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Synthesis and Evaluation of Macrocycles as Potential Antitumor Agents

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

in

Chemistry

by

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2012

The Dissertation of Chung-Mao Pan is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California, San Diego

San Diego State University

2012

DEDICATION

To mom and dad, and my An

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LIST OF ABBREVIATIONS

μM	Micromolar
nM	Nanomolar
Oxa	Oxazole
^{t}Bu	*tert-Butyl*
HOBt	1-Hydroxybenzotriazole
DIC	*N,N'-Diisopropylcarbodiimide*
Fmoc	9-Fluorenylmethyl chloroformate
v/v	Volume to volume
DAST	Diethylaminosulfur trifluoride
°C	Degree Celcius
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
BrCCl3	Bromotricchloromethane
K_2CO_3	Potassium carbonate
PLP	Protected Linear Pentapeptide
SAR	Structure-activity relationship
HDI	Histone deacetylase inhibitor
HAT	Histone acetyltransferase
HDAC	Histone deacetylase
SanA	Sansalvamide A
NCI	National Cancer Institute
Abu	Aminobutyric acid
IC_{50}	half maximal inhibitory concentration

Boc	*tert-butoxycarbonyl*
OMe	Methyl ester
OEt	Ethyl ester
TBTU	O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate
HATU	2-(1H-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronoium hexafluorphosphate
DEPBT	3-(Diethoxy-phosphoryloxy)-3H-benzo[d][1,2,3] triazin-4-one
DCM	Dichloromethane
ACN	Acetonitrile
Bn	Benzyl
Cbz	Carboxybenzyl
Pd-C	Palladium on carbon
LiOH	Lithium hydroxide
MeOH	Methanol
HCl	Hydrochloroic acid
NMR	Nuclear magnetic resonance
LC/MS	Liquid chromatography/mass spectrometry
RP-HPLC	Reversed phase-high performance liquid chromatography
TLC	Thin layer chromatography
DIPEA	*N,N-Diisopropylethylamine*
Mg	Milligram
g	Gram

mL	Milliliter
s	Singlet
d	Doublet
t	Triplet
q	Quartet
p	Pentet
m	Multiplet
br	Broad
dd	Doublet of doublet
dq	Doublet of quartet
Na_2SO_4	Sodium sulfate
Ala	Alanine
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
Cys	Cysteine
Glu	Glutamic acid
Gly	Glycine
Pro	Proline
Ser	Serine
Tyr	Tyrosine
His	Histidine
Ile	Isoleucine

Leu	Leucine
Lys	Lysine
Met	Methionine
Phe	Phenylalanine
Thr	Threonine
Trp	Tryptophan
Val	Valine
EA	Ethyl acetate
$NaHCO_3$	Sodium bicarbonate
Na2SO4	Sodium sulfate
TFA	Trifluoroacetic acid
min	Minute
h	hour
M	Molar
Hex	Hexane
DDLP	Double deprotected linear pentapeptide
THF	Tetrahydrofuran
mmol	Millimole
N	Normal
DMSO	Dimethyl sulfoxide
LR	Lawesson's reagent
$KHCO_3$	Potassium bicarbonate
OEt	Ethyl ester

EtOH	Ethanol
RT	Room temperature
SAHA	Suberoylanilide hydroxamic acid, Vorinostat
DME	1,2-Dimethoxyethane
EC_{100}	100% effective inhibiton concentration

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I cannot express my greatest gratitude to my mom and dad. You raised and supported me. Without your encouragement and love, I would never complete this journey. This dissertation is dedicated to you.

Chapter 2, in part, is a reprint of the material as it appears in "Design and synthesis of Hsp90 inhibitors": Exploring the SAR of Sansalvamide A derivatives" *Bioorganic Med. Chem.* **2010**, 18, 6822-6856 Sellers, R. P, Alexander, L. D, Johnson, V. A. Lin, C. –C, Savage, J. Corral, R, Moss, J, Slugocki, T. S, Singh, E. K, Davis, M. R, Ravula, S, Spicer, J. E, Oelrich, J. L, Thornquist, A, Pan, C. –M, and McAlpine, S. R. * The dissertation author was the primary investigator and author of this paper.

Chapter 3, in part, is a reprint of material as it appears in "Synthesis and biological evaluation of histone deacetylase inhibitors that are based on FR235222: A cyclic tetrapeptide scaffold." *Bioorganic & Medicinal Chemistry Letters*, **2008**, *18*, 2549-2554. Singh, E.K.; Ravula, S.; Pan, C-M; Pan, P-S; Vasko, R.C; Lapera, S.A.; Weerasinghe, S.; Pflum, M.K.H.; McAlpine, S.R. The dissertation author was the primary investigator and author of this paper.

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*Denotes PI on papers, order of authors indicates relative intellectual contributions, where first authors contributed the most after the PI contribution

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2. Synthesis and biological evaluation of Histone Deactylase inhibitors that are based on the FR235222 scaffold: A cyclic tetrapeptide scaffold
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FIELDS OF STUDY

Major Field: Organic Synthesis

Studies in Chemistry
Professor Shelli R. McAlpine

ABSTRACT OF THE DISSERTATION

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by

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Chapter 1

The first chapter discusses the background of peptides and their potential as drugs. The methodologies for peptide synthesis via solution and solid-phase as well as

macrocyclization strategies for cyclic peptides. Cyclic peptides' biological activity and the challenges for peptide drug development.

Chapter 2

This chapter emphasizes on the design, synthesis and Structural Activity Relationship (SAR) of San A derivatives. Six novel San A derivatives were designed based on the structural features of developed potent San A derivatives. The structural modifications of novel San A derivatives included: 1) D-amino acid incorporation, 2) insertion of *N*-Me-amino acid, and 3) Aliphatic size chain reduction. The cytotoxicity of synthesized San A derivatives were exanimated against pancreatic cancer cell line PL-45 using ^{3}H-Thymidine incorporation assay. The biological results and SAR studies of six novel San A derivatives were discussed in this chapter.

Chapter 3

Histone deacetylase (HDACs) are a class of enyzmes that are responsible for regulating the acetylation state of the histones. In many cancers, overexpression of HDACs is observed resulting hypoacetylation state of histones and improper gene transcriptions, which leads to tumor progression. Thus, the development of novel HDIs is critical for combating cancers. Chapter 3 focuses on the design and synthesis of novel Histone Deacetylase Inhibitors (HDIs) based on two cyclic tetrapeptide natural products: FR235222 and trapoxin B. The biological test results of developed novel HDIs and the discussion of structure-activity relationship are also presented.

Chapter 4

Urukthepelstatin A (Ustat A) is a macrocyclic compound, which composed of a heterocyclic backbone and a peptide segment. Ustat A exhibits potent anti-tumor activity (GI_{50} = 15.5 nM) and its biological mechanism varies from other structurally similar scaffolds. Thus, the development of Ustat A provides an opportunity to explore a novel cell-survival pathway. In chapter 4, a convergent synthetic approach for Ustat A and its derivatives was described. Ustat A derivatives were designed based on hetercycle placement strategy, which was never reported in literature. In this chapter, the synthesis of linear precursor and the cyclization attempt for Ustat A derivative Ustat A-2 were presented.

Chapter 1

Introduction

1.1 Introduction of Amino Acids

In nature, peptide molecules are constructed by utilizing amino acids as the basic building blocks. Peptide molecules are comprised of a *C*-terminus, *N*-terminus, and a side-chains (R). The character of an amino acid is generally determined by the properties of its side-chain. For example, serine is a polar amino acid because it contains a hydroxyl group attached to the β-carbon, while valine is a non-polar amino acid, because it contains an aliphatic side-chain.[1]

Besides dominating the characteristics of amino acids, side-chain groups are also used in peptide linkages and for polypeptide folding.[2] For instance, the thiol group of cysteine is widely used for connecting peptide sequences and for stabilizing proteins. Oxidation of two cysteines generates a disulfide bond creating a covalent link between amino acids.[3] Proline, a conformationally rigid amino acid, which consists of a nitrogen containing 5-membered ring, acts as a turn inducer in secondary protein structures and they are ususally found in the middle of α-helices or β-sheets.[4] Further, the stereochemistry of amino acid, L- or D-amino acids affects the overall 3-D conformation of peptide molecules and the molecular folding.[5]

1.2 Peptide as drugs

Utilizing structurally diverse amino acids nature generates biological active linear or cyclic peptide molecules.[6] The biological activities of these peptide molecules include antibodies,[7] peptide hormones,[8] and antibiotics.[9] Importantly, cyclic peptides are more resistant to enzymatic degradation than linear peptides.[10-12] In addition, reduced structural

freedom of the cyclic peptide structural conformation[13] leads to minimize the entropy penalty toward the binding affinity,[14] which translates into higher biological potency of the cyclic peptides. Macrocyclic peptides are effective drugs as exemplified by Vancomycin,[15] Colistin A,[16] and Cyclosporine A.[17] Moreover, peptide molecules can be assembled rapidly using commercially available chemically diverse amino acids in a relatively straightforward fashion.

1.3 Examples of Cyclic Peptide Drugs

Cyclic peptides are primarily derived from natural products found in microorganisms,[18,19] including marine sponge,[20] tunicates,[21] and fungi.[22] Those natural occurring cyclic peptides exhibit biological activity such as: antibacterial,[15] antiviral,[23] and immunosuppression,[17] and anticancer.[22]

Cyclosporine A,[17] a cyclic undecapeptide drug on the market (Neroal^{TM}) (**Figure 1.1**) was isolated from the metabolite of *Tolypocladium inflatum* in 1970 by Sandoz (now Novartis). Its potent immunosuppressant activity was discovered in 1976 and approved by FDA in 1983.[24] Cyclosporin A's biological mechanism involves interruption of the signaling pathway that activates T Cells.[25] Cyclosporin A is primarily prescribed for organ transplant patients, due to its ability to suppress the immune response and lower the risk of organ rejection. Two million annul prescriptions and 56 million U.S. dollars in saling indicate that cyclosporine A as one of the most successful drugs.[26]

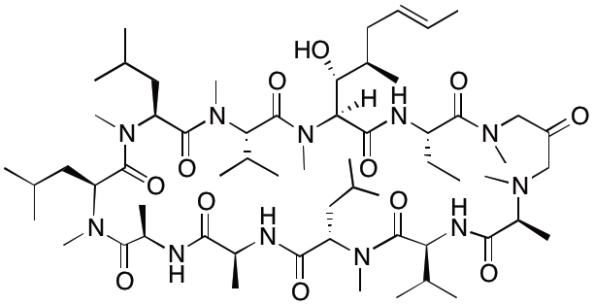


Figure 1.1 Structure of Cyclosporin A

The other well-recognized cyclic peptide drug is Vancomycin (Vancocin^{TM}) (**Figure 1.2**).[15] It was isolated in 1956 from the soil microbe *Amycolatopsis oreientalis* from the jungle of Borneo.[15] Vancomycin is a glycopeptide possessing antibiotic activity against Gram-positive bacteria and was introduced to the market by Eli Lilly in 1958.[27] The biological mechanism of Vancomycin involves interrupting the synthesis of the peptidoglycan layer of the bacteria, which consequently inhibits the growth of the Gram-positive bacteria because it inhibits cell wall synthesis.[27]

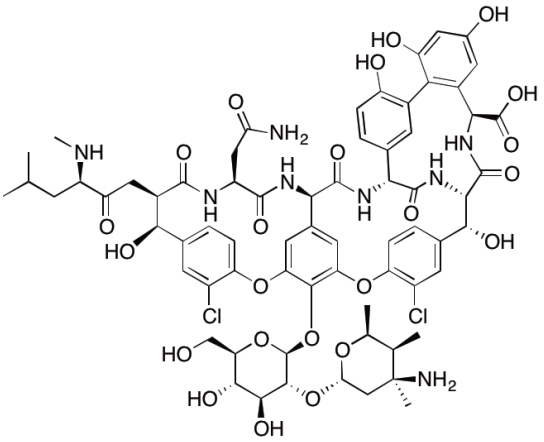


Figure 1.2 Structure of Vancomycin

Colistin A (**Figure 1.3**),[16] possesses potent antibiotic activity against Gram-positive bacteria and was isolated by Koyama and co-workers from *Bacillus polymyxa* in Japan.[28] It started being used therapeutically in the United States in the form of

colistimethate sodium in 1959 for the treatment of *Pseudomonas* infections.[29] Its mechanism of action involves displacement of phosphate ions from the membrane lipids by binding to lipopolysaccharides and phospholipids in the outer cell membrane of Gram-positive bacteria induces leakage of intracellular substances, eventually killing the bacteria.[30]

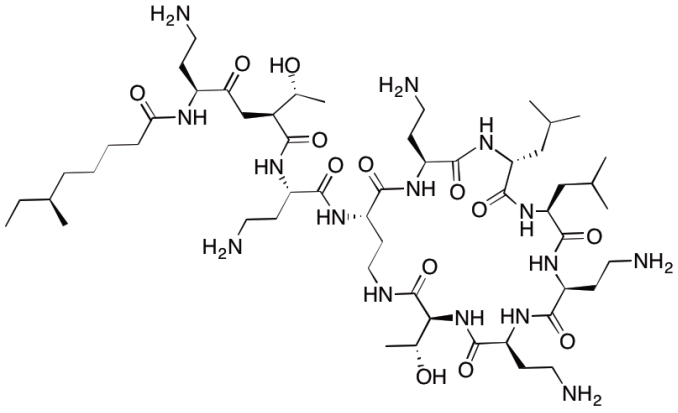
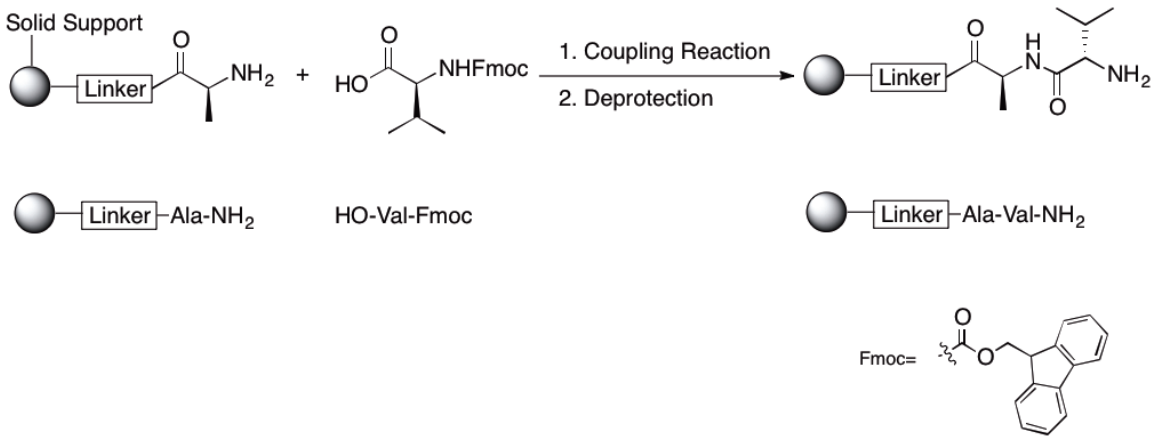


Figure 1.3 Structure of Colistin A

1.4 Amide Bond Formation Strategy

Chemists have done a magnificent job of developing strategies and reagents to synthesize peptide molecules for drug development. Solution phase synthesis is a classical approach to peptide synthesis and it allows chemists to monitor the products after each reaction. The other approach is solid phase synthesis making peptides (SPS), which was introduced by Merifield's group in 1963.[31] SPS strategy allows chemists performing reactions on solid supports (resins) and purifying molecules by washing off the excess reagents. Products remain attached to the solid support making purification straightforward. However, in SPS it is difficult to confirm that a reaction goes to completion, and challenging to identify the product before cleaving it from solid support. SPS is shown in **Scheme 1.1** where a free amine Ala-NH_2 is attached to a solid support

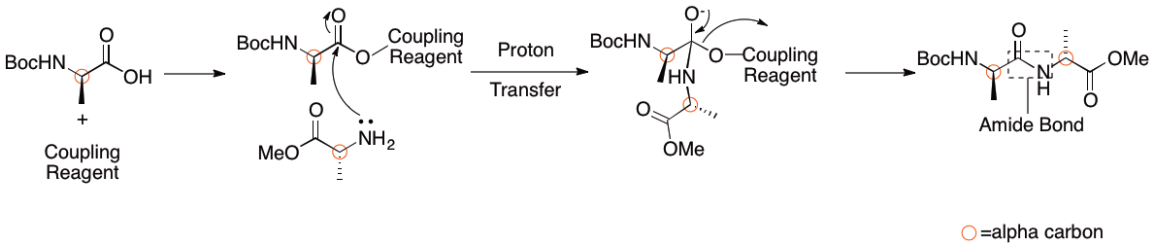
and was coupled to free acid HO-Val-Fmoc. The coupling is followed by a Fluorenylmethyloxylcarbonyl (Fmoc) removal reaction to obtain dipeptide free amine, which is made for further peptide elongation.



Scheme 1.1 Illustration of Solid Phase Peptide Synthesis

1.5 Peptide Coupling reagents and protecting groups

Amide bond formation is essential for building peptides and it is obtained through coupling. Utilizing coupling agents facilitates the reaction between the acid and amine Initiation of coupling involves activation of the free acid with coupling agents, followed by nucleophilic attack of the amine to the activated acid, generating an amide bond (**Scheme 1.2**).



Scheme 1.2 Mechanism of two Ala being coupled to form an amide bond

Numerous coupling reagents are available, and each offers advantages and disadvantages. In general, most coupling reagents are divided into 3 categories: carbodiimides, phosphonium-based reagents, and aminium-based reagents.

Carbodiimides

Dicyclohexylcarbodiimide (DCC, **Figure 1.4**), diisoprorylcarbodiimide (DIC), and ethyl-(N',N'-dimethylamino) propylcarbodiimide hydrochloride (EDC) are the most frequently used carbodiimides.

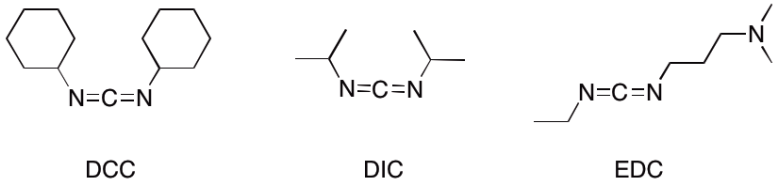
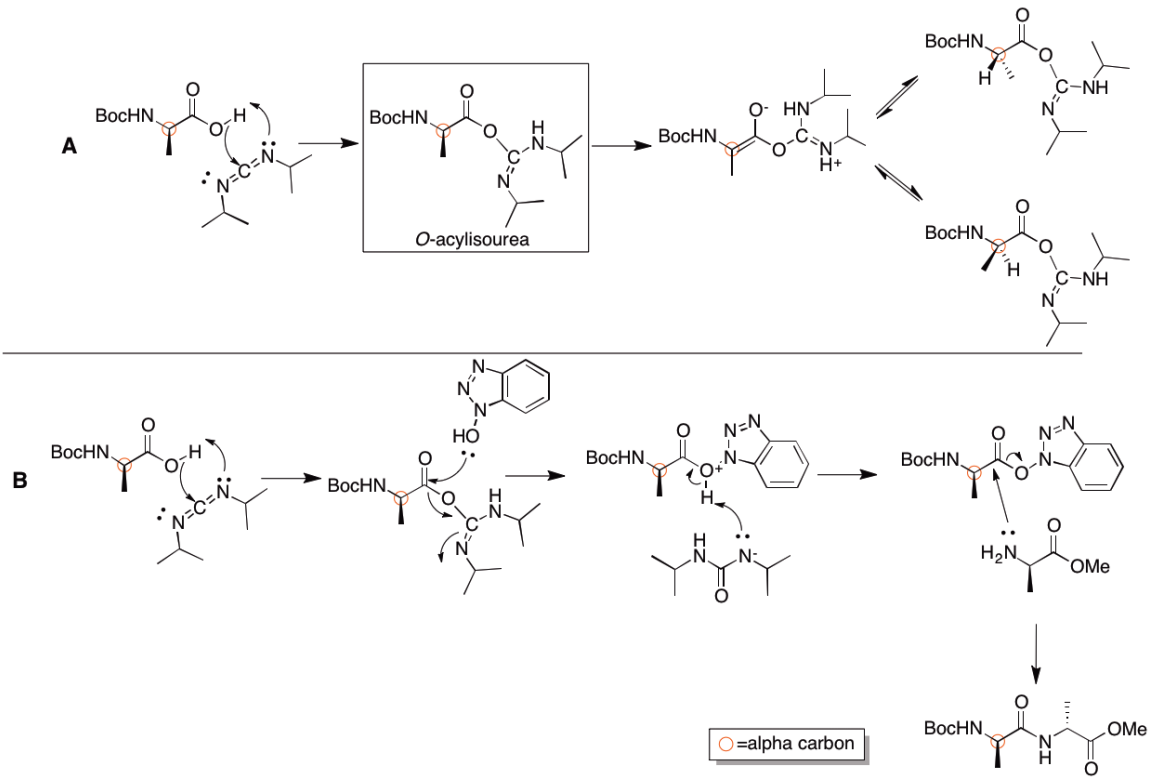


Figure 1.4 Structures of DCC, DIC, and EDC

The byproducts produced from these reagents, ureas, can be simply removed by aqueous extraction. During the peptide coupling reaction, the nitrogen atom on the *O*-acylisourea removes the proton on the α-carbon and intiates the racemization [32] (**Scheme 1.3A**). To minimize the undesired racemization, nucleophile 1-hydroxybenzotriazole (HOBt) is often used in conjunction with carbodiimides for acid activation. Benzotriazyl ester replaces *O*-acylisourea as acid activation group and minimizing this problem (**Scheme 1.3B**).[33]



Scheme 1.3 Mechanism of coupling reaction assisted by A) DIC, B) DIC and HOBt

Phosphonium-based reagents

The phosphonium-based coupling reagent was utilized with carbodiimide reagents to generate the benzotriazyl ester in situ, which reduces the racemization of the peptide coupling reaction. (Benzotriazol-1-yloxy)tris(dimethylamino) phosphonium hexafluorophosphate (BOP, **Figure 1.5**) is one of the first reagents[34] and it shows efficiency in facilitating peptide formation. However, the byproduct hexamethylphosphoramide (HMPA) of BOP is highly carcinogenic. (Benzotriazol-1-yloxy)tripyrrolidino-phosphonium hexafluorophosphate (PyBOP) is an alternative reagent. It shows comparable efficiency in facilitating peptide formation to BOP, and its byproduct is less toxic.

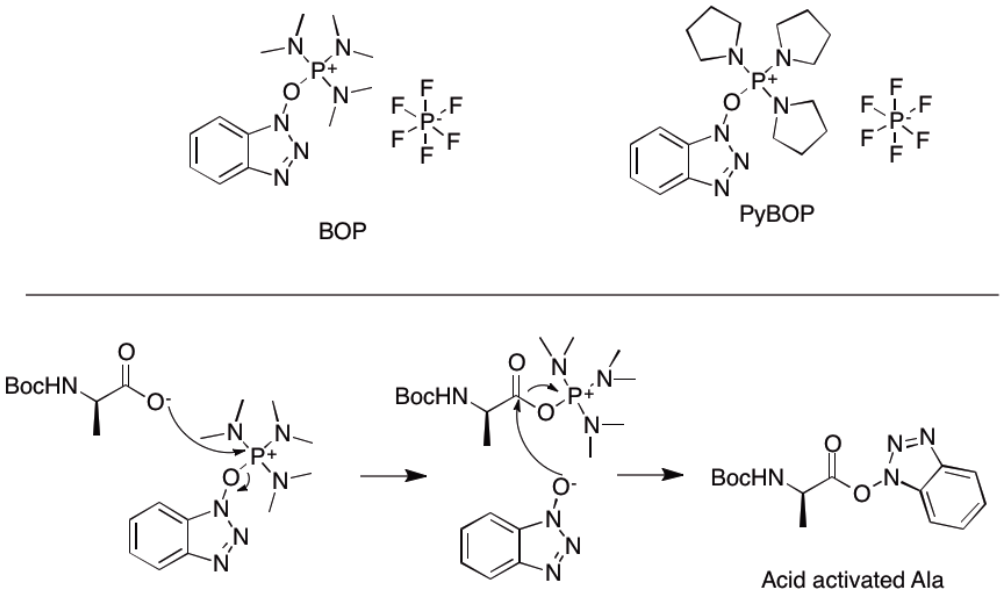


Figure 1.5 Structures of BOP and PyBOP and the acid activation mechanism of Ala using BOP

Aminium-based reagents

O-(Benzotriazol-1-yl)- N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU, **Figure 1.6**) is a popular coupling reagent that is very efficient in facilitating linear peptide coupling reaction and macrocyclizations. Furthermore, it has shown to a low racemization rate (d.e. = 97%).[35] The structure for O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) is similar to TBTU. However, the counter ion in HBTU is BF_4^- instead of PF_6^-. The other aminium-based coupling reagent is O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU). HATU is structurally similar to HBTU, where the benzotriazol group is replaced by an azabenzotriazol group. The incorporated nitrogen atom improves HATU's acid activation ability. During the coupling process activated azabenzotriaol ester is a better leaving group than than the benzotriazol ester. Thus, HATU is a more effective

coupling reagent than HBTU. It is also found to be the best coupling reagent for generating peptide bonds starting from secondary amines.

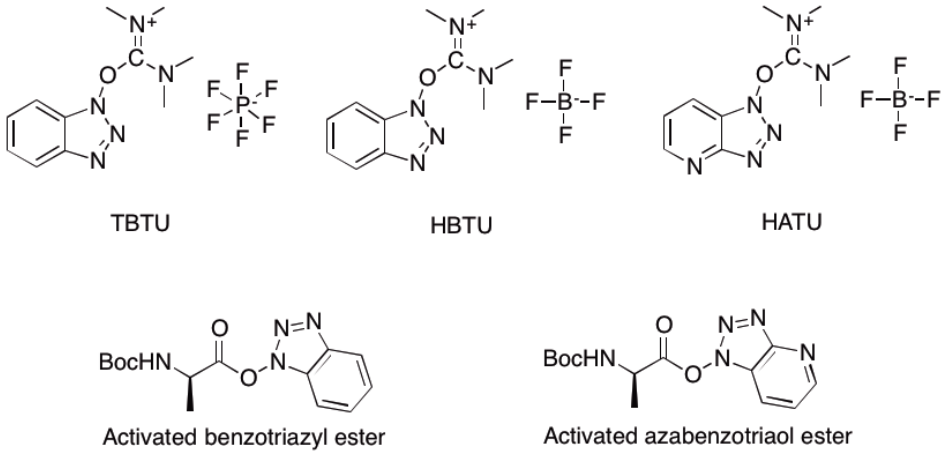


Figure 1.6 Structures of TBTU, HBTU, HATU, and the activated Ala benzotriazyl/azabenzotriaol ester

Other coupling reagent

3-(Diethylphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one (DEPBT) (**Figure 1.7**) is a coupling reagent that facilitates macrocyclization with little racemization (d.e. >98%).[36,37]

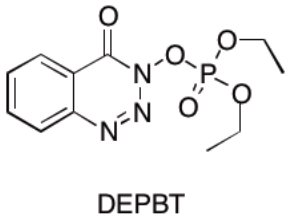


Figure 1.7 Structure of DEPBT

Though many coupling reagents are effective I have chosen to use 3 coupling reagents for my peptide synthesis: TBTU, HATU, and DEPBT. TBTU is cheap and efficient for simple di- and tri-peptide coupling reactions. The superior coupling

efficiency of DEPBT and HATU make them outstanding choices for complex coupling including N-methylated amines and cyclizations.

Protecting groups

In organic synthesis protecting groups are utilized to protect specific functional groups from a set of reaction conditions. Protecting groups need to be carefully selected to avoid being removed under specific reaction conditions. In the following chapters, I employed *tert*-butyloxycarbonyl (Boc, **Figure 1.8**) as an amine protecting group, methyl ester (OMe) or ethyl ester (OEt) for carboxylic acid, benzyl (OBn) and carbobenzyloxy (CBz) groups for hydroxyl and guanidine protecting groups respectively, and a ketal for carbonyl groups. Each protecting group is stable under the reactions conditions in which it is utilizaed.

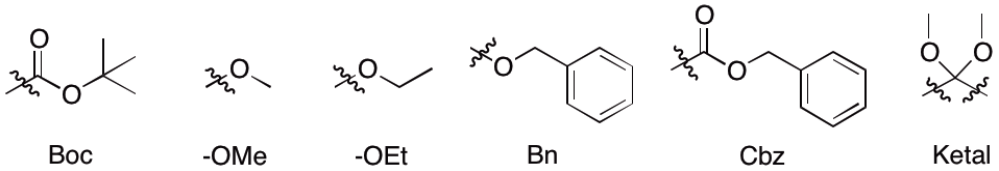


Figure 1.8 Structures of Boc, -OMe, -OEt, Bn, Cbz, and Ketal protecting groups

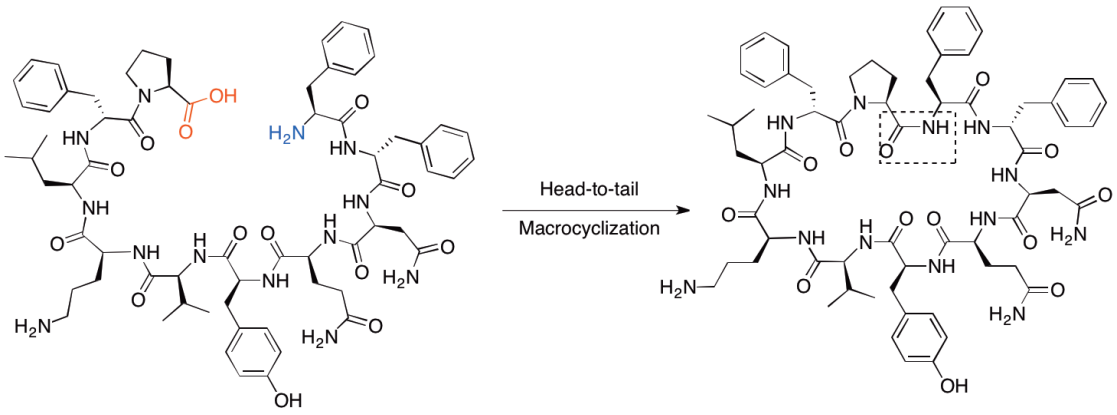
1.6 Cyclization Strategies for Cyclic Peptides

Macrocyclization (ring clousure) strategy can be classified into four categories: 1) Head-to-Tail,[38] 2) Head-to-Side Chain,[39] 3) Side Chain-to-Side Chain,[40] and 4) Side Chain-to-Tail.[41] Each strategy has been reported in literature and the following descriptions will provide examples of each strategy.

Head-to-Tail strategy

The head-to-tail cyclization is the most commonly used strategy in the synthesis of cyclic peptides. Connecting the *N*-termini and the *C*-termini of the linear peptide

precursor generates the macrocycle **(Scheme 1.4)**. Tyrocidine A[38] is a cyclic decapeptide with antibiotic activity and was synthesized in 1966 by Ohno group using a head-to-tail cyclization strategy.[39] The macrocyclization was carried out using a peptide coupling reaction between L-Phe and L-Pro residues. This was the strategy that I used on my molecules outlined in this thesis.



Scheme 1.4 Cyclization of Tyrocidine A via head-to-head strategy

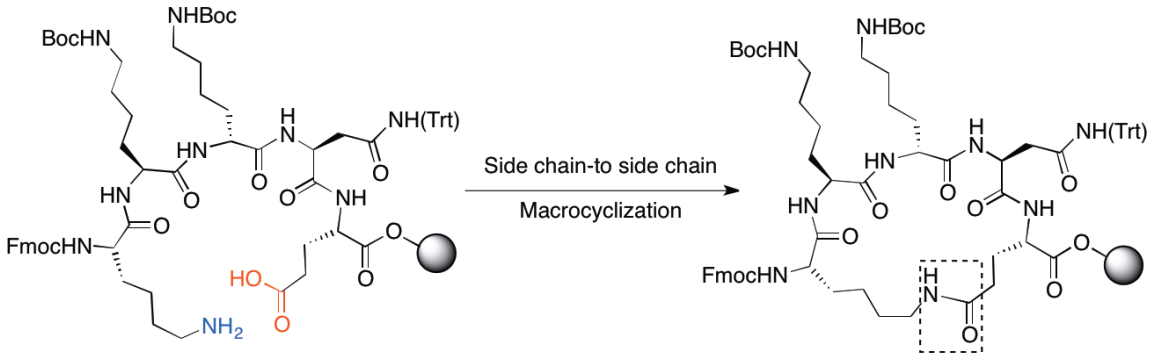
Head-to-Side-Chain strategy

This strategy is defined by connecting the side chain of an amino acid to the *N*-terminus of the peptide sequence. Yamasaki and co-workers reported a solution phase synthesis of an endothelin antagonis cyclic peptide RES-701-1[40] in 2003 (**Figure 1.9**) and the macrocyclization was carried out between the side chain of Asp and the *N*-terminus of Gly residue.

Figure 1.9 Macrocycle RES701-1 Synthesized via Head to Side Chain Strategy

Side-Chain to Side-Chain strategy

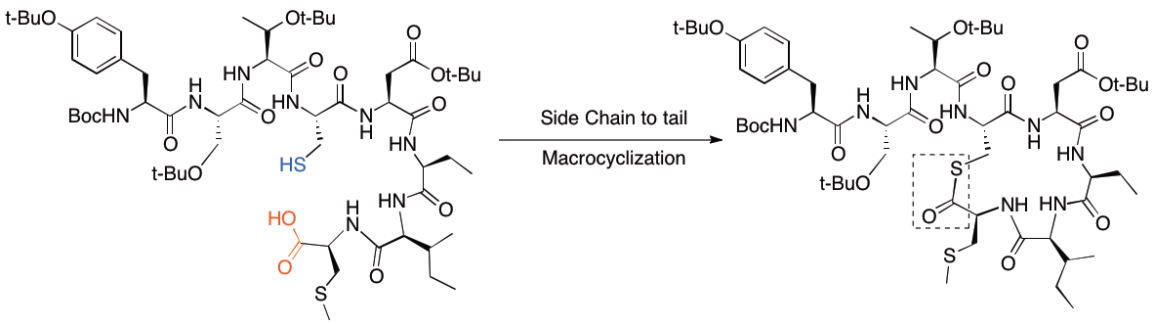
Utilizing the side-chain functionalities is another successful strategy for cyclizing. In 2000, Rana and co-workers completed the synthesis of the HIV active cyclic peptide shown in **Scheme 1.5**. This peptide macrocycle was synthesized by forming an amide bond between the side chain of Asp and Lys on solid support. This cyclic peptide inhibits HIV-1 gene expression by interrupting the HIV Tat-(TAR) RNA interaction.[24,41]



Scheme 1.5 Macrocycle synthesized via Side Chain-to-Side Chain Strategy

Side Chain-to-Tail strategy

Side chain to tail strategy is carried out by connecting the side chain and *C*-terminus. In 2003, Williams and co-workers[42] applied this strategy for the synthesis of cyclic thiolactone peptide. The macrocyclization was completed through thioester formation between the thiol group on Cys and the carboxyl group of the *C*-terminus (**Scheme 1.6**).



Scheme 1.6 Cyclic Thiolactone Peptide Synthesized via Head to Side Chain Strategy

1.7 Cyclic Peptide in Recent Drug Discovery and Focus of this Research

Many cyclic peptides have biological activity, including antibacterial,[15] antiviral,[23] immunosuppressive,[17] and anticancer.[22] Cyclic peptides provide a wide range of targets and valuable applications, making them excellent lead structures in the drug discovery. However, there is still a huge challenge to convert potent cyclic peptides into drugs. Thus, understanding cyclic peptides' structure activity relationship (SAR) and the role that conformations play in their biological activity are crucial for designing new drugs.

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Chapter 2

Synthesis of Sansalvamide A Derivatives

2.1 Discovery and Background of Natural Product Sansalvamide A

Sansalvamide A (San A) is a depsipeptide (**Figure 2.1**) isolated from a marine fungus (*Fusarium sp.*) in 1999 by Fenical and co-workers. [1-3] This marine fungus was found off the San Salvadore coast in the Bahamas. The depsipeptide displayed anti-cancer activity against National Cancer Institute (NCI) 60 cancer cell-lines panel (mean IC_{50} = 42.5 μM). The novelty and complexity of SanA's cyclic depsipeptide structure makes it a promising scaffold for development as anti-cancer agent.

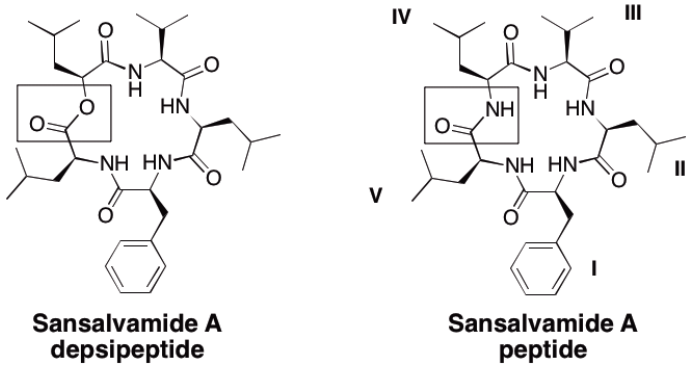


Figure 2.1 SansalvamideA Depsipeptide and Peptide

SanA was first synthesized by the Silverman group [4, 5] in 2000 and its anti-cancer activity was examined against pancreatic, colon, breast, prostate, and melanoma cancers. [6,7] SanA peptide (**Figure 2.1**), the peptide analogue of SanA, has an amino acid in position IV, was also synthesized by the Silverman group.[4] The peptide showed a 10-fold increase in anti-cancer potency compared to the San A depsipeptide, which is likely due to the higher stability of the peptide bond compared to the ester. During my Ph. D. study I

and my colleagues explored the structural activity relationship (SAR) of the SanA peptide as a potential anti-cancer therapeutic agent.

2.2 Retrosynthetic Strategy of SansalvamideA derivatives

I used a solution phase convergent synthetic approach involving two fragments (**Figure 2.2**) to generate SanA peptide derivatives.[8-10] SanA macrocycles were afforded from a cyclization reaction between the free acid (residue I) and free amine (residue V) of the double deprotected pentapeptide. The double deprotected linear pentapeptides were acquired via peptide coupling between the free acid (fragment B) and the free amine (fragment A) followed by double deprotection. The two fragments were generated using commercially available amino acids. This strategy allowed us to generate SanA peptide derivatives in a rapid and systematic fashion for extensive biological studies.

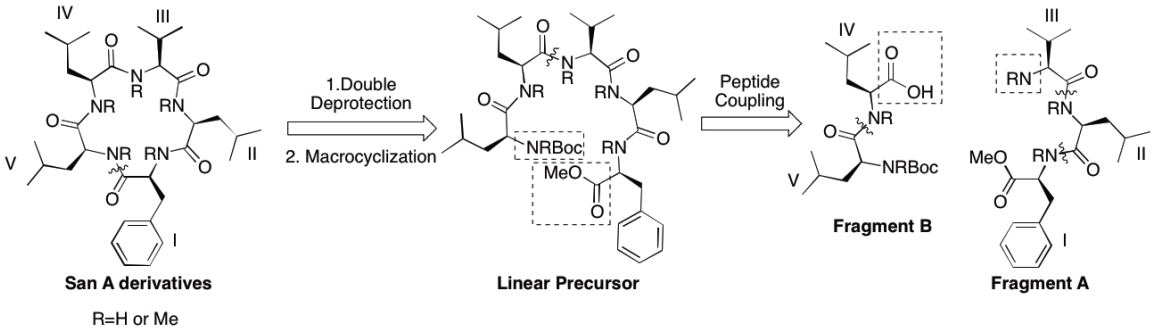


Figure 2.2 Retrosynthetic Strategy via a Convergent Approach for Sansalvamide A

Both L- and D-amino acids were used to develop our library of San A derivatives where these derivatives allowed us to study the molecules' SAR. I have contributed six SanA derivatives (**Figure 2.3**), which were numbered from SanA-**1** to SanA-**6** and colored in blue (**Figure 2.3**). These derivatives were developed for evaluating the cytotoxicity effect of three major structural features: (1) D-amino acid installation; (2) Aliphatic side chain modification and; (3) *N*-methylated acid incorporation. Combining

the structural features of the potent compounds in the library we designed a series of SanA analogues in other to evaluate additive and synergistic effects by that exist in our library.

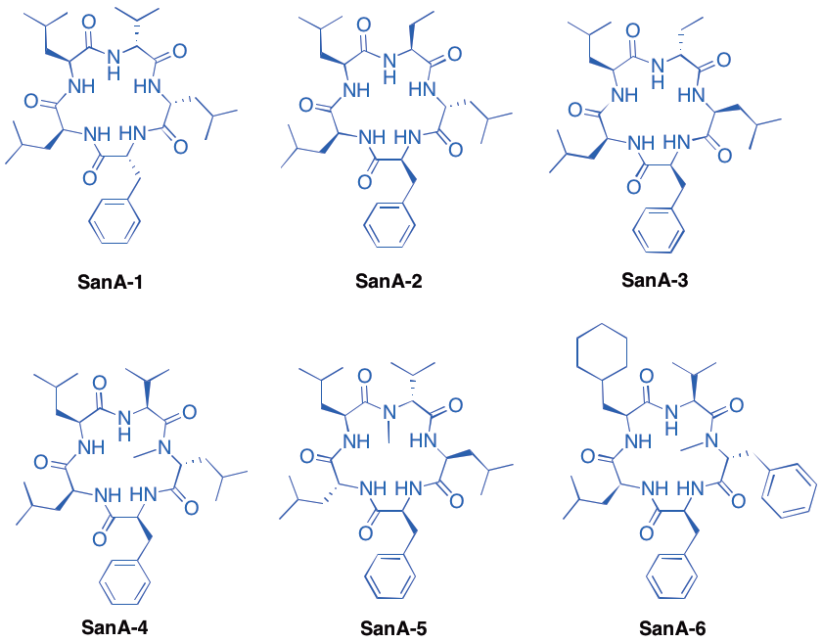


Figure 2.3 SanA derivatives that were synthesized by me

2.3 Rational Design of San A Derivatives

D-amino acid installation

D-amino acids are found in opioid peptides that are produced by microorganisms. The spatial orientations of D-amino acids is opposite from L-amino acids, thus, installing D-amino acids into SanA derivatives will change their 3D-conformations. Since the macrocycle conformation dictates biological activity, modifying the 3-D structure impacts the compounds' anticancer potency. Furthermore, adding D-amino acids to the peptide sequence can also reduce the enzymatic degradation by most proteases and ultimately prolong the compounds' half-life. SanA-**1**, has three consecutive D-amino acids introduced at positions I, II, and III. This compound was designed to explore the

effect of combining the features of potent molecules SanA-**7**, SanA-**8**, and SanA-**9** (**Figure 2.4**). These molecules exhibit potent growth inhibition against PL-45 pancreatic cancer cell line (**7**, 48%; **8**, 41%; **9**, 74%) at low micro-molar concentration (5 μM) and each had a single D-amino acid at positions I, II, and III, respectively.

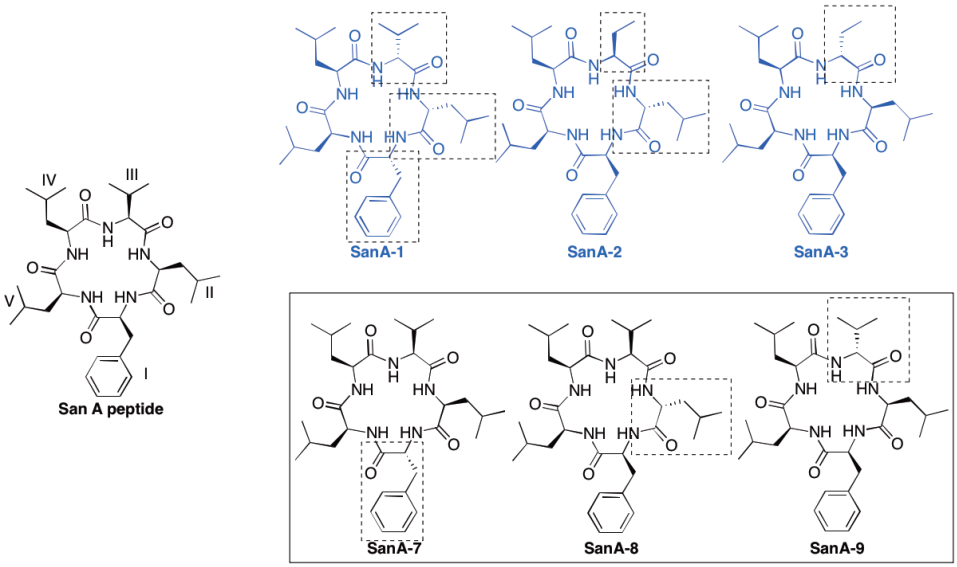


Figure 2.4 Rational Design of SanA-1, SanA-2, and SanA-3

Aliphatic side chain reduction

In SanA-**2** (**Figure 2.4**) D-Leu was incorporated at position II and distinguished itself from SanA-**8** by and replacing L-Val with a smaller L-Abu at position III. This molecule was generated to determine if reducing the aliphatic side chain size at position III impacted to cytotoxicity. The same rational was applied to SanA-**3**, which was derived from SanA-**9** by replacing D-Val with D-Abu at position III.

N-methylated D-amino acid incorporation

N-Me amino acids modify macrocycles by reducing their mobility. Kessler and co-workers have shown the addition of *N*-Me amino acids in cyclic pentapeptides reduced their conformation from 3 or 2 conformations to single conformation.[11-13] Thus,

N-methyl containing SanA-**4**, SanA-**5**, and SanA-**6** (**Figure 2.5**) were designed to study the impact of placing *N*-methylated acids at position II (**4**) and III (**5**, **6**) on cytotoxicity. SanA-**4** was designed from SanA-**10** (57% growth inhibition against PL-45) in order to evaluate weather *N*-Me-D-amino acid between position II and III improved to compounds' cytotoxicity relative to SanA peptide. SanA-**5**, which was derived from SanA-**10** (*N*-Me-D-Val at position III) by placing a D-Leu at position V, was designed to understand the cytotoxicity effect that occurred when combining D-amino acid at position IV with an *N*-methylated D-amino acid. Finally, SanA-**6**, was derived from SanA-**11** (57% growth inhibition against PL-45) by replacing the benzyl protected L-Ser with L-Leu same as San A peptide at position V while maintaining key structural feature of *N*-Me-D-Phe at position II and L-β-cyclohexylalanine at position IV.

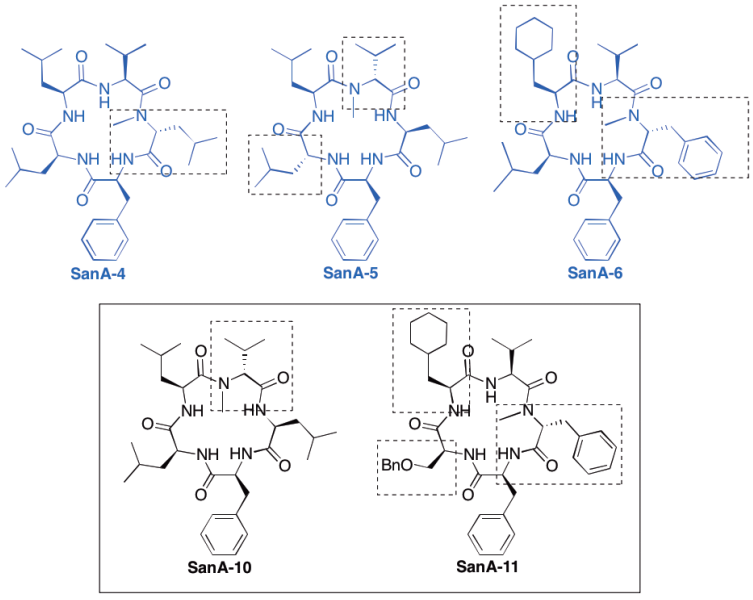
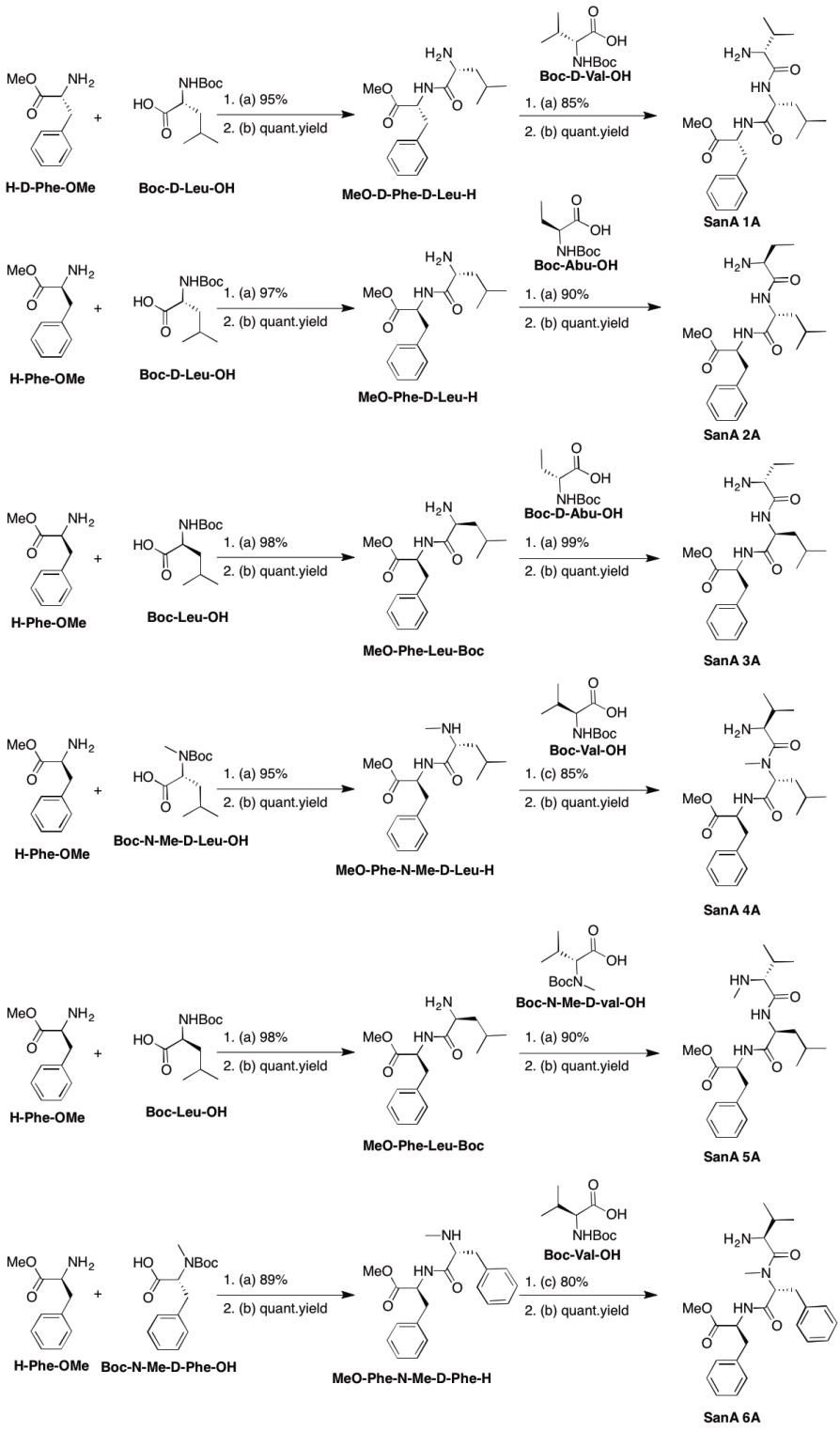


Figure 2.5 Rational Design of SanA-4, SanA-5, and SanA-6

2.4 Synthesis of San A Derivatives

2.4.1 Synthesis of Fragment A

The conditions for synthesizing deprotected tripeptides (fragment A) for the six derivatives are outlined in **Scheme 2.1**. Fragment A for SanA-**1** (SanA 1A) was synthesized via coupling reaction utilizing free acid Boc-D-Leu-OH (1.0 equivalent), free amine (1.1 equivalents), coupling reagent 2(1-H-benzotriazole-1-yl)-1,1,3 tetramethyluronium tetrafluoroborate (TBTU, 1.1 equivalents), and base diisopropylethylamine (DIPEA, 4.0 equivalents). The materials were dissolved in anhydrous methylene chloride (DCM, 0.1M). The reaction was monitored via thin-layer chromatography (TLC) and was complete in ~30 min. The crude reaction was diluted with DCM and washed with 10% (v/v) $HCl_{(aq.)}$ to remove excess free amine and DIPEA. The acid wash was followed by a basic wash using saturated aqueous sodium bicarbonate solution to remove the excess coupling agent. The organic layer was collected, dried over sodium sulfate, filtered, concentrated down *in vacuo*. The crude mixture was purified by flash column chromatography using silica gel and ethyl acetate-hexane as a gradient system. Pure dipeptide MeO-D-Phe-D-Leu-Boc was furnished in 95% yield. The structure and purity of MeO-D-Phe-D-Leu-Boc were confirmed using ^{1}H NMR spectroscopy. A similar coupling strategy was used to generate my other dipeptides: MeO-Phe-D-Leu-Boc (97% yield) for SanA-**2**; MeO-Phe-Leu-Boc (98% yield), which was a common building block for compounds SanA-**3** and SanA-**5**; MeO-Phe-*N*-Me-D-Leu-Boc (95% yield) for SanA-**4**; and MeO-Phe-*N*-Me-D-Phe-Boc (89% yield) used in SanA-**6**.



Conditions: a) TBTU (1.1 eq.), DIPEA (4.0 eq.), DCM (0.1 M); b) anisole (2.0 eq.), 20% (v/v) TFA:DCM=1:4 (0.1 M); c) HATU (1.1 eq.), DIPEA (4.0 eq.), DCM (0.1 M)

Scheme 2.1 Synthesis of Fragment A

t-butoxycarbonyl (Boc) was removed from dipeptide MeO-D-Phe-D-Leu-Boc was initiated using 20% (v/v) trifluoroacetic acid (TFA) in DCM (0.1 M) and anisole (2.0 equivalents). The reaction was monitored via TLC and was complete in ~30 min. Upon completion the reaction was concentrated *in vacuo* to remove excess of TFA and the resulting MeO-D-Phe-D-Leu-H was furnished in quantitative yield and subjected to the next coupling reaction without further purification. The same procedure was applied to synthesize other amine deprotected dipeptides: MeO-Phe-D-Leu-H, MeO-Phe-Leu-H, MeO-Phe-*N*-Me-D-Leu-H, and MeO-Phe-*N*-Me-D-Phe-H.

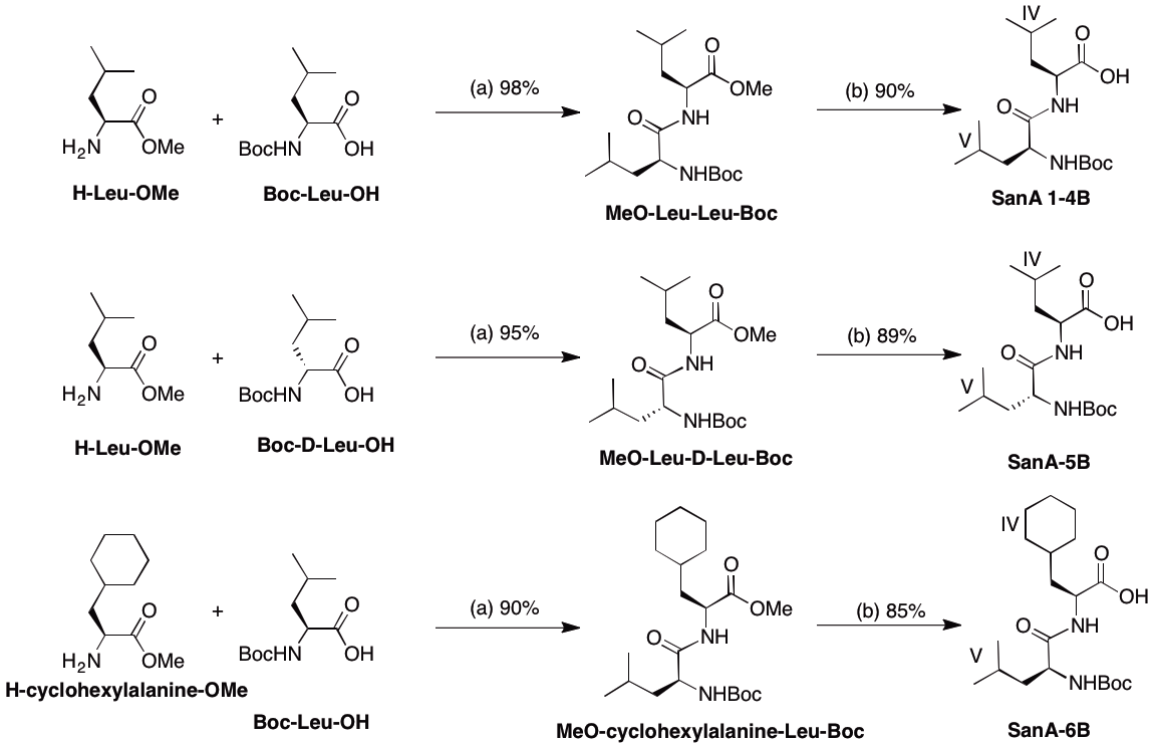
Diepeptide MeO-D-Phe-D-Leu-H was then coupled with free acid Boc-D-Val-OH to generate the protected tripeptide MeO-D-Phe-D-Leu-D-Val-Boc. The coupling reaction was performed utilizing free acid Boc-D-Val-OH (1.0 equivalent), free amine MeO-D-Phe-D-Leu-H (1.1 equivalents), coupling reagent TBTU (1.1 equivalents), and base DIPEA (4.0 equivalents). The reaction was monitored via TLC and was complete ~45 min. Upon completion, the crude reactions were diluted with DCM and washed with 10% (v/v) $HCl_{(aq.)}$ followed by a basic wash with saturated aqueous sodium bicarbonate. The organic layer was collected, dried over sodium sulfate, filtered, and concentrated *in vacuo*. The crude mixture purified via flash column chromatography technique. Pure tripeptide MeO-D-Phe-D-Leu-D-Val-Boc was furnished in 85% yield. The structure and purity of MeO-D-Phe-D-Leu-D-Val-Boc were confirmed using ^{1}H NMR spectroscopy. This strategy was applied to generate my other protected tripeptides: MeO-Phe-D-Leu-Abu-Boc (90% yield) for SanA-**2**, MeO-Phe-Leu-D-Abu-Boc (99% yield) for SanA-**3**, and MeO-Phe-Leu-*N*-Me-D-Val-Boc (90% yield) for SanA-**5**. The generation of tripeptide MeO-Phe-*N*-Me-D-Leu-Val-Boc (85% yield) for SanA-**4** and MeO-Phe-*N*-Me-

D-Phe-Val-Boc (80% yield) for SanA-**6** involved excess amount of 2-(H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU, 1.1 equivalents) as coupling reagent due to the presence of the secondary amine.

Removal of Boc from the tripeptides utilizing TFA:DCM (1:4, 0.1 M) and anisole (2.0 equivalents). Amine deprotection reactions were monitored via TLC and was complete in ~45 min. The crude reaction mixtures were then concentrated *in vacuo*, furnishing amine deprotected tripeptides (fragment A) in quantitative yields that were taken on without further purification.

2.4.2 Synthesis of Fragment B

Fragment B dipeptide MeO-Leu-Leu-Boc is common for compounds SanA **1-4** and it was synthesized using free acid Boc-L-Leu-OH (1.0 equivalent), free amine H-Leu-OMe (1.1 equivalents), coupling reagent TBTU (1.1 equivalents), and base DIPEA (4.0 equivalents) in anhydrous DCM (0.1 M) (**Scheme 2.2**). The reaction mixture was monitored via TLC and was complete ~30 minutes. After the reaction was complete, the organic mixture was diluted with DCM and washed using 10% (v/v) $HCl_{(aq.)}$ followed by a basic wash using saturated aqueous sodium bicarbonate solution. The organic layer was collected, dried over sodium sulfate, filtered, and concentrated *in vacuo*. The crude reaction mixture was purified via flash column chromatography yielding pure protected dipeptide MeO-Leu-Leu-Boc (98% yield). Structure confirmation and purity were verified using ^{1}H NMR spectroscopy. The similar synthesis procedure was used to generate other protected dipeptides: MeO-Leu-D-Leu-Boc (98% yield) for SanA-**5** and MeO-cyclohexylalanine-Leu-Boc (90% yield) for SanA-**6**.



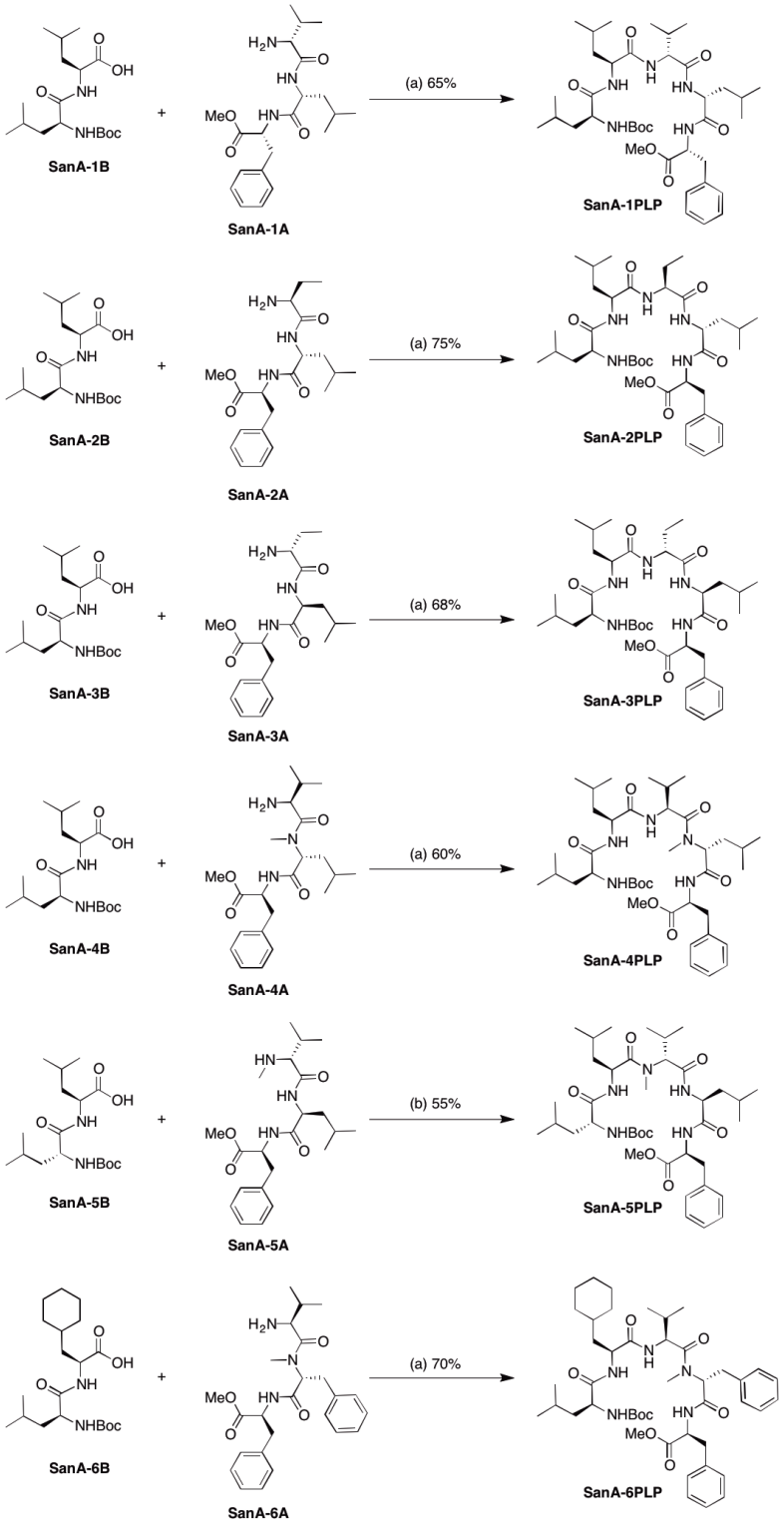
Conditions: a) TBTU (1.1 eq.), DIPEA (4.0 eq.), DCM (0.1 M); b) LiOH (8.0 eq.), MeOH (0.1 M)

Scheme 2.2 Synthesis of Fragment B

Acid deprotection of the dipeptides involved LiOH (8.0 equivalents) in MeOH (0.1 M) to hydrolyze the methyl ester on position IV (**Scheme 2.2**). The reaction was monitored via TLC and was complete ~8 hours. Upon completion the free acid dipeptide (fragment B) was worked up using 10% (v/v) $HCl_{(aq.)}$ to quench LiOH. The aqueous layer was extracted three times with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo* resulting in three acid deprotected dipeptides: Boc-Leu-Leu-OH (90% yield), Boc-D-Leu-Leu-OH (89% yield), and Boc-Leu-cyclohexylalanine-OH (85% yield).

