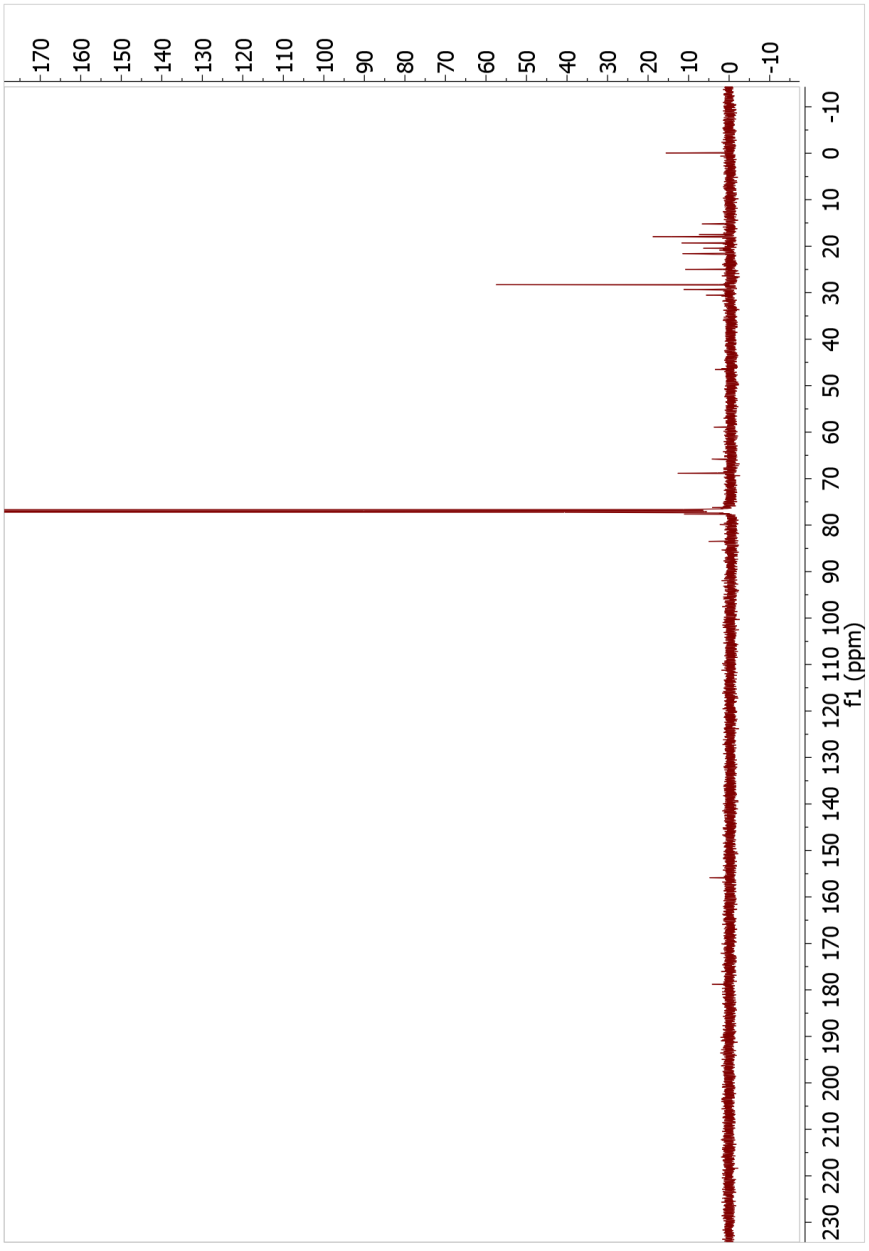

f1 (ppm)
175.64
172.17
155.44
152.58
136.14
129.47
128.87
127.12
83.74
75.41
68.77
66.38
58.50
58.05
49.54
37.31
31.16
28.59
28.35
28.15
25.08
20.56
20.31
19.32
18.02
17.25

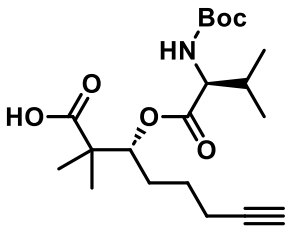
^{1}H and ^{13}C NMR and HRMS of Compound 1.42

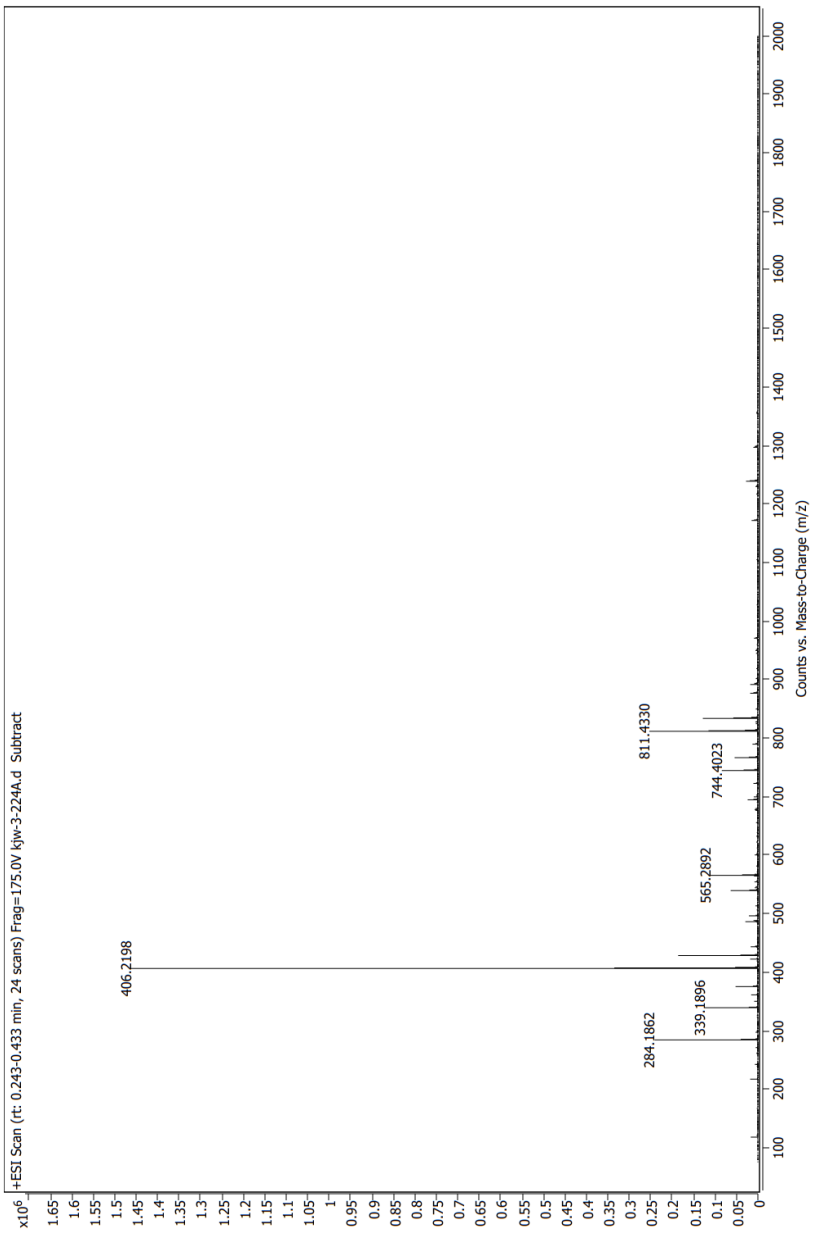




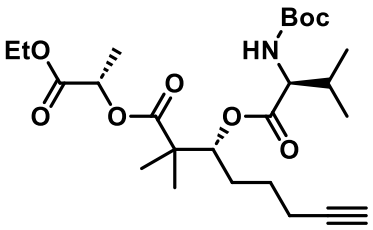

Boc
HN
HO
O
O
O

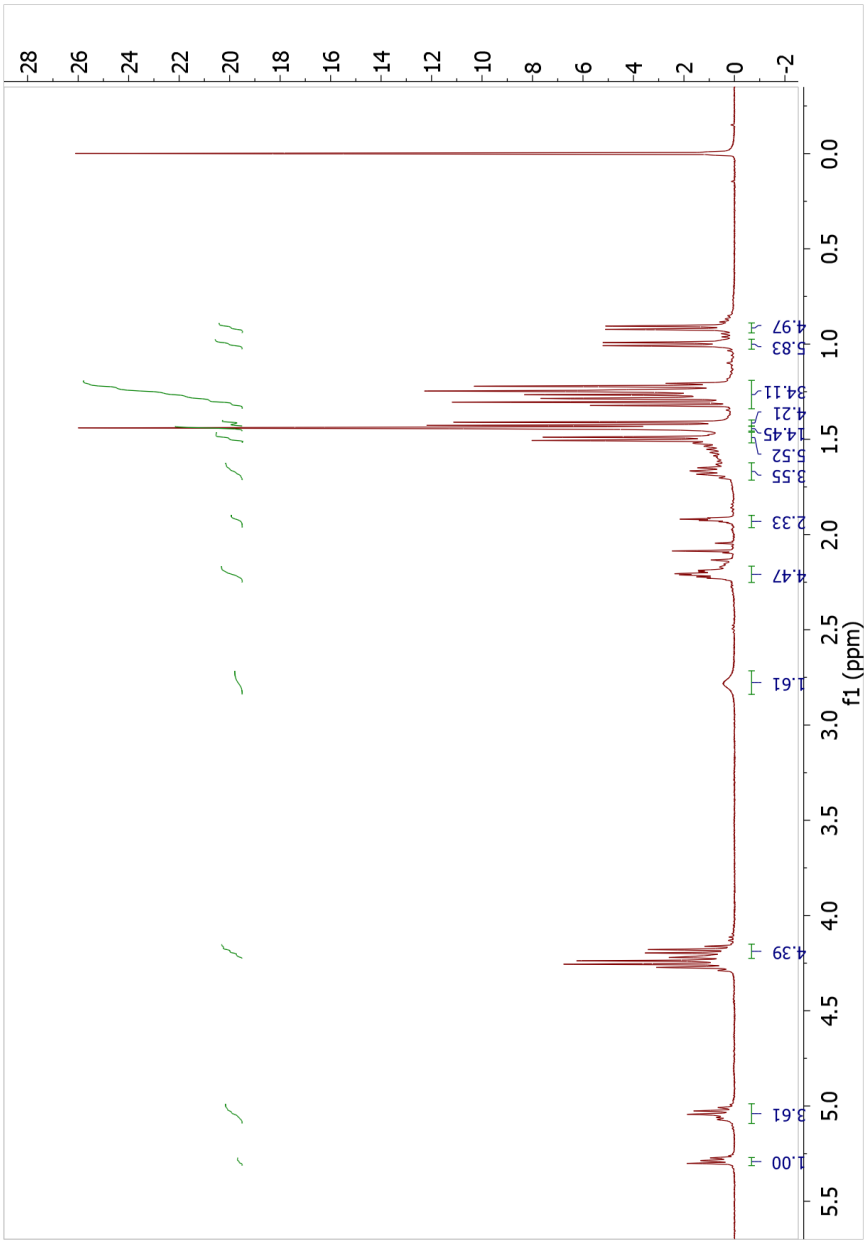

f1 (ppm)

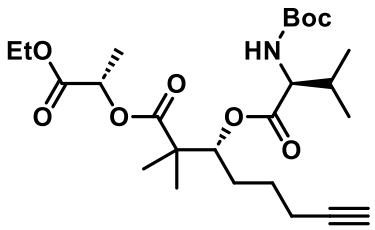

Boc
HN
HO
O
O
O

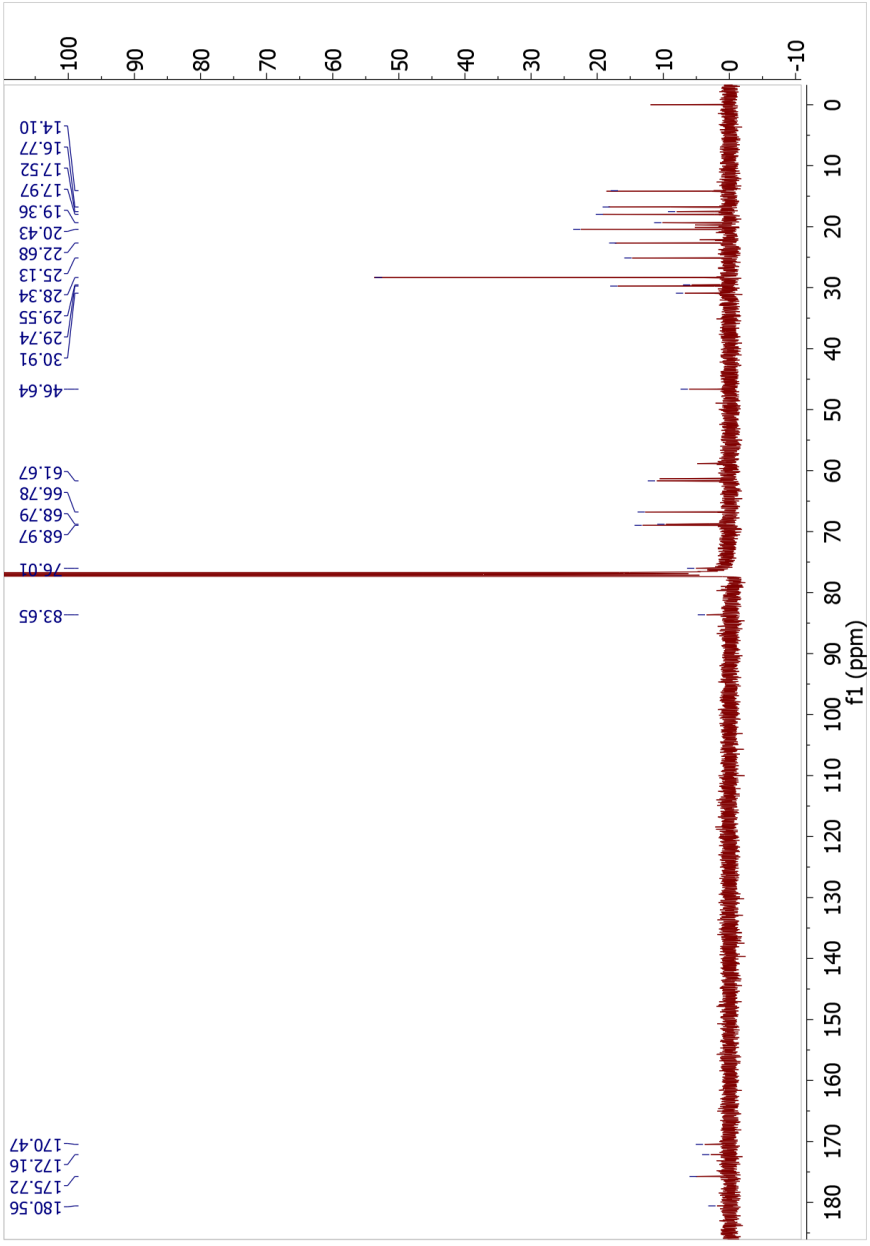

+ESI Scan (rt: 0.243-0.433 min, 24 scans) Frag=175.0V kjw-3-224A.d Subtract
x10^6
406.2198
811.4330
744.4023
565.2892
339.1896
284.1862
Counts vs. Mass-to-Charge (m/z)

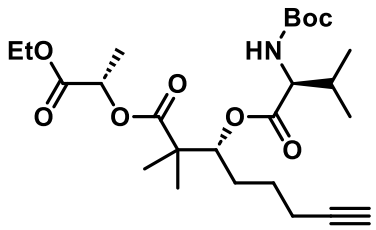
^{1}H and ^{13}C NMR and HRMS of Compound 1.43

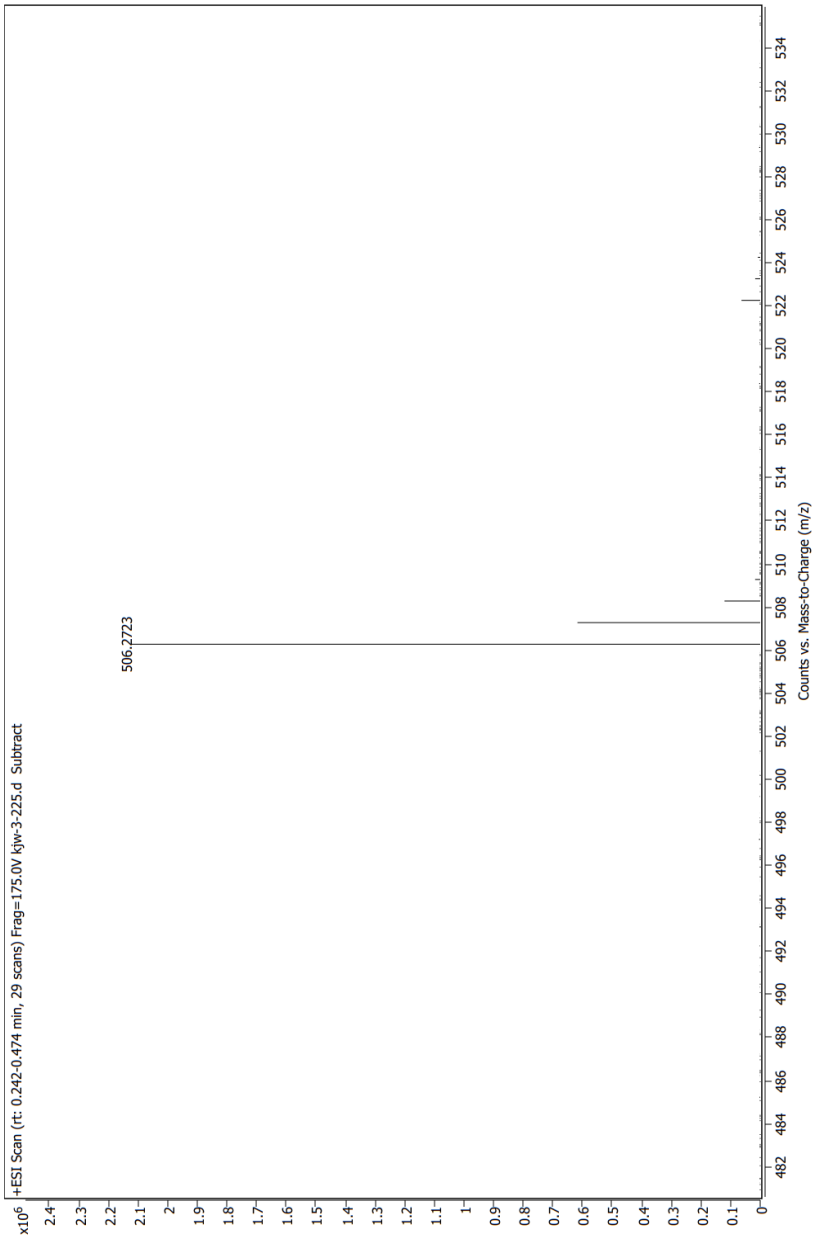




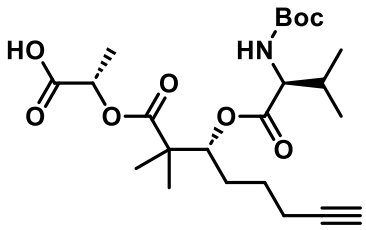

Boc
HN
EtO
O
O
O
O
O
O

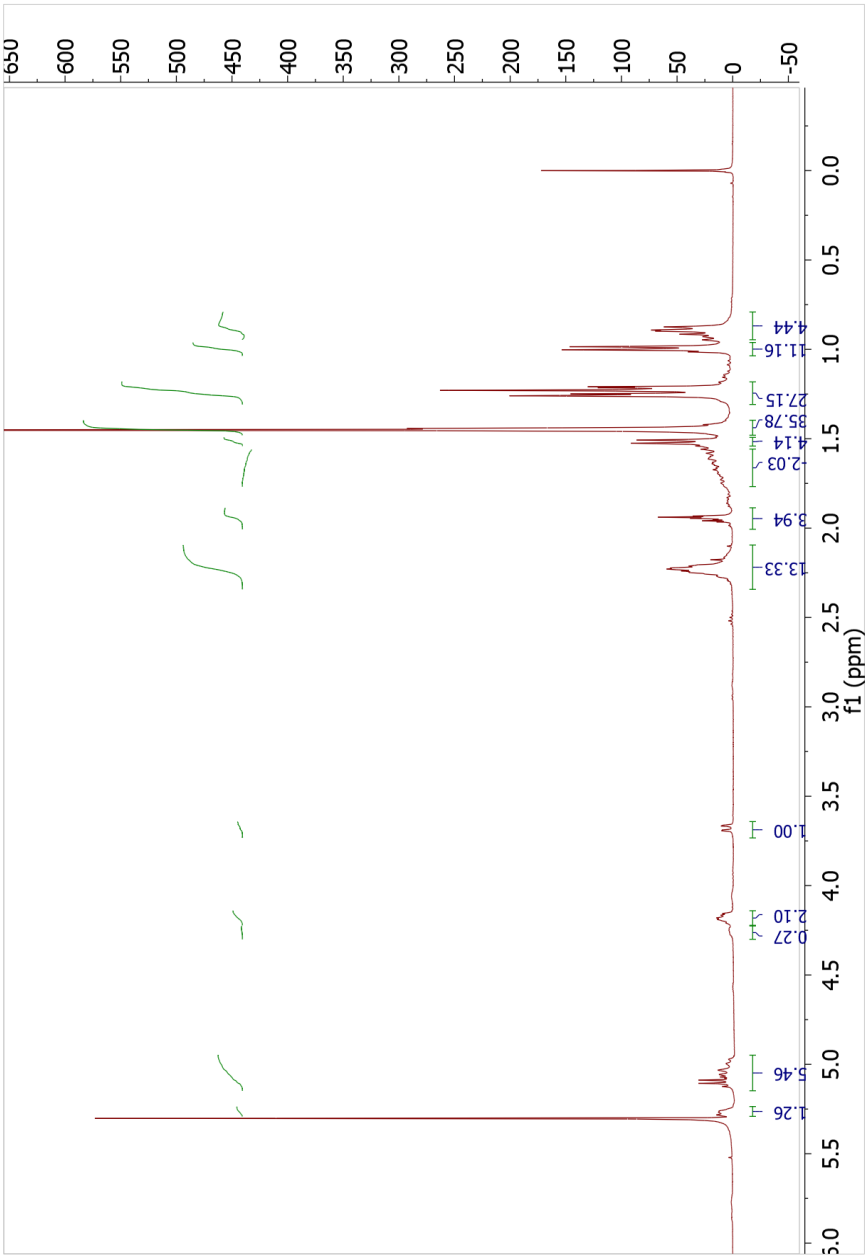

14.10
16.77
17.52
17.97
19.36
20.43
22.68
25.13
28.34
29.55
29.74
30.91
46.64
61.67
66.78
68.79
68.97
76.01
83.65
170.47
172.16
175.72
180.56
f1 (ppm)


