Synthesis of the western hemisphere of theonellamide C

Saroj Yadav

Louisiana State University and Agricultural and Mechanical College, syadav3@tigers.lsu.edu

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A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Chemistry

by
Saroj Yadav
M.Sc., Panjab University, 2008
December 2013
To My Family…

To my parents, Jagdish and Sushila Yadav, for believing in and supporting all my life pursuits.

To my husband Prathivind Bejgum, whose unconditional love inspires me to fulfill my academic milestones.
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<thead>
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<th>Description</th>
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<tbody>
<tr>
<td>Å</td>
<td>angstrom</td>
</tr>
<tr>
<td>AA</td>
<td>amino acid</td>
</tr>
<tr>
<td>AAA</td>
<td>α-amino adipic acid</td>
</tr>
<tr>
<td>Aboa</td>
<td>(3S,4S,5E,7E)-3-amino-8-(4-bromophenyl)-4-hydroxy-6-methylocta-5,7-dienoic acid</td>
</tr>
<tr>
<td>Ac*</td>
<td>Acetol</td>
</tr>
<tr>
<td>Ahad</td>
<td>(2S,4R)-2-amino-4-hydroxyadipic acid</td>
</tr>
<tr>
<td>Alloc</td>
<td>allyloxycarbonyl</td>
</tr>
<tr>
<td>Apoa</td>
<td>(3S,4S,5E,7E)-3-amino-4-hydroxy-6-methyl-8-phenylocta-5,7-dienoic acid</td>
</tr>
<tr>
<td>BCB</td>
<td>B-bromocatecholborane</td>
</tr>
<tr>
<td>BINOL</td>
<td>1,1′-Bi(2-naphthol)</td>
</tr>
<tr>
<td>Bn</td>
<td>benzyl</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-butyloxycarbonyl</td>
</tr>
<tr>
<td>BOP</td>
<td>benzotriazol-1-yloxytris(dimethylamino)-phosphoniumhexafluorophosphate</td>
</tr>
<tr>
<td>BORSM</td>
<td>based on recovered starting material</td>
</tr>
<tr>
<td>'Bu</td>
<td>tert-butyl</td>
</tr>
<tr>
<td>° C</td>
<td>degrees Celsius</td>
</tr>
<tr>
<td>Cbz</td>
<td>carbobenzyloxy</td>
</tr>
<tr>
<td>CDI</td>
<td>carbonyldimidazole</td>
</tr>
<tr>
<td>COSY</td>
<td>correlation spectroscopy</td>
</tr>
<tr>
<td>DCC</td>
<td>dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>d.e.</td>
<td>diastereomeric excess</td>
</tr>
<tr>
<td>DIEA</td>
<td>N,N-diisopropylethylamine</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>dimethylformamide</td>
</tr>
<tr>
<td>DPPA</td>
<td>diphenylphosphoryl azide</td>
</tr>
<tr>
<td>e.e.</td>
<td>enantiomeric excess</td>
</tr>
<tr>
<td>EDC</td>
<td>1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride</td>
</tr>
<tr>
<td>eHyAsn</td>
<td>erythro-hydroxyasparagine</td>
</tr>
<tr>
<td>ESI</td>
<td>electrospray ionization</td>
</tr>
<tr>
<td>FK463</td>
<td>micafungin</td>
</tr>
<tr>
<td>Fmoc</td>
<td>9-fluorenylmethoxycarbonyl</td>
</tr>
<tr>
<td>τ-HAL</td>
<td>τ-histidinoalanine</td>
</tr>
<tr>
<td>HATU</td>
<td>O-(7-azabenzotriazol-1yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate</td>
</tr>
<tr>
<td>HBTU</td>
<td>O-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate</td>
</tr>
<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
</tr>
<tr>
<td>HOBt</td>
<td>1-hydroxybenzotriazole</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>high resolution mass spectrometry</td>
</tr>
<tr>
<td>HyAsn</td>
<td>hydroxyasparagine</td>
</tr>
<tr>
<td>Hz</td>
<td>hertz</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>half maximal inhibitory concentration</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
</tr>
<tr>
<td>MOA</td>
<td>mode of action</td>
</tr>
<tr>
<td>MoBY-ORF</td>
<td>molecular barcoded yeast open reading frame</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>MVD1</td>
<td>mevalonate pyrophosphatase decarboxylase</td>
</tr>
<tr>
<td>NIS</td>
<td>N-iodosuccinimide</td>
</tr>
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</table>
NMR  nuclear magnetic resonance
nOe   nuclear Overhauser effect
PMA  phosphomolybdic acid
ppm parts per million
PPTS pyridinium p-toluenesulfonate
PyBOP benzotriaozol-1-yloxytri(pyrrolidino)phosphonium hexafluorophosphate
PyBroP bromotris(pyrrolidino)phosphonium hexafluorophosphate
\( R_f \)   retention factor
rt room temperature
s   singlet
q   quartet
SPPS solid phase peptide synthesis
TBAF tetra-\( n \)-butylammonium fluoride
TBS tert-butyldimethylsilyl
TBDDS tert-butyldiphenylsilyl
TFA trifluoroacetic acid
THF tetrahydrofuran
TLC thin layer chromatography
Trt triphenylmethyl
Troc 2,2,2-trichloroethoxycarbonyl
UV ultraviolet

Standard 3 letter codes are utilized throughout the document for amino acids
ABSTRACT

Theonellamides A-F were isolated from *Theonella swinhoei* by Fusetani and co-workers. Despite considerable synthetic effort to produce theonellamide F by the Shioiri group in the early 1990’s, the total synthesis of a theonellamide has yet to be reported. We report herein our efforts toward some of the required amino acid residues and construction of the western ring of theonellamide C.

We describe the synthesis of an uncoded amino acid, (2S,4R)-γ-hydroxy-α-amino adipic acid (Ahad), a building block for theonellamide C. We initially investigated the Corey-Lygo method for the catalytic asymmetric generation of the Cα stereocenter. Unfortunately, the alkylation of glycine benzophenone imines with a series of electrophiles, including [(S)-benzyl-3-(tert-butyldimethylsilyl)oxy]-4-iodobutanoate (104a), was unsuccessful due to low inherent reactivity and steric hindrance.

