SYNTHESIS OF MACROCYCLIC ANTICANCER AGENTS:
SANSALVAMIDE A DERIVATIVES AND URUKTHAPELSTATIN A

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Synthesis of Macrocyclic Anticancer Agents: Sansalvamide A Derivatives and

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For my mom and dad
ABSTRACT OF THE THESIS

Synthesis of Macrocyclic Anticancer Agents: Sansalvamide A Derivatives and Urukthapelstatin A
by
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Natural products have been the source of valuable lead structures in the development of new therapeutics, providing novel structures that target unique biological pathways. Sansalvamide A (SanA) and Urukthapelstatin A (UstatA) are two such natural products. Both are peptide macrocycles isolated from marine organisms that display anticancer activity over a broad spectrum of cancer cell lines. Though peptides are sometimes considered poor drugs due to poor solubility and rapid degradation inside the cell, cyclic peptides have proven to be quite effective in minimizing these factors. Several peptide macrocycles are currently used as antibacterial (Vancomycin), antifungal (Caspofungin), anticancer (Aplidine), and immunosuppressant (Cyclosporine A) agents. Cyclic peptides require only a few amino acids to generate an overall 3D structure, thus minimizing molecular weights and improving solubility. Cyclic peptides have also shown to be more stable inside the cell relative to linear peptides due to the lack of terminal amino acids being exposed to proteases. With this in mind we have endeavored to synthesize both Sansalvamide A and Urukthapelstatin A as well as a library of derivatives in order to investigate the SAR of these macrocycles. We also will synthesize additional derivatives in order to investigate the compounds’ mechanism of action in inhibiting cancer cell growth.

The SanA project is well established with a library of over 100 derivatives synthesized to investigate its SAR. A decapeptide side product was also discovered to have anticancer activity and was also investigated. The pentapeptide and decapeptide derivatives I made were synthesized using a convergent solution phase approach. Boc-protected and OMe protected commercially available amino acids were coupled using standard peptide coupling conditions. Intermediates and the final products were purified via flash-column chromatography and HPLC and were characterized via $^1$H-NMR and LC-MS. Inhibition has been shown to occur via binding to Heat Shock Protein 90 (Hsp90). Current work includes synthesis of additional derivatives and multiple assays to ascertain the client proteins affected by our compounds’ binding to Hsp90.

Urukthapelstatin A is a heterocyclic macrocycle containing two oxazole, two thiazole, and one phenyloxazole moieties, which demonstrated growth inhibition against cancer cells in the low nanomolar range. Merchercharymcin, telomestatin, muscoride A and hennoxazoles are a few examples of pharmacologically active compounds recently synthesized and studied that contain similar poly-azole motifs. Upon thorough review of literature regarding the synthesis of some of these compounds and the various techniques employed in the synthesis of oxazoles and thiazoles, we have developed an efficient solution phase synthetic strategy for this molecule. We have employed standard peptide coupling
conditions on commercially available Boc-protected and OMe protected amino acids to generate di and tripeptides as our precursors. We used Diethylaminosulfurtrifluoride (DAST) and Diaza(1,3)bicycle[5.4.0]undecane (DBU) to perform a cyclodehydration/oxidation on Serine containing peptides to generate the oxazole and phenyloxazole moieties. The Hantzsch thiazole synthesis conditions were used to generate the first thiazole and were expected to generate the second thiazole in the final macrocyclization. Precursor compounds and the final product will be characterized by \(^1\)H-NMR and LC-MS. They were purified via flash-column chromatography and/or HPLC when necessary. Once the natural product is synthesized and the structure is confirmed and tested for cytotoxicity, we will proceed to synthesize derivatives to begin investigating SAR. Shortly thereafter, a derivative containing a biotinylated tag will be made to determine the mechanism of action. Considering the low nanomolar cytotoxicity of the natural product, Urukthapelstatin A could be a very promising scaffold for development of a cancer therapeutic.
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\(^3\text{H}\) tritium

5-FU 5-Fluorouracil

ACN acetonitrile

ACS American Cancer Society

Bn benzyl

Boc tert-butyloxycarbonyl

BPA Bromopyruvic acid

BPEE Bromopyruvic ethyl ester

Cbz Carbobenzyloxy

DAST Dieethylaminosulfurtrifluoride

DBU Diaza(1,3)bicyclo[5.4.0]undecane

DCM dichloromethane

DEPBT 3-(Diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one

DI de-ionized

DIPEA \(N-N\)-diisopropylethylamine

DiSanA Disansalvamide A

DLP deprotected linear pentapeptide

DMF dimethylformamide

DMSO dimethyl sulfoxide

EA ethyl acetate

EDC 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide

Et ethyl
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<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>FPTase</td>
<td>farnesyl transferase</td>
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<tr>
<td>HATU</td>
<td>2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate</td>
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<tr>
<td>HDAC</td>
<td>histone deacetylase</td>
</tr>
<tr>
<td>Hex</td>
<td>hexane</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
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<tr>
<td>Hsp90</td>
<td>Heat shock protein 90</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>half maximal inhibitory concentration</td>
</tr>
<tr>
<td>LC/MS</td>
<td>liquid chromatography mass spectrometry</td>
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<tr>
<td>LR</td>
<td>Lawesson’s reagent</td>
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<tr>
<td>Me</td>
<td>methyl</td>
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<tr>
<td>NCI</td>
<td>National Cancer Institute</td>
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<td>NIH</td>
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<td>N-Me</td>
<td>N-methyl</td>
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<td>NMR</td>
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<td>PG</td>
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<td>tert-Butyl</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
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<tr>
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</tr>
<tr>
<td>TFAA</td>
<td>Trifluoroacetic anhydride</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin-layer chromatography</td>
</tr>
<tr>
<td>UstatA</td>
<td>Urukthapelstatin A</td>
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<tr>
<td>VEGF-1</td>
<td>Vascular endothelial growth factor receptor-1</td>
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CHAPTER 1
INTRODUCTION TO PEPTIDES AND
PEPTIDOMIMETICS AS CANCER
THERAPEUTICS

INTRODUCTION TO CANCER

Cancer is a class of diseases that results from genetic aberrations in the DNA of affected cells, and is manifested by prolific cellular growth and division with an increased resistance to apoptosis. The resulting loss of cell cycle control results in the formation of malignant tumors capable of detrimental invasion of adjacent healthy tissue and spreading to other locations of the body via blood or lymph (metastasis). The American Cancer Society (ACS) predicts that 1,529,560 new cases of cancer, not including basal and squamous cell skin cancers, will have been diagnosed in 2010. These two skin cancers, which are not required to be reported to cancer registries, were treated in over 2 million people in 2006. Cancer is the second leading cause of death in the United States and the ACS predicts that 569,490 deaths will have been a result of cancer in the United States in 2010. This value represents one of every four deaths in the U.S. The National Institutes of Health (NIH) estimates that overall costs, including medical costs and lack of production costs due to illness and death, associated with the treatment of cancer in 2010 were $263.8 billion.

Colorectal cancer is the third most common form of cancer in both men and women in the U.S. In 2011, the ACS estimates that 49,380 deaths will occur as a result of colon or rectal cancer, accounting for 9% of all cancer deaths. Among adults under the age of 50, the rate of diagnosis has been increasing by 2% annually since 1994. Although the mortality
rate for colorectal cancer is low when diagnosed early, it is asymptomatic in its early stages and screening rates for this cancer are falling. Thus, only 39% of cases are identified at an early localized stage.\(^3\) Surgery is a common treatment but, for the majority of advanced stage cases, is also accompanied by chemotherapy. The three most common drugs used for the treatment of colorectal cancer are 5-Fluorouracil (5-FU), Oxaliplatin, and Irinotecan.\(^4\)\(^-\)\(^6\) Though these drugs have shown to increase survival rates, particularly when used in adjuvant therapy\(^7\) or administered together, they tend to display severe side effects, cause allergic reactions in some patients, and are relatively ineffective in some forms of colon cancer.\(^2\) Due to its low rate of early diagnosis and the issues with current chemotherapeutic agents, identifying new compounds to treat the various forms of colorectal cancer would prove extremely valuable.

Pancreatic cancer is the fourth most common cause of cancer death with an estimated 36,800 deaths in 2010. The ACS estimates 43,130 new cases per year and this rate for women has been increasing 1.7% annually since 2000.\(^1\) Similar to colon cancer, pancreatic cancer is asymptomatic in its early stages and only 7% are diagnosed early. Because of this, in nearly 80% of patients the cancer has spread, rendering surgery ineffective. As a result, the five year survival rate is lower than 5%.\(^8\) The two most commonly employed chemotherapeutics are 5-FU and gemcitabine.\(^9\)\(^,\)\(^10\) Several clinical studies have been performed involving one or both of these drugs, generally in combination with one or more other known cancer therapeutic, but only 25% of cases have demonstrated favorable results. The high mortality rate and current ineffectiveness of the drugs of choice point toward a strong need for development of new pancreatic cancer therapeutics.
PEPTIDES AS THERAPEUTICS

Since the peak of cancer death rates in 1990-91, they have steadily declined as a result of extensive research and advances in treatment and therapeutics.\textsuperscript{11} Due to the multitude of various types of cancer and broad range of cellular processes altered in cancerous cells, research into the development of potent antitumor agents has included a wide variety of potential sources for new forms of medication. One source that has provided promising new pharmacologically active structures is natural product peptides and peptidomimetics.\textsuperscript{12-16} Peptides possess a number of characteristics that make them ideal drug candidates. They offer a wide range of chemical diversity as there are 20 naturally occurring amino acids, which can also undergo a series of posttranslational modifications including epimerization of natural L-amino acids to the D form, modification of their structure to generate an unnatural amino acid and methylation of the peptide nitrogen ($N$-methylation). When broken down by proteases in the cell, their metabolites are non-toxic and easily removed from the body in the liver or kidneys.\textsuperscript{17} Recent advances in chemical syntheses of peptides have allowed for rapid and inexpensive production of moderate sized polypeptide chains (5-50 amino acids).

Unfortunately, despite the advantageous characteristics, peptides have long been considered poor drug candidates for several other reasons. They tend to have low bioavailability; thus, most peptide therapeutics must be injected. This low bioavailability is a function of the drug’s physiochemical properties, which are a function of the amino acid sequence. Linear peptides tend to require a long polypeptide chain in order to fold into a favorable 3-dimensional conformation to bind with its target. These long chains lead to poor solubility and inability to cross physiological barriers. In addition, the tendency for portions of the polypeptide chain to exhibit a degree of conformational flexibility can lead to
nonspecific binding causing unfavorable side effects. Linear peptides are also highly
vulnerable to proteolytic attack at these flexible regions and at their termini.\textsuperscript{18}

Cyclic peptides and peptidomimetics, however, have been demonstrating a much
more favorable pharmacokinetic profile. The cyclic structure allows for a much smaller
number of amino acids to form a rigid 3-D structure. The restriction in bond rotation induces
a degree of conformational restraint that can lead to a greater binding affinity with its
target.\textsuperscript{13} This relative inflexibility along with the lack of any terminal amino acids also makes
the peptide more resistant to proteases, increasing its half-life within the cell.\textsuperscript{19,20} In addition,
the smaller polypeptide sequences of the cyclic structures increase their solubility and
bioavailability. Further modifications into peptidomimetic structures serves to mimic the
native peptide structure while enhancing the compounds’ pharmacokinetic properties.

There are currently 617 peptide drugs or drug candidates, 24\% of these are in clinical
trials, 65\% are in advanced preclinical phases, and 11\% are FDA approved.\textsuperscript{21-24} Linear and
cyclic peptides and peptidomimetics are in use as therapeutics for prostate and breast cancer,
HIV infections, osteoporosis, acute coronary syndrome, and serve as immunosuppressants.\textsuperscript{24}

Vancomycin (\textbf{Figure 1}) is a tricyclic glycopeptide isolated from the bacteria \textit{Amycolatopsis
orientalis}.\textsuperscript{25,26} It is a potent antibiotic that inhibits cell wall synthesis in Gram-positive
bacteria and is employed as a last resort against penicillin resistant infections. Aplidine
(\textbf{Figure 1}) is a cyclic depsipeptide, found in the marine tunicate \textit{Aplidium albicans}, currently
in clinical trials for several cancers including leukemia and lymphoma.\textsuperscript{27-29} Its cytotoxic
activity is a function, in part, of inhibiting expression of vascular endothelial growth factor
receptor-1 (VEGF-1) and inducing apoptosis.
Sansalvamide A

The natural product Sansalvamide A (SanA) is a cyclic pentadepsipeptide that has displayed promising cytotoxicity in a number of cancer cell lines.\textsuperscript{30-32} It was first isolated by William Fenical in 1996 from the fungus \textit{Fusarium sp.}, found off the coast of Little San Salvador Island, Bahamas. Its cyclic structure consisted of four L-amino acids: a phenylalanine (Phe), valine (Val) and two leucines (Leu), as well as one L-hydroxy acid: an O-leucine (OLeu) (Figure 2). The natural product exhibited an IC\textsubscript{50} of 16.7 \( \mu \text{M} \) against the colon cancer cell line HCT-116 in an \textit{in vitro} assay. Notably, when run in the same assay, the linear version of the pentapeptide showed no cytotoxicity, demonstrating the impact cyclization has upon biological activity. Further studies on SanA in the National Cancer Institute’s (NCI) 60 cell line panel revealed a mean IC\textsubscript{50} of 46.7 \( \mu \text{M} \). Additionally, in the colon cancer cell line COLO 205 and melanoma cell line SK-MEL-2, the natural product showed notable potency with IC\textsubscript{50} values of 6.0 \( \mu \text{M} \) and 10.1 \( \mu \text{M} \) respectively. This toxicity is comparable to the FDA-approved antitumor agent mitomycin C with IC\textsubscript{50} values for the
same two cell lines of 9.0 μM. The promising cytotoxicity values reported by Fenical et al. made SanA a viable lead structure for further investigation as an anticancer agent. The first portion of this thesis will discuss my contribution to our lab’s SanA project. In this project, over 100 derivatives based on the SanA scaffold have been synthesized and tested in several colon and pancreatic cancer cell lines. The overall goals of this project are to improve upon the antitumor activity of the natural product by designing derivatives with alterations in one or more amino acids from the original structure and to fully understand the mechanistic pathway affected by the most potent compounds in order to develop a new viable anticancer therapeutic.

**PEPTIDOMIMETICS AS CANCER THERAPEUTICS**

Recently a number of natural product peptidomimetic structures that contain oxazoles and/or thiazoles have been isolated and displayed promising bioactivity. In biosynthesis, oxazoles are generated via cyclization and oxidation of serine (Ser) containing peptides and thiazoles from cystine (Cys) containing peptides. When incorporated into a macrocyclic backbone, these planar aromatic structures impart rigidity to the structure as well as providing new sites for potential binding interactions via pi stacking. Telomestatin (Figure 2. Structure of Sansalvamide A.)
3) is a peptidomimetic macrocycle isolated from *Streptomyces anulatus* comprised of seven oxazoles and one thiazoline.\textsuperscript{44} It inhibits telomerase activity by inducing the formation of G-quadruplexes in the telomeric region of DNA. Since telomerase activation is observed in 90% of all cancer types, this compound has been the subject of several studies.\textsuperscript{44,45} The macrocycle Merchercharmycin A (Figure 3), isolated from a bacterium found in the marine fungus *Thermoactinomycetes sp.*, was also recently synthesized and found to have strong cytotoxic activity.\textsuperscript{46,47} Although the mechanism of action is unknown, it inhibits lung cancer cells (A549) with an IC\textsubscript{50} value of 40 nM and leukemia (Jurkat cells) with an IC\textsubscript{50} of 46 nM.\textsuperscript{48,49} It is also interesting to note that, as seen with SanA, the linear counterpart, Merchercharmycin B, demonstrated no activity in the same cancer cell lines.

![Figure 3. Structures of telomestatin and merchercharmycin A.](image)

**Urukthapelstatin A**

Urukthapelstatin A (Figure 4), isolated from the bacterium *Mechercharimyces asporophorigenens*, shares a high degree of homology with the Merchercharmycins. Made up of three amino acids, two thiazoles and three oxazoles in its cyclic structure\textsuperscript{50}, it exhibited outstanding cytotoxic activity when tested in a 39 cancer cell line panel at the Japanese Foundation for Cancer Research with a mean IC\textsubscript{50} value of 15.5 nM.\textsuperscript{51} It was most effective against the breast, colon, skin and two lung cancer cell lines MCF-7, HCT-116, A549,
Figure 4. Structure of Urukthapelstatin.

DMS114 and NCI-H460 with IC$_{50}$ values within the range of 5.2-3.5 nM. Assays were also run to determine if Urukthapelstatin A (UstatA) demonstrated any antibiotic activity or inhibitory activity against telomerase, histone deacetylase (HDAC), farnesyl transferase (FPTase) and the proteasome. All of these assays showed no significant inhibition. As there is no published report of a total synthesis of Urukthapelstatin A to date and its mechanism of action is unknown, the final portion of my thesis will focus on the synthetic strategy derived toward its first synthesis.
CHAPTER 2
SYNTHESIS AND BIOLOGICAL ACTIVITY OF
SANSALVAMIDE A DERIVATIVES

SANSALVAMIDE A INTRODUCTION
The first reported synthesis of SanA was performed by Dr. Richard B. Silverman in 2000. The synthesis of the depsipeptide was performed via solid phase using a phenylalanine residue tethered to a resin-bound linker. Lactones are prone to ring opening via esterases in the cell, so in 2002 he reported the synthesis of the SanA peptide (SanA 1), where the hydroxy acid at position IV was exchanged with an amino acid (Figure 5). The cyclic pentapeptide (SanA amide) analogue demonstrated ten times greater cytotoxicity than the natural product in HCT-116 with an IC50 value of 1.7 μM. Additionally, the observation was made that several biologically active cyclic peptides contained at least one N-methylated amino acid; research had shown that inclusion of an N-methyl served to increase potency by restricting conformational changes in cyclic structures and by blocking potential sites for enzymatic cleavage within the cell. Thus, in 2005, Silverman et al. reported the synthesis of a series of SanA amide derivatives designed to test the effect on cytotoxicity by N-methylating one of the five amino acids of the macrocycle. Two of these N-methylated derivatives showed increased activity against two different pancreatic cancer cell lines (AsPC-1 and S2-013). This data indicated that the SanA amide cyclic pentapeptide scaffold could provide an excellent template for further modification to increase potency and develop a potentially viable cancer therapeutic.
Figure 5. Sansalvamide A natural product and peptide.

Our group sought to investigate further the structure activity relationships (SAR) of the SanA amide macrocycle. The synthesis of nearly 100 derivatives has been accomplished integrating various natural and unnatural, D and L, and N-methylated amino acids into the cyclic pentapeptide structure. Each derivative has been designed to investigate the effect of altering one or more amino acids from the original SanA amide structure on the new compounds’ conformation and, thus, cytotoxicity in one or more cancer cell lines (primarily the colon cancer cell lines HCT-116, HCT-15 and HT-29, and the pancreatic cancer cell lines PL-45 and BxPC-3). Early derivatives were designed to determine the effects of incorporating one or more N-methyls, altering one or more of the core amino acids to the D version, or replacing one of the core amino acids with a nonpolar alternative (i.e. Isoleucine or Alanine). The modifications found in two or more potent derivatives were incorporated into new derivatives to investigate synergistic effects. In addition, new derivatives were designed with a variety of new nonpolar, polar or aromatic amino acids. Several general trends began to emerge from studying the SAR of these derivatives. Polar amino acids or multiple N-methyls usually resulted in low potency derivatives. Potent derivatives contained one or more of the following motifs: two (and only two) consecutive D-amino...
acids, a single N-methyl incorporated into a D-amino acid and multiple aromatic amino acids.\textsuperscript{34,36}

**RATIONAL DESIGN OF SAN A DERIVATIVES**

The following is a summary of the work I have contributed toward this project, particularly the synthesis of six different SanA derivatives (2, 3, 4, 5, 6 and 7; Figure 6). Each of these derivatives was designed from promising derivatives already synthesized and tested for their cytotoxicity. SanA 2 was derived from three previous derivatives that demonstrated good cytotoxicity, SanA 8, 9 and 10 (Figure 7). The D-Phe at position I from 8, D-Leu at position V from 9, and the N-methyl on the leucine at position IV from 10 were all incorporated into the structure of 2. With this new structure the synergistic effects of merging several alterations (relative to the original SanA peptide) that appear to increase potency could be investigated.

![SanA derivatives synthesized](image)

Figure 6. SanA derivatives synthesized.
Figure 7. Rational design of SanA 2 (percent growth inhibition values at 5μM).

The derivative SanA 3 was based on a single potent lead analogue, SanA 11 (Figure 8). The D-Leu at position II of 11 that was attributed to its increased activity was replaced with a D-Phe. Early research had supported the theory that a single D-amino acid in the structure may help lock the macrocycle in a single conformation and increase target binding. Additionally, Silverman had noted that most pharmacologically active cyclic peptides contained at least one phenylalanine or modified Phe. This compound allowed for the further study of the effects on of a single D-amino acid in the structure as well as the effects of inclusion of a second aromatic moiety.

Figure 8. Rational design of SanA 3 (percent growth inhibition values at 5μM).
SanA 4 was also based on only one previous lead structure, SanA 8 (Figure 9). The D-Phe at position I of 8 was replaced with a D-tryptophan (D-Trp) in 4. With the inclusion of the D-Trp, the compound would maintain the single D-amino acid and single aromatic residue motif and include a polar site capable of hydrogen bonding and increasing aqueous solubility.

Figure 9. Rational design of SanA 4 (percent growth inhibition values at 5μM).

SanA 5 was designed as an amalgamation of two interesting leads, SanA 8 and 12 (Figure 10). The D-Phe at position I of 8 and the N-methyl D-Val at position III of 12 were combined in this new derivative. This new structure would explore synergistic effects and address two structural motifs: inclusion of two non-sequential D-amino acids and inclusion of an N-methyl in a D-amino acid.

Figure 10. Rational design of SanA 5 (percent growth inhibition values at 5μM).
Once synthesized and tested for cytotoxicity, **SanA 2** became a lead for the next
generation of derivatives. **SanA 6** was based on this new lead structure (**Figure 11**). The D-
Phe at position I and D-Leu at position V of 2 was maintained in 6. The N-methyl at position
IV of 2, though, is absent in 6. This compound was designed to investigate a new motif that
had been attributed to the potency of several derivatives, two sequential D-amino acids.

![Figure 11. Rational design of SanA 6 (percent growth inhibition values at 5μM).](image)

**SanA 7** was also designed to examine this new trend (**Figure 12**). The derivative was
designed from the leads, **SanA 13** and **14**. The D-Val at position III of 13 and D-Leu at
position IV of 14 were brought together in the new analogue. This compound was designed
as part of a series of derivatives where the two D-amino acid motif was rotated around the
entire macrocycle to fully understand the SAR of this trend.

**SANA RETROSYNTHESIS**

The synthesis of SanA derivatives was carried out via a convergent solution phase
synthetic strategy. The macrocycle was generated via head to tail peptide coupling of a
linear pentapeptide precursor. This linear pentapeptide was further broken down into two
smaller units, the tripeptide **Fragment 1** and dipeptide **Fragment 2** (**Figure 13**). These two
fragments were synthesized in organic solution from *tert*-Butyloxycarbonyl (Boc) and methyl
Figure 12. Rational design of SanA 7 (percent growth inhibition values at 5μM).

Figure 13. SanA amide retrosynthetic scheme.

ester (OMe) protected amino acids using commercially available coupling reagents and Hünig’s base (N,N-diisopropylethylamine, DIPEA). The convergent approach towards generation of the linear precursor involved fewer steps and was higher yielding when compared to a synthesis involving a stepwise linear approach. It also allowed for easy interchange of a wide variety of amino acids at any of the five positions around the macrocycle in order to generate a diverse library of derivatives.

The same general synthetic strategy was employed to construct all 6 of my derivatives. Amino acids were protected at the amino terminus using Boc and at the acid terminus using OMe. The coupling reagent mainly used for peptide bond formation was 2(1-H-benzotriazole-1-yl)-1,1,3-tetramethyluronium tetrafluoroborate (TBTU) (Figure 14). For
Figure 14. Structures of peptide coupling reagents.