2.4.3 Synthesis of Protected Linear Pentapeptides

Synthesis of protected linear pentapeptides (PLP) for SanA-**1** were accomplished by coupling free acid SanA-**1**B and free amine SanA-**1**A (**Scheme 2.3**). Free acid dipeptide Boc-Leu-Leu-OH (SanA-**1**B, 1.0 equivalent) was coupled with the free amine tripeptide MeO-D-Phe-D-Leu-D-Val-H (SanA-**1**A, 1.1 equivalents) using coupling TBTU (1.1 equivalents), and base DIPEA (6.0 equivalents) in anhydrous DCM (0.1 M). This reaction was allowed to proceed at room temperature and monitored via liquid-chromatography mass spectroscopy (LC/MS) and TLC. The reaction was complete ~2 h. After the reaction was complete the crude reaction mixture was diluted with DCM and washed with 10% (v/v) $HCl_{(aq.)}$ twice. The acidic aqueous layer was extracted with ethyl acetate three times. The organic layers were combined and washed with a saturated aqueous sodium bicarbonate solution. After the basic wash, the basic aqueous layer was extracted with ethyl acetate. The organic layers were combined, dried over sodium sulfate, filtered, and concentrated down *in vacuo*. The crude material was purified via flash column chromatography using silica gel and ethyl acetate-hexane as a gradient system. Pure SanA-**1** protected linear pentapeptide (SanA-**1**PLP) MeO-D-Phe-D-Leu-D-Val-Leu-Leu-Boc was furnished in 65% yield and ^{1}H NMR was used to verify the products and the purity. A similar method was used to generate my other SanA-PLPs: MeO-Phe-D-Leu-Abu-Leu-Leu-Boc (SanA-**2**PLP, 75% yield), MeO-Phe-Leu-D-Abu-Leu-Leu-Boc (SanA-**3**PLP, 68% yield), MeO-Phe-*N*-Me-D-Leu-Val-Leu-Leu-Boc (SanA-**4**PLP, 60% yield), MeO-Phe-Leu-*N*-Me-D-Val-Leu-D-Leu-Boc (SanA-**5**PLP, 55% yield), and MeO-Phe-*N*-Me-D-Phe-Val-cyclohexylalanine-Leu-Boc (SanA-**6**PLP, 70% yield).



Conditions: a) TBTU (1.1 eq.), DIPEA (6.0 eq.), DCM (0.1 M); b) HATU (1.1 eq.), DIPEA (6.0 eq.), DCM (0.1 M)

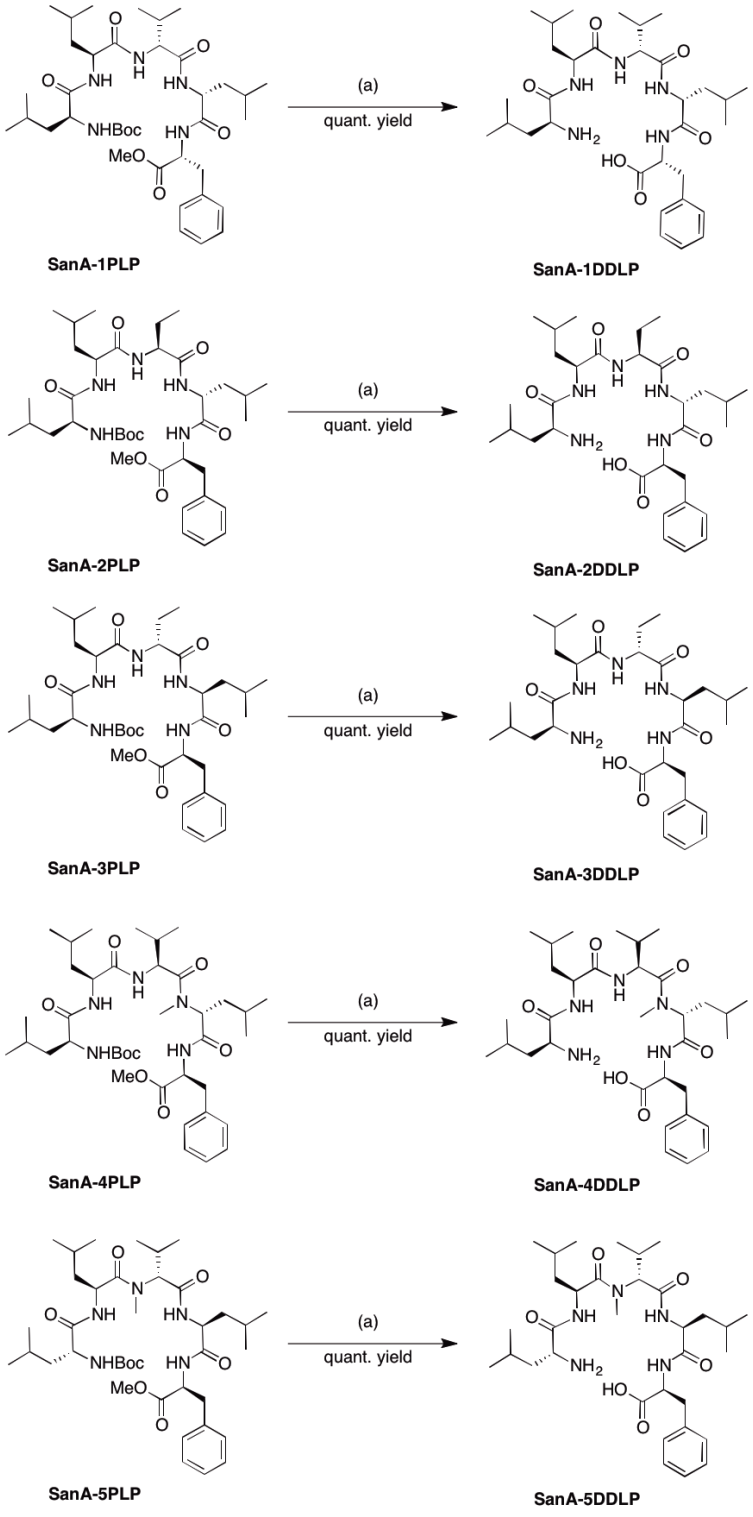
Scheme 2.3 Synthesis of Protected Linear Pentapeptides (SanA-PLPs)

2.4.4 Double Deprotection of Linear Pentapeptides

Remove Boc on position I and methyl ester on position V from the PLPs generated the double deprotected linear pentapeptides (DDLPs). Two different double deprotection strategies were utilized to generate the free amine at position I and free acid at position V in the synthesis of six San A derivatives.

***In Situ* Deprotection**[14]

SanA-**1**PLP MeO-D-Phe-D-Leu-D-Val-Leu-Leu-Boc, was acid and amine deprotected using 16 drops of concentrated HCl (8 drops per 0.3 mmols of linear pentapeptides) in tetrahydronfuran (THF, 0.05 M) and anisole (2 equivalents) (**Scheme 2.4**). The reaction was monitored via LC/MS and TLC. It was observed by LC/MS that 100% of the amine and ~50% of the methyl ester was removed from the pentapeptide after 2 days. On the third day, 6 drops of concentrated HCl (3 drops per 0.3 mmols) was then added to force the reaction to completion. The reaction was monitored through the presence of the free acid, free amine, and the disappearance of the SanA-**1**PLP. Upon completion the reaction was concentrated *in vacuo* resulting HO-D-Phe-D-Leu-D-Val-Leu-Leu-H (SanA-**1**DDLP) in quantitative yield. The *in situ* double deprotection strategy was used to obtain other SanA-DDLPs: HO-Phe-D-Leu-Abu-Leu-Leu-H (SanA-**2**DDLP), HO-Phe-Leu-D-Abu-Leu-Leu-H (SanA-**3**DDLP), HO-Phe-*N*-Me-D-Leu-Val-Leu-Leu-H (SanA-**4**DDLP), HO-Phe-Leu-*N*-Me-D-Val-Leu-D-Leu-H (SanA-**5**DDLP). These SanA-DDLPs are subjected to macrocyclization without purification or characterization.

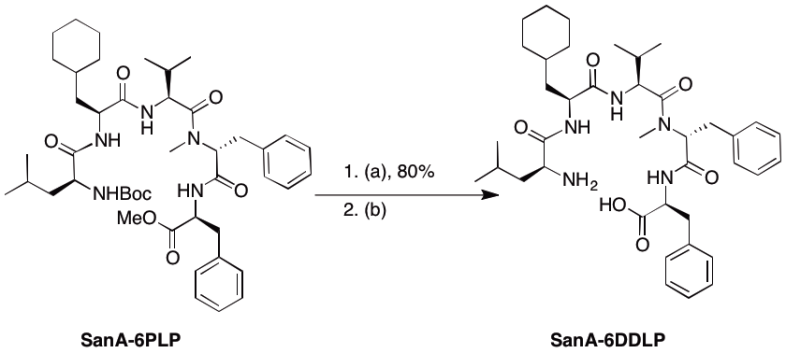


Condition: a) HCl (8 drops per 0.03 mmol for the first two days then 3 drops per 0.03 mmol for the following days), anisole (2.0 eq.), THF (0.05 M)

Scheme 2.4 In situ Double Deprotection Strategy

Step-wise Double Deprotection of Linear Pentapeptide

Although the *in-situ* double deprotection strategy was very straightforward, the reaction length was inefficient. Thus, an alternative method was developed and utilized for compound SanA-**6**PLP (**Scheme 2.5**). Double deprotection using a stepwise approach involved removing the methyl ester on position V of SanA-**6**PLP using LiOH (10.0 equivalents) in MeOH (0.05 M). The reaction was monitored via LC/MS and TLC and was complete ~24 h. Upon completion, the organic mixture was diluted with DCM and washed with 10% (v/v) $HCl_{(aq.)}$ to quench LiOH. The acidic aqueous layer was extracted with ethyl acetate three times. The combined organic layers were, dried over sodium sulfate, filtered, and concentrated *in vacuo*, where generated the acid deprotected SanA-**6** pentapeptide. The Boc group was then removed using 20% TFA (v/v) in DCM (0.05 M) and anisole (2.0 equivalents). The reaction was allowed to stir for an hour at room temperature and monitored via LCMS and TLC. Upon completion the reaction was concentrated *in vacuo* resulting SanA-**6**DDLP in quantitative yield without any further purification.

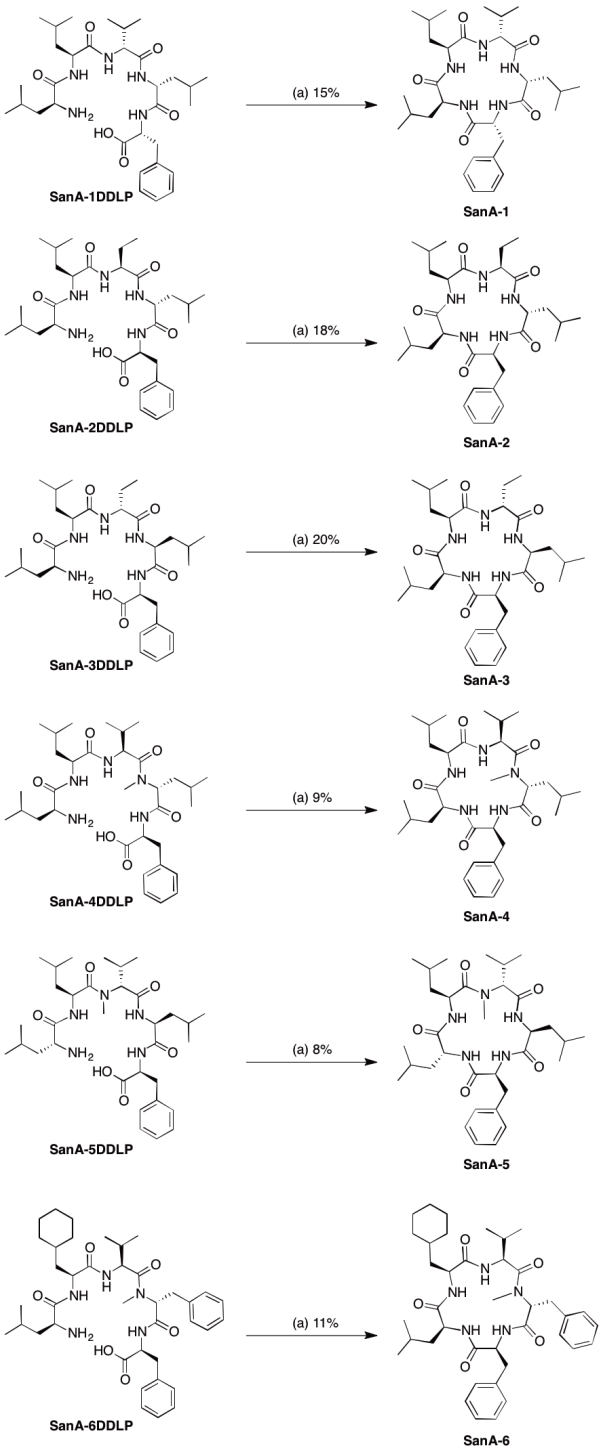


Conditions: a) LiOH (10.0 eq.), MeOH (0.05 M); b) 20% (v/v) TFA, anisole (2.0 eq.), DCM (0.05 M)

Scheme 2.5 Step-wise Double Deprotection Strategy

2.4.5 Macrocyclization of San A Derivatives

It has been reported in literature that coupling reagents exhibit selective efficacy in assisting amide bond formation between two amino acids. In order to maximize the yield of the cyclization I initiated the reaction of SanA-**1** by using a mixture of 3 coupling reagents: TBTU (0.7 equivalents), HATU (0.7 equivalents), and 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4-(3H)-one (DEPBT, 0.7 equivalents) along with base DIPEA (8.0 equivalents) in a 1:1 ratio of anhydrous DCM and ACN at 0.007 M (**Scheme 2.6**). Cyclization reaction was monitored via LC/MS and TLC and was complete ~12 hours. Upon completion, the organic mixture was diluted with DCM and washed with 10% (v/v) $HCl_{(aq.)}$ twice to remove excess free amine and DIPEA. The acidic aqueous layer was extracted three times using ethyl acetate. The combined organic layers were washed with saturated aqueous sodium bicarbonate and then extracted three times using ethyl acetate. Combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*, whereupon they were purified using flash column chromatography with silica gel and ethyl acetate-hexane as a gradient system. Pure SanA-**1** was furnished in 15% yield and its structure and purity were confirmed using ^{1}H NMR and LC/MS. The low yielding of SanA-**1** was caused by the undesired dimerization of SanA-**1**DDLP, which was observed in LC/MS. My other SanA macrocycles were made in a similar fashion: SanA-**2** (18% yield), SanA-**3** (20% yield), SanA-**4** (9%), SanA-**5** (8%), and SanA-**6** (11%). These compounds were added to the current SanA library for biological testing.



Condition: a) TBTU (0.7 eq.), HATU (0.7 eq.), DEPBT (0.7 eq.), DIPEA (8.0 eq.), DCM:ACN (1:1, 0.007 M)

Scheme 2.6 Cyclization of SanA 1-6

2.5 Biological Test Using ^{3}H-labeled thymidine incorporation assay

Utilizing a ^{3}H-labeled thymidine incorporation cell-based assay we examine the cytotoxicity of 12 SanA derivatives (6 of them were synthesized by me) against pancreatic cancer cell line PL-45. The biological assays were completed by my colleagues, Po-Shen Pan and Robert C. Vasko.

Pancreatic cancer is one of most deadly cancers in the U.S to date. Only 10% of patients can undergo a surgical procedure to remove the tumor and less than 20% of patients respond to the current drug of choice,[16] gemcitabine (2, 2-difluorodeoxycytidine).[17] Additionally, the five-year survival rate for pancreatic cancer patients is only 5%.[18] The frightening mortality rate of this cancer has shown that there is an immediate need for developing more effective chemotherapeutic agents.

2.5.1 Procedure of ^{3}H-labeled thymidine incorporation assay[19]

^{3}H-labeled thymidine incorporation assay provides information on cell proliferation. During the cell division DNA synthesis occurs, if cells are lived they will incorporate ^{3}H-labeled thymidine in to their DNA sequence. The amount of ^{3}H-labeled thymidine incorporated can be quantified using a detector that captures radioactivity. A low number of quantified ^{3}H-labled thymidine incorporated into a cell indicates a low cell proliferation rate, conveniently a high number of quantified ^{3}H-labled thymidine indicating low cytotoxicity of the SanA derivative. Seeding PL-45 pancreatic cancer cells on a 75mm^{2} tissue culture plate, and allowing cells to grow for 72 hours at 37^{o}C (5% CO_2) generated a sub-confluent monolayer. The cells were washed with 1x PBS then detached and suspended with 0.25% Trypsin-EDTA in fresh medium after 72 hours.

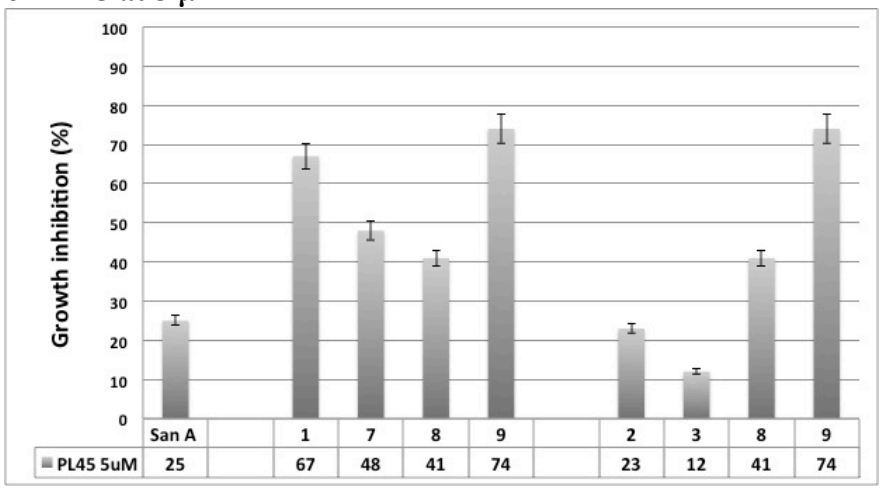
Cells were counted and seeded at 2500 cells per well onto a 96 well plate. The cells were fully attached to the plate after 6 h incubation at 37^{o}C (5% CO_2). SanA derivatives were then added to the plate at the chosen concentration using DMSO as buffer and the cells were incubated for 56 h at 37^{o}C (5% CO_2). 1μCi of ^{3}H-labeled thymidine was added to each well followed by incubation for 16 h at 37^{o}C (5% CO_2). The cells were harvested and captured on glass fiber filter paper using a Brandel 290 PHD multi-well cell harvester. The glass fiber filter paper for each well was then placed in 6.5 ml scintillation vial and 2 ml of ScinitVerse^{TM} was added to each vial. Each scintillation vial tritium content was then quantified using a Beckman LS 5000 TD. Count per minute (CPM) was used as the unit for quantification and converted to percent growth inhibition values when compared to controls where cells were treated with 1% DMSO 99% buffer. Twelve trials of each San A derivative were done by plating 3 sets of 4 experiments with each having consecutive wells Error is recorded as ±SEM, which lies within 5% of the growth inhibition value.

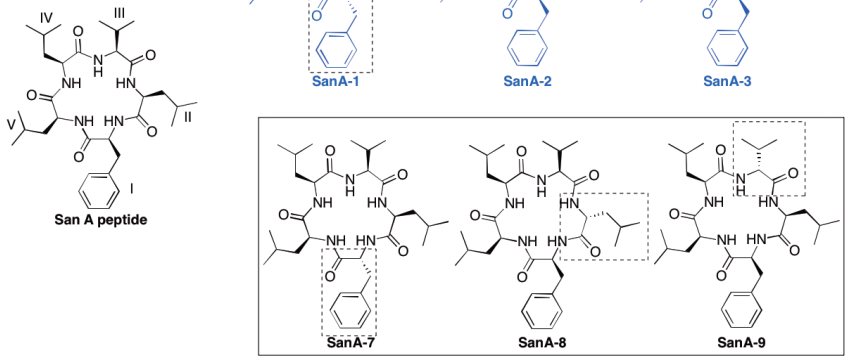
2.5.2 Cytotoxicity Results and SAR Studies

For any given series of medically relevant compounds it is important to understand their structural activity relationship (SAR). SanA molecules that were used as design elements and my six analogues were tested in biological assays. SanA-**1**, which combined the structural features of SanA-**7**, SanA-**8**, and SanA-**9** by incorporating three consecutive D-amino acids from position I to III showed promising growth inhibition 67% against PL-45 (**Table 2.1**). Based on the observed improved cytotoxicity of SanA-**1**, modifications to SanA-**1** altered the 3-D conformation of the structure, SanA-**9** is the most cytotoxic, thus, position III is likely the more favorable position for a D-amino acid

than positions I or II. Disappointingly, SanA-**1** exhibited lower growth inhibition against PL-45 compared to SanA-**9,** with a single D-amino acid. Thus the D-amino acids positions are not synergistic. SanA-**2** and SanA-**3**, which feature the modification of Val at position III, had essentially no cytotoxicity against the PL-45. In fact SanA-**2** and SanA-**3** exhibited lower potency than their origin molecules SanA-**7** and SanA-**8**.

Table 2.1 Growth Inhibition of Compound 1, 2, 3, 7, 8, and 9 against Pancreatic cancer cell line PL-45 at 5 µM

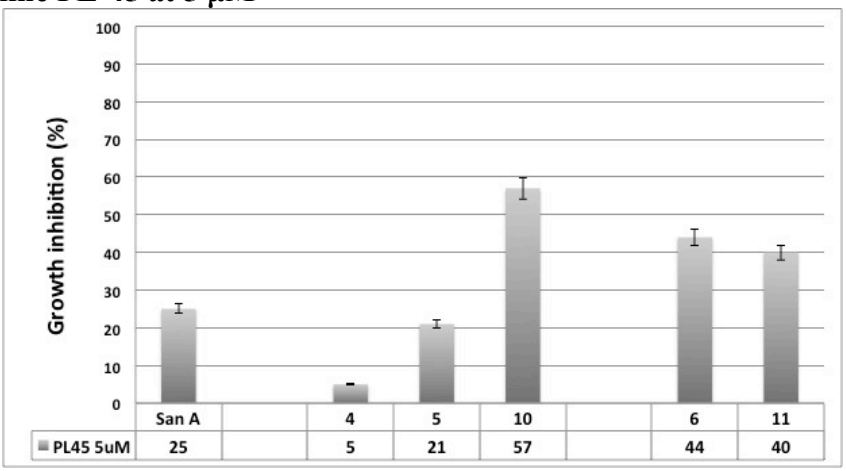


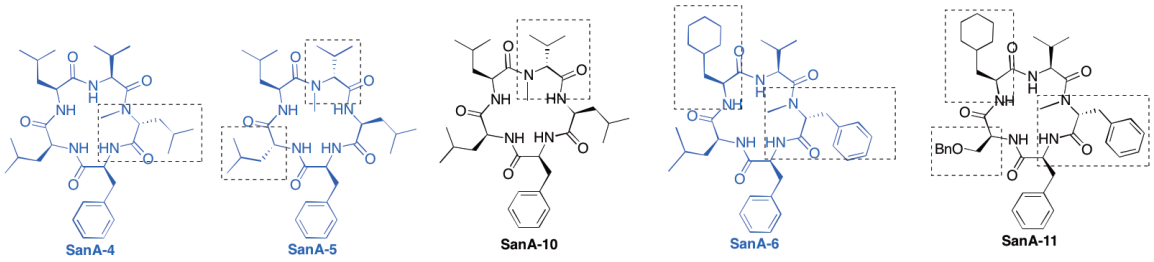


SanA-**10**, which contains an *N*-Me-D-Val at position III, showed better growth inhibition than SanA-**4** against PL-45 (**Table 2.2**). Furthermore SanA-**5**, which features a D-amino acid at position V and an *N*-Me-D-Val at position III, was not effective against

the PL-45. Rather, the cytotoxicity for SanA-**5** was dramatically decreased in PL-45. Lastly SanA-**6**, which was derived from SanA-**11,** showed no change on cytotoxicity in PL-45.

Table 2.2 Growth Inhibition of Compound 4, 5, 6, 10, and 11 against Pancreatic cancer cell line PL-45 at 5 µM





2.6 Conclusion

In conclusion, my convergent synthetic strategy has provided an efficient route to generate structurally diverse SanA analogues in a rapid fashion and provided sufficient material for biological testing. This work presents an efficient method for building cyclic pentapeptide libraries that can be used to explore the structure activity relationship (SAR) of SanA derivatives against the drug resistant pancreatic cancer cell line (PL-45). I synthesized six SanA molecules and my colleagues tested them in ^{3}H-labled thymidine incorporation assays against PL-45. The results from these assays coupled with data

generated from my colleagues' compounds provided valuable SAR. An *N*-Me-D-amino acid emerged as an essential element within the backbone. Further, it was clear from these data that each amino acid impacted the 3-D conformation of the ring. Noticeably, additive and synergistic effects did not occurred by combining potent SanA derivatives' structural features. The final preferred conformer was challenging to predict because it was a correlation of all 5 amino acids and their combined lowest energy configuration. The impact of structurally modification the backbone of each molecule cannot be predicted. Rather, each combination must be synthesized and tested for cytotoxicity. As we found through experiment, the final active compounds (**Figure 2.6**) had distinct features that did not overlap.

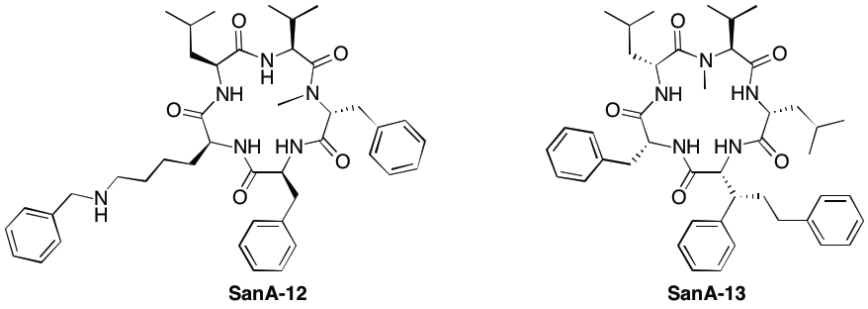


Figure 2.6 Structures of SanA-12 and SanA-13

Chapter 2, in part, is a reprint of the material as it appears in "Synthesis of second generation Sansalvamide A derivatives: Novel Templates as Potent Anti-tumor Agents." *Journal of Organic Chemistry*, **2007**, *72*, 1980-2002. Rodriguez, R.A.; Pan, P-S; Pan, C-M; Ravula, S.; Lapera, S.A.; Singh, E.K.; Styers, T.J.; Cajica, J.; Brown, J.D.; Parry, E.; McAlpine, S.R. The dissertation author was the primary investigator and author of this paper.

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Chapter 3

The synthesis of FR235222 and Trapoxin B Derivative as HDAC inhibitor

3.1 Acetylation and Deacetylation Process of Histones in DNA Transcription

In eukaryotic cells, a chromosome is responsible for packaging DNA into a smaller volume to ensure the DNA fits into nucleus. Chromosomes are made up of chromatin, which is composed of condensed nucleosomes (**Figure 3.1**).[1] Nucleosomes represents a DNA unit that wraps around the histone octamer. Histones are classified into five classes: H1, H2A, H2B, H3, and H4,[2] histones are positively charged due to a large number of Lys and Arg amino acids located within in their protein structure.[3] This allows histones to interact with the negatively-charged phosphate ions on the DNA backbone (**Figure 3.1**).[4] The electrostatic interaction between DNA and histones causes DNA to wrap around the histone core tightly making it inaccessible for gene transcription.

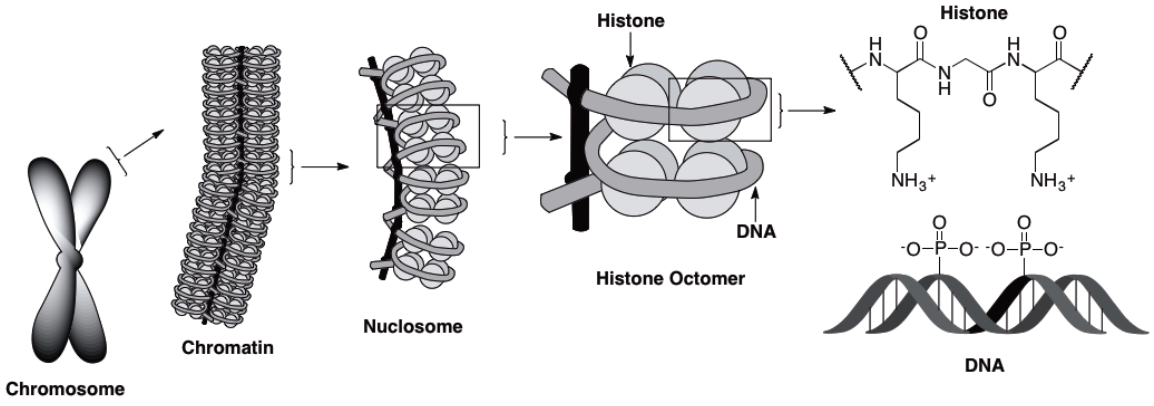


Figure 3.1 DNA-Nuclosomal Histones interaction

Acetylation and deacetylation of histones is used regulate the interaction between DNA and histone octamer for gene transcription. Histone acetyltransferases (HATs) are the enzymes that are responsible for acetylating the Lys residues on the histones core

(**Figure 3.2**).[4,5] Acetylated Lys residues lose the ability to interact with DNA. As a result, DNA unwinds from the histone, thus becoming accessible for gene transcription.

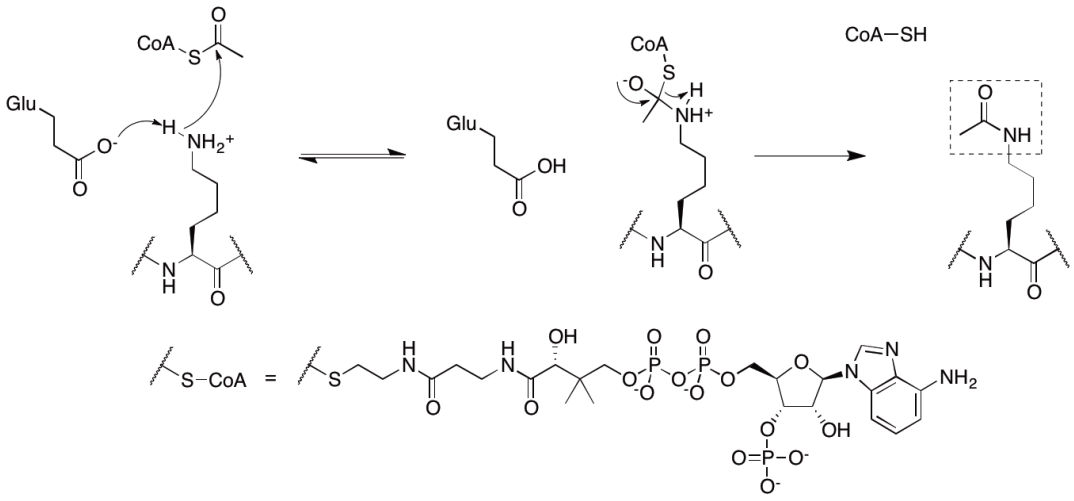


Figure 3.2 Acetylation of Histones Lysine Residue by Histone Acetyltransferase

In contrast to HATs, histone deacetylasess (HDACs)[6] remove the acetyl groups from Lys residues. (**Figure 3.3**) Deacetylated Lys becomes positively charged and capable of interacting with DNA.[6] Thus, deacetylation allows histones to re-establish the interaction with DNA and repackage the chromatin into a condensed structure. Over expressed HDACs lead to an undesired low histone acetylation level and this phenomenon is called hypoacetylation, which is observed in multiple cancers. Thus, it is important to regulate the deacetylation activity in cells in order to regulate the electrostatic interaction between DNA and histone to induce proper gene transcription process.

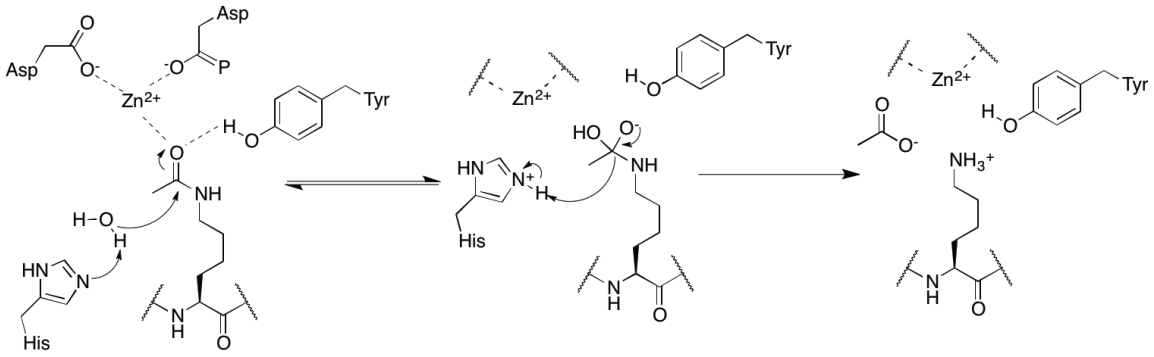


Figure 3.3 Deacetylation of Histone Lysine Residue by Histone Deacetylases (HDACs)

3.1 Background of HDACs

Overexpressed HDACs often lead to hypoacetylation of histones, which condensed the DNA tightly around the octamer. Tightly wrapped DNA makes genes inaccessible and they become silenced, unable to be transcribed. It is well documented that in cancer cells[7] overexpressed HDACs induce improper transcription of several tumor suppressor genes. These genes, p21 and p27, are involved in the cell division process and respond to anti-proliferative signals, which leads to apoptosis. Silencing these genes allows cells to continue growing despite the damage.[8,9] By silencing these cell-cycle regulators, cancers no longer respond to anti-growth signals and then continue to proliferate.[10] In addition, HDACs can also silence p53, which functions as a "molecular policeman"[11] in monitoring genome integrity.[12] Upon detecting DNA damage, p53 causes cell-cycle arrest, allowing either DNA repair or apoptosis.[13,14] By silencing p53, cancers are able to evade apoptosis.[10]

To date, there are a total of 18 human HDACs (**Table 3.1**) that have been identified and they are categorized into four classes based on the features of their sequence homology, size (number of amino acids), and location in the cell.[15-17] HDAC

class I, II, and IV contain Zn^{2+} in their catalytic site and they are considered potential targets for histone deacetylase inhibitors (HDIs). Class III HDACs, sirtuins, are a class of enzymes that possess protein deacetylase activity with the cooperation of NAD^+.[16] Its deacetylation mechanism involved coupling NAD^+ with acetylated Lys followed by hydrolysis to yield deacetylated Lys. In addition, sirtuins do not have Zn^{2+} within the catalytic site and most HDIs are inefficient at inhibiting their activity.[17]

Table 3.1 Classes of HDAC

Class	HDAC	Size (aa)	Expression in tumor
Class I	HDAC 1	483	Possible prognostic indicator for lung and breast cancer. Observed overexpression in prostate, gastric, colorectal, and hepatocellular carcinoma.
	HDAC 2	488	Overexpression observed in colorectal, prostate, and gastric cancers.
	HDAC 3	428	Over expressed in lung, gastric, prostate and colorectal cancers.
	HDAC 8	377	Knock down inhibits cell growth in lung, colorectal, and cervical cancer.
Class II	HDAC 4	1084	Upregulated in breast cancer compared to renal, bladder, and colorectal cancer.
	HDAC 5	1122	Upregulated in colorectal cancer compared to renal, bladder, and breast cancer.
	HDAC 6	1215	Highly expressed in squamous cell cancer.
	HDAC 7	855	Upregulated in colorectal cancer compared to renal, bladder, and breast cancer.
	HDAC 9	1011	Unknown
	HDAC 10	669	Unknown
Class III	SIRT1, SIRT2 SIRT3, SIRT3 SIRT4, SIRT5 SIRT6, SIRT7	355-747	Require NAD^+ to function as protein deacetylases
Class IV	HDAC 11	347	Unknown

(Class I, II, and IV HDACs are Zn^{2+} dependent. Class III, sirtuins, do not have metal within the catalytic site)

3.2 Histone Deacetylase Inhibitors (HDIs)

Since the overexpression of HDACs is associated with cancer development and progression, developing histone deacetylase inhibitors (HDIs) has become a goal in cancer therapy. Current HDIs target Zn^{2+} dependent classes I, II, and IV and are composed of three components (**Figure 3.4**) : 1) an metal binding unit, which interacts with the metal cations located in the catalytic site of the HDACs pocket. 2) The surface recognition unit, which is responsible for binding HDIs at the rim of the HDAC pocket and positioning the enzyme inactivating unit in a favorable orientation.[18,19] 3) The linker region, which serves as a connecter between the recognition unit and the enzyme inactivating unit (**Figure 3.4**).

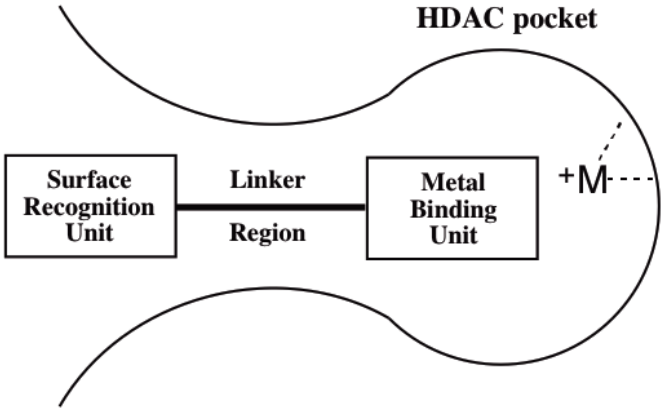
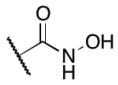
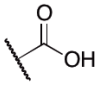
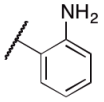
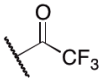
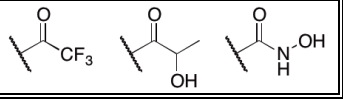


Figure 3.4 HDI Pharmacophore and HDAC pocket binding

Based on their structural features, current HDIs are organized into five classes[20]: small molecule hydroxamic acids, short chain fatty acids, benzamides, electrophilic ketones, and cyclic peptides (**Table 3.2**)

Table 3.2 Five classes of HDIs

Structural Class	Surface Rec. Domain	Linker Region	Enzyme Inactivating Unit
Small Molecule Hydroxamic acid	Hydrophobic group	Alkyl Chain	
Short Chain Fatty Acid	--	Alkyl Chain	
Benzamide	Aryl Group	Alkyl Chain	
Electrophilic Ketone	Aromatic	Ether/amide bond With Alkyl Chain	
Cyclic Peptide	Cyclic Peptide	Alkyl Chain	

The most notable HDI is Vorinostat® (Zolinza, SAHA), which is currently on the market as an anticancer agent (**Figure 3.5**). SAHA was developed by Merck® and approved by the FDA in 2006 for Cutaneous T-Cell Lymphoma (CTCL) treatment.[21] It exhibits potent Class I and II HDACs inhibition activity against T24 (bladder cancer) and MDA (breast cancer) cell lines at nanomolar concentration (IC_{50} < 86 nM).[22] The metal binding unit is a hydroxamic acid, which chelates to Zn^{2+} cations, the linker is a 7-atoms alkyl chain, and the surface recognition unit is a phenyl amide.[23] (**Figure 3.5**)

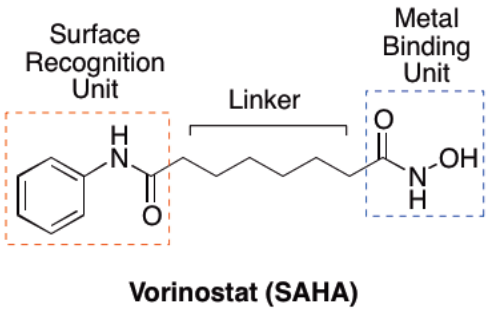


Figure 3.5 Structures of Small Molecules HDIs: Vorinostat® (SAHA)

The other important HDI class is cyclic peptides. The surface recognition unit of cyclic peptides creates a complex 3-D conformation. The cyclic peptide scaffold gives these HDIs a crucial advantage over other classes: The rim structure of the HDACs' catalytic pocket varies from one to another. Thus, developing HDAC specific HDIs by utilizing the cyclic peptide scaffold as surface recognition unit may allow selective HDAC inhibition. In addition, the surface recognition unit of cyclic peptide scaffold is capable of sealing the HDAC pocket after the metal binding unit chelates the metal cation within the HDAC catalytic site. In recent years three notable cyclic peptide natural products were discovered and their distinguished histone deacetylase inhibition activity has drawn attention to this HDI class.

Romidepsin (FK-228, **Figure 3.8**) is an anti-cancer drugs, which was isolated from the bacteria *chromobacterium violaceum* in 1994[24] and approved by FDA in 2009 for Cutaneous T-cell Lymphoma (CTCL) treatment. Upon reduction of its disulfide bond in the cell, the resulting thiol groups inactivate the Zn^{2+}. Trapoxin B (**Figure 3.8**), a cyclic tetrapeptide, which was isolated from a culture broth of *Helicoma ambiens* RF-1023 at Japan in 1990, exhibits low nanomolar cytotoxicity against several cancer cell lines.[25] Its mechanism of action involves the reduction of the α-epoxy moiety followed by

a covalent bond formation with the HDAC enzyme, resulting a irreversible HDAC inhibition. FR235222 (**Figure 3.8**) is a cyclic tetrapeptide natural product that was isolated from the fermentation broth of a fungus, *Acremonium sp* in 2003 by Mori and co-workers.[26] It showed promising potency against partially purified mammalian HDACs (IC_{50} = 22 nM).[26]

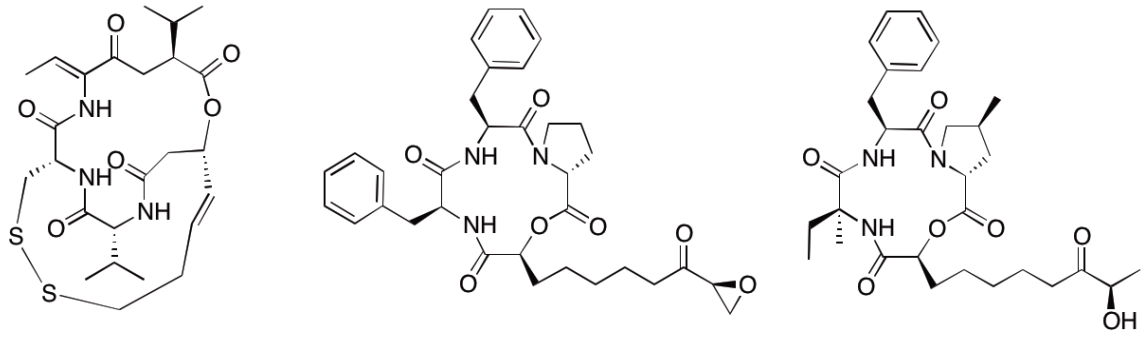


Figure 3.6 Structures of Cyclic Peptide HDIs: Romidepsin (FK-228), Trapoxin B, and FR235222

Natural products Trapoxin B and FR235222 are structurally similar to each other and they are both potent HDIs. Thus, our research group has designed a series of HDIs using both FR235222 and trapoxin B as templates.

3.4 Rational Design of FR235222 Derivatives

Using FR235222 and trapoxin B as templates, we designed six novel HDIs to investigate the SAR of the natural product. **Figure 3.7** illustrates the amino acids that were used in synthesizing designed HDIs. The amino acids that I used in the synthesis of my HDI (HDI-**4** in **Figure 3.8**) are colored in blue.

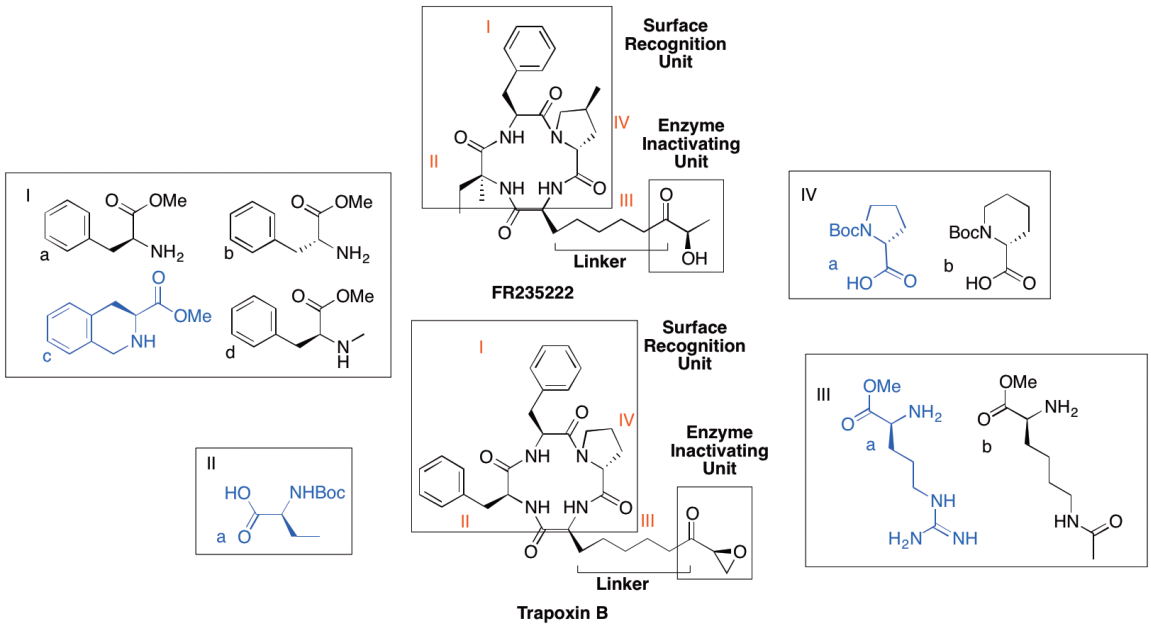


Figure 3.7 Amino acids used in the synthesis of FR235222 derivatives

We maintained an aromatic group at position I and explored changes in stereochemistry by inserting D-Phe in HDI-**2** and HDI-**3**. These molecules were designed to examine how the HDAC inhibition changed by altering the stereochemistry of the Phe residue. L-1,2,3,4 tetrahydroisoquinoline was incorporated in HDI-**4** to explore the effect on HDAC inhibition when a rigid aromatic side chain was inserted into the cyclic scaffold. An L-*N*-Me-Phe was introduced in HDI-**6** to establish how this reduced conformational freedom impacts potency. As observed by our lab and others an *N*-methyl reduces the conformational freedom of the macrocycle.[27-29] Position II, L-Abu was utilized in our HDIs and this structural change allows us to understand the effect on HDAC inhibition activity when the amino acid has a reduced size. In both natural products, position III, a 6-carbon linker and a metal binding unit. We choose to utilize L-Arg, which modified the linker length from 6 atoms in natural products to 4 atom in analogue HDI-**1** to HDI-**4**. Utilizing an L-acetylated-Lys in HDI-**5** and HDI-**6** generated a

linker with 5 atom length and different metal binding unit to verify the HDAC inhibition. The metal binding unit was a guanidine group in HDI **1**-**4** and acetyl group in HDI-**5** and **6**. A guanidine group has never been reported as a metal binding unit for HDIs, thus, it provided us an opportunity to explore a new metal binding unit. Acetylated-Lys is utilized to mimic the acetylated-Lys residue on the histones creating reversible HDIs. It is known that maintaining the D-amino acids at residue IV is critical for keeping the surface recognition domain in an optimal binding conformation and positioning the metal binding unit in a favorable orientation.[21] Hence we maintain the 5-membered ring system D-Pro moiety on trapoxin B for HDI-**1**, **2**, **4**, **5**, and **6**. In HDI-**3**, a D-pipecolic acid was incorporated to give an analogue with D-stereochemistry but as a 6-membered ring. These modifications would provide compounds useful in establishing SAR.

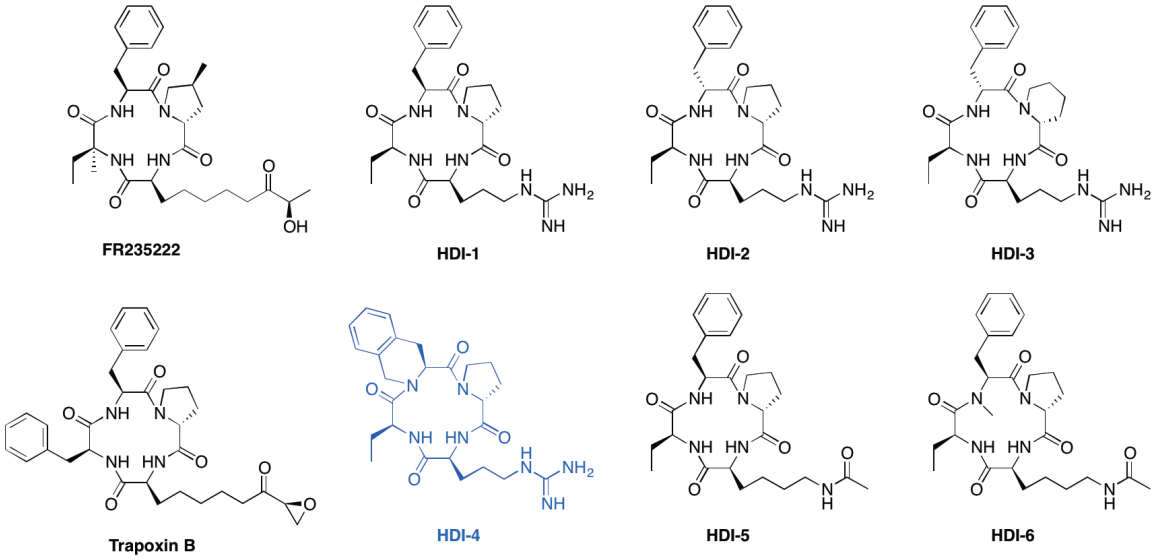
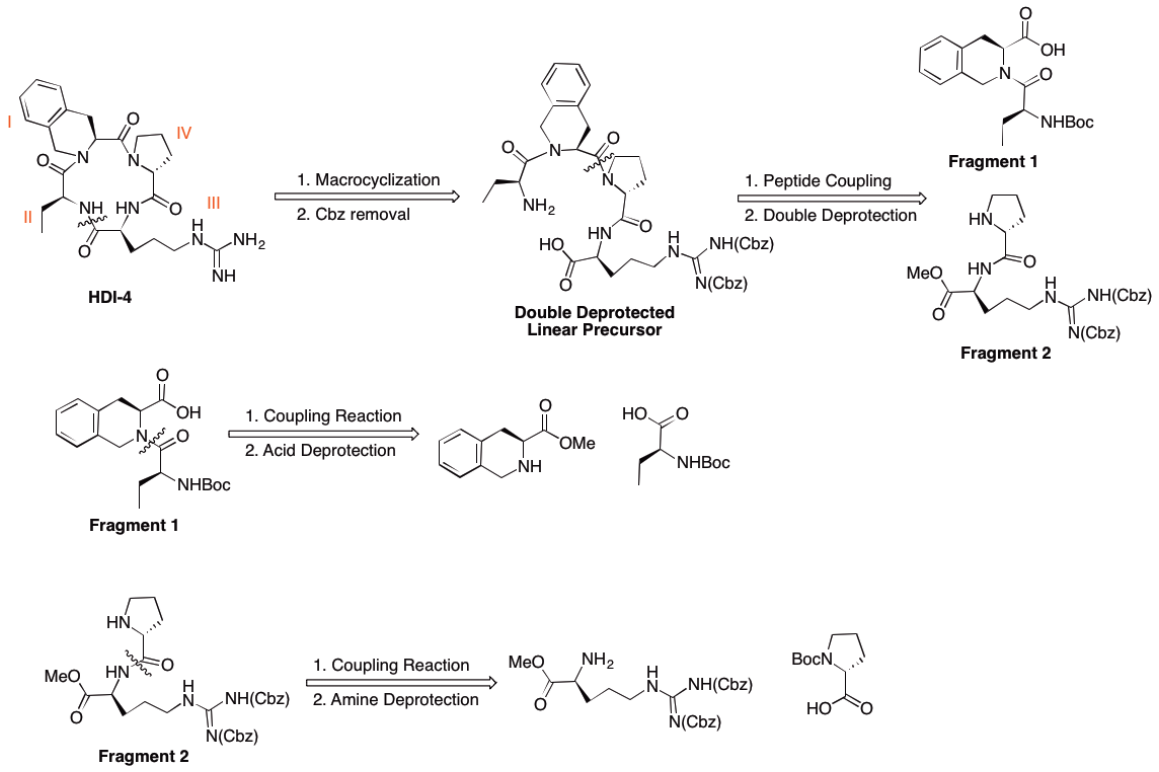


Figure 3.8 Structures of Natural Product FR235222 and its Derivatives

3.5 Retrosynthetic Strategy of HDI-4

A convergent approach involving two fragments was used to generate HDI-4 (**Scheme 3.1**). HDI-4 was afforded from a macrocyclization between free acid at position

III and free amine at position IV of the double deprotected linear tetrapeptide precursor. The double deprotected linear precursor was acquired via peptide coupling between free acid (fragment 1) and free amine (fragment 2) followed by *in-situ* double deprotection reaction. The two fragments were constructed via peptide coupling reaction in solution phase using commercially available protected amino acids.



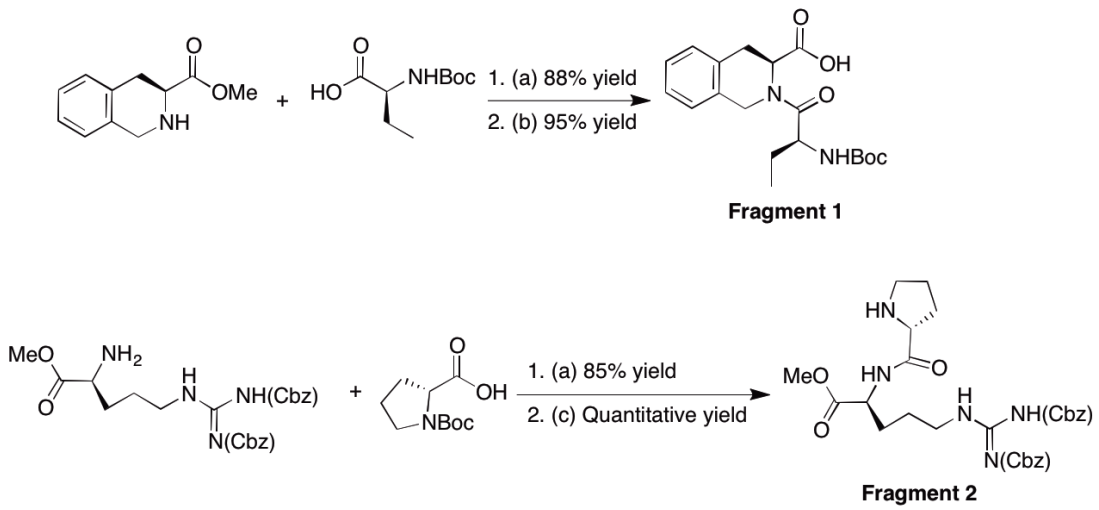
Scheme 3.1 Retrosynthetic Strategy of HDI-4

3.6 Synthesis of HDI-4

3.6.1 Synthesis of Fragment 1 and 2

Fragment 1 for HDI-4 was synthesized (**Scheme 3.2**) utilizing free acid L-2-Abutaric acid (1.0 equivalent), free amine 1,2,3,4-tetrahydro-3-isoquinoline (1.1 equivalents), coupling reagent TBTU (1.2 equivalents), and base DIPEA (4.0 equivalents). The materials were dissolved in anhydrous DCM (0.1 M). The reaction was

completed in 30 min and confirmed via TLC. Upon completion, the organic mixture was diluted with DCM and subjected to acid wash using 10% (v/v) $HCl_{(aq.)}$ to remove excess free amine and DIPEA, followed by a basic wash using aqueous saturated sodium bicarbonate solution to remove the excess coupling reagent. The organic layer was collected, dried over sodium sulfate, filtered, and concentrated down via rotary evaporator The material was purified by flash column chromatography using silica gel and ethyl acetate-hexane as the gradient solvent system. The pure protected fragment 1 dipeptide was furnished in 88% yield and its structure and purity was confirmed using ^{1}H NMR spectroscopy.



Conditions: a) TBTU (1.2 eq.), DIPEA (4.0 eq.), DCM (0.1 M); b) LiOH (8.0 eq.), MeOH (0.1 M); c) TFA : DCM = 1 : 3 (0.1 M)

Scheme 3.2 Synthesis of fragment 1 and 2

Methyl ester hydrolysis of the protected Fragment 1 was performed using LiOH (8.0 equivalents) in methanol at an overall concentration 0.1 M (**Scheme 3.2**). The reaction mixture was allowed to stir for 8 hours and was monitored via TLC. Upon completion the reaction was diluted with DCM and quenched with 10% (v/v) $HCl_{(aq.)}$ to

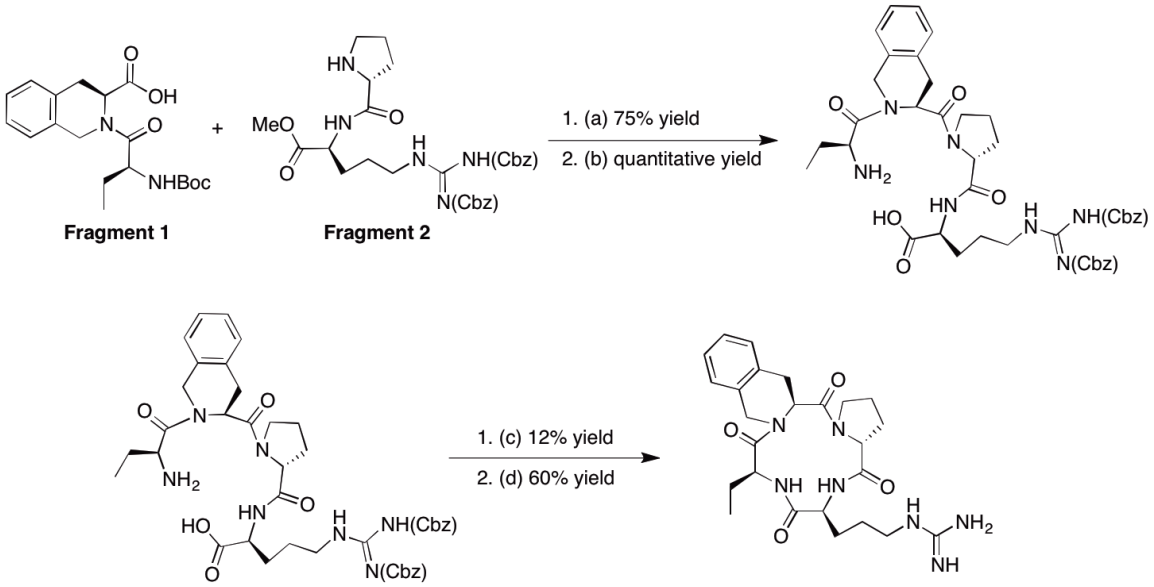
quench LiOH, and whereupon aqueous layer was back extracted three times with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered, and concentrated via rotary evaporator yielding pure free acid Fragment 1 in 95% yield.

Protected fragment 2 dipeptide was constructed by using free acid Boc-D-Pro (1.0 equivalent), free amine H-Arg(Cbz)-OMe (1.1 equivalents), coupling reagent TBTU (1.1 equivalents) and base DIPEA (4.0 equivalents) (**Scheme 3.2**). The materials were dissolved in anhydrous DCM at 0.1 M. The reaction was monitored via TLC and was completed in ~30min. Upon completion, the reaction mixture was diluted with DCM and washed using 10% (v/v) $HCl_{(aq.)}$ to remove the excess amine and DIPEA followed by basic extraction using saturated aqueous sodium bicarbonate solution to remove the excess coupling reagents. The organic layers were combined, dried over sodium sulfate, filtered, and concentrated *in vacuo*. The material was purified by flash column chromatography using silica gel and ethyl acetate-hexane as the gradient solvent system. The pure protected fragment 2 dipeptide was furnished in 85% yield and its structure and purity was confirmed using ^{1}H NMR spectroscopy.

Amine deprotection of the Boc-protected dipeptide fragment 2 was performed in TFA:DCM (1:4) solution (0.1 M) and addition anisole (2.0 equivalents) (**Scheme 3.2**). The reaction was monitored by TLC and was complete in ~45 min. Upon completion, the reaction was concentrated via rotary evaporator furnishing the free amine dipeptide in a quantitative yield, which was and taken on without further purification.

3.6.2 Synthesis of Protected Linear tetrapeptide, double deprotection, macrocyclization, and benzyl ester removal reaction

Protected linear tetrapeptide was synthesized utilizing free acid dipeptide (fragment 1, 1.0 equivalent), free amine dipeptide (fragment 2, 1.1 equivalents), coupling reagent TBTU (0.7 equivalents) and HATU (0.7 equivalents), and base DIPEA (6.0 equivalents) (**Scheme 3.3**). The materials were dissolved in anhydrous DCM to a concentration 0.1 M. The coupling reaction was monitored via TLC and LC/MS and was complete in ~3 hours. Upon completion, the organic mixture was diluted in DCM and washed using 10% (v/v) $HCl_{(aq.)}$ followed by a basic washed using aqueous saturated sodium bicarbonate solution. The aqueous layer was then extracted with ethyl acetate three times. The organic layers were combined, dried over sodium sulfate, filtered, and concentrated *in vacuo*. The linear peptide was purified via flash column chromatography using an ethyl acetate-hexane as gradient solvent system. The pure protected linear tetrapeptide was furnished in 75% yield and the structure and purity were confirmed via ^{1}H NMR and LC/MS.



Conditions: a) TBTU (0.7 eq.), HATU (0.7 eq.), DIPEA (6.0 eq.), DCM (0.1 M); b) HCl, Anisole (2.0 eq.), THF (0.05 M); c) TBTU (0.7 eq.), HATU (0.7 eq.), DEPBT (0.7 eq.), DIPEA (8.0 eq.), DCM (0.007 M); d) H_2, Pd-C (10%), MeOH (0.1M)

Scheme 3.3 Synthesis of Protected linear tetrapeptide, Double deprotection, Cyclization, and Hydrogenolysis of HDI-4

The double deprotection of protected linear tetrapeptide was completed using *in situ* strategy.[29] The acid and amine protecting groups were removed using concentrated HCl (8 drops per 0.03 mmole of double protected linear precursor) in THF at 0.05 M with the scavenging reagent anisole (2.0 equivalents). The reaction was allowed to stir at room temperature for approximately 3 days and monitored via TLC and LC/MS. Additional HCl (3 drops per 0.03 mmole of double protected linear precursor) was added to the reaction on the second day resulting in a total of 11 drops of concentrated HCl. Upon completion the reaction mixture was concentrated *in vacuo*, where LC/MS analysis showed double deprotected linear tetrapeptide was produced in a quantitative yield. The material was taken on to macrocyclization without further purification.

Cbz protected HDI-4 was cyclized using double deprotected linear tetrapeptide (1.0 equivalent), TBTU (0.7 equivalents), HATU (0.7 equivalents), DEPBT (0.7 equivalents), and base DIPEA (8.0 equivalents) in anhydrous DCM at 0.007 M (**Scheme3.3**). The reaction was monitored via TLC and LC/MS and was complete in ~8 hours. Upon completion, the organic mixture was diluted with DCM and washed with 10% (v/v) $HCl_{(aq.)}$ twice to remove DIPEA followed by a basic wash to remove excess coupling reagents. The basic aqueous layer was back extracted with ethyl acetate three times. The organic layers were combined, dried over sodium sulfate, filtered, and concentrated in vacuo. The crude cyclic tetrapeptide was purified via flash chromatography using an ethyl acetate and hexanes gradient solvent system and the semi-pure product was eluted at 80% ethyl acetate and 20% hexane gradient. The semi-pure product was further purified by RP-HPLC to give pure Cbz protected HDI-4 in a 12% yield. Structure and purity were verified via ^1H NMR and LC/MS.

Finally, the Cbz groups on Arg were removed via hydrogenolysis using Pd-C (10%) and H_2 in methanol at 0.1 M (**Scheme 3.3**). The reaction was monitored via LC/MS and was complete in ~8 hours. Upon completion the reaction mixture was filtered using celite and concentrated *in vacuo.* Pure HDI-4 was furnished in 60% yield.

3.7 Biological Examination of FR235222 and Trapoxin B Derivatives and SAR study

Once synthesized, we tested our HDIs for their ability to inhibit HDAC activity. Using a Fluor de Lys® fluorescent assay (**Figure 3.11**) our collaborator, Dr. Pflum at Wayne State University, evaluated HDAC activity. DMSO was the control and vehicle for HDI added to HeLa cell lysate, which was followed by the addition of Fluor de Lys® substrate (25 μL, for an overall 500 μM concentration). The substrate is comprised of an

acetylated Lys side-chain, which is deacetylated by HDACs in the HeLa Lysate. The reaction mixture was incubated for 1 hr at RT, followed by the addition of Fluor de Lys® developer (50 μL). The developer reacts with deacetylated Fluor de Lys® substrate within the reaction mixture and produces a fluorophore that is detected at 460 nm using a S6 Genios Fluorimeter. Percent of HDAC activity is determined by dividing of the lysate treated with HDI by the fluorescence of the lysate treated with DMSO.