A second approach was based on MacMillan’s enantioselective organocatalytic amine conjugate addition. Addition of CbzNHOTBS (136a) to (E)-benzyl-4-oxo-but-2-enoate (149), in the presence of catalytic (S)-2-diphenyl[trimethylsilyl]oxy)methylpyrrolidine (165) led to β-amino aldehyde (S)-benzyl 2-[(benzyloxy)carbonyl](tert-butyldimethylsilyloxy)amino-4-oxo-butanoate, (148a). A Wittig reaction between 148a and methyl-(triphenylphosphoranylidene)-acetate (142) afforded (2S,4E)-1-benzyl-2-[(benzyloxy)carbonyl](tert-butyldimethylsilyloxy)-amino-6-methyl-hex-4-enedioate (147a). The γ-hydroxy group was installed intramolecularly with 4:1 d.r. by treatment of 147a with TBAF. Hydrogenolysis was carried out to give unprotected Ahad.

The next challenge was to find the right combination of protecting groups for Ahad that would be compatible with the synthesis of the western hemisphere of theonellamide C. We performed model studies on α-amino adipic acid (α-AAA) which revealed that the δ-COOMe, introduced in the Wittig reaction, was incompatible with a Cα-acetol ester and a Nα-Allo
carbamate. α-AAA and Ahad bearing a δ-COO′Bu group were synthesized in an analogous fashion. The Ahad building block was synthesized in 11 steps and 6.5% overall yield. In readiness for coupling; the tert-butyl ester was cleaved to afford the free δ-acid.

Two tetrapeptides were assembled: Boc-Asn-HyAsn(OTBS)-Phe-β-Ala-OAll (249) and the simplified Fmoc-Asn(Trt)-Asn(Trt)-Phe-β-Ala-OAll (254). Removal of the N-terminal Fmoc-group from 254 and coupling with a suitably protected τ-histidinoalanine acid afforded a linear hexapeptide that is a precursor to the western hemisphere of theonellamide C.
1.1 Occurrence of Theonellamides

Sponges are widely found across the world and appeared more than six million years ago. Bergmann's pioneering work in the 1950s on the isolation of sterols and novel nucleosides made sponges a target for extensive studies to isolate new substances. These research activities led to the discovery of a variety of new compounds, often structurally novel and highly biologically active metabolites. Sponges of the family Lithistida, which include the genera *Discodermia* and *Theonella*, are a prominent source of bioactive secondary metabolites. These secondary metabolites include macrolides such as swinholide A, along with cyclic and linear peptides such as cyclotheonamides, onnamides and theonellamides.

Figure 1.1 Chemical structures of theonellamide F, theonegramide and theopalauamide.
Matsunaga et al. first reported the isolation and characterization of theonellamide F (1) (Figure 1.1) in 1989⁶ and then theonellamides A-E in 1995⁷ as bicyclic dodecapeptides from a *Theonella* sp. collected off Hachijo-jima Island in Japan. Subsequently, Bewley and Faulkner isolated the closely related compound theonegramide (2) in 1994,⁸ followed by theopalauamide (3) in 1998⁹ from the same marine species.

Early studies by Bewley and co-workers reported that a number of symbiotic bacteria live in association with *Theonella swinhoei*.¹⁰,¹¹ The sponge contains four cell populations: sponge cells, unicellular heterotrophic bacteria (eubacteria), unicellular cyanobacteria and filamentous heterotrophic bacteria (filaments). All populations of associated bacteria are located extracellularly. The unicellular cyanobacterium *Aphanocapsa feldmanni* has been the only species identified based on ultrastructural studies. The unicellular cyanobacteria occur only in the euctosome (cortical part of sponge) while the filamentous bacteria reside only in the endosome (membrane-bounded compartment inside the sponge). Heterotrophic eubacteria and sponge cells occur in both endosome and euctosome.

The separation of four cell populations was followed by dissociation and differential centrifugation of the cell suspension which led to cell types of >90% purity (Figure 1.2). Analysis of the four cell fractions by HPLC and ¹H NMR spectroscopy showed that the unicellular cyanobacteria and sponge cells lacked bioactive metabolites. The polyketide swinholide A (4) was present in the fraction of eubacteria and theopalauamide (3) occurred in the filamentous bacteria.

Theonellamides were isolated in limited quantities from nature. From 15 kilograms of sponge, Fusetani and coworkers isolated: theonegramides A (200 mg), B (19 mg), C (32 mg), D (14 mg), E (30 mg), and F (500 mg).⁶,⁷ Therefore, collaborative investigations have focused on theonellamides A and F in a variety of assays.
Figure 1.2 Swinholide A and theopalauamide are derived from different types of cells in *Theonella swinhoei*. Copyright 1998, John Wiley and Sons, reprinted with permission (p. 219).

1.2 Structural Characteristics of Theonellamides

Theonellamides A-F are characterized by a bridging \( \tau \)-histidinoalanine (\( \tau \)-HAL) residue. There are some other features common to all family members. All contain allo-threonine, serine and phenylalanine at positions AA1-AA3 respectively and serine, asparagine and erythro-\( \beta \)-hydroxyasparagine at positions AA5-AA7. Also, at the AA4 position there is \( (5E,7E) \)-3-amino-4-hydroxy-6-methyl-8-phenyl-5,7-octadienoic acid (Apoa) or its 4'-brominated derivative (Aboa). In position AA10 is \( (2S,4R) \)-\( \alpha \)-amino-\( \gamma \)-hydroxy adipic acid (Ahad) or \( \alpha \)-amino adipic acid (AAA).
There are further structural variations including glycosylation. Theonellamide A bears a \( \beta-D \)-galactose covalently linked to the imidazole \( \pi \)-nitrogen and isoserine at AA9 instead of \( \beta \)-alanine. Theonellamides D and E contain a \( \beta-D \)-arabinoside and \( \beta-D \)-galactoside respectively. There is \( p \)-bromophenylalanine (BPA) in theonellamides D-F and \( \beta \)-methyl-\( p \)-bromophenylalanine (BMPA) in theonellamides A and B at position AA8, whereas theonellamide C contains unmodified phenylalanine (Figure 1.3).

![Figure 1.3 Amino acid composition of theonellamides A-F, theonegramide and theopalauamide.](image)

Details of the structure elucidation of \( e \)HyAsn have been described in Douglas Wong’s dissertation.\(^{12}\) The origins and structure determination of Ahad will be detailed in Chapter 2.