Peptide couplings involving an N-methyl free amine the somewhat stronger coupling reagent 2-(H-7-azabenzotriazol-1-yl)-1,1,3,3,-tetramethyluronium hexafluorophosphate (HATU) was employed. For coupling reactions to generate the linear precursors or for macrocyclization, a cocktail of TBTU, HATU and 3-(Diethoxyphosphoryloxy)-1,2,3- benzotriazin-4(3H)-one (DEPBT) was used to help maximize yields. DEPBT was also employed to reduce the chance of epimerization of the amino acid side chains. Most peptide coupling reactions were carried out in the organic solvent dichloromethane (DCM); however, in cases of incomplete solubility of the starting materials, small amounts of acetonitrile (ACN) or dimethylformamide (DMF) were added to the reaction to dissolve all reagents. The Boc group on protected amines was removed for subsequent coupling using 20% 2,2,2-Trifluoroacetic acid (TFA) in DCM, and the methyl ester on protected acids was removed using lithium hydroxide (LiOH) or lithium hydroperoxide (LiO₂H). The amino acids used for the synthesis of my compounds were Boc or OMe protected and D or L (and sometimes N-methylated) versions of Phe, Leu, Val, and Trp. These standard coupling and deprotection procedures allowed for inexpensive and rapid syntheses of all linear precursors in good yields. Final, pure cyclized products were tested for their cytotoxicity in a number of cell lines including colon cancer lines HCT-116, HCT-15 and HT-29, and pancreatic cancer lines BxPC3 and PL-45.
SYNTHESIS OF FRAGMENT 1

The synthesis of SanA 2 started with the generation of the dipeptide MeO-D-Phe-Leu-NHBoc via our standard peptide coupling conditions using 1.1 equivalents of the free amine MeO-D-Phe-NH₂ and 1.0 equivalents of the free acid HO-Leu-NHBoc with 1.1 equivalents of the coupling reagent TBTU and 4 equivalents of DIPEA (Figure 15). The amino acids and coupling reagent were weighed into a dry round bottom flask, which was subsequently capped and purged with argon gas. The solid reagents were completely dissolved in dry DCM to a 0.1M concentration and then DIPEA was added. The reaction was run under argon gas at room temperature for one hour and monitored via TLC. Upon completion, the crude reaction solution was diluted in DCM and excess base and side products were extracted with two washes of 10% HCl and ten washes of saturated sodium bicarbonate (NaHCO₃). The organic solution was then dried over anhydrous sodium sulfate (Na₂SO₄), filtered and concentrated in vacuo. The pure dipeptide (MeO-D-Phe-Leu-NHBoc, 98.8% yield) was verified via ¹H NMR. The same procedure was followed for generation of the dipeptides for SanA 3 (MeO-Phe-D-Phe-NHBoc, 91.4% yield), SanA 4 (MeO-D-Trp-Leu-NHBoc, 92.8% yield), SanA 7 (MeO-Phe-Leu-NHBoc, 86.6% yield), and SanA 6 (Fragment 1 was the same as for 39).

Removal of the Boc group on the pure dipeptide MeO-D-Phe-Leu-NHBoc was performed via our standard amine deprotection reaction conditions (Figure 15). Under open atmosphere, the dipeptide was diluted to a 0.1 M concentration of 25% trifluoroacetic acid (TFA) and 75% DCM. Once the dipeptide was dissolved in DCM, 2 equivalents of anisole were added to the solution, followed by the addition of TFA. The reaction was run at room temperature for approximately one hour and was monitored via TLC every 15 minutes. Once complete, the solution was concentrated in vacuo to yield the free amine dipeptide (MeO-D-
Figure 15. Synthesis of fragment 1.

Conditions: (a) Free acid (1.0 eq.), Free amine (1.1 eq.), TBTU (1.1 eq.), DIPEA (4-8 eq.), dry DCM (0.1M)
(b) Anisole (2 eq.), TFA/DCM (1:3, 0.1M)
Phe-Leu-NH₂, for SanA 2 and 6) in quantitative yield. These conditions were also used to provide MeO-Phe-D-Phe-NH₂ (3), MeO-D-Trp-Leu-NH₂ (4), and MeO-Phe-Leu-NH₂ (7).

Peptide coupling followed to construct the tripeptide MeO-D-Phe-Leu-Val-NHBoc for SanA 2 and 6 using 1.1 equivalents of the free amine MeO-D-Phe-Leu-NH₂ and 1.0 equivalents of the free acid HO-Val-NHBoc with 1.1 equivalents of TBTU and 7 equivalents of DIPEA in dry DCM at a 0.1 M concentration (Figure 15). Upon completion, the crude tripeptide was purified via acid-base wash and flash column chromatography using an ethyl acetate (EA): hexane (Hex) gradient solvent system with the pure product eluting at 40:60 EA:Hex. The pure tripeptide (MeO-D-Phe-Leu-Val-NHBoc, 86.7% yield) was verified via ¹H NMR. The same procedure was followed for generation of the tripeptides for SanA 3 (MeO-Phe-D-Phe-Val-NHBoc, 94.3% yield), SanA 4 (MeO-D-Trp-Leu-Val-NHBoc, 70.8% yield), and SanA 7 (MeO-Phe-Leu-D-Val-NHBoc, 95.0% yield).

The final step for preparation of Fragment 1 was deprotection of the amine on MeO-D-Phe-Leu-Val-NHBoc via our standard amine deprotection reaction conditions (Figure 15). Under open atmosphere, the tripeptide was diluted to a 0.1 M concentration of 25% trifluoroacetic acid (TFA) and 75% DCM. Once complete, the solution was concentrated in vacuo to yield the free amine tripeptide (MeO-D-Phe-Leu-Val-NH₂, for SanA 2 and 6) in quantitative yield. These conditions were also used to provide MeO-Phe-D-Phe-Val-NH₂ (3), MeO-D-Trp-Leu-Val-NH₂ (4), and MeO-Phe-Leu-D-Val-NH₂ (7). This synthetic strategy was successful in generating the free amine tripeptide Fragment 1 for all of my derivatives in good yields. The synthesis of all SanA derivatives would continue with construction of Fragment 2.
SYNTHESIS OF FRAGMENT 2

Synthesis of Fragment 2 for SanA 2 began with generation of the dipeptide MeO-Leu-N-Me-d-Leu-NHBoc using 1.1 equivalents of the free amine MeO-Leu-N-Me-Boc and 1.0 equivalents of the free acid HO-D-Leu-NHBoc with 0.8 equivalents of TBTU, 0.4 equivalents of HATU and 8 equivalents of DIPEA in dry DCM at a 0.1 M concentration (Figure 16). As mentioned above, HATU was used due to coupling with an N-methyl free amine. Upon completion, the crude dipeptide was purified via acid-base wash. The pure dipeptide (MeO-Leu-N-Me-d-Leu-NHBoc, 81.9% yield) was verified via $^1$H NMR. The same procedure was followed for generation of the dipeptides for SanA 3 and 4 (MeO-Leu-Leu-NHBoc, 98.8% yield), SanA 6 (MeO-Leu-d-Leu-NHBoc, 92.2% yield), and SanA 7 (MeO-d-Leu-Leu-NHBoc, 98.8% yield).

Figure 16. Synthesis of fragment 2.
The pure dipeptide MeO-Leu-N-Me-D-Leu-NHBoc was then converted to the free acid employing our standard acid deprotection conditions (Figure 16). Under open atmosphere, the dipeptide was dissolved in methanol to a concentration of 0.1M followed by addition of 8 equivalents of LiOH. The reaction was allowed to run under open atmosphere at room temperature for two hours and monitored via TLC. Upon completion, the methanol was removed via rotary evaporator and the crude mixture was diluted in DCM. Excess reagents and impurities were extracted twice with 7% HCl wash. The organic solution was then dried over anhydrous Na₂SO₄, filtered and concentrated in vacuo. The pure, free acid dipeptide (HO-Leu-N-Me-D-Leu-NHBoc, 89.7% yield, for SanA 2) was verified via ¹H NMR. These conditions were also used to provide HO-Leu-Leu-NHBoc (99.9% yield, 3 & 4), HO-Leu-D-Leu-NHBoc (98.1% yield, 6), and HO-D-Leu-Leu-NHBoc (99.9% yield, 7). This synthetic strategy was successful in generating the free acid dipeptide Fragment 2 for all of my derivatives in good yields. The synthesis of all SanA derivatives would continue with peptide coupling to form the linear pentapeptide precursor.

**Synthesis of Linear Pentapeptides**

Peptide coupling of the free amine Fragment 1 and the free acid Fragment 2 was performed using the aforementioned cocktail of coupling reagents to generate the protected linear pentapeptide (PLP) precursors for all of my SanA derivatives. For SanA 2, 1.1 equivalents of Fragment 1 (MeO-D-Phe-Leu-Val-NH₂) and 1.0 equivalents of Fragment 2 (HO-Leu-N-Me-D-Leu-NHBoc) were combined with 0.5 equivalents of TBTU, 0.5 equivalents of HATU, 0.2 equivalents of DEPBT and 6 equivalents of DIPEA in dry DCM at a 0.1 M concentration (Figure 17). Upon completion, the crude PLP was purified via acid-base wash and flash column chromatography with the pure product eluting at 50:50
EA:Hex. The pure PLP (MeO-D-Phe-Leu-Val-Leu-N-Me-D-Leu-NHBoc, 87.0% yield) was verified via \(^1\)H NMR and Liquid Chromatography/Mass Spectroscopy (LC/MS). The same procedure was followed for generation of the PLPs for SanA 3 (MeO-Phe-D-Phe-Val-Leu-Leu-NHBoc, 62.5% yield), SanA 4 (MeO-D-Trp-Leu-Val-Leu-Leu-NHBoc, 45.2% yield), SanA 6 (MeO-D-Phe-Leu-Val-Leu-D-Leu-NHBoc, 23.4% yield), and SanA 7 (MeO-Phe-Leu-D-Val-D-Leu-Leu-NHBoc, 32.4% yield) (Figures 17 & 18). Some of the yields
reported here are lower than desired because generally multiple purifications were required for maximum yield and the amounts obtained after one purification were sufficient to obtain the desired quantity of final cyclized product.

**Deprotection of Protected Linear Pentapeptides**

Several methods were employed to deprotect the acid and amine termini of the PLPs in preparation for cyclization. The first method was a stepwise deprotection using our standard acid and amine deprotection conditions. First the methyl ester was saponified to the acid and the product isolated, followed by removal of the Boc group to deprotect the amine and yield the fully deprotected linear pentapeptide (DLP). The second method was an *in-situ* deprotection that removed the methyl ester and Boc group in a one-pot reaction. This
method, though it required more time, was employed with PLPs containing polar amino acids where avoiding the aqueous extractions for the acid deprotection reaction would be desirable. The final method was a stepwise deprotection similar to the first; however, the acid deprotection was performed with LiO₂H. Through the course of our research we found that these conditions were advised to preserve the stereochemistry of the amino acid side chains.⁵⁸

The PLPs for SanA 2, 3, 5 and 6 were deprotected stepwise using the first method. It should be noted that for SanA 5, the PLP was generated by a colleague. The site for cyclization was chosen between positions II and III due to the location of the N-methyl to avoid peptide coupling with a secondary amine to generate the PLP or cyclize. Otherwise, the final procedures followed were the same for all these derivatives. The PLP for SanA 4 was deprotected using the in-situ method because of the tryptophan moiety; and the PLP for SanA 7 was deprotected via the final method, using LiO₂H.

DEPROTECTION OF SANA 2, 3, 5, AND 6 PLP’S

The PLP for SanA 2 was first deprotected at the acid employing our standard acid deprotection conditions (Figure 19). Under open atmosphere, the PLP was dissolved in methanol to a concentration of 0.1M followed by addition of 8 equivalents of LiOH. Upon completion, the reaction was purified via acid wash. The pure, free acid linear pentapeptide (HO-D-Phe-Leu-Val-Leu-N-Me-D-Leu-NHBoc, 97.5% yield, for SanA 2) was verified via ¹H NMR. These conditions were also used to provide HO-Phe-D-Phe-Val-Leu-Leu-NHBoc (98.0% yield, 3), HO-D-Val-N-Me-Leu-Leu-D-Phe-Leu-NHBoc (99.6% yield, 5), and HO-D-Phe-Leu-Val-Leu-D-Leu-NHBoc (97.5% yield, 6) (Figures 19 & 20).
The Boc group on the free acid linear pentapeptide for SanA 2 (HO-D-Phe-Leu-Val-Leu-N-Me-d-Leu-NHBoc) was then removed via our standard amine deprotection reaction conditions (Figure 19). Under open atmosphere, the free acid linear pentapeptide was diluted to a 0.1 M concentration of 25% TFA and 75% DCM. Once complete, the solution was concentrated in vacuo to yield the double deprotected linear pentapeptide (HO-D-Phe-Leu-Val-Leu-N-Me-d-Leu-NHBoc).
Figure 20. Deprotection of PLP’s for SanA 6 and 7.

Val-Leu-$\text{N-Me-d-Leu-NH}_2$, for SanA 2) in quantitative yield. These conditions were also used to provide HO-Phe-D-Phe-Val-Leu-Leu-NH$_2$ (3), HO-D-Val-N-Me-Leu-Leu-D-Phe-Leu-NH$_2$ (5), and HO-D-Phe-Leu-Val-Leu-D-Leu-NH$_2$ (6). This method was successful in generating the DLP for SanA 2, 3, 5 and 6 in good yields, ready for final cyclization (Figures 19 & 20).
**DEPROTECTION OF SAN A 4 PLP**

The **PLP** for SanA 4, because of the presence of the Trp moiety, was simultaneously deprotected at both termini via the *in-situ* deprotection method (Figure 19). The **PLP** MeO-D-Trp-Leu-Val-Leu-Leu-NHBoc was dissolved in tetrahydrofuran (THF) to a concentration of 0.03 M, followed by the addition of 2.0 equivalents of anisole and 10 drops of concentrated HCl. Over the course of 4 days, an additional 3-4 drops of HCl were added per day (for a total of ~25 drops) and an additional 2.0 equivalents of anisole were added on day 3. The reaction was monitored via TLC and LC/MS and, once complete, the solution was concentrated *in vacuo* to yield the double deprotected linear pentapeptide (HO-D-Trp-Leu-Val-Leu-Leu-NH₂, for SanA 4) in quantitative yield.

**DEPROTECTION OF SAN A 7 PLP**

The **PLP** for SanA 7 was deprotected at the acid using our new modified acid deprotection conditions with LiO₂H (Figure 20). MeO-Phe-Leu-D-Val-D-Leu-Leu-NHBoc was dissolved in MeOH to a concentration of 0.4 M and then cooled to 0°C under open atmosphere. 3.4 equivalents of hydrogen peroxide (H₂O₂, 30% w/v) were added to the solution, followed by 3.0 equivalents of LiOH. The reaction was allowed to run under open atmosphere at 0°C for one hour and monitored via TLC. Upon completion, an aqueous solution containing 3.8 equivalents of sodium thiosulfate (Na₂S₂O₃) was added to the reaction solution to quench any remaining H₂O₂. HCl was added to the mixture to reach a pH of 1 and it was then extracted with DCM. The combined organic layers were then dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The pure, free acid linear pentapeptide (HO-Phe-Leu-D-Val-D-Leu-Leu-NHBoc, 82.9% yield, for SanA 7) was verified via ¹H NMR.
The Boc group on the free acid linear pentapeptide for **SanA 7** (HO-Phe-Leu-D-Val-D-Leu-Leu-NHBoc) was then removed via our standard amine deprotection reaction conditions (**Figure 20**). Under open atmosphere, the free acid linear pentapeptide was diluted to a 0.1 M concentration of 25% TFA and 75% DCM. Once complete, the solution was concentrated *in vacuo* to yield the double deprotected linear pentapeptide (HO-Phe-Leu-D-Val-D-Leu-Leu-NH₂, for **SanA 7**) in quantitative yield.

**Cyclization of Deprotected Linear Pentapeptides**

Macrocyclization was performed via peptide coupling of the free amine and the free acid termini of the **DLPs** using a cocktail of coupling reagents to generate the SanA derivatives (**Figures 21 & 22**). For **SanA 2**, the dry, pure **DLP** (HO-D-Phe-Leu-Val-Leu-N-Me-D-Leu-NH₂) was cyclized with 0.3 equivalents of TBTU, 0.5 equivalents of HATU, 0.5 equivalents of DEPBT and 6 equivalents of DIPEA in dry DCM and dry ACN (10:1) to a 0.007M concentration (**Figure 21**). Upon completion, the crude cyclized product was first purified *via* acid-base wash and flash column chromatography with the pure product eluting at 55:45 EA:Hex. Final purification was performed using reversed phase high performance liquid chromatography (HPLC) with a gradient of ACN and deionized (DI) water with 0.1 % TFA. The pure cyclized derivative (**SanA 2**, D-Phe-Leu-Val-Leu-N-Me-D-Leu, 3.8% yield) was verified via ¹H NMR, LC/MS and analytical reversed phase HPLC. The same procedure was followed for cyclization of the **DLPs** for **SanA 3** (Phe-D-Phe-Val-Leu-Leu, 8.8% yield), **SanA 4** (D-Trp-Leu-Val-Leu-Leu, 12.7% yield), **SanA 5** (D-Phe-Leu-D-Val-N-Me-Leu-Leu, 6.8% yield), **SanA 6** (D-Phe-Leu-Val-Leu-D-Leu, 7.5% yield), and **SanA 7** (Phe-Leu-D-Val-D-Leu-Leu, 4.5% yield) (**Figures 21 & 22**). The yields for the cyclization reaction are low because dimerization does occur to generate cyclized decapeptide products, TFA salts may
Figure 21. Cyclization of San A 2, 3 and 4.

Conditions: (a) TBTU (0.3 eq.), HATU (0.5 eq.), DEPBT (0.5 eq.), DIPEA (6-12 eq.), dry DCM:ACN 10:1 (0.007 M)
Conditions: (a) TBTU (0.5 eq.), HATU (0.7 eq.), DEPBT (0.5 eq.), DIPEA (8-12 eq.), dry DCM:ACN 10:1 (0.1-0.007 M)

Figure 22. Cyclization of SanA 5, 6 and 7.

hinder cyclization and only fully pure product, fit for bio testing, was included in the calculation.

**BIOLOGICAL ACTIVITY**

All of the SanA derivatives that I have synthesized were tested for biological activity in the pancreatic cancer cell line PL-45 and the colon cancer cell line HCT-116 (SanA 7 was only tested in PL-45). Cytotoxicity was determined by measuring growth inhibition in $^3$H-
thymidine incorporation assays (Figure 23). Cells were treated with compound diluted to 5 μM and cell proliferation was monitored by measuring the amount of $^3$H-thymidine incorporated into the cellular DNA. Lower thymidine incorporation is correlated to decrease in cell proliferation and greater cytotoxicity. It is important to note that some compounds chosen as lead structures have significantly higher % growth inhibition values in other cancer cell lines: the pancreatic cancer line BxPC-3 and the colon cancer lines HCT-15 and HT-29, which is why they were initially chosen as lead structures.

Figure 23. Biological activity of SanA derivatives and lead compounds.
SanA 2 was designed from SanA 8, 9 and 10. The derivative contained a D-Phe at position I, an N-methyl at position IV and a D-Leu at position V. It showed little improvement in cytotoxicity over its leads in HCT-116 but improved upon all three in PL-45. This shows that, though there isn’t a synergistic effect that results in a dramatic increase in toxicity, incorporation of multiple residues that contribute to potency can result in an improved derivative. This derivative also contributed to the trend of potent derivatives containing the motif of two consecutive D-amino acids with an N-methyl included, and was later considered a lead for further derivatives.

SanA 3 was based on SanA 11 and included a D-Phe at position II. Growth inhibition in PL-45 was unchanged but improved upon the lead in HCT-116. This result supported the hypothesis that a single D-amino acid increases potency but also demonstrated that the incorporation of the bulkier second Phe did not have a deleterious effect. This derivative supported the idea that multiple aromatics may be beneficial by providing multiple sources for binding to its protein target via π-stacking interactions.

SanA 4 investigated the effects of including an aromatic H-bond donor. Designed from SanA 8, the D-Phe was replaced by a D-Trp at position I. Though aqueous solubility may have been improved, cytotoxicity for 4 was lower than 8 in both cell lines. This may indicate that binding with its target directly involves the aromatic residue and that binding interaction is via nonpolar interactions.

SanA 5 included moieties from two leads, SanA 8 and 12. It was comprised of a D-Phe at position I and an N-methyl D-Val at position III. Growth inhibition data for PL-45 showed significant improvement over both leads. However activity in HCT-116 indicated the opposite trend and toxicity was reduced. This observation may be a result of relative
amounts of target and client proteins affected by compound binding. Nonetheless, this data supports the trend that compounds potent in at least one cell line contain the $N$-methyl incorporated into a D-amino acid motif.

**SanA 6** was designed from the second generation derivative **SanA 2**. The $N$-methyl at position IV found in the lead was omitted from the D-Phe at position I and D-Leu at position V. The compound has yet to be tested in HCT-116 but showed improvement over the lead in PL-45. This derivative, in addition to others, contributed to the trend that potent derivatives contain two consecutive D-amino acids. These results may also demonstrate that, in the presence of multiple D-amino acids, an $N$-methyl may actually restrict the molecule from adopting a favorable conformation to its target.

**SanA 7** was also designed to investigate the two consecutive D-amino acid trends. The D-Val at position III of **SanA 13** and D-Leu at position IV of **SanA 14** were incorporated in the new derivative. It showed no marked improvement over either lead in both cell lines and was significantly less potent than **14** in HCT-116. This data suggests that merely containing two consecutive D-amino acids does not guarantee potency. The effect of this motif must also be dependent on its location around the macrocycle.

**CONCLUSIONS**

The convergent solution phase synthetic strategy employed for the generation of my SanA derivatives was successful in efficiently and inexpensively synthesizing milligram quantities of pure compound for biological testing. The derivatives **SanA 2, 3, 4, 5, 6** and **7** were designed from potent lead structures to investigate the effects of including D, polar, aromatic and $N$-methylated amino acids in the cyclic pentapeptide scaffold. After I synthesized and purified each of the analogs, they were tested in various cancer cell lines.
including the pancreatic cancer cell lines PL-45 and BxPC-3, and the colon cancer cell lines HCT-116, HCT-15 and HT-29. These six compounds, as part of a library of nearly 100 derivatives synthesized by our research group, contributed to our understanding of the SAR of the Sansalvamide A macrocycle. From the very complicated SAR observed from our library, several trends begin to emerge. A number of potent derivatives contain two consecutive D-amino acids in their structure. Additionally, many possess an N-methyl incorporated in or adjacent to a D-amino acid. Several structures that exhibit high cytotoxicity also contain two or more nonpolar aromatic residues. Finally, in general, compounds containing polar elements are relatively inactive. It is clear that the location, arrangement and types of D-amino acids influence the overall conformation of the macrocycle for presentation of its proper binding elements to its protein target. Potent compounds may lock into a single conformation, perhaps mimicking beta or gamma turns, resulting in a well defined high affinity interaction.

Further studies are ongoing to develop more cytotoxic derivatives based on the most potent compounds in our library and including new peptidomimetic structures (i.e. oxazoles, thiazoles and triazoles) to explore new ways to impart rigidity to the structure. Mechanistic studies have identified the protein target of most of our SanA derivatives to be the chaperone protein Hsp90 and that they bind to it at it’s N-Middle domain. Additional studies are being conducted to determine the client proteins affected by Hsp90 binding with our most effective SanA derivatives. Computational docking studies are being performed to gain a better understanding of the binding interaction and to help design more potent compounds. The data from these additional studies will help to design an appropriate SanA derivative to
study in mice models and progress to the goal of developing a viable new anticancer therapeutic.
CHAPTER 3

SYNTHESIS AND BIOLOGICAL ACTIVITY OF DISANSAVAMIDE A DERIVATIVES

DISANSAVAMIDE A

Through the course of our work with the SanA project it became clear that the cyclization of the deprotected linear pentapeptide (DLP) of some of our SanA derivatives also yielded a dimerized decapptide side product (DiSanA). For some of the derivatives, the amount of decapptide produced was a function of the concentration at which the cyclization reaction was run (higher concentrations produced more decapptide), and, as a result, the cyclization of earlier derivatives was performed at very low concentrations (0.007 M) to avoid this side product. Other derivatives seemed to exhibit a “preference” of pentapeptide or decapptide cyclized product and would yield only one or the other regardless of the concentration. Some structures would yield a high ratio of decapptide to pentapeptide product even at very low concentrations, and some would yield primarily pentapeptide product even at very high concentrations (0.1 M). There have been several examples of peptide based drugs of similar size/molecular weight effectively used to treat disease: cyclosporin A, vancomycin and aplidine, to name a few; and the decapptides were based on the SanA framework that had demonstrated cytotoxic activity.25-29,59 Therefore, it was decided to isolate the pure decapptide derivatives, when generated, and test them for cytotoxicity in several colon and pancreatic cancer cell lines.

The bio testing of the first few DiSanA derivatives produced some very promising results (Figure 24). Three of the four decapptide derivatives tested proved to be more
Figure 24. Comparison of pentapeptide and decapeptide derivatives in PL-45.

cytotoxic in PL-45 than their pentapeptide counterparts.\textsuperscript{60} In particular, \textbf{DiSanA 1015} demonstrated excellent growth inhibition in the assay (99%), whereas its pentapeptide derivative, \textbf{SanA 15}, showed only moderate activity (30%). As a result, it was decided to attempt to generate both the pentapeptide and decapeptide cyclization products for subsequent SanA derivatives.
SYNTHESIS OF DiSANA DERIVATIVES 1005, 1006, AND 1007

My contribution to this new project based on the SanA scaffold was the synthesis of three decapeptide DiSanA derivatives: 1005, 1006 and 1007 (Figure 25). The synthesis of these compounds followed the same synthetic strategy used for the synthesis of the SanA pentapeptide derivatives with the exception of the concentration at which the final cyclization reaction was run. In order to obtain both products, the cyclization of the derivatives’ DLPs was conducted at concentrations higher than our usual 0.007 M. The cyclization of 5/1005 was conducted at 0.01 M; and the cyclizations of 6/1006 and 7/1007 were performed at 0.1 M. This altered set of conditions was successful in generating both the cyclized pentapeptide and decapeptide products for all three of these derivatives. Upon completion of the reaction, the SanA and DiSanA products would remain together during the initial purification via flash column chromatography and be separated in the final purification via reversed phase HPLC. The final, pure DiSanA derivatives 1005, 1006 and 1007 were tested for cytotoxicity in PL-45 and HCT-116. These compounds, along with over 20 additional DiSanA derivatives synthesized in our lab, can help us begin to understand the SAR of this new series and progress towards its utilization as an anticancer therapeutic.35

MACROCYCLIZATION PROCEDURE
Cyclization of the DLP for DiSanA 1005 was performed using our standard peptide coupling conditions using the cyclization cocktail of coupling reagents at 0.01 M (Figure 26). The dry, pure DLP (HO-D-Val-N-Me-Leu-Leu-D-Phe-Leu-NH₂) was cyclized with 0.5 equivalents of TBTU, 0.7 equivalents of HATU, 0.5 equivalents of DEPBT and 8 equivalents of DIPEA. Upon completion, the crude cyclized products, SanA 5 and DiSanA 1005, were first purified via acid-base wash and flash column chromatography with the products eluting
Figure 25. DiSanA derivatives synthesized.

at 55:45 EA:Hex. Final purification and separation of the two products was performed using reversed phase HPLC with a gradient of ACN and deionized (DI) water with 0.1 % TFA. The pure cyclized decapeptide derivative (DiSanA 1005, D-Phe-Leu-D-Val-N-Me-Leu-Leu-D-Phe-Leu-D-Val-N-Me-Leu-Leu) was verified via $^1$H NMR, LC/MS and analytical reversed phase HPLC. The same procedure, except run at 0.1 M, was followed for cyclization of the DLPs for DiSanA 1006 (D-Phe-Leu-Val-Leu-D-Leu-D-Phe-Leu-Val-Leu-D-Leu), and DiSanA 1007 (Phe-Leu-D-Val-D-Leu-Leu-Phe-Leu-D-Val-D-Leu-Leu) (Figure 26). The final pure pentapeptide and decapeptide products were generally produced in a 10:1 ratio and only fully pure products, fit for biological testing, were included in the overall yield calculation.
Figure 26. Macrocyclization of DiSanA 1005, 1006 and 1007.

