Total Synthesis of Apratoxin A Analogues

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

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2012

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ABSTRACT

Apratoxin A is a potent cytotoxic compound that was isolated from the marine cyanobacterium *Lyngbya majuscula* by Moore, Paul, and co-workers and reported in 2001. Structurally, this natural product is a macrocyclic depsipeptide that contains an unprecedented polyketide domain and a heavily methylated polypeptide domain. The biological mode of action of apratoxin A, to date, has not been fully elucidated. In addition, the mode of action cannot be correlated with any of the standard anti-cancer agents in the National Cancer Institute’s library, which suggests a unique mode of action.

Herein are reported our efforts towards discovering an apratoxin A analogue that retains its anti-cancer activity, but eliminates its action against normal cells. We have developed an efficient synthetic route towards the oxazoline analogue of apratoxin A that relies upon a late stage installation of the oxazoline moiety. The convergent nature of the synthetic route has provided us with access to several oxazoline analogues that will be assayed for anti-cancer activity. Also, a second generation synthesis of oxazoline analogues has been developed, which shortens the overall synthetic route and eliminates late stage epimerization problems seen in the original synthesis of oxazoline apratoxin A. In addition, we have developed a reliable synthetic route to an oxazoline analogue that contains a polyethylene glycol domain, which terminates with an azide. The azide has been successfully used in a 1,3-dipolar cycloaddition with a biotinylated alkyne, which
will be assayed to help determine the molecular targets of apratoxin A. Furthermore, the polyethylene glycol azide provides us with a handle to install additional biological tags.
This work is dedicated to my family
for their continual support throughout all of my endeavors
ACKNOWLEDGEMENTS

First and foremost, I have to thank my advisor, Professor Craig Forsyth, for giving me the opportunity to work in his group. The knowledge that I have acquired while in his lab has provided me with the toolset necessary to succeed in chemistry and everyday life. I will always be grateful for his advice and continued support throughout my graduate career.

My fondest memories of graduate school will be about the countless individuals that I have had the pleasure of working with. There is not enough space in this report to fully express my gratitude towards the influence all of you have had on my life.

I would like to start off by thanking Dr. Daniel Wherritt and Mr. Benjamin Wolfe for immediately making me feel a part of the group and all of the great discussions we had. I have to specially thank my great friend, Dr. Sean Butler, for all of the graduate school wisdom that you have shared with me and for making everyday in lab enjoyable.

My time in 4028 Evans Lab has provided me with many fond memories. To Dr. Bo Wang, I would like to thank you for all the technical advice and our many discussions on fish and history. I want to thank Dr. Feng Zhou, Dr. Yucheng Pang, and Dr. Dimao Wu for providing me with an enjoyable work environment. It was a real pleasure to work
with Mr. Patrick Page for several years and I appreciate all the challenging questions and dedication you brought to lab everyday. To Dr. Ting Wang, thank you for teaching me a great amount of chemistry and for putting up with my dry-ice jokes. I would like to also thank Daniel Adu-Ampratwum, Antony Okumu, and Krista Cunningham; you all have provided me a great work environment and lasting friendships. Finally, I have to thank another great friend, Mr. Nathan Line, for all of the great times we have had both inside and outside of lab.

I cannot fully put into words my thanks for my wife, Jamie. I am extremely lucky to have you in my life. Thank you for always being there for me. You are the love of my life and I am looking forward to spending the rest of my life with you.

I also want to thank Pat and Patty Wolf, as well as the entire Wolf family. You have always been supportive of me and cared for me like one of your own. I am proud to be part of the family and really look forward to all the good times to come.

I would not be where I am today without the love and support from my parents, Theresa and Dave, and my brother, Andy. Ever since I can remember, my parents have encouraged and supported me in all of my endeavors. I love you both very much and I hope that I have made, and will continue, to make you proud that I am your son. Finally, I would like to express a heartfelt thanks to my brother. You have been one of the biggest constants in my life and I couldn’t imagine a better person to grow up with.
VITA

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Major Field: Chemistry
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<td>Boc</td>
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<td>br</td>
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<td>$n$-$Bu$</td>
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<td>$s$-$Bu$</td>
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<tr>
<td>$t$-$Bu$</td>
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</table>
Bn  benzyl
Bz  benzoyl
°C  degrees Celsius
¹³C  carbon-13
calcd  calculated
cm⁻¹  wavenumbers (IR)
δ   delta
d  day(s); doublet (NMR)
DAST  diethylaminosulfur trifluoride
DCC  $N,N'$-dicyclohexylcarbodiimide
DDQ  2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIAD  diisopropyl azadicarboxylate
DIBAL-H  diisobutylaluminum hydride
DIC  $N,N'$-diisopropylcarbodiimide
DIEA  diisopropylethylamine
DIPCl  $B$-Chlorodiisopinocampheylborane
DMAP  4-($N,N$-dimethylamino)pyridine
DMF  $N,N$-dimethylformamide
DMP  Dess-Martin Periodinane; 2,2-dimethoxypropane
DMSO  dimethylsulfoxide
DPPA  diphenylphosphoryl azide
dr  diastereomeric ratio
Dt  3,7-dihydroxy-2,5,8,8-tetramethylnonanoic acid
EDCI  1-ethyl-3-(3-dimethylaminopropyl) carbodiimide

ee  enantiomeric excess

Et  ethyl

Et₂O  diethyl ether

ER  endoplasmic reticulum

ESI  electrospray ionization

FGFR  fibroblast growth factor receptor

Fmoc  9-fluorenylmethoxycarbonyl

γ  gamma

g  gram(s)

h  hour(s)

¹H  proton

HATU  O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate

HOBt  hydroxybenzotriazole

HPLC  high performance liquid chromatography

HRFABMS  high resolution fast atom bombardment mass spectrometry

Hünig’s base  diisopropylethylamine

Hz  hertz

IC₅₀  half maximal inhibitory concentration

Ile  isoleucine

imid  imidazole

IR  infrared
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<td>coupling constant in Hertz (NMR)</td>
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<td>L.</td>
<td><em>lyngbya</em></td>
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<td>LAH</td>
<td>lithium aluminum hydride</td>
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<tr>
<td>2,6-lutidine</td>
<td>2,6-dimethylpyridine</td>
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<tr>
<td>μ</td>
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<td>m</td>
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<td>M</td>
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<td>Mukaiyama’s reagent</td>
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<td>NaHMDS</td>
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<td>tertiary (tert)</td>
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<tr>
<td>t</td>
<td>triplet (NMR)</td>
</tr>
<tr>
<td>TBAF</td>
<td>tetra-butylammonium fluoride</td>
</tr>
<tr>
<td>TBS</td>
<td>tert-butyldimethylsilyl</td>
</tr>
<tr>
<td>TEMPO</td>
<td>(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl</td>
</tr>
<tr>
<td>TES</td>
<td>triethylsilyl</td>
</tr>
<tr>
<td>Tf</td>
<td>trifluoromethanesulfonyl</td>
</tr>
<tr>
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<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
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<tr>
<td>TMS</td>
<td>trimethylsilyl</td>
</tr>
<tr>
<td>TMSE</td>
<td>trimethylsilylethyl</td>
</tr>
<tr>
<td>TPAP</td>
<td>tetrapropylammonium perruthenate</td>
</tr>
<tr>
<td>$t_R$</td>
<td>retention time</td>
</tr>
<tr>
<td>Tr</td>
<td>trityl; triphenylmethyl</td>
</tr>
<tr>
<td>Troc</td>
<td>2,2,2-trichloroethoxycarbonyl</td>
</tr>
<tr>
<td>Ts</td>
<td>tosyl; p-toluene sulfonic acid</td>
</tr>
<tr>
<td>TS</td>
<td>transition state</td>
</tr>
<tr>
<td>TsOH</td>
<td>p-toluene sulfonic acid</td>
</tr>
<tr>
<td>Tyr</td>
<td>tyrosine</td>
</tr>
<tr>
<td>VEGF</td>
<td>vascular endothelial growth factor</td>
</tr>
</tbody>
</table>
CHAPTER 1

ISOLATION AND BIOLOGICAL SIGNIFICANCE OF THE APRATOXINS

1.1 Isolation and Structure Elucidation of Apratoxin A

Apratoxin A (1.1, Figure 1.1) is a potent cytotoxic natural product that was isolated from the marine cyanobacterium Lyngbya majuscula in by Moore, Paul, and coworkers and reported in 2001.¹ This natural product is a cyclodepsipeptide that originates from a mixed biogenesis, as indicated by the presence of a polyketide and polypeptide domain. Structurally, 1.1 features highly methylated amino acids (N-methyl isoleucine, N-methyl alanine, and O-methyl tyrosine) and a dihydroxylated fatty acid moiety, 3,7-dihydroxy-2,5,8,8-tetramethylnonanoic acid (Dtena). In addition, 1.1 contains a cysteine derived thiazoline and a proline ester linkage that unites the polyketide and polypeptide domains.

The gross structure of 1.1 was elucidated by various 1D and 2D NMR techniques coupled with HRFABMS data, which suggested a molecular formula of C₄₅H₆₉N₅O₈S. To determine the absolute configurations of the amino acid components in 1.1, the acid hydrolysis products were subjected to chiral HPLC analysis. The analysis revealed L
configurations for proline, \( N \)-methyl-isoleucine, \( N \)-methyl-alanine, and \( O \)-methyl-tyrosine, while the modified cysteine was determined to be the D configuration. The relative stereochemistry of the polyketide domain was determined by J-based configuration analysis utilizing \( ^3J_{H,H} \) and \( ^2J_{C,H} \) values, which was originally developed for acyclic systems with only staggered conformations. In the case of 1.1 and other large macrocycles, it is believed that only staggered conformations exist. Therefore this method is still applicable.\textsuperscript{2} The absolute stereochemistry of 1.1 (34\( S \), 35\( S \), 37\( S \), 39\( S \)) was determined by the modified Mosher method.\textsuperscript{3}

![Diagram of Apratoxin A](image)

**Figure 1.1.** Structure of apratoxin A (1.1)

### 1.2 Apratoxin Family

The apratoxin family currently consists of seven natural apratoxins and one semi-synthetic analogue. In 2001, Moore, Paul, and co-workers reported the isolation of apratoxins B (1.2) and C (1.3) in Guam and Palau from the marine cyanobacterium *Lyngbya* sp.\textsuperscript{4} Structurally, 1.2 is not \( N \)-methylated at the isoleucine residue, while 1.3 contains an *iso*-propyl group rather than *tert*-butyl. In addition to 1.2 and 1.3, a semi-
synthetic analogue, \((E)-34,35\text{-dehydroapratoxin A (1.4)}\), was discovered when compound 1.1 was exposed to acidic CDCl\(_3\) (Figure 1.2). Interestingly, the \(O\)-Me-Tyr–N-Me-Ala amide bond in 1.2 and 1.4 is in the \textit{cis} configuration (versus \textit{trans} in 1.1), as determined by NMR spectroscopy and modeling studies.

In 2008, the isolation of apratoxin D (1.5) was isolated from the marine cyanobacteria \textit{Lyngbya majuscula} and \textit{Lyngbya sordida} (both collected in Papua New Guinea) was reported by Gerwick and co-workers.\(^5\) This natural product features an extended polyketide moiety, 3,7-dihydroxy-2,5,8,10,10-pentamethylundecanoic acid. Later in 2008, Luesch and co-workers reported the isolation of apratoxin E (1.6) from the cyanobacterium \textit{Lyngbya bouillonii} (Guam), which features changes between both the polyketide and polypeptide domains (Figure 1.3). Interestingly, the Luesch group discovered that the structural diversity within the apratoxin family might be correlated with the depth at which the cyanobacteria were collected. For example, collection of \textit{L. bouillonii} from depths of 1–3 m and deeper waters of 12–14 m revealed vastly different amounts of 1.1 and 1.6 (Figure 1.4).\(^6\) It is known that filamentous cyanobacteria grow

\[ \text{Figure 1.2. Structure of apratoxin B (1.2), apratoxin C (1.3), and dehydroapratoxin (1.4)} \]
in patches and that each patch may have slight genetic differences. This indicates that collections of cyanobacteria may be genetically different, or at least chemically different, as a result of environmental stimuli that cause the transcription of different genes.\textsuperscript{6}

![Apratoxin D (1.5) and Apratoxin E (1.6)](image)

**Figure 1.3.** Structure of apratoxin D (1.5) and apratoxin E (1.6)

![Isolation depth versus apratoxin amounts](image)

**Figure 1.4.** Isolation depth versus apratoxin amounts

The final two apratoxins, apratoxin F (1.7) and apratoxin (1.8), were reported by the Gerwick group in 2008 as isolates from the marine cyanobacterium *Lyngbya bouillonii* (collected from Palmyra Atoll). These compounds feature the most structural
diversity, to date, within the apratoxin family. Both 1.7 and 1.8 feature a \(N\)-methyl alanine residue in place of proline, while 1.8 also contains a \(N\)-methyl valine instead of \(N\)-methyl isoleucine (Figure 1.5). Geographically, 1.7 and 1.8 were discovered thousands of miles away from the other apratoxins, which suggests an evolved biosynthesis in response to differing environmental conditions.

![Apratoxin F (1.7) and Apratoxin G (1.8)](image)

**Figure 1.5.** Structure of apratoxin F (1.7) and apratoxin G (1.8)

### 1.3 Biological Activity

The vast structural diversity among the apratoxin family is intriguing from a synthetic point of view, but the natural products also have a fascinating biological profile. Apratoxin A (1.1) exhibits incredible cytotoxicity in vitro with IC\(_{50}\) values of 0.52 nM against KB and 0.36 nM against LoVo cancer cells. In addition, 1.1 is extremely active in vivo, but is poorly tolerated, at best, in mice. The complete mode of action of these natural products currently remains unknown, though recent data have shed some light on the activity. Interestingly though, the biological activity of 1.1 cannot be correlated with any known modes of cytotoxicity, such as interacting with the microfilament
network, inhibition of microtubule polymerization/depolymerization, and interfering with topoisomerase I.

The rest of the apratoxin family has a large range in its cytotoxic profile, though the limited amounts of compounds 1.1–1.8 has provided scientists with incomplete data (Table 1.1). Based upon the range of cytotoxicity data, it is very apparent that small structural changes in the primary structure of the apratoxins can cause large conformational alterations, thus affecting the biological activity. Comparison of the cytotoxicity of apratoxin A (1.1) to apratoxin B (1.2) indicates that N-methylation at the isoleucine residue is relatively important to the overall active conformation of apratoxin. This is also supported by the observation the O-Me-Tyr–N-Me-Ala amide bond in 1.2 is cis rather than trans, as in 1.1. In addition, comparison between apratoxin A (1.1) to dehydroapratoxin A (1.4) shows that the C35 hydroxyl may be important to the active conformation and therefore the biological activity. These observations have been supported by molecular modeling studies, which indicate that the N-methyl group of the isoleucine and the C35 hydroxyl are in close proximity to each other. Alteration of the tert-butyl group (i.e. 1.3 and 1.5) seems to be well tolerated, but saturation of the C28-C29 olefin and dehydration of the C35 alcohol (apratoxin E (1.6)) does not appear to be tolerated. Interestingly, replacement of the proline residue with N-methyl alanine (apratoxin F (1.7) and apratoxin (1.8)) appears to be tolerated. This is not entirely unexpected, as it can be assumed that proline and N-methyl alanine may have very similar conformational profiles. Finally, though not a natural analogue of apratoxin A, the oxazoline analogue of apratoxin A (1.9) exhibits comparable activity to 1.1, which may provide an exciting new set of analogues to be studied (Figure 1.6). It should be
noted that the IC\textsubscript{50} (nM) values for apratoxin A (\textbf{1.1}) for the HeLa cells varies between 2.2 and 10 nM, depending upon the study\textsuperscript{6,12}.

<table>
<thead>
<tr>
<th>Apratoxin</th>
<th>KB (breast)</th>
<th>LoVo (colon)</th>
<th>H-460 (lung)</th>
<th>HT29 (colon)</th>
<th>HeLa (cervical)</th>
<th>U2OS (bone)</th>
<th>HCT116 (colon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (1.1)</td>
<td>0.52</td>
<td>0.36</td>
<td></td>
<td>1.4</td>
<td>10</td>
<td>10</td>
<td>1.21</td>
</tr>
<tr>
<td>B (1.2)</td>
<td>21.3</td>
<td>10.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (1.3)</td>
<td>1</td>
<td>0.73</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D (1.5)</td>
<td></td>
<td></td>
<td>2.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E (1.6)</td>
<td></td>
<td></td>
<td></td>
<td>21</td>
<td>72</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>F (1.7)</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td>36.7</td>
</tr>
<tr>
<td>G (1.8)</td>
<td></td>
<td></td>
<td></td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dehydro (1.4)</td>
<td></td>
<td></td>
<td>37.6</td>
<td>41</td>
<td>121</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>oxazoline (1.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>920</td>
</tr>
<tr>
<td>1.11</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;10000</td>
</tr>
<tr>
<td>1.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;10000</td>
</tr>
</tbody>
</table>

\textbf{Table 1.1.} Biological activity of apratoxins against various cell lines

Although Table 1.1 shows mostly natural analogues of \textbf{1.1}, several groups have designed synthetic analogues and have determined some interesting trends. In 2006, the Ma group reported that the oxazoline analogue (\textbf{1.9}) was nearly as active as \textbf{1.1}, as shown in Table 1.1. In addition, the Ma group also made the oxazoline analogue of apratoxin C (\textbf{1.10}), which showed an IC\textsubscript{50} value of 920 nM. This result indicates that the C40 methyl group might be important for the biological activity of \textbf{1.9}. This result was very interesting, as \textbf{1.1} and \textbf{1.3} have nearly the same activity, but subtle conformational changes can have large effects on activity, which was shown in Wipf’s report on lissoclinamide 7 (replaced thiazoline with oxazoline)\textsuperscript{13}. In the same study by the Ma group, the group made the C37 epimer of the oxazoline C analogue (\textbf{1.11}), as well as the
C37 desmethyl analogue (1.12) (Figure 1.6). They determined that the C37 position is necessary for retained biological activity, as both 1.11 and 1.12 had IC_{50} values greater than 10,000 nM (Table 1.1).

![Diagram of C37 desmethyl analogue](image)

Figure 1.6. Oxazoline analogue SAR study

### 1.4 Mode of Action Studies

The potent cytotoxicity against a variety of cancer cell lines and moderate activity against early-stage colon adenocarcinoma C38-derived tumors in vivo has motivated chemists to determine the mode of action and molecular target of 1.1. In 2006, the Luesch and Schultz group reported a functional genomics approach, which takes the wealth of data produced by various genomic projects and uses that to describe gene functions and interactions, to elucidate the mode of action. As determined by Moore, Paul, and co-workers, the Schultz group also confirmed that the mode of action of apratoxin A cannot be correlated with any of the standard anti-cancer agents in the NCI database, therefore suggesting a unique mode of action. The Schultz group discovered that apratoxin A (1.1) induces G1-phase cell cycle arrest and apoptosis, which is the part
of the cell cycle at which the cell is growing by building new organelles. In addition, 1.1 appears to act, in part, by blocking the fibroblast growth factor receptor (FGFR) signaling pathway, which is commonly involved in cancer. Also, compound 1.1 inhibited phosphorylation and activation of an important transcription factor, STAT3 (Signal Transducer and Activator of Transcription 3), which is a downstream effector of FGFR signaling. The significance of STAT3 activation is that it is often expressed and activated in a wide range of cancers, which makes it a very intriguing drug target.

The discovery that 1.1 potently inhibits STAT3 has provided scientists with a clue on how 1.1 may work, but the upstream events still remained unknown. In 2009, the Luesch group reported that 1.1 reversibly inhibits the secretory pathway for several cancer-associated receptors by interfering with co-translational translocation. The secretory pathway involves a series of steps that expels proteins out of the cells, which is a process known as secretion. Cotranslational translocation is the event of feeding a protein into the endoplasmic reticulum (ER) as it is being synthesized by a ribosome. In essence, synthesis of the proteins that enter the secretory pathway still occurs, but the proteins are diverted into the cytoplasm (rather than the ER). As a result of this diversion, the newly built protein is now destined for rapid degradation in the cytoplasm. The authors postulated that modulating the secretory pathway with apratoxin-based small molecules should present a new reversible tool to study the secretory pathway, therefore providing a new method to study cancer.

Following their initial lead regarding the secretory pathway, the Luesch group made several apratoxin analogues that altered the amino acids in the polypeptide domain...
(1.13–1.16) and an apratoxin A/E hybrid (1.17) (Figure 1.7). In addition, they inadvertently made two epimers of 1.1, 2-epi-1.1 (1.18) and 34-epi-1.1 (1.19).

Before testing their hypothesis that apratoxins effected growth factor secretion, the authors needed to determine which of the synthetic compounds retained their biological activity (Table 1.2). It was determined that the A/E hybrid (1.17) was the most potent analogue. In addition, it was discovered that replacing the proline with N-methyl alanine (1.13) and substituting the isoleucine for valine (1.15) resulted in little activity loss, while replacement of isoleucine for alanine resulted in a moderate decrease in activity. Also, the 2-epi-1.1 (1.18) saw a decrease in activity, but the C34-epi-1.1 (1.19) retained its activity.

![Figure 1.7. Apratoxin analogues](image-url)
The SAR studies were extended to determine the effects of the synthetic apratoxins on growth factor secretion by exploring their activity against an angiogenic drug target VEGF-A. As can be seen in Table 1.2, all of the tested apratoxins showed that they inhibited secretion of VEG-F, which supported their hypothesis that apratoxins affect the export of protein receptors. In addition, the authors discovered that compound 1.17 has some significant tumor selectivity (over normal cells) and increased tumor activity and potency. Furthermore, the SAR studies indicate that the activity of apratoxins may be tuned toward certain substrates for the secretion pathway, which could provide a new tool set to explore the secretion pathway as a viable anti-cancer target.16

Table 1.2. Activities of synthetic apratoxins on cell viability and secretion

<table>
<thead>
<tr>
<th>Apratoxin</th>
<th>IC_{50} (nM) cell viability</th>
<th>IC_{50} (nM) VEGF-A secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>5.97</td>
<td>1.49</td>
</tr>
<tr>
<td>1.4</td>
<td>184</td>
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<td>0.322</td>
</tr>
<tr>
<td>1.16</td>
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<td>340</td>
</tr>
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<td>1.17</td>
<td>1.14</td>
<td>0.308</td>
</tr>
<tr>
<td>1.18</td>
<td>258</td>
<td>112</td>
</tr>
<tr>
<td>1.19</td>
<td>1.58</td>
<td>0.391</td>
</tr>
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</table>
CHAPTER 2

PREVIOUSLY COMPLETED TOTAL SYNTHESES OF APRATOXIN A

2.1 Introduction to Prior Total Synthesis Efforts

The molecular complexity, obvious synthetic challenges, and remarkable biological activity of 1.1 have drawn significant interest from the synthetic community. One potential problem that all the groups had to overcome was the known acid sensitivity of 1.1, which dehydrated to 1.4 when exposed to acidic CDCl$_3$. In addition, the thiazoline’s 2-position substituent’s α-center (C34) and position 4 (C30) are susceptible to epimerization. The total synthesis of 1.1 has been conquered by the Forsyth$^{17,18}$, Ma$^{19}$, and Doi$^{20-22}$ groups. In addition, the oxazoline analogue 1.9 has been completed by Ma$^{23}$, Cavelier$^{24}$, and Doi$^{21}$. To date, only one synthesis of 1.4 has been completed$^{25}$ and a couple of synthetic efforts towards the polyketide domain have been described$^{26,27}$.

2.2 Forsyth Total Synthesis of Apratoxin A

The synthetic design of the Forsyth synthesis relied upon a unique, late stage, one-pot Staudinger reduction–intramolecular aza-Wittig (S-aW) reaction to install the sensitive thiazoline moiety of 1.1. Retrosynthetically, they disconnected 1.1 into essentially the polypeptide 2.1 and polyketide 2.2 domains. The two domains would be
united via a thioesterification reaction and the ultimate macrolactam would be closed at the isoleucine–proline amide. Fragment 2.1 was further disconnected to diamide 2.3 and acid 2.4, which represented a precursor to the moCys domain of 1.1. Acid 2.2 was envisioned to arise from a series of steps from lactone 2.5 (Scheme 2.1). It should be noted that the proline residue needed to be installed early because previous results indicated that the macrolactone could not be closed at the proline ester, therefore a revised synthetic approach needed to be taken.\textsuperscript{18}

Scheme 2.1. Forsyth Retrosynthesis.
In the forward direction, the synthesis began with a Brown allylation\(^{28}\) of pivaldehyde 2.6, which produced homoallylic alcohol 2.7 in good yield with moderate enantioselectivity. Homoallylic alcohol 2.7 was acylated with acrylic acid by utilizing Mukaiyama’s reagent (2-chloro-methylpyridinium iodide),\(^{29}\) which afforded ester 2.8. Ring closing metathesis of ester 2.8 with Grubbs’ first generation catalyst\(^{30}\) and addition of a higher order methyl cuprate to the resulting \(\alpha,\beta\)-unsaturated \(\delta\)-valeryl lactone generated lactone 2.5. The lactone was reductively opened with lithium aluminum hydride (LAH) and the resulting primary alcohol was selectively protected as the tertbutyldimethylsilyl (TBS) ether (Scheme 2.2).

![Scheme 2.2. Synthesis of silyl ether 2.9](image)

The robust proline residue was installed by an esterification reaction with alcohol 2.9 under Yamaguchi conditions.\(^{31}\) The primary TBS ether (2.10) was then cleaved with tetrabutylammonium fluoride (TBAF) and the resulting alcohol (2.11) was oxidized with tetrapropylammonium perruthenate (TPAP) to aldehyde 2.12. Aldehyde 2.12 was subjected to a Paterson anti-selective aldol reaction with \((R)-2\)-benzoyloxy-3-pentanone\(^{32}\) and the resulting \(\beta\)-hydroxyketone 2.13 was protected as TBS ether 2.14. The benzoate
in 2.14 was removed with potassium carbonate in methanol and the resulting α-hydroxy ketone was cleaved to carboxylic acid 2.2 with aqueous sodium periodate (Scheme 2.3).

The synthesis of the moCys moiety began with a Wittig reaction on (S)-glyceraldehyde acetonide 2.15, which gave α,β-unsaturated ester 2.16. Ester 2.16 was treated with p-toluenesulfonic acid (TsOH) in methanol to cleave the acetonide. The primary alcohol in 2.17 was regioselectively protected as the TBS ether and the secondary alcohol was converted to the p-methoxybenzyl (PMB) ether with p-methoxybenzyl trichloroacetimidate and triflic acid (TfOH). Finally, saponification of methyl ester 2.18 with lithium hydroxide (LiOH) produced carboxylic acid 2.4 (Scheme 2.4).
Diamide 2.3, which was assembled from a series of deprotection steps and amide bond formations, was subjected to trifluoroacetic acid (TFA) to remove the Boc protecting group. The crude ammonium trifluoroacetate salt was then coupled to carboxylic acid 2.4 with PyAOP via the in situ liberated free base (Et$_3$N), which afforded triamide 2.19. After removal of the TBS protecting group with HF-pyridine, the resulting alcohol was converted to thioester 2.20 under Mitsunobu conditions (Scheme 2.5).

Scheme 2.4. Synthesis of carboxylic acid 2.4.

Scheme 2.5. Preparation of $\alpha$-azido thioester 2.20.
The acetate group was removed from 2.20 to unveil thiol 2.1, which was coupled to the polyketide acid 2.2 with diphenylphosphoryl azide (DPPA) to yield thioester 2.21. After removal of the PMB with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), the resulting secondary alcohol (2.22) was converted to secondary azide 2.23 under Mitsunobu conditions. Based upon previous results, the authors knew that the C35 TBS ether could not be cleaved in the final step. Therefore, the C35 TBS ether was converted into a triethylsilyl (TES) ether by a deprotection with HF-pyridine followed by protection with triethylsilyl trifluoromethanesulfonate (TESOTf), which produced compound 2.24 (Scheme 2.6).
Thiazoline formation was accomplished by treating \( \alpha \)-azido thioester 2.24 with triphenylphosphine in THF under anhydrous conditions, which efficiently produced 2.25 (Scheme 2.7). The Boc protecting group in 2.25 was removed by a two-step procedure involving the initial formation of a silyl carbamate with \( \text{tert} \)-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) followed by degradation of the carbamate with TBAF. After saponification of methyl ester 2.26 with LiOH, the resulting amino acid was cyclized with PyAOP under high dilution conditions. Finally, the synthesis of 1.1

Scheme 2.6. Conversion to thioester 2.24
was completed with the removal of the TES group with buffered HF-pyridine (Scheme 2.7).

\[
\begin{align*}
\text{2.24} & \xrightarrow{\text{Ph}_3\text{P}} \text{THF, 50 °C} \quad 63\% \\
\text{2.25} \\
\text{1. TBSOTf, CH}_2\text{Cl}_2 & \text{2.6 lutidine} \\
\text{2. TBAF, THF, 0 °C} & 86\% (2\ steps) \\
\text{2.26} & \xleftarrow{\text{1. LiOH, t-BuOH}} \text{THF, H}_2\text{O} \\
& \text{2. PyAOP, iPr}_2\text{NEt}} \\
& \text{CH}_2\text{Cl}_2, 73\% (2\ steps) \\
& \text{3. HF-pyr, THF, 65\%} \\
\end{align*}
\]

Scheme 2.7. S-aW reaction and synthesis completion

\[\text{2.3 Doi Total Synthesis of Apratoxin A}\]

The Doi group decided to approach the total synthesis of 1.1 by disconnecting the molecule into compounds 2.27 and 2.28. Similar to the Forsyth synthesis, the group planned to close the macrolactam at the isoleucine–proline amide bond. Interestingly, they planned to install the sensitive thiazoline before coupling to the polypeptide domain,
which was potentially problematic due to the acid sensitivity and epimerization potential of the thiazoline ring. Thiazoline 2.28 was further disconnected to thioester 2.29, which was taken back moCys residue 2.30 and known carboxylic acid 2.2 (Scheme 2.8).20

Scheme 2.8. Doi Retrosynthesis

The synthesis began with a proline-catalyzed aldol reaction between acetone and pivaldehyde, which afforded β-hydroxy ketone 2.31.34,35 The secondary alcohol was protected as a PMB ether and the remaining ketone was attacked with allylmagnesium bromide. The resulting tertiary alcohol was protected as an acetate (2.33), which was then treated with palladium(II) to induce the acetoxy migration via π-allylpalladium complex. The acetate was then removed with potassium carbonate in methanol to yield
primary alcohol \textbf{2.34}. Compound \textbf{2.34} was subjected to a Ru(OAc)$_2$[(S)-binap]-catalyzed asymmetric hydrogenation under 100 atm of H$_2$ to generate alcohol \textbf{2.35}.\textsuperscript{36} After oxidation of \textbf{2.35} under Swern conditions, aldehyde \textbf{2.36} was subjected to the Paterson \textit{anti}-aldol reaction with (R)-2-benzyloxy-3-pentanone,\textsuperscript{32} which produced \textbf{2.37}. It should be noted that the Forsyth synthesis performed the aldol reaction with the proline already attached. The secondary alcohol was protected as a TBS ether with TBSOTf to give \textbf{2.38}. Appendage of the proline residue was accomplished by removing the PMB protecting group with DDQ, followed by esterification under Yamaguchi conditions to give \textbf{2.14}. Compound \textbf{2.14} was then converted to known carboxylic acid \textbf{2.2} by saponification of the benzoate ester and cleavage of the resulting \textit{\alpha}-hydroxy ketone with aqueous sodium periodate (Scheme 2.9).
The moCys moiety was assembled in several steps starting with carboxylic acid 2.39, which was derived from D-cysteine (Scheme 2.10). Carboxylic acid 2.39 was converted to its Weinreb amide, which was reduced with diisobutylaluminum hydride (DIBAL) to an intermediate aldehyde. The aldehyde was subjected to a Wittig reaction to produce α,β-unsaturated ester 2.40. The key intermediate 2.30 was prepared by: saponification of the ethyl ester with LiOH, esterification of the resulting carboxylic acid, and removal of the Boc protecting group with trimethylsilyl trifluorosulfonate (TMSOTf).
With the polyketide \textbf{2.2} and moCys \textbf{2.30} domains prepared, the two components were coupled with HATU to form amide \textbf{2.41} (Scheme 2.11). The authors next chose to manipulate their protecting groups to ensure safe removal at a later stage. First, they removed the TBS and Boc protecting groups with TBAF and TMSOTf/MeOH, respectively. Next, the resulting secondary amine was protected as the base sensitive fluorenylmethyloxycarbonyl (Fmoc) carbamate and the secondary alcohol was protected as a 2,2,2-trichloroethoxycarbonyl (Troc) ester (\textbf{2.29}). With the desired protecting groups in place, the authors used Kelly’s biomimetic method (Ph\textsubscript{3}PO, Tf\textsubscript{2}O)\textsuperscript{37} to efficiently install the thiazoline functionality. Unfortunately, attempts to purify the thiazoline product on silica gel resulted in significant $\beta$-elimination of the Troc group. Therefore, the thiazoline intermediate was immediately treated with Zn–NH\textsubscript{4}OAc, which did not affect the thiazoline ring or the adjacent epimerizable stereocenter, to remove the Troc group. Interestingly, the use of acetic acid (AcOH) resulted in partial hydrolysis of the thiazoline ring. Intermediate \textbf{2.42} was then treated with tetrakis(triphenylphosphine) palladium(0) (Pd(PPh\textsubscript{3})\textsubscript{4}) and $N$-methylaniline to convert the allyl ester to carboxylic acid.
The use of \(N\)-methylaniline as the allyl-trapping nucleophile was strategic because the typically used morpholine would have removed the Fmoc group.

\[
\text{Boc} \quad \text{TBS} \quad \text{H} \\ \text{TrtS} \quad \text{H} \quad \text{Allyl}
\]

\[
\text{R} \quad \text{X} \quad \text{OH} \\ \text{Fmoc} \quad \text{N} \quad \text{Allyl}
\]

Scheme 2.11. Thiazoline 2.28 assembly.

The allyl protected diamide 2.27 was coupled with carboxylic acid 2.28 using HATU and \(N,N\)-diisopropylethylamine (\(i\)-Pr\(_2\)NEt) to yield 2.43. The allyl and Fmoc protecting groups were then removed with Pd(PPh\(_3\))\(_4\)/\(N\)-methylaniline and diethylamine (Et\(_2\)NH), respectively. The resulting amino acid 2.44 was cyclized with HATU under high dilution to yield 1.1 in 53% yield over the final three steps (Scheme 2.12).

2.28
2.4 Ma Total Synthesis of Apratoxin A

The Ma group disconnected 1.1 into intermediates 2.45 and 2.46 with the intention of forming the macrolactam at the isoleucine–proline amide bond, as was done in the Forsyth total synthesis.\(^{17-19}\) Intermediate 2.45 was disconnected to its individual amino acids, while 2.46 was taken back to polyketide carboxylic acid 2.47 and moCys moiety 2.48 (Scheme 2.13). The Ma group decided to install the sensitive thiazoline moiety prior to coupling to the polypeptide domain, which presented possible issues with the stability of the thiazoline as well as the epimerizable \(\alpha\)-stereocenter.
The Ma group’s synthesis began with TBS protected β-hydroxy ketone 2.49, which was derived from a proline catalyzed aldol reaction and TBS protection. The ketone in 2.49 was non-selectively reduced with sodium borohydride (NaBH₄) to give 2.50. The resulting diastereomeric alcohols were converted to the mesylates and treated with potassium tert-butoxide (t-BuOK) to induce elimination, which yielded 2.51 in 71% over the four steps. The olefin in 2.51 was subjected to ozonolysis and the resulting aldehyde was attacked with the lithium enolate derived from ethyl acetate. Compound 2.52 was then treated with HF-pyridine to remove the TBS group and cyclization occurred to the β-hydroxy lactone. After treatment of the β-hydroxy lactone with methanesulfonyl chloride (MsCl) and base, lactone 2.53 was generated in moderate yield over the four steps (Scheme 2.14). It should be noted that 2.53 was made by a shorter
route by the Forsyth group, but the high cost of Grubbs’ catalyst rendered that route impractical for scale-up and analogue studies.\(^1\)

![Chemical reaction diagram](image)

**Scheme 2.14.** Synthesis of known lactone 2.53

As before, methylcupration of 2.53 with \(\text{Me}_2\text{Cu}(\text{CN})\text{Li}_2\) yielded 2.5, which was exhaustively reduced to the diol with LAH. The diol was bis-protected with acetic anhydride and then the primary alcohol was revealed with potassium carbonate in methanol. The resulting primary alcohol was oxidized with Dess-Martin periodinane (DMP) to aldehyde 2.54. To install the *anti*-aldol stereochemistry, the Ma group used Oppolzer’s methodology.\(^3\) \(N\)-Propionylsultam 2.55 was treated with \(\text{Et}_2\text{BOTf}\) and \(i\)-Pr\(_2\)NEt to generate the Z-enolate, which was treated with eight equivalents of TiCl\(_4\) followed by aldehyde 2.54 to afford the *anti*-selective aldol product 2.56. Next, removal of the chiral auxiliary and acetate group with LAH produced a triol. The 1,3-diol was protected as an acetonide and the remaining secondary alcohol was esterified with Fmoc protected proline by Yamaguchi’s procedure\(^3\) to give 2.57. The acetonide was then removed with TsOH/MeOH and the primary alcohol was chemoselectively oxidized over two-steps (TEMPO, NaClO; then NaH\(_2\)PO\(_4\)/NaClO\(_2\)) to yield the desired carboxylic acid 2.47 (Scheme 2.15).
The synthesis of the moCys moiety began with the conversion of protected cysteine 2.58 to Weinreb amide 2.59. The Weinreb amide was carefully reduced with LAH at $-78 \, ^\circ\text{C}$ and the resulting aldehyde was subjected to a Horner-Wadsworth-Emmons reaction with phosphonate 2.60 to produce $\alpha,\beta$-unsaturated ester 2.61. The Fmoc protecting group was then removed with Et$_2$NH to liberate free amine 2.48 (Scheme 2.16).
The polyketide acid 2.47 and moCys moiety 2.48 were united with coupling agent, HATU, to yield amide 2.62. The free secondary alcohol was acetylated with acetyl chloride and the thiazoline was installed with Kelly’s protocol (Ph3PO, Tf2O), which gave 2.63. Then, the prenyl group was removed with TMSOTf to produce key intermediate 2.46 (Scheme 2.17).

Scheme 2.16. Synthesis of moCys domain 2.48.
The Fmoc group in 2.45 was removed with Et₂NH and the resulting free amine was coupled to carboxylic acid 2.46 with HATU to give intermediate 2.64. The protected amine and carboxylic acid were freed by treatment with TBAF, which cleaved the Fmoc and trimethylsilylethyl (TMSE) ester in one-pot. The resulting amino acid was cyclized under high dilution with HATU to give 2.65. Finally, the acetate group was removed under mild conditions (KCN, EtOH) to yield compound 1.1 (Scheme 2.18).
Scheme 2.18. Completion of the synthesis of 1.1
CHAPTER 3

TOTAL SYNTHESIS OF THE OXAZOLINE ANALOGUE OF APRATOXIN A

3.1 Synthetic issues

The originality of the Forsyth total synthesis inspired us to design a synthesis of the oxazoline analogue of 1.1 by utilizing the unique Staudinger Aza-Wittig (S-aW) reaction to install the oxazoline ring. The oxazoline analogue 1.9 was an attractive target for further analogue studies because of the similar biological activity to 1.1, as well as the increased stability of the oxazoline to oxidation and hydrolysis versus the thiazoline ring (Figure 3.1).\(^\text{39}\) Also, the numerous methods to make oxazolines (versus thiazolines) provided us with multiple methods in the event that the S-aW was unsuccessful.\(^\text{40,41}\) In addition, the stability of the synthetic intermediates (the necessary sulfur intermediates may be easily susceptible to oxidation) made the oxazoline an attractive target.
With regards to analogue studies, the original synthesis needed alterations to make the route more scalable, cost affordable, and efficient. The first issue that needed to be resolved was the moderate enantioselectivity (83%) delivered by the Brown allylation, as well as the difficulty in removing the isopinocampheol by-product from the desired alcohol 2.7. The second issue in the synthesis is the early use of Grubbs’ catalyst to make lactone 2.53. The high cost of the first generation Grubbs’ catalyst renders this approach less than ideal for analogue study (Scheme 3.1).

Scheme 3.1. Early synthetic issues with original synthesis.
The third issue that needed to be resolved was the late stage incorporation of the azide group, which is required for the S-aW reaction. In the original synthesis, the azide was installed by a Mitsunobu reaction after coupling of the polyketide and polypeptide domains. From a synthetic viewpoint, it would be ideal to reduce the number of steps after coupling of the polyketide and polypeptide domains. The final issue regarding the original synthesis was the need to switch the TBS group for a TES group after the polyketide and polypeptide domains were united (Scheme 3.2). Previously, it was determined that if the TBS group was carried until the end the synthesis, it could not be successfully removed without degradation in the final step. As a result, the TBS protecting group had to be switched to a more labile one, such as TES, which could be removed to afford 1.1.

**Scheme 3.2.** Late-stage azide incorporation and protecting group shuffle
3.2 Retrosynthetic analysis

With the desire to exploit the S-aW to install the oxazoline, the overall synthetic route was designed to mirror the original approach towards 1.1. The resolution of the previously mentioned synthetic issues would provide a diverse, scalable, and analogue friendly synthetic scheme. Similar to all the synthetic endeavors thus far, the final synthetic steps were planned to be a macrolactamization at the isoleucine–proline amide bond and a liberation of the C35 hydroxyl group. Compound 1.9 was further disconnected into the polypeptide hydroxyl azide 3.1 and the polyketide acid 3.2. As discussed earlier, we wanted to incorporate the azide prior to coupling of the major fragments. Therefore, the polypeptide fragment was disconnected into diamide 2.3 and azide 3.3, which could arise from a diazo transfer reaction of D-serine methyl ester. In regards to the polyketide domain, the plan was to unite the polyketide and polypeptide domains without protecting the C35 alcohol. After coupling, the TES ether would then be installed for the S-aW reaction. Alternatively, incorporation of the TES protecting group prior to coupling to the polypeptide domain would also be a possible route. Compound 3.2 would arise from a Paterson\textsuperscript{32} anti-selective aldol reaction with aldehyde 2.12. The aldehyde was further disconnected to pivaldehyde 2.6 and acetone, which would be subjected to a proline-catalyzed asymmetric aldol reaction (Scheme 3.3).\textsuperscript{34,35} The proline catalyzed aldol reaction was expected to produce better overall results than the Brown allylation, especially in terms of enantioselectivity.\textsuperscript{28}
3.3 Synthesis of the Polyketide Domain

During the original synthetic planning for 1.9, the polyketide domain was designed to still involve lactone 2.53, which was previously made via ring-closing metathesis. The intentions were to apply an asymmetric vinylogous aldol reaction to set the C39 carbinol center. In addition, the use of a vinylogous addition would provide all the necessary carbons to quickly and efficiently form lactone 2.53.
Synthetically, a vinylogous aldol reaction between pivaldehyde 2.6 and silyloxydiene 3.4 with (S)-BINOL and titanium isopropoxide generated alcohol 3.5. Unfortunately, the reaction was not enantioselective. With the expectation that diastereomers could be separated at a later stage, the synthesis towards lactone 2.53 was continued. The next three steps were optimized to be a one-pot procedure, which provided attractiveness towards this route regardless of the enantioselectivity of the vinylogous aldol. First, alcohol 3.5 was treated with excess methanol in toluene and heated for 20 minutes at 125 °C under microwave irradiation (CEM Discover® microwave reactor). During the reaction, a retro Diels-Alder reaction occurred to give ketene 3.6, which immediately was trapped with methanol to give β-keto ester 3.7. Upon completion, the reaction mixture was cooled to 0 °C and sodium borohydride was added to reduce the ketone. After an acidic workup, the resulting crude β-hydroxy ester 3.8 was dissolved in toluene, treated with catalytic TsOH, and heated at 125 °C (microwave). After purification, the desired lactone (2.53) was isolated in 64% yield over the three steps (Scheme 3.4).
Following the original synthesis (Scheme 2.2 and Scheme 2.3), the diastereomeric compounds produced at the stage of intermediate 2.11 (proline attached and primary alcohol) were easily separable via column chromatography. At this stage though, it was realized that the current route was not suitable for analogue study because the microwave reactions could only be performed on a small scale. In addition, the lack of enantioselective control of the vinylogous aldol reaction further limited the amount of the desired synthetic intermediates. Therefore, the route needed revised to address enantioselectivity and scalability.

The vinylogous aldol route, though abandoned, made it very apparent that it was absolutely necessary to set the C39 carbinol stereocenter in an enantioselective fashion. As a result, the powerful proline-catalyzed aldol reaction was explored. According to literature sources, the proline aldol reaction on pivaldehyde generally produced moderate yield and extremely high enantiomeric excesses. When attempted, the reaction
of pivaldehyde 2.6 and acetone in the presence of 30 mol% D-proline produced alcohol 2.31 in a 68% yield and an enantioselectivity >99%. Although the reaction took nearly four days to reach completion, the acceptable yield and exceptional enantioselectivity was extremely useful. In addition, the reaction was performed on a 25 g scale without significant depreciation of the yield or enantioselectivity. Mechanistically, the proline amine condenses with acetone to form an enamine, which attacks the aldehyde via a chair-like transition state with the carboxylate controlling the facial bias. The bulky tert-butyl group has a strong preference to occupy the equatorial position, which results in the high enantioselectivity. After carbon-carbon bond formation, the resulting iminium ion gets hydrolyzed and releases proline to reenter the cycle (Scheme 3.5).
At this point, we determined that the next few reactions would follow the work, with slight modifications, of Cavelier.\textsuperscript{24} Therefore, $\beta$-hydroxy ketone 2.31 was diastereoselectively reduced with a Prasad reduction (Et\textsubscript{2}BOMe, NaBH\textsubscript{4}, THF/MeOH, $-78 \degree C$),\textsuperscript{45} which afforded 1,3-\textit{syn} diol 3.9 in a 76% yield and >19:1 dr. The resulting diol was converted into diastereomeric cyclic sulfites (3.10) with thionyl chloride (SOCl\textsubscript{2}). It should be noted that a slightly modified procedure was used; Cavelier used pyridine as the solvent, while the modified procedure used Et\textsubscript{2}O as the solvent with only 3 equivalents of pyridine. The cyclic sulfites were oxidized to cyclic sulfate 3.11 with RuCl\textsubscript{3} (5 mol\%) and sodium periodate as the co-oxidant in 90% over the two steps. One
operational advantage to these two reactions is that both 3.10 and 3.11 are easily purified crystalline solids. The resulting cyclic sulfate was then opened from the least hindered side in a S$_{N}$2 fashion with the cuprate derived from allylmagnesium bromide and CuI. The resulting crude sulfate was simply hydrolyzed during the workup by stirring with sulfuric acid for several hours. Alcohol 3.12 was ultimately obtained in 84% yield as a single diastereomer by $^1$H NMR spectroscopy (Scheme 3.6).

![Scheme 3.6. Synthesis of alcohol 3.12](image)

Rather than protect the alcohol (as the Cavelier group did), the secondary alcohol was esterified with N-Boc-L-proline by the use of diisopropylcarbodiimide (DIC) and 4-($N,N$-dimethylamino)pyridine (DMAP), which efficiently generated ester 3.13. In contrast to the Yamaguchi esterification,$^{31}$ the carbodiimide method was preferred due to scalability and cost, even though nearly five equivalents of proline and DIC were necessary to ensure complete conversion of the hindered secondary alcohol. The olefin in 3.13 was subjected to ozonolysis and following a reductive workup with triphenylphosphine (PPh$_3$), aldehyde 2.12 was formed in 87% yield. A highly anti-selective (>19:1 dr) Paterson aldol was performed on aldehyde 2.12 to generate $\beta$-
hydroxy ketone \textbf{2.13} (Scheme 3.7). During the course of the Paterson \textit{anti}-aldol reaction, it was determined that the reaction environment needed to be completely anhydrous to ensure complete conversion of the aldehyde to the desired product. The competing transition states for the \textit{anti} aldol reaction can be seen in Figure 3.2. In each case, there is a strong preference for the proton in the \textit{E}-enol boronates to eclipse the double bond to minimize 1,3-allylic strain. In transition state A, there is a likely destabilizing lone-pair interaction between the benzoate and enolate oxygen. In contrast, there may be a stabilizing formyl hydrogen bond between the benzoate oxygen and aldehyde proton in transition state B (Figure 3.2). \textsuperscript{46}

\textbf{Scheme 3.7.} Synthesis of \(\beta\)-hydroxy ketone \textbf{2.13}
To circumvent the late-stage TBS to TES protecting group shuffle (Scheme 3.2), the plan was to attempt to leave the C35 hydroxyl group unprotected for the coupling and then to protect it as a TES ether post coupling. Therefore, compound 2.13 was treated with potassium carbonate in methanol to remove the benzoate. The resulting α-hydroxy ketone 3.15 was oxidatively cleaved with sodium periodate in water and t-BuOH, which produced carboxylic acid 3.2 in good yield over the two steps (Scheme 3.8).