### 1.3 Initial Biological Studies

Initial studies by Matsunaga and co-workers on theonellamides A-F showed that they are antifungal and cytotoxic agents. They demonstrated cytotoxicity against P388 murine leukemia cells, with IC\(_{50}\) values of 5.0, 1.7, 2.5, 1.7, 0.9 and 2.7 \( \mu \)g/mL respectively.\(^{7}\) Theonellamide E (9) was shown to be the most cytotoxic. Glycosylation seems to have little effect on the cytotoxicity of the theonellamides. In addition, congener F was toxic to L120
leukemia cells with an IC₅₀ value of 3.2 µg/mL. Theonellamide F (1) also showed activity against fungi of *Candida* spp., *Trichophyton* spp., and *Aspergillus* spp.

In later studies, Watabe *et al.* reported that theonellamide F induced the formation of vacuoles (fluid-filled compartments in the cytoplasm) in 3Y1 rat embryonic fibroblasts.⁰¹³,⁰¹⁴ At higher concentrations, theonellamide F induced not only the formation of large vacuoles equivalent to the size of a cell, but also remarkable morphologic changes. The theonellamide F induced vacuoles contain an ATPase and thus maintain a slightly acidic pH. The disturbance of intralysosomal (within a lysosome) pH affects the function of lysosomal proteases that are responsible for autophagic degradation of organelles. The formation of vacuoles is a well-known marker of microbial activity that often precedes cell autophagy, although the mechanism is not well understood. Theonellamides are the first low molecular weight compounds to induce extraordinarily large vacuoles and thus are good molecular probes for studies on intracellular membrane structures.

The novelty of the molecular architecture of these theonellamides prompted Wada *et al.*⁰¹⁵ to probe whether they bind to proteins. They immobilized theonellamide A (5) to affinity gel beads and screened for binding to proteins in rabbit liver. As a prerequisite to this screening process, theonellamide A (5) was treated with sodium periodate, cleaving the vicinal 2,3- and 3,4-diols of the β-D-galactose and liberating aldehydic handles for the covalent attachment of the molecule to beads. Compound 10 was then reacted with hydrazine-containing Affi-Gel® beads to give TNM A-conjugated gel beads 11 (Scheme 1.1). Rabbit liver tissue extracts were exposed to the TNM A-conjugated gel beads for two hours and two major proteins were found to bind to the beads.
Scheme 1.1 Preparation of theonellamide A - conjugated gel beads.

The proteins which bound to the beads were identified as 17β-hydroxysteroid dehydrogenase IV and glutamate dehydrogenase (Scheme 1.2). 17β-Hydroxysteroid dehydrogenases mediate interconversion of estrone and estradiol and type IV of this enzyme oxidizes estradiol (12) to estrone (13). Theonellamide activates the conversion of α-ketoglutarate (15) from glutamate (14) by glutamate dehydrogenase, but has no effect on the reverse reaction.

Scheme 1.2 Function of 17β-hydroxysteroid dehydrogenase IV and glutamate dehydrogenase.

The effect of theonellamides on 17β-hydroxysteroid dehydrogenase IV might be the cause of its cytotoxicity. The studies described above, probing the origin of the metabolite, and its possible roles in biology, reflect the novelty of the molecular architecture.

1.4 More Recent Biological Studies: Chemical Genetics

A central problem in chemical biology is to determine the mode-of-action (MOA) of new compounds. Rich functional information has been obtained from scoring ~5000 viable yeast
haploid deletion mutant strains for hypersensitivity to a diverse set of compounds, a process termed chemical genetic profiling. Cells with gene deletions that are deemed hypersensitive to a specific compound can give clues about its MOA. In the yeast *Saccharomyces cerevisiae*, ~6000 potential genes have been characterized by the genome sequencing project.

With this genetic profiling method Boone *et al.* found that two extracts containing natural products stichloroside (16) (Figure 1.4) and theopalauamide (3) showed very similar chemical-genetic profiles (correlation coefficient of 0.892). Thus, these compounds share a common MOA in yeast and this illustrates that chemical-genetic profiling is an effective means of functional classification of natural product extracts. Moreover, stichloroside-resistant mutants also displayed theopalauamide resistance, suggesting that the compounds function similarly.

![Figure 1.4 Structure of stichloroside.](image)

There are limitations to these assays. Haploinsufficiency is the state of a diploid organism having only a single copy of a particular gene, the other copy being inactive due to mutation. An approach based on this status is unlikely to identify a drug target that is the product of an enzyme rather than the enzyme itself. Moreover, these protocols (one or the other) based upon clustering of chemical-genetic profiles are difficult to implement because they depend upon the assembly of a relatively large compendium of reference profiles. Hence, Ho *et al.* developed a new strategy by generating a library of molecular barcoded yeast open
reading frames (MoBY-ORF) to clone wild-type versions of mutant, drug-resistant genes by complementation using a minimal amount of bioactive compound. Each gene of this MoBY-ORF library is controlled by its native promoter and terminator. Cloning by complementation with the MoBY-ORF library is an assay that can be carried out with any *S. cerevisiae* strain.

Using the MoBY-ORF complementation assay, they identified an essential enzyme involved in an early step of the ergosterol biosynthesis pathway: mevalonate pyrophosphate decarboxylase (MVD1).

![Scheme 1.3 Ergosterol biosynthesis pathway.](image)

A theopalauamide-resistant mutant (*theo*<sup>R</sup>) was made to determine whether or not the compound targets a product of the MVD1 pathway. The *theo*<sup>R</sup> mutant was partially resistant to amphotericin B (a sterol-binding antifungal compound). Experiments showed that deletion
mutants erg2Δ, erg31Δ and erg32Δ, which participate in the second and third steps of ergosterol biosynthesis, were resistant to theopalauamide.

To test the hypothesis that theopalauamide (3) and stichloroside (16) bind to ergosterol (22), exogenous ergosterol was added to cultures containing toxic levels of these compounds alongside amphotericin B (23), a polyene antifungal drug, and ketoconazole (24) (Figure 1.5), another antifungal compound that inhibits Erg11p, different ergosterol biosynthetic enzyme. Exogenous ergosterol rescued the toxicity of theopalauamide, stichloroside and amphotericin B, but had no effect on ketoconazole’s toxicity. This observation suggested that, like amphotericin B, both theopalauamide and stichloroside may interact on a molecular level with ergosterol.

Figure 1.5 Structures of amphotericin B, ketoconzole and nystatin.