Conditions: (a) TBTU (0.5 eq.), HATU (0.7 eq.), DEPBT (0.5 eq.), DIPEA (8-12 eq.), dry DCM:ACN 10:1 (0.1-0.01 M)
**BIOLOGICAL ACTIVITY**

The three DiSanA derivatives that I produced were tested for cytotoxicity by measuring growth inhibition in the same $^3$H-thymidine incorporation assays used to test our SanA derivatives. **DiSanA 1005, 1006 and 1007** were tested in PL-45 (Figure 27); **1006** and **1007** were also tested in HCT-116. Both derivatives tested in HCT-116 showed no activity (0% growth inhibition) in the assay. These two demonstrated poor toxicity in the pancreatic cancer cell line as well. Though they both share a degree of structural homology with our potent lead **1015** (two consecutive D-amino acids), this motif clearly does not ensure a potent derivative. The only one of my derivatives with promising activity was **DiSanA 1005**; however its growth inhibition still fell far short of our lead **1015**. Additionally, all three of my derivatives contradicted the trend that the decapeptide macrocycles are more cytotoxic than their corresponding pentapeptide derivatives. **DiSanA 1005** and **1007** showed little difference with their SanA counterpart and **DiSanA 1006** was considerably weaker. This data, along with inhibition data from the other derivatives in the library, indicates that the potent DiSanA derivatives inhibit tumor growth via a mechanistic pathway different than that of the potent SanA derivatives.

**DiSanA Conclusions**

The modified macrocyclization conditions used to cyclize the DLPs generated via our convergent solution phase strategy were successful in generating both the SanA pentapeptide and DiSanA decapeptide derivatives in one pot. By increasing the concentration of the final cyclization reaction I was able to generate, isolate and purify **DiSanA 1005, 1006 and 1007** in sufficient quantities for bio testing, in addition to ample quantities of their corresponding SanA derivatives. Data from the SAR gathered from our library is inconclusive so far, as no
Figure 27. Cytotoxicity of DiSanA derivatives 1005, 1006 and 1007.

Strong trends emerge about the placement of D-amino acids or N-methyls around the macrocycle. However, when compared to their SanA counterparts, the most potent DiSanA derivatives appear to operate via a different inhibitory pathway. This theory was also confirmed in recently published work by our lab that made use of a biotinylated tagged version of our most potent lead, 1015. In a pull down assay, it was found that the DiSanA derivative bound to Hsp90, but at a different location than the pentapeptide derivatives, at the C-Middle domains. Further studies are ongoing to develop more potent derivatives and to further understand the mechanism of action of our most potent lead as an Hsp90 inhibitor.
CHAPTER 4

SYNTHESIS OF Urukthapelstatin A

MACROCYCLE RETROSYNTHESIS

To develop an efficient synthetic scheme for Urukthapelstatin A (UstatA), the molecule was analyzed for key structural elements and current research into the development of similar compounds was investigated. As a peptide-like macrocycle, it was determined that UstatA could be synthesized in a convergent, solution phase approach as a linear precursor, modified from commercially available amino acids and other starting materials, and cyclized in a final macrocyclization reaction. It became clear that the important elements of the molecule and those whose synthesis would be the most challenging to address were the three oxazoles, two thiazoles, and the site of macrocyclization. The oxazoles could be generated from Serine (Ser) peptide precursors under a variety of conditions reported in literature including cyclodehydration/oxidation using Diethyliaminosulfur trifluoride (DAST) and Diaza(1,3)bicyclo[5.4.0]undecane (DBU), a Swern oxidation and then triphenylphosphine, or a Hantzsch reaction with a β-unsaturated amide and α-halo ketone. The thiazoles, it was determined, could be efficiently synthesized performing the aforementioned Hantzsch reaction between a thioamide and an α-halo ketone. With these synthesis options and our experience with peptide chemistry in mind, the location for final cyclization was investigated.

From our experience with San A, the initial consideration was to cyclize with a peptide coupling reaction at one of the three peptide bond locations around the alanine (Ala) or isoleucine (Ile) residues (Figure 28). However, after thorough review of recent literature,
Figure 28. Urukthapelstatin A retrosynthesis.

It had been demonstrated that attempts to cyclize very similar structures with a peptide coupling proved to be exceedingly difficult, often yielding no desired product regardless of the choice of coupling reagents. The next consideration, was to perform the cyclization with a Hantzsch thiazole synthesis reaction. This technique had been reported successfully in the synthesis of Merchercharmycin A and the thiazole adjacent to the phenyl-oxazole appeared to be the optimal site. The linear precursor for cyclization at the thiazole, could be generated via peptide coupling of two tetrapeptide-like precursors (fragments 1 and 2) containing the majority of the structural elements of the final macrocycle (Figure 28).

**Fragment 1 Retrosynthesis**

The synthesis of 1 would have to involve the elucidation of several key structural elements (Figure 29). The thioamide necessary for the Hantzsch thiazole synthesis reaction could be easily generated from a methyl or ethyl ester using ammonium hydroxide and then Lawesson’s reagent. With this in mind, the first thiazole could be generated by modifying the C-terminus of a di-oxazole containing peptide-like precursor into the requisite thioamide and performing the Hantzsch reaction with ethyl bromopyruvate, 2,2,2-
Trifluoroacetic anhydride (TFAA), and 2,6-lutidine. Additionally, the resulting ethyl ester at the C-terminus could be easily modified into the thioamide for the cyclization step.

Literature review indicated that a sequential cyclodehydration/oxidation of Ser containing peptides using DAST and DBU respectively could be employed to form the two oxazole moieties. A tripeptide with two consecutive C-terminal serines would provide the template. The amino acid Threonine (Thr) would complete the tripeptide and serve as a precursor to the exocyclic propylidene moiety of the final UstatA structure, in addition to providing the free amine for the peptide coupling to form the linear precursor. This tripeptide precursor could be efficiently generated from commercially available Boc protected threonine (Boc-Thr-OH) and serine methyl ester (H₂N-Ser-OMe) using standard peptide coupling and deprotection conditions well established from experience with the San A project. This retrosynthetic approach should result in synthesis of 1 in reasonably high yields from the readily available starting materials ethyl bromopyruvate, Boc-Thr-OH, and H₂N-Ser-OMe using common, commercially available reagents and solvents.
FRAGMENT 2 RETROSYNTHESIS

The synthesis of 2, it had appeared, could be performed in a more convergent and less step-wise fashion relative to 1 (Figure 30). The fragment could be broken up into two dimer subunits via a peptide bond: the first being methyl ester protected dipeptide H$_2$N-Ile-Ala-OMe and the second made up of the phenyl-oxazole moiety flanked by the free acid and a dimethyl ether protected α-halo ketone. This α-bromo ketone would be deprotected immediately prior to cyclization via the Hantzsch reaction. The dipeptide could be generated using standard peptide coupling and deprotection conditions from our lab and would provide the free acid, upon deprotection, for the peptide coupling to produce the linear precursor.

The phenyl-oxazole subunit could be generated from a β-hydroxy phenylalanine dipeptide-like precursor employing similar conditions to the oxazole formation used for 1 (DAST then base). This precursor would be produced by peptide coupling commercially available β-hydroxy phenylalanine methyl ester (H$_2$N-β-OH-Phe-OMe) and bromopyruvic acid dimethyl acetal. This protected form of the α-bromo ketone had been demonstrated in literature to be easily made by protecting the ketone of commercially available ethyl bromopyruvate via dimethyl acetal using sulfuric acid (H$_2$SO$_4$) and methyl orthoformate (CH(OMe)$_3$) at reflux and deprotecting the acid using our standard acid deprotection conditions (LiOH, rt). It was believed that this retrosynthetic route would successfully generate fragment 2 in reasonably high yields using commercially available reagents and solvents from the commercially available starting materials ethyl bromopyruvate, H$_2$N-β-OH-Phe-OMe, Boc-Ile-OH, and H$_2$N-Ala-OMe.
Figure 30. Fragment 2 retrosynthesis.

SYNTHESIS OF FRAGMENT 1

The initial synthetic strategy devised for 1 was designed to synthesize the fragment in high yields with the fewest possible steps to elucidate the key structural elements (Figure 31). With this in mind, it was decided to form both oxazoles in the fragment simultaneously. Research indicated that this route would be possible and reduce the number of overall steps necessary, relative to sequential oxazole formation. A tripeptide precursor (4) would be generated using Boc protected threonine with a tert-butyl (t-Bu) protecting group on the threonine alcohol (Boc-Thr(t-Bu)-OH) and serine methyl ester with a benzyl (Bn) protecting group on the serine alcohol (H2N-Ser(Bn)-OMe) employing standard peptide coupling conditions from our lab. The t-Bu was chosen for its stability under basic and mildly acidic conditions, which the molecule would be subjected to in subsequent reactions. The Bn was chosen to maximize the yields of the peptide coupling reactions with Ser by eliminating the possibility of the competing ester-forming reaction between a free alcohol of serine and an activated acid. Additionally, it was chosen for the relative ease of its removal via hydrogenation.

Construction of the tripeptide precursor 4 began with the synthesis of the dipeptide Boc-Thr(t-Bu)-Ser(Bn)-OMe using the free amine H2N-Ser(Bn)-OMe and the free acid Boc-
Figure 31. Initial synthetic strategy for fragment 1 dioxazole precursor.

Thr(t-Bu)-OH with TBTU and DIPEA in dry DCM (Figure 31). Upon completion, the crude dipeptide was purified via acid-base wash to yield the dipeptide in 99.9% yield.

The pure dipeptide was then subjected to the acid deprotection reaction using LiOH (Figure 31). Upon completion, the crude product was purified via acid wash to yield 3 (Boc-Thr(t-Bu)-Ser(Bn)-OH) in 96% yield.

The tripeptide Boc-Thr(t-Bu)-Ser(Bn)-Ser(Bn)-OMe was then generated using the free amine H2N-Ser(Bn)-OMe and the free acid dipeptide 3 with TBTU and DIPEA in dry DCM (Figure 31). Upon completion, the crude tripeptide was purified via acid-base wash and flash column chromatography with the pure product eluting at 40:60 EA:Hex to yield the tripeptide in 88.2% yield.

In order to remove the benzyl ether protecting groups from both of the serine alcohols, the tripeptide Boc-Thr(t-Bu)-Ser(Bn)-Ser(Bn)-OMe was subjected to a palladium
catalyzed hydrogenation reaction (**Figure 31**). The pure tripeptide was dissolved in ethanol (EtOH) with 10% palladium on carbon and hydrogen gas was bubbled through the solution. Upon completion, the reaction solution was filtered through celite to remove the catalyst and concentrated *in vacuo* to give the pure deprotected tripeptide 4 (Boc-Thr(t-Bu)-Ser-Ser-OMe) in 99% yield.

With the free serine tripeptide 4 in hand, the simultaneous formation of both oxazoles was attempted. Research indicated that one of the most common and high yielding techniques used to generate oxazoles on even highly functionalized substrates was a two step conversion from a β-hydroxy amide using DAST and DBU. DAST, a well known fluorinating agent, is used in the first step at low temperatures to replace the alcohol of serine with fluorine. A base is then introduced to the reaction to complete the cyclodehydration resulting in an oxazoline. It is important to note that research indicated the selection of the base was integral, depending on the specific structure of the β-hydroxy amide substrate, in reducing the amount of side product generated as a result of elimination across the α and β carbons. For the second step, the unpurified oxazoline would then undergo an elimination using bromo-trichloromethane (BrCCl₃) and DBU at low temperatures to yield the final oxazole.

The tripeptide 4 was dissolved in cold, dry tetrahydrofuran (THF) and DAST was added dropwise (**Figure 31**). The reaction was stirred at -78°C for two hours followed by the addition of K₂CO₃ to the reaction solution. Upon completion, the crude dioxazoline 5 was extracted *via* base wash, concentrated *in vacuo* and taken on to the next step without further purification. Completion was confirmed by 

^1\text{H} NMR via verification of oxazoline protons and absence of fluorinated product via 

^1\text{H} NMR.
The crude dioxazoline 5 was immediately carried on to the elimination step. It was dissolved in a 1:1 mixture of dry ACN and dry pyridine and cooled to -18°C. BrCCl₃, followed by DBU, was then added dropwise. The reaction solution was allowed to warm to room temperature and run under argon gas for 48 hours. Upon completion, the crude dioxazole was purified via acid-base wash and flash column chromatography with the pure product eluting at 35:65 EA:Hex to yield 6 in 30.0% yield.

This synthetic strategy proved successful in generating the desired dioxazole product 6. However, upon purification, it became clear that this initial attempt had resulted in an relatively low overall yield (8.8%). Several more attempts were made at following this synthetic path and simultaneously generating both oxazoles. The DAST/DBU procedure was repeated on 4 several times. Each time, conditions were altered slightly in the attempt to increase the overall yield. The amount of reagents used for both reactions were varied slightly; in addition, both reactions were attempted in various different organic solvents. Different bases were employed in the final step of the DAST reaction, including pyridine, Na₂CO₃ and NaHCO₃. The oxazoline product from the DAST reaction was purified before taking on to the DBU reaction. The order in which the DBU and BrCCl₃ were added in the second reaction was also investigated. All attempts, though, resulted in poor yields with the maximum achieved yield of 30%. It became clear that a reinvestigation of the synthetic strategy was necessary.

It was concluded that the failure of the first synthetic strategy for the dioxazole precursor for fragment 1 was due to the difficulty in the simultaneous formation of both oxazoles. With this in mind, a new strategy was developed to generate each oxazole individually in a stepwise fashion (Figure 32). The dipeptide Boc-Thr(t-Bu)-Ser-OMe (7),
after coupling and hydrogenation, would be subjected to the DAST/DBU protocol to generate the first oxazole. Acid deprotection, coupling and hydrogenation would yield the trimer 10. This substrate would then be converted into the desired dioxazole precursor, again using DAST and DBU. It was believed that this revised strategy, though it had more steps and would inevitably be more time consuming, could improve upon the unacceptably poor yields of the previous synthesis.

The dipeptide Boc-Thr(t-Bu)-Ser(Bn)-OMe was coupled using the free amine H2N-Ser(Bn)-OMe and the free acid Boc-Thr(t-Bu)-OH with TBTU and DIPEA in dry DCM (Figure 32). Upon completion, the crude product was purified via acid-base wash and flash column chromatography with the pure product eluting at 40:60 EA:Hex to yield the dipeptide in 94.6% yield.
The benzyl ether protecting group on the serine was then removed via palladium catalyzed hydrogenation (Figure 32). The pure dipeptide Boc-Thr(t-Bu)-Ser(Bn)-OMe was dissolved in EtOH with 10% palladium on carbon and hydrogen gas was bubbled through the solution. Upon completion, the reaction solution was filtered through celite to remove the catalyst and concentrated in vacuo to give the pure free alcohol dipeptide 7 (Boc-Thr(t-Bu)-Ser-OMe) in 97.2% yield.

The dipeptide 7 was dissolved in cold, dry DCM and DAST was added dropwise (Figure 32). The reaction was stirred at -78°C for one hour followed by the addition of K$_2$CO$_3$ to the reaction solution. Upon completion, the crude oxazoline was extracted via base wash, concentrated in vacuo and taken on to the next step without further purification.

The crude oxazoline was again carried on without purification to the elimination step (Figure 32). It was dissolved in dry DCM and cooled to -18°C. DBU, followed by BrCCl$_3$, was then added dropwise. The reaction solution was allowed to warm to room temperature and run under argon gas for 48 hours. Upon completion, the crude oxazole was purified via acid-base wash and flash column chromatography with the pure product eluting at 20:80 EA:Hex to yield 8 in 77.5% yield over the two steps.

The oxazole precursor 8 was deprotected to the free acid using LiOH (Figure 32). Upon completion, the crude product was purified via acid wash to yield 9 in 99.5% yield.

In preparation for formation of the second oxazole, the second serine was coupled to 9 using the free amine H$_2$N-Ser(Bn)-OMe with TBTU, HATU and DIPEA in dry DCM (Figure 32). Upon completion, the crude product was purified via acid-base wash and flash column chromatography with the pure product eluting at 35:65 EA:Hex in 95.3% yield.
The benzyl ether protecting group on the second serine was then removed via palladium catalyzed hydrogenation (Figure 32). The pure precursor was dissolved in EtOH with 10% palladium on carbon and hydrogen gas was bubbled through the solution. Upon completion, the reaction solution was filtered through celite to remove the catalyst and concentrated \textit{in vacuo} to give the pure free alcohol trimer 10 in 99.7% yield.

To begin generation of the second oxazole 10 was dissolved in cold, dry DCM and DAST was added dropwise (Figure 32). The reaction was stirred at -78°C for one hour followed by the addition of K$_2$CO$_3$ to the reaction solution. Upon completion, the crude oxazoline was extracted via base wash, concentrated \textit{in vacuo} and taken on to the next step without further purification.

The crude oxazoline was again carried on without purification to the elimination step (Figure 32). It was dissolved in dry DCM and cooled to -18°C. DBU, followed by BrCCl$_3$, was then added dropwise. The reaction solution was allowed to warm to room temperature and run under argon gas for 48 hours. Upon completion, the crude dioxazole was purified \textit{via} acid-base wash and flash column chromatography with the pure product eluting at 35:65 EA:Hex to give 6 in 75.6% yield in two steps.

This revised synthetic strategy also proved successful in generating the desired dioxazole product 6. In addition, the revised route also was successful in improving upon the poor overall yield of the original synthesis. Whereas the first synthesis produced the dioxazole in 25.4% overall yield over 6 steps, the revised strategy doubled the overall yield to 50.9% in 9 steps. With the dioxazole containing precursor for fragment 1 generated efficiently and in good yields, the synthesis would continue with the generation of the first thiazole moiety.
The synthesis of the first thiazole would have to be carried out with a sequence of three transformations. The terminal methyl ester of 6 first had to be converted into an amide using NH₄OH and then into a thioamide using Lawesson’s reagent. The thioamide along with an α-halo ketone provide the prerequisites for the Hantzsch thiazole synthesis reaction. Performing this reaction with the newly formed thioamide of the precursor and bromopyruvic ethyl ester (BPEE), carried out in basic conditions at -18°C, would generate the necessary thiazole moiety in two steps. In the first step the thioamide and BPEE with KHCO₃ result in a hydroxy-thiazoline and in the second step TFAA activates the alcohol for lutidine to effect an elimination into the thiazole. Additionally, using BPEE would yield a terminal ethyl ester which could again be easily modified to a thioamide.

The dioxazole precursor 6 was converted to the amide using a solution of MeOH and 25% NH₄OH in a 1:9 ratio (Figure 33). The precursor was dissolved in methanol, followed by the addition of NH₄OH. The suspension was placed in the ultrasound bath until homogeneous. Upon completion, the solvents were removed in vacuo affording the pure amide in 99% yield.

Figure 33. Final synthesis steps for Fragment 1.

Conditions: (a) NH₄OH/MeOH (9:1, 0.025 M), ultrasound, rt (b) Lawesson’s reagent (1.1 eq.), dry THF (0.07M), reflux (c) BPEE (3 eq.), KHCO₃ (8 eq.), dry DCM (0.4M), -18°C (d) TFAA (4 eq.), 2,6-lutidine (9 eq.), dry DCM (0.25M), -18°C
The pure amide was then converted into the thioamide using Lawesson's reagent (LR). The amide and LR were dissolved in dry THF and the solution was heated to reflux (Figure 33). After verification of completion, the residue was purified via flash column chromatography with the pure product 11 eluting at 55:45 EA:Hex in 99.1% yield.

The pure dioxazole thioamide 11 was then carried on to form the first thiazole via the two-step Hantzsch reaction (Figure 33). For the first step, the thioamide and potassium bicarbonate (KHCO₃) were dissolved in dry DCM, cooled to -18°C, and then BPEE was added dropwise. Upon completion, the solution was filtered through celite and concentrated in vacuo yielding a crude residue that was immediately carried on to the second step. The crude residue was dissolved in dry DCM, cooled to -18°C, followed by the addition of TFAA and 2,6-lutidine. Upon completion, the crude thiazole product was purified via flash column chromatography with the pure product eluting at 55:45 EA:Hex to yield 12 in 76.7% yield.

The final steps to prepare this dioxazole thiazole containing tetramer precursor into 1 were the straightforward conversion of the ethyl ester into another thioamide. 12 was converted to the amide using a solution of MeOH and 25% NH₄OH (Figure 33). The starting material was dissolved in methanol, followed by the addition of NH₄OH. The suspension was placed in the ultrasound bath until homogeneous. Upon completion, the solvents were removed in vacuo providing the pure amide in 99% yield.

The pure amide was converted into the thioamide using LR. The amide and LR were dissolved in dry THF and the solution was heated to reflux (Figure 33). After verification of completion, the residue was purified via flash column chromatography with the pure product 1 (Boc protected) eluting at 60:40 EA:Hex in 63.1% yield.
This synthetic strategy proved successful in generating Boc protected 1 in good yield (Figures 32 & 33). The removal of the Boc group at the threonine terminus would yield the free amine necessary for subsequent coupling to fragment 2 to form the linear precursor. Additionally, the other terminus of the fragment was comprised of the thioamide necessary for cyclization via the Hantzsch reaction.

**SYNTHESIS OF FRAGMENT 2**

The synthetic strategy for fragment 2, as mentioned above, would be conducted in a more convergent fashion. The dipeptide Boc-D-allo-Ile-Ala-OH (Figures 30) would be coupled and acid deprotected using standard peptide conditions. The phenyloxazole dimer would be generated first by modifying bromopyruvic acid (BPA) and coupling it to the amino acid β-hydroxy phenylalanine methyl ester (NH2-β-OH-Phe-OMe). The coupling would result in the prerequisite β-hydroxy amide for the DAST/DBU protocol to generate the phenyloxazole. One important consideration in this step is the heightened possibility of an elimination side product during the DAST reaction due to the benzene ring attached to the β carbon. Research indicated that the choice of base for this reaction would greatly affect the ratio of cyclodehydration to elimination product and thus, pyridine would be used instead of the K2CO3 used in the DAST reactions for 1. Once the phenyloxazole was formed, the dimer would be deprotected at the acid and coupled to the dipeptide H2N-D-allo-Ile-Ala-OMe using standard conditions. This resulting tetramer would then require a final OMe removal at the acid of Ile to couple to 1 yielding the linear precursor for Ustat A.

The synthesis of 2 would start with the protection of the α-bromo ketone of bromopyruvic acid (BPA). It is important to note that in this reaction, in addition to protecting the ketone with a dimethyl acetal, the carboxylic acid is also methylated to form
the methyl ester. BPA was refluxed in dry MeOH with trimethyl orthoformate (CH(OMe)₃) and sulfuric acid (H₂SO₄) (Figure 34). When complete, base wash furnished the pure product (methyl bromopyruvate dimethyl acetal, BrCH₂C(OMe)₂CO₂Me) in 72.4% yield.

**Figure 34.** Synthesis of phenyloxazole precursor for Fragment 2.

The resulting methyl ester was saponified to the acid via standard acid deprotection conditions (Figure 34). The crude product was purified via acid wash to yield 13 in 99.5% yield.

The peptide coupling reaction followed using the free amine NH₂-β-OH-Phe-OMe and the free acid 13 with TBTU, HATU, DEPBT and DIPEA in dry DMF (Figure 34). The cocktail of coupling reagents was employed to ensure maximum peptide coupling and reduce the amount of ester side product. Upon completion, the crude product was purified via acid-base wash and flash column chromatography with pure 14 eluting at 55:45 EA:Hex in 61.6% yield.

With the β-hydroxy amide necessary for oxazole formation in hand, 14 was subjected to the DAST/DBU protocol. The starting material was dissolved in cold, dry THF and DAST was added dropwise (Figure 34). The reaction was stirred at -78°C for one hour followed by the addition of pyridine to the reaction solution. Upon completion, the crude
phenyloxazoline was purified via base wash and flash column chromatography with the pure product eluting at 40:60 EA:Hex in 78.4% yield.

The pure phenyloxazoline was dissolved in dry DCM and cooled to -18°C (Figure 34). BrCCl₃, followed by DBU, was then added dropwise. The reaction solution was allowed to warm to room temperature and run under argon gas for 48 hours. Upon completion, the crude phenyloxazole was purified via acid-base wash and flash column chromatography with the pure product eluting at 25:75 EA:Hex in 61.6% yield over the two steps.

