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Design and synthesis of bioactive macrocyclic natural products

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Erinprit K. Singh

Committee in charge:

University of California, San Diego

Professor Seth Cohen
Professor James Whitesell

San Diego State University

Professor Shelli R. McAlpine, Chair
Professor Douglas Grotjahn
Professor Paul Paolini

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The Dissertation of Erinprit K. Singh is approved, and it is acceptable in quality and form for publication electronically:

Chair

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San Diego State University

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DEDICATION

To Biji, Daddy, Mommy, Billy and Simrit.

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LIST OF ABBREVIATIONS AND SYMBOLS

° C	Degree Celsius
μM	Micromolar
μm	Micrometer
Å	Ångström; 10^{-10} m
Abu	Aminobutyric acid
Ac	Acetyl
ACN	Acetonitrile
ADME	Absorption, distribution, metabolism, and excretion
ADP	Adenosine 5′-diphosphate
Ala	Alanine
Ald	Aldehyde
Alk	Alkyne
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
ATP	Adenosine-5'-triphosphate
Az	Azide
BnBr	Benzyl bromide
Boc	*tert*-butoxycarbonyl
br	Broad
$BrCCl_3$	Bromotricchloromethane
Bzl	Benzyl

Cbz	Carboxybenzyl
CF	Cystic fibrosis
CTC	Chlorotrityl chloride
CTLC	Cytotoxic T Lymphocytes
$CuSO_4$	Copper (II) sulfate
Cys	Cysteine
d	Doublet
DAST	Diethylaminosulfur trifluoride
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
dd	Doublet of doublet
DDLP	Double deprotected linear pentapeptide
DEPBT	3-(Diethoxy-phosphoryloxy)-3H-benzo[d][1,2,3] triazin-4-one
DIC	*N*,*N*′-Diisopropylcarbodiimide
DIPEA	*N*,*N*-Diisopropylethylamine
DLP	Deprotected linear pentapeptide
DME	1,2-Dimethoxyethane
DMF	*N*,*N*-Dimethylmethanamide
DMSO	Dimethyl sulfoxide
dq	Doublet of quartet
EA	Ethyl acetate
ED_{50}	effective dose, for 50% of population
EtOH	Ethanol
Fmoc	9-Fluorenylmethyl chloroformate

FS	Free serine
g	Gram
GI	Growth inhibition
Glu	Glutamic acid
Gly	Glycine
HAT	Histone acetyltransferase
HATU	2-(1H-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronoium hexafluorphosphate
HBr	Hydrobromic acid
HCl	Hydrochloric acid
HDAC	Histone deacetylase
HDACI	Histone deacetylase inhibitor
Hex	Hexane
His	Histidine
HOBt	1-Hydroxybenzotriazole
hr	Hour
HSP90	Heat shock protein 90
IC_{50}	half maximal inhibitory concentration
Ile	Isoleucine
IPA	Ispropyl alcohol
K_2CO_3	Potassium carbonate
kDa	Kilodalton
$KHCO_3$	Potassium bicarbonate
L	Liter
LC/MS	Liquid chromatography/mass spectrometry

Leu	Leucine
LiOH	Lithium hydroxide
LP	Linear pentapeptide
LPP	Linear pseudopentapeptide
LR	Lawesson’s reagent
LT	Linear tetrapeptide
Lys	Lysine
m	Multiplet
M	Molar
MeOH	Methanol
Met	Methionine
mg	Milligram
min	Minute
mL	Milliliter
mm	Millimeter
mmol	Millimole
N	Normal
$Na_2S_2O_3$	Sodium thiosulfate
Na_2SO_4	Sodium sulfate
Na_2SO_4	Sodium sulfate
NaBr	Sodium bromide
NaH	Sodium hydride
$NaHCO_3$	Sodium bicarbonate
NaOCl	Sodium hypochlorite

NCI	National Cancer Institute
nM	Nanomolar
NMR	Nuclear magnetic resonance
OEt	Ethyl ester
OEt	Ethyl ester
OMe	Methyl ester
Ox	Oxazole
p	Pentet
Pd/C	Palladium on carbon
PEG	Polyethylene glycol
Phe	Phenylalanine
Pip	Piperidine-2-carboxylic acid
PPI	Peptidyl-prolyl isomerases
ppm	Parts per million
Pro	Proline
q	Quartet
RP-HPLC	Reversed phase-high performance liquid chromatography
rt	Room temperature
s	Singlet
SAHA	Suberoylanilide hydroxamic acid, Vorinostat
SanA	Sansalvamide A
SanB	Sanguinamide B
SAR	Structure-activity relationship
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis

Ser	Serine
t	Triplet
$T_{1/2}$	Half-life
TBTU	O-(Benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate
t-Bu	*tert*-Butyl
TEA	Triethylamine
TEMPO	2,2,6,6-Tetramethylpiperidine 1-oxyl, free radical
TFA	Trifluoroacetic acid
Tfa	Trifluoroacetyl
TFE	*2,2,2*-Trifluoroethanol
TfN_3	Trifluoromethanesulfonyl azide, triflic azide
Th	Thiazole
THF	Tetrahydrofuran
Thr	Threonine
TLC	Thin layer chromatography
TMSD	Trimethylsilyldiazomethane
Tri	Triazole
Trp	Tryptophan
Tyr	Tyrosine
v:v	Volume to volume
Val	Valine
Δ	Difference

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Dr. Pan, thank you both for your wealth of knowledge and great work ethic, you both greatly impacted the group and my research; I am so honored to have gotten to know the both of you. Finally, thank you to all of my former colleagues; you provided an enjoyable environment, in which I was able to learn and grow as a chemist and person. I am grateful for your encouragement and being there for me like a second family.

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Chapter 2, in part, is a reprint of the material as it appears in "A third generation of Sansalvamide A derivatives: Design and synthesis of Hsp90 inhibitors." *Bioorganic and Medicinal Chemistry* **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.; Thornquist, A.; Pan, C.-M.; McAlpine, S.R The dissertation author was a 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. Chapter 3, in part, is also a reprint of material as it appears in "Histone deacetylase inhibitors: synthesis of cyclic tetrapeptides and their triazole analogs." *Tetrahedron Letters* **2010** *51* 4357-4360. Singh, E.K.; Nazarova, L.A.; Lapera, S.A.; Alexander, L.D.; McAlpine , S.R. The dissertation author was the primary investigator and author of this paper.

Chapter 4, in part is a reprint of material as it appears in “Total Synthesis of Natural Product Sanguinamide B and Its Structurally Related Conformers.” *Organic Letters* **2012**, *14*, 1198-1201. Singh, E.K.; Ramsey, D.M.; McAlpine, S.R. The dissertation author was the primary investigator and author of this paper.

VITA

Education

Doctor of Philosophy, Chemistry,
University of California, San Diego, La Jolla, CA

Bachelor of Science, Bioengineering
University of California, San Diego, La Jolla, CA

Publications

1. Singh, E.K.; Ramsey, D.M.; McAlpine, S.R. Total Synthesis of Natural Product Sanguinamide B and Its Structurally Related Conformers. *Organic Letters* **2012**, *14*, 1198-1201.

2. Davis, M.R.; Singh, E.K.; Wahyudi, H.; Alexander, L.D.; Kunicki, J.B.; Fairweather, K.A.; Giltrap, A.; Jolliffe, K.A.; McAlpine, S.R. Synthesis of Sansalvamide A peptidomimetics: triazole, oxazole, thiazole, and pseudoproline containing compounds. *Tetrahedron* **2012**, *68*, 1029-1051.

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Presentations

1. *Synthesis of Sansalvamide A Peptidomimetic Derivatives.* Singh, E.K.; Davis, M.R.; Wahyudi, H.; Alexander, L.D.; Kunicki, J.B.; Fairweather, K.A.; Giltrap, A.; Jolliffe, K.A.; McAlpine, S.R. November 13, 2010 – Los Angeles, CA. *UCLA Glenn T. Seaborg Symposium,* Poster Presentation.

2. *Synthesis and biological evaluation of cyclic tetrapeptides serving as histone deacetylase inhibitors.* Singh, E.K.; Nazarova, L.A.; Lapera, S.A.; Alexander, L.D.; McAlpine, S.R. August 16, 2009 – Washington DC *238th ACS National Meeting, Medicinal Chemistry,* Poster Presentation.

3. *Synthesis and biological evaluation of histone deacetylase inhibitors that are based on FR235222.* 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. February 8, 2008 – San Diego, CA *Student Research Symposium: San Diego State University,* Oral Presentation.

4. *Synthesis of Sansalvamide A derivatives: Novel templates as potent anti-tumor agents.* Singh, E.K.; Rodriguez, R.A.; Pan, P-S; Pan, C-M; Ravula, S.; Lapera, S.; Styers, T.J.; Cajica, J.; Brown, J.D.; Parry, E.; McAlpine, S.R. March 25, 2007 - Chicago, Il *233rd ACS National Meeting, Medicinal Chemistry,* Poster Presentation.

ABSTRACT OF THE DISSERTATION

Design and synthesis of bioactive macrocyclic natural products

by

Erinprit K. Singh

Doctor of Philosophy in Chemistry

University of California, San Diego, 2012

San Diego State University, 2012

Professor Shelli R. McAlpine, Chair

Chapter 1 discusses the background of peptides and their potential as therapeutics; specifically as anti-cancer and antibacterial agents. The synthesis of linear peptides via solution and solid-phase is discussed as well as macrocyclization strategies.

Sansalvamide A, a macrocyclic depsipeptide, exhibits micromolar cytotoxicity activity against multiple cancer cell lines. Chapter 2 focuses on the synthesis and structure-activity relationship of Sansalvamide A derivatives. Some of the structural

features explored in the SAR include: D-amino acids, *N*-methyl amino acids, polar amino acids and heterocyclic moieties. This chapter includes a discussion of the synthesis for seven SanA derivatives and structure-activity relationship conclusions drawn from biological testing of these compounds.

Chapter 3 discusses the design, synthesis and evaluation of histone deacetylase inhibitors. More than half of all human cancers have non-functioning or mutated genes that are the result of overexpression of histone deacetylases; thus, the development of histone deacetylase inhibitors is critical for combating cancers. Chapter 3 describes the synthesis of six histone deacetylase inhibitors that I contributed to a library of 17 derivatives based on two macrocyclic tetrapeptide natural products: FR235222 and apicidin. Upon biological evaluation of these histone deacetylase inhibitors, a discussion of the structure-activity relationship is presented.

Chapter 4 describes the total synthesis of Sanguinamide B. This natural product contains two thiazoles and one oxazole, and is a modified octapeptide macrocycle; unlike other natural products isolated from this sponge, Sanguinamide B contains two proline residues, where these two residues are expected to control the conformation of the macrocycle. The potent cytotoxic and antibiotic properties of other macrolides isolated from the same nudibranch species, and the small microgram quantities of the compound that are available from the natural source, make this natural product very attractive to synthesize. The synthetic strategy to assemble this natural product and conformation of its structure is described in Chapter 4; as well as a discussion of Sanguinamide B's thermodynamic stability and biological activity.

Chapter 1 - Introduction

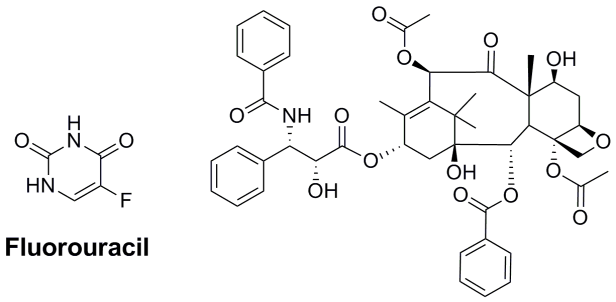
1.1 Cancer therapeutics

Cancer is a class of disease that results from genetic irregularities in the DNA of affected cells and is characterized by prolific cell growth and division, and an increased resistance to apoptosis.[1] The resulting loss of cell cycle control leads to the formation of malignant tumors that are capable of invading adjacent healthy tissues and spreading to other locations in the body.[1]

According to the American Cancer Society, nearly 60% of people diagnosed with cancer undergo surgery for treatment.[2] Surgery is used if there is a high likelihood of removing the tumor before it spreads, but rarely as a stand-alone treatment. Usually, cancer treatment is combined with radiation therapy and/or chemotherapy. Chemotherapeutic agents are an effective treatment method for cancer that has spread to other parts of the body and cannot be treated by any other means. Although a third of new drugs approved by the US FDA in 2011 are for the treatment of cancer, the American Cancer Society still predicts 577,190 cancer related deaths in the US this year; thus, development of novel chemotherapeutic agents is essential.[2]

Fluorouracil (trademarked as Adrucil, Carac, Efudix, Efudex, and Fluoroplex) has been used as chemotherapeutic agent for over 40 years.[3] This drug is a thymidylate synthase inhibitor and blocks synthesis of pyrimidine thymidine that is required for DNA replication; this machinery is heavily used by rapidly dividing cancerous cells.[3,4] Another widely used chemotherapeutic agent is Paclitaxel, which stops cells from undergoing mitosis by interfering with the breakdown of microtubules, thus preventing cancerous growth (**Figure 1.1**)[5-7]. This drug was developed commercially by Bristol-Myers Squibb under the trademark Taxol, and originally isolated from the bark of *Taxus brevifolia*, a Pacific yew tree.[3] Taxol is currently used to treat patients suffering from lung, ovarian,

breast, head and neck cancer and in over 500 clinical trials for treatment of several other types of cancer.[8]

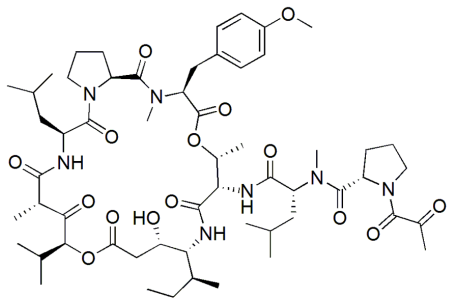


Fluorouracil **Paclitaxel**

Figure 1.1: Fluorouracil and Paclitaxel

1.2 Biologically active macrocyclic peptides

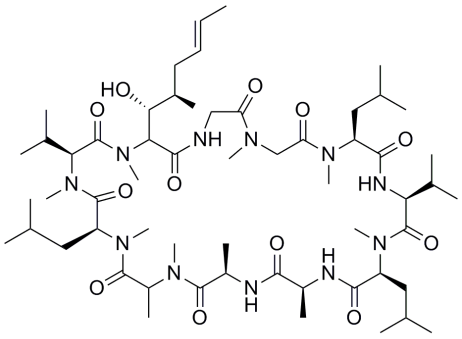
Cyclic peptides have been used in medicine for centuries and constitute a class of compounds that have made significant contributions to the treatment of several diseases, including cancer. Aplidine (**Figure 1.2**), a cyclic depsipeptide originally extracted from a sea squirt *Aplidium albicans*, shrinks tumors in pancreatic, stomach, bladder and prostate cancers.[9-11] Currently in phase II clinical trials in the US and with orphan drug status from the European Medicines Agency to treat acute lymphoma leukemia, Aplidine shows promise as a chemotherapeutic agent.



Aplidine

Figure 1.2: Aplidine

Also a macrocyclic peptide, Cyclosporine A, is one of the best-selling drugs worldwide and generates approximately 1 billion USD annually (**Figure 1.3**). Excluding the sale of synthetic insulin, Cyclosporine A corners pharmacological peptides sales for one third of the market.[12] This cyclic peptide is a metabolite produced by *Tolypocladium inflatum*, originally isolated at Sandoz (Novartis) in 1970.[13] Cyclosporine A has very potent immunosuppressant activity by interrupting a signaling pathway required to activate T cells. The US FDA approved Cyclosporine A as a drug in 1983 and it's now the primary drug of choice for organ transplant patients.[13]

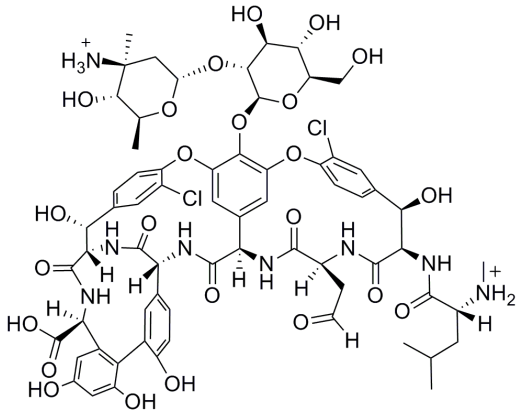


Cyclosporine A

Figure 1.3: Cyclosporine A

Vancomycin is another blockbuster macrocyclic peptide drug on the market (**Figure 1.4**). This antibiotic was isolated from a microbe *Amycolatopsis orientalis* in 1956 and inhibits cell wall synthesis in Gram-positive bacteria.[14] This drug is considered the drug of "last resort," used when other antibiotics have failed.[14]

There is a great need for the development of novel antibacterial agents as bacterial pathogens continue to evolve and build resistance to antibiotics currently in use. Macrocyclic peptides are one of three new classes of antibiotics in clinical use that have been discovered over the past 40 years.[15] An antibiotic, as its name suggests, is a



Vancomycin

Figure 1.4: Vancomycin

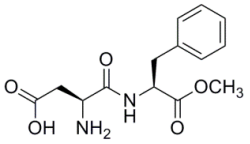
compound that stops or slows down bacterial growth. Commonly used antibiotics attack bacteria by: targeting the bacterial cell wall (penicillin), the cell membrane (polymixins), or interfering with essential bacterial enzymes (sulfonamides).[15] Further characterization of antibiotics is also based on specific types of bacteria: Gram-negative or Gram-positive, which have structurally different cell walls.

1.3 Background of peptides as therapeutics

Peptides are ideal therapeutics due to their low toxicity, specificity to receptors and protein targets and their straightforward synthesis with versatile building blocks. Synthetically made peptides can be as simple as the non-caloric sweetener aspartame, a dipeptide of aspartic acid and esterified phenylalanine (**Figure 1.5.a**), or as complex as the naturally occurring hormone insulin which is composed of 51 residues. (**Figure 1.5.b**)

Diabetes treatment was revolutionized when human insulin was synthetically manufactured for therapeutic purposes.[16] Insulin administration for the treatment of diabetes was originally only available as a purified peptide from an animal source.[17]

(a)



(b)

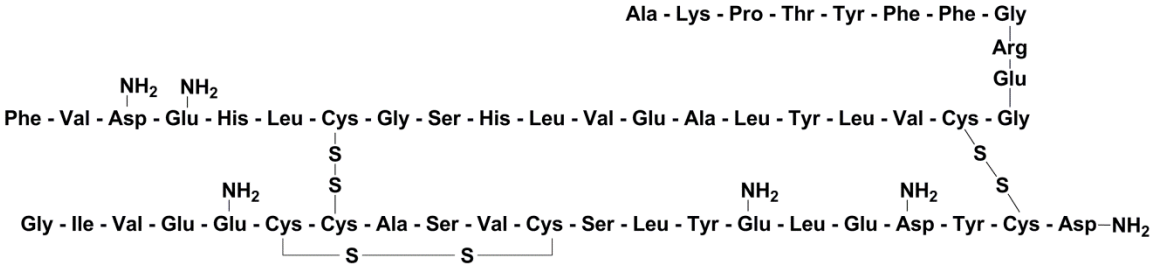


Figure 1.5: Peptide sequences (a) dipeptide aspartame; (b) 51-residue peptide insulin

Highly refined quantities of insulin were very limited and there were severe side effects associated with the impurities in animal-sourced insulin.[17] It wasn't until the 1950s (after nearly 30 years of administering animal-sourced insulin) that the structure of insulin was elucidated and a synthetic version of insulin was available. [17]

1.4. Solution phase synthesis of peptides

Solution phase synthesis is commonly used for large-scale peptide synthesis. Its main advantage is that intermediate products can be isolated and purified after each synthesis step. This method is also amenable to synthetic schemes that involve chemically modifying peptides.

Solution phase peptide coupling proceeds by activation of the carboxylic acid by converting the –OH of the acid to a good leaving group prior to treatment with the amine. Coupling reagents are used to activate carboxylic acids and I employed three different coupling reagents for peptide couplings in solution phase (**Figure 1.6**): *O*-(Benzotriazol-

1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU), *O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU), and 3-(Diethoxyphosphoryl-oxy)-1,2,3-benzotriazin-4(3H)-one (DEPBT). TBTU is a popular coupling reagent, thought at one point to have a uronium structure.[18] Crystal and solution structure studies revealed that TBTU has an aminium structure.[18] This coupling reagent is cost effective and very efficient with little racemization. HATU, also originally thought to be a uronium salt, is actually a guanidinum salt that is a very effective coupling reagent.[18] HATU reacts faster and serves better than TBTU when coupling at sterically hindered amines or for complex coupling reactions; however, it's a rather expensive coupling reagent. DEPBT is an organophosphorous coupling reagent that causes very little epimerization during peptide couplings and very efficient when used for macrocyclization of peptides.[19]

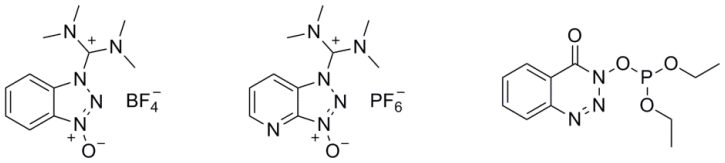


Figure 1.6: Coupling reagents used in solution phase peptide synthesis

1.5 Solid phase synthesis of peptides

Solid phase synthesis of peptides consists of elongation of a peptidic chain anchored to a solid matrix via successive additions of amino acids. This method was introduced by Merrifield in 1963 and was originally received with skepticism, as purification could only be performed once the peptide was cleaved from the resin.[20] However, this process has several advantages over solution phase synthesis; mainly, these reactions can be automated and what normally could be achieved in several days could be accomplished in a few hours.

Depending on the number and types of amino acids in a peptide sequence, various materials can be used to design a solid support resin. Key features that need to be considered when choosing a solid support resin are: using particles that are conventional and uniform size, mechanically robust, easily filterable, chemically inert, and chemically stable under the synthetic conditions designed to generate the desired peptide.[16, 21] The resin that I employed was a 1% divinylbenzene (DVB)-cross-linked polystyrene (PS) resin. Polystyrene is the most commonly used core resin in solid phase chemistry. These cross-linked resins are solvated and swollen in aprotic solvents (e.g. dimethylformamide (DMF), dichloromethane (DCM), toluene, etc.) where the resin swells 4-6 times its original volume.[21] Consequently, a resin that swells more has a higher diffusion rate of regents resulting in shorter reaction times and higher yielding couplings.

Solid phase peptide synthesis utilizes several coupling reagents that activate the carboxyl groups of amino acids in coupling reactions. Typically, uronium and phosphonium salts and 1-hydroxybenzotriazole (HOBt) are used because their high reactivity, high coupling yields and low cost. I utilized HOBt with diisopropylcarbodiimide (DIC) to couple my peptides using solid phase synthesis. DIC is a very common activating agent, however, the problem with carbodiimides is that they are too reactive and can cause racemization of the amino acid.[22, 23] So, to remedy this, triazoles such as HOBt were introduced to react with the O-acyl-urea that DIC forms and form an active ester that is less reactive and less susceptible to racemization.[23]

Once the desired peptide sequence is synthesized and the necessary protecting groups of the side chains are removed, the peptide can be cleaved from the resin. I used a 2-chlorotrityl chloride resin (CTC) as the linker between my peptide sequence

and resin (**Figure 1.7**). 2,2,2-trifluoroethanol (TFE) is used to free the peptide from support under mildly acidic conditions, generating a free carboxylic acid on the peptide.

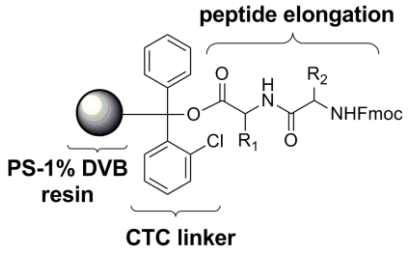


Figure 1.7: PS-1% DVB resin with a CTC linker for peptide elongation

1.6 Advantages of macrocycles over linear peptides

Peptides present several advantages as therapeutic agents, such as: low toxicity resulting from peptide degradation (byproducts are simply amino acids), minimized accumulation of peptides in tissues, and low cost of manufacturing.[24] However, a disadvantage of linear peptides is their low bioavailability, which is partly due to high degradation by gastrointestinal, plasma, and tissue peptidases.[24] Additionally, linear peptides possess many conformations in solution and this flexibility often relates to poor selectivity for biological targets.[25] One strategy to limit a peptide's flexibility is cyclization of linear peptides; this typically preserves biological properties of peptides and increases their resistance to degradation and elimination.[26] Cyclizing a linear peptide is a useful technique to reduce their conformational freedom, and potentially improve binding affinity towards a biological target.

Cilengitide is a cyclic pentapeptide currently with orphan drug status on the European market for the treatment of glioblastoma.[25] Kessler and co-workers found that the linear sequence Arg-Gly-Asp-Phe-Val successfully targeted integrins αvβ1, αvβ3, and αvβ5, which are involved in angiogenesis and metastasis of cancer cells from the primary solid tumor.[27] By cyclizing this linear peptide, Kessler saw an increase in

cytotoxicity of 1000 fold compared to the linear peptide.[26] (Figure 1.8). This cyclic pentapeptide was optimized to furnish Cilengitide.

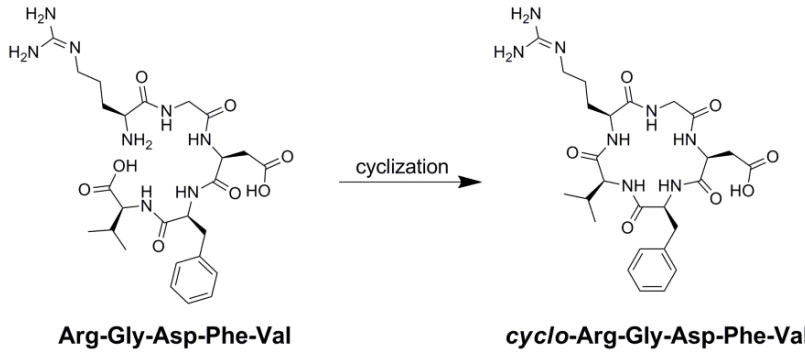


Figure 1.8: Linear precursor and cyclized Cilengitide analog

1.7 Synthesis of macrocyclic peptides

There are four possible ways that a peptide can be constrained as a macrocycle: head-to-tail (C-terminus to *N*-terminus), head-to-side chain, side chain-to-head, or side chain-to-side chain[27] (**Figure 1.9**). Researchers have used these methods to generate macrocycles that are reported in literature.

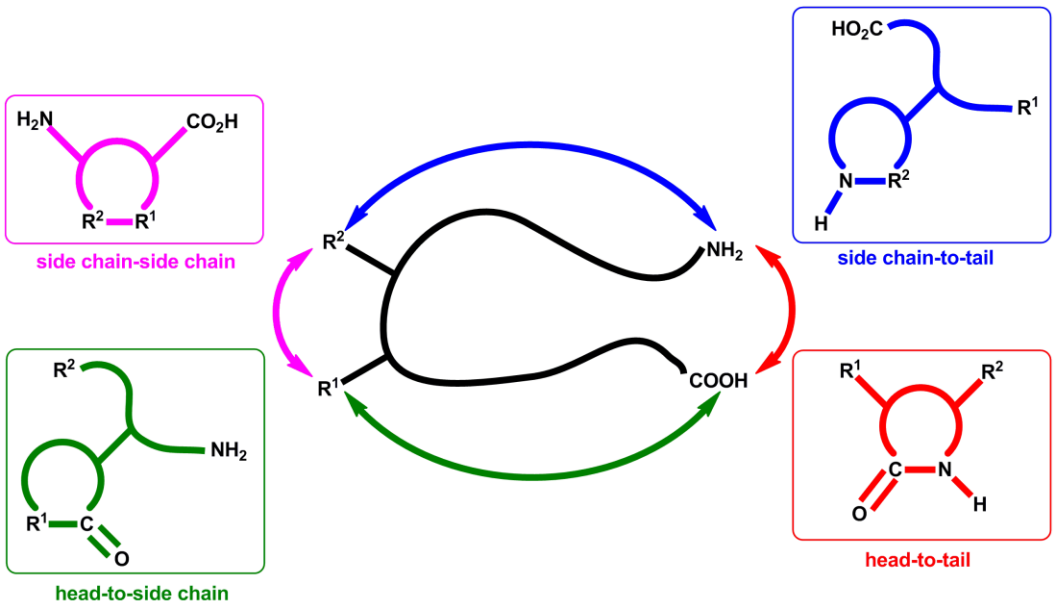


Figure 1.9: Four different ways to constrain a peptide into a macrocyclic conformation. Illustration adapted from reference[27]

The side chain-to-side chain strategy was used to generate a cyclic peptide that interrupts Tat-RNA interactions for the inhibition of HIV-1 gene expression.[28] The linear precursor was built on solid support and a peptide bond was formed between the side chains of Glutamic acid and Lysine (Figure 1.10).

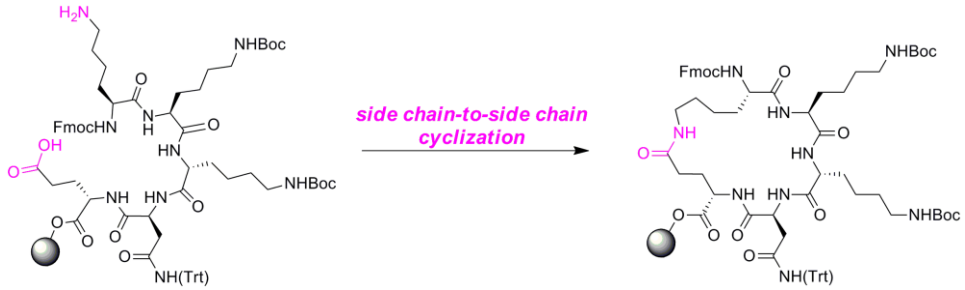


Figure 1.10: Tat-RNA interaction disruptor for inhibition of HIV-1 gene expression synthesized via side chain-to-tail cyclization

Aldrich and co-workers synthesized a library of cyclic dynorphin A analogs via a head-to-side chain cyclization.[29] Dynorphin A is a type of opioid peptide that modulates pain response and is released by many parts of the brain.[29] Cyclization to generate dynorphin A analogs involved a lactam bond formation between the *N*-terminal amine and the Glutamic acid side chain[30] (Figure 1.11).

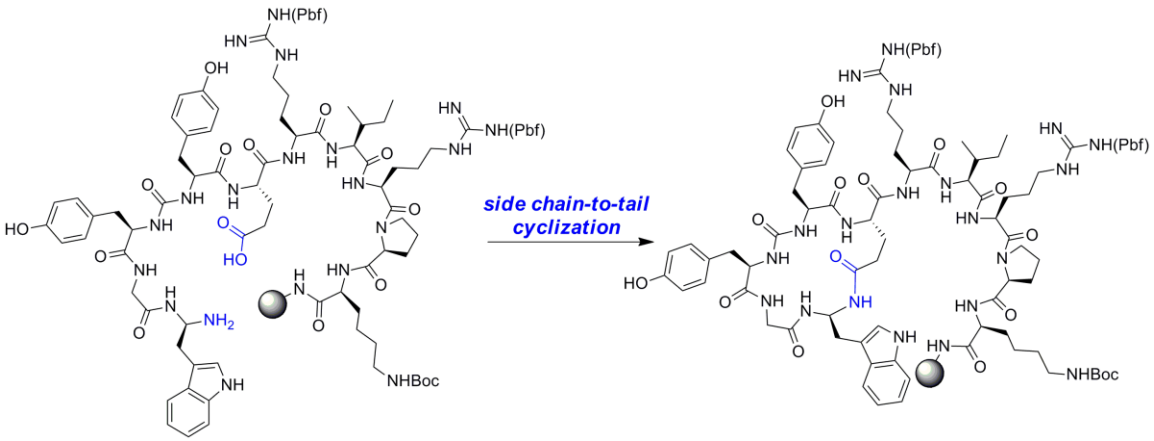


Figure 1.11: Cyclic dynorphin A analog synthesized via side chain-to-tail cyclization

Williams and co-workers synthesized a class of thiolactone cyclic pentapeptides responsible for inhibiting staphylococcal virulence using the side chain-to-tail strategy. The thiolactone linkage was the site of cyclization and the peptide was cyclized via a condensation of the Cysteine side chain (a sulfhydryl group) to the C-terminal carboxylic acid (Figure 1.12).[31]

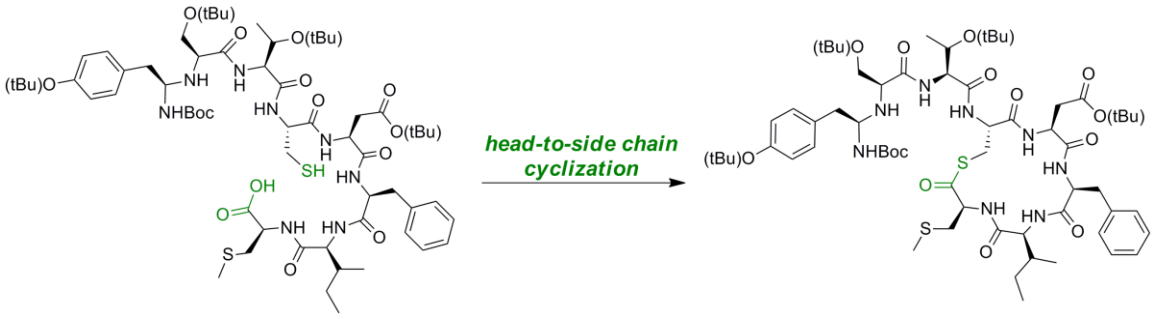


Figure 1.12: Thiolactone linkage to cyclize a staphylococcal virulence inhibitor via head-to-side chain cyclization

Head-to-tail macrocyclization is still the most commonly used strategy for cyclizing a peptide in solution. This method has been successful in cyclizing peptide sequences as long as 20 residues, though the success or failure of this cyclization is still mainly influenced by the conformation of the linear peptide precursors in solution. An example of a head-to-tail cyclization is seen with cyclic depsipeptide Halipeptin A.[32] This natural product was isolated from the marine sponge *Haliclona* sp., and possesses promising anti-inflammatory activity *in vivo*.[33] The Hamada group synthesized Halipeptin A, where macrocyclization occurred via peptide coupling at the *N*-Me-OH-Ile/Ala site[33] (Figure 1.13).

Figure 1.13: Head-to-tail cyclization to generate cyclic depsipeptide Halipeptin

1.7.1 Conformational elements to aid cyclization

The success or failure of macrocyclization is largely dependent on the ability of the linear precursor to prearrange its two reactive ends in close promixity to each other.[27] This prearrangement allows for an efficient macrocyclization with fewer by-products from intermolecular processes. Physically bringing the two reactive ends of a peptide closer together involves examining the secondary protein structure and introducing turns into the peptide sequence.

L- and D-stereochemistry greatly impacts the orientation of linear peptides because varying stereochemistry in a sequence induces β-turns. Cyclization is usually impossible or difficult when a peptide sequence has only all L- or D-amino acids because these β-turns are not present.[27] *N*-methyl amino acids and Prolines also induce this β-turn, as documented by Kessler and co-workers; these elements also position the linear peptide in orientation that facilitates macrocyclization.[34]

1.7.2 Coupling agent mediated cyclization

Coupling reagents are commonly used to cyclize linear peptides. Typical peptide couplings in solution phase employ a single coupling reagent and the amide bond formation is fairly straightforward. However, amide bond formation to cyclize linear peptides is more complex since a conformationally restricted structure is being generated.

Different coupling reagents are more/less effective in coupling different amino acids. For example, hexafluorophosphate uronium salts are less effective in amide bond formation involving Fmoc-Ile and Fmoc-Leu, while phosphonium salts are less effective in amide bond formation involving Fmoc-Tyr.[35] It has been reported in literature that mixing different types of coupling reagents improves yields significantly.[35] For

macrocyclizations that I performed via peptide bond formation, I employed a cocktail of coupling reagents; this ensured a successful coupling to generate the octa-, penta-, and tetrapeptides described in the following chapters.

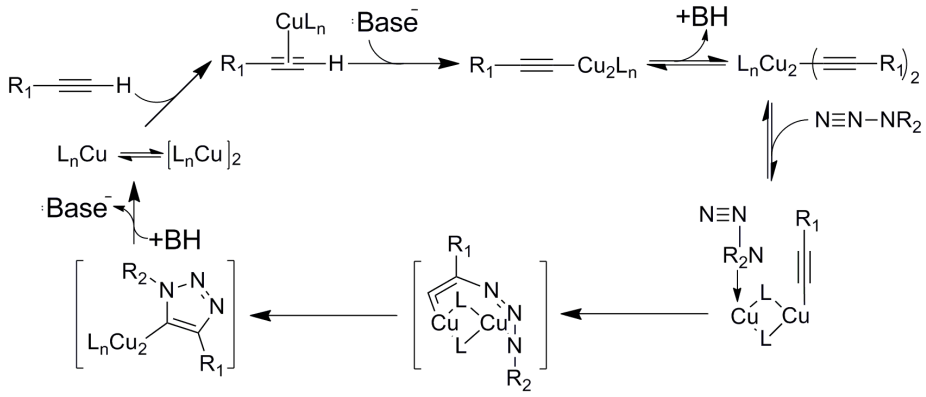
1.7.3 Alkyne-azide cycloaddition

There are numerous biologically active cyclic peptides with heterocyclic moieties embedded in their core structure. Specifically, oxazoles, thiazoles, and triazoles all impose conformational restrictions in macrocycles, making these cyclic peptides an interesting class of molecules.[27] 1,2,3-triazoles are particularly interesting as these heterocycles are both thermodynamically and physiologically stable.[27] Additionally, depending on the substitution pattern, triazoles can induce a *cis*- or *trans*- amide bond in the macrocycle.[36]

Triazoles are typically synthesized via a 1,3-dipolar cycloaddition between an alkyne and an azide, as shown by Huisgen. Recently, Meldal and Sharpless developed a regioselective way to generate the 1,4-disubstituted triazole under a Cu (I) catalyzed reaction under mild conditions.[37, 38] This macrocyclization tool was used by van Maarseveen and co-workers to synthesize a previously unobtainable cyclic tetrapeptide *cyclo*-Pro-Val-Pro-Tyr.[39, 40] Using a cycloaddition of an *N*-terminal azide and a C-terminal alkyne, they were able to synthesize a triazole containing macrocyclic analog that retained tyrosinase inhibitory activity.

This cycloaddition reaction proceeds by the Cu (I) species forming a pi complex with the triple bond of the terminal alkyne. Then, base deprotonates the terminal hydrogen from the alkyne to give a Cu acetylide intermediate. Another Cu atom activates the azide by coordinating with the electrons on the nitrogen atom. The azide displaces one Cu ligand to generate a Cu-azide-acetylide complex and then cyclization

takes place. Finally, after a protonation, the desired 1,4-disubstituted triazole is furnished by dissociation and the catalyst ligand complex is regenerated for further reaction cycles[39] (Scheme 1.1).



Scheme 1.1: Cu catalyzed alkyne-azide cycloaddition mechanism

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Chapter 1, in part, is a reprint of the material as it appears in "Conformational based design of macrocycles as antitumor agents." *Current Opinion in Drug Discovery & Development*, **2008**, *11* 544-552. Singh, E.K.; Sellers, R.P.; Alexander, L.D.; McAlpine, S.R. The dissertation author is the primary investigator and author of this paper.

Chapter 2 - Sansalvamide A

2.1 History of Sansalvamide A

Sansalvamide A was isolated from a marine fungus *Fusarium* sp. collected from the surface of a seagrass *Halodule wrightii* off the coast of Little San Salvador Island, Bahamas.[1] Fenical and co-workers determined that the structure of this natural product was a macrocyclic depsipeptide, which contained a L-Valine (Val), L-phenylalanine (Phe), two L-leucines (Leu), and a 2*S*-Leucic acid, with all five stereogenic centers having the *S*-configuration (**Figure 2.1**). When tested against the National Cancer Institute's (NCI's) 60 cell-line panel, this depsipeptide showed considerable cytotoxicity with a mean IC_{50} of 42.5 μM.[1]

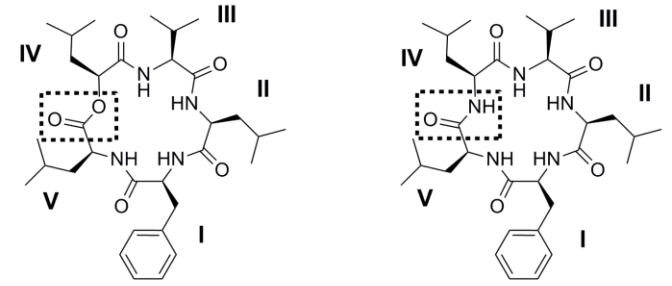


Figure 2.1: Natural product Sansalvamide A vs. macrocyclic pentapeptide SanA

The first total synthesis of this depsipeptide was reported by Lee and Silverman in 2000 in an efficient 10-step solid-phase synthesis.[2] In 2002, Silverman and co-workers replaced the lactone linkage between residues IV and V with a peptide bond, generating the first Sansalvamide A peptide [SanA, *cyclo*-Phe-Leu-Val-Leu-Leu, (**Figure 2.1**)][3]. Biological testing of SanA against HCT-116 human colon carcinoma, showed a 10-fold increase in potency compared to that of the natural product Sansalvamide A.[4]

There has been extensive research investigating the natural product depsipeptide Sansalvamide A and macrocyclic pentapeptide SanA as potential chemotherapeutic drugs.[5-8] Thorough exploration of the structure activity relationship

(SAR) of the SanA peptide was explored by our group with over 100 derivatives published to date.[4, 9-14] We have also determined the biological target of SanA and are now in the process of advancing SanA to the next stage of clinical development.[15]

2.2 Rational design of SanA derivatives

One common modification made when exploring the SAR of a lead peptide is *N*-methylation. Incorporating *N*-methyl amino acids into peptides can result in analogs with improved biological activity and pharmacological properties.[16] Secondary peptide bonds typically have a higher preference for the *trans*-peptide bond, whereas the presence of *N*-methyl on the amide nitrogen increases the propensity for *cis*-peptide bonds to occur about the *N*-alkylated amide bond.[17, 18] In fact, there is a significant effect on the backbone conformation of cyclic peptides when *N*-methyls are present, which results in an altered 3D structure.[17] Additionally, research has shown that the presence of a single *N*-methyl in a cyclic peptide minimizes the number of conformations that a macrocycle can adopt, thereby enhancing receptor selectivity by potentially locking the peptide into a single bioactive conformation.[17] Thus, the *N*-methyl moiety is an important tool in optimizing potency of cyclic peptides.

There are many natural product cyclic peptides that contain *N*-Methyl amino acids with notable pharmacological profiles including: IB-01212[19], Koshikamides[20] and Cyclosporine A[21], being the most prominent (**Figure 2.2**). Cyclosporine A (Neoral™) is a cyclic undecapeptide with seven *N*-methyls and is marketed as an immunosppresant. The natural product was isolated from a metabolite produced by *Tolypocladium inflatum* and is the primary drug of choice for organ transplant patients, as it suppresses the immune response and decreases the risk of the organ rejection. Though Cyclosporine A's metabolic stability is partly due to its cyclic structure, the presence of *N*-methyl

groups is critical for this peptide's oral availability.[22] The presence of an *N*-methyl abolishes the amino group's ability to H-bond and thus enhances the peptide's hydrophobicity, which results in improved intestinal absorption of the peptide by allowing transcellular membrane permeability across the epithelial layer.[23] Thus, during our exploration of SanA's SAR, we investigated the impact of incorporating *N*-methyl moieties into the macrocyclic peptide scaffold.

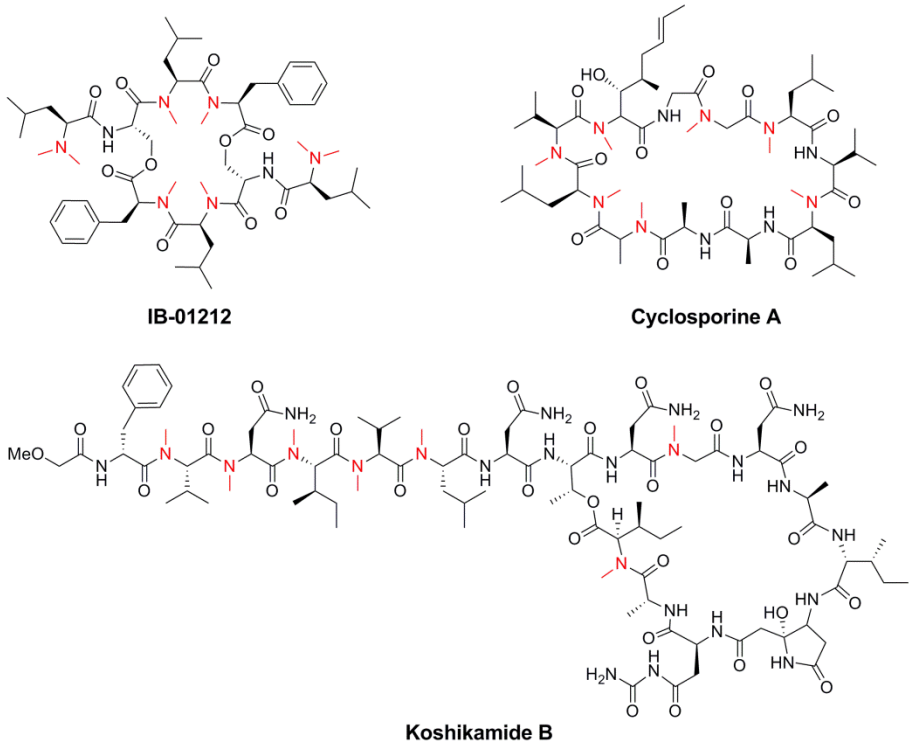


Figure 2.2: Cyclic peptides found in nature with *N*-methyl amino acids

The concept of incorporating a single *N*-methyl at each amino acid residue (*N*-methyl scan) was originally used by Sugano et al. in 1973[24, 25] in a successful effort to enhance the potency of an Eledoisin related peptide in a rabbit blood pressure assay (**Figure 2.3.a**) [24, 25]. Our group applied this concept and performed an *N*-methyl scan by incorporating a single *N*-methyl at each of the five residues found in SanA (**SanA 1-5**,

Figure 2.3.b). I synthesized two analogs in this series: **SanA 2**, with a single *N*-methyl at the II position and **SanA 4**, with a single *N*-methyl at the IV position.

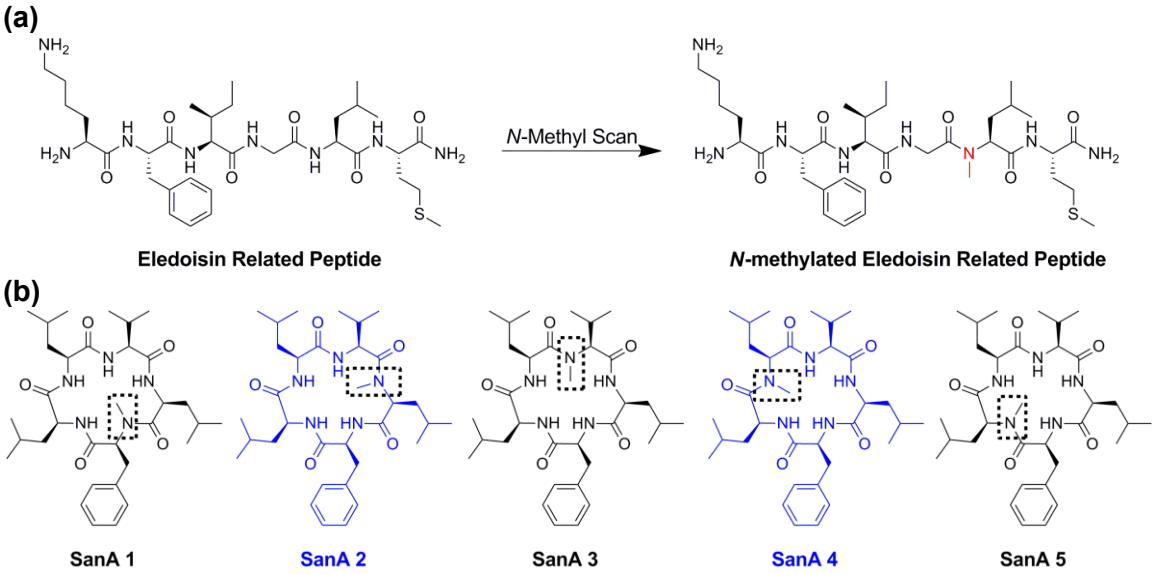


Figure 2.3: (a) Eledoisin related peptide and the most potent *N*-methyl derivative from an *N*-methyl scan; **(b)** *N*-methyl scan of SanA: Rational design for **SanA 2** and **SanA 4**

Another common modification made to peptides when exploring their SAR is exchanging L and D amino acids. The conformation of macrocyclic peptides with small ring sizes (e.g. five amino acids, 15 members) is dictated by the amino acid stereochemistry in the peptide sequence.[17] Additionally, including D-amino acids in a peptide sequence stabilizes the analog against enzymatic degradation.[26] However, generating the enantiomer of a peptide sequence has typically led to these peptides losing their biological activity. Many medicinal chemists' approach to incorporating D-AAs into their lead peptide sequence is by exchanging one residue at a time (D-scan). Recently, Kessler et al. discovered a potent compound by first cyclizing a linear pentapeptide sequence,[27] then optimizing the cyclic peptide's potency by performing a D-scan[27] and then an *N*-methyl scan[28] (**Figure 2.4**). The resulting cyclic pentapeptide, Cilengitide, is now in phase III clinical trials to treat glioblastomas as the first anti-angiogenic small molecule targeting the integrins αvβ3, αvβ5 and α5β1.[29]

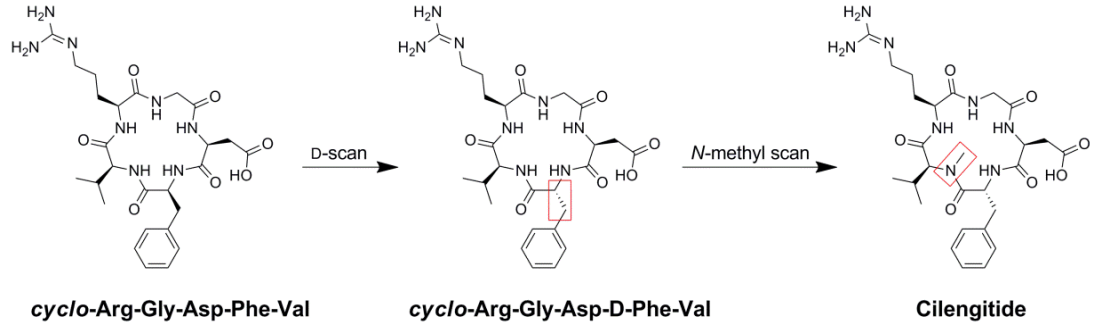


Figure 2.4: Rational design for the development of Cilengitide

For cyclic pentapeptides like SanA, a single D-amino acid substitution results in a γ-turn conformation of the D-amino acid and the two amino acids on either side. This γ-turn is in equilibrium with a βII'-turn that exists between the remaining two amino acids.[30] These adjustments to the macrocyclic backbone significantly impact whether or not the peptide locks into single a bioactive conformation. Thus, we performed a D-scan, exploring the effects that incorporating a single D-amino acid into our peptide sequence will impact the biological activity of SanA [**SanA 6-10** (**Figure 2.5**)].

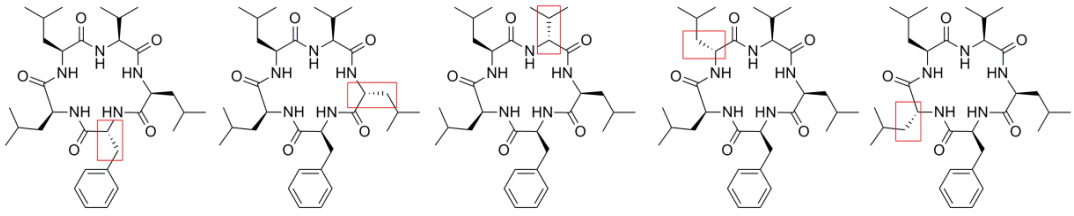


Figure 2.5: D-Scan of SanA

Compound **SanA 5** and **SanA 10** both showed reasonable cytotoxicity (IC_{50} = 10-20 μM) against colon cancer cell line HCT-116. To optimize potency, medicinal chemists have long used the concept of combining structural features of potent derivatives from individual compounds.[31-33] I synthesized compound **SanA 11** to investigate if there was a synergistic effect when combining the *N*-methyl and D-Leu at position V from parent compounds **SanA 5** and **SanA 10**, respectively (**Figure 2.6**).

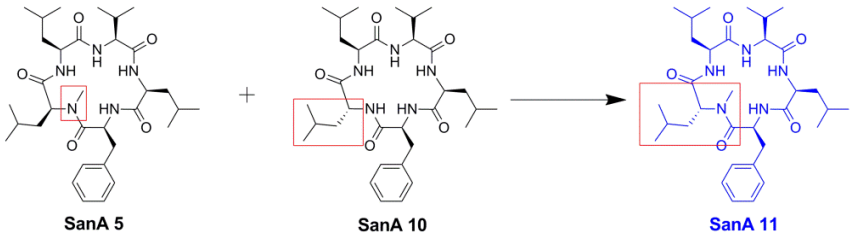


Figure 2.6: Rational design of **SanA 11** from parent compounds **SanA 5** and **SanA 10**

As part of our SAR analysis, I synthesized two compounds with polar amino acids: **SanA 13** and **SanA 15** (**Figure 2.7** and **Figure 2.8**, respectively). Balancing polar groups and non-polar groups within a drug is an important drug-development step, because polarity affects solubility, membrane permeability, and target affinity.[36] **SanA 13** was derived from parent compound **SanA 12**. In **SanA 12** the L-Val at position III was replaced with a D-Aminobutyric acid (D-Abu) (**Figure 2.7**). Incorporation of a smaller D-aliphatic group at position III proved a favorable modification to the SanA scaffold with a nearly 3-fold increase in % growth inhibition (GI) against pancreatic carcinoma (PL-45) compared to SanA (70% vs. 25%). By replacing D-Abu of **SanA 12** at position III with a D-Serine (D-Ser) we designed **SanA 13,** which contains a small but polar amino acid.

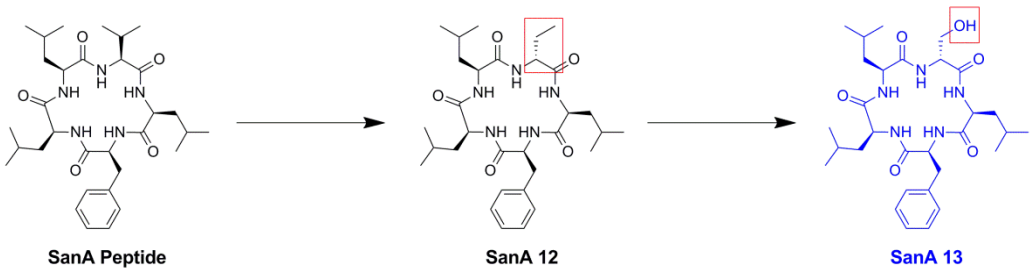


Figure 2.7: Rational design of **SanA 13** from parent compound **SanA 12**

I also synthesized **SanA 15**, which was derived from parent compound **SanA 14**. **SanA 14** contained a D-Tryptophan (D-Trp) at position I. Since the cytotoxicity of **SanA 14** was comparable to that of SanA (GI = 18% versus 25% for **SanA 14** versus SanA respectively, PL-45), we maintained the D-Trp at position I, while incorporating L-Arginine

(L-Arg) at position IV, generating **SanA 15** (**Figure 2.8**). Arg has a basic, charged side chain, and peptide sequences that include both basic and neutral amino acids significantly enhance membrane permeability via absorptive mediated endocytosis.[37] Additionally, the Arg guanidino group is a charged (cationic) species at physiological pH, which enhances peptide solubility in serum, a quality that is highly desirable for a potential drug.

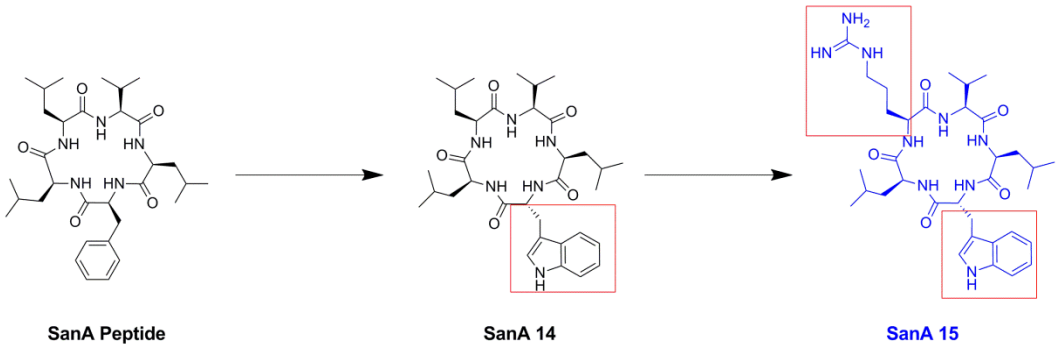
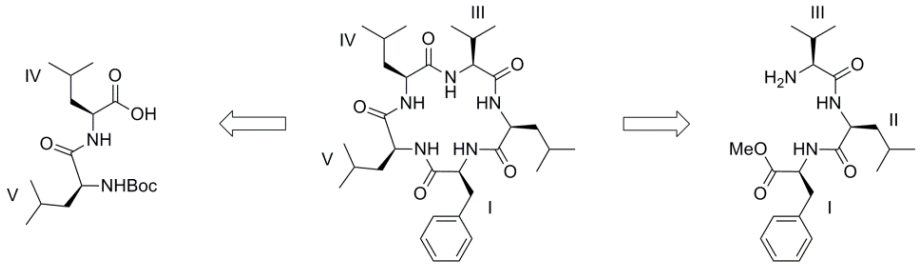


Figure 2.8: Rational design of **SanA 15** from parent compound **SanA 14**

2.3 Retrosynthetic approach: SanA peptide derivatives

My retrosynthetic analysis of the SanA macrocycle is shown in Scheme 2.1. The synthesis of SanA derivatives was carried out via a convergent solution phase synthetic strategy. The desired macrocyclic peptide was generated via a head-to-tail peptide coupling of the linear pentapeptide precursor. The linear pentapeptide was generated from two fragments: a tripeptide (fragment A) and a dipeptide (fragment B). Each fragment was synthesized in solution-phase using commercially available *tert*-butyloxycarbonyl (Boc) or methyl ester (OMe) protected amino acids, coupling reagents and Hünig's base (*N*,*N*-diisopropylethylamine, DIPEA). Generating the linear precursor via a convergent approach involved fewer steps and purifications compared to using a step-wise linear approach.



Fragment B **SanA Peptide** **Fragment A**

Scheme 2.1: Retrosynthesis of SanA derivatives

2.4 Synthesis of SanA derivatives

The same convergent synthetic strategy was used to construct all five of my SanA derivatives. The commercially available amino acids used were protected either at the *N*-terminus with a Boc group or at the C-terminus as an OMe. Coupling reagents were used to facilitate peptide bond formation. For straightforward peptide couplings, TBTU was used. In the case of more difficult couplings, which involved peptide bond formation at an *N*-methyl site or larger peptide fragments, HATU and/or DEPBT were employed. Macrocyclization of the linear pentapeptides involved a modified approach to the coupling reactions of two peptides, whereby we utilized a cocktail of three coupling reagents: TBTU, HATU and DEPBT. Most peptide coupling reactions were carried out in anhydrous dichloromethane (DCM). However, insoluble starting materials required the addition of anhydrous acetonitrile (ACN) and/or dimethylformamide (DMF) to dissolve all reagents.

Four different protecting groups were employed during the synthesis of my SanA derivatives: Boc to protect amines; OMe to protect acids; benzyl (Bzl) to protect the hydroxyl group of Ser; and two carboxybenzyl (Cbz) groups to protect the guanidinium moiety of Arg (**Figure 2.9**). The Boc group was removed using 20-25% 2,2,2-trifluroacetic acid (TFA) in DCM. Methyl ester hydrolysis to yield a free acid was achieved with lithium hydroxide (LiOH) in methanol (MeOH). Hydrogenolysis performed

in the presence of a catalytic amount of 10% Palladium on Carbon (Pd/C) was used to remove the Bzl protecting group on Ser and concentrated hydrobromic acid (HBr) was used to remove the Cbz protecting groups on Arg.

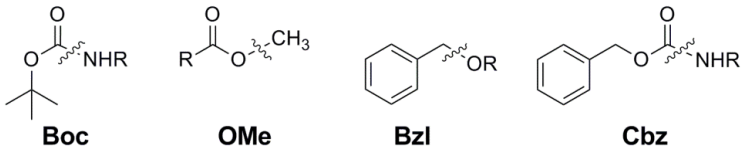


Figure 2.9: Protecting groups employed during SanA derivative synthesis.

Purification of intermediate dipeptide, tripeptide, and pentapeptide fragments was accomplished via acid-base extraction and/or column chromatography. No purification was necessary after removing the Boc or OMe protecting groups. However, both the Bzl and Cbz were removed at the final stage of synthesis, whereupon the products required purification. Purification using reverse-phase high-performance liquid chromatography (RP-HPLC) furnished final cyclized products in >98% purity, which were verified via ^{1}H Nuclear Magnetic Resonance (NMR) and liquid chromatography/mass spectrometry (LC/MS). Synthesized SanA derivatives were tested against a number of cancer cell lines including: colon (HCT-116), pancreatic (PL-45, BxPc3) and cervical (HeLa) to investigate our compounds' SAR.

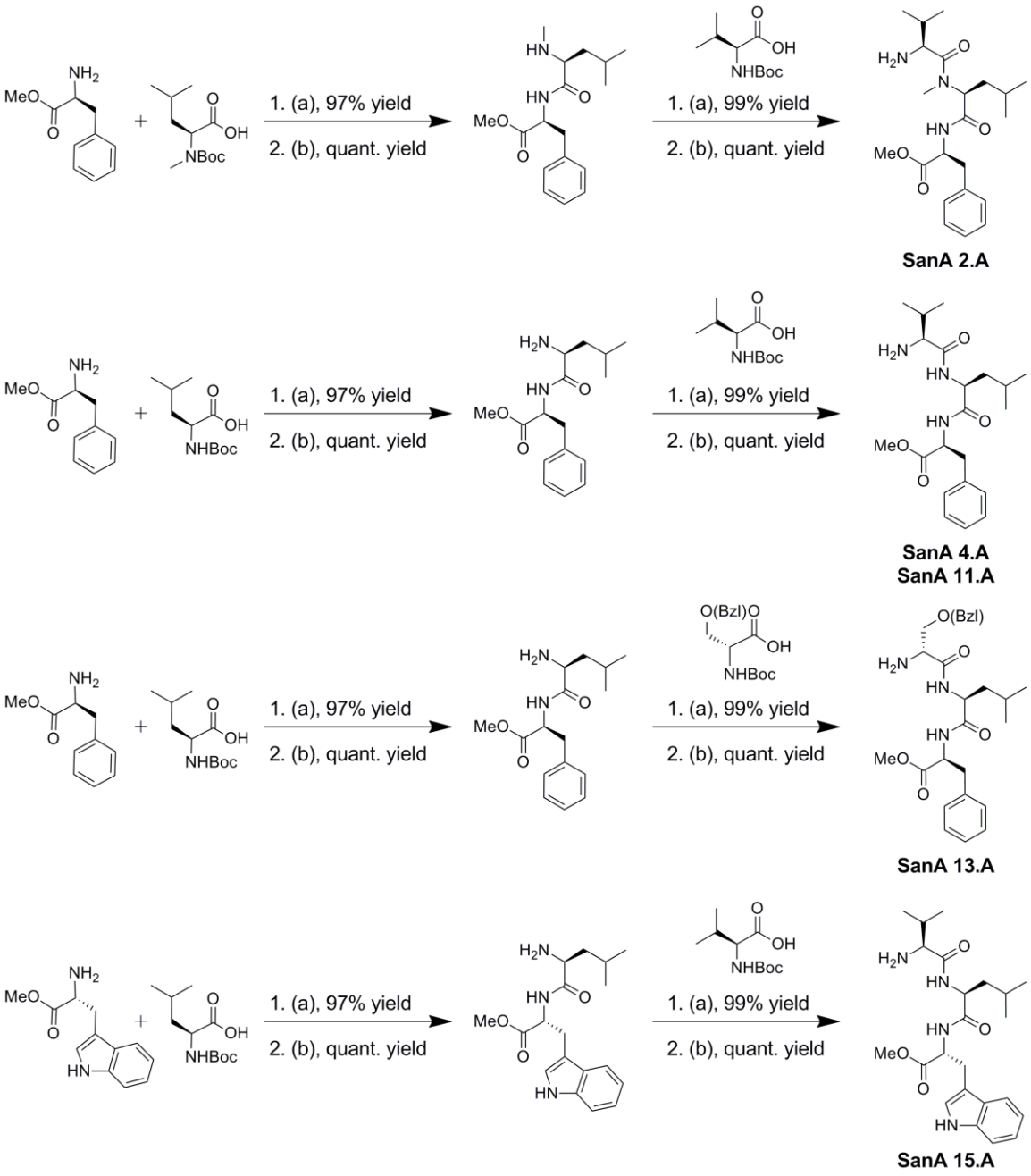
2.4.1 Synthesis of fragment A tripeptide

The synthesis of fragment A for **SanA 2** (**SanA 2.A**, **Scheme 2.2**) proceeded first with the generation of dipeptide MeO-Phe-Leu-N(Me)Boc using 1.1 equivalents of free amine MeO-Phe-NH_2, 1.0 equivalent of free acid HO-Leu-N(Me)Boc, 1.2 equivalents of coupling reagent TBTU and 4.0 equivalents of DIPEA. The amino acids and coupling reagent were added to a dry round bottom flask, which was subsequently fitted with a septum and purged with argon gas. The solid reagents were dissolved in DCM to a concentration of 0.1 M. Finally, DIPEA was added to the flask and the reaction was

allowed to proceed at room temperature for one hour and monitored via thin layer chromatography (TLC). Upon completion, the reaction was subjected to an acid-base extraction to purify the dipeptide. The crude reaction mixture was diluted with ethyl acetate (EA) and washed with 10% hydrochloric acid (HCl) solution, saturated sodium bicarbonate solution ($NaHCO_3$), and finally with brine. The organic layer was dried over sodium sulfate (Na_2SO_4), filtered, and concentrated *in vacuo*. The pure dipeptide (MeO-Phe-Leu-N(Me)Boc, 98% yield) was confirmed via ^1H NMR. A similar procedure, shown in **Scheme 2.2**, was followed for the generation of dipeptide MeO-Phe-Leu-NHBoc (95% yield), which was a common building block for compounds **SanA 4**, **SanA 11**, and **SanA 1,** and for dipeptide MeO-D-Trp-Leu-NHBoc (86% yield) used in **SanA 15**.

Removal of the Boc group from dipeptide MeO-Phe-Leu-N(Me)Boc for **SanA 2** was performed under open atmosphere in a 0.1 M solution of 25% TFA and 75% DCM. anisole (2.0 equivalents) was added to the reaction flask to act as a scavenger for the *tert*-butyl carbocation side product generated in the reaction. The reaction was run at room temperature and was monitored via TLC every 15 min. Upon completion (~1 hr), the solution was concentrated *in vacuo* to yield the free amine dipeptide MeO-Phe-Leu-N(Me)H in quantitative yield. These conditions were also applied to furnish free amines MeO-Phe-Leu-NH_2 (**SanA 4**, **SanA 11**, **SanA 13**) and MeO-D-Trp-Leu-NH_2 (**SanA 15**).

The tripeptide needed for **SanA 2** was generated by using 1.1 equivalents of free amine MeO-Phe-Leu-N(Me)H, 1.0 equivalent of free acid HO-Val-NHBoc, 0.6 equivalents of TBTU, 0.6 equivalents of HATU, and 4.0 equivalents of DIPEA in anhydrous DCM at 0.1 M concentration. HATU was necessary because peptide coupling occurred at an *N*-methyl site, which is sterically hindered site and slow to react. The reaction was run under argon at room temperature and was monitored via TLC. Upon



Scheme 2.2: Synthesis of fragment A. (a) TBTU and/or HATU (1.2 equivalents total), DIPEA (4-6.0 equivalents), DCM (0.1 M); (b) anisole (2.0 equivalents), TFA/DCM (1:4), 0.1 M

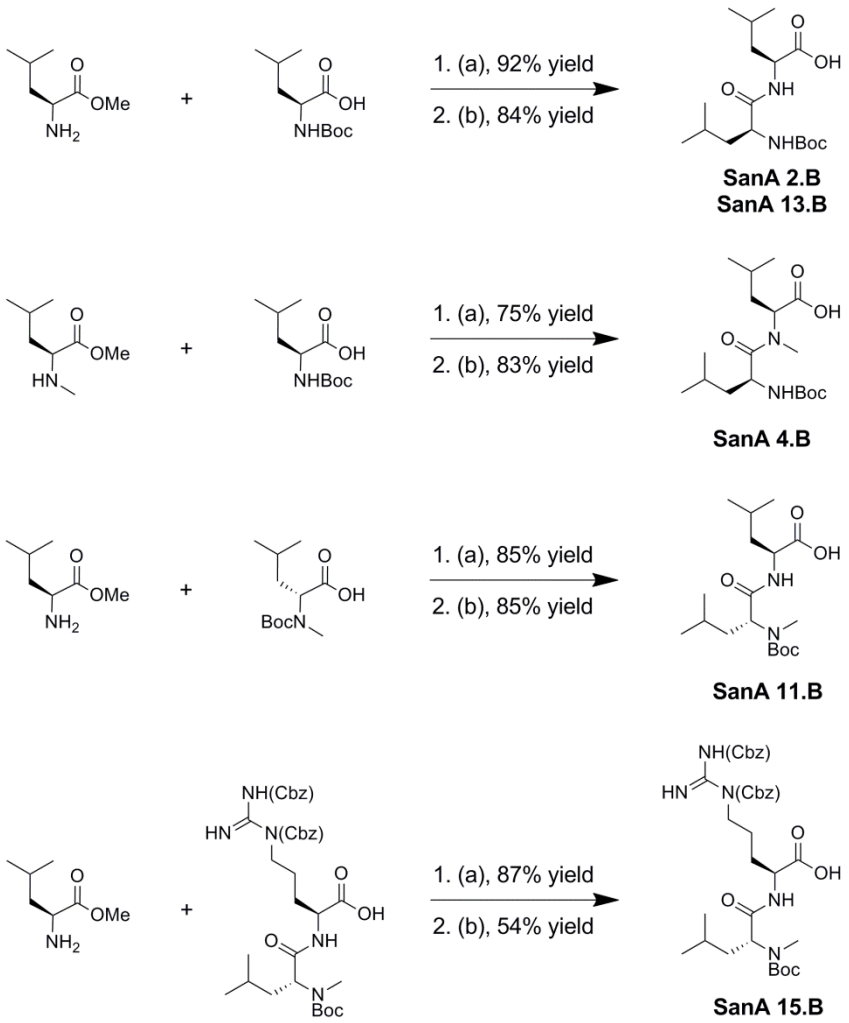
completion (~1 hr), the crude tripeptide was purified via acid-base extraction and tripeptide structure and purity were confirmed via ^{1}H NMR (MeO-Phe-Leu-N(Me)-Val-NHBoc, 94% yield). A similar set of conditions were used to generate the tripeptides for

SanA 4 and **SanA 11** (MeO-Phe-Leu-Val-NHBoc, 97% yield), **SanA 13** (MeO-Phe-Leu-Ser(Bzl)-NHBoc, 82% yield), and **SanA 15** (MeO-D-Trp-Leu-Val-NHBoc, 59% yield).

Deprotection of the amine on tripeptide MeO-Phe-Leu-N(Me)-Val-NHBoc was completed under open atmosphere. The tripeptide was diluted to 0.1 M concentration with 20% TFA and 80% DCM, with 2.0 equivalents of anisole. The reaction was monitored by TLC for disappearance of starting material and was complete within one hour. The solution was concentrated *in vacuo* to furnish **SanA 2.A** MeO-Phe-Leu-N(Me)-Val-NH_2 in quantitative yield. These conditions were also used to generate fragment A for all five of my compounds: **SanA 4.A** (MeO-Phe-Leu-Val-NH_2), **SanA 11.A** (MeO-Phe-Leu-Val-NH_2), **SanA 13.A** (MeO-Phe-Leu-D-Ser(Bzl)-NH_2), and **SanA 15.A** (MeO-D-Trp-Leu-Val-NH_2).

2.4.2 Synthesis of fragment B dipeptide

Synthesis of fragment B for **SanA 2** (**SanA 2.B**, **Scheme 2.3**) began with assembling dipeptide MeO-Leu-Leu-NHBoc using 1.1 equivalents of free amine MeO-Leu-NH_2, 1.0 equivalent of free acid HO-Leu-NHBoc, 1.2 equivalents of coupling reagent TBTU, and 4.0 equivalents of DIPEA. The reaction flask was purged with argon gas and diluted with anhydrous DCM to 0.1 M concentration. TLC monitoring showed that the coupling was complete after approximately one hour and an acid-base extraction of the reaction mixture furnished pure dipeptide. MeO-Leu-Leu-NHBoc was confirmed by ^{1}H NMR and afforded in 92% yield; this dipeptide was also used for **SanA 13**. The same procedure was applied to generate dipeptides for **SanA 4** (MeO-Leu-N(Me)-Leu-NHBoc, 75% yield), **SanA 11** (MeO-Leu-D-Leu-N(Me)Boc, 85% yield), and **SanA 15** (MeO-Arg(2Cbz)-Leu-NHBoc, 87% yield).



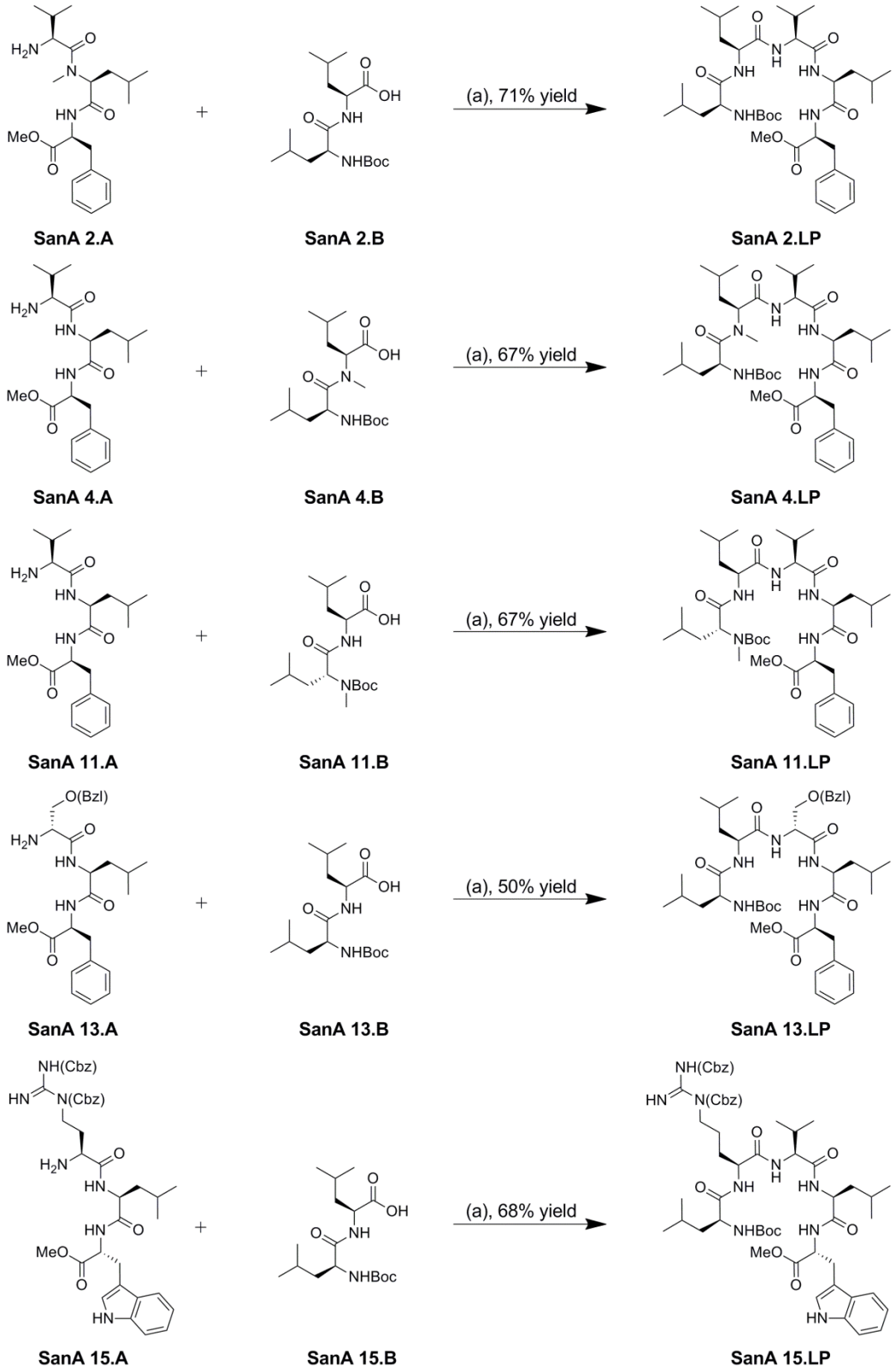
Scheme 2.3: Synthesis of fragment B (a) TBTU (1.2 equivalents), DIPEA (4.0 equivalents), DCM (0.1 M); (b) LiOH (8.0 equivalents), MeOH (0.1 M)

The pure dipeptide MeO-Leu-Leu-NHBoc was subjected to methyl ester hydrolysis to furnish free acid HO-Leu-Leu-NHBoc, fragment B (**SanA 2.B**, **Scheme 2.3**). The dipeptide was dissolved in MeOH to a concentration of 0.1M under open atmosphere, whereupon 8.0 equivalents of LiOH were added to the reaction mixture. This slurry was stirred until the TLC showed complete disappearance of starting material (~2 hrs). Upon completion, MeOH in the reaction was removed via rotary evaporator and the crude product was redissolved in EA. The reaction (diluted in EA) was extracted

with 7% HCl and then the aqueous layer was back-extracted with DCM. The organic layers were collected, dried over Na_2SO_4, filtered and concentrated *in vacuo*. HO-Leu-Leu-NHBoc (fragment B, **SanA 2.B** and **SanA 13.B**) was furnished in 84% yield and the structure was confirmed via ^{1}H NMR. The same conditions were applied to generate **SanA 4.B** (HO-Leu-N(Me)-Leu-NHBoc, 83% yield), **SanA 11.B** (HO-Leu-D-Leu-N(Me)Boc, 85% yield), and **SanA 15.B** (HO-Arg(2Cbz)-Leu-NHBoc, 54% yield).

2.4.3 Synthesis of linear pentapeptide

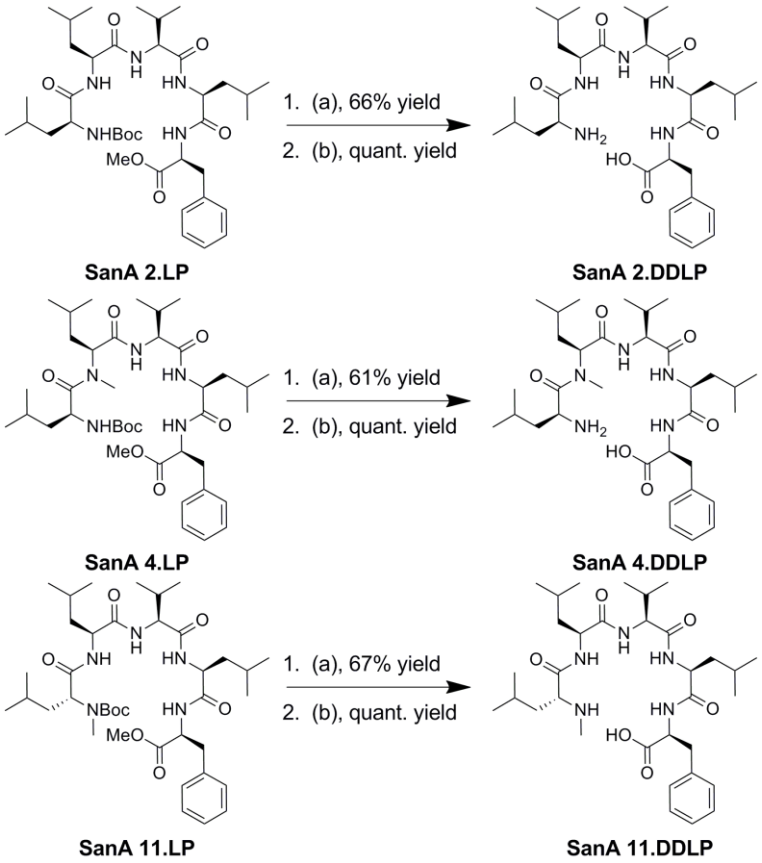
Coupling free amine fragment A and free acid fragment B (**Scheme 2.4**) generated SanA linear pentapeptides (LP). Generating linear pentapeptide **SanA 2** (MeO-Phe-Leu-N(Me)-Val-Leu-Leu-NHBoc, **SanA 2.LP**) was accomplished using 1.1 equivalents of free amine **SanA 2.A** (MeO-Phe-Leu-N(Me)-Val-NH_2), 1.0 equivalent of free acid **SanA 2.B** (HO-Leu-Leu-NHBoc), combined with 0.75 equivalents of TBTU, 0.75 equivalents of HATU, and 8.0 equivalents of DIPEA. The fragments and coupling reagents were purged with argon gas and dissolved in anhydrous ACN and DCM (2:3) for an overall concentration of 0.1 M. The reaction was monitored by TLC and upon completion (~4 hrs) crude **SanA 2.LP** underwent an acid-base extraction followed by flash column chromatography. Pure **SanA 2.LP** eluted at 50:50 Hex:EA and was furnished in 71% yield; the structure and purity were confirmed by ^{1}H NMR and LC/MS. Similar reaction conditions were applied to the synthesis of **SanA 4.LP** (MeO-Phe-Leu-Val-Leu-N(Me)-Leu-NHBoc, 67% yield), **SanA 11.LP** (MeO-Phe-Leu-Val-Leu-D-Leu-N(Me)Boc, 67% yield), **SanA 13.LP** (MeO-Phe-Leu-D-Ser(Bzl)-Leu-Leu-NHBoc, 50% yield), and **SanA 15.LP** (MeO-D-Trp-Leu-Val-Arg(2Cbz)-Leu-NHBoc, 68% yield). In the case of **SanA 13** multiple purifications via column chromatography were performed and only the purest fractions were taken on; this led to a less than desirable yield.



Scheme 2.4: Synthesis of linear pentapeptides. (a) TBTU, HATU, and/or DEPBT (1.2-1.8 equivalents total), DIPEA (6-8.0 equivalents), DCM/ACN (0.1 M)

2.4.4 Deprotection of linear pentapeptide

Two strategies were used to generate the double deprotected linear pentapeptide (**DDLP**). Method 1 involved a step-wise approach using standard acid and amine deprotection conditions; this strategy was used to generate three of my DDLP intermediates (**Scheme 2.5**). For **SanA 2.DDLP**, first methyl ester hydrolysis was performed to generate free acid HO-Phe-Leu-N(Me)-Val-Leu-Leu-NHBoc using LiOH in MeOH. The free acid was generated after 12 hrs of reaction time, and verified via LC/MS. Next, the Boc group was removed using TFA in DCM with anisole (2.0 equivalents) to furnish the double deprotected linear pentapeptide **SanA 2.DDLP** in quantitative yield. This step-wise method was also used to generate **SanA 4.DDLP** and **SanA 11.DDLP**.

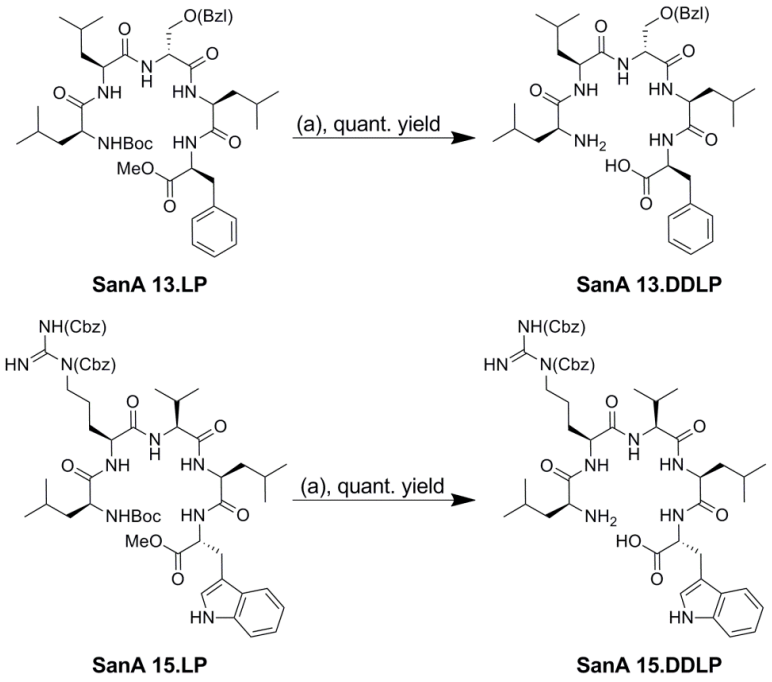


Scheme 2.5: Step-wise deprotection of linear pentapeptides (a) LiOH (8.0 equivalents), MeOH (0.1 M); (b) anisole (2.0 equivalents), TFA/DCM (1:4), 0.1 M

Method 2 involved deprotecting the acid and amine of the linear pentapeptides under acidic conditions. This strategy was used for two of my derivatives (**SanA 13** and **SanA 15**, Scheme 2.6). Though this method required more time (3 days compared to 1 day for method 1), this strategy was necessary for compounds where polar amino acids were present in order to avoid losing material in the aqueous washes that are required using method 1. **SanA 13.LP** (MeO-Phe-Leu-D-Ser(Bzl)-Leu-Leu-NHBoc) was dissolved in tetrahydrofuran (THF) to a concentration of 0.1 M and 2.0 equivalents of anisole were added to the reaction flask. 12 N HCl (8 drops per 0.3 mmol of **SanA 13.LP**) was added to the flask and the reaction stirred at room temperature under open atmosphere. The deprotection was monitored by LC/MS for the appearance of **SanA 13.DDLP**. Typically, reactions were 50% complete after the first day; however, two additional days were needed for reaction completion. An additional three drops of 12 N HCl per 0.3 mmol of **SanA 13.LP** were added to the reaction flask on both the second and third days, whereupon the reaction was complete. It was concentrated *in vacuo* and **SanA 13.DDLP** was afforded in quantitative yield. Method 2 was also used to furnish **SanA 15.DDLP** in quantitative yield.

2.4.5 Macrocyclization to generate SanA peptide derivatives

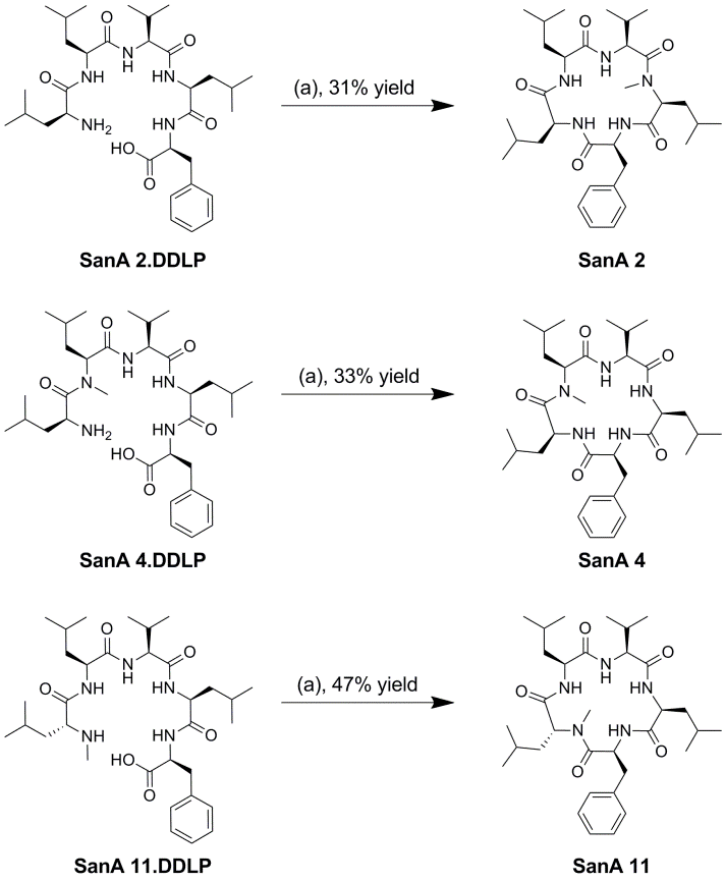
Cyclization reactions are atypical compared to coupling reactions that generate di-, tri- and pentapeptides because they are run under dilute concentrations (<0.01 M) to avoid oligomerization of our peptides. These dilute conditions slow down the reaction kinetics, and create challenges for generating macrocycles in a timely fashion with good yields. Thus, macrocyclization for each my SanA derivatives utilized a cocktail of coupling reagents and DIPEA in order to push the reaction to completion. Cyclization



Scheme 2.6: *In situ* deprotection of linear pentapeptide (a) 12 N HCl (8 drops/0.3 mmol), anisole (2.0 equivalents), THF (0.1 M), 24 h; then 3 drops/0.3 mmol 12 N HCl (day 2 and 3)

reactions were run at a concentration of 0.007 M, compared to the previously described couplings, which occurred at 0.1 M. To generate macrocycle **SanA 2** (*cyclo*- Phe-Leu-N(Me)-Val-Leu-Leu-NHBoc), 0.7 equivalents each of TBTU, HATU and DEPBT, and 8.0 equivalents of DIPEA were combined with **SanA 2.DDLP**. The double deprotected peptide and reagents were purged with argon gas and dissolved in anhydrous ACN and DCM (1:1) for an overall concentration of 0.007 M. The reaction was monitored by LC/MS for disappearance of starting material and was complete in 3 hours. Upon completion, crude **SanA 2** underwent an acid-base extraction followed by flash column chromatography. Pure **SanA 2** eluted at 95:5 EA:MeOH and was furnished in 31% yield; the structure and purity of the macrocycle was confirmed via LC/MS and ^{1}H NMR. Prior to biological testing of the SanA derivatives, RP-HPLC purification was performed. **SanA 4** (*cyclo*-Phe-Leu-Val-Leu-N(Me)-Leu, 33% yield) and **SanA 11** (*cyclo*-Phe-Leu-Val-Leu-D-Leu, 47% yield) were generated in a similar fashion and all compounds were

taken on for biological testing (Scheme 2.7). Macrocycles **SanA 13.M** (*cyclo*-Phe-Leu-D-Ser(Bzl)-Leu-Leu, 30% yield) and **SanA 15.M** (*cyclo*-D-Trp-Leu-Val-Arg(2Cbz)-Leu, 23% yield) were generated following the aforementioned procedure (Scheme 2.8), however an additional deprotection step was required to generated the desired final derivatives **SanA 13** and **SanA 15**.

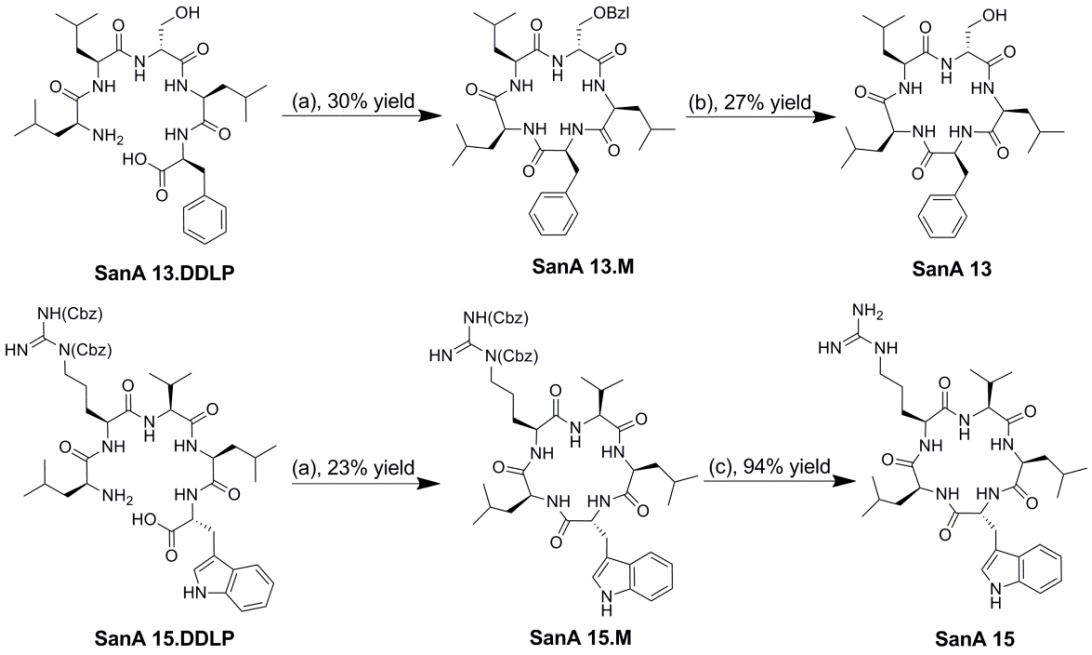


Scheme 2.7: Macrocyclization to yield **SanA 2**, **4**, and **11** (a) TBTU/HATU/DEPBT (1.8-2.0 equivalents total), DIPEA (6.0-10.0 equivalents), ACN and/or DCM (0.007 M)

2.4.6 Removal of Bzl and Cbz protecting groups

For **SanA 13**, hydrogenolysis was necessary after macrocyclization to remove the Bzl protecting group on the Ser residue. This was done by dissolving protected **SanA 13.M** in ethanol (EtOH) to 0.01 M concentration with a catalytic amount of Pd/C. Hydrogen gas was purged through the reaction solution and the deprotection was

monitored by LC/MS. After 12 hrs the reaction was complete and the mixture was filtered over Celite® to remove Pd/C (Scheme 2.8). The final compound **SanA 13** (*cyclo*-Phe-Leu-D-Ser-Leu Leu, 27% yield) was subjected to RP-HPLC purification prior to biological testing; its structure and purity were verified via ^{1}H NMR and LC/MS.



Scheme 2.8: Macrocyclization and subsequent deprotection to yield **SanA 13** and **15**. (a) TBTU/HATU/DEPBT (2.0-2.1 equivalents), DIPEA (8.0-12.0 equivalents), ACN, THF and/or DCM (0.007 M), 24 h; (b) Pd/C (cat.), EtOH (0.01-0.007 M); (c) 33% HBr in CH_3COOH (0.07 M)

To generate **SanA 15**, a deprotection was required to remove the two Cbz groups protecting the Arg residue. Typically, hydrogenolysis is used to remove the Cbz group, however, the milligram quantity of protected **SanA 15.M** macrocycle only allowed for the hydrogenolysis to be run at μM concentrations. These dilute conditions lead to long reactions times and often incomplete reactions. However, utilization of an alternative method involving 33% hydrogen bromide (HBr) in glacial acetic acid (0.07 M) furnished **SanA 15** after 2 hrs. The reaction solution was purified by extracting with ether

and filtering though cotton to furnish **SanA 15** (*cyclo*-D-Trp-Leu-Val-Arg-Leu, 94% yield). The final compound structure and purity were verified via ^{1}H NMR and LC/MS.

2.5 Biological investigation of SanA and derivatives

2.5.1 Biological activity

Cytotoxicity was determined using a thymidine uptake assay to measure cell proliferation (**Figure 2.10**). Carcinogenic cells were treated with an overall 5 μM concentration of SanA derivatives or with DMSO vehicle for a period of 48 hrs. Tritiated (^{3}H)-thymidine was added to the compound treated cells, where it was incorporated into the DNA of actively dividing cells. The amount of incorporated ^{3}H-thymidine is proportional to the amount of cell proliferation. Using a scintillation counter to compare treated cells to control cells (no SanA derivative, only 1% DMSO/99% buffer), we were able to calculate anti-proliferation (growth inhibition) activity of compounds. Lower thymidine incorporation was indicative of a decrease in cell proliferation and greater growth inhibition by the compound. Higher levels of thymidine incorporation indicated that the cell was successfully progressing through the cell cycle and the compound was not cytotoxic.

All SanA derivatives were tested for cytotoxicity against PL-45, a pancreatic cancer cell line and the trends appearing in SanA's SAR were examined based on growth inhibition data (**Figure 2.11**). **SanA 2** and **SanA 4** were a part of a series of SanA derivatives where a single *N*-methyl was placed at each of the five residues comprising the SanA pentapeptide. **SanA 2** had an *N*-methyl at position II and **SanA 4** had a single *N*-methyl at position IV. Growth inhibition data showed that placing a single *N*-methyl at positions I, IV and V were favorable in that these compounds were significantly more cytotoxic than the lead compound SanA.

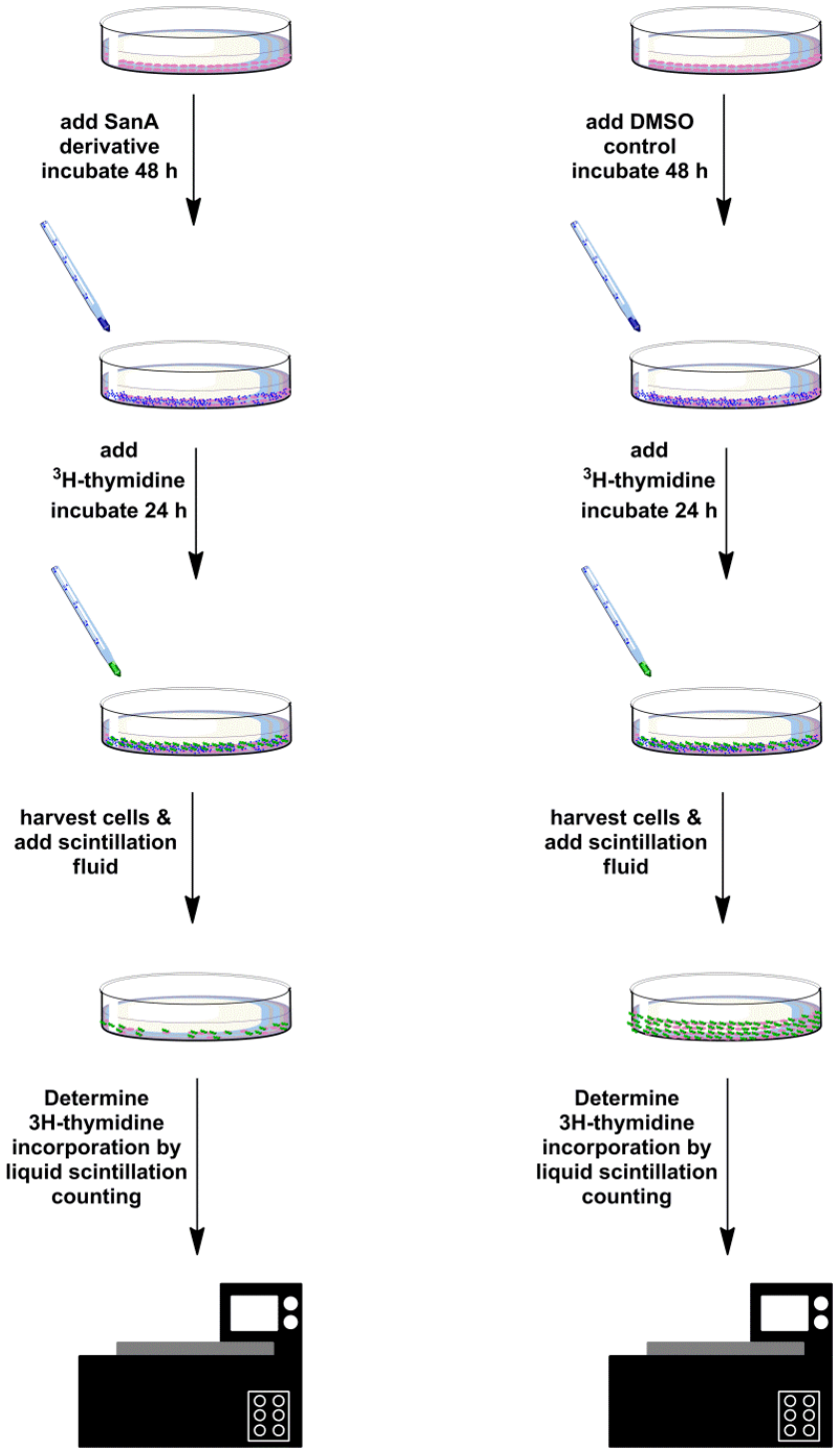


Figure 2.10: Tritiated thymidine incorporation assay

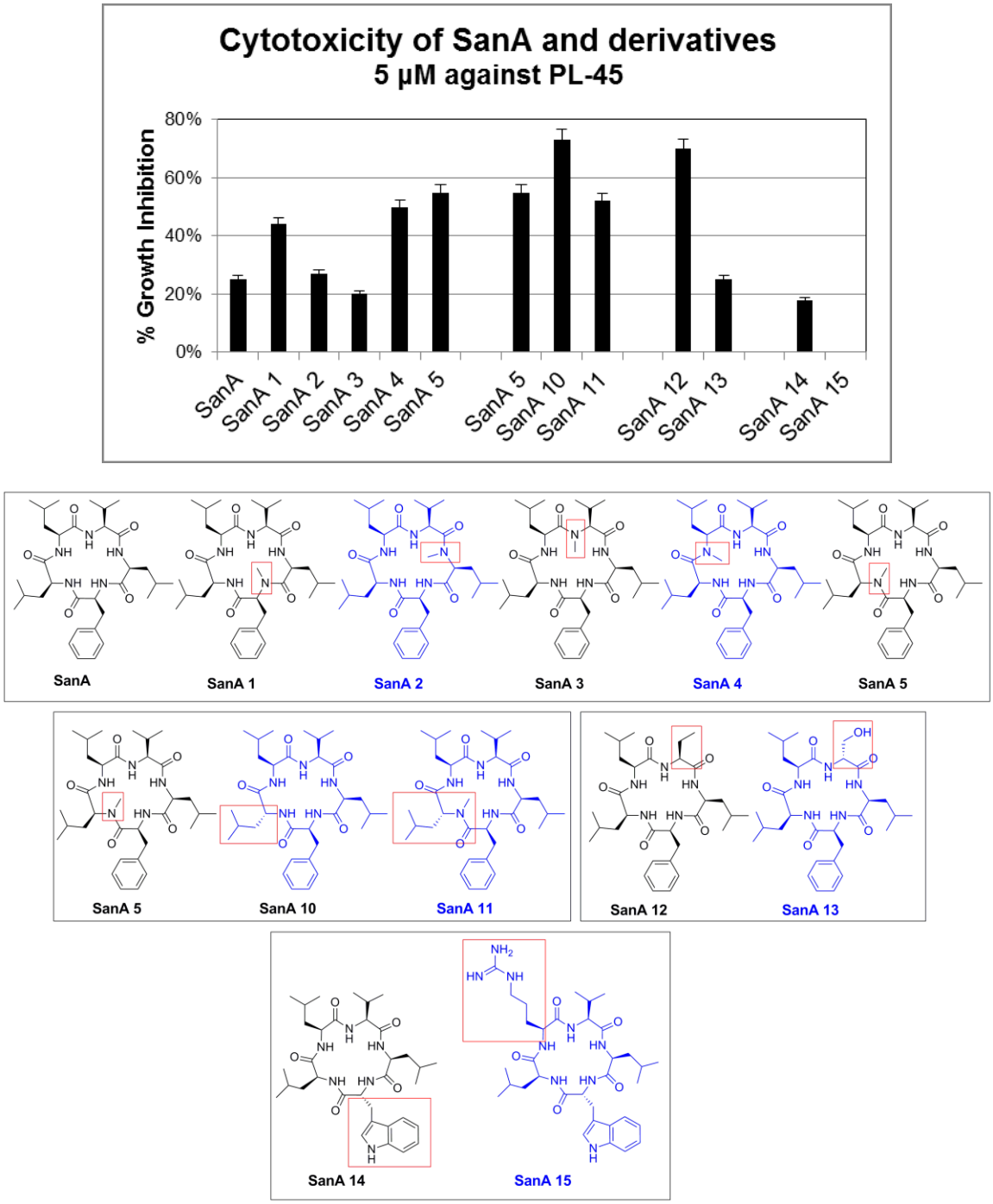


Figure 2.11: Biological activity of SanA peptide derivatives and parent compounds. Error is ±5% with compounds run in quadruplicate in 3 separate assays

As discussed earlier, we investigated the synergistic effect of incorporating the two features from **SanA5** and **SanA 10** (*N*-methyl and D-Leu at position V, respectively)

by synthesizing compound **SanA 11**. Growth inhibition data against PL-45 indicates that **SanA 11** was more potent than SanA peptide the two modifications did not have an additive effect. Incorporating polar groups generated compounds **SanA 13** and **SanA 15**, with the inclusion of a D-Ser at position III or a D-Trp at position I and an Arg at position IV respectively. Though the presence of these polar groups has improved solubility in aqueous media, growth inhibition data against PL-45 showed that cytotoxicity was adversely affected, where compounds **SanA13** and **SanA15** were significantly than the growth inhibition of SanA or parent compounds **SanA 12** and **SanA 14**, respectively. This is presumably related to the fact that polar peptides struggle to enter the cell via membrane diffusion, thus making them biologically ineffective.

2.5.2 Exploring the biological target of SanA

SanA's mechanism of action was determined using pull down assays. A SanA derivative was synthesized with an L-Lysine (L-Lys) at residue IV in order to generate a biotinylated derivative (**Figure 2.12**). Through our SAR,[4, 7, 9] we found that position IV was optimal for attaching a tag that could be used in pull-down assays.

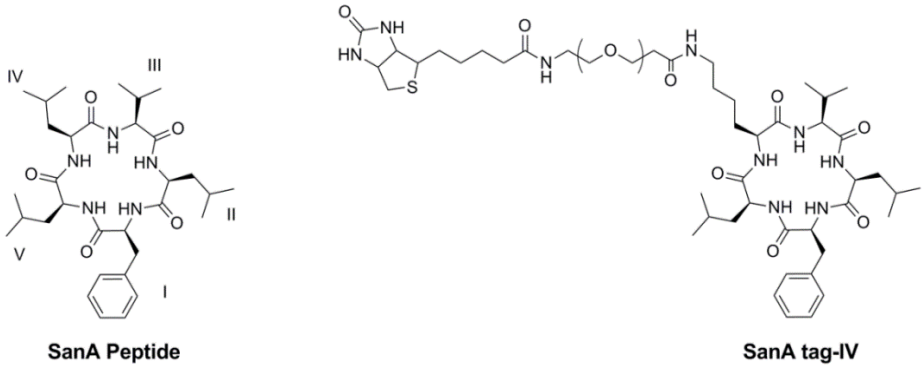
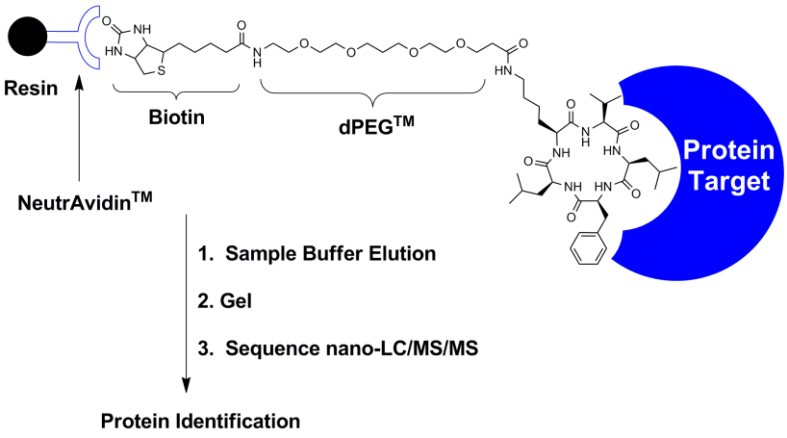


Figure 2.12: SanA biotinylated derivative (**SanA tag-IV**)

SanA tag-IV was incubated with colon cancer cell lysate (HCT-116), followed by addition of neutravadin-bound agarose beads to immobilize the compound with bound target protein(s). The beads were washed several times to remove non-specific binding

and target protein(s) were eluted using sample buffer. The eluted proteins were run on a sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) and visualized using a Coomassie blue stain. (**Figure 2.13.a**) Protein sequencing with nano-LC/MS/MS showed that five proteins were pulled down by **SanA tag-IV**: Heat shock protein 90 (HSP90), keratin, α-tubulin, β-tubulin, and actin. HSP90 was the most prominent band visualized on the gel and keratin, tubulin and actin are readily pulled down with HSP90.[15, 38, 39] A comparison of the band in the 90-95 kDa region for **SanA tag-IV** to negative controls (PEGylated biotin linker alone and vehicle) showed that there was indeed a specific interaction between HSP90 and SanA (**Figure 2.13.b**). Thus, we have determined that our SanA derivatives are HSP90 inhibitors.

(a)



(b)

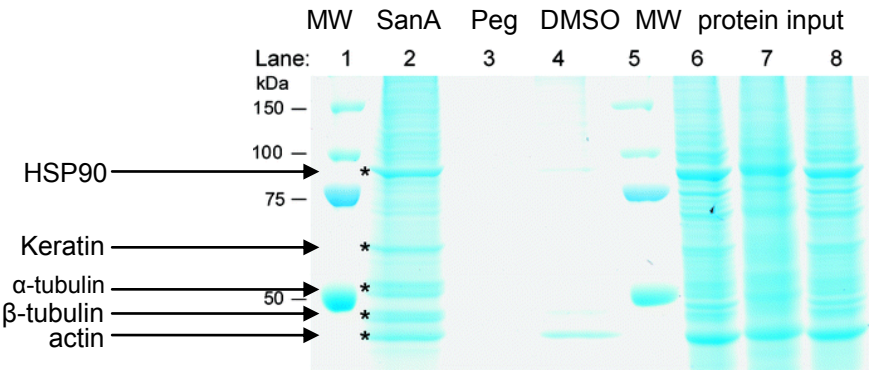


Figure 2.13: **(a)** Schematic diagram of pull-down assay **(b)** Bands isolated in the pull-down assay using HCT-116 colon cancer cell lysate. Lanes: 1 and 5, MW marker (kDa); 2, SanA tag-IV; 3, negative control (PEGlayted biotin linker); 4, DMSO control; and 6-8, protein input for lanes 2-4, respectively.

HSP90 belongs to a family of constitutively expressed molecular chaperones that are responsible for mediating proper protein folding, unfolding and stabilization.[40] HSPs are activated in response to protein denaturing stressors, which threaten cell survival (e.g. increased temperature, abnormal pH, nutrient deprivation, and hypoxia).[41] In these stressed environments, HSP expression increases as a response, ensuring that it refolds denatured proteins back to their native conformations.[41, 42 43] In normal cells, HSP90 constitutes 1-2% of total protein in the cell, and this amount increases two-fold in cancer cells, making HSP90 an attractive chemotherapeutic target.[44]

Termed for its 90kDa molecular weight, HSP90 exists predominantly as a homodimer; each homodimer is made up of a monomer that has three domains: 1) an N-terminal ATP-binding domain that is connected to 2) a middle domain by a flexible charged linker and 3) a C-terminal domain that is essential for dimerization (Figure 2.14).[44] Hydrolysis of ATP to ADP is necessary for the HSP90 dimer to switch from an open conformation, where only the C-terminal domains interact, to eventually a closed-and-twisted conformation, where both the N- and the C-terminal domains interact.[44] In a dimerized state, HSP90 is able to regulate the function of its client proteins, including those that are responsible for the progression of cancerous growth.

There are six hallmarks of cancer that are widely accepted[45]: 1) self-sufficiency in growth signals, 2) insensitivity to anti-growth signals, 3) evasion of apoptosis, 4) limitless replicative potential, 5) sustained angiogenesis, and 6) tissue invasion and metastasis. Given that HSP90 is up-regulated in cancer cells and plays an important role in regulating client proteins that contribute to these six hallmarks of cancer (**Table 2.1**), disrupting the function of this molecular chaperone may play a key role in combatting this disease.

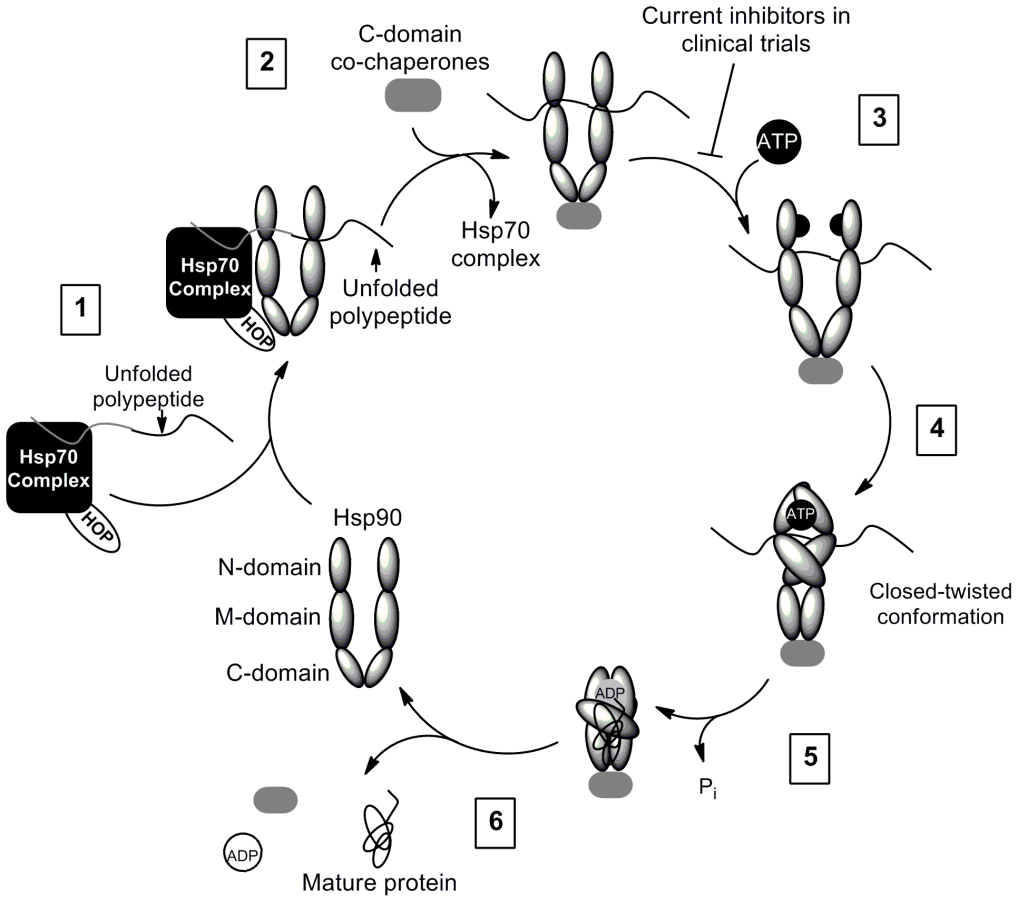


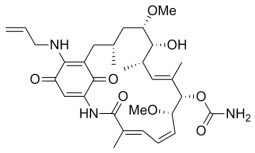
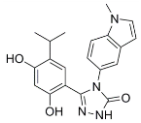
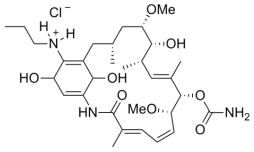
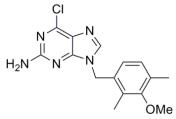
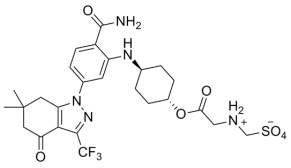
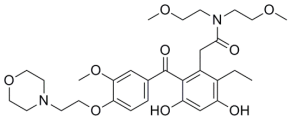
Figure 2.14: HSP90 folding cycle. Illustration adapted from reference.[44] The HSP90 cell cycle model currently proposes that client proteins and ATP bind to undimerized HSP90. ATP binding leads to dimerization of HSP90 and then a "closed-and-twisted" rearrangement of the protein where it can regulate the function of its client proteins, allowing for cell survival and ATP hydrolysis. Current HSP90 inhibitors prevent dimerization by blocking the ATP site.

Table 2.1: HSP90's role in regulating the six hallmarks of cancer

Hallmarks	Client Protein(s)
1. Self-sufficiency in growth signals	Raf-1, AKT, Her2, MEK, Bcr-Abl
2. Insensitivity to anti-growth signals	Plk, Weel, Mytl, CDK4, CDK6, Myt1
3. Evasion of apoptosis	RIP, AKT, mutant p53, c-Met, Apaf-1
4. Limitless replicative potential	Telomerase (h-Tert)
5. Sustained angiogenesis	FAK, AKT, Hif-1α, VEGFR, Flt-3,
6. Tissue invasion/metastasis	c-MET

All HSP90 inhibitors in clinical trials interact with HSP90 at the ATP binding pocket.[46] Blocking ATP prevents the chaperone from cycling between its ATP- and ADP-bound conformations, disabling HSP90's ability to facilitate proper protein folding, assembly, and stabilization of proteins.[44] There are currently 14 HSP90 inhibitors undergoing evaluation in humans, and these are involved in 41 clinical trials; 6 are in advanced clinical trials (> Phase I) as shown in **Table 2.2**.[47] Although, there are no approved HSP90 inhibitors currently on the market, there have been considerable advancements over the last decade, particularly with 17-AAG, which is undergoing Phase III clinical trials.[46]

Table 2.2: HSP90 inhibitors currently in advanced clinical trials (> Phase I)

Structure	Inhibitor	Phase	Route	Source
	17-AAG (tanespimycin, KOS-953)	II/III	Intravenous	BMS
	STA-9090 (ganetespib)	II/III	Intravenous	Synta
	IPI-504 (retaspimycin hydrochloride)	II	Intravenous	Infinity
	BIIB021 (CNF2024)	II	Oral	Biogen Idec
	AUY922 (erlotinib, OSI-774, tarceva)	I/II	Intravenous	Novartis
	KW-2478 (Velcade)	I/II	Intravenous	Kyowa Hakko Kirin

2.6 Rational design of SanA pseudo-peptide derivatives

The McAlpine group has synthesized and evaluated the biological activity of over 100 SanA derivatives. These compounds were tested against a variety of cancer cell lines including: pancreatic (PL-45, Bx-PC3), colon (HCT-116), and cervical (HeLa). From the cytotoxicity assays, we established an SAR for the SanA analogs, and three potent compounds were taken on to the next stage of development: **SanA 6**, **SanA 16**, and **SanA 17** (**Figure 2.15**).

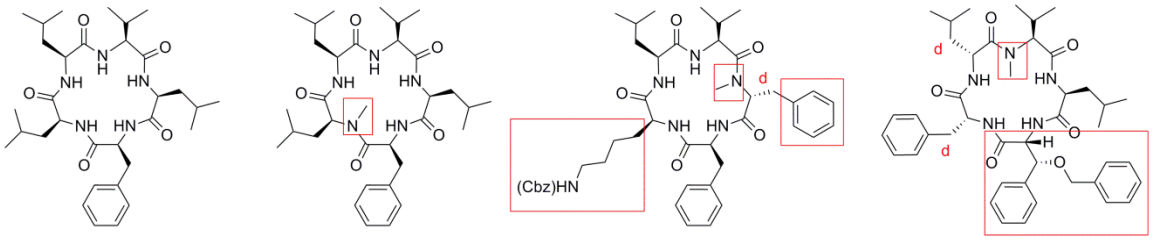


Figure 2.15: Potent SanA derivatives

The next stage of development for our potent SanA derivatives was to investigate their pharmacokinetic properties. Pharmacokinetics is divided into 4 areas, termed as ADME: absorption (process of the drug entering blood circulation), distribution (dispersion of the drug throughout the tissues in the body), metabolism (transformation of the drug into its metabolites) and excretion (the elimination of the drug from the body). We commissioned an outside agency, Biofocus Inc., to perform ADME studies on one of our lead compounds: **SanA 17**.

The solubility of **SanA 17** was assessed in a pH 7.4 phosphate buffered saline solution (with 2% DMSO) and found to be 7 μM (**Table 2.3**). Two reference compounds that are both orally bioavailable were also included in our studies: reserpine, an anti-hypertensive drug and hydrocortisone, an immunosuppressive drug. The aqueous solubility of **SanA 17** was equivalent to that of reserpine, indicating an acceptable

concentration of aqueous solubility for this derivative; however, it still had relatively low solubility compared to hydrocortisone.

Table 2.3: Kinetic solubility for **SanA 17**

Compound	Aqueous Solubility (μM)		
	Trial 1	Trial 2	Trial 3
SanA 17	7	7	7
Reserpine	7	6	7
Hydrocortisone	196	192	194

The stability of potential drugs in plasma is critical for maintaining appropriate drug concentration and half-life so that desirable pharmacological effects can be achieved. Unstable compounds in plasma tend to have rapid clearance and a short half-life, resulting in poor *in vivo* performance.[48] When **SanA 17** was tested for stability in human plasma, the compound reached the upper limit of the assay, with a half-life of over 200 min. When compared to the two controls: eucatropine (half-life = 14 min), an anti-muscarinic agent, and simvastatin, a hypolipidemic drug used to treat hypercholesterolemia (half-life = 188 min), **SanA 17** had acceptable stability in human plasma (**Table 2.4**). Since the liver is a major organ for drug metabolism, the metabolic stability of **SanA 17** in the liver was also investigated via a human hepatocyte stability study. Drugs that are rapidly metabolized (shorter half-life) typically require multiple dosing on continuous infusion, whereas a highly stable drug (longer half-life) indicates that the drug is not readily metabolized and eliminated, which may influence its safety.[49] It was found that **SanA 17** had a half-life of >172 min. When compared to midazolam, a sedative (half-life = 40 min) and hymecromone, an anti-spasmodic drug (half-life = 8 min), **SanA 17** was found to be very stable, but may not be readily cleared from the body (**Table 2.4**).

Table 2.4 Plasma and hepatocyte stability for **SanA 17**

Compound	Plasma stability: $T_{1/2}$ (min)			Hepatocyte stability: $T_{1/2}$ (min)		
	Trial 1	Trial 2	Average	Trial 1	Trial 2	Average
SanA 17	>200	>200	>200	172	>200	>172
Eucatropine	14	14	14	--	--	--
Simvastatin	>200	176	188	--	--	--
Midazolam	--	--	--	43	38	40
Hymecromone	--	--	--	8	8	8

Lastly, bi-directional Caco-2 permeability stability studies were performed to evaluate intestinal permeability (**Table 2.5**). Caco-2 cells are derived from human colon adenocarcinoma and possess many of the morphological and functional characteristics of the lining of the small intestine.[49] The permeability of a drug is measured from the apical (gut) to basolateral (blood) side (A→B) and in reverse (B→A). If compounds are passively transported across membranes, they will be equally permeable in both directions. The results for **SanA 17** showed that this compound was passively transported, with a low efflux ratio [(B→A) / (A→B) = 3]. Compared to vinblastine, an antimicrotubule drug used for the treatment of various cancers that is actively transported across membranes, **SanA 17** had a much lower efflux ratio (3 compared to 49 for vinblastine). The low efflux ratio is an excellent indicator that **SanA 17** would not be subject to the P-glycoprotein pump resistance mechanism, which pumps drugs out of the cell. This bodes well for SanA 17, and suggests it has potential as a lead Hsp90 inhibitor.

Given that **SanA 17** had poor solubility properties, we designed several derivatives of SanA based on our three potent compounds (**San A 6, 16** and **17**). Over half of all drugs on the market contain at least one heterocyclic component in their

Table 2.5: Bi-directional Caco-2 permeability assay for **SanA 17**

Compound	P_{AAP} (A→B)	P_{AAP} (B→A)	Efflux ratio
SanA 17	0.1	0.3	3
Vinblastine	1.1	55	49

structure.[50] Indeed, aromatic heterocycles are known to play a critical role in drug design as the presence of a heteroatom in a ring is known to increase interactions with biological targets and alter ADME profiles of potential drugs.[50] Therefore, our peptidomimetic analogs incorporated a single heteroaromatic ring. We focused on placing one of two heterocyclic moieties, oxazoles and thiazoles, into the peptide backbone. These two heterocycles introduce unique structural and chemical features within the macrocyclic backbone core.

We designed our pseudo-peptide derivatives using two approaches: 1) we incorporated a single oxazole or thiazole at position III to investigate the heterocycle's impact on **SanA 1**, **SanA 6**, and **SanA 16** cytotoxicity in cancer cells and 2) we walked a single oxazole around the macrocyclic backbone of **SanA 17** at positions I-III in order to investigate how the placement of a heterocycles will impact the molecule's potency. I synthesized two SanA pseudo-peptide derivatives **SanA 17-II-Ox** and **SanA 17-III-Ox** (**Figure 2.16**).

2.7 Retrosynthetic approach: SanA pseudo-peptide derivatives - Method 1

The retrosynthetic analysis (method 1) for **SanA 17-III-Ox** pseudo-peptide derivative involved constructing the oxazole moiety after macrocyclization (**Scheme 2.9**), where it was generated from a free serine. The macrocyclic precursor was formed via a head-to-tail peptide coupling of the linear precursor, followed by benzylation of the phenylserine residue. The linear precursor was built in a step-wise manner on solid-

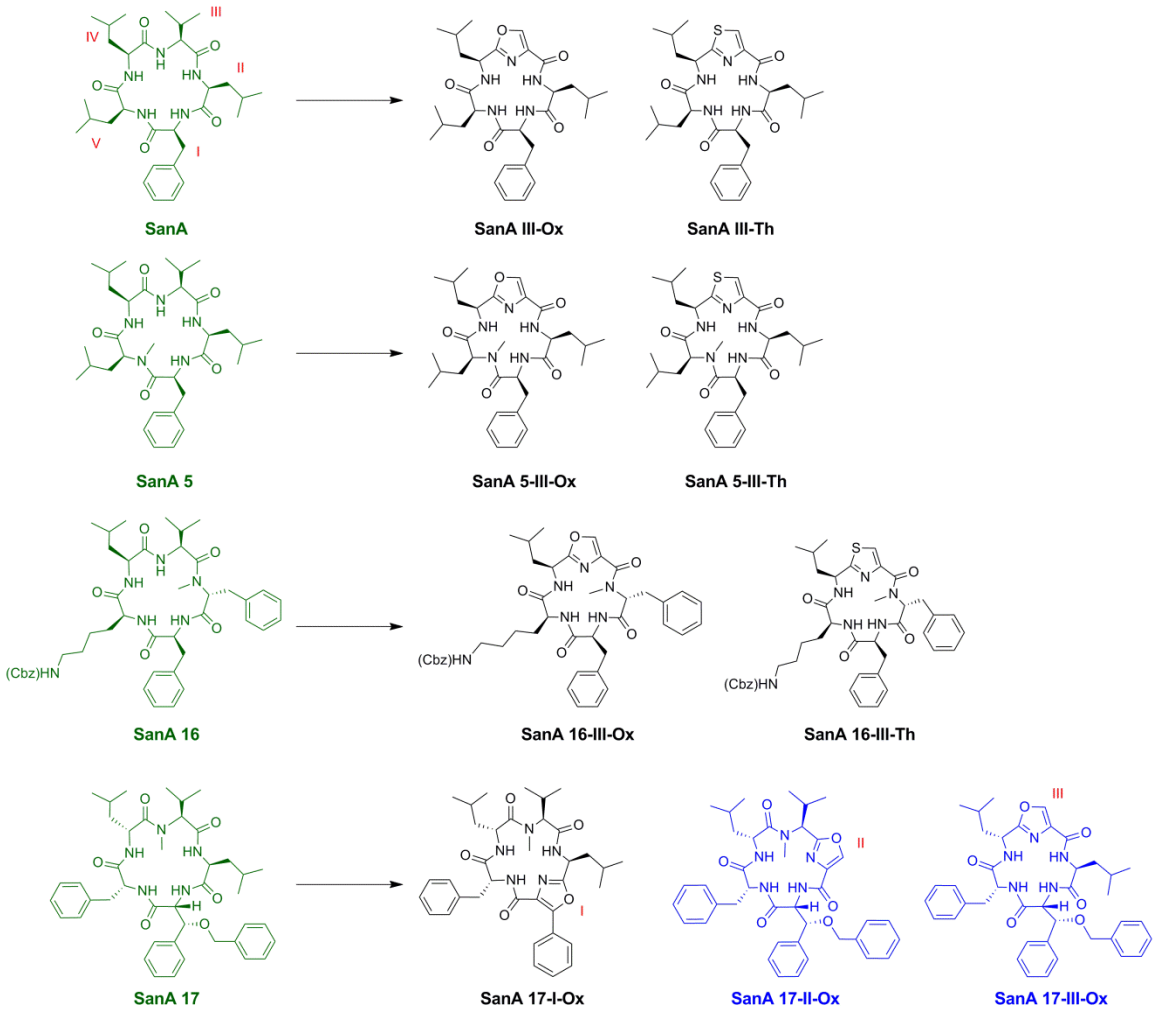
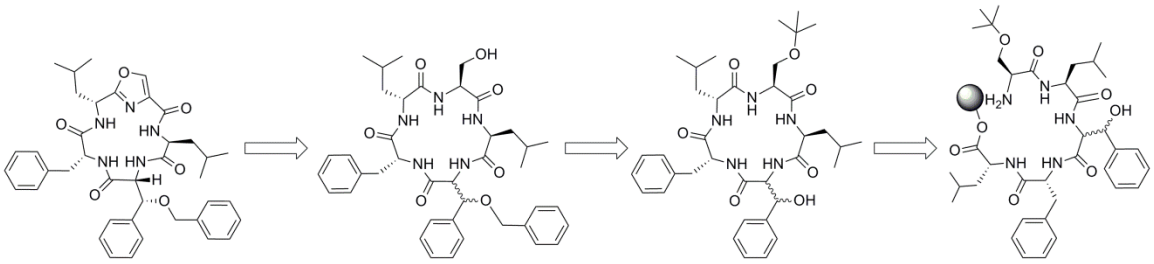


Figure 2.16: SanA pseudo-peptide derivatives. Lead compounds are colored in green and my analogs are denoted in blue. Naming of SanA pseudo-peptide derivatives follows the format: lead compound number – position of heterocyclic residue – type of heterocycle with 'Ox' for oxazole and 'Th' for thiazole

support using a chlorotrityl chloride resin pre-loaded with the first amino acid. The building blocks used to generate the linear precursor were commercially available Fmoc-protected amino acids using the coupling reagents diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole (HOBt). Upon the addition of all five amino acids, removal of the Fmoc protecting group and then cleavage of the pentapeptide from the resin, the linear precursor was primed for cyclization. This method was advantageous in that the linear precursor could be obtained in a period of 2-3 days without performing any purification steps. The macrocyclization and benzylation steps proceeded smoothly; however, due

to the steric constraints of the macrocyclic backbone, the oxazole moiety could not be generated. Thus, a second synthetic strategy was developed to avoid this problem.

