Devising a Scalable Synthesis to Probe the G-Protein Cell Receptor Signaling Pathway

Matthew Robert Medcalf
Washington University in St. Louis

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WASHINGTON UNIVERSITY IN ST. LOUIS

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Devising a Scalable Synthesis to Probe the G-Protein Cell Receptor Signaling Pathway

by

Matthew Robert Medcalf

A dissertation presented to
The Graduate School
of Washington University in
partial fulfillment of the
requirements for the degree
of Doctor of Philosophy

December 2019
St. Louis, Missouri
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List of Abbreviations

Ac$_2$O  acetic anhydride
Boc  tert-butoxycarbonyl
COMU  (1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate
DEAD  diethyl azodicarboxylate
DIPEA  N,N’-diisopropylethylamine
DMF  N,N’-dimethylformamide
DMSO  dimethyl sulfoxide
EDC  1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
FOS  Function-Oriented Synthesis
FRET  fluorescence resonance energy transfer
GDP  guanosine diphosphate
GPCR  G protein coupled receptor
GTP  guanosine triphosphate
HATU  1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
HOBt  hydroxybenzotriazole
IC$_{50}$  half maximal inhibitory concentration
LiHMDS  lithium bis(trimethylsilyl)amide
NaHMDS  sodium bis(trimethylsilyl)amide
NMM  N-methylmorpholine
NMR  nuclear magnetic resonance (spectroscopy)
TBS  tert-butyldimethylsilyl
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
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<tr>
<td>TBSOTf</td>
<td>tert-butylidimethylsilyl trifluoromethanesulfonate</td>
</tr>
<tr>
<td>TBTU</td>
<td>2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>TROC</td>
<td>trichloroethyl chloroformate</td>
</tr>
<tr>
<td>YM</td>
<td>YM-254890</td>
</tr>
<tr>
<td>WU</td>
<td>WU-07047</td>
</tr>
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Lastly, I’d like to thank my family and friends. I could not have done this without you all.

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Abstract of the Dissertation

Devising a Scalable Synthesis to Probe
the G-Protein Cell Receptor Signaling Pathway

By

Matthew Robert Medcalf

Doctor of Philosophy in Chemistry

Washington University in St. Louis, 2019

Professor Kevin D. Moeller, Chair

The G-protein coupled cell receptor signaling pathway is amongst the largest and most diverse class of cell-surface receptors in the body. Nearly 800 different genes encode for these cell membrane receptors which are responsible for mediating a variety of hormones, neurotransmitters, and sensory stimuli through the activation of intracellular G proteins. To date, roughly 34% of pharmaceuticals on the market target GPCR’s, but despite this fact, there are still many difficulties associated with targeting this family of receptors. The vast number of GPCR’s, disease states resulting from a dysregulation of multiple GPCR signaling pathways, and difficulties crystalizing and purifying the receptor—G-protein complex all pose significant challenges for targeting GPCR’s. Due to these challenges, in recent years there has been a growing interest in targeting the intracellular G protein as an alternative to the extracellular GPCR. For example, two known natural products YM-254890 and FR-900359, isolated from Chromobacterium sp. QS3666 and Ardisia Crenata respectively, potently and selectively inhibit signaling by Gq/11-class G-protein α subunits by trapping them in their inactive GDP-bound states. As part of an effort to better understand how these natural products operate and to develop
chemical probes for the Gq signaling pathway we have developed a scalable solution phase
synthesis of simplified analogs of YM/FR that retain important regions responsible for binding to
Gαq.

Through the use of this convergent synthesis with a longest linear sequence of eight steps
with an overall yield of 11.6%. We have successfully synthesized multiple analogs used to
probe the G-protein cell receptor signaling pathway. While the analogs synthesized to date are
not as potent as YM or FR, they do selectively bind to Gαq. With these results in place, we are
now probing the factors that are responsible for binding and potency of the analogs for Gq, along
with factors that determine the selectivity of the molecules for Gαq.

To this end, we have developed a new convergent synthesis, applying the lessons learned
from our second-generation synthesis, that returns functionality found in the natural products.
We believe the instillation of the “bottom bridge”, a dimer of an N-Me-O-Me-Thr and N-Ac-Thr,
will provide conformational constraint and return activity lost in the first generation of analogs.
Once more active analogs have been found, these molecules will be used as probes for
understanding the biological function of the Gq signaling pathway and as potential lead
compounds for the development of future therapeutics.
Chapter One: Introduction and Background

1.1 G Protein Cell Receptor Signaling Pathway

The G-protein cell receptor (GPCR) signaling pathway is the largest membrane protein family with nearly 800 receptors that regulate a wide variety of cellular or physiological processes, and a wide variety of disease states. This is reflected in the sheer number of pharmaceuticals that target the GPCR, accounting for over a third of the drugs currently available on the market. Every day, numerous neurotransmitters, chemokines, local mediators, and sensory stimuli exert their effects on a cell by binding to heptahelical membrane receptors coupled to heterotrimeric G proteins.

![G Protein Cell Signaling Pathway](image-url)

**Figure 1.1 – G Protein Cell Signaling Pathway**

In a healthy cell, the GPCR signaling cascade is represented in Figure 1.1. First, a ligand binds to a cell surface receptor (step B), promoting exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) on the Gα-subunit of the intracellular heterotrimeric G protein (Step C). The GTP bound Gα subunit then disassociates from the Gβγ dimer (step D). These subunits can then turn on a variety of downstream effectors. Upon completion of the cell signal, GTP is cleaved to GDP by the GTPase domain, one of the two domains within the Gα subunit (step E). The Gα subunit then re-associates with the Gβγ dimer, terminating the signal (step F), thus resetting the catalytic cell cycle.

While the process of GPCR regulated signaling is well understood, much less is known about the intracellular G proteins. The primary means by which the biochemistry community analyzes G protein function is through genetic manipulation where changes are made to individual amino acids within the G protein in order to examine the resulting changes in the signaling pathway. This approach is challenging due to the complexity of GPCR/G protein pairs and cellular adaptations to the genetic modifications, such as the rewiring of signaling pathways. These challenges, coupled with the knowledge that roughly 10-15% of all cancers are driven by a constitutively active mutant G protein α-subunit (step D), make alternative strategies for gaining insight into the GPCR signaling pathways attractive. Among these alternatives, molecules that selectively bind in a reversible manner and inhibit intracellular G proteins can serve as invaluable probes of specific G protein signaling pathways and as potential starting points for the development of new therapeutics. For this reason, such molecules are important synthetic targets.
1.2 Targeting Intracellular G Proteins

To date, only a handful of molecules directly target intracellular G proteins. Two such molecules are pertussis toxin and cholera toxin that covalently bind to Gαi and Gαs respectively and have been used to map these cell signaling pathways.\(^8,9\) In addition, the natural products YM-254890 (YM) and FR900359 (FR) (Figure 1.2) are known to potently and selectively bind to Gaq/11 in a pseudo-irreversible manner, trapping the α-subunit in its inactive GDP-bound state. These two natural products and their respective analogs are the only molecules known to behave in this manner.

![Figure 1.2 – YM-254890 & FR900359](image)

YM and FR were isolated from Chromobacterium sp. QS366 and the ornamental plant Ardisia crenata, respectively. They are both cyclic depsipeptides comprised of seven different amino acids, a twenty-two membered macrocycle, with eleven different stereocenters, and multiple ester, amide, and NMe amide bonds. The core structures of YM and FR differ in only two areas, highlighted red in Figure 1.2. FR contains a β-hydroxy leucine within the “bottom bridge” of macrocycle (compared to a threonine within YM) and a propionate derivative on the
\(\beta\)-hydroxy leucine tail of the macrocycle (compared to an acetyl residue found in YM). These two slight changes impart a three-fold increase in potency found in FR.\(^{11}\) While YM and FR are promising lead molecules, they are not isolated in high yields from natural sources, and efforts to synthesize these molecules in lab have primarily relied on solid state peptide chemistry. While a combination of solution phase and solid-state peptide chemistry has allowed for the completion of structure activity relationship (SAR) studies involving these molecules, it has not led to the generation of either of the natural products or their respective analogs in high yield.\(^{10-14}\) To this end, we propose a scalable synthesis of YM and FR analogs through a convergent route via exclusively solution-phase synthesis. Prior to the discussion of this synthesis, we should first focus on the knowledge available from SAR studies following isolation and synthesis of a variety of analogs of YM and FR. All available information is important for guiding the design and synthesis of new analogs. It is important to note that much of this information has become available during our synthetic efforts that will be discussed in Chapter 2.

1.3 What We Know So Far

To date, fifteen different analogs of FR have been synthesized or isolated, and more than 35 different analogs of YM.\(^{14}\) While no analogs have been found with improved potency and efficacy over that of the natural product FR, much has been learned about what portions of this molecule appear to be necessary for binding. We will discuss these changes from left to right in individual amino acids labeled in Figure 1.3.

Changes of the iso-propyl groups of \(\beta\)-HyLeu\(_1\) (often referred to as the \(\beta\)-hydroxy leucine tail) and \(\beta\)-HyLeu\(_2\) are not well tolerated and result in loss of potency and efficacy in comparison to the parent compound. However, in \(\beta\)-HyLeu\(_1\) multiple analogs with changes at
the acetyl group (R = CH₃, CH₂CH₃, CH₂SCH₃) have been isolated and screened with minimal observed change in activity.¹⁰,¹⁵

![Figure 1.3 – Labeled YM & FR Structure](image)

Changes within the N-MeAla₃ and Ala₄ have been shown to be tolerated. The methyl groups of the alanine have been substituted with phenyl rings resulting in no significant loss in activity. This information is consistent with the observation that the top bridge of the molecule is solvent exposed and resides outside of the binding region within Gαq. This is particularly relevant as these sites may prove suitable for future installation of cross-linking moieties to add conformational rigidity to simplified analogs or fluorescent groups for various biological assays.¹² The N-MeDha may provide a necessary conformational constraint, as illustrated by the subsequent loss in activity following the hydrogenation of the double bond. It is worth noting there is a significant disparity in activity between the resulting N-MeAla and N-Me-D-Ala. Hydrogenation leading to N-MeAla lead to a 16 fold loss in potency in comparison to the natural product YM, where a 160 fold loss in potency is observed in the case of N-Me-D-Ala.¹⁰,¹⁵ The difference between YM and FR, an Ac-Thr₇ and Ac-β-HyLeu₇, imparts three-fold more potency to FR. Lastly, changing the N-Me amide to a typical amide bond of the N-
MeThr(OMe) results in a nearly 500 fold loss in potency as compared to the natural product YM. With this knowledge in hand we can now discuss a scalable synthesis of YM and FR analogs through a convergent route via solution phase synthesis.

1.4 Scaling through Simplified Analogs

The use of a Function Oriented Synthesis was made popular by Paul Wender and coworkers in their synthesis of Bryostatin analogs, although this overall strategy has been employed by medicinal chemists for many years. While this idea will not be discussed at length here, the main concept is to retain the pharmacophore within a complex natural product, while stripping away some of its functionality, thus shortening the overall total synthesis. This was accomplished in the synthesis of simplified analogs of Bryostatin, shortening the synthesis of the molecule from more than seventy steps, to less than thirty.

![YM-254890 & WU-07047 Structures](image)

This strategy was first applied to the synthesis of simplified analogs of YM by Rensing et. al. from our group. Their approach is highlighted in Figure 1.4. The red regions represent regions of YM that were shown to bind to Gaq in an X-ray crystal structure of YM-Gaq.
complex. The asterisks represent direct points of contact within Gαq, and the numbers indicate intramolecular hydrogen bonds meant to stabilize the binding conformation of YM to Gαq. The intent was to simplify the groups bridging the points of contact with simplified alky chains and alkene units that would participate in a ring closing metathesis reaction.5

While the first simplified analogs were synthesized, leading to initial screens of biological activity, there were many challenges within the first-generation synthesis that still needed to be addressed. Namely a problematic β-elimination involving the side chain on the left-hand side of the molecule as drawn (sometimes referred to as the β-hydroxy leucine tail). This β-elimination resulted in a lowering of yields for every reaction in the synthesis following the introduction of the side chain via esterification to acyclic molecule, inevitably making the synthesis very difficult to repeat. With the intent of not only devising analogs that improve efficacy, but also scaling the synthesis of those analogs for use in animal models, it became clear that we must first address this elimination. The methods used to solve this problem, as well as efforts to improve the potency and efficacy of analogs that target Gαq, will be discussed in the following chapters. Greater detail about the initial approach and the issues encountered will be included below in connection with the chemistry used to address those issues.
1.5 References


Chapter 2: Synthesis of Simplified Analogs

2.1 First Generation Synthesis

Through the use of a convergent synthesis as outlined by Rensing et. al., the first simplified analog of YM-254890 (YM) was accomplished providing analog WU-07047 (WU) with a longest linear sequence of 10 steps and an overall yield of 6.4%. The retrosynthetic analysis from the first-generation synthesis discussed in chapter one is outlined in Figure 2.1.

![Figure 2.1 Retrosynthetic Analysis of WU-07047](image)

With this analog in hand, WU was screened for its ability to inhibit nucleotide exchange in Gαq and then compared with the activity associated with the natural product, YM. While this first-generation simplified analog retained selectivity towards Gαq, it was much less potent then the natural product and did not retain full efficacy. Still, the selectivity observed towards Gαq suggests that it may serve as a great starting point for the synthesis of future analogs. For our part, we are interested in synthesizing chemical probes for mapping the GPCR signaling pathways. To best probe this cell signaling pathway it is of paramount importance to obtain not only potent analogs, but analogs that retain selectivity and efficacy (evidence that the mechanism
of action of the simplified analog is similar to that of the natural product). With this in mind, a second look at our first-generation synthesis suggested that reproducing this synthetic route, varying the analogs made, and scaling the reactions to increase the amount of product isolated would prove quite challenging. The isolation of nearly 80 mgs of WU from the initial efforts was indeed a leap forward compared to the trace amounts (1.0 – 3.2 mgs) of material isolated through solid-state peptide chemistry$^{2-4}$, but despite literature precedent, the addition of the β-hydroxy leucine (highlighted red in Figure 2.1) side chain in the early stages of the synthesis led to significant losses in material along the way.

**Figure 2.2 A Problematic β-Elimination**

In the hands of an experienced synthetic organic chemist, it is possible to complete the synthesis of analog WU by rapidly assembling the macrocycle. However, this sequence is plagued with a β-elimination shown in Figure 2.2 at every step of the synthesis following incorporation of the β-hydroxyleucine side chain prior to the formation of the macrocycle. In order to continue to produce new analogs in a scalable fashion, it became evident we must first turn our attention to solving the problem of this β-elimination. It was apparent that this could be best accomplished by adding the β-hydroxy leucine sidechain last after synthesis of the intact macrocycle.
2.2 Avoiding the β-Elimination

Since the natural product could be isolated without any evidence of the undesired β-elimination, we believed it possible to add the β-hydroxy leucine sidechain in the final step of our total synthesis. This idea was solidified by the experimental observation that the β-elimination did not occur following formation of the macrocycle in the synthesis of the first simplified analog WU. Interestingly, a molecular dynamics minimization of both WU and YM using ChemBio 3D showed that in the macrocyclic products the proton responsible for this β-elimination is not antiperiplanar to the ester leaving group and hence not oriented in a fashion necessary for the elimination to readily occur (Figure 2.3). This arrangement is not observed in the acyclic molecules where free rotation allows for the necessary antiperiplanar arrangement of the bonds involved in the elimination reaction.

![Figure 2.3 – Molecular Dynamics Minimization of YM](image-url)
In the end, molecular dynamics calculations and the experimental observations appear to confirm our hypothesis that a strategy that places the β-hydroxyleucine side chain on last following synthesis of the intact macrocycle could be successful. This analysis has led to the retrosynthetic approach shown in Figure 2.4. One attractive feature of this approach is that it does not vary our synthetic strategy significantly from the synthesis accomplished by Rensing et al.

![figure]

**Figure 2.4 – Second Generation Retrosynthetic Analysis**

Upon removal of the final β-hydroxyleucine moiety in the retrosynthesis, the left-hand portion of the macrocycle (A) is still a β-hydroxy leucine derivative that is protected with a C-terminal ester and coupled to an extended amino acid derivative. The right-hand portion (B) of this molecule is the same coupled product of an allylglycine with a 2-hydroxy-3-phenylpropionic acid derivative that was used in the first-generation synthesis. The macrocycle would be constructed from a standard coupling of left- and right-hand portions of this macrocycle followed by the same olefin metathesis strategy used previously.
While this change may look trivial, there was no evidence in the literature of a synthetic approach that allows for the addition of the β-hydroxyleucine side chain last. We believe this is due to the N-Acetyl protecting group on the nitrogen of the β-hydroxyleucine side chain in the natural product. This acetyl protecting group is not orthogonal with standard coupling procedures. The challenges associated with this coupling will force us to either devise a new coupling strategy, find an alternative protecting group, or exchange a protecting group in the final steps of a total synthesis (the least appealing of the possible approaches). We began with the assembly of the macrocycle, before addressing the challenges associated with solving this β-elimination.

2.3 Right Hand Piece (RHP) Synthesis

![Scheme 2.1 – RHP Synthesis](image)

The synthesis of the right-hand portion of the molecule began with a commercially available (S) allyl-glycine (Scheme 2.1). The availability of this starting material removed the need to synthesize the allyl-glycine using an asymmetric Ireland Claisen rearrangement from the first-generation synthesis. The acylation of the amine afforded compound 2.1 in 73% percent yield. The carboxylic acid was then subjected to Mitsunobu coupling to a methyl (S)-2-hydroxy-3-phenylpropionate affording coupled product in a 93% yield. It is worth noting this Mitsunobu reaction serves two purposes. First, the reaction proceeds with stereo inversion since in the
mechanism the hydroxyl group is first converted to a leaving group and then displaced by the acid in an S$_{N}$2-type reaction. In addition, this coupling strategy is also compatible with the presence of the N-acetyl protecting group on the nitrogen. Under standard coupling conditions, the activated carboxylic acid intermediate is attacked by the nucleophilic oxygen of the acetyl group resulting primarily in oxazolone formation (Figure 2.5).

![Figure 2.5 – Mitsunobu vs. Standard Coupling](image)

Upon completion of the Mitsunobu reaction, the methyl ester was cleaved using LiI in refluxing THF as developed by Dr. Derek Rensing. This approach not only resulted in minimal racemization (confirmed by HNMR) but also provided a 69% yield of compound 2.3 that was then purified via recrystallization.

With this basic strategy in place, we turned our attention to making further analogs of this right-hand building block. To do so, we chose to protect the N-terminus of the allyl-glycine with an ethyl carbamate in place of the N-acetyl group found in the natural product. This alternate protection strategy would serve two purposes. First, it would improve yields relative to the acylation reaction that led to inconsistent yields during the production of compound 2.1. Second,
it would allow us to use an esterification reaction in place of the Mitsunobu coupling. As the esterification reaction will not invert the stereochemistry of the alcohol like the Mitsunobu reaction, this will provide an opportunity to probe how changes to this stereocenter might alter the biological behavior of the natural product. What role does this stereocenter play in the selectivity of the natural product for Gqα?