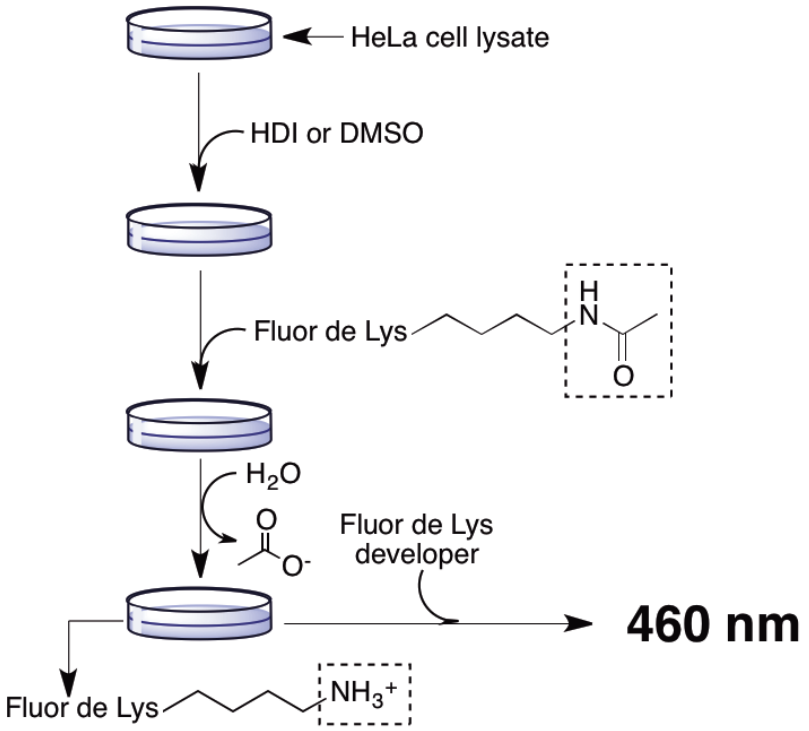
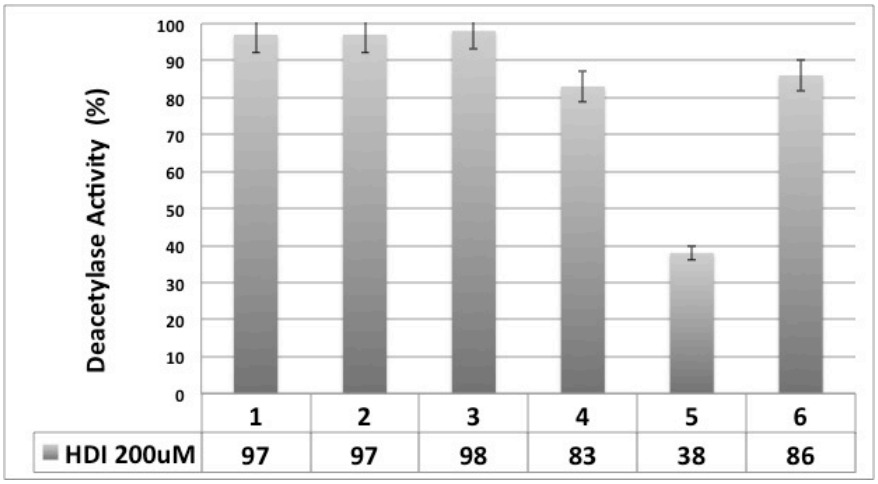


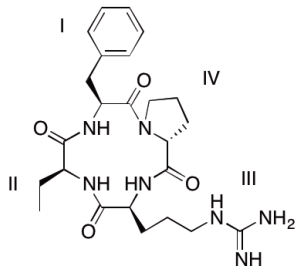
Figure 3.9 Procedure of HDAC inhibition assay

All HDIs were examined at an overall concentration of 200 μM. Our SAR, although not exceptional, showed that the stereochemistry at position I does not affect the HDAC inhibition activity. In addition, HDI-4, which incorporates a tetrahydroisoquinoline moiety at position I demonstrated better potency (83% HDAC activity) compare to HDI-1, 2 or 3. This information suggest that placing a rigid side-

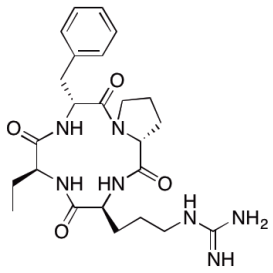
chain at position I is important for locking the surface recognition unit into a favorable conformation for HDAC binding. HDI-6, which contains a *N*-Me-Phe at position I showed lower potency than HDI-5. This result suggested that the surface recognition unit may be influenced by the *N*-methylated amino acid at position I and altered into an unfavorable binding conformation.

Table 3.3 Deacetylase Activity assay results

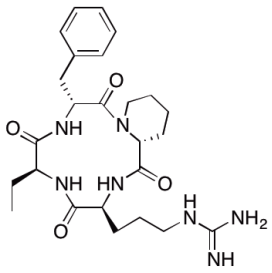




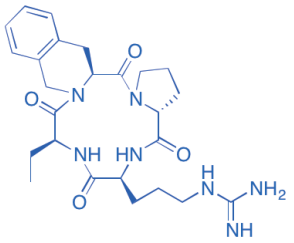
Compound 1



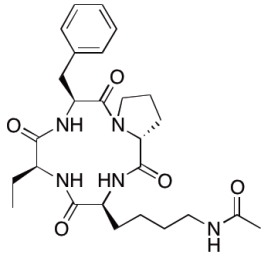
Compound 2



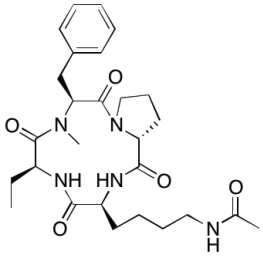
Compound 3



Compound 4



Compound 5



Compound 6

Further, in position III acetylated Lys containing HDIs (HDI-5 and 6) showed better potency than guanidine group containing HDIs (HDI-1 to 4) (**Figure 3.10**). The biological results can be translated into two possibilities 1) 5-carbon atoms length is more favorable than 4-carbon atoms length for linker region. 2) acetyl group is a better enzyme inactivating group than guanidine group. Finally, the HDAC inhibition activity does not affected by incorporating either 5-membered or 6-membered ring systems in position IV.

3.8 Conclusion

I have successfully synthesized one HDI based on the natural product FR235222 and trapoxin B. Its' biological activity has been tested along with the other HDIs that were completed by my colleagues.[30] We discovered that an acetylated Lys is a better choice for metal binding unit then the guanidine group. Also, it is beneficial to place a rigid side chain containing amino acid at position I for the surface recognition unit. However the incorporation of an *N*-Me-Phe at position I may alter the surface recognition unit into a unflavored conformation and leads to the decreased potency for this class of molecules. Finally, the biological activity was not affected by placing a 5-membered or 6-membered ring system at position IV.

Chapter 3, in part, is a reprint of material as it appears in "Synthesis and biological evaluation of histone deacetylase inhibitors that are based on FR235222: A cyclic tetrapeptide scaffold." *Bioorganic & Medicinal Chemistry Letters*, **2008**, *18*, 2549-2554. Singh, E.K.; Ravula, S.; Pan, C-M; Pan, P-S; Vasko, R.C; Lapera, S.A.; Weerasinghe, S.; Pflum, M.K.H.; McAlpine, S.R. The dissertation author was the primary investigator and author of this paper.

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Chapter 4

The Synthesis of Urukthapelstatin A

4.1 Background of Urukthapelstatin A

Nature products have provided a rich source of leads for drug discovery and synthetic challenging targets.[1] Thiazoles and oxazoles, as well their reduced variants, thiazoline and oxazoline are common structural features of many biologically active natural products.[2-4] Urukthapelstatin A (Ustat A), which contains oxazoles and thiazoles, (**Figure 4.1**) was isolated from cultured mycelia of marine-derived *Thermoactinomycetaceae* bacterium Mechercharimyces asporophorigenens YM11-542 by Prof. Matsuo and co-workers in 2007.[5,6] Ustat A exhibits potent cytotoxicity against human cancer cell lines at low nano-molar range (mean GI_{50} = 5.6 nM).[5] Its unique heterocycle-containing scaffold does not share structure homology with other current chemotherapeutic agents on the market. In addition, its unique biological activity profile distinguishes it from other structurally similar compounds, indicating that this unique scaffold may inhibit cancer growth through a novel cell-survival pathway. To investigate its biological mechanism of action, Ustat A was tested by Matsuo in several biological assays: telomerase, HDAC, farnesyl transferase, and proteasome inhibitory activity, however, it did not show activity in these assays indicating that its mechanism of action did not involves these proteins.[1] Ustat A's unknown mechanism of action and its complex structure mean that synthesis of Ustat A will be a challenging feat and provide an opportunity to explore alternative mechanisms for this natural products. Herein, we outline an efficient convergent synthetic strategy to generate chemically diverse Ustat A analogues.

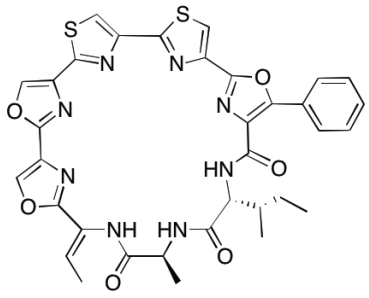


Figure 4.1 Structure of Urukthapelstatin A natural product

4.2 Biological activities and the synthesis of the other heterocycle-containing natural products

Described are three heterocycle-containing natural products that are structurally similar to Ustat A. These natural products all have interesting biological activity.

Telomestatin

Telomestatin (**Figure 4.2**) is a natural product that is composed of seven oxazoles and one thiazoline. This natural product was isolated in 2001 from a *Streptomyces anulatus* 3533-SV4.[7] This natural product possesses potent inhibitory activity against telomerase (IC_{50} = 5.0 nM). Its mechanism of action involves stabilizing the G-quadruplex on the DNA telomeres and preventing the telomerase from adding basep pairs to the DNA telomere ends. Since telomeres get shorter each replication cycle telomerase is essential to ensure the telomeres remain the same length. Loosing their base pairs without replacement leads to cell death. High telomerase activity is observed in cancer cells in order to maintain its immortality.[8] Thus, it is a viable strategy to prevent cancer progression by inhibiting the telomerase activity.

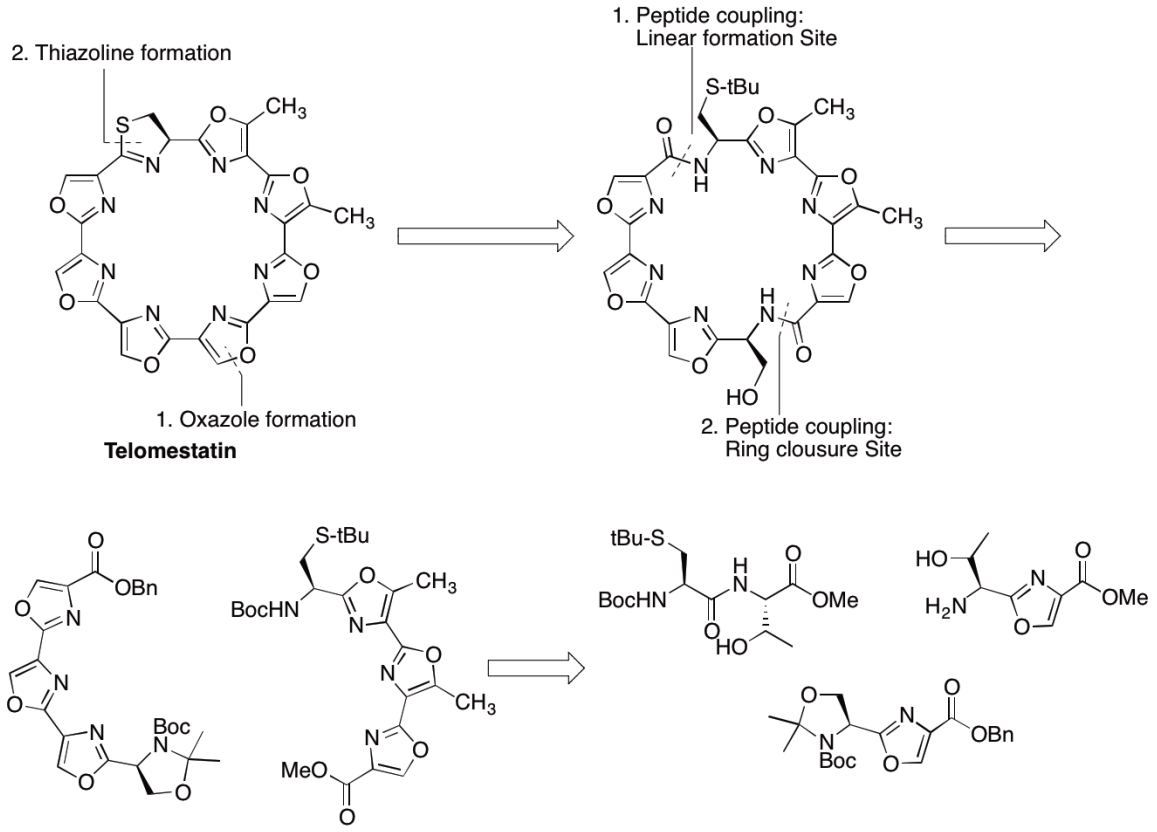


Figure 4.2 Structure and Synthetic Strategy of Telomestatin

Total synthesis of telomestatin was achieved by Dr. Takahashi and co-workers in 2006 (**Figure 4.2**).[9] The cyclization was done via a coupling reaction of the linear precursor followed by forming oxazole and thiazoline moiety sequentially. The linear precursor was afforded by coupling two trioxazole containing fragments, which were built from three synthetic units each containing a single oxazole. The synthetic units were acquired from a peptide coupling reaction followed by oxazole formation involving intramolecular cyclization and an oxidation reaction.

Thiangazole

Thiangazole (**Figure 4.3**),[10] a polythiazoline natural product, that is composed of three consecutive thiazoline moieties and one oxazole moiety. This natural product was

isolated from the gliding bacterium *Polyangium spec*. in 1992 and in a cell based assay showed 100% antiviral activity against the HIV-1 virus at low picomolar concentration (4.7 pM).[10] Interestingly, it showed no cell cytotoxicity at micromolar concentration, which indicates that it is virus specific. Although thiangazole showed promising potency as a mammalian HIV-1 inhibitor, its mechanism of action is still unknown.

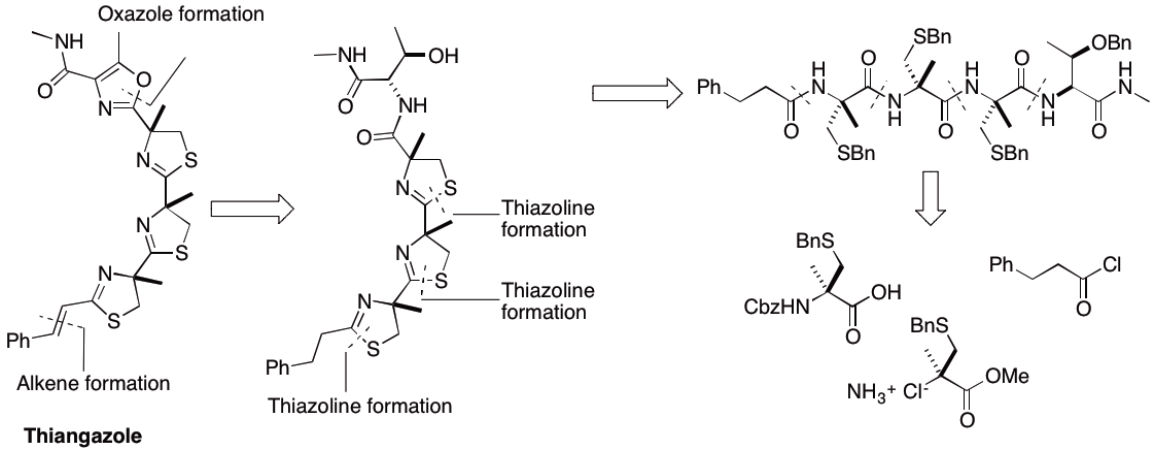


Figure 4.3 Structure and Synthetic strategy of Thiangazole

In 2006, Prof. Heathcock and co-workers completed the synthesis of Thiangazole (**Figure 4.3**).[11] This natural product was completed via oxazole formation followed by oxidation using DDQ to obtain the *Z*-alkene moiety. The trithiazoline moiety was acquired from intramolecular cyclization of the Cys residues on the linear tetrapeptide via $TiCl_4$ treatment. Finally, the tetrapeptide was acquired using a coupling reaction.[11]

Mechercharmycin A (IB-01211)

Mechercharmycin A (IB-01211, **Figure 4.4**)[12] was isolated from the marine microorganism strain ES7-008, which is phylogenetically similar to *Thermoactinomyces genus*. It possesses potent anticancer activity (0.03-0.09 μM) against various cancer cell lines (A-549, HT-29, and MDA-MB-231).[12] The structure of Mechercharmycin A is

comprised of five consecutive heterocycles (4 oxazoles and 1 thiazole) and a pseudo-tripeptide moiety. It was synthesized by Prof. Hernández and co-workers in 2007 (**Figure 4.4**).[13] In the following year, Prof. Hernández and co-workers completed a series of Mechercharmycin A derivatives. Synthesized Mechercharmycin A derivatives were tested against cancer cell lines (A-549, HT-29, and MDA-MB-231) to explore the SAR and its mechanism of action.[14] The Mechercharmycin A derivatives were designed by 1) reducing the alkene moiety to –H or $–CH_3$, 2) exchanging the hydrogen atoms on the oxazoles with methyl groups, and 3) replacing the alkene moiety with various PEG-tags. The cytotoxicity assay results showed that all the Mechercharmycin A derivatives possessed worse potency compared to Mechercharmycin A. Thus, the addition of methyl groups on the oxazole moieties and the reduction of the alkene are not favorable for cytotoxicity. In addition, the mechanistic study results of Mechercharmycin A displayed a cell cycle arrest at G2 phase. Although the biological pathway of this natural product has been discovered, its potential binding target is yet to be revealed.

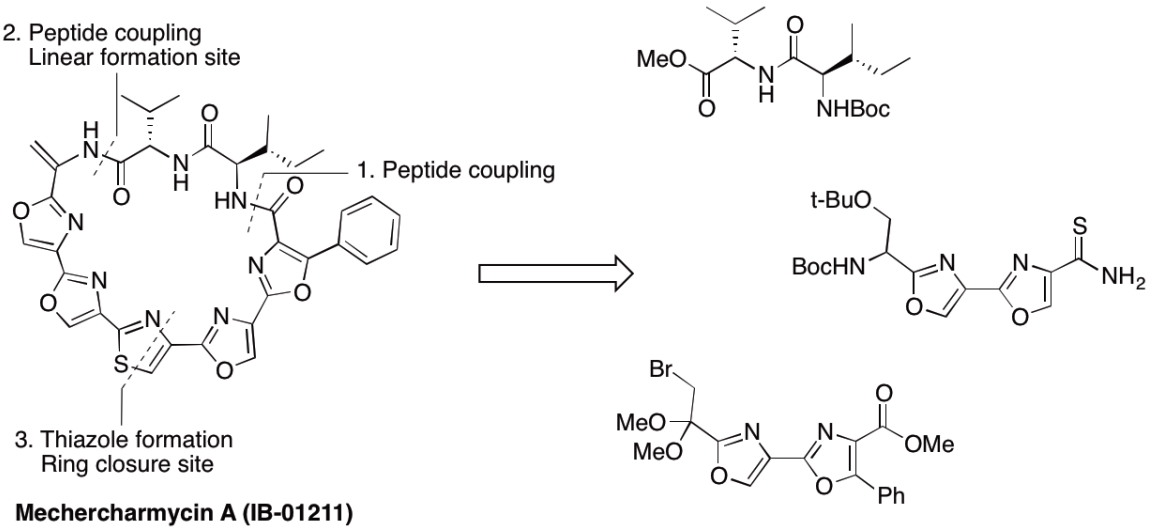


Figure 4.4 Structure and Synthetic Strategy of Mechercharmycin A

The total synthesis of mechercharmycin A was completed by Prof. Hernández and co-workers in 2007.[15] This heterocycle-containing a macrocycle was completed via thiazole formation between thioamide and bromoketone residues on the linear precursor. The linear precursor was constructed by coupling three fragments sequentially. Each fragment was completed by coupling reactions followed by oxazole formation and thioamide conversion.

From the biological perspective, described natural products all have a common structural feature that is consecutive heterocycle-containing backbone. However, the structural similarity does not translate into the same biological profile. Instead, these natural products exhibit their biological activities through various pathways. Hence, it is important to discover the role of the heterocyclic backbone in the biological system.

The convergent synthetic approach that used to generate telomestatin and mechercharmycin A is considered in Ustat A synthesis. This synthetic approach provide the possibility to generate chemically diverse Ustat A derivatives in a rapid and efficient fashion.

4.3 Rational Design of Ustat A Derivatives

The goals of this project are 1) synthesize Ustat A natural product and its derivatives and 2) explore the biological pathways against cancer cells. Herein, two Ustat A derivatives were designed to study the biological mechanism of this molecule. In this series of the Ustat A analogues, the heterocyclic backbone was modified by exchanging oxazoles and thiazoles in the various positions (heterocycle placement). In Ustat A-**2** thiazole at position III was exchanged with an oxazole, while maintaining a thiazole at

position IV. As for Ustat A-3, the thiazoles were incorporated at position I and II instead of oxazoles. The modifications were designed for understanding the biological potency pattern through the fundamental electronic change of the heterocycle backbone, where oxazoles form stronger hydrogen bond than thiazoles. In addition, ring closure capacity can also be observed through this series of analogues. Further, the heterocycle placement strategy has never been reported in any SAR or mechanistic study of this class of molecules. Therefore, developing a series of Ustat A derivatives is nessassary to reveal the impact of the heterocyclic backbone toward its potency. Ultimately reveal the potential biological target of Ustat A molecule.

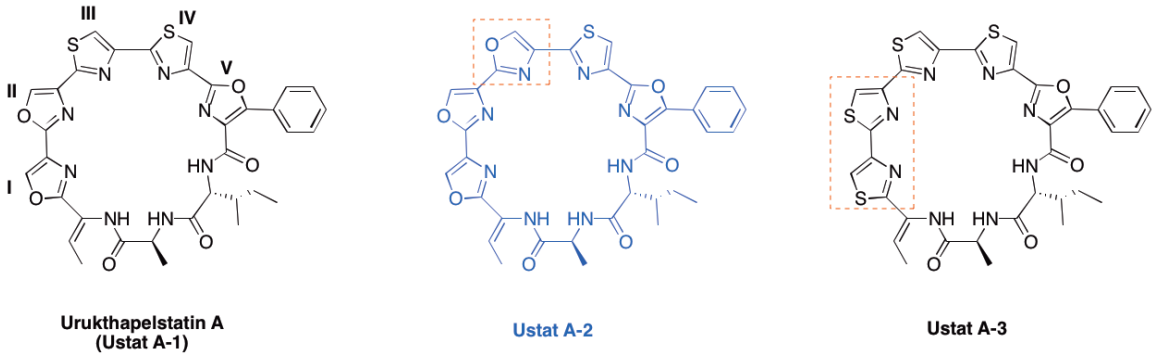


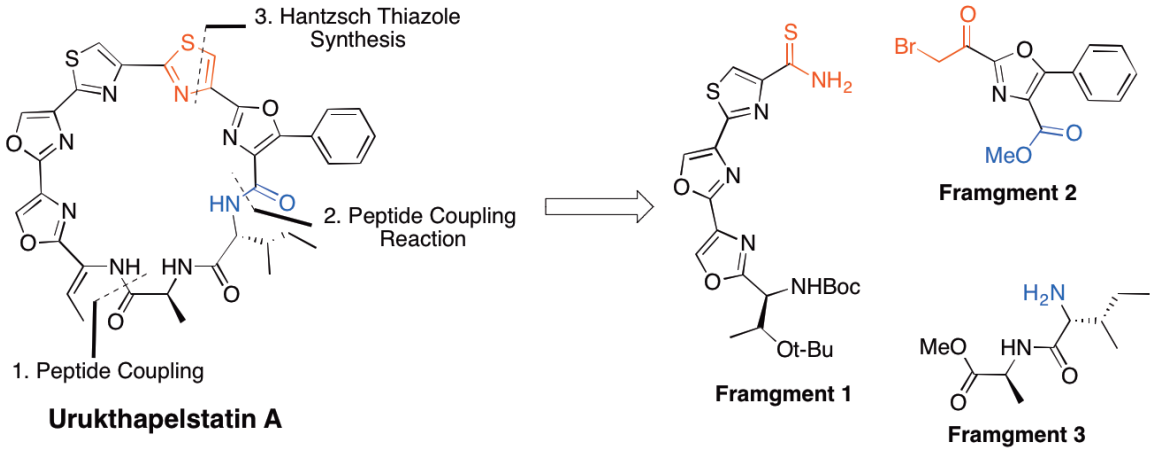
Figure 4.5 Structures of Urukthapelstatin A and two derivatives. (In this project, I am focusing on the blue colored Ustat A-2 derivative. Ustat A natural product and Ustat A-3 are currently synthesizing by my colleagues.)

4.4 Retrosynthetic Strategy of Ustat A-2

4.4.1 Convergent Synthetic Approach of Ustat A-2

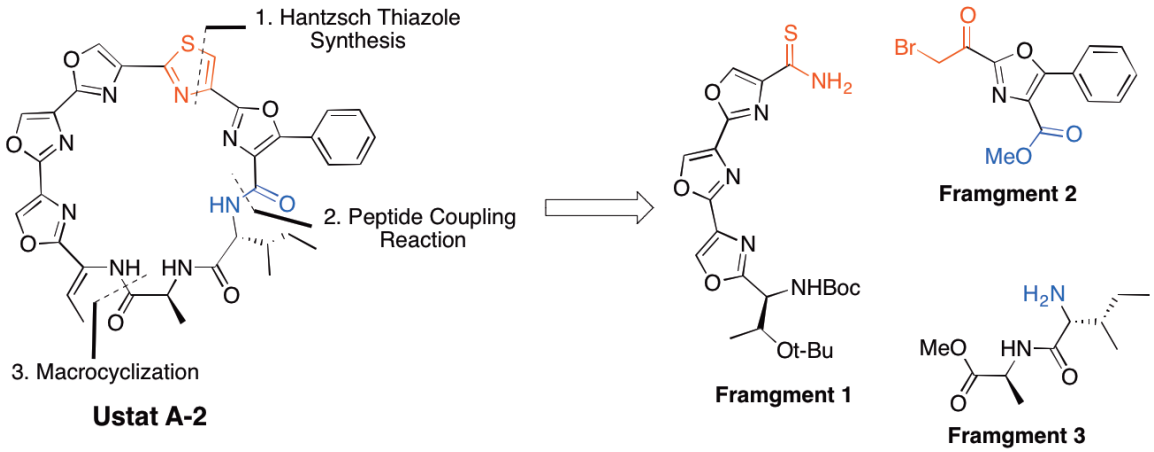
The original proposed convergent synthetic strategy (**Scheme 4.1**) for the natural product Ustat A involved three fragments. The natural product can be obtained through a base induced thiazole formation[16] at position IV from the linear precursor. The linear precursor was afforded by peptide coupling reaction between the free amine on fragment 1-3 and free acid on fragment 2. Fragment 1-3 was acquired via coupling reaction

between fragment 1 and 3. However, the ring closure thiazole formation of the linear precursor was unsuccessful. The decomposition of the linear precursor was observed during the bromoketone deprotection. Thus, a revised synthetic strategy was developed and applied for the synthesis of the Ustat A and its derivatives.



Scheme 4.1 Original Convergent Synthetic Strategy for Ustat A natural product

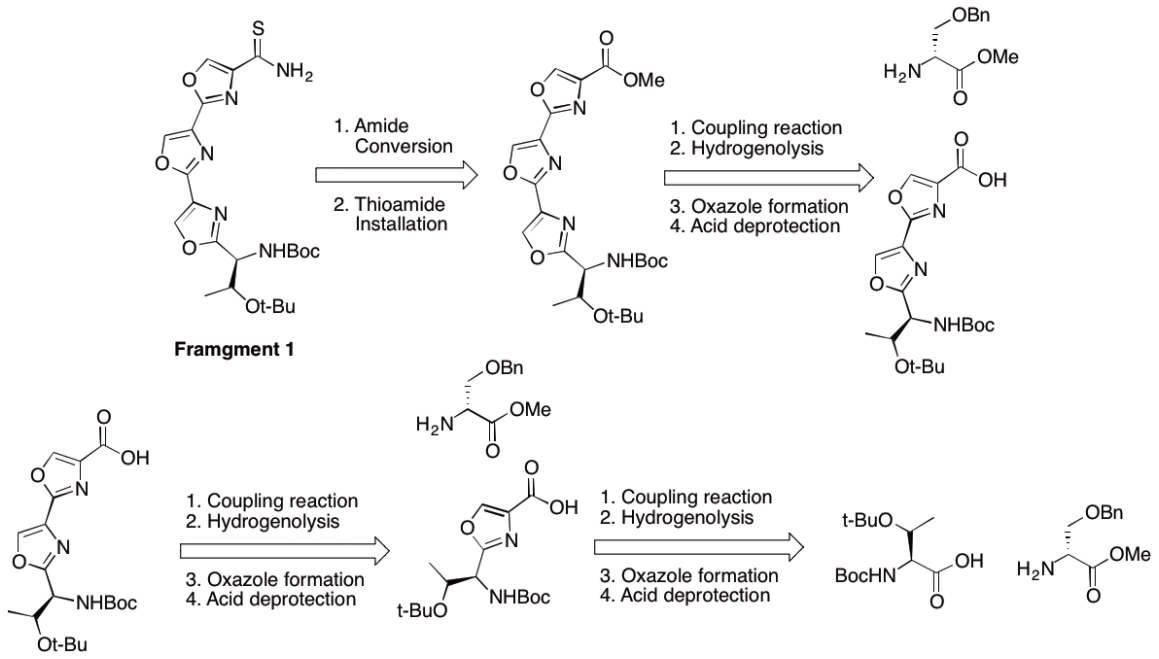
In the revised convergent synthetic approach (**Scheme 4.2**), three fragments were involved to generate Ustat A-**2**. Ustat A-**2** can be afforded from a macrocyclization between the free amine Thr residue and the free acid Ala residue of the double deprotected linear precursor followed by the elimination at the Thr residue to acquire *Z*-alkene moiety.[17] The double deprotected linear precursor was obtained through a peptide coupling reaction between the free acid of the pentaheterocycle fragment and the free amine of the dipeptide H-D-*allo*-Ile-Ala-OMe fragment 3. The heterocycle fragment was completed via Hantzsch thiazole synthesis between the thioamide moiety on the fragment 1 and the bromoketone moiety on the fragment 2. The retrosynthetic strategy of each fragment will be described individually in the following sections.



Scheme 4.2 Revised Convergent Synthetic Approach for Ustat A-2

4.4.2 Retrosynthetic Approach for Ustat-2 Fragment 1

Fragment 1 was afforded (**Scheme 4.3**) via two-step thioamide conversion from Boc-Thr(O^t-Bu)-triOxa-OMe involved amide conversion followed by sulfur installation. The trioxazole moiety was acquired from a dioxazole containing fragment Boc-Thr(O^t-Bu)-diOxa-Ser(OBn)-OMe by performing hydrogenolysis, intramolecular cyclization, and oxidation reaction sequentially. The dioxazole pseudopeptide Boc-Thr(O^t-Bu)-diOxa-Ser(OBn)-OMe was obtained by peptide coupling reaction between a free acid Boc-Thr(O^t-Bu)-diOxa-OH and a free amine H-Ser(OBn)-OMe. Futher, the dioxazole moiety of Boc-Thr(O^t-Bu)-diOxa-OH was acquired by utilizing peptide coupling reaction between a free acid Boc-Thr(O^t-Bu)-Oxa-OH and a free amine H-Ser(OBn)-OMe followed by hydrogenolysis, oxazole formation, and hydrolysis of methyl ester. Finally, the construction of free acid Boc-Thr(O^t-Bu)-Oxa-OH was achieved by coupling reaction between a free acid Boc-Thr(O^t-Bu-)-OH and a free amine H-Ser(OBn)-OMe followed by hydrogenolysis, oxazole formation, and an acid deprotection.

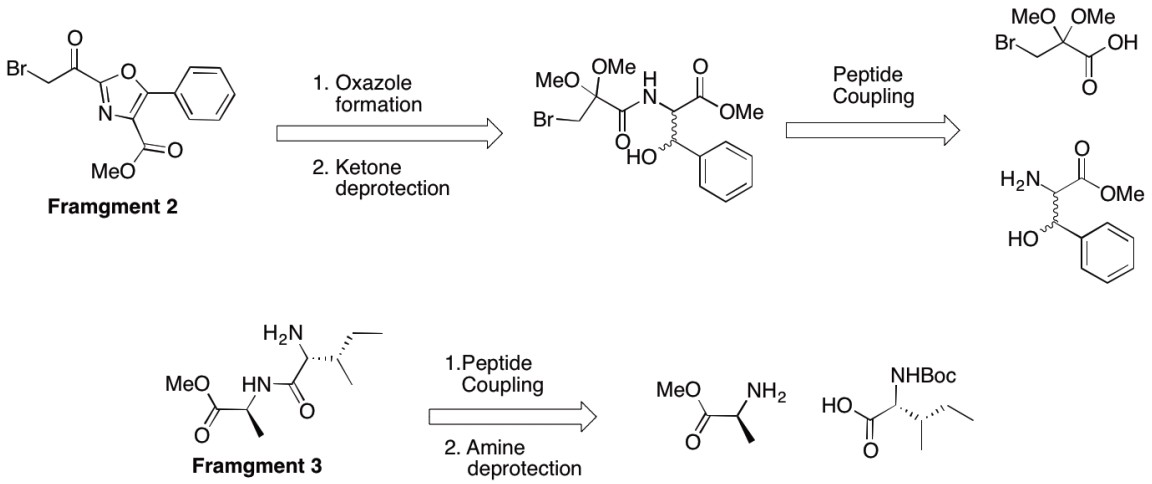


Scheme 4.3 Retrosynthesis of Ustat-2 Fragment 1

4.4.3 Retrosynthetic Approach for Ustat-2 Fragment 2 and 3

Fragment 2 was afforded (**Scheme 4.4**) by performing oxazole formation involving intramolecular cyclization and oxidation reaction followed by ketone deprotection on pseudopeptide *racemic* α-bromoketal-phenylserine-OMe. The *racemic* pseudopeptide was acquired from coupling a 3-bromo-2, 2-dimethoxypropionic acid and a free amine H-Phenylserine-OMe.

Fragment 3 was obtained (**Scheme 4.4**) by coupling a commercially available free acid Boc-D-*allo*-Ile-OH and a free amine H-Ala-OMe followed by amine deprotection.



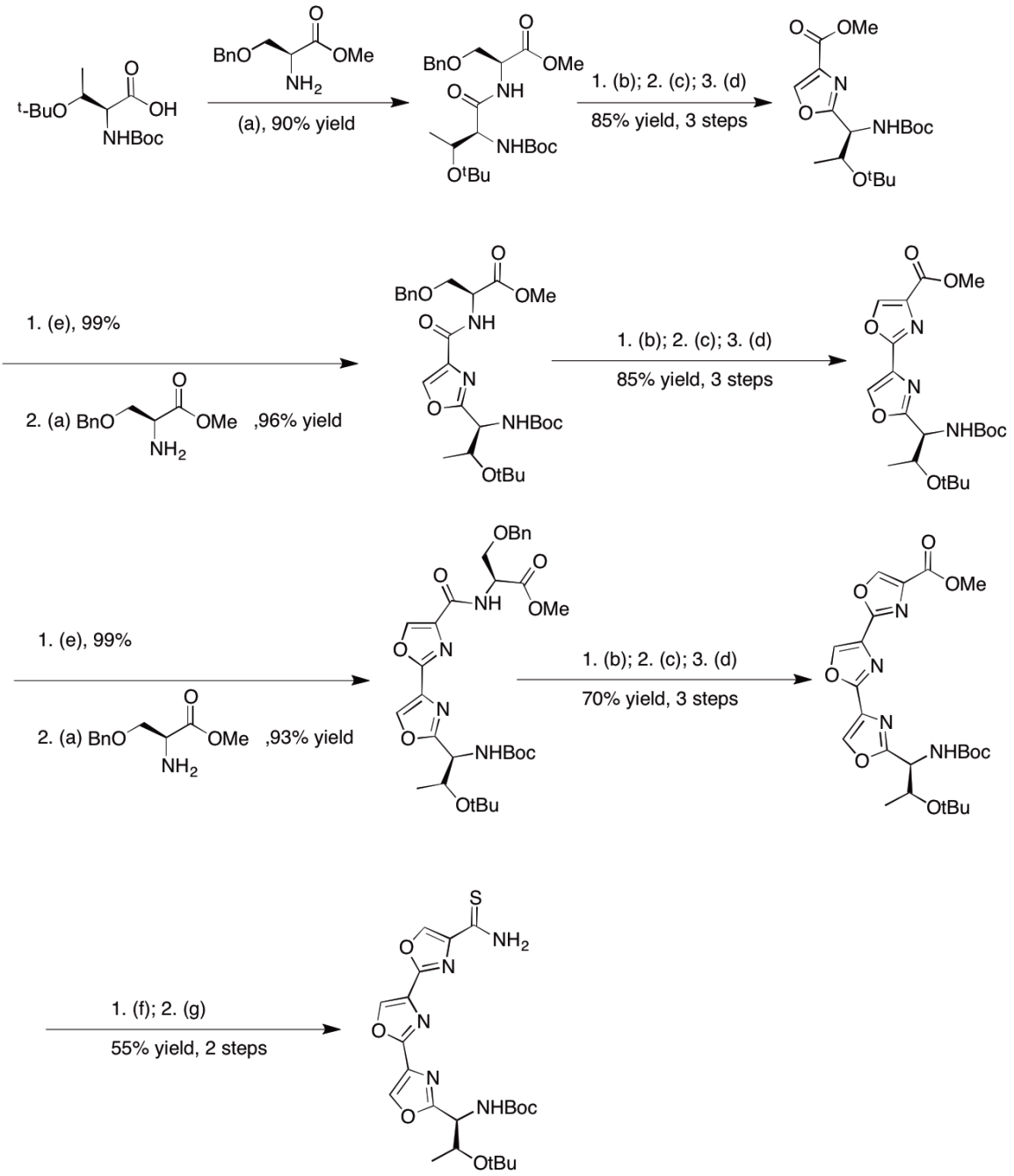
Scheme 4.4 Retrosynthesis of Ustat A-2 Fragment 2 and 3

4.5 Synthesis of Ustat A-2

4.5.1 Synthesis of Ustat A-2 Fragment 1

Protected dipeptide Boc-Thr(O^t-Bu)-Ser(OBn)-OMe (**Scheme 4.5**) was synthesized using free acid Boc-Thr(O^t-Bu)-OH (1.0 equivalent), free amine H-Ser(OBn)-OMe (1.1 equivalents), coupling reagent TBTU (1.1 equivalents), and base DIPEA (4.0 equivalents). The materials were dissolved in anhydrous DCM (0.1 M). The reaction was monitored via TLC and was complete in ~30 min and. Upon completion, the reaction mixture was diluted with DCM and washed with 10% (v/v) $HCl_{(aq.)}$ to remove the excess free amine and DIPEA. The organic layer was washed with saturated aqueous sodium bicarbonate to remove the excess coupling reagent. The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. The crude material was purified via flash column chromatography using silica gel and ethyl acetate-hexane as a gradient solvent system. The pure protected dipeptide Boc-Thr(O^t-Bu)-Ser(OBn)-OMe was furnished in 90% yield. Its structure and purity were confirmed via ^{1}H NMR

and ^{13}C spectroscopy. Pure protected dipeptide was taken on to perform the oxazole formation.



Conditions: a) TBTU (1.1 eq.), DIPEA (4.0 eq.), DCM (0.1 M); b) H_2, Pd-C (10%), EtOH (0.1 M); c) DAST (1.1 eq.), -78^{o}C 30 min then K_2CO_3 (2.0 eq.) 1h, -78^{o}C to RT, DCM (0.1 M); d) DBU (2.0 eq.) 10 min, -47^{o}C, $BrCCl_3$ (2.0 eq.), 12 h, -47^{o}C to RT; e) LiOH (8.0 eq.), MeOH (0.1 M); f) NH_4OH/MeOH (1/1, 0.005 M), ultrasound RT, 12 h; g) Lawesson's reagent (0.8 eq.) DME (0.1 M), 60^{o}C, 5 h

Scheme 4.5 Synthesis of Ustat A-2 fragment 1

Prior the oxazole moiety formation the benzyl ester on dipeptide Boc-Thr(O^t-Bu)-Ser(OBn)-OMe was removed (**Scheme 4.5**) via hydrogenolysis using catalytic amount of 10% Palladium on carbon (Pd-C) as a catalyst in EtOH (0.1 M). Hydrogen gas was purged though the reaction mixture several times. The reaction was monitored via TLC and was complete in ~8 h. Upon completion, the reaction mixture was filtered over Celite® to remove the catalyst and concentrated *in vacuo*. The dipeptide Boc-Thr(O^t-Bu)-Ser-OMe was dissolved in anhydrous DCM (0.1 M) and cooled to -78^{o}C. Fluorinating agent diethylaminosulfur trifluoride (DAST, 1.1 equivalents) was then added to the reaction. The reaction was monitored via TLC and allowed to stir for 30 min at -78^{o}C. Intramolecular cyclization was achieved to generate the oxazoline intermediate by adding K_2CO_3 (2.0 equivalents) to the solution and the organic mixture was stirred for an additional hour while warmed up to RT. Upon completion, the reaction was diluted with DCM and washed with saturated aqueous sodium bicarbonate. The collected organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The crude intermediate, oxazoline, was subjected to oxidation reaction without further purification. The oxidation of oxazoline was achieved by dissolving the oxazoline intermediate in anhydrous DCM (0.1 M) and cooled to -47^{o}C. Base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 2.0 equivalents) was added to the solution and allowed to stir for 10 min followed by adding $BrCCl_3$ (2.0 equivalents) and the reaction was monitored via TLC and allowed to proceed for 12 h while warmed up to RT. Upon completion, the reaction mixture was diluted with DCM and washed with 10% (v/v) $HCl_{(aq.)}$ to remove DBU. The collected organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The crude material was purified via flash column chromatography. The pure Boc-Thr(O^t-Bu)-

Oxa-OMe was furnished in 85% over 3 steps (hydrogenolysis, intramolecular cyclization, and oxidation) and its structure and purity was confirmed using ^{1}H and ^{13}C NMR spectroscopy.

Acid deprotection of Boc-Thr(O^{t}-Bu)-Oxa-OMe was completed using LiOH (8.0 equivalents) in MeOH (0.1 M). The reaction was monitored via TLC and was complete in ~8 h. Upon completion, the organic solution was washed with10% (v/v) $HCl_{(aq.)}$. The organic layer was collected, dried over sodium sulfate, filtered, and concentrated *in vacuo*. The free acid Boc-Thr(O^{t}-Bu)-Oxa-OH was furnished in 99% yield. The formation of Boc-Thr(O^{t}-Bu)-Oxa-Ser(OBn)-OMe was accomplished using free acid Boc-Thr(O^{t}-Bu)-Oxa-OH (1.0 equivalent), free amine H-Ser(OBn)-OMe (1.1 equivalents), coupling reagent TBTU (1.1 equivalents), and base DIPEA (4.0 equivalents). The reaction was completed in 30 min and confirmed via TLC. After the reaction was completed, the reaction was extracted using 10% (v/v) $HCl_{(aq.)}$ followed by a basic work up using saturated aqueous sodium bicarbonate solution. The collected organic layer was dried over sodium sulfate, and concentrated *in vacuo*. The crude material was purified via flash column chromatography using silica gel and ethyl acetate-hexane as a gradient solvent system. Purified pseudopeptide Boc-Thr(O^{t}-Bu)-Oxa-Ser(OBn)-OMe was furnished in 96% yield and its structure and purity were confirmed using ^{1}H and ^{13}C NMR spectroscopy. Pure mono-oxazole containing pseudopeptide was then taken on to form the second oxazole moiety. The benzyl ester on Boc-Thr(O^{t}-Bu)-Oxa-Ser(OBn)-OMe was removed via hydrogenlysis. The starting material was dissolved in EtOH (0.1 M) followed by the addition of Pd-C (10%) as catalyst. The reaction was purged with hydrogen gas three times and allowed to proceed for 8 h. Upon completion,

confirmed via TLC. The reaction mixture was filtered over Celite® and concentrated *in vacuo*. Boc-Thr(O^t-Bu)-Oxa-Ser-OMe was then dissolved in anhydrous DCM (0.1 M) and cooled to -78^{o}C followed by the addition of DAST (1.1 equivalents). The reaction was monitored via TLC and allowed to stir for 30 min at -78^{o}C. Intramolecular cyclization was furnished by adding K_2CO_3 (2.0 equivalents) to the solution and allowed to warm up to RT for an additional hour. Upon completion, the reaction was diluted with DCM and washed with saturated aqueous sodium bicarbonate. The collected organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The crude oxazoline intermediate was then dissolved in anhydrous DCM (0.1 M) and cooled to -47^{o}C. Base DBU (2.0 equivalents) was then added to the solution and stirred for 10 min followed by the addition of $BrCCl_3$ (2.0 equivalents) to the reaction mixture and allowed to proceed for 12 h while warmed up to RT. Upon completion, the reaction mixture was diluted with DCM and washed with 10% (v/v) $HCl_{(aq.)}$. The collected organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The purification was completed via flash column chromatography using silica gel and ethyl acetate-hexane as a gradient solvent system. Pure Boc-Thr(O^t-Bu)-diOxa-OMe was furnished in 85% over 3 steps and its structure and purity was confirmed using ^{1}H and ^{13}C NMR spectroscopy.

The acid deprotection of Boc-Thr(O^t-Bu)-diOxa-OMe was accomplished using LiOH (8.0 equivalents) in MeOH (0.1 M). The reaction was monitored via TLC and was complete ~8 h. Upon completion, the reaction was washed with 10% (v/v) $HCl_{(aq)}$ to quench LiOH. The collected organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The free acid Boc-Thr(O^t-Bu)-diOxa-OH was furnished in 99% yield. The pseudopeptide Boc-Thr(O^t-Bu)-diOxa-Ser(OBn)-OMe was synthesized using

free acid Boc-Thr(O^t-Bu)-diOxa-OH (1.0 equivalent), free amine H-Ser(OBn)-OMe (1.1 equivalents), coupling reagent TBTU (1.1 equivalents), and base DIPEA (4.0 equivalents). The reaction monitored via TLC and was complete in ~30 min. After the reaction was completed, the reaction mixture was washed with 10% (v/v) $HCl_{(aq.)}$ followed by a basic wash. The crude material was purified using flash column chromatography. Purified pseudopeptide Boc-Thr(O^t-Bu)-diOxa-Ser(OBn)-OMe was furnished in 93% yield and its structure and purity were confirmed using ^{1}H and ^{13}C NMR spectroscopy. The hydrogenolysis of the benzyl ester on Boc-Thr(O^t-Bu)-diOxa-Ser(OBn)-OMe was completed by dissolving the material in EtOH (0.1 M) followed by adding catalytic amount of Pd-C (10%). The reaction was purged with hydrogen gas and allowed to stir for 8 h. Upon completion, the reaction mixture was filtered over Celite® and concentrated *in vacuo*. Boc-Thr(O^t-Bu)-diOxa-Ser-OMe was then subjected two-step oxazole formation strategy to obtain a subsequent third oxazole moiety with 70% yield over 3 steps (hydrogenolysis, intramolecular cyclization, and oxidation) and its structure and purity was confirmed via ^{1}H and ^{13}C NMR spectroscopy.

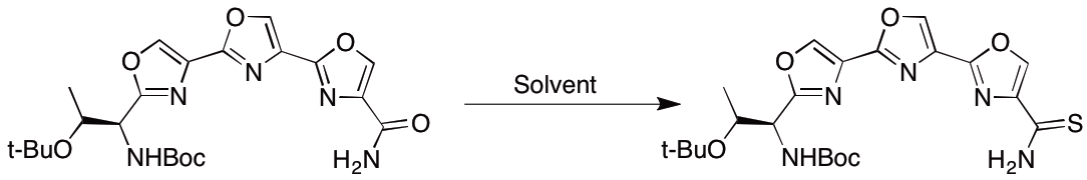
The amide conversion of Boc-Thr(O^t-Bu)-triOxa-OMe was initiated by dissolving a trioxazole containing pseudopeptide in ammonium hydroxide : methanol (1:1, 0.05 M). The reaction was ultrasonicated for 1 h and monitored via TLC and was complete in ~12 h. Upon completion, the reaction was concentrated *in vacuo*. The resulting amide was then subjected to thioamide synthesis without further purification. The thioamide conversion was initiated by dissolving Boc-Thr(O^t-Bu)-triOxa-$CONH_2$ (1.0 equivalent) and Lawesson's reagent (LR, 0.8 equivalents) in dimethoxyethane (DME, 0.1 M). The reaction was stirred at 60^{o}C, monitored via TLC, and was complete in ~5 h. Upon

completion, the reaction was dried *in vacuo* and purified via flash column chromatography using silica gel and ethyl acetate-hexane as a gradient solvent system. Boc-Thr(O^t-Bu)-triOxa-CSNH$_2$ was furnished in 55% yield over 2 steps and the structure and purity was confirmed using ^{1}H spectroscopy.

During the synthesis of fragment 1, I observed that it was more efficient to construct the oxazole moieties individually than building multiple oxazoles at once. Although it required more steps to obtain the trioxazole fragment, the overall yield was 10% better than building multiple oxazoles at once.

In addition, the solvent that was used for the thioamide conversion greatly affects the success of this reaction. My colleague and I found that using DME at 60^{o}C for thioamide conversion showed best yield compared to other types of solvent (**Table 4.1**) at the same temperature and the reaction period was also shortened using DME as a solvent.

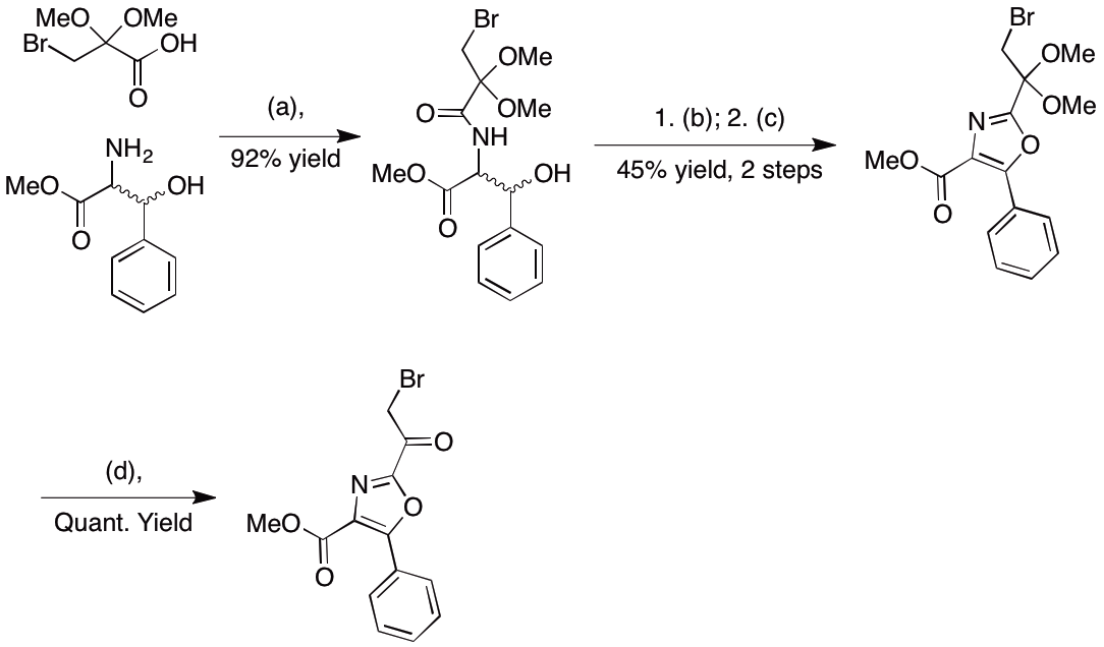
Table 4.1 Thioamide Conversion Results under various solvents



Solvent	Reaction Temp. (^{o}C)	Reaction Time (h)	Yield (%)
DCM	45	48	35
Benzene/Toluene (1/1)	110	12	40
Benzene	85	12	40
THF	70	12	35
DME	60	5	55

4.5.2 Synthesis of Ustat-2 Fragment 2

Pseudopeptide (2R, 3S) / (2S, 3R) *racemic* Bromoketal-*β*-hydroxyl-Phe-OMe was synthesized using free acid 3-bromo-2,2-dimethoxypropionic acid (1.0 equivalent), free amine (2R, 3S) / (2S, 3R) *racemic* NH_2-*β*-hydroxyl-Phe-OMe (1.1 equivalents), coupling reagent TBTU (1.1 equivalents), and base DIPEA (4.0 equivalents) in anhydrous DCM (0.1 M). The reaction was monitored via TLC and was complete in ~30 min. Upon completion, the reaction mixture was diluted with DCM and washed with 10% (v/v) $HCl_{(aq.)}$ followed by a basic wash using saturated aqueous sodium bicarbonate. The crude material was then purified via flash column chromatography using silica gel and ethyl acetate-hexane as a gradient solvent system. The pure dipeptide was furnished in 92% yield. The structure and purity were confirmed using ^{1}H and ^{13}C NMR spectroscopy.



Conditions: a) TBTU (1.1 eq.), DIPEA (4.0 eq.), DCM (0.1 M); b) DAST (1.1 eq.), 30 min, -78°C then pyridine (2.0 eq.) 1 h, -78°C to RT; c) DBU (2.0 eq.) 10 min, -47°C, $BrCCl_3$ (2.0 eq.), 12 h, -47°C to RT; d) Formic acid (0.1 M), 20 min, 60°C

Scheme 4.6 Synthesis of Ustat A-2 fragment 2

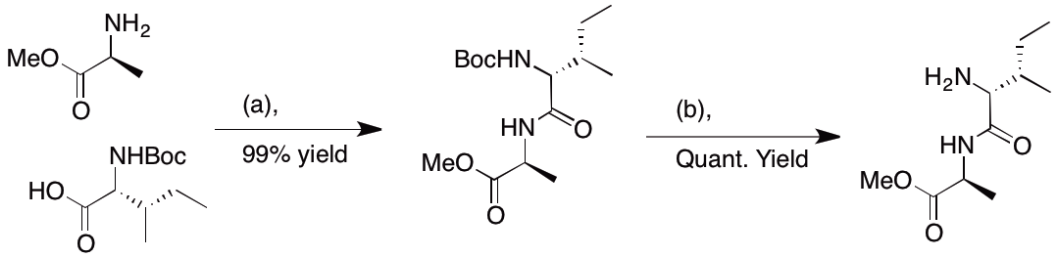
The pure pseudopeptide was transformed into bromoketalphenyloxazole-OMe via two-step oxazole synthesis procedure. The pseudopeptide *racemic* Bromoketal-*β*-hydroxyl-Phe-OMe (1.0 equivalent) was dissolved in anhydrous DCM (0.1 M) and cooled to -78^{o}C. DAST (1.1 equivalents) was then added to the reaction and allowed to stir for 30 min followed by the addition of base pyridine (2.0 equivalents) to furnish the intramolecular cyclization. The reaction was monitored and stirred for an additional hour while warmed up to RT. Upon completion, the reaction was washed with saturated aqueous sodium bicarbonate. The collected organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The crude material was purified via flash column chromatography using silica gel and ethyl acetate-hexane as a gradient solvent system. The phenyloxazoline intermediate obtained was then subjected to an oxidation reaction.

Phenyloxazoline was dissolved in anhydrous DCM (0.1 M) and cooled to -47^{o}C. Base DBU (2.0 equivalents) was added to the solution and the reaction was monitored via TLC and stirred for 10 min followed by the addition of $BrCCl_3$ (2.0 equivalents). The reaction was allowed to proceed for an additional 12 h while warmed up to RT. Upon completion, the reaction mixture was washed with 10% (v/v) $HCl_{(aq.)}$ followed by a basic wash with saturated aqueous sodium bicarbonate. The collected organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The crude material was purified via flash column chromatography using silica gel and ethyl acetate-hexane as a gradient solvent system. Ketone protected phenyloxazole moiety was furnished in 45% yield. Its structure and purity were confirmed using ^{1}H and ^{13}C NMR spectroscopy. The fragment 2 was finalized by removing the ketal groups on bromoketalphenyloxazole-OMe. The deprotection reaction of ketone was initiated by dissolving bromoketalphenyloxazole-

OMe in formic acid (0.1 M). The solution was heated from RT to 60°C for over 20 min and monitored via TLC. Upon completion, the reaction was washed with saturated aqueous sodium bicarbonate. The collected organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The bromophenyloxazole-OMe was then subjected to Hantzsch thiazole synthesis without further purification.

4.5.3 Synthesis of Ustat-2 Fragment 3

Dipeptide Boc-D-*allo*-Ile-Ala-OMe was synthesized using free acid Boc-D-*allo*-Ile-OH (1.0 equivalent), free amine H-Ala-OMe (1.1 equivalents), coupling reagent TBTU (1.1 equivalents) and base DIPEA (4.0 equivalents). The materials were dissolved in anhydrous DCM (0.1 M). The reaction was monitored via TLC and was complete in ~30 min. Upon completion, the reaction mixture was washed with 10% (v/v) $HCl_{(aq.)}$ followed by a basic wash to remove DIPEA, excess free amine and coupling reagent. The collected organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The crude material was purified via flash column chromatography using silica gel and an ethyl acetate-hexane as gradient solvent system. The pure dipeptide was furnished in 99% yield. Its structure and purity were confirmed using ^{1}H and ^{13}C NMR spectroscopy.



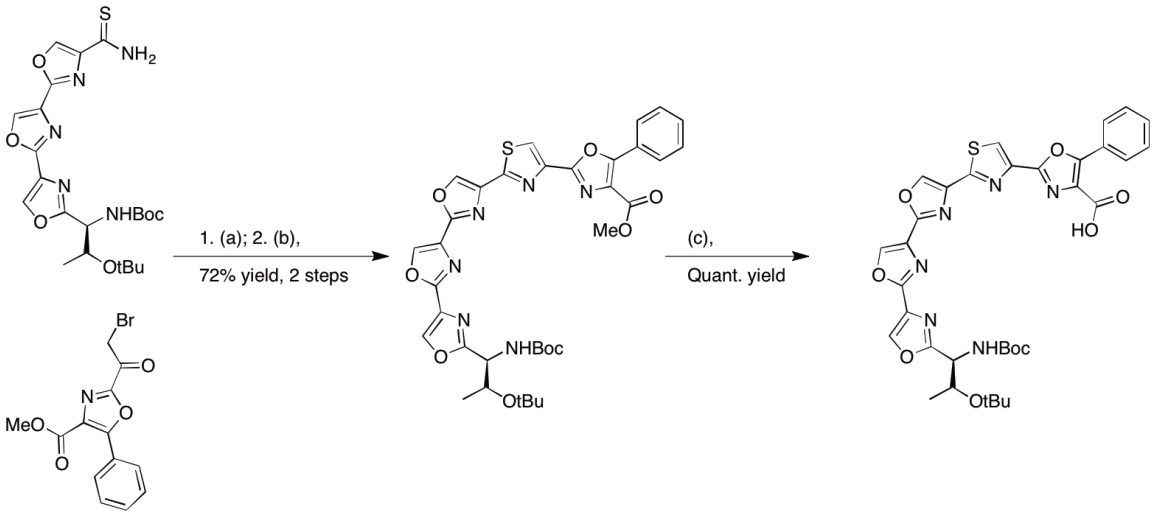
Conditions: a) TBTU (1.1 eq), DIPEA (4.0 eq), DCM (0.1 M); b) TFA/DCM (1/4, 0.1 M)
Scheme 4.7 Synthesis of Ustat A-2 fragment 3

The Boc removal reactions of dipeptide Boc-D-*allo*-Ile-Ala-OMe was completed in TFA:DCM (1:4, 0.1 M) solution with the addition of anisole (2.0 equivalents) in 0.1 M. The amine deprotection reactions was monitored via TLC and was complete in ~45 min. The reactions was concentrated *in vacuo* and free amine H-D-*allo*-Ile-Ala-OMe (fragment 3) was taken on without further purification or characterization.

4.5.4 Hantzsch Thiazole Synthesis of Ustat-2 Fragment 1 and 2

Boc-Thr(O^t-Bu)-triOxa-thiazole-phenyloxazole-OMe was synthesized via a two-step Hantzsch thiazole synthesis procedure involving thiazoline intermediate formation followed by dehydration reaction to furnish thiazole moiety. The thiazoline intermediate was synthesized by dissolving thioamide Boc-Thr(O^t-Bu)-triOxa-$CSNH_2$ (fragment 1, 1.0 equivalent) and base $KHCO_3$ (8.0 equivalents) in DME (90% of the calculated solvent volume, 0.05 M). The reaction was monitord via TLC and stirred for 10 min followed by the addition of α-bromophenyloxazole-OMe (3.0 equivalents) in DME (10% of the calculated solvent volume). The reaction was allowed to proceed for 16 h at RT. Upon completion, the crude reaction was diluted with chloroform and washed with brine. The collected organic layer was dried over sodium sulfate, filtered, concentrated *in vacuo.* Resulted crude intermediate thiazoline was subjected to dehydration reaction by dissolving the crude material in DME (0.01 M) and cooled to 0^{o}C. Pyridine (9.0 equivalents) was added to the solution for over 5 min followed by the addition of trifluoroacetic anhydride (TFAA, 4.0 equivalents). The reaction was stirred for 2 h at 0^{o}C then warmed to RT. Finally, the thiazole moiety was furnished by adding triethyl amine (TEA, 2.0 equivalents) to the solution and stirred for additional 3 h. Upon completion and confirmed via TLC, the solvent was diluted with chloroform and washed with brine. The

organic layer was dried over sodium sulfate, filtered, concentrated *in vacuo* and purified via flash column chromatography using silica gel and ethyl acetate-hexane as a gradient solvent system. Pure Boc-Thr(O^t-Bu)-triOxa-thiazole-OMe was furnished in 72% yield over 2 steps and its structure and purity was confirmed using ^{1}H and ^{13}C NMR spectroscopy. The obtained heterocycle fragment was then subjected to the ester hydrolysis.



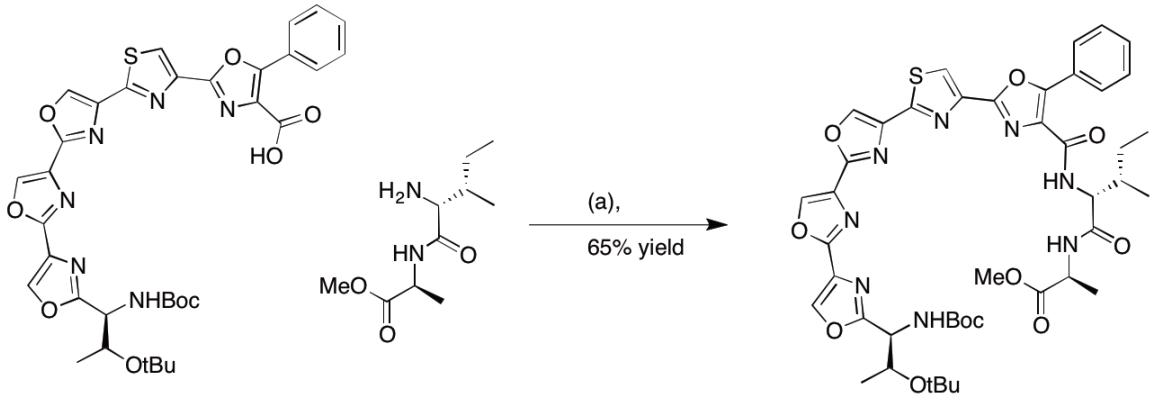
Conditions: a) $KHCO_3$ (8.0 eq.), DME (0.01 M), 16 h, RT; b) pyridine (9.0 eq.) 5 min 0^{o}C, TFAA (4.0 eq.) 2 h 0^{o}C to RT then TEA (2.0 eq.) 3 h. RT, DME (0.01 M); c) LiOH (8.0 eq.), H_2O (cat.), MeOH (0.1 M)

Scheme 4.8 Synthesis of Ustat A-2 fragment 1-2

The methyl ester of Boc-Thr(O^t-Bu)-triOxa-thiazole-OMe was removed via ester hydrolysis by dissolving Boc-Thr(O^t-Bu)-triOxa-thiazole-OMe (1.0 equivalent), LiOH (8.0 equivalents), and catalytic amount of water in MeOH (0.1 M). The reaction was monitored via TLC and stirred for 8 h. Upon completion, the reaction mixture was diluted with DCM and washed with 10% (v/v) $HCl_{(aq.)}$. The collected organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo* resulting in a free acid Boc-Thr(O^t-Bu)-triOxa-thiazole-OH. The fragment 1-3 free acid was coupled to fragment 2.

4.5.5 Synthesis of Ustat A-2 Protected Linear Precursor

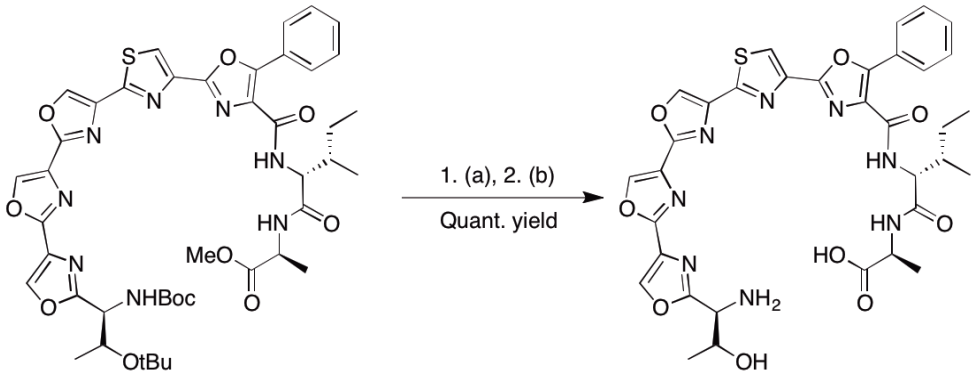
Protected Ustat A-**2** linear precursor was synthesized via a peptide coupling reaction using free acid Boc-Thr(O^t-Bu)-triOxa-thiazole-OH (1.0 equivalent), free amine D-*allo*-Ile-Ala-OMe (1.1 equivalents), coupling reagent TBTU (1.1 equivalents), and base DIPEA (4.0 equivalents). The materials were dissolved in anhydrous DCM (0.1 M). The reaction was monitored via TLC and LC/MS and was complete in ~2 h. Upon completion, the crude linear precursor was diluted with DCM and washed with 10% (v/v) $HCl_{(aq.)}$ followed by a basic wash using saturated aqueous sodium bicarbonate. The collected organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The crude material was purified via flash column chromatography using silica gel and ethyl acetate-hexane as a gradient solvent system. Pure protected linear precursor was furnished in 65% yield and its structure and purity of the pure protected linear precursor was confirmed via ^{1}H NMR, ^{13}C NMR.