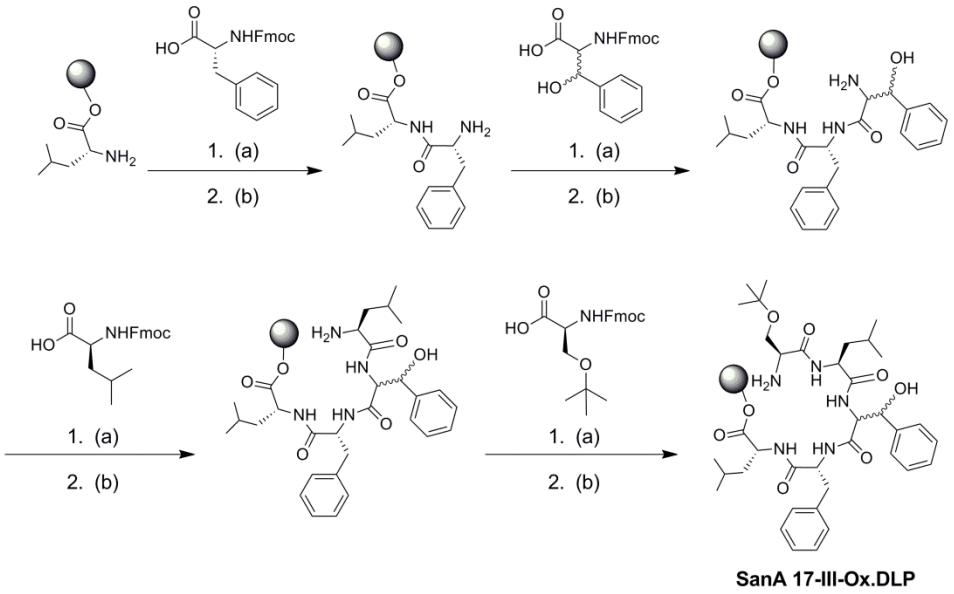


Scheme 2.9: ***Method 1 - Retrosynthetic strategy for SanA pseudo-peptide derivatives***

2.8 Synthesis of SanA pseudo-peptide derivatives – Method 1

2.8.1 Solid phase synthesis of linear pentapeptide

A linear stepwise approach was used to build the pentapeptide on solid support to generate **SanA 17-III-Ox** (**Scheme 2.10**). The synthesis of **SanA 17-III-Ox.DLP** began with commercially available chlorotrityl chloride (CTC) resin pre-loaded with D-Leu-NH_2. Prior to the first coupling, the pre-loaded resin was swollen in DMF for 30 min in a polypropylene solid-phase extraction cartridge fitted with a 20 μm polyethylene frit. HO-D-Phe-NHFmoc (3.0 equivalents) and 1-hydroxybenzotriazole (HOBt, 3.0 equivalents) were dissolved in DMF at a 0.2 M concentration. DMF used to swell the resin was drained from the cartridge and the solution of free acid and coupling reagent were added to the reaction. Diisopropylcarbamide (DIC, 6.0 equivalents) were added to the reaction vessel and the slurry shook for two hrs. Reaction completion was confirmed by a negative Kaiser test. The Kaiser test involves a common indicator, ninhydrin, which turns from yellow to blue in the presence of primary and secondary amines. Thus, a yellow color (negative Kaiser test) corresponds to complete peptide coupling, while a blue color corresponds to a reaction that has not gone to completion. The reaction mixture was drained to furnish Resin-O-D-Leu-D-Phe-NHFmoc in quantitative yield.



Scheme 2.10: Solid-phase synthesis of free amine linear pentapeptide on resin. (a) HOBt (3.0 equivalents), DIC (6.0 equivalents), DMF (0.2 M); (b) 20% Piperidine/DMF

The Fmoc protecting group of pure dipeptide Resin-O-D-Leu-D-Phe-NHFmoc was removed under basic conditions. The resin was treated with the following Fmoc removal washes (draining the solvent after the indicated shaking time): DMF (3 x 1 min), 20% piperidine in DMF (1 x 5 min, then 1 x 10 min), DMF (3 x 1 min), isopropanol (IPA) (1 x 1 min), DMF (1 x 1 min), IPA (1 x 1 min), and then DMF (3 x 1 min). After the washes were drained, free amine resin bound dipeptide Resin-O-D-Leu-D-Phe-NH_2 was furnished in quantitative yield.

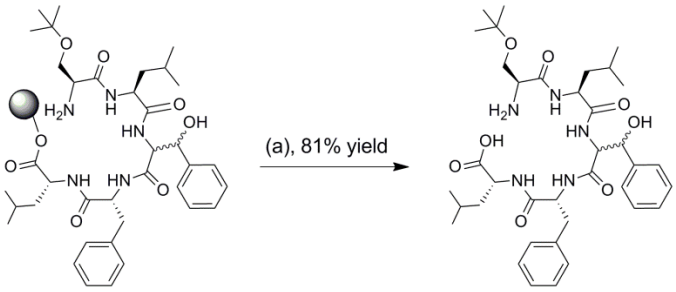
Tripeptide Resin-O-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHFmoc was generated using the same solid-phase coupling conditions and commercially available racemic free acid HO-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHFmoc (3.0 equivalents). The on resin peptide coupling took two hrs and was verified via a negative Kaiser test; the tripeptide was afforded in quantitative yield. To prepare the peptide for a subsequent coupling, the amine was deprotected using the Fmoc removal washes described above. Free amine tripeptide Resin-O-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NH_2 was furnished in quantitative yield.

The next coupling occurred with 3.0 equivalents of free acid HO-Leu-NHFmoc using standard solid-phase coupling conditions. The reaction was allowed to shake for 2 hrs, whereupon completion of the peptide coupling was verified via a negative Kaiser test. Tetrapeptide Resin-O-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-Leu-NHFmoc was afforded in quantitative yield and taken on for a subsequent amine deprotection. The Fmoc protecting group was removed from the resin bound tetrapeptide using the Fmoc removal washes. The free amine tetrapeptide Resin-O-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-Leu-NH_2 was afforded in quantitative yield and primed for a final solid phase peptide coupling.

Pentapeptide Resin-O-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-Leu-Set(*t*-Bu)-NHFmoc was synthesized using standard solid phase coupling conditions. 3.0 equivalents of free acid HO-Ser(*t*-Bu)-NHFmoc were used and the reaction was allowed to shake for 2 hrs. A negative Kaiser test confirmed reaction completion and the desired resin bound pentapeptide was furnished in quantitative yield. To prepare for macrocyclization, the amine was deprotected using the Fmoc removal washes described above and free amine pentapeptide Resin-O-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-Leu-Set(*t*-Bu)-NH_2 (**SanA 17-III-Ox.DLP**, **Scheme 2.10**) was furnished in quantitative yield.

2.8.2 Cleavage of linear pentapeptide from resin

SanA 17-III-Ox.DDLP was furnished by stirring free amine resin bound LP in a 1:1 (v:v) mixture of *2,2,2*-Trifluoroethanol (TFE) and DCM for 36 hrs. The slurry was poured over filter paper on a Buchner funnel and washed with DCM to fully isolate **SanA 17-III-Ox.DDLP**. The organic solution was concentrated *in vacuo* overnight to ensure complete removal of TFE. **SanA 17-III-Ox.DDLP** HO-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-Leu-Set(*t*-Bu)-NH_2 was afforded in 81% yield (**Scheme 2.11**).

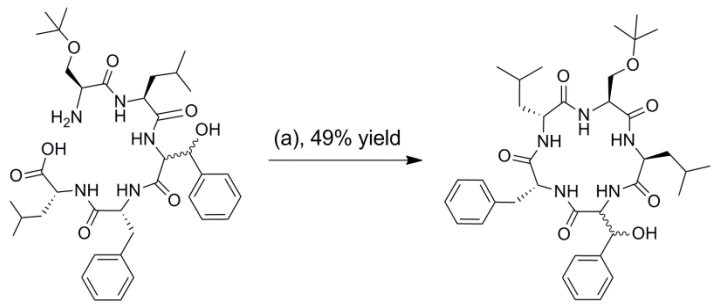


SanA 17-III-Ox.DLP **SanA 17-III-Ox.DDLP**

Scheme 2.11: Cleavage of linear pentapeptide from resin to yield SanA 17-III-ox.DDLP (a) TFE/DCM (1:1) 10 mL/g of resin

2.8.3. Macrocyclization of SanA pentapeptides

When cyclizing parent compound **SanA 17**, our lab found that this pentapeptide was prone to dimerization, even at very dilute conditions (0.007 M). To avoid this potential problem, **SanA 17-III-Ox.DDLP** was cyclized using a syringe pump in addition to very dilute macrocyclization conditions. TBTU (0.6 equivalents), HATU (0.8 equivalents) and DEPBT (0.6 equivalents) were placed in a round bottom flask, sealed with a rubber septum, and purged with argon gas. The coupling reagents were dissolved in 75% of the total volume of anhydrous solvent used in the reaction to attain an overall concentration of 0.007 M with 1:1:1 of ACN:DCM:DMF. **SanA 17-III-Ox.DDLP** was dissolved in the remaining anhydrous solvent and added to the reaction flask via syringe pump at a rate of 0.5 mL/min. The macrocyclization reaction stirred for 12 hours after all of **SanA 17-III-Ox.DDLP** was added to the flask, at which point reaction completion was confirmed by LC/MS. The reaction mixture was subjected to a wash with 10% HCl solution. The organic layer was collected, dried over sodium sulfate, filtered and concentrated *in vacuo*. The crude product was purified via automated flash chromatography; racemic **SanA 17-III-Ox.M.1** eluted at 100% EA and was furnished in 49% yield; the structure and purity was confirmed by LC/MS and ^{1}H NMR (Scheme 2.12).



SanA 17-III-Ox.DDLP **SanA 17-III-Ox.M.1**

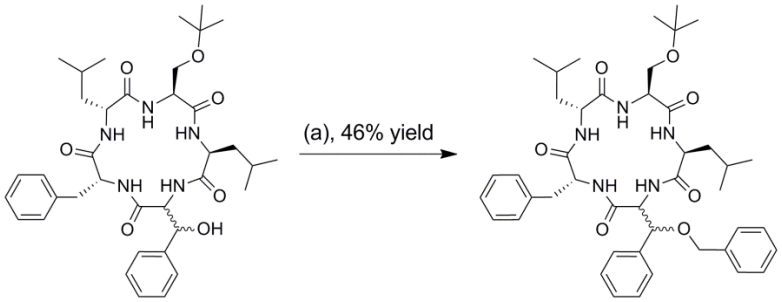
Scheme 2.12: Macrocyclization to generate **SanA 17-III-Ox.M.1** (a) TBTU (0.6 equivalents), HATU (0.6 equivalents), DEPBT (0.6 equivalents), DIPEA (6.0 equivalents), DCM (0.007 M)

2.8.4 Benzylation of macrocycle

Benzylation of the hydroxyphenylalanine residue of racemic *cyclo*-(2*R*,3*R*)/(2*S*,3*S*)-β-hydroxyphenylalanine-Leu-Ser(*t*-Bu)-D-Leu-D-Phe (**SanA 17-III-Ox.M.1**) was the next step in the generation of **SanA 17-III-Ox**. This was accomplished by dissolving **SanA 17-III-Ox.M** (1.0 Equivalent) and 2.0 equivalents of sodium hydride (NaH, 60% in mineral oil) in anhydrous THF at 0.01 M concentration in an oven dried round bottom flask. The reaction was run under argon atmosphere and stirred at -47 °C for 15 minutes prior to the dropwise addition of 2.0 equivalents of benzyl bromide (BnBr). The reaction was monitored by LC/MS and was complete in 12 hours. Upon completion, the excess reagents were quenched with water. The organic layer was collected, dried over sodium sulfate, filtered and concentrated *in vacuo*. Washed **SanA 17-III-Ox.Bz.1** was taken on for RP-HPLC purification and furnished in 46% yield; the structure and purity were confirmed via LC/MS and ^{1}H NMR (Scheme 2.13).

2.8.5 t-Bu removal

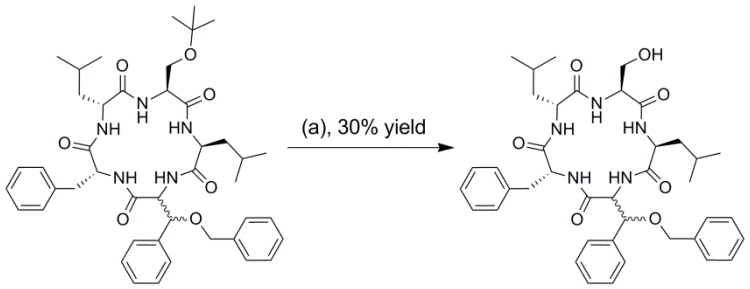
To prepare for oxazole formation, the *t*-Bu protecting group on the Ser residue of **SanA 17-III-Ox.Bz.1** was removed under acidic conditions. *Cyclo*-(2R,3R)/(2S,3S)-β-benzylphenylalanine-Leu-Ser(*t*-Bu)-D-Leu-D-Phe (**SanA 17-III-Ox.Bz.1,** 1.0 equivalent) and anisole (2.0 equivalents) were mixed with 50% TFA in DCM at 0.01 M



SanA 17-III-Ox.M.1 **SanA 17-III-Ox.Bz.1**

Scheme 2.13. Benzylation to generate **SanA 17-III-Ox.Bz.1** (a) NaH (2.0 equivalents), BnBr (2.0 equivalents), THF (0.01 M)

concentration. The deprotection reaction stirred until completion (2 hours), which was monitored by LC/MS. The crude mixture was concentrated *in vacuo* and subjected to further purification by RP-HPLC. Pure **SanA 17-III-Ox.FS.1** eluted at 100% ACN in 30% yield after RP-HPLC purification (Scheme 2.14).



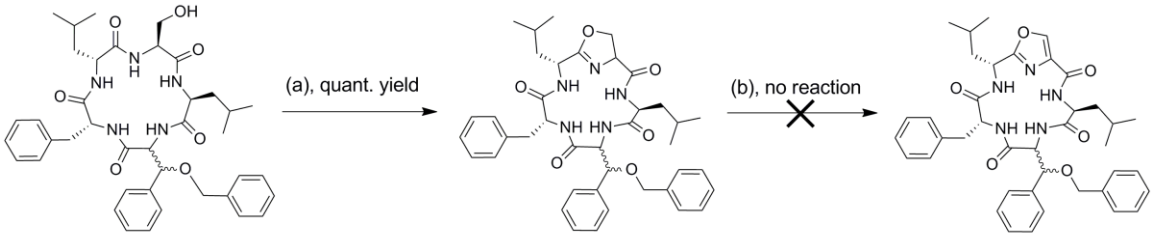
SanA 17-III-Ox.Bz.1 **SanA 17-III-Ox.FS**

Scheme 2.14: *t*-Bu removal to generate **SanA 17-III-Ox.FS.1** (a) anisole (2.0 equivalents), TFA/DCM (1:1) 0.01 M

2.8.6 Oxazole formation

The final step to generate the desired **SanA 17-III-Ox**, was to synthesize an oxazole moiety along the macrocyclic peptide backbone (Scheme 2.15) With the free serine in hand, oxazole formation proceeded by fluorination with diethylaminosulfur trifluoride (DAST). **SanA 17-III-Ox.FS.1** was dissolved in anhydrous DCM to 0.1 M concentration under argon gas at -78 °C. DAST (1.1 equivalents) were added to the reaction flask dropwise and the solution stirred for 20 min at -78 °C. Potassium carbonate (2.0 equivalents) was added to the reaction for cyclization to generate an

oxazoline intermediate. The reaction was brought to room temperature and stirred overnight. Upon completion, the crude reaction mixture was diluted with DCM and subjected to a base wash and dried over Na_2SO_4. Filtration and concentration of the reaction *in vacuo* furnished *cyclo*-(2*R*,3*R*)/(2*S*,3*S*)-β-benzylphenylalanine-Leu-oxazoline-D-Leu-D-Phe, which was confirmed by TLC, LC/MS and ^{1}H NMR. The oxazoline intermediate was then dissolved in anhydrous DCM at 0.1 M concentration under argon gas and cooled to -47 °C. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU, 2.0 equivalents) were added dropwise to the flask and the reaction stirred for 20 min. Bromotrichloromethane ($BrCCl_3$, 2.0 equivalents) were then added dropwise to the flask and the reaction stirred for -47 °C for 2 hrs. The reaction warmed to room temperature and stirred for an additional 12 hrs. The reaction was not complete by TLC or LC/MS, so the reaction stirred for an additional 24 hrs. After running the reaction a total of 36 hrs, no starting material remained, however, the desired oxazole was not formed and the peptide decomposed. Thus, an alternative synthetic route was developed to generate the desired SanA pseudo-peptide derivatives.



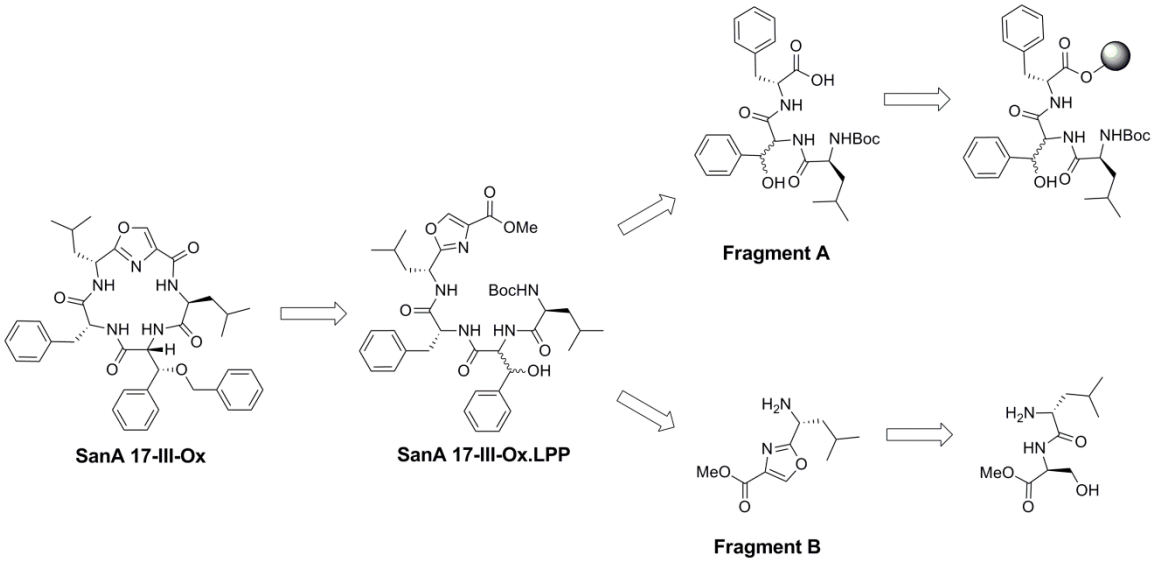
SanA 17-III-Ox.FS

Scheme 2.15: Failed attempt of oxazole formation to generate **SanA 17-III-Ox** (a) DAST (1.1 equivalents), K_2CO_3 (2.0 equivalents), DCM (0.1 M), -78 °C; (b) DBU (2.0 equivalents), $BrCCl_3$ (2.0 equivalents), DCM (0.1 M), -47 °C

2.9 Retrosynthetic approach: SanA pseudo-peptide derivatives - Method 2

A revised retrosynthetic analysis (method 2) to synthesize **SanA 17-III-Ox** pseudo-peptide derivative is shown in Scheme 2.16. Synthesis of **SanA 17-II-Ox** and **SanA 17-III-Ox** proceeded via a convergent strategy that incorporated both solution and

solid phase synthesis. The desired macrocyclic peptide was generated via a head-to-tail peptide coupling of the linear pseudopeptide precursor, which was subsequently benzylated. The linear pseudopeptide precursor was generated from two fragments: a tripeptide (fragment A) and an oxazole containing pseudo-dipeptide (fragment B). Fragment A was built on solid support synthesis using a combination of Fmoc and Boc amine protecting groups. Fragment B, which contained an oxazole moiety was generated from cyclization and oxidation with a free serine residue along the dipeptide backbone. In addition to the steric constraints of the macrocycle, which prevented the synthesis of the oxazole moiety on the macrocycle, we found that generating the oxazole on a smaller fragment (e.g. a dipeptide) gave high yields.



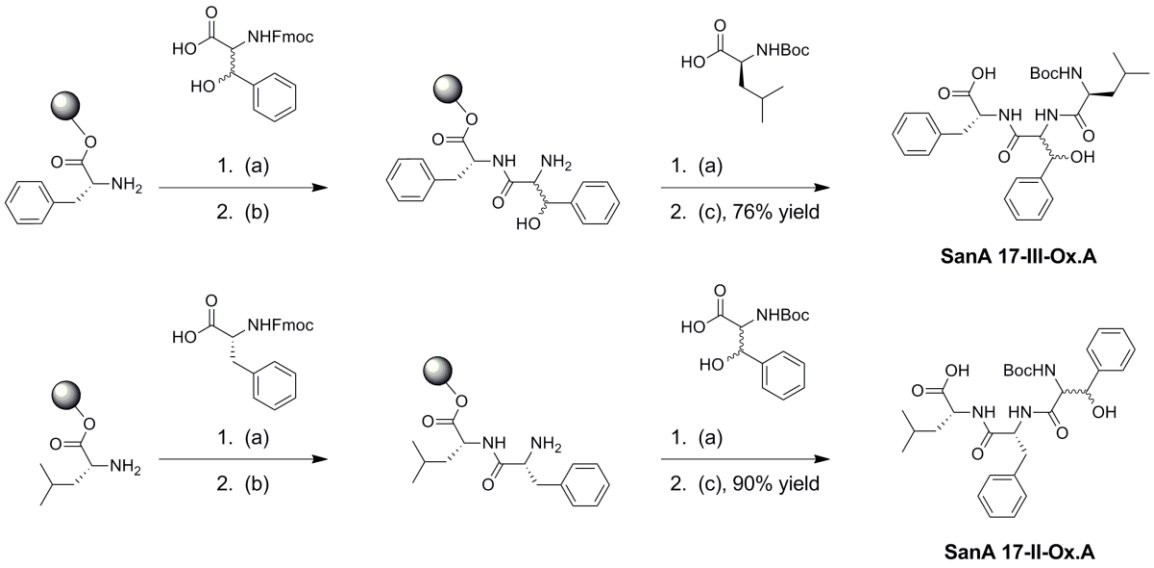
Scheme 2.16: Method 2 - Retrosynthetic strategy for SanA pseudo-peptide derivatives

2.10 Synthesis of SanA pseudo-peptide derivatives – Method 2

2.10.1 Solid phase synthesis of fragment A tripeptide

A solid phase approach was used to synthesize fragment A tripeptides to generate my two heterocycle containing compounds: **SanA 17-III-Ox** and **SanA 17-II-Ox** (**Scheme 2.17**). The synthesis of **SanA 17-III-Ox.A** began with commercially

available CTC resin pre-loaded with D-Phe-NH_2 (Resin-O-D-Phe-NH_2), which was swollen in DMF for 30 min in a cartridge. Racemic free acid HO-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHFmoc (3.0 equivalents) and HOBt (3.0 equivalents) were dissolved in DMF for an overall reaction concentration of 0.2 M. The free acid and coupling reagent solution were added to the swollen pre-loaded resin in a cartridge, followed by the addition of DIC (6.0 equivalents). The slurry was allowed to shake for two hours and reaction completion was confirmed by a negative Kaiser test. The reaction mixture was drained to furnish the resin bound Fmoc protected dipeptide Resin-O-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHFmoc in quantitative yield.



Scheme 2.17: Synthesis of fragment A on solid support. (a) HOBt (3.0 equivalents), DIC (6.0 equivalents), DMF (0.2 M); (b) 20% Piperidine/DMF; (c) TFE/DCM (1:1) 10 mL/g of resin

Removal of the Fmoc group from dipeptide Resin-O-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHFmoc was performed in a cartridge with two washes of 20% piperdine in DMF over a period of 15 min. Subsequent washes of IPA and DMF afforded the free amine Resin-O-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NH_2 ready for next coupling.

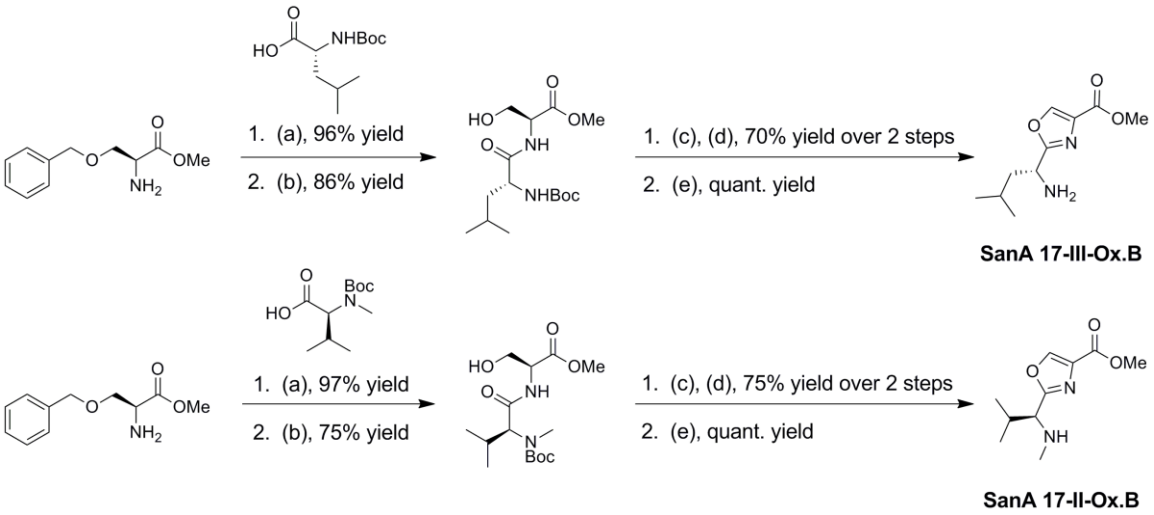
The tripeptide needed for Fragment A (**SanA 17-III-Ox**) was synthesized by adding a solution of free acid HO-Leu-NHBoc (3.0 equivalents) and HOBt (3.0

equivalents) in 0.2 M DMF to the cartridge containing Resin-O-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NH_2. DIC (6.0 equivalents) were added to the reaction cartridge and the vessel was allowed to shake for 2 hrs. A negative Kaiser test confirmed the reaction completion to yield tripeptide Resin-O-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-Leu-NHBoc.

The final step in the preparation of **SanA 17-III-Ox.A** was cleaving the tripeptide from the resin to yield free acid HO-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-Leu-NHBoc. After the final peptide coupling, the resin bound tripeptide was dried *in vacuo* overnight and a quantitative sample mass was determined. The resin bound tripeptide stirred for 36 hrs in a 1:1 ratio of TFE and DCM (10 mL per gram of resin). The slurry was filtered to separate the resin from the free acid tripeptide (**SanA 17-III-Ox.A**) and was concentrated *in vacuo* (75% yield). The tripeptide for Fragment A (**SanA 17-II-Ox.A**) was synthesized using the coupling method described above, deprotection and cleavage conditions furnished HO-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHBoc in 90% yield.

2.10.2 Solution phase synthesis of fragment B pseudo-dipeptide

A solution phase synthetic strategy was used to generate the oxazole containing pseudo-dipeptide **SanA 17-III-Ox.B** (**Scheme 2.18**). Free acid HO-D-Leu-NHBoc (1.0 equivalent), free amine MeO-Ser(Bzl)-NH_2 (1.1 equivalents) and TBTU (1.2 equivalents) were dissolved in anhydrous DCM under an atmosphere of argon. DIPEA (8.0 equivalents) was added to the reaction flask and the solution stirred at room temperature. Upon completion, confirmed by TLC (~1.5 hrs), the reaction was subjected to an acid-base extraction to furnish pure MeO-Ser(Bzl)-D-Leu-NHBoc in 96% yield. This same procedure was used to synthesize dipeptide MeO-Ser(Bzl)-Val-N(Me)Boc for **SanA 17-II-Ox.B** (97% yield).



Scheme 2.18: Synthesis of oxazole containing fragment B (a) TBTU (1.2 equivalents), DIPEA (8.0 equivalents), DCM (0.1 M); (b) H_2, Pd/C (cat.), EtOH (0.1 M); (c) DAST (1.1 equivalents), K_2CO_3 (2.0 equivalents), DCM (0.1 M), -78 °C; (d) DBU (2.0 equivalents), $BrCCl_3$ (2.0 equivalents), DCM (0.2 M), -47 °C to rt; (e) anisole (2.0 equivalents), TFA/DCM (1:4), 0.1 M

Dipeptide MeO-Ser(Bzl)-D-Leu-NHBoc was subjected to Pd-catalyzed hydrogenolysis to remove the benzyl protecting group. The protected dipeptide was dissolved in EtOH at 0.1 M concentration and a catalytic amount of Pd/C was added to the round bottom flask. Several balloons full of hydrogen gas were purged through the reaction flask at low atmospheric pressure. The reaction was monitored by TLC and upon completion the mixture was filtered over Celite® to yield pure dipeptide MeO-Ser-D-Leu-NHBoc in 86% yield. The same hydrogenolysis reaction conditions were used to furnish dipeptide MeO-Ser-D-Leu-NHBoc for **SanA 17-II-Ox.B** in 75% yield.

Formation of the oxazole moiety was accomplished by dissolving the dipeptide, MeO-Ser-D-Leu-NHBoc, in anhydrous DCM to a 0.1 M concentration under argon gas at -78 °C. DAST (1.1. equivalents) was added dropwise to the reaction flask to allow for fluorination of the serine hydroxyl and the solution stirred for 20 min at -78 °C. Cyclization to generate an oxazoline intermediate was accomplished by adding potassium carbonate (2.0 equivalents) to the reaction flask. The reaction was brought to room temperature as it was stirred overnight. Upon completion, the crude mixture was

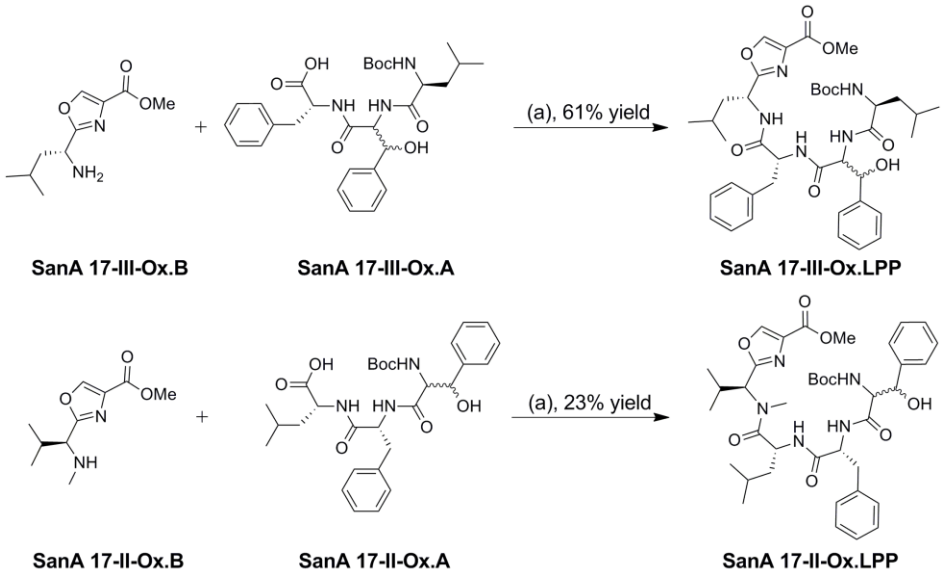
diluted with DCM and subjected to a base wash, dried over Na_2SO_4, filtered and concentrated *in vacuo* to furnish MeO-oxazoline-D-Leu-NHBoc. The washed oxazoline intermediate was dissolved in anhydrous DCM at 0.1M concentration under argon gas and cooled to -47 °C. DBU (2.0 equivalents) was added drop-wise to the flask and the reaction was allowed to stir for 20 min. Finally, $BrCCl_3$ (2.0 equivalents) was added drop-wise to the flask and the reaction stirred at -47 °C for 2 hrs. The reaction was warmed to room temperature and continued to proceed for an additional 12 hrs. Reaction completion was confirmed by TLC and crude MeO-Ox-D-Leu-NHBoc underwent an acid-base extraction followed by flash column chromatography. The pure compound was eluted at 3:7 Hex:EA in 70% yield over two steps; the structure and purity were confirmed by ^{1}H NMR. The same reaction conditions were used to synthesize the oxazole moiety of MeO-Ox-Val-N(Me)Boc for **SanA 17-II-Ox.B** in a 75% yield over two steps.

The final step in generating **SanA 17-III-Ox.B** was an amine deprotection with 20% TFA, anisole (2.0 equivalents) in 80% DCM to a 0.1 M concentration. The reaction stirred under open atmosphere for 30 min. Upon completion, confirmed by TLC, the reaction was concentrated *in vacuo* to furnish MeO-Ox-D-Leu-NH_2 (**SanA 17-III-Ox.B**) in quantitative yield. MeO-Ox-Val-N(Me)H (**SanA 17-II-Ox.B**) was synthesized using the same deprotection conditions in quantitative yield.

2.10.3 Synthesis of linear pseudo-pentapeptide

Synthesis of the linear pseudo-pentapeptides using method 2 (**LPP**) proceeded by coupling the free acid tripeptide (fragment A) and free amine pseudo-dipeptide (fragment B, **Scheme 2.19**). To generate **SanA 17-III-Ox.LPP** (MeO-Ox-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-Leu-NHBoc), free acid HO-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-Leu-NHBoc (1.0 equivalent, **SanA 17-III-Ox.A**) and free amine MeO-Ox-D-Leu-

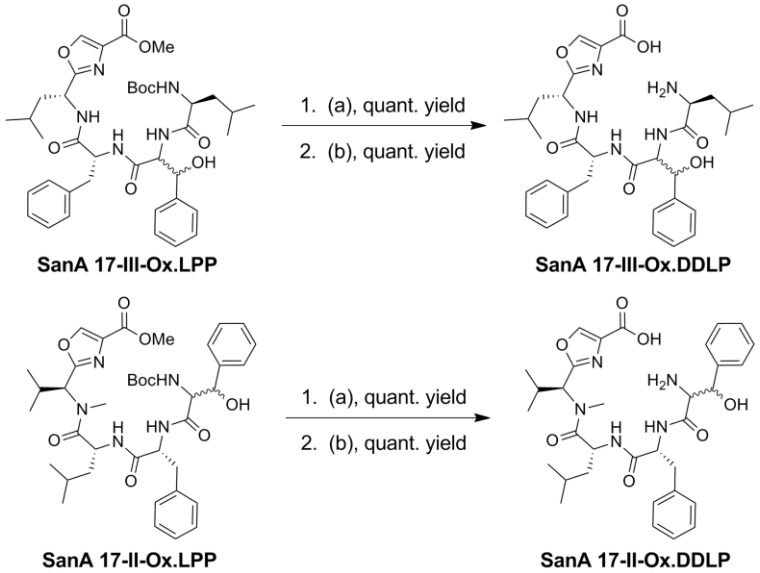
NHBoc (1.1 equivalents, **SanA 17-III-Ox.B**) were combined with TBTU (0.6 equivalents) and HATU (1.0 equivalents) in a round bottom flask. The fragments and coupling reagents were purged with argon gas and dissolved in ACN at a 0.1 M concentration. DIPEA (8.0 equivalents) was added to the solution and the reaction stirred at room temperature for 1.5 hrs. Upon completion, confirmed by TLC, crude **SanA 17-III-Ox.LPP** underwent an acid-base extraction followed by flash column chromatography. Pure **SanA 17-III-Ox.LPP** eluted at 1:9 Hex:EA and was furnished in 61% yield; the structure and purity were confirmed by LC/MS and ^{1}H NMR. The yield was lower than desired for this intermediate as coupling occurred at a sterically hindered site and more complex fragments were involved than a simple coupling of a dipeptide or tripeptide at a primary amine. Similar reaction conditions were applied to the synthesis of **SanA 17-II-Ox.LPP** (MeO-Ox-Val-N(Me)-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHBoc, 23% yield).



Scheme 2.19: Synthesis of **SanA 17-III-Ox.LPP** and **SanA 17-II-Ox.LPP** (a) TBTU and HATU (1.6-1.8 equivalents total), DIPEA (8.0-10.0 equivalents), ACN and/or DCM (0.1 M)

2.10.4 Deprotection of linear pseudo-pentapeptide

Deprotection of both LPPs proceeded via a stepwise fashion, deprotecting the acid first, and then the amine (**Scheme 2.20**). Methyl ester hydrolysis was achieved by dissolving MeO-Ox-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-Leu-NHBoc (**SanA 17-III-Ox.LPP**) in MeOH to a 0.4 M concentration and was cooled to 0 °C under open atmosphere. Hydrogen peroxide (3.4 equivalents, H_2O_2, 30% w/v) was added to the solution followed by LiOH (3.4 equivalents). The slurry was allowed to stir for 3 hrs at 0 °C and completion was verified by TLC and LC/MS. An acidic aqueous solution (pH 1 HCl solution) with sodium thiosulfate (3.8 equivalents, $Na_2S_2O_3$) was added to the reaction, and diluted with DCM to quench any remaining H_2O_2. The aqueous layer was back-extracted with EA and the organic layers were combined, filtered, and concentrated *in vacuo* to furnish HO-Ox-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-Leu-NHBoc in quantitative yield. Similar reaction conditions were applied to the synthesis of **SanA 17-II-Ox.DLPP** (HO-Ox-Val-N(Me)-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHBoc) in quantitative yield.



Scheme 2.20: Deprotection of **SanA 17-III-Ox.LPP** and **SanA 17-II-Ox.LPP** (a) LiOH (3.0 equivalents), H_2O_2 (1.1 equivalents), MeOH (0.4 M); (b) anisole (2.0 equivalents), TFA/DCM (1:3) (0.1 M)

The Boc protecting group was removed from HO-Ox-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-Leu-NHBoc using 25% TFA in DCM at 0.1 M concentration with anisole (2.0 equivalents). The reaction was monitored by LC/MS and was complete in 1 hour to yield a double deprotected linear pseudo-pentapeptide (DDLPP). **SanA 17-III-Ox.DDLPP** (HO-Ox-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-Leu-NH_2) was concentrated *in vacuo* and furnished in quantitative yield. The same stepwise synthetic procedure was used to generate **SanA 17-II-Ox.DDLPP** (HO-Ox-Val-N(Me)-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NH_2) in quantitative yield.

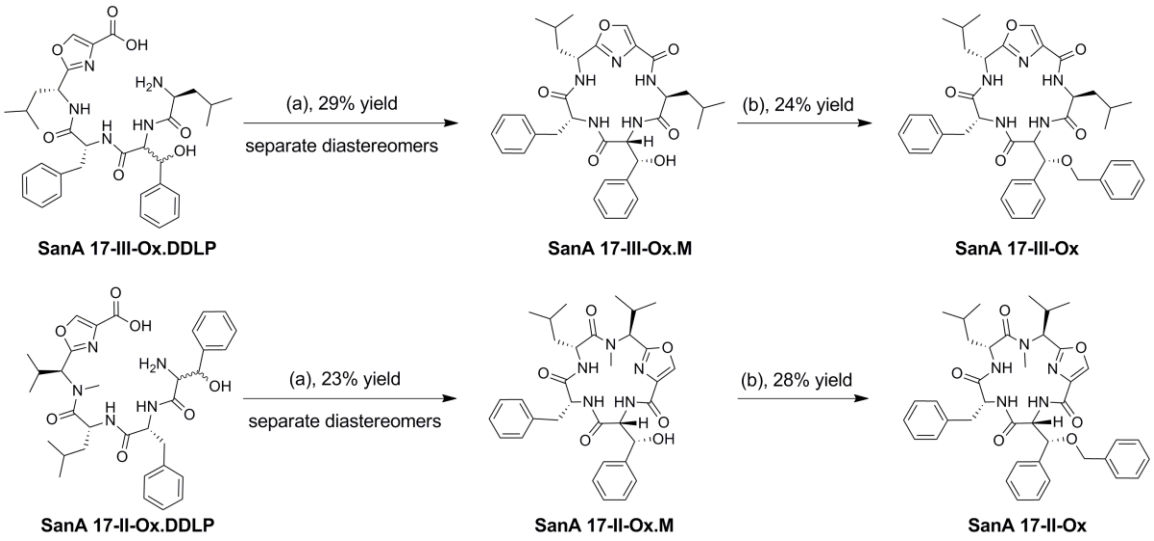
2.10.5 Macrocyclization to generate SanA pseudo-peptide derivatives

As described with method 1, **SanA 17-III-Ox.DDLPP** and **SanA 17-II-Ox.DDLPP** were cyclized using a syringe pump where the DLPPs were added to a round bottom flask with coupling reagents dissolved in dilute concentrations of solvent, respectively (**Scheme 2.21**). Macrocyclization of **SanA 17-III-Ox.DDLPP** proceeded with TBTU and HATU (1.0 equivalent each) in a round bottom flask purged with argon gas and sealed with a septa. The coupling reagents were dissolved in 50% of the total volume of anhydrous ACN and DCM (1:1) to achieve an overall concentration of 0.007 M and DIPEA (10.0 equivalents) was added to the solution of coupling reagents. **SanA 17-III-Ox.DDLPP** was dissolved in the remaining volume of anhydrous ACN and DCM and transferred to a syringe equipped with a 20-gauge long needle. Using a syringe pump, the DLPP was added dropwise to the reaction flask at a rate of 0.5mL/min. The reaction mixture was allowed to stir overnight upon the addition of all of the DLPP. LC/MS confirmed reaction completion and the crude mixture was concentrated *in vacuo* and redissolved in EA. The organic solution was subjected to an acid-base wash and then purification via flash chromatography. A racemic mixture of the cyclized product eluted at 100% EA and was furnished in 29% yield. Prior to benzylation, the diastereomers of

cyclo-(2*R*,3*R*)-β-OH-Phe-Leu-Ox-D-Leu-D-Phe were separated via RP-HPLC to give **SanA 17-III-Ox.M** (23% overall yield). The same procedure was used to cyclize and separate *cyclo*-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-Ox-Val-N(Me)-D-Leu-D-Phe (**SanA 17-II-Ox.M**) to furnish in 23% yield. Only the desired (2*R*, 3*R*) diastereomer was taken on for the benzylation step to generate **SanA 17-III-Ox** and **SanA 17-II-Ox**.

2.10.6 Benzylation of macrocycle

The final step to generate **SanA 17-III-Ox** was to benzylate the hydroxyphenylalanine residue of *cyclo*-(2*R*,3*R*)-β-OH-Phe-Leu-Ox-D-Leu-D-Phe (**SanA 17-III-Ox.M**). In an oven-dried round bottom flask, **SanA 17-III-Ox.M** (1.0 equivalent) and sodium hydride (2.0 equivalents, NaH, 60% in mineral oil) was dissolved in anhydrous THF to a concentration of 0.025 M. The reaction was run under argon atmosphere and stirred at -47 °C for 15 minutes prior to the drop-wise addition of benzyl bromide (4.0 equivalents, BnBr). Following the addition of all reagents, the reaction was monitored by LC/MS and was complete in 12 hrs. Upon completion, the excess reagents were quenched and washed with DI water. The organic layer was collected, dried over Na_2SO_4, filtered and concentrated *in vacuo* to furnish **SanA 17-III-Ox** in 24% yield. The final compound was subjected to RP-HPLC purification prior to biological testing; its structure and purity were verified via ^{1}H NMR, LC/MS, and HRMS. Benzylation of **SanA 17-II-Ox.M** was performed using the same reaction conditions to furnish **SanA 17-II-Ox** in 28% yield (**Scheme 2.21**). As is the case for all final compounds, RP-HPLC purification was performed prior to biological testing and structure and purity were verified via ^{1}H NMR, LC/MS, and HRMS.



Scheme 2.21: Macrocyclization and benzylation to generate **SanA 17-III-Ox** and **SanA 17-II-Ox** (a) TBTU and HATU (2.0-2.1 equivalents total), DIPEA (10.0 equivalents), DCM/ACN (1:1), 0.007 M; (b) NaH (2.0 equivalents), BnBr (2.0 equivalents), THF (0.1 M), -47 °C

2.11 Biological activity of SanA pseudo-peptide derivatives

All pseudopeptide SanA derivatives were evaluated for their ability to inhibit growth of HeLa cervical cancer cells. The bar graph in **Figure 2.17** shows that only one pseudopeptide derivative maintained potency of the parent compound: **SanA III-Th**. Interestingly, the thiazole containing pseudopeptide was not uniformly more potent than its oxazole counterpart, as seen by comparing **SanA 5-III-Ox** and **SanA 16-III-Ox** to their thiazole analogs. For the **SanA 17** pseudopeptide series, walking the oxazole around at residues I, II and III did not increase potency and in fact, the compounds had significantly diminished cytotoxicity compared to the parent compound.

Through examination of the biological data of our pseudopeptide derivatives, it was evident that each lead compound was affected differently by the inclusion of a heterocyclic moiety. No heterocycle proved superior and no position appeared to be optimal for placing a peptidomimetic moiety. Given this unique biological data, I believe that a trend is lacking because each heterocycle alters the macrocycle's 3D conformation differently. By incorporating a heterocylic moiety into the backbone of the

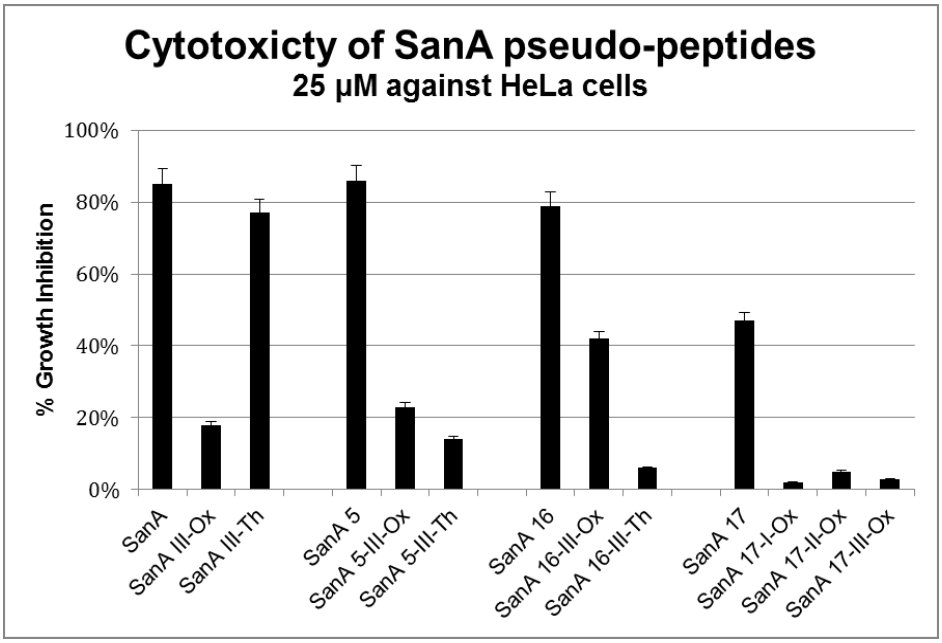


Figure 2.17: Biological activity of SanA pseudo-peptide derivatives and parent compounds. Error is ±5% with compounds run in triplicates.

macrocycle, the orientation of the side-chains is changed and their interaction with its protein target (HSP90) is altered. The side-chain orientation impacts the molecule's protein binding ability, potentially lowering its affinity for HSP90 with poor side chain orientation. Though one advantage of incorporating a heterocycle in the macrocyclic backbone is the possibility of locking the pseudopeptide into a single conformation, however, this conformation may not be bioactive. **Figure 2.18** illustrates the differences in 3D structure of the **SanA** series, in which the thiazole-containing derivative was more potent than the oxazole-containing derivative. Conversely, in the **SanA 16** series, the oxazole-containing derivative was the more potent than the thiazole-containing derivative. From these molecular models, it is clear that each heterocyclic moiety alters the backbone of the parent cyclic peptide differently and therefore interacting with its biological target differently as well.

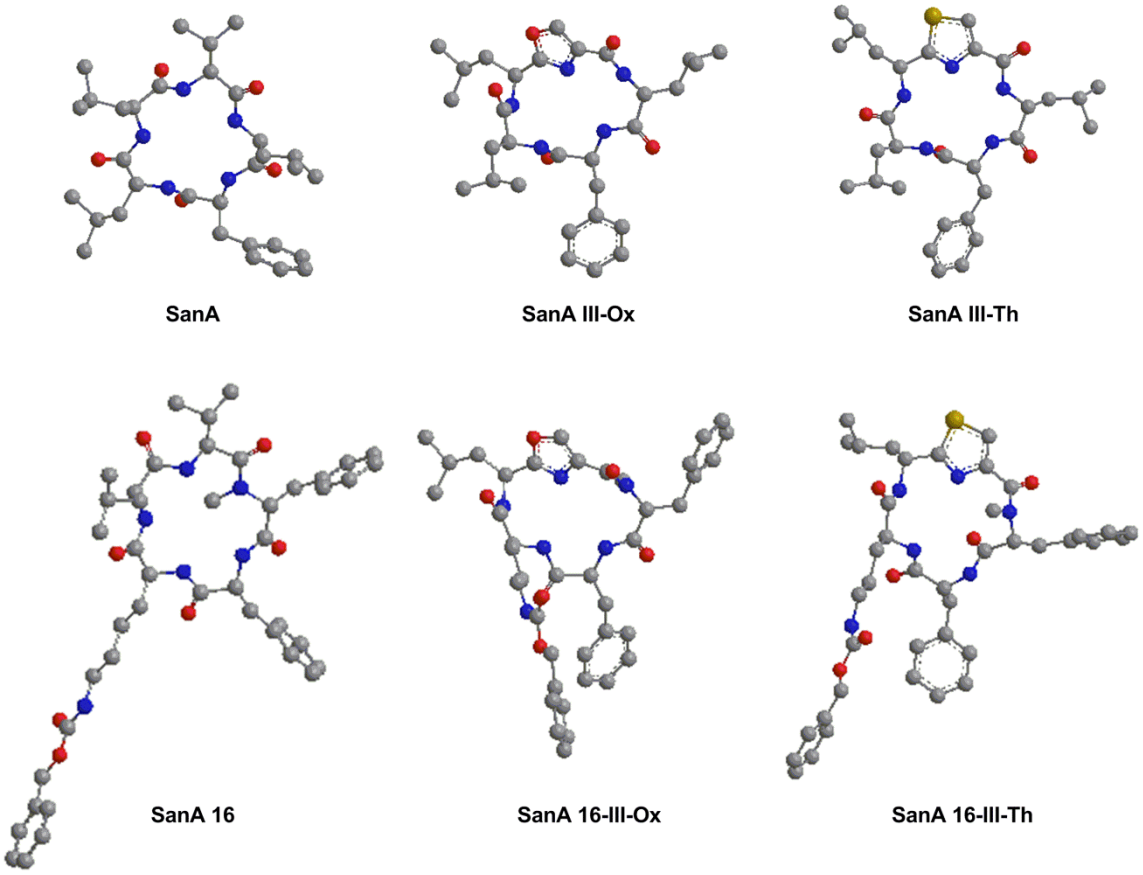


Figure 2.18. Molecular models of SanA, **SanA 16** and their respective pseudopeptide oxazole and thiazole derivatives. Energy was minimized using the Merck Molecular Force Field 94 (MMFF94) with ChemBio3D Ultra (version 12.0) available from CambridgeSoft. Convergence criteria: atomic root mean square force 0.01 kcal/mol; static energy 82.250e115.726 kcal/mol; 500 iterations.

2.12 Conclusions

Five SanA derivatives were synthesized in milligram quantities using a convergent solution phase approach. The synthesis of **SanA 2** and **SanA 4** showed that variation of a single *N*-methyl placed within the macrocyclic backbone can improve cytotoxicity. **SanA 11** showed that there was not a synergistic effect from the inclusion of multiple moieties that individually produced potent compounds. Finally, through **SanA 13** and **SanA 15** it was determined that polar amino acids were unfavorable, as these derivatives were significantly less potent than SanA. These five compounds were used as part of 100 derivatives that established the complex SAR that emerged for SanA.

The McAlpine lab observed several favorable trends including incorporating at least two non-polar aromatic residues or consecutive *N*-methyl-D-amino acids, which improved upon lead compound SanA's cytotoxicity. However, the location, arrangement and amino acid side chains were essential in influencing the overall 3D conformation of the macrocycle, thereby affecting how the derivatives positioned themselves for appropriate binding to HSP90.

Through exploration of SanA's SAR, an ideal location for placing a peg-biotin tag was determined and this biotinylated derivative was used to identify our target protein: HSP90. This molecular chaperone is a very attractive target as it is used by cancer cells to facilitate the function of numerous oncogenic proteins that induce cell growth. We progressed our compounds to the next stage of development and outsourced ADME studies on one of our most potent derivatives, **SanA 17**. Although **SanA 17** was stable in plasma, we found that its aqueous solubility could be improved and that the derivative was not readily cleared from the liver.

Given this data, we incorporated heterocyclic aromatic moieties (oxazole and thiazoles) into the backbone of the three most potent compounds: **SanA 5**, **SanA 16**, and **SanA 17**. We anticipated that oxazoles and thiazoles would impart rigidity to our compounds and improve their solubility. Our investigation with heterocyclic moieties allowed us to investigate two aspects: 1) the difference in biological activity between an oxazole versus a thiazole and 2) the impact of the position of the heterocycle within the backbone. Though only one derivative maintained the cytotoxicity of its parent compound, we discovered that both the type and position of the heterocycle impacted each derivative in a unique manner. We hypothesize that this is due to the 3D structure of each macrocycle locking into a bioactive or inactive conformation. We found that there is not one single change or moiety that must be present in a potent compound,

rather cytotoxicity depends on how the combination of structural changes affect the macrocycles overall 3D conformation.

2.13 References

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Chapter 3 – FR235222 and Apicidin

3.1 Packaging of DNA

Each eukaryotic chromosome has double stranded DNA that requires folding into an organized arrangement in order to fit into a cell's nucleus. Histones are essential proteins involved in correctly folding DNA.[1] There are five major classes of histones: H1, H2A, H2B, H3 and H4, all of which are positively charged under physiological conditions due to their high Arg and Lys content.[2] Negatively charged DNA wraps around a histone octamer cored composed of two pairs each of: H2A, H2B, H3, and H4.[3] Roughly 1.7 turns of DNA (about 147 base pairs) wrap around a single histone octamer core similar to thread wrapping around a spool to form a nucleosome (**a**, **Figure 3.1**).[4] Nucleosomes are linked together via repeating H1 histone proteins and approximately 20 base pairs to form a "beads on a string"-like structure (**b**, **Figure 3.1**).[5] The DNA-protein beaded string is rolled up into a cylindrical rope-like structure called super-coiled chromatin (**c**, **Figure 3.1**). In order to fit all the genetic information into cell's nucleus, the chromatin is further folded into a compact structure called chromosome (**d**, **Figure 3.1**).[5]

Proper organization of chromatin is critical for cellular processes, and changes in chromatin structure are directly influenced by post-translational modifications of the amino-terminal tails of histones (**Figure 3.2**). Though core histones are predominantly globular, their *N*-terminal tails are unstructured and vulnerable to modifications. There are eight modifications on histones: acetylation, methylation, sumoylation, ADP ribosylation, deamination, proline isomerization, and ubiquitination.[6]

The acetylation state of histones is the most well understood modification. Acetylation and deacetylation take place at the Ɛ-amino groups of Lys residues on histone proteins. Acetylations are catalyzed by enzymes called histone acetyltransferases (HATs) whereas histone deacetylases (HDACs) are responsible for deactylation.[6]

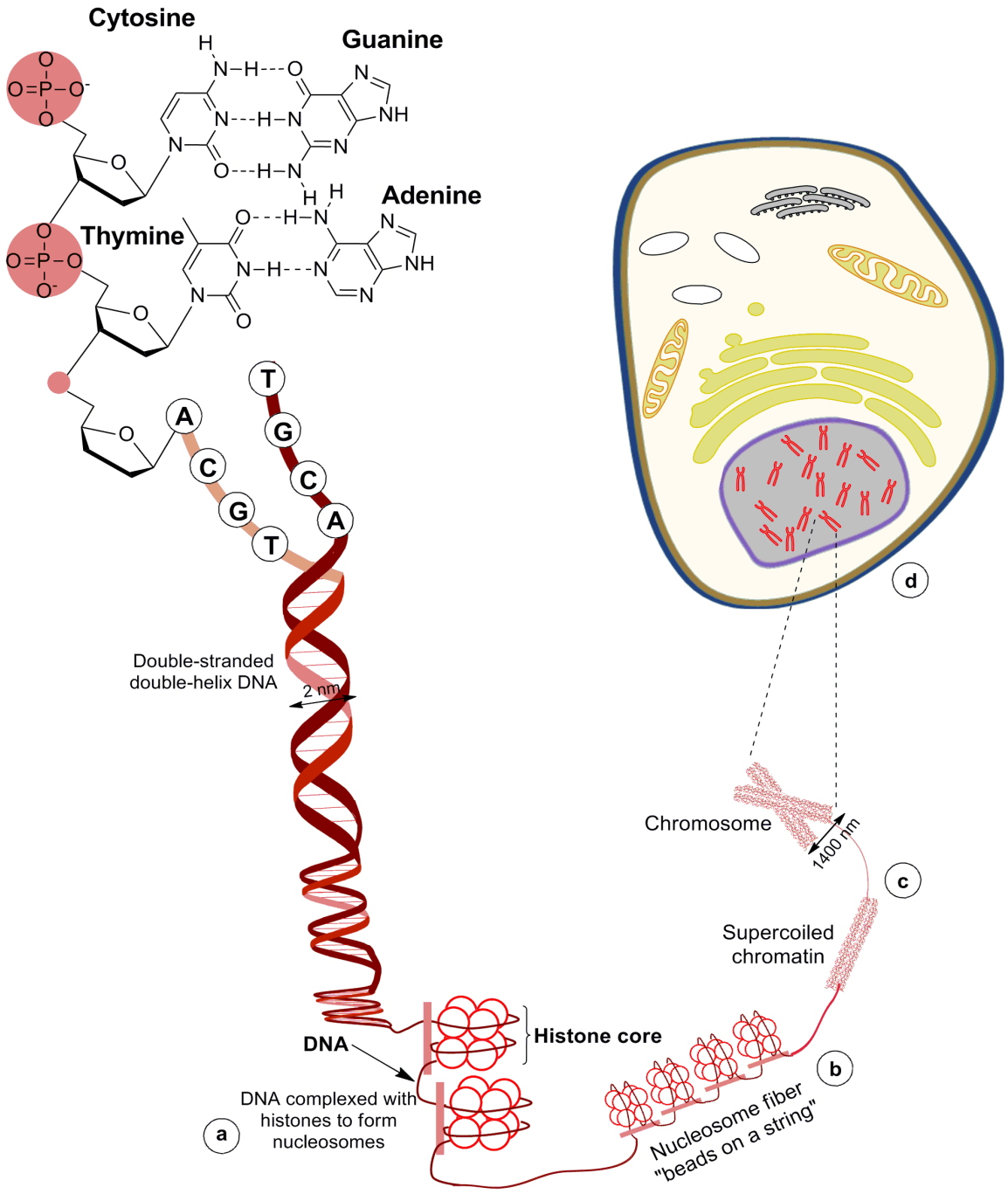


Figure 3.1: Double-stranded DNA organized into chromosomes. Illustration adapted from references.[7, 8] (a) shows DNA wrapped around histone octamer core, (b) nucleosomes come together to form “beads on a string” structure to compact DNA, (c) DNA is further condensed into supercoiled chromatin (d) to eventually fit genetic information into chromosomes that are present in the nucleus of the cell.

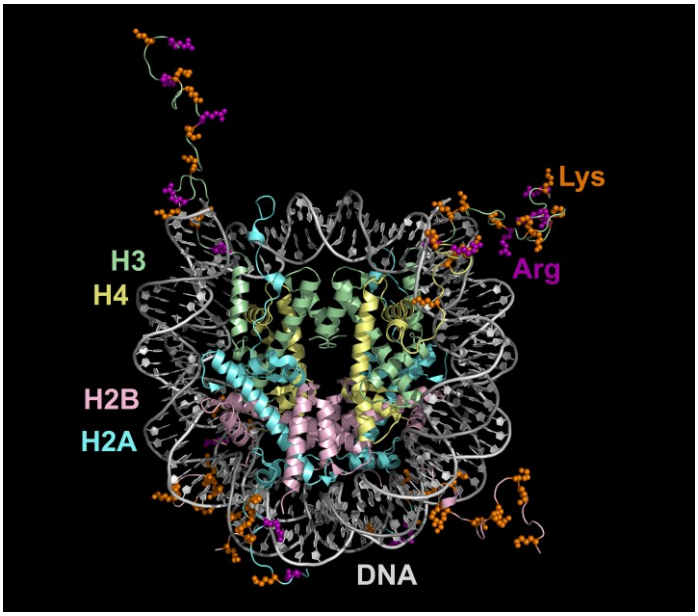


Figure 3.2: Arginine and Lysine residues at the amino-terminal tails in histones making up the nucleosome core particle. Crystal structure of the nucleosome core particle (Protein DataBank ID 1kx5; courtesy of Dr. Tom Huxford); Lysine (orange) and Arginine (purple).

When HATs acetylate histones, the positive charge on histones is removed, thereby removing histone's positively charged *N*-termini interaction with negatively charged phosphate groups of DNA.[9] Consequently, condensed chromatin is transformed into a relaxed state,[9] which is reversed by HDACs when they tightly repack DNA around the histone cores by removing capping acetyl groups (**Figure 3.3**).

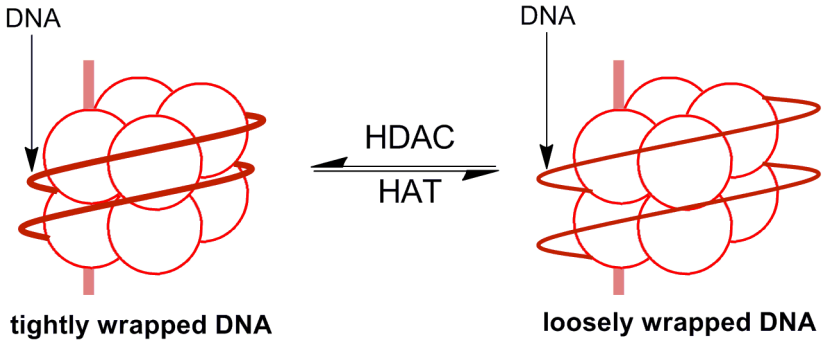


Figure 3.3: The acetylation state of histones controlled by HDACs and HATs resulting in tightly or loosely packed DNA around histones

Gene expression is regulated in part by HAT and HDAC activity. An overexpression of HDACs results in transcriptional suppression of genes due to nucleosomal inaccessibility. This gene silencing is associated with many diseases, including cancer. Many genes involved in tumor suppression and cell growth regulation are silenced via a HDAC-mediated epigenetic mechanism, including: p21, p27, p16, and p19.[10] The cyclin-dependent kinase inhibitor, p21, is responsible for inhibiting cell-cycle progression, and its expression is inactivated by hypoacetylation from HDACs.[11] Gui and co-workers found that treatment of a human multiple myeloma cell line with HDAC inhibitors (HDACIs) resulted in an increase in p21 gene expression and subsequent inhibition (suppression) of tumor-cell growth.[12]

HDACs can also silence genes by deacetylating non-histone proteins such as the oncosuppressor p53.[13, 14] Known as the "guardian of the genome," p53 prevents cancer through inducing apoptosis, destabilizing the genome, and inhibition of angiogenesis.[15] Under stress or when DNA is damaged, p53 induces cell cycle arrest at the G1/S checkpoint, which allows DNA repair and cell survival mechanisms to proceed, or causes apoptosis if DNA damage is irreparable. Treatment of carcinomas with HDACIs has been shown to restore the p53 pathway, activating this oncosuppressor, causing cell death.[16]

3.2 Histone deacetylases

A total of 18 human HDACs have been identified and they are grouped into four classes based on their sequence homology and size.[17] Classes I, II, and IV are Zn^{2+} dependent metalloproteins that are referred to as "classical" HDACs, while class III HDACs are called sirtuins and are NAD^{+} dependent.[18] Classical HDACs are promising anti-cancer targets and their over expression is observed in many types of cancers (**Table 3.1**). HDAC1-3 and 8 define class I; these HDACs are smaller in size and are

predominantly located in the nucleus.[17] Class II HDACs (HDAC4-7, 9, and 10) are found in both the cytoplasm and nucleus while HDAC11 is the sole member of class IV, found also in the cytoplasm and nucleus.[18]

Table 3.1: Classical family of HDACs organized by class

Class	HDAC	Size (aa)	Expression in tumor tissues
Class I	HDAC1	483	Possible prognostic indicator for lung and breast cancers. Over expressed in prostate cancers (hormone-refractory), gastric, colorectal, and hepatocellular carcinoma
	HDAC2	488	Loss of antigen presenting cells in colorectal cancers gave HDAC2 over expression. Over expressed in colorectal, prostate and gastric cancers.
	HDAC3	428	Over expressed in lung, gastric, prostate and colorectal cancers
	HDAC8	377	High HDAC8 expression correlates to childhood neuroblastoma and poor long term survival
Class II	HDAC4	1084	Upregulation of HDAC4 in breast cancer when compared with renal, bladder, colorectal cancer
	HDAC5	1122	Upregulation of HDAC5 in colorectal cancer when compared with renal, bladder, breast cancer
	HDAC6	1215	High expression and increased in advanced state in oral squamous cell cancer; inverse correlation of expression with survival and tumor cells
	HDAC7	855	High expression of HDAC7 in colorectal cancer when compared with renal, bladder, breast cancer
	HDAC9	1011	Unknown
	HDAC10	669	Unknown
Class IV	HDAC11	347	Unknown

3.3 HDAC Inhibitors

The first crystal structure of a mammalian HDAC enzyme (HDAC8) was reported in 2004 by scientists at Pharmacyclics and has provided essential information for designing HDACIs.[19] X-ray structures show that HDACs have an 11 Å deep catalytic

pocket with a hydrophobic outer rim.[17] At the bottom of this narrow pocket, there is a 14 Å long active site that harbors a metal cation (**Figure 3.4**). HDACIs bind inside the HDAC pocket and block substrate access, thereby inhibiting DNA deacetylation) and allow for proper gene transcription.

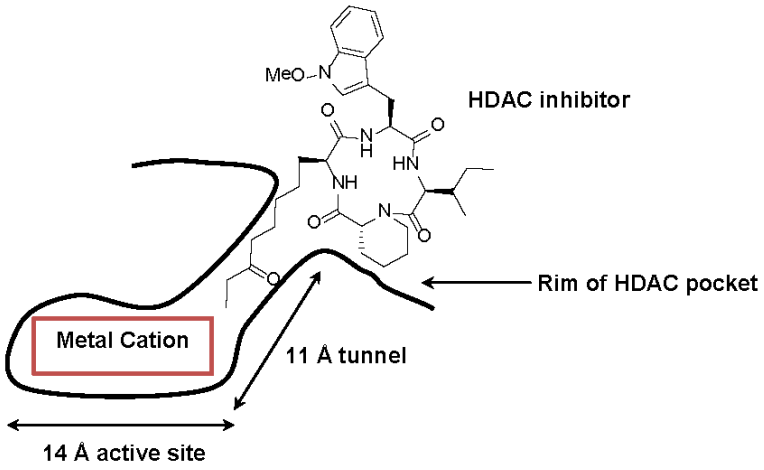
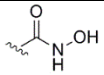
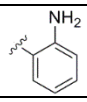
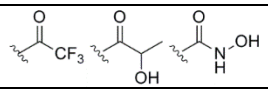


Figure 3.4: HDAC pocket; Illustration adapted from reference[20]

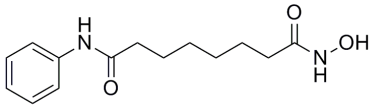
Most HDACIs follow a general pharmacophore model consisting of 1) an enzyme inactivation moiety that interacts with the metal cations or the amino acids within the HDAC pocket. The enzyme inactivation moiety is connected to 2) a lipophilic linker of 4-7 atoms in length, which is anchored to the rim via 3) a surface-recognition cap group that rests on the outside of the HDAC pocket (**Figure 3.5**).[20] There are five known structural families of HDACIs that follow this pharmacophore model: small molecule hydroxamic acids, short chain fatty acids, benzamides, electrophilic ketones, and cyclic peptides (**Table 3.2**).[21]

Figure 3.5: HDACI pharmacophore model

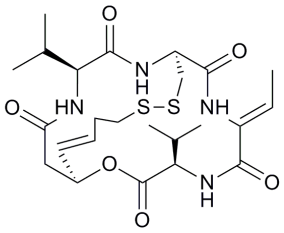
Table 3.2: Five structural classes of HDACIs

Structural Class	Surface Rec. Cap	Linker	Enzyme Inactivator
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 first identified HDACI to be approved by the US FDA for clinical use belongs to the small molecule hydroxamic acid family: suberoylanilide hydroxamic acid (SAHA, Vorinostat, or Zolinza, **Figure 3.6**). As its name suggests, SAHA has a hydroxamic acid that chelates Zn^{2+} ions in the HDAC pocket, which is linked to a hydrophobic aminobenzamide cap group with a six-carbon chain. SAHA was approved in 2006 for treatment of CTCL and there are currently over 200 clinical trials involving this HDACI.[22] A second HDACI approved by the FDA for treatment of CTCL is Romidepsin (FK228, **Figure 3.6**), which falls within the cyclic peptide family of HDACIs. FK228 is a natural product isolated from the bacteria *Chromobacterium violaceum*.[23] This natural product has a cyclic tetrapeptide as a surface recognition cap, connected with a four- carbon linker to a disulfide bridge. The disulfide bridge is reduced to a thiol metal chelator under intracellular conditions, which chelates inside the HDAC pocket to the metal cation.[24]



SAHA



FK-228

Figure 3.6: HDACIs currently on the market: SAHA and Romidepsin

There are approximately 50 clinical trials involving FK228 for treatment of numerous cancers.[22] Furthermore, nearly 400 ongoing investigations of HDACIs are being conducted in the US, supporting the fact that HDACs are promising cancer targets.[22] There are also 16 structurally unique HDACIs being tested in preclinical and early clinical trials (**Table 3.3**).[22] The large number of clinical trials with all the current HDACIs indicates the tremendous potential for HDACIs as chemotherapeutic agents.

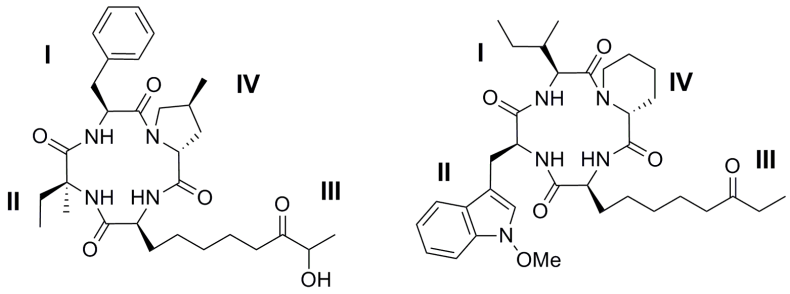
Table 3.3: Selected HDAC Inhibitors in Clinical Use or Development[25, 26]

Compound by Class	Manufacturer	Phase	Potency
Small molecule hydroxamic acid			
Vorinostat* (Zolinza, SAHA)	Merck	approved	μM
Panobinostat (LBH589)	Novartis	I/II	nM
Belinostat (PXD101)	CuraGen	II	μM
ITF2357	Italfarmaco SpA	II	μM
Short chain fatty acid			
Valproic acid	--	I/II	mM
Benzamide			
Entinostat	Syndax	II	μM
MGCD0103	Celgene, MethylGene	II	nM
Cyclic peptides			
Romidepsin (FK-228)	Gloucester	approved	nM

3.4 Macrocyclic HDACI scaffold

Given our expertise with macrocyclic peptides and the clinical relevance of Romidepsin, the cyclic peptide family of HDACIs was of particular interest to our research group. The unique feature of this compound class is the complex surface recognition cap that binds to the rim of the HDAC pocket.[27] The macrocyclic cap has hydrophobic amino acids and an alkyl linker connected to an enzyme inactivator. Two

natural products were used as templates in designing HDACIs that I synthesized: FR253222 and Apicidin (**Figure 3.7**).



FR235222 **Apicidin**

Figure 3.7: FR235222 and Apicidin natural products; Residues I, II, and IV comprise the surface recognition cap and residue III has the alkyl linker and enzyme inactivation moiety

FR235222 was isolated from the fermentation broth of a fungus, *Acremonium* sp. No. 27082.[28] Previous work by the Mori group reports that this cyclic tetrapeptide has an IC_{50} = 22 nM against human partially purified HDACs, thus demonstrating potential as an exciting lead for HDACIs.[29] Apicidin, another cyclic tetrapeptide, exhibits nanomolar activity against human partially purified HDACs (IC_{50} = 5 nM), making this natural product also a biologically interesting scaffold for designing cyclic peptide HDACIs.[30] Apicidin was isolated from fermentations of *Fusarium* spp. in Costa Rica.[31] Like FR235222, Apicidin is a cyclic tetrapeptide that includes an alkyl chain as a linker. This linker is connected to enzyme inactivation moieties: an α-hydroxy ketone in FR235222, and an ethyl ketone in Apicidin.

3.5 Rational design of HDACIs

Using FR235222 and Apicidin as templates, we designed 17 HDACIs to explore the SAR of these two natural products; **Figure 3.8** shows amino acids used at each residue with ones that I used indicated in blue Residue I in FR235222 is an L-Phe. In our synthesis we utilized an L-Phe as well as a D-Phe and an *N*-methylated L-Phe at position I. Residue I in Apicidin is an L-Ile, which we maintained in our synthetic analogs

of Apicidin. Residue II was replaced with an L-Abu, an L- or D-Trp amino acids, which is substituted for the iso-Valine in FR235222 or L-methyoxy-Trp in Apicidin, respectively.

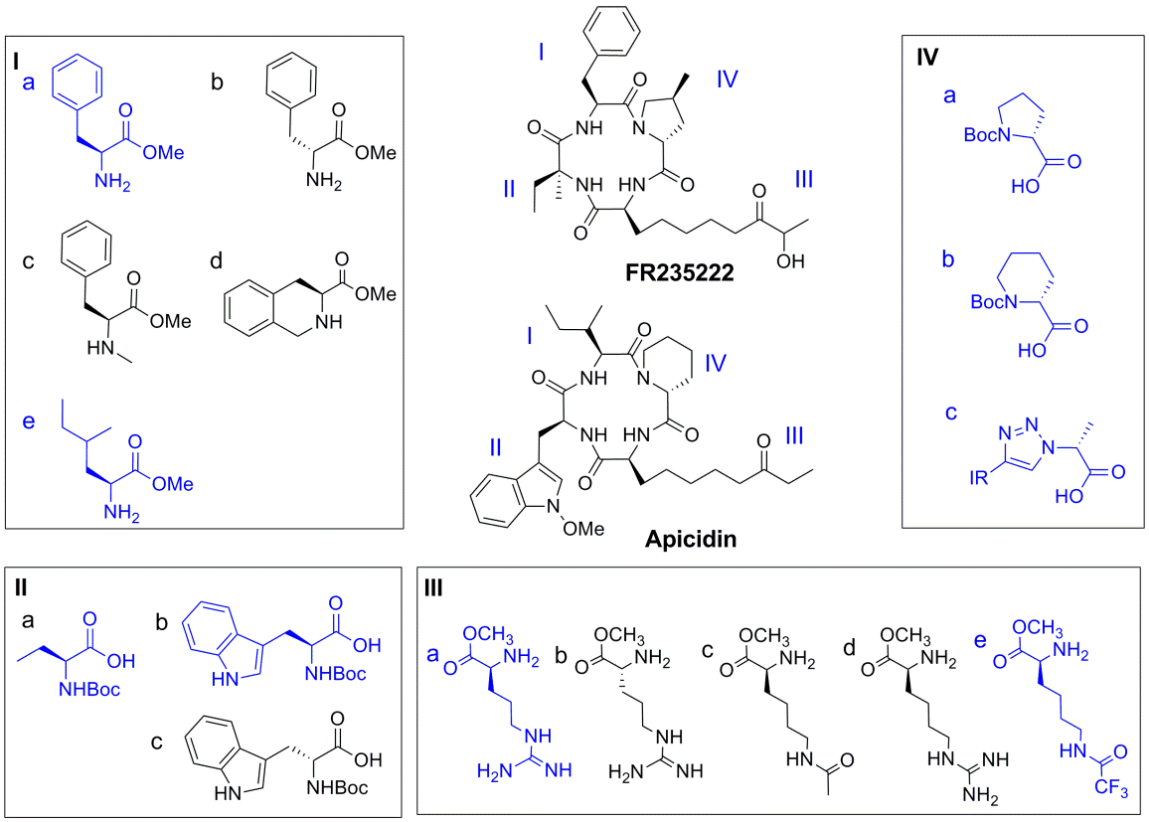


Figure 3.8: Amino acids used in designing HDACIs based on FR235222 and Apicidin.

Following the established pharmacophore model for HDACIs, residue III contained the linker and an enzyme inactivator moiety. We explored the importance of linker lengths by varying our linker from four or five atoms. Utilizing both L- or D-Arg, L-Arg homolog and L-Lys, we were able to modify the linker region, allowing it to vary from 3 Carbon atoms and the nitrogen from the guanidine unit (L- or D-Arg), to 4 carbon atoms (Lysine), to 4 carbon atoms and the nitrogen from the homoguanidine (L-homoArg, IIId). Further, these substitutions provided the opportunity to investigate how L- or D-stereochemistry in the linker residue will impact binding of the inhibitor. Three enzyme inactivator moieties were investigated: guanidine, acetyl, and a trifluoroacetyl. A guanidine moiety has never been reported in HDACIs and it may aid in the molecule's

solubility. Acetyl protected lysines are interesting, yet unexplored moieties in HDACIs because they mimic the acetyl group that HDACs deacetylate. Given that one major side effect in clinical trials is toxicity due to turning gene's permanently "on" with the inactivation of HDACs, utilizing a reversible inhibitor such as the acetyl protected lysine was provoking. Finally, precedence suggested that trifluoroacetyl groups successfully deactivate HDACs via chelating to Zn^{2+} cations in the HDAC pocket.[32]

It is well established that residue IV is critical to maintain a D-amino acid in order to ensure binding of the macrocycle to the HDAC binding site with the optimal orientation of the enzyme inactivator element.[33] Substituting in D-Proline (D-Pro) for D-methyl-Pro (FR235222) or the D-Pipecolic acid (Apicidin) provided the opportunity to examine the impact of alternative D-ring systems. In addition, a D-Alanine (D-Ala) 1,4-disubstituted triazole was placed at position IV to generate a rigid macrocyclic surface recognition cap.[34, 35] We anticipated that the triazole will rigidify the macrocyclic structure and restrict bond rotation, which will lead to a potent inhibitor.[34, 35] **Figure 3.9** shows all of the HDACIs that we synthesized based on FR235222 and Apicidin, where compounds that I synthesized are indicated in blue.

3.6 Retrosynthesis of peptide HDACIs

Retrosynthetic analysis of HDACI macrocyclic peptides (**Scheme 3.1**) involved a convergent solution phase synthesis strategy. The desired macrocyclic tetrapeptide was generated via a head-to-tail peptide coupling of the linear tetrapeptide precursor. The linear tetrapeptide was generated from two dipeptide fragments: fragment A and fragment B. Each fragment was synthesized in solution phase using commercially available Boc or OMe protected amino acids, coupling reagents and Hünig's base.

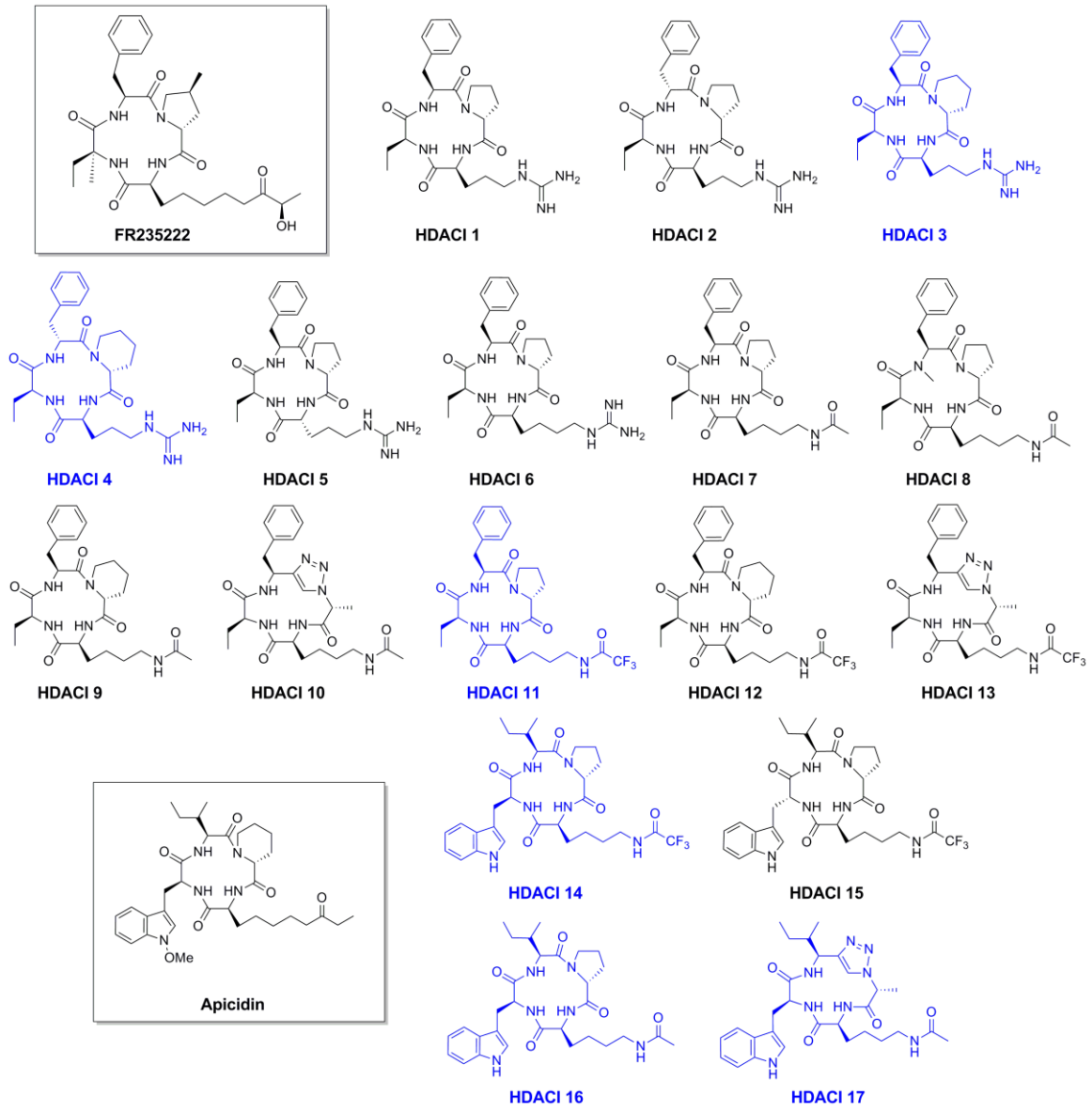
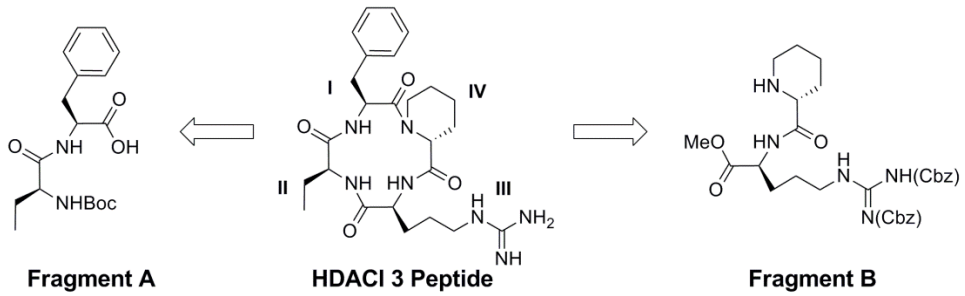


Figure 3.9: HDACIs designed based on FR235222 and Apicidin



Scheme 3.1: Retrosynthesis of HDACI macrocyclic peptides

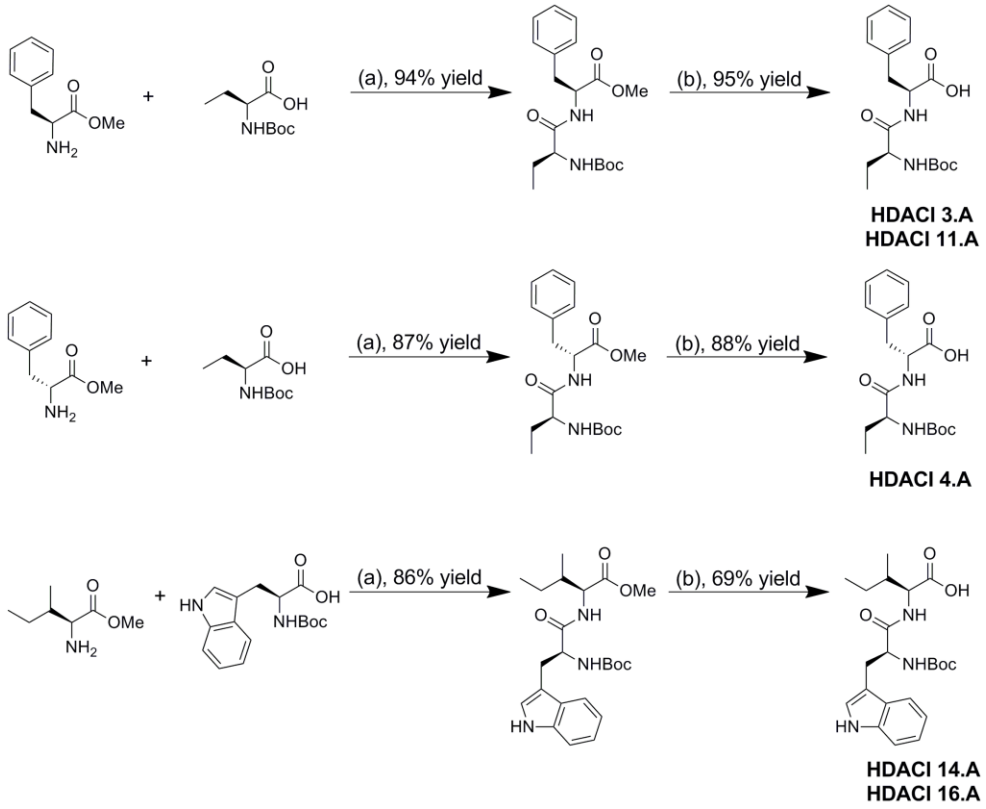
3.7 Synthesis of peptide HDACIs

The same convergent synthetic strategy was used to construct all five of my peptide HDACI derivatives. The commercially available amino acids used were protected either at the *N*-terminus with a Boc group or at the C-terminus as a methyl ester and 2 Cbz groups were used to protect the guanidinium moiety of Arg. Coupling reagents were used to facilitate peptide bond formation. Formation of dipeptides used TBTU as the coupling agent. However, more difficult couplings, (dipeptide bond formation from secondary amines or formation of the tetrapeptide) required HATU and/or DEPBT. Macrocyclization of the linear tetrapeptides utilized a cocktail of three coupling reagents: TBTU, HATU and DEPBT. Most peptide coupling reactions were carried out in anhydrous dichloromethane (DCM). However, some reactions required the addition of anhydrous acetonitrile (ACN) and/or dimethylformamide (DMF) to dissolve all reagents.

3.7.1 Synthesis of fragment A

The synthesis of fragment A for **HDACI 3** (**HDACI 3.A**, Scheme 3.2) proceeded first with the generation of dipeptide MeO-Phe-Abu-NHBoc using 1.1 equivalents of free amine MeO-Phe-NH_2, 1.0 equivalent of free acid HO-Abu-NHBoc, 1.2 equivalents of TBTU and 4.0 equivalents of DIPEA. The amino acids and TBTU were added to a dry round bottom flask, which was subsequently fitted with a septum and purged with argon. The reagents were dissolved in anhydrous DCM to a concentration of 0.1 M. Finally, DIPEA was added to the flask, the reaction stirred at RT and was monitored via TLC, whereupon it was complete after 1 hr. Upon completion, the reaction was subjected to an acid-base extraction to purify the dipeptide. The crude reaction mixture was diluted with ethyl acetate (EA) and washed with 10% HCl solution, then saturated $NaHCO_3$ solution, and finally with brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The pure dipeptide (MeO-Phe-Abu-NHBoc, 94% yield) was

characterized via ^{1}H NMR; this dipeptide was also used for **HDACI 11** (**Scheme 3.2**). A similar procedure was followed for the generation of dipeptide MeO-D-Phe-Abu-NHBoc (87% yield) for **HDACI 4** (scheme 3.2), and MeO-Trp-Ile-NHBoc (86% yield), which was a common building block for compounds **HDACI 14** and **HDACI 16** (**Scheme 3.2**).



Scheme 3.2: Synthesis of fragment A (a) TBTU (1.2 equivalents), DIPEA (4.0 equivalents), DCM (0.1 M); (b) LiOH (8.0 equivalents), MeOH (0.1 M)

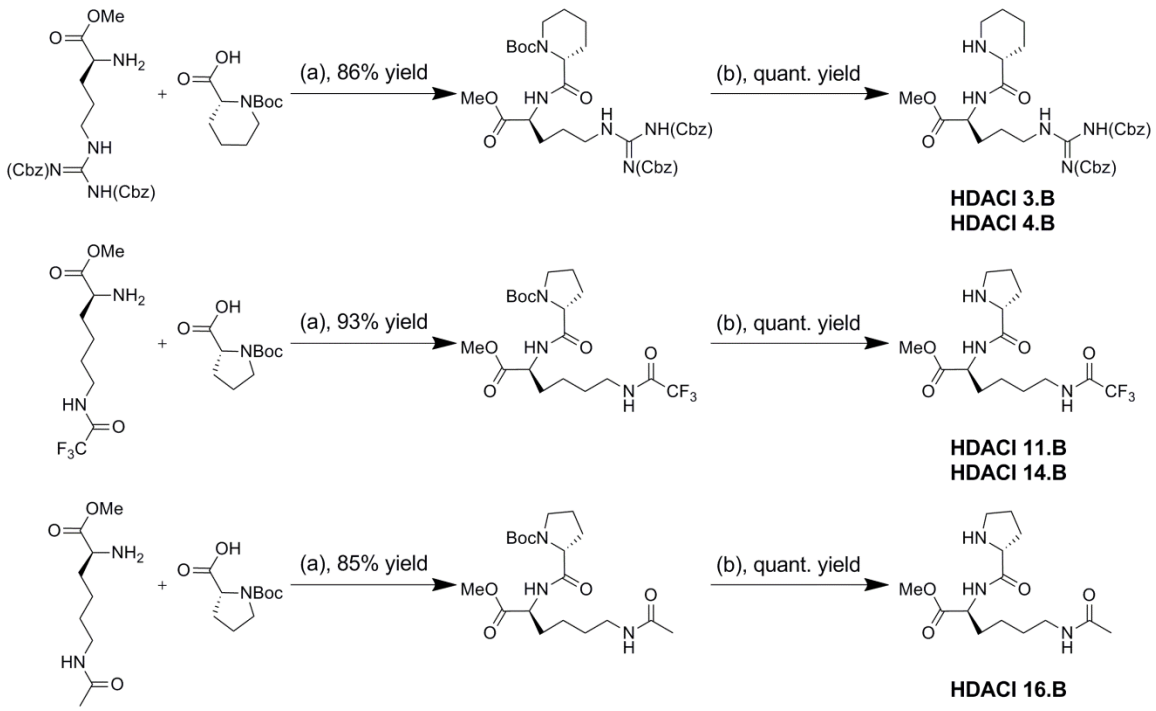
Dipeptide MeO-Phe-Abu-NHBoc was subjected to methyl ester hydrolysis to furnish free acid HO-Phe-Abu-NHBoc, fragment A (**HDACI 3.A, Scheme 3.2**). The dipeptide was dissolved in MeOH (0.1 M) under ambient conditions, 8.0 equivalents of LiOH were added to the reaction mixture. This slurry was stirred until the TLC showed complete disappearance of starting material (~2 hrs). Upon completion, the crude reaction was concentrated *in vacuo*, and then redissolved in EA. The reaction (diluted with EA) was washed with 7% HCl and the aqueous layer was back-extracted with DCM.

The organic layers were collected, dried over Na_2SO_4, filtered and concentrated *in vacuo*. HO-Phe-Abu-NHBoc (Fragment A, **HDACI 3.A** and **HDACI 11.A**) was furnished in 95% yield and the structure was confirmed via ^1H NMR. The same conditions were applied to generate **HDACI 4.A** (HO-D-Phe-Abu-NHBoc, 88% yield) as well as **HDACI 14.A** and **HDACI 16.A**, which shared the common fragment HO-Trp-Ile-NHBoc (69% yield).

3.7.2 Synthesis of fragment B

Synthesis of fragment B for **HDACI 3** (**HDACI 3.B**, **Scheme 3.3**) began with assembling dipeptide MeO-Arg(2Cbz)-D-Pip-NBoc using 1.1 equivalents of free amine MeO-Arg(2Cbz)-NH_2, 1.0 equivalent of free acid HO-D-Pip-NBoc, 1.2 equivalents TBTU, and 3.0 equivalents of DIPEA. The reaction mixture was purged with argon and diluted with anhydrous DCM (0.1 M). TLC monitoring showed that the coupling was complete after approximately two hrs. The crude reaction mixture was washed with saturated ammonium chloride solution (NH_4Cl) and then the organic fraction was concentrated and purified via flash column chromatography. Pure dipeptide MeO-Arg(2Cbz)-D-Pip-NHBoc eluted in 13:7 Hex/EA and was confirmed by ^1H NMR (86% yield); this dipeptide was also used for **HDACI 4** (scheme 3.3). The same procedure was applied to generate dipeptides for **HDACI 11** and **HDACI 14** (MeO-Lys(Tfa)-D-Pro-NBoc, 93% yield), and **HDACI 16** (MeO-Lys(Ac)-D-Pro-NBoc, 85% yield), both shown in **scheme 3.3**.

For amine deprotection of dipeptide MeO-Arg(2Cbz)-D-Pip-NBoc was performed under open atmosphere. The dipeptide was diluted to 0.1 M concentration in a mixture of TFA:DCM (1:4), with addition of 2.0 equivalents of anisole. The reaction was monitored by TLC for disappearance of starting material and was complete within 1 hr. The solution was concentrated *in vacuo* to furnish **HDACI 3.B** MeO-Arg(2Cbz)-D-Pip-NH in quantitative yield; this fragment was also used to generate **HDACI 4**. Similar conditions



Scheme 3.3: Synthesis of fragment B. (a) TBTU (1.2 equivalents), DIPEA (4.0-6.0 equivalents), DCM (0.1 M); (b) anisole (2.0 equivalents), TFA/DCM (1:4), 0.1 M; (c) TFA/DCM (1:4), 0.1 M

were also used to generate Fragment B for my remaining HDACI peptide compounds: **HDACI 11.B** and **HDACI 14.B** (MeO-Lys(Tfa)-D-Pro-NH) and **HDACI 16.B** (MeO-Lys(Ac)-D-Pro-NH).

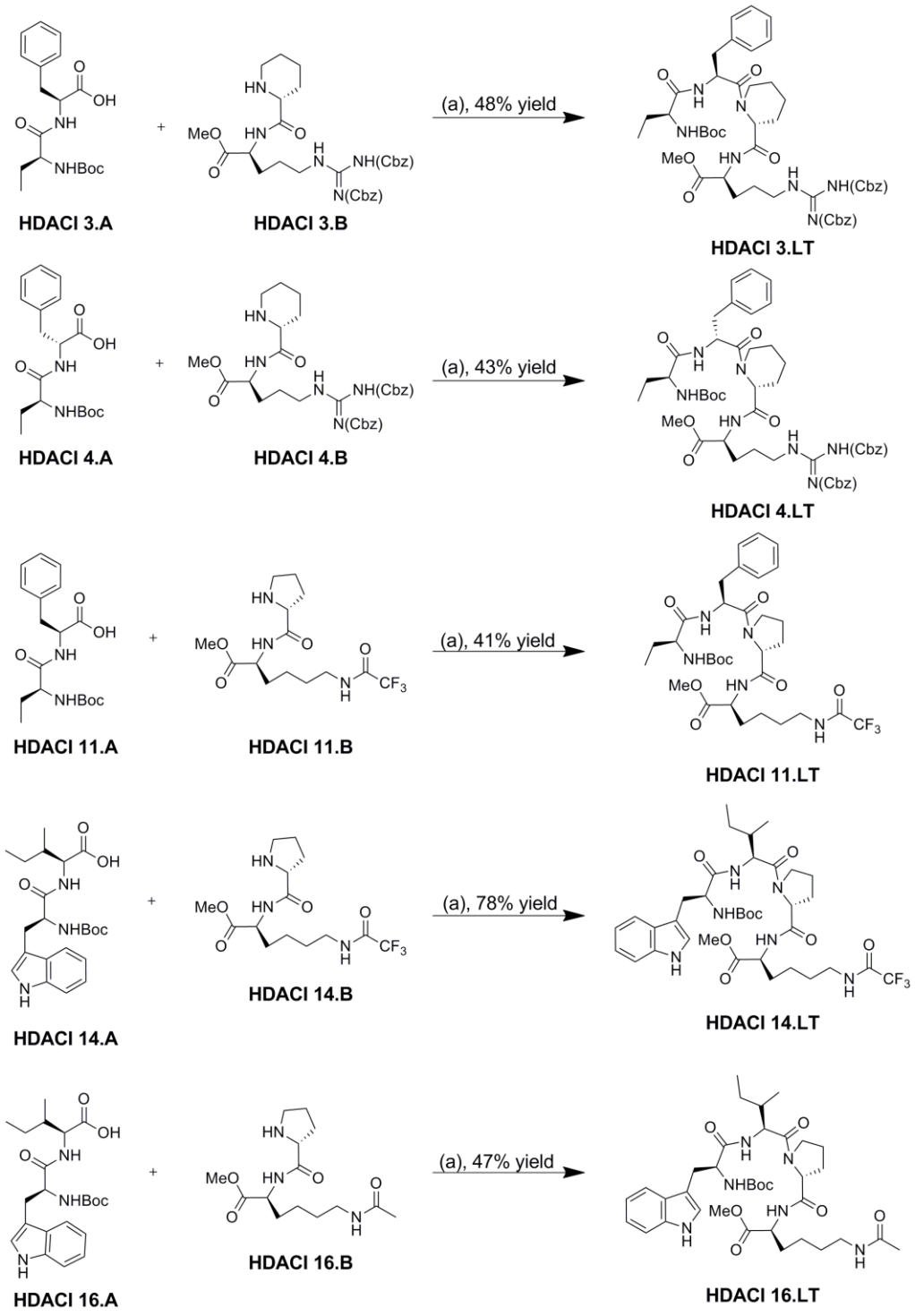
3.7.3 Synthesis of linear tetrapeptide

Coupling of free amine fragment A and free acid fragment B (**Scheme 3.4**) together generated HDACI linear tetrapeptides (LT). Generating linear tetrapeptide **HDACI 3.LT** (MeO-Arg(2Cbz)-D-Pip-Phe-Abu-NHBoc, **HDACI 3.LT**) was accomplished using 1.0 equivalent of free acid **HDACI 3.A** (HO-Phe-Abu-NHBoc), 1.1 equivalents of free amine **HDACI 3.B** (MeO-Arg(2Cbz)-D-Pip-NH), combined with TBTU (1.2 equivalents) and HATU (0.75 equivalents), and DIPEA (6.0 equivalents). The fragments and coupling reagents were dissolved in anhydrous DCM for an overall concentration of 0.1 M under argon atmosphere. The reaction was monitored by TLC and upon

completion (~ 4 hrs), crude **HDACI 3.LT** underwent an acid-base extraction followed by flash column chromatography. Pure **HDACI 3.LT** eluted at 13:7 Hex:EA and was furnished in 48% yield; the structure and purity were confirmed by ^{1}H NMR and LC/MS. In this case, two purifications via column chromatography resulted in a less than desirable yield. Similar reaction conditions were applied to the synthesis of **HDACI 4.LT** (MeO-Arg(2Cbz)-d-Pip-d-Phe-Abu-NHBoc, 43% yield), **HDACI 11.LT** (MeO-Lys(Tfa)-D-Pro-Phe-Abu-NHBoc, 41% yield), **HDACI 14.LT** (MeO-Lys(Tfa)-D-Pro-Ile-Trp-NHBoc, 78% yield), and **HDACI 16.LT** (MeO-Lys(Tfa)-D-Pro-Ile-Trp-NHBoc, 47% yield).

3.7.4 Deprotection of linear tetrapeptide

The acid and amine of the linear tetrapeptides were deprotected simultaneously under acidic conditions. This method required a longer reaction time than a step-wise deprotection strategy (3 days compared to 1 day), but the acid conditions worked well for all compounds, including those that had polar amino acids. The step-wise conditions did not work well for tryptophan containing compounds, as the base deprotonated the residue, and the compound went into the aqueous phase. **HDACI 3.LT** (MeO-Arg(2Cbz)-D-Pip-Phe-Abu-NHBoc) was dissolved in tetrahydrofuran (THF) to a concentration of 0.1 M and 2.0 equivalents of anisole were added to the reaction flask. 12 N HCl (8 drops per 0.3 mmol of **HDACI 3.LT**) was added to the flask and the reaction stirred at room temperature under open atmosphere. The deprotection was monitored by LC/MS for the appearance of **HDACI 3.DDLT**. Typically, reactions were 50% complete after the first day; however, two additional days were needed for full deprotection of both the amine and acid. An additional four drops of 12 N HCl per 0.3 mmol of **HDACI 3.LT** were added to the reaction flask on both the second and third days, whereupon the reaction was complete. It was concentrated *in vacuo* and **HDACI 3.DDLT** (HO-Arg(2Cbz)-D-Pip-Phe-Abu-NH_2) was afforded in quantitative yield. This *in*

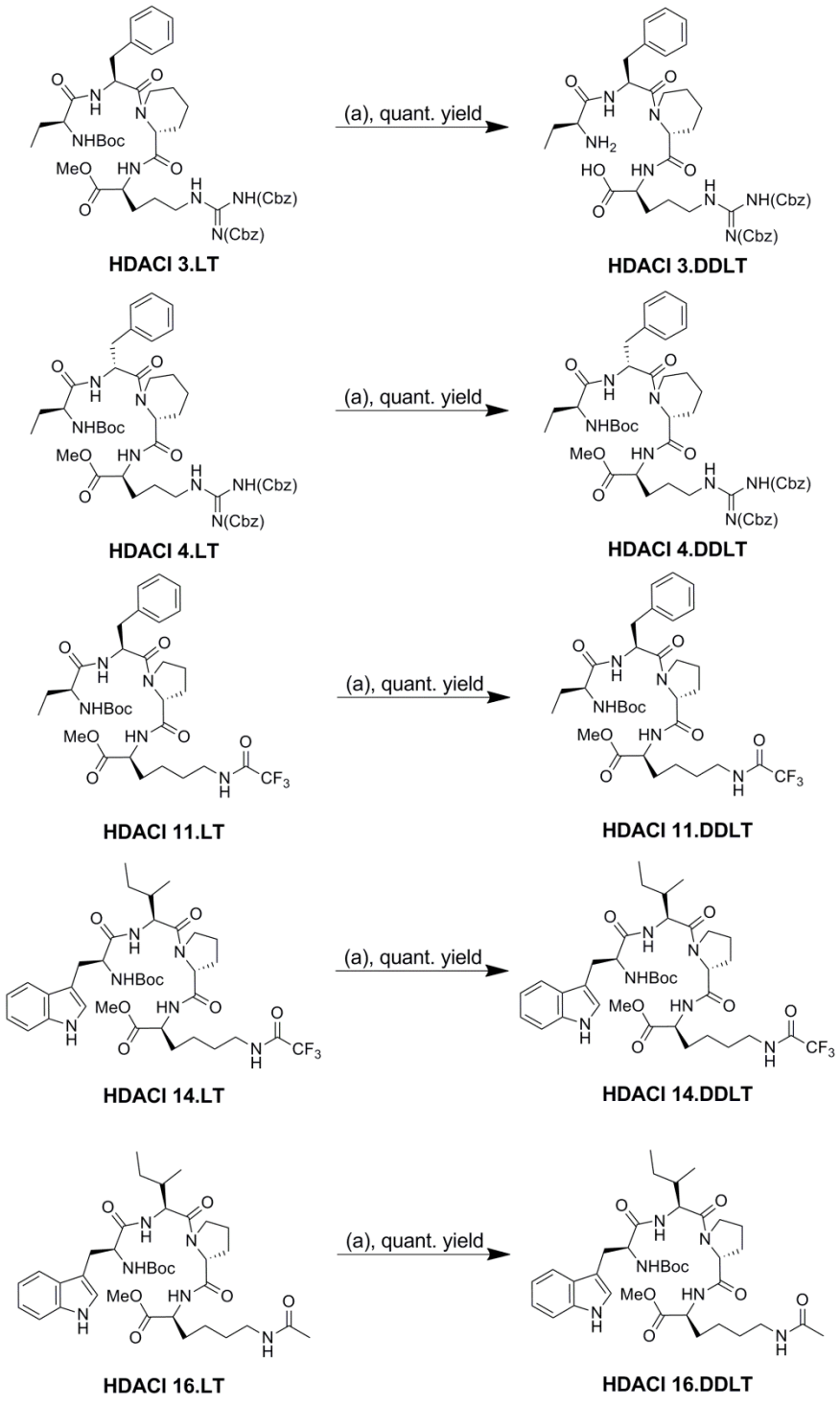


Scheme 3.4: Synthesis of linear tetrapeptide (a) TBTU/HATU/DEPBT (1.3–2.0 equivalents total), DIPEA (6.0–10.0 equivalents), ACN and/or DCM (0.1 M)

situ deprotection strategy was used to furnish **HDACI 4.DDLT** (HO-Arg(2Cbz)-D-Pip-D-Phe-Abu-NH_2), **HDACI 11.DDLT** (HO-Lys(Tfa)-D-Pro-Phe-Abu-NH_2), **HDACI 14.DDLT** (HO-Lys(Tfa)-D-Pro-Ile-Trp-NH_2), and **HDACI 16.DDLT** (HO-Lys(Tfa)-D-Pro-Ile-Trp-NH_2) in quantitative yields.

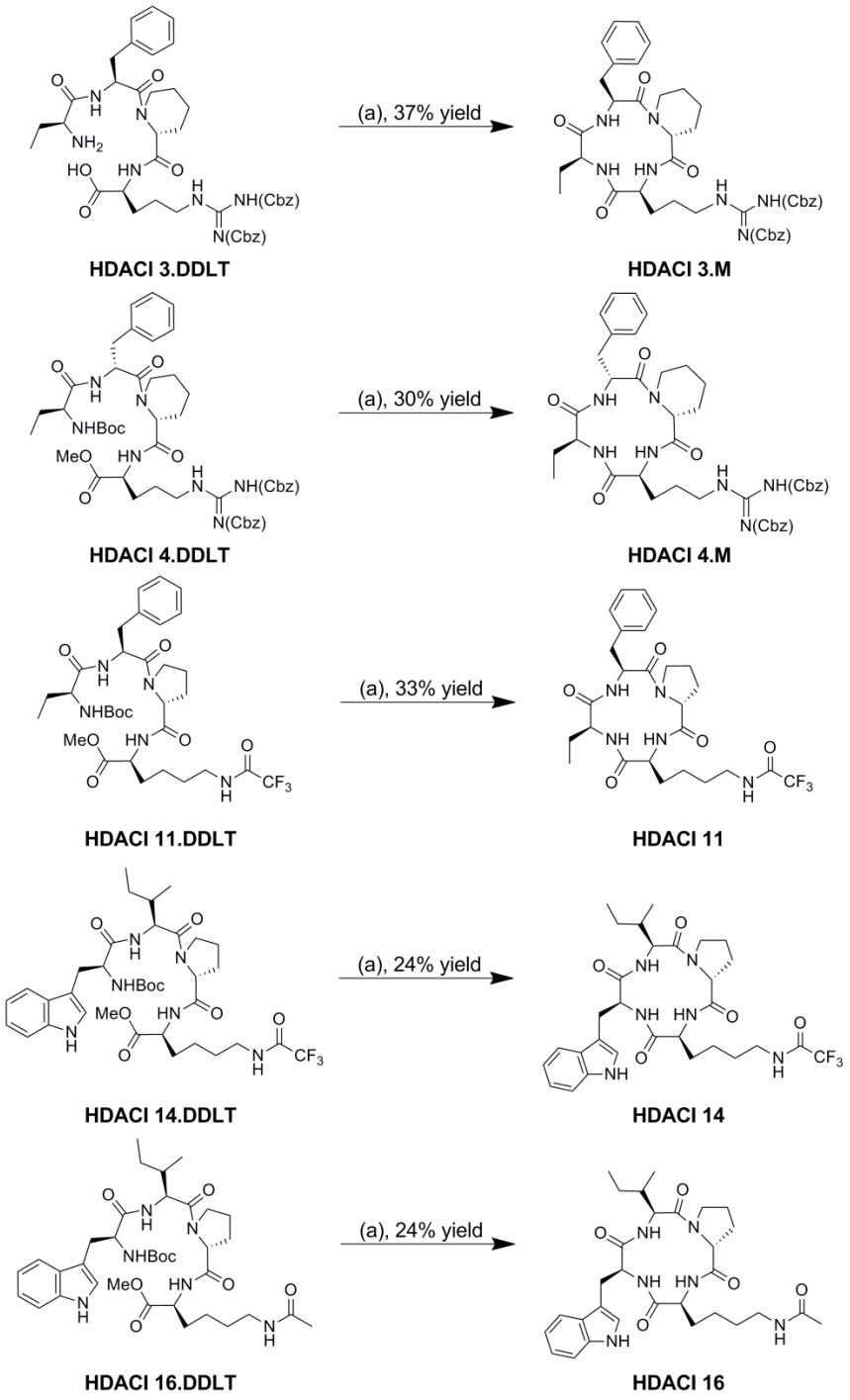
3.7.5 Macrocyclization to generate peptide HDACIs

Similar to the cyclization reactions carried out to furnish my SanA derivatives, HDACI derivatives were generated by running the macrocyclization under dilute concentrations (<0.01 M) to avoid oligomerization of my peptides. Macrocyclization for each HDACI derivative utilized a cocktail of coupling reagents and DIPEA to push the reaction to completion. Generating macrocycle **HDACI 3.M** (*cyclo*-Phe-Abu-Arg(2Cbz)-D-Pip) was accomplished using 0.7 equivalents each of TBTU, HATU and DEPBT, and 8.0 equivalents of DIPEA combined with **HDACI 3.DDLT**. Purging the flask with double deprotected peptide and reagents with argon and dissolving them in anhydrous ACN, DCM, THF (1:2:2) generated the ideal reaction conditions in an overall concentration of 0.007 M. Monitoring the reaction by LC/MS for disappearance of starting material indicated it was complete in ~4 hours. Upon completion, crude **HDACI 3.M** underwent an acid-base extraction followed by flash column chromatography. Pure **HDACI 3.M** eluted at 99:1 EA:MeOH and was furnished in 37% yield; the structure and purity of the macrocycle was confirmed via LC/MS and ^{1}H NMR. **HDACI 4.M** (*cyclo*-D-Phe-Abu-Arg(2Cbz)-D-Pip) was synthesized in a similar fashion and furnished in 30% yield. Both **HDACI 3.M** and **HDACI 4.M** required an additional deprotection step to remove the two Cbz groups protecting the guanidinum moiety of Arg. **HDACI 11** (*cyclo*-Phe-Abu-Lys(Tfa)-D-Pro, 33% yield), **HDACI 14** (*cyclo*-Ile-Trp-Lys(Tfa)-D-Pro, 24% yield) and **HDACI 16** (*cyclo*-Ile-Trp-Lys(Ac)-D-Pro, 24% yield) were generated following the



Scheme 3.5: Deprotection of linear tetrapeptide (a) 12 N HCl (8 drops/0.3 mmol), anisole (2.0 equivalents), THF (0.1 M), 24 h; then 3 drops/0.3 mmol 12 N HCl (day 2 and 3)

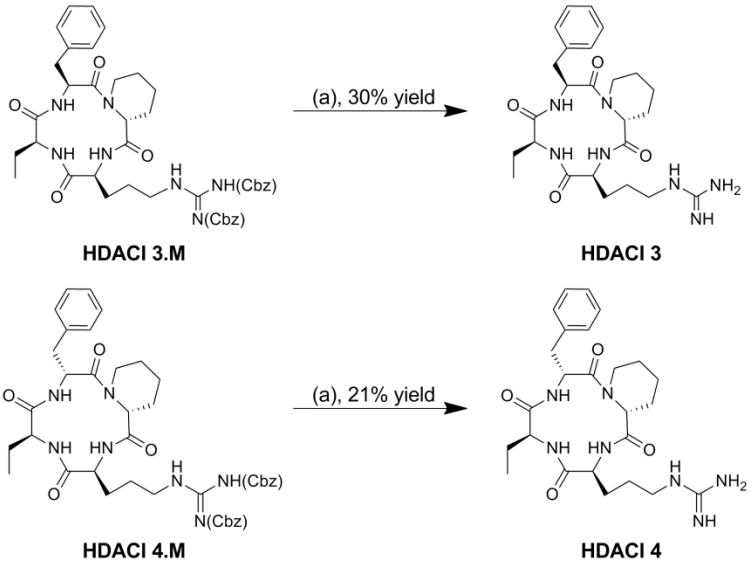
aforementioned procedure and taken on directly for biological testing after RP-HPLC purification (**Scheme 3.6**).



Scheme 3.6: Macrocyclization of peptide HDACI (a) TBTU/HATU/DEPBT (1.8-2.0 equivalents total), DIPEA (6.0-10.0 equivalents), ACN and/or DCM (0.007 M)

3.7.6 Removal of Cbz protecting groups

Hydrogenolysis was necessary to remove the two Cbz protecting groups on Arg to furnish **HDACI 3** and **HDACI 4**. This was done by dissolving protected **HDACI 3.M** in ethanol (EtOH) to 0.01 M concentration with a catalytic amount of Pd/C. Hydrogen gas was purged through the reaction solution and the deprotection was monitored by LC/MS. After 6 hrs the reaction was complete and the mixture was filtered over Celite® to remove Pd/C (Scheme 3.7). The final compound **HDACI 3** (*cyclo*-Phe-Abu-Arg-D-Pip, 30% yield) was subjected to RP-HPLC purification prior to biological testing; its structure and purity were verified via ^{1}H NMR and LC/MS. Hydrogenolysis was also performed on **HDACI 4.M** in a similar fashion to furnish the desired deprotected **HDACI 4** (*cyclo*-D-Phe-Abu-Arg-D-Pip, 21% yield).

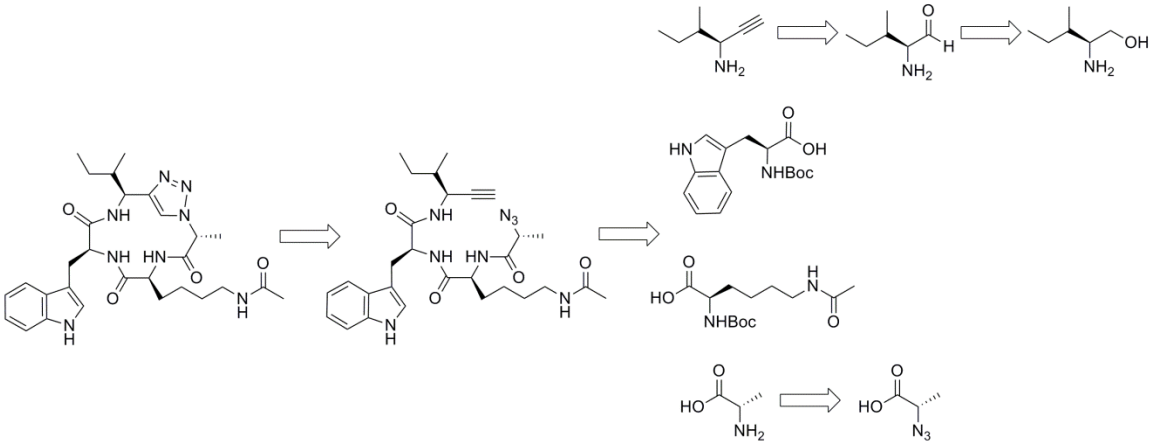


Scheme 3.7: Deprotection of guanidinium moiety in Arg (a) Pd/C (cat. amount), H_2 gas, EtOH (0.01 M)

3.8 Retrosynthesis of pseudopeptide HDACI

My retrosynthetic analysis of my pseudopeptide HDACI is shown in **Scheme 3.7**. The synthesis of my pseudopeptide HDACI derivative was carried out following a linear approach whereby each residue was sequentially added onto the molecule in solution-

phase. A linear approach for constructing this molecule was advantageous compared to a convergent approach because only Boc removal reactions were necessary in between couplings. The Boc removal reaction is typically complete in ~45 min whereas acid deprotections can take 3-4 hours, so the linear approach was a faster route than a convergent approach. The desired macrocyclic pseudo tetrapeptide was furnished by a 1,3-cycloadditon between an alkyne and azide moiety to generate a 1,4-triazole as described by Sharpless and co-workers.[36] The pseudo linear tetrapeptide contained a terminal alkyne and azide moiety and was furnished through sequential peptide couplings. Each amino acid used to generate the linear pseudo tetrapeptide was commercially available as a Boc or OMe protected residues. Azide HO-D-Ala-N_3 was synthesized via a diazo transfer from commercially available HO-D-Ala-NH_2. Terminal alkyne Alk-Ile-NHBoc was synthesized from aldehyde Ald-Ile-NHBoc via a Seyferth-Gilbert homologation of the aldehyde that was furnished from an oxidation of commercially available Isoleucinol-NHBoc.



Scheme 3.8: Retrosynthetic analysis for pseudopeptide HDACI

3.9 Forward synthesis of pseudopeptide HDACI

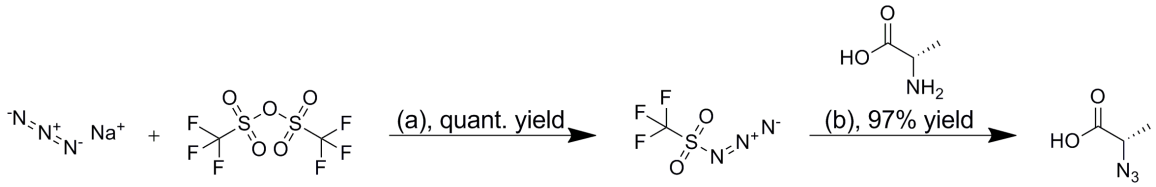
I synthesized one triazole containing HDACI. The commercially available amino acids and amino alcohol used were protected either at the *N*-terminus with a Boc group

or at the C-terminus as an OMe. The amino acid used to generate an azido moiety had free *N*- and C-termini. Coupling reagents were used to facilitate peptide bond formation. In the case of dipeptide couplings, TBTU was used. However, HATU was used for synthesizing the tripeptides and the tetrapeptide. Most peptide coupling reactions were carried out in anhydrous DCM. However, insoluble starting materials sometimes required the addition of anhydrous ACN to dissolve all reagents (see experimental for details).

3.9.1 Diazo transfer reaction

The 1,4-disubstitutted triazole present in **HDACI 17** was synthesized via a 1,3-cycloaddition between a terminal alkyne and an azide. I synthesized the azide of **HDACI 17.Az** (HO-D-Ala-N_3) by generating triflic azide (TfN_3) *in situ* first. First, sodium azide (NaN_3, 10.0 equivalents) was dissolved in water (6 M solution) and the solution was cooled to 0 °C for 10 min. Then DCM was added to the reaction flask generate an overall reaction concentration of 0.25 M. Triflic anhydride (Tf_2O, 2.0 equivalents) was added to the flask drop-wise (0.1 mL/min) to initiate formation of triflic azide. The biphasic mixture was monitored by TLC and complete transformation to TfN_3 occurred after ~2 hrs. The organic and aqueous layers were separated and the aqueous layer was back-extracted with DCM. All of the organic layers were collected and subjected to extraction with saturated sodium carbonate (Na_2CO_3), filtered, dried and kept in DCM. Next, HO-D-Ala-NH_2 (1.0 equivalent), potassium carbonate (K_2CO_3, 1.5 equivalents) and copper (II) sulfate ($CuSO_4 \cdot 5H_2O$, 0.01 equivalents) were combined with water and MeOH (1:1). The TfN_3 solution (in DCM) was added to the mixture for an overall concentration of 0.07 M (water:MeOH:DCM, 1:1:2). The reaction stirred at room temperature overnight and monitored for completion by TLC. Subsequently, the organic solvents were removed *in vacuo*, the aqueous slurry was diluted with water and acidified

to pH 6 with 12 N HCl and washed with EA. The organic layer was discarded and the aqueous layer was further acidified with 12 N HCl to pH 2 and washed with EA several times. These organic layers were dried, filtered and concentrated *in vacuo* to furnish pure **HDACI 17. Az** (HO-D-Ala-N_3) in 97% yield.

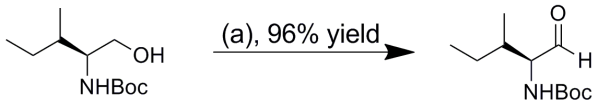


Scheme 3.9: Diazo-transfer reaction to generate azido alanine (a) NaN_3 (10.0 equivalents, 6 M in water), Tf_2O (2.0 equivalents), DCM (0.25 M), 0 °C; (b) HO-D-Ala-NH_2 (1.0 equivalent), K_2CO_3 (1.5 equivalents), CuSO4•5H2O (0.01 equivalents, water/MeOH/DCM (1:1:2, 0.07 M)

3.9.2 Oxidation of alcohol to aldehyde

Isoleucinal-NHBoc was not readily available, so the commercially available alcohol Isoleucinol-NHBoc was oxidized to the desired aldehyde;[37] the aldehyde was later transformed into a terminal alkyne. Isoleucinol-NHBoc (1.0 equivalent) was dissolved in biphasic mixture of Toluene, EA, and water (6:6:1) for 0.15 M overall reaction concentration. The reaction was cooled to 0 °C and a catalytic amount of 2,2,6,6-tetramethyl-l-piperidinyloxy (TEMPO) free radical and sodium bromide (NaBr, 0.02 equivalents) were added to the flask. The reaction continued to stir at 0 °C as an aqueous solution of sodium hypochlorite (NaOCl, 1.1 equivalents) and sodium bicarbonate ($NaHCO_3$, 2.9 equivalents) was added drop-wise (0.1 mL/min) over a period of 2 hrs. Upon monitoring the oxidation by TLC, the reaction was determined complete after ~4 hrs. The organic and aqueous layers were separated and the aqueous layer was back-extracted with ether. All organic layers were combined and washed with potassium iodide solution (KI, 0.04 equivalents) in 10% aqueous potassium hydrogen sulfate ($KHSO_4$) and then with brine. The organic layers were collected, dried, filtered

and concentrated *in vacuo*. The pure desired aldehyde **HDACI 17.Ald** was afforded in 97% yield; the structure and purity were confirmed by ^{1}H NMR (**Scheme 3.10**).

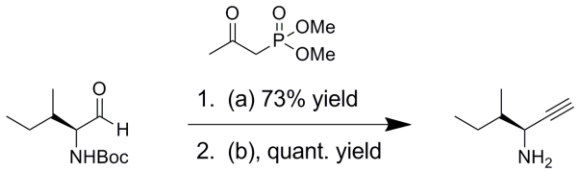


Scheme 3.10: Oxidation of Isoleucinol to Isoleucinal via TEMPO oxidation (a) TEMPO (cat. amount), NaBr (0.02 equivalents), NaOCl/$NaHCO_3$ buffer (1.1/2.9 equivalents, pH 8.9), Toluene/EA/water (6:6:1, 0.15 M)

3.9.3 Transformation of aldehyde to alkyne

Anhydrous K_2CO_3 (3.0 equivalents) was re-suspended in ACN (0.125 M) under argon atmosphere. Dimethyl (2-oxypropyl) phosphonate (3.0 equivalents) and *p*-tosyl azide (3.0 equivalents) were added drop-wise (0.1 mL/min) to the suspension to generate the Bestman-Ohira reagent. The reaction mixture was stirred at RT and was monitored via TLC for the disappearance of dimethyl (2-oxypropyl) phosphonate (~2 hrs), which indicated that the Bestmann-Ohira reagent was formed. **HDACI 17.Ald** (1.0 equivalent) was dissolved in calculated amount of dry methanol to bring the total reaction concentration to 0.25 M. Isoleucinal was added to the reaction flask drop-wise (0.1 mL/min) and left to stir overnight at rt. The reaction was complete the next day as confirmed by TLC. The crude reaction was concentrated *in vacuo* and then redissolved in EA and washed with saturated sodium bicarbonate and then brine. The extracted product (organic phase) was concentrated *in vacuo* and subjected to further purification by column chromatography and pure Alk-Ile-NHBoc eluted at 1:1 Hex:EA and was furnished in 73% yield; the structure and purity were confirmed by ^{1}H NMR.

To prepare the alkyne for installation to the next amino acid, the Boc protecting group was removed under acidic conditions. Alk-Ile-NHBoc was stirred under open atmosphere in a mixture of TFA:DCM (1:3) and 2.0 equivalents of anisole at 0.1 M concentration. The reaction was run at RT and was monitored via TLC every 15 min.

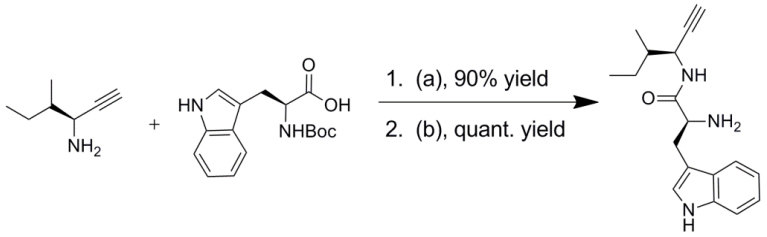


Scheme 3.11: Transforming aldehyde to alkyne (a) K_2CO_3 (3.0 equivalents), dimethyl (2-oxypropyl) phosphonate (3.0 equivalents) and *p*-tosyl azide (3.0 equivalents), ACN (0.125 M) then Ald-Ile-NHBoc (1.0 equivalent), MeOH (0.25 M); (b) Anisole (2.0 equivalents), TFA/DCM (1:3, 0.1 M)

Upon completion (~1 hr), the solution was concentrated *in vacuo* to yield the free amine Alk-Ile-NH_2 (**HDACI 17.Alk**) in quantitative yield.

3.9.4 Installation of Tryptophan

Next, residue II (Trp) was installed via peptide coupling. Free amine Alk-Ile-NH_2 (**HDAC 17.Alk**, 1.1 equivalents), free acid HO-Trp-NHBoc, and TBTU (1.2 equivalents) were dissolved in anhydrous DCM at 0.1 M concentration. DIPEA (6.0 equivalents) was added to the reaction flask, the reaction was run under argon at rt and was monitored via TLC. Upon completion (~1 hr), the crude pseudo dipeptide (Alk-Ile-Trp-NHBoc) was purified via acid-base extraction and its structure and purity were confirmed via ^{1}H NMR (**Scheme 3.12**, 90% yield).



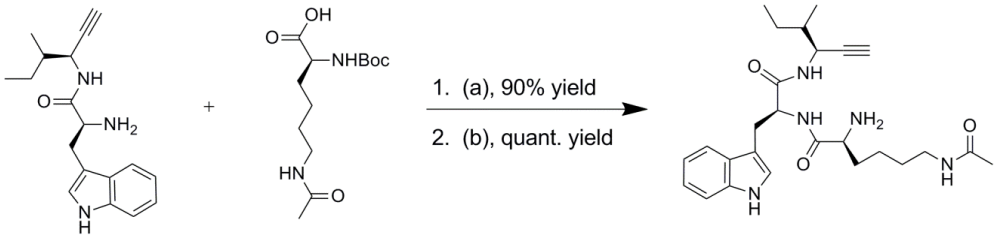
Scheme 3.12: Synthesis of pseudo dipeptide (a) TBTU (1.2 equivalents), DIPEA (6.0 equivalents), DCM (0.1 M); (b) anisole (2.0 equivalents), TFA/DCM (1:3, 0.1 M)

Deprotection of the amine on pseudo dipeptide Alk-Ile-Trp-NHBoc was completed under open atmosphere. The pseudo dipeptide was diluted to 0.1 M concentration with 20% TFA and 80% DCM, with 2.0 equivalents of anisole. The

reaction was monitored for disappearance of starting material by TLC and was complete within one hour. The solution was concentrated *in vacuo* to furnish **HDACI 17.Di** (Alk-Ile-Trp-NH_2) in quantitative yield.

3.9.5 Installation of N-acetyl Lysine

N-acetyl Lysine, was also installed via peptide coupling. Free amine Alk-Ile-Trp-NH_2 (**HDAC 17.Di**, 1.1 equivalents), free acid HO-Lys(Ac)-NHBoc, and TBTU (1.2 equivalents) were dissolved in anhydrous DCM at 0.1 M concentration. DIPEA (6.0 equivalents) was added to the reaction flask, the reaction was run under argon at room temperature and was monitored via TLC. Upon completion (~1 hr), the crude pseudo tripeptide (Alk-Ile-Trp-Lys(Ac)-NHBoc) was purified via acid-base extraction and then concentrated *in vacuo* for further purification via flash column chromatography. Pure pseudo tripeptide eluted at 100% EA and was furnished in 90% yield; the structure and purity was confirmed via LC/MS and ^{1}H NMR (**Scheme 3.13**).

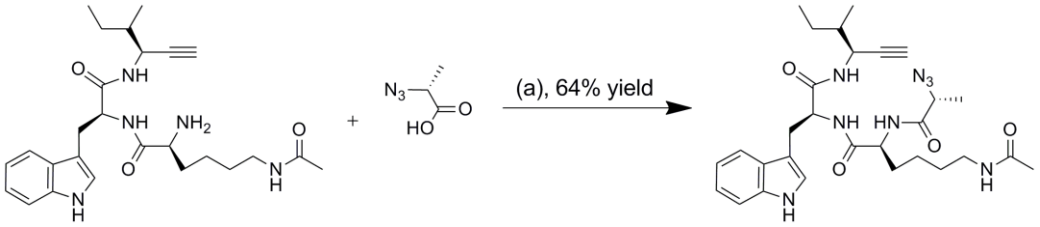


Scheme 3.13: Synthesis of pseudo tripeptide (a) TBTU (1.2 equivalents), DIPEA (6.0 equivalents), DCM (0.1 M); (b) anisole (2.0 equivalents), TFA/DCM (1:4, 0.1 M)

The Boc group was removed from Alk-Ile-Trp-Lys(Ac)-NHBoc under ambient conditions. The pseudo tripeptide was diluted to 0.1 M concentration in a mixture of TFA:DCM (1:4), with 2.0 equivalents of anisole. The reaction was monitored by TLC for disappearance of starting material and was complete within 1 hr. The solution was concentrated *in vacuo* to furnish **HDACI 17.Tri** (Alk-Ile-Trp-Lys(Ac)-NH_2) in quantitative yield.

3.9.6 Generation of pseudo tetrapeptide

Finally, HO-Ala-N_3 was coupled to pseudo tripeptide **HDACI 17.Tri** to install the azido moiety necessary for synthesizing a 1,4-disubstituted triazole. Free acid **HDACI 17.Az** (1.0 equivalents), free amine **HDACI 17.Tri** (1.1 equivalents) and 0.8 equivalents each of TBTU and HATU were dissolved in an anhydrous ACN and DCM (1:1) for 0.1 M overall concentration. DIPEA (10.0 equivalents) was added to the reaction flask under argon atmosphere and the reaction was monitored by TLC and LC/MS for completion. Upon completion (~2 hrs), the crude pseudo tetrapeptide (**HDACI 17.Tet**, Alk-Ile-Trp-Lys(Ac)-Ala-N_3) was purified via acid-base extraction and then concentrated *in vacuo* for further purification via flash column chromatography. Pure pseudo tripeptide eluted at 98:2 EA:MeOH and was furnished in 64% yield; the structure and purity was confirmed via LC/MS and ^{1}H NMR (**Scheme 3.14**).