Scheme 2.2 – RHP Synthesis Continued

Following the protection of the allyl glycine derivative with ethyl chloroformate (Scheme 2.2), compound 2.4 could then be subjected to standard esterification conditions with TBTU, resulting in an 89% yield of compound 2.5b, a product that retains the stereocenter found in the methyl (S)-2-hydroxy-3-phenylpropionate. To obtain the stereocenter found in the natural product we subjected compound 2.4 to Mitsunobu conditions, affording an 85% yield of compound 2.5a. Both methyl ester substrates were then treated with LiI in THF to afford the acid needed for the subsequent coupling reaction. It is worth noting the increase in yields, as well as an improvement in consistency of yields, across this sequence of reactions when the ethyl
carbamate protected amine was used in place of the acetamide. While the use of the ethyl carbamate may require exchanging protecting groups later in the synthesis, we hoped that analogs with the carbamate intact may retain sufficient activity as to make this additional transformation unnecessary.

With multiple derivates of the right-hand portion now available (and a synthetic route in place to make others), we turned our attention to the assembly of the building block containing both the simplified top bridge and the left-hand portion of the macrocycle (Scheme 2.3).

### 2.4 Left Hand Piece (LHP) Synthesis

The synthesis of the left-hand portion of the molecule prior to the addition of the sidechain did not vary from the first-generation synthesis (Scheme 2.3). To this end, the C-terminus of an extended alkyl chain amino acid derivative was activated with isobutyl chloroformate and N-methylmorpholine, and the resulting mixed anhydride treated with β-hydroxy leucine in 1 M NaOH, to afford the thermodynamic amide coupling product 2-11 in 93% yield. The new C-terminus of the molecule was then converted to the allyl ester providing the alkene necessary for the eventual ring closing metathesis. At this point we chose to leave the free hydroxyl group in compound 2-12 unprotected, hoping this would not interfere with the
coupling of our left- and right-hand portions of the molecule to make the precursor for the metathesis reaction. The idea was that amide bond formation would be preferred over the formation of an ester based on both the nucleophilicity of the amine and the stability of the amide product. With the building blocks of our simplified analog assembled, it was time to turn our attention to the synthesis of the macrocycle.

2.5 Macrocycle Synthesis

![Chemical structures](image)

Scheme 2.4 – Macrocycle Synthesis

The assembly of the macrocycle (Scheme 2.4) began with the treatment of the left-hand portion of our molecule (2-12) with standard Boc deprotection conditions using TFA. Following the Boc deprotection, the crude product was carried forward without isolation as the trifluoroacetate salt of the amine. The trifluoroacetate salt of compound 2-12 was treated with HATU, DIPEA in DMF, and the previously synthesized right-hand piece of the molecule (2-6a).
The use of these coupling conditions was chosen following a screening of the standard coupling conditions highlighted in Table 2.1.

Table 2.1 – Macrocycle Coupling Optimization

<table>
<thead>
<tr>
<th>Coupling Agent</th>
<th>Base</th>
<th>Solvent</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMU</td>
<td>2,2,6,6-TMP</td>
<td>DMF</td>
<td>40%</td>
</tr>
<tr>
<td>EDC, HOBl</td>
<td>NMM</td>
<td>DCM</td>
<td>35%</td>
</tr>
<tr>
<td>TBTU</td>
<td>Et₃N</td>
<td>DCM</td>
<td>Trace</td>
</tr>
<tr>
<td>HATU</td>
<td>Collidine</td>
<td>DMF</td>
<td>46%</td>
</tr>
<tr>
<td>HATU</td>
<td>DIPEA</td>
<td>DMF</td>
<td>64%</td>
</tr>
</tbody>
</table>

Following purification, compound 2-15a was isolated in a 64% yield. No evidence was obtained for an esterification product that was the result of a coupling reaction with the unprotected alcohol in compound 2-12. This acyclic molecule was then treated with Grubbs Generation II catalyst (Scheme 2.4) to afford compound 2-16a in 59% yield. With the macrocycle intact, we could now turn our attention to one of two strategies to add the final β-hydroxy leucine side chain.

2.6 β-Lactone Strategy

In order to couple the final β-hydroxyleucine moiety, we believed we could employ a similar approach to the “β-Lactam Synthon Method” used by Ojima and coworkers to complete the semi-synthesis of Taxol (Figure 2.6).²
The use of a similar β-lactone based strategy in our case was suggested for two reasons. First, we hoped that the use of a strained four membered ring may provide sufficient driving force for the coupling of a challenging ester bond in a way that allowed for more gentle reaction conditions, thus avoiding any chance of the β-elimination reaction. Second, the use of the lactone would allow us to avoid the juggling of protecting groups necessary for a standard esterification reaction in the presence of the alcohol in the β-hydroxyleucine sidechain (Figure 2.7).
This approach leads to a shorter synthetic strategy (Path A) that would save multiple steps relative to the more standard esterification and reduce the loss of the our most expensive substrate. The β-hydroxyleucine costs over $1,000 for 500 mgs, the common scale of material at the start of these protecting group sequences. Due to the significant cost of β-hydroxyleucine, we synthesize the material “in house” as highlighted in Scheme 2-8, which we will revisit later this chapter. Prior to this discussion, we will first focus on the synthesis of β-lactones.

Scheme 2.5 – β-Lactone Synthesis

The synthesis of this β-lactone began with the protection of the β-hydroxy leucine with methyl chloroformate affording compound 2-7 in 89% yield (Scheme 2.5). The use of a methyl carbamate as a protecting group was chosen in the hopes of avoiding the exchange of protecting groups to the acetyl group found in the natural product late in the synthesis. We believed there to be a high chance of biological tolerance due to the isolation of derivatives of the natural product with alternative protecting groups on the β-hydroxyleucine side chain. This material was isolated based upon its carboxylic acid and was carried forward without further purification. The synthesis of the β-lactone was accomplished with the coupling agent TBTU and triethylamine in DCM as outlined by Vitale et al. This reaction was run at very dilute conditions in order to avoid the bi-molecular esterification. The β-hydroxy lactone was then purified via flash column chromatography to afford 75% yield of expected product.
With the intent of making a variety of analogs we believed it to be possible to diversify in the last step of our synthesis. In order to probe the tolerance of protecting groups and side chains, a variety of β-lactones were synthesized (Table 2.2). These amino acids were chosen due to ease of synthesis and commercial availability. Following the synthesis of our macrocycle, this diverse array of β-lactones would allow us to rapidly make a variety of analogs via the final esterification reaction.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Protecting Group</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-21</td>
<td>iPro</td>
<td>Troc</td>
<td>71%</td>
</tr>
<tr>
<td>2-22</td>
<td>iPro</td>
<td>Acetyl</td>
<td>29%</td>
</tr>
<tr>
<td>2-24</td>
<td>iPro</td>
<td>Boc</td>
<td>77%</td>
</tr>
<tr>
<td>2-25</td>
<td>iPro</td>
<td>Methyl Carbamate</td>
<td>75%</td>
</tr>
<tr>
<td>2-27</td>
<td>iPro</td>
<td>Ethyl Carbamate</td>
<td>77%</td>
</tr>
<tr>
<td>2-28</td>
<td>Me</td>
<td>Cbz</td>
<td>64%</td>
</tr>
<tr>
<td>2-29</td>
<td>Ph</td>
<td>Boc</td>
<td>64%</td>
</tr>
</tbody>
</table>

**Table 2.2 – β-Lactones**

With these β-lactones in hand we turned our attention to devising a mock substrate system to screen conditions for the lactone opening. We hoped to avoid having to use the macrocycle as it was synthetically more advanced. Unfortunately, all mock substrates suffered from the same β-elimination seen in our first-generation synthesis (Figure 2.8).
This elimination proved quite challenging when trying to access the success of reaction. The screening of conditions began with previously synthesized compound 2-12. This material was chosen as it was readily available and would most closely resemble the macrocycle. Screening began with the use of NaHMDS, the same base used in the semi-synthesis of taxol. The use of an excess of a strong base, none to surprisingly, lead to exclusively β-elimination product, though pleasingly we found evidence of successful coupling in the proton NMR when the equivalents of base were decreased (Table 2.3). This led to the suggestion of the use of a catalytic base system in an attempt to slow the elimination. The use of DBU:Triazole in a catalytic fashion led to further evidence of product formation, however, rapid decomposition of the product due to the β-elimination led to problems isolating the desired product.

Table 2.3 – Condition Screening for Lactone Opening

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Lactone</th>
<th>Base</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound 2-12</td>
<td>Compound 2-24 (Boc)</td>
<td>NaHMDS (3 eq.)</td>
<td>Elimination</td>
</tr>
<tr>
<td>Compound 2-12</td>
<td>Compound 2-24 (Boc)</td>
<td>NaHMDS (1.2 eq.)</td>
<td>Elimination, Product (HNMR)</td>
</tr>
<tr>
<td>Compound 2-12</td>
<td>Compound 2-24 (Boc)</td>
<td>DBU; Triazole (0.33 eq.)</td>
<td>Elimination, Product (HNMR)</td>
</tr>
<tr>
<td>Compound 2-14 (Macrocycle)</td>
<td>Compound 2-24 (Boc)</td>
<td>DBU; Triazole (0.20 eq.)</td>
<td>Product (28%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound 2-14 (Macrocycle)</td>
<td>Compound 2-25 (Methyl Carbamate)</td>
<td>LiHMDS (1 eq.)</td>
<td>Compound 2-17 (15%)</td>
</tr>
</tbody>
</table>
This information led us to conclude that it would be necessary to test the esterification conditions using the intact macrocycle. Following the synthesis of the macrocycle, we were able to isolate trace amounts of the desired coupled product, but we were never able to bring obtain satisfactory yields of the product in a reproducible fashion. It is worth noting that during the screening of bases for the reaction, no evidence was found for the problematic β-elimination either in proton NMR of the crude material or in the products isolated following purification of the crude product. With no evidence of the β-elimination yet a very poor mass balance, we were quite troubled. It appears that the low mass balance is due to a polymerization of the ring opened β-lactone (Figure 2.9).

![Lactone Polymerization](image)

**Figure 2.9 – Lactone Polymerization**

To this end, opening of the β-lactone leads to a hydroxy anion is then capable of opening a second lactone. Of course, that ring opening would again lead to an alkoxide capable of opening a third lactone, and so forth and so on. Each product from the lactone opening can either continue this chain process or undergo a separate β-elimination. We made several attempts to control this polymerization. We began with varying the temperature of the reaction, monitoring completion of the reaction via TLC at -78 °C, 0 °C, and RT. This led to no observable change in yields. As we were able to recover our macrocycle starting material, we decided to use an excess of this starting material in an attempt to slow the polymerization. This, in conjunction with
addition of the lactone dropwise to the macrocycle over a 30-minute period did slightly improve yields. These yields which remained unacceptable from a synthetic standpoint are highlighted in Table 2.3. Unfortunately, it appears the β-hydroxyleucine side chain is less sterically hindered than the macrocycle, resulting in this polymerization because the extension of the chain is more rapid than the initial coupling reaction. As the struggles with this polymerization continued to occur, we chose to turn our attention to standard esterification conditions to add the β-hydroxyleucine side chain. This route may be longer, but if it can be consistently reproduced in higher yields, then it would be preferable compared to that of the inconsistent β-lactone approach. In addition to the protection strategy to be discussed below, future efforts to control the rate of this polymerization reaction include the use of a TBS trapping group, and acid catalysis.

2.7 β-Hydroxyleucine Protection Strategy

![Scheme 2.6 – β-Hydroxy Leucine Protection Strategy](image-url)
This less elegant strategy began with the use of methyl chloroformate to protect the free amine in our β-hydroxyleucine starting material to afford compound 2-7 in an 89% yield (Scheme 2.6). The carboxylic acid was then protected in the presence of the free hydroxyl group using benzyl bromide and cesium carbamate. This protecting group was chosen as opposed to the allyl ester in the first-generation synthesis due to its ease of removal.¹ The removal of the allyl protecting group relied on the use of a Pd(0) catalyst. The yields of this deprotection were inconsistent and depended highly on the quality of the catalyst that aged quickly. Yields could be improved with activation of the catalyst using n-BuLi, but inconsistency was still a significant challenge. Following the protection of the acid, the free hydroxyl group was then protected using TBDMS triflate, affording compound 2-9 in a 98% yield. Last, the benzyl group was cleaved using standard hydrogenation conditions. The resulting carboxylic acid was carried forward to the next step without further purification.

Scheme 2.7 – Macrocycle Esterification

The β-hydroxyleucine side chain was added to the macrocycle using the reaction sequence shown in Scheme 2.7. This was accomplished by treating the macrocycle (2-16a) with EDC, catalytic DMAP, and the crude carboxylic acid (2-10) as highlighted above. A catalytic amount of DMAP was used for the reaction in order to avoid conditions that were too basic.
Again, we were concerned with the possibility of a β-elimination reaction following the esterification, although there was no evidence of this elimination in either the isolated product or the proton NMR of the crude reaction product. Following purification, the esterification reaction afforded a 50% yield of compound 2-18a, which was then treated with TBAF to remove the TBDMS protecting group. After deprotection, the resulting alcohol, compound 2-19a, was isolated in a 55% yield.

In order to ensure this synthesis was as efficient as possible, we decided to determine the necessity of purification and isolation of intermediate 2-18a. Following the esterification reaction, the crude coupled product was then treated with TBAF. After purification, the desired product was isolated in a 40% yield over two steps. A comparison of yields seems to indicate it is unnecessary to isolate the coupled product prior to the subsequent TBDMS deprotection. At this point, the methyl carbamate was left in place and was not exchanged for the N-acetyl group found in the natural products. A topic that will be discussed in further detail in Chapter 3.

### 2.8 β-Hydroxyleucine Synthesis

![Scheme 2.8 – β-Hydroxyleucine](image)
The synthesis of the syn-β-hydroxyleucine was accomplished according to literature protocol (Scheme 2.8). The syn-β-hydroxyleucine was isolated in a 35% overall yield over five steps.

This concludes the discussion regarding the synthetic efforts made to complete the second-generation simplified analogs. We will now turn our attention to the lessons learned from this second-generation synthesis from both a synthetic and biological standpoint in Chapter 3.
2.9 References


2.10 Experimental Procedures

**General Procedures:**

**Methyl Ester Deprotection**

To a flame dried round bottom flask with stir bar was added lithium iodide (7.07 mmol) in THF (16 mL). The flask was then brought to reflux before addition of methyl ester (1.86 mmol) in THF (15 mL). The flask was then refluxed for 24-48h. After 24-48h, the reaction was allowed to cool to room temperature before concentrating. The resulting oil was dissolved in 50 mL chloroform and extracted with saturated NaHCO₃ (3x25 mL). The combined aqueous layers were then brought to pH=2 and extracted with chloroform (3x30 mL). The combined organic layers were then dried with MgSO₄ and concentrated *in vacuo*.

**Mitsunobu Esterification**

Carboxylic acid (1.80 mmol), was subjected to a benzene (30 mL) azeotropic distillation to remove any trace water. To this flask were added triphenylphosphine (1.80 mmol), followed by 4 mL THF. The flask was stirred at -30 °C before addition of alcohol (1.64 mmol) dissolved in an additional 4 mL THF, followed by dropwise addition of 0.84 mL (1.84 mmol) of a 40 wt % solution of diethyl azodicarboxylate in toluene. The temperature was maintained at -30 °C for 30 minutes and was then allowed to reach room temperature overnight. After 18 hours, the reaction
was concentrated *in vacuo*. The resulting oil was dissolved in 50 mL EtOAc and washed with saturated NaHCO₃ (3x25 mL). The aqueous layer was then back extracted with EtOAc (2x20 mL). The combined organic layers were then dried with MgSO₄ and concentrated *in vacuo*. The resulting oil was purified via flash column chromatography.

**Ring Closing Metathesis**

To a flame dried RBF was added the acyclic diene (0.25 mmol) in DCM (160 mL). The reaction was brought to reflux before addition of Grubbs gen. II Catalyst (0.05 mmol) in DCM (15 mL). After 20 hours at reflux, the reaction was allowed to cool to RT before concentration *in vacuo*. The resulting crude brown oil was then purified via flash column chromatography.

**Macrocycle Esterification**

To a flame dried RBF was added 0.1979 g (0.32 mmol) macrocycle with 0.0102 g (0.08 mmol) DMAP and 0.0917 g (0.48 mmol) EDC. The flask was cooled to 0 °C before addition of 0.1296 g (0.40 mmol) compound 7d in DCM (2.5 mL). The reaction was then stirred and allowed to reach RT overnight. After 48h the reaction was diluted with DCM (30 mL) and washed with saturated NaHCO₃ (3x25 mL). The aqueous layer was then back extracted with DCM (2x20 mL). The combined organic layers were then dried with MgSO₄ and concentrated *in vacuo*. The resulting oil was then purified via flash column chromatography.

**Amide Coupling (HATU)**

To a flame dried RBF was added 0.057 g (0.14 mmol) boc-protected amine, which was stirred at room temperature in dichloromethane (3 mL) with trifluoracetic acid (1 mL). After three hours the reaction was diluted with diethyl ether (20 mL) and concentrated *in vacuo*. This dilution and
concentration were repeated three times, followed by azeotropic distillation with benzene (25 mL) in order to remove any trace water. To this flask was then added 0.0520 g (0.17 mmol) carboxylic acid with 0.0798 g (0.21 mmol) HATU. The contents of the flask were then dissolved in DMF (1 mL), followed by addition of 0.06 mL (0.34 mmol) DIPEA. The reaction was then stirred at RT overnight. After 20 hours the reaction was diluted with EtOAc (30 mL) and washed with saturated NaHCO$_3$ (3x25 mL). The aqueous layer was then back extracted with EtOAc (2x20 mL). The combined organic layers were then dried with MgSO$_4$ and concentrated \textit{in vacuo}. The resulting oil was then purified via flash column chromatography.

**TBDMS Deprotection**

A stirring solution of 0.0135 g (0.015 mmol) TBS protected alcohol in THF (0.35 mL) was brought to 0 °C before addition of 0.05 mL (0.045 mmol) 1 M TBAF solution in THF. After 30 minutes, the reaction was quenched with saturated ammonium chloride (15 mL). The aqueous layer was then extracted with EtOAc (3x20 mL). The combined organic layers were then dried with MgSO$_4$ and concentrated \textit{in vacuo}. The resulting oil was then purified via flash column chromatography.

**Lactone Formation**

The β-hydroxy acid, 0.5258 g (2.13 mmol) was added to a flame dried flask with 0.8155 g (2.54 mmol) TBTU. The compounds were then dissolved in 100 mL DCM and stirred at room temperature prior to addition of 0.92 mL triethylamine. After 20 hours the reaction was washed with saturated NaHCO$_3$ (3x35 mL). The aqueous layer was then back extracted with DCM (2x30 mL). The combined organic layers were then dried with MgSO$_4$ and concentrated \textit{in vacuo}. The resulting oil was then purified via flash column chromatography.
[Compound 2-1] A stirring solution of 0.499 g (4.33 mmol) L-allylglycine in 15 mL H₂O was brought to 0 °C. The pH was adjusted to pH=10 with 1 M NaOH. 0.46 mL (4.74 mmol) freshly distilled Acetic Anhydride was then added dropwise to the stirring solution. The pH was re-adjusted to 10 and was allowed to stir and reach room temperature overnight. After 18 hours, the reaction was brought to pH=2 with 1 M HCl. The reaction was then extracted with EtOAc (3x30 mL). The organic layer was then dried with MgSO₄ and concentrated in vacuo to afford 0.5000 g (73% yield) of compound 2-1 as a white solid. This crude product was carried forward without any further purification.