Boc
HN
EtO
O
O
O
O
O
O

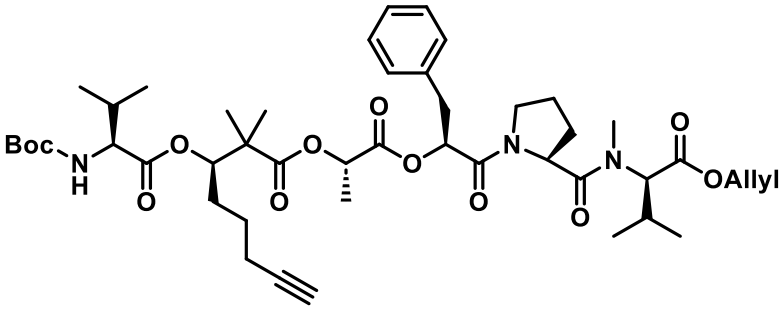

+ESI Scan (rt: 0.242-0.474 min, 29 scans) Frag=175.0V kjw-3-225.d Subtract
506.2723
Counts vs. Mass-to-Charge (m/z)
x10^6

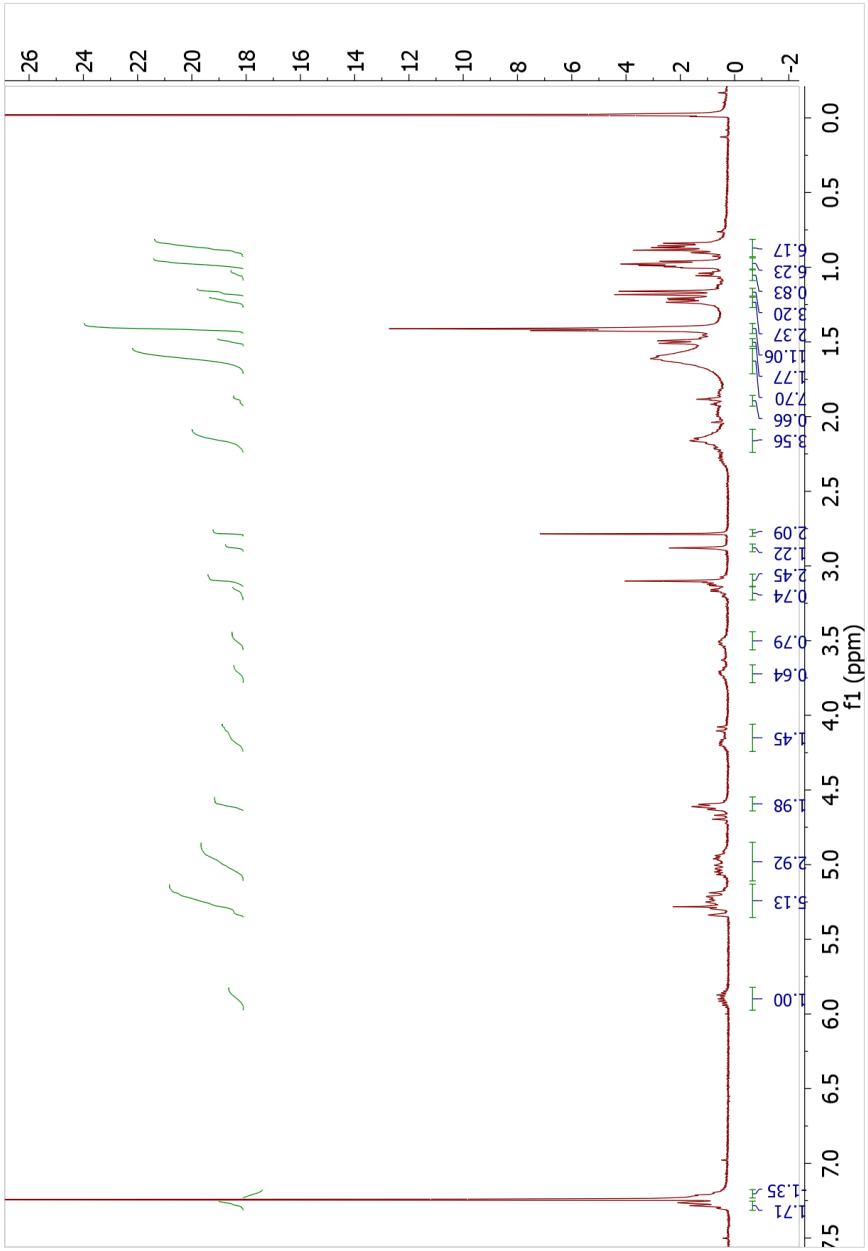
^{1}H of Compound 1.44

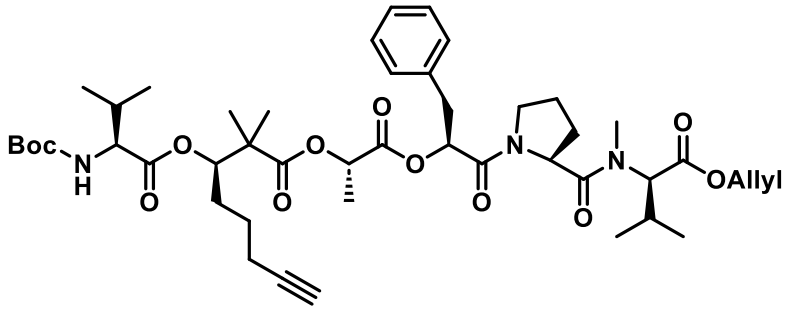


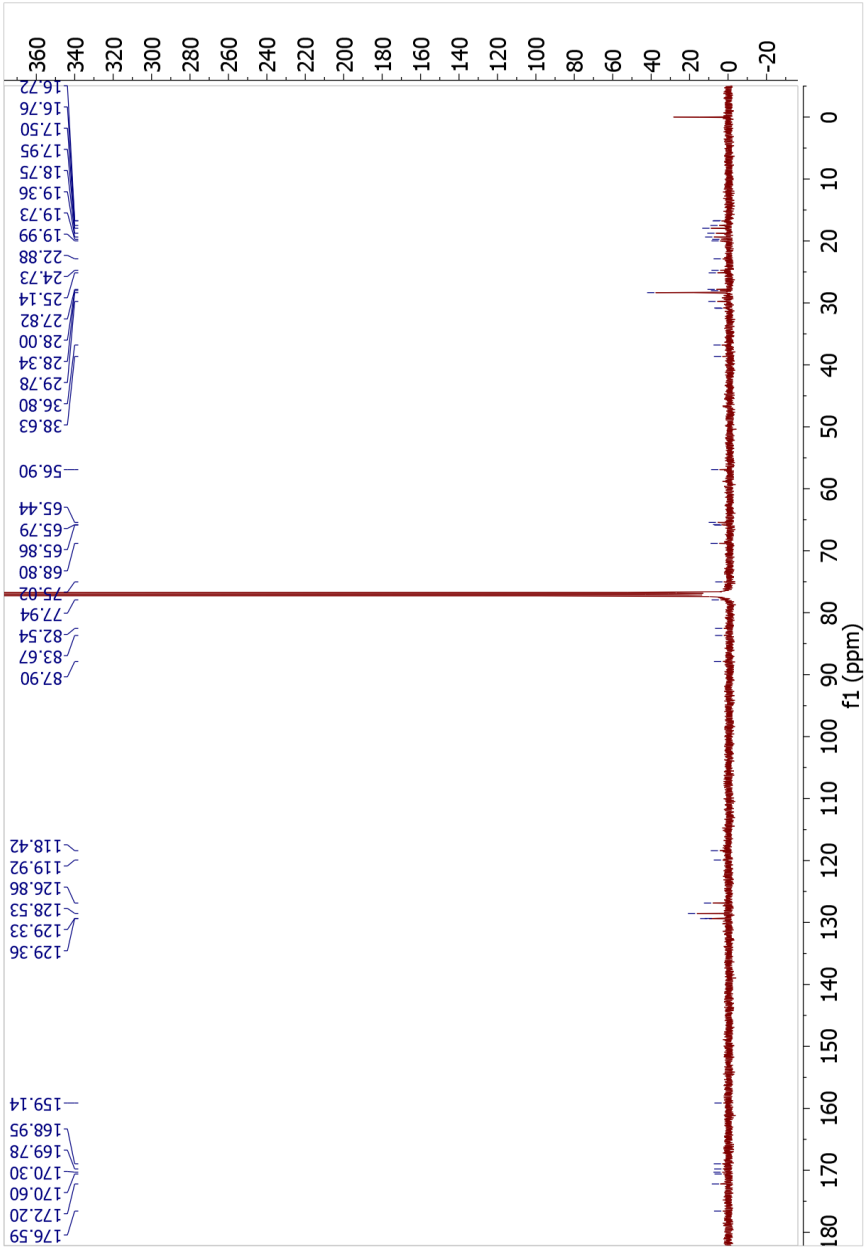


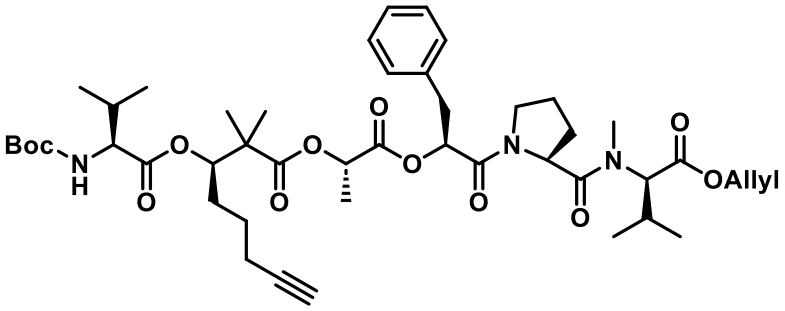
^{1}H and ^{13}C NMR and HRMS of Compound 1.45

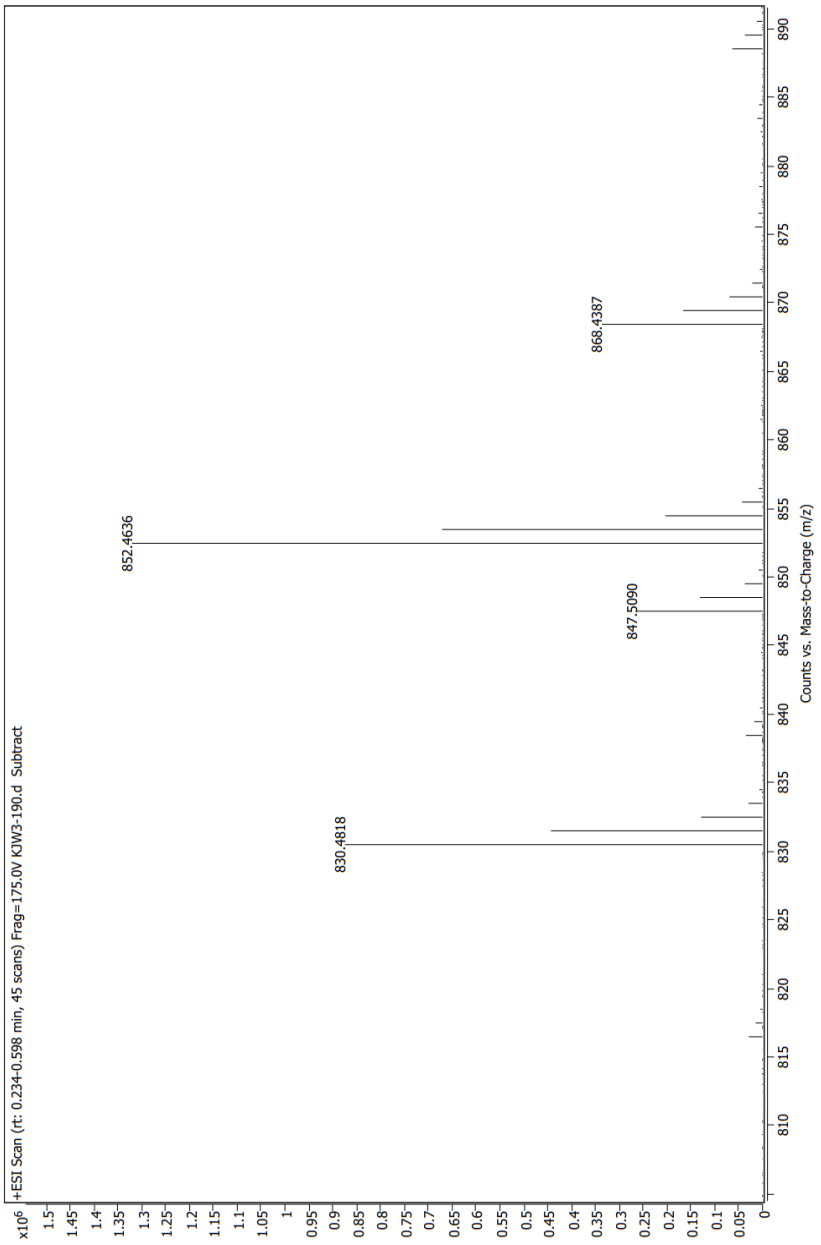




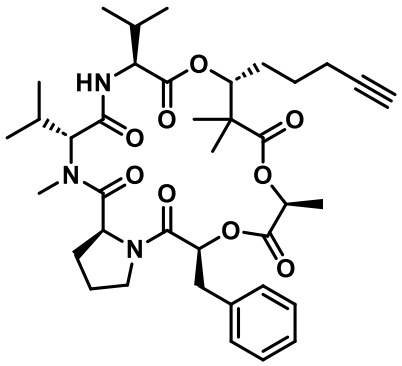

Boc
N
H
O
O
O
O
O
O
O
N
N
O
O
OAllyl

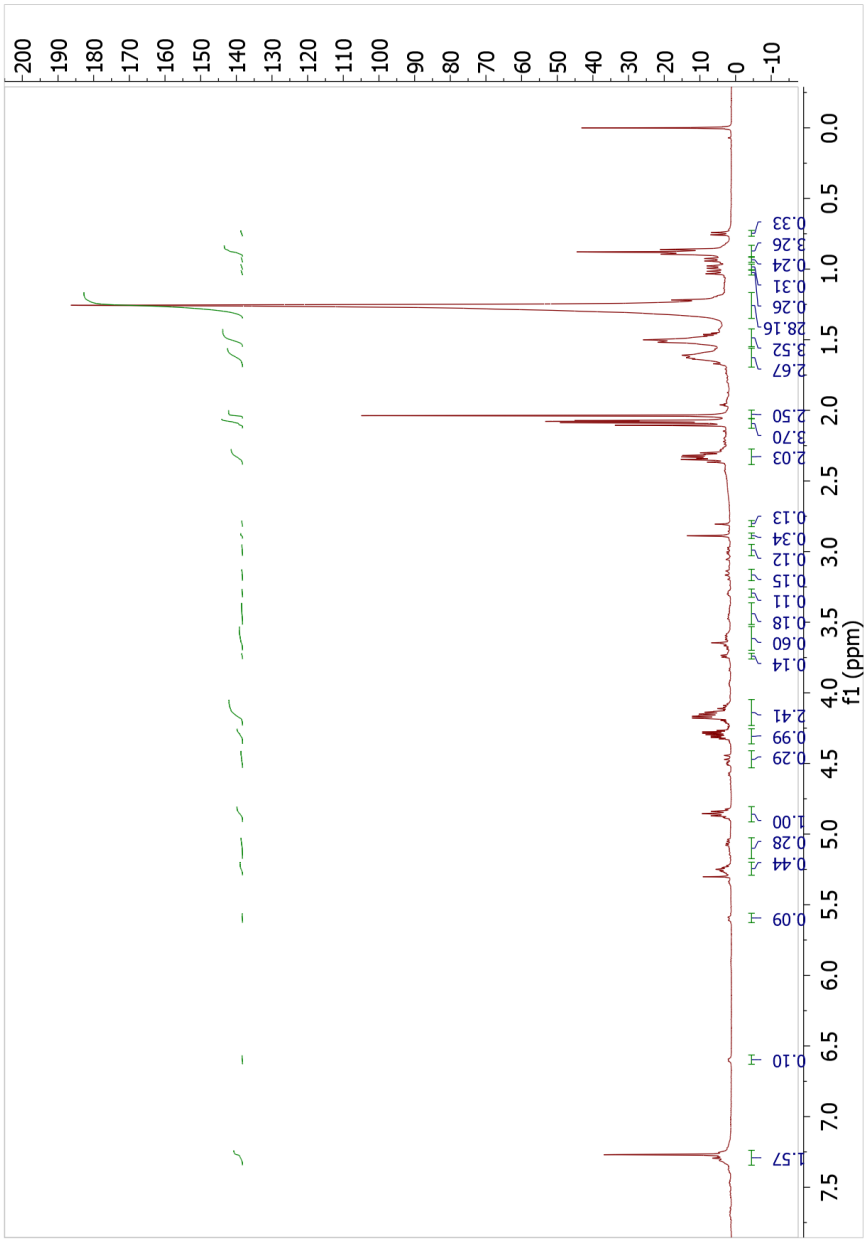

f1 (ppm)
16.72
16.76
17.50
17.95
18.75
19.36
19.73
19.99
22.88
24.73
25.14
27.82
28.00
28.34
29.78
36.80
38.63
56.90
65.44
65.79
65.86
68.80
75.02
77.94
82.54
83.67
87.90
118.42
119.92
126.86
128.53
129.33
129.36
159.14
168.95
169.78
170.30
170.60
172.20
176.59

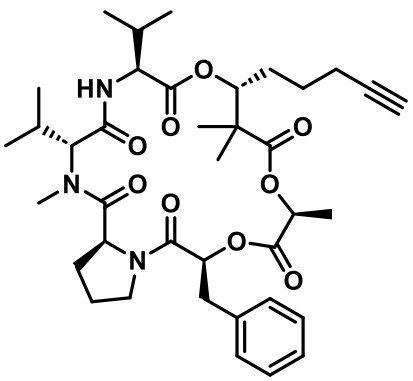

Boc
N
H
O
O
O
O
O
O
O
N
N
O
O
OAllyl

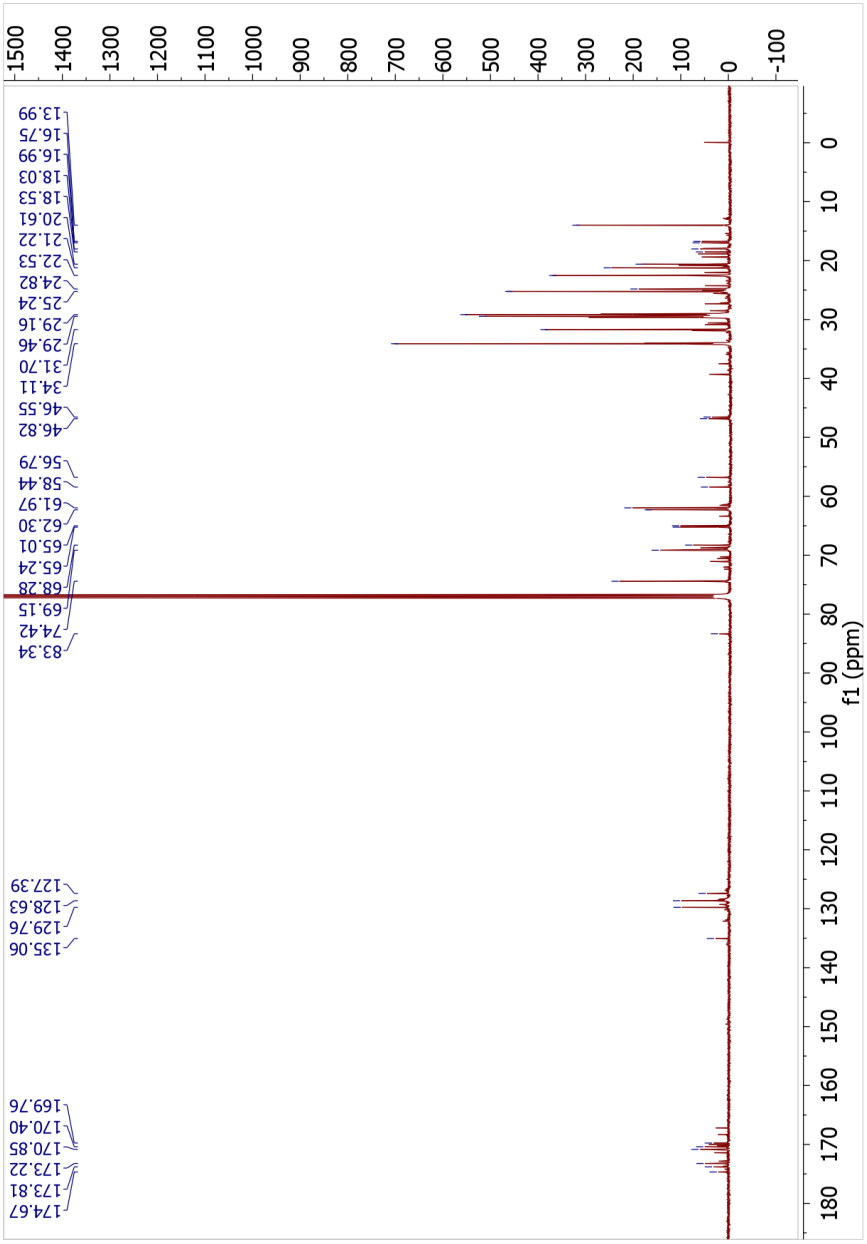

+ESI Scan (rt: 0.234-0.598 min, 45 scans) Frag=175.0V KIW3-190.d Subtract
x10^6
Counts vs. Mass-to-Charge (m/z)
868.4387
852.4636
847.5090
830.4818