Figure 3.2. Paterson anti-aldol transition states.
3.4 Synthesis of the Modified Serine and Polypeptide Domains

The synthesis of the modified serine moiety of 1.9 began with a diazotransfer reaction\(^\text{47}\) of D-serine methyl ester to produce azide 3.16. The primary alcohol was protected as a TBS ether with TBSCl, imidazole, and catalytic DMAP in 82% yield. After reduction of the methyl ester with DIBAL, a Wittig reaction with methyl 2-(triphenylphosphoranylidene)propionate on the resulting aldehyde gave \(\alpha,\beta\)-unsaturated ester 3.18. Finally, saponification of 3.18 was hydrolyzed with lithium hydroxide afforded carboxylic acid 3.3 (Scheme 3.9).
The next phase of the synthesis was to make the polypeptide chain and couple it to **3.3**. Starting from \(N\)-Boc-\(N\)-methyl-isoleucine methyl ester, the Boc blocking group was removed at 0 °C with trifluoroacetic acid. The resulting ammonium trifluoroacetate salt was coupled to \(N\)-Boc-\(N\)-methyl-alanine with the coupling agent PyAOP and \(i\)-Pr\(_2\)NEt in dichloromethane, which yielded amide **3.19**. Similarly, the Boc group was removed from amide **3.19** with trifluoroacetic acid and the ammonium salt was coupled to \(N\)-Boc-\(O\)-Me-tyrosine\(^{48}\) with PyAOP and \(i\)-Pr\(_2\)NEt to produce diamide **2.3** (Scheme 3.10). It should be noted that the original synthesis utilized DMF as the solvent for these amide bond formations, but it was determined that the reactions could be simply concentrated after completion, therefore dichloromethane was the more suitable solvent. Next, the modified serine acid **3.3** was coupled to diamide **2.3** (after Boc removal with trifluoroacetic acid) with PyAOP, which produce triamide **3.20**. Treatment of **3.20** with TsOH in methanol afforded the key azido alcohol (3.1) in 97% yield (Scheme 3.10).
3.5 Coupling of the Polyketide and Polypeptide Domains

With the polyketide carboxylic acid 3.2 and polypeptide 3.1 alcohol in hand, the next step was to couple them together via an esterification reaction to form 3.21. The free C35 alcohol was one of the main concerns in this reaction because it was possible that a β-lactone may form when the carboxylate was activated with the coupling agent. In addition, the electron withdrawing nature of the azide in 3.1 rendered the alcohol less nucleophilic, which suggested that conversion might be an issue.

The first coupling agent that was chosen was HATU. When the coupling was attempted in dichloromethane, there was very little conversion and both the 3.1 and 3.2 could be recovered. If the reaction was heated to reflux, elimination of 3.2 became a problem, but only trace amounts of desired 3.21 were formed. Next, the solvent was switched to DMF. Unfortunately though, there was still very little conversion. At this
point, it appeared that either HATU was not active enough for the esterification or the alcohol’s reduced nucleophilicity was, in fact, an issue. The next coupling agent that was employed was 1-ethyl-3-(3-dimethylamiopropyl)carbodiimide (EDCI). When the coupling was initially attempted, there was very little conversion at 0 °C, but a relatively rapid reaction occurred upon warming to room temperature. The carboxylic acid 3.2 was consumed, but the alcohol 3.1 was not. After purification, it was discovered that β-lactone 3.22 formed. It was postulated that because the acid must be activated prior to alcohol addition with EDCI, a better coupling agent might be PyAOP because both the acid and amine components can be placed in solution with the coupling agent and an amine base. In addition, PyAOP is slightly more activating than HATU, which showed little to no reactivity. When PyAOP was tested though, no coupling resulted, but β-lactone 3.22 formed slowly over longer reaction times. For completion sake, the coupling agent DIC was also attempted, but β-lactone 3.22 formed quickly (Table 3.1). As a consequence of these results, it was realized that the C35 alcohol in 3.2 needed to be protected.

As mentioned previously, one of the goals of the synthesis of 1.9 was to eliminate the C35 protecting group shuffle from TBS to TES post coupling. The logical idea was to simply install the TES group prior to coupling. As a result, compound 2.13 was treated with TESOTf and 2,6-lutidine at −78 °C. The reaction, though, started to remove substantial amounts of the Boc group in 2.13. Even when 1.0 equivalent of TESOTf was used, it was clear that the rate of Boc removal was similar to C35 TES protection. Therefore, the reaction conditions were changed to triethylsilyl chloride
(TESCl), imidazole, and catalytic DMAP, which produced the desired silylated alcohol 3.23 in 76% yield with no Boc removal (Scheme 3.11).

![Chemical Structures](image)

<table>
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<th>Solvent</th>
<th>Temperature</th>
<th>Result</th>
</tr>
</thead>
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<td>no coupling elimination at 40 °C</td>
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<tr>
<td>PyAOP</td>
<td>CH₂Cl₂</td>
<td>0 °C to 40 °C</td>
<td>no coupling, slight formation of 3.22 elimination at 40 °C</td>
</tr>
<tr>
<td>EDCI</td>
<td>CH₂Cl₂</td>
<td>0 °C to rt</td>
<td>3.22 formed rapidly at rt</td>
</tr>
<tr>
<td>DIC</td>
<td>CH₂Cl₂</td>
<td>0 °C to rt</td>
<td>3.22 formed rapidly at rt</td>
</tr>
</tbody>
</table>

Table 3.1. Coupling attempts
Compound 3.23 was then treated with potassium carbonate in methanol to remove the benzoate and the resulting $\alpha$-hydroxy ketone 3.25 was treated with sodium periodate in aqueous tert-butanol. However, the TES group was removed under these conditions, which was precedent. To determine if the sodium periodate was removing the TES group, lead tetraacetate (Pb(OAc)$_4$) was used to cleave the $\alpha$-hydroxy ketone, but the TES ether was cleaved and the $\alpha$-hydroxy group was partially oxidized. These results suggested that the silyl ether was being cleaved internally with intramolecular proton transfer via 3.26 (Scheme 3.12).

Scheme 3.11. TES protection of 2.13
Although one of the goals was to incorporate the TES group prior to coupling, the more robust TBS group would serve better. Compound 2.13, therefore, was treated with TBSOTf and 2,6-lutidine at –78 °C to give 2.14, but the Boc group was again removed with a comparable rate to hydroxyl silylation. In contrast to the TES group, the silyl carbamate 3.27 was stable and difficult to separate from desired 2.14. Similar to the TES group, the next conditions that were attempted were TBSCI, imidazole, and catalytic DMAP. In contrast to the use of TES reagents though, the C35 alcohol was not efficiently silylated at room temperature over 24 or 48 h in dichloromethane or dimethylformamide. In addition, heating the reaction mixture caused elimination of the C35 alcohol. The next reaction conditions attempted used TBSCI, silver nitrate, and 2,6-lutidine in dimethylformamide. Again though, the Boc group was competitively removed and elimination of the C35 alcohol occurred (Table 3.2).
In the end, the best approach to solve this problem was to treat 2.13 with TBSOTf at –78 °C, which gave a mixture of 2.14 and 3.27 in a combined 85% yield. The mixture was then treated with TBAF at 0 °C, which cleaved the silyl carbamate without removal of the C35 TBS group. The resulting free amine was then re-protected with a Boc group to give 2.14. Although not ideal, the yields of the steps were high enough to continue with the synthesis. With 2.14 available in large quantities, the desired carboxylic acid 2.2 was produced by first removal of the benzoate with potassium carbonate in methanol and then cleavage of the hydroxy ketone with sodium periodate (Scheme 3.13).

**Table 3.2.** Attempted TBS protection
With the preparation of the polyketide acid 2.2 and polypeptide alcohol 3.1 finished, the next step was to couple them together. Treatment of acid 2.2 with EDCI and DMAP at 0 °C and then addition of alcohol 3.1 efficiently produced the coupled product 3.28 in 86% yield. At this stage, the TBS group was removed with HF-pyridine and the resulting C35 alcohol was protected as a TES ether (3.29) (Scheme 3.14). Interestingly, the use of TESOTf did not remove the Boc group in this case. The stage was now set to attempt the key Staudinger aza-Wittig reaction.

Scheme 3.13. Synthesis of acid 2.2
3.6 Staudinger Aza-Wittig Reaction

The installation of the oxazoline via a Staudinger aza-Wittig (S-aW) reaction was the next step in the synthesis. Following the protocol in the original synthesis, azide 3.29 was treated with triphenylphosphine in anhydrous THF at 0 °C and the resulting mixture was heated to 45 °C. By TLC, the starting azide was consumed after 14 h, but the crude product revealed that azide reduction occurred to the iminophosphorane (by mass spectrometry) without formation of desired oxazoline 3.30. With that result, the mixture was heated to 65 °C in the next trial, but decomposition started to occur with no formation of the oxazoline. Since the problem seemed to be the aza-Wittig reaction and not the Staudinger reduction, smaller trialkylphosphines were explored. However, both tributylphosphine and trimethylphosphine did not afford the desired oxazoline 3.30, but rather formed the iminophosphorane and/or led to decomposition. At the time, contemporaneous research in the Forsyth group indicated that the Staudinger aza-Wittig reaction could be accelerated with the use of microwave irradiation for the formation of...
Therefore, the three trialkylphosphines were screened with the use of microwave irradiation as the heating method. Again though, no oxazoline was formed (Table 3.3). Several additional solvents (CH$_3$CN, toluene, CH$_2$Cl$_2$, CHCl$_3$) were attempted with the various trialkylphosphines, but still the desired oxazoline was not formed. It was postulated that reduced electrophilicity of the ester (versus a thioester) and the steric crowding in the context of 3.29 was simply too much to allow the aza-Wittig reaction to occur. Although oxazolines have been formed by the Staudinger aza-Wittig, it was clear at this point that the oxazoline would need to be formed by a different method.

![Diagram of 3.29 and 3.30 reactions](image)

<table>
<thead>
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<th>Trialkylphosphine</th>
<th>Temperature (°C)</th>
<th>Heating Method</th>
<th>Result</th>
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<td>0 to 45 to 65</td>
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<td>Iminophosphorane – no oxazoline</td>
</tr>
<tr>
<td>PBu$_3$</td>
<td>0 to 45 to 65</td>
<td>sealed tube</td>
<td>Iminophosphorane – no oxazoline</td>
</tr>
<tr>
<td>PMe$_3$</td>
<td>0 to 45 to 65</td>
<td>sealed tube</td>
<td>decomposition</td>
</tr>
<tr>
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<td>0 to 45 to 65</td>
<td>microwave</td>
<td>Iminophosphorane – no oxazoline</td>
</tr>
<tr>
<td>PBu$_3$</td>
<td>0 to 45 to 65</td>
<td>microwave</td>
<td>Iminophosphorane – no oxazoline</td>
</tr>
<tr>
<td>PMe$_3$</td>
<td>0 to 45 to 65</td>
<td>microwave</td>
<td>decomposition</td>
</tr>
</tbody>
</table>

Table 3.3. Staudinger Aza-Wittig Reaction Attempts
3.7 DAST Mediated Oxazoline Formation and Completion

With the failure of the Staudinger aza-Wittig to install the oxazoline, the retrosynthetic analysis had to be adjusted. Quickly though, it was realized that the entire synthesis did not have to re-engineered because azide 3.1 could still be used as a key intermediate. Rather than make the ester bond to couple the polyketide and polypeptide, it was realized that the amide bond could be just as easily formed. The two components would then be amine 3.31 and acid 3.2. Based upon the lessons learned in the esterification reaction to unite the polyketide and polypeptide domains, it was expected that the C35 alcohol may not have to be protected due to the increased nucleophilicity of the amine. As a result, the coupled product could then be treated with diethylaminosulfur trifluoride (DAST) to install the oxazoline (Scheme 3.15).\textsuperscript{55}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{scheme315.png}
\caption{Scheme 3.15. Revised retrosynthetic analysis}
\end{figure}

The key amino alcohol 3.31 was generated by a Staudinger reduction of the azide in 3.1. The Staudinger reduction with triphenylphosphine in THF/H\textsubscript{2}O did not produce encouraging results, as yields and conversion were low. Attempts to increase those yields and conversions, such as heating in a sealed tube and microwave irradiation, only
produced a maximum yield of 43%. To solve this problem, the smaller, more nucleophilic tributylphosphine was used. In addition to its more nucleophilic character, the resulting iminophosphorane intermediate was expected to be hydrolyzed at a much more rapid rate, which was suspected to be the problem with triphenylphosphine. Fortunately, it was discovered that treatment of azide 3.1 with tributylphosphine at 0 °C in THF/H₂O and warming to room temperature for 1 h caused efficient reduction to the iminophosphorane. After heating to 55 °C in the microwave reactor for 30 minutes, complete conversion was observed and a yield of 91% was obtained (Scheme 3.16).

![Scheme 3.16. Staudinger reduction of 3.1](image)

To couple amine 3.31 and carboxylic acid 3.2, the coupling agent, HATU, was chosen because, as previously observed during the esterification study (Table 3.1), β-lactone 3.22 would not be formed. It was expected that the more nucleophilic amine 3.31 would add into the activated carboxylate with no problems. When the reaction was attempted, the desired amide 3.32 was acquired in a 94% yield. Amide 3.32 was then treated with DAST at –78 °C and the desired oxazoline 3.33 was obtained in 78% yield (Scheme 3.17). It is worth noting that DAST can convert alcohols to alkyl fluorides, but usually warmer temperatures (0 °C to room temperature) are required. As a result, the
C35 alcohol did not have to be protected prior to oxazoline formation, which solved the original protecting group shuffle problem (TBS to TES).

![Chemical structures](image)

**Scheme 3.17.** Coupling and oxazoline formation

Compound **3.33** was treated with TMSOTf and 2,6-lutidine at 0 °C to remove the Boc group, which concurrently protected the C35 as a stable TMS ether. Though protection of the C35 alcohol was undesired, the TMS group was simply removed with TBAF at 0 °C to generate **3.34** in 68% yield over the two steps. The methyl ester in **3.34** was then saponified with lithium hydroxide and the resulting amino acid **3.35** was
cyclized to give **1.9** in 31% yield over two steps (Scheme 3.18). Originally, the low yield was attributed to the free C35 alcohol, but it was later discovered that the lithium hydroxide step epimerized the proton on C30, which produced an epimerized product that could not be separated from the HATU by-products on silica gel. With the synthesis of **1.9** completed, the attention of the project turned to the design and synthesis of analogues.

**Scheme 3.18.** Completion of **1.9**
CHAPTER 4

TOTAL SYNTHESIS OF ADDITIONAL ANALOGUES OF APRATOXIN A

4.1 Introduction to Synthetic Analogues

The total synthesis of 1.9 provided an efficient, tunable synthetic route for analogue studies. Overall, the project’s main goal is to discover a synthetic analogue that retains the prolific biological activity of 1.1, but reduces the toxicity towards normal cells. Without the key non-covalent interactions between 1.1 and the molecular target, the analogues were designed to probe how the activity would change with certain structural modifications. As seen in Figure 4.1, the targeted analogues focus on the moSer domain, the C35 alcohol region, and the tyrosine residue. We postulated that the polypeptide domain was responsible for the non-covalent interactions with the molecular target; therefore that region was necessary to retain. It was also hypothesized that the polyketide domain may be responsible for holding 1.1 into its active conformation, but it was unknown which functional groups were necessary for retention of anti-cancer activity. In addition, the C35 alcohol was believed to form an intramolecular hydrogen bond with the isoleucine residue, though this was only postulated with molecular modeling.¹ As a result, we wanted to determine the effect of
altering the C35 alcohol, as well as the region surrounding it. The moSer moiety, which may help provide conformational rigidity, was of interest because none the natural analogues of 1.1 only have alterations to that domain. Finally, the tyrosine methoxy group was not expected to be important for biological activity, therefore it seemed like the perfect place to attach an extended polyethylene glycol linkage that would provide a handle to attach biological probes.

Figure 4.1. Target Analogues
4.2 Allylated tyrosine analogue

To study the effect of altering the methoxy group in the tyrosine residue, it was decided to replace the –O-Me with an –O-Allyl group. The reason for this alteration was three fold: 1) it was necessary to determine if altering that region was important for biological activity, 2) the allyl group could be used to attach biological probes via ruthenium-catalyzed cross metathesis reactions, and 3) removal of the allyl group would produce a new analogue (phenol). Synthetically, the only change from the original route of 1.9 was to install the allyl group on tyrosine (Scheme 4.1).

![Scheme 4.1. Retrosynthesis of allylated 1.9](image-url)
In the forward synthetic direction, the polyketide domain 3.2 and moSer 3.3 were unaltered. For the polypeptide domain, the synthesis started with removal of the Boc group in 3.19 with trifluoroacetic acid at 0 °C. The resulting ammonium trifluoroacetate salt was coupled to N-Boc-O-allyl-tyrosine\(^{57}\) with PyAOP to generate diamide 4.3 in 84% over the two steps. The Boc group in 4.3 was removed again with TFA and the resulting ammonium salt was coupled to the moSer acid 3.3 with PyAOP, which produced triamide 4.4. Removal of the primary TBS with TsOH in methanol and Staudinger reduction of the resulting azido alcohol yielded amine 4.2 in 90% yield (Scheme 4.2).

\[
\begin{align*}
\text{Boc-} & \text{N} & \text{O} & \text{N} & \text{O} & \text{Me} \\
\text{3.19} & & & & & \\
1. \text{TFA, CH}_2\text{Cl}_2, \ 0 \ ^\circ\text{C}, \ 1 \ h & & & & & \\
2. \text{PyAOP, i-Pr}_2\text{NEt} & & & & & \\
N-\text{Boc-O-allyl-L-tyrosine} & & & & & \\
\text{CH}_2\text{Cl}_2, \text{rt, 12 h} & & & & & \\
84\% \ (2 \ steps) & & & & & \\
\end{align*}
\]

\[
\begin{align*}
\text{HO-} & \text{R} & \text{N} & \text{O} & \text{N} & \text{O} & \text{Me} \\
& & & & & & \\
\text{4.2} (R = \text{NH}_2) & & & & & & \\
\text{PBU}_3, \text{THF/H}_2\text{O} & & & & & & \\
0 \ ^\circ\text{C} \text{ to rt, 1 h} & & & & & & \\
\text{then microwave at } 55 \ ^\circ\text{C, 30 min} & & & & & & \\
90\% & & & & & & \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \text{Me} \\
\text{4.4} & & & & & & \\
\text{TsOH+H}_2\text{O} & & & & & & \\
\text{MeOH, rt, 2 h} & & & & & & \\
\text{TBS} & & & & & & \\
\text{4.5} (R = \text{N}_3) & & & & & & \\
\end{align*}
\]

\[
\begin{align*}
1. \text{TFA, CH}_2\text{Cl}_2, \ 0 \ ^\circ\text{C}, \ 1 \ h & & & & & \\
2. \text{3.3, PyAOP, i-Pr}_2\text{NEt} & & & & & \\
\text{CH}_2\text{Cl}_2, \text{rt, 5 h} & & & & & \\
78\% \ (2 \ steps) & & & & & \\
\end{align*}
\]

\textbf{Scheme 4.2}. Synthesis of amino alcohol 4.2
Amine 4.2 and carboxylic acid 3.2 were coupled with HATU/i-Pr₂NEt at room temperature to yield amide 4.6 in excellent yield (97%). Oxazoline 4.7 was then formed with DAST at low temperature and the resulting compound was treated with TMSOTf/2,6-lutidine to remove the Boc group. The crude TMS ether was removed with TBAF at 0 °C to yield amine 4.8. As seen in the synthesis of 1.9, the methyl ester was then hydrolyzed with lithium hydroxide and the resulting amino acid was cyclized with HATU, which generated 4.1 in 21% over two steps (Scheme 4.3). It should be noted the proton at C30 was epimerized during the methyl ester saponification, but the epimerized product was difficult to isolate in pure form from the reaction mixture via preparative thin layer chromatography. In addition, the approximately 10% less yield in the final steps of 4.1 (versus 1.9) can simply be attributed to experimental error and non-optimized conditions. Based upon ¹H NMR analysis, replacement of the methoxy group in the tyrosine residue for an allyl group does not appear to change the conformational preference, at least in CDCl₃.
4.3 Saturated MoSer Analogue

The next analogue that was sought after was the oxazoline analogue of an apratoxin A and apratoxin E hybrid (Figure 4.2). As previously mentioned, none of the natural analogues of 1.1 contain modifications only at the modified cysteine domain. Saturation of the C28–C29 olefin was expected to give 4.9 increased conformational freedom, which may enable 4.9 to bind tighter to its molecular target. In addition, it was
not expected that the C28–C29 olefin participated in binding to the molecular target, therefore saturating it should not decrease activity.

Retrosynthetically, 4.9 was disconnected into the known polyketide acid 3.2 and triamide 4.10. Triamide 4.10 was taken back to diamide 2.3 and the saturated modified serine domain 4.11, which could arise from L-glutamic acid (Scheme 4.4). In the forward direction, the key synthetic steps were expected to follow the synthesis of 1.9.
In comparison to the synthesis of 1.9, the only major change was to make a different moSer domain 4.11, which upon close inspection revealed that L-glutamic acid would be the perfect starting material. The mono-methyl ester of L-glutamic acid (4.12)\(^{58,59}\) was subjected to a diazo-transfer reaction,\(^{47}\) which proceeded with retention of configuration to give azide 4.13. The next step was to selectively reduce the carboxylic acid to the primary alcohol without affecting the azide or methyl ester. Initially, borane dimethylsulfide (BH\(_3\)·Me\(_2\)S) was attempted, but competitive reduction of the azide occurred. As a result, a two-step procedure was explored that involved activation of the carboxylic acid. First, the carboxylic acid was efficiently converted to the \(N\)-hydroxysuccinimide ester with the aid of \(N,N'\)-dicyclohexylcarbodiimide (DCC). The crude activated carboxylate was then reduced at 0 °C with sodium borohydride in
THF/EtOH, which yielded primary alcohol 4.14 in good yield over the two steps. The resulting primary alcohol was then protected as a TBS ether (4.15), and the remaining methyl ester was saponified with lithium hydroxide to yield the desired carboxylic acid 4.11 (Scheme 4.5).

The saturated moSer domain 4.11 was next coupled to the known diamide 2.3 by first removing the Boc group in 2.3 and then subjecting the acid and amine to a coupling reaction with PyAOP. The resulting triamide 4.16 was treated with TsOH in methanol to remove the primary silyl ether and the remaining azide (4.17) was reduced under Staudinger conditions to yield amino alcohol 4.10 (Scheme 4.6). At this point, it would have been possible to simply reduce the azide under hydrogenation conditions, but the recently developed microwave assisted Staudinger reduction worked satisfactorily.
At this stage, amine 4.10 and acid 3.2 were united with HATU/i-Pr₂NEt and, as expected, produced 4.19 with a high yield (90%). Next, the resulting amide (4.19) was converted into the oxazoline (4.20) with DAST at –78 °C. The Boc group was then removed with TMSOTf/2,6-lutidine and the resulting stable TMS ether was cleaved with TBAF at 0 °C, which generated 4.21 in good yield over the two steps. Finally, the methyl ester was saponified with lithium hydroxide and the resulting amino acid was cyclized with HATU/i-Pr₂NEt. Compound 4.9 was obtained in 51% yield over the two steps (Scheme 4.7). Interestingly, the final cyclization produced a much higher yield over two steps than 1.9 or 4.1, which both contain unsaturation in the moSer domain. It is worth noting that there was no significant epimerization of the proton at C30. The increased polarity (TLC analysis) of 4.9, in comparison to 1.9, suggests that the free secondary alcohol may be more available to interact with the silica gel, or that the conformation of the molecule on silica gel differs enough to increase interactions with the silica gel.
Scheme 4.7. Completion of 4.9
Though the $^1$H NMR and mass spectroscopic analysis confirmed the structure of 4.9, the possible participation of the unprotected C35 alcohol in the final macrolactamization was still in question. Therefore, it was decided that the C35 alcohol should be protected prior to cyclization to determine if the yield over the last two steps could be increased. As a result, compound 4.21 was treated with TESOTf/2,6-lutidine at −78 °C to protect the C35 alcohol as the TES ether (4.22). The remaining methyl ester was saponified with lithium hydroxide, but the TES ether was concurrently removed. The TES group was reinstalled to the crude amino acid with TESOTf and the resulting product was subjected to the macrolactamization conditions to give 4.23 in 38% yield over the three steps. Though the cyclization steps couldn’t be compared directly due to the unexpected TES removal, the opportunity to still finish the synthesis existed. Therefore, the TES ether was removed with HF-pyridine and the resulting product was purified with preparative thin layer chromatography to give the same product as before, 4.9 (Scheme 4.8).

Scheme 4.8. Protection/deprotection alternative to 4.9
4.4 Extended, Saturated MoSer Analogue

The saturated analogue 4.9 was expected to increase conformational freedom, which may enable the compound to achieve the necessary active conformation for binding to the molecular target. With that thought in mind, it was expected that increasing the methylene units by one in the moSer should give the compound even more conformational flexibility. As a result, the target analogue became 4.24, which could be put together similar to the previous analogues. The major change would arise within the moSer domain 4.26, as can be seen in the retrosynthesis (Scheme 4.9).

Scheme 4.9. Retrosynthesis of 4.24

For the synthesis of moSer domain 4.26, it was recognized that intermediate 4.16 (Scheme 4.5) could be used as the starting point. We envisioned that compound 4.26 could be made in two separate ways, both starting with a reduction of 4.16. Compound
4.16 was reduced with diisobutylaluminum hydride, which generated primary alcohol 4.27. Attempts to stop the reduction at the aldehyde stage were unsuccessful; therefore full reduction to the alcohol was necessary. At this point, the synthetic plan towards 4.26 was diverted in two separate routes. In the first route, the alcohol was converted to the alkyl iodide via an intermediate tosylate. The resulting iodide was then displaced with potassium cyanide, which produced nitrile 4.29. At this stage, the cyanide just needed to be hydrolyzed to produce the desired carboxylic acid 4.26. Attempts to hydrolyze the cyanide with both acidic and basic solutions only resulted in decomposition of the starting material. Therefore, the second route from 4.27 was explored. The primary alcohol was oxidized under Swern conditions to the aldehyde, which was subjected to a Wittig homologation. The resulting olefin was oxidized with a hydroboration/oxidation sequence to generate the homologated primary alcohol 4.29 in 47% yield over the three steps. The primary alcohol was then oxidized with TEMPO/BAIB\textsuperscript{60} to afford the desired carboxylic acid 4.26 (Scheme 4.10).
With the synthesis of 4.26 completed, the next step was to couple it to the amino acid chain. Therefore, the moSer carboxylic acid 4.26 was coupled to diamide 2.3 with PyAOP/\textit{i}-Pr\textsubscript{2}NEt, after the Boc group was removed from 2.3 with trifluoroacetic acid. The TBS ether in 4.30 was removed with TsOH in methanol, which afforded primary alcohol 4.31. When 4.31 was exposed to the microwave assisted Staudinger reduction, moderate to poor yields were consistently obtained. As a result, the azide was simply reduced by hydrogenation (Pd/C, 1 atm H\textsubscript{2}) to furnish the desired amine 4.25 in 87% yield (Scheme 4.11).
Activation of carboxylic acid 3.2 with HATU, followed by the addition of amine 4.25 led to the formation of amide 4.32 in excellent yield. The oxazoline (4.33) was formed with DAST and the Boc group was removed with trifluoroacetic acid, but again the resulting TMS ether was relatively stable. Therefore, the crude TMS ether was removed with TBAF at 0 °C, which generated the desired free amine 4.34. Treatment of 4.34 with lithium hydroxide in a mixture of t-butanol, THF, and water led to the formation of the cyclization precursor. The crude product was treated with HATU and i-Pr₂NEt in dichloromethane to yield the desired depsipeptide 4.24 in 47% yield over the two steps (Scheme 4.12). Similar to 4.9, the extended saturated analogue was more polar than 1.9, which again suggests different conformations on silica gel, which may involve increased availability of the C35 alcohol. In addition, epimerization of the proton at C30 was not a problem.
4.5 Oxazoline Analogue of (E)-34,35-dehydroapratoxin A

The semi-synthetic dehydrated analogue of apratoxin A (1.4) was discovered after exposing 1.1 to mildly acidic CDCl$_3$. Though not nearly as potent as 1.1, the dehydrated analogue still has remarkable activity. In addition, groups have avoided the dehydrated analogue most likely because of decreased activity, but the goal of this project
is to make an analogue that has selectivity for cancer cells, not only low nanomolar activity. As a result, the dehydrated analogue of 1.9 still seemed like a potentially valuable target compound. From a structure-activity point of view, the lack of the C35 hydroxyl group in 4.35 may help determine the necessity of the C35 alcohol in terms of cancer selectivity. As seen in the $^1$H NMR spectrum of 1.4, there is nearly a 1:1 ratio of conformers in CDCl$_3$, while 1.1 is mostly one conformer. This suggests that the C35 alcohol is, in fact, very important for conformational rigidity, at least in CDCl$_3$.

Retrosynthetically, 4.35 was disconnected similar to the other analogues. As seen in the syntheses of 1.9 and 4.1, the C30 proton was expected to be a problem due to its epimerizability. In the context of 4.35, the conjugation of the oxazoline and (E)-olefin would make the proton even more sensitive. Though problems were expected and the separation may be tough, we saw this as an opportunity to make an epimerized product and therefore an additional analogue. The polypeptide domain 3.31 remained the same as the in the total synthesis of 1.9. The polyketide domain, though, needed to be altered to install the (E)-olefin. The trisubstituted (E)-olefin would be incorporated via a Wittig reaction of aldehyde 2.12 (Scheme 4.13).
In the forward direction, the known aldehyde 2.12 was subjected to a Wittig reaction with methyl 2-(triphenylphosphoranylidene)propionate in toluene at 80 °C. The desired olefin 4.37 was isolated in 88% yield as a single diastereomer by \(^1\)H NMR spectroscopy. The methyl ester was saponified with lithium hydroxide, which produced the desired unsaturated polyketide carboxylic acid 4.36 in 88% yield (Scheme 4.14).

Scheme 4.13. Retrosynthesis of oxazoline analogue of (E)-34,35-dehydroapraotox A

Scheme 4.14. Polyketide carboxylic acid 4.36
With the unsaturated polyketide carboxylic acid 4.36 and polypeptide amine 3.31 in hand, the two components were coupled together with HATU. The resulting amide 4.38 was treated with DAST in dichloromethane, which formed the desired oxazoline (4.39) in moderate yield. At this stage, the Boc group was removed without any issues with TMSOTf in good yield (Scheme 4.15).

![Chemical structure](image)

Scheme 4.15. Synthesis of amine 4.40

Finally, the methyl ester was saponified with lithium hydroxide in t-butanol, THF, and water. The resulting amino acid was cyclized with HATU under high dilution conditions, which generated 4.35 as a 1:1 mixture of diastereomers. As expected, the
proton at C30 was easily epimerized during the saponification step (Scheme 4.16). Fortunately though, the two diastereomers were successfully separated, but the separation via preparative thin layer chromatography was challenging. If these unsaturated analogues are biologically relevant, a simple solution to access either of them would be to use an allyl ester in place of the methyl ester. The allyl ester could be simply removed with palladium and a non-basic amine, which should prevent the epimerization problem.

Scheme 4.16. Cyclization to 4.35

4.6 C34, C35 Saturated Analogue

The rationale of analogue synthesis, in part, is to determine what parts of the molecule are important for retention of the biological activity. Synthetically, it can be advantageous to simply the synthesis by removing stereocenters because it should increase the ease and efficiency of the synthesis. As a result, we chose to embark on the synthesis of an analogue that did not contain the C34 methyl group or C35 hydroxyl group. The resulting compound 4.41 should offer increased conformational flexibility and help determine the importance of the C34 methyl and C35 hydroxyl groups. Retrosynthetically, 4.41 could come from the known polypeptide domain 3.31 and a
saturated polyketide domain 4.42 (Scheme 4.17). Fortunately, aldehyde 2.12 could we used a key intermediate, which demonstrated the versatility of the synthesis of 1.9.

Starting with known aldehyde 2.12, a Wittig reaction was performed to install the necessary carbon atoms in the polyketide domain. The resulting α,β-unsaturated ester was reduced with hydrogen on palladium on carbon in methanol and the remaining methyl ester was saponified with lithium hydroxide, which generated the key polyketide carboxylic acid 4.42 in 67% yield over the three steps (Scheme 4.18). Originally, the three steps were attempted with purifications, but it was determined that simply filtering the Wittig reaction through silica gel and filtering the hydrogenation reaction through Celite® produced the most favorable results.
The polyketide acid 4.42 and amine 3.31 were united together by a HATU assisted amide bond formation, which generated amide 4.43 in good yield. Treatment of 4.43 with DAST in dichloromethane at −78 °C produced oxazoline 4.44 in 77% yield. The Boc protecting group was removed with TMSOTf and the intermediate silyl carbamate was hydrolyzed during the workup. Finally, the methyl ester was saponified with lithium hydroxide and the corresponding amino acid was cyclized with PyAOP to yield 4.41 as a mixture of diastereomers. Interestingly, the conformational flexibility was evident in the $^1$H NMR spectrum because each of the diastereomers existed in approximately a 2:1 ratio of rotamers (Scheme 4.19). At this point, it became clear that unsaturation between C28–C29 was the functionality causing the proton at C30 to be epimerizable under the lithium hydroxide conditions. Again though, the diastereomers represent new analogues to assay and if active, the analogues could be made by with an allyl ester protecting group rather than the methyl ester.
The simplest analogue that we targeted contained saturation in the moSer domain and removal of the C34 methyl and C35 hydroxyl groups. This analogue would be simple to make and would provide us with an insight on the importance of those domains, collectively. Additionally, the alterations would give the analogue the most
conformational flexibility. As can be seen in the retrosynthesis, the key polyketide and polypeptide intermediates would arise from the intermediates used in the synthesis of previous analogues 4.9 and 4.41 (Scheme 4.20). In addition, the use of previous intermediates to make new analogues highlights the flexibility and efficiency of the overall synthetic route.

In the forward direction, the carboxylic acid 4.42 was activated with HATU and then amine 4.10 was added to produce amide 4.47 in excellent yield. The resulting amide was treated with DAST to induce oxazoline (4.48) formation in moderate yield. The Boc group was removed with TMSOTf, as seen in the previous analogues, and the resulting amino acid was cyclized under the assistance of PyAOP, which generated analogue 4.46 in 58% yield over the two final steps (Scheme 4.21). Similar to earlier analogues, the $^1$H NMR spectrum indicated a nearly 1:1 ratio of rotamers, which suggests that the analogue has a lot of conformational freedom.
Scheme 4.21. Completion of 4.46
CHAPTER 5

REVISED SYNTHESIS OF THE POLYPEPTIDE DOMAIN AND
SYNTHESIS OF ADDITIONAL ANALOGUES

5.1 Synthetic Revision to the Polypeptide Domain

The synthetic route used, thus far, provided us with several analogues, but improvements were still needed to the overall synthetic plan. The epimerization of the proton at C30 in all of the unsaturated C28–C29 analogues during the saponification of the methyl ester greatly limited the efficiency of the synthesis. As a result, modifications of the synthetic route were required to ensure that epimerization would no longer be a problem. To solve the epimerization problem, we chose to install an allyl protecting group rather than the methyl ester. It was expected that the allyl ester could be converted to the carboxylic acid by treatment with palladium and a nucleophilic, non-basic amine to trap the allyl palladium intermediate.\(^{61,62}\) In addition, utilization of the azide as a masked amine, though useful originally, was not ideal. The ideal situation would be to protect the alcohol and amine in the polypeptide domain in such a way that the functional
groups could be revealed at the same time. This approach would help streamline the overall synthetic route.

Unfortunately, the installation of the methyl ester occurred at the very beginning of the synthesis. As a result, the revised synthesis began with \(N\)-methyl-\(N\)-Boc-isoleucine allyl ester (5.1), which is commercially available or obtainable by treatment of \(N\)-methyl-\(N\)-Boc-isoleucine with potassium carbonate and allyl bromide in DMF. The Boc blocking group was removed with trifluoroacetic acid and the resulting ammonium trifluoroacetate salt was coupled to \(N\)-Boc-\(N\)-methyl-alanine with PyAOP in dichloromethane, which produced amide 5.2 in great yield over the two steps. Amide 5.2 was treated with trifluoroacetic acid to remove the Boc group and the ammonium trifluoroacetate salt was coupled to \(N\)-Boc-\(O\)-Me-tyrosine, which yielded diamide 5.3 (Scheme 5.1).
In regards to the previous use of an azide, it was postulated that we could use acetonide and Boc protecting groups to mask the amino alcohol functionality (5.4) (Scheme 5.2).

This would provide an opportunity to remove both groups in one-pot and then couple the amine directly to the polyketide carboxylic acid. With the synthesis of diamide 5.3 completed, the next task was to design and execute the synthesis of the new modified serine domain 5.5. It was quickly realized that using Garner’s aldehyde (5.6)\(^{63}\), which can be conveniently made from D-serine, as a starting point would provide us with the desired functionality. Therefore, a Wittig reaction on Garner’s aldehyde with methyl 2-(triphenylphosphoranylidene)propionate\(^{19}\) was performed and the resulting unsaturated ester (5.7) was saponified with lithium hydroxide to give the new moSer carboxylic acid 5.5 (Scheme 5.3).
With the completion of diamide \(5.3\) and carboxylic acid \(5.5\), the next step was to couple them together. Therefore, the Boc protecting group in \(5.3\) was removed with trifluoroacetic acid and then coupled to acid \(5.5\) with PyAOP, which formed triamide \(5.4\) (Scheme 5.4).

With the synthesis of the new polypeptide domain \(5.4\) finished, new analogues were explored that involved the stereochemistry of the C34 methyl and C35 hydroxyl groups.

### 5.2 Synthesis of Stereoisomers at C34 and C35

The previous analogues have dealt with alterations of the oxidation state in the moSer and polyketide domains by removing functional groups and introducing or removing unsaturation. The next goal of our analogue study was to change the C34 and C35 stereochemistry to determine the importance of those stereocenters.
synthetic point of view, the different C34 and C35 stereocenters could be conveniently installed by use of asymmetric aldol reactions\textsuperscript{64,65} on aldehyde 2.12 (Scheme 5.5).

![Scheme 5.5. Installation of C34–C35 stereocenters](image)

We first targeted the analogue that would retain the natural relative \textit{anti} stereochemistry, but opposite absolute configurations (Figure 5.1).

![Figure 5.1 C34,C35–\textit{Anti} Analogue 5.11](image)
To install the desired *anti* stereochemistry at C34, C35, aldehyde 2.12 was subjected to a Paterson *anti*-aldol with (S)-2-benzoyloxy-3-pentanone.\(^{32}\) Interestingly, the diastereomeric ratio (\(^1{H}\) NMR analysis) was only 6:1, which is significantly less than the Paterson aldol using (R)-2-benzoyloxy-3-pentanone (\(>19:1\) dr). This suggests that the aldehyde \(\beta\)-methyl group must be influencing the selectivity and therefore creating a somewhat mismatched case. The resulting \(\beta\)-hydroxy ketone 5.12 was treated with potassium carbonate in methanol to free the secondary alcohol and the intermediate \(\alpha\)-hydroxy ketone was oxidatively cleaved to carboxylic acid 5.13 (Scheme 5.6). At this stage, the diastereomers produced from the aldol reaction were separable by column chromatography on silica gel.

![Chemical Structures](image)

**Scheme 5.6** Synthesis of polyketide acid 5.13

The next task was to remove the acetonide and Boc protecting groups in one-pot to free the amino alcohol from 5.4. This was accomplished by stirring 5.4 in a mixture of trifluoroacetic acid and water (4:1) followed by removing the excess trifluoroacetic acid.
acid. Exposure of acid 5.13 and the polypeptide domain to the coupling conditions produced 5.14 in 69% over two steps (Scheme 5.7). Though the yields of the coupling step are lower than previously with the azide route, the ability to free the amino alcohol in a single step without having to remove a silyl ether and Staudinger reduction outweighs the slightly lower yield. In addition, this coupling step is not optimized, but it was later discovered during the synthesis of other analogues that it was essential to generate the free amine by a basic workup during the TFA step to ensure high yields over the two steps.

Scheme 5.7. Coupling of 5.4 and 5.13

As seen with the previous analogues, amide 5.14 was treated with DAST in dichloromethane at –78 °C to form oxazoline 5.15. The Boc group was removed with TMSOTf and the resulting silyl ether was cleaved with TBAF at 0 °C, which generated amine 5.16 in good yield over the two steps (Scheme 5.8).
Finally, the allyl ester was converted to the carboxylic acid by treatment with catalytic \( \text{Pd}(\text{PPh}_3)_4 \) and excess morpholine.\(^6\) Upon completion, the reaction was concentrated and advanced to final macrolactamization step. The crude amino acid was cyclized with HATU under high dilution conditions, which produced analogue \( 5.11 \) in 47% yield over two steps as a single diastereomer (Scheme 5.9). As expected, the allyl ester solved the epimerization problem previously observed in the saponification of the methyl ester. In addition, the yield of the cyclization was 16% higher than the final two steps in the synthesis of \( 1.9 \).
Following the completion of 5.11, we next turned our attention toward an analogue that retained the C35 hydroxyl absolute configuration, but reversed the C34 methyl configuration. To accomplish this, aldehyde 2.12 was subjected to an Evans’ syn-selective aldol reaction with chiral auxiliary 5.17, which generated alcohol 5.18 in good yield and excellent diastereomeric selectivity. The chiral auxiliary was then removed under standard conditions (LiOH, H₂O₂). The desired carboxylic acid 5.19 was obtained as a single diastereomer by ¹H NMR analysis (Scheme 5.10).
With the success of the syn-aldol with 5.17, we wanted to examine the aldol reaction between aldehyde 2.12 and chiral auxiliary 5.20. This would provide us with an analogue that retains the C34 methyl absolute configuration, but reverses the C35 hydroxyl configuration. Unfortunately, the aldol reaction with aldehyde 2.12 and auxiliary 5.20 did not produce favorable results. The conversion was low and the yield was only moderate (67%), but the stereoselectivity was only 2.5:1 by $^1$H NMR analysis (Scheme 5.11). The use of a titanium enolate and Crimmins’ thiazolidinethione catalyst did not produce any better results.$^{67}$ As result, it was clear that this syn-aldol represented a somewhat mismatched case in regards to the β-methyl group of the aldehyde. This observation is similar the reduced diastereoselectivity of the anti-selective aldol discussed in Scheme 5.6. Though these results are not optimized, we decided to currently abandon 5.21 and move forward with compound 5.19.

![Scheme 5.11. Syn-selective aldol with 5.20](image)

The amino alcohol functionality in 5.4 was exposed by treatment with trifluoroacetic and water (4:1) and the resulting crude ammonium trifluoroacetate salt was coupled to acid 5.19 in only 65% yield. At this point, it was necessary to explore options to increase the efficiency of the deprotection/coupling sequence. It was discovered that the key-coupling step worked more efficiently if the free amine was exposed before subjecting it to the coupling conditions. This was accomplished by
treating the crude ammonium trifluoroacetate salt with aqueous sodium bicarbonate and then exhaustive extraction with chloroform. With this modification, the coupling step produced $5.22$ with consistent yields of around 85% over the two steps. With the key amide in hand, the oxazoline ($5.23$) was formed with DAST, the Boc protecting group was removed with TMSOTF, and the intermediate TMS silyl ether was cleaved with TBAF. Compound $5.24$ was then treated with catalytic palladium tetrakis and excess morpholine, which converted the allyl ester to the free carboxylic acid. The crude amino acid was cyclized with HATU, which produced analogue $5.25$ in 41% over the final two steps (Scheme 5.12).
Scheme 5.12. Completion of 5.25
CHAPTER 6

BIOTINYULATION OF THE OXAZOLINE ANALOGUE OF APRATOXIN A

6.1 Introduction to Biotin

Biotin (6.1) is an essential vitamin that is present in all living cells and is required for several important biological processes, such as the citric acid cycle and cell growth (Figure 6.1). Due to the small relative size (244.3 Daltons), biotin can be attached to proteins or small molecules without altering their inherent biological activity. The interaction between biotin and avidin/streptavidin has been described as the strongest, non-covalent, biological interaction known. Once the biotin becomes docked in the active site, the non-covalent interactions are unaffected by extremes in pH, temperature, organic solvents, and other denaturing agents. As a result, this strong interaction has
provided scientists with an efficient and robust assay for the interaction of small molecules with proteins. With regards to apratoxin, a biotinylated analogue could provide valuable insight into the molecular targets that apratoxin is interacting with.

6.2 Retrosynthetic Analysis of the Biotinylated Analogue

One of the early goals of the apratoxin project was to design and make an analogue that contained a biotin, which would be useful in determining which proteins apratoxin interacts with. From a retrosynthetic viewpoint, we planned to install the biotin via a late stage Huisgen azide-alkyne 1,3-dipolar cycloaddition, otherwise known as a “click” reaction, between alkyne 6.3 and a polyethylene glycol tethered azide 6.4, which would generate the desired biotinylated apratoxin 6.2. The advantage to installing the biotin via a late stage click reaction was that no further manipulations would be required after the azide-alkyne 1,3-dipolar cycloaddition. Alkyne 6.3 would arise from coupling of D-(+)-biotin and the corresponding amine. With the total synthesis of 1.9 completed, the same synthetic route would be followed for 6.4, but the polyethylene glycol ether would have to be installed in place of the methoxy group in 1.9. We planned to achieve this early in the synthesis before coupling of amino acids.
6.3 Synthesis of a polyethylene glycol tethered analogue

Based upon previous data, we reasoned that attachment of a polyethylene glycol linkage at the tyrosine residue should not influence biological activity. Early attempts to install the polyethylene glycol side chain via a Mitsunobu were achieved only with moderate success. Therefore, we turned to some previous chemistry that was used to methylate the tyrosine residue in 1.9. During the synthesis of 1.9, N-Boc-tyrosine was
chemoselectively methylated at the phenol over the carboxylic acid by formation of the dianion with sodium hydride, followed by addition of one equivalent of methyl iodide.\textsuperscript{48} Therefore, N-Boc-tyrosine (6.5) was treated with 2 equivalents of sodium hydride and then 1 equivalent of alkyl iodide 6.6,\textsuperscript{71} which generated 6.7 in good yield (Scheme 6.2).

Moving forward with the synthesis, the previously known amide 5.2 was treated with trifluoroacetic acid, which removed the Boc group. The resulting ammonium trifluoroacetate salt was coupled to 6.7 with PyAOP to yield diamide 6.8 in good yield. To complete the polypeptide domain, the Boc group was removed again with trifluoroacetic acid and the resulting crude product was coupled to carboxylic acid 5.5 with HATU (Scheme 6.3).
The key coupling of polypeptide domain 6.9 and the polyketide carboxylic acid 3.2 occurred in 78% over two steps with the coupling reagent HATU. The resulting amide was converted into oxazoline 6.10 with DAST at low temperature. Next, the Boc group was removed with TMSOTf and the relatively stable TMS ether was removed with TBAF at 0 °C (Scheme 6.4). The allyl ester was removed with Pd(PPh₃)₄ and excess morpholine and the crude amino acid was successfully cyclized with HATU, which generated 6.4 in 46% over the two steps (Scheme 6.5).