¹H NMR (500 MHz, Chloroform-d) δ 11.50 (br, 1H), 6.32 (d, J = 7.6 Hz, 1H), 5.66 (ddt, J = 16.5, 10.4, 7.2 Hz, 1H), 5.25 – 4.95 (d, 2H), 4.73 – 4.47 (m, 1H), 2.74 – 2.37 (m, 2H), 2.02 (s, 3H); ¹³C NMR (126 MHz, Chloroform-d) δ 173.40, 170.27, 131.02, 118.45, 50.85, 35.03, 21.84. Full characterization has been previously reported.¹

[Compound 2-2] The reaction was set up according to General Procedure: Mistunobu Esterification. The crude product was then purified by flash chromatography (silica gel, 100% Et₂O) to give 0.7390g (93% yield) of compound 2-2 as a white solid.
\[ \text{Compound 2-3} \] The reaction was set up according to general procedure, Methyl Ester Deprotection. After refluxing for 24 hours the reaction was worked up as seen in the general procedure, Methyl Ester Deprotection. 0.2389 g (69% yield) of compound 2-3 as a yellow oil. Recrystallization in EtOAc, followed by slow evaporation in MeOH affords pure diastereomer as clear crystals.

\[ \text{H NMR (500 MHz, Methanol-d}_4\text{) \& 7.34 - 7.19 (m, 5H), 5.61 (ddt, } J = 14.1, 10.3, 7.1 \text{ Hz, 1H), 5.19 (dd, } J = 9.4, 3.9, 1.9 \text{ Hz, 1H), 5.02 (dd, 1H) 4.98 - 4.91 (dd, 2H), 4.54 (td, } J = 7.5, 5.3, 2.1 \text{ Hz, 1H), 3.25 (dd, } J = 14.4, 3.8 \text{ Hz, 1H), 3.09 (dd, } J = 14.4, 9.3, 1.9 \text{ Hz, 1H), 2.44-2.31 (m, 2H), 1.93 (s, } J = 2.0 \text{ Hz, 3H); } ^{13}\text{C NMR (126 MHz, Methanol-d}_4\text{) \& 174.29, 173.72, 173.47, 138.93, 135.31, 135.26, 131.72, 130.77, 129.37, 129.26, 120.06, 76.22, 54.70, 39.50, 38.05, 23.58. Full characterization has been previously reported.} \]
To a flame dried round bottom flask were added 0.501 g (4.36 mmol) L-allylglycine, and 0.915 g (10.9 mmol) NaHCO$_3$. The flask was brought to 0 °C before addition of 17.5 mL THF, followed by 11.3 mL of H$_2$O. In two separate portions, 2.1 mL (21.8 mmol) of Ethyl chloroformate was added over thirty minutes. The flask was allowed to stir and reach room temperature overnight. After 20 hours, the reaction was diluted with H$_2$O (30 mL) and then washed with EtOAc (3x30 mL). The organic layer was then back extracted with saturated NaHCO$_3$ (2x25 mL). The combined aqueous layers were then acidified to pH = 2 with 1 M HCl and extracted with EtOAc (3x30 mL). The combined organic layers were then dried with MgSO$_4$ and concentrated in vacuo to afford 0.7313 g (90% yield) of compound 2-4 as a clear oil. This crude product was carried forward without any further purification.

FTIR (neat) 3324, 2983, 1694, 1519, 1419, 1381, 1339, 1220, 1096, 1054 cm$^{-1}$; $^1$H NMR (500 MHz, Chloroform-$d$) $\delta$ 10.73 (s, 1H), 5.74 (dq, $J$ = 16.9, 7.9 Hz, 1H), 5.38 (d, $J$ = 8.2 Hz, 1H), 5.16 (dd, $J$ = 13.7, 8.6 Hz, 2H), 4.46 (q, $J$ = 6.7 Hz, 1H), 4.15 (dq, $J$ = 21.6, 7.3 Hz, 2H), 2.58 (dt, $J$ = 40.4, 15.1, 6.9 Hz, 2H), 1.25 (t, $J$ = 7.2 Hz, 3H); $^{13}$C NMR (126 MHz, Chloroform-$d$) $\delta$ 176.12, 156.42, 132.04, 119.47, 61.48, 53.08, 36.42, 14.47; HRMS m/z calculated for C$_8$H$_{13}$O$_4$N$_1$ [M+Na]$^+$ 210.0737, 210.0751 observed.
[**Compound 2-5a**] The reaction was set up according to general procedure, Mitsunobu Esterification. The crude product was then purified by flash chromatography (silica gel, 80% Hexane: 20% EtOAc) to give 0.484 g (85% yield) of **compound 2-5a** as a white solid.

FTIR (neat) 3325, 2982, 1743, 1720, 1439, 1376, 2338, 1196, 1060 cm\(^{-1}\); \(^1\)H NMR (500 MHz, Chloroform-\(d\)) \(\delta\) 7.30 – 7.19 (m, 5H), 5.44 (m, \(J = 17.1, 7.8\) Hz, 1H), 5.25 (dd, \(J = 9.5, 4.0\) Hz, 1H), 5.11 (d, \(J = 8.4\) Hz, 1H), 4.98 (d, \(J = 10.2\) Hz, 1H), 4.87 (d, \(J = 17.0\) Hz, 1H), 4.51 (q, \(J = 6.4\) Hz, 1H), 4.10 (q, \(J = 7.2\) Hz, 2H), 3.74 (s, 3H), 3.23 (dd, \(J = 14.3, 4.0\) Hz, 1H), 3.09 (dd, \(J = 14.3, 9.4\) Hz, 1H), 2.45 (dt, \(J = 13.5, 6.3\) Hz, 1H), 2.35 (dt, \(J = 13.8, 6.6\) Hz, 1H), 1.24 (t, \(J = 7.2\) Hz, 3H); \(^{13}\)C NMR (126 MHz, Chloroform-\(d\)) \(\delta\) 171.09, 169.45, 155.73, 135.63, 131.71, 129.24, 128.58, 127.19, 119.33, 73.69, 61.14, 53.02, 52.49, 37.32, 36.53, 14.53; HRMS m/z calculated for C\(_{18}\)H\(_{23}\)O\(_6\)N\(_1\) [M+Na]\(^+\) 372.1418, 372.1413 observed.

![Compound 2-5a](image)

[**Compound 2-6a**] The reaction was set up according to general procedure, Methyl Ester Deprotection. After refluxing for 48 hours the reaction was worked up as seen in the general procedure, Methyl Ester Deprotection. 0.3516 g (82% yield) **compound 2-6a** as a clear oil was carried forward without any further purification.

FTIR (neat) 3324, 2982, 1717, 1519, 1437, 1377, 1339, 1187, 1061 cm\(^{-1}\); \(^1\)H NMR (500 MHz, Methanol-\(d_4\)) \(\delta\) 7.33 – 7.21 (m, 5H), 5.62 (m, \(J = 17.2, 10.3, 7.0\) Hz, 1H), 5.19 (t, \(J = 9.3, 3.8\) Hz, 1H), 5.02 – 4.92 (m, 2H), 4.29 (t, \(J = 8.2, 5.1\) Hz, 1H), 4.06 (t, \(J = 7.1, 2.6\) Hz, 2H), 3.25 (dd, \(J =
14.3, 3.9 Hz, 1H), 3.10 (dd, \( J = 14.3, 9.3 \) Hz, 1H), 2.46 – 2.41 (m, 1H), 2.30 (m, 1H), 1.25 (t, \( J = 14.1, 7.1 \) Hz, 3H); \(^{13}\)C NMR (126 MHz, Methanol-\( d_4 \)) \( \delta \) \(^{13}\)C NMR (126 MHz, cd\( 3\)od) \( \delta \) 173.88, 173.70, 159.76, 138.93, 135.42, 131.72, 131.72, 130.73, 130.73, 129.21, 120.01, 76.22, 63.31, 56.31, 39.52, 38.27, 16.15; HRMS m/z calculated for C\(_{17}\)H\(_{21}\)O\(_6\)N\(_1\) [M+Na]\(^+\) 358.1261, 358.1276 observed.

![Chemical Structure](image)

**[Compound 2-5b]** To a flame dried RBF were added 0.803 g (2.50 mmol) TBTU and 0.3784 g (2.10 mmol) Methyl L-3-phenyllactate. This this flask was transferred 0.430 g (2.30 mmol) **compound 2-4** in 15 mL Dichloromethane. The stirring solution was brought to 0 °C before dropwise addition of 0.91 mL (6.50 mmol) Triethylamine. The reaction was allowed to stir and reach RT overnight. After 20 hours the reaction was diluted with DCM (50 mL). The organic layer was then washed with saturated NaHCO\(_3\) (3x25 mL). The combined organic layer was then dried with MgSO\(_4\) and concentrated in vacuo. The resulting oil was then then purified by flash chromatography (silica gel, 80% Hexane: 20% EtOAc) to give 0.650 g (89% yield) of **compound 2-5b** as a clear oil.

FTIR (neat) 3324, 2982, 1721, 1523, 1439, 1379, 1341, 1191, 1064; \(^{1}\)H NMR (500 MHz, Chloroform-\( d \)) \( \delta \) 7.32 – 7.18 (m, 5H), 5.71 (m, 1H), 5.28 (dd, \( J = 8.1, 4.6 \) Hz, 1H), 5.21 (d, \( J = 8.5 \) Hz, 1H), 5.16 – 5.06 (m, 2H), 4.44 (m, 1H), 4.09 (q, \( J = 7.1 \) Hz, 2H), 3.68 (s, 3H), 3.12 (d, \( 1H \)), 2.97 (d, \( J = 6.5 \) Hz, 1H), 2.55 (dd, \( J = 7.2, 6.6 \) Hz, 2H), 1.21 (t, \( J = 7.1 \) Hz, 3H); \(^{13}\)C NMR (126 MHz, cdcl\(_3\)) \( \delta \) 177.18, 172.10, 158.60, 139.12, 134.79, 132.11, 131.93, 131.18, 131.17,
131.03, 122.07, 74.00, 63.79, 54.98, 43.22, 39.87, 39.13, 17.16; HRMS m/z calculated for 
C_{18}H_{23}O_{6}N_{1} [M+Na]^+ 372.1418, 372.1412 observed.

[Compound 2-6b] The reaction was set up according to general procedure, Methyl Ester 
Deprotection. After refluxing for 48 hours the reaction was worked up as seen in the general 
procedure, Methyl Ester Deprotection. After refluxing for 48 hours the reaction was worked up 
as seen in the general procedure, Methyl Ester Deprotection. 0.2454 g (63% yield) compound 2- 
6b as a clear oil was carried forward without any further purification.

FTIR (neat) 3362, 2947, 1836, 2506, 2074, 1700, 1439, 1379, 1275, 1188, 1118, 1023; $^1$H NMR 
(500 MHz, Methanol-$d_4$) $\delta$ 7.34 – 7.18 (m, 5H), 5.77 (m, $J = 18.5, 10.3, 7.7, 6.4$ Hz, 1H), 5.23 
(ddd, $J = 8.1, 4.3, 1.4$ Hz, 1H), 5.14 – 5.02 (m, 2H), 4.90 (s, 3H), 4.25 (dd, $J = 8.7, 5.0$ Hz, 1H), 
4.11 – 4.03 (m, 2H), 3.32 (dq, $J = 3.2, 1.6$ Hz, 1H), 3.23 (dd, $J = 14.4, 4.4$ Hz, 1H), 3.18 – 3.08 
(m, 1H), 2.58 (ddd, $J = 12.1, 9.2, 5.4$ Hz, 1H), 2.47 – 2.36 (m, 1H), 1.24 (tdd, $J = 7.0, 5.4, 1.3$ 
Hz, 3H); $^{13}$C NMR (126 MHz, cd$_{3}$od) $\delta$ 174.04, 173.52, 159.87, 138.75, 135.78, 131.78, 130.68, 
130.47, 129.21, 128.75, 119.98, 76.06, 63.33, 56.10, 39.43, 38.11, 16.16; HRMS m/z calculated 
for C_{17}H_{21}O_{6}N_{1} [M+Na]^+ 358.1261, 358.1277 observed.
[**Compound 2-7**] A stirred solution 0.5007 g (2.73 mmol) of the hydrochloride salt of β-hydroxy leucine with 0.6855 g (8.16 mmol) in 9 mL H₂O was brought to 0 °C. 9 mL THF was then added to stirring solution, followed by 1.2 mL (16.3 mmol) methyl chloroformate in three 0.4 mL portions over the next 30 minutes. Reaction was allowed to stir and reach room temperature overnight. After 20 hours, the reaction was diluted with H₂O (30 mL), and then washed with EtOAc (2x30 mL). The organic layer was then back extracted with saturated NaHCO₃ (3x25 mL). The combined aqueous layers were then acidified to pH = 2 with 1 M HCl and extracted with EtOAc (3x30 mL). The combined organic layers were then dried with MgSO₄ and concentrated en vacuo to give 0.4974 g (86% yield) **compound 2-7** as a white foaming oil that was used without further purification.

FTIR (neat) 3338, 2946, 2835, 2477, 2071, 1703, 1404, 1120, 1027 cm⁻¹; ¹H NMR (500 MHz, Chloroform-d) δ 6.03 (d, J = 9.6 Hz, 1H), 4.55 (d, J = 9.5 Hz, 1H), 3.85 – 3.77 (dd, 1H), 3.70 (s, 3H), 1.83 – 1.72 (m, 1H), 1.03 (d, J = 6.7 Hz, 3H), 0.93 (d, J = 7.7 Hz, 3H); ¹³C NMR (126 MHz, cdcl₃) δ 178.47, 160.44, 80.19, 58.97, 55.31, 33.36, 21.77, 21.50; HRMS m/z calculated for C₈H₁₅O₅N₁ [M+Na]⁺ 228.0842, 228.0852 observed.

![Structure](image)

[**Compound 2-8**] To a stirred solution at room temperature of 0.2873 g (1.08 mmol) **compound 2-7** with 0.4352 g (1.35mmol) Cs₂CO₃ in 4.4 mL DMF was added 0.19 mL (1.62 mmol) benzyl bromide drop-wise. Reaction was stirred at room temperature overnight. After 24 hours, the reaction was diluted with 50 mL EtOAc. The organic layer was then washed with saturated
NaHCO₃ (3x30 mL). The aqueous layer was then back extracted with EtOAc (2x20 mL). The combined organic layers were then dried with MgSO₄ and concentrated in vacuo. The crude product was then purified by flash chromatography (silica gel, 75% Hexane:25% EtOAc) to give 0.2588 g (81% yield) of compound 2-8 as a yellow oil.

FTIR (neat) 3392, 2961, 1703, 1523, 1456, 1380, 1340, 1274, 1210, 1166, 1117, 1060, 1001 cm⁻¹; ¹H NMR (500 MHz, Chloroform-d) δ 7.40 – 7.31 (m, 5H), 5.59 (d, J = 9.7 Hz, 1H), 5.20 (s, 2H), 4.61 – 4.56 (d, 1H), 3.76 – 3.65 (m, 4H), 1.82 – 1.71 (m, 1H), 1.01 (d, J = 6.7 Hz, 3H), 0.95 (d, J = 6.7 Hz, 3H); ¹³C NMR (126 MHz, cdcl₃) δ 174.54, 159.95, 138.04, 131.26, 131.26, 131.05, 130.76, 130.74, 80.16, 69.93, 58.94, 55.16, 33.47, 21.59, 21.53; HRMS m/z calculated for C₁₅H₂₁O₅N₁ [M+Na]⁺ 318.1312, 318.1320 observed.

[Compound 2-9] To a stirring solution of 0.2402 g (0.81 mmol) compound 2-8 in 5 mL DCM at 0 °C was added 0.47 mL (4.05 mmol) 2,6-Lutidine, followed by dropwise addition of 0.47 mL (2.03 mmol) TBDMS-triflate. Reaction was allowed to stir and reach RT overnight. After 24 hours, the reaction was quenched with 1 M HCl (20 mL). Reaction was then extracted with DCM (3x25 mL). The combined organic layers were then washed with 1 M KHSO₄(30 mL) and brine (30 mL). The organic layer was then dried with MgSO₄ and concentrated in vacuo. The crude product was then purified by flash chromatography (silica gel, 90% Hexane: 10% EtOAc) to give 0.2301 g (69% yield) of compound 2-9 as a clear oil.
FTIR (neat) 3450, 2956, 2857, 1728, 1500, 1463, 1337, 1252, 1202, 1165, 1081, 1059, 1003; $^1$H NMR (500 MHz, Chloroform-$d$) δ 7.42 – 7.29 (m, 5H), 5.39 (d, $J = 9.5$ Hz, 1H), 5.16 (s, $J = 1.7$ Hz, 2H), 4.48 (d, $J = 9.5$, 1.3 Hz, 1H), 3.97 (dd, $J = 6.6$, 1.3 Hz, 1H), 3.70 (s, 3H), 1.89 – 1.78 (m, 1H), 1.02 – 0.88 (m, 15H), 0.03 (s, 3H), -0.08 (s, 3H); $^{13}$C NMR (126 MHz, cdcl$_3$) δ 174.39, 159.51, 137.93, 131.26, 131.22, 130.99, 130.87, 130.85, 79.42, 69.89, 58.48, 55.00, 35.68, 28.57, 28.55, 28.31, 21.83, 20.79, -0.92, -1.63, -2.08; HRMS m/z calculated for C$_{21}$H$_{35}$O$_5$N$_1$Si$_1$ [M+Na]$^+$ 432.2177, 432.2177 observed.

[Compound 2-10] To a flame dried RBF containing 0.172 g (0.42 mmol) compound 2-9 was added 0.0150 g (0.13 mmol) Pd on C. The flask was put under positive pressure of H$_2$ and stirred vigorously in 1.8 mL MeOH. After 6 hours, the reaction was diluted with MeOH (25 mL) and passed through a plug column of celite. The reaction was then concentrated in vacuo to give 0.116 g (86% yield) compound 2-10 as a clear oil that was used without further purification.

FTIR (neat) 2957, 2930, 2858, 1715, 1511, 1465, 1362, 1253, 1213, 1080 cm$^{-1}$; $^1$H NMR (500 MHz, Chloroform-$d$) δ 5.40 (d, $J = 9.1$ Hz, 1H), 4.45 (d, $J = 9.1$, 1.5 Hz, 1H), 4.01 (dd, $J = 6.7$, 1.5 Hz, 1H), 3.71 (s, 3H), 1.90 – 1.77 (m, 1H), 0.95 – 0.89 (s, 15H), 0.08 (s, 3H), 0.01 (s, 3H); $^{13}$C NMR (126 MHz, cdcl$_3$) δ 179.54, 159.61, 79.67, 58.37, 55.17, 35.40, 28.56, 28.51, 28.26, 21.75, 20.96, 20.78, -1.69, -1.94; HRMS m/z calculated for C$_{14}$H$_{29}$O$_5$N$_1$Si$_1$ [M+Na]$^+$ 342.1707, 342.1706 observed.
[**Compound 2-11**] A stirred solution of 0.499 g (1.93 mmol) 8-(Boc-amino)octanoic acid and 0.42 mL (3.86 mmol) of 4-methylmorpholine in 5 mL THF was brought to -10 °C. To the stirring solution 0.25 mL isobutyl chloroformate was added and maintained at -10 °C for 30 minutes. The reaction was allowed to warm to 0 °C at which time 0.545 g (2.97 mmol) of the hydrochloride salt of β-hydroxyelucreine in 3.5 mL of 1 M NaOH was added. The reaction was allowed to warm to room temperature overnight and stirred for 24 hours. The reaction was then diluted with 30 mL H₂O, and washed with EtOAc (2x25 mL). The combined organic layers were then extracted with saturated NaHCO₃ (3x25 mL). All aqueous layers were combined and acidified to pH=2 with 1 M HCl and extracted with EtOAc (3x30 mL). The combined organic layers were then dried with MgSO₄ and concentrated *in vacuo* to give 0.696 g (88% yield) of **compound 2-11** as a tan foaming oil that was carried forward without further purification.