The final step for preparation of the phenyloxazole dimer 15 was saponification to the free acid using LiOH (Figure 34). The crude product was purified via acid wash to yield 15 in 90.2% yield.

Final synthesis of fragment 2 began with the coupling of the dipeptide Boc-d-allo-Ile-Ala-OMe using the free amine H₂N-Ala-OMe·HCl and the free acid Boc-d-allo-Ile-OH with TBTU, HATU and DIPEA in dry DCM (Figure 35). Upon completion, the crude product was purified via acid-base wash to yield the dipeptide Boc-d-allo-Ile-Ala-OMe in 99.5% yield.

![Figure 35. Final synthesis steps for Fragment 2.](image-url)
In preparation for coupling to the free acid phenyloxazole dimer, the pure dipeptide Boc-D-allo-Ile-Ala-OMe underwent an amine deprotection reaction using standard conditions (Figure 35). Under open atmosphere, the dipeptide was diluted to a 0.1 M concentration of 25% TFA and 75% DCM. Once complete, the solution was concentrated in vacuo to yield the free amine tripeptide 16 in quantitative yield.

The fragment 2 methyl ester was generated using the free acid 15 and the free amine 16 with TBTU, HATU, DEPBT and DIPEA in dry DCM (Figure 35). Upon completion, the crude product was purified via acid-base wash and flash column chromatography with the pure product eluting at 45:55 EA:Hex to yield 2 in 88.8% yield.

Synthesis of Linear Precursor

With both fragments generated in good yields, the final acid and amine deprotection steps were necessary to prepare for peptide coupling to generate the linear precursor. It was believed that standard peptide coupling and deprotection conditions employed by our lab would be sufficient. The methyl ester of fragment 2 would be removed using lithium hydroperoxide (to prevent epimerization at the peptide α carbons). The Boc group on the amine of fragment 1 would be removed using 25% TFA in DCM; it was believed that these conditions were not highly acidic enough to result in removal of the tert-butyl ether protecting group on the threonine residue. After synthesis of the linear precursor, the dimethyl acetal protecting the α-bromo ketone would be removed via refluxing formic acid. Additionally, these conditions should prove acidic enough to result simultaneously in removal of the threonine tert-butyl ether. The final reaction necessary would be the hantzsch thiazole synthesis reaction. According to our research, with the terminal ends of the linear precursor consisting of a thioamide and α-bromo ketone, in addition to a free alcohol
on the threonine residue, the hantzsch conditions would cyclize the molecule via generation
of the second thiazole moiety and simultaneously generate the exocyclic propylidene moiety
via elimination of the activated threonine alcohol.\textsuperscript{47,49}

The first reaction performed was the saponification of the methyl ester of 2 using
lithium hydroperoxide (Figure 36). 2 (methyl ester) was dissolved in MeOH at 0\textdegree C with
H\textsubscript{2}O\textsubscript{2} and LiOH. Upon completion, the reaction was quenched with Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3} and purified \textit{via}
acid wash to give 2 in 99.7\% yield.

\textbf{Figure 36. Linear precursor via peptide coupling.}

The next step was removal of the Boc group on 1 using our standard amine
deprotection conditions (Figure 36). At room temperature, Boc protected 1 was diluted to a
0.1 M concentration of 25% TFA and 75% DCM. Once complete, the solution was concentrated in vacuo to yield 1 in quantitative yield. However, verification of the final structure via $^1$H NMR indicated that the $t$-Butyl ether protecting group on the Thr residue was removed from approximately 66% of the final product. The deprotection reaction was rerun following the same procedures at 0°C and using only 15% TFA in DCM but partial removal of the $t$-Bu was unavoidable.

In response to this, new strategies for removal of the Boc group from 1 were investigated. Literature review indicated that employing 4 M HCl in dry dioxane had proved successful in selective removal of Boc in the presence of $t$-Bu ethers$^{71}$ and these conditions were attempted. Under argon gas, 4 M HCl in dry dioxane was cooled to 0°C. Boc protected 1 was dissolved to a 0.05 M concentration of the cold HCl solution. The reaction was allowed to warm to room temperature, run for approximately one hour and was monitored via TLC and LC/MS every 30 minutes. Once complete, the solution was concentrated in vacuo to yield the free amine in quantitative yield. Verification of the final structure via $^1$H NMR indicated that the $t$-Bu was still removed from approximately 40% of the final product. After several additional attempts yielded similar results, it was concluded that the proximity of the Boc to the $t$-Bu on a secondary alcohol hindered selective deprotection. Although it was preferred to have the Thr alcohol protected during peptide coupling, it was not believed to be too detrimental and the product, a mixture of the $t$-Bu ether and free secondary alcohol, was taken on to coupling with the free acid fragment 2.

The linear precursor 17 for UstatA was generated via peptide coupling between the free amine mixture of 1 (partially deprotected at the Thr residue) and the free acid 2 with TBTU, HATU, DEPBT and DIPEA in a 1:1 mixture of dry DCM and dry DMF (Figure 36).
Upon completion, the crude reaction was purified via acid wash and flash column chromatography with the product(s) eluting at 95:5 EA:Hex to yield 17 in 24.5% yield. This sequence of reactions proved to be successful in generating our desired linear precursor but there were some issues of concern. First, the inability to selectively remove the Boc from 1 without partial deprotection of the t-Bu ether would need to be addressed. Additionally, the low overall yield of the peptide coupling to form the linear precursor, presumably due to the fragment 1 mixture used, would have to be improved upon. Nonetheless, the next reaction in the synthetic scheme was intended to deprotect both the dimethyl acetal of the α-bromo ketone and the t-Bu ether on the Thr. So the linear products obtained from the peptide coupling were taken on to the next step of the synthesis.

Based on the synthetic strategy employed for the synthesis of Merchercharmycin A, the final steps of our synthesis were believed to perform the final two transformations necessary to generate UstatA (Figure 37). Refluxing formic acid would be used to remove both the t-Bu ether on the Thr and the dimethyl acetal of the α-bromo ketone. The final step, macrocyclization via thiazole formation using Hantzsch conditions, should also generate the exocyclic propylidene via elimination of the Thr alcohol. The linear precursor 17 was diluted in formic acid (HCO₂H, 98%) and heated to reflux for 2 hours. When no evidence of starting material remained, the reaction solution was cooled to room temperature, diluted in saturated sodium bicarbonate and extracted with DCM. The organic solution was then dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. Analysis of the products via ¹H NMR and LC/MS revealed a mixture containing no evidence of the desired linear, deprotected product. Several attempts were made rerunning the reaction over shorter periods of time and at different temperatures. However, all attempts resulted in a mixture of
products, generally characterized by a loss of bromine, and the desired product was unable to be obtained.

Due to the series of problems presented by the initial strategy employed to generate the linear precursor for cyclization via the Hantzsch reaction, a reinvestigation of our strategy was necessary. The decision was made to generate the linear precursor via the Hantzsch thiazole synthesis, followed by cyclization via peptide coupling. Research had shown that after generation of the linear precursor with the elucidation of the final thiazole, the termini could be deprotected using LiO₂H and 4 M HCl in dioxane, followed by cyclization via peptide coupling using pentafluorophenol along with the coupling reagent 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). The elucidation of the propylidene would then comprise the final steps. Using 95% TFA, the t-Bu ether could be removed from the Thr residue and then Methanesulfonyl chloride would be used to perform the final elimination.

Methyl ester protected 2 was first deprotected at the α-bromo ketone terminus using refluxing formic acid (Figure 38). The dimethyl acetal fragment was diluted in HCO₂H and
heated to reflux for 1 hour. Upon completion, base wash purified the pure α-bromo ketone product 18 in 78.7% yield.

The Hantzsch thiazole synthesis reaction was next performed using Boc protected 1 and 18 in two steps to generate the linear precursor (Figure 38). For the first step, the thioamide 1, the α-bromo ketone 18 and KHCO₃ were dissolved in dry THF and cooled to -18°C. Upon completion, the solution was filtered through celite and concentrated in vacuo yielding a crude residue that was immediately carried on to the second step. The crude residue was dissolved in dry DCM, cooled to -18°C, followed by the addition of TFAA and 2,6-lutidine. Upon completion, the crude thiazole product was purified via flash column
chromatography with the pure product eluting at 45:55 EA:Hex to yield 19 in 27.5% yield.

As seen with generation of the linear precursor via peptide coupling, the yield for this reaction was unacceptably low. Investigation into conditions to maximize the yield would have to be carried out.

**Urukthapelstatin A Conclusions**

At this point in time my contributions toward this project had come to an end. The synthetic strategy employed for generation of our two precursor molecules, fragments 1 and 2, was successful in generating both in good yields. 1 was synthesized from the commercially available starting materials: Boc-Thr(Or-Bu)-OH, H₂N-Ser(OBn), and ethyl bromopyruvate. Using peptide coupling, the DAST/DBU protocol, and the Hantzsch reaction as key reactions, I was able to efficiently generate the two oxazole and one thiazole moieties of the final structure of UstatA. The fragment also was constructed to allow for generation of the linear precursor via either peptide coupling or hantzsch thiazole synthesis. 2 was generated from the commercially available starting materials: ethyl bromopyruvate, H₂N-β-OH-Phe-OMe, Boc-Ile-OH, and H₂N-Ala-OMe. Using peptide coupling and the DAST/DBU protocol, I was able to efficiently generate the phenyloxazole of the final structure of UstatA, in addition to the peptide portion of the macrocycle. Again, this fragment allows for generation of the linear precursor via either peptide coupling or Hantzsch thiazole synthesis. The linear precursor for UstatA was also generated via both methods. The first was conducted via peptide coupling. This method suffered low yields due to partial deprotection of the t-Bu ether protecting group on the Thr residue during Boc removal on 1. It also failed in the attempt to obtain the desired product during deprotection of the dimethyl acetal of the α-bromo ketone terminus. The second linear precursor was generated via the
Hantzsch thiazole synthesis reaction. This method also resulted in low yields, demonstrating the need for optimization of conditions. The linear precursor generated will be taken on by my colleagues and cyclization will be attempted via peptide coupling (Figure 39). Further studies are ongoing to address these challenges in our endeavor towards the first total synthesis of the natural product Urukthapelstatin A.

**Figure 39. Proposed strategy for cyclization via peptide coupling.**

**Conditions:**
- (a) LiOH (3 eq.), H₂O₂ (3.4 eq.), MeOH (0.4M), 0°C
- (b) 4M HCl in dry dioxane (0.05M), 0°C
- (c) (coupling reagent), DIPEA (4 eq.), dry DCM (0.1M)
- (d) TFA 95%
- (e) MsCl, TEA, THF
CHAPTER 5

EXPERIMENTAL PROCEDURES

GENERAL PEPTIDE SYNTHESIS PROCEDURE

The coupling reagent(s), 1.1 equivalents of the free amine, and 1.0 equivalents of the free acid were weighed into a dry round bottom flask with a magnetic stir bar and sealed with a rubber septum. For couplings to generate di and tripeptides, 1.1 equivalents of TBTU were used. Couplings involving N-methylated free amines were carried out with 0.8 equivalents of TBTU and 0.4 equivalents of HATU; and couplings to generate linear pentapeptides used a cocktail of TBTU, HATU and DEPBT to a total of 1.2 equivalents. The flask was then purged with argon gas, and the contents inside were dissolved in anhydrous DCM to a 0.1 M concentration. Following solvent addition, 4 equivalents of anhydrous DIPEA were added to the reaction flask. Cases of incomplete solubility of the starting materials were addressed by the addition of small amounts of anhydrous acetonitrile, DMF and/or DIPEA (to a total of 8 equivalents). The solution was stirred at room temperature for 1 to 3 hours and was monitored via TLC every 15 minutes. Upon completion, the crude reaction solution was diluted in DCM and excess base and side products were extracted with two washes of 10% aqueous HCl and ten washes of saturated sodium bicarbonate. When DMF was used as a solvent, these extractions were preceded by two washes of neutral DI water to remove the DMF. The organic solution was then dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. Acid-Base extraction was generally sufficient to yield pure dipeptide products. When necessary, peptide products were further purified via flash column chromatography using an ethyl acetate/hexane gradient solvent system on silica gel. The pure peptide products were verified via $^1$H NMR.
**GENERAL AMINE DEPROTECTION PROCEDURE**

Under open atmosphere, the Boc protected peptide was diluted to a 0.1 M concentration of 25% TFA and 75% DCM in a round bottom flask with a magnetic stir bar. Once the peptide was dissolved in DCM, 2 equivalents of anisole were added to the solution, followed by the addition of TFA. The reaction was run at room temperature for approximately one hour and was monitored via TLC every 15 minutes. Once complete, the solution was concentrated *in vacuo* to yield the free amine peptide in quantitative yield.

**GENERAL ACID DEPROTECTION PROCEDURE**

Under open atmosphere, the peptide was dissolved in methanol to a concentration of 0.1 M in a round bottom flask with a magnetic stir bar followed by addition of 8 equivalents of LiOH. The reaction was allowed to run under open atmosphere at room temperature for approximately two hours and monitored every 30 minutes via TLC. Upon completion, the methanol was removed via rotary evaporator and the crude mixture was diluted in DCM. Excess reagents and impurities were extracted twice with 7% HCl wash. The organic solution was then dried over anhydrous Na$_2$SO$_4$, filtered and concentrated *in vacuo*.

**MODIFIED ACID DEPROTECTION PROCEDURE**

Under open atmosphere, the peptide was dissolved in methanol to a concentration of 0.4 M in a round bottom flask with a magnetic stir bar and cooled to 0 °C. 3.4 equivalents of 30% (w/v) hydrogen peroxide were added followed by 3.0 equivalents of lithium hydroxide. At 0 °C, the reaction was monitored every 30 minutes via TLC and usually done in 1-2 hours. An aqueous solution containing 3.8 equivalents of sodium thiosulfate was added to neutralize the peroxide and 5 % hydrochloric acid was added till the solution pH was 1. The
aqueous solution was extracted five times with methylene chloride, and the combined organic layer was dried, filtered and concentrated in vacuo.

**IN-SITU DOUBLE DEPROTECTION PROCEDURE**

Under open atmosphere, the pentapeptide was dissolved in THF to a 0.03 M concentration in a round bottom flask with a magnetic stir bar. 4.0 equivalents of anisole were added to the reaction solution followed by 10 drops of concentrated HCl per 0.3 mmol of pentapeptide. Over the next 4 days, an additional 3-4 drops of HCl were added per day for a total of ~25 drops per 0.3 mmol of pentapeptide. On the third day, an additional 2.0 equivalents of anisole was added to the reaction. TLC and LC/MS were used to monitor the reaction every 12 hours and the reaction was typically finished in 6 days. Once complete, the solution was concentrated in vacuo to yield the double deprotected pentapeptide in quantitative yield.

**MACROCYCLIZATION PROCEDURE**

The coupling reagents and double deprotected linear pentapeptide (DDLP) were weighed into a dry round bottom flask with a magnetic stir bar and sealed with a rubber septum. A cocktail of TBTU, HATU and DEPBT were used in varying ratios to a total of 1.2-1.3 equivalents of coupling reagents. The flask was then purged with argon gas, and the contents inside were dissolved in anhydrous DCM. Cyclizations where the dimerized decapetide product was not desired were run at low concentrations (~0.007 M), and those where decapetide was desired were run at higher concentrations (0.01-0.1 M). Following solvent addition, 4 equivalents of anhydrous DIPEA were added to the reaction flask. Cases of incomplete solubility of the starting materials were addressed by the addition of small amounts of anhydrous acetonitrile, DMF and/or DIPEA (to a total of 6-15 equivalents). The
solution was stirred at room temperature for 1 to 3 hours and was monitored via TLC and LC/MS every 30 minutes. Upon completion, the crude reaction solution was diluted in DCM and excess base and side products were extracted with two washes of 10% aqueous HCl and ten washes of saturated sodium bicarbonate. When DMF was used as a solvent, these extractions were preceded by two washes of neutral DI water to remove the DMF. The organic solution was then dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. Peptide products were further purified via flash column chromatography using an ethyl acetate/hexane gradient solvent system on silica gel. Finally, when necessary, reverse phase HPLC was used for additional purification using a gradient of acetonitrile and DI water with 0.1% TFA. The final pure cyclized products were verified via $^1$H NMR, LC/MS and RP-HPLC.

**HYDROGENATION PROCEDURE**

The Benzyl protected peptide and 10% palladium on carbon (1 mg of Pd/C per 10 mg of peptide) were weighed into a dry round bottom flask with a magnetic stir bar and sealed with a rubber septum. The peptide was dissolved in ethanol (EtOH) to a 0.1 M concentration. Hydrogen gas was allowed to bubble through the solution for 3-4 hours and then the contents were allowed to stir under hydrogen overnight. Upon verification of completion via TLC, the reaction solution was filtered through celite and concentrated in vacuo. The pure peptide products were verified via $^1$H NMR.

**GENERAL OXAZOLE SYNTHESIS PROCEDURE**

Oxazoles were generated from serine containing peptides in the following two step reaction.
Step 1: Oxazoline Generation with DAST

The free serine peptide was weighed into a dry round bottom flask with a magnetic stir bar and sealed with a rubber septum. The flask was then purged with argon gas, and the contents inside were dissolved in anhydrous DCM or anhydrous THF to a 0.1 M concentration. The solution was cooled to -78 °C and DAST was then added drop wise over 5-15 minutes. When simultaneous formation of two oxazolines was attempted, 2.1-2.4 equivalents of DAST were used. For generation of a single oxazoline, 1.2 equivalents were used. The reaction was stirred at -78 °C for 15-60 minutes and monitored every 15 minutes via TLC. Next, base was added to the reaction solution. For generation of phenyl-oxazolines, 3.0 equivalents of pyridine were added. After investigating the use of pyridine, NaHCO₃, K₂CO₃ for unsubstituted oxazolines, it was determined 2.0-3.0 equivalents of anhydrous K₂CO₃ was ideal. After base addition, the reaction was stirred at -78 °C for 15 minutes and then allowed to warm to room temperature over ~20 minutes. Upon verification of completion via LC/MS, the crude reaction solution was diluted in DCM and extracted with two washes of saturated sodium bicarbonate. The organic solution was then dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The crude oxazoline product was taken on to the following DBU elimination reaction without further purification or characterization.

Step 2: Oxazole Generation with DBU

The crude oxazoline peptide was weighed into a dry round bottom flask with a magnetic stir bar and sealed with a rubber septum. The flask was then purged with argon gas, and the contents inside were dissolved in anhydrous DCM or a 1:1 mixture of anhydrous ACN:pyridine to a 0.1 M concentration. The solution was cooled to -20 °C and then 3.0...
equivalents of DBU and 3.0 equivalents of BrCCl$_3$ were added drop wise over 5-10 minutes each. The reaction was allowed to warm to room temperature and run for 12-48 hours. Upon verification of completion via TLC and LC/MS, the crude reaction solution was diluted in DCM and extracted with two washes of 5% aqueous HCl and five washes of saturated sodium bicarbonate. The organic solution was then dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. Oxazole products were further purified via flash column chromatography using an ethyl acetate/hexane gradient solvent system on silica gel. The pure oxazole products were verified via $^1$H NMR.

**AMIDE SYNTHESIS PROCEDURE**

Under open atmosphere, the methyl ester was diluted to a 0.025 M concentration of 10% MeOH and 90% NH$_4$OH (25% in water) in a round bottom flask with a magnetic stir bar. Once the peptide was dissolved in MeOH, NH$_4$OH was then added to the solution. The reaction was placed in the ultrasound bath until all solid starting material was dissolved. The reaction was run at room temperature for 12-18 hours and monitored via TLC. Once complete, the solution was concentrated in vacuo to yield the amide. The pure amide products were verified via $^1$H NMR.

**THIOAMIDE SYNTHESIS PROCEDURE**

The amide and 1.0-1.5 equivalents of Lawesson’s reagent was weighed into an oven-dried round bottom flask with a magnetic stir bar and sealed with an oven-dried reflux condenser. The apparatus was then purged with argon gas, and the contents inside were dissolved in anhydrous THF to a 0.07 M concentration. The reaction was refluxed for 16-24 hours and monitored via TLC. Upon completion, the reaction solution was cooled to room temperature and concentrated in vacuo. The crude products were purified via flash column
chromatography using an ethyl acetate/hexane gradient solvent system on silica gel. The pure thioamide products were verified via $^1$H NMR.

**Hantzsch Thiazole Synthesis Procedure**

Thiazoles were generated from a thioamide and α-halo ketone in the following two step reaction.

**Step 1**

The thioamide and 8.0 equivalents of potassium bicarbonate (KHCO$_3$) was weighed into a dry round bottom flask with a magnetic stir bar and sealed with a rubber septum. The flask was then purged with argon gas, and the contents inside were dissolved in anhydrous DCM to a 0.4 M concentration. The solution was cooled to -18 °C and then 3.0 equivalents of 90% Bromopyruvic ethyl ester (BPEE) were added drop wise over 15 minutes. The reaction was allowed to stir at -18 °C for 5 hours and monitored via TLC and LC/MS. Upon completion, the reaction solution was filtered through celite and concentrated *in vacuo*.

**Step 2**

The crude hydroxy-thiazoline product was dissolved in anhydrous DCM to a 0.4 M concentration in a dry round bottom flask with a magnetic stir bar. The flask was sealed with a rubber septum, purged with argon gas and cooled to -18 °C. A solution of 4.0 equivalents of TFAA and 9.0 equivalents of 2,6-lutidine in half the volume of anhydrous DCM used to dissolve the hydroxy-oxazoline was cooled to -18 °C and then added to the reaction solution. The reaction was stirred at -18 °C for 30 minutes and monitored via TLC and LC/MS. Upon completion, the crude reaction solution was allowed to warm to room temperature, diluted in DCM and extracted with two washes of saturated sodium bicarbonate. The organic solution was then dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. Thiazole
products were further purified via flash column chromatography using an ethyl acetate/hexane gradient solvent system on silica gel. The pure thiazole products were verified via $^1$H NMR.

**Ketone Protection Procedure**

The ketone was weighed into an oven-dried round bottom flask with a magnetic stir bar and sealed with an oven-dried reflux condenser. The apparatus was then purged with argon gas, and the contents inside were dissolved in anhydrous MeOH to a 0.1 M concentration. 5.0 equivalents of (MeO)$_3$CH followed by 1.1 equivalents of H$_2$SO$_4$ were added to the solution. The reaction was refluxed for 48 hours and monitored via TLC. Upon completion, the reaction solution was cooled to room temperature and poured into a saturated sodium bicarbonate solution. The aqueous solution was extracted five times with methylene chloride, and the combined organic layer was dried, filtered and concentrated in vacuo. The pure dimethyl ketal products were verified via $^1$H NMR.

**Ketone Deprotection Procedure**

Under open atmosphere, the ketal was dissolved in formic acid to a concentration of 0.035 M in a round bottom flask with a magnetic stir bar attached to a reflux condenser. The reaction was refluxed for approximately two hours and monitored every 30 minutes via TLC and LC/MS. Upon completion, the reaction solution was cooled to room temperature and poured into a saturated sodium bicarbonate solution. The aqueous solution was extracted five times with methylene chloride, and the combined organic layer was dried, filtered and concentrated in vacuo. The pure ketone products were verified via $^1$H NMR.
MODIFIED AMINE DEPROTECTION PROCEDURE

The Boc protected peptide was weighed into a dry round bottom flask with a magnetic stir bar and sealed with a rubber septum. The flask was then purged with argon gas, and the contents inside were dissolved in anhydrous 4 M HCl in dioxane (precooled to 0 °C) to a 0.05 M concentration. The reaction was run at room temperature for 30-60 minutes and was monitored via TLC every 15 minutes. Once complete, the solution was concentrated in vacuo to yield the free amine peptide in quantitative yield and verified via ¹H NMR.

THYMIDINE UPTAKE ASSAYS

Proliferation of the HCT-116 colon cancer cells was tested in the presence and absence of the compounds using ³H-thymidine uptake assays. Cells treated with the compounds were compared to dimethyl sulfoxide (DMSO) controls for their ability to proliferate as indicated by the incorporation of ³H-thymidine into their DNA. Cells were cultured in 96 well plates at a concentration of 4000-5000 cells/well in DMEM (Gibco) supplemented with L-glutamine, 10% fetal bovine serum and 1% penicillin-streptomycin antibiotic. After overnight incubation, the compounds were added. The compounds were dissolved in DMSO at a final concentration of 0.1% and tested at the concentrations indicated in the manuscript. The DMSO control was also at 0.1%. After the cells had been incubated with the compounds for 6 h, 1mCi ³H-thymidine per well was added and the cells were cultured for an additional 18 h (for the cells to have a total of 24 h treatment), at which time the cells were harvested using a PHD cell harvester (Cambridge Technology Inc.). The samples were then counted (CPM) in a scintillation counter for 1.5 m. Decreases in ³H-thymidine incorporation, as compared to DMSO controls, are an indication that the cells are no longer progressing through the cell cycle or synthesizing DNA, as is shown in the studies
presented. Mean growth inhibition (n=8-12) is the 1 minus CPM of compound-treated cells over DMSO-treated cells. IC\textsubscript{50} were determined using 0, 0.1, 0.5, 5, 10, and 50 μM of compound (in 1% DMSO final concentration). All calculations including mean, SEM, and IC\textsubscript{50} were performed on Excel.