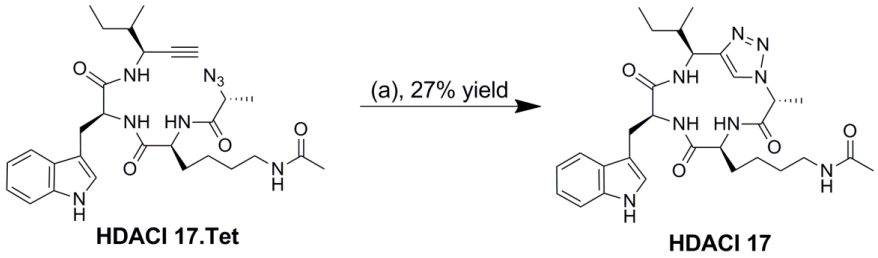


Scheme 3.14: Synthesis of pseudo tetrapeptide (a) TBTU (0.8 equivalents), HATU (0.8 equivalents), DIPEA (10.0 equivalents), ACN/DCM (1:1, 0.1 M)

3.9.7 Azide-alkyne cycloaddition of generate pseudopeptide HDACI

Sodium L-ascorbate (9.0 equivalents) was dissolved in 0.5 mL of water and put into a reaction flask. $CuSO_4$•$5H_2O$ (0.3 equivalents) was dissolved in an additional 0.5 mL of water and added to the flask. The reaction was run in a mixture of solvents MeOH and water (1:1) at 0.005 M overall concentration of linear pseudo-tetrapeptide and 10% of the solvent mixture was added to the flask. **HDACI 17.Tet** (1.0 equivalent) was dissolved in the remaining solvent mixture and added drop-wise via syringe pump to the reaction mixture (0.1 mL/min) and stirred overnight. Upon completion of the reaction,

confirmed by TLC and LC/MS, MeOH was removed *in vacuo*. The reaction was diluted with DCM, where the organic layer that contained crude **HDACI 17** (*cyclo*-Ile-Trp-Lys(Ac)-D-Ala-triazole) was collected and concentrated *in vacuo*. Further purification via flash column chromatography furnished pure **HDACI 17**, which eluted at 95:5 EA:MeOH and was furnished in 27% yield (**Scheme 3.15**). The final compound **HDACI 17** was subjected to RP-HPLC purification prior to biological testing; its structure and purity were verified via ^{1}H NMR and LC/MS.



Scheme 3.15: Synthesis of **HDACI 17** (a) $CuSO_4 \cdot 5H_2O$ (0.3 equivalents, 1.5 mM), sodium ascorbate (9.0 equivalents, 45 mM), MeOH:water (1:1, 0.005 M)

3.10 Biological data

HDAC activity was measured using Fluor de Lys® Fluorescent assay (Figure 3.9). HeLa lysates were added to DMSO vehicle or HDACI (5 mM). Fluor de Lys® substrate (25 µL, for an overall 500 µM concentration), comprised of an acetylated lysine side chain, was added to the reaction mixture and incubated for 1 hr at RT. Deacetylation of the substrate sensitized the substrate so that when the Fluor de Lys® developer (50 µL) was added, a fluorophore was produced. The developer was incubated with the reaction mixture for 10 min and fluorescence intensity was determined at 460 nm using a S6 Genios Fluorimeter. The deacetylase activity was the result of dividing the fluorescence intensity of the reaction in the presence of compound by the intensity in the absence of compound (Figure 3.10).

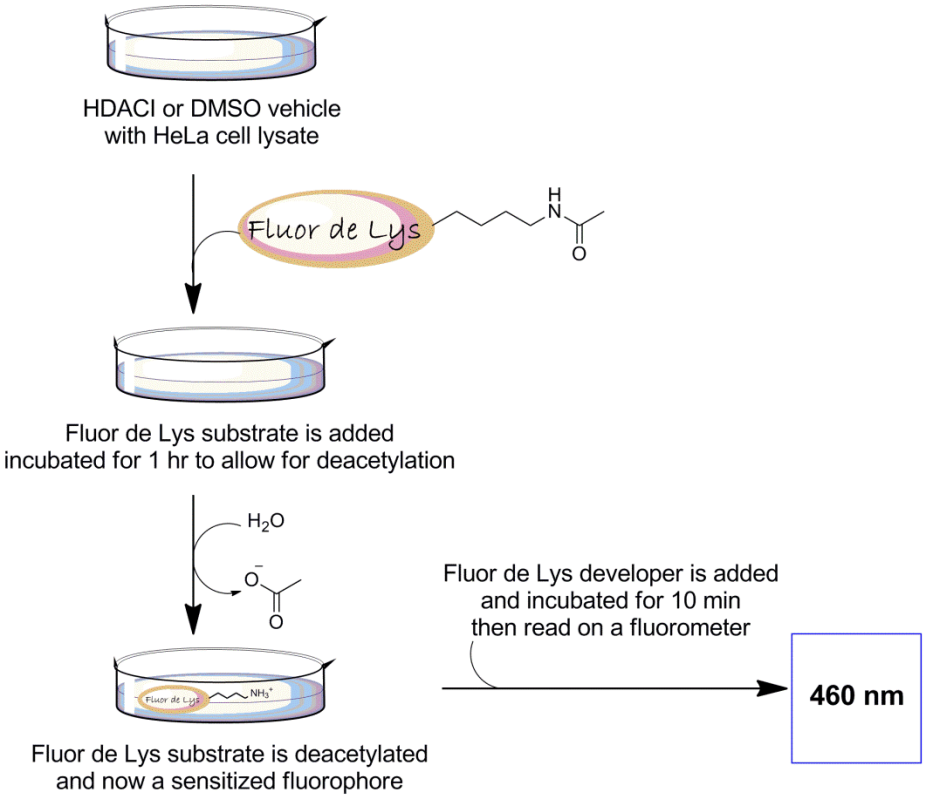


Figure 3.10: Schematic of Fluor de Lys HDAC inhibition activity assay

All HDACI derivatives were assayed at an overall concentration of 200 μM and the trends appearing in the HDACI's SAR were examined based on HDAC activity (Figure 3.11). We found some trends with each of the four residues. At residues I and II, we found that that L- or D-stereochemistry showed no significant difference in deacetylase activity, as seen with **HDACI 1** vs. **2**, **HDACI 3** vs. **4**, and **HDACI 14** vs. **15**. Additionally, **HDACI 7** and **8** only differ in the presence of an *N*-methyl at the residue I, suggesting that this single methyl group substitution influences potency by altering the conformation of the macrocyclic surface recognition unit.

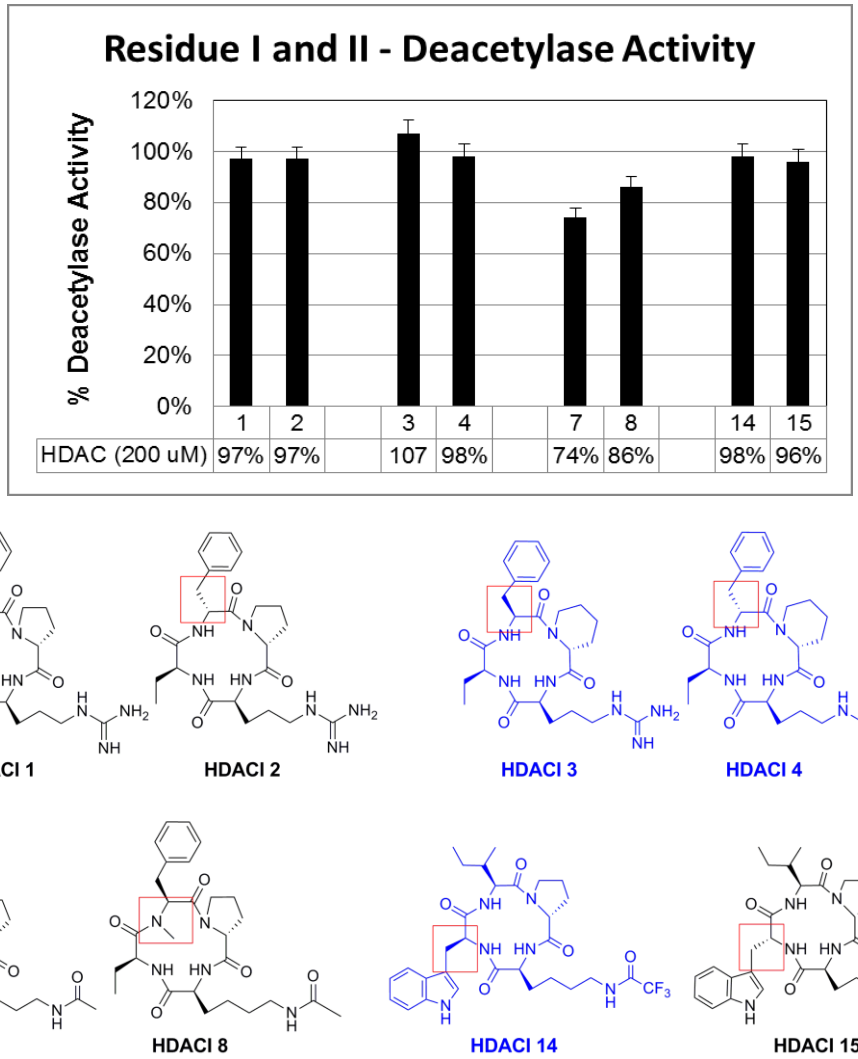
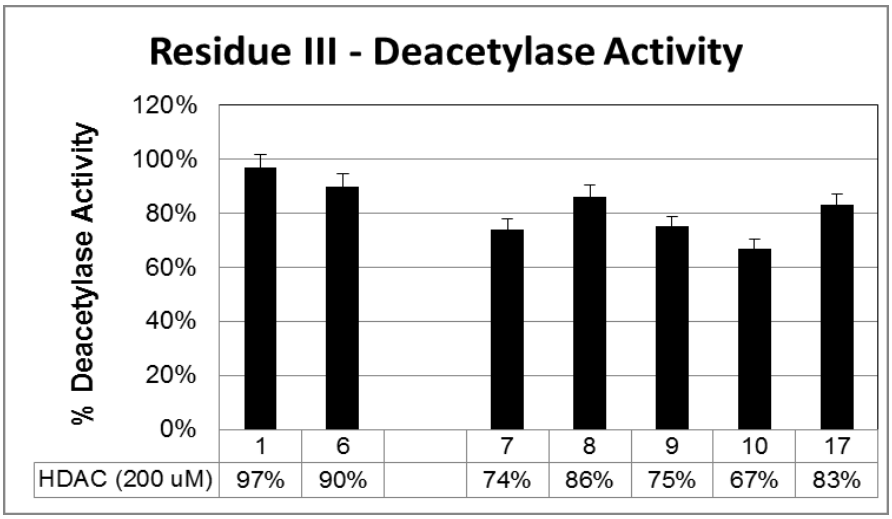


Figure 3.11: Investigating the stereochemistry at residues I and II, and the presence of an *N*-methyl at residue I. HDACIs were run at 200 μM

Residue III contained two elements of the basic HDACI pharmacophore model: 1) the linker and 2) the enzyme inhibition moiety. We explored linker lengths of four and five atoms in length. When **HDACI 1** (four atom linker) was compared to **HDACI 6** (five atom linker), the inhibitor with a five atom linker was only slightly better. Like residues I and II, having L- or D- stereochemistry did not show any significant difference. In terms of enzyme inactivation moieties, the acetyl group was generally most effective in HDACIs compared to HDACIs with a guanidyl or trifluoroacetyl (**Figure 3.12**).



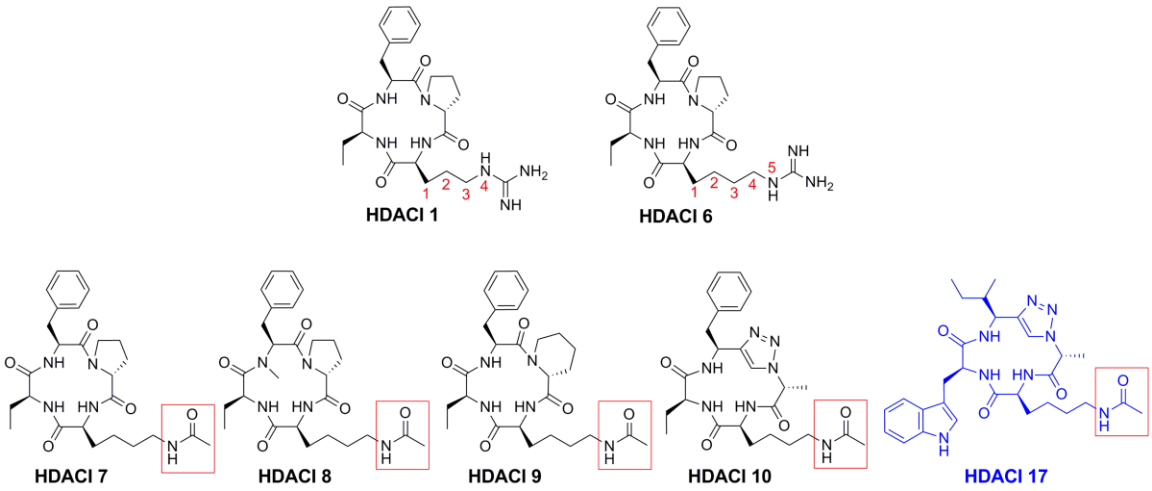


Figure 3.12: Investigating the linker length, stereochemistry of residue III and an acetyl enzyme inactivation moiety. HDACIs were run at 200 μM

Finally, at residue IV, we investigated the difference between having a five or six membered ring or a 1,4-disubstituted triazole. A direct comparison of all three of these cyclic moieties was made at residue IV with two series of HDACIs: **HDACI 7**, **9**, vs **10** and **HDACI 11**, **12**, vs **13** (five membered ring, six membered ring, triazole, respectively). In both series the five membered ring was better than the six membered ring, and the triazole was more effective HDACI in the series. With **HDACI 16** (five membered ring) vs. **HDACI 17** (triazole), the triazole containing compound was a more effective **HDACI** (**Figure 3.13**).

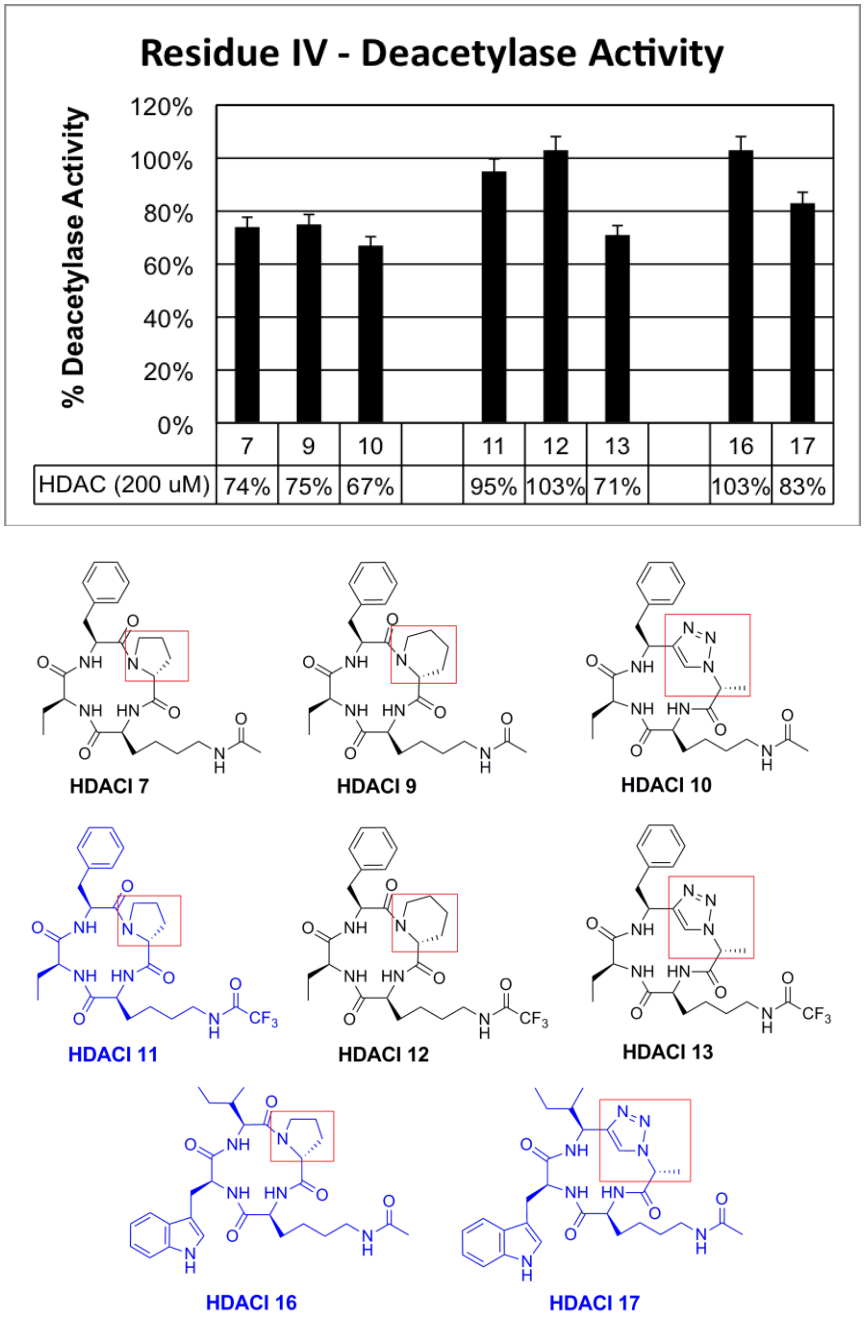


Figure 3.13: Investigating the ring at residue IV. Deacetylace activity of HDACIs; HDACIs were run at 200 μM.

3.11 Conclusions

I synthesized a total of six HDACI derivatives, one of which contained a triazole residue. These HDACIs were based on two natural product cyclic tetrapeptides. We discovered several trends that can be applied for future studies of optimizing cyclic

peptide HDACIs. The stereochemistry at residues I – III did not impact the activity of the HDACIs. Previous work showed that it was optimal to maintain a D-ring at residue IV. We found that having a 1,4-disubstituted triazole at residue IV was advantageous when comparing their activity to their peptide counterparts. Finally, we determined that the acetyl group was a more effective enzyme inactivator moiety than either the guanidine or trifluoroacetyl.

3.12 References

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Chapter 4 – Sanguinamide B

4.1 History of Sanguinamide B

Sanguinamide B (**SanB**) was isolated from a nudibranch collected in the Yasawa Island chain in Figi.[1] *Hexabranchus sanguineus,* literally meaning "six-gilled blood-colored" is a very large, colorful sea slug with a spongiverous diet.[2] *H. sanguineus* and its egg masses contain numerous bioactive polyketide macrolides including the kabiramides[3], ulapualides[4] and several trisoxazole-containing macrolides (**Figure 4.1**).

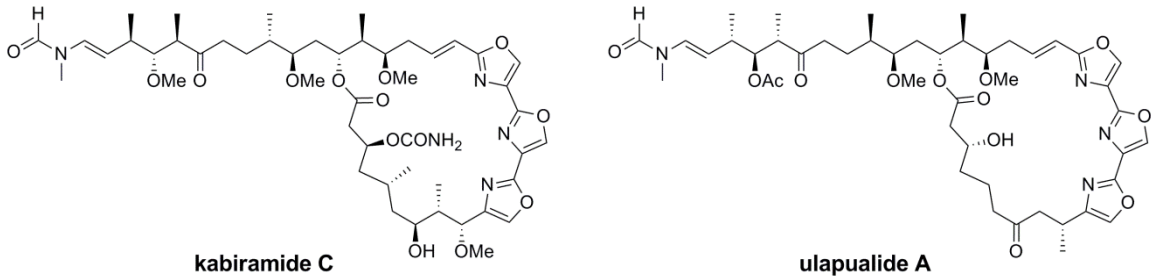


Figure 4.1: Examples of bioactive polyketide macrolides isolated from *H. sanguineus*

In 2009, Molinski and co-workers determined the structure of **SanB** (Figure 4.2) was a modified macrocyclic octapeptide that contained five L-configuration amino acids (Valine, two Prolines, Leucine, and Alanine), two thiazoles and one oxazole.[1] This was the first report of a thiazole-containing peptide isolated from *H. sanguineus*, which suggests the thiazole comes from either an unidentified dietary source or *de novo* biosynthesis .[1]

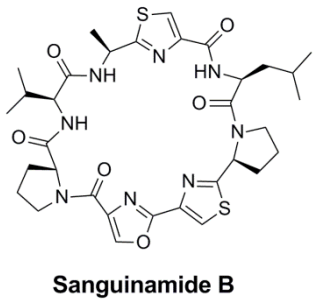


Figure 4.2: Sanguinamide B (SanB)

4.2 Structural features of Sanguinamide B

SanB falls in a unique class of natural products as it bears directly linked azoles: a 4,2-oxazole-thiazole moiety. Over the last two decades there has been a surge in discoveries of natural products with directly linked azoles and many of these compounds are now candidates for drug development.[5] Mixed 4,2-bisheterocycle tandem pairs, however, are extremely rare in peptides and only two natural products have been reported to date to contain an oxazole-thiazole subunit [5]: Leucamide A[6] and microcin B17[7] (**Figure 4.3**).

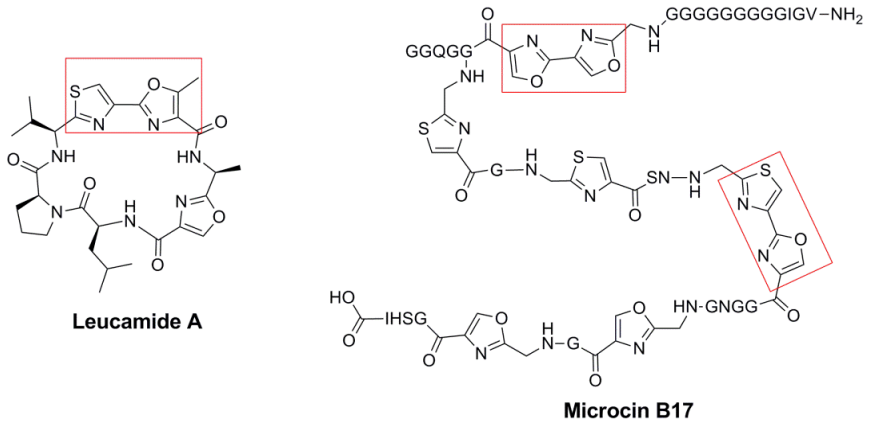


Figure 4.3: Natural products with tandem bisheterocycles pairs

Leucamide A is a cytotoxic modified heptapeptide with a methyloxazole and thiazole tandem pair isolated from an Australian marine sponge *Leucetta microraphis*.[6] The macrocycle was cytotoxic against several tumor cell lines in low micromolar concentrations.[6] Microcin B17 is a peptide found in some strains of *Escherichia coli* (*E. coli*) and has antibiotic activity, inhibiting bacterial DNA gyrase.[8] DNA gyrase is an enzyme found in prokaryotes and some eukaryotes that relieves strain while ds-DNA is being unwound by helicase. Given that this enzyme is not found in humans, it is a good target for antibiotics. There are two oxazole-thiazole units present in this 43-residue peptide antibiotic. Walsh and co-workers investigated the importance of the location and presence of the tandem bisheterocycle moieties in the peptide sequence and found that

altering or removing the two thiazole-oxazole pairs significantly reduced antibiotic activity.[7] Given the importance of the mixed tandem bisheterocycle moiety, performing the first total synthesis of SanB and exploring its pharmacological potential is very appealing.

The two prolines present in **SanB** also make this natural product very intriguing. Prolines, like *N*-methyl amino acids, are very common structural motifs in bioactive peptides that dramatically alter the 3D conformation of macrocycles.[9] Specifically, the rotation about prolyl amide bonds is restricted and, in peptides, the proline residue may adopt either a *cis*- or *trans*- conformation.[1] The difference between a *cis*- or *trans*- amide bond significantly alters the 3D shape of a macrocycle and may induce a bio-active or -inactive conformation. Both conformational isomers, *cis,cis*- and *trans,trans*-Ceratospongamide (Figure 4.4), were isolated from a symbiotic sponge (*Sigmadocia symbiotica)*, where its structure consists of a modified heptapeptide with a thiazole, methyl-oxazoline and two prolines.[10] When tested for anti-inflammatory activity, only the *trans,trans*- conformer exhibited potent inhibition of $sPLA_2$ expression (ED_{50}=32 nM), whereas the *cis,cis* isomer was inactive.[10] Given this precedence, the possibility of synthesizing multiple SanB conformational isomers with *cis* or *trans* about the two proline amide bonds will lead to a very interesting investigation, both synthetically and biologically.

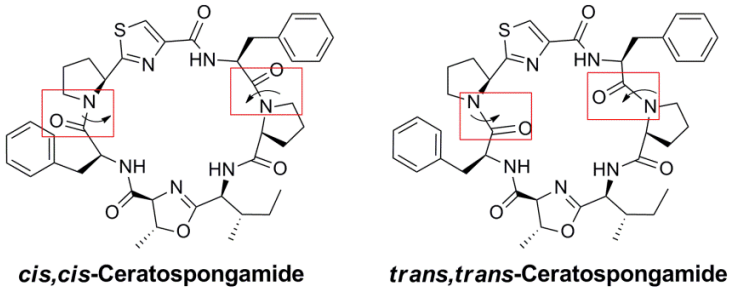


Figure 4.4: Conformational isomers *cis,cis*- and *trans,trans*-Ceratospongamide

SanB, a modified macrocyclic octapeptide resembles numerous biologically active natural products including ascidiacyclamide, patellamide D, and telomestatin, all of which are 24-atom macrocycles (**Figure 4.5**). Ascidiacyclamide is a cytotoxic cyclic peptide with thiazole and methyl-oxazoline rings isolated from Ascidiacea (sea squirt).[11] This natural product exhibited potent cytotoxic activity against P388 lymphocytic leukaemia cells (IC_{50}=14 μM).[12] Another biologically active 24-atom macrocycle is patellamide D, isolated from a marine tunicate *Lissaclinum patella.* Pattellamide D is a resistance-modifying agent that reverses resistance to anti-cancer agent Vinblastine in CEM/VLB_{100} cells.[13] Finally, the most investigated of these 24-atom macrocycles is telomestatin. Telomestatin stabilizes the G-quadruplex found in telomeric DNA and inhibits telomerase activity (IC_{50} = 5 nM) in numerous cancerous cells, inducing apoptosis.[14] The relatively potent biological activity of these 24-atom macrocycles emphasize the potential for **SanB** to have interesting biological activity**.**

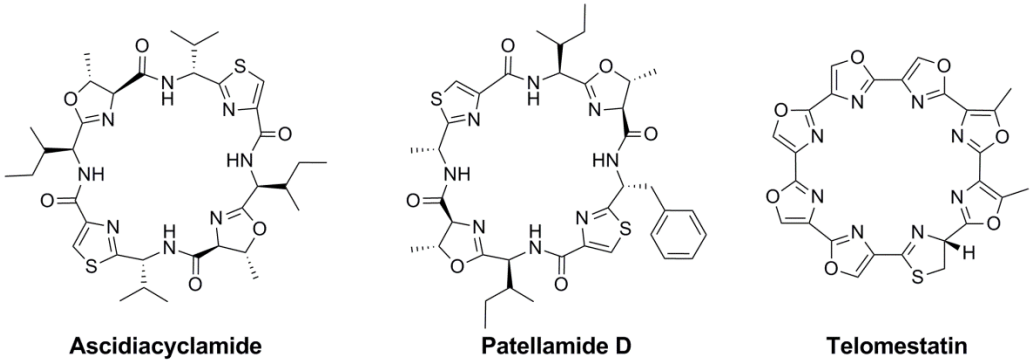
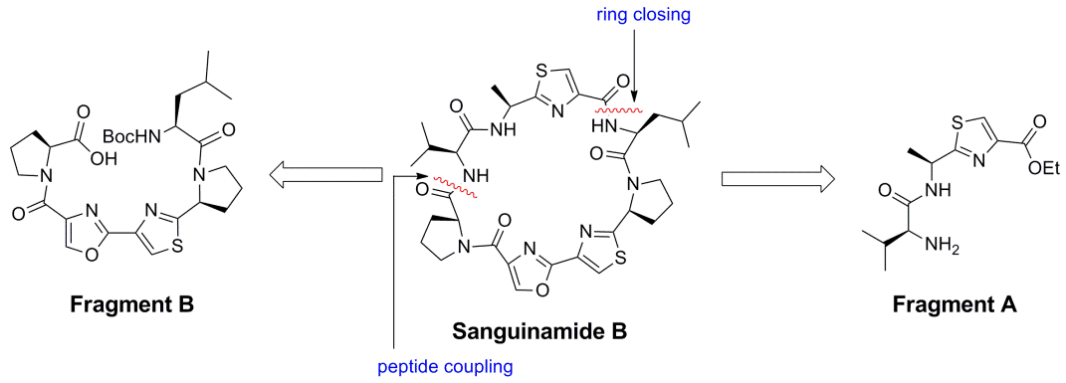


Figure 4.5: Biologically active 24-atom natural product macrocycles

4.3 Retrosynthetic approach

My retrosynthetic analysis for the synthesis of the SanB natural product (Scheme 4.1) involved a convergent solution-phase strategy, using a head-to-tail cyclization of a linear octapeptide precursor. The octapeptide was generated by coupling two

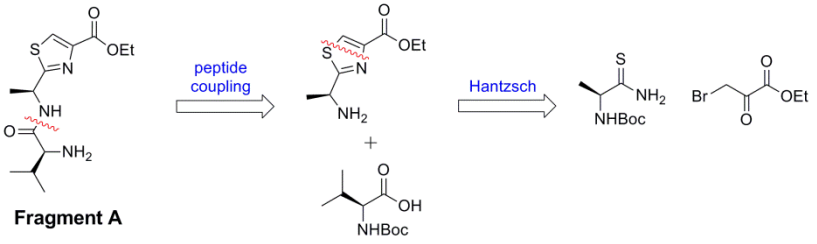
fragments: fragment A (a tripeptide containing a thiazole) and fragment B (a pentapeptide containing the tandem oxazole-thiazole).



Scheme 4.1: Retrosynthetic analysis of Sanguinamide B

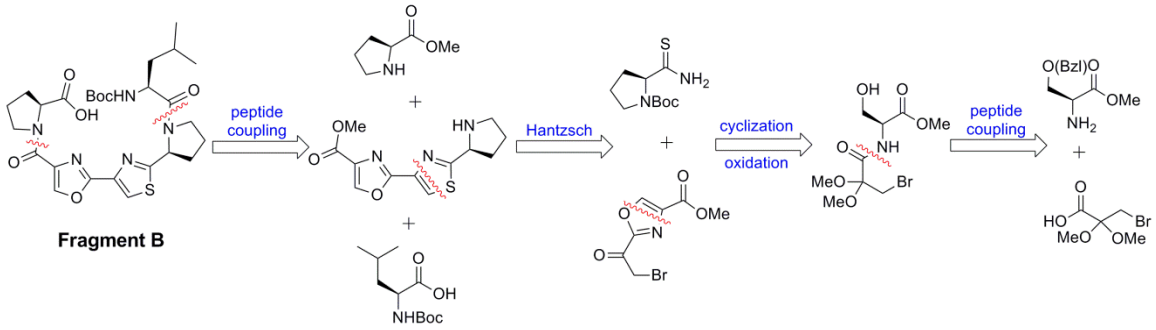
Fragment A was derived from a Hantzsch thiazole reaction between an Alanine thioamide and an (α)-bromoketone, followed by *N*-terminal extension with Valine (**Scheme 4.2.a**). Fragment B was also obtained via a Hantzsch reaction between an Ox-(α)-bromoketone and a Proline thioamide to form a diheterocyclic Ox-Th moiety. The Ox-(α)-bromoketone was obtained from the cyclization and oxidation of a Ser residue. Subsequent extension of the core Ox-Th moiety via peptide coupling to Proline and Leucine furnished Fragment B (**Scheme 4.2.b**). Each fragment was synthesized in solution-phase using commercially available Boc, OMe, or ethyl ester (OEt) protected starting materials.

(a)



Scheme 4.2: Retrosynthetic strategy for fragments A and B; (a) Retrosynthetic analysis for fragment A.

(b)



Scheme 4.2 (continued): Retrosynthetic strategy for fragments A and B; (b) Retrosynthetic analysis for fragment B

Generating the linear precursor via a convergent approach involved fewer steps and purifications than using a step-wise linear approach. Additionally, shorter fragments are more soluble in common organic solvents than longer fragments, thus promoting complete peptide bond formation and resulting in complete reactions and high yields.

4.4 Synthesis of Sanguinamide B

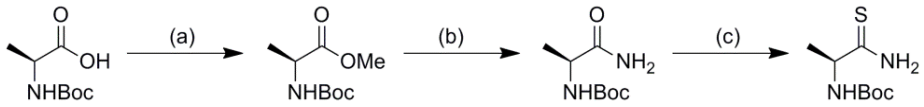
4.4.1 Synthesis of fragment A

Fragment A was comprised of a single thiazole and two amino acids: Alanine and Valine. The thiazole was generated first with an Ala residue, followed by *N*-terminal extension with Val.

4.4.1.1 Transformation of acid to thioamide

Synthesis of fragment A began by first converting the commercially available acid HO-Ala-NHBoc to ester MeO-Ala-NHBoc (**Scheme 4.3**). HO-Ala-NHBoc was dissolved in a 1:3 mixture of Benzene:MeOH (0.1 M) in a round-bottom flask, purged with argon. A solution of 2.0 M Trimethylsilyl diazomethane (TMSD, 10.0 equivalents), in diethyl ether, was added drop-wise to the reaction mixture. The reaction was stirred and monitored by TLC; upon completion (~30 min) the mixture was concentrated *in vacuo*.

The desired ester MeO-Ala-NHBoc was in confirmed via ^{1}H NMR, furnished in quantitative yield and taken on to the next transformation without further purification (**Scheme 4.3**).



Scheme 4.3: Acid to thioamide transformation (a) TMSD (10.0 equivalents), Benzene:MeOH (1:3, 0.1 M); (b) NH_4OH (25% in water):MeOH (1:3, 0.1 M); (c) LR (0.8 equivalents), DME (0.15 M)

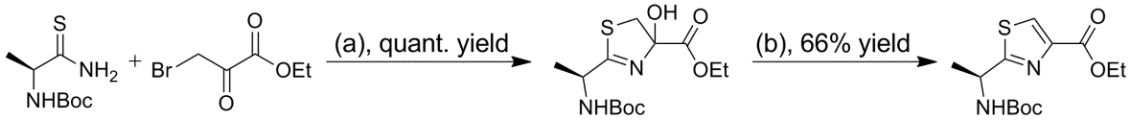
Next, ester MeO-Ala-NHBoc was converted to amide H_2N-Ala-NHBoc using ammonium hydroxide (**Scheme 4.3**) by dissolving the ester in a 0.1 M solution of ammonium hydroxide (25% in water) and MeOH. The reaction was monitored by TLC and upon completion (~12 hrs) the solution was concentrated *in vacuo*. The resulting amide was furnished in quantitative yield without further purification; structure and purity were verified by ^{1}H NMR.

Using Lawesson's Reagent (LR), a thiation agent, amide H_2N-Ala-NHBoc was converted to the desired thioamide H_2NS-Ala-NHBoc (**Scheme 4.3**). Amide H_2N-Ala-NHBoc (1.0 equivalent) and LR (0.8 equivalents) were placed in a dry round bottom flask, fitted with a septum and purged with argon. The amide and LR were dissolved in anhydrous 1,2-dimethoxyethane (DME) for an overall concentration of 0.15 M. The reaction mixture stirred and was monitored by TLC, upon completion (~12 hrs) the crude reaction mixture was purified via flash column chromatography. Pure thioamide H_2NS-Ala-NHBoc eluted in 1:1 Hex:EA and was furnished in 88% yield. The structure and purity were confirmed by ^{1}H NMR.

4.4.1.2 Thiazole formation

Thioamide H_2NS-Ala-NHBoc was subjected to modified Hantzsch thiazole synthesis conditions that preserved stereochemical integrity at Cα of the Ala residue

(**Scheme 4.4**).[15] H_2NS-Ala-NHBoc (1.0 equivalent) was dissolved in 50% of the total volume of anhydrous DME needed for an overall 0.1 M reaction concentration. Potassium bicarbonate ($KHCO_3$, 8.0 equivalents) was added to the reaction mixture and the slurry stirred for five min. Ethyl bromopyruvate (3.0 equivalents) was dissolved in the remaining DME and added to the reaction flask drop-wise using a syringe pump at a rate of 1.0 mL/min. The mixture stirred and was monitored by TLC (~16 hrs). The crude reaction was concentrated *in vacuo*, re-dissolved in EA and extracted with brine. The washed hydroxy-thiazoline intermediate was dried over sodium sulfate, filtered, concentrated *in vacuo* and taken on for a dehydration reaction without further purification or characterization.



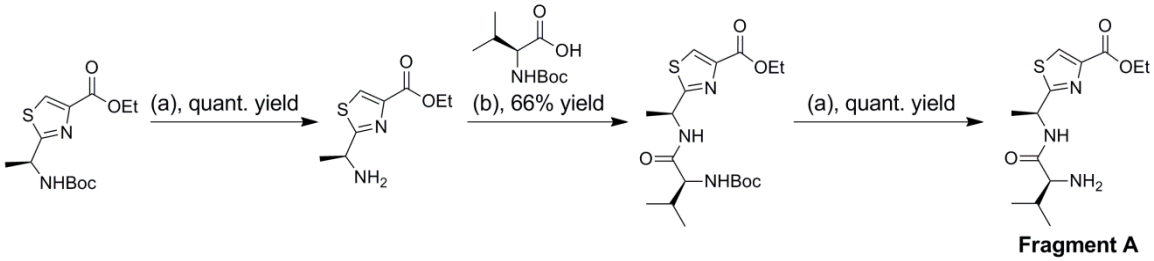
Scheme 4.4: Thiazole formation (a) $KHCO_3$ (8.0 equivalents), DME (0.1 M) 0 °C, 16 hrs; (b) pyridine (9.0 equivalents), DME (0.1M), 15 min; then TFAA (4.0 equivalents), 2 hrs; then TEA, 0 °C to RT, 12 hrs

Next, the hydroxy-thiazoline intermediate was dissolved in anhydrous DME for an overall concentration of 0.1 M under an atmosphere of argon. The solution stirred for 15 min at 0 °C, at which point pyridine (9.0 equivalents) was added to the reaction mixture drop-wise (0.1 ml/min) and stirred for an additional 15 min at 0 °C. Trifluoroacetic anhydride (TFAA, 4.0 equivalents) was added drop-wise (0.1 mL/min) to the reaction mixture and stirred at 0 °C for an additional two hrs. Finally, triethylamine (TEA, 2.0 equivalents) was added to the reaction flask drop-wise (0.1 mL/min) and stirred for 12 hrs as it warmed to room temperature. Reaction completion was confirmed by LC/MS and TLC; the mixture was concentrated *in vacuo*, re-dissolved in EA and subjected to an acid-base extraction followed by flash column chromatography. The pure OEt-Th-Ala-

NHBoc eluted in 9:1 Hex/EA and was furnished in 66% yield; its structure was confirmed by ^{1}H NMR.

4.4.1.3 Boc removals and valine installation to generate fragment A

With OEt-Th-Ala-NHBoc in hand, an amine deprotection was performed (Scheme 4.5) using open atmosphere, anisole (2.0 equivalents) and 20% TFA in DCM (0.1 M). The reaction was monitored by TLC for disappearance of starting material and was complete in one hr, at which point it was concentrated *in vacuo*. OEt-Th-Ala-NH_2 was furnished in quantitative yield without further purification or characterization.



Scheme 4.5: Synthesis of fragment A. (a) Anisole (2.0 equivalents), TFA/DCM (1:3), 0.1 M; (b) TBTU (1.2 equivalents), DIPEA (8.0 equivalents), DCM (0.1 M)

Next, by using 1.1 equivalents of commercially available acid HO-Val-NHBoc, 1.0 equivalent of free amine OEt-Th-Ala-NH_2, 1.2 equivalents of TBTU and 8.0 equivalents of DIPEA in anhydrous DCM (0.1 M) OEt-Th-Ala-Val-NHBoc was generated. The reaction mixture was stirred under an atmosphere of argon and upon completion (~3 hrs), where the product was confirmed by LC/MS and TLC, the mixture was concentrated *in vacuo*. The crude reaction was re-dissolved in EA and subjected to an acid-base extraction followed by flash column chromatography. Pure OEt-Th-Ala-Val-NHBoc eluted in 1:1 Hex/EA and was furnished in 66% yield; its structure was confirmed by ^{1}H NMR.

Finally, removal of the Boc protecting group from OEt-Th-Ala-Val-NHBoc was achieved with 20% trifluoroacetic acid (TFA) in 80% DCM (0.1 M overall concentration)

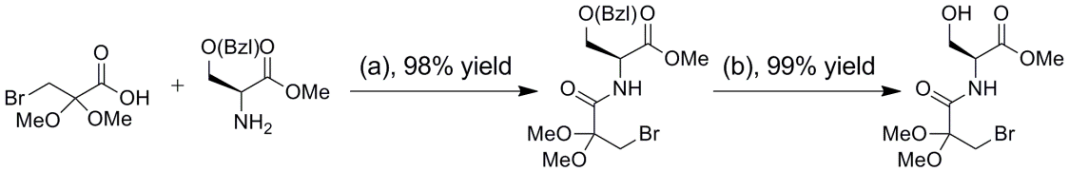
with 2.0 equivalents of anisole under open atmosphere. The reaction was monitored by TLC for disappearance of starting material and complete in one hr to furnish OEt-Th-Ala-Val-NH_2 (fragment A) in quantitative yield without further purification or characterization.

4.4.2 Synthesis of fragment B

Fragment B, comprised of two consecutive heterocycles (an oxazole and thiazole) and three amino acids, was synthesized by constructing the heterocycles first. Generating the oxazole and thiazole first, on smaller fragments has been shown to optimize the overall yield for this fragment.[16]

4.4.2.1 Synthesis of free serine pseudo dipeptide

Using standard peptide coupling conditions, amine MeO-Ser(Bzl)-NH_2 (1.1 equivalents), dimethoxyacetal bromopyruvic acid (1.0 equivalent), and TBTU (1.2 equivalents) with 6.0 equivalents DIPEA were dissolved in anhydrous DCM for an overall concentration of 0.1 M. The reaction was run under an atmosphere of argon and stirred for three hrs, at which point it was deemed complete by TLC. The crude reaction mixture was subjected to acid-base extraction and then further purified via flash column chromatography. The pure benzyl-protected pseudo-dipeptide MeO-Ser(Bzl)-bromoketal eluted in 1:1 Hex/EA and was furnished in 98% yield; structure and purity was confirmed via ^{1}H NMR (**Scheme 4.6**).



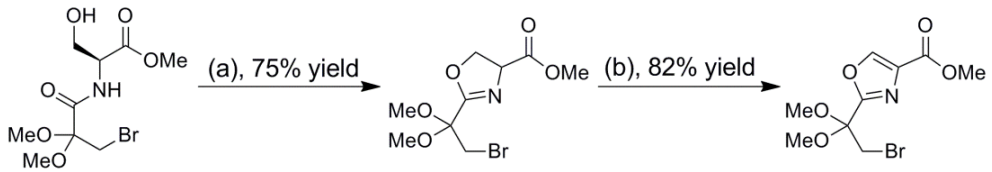
Scheme 4.6: (a) TBTU (1.2 equivalents), DIPEA (6.0 equivalents), DCM (0.1 M); (b) H_2, Pd/C (cat.), EtOH (0.1 M)

To prepare this pseudo-dipeptide for oxazole synthesis, the benzyl protecting group on Ser was removed via hydrogenolysis (**Scheme 4.6**). The benzyl-protected

pseudo-dipeptide MeO-Ser(Bzl)-bromoketal was dissolved in EtOH (0.1 M) and a catalytic amount of Pd/C was added to the solution. Hydrogen gas was purged through the solution at low atmospheric pressure and TLC was used to monitor the reaction. Upon completion (~5 hrs) the slurry was filtered over Celite®. Further purification was performed via flash column chromatography where the pure free hydroxyl containing pseudo-dipeptide MeO-Ser-bromoketal eluted in 7:13 Hex/EA and was furnished in 100% yield. Structure and purity was confirmed by ^{1}H NMR.

4.4.2.2 Oxazole generation

The first heterocycle for fragment B was accomplished by synthesizing an oxazole moiety along the backbone of the free hydroxyl in MeO-Ser-bromoketal (Scheme 4.7). The free hydroxyl pseudo-dipeptide was dissolved in anhydrous DCM (0.1 M) under argon at -78 °C. DAST (1.1 equivalents) was added dropwise (0.1 mL/min) to the reaction flask to fluorinate the serine hydroxyl as the solution stirred for 1 hr at -78 °C. Intramolecular cyclization to generate an oxazoline intermediate was accomplished by adding 2.0 equivalents of K_2CO_3 to the reaction flask; the reaction temperature was brought to RT as it stirred for 2 hrs. Upon completion, confirmed by TLC, the crude mixture was diluted with DCM and subjected to a base extraction and then further purified via flash column chromatography. Pure MeO-Oxazoline-bromoketal intermediate eluted at 3:7 Hex/EA in 75% yield and structure and purity were confirmed by ^{1}H NMR.

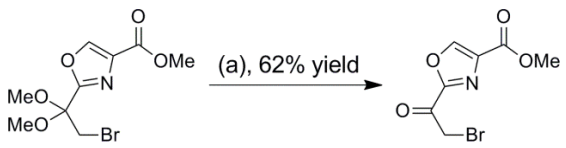


Scheme 4.7: Generation of oxazole moiety (a) DAST (1.1 equivalents), DCM (0.1 M), 1 hr, -78 °C; then K_2CO_3 (2.0 equivalents), 2 hrs, -78 °C to RT; (b) DBU (2.0 equivalents), DCM (0.1 M), 20 min, -47 °C; then $BrCCl_3$ (2.0 equivalents), 2 hrs, -47 °C to RT

The pure MeO-Oxazoline-bromoketal intermediate was dissolved in DCM at 0.1 M concentration under argon gas and cooled to -47 °C. DBU (2.0 equivalents) was added drop-wise (0.1 mL/min) to the reaction flask and stirred for 20 min. Next, $BrCCl_3$ (2.0 equivalents) was added to the reaction flask drop-wise (0.1 mL/min) and stirred for 2 hrs at -47 °C. Finally the reaction was allowed to warm to RT as it stirred for an additional 12 hrs. Reaction completion was confirmed by TLC, and the crude reaction was subjected to an acid-base extraction followed by additional purification via flash column chromatography. The pure MeO-Ox-bromoketal eluted in 1:1 Hex/EA and was furnished in 82% yield; structure and purity were confirmed by ^{1}H NMR.

4.4.2.3 Deprotection of ketone

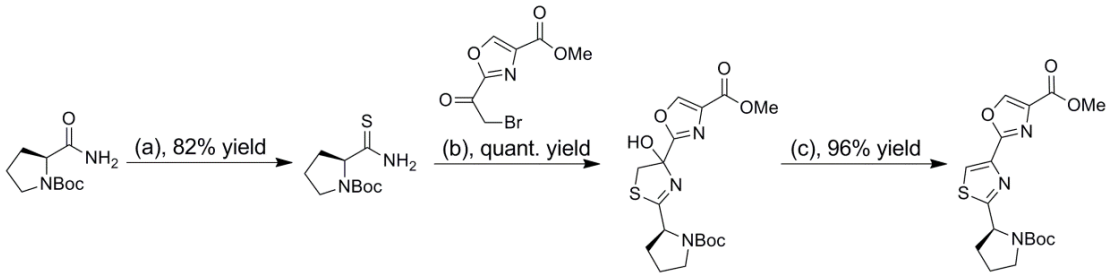
To prepare MeO-Ox-Bromoketal for installation of a thiazole, the ketone was deprotected with formic acid to furnish MeO-Ox-Bromoketone (**Scheme 4.8**). Deprotection proceeded by dissolving 1.0 equivalent of MeO-Ox-Bromoketal in formic acid (0.1 M) and heating the solution from RT to 60 °C. The reaction was monitored by TLC, after 1 hr, the reaction was complete, removed from the oil bath and diluted with EA. An aqueous solution of saturated sodium bicarbonate was slowly added to the diluted reaction in a separatory funnel to quench the reaction. Back-extraction of the aqueous layer with EA and DCM furnished the desired deprotected product upon concentration *in vacuo*. MeO-Ox-Bromoketone was furnished in 66% yield and its structure and purity were confirmed by ^{1}H NMR.



Scheme 4.8: Deprotection of ketone (a) Formic Acid (0.1 M), 1 hr, RT → 60 °C

4.4.2.4 Formation of oxazole-thiazole intermediate

The bromoketone moiety was converted to H_2NS-Pro-NHBoc in preparation for the modified Hantzsch thiazole synthesis (**Scheme 4.9**). Pro thioamide was generated by dissolving 1.0 equivalent of commercially available Pro amide (H_2N-Pro-NHBoc) and LR (0.8 equivalents) in DME (0.1 M) under argon gas. The reaction mixture stirred and upon completion (~12 hrs), verified by TLC, the crude reaction was purified via flash column chromatography. The desired thioamide (H_2NS-Pro-NHBoc) eluted in 1:1 Hex/EA and was furnished in 82% yield; the structure and purity were confirmed by ^1H NMR.



Scheme 4.9: (a) LR (0.8 equivalents), DME (0.1 M); (b) $KHCO_3$ (8.0 equivalents), DME (0.1 M) 0 °C, 16 hrs; (c) pyridine (9.0 equivalents), DME (0.1M), 15 min; then TFAA (4.0 equivalents), 2 hrs; then TEA, 0 °C to RT, 12 hrs

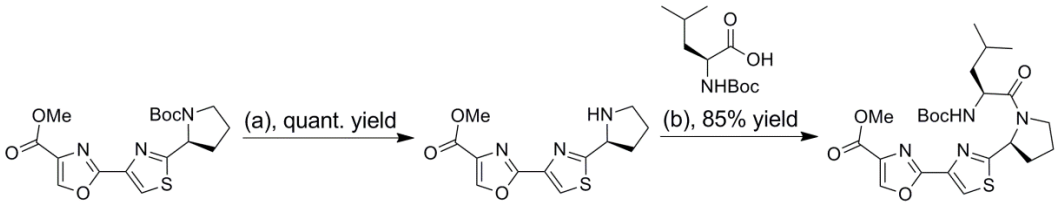
The thiazole was generated using the thioamide, H_2NS-Pro-NHBoc, (1.0 equivalent) and K_2CO_3 (8.0 equivalents), which were mixed with anhydrous DME (50% of the total volume needed for 0.1 M concentration) under an atmosphere of argon. The slurry was stirred at 0 °C for 5 min. MeO-Ox-Bromoketone (2.0 equivalents) was dissolved in the remaining DME and added drop-wise (1.0 mL/min) to the reaction flask. The reaction mixture was stirred overnight, where TLC confirmed reaction completion. The reaction was subjected to extraction with brine, and concentrated *in vacuo*, furnishing the desired hydroxy thiazoline intermediate in quantitative yield.

The thiazole was generated by dissolving MeO-Ox-Thiazoline-Pro-NBoc in

anhydrous DME (0.1 M) and stirred at 0 °C for 15 min. Pyridine (9.0 equivalents) was added to the reaction mixture drop-wise (0.1 mL/min) and the reaction stirred for an additional 15 min at 0 °C. Next, TFAA (4.0 equivalents) was added drop-wise to the reaction vessel (0.1 mL/min) and stirred at 0 °C for an additional 2 hrs. Finally, 2.0 equivalents of TEA were added drop-wise to the reaction (0.1 mL/min) and the reaction was warmed to RT overnight. Reaction completion was confirmed via TLC and LC/MS and the crude reaction was subjected to an acid-base extraction and then flash column chromatography for further purification. The desired di-heterocyclic fragment MeO-Ox-Th-Pro-NBoc eluted at 3:7 Hex/EA in 96% yield over two steps; purity and structure were confirmed by ^{1}H NMR and LC/MS (**Scheme 4.9**).

4.4.2.5 Installation of leucine residue

With both heterocycles installed, the Boc group of MeO-Ox-Th-Pro-NBoc was removed to prepare for coupling to HO-Leu-NHBoc. MeO-Ox-Th-Pro-NBoc was dissolved in 25% TFA and 75% DCM (0.1 M) with 2.0 equivalents of anisole. The reaction was monitored by TLC and upon completion (~1 hr), the mixture was concentrated *in vacuo*. MeO-Ox-Th-Pro-NH was furnished in quantitative yield without further purification (**Scheme 4.10**).



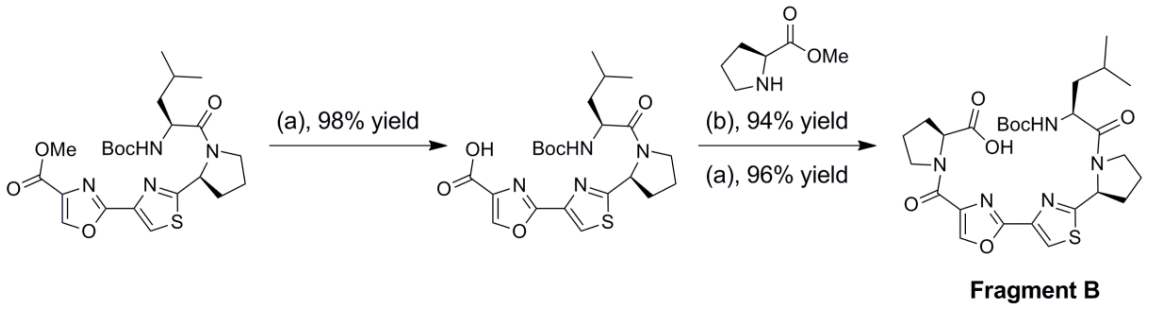
Scheme 4.10: Installation of Leu residue (a) anisole (2.0 equivalents), TFA/DCM (1:3) 0.1 M; (b) TBTU (0.8 equivalents), HATU (1.0 equivalent), DIPEA (8.0 equivalents), DCM (0.1 M)

Coupling to HO-Leu-NHBoc proceeded with 1.0 equivalent of free amine MeO-Ox-Th-Pro-NH, 1.1 equivalents of acid HO-Leu-NHBoc, 0.8 equivalents of TBTU, 1.0 equivalent of HATU and 8.0 equivalents of DIPEA dissolved in anyhydrous DCM (0.1 M)

under argon. The reaction mixture was allowed to stir for three hrs and upon completion the crude reaction mixture was subjected to an acid-base extraction followed by further purification via flash column chromatography. Pure MeO-Ox-Th-Pro-Leu-NHBoc eluted at 3:7 Hex/EA and was furnished in 85% yield; purity and structure were confirmed by LC/MS and ^{1}H, ^{13}C NMR (**Scheme 4.10**).

4.4.2.6 Hydrolysis and installation of proline to generate fragment B

Methyl ester hydrolysis of MeO-Ox-Th-Pro-Leu-NHBoc was necessary to prepare for the installation of the second Pro residue in fragment B (**Scheme 4.11**). MeO-Ox-Th-Pro-Leu-NHBoc (1.0 equivalent), LiOH•H_2O (8.0 equivalents) and hydrogen peroxide [H_2O_2 (30% in H_2O), 3.4 equivalents] were stirred in MeOH for 0.05 M overall concentration. The reaction was cooled to 0 °C and stirred overnight. Upon completion, confirmed by LC/MS, the reaction was diluted with DCM and washed with a pH 1 solution with 3.8 equivalents of sodium thiosulfate ($Na_2S_2O_3$) to quench the peroxide. After back-extraction of the aqueous layer with EA, the organic layers were combined, dried, filtered and concentrated *in vacuo*. Free acid HO-Ox-Th-Pro-Leu-NHBoc was furnished in 98% yield and its structure and purity were confirmed via ^{1}H and ^{13}C NMR and LC/MS.



Scheme 4.11: (a) LiOH•H_2O (8.0 equivalents), H_2O_2 (3.4 equivalents), MeOH (0.05 M), 0 °C; (b) HATU (1.4 equivalents), DIPEA (8.0 equivalents), DCM (0.1 M)

Coupling to MeO-Pro-NH proceeded with 1.0 equivalent of free acid HO-Ox-Th-

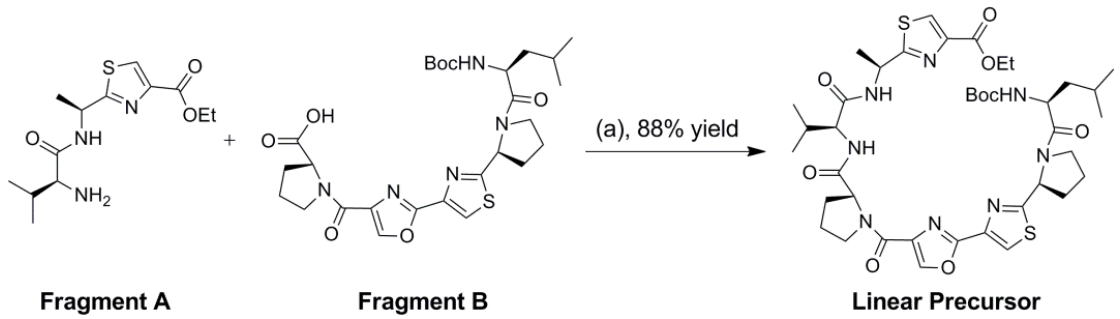
Pro-Leu-NHBoc, 1.1 equivalents of free amine MeO-Pro-NH, 1.4 equivalents of HATU and 8.0 equivalents of DIPEA in DCM (0.1 M), under argon gas. The reaction mixture stirred overnight and upon completion, confirmed by TLC and LC/MS, the crude reaction was subjected to an acid-base extraction and further purification via flash column chromatography. Pure MeO-Pro-Ox-Th-Pro-Leu-NHBoc eluted in 3:7 Hex/EA and was furnished in 94% yield; structure and purity was confirmed by ^{1}H and ^{13}C NMR and LC/MS.

Finally, MeO-Pro-Ox-Th-Pro-Leu-NHBoc (1.0 equivalent), $LiOH•H_2O$ (8.0 equivalents), and H_2O_2 (30% in H_2O, 3.4 equivalents) were combined in MeOH (0.05 M overall concentration) to generate free acid HO-Pro-Ox-Th-Pro-Leu-NHBoc (Fragment B, Scheme 4.11). The reaction was cooled to 0 °C and allowed to stir overnight. Reaction completion was confirmed by LC/MS, at which point the reaction was diluted with DCM and washed with a pH 1 solution with 3.8 equivalents of $Na_2S_2O_3$ to quench the peroxide. After back-extraction of the aqueous layer with EA, the organic layers were combined, dried, filtered and concentrated *in vacuo*. Pure fragment B HO-Pro-Ox-Th-Pro-Leu-NHBoc was afforded in 96% yield and its structure and purity were confirmed by LC/MS, ^{1}H and ^{13}C NMR.

4.4.3 Synthesis of linear precursor

Peptide coupling of the free amine Fragment A and the free acid fragment B produced a protected linear peptide (**Scheme 4.12**). EtO-Th-Ala-Val-Pro-Ox-Th-Pro-Leu-NHBoc was synthesized by coupling amine EtO-Thiazole-Ala-Val-NH_2 (Fragment A, 1.5 equivalents), to acid HO-Pro-Ox-Th-Pro-Leu-NHBoc (fragment B, 1.0 equivalent), using TBTU (0.8 equivalents), HATU (1.0 equivalent), and DIPEA (12.0 equivalents) dissolved in a 1:1 mixture of anhydrous ACN and DCM (0.1 M) under an atmosphere of argon. The reaction mixture stirred overnight and upon completion, the product was

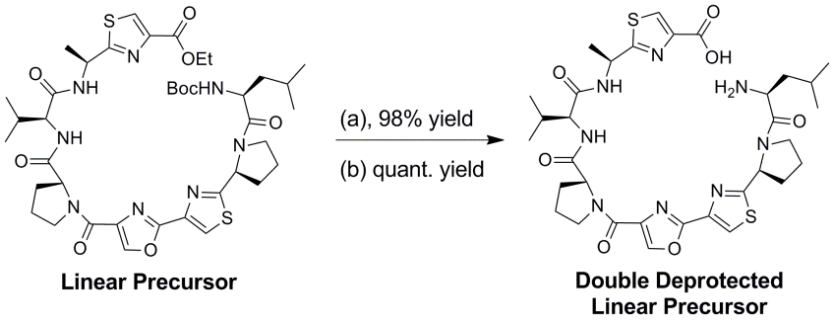
confirmed by LC/MS. The reaction mixture was subjected to an acid-base extraction, followed by further purification via flash column chromatography. The protected linear precursor eluted at 50:1 EA/MeOH to afford EtO-Th-Ala-Val-Pro-Ox-Th-Pro-Leu-NHBoc in 88% yield; structure and purity were confirmed by LC/MS and ^{1}H NMR.



Scheme 4.12: (a) TBTU (0.8 equivalents), HATU (1.0 equivalent), DIPEA (12.0 equivalents), ACN/DCM (1:1) 0.1 M

4.4.4 Deprotection of linear precursor

Acid and amine deprotections of EtO-Th-Ala-Val-Pro-Ox-Th-Pro-Leu-NHBoc were necessary to prepare the linear precursor for macrocyclization (**Scheme 4.13**). The acid was deprotected first by stirring EtO-Th-Ala-Val-Pro-Ox-Th-Pro-Leu-NHBoc (1.0 equivalent), LiOH•H_2O (10 equivalents) and H_2O_2 (30% in water, 3.4 equivalents) in EtOH (0.05 M). The reaction was cooled to 0 °C and stirred overnight. Upon completion, confirmed by LC/MS, the reaction was diluted with DCM and washed with a pH 1 solution with 3.8 equivalents of $Na_2S_2O_3$ to quench the peroxide. After back-extraction of the aqueous layer with EA, the organic layers were combined, dried, filtered and concentrated *in vacuo*. Pure HO-Th-Ala-Val-Pro-Ox-Th-Pro-Leu-NHBoc was afforded in 98% yield and taken on to the next deprotection without further purification; structure and purity was confirmed by LC/MS.



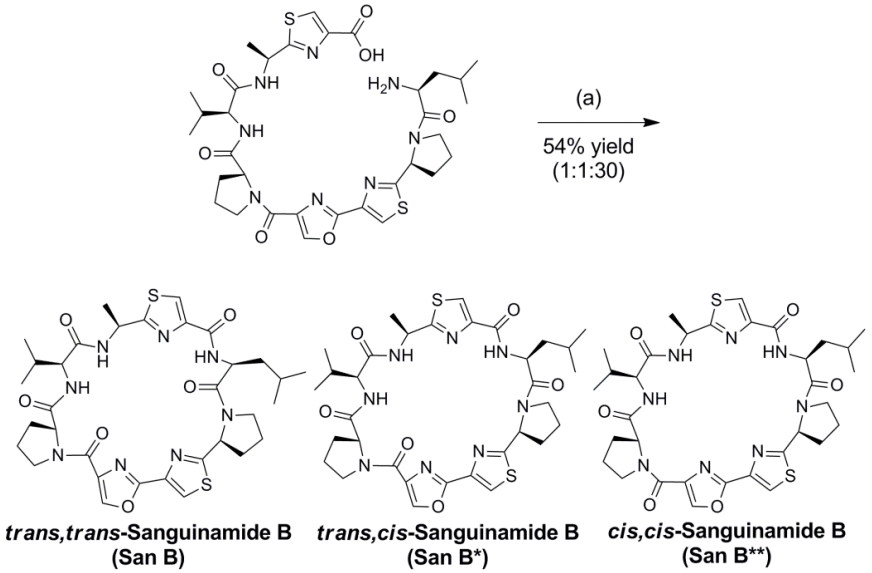
Scheme 4.13: Double deprotection of linear precursor. (a) LiOH•H_2O (8.0 equivalents), H_2O_2 (3.4 equivalents), EtOH (0.05 M), 0 °C; (b) anisole (2.0 equivalents), TFA/DCM (1:4) 0.1 M

Amine deprotection of HO-Th-Ala-Val-Pro-Ox-Th-Pro-Leu-NHBoc occurred by stirring the Boc-protected pseudo-peptide and 2.0 equivalents of anisole with 20% TFA in DCM (0.1 M). The reaction stirred for 45 min and upon completion, confirmed by LC/MS, the mixture was concentrated *in vacuo.* The double deprotected linear precursor HO-Th-Ala-Val-Pro-Ox-Th-Pro-Leu-NH_2 was furnished in quantitative yield and its structure and purity was confirmed by LC/MS.

4.4.5 Macrocyclization to generate Sanguinamide B

Macrocyclization of double deprotected HO-Th-Ala-Val-Pro-Ox-Th-Pro-Leu-NH_2 was performed under highly dilute conditions (0.007 M) to avoid oligomerization of the linear precursor (**Scheme 4.14**). Free acid and free amine containing HO-Th-Ala-Val-Pro-Ox-Th-Pro-Leu-NH_2, 1.2 equivalents each of TBTU and HATU, and 10.0 equivalents of DIPEA were dissolved in a 1:1 mixture of DCM/ACN for an overall concentration of 0.007 M. The cyclization reaction was run under an atmosphere of argon and was found complete after stirring overnight. LC/MS showed that three conformers were synthesized, whereby 3 peaks appeared close together, all with the same molecular weight. Purification of the crude reaction mixture by acid-base extraction and then flash column chromatography yielded all three conformers, which eluted in 19:1 EA/MeOH. Further purification via RP-HPLC was necessary to separate the three conformers. All

three conformers eluted in 100% ACN, where the natural product *trans,trans*-SanB (**SanB**) and *trans,cis*-SanB (**SanB***) eluted first as an inseparable mixture and the major product *cis,cis*-SanB (**SanB****) eluted as a separate peak. The **SanB** conformers were synthesized in an overall 54% yield with **SanB**:**SanB***:**SanB**** being produced in a 1:1:30 ratio.



Scheme 4.14: Cyclization to yield SanB conformers (a) TBTU (1.2 equivalents), HATU (1.2 equivalents), DIPEA (10.0 equivalents), ACN/DCM (1:1), 0.007M

The structure and purities of **SanB** and its conformers were established via ^{1}H and ^{13}C NMR, various 2D NMR experiments (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC), LC/MS and HRMS. Identification of the conformation of each **SanB** isomer involved examining the chemical shifts of their respective Proline β and γ carbons. It is well established that prolyl amide bonds adopting *cis*-/*trans*- conformation alter the difference in chemical shifts between β and γ carbons of proline residues (Δβγ).[17] A Proline amide bond that adopts a *cis*- conformation characteristically has a larger Δβγ than a Proline with an amide bond in the *trans*- conformation. The Δβγ for the natural product **SanB** (*trans,trans*-SanB) of Pro-1 and Pro-2 are 4.2 and 2.4 ppm, respectively.

These values and the spectroscopic data for *trans,trans*-San B are in good agreement with data for the natural product published by Molinski and co-workers (4.7 and 2.1 ppm, respectively)[1]. The Δβγ for San B* (3.8 and 13.5 ppm, respectively) are indicative of the *trans,cis*- conformation, and the Δβγ for San B** are 9.4 and 15.5 ppm, respectively, corresponds to the *cis,cis*- conformer.

4.5 Investigating the thermodynamic stability of Sanguinamide B

Given that the major product included was not the desired *trans,trans*-San B, a variable-temperature experiment was performed on the major product *cis,cis*-SanB to explore its ability to convert to the natural product *trans,trans*-SanB conformation. A similar experiment was performed by Gerwick and co-workers[10] and Deng and Tauton[18] in their investigations of ceratospongamide conformers and both groups successfully converted their major product *cis,cis*-ceratospongamide to the bioactive *trans,trans*-ceratospongamide.

The major product *cis,cis*-SanB (5.0 mg) was dissolved in DMSO and heated to 120 °C for a period of 1 hr. No change was observed via LC/MS or ^{1}H NMR and the conformer appeared to be stable at this temperature. Then, *cis*,*cis*-SanB in DMSO was heated to 170 °C over a period of 20 hours and a complete conversion to a 1:1 mixture of *trans,trans*-SanB and *trans,cis*-SanB occurred. This transformation was monitored by LC/MS (**Figure 4.6**).

Since no *cis,cis*- conformer was isolated from the *H. sanguineus*, the biosynthesis of the natural product *trans,trans*-SanB may be thermodynamically controlled. Given the high temperature required to convert the kinetically preferred product, *cis,cis*,- conformer to the natural product, it is plausible that peptidyl-prolyl isomerases (PPIs) are responsible for catalyzing the *cis*-*trans* isomerization.[19] PPIs are ubiquitous proteins that are expressed in both prokaryotic and eukaryotic cells, and their

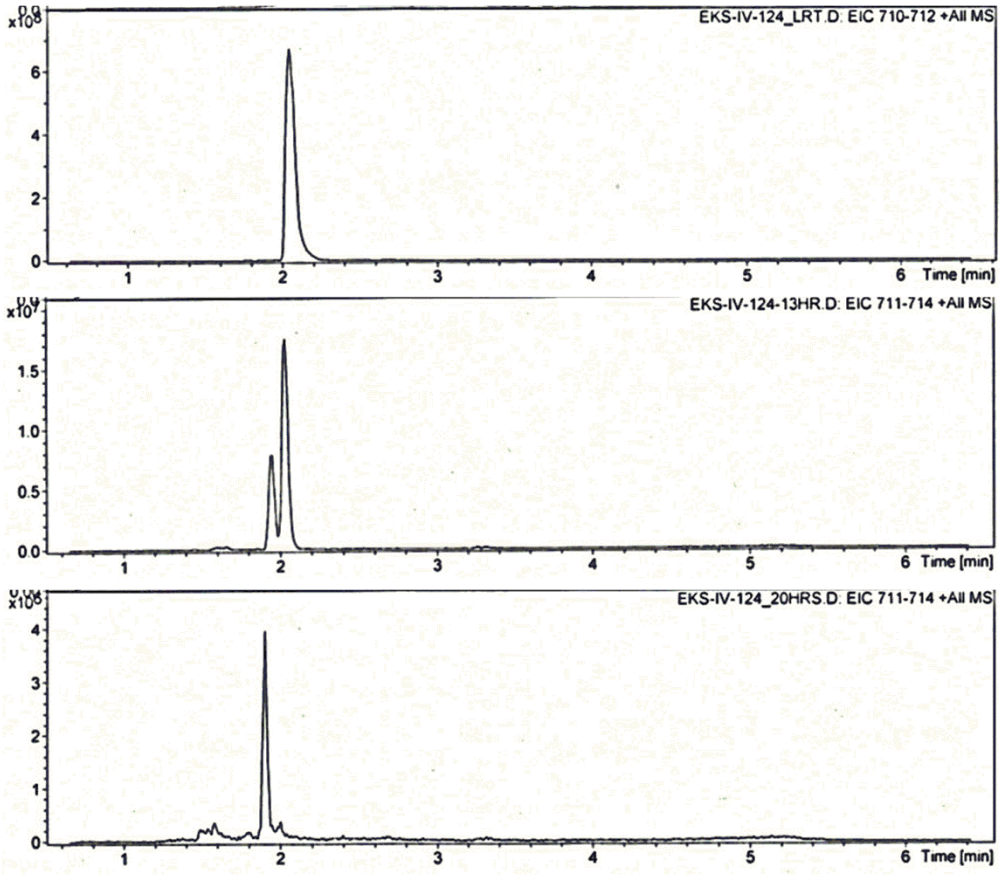


Figure 4.6: LC/MS chromatographs of *cis.cis*-SanB converting to a mixture of *trans,trans*-SanB and *trans,cis*-SanB taken at time 0 hr, 13 hrs, and 20 hrs

primary function is to facilitate *cis-trans* isomerization of peptide bonds *N*-terminal to Pro residues in polypeptide chains.[19]

4.6 Biological activity of Sanguinamide B and conformers

The difference in biological activity between the SanB conformers was examined against *Pseudomonas aeruginosa* bacteria. Macrocycles have been shown to exhibit antibacterial activity against respiratory pathogens[20, 21], and the use of macrolide antibiotics in the treatment of cystic fibrosis (CF) patients has produced promising results.

P. aeruginosa is a rod-shaped type of Gram-negative bacterium with unipolar motility. Motility is a critical feature of microbial life and requires significant cellular energy. Movement of bacteria is necessary for efficient nutrient acquisition, evasion of toxic substances, translocating to preferred hosts and accessing colonies within these hosts. Thus, preventing movement of bacteria is essential for an antibacterial agent. *P. aeruginosa* has two motility organelles: a single unsheathed polar flagellum and type IV pilli (**Figure 4.7**). The single, unsheathed polar flagellum has a long whip-like structure that is an important virulence factor responsible for spreading infections by facilitating swarming and swimming of the bacteria to new locations. Type IV pili are polar appendages with adhesion at their tips; these pili are important virulence factors that are responsible for adhesion to epithelium, which allows biofilm formation, swarming, and twitching motility. The effects of my SanB conformers were examined for their twitching motility on a type IV fimbriae-positive strain of *P. aeruginosa*.

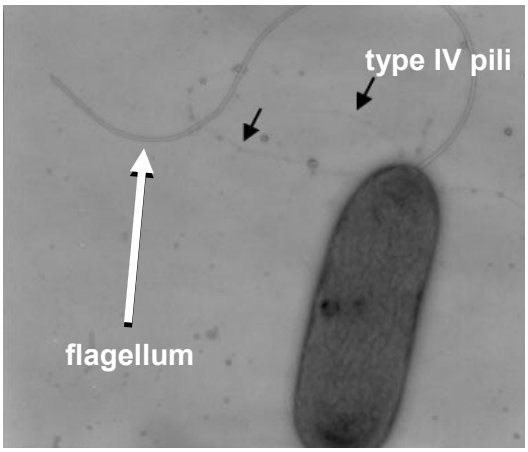


Figure 4.7: PAO1 (wild-type *P. aeruginosa*) bacteria harvested from plates cultured overnight, negatively stained using 2% uracyl acetate, and visualized using a Philips TEM 400.[22] Black arrows indicate type IV pili and white arrow shows flagellum

P. aeruginosa PAO1 or a *Staphylococcus aureus* (*S. aureus*) bacteria strain (positive control, non-modal bacteria) were stab-inoculated through a thin 1% LANS plate (10.0 g/L tryptone; 5.0 g/L yeast extract; 10.0 g/L agar), containing no compounds

(for negative control) or with drug: **SanB**/**SanB*** (inseparable) mixture or **SanB**** at 1.4 μM. Plates were incubated for 48 hrs at 37 °C under humidified conditions. The zone of motility at the agar/Petri-dish interface was measured, and the relative zone area was recorded in mm^2 (**Figure 4.8**).

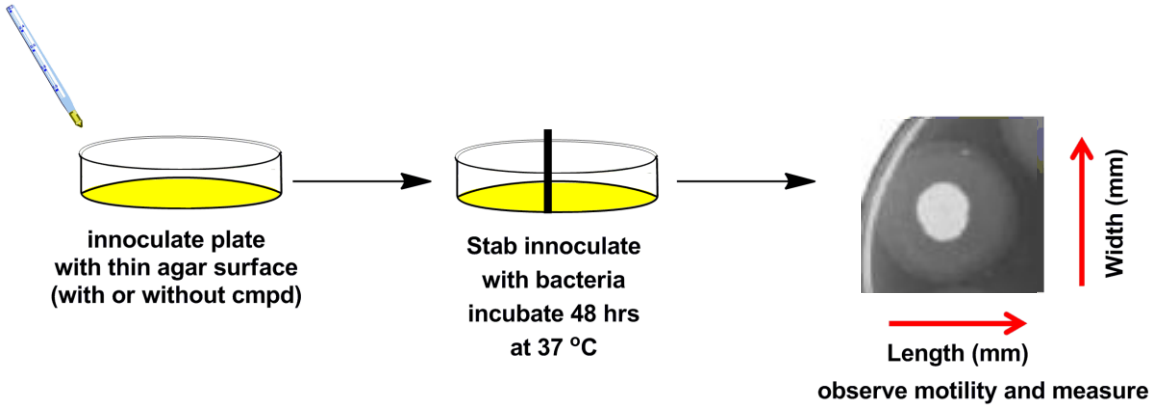


Figure 4.8: Twitching motility assay

The area of twitching motility was reduced in size by 24% when cells were treated with the *trans,trans*-SanB/*trans,cis*-SanB mixture at 1.4 μM. However, treatment with major product *cis,cis*-SanB at 1.4 μM showed no significant decrease in twitching motility area (Figure 4.9). Our data indicated that the mixture containing the natural product affected the twitching activity of this gram (-) bacteria, which is an important virulence determinant in *P. aeruginosa*. Presumably the lack of biological activity for the *cis,cis*-SanB structure was related to its conformation, and tied to the *cis* orientation of this molecule about the prolyl amide bonds.

4.7 Conclusions

I performed the first total synthesis of the natural product *trans,trans*-Sanguinamide B (SanB) and verified the structure that was proposed by Molinski and co-workers. Using a convergent solution phase approach, cyclization of the double deprotected linear precursor yielded three conformational isomers in milligram quantities: the natural

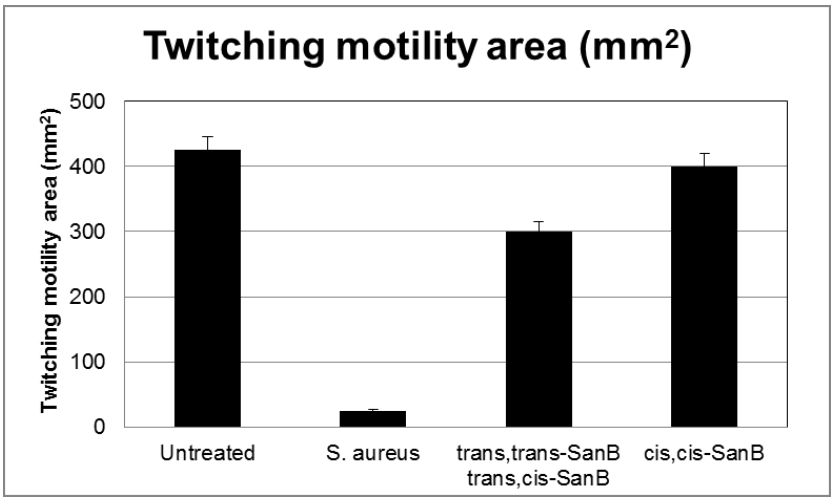


Figure 4.9: Twitching motility area (mm^2) for untreated *P. aeruginosa* (PAO1), nonmodal *S. aureus*, SanB/SanB* mixture (1.4 μM), and SanB** (1.4 μM)

product *trans,trans*-SanB with *trans,cis*-SanB and the major product *cis,cis*-SanB. The thermodynamic stability of *cis,cis*-SanB was investigated by dissolving it in DMSO and heating the macrocycle to 120 °C. The macrocycle was found to be stable up to 120 °C, however a complete conversion to a 1:1 inseparable mixture of the natural product *trans,trans*-SanB and *trans,cis*-SanB occurred upon heating at 170 °C for 20 hrs.

Testing the **SanB** conformers for antibacterial activity proved successful, where the *trans,trans*-SanB/*trans,cis*-SanB mixture decreased the twitching motility of type IV fimbriae by 24% compared to untreated of *P. aeurginosa*. *Cis,cis*-SanB did not show any activity in this assay, confirming that the orientation about the two prolyl bonds in **SanB** are critical for locking the macrocycle into a favorable and bioactive conformation.

4.8 References

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Chapter 4, in part is a reprint of material as it appears in “Total Synthesis of Natural Product Sanguinamide B and Its Structurally Related Conformers.” *Organic*

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Chapter 5 – Experimental methods

5.1 General remarks and procedures

All reactions were carried out under an argon atmosphere with dry solvents under anhydrous conditions, unless otherwise stated. Reagents were commercially obtained (Peptides International, ChemImpex, Aldrich, and Acros) at the highest quality and used without further purification.

Reactions were monitored via thin-layer chromatography (TLC) carried out on 250 μm Whatman silica gel plates (4861-820) using UV light as the visualizing agent and potassium permanganate in water as heat and developing agents.

SiliCycle SiliaFlash silica gel (60, particle size 40-63 μm) was used for flash chromatography. Automated column chromatography was performed on Teledyne CombiFlash Rf-200 using 12 g silica flash columns and 25 g solid sample cartridges. Yields refer to spectroscopically and/or chromatographically homogeneous materials.

5.1.1 General experimental procedures

5.1.1.1 Solution phase peptide coupling

Solution phase peptide coupling reactions were carried out under argon with anhydrous solvent, using ACN, DCM, or DMF, or a combination of the before mentioned solvents. The amine (1.1 equivalents) and acid (1.0 equivalent) were weighed into a dry flask along with 1.2-2.1 equivalents of a single coupling reagent or combination of TBTU, HATU and/or DEPBT. Amino acids and coupling reagent(s) were dissolved in anhydrous solvent to generate a 0.1 M solution and then DIPEA (3.0–10.0 equivalents) was added to the reaction flask. The solution was stirred at rt and reactions were monitored by TLC. Reactions were run for 45 min before checking for completion via TLC. If reaction was not complete after 3 hrs, additional coupling reagent was added to the reaction flask. Once the reaction was complete a work-up with acidic aqueous solution was done (either 10% aqueous HCl or saturated NH_4Cl solution) to remove

excess free amine. In some cases the crude reaction was washed with saturated $NaHCO_3$ to remove coupling agent by-products and excess coupling agent(s), and then with brine. Organic layers were combined, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Flash column chromatography using a gradient of Hex:EA was performed in some cases where further purification was necessary to give the desired peptide.

5.1.1.2 Boc removal

Boc groups were removed by dissolving protected peptides in DCM (75% for 0.1 M overall concentration), followed by the addition of 2.0 equivalents of anisole. TFA was added to the reaction flask (25% for 0.1 M overall concentration) and the reaction was run at rt under open atmosphere. The reactions were monitored by TLC after about 15 min, where the TLC sample was subjected to a mini aqueous workup (with DI water) on ~20 µL of the reaction mixture diluted with ~250 µL of DCM, in a syringe. Upon disappearance of starting material, the reaction mixture was concentrated *in vacuo* with several washes of DCM.

5.1.1.3 Methyl/ethyl ester hydrolysis

Acids were deprotected using one of two methods; with or without hydrogen peroxide. First, if no hydrogen peroxide was used, acids were deprotected by dissolving the protected peptide in MeOH to generate an overall concentration of 0.1 M in a round bottom flask. LiOH (8.0-10.0 equivalents) was weighed and placed into the flask and the reaction stirred at rt under open atmosphere. The hydrolysis reaction was monitored by TLC after about 1 hr, where the TLC sample was subjected to a mini acidic aqueous workup (with pH 1 HCl solution) on ~20 µL of the reaction mixture diluted with ~250 µL of DCM, in a syringe. Typically these reactions were complete in ~2 hrs, but in some cases, deprotections were run overnight. Upon completion, the reaction was acidifed using aqueous HCl solution to pH 1 and extracted with DCM 3 times. The aqueous layer

was back-extraccted with EA 3 times and then the combined organic layers were dried, filtered and concentrated *in vacuo*.

For deprotection where hydrogen peroxide was employed, the peptide was dissolved in methanol for a 0.1 M overall concentration and cooled to 0 °C. Hydrogen peroxide (3.4 equivalents) was added followed by LiOH (2-3.0 equivalents). The reaction was monitored by TLC as described above after ~1 hr. Typically these reactions were complete in ~2 hrs, but in some cases, deprotections were run overnight. The complete reaction was acidified with sodium thiosulfate (3.8 equivalents) in 5% HCl aqueous solution, which also neutralized the peroxide. The aqueous solution was extracted 5 times with DCM, and the combined organic layers were dried, filtered and concentrated *in vacuo*.

5.1.1.4 In situ deprotection

Peptides were acid and amine deprotected using 12 N HCl (8 drops per 0.3 mmols of peptide) in THF (0.05 M). Anisole (2.0 equivalents) was added to the flask and the reaction was stirred at rt under open atmosphere. The reaction typically took ~3 days, and LC/MS was used to monitor the reaction every 12 hrs. LC/MS data typically indicated the reaction was ~50% complete after the first day and ~75% by the second day. 3 drops of 12 N HCl per 0.3 mmol of peptide were added to the reaction for each additional day of stirring at rt. Upon verification of a fully double deprotected peptide (with free amine and free acid) and disappearance of the starting material permitted workup. The reaction was concentrated *in vacuo* with several washed of DCM to yield double deprotected linear peptide.

5.1.1.5 Peptide macrocyclization

Double deprotected peptides were cyclized using a combination of 2-3 coupling reagents (TBTU, HATU, DEPBT, 1.8-2.4 equivalents total). The dry double deprotected

peptide (free acid and free amine) and coupling agents were dissolved in anhydrous ACN, DCM, or DMF, or a combination of the before mentioned solvents for an overall concentration of 0.007 M under an atmosphere of argon. DIPEA (8-12.0 equivalents) was added to the reaction. TLC (macrocycle R_f similar to protected linear pentapeptide) and LC/MS were used to monitor the reaction which was usually finished in ~2 hrs. If reaction was not complete in 2 hrs, additional coupling agent(s) were added and it was left to stir overnight. Upon reaction completion, a work-up with acidic aqueous solution was done (either 10% aqueous HCl or saturated NH_4Cl solution) to remove excess free amine. In some cases the crude reaction was washed with saturated $NaHCO_3$ to remove coupling agent by-products and excess coupling agent(s), and then with brine. Organic layers were combined, dried over Na_2SO_4, filtered and concentrated *in vacuo*. All macrocycles were first purified by flash column chromatography using an Hex:EA gradient on silica gel. For macrocycles that did not require further deprotections, a final purification via RP-HPLC was performed prior to biological testing using a gradient of ACN and double-deionized water (each with 0.1% TFA).

5.1.1.6 Syringe pump macrocyclization

Double deprotected peptides were cyclized using a combination of 2-3 coupling reagents (TBTU, HATU, DEPBT, 1.8-2.4 equivalents total). These coupling agents were placed in a round bottom flask purged with argon and dissolved in ~50-75% of a calculated volume of anhydrous DCM that would give a 0.007 M overall concentration. The dry, double deprotected peptide (free acid and free amine) was dissolved in the remaining solvent volume and placed in a syringe equipped with a 20 G needle. DIPEA (8-10.0 equivalents) was then added to the reaction flask containing coupling reagents in solution. The double deprotected peptide was then added to the bulk solution drop-wise using a syringe pump at a rate of 30 mL/hr. The reaction was monitored via LCMS and

generally complete in ~2 hrs upon complete addition of the double deprotected peptide solution. Upon completion, the reaction was worked up by washing with aqueous 10% HCl solution, then saturated sodium bicarbonate and finally brine. After back extraction of aqueous layers with large quantities of DCM, the organic layers were combined, dried, filtered and concentrated *in vacuo*. All macrocycles were first purified by flash column chromatography using a Hex:EA gradient on silica gel. When necessary, RP-HPLC was used for additional purification using a gradient of ACN and double deionized water (each with 0.1% TFA).

5.1.1.7 Hydrogenation

The Bzl- or Cbz-protected compounds were dissolved in EtOH for an overall concentration of 0.05-0.1 M. A catalytic amount of Pd/C was added to the solution and the reaction flask was fitted with a septum. H_2 gas was bubbled into the solution at low atmospheric pressure using a balloon (5 times) and the reaction stirred at rt. After ~2 hrs, the reaction progression was monitored by TLC; typically, reactions were complete within 5 hrs. If, after 8 hours, the reaction was still not complete, additional H_2 gas was purged through the system and it was allowed to stir overnight. Upon completion, the solution was filtered over Celite® to remove the Pd/C. If necessary, the deprotected pepide was subjected to further purification via flash column chromatography using a Hex:EA gradient on silica gel and/or RP-HPLC using a gradient of ACN and double deionized water (each with 0.1% TFA).

5.1.1.8 Benzylation

The cyclized peptide was dissolved in THF or DCM to make a 0.1 M solution under an atmosphere of argon. NaH (60% in mineral oil) was used added to the reaction flask (1.1 equivalents) and dissolved in the 0.1 M solution. BnBr (2.0 equivalents) was then added drop-wise to the reaction. After ~2 hrs, LC/MS was used to monitor the

progression of the reaction. Upon complete disappearance of starting material, the reaction was diluted with 200 mL of EA and washed with deionized water. After that, the aqueous layer was back-extracted with 200 mL of DCM, organic layers were collected, dried, concentrated *in vacuo* and preliminarily purified by flash column chromatography using a Hex:EA gradient. RP-HPLC was used for further purification by using a gradient of ACN and double deionized water (each with 0.1% TFA) if necessary.

5.1.1.9 Oxazole synthesis

DAST (1.1 equivalents) was added (0.1 mL/min) to a solution of peptidyl-Ser (1.0 equivalent) in DCM for a 0.1 M concentration. The solution was cooled to -78 °C under argon atmosphere. The reaction mixture was stirred for 15 min and anhydrous K_2CO_3 (2.0 equivalents) was added to the reaction mixture to continue to stir at -78 °C for an additional 1 hr. The reaction mixture warmed to rt and stirred for an additional 1.5 hrs. Upon reaction completion, confirmed by TLC, the organic solution was poured into saturated aqueous sodium bicarbonate and extracted with DCM. After back extraction of the aqueous layer with EA, the organic layers were combined, dried over sodium sulfate, filtered, and concentrated *in vacuo* to give the oxazoline intermediate. The oxazoline was purified by flash column chromatography using a Hex:EA gradient.

Next, the dry oxazoline intermediate was dissolved in anhydrous DCM for an overall 0.1 M concentration and stirred at -47 °C for 15 min under argon. DBU (2.0 equivalents) was added drop-wise (0.1 mL/min) to the reaction flask and stirred for 20 min and $BrCCl_3$ (2.0 equivalents) was then added to the reaction mixture drop-wise (0.1 mL/min). The reaction continued to stir at -47 °C for an additional 2 hrs and then warmed to rt to stir an additional 12 hrs, or until deemed complete by TLC. Upon reaction completion, a work-up was done by extracting with aqueous 10% HCl solution. After back extraction of aqueous layers with large quantities of EA, organic layers were

combined, dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash column chromatography using a gradient of Hex:EA gave the desired peptidyl-oxazole.

5.1.1.10 Solid phase peptide coupling

In a vial, 2-3.0 equivalents of Fmoc-protected amino acid and 3.0 equivalents of HOBt were dissolved in DMF for an overall reaction concentration of 0.2 M. The amino acid and coupling reagent solution was poured into a cartridge fitted with a frit, followed by addition of DIC (6.0 equivalents). Couplings were allowed to shake, capped at rt for a minimum of ~2 hrs, and were tested for completeness via ninhydrin test. Once complete, the coupling reaction solution was drained. Note: Fmoc and *N*-methyl amino acids are coupled according to the cycle above, however for subsequent coupling onto the secondary amino terminus, HOBt was substituted for HOAt and the coupling was allowed to proceed overnight.

5.1.1.11 Fmoc removal

Following coupling completion, the resin-bound peptide was treated as follows for removal of the Fmoc protecting group: DMF wash (3 × 1 min), 20% Piperdine/DMF (1 × 5 min), 20% Piperdine/DMF (1 × 10 min), DMF wash (2 × 1 min), IPA wash (1 × 1 min), DMF was (1 × 1 min), IPA (1 × 1 min), DMF (3 × 1 min). A ninhydrin test was performed to verify Fmoc removal.

5.1.1.12 Cleaving the peptide from resin

The desired full-length, linear peptide was cleaved from the resin by swelling and shaking the peptide-resin for 24-36 hrs in a 1:1 (v/v) TFE/DCM (10 mL/g of dried resin). The cleavage solution was filtered through a Buchner filter, and the drained resin was washed several times with DCM (5 volumes/gram of initial dried peptide-resin) to fully extract the cleaved peptide from the resin. Solvents in the combined filtrates were evaporated by rotary evaporation and the solids dried *in vacuo* overnight. The solids

were then reconstituted in CH_2Cl_2, evaporated by rotary evaporation and dried *in vacuo* overnight again to remove residual entrapped TFE.

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

NMR spectra were obtained at 30°C on either a 600-MHz Varian NMR-S, 500-MHz INOVA, 400-MHz Varian NMR-S, 300-MHz Bruker NMR or 200-MHz Varian NMR-S using residual undeuterated solvent as an internal reference. NMR spectra for one compound were taken at -10 °C on 700-MHz Bruker NMR using an inverse-detect cryoprobe. The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, b = broad, bd = broad doublet, dd = doublet of doublet, and dq = doublet 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 DDI 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.

HRMS data were obtained at the Bioanalytical Mass Spectrometry Facility within the Mark Wainwright Analytical Centre of the University of New South Wales. HRMS data were recorded on a Thermo LTQ FT LC/MS/MS system. Subsidized access to this facility is gratefully acknowledged.

5.1.4 Notes for automated flash chromatography and 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) trifluoroacetic acid (solvent A) and DDI water with 0.1% (v/v) trifluoroacetic acid. 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 SanA 2

5.2.1.1 MeO-Phe-Leu-N(Me)Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-Leu-N(Me)Boc was synthesized utilizing 500 mg (2.04 mmol, 1.0 equivalent) of acid HO-Leu-N(Me)Boc, 484 mg (2.24 mmol, 1.1 equivalent) of amine OMe-Phe-NH_2, 786 mg (2.44 mmol, 1.2 equivalents) of TBTU, 1.42 mL (8.15 mmol, 4.0 equivalents) of DIPEA dissolved in 20.4 mL DCM, under argon. The reaction mixture stirred for 2 hrs and upon completion, the crude reaction mixture was diluted with 200 mL EA. The crude reaction was washed with 10% HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The pure desired peptide MeO-Phe-Leu-N(Me)Boc was afforded as a white solid (810 mg, 98% yield) R_f: 0.75 (1:1 Hex/EA). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.03 (t, *J*=6.9 Hz, 6H, $CH(C\underline{H}_3)_2$, 1.56 (s, 9H, $C(C\underline{H}_3)_3$), 1.67-1.73 (m, 1H, $CH_2CH(CH_3)_2$), 1.74-1.80 (m, 2H, $CHCH_2CH$, 3.13-3.21 (m, 1H, $CHC\underline{H}_aH_bPh$), 3.85 (s, 3H, $OC\underline{H}_3$), 4.65-4.84 (br, 1αH), 4.88-5.02 (q, *J*=6.9 Hz, 1αH), 7.15-7.25 (m, 2H, Ph), 7.34-7.45 (m, 3H, Ph).

5.2.1.2 MeO-Phe-Leu-N(Me)H

Following the ***Boc removal*** procedure: MeO-Phe-Leu-N(Me)H was synthesized by dissolving 810 mg (1.99 mmol, 1.0 equivalent) of MeO-Phe-Leu-N(Me)Boc in 14.0 mL DCM, followed by adding 435 µL (3.99 mmol, 2.0 equivalents) of anisole and then 5.98 mL of TFA. Boc removal was complete in 45 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 (595 mg, quantitative yield) as a light brown oil.

5.2.1.3 MeO-Phe-Leu-N(Me)-Val-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-Leu-N(Me)-Val-NHBoc was synthesized utilizing 394 mg (1.81 mmol, 1.0 equivalent) of acid HO-Val-NHBoc, 595 mg (1.99 mmol, 1.1 equivalent) of amine MeO-Phe-Leu-N(Me)H, 349 mg (1.09 mmol, 0.6 equivalents) of TBTU, 414 mg (1.09 mmol, 0.6 equivalents) of HATU, 1.27 mL (7.25 mmol, 4.0 equivalents) of DIPEA dissolved in 18.1 mL DCM, under argon. The reaction mixture stirred for 2 hrs and upon completion, the crude reaction mixture was diluted with 200 mL EA and then washed with 10% HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The pure desired peptide OMe-Phe-Leu-N(Me)-Val-NHBoc was afforded as a light yellow oil (886 mg, 94% yield). R_f: 0.55 (Hex/EA 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (m, 6H, CH(C$\underline{H}_3$)$_2$, 0.91 (d, *J*=6.8 Hz, 6H, CH(C$\underline{H}_3$)$_2$, 1.41 (s, 9H, C(C$\underline{H}_3$)$_3$), 1.59-1.69 (m, 2H, CHC$\underline{H}_2$CH), 1.73-1.87 (m, 2H, 2(C$\underline{H}$(CH$_3$)$_3$), 2.83 (s, 3H, NC$\underline{H}_3$), 3.09 (dd, *J*=13.3, 5.9 Hz, 2H, C$\underline{H}_2$Ph), 3.72 (s, 3H, OCH_3), 4.28-4.38 (m, 1αH), 4.81 (q, *J*=6.8 Hz, 1αH), 5.10-5.19 (m, 1αH), 6.37 (d, *J*=8.3 Hz, 1NH), 7.06-7.13 (m, 2H, Ph), 7.22-7.29 (m, 3H, Ph).

5.2.1.4 MeO-Phe-Leu-N(Me)-Val-NH_2

Following the ***Boc removal*** procedure: MeO-Phe-Leu-N(Me)-Val-NH_2 (**SanA 2.A**) was synthesized by dissolving 886 mg (1.71 mmol, 1.0 equivalent) of MeO-Phe-Leu-N(Me)-Val-NHBoc in 13.6 mL DCM, followed by adding 372 µL (3.41 mmol, 2.0 equivalents) of anisole and then 3.41 mL of TFA. Boc removal was complete in 45 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 (715 mg, quantitative yield) as a light brown oil.

5.2.1.5 MeO-Leu-Leu-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Leu-Leu-NHBoc was synthesized utilizing 500 mg (2.01 mmol, 1.0 equivalent) of acid HO-Leu-NHBoc, 401 mg (2.21 mmol, 1.1 equivalent) of amine OMe-Leu-NH_2, 774 mg (2.41 mmol, 1.2 equivalents) of TBTU, 1.40 mL (8.04 mmol, 4.0 equivalents) of DIPEA dissolved in 20.1 mL DCM, under argon. The reaction mixture stirred for 1.5 hrs and upon completion, the crude reaction mixture was diluted with 200 mL EA. The crude reaction was washed with 10% HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The pure desired peptide OMe-Leu-Leu-NHBoc was afforded as a white solid (664 mg, 92% yield); R_f: 0.65 (1:1 Hex:EA). ^1H NMR (200 MHz, $CDCl_3$): δ 0.81-1.03 (m, 12H, 2(CH($C\underline{H}_3$)$_2$), 1.45 (s, 9H, C($C\underline{H}_3$)$_3$), 1.49-1.56 (m, 1H, $C\underline{H}(CH_3)_2$, 1.58-1.70 (m, 4H, 2($CHC\underline{H}_2CH$), 1.71-1.78 (m, 1H, $C\underline{H}(CH_3)_2$, 3.73 (s, 3H, $OC\underline{H}_3$), 4.01-4.20 (m, 1αH), 4.54-4.70 (m, 1αH), 4.85 (d, *J*=8.3, 1NH), 6.43 (d, *J*=7.9, 1NH).

5.2.1.6 HO-Leu-Leu-NHBoc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-Leu-Leu-NHBoc was deprotected by utilizing 664 mg (1.86 mmol, 1.0 equivalent) of MeO-Leu-Leu-NHBoc and 623 mg (1.48 mmol, 8.0 equivalents) of $LiOH•H_2O$ in 18.6 mL MeOH. The peptide was dissolved in methanol and $LiOH•H_2O$ was added to the reaction flask and the reaction was run for 3 hrs. Upon completion, the reaction was diluted with 250 mL DCM and washed with pH 1 HCl solution (200 mL x 2). The aqueous layer was back-extracted with EA (100 mL x 2), and the combined organic layers were dried, filtered and concentrated *in vacuo* (184 mg, 84% yield) as a clear oil. The free acid HO-Leu-Leu-NHBoc (**SanA 2.B**) was taken on without any further purification or characterization.

5.2.1.7 MeO-Phe-Leu-N(Me)-Val-Leu-Leu-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-Leu-N(Me)-Val-Leu-Leu-NHBoc (**SanA 2.LP**) was synthesized utilizing 534 mg (1.55 mmol, 1.0 equivalent) of acid HO-Leu-Leu-NHBoc, 715 mg (1.71 mmol, 1.1 equivalent) of amine MeO-Phe-Leu-N(Me)-Val-NH_2, 373 mg (1.16 mmol, 0.75 equivalents) of TBTU, 0.442 mg (1.16 mmol, 0.75 equivalents) of HATU, 2.16 mL (12.4 mmol, 8.0 equivalents) of DIPEA dissolved in 9.3 mL DCM and 6.2 mL ACN, under argon. The reaction mixture stirred for 3 hrs and upon completion, the crude reaction mixture diluted with 200 mL EA. The crude reaction was washed with 10% HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed reaction underwent a final purification via column chromatography on silica gel (1:1 Hex/EA) to afford the desired peptide MeO-Phe-Leu-N(Me)-Val-Leu-Leu-NHBoc (814 mg, 71% yield) as a clear oil. R_f: 0.55 (Hex/EA 1:1). ^1H NMR (500 MHz, $CDCl_3$): δ 0.84-0.86 (m, 6H,

CH(C$\underline{H}_3$)$_2$), 0.87-0.89 (m, 6H, CH(C$\underline{H}_3$)$_2$), 0.91 (d, *J*=6.7 Hz, 6H, CH(C$\underline{H}_3$)$_2$), 0.94 (d, *J*=6.7 Hz, 6H, CH(C$\underline{H}_3$)$_2$), 1.45 (s, 9H, C(C$\underline{H}_3$)$_3$), 1.57-1.62 (m, 3H buried, 3(CH$_2$C$\underline{H}$(CH$_3$)$_2$)), 1.64-1.70 (m, 6H buried, 3(CHC$\underline{H}_2$CH), 1.87-1.93 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 2.84 (s, 3H, NC$\underline{H}_3$), 3.03 (dd, *J*=14.0, 6.9 Hz, 1H, CHC$\underline{H}_a$H$_b$Ph), 3.15 (dd, *J*=14.0, 6.9 Hz, 1H, CHCH$_a$$\underline{H}_b$Ph), 3.72 (s, 3H, OC$\underline{H}_3$), 4.05-4.11 (m, 1αH), 4.41-4.45 (m, 1αH), 4.68 (dd, *J*=9.1, 6.6 Hz, 1αH), 4.82 (q, *J*=6.7 Hz, 1αH), 5.12-5.17 (m, 1αH), 6.35 (d, *J*=7.6 Hz, 1NH), 6.35 (d, *J*=7.6 Hz, 1NH), 6.45 (d, *J*=8.0, 1NH), 6.63 (d, *J*=8.8, 1NH), 7.09-7.12 (m, 2H, Ph), 7.24-7.30 (m, 3H, Ph). LCMS: *m/z* called for $C_{38}H_{63}N_5O_8$ (M+Na$^+$) = 755.0, found 755.0.

5.2.1.8 HO-Phe-Leu-N(Me)-Val-Leu-Leu-NHBoc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-Phe-Leu-N(Me)-Val-Leu-Leu-NHBoc was deprotected by utilizing 814 mg (1.09 mmol, 1.0 equivalent) of MeO-Phe-Leu-N(Me)-Val-Leu-Leu-NHBoc and 366 mg (8.72 mmol, 8.0 equivalents) of LiOH•H_2O in 10.9 mL MeOH. The peptide was dissolved in methanol and LiOH•H_2O was added to the reaction flask and the reaction was run overnight. Upon completion, the reaction was diluted with 250 mL DCM. The aqueous layer was back-extracted with EA (100 mL x 2), and the combined organic layers were dried, filtered and concentrated *in vacuo* (487 mg, 61% yield) as a clear oil. The free acid HO-Phe-Leu-N(Me)-Val-Leu-Leu-NHBoc (**SanA 2.DLP**) was taken on without any further purification or characterization. LCMS: *m/z* called for $C_{38}H_{63}N_5O_8$ (M+1) = 718.5, found 718.2.

5.2.1.9 HO-Phe-Leu-N(Me)-Val-Leu-Leu-NH$_2$

Following the ***Boc removal*** procedure: HO-Phe-Leu-N(Me)-Val-Leu-Leu-NH_2 (**SanA 2.DDLP**) was synthesized by dissolving 487 mg (0.66 mmol, 1.0 equivalent) of HO-Phe-Leu-N(Me)-Val-NHBoc in 5.28 mL DCM, followed by adding 145 µL (1.32 mmol,

2.0 equivalents) of anisole and then 1.32 mL of TFA. Boc removal was complete in 45 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 (483 mg, quantitative yield) as a dark brown oil. LCMS: *m/z* called for $C_{33}H_{55}N_5O_6$ (M+1) = 617.8, found 618.9.

5.2.1.10 cyclo-Phe-Leu-N(Me)-Val-Leu-Leu

Following the ***macrocyclization*** procedure: *cyclo*-Phe-Leu-N(Me)-Val-Leu-Leu (**SanA 2**) was synthesized by dissolving 483 mg (0.66 mmol, 1.0 equivalent) of HO-Phe-Leu-N(Me)-Val-Leu-Leu-NH_2, 127 mg (0.40 mmol, 0.6 equivalents) of TBTU, 150 mg (0.40 mmol, 0.6 equivalents) of HATU and 78.9 mg (0.26 mmol, 0.4 equivalents) of DEPBT in 47.1 mL each of ACN and DCM. 1.15 mL of DIPEA (6.60 mmol, 10.0 equivalents) was added to the reaction flask and the solution stirred for 3 hrs. Upon completion, the crude reaction was diluted with 200 mL EA and washed with 10% HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed reaction underwent a final purification via column chromatography on silica gel to afford the desired. Pure *cyclo*-Phe-Leu-N(Me)-Val-Leu-Leu (**SanA 2**) eluted at 95:5 EA:MeOH and was furnished in 31% yield; the structure and purity of the macrocycle was confirmed via LC/MS and ^{1}H NMR. R_f: 0.40 (1:3 Hex/EA). A final purification via RP-HPLC was performed prior to biological evaluation. ^{1}H NMR (400 MHz, CD_3OD): δ 0.86 (dd, *J*=1.8, 6.8 Hz, 6H, CH($C\underline{H}_3$)$_2$, 0.89 (d, *J*=6.6 Hz, 3H, CH($C\underline{H}_3$), 0.92 (d, *J*=6.4 Hz, 3H, CH($C\underline{H}_3$), 0.97 (d, *J*=6.5 Hz, 6H, CH($C\underline{H}_3$)$_2$, 0.98 (d, *J*=6.4 Hz, 3H, CH($C\underline{H}_3$)), 1.00 (d, *J*=6.6 Hz, 3H, CH($C\underline{H}_3$), 1.35-1.45 (m, 2H buried, 2($C\underline{H}(CH_3)_2$)), 1.51-1.59 (m, 1H, $C\underline{H}(CH_3)_2$), 1.66-1.73 (m, 6H buried, 3($C\underline{H}CH_2(CH_3)_2$), 2.02-2.08 (m, 1H, $CHCH_a\underline{H}_bPh$), 2.99 (dd, *J*=14.4, 11.3 Hz, 1H, $CHC\underline{H}_aH_bPh$), 3.18 (s, 3H, $NC\underline{H}_3$), 3.89

(dd, *J*=10.4, 5.4 Hz, 1αH), 3.40 (dd, *J*=9.3, 6.2 Hz, 1αH), 4.40-4.45 (dd, *J*=10.4, 5.4 Hz, 1αH), 4.53 (dd, *J*=11.1, 4.3 Hz, 1αH), 4.59-4.63 (m, 1αH), 7.18-7.30 (m, 5H, Ph), 7.95 (d, *J*=7.9 Hz, 1NH). LCMS: *m/z* called for $C_{33}H_{53}N_5O_5$ ($M+Na^+$) = 622.8, found 623.2.

5.2.2 Experimental methods for SanA 4

5.2.2.1 MeO-Phe-Leu-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-Leu-NHBoc was synthesized utilizing 500 mg (2.01 mmol, 1.0 equivalent) of acid HO-Leu-NHBoc, 476 mg (2.21 mmol, 1.1 equivalent) of amine OMe-Phe-NH_2, 774 mg (2.41 mmol, 1.2 equivalents) of TBTU, 1.40 mL (8.04 mmol, 4.0 equivalents) of DIPEA dissolved in 20.1 mL DCM, under argon. The reaction mixture stirred for 1 hr and upon completion, the crude reaction mixture was diluted with 200 mL EA. The crude reaction was washed with 10% HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The pure desired peptide OMe-Leu-Leu-NHBoc was afforded as a white solid (766 mg, 97% yield); R_f: 0.75 (1:1 Hex:EA). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.04 (d, *J*=2.3 Hz, 3H, CHC$\underline{H}_3$), 1.07 (d, *J*=6.8 Hz, 3H, CHC$\underline{H}_3$), 1.58 (s, 9H, C(C$\underline{H}_3$)$_3$), 1.71-1.81 (m, 2H, CHC$\underline{H}_2$CH), 1.84-1.88 (m, 1H, $CH_2C\underline{H}(CH_3)_2$), 3.26 (t, *J*=6.5 Hz, 2H, CHC$\underline{H}_2$Ph), 3.85 (s, 3H, OC$\underline{H}_3$), 4.13-4.31 (m, 1αH), 4.92-5.06 (m, 1αH), 6.64 (d, *J*=7.98 Hz, 1NH), 7.21-7.29 (m, 2H, Ph), 7.36-7.45 (m, 3H, Ph).

5.2.2.2 MeO-Phe-Leu-NH_2

Following the ***Boc removal*** procedure: MeO-Phe-Leu-NH_2 was synthesized by dissolving 766 mg (1.95 mmol, 1.0 equivalent) of MeO-Phe-Leu-NHBoc in 15.6 mL DCM, followed by adding 425 µL (3.90 mmol, 2.0 equivalents) of anisole and then 3.90 mL of TFA. Boc removal was complete in 45 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 (570 mg, quantitative yield) as a light brown oil.

5.2.2.3 MeO-Phe-Leu-Val-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-Leu-Val-NHBoc was synthesized utilizing 385 mg (1.77 mmol, 1.0 equivalent) of acid HO-Val-NHBoc, 570 mg (1.95 mmol, 1.1 equivalent) of amine MeO-Phe-Leu-NH_2, 682 mg (2.12 mmol, 1.2 equivalents) of TBTU, 1.85 mL (10.6 mmol, 6.0 equivalents) of DIPEA dissolved in 17.7 mL DCM, under argon. The reaction mixture stirred for 1.5 hrs and upon completion, the crude reaction mixture was rotovapped down to dryness and redissolved in 200 mL EA. The crude reaction was washed with 10% HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The pure desired peptide OMe-Phe-Leu-Val-NHBoc was afforded as a light yellow oil (862 mg, 99% yield). R_f: 0.65 (1:1 Hex:EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.83-0.98 (m, 6H, $CH(C\underline{H}_3)_2$, 1.45 (s, 9H, $C(C\underline{H}_3)_3$), 1.58-1.73 (m, 2H, $CHC\underline{H}_2CH$), 2.04-2.18 (m, 1H, $CHC\underline{H}(CH_3)_2$), 3.11 (d, *J*=5.82 Hz, 2H, $C\underline{H}_2Ph$), 3.71 (s, 3H, $OC\underline{H}_3$), 3.79-3.91 (m, 1αH), 4.36-4.46 (m, 1αH), 4.76-4.88 (m, 1αH), 4.99 (d, *J*=8.6 Hz, 1NH), 6.31 (d, *J*=7.8 Hz, 1NH), 6.46 (d, *J*=7.4 Hz, 1NH), 7.07-7.14 (m, 2H, Ph), 7.22-7.34 (m, 3H, Ph). LCMS: *m/z* called for $C_{26}H_{41}N_3O_6$ (M+1) = 491.6, found 491.9.

5.2.2.4 MeO-Phe-Leu-Val-NH_2

Following the ***Boc removal*** procedure: MeO-Phe-Leu-Val-NH_2 was synthesized by dissolving 862 mg (1.75 mmol, 1.0 equivalent) of MeO-Phe-Leu-Val-NHBoc in 14.0 mL DCM, followed by adding 382 µL (3.50 mmol, 2.0 equivalents) of anisole and then 3.50 mL of TFA. Boc removal was complete in 45 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 (688 mg, quantitative yield) as a light brown oil.

5.2.2.5 MeO-Leu-N(Me)-Leu-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Leu-N(Me)-Leu-NHBoc was synthesized utilizing 500 mg (2.16 mmol, 1.0 equivalent) of acid HO-Leu-NHBoc, 379 mg (2.38 mmol, 1.1 equivalent) of amine OMe-Leu-N(Me)H, 518 mg (1.62 mmol, 0.75 equivalents) of TBTU, 616 mg (1.62 mmol, 0.75 equivalents) of HATU, 1.51 mL (8.64 mmol, 4.0 equivalents) of DIPEA dissolved in 21.6 mL DCM, under argon. The reaction mixture stirred for 1 hr and upon completion, the crude reaction mixture was diluted with 200 mL EA and washed with 10% HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The pure desired peptide OMe-Leu-N(Me)-Leu-NHBoc was afforded as a white solid (603 mg, 75% yield); R_f: 0.75 (1:1 Hex:EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.83 (d, *J*=6.6 Hz, 3H, CHC$\underline{H}_3$), 0.86-0.90 (m, 6H, CH(C$\underline{H}_3$)$_2$), 0.93 (d, *J*=6.6 Hz, 3H, CHC$\underline{H}_3$), 1.36 (s, 9H, C(C$\underline{H}_3$)$_3$), 1.40-1.45 (m, 2H buried, 2(CH_2C$\underline{H}$)), 1.60-1.69 (m, 4H buried, 2(C$\underline{H}_2$CH), 2.93 (s, 3H, NC$\underline{H}_3$), 3.63 (s, 3H, OC$\underline{H}_3$), 4.51-4.64 (m, 1αH), 5.09 (d, *J*=9.0 Hz, 1NH), 5.23-5.32 (m, 1αH).

5.2.2.6 HO-Leu-N(Me)-Leu-NHBoc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-Leu-N(Me)-Leu-NHBoc was deprotected by utilizing 603 mg (1.62 mmol, 1.0 equivalent) of MeO-Leu-N(Me)-Leu-NHBoc and 544 mg (12.9 mmol, 8.0 equivalents) of LiOH•H_2O in 16.2 mL MeOH. The peptide was dissolved in methanol and LiOH•H_2O was added to the reaction flask and the reaction was run for 3 hrs. Upon completion, the reaction was diluted with 250 mL DCM and washed with pH 1 HCl solution (200 mL x 2). The

aqueous layer was back-extracted with EA (100 mL x 2), and the combined organic layers were dried, filtered and concentrated *in vacuo* (481 mg, 83% yield) as a clear oil. The free acid HO-Leu-N(Me)-Leu-NHBoc (**SanA 4.B**) was taken on without any further purification or characterization.

5.2.2.7 MeO-Phe-Leu-Val-Leu-N(Me)-Leu-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-Leu-Val-Leu-N(Me)-Leu-NHBoc (**SanA 4.LP**) was synthesized utilizing 481 mg (1.34 mmol, 1.0 equivalent) of acid HO-Leu-N(Me)-Leu-NHBoc, 577 mg (1.47 mmol, 1.1 equivalent) of amine MeO-Phe-Leu-Val-NH_2, 322 mg (1.01 mmol, 0.75 equivalents) of TBTU, 382 mg (1.01 mmol, 0.75 equivalents) of HATU, 1.84 mL (10.7 mmol, 8.0 equivalents) of DIPEA dissolved in 6.7 mL each of DCM and ACN, under argon. The reaction mixture stirred for 3 hrs and upon completion, the crude reaction mixture diluted with 200 mL EA. The crude reaction was washed with 10% HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed reaction underwent a final purification via column chromatography on silica gel (1:1 Hex/EA) to afford the desired peptide MeO-Phe-Leu-Val-Leu-N(Me)-Leu-NHBoc (656 mg, 67% yield) as a clear oil. R_f: 0.50 (Hex/EA 1:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88-0.94 (m, 12H buried, 2(CH(C$\underline{H}_3$)$_2$)), 0.96-1.00 (m, 6H, CH(C$\underline{H}_3$)$_2$), 1.43 (s, 9H, C(C$\underline{H}_3$)$_3$), 1.53-1.62 (m, 3H buried, 3($CH_2C\underline{H}(CH_3)_2$)), 1.66-1.70 (m, 6H buried, 3(CHC$\underline{H}_2$CH), 1.75-1.81 (m, 1H, C$\underline{H}(CH_3)_2$), 2.95 (s, 3H, NC$\underline{H}_3$), 3.02 (d, *J*= 6.8 Hz, 2H, CHC$\underline{H}_2$Ph), 3.61 (s, 3H, OC$\underline{H}_3$), 4.05-4.15 (m, 1αH), 4.25-4.39 (m, 1αH), 4.44-4.60 (m, 1αH), 4.68-4.77 (m, 1αH), 4.91-4.98 (m, 1αH), 6.25 (d, *J*=7.9 Hz, 1NH), 6.39 (d, *J*=8.0 Hz, 1NH), 6.58 (d, *J*=8.2, 1NH), 6.65 (d, *J*=7.8, 1NH), 7.01-7.09 (m, 2H, Ph), 7.14-7.22 (m, 3H, Ph). LCMS: *m/z* called for $C_{38}H_{63}N_5O_8$ ($M+Na^+$) = 755.0, found 755.0.

5.2.2.8 HO-Phe-Leu-Val-Leu-N(Me)-Leu-NHBoc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-Phe-Leu-Val-Leu-N(Me)-Leu-NHBoc was deprotected by utilizing 656 mg (0.90 mmol, 1.0 equivalent) of MeO-Phe-Leu-N(Me)-Val-Leu-Leu-NHBoc and 57.6 mg (7.20 mmol, 8.0 equivalents) of LiOH•H_2O in 9.00 mL MeOH. The peptide was dissolved in methanol and LiOH•H_2O was added to the reaction flask and the reaction was run overnight. Upon completion, the reaction was diluted with 250 mL DCM. The aqueous layer was back-extracted with EA (100 mL x 2), and the combined organic layers were dried, filtered and concentrated *in vacuo* (394 mg, 61% yield) as a clear oil. The free acid HO-Phe-Leu-Val-Leu-N(Me)-Leu-NHBoc (**SanA 4.DLP**) was taken on without any further purification or characterization.

5.2.2.9 HO-Phe-Leu-Val-Leu-N(Me)-Leu-NH_2

Following the ***Boc removal*** procedure: HO-Phe-Leu-Val-Leu-N(Me)-Leu-NH_2 (**SanA 4.DDLP**) was synthesized by dissolving 394 mg (0.55 mmol, 1.0 equivalent) of HO-Phe-Leu-Val-Leu-N(Me)-Leu-NHBoc in 4.39 mL DCM, followed by adding 120 µL (1.10 mmol, 2.0 equivalents) of anisole and then 1.10 mL of TFA. Boc removal was complete in 45 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 (340 mg, quantitative yield) as a dark brown oil. LCMS: *m/z* called for $C_{33}H_{55}N_5O_6$ (M+1) = 618.8, found 619.7

5.2.2.10 cyclo-Phe-Leu-Val-Leu-N(Me)-Leu

Following the ***macrocyclization*** procedure: *cyclo*-Phe-Leu-Val-Leu-N(Me)-Leu (**SanA 4**) was synthesized by dissolving 226 mg (0.37 mmol, 1.0 equivalent) of HO-Phe-Leu-Val-Leu-N(Me)-Leu-NH_2, 82.0 mg (0.26 mmol, 0.7 equivalents) of TBTU, 98.0 mg (0.26 mmol, 0.7 equivalents) of HATU and 77.0 mg (0.26 mmol, 0.7 equivalents) of

DEPBT in 26.2 mL each of ACN and DCM. 384 µL of DIPEA (2.20 mmol, 6.0 equivalents) was added to the reaction flask and the solution stirred for 3 hrs. Upon completion, the crude reaction was diluted with 250 mL EA and washed with 10% HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed reaction underwent a final purification via column chromatography on silica gel to afford the desired. Pure *cyclo*-Phe-Leu-Val-Leu-N(Me)-Leu (**SanA 4**) eluted at 95:5 EA:MeOH and was furnished in 33% yield; the structure and purity of the macrocycle was confirmed via LC/MS and ^{1}H NMR. R_f: 0.35 (3:1 Hex:EA). A final purification via RP-HPLC was performed prior to biological evaluation. ^{1}H NMR (600 MHz, CD_3OD): δ 0.84 (t, *J*=6.8 Hz, 6H, $CH(C\underline{H}_3)_2$, 0.91 (d, *J*=6.6 Hz, 6H, $CH(C\underline{H}_3)_2$, 0.93 (d, *J*=6.8 Hz, 6H, $CH(C\underline{H}_3)_2$, 0.95-0.97 (m, 6H, $CH(C\underline{H}_3)_2$, 1.45-1.51 (m, 3H buried, $3(CHCH_2C\underline{H})$), 1.52-1.57 (m, 2H buried, $2(C\underline{H}_2CH_3)$), 1.64 (t, *J*=7.4 Hz, 2H, $CHC\underline{H}_2CH$), 1.78-1.83 (m, 1H, $C\underline{H}(CH_3)_2$), 1.94-1.99 (m, 1H, $CHCH_a\underline{H}_bPh$), 2.81 (s, 3H, $C\underline{H}_3N$), 3.10-3.17 (m, 1H, $CHC\underline{H}_aH_bPh$), 3.63-3.69 (m, 1αH), 4.13 (dd, *J*=9.5, 6.9 Hz, 1αH), 4.35-4.40 (m, 1αH), 4.81-4.86 (m, 1αH), 5.13 (t, *J*=7.7 Hz, 1αH), 7.22-7.31 (m, 5H, Ph), 7.40 (d, *J*=9.6 Hz, 1NH), 8.10 (d, *J*=8.6 Hz, 1NH), 8.49 (d, *J*=7.2 Hz, 1NH). LCMS: *m/z* called for $C_{33}H_{53}N_5O_5$ $(M+Na^+)$ = 622.8, found 622.5.

5.2.3 Experimental methods for SanA 11

5.2.3.1 MeO-Phe-Leu-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-Leu-NHBoc was synthesized utilizing 500 mg (2.01 mmol, 1.0 equivalent) of acid HO-Leu-NHBoc, 476 mg (2.21 mmol, 1.1 equivalent) of amine OMe-Phe-NH_2, 774 mg (2.41 mmol, 1.2 equivalents) of TBTU, 1.40 mL (8.04 mmol, 4.0 equivalents) of DIPEA

dissolved in 20.1 mL DCM, under argon. The reaction mixture stirred for 1 hr and upon completion, the crude reaction mixture was diluted with 200 mL EA and washed with 10% HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The pure desired peptide OMe-Leu-Leu-NHBoc was afforded as a white solid (766 mg, 97% yield); R_f: 0.75 (1:1 Hex:EA). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.04 (d, *J*=2.3 Hz, 3H, CHC$\underline{H}_3$), 1.07 (d, *J*=6.8 Hz, 3H, CHC$\underline{H}_3$), 1.58 (s, 9H, C(C$\underline{H}_3$)$_3$), 1.71-1.81 (m, 2H buried, CHC$\underline{H}_2$CH), 1.84-1.88 (m, 1H, $CH_2C\underline{H}(CH_3)_2$), 3.26 (t, *J*=6.5 Hz, 2H, CHC$\underline{H}_2$Ph), 3.85 (s, 3H, OC$\underline{H}_3$), 4.13-4.31 (m, 1αH), 4.92-5.06 (m, 1αH), 6.64 (d, *J*=7.98 Hz, 1NH), 7.21-7.29 (m, 2H, Ph), 7.36-7.45 (m, 3H, Ph).

5.2.3.2 MeO-Phe-Leu-NH_2

Following the ***Boc removal*** procedure: MeO-Phe-Leu-NH_2 was synthesized by dissolving 766 mg (1.95 mmol, 1.0 equivalent) of MeO-Phe-Leu-NHBoc in 15.6 mL DCM, followed by adding 425 µL (3.90 mmol, 2.0 equivalents) of anisole and then 3.90 mL of TFA. Boc removal was complete in 45 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 (570 mg, quantitative yield) as a light brown oil.

5.2.3.3 MeO-Phe-Leu-Val-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-Leu-Val-NHBoc was synthesized utilizing 385 mg (1.77 mmol, 1.0 equivalent) of acid HO-Val-NHBoc, 570 mg (1.95 mmol, 1.1 equivalent) of amine MeO-Phe-Leu-NH_2, 682 mg (2.12 mmol, 1.2 equivalents) of TBTU, 1.85 mL (10.6 mmol, 6.0 equivalents) of DIPEA dissolved in 17.7 mL DCM, under argon. The reaction mixture stirred for 1.5 hrs and upon completion, the crude reaction mixture was diluted with 200 mL EA. The crude reaction was washed with pH 1 HCl solution (100 mL x 2), then saturated $NaHCO_3$

solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The pure desired peptide OMe-Phe-Leu-Val-NHBoc was afforded as a light yellow oil (862 mg, 99% yield). R_f: 0.65 (1:1 Hex:EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.83-0.98 (m, 6H, CH(C<u>H</u>$_3$)$_2$, 1.45 (s, 9H, C(C<u>H</u>$_3$)$_3$), 1.58-1.73 (m, 2H, CHC<u>H</u>$_2$CH), 2.04-2.18 (m, 1H, CHC<u>H</u>(CH$_3$)$_2$), 3.11 (d, *J*=5.82 Hz, 2H, C<u>H</u>$_2$Ph), 3.71 (s, 3H, OC<u>H</u>$_3$), 3.79-3.91 (m, 1αH), 4.36-4.46 (m, 1αH), 4.76-4.88 (m, 1αH), 4.99 (d, *J*=8.6 Hz, 1NH), 6.31 (d, *J*=7.8 Hz, 1NH), 6.46 (d, *J*=7.4 Hz, 1NH), 7.07-7.14 (m, 2H, Ph), 7.22-7.34 (m, 3H, Ph). LCMS: *m/z* called for $C_{26}H_{41}N_3O_6$ (M+1) = 491.6, found 491.9.

5.2.3.4 MeO-Phe-Leu-Val-NH$_2$

Following the ***Boc removal*** procedure: MeO-Phe-Leu-Val-NH_2 was synthesized by dissolving 862 mg (1.75 mmol, 1.0 equivalent) of MeO-Phe-Leu-Val-NHBoc in 14.0 mL DCM, followed by adding 382 µL (3.50 mmol, 2.0 equivalents) of anisole and then 3.50 mL of TFA. Boc removal was complete in 45 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 (688 mg, quantitative yield) as a light brown oil.

5.2.3.5 MeO-Leu-D-Leu-N(Me)-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Leu-D-Leu-N(Me)Boc was synthesized utilizing 500 mg (2.01 mmol, 1.0 equivalent) of acid HO-D-Leu-N(Me)Boc, 407 mg (2.24 mmol, 1.1 equivalent) of amine OMe-Leu-NH_2, 785 mg (2.45 mmol, 1.2 equivalents) of TBTU, 1.42 mL (8.15 mmol, 4.0 equivalents) of DIPEA dissolved in 20.4 mL DCM, under argon. The reaction mixture stirred for 1 hr and upon completion, the crude reaction mixture was diluted with 200 mL EA and washed with 10% HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and

concentrated *in vacuo*. The pure desired peptide OMe-Leu-D-Leu-N(Me)Boc was afforded as a white solid (665 mg, 88% yield); R_f: 0.75 (1:1 Hex:EA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.90-0.98 (m, 12H, 2(CH($C\underline{H}_3$)$_2$), 3.72 (s, 3H, $OC\underline{H}_3$), 4.51-4.63 (br, 1αH), 4.65-4.81 (m, 1αH), 6.42-6.67 (br, 1NH).

5.2.3.6 HO-Leu-D-Leu-N(Me)-Boc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-Leu-D-Leu-N(Me)Boc was deprotected by utilizing 665 mg (1.79 mmol, 1.0 equivalent) of MeO-Leu-D-Leu-N(Me)Boc and 599 mg (1.43 mmol, 8.0 equivalents) of LiOH•H_2O in 17.9 mL MeOH. The peptide was dissolved in methanol and LiOH•H_2O was added to the reaction flask and the reaction was run for 1.5 hrs. Upon completion, the reaction was diluted with 250 mL DCM and washed with pH 1 HCl solution (200 mL x 2). The aqueous layer was back-extracted with EA (100 mL x 2), and the combined organic layers were dried, filtered and concentrated *in vacuo* (546 mg, 88% yield) as a clear oil. The free acid HO-Leu-D-Leu-N(Me)Boc (**SanA 11.B**) was taken on without any further purification or characterization.

5.2.3.7 MeO-Phe-Leu-Val-Leu-D-Leu-N(Me)-Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-Leu-Val-Leu-D-Leu-N(Me)Boc (**SanA 11.LP**) was synthesized utilizing 334 mg (0.93 mmol, 1.0 equivalent) of acid HO-Leu-Leu-NHBoc, 402 mg (1.03 mmol, 1.1 equivalent) of amine MeO-Phe-Leu-Val-NH_2, 359 mg (1.12 mmol, 1.2 equivalents) of TBTU, 976 µL (5.59 mmol, 6.0 equivalents) of DIPEA dissolved in 9.3 mL DCM, under argon. The reaction mixture stirred for 2 hrs and upon completion, the crude reaction mixture was diluted with 200 mL EA and washed with 10% HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed reaction underwent

a final purification via column chromatography on silica gel (Hex/EA 1:1) to afford the desired peptide MeO-Phe-Leu-Val-Leu-D-Leu-N(Me)Boc (473 mg, 70% yield) as a light yellow oil. R_f: 0.65 (1:4 Hex:EA). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (d, *J*=6.3 Hz, 3H, CH(C$\underline{H}_3$)), 0.89-0.93 (m, 6H, CH(C$\underline{H}_3$)$_2$), 0.93-0.96 (m, 12H buried, 2CH(C$\underline{H}_3$)$_2$), 0.97 (m, 3H, CH(C$\underline{H}_3$)), 1.49 (s, 9H, C(C$\underline{H}_3$)$_3$), 1.51-1.59 (m, 3H, 3(C$\underline{H}$(CH$_3$)$_2$)), 1.61-1.71 (m, 4H buried, 2(CH(C$\underline{H}$)$_2$)), 1.72-1.78 (m, 2H, CHC$\underline{H}_2$CH), 2.20-2.29 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 2.83 (s, 3H, C$\underline{H}_3$N), 3.04-3.19 (m, 2H, C$\underline{H}_2$Ph), 3.70 (s, 3H, OC$\underline{H}_3$), 4.19 (dd, *J*=6.0, 1.9 Hz, 1αH), 4.23-4.31 (m, 1αH), 4.44-4.50 (m, 1αH), 4.57-4.70 (br, 1αH), 4.76-4.85 (m, 1αH), 6.38 (br, 1NH), 6.66 (br, 1NH), 6.76 (br, 1NH), 6.87 (br, 1NH), 7.11-7.18 (m, 2H, Ph), 7.20-7.31 (m, 3H, Ph). LCMS: *m/z* called for $C_{39}H_{65}N_5O_8$ (M+1) = 732.0, found 732.2.