¹H NMR (500 MHz, Methanol- d₄) δ 4.69 (d, J = 2.5 Hz, 1H), 3.72 (dd, J = 9.1, 2.4 Hz, 1H), 3.03 (t, J = 7.0 Hz, 3H), 2.35 – 2.28 (m, 2H), 1.72 – 1.61 (m, 3H), 1.44 (s, 11H), 1.36 (q, J = 6.7, 5.7 Hz, 6H), 1.03 (d, J = 6.6 Hz, 3H), 0.91 (d, J = 6.7 Hz, 3H); ¹³C NMR (126 MHz, cd₃od) δ 177.70, 175.84, 159.79, 81.03, 79.36, 57.21, 42.63, 38.20, 36.20, 33.76, 32.17, 31.43, 31.35, 30.12, 29.02, 28.11, 27.28, 20.92, 20.70. Full characterization has been previously reported.¹

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![Chemical structure of Compound 2-11]
[**Compound 2-12**] To a stirring solution of 0.662 g (1.71 mmol) **compound 2-11** with 0.661 g NaHCO₃ (7.87 mmol) in 10 mL DMF was added 1.3 mL (15.8 mmol) Allyl Bromide in a dropwise fashion. The reaction was allowed to stir at room temperature overnight. After 20 hours the reaction was diluted with 30 mL H₂O, and extracted with EtOAc (3x30 mL). The combined organic layers were then washed with brine (1x20 mL). The organic layer was then dried with MgSO₄ and concentrated in vacuo. The crude product was then purified by flash chromatography (silica gel, 50% Hexane: 50% EtOAc) to give 0.4379 g (60% yield) of **compound 2-12** as a clear oil.

¹H NMR (300 MHz, CDCl₃) δ 6.75 (d, J=9.4 Hz, 1H), 5.78 (m, J=17.2, 10.4, 5.7 Hz, 1H), 5.20 - 5.11 (m, 2H), 4.69 (d, J=9.1, 2.1 Hz, 1H), 4.51 (d, J=5.3 Hz, 2H), 3.61 (dd, J=9.4, 1.8 Hz, 1H), 2.96 (dd, J=5.9 Hz, 2H), 2.14 (t, J=7.3 Hz, 2H), 1.42 - 1.70 (m, 4H), 1.31 (s, 11H), 1.20 (br, 6H), 0.89 (d, J=7.0 Hz, 3H), 0.79 (d, J=7.0 Hz, 3H); ¹³C NMR (126 MHz, cdcl₃) δ 176.20, 174.15, 158.72, 134.31, 121.25, 81.39, 80.04, 68.67, 56.97, 43.14, 39.02, 34.01, 33.75, 32.50, 31.45, 31.15, 31.07, 30.63, 29.18, 28.09, 21.74, 21.56. Full characterization has been previously reported.¹

![Chemical Structure](image)

[**Compound 2-13**] The reaction was set up according to General Procedure: Amide Coupling (HATU). The crude product was then purified by flash chromatography (silica gel, 10% Hexane: 90% EtOAc) to give 0.0631 g (77% yield) of **compound 2-13** as a yellow oil.
FTIR (neat) 3296, 3079, 2931, 2858, 1748, 1650, 1544, 1439, 1374, 1177, 1061 cm$^{-1}$; $^1$H NMR
(500 MHz, Chloroform-$d$) $\delta$ 7.24 – 7.11 (m, 5H), 6.90 – 6.79 (m, 1H), 5.90 – 5.80 (m, 2H), 5.38 – 5.30 (m, 2H), 5.30 – 5.15 (m, 2H), 5.00 – 4.88 (m, 3H), 4.58 (t, $J$ = 11.3, 5.7, 2.7, 1.4 Hz, 2H), 4.36 (ddd, $J$ = 7.1, 6.1, 0.9 Hz, 1H), 4.28 (dd, $J$ = 7.0, 1.1 Hz, 1H), 4.18 (dq, $J$ = 7.7, 6.3, 5.4 Hz, 1H), 3.32 (dd, $J$ = 14.5, 3.6 Hz, 1H), 3.19 – 3.12 (m, 2H), 2.96 (ddd, $J$ = 14.9, 9.6, 5.5 Hz, 1H), 2.29 – 2.10 (m, 4H), 1.95 – 1.89 (m, 4H), 1.78 (dq, $J$ = 13.3, 6.7 Hz, 1H), 1.57 (q, $J$ = 7.5 Hz, 2H), 1.45 – 1.33 (m, 2H), 1.31 – 1.12 (m, 5H), 0.96 (dd, $J$ = 6.6, 0.9 Hz, 1H), 0.90 – 0.83 (m, 6H); $^{13}$C NMR (126 MHz, cdc$_3$) $\delta$ 176.24, 174.01, 173.43, 173.22, 171.24, 139.24, 134.30, 132.07, 132.02, 131.17, 130.99, 129.69, 122.22, 121.37, 89.59, 77.70, 73.44, 68.64, 55.38, 42.12, 41.27, 40.44, 39.08, 37.55, 34.91, 33.84, 31.77, 31.53, 30.78, 29.22, 28.58, 25.30, 19.85. HRMS m/z calculated for C$_{33}$H$_{49}$O$_8$N$_3$ [M+Na]$^+$ 638.3412, 638.3385 observed.

[Compound 2-14] The reaction was set up according to general procedure: **Ring Closing Metathesis.** The crude product was then purified by flash chromatography (silica gel, 10% Hexane: 90% EtOAc) to give 0.0519 g (57% yield) of **compound 2-14** as a clear foaming oil.

FTIR (neat) 3294, 2931, 2858, 1746, 1653, 1542, 1439, 1375, 1176, 1061 cm$^{-1}$; $^1$H NMR (500 MHz, Chloroform-$d$) $\delta$ 7.32 – 7.20 (m, 7H), 6.72 (t, $J$ = 5.6 Hz, 1H), 6.15 (d, $J$ = 6.3 Hz, 1H), 5.54 – 5.49 (m, 2H), 5.37 (dd, $J$ = 9.3, 3.8 Hz, 1H), 4.64 (dd, $J$ = 13.1, 3.6 Hz, 1H), 4.49 – 4.42 (m, 2H), 4.41 – 4.35 (m, 1H), 4.34 – 4.28 (m, 1H), 3.43 – 3.34 (m, 2H), 3.23 (ddd, $J$ = 13.3, 8.7, 4.7 Hz, 1H), 2.98 (s, 3H), 2.28 (s, 3H), 2.11 (s, 3H), 1.99 (s, 3H), 1.90 (s, 3H), 1.83 (s, 3H). $^{13}$C NMR (126 MHz, cdc$_3$) $\delta$ 176.24, 174.01, 173.43, 173.22, 171.24, 139.24, 134.30, 132.07, 132.02, 131.17, 130.99, 129.69, 122.22, 121.37, 89.59, 77.70, 73.44, 68.64, 55.38, 42.12, 41.27, 40.44, 39.08, 37.55, 34.91, 33.84, 31.77, 31.53, 30.78, 29.22, 28.58, 25.30, 19.85. HRMS m/z calculated for C$_{33}$H$_{49}$O$_8$N$_3$ [M+Na]$^+$ 638.3412, 638.3385 observed.
3.7 Hz, 1H), 3.07 (dd, J = 14.4, 9.3 Hz, 1H), 2.27 (t, J = 7.5 Hz, 3H), 2.17 – 2.09 (m, 1H), 2.00 (s, 3H), 1.85 (dt, J = 13.3, 6.7 Hz, 1H), 1.70 – 1.50 (m, 4H), 1.39 – 1.20 (m, 4H), 0.94 (dd, J = 14.7, 6.8 Hz, 6H); 13C NMR (126 MHz, cdcl3) δ 178.30, 175.42, 174.97, 174.03, 172.89, 140.38, 133.18, 133.13, 132.20, 132.17, 131.95, 130.41, 130.07, 81.45, 64.73, 60.45, 57.22, 56.26, 42.60, 41.37, 39.02, 38.19, 34.44, 33.19, 29.23, 26.70, 22.93, 22.53, 22.33, 21.87, 18.16.HRMS m/z calculated for C_{31}H_{45}O_{8}N_{3} [M+Na]^+ 610.3099, 610.3068 observed.

![Compound structure](image)

[Compound 2-15a] The reaction was set up according General Procedure: Amide Coupling (HATU). The crude product was then purified via flash chromatography (silica gel, 50% Hexane: 50% EtOAc) to give 0.197 g (64% yield) of compound 2-15a as a clear oil.

FTIR (neat) 3306, 2931, 2858, 1747, 1699, 1650, 1536, 1440, 1372, 1259, 1156, 1156, 1058 cm\(^{-1}\); \(^1\)H NMR (500 MHz, Chloroform-\(d\)) δ 7.31 – 7.16 (m, 5H), 6.87 – 6.79 (m, 1H), 6.55 (d, J = 9.3 Hz, 1H), 5.89 (ddt, J = 17.2, 10.4, 5.7 Hz, 1H), 5.36 – 5.21 (m, 3H), 5.01 (dd, J = 16.8, 6.5, 3.2, 1.6 Hz, 2H), 4.84 (dd, J = 9.2, 2.2 Hz, 1H), 4.67 – 4.60 (m, 2H), 4.17 – 4.02 (m, 3H), 3.73 (dd, J = 9.0, 2.2 Hz, 1H), 3.35 (dd, J = 14.4, 3.6 Hz, 1H), 3.27 – 3.13 (m, 2H), 3.06 – 2.99 (m, 1H), 2.29 – 2.21 (m, 5H), 1.73 – 1.60 (m, 4H), 1.51 – 1.41 (m, 3H), 1.35 – 1.28 (m, 2H), 1.27 – 1.20 (m, 3H), 1.02 (d, J = 6.7 Hz, 3H), 0.96 – 0.92 (d, 3H); \(^13\)C NMR (126 MHz, cdcl3) δ 176.21, 174.17, 173.15, 171.41, 139.04, 134.35, 134.24, 132.06, 132.03, 131.00, 129.84, 129.49, 122.18, 121.20, 77.72, 73.41, 68.61, 64.17, 57.04, 56.40, 41.99, 41.26, 40.44, 39.04, 37.82, 33.81, 31.43,
31.23, 28.96, 27.99, 21.61, 19.83, 17.12; HRMS m/z calculated for C_{34}H_{51}O_{9}N_{3} [M+Na]^+ 668.3518, 668.3484 observed.

[Compound 2-16a] The reaction was set up according to general procedure: **Ring Closing Metathesis.** The crude product was then purified via flash chromatography (silica gel, 40% Hexane: 60% EtOAc) to give 0.108 g (59% yield) of **compound 2-16a** as a tan oil.

FTIR (neat) 3305, 2931, 1700, 1652, 1537, 1454, 1372, 1260, 1175, 1059 cm\(^{-1}\); \(^1\)H NMR (500 MHz, Chloroform-\(d\)) \(\delta\) 7.32 – 7.19 (m, 5H), 6.67 (d, \(J = 5.7 \) Hz, 1H), 6.47 (dd, \(J = 13.1, 8.9 \) Hz, 1H), 5.75 (d, \(J = 7.2 \) Hz, 1H), 5.56 (ddd, \(J = 16.9, 8.2, 6.2 \) Hz, 1H), 5.25 (dd, \(J = 9.3, 3.7 \) Hz, 1H), 5.15 (dt, \(J = 15.5, 5.0 \) Hz, 1H), 4.74 (dd, \(J = 8.7, 2.0 \) Hz, 1H), 4.62 (dd, \(J = 13.3, 5.1 \) Hz, 1H), 4.38 – 4.29 (m, 1H), 4.11 (dq, \(J = 11.4, 7.1 \) Hz, 2H), 3.74 (ddd, \(J = 8.9, 4.9, 1.9 \) Hz, 1H), 3.48 – 3.37 (m, 1H), 3.32 (dt, \(J = 14.5, 4.7 \) Hz, 1H), 3.19 – 3.03 (m, 2H), 2.87 (t, \(J = 5.1 \) Hz, 1H), 2.39 – 2.22 (m, 3H), 2.14 (dt, \(J = 14.2, 7.2 \) Hz, 0H), 1.78 – 1.69 (m, 1H), 1.68 – 1.60 (m, 1H), 1.40 – 1.19 (m, 4H), 1.02 (dd, \(J = 16.3, 6.6 \) Hz, 3H), 0.97 – 0.90 (m, 3H); \(^{13}\)C NMR (126 MHz, cdcl\(_3\)) \(\delta\) 176.77, 174.04, 173.32, 171.68, 159.20, 139.13, 131.03, 130.54, 130.26, 129.62, 129.54, 129.44, 78.22, 66.68, 64.04, 63.07, 57.07, 56.57, 41.75, 40.31, 38.63, 36.84, 33.72, 30.78, 30.45, 27.74, 26.60, 21.56, 19.82, 17.21, 16.86; HRMS m/z calculated for C_{32}H_{47}O_{9}N_{3} [M+Na]^+ 640.3205, 640.3181 observed.
Compound 2-15b] The reaction was set up according General Procedure: Amide Coupling (HATU). The crude product was then purified via flash chromatography (silica gel, 50% Hexane: 50% EtOAc) to give 0.164 g (68% yield) of compound 2-15b as a clear oil.

FTIR (neat) 3311, 2930, 2857, 1745, 1651, 1537, 1455, 1372, 1202, 1059 cm$^{-1}$; $^1$H NMR (500 MHz, Chloroform-$d$) $\delta$ 7.31 – 7.16 (m, 5H), 6.81 (d, $J = 6.8$ Hz, 1H), 6.53 (d, $J = 9.2$ Hz, 1H), 5.95 – 5.85 (m, 1H), 5.40 (td, $J = 10.3$, 9.1, 5.2 Hz, 2H), 5.35 – 5.09 (m, 5H), 4.85 (dd, $J = 9.2$, 2.1 Hz, 1H), 4.69 – 4.61 (m, 2H), 4.29 – 4.21 (m, 1H), 4.14 – 4.02 (m, 3H), 3.74 (d, $J = 9.0$ Hz, 1H), 3.28 (dd, $J = 14.4$, 4.1 Hz, 1H), 3.17 (dp, $J = 19.3$, 6.7 Hz, 2H), 3.07 (q, $J = 7.0$, 6.5 Hz, 1H), 2.44 (t, $J = 6.6$ Hz, 2H), 2.26 (t, $J = 7.6$ Hz, 2H), 1.67 (dp, $J = 30.0$, 7.5, 7.0 Hz, 3H), 1.43 (dt, $J = 14.6$, 8.5 Hz, 2H), 1.31 (qd, $J = 8.9$, 6.0, 5.0 Hz, 4H), 1.26 – 1.18 (m, 4H), 1.03 (d, $J = 6.6$ Hz, 3H), 0.94 (d, $J = 6.7$ Hz, 3H); $^{13}$C NMR (126 MHz, cdcl$_3$) $\delta$ 176.21, 174.17, 173.15, 171.41, 159.27, 139.04, 134.28, 132.08, 132.03, 131.97, 131.23, 131.00, 129.49, 122.18, 121.20, 77.72, 73.41, 68.61, 64.17, 57.04, 56.40, 41.99, 41.26, 40.44, 39.04, 37.82, 33.81, 31.43, 31.23, 28.96, 27.99, 21.61, 19.83, 17.12; HRMS m/z calculated for C$_{34}$H$_{51}$O$_{9}$N$_{3}$ [M+Na]$^+$ 668.3518, 668.3470 observed.
[**Compound 2-16b**] The reaction was set up according to general procedure: **Ring Closing Metathesis**. The crude product was then purified via flash chromatography (silica gel, 40% Hexane: 60% EtOAc) to give 0.092 g (58% yield) of **compound 2-16b** as a tan oil.

FTIR (neat) 3307, 2929, 2856, 1720, 1655, 1532, 1455, 1373, 1259, 1176, 1059 cm$^{-1}$; $^1$H NMR (500 MHz, Chloroform-$d$) δ 7.35 – 7.21 (m, 7H), 7.21 – 7.17 (m, 3H), 6.27 (d, $J$ = 9.0 Hz, 1H), 5.75 – 5.66 (m, 1H), 5.59 (dt, $J$ = 15.6, 5.5 Hz, 1H), 5.42 – 5.31 (m, 2H), 4.74 (dt, $J$ = 8.4, 3.2 Hz, 1H), 4.70 – 4.65 (m, 1H), 4.57 – 4.49 (m, 1H), 4.40 – 4.31 (m, 1H), 4.12 (dtdd, $J$ = 10.6, 7.7, 5.5, 3.0 Hz, 3H), 3.79 (dd, $J$ = 9.0, 2.0 Hz, 1H), 3.30 – 3.14 (m, 6H), 2.32 (dq, $J$ = 16.5, 9.9, 8.1 Hz, 2H), 2.19 (ddd, $J$ = 14.3, 8.3, 6.0 Hz, 1H), 1.72 (h, $J$ = 6.6 Hz, 7H), 1.57 (dd, $J$ = 14.4, 7.3 Hz, 1H), 1.41 (dq, $J$ = 18.6, 6.4, 5.6 Hz, 1H), 1.35 – 1.21 (m, 25H), 1.04 (d, $J$ = 6.5, 4.7 Hz, 3H), 0.99 – 0.93 (d, 3H); $^{13}$C NMR (126 MHz, cdcl$_3$) δ 173.65, 171.45, 170.23, 168.41, 158.00, 135.55, 129.88, 129.67, 128.46, 128.40, 128.01, 126.96, 126.93, 75.61, 64.70, 61.46, 54.52, 54.50, 53.53, 39.07, 38.62, 37.63, 36.26, 34.94, 31.21, 30.98, 28.47, 26.15, 25.03, 19.19, 18.86, 14.54; HRMS m/z calculated for C$_{32}$H$_{47}$O$_9$N$_3$ [M+Na]$^+$ 640.3205, 640.3157 observed.
The reaction was set up according to general procedure: **Macrocycle Esterification**. In an effort to confirm the necessity of purification prior to subsequent TBDMS deprotection crude material was carried forward. The crude material was then subjected to general procedure: **TBDMS Deprotection**. The crude product was then purified via flash chromatography (silica gel, 100% EtOAc) to give 8 mg (24% yield) of **compound 2-17** as a tan oil over two steps.