Condition: a) TBTU (1.1 eq.), DIPEA (4.0 eq.), DCM (0.1 M)
Scheme 4.9 Linear formation of Ustat A-2

4.5.6 Double Deprotection of Ustat A-2

The step-wise double deprotection of the linear precursor was initiated by dissolving the protected linear precursor in MeOH (0.1 M) with the addition of LiOH (8.0 equivalents) and H_2O (cat.). The reaction was monitored via TLC and LC/MS and was complete in ~12 h. Upon completion, the reaction was diluted with DCM and washed with 10% $HCl_{(aq.)}$, the acidic aqueous layer was extracted with ethyl acetate three times. The collected organic layers was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The furnished acid deprotected linear precursor was subjected to the Boc removal reaction without further purification.



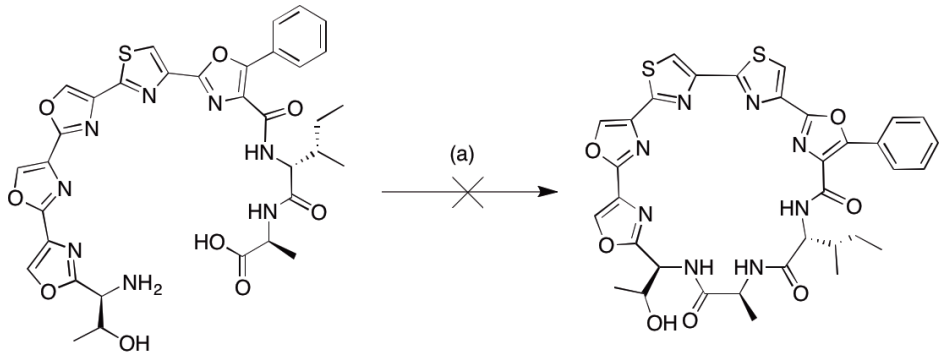
Conditions: 1) LiOH (8.0 eq.), H_2O (cat.), MeOH (0.1 M); b) TFA/DCM (1/4, 0.1 M), anisole (2.0 eq.)

Scheme 4.10 Double Deprotection Of Protected Linear Precursor

The Boc removal reaction of the linear precursor was completed in a TFA/DCM (1/4, 0.1 M) mixture with the addition of anisole (2.0 equivalents). The reaction monitored via LC/MS and was complete in ~45 min. The crude material was concentrated *in vacuo* and subjected to cyclization without further purification. In addition, the ^{t}Bu ester on the Thr residue was also removed under acidic conditions.

4.5.7 Macrocyclization of Ustat A-2

The macrocyclization for linear precursor was initiated by dissolving the double deprotected linear precursor of Ustat A-**2** (1.0 equivalent) and base DIPEA (6.0 equivalents) in anhydrous DCM using a round bottom flask. The mixture of coupling reagents, TBTU (0.7 equivalents), HATU (0.7 equivalents), and DEPBT (0.7 equivalents) were dissolved in anhydrous DCM in a separate round bottom flask then added to the double deprotected precursor solution over 2 h via a syringe pump and the final concentration of the reaction was at 0.001 M. The reaction was monitored via LC/MS and was stirred at RT for 5 h. After the 5 h, the molecular mass of the starting material was no longer observed in the LC/MS, however, the cyclized Ustat A-**2** molecular mass was never appeared in the LC/MS.

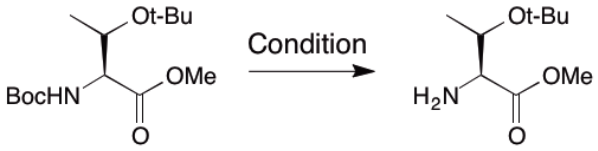


Condition: a) TBTU (0.7 eq.), HATU (0.7 eq.), DEPBT (0.7 eq.), DIPEA (6.0 eq.), DCM (0.001 M), 5 h

Scheme 4.11 Macrocyclization of Ustat A-2

The free hydroxyl group on the Thr residue was suspected to be the inference of the cyclization reaction as a nucleophilic competitor with free amine. Thus, it is necessary to deprotect the amine by removing Boc selectively while ^{t}Bu remain intact. I have successfully demonstrated a selective Boc removal[18] model reaction on a protected amino acid Boc-Thr(O^{t}-Bu)-OMe. The reaction (**Scheme 4.12**) was started by dissolving

the protected Thr in anhydrous HCl/dioxane (4 M) mixture to a concentration of 0.1 M at 0^{o}C. The reaction was monitored via TLC and LC/MS and was complete in ~25 min. Upon completion, the organic solution was concentrated *in vacuo* at 0^{o}C. The success of this reaction has strongly encouraged us to utilize this methodology in the future Ustat A derivatives synthesis.



Condition: HCl/dioxane (4 M), 0.1 M

Scheme 4.12 Selective Boc Removal Reaction

In addition, an alternative cyclization condition was considered for the future Ustat A derivatives macrocyclization, which is to pre-activate the free acid by using pentafluorophenol (PfpOH) and 1-Ethyl-3-3(dimethylaminopropyl)carbodiimide (EDC) to create an activated –Opfp ester followed by the selective Boc removal reaction then cyclizing the Ustat A derivatives under basic condition.[19] Also, other potential ring closure sites on the peptide backbone are also under investigation.

4.6 Conclusion

During the course of the Ustat A-**2** synthesis, I have learned that it is more efficient to build an oxazole moiety individually than constructing multiple oxazoles at once. In addtion, selective Boc removal reaction can be achieved under HCl/dioxane condition with the presence of the ^{t}Bu group. Finally, I have successfully conducted the synthesis of the linear precursor for Ustat A-**2** molecule and performed a cyclization reaction attempt upon the double deprotected Ustat A-**2** linear precursor. Although the

cyclization attempt was not successful, alternative cyclization conditions and ring closure sites are considered and will be investigate in the near future.

Chapter 4, in part, is a reprint of material as it appears in "Progress toward the synthesis of Urukthaplestatin A and two analogues." *Tetrahedron Letters*, **2012**, 53, 4065-4069. Pan, C–M; Lin, C–C; Kim, S. J; Sellers, R. P; McAlpine, S.R. The dissertation author was the primary investigator and author of this paper.

4.7 Reference

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Chapter 5

Experimental Methods

5.1 General Remarks and Procedures

Purchased chemicals were used as received from commercial suppliers (Novabiochem, Aldrich, Acros and Fisher scientific). Moisture and air sensitive reactions were conducted with anhydrous solvents under argon unless otherwise stated. Silica gel 60 (230-400 mesh) was used for thin-layer chromatography (TLC), and flash chromatographies. Reactions were monitored by LCMS or on TLC using UV light as visualizing method and $KMnO_4$, Bromocresol Green, and Ninhydrin as developing agents. ^{1}H and ^{13}C NMR spectra were recorded with instruments operating at 300MHz and 400MHz, respectively. Accurate mass spectra for the novel molecules were recorded high-resolution mass spectrometer, equipped with a conventional ESI source.

5.1.1.1 Solution Phase Peptide Coupling Procedure

Solution phase peptide coupling reactions were performed under argon with anhydrous solvents, DCM, ACN, THF or a combination of the therefore mentioned solvents. The free amine (1.1 equivalents) and free acid (1.0 equivalent) were weighted into a dry round bottom flask. 1.1 to 2.1 equivalents of single or combination of coupling reagents TBTU, HATU, and DEPBT were also weighted into the same flask. The materials were dissolved in anhydrous solvent to a concentration 0.1 M solution. Hünig base DIPEA (4.0-6.0 equivalents) were added to the reaction container. The reactions were proceeded in at RT and monitored by TLC every 30 min. Upon completion, the organic solution was diluted with DCM and a acidic aqueous work up was applied (10% (v/v) $HCl_{(aq)}$) to remove the excess free amine and DIPEA. The following basic work up

was done using saturated aqueous $NaHCO_3$ solution to remove the coupling reagent by-products and excess coupling reagent(s). The collected organic layers were combined, dried over Na_2SO_4, filtered, and concentrated *in vauco*. The purification of the crude material was done via flash column chromatography using silica gel and ethyl acetate-hexane as a gradient solvent system to elute desire peptide products.

5.1.1.2 Boc removal reaction (Amine deprotection)

Boc removal reaction was initiated by dissolving amine protected peptides in DCM (80% for 0.1 M overall concentration), followed by the addition of anisole (2.0 equivalents). TFA (20% for 0.1 M overall concentration) was then added to the reaction container and the reaction was allowed to proceed at RT under open atmosphere. The reaction was monitored via TLC every 30 min. Upon completion, the organic solution was concentrated *in vacuo* and wash with DCM (3 times) to remove solvent and TFA. Obtained crude material was subjected to the coupling reaction without further purification.

5.1.1.3 Methyl ester hydrolysis (Acid deprotection)

Methyl ester hydrolysis was initiated by dissolving acid protected peptide and in MeOH to generate an overall concentration of 0.1 M in a round bottom flask. Weighted LiOH (8.0 equivalents) was then added one portion to the reaction flask. The reaction was allowed to stir at RT under open atmosphere and monitored via TLC every 2 h. In some cases catalytic amount of deionized waster was added to the reaction if the hydrolysis was not completed in 8 h. Upon completion, the organic solution was diluted with DCM and a acidic work up was applied (10% (v/v) $HCl_{(aq.)}$) to remove LiOH. The acidic aqueous layer was back-extracted with DCM 3 times. The collected organic layers

were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Obtained acid deprotected peptide was subjected to peptide coupling reaction without further purification.

5.1.1.4 In situ double deprotection

The in situ double deprotection was started by dissolving double protected peptide in THF to a concentration 0.05 M. in a round bottom flask followed by the addition of anisole (2.0 equivalents). 12N HCl was then added drop-wise to the reaction mixture (8 drops per 0.3 mmoles of protected peptide) and the reaction was allowed to proceed at RT under open atmosphere. The in situ reaction typically took ~3 days and monitored via LC/MS every 12 h. Upon verification of a fully double deprotected peptide (with free amine and free acid) and disappearance of the starting material via LC/MS. The reaction was concentrated *in vacuo*.

5.1.1.5 Peptide macrocyclization

Double deprotected peptides were cyclized using mixture of coupling reagents (TBTU, HATU, DEPBT, 2.1-2.4 equivalents total). The double deprotected peptides and coupling reagents were dissolved in anhydrous DCM, ACN, or a combination of the before mentioned solvents for an overall concentration of 0.001-0.007 M under argon gas. Base DIPEA (6.0-8.0 equivalents) was then added to the reaction. TLC and LC/MS were used to monitor the progress of the reaction. The reaction was usually completed in ~3 h. Upon completion, the reaction mixture was diluted with DCM and a workup with acidic aqueous solution (10% (v/v) $HCl_{(aq.)}$) was done to remove the excess DIPEA and the excess free amine. The collected crude organic layer was washed with a basic solution using saturated $NaHCO_3$ solution. The basic aqueous layers were back washed 3 times with ethyl acetate. The collected organic layers were dried over Na_2SO_4, filtered,

and concentrated *in vacuo*. All macrocycles were purified via flash column chromatography using silica gel and ethyl acetate-hexane as a gradient system. In some cases, the macrocycles were further purified via RP-HPLC using ACN-double deionized H_2O (both with 0.1% TFA) as a gradient system.

5.1.1.6 Syringe pump macrocyclization

Double deprotected peptide was cyclized using a mixture of coupling reagents. (TBTU, HATU, DEPBT, 2.1-2.4 equivalents total) Double deprotected peptide placed in a round bottom flask and dissolved in 25% calculated volume of anhydrous DCM that would give a 0.001-0.007 overall concentration. The mixture coupling reagents were dissolved in the remaining 75% volume of the anhydrous solvent and purged with argon. DIPEA (4.0-8.0 equivalents) was then added to the round bottom flash containing coupling reagents. The double deprotected peptide was placed in a 30 mL syringe and transferred to the using reaction flask containing coupling reagents using syringe pump at a rate of 30 mL/h. The reaction was monitored via TLC and LC/MS every hour. The reaction was completed in 5 h. Upon completion, the organic mixture was diluted with DCM and a work-up with acidic aqueous solution was done using 10% (v/v) $HCl_{(aq.)}$. The organic layer was washed again with a basic aqueous solution (saturated $NaHCO_3$ aqueous solution). The basic aqueous layer was back washed with ethyl acetate 3 times. The organic layers were collected, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude material was purified via flash column chromatography using silica gel and ethyl acetate-hexane as a gradient system.

5.1.1.7 Hydrogenolysis

The Bn or Cbz protected compounds were dissolved in EtOH to an overall concentration of 0.1 M. A catalytic amount of Pd-C (10%) was added to the solution and reaction flask was fitted with septum. H_2 was purged through the reaction flask at low atmosphere pressure using a balloon (3 times). The reaction was allowed to stir at RT monitored via TLC every 2 h and usually completed in ~8-12 h. Upon completion, the reaction was filtered over Celite® to remove the catalyst Pd-C (10%). The filtered organic solution was concentrated *in vacuo*. Obtained material was not subjected to the further purification.

5.1.1.8 Oxazole formation

Oxazoline formation (K_2CO_3 as base)

Peptidyl-Ser was dissolved in anhydrous DCM to a concentration of 0.1 M and cooled to -78^{o}C. Fluorinating agent DAST (1.1 equivalents) was added to the solution drop-wise (0.1 mL/min). The reaction mixture was allowed to stir for 30 min followed by the addition of K_2CO_3 (2.0 equivalents) in one portion and the reaction was allowed to proceed for an additional hour. The reaction mixture warmed up to RT and stir for an additional 1.5 h. Upon reaction completion, confirmed by TLC, the organic solution was diluted with DCM and washed with basic solution (saturated $NaHCO_3$ aqueous solution). The collected organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*.

Oxazoline formation (Pyridine as base)

Peptidyl-Ser was dissolved in anhydrous DCM to a concentration of 0.1 M and cooled to -78^{o}C. Fluorinating agent DAST (1.1 equivalents) was added to the solution drop-wise (0.1 mL/min). The reaction mixture was allowed to stir for 30 min followed by

the addition of pyridine (2.0 equivalents) in one portion and the reaction was allowed to proceed for an additional hour. The reaction mixture warmed up to RT and stir for an additional 1.5 h. Upon reaction completion, confirmed by TLC, the organic solution was diluted with DCM and washed with basic solution (saturated $NaHCO_3$ aqueous solution). The collected organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Obtained crude oxazoline material was purified via flash column chromatography using silica gel and ethyl acetate-hexane as a gradient system.

Oxidation of Oxazoline

Peptidyl-oxazoline was dissolved in anhydrous DCM to a concentration of 0.1 M and cooled to -47^{o}C. Base DBU (2.0 equivalents) was added drop-wise (0.1 mL/min) to the reaction flask and stirred for 15 min. $BrCCl_3$ (2.0 equivalent) was added drop-wise (0.1 mL/min) to the reaction flask and stirred at -47oC for an additional 2 h and then allowed to warm up to RT to stir additional 8 h. The reaction was monitored via TLC every 2 h. Upon completion, confirmed via TLC, the reaction mixture was diluted with DCM and washed with acidic aqueous solution (10% (v/v) $HCl_{(aq.)}$). After the acidic work-up, the organic layer was washed with basic solution (saturated $NaHCO_3$ aqueous solution). The basic aqueous layer was back washed with ethyl acetate 3 times and the collected organic layers were dried over Na_2SO_4, filtered, concentrated *in vacuo*. Obtained crude oxazole material was purified via flash column chromatography using silica gel and ethyl acetate-hexane as a gradient system.

5.1.1.9 Amide conversion

Acid protected peptide was dissolved in a mixture of NH_4OH : MeOH (1:1~1:2) to a concentration of 0.1 M. The reaction was allowed to stirred at RT for 12 h and monitored via TLC. Upon completion, the solution was dried *in vacuo*.

5.1.1.10 Thioamide Conversion

Peptidyl-amide (1.0 equivalent) and the Lawesson's reagent (0.8 equivalents) were dissolved in anhydrous DME to a concentration of 0.1 M. The solution was heated to 60^oC and allowed to stir for 5 h and monitored via TLC. Upon completion, confirmed by TLC, the solvent was removed in vacuo. The crude material was purified via flash column chromatography using silica gel and ethyl acetate-hexane as a gradient system.

5.1.1.11 Thiazole formation

Peptidyl-thioamide (1.0 equivalent) was dissolved in DME to a concentration of 0.05 M followed by the addition of $KHCO_3$ (8.0 equivalents). The reaction mixture was stirred at RT for 15 min followed by the addition of peptidyl-α-bromoketone (3.0 equivalents). The reaction was stirred at RT for 16 h and monitored by TLC. Upon completion, the reaction mixture was concentrated *in vacuo*. The crude residues was dissolved in chloroform and washed with brine. The collected organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* resulted crude thiazoline. The crude thiazoline was re-dissolved in DME to a concentration of 0.05 M and cooled to 0^oC followed by the addition of pyridine (9.0 equivalents) drop-wise (0.1 mL/min) and the reaction mixture was allowed to stir for 10 min. TFAA (4.0 equivalents) was added to the reaction solution and stirred at 0^oC for additional 3 h. After 3 h, the reaction mixture was warmed up to RT and TEA (2.0 equivalents) was then added to the solution slowly (0.1

mL/min) and stirred for an additional 1 h. Upon completion, confirmed via TLC, the solvent was removed *in vacuo* and the crude residue was re-dissolved in chloroform followed by a acidic work-up (10% (v/v) $HCl_{(aq.)}$) to remove the pyridine and TEA. After the acidic work-up, the organic layer was washed using a basic solution (saturated $NaHCO_3$ aqueous solution) to remove the by-product of TFAA. The collected organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude material was purified via flash column chromatography using silica gel and ethyl acetate-hexane as a gradient system.

5.1.2 Notes for NMR used to verify structure and purity of compounds

NMR spectra were obtained at 30^{o}C on either a 600-MHz Varian, 400-MHz Varian NMR-S, 300-MHz Bruker NMR or 200-MHz Varian NMR-S using residual undeuterated solvent as an internal reference. The following abbreviation were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplete, b = broad, bd = broad doublet, dd = double of doublet, and dq = double of quartet.

5.1.3 Notes for MS used to verify structure and purity of compounds

LC/MS was recorded on an Agilent 1200 Series HPLC system (Zorbax Agilent SB-C18 column, 3.5μm, 2.1 x 30mm) connected to an Agilent 62440A LC/MS Trap running in the positive electrospray ionization (ESI+) mode. The mobile phase was composed of double-deionized water with 0.1% (v/v) formic acid (solvent A) and HPLC grade acetonitrile with 0.1% (v/v) formic acid (solvent B). The gradient elution was as follows: flow rate 1.0 mL/min; initial 80% solvent A, 20% solvent B; at 4.5 min 10% solvent A, 90% solvent B hold 0.1 min; at 7 min 85% solvent A, 15% solvent B

5.1.4 Notes for RP-HPLC purification

Semi-preparative reversed-phase HPLC was carried out on a Waters Flex inject system equipped with a Waters 2487 Dual λ Absorbance Detector (Phenomenex Symmetry C18 column, 3.5μm, 4.6 x 75mm). The mobile phase was composed of HPLC grade acetonitrile with 0.1% (v/v) TFA (solvent A) and double-deionized water with 0.1% (v/v) TFA (solvent B). The gradient elution was as follows: flow rate 2.0 mL/min; initial 70% solvent A, 30% solvent B; at 30 min 100% solvent B, hold for 15 min; at 48 min 70% solvent A, 30% solvent B, hold for 2 min.

5.2 Sansalvamide A derivatives

5.2.1 Experimental methods for San A-1

5.2.1.1 MeO-D-Phe-D-Leu-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-D-Phe-D-Leu-Boc was synthesized utilizing 500 mg (2.0 mmol, 1.0 equivalent) of free acid HO-D-Leu-Boc, 475.8 mg (2.2 mmol, 1.1 equivalents) of free amine H-D-Phe-OMe, 708 mg (2.2 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.4 mL (8.0 mmol, 4.0 equivalents) of DIPEA in 20.4 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 200 mL silica gel and ethyl acetate-hexane as a gradient system. Dipeptide MeO-D-Phe-D-Leu-Boc was eluted at the gradient of 75% of hexane and 25% of ethyl acetate. The pure dipeptide MeO-D-Phe-D-Leu-Boc was

afforded as a white solid (772 mg, 95% yield) R_f: 0.5 (3:1 Hex/EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81-1.03 (m, 6H), 1.45 (s, 9H), 1.58-1.79 (m, 3H), 3.01-3.21 (m, 2H), 3.73 (s, 3H), 4.01-4.20 (m, 1H), 4.75-4.90 (m, 2H), 6.43-6.53 (d, *J* = 8.4 Hz, 1H), 7.05-7.08 (m, 2H), 7.22-7.35 (m, 3H)

5.2.1.2 MeO-D-Phe-D-Leu-H

Following the ***Boc removal*** procedure MeO-D-Phe-D-Leu-H was synthesized by dissolving 772 mg of MeO-D-Phe-D-Leu-Boc in 16 mL of DCM, followed by adding 0.4 mL (3.8 mmol, 2.0 equivalents) of anisole and then 4 mL of TFA. Boc removal was completed in 30 min; the reaction mixture was concentrated *in vacuo* with DCM (250 mL x 3) and taken on to the next reaction without further purification or characterization (544 mg, quantitative yield) as a light yellow oil.

5.2.1.3 MeO-D-Phe-D-Leu-D-Val-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-D-Phe-D-Leu-D-Val-Boc was synthesized utilizing 455 mg (1.9 mmol, 1.0 equivalent) of free acid HO-D-Val-Boc, 544 mg (2.1 mmol, 1.1 equivalents) of free amine H-D-Phe-D-Leu-OMe, 674 mg (2.1 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.3 mL (7.6 mmol, 4.0 equivalents) of DIPEA in 19 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 150 mL silica gel and ethyl acetate-hexane as a gradient system. Tripeptide MeO-D-Phe-D-Leu-D-Val-Boc was eluted at the gradient of

70% of hexane and 30% of ethyl acetate. The pure tripeptide MeO-D-Phe-D-Leu-D-Val-Boc was afforded as a white solid (966 mg, 85% yield) R_f: 0.5 (7:3 Hex/EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81-1.03 (m, 12H), 1.45 (s, 9H), 1.41-1.61 (m, 3H), 1.95-2.05 (m, 1H), 2.97-3.02 (d, *J* = 8.1 Hz, 2H) 3.60 (s, 3H), 3.76-3.92 (m, 1H), 4.29-4.41 (m, 1H), 4.72-4.80 (q, *J* = 8.2 Hz, 1H), 4.9-5.0 (d, *J* = 8.3 Hz, 1H), 6.21-6.27 (d, *J* = 8.3 Hz, 1H), 6.39-6.42 (d, *J* = 8.3 Hz, 1H), 6.97-7.05 (m, 2H), 7.05-7.15 (m, 3H)

5.2.1.4 MeO-D-Phe-D-Leu-D-Val-H

Following the ***Boc removal*** procedure MeO-D-Phe-D-Leu-D-Val-H was synthesized by dissolving 966 mg of MeO-D-Phe-D-Leu-D-Val-Boc in 13.2 mL of DCM, followed by adding 0.3 mL (3.2 mmol, 2.0 equivalents) of anisole and then 3.3 mL of TFA. Boc removal was completed in 30 min; the reaction mixture was concentrated *in vacuo* with DCM (250 mL x 3) and taken on to the next reaction without further purification or characterization (766 mg, quantitative yield) as a light yellow oil.

5.2.1.5 MeO-Leu-Leu-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Leu-Leu-Boc was synthesized utilizing 500 mg (2.0 mmol, 1.0 equivalent) of free acid HO-Leu-Boc, 401 mg (2.2 mmol, 1.1 equivalents) of free amine H-Leu-OMe, 708 mg (2.1 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.4 mL (8.0 mmol, 4.0 equivalents) of DIPEA in 20 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column

chromatography using 150 mL silica gel and ethyl acetate-hexane as a gradient system. Dipeptide MeO-Leu-Leu-Boc was eluted at the gradient of 75% of hexane and 25% of ethyl acetate. The pure dipeptide MeO-Leu-Leu-Boc was afforded as a white solid (684 mg, 98% yield) R_f: 0.5 (3:1 Hex/EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81-1.03 (m, 12H), 1.45 (s, 9H), 1.58-1.57 (m, 4H), 1.71-1.78 (m, 1H), 3.73 (s, 3H), 4.01-4.20 (m, 1H), 4.54-4.70 (m, 1H), 4.85 (d, J = 8.3 Hz, 1H), 6.43 (d, J = 7.9 Hz, 1H)

5.2.1.6 HO-Leu-Leu-Boc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-Leu-Leu-Boc was deprotected by utilizing 684 mg (1.9 mmol, 1.0 equivalent) of MeO-Leu-Leu-Boc and 638 mg (15.2 mmol, 8.0 equivalents) of LiOH in 19 mL MeOH. The peptide was dissolved in MeOH and LiOH was added to the reaction flask and the reaction was run for 8 h. Upon completion, the reaction was diluted with 100 mL DCM and washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL). The acidic aqueous layer was back extracted with DCM (100 mL x 3). The organic layers were collected, dried over Na_2SO_4, filtered and concentrated *in vacuo* (588 mg, 90% yield) as a white solid. The free acid HO-Leu-Leu-Boc was taken on without any further purification or characterization.

5.2.1.7 MeO-D-Phe-D-Leu-D-Val-Leu-Leu-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-D-Phe-D-Leu-D-Val-Leu-Leu-Boc was synthesized utilizing 476 mg (1.46 mmol, 1.0 equivalent) of free acid HO-Leu-Leu-Boc, 766 mg (1.61 mmol, 1.1 equivalents) of free amine H-D-Phe-D-Leu-D-Val-OMe, 516 mg (1.61 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.0 mL (5.84 mmol, 4.0 equivalents) of DIPEA in 14.6 mL anhydrous DCM, under argon. The reaction was stirred for 45 min and upon completion, the crude reaction

mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 100 mL silica gel and ethyl acetate-hexane as a gradient system. Pentapeptide MeO-D-Phe-D-Leu-D-Val-Leu-Leu-Boc was eluted at the gradient of 65% of hexane and 35% of ethyl acetate. The pure pentapeptide MeO-D-Phe-D-Leu-D-Val-Leu-Leu-Boc was afforded as a white solid (682 mg, 65% yield) R_f: 0.5 (2:1 Hex/EA). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.78-0.93 (m, 24H), 1.43 (s, 9H), 1.50-1.60 (m, 4H), 1.61-1.70 (m, 2H), 1.71-1.77 (br, 1H), 2.12-2.2 (m, 1H), 3.02-3.17 (m, 2H), 3.73 (s, 3H), 4.22-4.33 (br, 1H), 4.4-4.5 (br, 1H), 4.6-4.82 (br, 3H), 5.31-5.40 (br. 1H), 6.92-7.02 (br, 1H), 7.13-7.3 (m, 5H)

5.2.1.8 HO-D-Phe-D-Leu-D-Val-Leu-Leu-H

Following ***in situ double deprotection*** procedure: HO-D-Phe-D-Leu-D-Val-Leu-Leu-H was synthesized by dissolving 682 mg (0.95 mmol 1.0 equivalent) of MeO-D-Phe-D-Leu-D-Val-Leu-Leu-Boc in 19 mL THF. 0.2 mL (1.9 mmol 2.0 equivalents) of anisole was added to the reaction solution followed by 24 drops of 12N HCl. The reaction was allowed to stir at RT for 3 d and monitored via LC/MS. Upon completion, the reaction mixture was concentrated *in vacuo* with DCM (250 mL x 3) and taken on to the next reaction without any further purification or characterization (572 mg, quantitative yield) as a brown oil.

5.2.1.9 *cyclo*-D-Phe-D-Leu-D-Val-Leu-Leu

Following the ***macrocyclization*** procedure: *cyclo*-D-Phe-D-Leu-D-Val-Leu-Leu was synthesized by utilizing 120 mg (0.2 mmol, 1.0 equivalent) of HO-D-Phe-D-Leu-D-Val-Leu-Leu-H, coupling reagents 45 mg (0.14 mmol, 0.7 equivalents) of TBTU, 53 mg (0.14 mmol, 0.7 equivalents) of HATU, 40 mg (0.14 mmol, 0.7 equivalents) of DEPBT and 0.28 mL (1.6 mmol, 8.0 equivalents) of DIPEA. The materials were dissolved in 14.5 mL of anhydrous DCM and 14.5 mL of anhydrous ACN. The reaction was allowed to stir under argon at RT and completed in 12 h. Upon completion, the reaction mixture was diluted with DCM (100 mL) and extracted with 10% (v/v) $HCl_{(aq.)}$. The organic layer was re-extracted with a saturated $NaHCO_3$ aqueous solution (100 mL x 3). The basic aqueous layer was back-washed with DCM (100 mL x 3) and ethyl acetate (100 mL x 2). The organic layers were collected, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude material was purified via flash column chromatography using 300 mL silica gel and ethyl acetate-hexane as a gradient system. *Cyclo*-D-Phe-D-Leu-D-Val-Leu-Leu was eluted at the gradient of 50% of hexane and 50% of ethyl acetate. The pure *cyclo*-D-Phe-D-Leu-D-Val-Leu-Leu was afforded as a light yellow solid (14 mg, 15% yield) R_f: 0.5 (1:2 Hex/EA). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.78-0.93 (m, 24H), 1.80-1.87 (m, 1H), 1.91-1.95 (m, 1H), 2.01-2.07 (m, 1H), 2.12-2.2 (m, 1H), 3.02-3.2 (br, 2H), 3.95-4.15 (m, 3H), 4.62-4.73 (br, 1H), 4.80-4.95 (br, 1H), 5.31-5.50 (br. 2H), 7.13-7.3 (m, 5H)

LCMS: *m/z* called for $C_{32}H_{51}N_5O_5$ (M+1) = 586.39, found 586.7

5.2.2 Experimental methods for San A 2

5.2.2.1 MeO-Phe-D-Leu-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-D-Leu-Boc was synthesized utilizing 500 mg (2.0 mmol, 1.0 equivalent) of free acid HO-D-Leu-Boc, 475.8 mg (2.2 mmol, 1.1 equivalents) of free amine H-Phe-OMe, 708 mg (2.2 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.4 mL (8.0 mmol, 4.0 equivalents) of DIPEA in 20.4 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 150 mL silica gel and ethyl acetate-hexane as a gradient system. Dipeptide MeO-Phe-D-Leu-Boc was eluted at the gradient of 75% of hexane and 25% of ethyl acetate. The pure dipeptide MeO-D-Phe-D-Leu-Boc was afforded as a white solid (788 mg, 97% yield) R_f: 0.5 (3:1 Hex/EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81-0.93 (d, J = 8.3 Hz, 6H), 1.42 (s, 9H), 1.50-1.70 (m, 3H), 3.01-3.21 (m, 2H), 3.73 (s, 3H), 4.01-4.20 (m, 1H), 4.70-4.95 (m, 2H), 6.47-6.59 (br, 1H), 7.05-7.18 (m, 2H), 7.22-7.35 (m, 3H)

5.2.2.2 MeO-Phe-D-Leu-H

Following the ***Boc removal*** procedure MeO-Phe-D-Leu-H was synthesized by dissolving 788 mg of MeO-Phe-D-Leu-Boc in 15.2 mL of DCM, followed by adding 0.4 mL (3.8 mmol, 2.0 equivalents) of anisole and then 3.8 mL of TFA. Boc removal was completed in 30 min; the reaction mixture was concentrated in vacuo with DCM (250 mL

x 3) and taken on to the next reaction without further purification or characterization (554 mg, quantitative yield) as a light yellow oil.

5.2.2.3 MeO-Phe-D-Leu-Abu-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-D-Leu-Abu-Boc was synthesized utilizing 435 mg (1.72 mmol, 1.0 equivalent) of free acid HO-Abu-Boc, 554 mg (1.9 mmol, 1.1 equivalents) of free amine H-Phe-D-Leu-OMe, 610 mg (1.9 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.3 mL (7.6 mmol, 4.0 equivalents) of DIPEA in 19 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 150 mL silica gel and ethyl acetate-hexane as a gradient system. Tripeptide MeO-Phe-D-Leu-Abu-Boc was eluted at the gradient of 75% of hexane and 25% of ethyl acetate. The pure tripeptide MeO-Phe-D-Leu-Abu-Boc was afforded as a white solid (860 mg, 90% yield) R_f: 0.5 (7:3 Hex/EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81-1.03 (m, 9H), 1.40 (s, 9H), 1.52-1.75 (m, 4H), 1.78-1.95 (m, 1H), 3.01-3.21 (m, 2H), 3.75 (s, 3H), 3.95-4.07 (q, *J* = 8.1 Hz, 1H), 4.38-4.45 (m, 1H), 4.77-4.85 (q, *J* = 8.1 Hz, 1H), 4.85-4.95 (br, 1H), 6.38-6.41 (d, *J* = 8.4 Hz, 1H), 6.58-6.72 (br, 1H), 7.05-7.08 (m, 2H), 7.22-7.35 (m, 3H)

5.2.2.4 MeO-Phe-D-Leu-Abu-H

Following the ***Boc removal*** procedure MeO-Phe-D-Leu-Abu-H was synthesized by dissolving 860 mg of MeO-Phe-D-Leu-Abu-Boc in 14 mL of DCM, followed by

adding 0.4 mL (3.5 mmol, 2.0 equivalents) of anisole and then 3.5 mL of TFA. Boc removal was completed in 30 min; the reaction mixture was concentrated *in vacuo* with DCM (250 mL x 3) and taken on to the next reaction without further purification or characterization (663 mg, quantitative yield) as a brown oil.

5.2.2.5 MeO-Leu-Leu-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Leu-Leu-Boc was synthesized utilizing 500 mg (2.0 mmol, 1.0 equivalent) of free acid HO-Leu-Boc, 401 mg (2.2 mmol, 1.1 equivalents) of free amine H-Leu-OMe, 708 mg (2.1 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.4 mL (8.0 mmol, 4.0 equivalents) of DIPEA in 20 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 100 mL silica gel and ethyl acetate-hexane as a gradient system. Dipeptide MeO-Leu-Leu-Boc was eluted at the gradient of 75% of hexane and 25% of ethyl acetate. The pure dipeptide MeO-Leu-Leu-Boc was afforded as a white solid (684 mg, 98% yield) R_f: 0.5 (3:1 Hex/EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81-1.03 (m, 12H), 1.45 (s, 9H), 1.58-1.57 (m, 4H), 1.71-1.78 (m, 1H), 3.73 (s, 3H), 4.01-4.20 (m, 1H), 4.54-4.70 (m, 1H), 4.85 (d, J = 8.3 Hz, 1H), 6.43 (d, J = 7.9 Hz, 1H)

5.2.2.6 HO-Leu-Leu-Boc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-Leu-Leu-Boc was deprotected by utilizing 684 mg (1.9 mmol, 1.0 equivalent) of MeO-Leu-

Leu-Boc and 638 mg (15.2 mmol, 8.0 equivalents) of LiOH in 19 mL MeOH. The peptide was dissolved in MeOH and LiOH was added to the reaction flask and the reaction was run for 8 h. Upon completion, the reaction was diluted with 100 mL DCM and washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL). The acidic aqueous layer was back extracted with DCM (100 mL x 3). The organic layers were collected, dried over Na_2SO_4, filtered and concentrated *in vacuo* (588 mg, 90% yield) as a white solid. The free acid HO-Leu-Leu-Boc was taken on without any further purification or characterization.

5.2.2.7 MeO-Phe-D-Leu-Abu-Leu-Leu-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-D-Leu-Abu-Leu-Leu-Boc was synthesized utilizing 502 mg (1.6 mmol, 1.0 equivalent) of free acid HO-Leu-Leu-Boc, 663 mg (1.76 mmol, 1.1 equivalents) of free amine H-Phe-D-Leu-Abu-OMe, 558 mg (1.9 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.1 mL (6.4 mmol, 4.0 equivalents) of DIPEA in 16 mL anhydrous DCM, under argon. The reaction was stirred for 40 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 100 mL silica gel and ethyl acetate-hexane as a gradient system. Pentapeptide MeO-Leu-Leu-Boc was eluted at the gradient of 65% of hexane and 35% of ethyl acetate. The pure pentapeptide MeO-Phe-D-Leu-Abu-Leu-Leu-Boc was afforded as a white solid (913 mg, 75% yield) R_f: 0.5 (2:1 Hex/EA). ^{1}H NMR (500 MHz, $CDOD_3$): δ 0.79-0.98 (m, 21H), 1.42 (s, 9H), 1.47-1.51 (m, 3H), 1.51-1.60 (m, 2H), 1.60-1.72 (m, 2H), 1.75-1.90 (m, 1H), 2.99-3.05 (m, 1H),

3.12-3.21 (m, 1H), 3.73 (s, 3H), 4.05-4.10 (br, 1H), 4.11-4.17 (m, 1H), 4.32-4.38 (m, 1H), 4.39-4.41 (br, 1H), 4.60-4.62 (m, 1H), 7.15-7.20 (m, 2H), 7.22-7.35 (m, 3H)

5.2.2.8 HO-Phe-D-Leu-Abu-Leu-Leu-H

Following ***in situ double deprotection*** procedure: HO-Phe-D-Leu-Abu-Leu-Leu-H was synthesized by dissolving 913 mg (1.3 mmol 1.0 equivalent) of MeO-Phe-D-Leu-Abu-Leu-Leu-Boc in 26 mL THF. 0.28 mL (2.6 mmol 2.0 equivalents) of anisole was added to the reaction solution followed by 32 drops of 12N HCl. The reaction was allowed to stir at RT for 3 d and monitored via LC/MS. Upon completion, the reaction mixture was concentrated *in vacuo* with DCM (250 mL x 3) and taken on to the next reaction without any further purification or characterization (765 mg, quantitative yield) as a brown oil.

5.2.2.9 *cyclo*-Phe-D-Leu-Abu-Leu-Leu

Following the ***macrocyclization*** procedure: *cyclo*-Phe-D-Leu-Abu-Leu-Leu was synthesized by utilizing 123 mg (0.21 mmol, 1.0 equivalent) of HO-Phe-D-Leu-Abu-Leu-Leu-H, coupling reagents 48 mg (0.15 mmol, 0.7 equivalents) of TBTU, 57 mg (0.15 mmol, 0.7 equivalents) of HATU, 44 mg (0.15 mmol, 0.7 equivalents) of DEPBT and 0.3 mL (1.68 mmol, 8.0 equivalents) of DIPEA. The materials were dissolved in 15 mL of anhydrous DCM and 15 mL of anhydrous ACN. The reaction was allowed to stir under argon at RT and completed in 10 h. Upon completion, the reaction mixture was diluted with DCM (100 mL) and extracted with 10% (v/v) $HCl_{(aq.)}$. The organic layer was re-extracted with a saturated $NaHCO_3$ aqueous solution (100 mL x 3). The basic aqueous layer was back-washed with DCM (100 mL x 3) and ethyl acetate (100 mL x 2). The organic layers were collected, dried over Na_2SO_4, filtered, and concentrated *in vacuo*.

The crude material was purified via flash column chromatography using 300 mL silica gel and ethyl acetate-hexane as a gradient system. *Cyclo*-Phe-D-Leu-Abu-Leu-Leu was eluted at the gradient of 55% of hexane and 45% of ethyl acetate. The pure *cyclo*-Phe-D-Leu-Abu-Leu-Leu was afforded as a light yellow solid (17 mg, 18% yield) R_f: 0.6 (1:2 Hex/EA). ^{1}H NMR (500 MHz, CD_3OD): δ 0.75-0.78 (dd, J = 8.1, 7.8 Hz, 3H), 0.80-1.01 (m, 18), 1.27-1.40 (m, 2H), 1.41-1.50 (m, 2H), 1.53-1.72 (m, 2H), 1.90-1.97 (m, 1H), 2.75-2.80 (m, 1H), 3.19-3.29 (m, 1H), 3.95-4.00 (m, 1H), 4.02-4.17 (m, 2H), 4.21-4.25 (m, 1H), 4.30-4.42 (m, 1H), 4.43-4.50 (br, 1H), 4.60-4.65 (m, 1H), 7.18-7.35 (m, 5H)

LCMS: *m/z* called for $C_{31}H_{49}N_5O_5$ (M + 1) = 572.37, found 572.6

5.2.3 Experimental methods for San A 3

5.2.3.1 MeO-Phe-Leu-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-Leu-Boc was synthesized utilizing 500 mg (2.0 mmol, 1.0 equivalent) of free acid HO-Leu-Boc, 475.8 mg (2.2 mmol, 1.1 equivalents) of free amine H-Phe-OMe, 708 mg (2.2 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.4 mL (8.0 mmol, 4.0 equivalents) of DIPEA in 20.4 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 100 mL silica gel and ethyl acetate-hexane as a gradient system. Dipeptide MeO-Phe-Leu-Boc was eluted at the gradient of 75% of hexane and 25% of ethyl acetate. The pure dipeptide MeO-Phe-Leu-Boc was afforded as a white

solid (790 mg, 98% yield) R_f: 0.5 (3:1 Hex/EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.85-0.98 (m, 6H), 1.42 (s, 9H), 1.52-1.76 (m, 4H), 3.01-3.21 (m, 2H), 3.75 (s, 3H), 4.02-4.17 (br, 1H), 4.77-4.85 (m, 2H), 6.48-6.53 (d, *J* = 8.3 Hz, 1H), 7.05-7.13 (m, 2H), 7.22-7.35 (m, 3H)

5.2.3.2 MeO-Phe-Leu-H

Following the ***Boc removal*** procedure MeO-Phe-Leu-H was synthesized by dissolving 790 mg of MeO-Phe-Leu-Boc in 16 mL of DCM, followed by adding 0.4 mL (3.7 mmol, 2.0 equivalents) of anisole and then 4 mL of TFA. Boc removal was completed in 30 min; the reaction mixture was concentrated in vacuo with DCM (250 mL x 3) and taken on to the next reaction without further purification or characterization (565 mg, quantitative yield) as a brown oil.

5.2.3.3 MeO-Phe-Leu-D-Abu-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-Leu-D-Abu-Boc was synthesized utilizing 435 mg (1.9 mmol, 1.0 equivalent) of free acid HO-D-Abu-Boc, 565 mg (2.1 mmol, 1.1 equivalents) of free amine H-Phe-Leu-OMe, 674 mg (2.1 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.3 mL (7.6 mmol, 4.0 equivalents) of DIPEA in 19 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 100 mL silica gel and ethyl acetate-hexane as a gradient system. Tripeptide MeO-Phe-Leu-D-Abu-Boc was eluted at the gradient of 70%

of hexane and 30% of ethyl acetate. The pure tripeptide MeO-Phe-Leu-D-Abu-Boc was afforded as a white solid (976 mg, 99% yield) R_f: 0.5 (7:3 Hex/EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81-0.97 (m, 9H), 1.42 (s, 9H), 1.55-1.61 (m, 5H), 1.78-1.95 (m, 1H), 3.01-3.19 (m, 2H), 3.75 (s, 3H), 3.90-4.41 (q, *J* = 8.1 Hz, 1H), 4.38-4.48 (m, 1H), 4.77-4.95 (br, 2H), 4.85-4.95 (br, 1H), 6.38-6.41 (d, *J* = 8.4 Hz, 1H), 6.52-6.68 (br, 1H), 7.05-7.08 (m, 2H), 7.22-7.35 (m, 3H)

5.2.3.4 MeO-Phe-Leu-D-Abu-H

Following the ***Boc removal*** procedure MeO-Phe-Leu-D-Abu-H was synthesized by dissolving 976 mg of MeO-Phe-Leu-D-Abu-Boc in 15.6 mL of DCM, followed by adding 0.4 mL (0.35 mmol, 2.0 equivalents) of anisole and then 4.4 mL of TFA. Boc removal was completed in 30 min; the reaction mixture was concentrated *in vacuo* with DCM (250 mL x 3) and taken on to the next reaction without further purification or characterization (776 mg, quantitative yield) as a light yellow oil.

5.2.3.5 MeO-Leu-Leu-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Leu-Leu-Boc was synthesized utilizing 500 mg (2.0 mmol, 1.0 equivalent) of free acid HO-Leu-Boc, 401 mg (2.2 mmol, 1.1 equivalents) of free amine H-Leu-OMe, 708 mg (2.1 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.4 mL (8.0 mmol, 4.0 equivalents) of DIPEA in 20 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column

chromatography using 100 mL silica gel and ethyl acetate-hexane as a gradient system. Dipeptide MeO-Leu-Leu-Boc was eluted at the gradient of 75% of hexane and 25% of ethyl acetate. The pure dipeptide MeO-Leu-Leu-Boc was afforded as a white solid (684 mg, 98% yield) R_f: 0.5 (3:1 Hex/EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81-1.03 (m, 12H), 1.45 (s, 9H), 1.58-1.57 (m, 4H), 1.71-1.78 (m, 1H), 3.73 (s, 3H), 4.01-4.20 (m, 1H), 4.54-4.70 (m, 1H), 4.85 (d, J = 8.3 Hz, 1H), 6.43 (d, J = 7.9 Hz, 1H)

5.2.3.6 HO-Leu-Leu-Boc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-Leu-Leu-Boc was deprotected by utilizing 684 mg (1.9 mmol, 1.0 equivalent) of MeO-Leu-Leu-Boc and 638 mg (15.2 mmol, 8.0 equivalents) of LiOH in 19 mL MeOH. The peptide was dissolved in MeOH and LiOH was added to the reaction flask and the reaction was run for 8 h. Upon completion, the reaction was diluted with 100 mL DCM and washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL). The acidic aqueous layer was back extracted with DCM (100 mL x 3). The organic layers were collected, dried over Na_2SO_4, filtered and concentrated *in vacuo* (588 mg, 90% yield) as a white solid. The free acid HO-Leu-Leu-Boc was taken on without any further purification or characterization.

5.2.3.7 MeO-Phe-Leu-D-Abu-Leu-Leu-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-Leu-D-Abu-Leu-Leu-Boc was synthesized utilizing 588 mg (1.69 mmol, 1.0 equivalent) of free acid HO-Leu-Leu-Boc, 776 mg (1.9 mmol, 1.1 equivalents) of free amine H-Phe-Leu-D-Abu-OMe, 610 mg (1.9 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.3 mL (7.6 mmol, 4.0 equivalents) of DIPEA in 19 mL anhydrous DCM, under argon. The reaction was stirred for 45 min and upon completion, the crude reaction mixture was diluted with

100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 150 mL silica gel and ethyl acetate-hexane as a gradient system. Pentapeptide MeO-Phe-Leu-D-Abu-Leu-Leu-Boc was eluted at the gradient of 65% of hexane and 35% of ethyl acetate. The pure pentapeptide MeO-Phe-Leu-D-Abu-Leu-Leu-Boc was afforded as a white solid (807 mg, 68% yield) R_f: 0.6 (2:1 Hex/EA). ^{1}H NMR (200 MHz, $CDOD_3$): δ 0.81-1.03 (m, 21H), 1.42 (s, 9H), 1.49-1.58 (m, 3H), 1.59-1.75 (m, 4H), 3.01-3.21 (m, 2H), 3.75 (s, 3H), 4.02-4.20 (m, 2H), 4.38-4.45 (m, 2H), 4.59-4.75 (m, 1H), 7.19-7.35 (m, 5H)

5.2.3.8 HO-Phe-Leu-D-Abu-Leu-Leu-H

Following ***in situ double deprotection*** procedure: HO-Phe-Leu-D-Abu-Leu-Leu-H was synthesized by dissolving 807 mg (1.14 mmol 1.0 equivalent) of MeO-D-Phe-D-Leu-D-Val-Leu-Leu-Boc in 22 mL THF. 0.25 mL (2.3 mmol 2.0 equivalents) of anisole was added to the reaction solution followed by 32 drops of 12N HCl. The reaction was allowed to stir at RT for 3 d and monitored via LC/MS. Upon completion, the reaction mixture was concentrated *in vacuo* with DCM (250 mL x 3) and taken on to the next reaction without any further purification or characterization (654 mg, quantitative yield) as a brown oil.

5.2.3.9 *cyclo*-Phe-Leu-D-Abu-Leu-Leu

Following the ***macrocyclization*** procedure: *cyclo*-Phe-Leu-D-Abu-Leu-Leu was synthesized by utilizing 100 mg (0.17 mmol, 1.0 equivalent) of HO-Phe-Leu-D-Abu-Leu-Leu-H, coupling reagents 39 mg (0.12 mmol, 0.7 equivalents) of TBTU, 46 mg (0.15

mmol, 0.7 equivalents) of HATU, 36 mg (0.15 mmol, 0.7 equivalents) of DEPBT and 0.2 mL (1.2 mmol, 8.0 equivalents) of DIPEA. The materials were dissolved in 12 mL of anhydrous DCM and 12 mL of anhydrous ACN. The reaction was allowed to stir under argon at RT and completed in 12 h. Upon completion, the reaction mixture was diluted with DCM (100 mL) and extracted with 10% (v/v) $HCl_{(aq.)}$. The organic layer was re-extracted with a saturated $NaHCO_3$ aqueous solution (100 mL x 3). The basic aqueous layer was back-washed with DCM (100 mL x 3) and ethyl acetate (100 mL x 2). The organic layers were collected, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude material was purified via flash column chromatography using 300 mL silica gel and ethyl acetate-hexane as a gradient system. *Cyclo*-Phe-Leu-D-Abu-Leu-Leu was eluted at the gradient of 55% of hexane and 45% of ethyl acetate. The pure *cyclo*-Phe-Leu-D-Abu-Leu-Leu was afforded as a light yellow solid (19 mg, 20% yield) R_f: 0.6 (1:2 Hex/EA). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.70-0.83 (d, J = 8.2 Hz, 3H), 0.85-1.01 (m, 18H), 1.49-1.58 (br, 3H), 1.59-1.62 (br, 4H), 1.68-1.78 (br, 2H), 1.91-1.95 (br, 1H), 2.75-2.81 (t, J = 7.6 Hz, 1H), 3.12-3.30 (d, J = 9.2 Hz, 1H), 3.96-3.98 (br, 1H), 4.10-4.15 (br, 1H), 4.20-4.30 (br, 1H), 4.49-4.55 (br, 1H), 4.6-4.65 (br, 1H), 5.30-5.38 (br, 1H), 7.17-7.32 (br, 5H), 7.35-7.40 (br, 1H), 7.50-7.70 (br, 1H), 7.79-7.85 (br, 1H), 8.53-8.62 (br, 1H) LCMS: *m/z* called for $C_{31}H_{48}N_5O_5$ (M+1) = 572.39, found 572.7

5.2.4 Experimental methods for San A 4

5.2.4.1 MeO-Phe-*N*-Me-D-Leu-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-*N*-Me-D-Leu-Boc was synthesized utilizing 520 mg (2.0 mmol, 1.0 equivalent) of free acid HO-*N*-Me-D-Leu-Boc, 495.8 mg (2.2 mmol, 1.1 equivalents) of free amine H-Phe-OMe, 708

mg (2.2 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.4 mL (8.0 mmol, 4.0 equivalents) of DIPEA in 20.4 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 100 mL silica gel and ethyl acetate-hexane as a gradient system. Dipeptide MeO-Phe-*N*-Me-D-Leu-Boc was eluted at the gradient of 75% of hexane and 25% of ethyl acetate. The pure dipeptide MeO-Phe-*N*-Me-D-Leu-Boc was afforded as a white solid (789 mg, 95% yield) R_f: 0.5 (3:1 Hex/EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.85-0.98 (m, 6H), 1.42 (s, 9H), 1.52-1.76 (m, 4H), 2.90 (s, 3H), 3.01-3.21 (m, 2H), 3.75 (s, 3H), 4.02-4.17 (br, 1H), 4.77-4.85 (m, 2H), 6.48-6.53 (d, J = 8.3 Hz, 1H), 7.05-7.13 (m, 2H), 7.22-7.35 (m, 3H)

5.2.4.2 MeO-Phe-*N*-Me-D-Leu-H

Following the ***Boc removal*** procedure MeO-Phe-*N*-Me-D-Leu-H was synthesized by dissolving 789 mg of MeO-Phe-*N*-Me-D-Leu-Boc in 16 mL of DCM, followed by adding 0.3 mL (3.6 mmol, 2.0 equivalents) of anisole and then 4 mL of TFA. Boc removal was completed in 30 min; the reaction mixture was concentrated in vacuo with DCM (250 mL x 3) and taken on to the next reaction without further purification or characterization (575 mg, quantitative yield) as a light yellow oil.

5.2.4.3 MeO-Phe-*N*-Me-D-Leu-Val-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-*N*-Me-D-Leu-Val-Boc was synthesized utilizing 444 mg (1.9 mmol, 1.0 equivalent) of free acid

HO-Val-Boc, 575 mg (2.1 mmol, 1.1 equivalents) of free amine H-Phe-*N*-Me-D-Leu-OMe, 701 mg (2.1 mmol, 1.1 equivalents) of coupling reagent HATU and 1.3 mL (7.6 mmol, 4.0 equivalents) of DIPEA in 19 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 150 mL silica gel and ethyl acetate-hexane as a gradient system. Tripeptide MeO-Phe-*N*-Me-D-Leu-Val-Boc was eluted at the gradient of 75% of hexane and 25% of ethyl acetate. The pure tripeptide MeO-Phe-*N*-Me-D-Leu-Val-Boc was afforded as a white solid (840 mg, 85% yield) R_f: 0.5 (7:3 Hex/EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81-1.02 (m, 12H), 1.45 (s, 9H), 1.51-1.75 (m, 4H), 2.19-2.39 (m, 1H), 2.79-2.83 (s, 3H), 3.01-3.21 (d, *J* = 8.2 Hz, 2H), 3.68 (s, 3H), 3.92-4.10 (m, 1H), 4.35-4.41 (m, 1H), 4.75-4.85 (q, *J* = 8.0 Hz, 1H), 6.48-6.64 (m, 2H), 7.09-7.15 (m, 2H), 7.22-7.33 (m, 3H)

5.2.4.4 MeO-Phe-*N*-Me-D-Leu-Val-H

Following the ***Boc removal*** procedure MeO-Phe-*N*-Me-D-Leu-Val-H was synthesized by dissolving 840 mg of MeO-Phe-*N*-Me-D-Leu-Val-Boc in 13.6 mL of DCM, followed by adding 0.3 mL (3.3 mmol, 2.0 equivalents) of anisole and then 3.4 mL of TFA. Boc removal was completed in 30 min; the reaction mixture was concentrated *in vacuo* with DCM (250 mL x 3) and taken on to the next reaction without further purification or characterization (720 mg, quantitative yield) as a brown oil.

5.2.4.5 MeO-Leu-Leu-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Leu-Leu-Boc was synthesized utilizing 500 mg (2.0 mmol, 1.0 equivalent) of free acid HO-Leu-Boc, 401 mg (2.2 mmol, 1.1 equivalents) of free amine H-Leu-OMe, 708 mg (2.1 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.4 mL (8.0 mmol, 4.0 equivalents) of DIPEA in 20 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 100 mL silica gel and ethyl acetate-hexane as a gradient system. Dipeptide MeO-Leu-Leu-Boc was eluted at the gradient of 75% of hexane and 25% of ethyl acetate. The pure dipeptide MeO-Leu-Leu-Boc was afforded as a white solid (684 mg, 98% yield) R_f: 0.5 (3:1 Hex/EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81-1.03 (m, 12H), 1.45 (s, 9H), 1.58-1.57 (m, 4H), 1.71-1.78 (m, 1H), 3.73 (s, 3H), 4.01-4.20 (m, 1H), 4.54-4.70 (m, 1H), 4.85 (d, J = 8.3 Hz, 1H), 6.43 (d, J = 7.9 Hz, 1H)

5.2.4.6 HO-Leu-Leu-Boc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-Leu-Leu-Boc was deprotected by utilizing 684 mg (1.9 mmol, 1.0 equivalent) of MeO-Leu-Leu-Boc and 638 mg (15.2 mmol, 8.0 equivalents) of LiOH in 19 mL MeOH. The peptide was dissolved in MeOH and LiOH was added to the reaction flask and the reaction was run for 8 h. Upon completion, the reaction was diluted with 100 mL DCM and washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL). The acidic aqueous layer was back

extracted with DCM (100 mL x 3). The organic layers were collected, dried over Na_2SO_4, filtered and concentrated *in vacuo* (588 mg, 90% yield) as a white solid. The free acid HO-Leu-Leu-Boc was taken on without any further purification or characterization.

5.2.4.7 MeO-Phe-*N*-Me-D-Leu-Val-Leu-Leu-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-*N*-Me-D-Leu-Val-Leu-Leu-Boc was synthesized utilizing 448 mg (1.45 mmol, 1.0 equivalent) of free acid HO-Leu-Leu-Boc, 720 mg (1.6 mmol, 1.1 equivalents) of free amine H-Phe-*N*-Me-D-Leu-Val-OMe, 513 mg (1.6 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.0 mL (5.8 mmol, 4.0 equivalents) of DIPEA in 14.5 mL anhydrous DCM, under argon. The reaction was stirred for 45 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 100 mL silica gel and ethyl acetate-hexane as a gradient system. Pentapeptide MeO-Phe-*N*-Me-D-Leu-Val-Leu-Leu-Boc was eluted at the gradient of 65% of hexane and 35% of ethyl acetate. The pure pentapeptide MeO-Phe-*N*-Me-D-Leu-Val-Leu-Leu-Boc was afforded as a white solid (701 mg, 60% yield) R_f: 0.6 (1:1 Hex/EA). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.79-1.00 (m, 24H), 1.40 (s, 9H), 1.50-1.79 (m, 4H), 1.95-2.10 (m, 1H), 2.94 (s, 3H), 3.00-3.22 (m, 2H), 3.75 (s, 3H), 4.02-4.18 (m, 1H), 4.39-4.50 (m, 1H), 4.51-4.62 (t, *J* = 8.0 Hz, 1H), 4.62-4.80 (m, 1H), 4.80-4.90 (br, 1H), 5.10-5.21 (dd, *J* = 7.8, 7.9 Hz, 1H), 6.41-6.50 (d, *J* = 8.1 Hz, 1H), 6.61-6.78 (d, *J* = 8.1 Hz, 1H), 6.84-6.92 (d, *J* = 8.1 Hz, 1H), 7.10-7.20 (m, 2H), 7.20-7.39 (m, 3H)

5.2.4.8 HO-Phe-*N*-Me-D-Leu-Val-Leu-Leu-H

Following ***in situ double deprotection*** procedure: HO-Phe-*N*-Me-D-Leu-Val-Leu-Leu-H was synthesized by dissolving 701 mg (0.95 mmol 1.0 equivalent) of MeO-Phe-*N*-Me-D-Leu-Val-Leu-Leu-Boc in 19 mL THF. 0.2 mL (1.9 mmol 2.0 equivalents) of anisole was added to the reaction solution followed by 24 drops of 12N HCl. The reaction was allowed to stir at RT for 3 d and monitored via LC/MS. After 3 days, 25% of the material was still acid protected, thus, 3 additional drop of 12N HCl was added to the reaction. The reaction was completed at 4^{th} day and confirmed via LC/MS. Upon completion, the reaction mixture was concentrated *in vacuo* with DCM (250 mL x 3) and taken on to the next reaction without any further purification or characterization (580 mg, quantitative yield) as a yellow oil.

5.2.4.9 *cyclo*-Phe-*N*-Me-D-Leu-Val-Leu-Leu

Following the ***macrocyclization*** procedure: *cyclo*-Phe-*N*-Me-D-Leu-Val-Leu-Leu was synthesized by utilizing 110 mg (0.18 mmol, 1.0 equivalent) of HO-Phe-*N*-Me-D-Leu-Val-Leu-Leu-H, coupling reagents 42 mg (0.13 mmol, 0.7 equivalents) of TBTU, 49 mg (0.13 mmol, 0.7 equivalents) of HATU, 39 mg (0.13 mmol, 0.7 equivalents) of DEPBT and 0.25 mL (1.44 mmol, 8.0 equivalents) of DIPEA. The materials were dissolved in 10.5 mL of anhydrous DCM and 10.5 mL of anhydrous ACN. The reaction was allowed to stir under argon at RT and completed in 12 h. Upon completion, the reaction mixture was diluted with DCM (100 mL) and extracted with 10% (v/v) $HCl_{(aq.)}$. The organic layer was re-extracted with a saturated $NaHCO_3$ aqueous solution (100 mL x 3). The basic aqueous layer was back-washed with DCM (100 mL x 3) and ethyl acetate (100 mL x 2). The organic layers were collected, dried over Na_2SO_4, filtered, and

concentrated *in vacuo*. The crude material was purified via flash column chromatography using 250 mL silica gel and ethyl acetate-hexane as a gradient system. *Cyclo*-Phe-*N*-Me-D-Leu-Val-Leu-Leu was eluted at the gradient of 50% of hexane and 50% of ethyl acetate. The pure *cyclo*-Phe-N-Me-D-Leu-Val-Leu-Leu was afforded as a light yellow solid (9.7 mg, 9% yield) R_f: 0.4 (1:2 Hex/EA). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.64-0.68 (m, 3H), 0.79-0.81 (d, J = 7.9 Hz, 3H), 0.88-0.95 (m, 18H), 1.25-1.40 (m, 4H), 1.61-1.63 (m, 2H), 1.75-1.81 (m, 2H), 1.99-2.02 (m, 1H), 2.75-2.81 (m, 2H), 3.05 (s, 3H), 3.11-3.20 (m, 1H), 3.22-3.27 (m, 1H), 3.60-3.65 (m, 2H), 4.21-4.22 (m, 1H), 4.38-4.40 (m, 2H), 4.50-4.65 (m, 2H), 7.20-7.31 (m, 5H)

LCMS: *m/z* called for $C_{33}H_{53}N_5O_5$ (M + 1) = 600.4, found 600.8

5.2.5 Experimental methods for San A 5

5.2.5.1 MeO-Phe-Leu-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-Leu-Boc was synthesized utilizing 500 mg (2.0 mmol, 1.0 equivalent) of free acid HO-Leu-Boc, 475.8 mg (2.2 mmol, 1.1 equivalents) of free amine H-Phe-OMe, 708 mg (2.2 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.4 mL (8.0 mmol, 4.0 equivalents) of DIPEA in 20.4 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 100 mL silica gel and ethyl acetate-hexane as a gradient system. Dipeptide MeO-Phe-Leu-Boc was eluted at the gradient of 75% of hexane and

25% of ethyl acetate. The pure dipeptide MeO-Phe-Leu-Boc was afforded as a white solid (790 mg, 98% yield) R_f: 0.5 (3:1 Hex/EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.85-0.95 (m, 6H), 1.42 (s, 9H), 1.53-1.75 (m, 5H), 3.01-3.21 (m, 2H), 3.75 (s, 3H), 4.02-4.19 (m, 2H), 4.75-4.95 (m, 2H), 6.51-6.55 (d, J = 8.4 Hz, 1H), 7.09-7.17 (m, 2H), 7.21-7.39 (m, 3H)

5.2.5.2 MeO-Phe-Leu-H

Following the ***Boc removal*** procedure MeO-Phe-Leu-H was synthesized by dissolving 790 mg of MeO-Phe-Leu-Boc in 16 mL of DCM, followed by adding 0.4 mL (0.37 mmol, 2.0 equivalents) of anisole and then 4 mL of TFA. Boc removal was completed in 30 min; the reaction mixture was concentrated in vacuo with DCM (250 mL x 3) and taken on to the next reaction without further purification or characterization (565 mg, quantitative yield) as a brown oil.

5.2.5.3 MeO-Phe-Leu-*N*-Me-D-Val-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-Leu-*N*-Me-D-Val-Boc was synthesized utilizing 465 mg (1.9 mmol, 1.0 equivalent) of free acid HO-*N*-Me-D-Val-Boc, 565 mg (2.1 mmol, 1.1 equivalents) of free amine H-Phe-Leu-OMe, 674 mg (2.1 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.3 mL (7.6 mmol, 4.0 equivalents) DIPEA in 19 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 100 mL silica gel and ethyl acetate-hexane as a

gradient system. Tripeptide MeO-Phe-Leu-*N*-Me-D-Val-Boc was eluted at the gradient of 70% of hexane and 30% of ethyl acetate. The pure tripeptide MeO-Phe-Leu-*N*-Me-D-Val-Boc was afforded as a white solid (915 mg, 90% yield) R_f: 0.5 (7:3 Hex/EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81-1.02 (m, 12H), 1.45 (s, 9H), 1.51-1.75 (m, 4H), 2.19-2.39 (m, 1H), 2.79-2.83 (s, 3H), 3.01-3.21 (d, J = 8.2 Hz, 2H), 3.68 (s, 3H), 3.92-4.10 (m, 1H), 4.35-4.41 (m, 1H), 4.75-4.85 (q, J = 8.0 Hz, 1H), 6.48-6.64 (m, 2H), 7.09-7.15 (m, 2H), 7.22-7.33 (m, 3H)

5.2.5.4 MeO-Phe-Leu-*N*-Me-D-Val-H

Following the ***Boc removal*** procedure MeO-Phe-Leu-*N*-Me-D-Val-H was synthesized by dissolving 915 mg of MeO-Phe-Leu-*N*-Me-D-Val-Boc in 15.6 mL of DCM, followed by adding 0.4 mL (0.35 mmol, 2.0 equivalents) of anisole and then 4.4 mL of TFA. Boc removal was completed in 30 min; the reaction mixture was concentrated *in vacuo* with DCM (250 mL x 3) and taken on to the next reaction without further purification or characterization (707 mg, quantitative yield) as a brown oil.