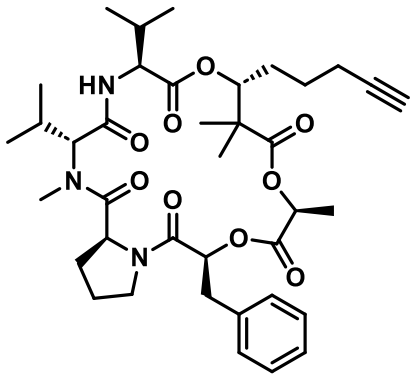
^{1}H and ^{13}C NMR and HRMS of Compound 1.46

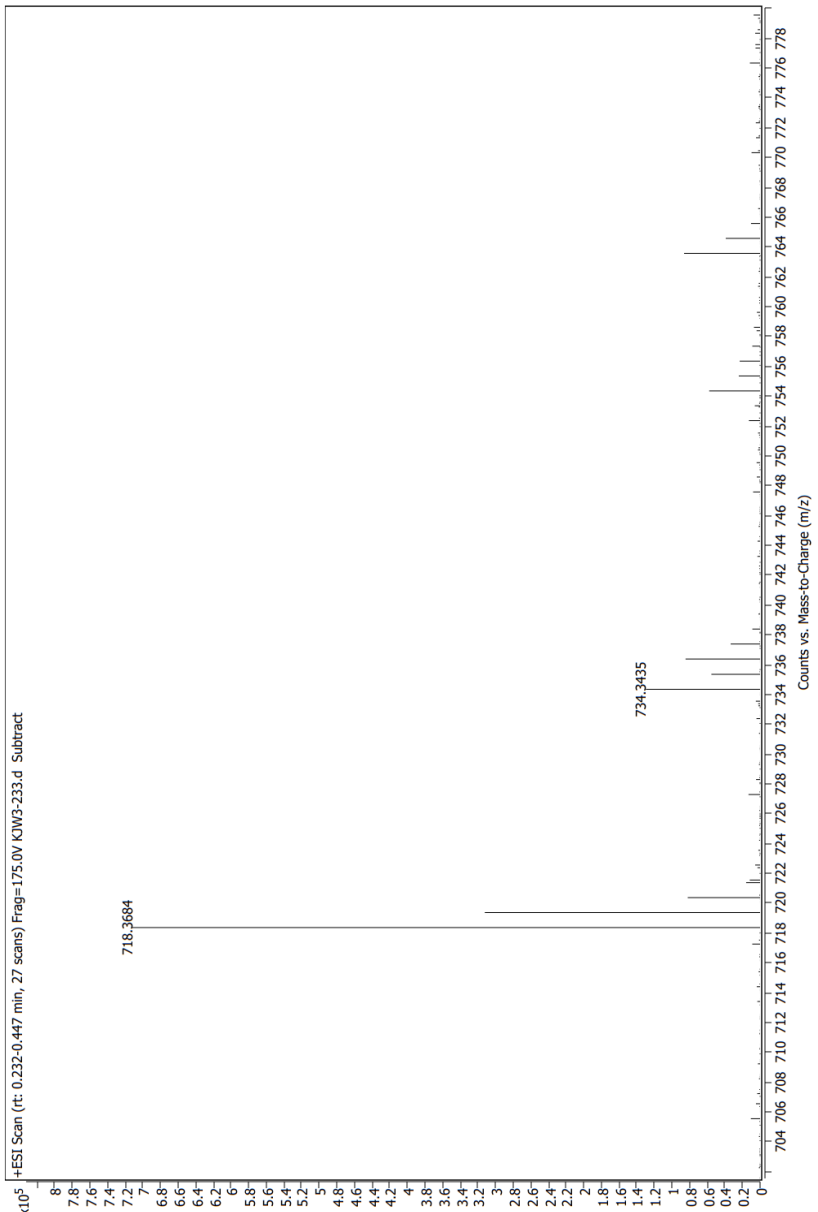




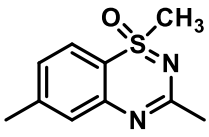

HN
O
O
O
O
O
O
O
N
O
N
O
O
O

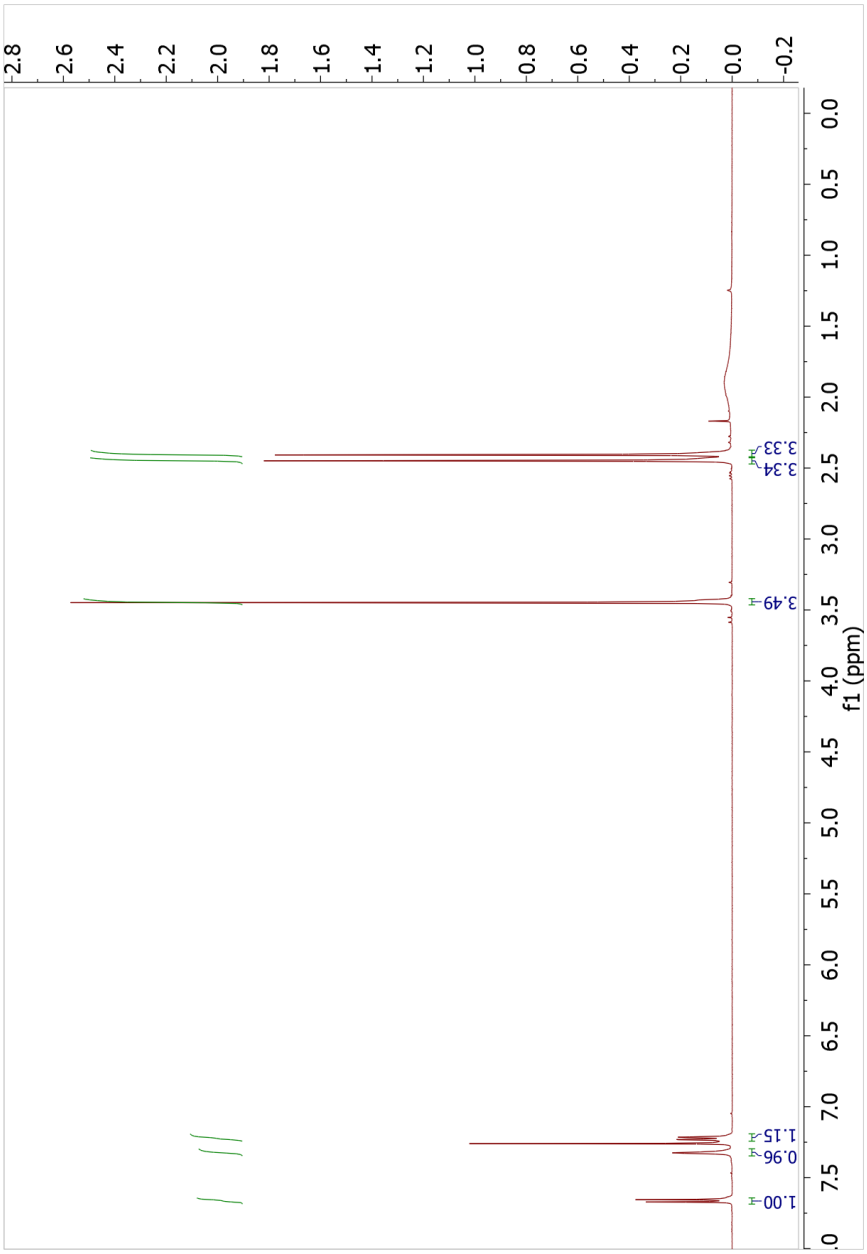

13.99
16.75
16.99
18.03
18.53
20.61
21.22
22.53
24.82
25.24
29.16
29.46
31.70
34.11
46.55
46.82
56.79
58.44
61.97
62.30
65.01
65.24
68.28
69.15
74.42
83.34
127.39
128.63
129.76
135.06
169.76
170.40
170.85
173.22
173.81
174.67
f1 (ppm)


HN
O
O
O
O
O
O
O
O
N
N
O
O
O
O

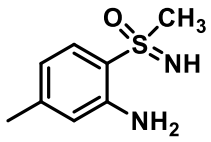

+ESI Scan (rt: 0.232-0.447 min, 27 scans) Frag=175.0V KJW3-233.d Subtract
x10^5
718.3684
734.3435
Counts vs. Mass-to-Charge (m/z)