**FTIR (neat)** 3306, 2924, 2854, 1745, 1652, 1536, 1456, 1375, 1264, 1172, 1060 cm$^{-1}$; **$^1$H NMR (500 MHz, Chloroform-d)** $\delta$ 7.38 – 7.24 (m, 8H), 7.19 (d, $J = 11.8$ Hz, 1H), 6.77 (dd, $J = 13.0$, 6.1 Hz, 1H), 6.38 (q, $J = 9.1$, 8.2 Hz, 1H), 5.63 – 5.55 (m, 1H), 5.36 – 5.26 (m, 2H), 5.00 (ddddd, $J = 28.4$, 13.7, 10.6, 6.0 Hz, 2H), 4.75 – 4.67 (m, 2H), 4.60 (d, $J = 15.3$ Hz, 1H), 4.49 (dq, $J = 12.8$, 5.8, 3.5 Hz, 2H), 4.36 (d, $J = 12.8$ Hz, 1H), 4.25 – 4.12 (m, 2H), 3.74 (tt, $J = 9.2$, 4.7 Hz, 5H), 3.53 – 3.37 (m, 2H), 3.22 (ddt, $J = 14.4$, 9.5, 4.3 Hz, 2H), 3.07 (tdd, $J = 14.1$, 10.4, 4.7 Hz, 1H), 2.41 – 2.26 (m, 4H), 2.11 – 1.95 (m, 7H), 1.90 – 1.71 (m, 5H), 1.47 – 1.20 (m, 14H), 1.17 – 1.10 (m, 3H), 1.08 – 0.85 (m, 10H). **$^{13}$C NMR (126 MHz, cdcl$_3$)** $\delta$ 177.81, 174.66, 174.00, 173.07, 173.05, 172.11, 159.63, 139.75, 132.25, 132.17, 131.09, 131.07, 129.53, 128.36, 128.34, 78.95, 67.40, 59.60, 59.57, 55.28, 55.23, 54.61, 41.67, 40.51, 37.98, 37.24, 33.60, 32.37, 32.34,
30.88, 30.86, 29.61, 29.45, 26.69, 25.30, 22.11, 21.63, 21.43, 21.02. HRMS m/z calculated for C_{39}H_{58}O_{12}N_{4} [M+Na]^+ 775.4124, 775.4089 observed.

[Compound 2-18a] The reaction was set up according to general procedure: **Macrocycle Esterification.** The crude product was then purified via flash chromatography (silica gel, 60% Hexane: 40% EtOAc) to give 0.0555 g (50% yield) of compound 2-18a as a clear foaming oil. Efforts were not made to fully characterize intermediate 2-18a as it was deemed unnecessary to purify material prior to subsequent TBDMS deprotection.

[Compound 2-19a] The reaction was set up according to general procedure: **TBDMS Deprotection.** The crude product was then purified via flash chromatography (silica gel, 70% Hexane: 30% EtOAc) to give 0.0113 g (58% yield) of compound 2-19a as a tan oil.
FTIR (neat) 3308, 2930, 1703, 1652, 1529, 1455, 1320, 1260, 1169, 1114, 1058 cm\(^{-1}\); \(^1\)H NMR (500 MHz, Chloroform-\(d\)) \(\delta\) 7.33 – 7.20 (m, 10H), 6.85 – 6.79 (m, 1H), 6.28 (d, \(J = 8.4\) Hz, 1H), 6.02 (d, \(J = 7.1\) Hz, 1H), 5.56 (d, \(J = 8.8\) Hz, 2H), 5.49 – 5.41 (m, 0H), 5.39 – 5.34 (m, 1H), 5.10 (dd, \(J = 10.1\), 3.3 Hz, 1H), 5.04 – 5.00 (m, 2H), 4.81 (dd, \(J = 12.0\), 7.4 Hz, 1H), 4.73 (d, \(J = 8.5\) Hz, 1H), 4.52 (dd, \(J = 13.9\), 4.7 Hz, 1H), 4.44 (ddd, \(J = 9.7\), 5.1, 1.7 Hz, 1H), 4.38 (q, \(J = 6.1\) Hz, 1H), 4.22 (d, \(J = 13.3\) Hz, 1H), 4.16 – 4.04 (m, 4H), 3.74 – 3.67 (m, 6H), 3.24 (dd, \(J = 14.3\), 3.3 Hz, 1H), 3.18 – 3.06 (m, 1H), 2.38 – 2.21 (m, 4H), 2.12 (td, \(J = 9.0\), 8.4, 4.7 Hz, 1H), 1.94 (ddt, \(J = 21.4\), 8.2, 6.7 Hz, 1H), 1.80 (ddd, \(J = 13.3\), 8.6, 6.5 Hz, 1H), 1.75 – 1.67 (m, 1H), 1.65 – 1.56 (m, 1H), 1.53 – 1.43 (m, 1H), 1.41 – 1.31 (m, 6H), 1.29 – 1.20 (m, 7H), 1.12 – 1.04 (m, 4H), 1.04 – 0.87 (m, 14H); \(^{13}\)C NMR (126 MHz, cdcl3) \(\delta\) 177.34, 174.52, 174.05, 173.11, 171.91, 159.64, 159.06, 139.47, 132.26, 132.21, 132.05, 131.04, 130.94, 129.48, 129.16, 80.51, 78.37, 67.55, 63.80, 63.03, 59.53, 56.29, 55.34, 41.69, 40.45, 38.13, 37.28, 33.53, 32.27, 30.66, 29.95, 27.10, 25.82, 23.69, 22.02, 21.62, 20.95, 17.24, 16.85, 16.05; HRMS m/z calculated for C\(_{40}\)H\(_{60}\)O\(_{13}\)N\(_4\) [M+Na]\(^{+}\) 827.4049, 827.3993 observed.

[Compound 2-18b] The reaction was set up according to general procedure: **Macrocycle Esterification.** The crude product was then purified via flash chromatography (silica gel, 70% Hexane: 30% EtOAc) to give 0.0555 g (63% yield) of compound 2-18a as a clear foaming oil.
Efforts were not made to fully characterize intermediate 2-18b as it was deemed unnecessary to purify material prior to subsequent TBDMS deprotection.

[Compound 2-19b] The reaction was set up according to general procedure: TBDMS Deprotection. The crude product was then purified via flash chromatography (silica gel, 70% Hexane: 30% EtOAc) to give 11 mg (64% yield) of compound 2-19a as a tan oil.

FTIR (neat) 3324, 2933, 1725, 1658, 1534, 1455, 1372, 1265, 1175, 1115, 1060 cm⁻¹; ¹H NMR (500 MHz, Chloroform-d) δ 7.32 – 7.14 (m, 6H), 6.27 (d, J = 9.4 Hz, 2H), 6.18 (s, 1H), 5.60 (dp, J = 27.5, 7.3, 6.4 Hz, 2H), 5.46 – 5.37 (m, 1H), 5.29 (d, J = 7.5 Hz, 1H), 5.06 (ddd, J = 11.2, 9.2, 1.9 Hz, 1H), 4.92 – 4.77 (m, 1H), 4.75 – 4.65 (m, 1H), 4.46 – 4.37 (m, 2H), 4.34 – 4.28 (m, 2H), 4.12 (dq, J = 10.5, 7.1 Hz, 4H), 3.75 – 3.63 (m, 5H), 3.62 (d, J = 8.8 Hz, 1H), 3.28 – 3.17 (m, 4H), 3.16 (dt, J = 14.4, 4.3 Hz, 3H), 2.55 (d, J = 14.7 Hz, 2H), 2.43 (q, J = 7.1 Hz, 1H), 2.32 (s, 2H), 2.20 (ddd, J = 14.5, 8.5, 6.0 Hz, 1H), 2.05 (s, 2H), 1.95 (dp, J = 9.2, 6.7 Hz, 1H), 1.75 (s, 7H), 1.58 – 1.51 (m, 1H), 1.42 (dd, J = 13.7, 7.0 Hz, 2H), 1.27 (ddd, J = 9.3, 6.1, 2.5 Hz, 13H), 1.11 – 0.96 (m, 8H), 0.93 (q, J = 6.0, 5.5 Hz, 10H); ¹³C NMR (126 MHz, cdcl₃) δ 176.67, 174.26, 174.01, 172.77, 171.06, 159.79, 159.02, 138.31, 132.32, 132.29, 131.34, 131.32, 131.00, 130.29, 129.56, 81.19, 78.03, 68.27, 64.11, 63.05, 59.33, 55.54, 55.18, 41.81, 40.33, 38.65,
HRMS m/z calculated for C_{40}H_{60}O_{13}N_{4} [M+Na]^+ 827.4049, 827.4000 observed.

[Compound 2-20] A stirred solution 0.5024 g (2.74 mmol) of the hydrochloride salt of β-hydroxy leucine with 0.6880 g (8.16 mmol) NaHCO₃ in 10 mL H₂O and 10 mL THF was brought to 0 °C. To this stirring solution was added 2.4 mL (17.2 mmol) 2,2,2-trichloroethoxycarbonyl chloride in three 0.8 mL portions over the next 30 minutes. Reaction was allowed to stir and reach room temperature overnight. After 24 hours, the reaction was diluted with H₂O (30 mL), and then washed with EtOAc (2x30 mL). The organic layer was then back extracted with saturated NaHCO₃ (3x25 mL). The combined aqueous layers were then acidified to pH = 2 with 1 M HCl and extracted with EtOAc (3x30 mL). The combined organic layers were then dried with MgSO₄ and concentrated in vacuo to give 0.6955 g (79% yield) compound 2-20 as a yellow foaming oil that was used without further purification.

¹H NMR (500 MHz, Chloroform-d) δ 6.22 (d, J = 9.5 Hz, 1H), 4.83 (d, J = 12.0 Hz, 1H), 4.69 (d, J = 12.0 Hz, 1H), 4.59 (dd, J = 9.6, 1.9 Hz, 1H), 3.85 (dd, J = 9.4, 1.9 Hz, 1H), 1.79 (m, J = 9.4, 6.6 Hz, 1H), 1.05 (d, J = 6.6 Hz, 3H), 0.95 (d, J = 6.6 Hz, 3H); ¹³C NMR (126 MHz, cdcl₃) δ 178.06, 157.95, 97.98, 80.19, 77.42, 59.08, 33.46, 21.82, 21.43; Full characterization has been previously reported.¹
[Compound 2-21] The reaction was set up according to General Procedure: **Lactone Formation.** The crude product was then purified by flash chromatography (silica gel, 80% Hexane: 20% EtOAc) to give 0.4222 g (71% yield) of **compound 2-21** as a white solid.

$^1$H NMR (500 MHz, Chloroform-$d$) $\delta$ 5.91 (d, $J = 9.3$ Hz, 1H), 5.53 (d, $J = 8.2$, 6.0, 2.1 Hz, 1H), 4.99 – 4.91 (d, 1H), 4.88 – 4.81 (d, 1H), 4.34 – 4.25 (dd, 1H), 1.93 (m, 1H), 1.15 – 1.04 (d, 3H), 0.96 (d, $J = 30.4$, 6.8, 2.1 Hz, 3H). $^{13}$C NMR (126 MHz, cdc$_3$) $\delta$ 171.17, 156.45, 85.17, 77.65, 77.49, 62.26, 31.41, 21.11, 19.91. Full characterization of **compound 2-21** was not obtained as this pathway was deemed unsuitable for future use.

[Compound 2-22] In a flame dried flask was added 0.222 g (0.73 mmol) **compound 2-21** which was then dissolved in 9.0 mL (94.9 mmol) acetic anhydride. This solution was then added via syringe to a flask charged with 0.8560 g (13.1 mmol) freshly activated Zinc. To this flask was added 0.18 mL (1.31 mmol) triethylamine. This reaction mixture was sonicated at 0°C for four hours after which the reaction contents were diluted with methanol (50 mL) and filtered to remove solid. The filtrate was then concentrated in vacuo. The crude product was then purified by flash chromatography (silica gel, 80% Hexane: 20% EtOAc) to give 0.0359 g (29% yield) of **compound 2-22** as a pale yellow oil.
$^1$H NMR (300 MHz, Chloroform-$d$) $\delta$ 6.29 (d, $J = 8.8$ Hz, 1H), 5.71 (d, $J = 8.8$, 5.9 Hz, 1H), 4.26 (dd, $J = 10.3, 5.9$ Hz, 1H), 2.07 (s, 3H), 1.94 – 1.81 (m, 1H), 1.08 (d, $J = 6.5$ Hz, 3H), 0.88 (d, 3H). Full characterization of compound 2-22 was not obtained as this pathway was deemed unsuitable for future use.

[Compound 2-23] A stirred solution 0.3074 g (2.63 mmol) of the hydrochloride salt of $\beta$-hydroxylycine with 0.4200 g (4.90 mmol) NaHCO$_3$ in 5.8 mL H$_2$O was brought to 0 °C. To this stirring solution was added 0.5836 g (2.67 mmol) Boc$_2$O dissolved in 2.2 mL THF. The reaction was allowed to stir and reach room temperature overnight. After 18 hours, the reaction was diluted with H$_2$O (30 mL), and then washed with EtOAc (2x30 mL). The organic layer was then back extracted with saturated NaHCO$_3$ (3x25 mL). The combined aqueous layers were then acidified to pH = 2 with 1 M HCl and extracted with EtOAc (3x30 mL). The combined organic layers were then dried with MgSO$_4$ and concentrated in vacuo to give 0.3613 g (87% yield) compound 2-23 as a white foaming oil that was used without further purification.

$^1$H NMR (500 MHz, Chloroform-$d$) $\delta$ 5.68 (d, $J = 9.4$ Hz, 1H), 4.44 (d, $J = 9.3$ Hz, 1H), 3.78 – 3.72 (dd, 1H), 1.78 – 1.71 (m, 1H), 1.43 (s, 9H), 1.01 (d, $J = 6.6$ Hz, 3H), 0.93 (d, $J = 14.2$, 6.7 Hz, 3H). $^{13}$C NMR (126 MHz, Chloroform-$d$) $\delta$ 178.67, 159.21, 82.80, 82.79, 58.67, 33.80, 33.40, 30.97, 30.66, 21.93, 21.53. Full characterization has been previously reported.$^1$
The reaction was set up according to General Procedure: \textbf{Lactone Formation}. The crude product was then purified by flash chromatography (silica gel, 80% Hexane: 20% EtOAc) to give 0.3739 g (77% yield) of \textbf{compound 2-24} as a white solid.

$^1$H NMR (300 MHz, Chloroform-$d$) $\delta$ 5.48 (dd, $J = 9.3, 5.7$ Hz, 1H), 5.41 (d, 1H), 4.22 (dd, $J = 10.4, 5.7$ Hz, 1H), 2.03 – 1.84 (m, 1H), 1.45 (s, 9H), 1.08 (d, $J = 6.5$ Hz, 3H), 0.91 (d, $J = 6.6$ Hz, 3H); $^{13}$C NMR (151 MHz, cdcl$_3$) $\delta$ 169.80, 154.43, 82.85, 81.13, 59.18, 28.64, 28.64, 28.10, 28.10, 18.41, 17.05. Full characterization of \textbf{compound 2-24} was not obtained as this pathway was deemed unsuitable for future use.

The reaction was set up according to General Procedure: \textbf{Lactone Formation}. The crude product was then purified by flash chromatography (silica gel, 80% Hexane: 20% EtOAc) to give 0.3412 g (75% yield) of \textbf{compound 2-25} as a white solid.

$^1$H NMR (300 MHz, Chloroform-$d$) $\delta$ 5.50 (d, $J = 5.8$ Hz, 2H), 4.25 (dd, $J = 10.2, 5.6$ Hz, 1H), 3.74 (s, 3H), 1.91 (dp, $J = 10.3, 6.6$ Hz, 1H), 1.09 (d, $J = 6.5$, 0.9 Hz, 3H), 0.92 (d, $J = 6.6$, 0.9 Hz, 3H). $^{13}$C NMR (151 MHz, Chloroform-$d$) $\delta$ 169.70, 154.43, 82.85, 81.13, 59.18, 28.64, 28.64, 28.10, 18.46, 17.26. Full characterization of \textbf{compound 2-25} was not obtained as this pathway was deemed unsuitable for future use.
[**Compound 2-26**] A stirred solution 0.3500 g (1.90 mmol) of the hydrochloride salt of β-hydroxyleucine with 0.4789 g (5.70 mmol) NaHCO₃ in 6 mL H₂O was brought to 0 °C. To this stirring solution was added 6 mL THF followed by 1.1 mL (11.4 mmol) ethyl chloroformate in three 0.35 mL portions over the next 30 minutes. The reaction was allowed to stir and reach room temperature overnight. After 20 hours, the reaction was diluted with H₂O (30 mL), and then washed with EtOAc (2x30 mL). The organic layer was then back extracted with saturated NaHCO₃ (3x25 mL). The combined aqueous layers were then acidified to pH = 2 with 1 M HCl and extracted with EtOAc (3x30 mL). The combined organic layers were then dried with MgSO₄ and concentrated *in vacuo* to give 0.0.3586 g (86% yield) **compound 2-26** as a clear foaming oil that was used without further purification.

¹H NMR (300 MHz, Chloroform-*) δ 6.04 (d, *J* = 9.4 Hz, 1H), 4.71 – 4.60 (d, 1H), 4.30 – 4.21 (q, 2H), 3.90 (dd, *J* = 9.5 Hz, 1H), 1.89 (m, 1H), 1.37 (t, *J* = 7.1 Hz, 3H), 1.17 – 1.11 (d, 3H), 1.04 (d, *J* = 6.6 Hz, 3H). ¹³C NMR (151 MHz, Chloroform-*) δ 175.71, 157.29, 77.42, 61.47, 56.13, 30.64, 19.13, 18.78, 14.35. Full characterization of **compound 2-26** was not obtained as this pathway was deemed unsuitable for future use.
[Compound 2-27] The reaction was set up according to General Procedure: Lactone Formation. The crude product was then purified by flash chromatography (silica gel, 80% Hexane: 20% EtOAc) to give 0.2545 g (77% yield) of compound 2-27 as a white solid.

$^1$H NMR (500 MHz, Chloroform-$d$) δ 5.65 (d, $J = 8.5$ Hz, 1H), 5.51 (d, $J = 9.4$, 5.9 Hz, 1H), 4.24 (dd, $J = 10.3$, 5.9 Hz, 1H), 4.20 – 4.12 (q, 2H), 1.92 (m, $J = 10.3$, 6.7 Hz, 1H), 1.30 – 1.23 (t, 3H), 1.08 (d, $J = 6.5$ Hz, 3H), 0.91 (d, $J = 6.6$ Hz, 3H). $^{13}$C NMR (151 MHz, Chloroform-$d$) δ 169.48, 155.47, 82.66, 62.03, 59.42, 28.63, 18.39, 17.19, 14.38. Full characterization of compound 2-27 was not obtained as this pathway was deemed unsuitable for future use.

\[
\begin{align*}
\text{O} & \quad \text{N} \\
\text{O} & \quad \text{C} \\
\text{O} & \quad \text{O} \\
\text{NHBoc} & \quad \text{NHboc}
\end{align*}
\]

[Compound 2-28] The reaction was set up according to General Procedure: Lactone Formation. The crude product was then purified by flash chromatography (silica gel, 80% Hexane: 20% EtOAc) to give 0.2991 g (64% yield) of compound 2-28 as a white solid.

$^1$H NMR (500 MHz, Chloroform-$d$) δ 7.39 – 7.29 (m, 5H), 5.86 (d, $J = 8.7$ Hz, 1H), 5.45 (dd, $J = 8.8$, 5.8 Hz, 1H), 5.12 (s, 2H), 4.83 (m, $J = 6.3$ Hz, 1H), 1.42 (d, $J = 6.1$ Hz, 3H). $^{13}$C NMR (126 MHz, cdcl$_3$) δ 171.63, 158.13, 138.21, 131.31, 131.31, 131.31, 130.90, 130.81, 77.52, 70.49, 63.01, 17.60. Full characterization of compound 2-28 was not obtained as this pathway was deemed unsuitable for future use.
[Compound 2-29]

The reaction was set up according to General Procedure: **Lactone Formation.** The crude product was then purified by flash chromatography (silica gel, 80% Hexane: 20% EtOAc) to give 0.2983 g (64% yield) of **compound 2-29** as a clear oil.