Scheme 6.4. Synthesis of amine 6.12
6.4 Biotinylated Analogue

Referring back to Scheme 6.1, the next task in the synthesis was to make the biotin containing compound 6.3. This was conveniently accomplished by coupling commercially available amine 6.13 and D- (+)-biotin (6.1) with EDCI in acetonitrile/methanol (Scheme 6.6). The limited solubility and high polarity of biotin and compound 6.3 caused inconsistent yields during this step, but a high of 74% yield was achieved.
With azide 6.4 and alkyne 6.3 prepared, the final step was unite them together with a 1,3-dipolar cycloaddition. After an extensive literature search, it was determined that the best condition to attempt, as material was limited, was the use of aqueous copper(II) sulfate with aqueous sodium ascorbate, which *in situ* generates copper(I). Fortunately, treatment of 1 equivalent of azide 6.4 and 1.5 equivalents of alkyne 6.3 with 20 mol% CuSO₄ and 40 mol% of sodium ascorbate in a mixture of water and t-butoanol generated the desired triazole 6.2 in an acceptable 57% yield (Scheme 6.7).
6.5 Future Work

The synthetic analogues of apratoxin A will be assayed for anti-cancer activity against various cancer cell lines. Once the cytotoxicity is determined for the analogues, the data will be analyzed to determine which structural features are necessary for retention of biological activity. Also, additional analogues will be targeted with the overall goal of retaining the potent cytotoxicity, while reducing the toxicity towards normal cells. In addition, the biotinylated analogue will be biologically studied to determine the mode of action and specific molecular targets of 1.1. Regarding the mode of action, additional biological tags will be appended via late stage click reactions.

6.6 Summary

Up to this point, we have developed a versatile route to several oxazoline analogues of the potent cytotoxic natural product, apratoxin A (1.1). During the synthesis of 1.9, we were able to replace the inefficient Brown allylation with a reliable proline catalyzed aldol and the early use of Grubbs’ metathesis was replaced with a sequence involving a Prasad reduction and opening of a cyclic sulfate via an alkyl cuprate. The coupling of the polyketide and polypeptide domain was efficiently performed without protection of the C35 alcohol, which eliminated the necessary protecting group shuffle in the Forsyth synthesis of 1.1. The key oxazoline moiety was initially attempted, without success, to be installed via a Staudinger aza-Wittig reaction. It was later discovered that the dehydrating agent, DAST, was an efficient reagent to install the oxazoline late stage, after coupling of the polyketide and polypeptide domains. The
synthetic route toward 1.9 provided us with a tunable route that enabled us to make several analogue of 1.9.

We focused on altering the moSer domain and C34 methyl and C35 hydroxyl groups. During the synthesis of some of the analogues, we discovered that epimerization of the proton at C30 was problematic during the saponification of the methyl ester. As a result, we modified the synthetic route to include a labile allyl ester, which was successfully removed with palladium and excess morpholine. In addition, the final two steps exhibited increased yields, over the final steps, with the replacement of the methyl ester for an allyl ester. Finally, a biotinylated analogue of 1.9 was completed via a late stage click reaction, which efficiently produced the desired analogue.
CHAPTER 7

EXPERIMENTAL DETAILS

**General Methods**: Unless other stated, all oxygen and moisture-sensitive reactions were performed under anhydrous conditions (over-dried glassware sealed under a dry argon atmosphere). Solutions and solvents sensitive to moisture were transferred using standard syringe and cannula techniques. All commercial reagents were purchased as reagent grade and, unless otherwise noted, used without further purification. All organic solvents were used dry: tetrahydrofuran (THF), diethyl ether (Et₂O), dichloromethane (CH₂Cl₂), dimethylformamide (DMF), and toluene were purified via a Pure Solv MD-6 Solvent Purification System; triethylamine (Et₃N), diisopropylamine, diisopropylethylamine (DIPEA), methanol, and acetonitrile were distilled from CaH₂; dimethyl sulfoxide (DMSO) was stored over freshly activated 4Å molecular sieves. Thin-layer chromatography was performed on Silicycle Glass Backed TLC Extra Hard Layer 60Å, 250 µm, F-254 TLC plates that were visualized via UV light (254 nm) or by p-anisaldehyde (PAA), phosphomolybdic acid (PMA), potassium permanganate (KMnO₄), or ceric ammonium molybdate (CAM) stains and the column chromatographic separations were performed using Silicycle SiliaFlash® P60 silica gel (40–63 µm). Melting points were measured on a Thomas Hoover (Uni-melt) capillary melting point.
apparatus. Optical rotations were measured by a Perkin-Elmer Model 241 Polarimeter at 589 nm with a sodium lamp and concentrations are reported in g/100 mL. Nuclear Magnetic Resonance (NMR) spectra were obtained for proton (\(^1\)H) and carbon (\(^{13}\)C) nuclei using Bruker DPX-400 and DRX-500 NMR spectrometers; residual solvent peak signals for CDCl\(_3\) were set at 7.26 and 77.16 ppm in the \(^1\)H and \(^{13}\)C spectra, respectively. A Perkin-Elmer 1600 Series FT-IR spectrometer was used to record infrared spectra and absorptions are reported in reciprocal centimeters. High-resolutions mass spectrometric data were obtained using a Bruker MicroTOF (ESI) Mass Spectrometer.
**Diol 3.9**

A solution of 2.31 (18.9 g, 131 mmol) in anhydrous THF (1050 mL) and MeOH (260 mL) was cooled to $-78 \, ^\circ\text{C}$ and MeOBEt$_2$ (36.1 mL, 144 mmol, 1 M solution in THF) was added dropwise. After 30 min, NaBH$_4$ (5.46 g, 144 mmol) was added in portions. The resulting mixture was stirred for 5 h at $-78 \, ^\circ\text{C}$ before a 30% H$_2$O$_2$ (60 mL) was carefully added. After the reaction warmed to rt and stirred for 12 h, the mixture was concentrated *in vacuo* and then dissolved in ethyl acetate. The ethyl acetate layer was washed with water, dried over MgSO$_4$, and concentrated. The resulting oil was purified via flash column chromatography (2:1 hexanes/EtOAc, v/v) on silica gel to afford diol 3.9 (14.6 g, 76%) as a colorless oil: $R_f$ 0.31 (2:1 hexanes/EtOAc, v/v); $[\alpha]_{D}^{25} = -2.2$ (c 1.13, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 4.06–3.98 (m, 1H), 3.50 (ddd, $J = 10.8, 2.4, 2.4$ Hz, 1H), 3.19 (s, 1H), 2.80 (d, $J = 3$ Hz, 1H), 1.62 (ddd, $J = 14.4, 2, 2$ Hz, 1H), 1.46–1.37 (m, 1H), 1.22 (d, $J = 6$ Hz, 3H), 0.90 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 81.2, 69.5, 38.9, 34.8, 25.5, 24.3; IR (neat) 3358, 2965, 1479, 1393, 1128 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_8$H$_{18}$NaO$_2$ [M+Na]$^+$ calcd 169.1199, found 169.1195.
Sulfate 3.11

A solution of diol 3.9 (0.117 g, 0.80 mmol) and pyridine (0.21 mL, 2.56 mmol) in Et₂O (8 mL) was cooled to 0 °C and SOCl₂ (88 µL, 1.21 mmol) was added dropwise. After 30 min at 0 °C, the reaction was diluted with water (10 mL) and allowed to warm to rt. The mixture was extracted with Et₂O (3 x 10 mL) and the combined organic extracts were dried over MgSO₄ filtered, and concentrated. The resulting diastereomeric cyclic sulfites 3.10 were dissolved in a mixture of H₂O (4 mL), CH₃CN (2 mL), and CCl₄ (2 mL). To the solution was added RuCl₃ (8.3 mg, 40 µmol) and NaIO₄ (0.26 g, 1.2 mmol). After stirring for 1 h at rt, the reaction mixture was diluted with Et₂O (20 mL), washed with H₂O (10 mL), saturated aqueous NaHCO₃ (10 mL), and saturated aqueous NaCl (10 mL). The organic phase was dried over MgSO₄ filtered, and concentrated. Purification via flash column chromatography (4:1 hexanes/EtOAc, v/v) on silica gel gave cyclic sulfate 3.11 (0.146 g, 90% over 2 steps) as a white solid: Rₛ 0.27 (5:1 hexanes/EtOAc, v/v); [α]²⁵_D = −3.1 (c 1.23, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 4.97–4.92 (m, 1H), 4.54 (dd, J = 5.2 Hz, 1H), 1.88–1.84 (m, 2H), 1.48 (d, J = 6 Hz, 3H), 1.01 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 91.8, 81.0, 34.3, 31.7, 25.3, 20.8; IR (neat) 2966, 1391, 1186, 1045, 893 cm⁻¹; HRMS (ESI) m/z for C₈H₁₆NaO₄S [M+Na]^+ calcd 231.0662, found 231.0667.
Alcohol 3.12

To a solution of CuI (9.4 g, 49.4 mmol) in THF (50 mL) was added a solution of cyclic sulfate 3.11 (8.4 g, 41.2 mmol) in THF (50 mL) at rt. The resulting mixture was cooled to −30 °C before allyl magnesium bromide (165 mL, 165 mmol, 1 M solution in Et₂O) was added dropwise. After stirring for 6 h at −30 °C, the reaction mixture was warmed to rt and concentrated. The resulting residue was dissolved in Et₂O (400 mL) and cooled to 0 °C, at which a 20% H₂SO₄ (150 mL) was carefully added. After stirring for 12 h at rt, the layers were separated and the aqueous layer was extracted with EtOAc (3 x 200 mL). The combined organic layers were washed saturated aqueous NaCl (150 mL), dried over Na₂SO₄, filtered, and concentrated. Purification via flash column chromatography on silica gel (8:1 hexanes/EtOAc, v/v) produced alcohol 3.12 (5.86 g, 84%) as a clear, colorless oil: Rₐ 0.45 (6:1 hexanes/EtOAc, v/v); [α]²⁵_D = −44.5 (c 1.42, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 5.83–5.73 (m, 1H), 5.06–5.02 (m, 2H), 3.34 (dd, J = 10.4, 1.6 Hz, 1H), 2.27–2.20 (m, 1H), 1.94–1.86 (m, 1H), 1.85–1.76 (m, 1H), 1.45 (ddd, J = 14.4, 9.6, 2 Hz, 1H), 1.36 (br s, 1H), 1.22 (ddd, J = 14, 10, 4 Hz, 1H), 0.98 (dd, J = 6.4 Hz, 3H), 0.91 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 137.1, 116.0, 77.6, 39.8, 38.5, 35, 29.9, 25.6, 20.9; IR (neat) 3995, 2955, 1640, 1366, 1069 cm⁻¹; HRMS (ESI) m/z for C₁₁H₂₂NaO [M+Na]⁺ calcd 193.1563, found 193.1557.
Ester 3.13

To a solution of N-Boc-L-proline (23 g, 106 mmol) in CH$_2$Cl$_2$ (330 mL) at 0 °C were added N,N’-diisopropylcarbodiimide (21.3 mL, 106 mmol) and DMAP (0.4 g, 3.5 mmol). After stirring for 10 min, a solution of alcohol 3.12 (5.1 g, 35.2 mmol) in CH$_2$Cl$_2$ (20 mL) was added and the resulting mixture was warmed to rt. After 24 h, the reaction mixture was filtered through a pad of Celite® and the filtrate was washed with saturated aqueous NaHCO$_3$ (200 mL). The resulting aqueous layer was extracted with CH$_2$Cl$_2$ (3 x 150 mL). The combined organic layers were washed with saturated aqueous NaCl (100 mL), dried over Na$_2$SO$_4$, filtered, and concentrated. Purification via flash column chromatography on silica gel (9:1 hexanes/EtOAc, v/v) gave ester 3.13 (10.6 g, 82%) as a clear, colorless oil: R$_f$ 0.24 (9:1 hexanes/EtOAc, v/v); $\left[\alpha\right]^{25}_D = +55.4$ (c 1.52, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$, mixture of carbamate rotamers) $\delta$ 5.78–5.68 (m, 1H); 5.03–4.94 (m, 2H), 4.88–4.48 (m, 1H), 4.34 (ddd, $J$ = 7.6, 7.6, 2.4 Hz, 1H), 3.55–3.42 (m, 1.6H), 3.40–3.34 (m, 0.4H), 2.27–2.14 (m, 2H), 2.06–1.75 (m, 4H), 1.57–1.28 (m, 3 H), 1.44 (s, 4H), 1.41 (s, 5H), 0.89 (s, 9H), 0.87 (d, 6.4 Hz, 3 H); $^{13}$C NMR (100 MHz, CDCl$_3$, mixture of carbamate rotamers) $\delta$ 173.0, 172.5, 154.1, 153.9, 136.7, 136.3, 116.5, 115.9, 79.9, 79.4, 79.1, 59.4, 59.3, 46.7, 46.1, 46.1, 39.4, 36.6, 36.5, 34.8, 34.6, 31.0, 30.8, 29.2, 28.9, 28.4, 28.3, 26.0, 25.9, 24.3, 23.1, 20.7; IR (neat) 2968, 1743, 1701, 1340, 1168; HRMS (ESI) m/z for C$_{21}$H$_{37}$NNaO$_4$ [M+Na]$^+$ calcd 390.2615, found 390.2603.
Aldehyde 2.12

A solution of olefin 3.13 (49.7 mg, 0.14 mmol) in CH$_2$Cl$_2$ (2.5 mL) and MeOH (0.5 mL) was cooled to –78 °C and ozone was bubbled through the solution until a blue color persisted. The excess ozone was removed by bubbling a stream of argon through the solution until the blue color dissipated, at which PPh$_3$ (90 mg, 0.34 mmol) was added. After stirring for 30 min at –78 °C, the reaction was warmed to rt and stirred for an additional 1 h. The reaction mixture was then concentrated under reduced pressure. Purification via flash column chromatography on silica gel (5:1 hexanes/EtOAc, v/v) to give aldehyde 2.12 as a clear, light yellow oil: R$_f$ 0.19 (6:1 hexanes/EtOAc, v/v); [α]$^\text{D}$_25 = –40.0 (c 1.24, CHCl$_3$) $^1$H NMR (400 MHz, CDCl$_3$, mixture of carbamate rotamers) δ 9.70 (d, $J$ = 2.4 Hz, 0.4H), 9.64 (d, $J$ = 3.6 Hz, 0.6H), 4.82 (dd, $J$ = 10.8, 1.6 Hz, 0.62H), 4.76 (dd, $J$ = 8.4, 3.6 Hz, 0.38H), 4.33 (ddd, $J$ = 8.4, 8.4, 2.4 Hz, 1H), 3.55–3.44 (m, 1.40H), 3.41–3.35 (m, 0.60H), 2.69 (ddd, $J$ = 15.2, 15.2, 2.8 Hz, 1H), 2.24–2.10 (m, 2H), 2.00–1.87 (m, 4H), 1.60 (ddd, $J$ = 14.4, 11.2, 3.6 Hz, 1H), 1.50 (m, 1H), 1.42 (s, 9H), 0.96 (d, $J$ = 6.8 Hz, 3H), 0.89 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$, mixture of carbamate rotamers) δ 203.9, 202.1, 173.4, 79.7, 79.1, 78.4, 59.4, 59.1, 49.1, 46.5, 46.2, 36.8, 34.6, 31.1, 30.1, 28.4, 28.4, 26.0, 26.0, 25.1, 24.4, 23.2, 21.3; IR (neat) 2967, 1726, 1697, 1398, 1163 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_2$$_{3}$H$_{39}$NNaO$_6$ [M+Na+MeOH]$^+$ calcd 424.2670, found 424.2670.
β-hydroxy ketone 2.13

To a –78 °C solution of dicyclohexylboron chloride (0.25 mL, 1.14 mmol) in Et₂O (3 mL) was added dimethylethylamine (0.19 mL, 1.71 mmol), followed by (R)-2-benzoyloxy-3-pentanone (0.18 g, 0.85 mmol) in Et₂O (2 mL) dropwise. The resulting mixture was warmed to 0 °C and stirred for 2 h. After re-cooling to –78 °C, a solution of aldehyde 2.12 (0.21 g, 0.57 mmol) in Et₂O (1 mL) was added via cannula and stirring was continued for 2 h at –78 °C. The reaction was held at –20 °C for an additional 14 h before warming to 0 °C. The reaction was slowly quenched with MeOH (3 mL), followed by the addition of pH 7 buffer (4 mL) and H₂O₂ (3 mL, 30% aqueous solution). After stirring at rt for 3 h, the mixture was partitioned between H₂O (10 mL) and CH₂Cl₂ (20 mL). The aqueous phase was extracted with CH₂Cl₂ (3 x 10 mL). The combined organic layers were dried of Na₂SO₄, filtered, and concentrated. Purification via flash column chromatography on silica gel (6:1 to 3:1 hexanes/EtOAc, v/v) yielded β-hydroxy ketone 2.13 (0.28 g, 85%) as a colorless oil: Rf 0.40 (2:1 hexanes/EtOAc, v/v); [α]²⁵_D = –53.3 (c 1.08, CHCl₃) ¹H NMR (400 MHz, CDCl₃, mixture of carbamate rotamers) δ 8.09 (m, 2H), 7.58–7.55 (m, 1H), 7.47–7.42 (m, 2H), 5.40 (q, J = 6.8 Hz, 1H), 4.86 (dd, J = 11.6, 1.6 Hz, 0.6H), 4.76 (dd, J = 10, 1.6 Hz, 0.4H), 4.33 (dd, J = 8.8, 2.4 Hz, 0.3H), 4.28 (dd, J = 8.8, 4 Hz, 0.7H), 3.90–3.79 (m, 1H), 3.53–3.34 (m, 2H), 3.00–2.87 (m, 2H), 2.22–2.15 (m, 1H), 2.02–1.95 (m, 1H), 1.94–1.84 (m, 2H), 1.76–1.69 (m, 2H), 1.67–1.55
(m, 4H), 1.45 (s, 6H), 1.43 (s, 3H), 1.35–1.27 (m, 2H), 1.21 (d, J = 7.2 Hz, 1H), 1.15 (d, J = 6.9 Hz, 2H), 0.96 (d, J = 6.8 Hz, 3H), 0.92 (s, 3H), 0.90 (s, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$, mixture of carbamate rotamers) δ 210.8, 172.9, 165.9, 133.1 129.8, 128.4, 128.4, 80.0, 78.5, 59.0, 50.0, 49.4, 46.7, 46.2, 40.0, 39.2, 37.8, 34.5, 30.0, 28.5, 28.3, 26.0, 25.0, 24.3, 20.8, 20.6, 15.5, 13.4; IR (neat) 3325, 2963, 1738, 1645, 1514, 1248 cm$^{-1}$; HRMS (ESI) m/z for C$_{32}$H$_{49}$NNO$_8$ [M+Na]$^+$ calcd 598.3350, found 598.3354.

**Carboxylic Acid 3.2**

To a solution of β-hydroxy ketone 2.13 (58.2 mg, 0.10 mmol) in MeOH (2 mL) at 0 °C was added K$_2$CO$_3$ (17 mg, 0.12 mmol) and the reaction was warmed to rt. After stirring at rt for 3, the mixture was diluted with CH$_2$Cl$_2$ (10 mL) and washed with H$_2$O (5 mL). The aqueous phase was extracted with CH$_2$Cl$_2$ (3 x 10 mL) and the combined organic layers were dried over Na$_2$SO$_4$, filtered, and concentrated to give crude α-hydroxy ketone 3.15. To a rt solution of crude α-hydroxy ketone 3.15 in t-BuOH (1.3 mL) and H$_2$O (0.7 mL) was added NaIO$_4$ (54 mg, 0.25 mmol). After 3 h, saturated aqueous NH$_4$Cl (1 mL) was added to the reaction mixture and the aqueous phase was extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over Na$_2$SO$_4$, filtered, and concentrated. Purification via flash column chromatography on silica gel (97:3 CH$_2$Cl$_2$/MeOH, v/v) gave β-hydroxy acid 3.2 (32 mg, 71%) as a white foam: R$_f$ 0.26 (92:8 CH$_2$Cl$_2$/MeOH, v/v); $[\alpha]^{25}_D = -44.2$ (c 1.25, CHCl$_3$) $^1$H NMR (400 MHz, CDCl$_3$)
MHz, CDCl$_3$, mixture of carbamate rotamers) $\delta$ 4.88 (dd, $J = 12$, 2 Hz, 1H); 4.26 (dd, $J = 8.4$, 4.4 Hz, 1H), 3.75 (ddd, $J = 10.4$, 8.4, 2 Hz, 1H), 3.54–3.48 (m, 1H), 3.43–3.36 (m, 1H), 2.43 (dddd, $J = 7.3$, 7.3, 7.3, 7.3 Hz, 1H), 2.28–2.19 (m, 1H), 2.02–1.93 (m, 2H), 1.92–1.79 (m, 3H), 1.71–1.63 (m, 2H), 1.43 (s, 9H), 1.33–1.25 (m, 2H), 1.17 (d, $J = 6.8$ Hz, 3H), 0.96 (d, $J = 6.8$ Hz, 3H), 0.88 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$, mixture of carbamate rotamers) $\delta$ 178.6, 172.6, 154.5, 80.5 78.1, 70.7, 58.9, 46.7, 46.5, 39.1, 37.3, 34.7, 34.6, 30.0, 28.4, 28.3, 26.0, 26.0, 24.9, 24.3, 20.3, 13.3; IR (neat) 3474, 2969, 1732, 1699, 1404, 1368 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_{23}$H$_{41}$NNaO$_7$ [M+Na]$^+$ calcd 466.2775, found 466.2788.

**Silyl ether 3.17**

![Reaction Scheme](image)

To a solution of alcohol 3.16 (1.8 g, 12.4 mmol) in CH$_2$Cl$_2$ (62 mL) was added imidazole (1.7 g, 24.8 mmol), followed by TBSCl (1.96 g, 13 mmol) at 0 °C. The reaction was warmed to rt and stirred for 1 h. The reaction was quenched with saturated aqueous NH$_4$Cl (30 mL) and diluted with Et$_2$O (100 mL). The layers were separated and the resulting aqueous layer was extracted with Et$_2$O (3 x 50 mL). The combined organic layers were washed with saturated aqueous NaCl (50 mL), dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (13:1 hexanes/EtOAc, v/v) yielded silyl ether 3.17 (2.62 g, 82%) as a clear, colorless oil: $R_f$ 0.34 (12:1 hexanes/EOAc, v/v); $[\alpha]_{D}^{25} = -28.8$ (c 1.0, CHCl$_3$) $^1$H NMR
(400 MHz, CDCl$_3$) $\delta$ 4.07–4.01 (m, 2H), 3.81–3.80 (m, 1H), 3.80 (s, 3H), 0.89 (s, 9H), 0.09 (s, 3H), 0.07 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 169.2, 64.5, 63.1, 52.5, 25.6, 18.1, −5.6, −5.8; IR (neat) 2955, 2106, 1749, 1258 cm$^{-1}$; HRMS (ESI) $m/z$ for $\text{C}_{19}\text{H}_{21}\text{N}_3\text{NaO}_3\text{Si} [\text{M+Na}]^+$ calcd 282.1244, found 282.1242.

**$\alpha,\beta$-unsaturated ester 3.18**

To a −78 °C solution of silyl ether 3.17 (0.17 g, 0.67 mmol) in toluene (7 mL) was added DIBAL-H (0.80 mL, 0.80 mmol, 1 M solution in toluene) dropwise over 15 min. After stirring at −78 °C for 2 h, the reaction was quenched with MeOH (0.1 mL) and a saturated aqueous Na/K tartrate solution (10 mL) was added. The reaction mixture was warmed to rt and diluted with Et$_2$O (20 mL). After stirring for 2 h, the layers were separated and the resulting aqueous layer was extracted with Et$_2$O (3 x 10 mL). The combined organic layers were dried over MgSO$_4$, filtered, and the Et$_2$O was removed under reduced pressure.

To the resulting solution of toluene (ca. 7 mL), methyl 2-(triphenylphosphoranylidene)propionate (0.39 g, 1.01 mmol) was added at 0 °C. The reaction warmed to rt and stirred for 12 h before it was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (14:1 hexanes/EtOAc, v/v) yielded ester 3.18 (0.18 g, 90% from ester 3.17) as a clear,
colorless oil: R\textsubscript{f} 0.37 (12:1 hexanes/EOAc, v/v); [\alpha]\textsubscript{D}\textsuperscript{25} = -25.5 (c 1.5, CHCl\textsubscript{3}) \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \delta 6.56 (dq, J = 8.8, 1 Hz, 1H), 4.25 (ddd, J = 11.2, 5.6, 5.6 Hz, 1H), 3.77 (s, 3H), 3.70–3.68 (m, 2H), 1.94 (d, J = 1.2 Hz, 3H), 0.91 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \delta 167.6, 134.9, 132.1, 65.4, 60.9, 52.1, 25.7, 18.2, 13.2, -5.4, -5.5; IR (neat) 2953, 2100, 1721, 1253, 1115 cm\textsuperscript{-1}; HRMS (ESI) m/z for C\textsubscript{13}H\textsubscript{25}N\textsubscript{3}NaO\textsubscript{3}Si [M+Na]\textsuperscript{+} calcd 322.1557, found 322.1558.

Carboxylic acid 3.3

![Chemical structure](image)

To a stirred solution of ester 3.18 (1.02 g, 3.40 mmol) in t-BuOH (17 mL), THF (8.5 mL), and H\textsubscript{2}O (8.5 mL) at 0 °C was added LiOH·H\textsubscript{2}O (1.43 g, 34 mmol) in one portion. After stirring at rt for 4 h, the reaction mixture was acidified with citric acid and diluted with EtOAc (50 mL). The separated aqueous layer was extracted with EtOAc (3 x 30 mL) and the combined organic layers were dried over Na\textsubscript{2}SO\textsubscript{4}, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (10:1 to 2:1 hexanes/EOAc, v/v) gave acid 3.3 (0.80 g, 82%) as light yellow oil: R\textsubscript{f} 0.29 (2:1 hexanes/EOAc, v/v); [\alpha]\textsubscript{D}\textsuperscript{25} = -0.9 (c 1.26, CHCl\textsubscript{3}) \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \delta 6.71 (dq, J = 9.2, 1.6 Hz, 1H), 4.27 (ddd, J = 9.2, 5.6, 5.6 Hz, 1H), 3.72–3.71 (m, 2H), 1.95 (d, J = 1.2 Hz, 3H), 0.91 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \delta 172.2, 137.4, 131.5, 65.3, 60.8, 25.8, 18.3, 12.9, -5.5; IR (neat) 2955,
2102, 1697, 1256 cm\(^{-1}\); HRMS (ESI) \(m/z\) for \(\text{C}_{12}\text{H}_{23}\text{N}_3\text{O}_3\text{Si} [\text{M+Na}]^+\) calcd 308.1401, found 308.1397.

**Amide 3.19**

To a solution of \(\text{N-Boc-}N\text{-methyl-L-isoleucine methyl ester 3.18}\) (1.4 g, 5.4 mmol) in \(\text{CH}_2\text{Cl}_2\) (27 mL) at 0 °C was added trifluoroacetic acid (27 mL). After stirring for 1 h at 0 °C, toluene (20 mL) was added and the mixture was concentrated under a stream of argon. The residue was dissolved in \(\text{CH}_2\text{Cl}_2\) (20 mL) and toluene (20 mL), concentrated to dryness, and placed under high vacuum for 2 h. To a solution of \(\text{N-Boc-}N\text{-methyl-alanine (0.99 g, 4.9 mmol)}\) in \(\text{CH}_2\text{Cl}_2\) (40 mL) was added PyAOP (2.6 g, 4.9 mmol) and \(i\text{-Pr}_2\text{NEt (2.6 mL, 15 mmol)}\) sequentially. The resulting mixture was stirred for 1 min at rt before a solution of the crude \(\text{N-methyl-L-isoleucine methyl ester ammonium trifluoroacetate in CH}_2\text{Cl}_2\) (10 mL) was added. After stirring at rt for 3 h, the reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (3:1 hexanes/EtOAc, v/v) yielded 3.19 (1.31g, 79% from 3.18) as a clear, colorless oil that solidified in the refrigerator: \(R_f\) 0.44 (2:1 hexanes/EOAc, v/v); \([\alpha]^{25}_D = −136\) (c 1.10, CHCl\(_3\)) \(^1\)H NMR (500 MHz, CDCl\(_3\), mixture of carbamate rotamers) \(δ\) 5.14–5.08 (m, 1H), 4.95 (d, \(J = 10.5\) Hz, 1H), 3.70 (s, 3H), 3.01 (s, 3H), 2.75 (s, 3H), 2.04–1.99 (m, 1H), 1.46–1.27 (m, 1H), 1.46 (s, 9H), 1.28 (d, \(J = 6.5\) Hz, 3H),
1.09–1.01 (m, 1H), 0.95 (d, \(J = 6.5\) Hz, 3H), 0.87 (t, \(J = 7.5\) Hz, 3H); \(^{13}\text{C}\) NMR (125 MHz, CDCl\(_3\), mixture of carbamate rotamers) \(\delta 172.5, 171.7, 171.7, 171.6, 171.1, 170.9, 170.8, 155.4, 155.0, 80.2, 63.5, 60.3, 52.3, 52.1, 51.7, 50.6, 50.5, 34.3, 34.2, 33.4, 33.2, 30.9, 30.6, 29.0, 28.7, 28.4, 25.4, 25.1, 24.8, 15.8, 15.7, 15.6, 14.7, 11.2, 10.7, 10.4, 10.3; IR (neat) 2972, 1740, 1647, 1556, 1319 cm\(^{-1}\); HRMS (ESI) \(m/z\) for C\(_{17}\)H\(_{32}\)N\(_2\)NaO\(_5\) [M+Na]\(^+\) calcd 367.2203, found 367.2202.

**Diamide 2.3**

![Reaction Scheme]

To a solution of amide 3.19 (98 mg, 0.28 mmol) in CH\(_2\)Cl\(_2\) (3 mL) at 0 °C was added trifluoroacetic acid (3 mL) and stirring was continued for 1 h at 0 °C. The reaction was diluted with toluene (3 mL) and concentrated to dryness under a stream of Ar. The resulting residue was dissolved in toluene (3 mL), concentrated to dryness, and placed under high vacuum for 2 h. To a solution of \(N\)-Boc-\(O\)-methyl-tyrosine (83 mg, 0.28 mmol) in CH\(_2\)Cl\(_2\) (4.5 mL) were added PyAOP (0.16 g, 0.31 mmol) and \(i\)-Pr\(_2\)NEt (0.15 mL, 0.84 mmol) at rt. The resulting mixture was stirred for 1 min before a solution of deprotected crude 3.19 in CH\(_2\)Cl\(_2\) (1 mL) was added. After stirring for 12 at rt, the reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (2:1 to 1:1 hexanes/EtOAc, v/v) gave diamide 2.3 (0.121 g, 83% from amide 3.19) as a white foam: R\(_f\) 0.23 (2:1 hexanes/EOAc, v/v); \([\alpha]^{25}_D = -77\) (c
0.93, CHCl$_3$) $^1$H NMR (500 MHz, CDCl$_3$, mixture of carbamate rotamers) δ 7.11 (d, $J$ = 8 Hz, 2H), 6.80 (d, $J$ = 8 Hz, 2H), 5.39 (q, $J$ = 7 Hz, 1H), 5.22 (d, $J$ = 8.5 Hz, 1H), 4.89 (d, $J$ = 10.5 Hz, 1H), 4.82 (m, 1H), 3.77 (s, 3H), 2.97 (dd, $J$ = 14, 7 Hz, 1H), 2.95 (s, 3H), 2.78 (dd, $J$ = 14, 7 Hz, 1H), 2.71 (s, 3H), 2.00–1.91 (m, 1H), 1.38 (s, 9H), 1.27 (d, $J$ = 7 Hz, 3H), 1.24–1.22 (m, 1H), 0.96 (m, 1H), 0.93 (d, $J$ = 6.5 Hz, 3H), 0.84 (t, $J$ = 7.5 Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$, mixture of carbamate rotamers) δ 171.8, 171.5, 158.6, 155.2, 130.4, 128.3, 120.5, 113.9, 79.9, 60.4, 55.2, 51.8, 49.9, 38.1, 33.2, 30.9, 30.5, 28.3, 28.2, 24.9, 15.7, 14.2, 10.5; IR (neat) 3308, 2972, 1740, 1641, 1514, HRMS (ESI) m/z for C$_{27}$H$_{43}$N$_3$NaO$_7$ [M+Na]$^+$ calcd 544.2993, found 544.2992.

**Triamide 3.20**

To a 0 °C solution of diamide 2.3 (90 mg, 0.18 mmol) in CH$_2$Cl$_2$ (2 mL) was added trifluoroacetic acid (2 mL). The reaction was stirred at 0 °C for 1 h before toluene (2 mL) was added. The mixture was concentrated under a stream of argon and additional toluene (2 mL) was added. After removing the toluene under a stream of argon, the viscous oil was placed under high vacuum for 2 h.

To a rt solution of acid 3.3 (54 mg, 0.19 mmol) in CH$_2$Cl$_2$ (3 mL) was added i-Pr$_2$NEt (0.09 mL, 0.20 mmol) followed by PyAOP (0.1 g, 0.20 mmol). After stirring for
1 min, a solution of deprotected diamide 2.3 in CH<sub>2</sub>Cl<sub>2</sub> (1 mL) was added and the reaction was stirred at rt for 8 h. The reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (2:1 to 1:1 hexanes/EtOAc, v/v) yielded triamide 3.20 (0.105 g, 88% from diamide 2.3) as a white foam: R<sub>f</sub> 0.23 (2:1 hexanes/EtOAc, v/v); [α]<sup>25</sup> = –79.3 (c 1.29, CHCl<sub>3</sub>)<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, mixture of rotamers) δ 7.06 (d, J = 8.8 Hz, 2H), 6.78 (d, J = 8.8 Hz, 2H), 6.42 (d, J = 8.4 Hz, 1H), 6.05–6.02 (m, 1H), 5.41 (q, J = 6.8 Hz, 1H), 5.19 (m, 1H), 4.91 (d, J = 10.4 Hz, 1H), 4.19 (m, 1H), 3.75 (s, 3H), 3.68 (s, 3H), 3.66–3.64 (m, 2H), 3.05 (dd, J = 13.6, 7.2 Hz), 2.99 (s, 3H), 2.88–2.82 (m, 2H), 2.79 (s, 3H), 1.98–1.94 (m, 1H), 1.88 (s, 3H), 1.27 (d, J = 6.8 Hz, 3H), 1.00–0.95 (m, 2H), 0.92 (d, J = 6.4 Hz, 3H), 0.89 (s, 9H), 0.85 (t, J = 7.2 Hz, 3H), 0.07 (s, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, mixture of rotamers) δ 171.9, 171.5, 171.3, 167.4, 135.7, 135.5, 130.4, 129.5, 129.3, 127.7, 127.7, 113.9, 65.6, 65.6, 60.9, 60.3, 55.2, 51.8, 50.5, 49.6, 37.7, 37.7, 33.3, 31.0, 30.6, 25.8, 25.0, 18.3, 15.7, 14.4, 13.6, 13.5, 10.6, –5.5; IR (neat) 3321, 2955, 2099, 1740, 1632, 1250 cm<sup>–1</sup>; HRMS (ESI) m/z for C<sub>34</sub>H<sub>56</sub>N<sub>6</sub>NaO<sub>7</sub>Si [M+Na]<sup>+</sup> calcd 711.3872, found 711.3854.
Primary alcohol 3.1

A solution of triamide 3.20 (0.12 g, 0.17 mmol) in MeOH (2 mL) was cooled to 0 °C and TsOH•H2O (5 mg, 0.03 mmol) was added. The reaction mixture was warmed to rt and stirring was continued for 1.5 h before Et3N (0.1 mmol) was added. The mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (98:2 to 96:4 CH2Cl2:MeOH) gave primary alcohol 3.1 (0.095 g, 97%) as a white foam: Rf 0.39 (14:1 CHCl3/MeOH, v/v); [α]25D = −96.8 (c 0.87, CHCl3) 1H NMR (500 MHz, CDCl3, mixture of rotamers) δ 7.08 (d, J = 9 Hz, 2H), 6.79 (d, J = 10.5 Hz, 2H), 6.57 (d, J = 8 Hz, 0.6H), 6.50 (d, J = 8.5 Hz, 0.4H), 6.12 (dq, J = 9, 1.5 Hz, 1H), 5.44 (q, J = 7 Hz, 1H), 5.20 (m, 1H), 4.93 (d, J = 10.5 Hz, 1H), 4.38–4.33 (m, 1H), 3.77 (s, 3H), 3.70 (s, 3H), 3.62–3.58 (m, 2H), 3.10–3.05 (m, 1H), 3.02 (s, 3H), 2.90–2.86 (m, 1H), 2.83 (s, 3H), 2.00–1.92 (m, 1H), 1.92 (d, J = 1.2 Hz, 3H), 1.68 (s, 3H), 1.30 (d, J = 6 Hz, 3H), 1.02–0.95 (m, 2H), 0.94 (d, J = 5.6 Hz, 3H), 0.87 (t, J = 6 Hz, 3H); 13C NMR (125 MHz, CDCl3, mixture of rotamers) δ 171.9, 171.8, 171.6, 171.4, 167.7, 167.5, 136.2, 136.0, 131.4, 130.3, 129.2, 129.0, 127.8, 127.6, 64.9, 61.0, 60.4, 55.2, 50.9, 50.7, 49.7, 37.5, 33.2, 30.9, 30.6, 24.9, 15.7, 14.3, 13.6, 13.5, 10.6, 10.5; IR (neat) 3408, 2963, 2101, 1740, 1628; HRMS (ESI) m/z for C28H42N6NaO7 [M+Na]+ calcd 597.3007, found 597.3006.
Amino alcohol 3.31

To a 0 °C solution of alcohol 3.1 (0.104 g, 0.18 mmol) in THF (3.4 mL) and H₂O (0.07 mL) was added tributylphosphine (0.07 mL, 0.27 mmol) dropwise. The reaction was warmed to rt and stirred for 30 min. The reaction was then heated to 50 °C under microwave irradiation for 30 min at which the reaction was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (98:2 to 90:10 CHCl₃:MeOH) yielded amino alcohol 3.31 (89.3 mg, 91%) as a white foam: Rₐ 0.10 (9:1 chloroform/MeOH, v/v); [α]²⁵ －97 (c 1.8, CHCl₃) ¹H NMR (400 MHz, CDCl₃, mixture of rotamers) δ 7.09 (d, J = 8.4 Hz, 2H), 6.79 (d, J = 8.8 Hz, 2H), 6.15–6.10 (m, 1H), 5.39 (q, J = 7.2 Hz, 1H), 5.22–5.15 (m, 1H), 4.91 (d, J = 10.4 Hz, 1H), 3.76 (s, 3H), 3.69 (s, 3H), 3.76–3.56 (m, 1H), 3.66–3.64 (m, 1H), 3.46–3.44 (m, 1H), 3.05 (dd, J = 14, 7.6 Hz, 1H), 2.99 (s, 3H), 2.90–2.83 (m, 4H), 2.76 (s, 3H), 1.99–1.92 (m, 1H), 1.87 (s, 3H), 1.28 (br s, 1H) 1.26 (d, J = 7.2 Hz, 3H), 0.99–0.96 (m, 2H), 0.93 (d, J = 6.4 Hz, 3H), 0.85 (t, J = 7.2 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃, mixture of rotamers) δ 171.7, 171.7, 171.6, 171.5, 168.3, 158.6, 130.4, 127.9, 113.9, 64.7, 64.6, 64.4, 60.4, 55.2, 51.8, 51.3, 50.8, 50.7, 49.9, 37.6, 37.5, 33.2, 30.9, 30.7, 29.7, 24.9, 19.1, 15.7, 15.6, 14.3, 13.7, 13.3,
10.5; IR (neat) 3328, 2959, 1738, 1656, 1514 cm⁻¹; HRMS (ESI) m/z for C₂₈H₄₅N₄O₇ [M+H]⁺ calcd 549.3283, found 549.3259.

**Amide 3.32**

To a rt solution of acid 3.2 (41.1 mg, 45.1 µmol) in CH₂Cl₂ (0.5 mL) was added i-Pr₂NEt (20 µL, 135 µmol), followed by HATU (18 mg, 47.4 µmol). Immediately after HATU addition, a solution of amino alcohol 3.31 (27 mg, 49.6 µmol) in CH₂Cl₂ (0.5 mL) was added. The resulting solution was stirred at rt for 12 h, at which the reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (98:2 to 96:4 CHCl₃/MeOH, v/v) gave amide 3.32 (41.1 mg, 94%) as a white foam: Rₜ 0.17 (24:1 chloroform/MeOH, v/v); [α]²⁵_D = −85.8 (c 0.68, CHCl₃) ¹H NMR (400 MHz, CDCl₃, mixture of rotamers) δ 7.08 (m, 2H), 6.78 (J = 8.4 Hz, 2H), 6.62 (d, J = 8 Hz, 0.5H), 6.46 (m, 1H), 6.31 (d, J = 8.4 Hz, 0.5), 6.22 (d, J = 9.2 Hz, 0.5H), 5.40 (q, J = 6.8 Hz, 1H), 5.23–5.16 (m, 1H), 4.90 (d, J = 10.4 Hz, 1H), 4.87–4.81 (m, 2H), 4.28 (ddd, J = 14.8, 8.8, 3.6 Hz, 1H), 3.76 (s, 3H), 3.68 (s, 3H), 3.63–3.57 (m, 2H), 3.53–3.48 (m, 2H), 3.45–3.34 (m, 2H), 3.04 (dd, J = 7.6, 13.2 Hz, 1H), 2.94 (s,
3H), 2.88–2.81 (m, 2H), 2.73 (s, 1.5H), 2.71 (s, 1.5H), 2.28–2.16 (m, 2H), 2.07–1.98 (m, 3H), 1.95 (br s, 1H), 1.89–1.86 (m, 3H), 1.91 (s, 3H), 1.67–1.55 (m, 2H), 1.38–1.32 (m, 1H), 1.46 (s, 4H), 1.44 (s, 5H), 1.25 (d, \(J = 6.8\) Hz, 3H), 1.23 (m, 2H), 1.13 (d, \(J = 6.8\) Hz, 3H), 0.98–0.91 (m, 6H), 0.87 (s, 9H), 0.85 (m, 4H), 0.83 (m, 1H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\), mixture of rotamers) \(\delta\) 176.2, 175.6, 173.6, 172.7, 171.8, 171.5, 171.4, 167.9, 167.7, 158.7, 158.6, 154.9, 154.7, 133.9, 133.5, 132.9, 132.3, 130.4, 128.0, 127.9, 113.9, 80.6, 80.2, 78.9, 78.5, 77.2, 71.7, 70.3, 64.7, 60.3, 59.0, 55.2, 51.8, 50.5, 50.3, 49.7, 49.3, 48.2, 47.4, 46.8, 46.7, 37.8, 37.6, 37.2, 34.8, 34.5, 33.2, 30.9, 30.5, 30.1, 30.0, 28.5, 26.0, 25.2, 24.9, 24.8, 24.4, 24.1, 20.7, 20.2, 16.4, 15.7, 14.4, 13.3, 10.5; IR (neat) 3408, 2962, 1735, 1621, 1250 cm\(^{-1}\); HRMS (ESI) \(m/z\) for C\(_{51}\)H\(_{83}\)N\(_{5}\)NaO\(_{13}\) [M+Na]\(^+\) calcd 996.5880, found 996.5853.

**Oxazoline 3.33**

![Diagram](image)

To a solution of amide 3.32 (27 mg, 27.7 \(\mu\)mol) in CH\(_2\)Cl\(_2\) (0.6 mL) at \(-78^\circ\)C was added DAST (3.7 \(\mu\)L, 27.7 \(\mu\)mol). After stirring for 1 h at \(-78^\circ\)C, additional DAST (1.8 \(\mu\)L, 13.9 \(\mu\)mol) was added and stirring was continued for 1 h. The reaction was
quenched at –78 °C with saturated aqueous NaHCO₃ (0.5 mL) and warmed to rt. The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (5 x 2 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (96:4 CH₂Cl₂/MeOH, v/v) gave oxazoline 3.33 (21.7 mg, 82%) as a white amorphous solid: Rₓ 0.13 (96:4 CH₂Cl₂/MeOH, v/v); [α]²⁵_D = –93.1 (c 0.47, CHCl₃) ¹H NMR (400 MHz, CDCl₃, mixture of rotamers) δ 7.08 (d, J = 8 Hz, 2H), 6.78 (d, J = 8.8 Hz, 2H), 6.59 (d, J = 8.8 Hz, 0.4H), 6.43 (d, J = 8.4 Hz, 0.6H), 6.15 (m, 1H), 5.40 (q, J = 6.4 Hz, 1H), 4.92–4.79 (m, 3H), 4.49–4.35 (m, 1H), 4.31 (m, 1H), 3.90–3.80 (m, 1H), 3.76 (s, 3H), 3.68 (s, 3H), 3.49–3.46 (m, 2H), 3.40–3.34 (m, 1H), 3.08 (m, 1H), 2.96 (s, 3H), 2.87–2.81 (m, 2H), 2.73 (s, 3H), 2.58–2.45 (m, 1H), 2.24–2.11 (m, 2H), 2.12–2.02 (m, 2H), 2.04–1.82 (m, 4H), 1.88 (s, 3H), 1.70–1.57 (m, 2H), 1.44 (s, 6H), 1.42 (s, 3H), 1.27–1.23 (m, 5H), 1.20–1.19 (m, 3H), 0.98–0.96 (m, 2H), 0.94–0.91 (m, 6H), 0.88 (s, 9H), 0.85–0.83 (m, 3H); ¹³C NMR (100 MHz, CDCl₃, mixture of rotamers) δ 173.0, 172.7, 171.8, 171.5, 171.4, 158.6, 154.2, 135.6, 135.3, 132.9, 130.4, 128.2, 113.9, 89.8, 80.1, 79.9, 77.2, 71.9, 71.8, 70.7, 63.5, 60.3, 59.7, 59.2, 55.2, 51.8, 50.6, 50.5, 49.7, 46.6, 46.2, 40.7, 40.6, 40.1, 37.9, 37.7, 37.6, 34.7, 33.2, 30.9, 30.5, 30.0, 29.7, 28.5, 28.4, 26.0, 25.1, 25.0, 24.4, 23.3, 20.5, 15.7, 14.4, 13.4, 10.6; IR (neat) 3471, 3342, 2967, 1730, 1403 cm⁻¹; HRMS (ESI) m/z for C₅₁H₈₁N₅NaO₁₂ [M+Na]⁺ calcd 978.5774, found 978.5743.
Amine 3.34

To a 0 °C solution of oxazoline 3.33 (24 mg, 25.1 µmol) in CH$_2$Cl$_2$ (0.3 mL) was added 2,6-lutidine (29 µL, 251 µmol) followed by trimethylsilyl trifluoromethanesulfonate (23 µL, 126 µmol) dropwise. The reaction was warmed to rt and stirred for 1 h. After cooling to 0 °C, saturated aqueous NaHCO$_3$ (0.3 mL) was added dropwise. The aqueous layer was extracted with CHCl$_3$ (10 x 1 mL) and the combined organic layers were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The residual 2,6-lutidine was removed under a stream of Ar and the crude residue was placed under high vacuum for 1 h.

To a 0 °C solution of crude trimethylsilyl ether in THF (0.3 mL) was added tetrabutylammonium fluoride (75 µL, 75 µmol, 1 M solution in THF). After stirring at 0 °C for 30 min, saturated aqueous NaHCO$_3$ (0.3 mL) was added and the mixture was warmed to rt. The aqueous layer was extracted with CHCl$_3$ (10 x 1 mL) and the combined organic layers were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (98:2 to 95:5 CHCl$_3$/MeOH, v/v) yielded amine 3.34 (14.7 mg, 68% from oxazoline 3.33) as a light yellow amorphous solid: $R_f$ 0.45 (9:1 CH$_3$Cl/MeOH, v/v); [α]$^25_D$ = −61.0 (c 0.74,
CHCl$_3$ $^1$H NMR (400 MHz, CDCl$_3$, mixture of rotamers) $\delta$ 7.09 (d, $J = 8.8$ Hz, 2H), 6.78 (d, $J = 8.4$ Hz, 2H), 6.52 (d, $J = 8.4$ Hz, 0.5H), 6.21 (dq, $J = 8.8$, 1.6 Hz, 0.2H), 6.16 (dq, $J = 8.4$, 1.2 Hz, 0.8H), 5.41 (q, $J = 7.2$ Hz, 1H), 5.23–5.17 (m, 1H), 4.97–4.85 (m, 3H), 4.48–4.40 (m, 1H), 3.91–3.83 (m, 2H), 3.76 (s, 3H), 3.73–3.70 (m, 1H), 3.68 (s, 3H), 3.10–3.00 (m, 4H), 2.97 (s, 2H), 2.95 (s, 1H), 2.88–2.82 (m, 2H), 2.74 (s, 2H), 2.67 (s, 1H), 2.47 (dddd, $J = 14.4$, 7.2, 7.2, 7.2 Hz, 1H), 2.19–2.10 (m, 1H), 1.98–1.91 (m, 2H), 1.88 (s, 3H), 1.84–1.72 (m, 3H), 1.63–1.53 (m, 2H), 1.39–1.33 (m, 2H), 1.27 (d, $J = 7.2$ Hz, 3H), 1.25–1.24 (m, 2H), 1.21 (d, $J = 7.2$ Hz, 3H), 1.06–0.96 (m, 2H), 0.93 (d, $J = 6.4$ Hz, 3H), 0.94–0.91 (m, 3H), 0.89 (s, 9H), 0.85 (t, $J = 3.6$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$, mixture of rotamers) $\delta$ 171.8, 171.7, 171.5, 171.4, 167.9, 158.7, 133.2, 130.4, 114.0, 79.8, 71.6, 70.8, 70.6, 63.5, 60.4, 60.0, 59.9, 58.9, 55.2, 51.8, 50.6, 49.8, 46.0, 40.5, 40.2, 37.7, 37.6, 36.2, 34.8, 33.3, 30.6, 25.9, 25.8, 25.3, 25.0, 24.7, 20.9, 19.7, 15.7, 15.6, 14.4, 13.5, 10.6, 10.5; IR (neat) 2963, 1738, 1645, 1514, 1248 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_{46}$H$_{74}$N$_5$O$_{10}$ [M+H]$^+$ calcd 856.5430, found 856.5425.
Depsipptide 1.9

To a 0 °C solution of amine 3.34 (7.0 mg, 8.2 µmol) in THF (40 µL) and t-BuOH (80 µL) was added lithium hydroxide (82 µL, 82 µmol, 1 M solution). The reaction was warmed to rt and stirred for 5 h before the reaction was concentrated under a stream of Ar. The residue was dissolved in CHCl₃ (0.2 mL) and neutralized with aqueous phosphate buffer (pH = 7, 0.1 mL). The separated aqueous phase was extracted with CHCl₃ (10 x 0.5 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The resulting residue was azeotropically dried with toluene twice and then placed under high vacuum for 2 h.