5.2.3.8 HO-Phe-Leu-Val-Leu-D-Leu-N(Me)-Boc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-Phe-Leu-Val-Leu-D-Leu-N(Me)Boc was deprotected by utilizing 473 mg (0.65 mmol, 1.0 equivalent) of MeO-Phe-Leu-Val-Leu-D-Leu-N(Me)Boc and 217 mg (5.17 mmol, 8.0 equivalents) of LiOH•H_2O in 6.47 mL MeOH. The peptide was dissolved in methanol and LiOH•H_2O was added to the reaction flask and the reaction was run overnight. Upon completion, the reaction was diluted with 250 mL DCM and washed with pH 1 HCl solution (200 mL x 2). The aqueous layer was back-extracted with EA (100 mL x 2), and the combined organic layers were dried, filtered and concentrated *in vacuo* (313 mg, 67% yield) as a clear oil. The free acid HO-Phe-Leu-Val-Leu-D-Leu-N(Me)Boc (**SanA 11.DLP**) was taken on without any further purification. LCMS: *m/z* called for $C_{38}H_{63}N_5O_8$ (M+1) = 717.9, found 718.2

5.2.3.9 HO-Phe-Leu-Val-Leu-D-Leu-N(Me)H

Following the ***Boc removal*** procedure: HO-Phe-Leu-Val-Leu-D-Leu-N(Me)H (**SanA 11.DDLP**) was synthesized by dissolving 313 mg (0.44 mmol, 1.0 equivalent) of

HO-Phe-Leu-Val-Leu-D-Leu-N(Me)Boc in 3.27 mL DCM, followed by adding 95.0 µL (0.87 mmol, 2.0 equivalents) of anisole and then 1.09 mL of TFA. Boc removal was complete in 1 hr; 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 (270 mg, quantitative yield) as a dark brown oil. LCMS: *m/z* called for $C_{33}H_{35}N_5O_6$ (M+1) = 617.8, found 617.8

5.2.3.10 *cyclo*-Phe-Leu-Val-Leu-D-Leu-N(Me)

Following the ***macrocyclization*** procedure: *cyclo*-Phe-Leu-Val-Leu-D-Leu-N(Me) (**SanA 11**) was synthesized by dissolving 270 mg (0.44 mmol, 1.0 equivalent) of HO-Phe-Leu-Val-Leu-D-Leu-N(Me)H, 59.0 mg (0.17 mmol, 0.4 equivalents) of TBTU, 133 mg (0.35 mmol, 0.8 equivalents) of HATU and 52.0 mg (0.17 mmol, 0.4 equivalents) of DEPBT in 31.2 mL of ACN and 15.6 mL each of DCM and THF. 609 µL of DIPEA (3.49 mmol, 8.0 equivalents) was added to the reaction flask and the solution stirred for 2 hrs. Upon completion, the crude reaction was diluted with 200 mL EA and washed with 10% HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed reaction underwent a final purification via column chromatography on silica gel to afford the desired. Pure *cyclo*-Phe-Leu-Val-Leu-D-Leu-N(Me) (**SanA 11**) eluted at 10:90 Hex:EA and was furnished in 47% yield; the structure and purity of the macrocycle was confirmed via LC/MS and ^{1}H NMR. Rf: 0.45 (1:3 Hex:EA). A final purification via RP-HPLC was performed prior to biological evaluation. ^{1}H NMR (600 MHz, CD_3OD): δ 0.82 (d, *J*=6.6 Hz, 6H, $CH(C\underline{H}_3)_2$, 0.91 (m, 6H, $CH(C\underline{H}_3)_2$, 0.93 (m, 6H, $CH(C\underline{H}_3)_2$, 0.97 (d, *J*=6.4 Hz, 6H, $CH(C\underline{H}_3)_2$, 1.13-1.21 (m, 1H, $CH_2C\underline{H}(CH_3)_2$), 1.46-1.52 (m, 1H, $CH_2C\underline{H}(CH_3)_2$), 1.53-1.63 (m, 6H buried, 3($CHC\underline{H}_2CH$)), 1.86-1.95 (m, 1H, $C\underline{H}(CH_3)_2$), 2.22-2.31 (m, 1H, $C\underline{H}(CH_3)_2$), 2.72 (s, 3H, $C\underline{H}_3N$), 2.92-3.00 (dd, *J*=19.7, 6.7

Hz, 1H, CHC$\underline{H}_a$H$_b$Ph), 3.10 (dd, *J*=13.1, 8.8 Hz, 1H, CHCH$_a\underline{H}_b$Ph), 3.69-3.76 (m, 1αH), 3.91-3.98 (m, 1αH), 4.35-4.43 (m, 1αH), 4.96-5.05 (m, 2αH buried), 7.18-7.22 (m, 2H, Ph), 7.23-7.27 (m, 3H, Ph), 7.82 (d, *J*=8.9 Hz, 1NH), 7.91 (d, *J*=7.8 Hz, 1NH), 8.47 (d, *J*=8.4 Hz, 1NH). LCMS: *m/z* called for $C_{33}H_{53}N_5O_5$ (M+1) = 599.8, found 600.3

5.2.4 Experimental methods for SanA 13

5.2.4.1 MeO-Phe-Leu-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-Leu-NHBoc was synthesized utilizing 500 mg (2.01 mmol, 1.0 equivalent) of acid HO-Leu-NHBoc, 476 mg (2.21 mmol, 1.1 equivalent) of amine OMe-Phe-NH_2, 774 mg (2.41 mmol, 1.2 equivalents) of TBTU, 1.40 mL (8.04 mmol, 4.0 equivalents) of DIPEA dissolved in 20.1 mL DCM, under argon. The reaction mixture stirred for 1 hr and upon completion, the crude reaction mixture was diluted with 200 mL EA and washed with 10% HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The pure desired peptide OMe-Leu-Leu-NHBoc was afforded as a white solid (766 mg, 97% yield); R_f: 0.75 (1:1 Hex:EA). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.04 (d, *J*=2.3 Hz, 3H, CHC$\underline{H}_3$), 1.07 (d, *J*=6.8 Hz, 3H, CHC$\underline{H}_3$), 1.58 (s, 9H, C(C$\underline{H}_3$)$_3$), 1.71-1.81 (m, 2H, CHC$\underline{H}_2$CH), 1.84-1.88 (m, 1H, $CH_2C\underline{H}(CH_3)_2$), 3.26 (t, *J*=6.5 Hz, 2H, CHC$\underline{H}_2$Ph), 3.85 (s, 3H, OC$\underline{H}_3$), 4.13-4.31 (m, 1αH), 4.92-5.06 (m, 1αH), 6.64 (d, *J*=7.98 Hz, 1NH), 7.21-7.29 (m, 2H, Ph), 7.36-7.45 (m, 3H, Ph).

5.2.4.2 MeO-Phe-Leu-NH_2

Following the ***Boc removal*** procedure: MeO-Phe-Leu-NH_2 was synthesized by dissolving 766 mg (1.95 mmol, 1.0 equivalent) of MeO-Phe-Leu-NHBoc in 15.6 mL DCM, followed by adding 425 µL (3.90 mmol, 2.0 equivalents) of anisole and then 3.90

mL of TFA. Boc removal was complete in 45 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 (570 mg, quantitative yield) as a light brown oil.

5.2.4.3 MeO-Phe-Leu-D-Ser(Bzl)-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-Leu-D-Ser(Bzl)-NHBoc was synthesized utilizing 366 mg (1.24 mmol, 1.0 equivalent) of acid HO-D-Ser(Bzl)-NHBoc, 400 mg (1.37 mmol, 1.1 equivalent) of amine MeO-Phe-Leu-NH_2, 478 mg (1.49 mmol, 1.2 equivalents) of TBTU, 867 µL (4.97 mmol, 4.0 equivalents) of DIPEA dissolved in 12.4 mL DCM, under argon. The reaction mixture stirred for 1.5 hrs and upon completion, the crude reaction mixture was rotovapped down to dryness and redissolved in 200 mL EA. The crude reaction was washed with pH 1 HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The pure desired peptide OMe-Phe-Leu-Val-NHBoc was afforded as a light yellow oil (570 mg, 90% yield). Rf: 0.65 (Hex/EA 1:1). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (d, *J*=6.2, 6H, CH($C\underline{H}_3$)$_2$, 1.47 (s, 9H, C($C\underline{H}_3$)$_3$), 1.53-1.71 (m, 2H, CHC$\underline{H}_2$CH), 1.74-1.93 (m, 1H, CHC$\underline{H}$(CH_3)$_2$), 2.96-3.16 (m, 2H, C$\underline{H}_2$Ph), 3.59 (dd, *J*=6.1, 3.5, 1H, CHC$\underline{H}_a$H$_b$O), 3.72 (s, 3H, OC$\underline{H}_3$), 3.88 (dd, *J*=8.8, 4.7, 1H, CHCH$_a$$\underline{H}_b$O), 4.51 (d, *J*=6.1, 2H, OC$\underline{H}_2$Ph), 4.78 (q, *J*=6.3, 1αH), 5.29-5.39 (br, 1αH), 6.52 (d, *J*=7.8, 1NH), 6.59 (d, *J*=8.1, 1NH), 7.08-7.13 (m, 1H, Ph), 7.20-7.29 (m, 5H, Ph), 7.29-7.37 (m, 3H, Ph).

5.2.4.4 MeO-Phe-Leu-D-Ser(Bzl)-NH_2

Following the ***Boc removal*** procedure: MeO-Phe-Leu-D-Ser(Bzl)-NH_2 was synthesized by dissolving 301 mg (0.53 mmol, 1.0 equivalent) of MeO-Phe-Leu-D-Ser(Bzl)-NHBoc in 3.70 mL DCM, followed by adding 122 µL (1.06 mmol, 2.0 equivalents) of anisole and then 1.58 mL of TFA. Boc removal was complete in 45 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 (248 mg, quantitative yield) as a light brown oil.

5.2.4.5 MeO-Leu-Leu-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Leu-Leu-NHBoc was synthesized utilizing 500 mg (2.01 mmol, 1.0 equivalent) of acid HO-Leu-NHBoc, 401 mg (2.21 mmol, 1.1 equivalent) of amine OMe-Leu-NH_2, 774 mg (2.41 mmol, 1.2 equivalents) of TBTU, 1.40 mL (8.04 mmol, 4.0 equivalents) of DIPEA dissolved in 20.1 mL DCM, under argon. The reaction mixture stirred for 1.5 hrs and upon completion, the crude reaction mixture was rotovapped down to dryness and redissolved in 200 mL EA. The crude reaction was washed with pH 1 HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The pure desired peptide OMe-Leu-Leu-NHBoc was afforded as a white solid (664 mg, 92% yield); Rf: 0.65 (Hex/EA 1:1). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81-1.03 (m, 12H, 2(CH($C\underline{H}_3$)$_2$), 1.45 (s, 9H, C($C\underline{H}_3$)$_3$), 1.49-1.56 (m, 1H, $C\underline{H}(CH_3)_2$, 1.58-1.70 (m, 4H, 2($CHC\underline{H}_2CH$), 1.71-1.78 (m, 1H, $C\underline{H}(CH_3)_2$, 3.73 (s, 3H, $OC\underline{H}_3$), 4.01-4.20 (m, 1αH), 4.54-4.70 (m, 1αH), 4.85 (d, *J*=8.3, 1NH), 6.43 (d, *J*=7.9, 1NH).

5.2.4.6 HO-Leu-Leu-NHBoc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-Leu-Leu-NHBoc was deprotected by utilizing 664 mg (1.86 mmol, 1.0 equivalent) of MeO-Leu-Leu-NHBoc and 623 mg (1.48 mmol, 8.0 equivalents) of LiOH•H_2O in 18.6 mL MeOH. The peptide was dissolved in methanol and LiOH•H_2O was added to the reaction flask and the reaction was run for 3 hrs. Upon completion, the reaction was diluted with 250 mL DCM. The aqueous layer was back-extracted with EA (100 mL x 2),

and the combined organic layers were dried, filtered and concentrated *in vacuo* (184 mg, 84% yield) as a clear oil. The free acid HO-Leu-Leu-NHBoc (**SanA 13.B**) was taken on without any further purification or characterization.

5.2.4.7 MeO-Phe-Leu-D-Ser(Bzl)-Leu-Leu-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-Leu-D-Ser(Bzl)-Leu-Leu-NHBoc (**SanA 13.LP**) was synthesized utilizing 174 mg (0.48 mmol, 1.0 equivalent) of acid HO-Leu-Leu-NHBoc, 248 mg (0.53 mmol, 1.1 equivalent) of amine MeO-Phe-Leu-D-Ser(Bzl)-NH_2, 154 mg (0.48 mmol, 1.0 equivalent) of TBTU, 91.0 mg (0.24 mmol, 0.50 equivalents) of HATU, 670 µL (3.84 mmol, 8.0 equivalents) of DIPEA dissolved in 2.4 each of DCM and ACN, under argon. The reaction mixture stirred for 3 hrs and upon completion, the crude reaction mixture was rotovapped down to dryness and redissolved in 200 mL EA. The crude reaction was washed with pH 1 HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed reaction underwent a final purification via column chromatography on silica gel (Hex/EA 1:1) to afford the desired peptide MeO-Phe-Leu-D-Ser(Bzl)-Leu-Leu-NHBoc (190 mg, 50% yield) as a white solid. Rf: 0.65 (Hex/EA 1:3). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81-0.89 (m, 6H, $CH(C\underline{H}_3)_2$), 0.90-0.96 (m, 12H buried, $2(CH(C\underline{H}_3)_2)$), 1.42 (s, 9H, $C(C\underline{H}_3)_3$), 1.46-1.54 (m, 3H buried, $3(CH_2CH(CH_3)_2)$), 1.56-1.72 (m, 6H buried, $3(CHC\underline{H}_2CH)$, 3.00-3.11 (m, 2H, $CHC\underline{H}_2O$), 3.65 (s, 3H, $OC\underline{H}_3$), 3.96 (dd, *J*=9.7, 4.5 Hz, 2H, $CHC\underline{H}_2Ph$), 4.00-4.12 (m, 1αH), 4.17-4.27 (m, 1αH), 4.50 (d, *J*=6.3 Hz, 2H, OCH_2Ph), 4.45-4.58 (m, 2αH buried), 4.75-4.88 (m, 1αH), 6.66 (d, *J*=6.7 Hz, 1NH), 6.83 (d, *J*=7.9 Hz, 1NH), 6.95 (d, *J*=6.7 Hz, 1NH), 7.03 (d, *J*=8.2 Hz, 1NH),7.09-7.15 (m, 2H, Ph), 7.20-7.23 (m, 3H, Ph), 7.24-7.31 (m, 5H, Ph). LCMS: *m/z* called for $C_{43}H_{65}N_5O_9$ (M+1) = 796.0, found 796.1

5.2.4.8 HO-Phe-Leu-D-Ser(Bzl)-Leu-Leu-NH_2

Following the ***in situ deprotection*** procedure: HO-Phe-Leu-D-Ser(Bzl)-Leu-Leu-NH_2 (**SanA 13.DDLP**) was synthesized by dissolving 157 mg (0.24 mmol, 1.0 equivalent) of MeO-Phe-Leu-D-Ser(Bzl)-Leu-Leu-NHBoc in 2.4 mL THF, followed by adding 52.0 µL (0.48 mmol, 2.0 equivalents) of anisole and then 8 drops of 12 N HCl. The reaction was monitored by LC/MS and 3 additional drops of 12 N HCl were added on days 2 and 3. By day 3, the C- and N-termini were fully deprotected; 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 (163 mg, quantitative yield) as a dark brown oil.

5.2.4.9 *cyclo*-Phe-Leu-D-Ser(Bzl)-Leu-Leu

Following the ***macrocyclization*** procedure: *cyclo*-Phe-Leu-D-Ser(Bzl)-Leu-Leu (**SanA 13.M**) was synthesized by dissolving 163 mg (0.24 mmol, 1.0 equivalent) of HO-Phe-Leu-D-Ser(Bzl)-Leu-Leu-NH_2, 38.0 mg (0.12 mmol, 0.5 equivalents) of TBTU, 38.0 mg (0.12 mmol, 0.5 equivalents) of HATU and 17.9 mg (0.12 mmol, 0.5 equivalents) of DEPBT in 17.1 mL each of ACN and DCM. 166 µL of DIPEA (0.95 mmol, 4.0 equivalents) was added to the reaction flask and the solution stirred overnight. Upon completion, the crude reaction was washed with pH 1 HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed reaction underwent a final purification via column chromatography on silica gel to afford the desired. Pure *cyclo*-Phe-Leu-D-Ser(Bzl)-Leu-Leu (**SanA 13.M**) eluted at 100% EA and was furnished in 30% yield; the structure and purity of the macrocycle was confirmed via LC/MS and ^{1}H NMR. R_f: 0.50 (100% EA). A final purification via RP-HPLC was

performed prior to biological evaluation. LCMS: *m/z* called for $C_{37}H_{53}N_5O_6$ (M+1) = 664.9, found 664.6.

5.2.4.10 *cyclo*-Phe-Leu-D-Ser-Leu-Leu

Following the ***hydrogenation*** procedure: *cyclo*-Phe-Leu-D-Ser-Leu-Leu (**SanA 13**) was furnished by dissolving 36.1 mg (54.5 µmol, 1.0 equivalent) of *cyclo*-Phe-Leu-D-Ser(Bzl)-Leu-Leu in 5.45 mL EtOH. A catalytic amount of 10% Pd/C was added to the reaction and H_2 gas was purged through the flask at low atmospheric pressure and the solution stirred overnight. Upon completion, confirmed by LC/MS, the reaction was concentrated *in vacuo* and the deprotected macrocycle underwent a final purification via column chromatography on silica gel to afford the desired product. Pure *cyclo*-Phe-Leu-D-Ser(Bzl)-Leu-D-Leu (**SanA 13**) eluted at 95:5 EA:MeOH and was furnished in 27% yield; the structure and purity of the macrocycle was confirmed via LC/MS and ^{1}H NMR. Rf: 0.35 (100% EA). A final purification via RP-HPLC was performed prior to biological evaluation. ^{1}H NMR (500 MHz, CD_3OD): δ 0.79-0.92 (m, 12H buried, 2(CH(C$\underline{H}_3$)$_2$)), 0.94-0.99 (m, 6H buried, CH(C$\underline{H}_3$)$_2$), 1.36-1.44 (m, 3H buried, 3($CH_2CH(CH_3)_2$)), 1.56-1.70 (m, 6H buried, 3(CHC$\underline{H}_2$CH)), 2.80-2.91 (m, 2H, CHC$\underline{H}_2$O), 3.52 (dd, *J*=9.0, 4.4 Hz, 2H, CHC$\underline{H}_2$Ph), 3.97-4.03 (m, 1αH), 4.15-4.20 (m, 1αH), 4.39-4.58 (m, 2αH buried), 7.20-7.39 (m, 5H, Ph). LCMS: *m/z* called for $C_{30}H_{47}N_5O_6$ ($M+Na^+$) = 597.7, found 596.4.

5.2.5 Experimental methods for SanA 15

5.2.5.1 MeO-D-Trp-Leu-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-D-Trp-Leu-NHBoc was synthesized utilizing 500 mg (2.01 mmol, 1.0 equivalent) of acid HO-Leu-NHBoc, 561 mg (2.21 mmol, 1.1 equivalent) of amine OMe-D-Trp-NH_2, 717 mg (2.39 mmol, 1.2 equivalents) of DEPBT, 1.40 mL (8.04 mmol, 4.0 equivalents) of DIPEA

dissolved in 20.1 mL DCM, under argon. The reaction mixture stirred for 1 hr and upon completion, the crude reaction mixture was rotovapped down to dryness and redissolved in 200 mL EA. The crude reaction was washed with pH 1 HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The pure desired peptide OMe-D-Trp-Leu-NHBoc was afforded as a white solid (650 mg, 82% yield); Rf: 0.65 (Hex/EA 1:1). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (d, *J*=6.2 Hz, 6H, $CH(C\underline{H}_3)_2$), 1.41 (s, 9H, $C(C\underline{H}_3)_3$), 1.46-1.60 (m, 2H, $CHC\underline{H}_2CH$), 1.69-1.73 (m, 1H, $CH_2C\underline{H}(CH_3)_2$), 3.31 (d, *J*=5.8, 2H, $CHC\underline{H}_2C$), 3.66 (s, 3H, $OC\underline{H}_3$), 4.80 (d, *J*=7.3, 1αH), 4.84-4.96 (m, 1αH), 6.65 (d, *J*=7.2, 1NH), 7.03 (d, *J*=7.0, 1NH), 7.06-7.24 (pd, *J*=8.0, 1.3, 2H, $CH(C\underline{H})_2CH$), 7.35 (d, J=7.3, 1H, $NHCC\underline{H}CH$), 7.54 (d, *J*=7.7, 1H, $CCC\underline{H}CH$), 8.11-8.21 (br, 1H, $CHN\underline{H}C$).

5.2.5.2 MeO-D-Trp-Leu-NH$_2$

Following the ***Boc removal*** procedure: MeO-D-Trp-Leu-NH_2 was synthesized by dissolving 650 mg (1.65 mmol, 1.0 equivalent) of MeO-D-Trp-Leu-NHBoc in 13.2 mL DCM, followed by adding 360 µL (3.30 mmol, 2.0 equivalents) of anisole and then 3.30 mL of TFA. Boc removal was complete in 45 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 (498 mg, quantitative yield) as a light brown oil.

5.2.5.3 MeO-D-Trp-Leu-Val-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-D-Trp-Leu-Val-NHBoc was synthesized utilizing 296 mg (1.36 mmol, 1.0 equivalent) of acid HO-Val-NHBoc, 498 mg (1.50 mmol, 1.1 equivalent) of amine MeO-D-Trp-Leu-NH_2, 488 mg (1.62 mmol, 1.2 equivalents) of DEPBT, 950 µL (5.44 mmol, 4.0 equivalents) of DIPEA dissolved in 13.6 mL DCM, under argon. The reaction mixture stirred for 1.5 hrs and

upon completion, the crude reaction mixture was rotovapped down to dryness and redissolved in 200 mL EA. The crude reaction was washed with pH 1 HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The pure desired peptide OMe-Phe-Leu-Val-NHBoc was afforded as a light yellow oil (488 mg, 67% yield). Rf: 0.55 (Hex/EA 1:1). ^1H NMR (200 MHz, $CDCl_3$): δ 0.83-0.98 (m, 6H, $CH(C\underline{H}_3)_2$, 1.45 (s, 9H, $C(C\underline{H}_3)_3$), 1.58-1.73 (m, 2H, $CHC\underline{H}_2CH$), 2.04-2.18 (m, 1H, $CHC\underline{H}(CH_3)_2$), 3.11 (d, *J*=5.82, 2H, $C\underline{H}_2Ph$), 3.71 (s, 3H, $OC\underline{H}_3$), 3.79-3.91 (m, 1αH), 4.36-4.46 (m, 1αH), 4.76-4.88 (m, 1αH), 4.99 (d, *J*=8.6, 1NH), 6.31 (d, *J*=7.8, 1NH), 6.46 (d, *J*=7.4, 1NH), 7.07-7.14 (m, 2H, Ph), 7.22-7.34 (m, 3H, Ph).

5.2.5.4 MeO-D-Trp-Leu-Val-NH_2

Following the ***Boc removal*** procedure: MeO-D-Trp-Leu-Val-NH_2 was synthesized by dissolving 488 mg (0.91 mmol, 1.0 equivalent) of MeO-D-Trp-Leu-Val-NHBoc in 7.28 mL DCM, followed by adding 199 µL (1.82 mmol, 2.0 equivalents) of anisole and then 1.82 mL of TFA. Boc removal was complete in 45 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 (357 mg, quantitative yield) as a light brown oil.

5.2.5.5 MeO-Arg(2Cbz)-Leu-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Arg(2Cbz)-Leu-NHBoc was synthesized utilizing 500 mg (2.16 mmol, 1.0 equivalent) of acid HO-Leu-NHBoc, 1.35 g (2.38 mmol, 1.1 equivalent) of amine OMe-Arg(2CBz)-NH_2, 832 mg (2.59 mmol, 1.2 equivalents) of TBTU, 1.51 mL (8.64 mmol, 4.0 equivalents) of DIPEA dissolved in 21.6 mL DCM, under argon. The reaction mixture stirred for 1.5 hrs and upon completion, the crude reaction mixture was rotovapped down to dryness and redissolved in 200 mL EA. The crude reaction was washed with pH 1 HCl solution (100

mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The pure desired peptide OMe-Leu-Leu-NHBoc was afforded as a white solid (1.26 g, 87% yield); R_f: 0.40 (Hex/EA 1:1). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.80-0.92 (m, 6H, $CH(C\underline{H}_3)_2$), 1.38 (s, 9H, $C(C\underline{H}_3)_3$), 1.41-1.50 (m, 1H, $C\underline{H}(CH_3)_2$, 1.51-1.67 (m, 4H buried, $CHC\underline{H}_2CH$, $CH_2C\underline{H}_2CH_2$), 1.73-1.89 (m, 2H, $CHC\underline{H}_2CH_2$), 3.30-3.42 (m, 2H, $NHC\underline{H}_2CH_2$), 4.01-4.15 (m, 1αH), 4.34-4.51 (m, 1αH), 5.20 (d, *J*=7.3 Hz, 1NH), 7.21-7.29 (m, 10H, 2Ph), 7.53 (d, *J*=7.8 Hz, 1NH).

5.2.5.6 HO-Arg(2Cbz)-Leu-NHBoc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-Arg(2CBz)-Leu-NHBoc was deprotected by utilizing 1.26 g (1.88 mmol, 1.0 equivalent) of MeO-Arg(2Cbz)-Leu-NHBoc and 630 mg (15.0 mmol, 8.0 equivalents) of $LiOH•H_2O$ in 18.8 mL MeOH. The peptide was dissolved in methanol and $LiOH•H_2O$ was added to the reaction flask and the reaction was run for 3 hrs. Upon completion, the reaction was diluted with 250 mL DCM. The aqueous layer was back-extracted with EA (100 mL x 2), and the combined organic layers were dried, filtered and concentrated *in vacuo* (665 mg, 54% yield) as a white solid. The free acid HO-Arg(2Cbz)-Leu-NHBoc (**SanA 15.B**) was taken on without any further purification or characterization

5.2.5.7 MeO-D-Trp-Leu-Val-Arg(2Cbz)-Leu-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-D-Trp-Leu-Val-Arg(2Cbz)-Leu-NHBoc (**SanA 15.LP**) was synthesized utilizing 934 mg (1.79 mmol, 1.0 equivalent) of acid HO-Arg(2Cbz)-Leu-NHBoc, 771 mg (1.97 mmol, 1.1 equivalents) of amine MeO-D-Trp-Leu-Val-NH_2, 344 mg (1.07 mmol, 0.6 equivalents) of TBTU, 407 mg (1.07 mmol, 0.6 equivalents) of HATU, 320 mg (1.07 mmol, 0.6 equivalents) of DEPBT, 3.12 mL (17.9 mmol, 10.0 equivalents) of DIPEA dissolved in 4.46 each of DCM

and ACN and 8.93 mL of THF, under argon. The reaction mixture stirred for 3.5 hrs and upon completion, the crude reaction mixture was rotovapped down to dryness and redissolved in 200 mL EA. The crude reaction was washed with pH 1 HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed reaction underwent a final purification via column chromatography on silica gel (Hex/EA 1:1) to afford the desired peptide MeO-D-Trp-Leu-Val-Arg(2Cbz)-Leu-NHBoc (777 mg, 47% yield) as a yellow solid. Rf: 0.50 (100% EA). ^{1}H NMR (400 MHz, CD_3OD): δ 0.80 (t, *J*=6.8 Hz, 6H, $CH(C\underline{H}_3)_2$), 0.89-0.97 (m, 12H buried, $2(CH(C\underline{H}_3)_2)$), 1.38-1.40 (m, 2H, $CHC\underline{H}_2CH_2$), 1.42 (s, 9H, $C(C\underline{H}_3)_3$), 1.45-1.48 (m, 4H buried, $2(CHC\underline{H}_2CH)$), 1.60-1.64 (m, 2H, $CH_2C\underline{H}_2CH_2$), 1.88-1.97 (m, 1H, $CH(C\underline{H}_3)_2$), 3.18-3.22 (m, 2H, $NHC\underline{H}_2CH_2$), 3.35 (s,1H, $CHC\underline{H}_aH_bC$), 3.38 (s,1H, $CHCH_a\underline{H}_bC$), 3.66 (s, 3H, $OC\underline{H}_3$), 4.11-4.18 (m, 2αH buried), 4.40-4.43 (m, 1αH), 4.45-4.53 (m, 2αH buried), 5.05 (s, 4H, $2C\underline{H}_2Ph$), 6.98-7.01 (m, 2H, Ph), 7.03-7.08 (m, 2H, Ph), 7.21-7.39 (m, 10H, 2Ph), 7.55 (d, *J*=7.8 Hz, 1NH). LCMS: *m/z* called for $C_{56}H_{77}N_9O_{12}$ (M+1) = 1068.3, found 1068.8

5.2.5.8 HO-D-Trp-Leu-Val-Arg(2Cbz)-Leu-NH_2

Following the ***in situ deprotection*** procedure: HO-D-Trp-Leu-Val-Arg(2Cbz)-Leu-NH_2 (**SanA 15.DDLP**) was synthesized by dissolving 777 mg (0.83 mmol, 1.0 equivalent) of MeO-D-Trp-Leu-Val-Arg(2Cbz)-Leu-NHBoc in 8.3 mL THF, followed by adding 181 µL (1.66 mmol, 2.0 equivalents) of anisole and then 14 drops of 12 N HCl. The reaction was monitored by LC/MS and 3 additional drops of 12 N HCl were added on days 2 and 3. By day 3, the C- and *N*-termini were fully deprotected; 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 (681 mg, quantitative yield) as a dark brown oil. LCMS: *m/z* called for $C_{50}H_{67}N_9O_{10}$ (M+1) = 955.1, found 955.6.

5.2.5.9 cyclo-D-Trp-Leu-Val-Arg(2Cbz)-Leu

Following the ***macrocyclization*** procedure: *cyclo*-D-Trp-Leu-Val-Arg(2Cbz)-Leu (**SanA 15.M**) was synthesized by dissolving 681 mg (0.83 mmol, 1.0 equivalent) of HO-D-Trp-Leu-Val-Arg(2Cbz)-Leu-NH_2, 186 mg (0.29 mmol, 0.7 equivalents) of TBTU, 190 mg (0.25 mmol, 0.6 equivalents) of HATU and 198 mg (0.33 mmol, 0.8 equivalents) of DEPBT in 29.7 mL each of ACN and DCM and 59.4 mL of THF. 1.73 mL of DIPEA (4.99 mmol, 12.0 equivalents) was added to the reaction flask and the solution stirred for 3.5 hrs. Upon completion, the crude reaction was washed with pH 1 HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed reaction underwent a final purification via column chromatography on silica gel to afford the desired. Pure *cyclo*-D-Trp-Leu-Val-Arg(2Cbz)-Leu (**SanA 15.M**) eluted at 10:90 to 0:100 Hex:EA and was furnished in 23% yield; the structure and purity of the macrocycle was confirmed via LC/MS and ^{1}H NMR. R_f: 0.40 (100% EA). LCMS: *m/z* called for $C_{50}H_{65}N_9O_9$ (M+Na) = 958.9, found 958.8.

5.2.5.10 cyclo-D-Trp-Leu-Val-Arg-Leu

Cyclo-D-Trp-Leu-Val-Arg-Leu (**SanA 15**) was furnished by dissolving 31.0 mg (33.1 µmol, 1.0 equivalent) of *cyclo*-D-Trp-Leu-Val-Arg(2Cbz)-Leu in 500 µL HBr (33% in glacial acetic acid). The reaction was run at room temperature under open atmosphere and found complete by LC/MS in 2 hrs. Upon completion, 10 mL of diethyl ether was added to the reaction flask, whereupon the pure product precipitated out of solution and filtered through cotton. This procedure was repeated 6 times and the desired product was collected and further concentrated *in vacuo.* Pure *cyclo*-D-Trp-Leu-Val-Arg-Leu

(**SanA 15**) was furnished in 94% yield; the structure and purity of the macrocycle was confirmed via LC/MS and ^{1}H NMR. Rf: 0.50 (98:2 EA:MeOH). A final purification via RP-HPLC was performed prior to biological evaluation. ^{1}H NMR (600 MHz, CD_3OD) δ 0.85 (m, 6H, $CH(C\underline{H}_3)_2$), 1.23 (d, *J* = 6.8 Hz, 6H, $CH(C\underline{H}_3)_2$), 1.26 (d, *J* = 6.4 Hz, 6H, $CH(C\underline{H}_3)_2$), 1.33 (d, *J* = 6.9 Hz, 6H, $CH(C\underline{H}_3)_2$), 1.40-1.52 (m, 6H buried, $CHC\underline{H}_2CH_2$ and 2($CHC\underline{H}_2CH$)), 1.60-1.64 (m, 2H, $CH_2C\underline{H}_2CH_2$), 2.12-2.20 (m, 1H, $CH(C\underline{H}_3)_2$), 3.18-3.22 (m, 2H, $NHC\underline{H}_2CH_2$), 3.98-4.02 (m, 1αH), 4.20-4.24 (m, 2αH buried), 4.35 (m, 1αH), 7.42-7.49 (m, 2H, Ph), 7.50-7.56 (m, 2H, Ph), 7.78 (d, *J*=7.8 Hz, 1NH), 7.90 (d, *J*=7.9 Hz, 1NH), 8.03 (d, *J*=7.1 Hz, 1NH), 8.21 (d, *J*=8.1 Hz, 1NH), 8.41 (d, *J*=7.9 Hz, 1NH). LCMS: *m/z* called for $C_{34}H_{53}N_9O_5$ (M+1) = 668.8, found 669.0.

5.2.6 Experimental methods for SanA 17-III-Ox

5.2.6.1 Resin-O-D-Phe-(2R,3R)/(2S,3S)-β-OH-Phe-NH_2

Prior to the first coupling, the D-Phe bound resin was swollen in 20 mL of DMF for 2 hrs. Solvent was removed and following the ***solution phase peptide coupling*** procedure: a mixture of 2.00 g Resin-O-D-Phe-NH_2 (1.44 mmol, 1.0 equivalent), 1.74 g HO-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHFmoc (4.32 mmol, 3.0 equivalents), 661 mg HOBt (4.32 mmol, 3.0 equivalents), and 1.34 mL of DIC (8.64 mmol, 6.0 equivalents) were shaken at room temperature for 3 hrs. Completion of the coupling reaction was verified by a negative ***ninhydrin test***. The reaction mixture was then drained to leave the amine-protected resin-bound dipeptide.

Following the ***Fmoc removal*** procedure: Resin-O-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NH_2 was furnished by washes with 20% Piperidine/DMF. A positive ***ninhydrin test*** served to verify Fmoc removal and gave the title compound.

5.2.6.2 HO-D-Phe-(2R,3R)/(2S,3S)-β-OH-Phe-Leu-NHBoc

Following the ***solution phase peptide coupling*** procedure: a mixture of Resin-O-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NH_2 (1.10 mmol, 1.0 equivalent), 923 mg HO-Leu-NHBoc residue (4.32 mmol, 3.0 equivalents), 661 mg HOBt (4.32 mmol, 3.0 equivalents), and 1.34 mL DIC (8.64 mmol, 6.0 equivalents) were stirred at room temperature for 3 hrs. Completion of the coupling reaction was verified by a negative ***ninhydrin test***.

The reaction mixture was then drained and dried *in vacuo* overnight to leave the Boc-protected resin-bound dipeptide. Following the ***cleaving the peptide from resin*** procedure: 2.49 g of dry resin bound tripeptide Resin-O-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHBoc was stirred with 10.3 mL each of TFE and DCM for 24 hrs. The slurry was filtered, washed with additional DCM, and dried *in vacuo* for 24 hrs to yield HO-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHBoc (596 mg, 76% yield).

5.2.6.3 MeO-Ser(Bzl)-D-Leu-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Ser(Bzl)-D-Leu-NHBoc was synthesized utilizing 767 mg (3.08 mmol, 1.0 equivalent) of acid HO-D-Leu-NHBoc, 709 mg (3.39 mmol, 1.1 equivalent) of amine OMe-Ser(Bzl)-NH_2, 1.19 g (3.69 mmol, 1.2 equivalents) of TBTU, 4.30 mL (24.6 mmol, 8.0 equivalents) of DIPEA dissolved in 30.8 mL DCM, under argon. The reaction mixture stirred for 2.5 hrs and upon completion, the crude reaction mixture was rotovapped down to dryness and redissolved in 200 mL EA. The crude reaction was washed with pH 1 HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The dipeptide was further purified by column chromatography; pure MeO-Ser(Bzl)-D-Leu-NHBoc eluted at 4:1 (Hex:EA). The structure and purity was confirmed via LC/MS and

^{1}H NMR (1.24 g, 96% yield). Rf: 0.78 (1:1 Hex:EA). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90-0.95 (m, 6H, $CH(C\underline{H}_3)_2$), 1.43 (s, 9H, $C(C\underline{H}_3)_3$), 1.63-1.73 (m, 2H, $CHC\underline{H}_2CH$), 3.65-3.70 (dd, 1H, dd, *J*=8.9, 2.8 Hz, $OC\underline{H}_aH_b$), 3.73 (s, 3H, $OC\underline{H}_3$), 3.86-3.91 (dd, 1H, *J*=9.0, 3.0 Hz, $OCH_a\underline{H}_b$), 4.18-4.26 (br, 1H, 1αH), 4.45-4.54 (q, 2H, *J*=11.9 Hz, $C\underline{H}_2Ph$), 4.70-4.75 (m, 1αH), 4.95-5.07 (br, 1NH), 7.01 (br, 1NH), 7.23-7.36 (m, 5H, Ph).

5.2.6.4 MeO-Ser-D-Leu-NHBoc

Following the ***hydrogenation*** procedure: MeO-Ser-D-Leu-NHBoc was furnished by dissolving 1.24 g (2.94 mmol, 1.0 equivalent) of MeO-Ser(Bzl)-D-Leu-NHBoc in 29.4 mL EtOH. A catalytic amount of 10% Pd/C was added to the reaction and H_2 gas was purged through the flask at low atmospheric pressure and the solution stirred overnight. Upon completion, confirmed by LC/MS, the reaction was filtered over Celite® and concentrated *in vacuo*. Pure MeO-Ser-D-Leu-NHBoc was furnished as a clear oil (839 mg, 86% yield); the dipeptide was taken on without further purification or characterization. R_f: 0.44 (1:1 Hex:EA).

5.2.6.5 MeO-Ox-D-Leu-NHBoc

Following the ***oxazole synthesis*** procedure: MeO-Ox-D-Leu-NHBoc was synthesized by dissolving 819 mg (2.46 mmols, 1.0 equivalent) of MeO-Ser-D-Leu-NHBoc in 24.6 mL of DCM, under argon. The reaction mixture was allowed to cool in a -78 °C (dry ice/acetone) bath for 15 min. 357 µL (2.71 mmols, 1.1 equivalents) of DAST was added to the reaction drop-wise (0.1 mL/min) and continued to stir at -78 °C for 1 hr. 664 mg (4.92 mmols, 2.0 equivalents) of K_2CO_3 was added to the reaction mixture and continued to stir for an additional hour at -78 °C. Finally, the reaction was warmed to room temperature and stirred for an additional 1.5 hrs to yield the MeO-Oxazoline-D-Leu-NHBoc intermediate. The intermediate was diluted with 100 mL DCM and washed with saturated $NaHCO_3$ solution (200 mL x 2), then back-extracted with EA (100 mL x 2).

The organic layers were collected, dried over Na_2SO_4, filtered and concentrated *in vacuo*. 773 mg (2.46 mmol, 1.0 equivalent) of MeO-Oxazoline-D-Leu-NHBoc was oxidized into the desired product by using 746 µL of DBU (4.92 mmols, 2.0 equivalents), 489 µL of $BrCCl_3$ (4.92 mmols, 2.0 equivalents), in 12.3 mL of DCM. The crude reaction was purified via column chromatography on silica gel (Hex/EA 3:2) to afford the desired oxazole MeO-Ox-D-Leu-NHBoc (516 mg, 70% yield over 2 steps) as a clear oil. R_f: 0.80 (Hex/EA 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (d, 3H, *J*=1.6 Hz, CHC$\underline{H}_3$), 0.87 (d, 3H, *J*=1.8 Hz, CHC$\underline{H}_3$), 1.35 (s, 9H, C(C$\underline{H}_3$)$_3$), 1.53-1.61 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 1.63-1.71 (m, 2H, CHC$\underline{H}_2$CH), 3.83 (s, 3H, OC$\underline{H}_3$), 4.87-4.95 (br, 1αH), 5.08-5.14 (br, 1NH), 8.11 (s, 1H, CC$\underline{H}$O).

5.2.6.6 MeO-Ox-D-Leu-NH$_2$

Following the ***Boc removal*** procedure: MeO-Ox-D-Leu-NH_2 was synthesized by dissolving 516 mg (1.65 mmol, 1.0 equivalent) of MeO-Ox-D-Leu-NHBoc in 13.2 mL DCM, followed by adding 360 µL (3.30 mmol, 2.0 equivalents) of anisole and then 3.30 mL of TFA. Boc removal was complete in 45 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 (350 mg, quantitative yield) as a light brown oil.

5.2.6.7 MeO-Ox-D-Leu-D-Phe-(2R,3R)/(2S,3S)-β-OH-Leu-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Ox-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Leu-NHBoc (**SanA 17-III-Ox.LP**) was synthesized utilizing 596 mg (1.10 mmol, 1.0 equivalent) of acid HO-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHBoc, 257 mg (1.21 mmol, 1.1 equivalents) of amine MeO-Ox-D-Leu-NH_2, 424 mg (1.32 mmol, 0.6 equivalents) of TBTU, 301 mg (0.79 mmol, 0.6 equivalents) of HATU, 2.11 mL (12.1 mmol, 10.0 equivalents) of DIPEA dissolved in 12.1 mL of DCM, under argon. The reaction mixture stirred for 2.5 hrs and upon completion, the crude reaction

mixture was rotovapped down to dryness and redissolved in 200 mL EA. The crude reaction was washed with pH 1 HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed reaction underwent a final purification via column chromatography on silica gel (Hex/EA 1:9) to afford the desired peptide MeO-Oxazole-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Leu-NHBoc (490 mg, 61% yield) as a light yellow oil; the structure and purity of the macrocycle was confirmed via LC/MS and ^{1}H NMR. Rf: 0.40 (Hex/EA 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.76-0.81 (m, 9H, 3(CHC$\underline{H}_3$)), 0.84 (d, *J*=4.0 Hz, 3H, CHC$\underline{H}_3$), 0.89 (d, *J*=3.4 Hz, 6H, 2(CHC$\underline{H}_3$)), 0.91 (d, *J*=3.5 Hz, 3H, CHC$\underline{H}_3$), 1.38 -1.42 (m, 4H buried, 2(C$\underline{H}$(CH_3)$_2$)), 1.39 (s, 9H, C(C$\underline{H}_3$)$_3$), 1.63 (m, 2H, CHC$\underline{H}_2$CH), 1.74 (m, 2H, CHC$\underline{H}_2$CH), 2.75 (dd, *J*=13.9, 6.6 Hz, 1H, PhC$\underline{H}_a$H$_b$), 3.21 (dd, *J*=13.8, 7.7 Hz, 1H PhCH$_a$$\underline{H}_b$), 3.63 (s, 3H, OC$\underline{H}_3$), 4.53 (m, 1αH), 4.63 (m, 2αH), 4.80 (m, 1αH), 4.88 (m, 1αH), 4.99 (m, 2αH), 5.20 (q, *J*=7.8 Hz, 1H, C$\underline{H}$OH), 5.30 (q, *J*=7.9 Hz, 1H, C$\underline{H}$OH), 6.55 (br, 1NH), 6.64 (d, *J*=8.8 Hz, 1NH), 6.95 (m, 2NH), 7.01 (d, *J*=8.5 Hz, 1NH), 7.07 (d, *J*=6.1 Hz, 1NH), 7.12-7.18 (m, 6H, 4(CC$\underline{H}$CH), 2(CHC$\underline{H}$CH)), 7.22-7.34 (m, 10H, Ph), 7.40 (m, 4H, 4(CHC$\underline{H}$CH)), 7.60 (d, *J*=7.2 Hz, 2NH), 8.19 (s, 2H, 2CC$\underline{H}$O). LCMS (ESI): m/z called for $C_{39}H_{53}N_5O_9$ (M+) = 735.4, found 735.8.

5.2.6.8 HO-Ox-D-Leu-D-Phe-(2R,3R)/(2S,3S)-β-OH-Leu-NHBoc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-Ox-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Leu-NHBoc was deprotected by utilizing 100 mg (0.14 mmol, 1.0 equivalent) of MeO-Ox-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Leu-NHBoc, 17.0 mg (0.41 mmol, 3.0 equivalents) of LiOH•H_2O and 14.0 µL 30% H_2O_2 in 340 µL MeOH. The peptide was dissolved in methanol and then H_2O_2 and LiOH•H_2O were added to the reaction flask. The reaction was run for 12 hrs, upon completion, the

reaction was diluted with 50 mL DCM and quenched with sodium thiosulfate (154 mg) in pH 1 hydrochloric acid solution (200 mL). The organic layer was separated and the aqueous layer was back extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo* to yield the deprotected acid HO-Ox-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Leu-NHBoc (76.0 mg, 77% yield). The free acid was taken on without any further purification.

5.2.6.9 HO-Ox-D-Leu-D-Phe-(2R,3R)/(2S,3S)-β-OH-Leu-NH$_2$

Following the ***Boc removal*** procedure: HO-Ox-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Leu-NH_2 was synthesized by dissolving 76.0 mg (0.11 mmol, 1.0 equivalent) of : HO-Ox-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Leu-NHBoc in 842 µL DCM, followed by adding 23.0 µL (0.21 mmol, 2.0 equivalents) of anisole and then 211 µL of TFA. Boc removal was complete in 45 min; the reaction mixture was concentrated *in vacuo* with DCM (50 mL x 3) and taken on to the next reaction without further purification (65.0 mg, quantitative yield) as a dark brown oil. LCMS (ESI): *m/z* called for $C_{33}H_{43}N_5O_7$ ($M+2H^+$) = 623.7, found 623.8.

5.2.6.10 cyclo-(2R,3R)/(2S,3S)-β-OH-Phe-Leu-Ox-D-Leu-D-Phe

Following the ***syringe pump macrocyclization*** procedure: *cyclo*-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-Leu-Ox-D-Leu-D-Phe (**SanA 17-III-Ox.M**) was synthesized by dissolving 41.0 mg (0.13 mmol, 0.9 equivalents) of TBTU, 36.0 mg (94.7 µmol, 0.9 equivalents) of HATU in 4.51 mL of ACN and 6.77 mL of DCM. 147 µL of DIPEA (0.84 mmol, 8.0 equivalents) was added to the reaction flask. The remaining solvent to achieve a 0.007 M overall reaction concentration (1.50 mL ACN and 2.26 mL of DCM) was used to dissolve 65.0 mg (0.11 mmol, 1.0 equivalent) of HO-Ox-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Leu-NH_2. The dissolved peptide was added drop-wise (0.25 mL/min) to the reaction flask using a syringe pump and upon addition of all of the

peptide, the solution was stirred overnight. Upon completion, the crude reaction was diluted with 200 mL DCM and washed with saturated NH_4Cl solution (200 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed reaction underwent a final purification via reverse phase-HPLC to yield the macrocycle (2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-Leu-Ox-D-Leu-D-Phe (19.0 mg, 29% yield). The structure and purity of the macrocycle was confirmed via LC/MS. Rf: 0.20 (7:13 Hex:EA). LCMS: *m/z* called for $C_{33}H_{41}N_5O_6$ (M+1) = 604.7, found 603.9.

5.2.6.11 cyclo-(2R,3R)-β-benzoxy-Phe-Leu-Ox-D-Leu-D-Phe

Following the ***benzylation*** procedure: a mixture with 18.0 mg of macrocycle (2*R*,3*R*)/(2*S*,3*S*)-β-benzoxy-Phe-Leu-Ox-D-Leu-D-Phe (0.298 μmol, 1.0 equivalent), 1.50 mg NaH (0.596 μmol, 2.0 equivalent) and 14.0 μL benzyl bromide (0.0119 mmol, 4.0 equivalents) in 1.19 mL of THF stirred at room temperature for 12 hrs under an atmosphere of argon. Upon completion, confirmed by LC/MS, the crude reaction was concentrated *in vacuo* and purified by reverse phase-HPLC to furnish (2*R*,3*R*)/(2*S*,3*S*)-β-benzoxy-Phe-Leu-Ox-D-Leu-D-Phe in 24% yield. Stereochemistry was assigned previously; the structure and purity of the macrocycle was confirmed via LC/MS and ^{1}H NMR. Rf: 0.40 (7:13 Hex/EA); >90% pure by HPLC. ^{1}H NMR (600 MHz, CD_3OD): δ 0.79 (d, *J*=6.6 Hz, 3H, CHC$\underline{H}_3$), 0.82 (d, *J*=6.5 Hz, 3H, CHC$\underline{H}_3$), 0.90 (d, *J*=6.4 Hz, 3H, CHC$\underline{H}_3$), 1.05 (d, *J*=6.6 Hz, 3H, CHC$\underline{H}_3$), 1.27-1.34 (m, 2H, 2(C$\underline{H}$(CH_3)$_2$)), 1.57-1.63 (m, 4H, 2(C$\underline{H}_2$CH)), 3.19 (m, 1H, C$\underline{H}_a$H$_b$Ph), 3.43 (m, 1H, CH$_a$$\underline{H}_b$Ph), 3.75-3.80 (m, 2H, OC$\underline{H}_2$Ph), 4.34-4.38 (m, 2αH buried), 4.70 (d, *J*=5.3 Hz, 1αH), 4.83 (m, 1αH), 5.06 (d, *J*=5.2 Hz, 1H, PhC$\underline{H}$O), 6.81 (d, *J*=8.7 Hz, 1NH), 7.14-7.34 (m, 10H, 2Ph), 7.37-7.47 (m, 5H, Ph), 7.58 (d, *J*=4.3 Hz, 1NH), 7.59 (d, *J*=4.7 Hz, 1NH), 8.42 (s, 1H, CC$\underline{H}$O), 8.52 (m, 1NH), 8.77 (m, 1NH). LCMS: m/z called for $C_{40}H_{47}N_5O_6$ (M+1) = 694.8, found 694.4; HRMS (ESI-TOF): MH^+, found 694.3592, $C_{40}H_{47}N_5O_6$ requires 694.3599.

5.2.7 Experimental methods for SanA 17-II-Ox

5.2.7.1 Resin-O-D-Leu-D-Phe-NH_2

Prior to the first coupling, the D-Phe bound resin was swollen in 20 mL of DMF for 2 hrs. Solvent was removed and following the ***solution phase peptide coupling*** procedure: a mixture of 1.50 g Resin-O-D-Leu-NH_2 (1.10 mmol, 1.0 equivalent), 1.27 g HO-D-Phe-NHFmoc (3.29 mmol, 3.0 equivalents), 503 mg HOBt (3.29 mmol, 3.0 equivalents), and 1.02 mL of DIC (6.57 mmol, 6.0 equivalents) were shaken at room temperature for 3 hrs. Completion of the coupling reaction was verified by a negative ***ninhydrin test***. The reaction mixture was then drained to leave the amine-protected resin-bound dipeptide.

Following the ***Fmoc removal*** procedure: Resin-O-D-Leu-D-Phe-NH_2 was furnished by washes with 20% Piperidine/DMF. A positive ***ninhydrin test*** served to verify Fmoc removal and gave the title compound.

5.2.7.2 HO-D-Leu-D-Phe-(2R,3R)/(2S,3S)-β-OH-Phe-NHBoc

Following the ***solution phase peptide coupling*** procedure: a mixture of Resin-O-D-Leu-D-Phe-NH_2 (1.10 mmol, 1.0 equivalent), 923 mg HO-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHBoc residue (3.29 mmol, 3.0 equivalents), 503 mg HOBt (3.29 mmol, 3.0 equivalents), and 1.02 mL DIC (6.57 mmol, 6.0 equivalents) were shaken at room temperature for 3 hrs. Completion of the coupling reaction was verified by a negative ***ninhydrin test***.

The reaction mixture was then drained and dried *in vacuo* overnight to leave the Boc-protected resin-bound tripeptide. Following the ***cleaving the peptide from resin*** procedure: 2.07 g of dry resin bound tripeptide Resin-O-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHBoc was stirred with 10.3 mL each of TFE and DCM for 24 hrs. The slurry

was filtered, washed with additional DCM, and dried *in vacuo* for 24 hrs to yield HO-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHBoc (534 mg, 90% yield).

5.2.7.3 MeO-Ser(Bzl)-Val-N(Me)Boc

Following the ***solution phase peptide coupling*** procedure: MeO-Ser(Bzl)-Val-N(Me)Boc was synthesized utilizing 712 mg (3.09 mmol, 1.0 equivalent) of acid HO-Val-N(Me)Boc, 709 mg (3.39 mmol, 1.1 equivalent) of amine OMe-Ser(Bzl)-NH_2, 1.19 g (3.69 mmol, 1.2 equivalents) of TBTU, 5.16 mL (24.6 mmol, 8.0 equivalents) of DIPEA dissolved in 30.8 mL DCM, under argon. The reaction mixture stirred for 2.5 hrs and upon completion, the crude reaction mixture was rotovapped down to dryness and redissolved in 200 mL EA. The crude reaction was washed with pH 1 HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The dipeptide was further purified by column chromatography; pure MeO-Ser(Bzl)-Val-N(Me)Boc eluted at 4:1 (Hex:EA). The structure and purity was confirmed via LC/MS and ^{1}H NMR (1.22 g, 97% yield). Rf: 0.80 (1:1 Hex:EA). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.82 (d, *J*=6.7 Hz, 3H, CHC$\underline{H}_3$), 0.95 (d, *J*=6.1 Hz, 3H, CHC$\underline{H}_3$), 1.41 (s, 9H, C(C$\underline{H}_3$)$_3$), 2.19-2.30 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 2.74 (s, 3H, NC$\underline{H}_3$), 3.58-3.62 (dd, *J*=9.4, 3.6 Hz, 1H, CHC$\underline{H}_a$H$_b$O), 3.70 (s, 3H, OC$\underline{H}_3$), 4.10 (br, 1αH), 3.77-3.82 (d, *J* =7.7 Hz, 1H, CHCH$_a$$\underline{H}_b$O), 4.40-4.56 (q, *J*=13.0 Hz, 2H, C$\underline{H}_2$Ph), 4.67 (m, 1αH), 6.93 (br, 1NH), 7.20-7.30 (m, 5H, Ph).

5.2.7.4 MeO-Ser-Val-N(Me)Boc

Following the ***hydrogenation*** procedure: MeO-Ser-Val-N(Me)Boc was furnished by dissolving 1.22 g (3.01 mmol, 1.0 equivalent) of MeO-Ser(Bzl)-Val-N(Me)Boc in 30.1 mL EtOH. A catalytic amount of 10% Pd/C was added to the reaction and H_2 gas was purged through the flask at low atmospheric pressure and the solution stirred overnight.

Upon completion, confirmed by LC/MS, the reaction was filtered over Celite® and concentrated *in vacuo*. Pure MeO-Ser-Val-N(Me)Boc was furnished as a clear oil (723 mg, 75% yield); the dipeptide was taken on without further purification or characterization. R_f: 0.50 (1:1 Hex:EA).

5.2.7.5 MeO-Ox-Val-N(Me)Boc

Following the ***oxazole synthesis*** procedure: MeO-Ox-Val-N(Me)Boc was synthesized by dissolving 700 mg (2.11 mmols, 1.0 equivalent) of MeO-Ser-Val-N(Me)Boc in 21.1 mL of DCM, under argon. The reaction mixture was allowed to cool in a -78 °C (dry ice/acetone) bath for 15 min. 306 µL (2.32 mmols, 1.1 equivalents) of DAST was added to the reaction drop-wise (0.1 mL/min) and continued to stir at -78 °C for 1 hr. 569 mg (4.21 mmols, 2.0 equivalents) of K_2CO_3 was added to the reaction mixture and continued to stir for an additional hour at -78 °C. Finally, the reaction was warmed to room temperature and stirred for an additional 1.5 hrs to yield the MeO-Oxazoline-Val-N(Me)Boc intermediate. The intermediate was diluted with 100 mL DCM and washed with saturated $NaHCO_3$ solution (200 mL x 2), then back-extracted with EA (100 mL x 2). The organic layers were collected, dried over Na_2SO_4, filtered and concentrated *in vacuo*. 773 mg (2.46 mmol, 1.0 equivalent) of MeO-Oxazoline-D-Leu-NHBoc was oxidized into the desired product by using 420 µL of DBU (4.21 mmols, 2.0 equivalents), 421 µL of $BrCCl_3$ (4.21 mmols, 2.0 equivalents), in 21.1 mL of DCM. The crude reaction was purified via column chromatography on silica gel (Hex/EA 3:2) to afford the desired oxazole MeO-Ox-D-Leu-NHBoc (494 mg, 75% yield over 2 steps) as a clear oil. R_f: 0.82 (Hex/EA 1:1). ^{1}H NMR (400 MHz, CDCl3): d 0.87 (d, *J*=6.7 Hz, 3H, CHC$\underline{H}_3$), 0.93 (d, *J*=5.6 Hz, 3H, CHC$\underline{H}_3$), 1.43 (s, 9H, C(C$\underline{H}_3$)$_3$)), 2.41-2.52 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 2.76 (s, 3H, NC$\underline{H}_3$), 3.84 (s, 3H, OC$\underline{H}_3$), 4.86 (d, *J*=10.5 Hz, 1αH), 5.12 (d, *J*=10.9 Hz, 1NH), 8.19 (s, 1H, CC$\underline{H}$O).

5.2.7.6 MeO-Ox-Val-N(Me)H

Following the ***Boc removal*** procedure: MeO-Ox-Val-N(Me)H was synthesized by dissolving 494 mg (1.58 mmol, 1.0 equivalent) of MeO-Ox-Val-N(Me)Boc in 12.6 mL DCM, followed by adding 345 µL (3.16 mmol, 2.0 equivalents) of anisole and then 3.16 mL of TFA. Boc removal was complete in 45 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 (335 mg, quantitative yield) as a light brown oil.

5.2.7.7 MeO-Ox-Val-N(Me)-D-Leu-D-Phe-(2R,3R)/(2S,3S)-β-OH-Phe-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Ox-Val-N(Me)-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHBoc (**SanA 17-II-Ox.LP**) was synthesized utilizing 534 mg (0.99 mmol, 1.0 equivalent) of acid HO-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHBoc, 230 mg (1.09 mmol, 1.1 equivalents) of amine MeO-Ox-Val-N(Me)H, 189 mg (0.59 mmol, 0.6 equivalents) of TBTU, 375 mg (0.99 mmol, 1.0 equivalent) of HATU, 1.38 mL (7.89 mmol, 8.0 equivalents) of DIPEA dissolved in 9.86 mL of ACN, under argon. The reaction mixture stirred for 1.5 hrs and upon completion, the crude reaction mixture was rotovapped down to dryness and redissolved in 200 mL EA. The crude reaction was washed with pH 1 HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed reaction underwent a final purification via column chromatography on silica gel (Hex/EA 1:9) to afford the desired peptide MeO-Ox-Val-N(Me)-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHBoc (158 mg, 22% yield) as a light yellow oil; the structure and purity of the macrocycle was confirmed via LC/MS and ^{1}H NMR. Rf: 0.30 (Hex/EA 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.76 (d, *J*=2.7 Hz, 6H, $CH(C\underline{H}_3)_2$), 0.78 (d, *J*=1.7 Hz, 6H, $CH(C\underline{H}_3)_2$), 0.81-0.79 (d, *J*=3.5 Hz, 6H, $CH(C\underline{H}_3)_2$), 0.87-0.83 (m, 6H, $2(CH(C\underline{H}_3)_2)$), 1.14 (s, 9H, $C(C\underline{H}_3)_3$), 1.22 (s,

9H, C(C<u>H</u>$_3$)$_3$), 1.32-1.44 (br, 2H, C<u>H</u>(CH$_3$)$_2$), 2.32-2.44 (m, 4H, 2(CHC<u>H</u>$_2$CH)), 2.73 (m, 2H, 2(C<u>H</u>(CH$_3$)$_2$)), 2.84-2.90 (m, 6H, 2NC<u>H</u>$_3$), 3.78 (s, 3H, OC<u>H</u>$_3$), 3.79 (s, 3H, OC<u>H</u>$_3$), 3.82 (br, 4H, 2(C<u>H</u>$_2$Ph)), 4.09-4.21 (m, 2αH), 4.44-4.52 (q, *J*=6.4 Hz, 1αH), 4.51-4.65 (m, 1αH), 4.78 (br, 2αH), 4.86 (m, 1αH), 4.90 (m, 1αH), 5.32 (d, *J*=8.6 Hz, 1H, C<u>H</u>OH), 5.42 (t, *J*=12.3 Hz, 1H, C<u>H</u>OH), 6.00 (d, *J*=6.7 Hz, 2NH), 6.59 (d, *J*=8.4 Hz, 2NH), 6.83-6.87 (m, 2NH), 7.02-7.32 (m, 20H, 4Ph), 8.05 (s, 1H, CC<u>H</u>O), 8.10 (s, 1H, CC<u>H</u>O).

5.2.7.8 HO-Ox-Val-N(Me)-D-Leu-D-Phe-(2R,3R)/(2S,3S)-β-OH-Phe-NHBoc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-Ox-Val-N(Me)-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHBoc was deprotected by utilizing 158 mg (0.21 mmol, 1.0 equivalent) of MeO-Ox-Val-N(Me)-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHBoc, 18.0 mg (0.43 mmol, 2.0 equivalents) of LiOH•H_2O and 70.0 µL 30% H_2O_2 in 403 µL MeOH and 134 µL of water. The peptide was dissolved in methanol and then water, H_2O_2 and LiOH•H_2O were added to the reaction flask. The reaction was run for 12 hrs, upon completion, the reaction was diluted with 50 mL DCM and quenched with sodium thiosulfate (175 mg) in pH 1 hydrochloric acid solution (200 mL). The organic layer was separated and the aqueous layer was back extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo* to yield the deprotected acid HO-Ox-Val-N(Me)-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHBoc (155 mg, quantitative yield). The free acid was taken on without any further purification.

5.2.7.9 HO-Ox-Val-N(Me)-D-Leu-D-Phe-(2R,3R)/(2S,3S)-β-OH-Phe-NH$_2$

Following the ***Boc removal*** procedure: HO-Ox-Val-N(Me)-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NH_2 was synthesized by dissolving 155 mg (0.22 mmol, 1.0 equivalent) of HO-Ox-Val-N(Me)-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHBoc in 1.61 mL DCM, followed by adding 50.0 µL (0.43 mmol, 2.0 equivalents) of anisole and

then 537 µL of TFA. Boc removal was complete in 45 min; the reaction mixture was concentrated *in vacuo* with DCM (200 mL x 3) and taken on to the next reaction without further purification (134 mg, quantitative yield) as a dark brown oil. LCMS (ESI): *m/z* called for $C_{33}H_{43}N_5O_7$ ($M+H^+$) = 621.7, found 621.8.

5.2.7.10 cyclo-(2R,3R)/(2S,3S)-β-OH-Phe-Ox-Val-N(Me)-D-Leu-D-Phe

Following the ***syringe pump macrocyclization*** procedure: *cyclo*-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-Ox-Val-N(Me)-D-Leu-D-Phe (**SanA 17-II-Ox.M**) was synthesized by dissolving 69.0 mg (0.22 mmol, 1.0 equivalent) of TBTU, 82.0 mg (0.22 mmol, 1.0 equivalent) of HATU in 7.68 mL each of ACN and DCM. 375 µL of DIPEA (2.15 mmol, 10.0 equivalents) was added to the reaction flask. The remaining solvent to achieve a 0.007 M overall reaction concentration (7.68 mL each of ACN and DCM) was used to dissolve 134 mg (0.22 mmol, 1.0 equivalent) of HO-Ox-Val-N(Me)-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NH_2. The dissolved peptide was added drop-wise (0.25 mL/min) to the reaction flask using a syringe pump and upon addition of all of the peptide, the solution was stirred overnight. Upon completion, the crude reaction was diluted with 500 mL EA and washed with 10% HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed reaction underwent a final purification via reverse phase-HPLC to yield the macrocycle *cyclo*-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-Ox-Val-N(Me)-D-Leu-D-Phe (29.0 mg, 23% yield). The structure and purity of the macrocycle was confirmed via LC/MS. Rf: 0.20 (7:13 Hex:EA). LCMS: *m/z* called for $C_{33}H_{41}N_5O_6$ (M+1) = 604.7, found 603.9.

5.2.7.11 cyclo-(2R,3R)-β-benzoxy-Phe-Ox-Val-N(Me)-D-Leu-D-Phe

Following the ***benzylation*** procedure: a mixture with 18.0 mg of macrocycle *cyclo*-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-Ox-Val-N(Me)-D-Leu-D-Phe (0.298 µmol, 1.0

equivalent), 1.50 mg NaH (0.596 μmol, 2.0 equivalent) and 14.0 μL benzyl bromide (0.0119 mmol, 4.0 equivalents) in 1.19 mL of THF stirred at room temperature for 12 hrs under an atmosphere of argon. Upon completion, confirmed by LC/MS, the crude reaction was concentrated *in vacuo* and purified by reverse phase-HPLC to furnish (2*R*,3*R*)/(2*S*,3*S*)-β-benzoxy-Phe-Leu-Ox-D-Leu-D-Phe in 28% yield. Stereochemistry was assigned previously; the structure and purity of the macrocycle was confirmed via LC/MS and ^{1}H NMR. Rf: 0.40 (7:13 Hex/EA); >90% pure by HPLC. ^{1}H NMR (600 MHz, CD_3OD): δ 0.79 (d, *J*=6.4 Hz, 3H, CHC$\underline{H}_3$), 0.82 (d, *J*=6.4 Hz, 3H, CHC$\underline{H}_3$), 1.04 (d, *J*=6.8 Hz, 3H, $CHCH_3$), 1.37 (d, *J*=7.3 Hz, 3H, $CHCH_3$), 1.49 (m, 1H, C$\underline{H}(CH_3)_2$), 1.93 (m, 2H, C$\underline{H}_2$CH), 2.92 (m, 1H, C$\underline{H}(CH_3)_2$), 3.18 (m, 1H, C$\underline{H}_a$H$_b$Ph), 3.32 (s, 3H, NC$\underline{H}_3$), 3.42 (m, 1H, CH$_a\underline{H}_b$Ph), 3.74 (m, 2H, OC$\underline{H}_2$Ph), 4.69 (d, *J*=5.6 Hz, 1H, OC$\underline{H}$), 4.90 (m, 1αH), 5.04 (m, 1αH), 6.64 (m, 1αH), 6.81 (m, 1αH), 7.12-7.20 (m, 5H, Ph), 7.23-7.31 (m, 10H, 2Ph), 7.43 (d, *J*=8.8 Hz, 1NH), 7.71 (d, *J*=8.8 Hz, 1NH), 8.41 (s, 1H, CC$\underline{H}$O), 8.70 (d, *J*=10.3 Hz, 1NH). LCMS: m/z called for $C_{40}H_{47}N_5O_6$ (M+1) = 694.8, found 694.0; HRMS (ESI-TOF): MH^+, found 694.3570, $C_{40}H_{47}N_5O_6$ requires 694.3599.

5.3 FR235222 and Apicidin based HDACI derivatives

5.3.1 Experimental methods for HDACI 3

5.3.1.1 MeO-Phe-Abu-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-Abu-NHBoc was synthesized utilizing 577 mg (2.84 mmol, 1.0 equivalent) of acid HO-Abu-NHBoc, 674 mg (3.12 mmol, 1.1 equivalent) of amine OMe-Phe-NH_2, 1.09 g (3.41 mmol, 1.2 equivalents) of TBTU, 1.98 mL (11.4 mmol, 4.0 equivalents) of DIPEA dissolved in 28.4 mL DCM, under argon. The reaction mixture stirred for 1 hr and upon completion, the crude reaction mixture was rotovapped down to dryness and redissolved in 200 mL

EA. The crude reaction was washed with pH 1 HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The pure desired peptide OMe-Phe-Abu-NHBoc was afforded as a white solid (960 mg, 93% yield); R_f: 0.67 (Hex/EA 1:1). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.92 (t, *J*=7.4 Hz, 3H, $CH_2C\underline{H}_3$), 1.45 (s, 9H, $C(C\underline{H}_3)_3$), 1.69-1.73 (m, 1H, $CH_2C\underline{H}(CH_3)_2$), 1.74-1.82 (br, 2H, $C\underline{H}_2CH_3$), 3.10 (m, 1H, $C\underline{H}_aH_bPh$), 3.73 (s, 3H, $OC\underline{H}_3$), 3.96-4.07 (br, 1H, $CH_a\underline{H}_bPh$), 4.83-4.93 (m, 1αH), 4.95-5.06 (br, 1αH), 6.48 (d, *J*=7.3 Hz, 1NH), 7.08-7.14 (m, 2H, Ph), 7.25-7.31 (m, 3H, Ph).

5.3.1.2 HO-Phe-Abu-NHBoc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-Phe-Abu-NHBoc was deprotected by utilizing 911 mg (2.50 mmol, 1.0 equivalent) of MeO-Phe-Abu-NHBoc and 840 mg (20.0 mmol, 8.0 equivalents) of $LiOH•H_2O$ in 25.0 mL MeOH. The peptide was dissolved in methanol and $LiOH•H_2O$ was added to the reaction flask and the reaction was run for 1.5 hrs. Upon completion, the reaction was diluted with 250 mL DCM. The aqueous layer was back-extracted with EA (100 mL x 2), and the combined organic layers were dried, filtered and concentrated *in vacuo* (830 mg, 95% yield) as a white solid. The free acid HO-Phe-Abu-NHBoc (**HDACI 3.A**) was taken on without any further purification or characterization.

5.3.1.3 MeO-Arg(2Cbz)-D-Pip-NBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Arg(2Cbz)-D-Pip-NHBoc was synthesized utilizing 767 mg (3.35 mmol, 1.0 equivalent) of acid HO-D-Pip-NHBoc, 1.68 g (3.68 mmol, 1.1 equivalent) of amine OMe-Arg(2Cbz)-NH_2, 1.29 g (4.01 mmol, 1.2 equivalents) of TBTU, 1.75 mL (10.0 mmol, 3.0 equivalents) of DIPEA dissolved in 33.5 mL DCM, under argon. The reaction mixture stirred for 2 hrs and upon completion, the crude reaction mixture was diluted with 200 mL DCM and washed with

saturated NH_4Cl aqueous solution (200 mL x 2). The organic layers were collected and dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed dipeptide was further purified via column chromatography and the pure peptide OMe-Arg(2Cbz)-D-Pip-NHBoc eluted in 13:7 (Hex:EA) as a white solid (1.97 g, 86% yield); Rf: 0.65 (Hex/EA 1:1). ^1H NMR (200 MHz, $CDCl_3$): δ 1.47 (s, 9H, $C(C\underline{H}_3)_3$), 1.58-1.60 (m, 2H, $CH_2C\underline{H}_2CH_2NH$), 1.63-1.72 (m, 6H buried, $CH_2C\underline{H}_2CH_2CH_2$),1.80-1.89 (m, 2H, $CHC\underline{H}_2CH_2$), 2.23-2.28 (br, 1NH), 2.88 (t, *J*=12.4, 2H, $C\underline{H}_2NHC$), 3.64 (s, 3H, $OC\underline{H}_3$), 3.97-4.07 (m, 2H, $NC\underline{H}_2CH_2$), 4.73-4.84 (br, 1αH), 4.57-4.63 (m, 1αH), 5.15 (s, 2H, $OC\underline{H}_2Ph$), 5.26 (s, 2H, $OC\underline{H}_2Ph$), 7.32-7.43 (m, 10H, 2Ph).

5.3.1.4 MeO-Arg(2Cbz)-D-Pip-NH

Following the ***Boc removal*** procedure: OMe-Arg(2Cbz)-D-Pip-NH was synthesized by dissolving 986 mg (1.48 mmol, 1.0 equivalent) of: OMe-Arg(2Cbz)-D-Pip-NHBoc in 11.8 mL DCM, followed by adding 320 µL (2.93 mmol, 2.0 equivalents) of anisole and then 2.95 mL of TFA. Boc removal was complete in 45 min; the reaction mixture was concentrated *in vacuo* with DCM (500 mL x 3) and taken on to the next reaction without further purification (838 mg, quantitative yield) as a dark brown oil.

5.3.1.5 MeO-Arg(2Cbz)-D-Pip-Phe-Abu-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Arg(2Cbz)-D-Pip-Phe-Abu-NHBoc was synthesized utilizing 235 mg (0.67 mmol, 1.0 equivalent) of acid HO-Phe-Abu-NHBoc, 419 mg (0.74 mmol, 1.1 equivalent) of amine OMe-Arg(2Cbz)-D-Pip-NH, 259 mg (0.81 mmol, 1.2 equivalents) of TBTU, 191 mg (0.50 mmol, 0.75 equivalents), 468 µL (2.68 mmol, 4.0 equivalents) of DIPEA dissolved in 6.71 mL DCM, under argon. The reaction mixture stirred for 3 hrs and upon completion, the crude reaction mixture was diluted with 50 mL DCM and washed with saturated NH_4Cl aqueous solution (100 mL x 2). The organic layers were collected and dried over

Na_2SO_4, filtered and concentrated *in vacuo*. The washed dipeptide was further purified via column chromatography and the pure peptide OMe-Arg(2Cbz)-D-Pip-Phe-Abu-NHBoc eluted in 1:1 (Hex:EA) as a white solid (294 mg, 48% yield); Rf: 0.50 (Hex/EA 1:1). ^{1}H NMR (500 MHz, $CDCl_3$): 0.92-0.99 (m, 3H, $CH_2C\underline{H}_3$), 1.43 (s, 9H, $C(C\underline{H}_3)_3$), 1.58-1.60 (m, 2H, $CH_2C\underline{H}_2CH_2NH$), 1.53-1.62 (m, 6H buried, $CH_2C\underline{H}_2CH_2CH_2$), 1.74-1.82 (m, 2H, $C\underline{H}_2CH_3$), 1.83-1.89 (br, 2H, $CHC\underline{H}_2CH_2$), 2.60 (m, 2H, $C\underline{H}_2NHC$), 3.19-3.24 (m, 2H, $NC\underline{H}_2CH_2$), 3.63 (s, 3H, $OC\underline{H}_3$), 3.86-3.98 (br, 1αH), 3.99-4.08 (br, 1αH), 4.38-4.47 (m, 2αH buried), 5.17 (d, *J*=6.5 Hz, 2H, $OC\underline{H}_2Ph$), 5.23 (d, *J*=6.3 Hz, 2H, $OC\underline{H}_2Ph$), 7.19 (d, *J*=7.7 Hz, 1NH), 7.24 (d, *J*=7.1 Hz, 1NH), 7.27 (d, *J*=8.1 Hz, 1NH), 7.28-7.44 (m, 15H, 3Ph). LCMS: *m/z* called for $C_{47}H_{61}N_7O_{11}$ (M+1) = 900.0, found 900.4.

5.3.1.6 HO-Arg(2Cbz)-D-Pip-Phe-Abu-NH$_2$

Following the ***in situ deprotection*** procedure: HO-Arg(2Cbz)-D-Pip-Phe-Abu-NH_2 (**HDACI 3.DDLP**) was synthesized by dissolving 147 mg (0.16 mmol, 1.0 equivalent) of MeO-Arg(2Cbz)-D-Pip-Phe-Abu-NHBoc in 1.6 mL THF, followed by adding 36.0 µL (0.33 mmol, 2.0 equivalents) of anisole and then 8 drops of 12 N HCl. The reaction was monitored by LC/MS and 3 additional drops of 12 N HCl were added on days 2 and 3. By day 3, the C- and *N*-termini were fully deprotected; the reaction mixture was concentrated *in vacuo* with DCM (150 mL x 3) and taken on to the next reaction without further purification or characterization (129 mg, quantitative yield) as a dark brown oil. LCMS: *m/z* called for $C_{41}H_{51}N_7O_9$ (M+1) = 786.9, found 788.5.

5.3.1.7 cyclo-Phe-Abu-Arg(2Cbz)-D-Pip

Following the ***macrocyclization*** procedure: *cyclo*-Phe-Abu-Arg(2Cbz)-D-Pip (**HDACI 3.M**) was synthesized by dissolving 129 mg (0.16 mmol, 1.0 equivalent) of HO-Arg(2Cbz)-D-Pip-Phe-Abu-NH_2, 36.7 mg (0.11 mmol, 0.7 equivalents) of TBTU, 43.5 mg (0.11 mmol, 0.7 equivalents) of HATU and 34.2 mg (0.11 mmol, 0.7 equivalents) of

DEPBT in 4.66 mL of ACN and 9.32 mL each of DCM and THF. 171 µL of DIPEA (0.98 mmol, 6.0 equivalents) was added to the reaction flask and the solution stirred for 3.5 hrs. Upon completion, the crude reaction the crude reaction mixture was diluted with 50 mL DCM and washed with saturated NH_4Cl aqueous solution (100 mL x 2). The organic layers were collected and dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed macrocycle was further purified via column chromatography and the pure protected macrocycle *cyclo*-Phe-Abu-Arg(2Cbz)-D-Pip (**HDACI 3.M**) eluted at 1:3 Hex:EA and was furnished in 43% yield; the structure and purity of the macrocycle was confirmed via LC/MS and ^{1}H NMR. R_f: 0.40 (100% EA). LCMS: *m/z* called for $C_{41}H_{49}N_7O_8$ (M+1) = 767.4, found 767.2

5.3.1.8 cyclo-Phe-Abu-Arg-D-Pip

Following the ***hydrogenation*** procedure: *cyclo*-Phe-Abu-Arg-D-Pip (**HDACI 3**) was furnished by dissolving 26.0 mg (33.9 µmol, 1.0 equivalent) of *cyclo*-Phe-Abu-Arg(2Cbz)-D-Pip in 3.39 mL EtOH. A catalytic amount of 10% Pd/C was added to the reaction and H_2 gas was purged through the flask at low atmospheric pressure and the solution stirred overnight. Upon completion, confirmed by LC/MS, the reaction was concentrated *in vacuo* and the deprotected macrocycle underwent a final purification via column chromatography on silica gel to afford the desired product. Pure *cyclo*-Phe-Abu-Arg-D-Pip (**HDACI 3**) eluted at 100% EA and was furnished in 30% yield; the structure and purity of the macrocycle was confirmed via LC/MS and ^{1}H NMR. Rf: 0.30 (100% EA). A final purification via RP-HPLC was performed prior to biological evaluation. ^{1}H NMR (400 MHz, CD_3OD): δ 0.90-1.00 (t, *J*=6.8 Hz, 3H, $CH_2C\underline{H}_3$), 1.21-1.36 (m, 4H buried, $C\underline{H}_2CH_3$ and $CH_2C\underline{H}_2CH_2NH$), 1.53-1.60 (m, 6H buried, $CH_2C\underline{H}_2CH_2CH_2$), 1.75-1.81 (m, 2H, $CHC\underline{H}_2CH_2$), 1.97 (m, 1NH), 2.14-2.21 (m, 2H, $C\underline{H}_2NHC$), 3.04-3.09 (m, 2H, $C\underline{H}_2Ph$), 3.26-3.38 (m, 2H, $CH_2CH_2C\underline{H}_2NH$), 3.56-3.60 (m, 2αH buried), 3.61-3.65 (m,

1αH), 3.61-3.65 (m, 1αH), 4.53 (m, 1αH), 5.77 (dd, *J*=10.4, 6.3 Hz, 1NH), 7.19 (d, *J*=7.9 Hz, 1NH), 7.21 (d, *J*=7.5 Hz, 1NH), 7.23-7.35 (m, 5H, Ph). LCMS: *m/z* called for $C_{25}H_{37}N_7O_4$ ($M+Na^+$) = 523.6, found 525.3.

5.3.2 Experimental methods for HDACI 4

5.3.2.1 MeO-D-Phe-Abu-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-D-Phe-Abu-NHBoc was synthesized utilizing 500 mg (2.46 mmol, 1.0 equivalent) of acid HO-Abu-NHBoc, 584 mg (2.71 mmol, 1.1 equivalent) of amine OMe-D-Phe-NH_2, 948 mg (2.95 mmol, 1.2 equivalents) of TBTU, 1.29 mL (7.38 mmol, 3.0 equivalents) of DIPEA dissolved in 24.6 mL DCM, under argon. The reaction mixture stirred for 1 hr and upon completion, the crude reaction mixture was diluted with 200 mL DCM and washed with saturated NH_4Cl aqueous solution (200 mL x 2). The organic layers were collected and dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed dipeptide was further purified via column chromatography and the pure peptide OMe-Arg(2Cbz)-D-Pip-NHBoc eluted in 13:7 (Hex:EA) as a white solid (782 mg, 87% yield); Rf: 0.65 (Hex/EA 1:1). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (t, *J*=7.5 Hz, 3H, $CH_2C\underline{H}_3$), 1.45 (s, 9H, $C(C\underline{H}_3)_3$), 1.57 (q, *J*=7.5 Hz, 1H, $C\underline{H}_aH_bCH_3$), 1.83 (q, *J*=6.7 Hz, 1H, $CH_a\underline{H}_bCH_3$), 3.08-3.15 (m, $C\underline{H}_2Ph$), 3.73 (s, 3H, $OC\underline{H}_3$), 3.98-4.10 (br, 1αH), 4.82-4.95 (m, 1αH), 6.51 (d, *J*=7.9, 1NH), 7.08-7.15 (m, 2H, Ph), 7.23-7.31 (m, 3H, Ph).

5.3.2.2 HO-D-Phe-Abu-NHBoc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-D-Phe-Abu-NHBoc was deprotected by utilizing 782 mg (2.15 mmol, 1.0 equivalent) of MeO-D-Phe-Abu-NHBoc and 361 mg (8.60 mmol, 4.0 equivalents) of $LiOH•H_2O$ in 21.5 mL MeOH. The peptide was dissolved in methanol and $LiOH•H_2O$ was added to the

reaction flask and the reaction was run for 3 hrs. Upon completion, the reaction was diluted with 250 mL DCM. The aqueous layer was back-extracted with EA (100 mL x 2), and the combined organic layers were dried, filtered and concentrated *in vacuo* (662 mg, 88% yield) as a white solid. The free acid HO-D-Phe-Abu-NHBoc (**HDACI 4.A**) was taken on without any further purification or characterization

5.3.2.3 MeO-Arg(2Cbz)-D-Pip-NBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Arg(2Cbz)-D-Pip-NHBoc was synthesized utilizing 767 mg (3.35 mmol, 1.0 equivalent) of acid HO-D-Pip-NHBoc, 1.68 g (3.68 mmol, 1.1 equivalent) of amine OMe-Arg(2Cbz)-NH_2, 1.29 g (4.01 mmol, 1.2 equivalents) of TBTU, 1.75 mL (10.0 mmol, 3.0 equivalents) of DIPEA dissolved in 33.5 mL DCM, under argon. The reaction mixture stirred for 2 hrs and upon completion, the crude reaction mixture was diluted with 200 mL DCM and washed with saturated NH_4Cl aqueous solution (200 mL x 2). The organic layers were collected and dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed dipeptide was further purified via column chromatography and the pure peptide OMe-Arg(2Cbz)-D-Pip-NHBoc eluted in 13:7 (Hex:EA) as a white solid (1.97 g, 86% yield); Rf: 0.65 (Hex/EA 1:1). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.47 (s, 9H, $C(C\underline{H}_3)_3$), 1.58-1.60 (m, 2H, $CH_2C\underline{H}_2CH_2NH$), 1.63-1.72 (m, 6H buried, $CH_2C\underline{H}_2CH_2CH_2$),1.80-1.89 (m, 2H, $CHC\underline{H}_2CH_2$), 2.23-2.28 (br, 1NH), 2.88 (t, *J*=12.4, 2H, $C\underline{H}_2NHC$), 3.64 (s, 3H, $OC\underline{H}_3$), 3.97-4.07 (m, 2H, $NC\underline{H}_2CH_2$), 4.73-4.84 (br, 1αH), 4.57-4.63 (m, 1αH), 5.15 (s, 2H, $OC\underline{H}_2Ph$), 5.26 (s, 2H, $OC\underline{H}_2Ph$), 7.32-7.43 (m, 10H, 2Ph).

5.3.2.4 MeO-Arg(2Cbz)-D-Pip-NH

Following the ***Boc removal*** procedure: OMe-Arg(2Cbz)-D-Pip-NH was synthesized by dissolving 986 mg (1.48 mmol, 1.0 equivalent) of: OMe-Arg(2Cbz)-D-Pip-NHBoc in 11.8 mL DCM, followed by adding 320 µL (2.93 mmol, 2.0 equivalents) of

anisole and then 2.95 mL of TFA. Boc removal was complete in 45 min; the reaction mixture was concentrated *in vacuo* with DCM (500 mL x 3) and taken on to the next reaction without further purification (838 mg, quantitative yield) as a dark brown oil.

5.3.2.5 MeO-Arg(2Cbz)-D-Pip-D-Phe-Abu-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Arg(2Cbz)-D-Pip-D-Phe-Abu-NHBoc (**HDACI 4.LT**) was synthesized utilizing 235 mg (0.67 mmol, 1.0 equivalent) of acid HO-D-Phe-Abu-NHBoc, 419 mg (0.74 mmol, 1.1 equivalent) of amine OMe-Arg(2Cbz)-D-Pip-NH, 259 mg (0.81 mmol, 1.2 equivalents) of TBTU, 191 mg (0.50 mmol, 0.75 equivalents) of HATU, and 468 µL (2.68 mmol, 4.0 equivalents) of DIPEA dissolved in 6.71 mL DCM, under argon. The reaction mixture stirred for 3 hrs and upon completion, the crude reaction mixture was diluted with 50 mL DCM and washed with saturated NH_4Cl aqueous solution (100 mL x 2). The organic layers were collected and dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed peptide was further purified via column chromatography and the pure linear tetrapeptide OMe-Arg(2Cbz)-D-Pip-D-Phe-Abu-NHBoc eluted in 1:1 (Hex:EA) as a white solid (260 mg, 43% yield); Rf: 0.50 (Hex/EA 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.82 (d, *J*=7.2 Hz, 3H, $CH_2C\underline{H}_3$), 1.45 (s, 9H, $C(C\underline{H}_3)_3$), 1.57-1.67 (m, 2H, $CHCH_2C\underline{H}_2CH_2NH$), 1.69-1.80 (m, 2H, $CHC\underline{H}_2CH_3$), 1.81-1.87 (m, 1H, $CHC\underline{H}_aH_bCH_2$), 1.88-1.98 (m, 1H, $CHCH_a\underline{H}_bCH_2$), 2.11-2.17 (m, 2H, $CHCH_2C\underline{H}_2$), 2.84-2.89 (m, 2H, $NCH_2C\underline{H}_2$), 2.99 (dd, *J*=14.8, 9.7 Hz, 2H, $NC\underline{H}_2CH_2$), 3.08-3.15 (m, 2H, $CHC\underline{H}_2CH_2$), 3.20-3.26 (m, 2H, $C\underline{H}_2NHC$), 3.62 (s, 3H, $OC\underline{H}_3$), 3.76-3.80 (m, 2H, $CHC\underline{H}_2Ph$), 3.96-4.06 (m, 2H, $HNC\underline{H}_2CH_2$), 4.45-4.51 (m, 1αH), 4.53-4.60 (m, 1αH), 4.80-4.90 (br, 1αH), 5.02-5.05 (br, 1αH), 5.10 (s, 1H, $OC\underline{H}_aH_bPh$), 5.14 (s, 1H, $OCH_a\underline{H}_bPh$), 5.19 (s, 1H, $OC\underline{H}_aH_bPh$), 5.26 (s, 1H, $OCH_a\underline{H}_bPh$), 6.49 (d, *J*=8.1 Hz, 1NH), 6.77 (d, *J*=7.9 Hz, 1NH), 7.02 (br, 1NH), 7.16-7.21 (m, 2H, Ph), 7.24-7.30 (m, 3H, Ph),

7.32-7.43 (m, 10H, 2Ph), 7.72 (br, 1NH), 8.26 (d, *J*=7.7 Hz, 1NH). LCMS: *m/z* called for $C_{47}H_{61}N_7O_{11}$ (M+1) = 900.0, found 900.4.

5.3.2.6 HO-Arg(2Cbz)-D-Pip-D-Phe-Abu-NH₂

Following the ***in situ deprotection*** procedure: tetrapeptide HO-Arg(2Cbz)-D-Pip-D-Phe-Abu-NH_2 (**HDACI 4.DDLP**) was synthesized by dissolving 260 mg (0.29 mmol, 1.0 equivalent) of tetrapeptide OMe-Arg(2Cbz)-D-Pip-D-Phe-Abu-NHBoc in 2.9 mL THF, followed by adding 63.0 µL (0.58 mmol, 2.0 equivalents) of anisole and then 8 drops of 12 N HCl. The reaction was monitored by LC/MS and 3 additional drops of 12 N HCl were added on days 2 and 3. By day 3, the C- and *N*-termini were fully deprotected; 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 (228 mg, quantitative yield) as a dark brown oil. LCMS: *m/z* called for $C_{41}H_{51}N_7O_9$ (M+1) = 786.9, found 788.8.

5.3.2.7 cyclo-D-Phe-Abu-Arg(2Cbz)-D-Pip

Following the ***macrocyclization*** procedure: *cyclo*-D-Phe-Abu-Arg(2Cbz)-D-Pip (**HDACI 4.M**) was synthesized by dissolving 228 mg (0.29 mmol, 1.0 equivalent) of HO-Arg(2Cbz)-D-Pip-D-Phe-Abu-NH_2, 65.0 mg (0.20 mmol, 0.7 equivalents) of TBTU, 76.9 mg (0.20 mmol, 0.7 equivalents) of HATU and 60.5 mg (0.20 mmol, 0.7 equivalents) of DEPBT in 16.5 mL each of THF and DCM and 8.26 mL of ACN. 303 µL of DIPEA (1.74 mmol, 6.0 equivalents) was added to the reaction flask and the solution stirred for 3.0 hrs. Upon completion, the crude reaction mixture was diluted with 100 mL DCM and washed with saturated NH_4Cl aqueous solution (100 mL x 2). The organic layers were collected and dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed peptide was further purified via column chromatography and the pure protected cyclized macrocycle *cyclo*-D-Phe-Abu-Arg(2Cbz)-D-Pip (**HDACI 4.M**) eluted in 1:3 (Hex:EA) in 30% yield; the structure and purity of the macrocycle was confirmed via LC/MS and ^1H

NMR. R_f: 0.50 (1:3 Hex:EA) LCMS: *m/z* called for $C_{41}H_{49}N_7O_8$ (M+1) = 767.9, found 767.1.

5.3.2.8 cyclo-D-Phe-Abu-Arg-D-Pip

Following the ***hydrogenation*** procedure: *cyclo*-D-Phe-Abu-Arg-D-Pip (**HDACI 4**) was furnished by dissolving 32.7 mg (42.6 µmol, 1.0 equivalent) of *cyclo*-D-Phe-Abu-Arg(2Cbz)-D-Pip in 5.65 mL EtOH. A catalytic amount of 10% Pd/C was added to the reaction and H_2 gas was purged through the flask at low atmospheric pressure and the solution stirred for 5.5 hrs. Upon completion, confirmed by LC/MS, the reaction was filtered over Celite® concentrated *in vacuo* and the deprotected macrocycle underwent a final purification via RP-HPLC to afford the desired product *cyclo*-Phe-Abu-Arg-D-Pip (**HDACI 4**) in 21% yield; the structure and purity of the macrocycle was confirmed via LC/MS and ^{1}H NMR. Rf: 0.30 (100% EA). A final purification via RP-HPLC was performed prior to biological evaluation. ^{1}H NMR (500 MHz, CD_3OD): δ 0.84 (m, 3H, $CH_2C\underline{H}_3$), 0.95-1.03 (m, 2H, $CHCH_2C\underline{H}_2CH_2NH$), 1.19-1.26 (m, 2H, $CHC\underline{H}_2CH_3$), 1.42 (m, 1H, $CHC\underline{H}_aH_bCH_2$), 1.50-1.60 (m, 3H buried, $CHCH_a\underline{H}_bCH_2$ and $CHCH_2C\underline{H}_2$), 1.80-1.86 (m, 2H, $NCH_2C\underline{H}_2$), 3.01 (m, 2H, $NC\underline{H}_2CH_2$), 3.40 (m, 2H, $CHC\underline{H}_2CH_2$), 3.42-3.46 (m, 2H, $C\underline{H}_2NHC$), 3.56-3.60 (m, 2H, $CHC\underline{H}_2Ph$), 3.80-3.84 (m, 2H, $HNC\underline{H}_2CH_2$), 3.95-4.12 (m, 2αH), 5.05 (d, *J*=6.7 Hz, 1αH), 5.19 (d, *J*=6.4 Hz, 1αH), 6.98 (d, *J*=7.1 Hz, 1NH), 7.02 (d, *J*=7.9 Hz, 1NH), 7.10-7.19 (m, 2H, Ph), 7.20-7.30 (m, 3H, Ph), 7.39 (d, *J*=7.7 Hz, 1NH). LCMS: *m/z* called for $C_{25}H_{37}N_7O_4$ (M+1) = 500.3, found 502.5

5.3.3 Experimental methods for HDACI 11

5.3.3.1 MeO-Phe-Abu-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Phe-Abu-NHBoc was synthesized utilizing 577 mg (2.84 mmol, 1.0 equivalent) of acid HO-Abu-

NHBoc, 674 mg (3.12 mmol, 1.1 equivalent) of amine OMe-Phe-NH_2, 1.09 g (3.41 mmol, 1.2 equivalents) of TBTU, 1.98 mL (11.4 mmol, 4.0 equivalents) of DIPEA dissolved in 28.4 mL DCM, under argon. The reaction mixture stirred for 1 hr and upon completion, the crude reaction mixture was rotovapped down to dryness and redissolved in 200 mL EA. The crude reaction was washed with pH 1 HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The pure desired peptide OMe-Phe-Abu-NHBoc was afforded as a white solid (960 mg, 93% yield); R_f: 0.67 (Hex/EA 1:1). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.92 (t, *J*=7.4 Hz, 3H, $CH_2C\underline{H}_3$), 1.45 (s, 9H, $C(C\underline{H}_3)_3$), 1.69-1.73 (m, 1H, $CH_2C\underline{H}(CH_3)_2$), 1.74-1.82 (br, 2H, $C\underline{H}_2CH_3$), 3.10 (m, 1H, $C\underline{H}_aH_bPh$), 3.73 (s, 3H, $OC\underline{H}_3$), 3.96-4.07 (br, 1H, $CH_a\underline{H}_bPh$), 4.83-4.93 (m, 1αH), 4.95-5.06 (br, 1αH), 6.48 (d, *J*=7.3 Hz, 1NH), 7.08-7.14 (m, 2H, Ph), 7.25-7.31 (m, 3H, Ph).

5.3.3.2 HO-Phe-Abu-NHBoc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-Phe-Abu-NHBoc was deprotected by utilizing 911 mg (2.50 mmol, 1.0 equivalent) of MeO-Phe-Abu-NHBoc and 840 mg (20.0 mmol, 8.0 equivalents) of $LiOH•H_2O$ in 25.0 mL MeOH. The peptide was dissolved in methanol and $LiOH•H_2O$ was added to the reaction flask and the reaction was run for 1.5 hrs. Upon completion, the reaction was diluted with 250 mL DCM. The aqueous layer was back-extracted with EA (100 mL x 2), and the combined organic layers were dried, filtered and concentrated *in vacuo* (830 mg, 95% yield) as a white solid. The free acid HO-Phe-Abu-NHBoc (**HDACI 3.A**) was taken on without any further purification or characterization.

5.3.3.3 MeO-Lys(Tfa)-D-Pro-NBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Lys(Tfa)-D-Pro-NBoc was synthesized utilizing 141 mg (0.65 mmol, 1.0 equivalent) of acid HO-D-

Pro-NBoc, 184 mg (0.72 mmol, 1.1 equivalent) of amine OMe-Lys(Tfa)-NH_2, 252 mg (0.78 mmol, 1.2 equivalents) of TBTU, 684 µL (3.92 mmol, 6.0 equivalents) of DIPEA dissolved in 6.54 mL DCM, under argon. The reaction mixture stirred for 2.5 hrs and upon completion, the crude reaction mixture was diluted with 200 mL EA. The crude reaction was washed with pH 1 HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The pure desired peptide OMe-Lys(Tfa)-D-Pro-NBoc was afforded as a white solid (276 mg, 96% yield); Rf: 0.40 (Hex/EA 1:1). ^1H NMR (200 MHz, $CDCl_3$): δ 1.30-1.35 (m, 2H, $CH_2C\underline{H}_2CH_2CH_2$), 1.41 (s, 9H, $C(C\underline{H}_3)_3$), 1.51-1.61 (m, 2H, $CH_2CH_2C\underline{H}_2CH_2$), 1.77-1,84 (m, 2H, $CHC\underline{H}_2CH_2$), 1.92-2.06 (br, 1H, $CHC\underline{H}_aH_b$), 2.11-2.23 (br, 1H, $CHCH_a\underline{H}_b$), 3.21 (br, 1H, $NC\underline{H}_aH_bCH_2$), 3.27-3.35 (m, 2H, $CH_2C\underline{H}_2NH$), 3.41 (br, 1H, $NCH_a\underline{H}_bCH_2$), 3.66 (s, 3H, $OC\underline{H}_3$), 4.22 (br, 1αH), 4.54 (td, *J*=8.5, 4.3 Hz, 1αH), 7.03-7.10 (br, 1NH).

5.3.3.4 MeO-Lys(Tfa)-D-Pro-NH

Following the ***Boc removal*** procedure MeO-Lys(Tfa)-D-Pro-NH was synthesized by dissolving 238 mg (0.53 mmol, 1.0 equivalent) of MeO-Lys(Tfa)-D-Pro-NBoc in 3.94 mL DCM, followed by adding 115 µL (1.05 mmol, 2.0 equivalents) of anisole and then 1.31 mL of TFA. Boc removal was complete in 45 min; the reaction mixture was concentrated *in vacuo* with DCM (100 mL x 3) and taken on to the next reaction without further purification (186 mg, quantitative yield) as a dark brown oil.

5.3.3.5 MeO-Lys(Tfa)-D-Pro-Phe-Abu-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Lys(Tfa)-D-Pro-Phe-Abu-NHBoc was synthesized utilizing 389 mg (1.11 mmol, 1.0 equivalent) of acid HO-Phe-Abu-NHBoc, 553 mg (1.22 mmol, 1.1 equivalent) of amine OMe-Lys(Tfa)-D-Pro-NH, 213 mg (0.66 mmol, 0.6 equivalents) of TBTU, 252 mg (0.66 mmol, 0.6

equivalents), 165 mg (0.55 mmol, 0.5 equivalents) of DEPBT, 1.93 mL (11.1 mmol, 10.0 equivalents) of DIPEA dissolved in 5.54 mL each of DCM and ACN, under argon. The reaction mixture stirred for 2 hrs and upon completion, the crude reaction mixture was diluted with 150 mL DCM. The crude reaction was washed with saturated NH_4Cl aqueous solution (75 mL x 2). The organic layers were collected and dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed peptide was further purified via column chromatography and the pure protected linear tetrapeptide MeO-Lys(Tfa)-D-Pro-Phe-Abu-NHBoc (**HDACI 11.LT**) eluted in 1:3 (Hex:EA) in 84% yield; the structure and purity was confirmed via LC/MS and ^{1}H NMR. Rf: 0.44 (Hex/EA 1:3). ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.96 (t, *J*=6.6 Hz, 3H, $CH_2C\underline{H}_3$), 1.34-1.40 (m, 2H, $CH_2C\underline{H}_2CH_2CH_2$), 1.42 (s, 9H, $C(C\underline{H}_3)_3$), 1.52-1.61 (m, 4H buried, $CH_2C\underline{H}_2C\underline{H}_2CH_2$), 1.90-1.96 (br, 2H, $C\underline{H}_2CH_3$), 2.18-2.21 (br, 2H, $NCHC\underline{H}_2$), 2.59 (m, 2H, $NCH_2C\underline{H}_2$), 3.07 (m, 2H, $C\underline{H}_2Ph$), 3.17-3.21 (m, 2H, CH_2NHC), 3.62 (br, 2H, $NC\underline{H}_2CH_2$), 3.72 (s, 3H, $OC\underline{H}_3$), 4.01 (br, 1αH), 4.43 (d, *J*=7.1 Hz, 1αH), 4.68 (br, 1αH), 4.93 (d, *J*=6.8 Hz, 1αH), 6.71 (br, 1NH), 7.18 (d, *J*=8.0 Hz, 1NH), 7.20-7.25 (m, 2H, Ph), 7.28-7.33 (m, 3H, Ph), 7.60 (br, 1NH). LCMS: *m/z* called for $C_{32}H_{46}F_3N_5O_8$ (M+1) = 686.7, found 686.5.

5.3.3.6 HO-Lys(Tfa)-D-Pro-Phe-Abu-NH$_2$

Following the ***in situ deprotection*** procedure: HO-Lys(Tfa)-D-Pro-Phe-Abu-NH_2 (**HDACI 11.DDLP**) was synthesized by dissolving 448 mg (0.65 mmol, 1.0 equivalent) of MeO-Lys(Tfa)-D-Pro-Phe-Abu-NHBoc in 6.53 mL DCM, followed by adding 143 µL (1.31 mmol, 2.0 equivalents) of anisole and then 8 drops of 12 N HCl. The reaction was monitored by LC/MS and 4 additional drops of 12 N HCl were added on days 2 and 3. By day 3, the C- and *N*-termini were fully deprotected; 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 (374 mg, quantitative yield) as a dark brown oil. LCMS: *m/z* called for $C_{26}H_{36}F_3N_5O_6$ (M+1) = 572.6, found 572.4

5.3.3.7 cyclo-Phe-Abu-Lys(Tfa)-D-Pro

Following the ***macrocyclization*** procedure: *cyclo*-Phe-Abu-Lys(Tfa)-D-Pro (**HDACI 11**) was synthesized by dissolving 374 mg (0.65 mmol, 1.0 equivalent) of HO-Lys(Tfa)-D-Pro-Phe-Abu-NH_2, 189 mg (0.59 mmol, 0.9 equivalents) of TBTU, 124 mg (0.33 mmol, 0.5 equivalents) of HATU and 98 mg (0.33 mmol, 0.5 equivalents) of DEPBT in 46.7 mL each of ACN and DCM. 913 µL of DIPEA (5.23 mmol, 8.0 equivalents) was added to the reaction flask and the solution stirred for 2 hrs. Upon completion, the crude reaction was washed with saturated NH_4Cl aqueous solution (200 mL x 2). The organic layers were collected and dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed peptide was further purified via column chromatography and the pure protected linear tetrapeptide MeO-Lys(Tfa)-D-Pro-Phe-Abu-NHBoc (**HDACI 11**) eluted in 100% EA in 33% yield; the structure and purity was confirmed via LC/MS and ^{1}H NMR. Rf: 0.44 (Hex/EA 1:3). The structure and purity of the macrocycle was confirmed via LC/MS and ^{1}H NMR. R_f: 0.33 (1:4 Hex:EA). A final purification via RP-HPLC was performed prior to biological evaluation. ^{1}H NMR (600 MHz, CD_3OD): δ 0.98 (t, *J*=6.2 Hz, 3H, $CH_2C\underline{H}_3$), 1.08 (m, 2H, $CHC\underline{H}_2CH_2CH_2CH_2$), 1.18 (m, 2H, $CH_2CH_2C\underline{H}_2CH_2$), 1.22-1.39 (m, 2H, $CH_2CH_2C\underline{H}_2CH_2$), 1.47-1.62 (m, 2H, $NCHC\underline{H}_2$), 1.75-1.83 (m, 2H, $C\underline{H}_2CH_3$), 1.85-2.00 (m, 2H, $NCHCH_2C\underline{H}_2$), 2.20-2.34 (m, 2H, ($CH_2CH_2C\underline{H}_2CH_2NH$), 2.80 (m, 1H, $NC\underline{H}_aH_b$), 2.98 (dd, *J*=6.4 Hz, 2H, $C\underline{H}_2Ph$), 3.59 (m, 1H, $NC\underline{H}_aH_b$), 3.62 (m, 2H, $(CH_2)_3C\underline{H}_2NH$), 4.21 (d, *J*=6.6 Hz, 1αH), 4.32 (m, 1αH), 4.58(m, 1αH), 4.98-5.03 (m, 1αH), 7.23 (d, *J*=8.1 Hz, 1NH), 7.20 (m, 1NH), 7.23-7.34 (m, 5H, Ph), 7.38 (d, *J*=7.8 Hz, 1NH), 7.80 (d, *J*=7.4 Hz, 1NH). LCMS: *m/z* called for $C_{26}H_{34}F_3N_5O_5$ (M+1) = 554.6, found 554.6.

5.3.4 Experimental methods for HDACI 14

5.3.4.1 MeO-Ile-Trp-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Ile-Trp-NHBoc was synthesized utilizing 1.20 g (3.94 mmol, 1.0 equivalent) of acid HO-Trp-NHBoc, 630 mg (4.34 mmol, 1.1 equivalent) of amine OMe-Ile-NH_2, 1.52 g (4.73 mmol, 1.2 equivalents) of TBTU, 2.75 mL (15.8 mmol, 4.0 equivalents) of DIPEA dissolved in 10.0 mL of ACN and 29.4 of DCM, under argon. The reaction mixture stirred for 1.5 hrs and upon completion, the crude reaction mixture was diluted with 200 mL DCM and washed with saturated NH_4Cl aqueous solution (100 mL x 2). The organic layers were collected and dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed dipeptide was further purified via column chromatography and the pure peptide OMe-Ile-Trp-NHBoc eluted in 1:1 (Hex:EA) as a white solid (1.46 g, 86% yield); Rf: 0.50 (Hex/EA 1:1). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.86 (d, *J*=7.0 Hz, 3H, $CH_2C\underline{H}_3$), 0.97 (d, *J*=7.5 Hz, 3H, $CH_2C\underline{H}_3$), 1.07-1.25 (m, 2H, $CH_3C\underline{H}_2CH$), 1.33-1.47 (m, 1H, $CH_3CH_2C\underline{H}CH_3$), 1.56 (s, 9H, $C(C\underline{H}_3)_3$), 3.36 (qd, *J*=14.5, 6.2 Hz, 2H, $C\underline{H}_2CNH$), 3.74 (s, 3H, $OC\underline{H}_3$), 4.53-4.60 (m, 1αH), 5.25-5.40 (br, 1αH), 6.45 (d, *J*=8.6 Hz, 1NH), 7.17-7.40 (m, 4H, $C(CH)_4C$), 7.47 (d, *J*=8.2 Hz, 1H, $CC\underline{H}C$), 7.78 (d, J=7.2, 1NH), 8.40 (br, 1NH)

5.3.4.2 HO-Ile-Trp-NHBoc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-Ile-Trp-NHBoc was deprotected by utilizing 163 mg (0.38 mmol, 1.0 equivalent) of MeO-Ile-Trp-NHBoc and 127 mg (3.02 mmol, 8.0 equivalents) of $LiOH•H_2O$ in 3.78 mL MeOH. The peptide was dissolved in methanol and $LiOH•H_2O$ was added to the reaction flask and the reaction was run overnight. Upon completion, the reaction was diluted with 150 mL DCM and washed with pH 1 HCl solution (200 mL x 2). The aqueous layer was back-extracted with EA (200 mL x 2), and the combined organic layers were dried,

filtered and concentrated *in vacuo* (108 mg, 69% yield) as a white solid. The free acid HO-Ile-Trp-NHBoc (**HDACI 14.A**) was taken on without any further purification or characterization

5.3.4.3 MeO-Lys(Tfa)-D-Pro-NBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Lys(Tfa)-D-Pro-NBoc was synthesized utilizing 141 mg (0.65 mmol, 1.0 equivalent) of acid HO-D-Pro-NBoc, 184 mg (0.72 mmol, 1.1 equivalent) of amine OMe-Lys(Tfa)-NH_2, 252 mg (0.78 mmol, 1.2 equivalents) of TBTU, 684 µL (3.92 mmol, 6.0 equivalents) of DIPEA dissolved in 6.54 mL DCM, under argon. The reaction mixture stirred for 2.5 hrs and upon completion, the crude reaction mixture was diluted with 200 mL EA. The crude reaction was washed with pH 1 HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The pure desired peptide OMe-Lys(Tfa)-D-Pro-NBoc was afforded as a white solid (276 mg, 96% yield); Rf: 0.40 (Hex/EA 1:1). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.30-1.35 (m, 2H, $CH_2C\underline{H}_2CH_2CH_2$), 1.41 (s, 9H, $C(C\underline{H}_3)_3$), 1.51-1.61 (m, 2H, $CH_2CH_2C\underline{H}_2CH_2$), 1.77-1.84 (m, 2H, $CHC\underline{H}_2CH_2$), 1.92-2.06 (br, 1H, $CHC\underline{H}_aH_b$), 2.11-2.23 (br, 1H, $CHCH_a\underline{H}_b$), 3.21 (br, 1H, $NC\underline{H}_aH_bCH_2$), 3.27-3.35 (m, 2H, $CH_2C\underline{H}_2NH$), 3.41 (br, 1H, $NCH_a\underline{H}_bCH_2$), 3.66 (s, 3H, $OC\underline{H}_3$), 4.22 (br, 1αH), 4.54 (td, *J*=8.5, 4.3 Hz, 1αH), 7.03-7.10 (br, 1NH).

5.3.4.4 MeO-Lys(Tfa)-D-Pro-NH

Following the ***Boc removal*** procedure MeO-Lys(Tfa)-D-Pro-NH was synthesized by dissolving 238 mg (0.53 mmol, 1.0 equivalent) of MeO-Lys(Tfa)-D-Pro-NBoc in 3.94 mL DCM, followed by adding 115 µL (1.05 mmol, 2.0 equivalents) of anisole and then 1.31 mL of TFA. Boc removal was complete in 45 min; the reaction mixture was

concentrated *in vacuo* with DCM (100 mL x 3) and taken on to the next reaction without further purification (186 mg, quantitative yield) as a dark brown oil.

5.3.4.5 MeO-Lys(Tfa)-D-Pro-Ile-Trp-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Lys(Tfa)-D-Pro-Ile-Trp-NHBoc was synthesized utilizing 108 mg (0.26 mmol, 1.0 equivalent) of acid HO-Ile-Trp-NHBoc, 101 mg (0.29 mmol, 1.1 equivalent) of amine OMe-Lys(Tfa)-D-Pro-NH, 38.0 mg (0.12 mmol, 0.45 equivalents) of TBTU, 74.0 mg (0.20 mmol, 0.75 equivalents), 272 µL (1.56 mmol, 6.0 equivalents) of DIPEA dissolved in 1.3 mL each of DCM and ACN, under argon. The reaction mixture stirred for 2 hrs and upon completion, the crude reaction mixture was diluted with 200 mL DCM. The crude reaction was washed with saturated NH_4Cl aqueous solution (100 mL x 2). The organic layers were collected and dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed peptide was further purified via column chromatography and the pure protected linear tetrapeptide MeO-Lys(Tfa)-D-Pro-Ile-Trp-NHBoc (**HDACI 14.LT**) eluted in 7:13 (Hex:EA) in 78% yield; the structure and purity was confirmed via LC/MS and ^{1}H NMR. Rf: 0.40 (Hex/EA 1:9). ^{1}H NMR (500 MHz, CD_3OD): δ 0.98 (d, *J*=6.1 Hz, 3H, $C\underline{H}_3$), 1.00 (d, *J*=6.4 Hz, 3H, $C\underline{H}_3$), 1.14 (m, 2H, $CH_3C\underline{H}_2CH$), 1.26-1.32 (m, 2H, $CH_2C\underline{H}_2CH_2CH_2$), 1.38 (s, 9H, $C(C\underline{H}_3)_3$), 1.45-1.60 (m, 2H, $CH_2CH_2C\underline{H}_2CH_2$), 1.62-1.71 (m, 2H, $CHC\underline{H}_2CH_2$), 1.88-1.92 (m, 2H, $CHC\underline{H}_2CH_2$), 1.94 (m, 1H, $CH_3CH_2C\underline{H}CH_3$), 2.76-2.80 (m, 2H, $NCH_2C\underline{H}_2$), 3.20 (dd, *J*=9.1, 4.2 Hz, 2H, $C\underline{H}_2CCHC$), 3.42 (dd, *J*=6.2, 4.3 Hz, 2H, $NC\underline{H}_2$), 3.59-3.63 (m, 2H, $C\underline{H}_2NHC$), 3.79 (s, 3H, $OC\underline{H}_3$), 3.81 (d, *J*=6.3 Hz, 1αH), 4.00 (dd, *J*=7.3, 4.1 Hz, 1αH), 4.20 (dd, *J*=6.1, 3.2 Hz, 1αH), 4.43 (d, *J*=6.4 Hz, 1αH), 6.91-7.03 (m, 2H, $CC\underline{H}(CH)_2C\underline{H}$), 7.04-7.09 (m, 2H, $CCH(C\underline{H})_2CH$), 7.21 (t, *J*=5.0 Hz, 1H, $CC\underline{H}C$), 7.30 (d, *J*=7.2 Hz, 1NH), 7.41 (d, *J*=7.0 Hz, 1NH), 7.43 (d, *J*=8.0 Hz, 1NH). LCMS: *m/z* called for $C_{36}H_{51}F_3N_6O_8$ (M+1) = 753.8, found 755.3

5.3.4.6 HO-Lys(Tfa)-D-Pro-Ile-Trp-NH_2

Following the ***in situ deprotection*** procedure: HO-Lys(Tfa)-D-Pro-Ile-Trp-NH_2 (**HDACI 14.DDLP**) was synthesized by dissolving 153 mg (0.20 mmol, 1.0 equivalent) of MeO-Lys(Tfa)-D-Pro-Ile-Trp-NHBoc in 2.03 mL THF, followed by adding 22.0 µL (0.41 mmol, 2.0 equivalents) of anisole and then 8 drops of 12 N HCl. The reaction was monitored by LC/MS and 4 additional drops of 12 N HCl were added on days 2 and 3. By day 3, the C- and *N*-termini were fully deprotected; 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 (129 mg, quantitative yield) as a dark brown oil. LCMS: *m/z* called for $C_{30}H_{41}F_3N_6O_6$ (M+1) = 638.7, found 639.3

5.3.4.7 cyclo-Ile-Trp-Lys(Tfa)-D-Pro

Following the ***macrocyclization*** procedure: *cyclo*-Ile-Trp-Lys(Tfa)-D-Pro (**HDACI 14**) was synthesized by dissolving 129 mg (0.54 mmol, 1.0 equivalent) of HO-Lys(Tfa)-D-Pro-Ile-Trp-NH_2, 86.0 mg (0.27 mmol, 0.5 equivalents) of TBTU, 102 mg (0.27 mmol, 0.5 equivalents) of HATU and 80.0 mg (0.27 mmol, 0.5 equivalents) of DEPBT in 26.9 mL each of ACN and DCM. 750 µL of DIPEA (4.30 mmol, 8.0 equivalents) was added to the reaction flask and the solution stirred for 2 hrs. Upon completion, the crude reaction was washed with saturated NH_4Cl aqueous solution (200 mL x 2). The organic layers were collected and dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed peptide was further purified via column chromatography and the pure protected linear tetrapeptide MeO-Lys(Tfa)-D-Pro-Phe-Abu-NHBoc (**HDACI 14**) eluted in 98:2 EA:MeOH in 24% yield; the structure and purity was confirmed via LC/MS and ^{1}H NMR. Rf: 0.44 (Hex/EA 1:3). The structure and purity of the macrocycle was confirmed via LC/MS and ^{1}H NMR. Rf: 0.30 (100% EA). A final purification via RP-HPLC was performed prior to biological evaluation. ^{1}H NMR (500 MHz, CD_3OD): δ 0.98 (t, *J*=6.3 Hz, 3H, $CH_2C\underline{H}_3$),

1.18 (m, 2H, CHC$\underline{H}_2$CH$_2$CH$_2$CH$_2$), 1.38 (m, 4H buried, CH$_2$C$\underline{H}_2$C$\underline{H}_2$CH$_2$), 1.56 (m, 2H, C$\underline{H}_2$CH$_3$), 1.63-1.72 (m, 2H, NCCH$_2$C$\underline{H}_2$), 1.73-1.82 (m, 2H, NCHC$\underline{H}_2$CH$_2$), 2.84 (br, 2H, (CH$_2$)$_3$C$\underline{H}_2$NH), 3.19 (m, 2H, C$\underline{H}_2$CNHC), 3.98 (m, 2αH buried), 4.43 (m, 2αH buried), 7.39-7.45 (m, 4H, C(C$\underline{H}_2$)$_4$C), 7.47 (m, 1H, CC$\underline{H}$C).

5.3.5 Experimental methods for HDACI 16

5.3.5.1 MeO-Ile-Trp-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Ile-Trp-NHBoc was synthesized utilizing 1.20 g (3.94 mmol, 1.0 equivalent) of acid HO-Trp-NHBoc, 630 mg (4.34 mmol, 1.1 equivalent) of amine OMe-Ile-NH$_2$, 1.52 g (4.73 mmol, 1.2 equivalents) of TBTU, 2.75 mL (15.8 mmol, 4.0 equivalents) of DIPEA dissolved in 10.0 mL of ACN and 29.4 of DCM, under argon. The reaction mixture stirred for 1.5 hrs and upon completion, the crude reaction mixture was diluted with 200 mL DCM and washed with saturated NH$_4$Cl aqueous solution (100 mL x 2). The organic layers were collected and dried over Na$_2$SO$_4$, filtered and concentrated *in vacuo*. The washed dipeptide was further purified via column chromatography and the pure peptide OMe-Ile-Trp-NHBoc eluted in 1:1 (Hex:EA) as a white solid (1.46 g, 86% yield); Rf: 0.50 (Hex/EA 1:1). ^{1}H NMR (200 MHz, CDCl$_3$): δ 0.86 (d, *J*=7.0 Hz, 3H, CH$_2$C$\underline{H}_3$), 0.97 (d, *J*=7.5 Hz, 3H, CH$_2$C$\underline{H}_3$), 1.07-1.25 (m, 2H, CH$_3$C$\underline{H}_2$CH), 1.33-1.47 (m, 1H, CH$_3$CH$_2$C$\underline{H}$CH$_3$), 1.56 (s, 9H, C(C$\underline{H}_3$)$_3$), 3.36 (qd, *J*=14.5, 6.2 Hz, 2H, C$\underline{H}_2$CNH), 3.74 (s, 3H, OC$\underline{H}_3$), 4.53-4.60 (m, 1αH), 5.25-5.40 (br, 1αH), 6.45 (d, *J*=8.6 Hz, 1NH), 7.17-7.40 (m, 4H, C(CH)$_4$C), 7.47 (d, *J*=8.2 Hz, 1H, CC$\underline{H}$C), 7.78 (d, J=7.2, 1NH), 8.40 (br, 1NH)

5.3.5.2 HO-Ile-Trp-NHBoc

Following the ***methyl ester hydrolysis*** procedure: The acid of peptide MeO-Ile-Trp-NHBoc was deprotected by utilizing 163 mg (0.38 mmol, 1.0 equivalent) of MeO-Ile-

Trp-NHBoc and 127 mg (3.02 mmol, 8.0 equivalents) of LiOH•H_2O in 3.78 mL MeOH. The peptide was dissolved in methanol and LiOH•H_2O was added to the reaction flask and the reaction was run overnight. Upon completion, the reaction was diluted with 150 mL DCM and washed with pH 1 HCl solution (200 mL x 2). The aqueous layer was back-extracted with EA (200 mL x 2), and the combined organic layers were dried, filtered and concentrated *in vacuo* (108 mg, 69% yield) as a white solid. The free acid HO-Ile-Trp-NHBoc (**HDACI 16.A**) was taken on without any further purification or characterization

5.3.5.3 MeO-Lys(Ac)-D-Pro-NBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Lys(Ac)-D-Pro-NBoc was synthesized utilizing 322 mg (1.50 mmol, 1.0 equivalent) of acid HO-D-Pro-NBoc, 333 mg (1.65 mmol, 1.1 equivalent) of amine OMe-Lys(Ac)-NH_2, 577 mg (1.80 mmol, 1.2 equivalents) of TBTU, 2.09 mL (1.20 mmol, 8.0 equivalents) of DIPEA dissolved in 15.0 mL DCM, under argon. The reaction mixture stirred for 1.5 hrs and upon completion, the crude reaction mixture was diluted with 200 mL EA. The crude reaction was washed with pH 1 HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The pure desired peptide OMe-Lys(Ac)-D-Pro-NBoc was afforded as a white solid (502 mg, 84% yield); Rf: 0.45 (Hex/EA 7:13 ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.30-1.35 (m, 2H, $CH_2C\underline{H}_2CH_2CH_2$), 1.41 (s, 9H, $C(C\underline{H}_3)_3$), 1.51-1.61 (m, 2H, $CH_2CH_2C\underline{H}_2CH_2$), 1.77-1.84 (m, 2H, $CHC\underline{H}_2CH_2$), 1.92-2.06 (br, 1H, $CHC\underline{H}_aH_b$), 2.11-2.23 (br, 1H, $CHCH_a\underline{H}_b$), 3.21 (br, 1H, $NC\underline{H}_aH_bCH_2$), 3.27-3.35 (m, 2H, $CH_2C\underline{H}_2NH$), 3.41 (br, 1H, $NCH_a\underline{H}_bCH_2$), 3.66 (s, 3H, $OC\underline{H}_3$), 4.22 (br, 1αH), 4.54 (td, *J*=8.5, 4.3 Hz, 1αH), 7.03-7.10 (br, 1NH).

5.3.5.4 MeO-Lys(Ac)-D-Pro-NH

Following the ***Boc removal*** procedure MeO-Lys(Ac)-D-Pro-NH was synthesized by dissolving 300 mg (0.75 mmol, 1.0 equivalent) of MeO-Lys(Ac)-D-Pro-NBoc in 6.00 mL DCM, followed by adding 164 µL (1.50 mmol, 2.0 equivalents) of anisole and then 1.50 mL of TFA. Boc removal was complete in 45 min; the reaction mixture was concentrated *in vacuo* with DCM (250 mL x 3) and taken on to the next reaction without further purification (225 mg, quantitative yield) as a dark brown oil.

5.3.5.5 MeO-Lys(Ac)-D-Pro-Ile-Trp-NHBoc

Following the ***solution phase peptide coupling*** procedure: MeO-Lys(Ac)-D-Pro-Ile-Trp-NHBoc was synthesized utilizing 285 mg (0.68 mmol, 1.0 equivalent) of acid HO-Ile-Trp-NHBoc, 300 mg (0.75 mmol, 1.1 equivalent) of amine OMe-Lys(Ac)-D-Pro-NH, 131 mg (0.41 mmol, 0.6 equivalents) of TBTU, 207 mg (0.55 mmol, 0.8 equivalents), 953 µL (5.46 mmol, 8.0 equivalents) of DIPEA dissolved in 3.41 mL each of DCM and ACN, under argon. The reaction mixture stirred for 2 hrs and upon completion, the crude reaction mixture was diluted with 200 mL DCM. The crude reaction was washed with saturated NH_4Cl aqueous solution (100 mL x 2). The organic layers were collected and dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed peptide was further purified via column chromatography and the pure protected linear tetrapeptide MeO-Lys(Ac)-D-Pro-Ile-Trp-NHBoc (**HDACI 16.LT**) eluted in 1:3 (Hex:EA) in 47% yield; the structure and purity was confirmed via LC/MS and ^{1}H NMR. Rf: 0.45 (Hex/EA 1:9). ^{1}H NMR (500 MHz, CD_3OD): δ 0.98 (d, *J*=6.1 Hz, 3H, CH_3), 1.00 (d, *J*=6.4 Hz, 3H, CH_3), 1.14 (m, 2H, CH_3CH_2CH), 1.26-1.32 (m, 2H, $CH_2CH_2CH_2CH_2$), 1.38 (s, 9H, $C(CH_3)_3$), 1.45-1.60 (m, 2H, $CH_2CH_2CH_2CH_2$), 1.62-1.71 (m, 2H, $CHCH_2CH_2$), 1.88-1.92 (m, 2H, $CHCH_2CH_2$), 1.94 (m, 1H, $CH_3CH_2CHCH_3$), 2.76-2.80 (m, 2H, NCH_2CH_2), 3.20 (dd, *J*=9.1, 4.2 Hz, 2H, CH_2CCHC), 3.42 (dd, *J*=6.2, 4.3 Hz, 2H,

NC$\underline{H}_2$), 3.59-3.63 (m, 2H, C$\underline{H}_2$NHC), 3.79 (s, 3H, OC$\underline{H}_3$), 3.81 (d, *J*=6.3 Hz, 1αH), 4.00 (dd, *J*=7.3, 4.1 Hz, 1αH), 4.20 (dd, *J*=6.1, 3.2 Hz, 1αH), 4.43 (d, *J*=6.4 Hz, 1αH), 6.91-7.03 (m, 2H, CC$\underline{H}$(CH)$_2$C$\underline{H}$), 7.04-7.09 (m, 2H, CCH(C$\underline{H}$)$_2$CH), 7.21 (t, *J*=5.0 Hz, 1H, CC$\underline{H}$C), 7.30 (d, *J*=7.2 Hz, 1NH), 7.41 (d, *J*=7.0 Hz, 1NH), 7.43 (d, *J*=8.0 Hz, 1NH).

5.3.5.6 HO-Lys(Ac)-D-Pro-Ile-Trp-NH$_2$

Following the ***in situ deprotection*** procedure: HO-Lys(Ac)-D-Pro-Ile-Trp-NH$_2$ (**HDACI 16.DDLP**) was synthesized by dissolving 222 mg (0.32 mmol, 1.0 equivalent) of MeO-Lys(Ac)-D-Pro-Ile-Trp-NHBoc in 3.17 mL DCM, followed by adding 60.0 µL (0.63 mmol, 2.0 equivalents) of anisole and then 8 drops of 12 N HCl. The reaction was monitored by LC/MS and 4 additional drops of 12 N HCl were added on days 2 and 3. By day 3, the C- and *N*-termini were fully deprotected; 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 (185 mg, quantitative yield) as a dark brown oil.

5.3.5.7 cyclo-Ile-Trp-Lys(Ac)-D-Pro

Following the ***macrocyclization*** procedure: *cyclo*-Ile-Trp-Lys(Ac)-D-Pro (**HDACI 16**) was synthesized by dissolving 185 mg (0.32 mmol, 1.0 equivalent) of HO-Lys(Ac)-D-Pro-Ile-Trp-NH$_2$, 61.0 mg (0.19 mmol, 0.6 equivalents) of TBTU, 72.0 mg (0.19 mmol, 0.6 equivalents) of HATU and 47.0 mg (0.16 mmol, 0.5 equivalents) of DEPBT in 15.9 mL each of ACN and DCM. 553 µL of DIPEA (3.17 mmol, 10.0 equivalents) was added to the reaction flask and the solution stirred for 2 hrs. Upon completion, the crude reaction was washed with saturated NH_4Cl aqueous solution (200 mL x 2). The organic layers were collected and dried over Na_2SO_4, filtered and concentrated *in vacuo*. The washed peptide was further purified via column chromatography and the pure protected linear tetrapeptide MeO-Lys(Tfa)-D-Pro-Phe-Abu-NHBoc (**HDACI 14**) eluted in 100% EA in 24% yield; the structure and purity was confirmed via LC/MS and ^{1}H NMR. Rf: 0.30

(100% EA). A final purification via RP-HPLC was performed prior to biological evaluation. ^{1}H NMR (600 MHz, CD_3OD): δ 0.98 (t, *J*=6.2 Hz, 3H, $CH_2C\underline{H}_3$), 1.08 (m, 2H, $CHC\underline{H}_2CH_2CH_2CH_2$), 1.18 (m, 2H, $CH_2CH_2C\underline{H}_2CH_2$), 1.22-1.39 (m, 2H, $CH_2CH_2C\underline{H}_2CH_2$), 1.47-1.62 (m, 2H, $NCHC\underline{H}_2$), 1.75-1.83 (m, 2H, $C\underline{H}_2CH_3$), 1.85-2.00 (m, 2H, $NCHCH_2C\underline{H}_2$), 2.20-2.34 (m, 2H, ($CH_2CH_2C\underline{H}_2CH_2NH$), 2.80 (m, 1H, $NC\underline{H}_aH_b$), 2.98 (dd, *J*=6.4 Hz, 2H, $C\underline{H}_2Ph$), 3.59 (m, 1H, $NC\underline{H}_aH_b$), 3.62 (m, 2H, $(CH_2)_3C\underline{H}_2NH$), 4.21 (d, *J*=6.6 Hz, 1αH), 4.32 (m, 1αH), 4.58(m, 1αH), 4.98-5.03 (m, 1αH), 7.23 (d, *J*=8.1 Hz, 1NH), 7.20 (m, 1NH), 7.23-7.34 (m, 5H, Ph), 7.38 (d, *J*=7.8 Hz, 1NH), 7.80 (d, *J*=7.4 Hz, 1NH). LCMS: *m/z* called for $C_{30}H_{42}N_6O_5$ (M+1) = 567.7, found 568.2

5.3.6 Experimental methods for HDACI 17

5.3.6.1 HO-D-Ala-N_3 HDACI 17. Az

Azide HO-d-Ala-N_3 was synthesized utilizing 500 mg (4.27 mmol) of HO-d-Ala-NH_2, 2.82 g (8.54 mmol) of triflic anhydride, 2.78 g (42.7 mmol) of sodium azide, 885 mg (6.41 mmol) of potassium carbonate, 10.6 mg (42.5 mmol) of $CuSO_4$•$5H_2O$, in 48 mL of DCM/MeOH/H_2O (2:1:1) solvent system. The crude reaction was purified using an aqueous acidic wash to yield the pure monomer (600 mg, 97% yield).

5.3.6.2 Alk-Ile-NHBoc

Dry K_2CO_3 (3.0 equivalents, 401 mg) was weighed into a round bottom flask under argon atmosphere. 1.93 mL of anhydrous ACN was added to the flask followed by the addition of 439 µL of *p*-TsN_3 (3.0 equivalents) and 396 µL of dimethyl (2-oxypropyl) phosphonate (3.0 equivalents). The solution stirred at rt for ~2 hrs to generate the desired Bestmann-Ohira reagent. Once the Bestmann-Ohira regaent was generated, confirmed by TLC, 208 mg of Isoleucinal-NHBoc (3.0 equivalents) dissolved in 1.93 mL of anhydrous MeOH was added to the reaction mixture; this brought the

overall reaction concentration to 0.25 M. The reaction mixture was left to stir at rt overnight. Upon completion, confirmed by TLC, the reaction was concentrated *in vacuo*. The crude dried product was dissolved in 200 mL of EA and washed with saturated sodium bicarbonate (150 mL x 2) and then with brine (100 mL x 2). The organic layer was collected, dried over sodium sulfate, and concentrated *in vacuo*. Flash column chromatography with a gradient of hex/EA was performed to purify the desired alkyne Alk-Ile-NHBoc (**HDACI 17.Alk**), which eluted in 3:1 (Hex:EA) in 73% yield; the structure and purity was confirmed via ^{1}H NMR. R_f: 0.45 (Hex/EA 3:1). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.90-0.97 (m, 6H, 2C$\underline{H}_3$), 1.17-1.31 (m, 2H, $CH_3C\underline{H}_2$), 1.45 (s, 9H, $C(C\underline{H}_3)_3$), 1.60-1.69 (m, 1H, $CH_3CH_2C\underline{H}$), 2.22 (s, 1H, $CC\underline{H}$), 4.44 (br, 1αH), 4.75 (br, 1NH).

5.3.6.3 Alk-Ile-NH$_2$

Following the ***Boc removal*** procedure Alk-Ile-NH_2 was synthesized by dissolving 217 mg (1.03 mmol, 1.0 equivalent) of Alk-Ile-NHBoc in 8.23 mL DCM, followed by adding 224 µL (2.06 mmol, 2.0 equivalents) of anisole and then 2.06 mL of TFA. Boc removal was complete in 45 min; the reaction mixture was concentrated *in vacuo* with DCM (250 mL x 3) and taken on to the next reaction without further purification (114 mg, quantitative yield) as a dark brown oil.

5.3.6.4 Alk-Ile-Trp-NHBoc

Following the ***solution phase peptide coupling*** procedure: Alk-Ile-Trp-NHBoc was synthesized utilizing 284 mg (0.94 mmol, 1.0 equivalent) of acid HO-Trp-NHBoc, 114 mg (1.03 mmol, 1.1 equivalent) of amine Alk-Ile-NH_2, 360 mg (1.12 mmol, 1.2 equivalents) of TBTU, 980 µL (5.61 mmol, 6.0 equivalents) of DIPEA dissolved in 9.36 mL of DCM, under argon. The reaction mixture stirred for 1.5 hrs and upon completion, the crude reaction mixture was diluted with 100 mL DCM and washed with 10% HCl solution (50 mL x 2), then saturated $NaHCO_3$ solution (50 mL x 5), and finally brine (50

mL x 2). The organic layers were collected and dried over Na_2SO_4, filtered and concentrated *in vacuo*. The pure peptide Alk-Ile-Trp-NHBoc was furnished as a white solid (334 mg, 90% yield); Rf: 0.30 (Hex/EA 3:1).

5.3.6.5 Alk-Ile-Trp-NH_2

Following the ***Boc removal*** procedure Alk-Ile-Trp-NH_2 was synthesized by dissolving 334 mg (0.84 mmol, 1.0 equivalent) of Alk-Ile-Trp-NHBoc in 6.30 mL DCM, followed by adding 183 µL (1.68 mmol, 2.0 equivalents) of anisole and then 2.10 mL of TFA. Boc removal was complete in 45 min; the reaction mixture was concentrated *in vacuo* with DCM (100 mL x 3) and taken on to the next reaction without further purification (250 mg, quantitative yield) as a dark brown oil.