5.2.5.5 MeO-Leu-D-Leu-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Leu-D-Leu-Boc was synthesized utilizing 500 mg (2.0 mmol, 1.0 equivalent) of free acid HO-D-Leu-Boc, 401 mg (2.2 mmol, 1.1 equivalents) of free amine H-Leu-OMe, 708 mg (2.1 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.4 mL (8.0 mmol, 4.0 equivalents) of DIPEA in 20 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried

over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 100 mL silica gel and ethyl acetate-hexane as a gradient system. Dipeptide MeO-Leu-D-Leu-Boc was eluted at the gradient of 75% of hexane and 25% of ethyl acetate. The pure dipeptide MeO-Leu-D-Leu-Boc was afforded as a white solid (674 mg, 95% yield) R_f: 0.5 (3:1 Hex/EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81-1.03 (m, 12H), 1.45 (s, 9H), 1.58-1.57 (m, 4H), 1.71-1.78 (m, 1H), 3.73 (s, 3H), 4.01-4.20 (m, 1H), 4.54-4.70 (m, 1H), 4.85 (d, J = 8.3 Hz, 1H), 6.43 (d, J = 7.9 Hz, 1H)

5.2.5.6 HO-Leu-D-Leu-Boc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-Leu-D-Leu-Boc was deprotected by utilizing 674 mg (1.9 mmol, 1.0 equivalent) of MeO-Leu-Leu-Boc and 638 mg (15.2 mmol, 8.0 equivalents) of LiOH in 19 mL MeOH. The peptide was dissolved in MeOH and LiOH was added to the reaction flask and the reaction was run for 8 h. Upon completion, the reaction was diluted with 100 mL DCM and washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL). The acidic aqueous layer was back extracted with DCM (100 mL x 3). The organic layers were collected, dried over Na_2SO_4, filtered and concentrated *in vacuo* (580 mg, 89% yield) as a white solid. The free acid HO-Leu-D-Leu-Boc was taken on without any further purification or characterization.

5.2.5.7 MeO-Phe-Leu-*N*-Me-D-Val-Leu-D-Leu-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-Leu-*N*-Me D-Val-Leu-D-Leu-Boc was synthesized utilizing 560 mg (1.6 mmol, 1.0 equivalent) of free acid HO-Leu-D-Leu-Boc, 707 mg (1.76 mmol, 1.1 equivalents) of free amine H-Phe-Leu-*N*-Me-D-Val-OMe, 513 mg (1.76 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.1 mL (6.4 mmol, 4.0 equivalents) of DIPEA in 16 mL anhydrous DCM, under

argon. The reaction was stirred for 45 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 100 mL silica gel and ethyl acetate-hexane as a gradient system. Pentapeptide MeO-Phe-Leu-*N*-Me-D-Val-Leu-D-Leu-Boc was eluted at the gradient of 55% of hexane and 45% of ethyl acetate. The pure pentapeptide MeO-Phe-Leu-D-Abu-Leu-Leu-Boc was afforded as a white solid (837 mg, 55% yield) R_f: 0.4 (2:1 Hex/EA). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.80-1.00 (m, 24H), 1.41 (s, 9H), 1.50-1.78 (m, 5H), 2.82 (s, 3H), 3.01-3.15 (m, 2H), 3.75 (s, 3H), 4.02-4.18 (m, 1H), 4.29-4.38 (m, 1H), 4.52-4.57 (d, J = 7.9 Hz, 1H), 4.75-4.81 (m, 1H), 4.74-4.99 (br, 2H), 6.57-6.67 (m, 1H), 7.12-7.18 (m, 2H), 7.21-7.30 (m, 3H)

5.2.5.8 HO-Phe-Leu-*N*-Me-D-Abu-Leu-Leu-H

Following ***in situ double deprotection*** procedure: HO-Phe-Leu-*N*-Me-D-Val-Leu-D-Leu-H was synthesized by dissolving 837 mg (1.1 mmol 1.0 equivalent) of MeO-Phe-Leu-*N*-Me-D-Val-Leu-D-Leu-Boc in 22 mL THF. 0.23 mL (2.2 mmol 2.0 equivalents) of anisole was added to the reaction solution followed by 32 drops of 12N HCl. The reaction was allowed to stir at RT for 2 d and monitored via LC/MS. Upon completion, the reaction mixture was concentrated *in vacuo* with DCM (250 mL x 3) and taken on to the next reaction without any further purification or characterization (599 mg, quantitative yield) as a brown oil.

5.2.5.9 *cyclo*-Phe-Leu-*N*-Me-D-Val-Leu-D-Leu

Following the ***macrocyclization*** procedure: *cyclo*-Phe-Leu-*N*-Me-D-Val-Leu-D-Leu was synthesized by utilizing 100 mg (0.16 mmol, 1.0 equivalent) of HO-Phe-Leu-*N*-Me-D-Val-Leu-D-Leu-H, coupling reagents 35 mg (0.11 mmol, 0.7 equivalents) of TBTU, 42 mg (0.11 mmol, 0.7 equivalents) of HATU, 33 mg (0.15 mmol, 0.7 equivalents) of DEPBT and 0.23 mL (1.28 mmol, 8.0 equivalents) of DIPEA. The materials were dissolved in 11.5 mL of anhydrous DCM and 11.5 mL of anhydrous ACN. The reaction was allowed to stir under argon at RT and completed in 12 h. Upon completion, the reaction mixture was diluted with DCM (100 mL) and extracted with 10% (v/v) $HCl_{(aq.)}$. The organic layer was re-extracted with a saturated $NaHCO_3$ aqueous solution (100 mL x 3). The basic aqueous layer was back-washed with DCM (100 mL x 3) and ethyl acetate (100 mL x 2). The organic layers were collected, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude material was purified via flash column chromatography using 300 mL silica gel and ethyl acetate-hexane as a gradient system. *Cyclo*-Phe-Leu-*N*-Me-D-Val-Leu-D-Leu was eluted at the gradient of 30% of hexane and 70% of ethyl acetate. The pure *cyclo*-Phe-Leu-N-Me-D-Val-Leu-D-Leu was afforded as a white solid (7 mg, 8% yield) R_f: 0.3 (1:2 Hex/EA). ^{1}H NMR (500 MHz, $CDOD_3$): δ 0.64-0.68 (m, 2H), 0.79-0.81 (d, *J* = 7.9 Hz, 2H), 0.90-0.95 (m, 5H), 1.05-1.37 (m, 2H), 1.41 (s, 9H), 1.60-1.72 (m, 5H), 2.82-2.95 (m, 2H), 2.95 (s, 3H), 3.00-3.10 (m, 1H), 3.11-3.20 (m, 1H), 3.29-3.41 (m, 2H), 3.75 (s, 3H), 4.05-4.10 (m, 1H), 4.32-4.41 (m, 2H), 4.75-4.85 (m, 2H), 5.50-5.60 (m, 2H), 6.39-6.41 (d, *J* = 7.9 Hz, 1H), 6.59-6.61 (d, *J* = 7.9 Hz, 1H), 6.68-6.69 (d, *J* = 7.9 Hz, 1H), 6.99-7.01 (d, *J* = 7.9 Hz, 1H), 7.09-7.38 (m, 10H) LCMS: *m/z* called for $C_{32}H_{52}N_5O_6$ (M + 1) = 602.83, found 603.8

5.2.6 Experimental methods for San A 6

5.2.6.1 MeO-Phe-*N*-Me-D-Phe-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-*N*-Me-D-Phe-Boc was synthesized utilizing 560 mg (2.0 mmol, 1.0 equivalent) of free acid HO-*N*-Me-D-Phe-Boc, 475.8 mg (2.2 mmol, 1.1 equivalents) of free amine H-Phe-OMe, 708 mg (2.2 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.4 mL (8.0 mmol, 4.0 equivalents) of DIPEA in 20.4 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 100 mL silica gel and ethyl acetate-hexane as a gradient system. Dipeptide MeO-Phe-*N*-Me-D-Phe-Boc was eluted at the gradient of 80% of hexane and 20% of ethyl acetate. The pure dipeptide MeO-Phe-*N*-Me-D-Phe-Boc was afforded as a white solid (820 mg, 89% yield) R_f: 0.5 (3:1 Hex/EA). ^{1}H NMR (200 MHz, $CDCl_3$): ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.20-1.30 (m, 5H), 1.38 (s, 9H), 1.40-1.52 (m, 5H), 2.62-2.78 (d, *J* = 8.4 Hz, 2H), 3.01-3.16 (m, 2H), 3.21-3.45 (m, 1H), 3.75-3.81 (d, *J* = 8.2 Hz, 3H), 4.02-4.19 (q, *J* = 8.0 Hz, 1H), 4.65-4.99 (m, 2H), 6.22-6.39 (d, *J* = 8.1 Hz, 1H), 6.59-6.68 (d, *J* = 8.1 Hz, 1H), 6.95-7.05 (br, 2H), 7.13-7.38 (m, 8H)

5.2.6.2 MeO-Phe-*N*-Me-D-Phe-H

Following the ***Boc removal*** procedure MeO-Phe-*N*-Me-D-Phe-H was synthesized by dissolving 820 mg of MeO-Phe-*N*-Me-D-Phe-Boc in 15.5 mL of DCM, followed by adding 0.4 mL (0.37 mmol, 2.0 equivalents) of anisole and then 3.5 mL of TFA. Boc

removal was completed in 30 min; the reaction mixture was concentrated *in vacuo* with DCM (250 mL x 3) and taken on to the next reaction without further purification or characterization (565 mg, quantitative yield) as a yellow oil.

5.2.6.3 MeO-Phe-*N*-Me-D-Phe-Val-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-*N*-Me-D-Phe-Val-Boc was synthesized utilizing 465 mg (1.9 mmol, 1.0 equivalent) of free acid HO-*N*-Me-D-Val-Boc, 565 mg (2.1 mmol, 1.1 equivalents) of free amine H-Phe-Leu-OMe, 690 mg (2.1 mmol, 1.1 equivalents) of coupling reagent HATU and 1.3 mL (7.6 mmol, 4.0 equivalents) of DIPEA in 19 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 100 mL silica gel and ethyl acetate-hexane as a gradient system. Tripeptide MeO-Phe-*N*-Me-D-Phe-Val-Boc was eluted at the gradient of 70% of hexane and 30% of ethyl acetate. The pure tripeptide MeO-Phe-*N*-Me-D-Phe-Val-Boc was afforded as a white solid (900 mg, 80% yield) R_f: 0.6 (7:3 Hex/EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.50-0.65 (d, *J* = 8.4 Hz, 6H), 1.42 (s, 9H), 1.60 (s, 6H), 2.82-2.95 (m, 4H), 3.01-3.20 (m, 3H), 3.29-3.41 (m, 1H), 3.73 (s, 1H), 4.17-4.22 (dd, *J* = 6.4, 8.0 Hz, 1H), 4.65-4.81 (q, *J* = 8.3 Hz, 1H), 4.99-5.08 (d, *J* = 8.3 Hz, 1H), 5.47-5.55 (q, *J* = 8.0 Hz, 1H), 6.75-6.82 (d, *J* = 8.4 Hz, 1H), 7.04-7.39 (m, 10H)

5.2.6.4 MeO-Phe-*N*-Me-D-Phe-Val-H

Following the ***Boc removal*** procedure MeO-Phe-*N*-Me-D-Phe-Val-H was synthesized by dissolving 900 mg of MeO-Phe-*N*-Me-D-Phe-Val-Boc in 15.6 mL of DCM, followed by adding 0.4 mL (0.35 mmol, 2.0 equivalents) of anisole and then 4.4 mL of TFA. Boc removal was completed in 30 min; the reaction mixture was concentrated *in vacuo* with DCM (250 mL x 3) and taken on to the next reaction without further purification or characterization (727 mg, quantitative yield) as a yellow oil.

5.2.6.5 MeO-β-cyclohexylalanine-Leu-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-β-cyclohexylalanine-Leu-Boc was synthesized utilizing 500 mg (2.0 mmol, 1.0 equivalent) of free acid HO-Leu-Boc, 560 mg (2.2 mmol, 1.1 equivalents) of free amine H-β-cyclohexylalanine-OMe, 708 mg (2.1 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.4 mL (8.0 mmol, 4.0 equivalents) of DIPEA in 20 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 150 mL silica gel and ethyl acetate-hexane as a gradient system. Dipeptide MeO-β-cyclohexylalanine-Leu-Boc was eluted at the gradient of 75% of hexane and 25% of ethyl acetate. The pure dipeptide MeO-β-cyclohexylalanine-Leu-Boc was afforded as a white solid (990 mg, 90% yield) R_f: 0.4 (3:1 Hex/EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.95-1.01 (m, 6H), 1.05-1.20 (m, 4H), 1.43 (s, 9H), 1.60-1.81 (m, 9H), 3.78 (s, 3H), 4.02-

4.19 (q, *J* = 8.0 Hz, 1H), 4.59-4.75 (m, 1H), 4.80-4.96 (br, 1H), 6.39-6.42 (d, *J* = 8.2 Hz, 1H)

5.2.6.6 HO-β-cyclohexylalanine-Leu-Boc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-β-cyclohexylalanine-Leu-Boc was deprotected by utilizing 990 mg (1.9 mmol, 1.0 equivalent) of MeO-β-cyclohexylalanine-Leu-Boc and 638 mg (15.2 mmol, 8.0 equivalents) of LiOH in 19 mL MeOH. The peptide was dissolved in MeOH and LiOH was added to the reaction flask and the reaction was run for 8 h. Upon completion, the reaction was diluted with 100 mL DCM and washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL). The acidic aqueous layer was back extracted with DCM (100 mL x 3). The organic layers were collected, dried over Na_2SO_4, filtered and concentrated *in vacuo* (840 mg, 85% yield) as a white solid. The free acid HO-β-cyclohexylalanine-Leu-Boc was taken on without any further purification or characterization.

5.2.6.7 MeO-Phe-*N*-Me-D-Phe-Val-β-cyclohexylalanine-Leu-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-*N*-Me-D-Phe-Val-β-cyclohexylalanine-Leu-Boc was synthesized utilizing 499 mg (1.3 mmol, 1.0 equivalent) of free acid HO-β-cyclohexylalanine-Leu-Boc, 727 mg (1.42 mmol, 1.1 equivalents) of free amine H-Phe-*N*-Me-D-Phe-Val-OMe, 610 mg (1.8 mmol, 1.1 equivalents) of coupling reagent TBTU and 0.9 mL (5.2 mmol, 4.0 equivalents) of DIPEA in 19 mL anhydrous DCM, under argon. The reaction was stirred for 45 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried

over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 100 mL silica gel and ethyl acetate-hexane as a gradient system. Pentapeptide MeO-Phe-*N*-Me-D-Phe-Val-β-cyclohexylalanine-Leu-Boc was eluted at the gradient of 60% of hexane and 40% of ethyl acetate. The pure pentapeptide MeO-Phe-*N*-Me-D-Phe-Val-β-cyclohexylalanine-Leu-Boc was afforded as a white solid (732 mg, 70% yield) R_f: 0.4 (2:1 Hex/EA). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.44-0.52 (dd, *J* = 7.9, 8.4 Hz, 2H), 0.61-0.75 (dd, *J* = 7.9, 8.4 Hz, 2H), 0.90-0.95 (m, 5H), 1.05-1.37 (m, 2H), 1.41 (s, 9H), 1.60-1.72 (m, 5H), 2.82-2.95 (m, 2H), 2.95 (s, 3H), 3.00-3.10 (m, 1H), 3.11-3.20 (m, 1H), 3.29-3.41 (m, 2H), 3.75 (s, 3H), 4.05-4.10 (m, 1H), 4.32-4.41 (m, 2H), 4.75-4.85 (m, 2H), 5.50-5.60 (m, 2H), 6.39-6.41 (d, *J* = 7.9 Hz, 1H), 6.59-6.61 (d, *J* = 7.9 Hz, 1H), 6.68-6.69 (d, *J* = 7.9 Hz, 1H), 6.99-7.01 (d, *J* = 7.9 Hz, 1H), 7.09-7.38 (m, 10H)

5.2.6.8 HO-Phe-*N*-Me-D-Phe-Val-β-cyclohexylalanine-Leu-H

Following the ***methyl ester hydrolysis*** procedure: The acid of pentapeptide MeO-Phe-*N*-Me-D-Phe-Val-β-cyclohexylalanine-Leu-Boc was deprotected by utilizing 732 mg (1.2 mmol, 1.0 equivalent) of MeO-Phe-*N*-Me-D-Phe-Val-β-cyclohexylalanine-Leu-Boc and 403 mg (9.6 mmol, 8.0 equivalents) of LiOH in 24 mL MeOH. The peptide was dissolved in MeOH and LiOH was added to the reaction flask and the reaction was run for 24 h. Upon completion, the reaction was diluted with 100 mL DCM and washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL). The acidic aqueous layer was back extracted with DCM (100 mL x 3). The organic layers were collected, dried over Na_2SO_4, filtered and concentrated *in vacuo* (575 mg, 80% yield) as a light yellow solid. The free acid HO-Phe-*N*-Me-D-Phe-Val-β-cyclohexylalanine-Leu-Boc was taken on to Boc removal reaction without any further purification or characterization.

Following the ***Boc removal*** procedure HO-Phe-*N*-Me-D-Phe-Val-β-cyclohexylalanine-Leu-H was synthesized by dissolving 575 mg of HO-Phe-*N*-Me-D-Phe-Val-β-cyclohexylalanine-Leu-Boc in 15.2 mL of DCM, followed by adding 0.2 mL (1.9 mmol, 2.0 equivalents) of anisole and then 3.8 mL of TFA. Boc removal was completed in 30 min; the reaction mixture was concentrated *in vacuo* with DCM (250 mL x 3) and taken on to the next reaction without further purification or characterization (470 mg, quantitative yield) as a brown oil.

5.2.6.9 *cyclo*-Phe-*N*-Me-D-Phe-Val-β-cyclohexylalanine-Leu

Following the ***macrocyclization*** procedure: *cyclo*-Phe-*N*-Me-D-Phe-Val-β-cyclohexylalanine-Leu was synthesized by utilizing 125 mg (0.18 mmol, 1.0 equivalent) of HO-Phe-*N*-Me-D-Phe-Val-β-cyclohexylalanine-Leu-H, coupling reagents 46 mg (0.15 mmol, 0.7 equivalents) of TBTU, 54 mg (0.15 mmol, 0.7 equivalents) of HATU, 42 mg (0.15 mmol, 0.7 equivalents) of DEPBT and 0.25 mL (1.44 mmol, 8.0 equivalents) of DIPEA. The materials were dissolved in 12.5 mL of anhydrous DCM and 12.5 mL of anhydrous ACN. The reaction was allowed to stir under argon at RT and completed in 16 h. Upon completion, the reaction mixture was diluted with DCM (100 mL) and extracted with 10% (v/v) $HCl_{(aq.)}$. The organic layer was re-extracted with a saturated $NaHCO_3$ aqueous solution (100 mL x 3). The basic aqueous layer was back-washed with DCM (100 mL x 3) and ethyl acetate (100 mL x 2). The organic layers were collected, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude material was purified via flash column chromatography using 250 mL silica gel and ethyl acetate-hexane as a gradient system. *Cyclo*-Phe-*N*-Me-D-Phe-Val-β-cyclohexylalanine-Leu was eluted at the gradient of 35% of hexane and 65% of ethyl acetate. The pure *cyclo*-Phe-*N*-Me-D-Phe-

Val-β-cyclohexylalanine-Leu was afforded as a white solid (13 mg, 11% yield) R_f: 0.4 (1:2 Hex/EA). ^{1}H NMR (500 MHz, CD_3OD): δ 0.54-0.60 (dd, *J* = 7.9, 8.4 Hz, 2H), 0.73-0.75 (d, *J* = 7.9 Hz, 2H), 0.80-1.02 (m, 10H), 1.12-1.22 (m, 2H), 1.31-1.51 (m, 6H), 1.60-1.80 (m, 5H), 1.82-2.05 (m, 2H), 2.90-3.01 (m, 6H), 3.05 (s, 3H), 4.05-4.10 (m, 1H), 4.20-4.31 (m, 2H), 4.40-4.41 (d, *J* = 7.9 Hz, 1H), 4.42-4.58 (m, 2H), 4.60-4.71 (m, 2H), 5.15-5.22 (m, 2H), 7.10-7.35 (m, 10H), LCMS: *m/z* called for $C_{39}H_{55}N_5O_5$ (M+1) = 674.42, found 673.6

5.3 FR235222 and Trapoxin B derivative

5.3.1 Experimental method for HDI-4

5.3.1.1 MeO-1,2,3,4-tetrahydro-3-isoquinoline-Abu-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-1,2,3,4-tetrahydro-3-isoquinoline-Abu-Boc was synthesized utilizing 406 mg (2.0 mmol, 1.0 equivalent) of free acid HO-Abu-Boc, 420 mg (2.2 mmol, 1.1 equivalents) of free amine H-1,2,3,4-tetrahydro-3-isoquinoline-OMe, 708 mg (2.2 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.4 mL (8.0 mmol, 4.0 equivalents) of DIPEA in 20.4 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 100 mL silica gel and ethyl acetate-hexane as a gradient system. Dipeptide MeO-1,2,3,4-tetrahydro-3-isoquinoline-Abu-Boc was eluted at the gradient of 80% of hexane and 20% of ethyl acetate. The pure dipeptide MeO-1,2,3,4-tetrahydro-3-isoquinoline-

Abu-Boc was afforded as a light yellow solid (660 mg, 88% yield) R_f: 0.5 (3:1 Hex/EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89-0.98 (m, 6H), 1.41 (s, 9H), 1.50-1.70 (m, 1H), 1.75-1.90 (m, 1H), 2.98-3.21 (m, 2H), 3.78 (s, 3H), 3.90-4.01 (m, 1H), 4.79-4.99 (m, 2H), 6.55-6.61 (d, *J* = 8.0 Hz, 1H), 6.95-7.01 (dd, *J* = 7.3, 8.4 Hz 1H), 7.20-7.30 (d, *J* = 8.2 Hz, 2H), 7.31-7.40 (d, *J* = 8.3 Hz, 1H)

5.3.1.2 HO-1,2,3,4-tetrahydro-3-isoquinoline-Abu-Boc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-1,2,3,4-tetrahydro-3-isoquinoline-Abu-Boc was deprotected by utilizing 660 mg (1.76 mmol, 1.0 equivalent) of MeO-1,2,3,4-tetrahydro-3-isoquinoline-Abu-Boc and 591 mg (15.2 mmol, 8.0 equivalents) of LiOH in 17.6 mL MeOH. The peptide was dissolved in MeOH and LiOH was added to the reaction flask and the reaction was run for 8 h. Upon completion, the reaction was diluted with 100 mL DCM and washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL). The acidic aqueous layer was back extracted with DCM (100 mL x 3). The organic layers were collected, dried over Na_2SO_4, filtered and concentrated *in vacuo* (604 mg, 95% yield) as a white solid. The free acid HO-1,2,3,4-tetrahydro-3-isoquinoline-Abu-Boc was taken on without any further purification or characterization.

5.3.1.3 MeO-Arg(Cbz)-D-Pro-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Arg(Cbz)-D-Pro-Boc was synthesized utilizing 430 mg (2.0 mmol, 1.0 equivalent) of free acid HO-D-Pro-Boc, 957 mg (2.2 mmol, 1.1 equivalents) of free amine H-Arg(Cbz)-OMe, 674 mg (2.2 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.3 mL (8.0 mmol, 4.0 equivalents) of DIPEA in 20 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 100 mL

DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 100 mL silica gel and ethyl acetate-hexane as a gradient system. Dipeptide MeO-Arg(Cbz)-D-Pro-Boc was eluted at the gradient of 70% of hexane and 30% of ethyl acetate. The pure dipeptide MeO-Arg(Cbz)-D-Pro-Boc was afforded as a white solid (1.1 g, 85% yield) R_f: 0.6 (1:1 Hex/EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.80-0.98 (br, 1H), 1.43 (s, 9H), 1.60-1.95 (br, 8H), 3.15-3.57 (br, 2H), 3.61 (s, 3H), 3.85-4.10 (s, 2H), 4.13-4.35 (br, 2H), 4.55-4.70 (br, 2H), 5.18 (s, 2H), 5.22 (s, 2H), 7.30-7.50 (m, 10H)

5.3.1.4 MeO-Arg(Cbz)-D-Pro-H

Following the ***Boc removal*** procedure MeO-Arg(Cbz)-D-Pro-H was synthesized by dissolving 1.1 g of MeO-Arg(Cbz)-D-Pro-Boc in 13.6 mL of DCM, followed by adding 0.4 mL (3.4 mmol, 2.0 equivalents) of anisole and then 3.4 mL of TFA. Boc removal was completed in 30 min; the reaction mixture was concentrated *in vacuo* with DCM (250 mL x 3) and taken on to the next reaction without further purification or characterization (943 mg, quantitative yield) as a brown oil.

5.3.1.5 MeO-Arg(Cbz)-D-Pro-1,2,3,4-tetrahydro-3-isoquinoline-Abu-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Arg(Cbz)-D-Pro-1,2,3,4-tetrahydro-3-isoquinoline-Abu-Boc was synthesized utilizing 524 mg (1.54 mmol, 1.0 equivalent) of free acid HO-1,2,3,4-tetrahydro-3-isoquinoline-Abu-Boc, 943 mg (1.7 mmol, 1.1 equivalents) of free amine MeO-Arg(Cbz)-D-Pro-H, 375 mg (1.1 mmol, 0.7 equivalents) of TBTU, 444 mg (1.1 mmol, 0.7 equivalents) of HATU, and 1.8

mL (9.5 mmol, 6.0 equivalents) of DIPEA in 16.7 mL anhydrous DCM, under argon. The reaction was stirred for 45 min and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 100 mL silica gel and ethyl acetate-hexane as a gradient system. Tetrapeptide MeO-Arg(Cbz)-D-Pro-1,2,3,4-tetrahydro-3-isoquinoline-Abu-Boc was eluted at the gradient of 50% of hexane and 50% of ethyl acetate. The pure tetrapeptide MeO-Arg(Cbz)-D-Pro-1,2,3,4-tetrahydro-3-isoquinoline-Abu-Boc was afforded as a white solid (1.03 g, 75% yield) R_f: 0.3 (1:1 Hex/EA). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.80-0.98 (br, 3H), 1.40 (s, 9H), 1.60-1.75 (br, 8H), 1.78-1.85 (br, 3H), 1.95-2.05 (br, 4H), 2.92-3.05 (m, 2H), 3.15-3.48 (br, 2H), 3.72 (s, 3H), 3.85-3.95 (br, 1H), 4.05-4.15 (br, 1H), 4.18-4.23 (m, 2H), 4.45-4.55 (br, 1H), 4.62-4.65 (br, 1H), 5.08-5.13 (br, 1H), 5.25 (s, 4H), 6.99-7.10 (br, 2H), 7.22-7.41 (m, 9H), 7.45-7.52 (br, 1H), 7.58-7.62 (br, 1H)

5.3.1.6 HO-Arg(Cbz)-D-Pro-1,2,3,4-tetrahydro-3-isoquinoline-Abu-H

Following ***in situ double deprotection*** procedure: HO-Arg(Cbz)-D-Pro-1,2,3,4-tetrahydro-3-isoquinoline-Abu-H was synthesized by dissolving 1.03 g (1.15 mmol 1.0 equivalent) of MeO-Arg(Cbz)-D-Pro-1,2,3,4-tetrahydro-3-isoquinoline-Abu-Boc in 23 mL THF. 0.2 mL (2.3 mmol 2.0 equivalents) of anisole was added to the reaction solution followed by 32 drops of 12N HCl. The reaction was allowed to stir at RT for 2 d and monitored via LC/MS. Upon completion, the reaction mixture was concentrated *in*

vacuo with DCM (250 mL x 3) and taken on to the next reaction without any further purification or characterization (910 mg, quantitative yield) as a brown oil.

5.3.1.7 *cyclo*-Arg(Cbz)-D-Pro-1,2,3,4-tetrahydro-3-isoquinoline-Abu

Following the ***macrocyclization*** procedure: *cyclo*-Arg(Cbz)-D-Pro-1,2,3,4-tetrahydro-3-isoquinoline-Abu was synthesized by utilizing 137 mg (0.13 mmol, 1.0 equivalent) of HO-Arg(Cbz)-D-Pro-1,2,3,4-tetrahydro-3-isoquinoline-Abu-H, coupling reagents 29 mg (0.09 mmol, 0.7 equivalents) of TBTU, 34 mg (0.09 mmol, 0.7 equivalents) of HATU, 27 mg (0.09 mmol, 0.7 equivalents) of DEPBT and 0.18 mL (1.04 mmol, 8.0 equivalents) of DIPEA. The materials were dissolved in 7.5 mL of anhydrous DCM and 7.5 mL of anhydrous ACN. The reaction was allowed to stir under argon at RT and completed in 8 h. Upon completion, the reaction mixture was diluted with DCM (100 mL) and extracted with 10% (v/v) $HCl_{(aq.)}$. The organic layer was re-extracted with a saturated $NaHCO_3$ aqueous solution (100 mL x 3). The basic aqueous layer was back-washed with DCM (100 mL x 3) and ethyl acetate (100 mL x 2). The organic layers were collected, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude material was purified via flash column chromatography using 250 mL silica gel and ethyl acetate-hexane as a gradient system. *Cyclo*-Arg(Cbz)-D-Pro-1,2,3,4-tetrahydro-3-isoquinoline-Abu was eluted at the gradient of 30% of hexane and 60% of ethyl acetate. The *cyclo*-Arg(Cbz)-D-Pro-1,2,3,4-tetrahydro-3-isoquinoline-Abu was further purified via RP-HPLC to yield pure *cyclo*-Arg(Cbz)-D-Pro-1,2,3,4-tetrahydro-3-isoquinoline-Abu as a white solid (10.6 mg, 12% yield) R_f: 0.5 (1:4 Hex/EA). ^{1}H NMR (500 MHz, CD_3OD): δ 0.80-0.98 (m, 3H), 1.28-1.40 (m, 12H), 1.58-1.71 (br, 3H), 1.78-1.91 (m, 3H), 1.95-2.10 (br, 2H), 2.95-3.10 (m, 2H), 3.58-3.70 (m, 2H), 3.72-3.90 (m, 1H), 4.05-4.15 (m, 2H),

4.13-4.19 (m, 1H), 4.48-4.60 (m, 1H), 5.15-5.39 (m, 2H), 7.15-7.21 (m, 1H), 7.24-7.35 (m, 1H), 7.32-7.42 (m, 7H), 7.59-7.72 (m, 1H)

5.3.1.8 Hydrogenolysis of *Cyclo*-Arg(Cbz)-D-Pro-1,2,3,4-tetrahydro-3-isoquinoline-Abu

Following the ***hydrogenolysis*** procedure: *Cyclo*-Arg-D-Pro-1,2,3,4-tetrahydro-3-isoquinoline-Abu was synthesized by dissolving 10.6 mg (0.14 mmol, 1.0 equivalent) of *cyclo*-Arg(Cbz)-D-Pro-1,2,3,4-tetrahydro-3-isoquinoline-Abu in 1.4 mL of EtOH followed by adding 16 mg of Pd-C (10%) to the reaction solution. H_2 was purged through the reaction flask at low atmosphere pressure using a balloon (3 times). The reaction was allowed to stir at RT monitored via TLC every 2 h and completed in 8 h. Upon completion, the reaction was filtered over Celite® to remove the catalyst Pd-C (10%). The filtered organic solution was concentrated *in vacuo* to yield *cyclo*-Arg-D-Pro-1,2,3,4-tetrahydro-3-isoquinoline-Abu as a white solid (4 mg, 60% yield). R_f: 0.4 (1:9 Hex/EA). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.80-0.98 (m, 3H), 1.28-1.40 (m, 12H), 1.58-1.71 (br, 3H), 1.78-1.91 (m, 3H), 1.95-2.10 (br, 2H), 2.95-3.10 (m, 2H), 3.58-3.70 (m, 2H), 3.72-3.90 (m, 1H), 4.05-4.15 (m, 2H), 4.13-4.19 (m, 1H), 4.48-4.60 (m, 1H), 5.15-5.39 (m, 2H), 7.15-7.21 (m, 1H), 7.24-7.35 (m, 1H), 7.32-7.42 (m, 7H), 7.59-7.72 (m, 1H)

LCMS: *m/z* called for $C_{25}H_{35}N_7O_4$ (M+1) = 540.28, found 540.7

5.4 Synthesis of Urukthapelstatin A

5.4.1 Experimental method for Ustat A-2 fragment 1

5.4.1.1 MeO-Ser(Bn)-Thr(O^{t}Bu)-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Ser(Bn)-Thr(O^{t}Bu)-Boc was synthesized utilizing 1.2 g (4.33 mmol, 1.0 equivalent) of free acid HO-Thr(O^{t}Bu)-Boc, 1 g (4.76 mmol, 1.1 equivalents) of free amine H-Ser(Bn)-OMe, 1.53 g (4.76 mmol, 1.1 equivalents) of coupling reagent TBTU and 3.02 mL (17.2 mmol, 4.0 equivalents) of DIPEA in 43 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 200 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL x 2) followed by basic extraction using saturated $NaHCO_3$ (200 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 400 mL silica gel and ethyl acetate-hexane as a gradient system. Dipeptide MeO-Ser(Bn)-Thr(O^{t}Bu)-Boc was eluted at the gradient of 80% of hexane and 20% of ethyl acetate. The pure dipeptide MeO-Ser(Bn)-Thr(O^{t}Bu)-Boc was afforded as a yellow solid (1.82 g, 90% yield) R_f: 0.5 (3:1 Hex/EA). ^{1}H NMR: ($CDCl_3$, 300 MHz): δ = 7.96 (d, J = 5.4 Hz, 1H), 7.34-7.21 (m, 5H), 5.60 (br, 1H), 4.70-4.67 (m, 1H), 4.49 (q, J = 19.2 Hz, 2H), 4.14-4.10 (m, 2H), 3.82 (dd, J = 5.7, 2.4 Hz, 1H), 3.74 (s, 3H), 3.67 (dd, J = 2.4, 2.6 Hz, 1H), 1.45 (s, 9H), 1.27 (s, 9H), 1.12 ppm (d, J = 4.8 Hz, 3H). ^{13}C NMR: ($CDCl_3$, 400 MHz) δ = 170.60, 170.16, 155.67, 137.40, 128.34, 127.79, 127.66, 127.60, 79.92, 75.13, 73.20, 69.37, 67.08, 58.43, 52.94, 52.38, 28.29, 28.15, 17.22 ppm ; HRMS(ESI): calcd for $C_{24}H_{38}N_2O_7Na^+$ [M + Na^+] 489.2577, found 489.2561.

5.4.1.2 MeO-Ser-Thr(O^{t}Bu)-Boc

Following the ***hydrogenolysis*** procedure: MeO-Ser-Thr(O^{t}Bu)-Boc was synthesized by dissolving 1.83 g (3.9 mmol, 1.0 equivalent) of MeO-Ser(Bn)-Thr(O^{t}Bu)-Boc in 39 mL of EtOH followed by adding 183 mg of Pd-C (10%) to the reaction solution. H_2 was purged through the reaction flask at low atmosphere pressure using a balloon (3 times). The reaction was allowed to stir at RT monitored via TLC every 2 h and completed in 8 h. Upon completion, the reaction was filtered over Celite® to remove the catalyst Pd-C (10%). The filtered organic solution was concentrated *in vacuo* in quantitative yield MeO-Ser-Thr(O^{t}Bu)-Boc as a colorless oil. The material was taken on to the oxazole formation without further purification and characterization.

5.4.1.3 MeO-Oxa-Thr(O^{t}Bu)-Boc

Oxazoline formation (K_2CO_3 as base)

1.46 g (3.9 mmol, 1.0 equivalent) of MeO-Ser-Thr(O^{t}Bu)-Boc was dissolved in 39 mL of anhydrous DCM and cooled to -78^{o}C. 0.6 mL (4.3 mmol, 1.1 equivalents) of fluorinating agent DAST was added to the solution drop-wise (0.1 mL/min). The reaction mixture was allowed to stir for 30 min followed by the addition of 884 mg (7.8 mmol, 2.0 equivalents) of K_2CO_3 in one portion and the reaction was allowed to proceed for an additional hour at -78^{o}C. The reaction mixture was allowed to warm up to RT and stir for an additional 1.5 h. Upon reaction completion, confirmed by TLC, the organic solution was diluted with 100 mL of DCM and washed with saturated $NaHCO_3$ aqueous solution (200 mL x 2). The collected organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to yield 1.75 g MeO-Oxazoline-Thr(O^{t}Bu)-Boc as a yellow oil.

The material was taken to the oxidation reaction without further purification and characterization.

Oxidation of Oxazoline

1.75 g of MeO-Oxazoline-Thr(O^{t}Bu)-Boc was dissolved in anhydrous 16 mL of anhydrous DCM and cooled to -47^{o}C. 0.97 mL (7.8 mmol, 2.0 equivalents) of base DBU was added drop-wise (0.1 mL/min) to the reaction flask and stirred for 15 min. 0.63 mL (7.8 mmol, 2.0 equivalents) of $BrCCl_3$ was added drop-wise (0.1 mL/min) to the reaction flask and stirred at -47^{o}C for an additional 2 h and then allowed to warm up to RT to stir additional 8 h. The reaction was monitored via TLC every 2 h. Upon completion, confirmed via TLC, the reaction mixture was diluted with 100 mL of DCM and washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL x 2). After the acidic work-up, the organic layer was washed with saturated $NaHCO_3$ aqueous solution (100 mL x 2). The basic aqueous layer was back washed with ethyl acetate (100 mL x 3) and the collected organic layers were dried over Na_2SO_4, filtered, concentrated *in vacuo*. Obtained crude oxazole material was purified via flash column chromatography using 200 mL of silica gel and ethyl acetate-hexane as a gradient system. MeO-Oxa-Thr(O^{t}Bu)-Boc was eluted at the gradient of 75% of hexane and 25% of ethyl acetate. The pure dipeptide MeO-Oxa-Thr(O^{t}Bu)-Boc was afforded as a white solid (973 mg, 85% yield for 3 steps) R_f: 0.74 (1:1 Hex/EA). ^{1}H NMR: ($CDCl_3$, 300 MHz) δ = 8.21 (s, 1H), 5.58 (d, *J* = 6.3 Hz, 1H), 4.85 (d, *J* = 6.9 Hz, 1H), 3.98 (s, 3H), 1.61 (s, 9H), 1.48 (d, *J* = 4.8 Hz, 3H), 0.98 ppm (s, 9H). 13C NMR: ($CDCl_3$, 400 MHz) δ = 164.22, 161.97, 155.89, 143.90, 133.87, 79.92, 74.35, 68.22, 55.34, 52.13, 28.81, 28.78, 20.13 ppm ; HRMS(ESI): calcd for $C_{17}H_{28}N_2O_6Na^+$ [M + Na^+] 379.1845, found 379.1832.

5.4.1.4 HO-Oxa-Thr(O^{t}Bu)-Boc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-Oxa-Thr(O^{t}Bu)-Boc was deprotected by utilizing 973 mg (2.72 mmol, 1.0 equivalent) of MeO-Oxa-Thr(O^{t}Bu)-Boc and 913 mg (21.76 mmol, 8.0 equivalents) of LiOH in 27.2 mL MeOH. The peptide was dissolved in MeOH and LiOH was added to the reaction flask and the reaction was run for 8 h. Upon completion, the reaction was diluted with 150 mL DCM and washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL x 2). The acidic aqueous layer was back extracted with DCM (100 mL x 3). The organic layers were collected, dried over Na_2SO_4, filtered and concentrated *in vacuo* (920 mg, 99% yield) as a white solid. The free acid HO-Oxa-Thr(O^{t}Bu)-Boc was taken on without any further purification or characterization.

5.4.1.5 MeO-Ser(Bn)-Oxa-Thr(O^{t}Bu)-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Ser(Bn)-Oxa-Thr(O^{t}Bu)-Boc was synthesized utilizing 920 g (2.69 mmol, 1.0 equivalent) of free acid HO-Oxa-Thr(O^{t}Bu)-Boc, 613 mg (2.94 mmol, 1.1 equivalents) of free amine H-Ser(Bn)-OMe, 944 g (2.94 mmol, 1.1 equivalents) of coupling reagent TBTU and 2.05 mL (10.7 mmol, 4.0 equivalents) of DIPEA in 27 mL anhydrous DCM, under argon. The reaction was stirred for 30 min and upon completion, the crude reaction mixture was diluted with 200 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL x 2) followed by basic extraction using saturated $NaHCO_3$ (200 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 400 mL silica gel and ethyl acetate-hexane as a gradient system. MeO-Ser(Bn)-Oxa-Thr(O^{t}Bu)-Boc was eluted at the

gradient of 80% of hexane and 20% of ethyl acetate. The pure dipeptide MeO-Ser(Bn)-Oxa-Thr(O^{t}Bu)-Boc was afforded as a yellow solid (1.35 g, 95% yield) R_f: 0.71 (1:1 Hex/EA). ^{1}H NMR: ($CDCl_3$, 300 MHz) δ = 8.12 (s, 1H), 7.66-7.64 (m, 1H), 7.35-7.25 (m, 1H), 5.47 (d, *J* = 6.3 Hz, 1H), 4.89 (m, 1H), 4.81 (d, *J* = 6.9 Hz, 1H), 4.54 (q, *J* = 17.8 Hz, 2H), 4.14 (br, 1H), 3.98-3.95 (m, 1H), 3.76 (s, 3H), 1.44 (s, 9H), 1.30 (d, *J* = 4.8 Hz, 3H), 0.97 ppm (s, 9H). ^{13}C NMR: ($CDCl_3$, 400 MHz) δ = 170.26, 163.49, 160.34, 141.07, 137.48, 135.74, 128.34, 127.79, 127.60, 80.11, 74.39, 73.27, 69.54, 68.38, 54.95, 52.52, 52.28, 28.30, 28.08, 20.23 ppm ; HRMS(ESI): calcd for $C_{27}H_{39}N_3O_8Na^+$ [M + Na^+] 556.2635, found 556.2616.

5.4.1.6 MeO-Ser-Oxa-Thr(O^{t}Bu)-Boc

Following the ***hydrogenolysis*** procedure: MeO-Ser-Oxa-Thr(O^{t}Bu)-Boc was synthesized by dissolving 1.35 g (2.5 mmol, 1.0 equivalent) of MeO-Ser(Bn)-Oxa-Thr(O^{t}Bu)-Boc in 25 mL of EtOH followed by adding 135 mg of Pd-C (10%) to the reaction solution. H_2 was purged through the reaction flask at low atmosphere pressure using a balloon (3 times). The reaction was allowed to stir at RT monitored via TLC every 2 h and completed in 8 h. Upon completion, the reaction was filtered over Celite® to remove the catalyst Pd-C (10%). The filtered organic solution was concentrated *in vacuo* in quantitative yield MeO-Ser-Oxa-Thr(O^{t}Bu)-Boc as a white solid. The material was taken on to the oxazole formation without further purification and characterization.

5.4.1.7 MeO-diOxa-Thr(O^{t}Bu)-Boc

Oxazoline formation (K_2CO_3 as base)

1.32 g (2.5 mmol, 1.0 equivalent) of MeO-Ser-Thr(O^{t}Bu)-Boc was dissolved in 25 mL of anhydrous DCM and cooled to -78^{o}C. 0.4 mL (2.75 mmol, 1.1 equivalents) of fluorinating agent DAST was added to the solution drop-wise (0.1 mL/min). The reaction mixture was allowed to stir for 30 min followed by the addition of 667 mg (5.0 mmol, 2.0 equivalents) of K_2CO_3 in one portion and the reaction was allowed to proceed for an additional hour at -78^{o}C. The reaction mixture was allowed to warm up to RT and stir for an additional 1.5 h. Upon reaction completion, confirmed by TLC, the organic solution was diluted with 100 mL of DCM and washed with saturated $NaHCO_3$ aqueous solution (200 mL x 2). The collected organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to yield 1.1 g MeO-Oxazoline-Oxa-Thr(O^{t}Bu)-Boc as a yellow oil. The material was taken to the oxidation reaction without further purification and characterization.

Oxidation of Oxazoline

1.1 g of MeO-Oxazoline-Oxa-Thr(O^{t}Bu)-Boc was dissolved in anhydrous 13 mL of anhydrous DCM and cooled to -47^{o}C. 0.75 mL (5.0 mmol, 2.0 equivalents) of base DBU was added drop-wise (0.1 mL/min) to the reaction flask and stirred for 15 min. 0.5 mL (5.0 mmol, 2.0 equivalents) of $BrCCl_3$ was added drop-wise (0.1 mL/min) to the reaction flask and stirred at -47^{o}C for an additional 2 h and then allowed to warm up to RT to stir additional 8 h. The reaction was monitored via TLC every 2 h. Upon completion, confirmed via TLC, the reaction mixture was diluted with 100 mL of DCM and washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL x 2). After the acidic work-up, the organic

layer was washed with saturated $NaHCO_3$ aqueous solution (100 mL x 2). The basic aqueous layer was back washed with ethyl acetate (100 mL x 3) and the collected organic layers were dried over Na_2SO_4, filtered, concentrated *in vacuo*. Obtained crude oxazole material was purified via flash column chromatography using 250 mL of silica gel and ethyl acetate-hexane as a gradient system. MeO-diOxa-Thr(O^{t}Bu)-Boc was eluted at the gradient of 75% of hexane and 25% of ethyl acetate. The pure MeO-diOxa-Thr(O^{t}Bu)-Boc was afforded as a white solid (882 mg, 85% yield for 3 steps) R_f: 0.43 (1:1 Hex/EA). ^{1}H NMR: ($CDCl_3$, 300 MHz) δ = 8.23 (d, *J* = 2.4 Hz, 2H), 5.54 (d, *J* = 6.3 Hz, 1H), 4.87 (d, *J* = 6.9 Hz, 1H), 4.10 (d, *J* = 2.4 Hz, 1H), 3.87 (s, 3H), 1.39 (s, 9H), 1.21 (s, 3H), 0.97 ppm (s, 9H). ^{13}C NMR: ($CDCl_3$, 400 MHz) δ = 165.03, 161.29, 155.76, 155.67, 143.65, 139.25, 134.29, 129.81, 80.03, 74.38, 68.60, 54.99, 52.22, 28.27, 28.00, 20.23 ppm ; HRMS(ESI): calcd for $C_{20}H_{29}N_3O_7Na^+$ [M + Na^+] 446.1904, found 446.1891.

5.4.1.8 HO-diOxa-Thr(O^{t}Bu)-Boc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-diOxa-Thr(O^{t}Bu)-Boc was deprotected by utilizing 882 mg (2.12 mmol, 1.0 equivalent) of MeO-Oxa-Thr(O^{t}Bu)-Boc and 714 mg (17 mmol, 8.0 equivalents) of LiOH in 21.2 mL MeOH. The peptide was dissolved in MeOH and LiOH was added to the reaction flask and the reaction was run for 8 h. Upon completion, the reaction was diluted with 150 mL DCM and washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL x 2). The acidic aqueous layer was back extracted with DCM (100 mL x 3). The organic layers were collected, dried over Na_2SO_4, filtered and concentrated *in vacuo* (856 mg, 99% yield) as a white solid. The free acid HO-diOxa-Thr(O^{t}Bu)-Boc was taken on without any further purification or characterization.

5.4.1.9 MeO-Ser(Bn)-diOxa-Thr(O^{t}Bu)-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Ser(Bn)-diOxa-Thr(O^{t}Bu)-Boc was synthesized utilizing 856 g (2.10 mmol, 1.0 equivalent) of free acid HO-diOxa-Thr(O^{t}Bu)-Boc, 482 mg (2.31 mmol, 1.1 equivalents) of free amine H-Ser(Bn)-OMe, 741 g (2.31 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.43 mL (8.4 mmol, 4.0 equivalents) of DIPEA in 21 mL anhydrous DCM, under argon. The reaction was stirred for 40 min and upon completion, the crude reaction mixture was diluted with 200 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL x 2) followed by basic extraction using saturated $NaHCO_3$ (200 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 300 mL silica gel and ethyl acetate-hexane as a gradient system. MeO-Ser(Bn)-diOxa-Thr(O^{t}Bu)-Boc was eluted at the gradient of 70% of hexane and 30% of ethyl acetate. The pure dipeptide MeO-Ser(Bn)-diOxa-Thr(O^{t}Bu)-Boc was afforded as a yellow solid (1.17 g, 93% yield) R_f: 0.68 (1:1 Hex/EA). ^{1}H NMR: ($CDCl_3$, 400 MHz): δ = 8.3 (s,1H), 8.22 (s, 1H), 7.81 (d, *J* = 8.7 Hz, 1H), 7.38-7.26 (m, 5H), 5.65 (d, *J* = 9.2 Hz, 1H), 4.94 (dt, *J* = 8.7, 3.8 Hz, 1H), 4.92 (d, *J* = 8.9 Hz, 1H), 4.56 (dd, *J* = 35.2, 12.2 Hz, 2H), 4.21 (q, *J* = 5.8 Hz, 1H), 3.97 (dd, *J* = 9.7, 3.7 Hz, 1H), 3.77 (s, 3H), 3.76 (dd, *J* = 9.7, 4.1 Hz, 1H), 1.47 (s, 9H), 1.26 (dd, *J* = 14.0, 6.3 Hz, 3H), 0.98 ppm (s, 9H). HRMS(ESI): calcd for $C_{30}H_{40}N_4O_9Na^+$ [M + Na^+] 623.2693, found 623.2675.

5.4.1.10 MeO-Ser-diOxa-Thr(O^{t}Bu)-Boc

Following the ***hydrogenolysis*** procedure: MeO-Ser-diOxa-Thr(O^{t}Bu)-Boc was synthesized by dissolving 1.17 g (1.95 mmol, 1.0 equivalent) of MeO-Ser(Bn)-diOxa-

Thr(O^{t}Bu)-Boc in 20 mL of EtOH followed by adding 117 mg of Pd-C (10%) to the reaction solution. H_2 was purged through the reaction flask at low atmosphere pressure using a balloon (3 times). The reaction was allowed to stir at RT monitored via TLC every 2 h and completed in 8 h. Upon completion, the reaction was filtered over Celite® to remove the catalyst Pd-C (10%). The filtered organic solution was concentrated *in vacuo* in quantitative yield MeO-Ser-diOxa-Thr(O^{t}Bu)-Boc as a colorless solid. The material was taken on to the oxazole formation without further purification and characterization.

5.4.1.11 MeO-triOxa-Thr(O^{t}Bu)-Boc

Oxazoline formation (K_2CO_3 as base)

984 mg (1.95 mmol, 1.0 equivalent) of MeO-Ser-Thr(O^{t}Bu)-Boc was dissolved in 20 mL of anhydrous DCM and cooled to -78^{o}C. 0.25 mL (2.15 mmol, 1.1 equivalents) of fluorinating agent DAST was added to the solution drop-wise (0.1 mL/min). The reaction mixture was allowed to stir for 30 min followed by the addition of 575 mg (3.9 mmol, 2.0 equivalents) of K_2CO_3 in one portion and the reaction was allowed to proceed for an additional hour at -78^{o}C. The reaction mixture was allowed to warm up to RT and stir for an additional 1.5 h. Upon reaction completion, confirmed by TLC, the organic solution was diluted with 100 mL of DCM and washed with saturated $NaHCO_3$ aqueous solution (200 mL x 2). The collected organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to yield 906 mg MeO-Oxazoline-diOxa-Thr(O^{t}Bu)-Boc as a light yellow oil. The material was taken to the oxidation reaction without further purification and characterization.

Oxidation of Oxazoline

906 mg of MeO-Oxazoline-diOxa-Thr(O^{t}Bu)-Boc was dissolved in anhydrous 10 mL of anhydrous DCM and cooled to -47^{o}C. 0.48 mL (3.9 mmol, 2.0 equivalents) of base DBU was added drop-wise (0.1 mL/min) to the reaction flask and stirred for 15 min. 0.4 mL (3.9 mmol, 2.0 equivalents) of $BrCCl_3$ was added drop-wise (0.1 mL/min) to the reaction flask and stirred at -47^{o}C for an additional 2 h and then allowed to warm up to RT to stir additional 8 h. The reaction was monitored via TLC every 2 h. Upon completion, confirmed via TLC, the reaction mixture was diluted with 100 mL of DCM and washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL x 2). After the acidic work-up, the organic layer was washed with saturated $NaHCO_3$ aqueous solution (100 mL x 2). The basic aqueous layer was back washed with ethyl acetate (100 mL x 3) and the collected organic layers were dried over Na_2SO_4, filtered, concentrated *in vacuo*. Obtained crude oxazole material was purified via flash column chromatography using 250 mL of silica gel and ethyl acetate-hexane as a gradient system. MeO-triOxa-Thr(O^{t}Bu)-Boc was eluted at the gradient of 65% of hexane and 35% of ethyl acetate. The pure MeO-triOxa-Thr(O^{t}Bu)-Boc was afforded as a white solid (668 mg, 70% yield for 3 steps) R_f: 0.40 (1:1 Hex/EA). ^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 8.42 (s, 1H), 8.31 (s, 1H), 8.30 (s, 1H), 5.60 (d, *J* = 9.3 Hz, 1H), 4.90 (d, *J* = 9.3 Hz, 1H), 4.19 (q, *J* = 6.9 Hz, 1H), 3.95 (s, 3H), 1.47 (s, 9H) 1.27 (dd, *J* = 6.0, 1.2 Hz, 3H), 0.98 ppm (s, 9H). HRMS(ESI): calcd for $C_{23}H_{30}N_4O_8Na^+$ [M + Na^+] 513.1962, found 513.1951.

5.4.1.12 Boc-Thr(O^{t}Bu)-triOxa-CONH$_2$

Following ***Amide Conversion*** Procedure: 668 mg (1.37 mmol, 1.0 equivalent) acid protected MeO-triOxa-Thr(O^{t}Bu)-Boc was dissolved in a 274 mL of a mixture of

NH_4OH : MeOH (1:1). The organic solution was allowed to stirred at RT for 12 h and monitored via TLC. Upon completion, the solution was dried *in vacuo* to yield 650 mg Boc-Thr(O^{t}Bu)-triOxa-$CONH_2$ in quantitative yield as a white solid. The material was taken to the thioamide conversion without further purification and characterization.

5.4.1.13 Boc-Thr(O^{t}Bu)-triOxa-$CSNH_2$

Following ***Thioamide Conversion*** Procedure: Boc-Thr(O^{t}Bu)-triOxa-$CSNH_2$ was synthesized by utilizing 650 (1.37 mmol, 1.0 equivalent) of Boc-Thr(O^{t}Bu)-triOxa-$CONH_2$ and 443 mg (1.1 mmol, 0.8 equivalents) of Lawesson's reagent. The materials were dissolved in 13.7 mL of anhydrous DME. The reaction solution was heated to 60^{o}C and allowed to stir for 5 h and monitored via TLC. Upon completion, confirmed by TLC, the solvent was removed *in vacuo*. The crude material was purified via flash column chromatography using 200 mL of silica gel and ethyl acetate-hexane as a gradient system. Boc-Thr(O^{t}Bu)-triOxa-$CSNH_2$ was eluted at the gradient of 65% of hexane and 35% of ethyl acetate. The pure Boc-Thr(O^{t}Bu)-triOxa-$CSNH_2$ was afforded as a white solid (370 mg, 55% yield for 2 steps) R_f: 0.45 (1:1 Hex/EA). ^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 8.40 (s, 1H), 8.31 (s, 1H), 8.28 (s, 1H), 8.23 (s, 1H), 8.12 (br, 1H), 5.60 (d, J = 9.3 Hz, 1H), 5.54 (br, 1H), 4.89 (d, J = 9.1 Hz, 1H), 4.18 (q, J = 5.6 Hz, 1H), 1.46 (s, 9H), 1.26 (d, J = 6.3 Hz, 3H), 0.96 ppm (s, 9H) ; HRMS(ESI): calcd for $C_{22}H_{29}N_5O_8SNa^+$ [M + Na^+] 514.1737, found 513.1950.

5.4.2 Experimental method for Ustat A-2 fragment 2

5.4.2.1 (2R, 3S) / (2S, 3R) *racemic* Bromoketal-*β*-hydroxyl-Phe-OMe

Following the ***solution phase peptide coupling*** procedure: (2R, 3S) / (2S, 3R) *racemic* Bromoketal-*β*-hydroxyl-Phe-OMe was synthesized utilizing 508 g (2.38 mmol,

1.0 equivalent) of 3-bromo-2,2-dimethoxypropionic acid, 546.6 mg (2.8 mmol, 1.1 equivalents) of free amine *racemic-β*-hydroxyl-Phe-OMe, 918 g (2.8 mmol, 1.1 equivalents) of coupling reagent TBTU and 1.66 mL (9.54 mmol, 4.0 equivalents) of DIPEA in 24 mL anhydrous DCM, under argon. The reaction was stirred for 35 min and upon completion, the crude reaction mixture was diluted with 200 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL x 2) followed by basic extraction using saturated $NaHCO_3$ (200 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 400 mL silica gel and ethyl acetate-hexane as a gradient system. (2R, 3S) / (2S, 3R) *racemic* Bromoketal-*β*-hydroxyl-Phe-OMe was eluted at the gradient of 65% of hexane and 35% of ethyl acetate. The pure (2R, 3S) / (2S, 3R) *racemic* Bromoketal-*β*-hydroxyl-Phe-OMe was afforded as a white solid (852 mg, 92% yield) R_f: 0.46 (1:1 Hex/EA). ^{1}H NMR: ($CDCl_3$, 400 MHz): δ = 7.50 (d, *J* = 8.9 Hz, 1H), 7.37-7.14 (m, 5H), 5.36 (t, *J* = 3.4 Hz,1H), 4.86 (dd, *J* = 9.2, 3.1 Hz, 1H), 3.71 (s, 3H), 3.44 (s, 2H), 3.22 (s, 3H), 2.92 (s, 3H), 2.62 ppm (d, *J* = 4.0 Hz, 1H). ^{13}C NMR: ($CDCl_3$, 400 MHz) δ = 170.38, 167.44, 139.45, 128.38, 127.91, 125.78, 100.28, 72.95, 58.02, 52.59, 50.18, 49.97, 29.59 ppm ; HRMS(ESI): calcd for $C_{15}H_{20}BrNO_6Na^+$ [M + Na^+] 412.0372, found 412.0361.

5.4.2.2 α-bromoketalphenyloxazole-OMe

Oxazoline formation (Pyridine as base)

852 mg (2.12 mmol, 1.0 equivalent) of (2R, 3S) / (2S, 3R) *racemic* α-Bromoketal-*β*-hydroxyl-Phe-OMe was dissolved in 21.2 mL of anhydrous DCM and cooled to -78^{o}C. 0.4 mL (2.33 mmol, 1.1 equivalents) of fluorinating agent DAST was added to the

solution drop-wise (0.1 mL/min). The reaction mixture was allowed to stir for 30 min followed by adding 0.35 mL (4.24 mmol, 2.0 equivalents) pyridine drop-wise (0.1 mL/min) to the reaction. And the reaction was allowed to proceed for an additional 30 min at -78^{o}C. The reaction mixture warmed up to RT and stir for an additional 1.5 h. Upon reaction completion, confirmed by TLC, the organic solution was diluted with DCM and washed with basic solution (saturated $NaHCO_3$ aqueous solution). The collected organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Obtained crude oxazoline material was purified via flash column chromatography using 150 mL silica gel and ethyl acetate-hexane as a gradient system. α-bromoketal-phenyloxazoline-OMe was eluted at the gradient of 60% of hexane and 40% of ethyl acetate. The pure α-bromoketal-phenyloxazoline-OMe was afforded as a white solid (707 mg, 90% yield) R_f: 0.46 (1:1 Hex/EA). ^{1}H NMR (400 MHz, $CDCl_3$):

Oxidation of Oxazoline

707 mg (1.91 mmol, 1.0 equivalent) of α-bromoketal-phenyloxazoline-OMe was dissolved in anhydrous 19 mL of anhydrous DCM and cooled to -47^{o}C. 0.4 mL (3.9 mmol, 2.0 equivalents) of base DBU was added drop-wise (0.1 mL/min) to the reaction flask and stirred for 15 min. 0.2 mL (3.9 mmol, 2.0 equivalents) of $BrCCl_3$ was added drop-wise (0.1 mL/min) to the reaction flask and stirred at -47^{o}C for an additional 2 h and then allowed to warm up to RT to stir additional 8 h. The reaction was monitored via TLC every 2 h. Upon completion, confirmed via TLC, the reaction mixture was diluted with 100 mL of DCM and washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL x 2). After the acidic work-up, the organic layer was washed with saturated $NaHCO_3$ aqueous solution (100 mL x 2). The basic aqueous layer was back washed with ethyl acetate (100 mL x 3) and

the collected organic layers were dried over Na_2SO_4, filtered, concentrated *in vacuo*. Obtained crude oxazole material was purified via flash column chromatography using 250 mL of silica gel and ethyl acetate-hexane as a gradient system. α-bromoketal-phenyloxazole-OMe was eluted at the gradient of 60% of hexane and 40% of ethyl acetate. The pure α-bromoketal-phenyloxazole-OMe was afforded as a white solid (352 mg, 70% yield for 2 steps) R_f: 0.40 (1:1 Hex/EA). ^{1}H NMR: ($CDCl_3$, 400 MHz): δ = 8.06-8.00 (m, 2H), 7.45-7.38 (m, 3H), 3.87 (s, 3H), 3.82 (s, 2H), 3.30 ppm (s, 6H). ^{13}C NMR: ($CDCl_3$, 400 MHz) δ = 162.38, 157.99, 156.11, 130.59, 128.57, 128.42, 126.99, 126.51, 99.12, 52.28, 52.20, 52.13, 50.20, 50.16, 50.13, 31.68, 31.59, 31.51 ppm ; HRMS(ESI): calcd for $C_{15}H_{16}BrNO_5Na^+$ [M + Na^+] 392.0110, found 392.0101.

5.4.2.3 α-Bromo-Phenyloxazole-OMe

α-bromoketal-phyenyloxazole-OMe was deprotected by dissolving 352 mg (0.95 mmol, 1.0 equivalent) of α-bromoketal-phyenyloxazole-OMe in 9.5 mL of formic acid. The solution was heated up to 60^{o}C and allowed to stir for 20 min. Upon completion, confirmed via TLC. The organic solution was diluted with 200 mL DCM and washed with saturated aqueous $NaHCO_3$ solution (300 mL x 2). The collected organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to yield α-bromo-phenyloxazole-OMe (305 mg, quantitative yield) as a light yellow oil. The material was taken on to the thiazole formation without further purification or characterization.

5.4.3 Experimental method for Ustat A-2 fragment 3

5.4.3.1 MeO-Ala-D-*allo*-Ile-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Ala-D-*allo*-Ile-Boc was synthesized utilizing 1.0 g (4.32 mmol, 1.0 equivalent) of free acid HO-D-*allo*-

Ile-Boc, 664 mg (4.76 mmol, 1.1 equivalents) of free amine H-Ala-OMe, 1.53 g (4.76 mmol, 1.1 equivalents) of coupling reagent TBTU and 3.0 mL (17.28 mmol, 4.0 equivalents) of DIPEA in 43 mL anhydrous DCM, under argon. The reaction was stirred for 40 min and upon completion, the crude reaction mixture was diluted with 300 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL x 2) followed by basic extraction using saturated $NaHCO_3$ (200 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 300 mL silica gel and ethyl acetate-hexane as a gradient system. MeO-Ala-D-*allo*-Ile-Boc was eluted at the gradient of 75% of hexane and 25% of ethyl acetate. The pure dipeptide MeO-Ala-D-*allo*-Ile-Boc was afforded as a yellow oil (1.35 g, 99% yield) R_f: 0.50 (1:1 Hex/EA). ^{1}H NMR: ($CDCl_3$, 400 MHz): δ = 6.53 (d, *J* = 6.5 Hz, 1H), 4.92 (br, 1H), 4.61 (m, 1H), 4.14 (br, 1H), 3.77 (s, 3H), 2.10-1.95 (m, 1H), 1.52-1.37 (m, 1H), 1.47 (s, 9H), 1.42 (d, *J* = 7.1 Hz, 3H) 1.31-1.16 (m, 1H), 0.95 (t, *J* = 7.3 Hz, 3H), 0.87 ppm (d, *J* = 7.1 Hz, 3H). ^{13}C NMR: ($CDCl_3$, 400 MHz) δ = 173.20, 171.39, 155.79, 79.89, 57.96, 52.32, 47.89, 37.10, 28.21, 26.23, 18.14, 14.09, 11.60 ppm ; HRMS(ESI): calcd for $C_{15}H_{28}N_2O_5Na^+$ [M + Na^+] 339.1896, found 339.1884.