The crude amino acid (3.35) was dissolved in CH₂Cl₂ (8 mL, 0.001 M) and cooled to 0 °C. To the cooled solution was added i-Pr₂NEt (7.1 µL, 41 µmol) followed by PyAOP (8.6 mg, 16.4 µmol) and the reaction mixture was warmed to rt. After stirring at rt for 48 h, the reaction mixture was concentrated under reduced pressure. Purification via preparative thin layer chromatography on silica gel (98:2 EtOAc/MeOH, v/v) yielded cyclic depsipeptide 1.9 (2.9 mg, 43% from amine 3.34) as a white amorphous solid: Rᵣ 0.61 (9:1 CHCl₃/MeOH, v/v); [α]°D₂₅ = −102.3 (c 0.30, MeOH), lit.°D₂₅ = −167 (c 0.10, MeOH), lit.°D₂₅ = −117.5 (c 0.20, CHCl₃); ¹H NMR (500 MHz, CDCl₃, mixture
of rotamers) δ 7.16 (d, J = 8.6 Hz, 2H), 6.79 (d, J = 8.6 Hz, 2H), 6.18 (dq, J = 8.0, 1.2 Hz, 1H), 5.98 (d, J = 9.4 Hz, 1H), 5.23 (d, J = 11.6 Hz, 1H), 5.06 (ddd, J = 10.4, 10.4, 4.8 Hz, 1H), 4.97 (dd, J = 12.5, 1.7 Hz, 1H), 4.78 (ddd, J = 9.3, 9.1, 5.7 Hz, 1H), 4.69 (d, J = 10.6 Hz, 1H), 4.34 (t, J = 8 Hz, 1H), 4.24–4.14 (m, 3H), 3.77 (s, 3H), 3.70–3.61 (m, 2H), 3.32–3.27 (m, 1H), 3.11 (dd, J = 11.7 Hz, 1H), 2.85 (dd, J = 12.7, 4.8 Hz, 1H), 2.81 (s, 3H), 2.74 (s, 3H), 2.42 (dd, J = 11.1, 6.4 Hz, 1H), 2.36–2.31 (m, 1H), 2.27–2.23 (m, 1H), 2.18–2.13 (m, 2H), 2.09–2.04 (m, 2H), 1.95–1.86 (m, 2H), 1.91 (d, J = 1.5 Hz, 3H), 1.79 (ddd, J = 14, 14, 4 Hz, 1H), 1.43–1.37 (m, 2H), 1.30–1.28 (m, 1H), 1.23 (d, J = 6 Hz, 3H), 1.14–1.08 (m, 1H), 1.06 (d, J = 5.1 Hz, 3H), 1.04–1.0 (m, 1H) 0.98 (d, J = 6.7 Hz, 3H), 0.97–0.92 (m, 1H), 0.94 (t, J = 8.4 Hz, 3H), 0.84–0.81 (m, 1H), 0.87 (s, 9H); IR (neat) 3394, 2965, 1739, 1635, 1252 cm⁻¹; HRMS (ESI) m/z for C₄₅H₇₀N₅O₉ [M+H]⁺ calcd 824.5168, found 824.5167.

**Diamide 4.3**

![Reaction Scheme](image)

To a 0 °C of amide 3.19 (0.34 g, 0.99 mmol) in CH₂Cl₂ (5 mL) was added trifluoroacetic acid (5 mL) dropwise. The solution was stirred at 0 °C for 1 h before toluene (5 mL) was added. The reaction was concentrated under a stream of argon and additional toluene was added (5 mL). After concentrating the reaction mixture under a stream of argon, the crude product was placed under high vacuum for 2 h.
To a rt solution of N-Boc-O-allyl-L-tyrosine (0.29 g, 0.90 mmol) in CH₂Cl₂ (8 mL) were added i-Pr₂NEt (0.47 mL, 2.7 mmol) and PyAOP (0.50 g, 0.95 mmol) sequentially. After stirring for 2 min at rt, a solution of crude deprotected amide 3.19 in CH₂Cl₂ (2 mL) was added and the reaction was stirred for 12 h at rt. Upon completion, the reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (2:1 to 1:1 hexanes/EtOAc, v/v) yielded diamide 4.3 (0.41 g, 84% from amide 3.19) as a white amorphous solid: Rf 0.30 (1:1 hexanes/EOAc, v/v); [α]²⁵_D = −113.3 (c 0.92, CHCl₃) ¹H NMR (400 MHz, CDCl₃, mixture of carbamate rotamers) δ 7.10 (d, J = 8.4 Hz, 2H), 6.80 (d, J = 8.8 Hz, 2H), 6.07–5.98 (m, 1H), 5.41–5.36 (m, 1H), 5.38 (dd, J = 17.6, 1.6 Hz, 1H), 5.26 (dd, J = 10.4, 1.6 Hz, 1H), 5.17 (d, J = 8.8 Hz, 1H), 4.88 (d, J = 10.4 Hz, 1H), 4.81 (dd, J = 6.8 Hz, 1H), 4.48 (dd, J = 5.2, 5.2 Hz, 2H), 3.67 (s, 3H), 2.97 (dd, J = 13.2, 7.2 Hz, 1H), 2.92 (s, 3H), 2.79–2.75 (m, 1H), 2.68 (s, 3H), 1.98–1.90 (m, 1H), 1.38 (s, 9H), 1.25 (d, J = 6.8 Hz, 3H), 0.97–0.94 (m, 2H), 0.91 (d, J = 6.8 Hz, 3H), 0.83 (t, J = 7.2 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃, mixture of carbamate rotamers) δ 171.7, 171.7, 171.5, 157.6, 155.1, 133.3, 130.4, 128.6, 117.6, 114.7, 79.8, 68.8, 68.7, 60.3, 52.1, 51.8, 51.8, 51.7, 49.8, 38.3, 33.2, 30.8, 28.3, 28.2, 24.9, 15.7, 14.3, 11.6, 10.5; IR (neat) 3302, 2972, 1740, 1709, 1644, 1510 cm⁻¹; HRMS (ESI) m/z for C₂₉H₄₅N₃NaO₇ [M+Na]^+ calcd 570.3150, found 570.3153.
**Triamide 4.4**

To a solution of diamide **4.3** (0.41 g, 0.75 mmol) in CH$_2$Cl$_2$ (4 mL) at 0 °C was added trifluoroacetic acid (4 mL) dropwise. After stirring for 1 h at 0 °C, toluene (4 mL) was added and the reaction was concentrated under a stream of Ar. Toluene (4 mL) was again added and the mixture was concentrated under a stream of argon, at which the crude deprotected diamide **4.3** was placed under high vacuum for 2 h.

To a solution of acid **3.3** (0.19 g, 0.68 mmol) in CH$_2$Cl$_2$ (5 mL) at rt was added i-Pr$_2$NEt (0.36 mL, 2.04 mmol), followed by PyAOP (0.38 g, 0.72 mmol). After stirring for 2 min, a solution of crude deprotected diamide **4.3** in CH$_2$Cl$_2$ (2 mL) was added dropwise and the mixture was stirred for 5 h at rt. The reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (2:1 hexanes/EtOAc, v/v) gave triamide **4.4** (0.38 g, 79% from diamide **4.3**) as a white amorphous solid: R$_f$ 0.29 (2:1 hexanes/EOAc, v/v); [α]$^2_{D} = -78.2$ (c 1.13, CHCl$_3$) $^1$H NMR (500 MHz, CDCl$_3$, mixture of rotamers) δ 7.06 (d, J = 8.5 Hz, 2H), 6.81–6.79 (m, 2H), 6.41 (d, J = 8.5 Hz, 1H), 6.06–5.99 (m, 2H), 5.44–5.37 (m, 2H), 4.91 (d, J = 10.5 Hz, 1H), 4.48 (d, J = 5.5 Hz, 2H), 4.23–4.17 (m, 1H), 3.69 (s, 3H), 3.66–3.63 (m, 2H), 3.05 (dd, J = 14, 7.5 Hz, 1H), 2.99 (s, 3H), 2.87–2.82 (m, 2H), 2.80 (s, 3H), 2.02–1.92 (m, 1H), 1.89 (s, 3H), 1.29–1.25 (m, 1H), 1.28 (d, J = 6.5 Hz, 3H), 1.02–0.96 (m, 2H), 0.93 (d, J = 6.5 Hz, 3H), 0.90 (s, 9H), 0.86 (t, J = 7.5 Hz, 3H), 0.08 (s, 3H), 0.07 (s, 3H); $^{13}$C NMR
(125 MHz, CDCl$_3$, mixture of rotamers) δ 171.9, 171.5, 171.4, 167.4, 157.7, 135.7, 133.2, 130.4, 130.2, 129.5, 129.3, 127.9, 127.9, 117.7, 114.8, 68.8, 65.6, 60.9, 60.4, 51.8, 50.5, 49.7, 49.0, 37.7, 37.7, 33.3, 31.0, 30.6, 25.0, 18.3, 15.7, 14.4, 13.6, 13.5, 10.6, –5.5; IR (neat) 2949, 2101, 1739, 1642, 1538 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_36$H$_{58}$N$_6$NaO$_7$Si $[\text{M+Na}]^+$ calcd 737.4028, found 737.4030.

**Primary alcohol 4.5**

![Chemical structure](image)

To a 0 °C solution of triamide 4.4 (0.38 g, 0.55 mmol) in MeOH (6 mL) was added TsOH$\cdot$H$_2$O (20 mg, 0.10 mmol) and the reaction was warmed to rt. After stirring for 2 h at rt, Et$_3$N was added to quench the residual acid. The reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (95:5 CHCl$_3$/MeOH, v/v) to give alcohol 4.5 (0.30 g, 95%) as a white amorphous solid: R$_f$ 0.12 (96:4 CHCl$_3$/MeOH, v/v); [$\alpha$]$^2$$_D$ = –81.1 (c 1.13, CHCl$_3$) $^1$H NMR (500 MHz, CDCl$_3$, mixture of rotamers) δ 7.07–7.05 (m, 2H), 6.80–6.78 (m, 2H), 6.71 (d, $J$ = 8.5 Hz, 0.4H), 6.57 (d, $J$ = 8 Hz, 0.4H), 6.13–6.10 (m, 1H), 6.05–5.98 (m, 1H), 5.44–5.36 (m, 1H), 5.38 (dd, $J$ = 17.5, 1.5 Hz, 1H), 5.26 (dd, $J$ = 10.5, 1.5, 1H), 5.18 (ddd, $J$ = 12.5, 6.5, 6.5 Hz,1H), 4.90 (d, $J$ = 10.5 Hz, 1H), 4.47 (dt, $J$ = 5, 1 Hz, 2H), 4.35–4.30 (m, 1H), 3.68 (s, 3H), 3.60–3.57 (m, 2H), 3.06 (dd, $J$ = 14.5, 7.5 Hz, 1H), 3.01
(s, 3H), 2.88–2.83 (m, 2H), 2.81 (s, 3H), 2.00–1.92 (m, 1H), 1.89 (d, J = 1 Hz, 3H), 1.29 (d, J = 7 Hz, 3H), 1.01–0.95 (m, 2H), 0.92 (d, J = 6.5 Hz, 3H), 0.86–0.82 (m, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$, mixture of rotamers) δ 171.9, 171.9, 171.6, 171.5, 171.4, 167.5, 167.4, 157.8, 157.8, 136.5, 136.2, 133.2, 130.4, 130.4, 129.0, 128.8, 127.9, 127.9, 117.7, 114.8, 114.8, 77.3, 77.0, 68.8, 64.3, 61.1, 61.1, 60.4, 51.9, 50.8, 50.6, 49.7, 37.6, 33.2, 31.0, 30.7, 30.6, 25.0, 15.7, 14.4, 13.6, 13.6, 10.6, 10.6; IR (neat) 3408, 3327, 2967, 2101, 1738, 1614 cm$^{-1}$; HRMS (ESI) m/z for C$_{30}$H$_{44}$N$_6$NaO$_7$ [M+Na]$^+$ calcld 623.3164, found 623.3163.

**Amine 4.2**

![Diagram](image)

To a 0 °C solution of alcohol 4.5 (0.30 g, 0.49 mmol) in a mixture of THF (5 mL) and H$_2$O (0.1 mL) was added PBu$_3$ (0.24 mL, 0.98 mmol) dropwise. After warming to rt and stirring for 1 h, the reaction was heat under microwave irradiation at 55 °C for 30 min. The reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (96:4 to 9:1 CHCl$_3$/MeOH, v/v) gave amino alcohol 4.2 (0.25 g, 90%) as a white amorphous solid: $R_f$ 0.12 (9:1 CHCl$_3$/MeOH, v/v); $[\alpha]_D^{25} = -109.0$ (c 1.03, CHCl$_3$) $^1$H NMR (500 MHz, CDCl$_3$, mixture of rotamers) δ 7.07 (d, J = 8.5 Hz, 2H), 6.79 (d, J = 9 Hz, 2H), 6.62 (d, J = 8.5 Hz, 0.4H), 6.60 (d, J = 8 Hz, 2H), 6.57 (s, 1H), 6.49 (d, J = 8 Hz, 2H), 5.71 (s, 1H), 4.68 (q, J = 7 Hz, 2H), 4.27 (t, J = 7 Hz, 2H), 4.10 (q, J = 7 Hz, 2H), 3.93 (s, 3H), 3.11 (s, 3H), 2.00–1.92 (m, 1H), 1.89 (d, J = 1 Hz, 3H), 1.29 (d, J = 7 Hz, 3H), 1.01–0.95 (m, 2H), 0.92 (d, J = 6.5 Hz, 3H), 0.86–0.82 (m, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$, mixture of rotamers) δ 171.9, 171.9, 171.6, 171.5, 171.4, 167.5, 167.4, 157.8, 157.8, 136.5, 136.2, 133.2, 130.4, 130.4, 129.0, 128.8, 127.9, 127.9, 117.7, 114.8, 114.8, 77.3, 77.0, 68.8, 64.3, 61.1, 61.1, 60.4, 51.9, 50.8, 50.6, 49.7, 37.6, 33.2, 31.0, 30.7, 30.6, 25.0, 15.7, 14.4, 13.6, 13.6, 10.6, 10.6; IR (neat) 3408, 3327, 2967, 2101, 1738, 1614 cm$^{-1}$; HRMS (ESI) m/z for C$_{30}$H$_{44}$N$_6$NaO$_7$ [M+Na]$^+$ calcld 623.3164, found 623.3163.
0.4H), 6.09 (dq, J = 9, 9 Hz, 1H), 6.05–5.98 (m, 1H), 5.41–5.35 (m, 1H), 5.37 (dd, J = 9, 1.5 Hz, 1H), 5.26 (dd, J = 10.5, 1 Hz, 1H), 5.18 (q, J = 7 Hz, 1H), 4.89 (d, J = 10.5 Hz, 1H), 4.47 (d, J = 5 Hz, 2H), 3.72 (ddd, J = 8, 8, 4.5 Hz, 1H), 3.67 (s, 3H), 3.53 (ddd, J = 10.5, 4, 1 Hz, 1H), 3.38 (dd, J = 11, 8 Hz, 1H), 3.03 (dd, J = 14, 7.5 Hz, 1H), 2.97 (s, 3H), 2.87–2.81 (m, 2H), 2.74 (s, 3H), 2.21 (m, 3H), 1.98–1.90 (m, 1H), 1.85 (s, 3H), 1.26 (d, J = 7 Hz, 3H), 0.99–0.97 (m, 2H), 0.91 (d, J = 10 Hz, 3H), 0.84 (t, J = 7.5 Hz, 3H); ^13^C NMR (125 MHz, CDCl$_3$, mixture of rotamers) δ 171.8, 171.6, 171.6, 171.5, 168.3, 168.2, 157.7, 136.2, 161.2, 133.3, 133.2, 132.3, 132. 2, 130.4, 130.2, 128.0, 117.7, 114.8, 77.3, 76.7, 68.8, 65.5, 60.4, 51.8, 51.2, 51.2, 50.6, 49.8, 37.7, 37.7, 33.2, 30.9, 30.6, 25.0, 16.1, 15.7, 14.4, 13.2, 11.6, 10.6; IR (neat) 3350, 2965, 1738, 1614, 1505 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_{30}$H$_{47}$N$_4$O$_7$ [M+H]$^+$ calcd 575.3439, found 575.3444.

**Amide 4.6**

![Amide 4.6](image)

To a rt solution of acid 3.2 (19.8 mg, 44.7 µmol) in CH$_2$Cl$_2$ (0.4 mL) was added i-Pr$_2$NEt (23.4 µL, 134 µmol), followed by HATU (17.9 mg, 44.7 µmol). Immediately after HATU addition, a solution of amino alcohol 4.2 (28.3 mg, 49.2 µmol) in CH$_2$Cl$_2$
(0.1 mL) was added. The resulting solution was stirred at rt for 12 h, at which the reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (98:2 to 96:4 CHCl₃/MeOH, v/v) gave amide 4.6 (43.7 mg, 97%) as a white foam: Rᵣ 0.21 (96:4 CHCl₃/MeOH, v/v); [α]²⁵ₒ = −75.4 (c 0.85, CHCl₃)

¹H NMR (500 MHz, CDCl₃, mixture of carbamate rotamers) δ 7.10–7.07 (m, 2H), 6.80 (d, J = 8.5 Hz, 2H), 6.61 (d, J = 7.5 Hz, 0.3H), 6.45 (d, J = 8 Hz, 0.3H), 6.42 (d, J = 8 Hz, 0.3H), 6.32 (dq, J = 8.5, 1 Hz, 0.5H), 6.22 (dq, J = 9, 1.5 Hz, 0.5H), 6.07–6.00 (m, 1H), 5.41–5.37 (m, 1H), 5.39 (dd, J = 17.5, 1.5 Hz, 1H), 5.27 (dd, J = 10.5, 1 Hz, 1H), 5.19 (dddd, J = 8, 8, 8 Hz, 1H), 4.90 (d, J = 10.5 Hz, 1H), 4.87–4.81 (m, 2H), 4.67 (dt, J = 5, 1 Hz, 2H), 4.28 (ddd, J = 18, 8.5, 3.5 Hz, 1H), 3.77–3.66 (m, 2H), 3.68 (s, 3H), 3.63–3.58 (m, 2H), 3.55–3.48 (m, 2H), 3.45–3.37 (m, 1H), 3.04 (ddd, J = 13.5, 7.5, 3 Hz, 1H), 2.94 (s, 3H), 2.88–2.84 (m, 1H), 2.71 (s, 3H), 2.28–2.18 (m, 2H), 2.04–1.99 (m, 1H), 1.97–1.92 (m, 2H), 1.90 (s, 3H), 1.90–1.82 (m, 2H), 1.47 (s, 9H), 1.49–1.42 (m, 5H), 1.26 (d, J = 6.5 Hz, 3H), 1.13 (d, J = 7 Hz, 3H), 0.96–0.91 (m, 7H), 0.87 (s, 9H), 0.85 (t, J = 7.5 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃, mixture of carbamate rotamers) δ 176.2, 173.7, 172.8, 171.8, 171.5, 171.5, 171.4, 171.4, 170.2, 167.9, 167.7, 165.9, 157.7, 157.7, 154.9, 154.8, 150.0, 133.9, 133.5, 133.2, 133.2, 132.9, 132.2, 130.4, 128.2, 128.1, 124.7, 121.9, 117.7, 117.7, 114.8, 110.8, 109.6, 80.6, 80.2, 78.9, 78.5, 78.1, 77.6, 77.5, 77.2, 77.2, 71.7, 70.4, 68.8, 64.8, 64.7, 60.4, 60.3, 59.0, 59.0, 55.7, 51.8, 50.6, 50.5, 50.3, 49.7, 49.3, 48.2, 47.4, 46.9, 46.7, 43.7, 40.8, 40.1, 37.9, 37.8, 37.6, 37.3, 34.8, 34.5, 33.2, 30.9, 30.9, 30.6, 30.2, 30.0, 28.5, 28.5, 28.4, 26.0, 26.0, 26.0 25.2 25.0, 25.0, 24.8, 24.4, 24.2, 20.8, 20.2, 18.6, 18.6, 17.2, 16.5, 15.7, 14.4, 14.4, 13.3, 12.5, 11.6, 10.6, 10.5; IR (neat) 3408, 3390, 2936,
1738, 1645 cm\(^{-1}\); HRMS (ESI) \(m/z\) for C\(_{53}\)H\(_{85}\)N\(_3\)O\(_{13}\) [M+Na]\(^+\) calcd 1022.6036, found 1022.6041.

**Oxazoline 4.7**

To a \(-78^\circ\text{C}\) solution of alcohol 4.6 (43.7 mg, 43.7 \(\mu\)mol) in CH\(_2\)Cl\(_2\) (0.9 mL) was added DAST (8.7 \(\mu\)L, 65.6 \(\mu\)mol) dropwise. After stirring for 1 h at \(-78^\circ\text{C}\), additional DAST (4.4 \(\mu\)L, 32.8 \(\mu\)mol) was added and stirring continued for 30 min. The reaction was quenched with aqueous saturated NaHCO\(_3\) (1 mL) at \(-78^\circ\text{C}\) and the solution was warmed to rt. The layers were separated and the aqueous layer was extracted with CH\(_2\)Cl\(_2\) (5 x 5 mL). The combined organic layers were dried over Na\(_2\)SO\(_4\), filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (97:3 to 96:4 CHCl\(_3\)/MeOH, v/v) yielded oxazoline 4.7 (35 mg, 81%) as an amorphous solid: \(R_f\) 0.25 (96:4 CHCl\(_3\)/MeOH, v/v); \([\alpha]^{25}_D = -81.9\) (c 1.22, CHCl\(_3\)) \(^1\)H NMR (500 MHz, CDCl\(_3\), mixture of carbamate rotamers) \(\delta\) 7.08 (d, \(J = 8.5\) Hz, 2H), 6.79 (d, \(J = 8.5\) Hz, 2H), 6.61 (d, \(J = 8.5\) Hz, 0.3H), 6.43 (d, \(J = 8.5\) Hz, 0.3H), 6.16 (dq, \(J = 9\) Hz, 1H), 6.07–5.99 (m, 1H), 5.39 (dd, \(J = 17, 1.5\) Hz, 1H), 5.41–
5.37 (m, 1H), 5.27 (dd, J = 10.5, 1.5 Hz, 1H), 5.20 (ddd, J = 13.5, 6, 6 Hz, 1H), 4.94–4.79 (m, 3H), 4.48 (dt, J = 5.5, 1.5 Hz, 2H), 4.45–4.30 (m, 2H), 3.90–3.81 (m, 1H), 3.78–3.71 (m, 1H), 3.68 (s, 3H), 3.49–3.47 (m, 1H), 3.39–3.34 (m, 1H), 3.07–3.02 (m, 1H), 2.96 (s, 3H), 2.87–2.83 (m, 2H), 2.70 (s, 3H), 2.56–2.51 (m, 1H), 2.21–2.15 (m, 1H), 2.06–2.00 (m, 1H), 1.97–1.84 (m, 3H), 1.88 (s, 3H), 1.74 (br s, 2H), 1.70–1.58 (m, 2H), 1.45 (s, 9H), 1.26 (d, J = 7 Hz, 3H), 1.20 (d, J = 6.5 Hz, 3H), 1.01–0.97 (m, 2H), 0.95–0.91 (m, 6H), 0.87 (s, 9H), 0.86–0.83 (m, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$, mixture of carbamate rotamers) δ 173.5, 172.7, 172.6, 171.5, 171.5, 171.0, 171.0, 169.8, 167.9, 167.7, 164.8, 157.5, 157.5, 1549, 154.8, 149.6, 149.5, 133.8, 133.8, 133.8, 133.2, 132.9, 132.2, 130.1, 128.2, 124.7, 121.8, 117.1, 117.1, 114.8, 110.8, 108.6, 80.2, 80.1, 78.8, 78.5, 78.1, 77.7, 77.6, 77.5, 70.4, 69.0, 64.8, 64.8, 60.5, 59.0, 55.7, 53.7, 51.8, 50.6, 50.4, 50.3, 49.8, 49.2, 48.8, 47.4, 46.8, 43.2, 40.3, 40.1, 38.0, 37.6, 37.3, 34.5, 33.0, 30.9, 30.8, 30.6, 30.2, 30.1, 28.5, 28.5, 28.4, 26.4, 26.2, 26.1, 25.0, 24.9, 24.8, 24.8, 24.8, 24.8, 20.5, 20.3, 18.6, 17.2, 16.6, 15.3, 14.3, 13.2, 13.2, 11.9, 11.6, 10.5; IR (neat) 3384, 2942 1742, 1643, 1207 cm$^{-1}$; HRMS (ESI) m/z for C$_{53}$H$_{83}$N$_5$NaO$_{12}$ [M+Na]$^+$ calcd 1004.5930, found 1004.5928.
Amine 4.8

To a 0 °C solution of oxazoline 4.7 (40 mg, 40.7 µmol) in CH₂Cl₂ (0.5 mL) was added 2,6-lutidine (38 µL, 326 µmol) followed by trimethylsilyl trifluoromethanesulfonate (37 µL, 204 µmol) dropwise. The reaction was warmed to rt and stirred for 1 h. After cooling to 0 °C, saturated aqueous NaHCO₃ (0.5 mL) was added dropwise. The aqueous layer was extracted with CHCl₃ (10 x 1 mL) and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residual 2,6-lutidine was removed under a stream of Ar and the crude residue was placed under high vacuum for 1 h.

To a 0 °C solution of crude trimethylsilyl ether in THF (0.5 mL) was added tetrabutylammonium fluoride (120 µL, 122 µmol, 1 M solution in THF). After stirring at 0 °C for 30 min, saturated aqueous NaHCO₃ (0.5 mL) was added and the mixture was warmed to rt. The aqueous layer was extracted with CHCl₃ (10 x 1 mL) and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (98:2 to 95:5 CHCl₃/MeOH, v/v) yielded amine 4.8 (32 mg, 81% from oxazoline 4.7) as a light yellow amorphous solid: Rf 0.39 (10:1 CH₂Cl₂/MeOH, v/v); [α]²⁵° d = −80.7 (1.14, CHCl₃);
$^1$H NMR (500 MHz, CDCl$_3$, mixture of carbamate rotamers) δ 7.11–7.08 (m, 2H), 6.81–6.79 (m, 2H), 6.75 (d, $J = 8$ Hz, 1H), 6.24 (dq, $J = 8.5$, 1.5 Hz, 1H), 6.07–5.99 (m, 1H), 5.39 (dd, $J = 17.5$, 1.5 Hz, 1H), 5.40–5.36 (m, 1H), 5.27 (d, $J = 10.5$ Hz, 1H), 5.20 (ddd, $J = 7.5$ Hz, 1H), 5.01–4.93 (m, 2H), 4.89 (dd, $J = 10.5$, 2 Hz, 1H), 4.48 (dd, $J = 5$, 1.5 Hz, 2H), 4.52–4.42 (m, 2H), 4.33–4.29 (m, 1H), 4.27–4.24 (m, 1H), 3.95 (t, $J = 8.5$ Hz, 1H), 3.72–3.66 (m, 1H), 3.68 (s, 3H), 3.31–3.27 (m, 1H), 3.25–3.20 (m, 1H), 3.17–3.12 (m, 1H), 3.07–2.97 (m, 2H), 2.96 (s, 3H), 2.91–2.81 (m, 2H), 2.72 (s, 3H), 2.45–2.41 (m, 1H), 2.33–2.26 (m, 1H), 2.05–1.91 (m, 5H), 1.88 (s, 3H), 1.70–1.62 (m, 2H), 1.55–1.44 (m, 2H), 1.38–1.29 (m, 2H), 1.29–1.25 (m, 4H), 1.25–1.19 (m, 3H), 0.97–0.96 (m, 2H), 0.94–0.91 (m, 4H), 0.89 (s, 9H), 0.86–0.83 (m, 2H); $^{13}$C NMR (125 MHz, CDCl$_3$, mixture of carbamate rotamers) δ 172.5, 171.8, 171.7, 171.5, 171.4, 168.2, 157.7, 135.4, 133.2, 133.1, 130.4, 128.2, 117.7, 114.7, 79.7, 79.5, 71.9, 71.8, 70.9, 70.6, 68.8, 63.4, 60.3, 59.8, 51.8, 50.7, 50.6, 49.8, 46.2, 46.0, 40.7, 40.5, 38.6, 37.7, 36.5, 34.8, 33.2, 30.9, 30.8, 30.6, 29.7, 29.5, 29.4, 25.9, 25.9, 25.4, 25.3, 25.0, 24.8, 20.9, 20.9, 16.1, 15.7, 15.2, 15.0, 14.4, 13.5, 13.4, 10.5; IR (neat) 3368, 2929, 1728, 1630, 1508 cm$^{-1}$; HRMS (ESI) m/z for C$_{48}$H$_{76}$N$_{5}$O$_{10}$ [M+H]$^+$ calcd 882.5574, found 882.5587.
Depsipeptide 4.1

To a 0 °C solution of amine 4.8 (8.9 mg, 10.1 µmol) in THF (0.10 mL), H₂O (0.10 mL), and t-BuOH (0.20 mL) was added lithium hydroxide (4.2 mg, 101 µmol). The reaction was warmed to rt and stirred for 6 h before the reaction was concentrated under a stream of argon. The residue was dissolved in CHCl₃ (0.5 mL) and neutralized with aqueous phosphate buffer (pH = 7, 0.3 mL). The separated aqueous phase was extracted with CHCl₃ (10 x 0.5 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The resulting residue was azeotropically dried with toluene twice and then placed under high vacuum for 2 h.

The crude amino acid was dissolved in CH₂Cl₂ (10 mL, 0.001 M) and cooled to 0 °C. To the cooled solution was added i-Pr₂NEt (9 µL, 51 µmol) followed by PyAOP (10.5 mg, 20.2 µmol) and the reaction mixture was warmed to rt. After stirring at rt for 48 h, the reaction mixture was concentrated under reduced pressure. Purification via preparative thin layer chromatography on silica gel (98:2 EtOAc/MeOH, v/v) yielded cyclic depsipeptide 4.1 (3.5 mg, 41% from amine 4.8) as a white amorphous solid:
[α]$_D^{25}$ = −143.3 (c 0.24, MeOH); $^1$H NMR (500 MHz, CDCl$_3$, mixture of carbamate rotamers) δ 7.15 (d, $J = 8.6$ Hz, 2H), 6.81 (d, $J = 8.6$ Hz, 2H), 6.18 (d, $J = 9.1$ Hz, 1H), 6.07–5.99 (m, 1H), 5.96 (d, $J = 9.4$ Hz, 1H), 5.39 (ddd, $J = 17.3$, 1.5 Hz, 1H), 5.27 (dd, $J = 10.6$, 1.4 Hz, 1H), 5.24 (d, $J = 11.7$ Hz, 1H), 5.06 (ddd, $J = 10$, 10, 4.9 Hz, 1H), 4.97 (dd, $J = 13.6$, 2.5, Hz, 1H), 4.78 (ddd, $J = 9$, 9, 5.6 Hz, 1H), 4.69 (d, $J = 10.6$ Hz, 1H), 4.50 (dt, $J = 5$, 1.2 Hz, 2H), 4.34 (dd, $J = 8.7$, 8.7 Hz, 1H), 4.25–4.21 (m, 1H), 4.19–4.15 (m, 2H), 3.69–3.60 (m, 4H), 3.32–3.27 (m 1H), 3.11 (dd, $J = 11.8$ Hz, 1H), 2.86 (dd, $J = 12.8$, 4.9 Hz, 1H), 2.81 (s, 3H), 2.74 (s, 3H), 2.42 (dd, $J = 10.2$, 6.7 Hz, 1H), 2.37–2.32 (m, 1H), 2.27–2.22 (m, 1H), 2.18–2.12 (m, 1H), 2.09–2.04 (m, 1H), 1.96–1.86 (m, 2H), 1.91 (s, 3H), 1.79 (ddd, $J = 13.5$, 13.5, 3.4 Hz, 1H), 1.45–1.39 (m, 2H), 1.22 (d, $J = 6.7$ Hz, 3H), 1.14–1.11 (m, 1H), 1.08–1.02 (m, 1H), 1.06 (d, $J = 6.9$ Hz, 3H), 0.98 (d, $J = 6.6$ Hz, 3H), 0.95 (t, $J = 7.4$ Hz, 3H), 0.99–0.93 (m, 1H), 0.87 (s, 9H); HRMS (ESI) m/z for C$_{47}$H$_{72}$N$_5$O$_9$ [M+H]$^+$ calcd 850.5325, found 850.5329.

**Azide 4.13**

![Chemical Structure](image)

To a rt solution of amine 4.12 (1.51 g, 7.6 mmol) in MeOH (40 mL) were added potassium carbonate (3.5 g, 25 mmol) and CuSO$_4$·5H$_2$O (0.28 g, 1.14 mmol) followed by diazo transfer reagent (2.4 g, 11.4 mmol) portion wise. After stirring for 6 h at rt, the reaction was concentrated under reduced pressure. The residue was dissolved in 50 mL
of H$_2$O and acidified to pH 3 with concentrated HCl. The aqueous layer was extracted with EtOAc (3 x 70 mL) and the combined organic layers were dried over MgSO$_4$, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (2:1 to 1:1 hexanes/EtOAc, v/v) gave azide 4.13 (0.97 g, 68%) as a clear, colorless oil: $R_f$ 0.21 (1:1 hexanes/EtOAc, v/v); $[\alpha]_{D}^{25} = -71.9$ (c 1.18, CHCl$_3$) $^{1}$H NMR (500 MHz, CDCl$_3$) $\delta$ 9.71 (br s, 1H), 4.09 (dd, $J$ = 8.5, 5 Hz, 1H), 3.69 (s, 3H), 2.52–2.49 (m, 2H), 2.26–2.19 (m, 1H), 2.08–2.01 (m, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 175.2, 173.0, 60.8, 52.0, 29.8, 26.4; IR (neat) 3551, 3370, 2117, 1732, 1255 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_6$H$_9$N$_3$NaO$_4$ [M+Na]$^+$ calcd 210.0485, found 210.0487.

**Primary alcohol 4.14**

![Chemical structure](Image)

To a 0 °C of azide 4.13 (2.82 g, 15.1 mmol) in EtOAc (50 mL) was added DCC (2.08 g, 18.1 mmol). After stirring for 5 min, $N$-hydroxysuccinimide (3.7 g, 18.1 mmol) was added in one portion and the reaction was warmed to rt. After stirring at rt for 2 h, the reaction was diluted with EtOAc (50 ml) and filtered through a pad of Celite®. The filtrate was washed with saturated aqueous NaHCO$_3$ (100 mL) and the layers were separated. The aqueous layer was extracted with EtOAc (3 x 50 mL) and the combined organic layers were washed with saturated aqueous NaCl, dried over MgSO$_4$, filtered, and concentrated under reduced pressure.
The resulting oil was dissolved in THF (45 mL) and the solution was cooled to 0 °C. Sodium borohydride (0.60 g, 15.9 mmol) was added in one portion followed by EtOH (15 mL). After stirring for 10 min at 0 °C, the reaction was quenched with saturated aqueous NH$_4$Cl (30 mL) and diluted with EtOAc (50 mL). The aqueous layer was separated and extracted with EtOAc (3 x 50 mL). The combined organic layers were wash with saturated aqueous NaCl, dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (3:1 to 1:1 hexanes/EtOAc, v/v) gave primary alcohol 4.14 (2.2 g, 84% from azide 4.13): R$_f$ 0.34 (1:1 hexanes/EtOAc, v/v); [$\alpha$]$^25_D$ = –19 (c 0.90, CHCl$_3$) $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 3.72–3.66 (m, 1H), 3.65 (s, 3H), 3.60–3.54 (m, 1H), 3.51–3.45 (m, 1H), 2.90 (t, $J$ = 5.2 Hz, 1H), 2.50–2.36 (m, 2H), 1.89–1.80 (m, 1H), 1.75–1.66 (m, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 173.6, 64.9, 63.2, 51.9, 30.2, 25.7; IR (neat) 3418, 2953, 2104, 1732, 1269 cm$^{-1}$; HRMS (ESI) m/z for C$_6$H$_{11}$N$_3$NaO$_3$ [M+Na]$^+$ calcd 196.0693, found 196.0692.

**Silyl ether 4.15**

![Chemical structure](image)

To a 0 °C solution of alcohol 4.14 (0.29 g, 1.67 mmol) in CH$_2$Cl$_2$ (17 mL) were added DMAP (21 mg, 0.17 mmol), imidazole (0.23 g, 3.34 mmol), and TBSCl (0.28 g, 1.84 mmol), sequentially. The reaction was warmed to rt and stirred for 30 min at which saturated aqueous NH$_4$Cl (10 mL) was added. The mixture was diluted with Et$_2$O (40 mL) and the layers were separated. The aqueous layer was extracted with Et$_2$O (3 x 25
mL) and the combined organic layers were washed with saturated aqueous NaCl, dried over MgSO<sub>4</sub>, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (12:1 hexanes/EtOAc, v/v) gave silyl ether 4.15 (0.42 g, 88%) as a clear, colorless oil: R<sub>f</sub> 0.44 (9:1 hexanes/EtOAc, v/v); [α]<sup>25</sup><sub>D</sub> = −38.3 (c 1.39, CHCl<sub>3</sub>)<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 3.72 (dd, <i>J</i> = 10.5, 4 Hz, 1H), 3.65 (s, 3H), 3.60 (dd, <i>J</i> = 10.5, 7 Hz, 1H), 3.41–3.37 (m, 1H), 2.47–2.36 (m, 2H), 1.83–1.76 (m, 1H), 1.68–1.60 (m, 1H), 0.88 (s, 9H), 0.06 (s, 6H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 173.2, 66.3, 62.8, 51.6, 30.4, 25.7, 25.6, 18.2, −5.6; IR (neat) 2953, 2102, 1742, 1437, 1258 cm<sup>−1</sup>; HRMS (ESI) <i>m/z</i> for C<sub>12</sub>H<sub>25</sub>N<sub>3</sub>NaO<sub>3</sub>Si [M+Na]<sup>+</sup> calcd 310.1557, found 310.1553.

**Carboxylic acid 4.11**

![](image)

To a rt solution of silyl ether 4.15 (1.5 g, 5.2 mmol) in t-BuOH (35 mL), THF (9 mL), and H<sub>2</sub>O (9 mL) was added LiOH•H<sub>2</sub>O (2.2 g, 52 mmol). After stirring for 3 h at rt, the reaction was acidified with citric acid and diluted with EtOAc (75 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 x 50 mL). The combined organic layers were dried of Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (6:1 to 4:1 hexanes/EtOAc, v/v) yielded carboxylic acid 4.11 (1.2 g, 86%) as viscous oil that partially solidified in the freezer: R<sub>f</sub> 0.21 (4:1 hexanes/EtOAc, v/v); [α]<sup>25</sup><sub>D</sub> = −37.3 (c 1.44, CHCl<sub>3</sub>)<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.76 (dd, <i>J</i> = 10.4, 4 Hz, 1H), 3.65 (dd, <i>J</i> =
10.4, 6.8 Hz, 1H), 3.48–3.42 (m, 1H), 2.58–2.44 (m, 2H), 1.88–1.80 (m, 1H), 1.73–1.64 (m, 1H), 0.91 (s, 9H), 0.09 (s, 6H); \(^1^C\) NMR (100 MHz, CDCl\(_3\)) \(\delta\) 178.6, 66.32, 62.6, 30.4, 25.4, 18.2, –5.6; IR (neat) 2955, 2101, 1713, 1472 cm\(^{-1}\); HRMS (ESI) \(m/z\) for C\(_{11}\)H\(_{23}\)N\(_3\)NaO\(_3\)Si [M+Na]\(^+\) calcld 296.1401, found 296.1400.

**Triamide 4.16**

![Triamide 4.16](image)

To a 0 °C solution of carbamate 2.3 (0.31 g, 0.58 mmol) in CH\(_2\)Cl\(_2\) (5.8 mL) was added trifluoroacetic acid (5.8 mL) and stirring was continued at 0 °C for 1 h. After 1 h, toluene (5 mL) was added to the reaction mixture and the mixture was concentrated under a stream of argon. Toluene (5 mL) was added again, the mixture was concentrated under a stream of argon, and placed under high vacuum for 1 h.

To a rt solution of carboxylic acid 4.11 (0.15 g, 0.55 mmol) in CH\(_2\)Cl\(_2\) (4 mL) was added \(i\)-Pr\(_2\)NEt (0.29 mL, 1.65 mmol) and PyAOP (0.29 g, 0.55 mmol), sequentially. After 1 min, a solution of crude deprotected 2.3 in CH\(_2\)Cl\(_2\) (2 mL) was added. After stirring for 8 h at rt, the reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (2:1 hexanes/EtOAc, v/v) gave triamide 4.16 (0.31 g, 80% from diamide 2.3) as a white amorphous solid: \(R_f\) 0.27 (2:1 hexanes/EtOAc, v/v); \([\alpha]^{25}_D = –75.2\) (c 1.15, CHCl\(_3\)) \(^1^H\) NMR (400 MHz, CDCl\(_3\)) \(\delta\)
7.09 (d, \( J = 8.8 \) Hz, 2H), 6.79 (d, \( J = 8.8 \) Hz, 2H), 6.25 (d, \( J = 8.8 \) Hz, 1H), 5.40 (q, \( J = 6.8 \) Hz, 1H), 5.18 (dd, \( J = 8.4, 6.8, 6.8 \) Hz, 1H), 4.90 (d, \( J = 10.4 \) Hz, 1H), 3.76 (s, 3H), 3.71 (dd, \( J = 10.4, 3.6 \) Hz, 1H), 3.68 (s, 3H), 3.36 (dd, \( J = 10.4, 6.8 \) Hz, 1H), 3.28–3.22 (m, 1H), 3.02 (dd, \( J = 14, 7.2 \) Hz, 1H), 2.98 (s, 3H), 2.81 (dd, \( J = 15.2, 8 \) Hz, 1H), 2.76 (s, 3H), 2.32–2.16 (m, 2H), 2.00–1.89 (m, 1H), 1.81–1.73 (m, 1H), 1.61–1.52 (m, 1H), 1.27 (d, \( J = 6.8 \) Hz, 3H), 1.28–1.22 (m, 1H), 1.00–0.94 (m, 1H), 0.92 (d, \( J = 6.8 \) Hz, 3H), 0.89 (s, 9H), 0.84 (t, \( J = 7.6 \) Hz, 3H), 0.06 (s, 6H); \(^{13}\text{C} \text{NMR} \ (100 \text{ MHz}, \text{CDCl}_3) \ \delta \ 171.9, 171.5, 171.2, 158.7, 130.3, 130.2, 127.9, 113.9, 66.5, 62.7, 60.3, 55.2, 51.8, 50.2, 49.7, 37.9, 33.2, 32.5, 30.9, 30.5, 25.9, 25.8, 24.9, 18.2, 15.7 14.3, 10.5, −5.6; IR (neat) 3301, 2928, 2150, 1747, 1652 cm\(^{-1}\); HRMS (ESI) \( m/z \) for C\(_{33}\)H\(_{56}\)N\(_6\)NaO\(_7\)Si [M+Na]\(^+\) calcd 699.3872, found 699.3871.

**Primary alcohol 4.17**

To a 0 °C solution of triamide 4.16 (0.24 g, 0.35 mmol) in MeOH (3.5 mL) was added TsOH•H\(_2\)O (9.5 mg, 0.05 mmol) and the reaction was warmed to rt. After stirring for 1 h at rt, Et\(_3\)N (~0.07 mmol) was added to quench the reaction. The reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (95:5 CHCl\(_3\)/MeOH, v/v) to give alcohol 4.17 (0.19 g, 94%) as a white amorphous solid: \( R_f \) 0.40 (14:1 CHCl\(_3\)/MeOH, v/v); \([\alpha]^{25}_D = −82.2 \) (c 0.90, CHCl\(_3\)) \(^1\text{H}\)
NMR (400 MHz, CDCl$_3$) δ 7.08 (d, $J = 8.8$ Hz, 2H), 6.79 (d, $J = 8.8$ Hz, 2H), 6.48 (d, $J = 8.4$ Hz, 1H), 5.39 (q, $J = 6.8$ Hz, 1H), 5.17 (ddd, $J = 8.4, 7.2, 7.2$ Hz, 1H), 4.89 (d, $J = 10.4$ Hz, 1H), 3.75 (s, 3H), 3.67 (s, 3H), 3.60 (m, 1H), 3.52–3.48 (m, 1H), 3.32–3.26 (m, 1H), 3.03 (dd, $J = 14, 6.8$ Hz, 1H), 2.98 (s, 3H), 2.79 (dd, $J = 12, 4.8$ Hz, 1H), 2.76 (s, 3H), 2.35–2.18 (m, 2H), 2.02–1.92 (m, 1H), 1.88–1.80 (m, 2H), 1.73–1.60 (m, 1H), 1.27–1.21 (m, 1H), 1.27 (d, $J = 7.2$ Hz, 3H), 0.99–0.93 (m, 2H), 0.91 (d, $J = 6.4$ Hz, 3H), 0.83 (t, $J = 7.2$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 171.8, 171.6, 171.6, 171.5, 158.7, 130.4, 127.8, 114.0, 64.6, 62.9, 60.4, 55.2, 51.9, 50.3, 49.8, 37.7, 33.2, 31.9, 30.9, 30.6, 25.7, 24.9, 15.7, 14.3, 10.5; IR (neat) 3306, 2965, 2099, 1732, 1614 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_{27}$H$_{42}$N$_6$O$_7$ [M+Na]$^+$ calcd 585.3007, found 585.3009.

**Amino alcohol 4.10**

![Chemical structure](image)

To a 0 °C solution of alcohol 4.17 (0.15 g, 0.26 mmol) in a mixture of THF (3 mL) and H$_2$O (0.06 mL) was added PBu$_3$ (0.13 mL, 0.52 mmol) dropwise. After warming to rt and stirring for 1 h, the reaction was heat under microwave irradiation at 55 °C for 30 min at which the solution became nearly colorless. The reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (96:4 to 9:1 CHCl$_3$/MeOH, v/v) gave amino alcohol 4.10 (0.11 g, 79%) as a white amorphous solid: $R_f$ 0.10 (9:1 CHCl$_3$/MeOH, v/v); $[\alpha]^{25}_D = -102.9$ (c 1.28, CHCl$_3$)
$^1$H NMR (500 MHz, CDCl$_3$) δ 7.09 (d, $J = 8.5$ Hz, 2H), 6.78 (d, $J = 8.5$ Hz, 2H), 5.38 (q, $J = 7$ Hz, 1H), 5.14 (ddd, $J = 7.5$ Hz, 1H), 4.88 (d, $J = 10.5$ Hz, 1H), 3.75 (s, 3H); 3.67 (s, 3H), 3.51 (dd, $J = 11$, 4 Hz, 1H), 3.28 (dd, $J = 11$, 7 Hz, 1H), 3.00 (dd, $J = 14.5$, 7.5 Hz, 1H), 2.97 (s, 3H), 2.83–2.78 (m, 2H), 2.72 (s, 3H), 2.34–2.22 (m, 2H), 1.96–1.90 (m, 1H), 1.74 (dddd, $J = 13$, 7.5, 7.5, 7.5 Hz, 1H), 1.55 (dddd, $J = 14.5$, 7, 7, 7 Hz, 1H), 1.27–1.20 (m, 1H), 1.25 (d, $J = 7$ Hz, 3H), 0.97–0.93 (m, 2H), 0.91 (d, $J = 6.5$ Hz, 3H), 0.83 (t, $J = 7.5$ Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 172.3, 171.7, 171.7, 171.5, 158.7, 130.4, 128.0, 113.9, 66.2, 60.3, 55.2, 52.5, 51.8, 50.4, 49.8, 37.6, 33.2, 32.7, 30.9, 30.6, 28.9, 24.9, 15.7, 14.3, 10.5; IR (neat) 3292, 2965, 1738, 1614, 1248 cm$^{-1}$; HRMS (ESI) m/z for C$_{27}$H$_{45}$N$_4$O$_7$ [M+H]$^+$ calcd 537.3283, found 537.3281.