5.3.6.6 Alk-Ile-Trp-Lys(Ac)-NHBoc

Following the ***solution phase peptide coupling*** procedure: Alk-Ile-Trp-Lys(Ac)-NHBoc was synthesized utilizing 220 mg (0.76 mmol, 1.0 equivalent) of acid HO-Lys(Ac)-NHBoc, 250 mg (0.84 mmol, 1.1 equivalent) of amine Alk-Ile-Trp-NH_2, 294 mg (0.92 mmol, 1.2 equivalents) of TBTU, 1.07 mL (6.11 mmol, 8.0 equivalents) of DIPEA dissolved in 7.64 mL of DCM, under argon. The reaction mixture stirred for 2 hrs and upon completion, the crude reaction mixture was diluted with 100 mL DCM and washed with 10% HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 5), and finally brine (100 mL x 2). The organic layers were collected and dried over Na_2SO_4, filtered and concentrated *in vacuo*. Further purification was performed via column chromatography and the pure peptide eluted in 100% EA in 90% yield. Rf: 0.40 (100% EA).

5.3.6.7 Alk-Ile-Trp-Lys(Ac)-NH_2

Following the ***Boc removal*** procedure Alk-Ile-Trp-Lys(Ac)-NH_2 was synthesized by dissolving 150 mg (0.26 mmol, 1.0 equivalent) of Alk-Ile-Trp-Lys(Ac)-NHBoc in 1.99

mL DCM, followed by adding 58.0 µL (0.53 mmol, 2.0 equivalents) of anisole and then 662 µL of TFA. Boc removal was complete in 45 min; the reaction mixture was concentrated *in vacuo* with DCM (100 mL x 3) and taken on to the next reaction without further purification (124 mg, quantitative yield) as a dark brown oil.

5.3.6.8 Alk-Ile-Trp-Lys(Ac)-D-Ala-N_3

Following the ***solution phase peptide coupling*** procedure: Alk-Ile-Trp-Lys(Ac)-d-Ala-N_3 was synthesized utilizing 28.0 mg (0.24 mmol, 1.0 equivalent) of acid HO-D-Ala-N_3, 124 mg (0.26 mmol, 1.1 equivalent) of amine Alk-Ile-Trp-Lys(Ac)-NH_2, 62.0 mg (0.19 mmol, 1.2 equivalents) of TBTU, 420 µL (2.41 mmol, 10.0 equivalents) of DIPEA dissolved in 1.32 mL each of ACN and DCM, under argon. The reaction mixture stirred for 2 hrs and upon completion, the crude reaction mixture was diluted with 100 mL DCM and washed with 10% HCl solution (100 mL x 2), then saturated $NaHCO_3$ solution (100 mL x 5), and finally brine (100 mL x 2). The organic layers were collected and dried over Na_2SO_4, filtered and concentrated *in vacuo*. The pure tetrapeptide was furnished in 59% yield (80.0 mg). Rf: 0.50 (98:2 EA:MeOH).

5.3.6.9 cyclo-Ile-Trp-Lys(Ac)-D-Ala-Tr

The reaction was run in a mixture of solvents methanol:water (1:1 ratio) at 0.005 M overall concentration of linear tetrapeptide. Sodium ascorbate was dissolved in 0.5 mL of water and put into round bottom flask. Copper sulphate was dissolved in 0.5 mL of water and added to the flask 10% of the solvent mixture was added to the flask. The linear tetrapeptide was dissolved in the remaining solvent mixture and added dropwise via syringe pump to the reaction flask mixture overnight. The concentration of copper was 1.5 mM and concentration of sodium ascorbate was 45 mM for the overall reaction. Upon completion of the reaction, methanol was removed under reduced pressure and the reaction mixture was diluted with 100 mL of dichloromethane. Organic layer was

collected and concentrated in vacuo. Flash chromatography with a gradient of ethyl acetate – hexane was performed to purify the desired derivative. Finally, RP- HPLC was used for additional purification using a gradient of acetonitrile and distilled water with 0.1% TFA. ^{1}H NMR (600 MHz, CDCl3): δ 0.81 (m, 6H, 2C<u>H</u>3), 1.21 (m, 2H, CHC<u>H</u>$_2$CH$_2$), 1.25-1.30 (m, 2H, CHCH$_2$C<u>H</u>$_2$), 1.35 (m, 2H, CH$_3$C<u>H</u>$_2$), 1.40-1.56 (m, 3H, OCC<u>H</u>), 1.87 (s, 3H, CHC<u>H</u>$_3$), 2.65 (br, 1H, CH$_3$CH$_2$C<u>H</u>), 3.05-3.20 (br, 4H, C<u>H</u>$_2$C, C<u>H</u>$_2$NH), 3.73 (m, 1αH), 3.86 (m, 1αH), 4.30 (m, 1αH), 4.93 (m, 1αH), 6.89-7.18 (m, 4H, C(CH2)4C), 7.39 (s, 1H, CC<u>H</u>CNNN), 78.56 (m, 1NH), 7.93 (m, 1NH). LCMS: *m/z* called for $C_{30}H_{42}N_6O_5$ (M+1) = 568.7, found 586.2

5.4 Sanguinamide B

5.4.1 Experimental methods for Sanguinamide B

5.4.1.1 MeO-Ala-NHBoc

MeO-Ala-NHBoc was synthesized utilizing 1.50 g (7.93 mmol, 1.0 equivalent) of HO-Ala-NHBoc dissolved in 59.5 mL of MeOH and 19.3 mL of benzene. In total, 7.00 mL of (trimethylsilyl) diazomethane, 2.0 M in diethyl ether was added drop-wise to the reaction, and the reaction was stirred for 30 min. The methyl ester was concentrated *in vacuo* and taken on to the next reaction without further purification (1.61 g, quantitative yield) as clear crystals. Physical and spectroscopic data are consistent with those reported in the literature. Rf: 0.89 (Hex/EA 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.40 (d, J=7.4 Hz, 3H, C<u>H</u>$_3$CH); 1.50 (s, 9H, C(C<u>H</u>$_3$)$_3$); 3.79 (s, 3H, OC<u>H</u>$_3$); 4.22-4.40 (br, 1α<u>H</u>); 5.04 (br, 1N<u>H</u>).

5.4.1.2 H_2N-Ala-NHBoc

H_2N-Ala-NHBoc was synthesized utilizing 1.61 g (7.93 mmol, 1.0 equivalents) of OMe-Ala-NHBoc in 285 mL of ammonium hydroxide (25% in water) and 32.0 mL of

methanol. The reaction stirred overnight and concentrated *in vacuo*; the resulting amide was taken on to the next reaction without further purification (1.49 g, quantitative yield) as a white powder. R_f: 0.15 (Hex/EA 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.33 (d, *J*=7.2 Hz, 3H, C$\underline{H}_3$CH); 1.41 (s, 9H, C(C$\underline{H}_3$)$_3$); 4.00-4.30 (br, 1αH); 4.82-5.12 (br, 1H, N$\underline{H}$); 5.34-5.64 (br, 1H, N$\underline{H}_2$); 5.99-6.28 (br, 1H, N$\underline{H}_2$).

5.4.1.3 H_2NS-Ala-NHBoc

Thioamide H_2NS-Ala-NHBoc was synthesized utilizing 722 mg (3.84 mmol, 1.0 equivalent) of H_2N-Ala-NHBoc and 1.24 g (3.07 mmol, 1.0 equivalent) of L.R. dissolved in 5.49 mL of DME. The reaction mixture stirred overnight and upon completion, the crude reaction mixture was rotovapped down to dryness and purified via column chromatography on silica gel (Hex/EA 1:1 to 3:7) to afford the desired thioamide (691 mg, 88% yield) as a white film. R_f: 0.6 (Hex/EA 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (s, 9H, C(C$\underline{H}_3$)$_3$), 1.46 (d, *J*=6.9 Hz, 9H, CHC$\underline{H}_3$), 4.52-4.62 (m, 1αH), 4.52-4.62 (m, 1αH), 5.35-5.47 (br, 1H, N$\underline{H}$), 7.74-7.79 (br, 1H, N$\underline{H}_a$H$_b$), 8.05-8.19 (br, 1H, NH$_a\underline{H}_b$).

5.4.1.4 OEt-Th-Ala-NHBoc

OEt-Th-Ala-NHBoc was synthesized utilizing 691 mg (3.38 mmol, 1.0 equivalent) of H_2NS-Ala-NHBoc dissolved in 33.8 mL of DME, under argon. 2.70 g (27.0 mmol, 8.0 equivalents) of potassium bicarbonate was added to the reaction and the mixture stirred for 5 min. 1.27 mL of ethyl bromopyruvate (10.1 mmol, 3.0 equivalents) was dissolved in an additional 33.8 mL of DME and added drop-wise (1.0 mL/min) to the reaction vessel. The reaction mixture stirred overnight and upon completion the desired thiazoline intermediate was concentrated *in vacuo*, redissolved in EA, extracted with brine, dried over sodium sulfate, filtered and concentrated *in vacuo*. The crude thizaoline intermediate was dissolved in DME and stirred at 0 °C for 15 min. 2.46 mL (30.4 mmol, 9.0 equivalents) of pyridine was added to reaction (0.1 mL/min) and stirred at 0 °C for an

additional 15 min. 1.88 mL (13.5 mmol, 4.0 equivalents) TFAA was added to the reaction mixture (0.1 mL/min) and stirred at 0 °C for an additional 2 hrs. Finally, 940 µL (6.76 mmol, 2.0 equivalents) TEA was added drop-wise (0.1 mL/min) and the reaction ran from 0 °C to room temperature overnight. The reaction was concentrated *in vacuo* and redissolved in 200 mL EA. The crude reaction was washed with pH 1 hydrochloric acid solution (100 mL x 2), then saturated sodium bicarbonate solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over sodium sulfate, filtered and concentrated *in vacuo*. The washed reaction underwent a purification via column chromatography on silica gel (Hex/EA 9:11 to 2:3) to afford the desired thiazole (665 mg, 66% yield) as a light yellow oil. R_f: 0.78 (Hex/EA 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.35 (t, *J*=7.1 Hz, 3H, $CH_2C\underline{H}_3$); 1.42 (br, 9H, $C(C\underline{H}_3)_3$); 1.60 (d, *J*=6.8 Hz, 3H, $C\underline{H}_3CH$); 4.50 (q, *J*=7.2 Hz, 2H, $C\underline{H}_2CH_3$); 5.12 (br, 1αH); 5.20 (br, 1H, N<u>H</u>); 8.11 (s, 1H, SC<u>H</u>C).

5.4.1.5 OEt-Th-Ala-NH$_2$

OEt-Th-Ala-NH_2 was synthesized by dissolving 665 mg (2.20 mmol, 1.0 equivalent) of OEt-Th-Ala-NHBoc in 17.8 mL DCM, followed by adding 480 µL (4.43 mmol, 2.0 equivalents) of anisole and then 4.40 mL of TFA. Boc removal was complete in 45 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 (445 mg, quantitative yield) as a light brown oil.

5.4.1.6 OEt-Th-Ala-Val-NHBoc

OEt-Th-Ala-Val-NHBoc was synthesized utilizing 420 mg (2.10 mmol, 1.0 equivalent) of amine OEt-Th-Ala-NH_2, 732 mg (2.28 mmol, 1.2 equivalents) of TBTU, 2.67 mL (1.53 mmol, 8.0 equivalents) of DIPEA dissolved in 19.1 mL DCM, under argon. The reaction mixture stirred for 3 hrs and upon completion, the crude reaction mixture was rotovapped down to dryness and redissolved in 200 mL EA. The crude reaction

was washed with pH 1 hydrochloric acid solution (100 mL x 2), then saturated sodium bicarbonate solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over sodium sulfate, filtered and concentrated *in vacuo*. The washed reaction underwent a final purification via column chromatography on silica gel (Hex/EA 1:1 to 1:4) to afford the desired peptide (500 mg, 66% yield) as a yellow oil. R_f : 0.55 (hexanes/ethyl acetate 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (d, *J*=6.9 Hz, 3H, $CH_2C\underline{H}_3$), 0.94 (d, *J*=6.8 Hz, 3H, $CH_2C\underline{H}_3$), 1.37 (t, *J*=7.1 Hz, 3H, $C\underline{H}_3CH$), 1.41 (s, 9H, $C(C\underline{H}_3)_3$), 1.63 (d, *J*=7.0 Hz, 3H, $C\underline{H}_3CH_2$), 2.10-2.20 (m, 1H, $CHC\underline{H}(CH_3)_2$), 3.95 (br, 1αH), 4.38 (q, *J*=7.1 Hz, 2H, $C\underline{H}_2CH_3$), 4.99-5.09 (br, 1H, N<u>H</u>), 5.40 (p, *J*=7.3 Hz, 1αH), 6.77 and 6.84 (d, *J*=7.9 Hz, 1H, N<u>H</u>), 8.13 (s, 1H, CC<u>H</u>S). ^{13}C NMR (300 MHz, $CDCl_3$): δ 14.4, 17.6, 19.5, 21.4, 28.3 (3C), 30.6, 47.3, 60.0, 61.5, 80.5, 127.5, 147.2, 155.5, 161.5, 171.3, 173.0. HRMS (ESI-TOF): $M+H^+$, found 400.1896 $C_{18}H_{29}N_3O_5S$ requires 400.1906.

5.4.1.7 OEt-Th-Ala-Val-NH$_2$

OEt-Th-Ala-Val-NH_2 was synthesized by dissolving 500 mg (1.26 mmol, 1.0 equivalent) of OEt-Th-Ala-Val-NHBoc in 9.42 mL DCM, followed by adding 274 µL (2.51 mmol, 2.0 equivalents) of anisole and then 3.14 mL of TFA. Boc removal was complete in 45 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 (376 mg, quantitative yield) as a light brown oil.

5.4.1.8 3-bromo-2,2-dimethoxypropanoic acid

5.00 g of 3-bromopyruvic acid was dissolved in 10 mL (90.0 mmol, 3.0 equivalents) of trimethyl orthoformate and 400 µL (7.50 mmol, 0.25 equivalents) of sulfuric acid was added to the solution. The reaction mixture stirred overnight and, upon completion, was diluted with 200 mL of DCM, washed with pH 1 water and back

extracted with EA (200 mL x 2). All organic layers were combined, dried over sodium sulfate, filtered and concentrated *in vacuo* to afford the desired bromoketal acid (3.61 g, 56% yield) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.41 (s, 6H, 2OC$\underline{H}_3$), 3.69 (s, 2H, BrC$\underline{H}_2$).

5.4.1.9 MeO-Ser(Bzl)-Bromoketal

MeO-Ser(Bzl)-Bromoketal was synthesized utilizing 708 mg (3.39 mmol, 1.0 equivalent) of amine MeO-Ser(Bzl)-NH_2, 794 mg (7.37 mmol, 1.1 equivalents) of 3-bromo-2,2-dimethoxypropanoic acid, 1.31 g (4.07 mmol, 1.2 equivalents) of TBTU, 3.55 mL (20.3 mmol, 6.0 equivalents) of DIPEA dissolved in 33.9 mL DCM, under argon. The reaction mixture stirred for 3 hrs and upon completion, the crude reaction mixture was rotovapped down to dryness and redissolved in 400 mL EA. The crude reaction was washed with pH 1 hydrochloric acid solution (200 mL x 2), then saturated sodium bicarbonate solution (200 mL x 10) and finally brine (200 mL x 2). The organic layer was dried over sodium sulfate, filtered and concentrated *in vacuo*. The washed reaction underwent a final purification via column chromatography on silica gel (Hex/EA 1:1 to 2:3) to afford the desired peptide (1.34 g, 98% yield) as a clear oil. R_f: 0.71 (Hex/EA 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.31 (s, 3H, OC$\underline{H}_3$), 3.33 (s, 3H, OC$\underline{H}_3$), 3.55-3.66 (q, *J*=11.4 Hz, 2H, BrC$\underline{H}_2$C), 3.69-3.72 (dd, *J*=3.5, 9.1 Hz, 1H, CHC$\underline{H}_a$H$_b$O), 3.75 (s, 3H, OC$\underline{H}_3$), 3.92-3.95 (dd, *J*=3.3, 9.7 Hz, 1H, CHCH$_a$$\underline{H}_b$O), 4.46-4.61 (q, *J*=5.7 Hz, 2H, PhC$\underline{H}_2$O), 4.85 (m, 1αH), 7.14-7.43 (m, 5H, $\underline{Ph}$), 7.77 (d, *J*=8.0 Hz, 1H, N$\underline{H}$). ^{13}C NMR (400 MHz, $CDCl_3$): δ 29.8 (3C), 49.6, 50.8, 52.6, 69.4, 73.2, 100.3, 127.6, 127.7, 127.8, 128.4 (2C), 137.4, 166.9, 170.1. HRMS (ESI-TOF): M+Na^+ found 426.0514 $C_{16}H_{22}BrNO_6$ requires 426.0529.

5.4.1.10 MeO-Ser-Bromoketal

MeO-Ser-Bromoketal was synthesized by dissolving 1.34 g (3.32 mmol) of MeO-Ser(Bzl)-Bromoketal in 33.2 mL EtOH and adding a catalytic amount of 10% Pd/C. The reaction was purged several times with H_2 gas and stirred overnight. Upon completion, the reaction mixture was filtered over Celite® and concentrated *in vacuo*. The crude reaction was purified via column chromatography on silica gel (Hex/EA 7:13 to 0:1) to afford the desired peptide (1.06 g, 100% yield) as a clear oil. R_f: 0.33 (Hex/EA 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.20-2.40 (br, 1H, O<u>H</u>), 3.30 (s, 3H, OC<u>H</u>$_3$), 3.33 (s, 3H, OC<u>H</u>$_3$), 3.55-3.66 (q, *J*=11.3 Hz, 2H, BrC<u>H</u>$_2$C), 3.79 (s, 3H), 3.93-4.04 (dq, *J*=3.6 Hz, 11.6, 2H, CHC<u>H</u>$_2$O), 4.68-4.72 (m, 1αH), 7.64-7.72 (br, 1H, N<u>H</u>). ^{13}C NMR (300 MHz, $CDCl_3$): δ 29.9, 49.8, 50.9, 52.8, 54.7, 62.4, 100.2, 166.5, 170.2. HRMS (ESI-TOF): M+Na$^+$, found 336.0054 $C_9H_{16}BrNO_6$ requires 336.0059.

5.4.1.11 MeO-Ox-Bromoketal

MeO-Ox-Bromoketal was synthesized by dissolving 1.05 g (3.30 mmols, 1.0 equivalent) of MeO-Ser-Bromoketal in 33.0 mL of DCM, under argon. The reaction mixture was allowed to cool in a -78 °C (dry ice/acetone) bath for 15 min. 480 µL (3.63 mmols, 1.1 equivalents) of DAST was added to the reaction drop-wise (0.1 mL/min) and continued to stir at -78°C for 1 hr. 892 mg (6.60 mmols, 2.0 equiv.) of K_2CO_3 was added to the reaction mixture and continued to stir for an additional hour at -78 °C. Finally, the reaction was warmed to room temperature and stirred for an additional 1.5 hrs to yield the MeO-Oxazoline-Bromoketal intermediate, which was purified via column chromatography on silica gel (Hex/EA 3:7 to 1:9). 727 mg (2.46 mmol, 1.0 equivalent) of MeO-Oxazoline-Bromoketal was oxidized into the desired product by using 746 µL of DBU (4.92 mmols, 2.0 equivalents), 488 µL of $BrCCl_3$ (4.92 mmols, 2.0 equivalents), in 12.3 mL of DCM. The crude reaction was purified via column chromatography on silica

gel (Hex/EA 1:1 to 2:3) to afford the desired oxazole (595 mg, 61% yield over 2 steps) as a clear oil. R_f: 0.6 (Hex/EA 1:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.30 (s, 6H, 2OC$\underline{H}_3$), 3.83 (s, 2H, BrC$\underline{H}_2$C), 3.90 (s, 3H, OC$\underline{H}_3$), 8.28 (s, 1H, CC$\underline{H}$O). ^{13}C NMR (400 MHz, $CDCl_3$): δ 31.7, 50.1, 50.2, 52.1, 99.2, 133.7, 144.5, 160.9, 161.1. HRMS (ESI-TOF): M+H$^+$, found 293.9971 $C_9H_{12}BrNO_5$ requires 293.9977.

5.4.1.12 MeO-Ox-Bromoketone

MeO-Ox-Bromoketone was synthesized by dissolving 595 mg (2.02 mmol, 1.0 equivalent) of MeO-Ox-Bromoketal in 20.3 mL of formic acid. The reaction was run while heated from room temperature to 60 °C. Upon reaching 60 °C, the reaction was removed from the oil bath and confirmed complete by TLC. The reaction was dissolved in 300 mL of DCM and placed in a separatory funnel. A total of 160 mL of saturated sodium bicarbonate was added slowly to the separatory funnel to quench the reaction. The aqueous layer was back-extracted with EA (250 mL x 2) and all organic layers were combined, dried over sodium sulfate, filtered and concentrated *in vacuo*. The reaction was taken on without further purification (312 mg, 62% yield) as a white powder. R_f: 0.75 (Hex/EA 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.02 (s, 3H, OC$\underline{H}_3$), 4.70 (s, 2H, BrC$\underline{H}_2$C), 8.48 (s, 1H, CC$\underline{H}$O). ^{13}C NMR (300 MHz, $CDCl_3$): δ 30.3, 52.8, 134.6, 147.0, 155.4, 160.1, 179.0. HRMS (ESI-TOF): M+Na$^+$, found 269.9371 $C_7H_6BrNO_4$ requires 269.9378.

5.4.1.13 H_2NS-Pro-NBoc

H_2NS-Pro-NBoc was synthesized utilizing 1.25 g (5.84 mmol, 1.0 equivalent) of H_2N-Pro-NBoc and 1.88 g (4.65 mmol, 0.8 equivalents) of L.R. dissolved in 9.0 mL of DME, under argon. The reaction mixture stirred overnight and upon completion, the crude reaction mixture was purified via column chromatography on silica gel (Hex/EA 1:1 to 1:3) to afford the desired thioamide (1.10 g, 82% yield) as a white powder. R_f: 0.55

(Hex/EA 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.40 (s, 9H, $C(C\underline{H}_3)_3$), 1.52-1.58 (br, 2H buried, $CHC\underline{H}_aH_bC\underline{H}_aH_bCH_2$), 1.78-1.85 (m, 2H, $CHCH_a\underline{H}_bCH_a\underline{H}_bCH_2$), 3.37-3.42 (br, 2H, $C\underline{H}_2$NBoc), 4.58 (m, 1αH), 7.32-7.38 (br, 1H, $N\underline{H}_2$).

5.4.1.14 MeO-Ox-Th-Pro-NBoc

MeO-Ox-Th-Pro-NBoc was synthesized utilizing 145 mg (0.63 mmol, 1.0 equivalent) of H_2NS-Pro-NBoc and 503 mg (5.03 mmol, 8.0 equivalents) of potassium carbonate, dissolved in 6.30 mL of DME and was stirred for 5 min. 312 mg of MeO-Ox-Bromoketone (1.26 mmol, 2.0 equivalents) was dissolved in an additional 6.30 mL of DME and added drop-wise (1.0 mL/min) to the reaction vessel. The reaction mixture was stirred overnight and upon completion the desired thiazoline intermediate was concentrated *in vacuo*. The crude reaction was redissolved in EA and extracted with brine, dried over sodium sulfate, filtered and concentrated *in vacuo*. The crude thizaoline intermediate was dissolved in DME and stirred at 0 °C for 15 min. 457 µL (5.66 mmol, 9.0 equivalents) of pyridine was added to reaction mixture (0.1 mL/min) and stirred at 0 °C for an additional 15 min. 350 µL (2.52 mmol, 4.0 equivalents) TFAA was added to the reaction mixture (0.1 mL/min) and stirred at 0 °C for an additional 2 hrs. Finally, 175 µL (1.26 mmol, 2.0 equivalents) TEA was added to the reaction drop-wise and ran at 0 °C to room temperature overnight. The reaction concentrated *in vacuo* and dissolved in 200 mL EA. The crude reaction was purified via acid-base extraction and then column chromatography on silica gel (Hex/EA 3:7 to 3:17) to afford the desired thiazole (229 mg, 96% yield) as a light yellow oil. R_f: 0.37 (Hex/EA 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26-1.50 (bd, 9H, $C(C\underline{H}_3)_3$), 1.88-1.96 (m, 2H, $CH_2C\underline{H}_2CH_2$), 2.20-2.42 (bd, 2H, $CHC\underline{H}_2CH_2$), 3.40-3.64 (bd, 2H, $C\underline{H}_2$NBoc), 3.92 (s, 3H, $OC\underline{H}_3$), 5.14-5.30 (bd, 1αH), 8.04 (s, 1H, $CC\underline{H}S$), 8.26 (s, 1H, $CC\underline{H}O$). ^{13}C NMR (300 MHz, $CDCl_3$): δ 23.2, 28.2

(3C), 33.9, 46.8, 52.2, 59.4, 80.4, 120.8, 134.2, 142.3, 143.7, 154.0, 157.5, 161.4, 177.6. HRMS (ESI-TOF): $M+Na^+$, found 402.1085 $C_{17}H_{21}N_3O_5S$ requires 402.1100.

5.4.1.15 MeO-Ox-Th-Pro-NH

MeO-Ox-Th-Pro-NH was synthesized by dissolving 175 mg (0.46 mmol, 1.0 equivalent) of MeO-Ox-Th-Pro-NBoc in 3.7 mL DCM, followed by adding 101 µL (0.92 mmol, 2.0 equivalents) of anisole and then 3.70 mL of TFA. Boc removal was complete in 45 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 (129 mg, 100% yield) as a light brown oil.

5.4.1.16 MeO-Ox-Th-Pro-Leu-NHBoc

MeO-Ox-Th-Pro-Leu-NHBoc was synthesized by first utilizing 129 mg (0.46 mmol, 1.0 equivalent) of amine MeO-Ox-Th-Pro-NH, 117 mg (0.51 mmol, 1.1 equivalents) of acid HO-Leu-NHBoc, 118 mg (0.37 mmol, 0.8 equivalents) of TBTU, 175 mg (0.46 mmol, 1.0 equivalent) of HATU, 644 µL (3.69 mmol, 8.0 equivalents) of DIPEA dissolved in 4.6 mL DCM, under argon. The reaction mixture stirred for 3 hrs and upon completion, the crude reaction mixture was rotovapped down to dryness and redissolved in 200 mL EA. The crude reaction was washed with pH 1 hydrochloric acid solution (100 mL x 2), then saturated sodium bicarbonate solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over sodium sulfate, filtered and concentrated *in vacuo*. The washed reaction underwent a final purification via column chromatography on silica gel (Hex/EA 3:7 to 0:1) to afford an acid and amine protected peptide (193 mg, 85% yield) as a light yellow oil. R_f: 0.44 (Hex/EA 0:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97 (d, *J*=6.8 Hz, 3H, CHC$\underline{H}_3$), 1.01 (d, *J*=6.5 Hz, 3H, CHC$\underline{H}_3$), 1.43 (s, 9H, C(C$\underline{H}_3$)$_3$), 1.49-1.58 (m, 2H, CHC$\underline{H}_2$CH), 1.76-1.84 (m, 1H, CHC$\underline{H}_a$H$_b$CH$_2$), 2.12-2.18 (m, 1H, CHCH$_a\underline{H}_b$CH$_2$), 2.31-2.40 (m, 1H, C$\underline{H}_a$H$_b$N), 2.43-2.49 (m, 1H, CH$_a\underline{H}_b$N), 3.71-3.78 (m,

1H, C$\underline{H}_a$H$_b$N), 3.83-3.92 (m, 1H, CH$_a\underline{H}_b$N), 3.95 (s, 3H, OC$\underline{H}_3$), 4.52-4.60 (m, 1αH), 5.14 (d, *J*=9.3 Hz, 1H, N$\underline{H}$), 5.58 (dd, *J*=2.9, 8.2 Hz, 1αH), 8.06 (s, 1H, CC$\underline{H}$S), 8.32 (s, 1H, CC$\underline{H}$O). ^{13}C NMR (300 MHz, $CDCl_3$): δ 21.1, 21.9, 23.6, 24.8, 28.5 (3C), 31.8, 42.4, 47.2, 50.5, 52.5, 58.9, 79.8, 121.6, 134.4, 142.5, 143.7, 155.9, 158.0, 161.7, 172.9, 174.3. HRMS (ESI-TOF): M+H$^+$, found 493.2108 $C_{23}H_{32}N_4O_6S$ requires 493.2121.

5.4.1.17 HO-Ox-Th-Pro-Leu-NHBoc

The acid of peptide MeO-Ox-Th-Pro-Leu-NHBoc was deprotected by utilizing 193 mg (0.39 mmol, 1.0 equivalent) of MeO-Ox-Th-Pro-Leu-NHBoc, 132 mg (3.14 mmol, 8.0 equivalents) of LiOH•H_2O and 41.0 µL (1.33 mmol, 3.4 equivalents) of H_2O_2 (30% in water) in 7.80 mL MeOH. The peptide was dissolved in methanol and cooled to 0 °C. H_2O_2 was added the reaction flask, followed by LiOH•H_2O and the reaction was run overnight. Upon completion, the reaction was diluted with 100 mL methylene chloride. Sodium thiosulfate (3.8 equivalents) was added to a 5% hydrochloric acid pH 1 solution in order to neutralize the peroxide, the reaction was washed (100 mL x 2). The aqueous layer was back-extracted with ethyl acetate (100 mL x 2), and the combined organic layers were dried, filtered and concentrated *in vacuo* (184 mg, 98% yield) as a clear oil. The free acid was taken on without any further purification or characterization.

5.4.1.18 MeO-Pro-Ox-Th-Pro-Leu-NHBoc

MeO-Pro-Ox-Th-Pro-Leu-NHBoc was synthesized utilizing 184 mg (0.38 mmol, 1.0 equivalent) of acid HO-Ox-Th-Pro-Leu-NHBoc, 70.0 mg (0.42 mmol, 1.1 equivalents) of amine MeO-Pro-NBoc, 204 mg (0.54 mmol, 1.4 equivalents) of HATU, 537 µL (3.07 mmol, 8.0 equivalents) of DIPEA dissolved in 7.69 mL DCM, under argon. The reaction mixture stirred overnight and upon completion, the reaction mixture was rotovapped down to dryness and redissolved in 200 mL EA. The crude reaction was washed with pH 1 hydrochloric acid solution (100 mL x 2), then saturated sodium bicarbonate solution

(100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over sodium sulfate, filtered and concentrated *in vacuo*. The washed reaction underwent a final purification via column chromatography on silica gel (Hex/EA 3:7 to 0:1) to afford the desired peptide (213 mg, 94% yield) as a 1:1 mixture of rotamers. R_f: 0.35 (Hex/EA 0:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.92 (d, *J*=6.5 Hz, 3H, $CHC\underline{H}_3$), 0.97 (d, *J*=6.4 Hz, 3H, $CHC\underline{H}_3$), 1.40 (s, 9H, $C(C\underline{H}_3)_3$), 1.46 (m, 2H, $CHC\underline{H}_2CH$), 1.75 (m, 1H, $C\underline{H}(CH_3)_2$), 1.90 (m, 1H, $CHCH_2C\underline{H}_aH_bCH_2$), 2.00 (br, 1H buried, $CHCH_2CH_a\underline{H}_bCH_2$), 2.10 (m, 2H, $CHCH_2C\underline{H}_2CH_2$), 2.21 (m, 1H $CHC\underline{H}_aH_bCH_2$), 2.30 (m, 2H buried, $CHCH_a\underline{H}_bCH_2$, $CHCH_a\underline{H}_bCH_2$), 2.44 (m, 1H, $CHC\underline{H}_aH_bCH_2$), 3.66 (br, 2H, $C\underline{H}_2N$), 3.72 (s, 3H, OCH_3), 3.82 (m, 1H, $C\underline{H}_aH_bN$), 4.16 (m, 1H, $CH_a\underline{H}_bN$), 4.50 (m, 1αH), 5.15 (m, 1αH), 5.30 and 4.63 (dd, *J*=2.9, 9.1 Hz, 1H), 5.52 (m, 1αH), 7.82 and 7.88 (s, 1H, CCHS), 8.22 and 8.25 (s, 1H, CCHO). ^{13}C NMR (300 MHz, $CDCl_3$): δ 21.5, 22.1, 22.5 (2C), 23.4, 28.1 (3C), 29.3, 29.5, 34.8, 46.5 (2C), 52.0 (2C), 58.4, 59.4, 77.5, 120.7, 137.6, 142.6, 143.0, 156.1, 156.4, 160.2, 170.9, 172.6, 174.1. HRMS (ESI-TOF): $M+H^+$, found 590.2634 $C_{28}H_{39}N_5O_7S$ requires 590.2648.

5.4.1.19 HO-Pro-Ox-Th-Pro-Leu-NHBoc

HO-Pro-Ox-Th-Pro-Leu-NHBoc (fragment B) was synthesized utilizing 213 mg (0.36 mmol, 1.0 equivalent) of MeO-Pro-Ox-Th-Pro-Leu-NHBoc, 121 mg (2.89 mmol, 8.0 equivalent) of $LiOH•H_2O$ and 38.0 µL (1.23 mmol, 3.4 equivalents) of H_2O_2 (30% in water) in 7.20 mL MeOH. The peptide was dissolved in methanol and cooled to 0 °C. H_2O_2 was added the reaction flask, followed by $LiOH•H_2O$ and the reaction was run overnight. Upon completion, the reaction was diluted with 100 mL methylene chloride. Sodium thiosulfate (3.8 equivalents) was added to a 5 % hydrochloric acid pH 1 solution in order to neutralize the peroxide, the reaction was washed (100 mL x 2). The aqueous layer was back-extracted with ethyl acetate (100 mL x 2), and the combined organic

layers were dried, filtered and concentrated *in vacuo* and taken on to the next reaction without further purification (199 mg, 96% yield) as a clear oil.

5.4.1.20 EtO-Th-Ala-Val-Pro-Ox-Th-Pro-Leu-NHBoc

EtO-Th-Ala-Val-Pro-Ox-Th-Pro-Leu-NHBoc was synthesized utilizing 199 mg (0.35 mmol, 1.0 equivalent) of acid HO-Pro-Ox-Th-Pro-Leu-NHBoc (fragment B), 155 mg (0.52 mmol, 1.5 equivalents) of amine EtO-Th-Ala-Val-NH_2 (fragment A), 89.0 mg (0.28 mmol, 0.8 equivalents) of TBTU, 131 mg (0.35 mmol, 1.0 equivalents) of HATU, 1.21 mL (6.91 mmol, 20.0 equivalents) of DIPEA dissolved in 3.46 mL DCM and 3.46 mL ACN, under argon. The reaction mixture stirred overnight and upon completion, the reaction mixture was rotovapped down to dryness and redissolved in 200 mL EA. The crude reaction was washed with pH 1 hydrochloric acid solution (100 mL x 2), then saturated sodium bicarbonate solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was dried over sodium sulfate, filtered and concentrated *in vacuo*. The washed reaction underwent a final purification via column chromatography on silica gel (EA/MeOH 50:1) to afford the desired peptide (262 mg, 88% yield). R_f: 0.30 (EA/MeOH 19:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.80 (d, *J*=6.4 Hz, 3H, CHC$\underline{H}_3$), 0.86 (d, *J*=7.4 Hz, 3H, CHC$\underline{H}_3$), 0.90 (d, *J*=7.7 Hz, 3H, CHC$\underline{H}_3$), 0.94 (d, *J*=6.5 Hz, 3H, CHC$\underline{H}_3$), 0.99 (t, *J*=7.2 Hz, 3H, $OCH_2C\underline{H}_3$), 1.37 (s, 9H, $C(C\underline{H}_3)_3$), 1.40-1.47 (br, 3H buried, $CH_\alpha C\underline{H}_2CH_2$, $C\underline{H}(CH_3)_2$), 1.54 (m, 2H, $CH_2C\underline{H}_2CH_2$), 1.59 (d, *J*=7.0 Hz, 3H, $C\underline{H}_3CH_\alpha$), 1.65-1.80 (br, 2H buried, $CH_\alpha CH_2CH$), 2.20-2.25 (m, 1H, $CH(CH_3)_2$), 2.25-2.32 (m, 3H buried, $CH_2C\underline{H}_2CH_2$, $CH_2C\underline{H}_aH_bCH_\alpha$), 2.35-2.42 (m, 1H, $CH_2CH_a\underline{H}_bCH_\alpha$), 3.66 (m, 1H, $NC\underline{H}_aH_bCH_2$), 3.80 (m, 1H, $NCH_a\underline{H}_bCH_2$), 4.12-4.18 (br, 1H, $NC\underline{H}_aH_bCH_2$), 4.20-4.24 (m, 1H, $NCH_a\underline{H}_bCH_2$), 4.30-4.38 (m, 2H, $OC\underline{H}_2CH_3$), 4.44-4.50 (br, 1αH), 4.68-4.72 (br, 1αH), 5.08 (d, *J*=9.0 Hz, 1αH), 5.34 (m, 1αH), 5.50 (m, 1αH), 6.75 and 6.88 (br, 1H, N$\underline{H}$), 7.04 (d, *J*=10.2 Hz, 1H, N$\underline{H}$), 7.87 and 7.94 (s, 1H, CC$\underline{H}$S), 7.99 and 8.02 (s, 1H, CC$\underline{H}$S),

8.19 and 8.22 (s, 1H, CC<u>H</u>O). LC/MS (ESI): *m/z* called for $C_{40}H_{56}N_8O_9S_2$ ($M+H^+$) = 857.4, found 857.4. HRMS (ESI-TOF): $M+H^+$, found 857.3685 $C_{40}H_{56}N_8O_9S_2$ requires 856.3612.

5.4.1.21 HO-Th-Ala-Val-Pro-Ox-Th-Pro-Leu-NHBoc

HO-Th-Ala-Val-Pro-Ox-Th-Pro-Leu-NHBoc was synthesized utilizing 262 mg (0.36 mmol, 1.0 equivalent) of EtO-Th-Ala-Val-Pro-Ox-Th-Pro-Leu-NHBoc, 128 mg (3.06 mmol, 10.0 equivalents) of $LiOH•H_2O$ and 32.0 µL (1.04 mmol, 3.4 equivalents) of H_2O_2 (30% in water) in 6.10 mL EtOH. The peptide was dissolved in methanol and cooled to 0 °C. H_2O_2 was added the reaction flask, followed by $LiOH•H_2O$ and the reaction was run overnight. Upon completion, the reaction was diluted with 100 mL methylene chloride. Sodium thiosulfate (3.8 equivalents) was added to a 5% hydrochloric acid pH 1 solution in order to neutralize the peroxide, the reaction was washed (100 mL x 2). The aqueous layer was back-extracted with ethyl acetate (100 mL x 2), and the combined organic layers were dried, filtered and concentrated *in vacuo* and taken on to the next reaction without further purification (250 mg, 98% yield). LC/MS (ESI): *m/z* called for $C_{38}H_{52}N_8O_9S_2$ ($M+H^+$) = 829.3, found 829.6.

5.4.1.22 HO-Th-Ala-Val-Pro-Ox-Th-Pro-Leu-NH$_2$

HO-Th-Ala-Val-Pro-Ox-Th-Pro-Leu-NH_2 was synthesized by dissolving 250 mg (0.30 mmol, 1.0 equivalent) of HO-Th-Ala-Val-Pro-Ox-Th-Pro-Leu-NHBoc in 4.80 mL DCM, followed by adding 65.0 µL (6.02 mmol, 2.0 equivalents) of anisole and then 1.20 mL of TFA. Boc removal was complete in 45 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 (219 mg, quantitative yield). LC/MS (ESI): *m/z* called for $C_{33}H_{44}N_8O_7S_2$ ($M+H^+$) = 729.3, found 728.8.

5.4.1.23 trans,trans-, cis,cis-, and trans,cis-Sanguinamide B

Sanguinamide B and its conformers were synthesized by dissolving 219 mg (0.301 mmol, 1.0 equivalent) of HO-Th-Ala-Val-Pro-Ox-Th-Pro-Leu-NH_2, 97.0 mg (0.301 mmol, 1.0 equivalent) of TBTU, 137 mg (0.361 mmol, 1.2 equivalent) of HATU, with 525 µL (3.01 mmol, 10.0 equivalents) of DIPEA in 21.5 mL of DCM and 21.5 mL of ACN (0.007 M concentration overall), under argon. The reaction mixture stirred overnight and upon completion, it was rotovapped down to dryness and redissolved in 200 mL EA. The crude reaction was washed with pH 1 hydrochloric acid solution (100 mL x 2), then saturated sodium bicarbonate solution (100 mL x 5) and finally brine (100 mL x 2). The organic layer was dried over sodium sulfate, filtered and concentrated *in vacuo*. The washed reaction underwent an initial purification via column chromatography on silica gel (EA/MeOH 19:1) and the resulting semi-pure residue was subjected to reversed-phase HPLC purification to afford a 1:1 inseparable mixture of *trans,trans*-Sanguinamide B (natural product) with *trans,cis*-Sanguinamide B (1.50 mg total) and the single conformer *cis,cis*-Sanguinamide B (24.5 mg) in a 54% overall yield prior to RP-HPLC purification.

***cis,cis*-Sanguinamide B (SanB**):** ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.87 (d, *J*=6.8, 3H, CHC$\underline{H}_3$), 0.93 (d, *J*=6.4, 3H, CHC$\underline{H}_3$), 0.99 (m, 6H buried, 2CHC$\underline{H}_3$), 1.56 (m, 1H, C$\underline{H}$(CH_3)$_2$), 1.58 (m, 2H, CHC$\underline{H}_2$CH), 1.79 (m, 1H, $CH_2C\underline{H}_aH_bCH_2$), 1.94 (m, 3H, CHC$\underline{H}_3$), 1.97 (m, 1H, $CH_2CH_a\underline{H}_bCH_2$), 2.01 (m, 2H, CHC$\underline{H}_2CH_2$), 2.13 (m, CHC$\underline{H}_aH_bCH_2$), 2.30 (m, 2H, $CH_2C\underline{H}_2CH_2$), 2.34 (m, 1H, $CHCH_a\underline{H}_bCH_2$), 2.88 (m, 1H, C$\underline{H}$($CH_3$)$_2$), 3.80 (m, 2H, C$\underline{H}_2$CH), 3.89 (m, 2H, C$\underline{H}_2$CH), 4.79 (m, 1H buried, 1αH), 4.80 (m, 1H buried, 1αH), 5.11 (m, 2H buried, 2αH), 5.47 (t, *J*=6.7 Hz, 1αH), 6.37 (d, *J*=10.4 Hz, 1H, N$\underline{H}$), 7.15 (d, *J*=10.7 Hz, 1H, N$\underline{H}$), 7.19 (d, *J*=8.4 Hz, 1H, N$\underline{H}$), 7.45 (s, 1H, CC$\underline{H}$S), 7.94 (s, 1H, CC$\underline{H}$S), 8.38 (s, 1H, CC$\underline{H}$O). ^{13}C NMR (600 MHz, $CDCl_3$): δ 16.1,

16.4, 19.7, 20.2 (C_γ Pro-2), 22.2, 22.5 (C_γ Pro-1), 23.3, 24.3, 28.3, 31.9 (C_β Pro-1), 35.6 (C_β Pro-2), 41.3, 45.7, 46.8, 48.1, 49.0, 56.9, 59.0, 64.1, 122.5, 125.9, 136.5, 140.5, 143.7, 146.3, 157.6, 160.1, 162.2, 169.2, 170.8, 170.9, 172.7, 173.8. LC/MS (ESI): *m/z* called for $C_{38}H_{52}N_8O_9S_2$ ($M+H^+$) = 711.3, found 711.5. HRMS (ESI-TOF): MNa^+, found 733.2544 $C_{33}H_{42}N_8O_6S_2$ requires 733.2567.

***trans,cis*-Sanguinamide B (SanB*)** and ***trans,trans*-Sanguinamide B (SanB):** A 1:1 mixture of SanB* and SanB was characterized using a 700 MHz NMR 5mm ^{1}H inverse-detected cryoprobe cooled to -10 °C. Chemical shifts of all but 5 carbons were resolved for each conformer and * is used to denote shifts for SanB*. ^{1}H NMR (700 MHz, $CDCl_3$): δ 0.82 (br, 3H, CHC$\underline{H}_3$), 0.87* (br, 3H buried, CHC$\underline{H}_3$), 0.89* (m, 3H buried, CHC$\underline{H}_3$), 0.95* (br, 3H, CHC$\underline{H}_3$), 0.99 (d, *J*=7.0, 3H, CHC$\underline{H}_3$), 1.05 (d, *J*=6.9, 3H, CHC$\underline{H}_3$), 1.10* (d, *J*=6.3, 3H, CHC$\underline{H}_3$), 1.15 (d, *J*=3.5, 3H, CHC$\underline{H}_3$), 1.40* (m, 2H, CHC$\underline{H}_2$CH), 1.47* (m, 1H, C$\underline{H}(CH_3)_2$), 1.58 (m, 1H, C$\underline{H}(CH_3)_2$), 1.61* (d, *J*=6.3, 3H, CHC$\underline{H}_3$), 1.67 (d, *J*=5.6, 3H, CHC$\underline{H}_3$), 1.69 (br, 1H, C$\underline{H}(CH_3)_2$), 1.83 (br, 1H buried, $CH_2C\underline{H}_aH_bCH_2$), 1.85* (br, 1H buried, $CH_2C\underline{H}_aH_bCH_2$), 1.94* (br, 1H buried, $CH_2C\underline{H}_aH_bCH_2$), 2.02* (m, 1H buried, $CHC\underline{H}_aH_bCH_2$), 2.04* (m, 1H buried, $CH_2CH_a\underline{H}_bCH_2$), 2.05 (m, 1H buried, $CHC\underline{H}_aH_bCH_2$), 2.06 (m, 1H buried, $CH_2CH_a\underline{H}_bCH_2$), 2.07 (m, 1H buried, $CH_2C\underline{H}_aH_bCH_2$), 2.15 (m, 1H, $CHC\underline{H}_aH_bCH_2$), 2.17* (m, 1H $CH_2CH_a\underline{H}_bCH_2$), 2.26* (m, 1H, $CHC\underline{H}_aH_bCH_2$), 2.35* (m, 1H, $CHCH_a\underline{H}_bCH_2$), 2.54 (m, 1H buried, $CH_2CH_a\underline{H}_bCH_2$), 2.55 (m, 1H buried, $CHCH_a\underline{H}_bCH_2$), 2.62* (m, 1H, $CHCH_a\underline{H}_bCH_2$), 2.74* (m, 1H, CHC$\underline{H}(CH_3)_2$), 2.77 (m, 1H, CC$\underline{H}(CH_3)_2$), 2.95 (m, $CHCH_a\underline{H}_bCH_2$), 3.40* (m, 1H, $CH_2C\underline{H}_aH_bC$), 3.55 (m, 1H, $CH_2C\underline{H}_aH_bC$), 3.75* (m, 1H, $CH_2C\underline{H}_aH_bC$), 3.79 (m, 1H, $CH_2C\underline{H}_aH_bC$), 3.85* (m, 1H, $CH_2CH_a\underline{H}_bC$), 4.00 (m, 1H buried, $CH_2CH_a\underline{H}_bC$), 4.05 (m, 1H buried, $CH_2CH_a\underline{H}_bC$), 4.25* (m, 1H, $CH_2CH_a\underline{H}_bC$), 4.32 (m, 1H, 1αH), 4.50 (m, 1H, 1αH), 4.68 (d, *J*=7.0, 1H, 1αH),

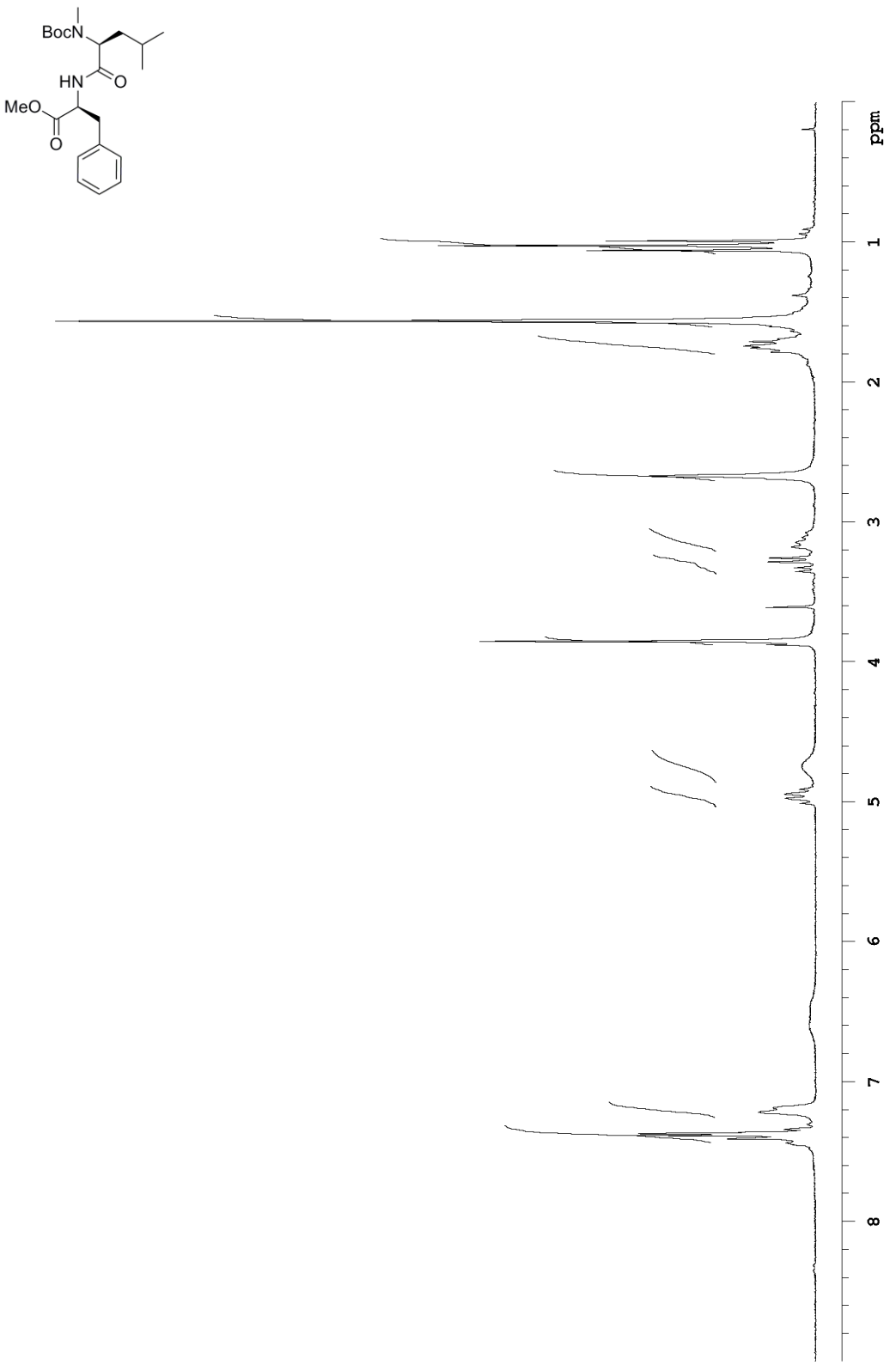
4.78* (m, 1H, 1αH), 4.91* (m, 1H, 1αH), 5.38 (m, 1H, 1αH), 5.45* (m, 1H, 1αH), 5.48 (m, 1H, 1αH), 5.65* (d, *J*=6.3, 1H, 1αH), 5.87* (d, *J*=7.0, 1H, 1αH), 6.95* (d, *J*=8.4, 1H, NH), 7.25 (d, *J*=10.5, 1H, NH), 7.44* (s, 1H, CCHS), 7.52* (d, *J*=10.5, 1H, NH), 7.78* (s, 1H, CCHO), 7.92* (s, 1H, CCHS), 7.92 (s, 1H, CCHS), 8.02 (s, 1H, CCHS), 8.13 (d, *J*=10.5, 1H, NH), 8.23 (s, 1H, CCHO), 8.25 (d, *J*=6.3, 1H, NH), 8.55 (d, *J*=11.9, 1H, NH). ^{13}C NMR (600 MHz, $CDCl_3$): δ 15.0*, 17.5, 19.5, 21.3, 21.4* (C_γ Pro-2), 21.5*, 22.3* (C_γ Pro-1), 22.4, 22.5*, 23.5*, 23.7*, 24.0, 24.5*, 25.0, 25.8 (C_γ Pro-1), 26.1* (C_β Pro-1), 28.2, 30.5 (C_β Pro-1), 32.4 (C_γ Pro-2), 34.5 (C_β Pro-2), 34.9* (C_β Pro-2), 41.0*, 42.3*, 43.0, 45.2, 46.1, 46.6, 47.0, 48.0, 48.2*, 50.3*, 56.8*, 57.7, 58.5, 59.8*, 60.6*, 60.7*, 62.8, 121.5, 121.7*, 122.7*, 123.7, 140.3*, 141.0, 141.5*, 143.5, 148.5, 149.2*, 155.3, 157.3*, 158.7*, 171.2, 172.0, 172.3, 172.8, 172.9*, 174.2*. LC/MS (ESI): *m/z* called for $C_{38}H_{52}N_8O_9S_2$ ($M+H^+$) = 711.3, found 711.5. HRMS (ESI-TOF): MH^+: found 711.2730 $C_{33}H_{42}N_8O_6S_2$ requires 711.2749.

Structure elucidation of SanB and SanB* mixture: A total of 12 Proline methylenes are present in the SanB and SanB* mixture; their identities were revealed by analysis of HMBC and HSQC data. Pro-1 methylenes had HMBC correlations to the adjacent oxazole (10 to 13; 8 and 9 to 10). Pro-2 methylenes had HMBC correlations to the adjactent thiazole (19 to 16; 20 and 21 to 19).

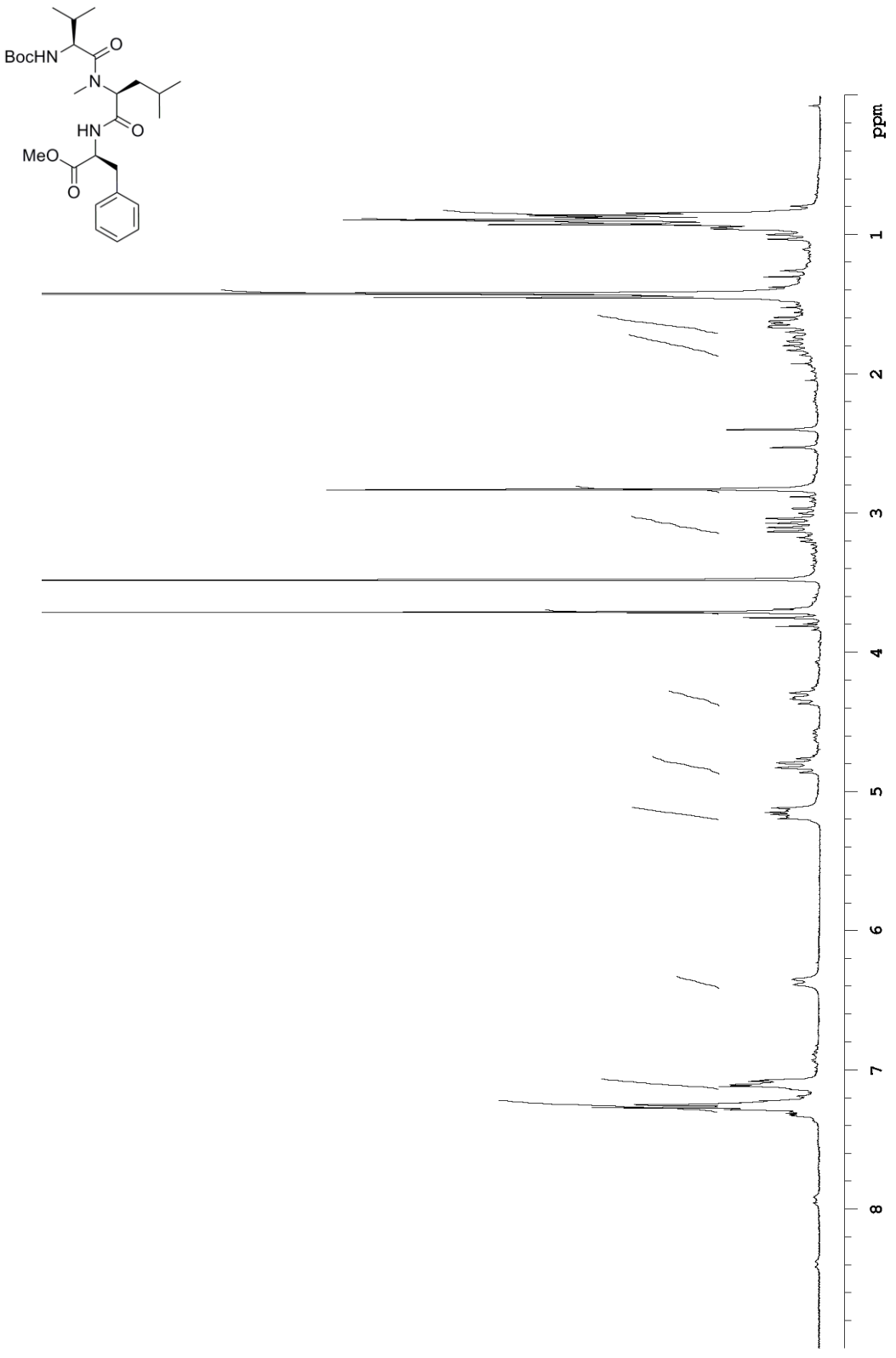
Appendices

Appendix A – Supporting spectra for Chapter 2

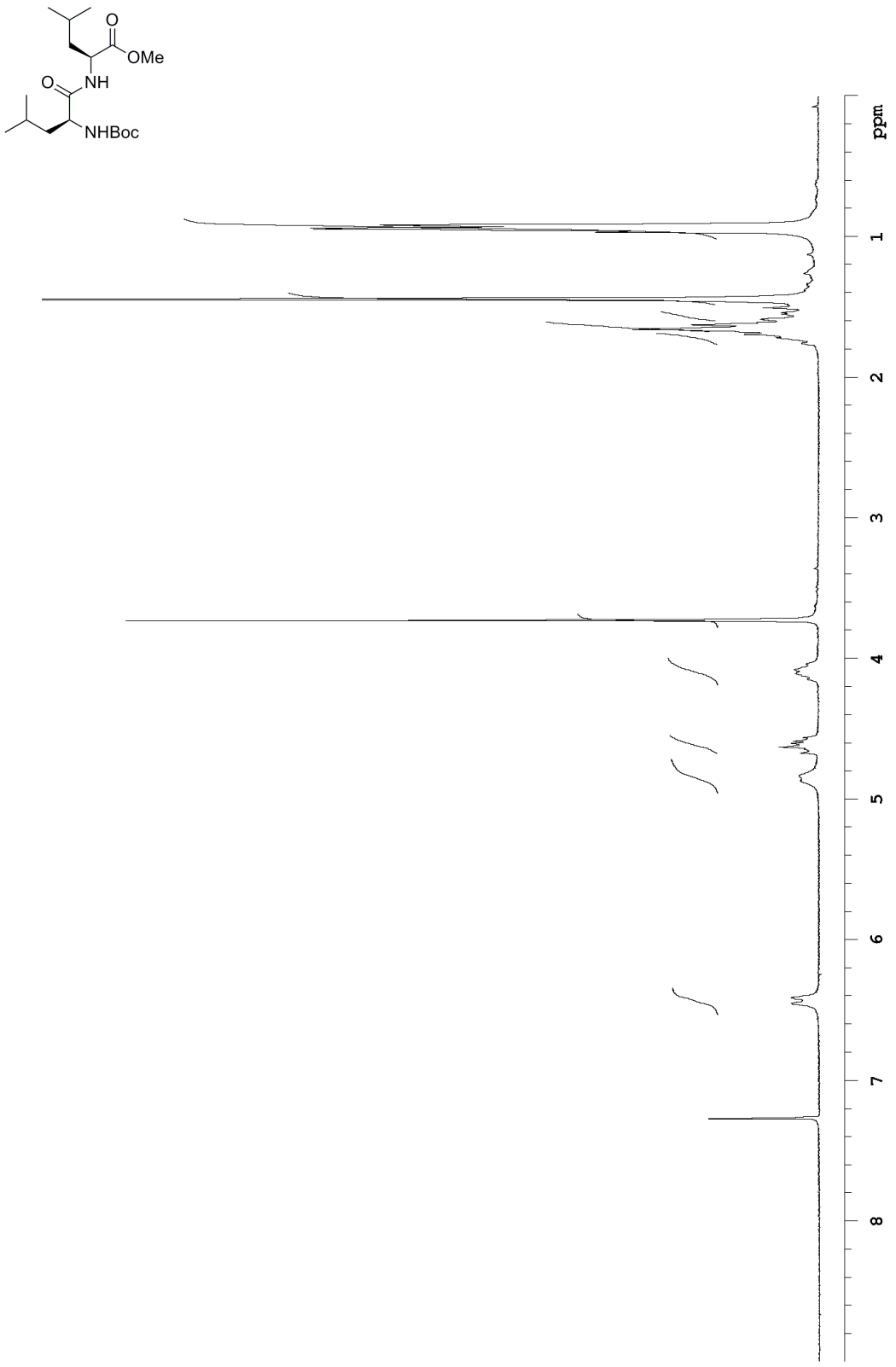
Supporting spectra for SanA 2



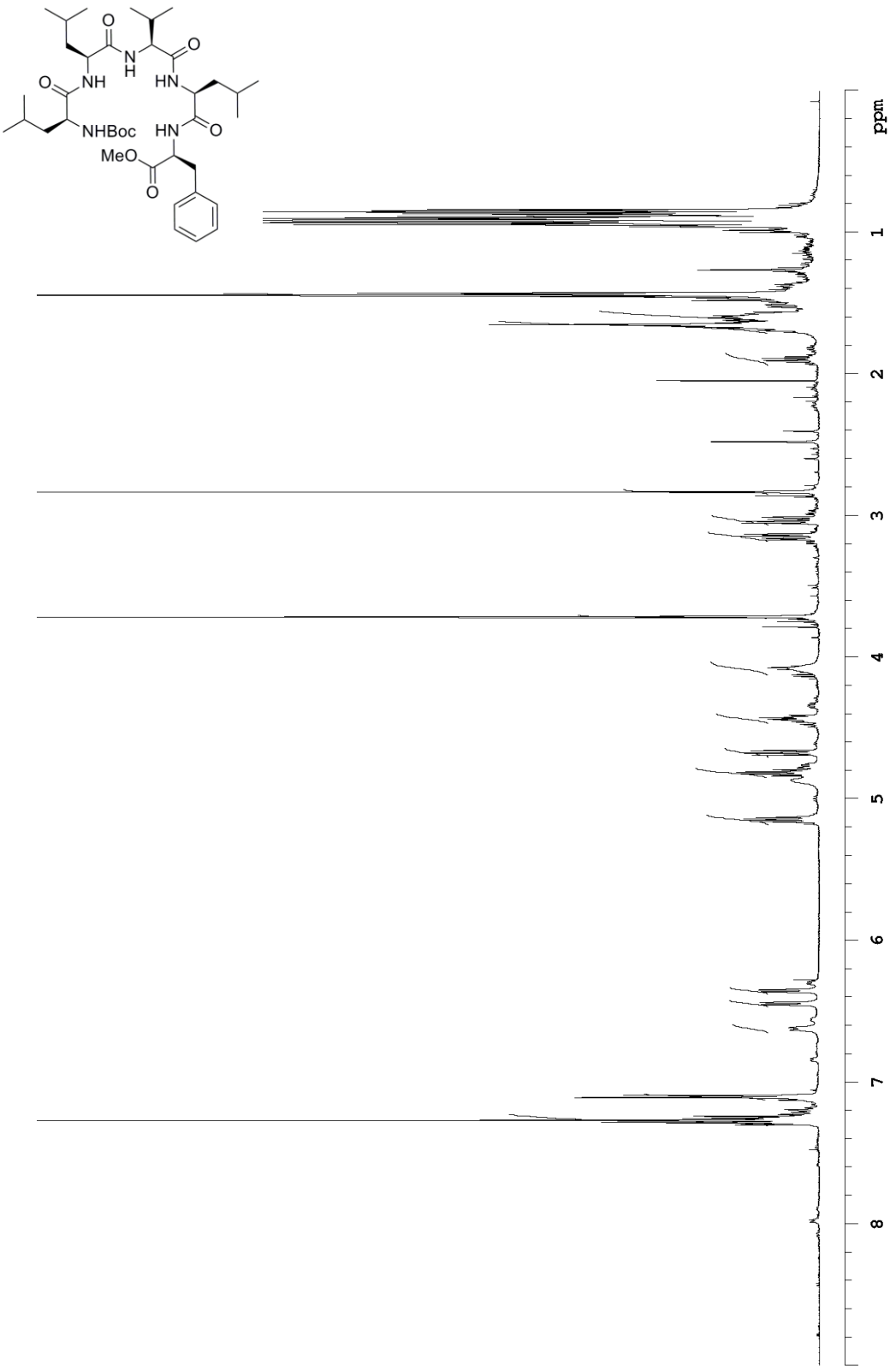
^{1}H NMR MeO-Phe-Leu(NMe)Boc



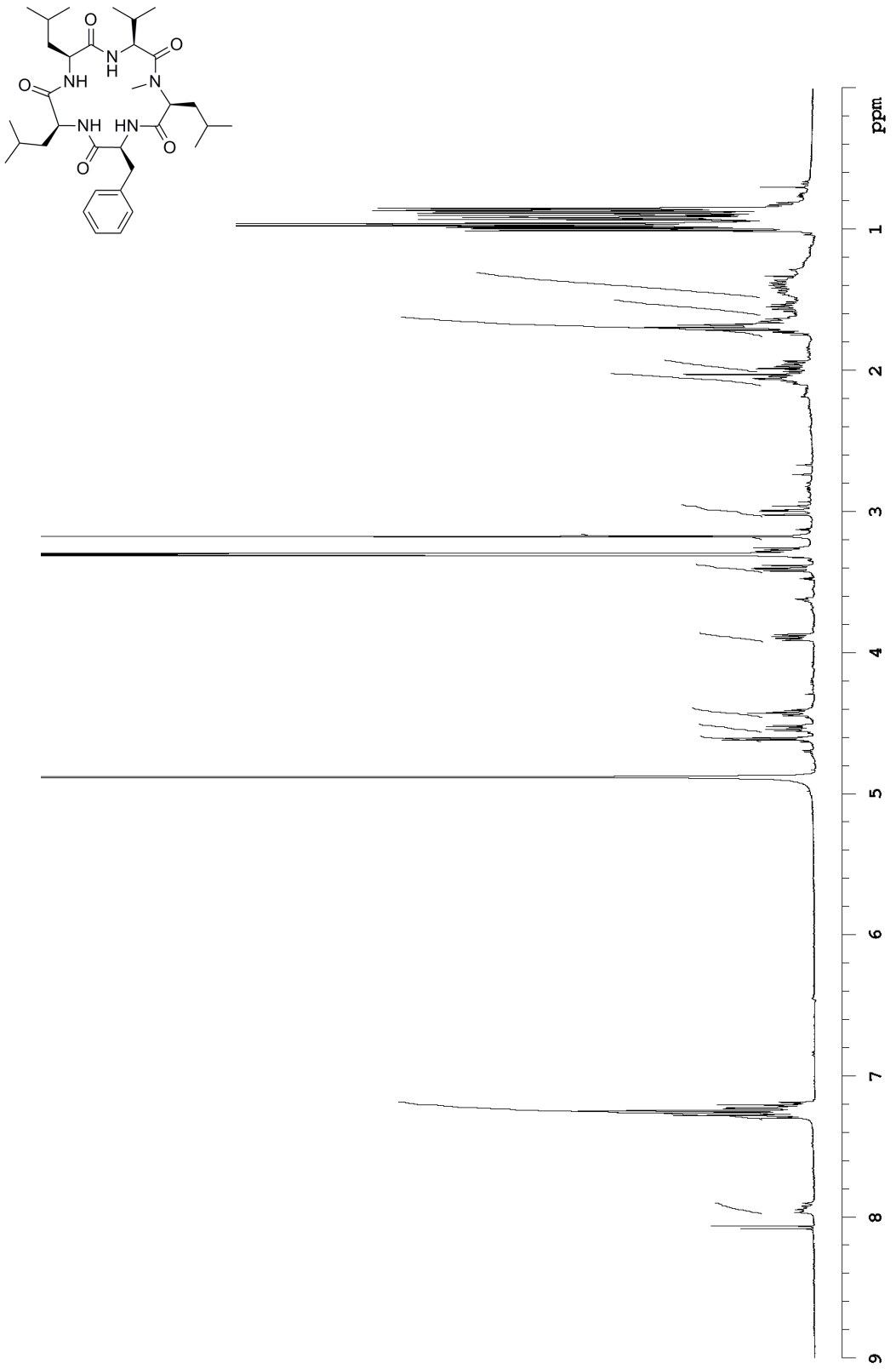
^{1}H NMR MeO-Phe-Leu(NMe)Val-NHBoc



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



^{1}H NMR MeO-Phe-Leu(NMe)Val-Leu-Leu-NHBoc



^{1}H NMR *cyclo*-Phe-Leu(NMe)Val-Leu-Leu

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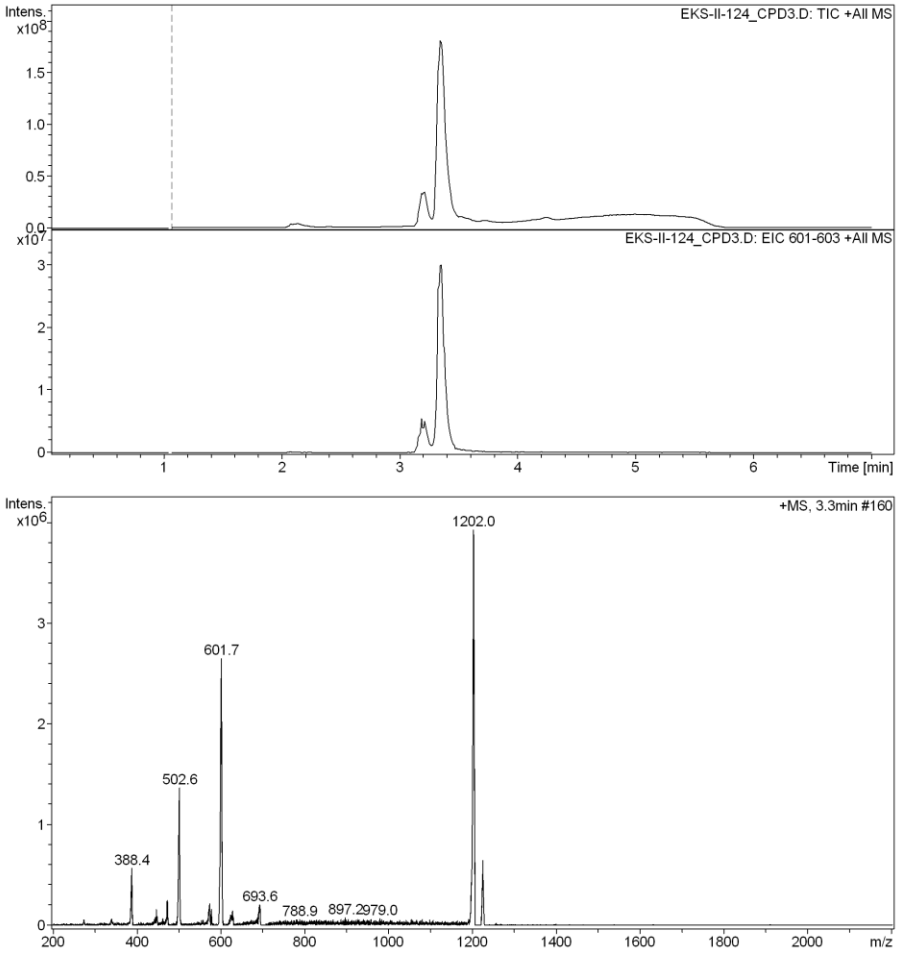
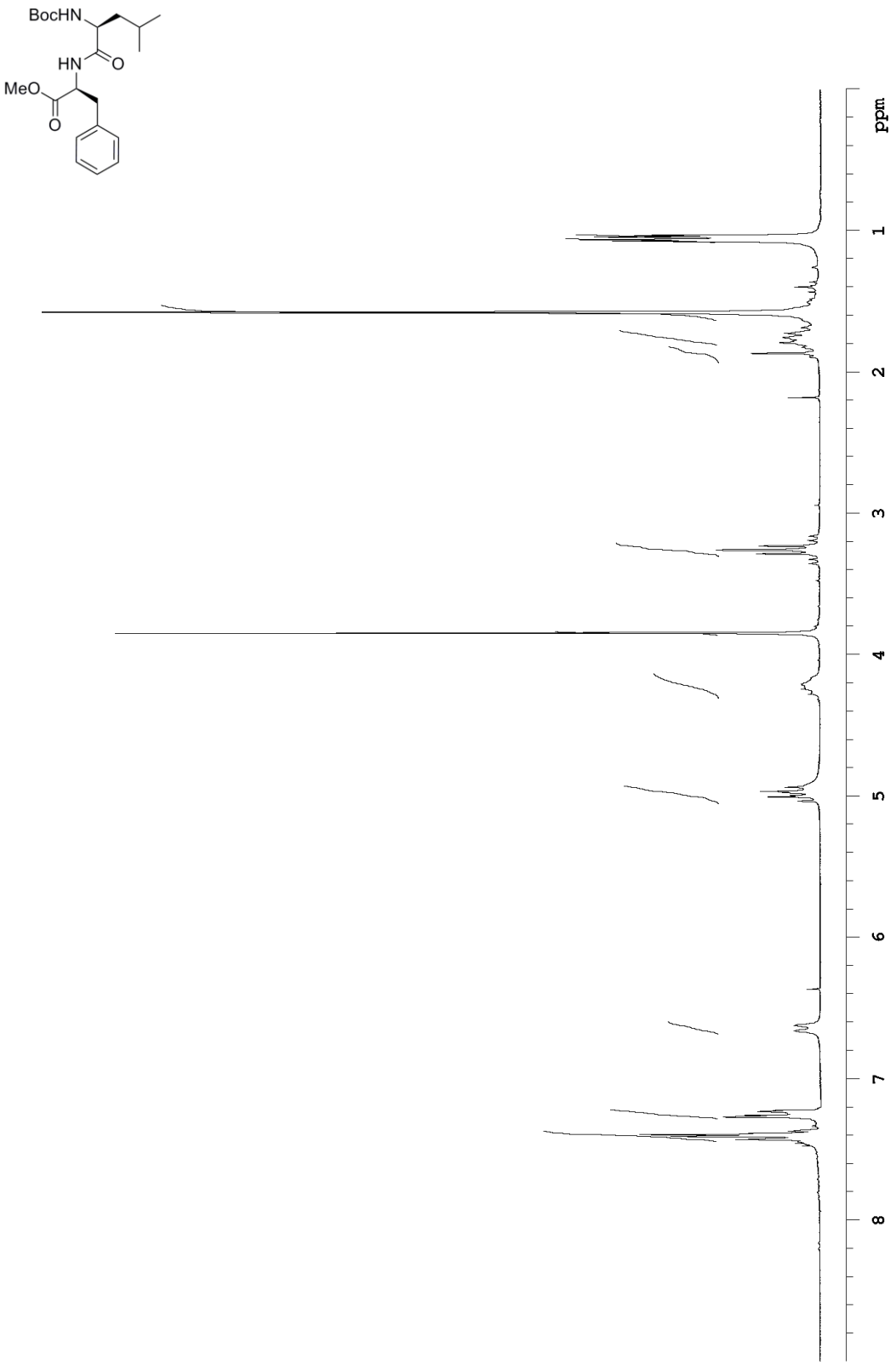
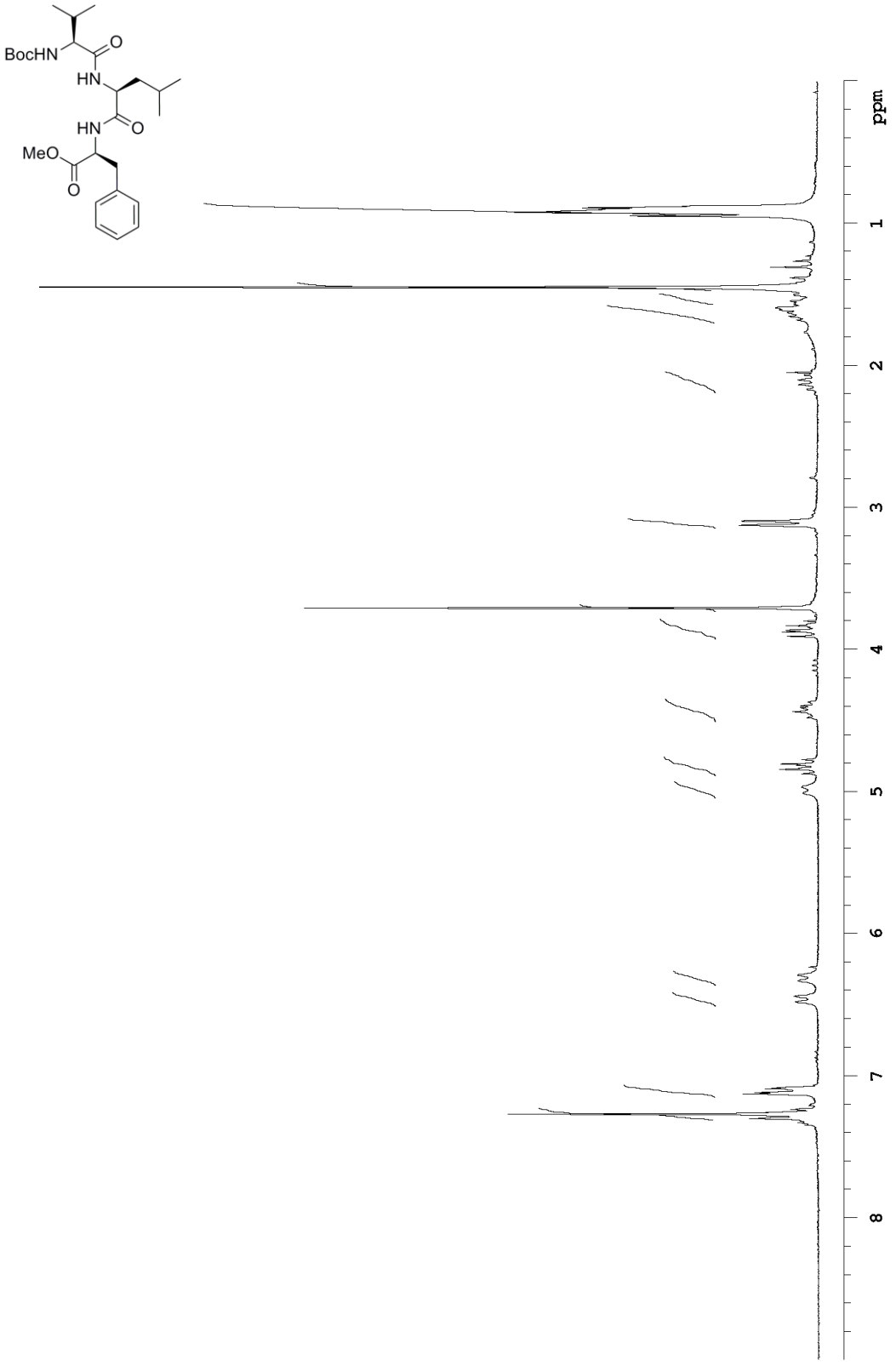


LC/MS *cyclo*-Phe-Leu(NMe)Val-Leu-Leu

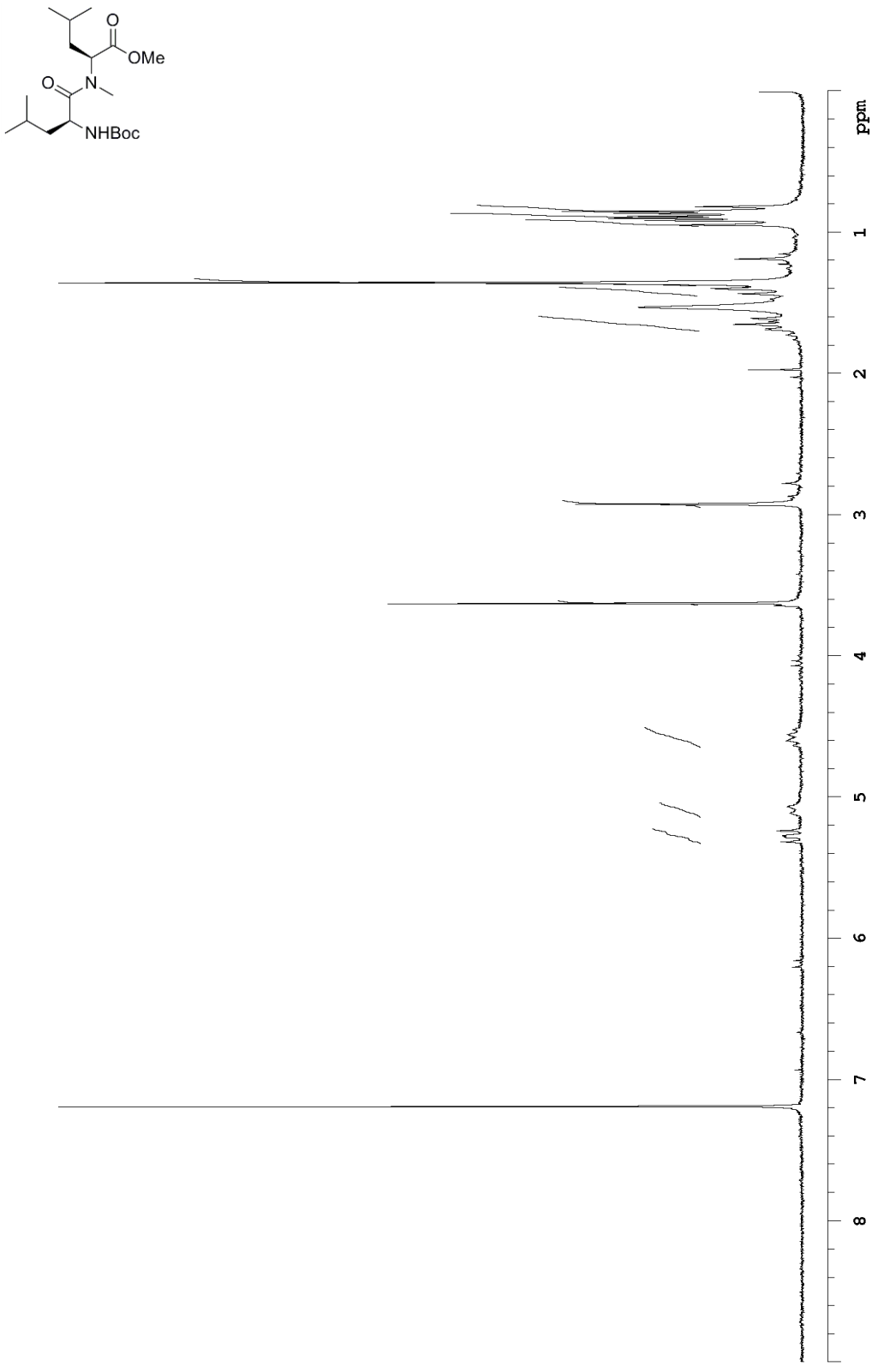
Supporting spectra for SanA 4



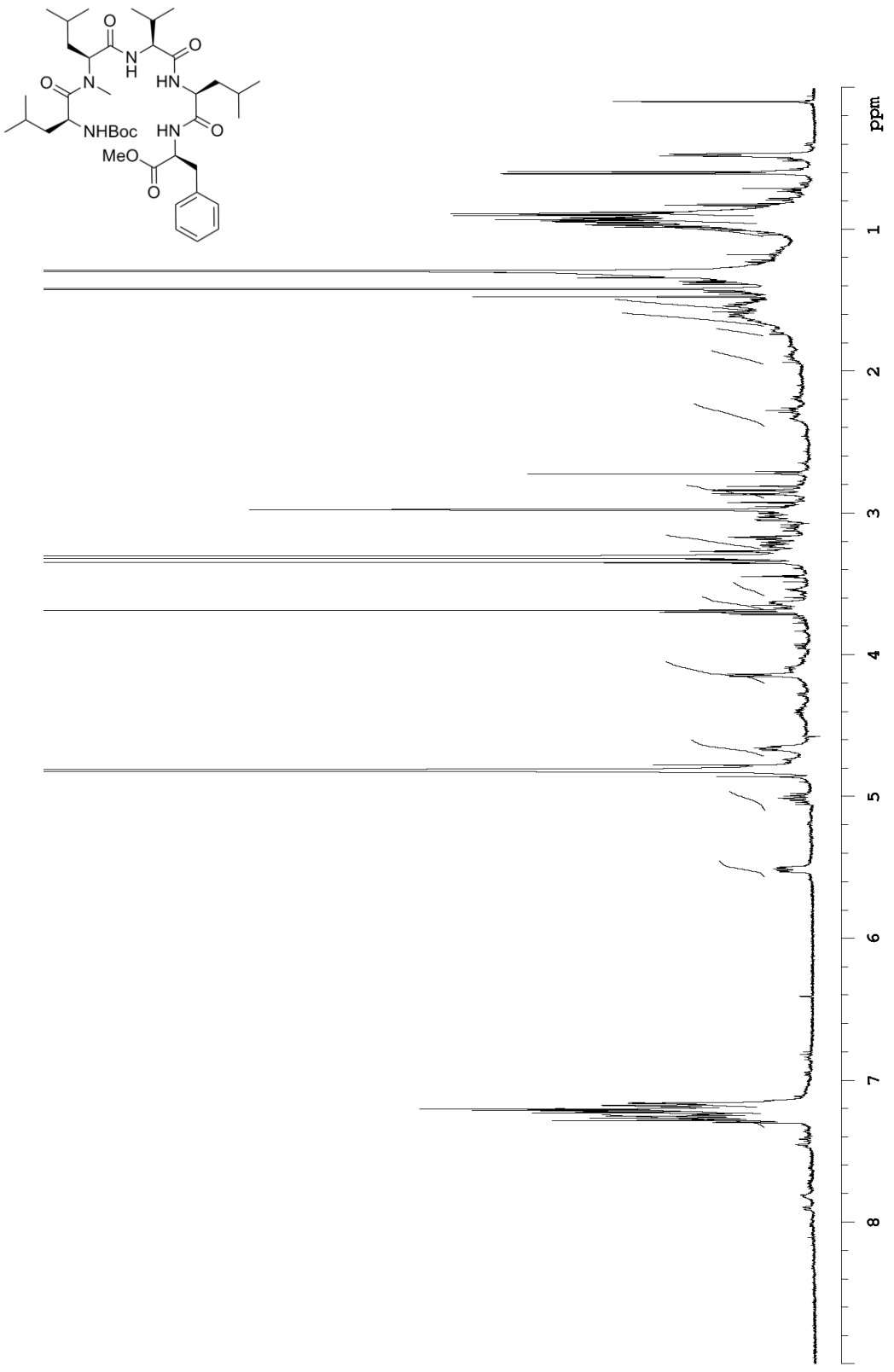
^{1}H NMR MeO-Phe-Leu-NHBoc



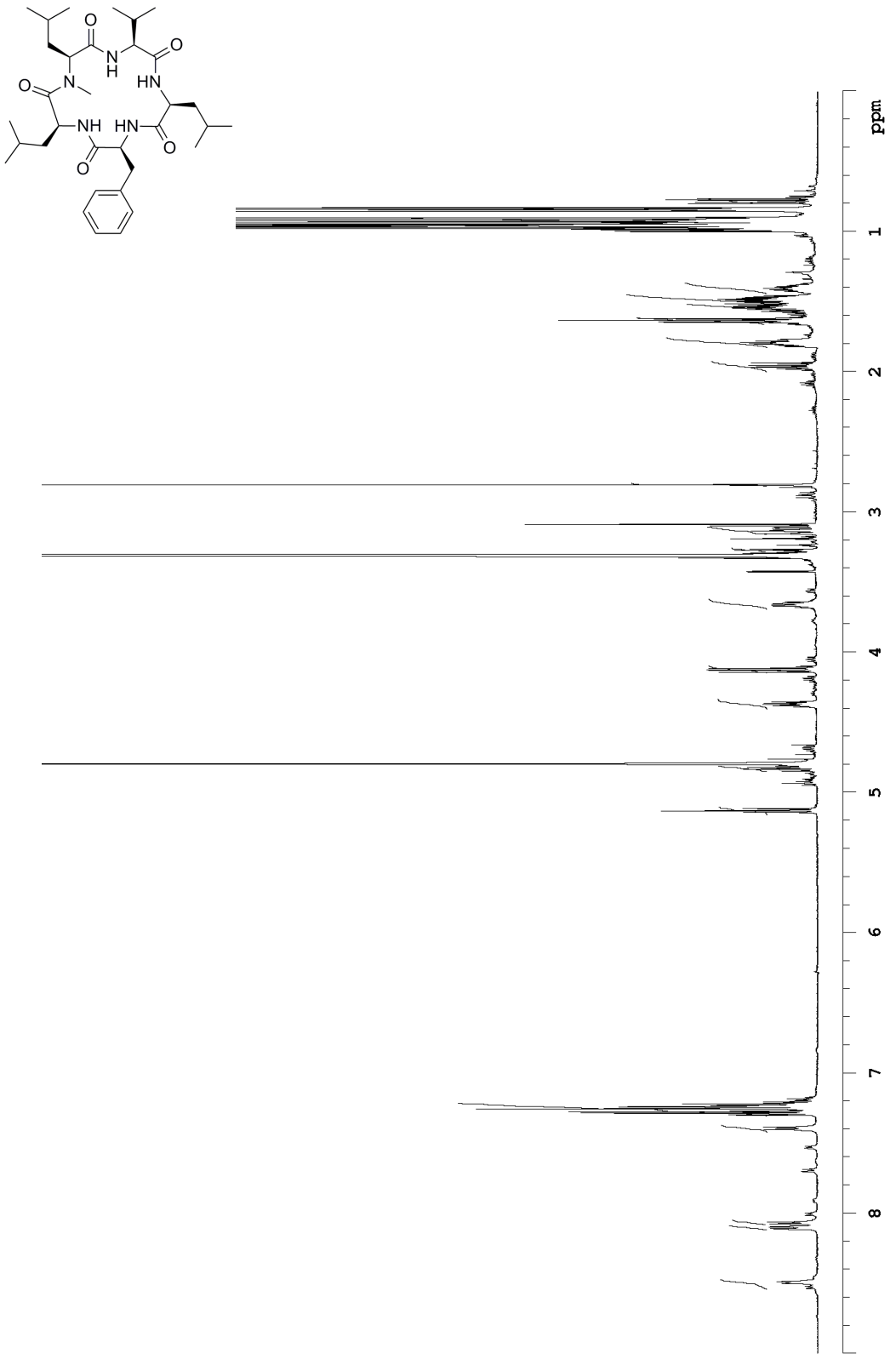
^{1}H NMR MeO-Phe-Leu-Val-NHBoc



^{1}H NMR MeO-Leu(NMe)Leu-NHBoc



^{1}H NMR MeO-Phe-Leu-Val-Leu-NMe-Leu-NHBoc



^{1}H NMR *cyclo*-Phe-Leu-Val-Leu(NMe)-Leu

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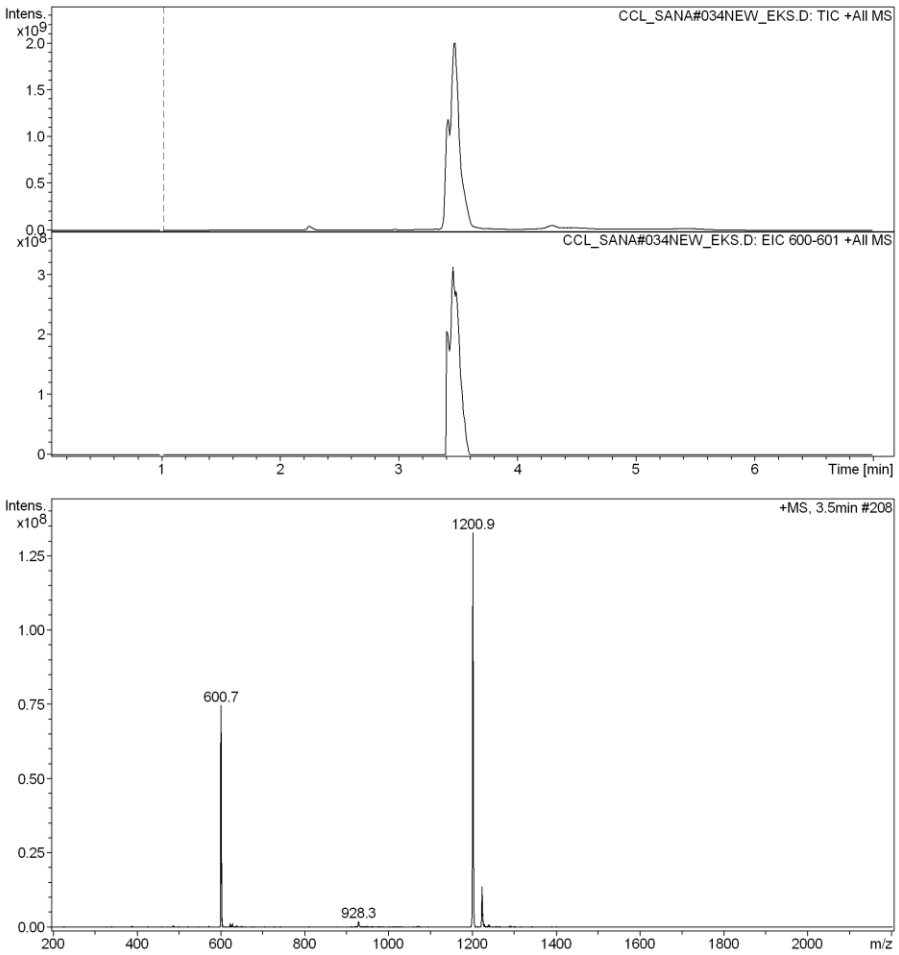
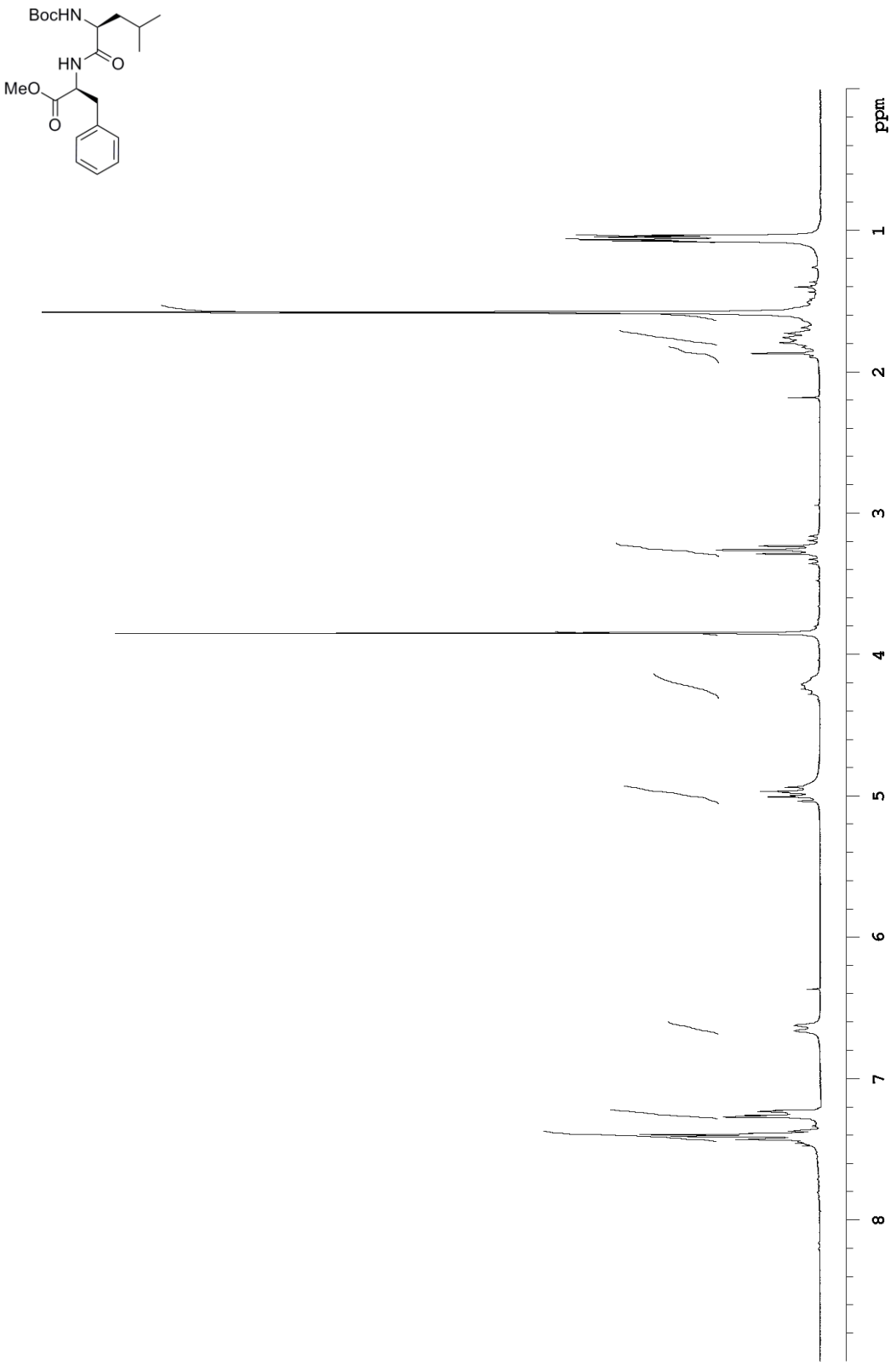
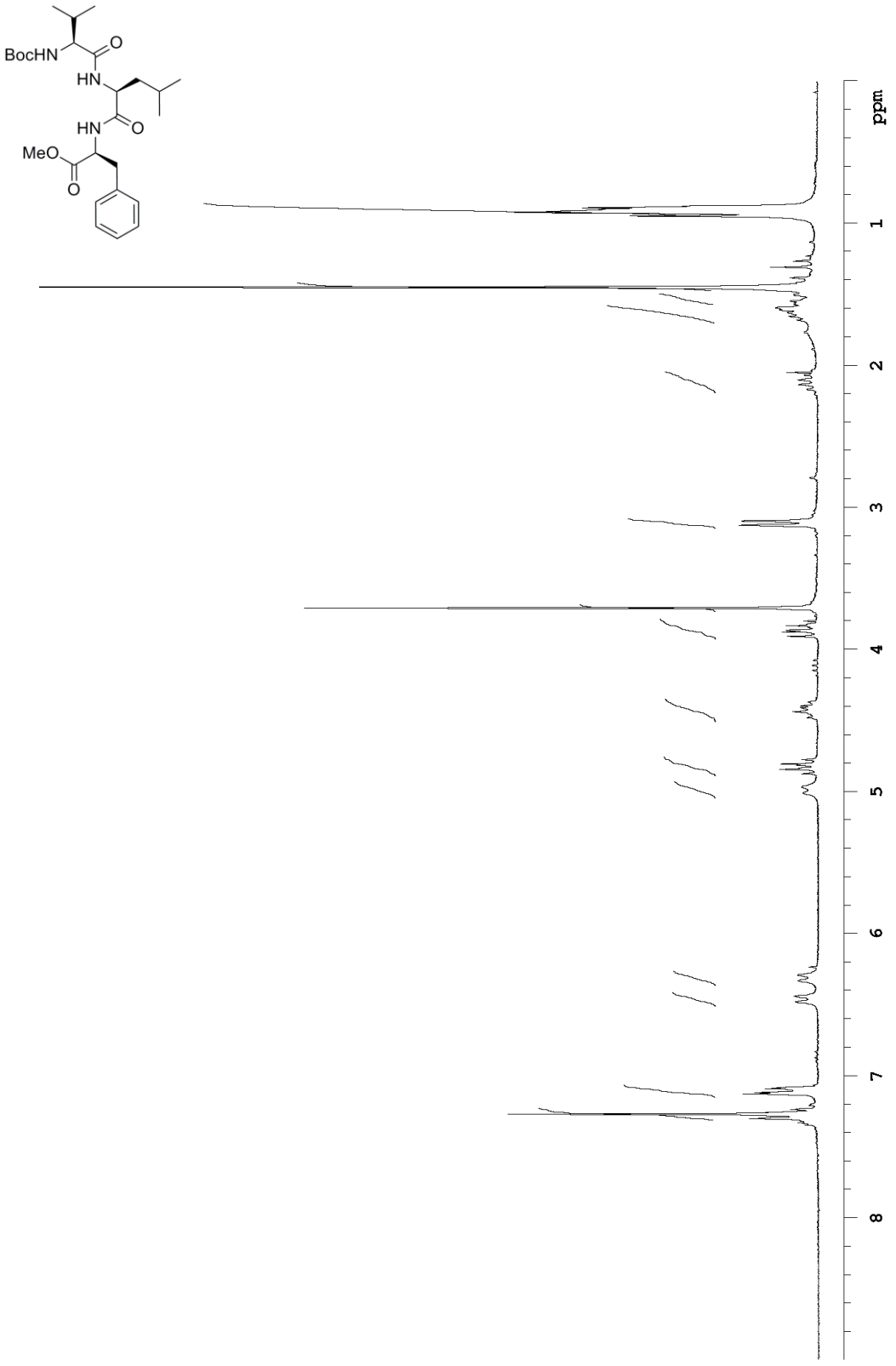


LC/MS *cyclo*-Phe-Leu-Val-Leu(NMe)-Leu

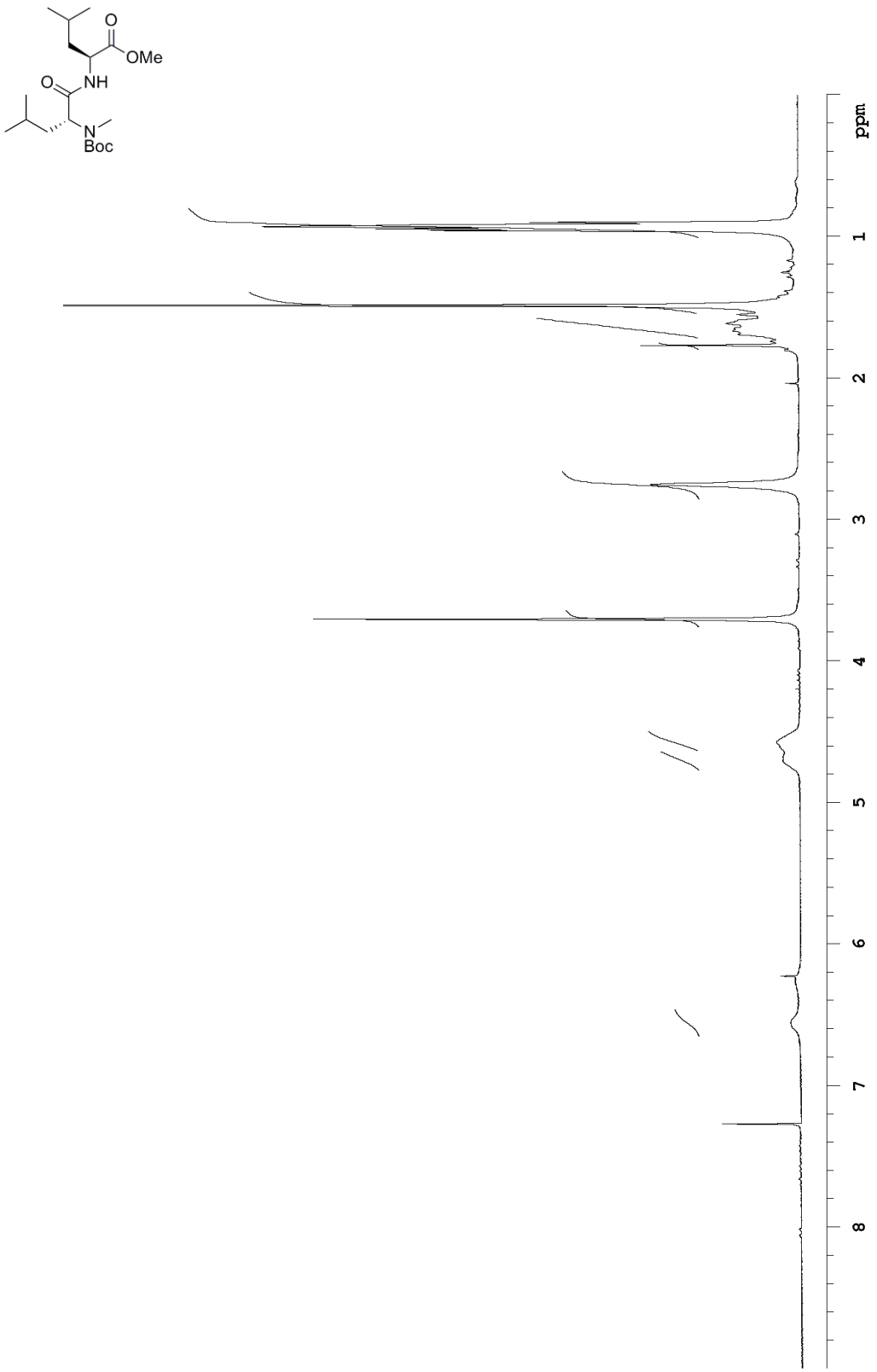
Supporting spectra for SanA 11



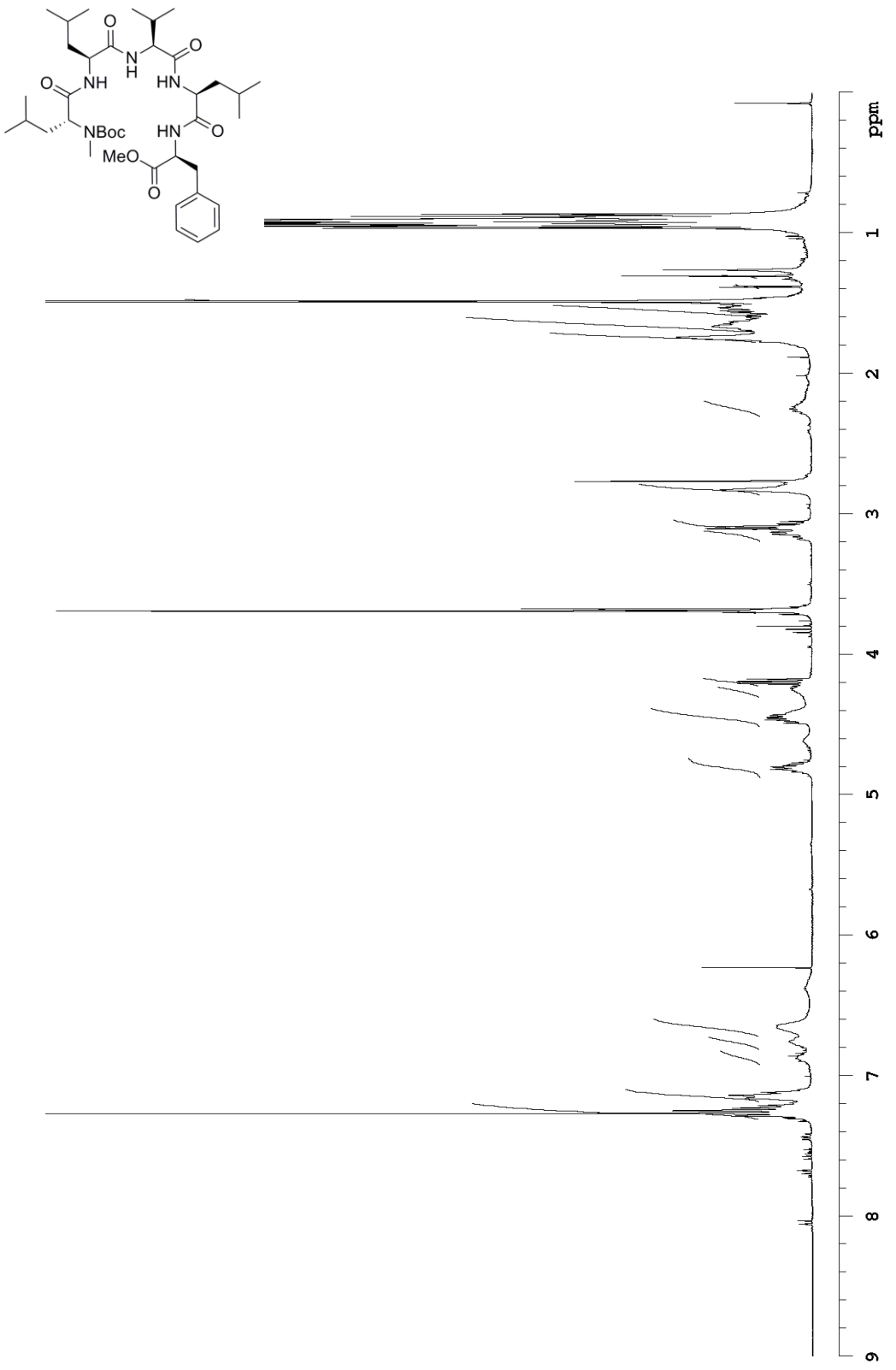
^{1}H NMR MeO-Phe-Leu-NHBoc



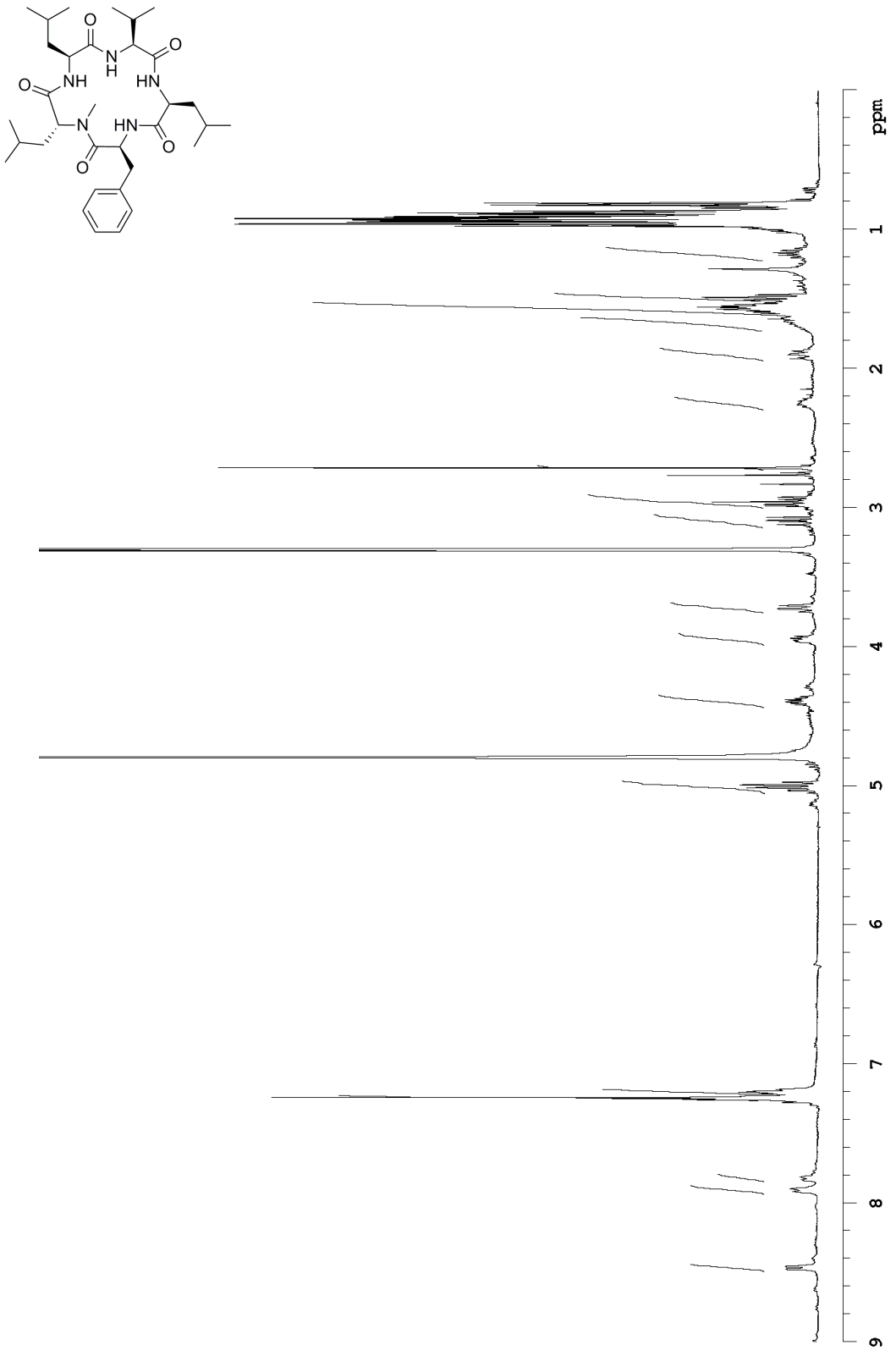
^{1}H NMR MeO-Phe-Leu-Val-NHBoc



^{1}H NMR MeO-Leu-D-Leu(NMe)Boc

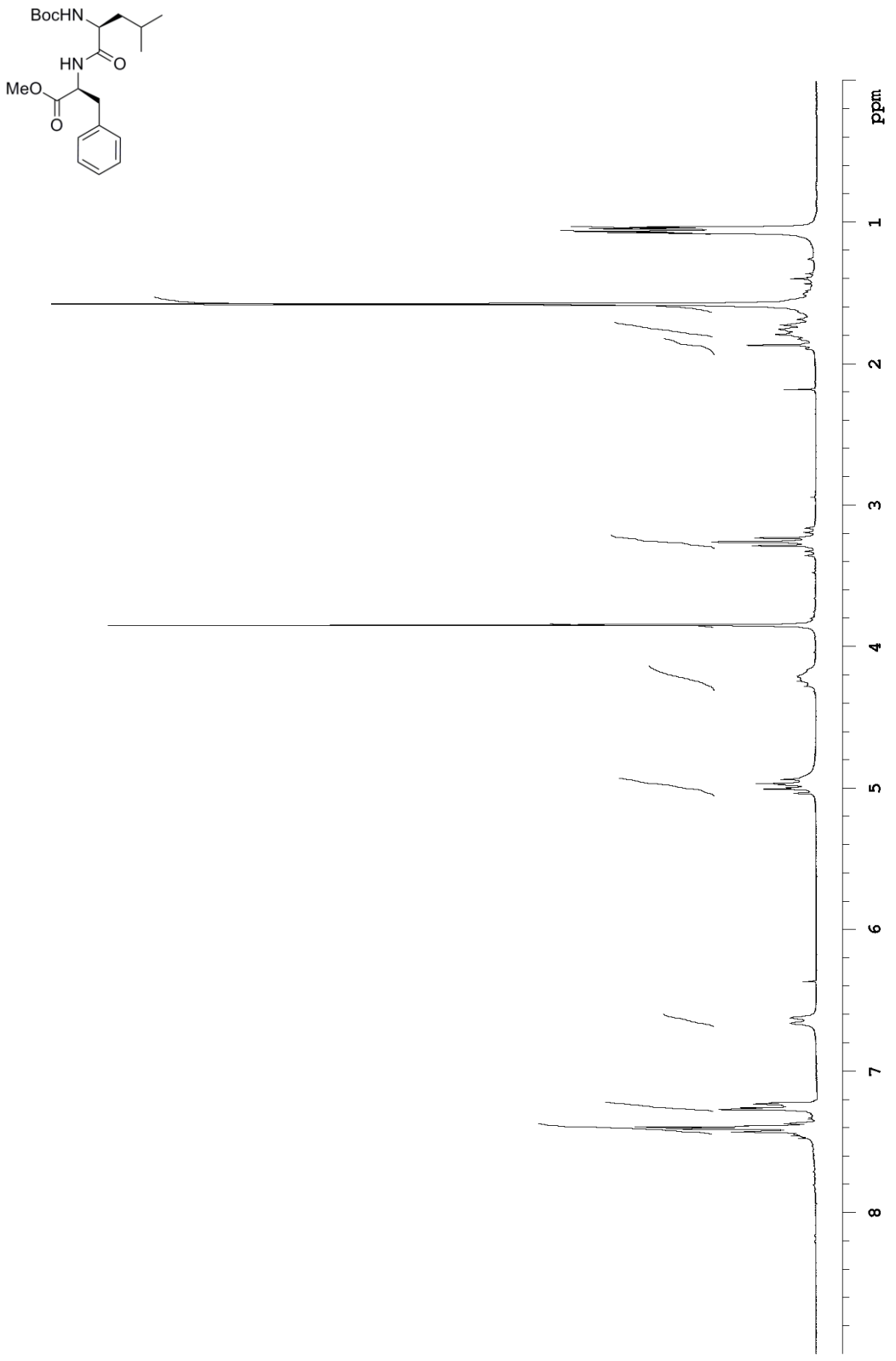


^{1}H NMR MeO-Phe-Leu-Val-Leu-**D**-Leu(NMe)Boc

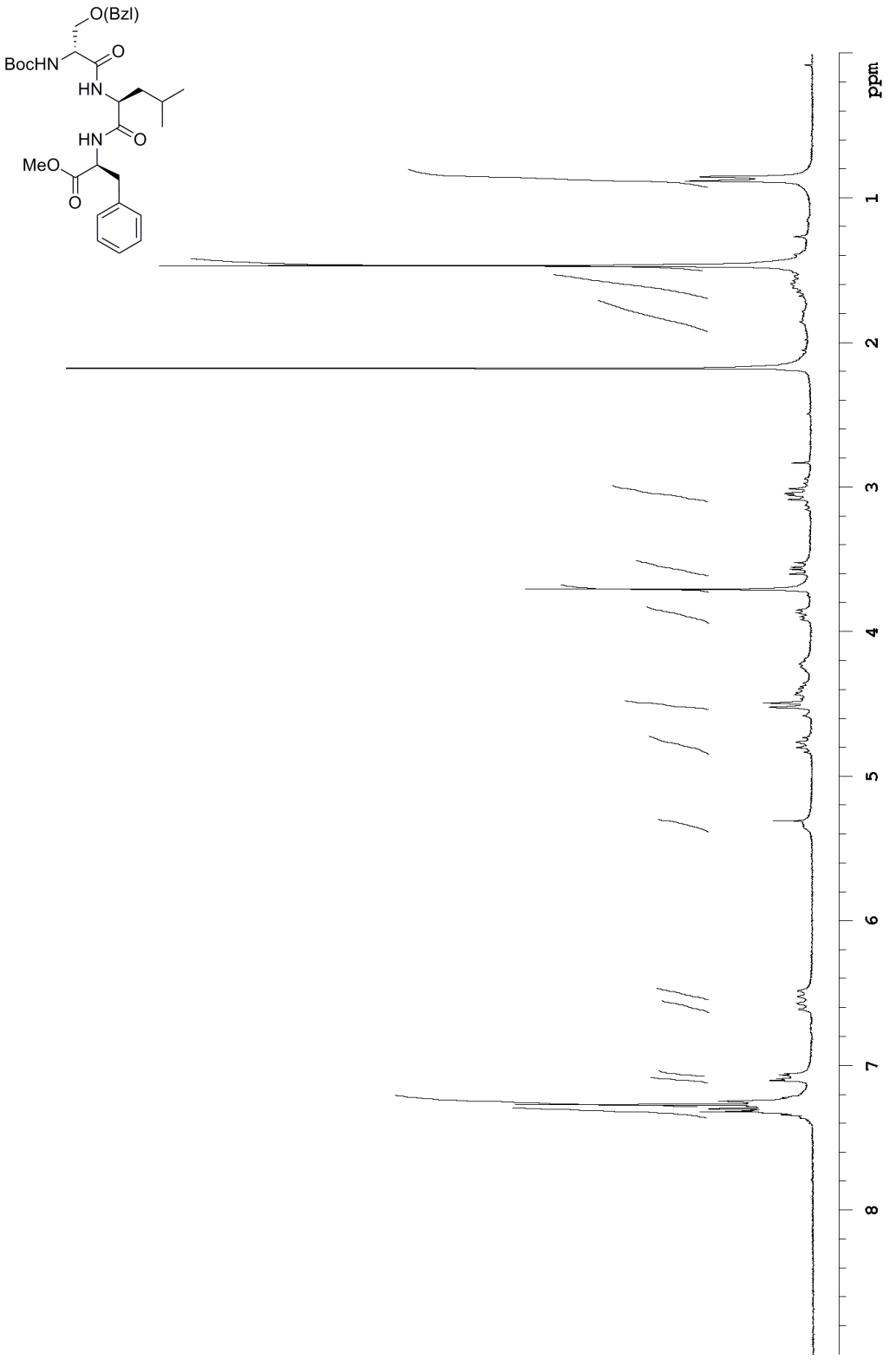


^{1}H NMR *cyclo*-Phe-Leu-Val-Leu-D-Leu(NMe)

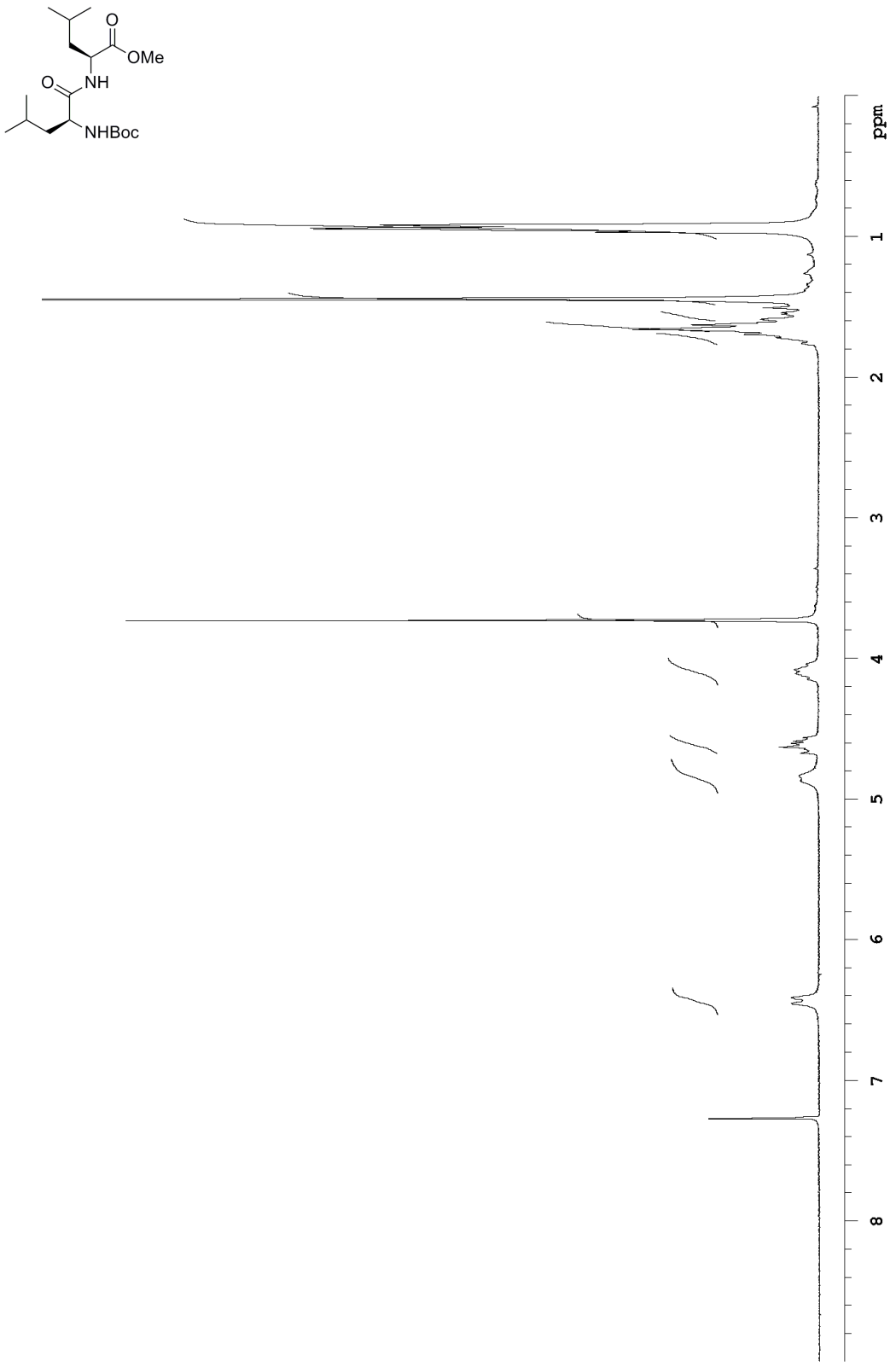
Supporting spectra for SanA 13



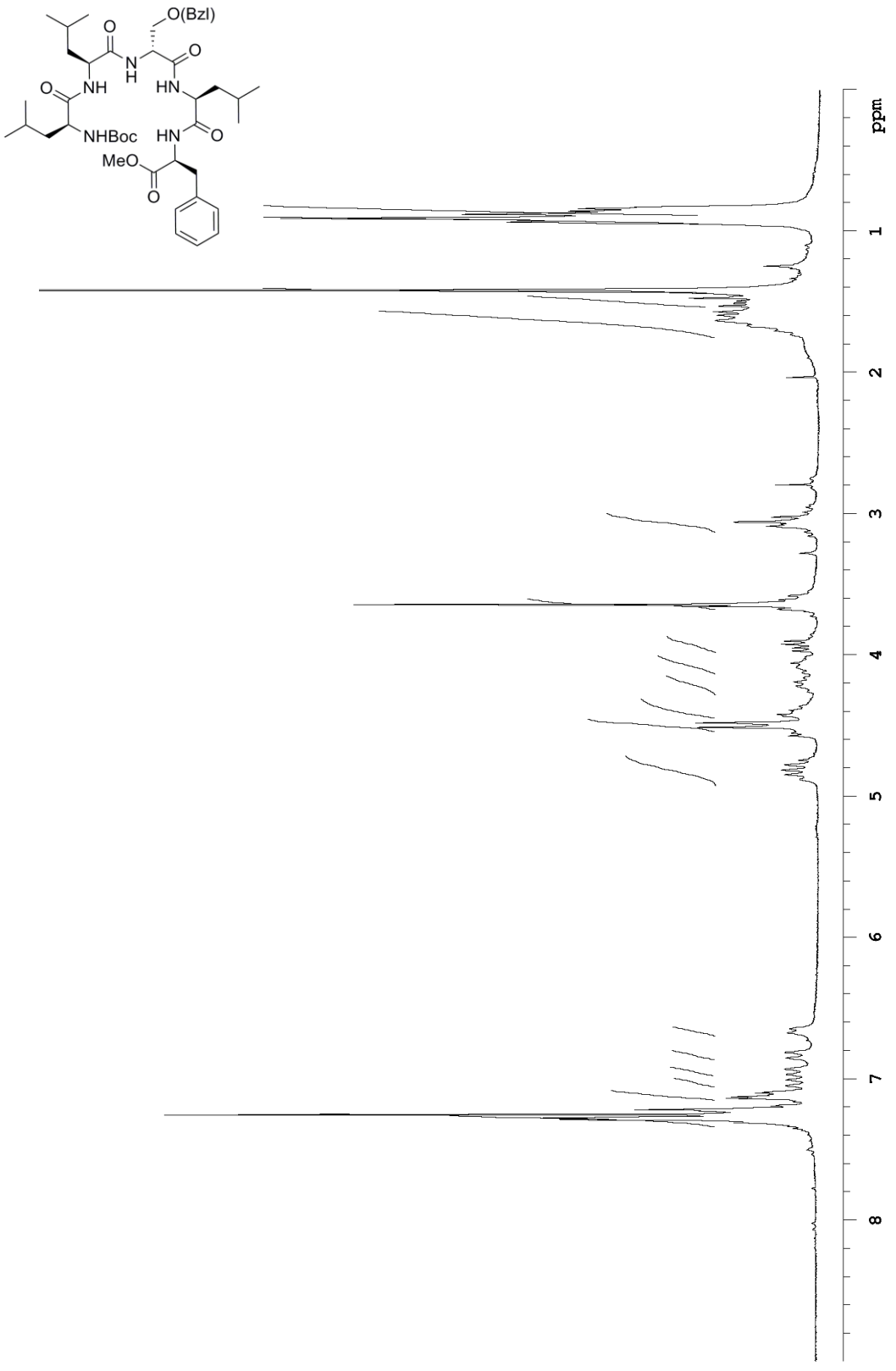
^{1}H NMR MeO-Phe-Leu-NHBoc



^{1}H NMR MeO-Phe-Leu-D-Ser(Bzl)-NHBoc



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



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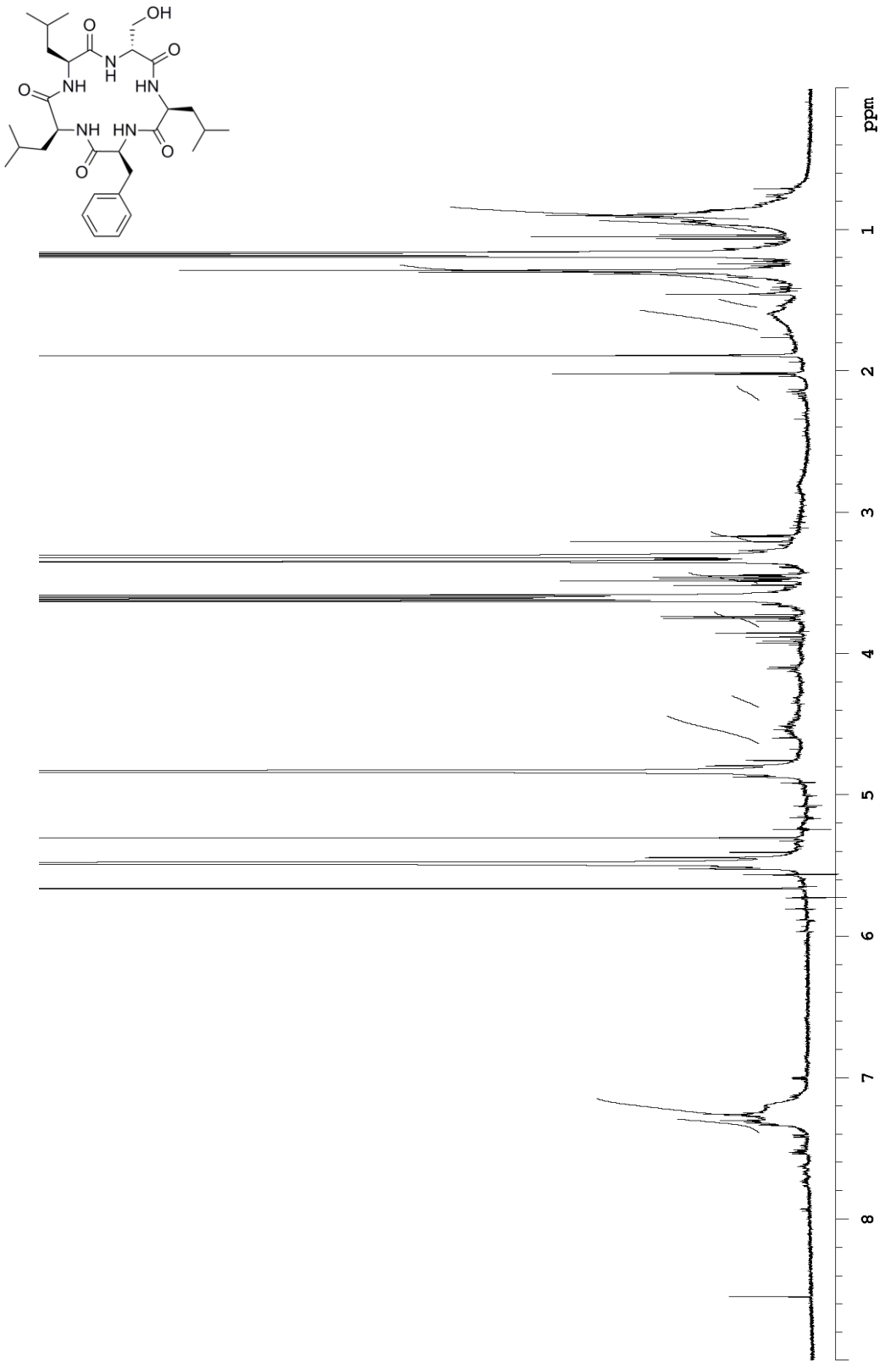
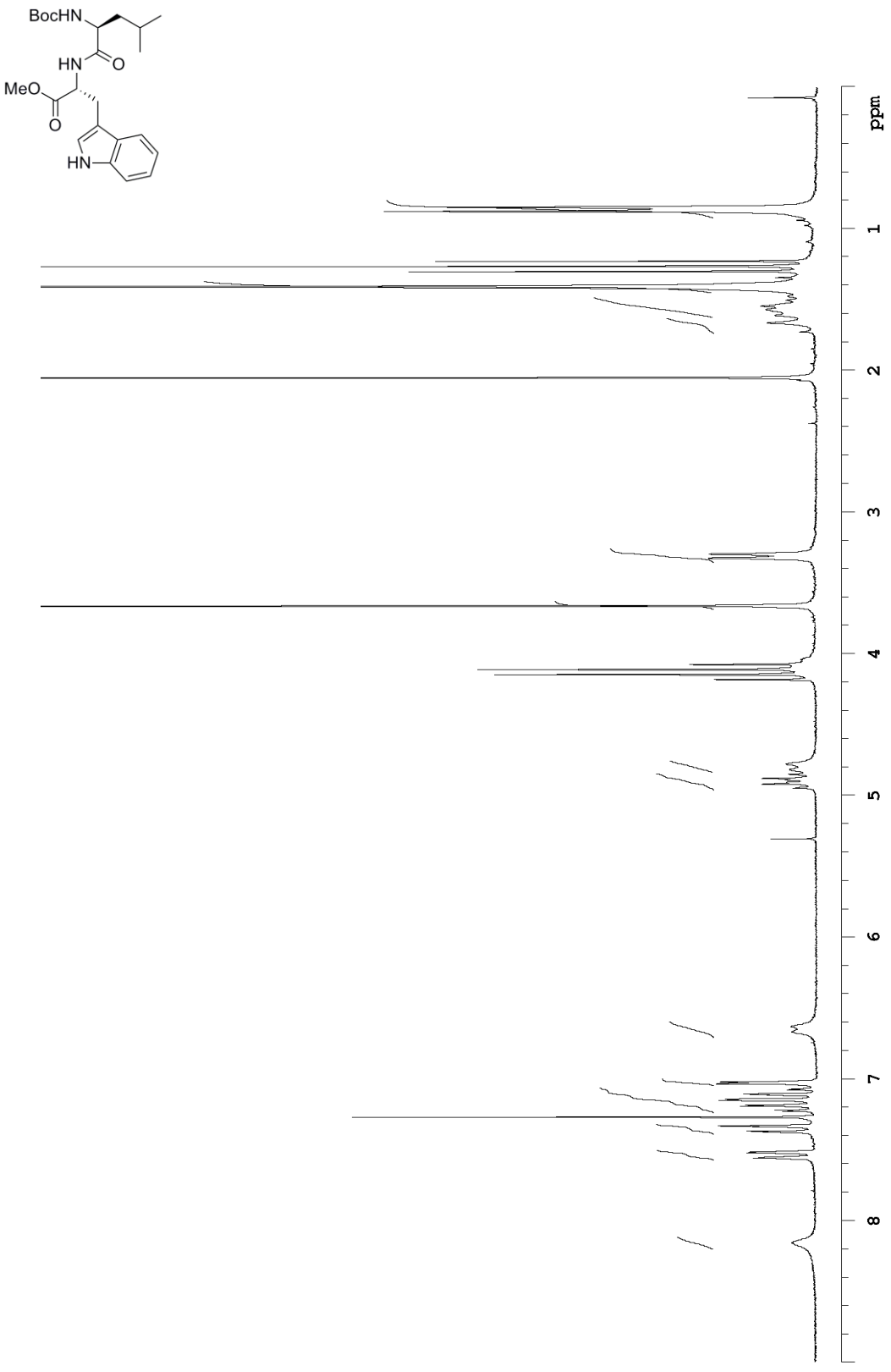
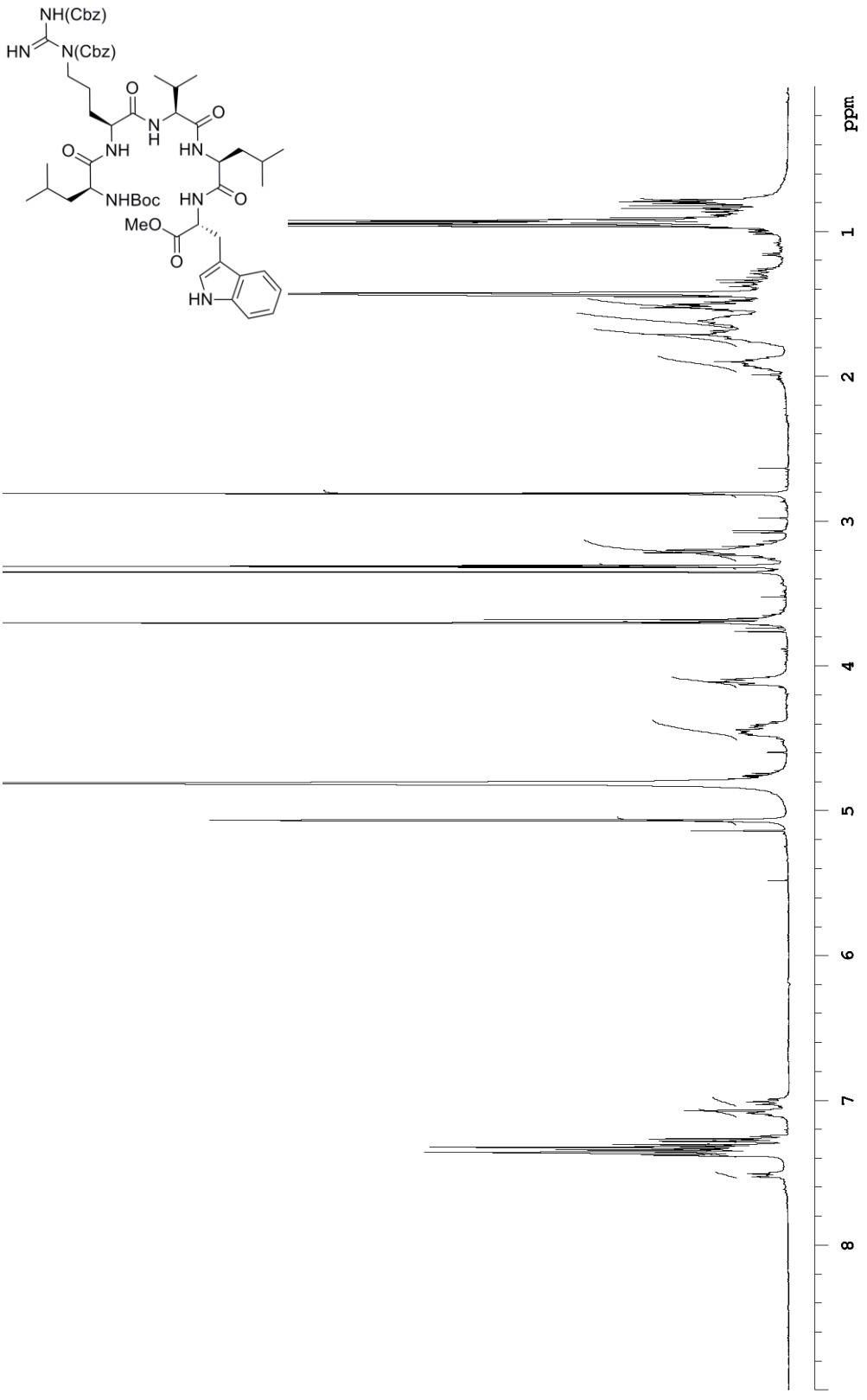