5.4.3.2 MeO-Ala-D-*allo*-Ile-H

Following the ***Boc removal*** procedure MeO-Ala-D-*allo*-Ile-H was synthesized by dissolving 1.35 g (4.27 mmol, 1.0 equivalent) of MeO-Ala-D-*allo*-Ile-Boc in 35.6 mL of DCM, followed by adding 0.92 mL (8.54 mmol, 2.0 equivalents) of anisole and then 8.6 mL of TFA. Boc removal was completed in 30 min; the reaction mixture was concentrated *in vacuo* with DCM (350 mL x 3) and taken on to the next reaction without

further purification or characterization (922 mg, quantitative yield) as a yellow oil.

5.4.4 Experimental method for Ustat A-2 Protected Linear Precursor

5.4.4.1 Boc-Thr(O^{t}Bu)-triOxa-thiazole-OMe

Following ***Thiazole Formation*** Procedure: 272.3 mg (0.54 mmol, 1.0 equivalent) of Boc-Thr(O^{t}Bu)-triOxa-$CSNH_2$ (1.0 equivalent) was dissolved in 54 DME followed by the addition of 596 mg (4.32 mmol, 8.0 equivalents) of $KHCO_3$. The reaction mixture was stirred at RT for 15 min followed by the addition of 599 mg (1.62 mmol, 3.0 equivalents) of α-bromo-phenyloxazole-OMe. The reaction was stirred at RT for 16 h and monitored by TLC. Upon completion, the reaction mixture was concentrated *in vacuo.* The crude residues was dissolved in chloroform and washed with brine. The collected organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* resulted crude Boc-Thr(O^{t}Bu)-triOxa-thiazoline-OMe. The crude Boc-Thr(O^{t}Bu)-triOxa-thiazoline-OMe was re-dissolved in 54 mL of DME and cooled to 0^{o}C followed by adding 0.4 mL (4.0 mmol, 9.0 equivalents) of pyridine drop-wise (0.1 mL/min) to the solution and the reaction mixture was allowed to stir for 10 min. 0.3 mL (2.2 mmol, 4.0 equivalents) of TFAA was added to the reaction solution and stirred at 0^{o}C for additional 3 h. After 3 h, the reaction mixture was warmed up to RT and 0.2 mL (1.08 mmol, 2.0 equivalents) of TEA was then added to the solution slowly (0.1 mL/min) and stirred for an additional 1 h. Upon completion, confirmed via TLC, the solvent was removed *in vacuo* and the crude residue was re-dissolved in 100 mL of chloroform followed by a acidic work-up using 10% (v/v) $HCl_{(aq.)}$ (100 mL x 2) to remove the pyridine and TEA. After the acidic work-up, the organic layer was washed using a saturated $NaHCO_3$ aqueous solution (200 mL x 2) to remove the by-product of TFAA. The collected organic layer was dried over Na_2SO_4,

filtered, and concentrated *in vacuo*. The crude material was purified via flash column chromatography using 200 mL of silica gel and ethyl acetate-hexane as a gradient system. Boc-Thr(O^{t}Bu)-triOxa-thiazole-OMe was eluted at the gradient of 70% of hexane and 30% of ethyl acetate. The pure Boc-Thr(O^{t}Bu)-triOxa-thiazole-OMe was afforded as a yellow oil (278 mg, 72% yield for 2 steps) R_f: 0.3 (1:1 Hex/EA). ^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 8.50 (s, 1H), 8.40 (s, 1H), 8.38 (s, 1H), 8.23 (s, 1H), 8.21-8.17 (m, 2H), 7.55-7.47 (m, 3H), 5.62 (d, *J* = 9.8 Hz, 1H), 4.92 (d, *J* = 8.9 Hz, 1H), 4.20 (q, *J* = 7.0 Hz, 1H), 3.99 (s, 3H), 1.42 (s, 9H), 1.21 (d, *J* = 6.4 Hz, 3H), 0.98 ppm (s, 9H) ; HRMS(ESI): calcd for $C_{35}H_{37}N_6O_9SH^+$ [M + 1] 717.2343, found 717.1958.

5.4.4.2 Boc-Thr(O^{t}Bu)-triOxa-thiazole-OH

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide Boc-Thr(O^{t}Bu)-triOxa-thiazole-OMe was deprotected by utilizing 278 mg (0.39 mmol, 1.0 equivalent) of Boc-Thr(O^{t}Bu)-triOxa-thiazole-OMe and 131 mg (3.12 mmol, 8.0 equivalents) of LiOH in 3.9 mL MeOH. The peptide was dissolved in MeOH and LiOH was added to the reaction flask and the reaction was run for 8 h. Upon completion, the reaction was diluted with 100 mL DCM and washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL). The acidic aqueous layer was back extracted with DCM (100 mL x 3). The organic layers were collected, dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield 273 mg (quantitative yield) of Boc-Thr(O^{t}Bu)-triOxa-thiazole-OH as a white solid. The free acid Boc-Thr(O^{t}Bu)-triOxa-thiazole-OH was taken on without any further purification or characterization.

5.4.4.3 Boc-Thr(O^{t}Bu)-triOxa-thiazole-D-*allo*-Ile-Ala-OMe

Following the ***solution phase peptide coupling*** procedure: Boc-Thr(O^{t}Bu)-triOxa-thiazole-D-*allo*-Ile-Ala-OMe was synthesized utilizing 274 mg (0.38 mmol, 1.0 equivalent) of free acid Boc-Thr(O^{t}Bu)-triOxa-thiazole-OH, 90 mg (0.42 mmol, 1.1 equivalents) of free amine H-D-*allo*-Ile-Ala-OMe, 134.8 mg (0.42 mmol, 1.1 equivalents) of coupling reagent TBTU and 0.3 mL (1.68 mmol, 4.0 equivalents) of DIPEA in 4 mL anhydrous DCM, under argon. The reaction was stirred for 2 h and upon completion, the crude reaction mixture was diluted with 100 mL DCM. The organic solution was washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL) followed by basic extraction using saturated $NaHCO_3$ (100 mL x 2) aqueous solution. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and purified via flash column chromatography using 100 mL silica gel and ethyl acetate-hexane as a gradient system. Boc-Thr(O^{t}Bu)-triOxa-thiazole-D-*allo*-Ile-Ala-OMe was eluted at the gradient of 50% of hexane and 50% of ethyl acetate. The pure Boc-Thr(O^{t}Bu)-triOxa-thiazole-D-*allo*-Ile-Ala-OMe was afforded as a white solid (222 mg, 65% yield) R_f: 0.3 (2:3 Hex/EA). ^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 8.52 (s, 1H), 8.38 (s, 1H), 8.38(s, 1H), 8.33 (s, 1H), 8.33 (dd, *J* = 8.2, 2.0 Hz, 2H), 7.81 (d, *J* = 9.0 Hz, 1H), 7.51-7.42 (m, 3H), 6.60 (d, *J* = 7.4 Hz, 1H), 5.62 (d, *J* = 9.8 Hz, 1H), 4.89 (d, *J* = 9.4, 1H), 4.63 (dd, *J* = 10.0, 5.5 Hz, 1H), 4.59 (q, *J* = 7.3 Hz, 1H), 4.18 (q, *J* = 7.1 Hz, 1H), 3.71 (s, 3H), 2.22-2.18 (m, 1H), 1.48 (s, 9H), 1.41 (d, *J* = 7.2 Hz, 3H), 1.25 (t, *J* = 14.1 Hz, 2H), 1.24 (t, *J* = 6.2 Hz, 3H), 1.03 (d, *J* = 6.8 Hz, 3H), 0.97 (s, 9H), 0.93 ppm (d, *J* = 7.4 Hz, 3H). ^{13}C NMR: ($CDCl_3$, 400 MHz) δ = 173.1, 170.7, 165.2, 161.4, 161.0, 156.3, 155.9, 155.3, 154.1, 153.1, 143.6, 139.4, 139.1, 136.6, 130.1, 130.3, 129.8, 129.6, 128.4, 126.9, 121.6, 80.1, 74.5, 68.7,

57.0, 55.1, 52.5, 48.2, 37.1, 28.3, 28.1, 26.4, 20.3, 18.2, 14.7, 11.6 ppm; HRMS(ESI): calcd for $C_{44}H_{52}N_8O_{11}SNa^+$ [M + Na^+] 923.3368, found 923.3339.

5.4.4.4 Boc-Thr(O^{t}Bu)-triOxa-thiazole-D-*allo*-Ile-Ala-OH

Following the ***methyl ester hydrolysis*** procedure: The acid of Boc-Thr(O^{t}Bu)-triOxa-thiazole-D-*allo*-Ile-Ala-OMe was deprotected by utilizing 222 mg (0.25 mmol, 1.0 equivalent) of Boc-Thr(O^{t}Bu)-triOxa-thiazole-D-*allo*-Ile-Ala-OMe and 84 mg (2.0 mmol, 8.0 equivalents) of LiOH in 5.0 mL MeOH. The peptide was dissolved in MeOH and LiOH was added to the reaction flask. Catalytic amount of deionized-water was then added to the solution and the reaction was allow to run for 8 h. Upon completion, the reaction was diluted with 100 mL DCM and washed with 10% (v/v) $HCl_{(aq.)}$ (100 mL). The acidic aqueous layer was back extracted with DCM (100 mL x 3). The organic layers were collected, dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield 200 mg (quantitative yield) of Boc-Thr(O^{t}Bu)-triOxa-thiazole-D-*allo*-Ile-Ala-OH as a white solid. The free acid Boc-Thr(O^{t}Bu)-triOxa-thiazole-D-*allo*-Ile-Ala-OH was taken on without any further purification or characterization.

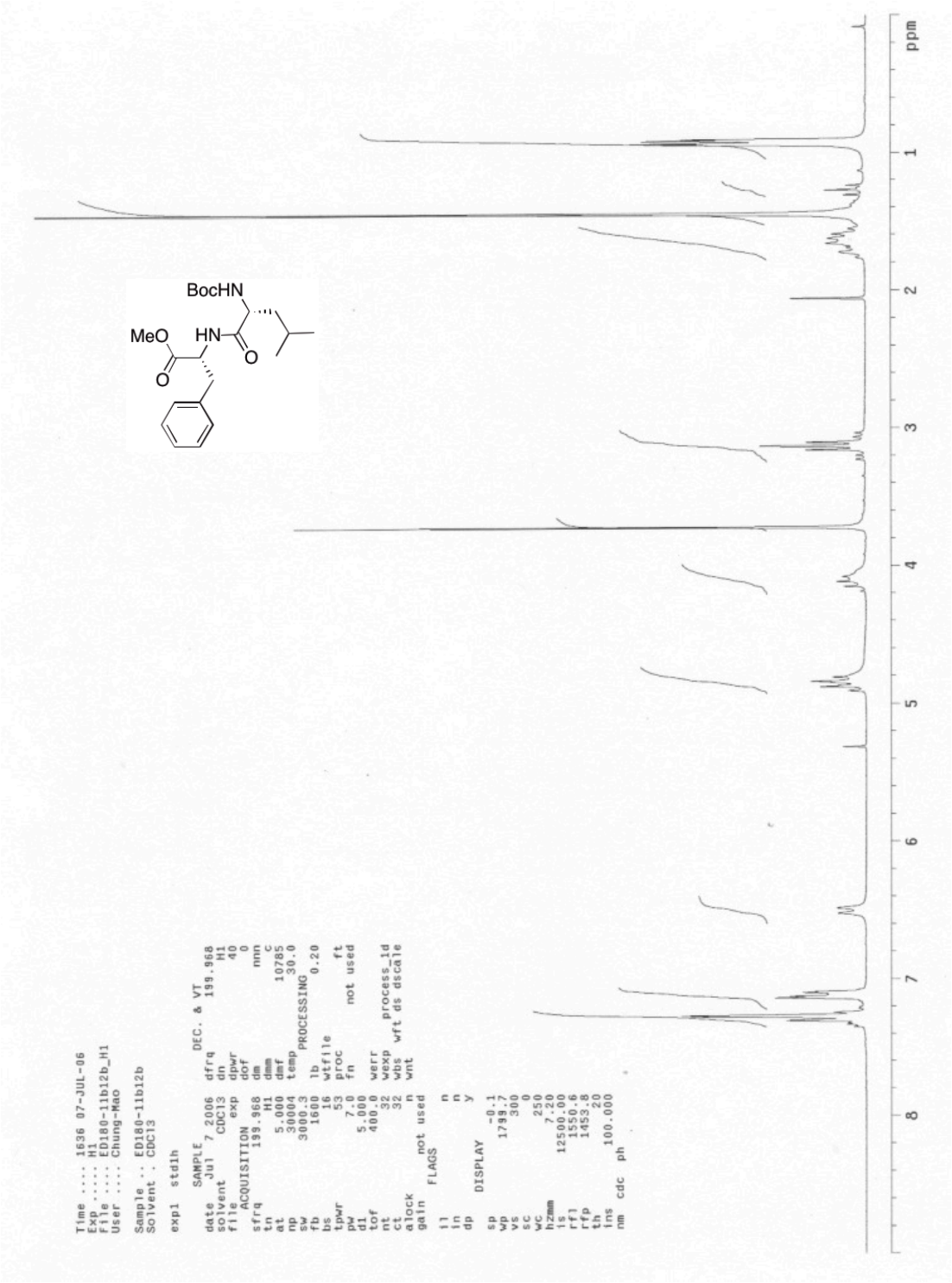
5.4.4.5 H-Thr-triOxa-thiazole-D-*allo*-Ile-Ala-OH

Following the ***Boc removal*** procedure: H-Thr-triOxa-thiazole-D-*allo*-Ile-Ala-OH was synthesized by dissolving 200 mg (0.25 mmol, 1.0 equivalent) of Boc-Thr(O^{t}Bu)-triOxa-thiazole-D-*allo*-Ile-Ala-OH in 5.0 mL of DCM, followed by adding 0.1 mL (0.50 mmol, 2.0 equivalents) of anisole and then 8.6 mL of TFA. Boc removal was completed in 30 min; the reaction mixture was concentrated *in vacuo* with DCM (350 mL x 3) and taken on to the next reaction without further purification or characterization (120 mg, quantitative yield) as a yellow oil.

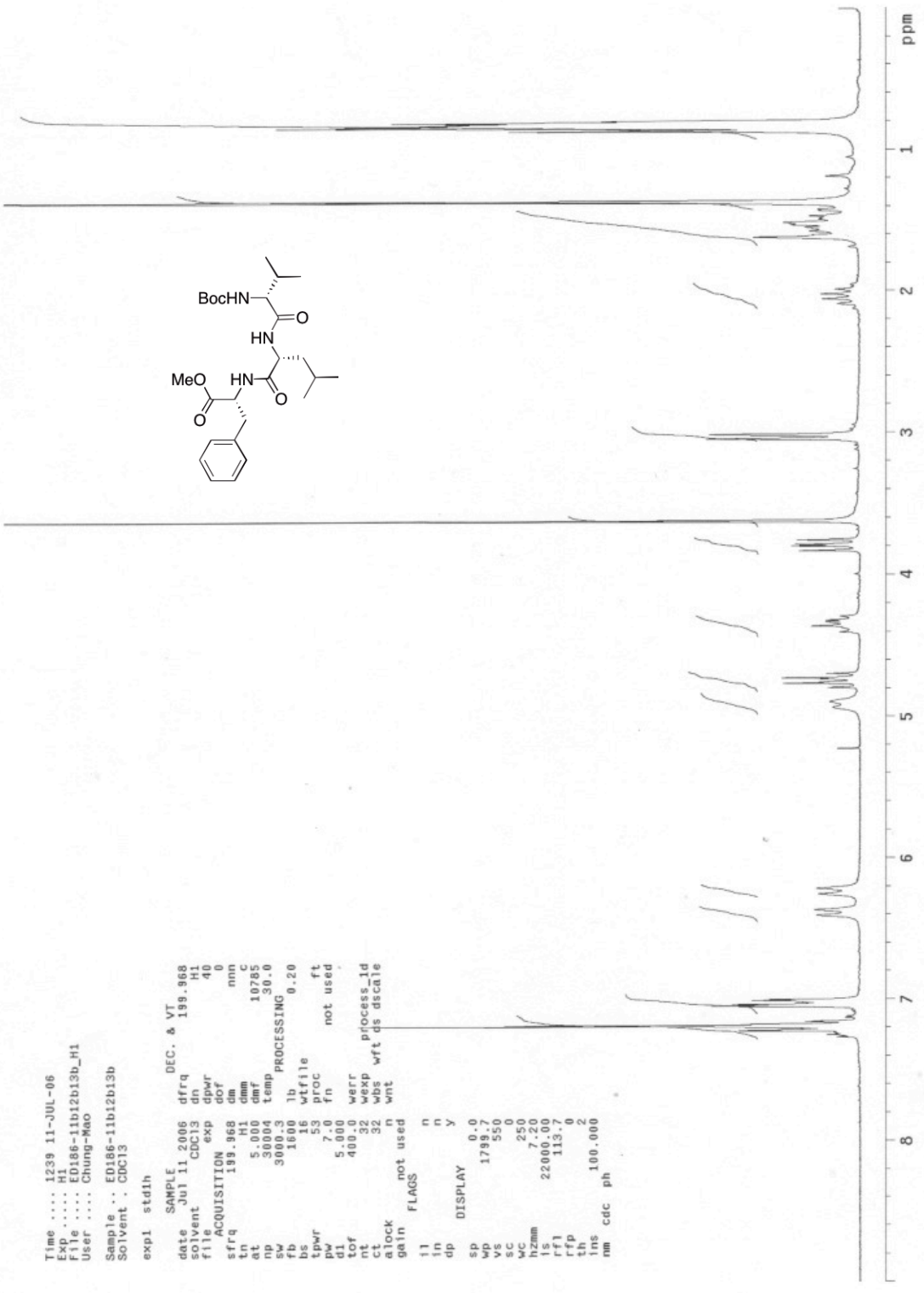
5.4.4.6 Cyclization of *Cyclo*-Thr-triOxa-thiazole-D-*allo*-Ile-Ala

Following **Syringe Pump Cyclization Procedure**: 120 mg (0.25 mmol) of H-Thr-triOxa-thiazole-D-*allo*-Ile-Ala-OH was cyclized using a mixture of coupling reagents: 56 mg of TBTU (0.18 mmol, 0.8 equivalents), 66 mg of HATU (0.18 mmol, 0.8 equivalents), and 52 mg of DEPBT (0.18 mmol, 0.8 equivalents). Double deprotected H-Thr-triOxa-thiazole-D-*allo*-Ile-Ala-OH was placed in a round bottom flask and dissolved in 62.5 mL (25% calculated volume) of anhydrous. The mixture coupling reagents were dissolved in the remaining 187.5 mL (75% volume of the anhydrous DCM) and purged with argon. 0.2 mL of DIPEA (1.0 mmol, 8.0 equivalents) was then added to the round bottom flash containing coupling reagents. H-Thr-triOxa-thiazole-D-*allo*-Ile-Ala-OH was placed in a 30 mL syringe and transferred to the using reaction flask containing coupling reagents using syringe pump at a rate of 30 mL/h. The reaction was monitored via TLC and LC/MS every hour and was complete in 5 h. Upon completion, the organic mixture was diluted with DCM and washed with acidic aqueous solution using 10% (v/v) $HCl_{(aq.)}$ (100 mL x 2). The organic layer was washed again with a saturated aqueous $NaHCO_3$ solution (200 mL x 2). The basic aqueous layer was extracted with ethyl acetate (100 mL x 3). Combined organic layers were, dried over Na_2SO_4, filtered, and concentrated *in vacuo*.

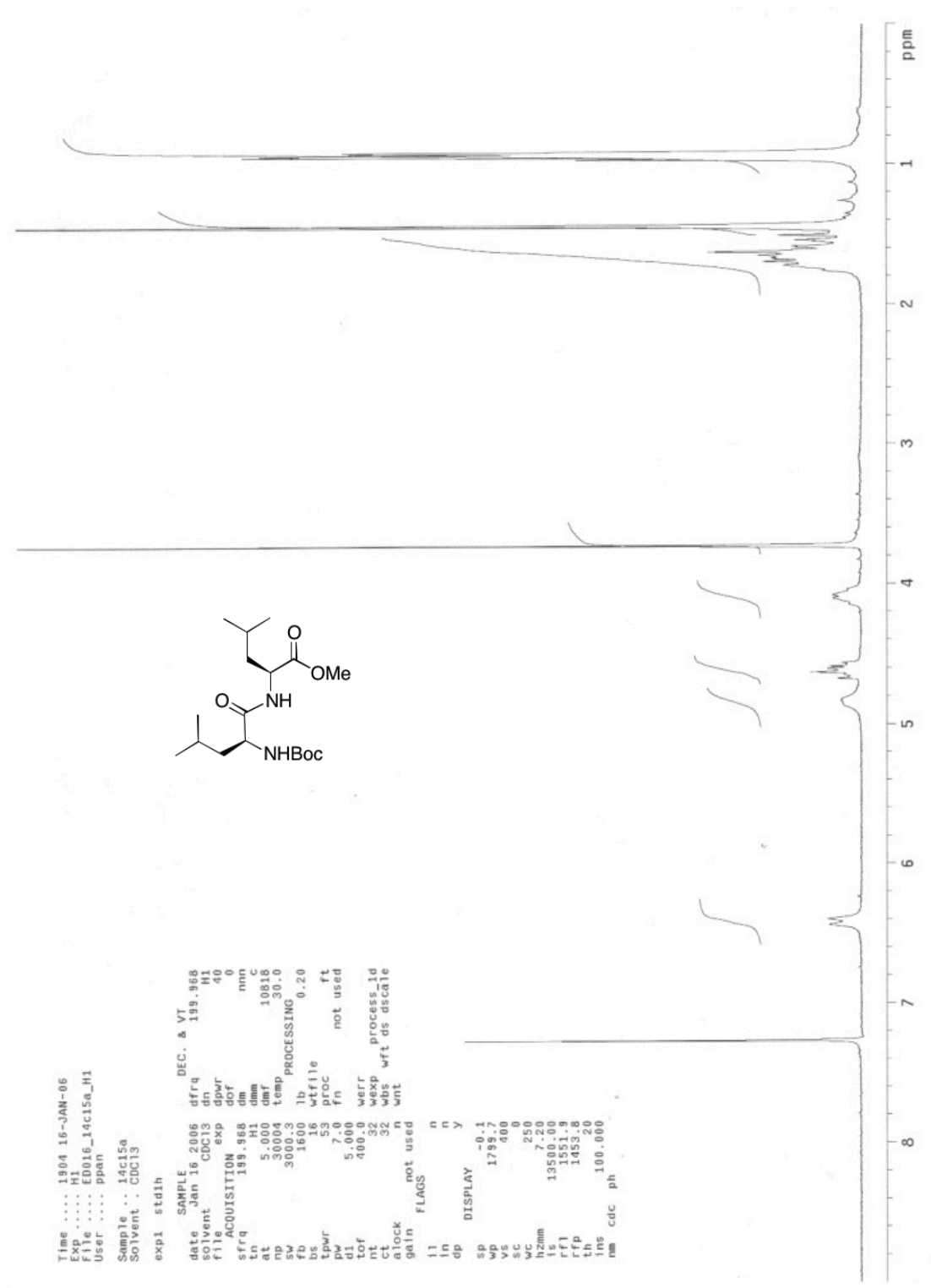
5.5.1 Supporting Spectra for Chapter 2 (SanA-1)



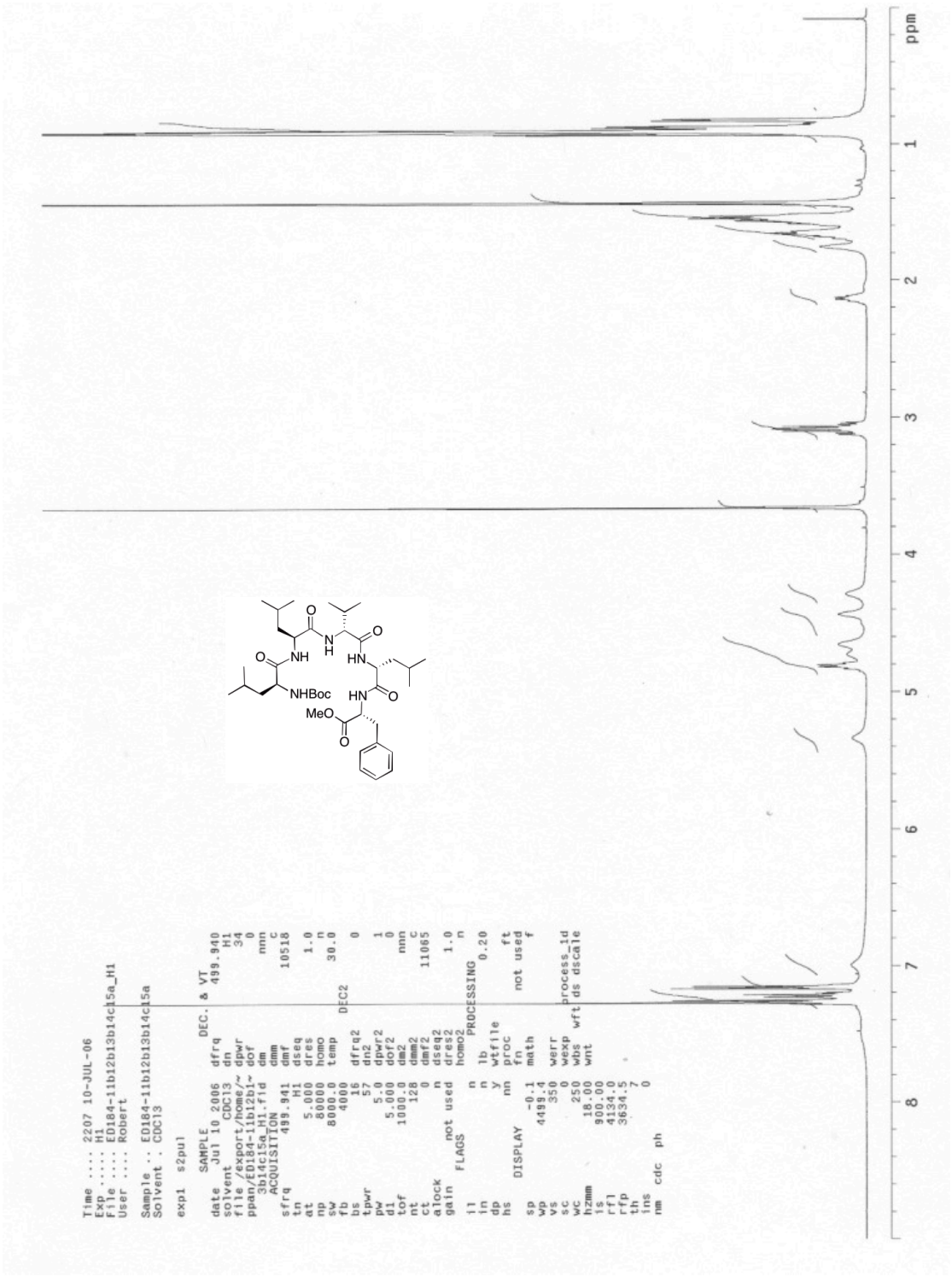
^{1}H NMR MeO-D-Phe-D-Leu-Boc



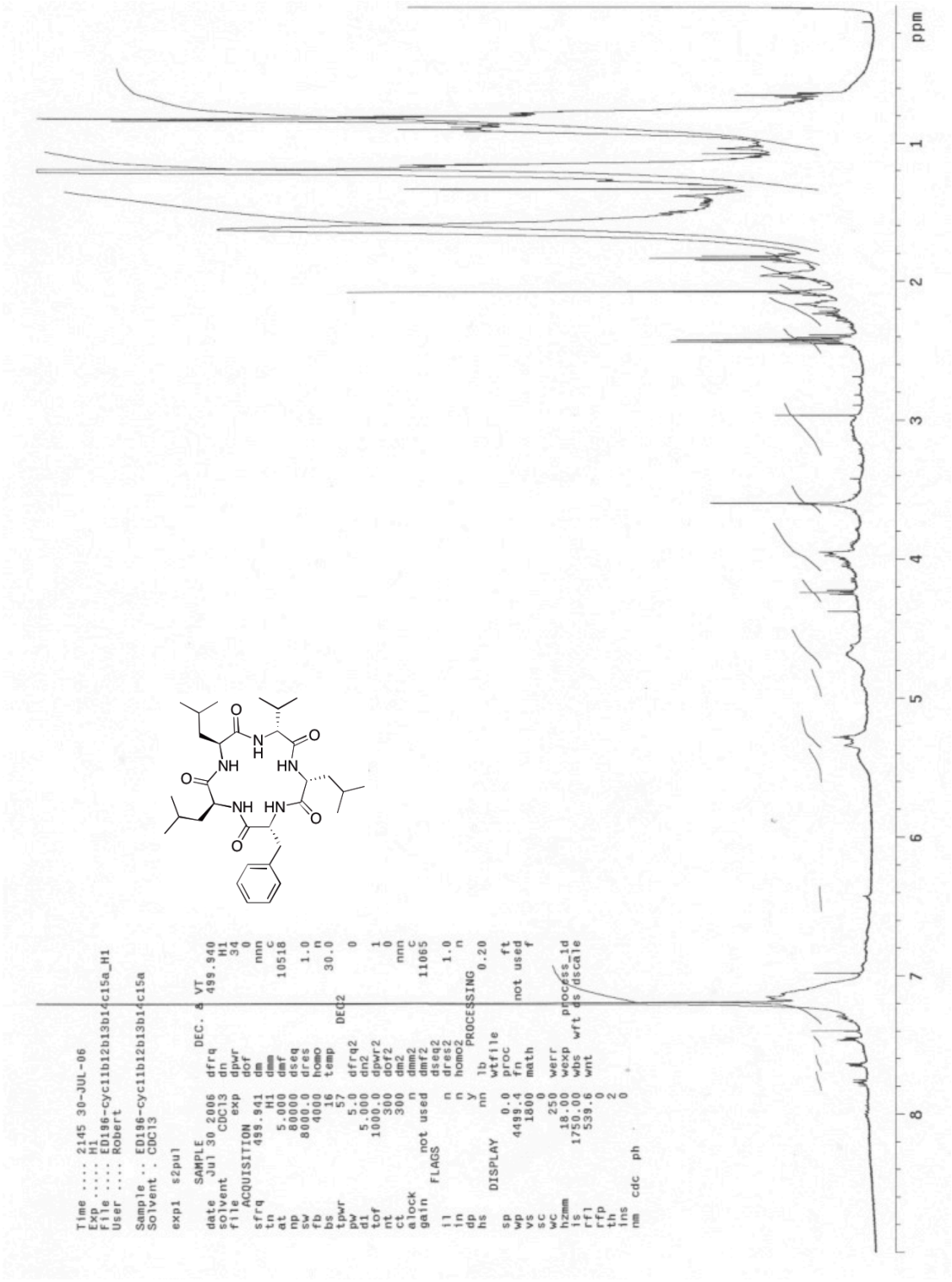
^{1}H NMR MeO-D-Phe-D-Leu-D-Val-Boc



^{1}H NMR MeO-Leu-Leu-Boc

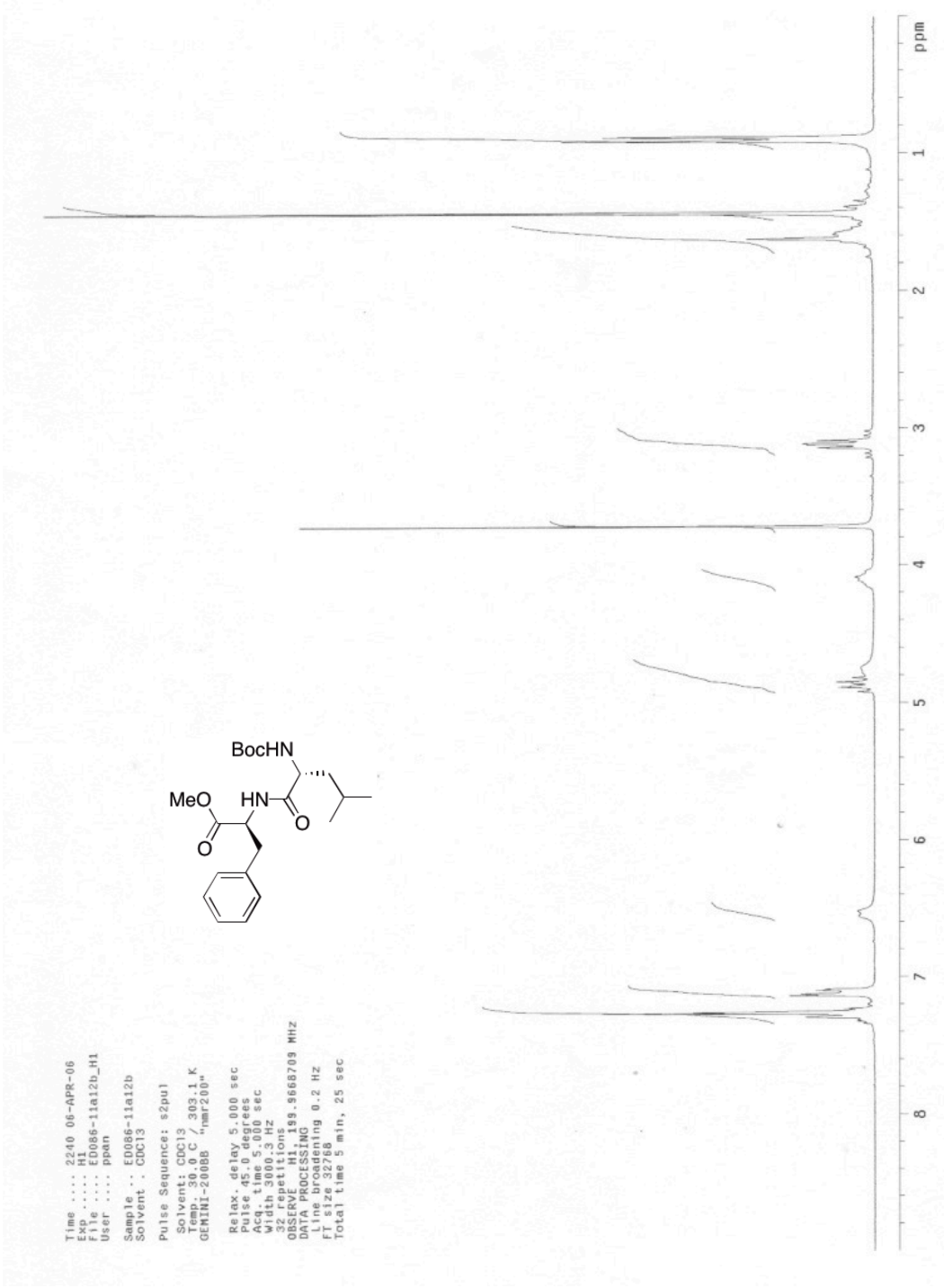


[1]H MeO-D-Phe-D-Leu-D-Val-Leu-Leu-Boc

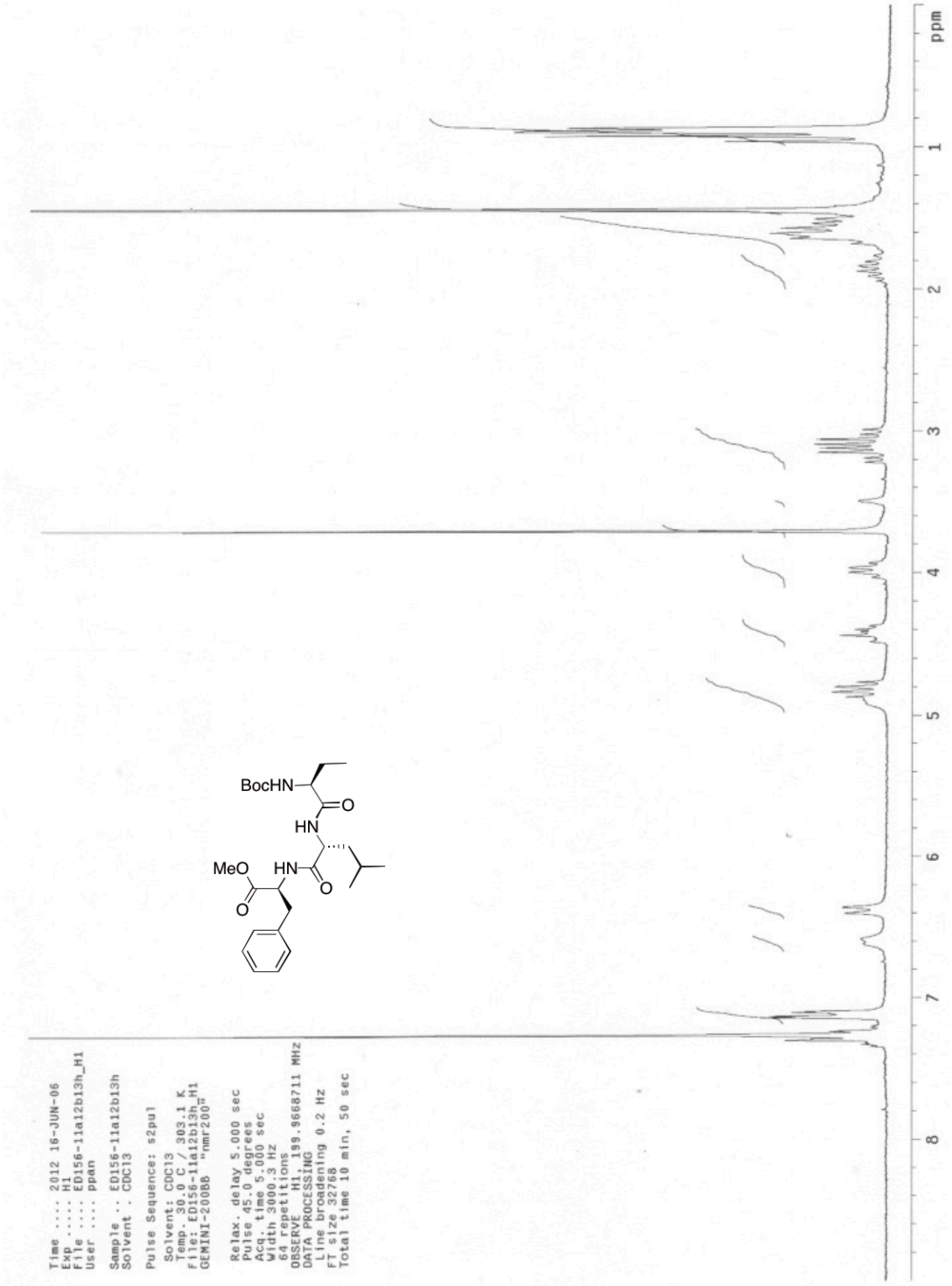


^{1}H *Cyclo*-D-Phe-D-Leu-D-Val-Leu-Leu

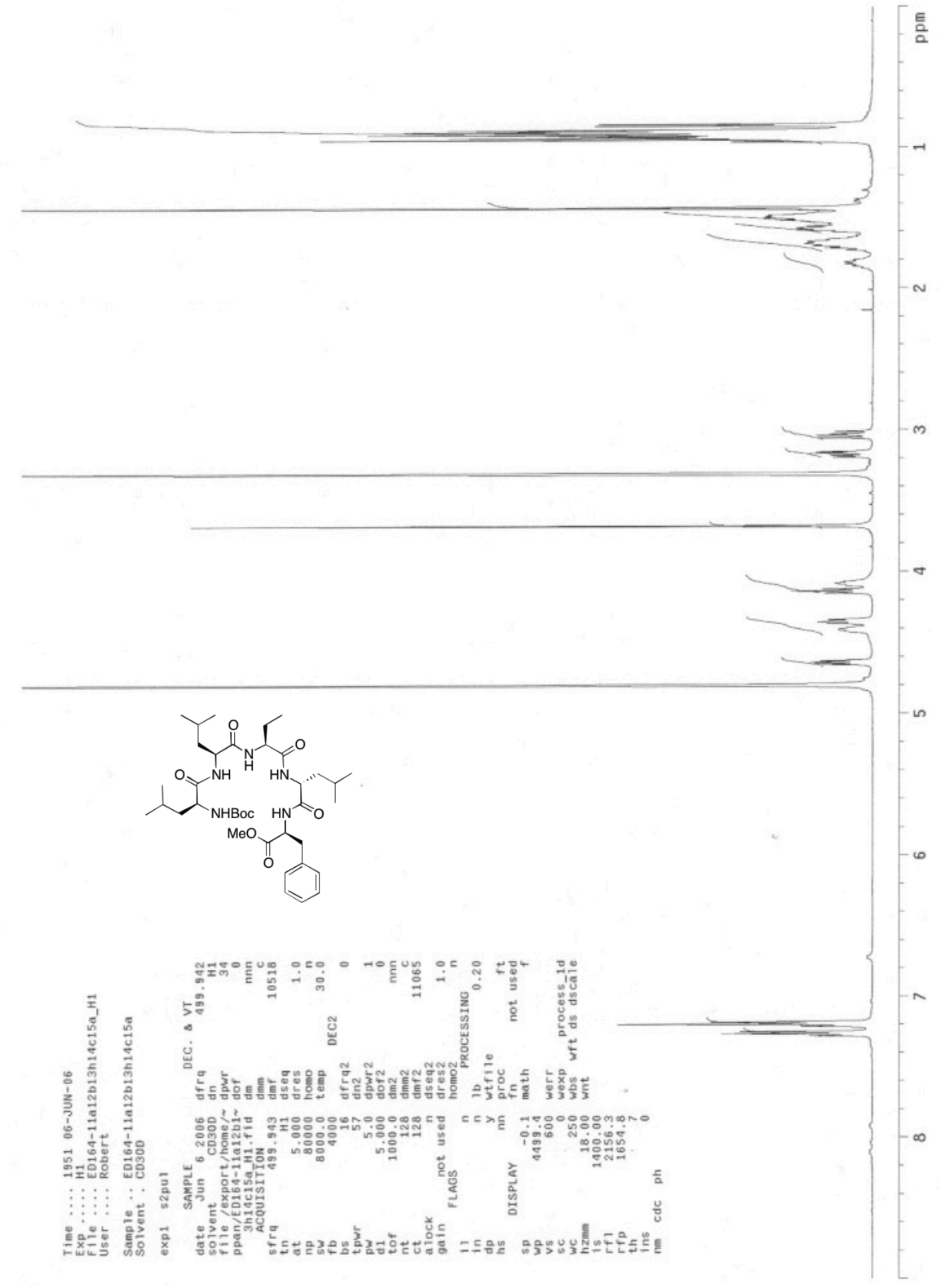
5.5.2 Supporting Spectra for Chapter 2 (SanA-2)



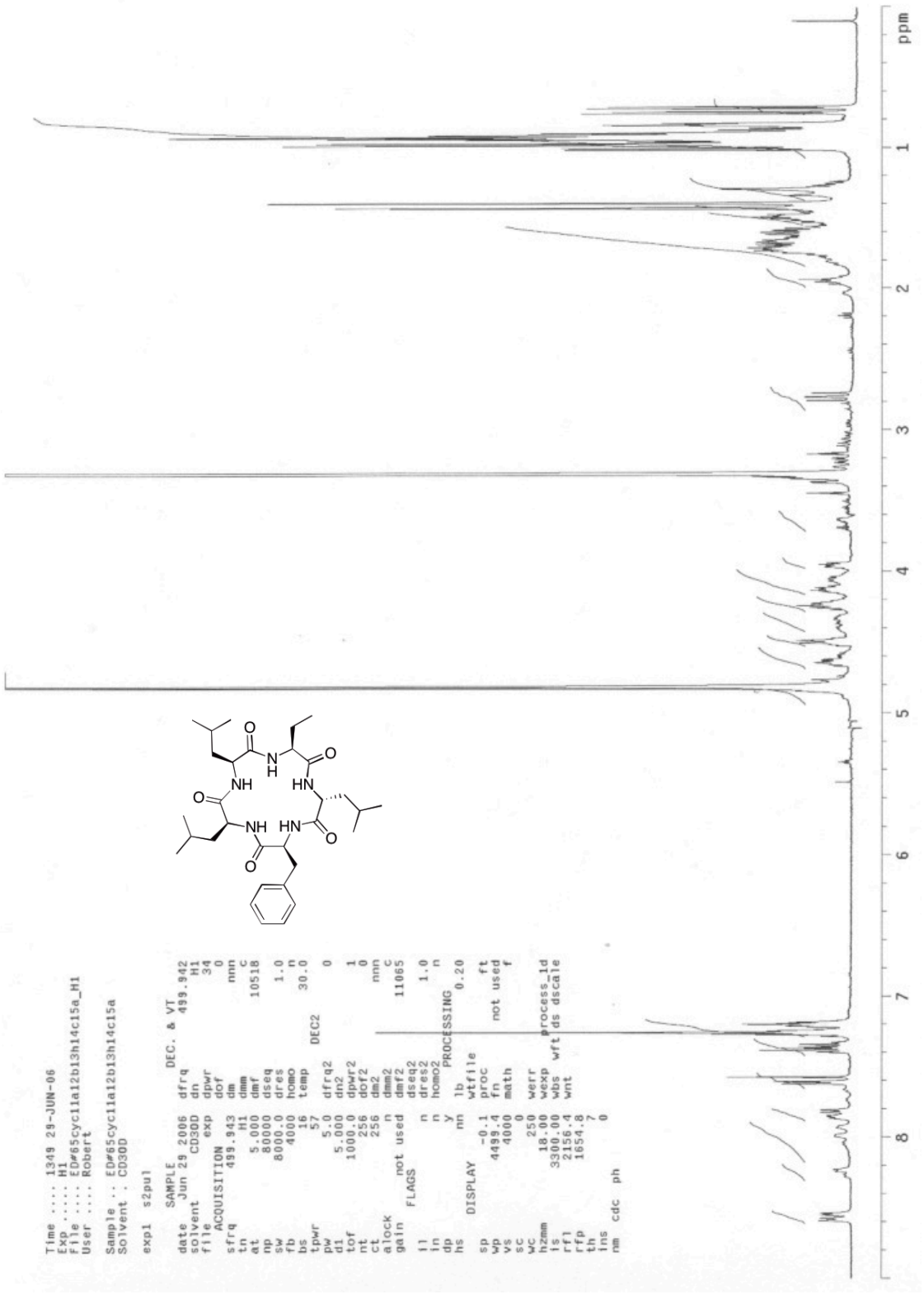
^{1}H MeO-Phe-D-Leu-Boc



^{1}H MeO-Phe-D-Leu-Abu-Boc

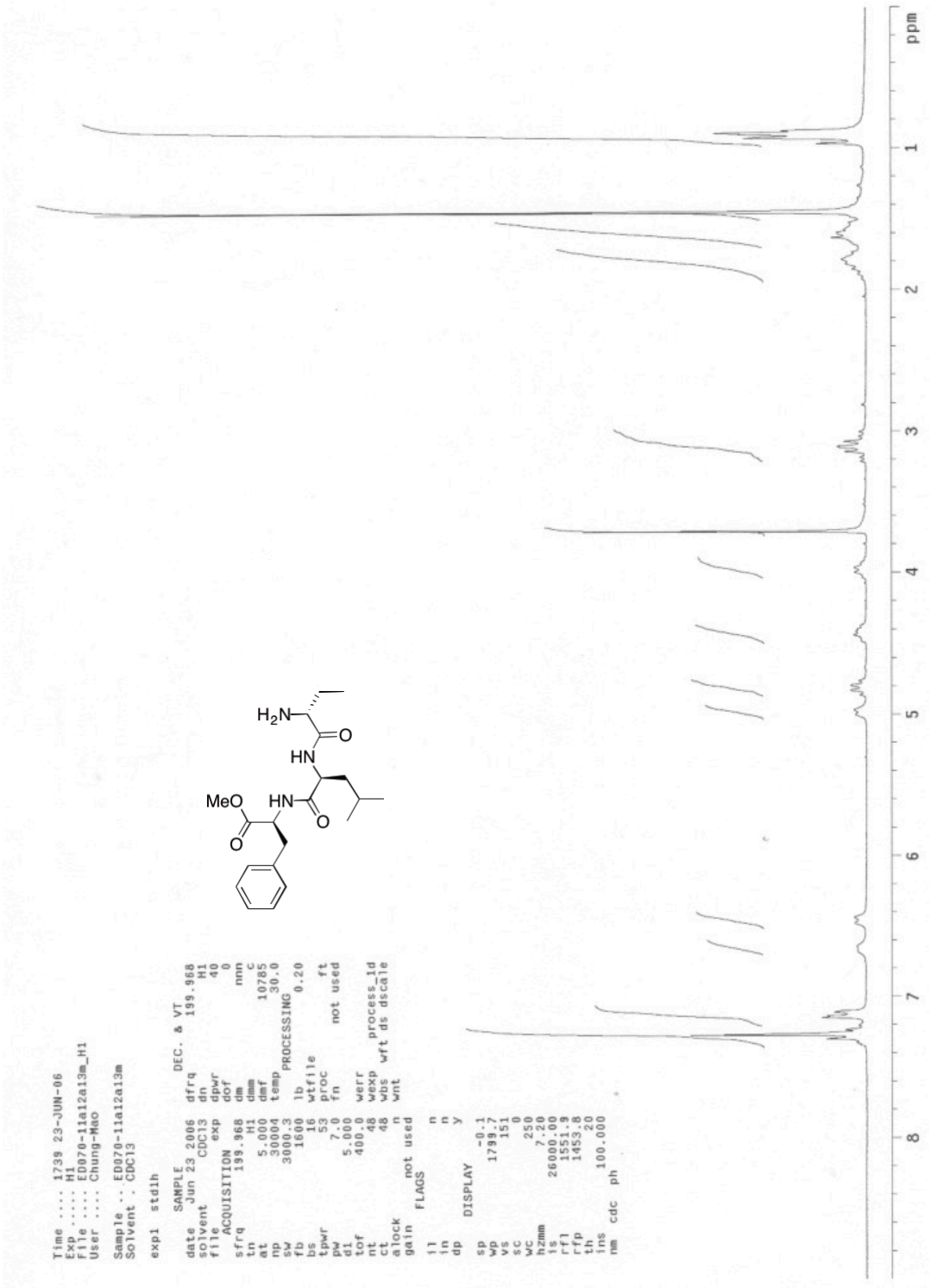


[1]H MeO-Phe-D-Leu-Abu-Leu-Leu-Boc

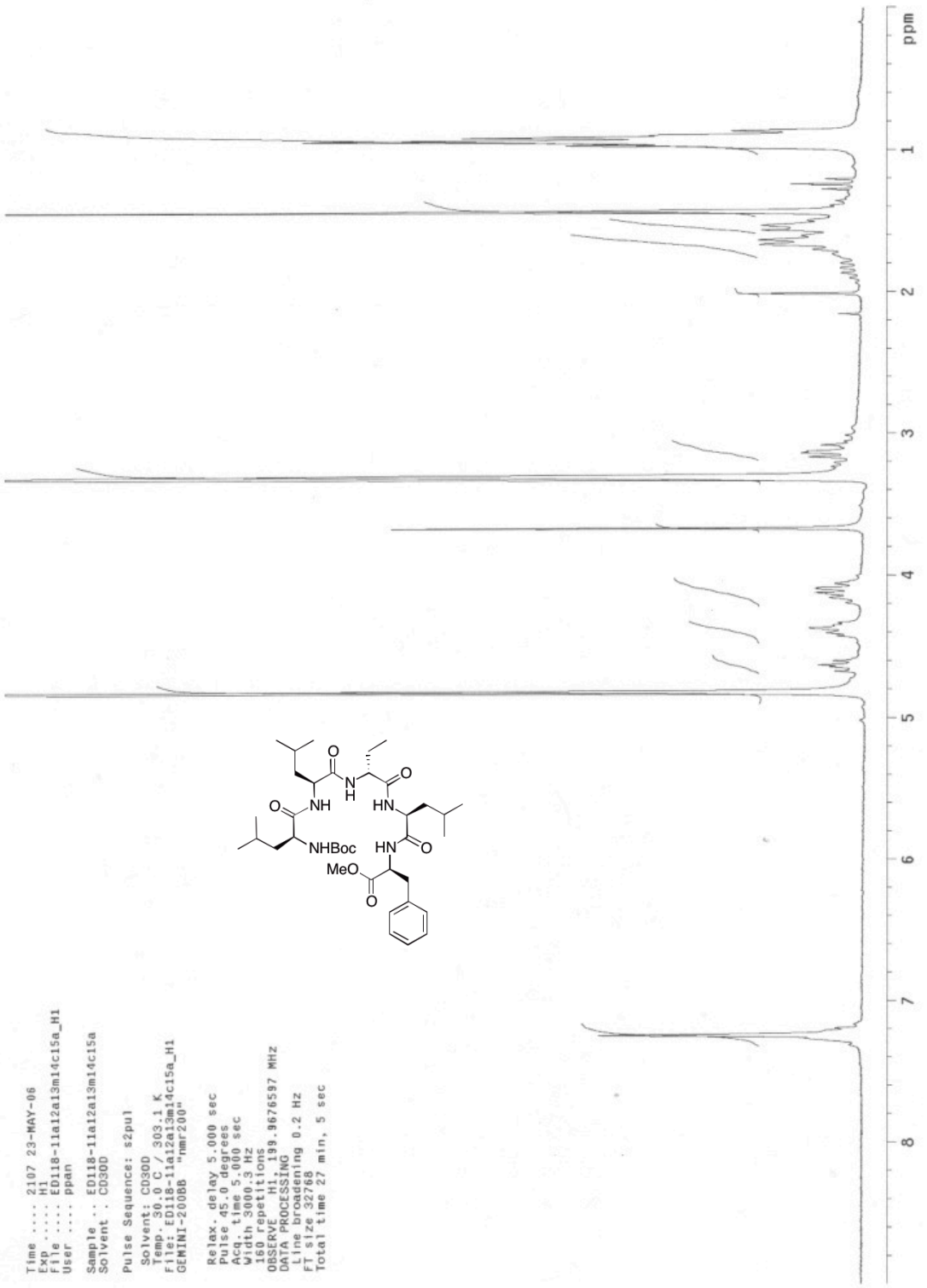


^{1}H *Cyclo*-Phe-D-Leu-Abu-Leu-Leu-Boc

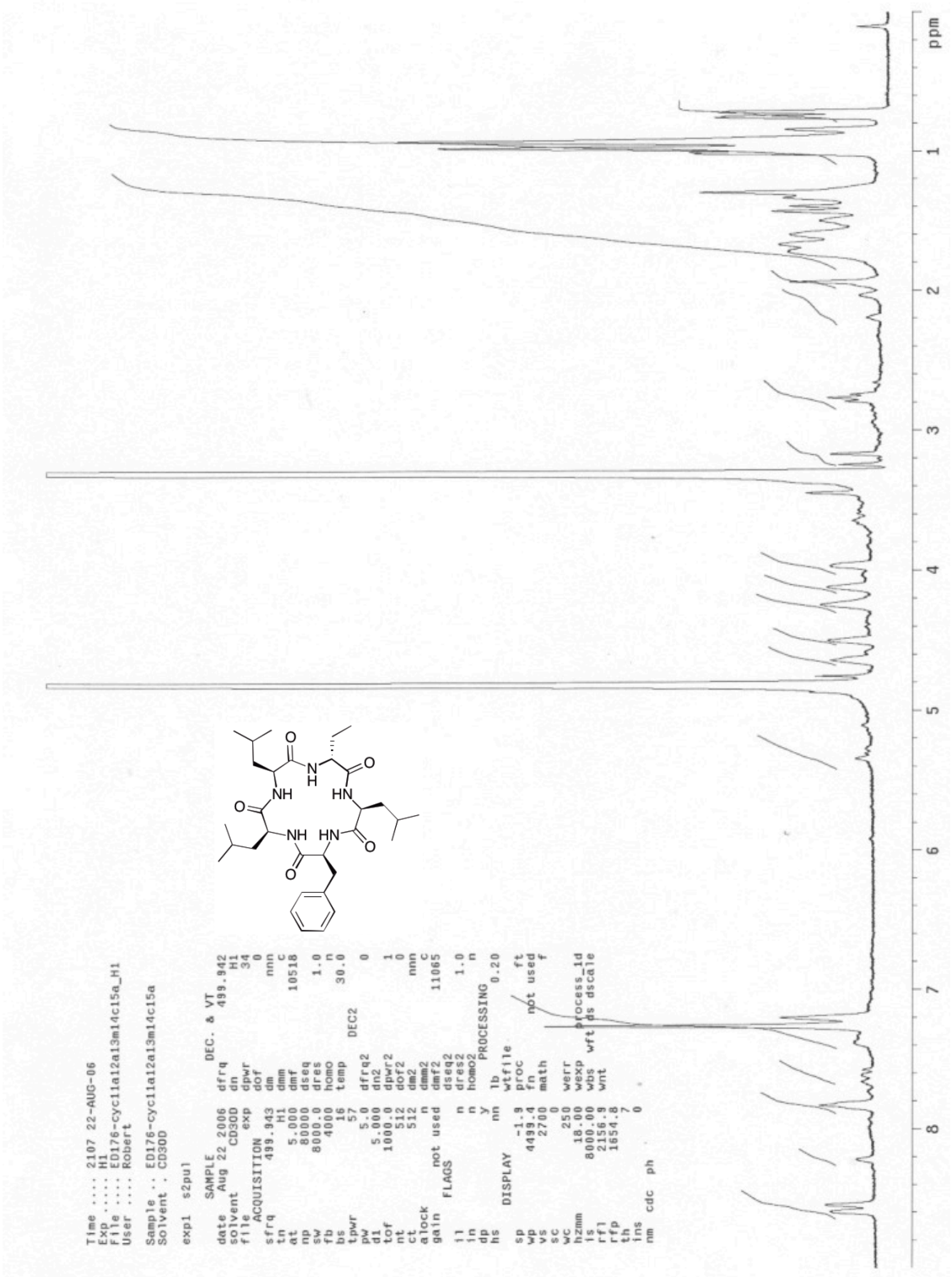
5.5.3 Supporting Spectra for Chapter 2 (SanA-3)



^{1}H NMR MeO-Phe-Leu-D-Abu-Boc

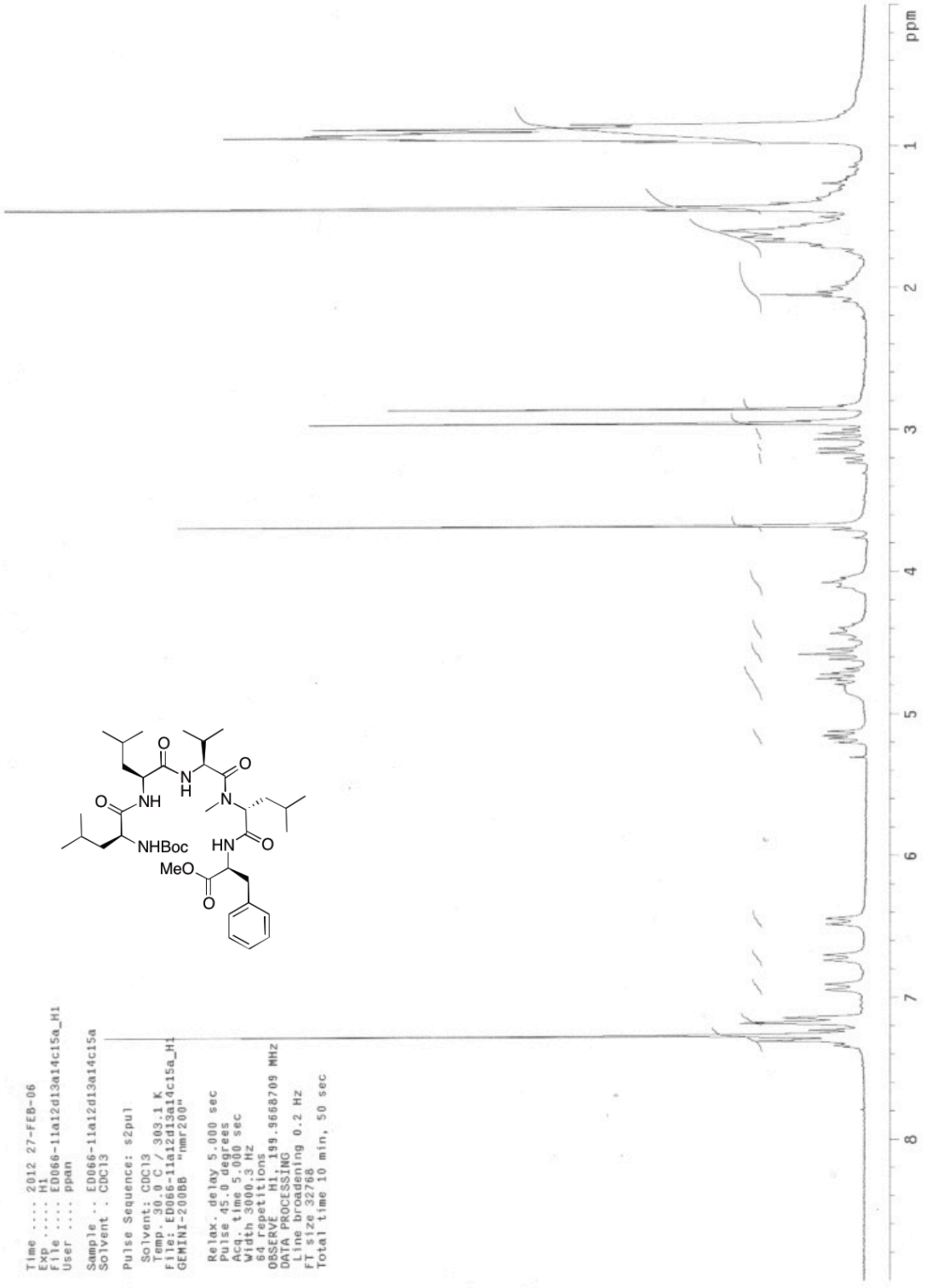


^{1}H NMR MeO-Phe-Leu-D-Abu-Leu-Leu-Boc

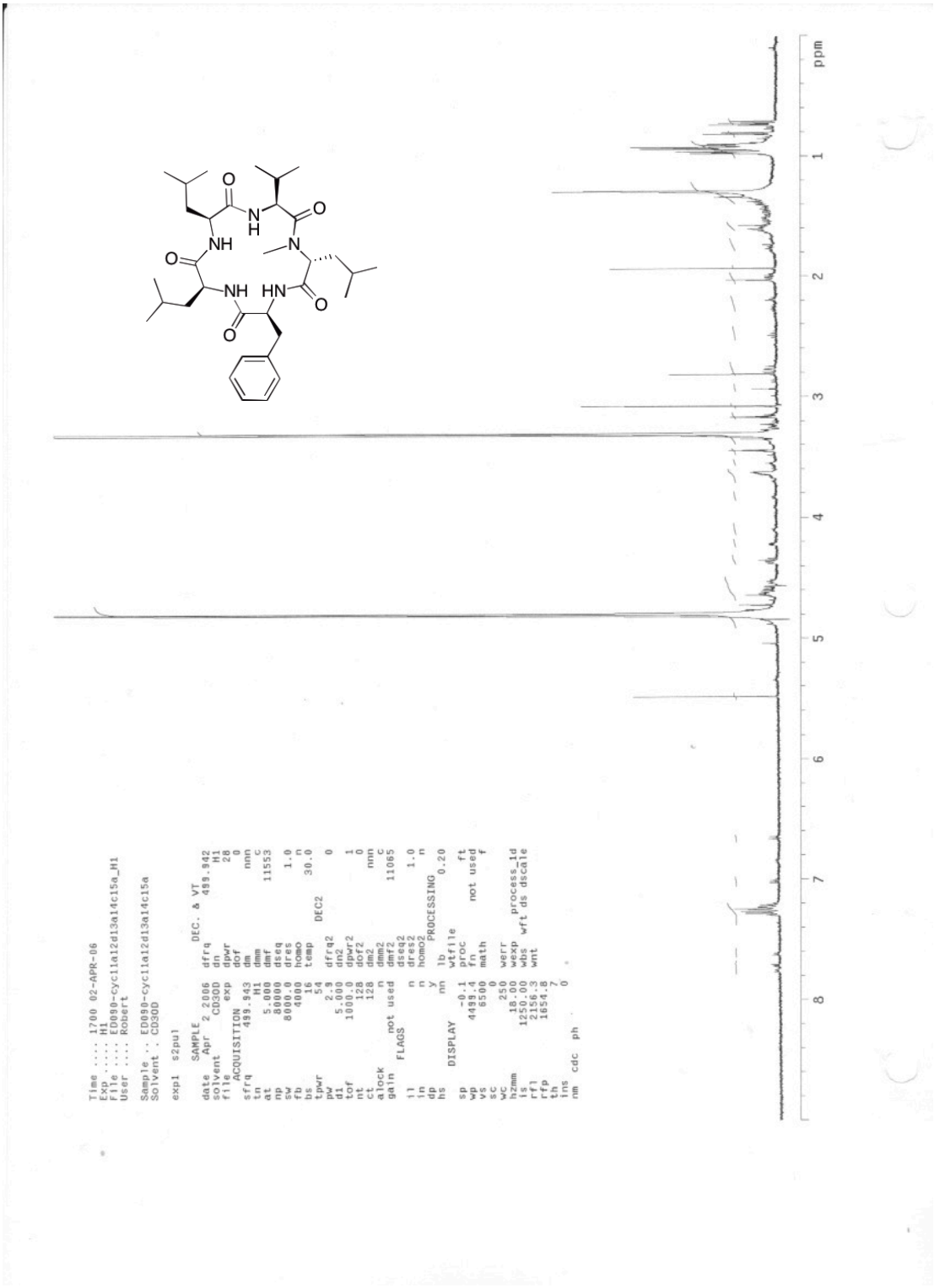


^{1}H NMR *Cyclo*-Phe-Leu-D-Abu-Leu-Leu

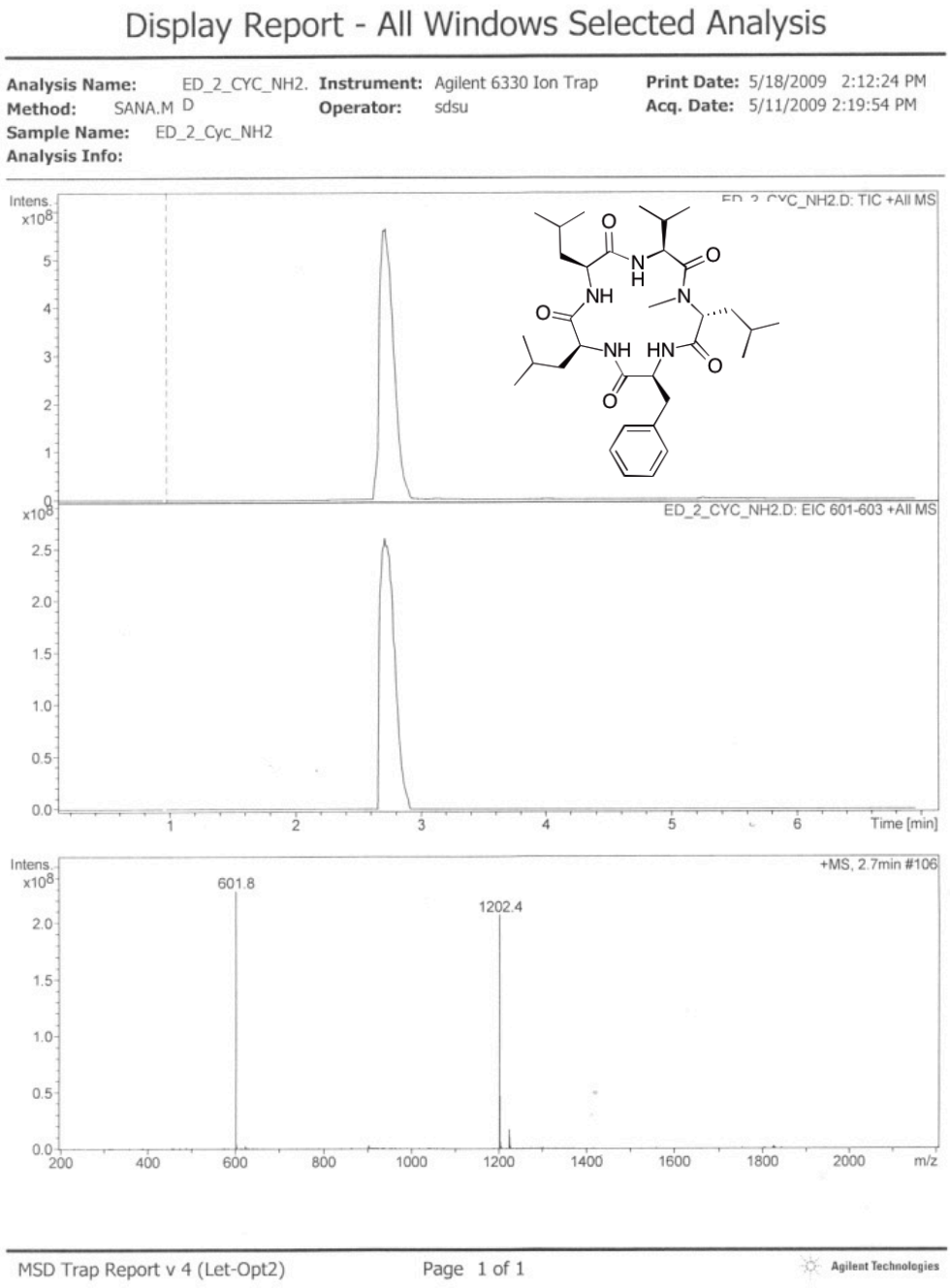
5.5.4 Supportin Spectra for Chapter 2 (SanA-4)