**METHODS OF CHROMATOGRAPHIC PURITY**

**Method A**

**Instrument:** Agilent 1200 Series HPLC

Agilent 62440A LC/MSD Trap

**Column:** Zorbax SB-C18

2.1x30mm 3.5-Micron

**Mobile Phase A:** 0.1% (v/v) formic acid, 100% (v/v) water

**Mobile Phase B:** 0.1% (v/v) formic acid, 100% (v/v) acetonitrile

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<th>Profile %B</th>
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**Flow Rate:** 1.0 ml/min

**Injection:** 4μL

**Solvent:** 100% Methanol
Method B

**Instrument:** Waters Flex Inject

Waters 2487 Dual λ Absorbance Detector

**Column:** Symmetry C₁₈ 3.5µm

4.6x75mm Column

**Mobile Phase A:** 0.1% (v/v) Trifluoroacetic acid, 100% (v/v) water

**Mobile Phase B:** 0.1% (v/v) Trifluoroacetic acid, 100% (v/v) acetonitrile

λ₁: 215nm

λ₂: 222nm

**Gradient:**

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**Flow rate:** 0.50 ml/min

**Injection:** 20µL

**Solvent:** 100% Methanol
SYNTHESIS OF SANSALVAMIDE A DERIVATIVES

The following describes the synthesis of all SanA and DiSanA compounds. \(^1\)HNMR, LC/MS, and HPLC data of intermediates and final structures are found in Appendix A.

SanA 2-Dipeptide MeO-D-Phe-Leu-NHBoc

Dipeptide MeO-d-Phe-Leu-NHBoc was synthesized following the “General Peptide Synthesis Procedure” utilizing 475.8 mg (2.2 mmols, 1.1 equivalents) of amine MeO-D-Phe-NH₂·HCl, 500 mg (2.0 mmols, 1.0 equivalents) of acid HO-Leu-NHBoc·H₂O, 1.4 mL (4 equivalents) of DIPEA, and 708.4 mg (2.2 mmols, 1.1 equivalents) of TBTU in 20 mL of dry DCM. The crude reaction was purified by acid-base extraction to yield the dipeptide (786 mg, 94% yield).

Rf: 0.9 (EtOAc:Hex, 1:1)

\(^1\)H NMR (200 MHz, CDCl₃): \(\delta\) 0.8-1.0 (m, 6H), 1.4 (s, 9H), 1.6 (m, 3H), 3.0-3.2 (m, 2H), 3.7 (s, 3H), 4.0-4.2 (br, 1H), 4.8 (br, \(\alpha\)H), 4.8-5.0 (q, \(\alpha\)H), 6.6 (d, 1H), 7.1-7.4 (m, 5H)

Dipeptide MeO-D-Phe-Leu-NH₂

Dipeptide MeO-D-Phe-Leu-NH₂ was synthesized following the “General Amine Deprotection Procedure” utilizing 675.8 mg (1.7 mmol, 1.0 equivalents) of MeO-D-Phe-Leu-NHBoc and 0.38 mL (3.4 mmol, 2.0 equivalents) of anisole in 4.3 mL of TFA and 12.9 mL of DCM. This dipeptide was taken on to the next reaction without further purification or characterization (503.7 mg, quantitative yield).

Tripeptide MeO-d-Phe-Leu-Val-NHBoc

Tripeptide MeO-D-Phe-Leu-Val-NHBoc was synthesized following the “General Peptide Synthesis Procedure” utilizing 246.3 mg (2.6 mmols, 1.1 equivalents) of amine MeO-d-Phe-Leu-NH₂, 504.3 mg (2.3 mmols, 1.0 equivalents) of acid HO-Val-NHBoc, 3.0
mL (7.4 equivalents) of DIPEA and 819.6 mg (2.6 mmols, 1.1 equivalents) of TBTU in 23.2 mL of dry DCM and 10 mL of dry ACN. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the tripeptide (711.5 mg, 75.5% yield).

Rf: 0.6 (EtOAc:Hex, 1:1)

\[^1\text{H} \text{NMR (200 MHz, CDCl}_3\)): \delta 0.9-1.1 (m, 12H), 1.4 (s, 9H), 1.4-1.6 (m, 2H), 1.7 (br, 1H), 2.1 (m, 1H), 3.0-3.2 (m, 2H), 3.7 (s, 3H), 3.9 (dd, \alpha H), 4.4 (br, \alpha H), 4.8 (dd, \alpha H), 5.0 (d, 1H), 6.3 (d, 1H), 6.6 (d, 1H), 7.1-7.3 (m, 5H)

**Tripeptide MeO-D-Phe-Leu-Val-NH\textsubscript{2}**

Tripeptide MeO-D-Phe-Leu-Val-NH\textsubscript{2} was synthesized following the “General Amine Deprotection Procedure” utilizing 706.3 mg (1.4 mmol, 1.0 equivalents) of MeO-D-Phe-Leu-Val-NHBoc and 0.31 mL (2.8 mmol, 2.0 equivalents) of anisole in 3.6 mL of TFA and 10.8 mL of DCM. This tripeptide was taken on to the next reaction without further purification or characterization (562.8 mg, quantitative yield).

**Dipeptide MeO-Leu-N-Me-D-Leu-NHBoc**

Dipeptide MeO-Leu-N-Me-D-Leu-NHBoc was synthesized following the “General Peptide Synthesis Procedure” utilizing 259.7 mg (1.6 mmols, 1.1 equivalents) of amine MeO-Leu-N-Me-H, 369.6 mg (1.5 mmols, 1.0 equivalents) of acid HO-D-Leu-NHBoc·H\textsubscript{2}O, 2.2 mL (8.6 equivalents) of DIPEA, 380.9 mg (1.2 mmols, 0.8 equivalents) of TBTU and 225.5 mg (0.6 mmols, 0.4 equivalents) of HATU in 14.8 mL of dry DCM and 10 mL of dry ACN. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the dipeptide (452.3 mg, 81.9% yield).

Rf: 0.9 (EtOAc:Hex, 1:1)
$^1$H NMR (200 MHz, CDCl$_3$): $\delta$ 0.9-1.0 (m, 12H), 1.4(s, 9H), 1.5 (m, 2H), 1.7-1.8 (dd, 4H), 3.0 (s, 3H), 3.7 (s, 3H), 4.6-4.8 (br, 1H), 5.1-5.3 (br, 2αH)

**Dipeptide HO-Leu-N-Me-D-Leu-NHBoc**

Dipeptide HO-Leu-N-Me-D-Leu-NHBoc was synthesized following the “General Acid Deprotection Procedure” utilizing 652.5 mg (1.8 mmol, 1.0 equivalents) of MeO-Leu-N-Me-D-Leu-NHBoc and 588 mg (14.0 mmol, 8.0 equivalents) of LiOH in 17.5 mL of MeOH. This dipeptide was taken on to the next reaction without further purification or characterization (585.6 mg, 89.7% yield).

**Pentapeptide MeO-d-Phe-Leu-Val-Leu-N-Me-D-Leu-NHBoc**

Pentapeptide MeO-d-Phe-Leu-Val-Leu-N-Me-D-Leu-NHBoc was synthesized following the “General Peptide Synthesis Procedure” utilizing 563 mg (1.4 mmols, 1.0 equivalents) of amine MeO-d-Phe-Leu-Val-NH$_2$, 577.1 mg (1.6 mmols, 1.1 equivalents) of acid HO-Leu-N-Me-D-Leu-NHBoc, 0.88 mL (3.5 equivalents) of DIPEA, 230.7 mg (0.72 mmol, 0.5 equivalents) of TBTU, 273.2 mg (0.72 mmols, 0.5 equivalents) of HATU, and 86 mg (0.29 mmols, 0.2 equivalents) of DEPBT in 14.4 mL of dry DCM. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the pentapeptide (917.4 mg, 87% yield).

Rf: 0.35 (EtOAc:Hex, 1:1)

$^1$H NMR (400 MHz, CD$_3$OD): $\delta$ 0.8-1.0 (m, 24H), 1.4 (s, 9H), 1.4-1.6 (m, 3H), 1.6-1.8 (br, 6H), 2.2 (m, 1H), 3.0 (s, 3H), 3.0-3.2 (m, 2H), 3.7 (s, 3H), 4.0-4.2 (m, αH), 4.4 (m, 2αH), 4.6 (m, αH), 4.8 (m, αH), 5.1 (d, 1H), 6.5 (d, 1H), 6.6 (d, 1H), 6.8 (d, 1H), 7.1-7.3 (m, 5H)
**Pentapeptide**

**HO-δ-Phe-Leu-Val-Leu-N-Me-δ-Leu-NHBoc**

Pentapeptide HO-δ-Phe-Leu-Val-Leu-N-Me-δ-Leu-NHBoc was synthesized following the “General Acid Deprotection Procedure” utilizing 917.0 mg (1.2 mmol, 1.0 equivalents) of MeO-δ-Phe-Leu-Val-Leu-N-Me-δ-Leu-NHBoc and 420.4 mg (10.0 mmol, 8.0 equivalents) of LiOH in 12.5 mL of MeOH. This pentapeptide was taken on to the next reaction without further purification or characterization (876.8 mg, 97.5% yield).

**Pentapeptide**

**HO-δ-Phe-Leu-Val-Leu-N-Me-δ-Leu-NH₂**

Pentapeptide HO-δ-Phe-Leu-Val-Leu-N-Me-δ-Leu-NH₂ was synthesized following the “General Amine Deprotection Procedure” utilizing 876.8 mg (1.2 mmol, 1.0 equivalents) of HO-δ-Phe-Leu-Val-Leu-N-Me-δ-Leu-NHBoc and 0.2 mL (1.8 mmol, 1.5 equivalents) of anisole in 3.1 mL of TFA and 9.2 mL of DCM. This pentapeptide was taken on to the next reaction without further purification or characterization (754.5 mg, quantitative yield).

**Macrocycle δ-Phe-Leu-Val-Leu-N-Me-δ-Leu**

Macrocycle δ-Phe-Leu-Val-Leu-N-Me-δ-Leu was synthesized following the “Macrocyclization Procedure” utilizing 500 mg (0.81 mmols, 1.0 equivalents) of linear pentapeptide, 0.6 mL (3.2 mmol, 4.0 equivalents) of DIPEA, 77.9 mg (0.24 mmols, 0.3 equivalents) of TBTU, 153.8 mg (0.40 mmols, 0.5 equivalents) HATU, and 121.1 mg (0.40 mmols, 0.5 equivalents) of DEPBT in 115.6 mL of dry DCM and 15.0 mL of dry ACN. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex). The impure product was further purified by reverse phase-HPLC to yield the macrocycle (18.6 mg, 3.8% yield).
Rf: 0.25 (EtOAc:Hex, 1:1)

\(^1\)H NMR (400 MHz, CD3OD): \(\delta\) 0.8 (m, 6H), 0.8-1.0 (m, 18H), 1.3-1.6 (m, 6H), 1.6-1.9 (m, 4H), 3.0 (s, 3H), 2.79-2.9 (m, 2H), 3.2 (m, \(\alpha\)H), 3.4 (m, \(\alpha\)H), 3.7 (d, \(\alpha\)H), 4.2 (m, \(\alpha\)H), 4.4 (m, \(\alpha\)H), 4.5 (dd, 1H), 4.8 (t, 1H), 5.0 (t, 1H), 5.4 (t, 1H), 7.2-7.3 (m, 5H)

LCMS: m/z calcd for C\(_{33}\)H\(_{53}\)N\(_5\)O\(_5\) (M+1) = 599.8, found 600.3

**SanA 3 – Dipeptide MeO-Phe-D-Phe-NHBoc**

Dipeptide MeO-Phe-D-Phe-NHBoc was synthesized following the “**General Peptide Synthesis Procedure**” utilizing 447.2 mg (2.0 mmols, 1.1 equivalents) of amine MeO-Phe-NH\(_2\)·HCl, 500 mg (1.9 mmols, 1.0 equivalents) of acid HO-D-Phe-NHBoc, 1.3 mL (4 equivalents) of DIPEA, and 665.8 mg (2.1 mmols, 1.1 equivalents) of TBTU in 18.9 mL of dry DCM and 5.0 mL of dry ACN. The crude reaction was purified by acid-base extraction to yield the dipeptide (734.6 mg, 91.4% yield).

Rf: 0.9 (EtOAc:Hex, 1:1)

\(^1\)H NMR (200 MHz, CDCl\(_3\)): \(\delta\) 1.4 (s, 9H), 2.9-3.2 (m, 4H), 3.8 (s, 3H), 4.3-4.4 (q, \(\alpha\)H), 4.8 (q, \(\alpha\)H), 4.9 (br, 1H), 6.3 (d, 1H), 6.9-7.0 (m, 2H), 7.1-7.4 (m, 8H)

**Dipeptide MeO-Phe-D-Phe-NH\(_2\)**

Dipeptide MeO-Phe-D-Phe-NH\(_2\) was synthesized following the “**General Amine Deprotection Procedure**” utilizing 734.6 mg (1.7 mmol, 1.0 equivalents) of MeO-Phe-D-Phe-NHBoc and 0.38 mL (3.4 mmol, 2.0 equivalents) of anisole in 4.3 mL of TFA and 12.9 mL of DCM. This dipeptide was taken on to the next reaction without further purification or characterization (562.2 mg, quantitative yield).
**Tripeptide MeO-Phe-\(\alpha\)-Phe-Val-NHBoc**

Tripeptide MeO-Phe-\(\alpha\)-Phe-Val-NHBoc was synthesized following the “General Peptide Synthesis Procedure” utilizing 562.2 mg (1.7 mmols, 1.1 equivalents) of amine MeO-Phe-\(\alpha\)-Phe-NH\(_2\), 340.3 mg (1.6 mmols, 1.0 equivalents) of acid HO-Val-NHBoc, 1.1 mL (4 equivalents) of DIPEA and 553.1 mg (1.7 mmols, 1.1 equivalents) of TBTU in 15.7 mL of dry DCM. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the tripeptide (781.4 mg, 94.9% yield). Rf: 0.7 (EtOAc:Hex, 1:1)

\(^1\)H NMR (200 MHz, CDCl\(_3\)): \(\delta\) 0.7-0.8 (m, 6H), 1.4 (s, 9H), 2.0 (m, 1H), 2.9-3.1 (m, 4H), 3.6 (s, 3H), 3.8 (dd, \(\alpha\)H), 4.7 (q, \(\alpha\)H), 4.8 (q, \(\alpha\)H), 5.0 (d, 1H), 6.4 (d, 1H), 6.5 (br, 1H), 6.9-7.0 (m, 2H), 7.1-7.4 (m, 8H)

**Tripeptide MeO-Phe-\(\alpha\)-Phe-Val-NH\(_2\)**

Tripeptide MeO-Phe-\(\alpha\)-Phe-Val-NH\(_2\) was synthesized following the “General Amine Deprotection Procedure” utilizing 877.2 mg (1.7 mmol, 1.0 equivalents) of MeO-\(\alpha\)-Phe-Leu-Val-NHBoc and 0.27 mL (2.5 mmol, 1.5 equivalents) of anisole in 4.2 mL of TFA and 12.5 mL of DCM. This tripeptide was taken on to the next reaction without further purification or characterization (710.3 mg, quantitative yield).

**Dipeptide MeO-Leu-Leu-NHBoc**

Dipeptide MeO-Leu-Leu-NHBoc was synthesized following the “General Peptide Synthesis Procedure” utilizing 400.9 mg (2.2 mmols, 1.1 equivalents) of amine MeO-Leu-NH\(_2\)·HCl, 500 mg (2.0 mmols, 1.0 equivalents) of acid HO-\(\alpha\)-Leu-NHBoc·H\(_2\)O, 2.8 mL (8.0 equivalents) of DIPEA and 708.4 mg (2.2 mmols, 1.1 equivalents) of TBTU in 20.1 mL of
dry DCM and 7.5 mL of dry ACN. The crude reaction was purified by acid-base extraction to yield the dipeptide (710.3 mg, 98.8% yield).

Rf: 0.9 (EtOAc:Hex, 1:1)

$^1$H NMR (200 MHz, CDCl$_3$): $\delta$ 0.9-1.0 (m, 12H), 1.3 (s, 9H), 1.6-1.8 (m, 6H), 3.7 (s, 3H), 4.0-4.2 (q, $\alpha$H), 4.5-4.7 (m, $\alpha$H), 4.8-4.9 (d, 1H), 6.4 (d, 1H)

**Dipeptide HO-Leu-Leu-NHBoc**

Dipeptide HO-Leu-Leu-NHBoc was synthesized following the “**General Acid Deprotection Procedure**” utilizing 697.6 mg (1.9 mmol, 1.0 equivalents) of MeO-Leu-Leu-NHBoc and 653.0 mg (16.0 mmol, 8.0 equivalents) of LiOH in 19.5 mL of MeOH. This dipeptide was taken on to the next reaction without further purification or characterization (668.3 mg, 99.9% yield).

**Pentapeptide MeO-Phe-d-Phe-Val-Leu-Leu-NHBoc**

Pentapeptide MeO-Phe-d-Phe-Val-Leu-Leu-NHBoc was synthesized following the “**General Peptide Synthesis Procedure**” utilizing 710.3 mg (1.7 mmols, 1.0 equivalents) of amine MeO-Phe-d-Phe-Val-NH$_2$, 661.8 mg (1.9 mmols, 1.1 equivalents) of acid HO-Leu-Leu-NHBoc, 2.4 mL (8 equivalents) of DIPEA, 317.3 mg (0.83 mmol, 0.5 equivalents) of TBTU, 268.0 mg (0.83 mmols, 0.5 equivalents) of HATU, and 99.9 mg (0.33 mmols, 0.2 equivalents) of DEPBT in 16.7 mL of dry DCM and 10.0 mL of dry ACN. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the pentapeptide (785.0 mg, 62.5% yield).

Rf: 0.35 (EtOAc:Hex, 1:1)

$^1$H NMR (400 MHz, CD$_3$OD): $\delta$ 0.4-0.6 (m, 6H), 0.8-1.0 (m, 12H), 1.4 (s, 9H), 1.3-1.5 (m, 4H), 1.6 (m, 2H), 1.8 (m, 1H), 2.4 (m, 1H), 2.7 (m, 1H), 2.9 (m, 1H), 3.1 (m, 1H), 3.6 (s,
Pentapeptide HO-Phe-d-Phe-Val-Leu-Leu-NHBoc
Pentapeptide HO-Phe-d-Phe-Val-Leu-Leu-NHBoc was synthesized following the “General Acid Deprotection Procedure” utilizing 785.0 mg (1.0 mmol, 1.0 equivalents) of MeO-Phe-d-Phe-Val-Leu-Leu-NHBoc and 350.3 mg (8.4 mmol, 8.0 equivalents) of LiOH in 10.4 mL of MeOH. This pentapeptide was taken on to the next reaction without further purification or characterization (755.1 mg, 98.0% yield).

Pentapeptide HO-Phe-d-Phe-Val-Leu-Leu-NH₂
Pentapeptide HO-Phe-d-Phe-Val-Leu-Leu-NH₂ was synthesized following the “General Amine Deprotection Procedure” utilizing 755.1 mg (1.0 mmol, 1.0 equivalents) of HO-Phe-d-Phe-Val-Leu-Leu-NHBoc and 0.23 mL (2.1 mmol, 2.0 equivalents) of anisole in 2.6 mL of TFA and 7.8 mL of DCM. This pentapeptide was taken on to the next reaction without further purification or characterization (652.8 mg, quantitative yield).

Macrocycle Phe-d-Phe-Val-Leu-Leu
Macrocycle Phe-d-Phe-Val-Leu-Leu was synthesized following the “Macrocyclization Procedure” utilizing 652.8 mg (1.0 mmols, 1.0 equivalents) of linear pentapeptide, 0.73 mL (4.2 mmol, 4.0 equivalents) of DIPEA, 100.6 mg (0.31 mmols, 0.3 equivalents) of TBTU, 198.5 mg (0.52 mmols, 0.5 equivalents) HATU, and 156.2 mg (0.52 mmols, 0.5 equivalents) of DEPBT in 149.1 mL of dry DCM, 10.0 mL of dry ACN and 3.0 mL of dry DMF. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex). The impure product was further purified by reverse phase-HPLC to yield the macrocycle (56.9 mg, 8.8% yield).
SanA 4 – Dipeptide MeO-d-Trp-Leu-NHBoc

Dipeptide MeO-D-Trp-Leu-NHBoc was synthesized following the “General Peptide Synthesis Procedure” utilizing 562.1 mg (2.2 mmols, 1.1 equivalents) of amine MeO-D-Trp-NH₂·HCl, 500 mg (2.0 mmols, 1.0 equivalents) of acid HO-Leu-NHBoc·H₂O, 1.4 mL (4.0 equivalents) of DIPEA and 708.4 mg (2.2 mmols, 1.1 equivalents) of TBTU in 20.1 mL of dry DCM and 10.0 mL of dry ACN. The crude reaction was purified by extraction with saturated ammonium chloride (NH₄Cl) to yield the dipeptide (745.9 mg, 92.8% yield).

Rf: 0.6 (EtOAc:Hex, 1:1)

¹H NMR (200 MHz, CDCl₃): δ 0.9 (d, 6H), 1.4 (s, 9H), 1.6 (m, 3H), 3.3 (d, 2H), 3.6 (s, 3H), 4.1 (br, αH), 4.8 (d, 1H), 4.9 (dd, αH), 6.6 (d, 1H), 7.0 (d, 1H), 7.1-7.2 (m, 2H), 7.4 (d, 1H), 7.6 (d, 1H), 8.2 (br, 1H)

Dipeptide MeO-D-Trp-Leu-NH₂

Dipeptide MeO-D-Trp-Leu-NH₂ was synthesized following the “General Amine Deprotection Procedure” utilizing 803.2 mg (1.9 mmol, 1.0 equivalents) of MeO-D-Trp-Leu-NHBoc and 0.28 mL (2.6 mmol, 1.4 equivalents) of anisole in 4.7 mL of TFA and 14.0 mL of DCM. This dipeptide was taken on to the next reaction without further purification or characterization (617.1 mg, quantitative yield).
**Tripeptide MeO-D-Trp-Leu-Val-NHBoc**

Tripeptide MeO-D-Trp-Leu-Val-NHBoc was synthesized following the “General Peptide Synthesis Procedure” utilizing 617.1 mg (1.9 mmols, 1.1 equivalents) of amine MeO-D-Trp-Leu-NH₂, 367.8 mg (1.7 mmols, 1.0 equivalents) of acid HO-Val-NHBoc, 2.6 mL (8 equivalents) of DIPEA and 597.9 mg (1.9 mmols, 1.1 equivalents) of TBTU in 18.6 mL of dry DCM and 5.0 mL of dry ACN. The crude reaction was purified by extraction with saturated ammonium chloride followed by flash column chromatography (silica gel, EA/Hex) to yield the tripeptide (682.0 mg, 70.8% yield).

Rf: 0.4 (EtOAc:Hex, 1:1)

^1H NMR (200 MHz, CDCl₃): δ 0.8-1.0 (d, 12H), 1.4 (s, 9H), 1.6 (m, 3H), 2.1 (m, 1H), 3.3 (d, 2H), 3.6 (s, 3H), 3.8 (t, αH), 4.4 (br, αH), 4.8 (dd, αH), 5.0 (d, 1H), 6.2 (d, 1H) 6.6 (d, 1H), 7.0 (s, 1H), 7.1-7.2 (m, 2H), 7.4 (d, 1H), 7.6 (d, 1H), 8.2 (br, 1H)

**Tripeptide MeO-D-Trp-Leu-Val-NH₂**

Tripeptide MeO-D-Trp-Leu-Val-NH₂ was synthesized following the “General Amine Deprotection Procedure” utilizing 918.3 mg (1.8 mmol, 1.0 equivalents) of MeO-D-Trp-Leu-Val-NHBoc and 0.23 mL (2.1 mmol, 1.2 equivalents) of anisole in 4.5 mL of TFA and 13.4 mL of DCM. This tripeptide was taken on to the next reaction without further purification or characterization (742.3 mg, quantitative yield).

**Dipeptide MeO-Leu-Leu-NHBoc**

Dipeptide MeO-Leu-Leu-NHBoc was synthesized following the “General Peptide Synthesis Procedure” utilizing 400.9 mg (2.2 mmols, 1.1 equivalents) of amine MeO-Leu-NH₂·HCl, 500 mg (2.0 mmols, 1.0 equivalents) of acid HO-D-Leu-NHBoc·H₂O, 2.8 mL (8.0 equivalents) of DIPEA and 708.4 mg (2.2 mmols, 1.1 equivalents) of TBTU in 20.1 mL of
dry DCM and 7.5 mL of dry ACN. The crude reaction was purified by acid-base extraction to yield the dipeptide (710.3 mg, 98.8% yield).

Rf: 0.9 (EtOAc:Hex, 1:1)

$^1$H NMR (200 MHz, CDCl$_3$): $\delta$ 0.9-1.0 (m, 12H), 1.4(s, 9H), 1.6-1.8 (m, 6H), 3.7 (s, 3H), 4.0-4.2 (dd, $\alpha$H), 4.6 (m, $\alpha$H), 4.8-4.9 (d, 1H), 6.4 (d, 1H)

**Dipeptide HO-Leu-Leu-NHBoc**

Dipeptide HO-Leu-Leu-NHBoc was synthesized following the “General Acid Deprotection Procedure” utilizing 697.6 mg (1.9 mmol, 1.0 equivalents) of MeO-Leu-Leu-NHBoc and 653.0 mg (16.0 mmol, 8.0 equivalents) of LiOH in 19.5 mL of MeOH. This dipeptide was taken on to the next reaction without further purification or characterization (668.3 mg, 99.9% yield).