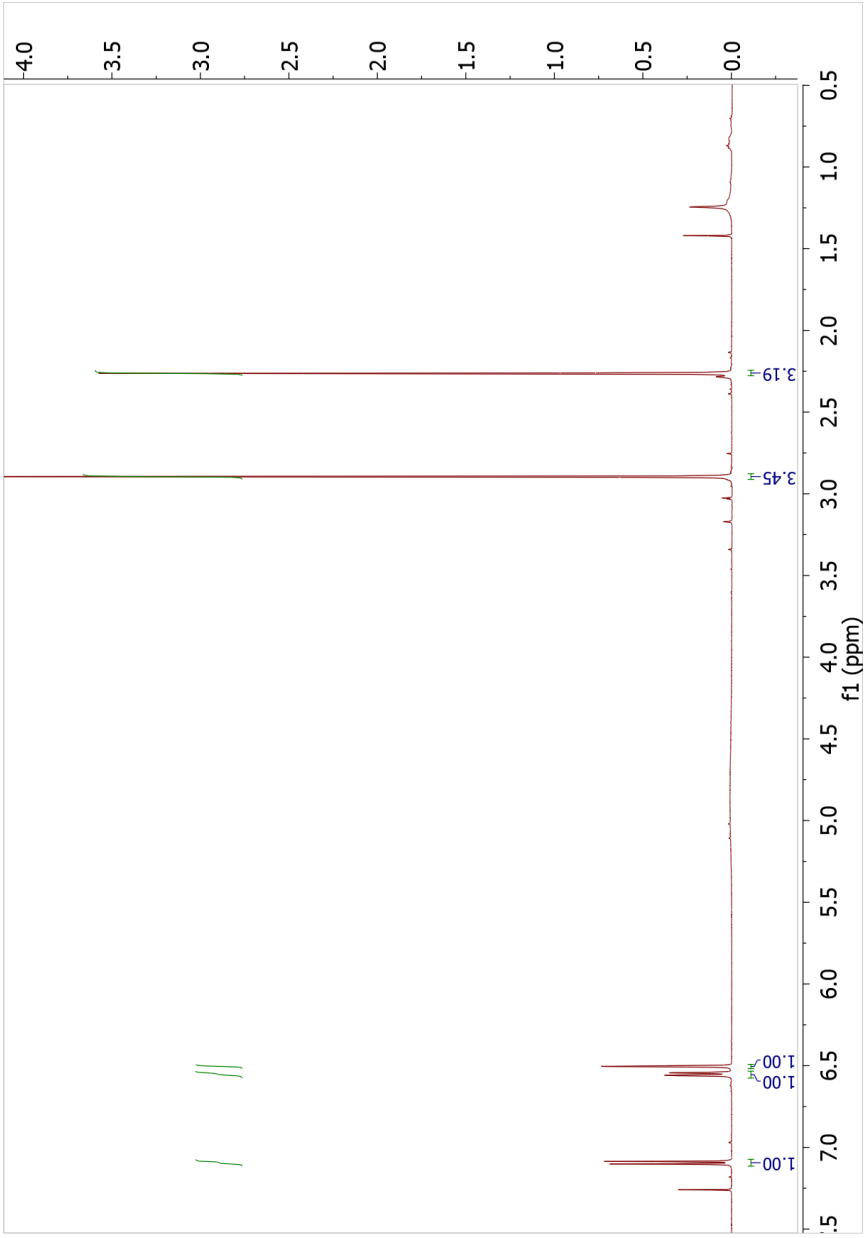
^{1}H NMR of Compound 3.2



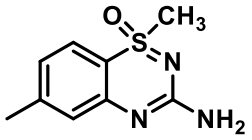


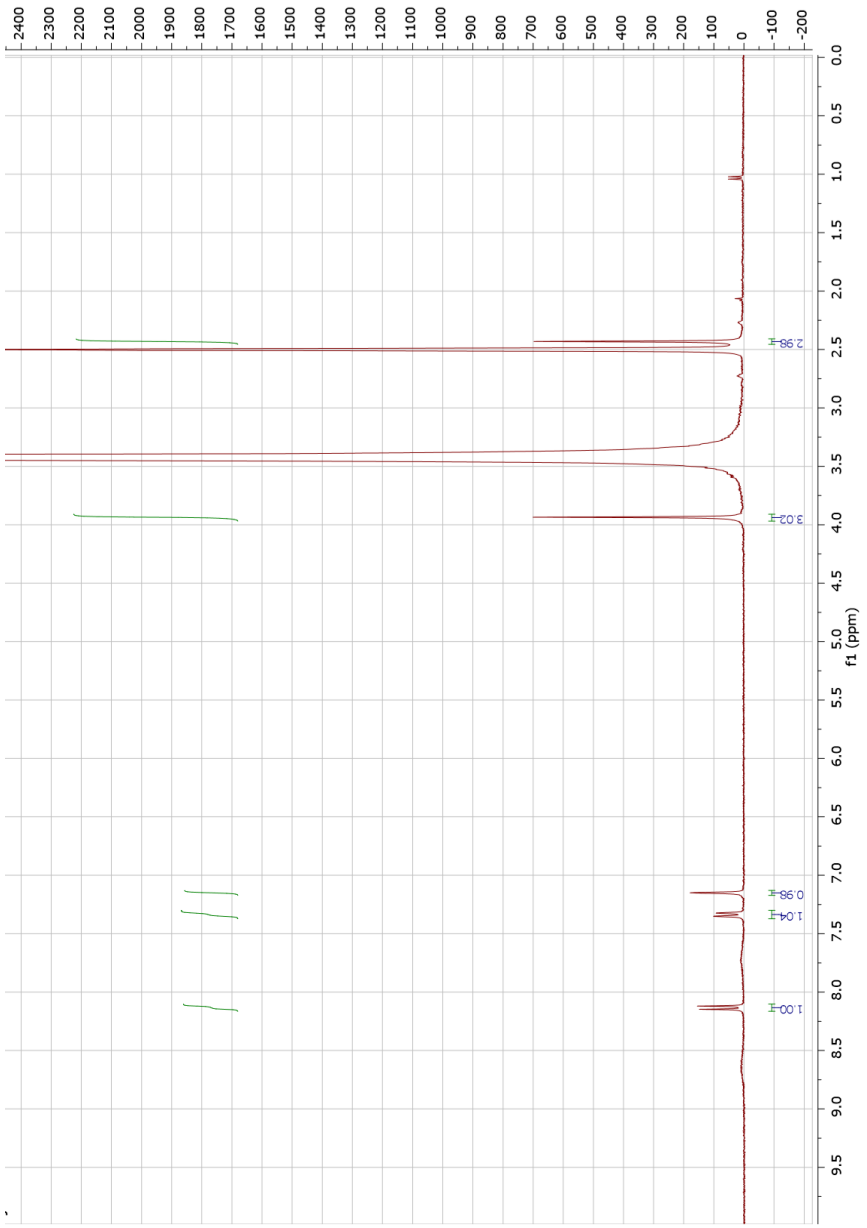
^{1}H NMR of Compound 3.3



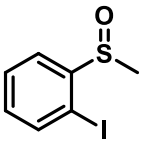


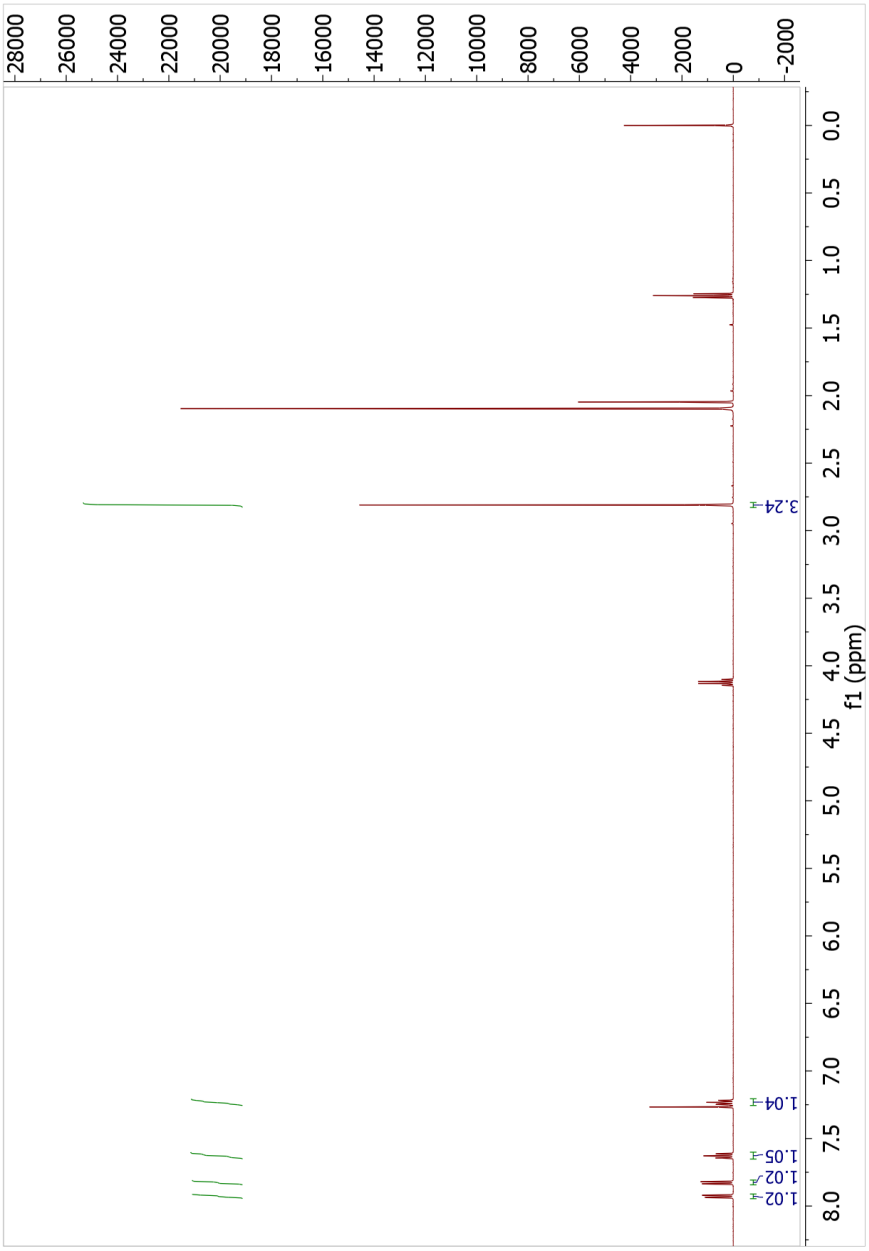
^{1}H NMR of Compound 3.4



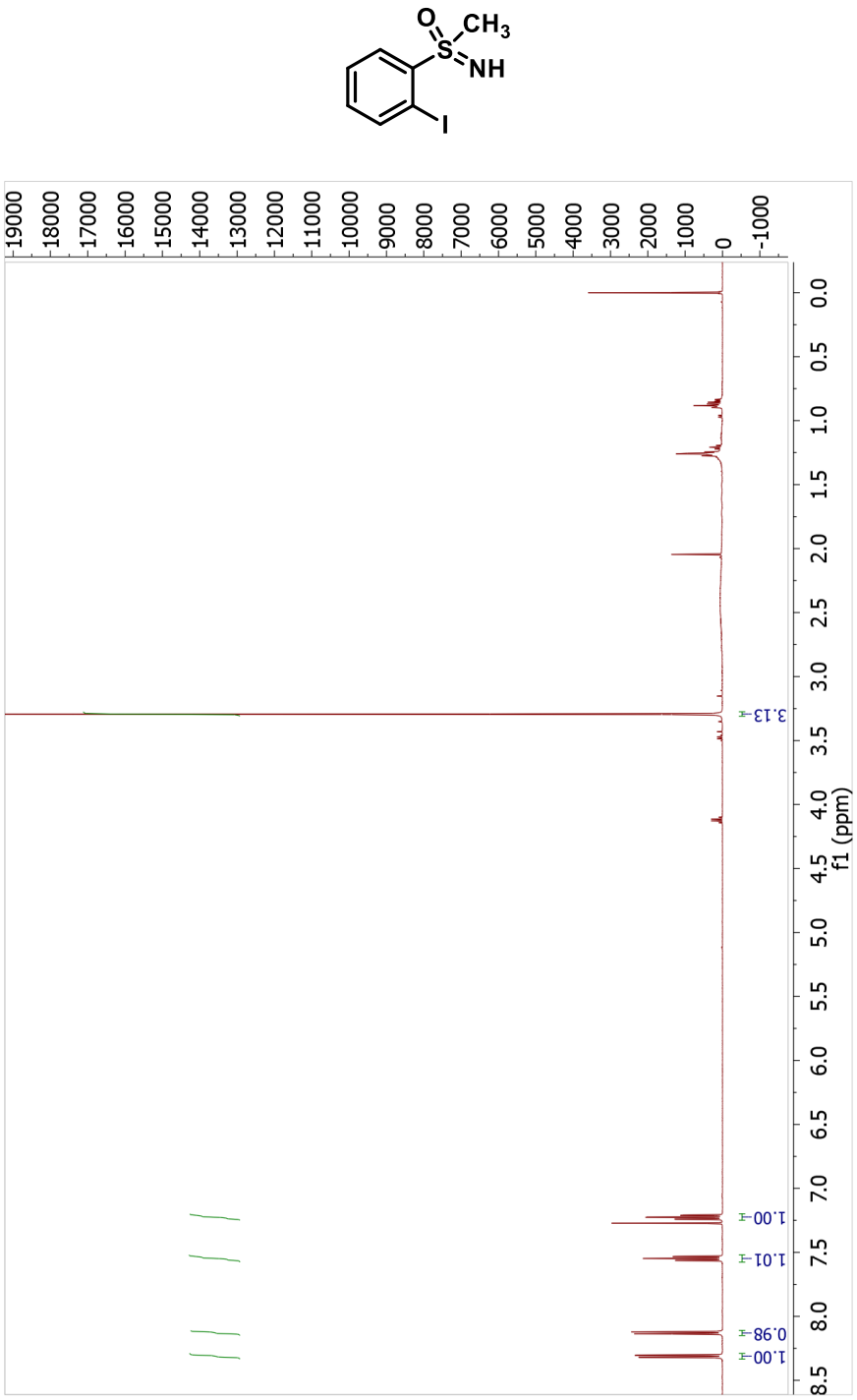


^{1}H NMR of Compound 3.6

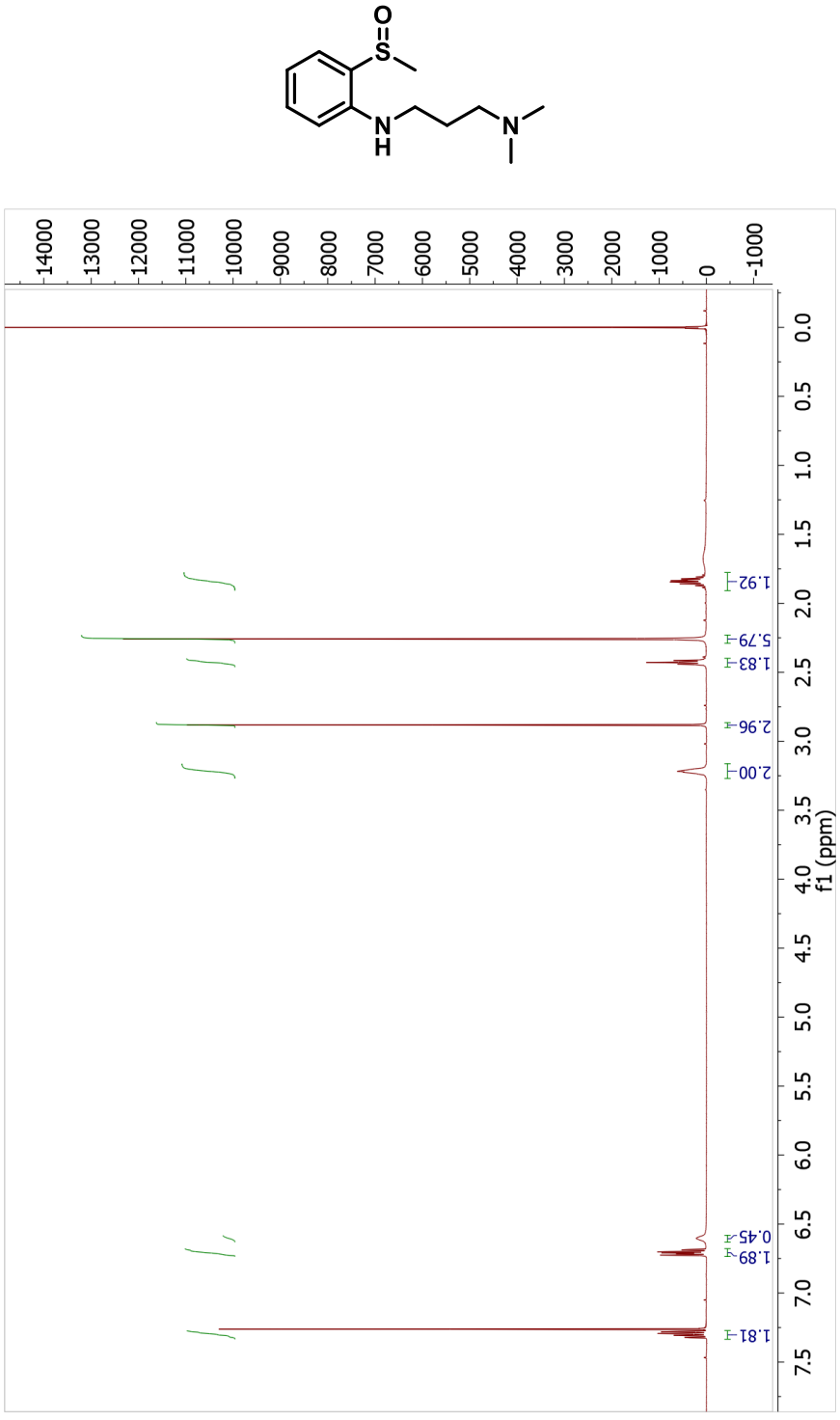




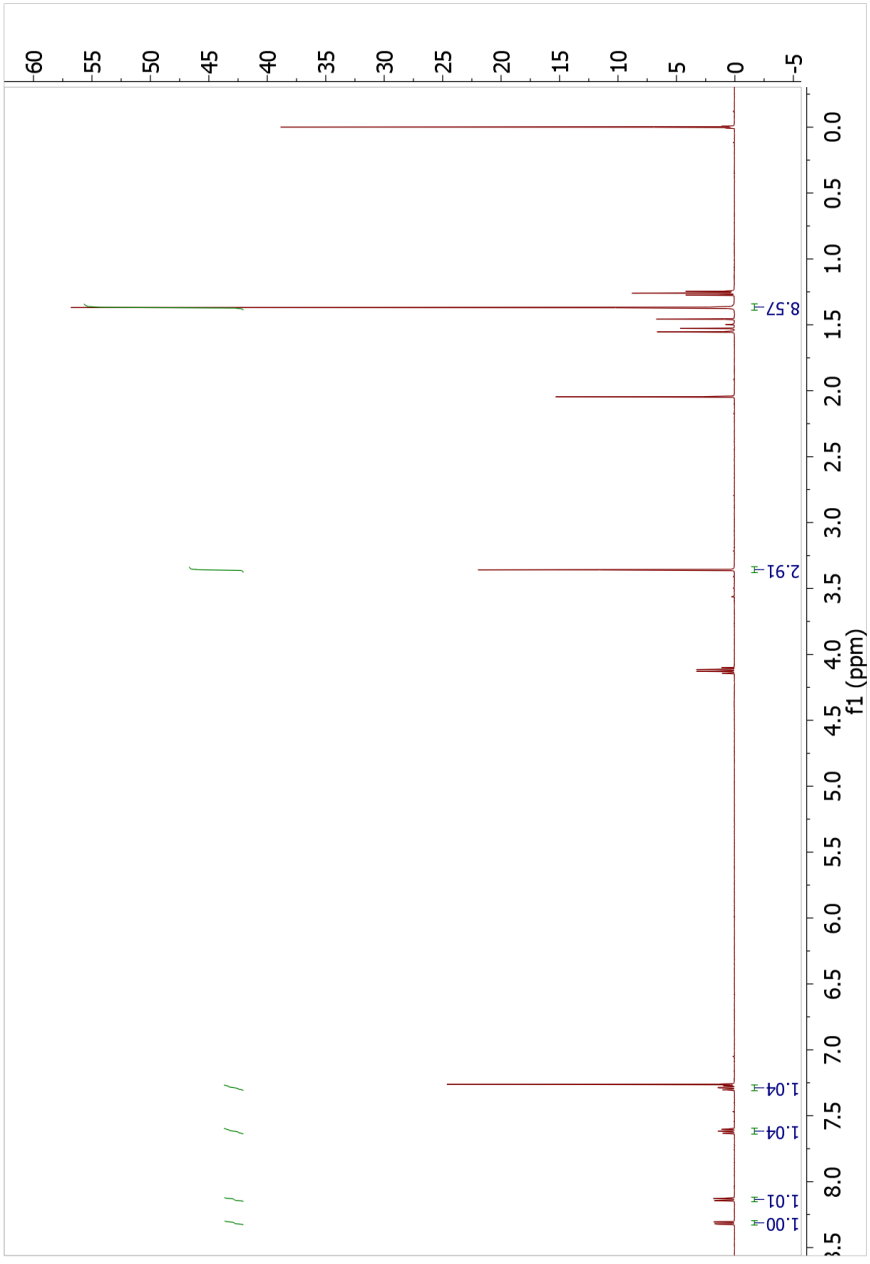
^{1}H NMR of Compound 3.7



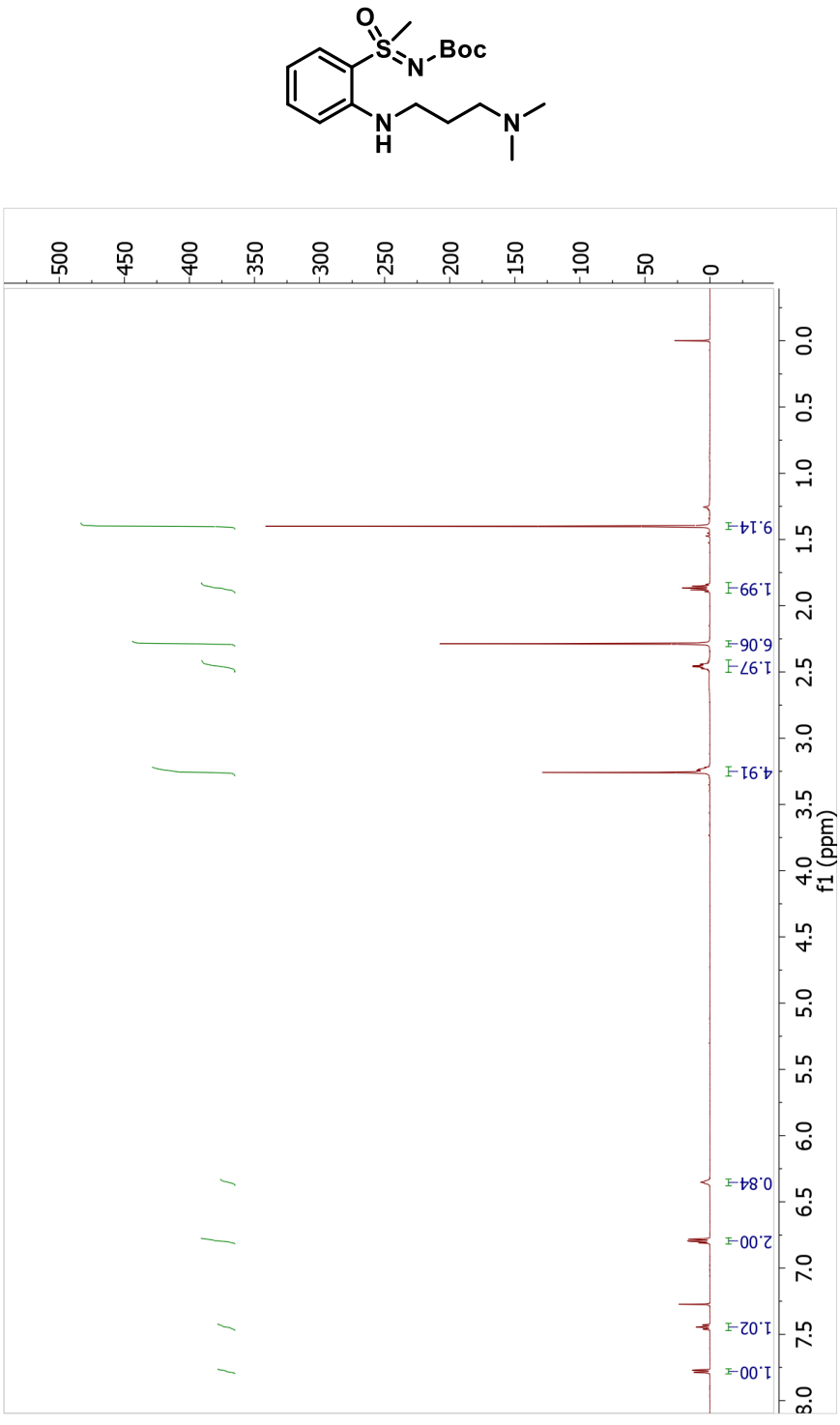
^{1}H NMR of Compound 3.8



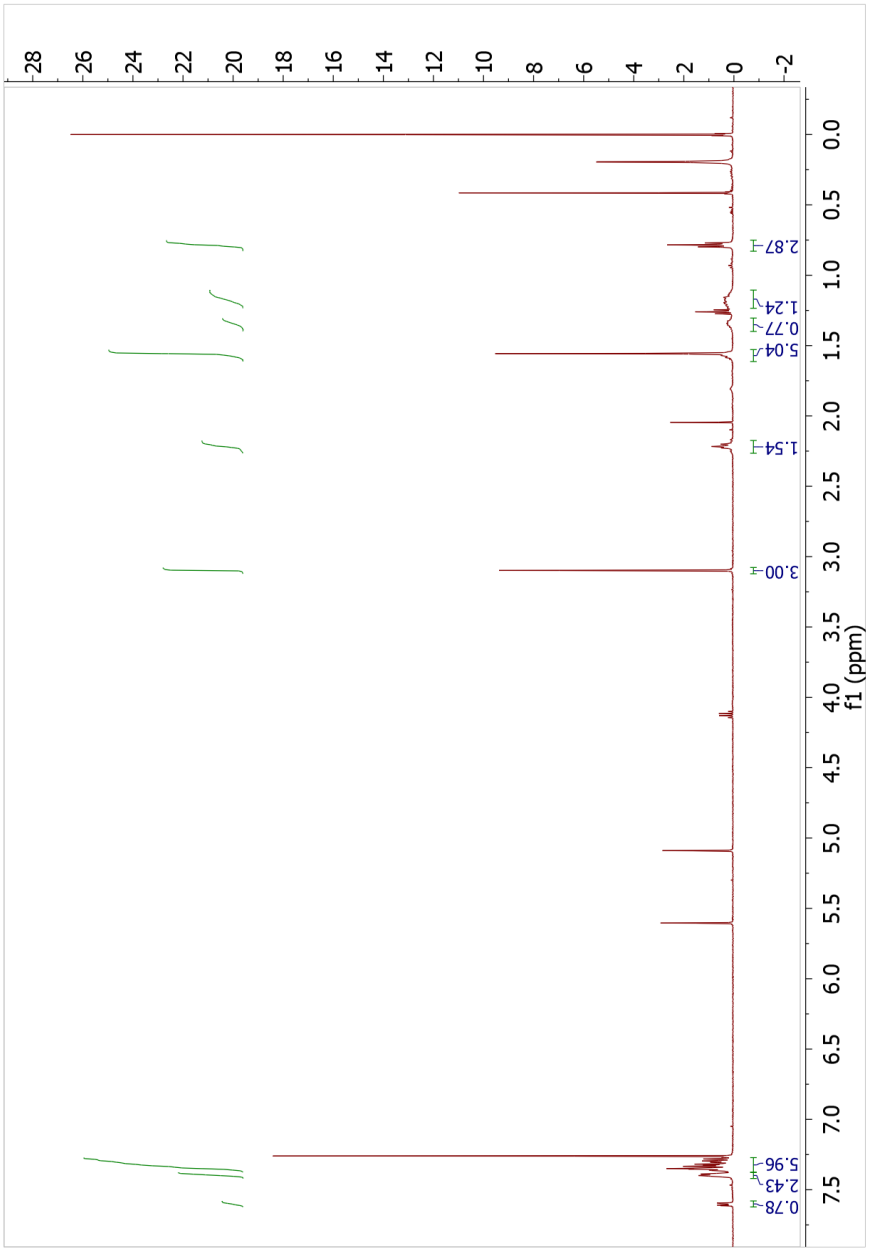
^{1}H NMR of Compound 3.9



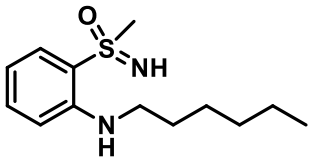
^{1}H NMR of Compound 3.10

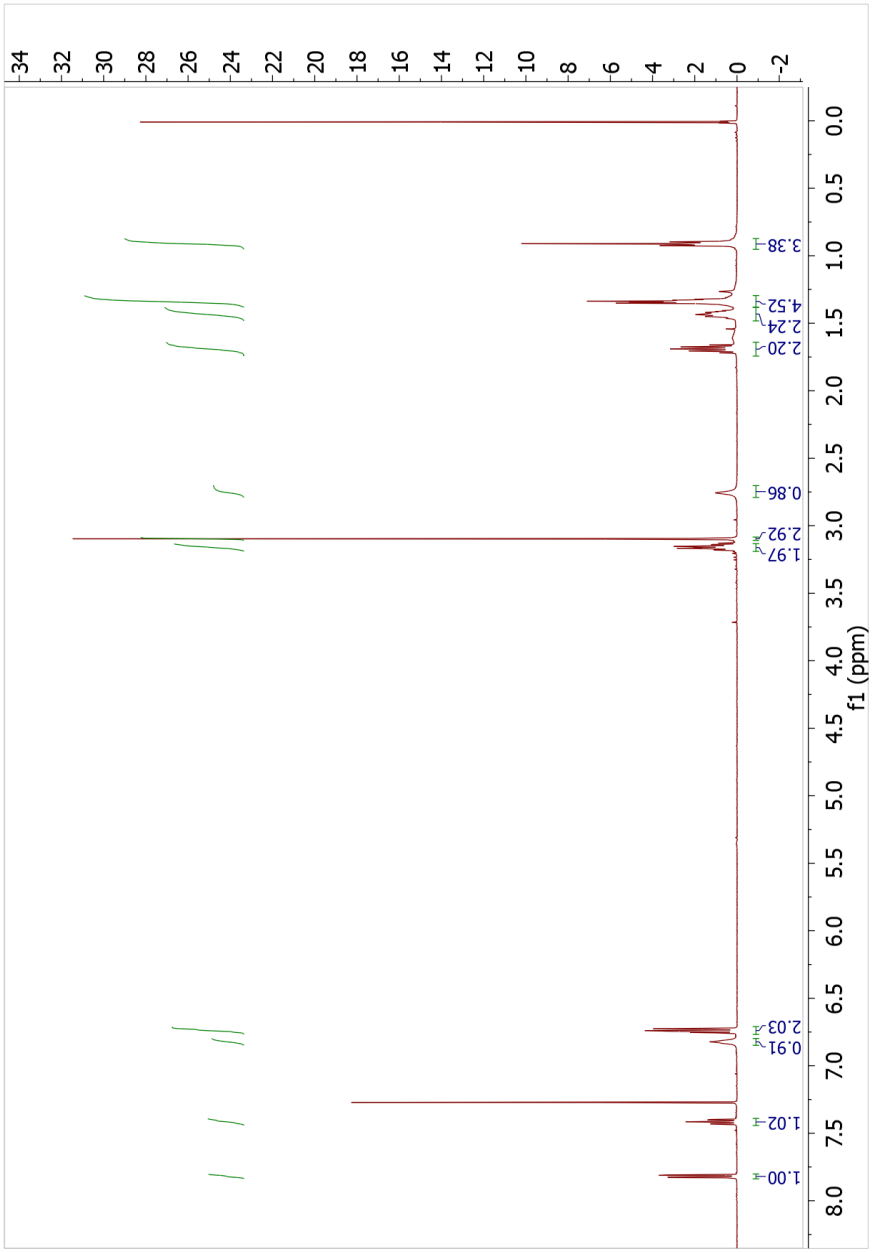