^{1}H NMR MeO-Phe-*N*-Me-D-Leu-Val-Leu-Leu-Boc

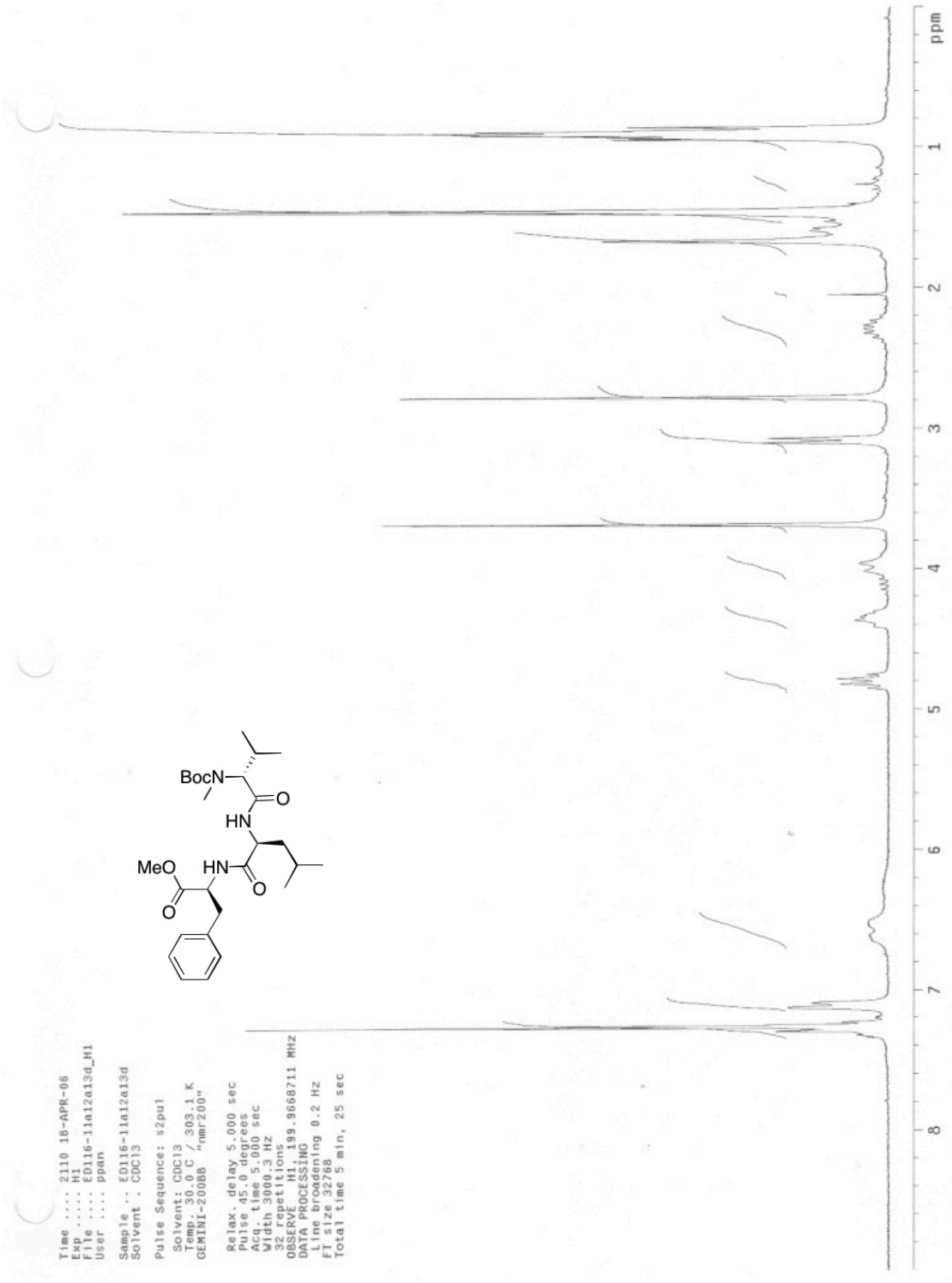


^{1}H NMR *Cyclo*-Phe-*N*-Me-D-Leu-Val-Leu-Leu

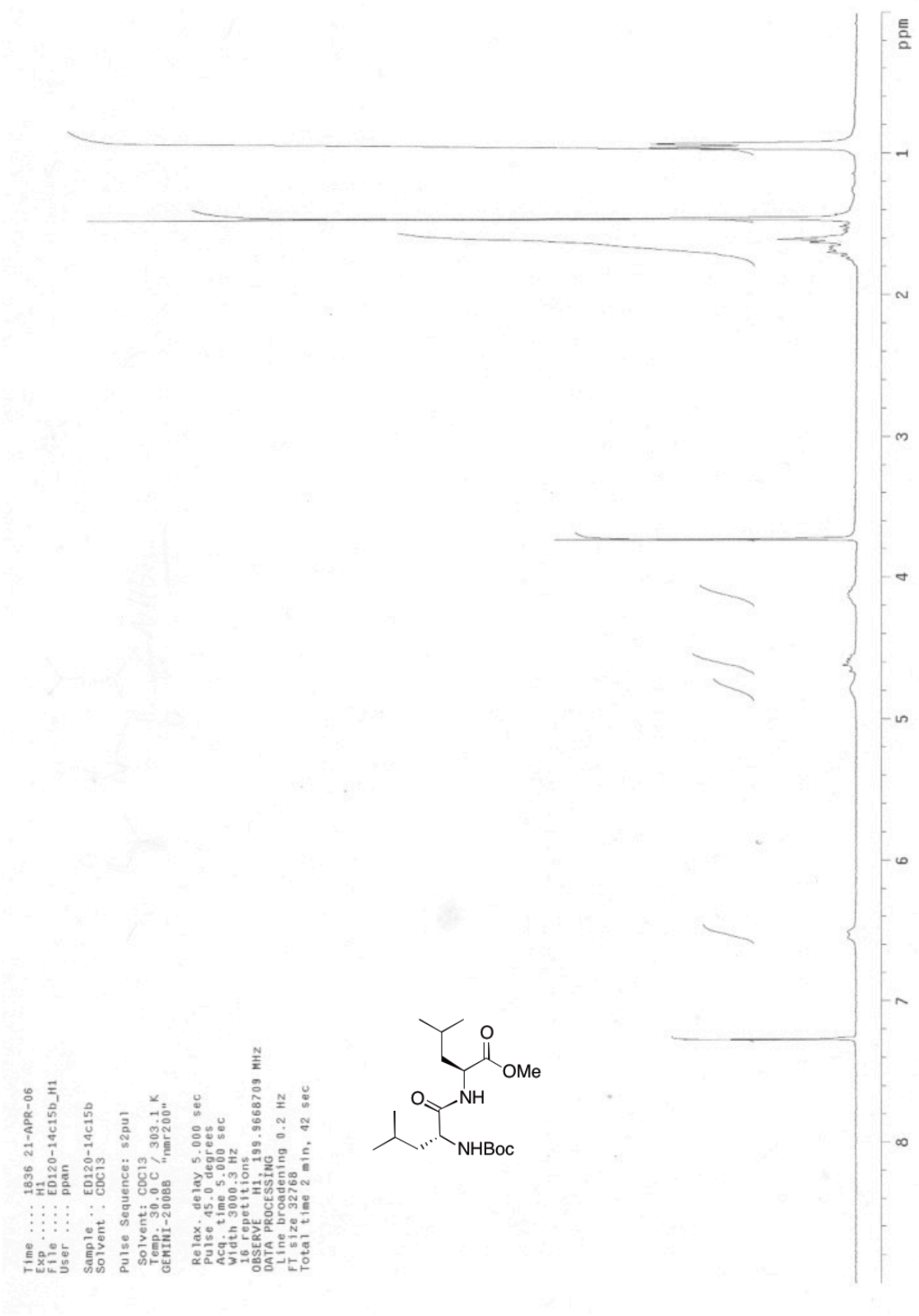


LC/MS *Cyclo*-Phe-*N*-Me-D-Leu-Val-Leu-Leu

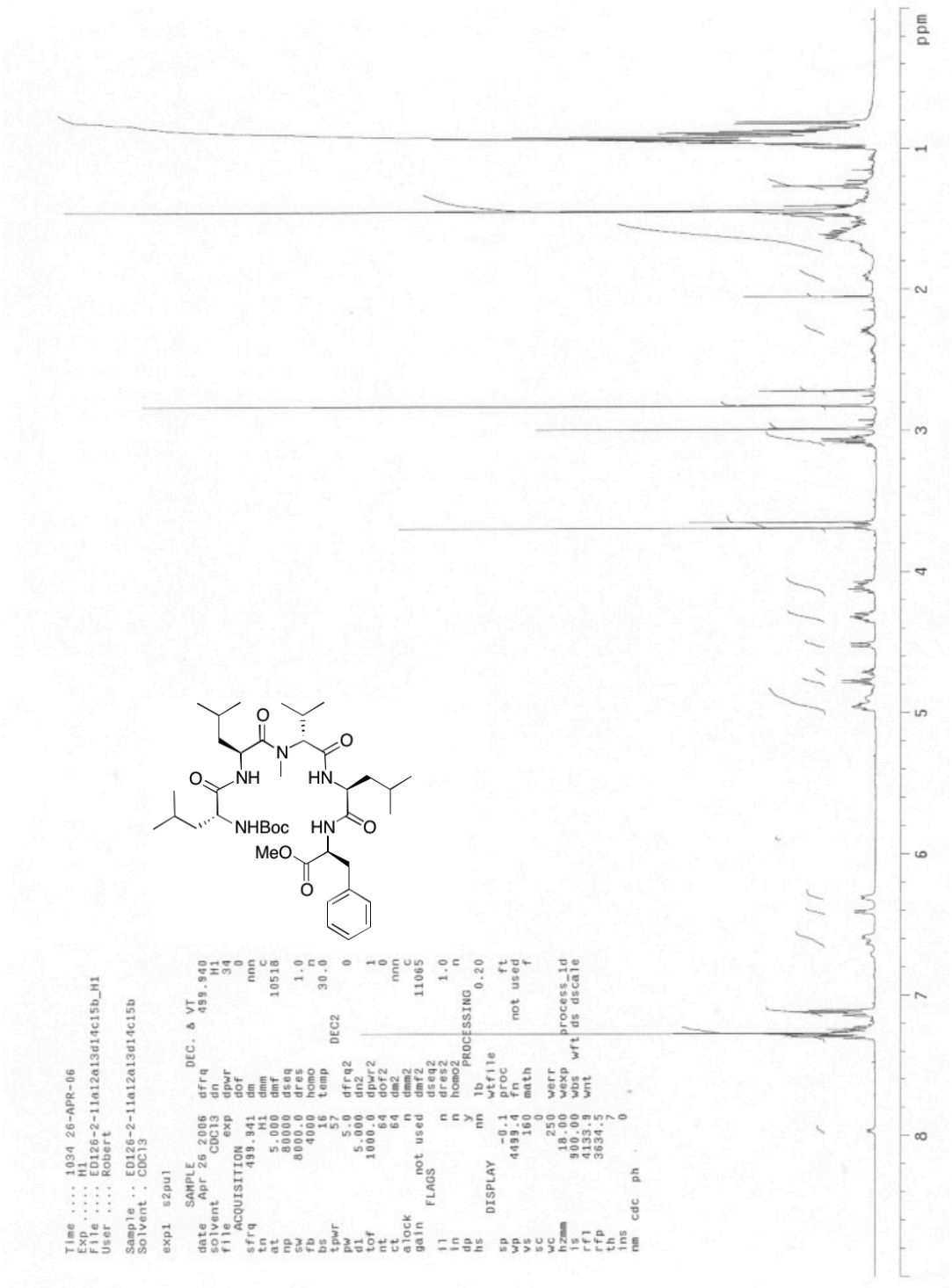
5.5.5 Supporting Spectra for Chapter 2 (SanA-5)



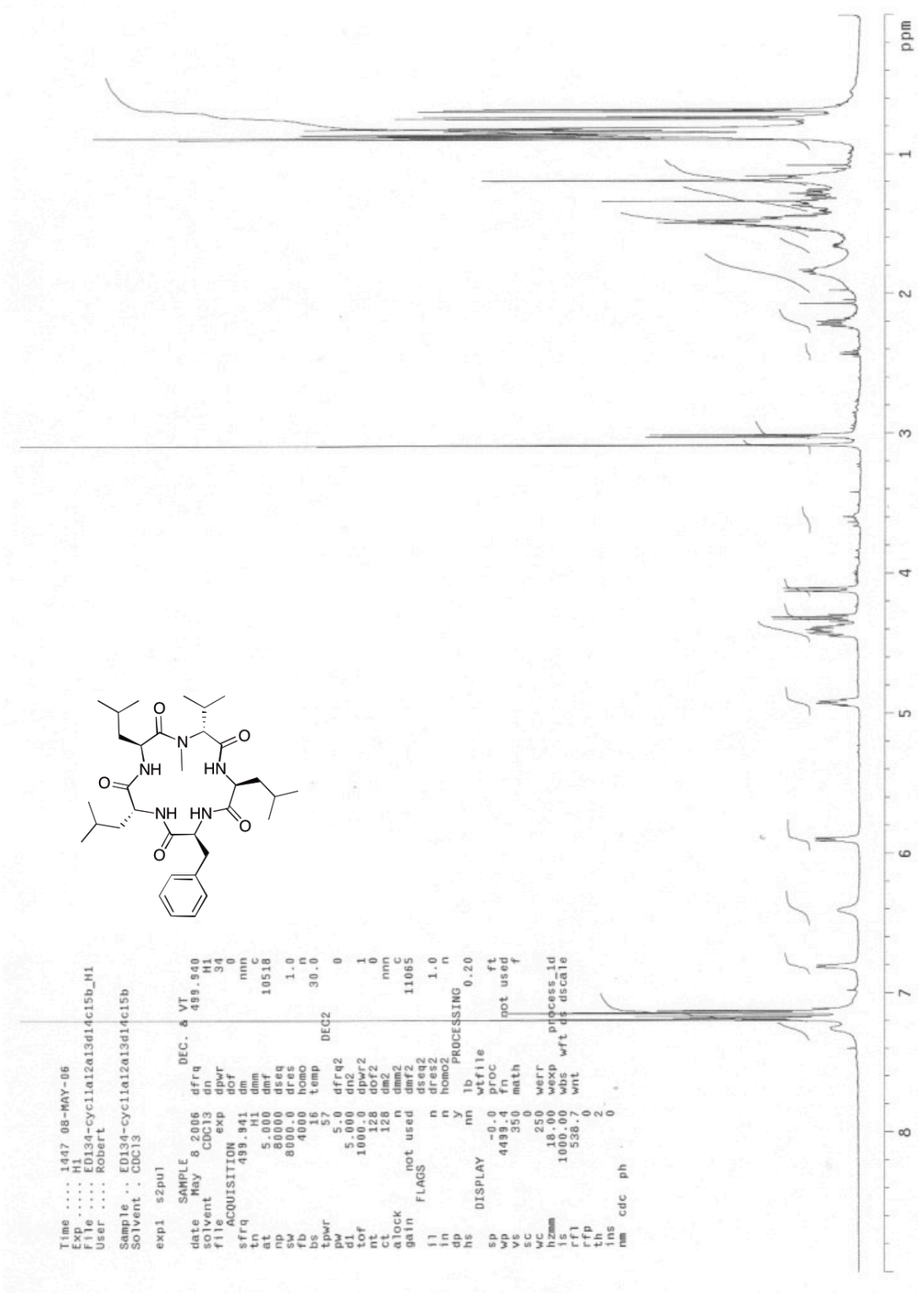
^{1}H MeO-Phe-Leu-N-Me-D-Val-Boc



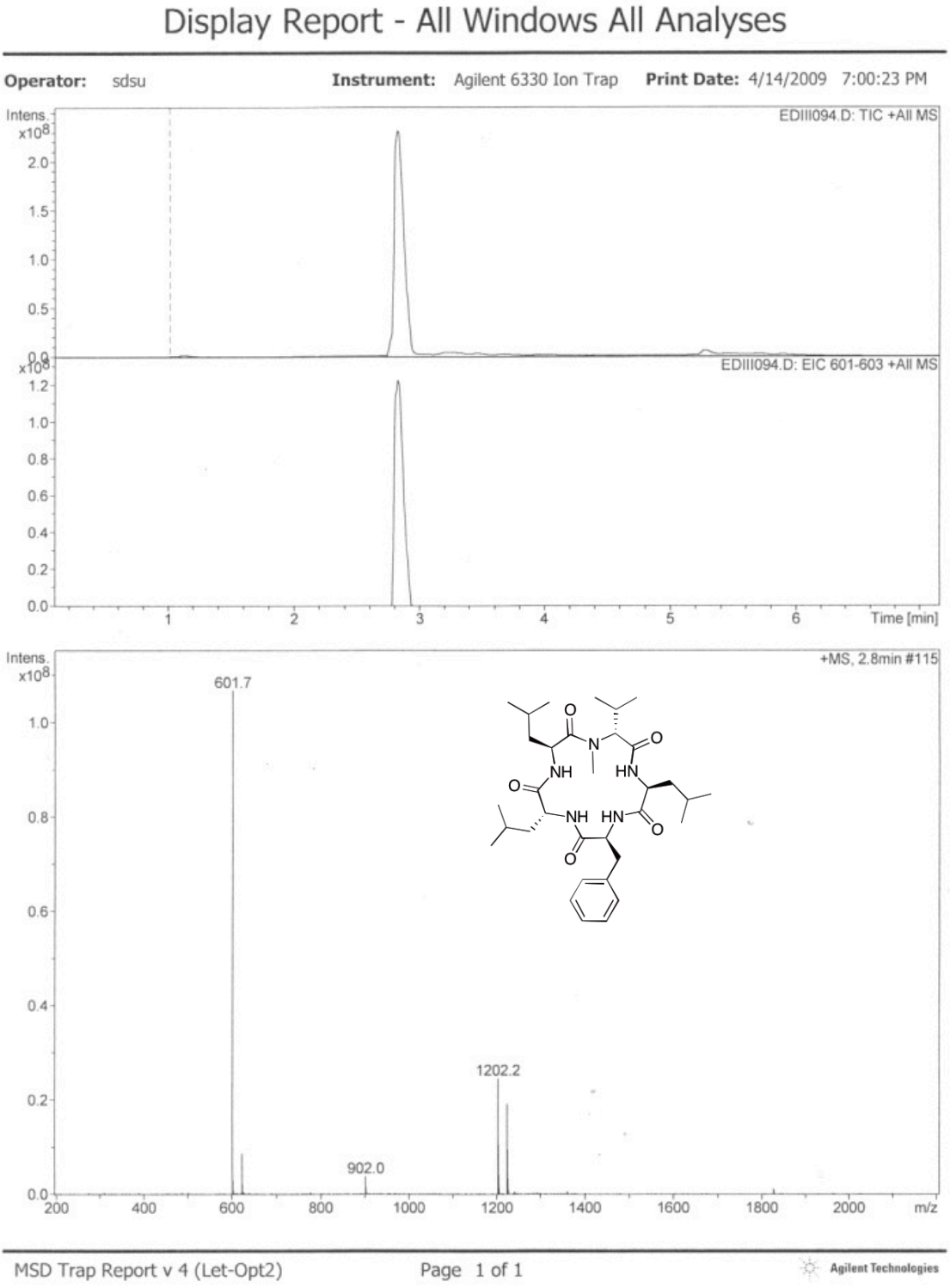
^{1}H NMR MeO-Leu-D-Leu-Boc



^{1}H NMR MeO-Phe-Leu-*N*-Me-D-Val-Leu-D-Leu-Boc

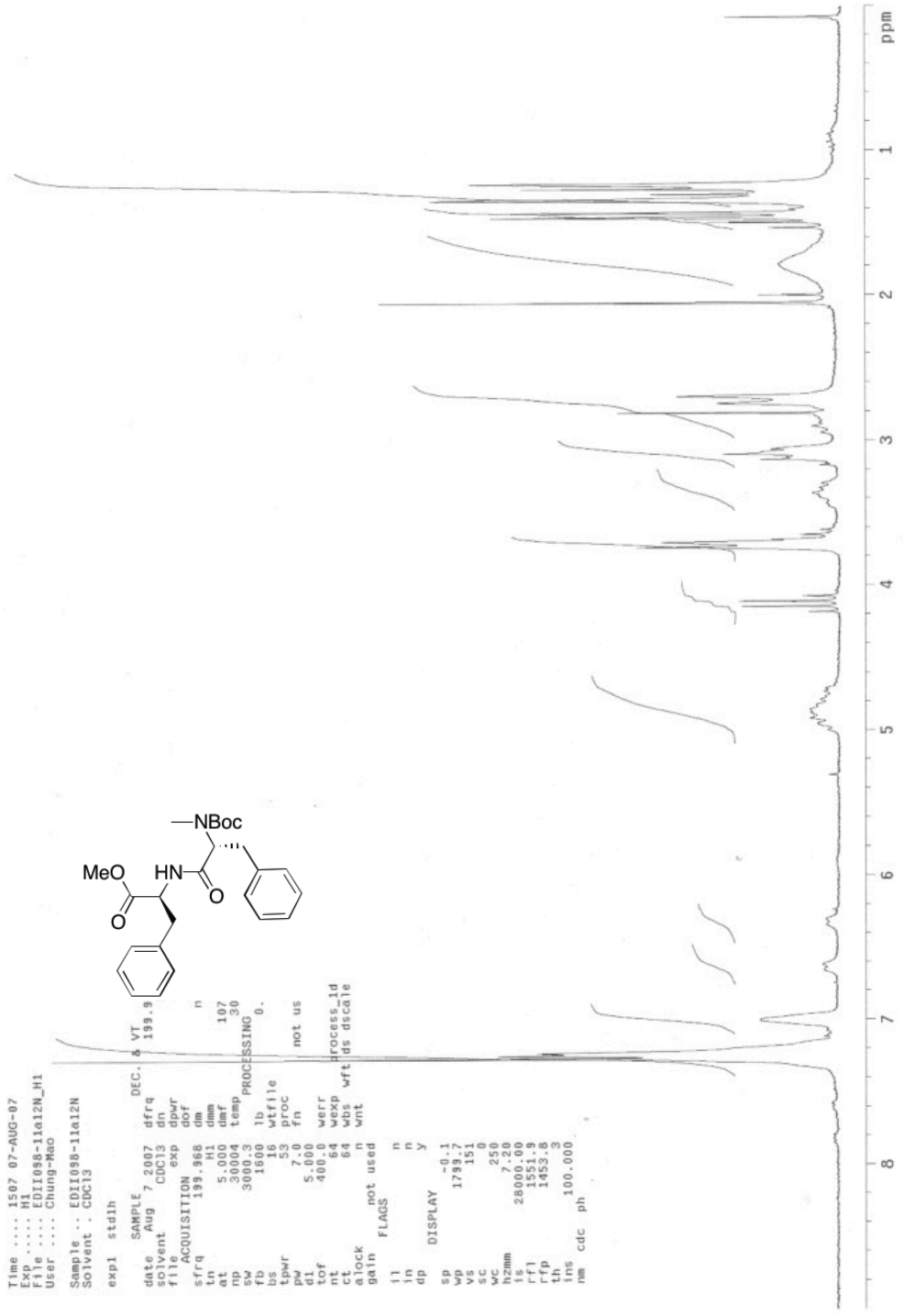


^{1}H NMR *Cyclo*-Phe-Leu-*N*-Me-D-Val-Leu-D-Leu

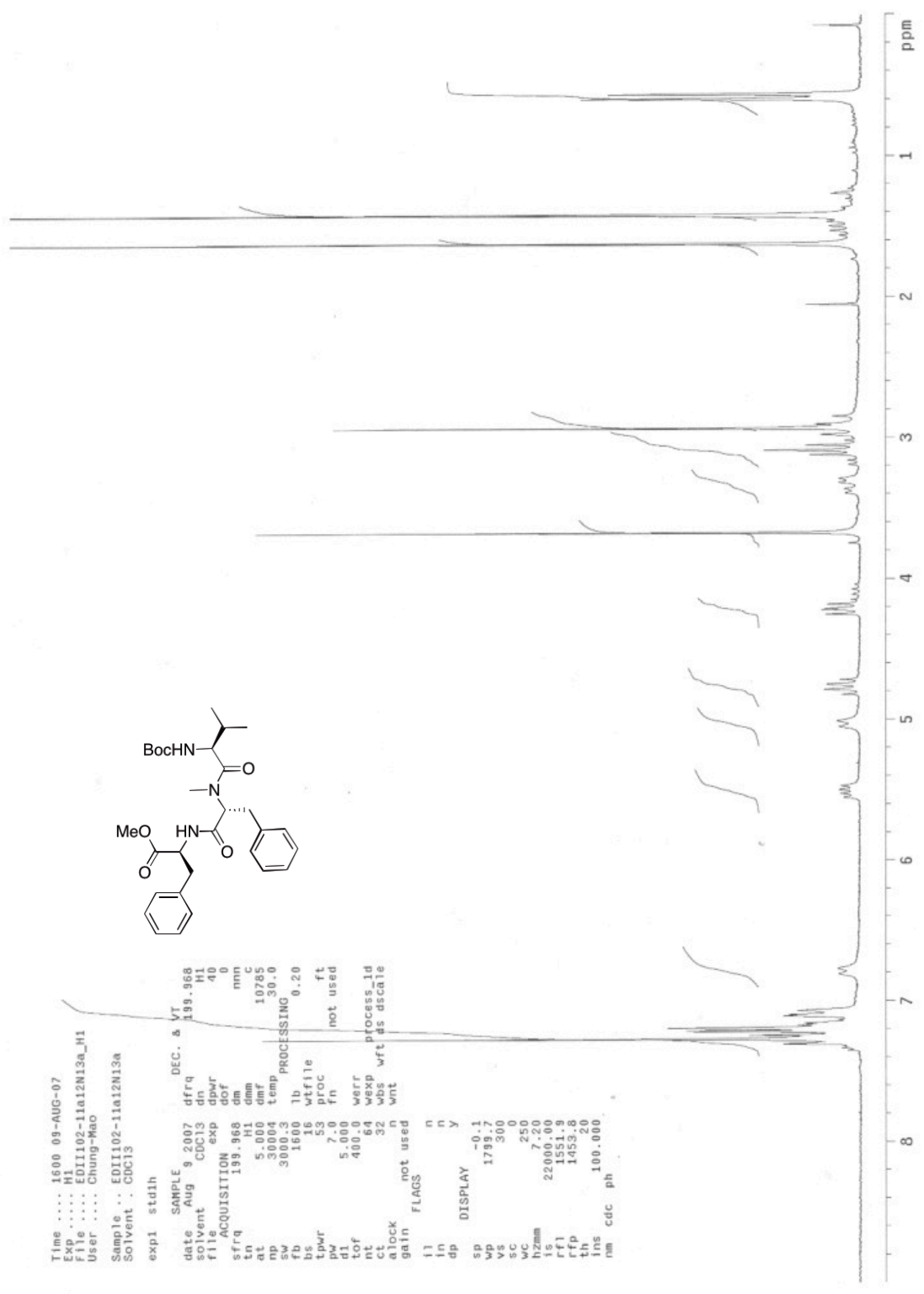


LC/MS *Cyclo*-Phe-Leu-*N*-Me-D-Val-Leu-D-Leu

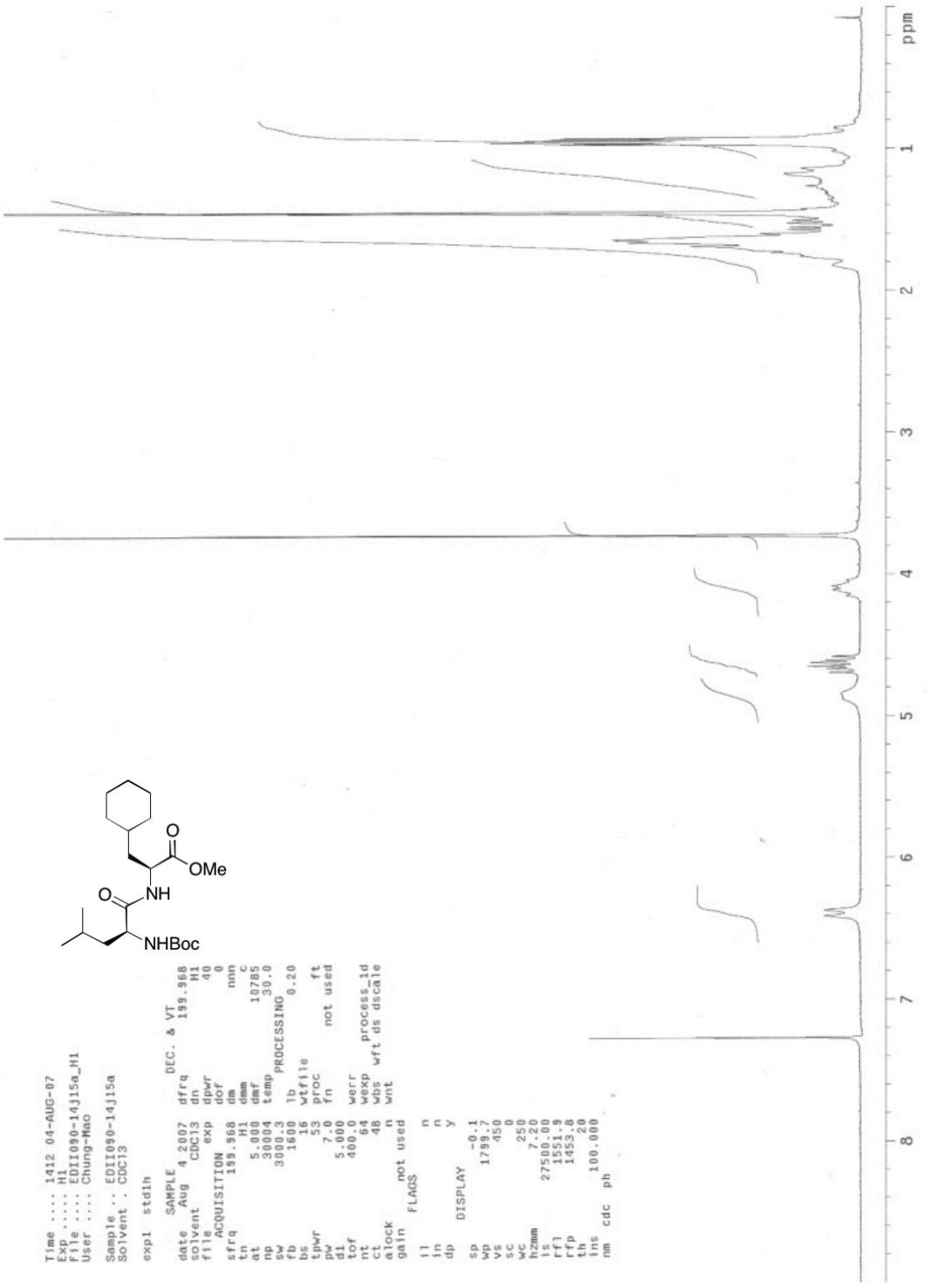
5.5.6 Supporting Spectra for Chapter 2 (SanA-6)



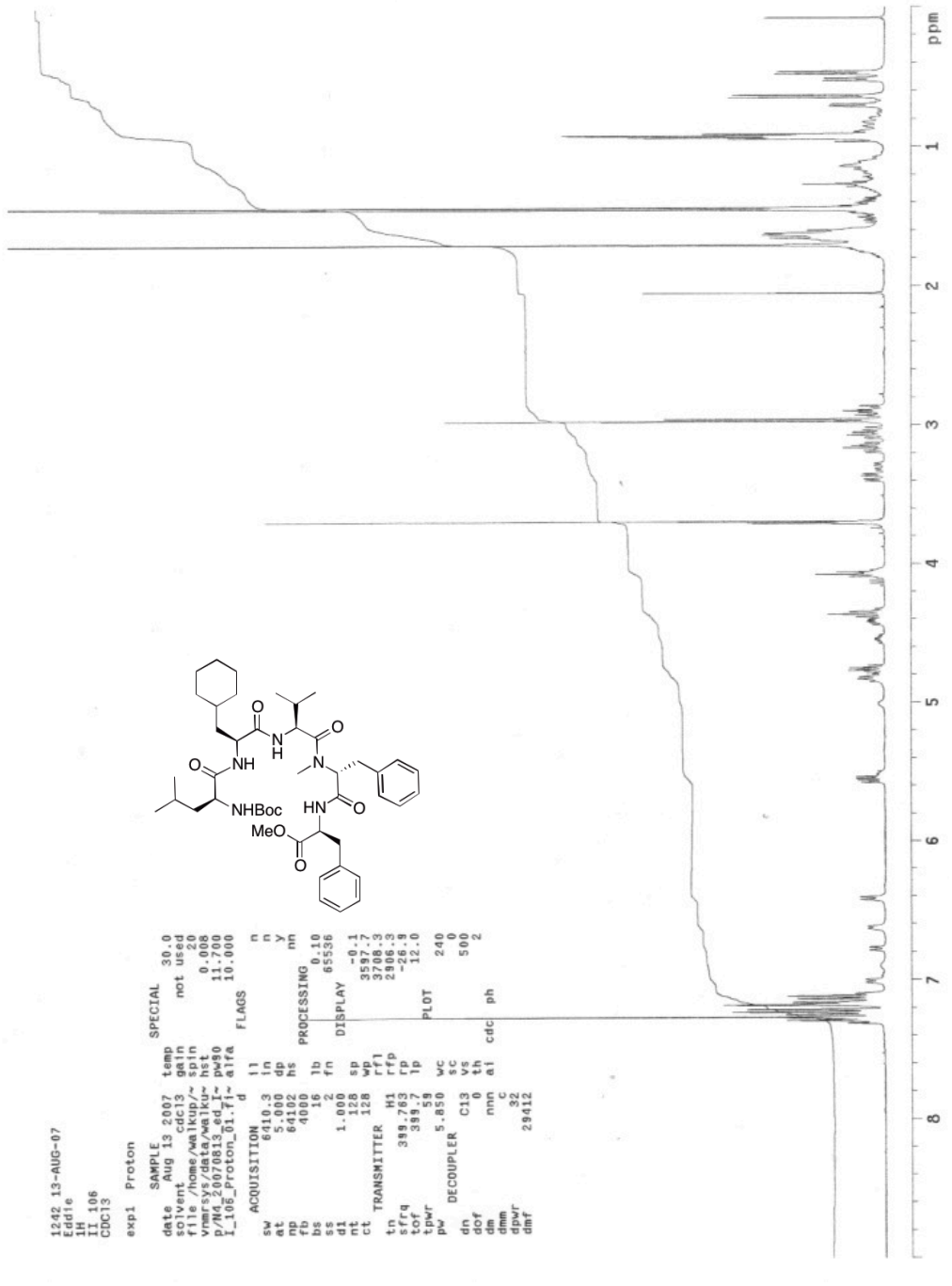
^{1}H NMR MeO-Phe-*N*-Me-D-Phe-Boc



^{1}H NMR MeO-Phe-*N*-Me-D-Phe-Val-Boc



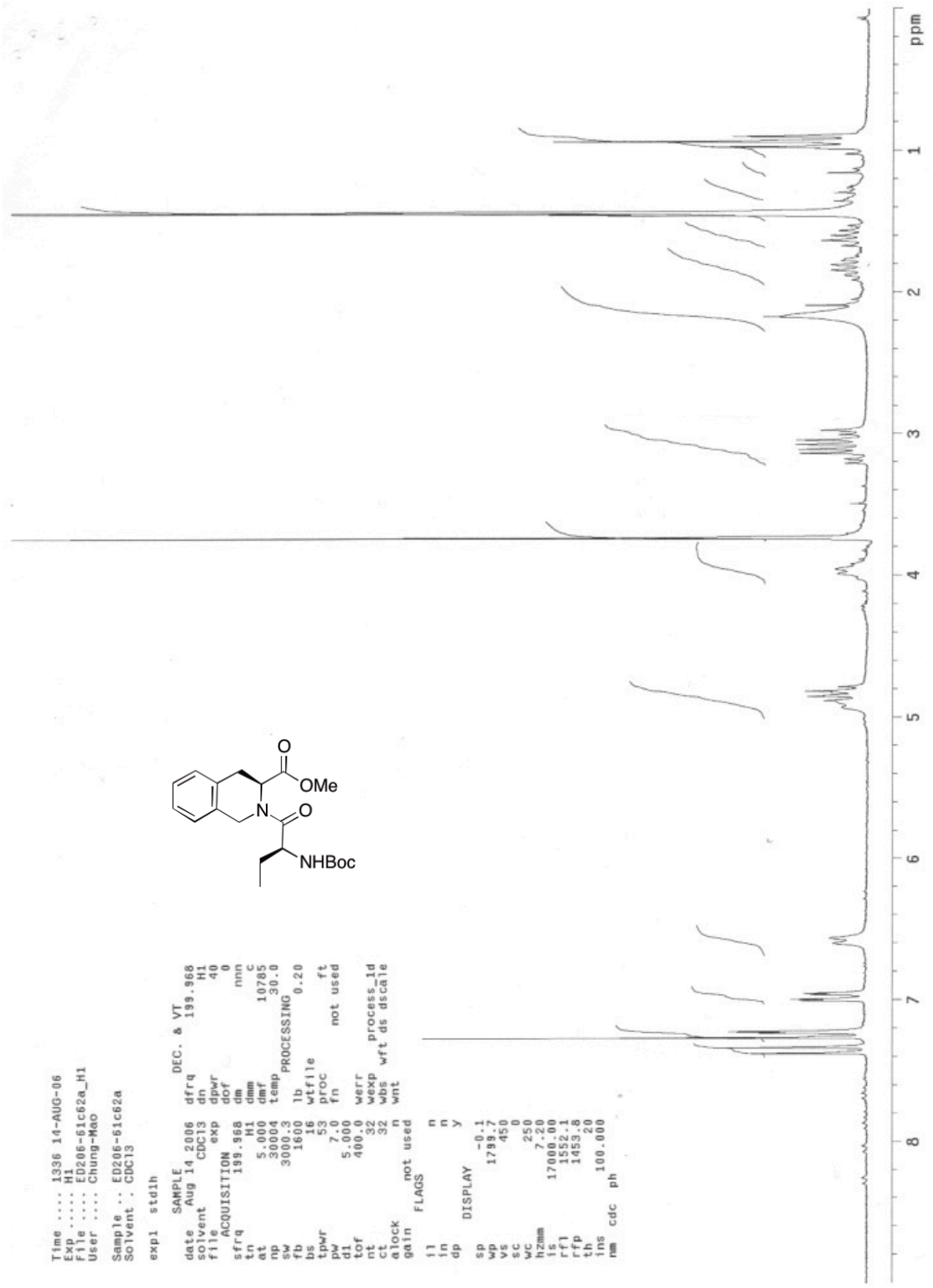
^{1}H NMR MeO-Leu-cyclohexylalanine-Boc



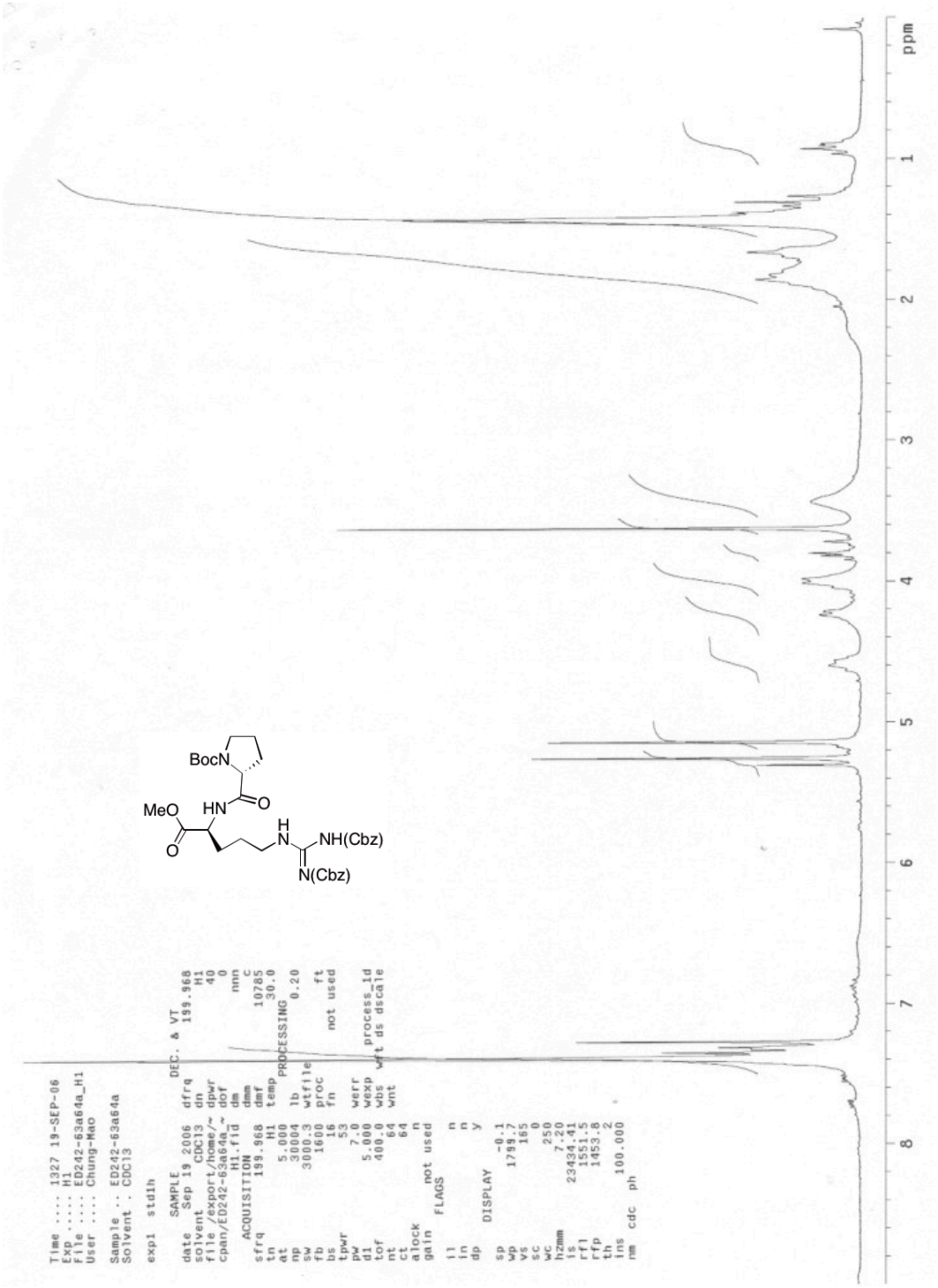
^{1}H NMR MeO-Phe-*N*-Me-D-Phe-Val-cyclohexylalanine-Leu-Boc

^{1}H NMR Cyclo-Phe-*N*-Me-D-Phe-Val-cyclohexylalanine-Leu

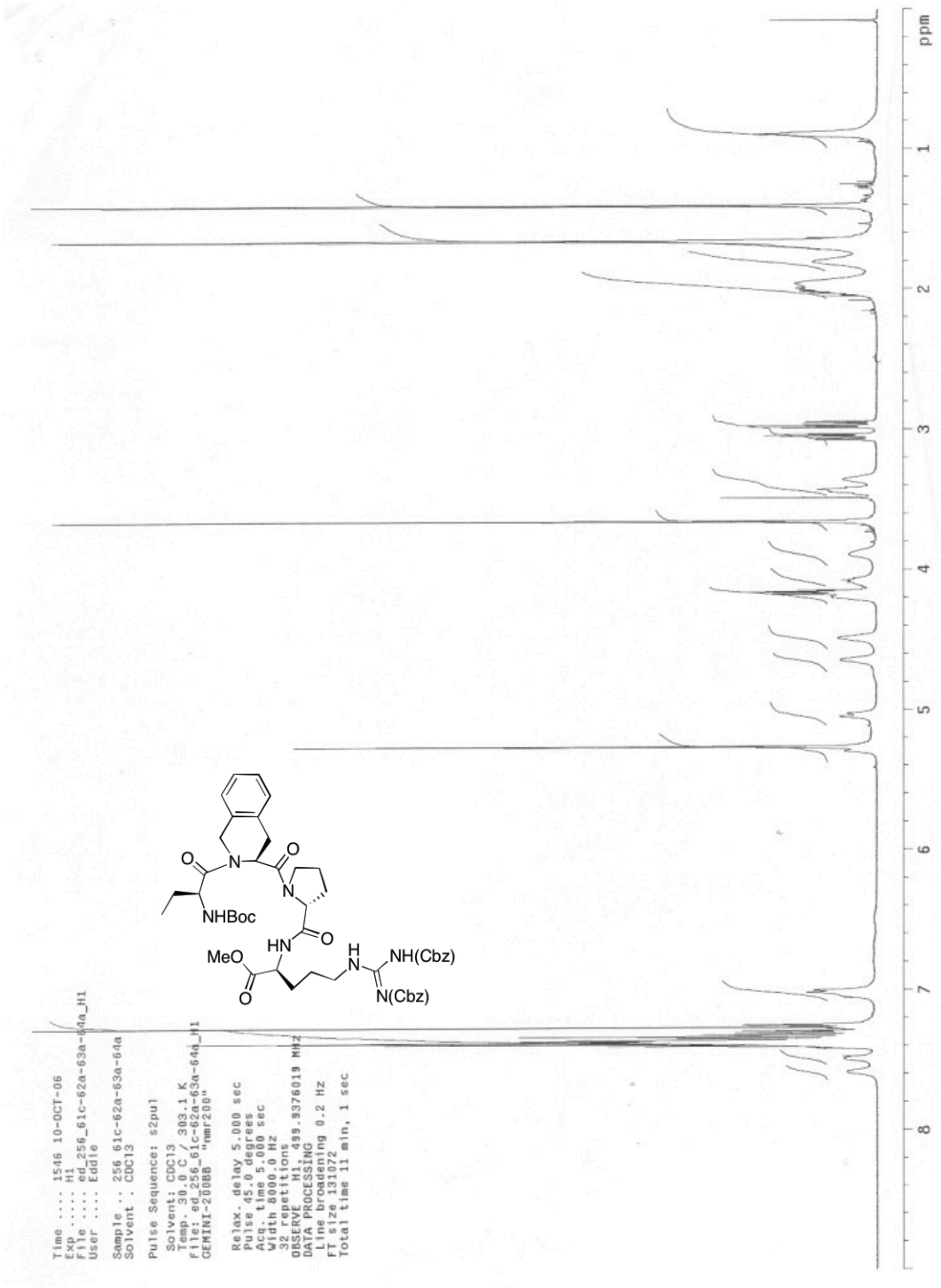
5.6 Supporting Spectra for Chapter 3



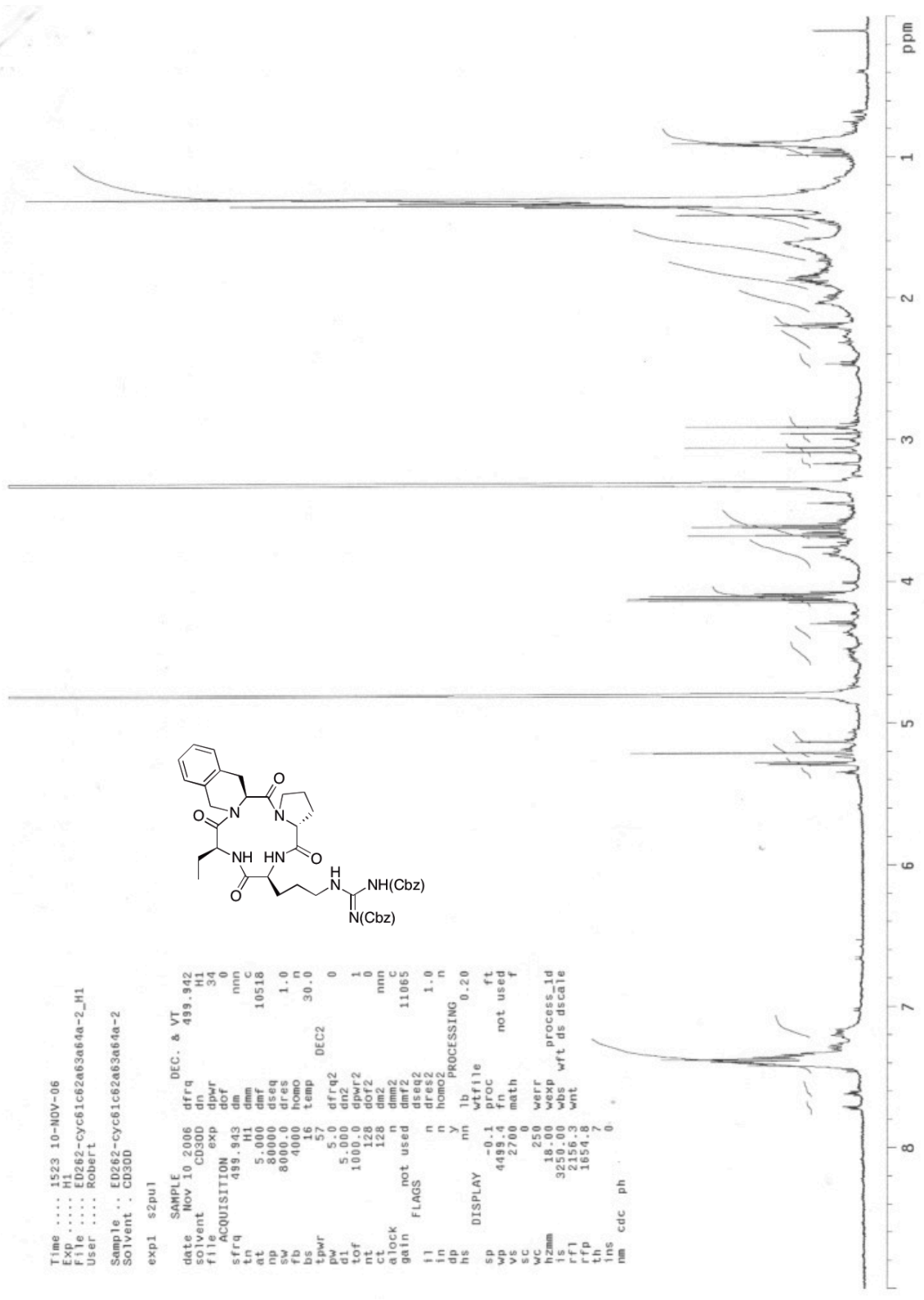
[1]H NMR MeO-1,2,3,4-tetrahydro-3-isoquinoline-Abu-Boc



1H NMR MeO-Arg(Cbz)-D-Pro-Boc

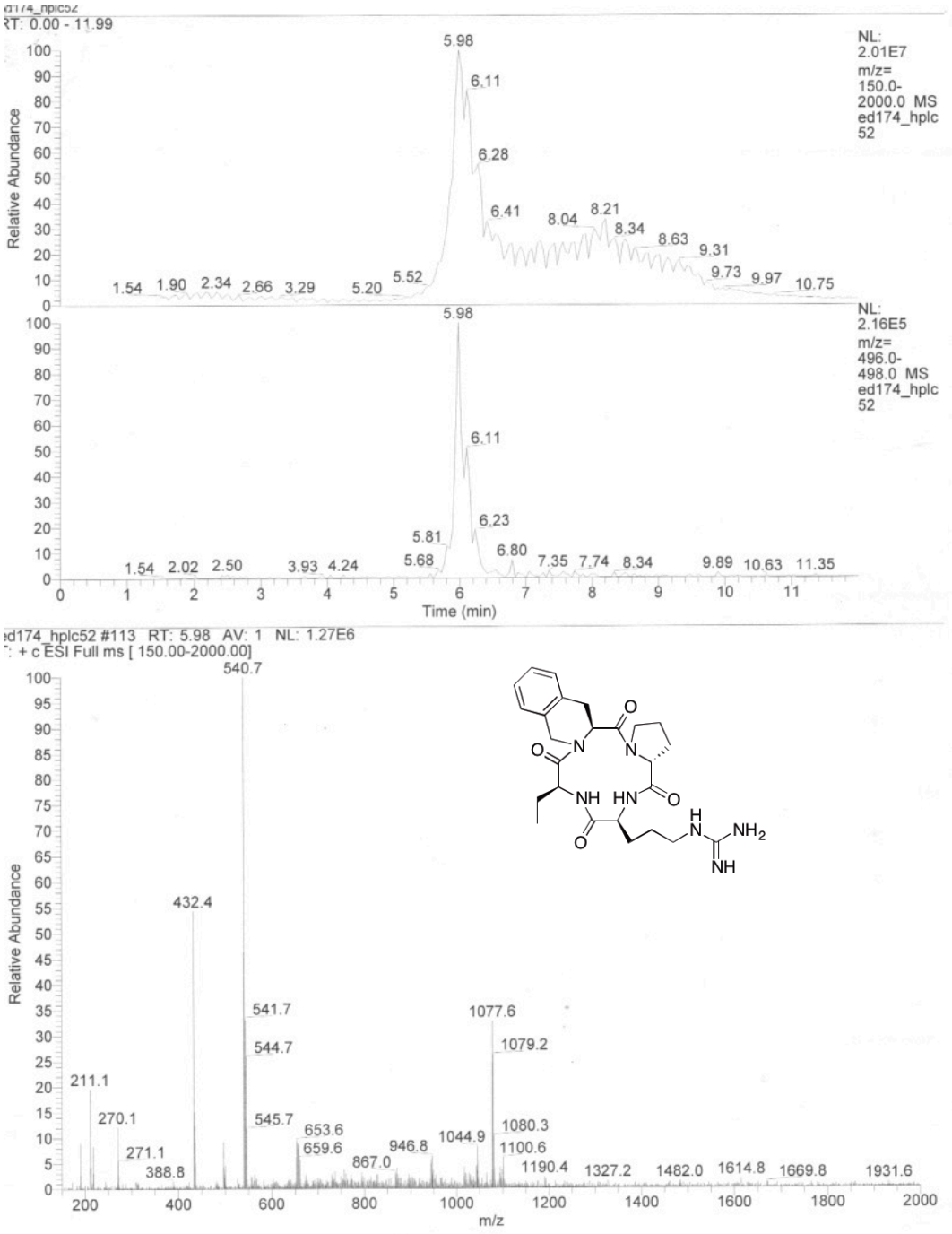


[1]H-NMR MeO-1,2,3,4-tetrahydro-3-isoquinoline-Abu-D-Pro-Arg(Cbz)-Boc



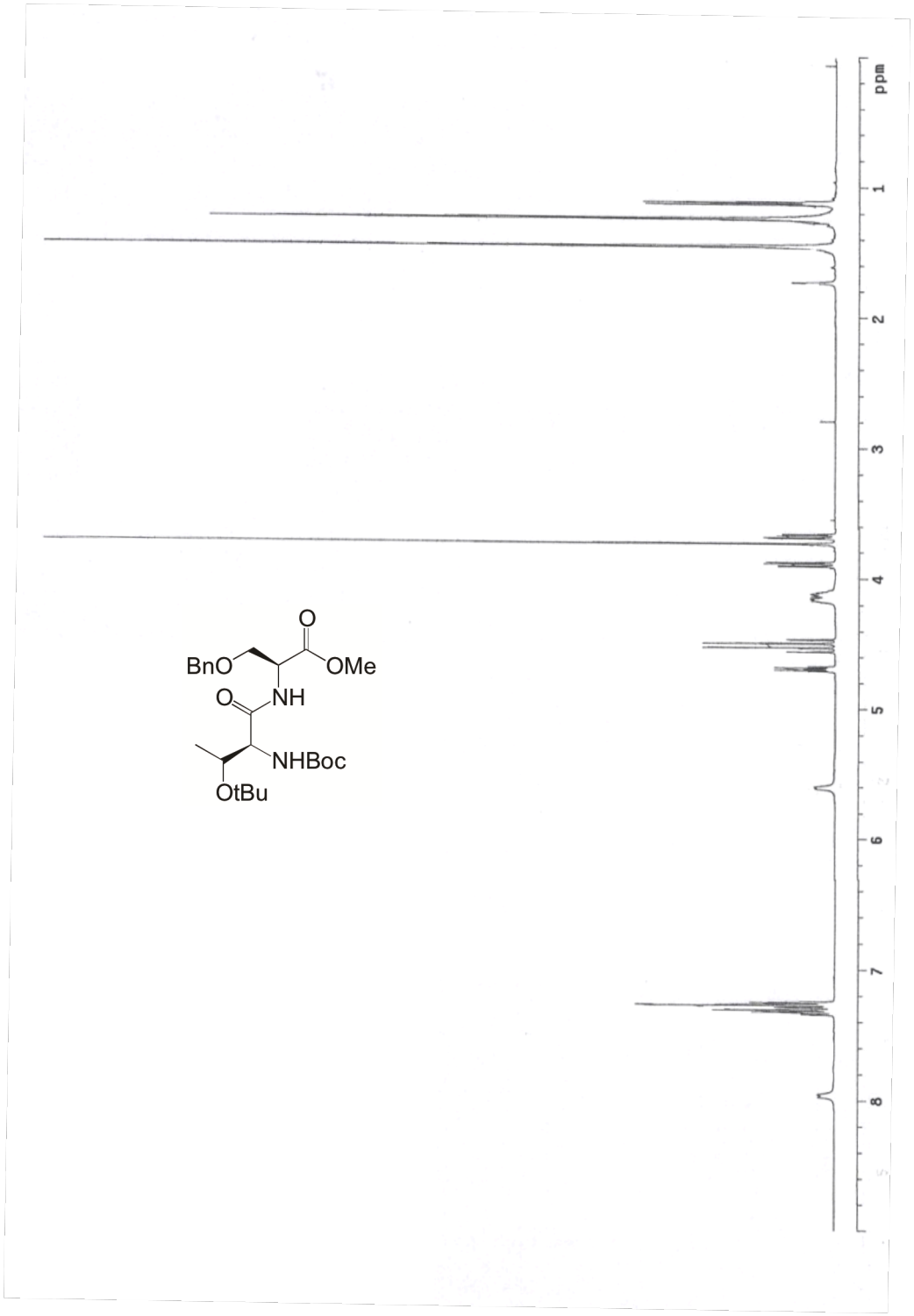
^{1}H NMR *Cyclo*-1,2,3,4-tetrahydro-3-isoquinoline-Abu-D-Pro-Arg(Cbz)