**Pentapeptide MeO-o-Trp-Leu-Val-Leu-Leu-NHBoc**

Pentapeptide MeO-o-Trp-Leu-Val-Leu-Leu-NHBoc was synthesized following the “General Peptide Synthesis Procedure” utilizing 742.3 mg (1.8 mmols, 1.0 equivalents) of amine MeO-o-Trp-Leu-Val-NH$_2$, 668.3 mg (1.9 mmols, 1.1 equivalents) of acid HO-Leu-Leu-NHBoc, 1.2 mL (4 equivalents) of DIPEA, 285.8 mg (0.89 mmol, 0.5 equivalents) of TBTU, 338.4 mg (0.89 mmols, 0.5 equivalents) of HATU, and 106.5 mg (0.36 mmols, 0.2 equivalents) of DEPBT in 17.8 mL of dry DCM. The crude reaction was purified by extraction with saturated ammonium chloride followed by flash column chromatography (silica gel, EA/Hex) to yield the pentapeptide (609.1 mg, 45.2% yield).

Rf: 0.25 (EtOAc: Hex 1:1)
\[ ^1 \text{H NMR (200 MHz, CDCl}_3\text{): } \delta \text{ 0.8 (d, 6H), 0.9-1.0 (m, 18H), 1.3 (m, 3H), 1.4 (s, 9H), 1.6 (m, 6H), 2.2 (m, 1H), 3.1 (m, 2H), 3.6 (s, 3H), 4.1 (m, 2\alpha H), 4.4 (br, 2\alpha H), 4.7 (dd, \alpha H), 7.0 (s, 1H), 7.1-7.2 (m, 2H), 7.4 (d, 1H), 7.6 (d, 1H) } \]

**Pentapeptide HO-\text{d-Trp-Leu-Val-Leu-Leu-NH}_2**

Pentapeptide HO-\text{d-Trp-Leu-Val-Leu-Leu-NH}_2 was synthesized following the “**In-situ Double Deprotection Procedure**” utilizing 181.0 mg (0.24 mmol, 1.0 equivalents) of MeO-\text{d-Trp-Leu-Val-Leu-Leu-NHBoc} and 0.10 mL (2.1 mmol, 4.0 equivalents) of anisole in 8.0 mL of THF. Once dissolved, 10 drops of concentrated HCl were added to the solution and, over 4 additional days, 3-4 drops were added for a total of 24 drops. The reaction was complete in 6 days and the pentapeptide was taken on to the next reaction without further purification or characterization (153.5 mg, quantitative yield).

**Macrocycle \text{d-Trp-Leu-Val-Leu-Leu}**

Macrocycle \text{d-Trp-Leu-Val-Leu-Leu} was synthesized following the “**Macrocyclization Procedure**” utilizing 191.1 mg (0.30 mmols, 1.0 equivalents) of linear pentapeptide, 0.62 mL (3.6 mmol, 12.0 equivalents) of DIPEA, 28.6 mg (0.09 mmols, 0.3 equivalents) of TBTU, 56.5 mg (0.15 mmols, 0.5 equivalents) HATU, and 44.5 mg (0.52 mmols, 0.5 equivalents) of DEPBT in 42.5 mL of dry DCM and 7.0 mL of dry ACN. The crude reaction was purified by extraction with saturated ammonium chloride followed by flash column chromatography (silica gel, EA/Hex). The impure product was further purified by reverse phase-HPLC to yield the macrocycle (23.6 mg, 12.7% yield).

Rf: 0.1 (EtOAc:Hex, 1:1)
San A 5 and DiSan A 5- Dipeptide
MeO-D-Phe-Leu-NHBoc

Dipeptide MeO-D-Phe-Leu-NHBoc was synthesized following the “General Peptide Synthesis Procedure” utilizing 480 mg (2.2 mmols, 1.1 equivalents) of amine MeO-D-Phe-NH₂, 505 mg (2.0 mmols, 1.0 equivalents) of acid HO-Leu-NHBoc, 1.4 mL (4 equivalents) of DIPEA and 772 mg (2.4 mmols, 1.2 equivalents) of TBTU in 22.3 mL of dry DCM. The crude reaction was purified by acid-base extraction to yield the dipeptide (786 mg, 94% yield).

Rf: 0.9 (EtOAc:Hex, 1:1)

Dipeptide HO-D-Phe-Leu-NHBoc

Dipeptide HO-D-Phe-Leu-NHBoc was synthesized following the “General Acid Deprotection Procedure” utilizing 736.4 mg (1.9 mmol, 1.0 equivalents) of MeO-D-Phe-Leu-NHBoc and 629.6 mg (15.0 mmol, 8.0 equivalents) of LiOH in 18.8 mL of MeOH. This dipeptide was taken on to the next reaction without further purification or characterization (632 mg, 89% yield).
**Dipeptide MeO-Leu-Leu-NHBoc**

Dipeptide MeO-Leu-Leu-NHBoc was synthesized following the “General Peptide Synthesis Procedure” utilizing 801 mg (4.4 mmols, 1.1 equivalents) of amine MeO-Leu-NH₂, 1.0 g (4.0 mmols, 1.0 equivalents) of acid HO-Leu-NHBoc, 2.8 mL (4 equivalents) of DIPEA and 1.5 g (4.8 mmols, 1.2 equivalents) of TBTU in 40.1 mL of dry DCM. The crude reaction was purified by acid-base extraction to yield the dipeptide (1.43 g, 99% yield).

Rf: 0.9 (EtOAc:Hex, 1:1)

¹H NMR (200 MHz, CDCl₃): δ 0.9-1.0 (m, 12H), 1.4(s, 9H), 1.6-1.8 (t, 6H), 3.7 (s, 3H), 4.0-4.1 (dd, αH), 4.5-4.7 (m, αH), 4.8-4.9 (br, 1H), 6.4 (d, 1H)

**Dipeptide HO-Leu-Leu-NHBoc**

Dipeptide HO-Leu-Leu-NHBoc was synthesized following the “General Acid Deprotection Procedure” utilizing 1.43 g (4.0 mmol, 1.0 equivalents) of MeO-Leu-Leu-NHBoc and 1.35 g (32.1 mmol, 8.0 equivalents) of LiOH in 40.1 mL of MeOH. This dipeptide was taken on to the next reaction without further purification or characterization (1.2 g, 82% yield).

**Tripeptide MeO-d-Val-N-Me-Leu-Leu-NHBoc**

Tripeptide MeO-d-Val-N-Me-Leu-Leu-NHBoc was synthesized following the “General Peptide Synthesis Procedure” utilizing 258 mg (2.3 mmols, 1.1 equivalents) of amine MeO-d-Val-N-Me-H, 563 mg (1.6 mmols, 1.0 equivalents) of acid HO-Leu-Leu-NHBoc, 1.1 mL (4 equivalents) of DIPEA and 620 mg (1.6 mmols, 1.0 equivalents) of HATU in 15.7 mL of dry DCM. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the tripeptide (341 mg, 40% yield).
Rf: 0.5 (EtOAc:Hex, 1:1)

$^1$H NMR (200 MHz, CDCl$_3$): $\delta$ 0.9-1.1 (m, 18H), 1.5 (s, 9H), 1.6-1.8 (m, 1H), 2.1 (m, 2H), 2.8 (s, 1H), 3.0 (d, 3H), 3.7 (s, 3H), 4.0-4.2 (br, 2H), 4.2 (m, $\alpha$H), 5.0 (s, $\alpha$H), 5.1 (s, $\alpha$H), 5.2-5.4 (br, $\alpha$H), 5.5 (m, 1H), 6.8 (d, 1H)

**Tripeptide MeO-$\beta$-Val-$\eta$-Me-Leu-Leu-NH$_2$**

Tripeptide MeO-$\beta$-Val-$\eta$-Me-Leu-Leu-NH$_2$ was synthesized following the “General Amine Deprotection Procedure” utilizing 341 mg (0.62 mmol, 1.0 equivalents) of MeO-$\beta$-Val-$\eta$-Me-Leu-Leu-NHBoc and 0.22 mL (1.3 mmol, 2.0 equivalents) of anisole in 1.6 mL of TFA and 4.7 mL of DCM. This tripeptide was taken on to the next reaction without further purification or characterization (278 mg, quantitative yield).

**Pentapeptide MeO-$\beta$-Phe-Leu-$\beta$-Val-$\eta$-Me-Leu-Leu-NHBoc**

Pentapeptide MeO-$\beta$-Phe-Leu-$\beta$-Val-$\eta$-Me-Leu-Leu-NHBoc was synthesized following the “General Peptide Synthesis Procedure” utilizing 278 mg (0.63 mmols, 1.1 equivalents) of amine MeO-$\beta$-Val-$\eta$-Me-Leu-Leu-NH$_2$, 217 mg (0.57 mmols, 1.0 equivalents) of acid HO-$\beta$-Phe-Leu-NHBoc, 0.40 mL (5.0 equivalents) of DIPEA, 172 mg (0.57 mmols, 1.0 equivalents) of DEPBT and 45 mg (0.11 mmols, 0.2 equivalents) HATU in 4.1 mL of dry DCM. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the tripeptide (229 mg, 55% yield).

Rf: 0.35 (EtOAc:Hex, 1:1)
$^1$H NMR (400 MHz, CD$_3$OD): $\delta$ 0.7-0.9 (m, 24H), 1.4 (s, 9H), 1.5-1.7 (m, 6H), 1.8 (m, 4H), 2.2 (m, 2H), 3.1 (s, 3H), 3.5 (s, 3H), 4.1 (m, aH), 4.5 (m, 2aH), 4.7-4.9 (m, 2aH), 5.1 (d, 1H), 6.7 (d, 1H), 6.8 (m, 1H), 7.0 (d, 1H), 7.1-7.3 (m, 5H)

**Pentapeptide**

**HO-d-Phe-Leu-d-Val-N-Me-Leu-Leu-NHBoc**

Pentapeptide HO-d-Phe-Leu-d-Val-N-Me-Leu-Leu-NHBoc was synthesized following the “General Acid Deprotection Procedure” utilizing 229.0 mg (0.41 mmol, 1.0 equivalents) of MeO-d-Phe-Leu-d-Val-N-Me-Leu-Leu-NHBoc and 137.1 mg (3.3 mmol, 8.0 equivalents) of LiOH in 8.0 mL of MeOH. This pentapeptide was taken on to the next reaction without further purification or characterization (224.0 mg, 99.6% yield).

**Pentapeptide**

**HO-d-Phe-Leu-d-Val-N-Me-Leu-Leu-NH$_2$**

Pentapeptide HO-d-Phe-Leu-d-Val-N-Me-Leu-Leu-NH$_2$ was synthesized following the “General Amine Deprotection Procedure” utilizing 224.0 mg (0.31 mmol, 1.0 equivalents) of HO-d-Phe-Leu-d-Val-N-Me-Leu-Leu-NHBoc and 0.05 mL (0.47 mmol, 1.5 equivalents) of anisole in 0.78 mL of TFA and 2.34 mL of DCM. This pentapeptide was taken on to the next reaction without further purification or characterization (193.2 mg, quantitative yield).

**Macrocycle d-Phe-Leu-d-Val-N-Me-Leu-Leu (SanA 5)**

and **d-Phe-Leu-d-Val-N-Me-Leu-Leu-d-Phe-Leu-d-Val-N-Me-Leu-Leu (DiSanA 1005)**

Macrocycles D-Phe-Leu-D-Val-N-Me-Leu-Leu and D-Phe-Leu-D-Val-N-Me-Leu-Leu-D-Phe-Leu-D-Val-N-Me-Leu-Leu were synthesized following the “Macrocyclization Procedure” utilizing 193.2 mg (0.31 mmols, 1.0 equivalents) of linear pentapeptide, 0.6 mL
(11.0 equivalents) of DIPEA, 50.3 mg (0.16 mmols, 0.5 equivalents) of TBTU, 83.3 mg (0.22 mmols, 0.7 equivalents) HATU and 46.8 mg (0.16 mmols, 0.5 equivalents) of DEPBT in 3.0 mL of dry DCM and 1.0 mL of dry ACN. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex). The impure products were further purified by reverse phase-HPLC to yield the macrocycles SanA 5 (12.8 mg, 6.8% yield) and DiSanA 1005 (2.2 mg, 1.2% yield).

**SanA 5**
Rf: 0.25 (EtOAc:Hex, 1:1)

$^1$H NMR (400 MHz, CD3OD): $\delta$ 0.8-1.0 (m, 24H), 1.3-1.8 (m, 9H), 2.2 (m, 1H), 2.8 (s, 3H), 2.9-3.1 (m, 2H), 4.0 (m, $\alpha$H), 4.3 (m, $\alpha$H), 4.5 (m, 2$\alpha$H), 4.8 (m, $\alpha$H), 7.0 (d, 1H), 7.2-7.3 (m, 5H), 7.6 (d, 1H), 8.3 (d, 1H), 8.7 (d, 1H)

LCMS: m/z calcd for C$_{33}$H$_{53}$N$_5$O$_5$ (M+Na$^+$) = 622.8, found 622.5

**Disana 1005**
Rf: 0.25 (EtOAc:Hex, 1:1)

$^1$H NMR (400 MHz, CD3OD): $\delta$ 0.8-1.0 (m, 48H), 1.3-1.8 (m, 18H), 2.2 (m, 2H), 2.8 (s, 6H), 2.9-3.1 (m, 4H), 4.0 (m, 2$\alpha$H), 4.3 (m, 2$\alpha$H), 4.5 (m, 4$\alpha$H), 4.8 (m, 2$\alpha$H), 7.0 (d, 2H), 7.2-7.3 (m, 10H), 7.6 (d, 2H), 8.3 (m, 2H), 8.7 (s, 2H)

LCMS: m/z calcd for C$_{66}$H$_{106}$N$_{10}$O$_{10}$ (M+Na$^+$) = 1222.6, found 1222.5

**SanA 6 and DiSanA 1006- Dipeptide MeO-d-Phe-Leu-NHBoc**

Dipeptide MeO-d-Phe-Leu-NHBoc was synthesized following the “General Peptide Synthesis Procedure” utilizing 380.7 mg (1.8 mmols, 1.1 equivalents) of amine OMe-d-Phe-NH$_2$·HCl, 400 mg (1.6 mmols, 1.0 equivalents) of acid HO-Leu-NHBoc·H$_2$O, 3.0 mL
(11.0 equivalents) of DIPEA and 566.7 mg (1.8 mmols, 1.1 equivalents) of TBTU in 16 mL of dry DCM and 5.0 mL of dry ACN. The crude reaction was purified by acid-base extraction to yield the dipeptide (622.1 mg, 98.8% yield).

Rf: 0.9 (EtOAc:Hex, 1:1)

$^1$H NMR (200 MHz, CDCl$_3$): $\delta$ 0.8-1.0 (m, 6H), 1.4 (s, 9H), 1.4-1.7 (m, 3H), 3.0-3.2 (m, 2H), 3.7 (s, 3H), 4.0-4.2 (br, $\alpha$H), 4.8 (d, 1H), 4.8-5.0 (q, $\alpha$H), 6.6 (d, 1H), 7.1-7.4 (m, 5H)

**Dipeptide MeO- D-Phe-Leu-NH$_2$**

Dipeptide MeO-D-Phe-Leu-NH$_2$ was synthesized following the “General Amine Deprotection Procedure” utilizing 622.1 mg (1.6 mmol, 1.0 equivalents) of MeO-D-Phe-Leu-NHBoc and 0.22 mL (2.1 mmol, 1.3 equivalents) of anisole in 4.0 mL of TFA and 11.9 mL of DCM. This dipeptide was taken on to the next reaction without further purification or characterization (464.0 mg, quantitative yield).

**Tripeptide MeO-D-Phe-Leu-Val-NHBoc**

Tripeptide MeO-D-Phe-Leu-Val-NHBoc was synthesized following the “General Peptide Synthesis Procedure” utilizing 464.0 mg (1.6 mmols, 1.1 equivalents) of amine MeO-D-Phe-Leu-NH$_2$, 313.1 mg (1.4 mmols, 1.0 equivalents) of acid HO-Val-NHBoc, 2.6 mL (10.0 equivalents) of DIPEA and 508.9 mg (1.6 mmols, 1.1 equiv.) of TBTU in 12.0 mL of DCM and 3.0 mL of ACN. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the tripeptide (614.4 mg, 86.7% yield).

Rf: 0.5 (EtOAc:Hex, 1:1)
$^1$H NMR (200 MHz, CDCl$_3$): δ 0.8-1.0 (m, 12H), 1.4 (s, 9H), 1.4-1.6 (m, 2H), 1.7 (s, 1H),
2.1 (m, 1H), 3.0-3.2 (m, 2H), 3.7 (s, 3H), 3.8 (dd, αH), 4.4 (dd, αH), 4.8 (dd, αH), 5.0 (d, 1H), 6.3 (d, 1H), 6.6 (d, 1H), 7.1-7.3 (m, 5H)

**Tripeptide MeO-δ-Phe-Leu-Val-NH$_2$**
Tripeptide MeO-δ-Phe-Leu-NH$_2$ was synthesized following the “**General Amine Deprotection Procedure**” utilizing 614.4 mg (1.2 mmol, 1.0 equivalents) of MeO-D-Phe-Leu-NHBoc and 0.25 mL (2.2 mmol, 1.8 equivalents) of anisole in 3.1 mL of TFA and 9.4 mL of DCM. This tripeptide was taken on to the next reaction without further purification or characterization (489.3 mg, quantitative yield).

**Dipeptide MeO-Leu-δ-Leu-NHBoc**
Dipeptide MeO-Leu-δ-Leu-NHBoc was synthesized following the “**General Peptide Synthesis Procedure**” utilizing 320.7 mg (1.8 mmols, 1.1 equivalents) of amine MeO-Leu-NH$_2$·HCl, 400 mg (1.6 mmols, 1.0 equivalents) of acid HO-D-Leu-NHBoc, 2.2 mL (8.0 equivalents) of DIPEA and 566.7 mg (1.8 mmols, 1.1 equivalents) of TBTU in 16.0 mL of dry DCM and 4 mL of dry ACN. The crude reaction was purified by acid-base extraction to yield the dipeptide (530.3 mg, 92.2% yield).

Rf: 0.8 (EtOAc:Hex, 1:1)

$^1$H NMR (200 MHz, CDCl$_3$): δ 0.9-1.0 (d, 12H), 1.4 (s, 9H), 1.4-1.8 (m, 6H), 3.7 (s, 3H), 4.1 (br, αH), 4.6 (br, αH), 4.8 (br, 1H), 6.6 (d, 1H).

**Dipeptide HO-Leu-δ-Leu-NHBoc**
Dipeptide HO-Leu-δ-Leu-NHBoc was synthesized following the “**General Acid Deprotection Procedure**” utilizing 521.2 mg (1.5 mmol, 1.0 equivalents) of MeO-Leu-δ-Leu-NHBoc and 487.9 mg (11.6 mmol, 8.0 equivalents) of LiOH in 14.5 mL of MeOH. This
Pentapeptide MeO-D-Phe-Leu-Val-Leu-D-Leu-NHBoc
Pentapeptide MeO-D-Phe-Leu-Val-Leu-D-Leu-NHBoc was synthesized following the “General Peptide Synthesis Procedure” utilizing 489.3 mg (1.3 mmols, 1.0 equivalents) of amine MeO-D-Phe-Leu-Val-NH₂, 491.4 mg (1.4 mmols, 1.1 equivalents) of acid HO-Leu-D-Leu-NHBoc, 1.0 mL (5.0 equivalents) of DIPEA, 200.6 mg (0.63 mmols, 0.5 equivalents) of TBTU, 237.6 mg (0.63 mmols, 0.5 equivalents) of HATU and 74.8 mg (0.25 mmols, 0.2 equivalents) of DEPBT in 12.5 mL of methylene chloride and 2.0 mL acetonitrile. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the pentapeptide (201.3 mg, 23.4% yield).

Rf: 0.4 (EtOAc:Hex, 1:1)

^1H NMR (400 MHz, CD₃OD): δ 0.8-1.0 (m, 24H), 1.3 (m, 2H), 1.4 (s, 9H), 1.5 (m, 3H), 1.6 (m, 2H), 1.7 (m, 2H), 2.2 (m, 1H), 2.9-3.2 (m, 2H), 3.7 (s, 3H), 4.1 (m, αH), 4.2 (m, αH), 4.4 (m, 2αH), 4.6 (m, αH), 7.5-7.7 (m, 5H)

Pentapeptide HO-D-Phe-Leu-Val-Leu-D-Leu-NHBoc
Pentapeptide HO-D-Phe-Leu-Val-Leu-D-Leu-NHBoc was synthesized following the “General Acid Deprotection Procedure” utilizing 201.3 mg (0.28 mmol, 1.0 equivalents) of MeO-D-Phe-Leu-Val-Leu-D-Leu-NHBoc and 94.1 mg (2.2 mmol, 8.0 equivalents) of LiOH in 2.8 mL of MeOH and 2.0 mL of DMF. This pentapeptide was taken on to the next reaction without further purification or characterization (197.4 mg, 99.9% yield).
**Pentapeptide HO-\textit{d}-Phe-Leu-Val-Leu-\textit{d}-Leu-NH\textsubscript{2}**

Pentapeptide HO-\textit{d}-Phe-Leu-Val-Leu-\textit{d}-Leu-NH\textsubscript{2} was synthesized following the “**General Amine Deprotection Procedure**” utilizing 197.4 mg (0.28 mmol, 1.0 equivalents) of HO-\textit{d}-Phe-Leu-Val-Leu-\textit{d}-Leu-NHBoc and 0.04 mL (0.39 mmol, 1.4 equivalents) of anisole in 0.7 mL of TFA and 2.1 mL of DCM. This pentapeptide was taken on to the next reaction without further purification or characterization (169.3 mg, quantitative yield).

**Macrocycle \textit{d}-Phe-Leu-Val-Leu-\textit{d}-Leu (SanA 6) and \textit{d}-Phe-Leu-Val-Leu-\textit{d}-Leu-\textit{d}-Phe-Leu-Val-Leu-\textit{d}-Leu (DiSanA 1006)**

Macrocycles \textit{d}-Phe-Leu-Val-Leu-\textit{d}-Leu and \textit{d}-Phe-Leu-Val-Leu-\textit{d}-Leu-\textit{d}-Phe-Leu-Val-Leu-\textit{d}-Leu were synthesized following the “**Macrocyclization Procedure**” utilizing 169.3 mg (0.28 mmols, 1.0 equivalents) of linear pentapeptide, 0.7 mL (15.0 equivalents) of DIPEA, 45.0 mg (0.14 mmols, 0.5 equivalents) of TBTU, 74.6 mg (0.2 mmols, 0.7 equivalents) HATU, and 41.9 mg (0.14 mmols, 0.5 equivalents) of DEPBT in 15 mL methylene chloride, 4 mL acetonitrile and 2 mL dimethyl formamide. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex). The impure products were further purified by reverse phase-HPLC to yield the macrocycles **SanA 6** (12.3 mg, 7.4% yield) and **DiSanA 1006** (1.5 mg, 0.5% yield).

**SanA 6**

Rf: 0.25 (EtOAc:Hex, 1:1)

\(^1\)H NMR (400 MHz, CD\textsubscript{3}OD): \(\delta\) 0.7-1.0 (m, 24H), 1.2-1.8 (m, 9H), 2.0 (m, 1H), 2.9-3.1 (m, 2H), 3.6 (m, \(a\)H), 3.8 (m, \(a\)H), 4.2 (m, \(a\)H), 4.5 (m, \(a\)H), 4.6 (m, \(a\)H), 7.1-7.3 (m, 5H), 6.6 (d, 1H), 7.0 (d, 1H), 7.5 (d, 1H), 7.6 (d, 1H), 8.0 (d, 1H)

LCMS: m/z calcd for C\textsubscript{32}H\textsubscript{51}N\textsubscript{5}O\textsubscript{5} (M+1) = 586.4, found 587.5
**DiSANA 1006**
Rf: 0.25 (EtOAc:Hex, 1:1)

$^1$H NMR (400 MHz, CD$_3$OD): δ 0.7-1.0 (m, 48H), 1.3-1.8 (m, 18H), 2.3 (m, 2H), 2.9-3.1 (m, 4H), 3.6 (m, 2αH), 3.8 (m, 2αH), 4.2 (m, 2αH), 4.5 (m, 2αH), 4.6 (m, 2αH), 7.1-7.3 (m, 10H), 7.6 (d, 4H), 7.9 (d, 2H), 8.3 (d, 2H), 8.4 (s, 2H)

LCMS: m/z calcd for C$_{64}$H$_{102}$N$_{10}$O$_{10}$ (M+1) = 1171.78, found 1172.8

**SanA 7 and 1007- Dioeotide MeO-Phe-Leu-NHBoc**

Dipeptide MeO-Phe-Leu-NHBoc was synthesized following the “General Peptide Synthesis Procedure” utilizing 475.8 mg (2.2 mmols, 1.1 equivalents) of amine MeO-Phe-NH$_2$·HCl, 500 mg (2.0 mmols, 1.0 equivalents) of acid HO-Leu-NHBoc·H$_2$O, 1.4 mL (4.0 equivalents) of DIPEA and 708.4 mg (2.2 mmols, 1.1 equivalents) of TBTU in 20.1 mL of dry DCM. The crude reaction was purified by acid-base extraction to yield the dipeptide (682.1 mg, 86.6% yield).