The Ho Group also studied the effect of these compounds using fluorescent markers released from phosphatidylcholine liposomes that contained various levels of ergosterol to show that theopalauamide and stichloroside interact with ergosterol. Theopalauamide (10 µg/mL) caused a maximal 30% leakage of calcein from liposomes containing 20% ergosterol in their membrane, whereas it had no effect on liposomes lacking ergosterol. Stichloroside (16) and nystatin (25) also caused ergosterol-enhanced leakage, and were more potent than theonellamide. Thus, theopalauamide, stichloroside and nystatin disrupt ergosterol-containing membranes specifically.
A similar experiment showed that fluorescently labeled theonellamide A bound to ergosterol specifically in an in vitro lipid-binding assay. Theonellamide A (5) was conjugated to AMCA to give compounds described by structure 26 (Figure 1.6). Aminomethyl coumarin acetate (AMCA) is a blue-fluorescent dye used to label antibodies, proteins and other molecules for fluorescence imaging.

Figure 1.6 Structure of theonellamide A-fluorophore conjugates.

The theopalauamide-resistant yeast strain (theo\(^R\)) was also resistant to theonellamide A, previously established to be toxic to wild-type \(S.\) \(cerevisiae\).\(^{16,17}\) Ho \(et\) \(al.\)\(^{17}\) demonstrated that fluorescently labeled theonellamide A stained the cell surface of wild-type budding yeast, but only weakly stained the plasma membrane of theo\(^R\) mutant cells. All these studies suggest that theopalauamid e and theonellamide A represent a novel class of sterol-binding compound and behave similarly.

The specificity of lipid-binding properties and the mechanism of their antifungal activity remained ambiguous. In 2010 Yoshida and co-workers\(^{18}\) reported on the production of theonellamide affinity beads by a photo-affinity immobilization method (similar to the earlier work by the Wada group, Scheme 1.1) and tried to identify the binding proteins from fusion yeast cells. They were unable to detect any proteins that specifically bound to theonellamide F. Therefore, they decided to search for chemical-genetic interactions using a set of strains expressing all 5000 protein-coding ORFs of the fission yeast \(Schizosaccharomyces pombe\),
which might provide insights into the genes and pathways targeted by general bioactive metabolites.

They generated a chemical-genomic profile of theonellamide F using a collection of fission yeast strains in which each open reading frame is expressed under the control of an inducible promoter. Strains showing a significantly altered sensitivity compared to the control strain were selected. A total of 20 profiles were obtained and analyzed by 2D-hierarchical clustering analysis. They found a weak correlation between amphotericin B (23) and nystatin (25) (correlation = 0.18 for each of these compounds with respect to theonellamide F), implying that the MOA of theonellamide F is only partially shared with these sterol-binders. The most sensitive strain of yeast overexpressed *SPAC26A3.09*, which encodes a homolog of Rho-type GTPase activating protein Rga2, a small GTPase that plays a role in a signaling pathway of cell polarity and in 1,3-β-D-glucan synthesis in fission yeast. The second most sensitive strain overexpressed *pck1*, which encodes a protein kinase C homolog that regulates 1,3-β-D-glucan synthesis. 2D-Hierarchical clustering analysis showed a modest linkage (correlation coefficient = 0.35) between theonellamide F and FK463 (28) (Figure 1.7), a frontline clinical antifungal drug (common name Micafungin™) that inhibits the synthesis of 1,3-β-D-glucan.

Figure 1.7 Chemical structure of FK463 (28).
Of the 32 genes from generated chemical-genomic profile of theonellamide F, 12 were in common with those affected by FK463, suggesting that the MOA of theonellamide F and FK463 are functionally related. They compared the morphology of cells after exposure to each drug. Cell lysis was observed at the growing ends in fungi treated with FK463, whereas theonellamide F did not lead to any signs of cell lysis. Unexpectedly, calcofluor white (Cfw) staining showed strong signals due to increased 1,3-β-D-glucan at the growing ends and/or the medial region of the cell treated with theonellamide F. Thus, theonellamide F appears to counteract FK463 action by enhancing 1,3-β-D-glucan synthesis.

Further experimental results suggested that the major pathway for 1,3-β-D-glucan synthesis upon theonellamide F treatment is the direct activation of Bgs1 (1,3-β-D-glucan-UDP glucosyltransferase) by Rho1. Theonellamide A (5) was fluorescently labeled as its TNMF-BF derivative 27 (TNMF-BF = theonellamide F-boron-dipyrromethane conjugate) by Yoshida and co-workers to determine the subcellular localization of the theonellamide-binding molecule (Figure 1.6). The in vitro binding assay revealed that TNM-BF (27, Figure 1.6) recognizes ergosterol (22), cholesterol (29), cholestanol (30) and 5α-cholest-7-en-3β-ol (31) (Figure 1.8). Thus, theonellamide F recognizes 3β-hydroxyestersols, a class of lipid molecules, rather than a protein.

![Figure 1.8 Structures of 3β-Hydroxyestersols.](image)

Mutations to genes in the ergosterol biosynthetic pathway (Scheme 1.3) have shown sensitivity to polyene antibiotics in yeast. Mutants lacking the enzymes Erg2, or simultaneous deletion of Erg31 and Erg32, displayed high tolerance to theonellamide F and a decreased
ability of the cells to bind theonellamide F. Deletion of other enzymes (Erg5, Δsts1/Erg4), resulted in slight resistance to theonellamide F. Calcein (a fluorescent dye) was added to S. pombe cells that had been treated with theonellamide F for nine hours. Entry of calcein over the plasma membrane was observed, suggesting that cells do not retain their membrane integrity in the presence of thenellamide F. Moreover, calcein diffusion into theonellamide F treated cells gradually increased over time and in a dose-dependent manner.

The MOA of theonellamide F is different from that of the polyene antibiotics. A typical morphological change in yeast cells after treatment with polyene antibiotics is enlargement of vacuoles whereas the vacuoles of theonellamide F-treated cells became marginally fragmented. This finding is in contrast with earlier rat fibroblast experiments where theonellamides caused the formation of extremely large vacuoles (Section 1.3). The other characteristic aspect of polyene antibiotics is their acute fungicidal effect while theonellamide F showed gradual time dependent toxicity. In agreement with Ho and co-workers, Yoshida concluded that the theonellamides represent a new class of sterol-binding agents.