$^1$H NMR (500 MHz, Chloroform-$d$) $\delta$ 7.46 – 7.38 (m, 3H), 7.28 (m, $J = 6.1$ Hz, 2H), 5.81 – 5.72 (m, 2H), 4.86 (d, $J = 9.4$ Hz, 1H), 1.33 (s, 9H). $^{13}$C NMR (126 MHz, cdcl$_3$) $\delta$ 171.92, 156.73, 135.80, 131.68, 131.61, 131.57, 128.43, 83.76, 80.35, 64.95, 30.98, 30.90, 30.67, 29.94. Full characterization of **compound 2-29** was not obtained as this pathway was deemed unsuitable for future use.

![Structure of compound 2-29](image)

[Compound 2-30]

The reaction was set up according to General Procedure: **Lactone Formation.** The crude product was then purified by flash chromatography (silica gel, 80% Hexane: 20% EtOAc) to give 0.1144 g (55% yield) of **compound 2-30** as a white solid.

$^1$H NMR (500 MHz, Chloroform-$d$) $\delta$ 5.51 (d, $J = 8.3$ Hz, 1H), 5.42 (dd, $J = 8.7$, 5.9 Hz, 1H), 4.85 (dq, $J = 6.2$ Hz, 1H), 1.45 (s, 12H). $^{13}$C NMR (151 MHz, Chloroform-$d$) $\delta$ 169.46, 154.58, 81.10, 74.97, 60.00, 28.09, 14.86. Full characterization of **compound 2-30** was not obtained as this pathway was deemed unsuitable for future use.
Chapter Three: Simplified Analogs
Conclusions and Future Directions

3.1 First Generation Synthesis

![FR900359 and WU-07047](image)

Figure 3.1 – WU-07047 & FR900359

This project began with the successful synthesis of analog WU-07047 (Figure 3.1), also highlighted in Figure 1.4. This analog was then screened in a receptor-assisted GTPγS nucleotide exchange assay to determine its inhibitory activity in comparison to the natural product FR.1-3 In this assay the uptake of GTP was observed using GTPγS, a γ-phosphate radiolabeled with 35S. As an inactive G protein is bound to GDP (Figure 1.1), observing an uptake in GTP is indicative of the activation of this cell signaling pathway. We hoped to observe less of an uptake of GTP, indicating the successful binding and subsequent inactivation of this cell signaling pathway. When compared to the commercially available compound UBO-QIC, now referred to as FR900359 (Figure 3.1) there are two noteworthy observations regarding WU-07047. First, WU retained selectivity towards only one G protein and successfully inhibited nucleotide exchange on Gaq in a concentration dependent manner. Second, while WU was able
to inhibit nucleotide exchange with similar efficacy as FR (up to 40% inhibition of nucleotide exchange) it was much less potent (Figure 3.2).  

![Figure 3.2 – Receptor-assisted GTPγS Nucleotide Exchange Assay](image)

We believe this loss is due to the simplification of the top and bottom bridges to alkyl chains. Efforts were first made to install an intramolecular hydrogen bond found in the natural product to the simplified analog.

### 3.2 Top Bridge Amide Bond Installation

In the first attempt to return biological activity to a simplified analog, Dr. Derek Rensing chose to install an amide bond within the simplified alkyl chain of the top bridge in WU-07047. This decision was made based on an intramolecular hydrogen bond that appears to be important in the crystal structure of YM bound to its active site in Gαq. This hydrogen bond donor is highlighted red in both FR and the new, simplified analog WU-09060 (Figure 3.3). The intent was to stabilize the bound conformation of the analog by returning this intramolecular hydrogen bond and in doing so improve binding of the analog to the receptor.
Significant challenges were faced when assembling this analog, inevitably leading to the analog WU-09060 in only a 1.8% overall yield, with a longest linear reaction sequence of 10 steps. When tested for its ability to inhibit exchange of GDP for GTP on Gαq the analog installed with the intramolecular hydrogen bond proved to be worse than the first simplified analog WU-07047 (Figure 3.5). Due to the difficulties associated with the synthesis of WU-09060 and the loss of potency and efficacy in the biological assay, this analog is no longer being pursued. The observation that a change within the top bridge of the simplified analogs did not lead to a significant change in biology is consistent with the suggestion that the top bridge of the molecule may not play a significant role in binding. As mentioned in Section 1.2, this portion of the molecule appears to reside outside of the active site in the natural product – receptor complexes forwarded to date. This knowledge, in addition to information currently available in the literature, has led to the decision to install the “bottom bridge” of the macrocycle (discussed in Chapter 4) in an effort to improve the binding and efficacy of the simplified analogs.
3.3 Inhibition of Vasoconstriction by YM & WU

In addition to the biological studies underway in our collaboration with the Blumer lab studying G protein signaling and its relationship to uveal melanoma, our collaborators in the Osei-Owusu lab are exploring the use of our simplified analogs in connection with the role of Gαq/11 in vasoconstriction. 6 Alone these lines, calcium influx through voltage-gated L-type calcium channels (LTCC) and receptor-operated calcium channels are critical for vasoconstriction, and it appears that Gαq/11 plays a critical role in regulating these cell signaling events. As such, efforts have been made to determine the ability of Gαq/11 inhibitors to block the LTCC. This has led to a unique observation. Both YM and WU exhibit an inhibitory effect on calcium influx by partially targeting the LTCC itself, in addition to Gαq/11, thereby blocking vasoconstriction. This is in contrast to FR, which blocks vasoconstriction by only targeting Gαq/11, with no off-target binding at the LTCC. Efforts to synthesize new analogs to probe this difference between activity are currently underway and will be discussed in Section 3.6.

3.4 Alternative Binding Mode of WU

The observation that YM and WU partially block vasoconstriction through targeting the LTCC led to a molecular modeling study comparing the critical residues within the hydrophobic pocket of Gαq/11 to that of the LTCC. It was found that there was significant homology between the two receptors, potentially accounting for YM and WU’s ability to block vasoconstriction by binding to the LTCC. Interestingly, while both YM and WU bind to the LTCC, molecular modeling suggests that the two molecules bind the receptor with distinct binding motifs (Figure 3.4). 6 In fact, relative to YM, the WU analog is completely inverted in the binding pocket.
In the case of YM, the phenyl ring of the phenylacetic acid (DPla6) binds in a hydrophobic pocket near amino acid residue Y168. In comparison, the bound WU analog places the phenyl ring towards amino acid residue F203, and the isopropyl group of the β-hydroxy leucine tail is oriented towards Y168. These observations have led to the projection of a variety of analogs to probe this unique binding motif. Is it possible to exploit the binding motif displayed above to develop an analog that is selective towards the LTCC receptor in the same fashion that FR is selective towards only Gαq? These analogs will be discussed further detail in Section 3.6.

3.5 Methyl Carbamate Protecting Group

The use of a methyl chloroformate as a replacement for the acetate group on the β-hydroxy leucine tail was chosen in the hopes that it would be biologically tolerated thereby eliminating the need exchange protecting groups in the final steps of the synthesis. As previously mentioned, the use of an acetyl protecting group on an amino acid derivative is not compatible
with an esterification reaction of the acid and hence cannot be used to add the β-hydroxy leucine side chain. The hypothesis that the methyl carbamate would be tolerated was based upon the observation that one of the two differences between YM and FR occurs at this site. The FR natural product has a propionate group at this position, and a variety of analogs of FR have been isolated with groups larger than a propionate appended to the amine in the β-hydroxy leucine side chain.7

![Comparison of FR and WU compounds](image)

**Figure 3.5 – Comparison of FR to WU Analogs**

Fortunately, not only did the use of a methoxycarbamate protecting group result in an improvement of overall yields, but it also improved the efficacy and potency of the analog relative to the first simplified analog WU-07047. This new analog, **Compound 2-17**, is labeled WU_06047 in **Figure 3.5**. In this case, the efficacy of the simplified analogs was compared to that of FR using an agonist-induced Ca²⁺ flux assay.8 In this assay HEK2923 cells were transfected with a Twitch 2B Ca²⁺ Fret reporter. The cells were then treated with FR, an FR analog, or a vehicle for three hours, and then stimulated with the Gq-coupled GPCR agonist
carbachol. The recorded changes in Twitch 2B fluorescence and FRET are reported in Figure 3.5. While the analog WU_06047 is a step in the right direction, we are still far from the efficacy found in the natural product FR.

### 3.6 Simplified Analogs from Molecular Modeling

Based upon some of the observations discussed in Section 3.4, there are a handful of analogs of interest that are currently in development. There is significant interest in probing the hydrophobic pocket the phenyl ring within the right-hand portion of the molecules (highlighted red in Figure 3.6). Analogs in development will have hydroxy or methoxy substituents in either the ortho, meta, or para positions.

![Figure 3.6 – Simplified Analogs from Molecular Modeling](image)

Due to the unique inverted binding mode to the LTCC predicted for analog WU-07047, an analog containing a phenyl group in place of an isopropyl group of the β-hydroxyleucine tail is being synthesized by Dr. Ruby Krueger in our lab. Dr. Krueger has been able to rapidly assemble the core ring scaffold and is currently at the final esterification reaction necessary to add the β-hydroxy leucine side-chain. Her ability to rapidly reach this point in the synthesis
within several weeks of joining our lab is further evidence of the success and reproducibility of our second-generation synthesis.

3.7 Simplified Analogs to Improve Efficacy

The second-generation synthesis has given us rapid access to analogs to probe distinct portions of the simplified analogs. Two such analogs that may significantly improve activity with few changes in our synthetic strategy are outlined in Figure 3.7. According to current SAR studies, the exchange of the NMe-amide labeled 1 to an amide results in a 670-fold loss in potency. Similarly, the exchange of the NMe-amide labeled 2 resulted in a nearly 500-fold loss in potency.9

![Figure 3.7 – Recommended Analogs Based on SAR](image)

The installation of the NMe-amides within the structure should in theory require only one additional step to install each NMe-amine (Figure 3.8). The methylation of the amine within the “top bridge” labeled 1 can be synthesized by treating Boc-8-aminocaprylic acid with methyl iodide and sodium hydride. These conditions will mimic the methylation conditions used in the synthesis of NMe-OMe-Thr in our central building block (Scheme 3.1).9,10 The installation of the NMe-amide within the “bottom bridge” labeled 2 could be readily synthesized from
Compound 2-11. In our second-generation synthesis Compound 2-11 was protected with an allyl ester (Scheme 2.3). In this case, it may be plausible to use standard coupling conditions to couple the carboxylic acid with an N-allylmethylamine. Upon synthesis of these two molecules, the synthetic route to the final product would not vary from our second-generation synthesis.

![Scheme 2.3](image)

**Figure 3.8 – N-Methyl Synthetic Strategy**

### 3.8 Conclusions from Simplified Analogs

![Figure 3.9](image)

**Figure 3.9 – Second Generation Synthesis**

The use of our second-generation synthesis has played a key role in the development and scaling of new analogs. The key step, the addition of the β-hydroxy leucine to the macrocycle last (Figure 3.9), provided the first evidence that this esterification can be accomplished in the final stage of a synthesis. A key component of this second-generation synthesis is the use of a methyl carbamate protecting group in place of the acetyl group located on the β-hydroxyleucine.
tail in the natural product. At present, this synthetic route has produced three different analogs, and is still in use for the rapid synthesis of chemical probes (some in as little as 2-3 weeks). This overall strategy will continue to be employed for the development of all new analogs to probe the GPCR many of which were discussed above. With the second-generation strategy in place, we have turned our attention to returning efficacy and potency to our simplified analogs and the development of analogs that are selective inhibitors of voltage-gated L-type calcium channels.
3.9 References


Chapter Four: Returning Activity through Installation of the Bottom Bridge

4.1 A New Retrosynthetic Analysis

As more information has become available, the X-ray crystal driven hypothesis that only the left- and right-hand portions of the natural product that make direct contact with Gaq are necessary for the activity of the molecule has been shown to be incorrect. This has been confirmed through the synthesis of our simplified analogs that retain selectivity towards Gaq, but do not have either the same potency or efficacy as the natural product. This knowledge combined with the work of others suggests the importance of the “bottom bridge”.\textsuperscript{1,2} In these structure activity studies, changes within the bottom bridge have had a significant impact on the potency and efficacy of the analog. For example, exchanging the NMe-OMe-Thr for a threonine within central building block (\textbf{Figure 4.1}) resulted in nearly a 500-fold loss in potency. Whether through direct contact with the active site, or through conformational constraint, it appears that incorporation of an intact portion of the macrocycle (stretching from DhAla to $\beta$-hydroxyleucine) will be necessary to improve binding the activity of the analogs being scaled.

\textbf{Figure 4.1} – “Bottom Bridge” Retrosynthetic Analysis
From the lessons learned from our second-generation synthesis discussed in Chapter 2, the plan for construction of a more functionalized analog called for the addition of the β-hydroxy leucine tail last, and a ring closing metathesis strategy to tie together the macrocycle (Figure 4.1). In the retrosynthetic analysis, following removal of the β-hydroxy leucine tail, the synthesis breaks down to the construction and subsequent assembly of three major building blocks. The right-hand portion is a coupled product of N-allylamine and a phenylacetic acid derivative. The central portion is a known building block that is a dipeptide made from a demethylated threonine and an N-acylated threonine. The left-hand portion of our molecule is a coupled product of a β-hydroxy leucine to a carboxylic acid derivative containing a terminal alkene. The plan called for assembly of these three molecules required standard coupling or esterification procedures, with the formation of the macrocycle using a ring closing metathesis reaction. This convergent route will give us rapid access to multiple analogs. With that backdrop, we begin with the reported synthesis of the bottom bridge and the challenges associated with its assembly.

### 4.2 Synthesis of the Bottom Bridge

Scheme 4.1 – Central Building Block Synthesis
The synthesis of the central building block was repeated following current literature protocol.\textsuperscript{1,2} The synthesis began with Boc protected threonine that was treated with an excess of sodium hydride (5 eq.) and methyl iodide (10 eq.). This afforded compound 3-3 as an inseparable mixture of NMe-OMe-Thr and β-elimination product in a ratio between [3-1] and [4-1] determined by proton NMR integration. This material was purified following coupling to previously synthesized compound 3-1. In our hands, this product could be synthesized in a 27% isolated yield over three steps (Scheme 4.1). While the structure of the product was confirmed, it could not be readily carried forward in subsequent reactions to assemble the larger acyclic structure. Yet another β-elimination plagued synthetic transformations using this building block as starting material (Figure 4.2). For example, while compound 3-4 can be stored below room temperature to significantly slow the β-elimination, this decomposition occurs during purification, and in the presence of base in subsequent reactions.

\begin{center}
\includegraphics[width=0.5\textwidth]{figure4.2.png}
\end{center}

**Figure 4.2 – β-Elimination Decomposition Products**

Despite the evidence of this decomposition pathway efforts were made to push forward in the synthesis. While we were able to handle these molecules gently enough to minimize this decomposition as evidence of the synthesis of compound 3-13 in 66% yield across two steps (Scheme 3.2). As we carried compound 3-13 forward to the subsequent Boc deprotection and coupling to the left-hand portion we isolated further evidence of β-elimination (Figure 4.3).
At this point it had become clear that the β-elimination needed to be addressed immediately because similar reactions will occur in all subsequent steps of this reaction sequence prior to the macrocyclization. One simple option that we hoped would slow this β-elimination, was to change the N-acetyl protecting group so that the nitrogen would not be as electron-withdrawing, a change that would decrease the acidity of the α-proton involved in the elimination. Given our prior experience with the second-generation synthesis, we chose to protect this amine with a methyl carbamate, at the time hoping this change in the final analog would once again be tolerated biologically. This will allow us to avoid an exchange of protecting groups to that of the acetate found in the natural product late in the synthesis.
4.3 Avoiding the β-Elimination of the Bottom Bridge

The synthesis of the modified central building block (the “bottom bridge” in the natural product) began in a similar fashion as reported above (Scheme 4.3).1,2 The first modified amino acid required for the central building block was synthesized from the commercially available benzyl ester of the threonine oxalate salt. This substrate was chosen due to difficulties isolating the methyl carbamate protected acid of threonine itself from the aqueous layer following work up. The methyl carbamate protected compound 3-2 was isolated in an 84% yield underwent the subsequent esterification reaction with the previously synthesized NMe-OMe-Thr (compound 3-3), using catalytic DMAP and no additional stoichiometric base in an effort to reduce the amount of β-elimination side product generated. This reaction afforded a 75% yield of compound 3-5. When compared to the overall yield across the same steps containing the N-acyl amine, there was an improvement in yield observed from 27% to 42% yield over three steps, with less evidence of elimination product. It appears that the use of the modified protecting group strategy did aid the synthesis. With that, attention was turned toward the assembly of the rest of the macrocycle.
4.4 Synthesis of the Right-Hand Building Block

Scheme 4.4 – Right-Hand Piece Synthesis

The synthesis of the right-hand building block was accomplished through a standard amide coupling procedure with HATU and DIPEA (Scheme 4.4). In this case, the thermodynamic amide product, compound 3-6, was isolated in an 82% yield. A slight excess of our phenylacetic acid derivative (1.2 eq.) was used, as any uncoupled material would be lost in aqueous work up. With the right-hand building block in hand we could now turn our attention to the final piece of the macrocycle, the left-hand building block.

4.5 Synthesis of the Left-Hand Building Block

Scheme 4.5 – Left-Hand Building Block Synthesis

They synthesis of the left-hand building block began in a similar fashion to our previous synthesis (Scheme 4.5). To this end, we were able to activate the carboxylic acid derivative as a
mixed anhydride. Upon treatment of the mixed anhydride with β-hydroxy leucine in 1 M NaOH, **compound 3-7** was generated in an 81% yield. The intent was to carry this material forward to the coupling of the NMe-threonine within the central building block. However, this coupling reaction resulted in poor yields of the desired amide product.

![Chemical Structures](image)

**Figure 4.4 – Evidence of Esterification**

In our second-generation synthesis, it was possible to isolate the thermodynamic amide product in the presence of a free hydroxyl group (**Scheme 2.4**). In this case however, it appears that the acylation of the methyl amine is much too slow. As evidence, the major product isolated shown in **Figure 4.4** was clearly evidence of the β-elimination we have seen time and time again. For this β-elimination reaction to occur, the free hydroxyl group must first be converted into a leaving group, in this case an ester that is a dimer of **compound 3-7**. Evidence of precisely this β-elimination was first highlighted in **Figure 2.2**. The isolation of this product made it clear we must devise a protecting group strategy as the free hydroxyl group is no longer an innocent bystander.
Upon synthesis of compound 3.7, the protecting group strategy began with treating the carboxylic acid with NaHCO₃ and allyl bromide to afford the allyl ester (Scheme 4.5). This allyl ester protecting group was chosen over that of a benzyl protection group used earlier in the synthesis, due to the deprotection conditions. The hydrogenation reaction most typically used to remove a benzyl group would also reduce the alkene necessary for the future ring closing metathesis, where deprotection of the alloc group with Pd(Ph₃)₄ should not touch the alkene. Following protection of the carboxylic acid, we protected the free hydroxyl group with TBDMS-triflate, followed by subsequent deprotection of the alloc group. This afforded our appropriately protected left hand piece, compound 3.10, in a 71% yield. With the three major pieces of our synthetic route in hand, we could now turn our attention to the assembly of the macrocycle.
4.6 Assembling the Macrocycle

With the three major components of our macrocycle in hand, we had a choice to make. Should we build the macrocycle from left to right, or right to left? The decision was made to couple the central building block to the right-hand piece first, due challenges removing triphenylphosphine oxide (a byproduct of the Mitsunobu reaction) from the desired product. We felt that it was best to ensure this by-product was removed before assembly of the whole molecule.