Rf: 0.9 (EtOAc:Hex, 1:1)

$^1$H NMR (200 MHz, CDCl$_3$): δ 0.8-1.0 (m, 6H), 1.4 (s, 9H), 1.6 (m, 3H), 3.0-3.2 (m, 2H), 3.7 (s, 3H), 4.0-4.2 (br, 1H), 4.8 (br, αH), 4.8-5.0 (q, αH), 6.5 (m, 1H), 7.1-7.4 (m, 5H)

**Dipeptide MeO-Phe-Leu-NH$_2$**

Dipeptide MeO-Phe-Leu-NH$_2$ was synthesized following the “General Amine Deprotection Procedure” utilizing 963.4 mg (2.5 mmol, 1.0 equivalents) of MeO-Phe-Leu-NHBoc and 0.37 mL (3.4 mmol, 1.4 equivalents) of anisole in 6.1 mL of TFA and 18.4 mL of DCM. This dipeptide was taken on to the next reaction without further purification or characterization (718.0 mg, quantitative yield).
**Tripeptide MeO-Phe-Leu-D-Val-NHBoc**

Tripeptide MeO-Phe-Leu-D-Val-NHBoc was synthesized following the “General Peptide Synthesis” Procedure utilizing 960.3 mg (2.5 mmols, 1.1 equivalents) of amine MeO-Phe-Leu-NH₂, 484.8 mg (2.2 mmols, 1.0 equivalents) of acid HO-D-Val-NHBoc, 1.6 mL (4 equivalents) of DIPEA and 788.0 mg (2.5 mmols, 1.1 equivalents) of TBTU in 24.5 mL of dry DCM. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the tripeptide (950.9 mg, 86.7% yield). Rf: 0.7 (EtOAc:Hex, 1:1)

1H NMR (200 MHz, CDCl₃): δ 0.9-1.1 (m, 12H), 1.5 (s, 9H), 1.6-1.7 (m, 2H), 1.8 (s, 1H), 2.1 (m, 1H), 3.1 (m, 2H), 3.7 (s 3H), 3.9 (dd, 1H), 4.4 (br, αH), 4.8 (dd, αH), 5.0 (d, αH), 6.3 (s, 1H), 6.6 (d, 1H), 7.1-7.3 (m, 5H)

**Tripeptide MeO-Phe-Leu-D-Val-NH₂**

Tripeptide MeO-Phe-Leu-D-Val-NH₂ was synthesized following the “General Amine Deprotection Procedure” utilizing 950.9 mg (1.9 mmol, 1.0 equivalents) of MeO-Phe-Leu-D-Val-NHBoc and 0.3 mL (2.7 mmol, 1.4 equivalents) of anisole in 4.8 mL of TFA and 14.5 mL of DCM. This tripeptide was taken on to the next reaction without further purification or characterization (757.4 mg, quantitative yield).

**Dipeptide MeO-D-Leu-Leu-NHBoc**

Dipeptide MeO-D-Leu-Leu-NHBoc was synthesized following the “General Peptide Synthesis Procedure” utilizing 801 mg (4.4 mmols, 1.1 equivalents) of amine MeO-D-Leu-NH₂·HCl, 1.0 g (4.0 mmols, 1.0 equivalents) of acid HO-Leu-NHBoc·H₂O, 2.8 mL (4 equivalents) of DIPEA and 1.5 g (4.8 mmols, 1.2 equivalents) of TBTU in 22.1 mL of dry
DCM and 3.0 mL of dry ACN. The crude reaction was purified by acid-base extraction to yield the dipeptide (1.4 g, 99% yield).

Rf: 0.9 (EtOAc:Hex, 1:1)

$^1$H NMR (200 MHz, CDCl$_3$): δ 0.9-1.0 (m, 12H), 1.4 (s, 9H), 1.6-1.8 (t, 6H), 3.7 (s, 3H), 4.0-4.1 (dd, $\alpha$H), 4.5-4.7 (m, $\alpha$H), 4.8-4.9 (br, 1H), 6.4 (d, 1H)

**Dipeptide HO-d-Leu-Leu-NHBoc**

Dipeptide HO-d-Leu-Leu-NHBoc was synthesized following the “General Acid Deprotection Procedure” utilizing 705.6 mg (2.0 mmol, 1.0 equivalents) of MeO-D-Leu-Leu-NHBoc and 660.3 mg (15.7 mmol, 8.0 equivalents) of LiOH in 19.7 mL of MeOH. This dipeptide was taken on to the next reaction without further purification or characterization (666.6 mg, 98.3% yield).

**Pentapeptide MeO-Phe-Leu-d-Val-d-Leu-Leu-NHBoc**

Pentapeptide MeO-Phe-Leu-d-Val-d-Leu-Leu-NHBoc was synthesized following the “General Peptide Synthesis Procedure” utilizing 758 mg (1.9 mmols, 1.0 equivalents) of amine MeO-Phe-Leu-d-Val-NH$_2$, 666.6 mg (1.9 mmols, 1.0 equivalents) of acid HO-D-Leu-Leu-NHBoc, 3.6 mL (11 equivalents) of DIPEA, 372.6 mg (1.2 mmol, 0.6 equivalents) of TBTU, 115.7 mg (0.38 mmols, 0.2 equivalents) of DEPBT and 441.2 mg (1.1 mmols, 0.6 equivalents) of HATU in 19.3 mL of dry DCM and 5.0 mL of dry ACN. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the pentapeptide (378.7 mg, 27% yield).

Rf: 0.4 (EtOAc:Hex, 1:1)
Pentapeptide HO-Phe-Leu-D-Val-D-Leu-Leu-NHBoc

Pentapeptide HO-Phe-Leu-D-Val-D-Leu-Leu-NHBoc was synthesized following the “Modified Acid Deprotection Procedure” utilizing 368.7 mg (0.28 mmol, 1.0 equivalents) of MeO-Phe-Leu-D-Val-D-Leu-Leu-NHBoc, 64.6 mg (1.5 mmol, 3.0 equivalents) of LiOH and 0.20 mL of H₂O₂ (30% w/v) in 1.3 mL of MeOH. This pentapeptide was taken on to the next reaction without further purification or characterization (299.5 mg, 82.9% yield).

Pentapeptide HO-Phe-Leu-D-Val-D-Leu-Leu-NH₂

Pentapeptide HO-Phe-Leu-D-Val-D-Leu-Leu-NH₂ was synthesized following the “General Amine Deprotection Procedure” utilizing 299.5 mg (0.43 mmol, 1.0 equivalents) of HO-Phe-Leu-D-Val-D-Leu-Leu-NHBoc and 0.06 mL (0.60 mmol, 1.4 equivalents) of anisole in 1.1 mL of TFA and 3.2 mL of DCM. This pentapeptide was taken on to the next reaction without further purification or characterization (257.0 mg, quantitative yield).

Macrocycles Phe-Leu-D-Val-D-Leu-Leu (SanA 7) and Phe-Leu-D-Val-D-Leu-Leu-Phe-Leu-D-Val-D-Leu-Leu (DiSanA 1007)

Macrocycles Phe-Leu-D-Val-D-Leu-Leu and Phe-Leu-D-Val-D-Leu-Leu-Phe-Leu-D-Val-D-Leu-Leu were synthesized following the “Macrocyclization Procedure” utilizing 257.0 mg (0.43 mmols, 1.0 equivalents) of linear pentapeptide, 0.9 mL (12 equivalents) of DIPEA, 68.3 mg (0.21 mmols, 0.5 equivalents) of TBTU, 113.2 mg (0.30 mmols, 0.7 equivalents) of HATU and 63.7 mg (0.21 mmols, 0.5 equivalents) of DEPBT in 4.3 mL of dry DCM and 6.0 mL of dry DMF. The crude reaction was purified by acid-base extraction
followed by flash column chromatography (silica gel, EA/Hex). The impure products were further purified by reverse phase-HPLC to yield the macrocycles \textbf{SanA 7} (11.2 mg, 4.5% yield) and \textbf{DiSanA 1007} (1.3 mg, 0.5% yield).

\textbf{SanA 7}
Rf: 0.25 (EtOAc:Hex, 1:1)

$^1$H NMR (400 MHz, CD3OD): $\delta$ 0.7-1.0 (m, 24H), 1.3-1.8 (m, 9H), 2.0 (m, 1H), 2.9-3.1 (m, 2H), 3.6 (m, $\alpha$H), 3.8 (m, $\alpha$H), 4.2 (m, $\alpha$H), 4.5 (m, $\alpha$H), 4.6 (m, $\alpha$H), 7.1-7.3 (m, 5H), 7.2 (d, 1H), 7.6 (d, 1H), 8.2 (d, 1H), 8.6 (d, 1H), 8.7 (d, 1H)

LCMS: m/z calcd for C$_{32}$H$_{51}$N$_{5}$O$_{5}$ (M+1) = 586.4, found 587.5

\textbf{DiSanA 1007}
Rf: 0.25 (EtOAc:Hex, 1:1)

$^1$H NMR (400 MHz, CD3OD): $\delta$ 0.7-1.0 (m, 48H), 1.3-1.8 (m, 18H), 2.0 (m, 2H), 2.9-3.1 (m, 4H), 3.6 (m, 2$\alpha$H), 3.8 (m, 2$\alpha$H), 4.2 (m, 2$\alpha$H), 4.5 (m, 2$\alpha$H), 4.6 (m, 2$\alpha$H), 7.1-7.3 (m, 10H), 7.2 (d, 2H), 7.6 (d, 2H), 8.2 (d, 2H), 8.6 (d, 2H), 8.7 (s, 2H)

LCMS: m/z calcd for C$_{64}$H$_{102}$N$_{10}$O$_{10}$ (M+1) = 1171.78, found 1172.9

\textbf{SYNTHESIS OF URUKTHAPELSTATIN A}

The following describes the synthesis of all UstatA precursor compounds. $^1$HNMR data of structures are found in Appendix B.

\textbf{Initial Synthesis of Fragment 1: Dipeptide BocNH-Thr(t-Bu)-Ser(Bn)-OMe}
Dipeptide BocNH-Thr(t-Bu)-Ser(Bn)-OMe was synthesized following the “\textbf{General Peptide Synthesis Procedure}” utilizing 601.0 mg (1.8 mmols, 1.1 equivalents) of amine MeO-Ser(Bn)-NH$_2$, 441.4 mg (1.6 mmols, 1.0 equivalents) of acid HO-Thr(t-Bu)-NHBoc,
2.2 mL (8.0 equivalents) of DIPEA, 617.7 mg (1.9 mmols, 1.2 equivalents) of TBTU in 16.0 mL of dry DCM and 3.0 mL of dry ACN. The crude reaction was purified by acid-base extraction to yield the dipeptide (821.7 mg, 99.9% yield).

Rf: 0.8 (EtOAc:Hex, 1:1)

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.2-1.3 (d, 3H), 1.3 (s, 9H), 1.5 (s, 9H), 3.7-3.9 (dd, 2H), 3.8 (s, 3H), 4.2 (m, 2αH), 4.5-4.6 (q, 2H), 4.7 (m, 1H), 5.6 (d, 1H), 7.2-7.4 (m, 5H), 8.0 (d, 1H)

Diepeptide Compound 3

Dipeptide BocNH-Thr(t-Bu)-Ser(Bn)-OH was synthesized following the “**General Acid Deprotection Procedure**” utilizing 747.9 mg (1.6 mmol, 1.0 equivalents) of BocNH-Thr(t-Bu)-Ser(Bn)-OMe and 538.0 mg (12.8 mmol, 8.0 equivalents) of LiOH in 22.0 mL of MeOH. This dipeptide was taken on to the next reaction without further purification or characterization (699.5 mg, 96.4% yield).

**Tripeptide BocNH-Thr(t-Bu)-Ser(Bn)-Ser(Bn)-OMe**

Tripeptide BocNH-Thr(t-Bu)-Ser(Bn)-Ser(Bn)-OMe was synthesized following the “**General Peptide Synthesis Procedure**” utilizing 573.3 mg (1.7 mmols, 1.1 equivalents) of amine H$_2$N-Ser(Bn)-OMe, 699.5 mg (1.5 mmols, 1.0 equivalents) of acid BocNH-Thr(t-Bu)-Ser(Bn)-OH, 1.1 mL (4.0 equivalents) of DIPEA and 595.7 mg (1.9 mmols, 1.2 equivalents) of TBTU in 15.5 mL of dry DCM. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the tripeptide (938.6 mg, 88.2% yield).

Rf: 0.7 (EtOAc:Hex, 1:1)
$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.2-1.3 (d, 3H), 1.3 (s, 9H), 1.5 (s, 9H), 3.6-3.9 (m, 4H), 3.8 (s, 3H), 4.2 (m, 2H), 4.4-4.5 (q, 2H), 4.6 (s, 2H), 4.7 (m, 1H), 4.8 (m, 2H), 5.6 (d, 1H), 7.2-7.4 (m, 10H), 7.6 (d, 1H), 7.8 (d, 1H)

**Tripeptide Compound 4**

Tripeptide BocNH-Thr(\text{-}Bu)-Ser-Ser-OMe was synthesized following the “Hydrogenation Procedure” utilizing 245.3 mg (0.38 mmols, 1.0 equivalents) of tripeptide BocNH-Thr(\text{-}Bu)-Ser-Ser-OMe and 25.0 mg of 10% Pd/C in 3.8 mL of ethanol. The crude reaction was filtered through celite and concentrated *in vacuo* to yield the tripeptide (175.7 mg, 99.5% yield).

Rf: 0.05 (EtOAc:Hex, 1:1)

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.2-1.3 (d, 3H), 1.3 (s, 9H), 1.5 (s, 9H), 3.6-3.9 (m, 4H), 3.8 (s, 3H), 4.2 (m, 2H), 4.5 (m, 2H), 4.6 (m, 1H), 5.6 (d, 1H), 7.6 (d, 1H), 7.7 (d, 1H)

**Dioxazole Precursor Compound 6**

The dioxazole precursor 6 was synthesized following the “General Oxazole Synthesis Procedure” using 821.0 mg (1.8 mmol, 1.0 equivalents) of tripeptide 4, 0.49 mL (3.7 mmol, 2.1 equivalents) of DAST and 783.3 mg (5.7 mmol, 3.2 equivalents) of K$_2$CO$_3$ in 17.7 mL of dry THF for the first step. After filtration through celite, the second step was performed with 0.45 mL (4.5 mmol, 4.0 equivalents) of BrCCl$_3$ and 0.68 mL (4.5 mmol, 4.0 equivalents) of DBU in 11.4 mL of dry DCM. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the dioxazole precursor (224.9 mg, 30.0% yield).

Rf: 0.6 (EtOAc:Hex, 1:1)
Revised Synthesis of Fragment 1:
Dipeptide BocNH-Thr(t-Bu)-Ser(Bn)-OMe

Dipeptide BocNH-Thr(t-Bu)-Ser(Bn)-OMe was synthesized following the “General Peptide Synthesis Procedure” utilizing 2.360 g (11.3 mmols, 1.1 equivalents) of amine MeO-Ser(Bn)-NH2, 2.826 g (10.0 mmols, 1.0 equivalents) of acid HO-Thr(t-Bu)-NHBoc, 7.2 mL (4.0 equivalents) of DIPEA, 3.954 g (12.3 mmols, 1.2 equivalents) of TBTU in 112.9 mL of dry DCM. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the dipeptide (4.981 g, 94.6% yield).

Rf: 0.8 (EtOAc:Hex, 1:1)

1H NMR (400 MHz, CDCl3): δ 1.1 (d, 3H), 1.2 (s, 9H), 1.4 (s, 9H), 3.7-3.9 (dd, 2βH), 3.8 (s, 3H), 4.1 (m, βH), 4.1-4.2 (d, αH), 4.5-4.6 (q, 2H), 4.7 (m, αH), 5.6 (br, 1H), 7.2-7.4 (m, 5H), 8.0 (d, 1H)

Dieptptide Compound 7

Dipeptide BocNH-Thr(t-Bu)-Ser-OMe was synthesized following the “Hydrogenation Procedure” utilizing 4.385 g (9.4 mmols, 1.0 equivalents) of dipeptide BocNH-Thr(t-Bu)-Ser-OMe and 438.5.0 mg of 10% Pd/C in 100.0 mL of ethanol. The crude reaction was filtered through celite and concentrated in vacuo to yield the tripeptide (3.440 g, 97.2% yield).

Rf: 0.4 (EtOAc:Hex, 1:1)

1H NMR (400 MHz, CDCl3): δ 1.1 (d, 3H), 1.2 (s, 9H), 1.4 (s, 9H), 3.7 (q, OH), 3.8 (s, 3H), 3.9-4.0 (q, 2βH), 4.1 (m, βH), 4.1-4.2 (d, αH), 4.6 (br, αH), 5.6 (br, 1H), 7.9 (br, 1H)
Oxazole Dimer Compound 8

The oxazole precursor 8 was synthesized following the “General Oxazole Synthesis Procedure” using 3.440 g (9.1 mmol, 1.0 equivalents) of dipeptide 7, 1.45 mL (11.0 mmol, 1.2 equivalents) of DAST and 2.526 g (18.3 mmol, 2.0 equivalents) of K₂CO₃ in 91.4 mL of dry DCM for the first step. After filtration through celite, the second step was performed with 2.7 mL (27.4 mmol, 3.0 equivalents) of BrCCl₃ and 4.1 mL (27.4 mmol, 3.0 equivalents) of DBU in 91.4 mL of dry DCM. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the oxazole precursor (2.523 g, 77.5% yield).

Rf: 0.8 (EtOAc:Hex, 1:1)

¹H NMR (400 MHz, CDCl₃): δ 1.0 (s, 9H), 1.2 (d, 3H), 1.4 (s, 9H), 3.9 (s, 3H), 4.2 (m, βH), 4.8 (d, αH), 5.6 (d, 1H), 8.2 (s, 1H)

Oxazole Dimer Compound 9

Precursor 9 was synthesized following the “General Acid Deprotection Procedure” utilizing 2.523 g (7.1 mmol, 1.0 equivalents) of 8 and 1.188 g (28.3 mmol, 4.0 equivalents) of LiOH in 70.1 mL of MeOH. This dipeptide was taken on to the next reaction without further purification or characterization (2.412 g, 99.5% yield).

Oxazole Trimer BocNH-Thr(t-Bu)-Ox-Ser(Bn)-OMe

Precursor BocNH-Thr(t-Bu)-Ox-Ser(Bn)-OMe was synthesized following the “General Peptide Synthesis Procedure” utilizing 2.126 g (10.2 mmols, 1.4 equivalents) of amine H₂N-Ser(Bn)-OMe, 2.412 g (7.1 mmols, 1.0 equivalents) of acid 9, 4.9 mL (4.0 equivalents) of DIPEA, 1.591 g (5.0 mmols, 0.7 equivalents) of TBTU, and 1.346 g (3.5 mmol, 0.5 equivalents) of HATU in 70.8 mL of dry DCM. The crude reaction was purified
by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the trimer (3.601 g, 95.3% yield).

Rf: 0.6 (EtOAc:Hex, 1:1)

$^1$H NMR (400 MHz, CDCl$_3$): δ 1.0 (s, 9H), 1.2 (d, 3H), 1.5 (s, 9H), 3.8 (s, 3H), 3.8-4.0 (ddd, 2βH), 4.2 (m, βH), 4.6 (q, 2H), 4.8 (d, αH), 4.9 (dt, αH), 5.5 (d, 1H), 7.3-7.4 (m, 5H), 7.6 (d, 1H), 8.2 (s, 1H)

**Oxazole Trimer Compound 10**

Trimer 10 was synthesized following the “Hydrogenation Procedure” utilizing 2.944 g (5.5 mmols, 1.0 equivalents) of trimer BocNH-Thr(t-Bu)-Ox-Ser(Bn)-OMe and 294.0 mg of 10% Pd/C in 55.2 mL of ethanol. The crude reaction was filtered through celite and concentrated in vacuo to yield the tripeptide (2.443 g, 99.7% yield).

Rf: 0.3 (EtOAc:Hex, 1:1)

$^1$H NMR (400 MHz, CDCl$_3$): δ 1.0 (s, 9H), 1.2 (d, 3H), 1.5 (s, 9H), 3.7 (q, OH), 3.8 (s, 3H), 4.0-4.1 (q, 2βH), 4.2 (br, βH), 4.8 (br, 2αH), 5.5 (d, 1H), 7.7 (d, 1H), 8.2 (s, 1H)

**Dioxazole Precursor Compound 6**

The dioxazole precursor 6 was synthesized following the “General Oxazole Synthesis Procedure” using 2.443 g (5.5 mmol, 1.0 equivalents) of trimer 10, 0.88 mL (6.6 mmol, 1.2 equivalents) of DAST and 1.523 g (11.0 mmol, 2.0 equivalents) of K$_2$CO$_3$ in 55.1 mL of dry DCM for the first step. After filtration through celite, the second step was performed with 1.64 mL (16.5 mmol, 3.0 equivalents) of BrCCl$_3$ and 2.47 mL (16.5 mmol, 3.0 equivalents) of DBU in 55.1 mL of dry DCM. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the dioxazole precursor (1.762 g, 75.6% yield).
1H NMR (400 MHz, CDCl₃): δ 1.0 (s, 9H), 1.3 (d, 3H), 1.5 (s, 9H), 4.0 (s, 3H), 4.2 (m, βH), 4.9 (d, αH), 5.6 (d, 1H), 8.3 (d, 2H)

**Dioxazole Amide BocNH-Thr(t-Bu)-Ox-Ox-CONH₂**

Amide precursor BocNH-Thr(t-Bu)-Ox-Ox-CONH₂ was synthesized following the “Amide Synthesis Procedure” utilizing 1.411 g (3.3 mmol, 1.0 equivalents) of 6 in 13.3 mL of MeOH and 120.0 mL of 25% NH₄OH. This amide was taken on to the next reaction without further purification (1.360 g, 99.9% yield).

Rf: 0.1 (EtOAc:Hex, 1:1)

1H NMR (400 MHz, CDCl₃): δ 1.0 (s, 9H), 1.3 (d, 3H), 1.5 (s, 9H), 4.2 (m, βH), 4.9 (d, αH), 5.6 (d, 1H), 5.9 (br, 1H), 7.0 (br, 1H), 8.2 (s, 1H), 8.3 (s, 1H)

**Dioxazole Thioamide Compound 11**

Thioamide precursor 11 was synthesized following the “Thioamide Synthesis Procedure” utilizing 1.360 g (3.3 mmol, 1.0 equivalents) of BocNH-Thr(t-Bu)-Ox-Ox-CONH₂ and 1.348 g (3.3 mmol, 1.0 equivalents) of Lawesson’s reagent in 47.6 mL of dry THF. The crude reaction was purified by flash column chromatography (silica gel, EA/Hex) to yield the thioamide (1.402g, 99.1% yield).

Rf: 0.6 (EtOAc:Hex, 1:1)

1H NMR (400 MHz, CDCl₃): δ 1.0 (s, 9H), 1.3 (d, 3H), 1.5 (s, 9H), 4.2 (m, βH), 4.9 (d, αH), 5.7 (d, 1H), 7.8 (br, 1H), 8.2 (s, 1H), 8.3 (br, 1H), 8.3 (s, 1H)

**Dioxazole Thiazole Tetramer Compound 12**

The thiazole for the tetramer 12 was synthesized following the “Hantzsch Thiazole Synthesis Procedure” using 1.402 g (3.3 mmol, 1.0 equivalents) of thioamide 11, 1.39 mL
(9.9 mmol, 3.0 equivalents) of BPEE and 2.645 g (26.4 mmol, 8.0 equivalents) of KHCO₃ in 8.26 mL of dry DCM for the first step. After filtration through celite, the second step was performed with 1.84 mL (13.2 mmol, 4.0 equivalents) of TFAA and 3.46 mL (30.0 mmol, 9.0 equivalents) of 2,6-lutidine in 13.0 mL of dry DCM. The crude reaction was purified by base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the thiazole tetramer (1.319 g, 76.7% yield).

Rf: 0.5 (EtOAc:Hex, 1:1)

1H NMR (400 MHz, CDCl₃): \( \delta \) 1.0 (s, 9H), 1.3 (d, 3H), 1.4 (t, 3H), 1.5 (s, 9H), 4.2 (m, \( \beta \)H), 4.4 (q, 2H), 4.9 (d, \( \alpha \)H), 5.6 (d, 1H), 8.2 (s, 1H), 8.3 (s, 1H), 8.4 (s, 1H)

Dioxazole Thiazole Amide

**BocNH-Thr(t-Bu)-Ox-Ox-Th-CONH₂**

Amide precursor BocNH-Thr(t-Bu)-Ox-Ox-Th-CONH₂ was synthesized following the “Amide Synthesis Procedure” utilizing 1.319 g (2.5 mmol, 1.0 equivalents) of 12 in 10.1 mL of MeOH and 91.2 mL of 25% NH₄OH. This amide was taken on to the next reaction without further purification (1.244 g, 99.9% yield).

Rf: 0.1 (EtOAc:Hex, 1:1)

1H NMR (400 MHz, CD₃OD): \( \delta \) 1.0 (s, 9H), 1.2 (d, 3H), 1.5 (s, 9H), 4.2 (m, \( \beta \)H), 4.9 (br, \( \alpha \)H), 6.6 (d, 1H), 8.3 (s, 1H), 8.6 (s, 1H), 8.6 (s, 1H)

**Fragment 1 BocNH-Thr(t-Bu)-Ox-Ox-Th-CSNH₂**

Fragment 1 was synthesized following the “Thioamide Synthesis Procedure” utilizing 1.244 g (2.5 mmol, 1.0 equivalents) of BocNH-Thr(t-Bu)-Ox-Ox-Th-CONH₂ and 1.025 g (2.5 mmol, 1.0 equivalents) of Lawesson’s reagent in 36.2 mL of dry THF. The
crude reaction was purified by flash column chromatography (silica gel, EA/Hex) to yield the thioamide (811.7 mg, 63.1% yield).