In 2013, Yoshida et al. published new results from surface plasmon resonance (SPR) and solid-state $^2$H NMR experiments leading them to postulate a mechanism for sterol recognition by theonellamide F in lipid bilayers. $^{19}$ Earlier studies by the same research group had evaluated the binding of amphotericin B (23) $^{20}$ and amphidinol $^{21}$ to palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (32, POPC) liposomes. They performed analogous experiments with theonellamide A, studying the interaction of theonellamide A with POPC liposomes containing ergosterol (22), cholesterol (29), or epicholesterol (33) (Figure 1.9). Surface plasmon resonance (SPR) revealed that the incorporation of 10 mole percent cholesterol or ergosterol into POPC membranes significantly enhances the affinity of theonellamide F for the membrane, particularly initial binding to the membrane surface. Conversely, binding of the peptide to epicholesterol (diastereomer of cholesterol, varying in configuration at C3) liposomes and pure POPC
liposomes was weak by comparison. This demonstrated the preference of the peptide for the 3β-hydroxysterol-containing membranes.

Figure 1.9 Chemical structure of POPC, cholesterol, ergosterol and epicholesterol.

Yoshida et al. also conducted solid-state $^2$H NMR experiments using cholesterol, ergosterol and epicholesterol. These compounds were labeled by deuterium at C3 and incorporated into POPC liposomes in the absence and presence of theonellamide A. The axial rotation in NMR for sterol molecules in lipid bilayers undergoes fast lateral diffusion and gives quadrupolar splitting which depends on the tilt angle of the C−$^2$H bond with respect to the rotation axis and the wobbling of the molecule. Experiments conducted using 3-$d$-cholesterol (29D) and 3-$d$-ergosterol (22D) showed similar results. In the absence of peptide (Figure 1.10 A and C), a characteristic triplet peak was observed, indicating fast rotational motion of the sterol in the POPC bilayers.

However, a stark attenuation of the splitting signal was observed upon addition of the peptide (Figure 1.10 B and D), suggesting that the molecular rotation falls into an intermediate motional speed with correlation times of $10^{-4}$ to $10^{-5}$ seconds. On the contrary, the characteristic splitting didn’t change much for 3-$d$-epicholesterol (33D) containing POPC liposomes (Figure 1.10 E and F). The $^2$H NMR experiments showed that theonellamide A inhibits the fast rotational motion of cholesterol and ergosterol.
On this background of intriguing biological activity, the theonellamides present a worthy target for total synthesis. They present a formidable chemical challenge that requires the asymmetric synthesis of unusual amino acids and their assembly into a demanding bicyclic array.

1.5 Previous Studies Directed Toward the Synthesis of Theonellamides

Nearly two decades ago, Tohdo, Hamada and Shioiri reported progress towards the synthesis of theonellamide F in the form of conference proceedings and communications.\textsuperscript{22,23,24,25} The synthesis has never been completed. They formed the two rings independently but never described plans for the formation of the bicycle. They presumably targeted theonellamide F because it was the first structure to be revealed; none of the other theonellamides have been the subject of synthetic studies.
In 1994, Tohdo et al. published a short letter on their efforts toward the synthesis of what they termed as the northern hemisphere* of theonellamide F. The synthesis of pertinent building blocks will be discussed in relevant chapters of this dissertation. The construction of the western hemisphere was carried out by a fragment condensation approach (Scheme 1.4).

Synthesis of the Ahad-HAL tripeptide is illustrated on the left hand side of Scheme 1.4. At the outset, the protecting group of the Ahad α-amine was switched from Boc (compound 34) to Troc (compound 35). This was done in order that both protecting groups could be removed simultaneously from linear precursor 47 prior to cyclization. The δ-COOH of Ahad lactone 35 was coupled with the free amine of HAL 36 to give tripeptide 37. In readiness for coupling, the tert-butyl ester was cleaved under acidic conditions to afford acid 38.

The remaining tetrapeptide was assembled as shown in the right hand side of Scheme 1.4. p-Bromophenylalanine derivative 39 was coupled with the trichloroethyl (TCE) ester of β-alanine (40) to give dipeptide 41. Elongation of the peptide in the C→N terminal direction was accomplished by Boc deprotection and homologation with eHyAsn building block 42, giving tripeptide 43. Analogous deprotection and coupling with Boc-Asn-OH (44) was accompanied by loss of the TBS ether under acidic conditions. The β-OH of the eHyAsn residue was reprotected as the more robust TBDPS ether to obtain compound 45. In readiness for coupling, the tert-butyloxy carbonyl group was cleaved under Lewis acidic conditions to afford amine 46. They used diphenylphosphoryl azide [DPPA, (C₆H₅O)₂P(O)N₃ (49)] and diethyl phosphorocyanidate [DEPC, (C₂H₅O)₂P(O)CN) (50)] for the various coupling reactions. These coupling reagents were introduced by Shioiri in the 1970’s. In readiness for coupling, the tert-butyloxy carbonyl group was cleaved under Lewis acidic conditions to afford amine 46.

*They drew the molecule rotated 90° relative to representations in this dissertation. Thus their references to northern and southern hemispheres correlate with our western and eastern hemispheres respectively.
Scheme 1.4 Assembly of the western hemisphere of theonellamide F.
The tripeptide acid 38 was coupled with tetrapeptide amine 46 by using DEPC. The linear precursor 47 was treated with lithium hydroxide followed by cyclization with DPPA to give the western hemisphere of theonellamide F, viz. compound 48. While these coupling reagents gave reasonable results for small peptides, more hindered couplings and the key cyclization steps were low yielding. The synthesis involves 10 steps (longest linear sequence) with an overall yield of 7.9%. Moreover, they incorporated the Ahad residue into the cyclic peptide in its lactone form. We believe that the Ahad residue should be incorporated into the cyclic peptides in the open chain form and that the rigidity of the lactone may have a contributing factor to the low-yielding cyclization of the western ring in the synthetic effort of Tohdo et al.

1.6 Research Goals of This Dissertation

Despite considerable synthetic effort to produce theonellamide F by the Shioiri group in the early 1990s, a total synthesis of a theonellamide has yet to be reported. An efficient chemical synthesis of these compounds offers benefits such as an alternative to cultivation from natural sources and the potential to produce analogs and explore new ideas about their emerging biological role. We chose to synthesize theonellamide C (7) in our laboratory as it is the simplest congener to make, requiring the synthesis of the least number of amino acids, prior to fragment condensations and cyclizations.

My goal was to synthesize the western hemisphere of theonellamide C (7). In common with the other theonellamide congeners, theonellamide C (7) has ten amino acids and one bis-amino acid in total. The two hemispheres of theonellamide C (7), designated east and west, each have five amino acids and the bicycle is bridged by a \( \tau \)-histidinoalanine (\( \tau \)-HAL) residue.