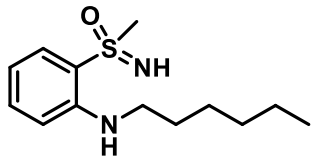
^{1}H NMR of Compound 3.11

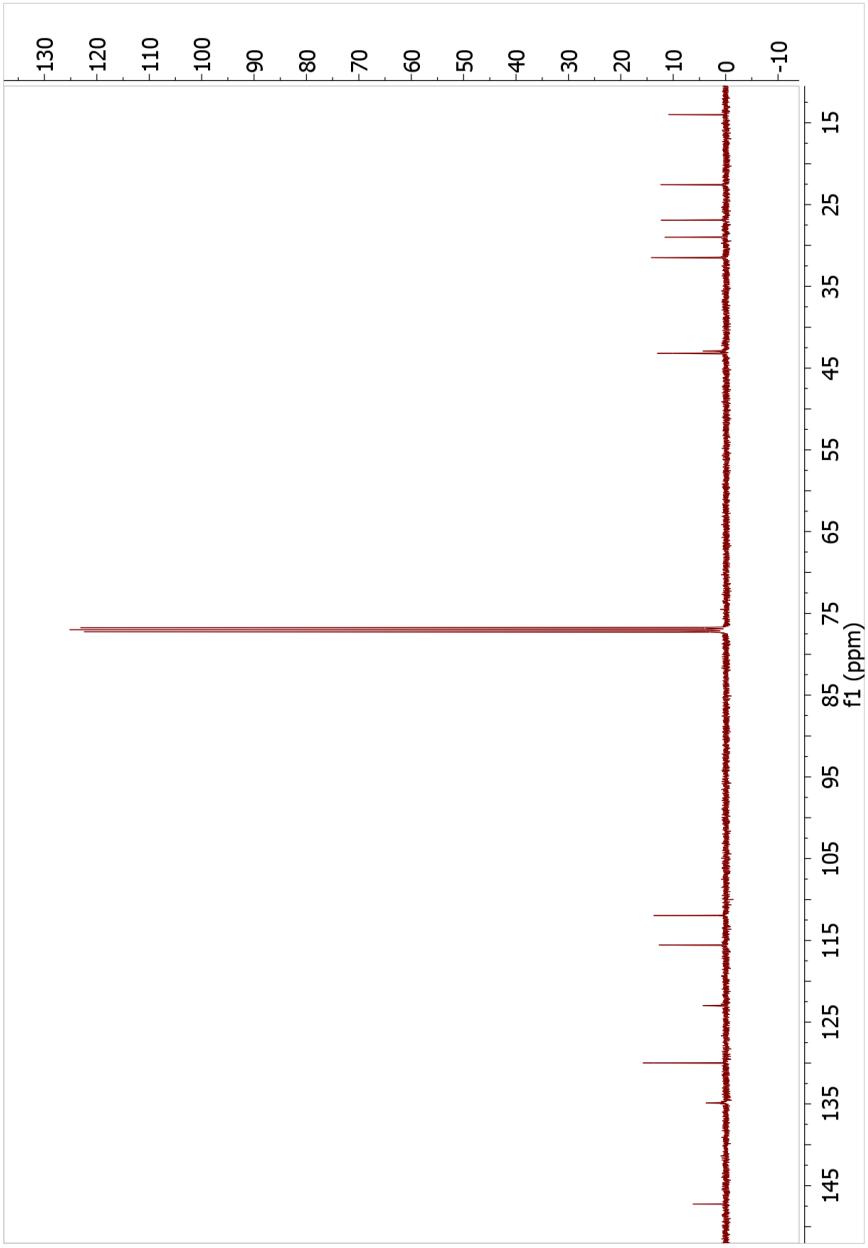


^{1}H and ^{13}C NMR of Compound 3.12

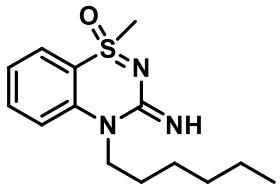


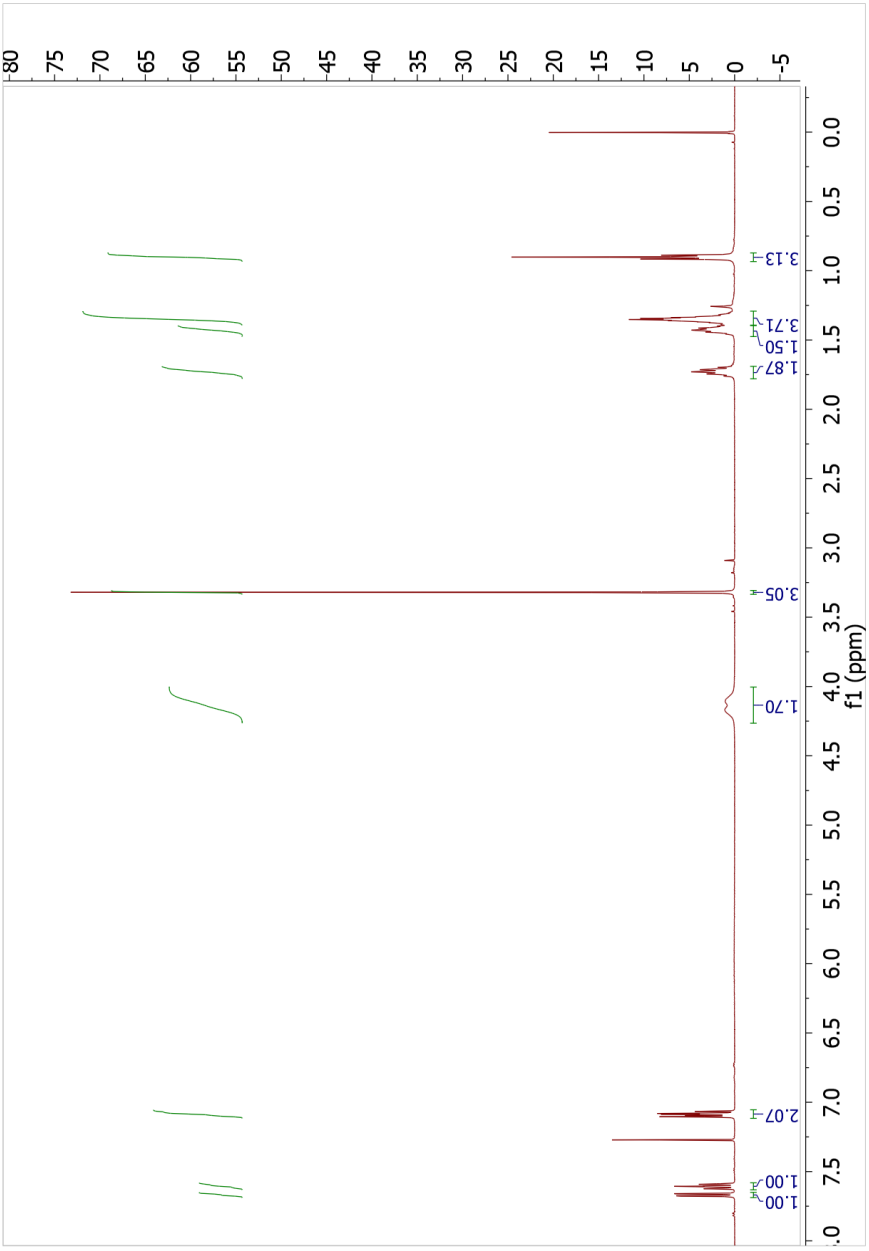


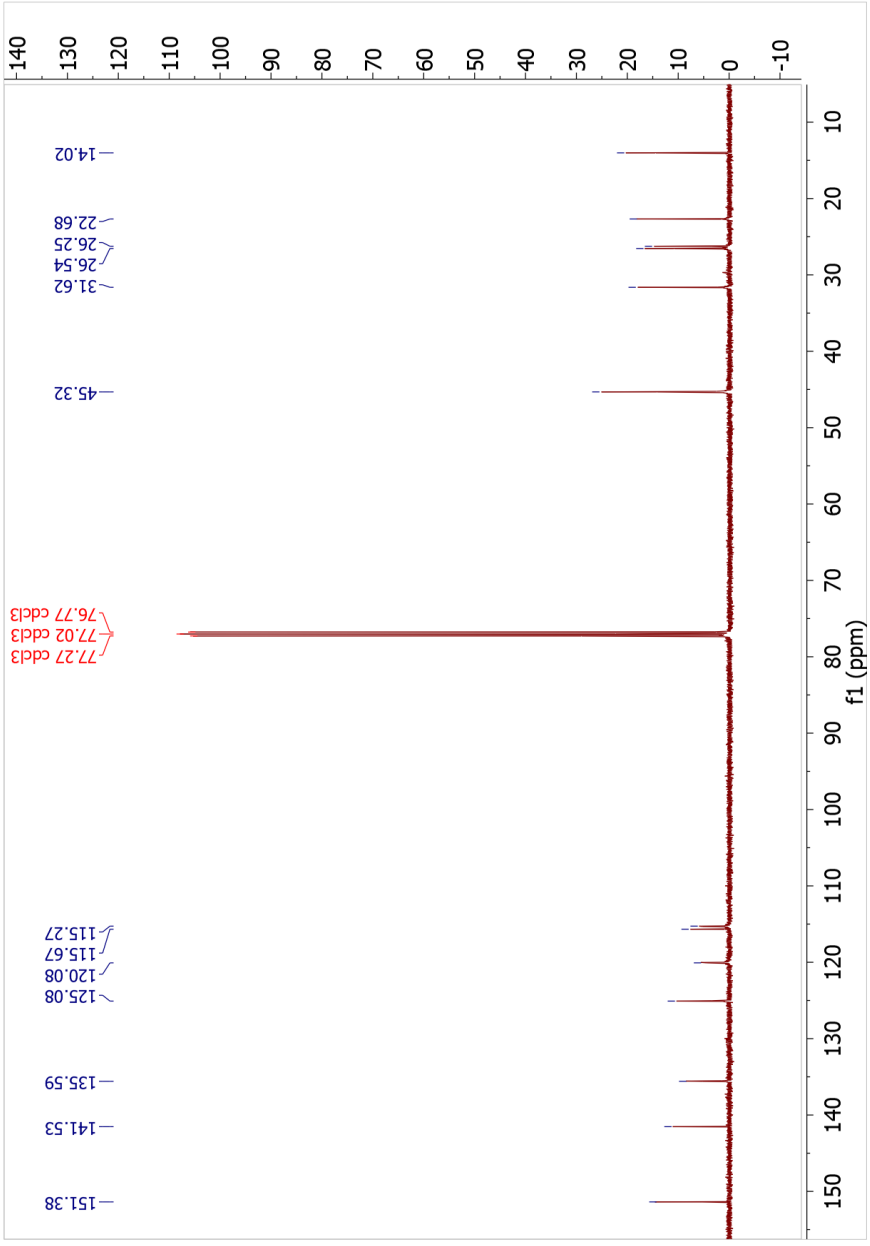

O
S
NH
N
H


130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
15
25
35
45
55
65
75
85
95
105
115
125
135
145
f1 (ppm)

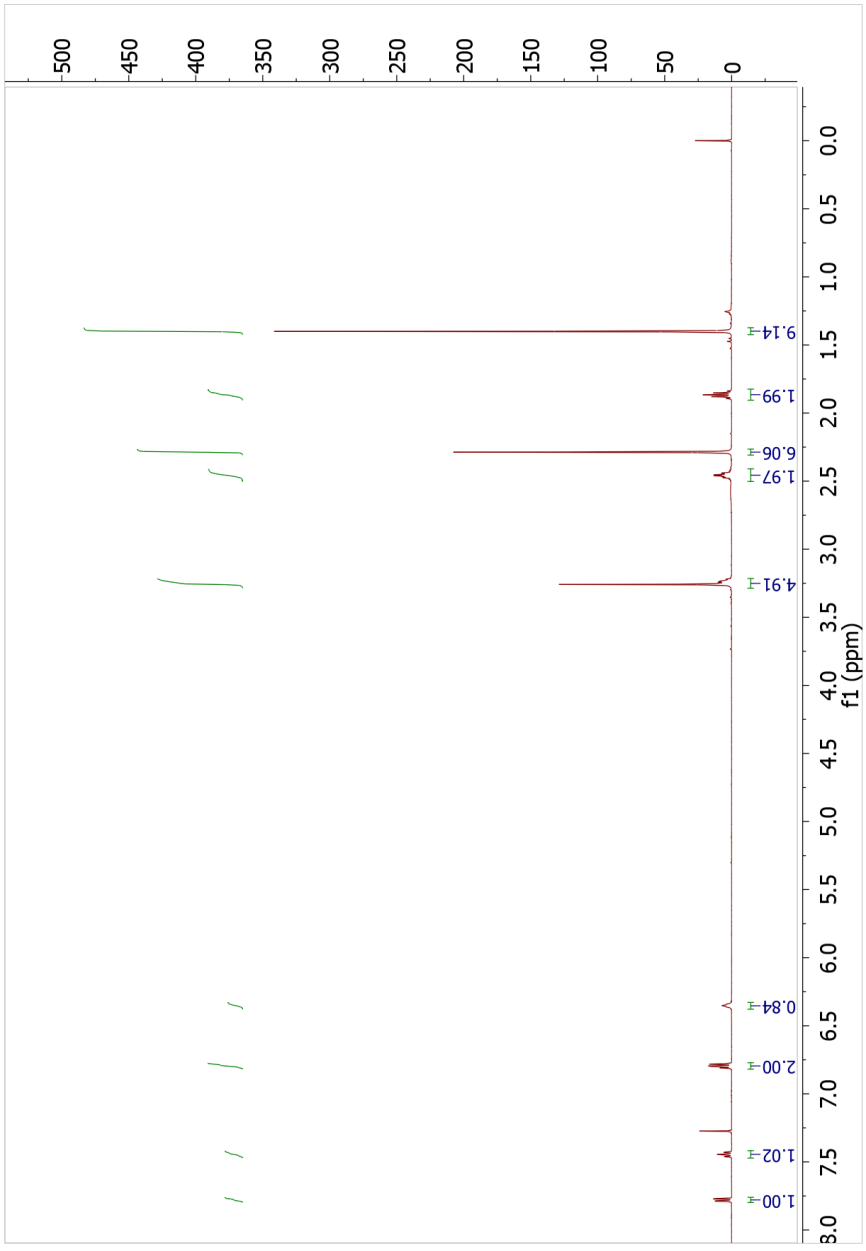
^{1}H and ^{13}C NMR of Compound 3.13



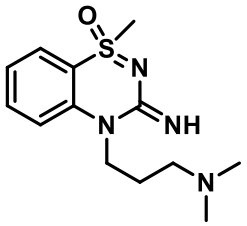


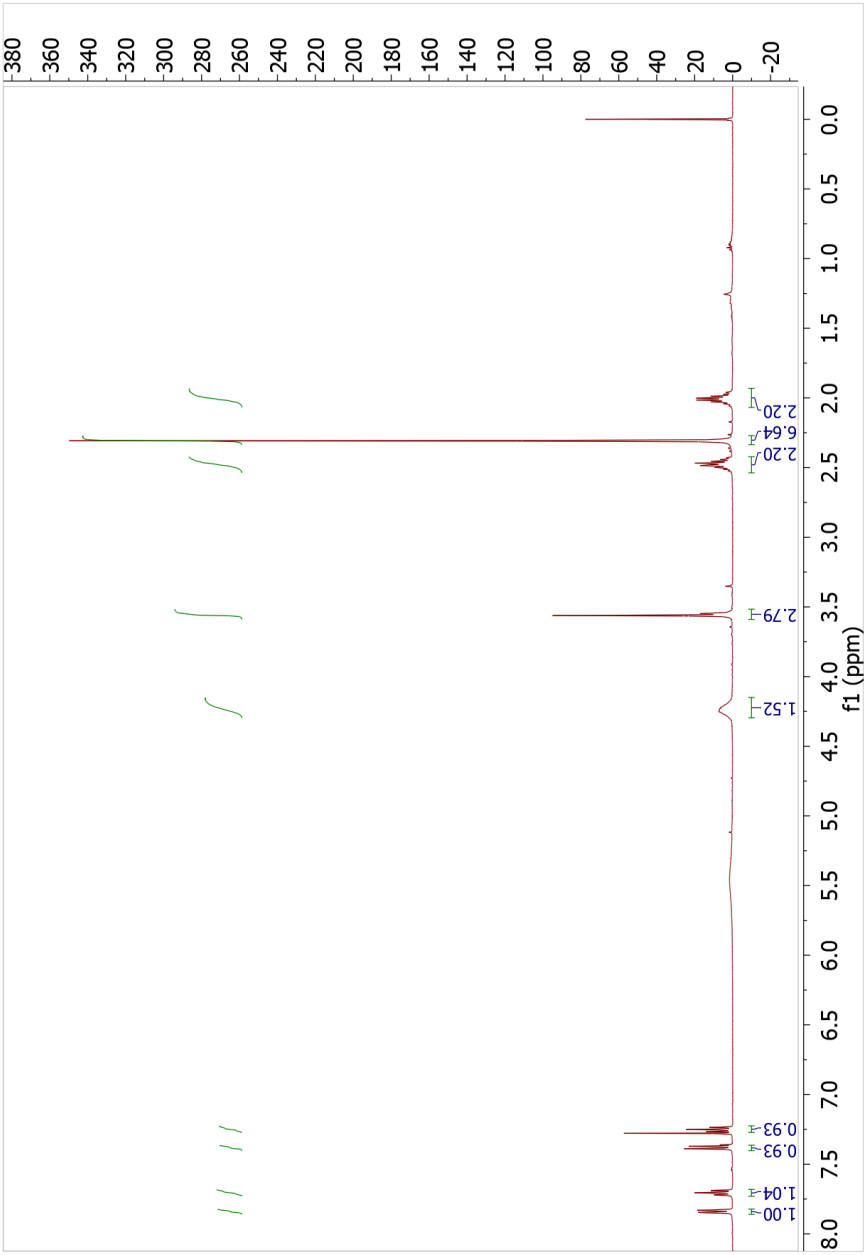

O
S
N
N
NH
f1 (ppm)
151.38
141.53
135.59
125.08
120.08
115.67
115.27
77.27 cdcl3
77.02 cdcl3
76.77 cdcl3
45.32
31.62
26.54
26.25
22.68
14.02