**Amide 4.19**

![Diagram of Amide 4.19]

To a rt solution of carboxylic acid 3.2 (51.7 mg, 0.12 mmol) in CH$_2$Cl$_2$ (1.8 mL) was added i-Pr$_2$NET (63 µL, 0.36 mmol), followed by HATU (48 mg, 0.126 mmol). Immediately after HATU addition, a solution of amino alcohol 4.10 (70 mg, 0.13 mmol) in CH$_2$Cl$_2$ (0.4 mL) was added. The resulting yellow solution was stirred at rt for 12 h, at
which the reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (98:2 to 96:4 CHCl₃/MeOH, v/v) gave amide 4.19 (0.10 g, 90%) as a white foam: Rᵋ 0.18 (96:4 CHCl₃/MeOH, v/v); [α]ᵋ²⁵_D = −78.3 (c 0.81, CHCl₃) ¹H NMR (500 MHz, CDCl₃, mixture of carbamate rotamers) δ 7.11 (d, J = 8.5 Hz, 2H), 6.78 (d, J = 8.5 Hz, 2H), 5.40 (q, J = 7 Hz, 1H), 5.15 (ddd, J = 7 Hz, 1H), 4.89 (d, J = 10.5 Hz, 1H), 4.84 (d, J = 10 Hz, 1H), 4.29 (dd, J = 9, 4 Hz, 1H), 3.84–3.79 (m, 1H), 3.76 (s, 3H), 3.71 (dd, J = 7 Hz, 1H), 3.67 (s, 3H), 3.64–3.62 (m, 2H), 3.58–3.53 (m, 1H), 3.53–3.47 (m, 1H), 3.41–3.37 (m, 2H), 3.31–3.29 (m, 1H), 3.17 (ddd, J = 7.5, 7.5, 7.5 Hz, 1H), 3.01 (dd, J = 13.5, 7 Hz, 1H), 2.95 (s, 3H), 2.85–2.79 (m, 2H), 2.69 (s, 3H), 2.30–2.15 (m, 2H), 2.05–1.98 (m, 2H), 1.95–1.86 (m, 2H), 1.82–1.75 (m, 2H), 1.75–1.67 (m, 1H), 1.63–1.57 (m, 2H), 1.48–1.41 (m, 3H), 1.44 (s, 9H), 1.37–1.31 (m, 1H), 1.26–1.20 (m, 1H), 1.25 (d, J = 7 Hz, 3H), 1.16 (d, J = 7 Hz, 3H), 1.13–1.08 (m, 1H), 0.94–0.92 (m, 5H), 0.88 (s, 9H), 0.83 (t, J = 7.5 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃, mixture of carbamate rotamers) δ 176.8, 172.9, 172.3, 171.7, 171.5, 158.6, 154.7, 130.4, 130.3, 128.2, 113.9, 80.2 78.6, 71.4, 64.8, 60.4, 59.1, 55.7, 55.2, 51.8, 51.4, 50.5, 49.8, 47.6, 46.7, 43.6, 40.4, 37.6, 37.4, 34.7, 33.2, 32.6, 30.9, 30.5, 30.0, 28.5, 25.9, 24.9, 24.2, 20.4, 15.7, 14.3, 12.5, 10.5; IR (neat) 3408, 2967, 1732, 1634, 1514 cm⁻¹; HRMS (ESI) m/z for C₅₀H₈₃N₅NaO₁₃ [M+Na]⁺ calcd 984.5880, found 984.5881.
Oxazoline 4.20

To a –78 °C solution of alcohol 4.19 (0.102 g, 0.106 mol) in CH₂Cl₂ (2 mL) was added DAST (14 µL, 0.11 mmol) dropwise. After stirring for 1 h at –78 °C, additional DAST (14 µL, 0.11 mmol) was added and stirring continued for 30 min. The reaction was quenched with aqueous saturated NaHCO₃ (1.5 mL) at –78 °C and the solution was warmed to rt. The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (10 x 5 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (97:3 CHCl₃/MeOH, v/v) yielded oxazoline 4.20 (86 mg, 86%) as an amorphous white solid: Rᶠ 0.23 (96:4 CHCl₃/MeOH, v/v); [α]²⁵D = –115.0 (c 1.08, CHCl₃) ¹H NMR (500 MHz, CDCl₃, mixture of carbamate rotamers) δ 7.10 (d, J = 8.5 Hz, 2H), 6.75 (d, J = 8.5 Hz, 2H), 5.38 (q, J = 6.5 Hz, 1H), 5.18–5.09 (m, 1H), 4.88–4.77 (m, 2H), 4.31 (dd, J = 8.5, 3.5 Hz, 1H), 4.23–4.11 (m, 1H), 3.97–3.91 (m, 1H), 3.79–3.76 (m, 1H), 3.74 (s, 3H), 3.70–3.61 (m, 1H), 3.66 (s, 3H), 3.49–3.45 (m, 2H), 3.39–3.33 (m, 1H), 3.00–2.97 (m, 1H), 2.94 (s, 3H), 2.78 (dd, J = 13.5, 7 Hz, 1H), 2.60 (s, 3H), 2.49 (t, J = 6.5 Hz, 1H), 2.35–2.14 (m, 3H), 2.05–1.99 (m, 1H), 1.94–1.83 (m, 5H), 1.73–1.56 (m, 3H), 1.47–1.39 (m, 1H), 1.43 (s, 9H), 1.33–1.27 (m, 1H), 1.26–1.23 (m, 1H), 1.23 (d, J =
7 Hz, 3H), 1.15 (d, J = 7 Hz, 3H), 1.07–0.99 (m, 1H), 0.97–0.93 (m, 4H), 0.89 (d, J = 6.5 Hz, 3H), 0.86 (s, 9H), 0.82 (t, J = 7 Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$, mixture of carbamate rotamers) δ 172.8, 172.3, 171.7, 171.3, 158.6, 154.2, 130.3, 128.3, 113.8, 113.8, 79.9, 78.2, 71.7, 71.5, 70.7, 64.7, 60.3, 59.6, 59.1, 55.6, 55.2, 51.8, 50.3, 50.2, 49.8, 46.6, 46.2, 41.0, 40.9, 40.4, 40.2, 39.4, 38.1, 37.8, 37.7, 37.6, 34.7, 33.2, 31.0, 30.9, 30.8, 30.5, 28.5, 28.4, 25.1, 24.9, 24.3, 23.3, 20.7, 20.5, 15.7, 14.9, 14.6, 14.2, 10.5; IR (neat) 3466, 2967, 1738, 1634, 1514 cm$^{-1}$; HRMS (ESI) m/z for C$_{50}$H$_{81}$N$_5$NaO$_{12}$ [M+Na]$^+$ calcd 966.5774, found 966.5773.

**Amine 4.21**

![Chemical structure of 4.20 and 4.21](image)

To a 0 °C solution of oxazoline 4.20 (86 mg, 91 µmol) in CH$_2$Cl$_2$ (1.0 mL) was added 2,6-lutidine (85 µL, 0.73 mmol) followed by trimethylsilyl trifluoromethanesulfonate (83 µL, 0.46 mmol) dropwise. The reaction was warmed to rt and stirred for 1 h. After cooling to 0 °C, saturated aqueous NaHCO$_3$ (1.0 mL) was added and the mixture was warmed back to rt. The aqueous layer was extracted with CHCl$_3$ (10 x 1 mL) and the combined organic layers were dried over Na$_2$SO$_4$, filtered,
and concentrated under reduced pressure. The residual 2,6-lutidine was removed under a stream of argon and the crude residue was placed under high vacuum for 1 h.

To a 0 °C solution of crude trimethylsilyl ether in THF (1.0 mL) was added tetrabutylammonium fluoride (0.27 mL, 0.27 mmol, 1 M solution in THF). After stirring at 0 °C for 30 min, saturated aqueous NaHCO₃ (1.0 mL) was added and the mixture was warmed to rt. The aqueous layer was extracted with CHCl₃ (10 x 1 mL) and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (98:2 to 95:5 CHCl₃/MeOH, v/v) yielded amine 4.21 (61 mg, 79% from oxazoline 4.20) as a light yellow amorphous solid: Rᵥ 0.12 (9:1 CHCl₃/MeOH, v/v); [α]²⁵ºD = −117 (c 0.88, CHCl₃); ¹H NMR (500 MHz, CDCl₃, mixture of rotamers) δ 7.19 (d, J = 8.5 Hz, 2H), 6.77 (d, J = 8.5 Hz, 2H), 5.76 (dd, J = 9.5, 6 Hz, 1H), 5.53 (q, J = 6.5 Hz, 1H), 4.91 (d, J = 11.5 Hz, 1H), 4.81 (d, J = 10 Hz, 1H), 4.20 (m, 2H), 3.75 (s, 3H), 3.66 (s, 3H), 3.51 (dd, J = 12.5, 4.5 Hz, 1H), 3.36 (dd, J = 12.5, 7 Hz, 1H), 3.24–3.21 (m, 4H), 2.79 (s, 3H), 2.61–2.54 (m, 1H), 2.46 (s, 3H), 2.35–2.27 (m, 1H), 2.34–2.20 (m, 1H), 2.01–1.84 (m, 6H), 1.66–1.57 (m, 6H), 1.46–1.39 (m, 3H), 1.37–1.31 (m, 1H), 1.23 (d, J = 7 Hz, 3H), 1.16 (d, J = 7 Hz, 3H), 1.01–0.99 (m, 3H), 0.92–0.89 (m, 2H), 0.92 (d, J = 6 Hz, 3H), 0.88 (s, 9H), 0.79 (t, J = 7 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃, mixture of rotamers) δ 175.6, 171.5, 171.4, 170.1, 169.6, 168.9, 158.2, 130.7, 130.6, 130.5, 128.8, 113.8, 113.7, 79.9, 70.9, 63.6, 60.5, 59.8, 59.7, 58.8, 55.2, 53.8, 52.0, 51.8, 47.5, 46.3, 43.7, 40.2, 36.9, 36.9, 34.6, 34.3, 33.7, 33.3, 30.7, 30.1, 29.8, 29.6, 29.5, 29.4, 26.1, 25.9, 25.9, 25.6, 25.5, 24.9, 24.8, 24.5, 24.0, 20.8, 19.7, 15.8, 15.7, 14.5, 13.6, 11.3, 10.4; IR (neat) 3306,
2965, 1738, 1634, 1248 cm\(^{-1}\); HRMS (ESI) \(m/z\) for C\(_{45}\)H\(_{74}\)N\(_5\)O\(_{10}\) [M+H]\(^+\) calcd 844.5430 found 844.5429.

**Depsipeptide 4.9**

![Diagram of Depsipeptide 4.9](image)

To a 0 °C solution of amine 4.21 (5.7 mg, 6.8 µmol) in THF (30 µL), and \(t\)-BuOH (70 µL) was added a solution of lithium hydroxide (68 µL, 68 µmol, 1 M in H\(_2\)O). The reaction was warmed to rt and stirred for 6 h before the reaction was concentrated under a stream of argon. The residue was dissolved in CHCl\(_3\) (0.2 mL) and neutralized with aqueous phosphate buffer (pH = 7, 0.2 mL). The separated aqueous phase was extracted with CHCl\(_3\) (10 x 0.5 mL). The combined organic layers were dried over Na\(_2\)SO\(_4\), filtered, and concentrated under reduced pressure. The resulting residue was azeotropically dried with toluene twice and then placed under high vacuum for 2 h.

The crude amino acid was dissolved in CH\(_2\)Cl\(_2\) (6.8 mL, 0.001 M) and cooled to 0 °C. To the cooled solution was added \(i\)-Pr\(_2\)NEt (3.6 µL, 20.4 µmol) followed by PyAOP (3.7 mg, 20.4 µmol) and the reaction mixture was warmed to rt. After stirring at rt for 24 h, the reaction mixture was concentrated under reduced pressure. Purification
via preparative thin layer chromatography on silica gel (95:5 EtOAc/MeOH, v/v) yielded cyclic depsipeptide \textbf{4.9} (2.8 mg, 51% from amine \textbf{4.21}) as a white amorphous solid: $[\alpha]_{D}^{25} = -107.9$ (c 0.24, MeOH); $^1$H NMR (500 MHz, CDCl$_3$, mixture of carbamate rotamers) $\delta$ 7.15 (d, $J = 8.5$ Hz, 2H), 6.81 (d, $J = 8.5$ Hz, 2H), 5.71 (dd, $J = 8.9$, 6.4 Hz, 1H), 5.21 (d, $J = 6.4$ Hz, 1H), 5.03 (q, $J = 6.9$ Hz, 1H), 4.61 (d, $J = 12$ Hz, 1H), 4.52 (d, $J = 11$ Hz, 1H), 4.48 (d, $J = 7.3$ Hz, 1H), 3.86–3.82 (m, 1H), 3.77 (s, 3H), 3.66–3.65 (m, 1H), 3.58–3.51 (m, 2H), 3.47 (d, $J = 12$ Hz, 1H), 3.35 (dt, $J = 12$, 4, 4 Hz, 1H), 3.25–3.21 (m, 1H), 3.14 (dd, $J = 14$, 6.8 Hz, 1H), 3.09 (s, 3H), 3.07 (s, 3H), 2.97–2.88 (m, 1H), 2.66 (dd, $J = 8.8$, 7.0 Hz, 1H), 2.48–2.45 (m, 1H), 2.32 (dd, $J = 10.5$, 3.2 Hz, 1H), 2.78–2.18 (m, 2H), 2.00–1.89 (m, 2H), 1.87–1.82 (m, 2H), 1.72–1.67 (m, 2H), 1.59 (d, $J = 6.9$ Hz, 3H), 1.49–1.47 (m, 2H), 1.36–1.31 (m, 2H), 1.15–1.07 (m, 2H), 1.10 (d, $J = 6.8$ Hz, 3H), 0.99 (d, $J = 6.4$ Hz, 3H), 0.95–0.91 (m, 3H), 0.87 (s, 9H), 0.83 (t, $J = 6.6$ Hz, 3H); HRMS (ESI) $m/z$ for $\text{C}_{44}\text{H}_{69}\text{N}_5\text{NaO}_9$ [M+Na]$^+$ calcd 834.4987, found 834.4988.

**Primary alcohol 4.27**

![Chemical structure](image)

To a $-78$ °C solution of ester \textbf{4.15} (1.6 g, 5.6 mmol) in CH$_2$Cl$_2$ (50 mL) was added DIBAI-H (12.3 mL, 12.3 mmol, 1 M solution in toluene) dropwise down the side of the flask. After stirring for 30 min at $-78$ °C, the solution was warmed to 0 °C and stirred for an additional 1.5 h. The reaction was quenched with MeOH and a saturated aqueous Na/K tartrate solution (50 mL) was added. After warming to rt, Et$_2$O (100 mL)
was added and the cloudy mixture was stirred until the layers were clear and colorless (~1 h). The separated aqueous layer was extracted with Et₂O (3 x 50 mL) and the combined organic layers were dried over MgSO₄, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (2:1 hexanes/EtOAc, v/v) yielded alcohol **4.27** (1.21 g, 83%) as a clear, colorless oil: R₇ 0.35 (2:1 hexanes/EtOAc, v/v); [α]²⁵/D = −24.5 (c 0.98, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 3.72 (dd, J = 10.5, 3.5 Hz, 1H), 3.65–3.59 (m, 3H), 3.39–3.34 (m, 1H), 1.96 (br s, 1H), 1.72–1.65 (m, 1H), 1.64–1.53 (m, 2H), 1.49–1.41 (m, 1H), 0.89 (s, 9H), 0.07 (s, 6H); ¹³C NMR (125 MHz, CDCl₃) δ 66.5, 63.6, 62.3, 29.2, 26.8, 25.8, 18.2, −5.5; IR (neat) 3348, 2106, 1472, 1254 cm⁻¹; HRMS (ESI) m/z for C₁₁H₂₅N₃NaO₂Si [M+Na]⁺ calcd 282.1608 found 282.1603.

**Primary alcohol 4.29**

![Chemical structure](image)

To a −78 °C solution of CH₂Cl₂ (14 mL) and DMSO (1.2 mL, 16.8 mmol) was added oxalyl chloride (0.72 mL, 8.4 mmol) dropwise. After 15 min, a solution of alcohol **4.27** (1.1 g, 4.2 mmol) in CH₂Cl₂ (4.2 mL) was added dropwise and stirring was continued at −78 °C for 45 min, at which Et₃N (4.7 mL, 33.6 mmol) was added. After slowly warming to rt, the mixture was quenched with saturated aqueous NH₄Cl (10 mL) and the resulting mixture was extracted with Et₂O (3 x 30 mL). The combined organic
layers were washed with saturated aqueous NaCl (10 mL), dried over MgSO$_4$, filtered, and concentrated under reduced pressure to give a crude aldehyde that was taken on to the next step without further purification.

To a 0 °C solution of Ph$_3$PCH$_3$Br (2.1 g, 5.9 mmol) in THF (30 mL) was added NaHMDS (5.0 mL, 5.04 mmol, 1 M solution in THF). After stirring for 1 h at 0 °C, a solution of the crude aldehyde (ca. 4.2 mmol) in THF (10 mL) was added dropwise and the reaction mixture was warmed to rt for 1 h. The reaction was quenched with saturated aqueous NH$_4$Cl (20 mL) and diluted with Et$_2$O (20 mL). The separated aqueous layer was extracted with Et$_2$O (3 x 30 mL) and the combined organic layers were washed with saturated aqueous NaCl, dried over MgSO$_4$, filtered, and concentrated under reduced pressure to give a crude olefin that was taken on to the next step without further purification.

To a 0 °C solution of the crude olefin (ca. 4.2 mmol) in THF (20 mL) was added BH$_3$•Me$_2$S (0.6 mL, 6.3 mmol) dropwise. The reaction was stirred for 1.5 h at 0 °C and then carefully quenched with 3N NaOH (10 mL) and H$_2$O$_2$ (10 mL, 30% aqueous solution). The resulting solution was stirred overnight at rt. The reaction mixture was diluted with Et$_2$O (20 mL) and the layers were separated. The resulting aqueous layer was extracted with additional Et$_2$O (3 x 20 mL) and the combined organic layers were washed with saturated aqueous NaCl, dried over MgSO$_4$, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (4:1 to 3:1 hexanes/EtOAc, v/v) produced alcohol 4.29 (0.54 g, 47% from alcohol 4.27) as a clear, colorless oil: R$_f$ 0.36 (2:1 hexanes/EtOAc, v/v); [$\alpha$]$^\text{D}{25} = -21.8$ (c 1.18, CHCl$_3$); $^1$H NMR
(500 MHz, CDCl$_3$) $\delta$ 3.71 (dd, $J = 10.5$, 4 Hz, 1H), 3.65–3.59 (m, 3H), 3.33 (dddd, $J = 8$, 8, 4, 4 Hz, 1H), 1.67 (br s, 1H), 1.61–1.40 (m, 6H), 0.90 (s, 9H), 0.07 (s, 6H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 66.4, 63.8, 62.6, 32.4, 30.1, 25.8, 22.4, 18.2, $-5.5$; IR (neat) 3347, 2947, 2101, 1462 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_{12}$H$_{27}$N$_3$NaO$_2$Si [M+Na]$^+$ calcd 296.1765 found 296.1762.

**Carboxylic acid 4.26**

![Chemical structure of carboxylic acid 4.26]

To a rt solution of alcohol 4.29 (0.42 g, 1.5 mmol) in a mixture of CH$_3$CN (10 mL) and H$_2$O (5 mL) was added BAIB (1.06, 3.3 mmol) followed by TEMPO (23 mg, 0.15 mmol). After 4 h at rt, the reaction mixture was quenched with saturated aqueous NH$_4$Cl (10 mL) and diluted with EtOAc (20 mL). The separated aqueous layer was extracted with EtOAc (4 x 20 mL) and the combined organic layers were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (2:1 hexanes/EtOAc, v/v) yielded carboxylic acid 4.26 (0.38 g, 88%) as a viscous oil that partially solidified in the freezer: R$_f$ 0.31 (2:1 hexanes/EtOAc, v/v); $[\alpha]^{25}_{D} = -25.5$ (c 1.1, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 3.73 (dd, $J = 10.5$, 4 Hz, 1H), 3.63 (dd, $J = 10.5$, 7 Hz, 1H), 3.35 (m, 1H), 2.40 (t, $J = 7.5$ Hz, 2H), 1.81 (m, 1H), 1.70 (m, 1H), 1.54 (m, 1H), 1.45 (m, 1H), 0.91 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 179.4, 66.3, 63.4, 33.6, 29.7, 25.8, 21.3,
18.2, –5.5; IR (neat) 3040, 2114, 1720, 1464 cm\(^{-1}\); HRMS (ESI) \(m/z\) for \(\text{C}_{12}\text{H}_{25}\text{N}_{3}\text{NaO}_{3}\text{Si}^{+}\) calcld 310.1557 found 310.1554.

**Triamide 4.30**

![Diagram of triamide 4.30]

To a 0 °C solution of carbamate 2.3 (0.21 g, 0.41 mmol) in \(\text{CH}_2\text{Cl}_2\) (4.1 mL) was added trifluoroacetic acid (4.1 mL) and stirring was continued at 0 °C for 1 h. After 1 h, toluene (5 mL) was added to the reaction mixture and the mixture was concentrated under a stream of argon. Toluene (5 mL) was added again, the mixture was concentrated under a stream of argon, and placed under high vacuum for 1 h.

To a rt solution of acid 4.26 (0.13 g, 0.45 mmol) in \(\text{CH}_2\text{Cl}_2\) (3 mL) was added \(i\)-Pr\(_2\)NEt (0.21 mL, 1.23 mmol) and PyAOP (0.26 g, 0.47 mmol), sequentially. After 1 min, a solution of crude deprotected 2.3 in \(\text{CH}_2\text{Cl}_2\) (1 mL) was added and the solution turned yellow. After stirring for 8 h at rt, the reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (2:1 hexanes/EtOAc, v/v) gave triamide 4.30 (0.20 g, 71% from diamide 2.3) as a white amorphous solid: \(R_f\) 0.30 (2:1 hexanes/EtOAc, v/v); \([\alpha]^{25}\text{D} = -92.6\) (c 0.90, CHCl\(_3\)); \(^1\text{H}\) NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.08 (d, \(J = 8.5\) Hz, 2H), 6.79 (d, \(J = 8.5\) Hz, 2H), 6.13 (d, \(J = 8.5\) Hz, 1H), 5.39 (q, \(J = 13.5\), 6.5 Hz, 1H), 5.18 (ddd, \(J = 7, 7, 7\) Hz, 1H), 4.91 (d, \(J = 10.5\) Hz, 1H), 3.76 (s,
3H), 3.70–3.64 (m, 1H), 3.68 (s, 3H), 3.57 (dd, $J = 10.5, 7$ Hz, 1H), 3.32–3.27 (m, 1H), 3.02 (dd, $J = 14, 7.5$ Hz, 1H), 2.97 (s, 3H), 2.82–2.77 (m, 1H), 2.76 (s, 3H), 2.16 (ddd, $J = 8, 8, 2$ Hz, 1H), 1.98–1.92 (m, 1H), 1.76–1.68 (m, 1H), 1.66–1.57 (m, 1H), 1.45–1.38 (m, 1H), 1.37–1.30 (m, 1H), 1.27 (d, $J = 7$ Hz, 3H), 1.00–0.95 (m, 2H), 0.92 (d, $J = 6.5$ Hz, 3H), 0.90 (s, 9H), 0.85 (t, $J = 7.5$ Hz, 3H), 0.07 (s, 6H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 171.8, 171.6, 171.6, 171.5, 158.7, 130.4, 127.9, 113.9, 66.4, 63.6, 60.3, 55.2, 51.8, 50.1, 49.7, 37.8, 35.9, 33.3, 30.9, 30.6, 29.8, 25.8, 24.9, 22.1, 18.2, 15.7, 14.3, 10.6, –5.5; IR (neat) 3304, 2955, 2104, 1740, 1635 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_{34}$H$_{58}$N$_6$NaO$_7$Si [M+Na]$^+$ calcd 713.4028, found 713.4028.

**Primary alcohol 4.31**

![Diagram](image)

To a 0 °C solution of triamide 4.30 (0.23 g, 0.33 mmol) in MeOH (3.3 mL) was added TsOH•H$_2$O (9.5 mg, 0.05 mmol) and the reaction was warmed to rt. After stirring for 1 h at rt, Et$_3$N (~0.07 mmol) was added to quench the reaction. The reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (95:5 CHCl$_3$/MeOH, v/v) to give alcohol 4.31 (0.18 g, 92%) as a white amorphous solid: $R_f$ 0.39 (14:1 CHCl$_3$/MeOH, v/v); $[\alpha]_{D}^{25}$ = –77.8 (c 0.95, CHCl$_3$) $^1$H NMR (500 MHz, CDCl$_3$) δ 7.08 (d, $J = 8.5$ Hz, 2H), 6.79 (d, $J = 8.5$ Hz, 2H), 6.30 (d, $J = 8.5$ Hz, 1H), 5.38 (q, $J = 6.5$ Hz, 1H), 5.17 (ddd, $J = 7, 7$, 7 Hz, 1H), 4.90 (d, $J = 10.5$ Hz, 1H), 3.02 (s, 3H), 2.76 (s, 3H), 2.16 (ddd, $J = 8, 8, 2$ Hz, 1H), 1.98–1.92 (m, 1H), 1.76–1.68 (m, 1H), 1.66–1.57 (m, 1H), 1.45–1.38 (m, 1H), 1.37–1.30 (m, 1H), 1.27 (d, $J = 7$ Hz, 3H), 1.00–0.95 (m, 2H), 0.92 (d, $J = 6.5$ Hz, 3H), 0.90 (s, 9H), 0.85 (t, $J = 7.5$ Hz, 3H), 0.07 (s, 6H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 171.8, 171.6, 171.6, 171.5, 158.7, 130.4, 127.9, 113.9, 66.4, 63.6, 60.3, 55.2, 51.8, 50.1, 49.7, 37.8, 35.9, 33.3, 30.9, 30.6, 29.8, 25.8, 24.9, 22.1, 18.2, 15.7, 14.3, 10.6, –5.5; IR (neat) 3304, 2955, 2104, 1740, 1635 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_{34}$H$_{58}$N$_6$NaO$_7$Si [M+Na]$^+$ calcd 713.4028, found 713.4028.
1H), 3.76 (s, 3H), 3.68 (s, 3H), 3.66–3.64 (m, 1H), 3.58–3.54 (m, 1H), 3.41–3.36 (m, 1H), 3.03–2.99 (m, 1H), 2.98 (s, 3H), 2.80 (dd, $J = 3.5$ Hz, 1H), 2.76 (s, 3H), 2.70–2.61 (m, 1H), 2.17 (t, $J = 7$ Hz, 2H), 1.97–1.90 (m, 1H), 1.76–1.69 (m, 1H), 1.65–1.58 (m, 1H), 1.52–1.45 (m, 1H), 1.44–1.38 (m, 1H), 1.28–1.22 (m, 1H), 1.27 (d, $J = 7$ Hz, 3H), 0.99–0.94 (m, 1H), 0.92 (d, $J = 6.5$ Hz, 3H), 0.84 (t, $J = 7$ Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 171.8, 171.7, 171.5, 158.7, 130.4, 130.2, 127.9, 114.0, 64.9, 63.8, 60.4, 55.2, 51.9, 50.2, 49.8, 37.7, 35.7, 33.3, 30.9, 30.6, 29.8, 25.0, 21.7, 15.7, 14.3, 10.5; IR (neat) 3306, 2963, 2104, 1732, 1643 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_{28}$H$_{44}$N$_6$NaO$_7$ [M+Na]$^+$ calcd 599.3164, found 599.3161.

**Amino alcohol 4.25**

![Chemical structure of azide 4.31 and amino alcohol 4.25]

To a argon flushed solution of azide 4.31 (0.16 g, 0.27 mmol) was added 10% Pd/C (30 mg, 20% by weight of azide). A balloon of H$_2$ was bubbled through the solution and then the reaction was placed under 1 atm of H$_2$ for 24 h. After completion, the flask was flushed with argon and the heterogeneous mixture was filtered through a pad of Celite®. The filtrate was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (9:1 CHCl$_3$/MeOH, v/v) yielded amine 4.25 (0.13 g, 87%) as a white amorphous solid: $R_f$ 0.08 (9:1 CHCl$_3$/MeOH, v/v); $[\alpha]^25_D = -94.9$ (c 1.2, CHCl$_3$) $^1$H NMR (500 MHz, CDCl$_3$) δ 7.07 (d, $J = 8.5$ Hz, 2H), 6.77 (d, $J =$ 161
8.5 Hz, 2H), 6.54 (d, J = 7.5 Hz, 1H), 5.36 (q, J = 7 Hz, 1H), 5.17 (ddd, J = 7, 7, 7 Hz, 1H), 4.88 (d, J = 10.5 Hz, 1H), 3.74 (s, 3H), 3.66 (s, 3H), 3.52 (dd, J = 10.5, 3.5 Hz, 1 H), 3.28–3.24 (m, 1H), 2.99 (dd, J = 11, 7 Hz, 1H), 2.96 (s, 3H), 2.82–2.77 (m, 2H), 2.70 (s, 3H), 2.56–2.46 (m, 1H), 2.21–2.09 (m, 2H), 1.95–1.89 (m, 1H), 1.70–1.64 (m, 1H), 1.62–1.55 (m, 1H), 1.41–1.33 (m, 2H), 1.27–1.19 (m, 2H), 1.24 (d, J = 6.5 Hz, 3H), 0.95–0.92 (m, 2H), 0.90 (d, J = 6.5 Hz, 3H), 0.82 (t, J = 7 Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 172.2, 171.7, 171.5, 158.6, 130.4, 128.0, 113.9, 66.4, 60.3, 55.2, 52.5, 51.8, 50.2, 49.8, 37.7, 36.0, 33.3, 33.2, 30.9, 30.6, 25.0, 21.9, 15.7, 14.3, 10.5; IR (neat) 3345, 2962, 1738, 1657, 1514 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_{28}$H$_{47}$N$_4$O$_7$ [M+H]$^+$ calcd 551.3439, found 551.3435.

**Amide 4.32**

To a rt solution of carboxylic acid 3.2 (69 mg, 0.155 mmol) in CH$_2$Cl$_2$ (1 mL) was added $i$-Pr$_2$NEt (80 $\mu$L, 0.47 mmol), followed by HATU (62 mg, 0.16 mmol). After approximately 1 min, a solution of amino alcohol 4.25 (94 mg, 0.17 mmol) in CH$_2$Cl$_2$ (1 mL) was added. The resulting yellow solution was stirred at rt for 12 h, at which the
reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (98:2 to 96:4 CHCl₃/MeOH, v/v) gave amide 4.32 (0.14 g, 91%) as a white foam: Rₓ 0.20 (96:4 CHCl₃/MeOH, v/v); [α]₂⁵³ₒ = −85.0 (c 0.92, CHCl₃)

¹H NMR (500 MHz, CDCl₃, mixture of carbamate rotamers) δ 7.09 (d, J = 8.5 Hz, 2H), 6.78 (d, J = 8.5 Hz, 2H), 6.49 (d, J = 8 Hz, 1H), 6.36 (d, J = 8.5 Hz, 1H), 5.38 (q, J = 7 Hz, 1H), 5.13 (ddd, J = 7.5, 7.5, 7.5 Hz, 1H), 4.89 (d, J = 10.5 Hz, 1H), 4.83 (d, J = 11 Hz, 1H), 4.28 (dd, J = 8.5, 4 Hz, 1H), 3.93–3.89 (m, 1H), 3.76 (s, 3H), 3.67 (s, 3H), 3.66–3.61 (m, 3H), 3.51–3.43 (m, 2H), 3.39–3.34 (m, 2H), 3.01–2.97 (m, 1H), 2.96 (s, 3H), 2.83–2.77 (m, 1H), 2.71 (s, 3H), 2.25–2.17 (m, 3H), 2.13 (ddd, J = 7.5, 7.5, 7.5 Hz, 1H), 2.06–1.98 (m, 1H), 1.96–1.91 (m, 1H), 1.90–1.85 (m, 2H), 1.81–1.75 (m, 1H), 1.70–1.65 (m, 1H), 1.62–1.54 (m, 3H), 1.49–1.32 (m, 4H), 1.44 (s, 9H), 1.25 (d, J = 6.5 Hz, 3H), 1.27–1.21 (m, 1H), 0.98–0.95 (m, 2H), 0.93–0.90 (m, 6H), 0.86 (s, 9H), 0.83 (t, J = 7 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃, mixture of carbamate rotamers) δ 176.8, 172.9, 172.2, 171.8, 171.6, 171.5, 158.6, 154.7, 130.4, 128.1, 113.9, 80.1, 78.6, 71.5, 65.3, 60.3, 59.1, 55.2, 51.5, 50.3, 49.8, 47.6, 46.7, 40.3, 37.7, 37.4, 35.6, 34.7, 33.2, 30.9, 30.5, 30.0, 28.5, 28.3, 26.0, 26.0, 25.9, 25.3, 25.3, 25.0, 24.2, 21.9, 20.3, 15.7, 14.8, 14.8, 14.3, 10.5; IR (neat) 3316, 2965, 1732, 1645, 1402 cm⁻¹; HRMS (ESI) m/z for C₅₁H₈₅N₅NaO₁₃ [M+Na]⁺ calcd 998.6036, found 998.6035.
Oxazoline 4.33

To a −78 °C solution of alcohol 4.32 (70 mg, 0.072 mmol) in CH$_2$Cl$_2$ (1.5 mL) was added DAST (9.5 µL, 0.072 mmol) dropwise. After stirring for 1 h at −78 °C, additional DAST (4.8 µL, 0.036 mmol) was added and stirring continued for 30 min. The reaction was quenched with aqueous saturated NaHCO$_3$ (1 mL) at −78 °C and the solution was warmed to rt. The layers were separated and the aqueous layer was extracted with CH$_2$Cl$_2$ (10 x 1 mL). The combined organic layers were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (97:3 CHCl$_3$/MeOH, v/v) yielded oxazoline 4.33 (58 mg, 84%) as an amorphous white solid: $R_f$ 0.29 (96:4 CHCl$_3$/MeOH, v/v); $\left[\alpha\right]_{D}^{25}$ = −102 (c 1.0, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$, mixture of carbamate rotamers) $\delta$ 7.08 (d, $J$ = 8.5 Hz, 2H), 6.77 (d, $J$ = 8.5 Hz, 2H), 6.29 (d, $J$ = 8.5 Hz, 1H), 5.37 (q, $J$ = 7 Hz, 1H), 5.19–5.14 (m, 1H), 4.89 (d, $J$ = 10 Hz, 1H), 4.83 (d, $J$ = 11.5 Hz, 1H), 4.31 (ddd, $J$ = 8, 8, 4 Hz, 1H), 4.24–4.20 (m, 1H), 4.04–3.98 (m, 1H), 3.79 (dd, $J$ = 16, 6 Hz, 1H), 3.75 (s, 3H), 3.67 (s, 3H), 3.71–3.69 (m, 1H), 3.49–3.45 (m, 1H), 3.38–3.30 (m, 1H), 3.00 (dd, $J$ = 13.5, 7.5 Hz, 1H), 2.79 (dd, $J$ = 12.5, 6.5 Hz, 1H), 2.70 (s, 3H), 2.49–2.40 (m, 1H), 2.20–2.07 (m, 4H), 2.04–1.99 (m, 2H), 1.96–1.83 (m, 4H), 1.83–1.72 (m, 1H), 1.70–1.47
(m, 6H), 1.43 (s, 9H), 1.33–1.28 (m, 2H), 1.24 (d, J = 7 Hz, 3H), 1.15 (d, J = 7.5 Hz, 3H), 1.09–1.01 (m, 1H), 0.99–0.95 (m, 2H), 0.95–0.90 (m, 6H), 0.86 (s, 9H), 0.83 (t, J = 7 Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$, mixture of carbamate rotamers) δ 173.0, 172.7, 172.0, 172.0, 171.8, 171.7, 171.5, 171.3, 169.8, 169.6, 158.6, 154.2, 153.9, 130.4, 130.3, 128.1, 128.0, 114.1, 113.9, 80.1, 79.8, 79.2, 78.3, 71.8, 70.6, 65.5, 60.3, 59.6, 59.2, 55.2, 51.2, 50.1, 49.7, 46.6, 46.2, 40.6, 39.4, 38.1, 37.8, 37.7, 36.1, 35.0, 34.9, 34.7, 33.2, 31.0, 30.9, 30.5, 30.0, 28.5, 28.5, 28.4, 26.1, 26.0, 25.9, 25.2, 24.9, 24.3, 23.3, 21.9, 20.7, 20.5, 15.7, 14.7, 14.3, 10.5; IR (neat) 3304, 2967, 1739, 1645, 1402 cm$^{-1}$; HRMS (ESI) m/z for C$_{51}$H$_{83}$N$_5$NaO$_{12}$ [M+Na]$^+$ calcd 980.5930, found 980.5931.

**Amine 4.34**

![Amine 4.33](image)

To a 0 °C solution of oxazoline 4.33 (99 mg, 0.10 mmol) in CH$_2$Cl$_2$ (2 mL) was added 2,6-lutidine (90 µL, 0.50 mmol) followed by trimethylsilyl trifluoromethanesulfonate (90 µL, 0.80 mmol) dropwise. The reaction was warmed to rt and stirred for 1 h. After cooling to 0 °C, saturated aqueous NaHCO$_3$ (1 mL) was added and the mixture was warmed back to rt. The aqueous layer was extracted with CHCl$_3$ (10
x 1 mL) and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residual 2,6-lutidine was removed under a stream of Ar and the crude residue was placed under high vacuum for 1 h.

To a 0 °C solution of crude trimethylsilyl ether in THF (2 mL) was added tetrabutylammonium fluoride (0.30 mL, 0.30 mmol, 1 M solution in THF). After stirring at 0 °C for 30 min, saturated aqueous NaHCO₃ (1 mL) was added and the mixture was warmed to rt. The aqueous layer was extracted with CHCl₃ (10 x 1 mL) and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (98:2 to 95:5 CHCl₃/MeOH, v/v) yielded amine 4.34 (56.2 mg, 66% from oxazoline 4.33) as a yellow amorphous solid: Rₐ 0.13 (9:1 CHCl₃/MeOH, v/v); [α]²⁵D = −89.7 (c 0.88, CHCl₃); ¹H NMR (500 MHz, CDCl₃, mixture of rotamers) δ 7.11 (d, J = 8.5 Hz, 2H), 6.78 (d, J = 8.5 Hz, 2H), 5.34 (q, J = 6.5 Hz, 1H), 5.13–5.08 (m, 1H), 4.95 (dd, J = 11.5, 3 Hz, 1H), 4.89 (d, J = 10.5 Hz, 1H), 4.33 (dd, J = 8.5, 4.5 Hz, 1H), 4.23 (dd, J = 9 Hz, 1H), 4.14–4.06 (m, 1H), 3.86 (dd, J = 7.5 Hz, 1H), 3.76 (s, 3H), 3.67 (s, 3H), 3.41–3.28 (m, 2H), 3.00 (dd, J = 14.0, 7.5 Hz, 1H), 2.96 (s, 3H), 2.89 (dd, J = 12, 5.5 Hz, 1H), 2.79–2.69 (m, 1H), 2.66 (s, 3H), 2.44–2.34 (m, 2H), 2.32–2.26 (m, 1H), 2.21–2.13 (m, 1H), 2.06–1.85 (m, 5H), 1.67–1.53 (m, 4H), 1.50–1.40 (m, 2H), 1.35–1.29 (m, 2H), 1.28–1.20 (m, 2H), 1.25 (d, J = 6.5 Hz, 3H), 1.16 (d, J = 7 Hz, 3H), 1.00–0.94 (m, 3H), 0.94–0.90 (m, 3H), 0.91 (d, J = 6 Hz, 3H), 0.88 (s, 9H), 0.83 (t, J = 7 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃, mixture of rotamers) δ 172.5, 171.8, 171.6, 171.5, 169.9, 158.6, 130.4, 130.3, 128.2, 113.9, 79.7, 71.6, 70.6, 65.3, 60.3, 59.8, 55.2, 51.8, 50.5, 50.1, 46.0, 40.5,
38.8, 37.6, 36.4, 35.8, 34.8, 34.7, 33.2, 30.8, 30.7, 29.4, 26.0, 25.9, 25.2, 25.0, 24.5, 21.7, 21.0, 15.7, 15.0, 14.2, 10.5; IR (neat) 3306, 2936, 1732, 1645, 1514 cm\(^{-1}\); HRMS (ESI) \(m/z\) for C\(_{46}\)H\(_{76}\)N\(_5\)O\(_{10}\) \([\text{M+H}]^+\) calcld 858.5587 found 858.5583.

**Depsideptide 4.24**

![Depsideptide 4.24 diagram](image)

To a 0 °C solution of amine 4.34 (6.6 mg, 7.7 µmol) in THF (38 µL), and \(t\)-BuOH (77 µL) was added a solution of lithium hydroxide (77 µL, 77 µmol, 1 M in H\(_2\)O). The reaction was warmed to rt and stirred for 4 h before the reaction was concentrated under a stream of argon. The residue was dissolved in CHCl\(_3\) (1 mL) and neutralized with aqueous phosphate buffer (pH = 7, 0.5 mL). The separated aqueous phase was extracted with CHCl\(_3\) (10 x 0.5 mL). The combined organic layers were dried over Na\(_2\)SO\(_4\), filtered, and concentrated under reduced pressure. The resulting residue was azeotropically dried with toluene twice and then placed under high vacuum for 2 h.

The crude amino acid was dissolved in CH\(_2\)Cl\(_2\) (7.7 mL, 0.001 M) and cooled to 0 °C. To the cooled solution was added \(t\)-Pr\(_2\)NEt (6.8 µL, 39 µmol) followed by PyAOP (8.0 mg, 15.4 µmol) and the reaction mixture was warmed to rt. After stirring at rt for 24
h, the reaction mixture was concentrated under reduced pressure. Purification via preparative thin layer chromatography on silica gel (95:5 EtOAc/MeOH, v/v) yielded cyclic depsipeptide \( \text{4.24} \) (3.0 mg, 47% from amine \( \text{4.34} \)) as a white amorphous solid: \([\alpha]^{25}_D = -83.5 \) (c 0.23, MeOH); \(^1\)H NMR (500 MHz, CDCl\(_3\), mixture of carbamate rotamers) \( \delta \) 7.11 (d, \( J = 8.5 \) Hz, 2H), 6.82 (d, \( J = 8.6 \) Hz, 2H), 6.32 (d, \( J = 8.6 \) Hz, 0.6H), 6.04 (d, \( J = 8.6 \) Hz, 0.4H), 5.31 (m, 0.6H), 5.22 (d, \( J = 11.5 \) Hz, 0.4H), 5.09 (ddd, \( J = 12, 6.8, 6.8 \) Hz, 0.4H), 4.97 (d, \( J = 13, 1.7 \) Hz, 0.3H), 4.91 (dd, \( J = 13, 2.8 \) Hz, 0.7H), 4.83 (d, \( J = 11.4 \) Hz, 0.7H), 4.72–4.66 (m, 0.7H), 4.58 (d, \( J = 10 \) Hz, 0.3H), 4.36 (dd, \( J = 8.8, 6 \) Hz, 1H), 4.31–4.27 (m, 1H), 4.23–4.19 (m, 1H), 4.17 (d, \( J = 7.6 \) Hz, 1H), 4.08 (dd, \( J = 6.8, 6.8 \) Hz, 0.3H), 4.02–3.96 (m, 0.7H), 3.91–3.83 (m, 1H), 3.77 (s, 3H), 3.68–3.60 (m, 2H), 2.96 (dd, \( J = 11.4, 6.2 \) Hz, 1H), 2.92 (s, 2H), 2.78 (s, 1H), 2.72 (s, 1H), 2.68 (s, 2H), 2.43–2.33 (m, 2H), 2.29–2.21 (m, 2H), 2.09–2.03 (m, 1H), 1.99–1.83 (m, 3H), 1.80 (dd, \( J = 13.3, 3.3 \) Hz, 1H), 1.76–1.74 (m, 1H), 1.69–1.66 (m, 1H), 1.47–1.37 (m, 3H), 1.34–1.23 (m, 4H), 1.18 (d, \( J = 6.7 \) Hz, 1H), 1.15–1.11 (m, 1H), 1.07–1.04 (m, 5H), 1.02 (d, \( J = 6.8 \) Hz, 2H), 0.97 (d, \( J = 6.5 \) Hz, 3H), 0.98–0.94 (m, 2H), 0.88 (s, 9H), 0.85 (t, \( J = 7.3 \) Hz, 3H); HRMS (ESI) \( m/z \) for C\(_{45}H\(_{71}\)N\(_5\)NaO\(_9\) [M+Na]\(^+\) calcd 848.5144, found 848.5143.
To a solution of aldehyde 2.12 (69 mg, 0.19 mmol) in toluene (2 mL) was added methyl 2-(triphenylphosphoranylidene)propionate (111 mg, 0.29 mmol) and the mixture was heated to 80 °C. After 6 h, the reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (9:1 to 6:1 hexanes/EtOAc, v/v) yielded α,β-unsaturated ester 4.37 (74 mg, 88%) as a colorless, viscous oil: Rf 0.43 (6:1 hexanes/EOAc, v/v); [α]$_{D}^{25}$ = −61.6 (c 1.22, CHCl$_3$) $^{1}$H NMR (500 MHz, CDCl$_3$, mixture of carbamate rotamers) δ 6.72 (ddd, $J$ = 7.5, 7.5, 7.5 Hz, 1H), 4.84–4.79 (m, 1H), 4.31 (dd, $J$ = 8.5, 2.5 Hz, 1H), 3.69 (s, 3H), 3.53–3.33 (m, 2H), 2.35–2.31 (m, 1H), 2.23–2.11 (m, 1H), 2.05–1.98 (m, 1H), 1.96–1.90 (m, 2H), 1.89–1.84 (m, 2H), 1.82 (s, 3H), 1.70–1.65 (m, 0.5H), 1.54–1.52 (m, 0.5H), 1.48–1.36 (m, 1H) 1.42 (s, 9H), 0.89 (d, $J$ = 6 Hz, 3H), 0.86 (s, 9H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 173.1, 172.6, 168.6, 168.5, 154.2, 153.9, 141.0, 140.1, 128.7, 128.5, 79.9, 79.5, 79.3, 78.8, 59.3, 59.2, 51.6, 51.5, 46.4, 46.1, 37.0, 34.7, 34.6, 33.9, 31.0, 30.0, 29.4, 29.1, 28.4, 28.3, 25.9, 25.9, 24.3, 23.2, 20.9, 12.6; IR (neat) 2959, 1701, 1401, 1383, 1163 cm$^{-1}$; HRMS (ESI) m/z for C$_{24}$H$_{41}$NNaO$_6$ [M+Na]$^+$ calcd 462.2826, found 462.2824.
Carboxylic acid 4.36

\[
\begin{array}{c}
\text{Boc} \quad \text{O} \\
\text{N} \\
\text{Boc} \quad \text{O} \\
\text{H} \quad \text{O} \\
\text{4.37} \\
\end{array} \quad \text{LiOH}\cdot\text{H}_2\text{O} \quad \text{t-BuOH/H}_2\text{O}/\text{THF, rt, 3 h} \quad \text{Boc} \quad \text{O} \\
\text{N} \\
\text{Boc} \quad \text{O} \\
\text{H} \quad \text{O} \\
\text{4.36} \\
\end{array}
\]

To a 0 °C solution of ester 4.37 (0.119 g, 0.27 mmol) in a mixture of t-BuOH (1.4 mL), THF (0.7 mL), and H₂O (0.7 mL) was added LiOH•H₂O (0.113 g, 2.7 mmol) and the reaction was warmed to rt. After stirring at rt for 3 h, the mixture was acidified with 10% citric acid (pH ~ 3) and the aqueous layer was extracted with EtOAc (5 x 5 mL). The combined organic layers were dried of Na₂SO₄, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (3:1 to 1:1 hexanes/EtOAc, v/v) produced acid 4.36 (0.103 g, 90%) as a white foam: Rₜ 0.17 (2:1 hexanes/EOAc, v/v); [α]²⁵_D = −57.5 (c 0.96, CHCl₃); ¹H NMR (500 MHz, CDCl₃, mixture of carbamate rotamers) δ 5.80 (m, 1H), 4.86–4.81 (m, 1H), 4.34 (d, J = 9.5 Hz, 1H), 3.56–3.36 (m, 2H), 2.39–2.35 (m, 1H), 2.26–2.16 (m, 1H), 2.13–1.93 (m, 4H), 1.85 (s, 3H), 1.76–1.71 (m, 0.5H), 1.61–1.58 (m, 0.5H), 1.53–1.46 (m, 2H), 1.44 (s, 9H), 0.92 (dd, J = 6.5, 6.5 Hz, 1H), 0.89 (s, 9H); ¹³C NMR (125 MHz, CDCl₃) δ 173.2, 173.1, 173.0, 172.7, 154.2, 154.0, 143.6, 142.5, 128.4, 127.8, 80.1, 79.7, 79.3, 78.8, 59.4, 59.2, 46.5, 46.2, 39.9, 34.8, 34.7, 34.1, 31.1, 30.1, 29.5, 29.1, 28.4, 28.4, 26.0, 25.9, 24.4, 23.2, 21.0, 20.9, 12.3, 12.2; IR (neat) 3183, 2936, 1732, 1697, 1398 cm⁻¹; HRMS (ESI) m/z for C₂₃H₃₉NNaO₆ [M+Na]^+ calcd 448.2670, found 448.2669.
Amide 4.38

To a rt solution of acid 4.36 (68 mg, 0.16 mmol) in CH₂Cl₂ (1.5 mL) was added i-Pr₂NEt (84 µL, 0.48 mmol), followed by HATU (61 mg, 0.16 mmol). After approximately 1 min, a solution of amino alcohol 3.31 (90 mg, 0.17 mmol) in CH₂Cl₂ (0.5 mL) was added. The resulting yellow solution was stirred at rt for 12 h, at which the reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (99:1 to 98:2 CH₂Cl₂/MeOH, v/v) gave amide 4.38 (0.132 g, 86%) as a white foam: Rᵢ 0.31 (97:3 CH₂Cl₂/MeOH, v/v); [α]²⁵_D = −90.5 (c 1.1, CHCl₃); ¹H NMR (500 MHz, CDCl₃, mixture of carbamate rotamers) δ 7.08 (d, J = 8.5 Hz, 2H), 6.78 (d, J = 8 Hz, 2H), 6.61–6.55 (m, 1H), 6.47 (d, J = 8.5 Hz, 1H), 6.29–6.24 (m, 2H), 5.39 (q, J = 7 Hz, 1H), 5.19 (ddd, J = 8, 8, 8 Hz, 1H), 4.90 (d, J = 10.5 Hz, 1H), 4.91–4.89 (m, 1H), 4.83–4.76 (m, 1H), 4.38–4.34 (m, 1H), 3.75 (s, 3H), 3.72–3.65 (m, 2H), 3.68 (s, 3H), 3.56–3.44 (m, 2H), 3.04 (dd, J = 13.5, 7.5 Hz, 1H), 2.94 (s, 3H), 2.87–2.81 (m, 2H), 2.72 (s, 3H), 2.27–2.16 (m, 2H), 2.11–2.02 (m, 2H), 1.98–1.89 (m, 3H), 1.94 (s, 3H), 1.84 (s, 3H), 1.74–1.65 (m, 1H), 1.58–1.53 (m, 2H), 1.47 (ddd, J = 11.5, 4.5, 4.5 Hz, 1H), 1.43 (s, 9H), 1.26 (d, J = 7 Hz, 3H), 0.98–0.94 (m, 1H), 0.96 (d, J = 6.5 Hz, 3H), 0.92 (d, J = 6.5 Hz, 3H), 0.87–0.83 (m, 3H), 0.86 (s, 9H); ¹³C NMR (125 MHz, 171
CDCl₃, mixture of carbamate rotamers) δ 174.1, 174.0, 171.7, 171.5, 171.4, 169.6, 169.4, 168.0, 158.6, 154.2, 153.9, 153.5, 135.5, 134.8, 133.4, 131.5, 131.3, 131.0, 130.4, 128.0, 113.9, 80.2, 79.8, 64.9, 60.3, 59.3, 55.2, 51.8, 49.7, 46.5, 46.2, 39.9, 39.8, 34.7, 33.2, 31.0, 30.9, 30.5, 30.1, 29.3, 29.2, 28.4, 25.9, 25.8, 25.8, 24.9, 18.6, 17.2, 15.7, 14.3, 13.3, 12.9, 10.5; IR (neat) 3364, 2965, 1732, 1645, 1514 cm⁻¹; HRMS (ESI) m/z for C₅₁H₈₁N₅NaO₁₂ [M+Na]⁺ calcd 978.5774, found 978.5773.