^{1}H NMR *Cyclo*-1,2,3,4-tetrahydro-3-isoquinoline-Abu-D-Pro-Arg

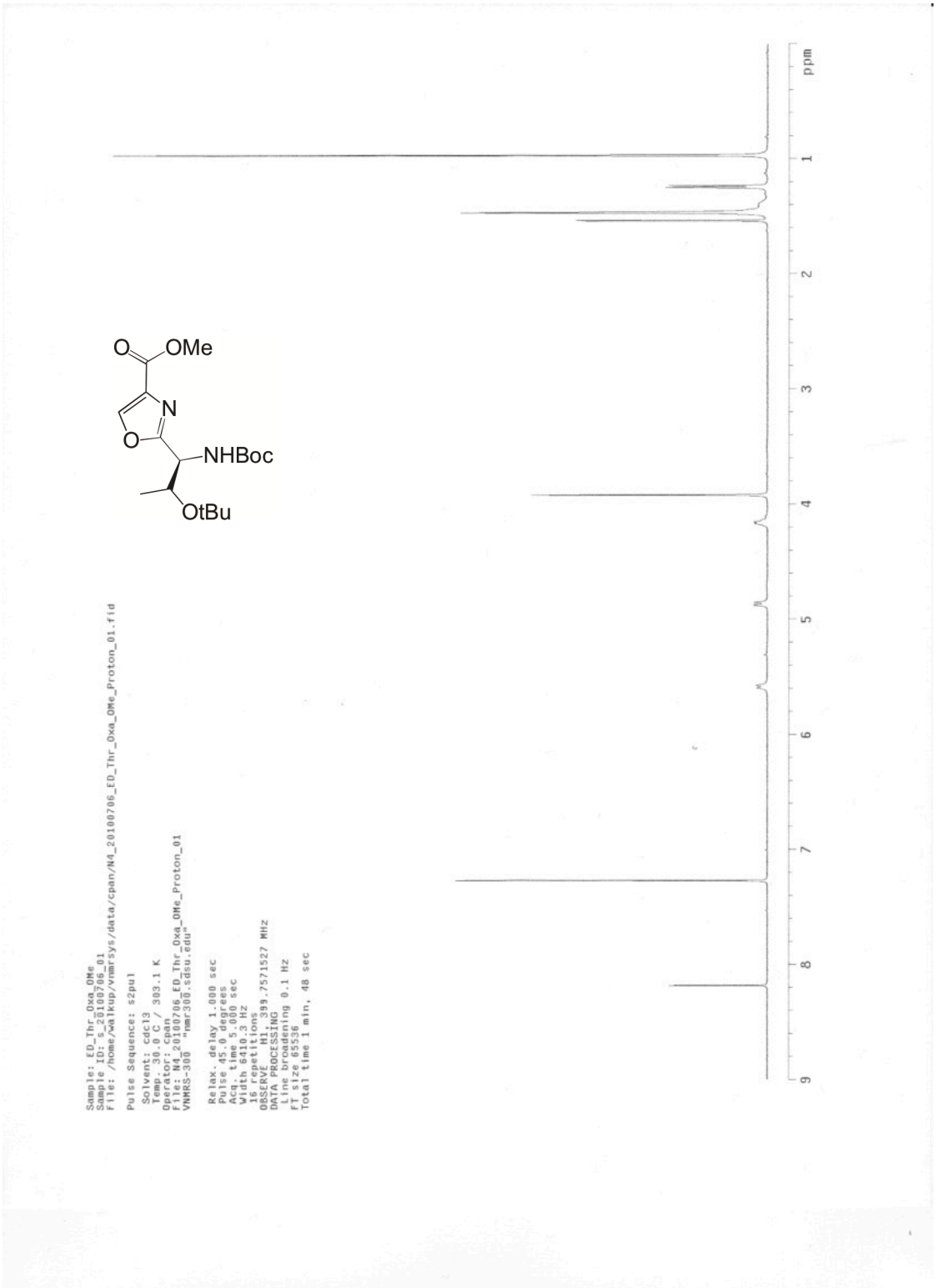


LC/MS *Cyclo*-1,2,3,4-tetrahydro-3-isoquinoline-Abu-D-Pro-Arg

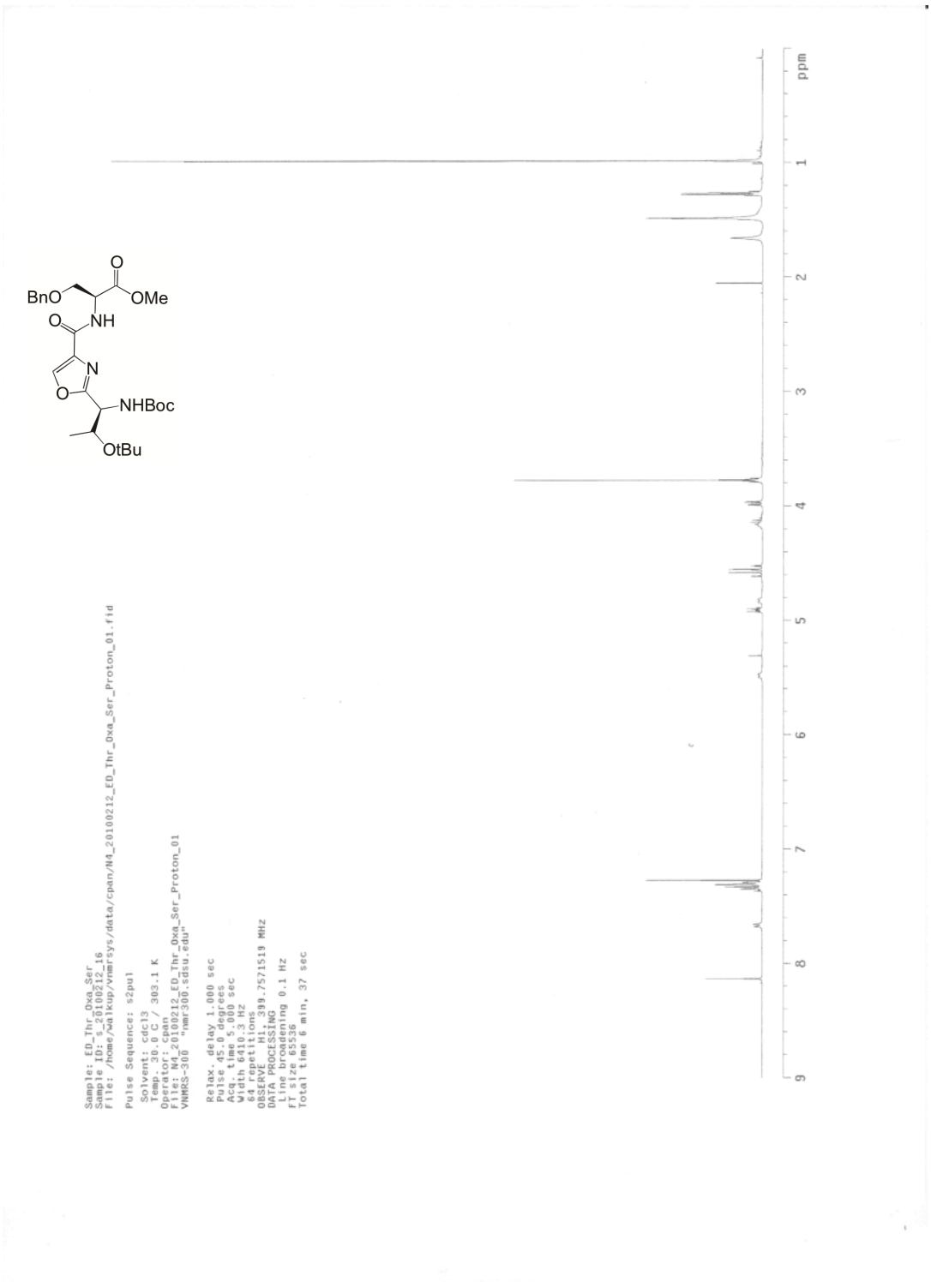
5.7 Supporting Spectra for Chapter 4



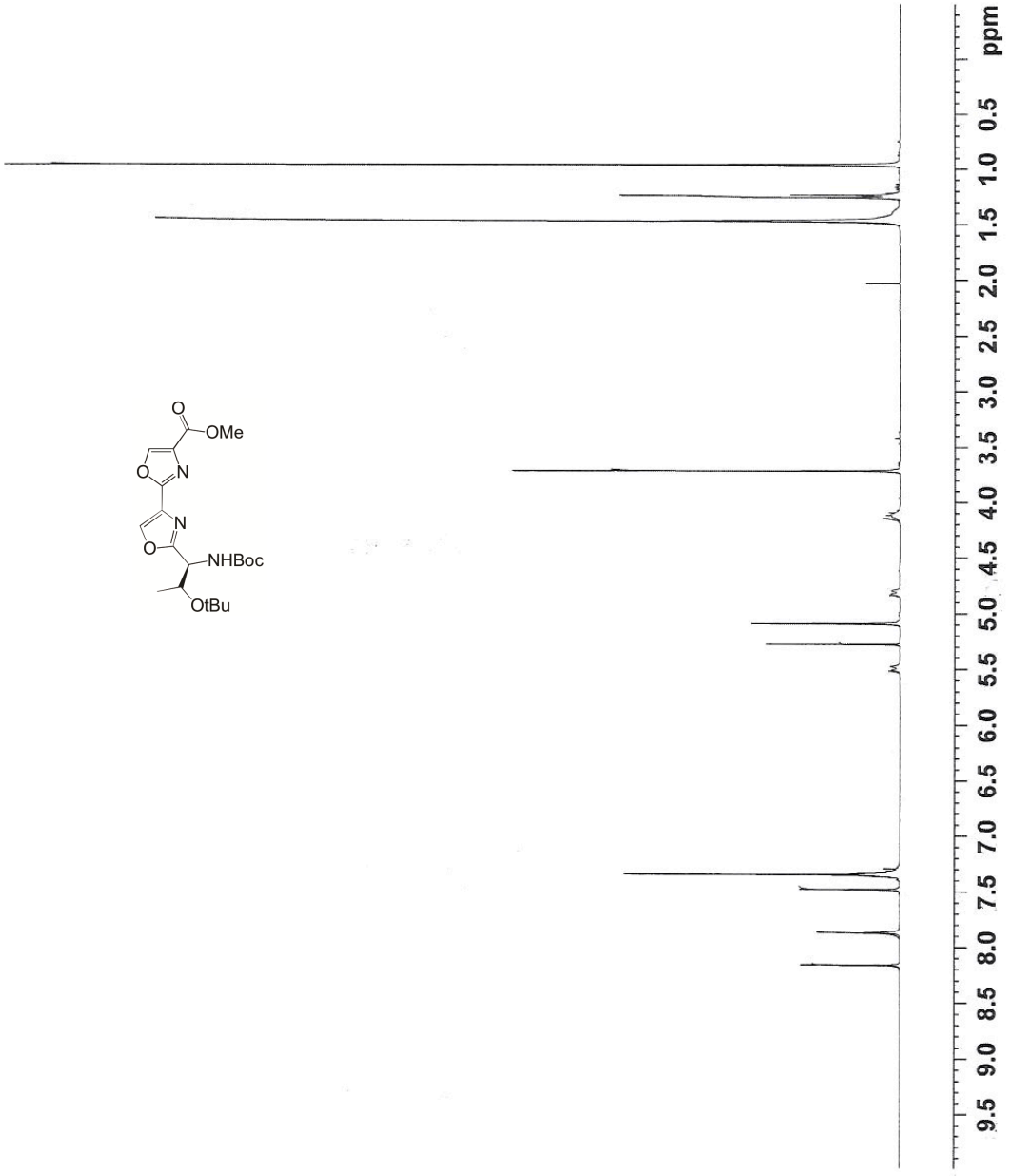
^{1}H NMR MeO-Ser(OBn)-Thr(O^{t}Bu)-Boc



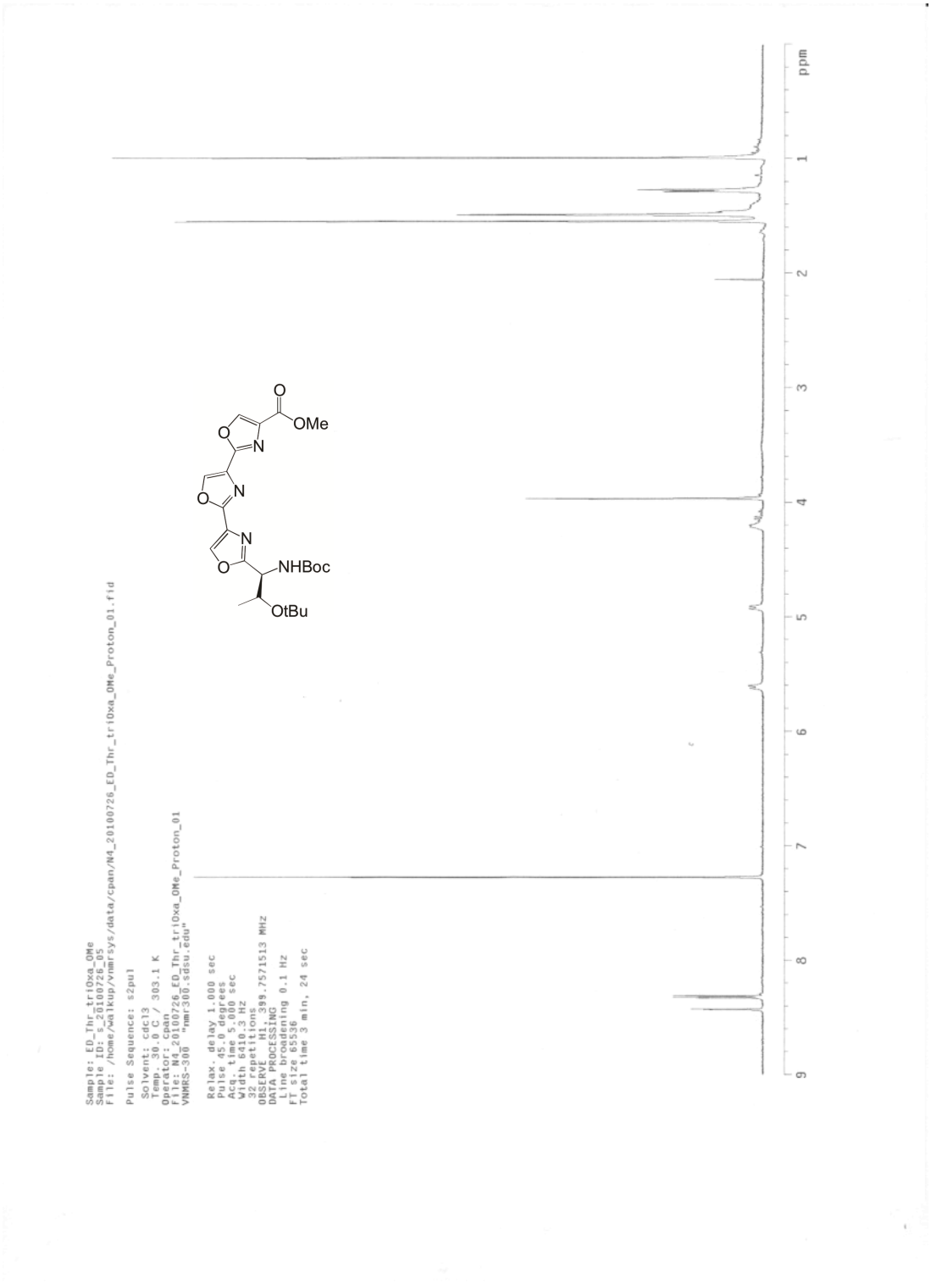
^{1}H NMR MeO-Ser(OBn)-Oxa-Boc



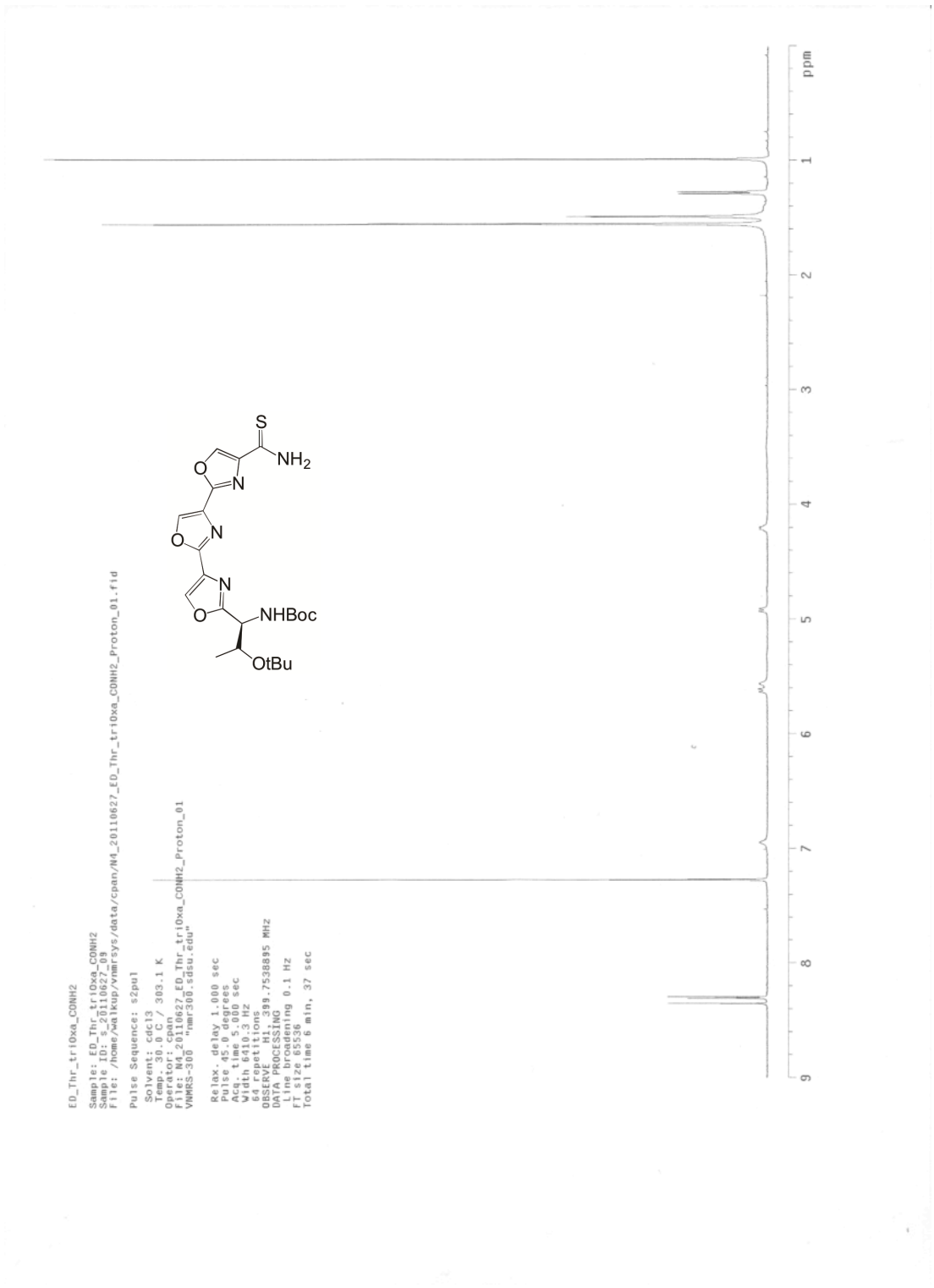
^{1}H NMR MeO-Ser(OBn)-Oxa-Thr(O^{t}Bu)-Boc



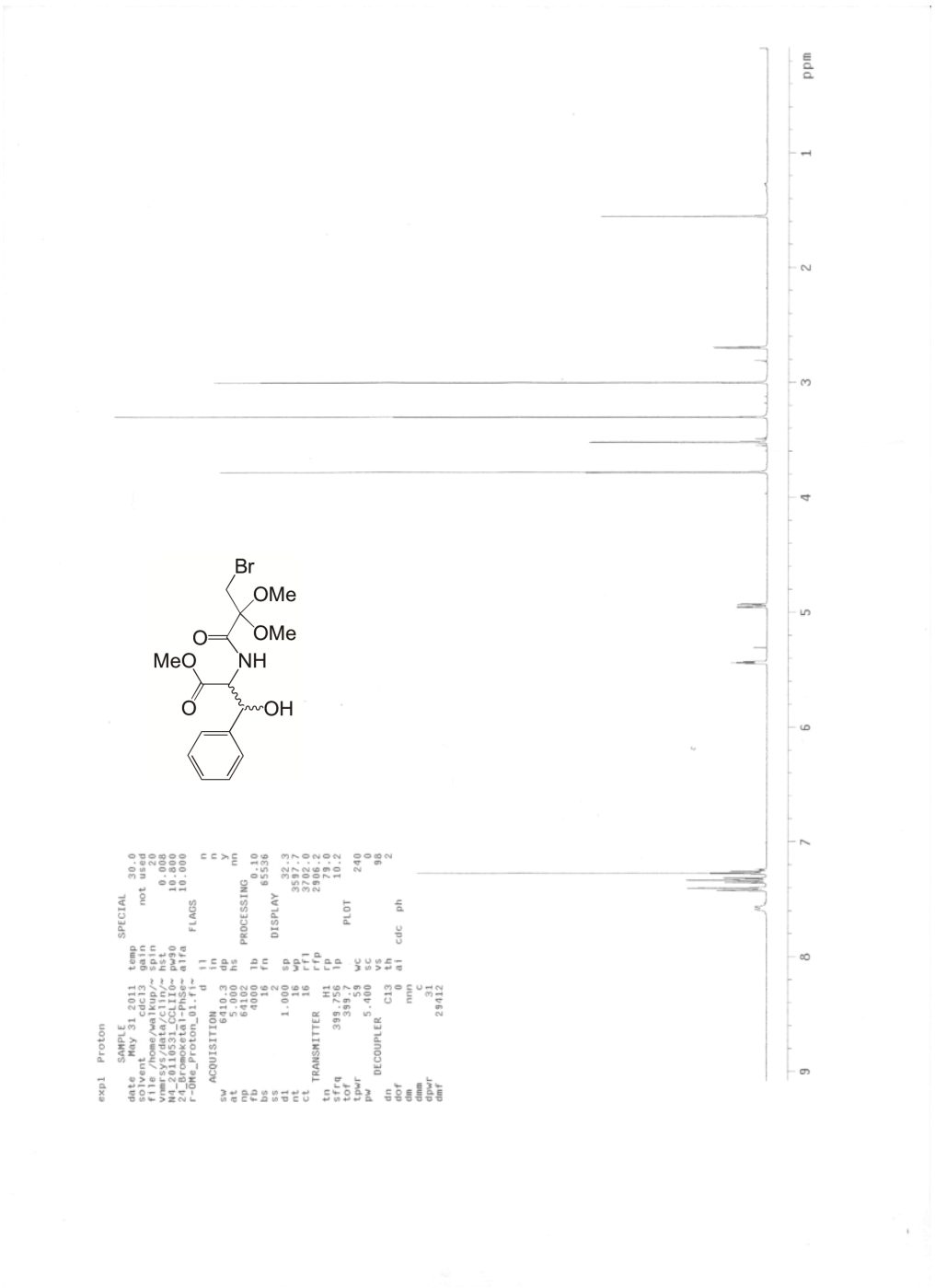
1H NMR MeO-Ser(OBn)-diOxa-Thr(O^{t}Bu)-Boc



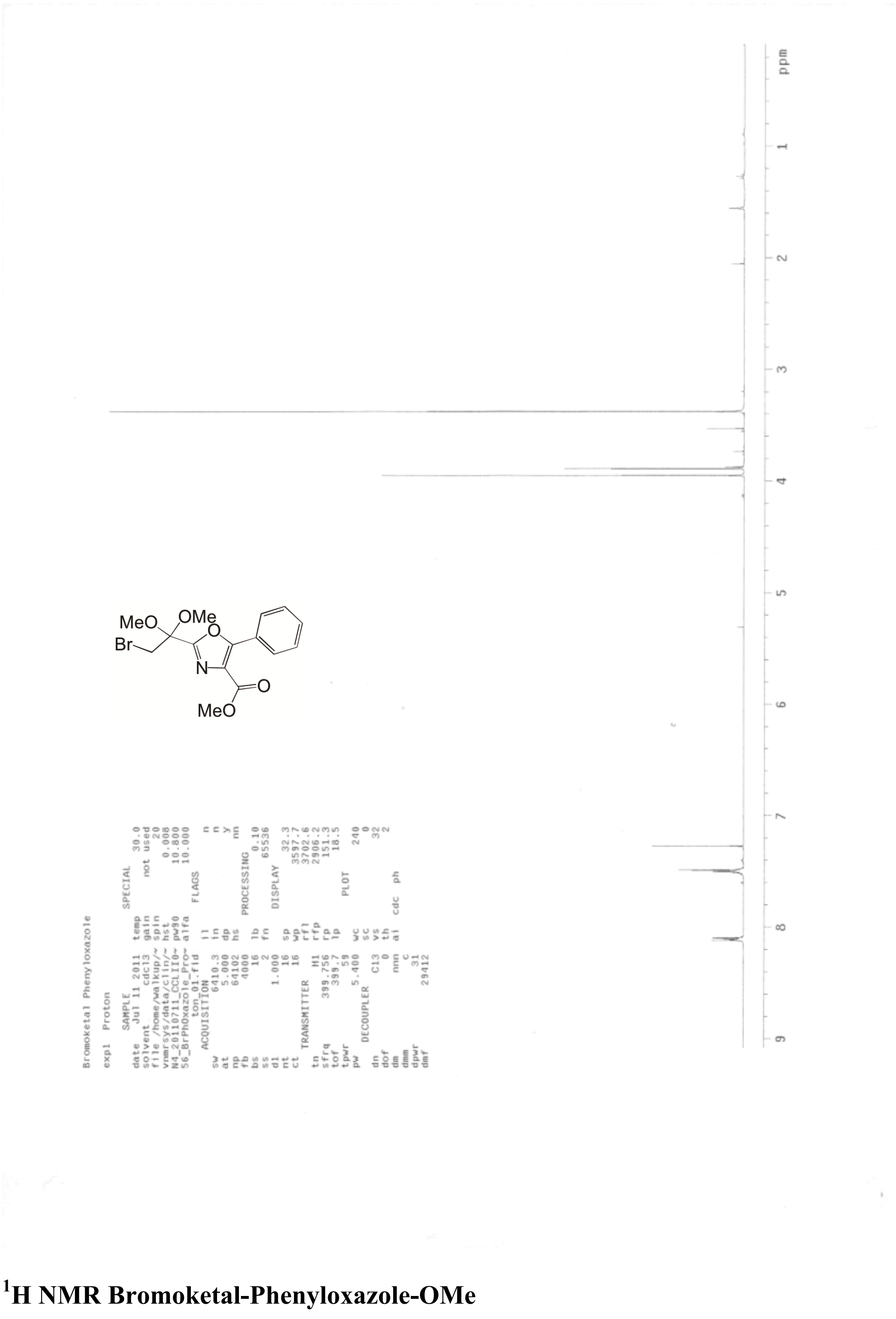
^{1}H NMR MeO-triOxa-Thr(O^{t}Bu)-Boc



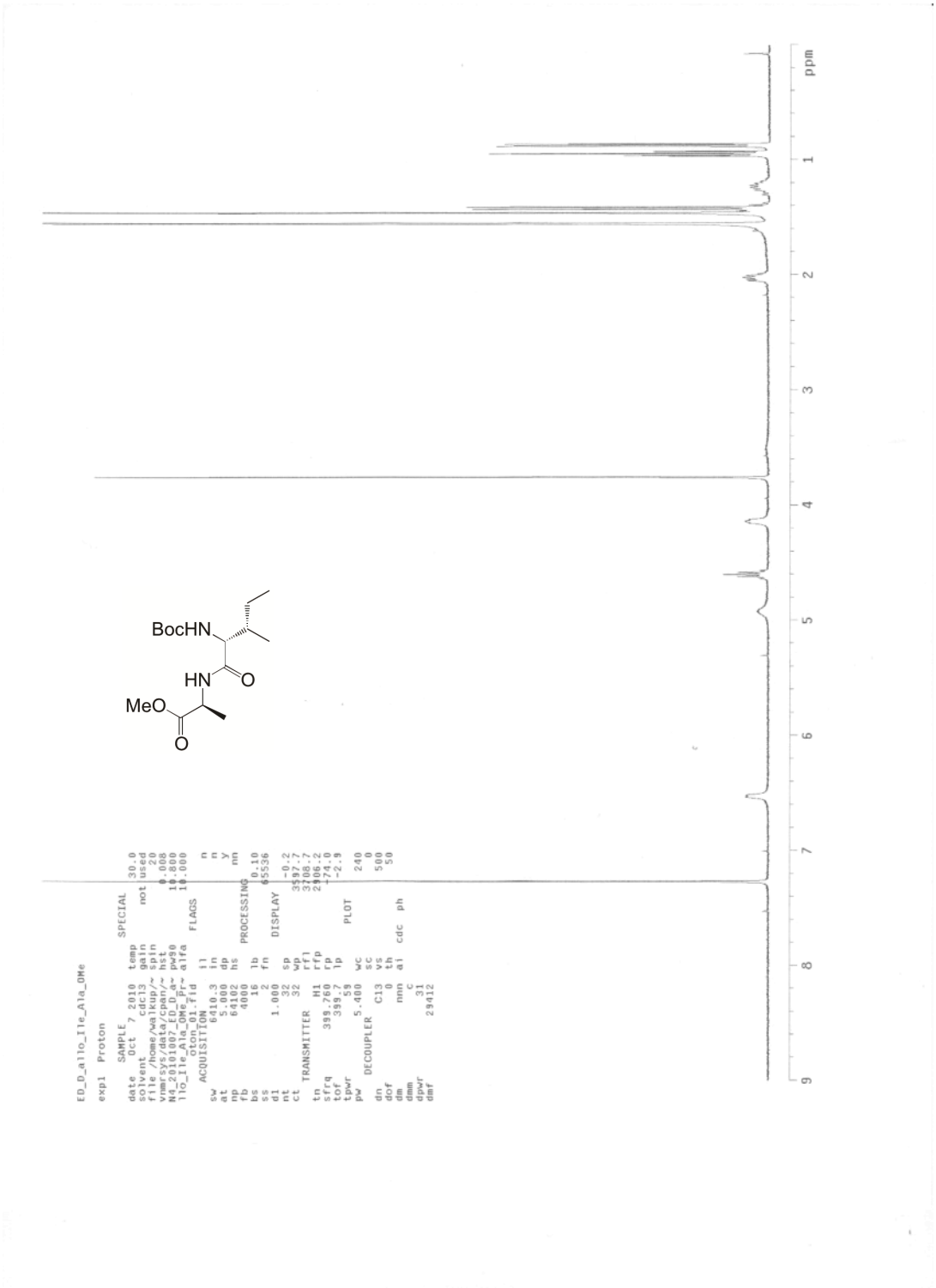
^{1}H NMR NH_2SC-triOxa-Thr($O^{t}Bu$)-Boc



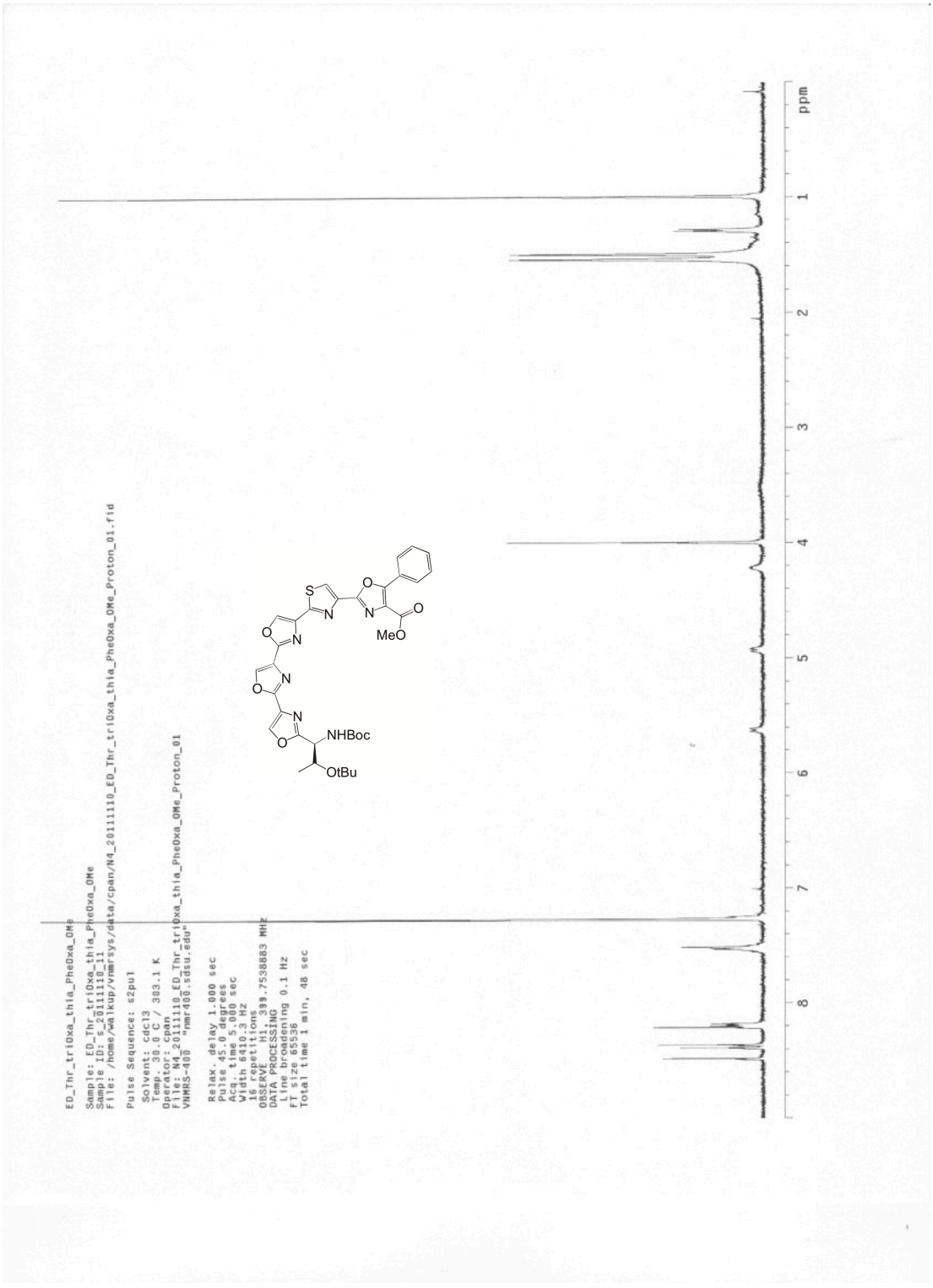
^{1}H NMR *Racemic*-Bromoketal-*β*-hydroxyl-Phe-OMe



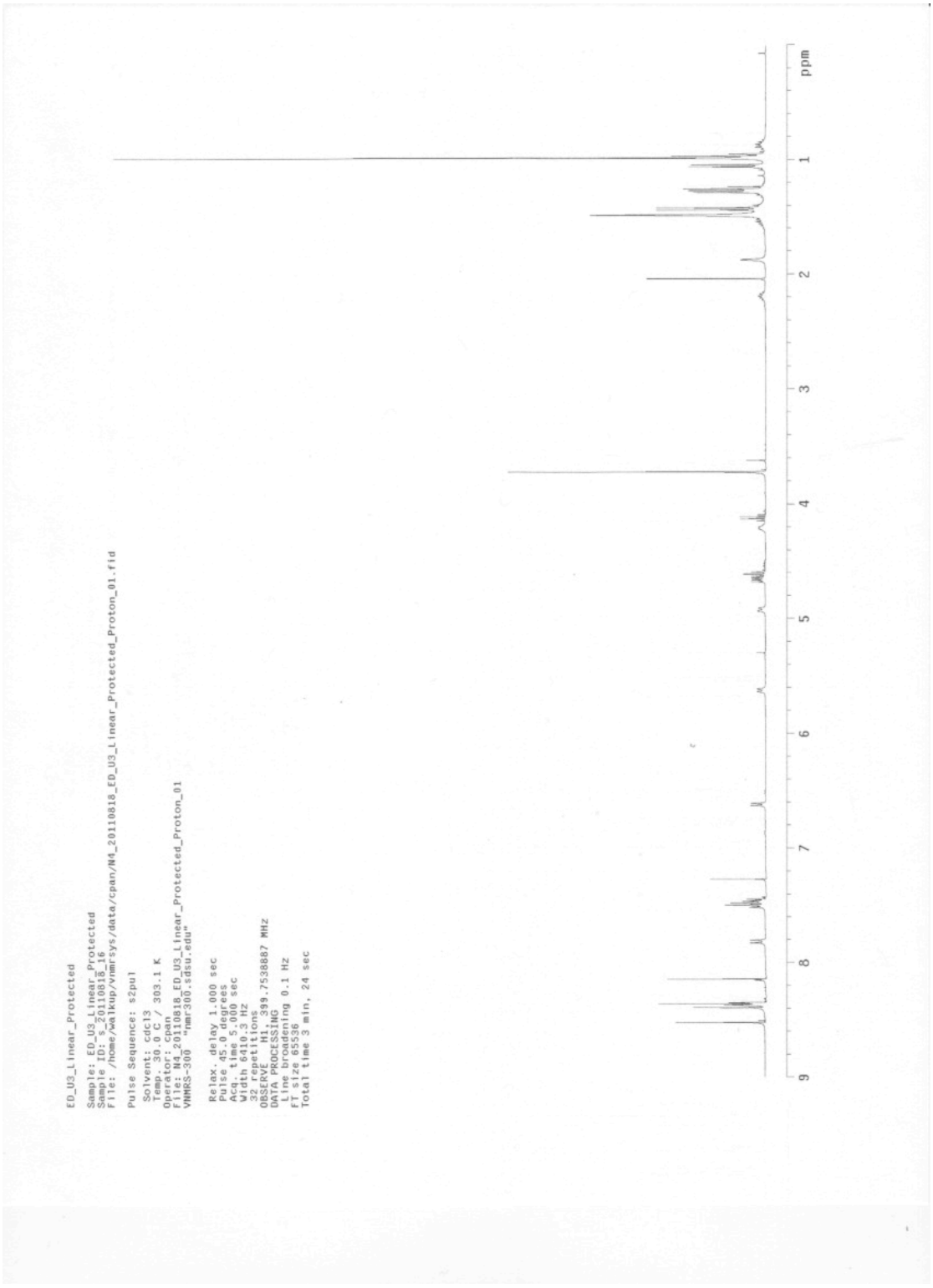
¹H NMR Bromoketal-Phenyloxazole-OMe



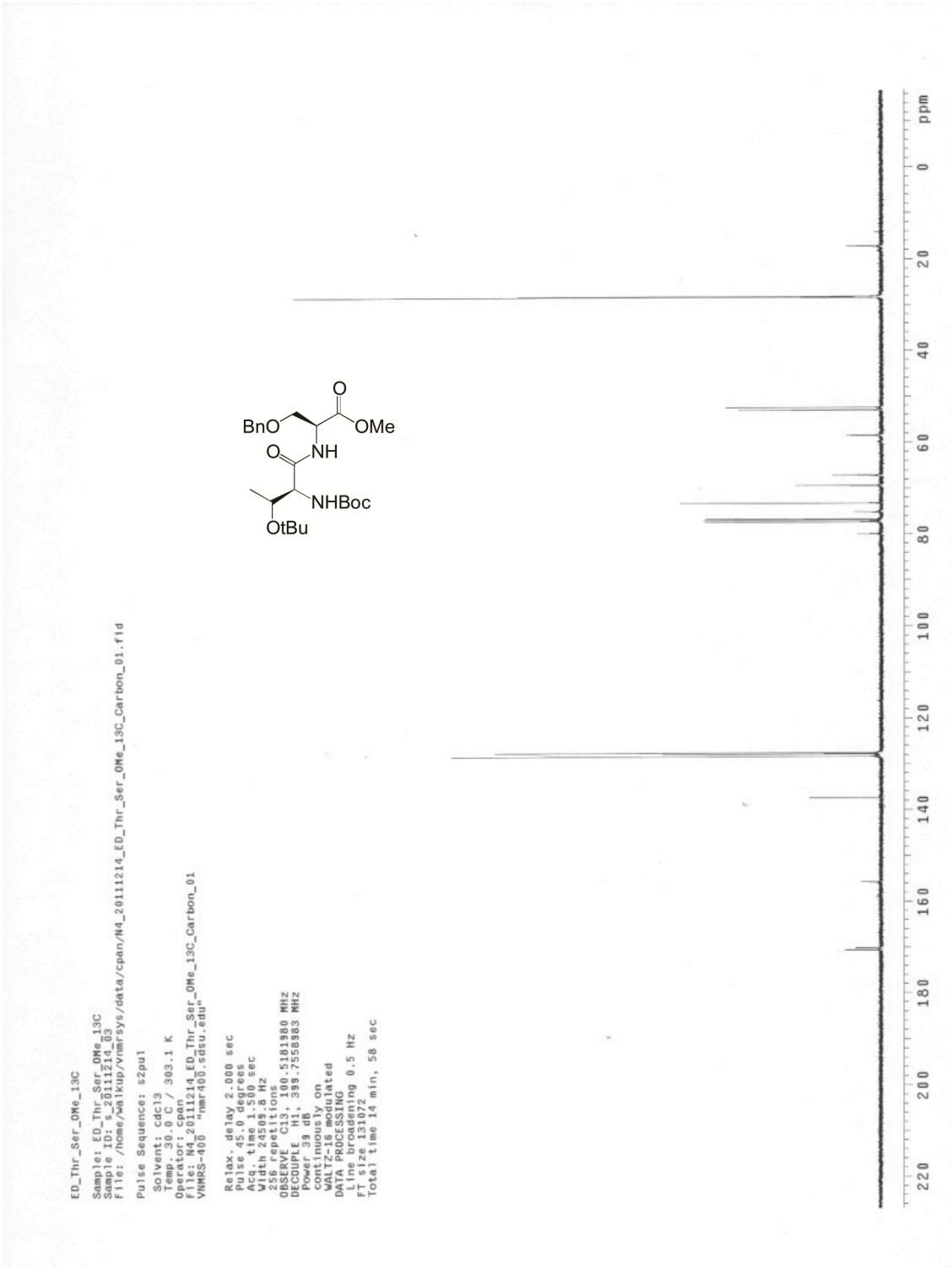
^{1}H NMR MeO-Ala-D-*allo*-Ile-Boc



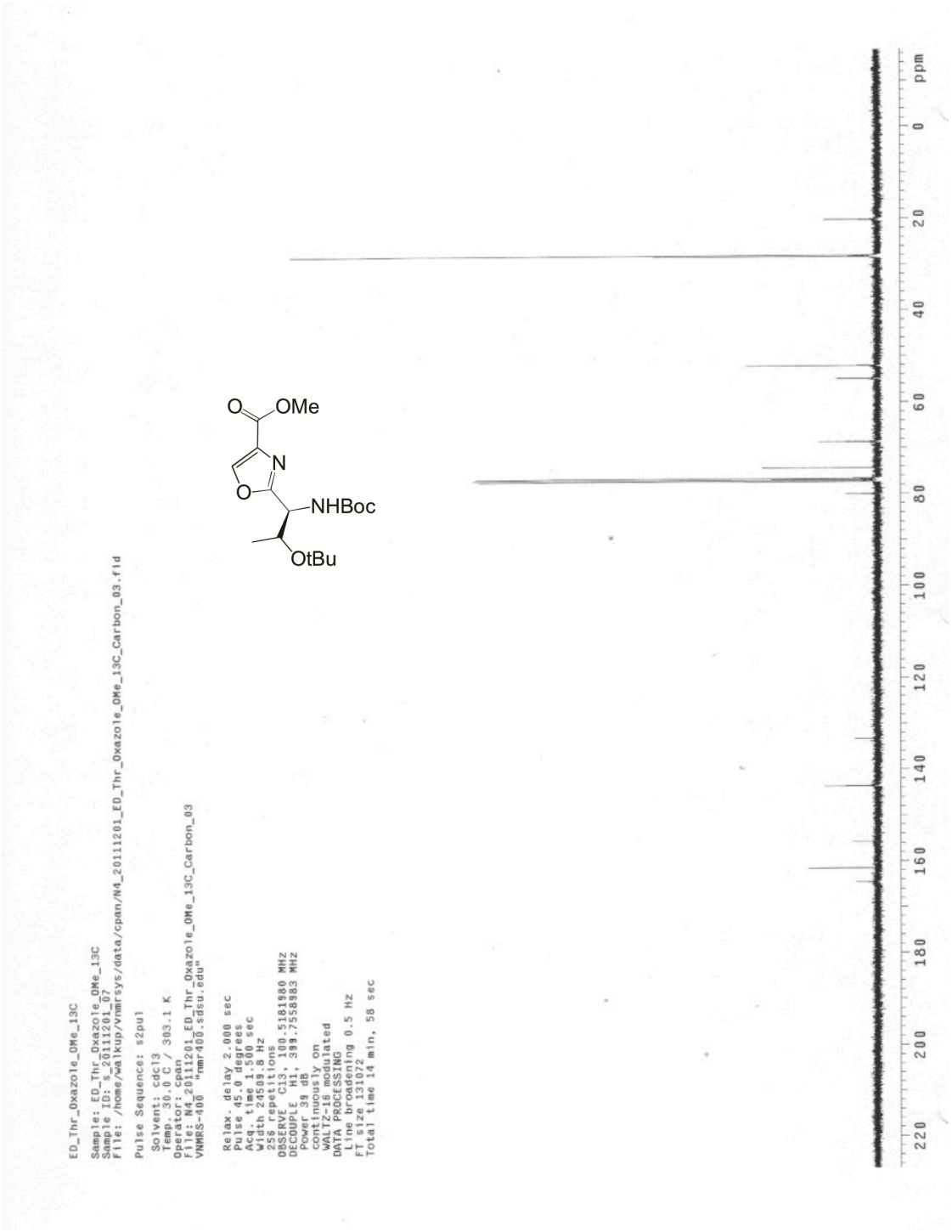
^{1}H NMR MeO-Phenyloxazole-Thiazole-triOxa-Thr(O^{t}Bu)-Boc



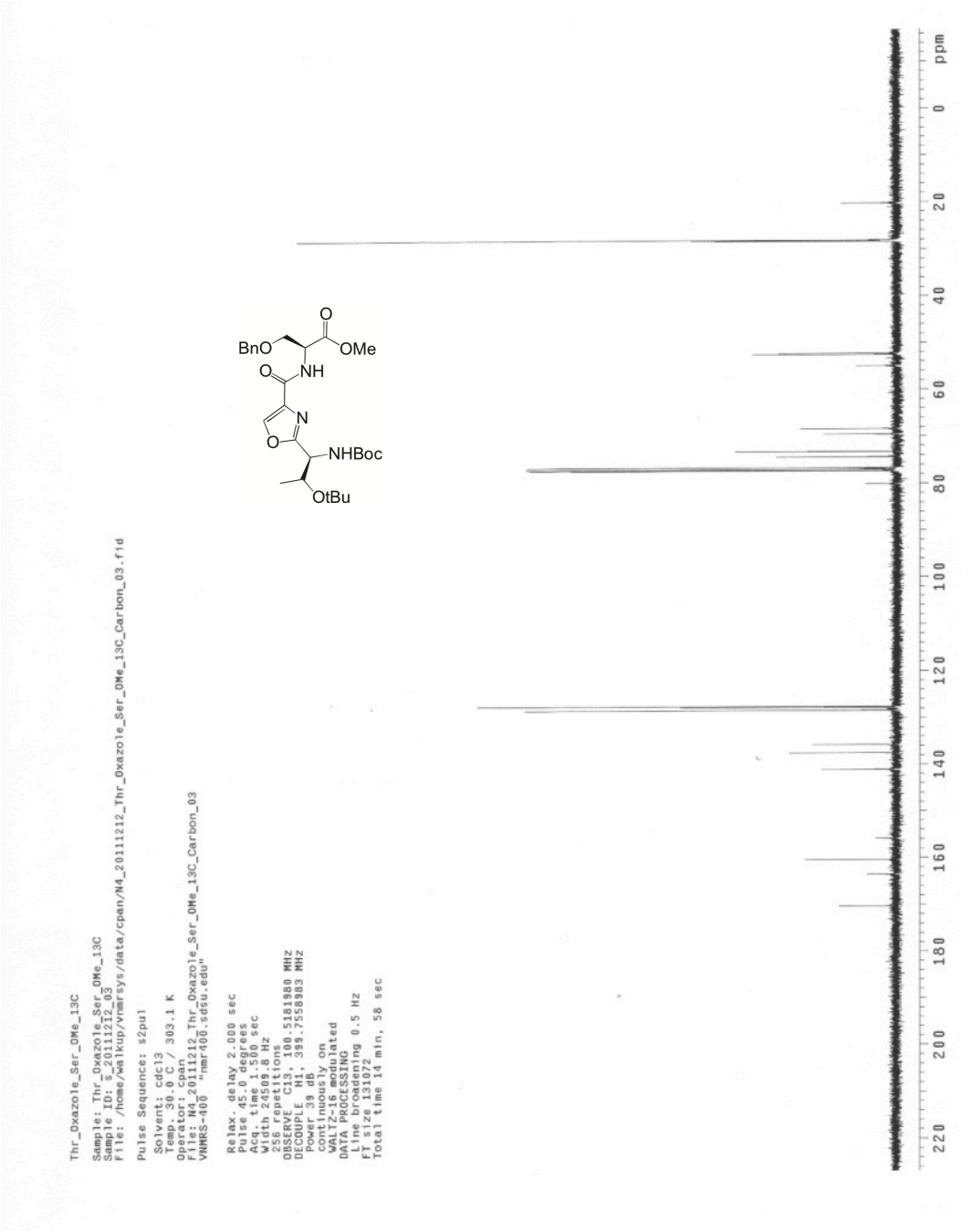
^{1}H NMR MeO-Ala-D-allo-Ile-Phenyloxazole-Thiazole-triOxa-Thr(O^{t}Bu)-Boc



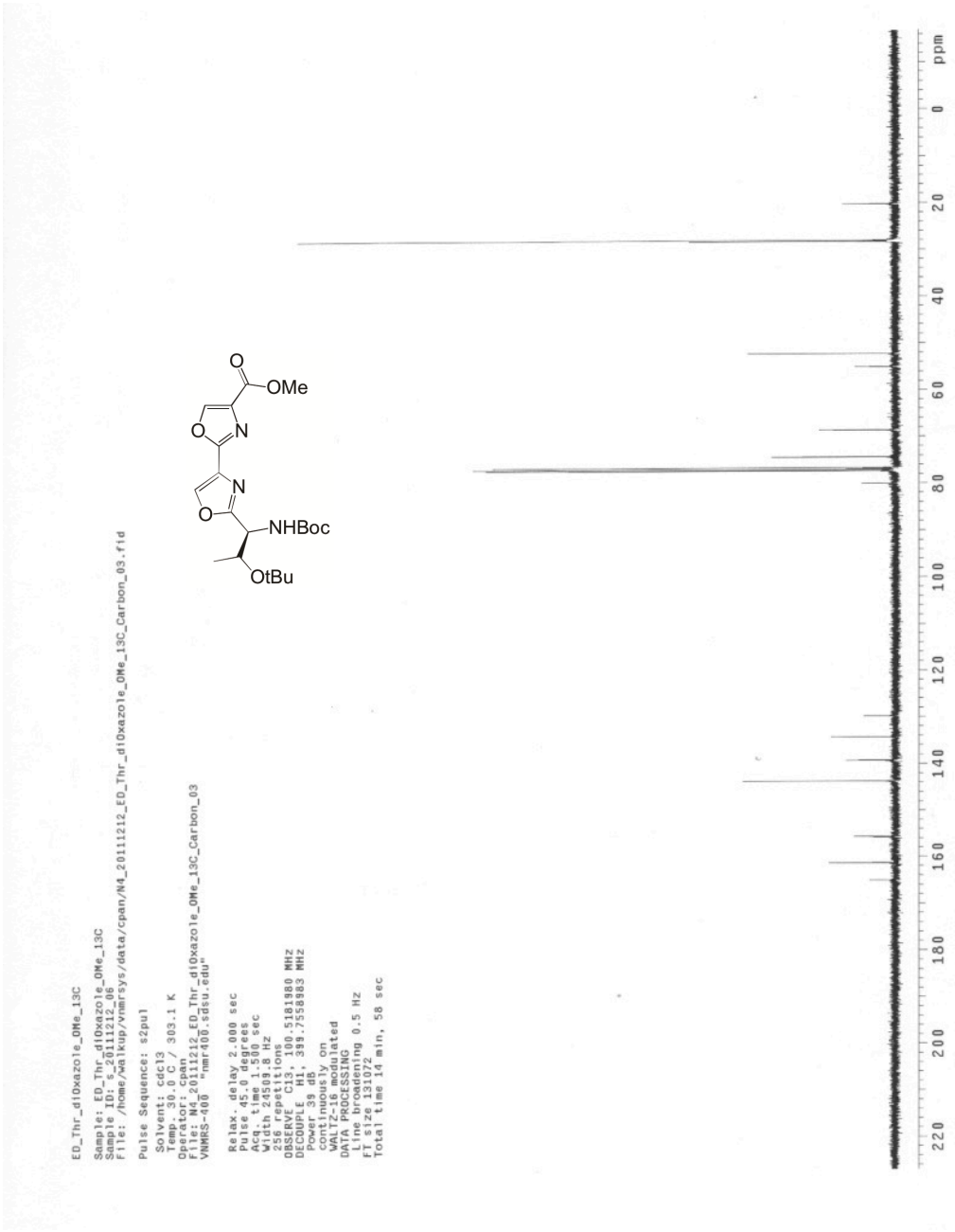
^{13}C NMR MeO-Ser(OBn)-Thr(O^{t}Bu)-Boc



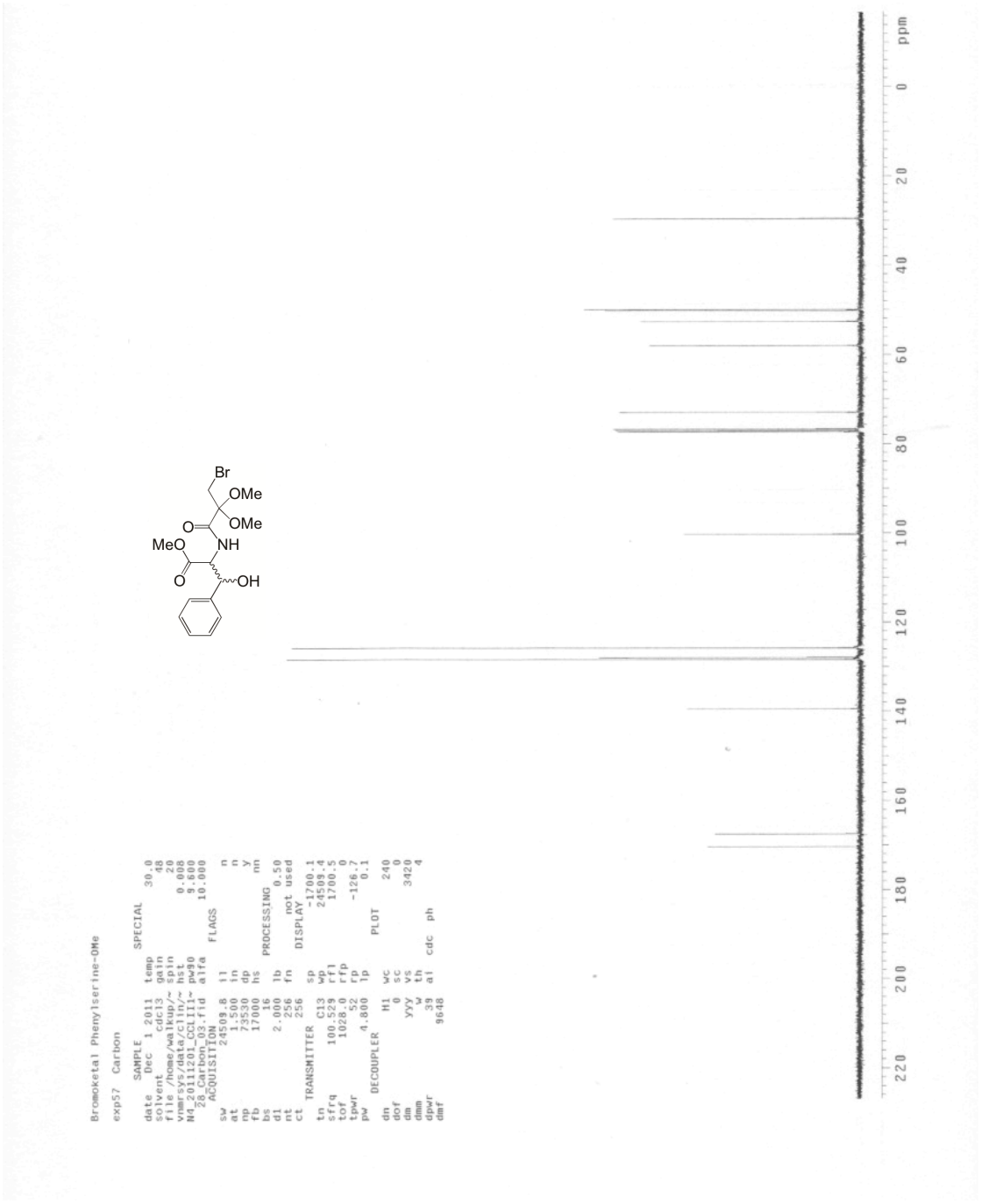
^{13}C NMR MeO-Oxa-Thr(O^{t}Bu)-Boc



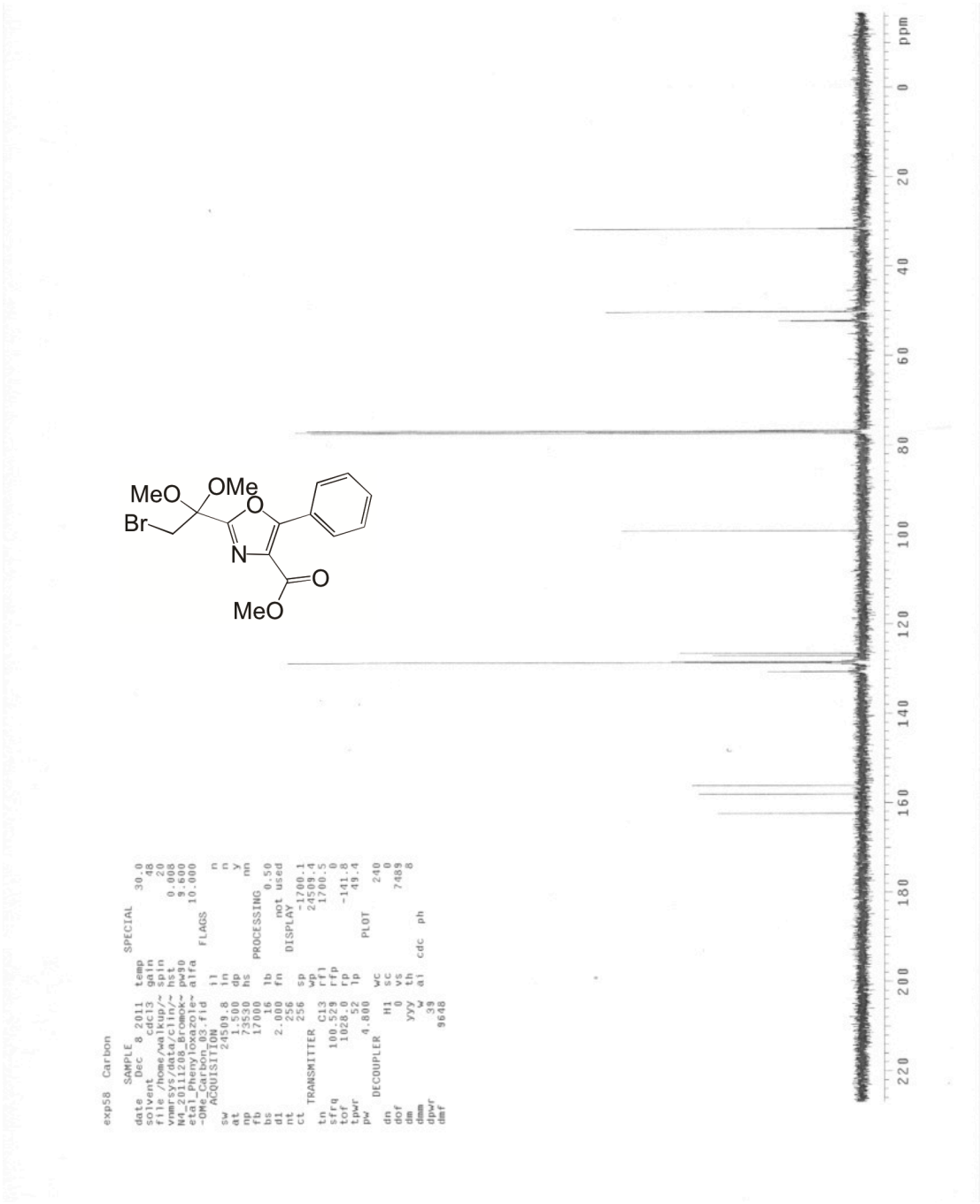
^{13}C NMR MeO-Ser(OBn)-Oxa-Thr(O^{t}Bu)-Boc



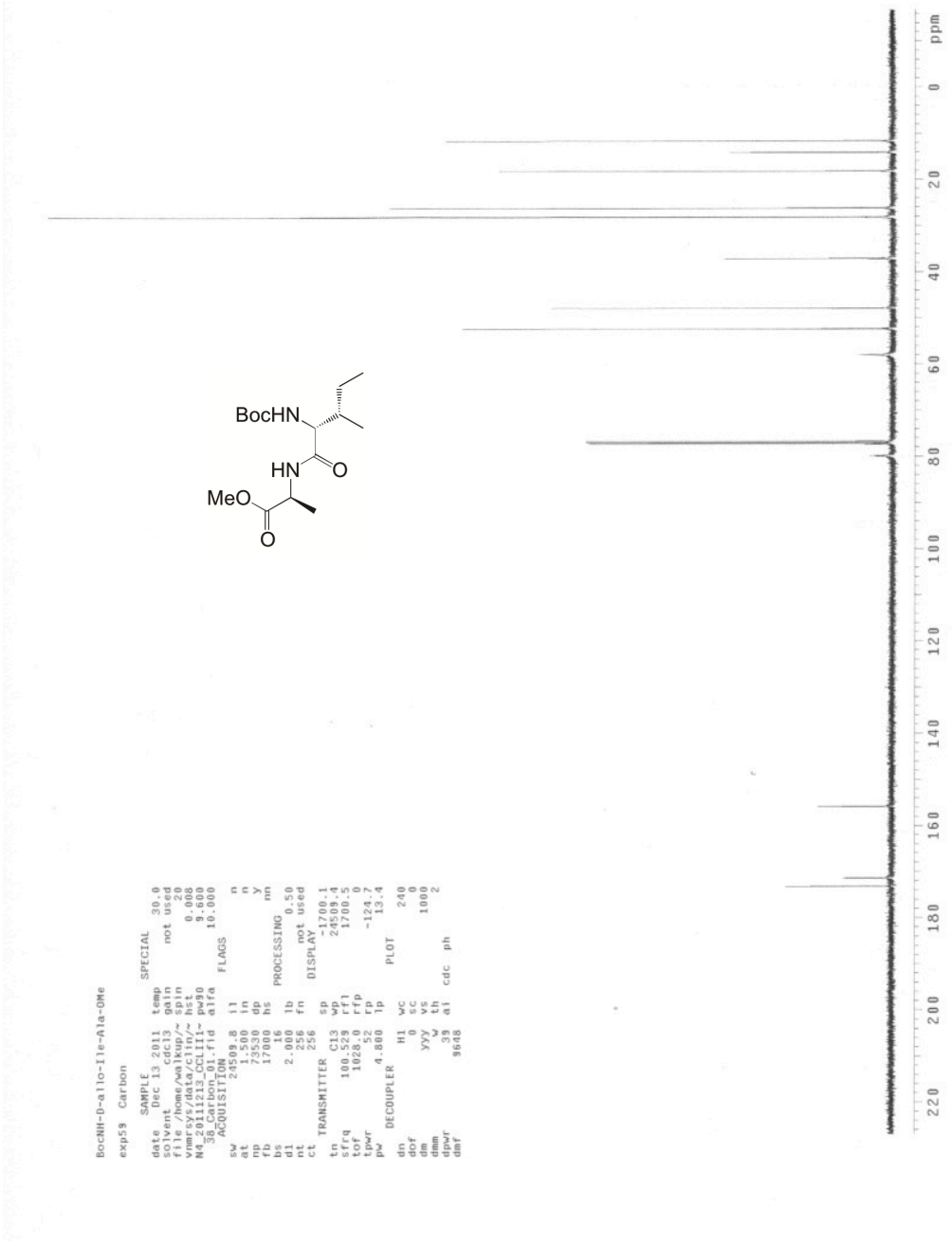
^{13}C NMR MeO-diOxa-Thr(O^{t}Bu)-Boc



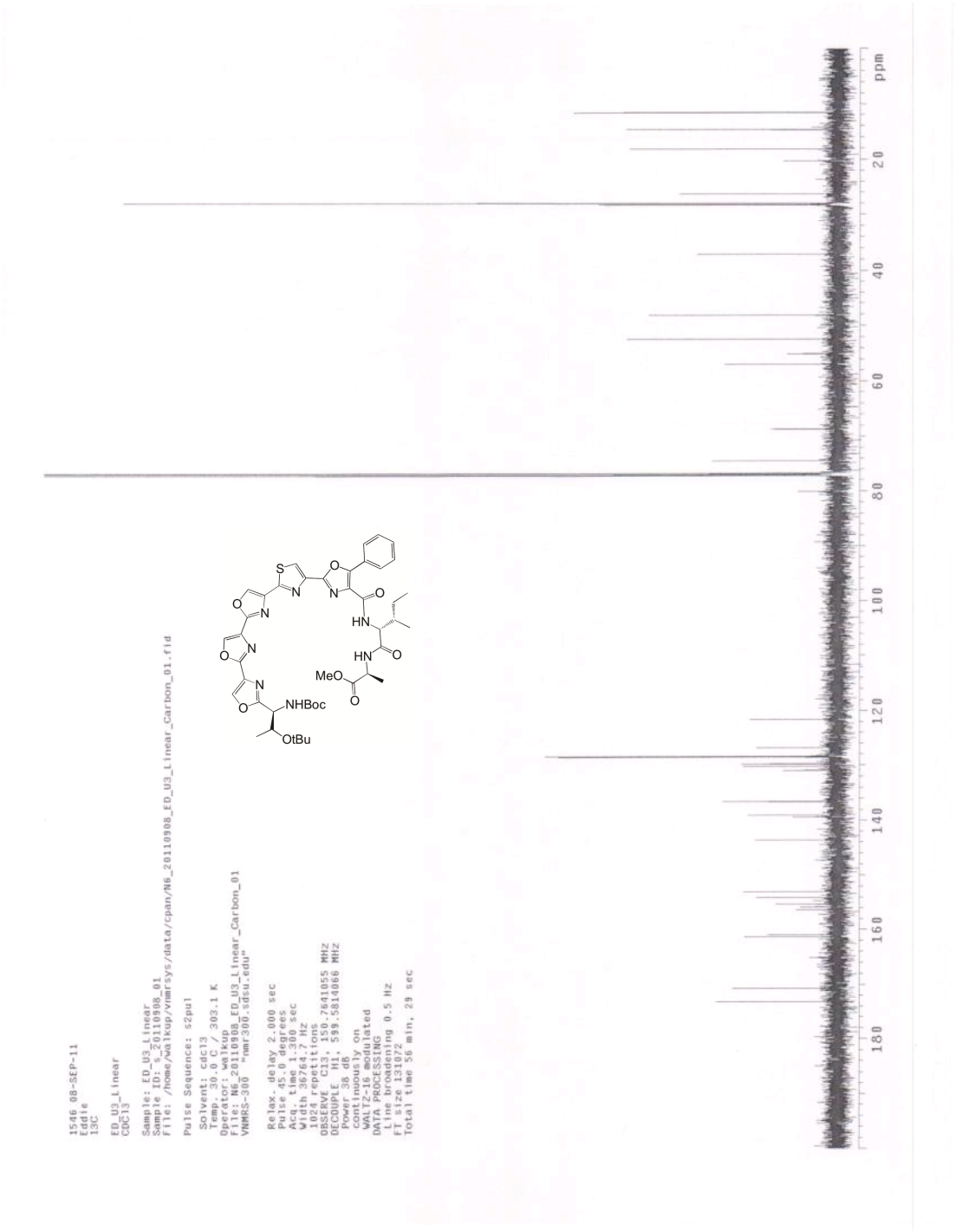
^{13}C NMR *Racemic*-Bromoketal-*β*-hydroxyl-Phe-OMe



^{13}C NMR Bromoketal-Phenyloxazole-OMe



^{13}C NMR MeO-Ala-D-*allo*-Ile-Boc



^{13}C NMR MeO-Ala-D-*allo*-Ile-Phenyloxazole-Thiazole-triOxa-Thr(O^{t}Bu)-Boc