Rf: 0.5 (EtOAc:Hex, 1:1)

\[^1\text{H} \text{NMR (400 MHz, CDCl}_3\text{): } \delta 1.0 \text{ (s, 9H), 1.3 (d, 3H), 1.5 (s, 9H), 4.2 (m, } \beta \text{H), 4.9 (d, } \alpha \text{H), 5.6 (d, 1H), 7.9 (d, 1H), 8.2 (s, 1H), 8.2 (s, 1H), 8.5 (s, 1H), 8.7 (d, 1H)}\]

**Synthesis of Fragment 2: Bromopyruvic Methyl Ester Dimethyl Ketal BrCH\textsubscript{2}C(OMe\textsubscript{2})CO\textsubscript{2}Me**

The ketone and acid of BrCH\textsubscript{2}COCO\textsubscript{2}H (BPA) were protected following the “Ketone Protection Procedure” utilizing 4.00 g (23.5 mmol, 1.0 equivalents) of 90% BPA, 12.8 mL (0.12 mol, 5.0 equivalents) of CH\textsubscript{2}CO\textsubscript{3}H\textsubscript{3} and 1.38 mL (25.8 mmol, 1.1 equivalents) of H\textsubscript{2}SO\textsubscript{4} in 47.0 mL of dry MeOH. The product BrCH\textsubscript{2}C(OMe\textsubscript{2})CO\textsubscript{2}Me was taken on to the next reaction without further purification (3.858 g, 72.4% yield).

Rf: 0.5 (EtOAc:Hex, 1:1)

\[^1\text{H} \text{NMR (400 MHz, CDCl}_3\text{): } \delta 3.3 \text{ (s, 6H), 3.6 (s, 2H), 3.9 (s, 3H)}\]

**Bromopyruvic Acid Dimethyl Ketal Compound 13**

Precursor 13 was synthesized following the “General Acid Deprotection Procedure” utilizing 1.815 g (8.0 mmol, 1.0 equivalents) of BrCH\textsubscript{2}C(OMe\textsubscript{2})CO\textsubscript{2}Me and 2.683 g (64.0 mmol, 8.0 equivalents) of LiOH in 80.1 mL of MeOH. The free acid was taken on to the next reaction without further purification (649 mg, 64.2% yield).

Rf: 0.0 (EtOAc:Hex, 1:1)

\[^1\text{H} \text{NMR (400 MHz, CDCl}_3\text{): } \delta 3.4 \text{ (s, 6H), 3.6 (s, 2H)}\]
**Dimer Compound 14**

The dimer 14 was synthesized following the “General Peptide Synthesis Procedure” utilizing 694.0 mg (3.6 mmols, 1.0 equivalents) of amine \(\text{H}_2\text{N-β-OH-Phe-OMe}\), 833.1 mg (3.9 mmols, 1.1 equivalents) of acid 13, 3.7 mL (6.0 equivalents) of DIPEA, 913.2 mg (2.8 mmols, 0.8 equivalents) of TBTU, 1.081 g (2.8 mmol, 0.8 equivalents) of HATU and 850.9 mg (2.8 mmol, 0.8 equivalents) of DEPBT in 9.0 mL of dry DCM, 1.2 mL of dry DMF and 1.0 mL of dry ACN. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the dimer (854.6 mg, 61.6% yield).

Rf: 0.5 (EtOAc:Hex, 1:1)

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 3.0 (s, 3H), 3.3 (s, 3H), 3.5 (s, 2H), 3.8 (s, 3H), 4.9 (dd, \(\alpha\)H), 5.4 (d, \(β\)H), 7.2-7.4 (m, 5H), 7.6 (d, 1H)

**Phenyloxazole Precursor**

\(\text{BrCH}_2\text{C(OMe)}_2\text{-PheOx-CO}_2\text{Me}\)

The phenyl-oxazole \(\text{BrCH}_2\text{C(OMe)}_2\text{-PheOx-CO}_2\text{Me}\) was synthesized following the “General Oxazole Synthesis Procedure” using 786.9 g (2.0 mmol, 1.0 equivalents) of dimer 14, 0.32 mL (2.4 mmol, 1.2 equivalents) of DAST and 4.87 mL (6.1 mmol, 3.0 equivalents) of pyridine in 20.2 mL of dry THF for the first step. The crude phenyloxazoline was filtered through celite and purified by flash column chromatography (silica gel, EA/Hex) to yield the semi-pure product (566.3 mg, 78.4% yield).

The second step was performed with 0.45 mL (4.6 mmol, 3.0 equivalents) of \(\text{BrCCl}_3\) and 0.68 mL (4.6 mmol, 3.0 equivalents) of DBU in 15.2 mL of dry DCM. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the dioxazole precursor (442.2 mg, 78.6% yield).
Phenyloxazole Precursor Compound 15

Precursor 15 was synthesized following the “General Acid Deprotection Procedure” utilizing 442.2 mg (1.2 mmol, 1.0 equivalents) of BrCH2C(OMe)2-PheOx-CO2Me and 200.5 mg (4.8 mmol, 4.0 equivalents) of LiOH in 12.0 mL of MeOH. The free acid was taken on to the next reaction without further purification (384.2 mg, 90.2% yield).

Rf: 0.05 (EtOAc:Hex, 1:1)

$^1$H NMR (400 MHz, CDCl$_3$): δ 3.4 (s, 6H), 3.9 (s, 2H), 7.5 (m, 3H), 8.1 (m, 2H)

Dipeptide NHBoc-D-\textit{allo}-Ile-Ala-OMe

Dipeptide NHBoc-D-\textit{allo}-Ile-Ala-OMe was synthesized following the “General Peptide Synthesis Procedure” utilizing 663.9 mg (4.8 mmols, 1.1 equivalents) of amine H$_2$N-Ala-OMe·HCl, 1.00 g (4.3 mmols, 1.0 equivalents) of acid NHBoc-D-\textit{allo}-Ile-OH, 3.0 mL (4.0 equivalents) of DIPEA, 971.8 mg (3.0 mmols, 0.7 equivalents) of TBTU and 821.9 mg (2.2 mmol, 0.5 equivalents) of HATU in 43.2 mL of dry DCM. The crude reaction was purified by acid-base extraction to yield the dipeptide (1.361 g, 99.5% yield).

Rf: 0.6 (EtOAc:Hex, 1:1)

$^1$H NMR (400 MHz, CDCl$_3$): δ 0.8-0.9 (d, 3H), 0.9 (t, 3H), 1.2-1.4 (dm, 2H), 1.4 (d, 3H), 1.4-1.5 (s, 9H), 2.0 (m, 1H), 3.7 (s, 3H), 4.1 (br, αH), 4.6 (m, αH), 4.9 (br, 1H), 6.4 (d, 1H).

Dipeptide Compound 16

Dipeptide 16 was synthesized following the “General Amine Deprotection Procedure” utilizing 1.361 g (4.3 mmol, 1.0 equivalents) of NHBoc-D-\textit{allo}-Ile-Ala-OMe and 0.70 mL (6.1 mmol, 1.4 equivalents) of anisole in 10.8 mL of TFA and 32.4 mL of DCM.
This dipeptide was taken on to the next reaction without further purification or characterization (931.0 mg, quantitative yield).

**Fragment 2**

BrCH$_2$C(OMe)$_2$-PheOx-d-**allo**-Ile-Ala-OMe

Fragment 2 BrCH$_2$C(OMe)$_2$-PheOx-d-**allo**-Ile-Ala-OMe was synthesized following the “**General Peptide Synthesis Procedure**” utilizing 284.3 mg (1.3 mmols, 1.1 equivalents) of amine 16, 425.7 mg (1.2 mmols, 1.0 equivalents) of acid 15, 0.83 mL (4.0 equivalents) of DIPEA, 191.8 mg (0.60 mmols, 0.5 equivalents) of TBTU, 227.2 mg (0.60 mmol, 0.5 equivalents) of HATU and 107.3 mg (0.36 mmol, 0.3 equivalents) of DEPBT in 12.0 mL of dry DCM and 4.0 mL of dry ACN. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the trimer (588.6 mg, 88.8% yield).

Rf: 0.5 (EtOAc:Hex, 1:1)

$^1$H NMR (400 MHz, CDCl$_3$): δ 0.9 (t, 3H), 1.0 (d, 3H), 1.2-1.5 (dm, 2H), 1.4(d, 3H), 2.2 (m, 1H), 3.4 (s, 6H), 3.7 (s, 3H), 3.9 (s, 2H), 4.6 (m, 2αH), 6.6 (d, 1H), 7.4 (m, 3H), 7.6 (d, 1H), 8.3 (d, 2H).

**Synthesis of Linear Precursor: Fragment 2 Free Acid**

BrCH$_2$C(OMe)$_2$-PheOx-d-**allo**-Ile-Ala-OH

Deprotected fragment 2 BrCH$_2$C(OMe)$_2$-PheOx-d-**allo**-Ile-Ala-OH was synthesized following the “**Modified Acid Deprotection Procedure**” utilizing 588.6 mg (1.1 mmol, 1.0 equivalents) of BrCH$_2$C(OMe)$_2$-PheOx-d-**allo**-Ile-Ala-OMe, 133.6 mg (3.2 mmol, 3.0 equivalents) of LiOH and 0.41 mL of H$_2$O$_2$ (30% w/v) in 2.7 mL of MeOH. The reaction was purified via acid extraction to yield the free acid (572.0 mg, 99.7% yield).

Rf: 0.1 (EtOAc:Hex, 1:1)
1^H NMR (400 MHz, CDCl₃): δ 1.0 (m, 6H), 1.2-1.6 (dm, 2H), 1.5 (d, 3H), 2.0 (m, 1H), 3.4 (s, 6H), 3.9 (s, 2H), 4.6 (m, αH), 4.9 (dd, αH), 7.2 (d, 1H), 7.4 (m, 3H), 7.9 (d, 1H), 8.1 (m, 2H)

**Fragment 1 Free Amine**

H₂N-Thr(t-Bu)-Ox-Ox-Th-CSNH₂

Deprotected fragment 1 H₂N-Thr(t-Bu)-Ox-Ox-Th-CSNH₂ was synthesized following the “Modified Amine Deprotection Procedure” utilizing 113.1 mg (0.22 mmol, 1.0 equivalents) of BocNH-Thr(Ot-Bu)-Ox-Ox-Th-CSNH₂ in 4.5 mL of 4 M HCl in dioxane. The fragment was taken on to the next reaction without further purification (90.8 mg, quantitative yield).

Rf: 0.0 (EtOAc:Hex, 1:1)

1^H NMR (400 MHz, CD₃OD): δ 1.0 (s, ~6H), 1.4 (d, 3H), 4.4 (m, βH), 4.7 (d, αH), 8.4 (br, 1H), 8.6 (br, 1H), 8.8 (br, 1H)

**Linear Precursor via Peptide Coupling Compound 17**

The linear precursor 17 was synthesized following the “General Peptide Synthesis Procedure” utilizing 90.8 mg (0.22 mmols, 1.0 equivalents) of amine 1, 126.4 mg (0.23 mmols, 1.05 equivalents) of acid 2, 0.46 mL (12.0 equivalents) of DIPEA, 35.8 mg (0.11 mmols, 0.5 equivalents) of TBTU, 84.8 mg (0.22 mmol, 1.0 equivalents) of HATU and 33.3 mg (0.11 mmol, 0.5 equivalents) of DEPBT in 3.2 mL of dry DCM, 3.0 mL of dry DMF and 2.0 mL of dry ACN. The crude reaction was purified by acid-base extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the linear precursor (49.5 mg, 24.5% yield).

Rf: 0.3 (EtOAc:Hex, 1:1)
$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 0.9 (t, 3H), 1.0 (m, ~9H), 1.2-1.6 (m, 9H), 2.2 (m, 1H), 3.4 (s, 6H), 3.9 (s, 2H), 4.1 (m, 3H), 4.6 (m, 1H), 5.2 (d, 1H), 7.4 (m, 3H), 7.6 (m, 2H), 8.2 (m, 4H), 8.5 (s, 1H), 8.6 (br, 1H)

**Linear Precursor BrCH$_2$CO-PheOx-\textit{d-allo}-Ile-Ala-Thr(\textit{t}-Bu)-Ox-Ox-Th-CSNH$_2$**

The linear precursor BrCH$_2$CO-PheOx-\textit{d-allo}-Ile-Ala-Thr(\textit{t}-Bu)-Ox-Ox-Th-CSNH$_2$ was attempted following the “Ketone Deprotection Procedure” utilizing 49.5 mg (0.06 mmol, 1.0 equivalents) of 17 in 1.62 mL of HCO$_2$H. The desired product was not successfully isolated.

**Fragment 2 Ketone Deprotection Compound 18**

18 was synthesized following the “Ketone Deprotection Procedure” utilizing 51.0 mg (0.09 mmol, 1.0 equivalents) of BrCH$_2$C(OMe)$_2$-PheOx-\textit{d-allo}-Ile-Ala-OMe in 2.63 mL of HCO$_2$H. The fragment was taken on to the next reaction without further purification (36.8 mg, 78.7% yield).

Rf: 0.5 (EtOAc:Hex, 1:1)

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.0 (m, 6H), 1.2-1.6 (dm, 2H), 1.6 (d, 3H), 2.0 (m, 1H), 3.7 (s, 3H), 4.6 (m, 4H), 6.6 (d, 1H), 7.5 (m, 3H), 7.7 (d, 1H), 8.4 (m, 2H)

**Linear Precursor via Hantzch Compound 19**

The linear precursor 19 was attempted following the “Hantzsch Thiazole Synthesis Procedure” using 108.7 mg (0.21 mmol, 1.0 equivalents) of thioamide BocNH-Thr(\textit{t}-Bu)-Ox-Ox-Th-CSNH$_2$ (1), 107.3 mg (0.21 mmol, 1.0 equivalents) of 18 and 168.9 mg (1.7 mmol, 8.0 equivalents) of KHCO$_3$ in 0.5 mL of dry THF for the first step. After filtration through celite, the second step was performed with 0.12 mL (0.84 mmol, 4.0 equivalents) of
TFAA and 0.22 mL (1.9 mmol, 9.0 equivalents) of 2,6-lutidine in 0.8 mL of dry THF. The crude reaction was purified by aqueous extraction followed by flash column chromatography (silica gel, EA/Hex) to yield the linear precursor (53.2 mg, 27.5% yield).

Rf: 0.4 (EtOAc:Hex, 1:1)

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.0 (m, 12H), 1.1 (d, 3H), 1.3 (d, 6H), 1.5 (m, 11H), 2.2 (m, 1H), 3.7 (s 3H), 4.2 (d, 1H), 4.6 (m, 2H), 4.9 (d, 1H), 5.6 (d, 1H), 6.6 (d, 1H), 7.5 (m, 4H), 7.8 (d, 1H), 8.2 (s, 1H), 8.3 (d, 2H), 8.4 (s, 1H), 8.4 (d, 1H)
REFERENCES


APPENDIX A

$^1$H NMR, LC/MS, AND HPLC SPECTRA FOR SANA AND DISANA DERIVATIVES
SanA 2 NMR Dipeptide MeO-D-Phe-Leu-NHBoc
SanA 2 NMR Tripeptide MeO-D-Phe-Leu-Val-NHBoc
SanA 2 NMR Linear Pentapeptide MeO-d-Phe-Leu-Val-N-Me-Leu-NHBoc
SanA 2 LCMS Linear Pentapeptide HO-d-Phe-Leu-Val-N-Me-Leu-NH₂ (MW=617.8)
SanA 2 NMR Cyclized Pentapeptide d-Phe-Leu-Val-Leu-N-Me-Leu
SanA 2 LCMS Cyclized Pentapeptide D-Phe-Leu-Val-Leu-N-Me-Leu (MW=599.8)
SanA 3 NMR Dipeptide MeO-Phe-δ-Phe-NHBoc
SanA 3 NMR Tripeptide MeO-Phe-d-Phe-Val-NHBoc
SanA 3 NMR Dipeptide MeO-Leu-Leu-NHBoc
SanA 3 NMR Linear Pentapeptide MeO-Phe-δ-Phe-Val-Leu-Leu-NHBoc
SanA 3 LCMS Linear Pentapeptide HO-Phe-d-Phe-Val-Leu-Leu-NH₂ (MW = 637.8)
SanA 3 NMR Cyclized Pentapeptide Phe-d-Phe-Val-Leu-Leu
SanA 3 LCMS Cyclized Pentapeptide Phe-D-Phe-Val-Leu-Leu (MW = 619.8)
SanA 3 HPLC Cyclized Pentapeptide Phe-D-Phe-Val-Leu-Leu
SanA 4 NMR Dipeptide MeO-d-Trp-Leu-NHBoc
SanA 4 NMR Tripeptide MeO-d-Trp-Leu-Yal-NHBoc
SanA 4 NMR Dipeptide MeO-Leu-Leu-NHBoc

Reduction

Sample: Dipeptide MeO-Leu-Leu-NHBoc
Solvent: DMSO-d6

NMR Spectrogram
SanA 4 NMR Linear Pentapeptide MeO-D-Trp-Leu-Val-Leu-Leu-NH{Boc}
SanA 4 LCMS Linear Pentapeptide HO-d-Trp-Leu-Val-Leu-Leu-NH₂ (MW = 642.8)
SanA 4 NMR Cyclized Pentapeptide d-Trp-Leu-Val-Leu-Leu
SanA 4 LCMS Cyclized Pentapeptide d-Trp-Leu-Val-Leu-Leu (MW = 624.8)
SanA 4 HPLC Cyclized Pentapeptide d-Trp-Leu-Val-Leu-Leu
SanA 5/DiSanA 1005 NMR Dipeptide MeO-d-Phe-Leu-NHBoc
SanA 5/DiSanA 1005 NMR Tripeptide MeO-d-Val-N-Me-Leu-Leu-NHBoc
SanA 5/DiSanA 1005 LCMS Linear Pentapeptide HO-D-Val-N-Me-Leu-Leu-D-Phe-Leu-NH₂ (MW = 617.8)
SanA 5 NMR Cyclized Pentapeptide D-Phe-Leu-D-Val-N-Me-Leu-Leu
SanA 5 LCMS Cyclized Pentapeptide D-Phe-Leu-D-Val-N-Me-Leu-Leu (MW = 599.8)
SanA 5 HPLC Cyclized Pentapeptide d-Phe-Leu-d-Val-N-Me-Leu-Leu
DiSanA 1005 NMR Cyclized Decapeptide d-Phe-Leu-d-Val-N-Me-Leu-Leu-d-Phe-Leu-d-Val-N-Me-Leu-Leu
SanA 6/DiSanA 1006 NMR Dipeptide MeO-Leu-D-Leu-NHBoc

1H NMR (CDCl3, ppm): 3.00 (s, 3H), 4.50 (s, 2H), 5.50 (s, 1H), 6.50 (s, 1H), 7.50 (s, 1H), 8.50 (s, 1H).

Other spectral details:
- δ = 1.00 (Me), 2.00 (CH), 3.00 (NHBoc), 4.00 (ester), 5.00 (olefinic), 6.00 (aromatic).
- J = 8.00 Hz (coupling constant).
- Spectral width: 1000 Hz.
- Spectral resolution: 0.01 ppm.

Additional notes:
- Solvent: CDCl3.
- Acquisition parameters: 400 MHz, 8K data points, 0.3 s acquisition time.
SanA 6/DiSanA 1006 NMR Linear Pentapeptide MeO-D-Phe-Leu-Val-Leu-D-Leu-NHBoc
SanA 6/DiSanA 1006 LCMS Linear Pentapeptide HO-D-Phe-Leu-Val-Leu-D-Leu-NH₂
(MW = 603.8)
SanA 6 NMR Cyclized Pentapeptide D-Phe-Leu-Val-Leu-D-Leu
SanA 6 LCMS Cyclized Pentapeptide D-Phe-Leu-Val-Leu-D-Leu (MW = 586.4)
SanA 6 HPLC Cyclized Pentapeptide  D-Phe-Leu-Val-Leu-D-Leu
DiSanA 1006 NMR Cyclized Decapeptide  D-Phe-Leu-Val-Leu-D-Leu-D-Phe-Leu-Val-Leu-D-Leu
DiSanA 1006 LCMS Cyclized Decapeptide  D-Phe-Leu-Val-Leu-D-Leu-D-Phe-Leu-Val-Leu-D-Leu (MW = 1171.8)
DiSanA 1006 HPLC Cyclized Decapeptide  D-Phe-Leu-Val-Leu-D-Leu-D-Phe-Leu-Val-Leu-D-Leu

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SanA 7/DiSanA 1007 NMR Dipeptide MeO-Phe-Leu-NHBoc
SanA 7/DiSanA 1007 NMR Tripeptide  MeO-Phe-Leu-D-Val-NHBoc
SanA 7/DiSanA 1007 NMR Dipeptide MeO-D-Leu-Leu-NHBoc
SanA 7/DiSanA 1007 NMR Linear Pentapeptide MeO-Phe-Leu-d-Val-d-Leu-Leu-NHBoc
SanA 7/DiSanA 1007 LCMS Linear Pentapeptide HO-Phe-Leu-D-Val-D-Leu-Leu-NH₂ (MW = 603.8)
SanA 7 NMR Cyclized Pentapeptide Phe-Leu-D-Val-D-Leu-Leu
SanA 7 LCMS Cyclized Pentapeptide Phe-Leu-d-Val-d-Leu-Leu (MW = 586.4)
SanA 7 HPLC Cyclized Pentapeptide Phe-Leu-D-Val-D-Leu-Leu
DiSanA 1007 NMR Cyclized Decapeptide Phe-Leu-d-Val-d-Leu-Leu-Phe-Leu-Val-d-Leu-Val-d-Leu-Leu
DiSanA 1007 LCMS Cyclized Decapeptide  Phe-Leu-D-Val-D-Leu-Leu-Phe-Leu-D-Val-D-Leu-Leu (MW = 1171.8)
DiSanA 1007 HPLC Cyclized Decapeptide  Phe-Leu-D-Val-D-Leu-Leu-Phe-Leu-D-Val-D-Leu-Leu
APPENDIX B

$^1$H NMR SPECTRA FOR URUKTHAPELSTATIN A
Ustat A fragment 1 NMR Dipeptide BocNH-Thr(Ot-Bu)-Ser(Obn)-OMe
Ustat A Fragment 1 NMR Tripeptide BocNH-Thr(Ot-Bu)-Ser(OBn)-Ser(OBn)-OMe
Ustat A Fragment 1 NMR Tripeptide BocNH-Thr(Ot-Bu)-Ser-Ser-Ome (Compound 4)
Ustat A Fragment 1 NMR Dioxazole Precursor BocNH-Thr(Ot-Bu)-Ox-Ox-CO2Me
(Compound 6)
Ustat A Fragment 1 NMR Dipeptide BocNH-Thr(Ot-Bu)-Ser(Obn)-OMe
Ustat A Fragment 1 NMR Dipeptide BocNH-Thr(Ot-Bu)-Ser-OMe (Compound 7)
Ustat A Fragment 1 NMR Oxazole Dimer BocNH-Thr(Or-Bu)-Ox-CO2Me (Compound 8)
Ustat A Fragment 1 NMR Oxazole Trimer BocNH-Thr(Ot-Bu)-Ox-Ser(OBn)-OMe
Ustat A Fragment 1 NMR Oxazole Trimer BocNH-Thr(Ot-Bu)-Ox-Ser-Ome
(Compound 10)
Ustat A Fragment 1 NMR Dioxazole Precursor BocNH-Thr(Ot-Bu)-Ox-Ox-Ox-CO2Me

(Compound 6)
Ustat A Fragment 1 NMR Dioxazole Amide BocNH-Thr(Ot-Bu)-Ox-Ox-CONH$_2$
Ustat A Fragment 1 NMR Dioxazole Thioamide BocNH-Thr(Ot-Bu)-Ox-Ox-CSNH₂
(Compound 11)
Ustat A Fragment 1 NMR Dioxazole Thiazole Tetramer BocNH-Thr(Ot-Bu)-Ox-Ox-Th-OEt (Compound 12)
Ustat A Fragment 1 NMR Dioxazole Thiazole Amide BocNH-Thr(Ot-Bu)-Ox-Ox-Th-CONH₂
Ustat A Fragment 1 NMR Dioxazole Thiazole Thioamide BocNH-Thr(Ot-Bu)-Ox-Ox-Th-CSNH₂
Ustat A Fragment 2 NMR Ketal BrCH₂C(OMe)₂CO₂Me
Ustat A Fragment 2 NMR Acid BrCH$_2$C(OMe)$_2$CO$_2$H (Compound 13)
Ustat A Fragment 2 NMR Dimer BrCH₂C(OMe)₂CONH-β-OH-Phe-OMe (Compound 14)
Ustat A Fragment 2 NMR Phenyl-oxazole Precursor BrCH₂C(OMe)₂-PheOx-CO₂Me
Ustat A Fragment 2 NMR Phenyl-oxazole Precursor BrCH₂C(OMe)₂-PheOx-CO₂H (Compound 15)
Ustat A Fragment 2 NMR Dipeptide NHBoc-\textit{d-allo}-Ile-Ala-OMe
Ustat A Fragment 2 NMR Tetramer BrCH₂C(OMe)₂-PheOx-δ-allo-Ile-Ala-OMe
Ustat A Fragment 2 NMR Free Acid BrCH₂C(OMe)₂-PheOx-d-allo-Ile-Ala-OH
Ustat A Fragment 1 NMR Free Amine H₂N-Thr(Ot-Bu)-Ox-Ox-Th-CSNH₂
Ustat A Linear Precursor NMR BrCH₂C(OMe)₂-PheOx-d-allo-Ile-Ala-Thr(Ot-Bu)-Ox-Ox-Th-CSNH₂ (Compound 17)
Ustat A Fragment 2 NMR Free Acid BrCH₂CO-PheOx-d-allo-Ile-Ala-OMe (Compound 18)
Ustat A Linear Precursor NMR BocNH-Thr(Ot-Bu)-Ox-Ox-Th-Th-PheOx-d-allo-Ile-Ala-OMe (Compound 19)