1.7 Retrosynthetic Analysis

There are four amino acids in theonellamide C (7) that are not commercially available. These are Aboa ④, eHyAsn ⑦, Ahad ⑩, and \( \tau \)-HAL. Since the major element of
conformational restraint in the molecule is the \( \tau \)-HAL residue, we plan to construct this bis-amino acid and build on the two rings sequentially. It is desirable to perform fragment couplings with carboxy components that are not prone to epimerization at C\( \alpha \); three residues provide this opportunity: \( \beta \)-Ala \( \textcircled{9} \) and Ahad \( \textcircled{10} \) in the western ring and Aboa \( \textcircled{5} \) in the eastern ring. Capitalizing on the opportunities provided by these non-epimerizable residues, the major cyclization disconnection for the western hemisphere is between residues \( \textcircled{9} \) and \( \textcircled{10} \) (Scheme 1.5). This leads to cyclization precursor 49. The deprotection of the Alloc and Allyl groups will be accomplished simultaneously by using Pd(0) catalysis, followed by subsequent cyclization. Further disconnection of compound 49 leads to tetrapeptide 52, \( \tau \)-HAL (53) and Ahad (54) residues.

Scheme 1.5 Retrosynthesis of the western hemisphere of theonellamide C.

This dissertation will highlight the synthesis of the Ahad residue (Chapters 2, 3 and 4) and the \( \textcircled{6} - \textcircled{9} \) tetrapeptide (western hemisphere, Chapter 5) (both building blocks shown in red boxes) and peptide assembly for the western hemisphere of theonellamide C.
CHAPTER 2: INITIAL APPROACH TO (2S,4R)-α-AMINO-γ-HYDROXY ADIPIC ACID (AHAD): COREY-LYGO METHOD

2.1 Structural Determination of (2S,4R)-α-Amino-γ-Hydroxy Adipic Acid (Ahad)

Theonellamide F (1) showed UV maxima at 283 nm, 294 nm and 315 nm. Upon acid hydrolysis with 6N HCl at 110 °C for 16 hours compound 1 generated several ninhydrin-positive spots on TLC. The hydrolysate was dissolved in triethylamine/formic acid buffer (pH 3.41) and subjected to HPLC. This HPLC analysis of the hydrolysate indicated the presence of asparagine (Asn), allo-threonine (aThr), phenylalanine (Phe), β-alanine (β-Ala) and two residues of serine (Ser). The stereochemistry of the standard amino acid residues was determined by chiral GC-MS analysis of derivatives.

![Scheme 2.1](image)

Scheme 2.1 Ahad structure elucidation from theonellamide F degradation.

Preparative ion-exchange chromatography showed a single peak corresponding to compounds 55a and 55b (Scheme 2.1) which eluted after β-Ala in the acid hydrolysate HPLC analysis. The (M+H)^+ ion at m/z 178 was observed in the fast atom bombardment (FAB) mass spectrum of the amino acid derivatives, suggesting that 55a and 55b were a diastereomeric mixture of aminohydroxyadipic acids. According to ^1^H and ^13^C NMR analysis, these diastereomeric mixtures were isolated as an equilibrium mixture of γ-lactones 57a, 57b and triethylammonium salts of dicarboxylic acids 56a, 56b formed during evaporation of
triethylammonium formate buffer. The isolated mixture was treated with 2-((tert-butoxycarbonyloxyimino)-2-phenylacetonitrile (BocON) and triethylamine, followed by acidification to promote lactonization and esterification with diazomethane. The two diastereomers were separated by normal phase HPLC to afford lactones 58a and 58b (Scheme 2.1).

When the acidic hydrolysate was analyzed after a shorter reaction time, the (2S,4R) isomer (trans) was detected as the major isomer. The ratios of 4S to 4R isomers were 1:4, 1:2, and 1:1.5, upon acidic hydrolysis for 2, 4, and 8 hours, respectively, as analyzed by chiral GC-MS analysis. The CD spectrum of 58a, showed a positive Cotton effect at 217 nm.27 Therefore the configuration at C2 of both isomers was deemed to be S. The two diastereomers were distinguished based on the 1H NMR signals for H3 and H3'. For cis-2,4-disubstituted γ-lactones, two separate signals are observed; a single signal is observed for the trans diastereomer (Figure 2.1). Proton NMR studies showed that the isomer 58a appeared to be the cis-lactone, by comparison with the data on synthetic model compounds prepared by Stoddart and coworkers.28

![Figure 2.1 Comparison of H3 signals 1H NMR of cis- and trans-disubstituted lactones.](image)

Accordingly, the cis-lactone 58a had (2S,4S) stereochemistry, while the trans-lactone 58b, had the (2S,4R) stereochemistry, as deduced from the 1H NMR spectrum. The C-4 hydroxyl group might have been epimerized through a backside attack of the carboxyl group during lactonization (Scheme 2.2). Moreover, equilibration to the more stable cis-2,4-disubstituted γ-lactone may have enhanced the (2S,4S) diastereomer.
Therefore, Matsunaga and co-workers concluded that (2S,4R)-2-amino-4-hydroxyadipic acid (55a) was the constituent of theonellamide F (1), while the (2S,4S) isomer was an artifact of the isolation procedure.

2.2 Biogenesis of Ahad and α-AAA

Structure elucidation of the theonellamides showed that they contain (2S,4R)-α-amino-γ-hydroxy adipic acid (Ahad) (55a) (Schemes 2.1 and 2.3).
The onegramide and theopalauamide contain (2S)-α-amino-adipic acid (α-AAA, 64, Scheme 2.3), which lacks the γ-hydroxy group. In mammals and higher plants metabolic degradation of lysine\(^{29}\) gives saccharopine (62), formed by condensation of lysine (60) and α-ketoglutarate (61). In eukaryotes, normal metabolic degradation of lysine (60) gives α-AAA (64). Whereas, metabolic degradation of adipic acid (65) gives (3R)-3-hydroxyadipic acid (66) which is excreted in the urine and observed in the serum of ketoacidosis patients.\(^{30}\) α-Amino-γ-hydroxy adipic acid (Ahad, 55a) would appear to be of novel, or mixed, biogenesis. Aside from the appearance in theonellamides (vide supra), there has been only one other report of Ahad by Blass and Macheboeuf who proposed the occurrence of Ahad in cultures of Vibrio cholera, based on elemental analysis but without spectroscopic evidence.\(^{31}\)

### 2.3 Previous Syntheses of (2S,4R)-γ-Hydroxy-α-Amino Adipic Acid (Ahad)

In 1957 Benoiton and co-workers synthesized γ-hydroxyglutamic acids.\(^{32}\) By analogy, Kristensen et al. reported the synthesis of a mixture of all four stereoisomers of Ahad in 1980\(^{33}\) (Scheme 2.4). Condensation of the anion of ethyl acetamidocyanacetate (68) with 3-acetoxy-4-chlorobutynitrile (67), followed by hydrolysis and decarboxylation, afforded a mixture of four isomers of 55 which was separated into racemates of the two diastereoisomers by ion-exchange chromatography and preparative high voltage electrophoresis.