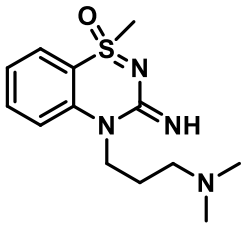
^{1}H NMR of Compound 3.14

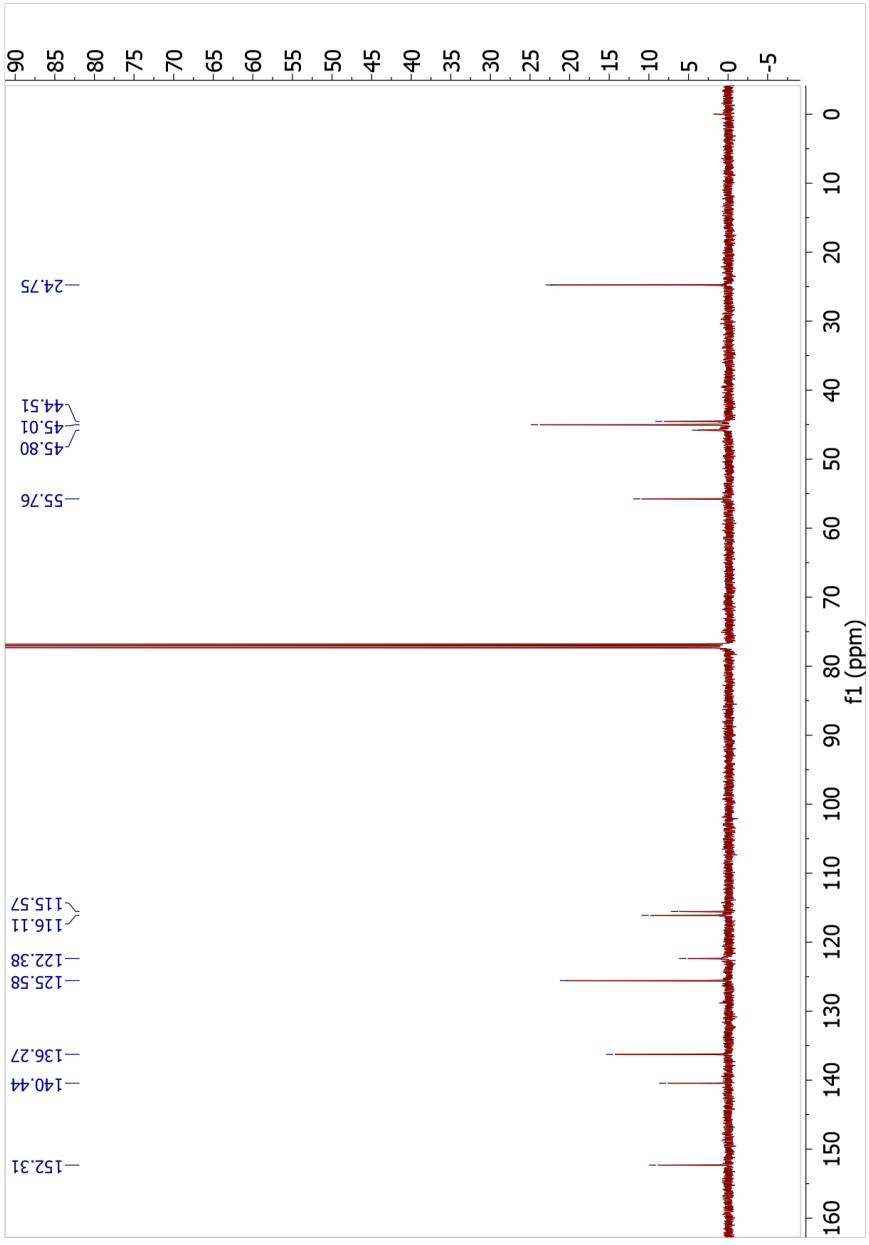


^{1}H and ^{13}C NMR of Compound 3.15

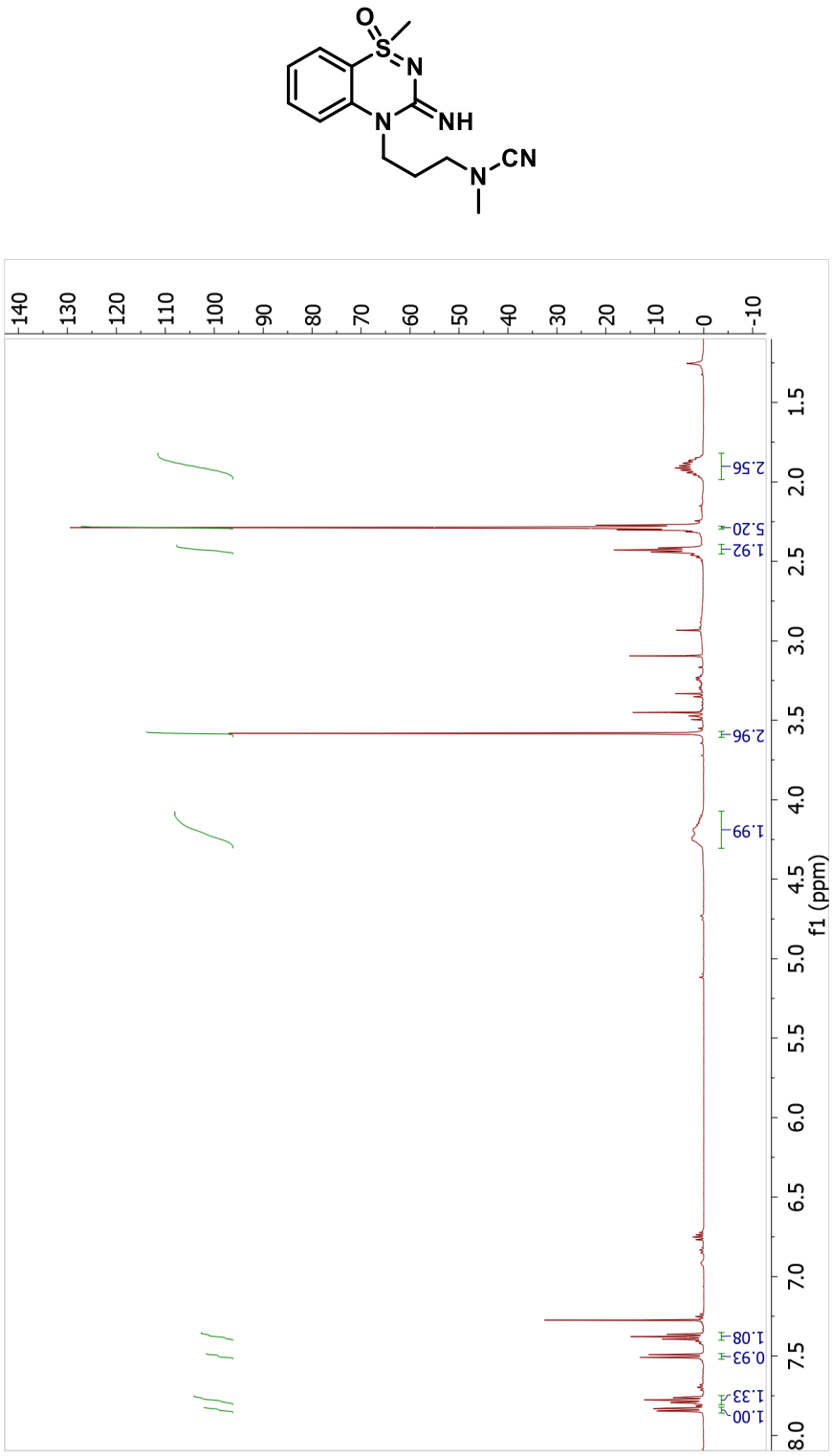




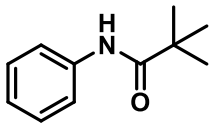

O
S
N
N
NH
N

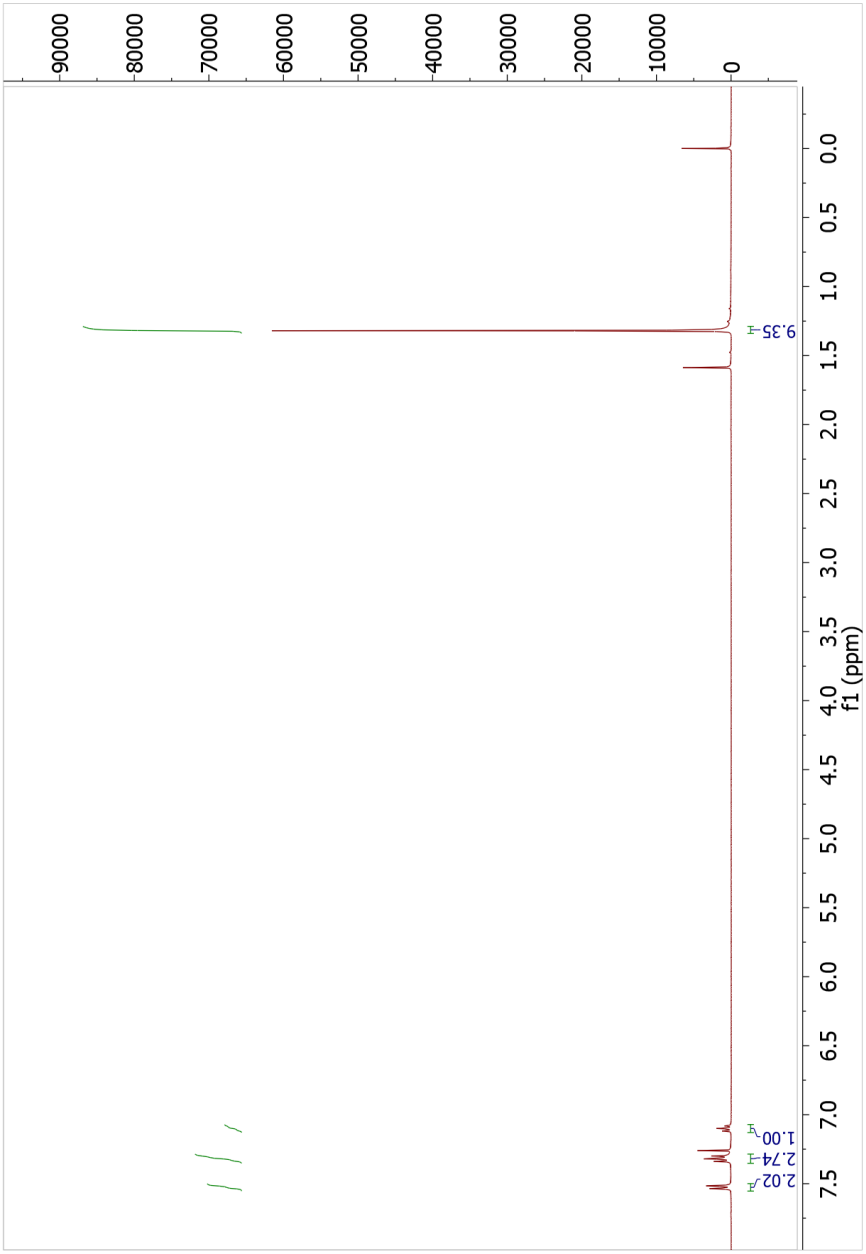

24.75
44.51
45.80
45.01
55.76
115.57
116.11
122.38
125.58
136.27
140.44
152.31
f1 (ppm)

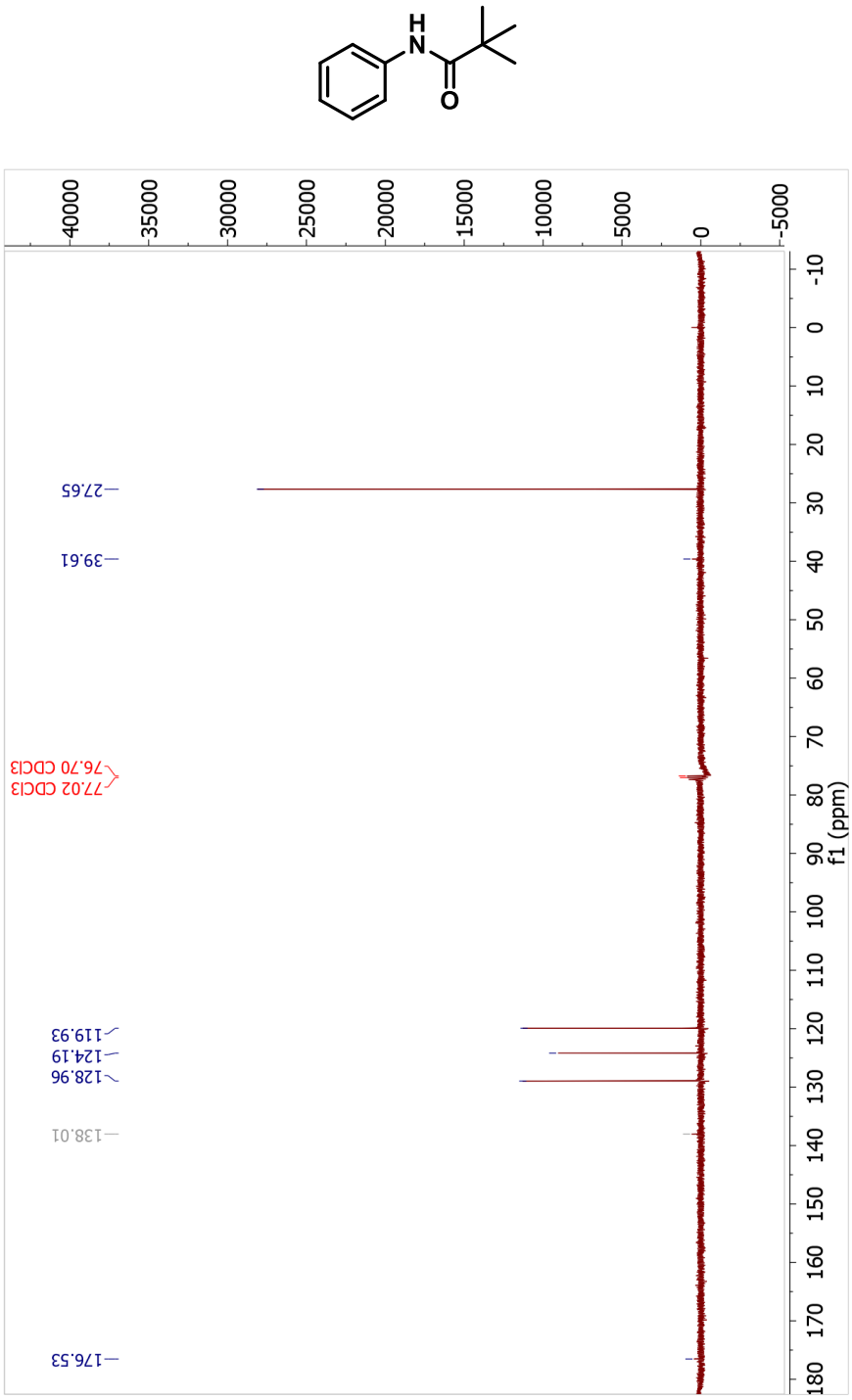
^{1}H NMR of Compound 3.16



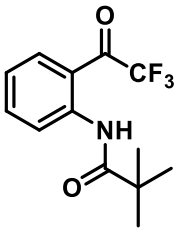
^{1}H and ^{13}C NMR of Compound 3.19

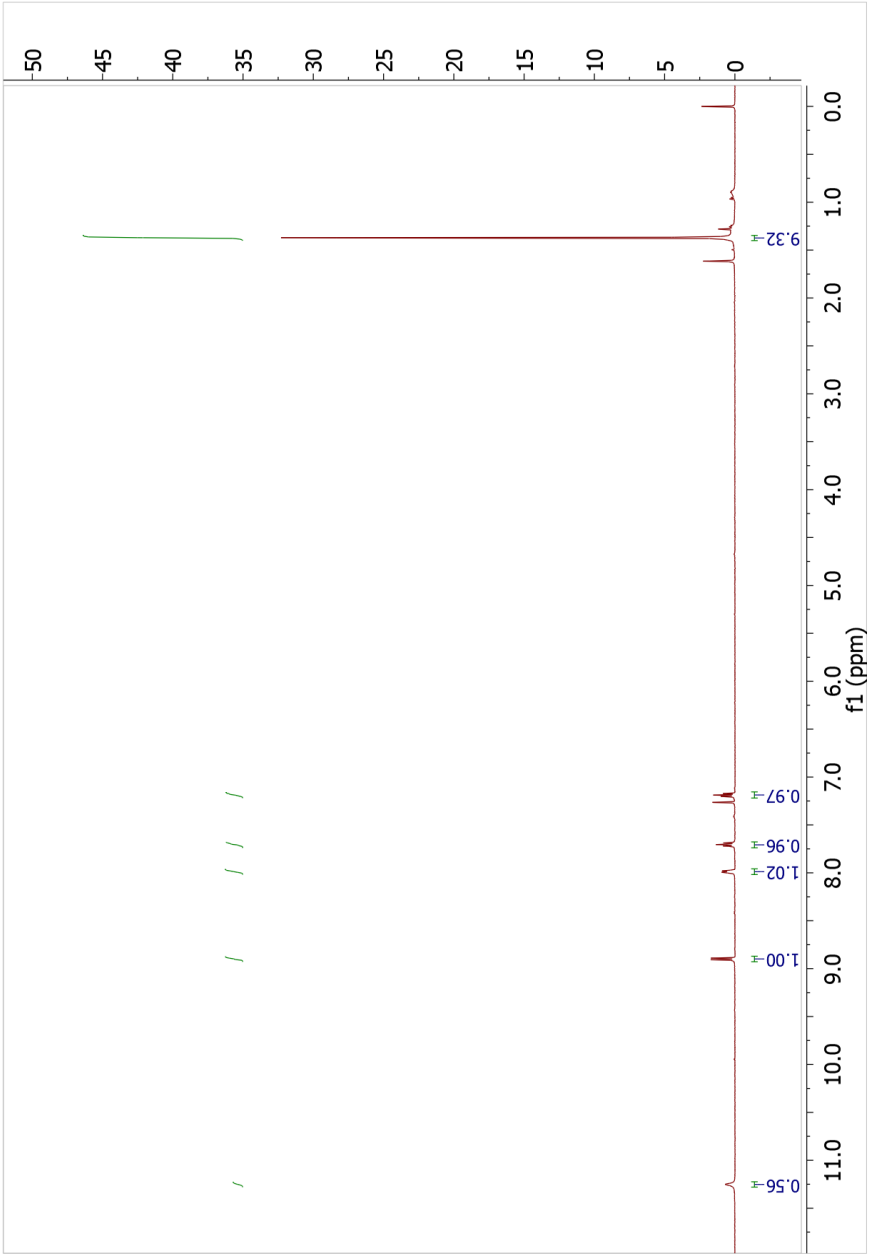


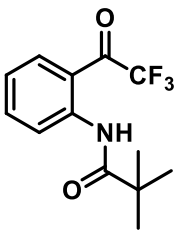


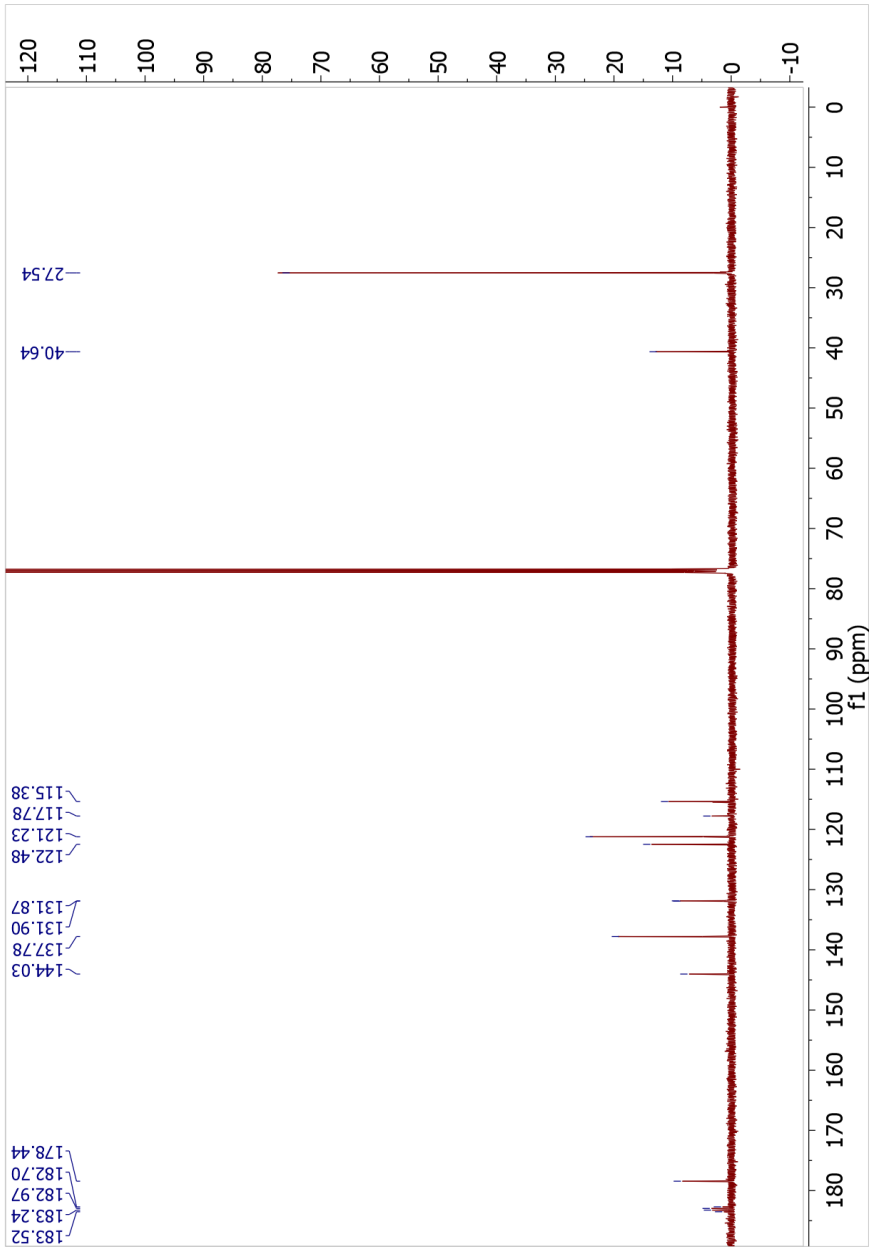

H
N
O
176.53
138.01
128.96
124.19
119.93
77.02 CDCl3
76.70 CDCl3
39.61
27.65
f1 (ppm)

^{1}H and ^{13}C NMR of Compound 3.20

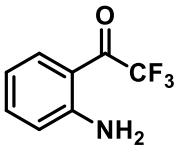


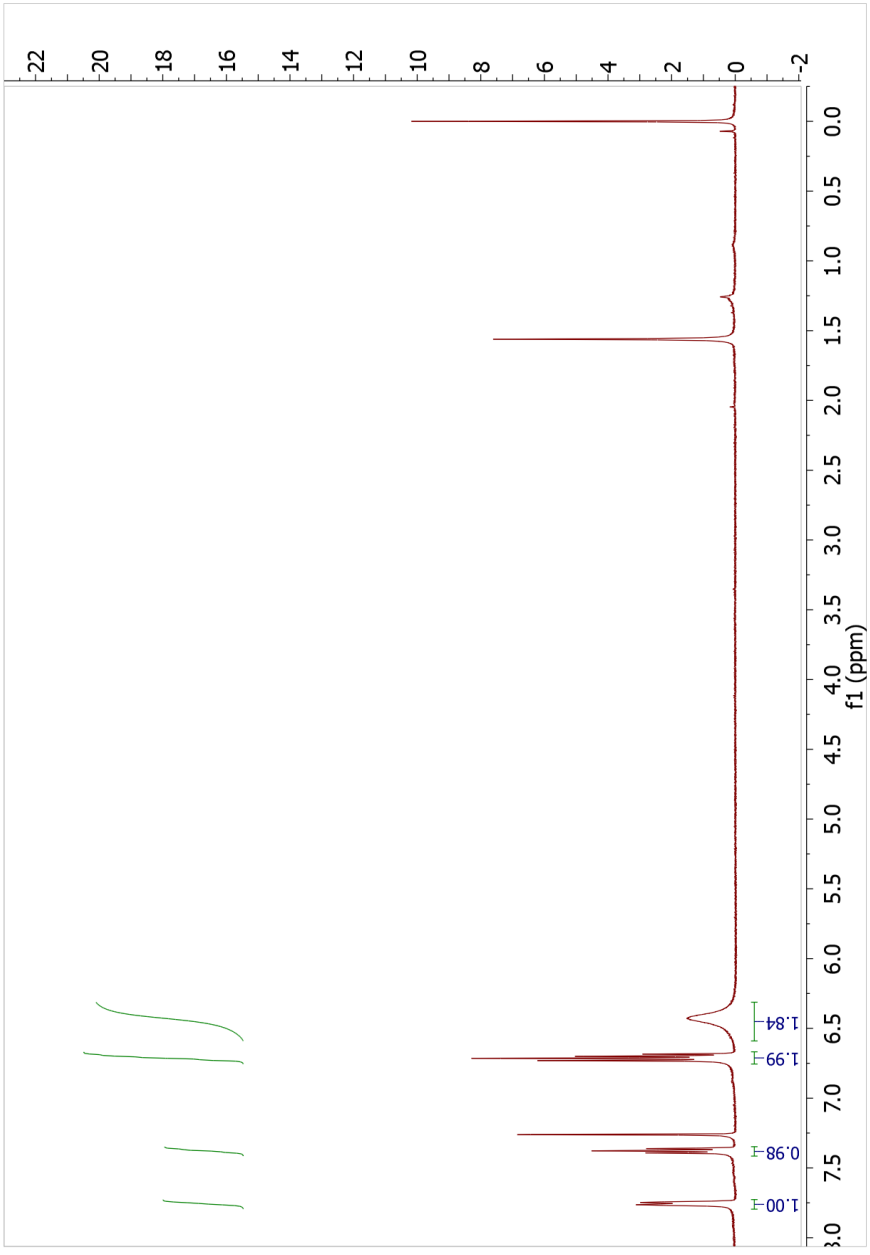



O
CF3
NH
O

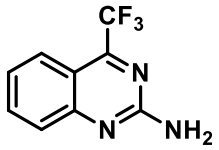

27.54
40.64
115.38
117.78
121.23
122.48
131.87
131.90
137.78
144.03
178.44
182.70
182.97
183.24
183.52
f1 (ppm)