**Oxazoline 4.39**

![Diagram of oxazoline 4.39]

To a −78 °C solution of alcohol 4.38 (51 mg, 52.3 µmol) in CH₂Cl₂ (5 mL) was added DAST (7.0 µL, 52.3 µmol) dropwise. After stirring for 1 h at −78 °C, additional DAST (3.5 µL, 26.7 µmol) was added and stirring continued for 30 min. The reaction was quenched with aqueous saturated NaHCO₃ (1.5 mL) at −78 °C and the solution was warmed to rt. The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (10 x 1.5 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (97:3 CH₂Cl₂/MeOH, v/v) yielded oxazoline 4.39 (38 mg, 76%) as an
amorphous white solid:  \( R_f \) 0.34 (97:3 CH\(_2\)Cl\(_2\)/MeOH, v/v); \( [\alpha]^{25}_D = -77.8 \) (c 1.13, CHCl\(_3\)); \(^1\)H NMR (500 MHz, CDCl\(_3\), mixture of carbamate rotamers) \( \delta \) 7.08 (d, \( J = 8.5 \) Hz, 2H), 6.77 (d, \( J = 8.5 \) Hz, 2H), 6.44–6.43 (m, 2H), 6.12 (d, \( J = 8.5 \) Hz, 1H), 5.40 (q, \( J = 7 \) Hz, 1H), 5.20 (ddd, \( J = 7.5 \) Hz, 1H), 4.96–4.82 (m, 2H), 4.90 (d, \( J = 10.5 \) Hz, 1H), 4.42 (dd, \( J = 9, 9 \) Hz, 1H), 4.34 (d, \( J = 9 \) Hz, 1H), 3.91–3.86 (m, 1H), 3.76 (s, 3H), 3.68 (s, 3H), 3.55–3.44 (m, 1.5H), 3.40–3.35 (m, 0.5H), 3.04 (dd, \( J = 13.5, 7.5 \) Hz, 1H), 2.96 (s, 3H), 2.87–2.81 (m, 2H), 2.74 (s, 3H), 2.38–2.33 (m, 1H), 2.23–2.16 (m, 1H), 2.07–2.02 (m, 2H), 2.00–1.96 (m, 2H), 1.96–1.84 (m, 3H), 1.93 (s, 3H), 1.89 (s, 3H), 1.76 (br s, 1H), 1.48–1.42 (m, 2H), 1.44 (s, 9H), 1.26 (d, \( J = 7 \) Hz, 3H), 0.99–0.91 (m, 2H), 0.92 (d, \( J = 6.5 \) Hz, 3H), 0.89 (s, 9H), 0.84 (t, \( J = 7.5 \) Hz, 3H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\), mixture of carbamate rotamers) \( \delta \) 173.1, 172.7, 171.8, 171.5, 171.4, 171.4, 158.7, 154.2, 154.0, 132.8, 130.4, 130.4, 127.9, 114.0, 113.9, 80.0, 79.6, 79.4, 78.9, 71.9, 64.1, 64.1, 60.4, 59.4, 59.2, 55.2, 51.8, 50.5, 50.4, 49.7, 46.4, 46.2, 37.8, 37.8, 37.1, 37.0, 34.8, 34.7, 33.9, 33.8, 33.3, 31.0, 30.9, 30.5, 30.1, 29.7, 29.3, 28.5, 28.4, 28.3, 26.0, 25.9, 25.0, 24.4, 23.2, 21.0, 20.9, 15.7, 14.4, 14.4, 13.6, 13.4, 13.4, 10.6; IR (neat) 2965, 1738, 1699, 1628, 1400 cm\(^{-1}\); HRMS (ESI) \( m/z \) for \( \text{C}_{51}\text{H}_{86}\text{N}_{13}\text{O}_{11} \) [M+H]\(^+\) calcd 938.5849, found 938.5849.
To a 0 °C solution of oxazoline \( \text{4.39} \) (38 mg, 40.5 µmol) in CH\(_2\)Cl\(_2\) (1 mL) was added 2,6-lutidine (38 µL, 324 µmol) followed by trimethylsilyl trifluoromethanesulfonate (37 µL, 203 µmol) dropwise. The reaction was warmed to rt and stirred for 1 h. After cooling to 0 °C, saturated aqueous NaHCO\(_3\) (1 mL) was added and the mixture was warmed back to rt. The aqueous layer was extracted with CHCl\(_3\) (10 x 1 mL) and the combined organic layers were dried over Na\(_2\)SO\(_4\), filtered, and concentrated under reduced pressure. The residual 2,6-lutidine was removed under a stream of argon and the crude residue was placed under high vacuum for 1 h. Purification via flash column chromatography on silica gel (98:2 to 95:5 CHCl\(_3\)/MeOH, v/v) yielded amine \( \text{4.40} \) (27 mg, 79%) as a light yellow amorphous solid: \( R_f \) 0.18 (95:5 CHCl\(_3\)/MeOH, v/v); \([\alpha]^{25}_D = -94.8\) (c 0.93, CHCl\(_3\)); \(^1\)H NMR (500 MHz, CDCl\(_3\), mixture of carbamate rotamers) \( \delta \) 7.08 (d, \( J = 8.5 \text{ Hz}, 2\)H), 6.77 (d, \( J = 8.5 \text{ Hz}, 2\)H), 6.45–6.40 (m, 2H), 6.11 (t, \( J = 8 \text{ Hz}, 1\)H), 5.40 (q, \( J = 6.5 \text{ Hz}, 1\)H), 5.20 (ddd, \( J = 7.5, 7.5, 7.5 \text{ Hz}, 1\)H), 4.94 (dd, \( J = 9.5, 3.5 \text{ Hz}, 1\)H), 4.90 (d, \( J = 10.5 \text{ Hz}, 1\)H), 4.88 (d, \( J = 6 \text{ Hz}, 1\)H), 4.43 (dd, \( J = 9.5, 9.5 \text{ Hz}, 1\)H), 3.92–3.87 (m, 1H), 3.79 (dd, \( J = 8.5, 6 \text{ Hz}, 1\)H), 3.76 (s, 3H), 3.68 (s, 3H), 3.12–3.08 (m, 1H), 3.04 (dd, \( J = 14, 7.5 \text{ Hz}, 1\)H), 2.98–2.91 (m, 1H), 174
2.96 (s, 3H), 2.87–2.81 (m, 1H), 2.74 (s, 3H), 2.35–2.30 (m, 1H), 2.21–2.13 (m, 1H), 2.02 (dd, \( J = 14.5, 7, 7, 7 \) Hz, 1H), 1.92 (s, 3H), 1.89 (s, 3H), 1.86–1.70 (m, 5H), 1.57–1.52 (m, 1H), 1.48 (dd, \( J = 6, 6 \) Hz, 2H), 1.27–1.21 (m, 2H), 1.26 (d, \( J = 6.5 \) Hz, 3H), 0.99–0.95 (m, 1H), 0.92 (d, \( J = 6.5 \) Hz, 3H), 0.91–0.90 (m, 2H), 0.89 (s, 9H), 0.85 (t, \( J = 7.5 \) Hz, 3H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\), mixture of carbamate rotamers) \( \delta 175.0, 171.8, 171.5, 171.4, 168.1, 168.0, 166.8, 158.7, 137.0, 136.9, 135.5, 135.2, 133.1, 132.9, 130.4, 128.0, 127.9, 125.0, 125.0, 113.9, 113.9, 79.5, 71.9, 64.1, 64.1, 60.3, 60.1, 55.2, 51.8, 50.5, 50.4, 49.7, 46.9, 37.8, 36.8, 36.7, 34.8, 34.0, 33.3, 30.9, 30.6, 30.4, 29.8, 26.0, 25.4, 25.0, 20.9, 20.8, 15.7, 14.4, 14.4, 13.6 13.4, 13.4, 10.6; IR (neat) 3327, 2965, 1734, 1630, 1514 cm\(^{-1}\); HRMS (ESI) \( m/z \) for C\(_{46}\)H\(_{72}\)N\(_5\)O\(_9\) [M+Na]\(^+\) calcd 838.5325, found 838.5333.

**Depsipептид 4.35**

![Chemical Structure Image]

To a 0 °C solution of amine 4.40 (4.2 mg, 5.0 µmol) in THF (20 µL), and t-BuOH (50 µL) was added a solution of lithium hydroxide (50 µL, 50 µmol, 1 M in H\(_2\)O). The reaction was warmed to rt and stirred for 5 h before the reaction was concentrated
under a stream of argon. The residue was dissolved in CHCl$_3$ (1 mL) and neutralized with aqueous phosphate buffer (pH = 7, 0.5 mL). The separated aqueous phase was extracted with CHCl$_3$ (10 x 0.5 mL). The combined organic layers were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The resulting residue was azeotropically dried with toluene twice and then placed under high vacuum for 2 h.

The crude amino acid was dissolved in CH$_2$Cl$_2$ (5 mL, 0.001 M) and cooled to 0 °C. To the cooled solution was added $i$-Pr$_2$NEt (5.2 µL, 30.0 µmol) followed by HATU (3.8 mg, 10.0 µmol) and the reaction mixture was warmed to rt. After stirring at rt for 16 h, the reaction mixture was concentrated under reduced pressure. Purification via preparative thin layer chromatography on silica gel (4:1 EtOAc/petroleum ether, v/v) yielded cyclic depsipeptides 4.35 (2.2 mg, 55% from amine 4.40, 1:1 mixture of separable diastereomers) as a white amorphous solids: **TOP Diastereomer** [α]$^{25}_D$ = –160 (c 0.20, MeOH); $^1$H NMR (500 MHz, CDCl$_3$, ~ 2:1 mixture of carbamate rotamers) δ 7.16 (d, $J$ = 8.6 Hz, 2H), 6.81 (d, $J$ = 8.6 Hz, 2H), 6.62–6.55 (m, 1H), 6.32 (d, $J$ = 10.3 Hz, 0.6 H), 6.25 (d, $J$ = 9.3 Hz, 0.6H), 6.07 (d, $J$ = 9.5 Hz, 0.4H), 5.96 (d, $J$ = 9.1 Hz, 0.4H), 5.32 (ddd, $J$ = 9.5, 9.5, 4 Hz, 1H), 5.16 (d, $J$ = 11.5 Hz, 0.5H), 5.12–5.08 (m, 1H), 5.00 (d, $J$ = 10.3 Hz, 0.5H), 4.94 (d, $J$ = 11.6 Hz, 1H), 4.93–4.87 (m, 1H), 4.84 (11.4 Hz, 1H), 4.73 (ddd, $J$ = 6.8 Hz, 1H), 4.39–4.33 (m, 1H), 4.26 (dd, $J$ = 8.4, 8.4 Hz, 1H), 4.23–4.17 (m, 1H), 4.13–4.11 (m, 0.6H), 4.07–4.01 (m, 1H), 3.77 (s, 3H), 3.66–3.59 (m, 1H), 3.35–3.32 (m, 1H), 3.22 (dd, $J$ = 12.8, 10.3 Hz, 0.7H), 3.15 (dd, $J$ = 12.3, 10.1 Hz, 0.3H), 2.91 (dd, $J$ = 12.5, 4.4 Hz, 1H), 2.83 (s, 3H), 2.60 (s, 3H), 2.57–2.53 (m, 1H), 2.29–2.24 (m, 1H), 1.94 (s, 3H), 1.66–1.57 (m, 2H), 1.40–1.31 (m, 3H), 1.25 (d, $J$ = 7 Hz, 3H), 1.11 (d, $J$ = 6.7 Hz, 3H), 0.99 (d, $J$ = 6.1 Hz, 3H), 0.97–0.91 (m, 2H), 0.89 (s, 9H), 0.83 (t, $J$ = 176 Hz).
5.4 Hz, 3H), 0.67 (d, $J = 6.6$ Hz, 2H); HRMS (ESI) $m/z$ for $\text{C}_{45}\text{H}_{68}\text{N}_5\text{O}_8 [\text{M+H}]^+$ calcd 806.5062, found 806.5086.

**Bottom Diastereomer** $[\alpha]^{25}_D = -119$ (c 0.16, MeOH); $^1$H NMR (500 MHz, CDCl$_3$, ~1:1 mixture of carbamate rotamers) δ 7.19 (d, $J = 8.5$ Hz, 1H), 7.15 (d, $J = 8.5$ Hz, 1H), 6.81 (d, $J = 8.6$ Hz, 1H), 6.79 (d, $J = 8.6$ Hz, 1H), 6.77–6.74 (m, 0.5 H), 6.67–6.64 (m, 0.5H), 6.21 (d, $J = 8.7$ Hz, 0.5H), 5.93 (d, $J = 10.3$ Hz, 0.5H), 5.88 (d, $J = 8.4$ Hz, 0.5H), 5.58 (d, $J = 7.6$ Hz, 0.5H), 5.36–5.33 (m, 0.4H), 5.22 (d, $J = 11.1$ Hz, 0.5H), 5.16–5.11 (m, 1.6H), 4.99 ($J = 12.4$ Hz, 1H), 4.95–4.92 (m, 1H), 4.84 (d, $J = 11.3$ Hz, 1H), 4.63 (ddd, $J = 6.4$, 6.4, 6.4 Hz, 0.5H), 4.49 (dd, $J = 9.2$, 9.2 Hz, 0.5H), 4.34 (t, $J = 8.8$ Hz, 0.5H), 4.27 (dd, $J = 8.1$, 6.6 Hz, 0.5H), 4.23 (dd, $J = 7.4$ Hz, 0.5H), 4.06–4.03 (m, 1.5H), 3.94 (dd, $J = 8.3$ Hz, 0.5H), 3.77 (s, 3H), 3.69–3.60 (m, 1H), 3.39–3.35 (m, 1H), 3.18–3.10 (m, 1H), 3.04 (dd, $J = 12.9$, 4.3 Hz, 1H), 2.97 (s, 1.5H), 2.86 (s, 1.5H), 2.85 (s, 1.5H), 2.83–2.80 (m, 1H), 2.61 (s, 1.5H), 2.46–2.28 (m, 2H), 2.28–2.17 (m, 2H), 2.02 (s, 3H), 1.91 (s, 3H), 1.75–1.68 (m, 1H), 1.66–1.60 (m, 1H), 1.32–1.26 (m, 3H), 0.96 (d, $J = 6.3$ Hz, 2H), 0.94–0.90 (m, 3H), 0.88 (s, 9H), 0.84–0.81 (m, 3H), 0.60 (d, $J = 7$ Hz, 1H); HRMS (ESI) $m/z$ for $\text{C}_{45}\text{H}_{68}\text{N}_5\text{O}_8 [\text{M+H}]^+$ calcd 806.5062, found 806.5082.
**Carboxylic acid 4.42**

To a solution of aldehyde **2.12** (0.147 g, 0.40 mmol) in toluene at was added methyl (triphenylphosphoranylidene)acetate (0.27 g, 0.80 mmol). The mixture was heated to 80 °C for 4 h. After cooling to rt, the reaction was concentrated under reduced pressure. The resulting crude mixture was passed through a pad of silica to remove remaining salts and triphenylphosphine oxide.

To an argon flushed solution of the crude α,β-unsaturated ester (ca. 0.40 mmol) in EtOH was added 10% Pd/C (20 mg, ~10% by weight of ester). After flushing the reaction mixture with a balloon of H₂, the reaction was placed under 1 atm of H₂ for 1 h. After completion, the reaction mixture was flushed with argon. The reaction mixture was filtered through a pad of Celite® and taken on to the next step without further purification.

To a 0 °C solution of the crude ester (ca. 0.40 mmol) in a mixture of t-BuOH (1.6 mL), THF (0.8 mL), and H₂O (0.8 mL) was added LiOH•H₂O (68 mg, 1.6 mmol) and the reaction was warmed to rt. After stirring at rt for 4 h, the mixture was acidified with 10% citric acid (pH ~ 3) and the aqueous layer was extracted with EtOAc (5 x 10 mL). The combined organic layers were dried of Na₂SO₄, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (2:1 to 1:1 hexanes/EtOAc, v/v) produced acid **4.42** (88 mg, 67% from aldehyde **2.12**) as a white foam: Rf 0.26 (1:1 hexanes/EtOAc, v/v); [α]²⁵_D = −59 (c 1.12, CHCl₃); ¹H NMR (500
MHz, CDCl$_3$, mixture of carbamate rotamers) δ 4.83 (d, $J= 11$ Hz, 1H), 4.37 (dd, $J= 8.5$, 3 Hz, 1H), 3.54–3.44 (m, 2H), 2.38–2.26 (m, 2H), 2.22–2.13 (m, 1H), 2.05–2.00 (m, 1H), 1.91–1.86 (m, 2H), 1.73–1.72 (m, 1H), 1.59–1.47 (m, 3H), 1.44–1.33 (m, 3H), 1.44 (s, 9H), 0.90 (d, $J= 7$ Hz, 3H), 0.87 (s, 9H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 178.1, 172.7, 172.6, 154.5, 154.0, 80.0, 79.9, 79.6, 79.0, 59.4, 59.1, 46.4, 46.2, 37.4, 37.4, 34.8, 34.2, 34.1, 31.0, 30.0, 29.4, 28.5, 28.4, 26.0, 24.2, 23.2, 21.9, 21.8, 20.7, 20.6; IR (neat) 3165, 2934, 1748, 1651, 1454 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_{22}$H$_{39}$NNaO$_6$ [M+Na]$^+$ calcd 436.2670, found 436.2674.

**Amide 4.43**

![Chemical structure of amide 4.43](image)

To a rt solution of acid 4.42 (49 mg, 0.118 mmol) in CH$_2$Cl$_2$ (0.8 mL) was added i-Pr$_2$NEt (62 µL, 0.35 mmol), followed by HATU (47 mg, 0.124 mmol). After approximately 1 min, a solution of amino alcohol 3.31 (71.2 mg, 0.13 mmol) in CH$_2$Cl$_2$ (0.4 mL) was added. The resulting yellow solution was stirred at rt for 12 h, at which the reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (98:2 CH$_2$Cl$_2$/MeOH, v/v) gave amide 4.43 (98.7 mg, 89% yield).
89%) as a white foam: \( R_f 0.35 \) (97:3 \( \text{CH}_2\text{Cl}_2/\text{MeOH}, \text{v/v} \)); \([\alpha]^{25}_D = -81.4 \) (c 1.06, \( \text{CHCl}_3 \)); \(^1\)H NMR (500 MHz, \( \text{CDCl}_3 \), mixture of carbamate rotamers) \( \delta \) 7.08 (d, \( J = 8.5 \) Hz, 2H), 6.77 (d, \( J = 8.4 \) Hz, 2H), 6.65–6.48 (m, 1H), 6.25 (d, \( J = 8.4 \) Hz, 1H), 5.39–5.37 (m, 1H), 5.18 (ddd, \( J = 6.2, 6.2, 6.2 \) Hz, 1H), 4.89 (d, \( J = 10.4 \) Hz, 1H), 4.83–4.74 (m, 2H), 4.35–4.33 (m, 1H), 3.75 (s, 3H), 3.72–3.62 (m, 2H), 3.67 (s, 3H), 3.50–3.49 (m, 1H), 3.40–3.39 (m, 1H), 3.05–3.01 (m, 1H), 2.94 (s, 3H), 2.87–2.80 (m, 2H), 2.72 (s, 3H), 2.22–2.14 (m, 3H), 2.07–2.00 (m, 2H), 1.95–1.86 (m, 3H), 1.91 (s, 3H), 1.81–1.68 (m, 1H), 1.52–1.50 (m, 2H), 1.47–1.37 (m, 2H), 1.42 (s, 9H), 1.34–1.25 (m, 2H), 1.25 (d, \( J = 6.9 \) Hz, 3H), 0.97–0.94 (m, 2H), 0.91 (d, \( J = 6.6 \) Hz, 3H), 0.89–0.86 (m, 3H), 0.86 (s, 9H), 0.84 (t, \( J = 7.4 \) Hz, 3H); \(^{13}\)C NMR (125 MHz, \( \text{CDCl}_3 \), mixture of carbamate rotamers) \( \delta \) 173.3, 171.7, 171.7, 171.4, 171.4, 165.7, 158.6, 154.4, 154.4, 132.7, 130.4, 130.3, 130.3, 128.0, 113.9, 80.2, 79.8, 79.8, 64.7, 60.4, 59.4, 59.4, 59.3, 55.2, 51.8, 50.7, 50.4, 50.0, 49.8, 49.8, 46.5, 46.2, 43.5, 38.6, 37.7, 37.6, 30.9, 30.5, 30.1, 30.1, 28.4, 28.4, 28.4, 28.2, 25.9, 25.0, 24.3, 24.2, 23.1, 22.6, 20.8, 20.6, 17.0, 17.0, 15.7, 14.3, 13.3, 12.7, 10.5; IR (neat) 3333, 2965, 1732, 1644, 1402 cm\(^{-1}\); HRMS (ESI) \( m/z \) for \( \text{C}_{50}\text{H}_{81}\text{N}_5\text{NaO}_{12} \) [M+Na]\(^+ \) calcd 966.5774, found 966.5775.
Oxazoline 4.44

To a \(-78 \, ^\circ\text{C}\) solution of alcohol 4.43 (98.7 mg, 0.105 mmol) in CH\(_2\)Cl\(_2\) (2.1 mL) was added DAST (21 \(\mu\)L, 0.158 mmol) dropwise. After stirring for 1 h at \(-78 \, ^\circ\text{C}\), the reaction was quenched with aqueous saturated NaHCO\(_3\) (1.5 mL) at \(-78 \, ^\circ\text{C}\) and the solution was warmed to rt. The layers were separated and the aqueous layer was extracted with CH\(_2\)Cl\(_2\) (10 x 2 mL). The combined organic layers were dried over Na\(_2\)SO\(_4\), filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (97:3 CH\(_2\)Cl\(_2\)/MeOH, v/v) yielded oxazoline 4.44 (75 mg, 77\%) as an amorphous white solid: \(R_f\) 0.37 (97:3 CH\(_2\)Cl\(_2\)/MeOH, v/v); \([\alpha]^{25}_D = -97.6\) (c 0.97, CHCl\(_3\)); \(^1\)H NMR (500 MHz, CDCl\(_3\), mixture of carbamate rotamers) \(\delta\) 7.06 (d, \(J = 8.2\) Hz, 2H), 6.76 (d, \(J = 8.5\) Hz, 2H), 6.47–6.43 (m, 1H), 6.10 (m, 1H), 5.38 (q, \(J = 6.9\) Hz, 1H), 5.128 (ddd, \(J = 6.8, 6.8, 6.8\) Hz, 1H), 4.88 (d, \(J = 10.5\) Hz, 1H), 4.82–4.78 (m, 2H), 4.41–4.36 (m, 1H), 4.34–4.30 (m, 1H), 3.88–3.81 (m, 1H), 3.74 (s, 3H), 3.66 (s, 3H), 3.51–3.32 (m, 2H), 3.05 (m, 1H), 2.94 (s, 3H), 2.84–2.80 (m, 2H), 2.71 (s, 3H), 2.30–2.13 (m, 3H), 2.04–1.99 (m, 1H), 1.98–1.89 (m, 2H), 1.86 (d, \(J = 5\) Hz, 3H), 1.68–1.62 (m, 1H), 1.56–1.48 (m, 2H), 1.42 (s, 9H), 1.42–1.39 (m, 2H), 1.26–1.21 (m, 1H), 1.24 (d, \(J = 7.1\) Hz, 3H), 1.14–1.08 (m, 1H), 1.05–0.98 (m, 1H), 0.98–0.92 (m, 2H), 0.90 (d, \(J = 181\)
7.2 Hz, 3H), 0.88–0.87 (m, 2H), 0.86 (s, 9H), 0.83 (t, J = 7.7 Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$, mixture of carbamate rotamers) $\delta$ 172.9, 172.6, 171.8, 171.5, 171.4, 171.3, 169.6, 169.3, 169.3, 168.1, 168.0, 158.6, 154.1, 153.9, 135.7, 135.4, 133.1, 133.1, 130.4, 130.4, 128.0, 127.9, 122.9, 113.9, 113.9, 79.9, 79.5, 79.0, 72.2, 72.1, 63.7, 63.7, 60.3, 59.4, 59.3, 55.2, 51.8, 50.5, 50.5, 49.7, 46.4, 46.2, 37.8, 37.4, 37.3, 34.9, 34.8, 34.7, 34.6, 33.2, 31.1, 30.9, 30.5, 30.1, 29.4, 29.4, 29.0, 28.5, 28.4, 28.3, 26.0, 25.9, 24.3, 23.2, 23.1, 22.9, 20.7, 15.7, 14.4, 14.3, 13.4, 13.3, 10.5; IR (neat) 3321, 2965, 1732, 1694, 1514 cm$^{-1}$; HRMS (ESI) m/z for C$_{50}$H$_{79}$N$_5$NaO$_{11}$ [M+Na]$^+$ calcd 948.5668, found 948.5666.

**Amine 4.45**

To a 0 °C solution of oxazoline 4.44 (76.4 mg, 82 µmol) in CH$_2$Cl$_2$ (1 mL) was added 2,6-lutidine (77 µL, 0.66 mmol) followed by trimethylsilyl trifluoromethanesulfonate (74 µL, 0.41 mmol) dropwise. The reaction was warmed to rt and stirred for 1 h. After cooling to 0 °C, saturated aqueous NaHCO$_3$ (1 mL) was added and the mixture was warmed back to rt. The aqueous layer was extracted with CHCl$_3$ (10 x 1 mL) and the combined organic layers were dried over Na$_2$SO$_4$, filtered, and
concentrated under reduced pressure. The residual 2,6-lutidine was removed under a stream of argon and the crude residue was placed under high vacuum for 1 h. Purification via flash column chromatography on silica gel (98:2 to 95:5 CHCl₃/MeOH, v/v) yielded amine 4.45 (56.0 mg, 83%) as a white amorphous solid: Rₚ 0.15 (95:5 CHCl₃/MeOH, v/v); [α]²⁵ᵣ = −95.7 (c 0.95, CHCl₃); ¹H NMR (500 MHz, CDCl₃, mixture of carbamate rotamers) δ 7.08 (d, J = 7.8 Hz, 2H), 6.77 (d, J = 7.3 Hz, 2H), 6.56–6.54 (m, 1H), 6.13–6.11 (m, 1H), 5.39 (q, J = 6.8 Hz, 1H), 5.19 (ddd, J = 6.4, 6.4, 6.4 Hz, 1H), 4.89 (d, J = 10.5 Hz, 1H), 4.86–4.82 (m, 2H), 4.40 (dd, J = 9.6 Hz, 1H), 3.87 (ddd, J = 8.1 Hz, 1H), 3.82–3.78 (m, 1H), 3.75 (s, 3H), 3.67 (s, 3H), 3.11–3.06 (m, 2H), 3.02 (dd, J = 13.8, 7.2 Hz, 1H), 2.95 (s, 3H), 2.86–2.78 (m, 2H), 2.71 (s, 3H), 2.29–2.22 (m, 2H), 2.20–2.13 (m, 1H), 1.96–1.89 (m, 2H), 1.87 (d, J = 4.6 Hz, 3H), 1.83–1.77 (m, 1H), 1.76–1.71 (m, 1H), 1.69–1.63 (m, 1H), 1.55–1.48 (m, 2H), 1.43–1.35 (m, 3H), 1.27–1.20 (m, 1H), 1.25 (d, J = 6.7 Hz, 3H), 1.10–1.03 (m, 1H), 0.98–0.93 (m, 1H), 0.91 (d, J = 6.7 Hz, 3H), 0.88–0.86 (m, 3H), 0.87 (s, 9H), 0.84 (t, J = 7.2 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃, mixture of carbamate rotamers) δ 172.9, 172.6, 171.8, 171.5, 171.4, 171.3, 169.6, 169.3, 168.1, 158.6, 154.1, 153.9, 135.4, 133.1, 130.4, 128.0, 127.9, 113.9, 79.9, 79.5, 72.2, 63.7, 63.7, 60.3, 59.4, 59.3, 55.2, 51.8, 50.5, 49.7, 46.4, 46.2, 37.8, 34.8, 34.7, 33.2, 31.0, 30.9, 30.1, 28.5, 28.4, 26.0, 25.9, 25.0, 24.3, 23.2, 20.7, 15.7, 14.4, 14.3, 13.4, 13.3, 10.5; IR (neat) 3325, 2963, 1732, 1659, 1514 cm⁻¹; HRMS (ESI) m/z for C₄₅H₇₂N₅O₉ [M+H]⁺ calcd 826.5325, found 826.5322.
**Depsipipeptide 4.41**

To a 0 °C solution of amine 4.45 (6.7 mg, 8.1 µmol) in THF (40 µL), and t-BuOH (81 µL) was added a solution of lithium hydroxide (81 µL, 81 µmol, 1 M in H₂O). The reaction was warmed to rt and stirred for 4 h before the reaction was concentrated under a stream of argon. The residue was dissolved in CHCl₃ (1 mL) and neutralized with aqueous phosphate buffer (pH = 7, 0.5 mL). The separated aqueous phase was extracted with CHCl₃ (10 x 0.5 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The resulting residue was azeotropically dried with toluene twice and then placed under high vacuum for 2 h.

The crude amino acid was dissolved in CH₂Cl₂ (8.1 mL, 0.001 M) and cooled to 0 °C. To the cooled solution was added i-Pr₂NEt (7.1 µL, 40.5 µmol) followed by PyAOP (8.4 mg, 16.2 µmol) and the reaction mixture was warmed to rt. After stirring at rt for 24 h, the reaction mixture was concentrated under reduced pressure. Purification via preparative thin layer chromatography on silica gel (EtOAc, v) yielded cyclic depsipeptides 4.41 (3.7 mg, 55% from amine 4.45, 2.1 mg of *top diastereomer* 1.6 mg of *bottom diastereomer*) as a white amorphous solids: **TOP Diastereomer** [α]°D = –56.4 (c 0.11, MeOH); ¹H NMR (500 MHz, CDCl₃, ~ 1:1 mixture of carbamate 184
rotamers) δ 7.19 (d, J = 8.6 Hz, 1H), 7.16 (d, J = 8.5 Hz, 1H), 6.81 (d, J = 8.6 Hz, 1H), 6.79 (d, J = 8.6 Hz, 1H), 6.18 (d, J = 8.6 Hz, 0.5H), 5.90 (d, J = 9.7 Hz, 0.5H), 5.76 (dq, J = 8.8 Hz, 0.5H), 5.53 (dq, J = 8.2 Hz, 0.5H), 5.36–5.33 (m, 1H), 5.27 (d, J = 11.2 Hz, 1H), 5.13 (ddd, J = 10.0, 10.0, 5.0, 0.6H), 5.06 (ddd, J = 9.7, 9.7, 4 Hz, 0.4H), 5.00 (d, J = 10.6 Hz, 0.5H), 4.92–4.87 (m, 1.5H), 4.79–4.75 (m, 0.5H), 4.60 (ddd, J = 7.8, 7.8, 7.8 Hz, 0.5H), 4.40 (ddd, J = 10, 9 Hz, 0.5H), 4.32 (dd, J = 9, 6 Hz, 0.5H), 4.23 (ddd, J = 8, 8, 8 Hz, 1H), 4.12 (ddd, J = 7.1, 7.1, 7.1 Hz, 0.5H), 4.04–4.00 (m, 1H), 3.93 (dd, J = 8.5, 6.5 Hz, 0.5H), 3.77 (s, 3H), 3.68–3.59 (m, 1H), 3.37 (m, 0.5H), 3.18–3.07 (m, 1.5H), 3.00 (s, 1.5H), 2.87 (s, 1.5H), 2.85 (s, 1.5H), 2.84–2.80 (m, 1H), 2.62 (s, 1.5H), 2.28–2.25 (m, 1H), 2.21 (dd, J = 7.4 Hz, 1H), 2.19–2.15 (m, 1H), 2.13–2.07 (m, 1H), 2.03–1.99 (m, 2H), 1.92–1.85 (m, 2H), 1.91 (s, 3H), 1.68–1.61 (m, 2H), 1.31–1.24 (m, 2H), 1.27 (d, J = 8.0 Hz, 3H), 1.08 (d, J = 7 Hz, 1.5H), 1.01 (d, J = 6.4 Hz, 1.5H), 0.92 (d, J = 7.2 Hz, 1.5H), 0.89–0.88 (m, 2H), 0.86 (s, 9H), 0.76–0.72 (m, 1H), 0.60 (d, J = 7.2 Hz, 1.5H); HRMS (ESI) m/z for C_{44}H_{68}N_{5}O_{8} [M+H]^+ calcd 794.5062, found 794.5063.

**Bottom Diastereomer** [α]^{25}_D = –125 (c 0.06, MeOH); ^1H NMR (500 MHz, CDCl₃, mixture of carbamate rotamers) δ 7.17 (d, J = 8.5 Hz, 1H), 7.16 (d, J = 7.4 Hz, 1H), 6.82 (d, J = 8.7 Hz, 1H), 6.81 (d, J = 8.5 Hz, 1H), 6.30–6.28 (m, 1H), 6.12 (d, J = 9.6 Hz, 0.5H), 6.08 (d, J = 9.4 Hz, 0.5H), 5.36 (m, 0.3H), 5.22 (d, J = 11.4 Hz, 1H), 5.10 (ddd, J = 10.5, 10.5, 4.7 Hz, 0.7H), 4.99 (d, J = 12 Hz, 1H), 4.85 (d, J = 11 Hz, 1H), 4.83–4.75 (m, 1H), 4.33–4.25 (m, 1H), 4.21 (dd, J = 7.5, 7.5 Hz, 1H), 4.15–4.02 (m, 2H), 3.78 (s, 3H), 3.67 (dd, J = 16, 6.9 Hz, 1H), 3.64–3.58 (m, 0.5H), 3.32–3.27 (m, 0.5H), 3.19 (dd, J = 12 Hz, 0.3H), 3.13 (dd, J = 12 Hz, 0.7H), 3.00–2.98 (m, 0.3H), 2.95–2.91 (m, 1H), 185
2.89–2.87 (m, 0.7H), 2.85 (s, 3H), 2.81 (s, 2H), 2.63 (s, 1H), 2.40–2.15 (m, 4H), 2.09–2.02 (m, 2H), 1.94 (s, 3H), 1.94–1.85 (m, 3H), 1.72–1.59 (m, 4H), 1.28–1.21 (m, 2H), 1.23 (d, $J = 6.9$ Hz, 3H), 1.07 (d, $J = 6.9$ Hz, 1H), 1.03 (d, $J = 6.5$ Hz, 1H), 1.00 (d, $J = 6.9$ Hz, 2H), 0.94–0.92 (m, 2H), 0.90 (d, $J = 6.0$ Hz, 3H), 0.86 (s, 9H), 0.66 (d, $J = 7.4$ Hz, 1H); HRMS (ESI) $m/z$ for C$_{44}$H$_{68}$N$_5$O$_8$ [M+H]$^+$ calcd 794.5062, found 794.5081.

**Amide 4.47**

![Chemical structure of amide 4.47](image)

To a rt solution of acid 4.42 (51.3 mg, 0.124 mmol) in CH$_2$Cl$_2$ (2 mL) was added $i$-Pr$_2$NET (65 µL, 0.37 mmol), followed by HATU (50 mg, 0.13 mmol). After approximately 1 min, a solution of amino alcohol 4.10 (73 mg, 0.136 mmol) in CH$_2$Cl$_2$ (0.5 mL) was added. The resulting yellow solution was stirred at rt for 12 h, at which the reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (97:3 CH$_2$Cl$_2$/MeOH, v/v) gave amide 4.47 (0.11 g, 95%) as a white foam: $R_f$ 0.32 (97:3 CH$_2$Cl$_2$/MeOH, v/v); $[\alpha]^{25}_D = -90.6$ (c 1.38, CHCl$_3$); $^1$H NMR (500 MHz, CDC$_3$, mixture of carbamate rotamers) $\delta$ 7.09 (d, $J = 7.2$ Hz, 2H), 6.75 (d, $J = 8.5$ Hz, 2H), 5.38 (q, $J = 6.8$ Hz, 1H), 5.13 (ddd, $J = 7.4, 7.4, 7.4$ Hz, 1H), 4.86 (d, 186
$J = 10.5 \text{ Hz, 1H}$, 4.79–4.74 (m, 1H), 4.33–4.31 (m, 1H), 3.77–3.71 (m, 1H), 3.73 (s, 3H), 3.65 (s, 3H), 3.52–3.41 (m, 4H), 3.38–3.30 (m, 2H), 3.00 (dd, $J = 14.2, 6.9 \text{ Hz, 1H}$), 2.94 (s, 3H), 2.87–2.77 (m, 2H), 2.68 (s, 3H), 2.26–2.11 (m, 5H), 2.01–1.84 (m, 5H), 1.81–1.76 (m, 1H), 1.72–1.67 (m, 2H), 1.57–1.33 (m, 5H), 1.41 (s, 9H), 1.25–1.18 (m, 1H), 1.23 (d, $J = 6.6 \text{ Hz, 3H}$), 0.94–0.91 (m, 2H), 0.88 (d, $J = 6.9 \text{ Hz, 3H}$), 0.89–0.86 (m, 2H), 0.84 (s, 9H), 0.81 (t, $J = 7.4 \text{ Hz, 3H}$); $^{13}$C NMR (125 MHz, CDCl$_3$, mixture of carbamate rotamers) δ 174.5, 173.8, 173.5, 172.4, 171.7, 171.5, 171.4, 158.6, 158.6, 154.3, 154.0, 130.4, 128.2, 113.9, 80.2, 80.0, 79.8, 79.5, 64.7, 60.4, 59.2, 55.2, 51.8, 51.2, 50.5, 49.8, 46.5, 37.6, 36.0, 34.7, 34.3, 33.2, 32.6, 31.0, 30.9, 30.5, 30.1, 28.9, 28.5, 28.4, 28.1, 26.9, 26.0, 25.9, 24.9, 24.3, 23.2, 22.7, 20.9, 20.7, 15.7, 14.3, 12.5, 11.5, 10.5; IR (neat) 3310, 2925, 1732, 1694, 1543 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_{49}$H$_{81}$N$_{5}$NaO$_{12}$ [M+Na]$^+$ calcd 954.5774, found 954.5769.

**Oxazoline 4.48**

$
\begin{align*}
\text{To a } -78 ^\circ \text{C solution of alcohol 4.47 (0.110 g, 0.118 mmol) in CH}_2\text{Cl}_2 (2.4 \text{ mL}) \\
\text{was added DAST (23 } \mu \text{L, 0.177 mmol) dropwise. After stirring for 1.5 h at } -78 ^\circ \text{C, the}
\end{align*}$
reaction was quenched with aqueous saturated NaHCO₃ (1.5 mL) at −78 °C and the solution was warmed to rt. The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (10 x 2 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (97:3 CH₂Cl₂/MeOH, v/v) yielded oxazoline 4.48 (82.5 mg, 76%) as an amorphous white solid: R_f 0.35 (97:3 CH₂Cl₂/MeOH, v/v); [α]²⁵_D = −128.7 (c 1.03, CHCl₃); ¹H NMR (500 MHz, CDCl₃, mixture of carbamate rotamers) δ 7.07 (d, J = 8.5 Hz, 2H), 6.75 (d, J = 9 Hz, 2H), 6.49–6.45 (m, 1H), 5.37 (q, J = 6.5 Hz, 1H), 5.15 (ddd, J = 7.5, 7.5, 7.5 Hz, 1H), 4.87 (d, J = 10.5 Hz, 1H), 4.82–4.77 (m, 1H), 4.32 (dd, J = 9, 9 Hz, 1H), 4.19 (dd, J = 15.5, 8.5 Hz, 1H), 3.88–3.85 (m, 1H), 3.73 (s, 3H), 3.65 (s, 3H), 3.51–3.32 (m, 2H), 2.98 (dd, J = 13.5, 7 Hz, 1H), 2.93 (s, 3H), 2.77 (dd, J = 13.5, 7.5 Hz, 1H), 2.69 (s, 3H), 2.30–2.13 (m, 5H), 2.02–1.80 (m, 5H), 1.66–1.59 (m, 2H), 1.41 (s, 9H), 1.51–1.34 (m, 6H), 1.26–1.17 (m, 3H), 1.23 (d, J = 7 Hz, 3H), 1.12–0.97 (m, 2H), 0.95–0.92 (m, 2H), 0.89 (d, J = 6.5 Hz, 3H), 0.86 (s, 9H), 0.81 (t, J = 7.5 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃, mixture of carbamate rotamers) δ 172.9, 172.6, 171.9, 171.9, 171.7, 171.5, 171.4, 168.2, 167.9, 158.6, 154.1, 153.9, 150.4, 130.3, 128.1, 128.1, 113.9, 79.9, 79.5, 79.4, 79.1, 72.0, 72.0, 65.1, 60.3, 59.4, 59.3, 55.1, 51.8, 50.2, 49.7, 46.4, 46.1, 37.8, 34.9, 34.8, 34.7, 34.6, 33.2, 32.8, 31.5, 31.0, 30.8, 30.5, 30.1, 29.7, 29.4, 29.0, 28.5, 28.4, 28.3, 26.0, 25.9, 24.9, 24.3, 23.2, 23.1, 20.7, 15.7, 14.3, 10.5; IR (neat) 3306, 2963, 1746, 1643, 1447 cm⁻¹; HRMS (ESI) m/z for C₄₉H₈₀N₅O₁₁ [M+H]^⁺ calcd 914.5849, found 914.5851.
Amine 4.49

To a 0 °C solution of oxazoline 4.48 (82 mg, 89.7 µmol) in CH₂Cl₂ (1.5 mL) was added 2,6-lutidine (84 µL, 0.718 mmol) followed by trimethylsilyl trifluoromethanesulfonate (81 µL, 0.449 mmol) dropwise. The reaction was warmed to rt and stirred for 1 h. After cooling to 0 °C, saturated aqueous NaHCO₃ (1 mL) was added and the mixture was warmed back to rt. The aqueous layer was extracted with CHCl₃ (10 x 1 mL) and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residual 2,6-lutidine was removed under a stream of argon and the crude residue was placed under high vacuum for 1 h. Purification via flash column chromatography on silica gel (98:2 to 95:5 CHCl₃/MeOH, v/v) yielded amine 4.49 (56.0 mg, 80%) as a white amorphous solid: Rᵢ 0.15 (95:5 CHCl₃/MeOH, v/v); [α]²⁵<sup>D</sup> = −103.7 (c 0.98, CHCl₃); <sup>1</sup>H NMR (500 MHz, CDCl₃, mixture of carbamate rotamers) δ 7.17 (d, J = 8.5 Hz, 2H), 6.77 (d, J = 8.5 Hz, 2H), 6.25 (d, J = 7 Hz, 1H), 5.81 (dd, J = 9, 6.5 Hz, 1H), 5.38 (q, J = 7 Hz, 1H), 4.82 (d, J = 10.5 Hz, 1H), 4.21–4.14 (m, 1H), 3.79–3.77 (m, 1H), 3.74 (s, 3H), 3.65 (s, 3H), 3.53 (dd, J = 12.5, 4.5 Hz, 1H), 3.28–3.09 (m, 5H), 2.95–2.90 (m, 1H), 2.84 (s, 3H), 2.81–2.70 (m, 3H), 2.52 (s, 3H), 2.48–2.41 (m, 2H), 2.22–2.15 (m, 1H), 2.07–2.00 (m, 1H), 1.98–1.92 (m, 2H), 1.89
1.89–1.68 (m, 6H), 1.49–1.39 (m, 3H), 1.37–1.32 (m, 2H), 1.29–1.24 (m 1H); 1.25 (d, \(J = 6.5\) Hz, 3H), 1.17–1.11 (m, 1H), 1.03–0.95 (m, 2H), 0.91 (d, \(J = 7.5\) Hz, 3H), 0.87 (s, 9H), 0.79 (t, \(J = 7\) Hz, 3H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\), mixture of carbamate rotamers) \(\delta\) 175.1, 175.0, 173.0, 171.6, 171.5, 171.0, 170.1, 168.9, 168.6, 168.3, 158.5, 158.3, 130.5, 130.4, 128.7, 113.8, 79.9, 63.6, 60.4, 60.3, 55.2, 53.9, 53.5, 52.0, 51.8, 49.9, 47.2, 46.9, 43.7, 37.2, 35.9, 34.7, 34.2, 34.1, 33.8, 33.3, 30.7, 30.6, 29.7, 29.6, 29.4, 28.5, 26.4, 25.9, 25.6, 25.0, 24.5, 22.5, 22.4, 20.8, 15.8, 15.7, 14.8, 14.5, 11.3, 10.5; IR (neat) 3277, 2963, 1734, 1629, 1513 cm\(^{-1}\); HRMS (ESI) \(m/z\) for \(C_{44}H_{72}N_5O_9\) [M+H\(^{+}\)]\(^{\text{c}}\) calcd 814.5325, found 814.5328.