![Scheme 2.4 Synthesis of four stereoisomers of Ahad by Kristensen et al.](image)

In 1994, Tohdo et al.\(^{22,23}\) reported the synthesis of Ahad in optically active form (Scheme 2.5). They took commercially available \(N\text{-}\text{tert}-\text{butoxycarbonyl-}(S)\)-aspartic acid β-benzyl ester (70) and converted it to Boc-\((S)\)-Asp-OMe (71). The β-carboxyl group of 71 was activated with carbonyldiimidazole (CDI) and the resulting imidazolide was reacted with the
magnesium enolate of ethyl hydrogen malonate to yield β-keto ester 72 which was reduced stereoselectively. The yield and diastereomeric excess of the reduced products 73a and 73b were measured following acid-catalyzed lactonization. The best result was obtained by asymmetric hydrogenation with Noyori's Ru-(R)-BINAP catalyst to give a mixture of 73a and 73b that was converted to the γ-lactones 74a and 74b (ratio of 27:1) by treating with a catalytic amount of trifluoroacetic acid. Hydrolysis of compound 74a with lithium hydroxide followed by relactonization with acid gave the lactone 75a. Tohdo et al. used this γ-lactone functionality as "intramolecular protection" for their Ahad building block as discussed in Chapter 1, Section 1.5.

Scheme 2.5 Synthesis of the Ahad lactone by Tohdo et al.

The α-carboxylic acid is not part of the peptide backbone and may be important for stabilization of an imidazolium cation in the full theonellamide structure. As described earlier, Ahad 55a was isolated as the corresponding γ-lactone 57a following acidic degradation of the theonellamides. The work of Benson Edagwa in the Taylor Laboratory with 4,5-dihydroxyleucine alert us to the fact that there would be a high risk of epimerization at Cα while opening the γ-lactone. Michl had shown earlier that epimerization of Nα-acyl and Nα-carbamoyl γ-lactones occurs through oxazolone formation. On this basis, we have concerns
for the stereochemical integrity of 75a in Scheme 2.5. For this reason, we wanted to avoid the γ-lactone formation moiety throughout our synthesis.

2.4 Retrosynthesis of Ahad via Alkylation of a Glycine Ester Enolate

Our Ahad building block has two stereocenters: Cα and Cγ (Scheme 2.6). We planned to establish the Cγ configuration in an electrophilic reaction partner and focus our attention on the Corey-Lygo method for the catalytic asymmetric generation of the Cα stereocenter. Corey and Lygo independently developed a new class of asymmetric phase-transfer catalyst derived from Cinchona alkaloids36 and applied them to the enantioselective synthesis of α-amino acids.

Retrosynthetically, it can be imagined that an Ahad building block might be arrived at via stereoselective alkylation of the enolate of glycine derivative 76 (commercially available) with an electrophile (Scheme 2.6).

Scheme 2.6 Retrosynthesis of Ahad.

2.5 Phase-Transfer Catalysis (PTC) and the Corey Lygo Approach

2.5.1 The Concept

Phase-transfer catalysis (PTC)37 facilitates the migration of a reactant in a heterogeneous system from one phase into another phase where a reaction can take place.38 An inexpensive, commercially available source of effective chiral phase-transfer catalysts is the family of Cinchona alkaloids (Figure 2.2). These alkaloids can be transformed into quaternary
ammonium salt catalysts in one or two steps. Furthermore, these alkaloids are available as pseudoenantiomers (quinine and quinidine, e.g., 77 and 78) that can achieve similar levels of asymmetric induction with the opposite sense of enantioselectivity. An interfacial mechanism is implicated for lipophilic quaternary ammonium catalysts (Q’X) when basic conditions are employed in PTC, as illustrated in Scheme 2.7. The interfacial mechanism involves formation of a contact ion pair between the quaternary ammonium cation (Q+) and the anionic organic nucleophile (enolate, M+) that is generated at the interface by an inorganic base. The generated quaternary ammonium cation complex (enolate, Q+) transfers the anionic organic nucleophile into the organic phase, where it can react with the organic electrophile (R-X).

![Scheme 2.7 Phase transfer alkylation of imine.](image)

Fig 2.2 Cinchona-derived chiral phase-transfer catalysts.

One reason for the enantioselectivities obtained in the presence of quaternary ammonium salts would be reaction via an ion-pair arrangement 81 (Scheme 2.8). In this
arrangement the preferential attack would occur via the Si-face as the Re-face of the enolate carbon is blocked by the quinoline ring of the quaternary ammonium salt.

Scheme 2.8 Possible ion pair arrangement that explains facial selectivity.

2.5.2 Previous Examples

Previous examples of the alkylation of esters of glycine benzophenone imine with relatively complex electrophiles encouraged us to pursue such an approach to the stereoselective formation of Cα in Ahad. Zhu and coworkers used the Corey-Lygo method for the synthesis of (-)-quinocarcin (85)\(^{42}\) and lemonomycinone amide (87) (Scheme 2.9).\(^ {43}\)

Scheme 2.9 Application of Corey-Lygo alkylation to complex molecules.

The preparation of intermediate 84 for the total synthesis of (-)-quinocarcin (85) is shown in Scheme 2.9. Following Corey’s procedure, the enantioselective alkylation of N-(diphenylmethylene)glycine tert-butyl ester 76b was performed with 2-bromo-5-tert-
butyldimethylsilyloxybenzyl bromide (82) in the presence of O-allyl-N-(9-anthracenylmethyl) cinchonidinium bromide (86), followed by chemoselective hydrolysis of the imine functionality gave the amino ester 83 in 87% yield. Cleavage of the silyl ether gave amino phenol 84 in 91% yield.