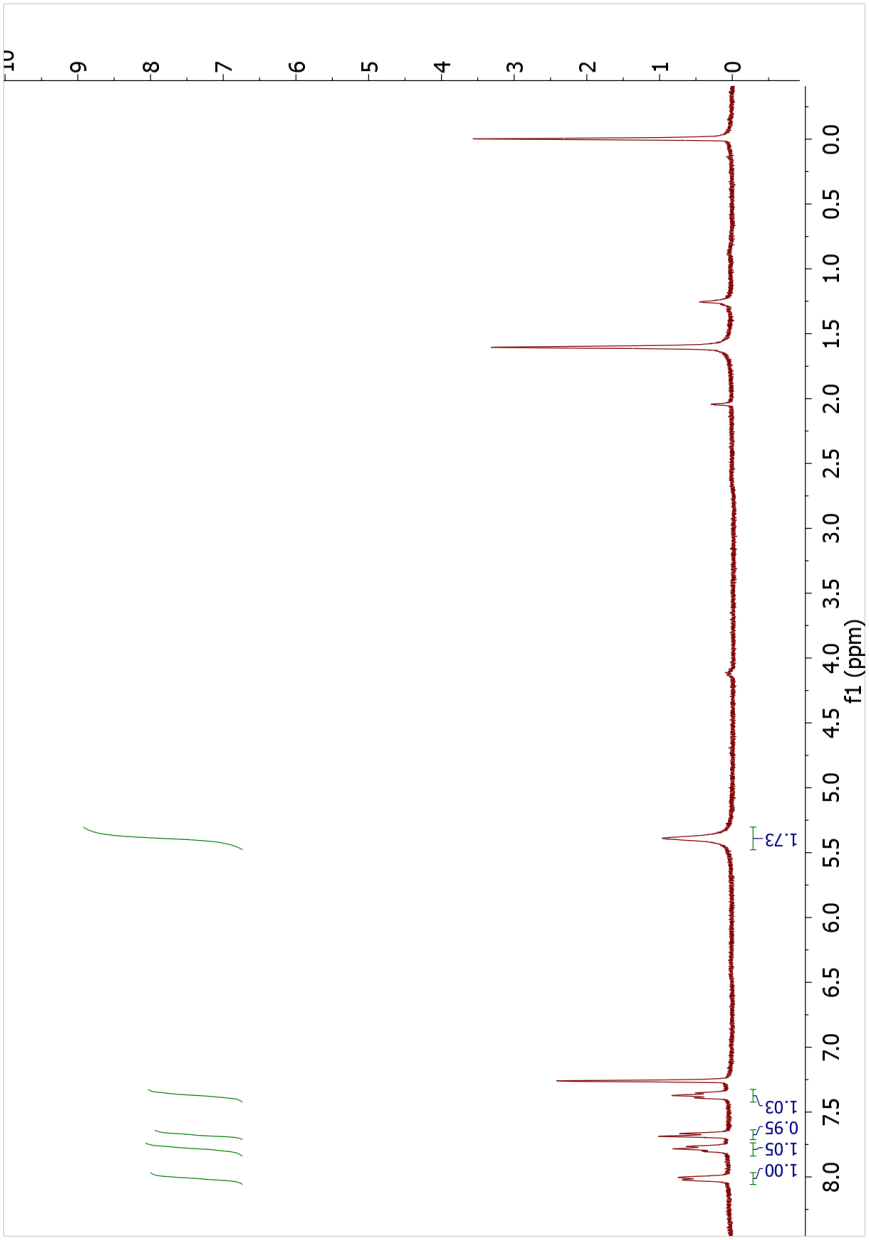
^{1}H NMR of Compound 3.21

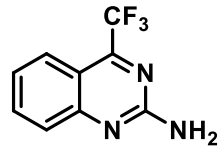


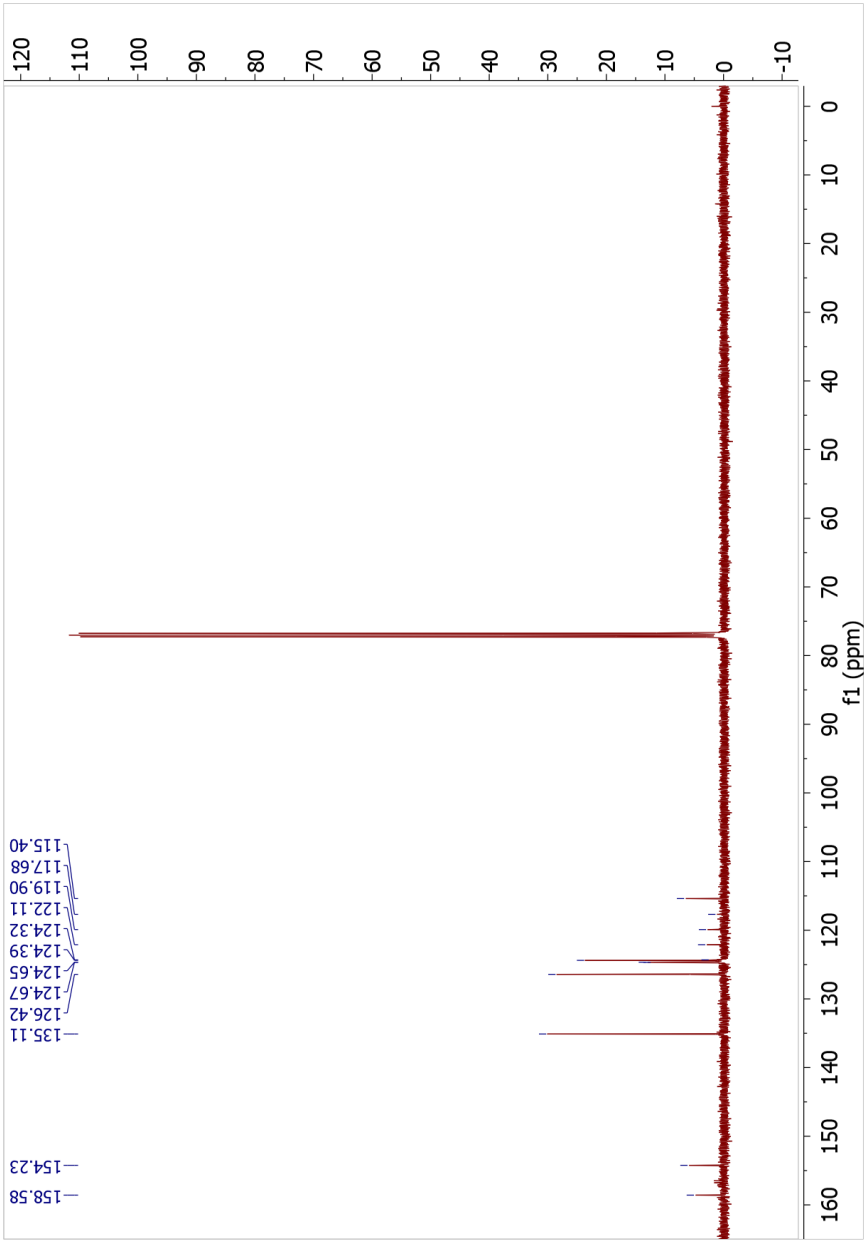


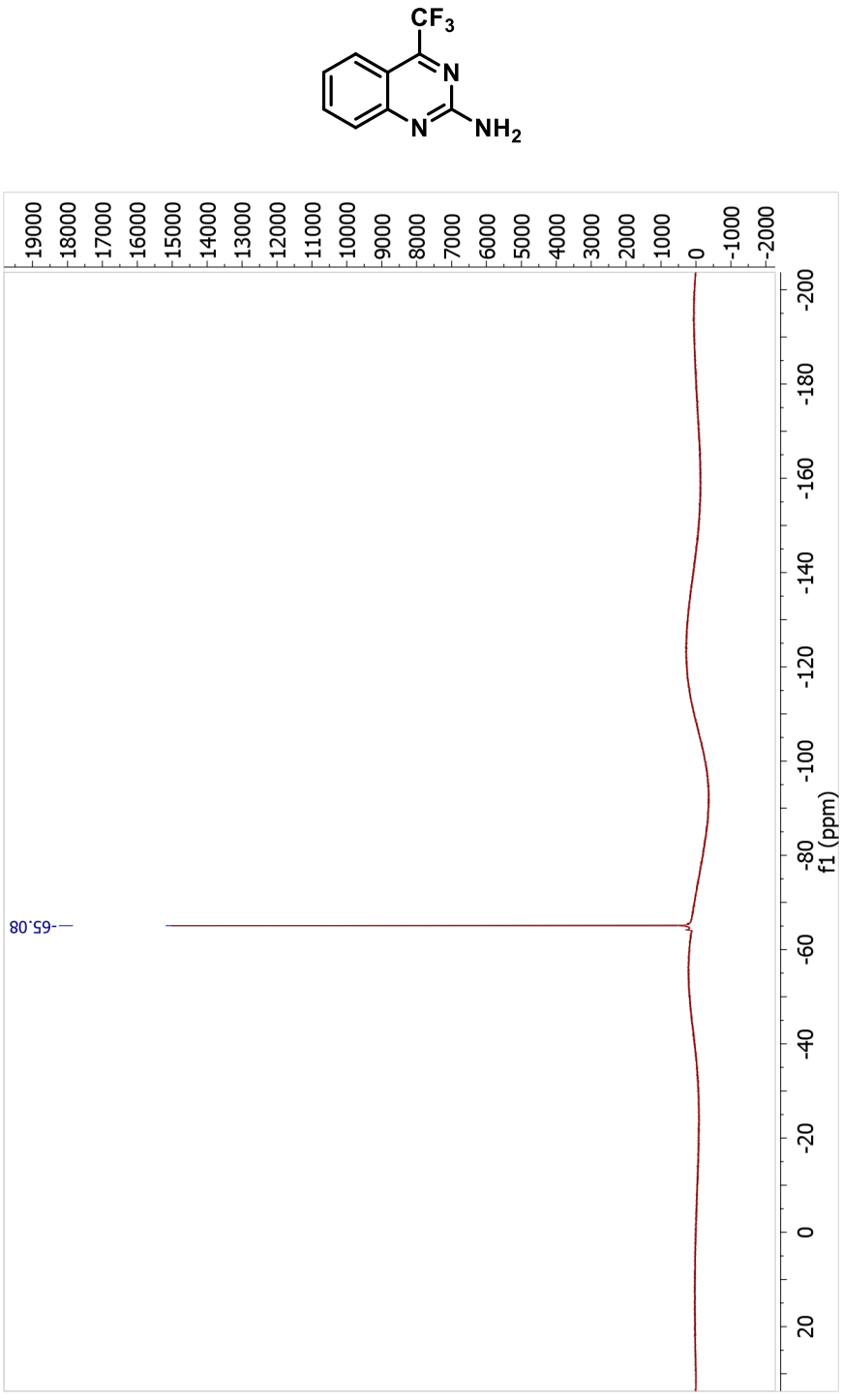
^{1}H, ^{13}C, and ^{19}F NMR of Compound 3.22



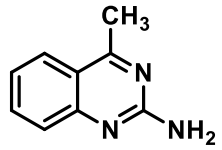


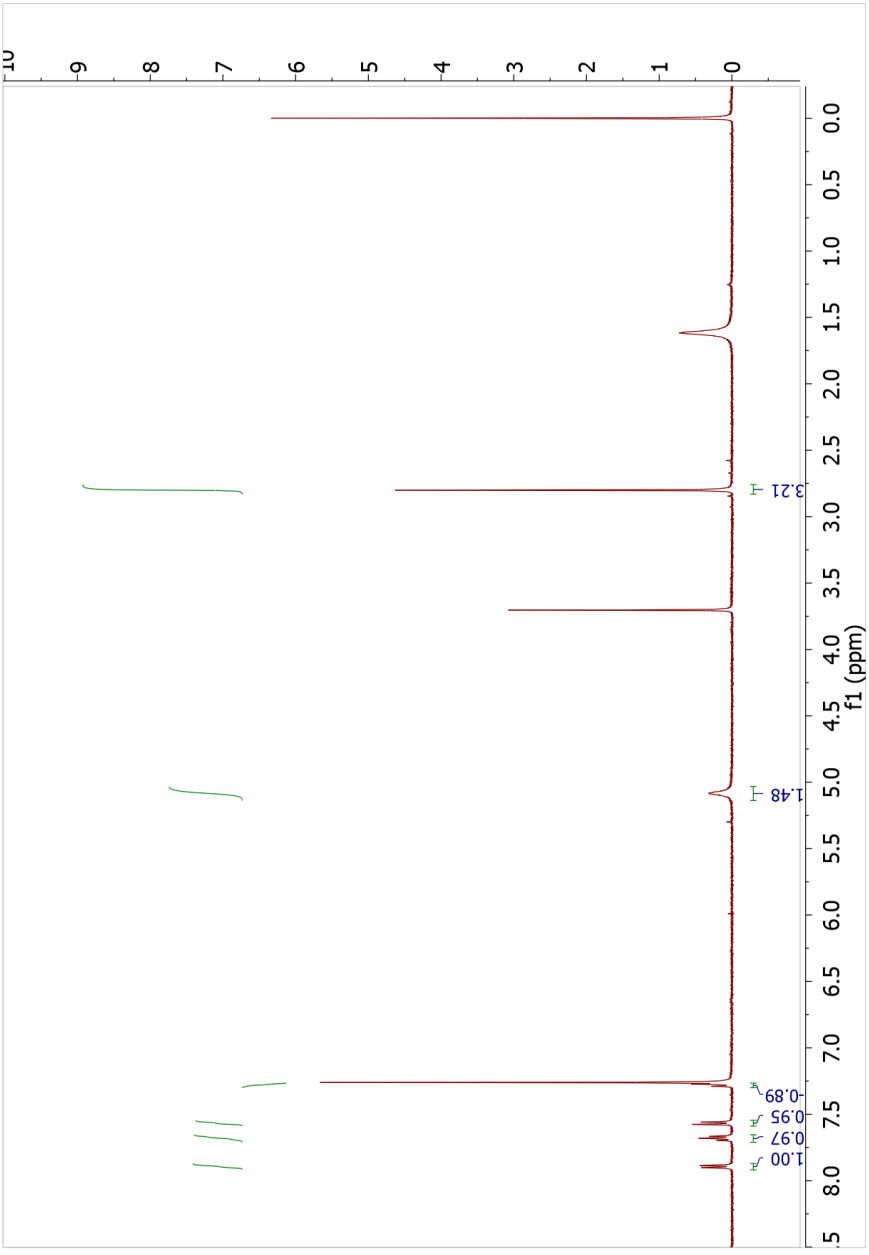

CF3
N
N
NH2


158.58
154.23
135.11
126.42
124.67
124.65
124.39
124.32
122.11
119.90
117.68
115.40
f1 (ppm)

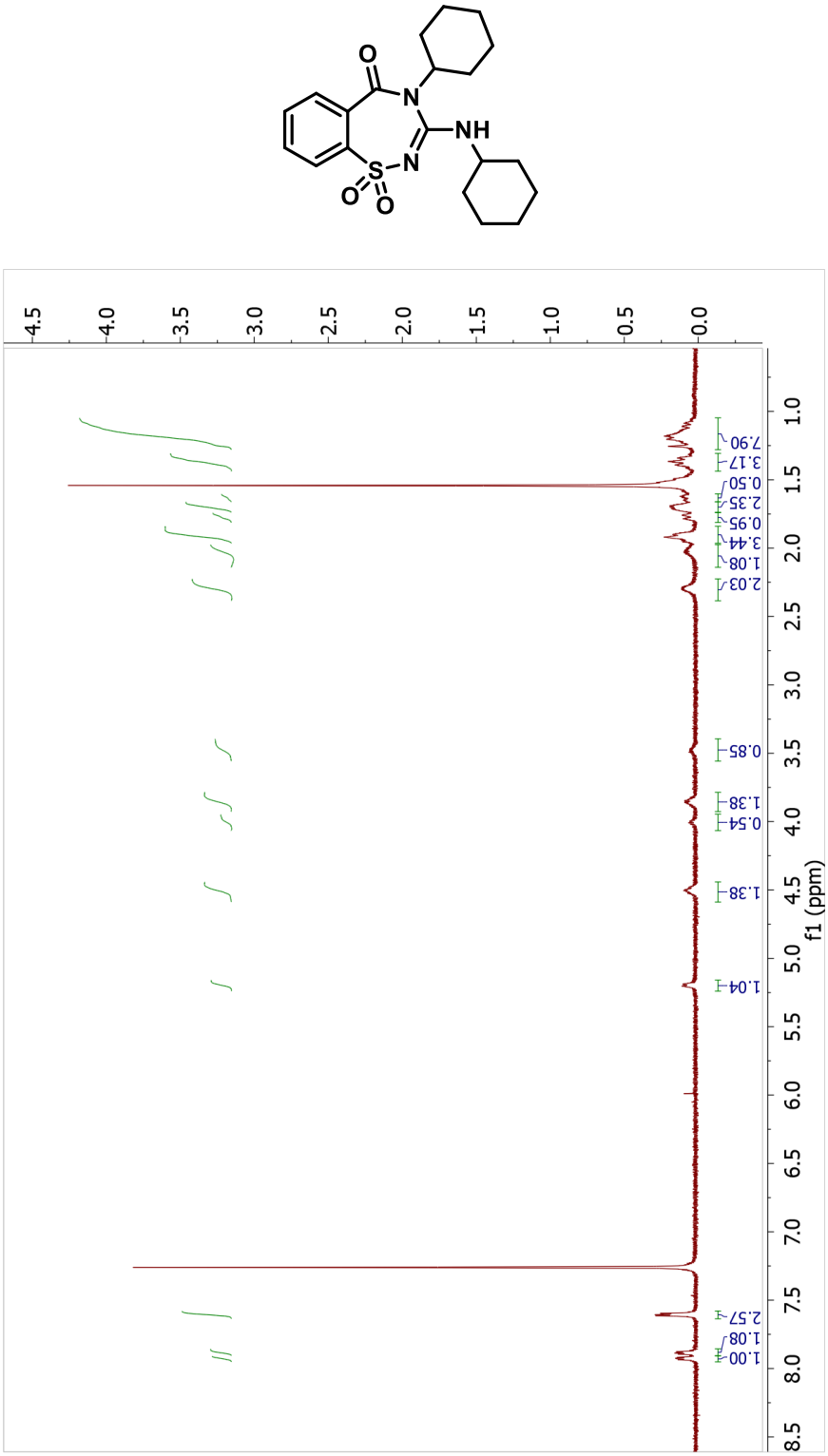

CF3
N
N
NH2
-65.08
f1 (ppm)