**Depsipeptide 4.46**

![Depsipeptide 4.46](image)

To a 0 °C solution of amine **4.49** (6.6 mg, 8.1 µmol) in THF (40 µL), and \(t\)-BuOH (80 µL) was added a solution of lithium hydroxide (81 µL, 81 µmol, 1 M in H\(_2\)O). The reaction was warmed to rt and stirred for 6 h before the reaction was concentrated under a stream of argon. The residue was dissolved in CHCl\(_3\) (1 mL) and neutralized with aqueous phosphate buffer (pH = 7, 0.5 mL). The separated aqueous phase was
extracted with CHCl₃ (10 x 0.5 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The resulting residue was azeotropically dried with toluene twice and then placed under high vacuum for 2 h.

The crude amino acid was dissolved in CH₂Cl₂ (8.1 mL, 0.001 M) and cooled to 0 °C. To the cooled solution was added i-Pr₂NEt (7.1 µL, 40.5 µmol) followed by PyAOP (8.4 mg, 16.2 µmol) and the reaction mixture was warmed to rt. After stirring at rt for 12 h, the reaction mixture was concentrated under reduced pressure. Purification via preparative thin layer chromatography on silica gel (95:5 EtOAc/MeOH, v/v) yielded cyclic depsipeptide 4.46 (2.8 mg, 58% from amine 4.49) as a white amorphous solid: [α]²⁵D = −89.4 (c 0.17, MeOH); ¹H NMR (500 MHz, CDCl₃, mixture of carbamate rotamers) δ 7.15 (d, J = 8.5 Hz, 2H), 6.80 (d, J = 8.5 Hz, 2H), 5.71 (dd, J = 9.1, 6.9 Hz, 0.5H), 5.68–5.63 (m, 0.5H), 5.17 (d, J = 7.5 Hz, 0.4H), 4.93–4.89 (m, 0.6H), 4.86–4.84 (m, 0.5H), 4.74 (d, J = 10.7 Hz, 0.5H), 4.66 (dd, J = 11.1, 2.0 Hz, 0.5H), 4.59–4.57 (m, 0.5H), 4.54 (d, J = 7.2 Hz, 0.5H), 4.45 (d, J = 7.6 Hz, 0.5H), 3.77 (s, 3H), 3.73–3.61 (m, 1H), 3.57–3.52 (m, 1H), 3.50–3.44 (m, 1H), 3.35 (ddd, J = 12, 3.2, 3.2 Hz, 1H), 3.23–3.19 (m, 1H), 3.19 (dd, J = 14.3, 6.4 Hz, 1H), 3.09–3.06 (m, 1H), 3.05 (s, 3H), 3.02 (s, 3H), 2.99–2.98 (m, 1H), 2.94–2.86 (m, 1H), 2.84 (br s, 1H), 2.72 (br s, 1H), 2.63–2.57 (m, 0.5H), 2.55–2.51 (m, 0.5H), 2.43–2.38 (m, 1H), 2.36–2.15 (m, 3H), 2.07–1.91 (m, 4H), 1.78–1.68 (m, 3H), 1.37 (d, J = 7.3 Hz, 1H), 1.29–1.26 (m, 2H), 1.18 (d, J = 6.3 Hz, 1H), 1.14 (m, 2H), 1.07–1.03 (m, 1H), 1.00–0.95 (m, 2H), 0.93–0.91 (m, 6H), 0.87–0.86 (m, 3H), 0.85 (s, 9H); HRMS (ESI) m/z for C₄₃H₆₇N₅O₈ [M+Na]⁺ calcd 804.4882, found 804.4885.
Amide 5.2

To a 0 °C of amide 5.1 (0.56 g, 1.96 mmol) in CH$_2$Cl$_2$ (20 mL) was added trifluoroacetic acid (20 mL) dropwise. After stirring for 1 h at 0 °C, toluene (20 mL) was added and the reaction mixture was concentrated under a stream of argon. The resulting residue was azeotropically dried with toluene (20 mL) twice and then placed under high vacuum for 1 h. The resulting crude de-protected product was advanced to the next step without further purification.

To a rt solution of N-Boc-N-methyl alanine (0.38 g, 1.87 mmol) in CH$_2$Cl$_2$ (16 mL) were added i-Pr$_2$NEt (0.98 mL, 5.61 mmol) and PyAOP (1.07 g, 2.06 mmol) sequentially. After stirring for 2 min at rt, a solution of crude deprotected amide 5.1 in CH$_2$Cl$_2$ (4 mL) was added and the reaction was stirred for 4 h at rt. Upon completion, the reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (1:1 hexanes/EtOAc, v/v) yielded amide 5.2 (0.61 g, 88% from amide 5.1) as a white gel that solidified in the refrigerator: R$_f$ 0.33 (1:1 hexanes/EOAc, v/v); [$\alpha$]$^\text{25D}$ = −145 (c 1.07, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$, mixture of carbamate rotamers) $\delta$ 5.89–5.84 (m, 1H), 5.31–5.27 (m, 1H), 5.24–5.20 (m, 1H), 5.10 (q, $J$ = 11.5 Hz, 1H), 4.98–4.95 (m, 1H), 4.67–4.51 (m, 2H), 3.00 (s, 3H), 2.75 (s, 3H), 2.03–1.98 (m 1H), 1.45 (s, 9H), 1.27 (d, $J$ = 6.5 Hz, 3H), 0.95 (d, $J$ = 7 Hz, 3H), 0.97–0.90 (m, 3H), 0.87–0.84 (m, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$, mixture of carbamate rotamers) $\delta$ 172.6, 170.9, 155.5, 131.8, 118.5, 80.0, 65.9, 65.3, 63.9, 60.6, 34.3, 33.3,
31.0, 29.7, 29.5, 28.5, 28.4, 28.3, 25.0, 24.8, 15.8, 15.7, 14.8, 14.5, 11.4, 10.5; IR (neat) 2972, 1739, 1693, 1651, 1391 cm\(^{-1}\); HRMS (ESI) \(m/z\) for C\(_{19}\)H\(_{34}\)N\(_2\)NaO\(_5\) [M+Na]\(^+\) calcd 393.2360, found 393.2361.

**Diamide 5.3**

To a 0 °C of amide 5.2 (1.02 g, 2.75 mmol) in CH\(_2\)Cl\(_2\) (28 mL) was added trifluoroacetic acid (28 mL) dropwise. The reaction was stirred at 0 °C for 1.5 h before toluene (20 mL) was added. The resulting mixture was concentrated under a stream of argon and additional toluene was added (20 mL). After concentrating the reaction mixture under a stream of argon, the crude product was placed under high vacuum for 2 h.

To a rt solution of N-Boc-O-methyl-L-tyrosine (0.68 g, 2.29 mmol) in CH\(_2\)Cl\(_2\) (23 mL) were added i-Pr\(_2\)NEt (1.2 mL, 6.87 mmol) and PyAOP (1.19 g, 2.29 mmol). After stirring for 1 min at rt, a solution of crude deprotected amide 5.2 in CH\(_2\)Cl\(_2\) (5 mL) was added dropwise and stirring was continued at rt. After 12 h, the reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (3:1 hexanes/EtOAc, v/v) yielded diamide 5.3 (0.86 g, 67% from amide 5.2) as a white amorphous solid: \(R_f\) 0.51 (2:1 hexanes/EOAc, v/v); \([\alpha]^{25}_D = -96.6\) (c 1.16, CHCl\(_3\)); \(^1\)H NMR (500 MHz, CDCl\(_3\), mixture of carbamate rotamers) \(\delta\) 7.09 (d, \(J = 8.5\) Hz, 2H), 6.76 (d, \(J = 8.5\) Hz, 2H), 5.89–5.81 (m, 1H), 5.37 (q, \(J = 6.5\) Hz, 1H), 5.26 (dd, \(J = 193\)
= 18.5 Hz, 1H), 5.19 (dd, 10, 1 Hz, 1H), 4.89 (d, J = 10 Hz, 1H), 4.79 (ddd, J = 7.5, 7.5, 7.5 Hz, 1H), 4.56–4.55 (m, 2H), 3.73 (s, 3H), 2.96 (dd, J = 13, 7 Hz, 1H), 2.91 (s, 3H), 2.76 (dd, J = 13.5, 6 Hz, 1H), 2.66 (s, 3H), 2.00–1.88 (m, 1H), 1.36 (s, 9H), 1.23 (d, J = 7 Hz, 3H), 0.97–0.90 (m, 2H), 0.90 (d, J = 6.5 Hz, 3H), 0.81 (t, J = 7.5 Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$, mixture of carbamate rotamers) $\delta$ 171.7, 171.7, 170.6, 158.5, 155.1, 131.7, 130.4, 128.4, 118.6, 113.9, 79.8, 65.3, 60.5, 55.2, 49.7, 38.2, 33.3, 30.9, 30.4, 28.3, 24.9, 15.7, 14.3, 10.3; IR (neat) 3304, 2970, 1738, 1643, 1248 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_{29}$H$_{45}$N$_3$O$_7$ [M+Na]$^+$ calcd 570.3150, found 570.3151.

**$\alpha,\beta$-unsaturated carboxylic acid 5.5**

![Chemical structure](image)

To a 0 °C solution of ester 5.7 (1.01 g, 3.37 mmol) in a mixture of $t$-BuOH (17 mL), THF (8.5 mL), and H$_2$O (8.5 mL) was added LiOH·H$_2$O (0.28 g, 6.74 mmol). After warming to rt and stirring for 4 h, the reaction mixture was acidified to pH = 3 with 1N HCl. The aqueous layer was extracted with EtOAc (5 x 20 mL) and the combined organic layers were washed with saturated aqueous NaCl, dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (96:4 CHCl$_3$/MeOH, v/v) yielded acid 5.5 (0.84 g, 88%) as a colorless viscous oil: $R_f$ 0.32 (96:4 CHCl$_3$/MeOH, v/v); [$\alpha$]$^2_{D}^{25}$ = +9.6 (c 1.0, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$, mixture of carbamate rotamers) $\delta$ 6.77 (d, J = 8 Hz, 1H), 4.73–4.60...
(m, 1H), 4.11 (dd, J = 8.5, 6.5 Hz, 1H), 3.70 (dd, J = 9, 3.5 Hz, 1H), 1.87 (s, 3H), 1.62 (s, 3H), 1.54 (s, 3H), 1.39 (s, 9H); $^{13}$C NMR (125 MHz, CDCl$_3$, mixture of carbamate rotamers) δ 172.9, 151.7, 143.3, 127.4, 94.6, 80.3, 67.5, 55.4, 28.4, 26.2, 24.2, 12.2; IR (neat) 3157, 2980, 1713, 1384, 1252 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_{14}$H$_{23}$NNaO$_5$ [M+Na]$^+$ calcd 308.1468, found 308.1469.

### Triamide 5.4

![Triamide 5.4](image)

To a solution of diamide 5.3 (0.217 g, 0.40 mmol) in CH$_2$Cl$_2$ (4 mL) at 0 °C was added trifluoroacetic acid (4 mL) dropwise. After stirring for 1 h at 0 °C, toluene (4 mL) was added and the reaction was concentrated under a stream of argon. Additional toluene (4 mL) was added and the mixture was concentrated under a stream of argon, at which the crude deprotected diamide 5.3 was place under high vacuum for 2 h.

To a solution of acid 5.5 (0.103 g, 0.36 mmol) in CH$_2$Cl$_2$ (3 mL) at rt was added $i$-Pr$_2$NEt (0.19 mL, 1.08 mmol), followed by PyAOP (0.19 g, 0.36 mmol). After stirring for 1 min, a solution of crude deprotected diamide 5.3 in CH$_2$Cl$_2$ (1 mL) was added and the mixture was stirred for 8 h at rt. The reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (3:1 to 1:1 hexanes/EtOAc, v/v) gave triamide 5.4 (0.191g, 74% from diamide 5.3) as a white
amorphous solid: R_f 0.26 (1:1 hexanes/EOAc, v/v); [α]^{25}_D = −76.7 (c 1.04, CHCl_3); \(^1\)H NMR (500 MHz, CDCl_3, mixture of rotamers) \(\delta\) 7.07 (d, \(J = 7\) Hz, 2H), 6.76 (d, \(J = 7\) Hz, 2H), 6.44–6.42 (m, 0.6H), 6.10–6.08 (m, 0.4H), 5.91–5.84 (m, 1H), 5.41 (m, 1H), 5.29 (dd, \(J = 17.5, 1.5\) Hz, 1H), 5.22 (dd, \(J = 10.5, 1\) Hz, 1H), 5.23–5.18 (m, 1H), 4.95–4.93 (m, 1H), 4.62–4.54 (m, 2H), 4.07 (dd, \(J = 9, 6.5\) Hz, 1H), 3.74 (s, 3H), 3.67–3.65 (m, 1H), 3.10–2.97 (m, 4H), 2.89–2.76 (m, 4H), 2.01–1.95 (m, 1H), 1.90–1.78 (m, 3H), 1.61 (m, 3H), 1.51 (s, 9H), 1.36 (m, 5H), 1.28 (m, 4H), 0.97–0.90 (m, 1H), 0.94 (d, \(J = 6.5\) Hz, 3H), 0.86 (t, \(J = 7.5\) Hz, 3H); \(^13\)C NMR (125 MHz, CDCl_3, mixture of rotamers) \(\delta\) 171.9, 171.6, 170.6, 169.6, 168.1, 158.6, 151.7, 135.1, 131.7, 130.4, 119.8, 118.7, 114.1, 114.0, 94.4, 93.8, 80.4, 80.0, 67.8, 66.0, 65.4, 60.5, 55.1, 50.4, 49.7, 33.3, 31.0, 30.4, 29.9, 28.4, 26.4, 24.0, 16.1, 15.8, 15.7, 14.9, 12.8, 11.6, 10.6; IR (neat) 2976, 1738, 1694, 1645, 1514 cm\(^{-1}\); HRMS (ESI) \(m/z\) for C\(_{38}\)H\(_{58}\)N\(_4\)NaO\(_9\) [M+Na]\(^+\) calcd 737.4096, found 737.4096.

**β-hydroxy ketone 5.12**

![Diagram of synthesis](image)

To a −78 °C solution of dicyclohexylboron chloride (0.83 mL, 3.81 mmol) in Et\(_2\)O (2 mL) was added dimethylethylamine (0.50 mL, 4.62 mmol) dropwise, followed by (S)-2-benzoyloxy-3-pentanone (0.53 g, 2.6 mmol) in Et\(_2\)O (10 mL) dropwise. The resulting mixture was stirred at −78 °C for 10 min, then warmed to 0 °C and stirred for 2
h. After re-cooling to $-78 \, ^\circ\text{C}$, a solution of aldehyde 2.12 (0.50 g, 1.36 mmol) in Et$_2$O (1.8 mL) was added via cannula and stirring was continued for 4 h at $-78 \, ^\circ\text{C}$. The reaction was held at $-20 \, ^\circ\text{C}$ for an additional 16 h before warming to 0 °C. The reaction was slowly quenched with MeOH (10 mL), followed by the addition of pH 7 buffer (10 mL) and H$_2$O$_2$ (10 mL, 30% aqueous solution). After stirring at 0 °C for 1 h, the mixture was partitioned between H$_2$O (10 mL) and EtOAc (20 mL). The aqueous phase was extracted with EtOAc (3 x 20 mL). The combined organic layers were dried of Na$_2$SO$_4$, filtered, and concentrated. Purification via flash column chromatography on silica gel (6:1 to 4:1 to 3:1 hexanes/EtOAc, v/v) yielded β-hydroxy ketone 5.12 (0.65 g, 83%) as a colorless oil: $R_f$ 0.41 (2:1 hexanes/EtOAc, v/v); $[\alpha]^25_D = -18.5$ (c 0.99, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$, mixture of carbamate rotamers) $\delta$ 8.07 (d, $J = 8$ Hz, 2H), 7.59–7.55 (m, 1H), 7.47–7.43 (m, 2H), 5.43 (q, $J = 7$ Hz, 1H), 4.81 (dd, $J = 9.5$, 2.5 Hz, 1H), 4.33–4.30 (m, 1H), 3.85–3.83 (m, 1H), 3.53–3.44 (m, 1.5H), 3.40–3.35 (m, 0.5H), 3.20 (d, $J = 6$ Hz, 1H), 2.88 (dq, $J = 14.5$, 14.5, 7.5, 7.5 Hz, 1H), 2.22–2.14 (m, 1H), 2.05–1.94 (m, 1H), 1.92–1.84 (m, 2H), 1.75–1.67 (m, 1H), 1.59–1.52 (m, 1H), 1.57 (d, $J = 7$ Hz, 3H), 1.48–1.46 (m, 1H), 1.44 (s, 5H), 1.42 (s, 4H), 1.37–1.31 (m, 1H), 1.29–1.22 (m, 1H), 1.22 (d, $J = 7$ Hz, 3H), 0.99 (d, $J = 7$ Hz, 3H), 0.88 (s, 4H), 0.87 (s, 5H); $^{13}$C NMR (125 MHz, CDCl$_3$, mixture of carbamate rotamers) $\delta$ 211.5, 173.1, 165.9, 154.4, 133.3, 129.9, 128.4, 109.5, 80.6, 79.7, 74.9, 72.4, 70.4, 59.1, 48.9, 46.5, 41.7, 37.9, 35.5, 34.8, 28.5, 25.9, 25.5, 24.1, 15.8, 14.5; IR (neat) 3466, 2969, 1741, 1699, 1452 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_{32}$H$_{49}$NNaO$_8$ [M+Na]$^+$ calcd 598.3350, found 598.3356.
**Carboxylic acid 5.13**

To a 0 °C solution of β-hydroxy ketone 5.12 (0.31 g, 0.54 mmol) in MeOH (5.4 mL) was added K$_2$CO$_3$ (97 mg, 0.70 mmol). After stirring at 0 °C for 3 h, the reaction mixture was diluted with EtOAc (20 mL) and washed with H$_2$O (10 mL). The aqueous layer was extracted with EtOAc (3 x 10 mL) and the combined organic layers were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The crude product was advanced to next step without further purification.

To a rt solution of the crude α-hydroxy ketone in t-BuOH (7.3 mL) and H$_2$O (3.7 mL) was added NaIO$_4$ (0.23 g, 1.08 mmol) in several portions. After stirring at rt for 3 h, the reaction was quenched with saturated aqueous NH$_4$Cl and the aqueous layer was extracted with EtOAc (3 x 15 mL). The combined organic layers were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (98:2 to 97:3 CH$_2$Cl$_2$/MeOH, v/v) to produce acid 5.13 (0.177 g, 74% from ketone 5.12) as a white foam: $R_f$ 0.27 (98:2, CH$_2$Cl$_2$/MeOH, v/v); $[\alpha]^{25}_D = -45.0$ (c 1.02, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$, mixture of carbamate rotamers) δ 4.85 (dd, $J = 11.5$, 1 Hz, 1H), 4.38 (dd, $J = 8.5$, 3 Hz, 1H), 3.73 (ddd, $J = 7$, 7, 4 Hz, 1H), 3.53–3.46 (m, 1H), 3.40–3.35 (m, 1H), 2.57–2.50 (m, 1H), 2.23–2.15 (m, 1H), 2.14–2.08 (m, 1H), 1.93–1.79 (m, 2H), 1.72–1.67 (m, 1H), 1.64–1.56 (m, 2H), 1.46 (s, 9H), 1.39–1.34 (m, 1H), 1.28 (d, $J = 7.5$ Hz, 3H), 0.97 (dd, $J = 10$, 5.5 Hz, 1H), 0.94 (d, $J$
= 6.5 Hz, 3H), 0.89–0.86 (m, 1H), 0.86 (s, 9H); $^{13}$C NMR (125 MHz, CDCl$_3$, mixture of carbamate rotamers) δ 177.5, 171.9, 155.1, 79.1, 71.8, 59.0, 46.0, 43.5, 41.5, 38.0, 35.0, 29.9, 26.0, 25.9, 25.6, 24.1, 22.1, 14.8; IR (neat) 3448, 2970, 1701, 1478, 1400 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_{23}$H$_{41}$NNaO$_7$ [M+Na]$^+$ calcd 466.2775, found 466.2777.

Amide 5.14

To a 0 °C solution of triamide 5.4 (0.142 g, 0.199 mmol) H$_2$O (0.5 mL) was added trifluoroacetic acid (1.5 mL) and the resulting mixture was warmed to rt. After stirring at rt for 2 h, the reaction was concentrated under reduced pressure, azeotropically dried with toluene (2 x 2 mL), and placed under high vacuum for 2 h. The resulting residue was advanced to the next step without further purification.

To a 0 °C solution of acid 5.13 (58.1 mg, 0.13 mmol) in CH$_2$Cl$_2$ (2 mL) was added $i$-Pr$_2$NEt (68 µL, 0.39 mmol), followed by HATU (53 mg, 0.14 mmol). After approximately 1 min, a solution of crude deprotected amino alcohol 5.4 (ca. 0.199 mmol) in CH$_2$Cl$_2$ (0.6 mL) was added. The resulting yellow solution was stirred at rt for 12 h, at which the reaction mixture was concentrated under reduced pressure.
Purification via flash column chromatography on silica gel (96:4 CH₂Cl₂/MeOH, v/v) gave amide 5.14 (91 mg, 69% from triamide 5.4) as a white foam: Rf 0.21 (24:1 chloroform/MeOH, v/v); [α]²⁵²⁰ = −51.8 (c 1.2, CHCl₃); ¹H NMR (500 MHz, CDCl₃, mixture of carbamate rotamers) δ 7.07 (d, J = 8.5 Hz, 2H), 6.77 (d, J = 8.5 Hz, 2H), 6.59 (d, J = 8.5 Hz, 0.5H), 6.30 (d, J = 8.5 Hz, 0.5H), 5.92–5.83 (m, 1H), 5.37 (q, J = 6.5 Hz, 1H), 5.33–5.25 (m, 1H), 5.21 (d, J = 10 Hz, 1H), 5.19–5.13 (m, 1H), 4.91 (d, J = 10.5 Hz, 1H), 4.72 (ddd, J = 8.5, 8.5, 8.5 Hz, 1H); 4.67–4.65 (m, 1H), 4.58 (dd, J = 6, 1.5 Hz, 1H), 4.27 (dd, J = 8.5, 4 Hz, 1H), 3.74 (s, 3H), 3.72–3.66 (m, 4H), 3.61–3.58 (m, 1H), 3.51–3.48 (m, 2H), 3.43–3.48 (m, 1H), 3.31 (d, J = 5 Hz, 1H), 3.01 (dd, J = 14, 7.5 Hz, 1H), 2.94 (s, 3H), 2.87–2.78 (m, 1H), 2.72 (s, 3H), 2.50–2.40 (m, 1H), 2.26–2.20 (m, 1H), 2.04–1.94 (m, 2H), 1.90 (s, 3H), 1.88–1.79 (m, 2H), 1.58–1.51 (m, 2H), 1.45–1.34 (m, 5H), 1.41 (s, 9H), 1.29–1.22 (m, 2H), 1.25 (d, J = 7.5 Hz, 3H), 0.95 (d, J = 6 Hz, 3H), 0.99–0.90 (m, 6H), 0.85 (s, 9H); ¹³C NMR (125 MHz, CDCl₃, mixture of carbamate rotamers) δ 176.7, 173.6, 171.8, 171.4, 170.6, 167.9, 158.7, 154.8, 131.7, 131.5, 130.4, 119.3, 118.7, 114, 80.2, 80.1, 72.5, 67.3, 65.9, 65.4, 64.8, 60.5, 59.2, 55.2, 50.8, 50.7, 49.7, 46.8, 43.7, 42.5, 38.0, 37.8, 37.5, 34.8, 34.7, 33.3, 31.0, 30.6, 30.2, 28.5, 28.4, 26.2, 25.0, 24.4, 22.2, 16.4, 15.8, 15.0, 14.4, 13.3, 12.7, 11.5, 10.6; IR (neat) 3410, 2968, 1732, 1643, 1634 cm⁻¹; HRMS (ESI) m/z for C₅₃H₈₅N₅O₁₃ [M+Na]⁺ calcd 1022.6036, found 1022.6035.
Oxazoline 5.15

To a –78 °C solution of amide 5.14 (68.5 mg, 0.068 mmol) in CH₂Cl₂ (1.4 mL) was added DAST (9.0 μL, 0.068 mmol) dropwise and stirring was continued for 1 h at –78 °C. Additional DAST (4.5 μL, 0.034 mmol) was added dropwise. After 0.5 h, the reaction was quenched at a –78 °C with saturated aqueous NaHCO₃ (1 mL) and allowed to warm to rt. The aqueous phase was extracted with CHCl₃ (10 x 1 mL) and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (97:3, CHCl₃/MeOH, v/v) yielded oxazoline 5.15 (54.2 mg, 81%) as a white amorphous solid: Rf 0.28 (24:1 chloroform/MeOH, v/v); [α]²⁵D = –71.6 (c 0.99, CHCl₃); ¹H NMR (500 MHz, CDCl₃, mixture of carbamate rotamers) δ 7.08 (d, J = 8.5 Hz, 2H), 6.78 (d, J = 8.5 Hz, 2H), 6.18 (d, J = 8.5 Hz, 1H), 5.97–5.81 (m, 1H), 5.40 (q, J = 6.5 Hz, 1H), 5.34 (dd, J = 17, 1.5 Hz, 1H), 5.31–5.28 (m, 1H), 5.25 (dd, J = 10.5, 1 Hz, 1H), 5.21–5.18 (m, 1H), 4.93 (ddd, J = 9, 9, 9 Hz, 1H), 4.88–4.81 (m, 2H), 4.63 (dd, J = 6, 1.5 Hz, 1H), 4.59 (dd, J = 6, 1 Hz, 1H), 4.43–4.33 (m, 2H), 3.89 (ddd, J = 8.5, 8.5, 3 Hz, 1H), 3.76 (s, 3H), 3.72–3.65 (m, 1H), 3.53–3.35 (m, 2H), 3.07 (m, 1H), 2.96 (s, 3H), 2.88–2.79 (m, 2H), 2.73 (s, 3H), 2.54–2.46 (m, 1H), 2.23–2.14 (m, 1H), 2.06–1.81 (m, 6H), 1.87 (s, 3H), 1.69–1.51
(m, 3H), 1.44 (s, 9H), 1.32–1.31 (m, 5H), 1.22 (d, J = 7 Hz, 3H), 0.99 (d, J = 6.5 Hz, 3H), 0.94 (d, J = 7 Hz, 3H), 0.90–0.81 (m, 3H), 0.89 (s, 9H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\), mixture of carbamate rotamers) δ 174.6, 171.8, 171.5, 171.4, 170.7, 158.7, 135.2, 133.2, 131.7, 130.4, 118.7, 118.6, 113.9, 80.1, 80.0, 76.6, 79.4, 79.4, 72.3, 68.1, 65.4, 65.1, 63.5, 60.5, 59.3, 56.9, 55.2, 50.6, 49.7, 49.6, 46.5, 46.2, 41.0, 39.3, 38.3, 38.0, 37.9, 37.7, 35.3, 34.9, 34.8, 33.3, 31.1, 30.6, 30.1, 28.5, 28.4, 28.2, 27.5, 26.0, 25.9, 25.7, 25.1, 24.3, 23.2, 22.1, 21.9, 15.5, 15.6, 15.2; IR (neat) 3337, 2967, 1734, 1699, 1400 cm\(^{-1}\); HRMS (ESI) \(m/z\) for C\(_{53}\)H\(_{84}\)N\(_5\)O\(_{12}\) [M+H]\(^+\) calcd 982.6111, found 982.6110.

**Amine 5.16**

To a 0 °C solution of oxazoline 5.15 (34 mg, 35 µmol) in CH\(_2\)Cl\(_2\) (0.7 mL) was added 2,6-lutidine (33 µL, 280 µmol) followed by trimethylsilyl trifluoromethanesulfonate (32 µL, 175 µmol) dropwise. The reaction was warmed to rt and stirred for 1 h. After cooling to 0 °C, saturated aqueous NaHCO\(_3\) (1 mL) was added dropwise. The aqueous layer was extracted with CHCl\(_3\) (10 x 1 mL) and the combined organic layers were dried over Na\(_2\)SO\(_4\), filtered, and concentrated under reduced pressure.
The residual 2,6-lutidine was removed under a stream of argon and the crude residue was placed under high vacuum for 1 h.

To a 0 °C solution of crude trimethylsilyl ether in THF (0.7 mL) was added tetrabutylammonium fluoride (0.11 mL, 110 µmol, 1 M solution in THF). After stirring at 0 °C for 30 min, saturated aqueous NaHCO₃ (1 mL) was added and the mixture was warmed to rt. The aqueous layer was extracted with CHCl₃ (10 x 1 mL) and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (98:2 to 95:5 CHCl₃/MeOH, v/v) yielded amine 5.16 (21.1 mg, 68% from oxazoline 5.15) as a light yellow amorphous solid: Rf 0.14 (9:1 CHCl₃/MeOH, v/v); [α]₂⁵ D = −114.9 (c 1.01, CHCl₃); ¹H NMR (500 MHz, CDCl₃, mixture of rotamers) δ 7.08 (d, J = 8 Hz, 2H), 6.78 (d, J = 8 Hz, 2H), 6.72 (d, J = 9 Hz, 1H), 6.22 (d, J = 8.5 Hz, 2H), 5.92–5.83 (m, 1H), 5.39 (q, J = 7 Hz, 1H), 5.29 (d, J = 17 Hz, 1H), 5.22–5.19 (m, 2H), 4.94–4.87 (m, 3H), 4.59 (m, 2H), 4.40 (dd, J = 9 Hz, 1H), 3.95 (dd, J = 8.5 Hz, 1 H), 3.76 (s, 3H), 3.68–3.64 (m, 1H), 3.25–3.22 (m, 1H), 3.13–3.09 (m, 1H), 3.02 (dd, J = 14, 7.5 Hz, 1H), 2.96 (s, 3H), 2.93–2.88 (m, 2H), 2.85–2.81 (m, 2H), 2.71 (s, 3H), 2.48–2.46 (m, 1H), 2.19–2.12 (m, 1H), 1.99–1.91 (m, 2H), 1.88 (s, 3H), 1.81–1.74 (m, 3H), 1.65–1.57 (m, 3H), 1.43 (m, 2H), 1.36–1.30 (m, 2H), 1.29–1.25 (m, 3H), 1.25 (d, J = 5 Hz, 3H), 1.02–0.99 (m, 2H), 0.97–0.96 (m, 2H), 0.93 (d, J = 6.5 Hz, 3H), 0.88 (s, 9H), 0.85 (t, J = 7 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃, mixture of rotamers) δ 175, 174.1, 171.8, 171.5, 171.3, 170.7, 170.6, 169.6, 168.0, 167.7, 166.5, 165.7, 163.2, 162.0, 158.7, 157.5, 156.7, 156.1, 155.8, 154.1, 151.9, 150.7, 135.2, 133.2, 131.7, 130.4, 128.1, 118.7, 114.0, 79.6, 72.1, 71.7, 65.4,
63.6, 60.5, 60.3, 55.2, 50.6, 49.7, 40.7, 38.6, 37.7, 37.3, 34.9, 33.3, 30.9, 30.4, 27.6, 26.0, 25.5, 25.1, 24.0, 21.4, 19.7, 15.8, 14.0, 13.5, 10.6; IR (neat) 3348, 2967, 1730, 1630, 1508 cm\(^{-1}\); HRMS (ESI) m/z for C\(_{46}H_{76}N_{10}O_{10}\) [M+H]\(^{+}\) calcd 882.5587 found 882.5589.

**Depsipeptide 5.11**

To a rt solution of amine 5.16 (7.8 mg, 8.8 \(\mu\)mol) in THF (0.18 mL) was added morpholine (9.2 \(\mu\)L, 106 \(\mu\)mol), followed by Pd(PPh\(_3\))\(_4\) (1.5 mg, 1.32 \(\mu\)mol). After stirring for 1 h at rt, the reaction mixture was concentrated under reduced pressure. The resulting crude solid was azeotropically dried with toluene twice and then place under high vacuum for 2 h.

The crude amino acid was dissolved in CH\(_2\)Cl\(_2\) (9 mL, 0.001 M) and cooled to 0 °C. To the cooled solution was added i-Pr\(_2\)NEt (9.3 \(\mu\)L, 53 \(\mu\)mol) followed by HATU (6.7 mg, 17.6 \(\mu\)mol) and the reaction mixture was warmed to rt. After stirring at rt for 24 h, the reaction mixture was concentrated under reduced pressure. Purification via preparative thin layer chromatography on silica gel (99:1 EtOAc/MeOH, v/v) yielded cyclic depsipeptide 5.11 (3.4 mg, 47% from amine 5.16) as a white amorphous solid:
[\alpha]^{25}_{D} = -126.0 (c 0.2, MeOH); $^1$H NMR (500 MHz, CDCl$_3$, mixture of rotamers) $\delta$ 7.13 (d, $J = 8.5$ Hz, 2H), 6.81 (d, $J = 8.5$ Hz, 2H), 6.29–6.25 (m, 2H), 5.34 (ddd, $J = 9.8, 9.8, 4.9$ Hz, 1H), 5.01 (d, $J = 11.4$ Hz, 1H), 4.88–4.83 (m, 3H), 4.32 (dd, $J = 8.8$ Hz, 1H), 4.29 (dd, $J = 8.4, 4.4$ Hz, 1H), 4.08–4.03 (m, 2H), 3.84–3.81 (m, 1H), 3.77 (s, 3H), 3.67–3.62 (m, 2H), 3.21 (dd, $J = 12.5, 9.6$ Hz, 1H), 2.88 (dd, $J = 13, 9$ Hz, 1H), 2.85–2.80 (m, 1H), 2.77 (s, 3H), 2.60 (s, 3H), 2.29–2.25 (m, 2H), 2.01–1.89 (m, 6H), 1.91 (s, 3H), 1.68–1.64 (m, 1H), 1.36–1.23 (m, 4H), 1.18 (d, $J = 7.1$ Hz, 3H), 1.09–1.05 (m, 2H), 0.99 (d, $J = 6.6$ Hz, 3H), 0.95 (d, $J = 8.6$ Hz, 3H), 0.86 (s, 9H), 0.81 (t, $J = 6.6$ Hz, 3H) HRMS (ESI) m/z for C$_{45}$H$_{70}$N$_5$O$_9$ [M+H]$^+$ calcld 824.5168, found 824.5167.

**β-hydroxy alcohol 5.18**

![Reaction Scheme](image)

To a $-78 \, ^{\circ}$C solution of oxazolidinone 5.17 (0.30 g, 1.3 mmol) in CH$_2$Cl$_2$ (4 mL) was added n-BuBOTf (1.5 mL, 1.54 mmol, 1 M solution in CH$_2$Cl$_2$). After 10 min, i-$\text{Pr}_2\text{NEt}$ (0.41 mL, 2.34 mmol) was added dropwise and stirring was continued for 1 h. The reaction mixture was warmed to 0 °C and stirred for 30 min, followed by cooling back to $-78 \, ^{\circ}$C. A solution of aldehyde 2.12 (0.24 g, 0.65 mmol) in CH$_2$Cl$_2$ (2.5 mL) was added dropwise to the reaction mixture over 10 min. After stirring at $-78 \, ^{\circ}$C for 1 h, the reaction was warmed to 0 °C and stirred for an additional 1 h. Upon completion, aqueous phosphate buffer (3 mL, pH = 7) and methanol (6 mL) were added carefully,
followed by dropwise addition of $\text{H}_2\text{O}_2$ (3 mL, 35% aqueous solution). The resulting mixture was stirred at 0 °C for 1 h, then the volatiles were removed under reduced pressure. The aqueous layer was extracted with $\text{Et}_2\text{O}$ (5 x 10 mL) and the combined organic layers were washed with saturated aqueous NaHCO$_3$ (10 mL), dried over MgSO$_4$, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (2:1 hexanes/EtOAc, v/v) yielded alcohol 5.18 (0.34 g, 87%) as a thick, colorless oil: $\left[\alpha\right]_{D}^{25} = -73.6$ (c 1.03, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$, mixture of carbamate rotamers) $\delta$ 7.32–7.29 (m, 2H), 7.27–7.22 (m, 2H), 7.20 (d, $J = 7.1$ Hz, 1H), 4.87 (dd, $J = 14.2$, 2.6 Hz, 1H), 4.70–4.65 (m, 1H), 4.34–4.27 (m, 1H), 4.20 (dd $J = 7$, 7 Hz, 1H), 4.17–4.11 (m, 1H), 3.97–3.86 (m, 1H), 3.63 (dddd, $J = 7.3$, 7.3, 7.3, 7.3 Hz, 1H), 3.52–3.43 (m, 1H), 3.41–3.37 (m, 1H), 3.27 (dd, $J = 13.4$, 3.3 Hz, 1H), 2.75 (dd, $J = 13.1$, 9.5 Hz, 1H), 2.21–2.13 (m, 1H), 2.01–1.96 (m, 1H), 1.92–1.84 (m, 3H), 1.76–1.58 (m, 2H), 1.46 (s, 5H), 1.42 (s, 4H), 1.28 (d, $J = 6.9$ Hz, 3H), 1.15 (dd, $J = 16$, 6.9 Hz, 1H), 1.01–0.94 (m, 2H), 0.98 (d, $J = 6.6$ Hz, 3H), 0.87 (s, 9H); $^{13}$C NMR (125 MHz, CDCl$_3$, mixture of carbamate rotamers) $\delta$ 175.8, 172.7, 154.4, 153.0, 135.5, 129.5, 128.9, 127.2, 127.0, 80.2, 78.1, 69.7, 66.0, 58.9, 55.4, 46.6, 44.8, 39.8, 37.7, 37.4, 34.7, 30.0, 28.5, 28.3, 28.3, 26.0, 25.9, 25.2, 24.3, 23.3, 20.3, 12.2; IR (neat) 3446, 2969, 1775, 1701, 1400 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_{33}$H$_{50}$N$_2$NaO$_8$ [M+Na]$^+$ calcd 625.3459, found 625.3459.
Carboxylic acid 5.19

To a 0 °C solution of imide 5.18 (47.5 mg, 0.079 mmol) in THF (3.3 mL) and H₂O (0.7 mL) was added H₂O₂ (56 µL, 0.63 mmol), followed by LiOH•H₂O (6.7 mg, 0.16 mmol). After stirring for 1 h at 0 °C, Na₂SO₃ (2 mL, 2 M aqueous solution) was added and vigorous stirring was continued for 10 min. The reaction mixture was acidified with citric acid (pH ~ 3) and the resulting mixture was extracted with EtOAc (5 x 10 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (97:3 to 95:5 CHCl₃/MeOH, v/v) gave acid 5.19 (30 mg, 86%) as a white amorphous solid: [α]²⁵D = −78.6 (c 1.03, CHCl₃); ¹H NMR (500 MHz, CDCl₃, mixture of carbamate rotamers) δ 4.87 (dd, J = 11.8, 1.9 Hz, 1H), 4.27 (dd, J = 8.4, 4.5 Hz, 1H), 3.89 (dd, J = 10.8, 5.2 Hz, 1H), 3.53–3.47 (m, 1H), 3.42–3.37 (m, 1H), 2.57–2.49 (m, 1H), 2.27–2.16 (m, 1H), 2.07–1.96 (m, 1H), 1.94–1.85 (m, 2H), 1.77–1.72 (m, 1H), 1.69–1.50 (m, 2H), 1.48–1.37 (m, 2H), 1.44 (s, 9H), 1.32–1.25 (m, 2H), 1.20 (d, J = 8.1 Hz, 3H), 0.95 (d, J = 6.8 Hz, 3H), 0.88 (s, 9H); ¹³C NMR (125 MHz, CDCl₃, mixture of carbamate rotamers) δ 177.9, 172.8, 154.7, 80.6, 80.4, 78.3, 70.9, 69.8, 59.0, 46.8, 46.7, 46.1, 45.5, 39.6, 38.6, 37.2, 34.9, 34.5, 30.0, 29.7, 28.5, 28.3, 25.9, 25.1, 24.9, 24.3, 24.2, 20.7, 20.4; IR (neat) 3349, 2968, 1739, 1701, 1400 cm⁻¹; HRMS (ESI) m/z for C₂₃H₄₁NNaO₇ [M+Na]⁺ calcd 466.2775, found 466.2775.
Amide 5.22

To a 0 °C solution of triamide 5.4 (0.12 g, 0.17 mmol) H$_2$O (0.4 mL) was added trifluoroacetic acid (1.3 mL) and the resulting mixture was warmed to rt. After stirring at rt for 2 h, the reaction was concentrated under reduced pressure, azeotropically dried with toluene (2 x 2 mL), and placed under high vacuum for 2 h. The resulting residue was advanced to the next step without further purification.

To a 0 °C solution of acid 5.19 (29.4 mg, 66.3 µmol) in CH$_2$Cl$_2$ (1.0 mL) was added i-Pr$_2$NEt (58 µL, 0.33 mmol), followed by HATU (46 mg, 0.12 mmol). After approximately 1 min, a solution of crude deprotected amino alcohol 5.4 (ca. 0.17 mmol) in CH$_2$Cl$_2$ (0.3 mL) was added. The resulting yellow solution was stirred at rt for 12 h, at which the reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (96:4 CH$_2$Cl$_2$/MeOH, v/v) yielded amide 5.22 (93.2 mg, 85% from triamide 5.4) as a white foam: [α]$^2$$_D$ = −48.6 (c 1.17, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$, mixture of carbamate rotamers) δ 7.06 (d, $J$ = 8.3 Hz, 2H), 6.76 (d, $J$ = 8.5 Hz, 2H), 6.55 (d, $J$ = 8.6 Hz, 1H), 6.23 (d, $J$ = 8.8 Hz, 1H), 5.90–5.82 (m, 1H), 5.37 (q, $J$ = 6.6 Hz, 1H), 5.28 (dd, $J$ = 17.1, 1.3 Hz, 1H), 5.21 (dd, $J$ = 10.3, 1.6 Hz,
1H), 5.15 (dd, J = 14.5, 7 Hz, 1H), 4.91 (d, J = 10.6 Hz, 1H), 4.84–4.80 (m, 1H), 4.74–4.68 (m, 1H), 4.58–4.57 (m, 2H), 4.26 (dd, J = 8.4, 3.9 Hz, 1H), 3.88–3.83 (m, 1H), 3.74 (s, 3H), 3.66–3.57 (m, 2H), 3.51–3.44 (m, 2H), 3.40–3.35 (m, 1H), 3.02 (dd, J = 14.2, 8.4 Hz, 1H), 2.93 (s, 3H), 2.88–2.81 (m, 1H), 2.72 (s, 3H), 2.33–2.28 (m, 1H), 2.23–2.16 (m, 1H), 2.05–1.92 (m, 1H), 1.91–1.83 (m, 2H), 1.88 (s, 3H), 1.75–1.57 (m, 2H), 1.45–1.37 (m, 4H), 1.42 (s, 9H), 1.28–1.22 (m, 1H), 1.24 (d, J = 7.4 Hz, 3H), 1.13 (d, J = 7.1 Hz, 3H), 0.98–0.90 (m, 4H), 0.92 (d, J = 7.4 Hz, 3H), 0.86 (s, 9H), 0.84 (t, J = 7.1 Hz, 3H); ^13^C NMR (125 MHz, CDCl₃, mixture of carbamate rotamers) δ 176.7, 173.1, 171.9, 171.5, 170.6, 167.9, 158.8, 154.7, 134.2, 132.2, 131.7, 130.6, 128.1, 118.6, 114.1, 80.3, 78.5, 69.6, 64.5, 60.5, 59.2, 55.7, 55.0, 50.6, 49.9, 47.1, 46.7, 43.7, 39.1, 37.7, 37.3, 34.6, 34.4, 33.3, 31.0, 30.5, 30.1, 28.5, 28.3, 25.9, 25.0, 24.3, 20.7, 15.8, 14.4, 13.4, 13.3, 12.6, 11.6, 10.6; IR (neat) 3412, 2968, 1734, 1629, 1406 cm⁻¹; HRMS (ESI) m/z for C₅₃H₈₅N₅NaO₁₃ [M+Na]^+ calcd 1022.6036, found 1022.6034.
Oxazoline 5.23

To a −78 °C solution of amide 5.22 (90.3 mg, 90 µmol) in CH₂Cl₂ (2 mL) was added DAST (13 µL, 90 µmol) dropwise and stirring was continued for 1 h at −78 °C. Additional DAST (9.5 µL, 72 µmol) was added dropwise. After 1 h, the reaction was quenched at a −78 °C with saturated aqueous NaHCO₃ (1 mL) and allowed to warm to rt. The aqueous phase was extracted with CHCl₃ (10 x 1 mL) and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (97:3, CHCl₃/MeOH, v/v) yielded oxazoline 5.23 (74 mg, 84%) as a white amorphous solid: [α]²⁵_D = −85.0 (c 1.0, CHCl₃); ¹H NMR (500 MHz, CDCl₃, mixture of carbamate rotamers) δ 7.07 (d, J = 8.4 Hz, 2H), 6.77 (d, J = 8.3 Hz, 2H), 6.42 (d, J = 8.1 Hz, 1H), 6.13 (d, J = 8.4 Hz, 1H), 5.91–5.85 (m, 1H), 5.39 (q, J = 6.6 Hz, 1H), 5.29 (d, J = 17 Hz, 1H), 5.21 (d, J = 11.2 Hz, 1H), 5.20–5.17 (m, 1H), 4.92 (d, J = 9.7 Hz, 1H), 4.88–4.78 (m, 2H), 4.58 (d, J = 12.8 Hz, 2H), 4.46–4.36 (m, 1H), 4.33–4.28 (m, 1H), 3.89–3.82 (m, 1H), 3.75 (s, 3H), 3.50–3.45 (m, 1H), 3.36 (dd, J = 17, 8.3 Hz, 1H), 3.03 (dd, J = 14. 7.4 Hz, 1H), 2.96 (s, 3H), 2.87–2.81 (m, 2H), 2.74 (s, 3H), 2.52–2.45 (m, 1H), 2.21–2.14 (m, 1H), 2.03–1.80 (m, 4H), 1.86 (s, 3H), 1.70–1.48 (m, 2H), 1.48–1.38 (m, 2H), 1.43 (s, 9H), 1.32–1.17 (m, 6H), 1.25 (d, J = 210
7.2 Hz, 3H), 0.99–0.87 (m, 4H), 0.93 (d, J = 7.2 Hz, 3H), 0.87 (s, 9H), 0.84 (t, J = 5.5 Hz, 3H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\), mixture of carbamate rotamers) \(\delta\) 172.6, 171.8, 171.6, 171.4, 170.6, 167.9, 158.6, 154.3, 135.4, 135.3, 133.0, 131.7, 130.4, 127.9, 11.8.7, 113.9, 80.0, 78.2, 71.9, 70.2, 65.4, 63.5, 60.5, 59.6, 59.0, 55.2, 50.5, 49.6, 46.6, 40.9, 40.1, 39.9, 37.4, 34.7, 33.3, 31.0, 30.5, 28.5, 26.1, 25.3, 25.1, 24.3, 23.2, 20.5, 16.1, 15.8, 14.4, 13.5, 13.4, 12.2, 11.6, 10.6; IR (neat) 3447, 2968, 1736, 1629, 1400 cm\(^{-1}\); HRMS (ESI) \(m/z\) for C\(_{53}\)H\(_{84}\)N\(_5\)O\(_{12}\) [M+H]\(^{+}\) calcd 982.6111, found 982.6109.