^{1}H NMR *cyclo*-Phe-Leu-D-Ser-Leu-Leu

Supporting spectra for SanA 15



^{1}H NMR MeO-D-Trp-Leu-NHBoc



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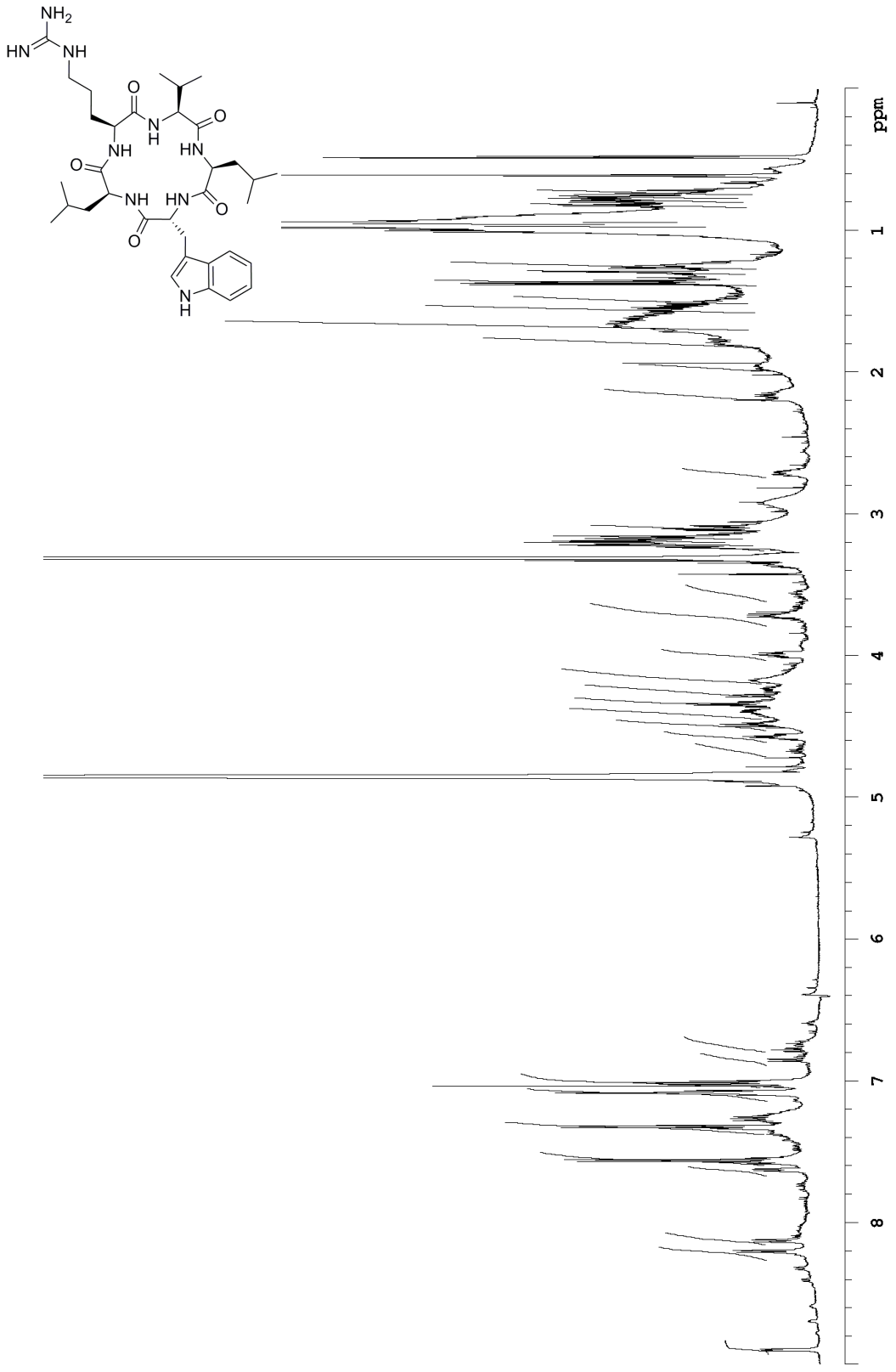

^{1}H NMR *cyclo*-D-Trp-Leu-Val-Arg-Leu

Display Report - All Windows Selected Analysis

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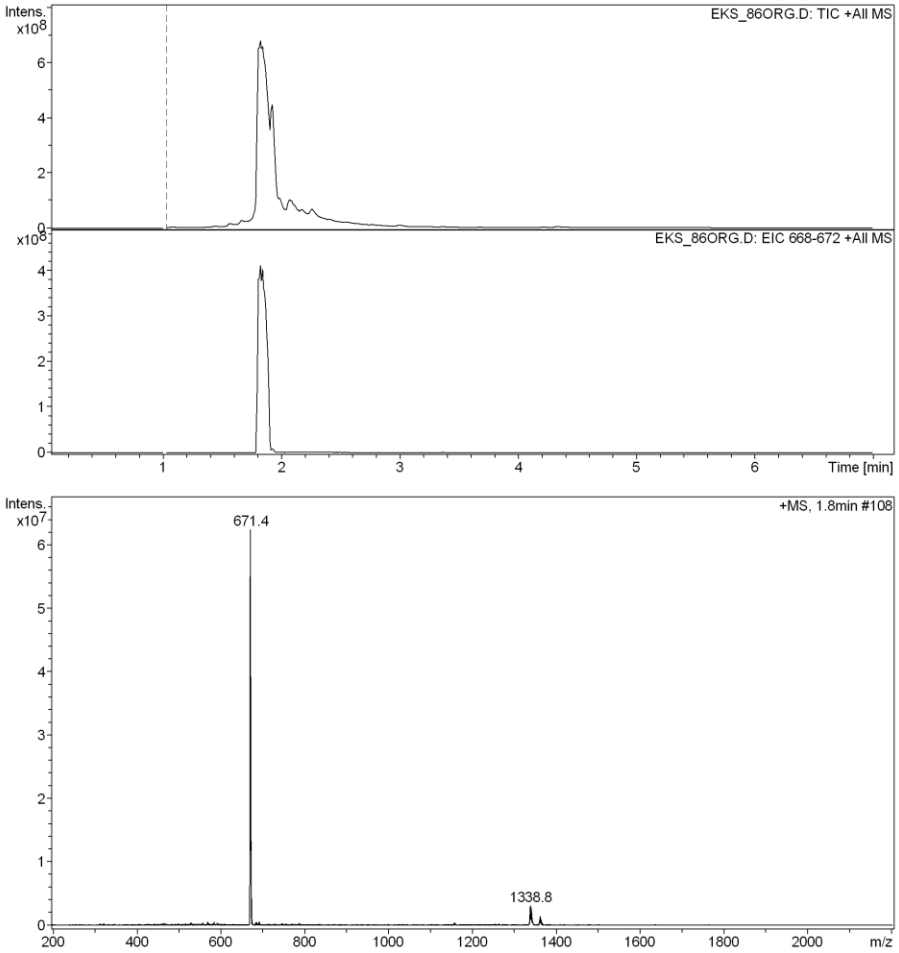
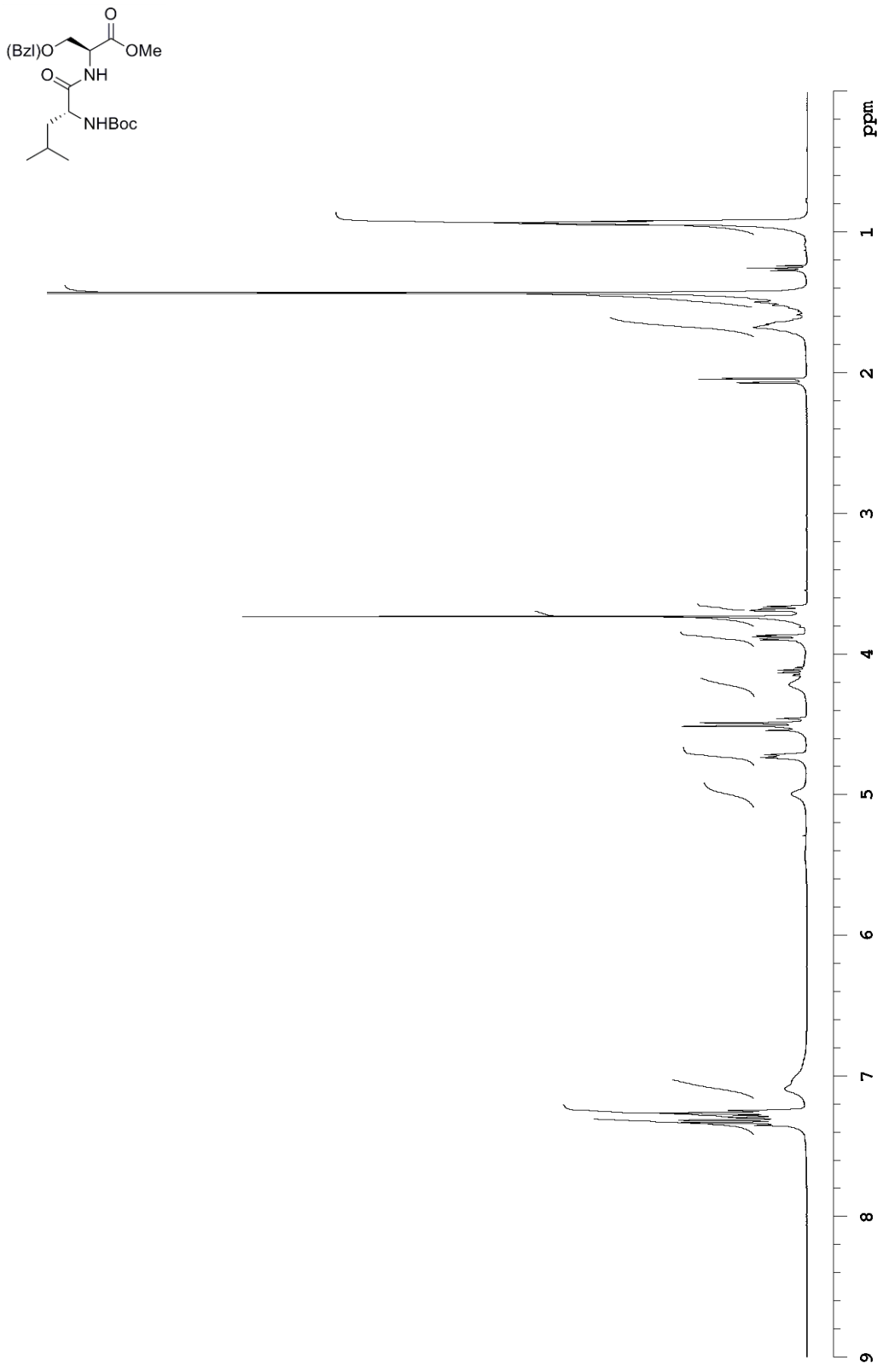
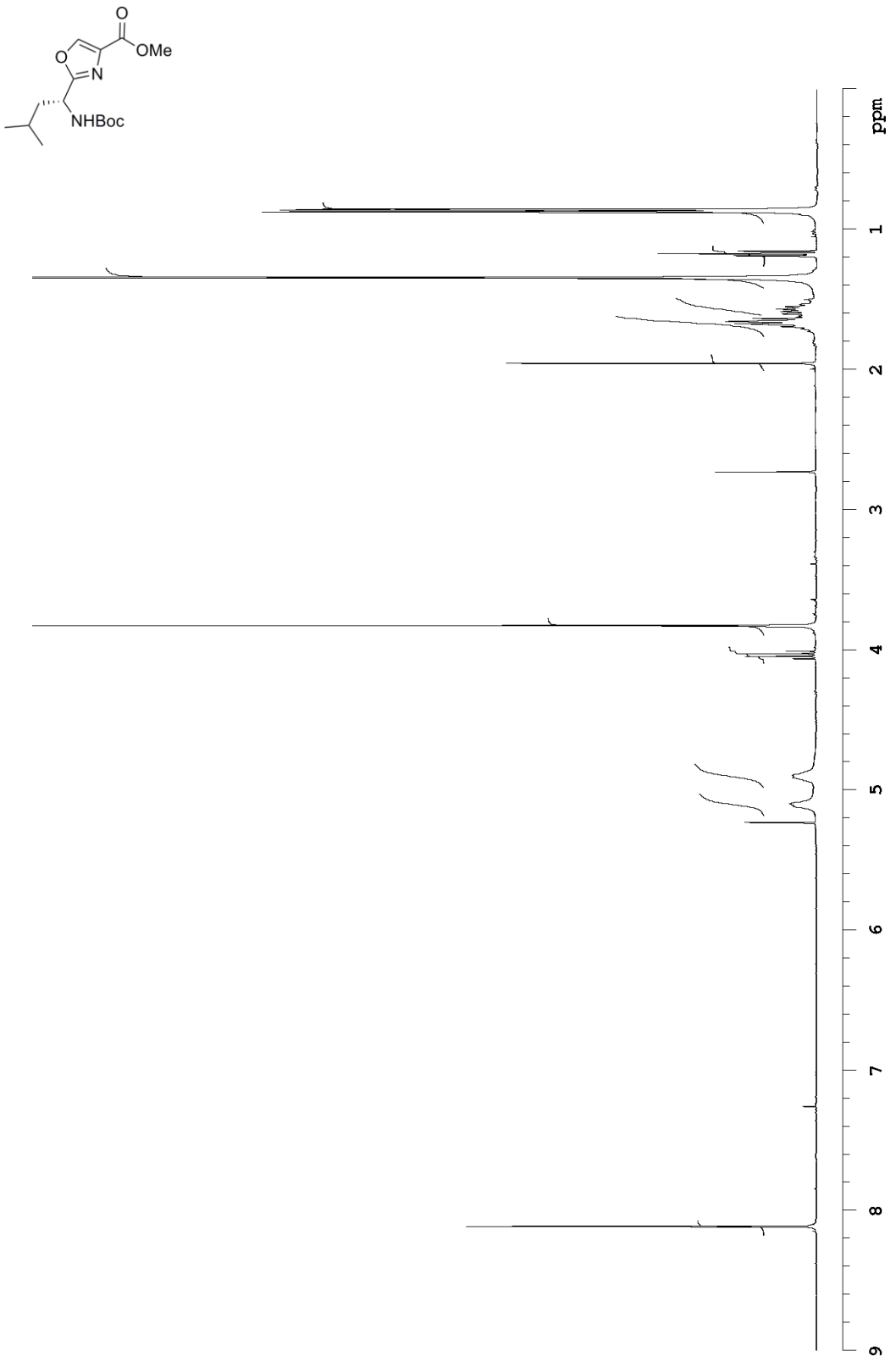


LC/MS *cyclo*-D-Trp-Leu-Val-Arg-Leu

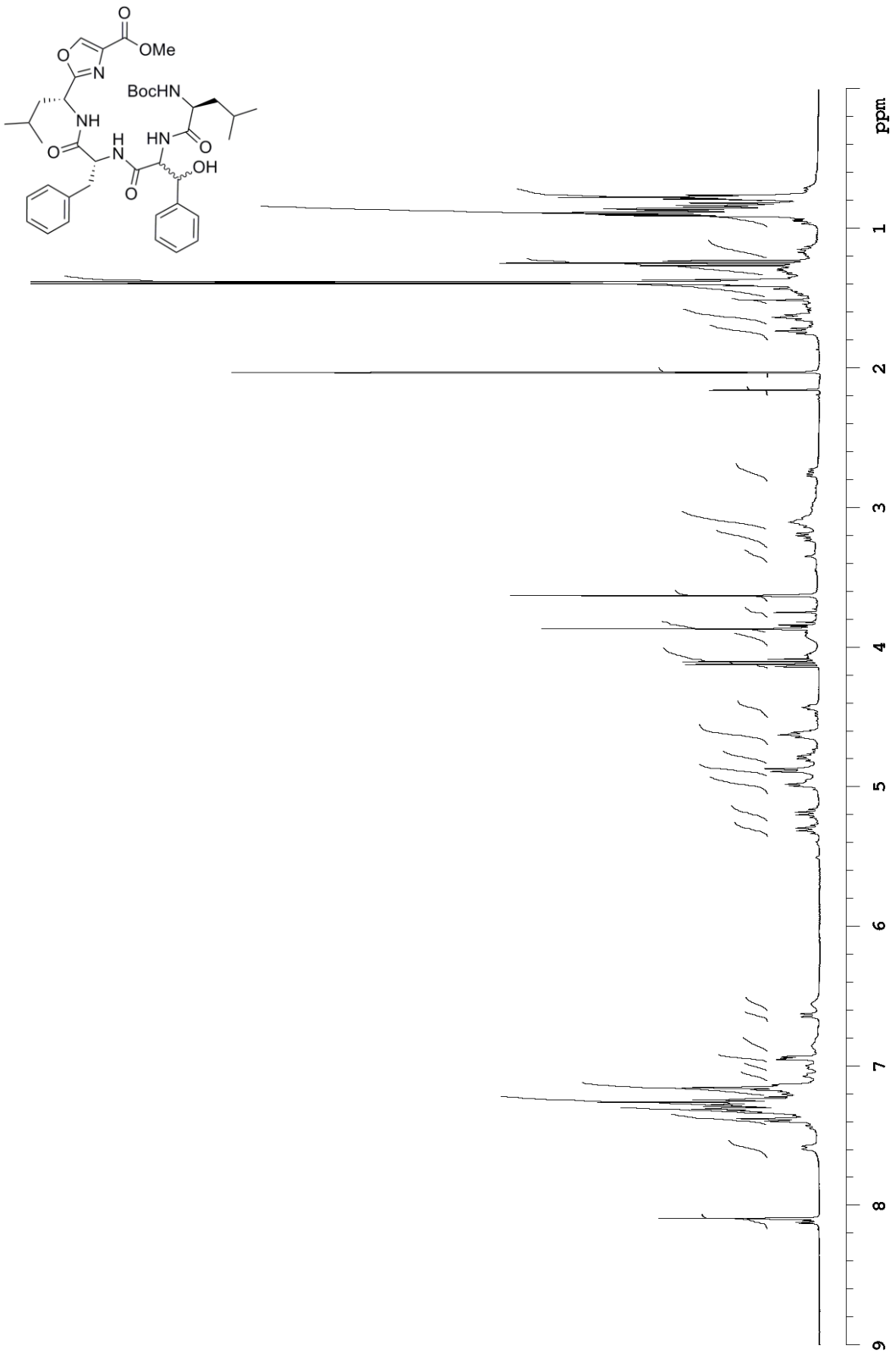
Supporting spectra for SanA 17-III-Ox



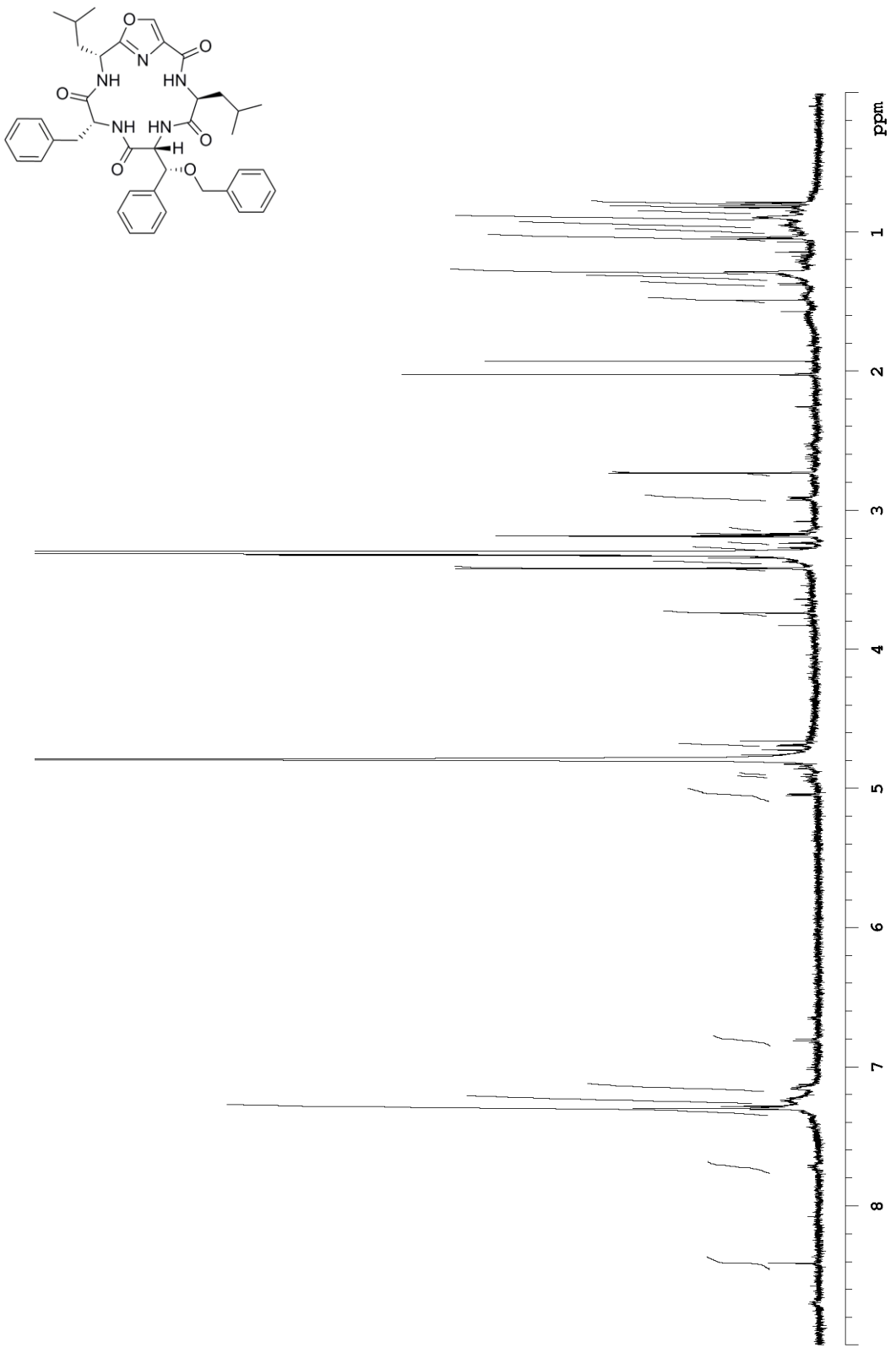
^{1}H NMR MeO-Ser(Bzl)-D-Leu-NHBoc



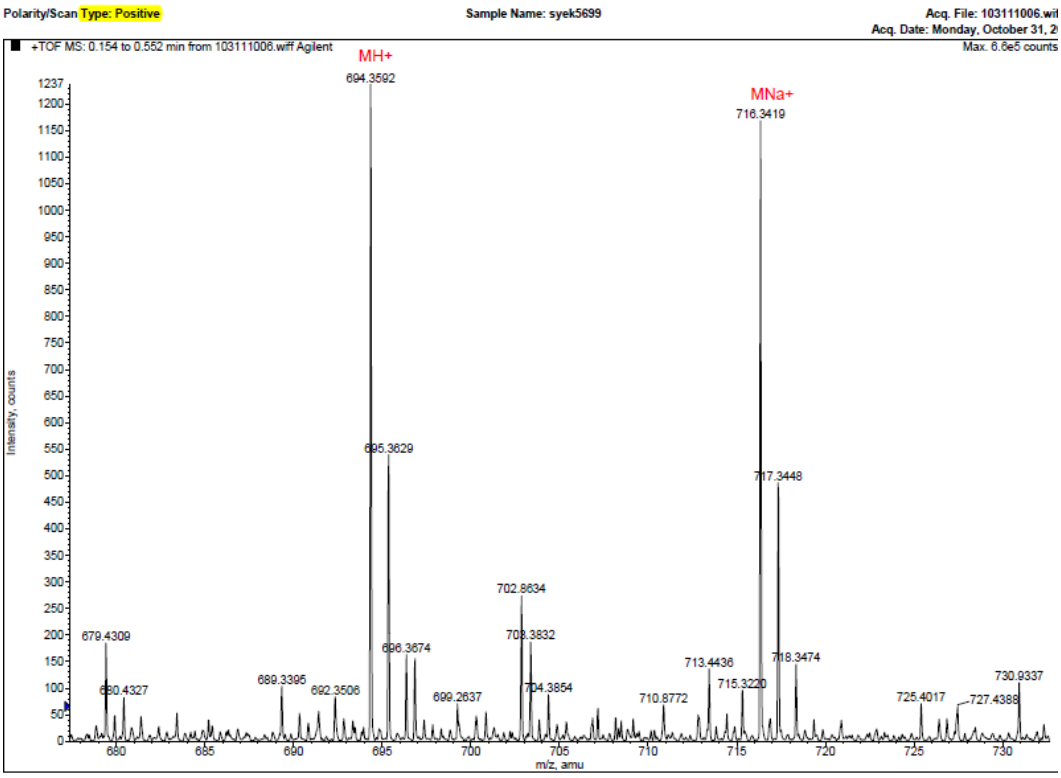
^{1}H NMR MeO-Ox-D-Leu-NHBoc



^{1}H NMR MeO-Ox-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-Leu-NHBoc

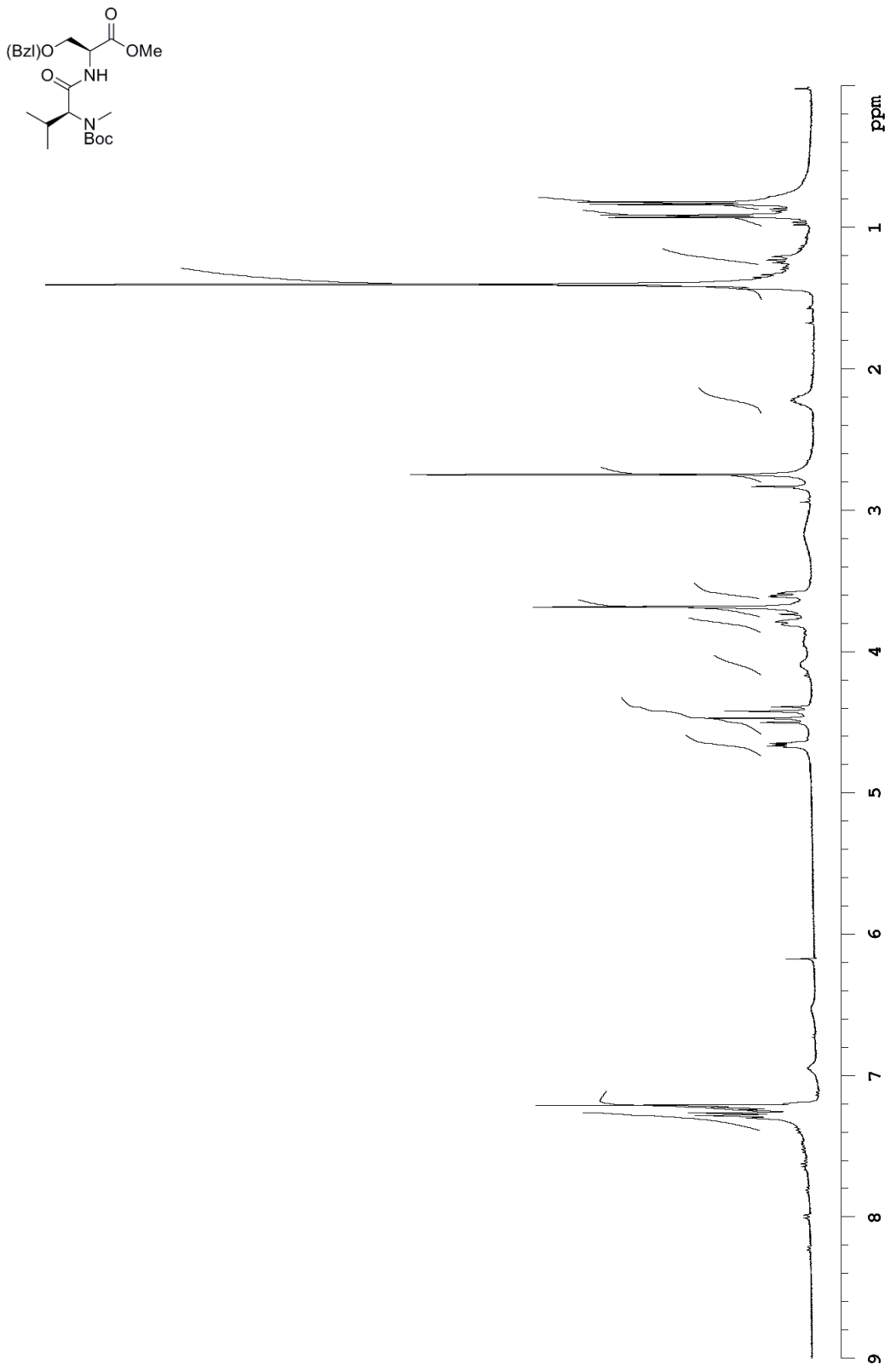


^{1}H NMR *cyclo*-(2*R*,3*R*)-β-benzoxy-Phe-Leu-Ox-D-Leu-D-Phe

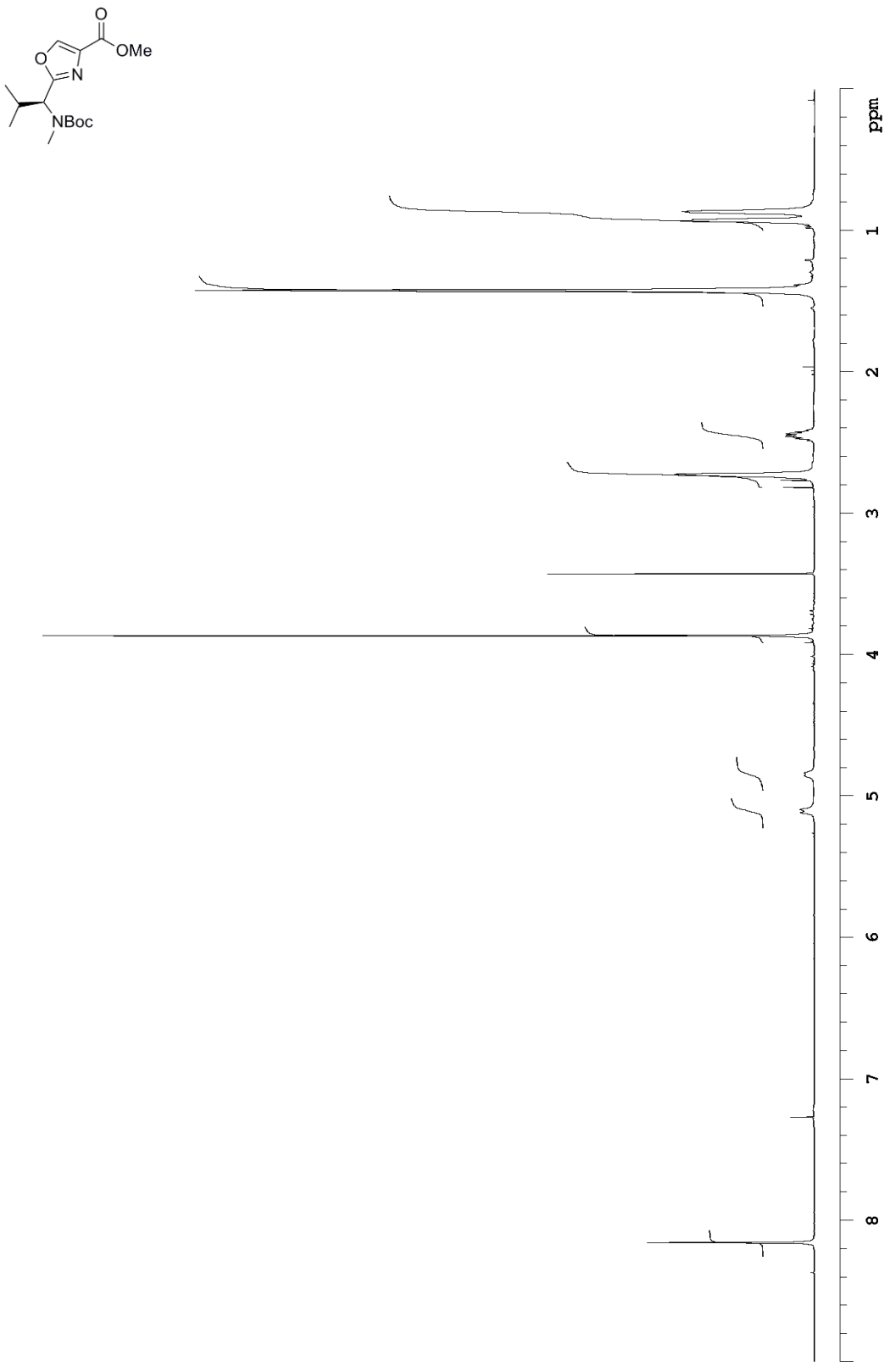


HRMS *cyclo*-(2*R*,3*R*)-β-benzoxy-Phe-Leu-Ox-D-Leu-D-Phe

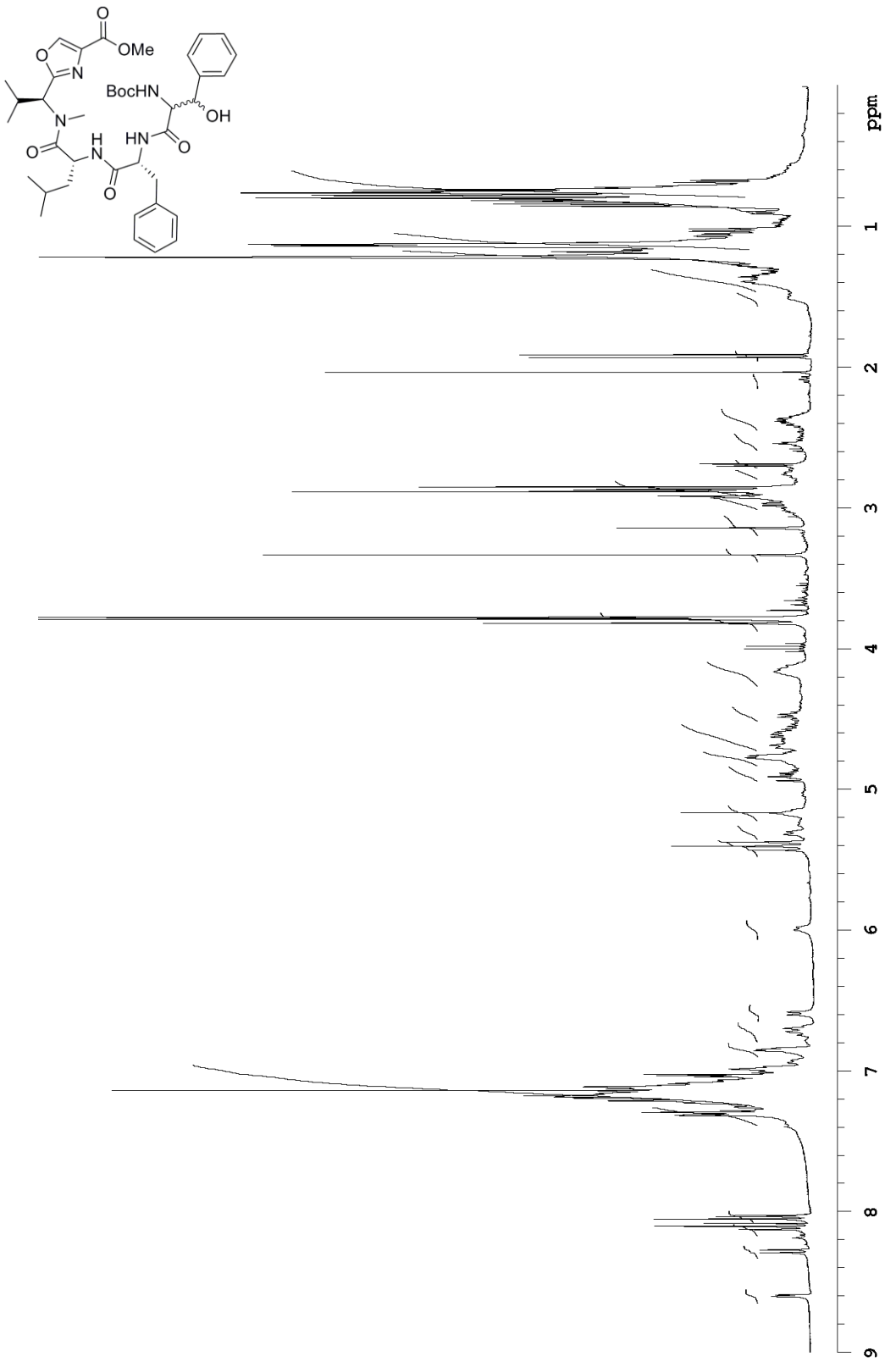
Supporting spectra for SanA 17-II-Ox



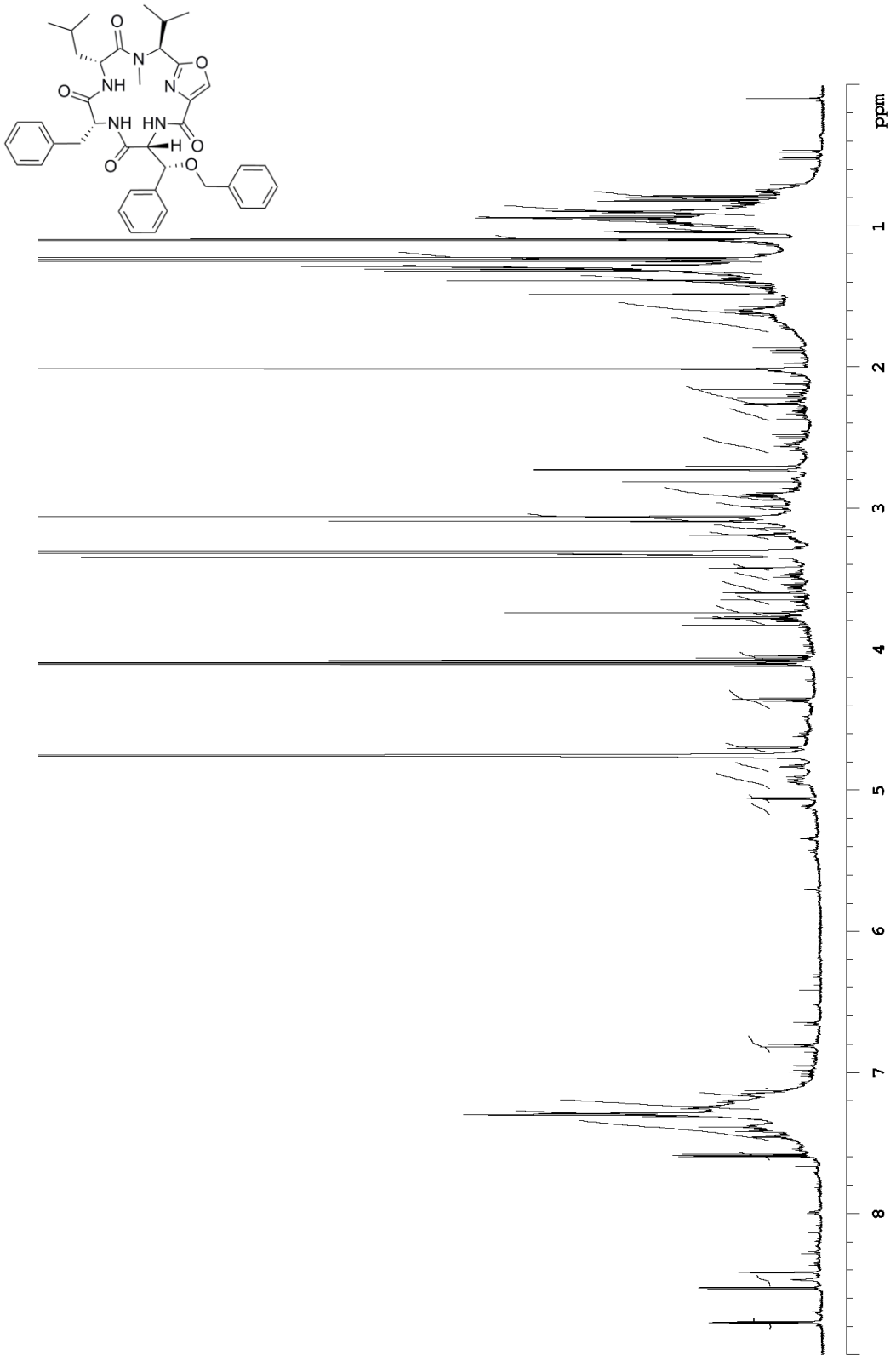
^{1}H NMR MeO-Ser(Bzl)-Val-N(Me)Boc



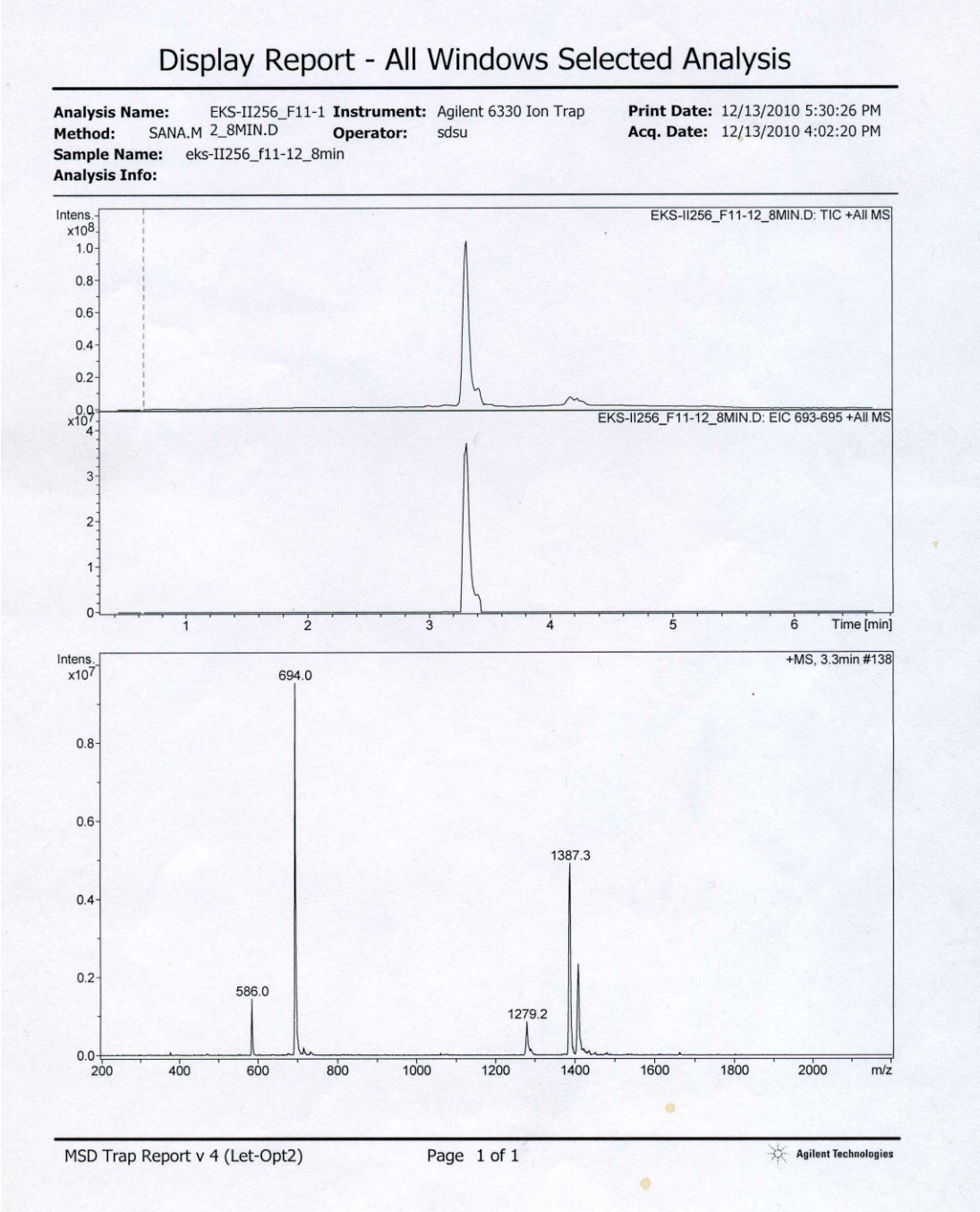
^{1}H NMR MeO-Ox-Val-N(Me)Boc



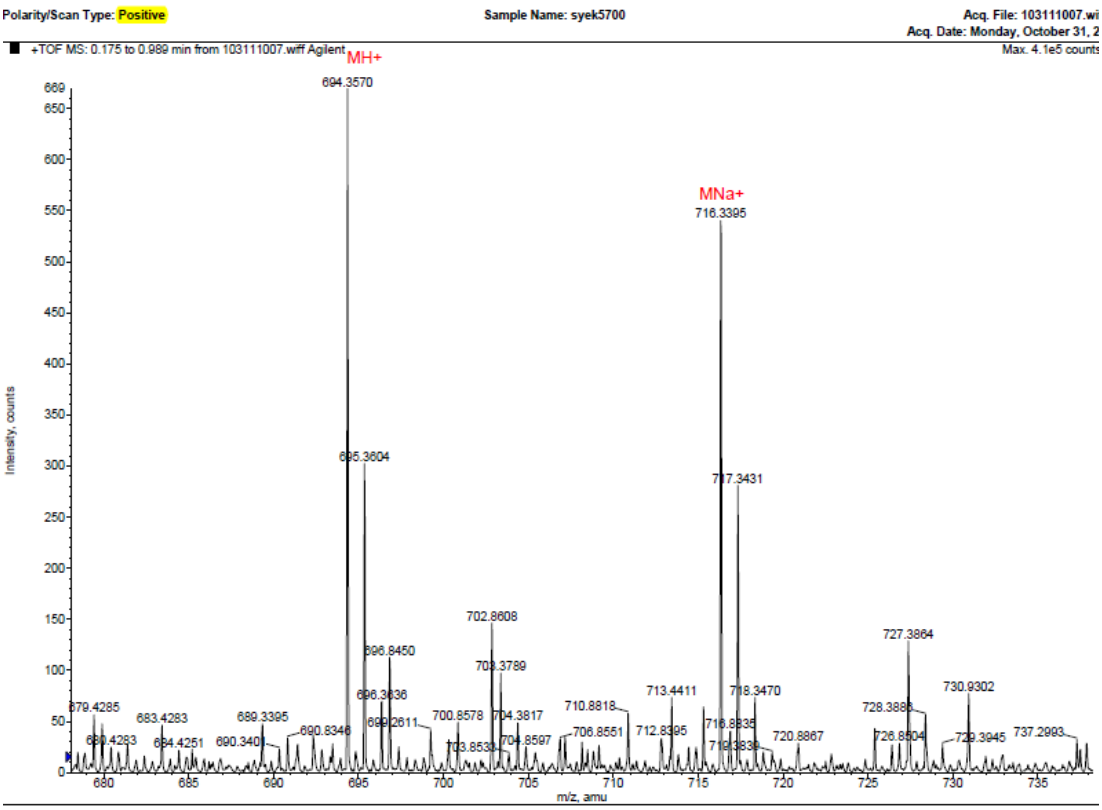
^{1}H NMR MeO-Ox-Val-N(Me)-D-Leu-D-Phe-(2*R*,3*R*)/(2*S*,3*S*)-β-OH-Phe-NHBoc



^{1}H NMR *cyclo*-(2*R*,3*R*)-β-benzoxy-Phe-Ox-Val-N(Me)-D-Leu-D-Phe



LC/MS *cyclo*-(2*R*,3*R*)-β-benzoxy-Phe-Ox-Val-N(Me)-D-Leu-D-Phe



HRMS *cyclo*-(2*R*,3*R*)-β-benzoxy-Phe-Ox-Val-N(Me)-D-Leu-D-Phe

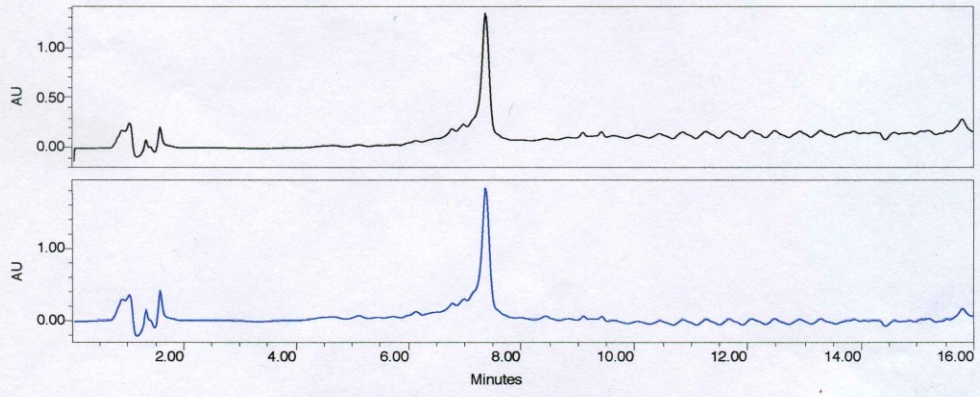
SDSU

Project Name: Defaults
Reported by User: System

Breeze

SAMPLE INFORMATION

Sample Name:	eks-III-252_ERT-208	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	
Vial:	1	Acq. Method:	primary_sanA_ss_ACN
Injection #:	42	Date Acquired:	12/7/2010 4:31:57 PM
Run Time:	16.00 Minutes	Injection Volume:	75.00 ul



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Channel: 2487Channel 2 Channel Desc.: Processing Method: *

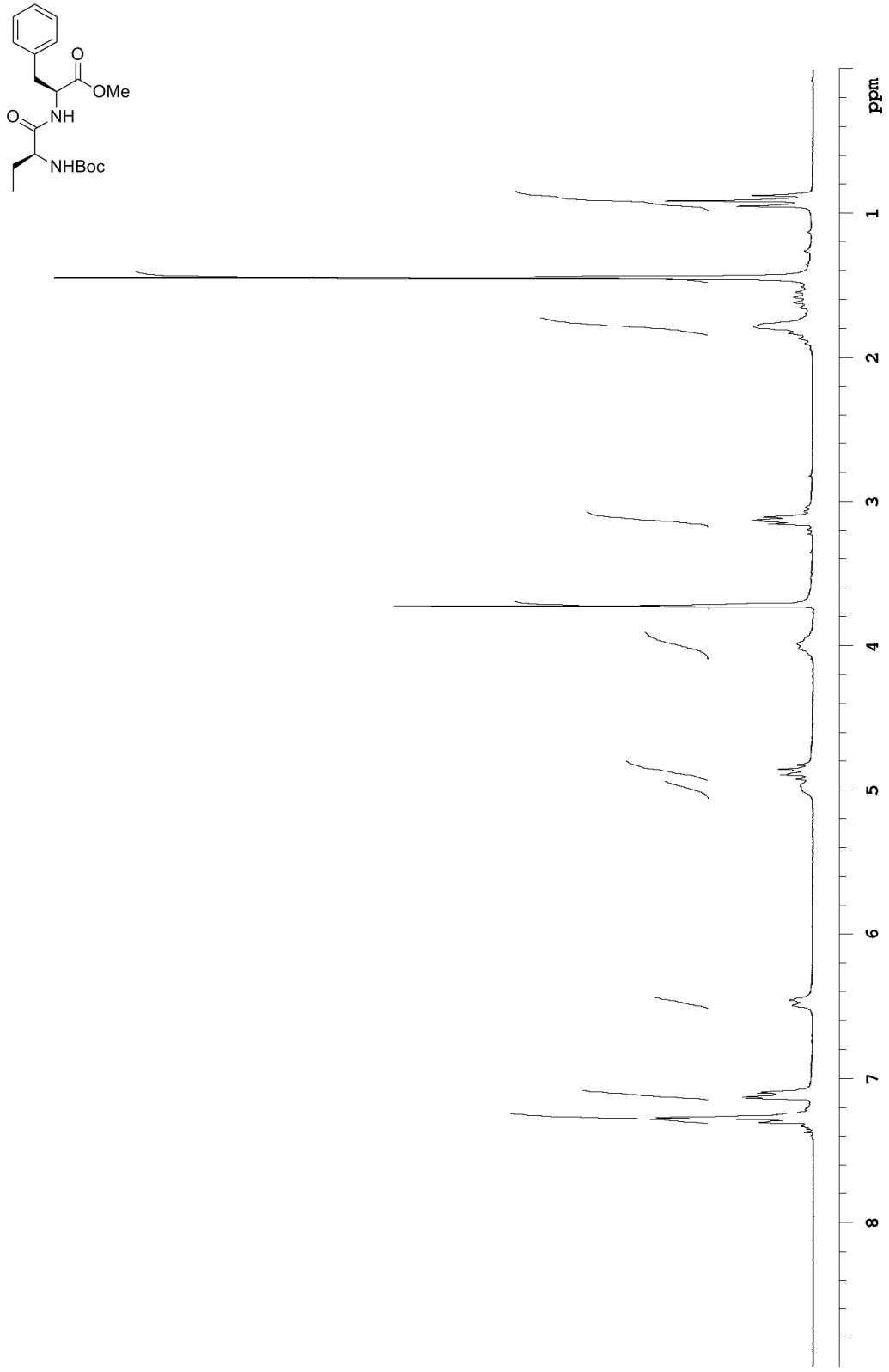
	Peak Name	RT (min)	Area (V*sec)	% Area	Height (V)	Amount	Units
1	****	****	****	****	****	****	****
2	****	****	****	****	****	****	****

Report Method: Injection Summary Report Printed 4:48:11 PM 12/7/2010 Page: 1 of 1

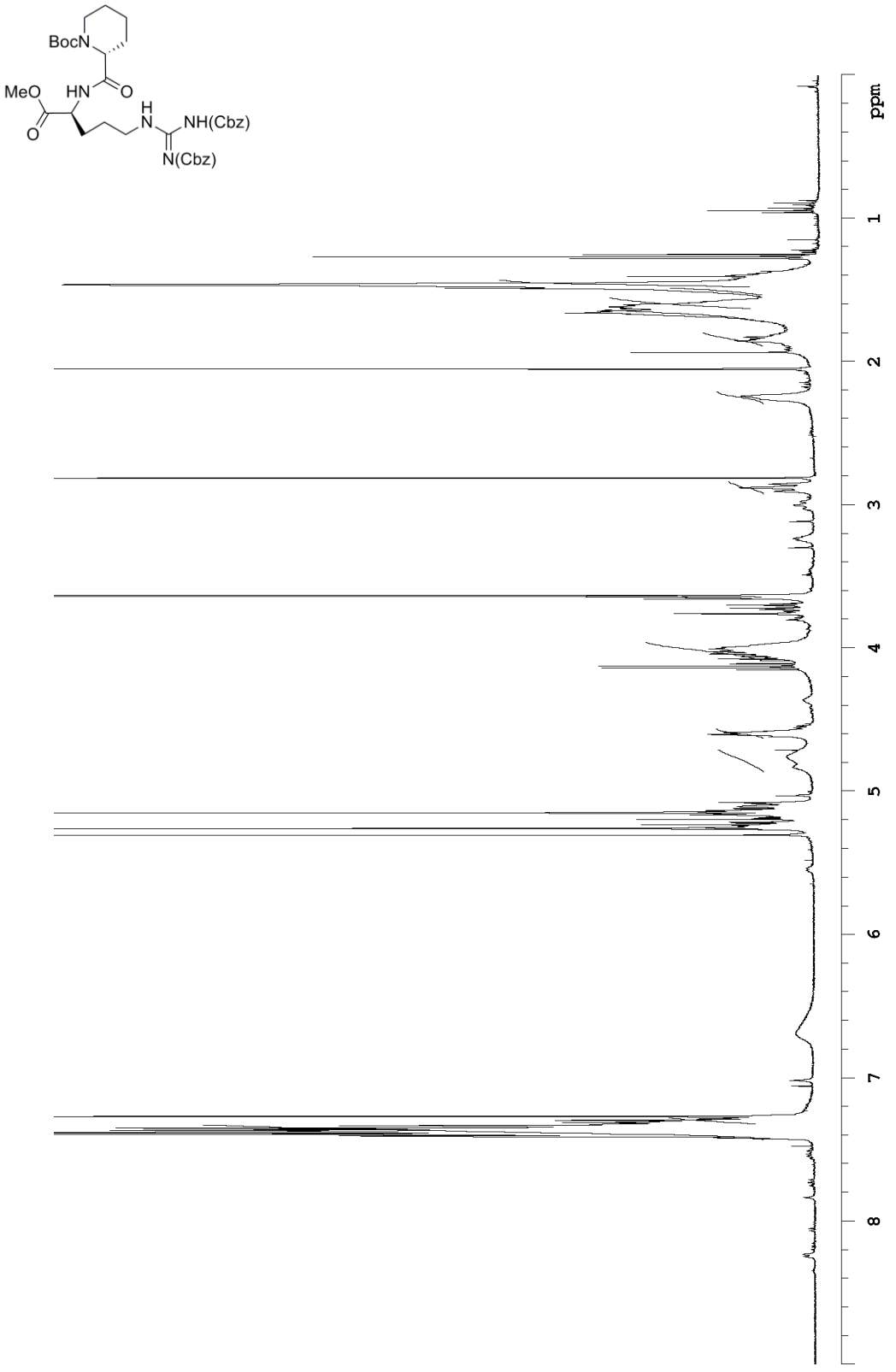
HPLC *cyclo*-(2*R*,3*R*)-β-benzoxy-Phe-Ox-Val-N(Me)-D-Leu-D-Phe

Appendix B – Supporting spectra for Chapter 3

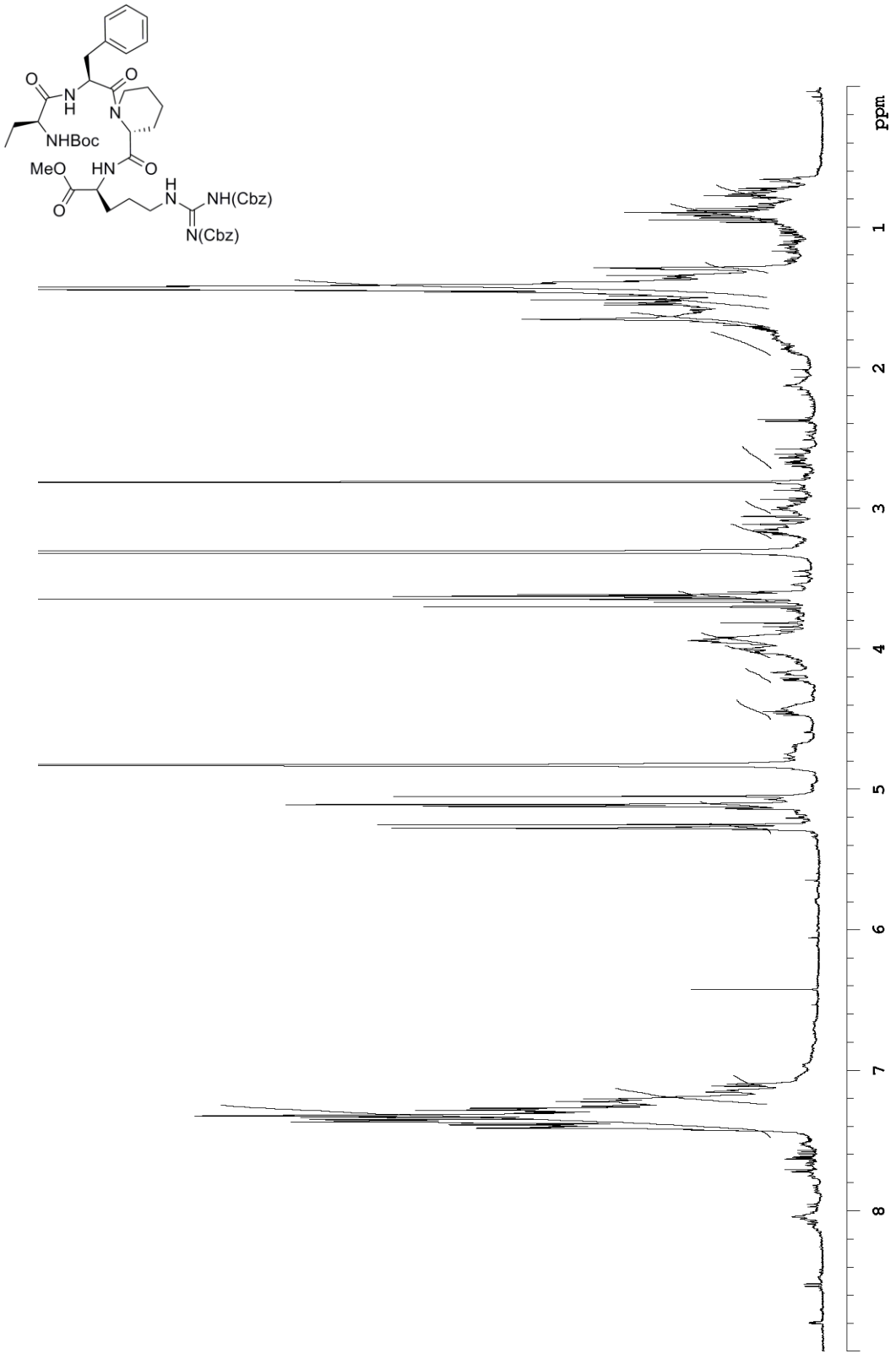
Supporting spectra for HDACI 3



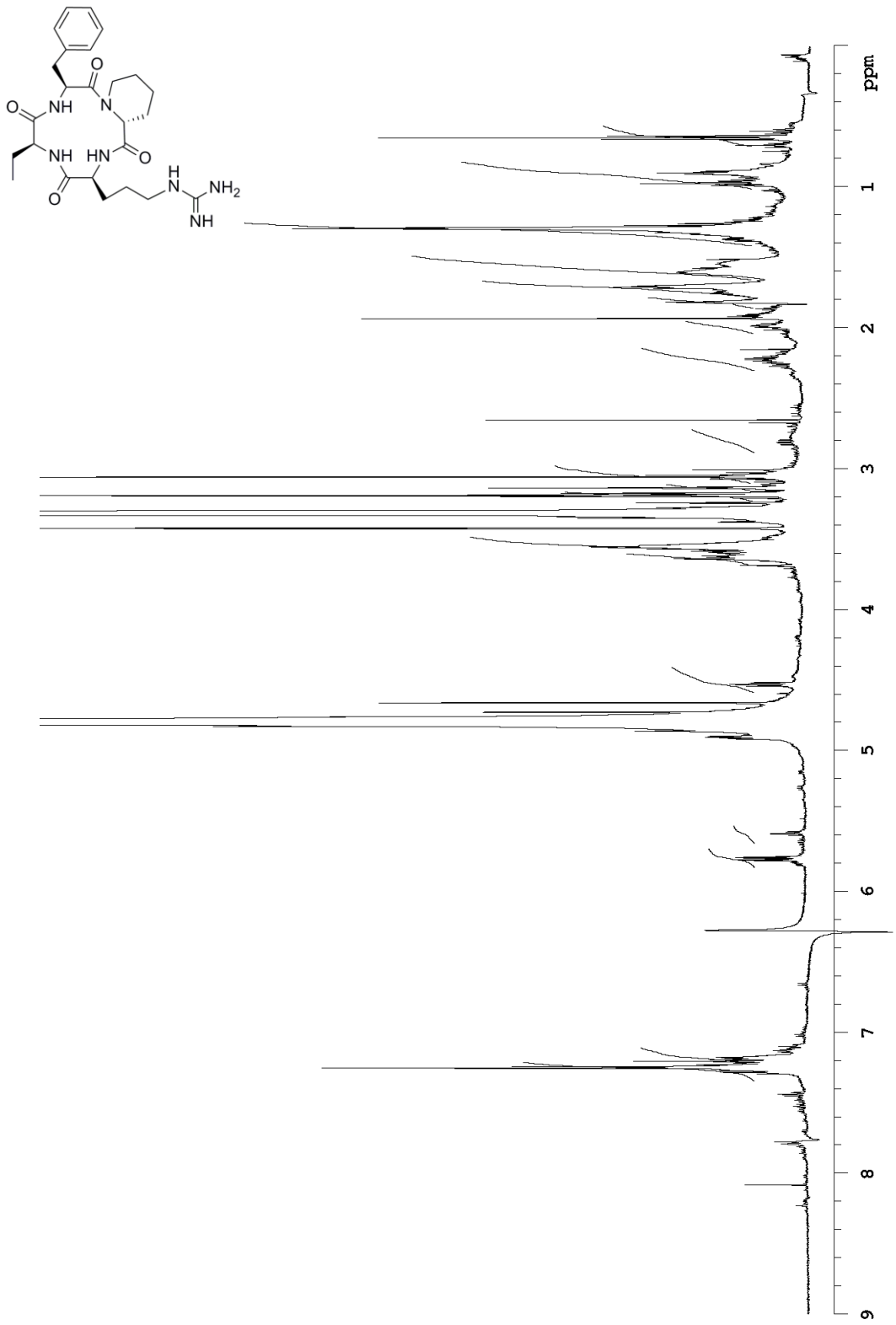
^{1}H NMR MeO-Phe-Abu-NHBoc



^{1}H NMR MeO-Arg(2Cbz)-D-Pip-NBoc

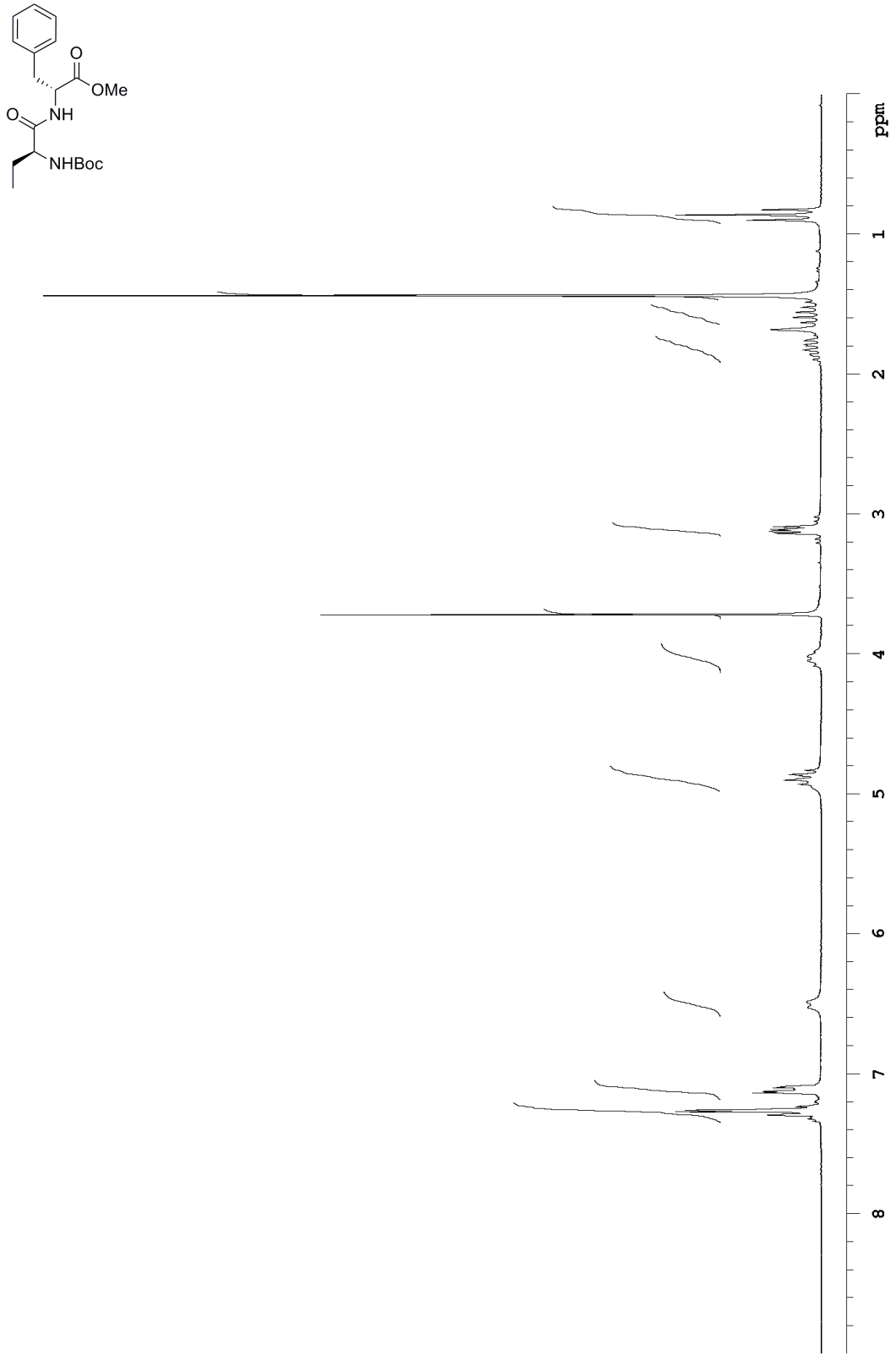


^{1}H NMR MeO-Arg(2Cbz)-D-Pip-Phe-Abu-NHBoc

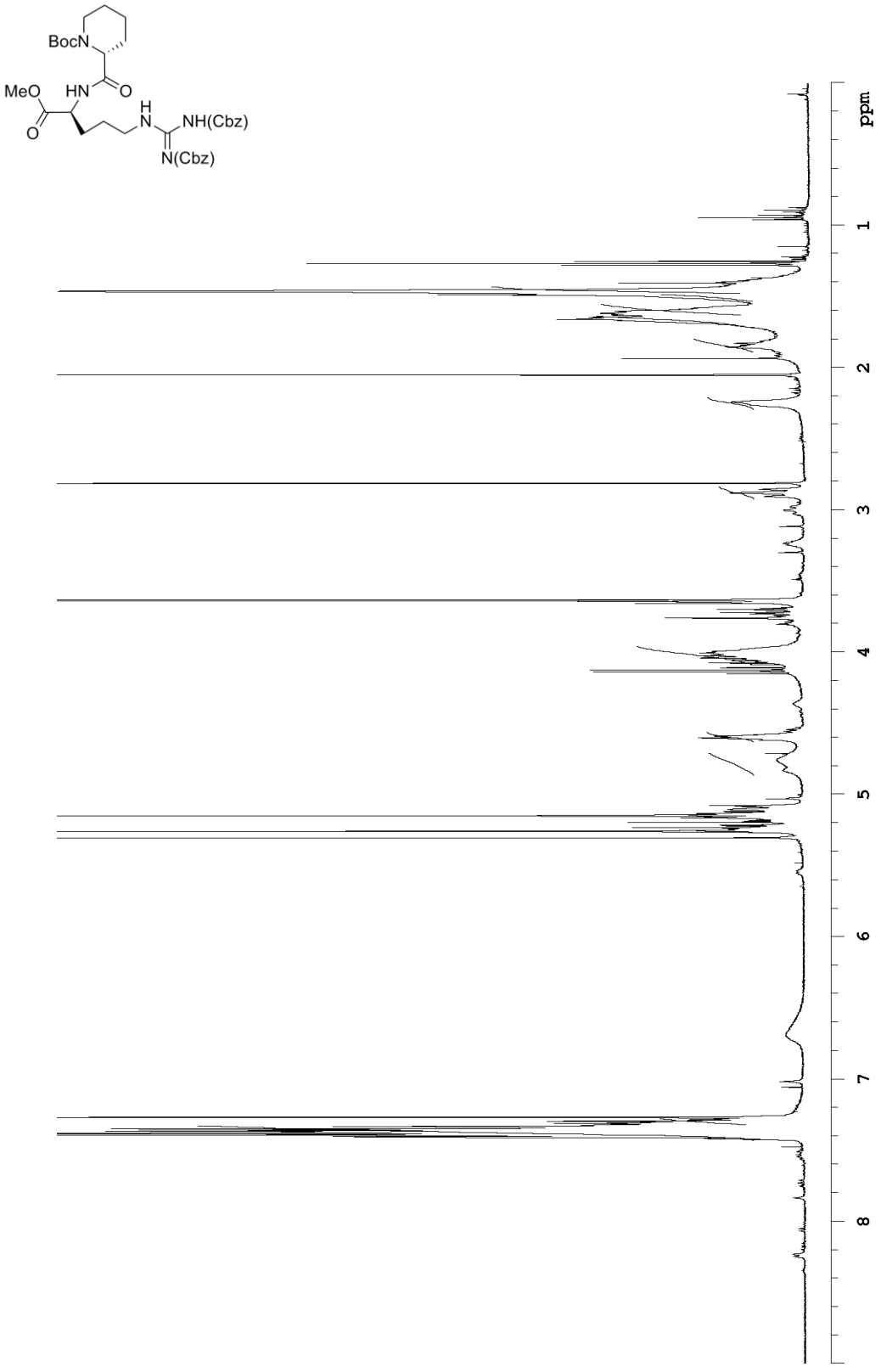


^{1}H NMR *cyclo*-Phe-Abu-Arg-D-Pip

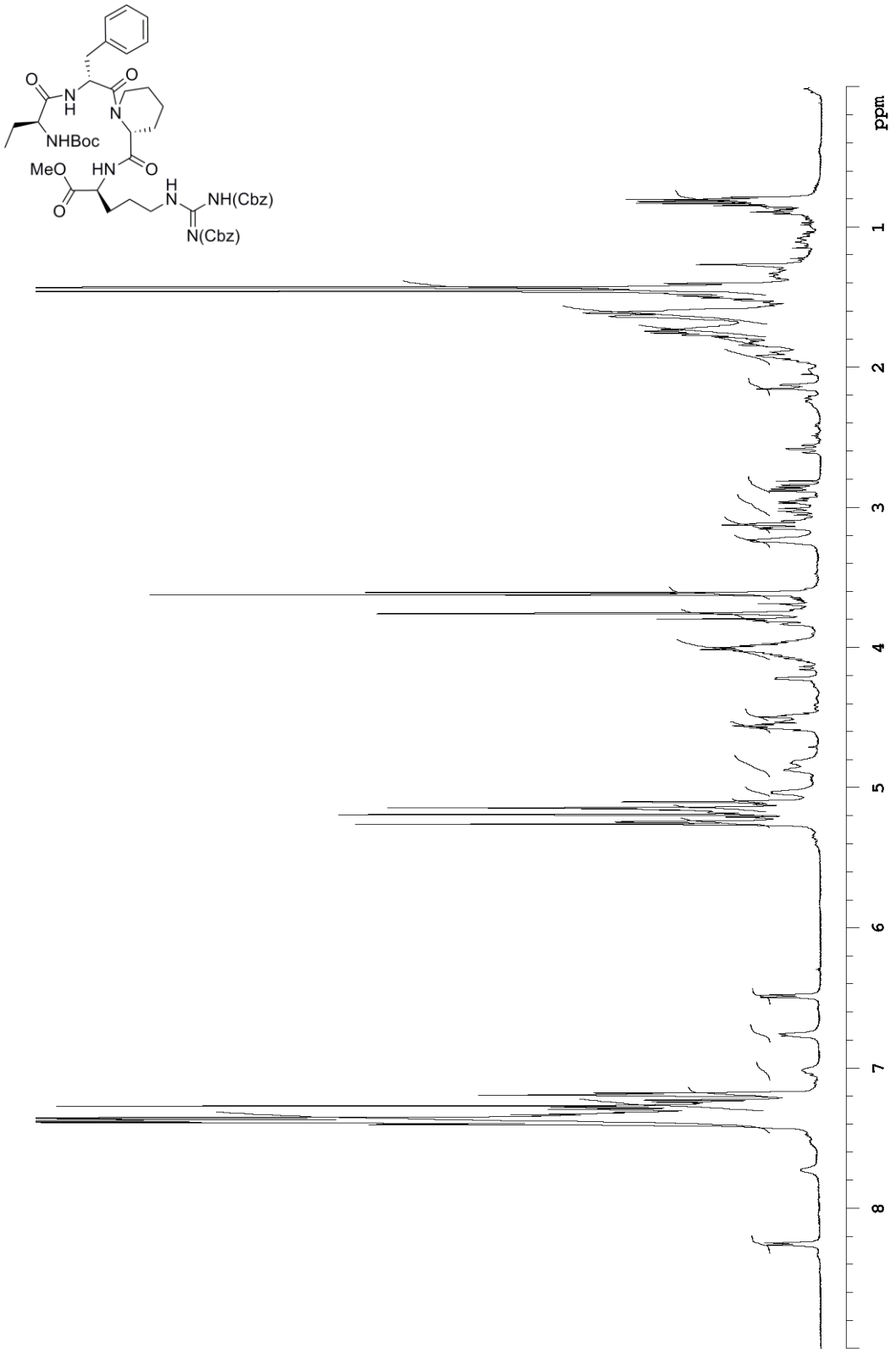
Supporting spectra for HDACI 4



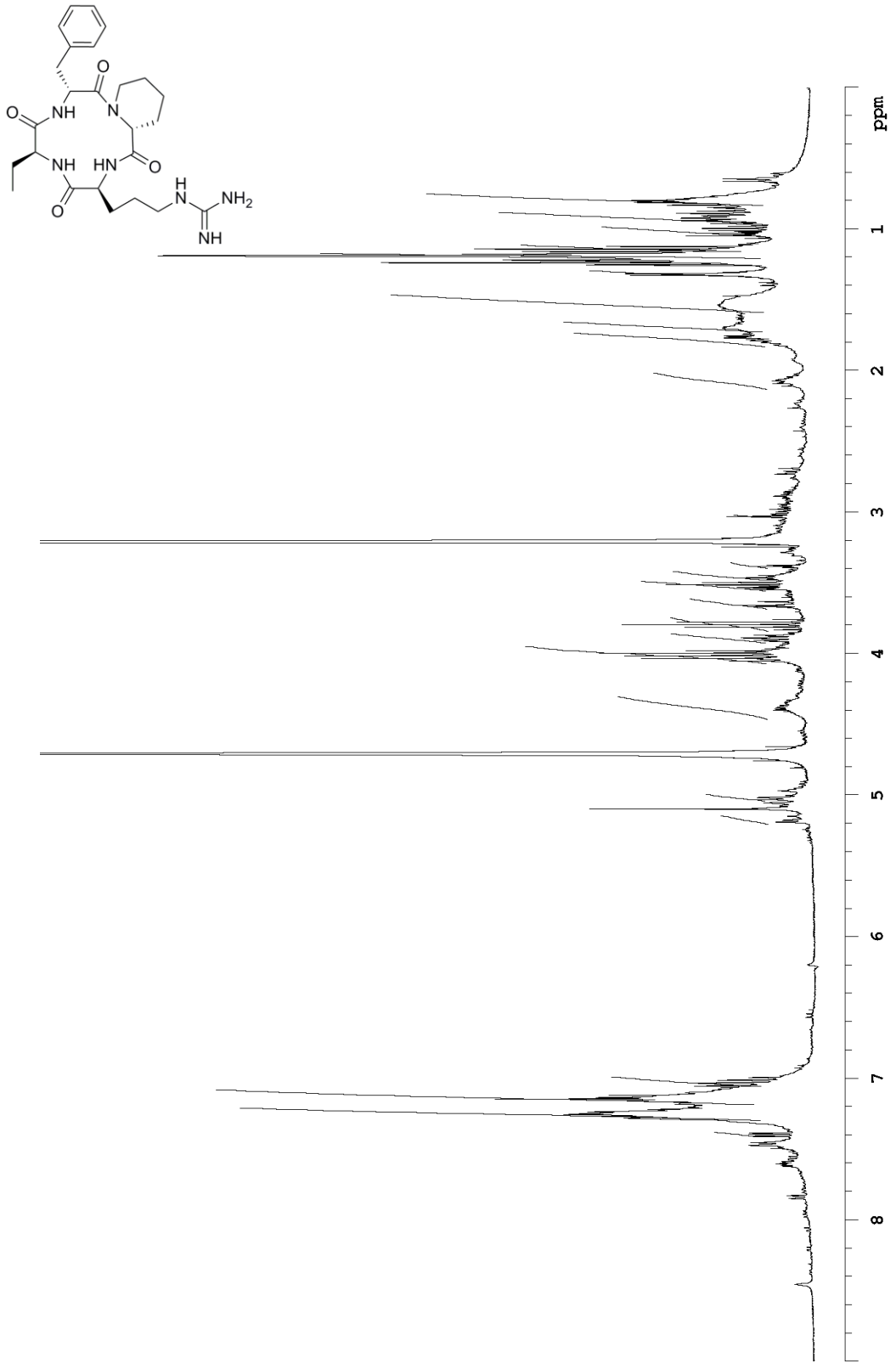
^{1}H NMR MeO-D-Phe-Abu-NHBoc



^{1}H NMR MeO-Arg(2Cbz)-D-Pip-NBoc

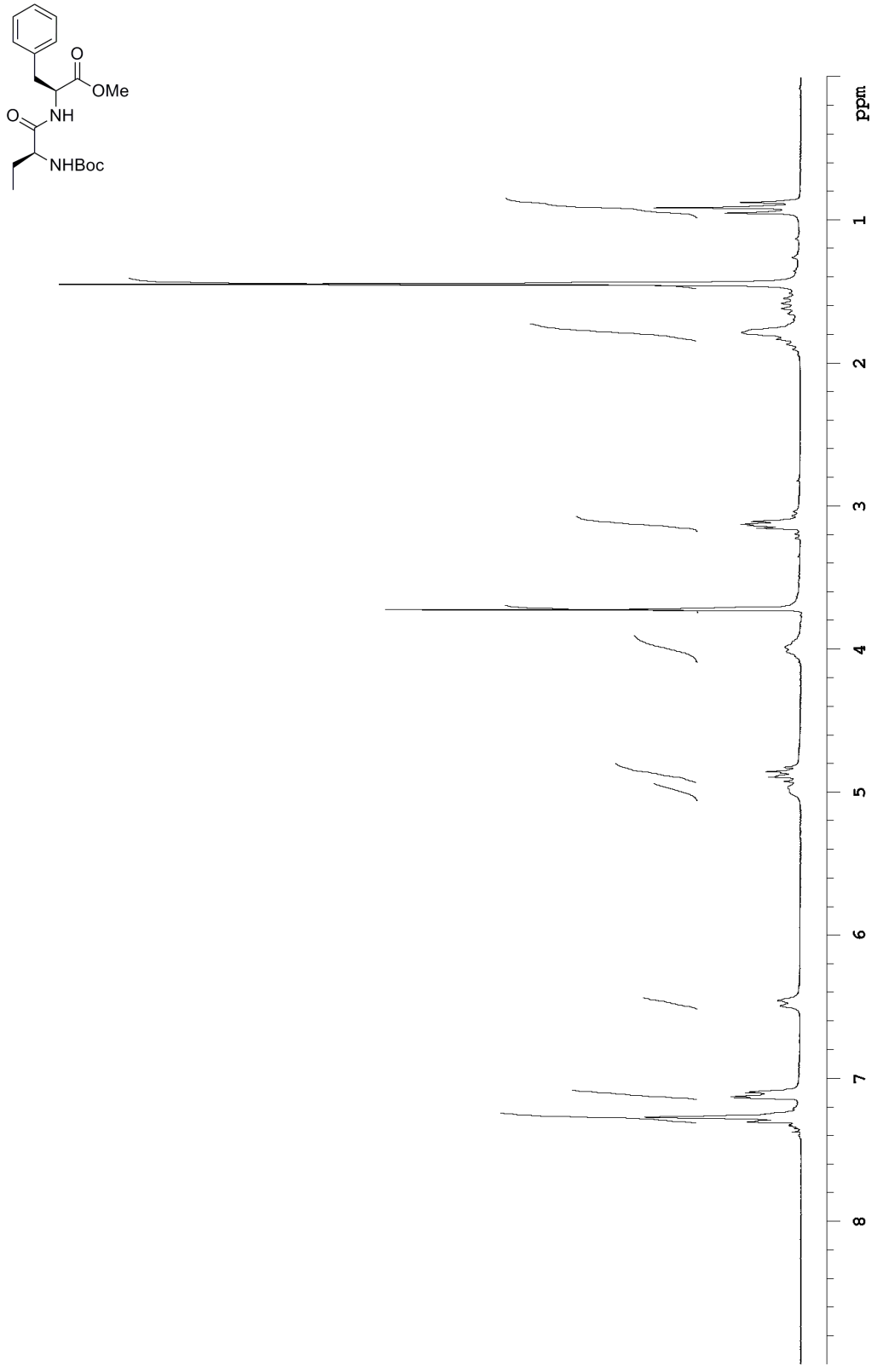


^{1}H NMR MeO-Arg(2Cbz)-D-Pip-D-Phe-Abu-NHBoc

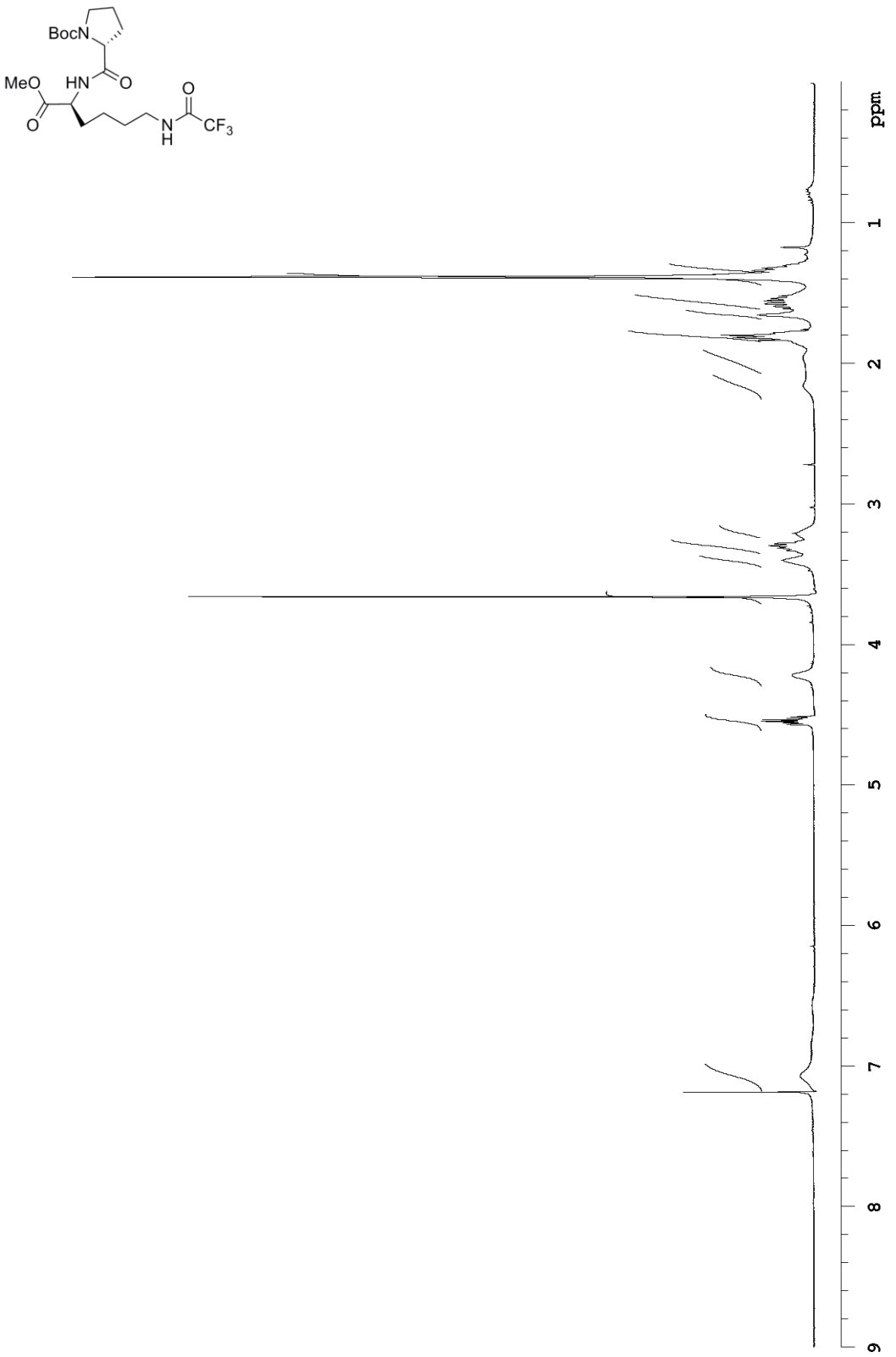


^{1}H NMR *cyclo*-D-Phe-Abu-Arg-D-Pip

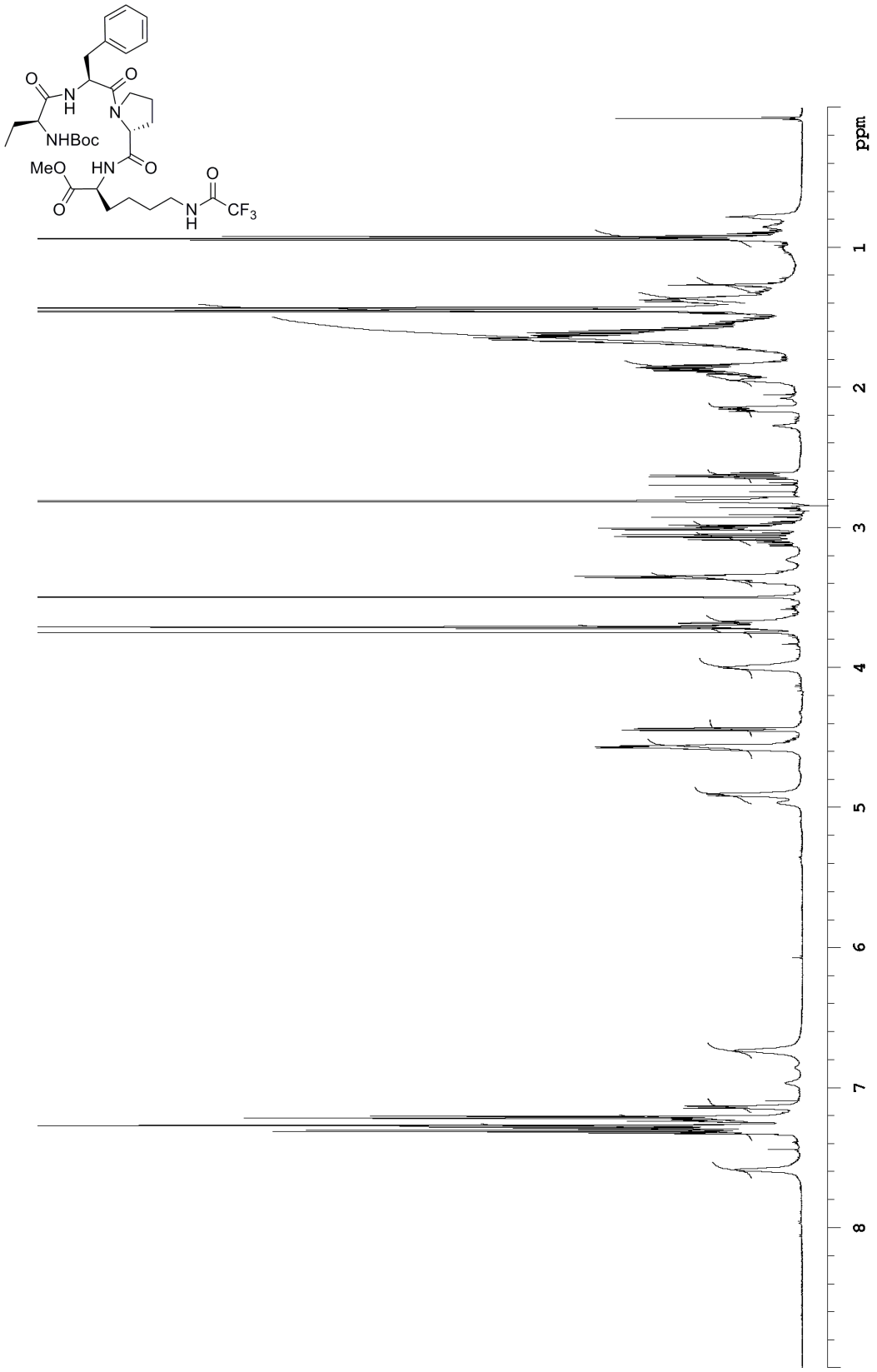
Supporting spectra for HDACI 11



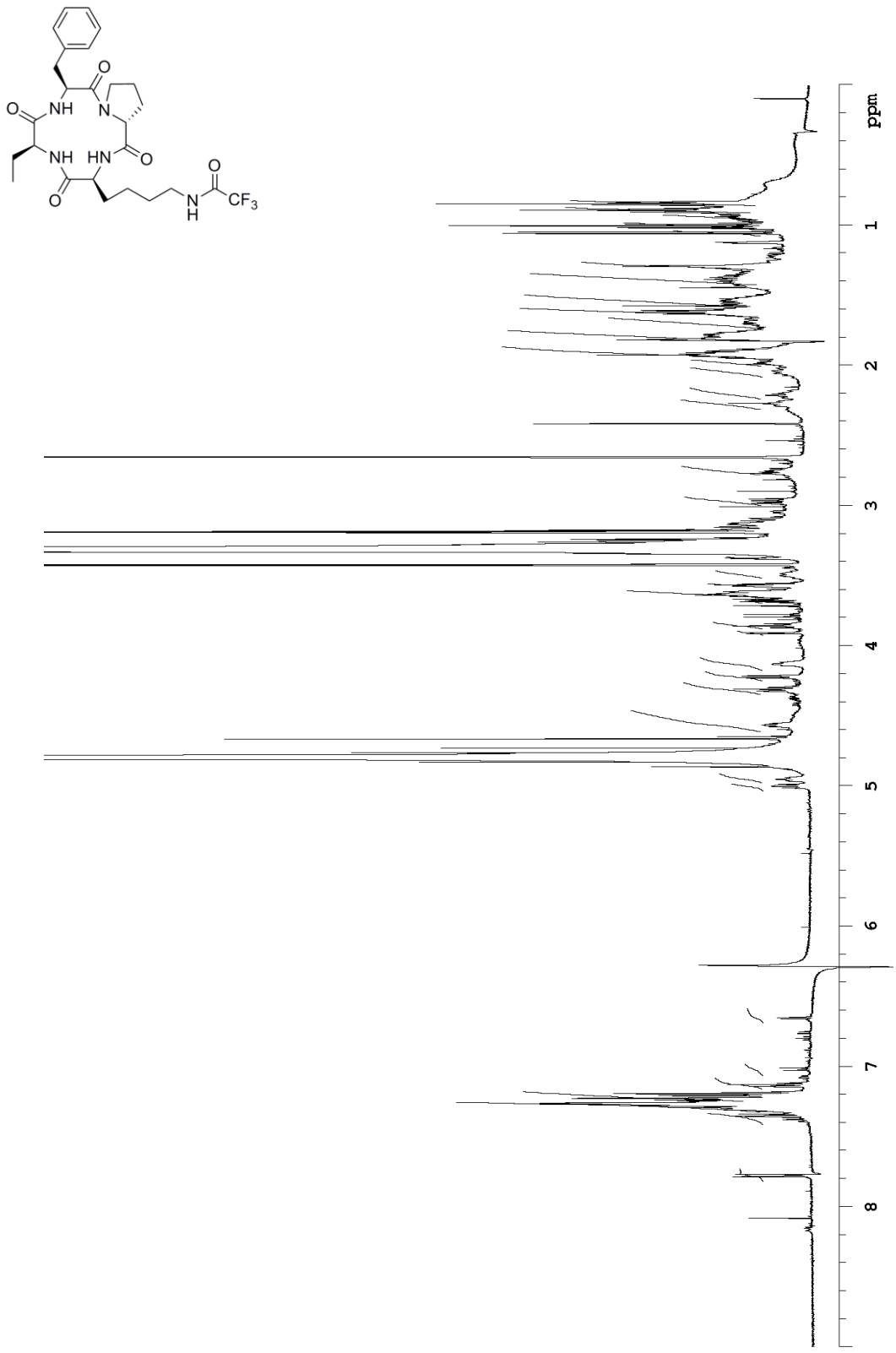
^{1}H NMR MeO-Phe-Abu-NHBoc



^{1}H NMR MeO-Lys(Tfa)-D-Pro-NBoc

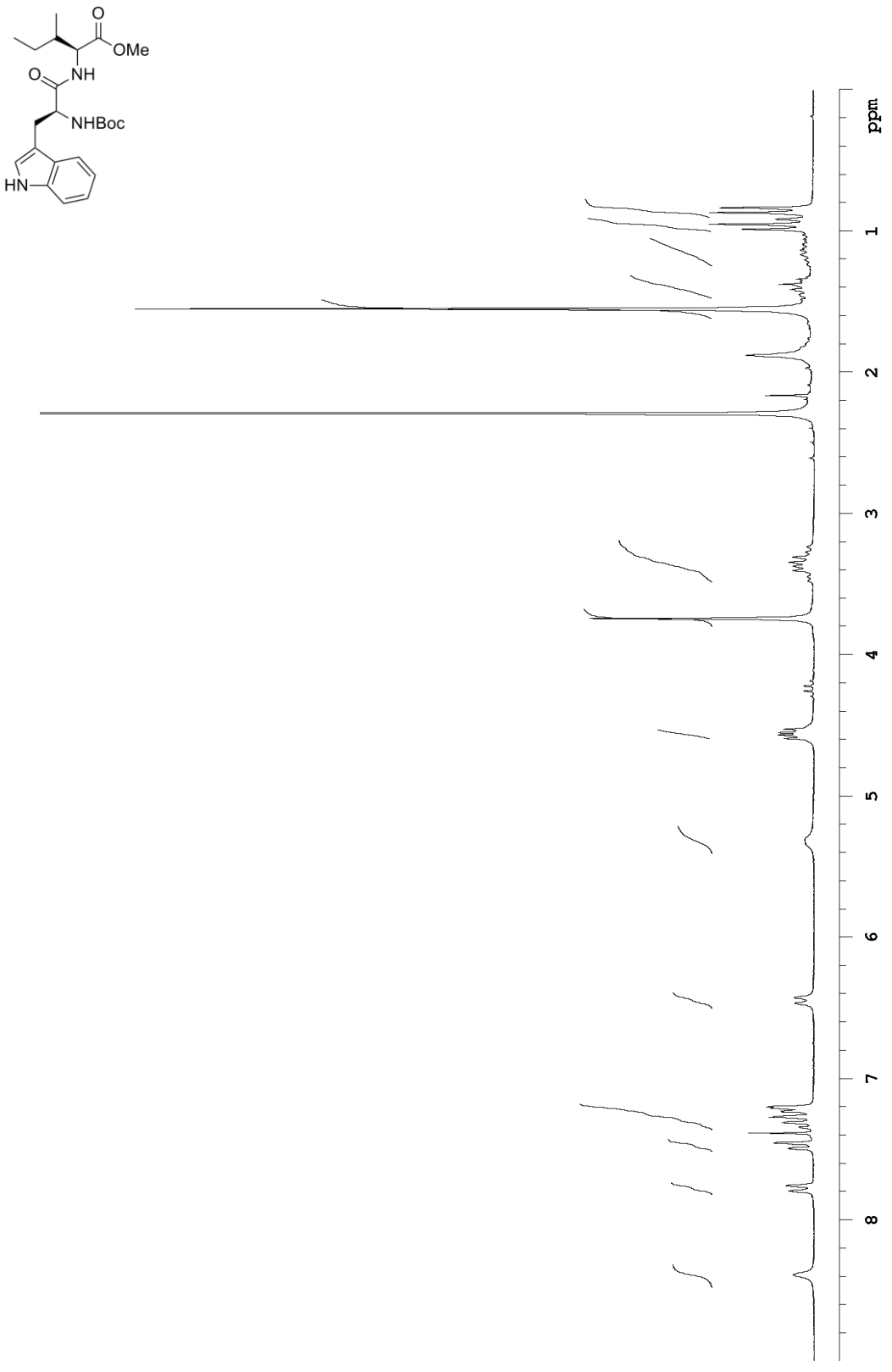


^{1}H NMR MeO-Lys-(Tfa)-D-Pro-Phe-Abu-NHBoc

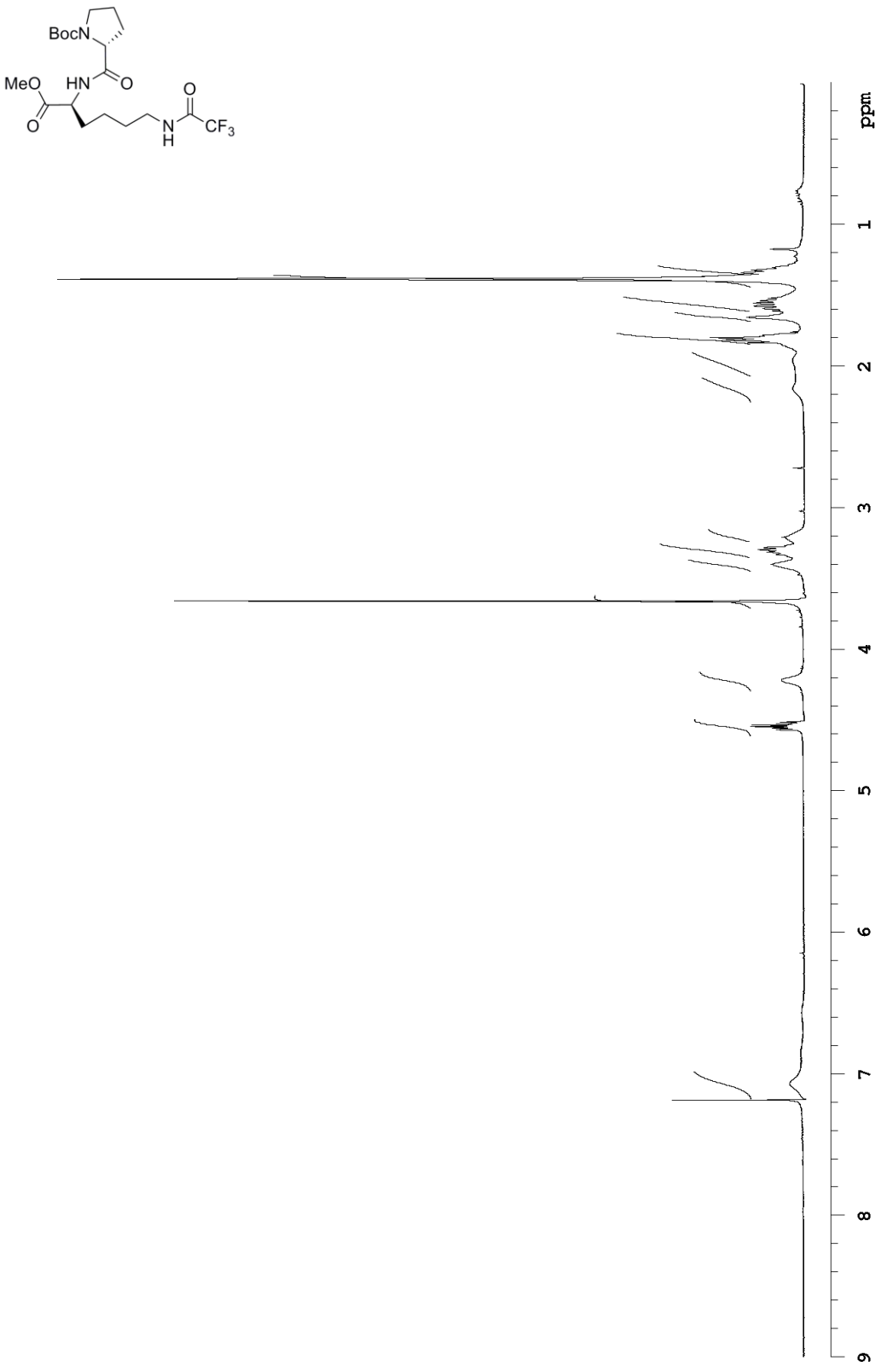


^{1}H NMR *cyclo*-Phe-Abu-Lys(Tfa)-D-Pro

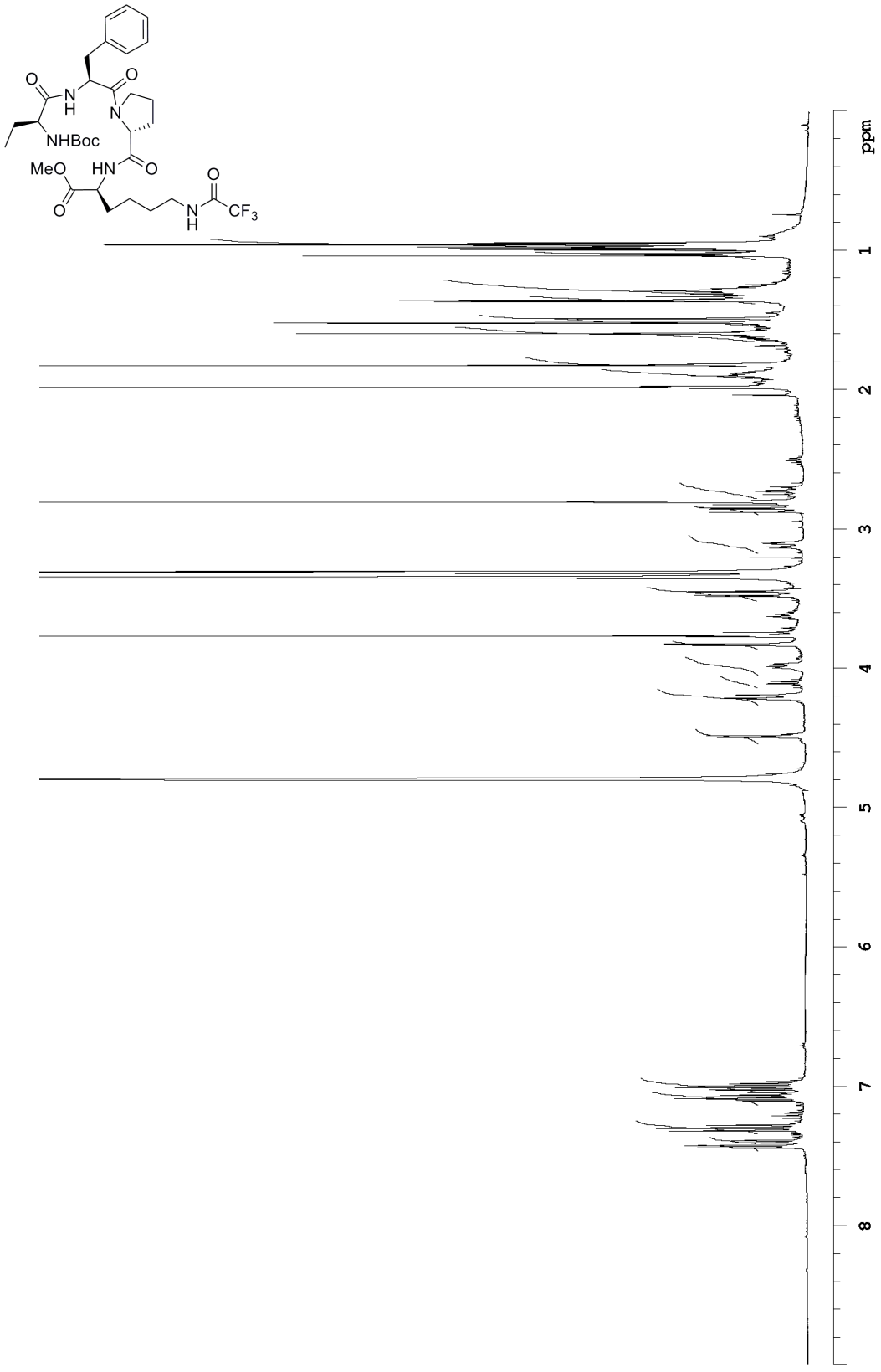
Supporting spectra for HDACI 14



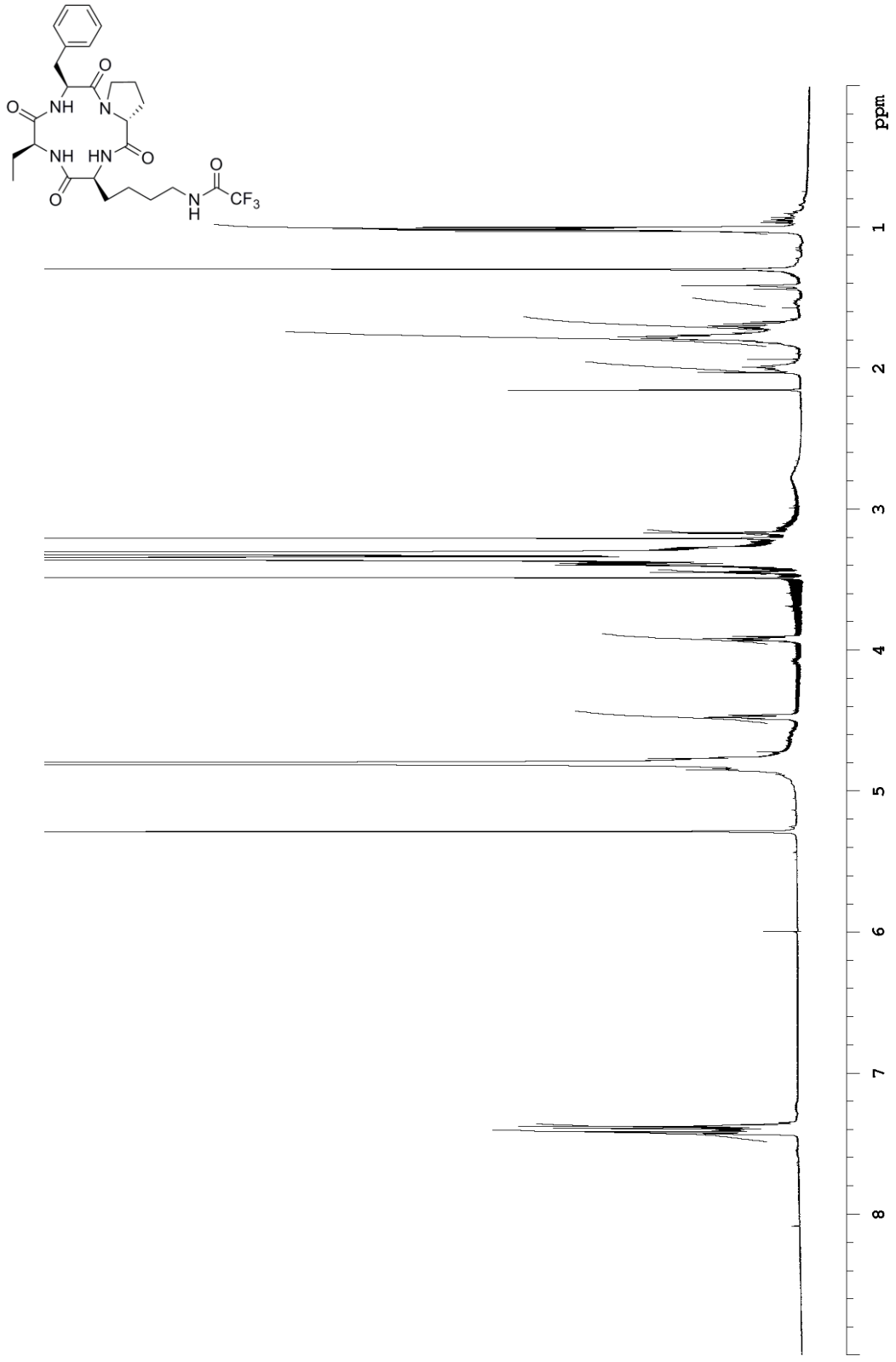
^{1}H NMR MeO-Ile-Trp-NHBoc



^{1}H NMR MeO-Lys(Tfa)-D-Pro-NBoc



^{1}H NMR MeO-Lys(Tfa)-D-Pro-Phe-Abu-NHBoc



^{1}H NMR *cyclo*-Phe-Abu-Lys(Tfa)-D-Pro

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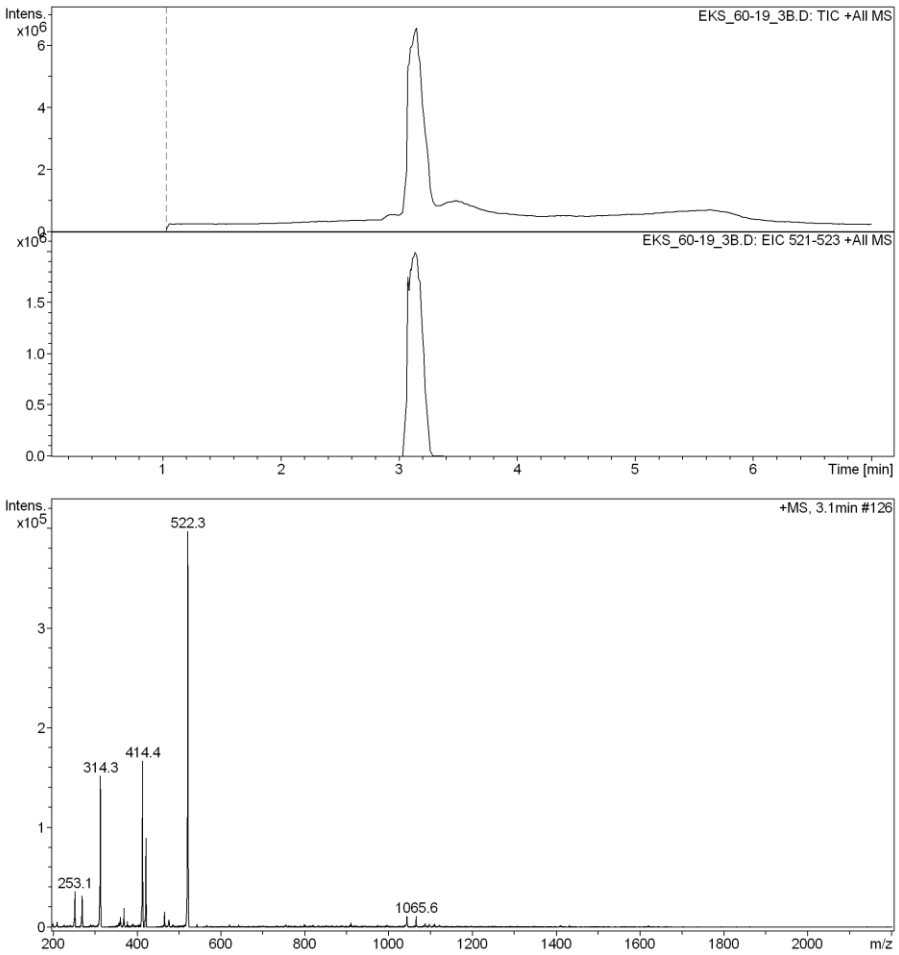
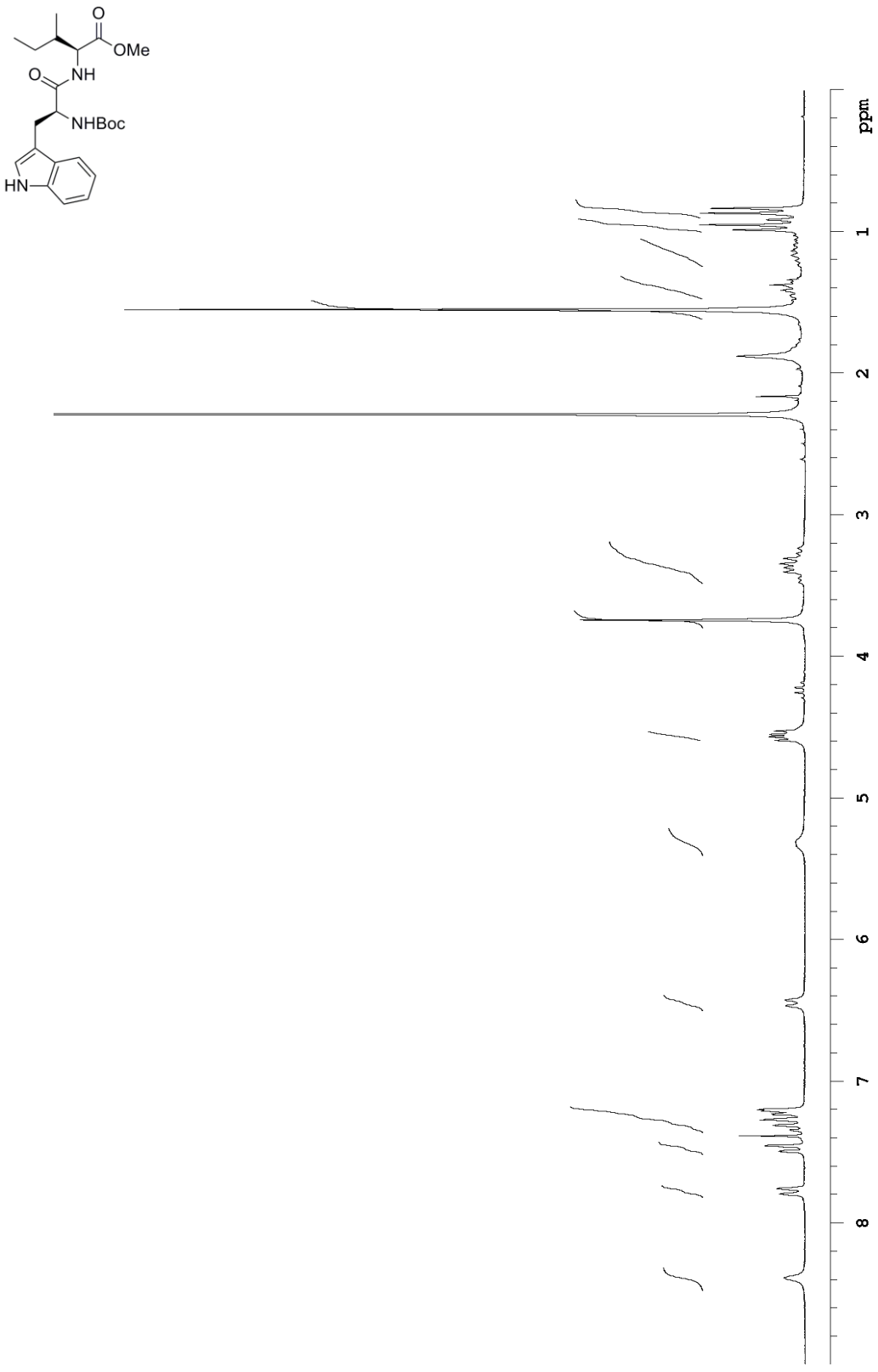
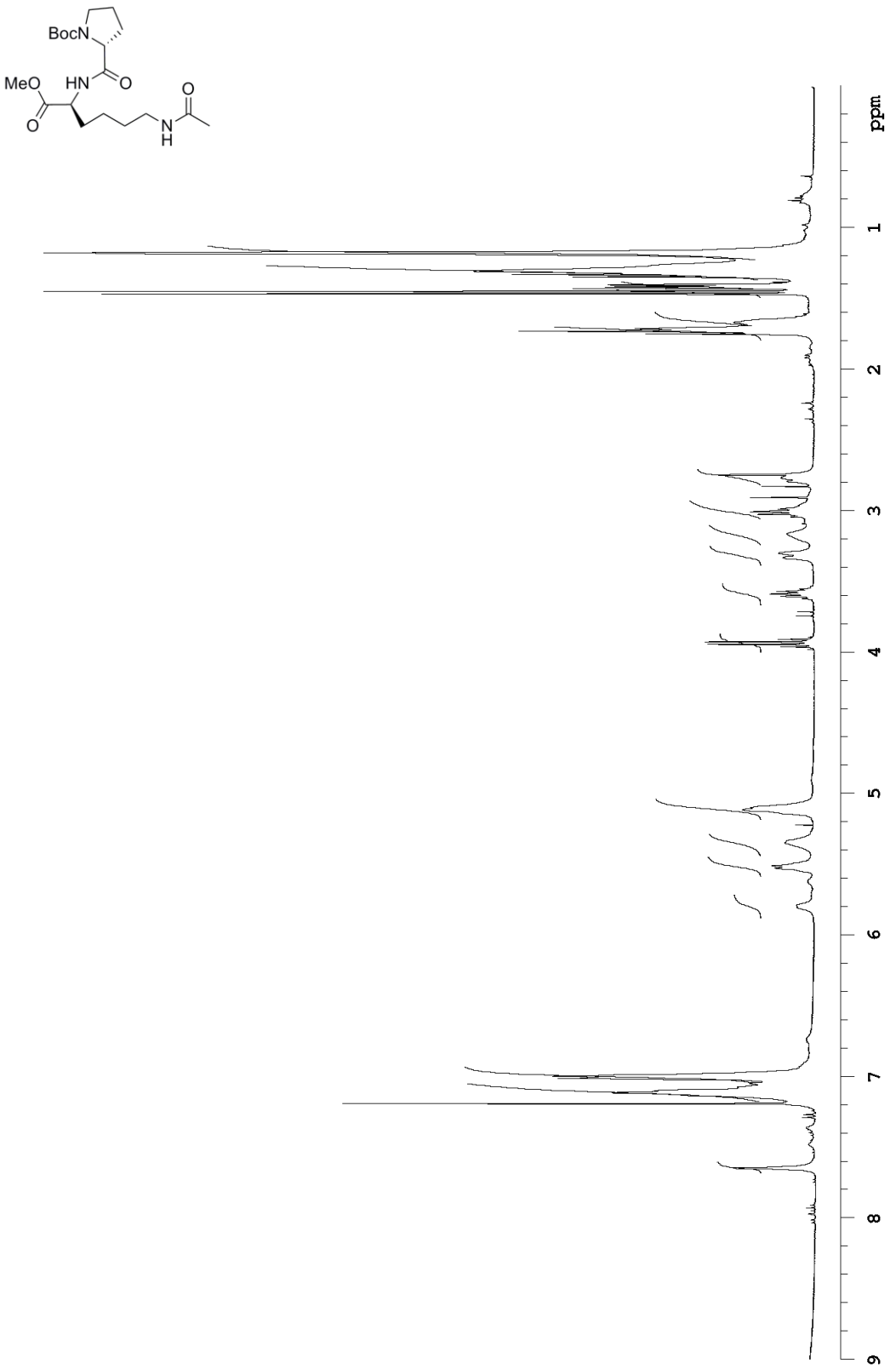