Scheme 4.7 – Assembly of the Macrocycle

With this in mind, the previously synthesized central building block was treated with standard hydrogenation conditions to cleave the benzyl protecting group, affording a 90% yield of the acid that was carried forward without further purification. The isolated carboxylic acid then underwent the Mitsunobu reaction with the previously synthesized right-hand piece, affording compound 3-14 in an 88% yield, with no evidence of elimination product present. It appears without the presence of a strong base, the β-elimination does not occur as readily with the use of the methylcarbamate protecting group.
Figure 4.5 – Challenges Faced with the N-Methyl Coupling

This is the current stopping point for this approach to the synthesis due to significant challenges faced in coupling the N-Me amine (following Boc deprotection) to the left-hand portion of the molecule (Figure 4.5). Despite our efforts to devise a suitable protection strategy for the left-hand portion of our molecule, further evidence of the problematic β-elimination was isolated, with only trace evidence of coupled product observed in the proton NMR. Future strategies that may be used to successfully assemble this molecule will be discussed Chapter 5.
4.7 References


4.8 Experimental Procedures

[Compound 3-1] To a flame dried round bottom flask was added 0.5041 g (1.67 mmol) L-Threonine benzyl ester oxalate with 0.1485 g (1.75 mmol) NaHCO₃. The flask was then brought to 0 °C and stirred in 5 mL THF with 5 mL H₂O. To the stirring solution was added 0.16 mL (1.67 mmol) Acetic anhydride. The pH of the reaction was then adjusted to 10 using 1 M NaOH solution, and was allowed to stir and reach room temperature overnight. The reaction was then diluted with 30 mL H₂O and extracted with EtOAc (3x35 mL). The combined organic layers were then dried with MgSO₄ and concentrated in vacuo to afford 0.3118 g (74% yield) of compound 3-1 as a white solid. This product was carried forward without any further purification.

¹H NMR (300 MHz, Chloroform-d) δ 7.33 (d, J = 2.7 Hz, 5H), 6.67 (d, J = 8.9 Hz, 1H), 5.16 (s, 2H), 4.60 (dd, J = 9.0, 2.5 Hz, 1H), 4.34 (dq, J = 6.4, 3.2 Hz, 1H), 2.03 (s, 3H), 1.17 (d, J = 6.4 Hz, 3H); ¹³C NMR (126 MHz, cdcl₃) δ 170.97, 170.78, 135.22, 128.66, 128.66, 128.51, 128.19, 128.19, 77.28, 77.03, 76.77, 68.06, 67.36, 57.35, 23.09, 20.02. Full characterization of compound 3-1 was not obtained as this pathway was deemed unsuitable for future use.
[Compound 3-2] To a flame dried round bottom flask was added 0.499 g (1.67 mmol) L-Threonine benzyl ester oxalate with 0.3506 g (4.17 mmol) NaHCO₃. The flask was then brought to 0 °C and stirred in 6 mL THF with 6 mL H₂O. To this stirring solution was added 0.65 mL (8.35 mmol) Methyl chloroformate dropwise. The reaction was allowed to stir and reach room temperature overnight. After 20 hours, the reaction was diluted with 50 mL EtOAc and washed with saturated NaHCO₃ (3x30 mL). The combined aqueous layers were then back extracted with EtOAc (2x20 mL). All organic layers were then dried with MgSO₄ and concentrated in vacuo to afford 0.3755 g (84% yield) of compound 3-2 as a clear oil. This product was carried forward without any further purification.

¹H NMR (500 MHz, Chloroform-d) δ 7.37 – 7.25 (m, 5H), 5.99 (d, J = 9.3 Hz, 1H), 5.15 (s, 2H), 4.37 – 4.28 (m, 2H), 3.64 (s, 3H), 1.20 (d, J = 6.5 Hz, 3H); ¹³C NMR (126 MHz, Chloroform-d) δ 173.93, 160.30, 137.99, 131.24, 131.04, 130.98, 130.78, 130.78, 80.17, 79.91, 79.65, 70.55, 69.92, 62.22, 55.19, 22.55. Full characterization of compound 3-2 was not pursued as this synthetic route is still in development.

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[Compound 3-3] To a flame dried round bottom flask was added 1.0010 g (4.56 mmol) Boc-L-threonine with 0.90522 g (22.6 mmol) NaH 60% dispersion in mineral oil. The flask was then brought to 0 °C before addition of 28 mL THF. To this stirring solution 2.8 mL Iodomethane was then carefully added. The reaction was allowed to stir and reach room temperature overnight.
After 24 hours, the flask was diluted with 50 mL DI H₂O and concentrated *in vacuo*. The remaining aqueous layer was then brought to pH=3 with 1 M HCl, and was then extracted with EtOAc (3x35 mL). The crude product was then purified by flash chromatography (silica gel, 70% Hexane: 30% EtOAC) to afford 0.7574 g (67% yield) of **compound 3-3** as a clear oil. Trace impurity inseparable and was carried forward to the subsequent esterification.

$^1$H NMR (500 MHz, Chloroform-$d$) $\delta$ 7.03 – 6.83 (q, 1H), 4.95 – 4.61 (d, 1H), 4.02 (dq, $J = 39.4$, 5.9 Hz, 1H), 3.36 – 3.29 (s, 3H), 3.01 – 2.94 (s, eH), 1.81 (d, $J = 7.1$, 2.4 Hz, 1H), 1.53 – 1.40 (s, 9H), 1.18 (d, $J = 10.2$, 6.3 Hz, 3H); $^{13}$C NMR (126 MHz, ccdl$_3$) $\delta$ 178.23, 159.80, 83.13, 79.12, 66.04, 59.90, 37.99, 30.96, 30.94, 30.80, 17.85. Full characterization of **compound 3-3** has been previously reported.$^1$

![Compound 3-3](image)

**[Compound 3-4]** A flame dried flask was brought to 0 °C with 0.2030 g (1.10 mmol) **compound 3-1**, 0.3789 g (1.98 mmol) EDC, and 0.0273 g (0.22 mmol) DMAP. To this flask was added 0.3264 g (1.32 mmol) of compound 3-3 in 16 mL DCM. The reaction was allowed to stir and reach room temperature overnight. After 48 hours, the reaction was diluted with 50 mL DCM. The organic layer was washed with saturated NaHCO$_3$ (3x30 mL). The combined aqueous layers were then back extracted with DCM (2x20 mL). All organic layers were then dried with MgSO$_4$ and concentrated *in vacuo*. The crude product was then purified by flash chromatography (silica gel, 50% Hexane: 50% EtOAC) to afford 0.2710 g (54% yield) of **compound 3-4** as a clear oil.
\[ \text{H NMR (300 MHz, Chloroform-d) } \delta 7.32 – 7.24 \text{ (m, 5H), 5.39 (dq, } J = 6.3, 2.8 \text{ Hz, 1H), 5.13 – 5.00 \text{ (m, 3H), 4.87 (d, 1H), 3.85 (dq, } J = 6.2 \text{ Hz, 1H), 3.20 (s, 3H), 2.82 (s, 3H), 2.01 (s, 3H), 1.39 (s, 9H), 1.27 (d, 3H), 1.08 (d, } J = 6.1 \text{ Hz, 3H). Full characterization of compound 3-4 was not obtained as this pathway was deemed unsuitable for future use.} \]

\[ \text{[Compound 3-5] A flame dried flask was brought to 0 °C with 0.6868 g (2.57 mmol) compound 3-2, 0.6940 g (3.63 mmol) EDC, and 0.0785 g (0.64 mmol) DMAP. To this flask was added 0.8972 g (3.63 mmol) of compound 3-3 in 16 mL DCM. The reaction was allowed to stir and reach room temperature overnight. After 48 hours, the reaction was diluted with 50 mL DCM. The organic layer was washed with saturated NaHCO}_3 (3x30 mL). The combined aqueous layers were then back extracted with DCM (2x20 mL). All organic layers were then dried with MgSO}_4 and concentrated in vacuo. The crude product was then purified by flash chromatography (silica gel, 80% Hexane: 20% EtOAC) to afford 0.9616 g (75% yield) of compound 3-5 as a clear oil.} \]

\[ \text{H NMR (500 MHz, Chloroform-d) } \delta 7.40 – 7.30 \text{ (m, 5H), 5.56 – 5.42 \text{ (m, 2H), 5.14 \text{ (s, 2H), 4.86 \text{ (d, } J = 5.0 \text{ Hz, 1H), 3.91 \text{ (dq, } J = 17.7, 11.9, 6.1 \text{ Hz, 1H), 3.71 \text{ (s, 3H), 3.26 \text{ (s, 3H), 2.90 \text{ (s, } 3H), 1.46 \text{ (s, 9H), 1.36 \text{ (d, 3H), 1.14 \text{ (d, } J = 6.6 \text{ Hz, 3H); 13C NMR (126 MHz, cdcl}_3 \delta 172.19, 171.78, 159.80, 159.44, 137.63, 131.28, 131.26, 131.15, 131.10, 131.06, 82.66, 73.87, 70.42, 70.26, 64.72, 60.29, 59.62, 55.22, 35.44, 30.95, 30.95, 30.81, 19.59, 17.57. Full characterization of compound 3-5 was not pursued as this synthetic route is still in development.} \]
[Compound 3-6] To a flame dried flask was added 0.5015 g (3.00 mmol) Phenylactic acid with 1.4050 g (3.70 mmol) HATU which were then set to stir in 20 mL DMF. To this stirring solution was added 0.20 mL (2.50 mmol) N-allyl amine and 1.0 mL (6.00 mmol) DIPEA. The reaction was allowed to stir at room temperature overnight. After 20 hours, the reaction was diluted with 50 mL EtOAc and washed with saturated NaHCO₃ (3x30 mL). The combined aqueous layers were then back extracted with EtOAc (2x20 mL). All organic layers were then dried with MgSO₄ and concentrated in vacuo. The crude product was then purified by flash chromatography (silica gel, 60% Hexane: 40% EtOAC) to afford 0.4493 g (82% yield) of compound 3-6 as a clear oil.

1H NMR (300 MHz, Chloroform-d) δ 7.34 – 7.18 (m, 5H), 6.79 (t, J = 6.1 Hz, 1H), 5.73 (m, 1H), 5.14 – 5.04 (m, 2H), 4.26 (t, J = 8.5, 4.9, 3.8 Hz, 1H), 3.81 (m, J = 6.0, 4.6, 1.6 Hz, 2H), 3.18 (dd, J = 13.9, 3.8 Hz, 1H), 2.84 (dd, J = 13.9, 8.3 Hz, 1H); 13C NMR (126 MHz, cdcl₃) δ 176.42, 140.26, 136.46, 132.32, 131.97, 130.86, 129.11, 118.76, 75.33, 43.91, 41.18. Full characterization of compound 3-6 was not pursued as this synthetic route is still in development.

[Compound 3-7] A stirred solution of 0.37 mL (2.73 mmol) 6-Heptenoic acid and 0.33 mL (2.97 mmol) of 4-methylmorpholine in 8 mL THF was brought to -10 °C. To the stirring solution 0.37 mL (2.86 mmol) isobutyl chloroformate was added and maintained at -10 °C for 30
minutes. The reaction was allowed to warm to 0 °C at which time 0.6423 g (3.51 mmol) of the hydrochloride salt of β-hydroxyleucine in 5.5 mL of 1 M NaOH was added. The reaction was allowed to warm to room temperature overnight and stirred for 24 hours. The reaction was then diluted with 30 mL H<sub>2</sub>O, and washed with EtOAc (2x25 mL). The combined organic layers were then extracted with saturated NaHCO<sub>3</sub> (3x25 mL). All aqueous layers were combined and acidified to pH=2 with 1 M HCl and extracted with EtOAc (3x30 mL). The combined organic layers were then dried with MgSO<sub>4</sub> and concentrated in vacuo to give 0.5695 g (81% yield) of compound 3-7 as a white foaming oil that was carried forward without further purification.

\[
\begin{align*}
\text{H NMR (500 MHz, Chloroform-d)} & \delta 6.87 (d, J = 7.8 Hz, 1H), 5.84 – 5.75 (m, 1H), 5.04 – 4.94 (m, 2H), 4.75 (d, J = 8.9, 1.9 Hz, 1H), 3.82 (dd, J = 9.3, 1.9 Hz, 1H), 2.33 (t, J = 27.5, 7.4 Hz, 2H), 2.12 – 2.02 (m, 2H), 1.74 – 1.62 (m, 3H), 1.49 – 1.38 (m, 2H), 1.02 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.7 Hz, 3H). \\
\text{C NMR (126 MHz, cdcl<sub>3})} & \delta 181.27, 177.72, 140.99, 117.38, 57.18, 38.74, 36.00, 33.45, 31.00, 27.76, 26.80, 21.85, 21.45. 
\end{align*}
\]

Full characterization of compound 3-7 was not pursued as this synthetic route is still in development.

[Compound 3-8] To a stirring solution of 0.3123 g (1.21 mmol) compound 3-7 with 0.4074 g NaHCO<sub>3</sub> (4.85 mmol) in 5.8 mL DMF was added 0.89 mL (10.3 mmol) Allyl bromide in a dropwise fashion. The reaction was allowed to stir at room temperature overnight. After 20 hours the reaction was diluted with 30 mL H<sub>2</sub>O, and extracted with EtOAc (3x30 mL). The combined organic layers were then washed with saturated NaHCO<sub>3</sub> (2x20 mL) then brine (1x20 mL). The
organic layer was then dried with MgSO₄ and concentrated in vacuo. The crude product was then purified by flash chromatography (silica gel, 60% Hexane: 40% EtOAc) to give 0.2074 g (57% yield) of compound 3-8 as a clear oil.

1H NMR (300 MHz, Chloroform-d) δ 6.48 (d, J = 9.2 Hz, 1H), 6.01 – 5.68 (m, 2H), 5.42 – 5.19 (m, 2H), 5.10 – 4.85 (m, 2H), 4.83 (dt, J = 9.2, 2.0 Hz, 1H), 4.64 (dd, J = 5.8, 1.3 Hz, 2H), 3.74 (dd, J = 9.0, 2.1 Hz, 1H), 2.27 (t, J = 7.5, 1.9 Hz, 2H), 2.14 – 1.99 (m, 2H), 1.67 (m, 3H), 1.51 – 1.34 (m, 2H), 1.01 (d, J = 6.6, 1.9 Hz, 3H), 0.92 (d, J = 6.8, 2.0 Hz, 3H). 13C NMR (126 MHz, Chloroform-d) δ 176.07, 174.18, 141.08, 134.23, 121.43, 117.31, 80.09, 68.77, 56.77, 39.01, 36.06, 33.63, 31.04, 27.73, 21.55, 21.51. Full characterization of compound 3-8 was not pursued as this synthetic route is still in development.

[Compound 3-9] To a stirring solution of 0.1362 g (0.45 mmol) compound 3-8 in 2.5 mL DCM at 0 °C was added 0.26 mL (2.29 mmol) 2,6-Lutidine, followed by dropwise addition of 0.26 mL (1.14 mmol) TBDMS-triflate. The reaction was allowed to stir and reach room temperature overnight. After 24 hours, the reaction was quenched with 1 M HCl (20 mL). Reaction was then extracted with DCM (3x25 mL). The combined organic layers were then washed with 1 M KHSO₄ (30 mL) and brine (30 mL). The organic layer was then dried with MgSO₄ and concentrated in vacuo. The crude product was then purified by flash chromatography (silica gel, 90% Hexane: 10% EtOAc) to give 0.1736 g (92% yield) of compound 3-9 as a clear oil.
$^1$H NMR (500 MHz, Chloroform-$d$) $\delta$ 6.18 (d, $J = 9.1$ Hz, 1H), 5.92 (m, 1H), 5.85 – 5.76 (m, 1H), 5.40 – 5.25 (m, 2H), 5.08 – 4.94 (m, 2H), 4.77 – 4.70 (d, 1H), 4.62 (dd, $J = 4.5$, 2.9, 1.4 Hz, 2H), 4.00 (dd, $J = 6.5$, 3.3, 1.3 Hz, 1H), 2.33 – 2.25 (t, 2H), 2.14 – 2.06 (m, 2H), 1.86 – 1.76 (m, 2H), 1.72 – 1.66 (m, 2H), 1.53 – 1.44 (m, 2H), 1.02 – 0.88 (m, 15H), 0.10 (s, 3H), 0.02 – -0.03 (s, 3H). $^{13}$C NMR (126 MHz, cdc13) $\delta$ 175.33, 173.93, 141.06, 134.22, 121.43, 117.32, 79.60, 68.70, 56.15, 39.15, 36.07, 35.91, 30.99, 28.58, 28.31, 27.63, 21.77, 20.66, -0.93, -1.55, -2.13.

Full characterization of compound 3-9 was not pursued as this synthetic route is still in development.

[Compound 3-10] A stirred solution of 0.0463 g (0.04 mmol) Pd(PPh3)4 in 0.7 mL of THF was cooled to -78°C. To this was added 0.02 mL n-BuLi (0.04 mmol of a 2.5 molar solution in hexanes) and the solution was stirred at -78 for 1 hour at which time it was added to a separate stirred solution of 0.1696 g (0.41 mmol) compound 3-9 in 0.7 mL THF. Added immediately to this stirring solution was 0.35 mL (4.0 mmol) Morpholine. The reaction was stirred at room temperature for 2 hours at which time the reaction was diluted with 15 mL of diethyl ether and organic layer washed with 1 M KHSO4 (3x15 mL) and brine (15 mL). The organic layer was dried over MgSO4 and concentrated in vacuo. The crude product was then purified by flash chromatography (silica gel, 70% Hexane: 30% EtOAc) to give 0.072 g (71% yield) of compound 3-10 as a clear oil.
\[ \text{H NMR (500 MHz, Chloroform-}d) \delta 6.27 (dd, J = 8.6, 2.5 Hz, 1H), 5.81 – 5.71 (m, 1H), 5.01 – 4.88 (m, 2H), 4.62 (ddt, J = 8.6, 2.7, 1.3 Hz, 1H), 4.05 – 4.00 (m, 1H), 2.26 (td, J = 7.7, 2.9 Hz, 2H), 2.03 (qdd, J = 7.0, 3.7, 1.4 Hz, 2H), 1.80 – 1.70 (m, 1H), 1.66 – 1.59 (m, 2H), 1.41 (dtd, J = 15.1, 7.3, 3.9 Hz, 2H), 0.99 (ddd, J = 9.2, 4.8, 2.1 Hz, 1H), 0.92 (dt, J = 5.1, 2.6 Hz, 2H), 0.88 (dt, J = 3.8, 2.0 Hz, 12H), 0.06 (dt, J = 2.8, 1.3 Hz, 3H), 0.00 (dd, J = 3.1, 1.1 Hz, 3H). \]

\[ \text{13C NMR (126 MHz, cde}l_3) \delta 179.42, 178.33, 142.92, 119.33, 81.23, 58.30, 41.00, 37.97, 37.72, 32.87, 30.56, 30.24, 29.53, 23.60, 22.80, 22.74, 0.95, 0.24, 0.00. \]

Full characterization of compound 3-10 was not pursued as this synthetic route is still in development.

[Compound 3-11] To a flame dried flak were added 0.2470 g (0.51 mmol) compound 3-4 and 0.0180 g (0.17 mmol) Pd on Carbon in 2.3 mL MeOH. The flask was then placed under positive pressure of H\(_2\) and stirred vigorously and monitored by TLC until completion, between 6-8 hours, at which time the reaction was diluted with 50 mL MeOH. The solution was then filtered through celite and concentrated \textit{in vacuo}. The crude product, 0.1966 g (98%) compound 3-11, was carried forward without any further purification.