**Amine 5.24**

![Diagram of chemical structure](image)

To a 0 °C solution of oxazoline 5.23 (61 mg, 62 µmol) in CH\(_2\)Cl\(_2\) (1 mL) was added 2,6-lutidine (58 µL, 0.50 mmol) followed by trimethylsilyl trifluoromethanesulfonate (56 µL, 0.31 mmol) dropwise. The reaction was warmed to rt and stirred for 1 h. After cooling to 0 °C, saturated aqueous NaHCO\(_3\) (1 mL) was added dropwise. The aqueous layer was extracted with CHCl\(_3\) (10 x 1 mL) and the combined organic layers were dried over Na\(_2\)SO\(_4\), filtered, and concentrated under reduced pressure.
The residual 2,6-lutidine was removed under a stream of Ar and the crude residue was placed under high vacuum for 1 h.

To a 0 °C solution of crude trimethylsilyl ether in THF (1.2 mL) was added tetrabutylammonium fluoride (0.19 mL, 0.19 mmol, 1 M solution in THF). After stirring at 0 °C for 30 min, saturated aqueous NaHCO₃ (1 mL) was added and the mixture was warmed to rt. The aqueous layer was extracted with CHCl₃ (10 x 1 mL) and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (98:2 to 95:5 CHCl₃/MeOH, v/v) yielded amine **5.24** (28 mg, 51% from oxazoline **5.23**) as a light yellow amorphous solid: [α]ᵢ²⁵ᵣ = –109.0 (c 0.96, CHCl₃); ¹H NMR (500 MHz, CDCl₃, mixture of carbamate rotamers) δ 7.08 (d, J = 7.9 Hz, 2H), 6.78 (d, J = 8.4 Hz, 2H), 6.54 (d, J = 8.4 Hz, 1H), 6.16 (d, J = 8.4 Hz, 1H), 5.91–5.84 (m, 1H), 5.40 (q, J = 7.9 Hz, 1H), 5.29 (dd, J = 16.9, 1.3 Hz, 1H), 5.22 (d, J = 10.1 Hz, 1H), 5.21–5.17 (m, 1H), 4.93 (d, J = 11.1 Hz, 1H), 4.91–4.85 (m, 2H), 4.59 (d, J = 5.9 Hz, 2H), 4.46–4.39 (m, 1H), 3.93–3.88 (m, 2H), 3.87–3.82 (m, 1H), 3.76 (s, 3H), 3.29–3.17 (m, 2H), 3.10 (dd, J = 9.8, 6.5 Hz, 1H), 3.08–3.07 (m, 1H), 3.05 (dd, J = 5.2, 2.0 Hz, 1H), 2.97 (s, 3H), 2.86 (dd, J = 11.4, 5.8 Hz, 1H), 2.88–2.82 (m, 1H), 2.74 (s, 3H), 2.52–2.42 (m, 1H), 2.16 (dddd J = 16, 8.7, 8.7, 8.7 Hz, 1H), 1.98–1.90 (m, 2H), 1.88 (s, 3H), 1.82–1.76 (m, 3H), 1.76–1.73 (m, 1H), 1.56 (dd, J = 12 Hz, 1H), 1.37 (dd, J = 12.1 Hz, 1H), 1.30–1.24 (m, 1H), 1.26 (d, J = 7.5 Hz, 3H), 1.19 (d, J = 6.9 Hz, 3H), 1.01–0.96 (m, 2H), 0.96–0.92 (m, 3H), 0.94 (d, J = 6.3 Hz, 3H), 0.89 (s, 3H), 0.85 (t, J = 8.6 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃, mixture of carbamate rotamers) δ 174.8, 171.8, 171.4, 170.7, 168.0, 158.6, 135.3, 133.1,
131.7, 130.4, 127.9, 118.8, 113.9, 79.0, 78.9, 72.0, 71.8, 70.6, 69.7, 65.4, 63.5, 60.5, 60.0, 55.2, 50.5, 49.6, 46.6, 40.3, 40.0, 39.2, 37.2, 34.7, 33.3, 31.0, 30.6, 29.9, 26.0, 25.8, 25.6, 25.2, 25.0, 20.7, 15.8, 14.8, 14.4, 13.4, 12.2, 10.6; IR (neat) 3310, 2965, 1734, 1647, 1514 cm\(^{-1}\); HRMS (ESI) \(m/z\) for C\(_{48}\)H\(_{76}\)N\(_{5}\)O\(_{10}\) [M+H]\(^+\) calcd 882.5587, found 882.5589.

**Depsideptide 5.25**

![Depsideptide 5.25 diagram](image)

To a rt solution of amine 5.24 (15 mg, 17 \(\mu\)mol) in THF (0.2 mL) was added morpholine (19 \(\mu\)L, 204 \(\mu\)mol), followed by Pd(PPh\(_3\))\(_4\) (2.9 mg, 2.6 \(\mu\)mol). After stirring for 1 h at rt, the reaction mixture was concentrated under reduced pressure. The resulting crude solid was azeotropically dried with toluene twice and then place under high vacuum for 2 h.

The crude amino acid was dissolved in CH\(_2\)Cl\(_2\) (17 mL, 0.001 M) and cooled to 0 \(^\circ\)C. To the cooled solution was added \(i\)-Pr\(_2\)NEt (24 \(\mu\)L, 136 \(\mu\)mol) followed by HATU (12.9 mg, 34 \(\mu\)mol) and the reaction mixture was warmed to rt. After stirring at rt for 24 h, the reaction mixture was concentrated under reduced pressure. Purification via preparative thin layer chromatography on silica gel (99:1 EtOAc/MeOH, v/v) yielded
cyclic depsipeptide 5.25 (5.8 mg, 41% from amine 5.24) as a white amorphous solid: 
\([\alpha]_{D}^{25} = -117.1 (c 0.21, \text{MeOH})\); \(^1\)H NMR (500 MHz, CDCl\(_3\), mixture of rotamers) \(\delta \) 7.14 (d, \(J = 8.5\) Hz, 2H), 6.81 (d, \(J = 8.6\) Hz, 2H), 6.26 (dq, \(J = 10.2, 1.3\) Hz, 1H), 5.33 (ddd, \(J = 10, 10, 4.6\) Hz, 1H), 5.23 (dd, \(J = 11.6, 4.4\) Hz, 0.3H), 5.10–5.04 (m, 0.3H), 5.01–4.96 (m, 0.3H), 4.93 (dd, \(J = 12.9, 3.4\) Hz, 0.7H), 4.89 (d, \(J = 11.8\) Hz, 1H), 4.83–4.78 (m, 1H), 4.75 (q, \(J = 7\) Hz, 1H), 4.40 (dd, \(J = 8.4, 5.6\) Hz, 0.7H), 4.37–4.32 (m, 0.3H), 4.28 (dd, \(J = 7.9, 7.9\) Hz, 1H), 4.22 (dd, \(J = 8.2, 4.2\) Hz, 1H), 4.19–4.16 (m, 1H), 4.13–4.09 (m, 2H), 3.77 (s, 3H), 3.70–3.60 (m, 1H), 3.35–3.29 (m, 0.3H), 3.20 (dd, \(J = 12.9, 10.7\) Hz, 0.7H), 3.15–3.08 (m, 0.3H), 2.91 (dd, \(J = 12.3, 4.8\) Hz, 1H), 2.91 (s, 1H), 2.82 (d, \(J = 4.8\) Hz, 1H), 2.80 (s, 2H), 2.74 (s, 1H), 2.59 (s, 2H), 2.41–2.34 (m, 1H), 2.29–2.24 (m, 1H), 2.11–2.05 (m, 1H), 1.99–1.87 (m, 2H), 1.93 (s, 3H), 1.77 (ddd, \(J = 13.5, 13.5, 2.5\) Hz, 1H), 1.73–1.67 (m, 1H), 1.30–1.22 (m, 2H), 1.13 (d, \(J = 6.7\) Hz, 3H), 1.08 (d, \(J = 7.1\) Hz, 3H), 1.03–0.97 (m, 2H), 0.99 (d, \(J = 6.7\) Hz, 3H), 0.96–0.92 (m, 2H), 0.88 (s, 9H), 0.82 (t, \(J = 7.9\) Hz, 3H); HRMS (ESI) \(m/z\) for C\(_{45}\)H\(_{69}\)N\(_5\)NaO\(_9\) [M+Na]\(^+\) calcd 846.4987, found 846.4997.

**Tyrosine 6.7**

![Chemical Reaction](image)

To a 0 °C solution of \(N\)-Boc-L-tyrosine (6.5) (1.13 g, 4.0 mmol) in dry DMF (16 mL) was added NaH (0.37 g, 9.2 mmol, 60% in mineral oil) in a single portion. After
stirring at 0 °C for 1 h, alkyl iodide \textbf{6.6} (1.13 g, 4.0 mmol) was added as a solution in DMF (2 mL). The resulting mixture was stirred for 3 h at 0 °C, at which cooled \(\text{H}_2\text{O}\) (15 mL) was added. The aqueous layer was extracted with EtOAc (3 x 10 mL) and the extracted aqueous phase was acidified with 6N HCl (pH ~ 3) with cooling. The acidified aqueous layer was extracted with EtOAc (5 x 15 mL) and the combined organic layers were dried over \(\text{Na}_2\text{SO}_4\), filtered, and concentrated under reduced pressure to give a crude white solid. Purification via flash column chromatography on silica gel (95:5 \(\text{CHCl}_3/\text{MeOH}\), v/v) yielded acid \textbf{6.7} (1.39 g, 79%) as a white gel-like solid: \(R_f\) 0.24 (95:5 \(\text{CHCl}_3/\text{MeOH}\), v/v); \(\left[\alpha\right]^{25}_D\) = +20.9 (c 1.39, \(\text{CHCl}_3\)); \(^1\text{H} \text{NMR} (500 MHz, \text{CDCl}_3,\) mixture of rotamers) \(\delta\) 7.08 (d, \(J = 8.5\) Hz, 2H), 6.85 (d, \(J = 9\) Hz, 2H), 4.95–4.93 (m, 0.6H), 4.54–4.53 (m, 0.6H), 4.11 (dd, \(J = 5, 5\) Hz, 2H), 3.86 (dd, \(J = 5, 5\) Hz, 2H), 3.74 (dd, \(J = 4, 4\) Hz, 1H), 3.73 (m, 1H), 3.70–3.67 (m, 4H), 3.38 (t, \(J = 5\) Hz, 2H), 3.11 (dd, \(J = 14.5, 5\) Hz, 1H), 3.02 (dd, \(J = 13.5, 5\) Hz, 1H), 1.42 (s, 9H); \(^{13}\text{C} \text{NMR} (125 MHz, \text{CDCl}_3,\) mixture of rotamers) \(\delta\) 175.9, 157.9, 155.4, 130.4, 128.1, 114.8, 80.2, 70.8, 70.7, 69.9, 67.4, 54.4, 50.7, 37.0, 28.3; IR (neat) 2974, 2108, 1713, 1613, 1512 cm\(^{-1}\); HRMS (ESI) \(m/z\) for \(\text{C}_{20}\text{H}_{30}\text{N}_4\text{NaO}_7\) \([\text{M+Na}]^+\) calcd 461.2007, found 461.2008.
Diamide 6.8

To a solution of amide 5.2 (0.583 g, 1.57 mmol) in CH₂Cl₂ (16 mL) at 0 °C was added trifluoroacetic acid (16 mL) dropwise and stirring was continued for 1 h at 0 °C. The reaction was diluted with toluene (15 mL) and concentrated to dryness under a stream of argon. The resulting residue was re-dissolved in toluene (15 mL), concentrated to dryness, and placed under high vacuum for 2 h.

To a solution of acid 6.7 (0.63 g, 1.43 mmol) in CH₂Cl₂ (12 mL) were added PyAOP (0.75 g, 1.43 mmol) and i-Pr₂NEt (0.75 mL, 4.29 mmol) at rt. The resulting mixture was stirred for 1 min before a solution of deprotected crude 5.2 in CH₂Cl₂ (4 mL) was added. After stirring for 12 at rt, the reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (1:1 hexanes/EtOAc, v/v) gave diamide 6.8 (0.732 g, 74% from amide 5.2) as a white foam: Rf 0.34 (1:1 hexanes/EOAc, v/v); [α]²⁵ᵦ = −51.0 (c 1.11, CHCl₃); ¹H NMR (500 MHz, CDCl₃, mixture of carbamate rotamers) δ 7.05 (d, J = 8.5 Hz, 2H), 6.75 (d, J = 8 Hz, 2H), 5.86–5.78 (m, 1H), 5.34 (q, J = 6.5 Hz, 1H), 5.26–5.27 (m, 2H), 5.17 (d, J = 10.5 Hz, 1H), 4.86 (d, J = 10.5 Hz, 1H), 4.76 (ddd, J = 8.5, 7, 7 Hz, 1H), 4.54–4.52 (m, 2H), 4.03 (dd, J = 4.5, 4.5 Hz, 2H), 3.79 (dd, J = 4.5, 4.5 Hz, 2H), 3.67–3.66 (m, 2H), 3.62 (dd, J = 5 Hz, 4H), 3.32 (t, J = 4.5 Hz, 2H), 2.92 (dd, J = 14, 8 Hz, 1H), 2.89 (s, 3H), 2.74–2.71 (m, 1H), 2.67 (s, 3H), 1.97–1.88 (m, 1H), 1.33 (s, 9H), 1.21 (d, J = 6.5 Hz, 3H), 0.94–0.87 (m, 2H), 216
0.88 (d, J = 6.5 Hz, 3H), 0.79 (t, J = 7.5 Hz, 3H); $^1$H NMR (125 MHz, CDCl$_3$, mixture of carbamate rotamers) δ 171.7, 171.7, 170.6, 157.7, 155.1, 131.7, 131.4, 130.4, 130.3, 128.6, 118.6, 114.6, 79.7, 70.8, 70.7, 70.1, 69.8, 67.4, 65.3, 60.5, 51.8, 50.7, 49.7, 38.2, 34.2, 33.2, 30.9, 30.4, 28.3, 24.9, 15.7, 14.3, 11.6, 10.5; IR (neat) 3302, 2934, 2104, 1701, 1508; HRMS (ESI) m/z for C$_{34}$H$_{54}$N$_6$NaO$_9$ [M+Na]$^+$ calcd 713.3844, found 713.3845.

**Triamide 6.9**

![Diagram of triamide 6.9](image)

To a 0 °C solution of diamide 6.8 (0.317 g, 0.459 mmol) in CH$_2$Cl$_2$ (4.6 mL) was added trifluoroacetic acid (4.6 mL). The reaction was stirred at 0 °C for 1 h before toluene (2 mL) was added. The mixture was concentrated under a stream of argon and additional toluene (2 mL) was added. After removing the toluene under a stream of argon, the viscous oil was placed under high vacuum for 2 h.

To a rt solution of acid 5.5 (120 mg, 0.42 mmol) in CH$_2$Cl$_2$ (3 mL) was added i-Pr$_2$NEt (0.22 mL, 1.26 mmol) followed by HATU (167 g, 0.44 mmol). After stirring for 1 min, a solution of deprotected diamide 6.8 in CH$_2$Cl$_2$ (2 mL) was added dropwise and the reaction was stirred at rt for 8 h. The reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (1:1 hexanes/EtOAc, v/v) yielded triamide 6.9 (0.319 g, 89% from diamide 6.8) as a white
foam: Rf 0.31 (1:1 hexanes/EOAc, v/v); [α]$_D^{25}$ = −46.4 (c 1.05, CHCl$_3$); $^1$H NMR (500 MHz, CD$_3$OD, mixture of rotamers) δ 7.19 (d, $J = 8.5$ Hz, 2H), 6.85 (d, $J = 8.5$ Hz, 2H), 6.15 (dq, $J = 9.5$, 1.5 Hz, 1H), 5.97–5.88 (m, 1H), 5.33–5.22 (m, 3H), 5.10–5.07 (m, 1H), 4.67–4.58 (m, 2H), 4.60 (d, $J = 5.5$ Hz, 1H), 4.14 (dd, $J = 9$, 6.5 Hz, 1H), 4.09 (dd, $J = 4$ Hz, 2H), 3.72–3.70 (m, 3H), 3.68–3.66 (m, 4H), 3.36 (t, $J = 5$ Hz, 2H), 3.13–2.89 (m, 5H), 2.83 (br s, 1H), 2.74–2.70 (m, 2H), 2.06–1.97 (m, 1H), 1.86–1.82 (m, 3H), 1.61 (s, 3H), 1.52 (s, 3H), 1.48 (br s, 3H), 1.38–1.29 (m, 3H), 1.34 (s, 6H), 1.25–1.22 (m, 3H), 0.98 (d, $J = 7$ Hz, 2H), 0.95 (d, $J = 7$ Hz, 3H), 0.86 (t, $J = 7$ Hz, 3H); $^{13}$C NMR (125 MHz, CD$_3$OD, mixture of rotamers) δ 173.3, 173.0, 172.7, 171.2, 170.3, 159.0, 158.7, 136.5, 132.9, 132.9, 132.6, 131.2, 131.1, 129.7, 119.9, 118.5, 115.4, 95.1, 95.0, 81.0, 80.9, 71.4, 71.2, 70.8, 70.5, 68.5, 68.2, 66.8, 66.1, 62.1, 56.3, 56.2, 52.4, 51.7, 51.4, 33.9, 31.7, 31.2, 28.4, 25.8, 15.9, 14.6, 12.8, 10.4; IR (neat) 2976, 2108, 1734, 1695, 1512 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_{43}$H$_{67}$N$_7$NaO$_{11}$ [M+Na]$^+$ calcd 880.4791, found 880.4791.
Amide 6.10

To a 0 °C solution of triamide 6.9 (0.107 g, 0.125 mmol) H₂O (0.3 mL) was added trifluoroacetic acid (0.95 mL) and the resulting mixture was warmed to rt. After stirring at rt for 2 h, the reaction was concentrated under reduced pressure, azeotropically dried with toluene (2 x 2 mL), and placed under high vacuum for 2 h. The resulting residue was advanced to the next step without further purification.

To a 0 °C solution of acid 3.2 (29.4 mg, 66.3 µmol) in CH₂Cl₂ (1 mL) was added i-Pr₂NET (35 µL, 0.20 mmol), followed by HATU (26.5 mg, 69.6 µmol). After approximately 1 min, a solution of crude deprotected amino alcohol 6.9 (ca. 0.125 mmol) in CH₂Cl₂ (0.3 mL) was added. The resulting yellow solution was stirred at rt for 12 h, at which the reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (99:1 to 98:2 EtOAc/MeOH, v/v) gave amide 6.10 (59.0 mg, 78% from triamide 6.9) as a white foam: [α]²⁵_D = −56.9 (c 1.18, CHCl₃); ¹H NMR (500 MHz, CDCl₃, mixture of rotamers) δ 7.06 (d, J = 8.5 Hz, 2H), 6.79 (d, J = 8.5 Hz, 2H), 6.62 (d, J = 7.5 Hz, 0.5H), 6.50 (d, J = 8 Hz, 0.5H), 6.21 (d,
$J = 8.5 \text{ Hz, 1H}$, 5.91–5.83 (m, 1H), 5.38 (q, $J = 7 \text{ Hz, 1H}$), 5.29 (dd, $J = 17.5, 1.5 \text{ Hz, 1H}$), 5.21 (dd, $J = 10.5, 1 \text{ Hz, 1H}$), 5.16 (ddd, $J = 7, 7, 7 \text{ Hz, 1H}$), 4.91 (d, $J = 10.5 \text{ Hz, 1H}$), 4.86–4.79 (m, 2H), 4.58 (dd, $J = 5.5, 1 \text{ Hz, 2H}$), 4.29 (dd, $J = 8.5, 3.5 \text{ Hz, 1H}$), 4.07 (t, $J = 4.5 \text{ Hz, 2H}$), 3.83 (t, $J = 5 \text{ Hz, 2H}$), 3.73–3.71 (m, 3H), 3.68–3.59 (m, 3H), 3.67 (t, $J = 5 \text{ Hz, 2H}$), 3.51–3.48 (m, 2H), 3.67 (dd, $J = 5, 5 \text{ Hz, 2H}$), 3.02 (dd, $J = 13.5, 7 \text{ Hz, 1H}$), 2.95 (s, 3H), 2.87–2.81 (m, 1H), 2.78 (s, 3H), 2.24–2.16 (m, 3H), 2.05–1.92 (m, 2H), 1.92–1.83 (m, 2H), 1.88 (s, 3H), 1.79–1.75 (m, 1H), 1.63–1.52 (m, 2H), 1.45–1.40 (m, 1H), 1.43 (s, 9H), 1.37–1.32 (m, 2H), 1.32–1.21 (m, 1H), 1.25 (d, $J = 7 \text{ Hz, 3H}$), 1.16–1.08 (m, 1H), 1.11 (d, $J = 7 \text{ Hz, 3H}$), 0.99–0.95 (m, 2H), 0.95–0.91 (m, 6H), 0.89–0.83 (m, 3H), 0.87 (s, 9H); $^{13}$C NMR (125 MHz, CDCl$_3$, mixture of rotamers) $\delta$ 176.2, 172.9, 171.9, 171.5, 170.6, 167.7, 157.9, 154.8, 133.9, 132.1, 131.7, 130.4, 128.2, 118.7, 114.7, 80.3, 78.7, 71.7, 69.8, 67.4, 65.4, 64.6, 60.6, 59.1, 50.7, 50.2, 49.7, 47.4, 46.7, 40.1, 38.6, 37.5, 37.3, 34.7, 33.3, 31.1, 30.6, 29.9, 28.5, 28.3, 26.0, 25.9, 25.2, 25.1, 24.2, 20.3, 15.8, 14.5, 14.4, 13.3, 10.6; IR (neat) 3416, 2965, 2108, 1732, 1614, 1454 cm$^{-1}$; HRMS (ESI) $m/z$ for C$_{58}$H$_{94}$N$_8$NaO$_{15}$ [M+Na]$^+$ calcd 1165.6731, found 1165.6730.
Oxazoline 6.11

To a −78 °C solution of amide 6.10 (59 mg, 51.6 µmol) in CH₂Cl₂ (1.0 mL) was added DAST (6.8 µL, 51.6 µmol) dropwise and stirring was continued for 1.5 h at −78 °C. Additional DAST (3.4 µL, 25.8 µmol) was added dropwise. After 0.5 h, the reaction was quenched at a −78 °C with saturated aqueous NaHCO₃ (1 mL) and allowed to warm to rt. The aqueous phase was extracted with CHCl₃ (10 x 1 mL) and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (100:1, EtOAc/MeOH, v/v) yielded oxazoline 6.11 (42 mg, 72%) as a white amorphous solid: [α]²⁵_D = −97.6 (c 1.03, CHCl₃); ¹H NMR (500 MHz, CDCl₃, mixture of rotamers) δ 7.06 (d, J = 8.4 Hz, 2H), 6.79 (d, J = 8.2 Hz, 2H), 6.44 (d, J = 8.5 Hz, 1H), 6.15 (d, J = 8.4 Hz, 1H), 5.91–5.83 (m, 1H), 5.40 (q, J = 7.9 Hz, 1H), 5.29 (dd, J = 17.2, 1.2 Hz, 1H), 5.22 (dd, J = 10.5, 1 Hz, 1H), 5.21–5.17 (m, 1H), 4.93 (m, 2H), 4.86 (d, J = 12.7 Hz, 1H), 4.58 (d, J = 5.7 Hz, 2H), 4.46–4.37 (m, 1H), 4.33–4.29 (m, 1H), 4.07 (t, J = 3.5 Hz, 2H), 3.90–3.86 (m, 1H), 3.84 (t, J = 4.1 Hz, 2H), 3.73–3.71 (m, 3H), 3.67 (t, J = 5.2 Hz, 4H), 3.50–3.45 (m, 1H), 3.38 (t, J = 5.2 Hz, 3H), 3.03 (dd, J = 13.8, 7.5 Hz, 1H), 2.96 (s, 3H), 2.87–2.81 (m, 1H), 2.77
(s, 3H), 2.54–2.45 (m, 1H), 2.20–2.11 (m, 1H), 2.03–1.80 (m, 6H), 1.87 (s, 3H), 1.68–1.57 (m, 2H), 1.48–1.39 (m, 1H), 1.44 (s, 9H), 1.30–1.25 (m, 5H), 1.18 (d, \(J = 8.0\) Hz, 3H), 1.01–0.97 (m, 2H), 0.97–0.93 (m, 6H), 0.87 (s, 9H), 0.85 (t, \(J = 7.2\) Hz, 3H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\), mixture of rotamers) \(\delta\) 171.9, 171.7, 171.5, 170.6, 168.0, 157.9, 154.2, 153.9, 135.6, 135.3, 132.9, 131.7, 130.4, 130.2, 128.2, 119.9, 118.7, 114.6, 80.1, 79.9, 79.1, 78.1, 70.9, 70.7, 70.1, 69.8, 67.4, 65.4, 63.5, 60.5, 59.6, 59.1, 50.7, 50.5, 49.6, 46.6, 46.2, 40.5, 40.1, 39.0, 38.0, 37.8, 37.6, 34.7, 33.3, 31.0, 30.5, 30.0, 28.5, 28.3, 26.1, 26.0, 25.8, 25.1, 25.0, 24.3, 23.2, 20.7, 20.4, 15.8, 14.7, 14.4, 14.3, 13.4, 10.6; IR (neat) 3472, 2967, 2106, 1732, 1402 cm\(^{-1}\); HRMS (ESI) \(m/z\) for C\(_{58}\)H\(_{92}\)N\(_8\)NaO\(_{14}\) [M+Na]\(^+\) calcd 1147.6625, found 1147.6623.

**Amine 6.12**

![Diagram](image)

To a 0 °C solution of oxazoline **6.11** (41 mg, 36 µmol) in CH\(_2\)Cl\(_2\) (0.4 mL) was added 2,6-lutidine (34 µL, 291 µmol) followed by trimethylsilyl trifluoromethanesulfonate (33 µL, 182 µmol) dropwise. The reaction was warmed to rt
and stirred for 1 h. After cooling to 0 °C, saturated aqueous NaHCO₃ (1 mL) was added dropwise. The aqueous layer was extracted with CHCl₃ (10 x 1 mL) and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residual 2,6-lutidine was removed under a stream of argon and the crude residue was placed under high vacuum for 1 h.

To a 0 °C solution of crude trimethylsilyl ether in THF (0.5 mL) was added tetrabutylammonium fluoride (0.11 mL, 109 µmol, 1 M solution in THF). After stirring at 0 °C for 30 min, saturated aqueous NaHCO₃ (1 mL) was added and the mixture was warmed to rt. The aqueous layer was extracted with CHCl₃ (10 x 1 mL) and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification via flash column chromatography on silica gel (98:2 to 95:5 CHCl₃/MeOH, v/v) yielded amine 6.12 (33 mg, 89% from oxazoline 6.11) as a light yellow amorphous solid: [α]²⁵⁰ = –92.7 (c 1.1, CHCl₃); ¹H NMR (500 MHz, CDCl₃, mixture of rotamers) δ 7.08 (d, J = 8.5 Hz, 2H), 6.80 (d, J = 8.6 Hz, 2H), 6.69 (d, J = 7.8 Hz, 1H), 6.22 (d, J = 9.8 Hz, 1H), 5.91–5.84 (m, 1H), 5.40 (q, J = 6.2 Hz, 1H), 5.29 (dd, J = 17.2, 1.5 Hz, 1H), 5.22 (dd, J = 10.5, 1.2 Hz, 1H), 5.18 (dd, J = 14.9, 7.3 Hz, 1H), 4.96 (dd, J = 17.5, 9.1 Hz, 1H), 4.92 (dd, J = 10.5, 3.2 Hz, 2H), 4.59 (dd, J = 5.8, 2 Hz, 2H), 4.43 (dd, J = 9.2, 9.2 Hz, 2H), 4.16–4.13 (m, 1H), 4.07 (t, J = 4.8 Hz, 2H), 3.92 (dd, J = 8.5, 8.5 Hz, 1H), 3.84 (t, J = 4.8 Hz, 2H), 3.73–3.71 (m, 2H), 3.67 (t, J = 5.1 Hz, 4H), 3.78 (t, J = 5.1 Hz, 2H), 3.19–3.10 (m, 2H), 3.04 (dd, J = 13.9, 7.5 Hz, 1H), 2.97 (s, 3H), 2.86 (dd, J = 15, 8.2 Hz, 1H), 2.76 (s, 3H), 2.43 (dddd, J = 6.8, 6.8, 6.8, 6.8 Hz, 1H), 2.28–2.21 (m, 1H), 2.01–1.91 (m, 4H), 1.88 (s, 3H), 1.86–1.79 (m, 2H), 1.62 (ddd, J = 14.3, 11, 3.8 Hz, 1H), 1.50 (dd, J = 12.2 Hz, 1H), 1.33 (ddd, J = 14.6, 11.6, 3.4 Hz, 1H), 2.23
1.28–1.25 (m, 1H), 1.27 (d, $J = 7.5$ Hz, 3H), 1.21 (d, $J = 7.5$ Hz, 3H), 1.01–0.97 (m, 2H), 0.94 (d, $J = 7$ Hz, 3H), 0.92 (d, $J = 7.5$ Hz, 3H), 0.88 (s, 9H), 0.85 (t, $J = 6.8$ Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$, mixture of rotamers) δ 171.8, 171.5, 171.4, 170.6, 168.0, 135.4, 133.0, 131.7, 131.3, 130.4, 128.3, 118.7, 114.6, 79.4, 71.8, 70.9, 70.7, 70.6, 70.1, 69.8, 67.4, 65.4, 63.5, 60.5, 59.8, 50.7, 49.7, 46.2, 40.4, 38.9, 37.7, 36.6, 34.7, 33.3, 31.0, 30.6, 29.6, 25.9, 25.4, 25.0, 24.9, 20.8, 15.8, 15.0, 14.4, 13.4, 10.6; IR (neat) 3337, 2945, 2104, 1736, 1630 cm$^{-1}$; HRMS (ESI) m/z for C$_{53}$H$_{85}$N$_8$O$_{12}$ [M+H]$^+$ calcd 1025.6281, found 1025.6277.

**Depsideptide 6.4**

To a rt solution of amine 6.12 (7.6 mg, 7.4 µmol) in THF (0.7 mL) was added morpholine (7.0 µL, 81 µmol), followed by Pd(PPh$_3$)$_4$ (1.0 mg, 0.86 µmol). After stirring for 1 h at rt, the reaction mixture was concentrated under reduced pressure. The resulting crude solid was azeotropically dried with toluene twice and then placed under high vacuum for 2 h.
The crude amino acid was dissolved in CH$_2$Cl$_2$ (7.4 mL, 0.001 M) and cooled to 0 °C. To the cooled solution was added $i$-Pr$_2$NEt (7.8 µL, 44 µmol) followed by HATU (5.6 mg, 14.8 µmol) and the reaction mixture was warmed to rt. After stirring at rt for 24 h, the reaction mixture was concentrated under reduced pressure. Purification via preparative thin layer chromatography on silica gel (98:2 EtOAc/MeOH, v/v) yielded cyclic depsipeptide 6.4 (3.3 mg, 46% from amine 6.12) as a white amorphous solid: $[\alpha]^{25}_D = -81.3$ (c 0.15, MeOH); $^1$H NMR (500 MHz, CDCl$_3$, mixture of rotamers) δ 7.15 (d, $J = 8$ Hz, 2H), 6.81 (d, $J = 8.4$ Hz, 2H), 6.18 (d, $J = 9.1$ Hz, 1H), 6.01 (d, $J = 9.3$ Hz, 1H), 5.23 (d, $J = 10.8$ Hz, 1H), 5.06 (ddd, $J = 10.2$, 10.2, 5.1 Hz, 1H), 4.97 (d, $J = 12.1$ Hz, 1H), 4.79 (ddd, $J = 9$, 9, 5.1 Hz, 1H), 4.70 (d, $J = 10.1$ Hz, 1H), 4.34 (dd, $J = 9.0$, 9.0 Hz, 1H), 4.25–4.21 (m, 2H), 4.17 (dd, $J = 15.1$, 8 Hz, 1H), 4.09 (t, $J = 4.7$ Hz, 2H), 3.85 (t, $J = 4.3$ Hz, 2H), 3.74–3.73 (m, 2H), 3.69–3.67 (m, 5H), 3.39 (t, $J = 5.2$ Hz, 2H), 3.32–3.28 (m, 1H), 3.11 (dd, $J = 9.9$, 9.9 Hz, 1H), 2.85 (dd, $J = 11.8$, 5.7 Hz, 1H), 2.80 (s, 3H), 2.74 (s, 3H), 2.67–2.62 (m, 1H), 2.42 (dd, $J = 9.4$, 7.1 Hz, 1H), 2.37–2.31 (m, 1H), 2.26–2.23 (m, 1H), 2.19–2.12 (m, 1H), 2.09–2.04 (m, 2H), 1.95–1.86 (m, 2H), 1.91 (s, 3H), 1.79 (dd, $J = 12.7$, 12.7 Hz, 1H), 1.43–1.36 (m, 2H), 1.29–1.26 (m, 2H), 1.23 (d, $J = 7.6$ Hz, 3H), 1.14–1.08 (m, 2H), 1.06 (d, $J = 6.6$ Hz, 3H), 0.98 (d, $J = 7.6$ Hz, 3H), 0.94 (t, $J = 7.6$ Hz, 3H), 0.94–0.93 (m, 1H), 0.87 (s, 9H); HRMS (ESI) $m/z$ for C$_{50}$H$_{78}$N$_8$NaO$_{11}$ [M+Na]$^+$ calcd 989.5682, found 989.5681.
Alkyne 6.3

To a rt solution of D- (+)-biotin (6.1) (56.3 mg, 0.23 mmol) in CH₃CN (3 mL) and MeOH (1 mL) was added EDCI (49 mg, 0.25 mmol) and the resulting mixture was stirred for 5 min. A solution of amine 6.13 (29.1 mg, 0.35 mmol) in CH₃CN (0.5 mL) was then added and the resulting mixture was stirred at rt for 12 h. After completion, the reaction mixture was concentrated under reduced pressure. Purification via flash column chromatography on silica gel (10:1, CHCl₃/MeOH, v/v) yielded alkyne 6.3 (53 mg, 74%) as an off white solid: [α]₂⁵²D = +61.1 (c 0.96, MeOH); ¹H NMR (500 MHz, MeOH) δ 4.45 (ddd, J = 7.9, 5.0, 0.9 Hz, 1H), 4.27 (dd, J = 7.7, 4.6 Hz, 1H), 3.27 (ddd, J = 2, 2, 2 Hz, 1H), 3.22 (t, J = 6.6 Hz, 2H), 3.17 (ddd, J = 10.4, 5.9, 4.6 Hz, 1H), 2.89 (dd, J = 13, 5.2 Hz, 1H), 2.67 (d, J = 12.6 Hz, 1H), 2.20–2.15 (m, 5H), 1.74–1.52 (m, 6H), 1.43–1.37 (m, 2H); ¹³C NMR (125 MHz, MeOH) δ 174.7, 164.7, 82.8, 68.6, 62.0, 60.2, 55.6, 39.6, 38.1, 35.4, 28.4, 28.1, 28.0, 25.5, 15.3; IR (neat) 2927, 1701, 1641, 1545 cm⁻¹; HRMS (ESI) m/z for C₁₅H₂₃N₃NaO₂S [M+Na]⁺ calcd 332.1403, found 332.1403.
Triazole 6.2

To a solution of azide 6.4 (1.2 mg, 1.24 µmol) and alkyne 6.3 (0.4 mg, 1.24 µmol) in t-BuOH (15 µL) and H₂O (15 µL) was added CuSO₄•5H₂O (0.1 µL, 0.12 µmol, 1 M solution in H₂O) and sodium ascorbate (0.5 µL, 0.24 µmol, 2 M solution in H₂O). After stirring for 15 h at rt, the reaction mixture was concentrated under a stream of argon.

Purification via preparative thin layer chromatography on silica gel (9:1 to 3:1, EtOAc/MeOH, v/v) yielded biotinylated apratoxin 6.2 (0.9 mg, 57%) as a white amorphous solid: \([\alpha]^{25}_D = -51.2\) (c 0.43, MeOH); \(^1\)H NMR (500 MHz, CDCl₃, mixture of rotamers) \(\delta\) 7.52 (s, 1H), 7.13 (d, \(J = 8.5\) Hz, 2H), 6.80 (d, \(J = 8.6\) Hz, 2H), 6.37–6.35 (m, 1H), 6.21 (d, \(J = 10.0\) Hz, 1H), 5.41 (m, 1H), 5.20 (d, \(J = 14.2\) Hz, 1H), 5.10–5.05 (m, 1H), 5.02 (d, \(J = 2.6\) Hz, 1H), 4.97 (dd, \(J = 12.5, 3.1\) Hz, 1H), 4.81–4.76 (m, 2H), 4.63 (d, \(J = 10.4\) Hz, 1H), 4.51 (t, \(J = 5.2\) Hz, 3H), 4.37–4.35 (m, 1H), 4.31 (dd, \(J = 7.5, 7.5\) Hz, 1H), 4.22–4.16 (m, 3H), 4.08 (dd, \(J = 6.4, 3.7\) Hz, 2H), 3.86 (t, \(J = 5.3\) Hz, 2H), 3.80 (t, \(J = 5.4\) Hz, 2H), 3.69–3.66 (m, 4H), 3.64–3.62 (m, 2H), 3.35–3.27 (m, 2H), 3.23–3.16 (m, 2H), 3.12 (dd, \(J = 12.7, 10.8\) Hz, 1H), 2.93 (dd, \(J = 13, 5.1\) Hz, 1H), 2.86 (s, 3H), 2.74 (s,
3H), 2.70 (dd, J = 7.3, 7.3 Hz, 2H), 2.42 (dd, J = 10.2, 6.7 Hz, 1H), 2.35–2.28 (m, 1H), 2.28–2.27 (m, 1H), 2.22–2.19 (m, 1H), 2.18–2.13 (m, 2H), 2.10–2.06 (m, 2H), 1.95 (d, J = 1.3 Hz, 3H), 1.92–1.81 (m, 4H), 1.80–1.78 (m, 1H), 1.77–1.75 (m, 1H), 1.71–1.63 (m, 3H), 1.41–1.35 (m, 1H), 1.33–1.29 (m, 1H), 1.26 (m, 4H), 1.18 (d, J = 6.3 Hz, 3H), 1.15–1.13 (m, 1H), 1.12–1.11 (m, 1H), 1.10–1.09 (m, 1H), 1.06 (d, J = 7.3 Hz, 3H), 0.99–0.96 (m, 4H), 0.94 (d, J = 8 Hz, 3H), 0.93–0.92 (m, 2H), 0.88 (s, 9H); HRMS (ESI) m/z for C₆₅H₁₀₁N₁₁NaO₁₃S [M+Na]+ calcd 1298.7193, found 1298.7196.
LIST OF REFERENCES


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APPENDIX A:

$^1$H NMR SPECTRA
$^{1}\text{H NMR Spectrum of 3.9 (400 MHz, CDCl}_3\text{)}$
$^1$H NMR Spectrum of 3.11 (400 MHz, CDCl$_3$)
$^{1}\text{H NMR Spectrum of 3.12 (400 MHz, CDCl}_3\text{)}$
$^1$H NMR Spectrum of 3.13 (400 MHz, CDCl$_3$)
$^1$H NMR Spectrum of 2.12 (400 MHz, CDCl$_3$)
$^1\text{H NMR Spectrum of 2.13 (400 MHz, CDCl$_3$)}$
$^1$H NMR Spectrum of 3.2 (400 MHz, CDCl$_3$)
$^1$H NMR Spectrum of 3.17 (400 MHz, CDCl$_3$)
H NMR Spectrum of 3.18 (400 MHz, CDCl$_3$)

$^1$H NMR Spectrum of 3.18 (400 MHz, CDCl$_3$)
$\text{H NMR Spectrum of 3.3 (400 MHz, CDCl}_3)$

TBSO

$\text{O}$

$\text{CH}$

$\text{3.3}$
$^1$H NMR Spectrum of 3.19 (500 MHz, CDCl$_3$)
\[ ^1H \text{NMR Spectrum of 2,3 (500 MHz, CDCl}_3 \]
H NMR Spectrum of 3.20 (400 MHz, CDCl₃)
$^1$H NMR Spectrum of 3.1 (500 MHz, CDCl$_3$)
$^1$H NMR Spectrum of 3.31 (400 MHz, CDCl$_3$)
$^1$H NMR Spectrum of 3.32 (400 MHz, CDCl$_3$)
$^1$H NMR Spectrum of 3.33 (400 MHz, CDCl$_3$)
$^1$H NMR Spectrum of 3.34 (400 MHz, CDCl$_3$)
$^1\text{H NMR Spectrum of 1.9 (500 MHz, CDCl}_3\text{)}$
$\text{H NMR Spectrum of 4.3 (400 MHz, CDCl}_3$)
$^1$H NMR Spectrum of 4.4 (500 MHz, CDCl$_3$)
Figure: 

**H NMR Spectrum of 4.5 (500 MHz, CDCl₃)**

- **1.0 ppm**
- **1.5 ppm**
- **2.0 ppm**
- **2.5 ppm**
- **3.0 ppm**
- **3.5 ppm**
- **4.0 ppm**
- **4.5 ppm**
- **5.0 ppm**
- **5.5 ppm**
- **6.0 ppm**
- **6.5 ppm**
- **7.0 ppm**

Chemical structure of compound 4.5.
H NMR Spectrum of 4.2 (500 MHz, CDCl3)
\textsuperscript{1}H NMR Spectrum of 4.6 (500 MHz, CDCl\textsubscript{3})
H NMR Spectrum of 4.7 (500 MHz, CDCl₃)
$^1$H NMR Spectrum of 4.8 (500 MHz, CDCl$_3$)
$^1$H NMR Spectrum of 4.1 (500 MHz, CDCl$_3$)
H NMR Spectrum of 4.13 (500 MHz, CDCl3)
$^{1}$H NMR Spectrum of 4.14 (400 MHz, CDCl$_3$)
H NMR Spectrum of 4.15 (500 MHz, CDCl$_3$)
$\text{H NMR Spectrum of 4.11 (400 MHz, CDCl}_3$)
\textsuperscript{1}H NMR Spectrum of 4.16 (400 MHz, CDCl\textsubscript{3})
H NMR Spectrum of 4.17 (400 MHz, CDCl₃)
$\text{H NMR Spectrum of 4.10 (500 MHz, CDCl}_3\text{)}$
$^{1}$H NMR Spectrum of 4.19 (500 MHz, CDCl$_3$)
$\text{H NMR Spectrum of } 4.20 \ (500 \text{ MHz, CDCl}_3)$
$^1$H NMR Spectrum of 421 (500 MHz, CDCl$_3$)
$^{1}$H NMR Spectrum of 4.9 (500 MHz, CDCl$_3$)
$^1$H NMR Spectrum of 4.27 (500 MHz, CDCl$_3$)
H NMR Spectrum of **4.29** (500 MHz, CDCl₃)
$^1$H NMR Spectrum of 4.26 (500 MHz, CDCl$_3$)
\[ \text{H NMR Spectrum of 4.30 (500 MHz, CDCl}_3) \]
$^1\text{H NMR Spectrum of 4.31 (500 MHz, CDCl}_3\text{)}$
$^{1}$H NMR Spectrum of 4.25 (500 MHz, CDCl$_3$)
$^{1}$H NMR Spectrum of 4.32 (500 MHz, CDCl$_3$)
$\text{H NMR Spectrum of 4.33 (500 MHz, CDCl}_3\text{)}$
\( ^1H \) NMR Spectrum of 4.34 (500 MHz, CDCl₃)
\[ \text{\hspace{1cm} H NMR Spectrum of 4.24 (500 MHz, CDCl}_3) \]
$^1$H NMR Spectrum of 4.37 (500 MHz, CDCl$_3$)
$^1$H NMR Spectrum of 4.36 (500 MHz, CDCl$_3$)
H NMR Spectrum of 4.38 (500 MHz, CDCl₃)
H NMR Spectrum of 4.39 (500 MHz, CDCl₃)

Carbon-13 NMR Spectrum of 4.39 (125 MHz, CDCl₃)
$\text{H NMR Spectrum of 4.40 (500 MHz, CDCl}_3$)

4.40
$^1$H NMR Spectrum of 4.35 TOP (500 MHz, CDCl$_3$)
$^1$H NMR Spectrum of **4.35 BOTTOM** (500 MHz, CDCl$_3$)
$^1$H NMR Spectrum of 4.42 (500 MHz, CDCl$_3$)
H NMR Spectrum of 4.43 (500 MHz, CDCl₃)

^1H NMR Spectrum of 4.43 (500 MHz, CDCl₃)
$^1$H NMR Spectrum of 4.44 (500 MHz, CDCl$_3$)
H NMR Spectrum of 4.45 (500 MHz, CDCl₃)

\[ \text{H NMR Spectrum of 4.45 (500 MHz, CDCl₃)} \]
\[ { }^1 \text{H} \text{NMR Spectrum of 4.41 TOP (500 MHz, CDCl}_3) \]

H NMR Spectrum of 4.41 TOP (500 MHz, CDCl3)
HNMR Spectrum of 4.41 BOTTOM (500 MHz, CDCl3)
$^1$H NMR Spectrum of 4.47 (500 MHz, CDCl$_3$)
$^1$H NMR Spectrum of 4.48 (500 MHz, CDCl$_3$)
$\text{H NMR Spectrum of 4.49 (500 MHz, CDCl}_3\text{)}$
$^{1}$H NMR Spectrum of 4.46 (500 MHz, CDCl$_3$)
$^1$H NMR Spectrum of 5.2 (500 MHz, CDCl$_3$)
$^{1}\text{H NMR Spectrum of 5.3 (500 MHz, CDCl}_3\text{)}$

$\text{MeO}$

$\text{BocHN}$

$\text{N}$

$\text{O}$

$\text{O}$

$\text{Allyl}$

$\text{5.3}$
H NMR Spectrum of 5.5 (500 MHz, CDCl$_3$)

$^1$H NMR Spectrum of 5.5 (500 MHz, CDCl$_3$)
$^{1}$H NMR Spectrum of 5.4 (500 MHz, CDCl$_3$)
$^1$H NMR Spectrum of 5.12 (500 MHz, CDCl$_3$)
$^1$H NMR Spectrum of 5.13 (500 MHz, CDCl$_3$)
$^1$H NMR Spectrum of 5.14 (500 MHz, CDCl$_3$)
$^1$H NMR Spectrum of 5.15 (500 MHz, CDCl$_3$)
$^{1}$H NMR Spectrum of 5.16 (500 MHz, CDCl$_3$)
$^1$H NMR Spectrum of 5.11 (500 MHz, CDCl$_3$)
$^{1}$H NMR Spectrum of 5.18 (500 MHz, CDCl$_3$)
$^1$H NMR Spectrum of 5.19 (500 MHz, CDCl$_3$)
H NMR Spectrum of 5.22 (500 MHz, CDCl₃)
H NMR Spectrum of 5.23 (500 MHz, CDCl₃)

1H NMR Spectrum of 5.23 (500 MHz, CDCl₃)
$^{1}\text{H NMR Spectrum of 5.24 (500 MHz, CDCl}_{3})$
$^1$H NMR Spectrum of 5.25 (500 MHz, CDCl$_3$)

![Chemical structure of 5.25]

Peaks are observed at various ppm values.

Note: The chemical structure and spectrum show the presence of functional groups and expected chemical shifts.
NMR Spectrum of 6.7 (500 MHz, CDCl$_3$)

1HNMR Spectrum of 6.7 (500 MHz, CDCl$_3$)
$^1$H NMR Spectrum of 6.8 (500 MHz, CDCl$_3$)
$^{1}$H NMR Spectrum of 6.9 (500 MHz, CD$_3$OD)
$^1$H NMR Spectrum of 6.10 (500 MHz, CDCl$_3$)
H NMR Spectrum of 6.11 (500 MHz, CDCl$_3$)

$^1$H NMR Spectrum of 6.11 (500 MHz, CDCl$_3$)
$^{1}$H NMR Spectrum of 6.12 (500 MHz, CDCl$_3$)
H NMR Spectrum of 6.4 (500 MHz, CDCl₃)

1H NMR Spectrum of 6.4 (500 MHz, CDCl₃)
$^{1}H\text{ NMR Spectrum of 6.3 (500 MHz, CD}_{3}\text{OD)}$
$\text{H NMR Spectrum of 6.2 (500 MHz, CDCl}_3$)