^{1}H NMR of Compound 3.24

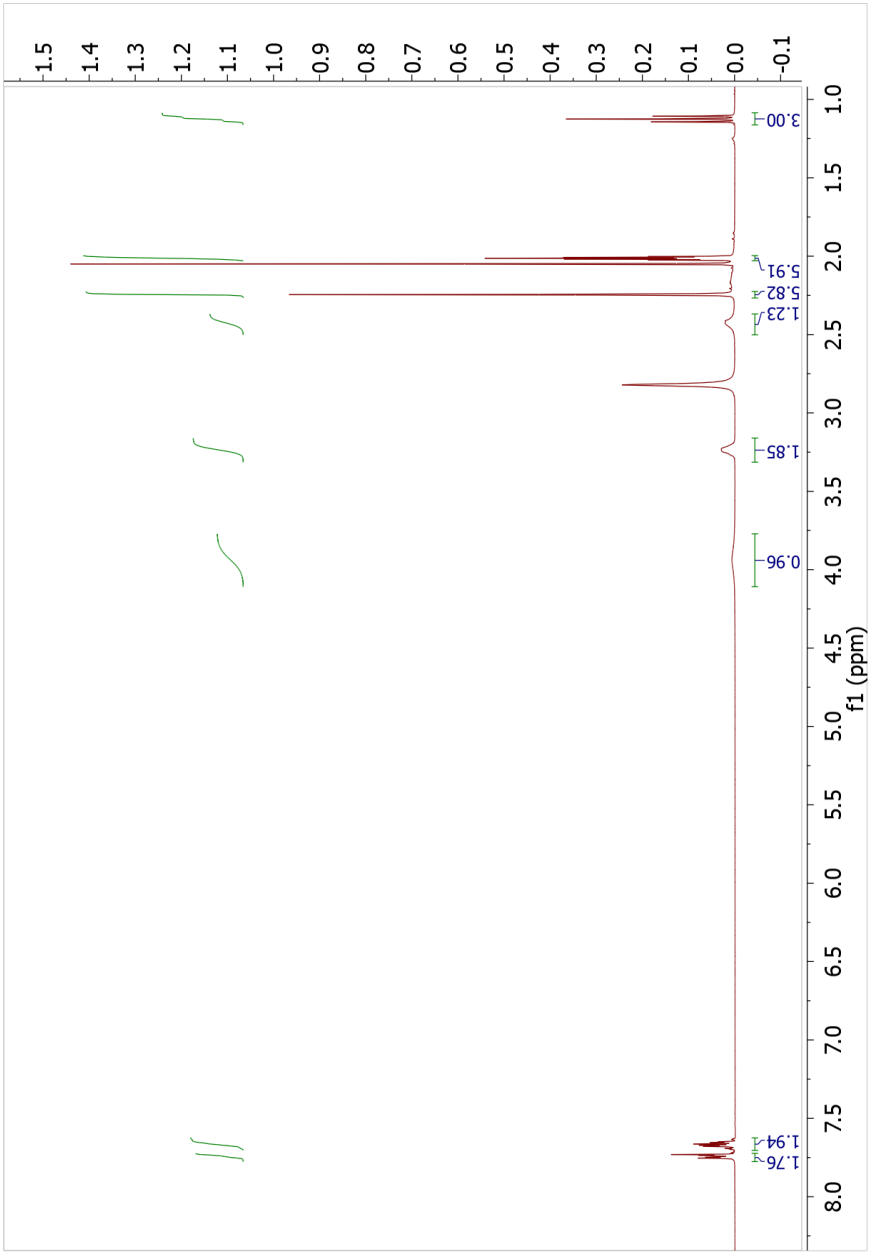




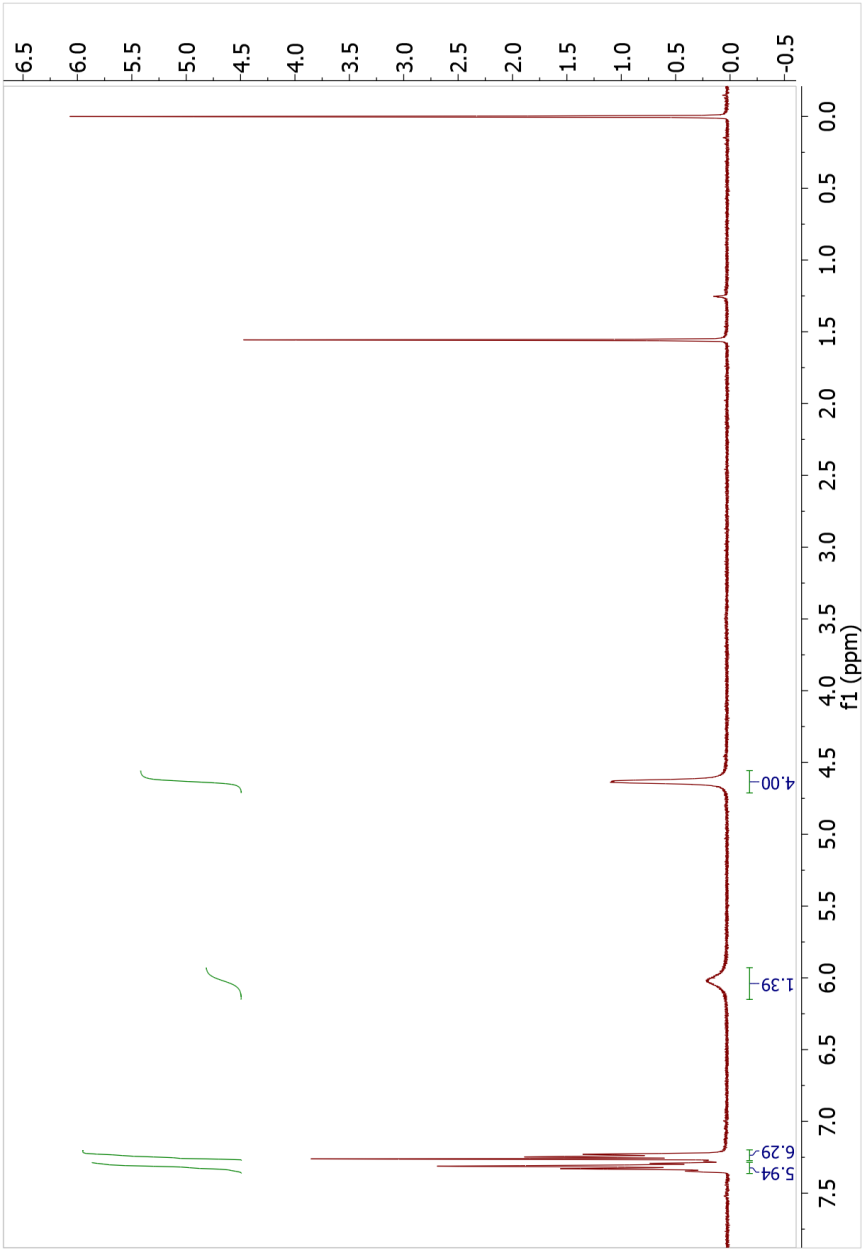
^{1}H NMR of Compound 3.27



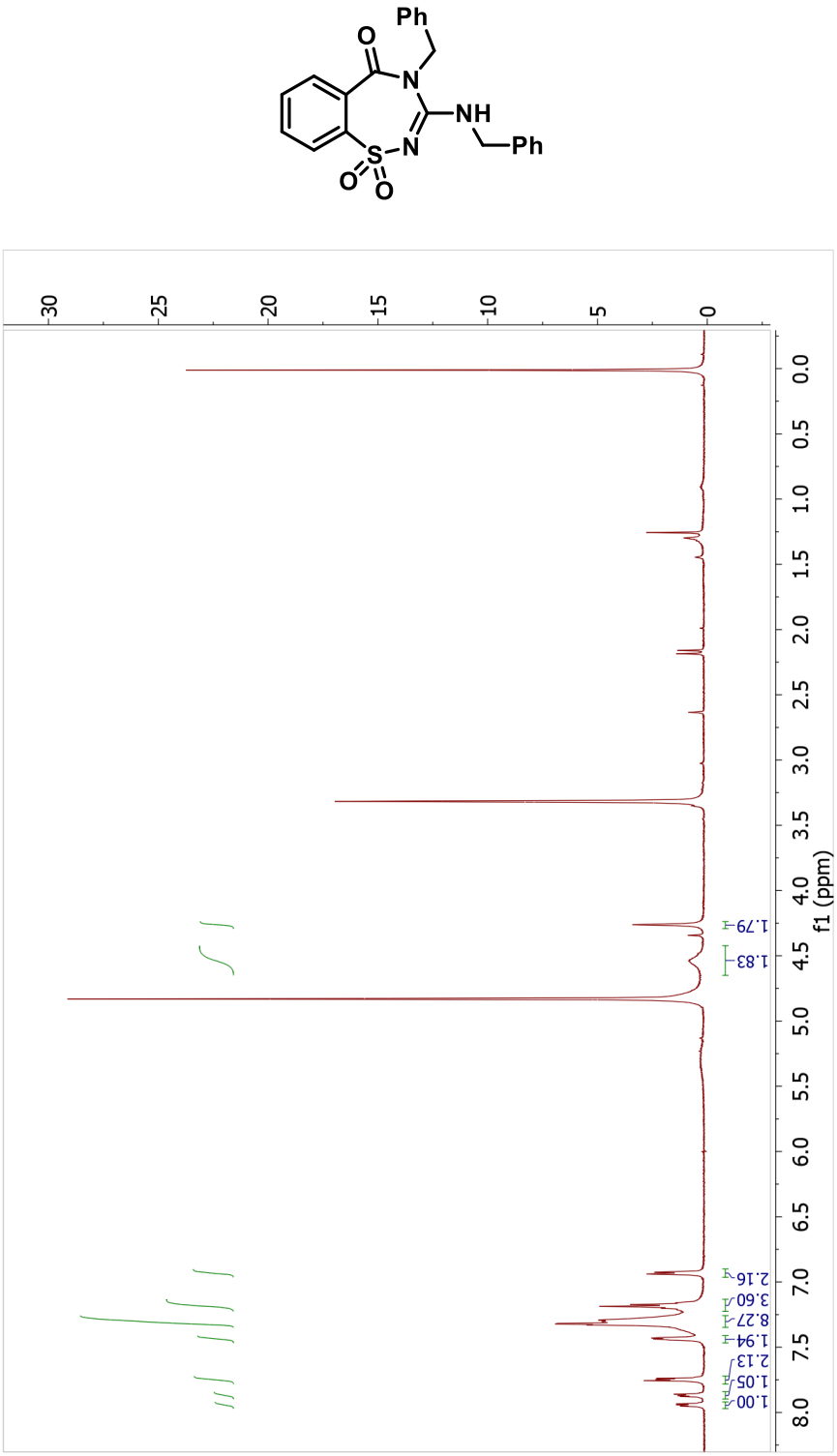
^{1}H NMR of Compound 3.28



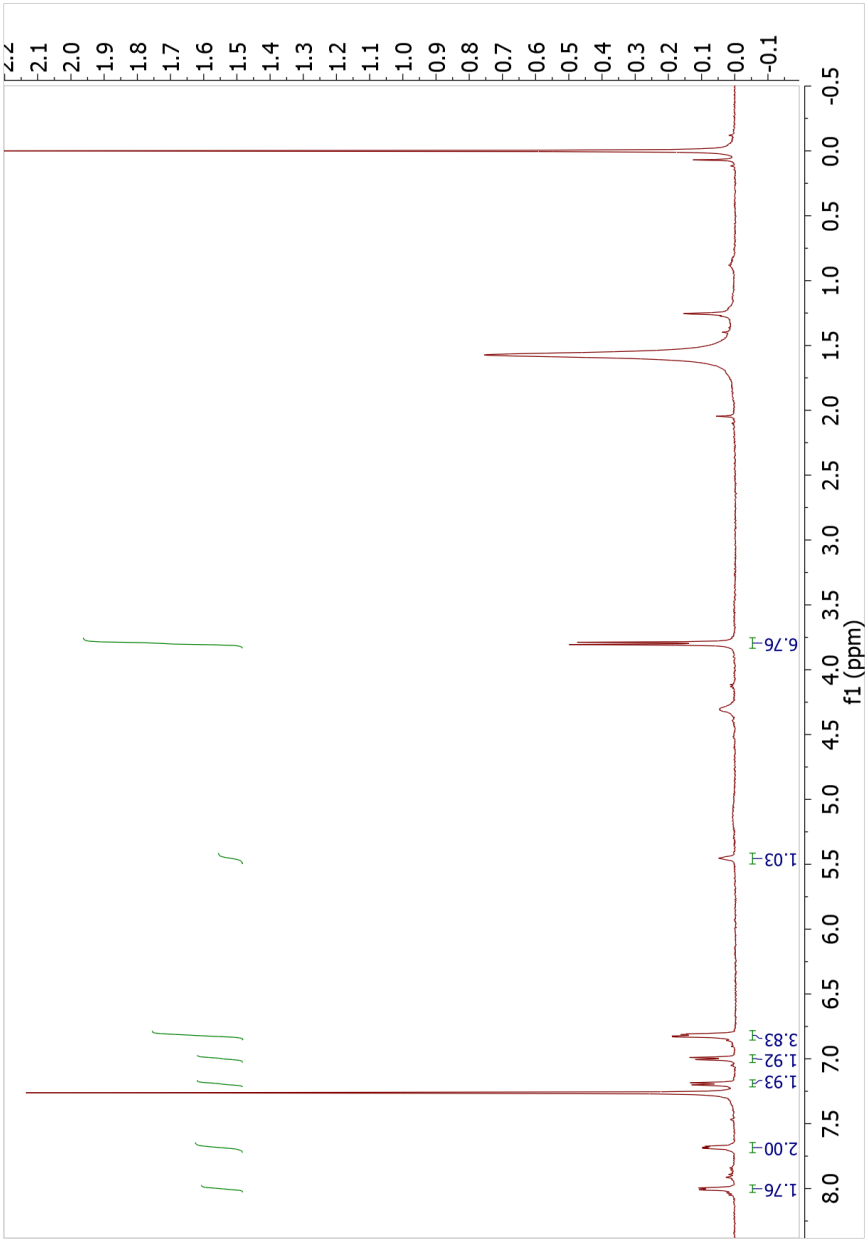
^{1}H NMR of Compound 3.29



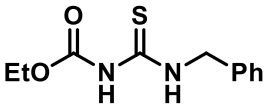
^{1}H NMR of Compound 3.30

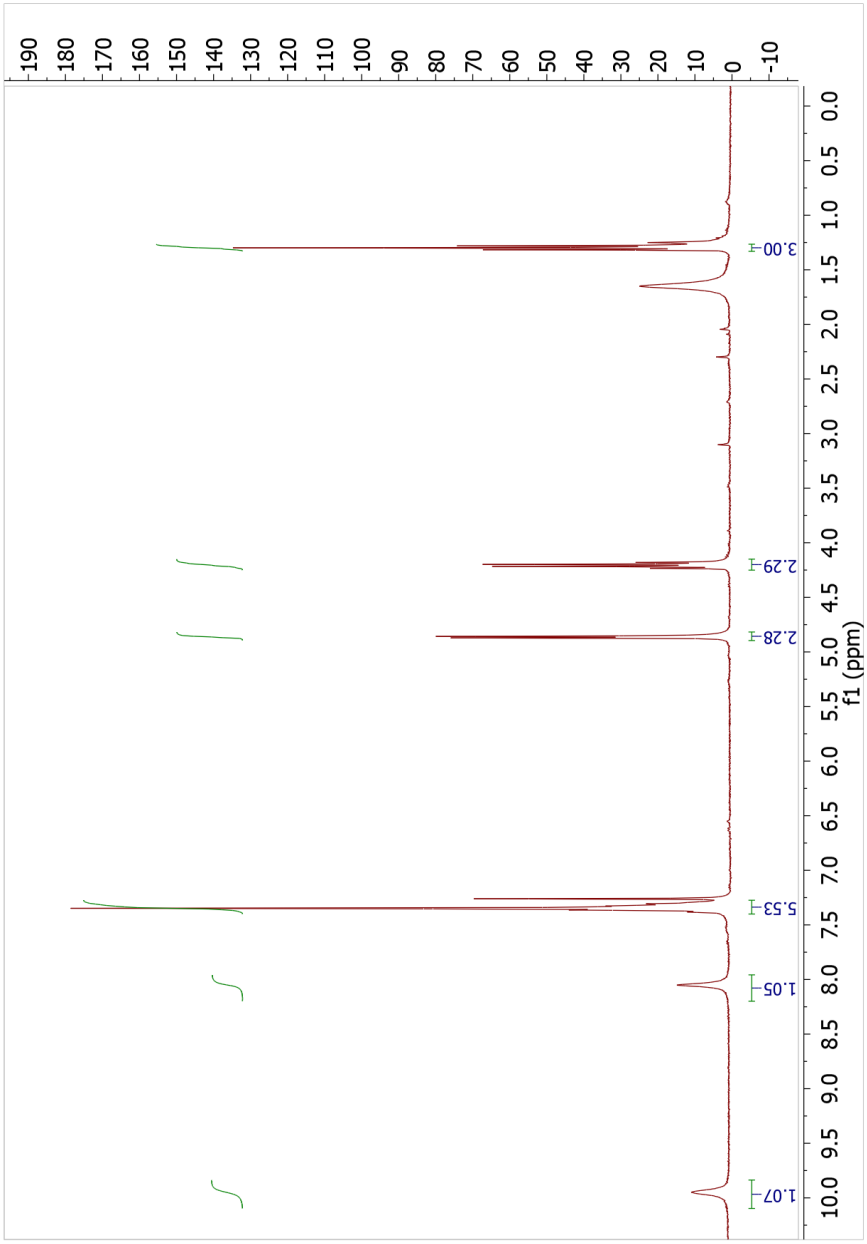


^{1}H NMR of Compound 3.33

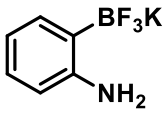


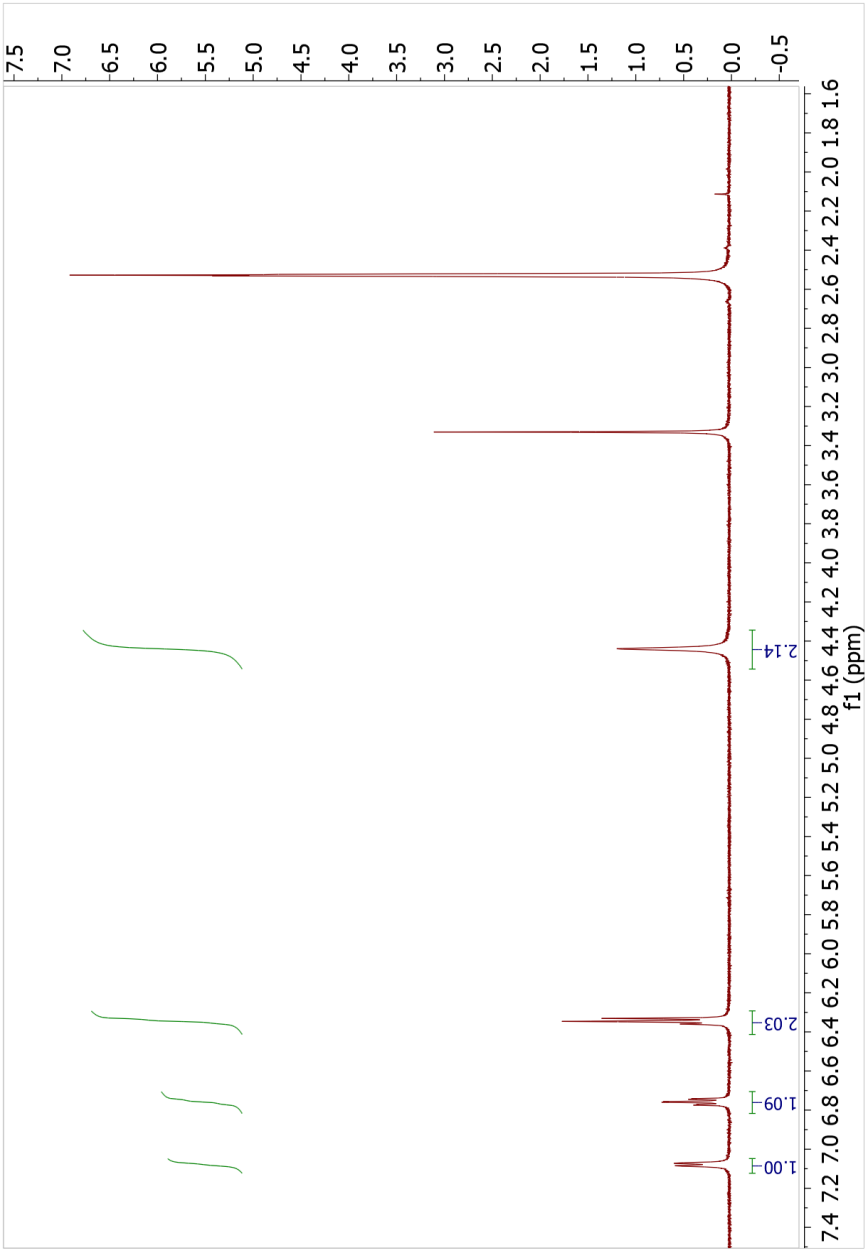
^{1}H NMR of Compound 3.34



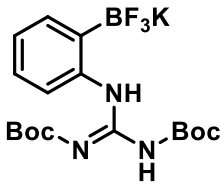


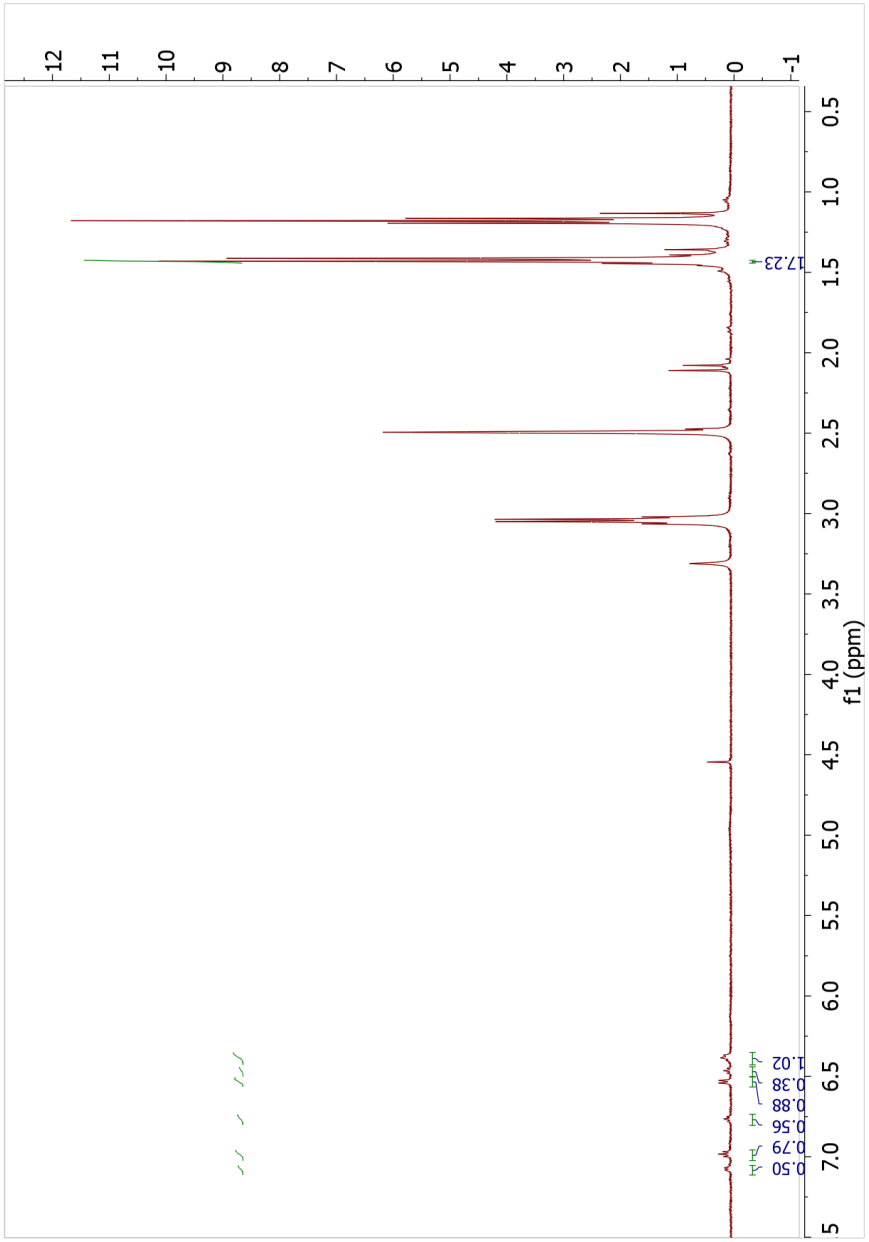
^{1}H NMR of Compound 3.40





^{1}H NMR of Compound 3.41





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