LC/MS *cyclo*-Phe-Abu-Lys(Tfa)-D-Pro

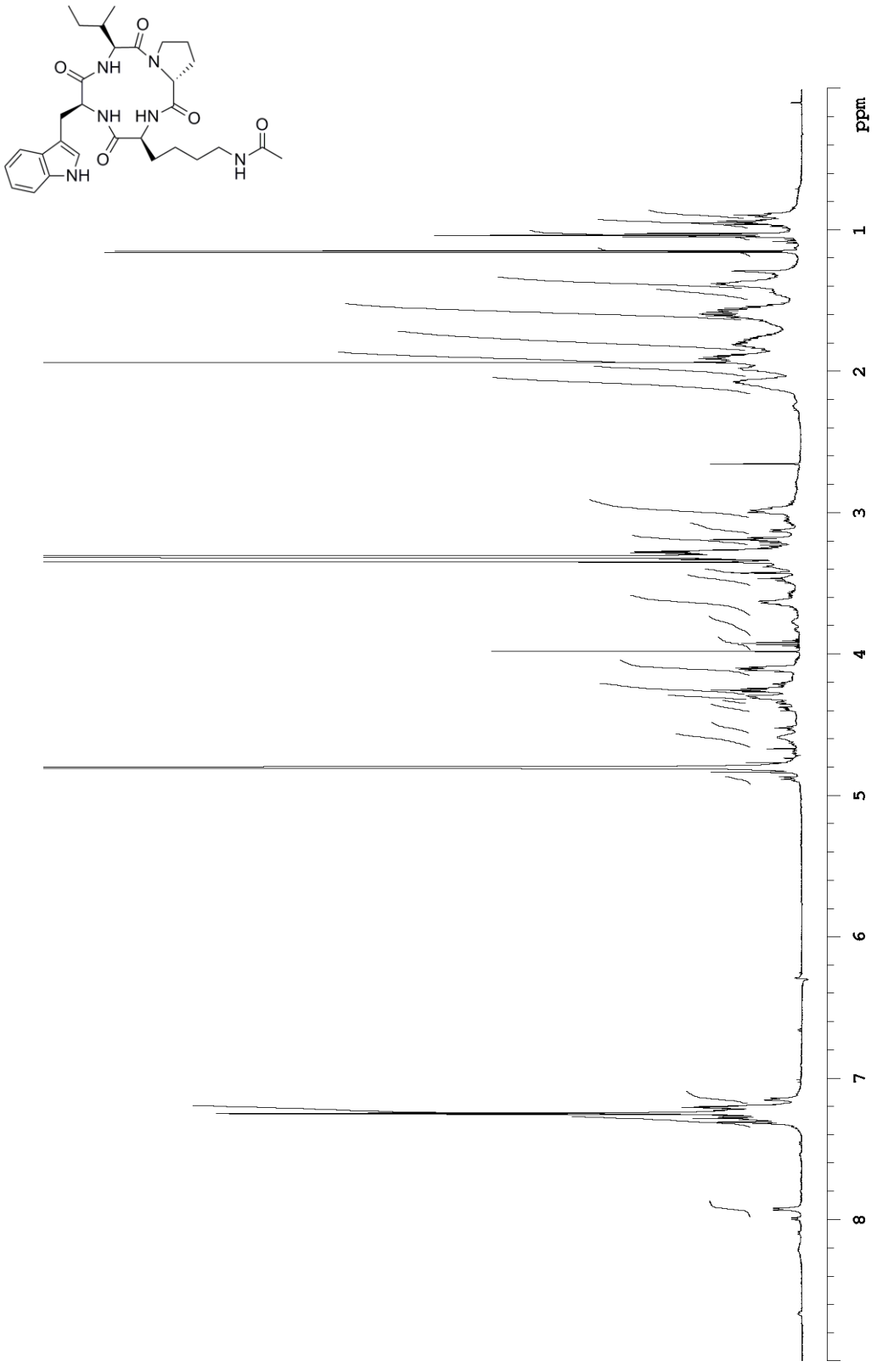
Supporting spectra for HDACI 16



^{1}H NMR MeO-Ile-Trp-NHBoc



^{1}H NMR MeO-Lys(Ac)-D-Pro



^{1}H NMR *cyclo*-Phe-Abu-Lys(Ac)-D-Pro

Display Report - All Windows Selected Analysis

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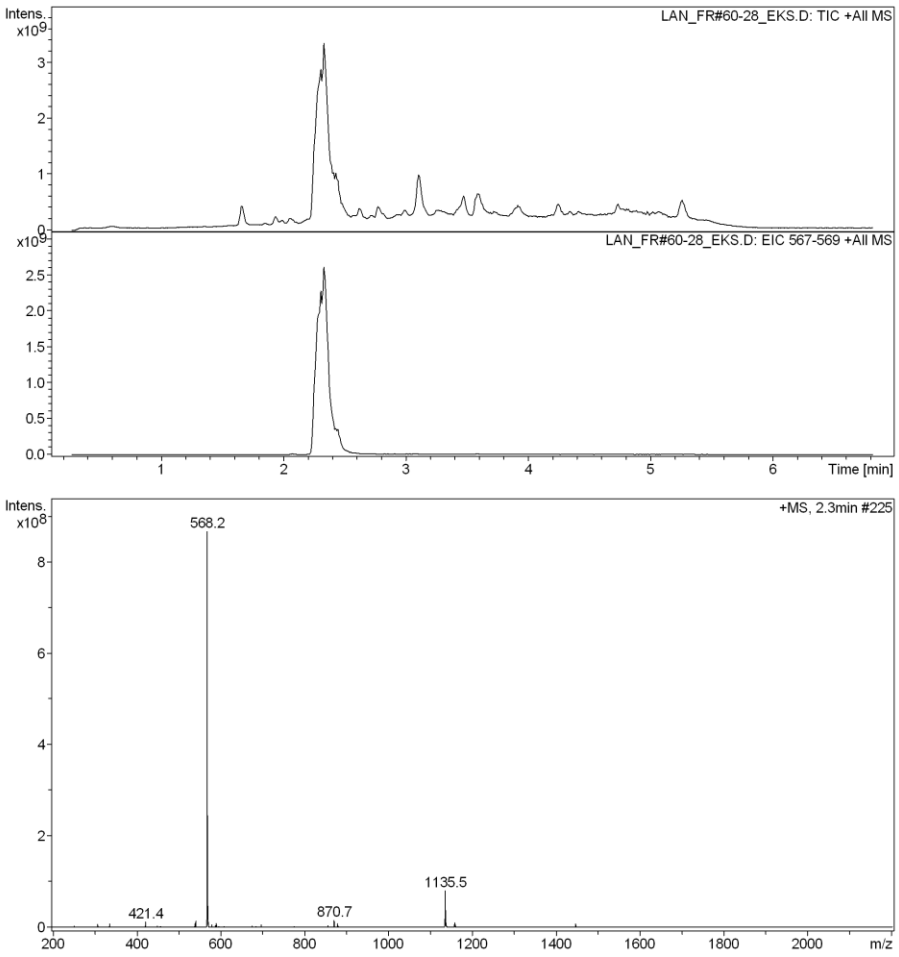
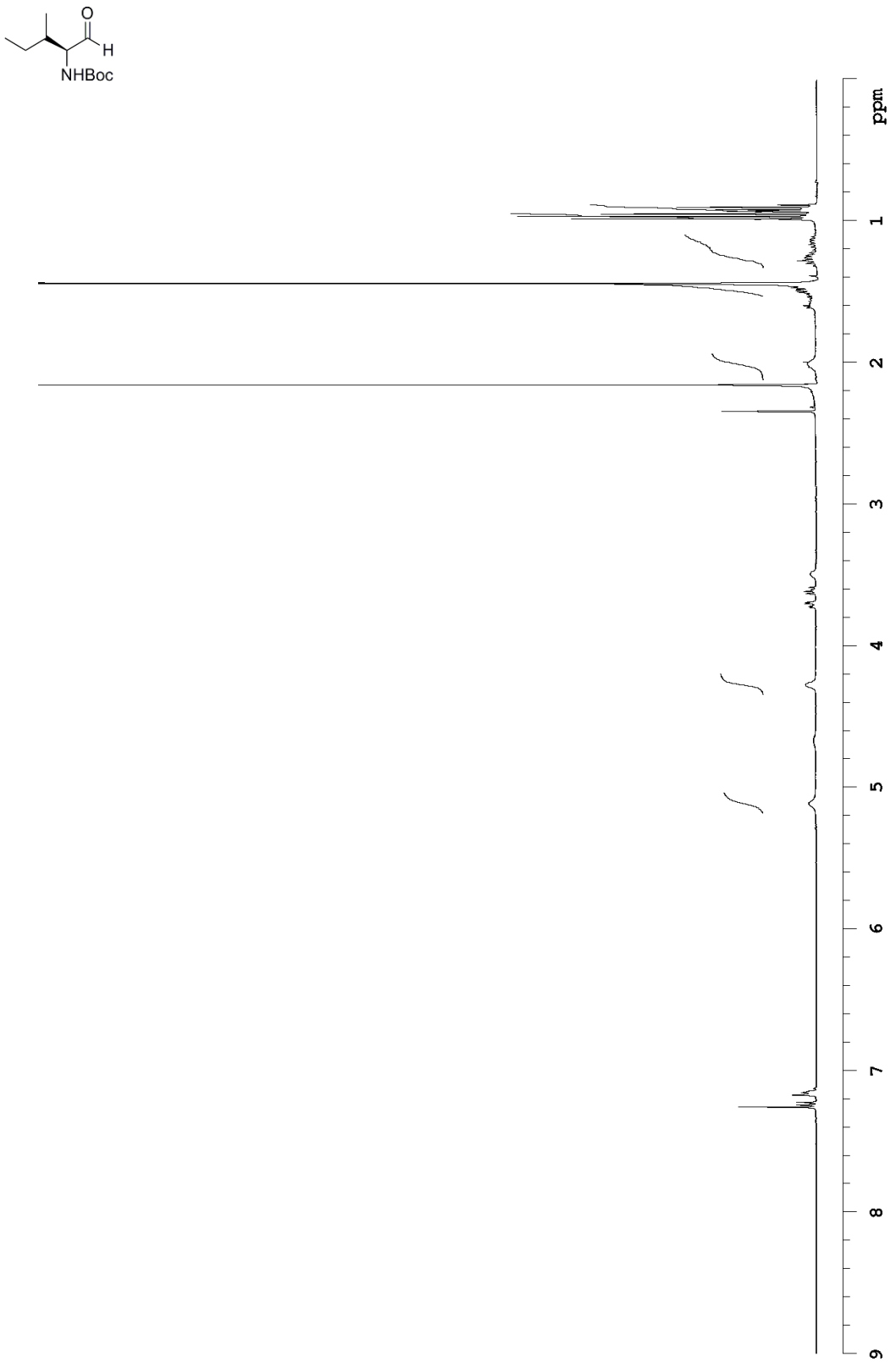
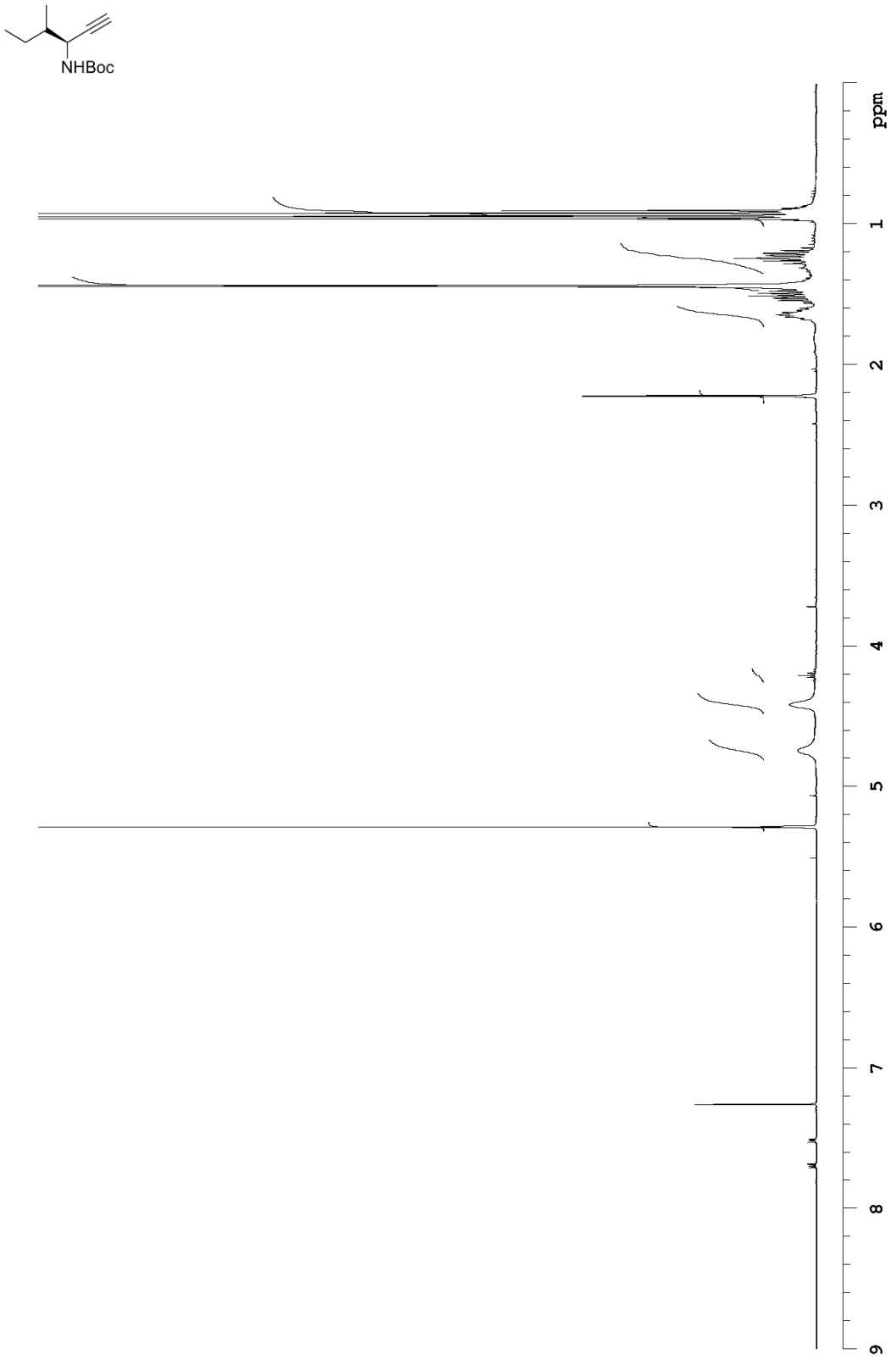


LC/MS *cyclo*-Phe-Abu-Lys(Ac)-D-Pro

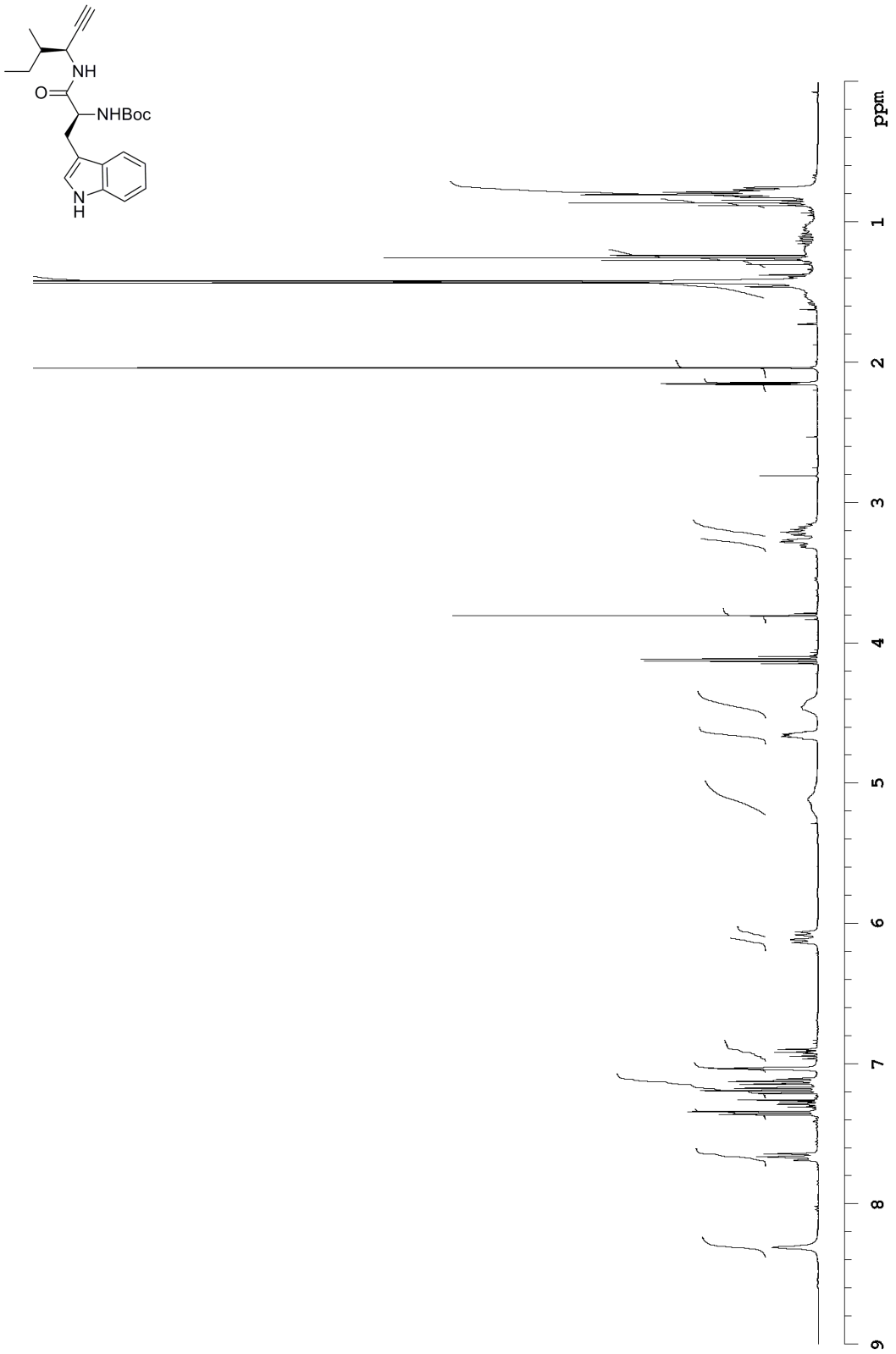
Supporting spectra for HDACI 17



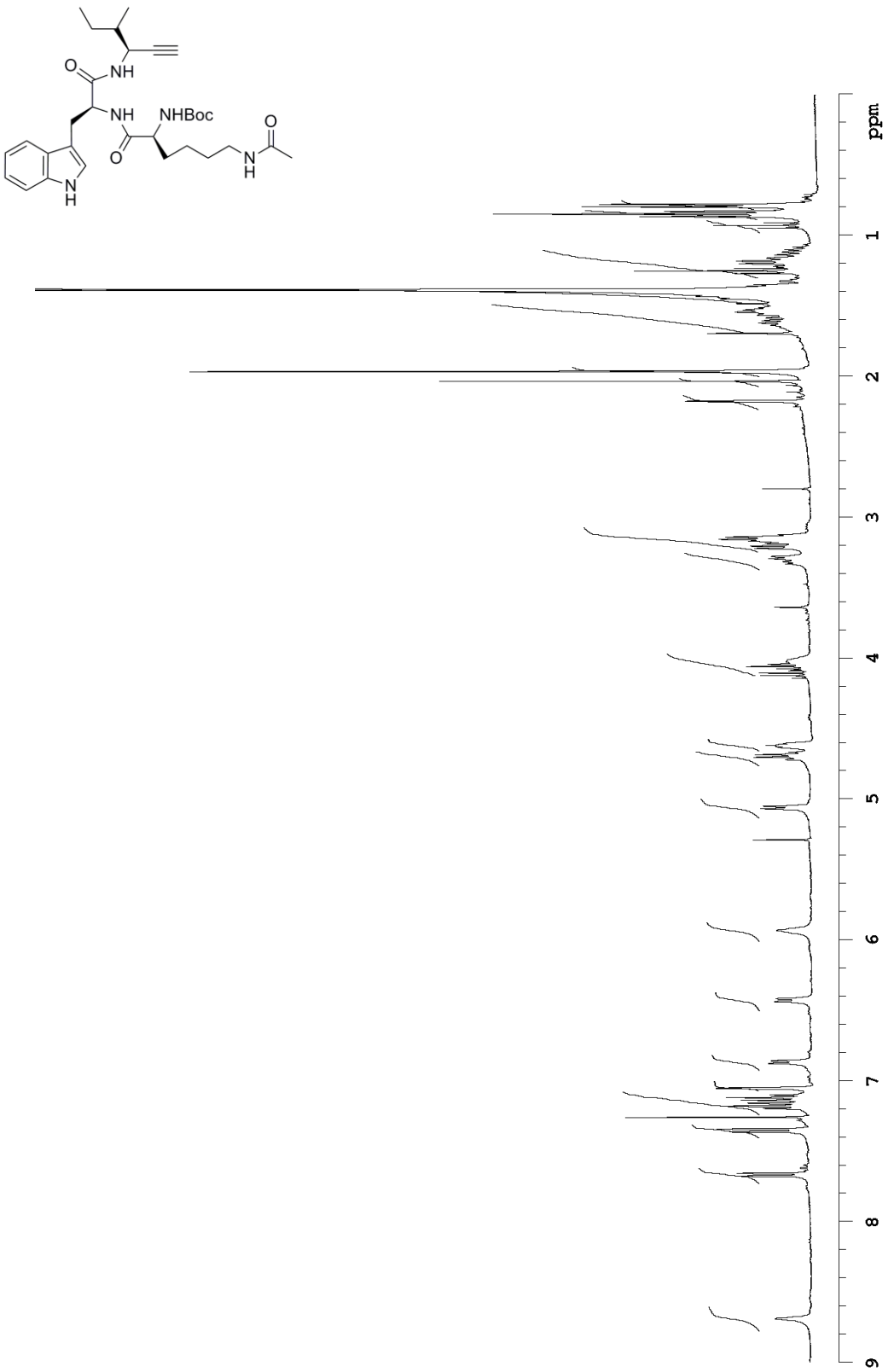
^{1}H NMR Isoleuicinal-NHBoc



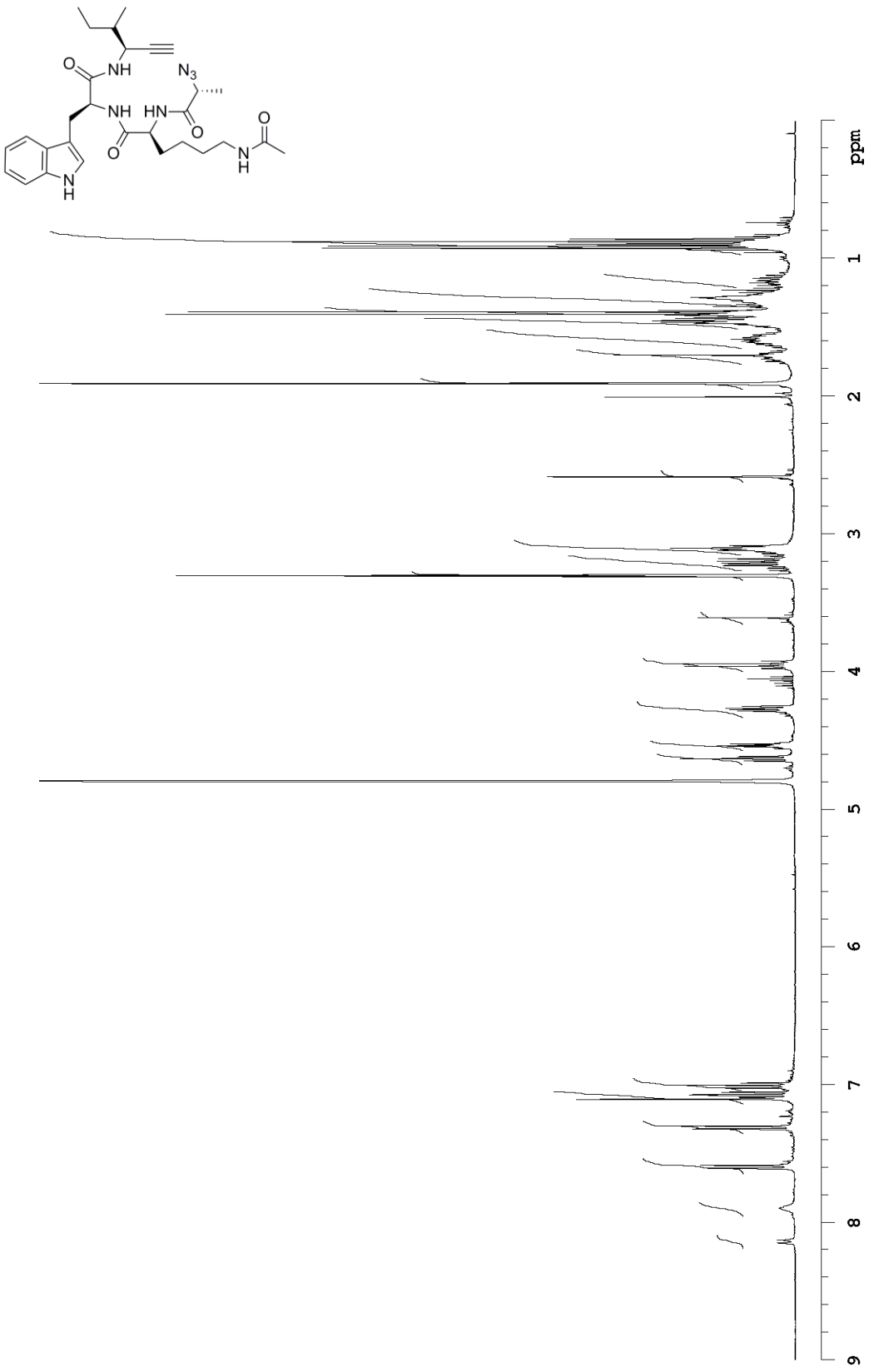
^{1}H NMR Alkyne-Ile-NHBoc



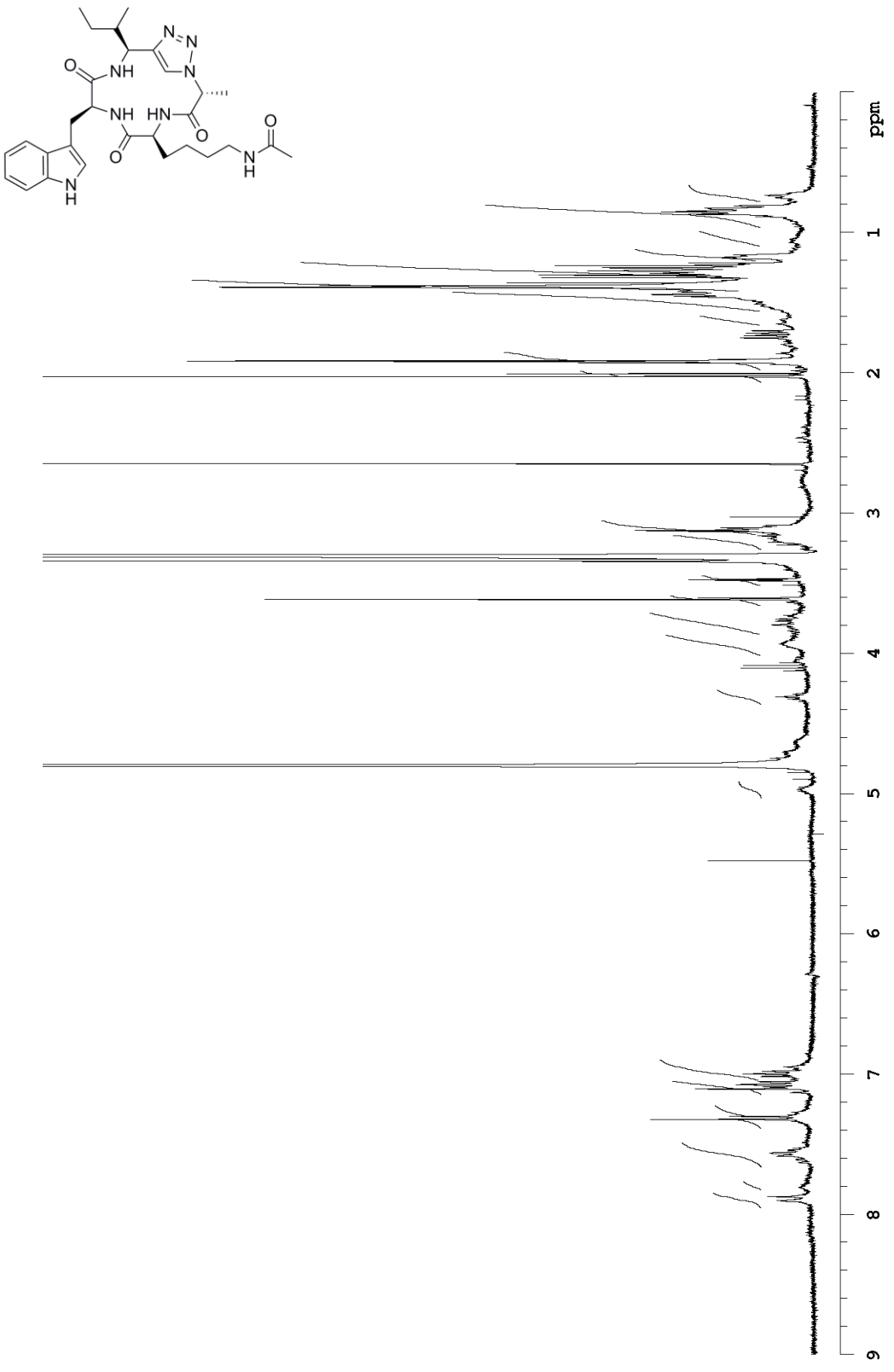
^{1}H NMR Alkyne-Ile-Trp-NHBoc



^{1}H NMR Alkyne-Ile-Trp-Lys(Ac)-NHBoc



^{1}H NMR Alkyne-Ile-Trp-Lys(Ac)-D-Ala-N_3



^{1}H NMR *cyclo*-Ile-Trp-Lys(Ac)-D-Ala-Tr

Display Report - All Windows Selected Analysis

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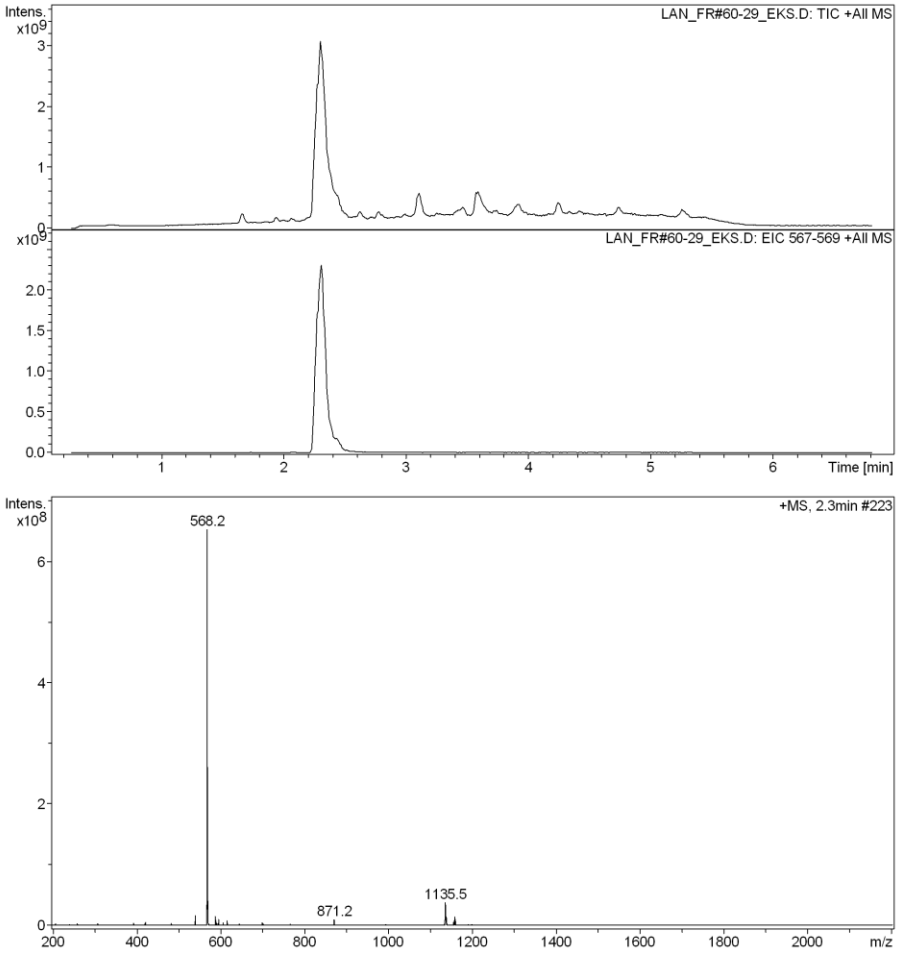
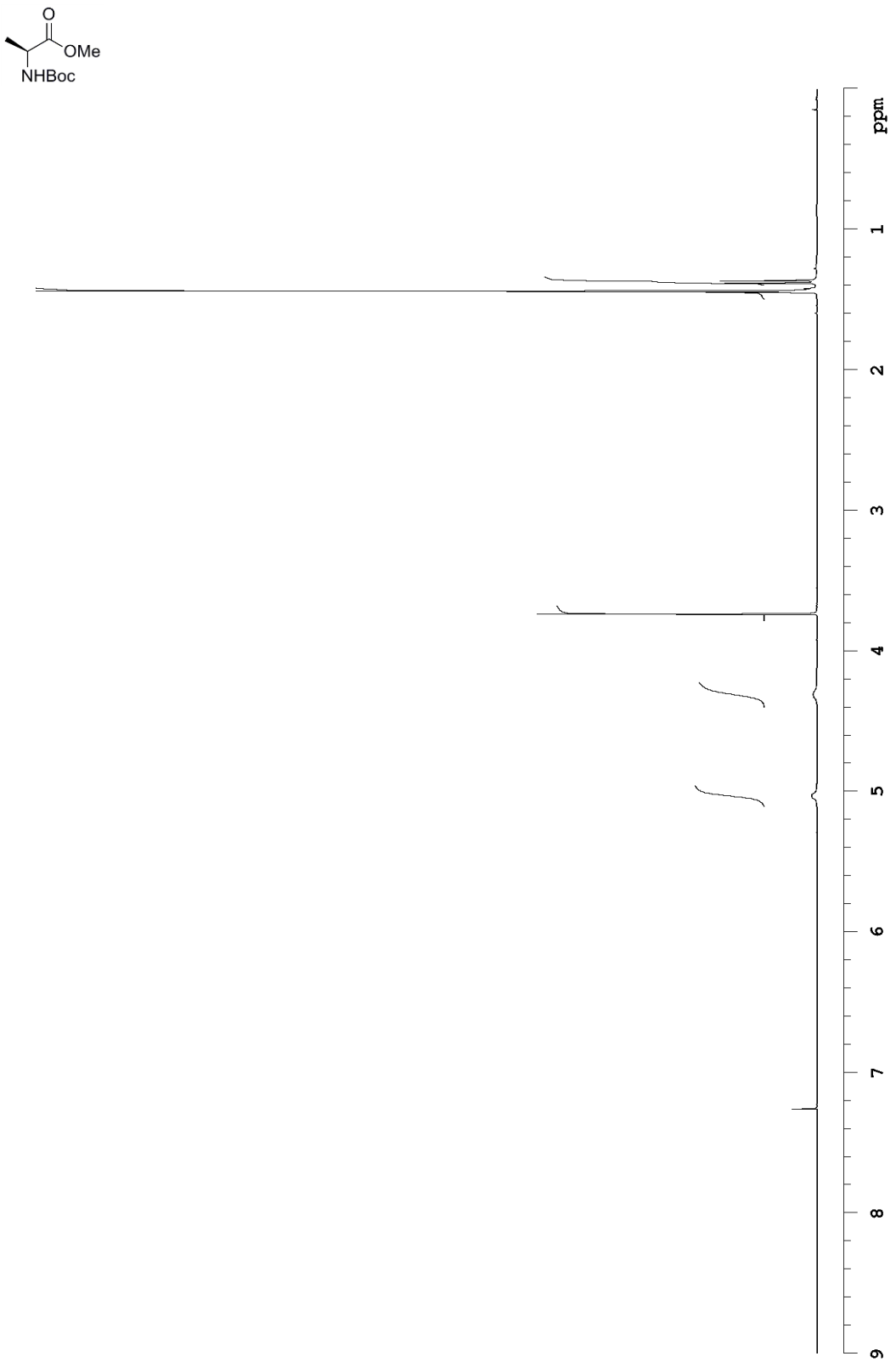


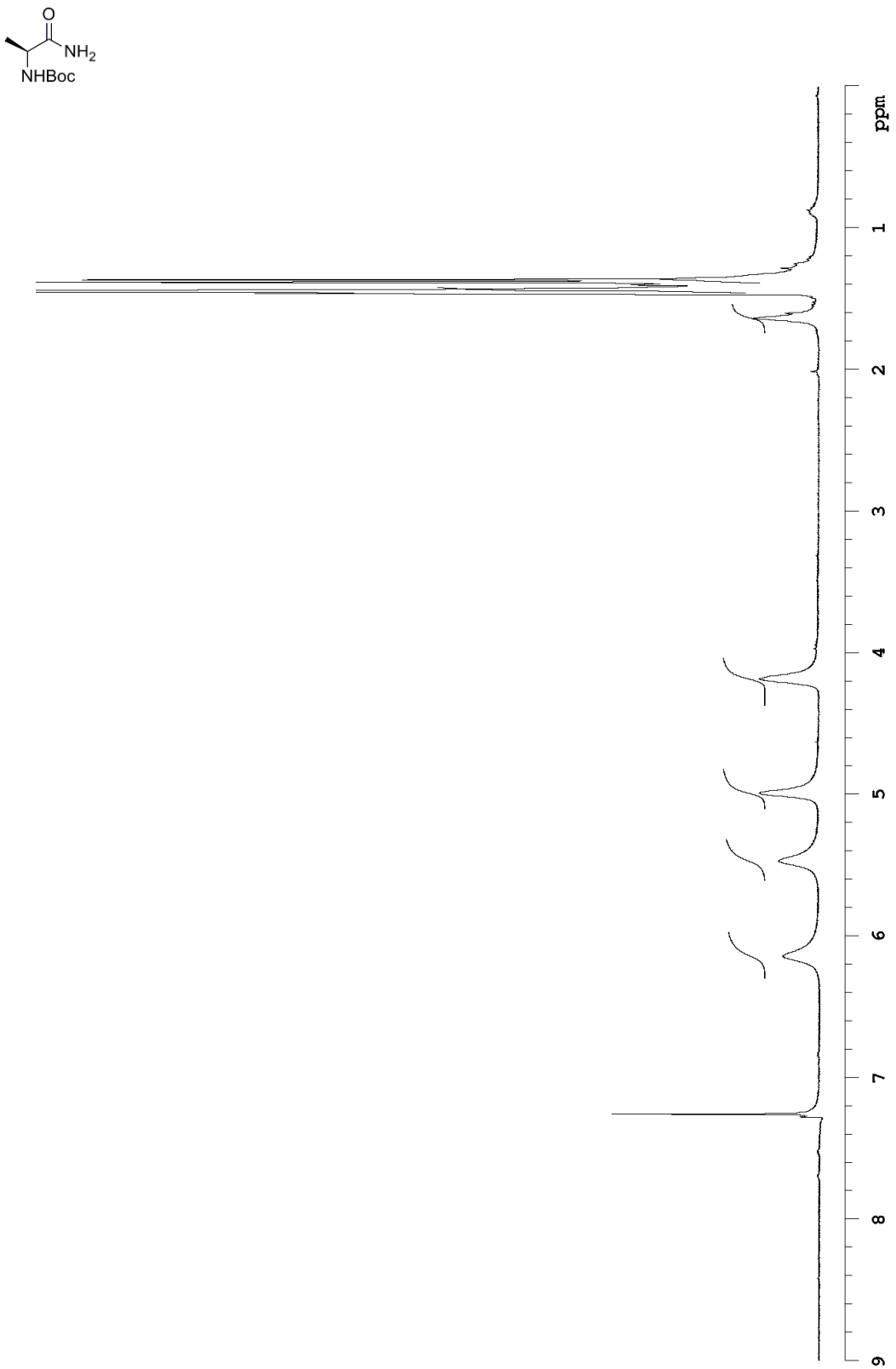
LC/MS *cyclo*-Ile-Trp-Lys(Ac)-D-Ala-Tr

Appendix C – Supporting spectra for Chapter 4

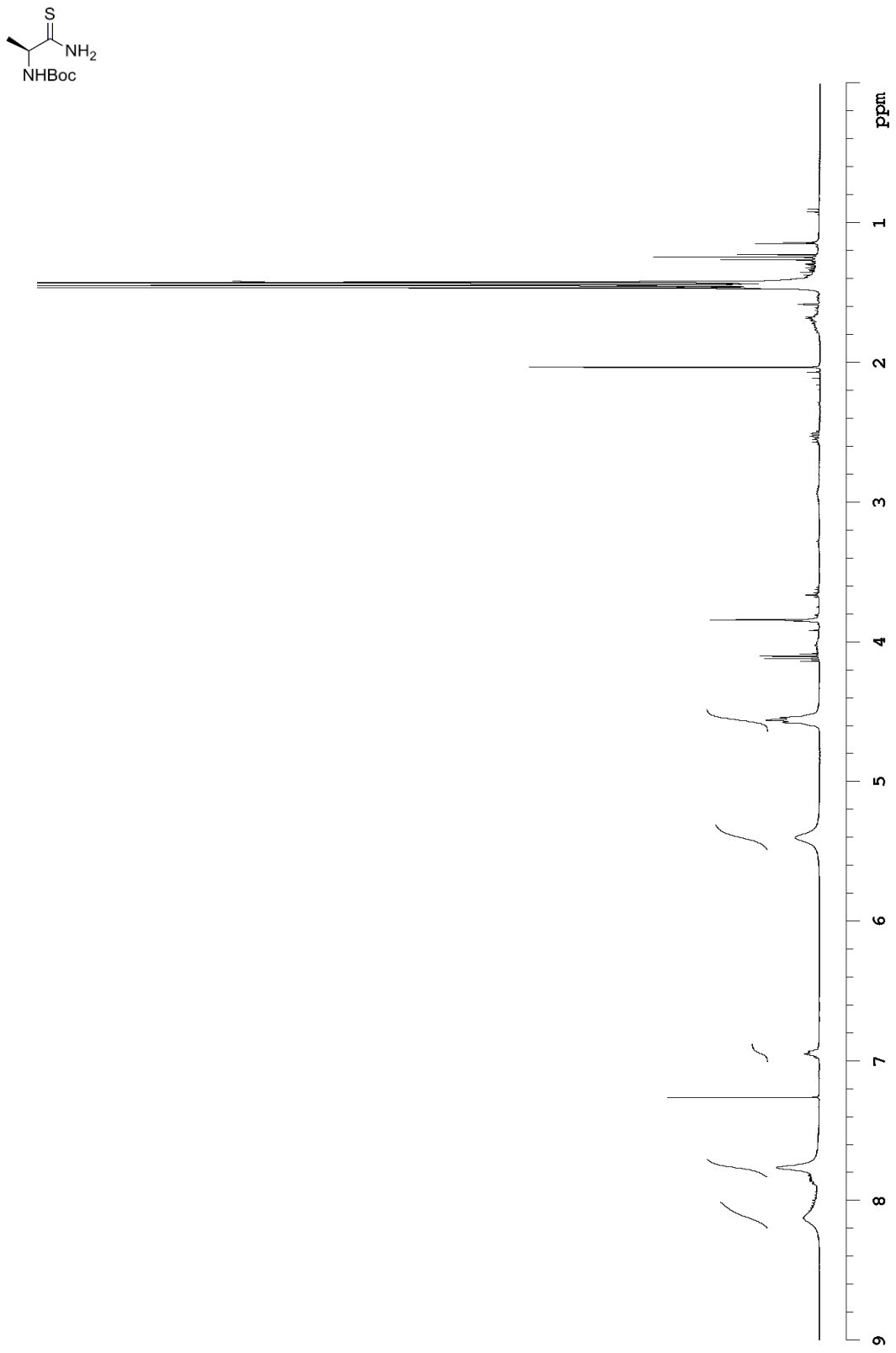
Supporting spectra for Sanguinamide B



^{1}H NMR MeO-Ala-NHBoc



^{1}H NMR $H_2N(=O)C$-Ala-NHBoc



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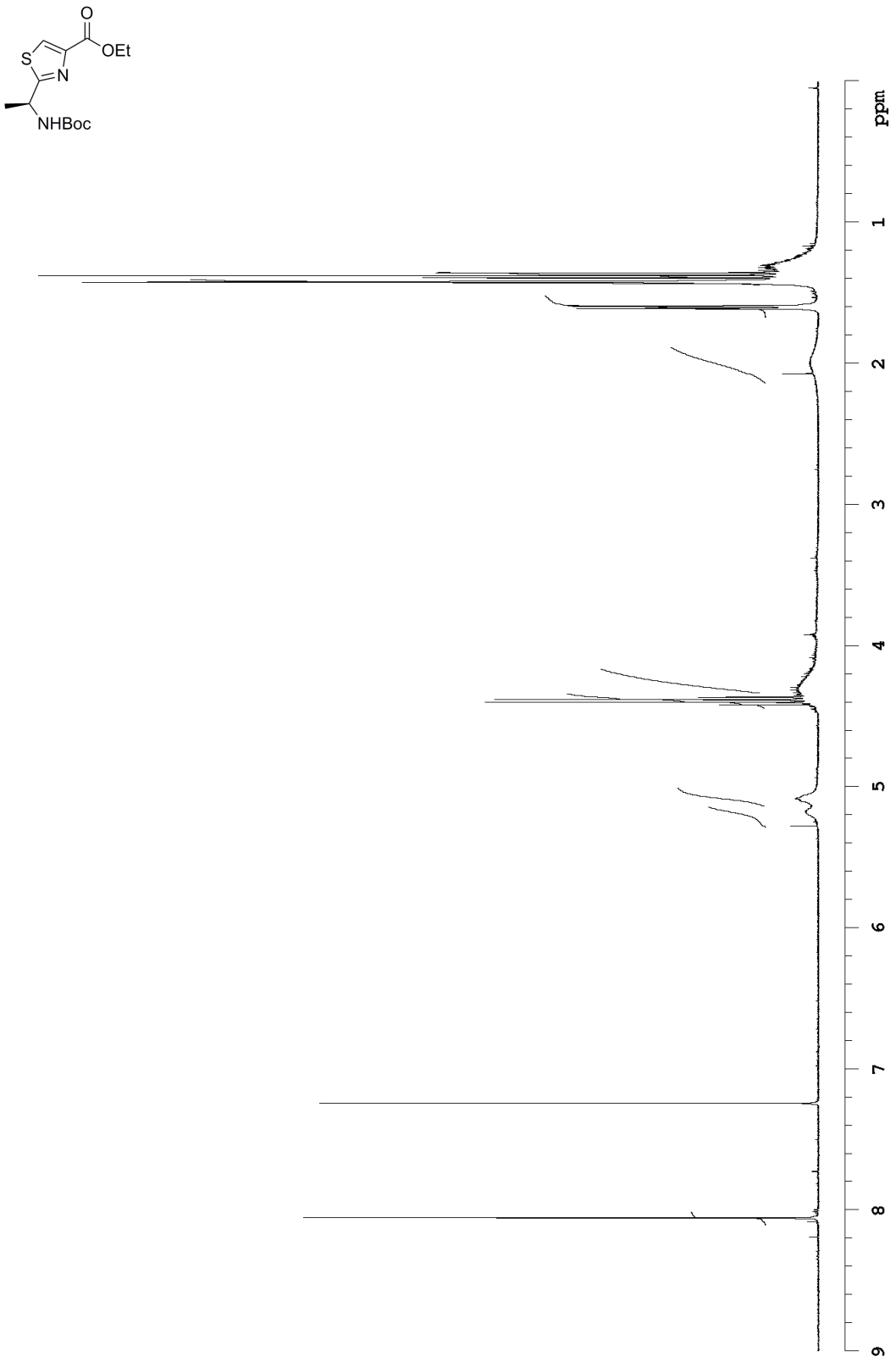

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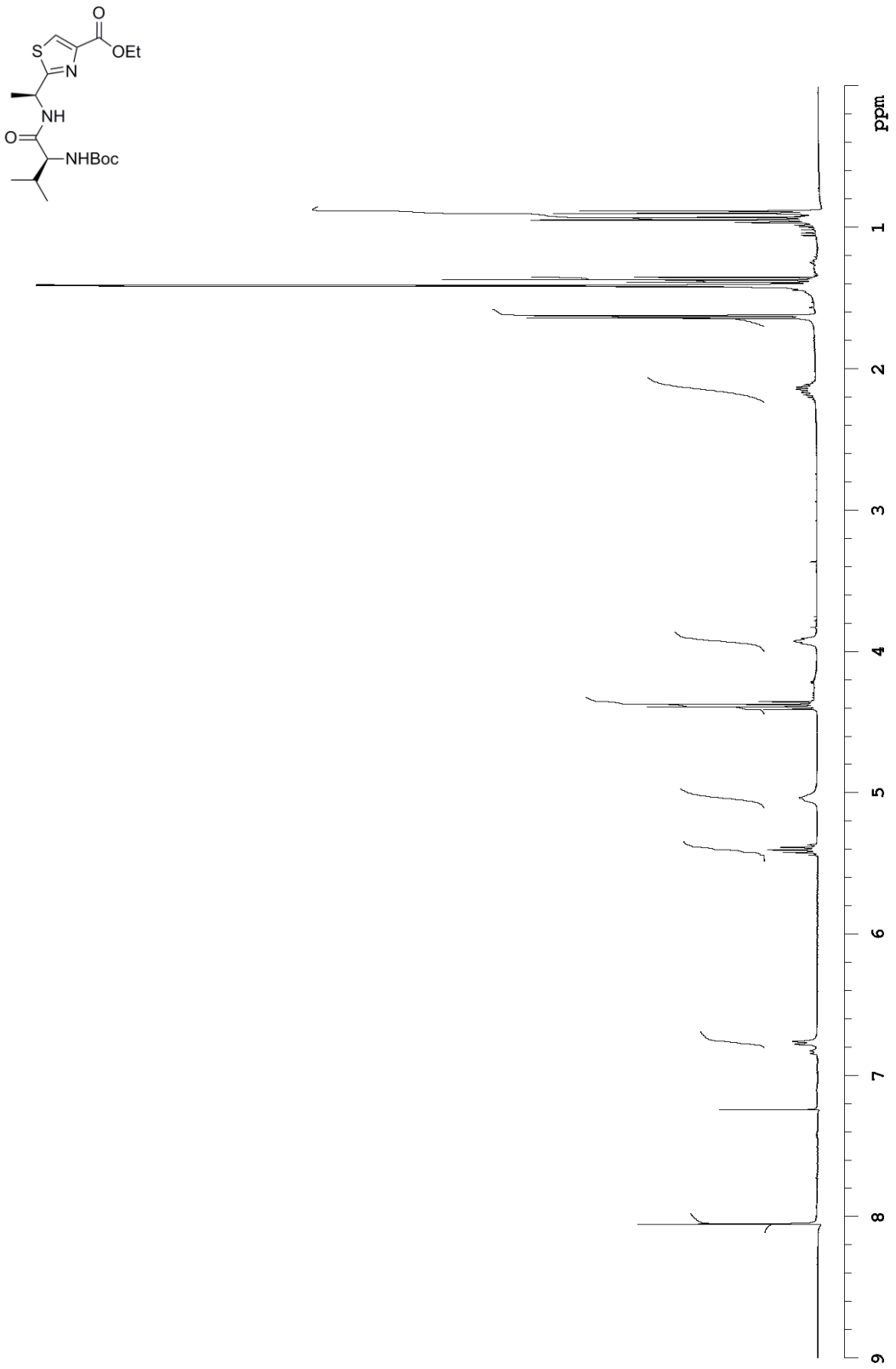

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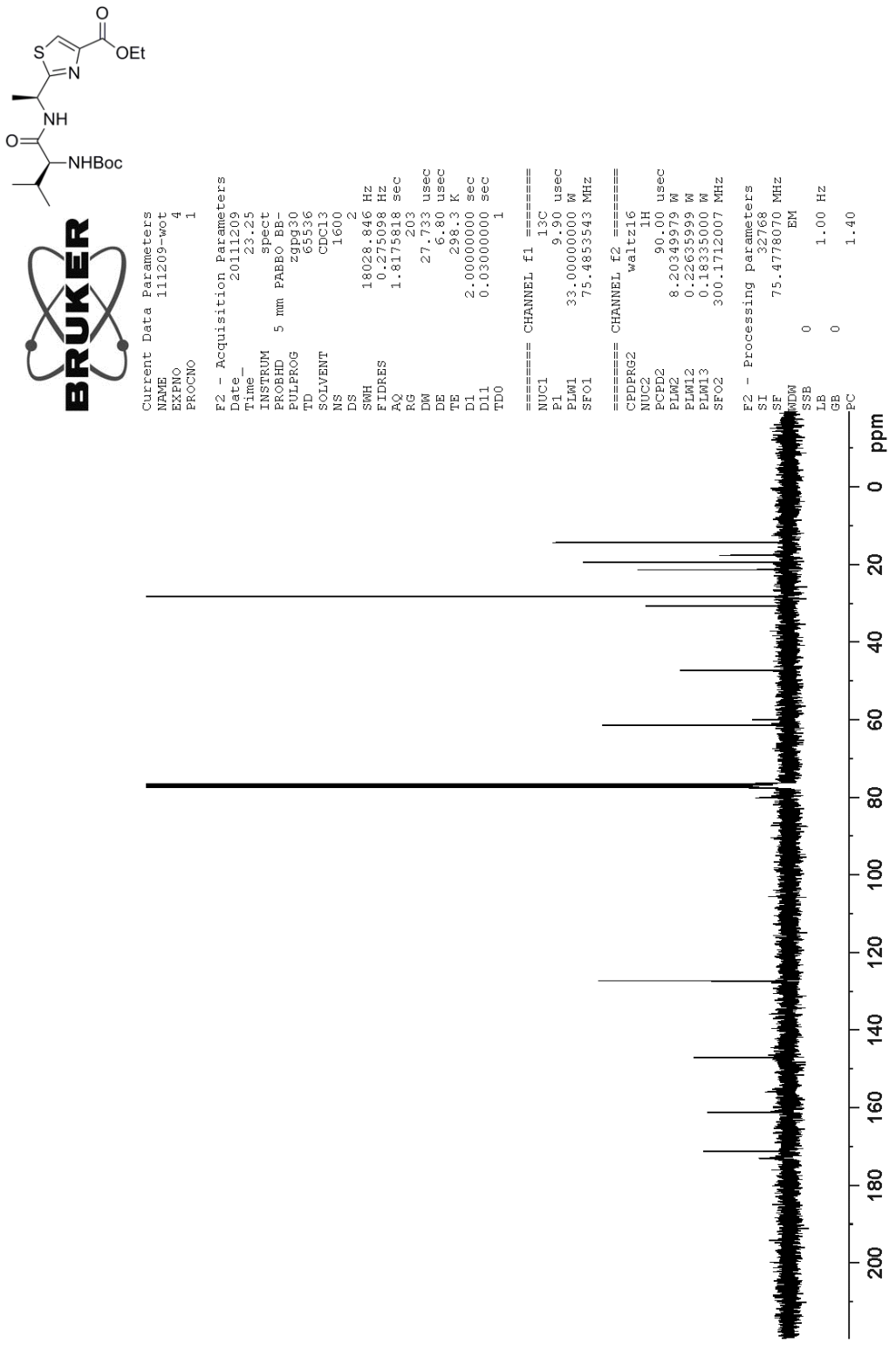

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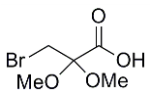

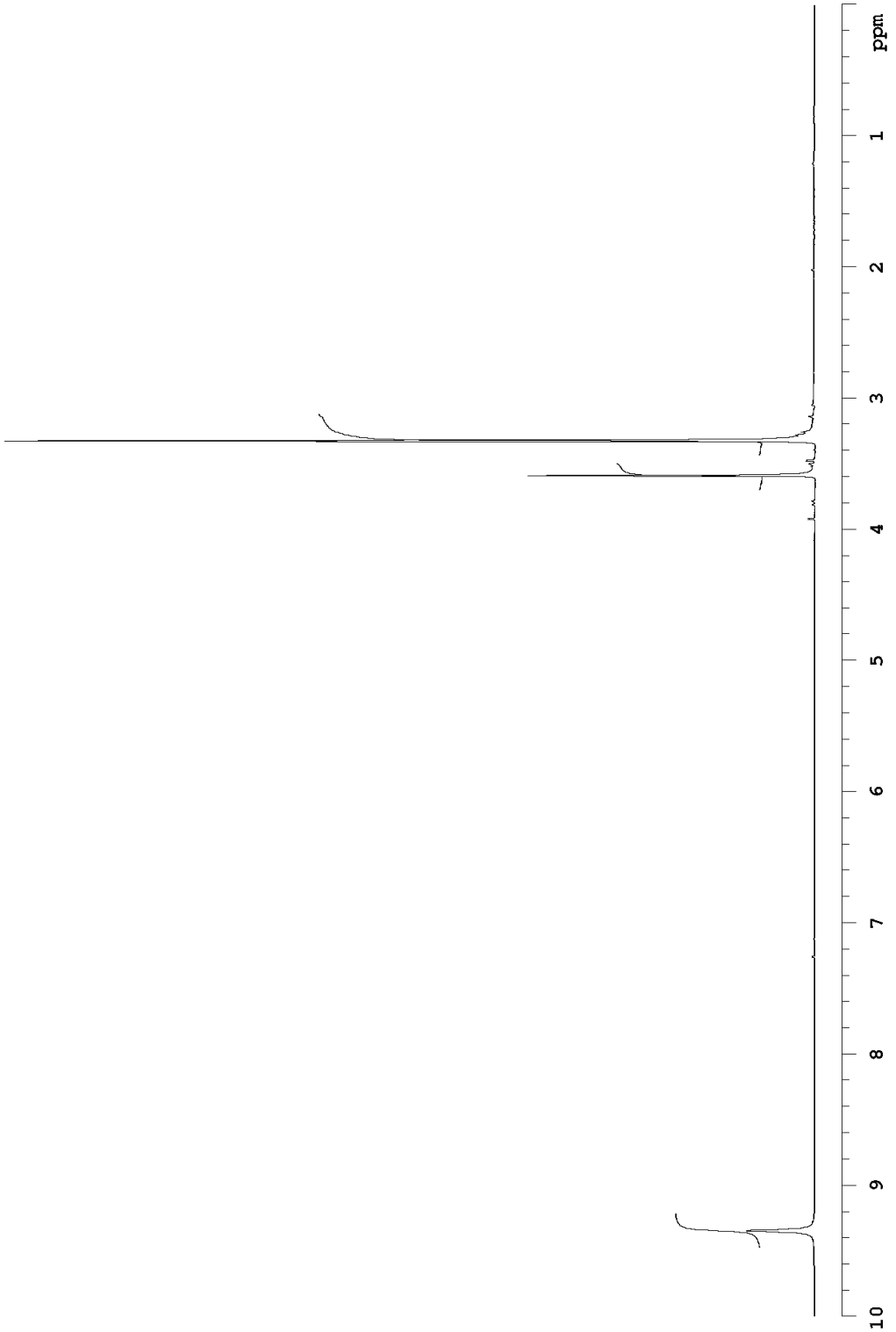

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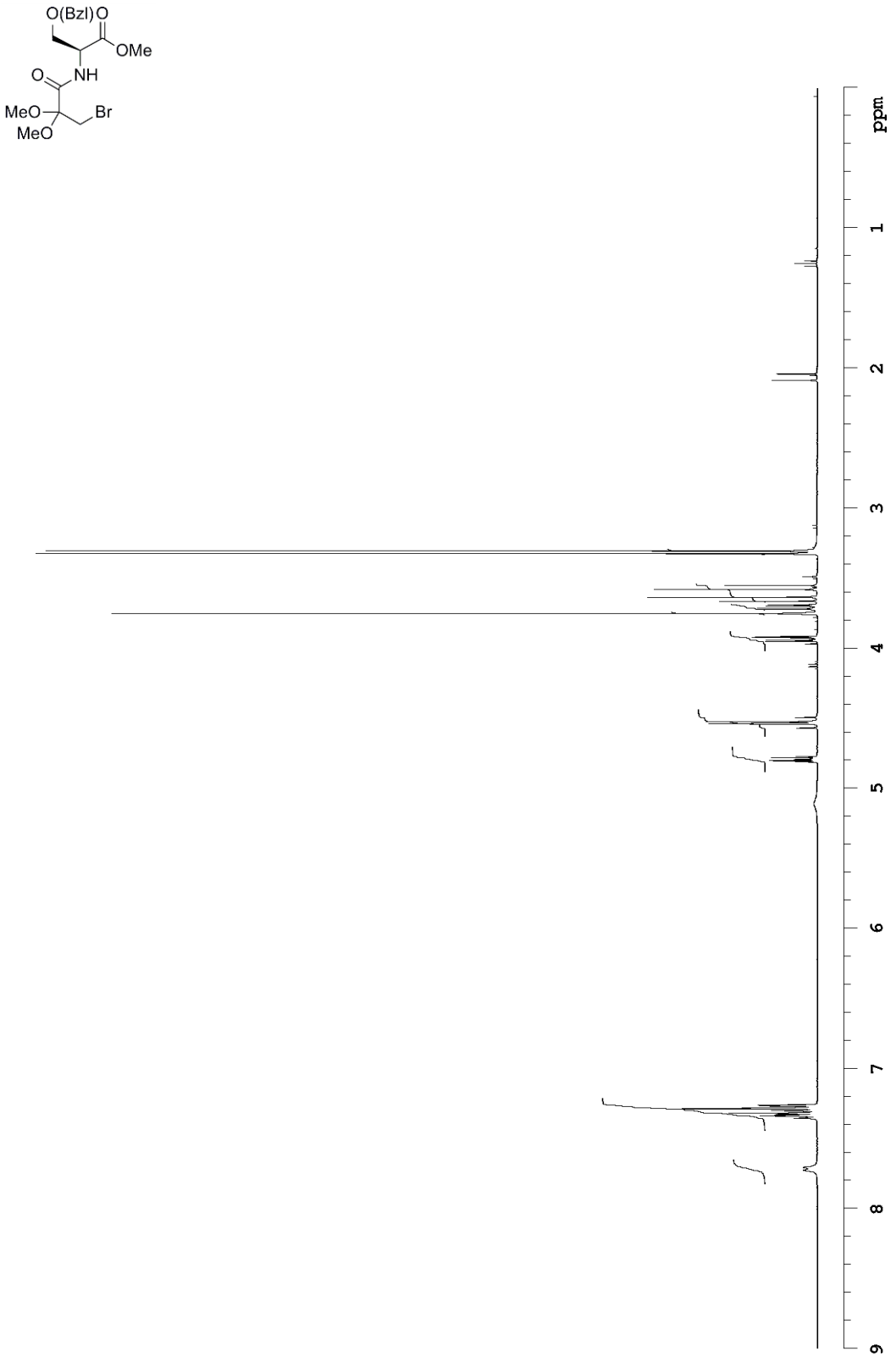
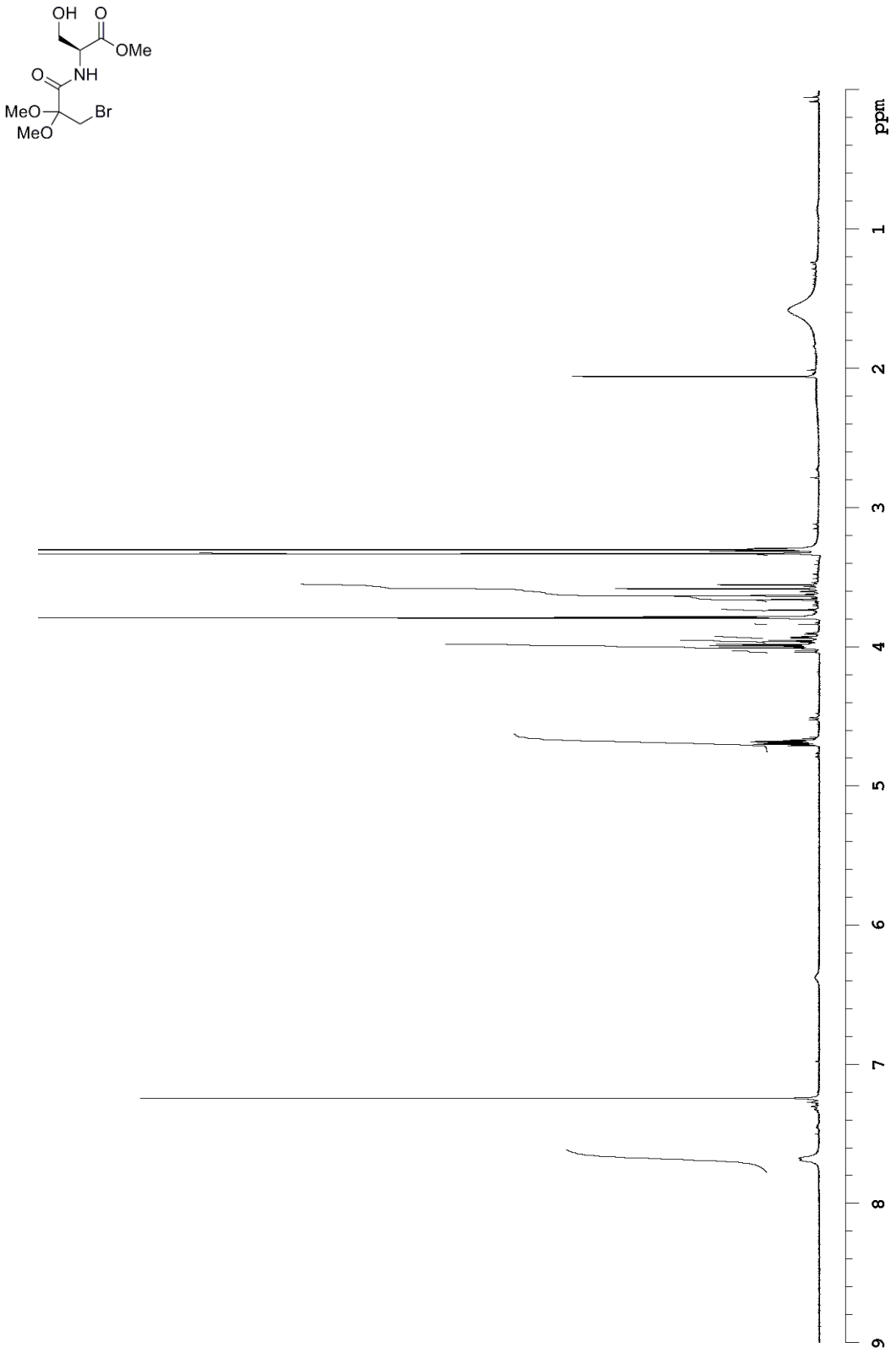


^{1}H NMR MeO-Ser(Bzl)-Bromoketal



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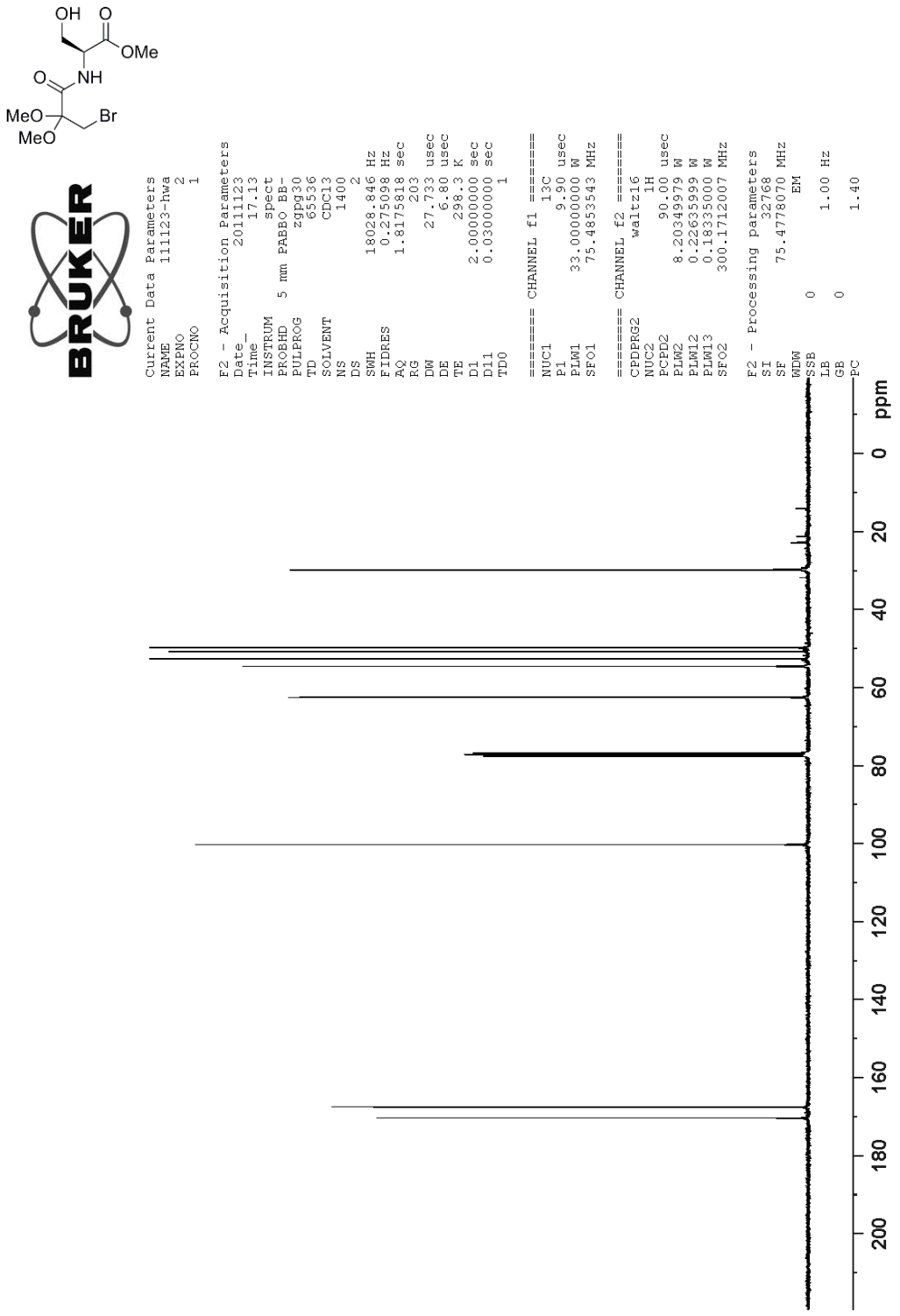

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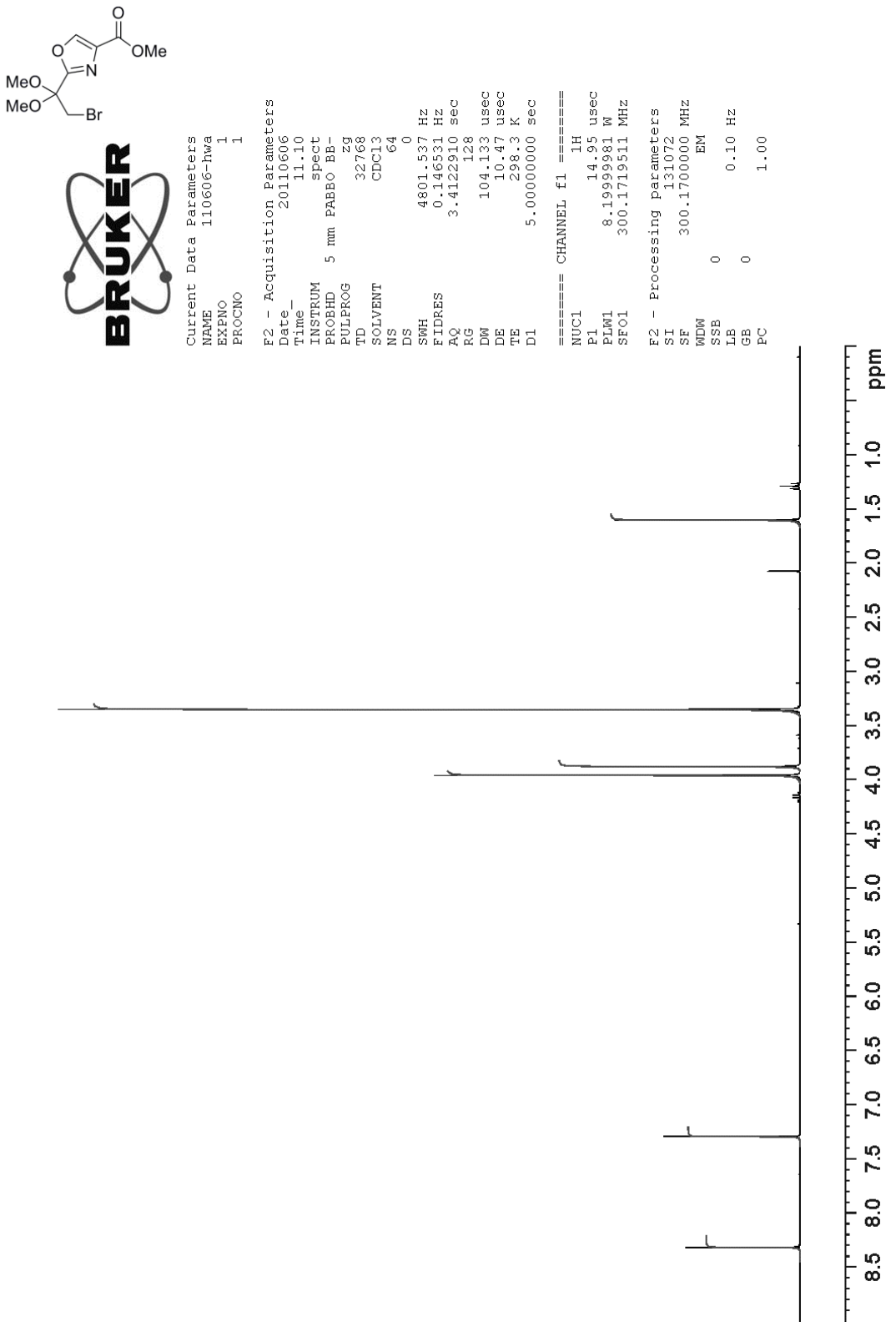

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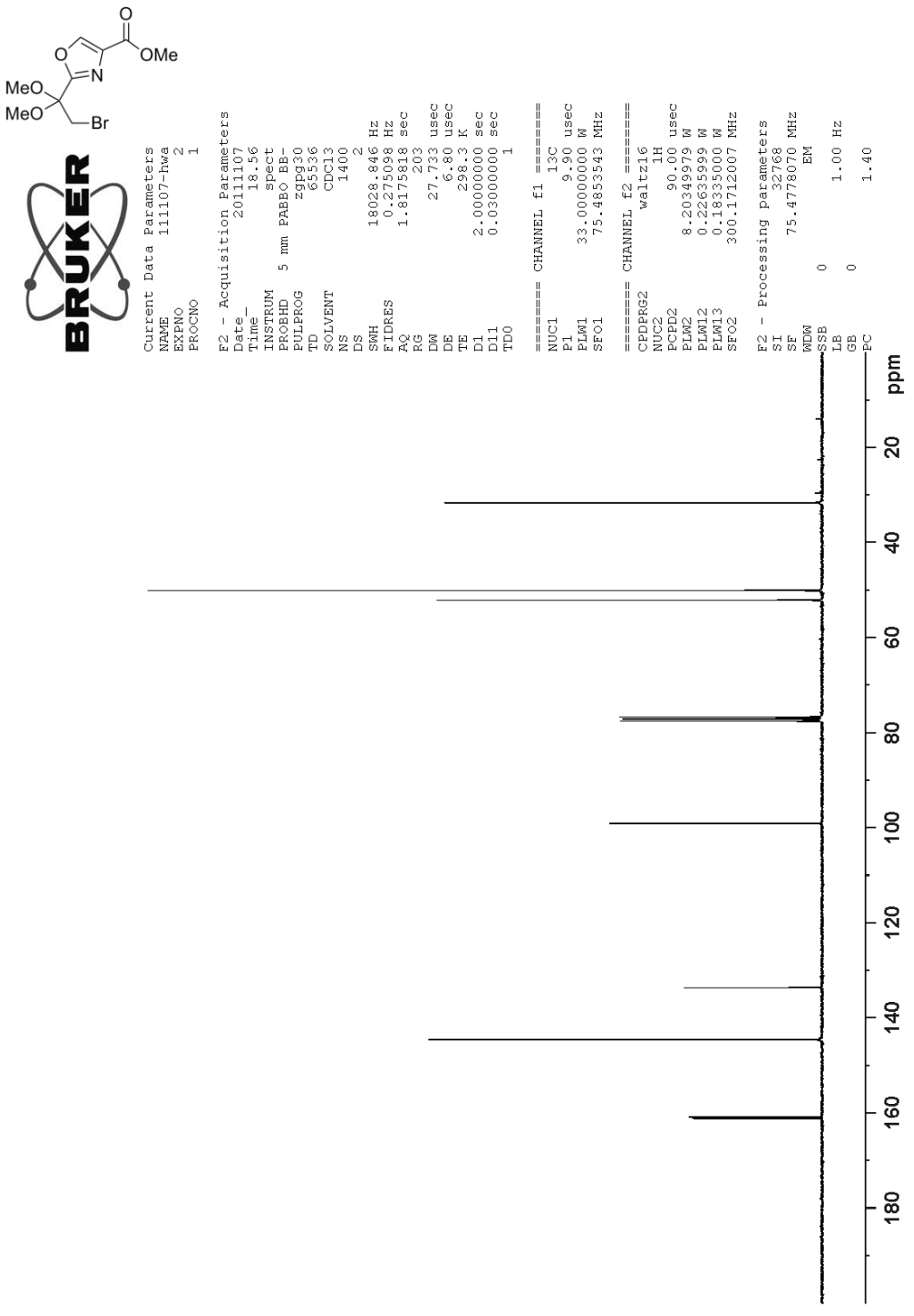

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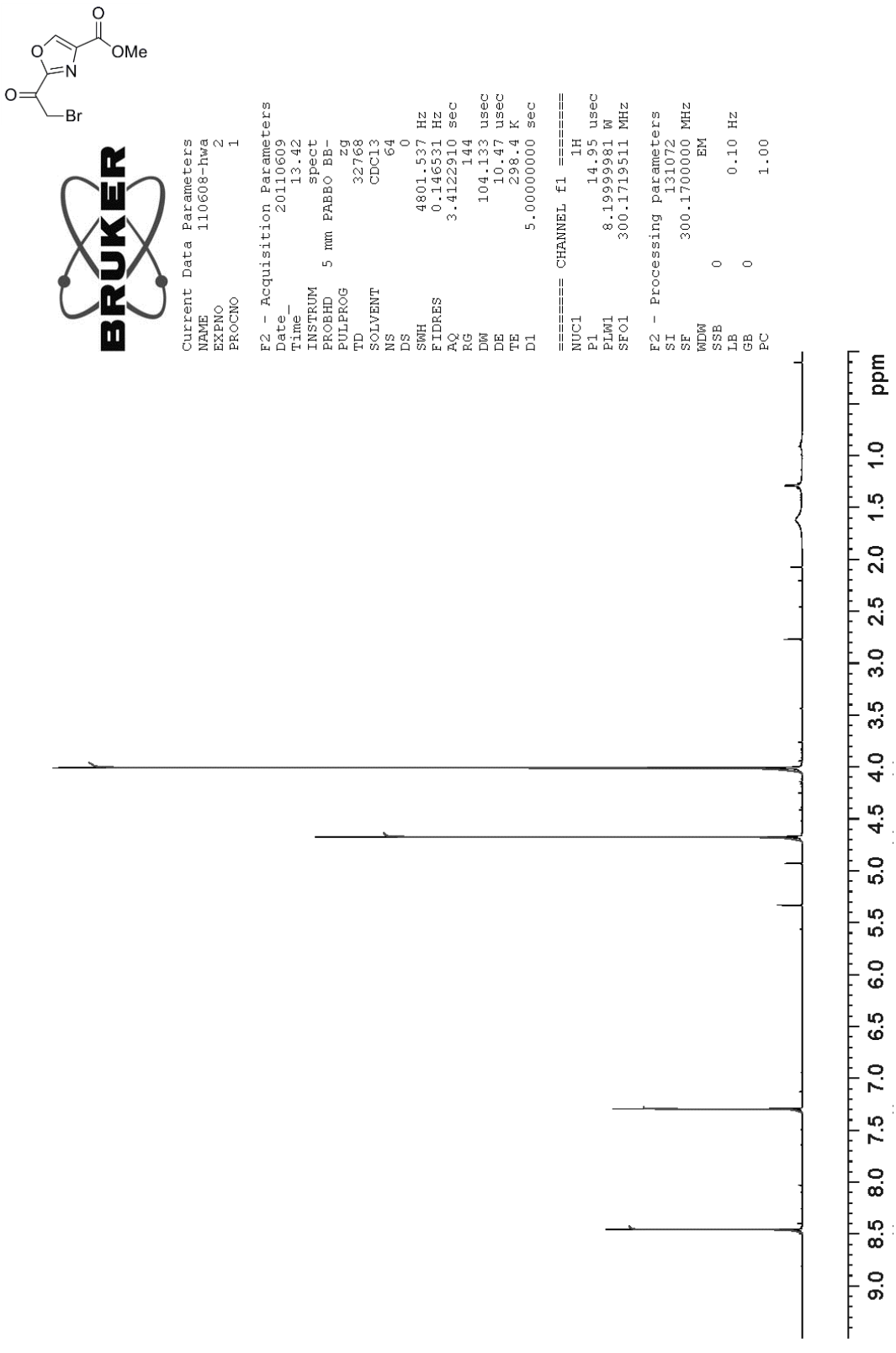

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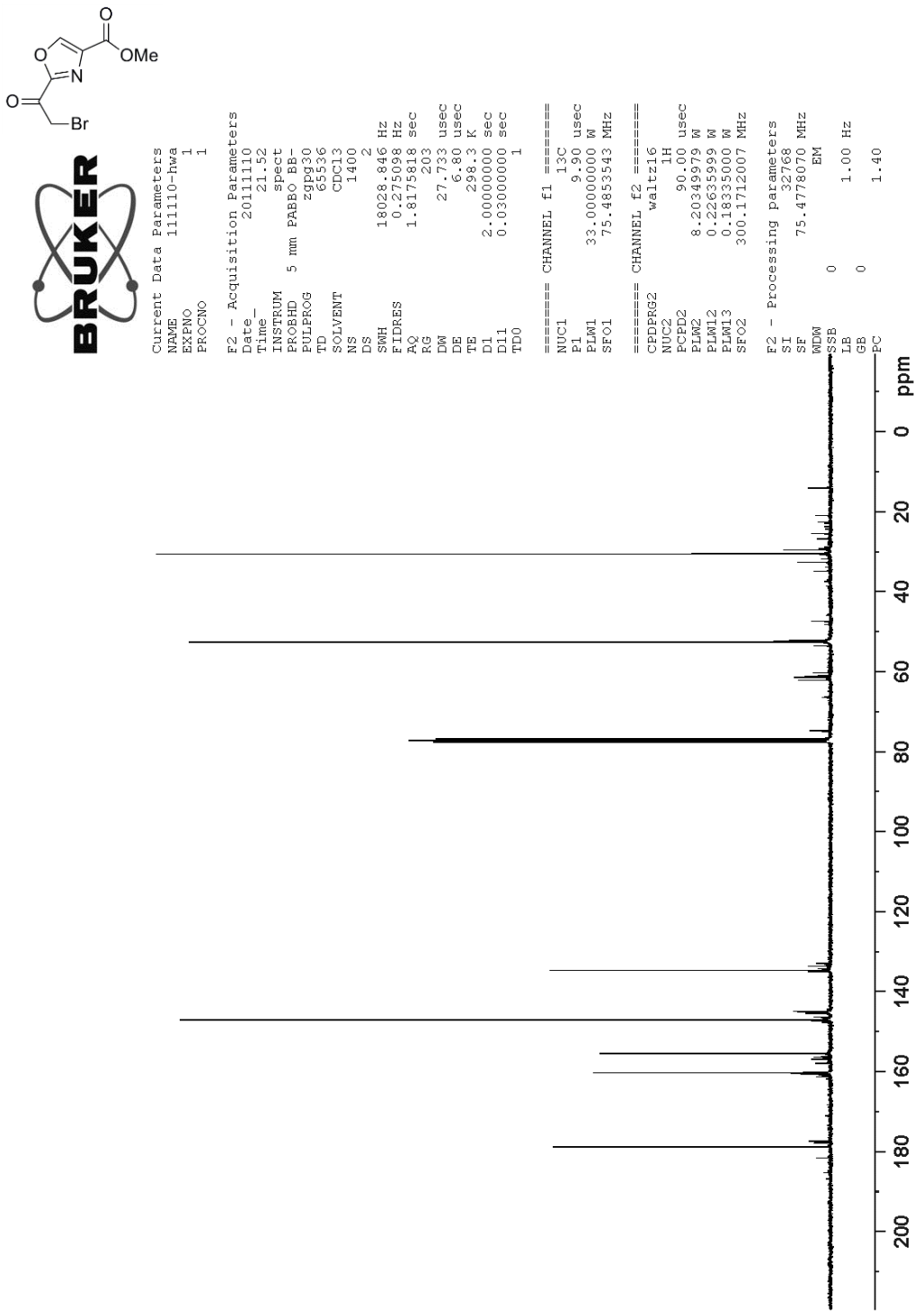

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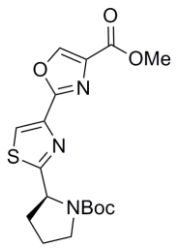
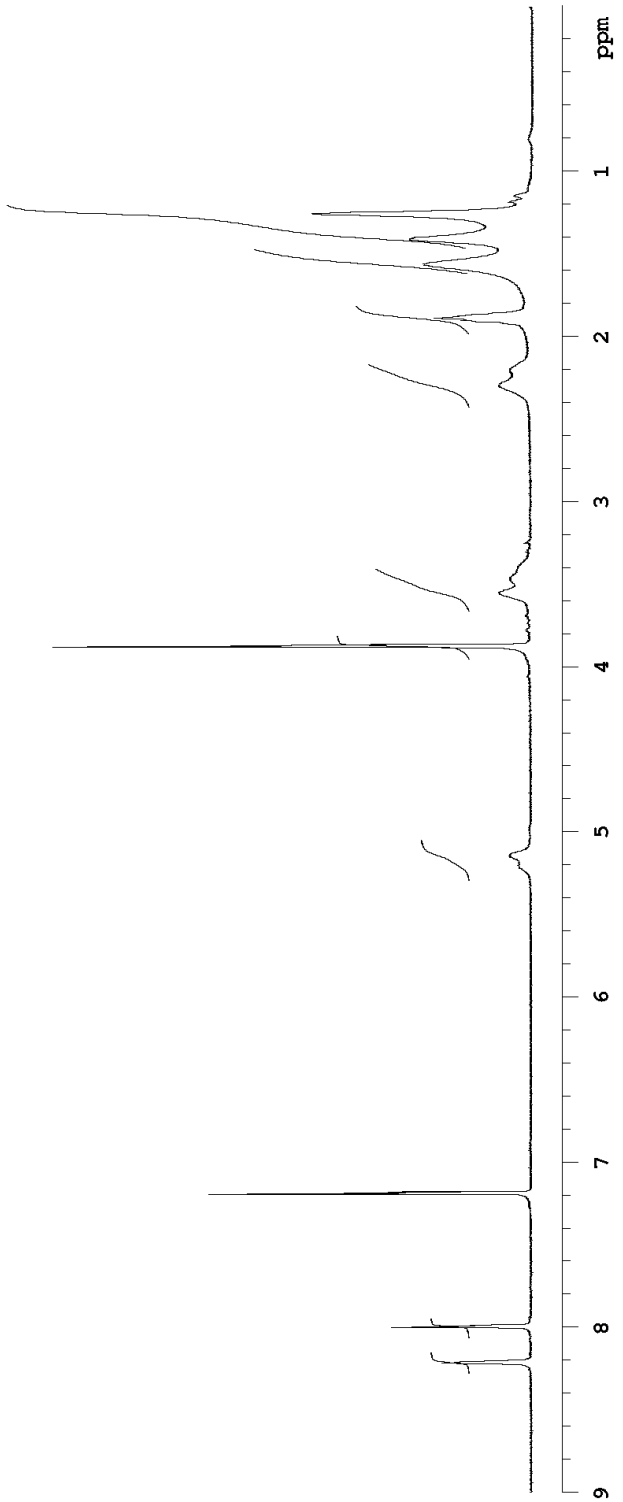
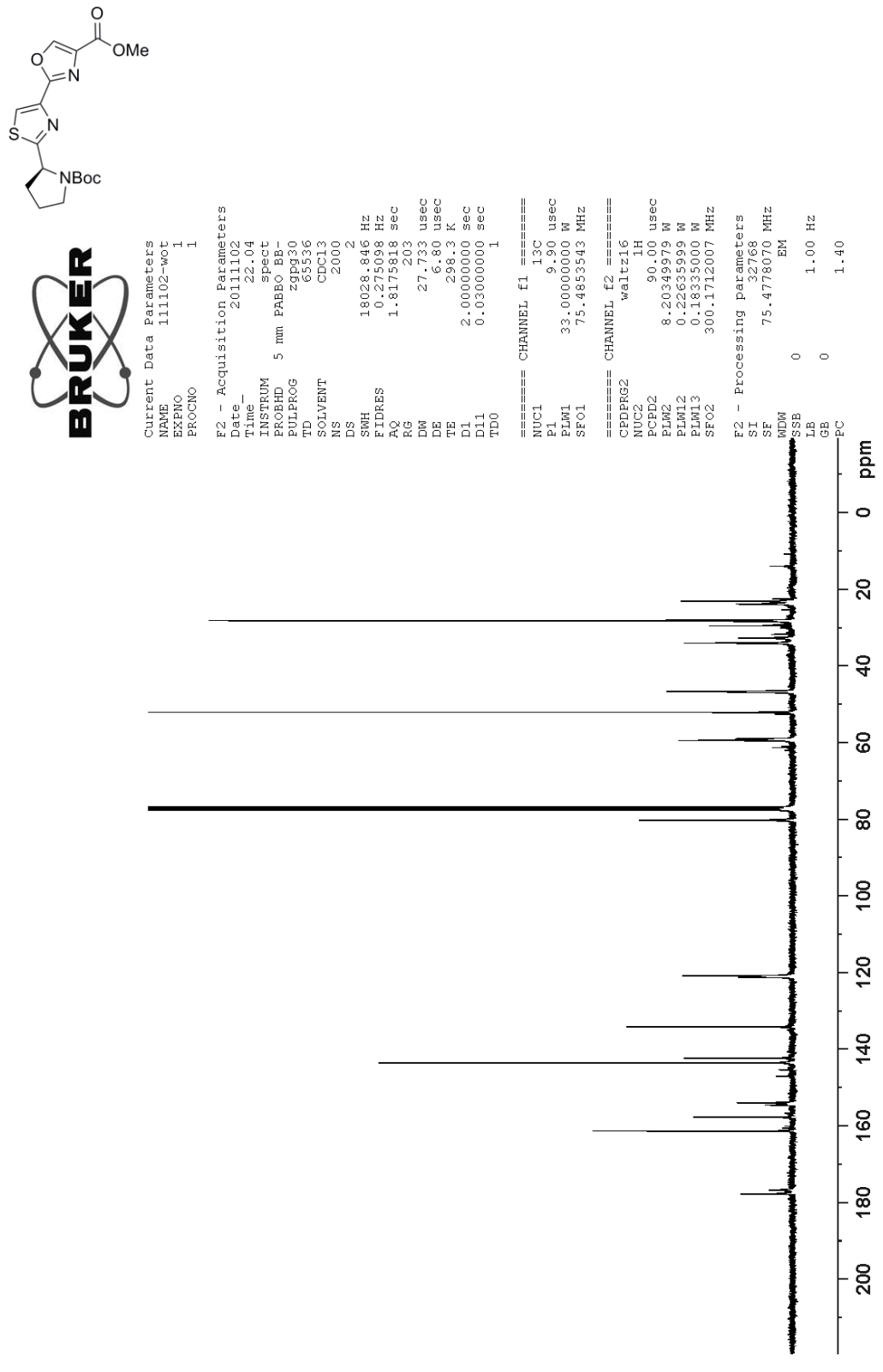




1H NMR MeO-Ox-Th-Pro-NBoc



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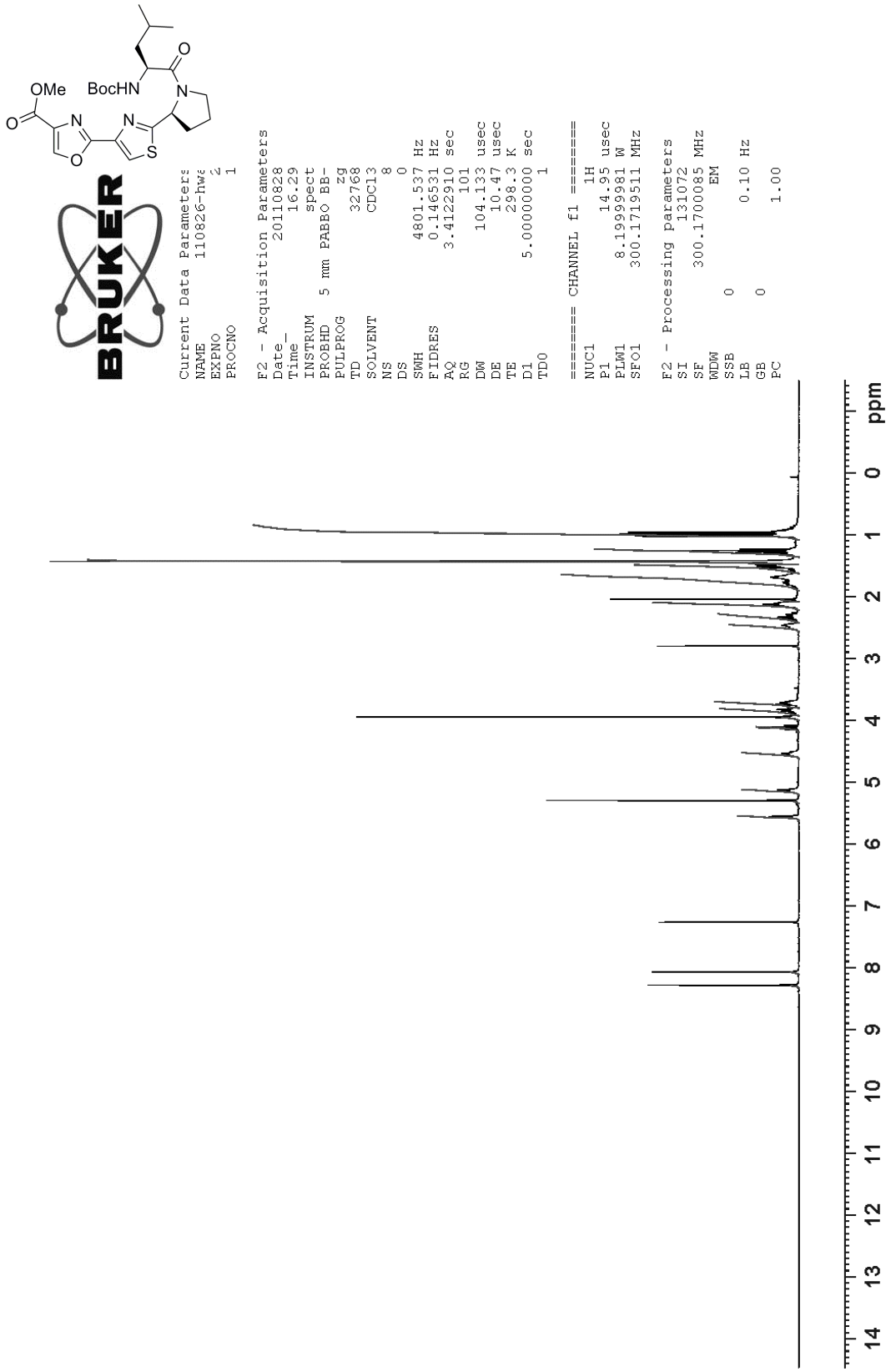

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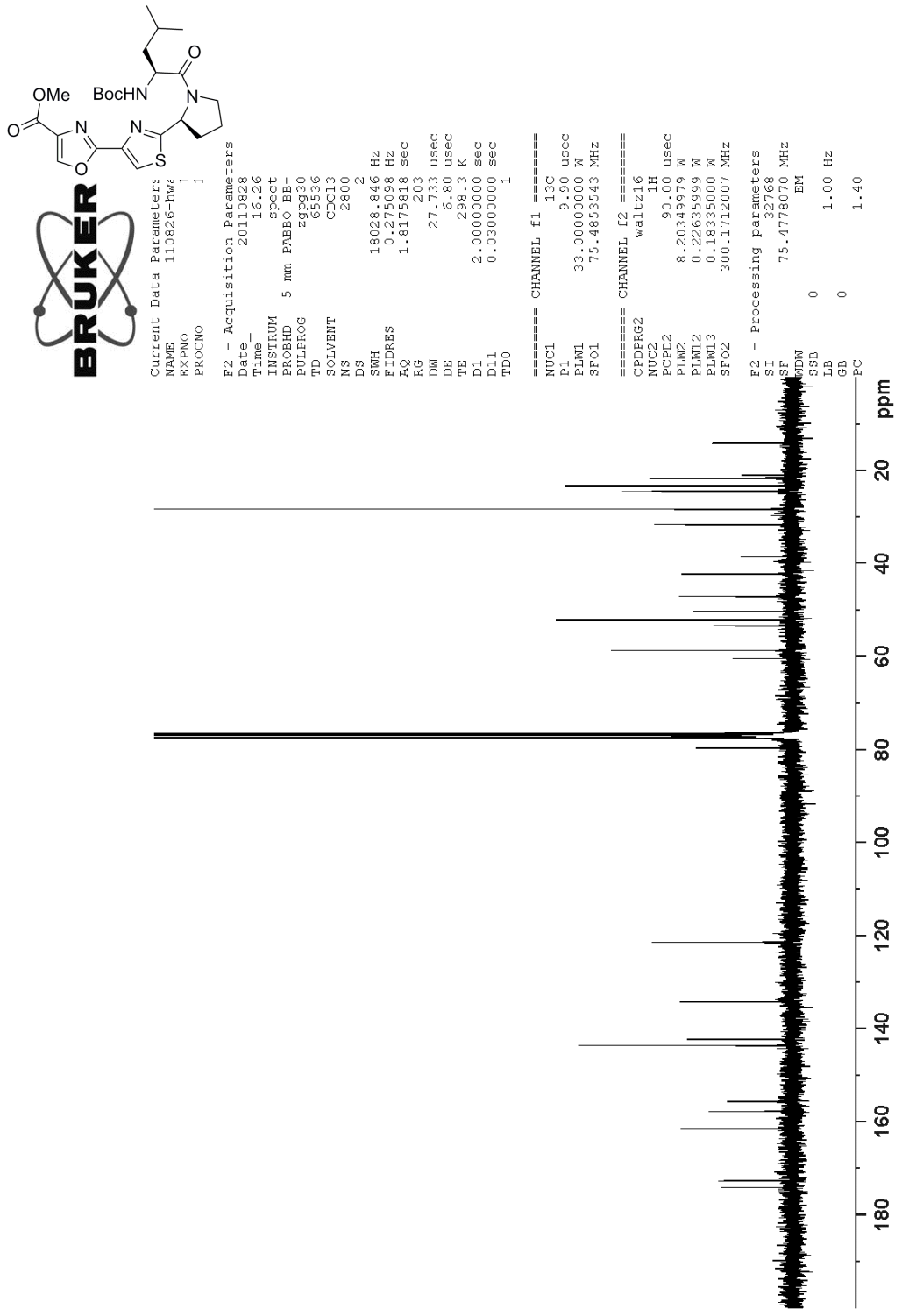

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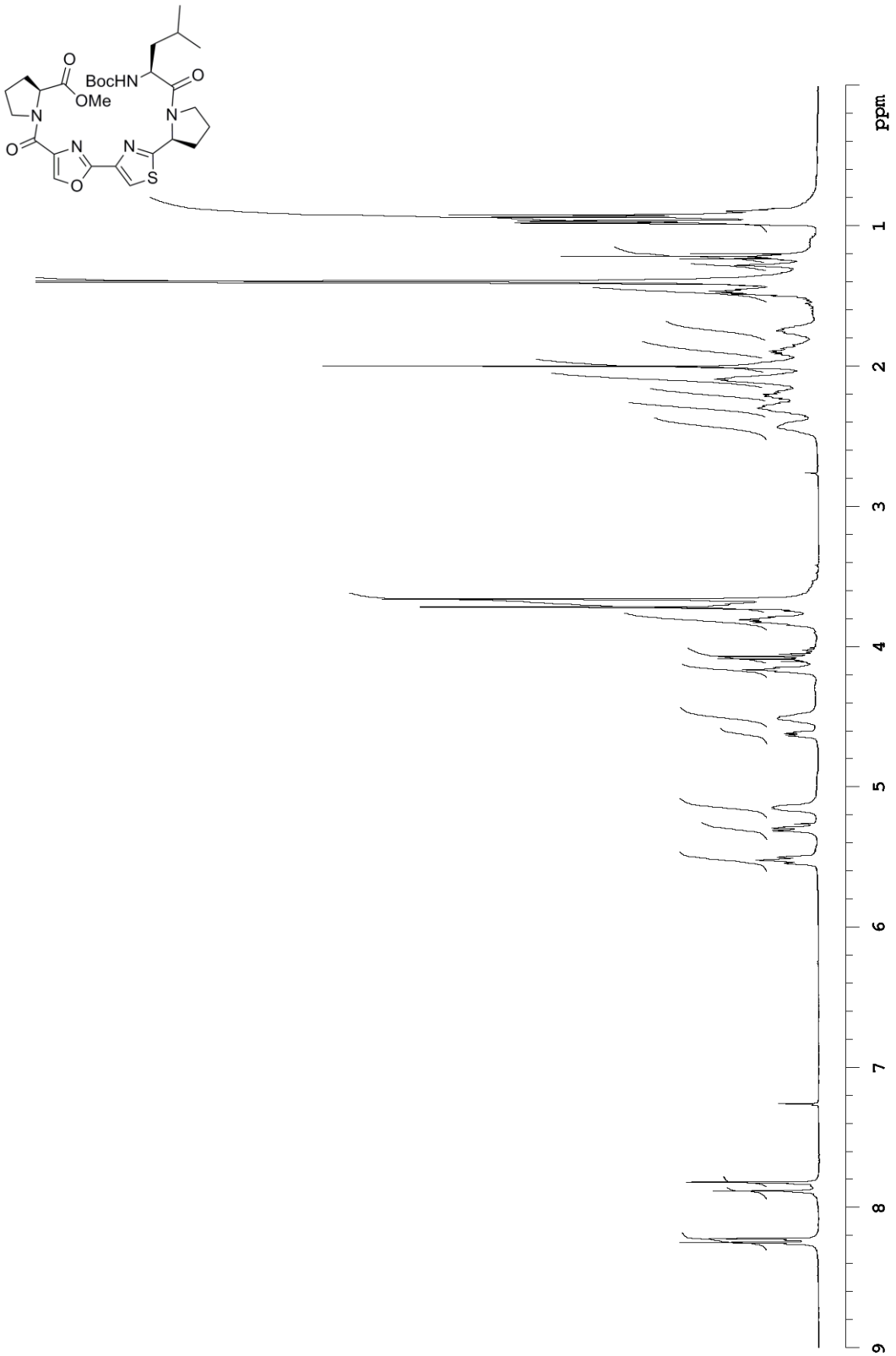

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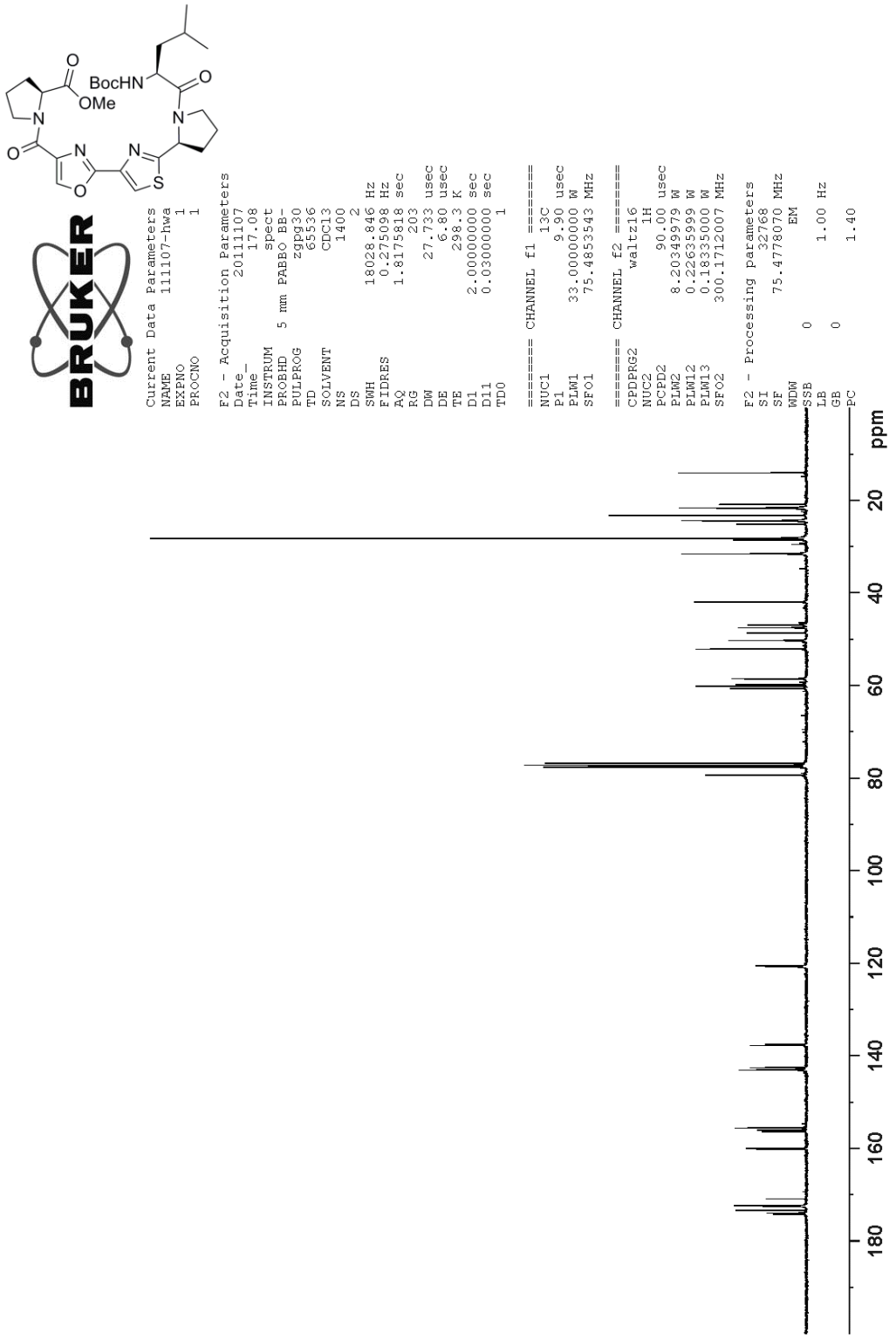
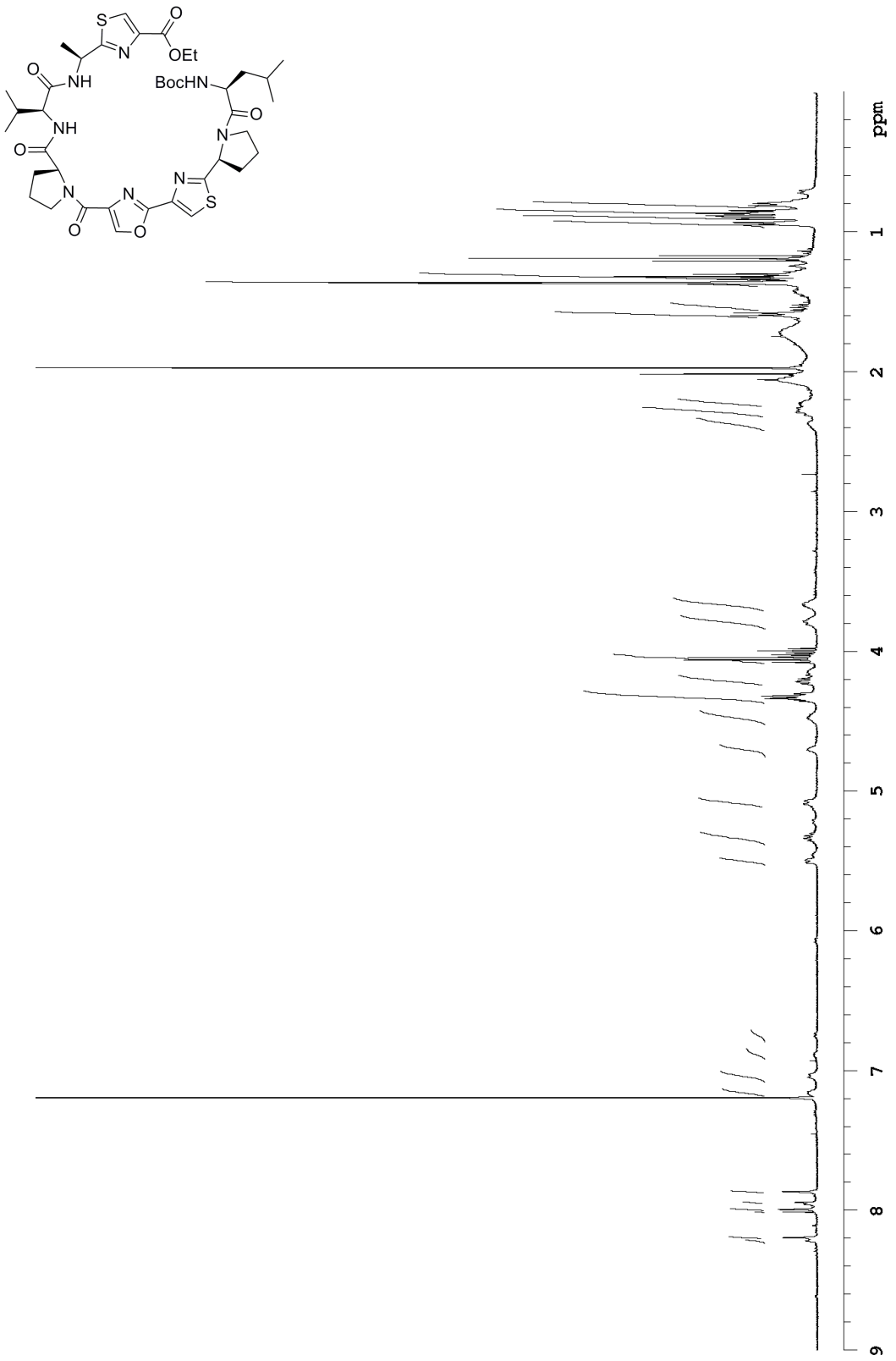


^{13}C NMR MeO-Pro-Ox-Th-Pro-Leu-NHBoc



^{1}H NMR EtO-Th-Ala-Val-Pro-Ox-Th-Pro-Leu-NHBoc

Display Report - All Windows Selected Analysis

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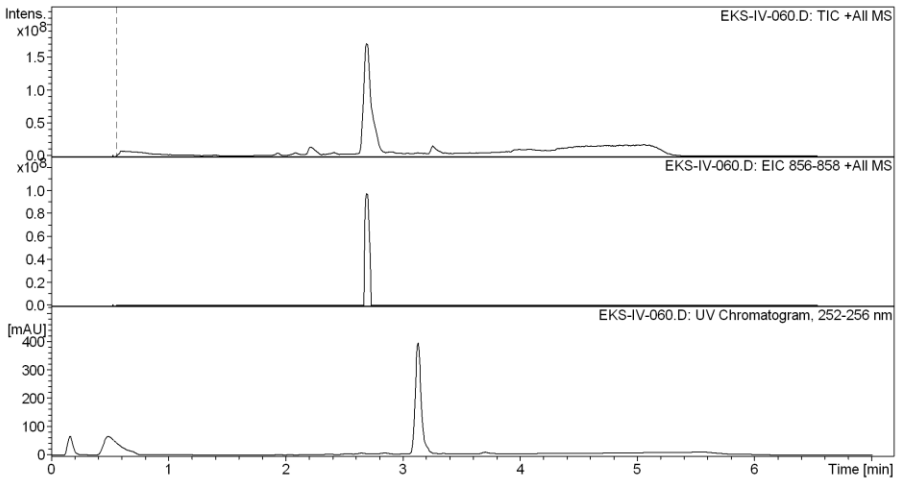
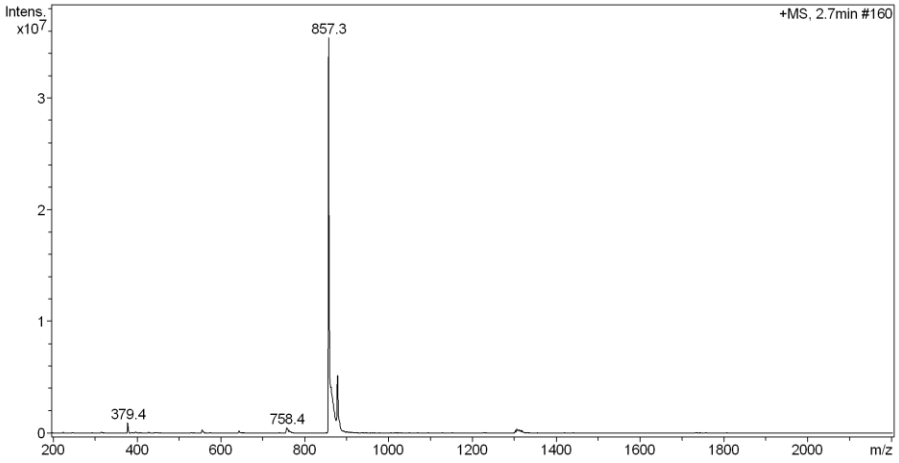
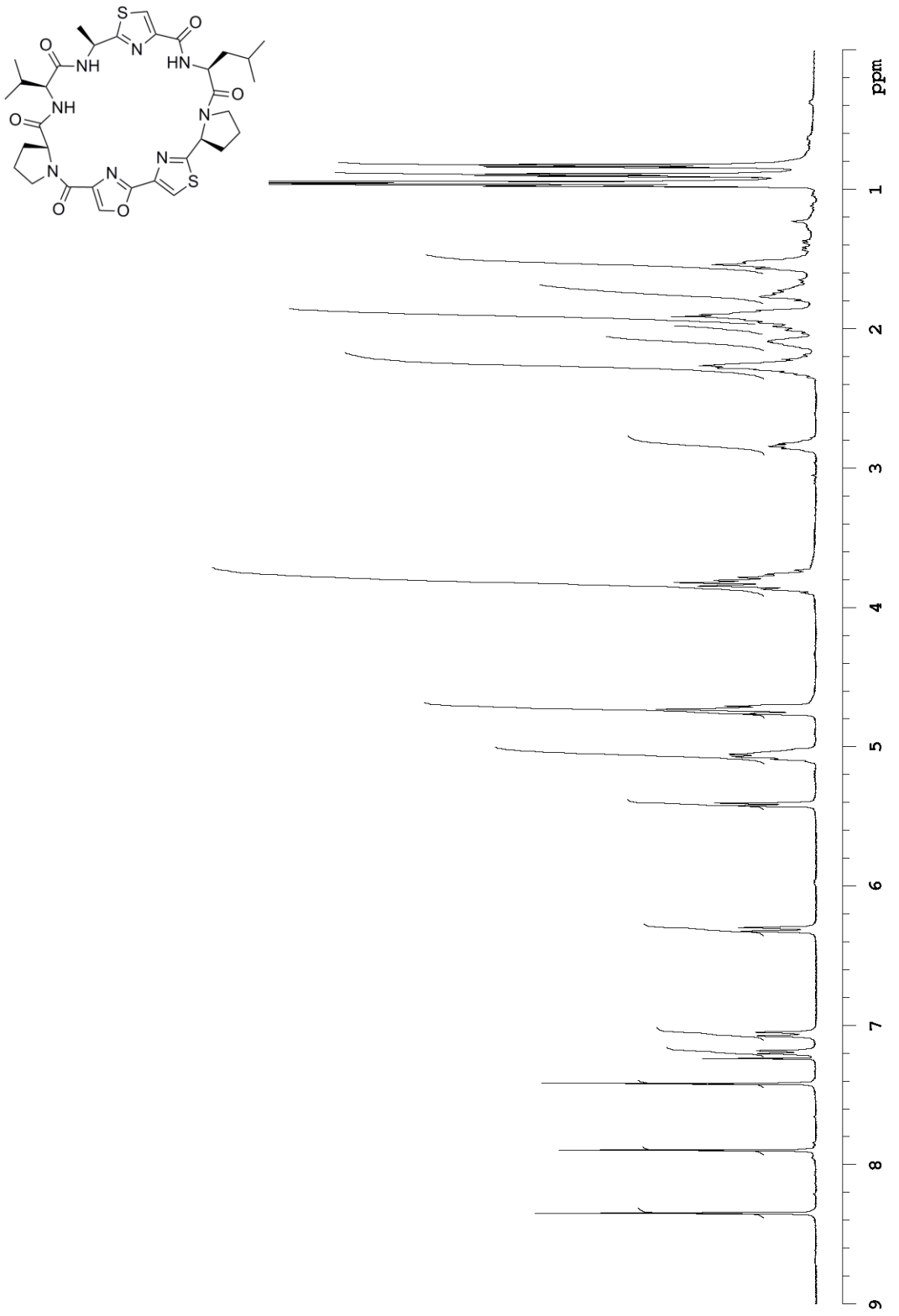
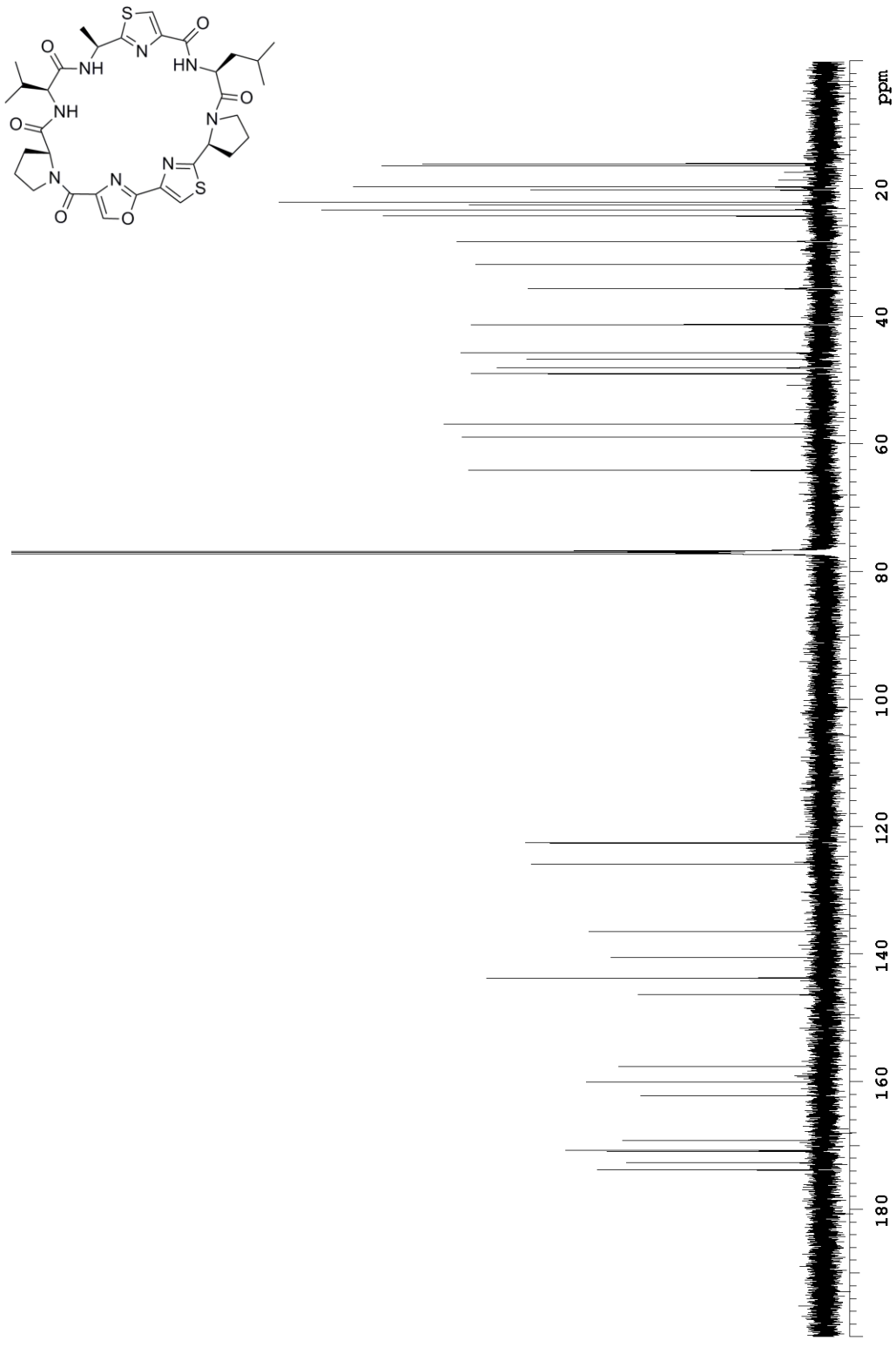




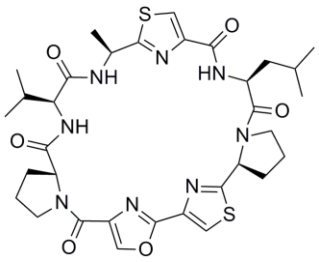
LCMS EtO-Th-Ala-Val-Pro-Ox-Th-Pro-Leu-NHBoc