[Compound 3-12] To a flame dried flak were added 0.4133 g (0.83 mmol) compound 3-5 and 0.0306 g (0.27 mmol) Pd on Carbon in 4 mL MeOH. The flask was then placed under positive
pressure of H₂ and stirred vigorously for The reaction was monitored by TLC until completion, between 6-8 hours, at which time the reaction was diluted with 50 mL MeOH. The solution was then filtered through celite and concentrated in vacuo. The crude product, 0.3280 g (97%) compound 3-12, was carried forward without any further purification.

[Compound 3-13] In a flame dried round bottom flask 0.2007 g (0.51 mmol) compound 3-11 was subjected to a benzene (30 mL) azeotropic distillation to remove any trace water. To this flask was added 0.1298 g (0.49 mmol) triphenylphosphine, followed by 1.8 mL THF. The flask was stirred at -30 °C before addition of 0.0965 g (0.47 mmol) compound 3-6 dissolved in an additional 1.8 mL THF, followed by dropwise addition of 0.24 mL (0.52 mmol) of a 40 wt % solution of Diethyl azodicarboxylate in toluene. The temperature was maintained at -30 °C for 30 minutes and was then allowed to reach room temperature overnight. After 18 hours, the reaction was concentrated in vacuo. The resulting oil was dissolved in 50 mL EtOAc and washed with saturated NaHCO₃ (3x25 mL). The aqueous layer was then back extracted with EtOAc (2x20 mL). The combined organic layers were then dried with MgSO₄ and concentrated in vacuo. The resulting oil was purified via flash column chromatography (silica gel, 100% Et₂O) to afford 0.2007 g (68% yield) of compound 3-13 as a clear foaming oil.

¹H NMR (300 MHz, Chloroform-d) δ 7.29 – 7.15 (m, 5H), 6.82 (d, J = 5.7 Hz, 1H), 5.82 – 5.62 (m, 1H), 5.42 (dd, J = 9.1, 3.8 Hz, 1H), 5.10 – 5.00 (m, 2H), 4.69 (d, J = 5.7 Hz, 1H), 4.30 (dd, J = 7.4, 5.6 Hz, 1H), 3.96 – 3.79 (m, 2H), 3.24 (s, J = 2.3 Hz, 3H), 3.20 – 3.14 (m, 1H), 3.12 –
3.02 (m, 1H), 2.87 (s, $J = 11.4$ Hz, 3H), 1.95 (s, $J = 6.3$ Hz, 3H), 1.50 – 1.38 (s, 9H), 1.17 – 1.11 (d, 3H), 0.81 (d, $J = 6.4$ Hz, 3H). Full characterization of **compound 3-13** was not obtained as this pathway was deemed unsuitable for future use.

![Compound 3-13](image)

**[Compound 3-14]** In a flame dried round bottom flask 0.3186 g (0.78 mmol) **compound 3-12** was subjected to a benzene (30 mL) azeotropic distillation to remove any trace water. To this flask was added 0.1975 g (0.75 mmol) triphenylphosphine, followed by 2.8 mL THF. The flask was stirred at -30 °C before addition of 0.1900 g (0.93 mmol) **compound 3-6** dissolved in an additional 2.8 mL THF, followed by dropwise addition of 0.37 mL (0.82 mmol) of a 40 wt % solution of Diethyl azodicarboxylate in toluene. The temperature was maintained at -30 °C for 30 minutes and was then allowed to reach room temperature overnight. After 18 hours, the reaction was concentrated in vacuo. The resulting oil was dissolved in 50 mL EtOAc and washed with saturated NaHCO$_3$ (3x25 mL). The Aqueous layer was then back extracted with EtOAc (2x20 mL). The combined organic layers were then dried with MgSO$_4$ and concentrated in vacuo. The resulting oil was purified via flash column chromatography (silica gel, 50% Hexane: 10% EtOAc: 40% Et$_2$O) to afford 0.4097 g (88% yield) of **compound 3-14** as a clear foaming oil.

$^1$H NMR (500 MHz, Chloroform-d) $\delta$ 7.29 – 7.20 (m, 6H), 6.87 (t, $J = 5.9$ Hz, 1H), 5.78 – 5.69 (m, 1H), 5.45 (dd, $J = 8.2$, 4.3 Hz, 1H), 5.18 (dd, $J = 13.4$, 6.7 Hz, 1H), 5.05 (dq, $J = 14.0$, 1.8 Hz, 2H), 4.77 (d, $J = 5.4$ Hz, 1H), 4.27 (t, $J = 6.5$ Hz, 1H), 3.92 (p, $J = 6.2$ Hz, 1H), 3.88 – 3.80 (m, 1H), 3.65 (s, 3H), 3.38 – 3.31 (m, 1H), 3.25 (s, 2H), 3.12 (dt, $J = 14.3$, 7.1 Hz, 1H), 2.91 (s,
2H), 1.44 (d, $J = 17.3$ Hz, 9H), 1.15 (t, $J = 7.1$ Hz, 3H), 0.99 (d, $J = 6.2$ Hz, 3H); $^{13}$C NMR (126 MHz, cdcl$_3$) $\delta$ 172.64, 172.06, 170.91, 159.87, 159.50, 138.70, 136.37, 132.35, 132.13, 131.09, 130.82, 118.76, 82.93, 79.08, 78.43, 72.83, 65.17, 61.47, 59.46, 55.27, 44.35, 40.30, 35.70, 30.92, 30.77, 30.76, 23.66, 19.50, 17.60. Full characterization of compound 3-14 was not pursued as this synthetic route is still in development.
Chapter Five: Conclusions and Future Directions

5.1 Conclusions

![Chemical Structure](image)

Figure 5.1 – β-Elimination of the Bottom Bridge Analog

The effort to improve efficacy and potency of our simplified analogs is continuing with the development of a convergent synthesis of analogs that contain an intact central building block, or “bottom bridge” of the macrocycle. In this case, another pair of β-elimination reactions continue to interfere with our progress. These β-elimination reactions involve the protons highlighted in red in Figure 5.1. One destabilizes the functionalized bottom bridge, and the other complicates the coupling of the N-methylamine within our central building block to the carboxylic acid of left-hand portion of the macrocycle. While the exchange of the acetyl protecting group to a methyl carbamate within the central building block improved yields and slowed the β-elimination reaction, it is still problematic. The challenges with the formation of the N-methyl amide have yet to be completely resolved, although progress is currently being made by Dr. Yu Zhu in our group. The current hypothesis as to the challenges with the coupling
reaction are highlighted in Figure 5.2. We propose that the challenge is related to problems associated with coupling a NAcyl amino acid that were previously highlighted in Figure 2.5.

![Figure 5.2 – Plausible Oxazolone Formation](image)

To be more specific, it is possible, regardless of the size of the R group, that the intramolecular nucleophilic trapping by the acyl amine of the activated ester necessary for the desired coupling is faster than the bimolecular coupling. Following the displacement of the leaving group for the activated ester, deprotonation of the proton involved in the β-elimination results in an aromatic intermediate that rapidly undergoes the elimination of the TBS-alcohol. While the ring opening of the oxazolone may be reversible, the β-elimination reaction is not. It possible the opening of the oxazolone ring could lead to coupled product or be lost upon aqueous work up. Efforts to solve this problem will be discussed in Section 5.2, though our experience from the first-generation synthesis of WU-07047 suggests that at the very least the the β-elimination at the core of the bottom bridge will continue to complicate the synthesis until the macrocycle is assembled.

**Despite this decomposition pathway**, we chose to push forward in an attempt to determine if the efficacy and potency would be improved through the installation of the bottom bridge. We knew moving forward that this particular reaction sequence would not be scalable in the future, but we hoped that positive result on the biological front would provide a foundation for the synthetic efforts necessary to overcome the problems we have encountered. However,
while trace amounts of material have been isolated from the coupling to the left-hand portion, the amount of material generated have not been in sufficient yield to determine if the ring closing metathesis strategy will be successful moving forward. The decision was made to carry trace amounts of crude product forward to the ring closing metathesis (in order to reduce the risk of β-elimination) but these efforts were inconclusive. Following purification after the metathesis reaction, it was evident that polymeric product had been generated. We hypothesize that the refluxing conditions necessary for the ring closing metathesis may increase the rate of the problematic β-elimination. However, we need to be cautious drawing conclusions regarding the metathesis reaction since the substrate for the reaction has not been characterized with respect to purity.

The combined problems encountered with this route suggest that we should be devising alternative strategies for the synthesis of more the functionalized analogs. What follows is a brief discussion of future more functionalized analogs, and possible options aimed at avoiding the issues discussed above.

### 5.2 Future Directions

Looking forward, if we intend to use the convergent approach outlined in Figure 4.1 to synthesize more complex analogs, we must first address the challenges of coupling the central building block to the left-hand piece. There is literature evidence of the success of a macrolactamization of the NMe-amine of threonine (of our central building block) to the β-hydroxyleucine (of our left-hand piece), although no yield is reported for this reaction. With the knowledge that this coupling can be successful, there are two possible protecting group strategies that may remedy the issue that we have observed to date. It may be plausible to find a suitable
protecting group for the hydroxyl group in our left-hand portion that will avoid the β-elimination, although we chose the TBDMS ether in an effort to do just that (Figure 5.3). If this approach continues to be unsuccessful, we could alter the protecting group on the neighboring amine of the left-hand piece to that of a carbamate group. We have already observed that a change from an acyl group to a carbamate protecting group on an amine of an amino acid can reduce or eliminate oxazolone formation in subsequent coupling reactions. There is evidence from Dr. Yu Zhu in our group that such an approach can be successful. However, here we will emphasize the protecting group strategy involving the hydroxy group of the left-hand piece. Solving the problem in this manner will result in a shorter synthesis overall, eliminating the need to juggle protection groups in the steps after the desired coupling reaction. Remember that the length of the synthesis may be incredibly important because of the potential loss of material due to the β-elimination within the bottom bridge during each subsequent reaction (Figure 4.2).

**Figure 5.3 – Protection Strategies that Reduce the β-Elimination**

There are two possible approaches when choosing a protecting group for the left-hand piece. We can either find a protecting group that would result in the oxygen being an unstable leaving group, or a protecting group large enough to prevent any base from accessing the proton (highlighted red) responsible for the β-elimination. A few such options to consider would be a
TIPS, trityl, or benzyl protecting group. In each case, the hope is that the protecting group would be more stable than the TBS group. This is based upon the evidence of the β-elimination in both the TBS protected left-hand piece, the left-hand piece with the unprotected hydroxyl group (Figure 4.4-5) and the plausible oxazolone formation mechanism highlighted in Figure 5.2.

![Figure 5.4 – Ring Closing Macrolactamization](image)

This protecting group strategy will be necessary for any analog synthesized based on the current convergent approach (Figure 5.4). Once the problem of the coupling reaction has been resolved (there is precedence that indicates it is a solvable problem), we will need to turn our attention to formation of the macrocycle. For this transformation, it may be necessary to avoid the elevated temperatures typically associated with the ring closing metathesis reaction. These harsher conditions may trigger the unwanted β-elimination reactions. One approach that would avoid these harsh conditions would use a ring closing macrolactamization (highlighted red) to complete the synthesis of macrocycle. Once the macrocycle has been generated, the β-elimination within the bottom bridge should no longer be possible. We can then add the β-hydroxy leucine tail in a similar manner as the second-generation synthesis.

If the methods that discussed above that attempt to reduce the β-elimination are not successful, it is possible to synthesize analogs that will avoid the β-elimination in its entirety (Figure 5.5).
Figure 5.5 – Analogs that Avoid the β-Elimination

There are several approaches we have considered to avoid the β-elimination within our central building block, two of which are actively being pursued in our lab. In the first analog shown above (left, Figure 4.5), the carbonyl of the threonine of our central building block has been reduced (highlighted red). This change would make the molecule more stable by significantly reducing the leaving group ability of the portion of the molecule eliminated in the unwanted side-reaction.

Scheme 5.1 – Threonine Reduction

The synthesis of this analog can in principle be accomplished by reduction of the carboxylic acid in the original demethylated amino acid, conversion of the resulting hydroxy group to a leaving group, and an S_N2 type displacement of the leaving group by the secondary alcohol of the threonine (Scheme 5.1).
It is also possible to prevent the problematic β-elimination entirely through removal of the problematic acidic proton via methylation (highlighted red) of the α-carbon of the right-hand threonine (right, Figure 5.5). Amino acids of this nature have been synthesized. While these options are plausible synthetically, the strategies may not be tolerated from the standpoint of biological activity.

In addition, one could consider the use of a ring closing macrolactonization (highlighted red) to complete the synthesis of our macrocycle (right, Figure 5.5). While this will require an alternative coupling approach to assemble the acyclic molecule, by ring closing at the threonine dimer, we would entirely avoid the chance of the β-elimination prior to forming the macrocycle while still retaining the bottom bridge found in the natural products.

One final approach to consider is the use of a carbamate within the top bridge (Figure 5.6). The use of a carbamate in place of the amide found in the natural product should help avoid oxazolone formation under standard coupling conditions. The installation of this the top bridge with a carbamate will allow us to use the same building block approach to piece together the linear molecule prior to the use of a ring closing metathesis to tie together the macrocycle. If this method is unsuccessful, one could envision assembling the linear molecule in a stepwise fashion (Scheme 5.2)
Scheme 5.2: Carbamate Coupling Strategy

Efforts are currently underway in our lab if assembly of this molecule in a linear fashion will avoid carbamate formation. Evidence found by Dr. Yu Zhu in our lab indicates that the coupling between the NMe-OMe-Thr of the central building block, to the Boc protected β-hydroxy leucine of the left hand piece is possible.

Figure 5.7 – Bioorthogonal Handle Analog

As we work to improve the potency and efficacy of our simplified analogs, it is becoming clear we need to devise a rapid way to screen molecules for activity prior to the use of expensive and time-consuming whole cell assays that are currently used. To this end, we are interested installing a biorthogonal handle (R) such as an alkyne or an aryl bromide (Figure 5.7). This will
serve two purposes, first, both groups have been used to place molecules onto microelectrode
arrays to monitor for binding to biological targets. Second, this synthetic addition will also
provide our medical school collaborators a handle for labeling the analogs in connection with
future biological assays. Both handles can be used to add several probes to previously
synthesized analogs. In Figure 5.7, I have suggested a site (highlighted red) where this handle
could potentially be installed. This site was chosen because the inclusion of larger groups at this
position in the natural product have been tolerated. For example, as discussed in Section 1.3, the
substitution of a phenyl group for the methyl group found in the natural product has been
tolerated with no significant loss in activity. The installation of an amino acid with a
bioorthogonal handle can be accomplished through standard coupling reactions.

All this work is currently underway in the Moeller group. These efforts seek to improve
potency and efficacy towards that found in the natural products, the development of methods for
the rapid screening of newly synthesized analogs, and the scaling of the synthesis for building
YM- and FR-analogs. These analogs will prove invaluable for probing the G protein signaling
pathways, as well as providing potential lead compounds for the development of therapeutics in
the future.
5.3 – References


Appendix: Spectral Data

$^1$HNMR Compound 2-1
$^{13}$CNMR Compound 2-1

102
1H NMR Compound 2-2
$^{13}$CNMR Compound 2-2
$^{13}$CNMR Compound 2-3
$^1$HNMR Compound 2-4
\[^{13}\text{CNMR Compound 2-4}\]
$^1$HNMR Compound 2-5a
MRM.07.014

\[ \text{CNMR Compound 2-5a} \]

110
$^1$HNMR Compound 2-6a
\(^{13}\text{CNMR Compound 2-6a}\)
1H NMR Compound 2-5b
$^{13}$CNMR Compound 2-5b
$^1$HNMR Compound 2-6b
MRM.07.054

$^{13}$CNMR Compound 2-6b

116
MRM.07.011

\[ \begin{align*}
13^\text{CNMR Compound 2-7} \\
118
\end{align*} \]
1H NMR Compound 2-8
$^{13}$CNMR Compound 2-8
MRM.07.045

\[ \text{HNMR Compound 2-9} \]
HNMR Compound 2-10
MRM.07.03

CNMR Compound 2-10

124
$^1$HNMR Compound 2-11

125
$^1$HNMR Compound 2-12
CNMR Compound 2-12

13
$^1$HNMR Compound 2-13
$^{13}$CNMR Compound 2-14

132
MRM.07.040

\[ ^1H NMR \text{ Compound 2-15a} \]
MRM.07.040

$^{13}$CNMR Compound 2-15a

134
$^1$HNMR Compound 2-16a
$^{13}$CNMR Compound 2-16a
1H NMR Compound 2-15b
MRM.07.017

\[ \begin{array}{c}
\text{CNMR Compound 2-15b} \\
138
\end{array} \]
$^1$HNMR Compound 2-16b
$^{13}$CNMR Compound 2-16b
$^1$HNMR Compound 2-17
$^1$H NMR Compound 2-17 (Scan)

143
1HNMR Compound 2-19a
$^1$HNMR Compound 2-19b
$^1$H NMR Compound 2-19b

147
\[ ^{13}\text{CNMR Compound 2-19b (Scan)} \]
$^1$HNMR Compound 2-20
13CNMR Compound 2-20

150
$^1$HNMR Compound 2-21 (Crude)
$^1$HNMR Compound 2-21 (Pure)

152
$^{13}$CNMR Compound 2-21
HNMR

Compound 2-22

1H NMR
$^{1}$HNMR Compound 2-23
$^{13}$CNMR Compound 2-23
\[ ^1H \text{NMR Compound 2-24} \]
$^1$HNMR Compound 2-25

159
13C NMR Compound 2-25
MRM.06.073

H₂C
\(\text{CH}_3\)
\(\text{CH}_3\)
\(\text{N}\)  \(\text{O}\)
\(\text{O}\)
\(\text{CH}_3\)

\[^1\text{HNMR Compound 2-26}\]

161
$^{1}$HNMR Compound 2-27

163
MRM.06.073

\[
\begin{align*}
\text{CH}_3 & \quad \text{H}_3\text{C} \\
\text{O} & \quad \text{O} \\
\text{C} & \quad \text{NH} \\
\text{O} & \quad \text{O} \\
\text{CH}_3 &
\end{align*}
\]

\[13^\text{CNMR Compound 2-27}\]

164
$^1$HNMR Compound 2-28

165
$^{13}$CNMR Compound 2-28
MRM.06.089

$^1$HNMR Compound 2-29
\(^1\)HNMR Compound 2-30

169
$^{13}$CNMR Compound 2-30
$\text{HNMR}$ Compound 3-1

171
$^{13}$CNMR Compound 3-1
Compound (3-3)

$^1$HNMR Compound 3-3

175
$^{13}$CNMR Compound 3-3
$^1$HNMR Compound 3-4
MRM.08.035

13CNMR Compound 3-5
$^1$HNMR Compound 3-6
$^1$HNMR Compound 3-7

182
13CNMR Compound 3-7
$^1$HNMR Compound 3-8
184
13CNMR Compound 3-8
$^1$HNMR Compound 3-9
$^{13}$CNMR Compound 3-9
$^{1}$$^{1}$HNMR Compound 3-10

188
MRM.08.045

13CNMR Compound 3-10

189
Compound (3-13)

$^1$HNMR Compound 3-13

190
$^{13}$CNMR Compound 3-14