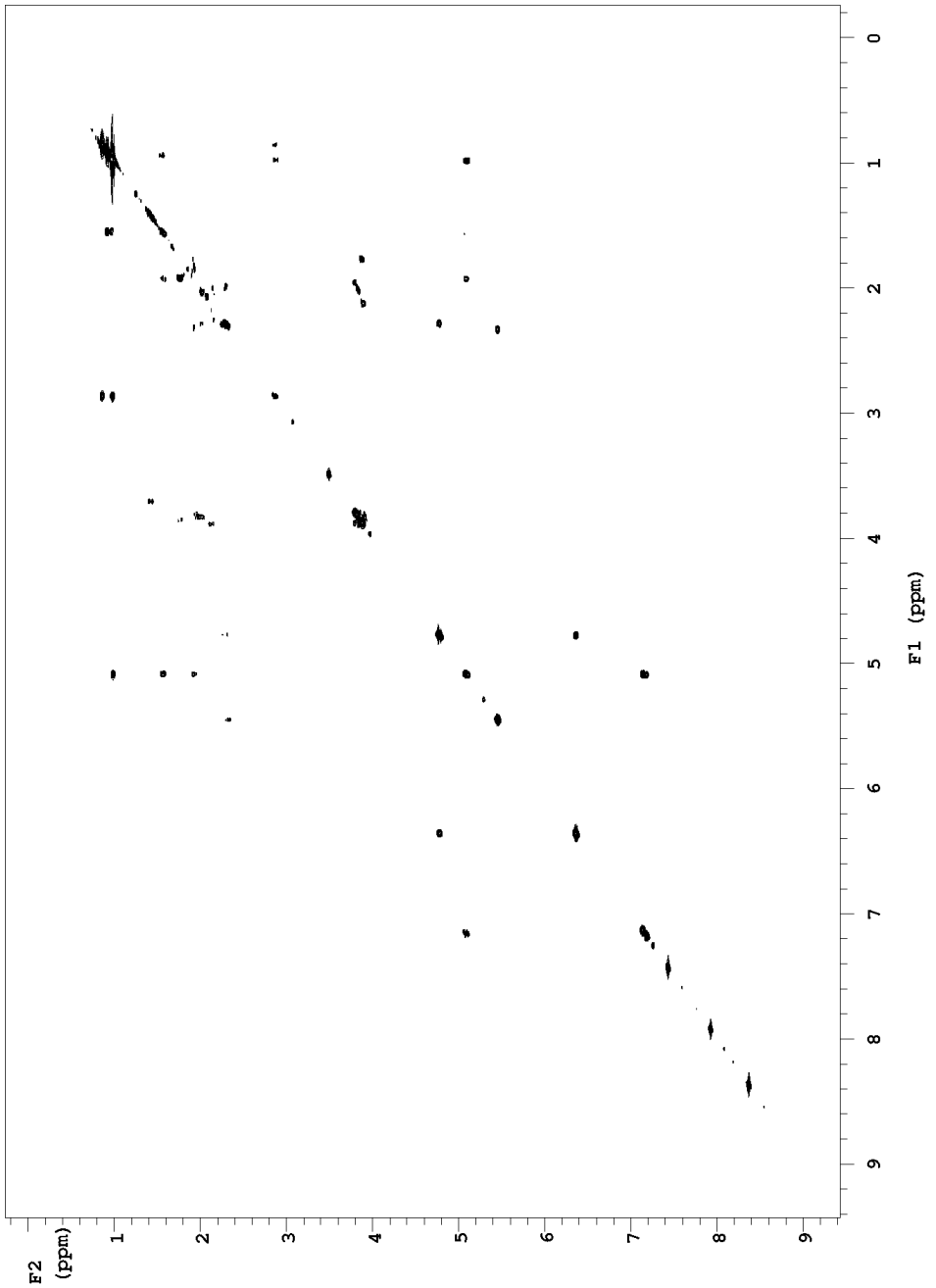


^{1}H NMR *cis,cis*-SanB

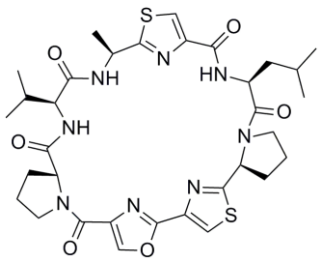


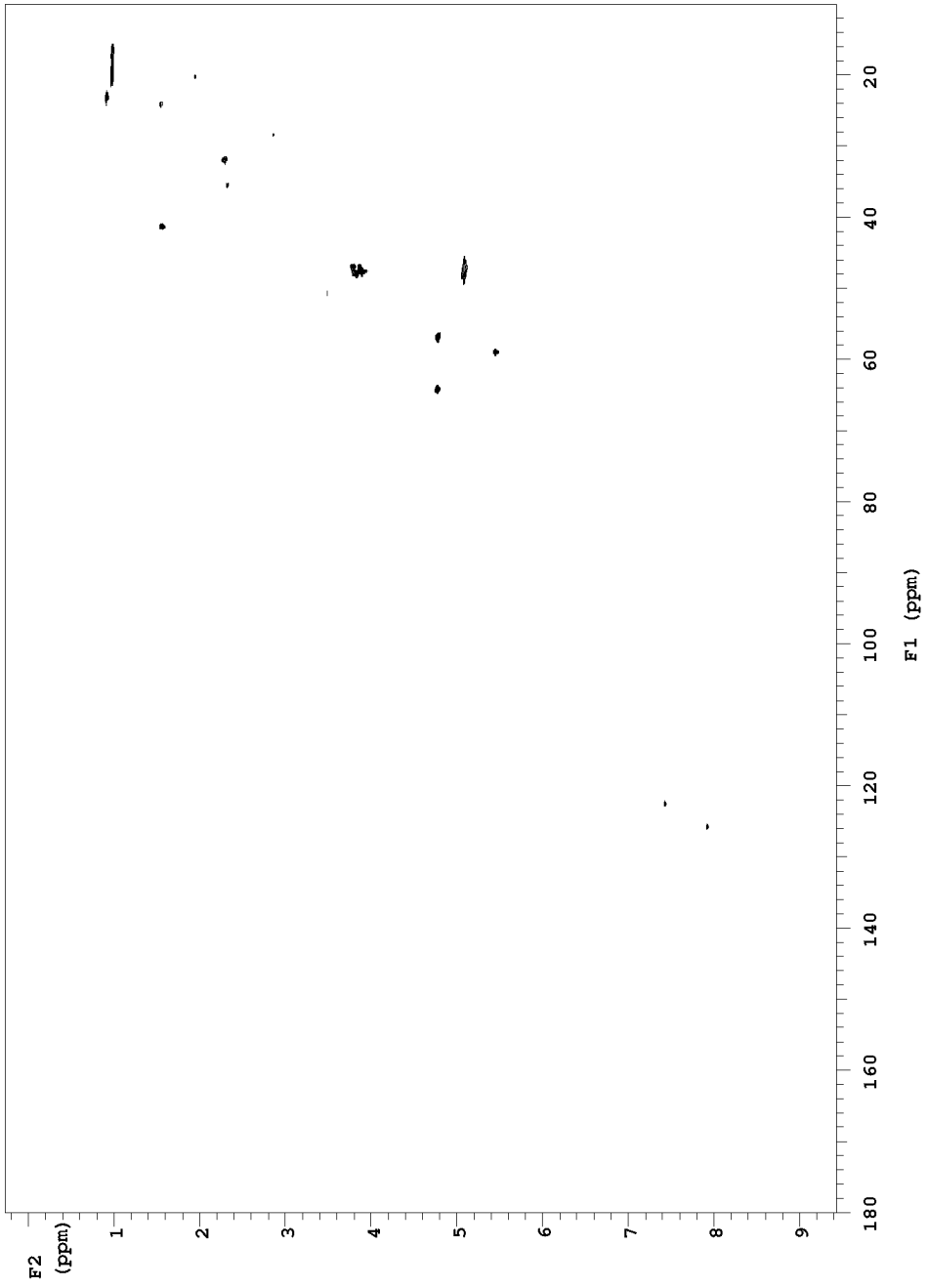
^{13}C NMR *cis,cis*-SanB



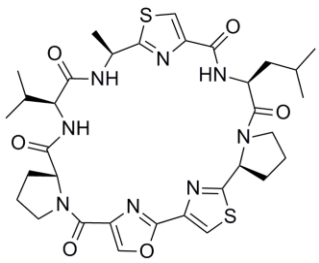


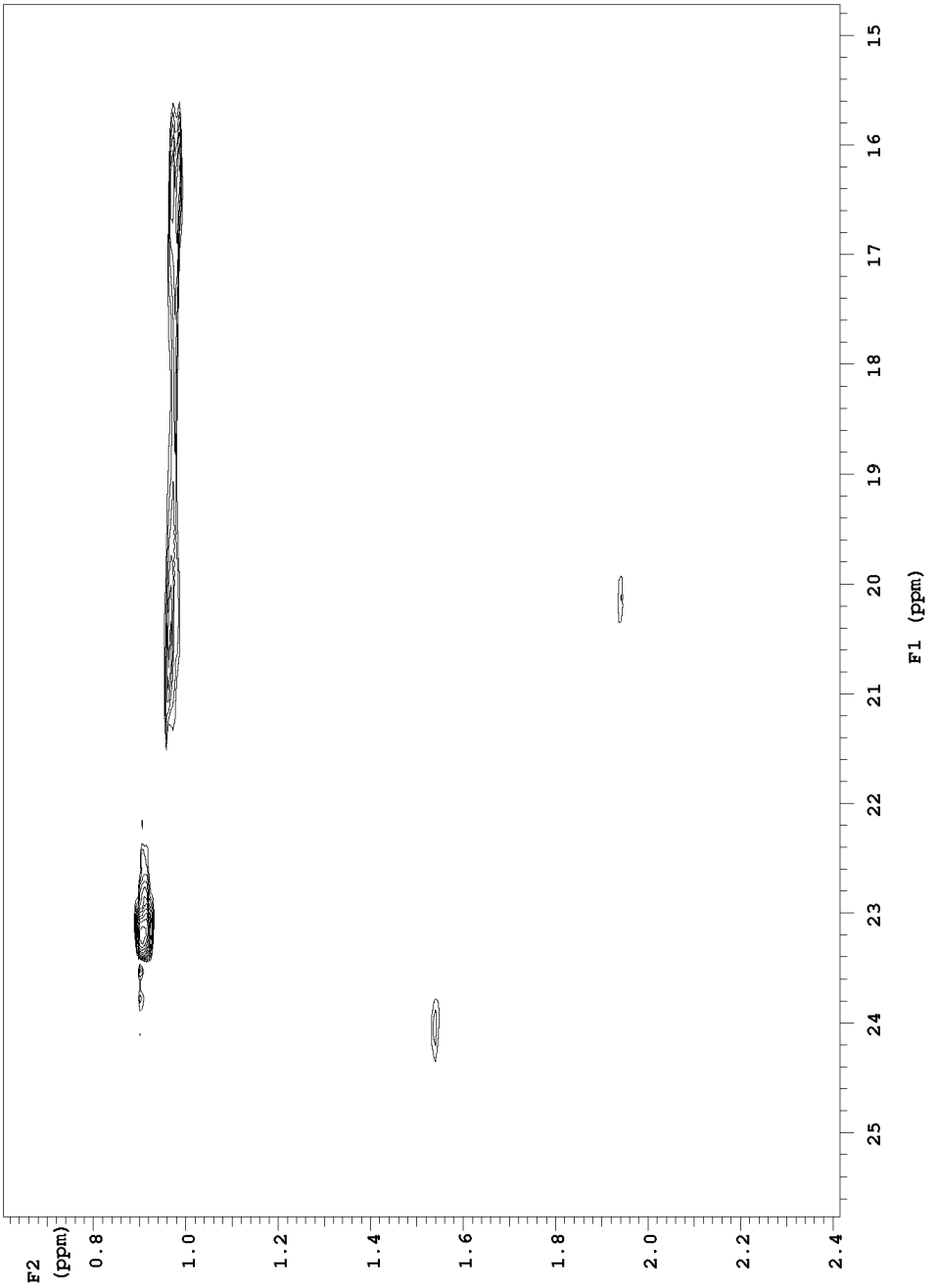
^{1}H-^{1}H COSY NMR *cis*,*cis*-SanB



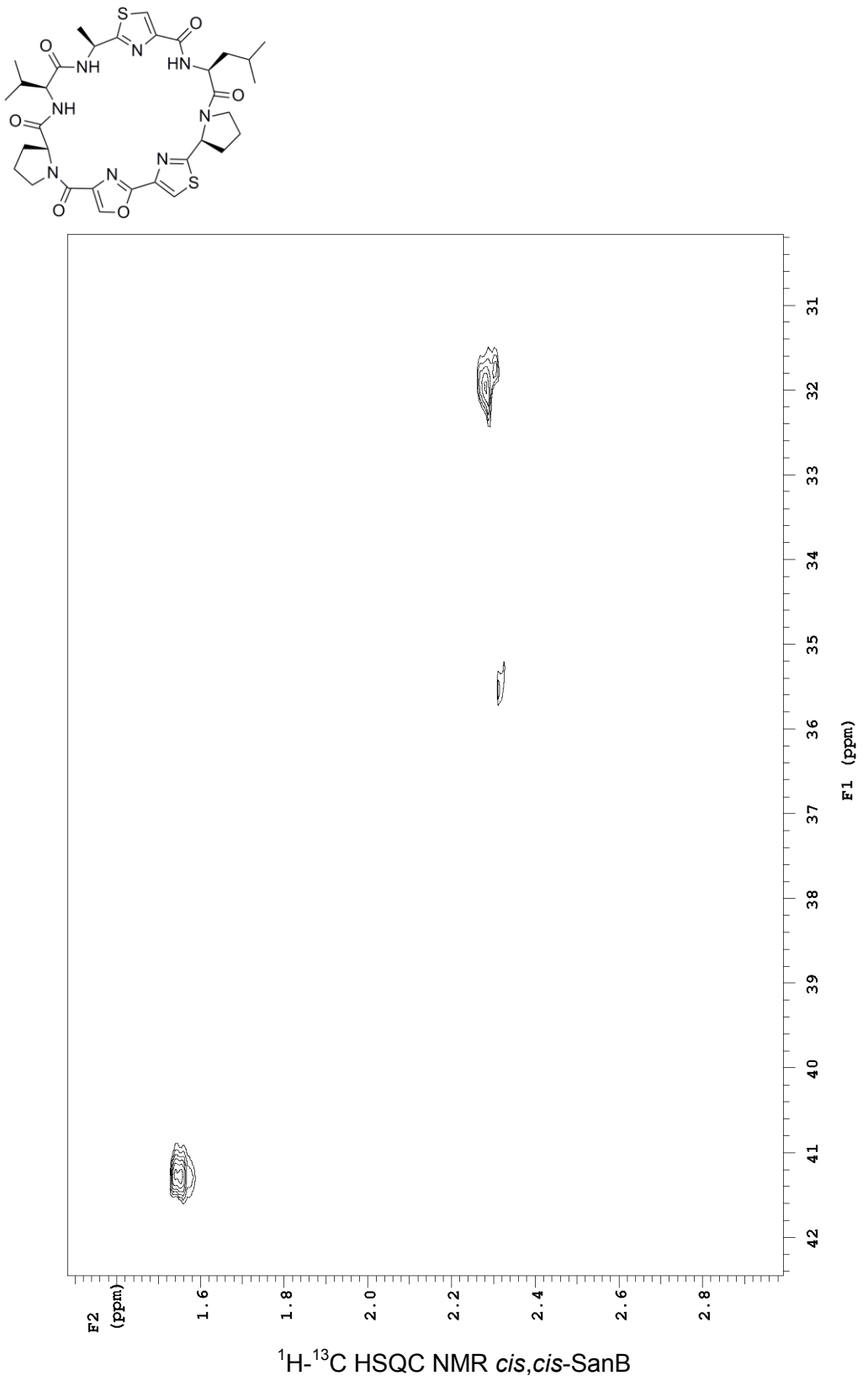


^{1}H-^{13}C HSQC NMR *cis*,*cis*-SanB

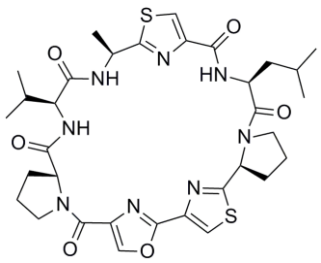


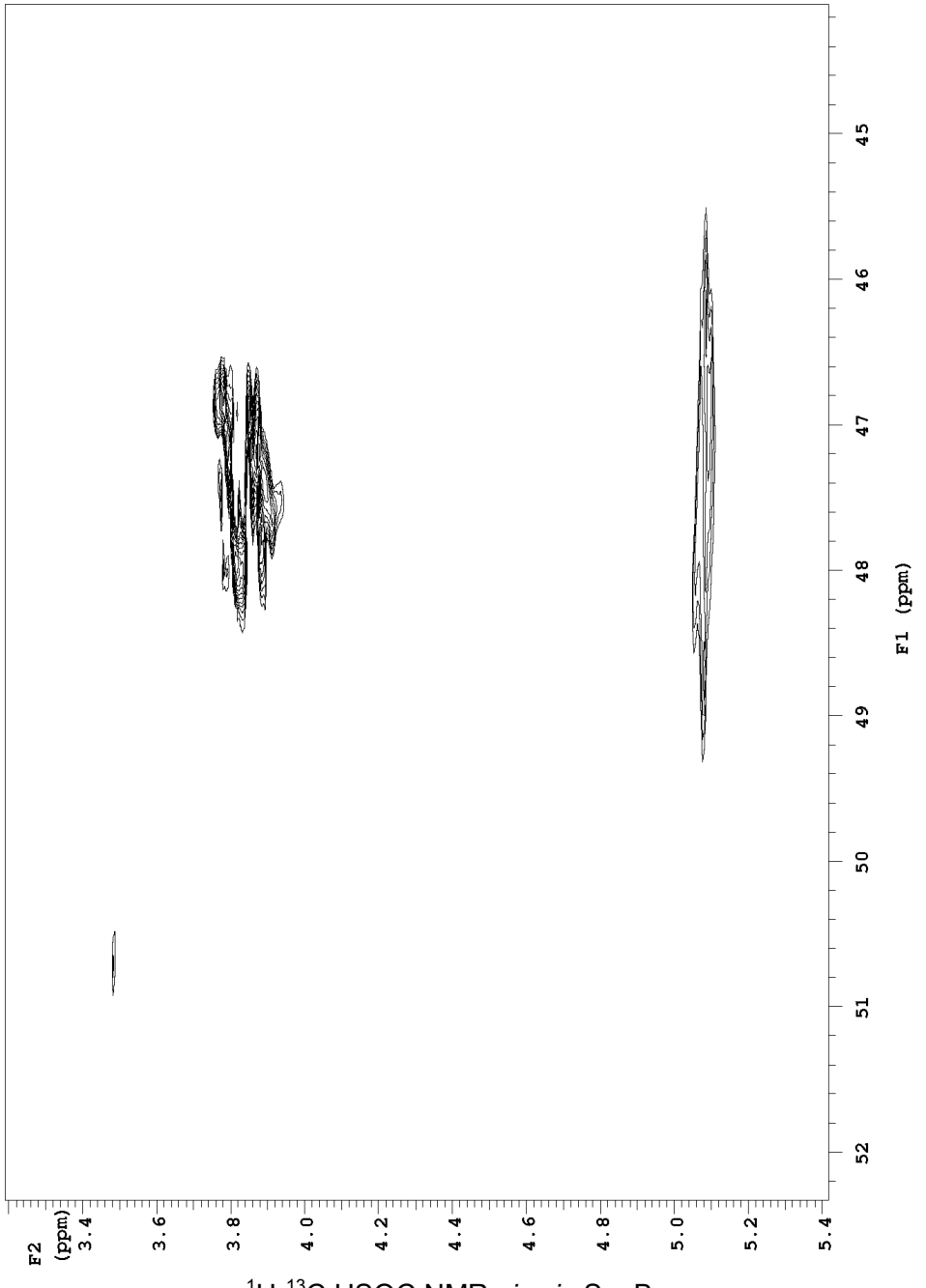


^{1}H-^{13}C HSQC NMR *cis*,*cis*-SanB

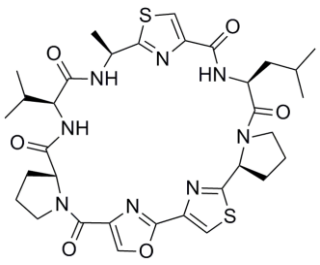


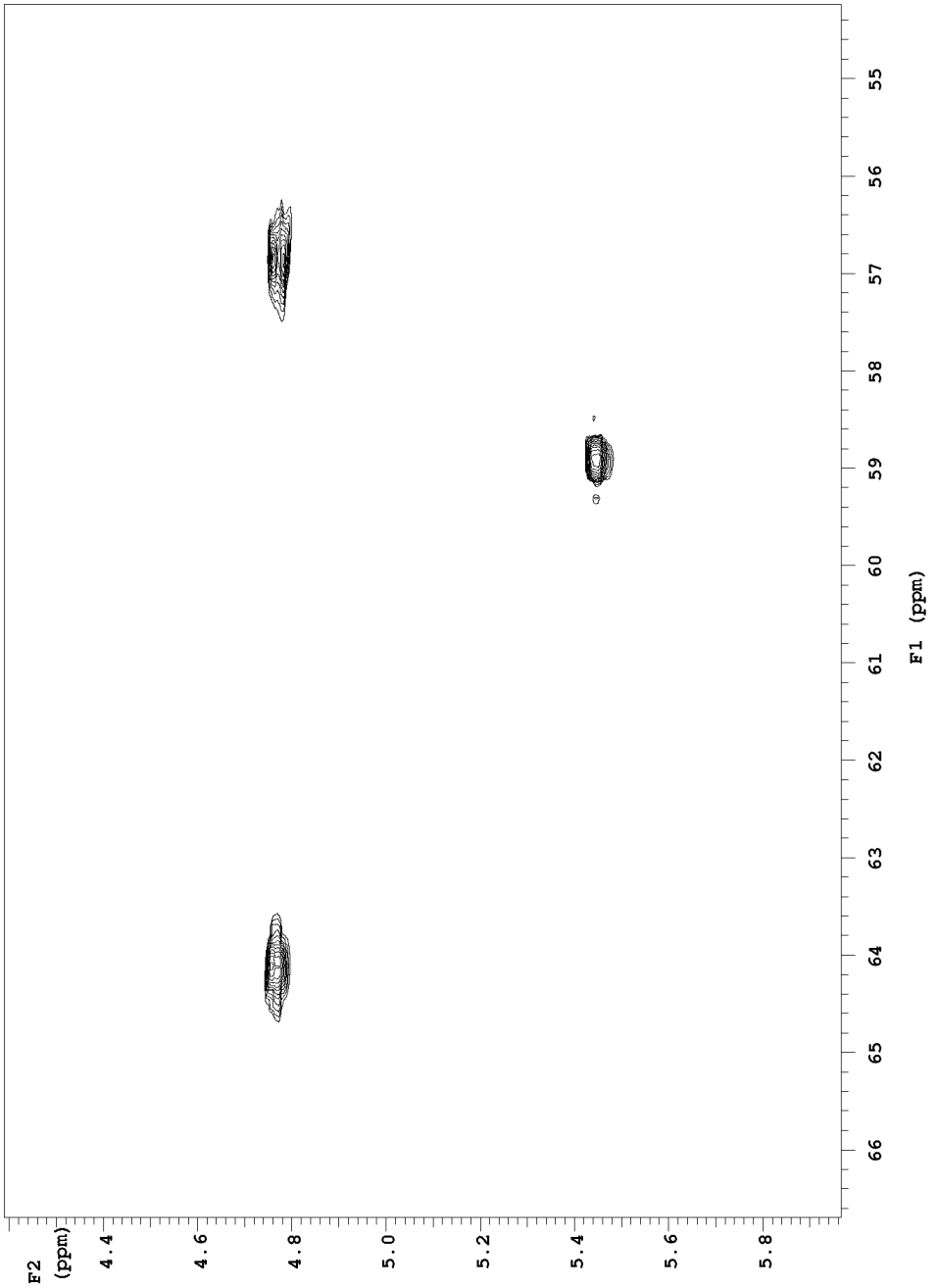
^{1}H-^{13}C HSQC NMR *cis*,*cis*-SanB



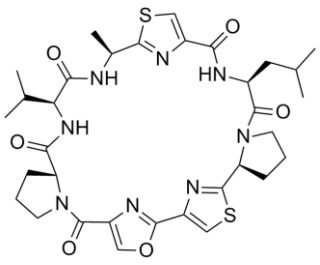


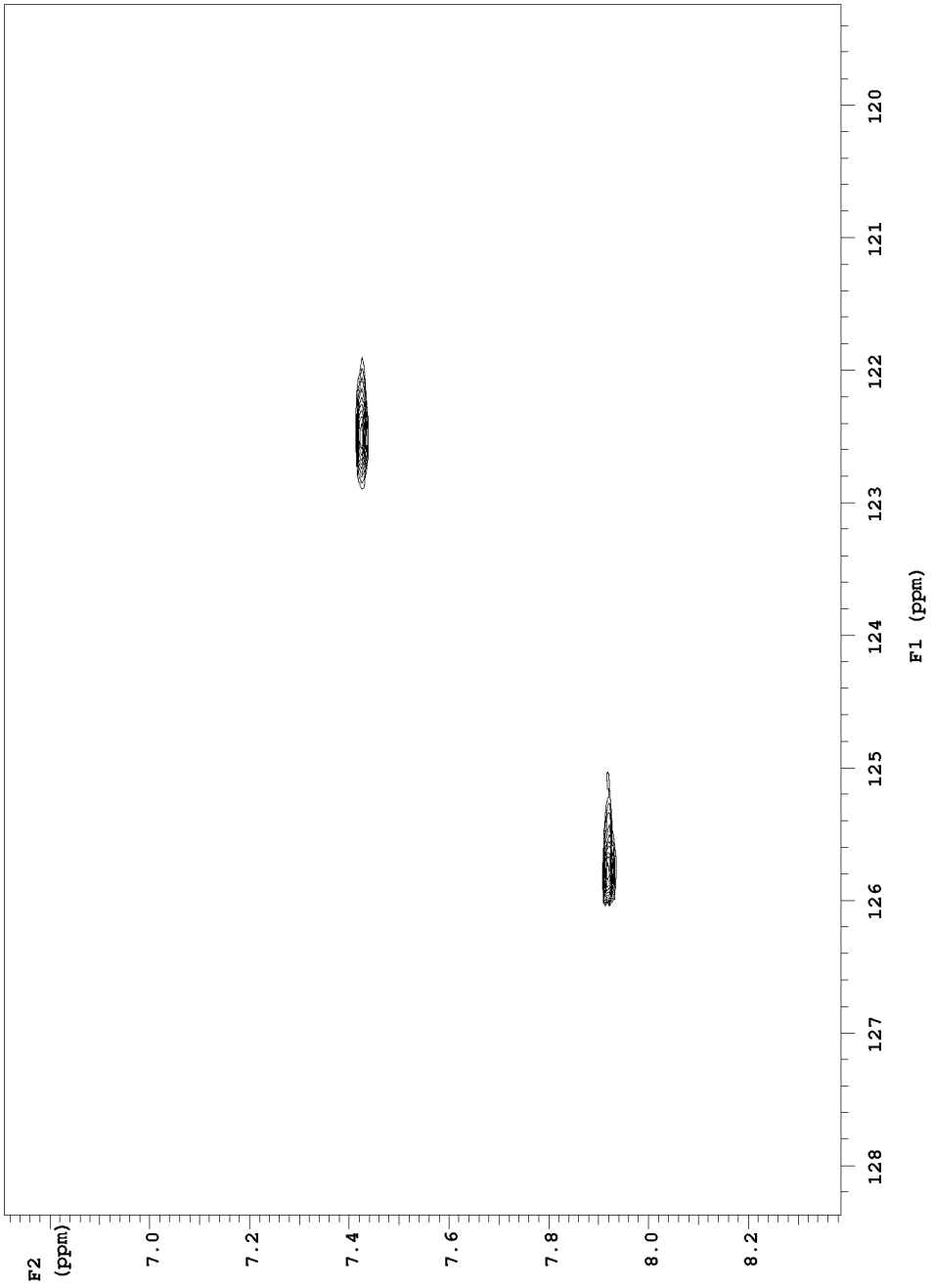
^{1}H-^{13}C HSQC NMR *cis*,*cis*-SanB



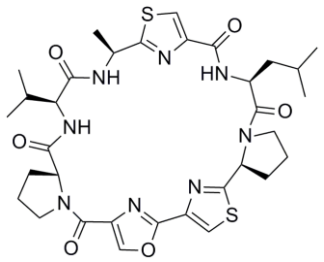


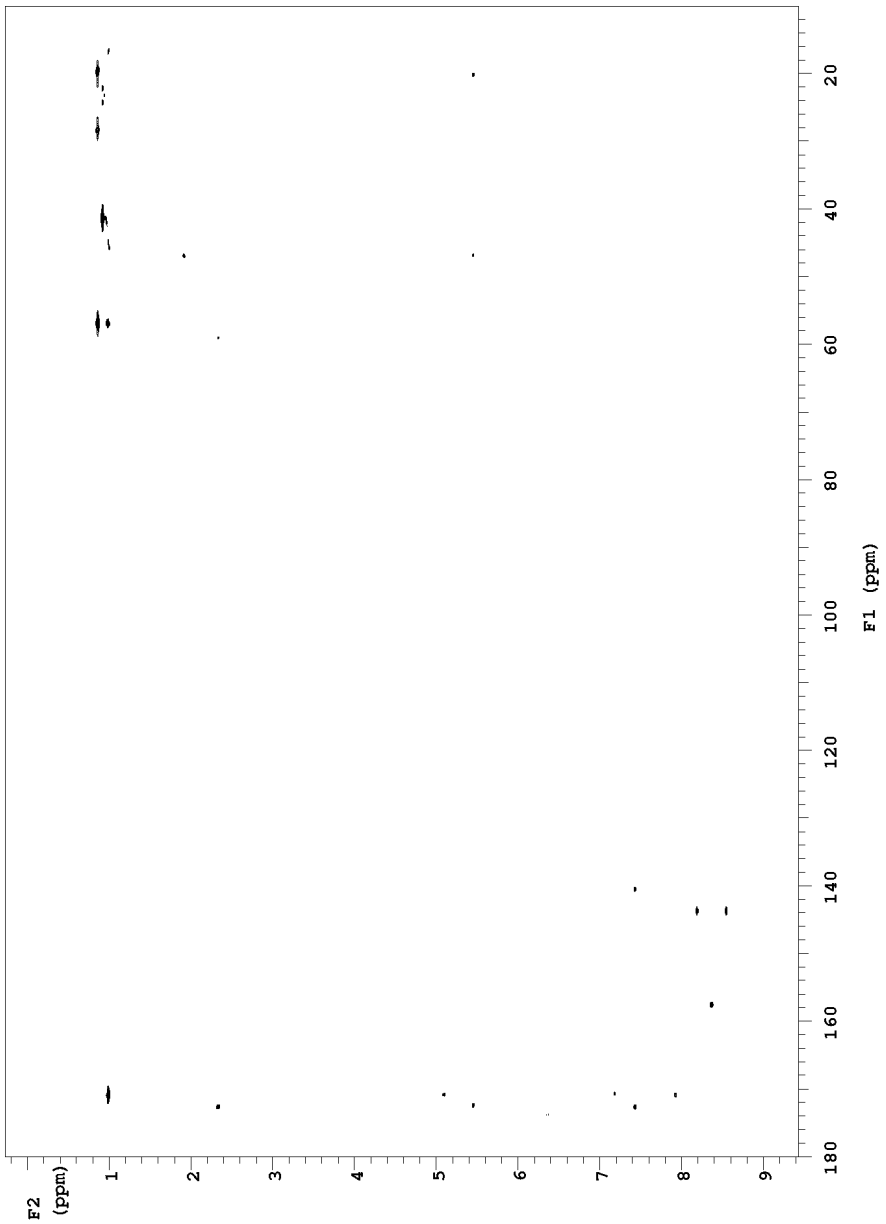
^{1}H-^{13}C HSQC NMR *cis*,*cis*-SanB



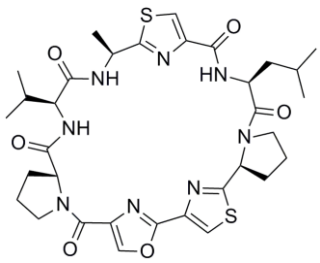


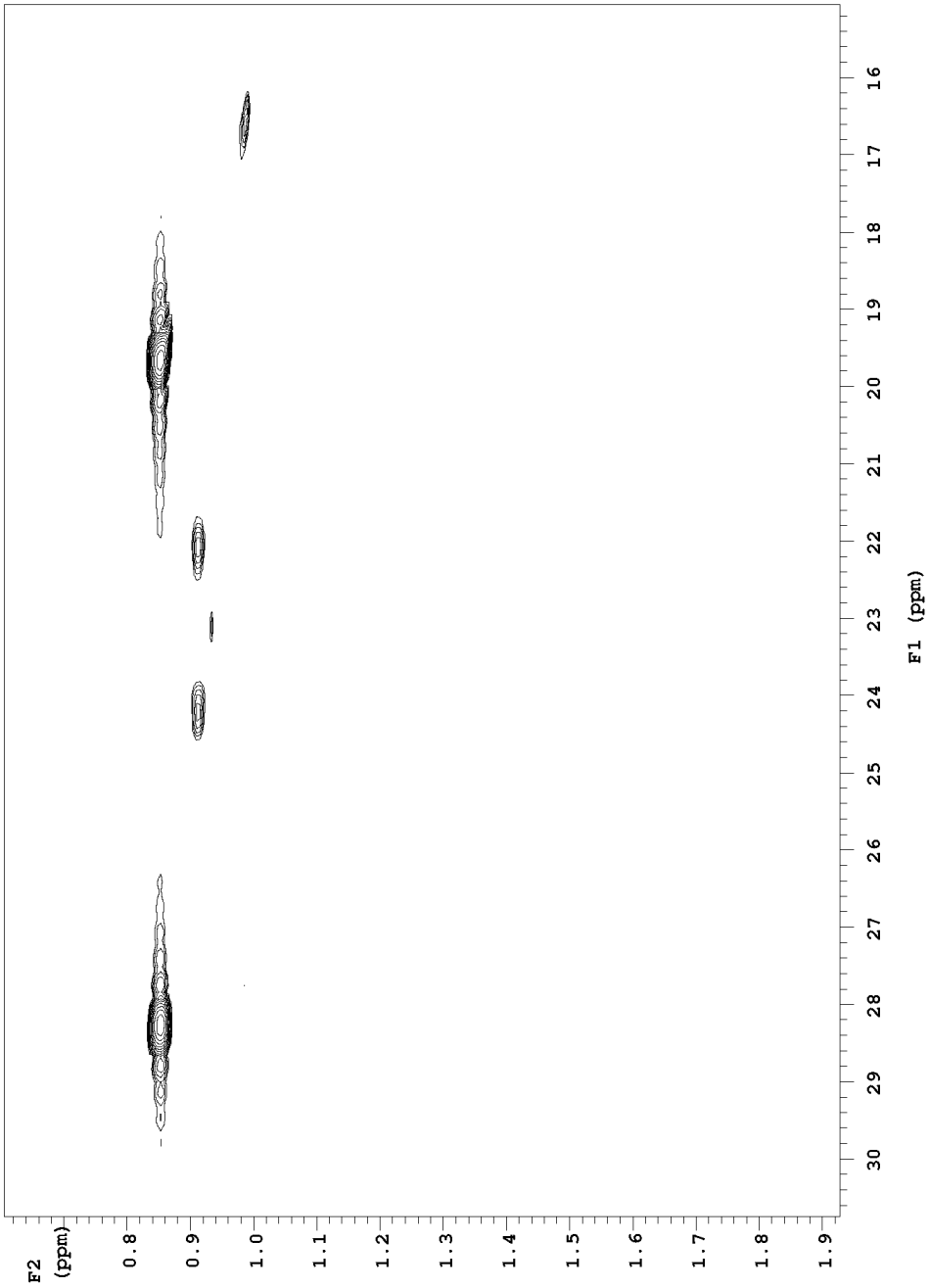
^{1}H-^{13}C HSQC NMR *cis*,*cis*-SanB



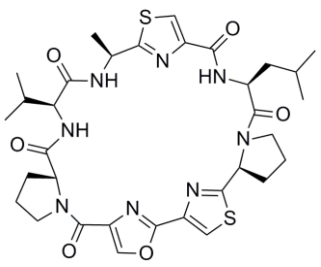


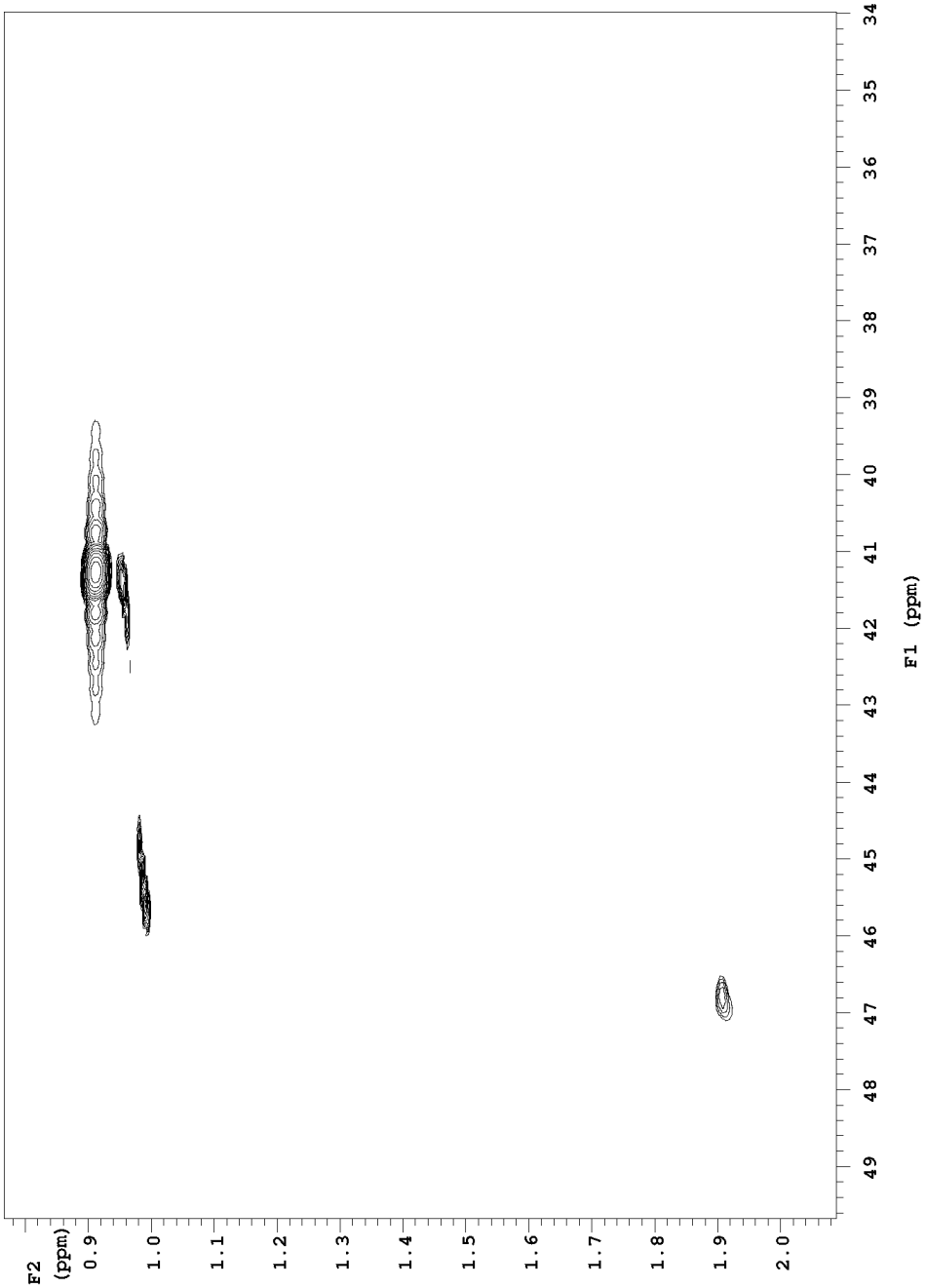
^{1}H-^{13}C HMBC NMR *cis*,*cis*-SanB



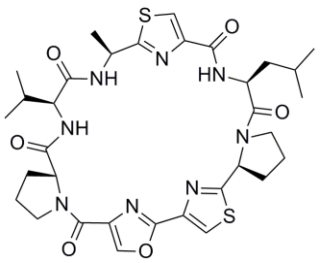


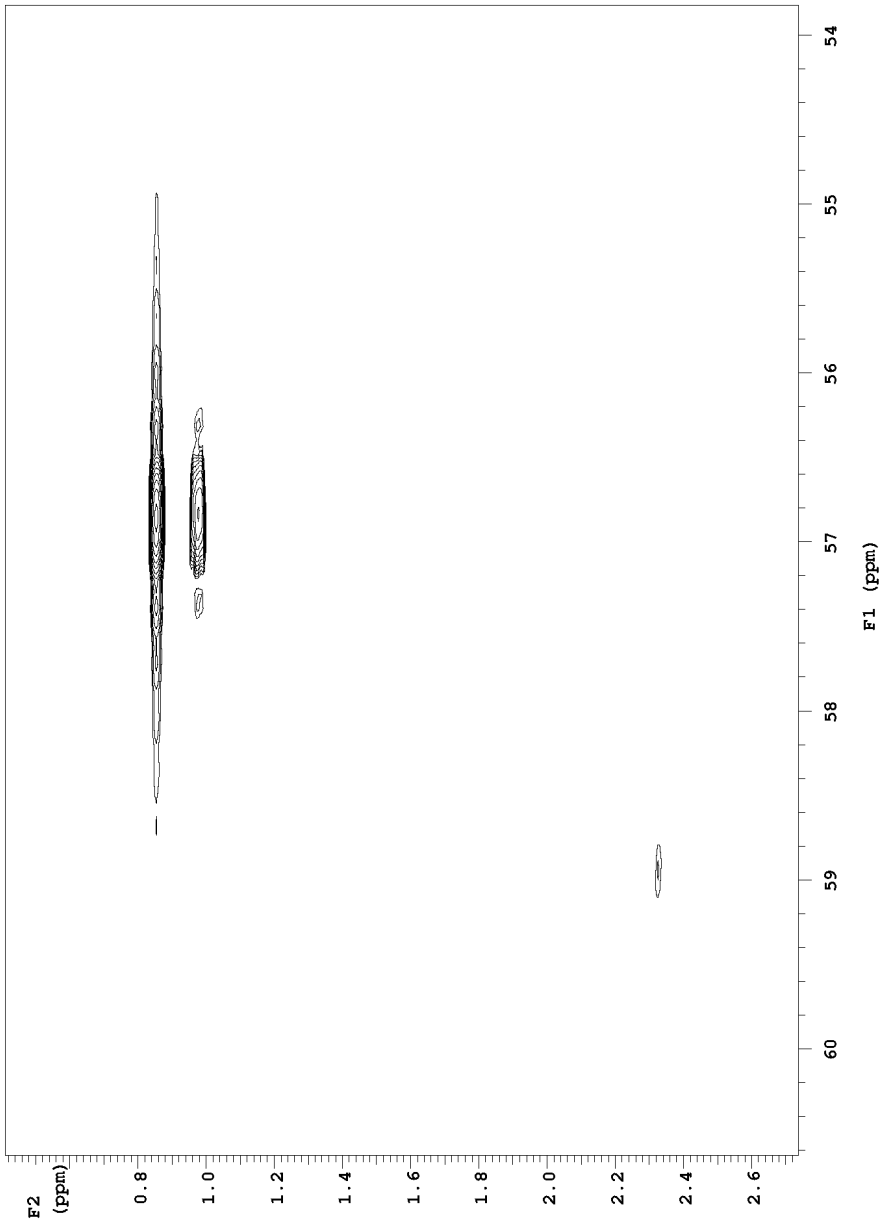
^{1}H-^{13}C HMBC NMR *cis*,*cis*-SanB



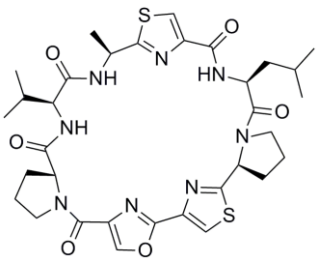


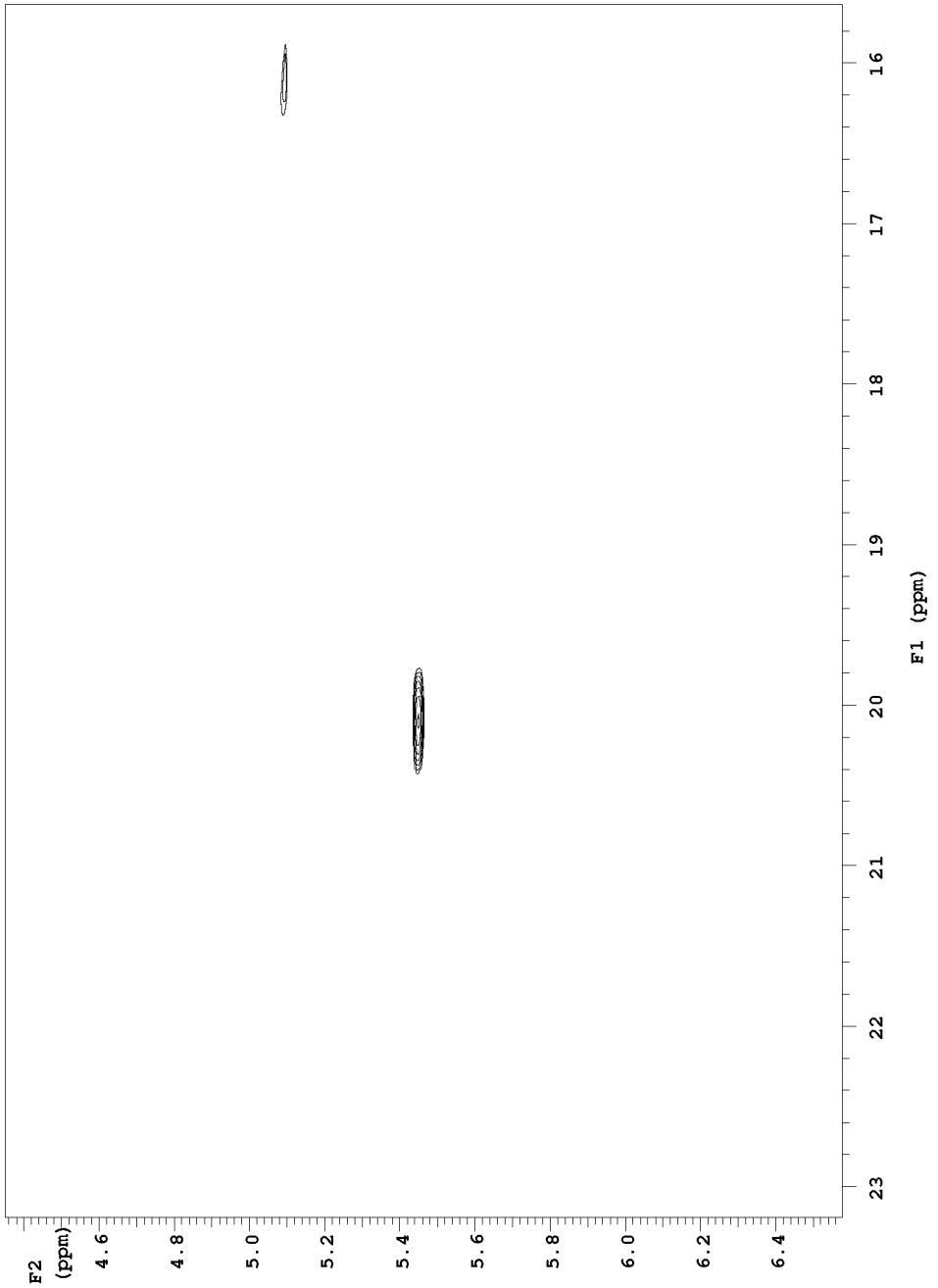
^{1}H-^{13}C HMBC NMR *cis*,*cis*-SanB



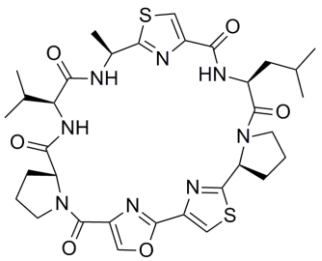


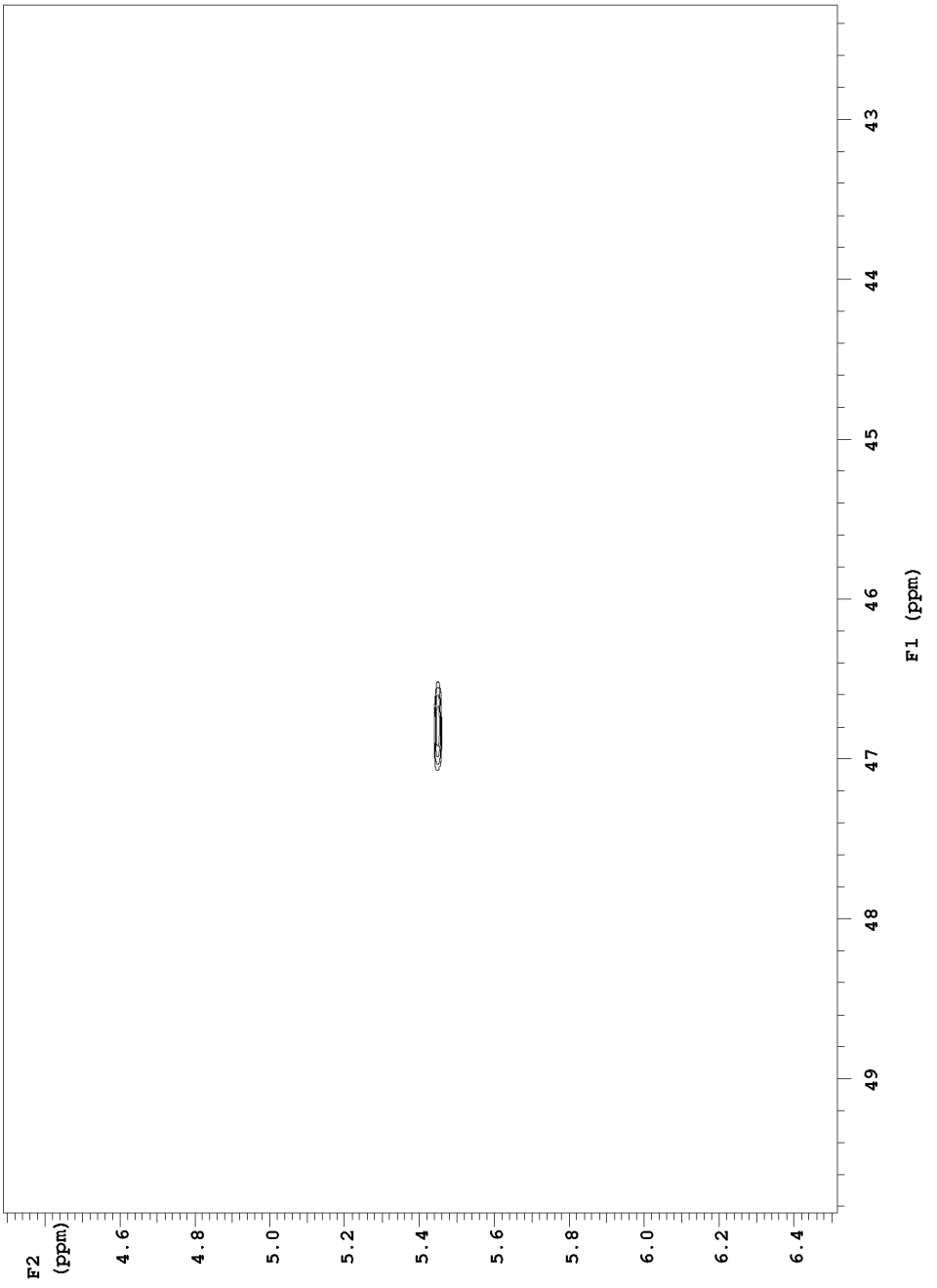
^{1}H-^{13}C HMBC NMR *cis*,*cis*-SanB



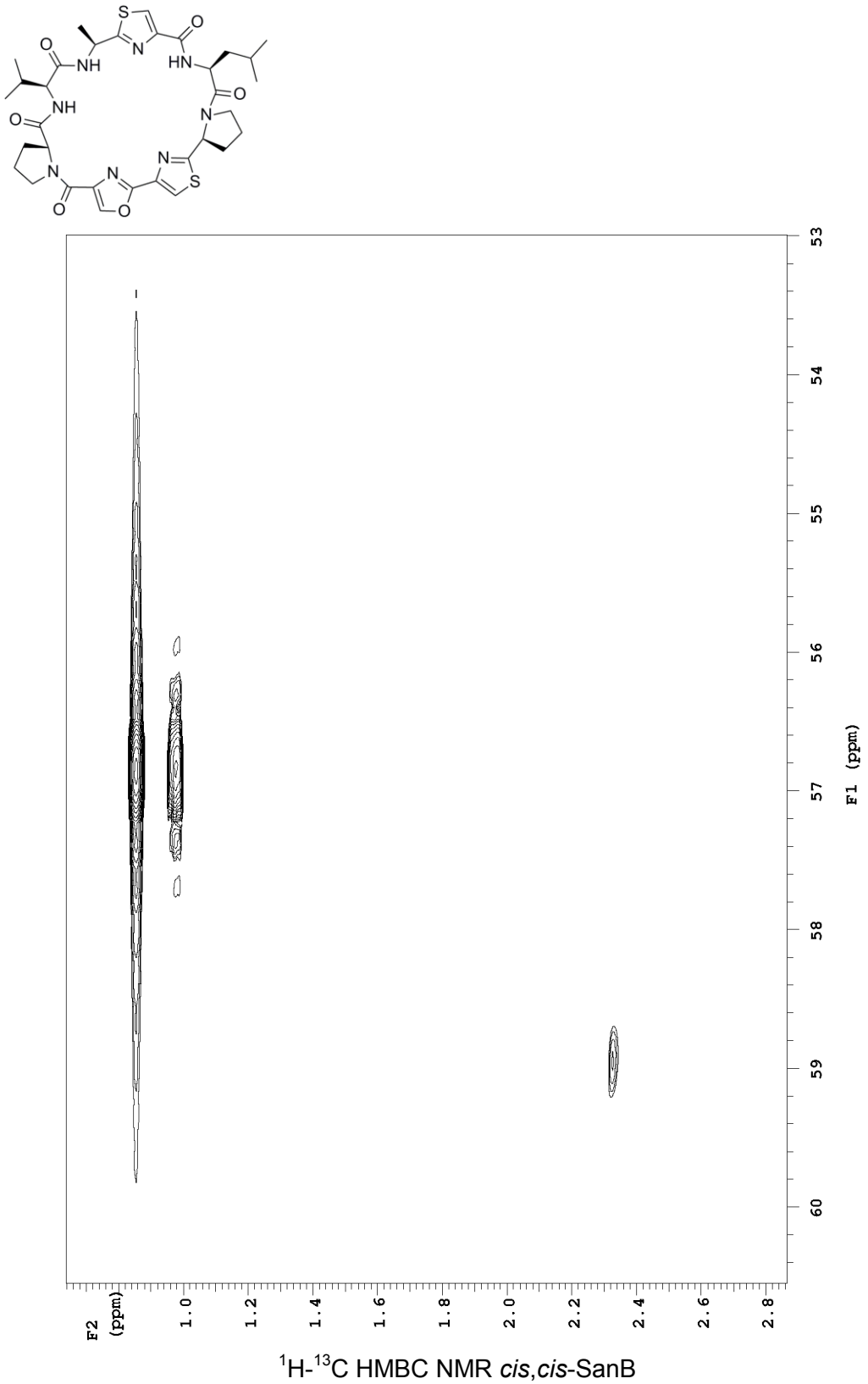


^{1}H-^{13}C HMBC NMR *cis*,*cis*-SanB

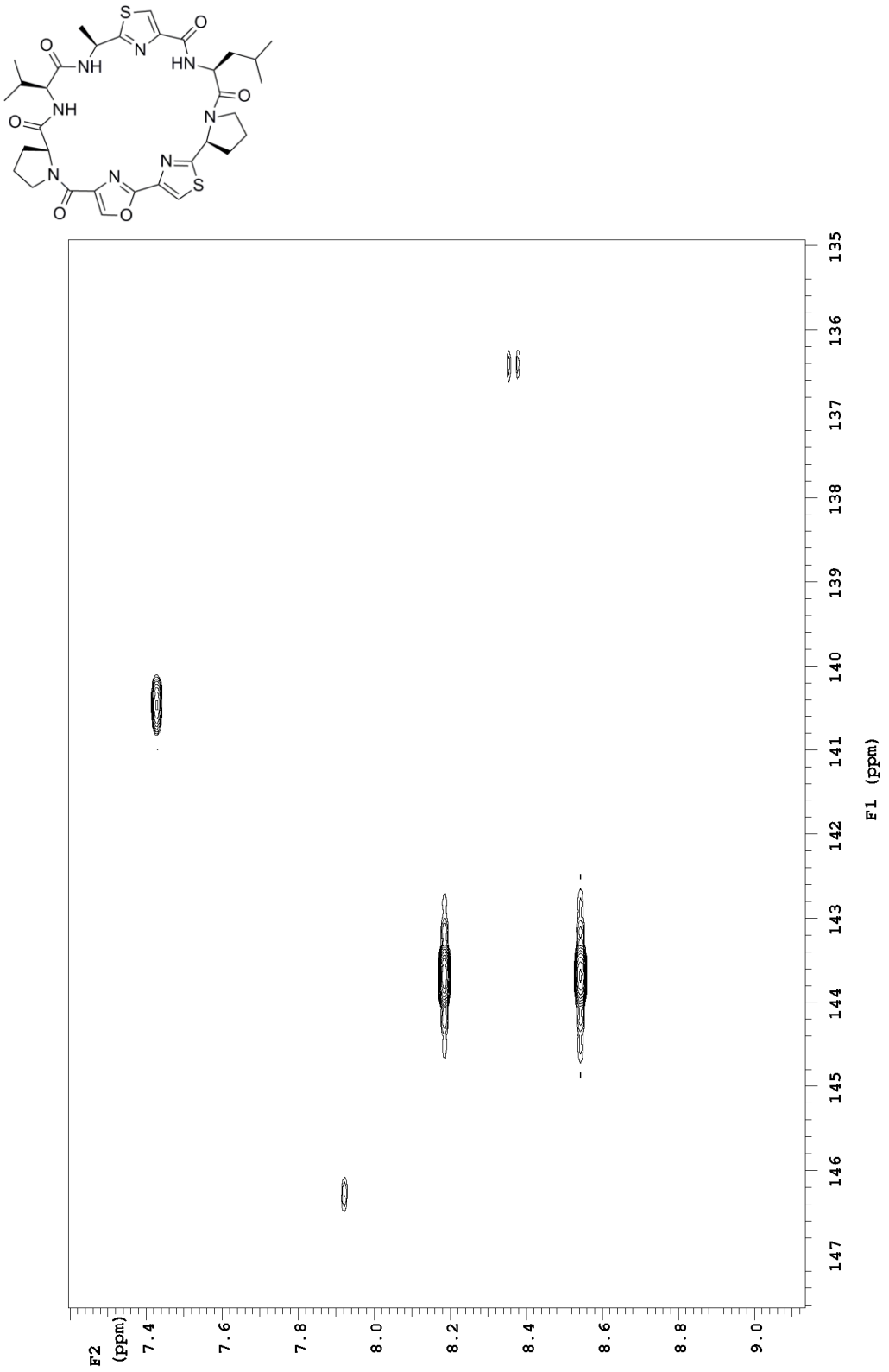




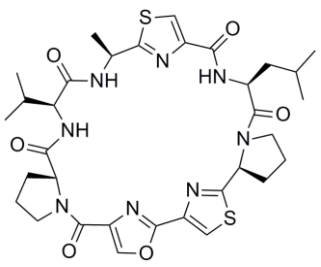
^{1}H-^{13}C HMBC NMR *cis*,*cis*-SanB

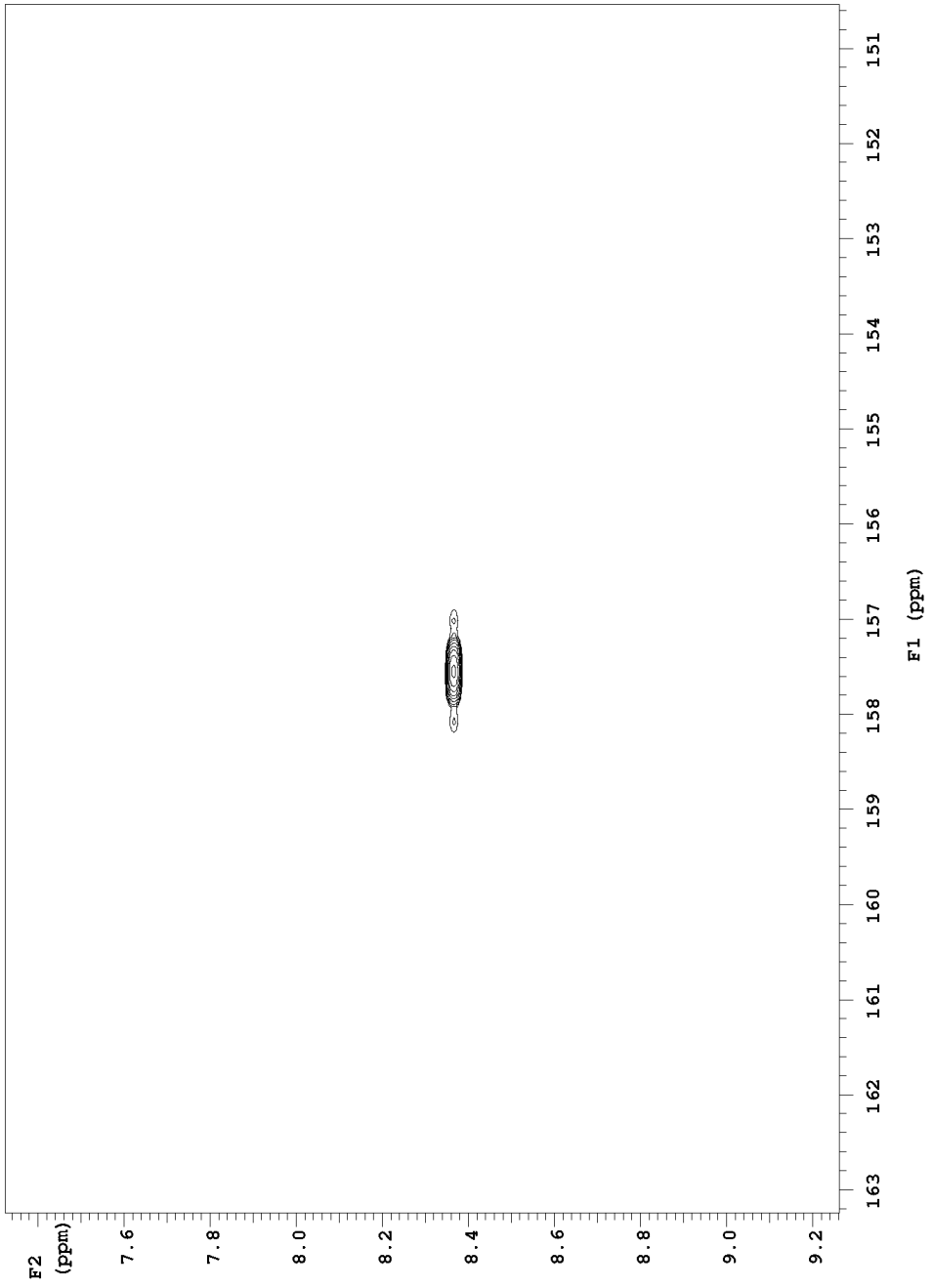


^{1}H-^{13}C HMBC NMR *cis*,*cis*-SanB

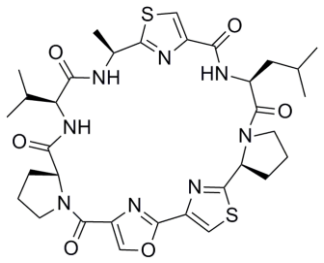


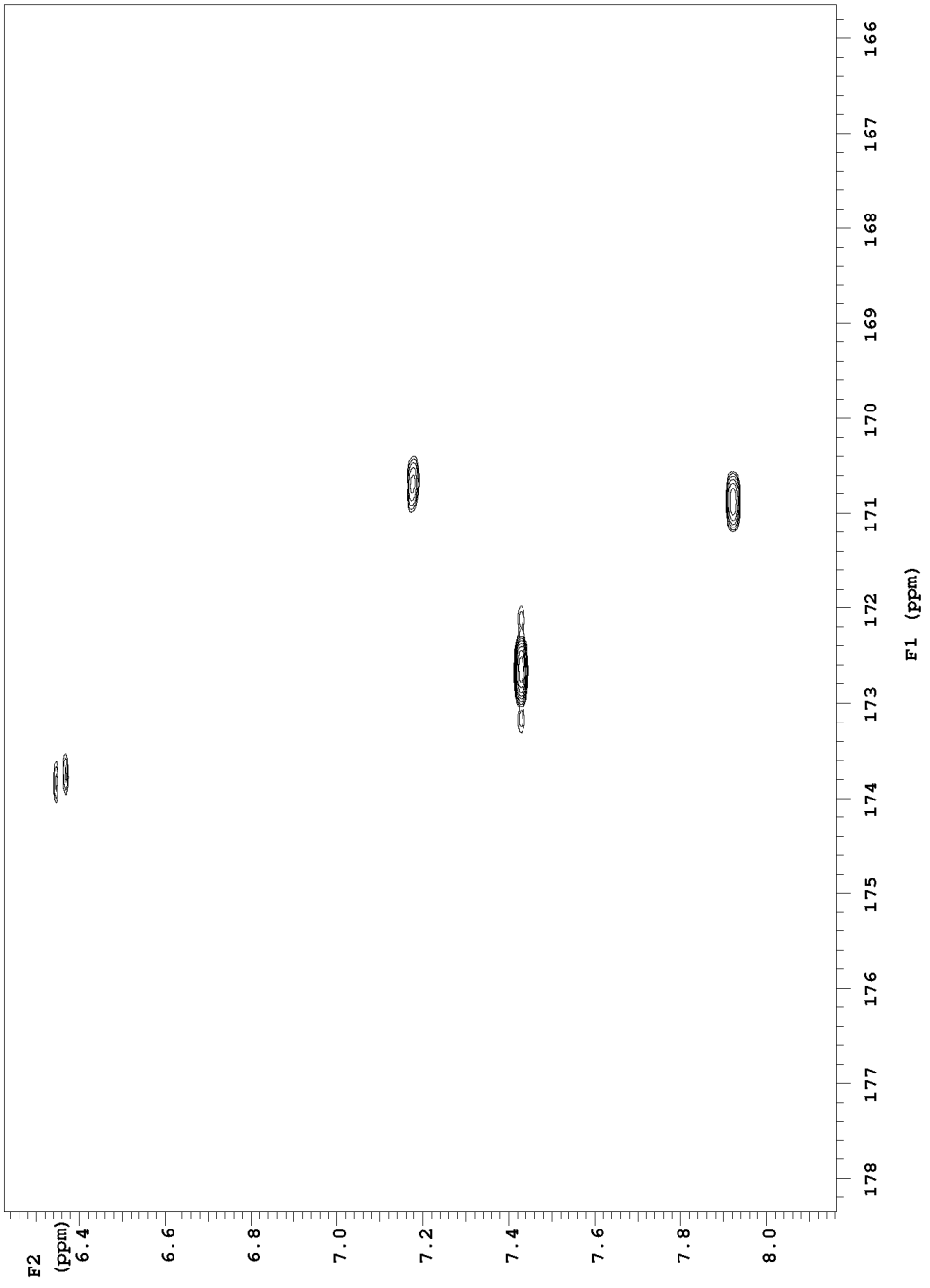
^{1}H-^{13}C HMBC NMR *cis*,*cis*-SanB



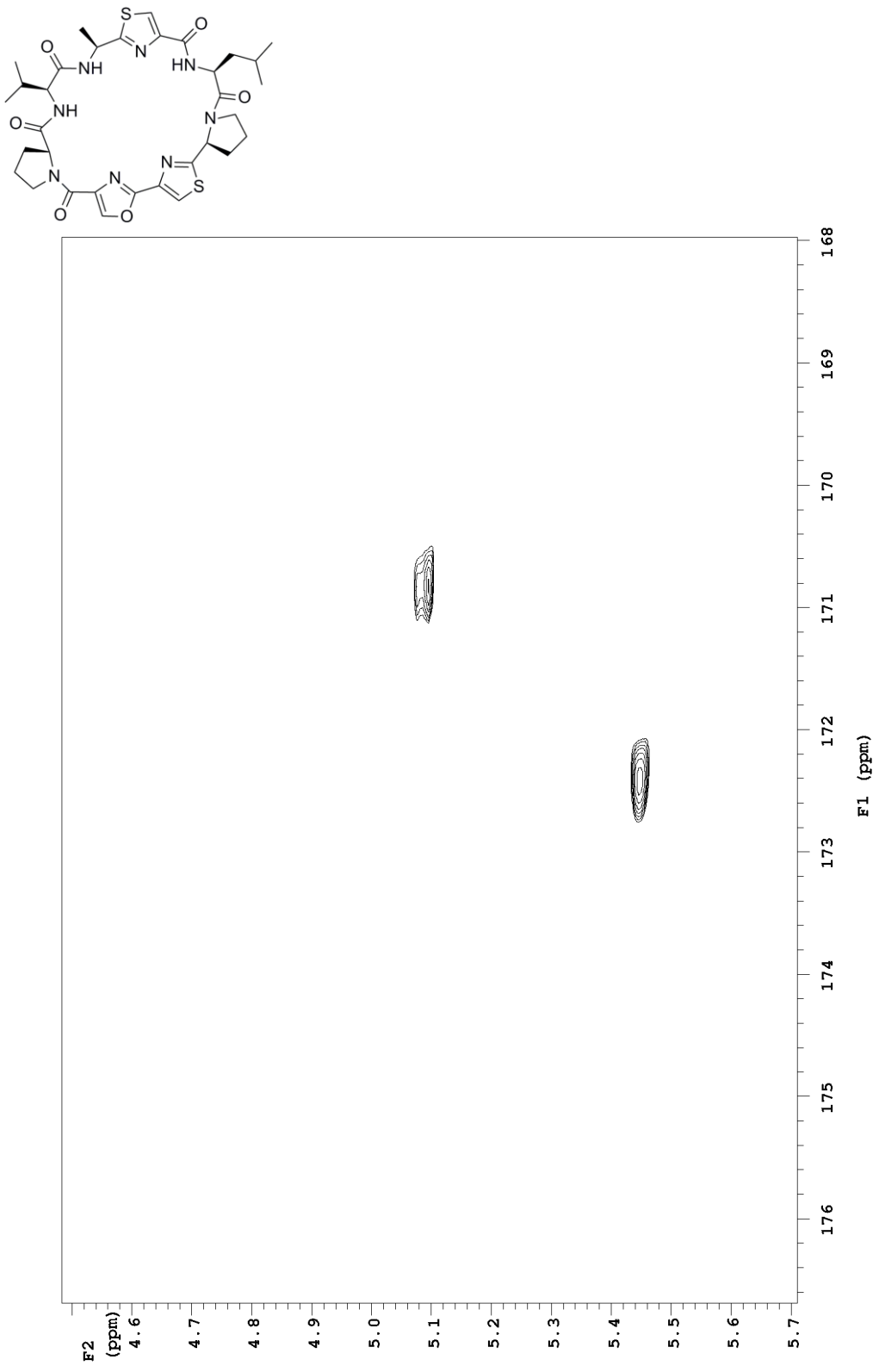


^{1}H-^{13}C HMBC NMR *cis*,*cis*-SanB

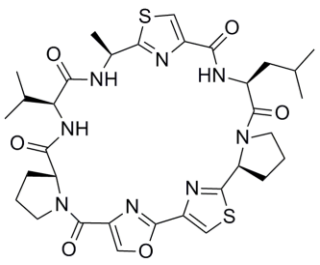


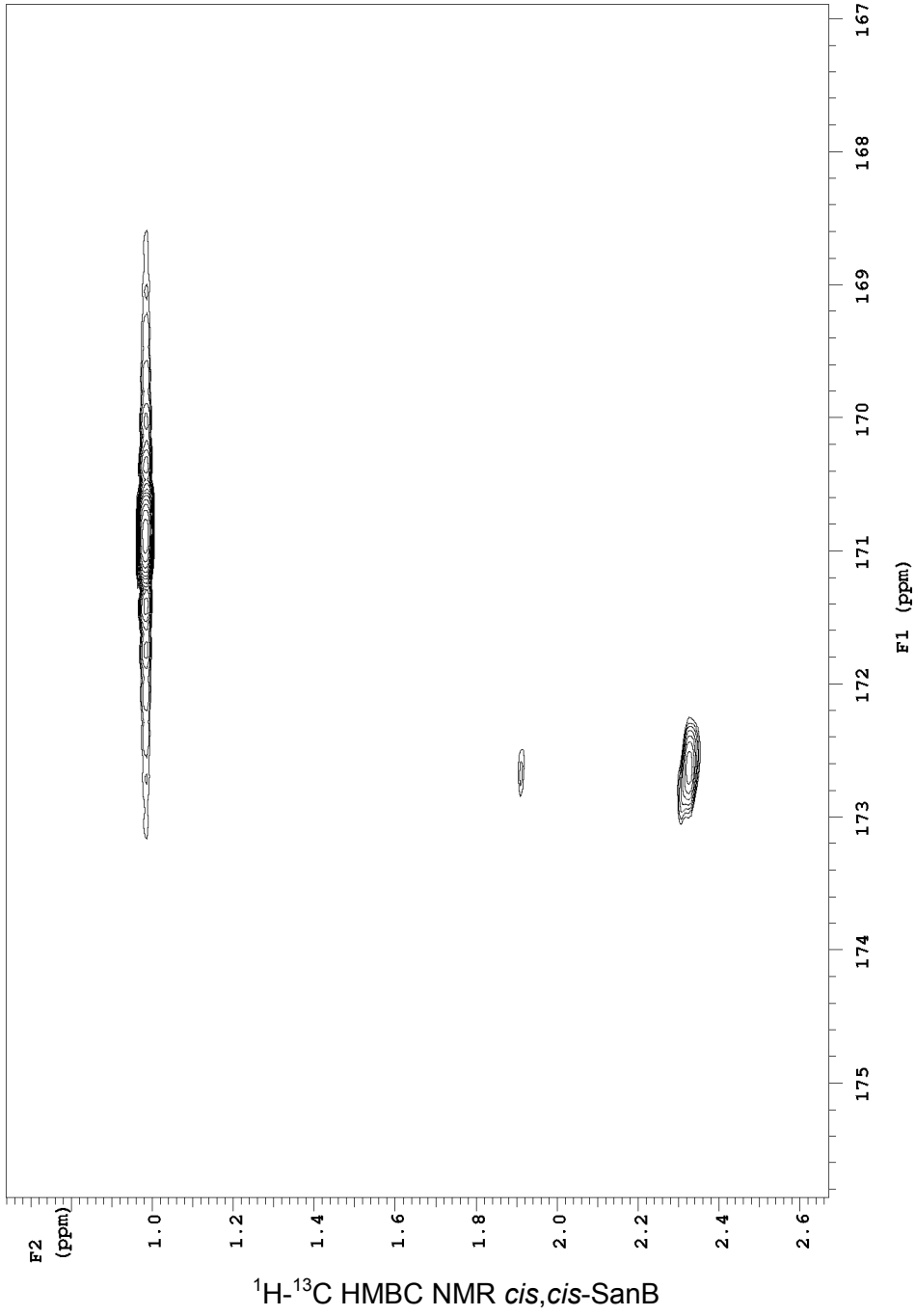


^{1}H-^{13}C HMBC NMR *cis*,*cis*-SanB



^{1}H-^{13}C HMBC NMR *cis*,*cis*-SanB

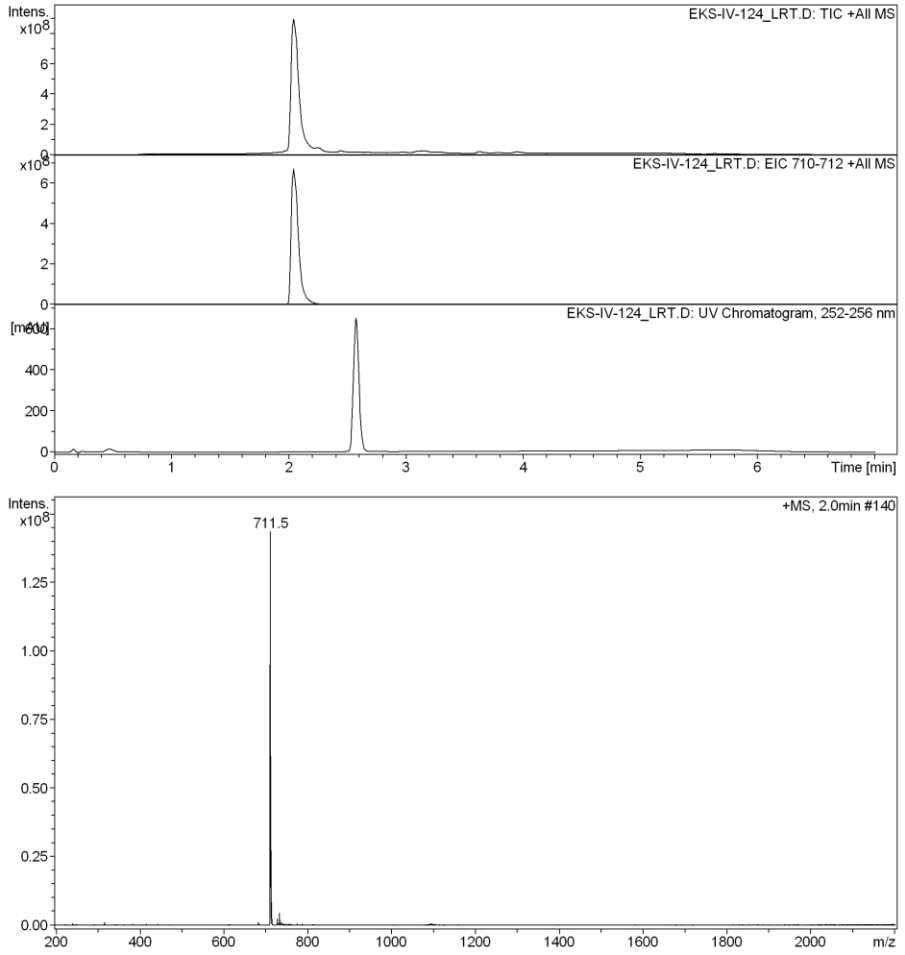




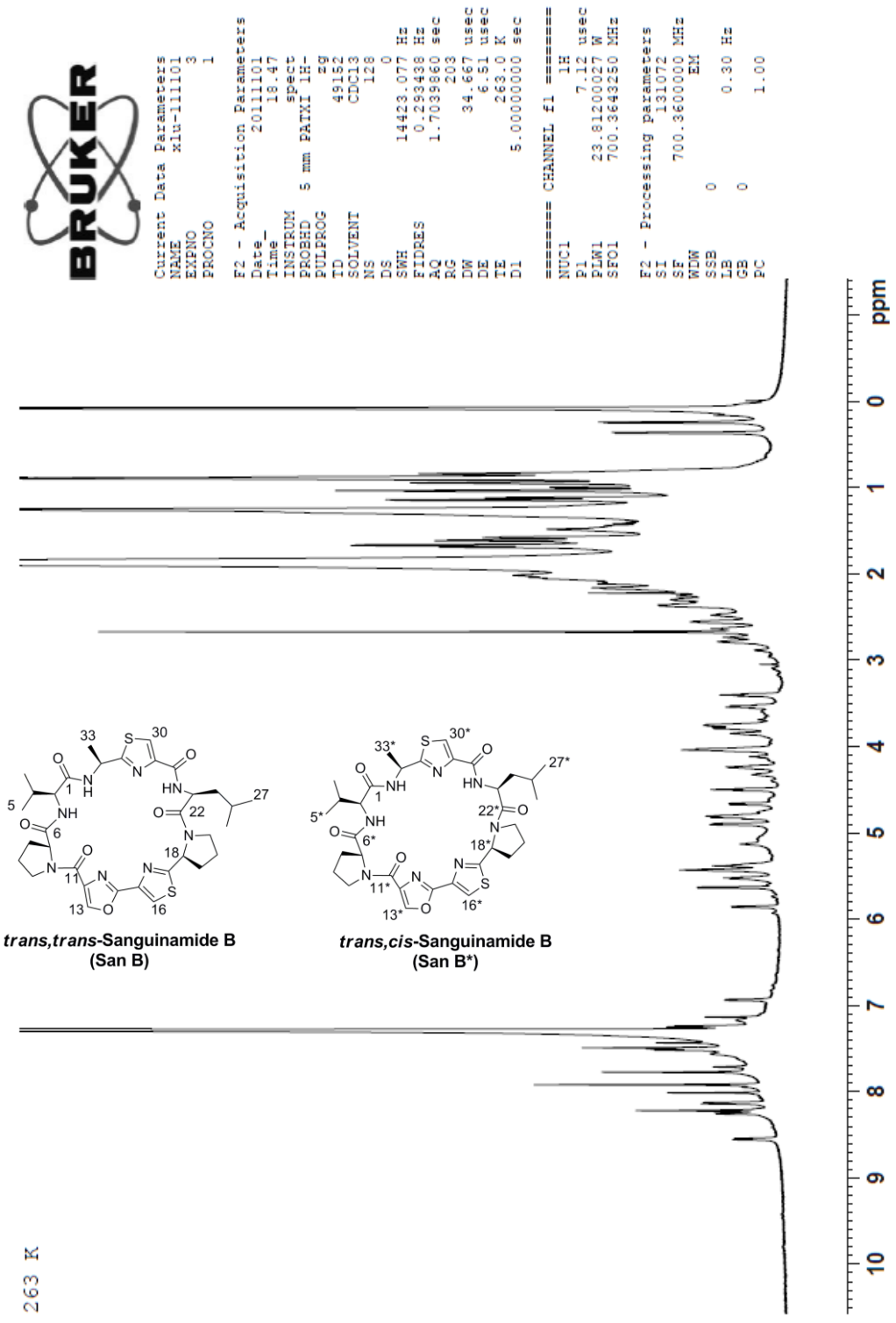
^{1}H-^{13}C HMBC NMR *cis*,*cis*-SanB

Display Report - All Windows Selected Analysis

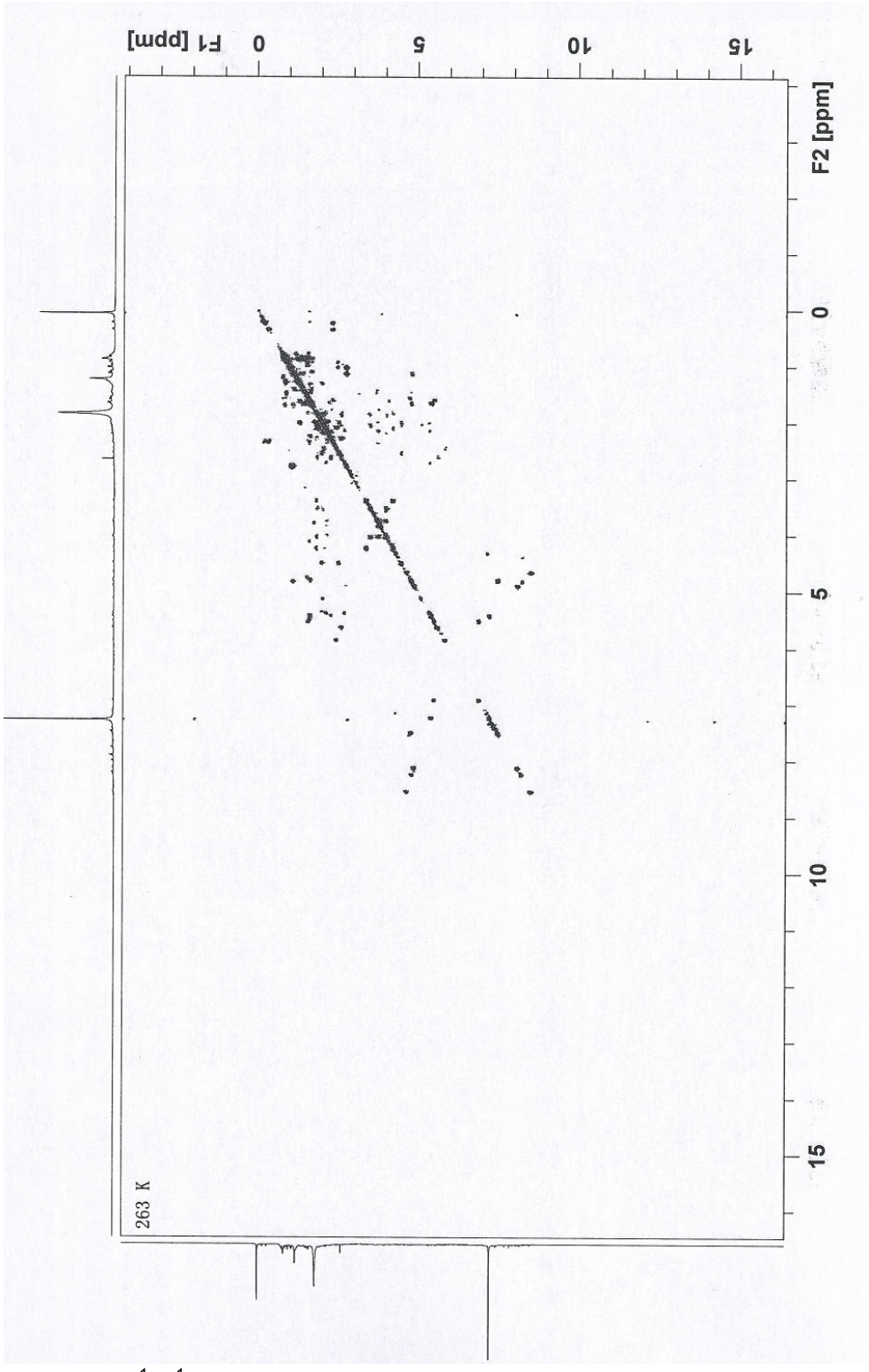
Analysis Name: EKS-IV-124_LRT. **Instrument:** Agilent 6330 Ion Trap **Print Date:** 6/6/2012 1:11:00 PM
Method: DI SANA.M **Operator:** sdsu **Acq. Date:** 9/19/2011 12:50:42 PM
Sample Name: eks-IV-124_lrt
Analysis Info:



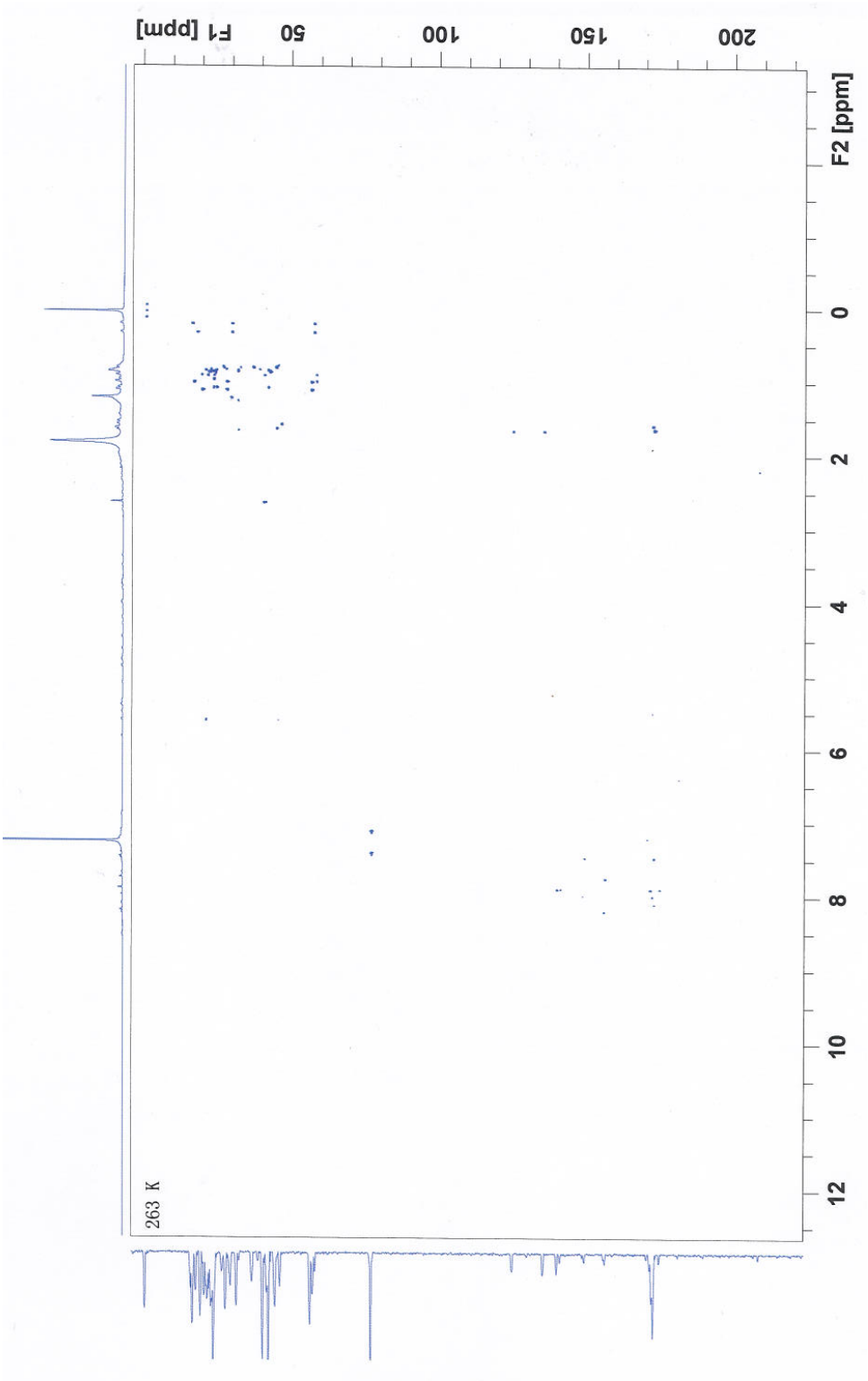
LC/MS *cis*,*cis*-SanB



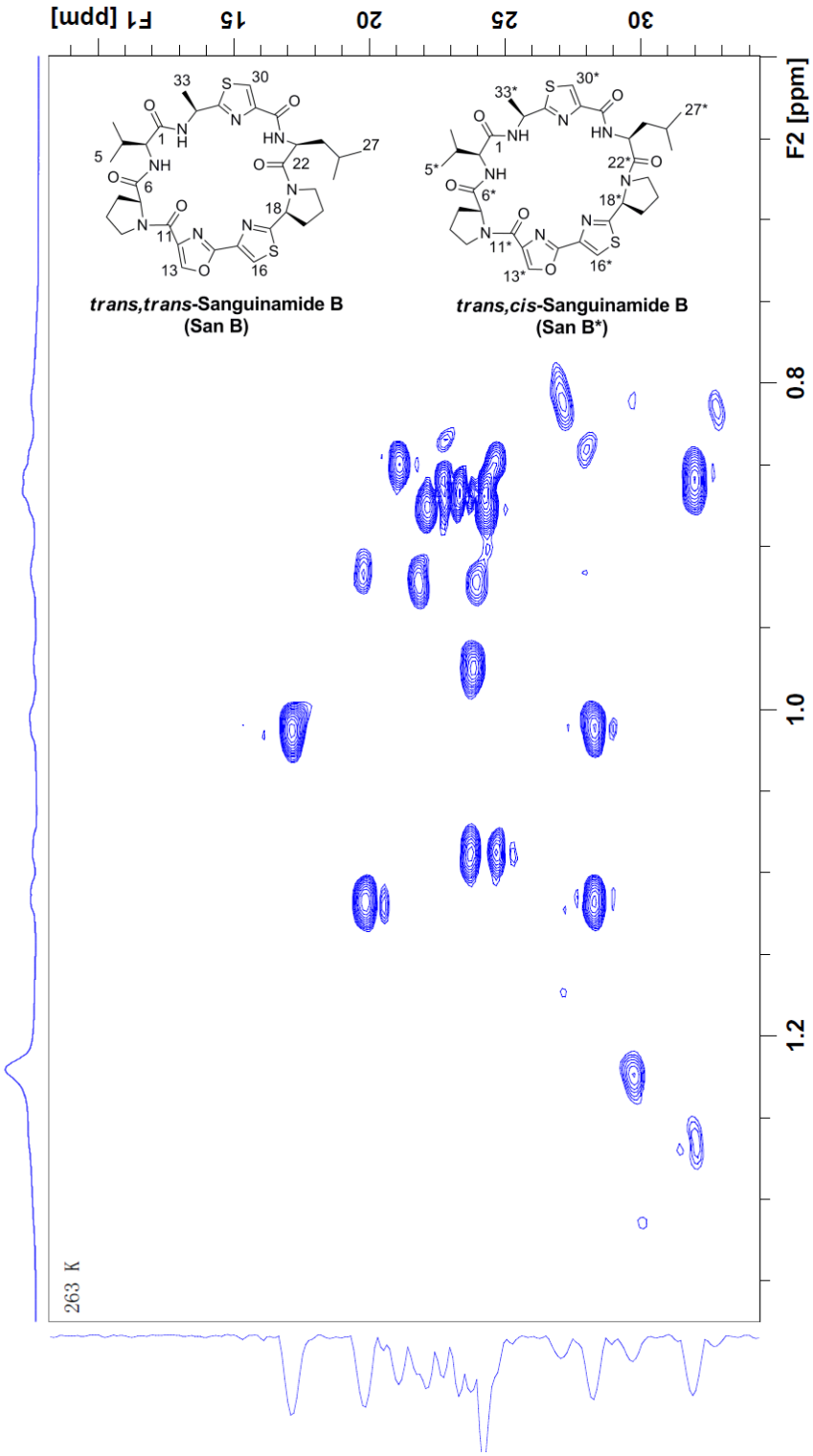
^{1}H NMR *trans*,*cis*-SanB/*trans*,*trans*-SanB



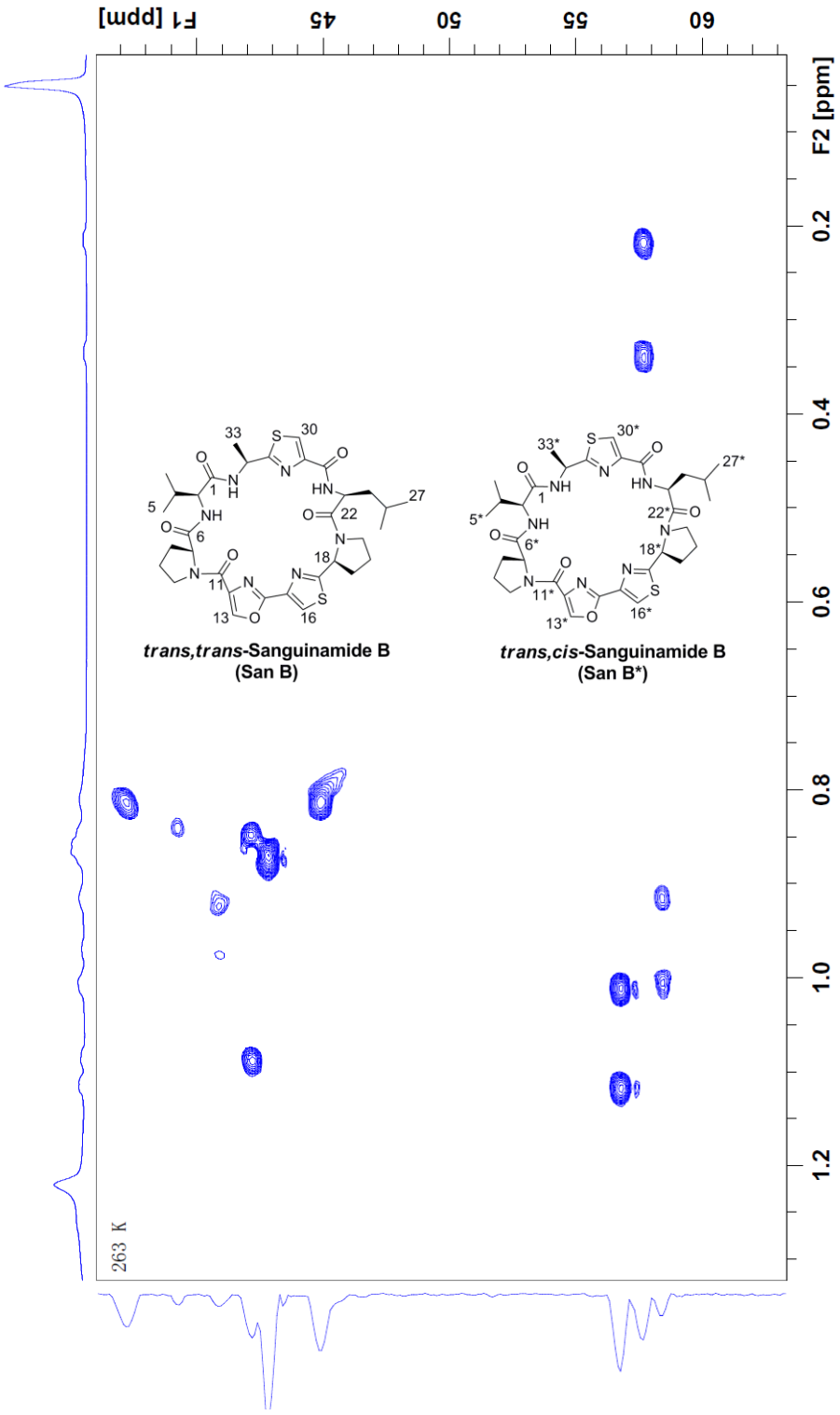
^{1}H-^{1}H COSY NMR *trans*,*cis*-SanB/*trans*,*trans*-SanB



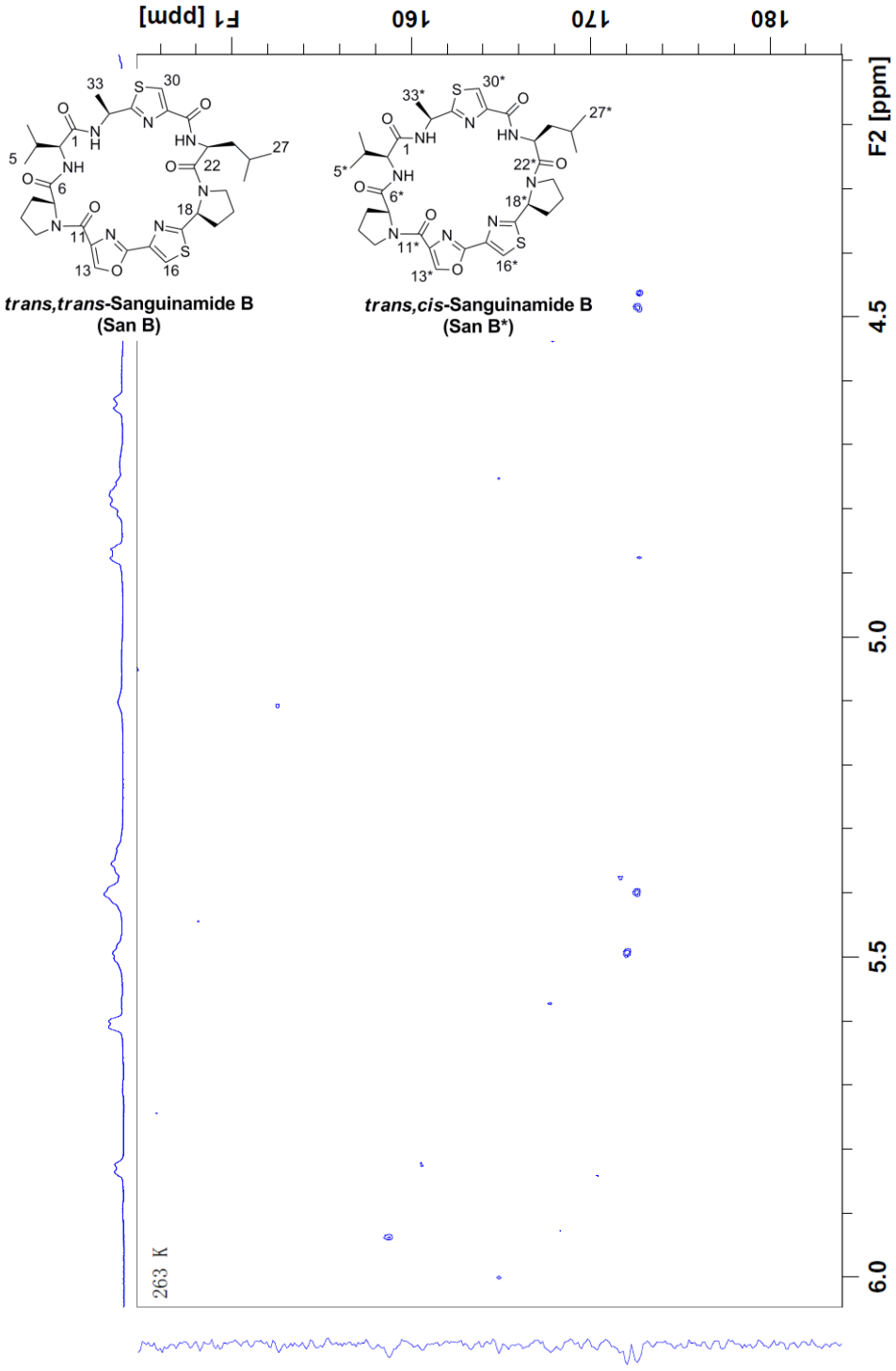
^{1}H-^{13}C HMBC NMR *trans*,*cis*-SanB/*trans*,*trans*-SanB



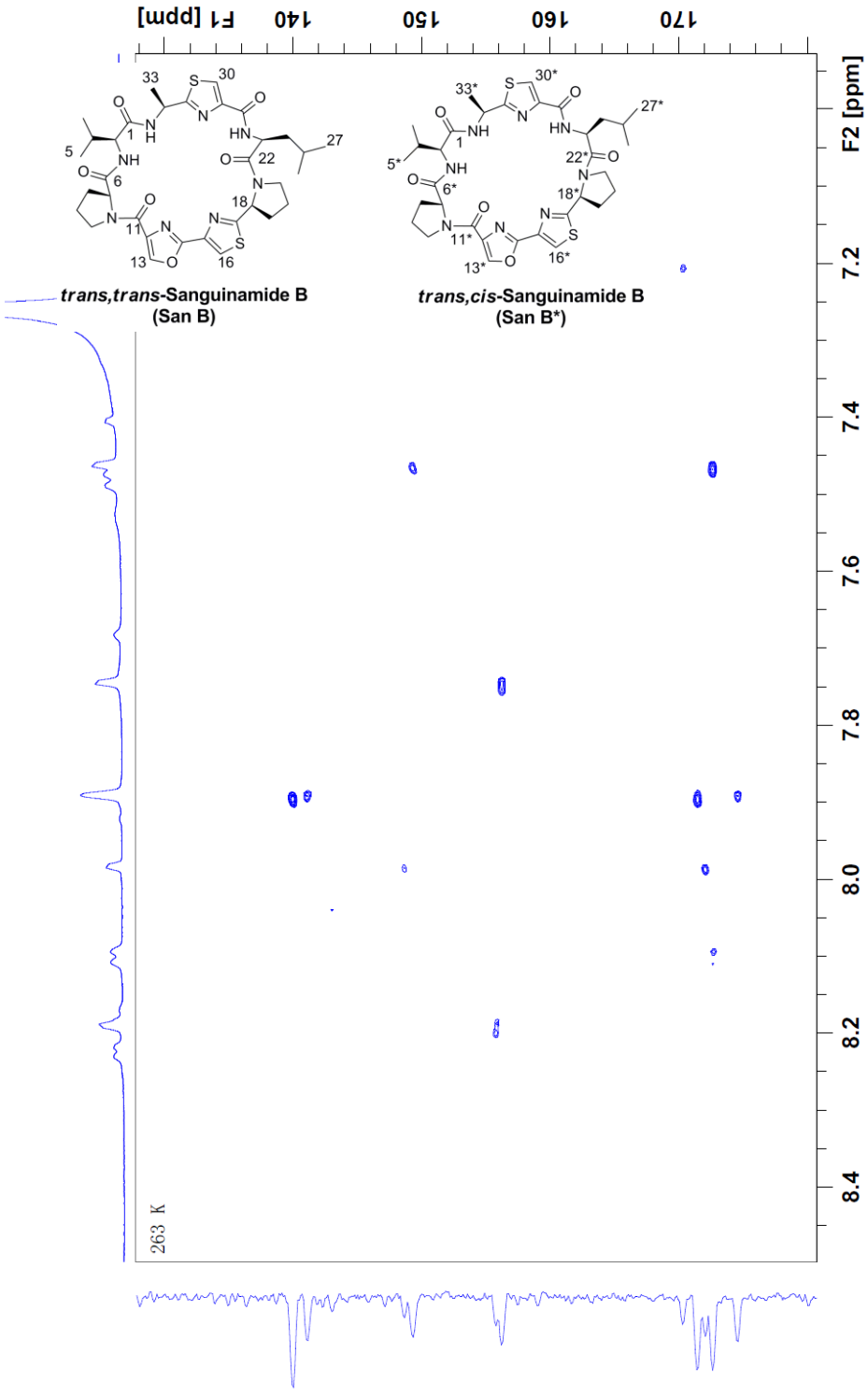
^{1}H-^{13}C HMBC NMR *trans*,*cis*-SanB/*trans*,*trans*-SanB
C3, C3*, C4, C4*, C5, C5*, C26, C26*, C27, C27*



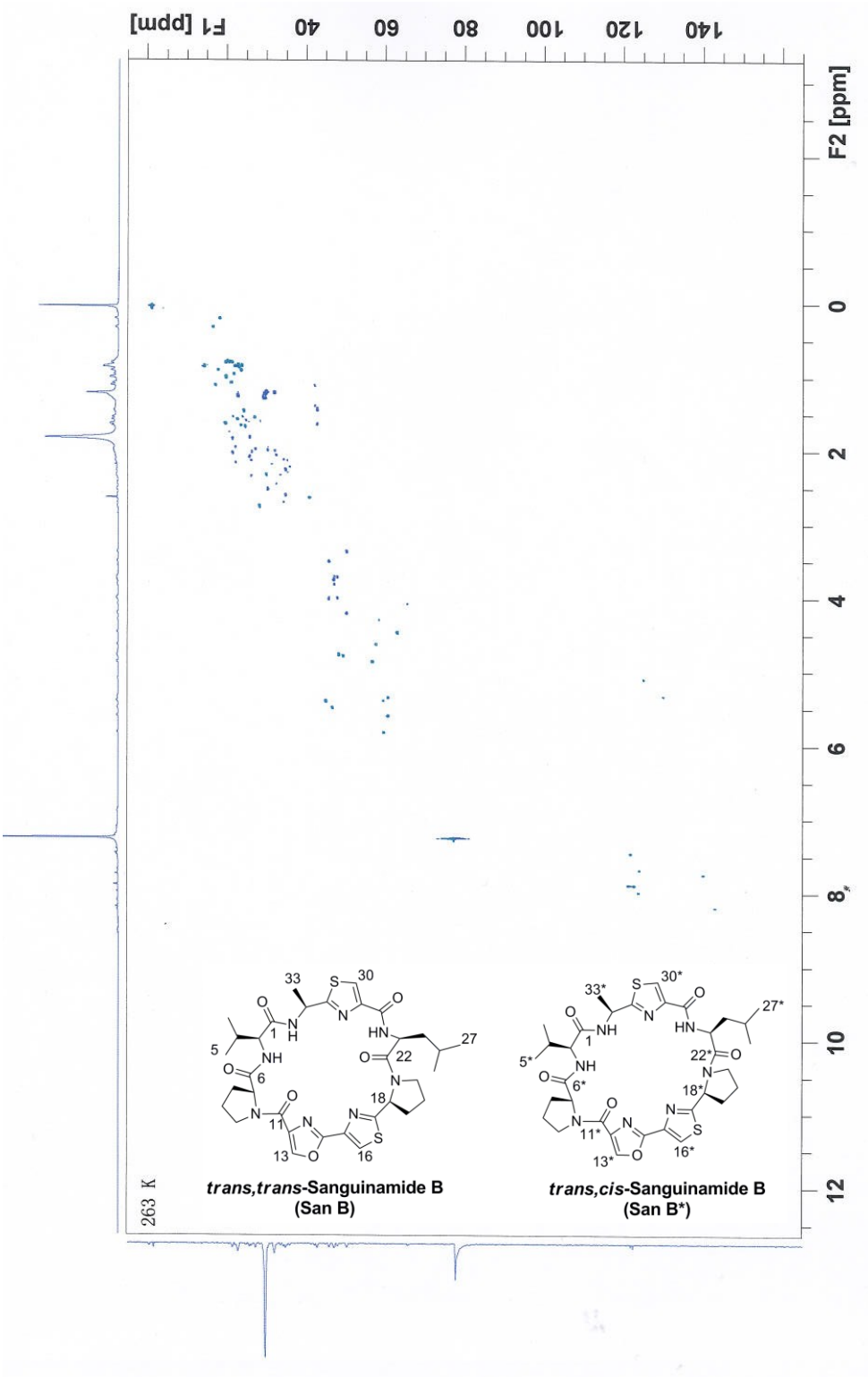
^{1}H-^{13}C HMBC NMR *trans*,*cis*-SanB/*trans*,*trans*-SanB
C2, C2*, C10, C10*, C18, C18*, C24, C24*



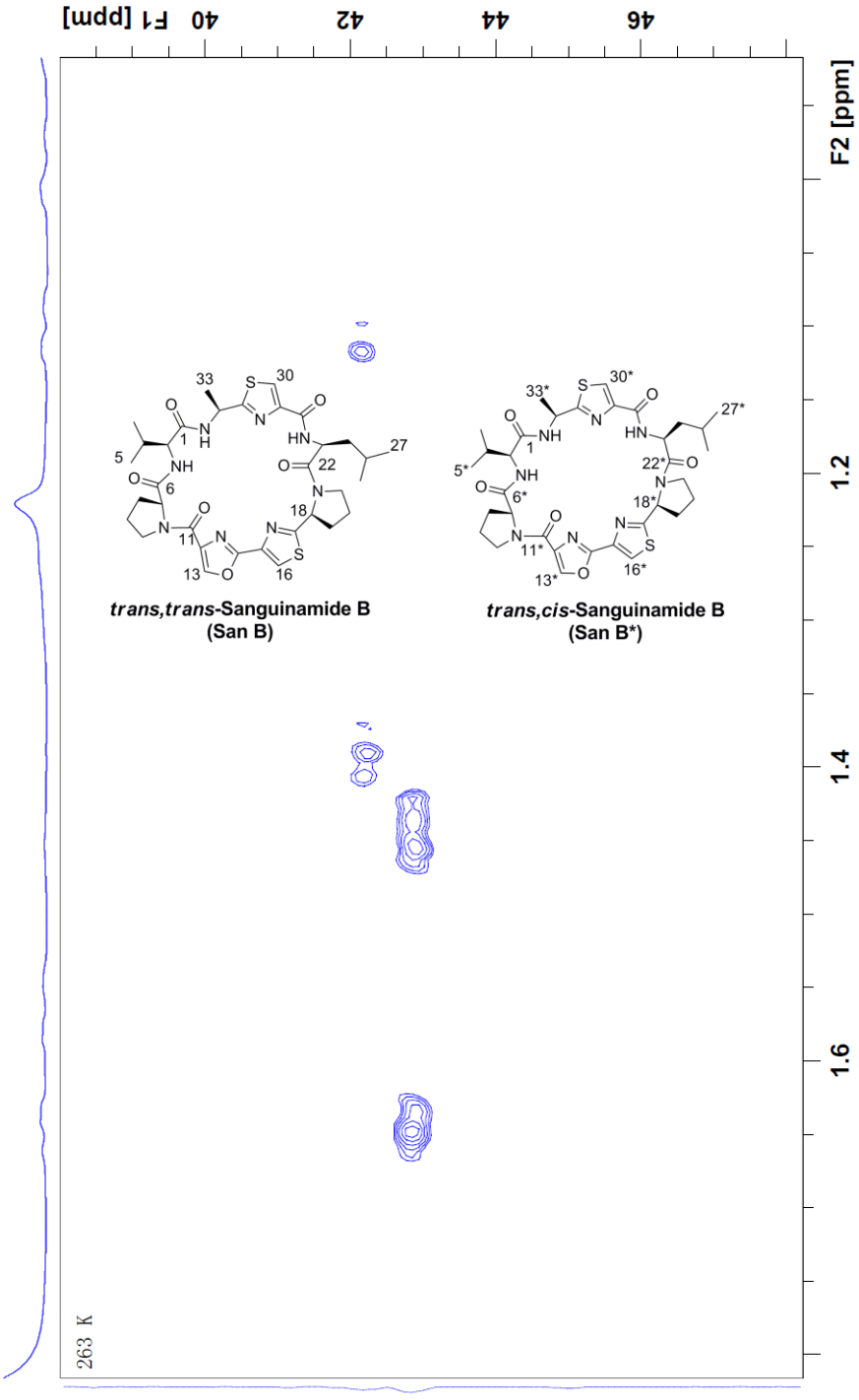
^{1}H-^{13}C HMBC NMR *trans*,*cis*-SanB/*trans*,*trans*-SanB
C1, C1*, C17, C17*, C31, C31*



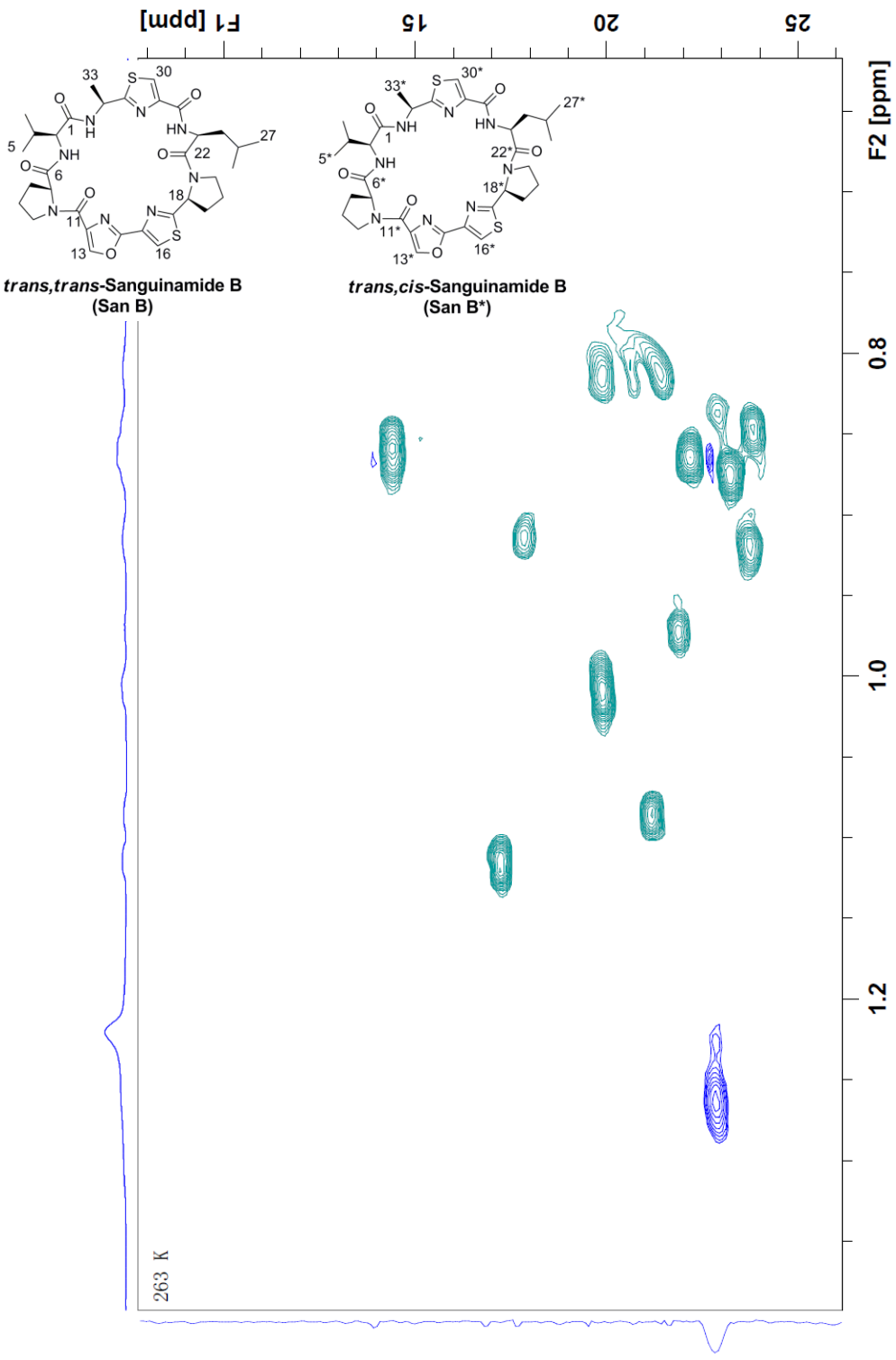
^{1}H-^{13}C HMBC NMR *trans*,*cis*-SanB/*trans*,*trans*-SanB
C14, C14*, C15, C15*, C17, C17*, C29, C29*, C31, C31*



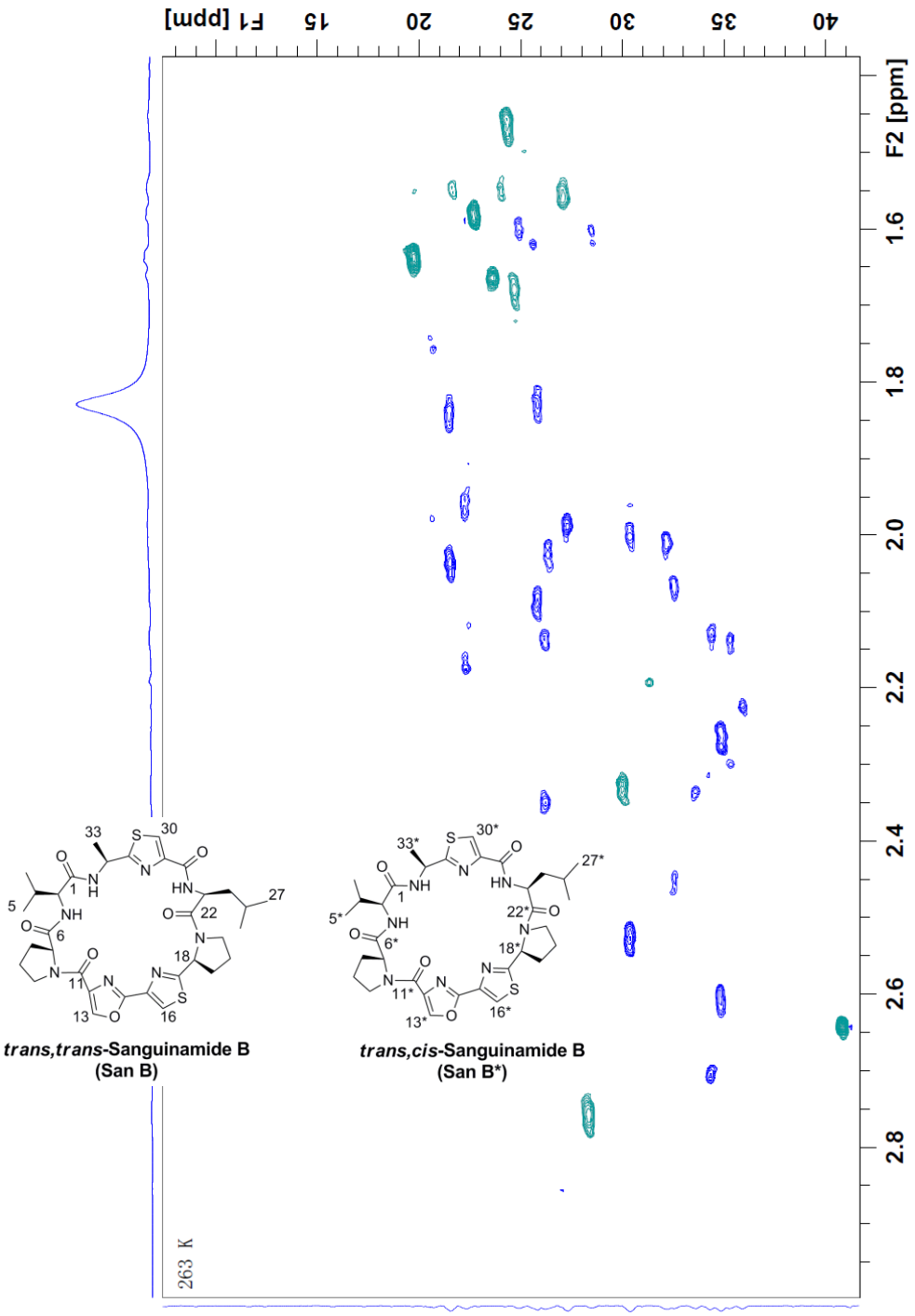
^{1}H-^{13}C HSQC NMR *trans*,*cis*-SanB/*trans*,*trans*-SanB



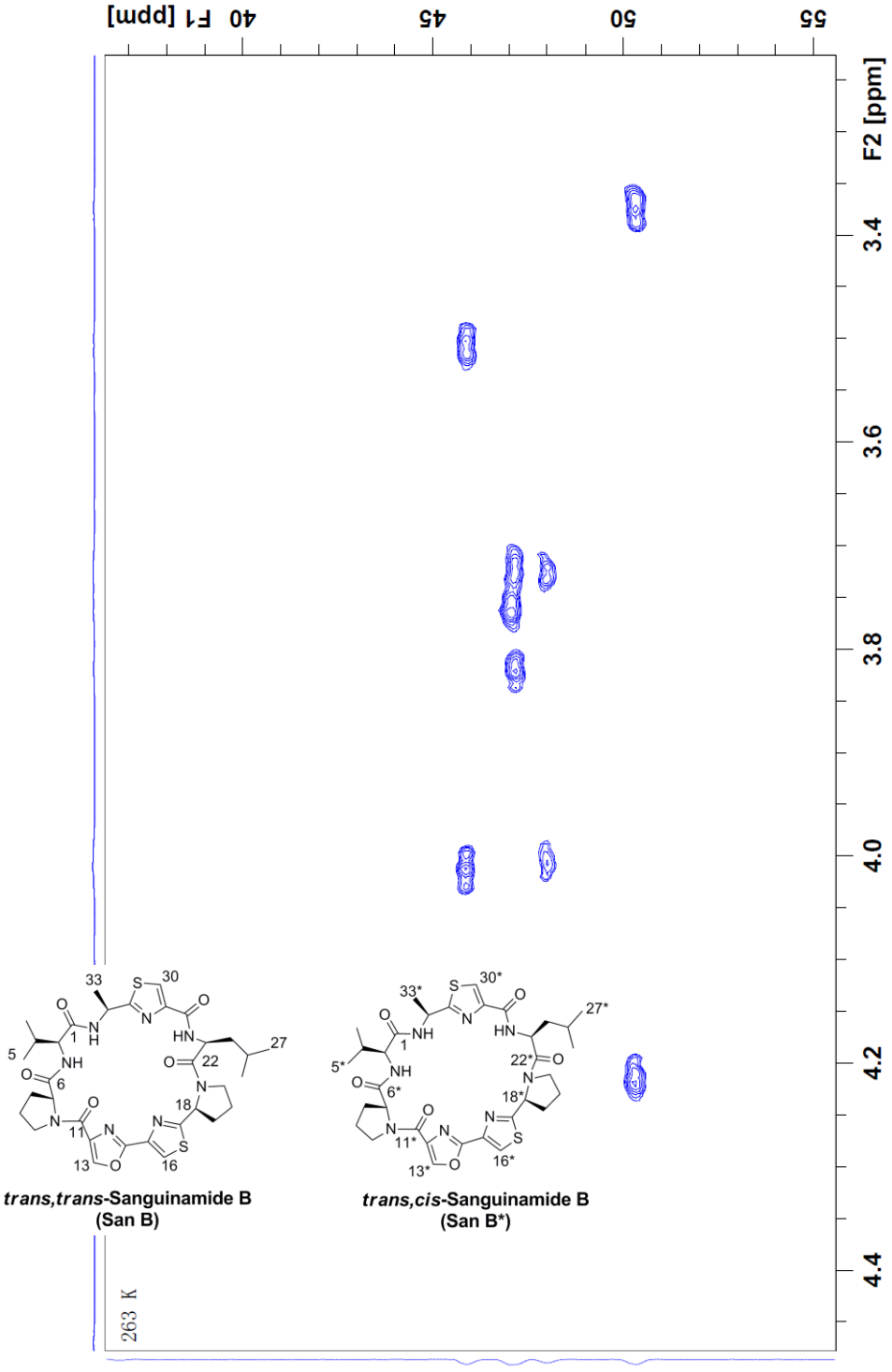
^{1}H-^{13}C HSQC NMR *trans*,*cis*-SanB/*trans*,*trans*-SanB
C24, C24*



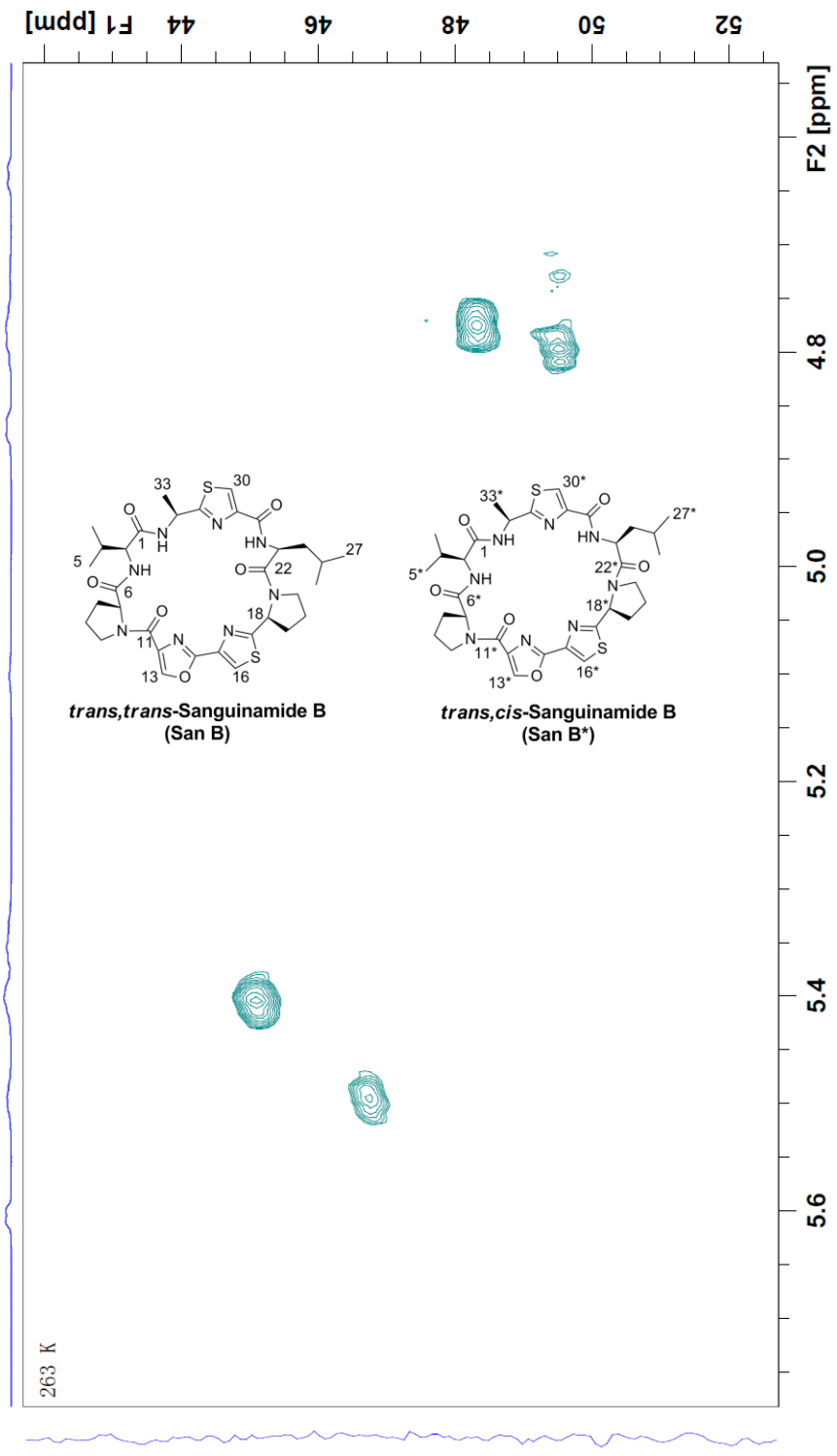
^{1}H-^{13}C HSQC NMR *trans*,*cis*-SanB/*trans*,*trans*-SanB
C4, C4*, C5, C5*, C25, C25*, C26, C26*, C27, C27*



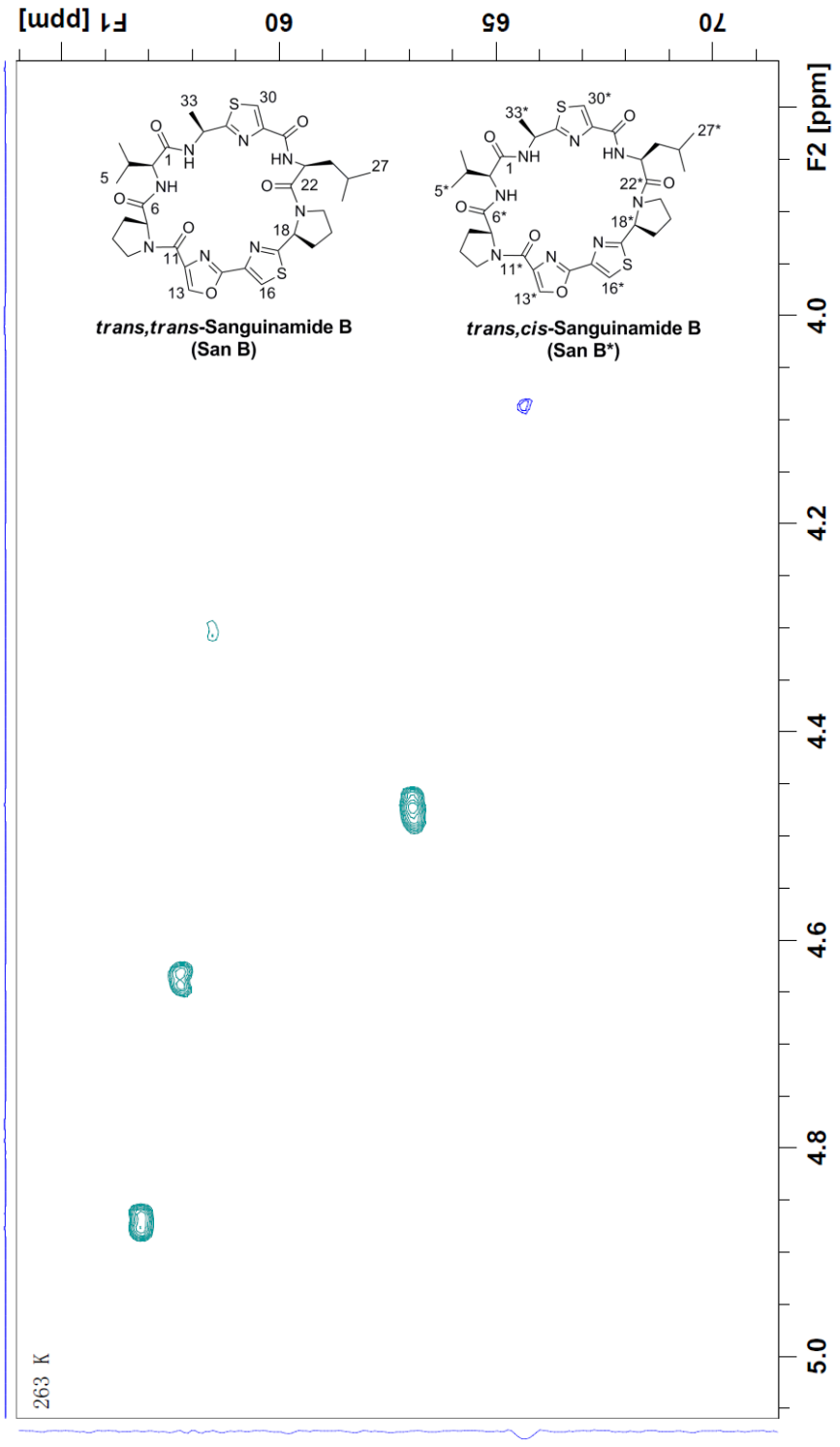
^{1}H-^{13}C HSQC NMR *trans*,*cis*-SanB/*trans*,*trans*-SanB
C8, C8*, C9, C9*, C19, C19*, C20, C20*



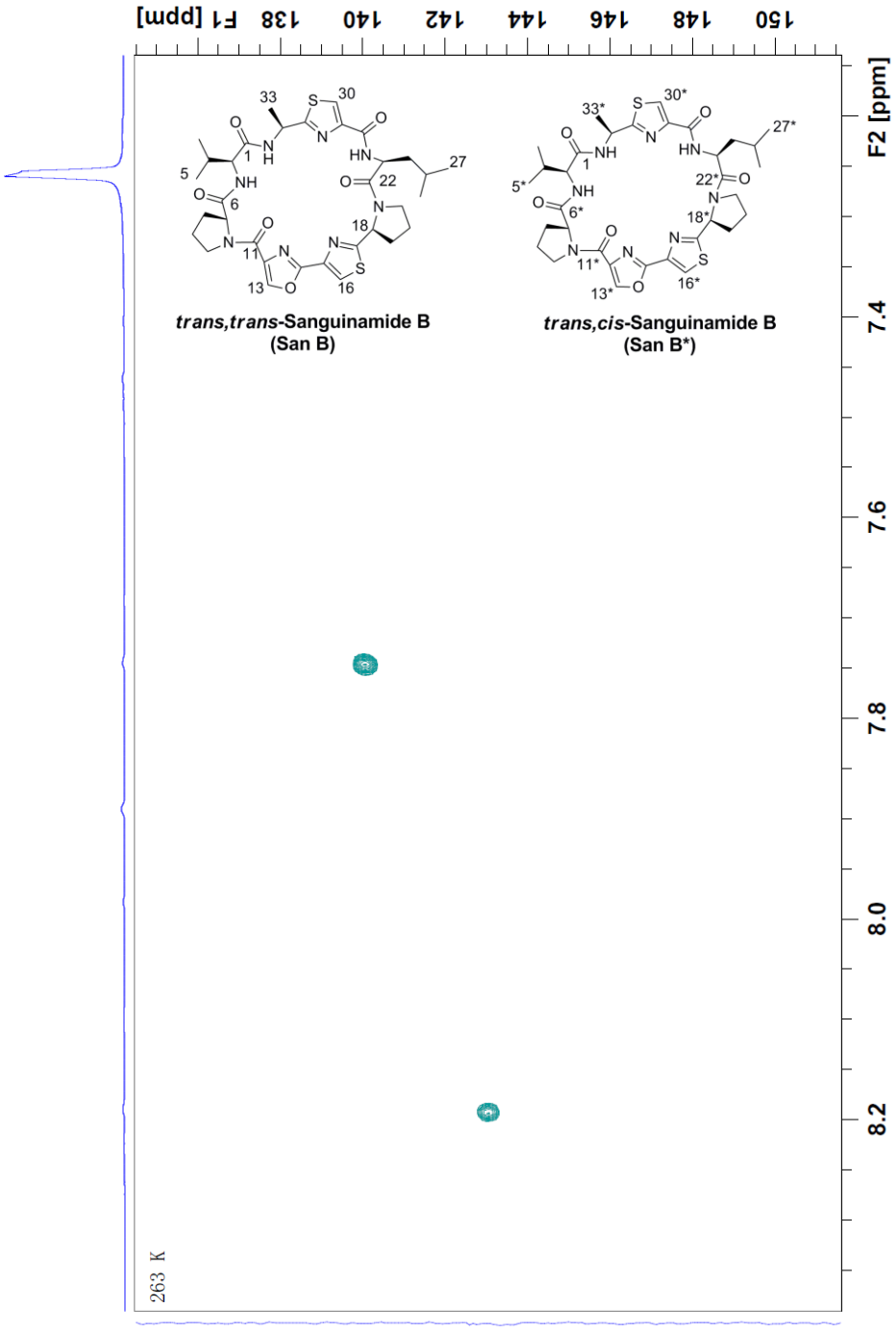
^{1}H-^{13}C HSQC NMR *trans*,*cis*-SanB/*trans*,*trans*-SanB
C10, C10*, C21, C21*



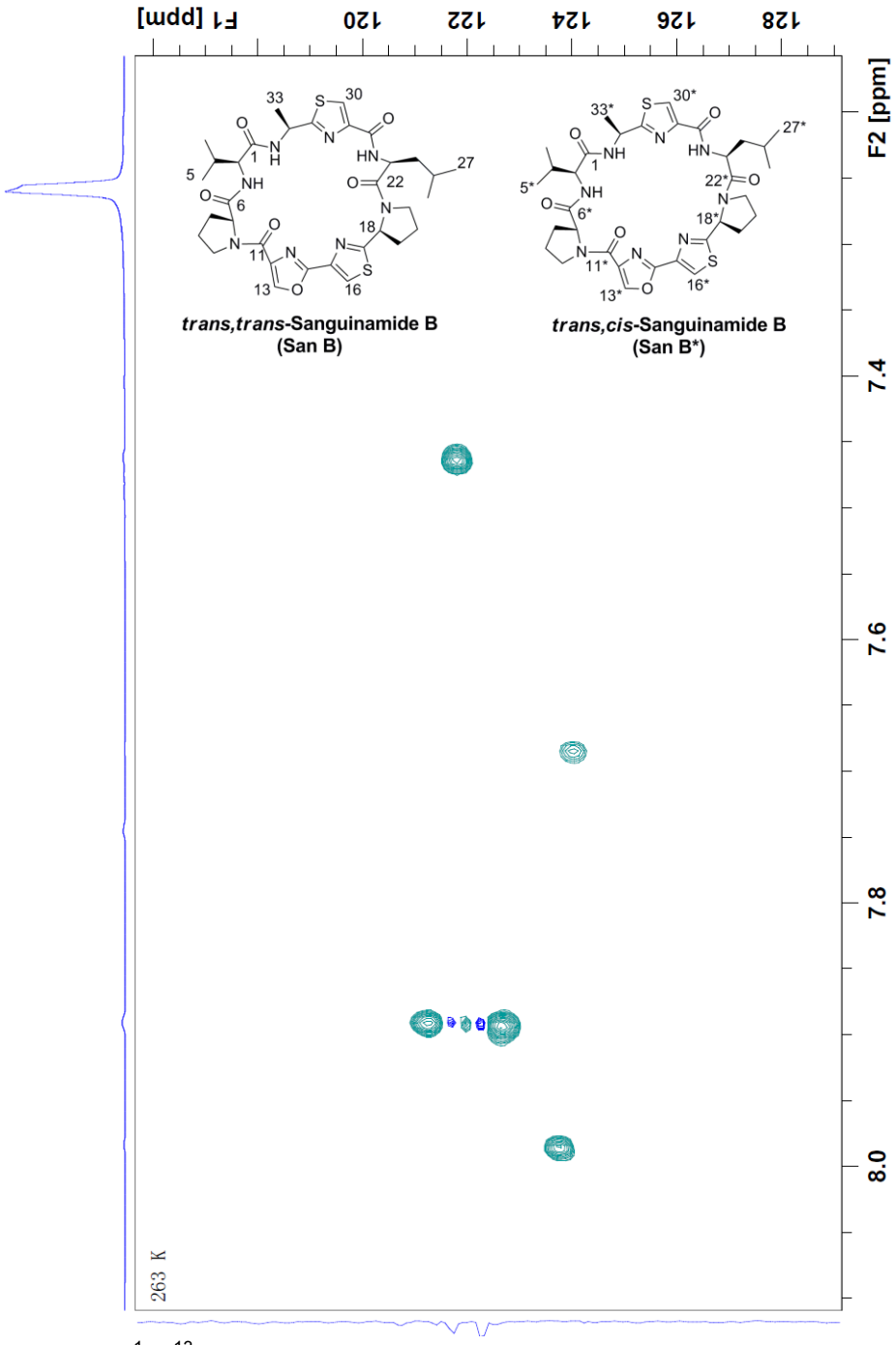
^{1}H-^{13}C HSQC NMR *trans*,*cis*-SanB/*trans*,*trans*-SanB
C32, C32*, C23, C23*



^{1}H-^{13}C HSQC NMR *trans*,*cis*-SanB/*trans*,*trans*-SanB
C2, C2*, C18, C7



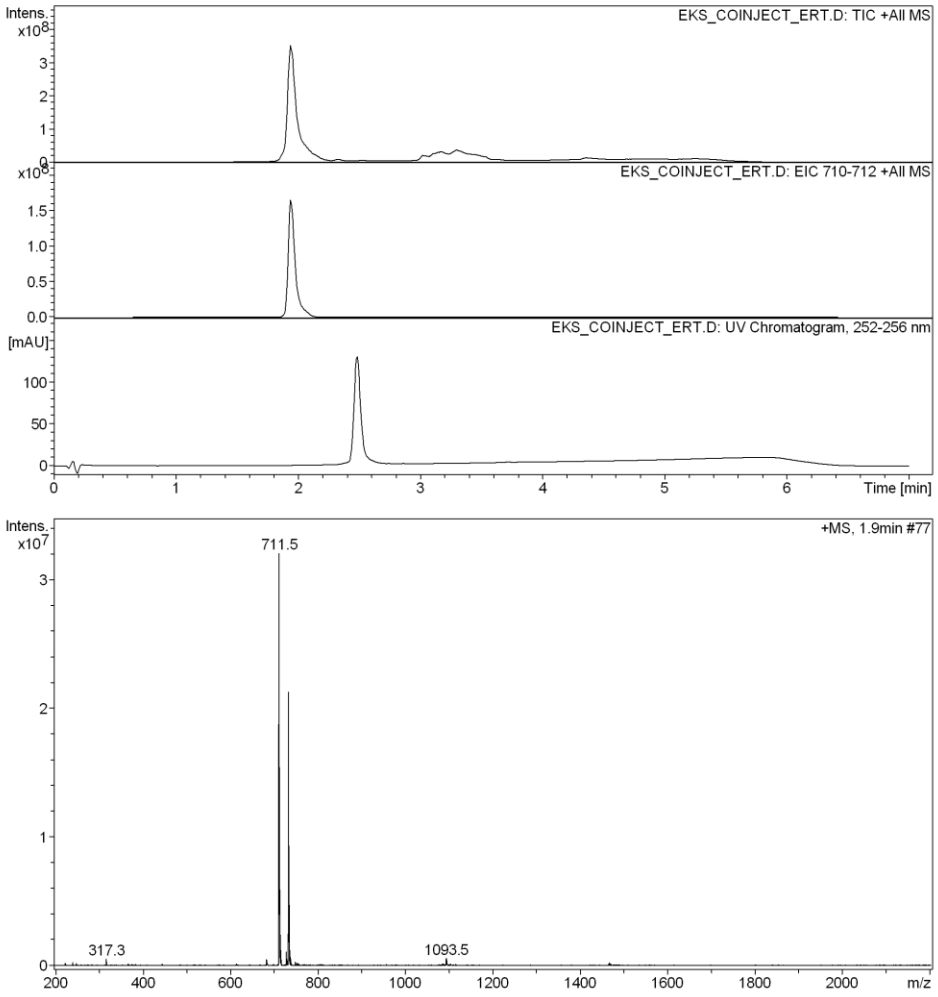
^{1}H-^{13}C HSQC NMR *trans*,*cis*-SanB/*trans*,*trans*-SanB
C13, C13*



^{1}H-^{13}C HSQC NMR *trans*,*cis*-SanB/*trans*,*trans*-SanB
C16, C16*, C30, C30*

Display Report - All Windows Selected Analysis

Analysis Name: EKS_COINJECT_ERT.D **Instrument:** Agilent 6330 Ion Trap **Print Date:** 6/6/2012 1:12:42 PM
Method: DI SANA.M **Operator:** sdsu **Acq. Date:** 10/14/2011 12:19:23 PM
Sample Name: eks_coinject_ert
Analysis Info:



LC/MS *trans*,*cis*-SanB/*trans*,*trans*-SanB