Also, Tohdo et al. utilized Nq-benzylcinchonidinium chloride (90) as a phase transfer catalyst for the preparation of Na-Boc-(S)-p-bromophenylalanine (39) which occurs in theonellamide F. The asymmetric alkylation of the Schiff base 76b was carried out with p-bromobenzyl bromide (88) using catalyst 90 to obtain 89 (Scheme 2.10). Acid hydrolysis followed by neutralization and treatment with Boc₂O afforded the required Boc derivative 39.

Scheme 2.10 Preparation of (S)-Na-Boc-p-bromophenylalanine by Tohdo et al.

2.6 Synthesis of an Electrophile for Ahad Synthesis

As depicted in Scheme 2.6, we required an appropriate electrophile. The initial steps in the synthesis of this electrophile were accomplished using an established procedure reported independently by Lee et al. and Bellamy et al. Bellamy described the synthesis of (R)-carnitine (95) according to Scheme 2.11. Starting from D-malic acid (91), the formation of dibenzyl ester 92 was performed. Chemoselective reduction of the α-hydroxyester of compound 92 was carried out by using BH₃.SMe₂ to obtain diol 93. This chemoselectivity was first observed by Moriwake. The reaction involves the formation of a boroxolane-type intermediate 96 (Scheme 2.11). The chemoselectivity observed in the reduction might be derived from the fact that relatively short B-O bond length in 96 vs. 97 and low entropy factor for the more feasible
formation of 96 than that of boroxane-type intermediate 97. Monotosylation of the diol 93 led to compound 94, a key intermediate en route to the synthesis of (R)-carnitine (95). Similar reactions were carried out by Lee et al. for the synthesis of (2S,3R)-2-benzyl-3,4-epoxybutanoic acid (BEBA) (98) (shown in a box in Scheme 2.11).  

Scheme 2.11 Synthetic manipulations of malic acid by Lee et al. and Bellamy et al.

Kocienski et al. synthesized the analogous methyl ester 101 in their efforts toward the total synthesis of (-)-lipstatin. Enantiomerically pure dimethyl (S)-(-)-malate (99) was selectively reduced to diol 100. The primary hydroxyl function of the resultant diol 100 was converted to the tosylate 101.

Scheme 2.12 Synthesis of intermediate electrophile for total synthesis of (-)-lipstatin by Kocienski et al.

We repeated the reactions as shown in Scheme 2.11 using D-malic acid (91). However, we obtained only low yields of tosylate 94 under the conditions reported by Kocienski et al. Galvez and coworkers reported that conversion of a diol to a stannylene acetal enhanced the nucleophilicity of the oxygen, while still promoting regioselectivity (shown in a box in Scheme
2.13.\textsuperscript{42} Diol \textbf{93} was successfully monotosylated using this strategy. Protection of the remaining secondary alcohol as both TBS ether \textbf{103a} and TBDPS (more hindered) ether \textbf{103b} was achieved. The primary tosylates \textbf{103a} and \textbf{103b} were each treated with sodium iodide/acetone to obtain another pair of electrophiles \textbf{104a} and \textbf{104b} with iodide as a good leaving group. Moreover, another less hindered electrophile was synthesized without the protection of the secondary alcohol, \textit{viz.} \textbf{102}.

![Scheme 2.13 Synthesis of electrophiles.](image)

In conclusion, we made a series of potential electrophiles to do the alkylation from less sterically hindered \textbf{102} to more bulky group on secondary alcohol \textbf{103} and from good leaving group tosylate \textbf{94} to better leaving group iodide \textbf{104}.

\textbf{2.7 Alkylation of Glycine Ester Enolates}

The next step was to alkylate a glycine anion synthon with the electrophiles synthesized in Scheme 2.13. According to the literature, glycine benzophenone imine \textit{tert}-butyl ester \textbf{76b} has typically been used in these reactions; the \textit{tert}-butyl ester can be cleaved under the same conditions as the benzophenone imine in a single step. For our synthesis we chose the corresponding ethyl ester \textbf{76a} as it is less expensive and simple examples demonstrate that the ethyl ester is equally effective (Table 2.1).
In order to develop some experience of the asymmetric phase-transfer alkylation of imine 76 we repeated some known examples from the literature.

There are two sets of conditions employed for alkylation with some differences.

1. Corey’s conditions: The conditions are ten equivalents of CsOH·H₂O, five equivalents of electrophile, CH₂Cl₂, -60 °C. An example was shown earlier in Zhu’s synthesis of (-) quinocarcin (85) (Scheme 2.9).

2. Lygo’s conditions: 50 percent aqueous KOH/NaOH, water, five equivalents of electrophile, toluene, -60 °C to room temperature. An example was shown earlier in Tohdo’s synthesis of p-bromophenylalanine (39) (Scheme 2.10).

We initially investigated the alkylation of glycine benzophenone imine ethyl ester (76a) with allyl bromide as alkylating agent employing 10 equivalents of base (Table 2.1, Entry 1). The same reaction conditions were applied with glycine benzophenone imine tert-butyl ester 76b, leading to a comparable yield of the product (Entry 2). Corey reported 97% e.e. for the latter alkylation. The enantioselectivity of the product from Entry 2 was determined to be 92% e.e. by chiral HPLC. We applied the same reaction conditions to a commercially available iodobutane, a linear aliphatic alkyl halide, and the yields from both the glycine derivatives were comparable (Entries 3 and 4). Lygo’s conditions were also tested and the yield was much the same for alkylation of both the glycine enolates (Entry 5 and 6). Furthermore, we tried the Lygo conditions using a mixture of solvents (Entry 7). The low yield suggested that the ethyl ester hydrolysis was competing kinetically with enolate formation and alkylation under the strongly basic conditions.

Excited that we could achieve the alkylation in good yield under Corey’s conditions, we subjected our more complex electrophile 103b to the reaction conditions; no product was isolated (Entry 8). Therefore, we switched from the tosylate 103b, to the iodide 104b as a superior leaving group (Entry 9). Again no product was isolated. One reason for this could be...
that the electrophile is unreactive due to the adjacent, bulky TBDPS ether. Therefore, we reduced the size of the adjacent protecting group, to the smaller TBS ether. No product was isolated from the attempted alkylation using 104a (Entry 10). We raised the temperature to room temperature; but again no desired product was formed (Entry 11). We presume that the TBS group also provides steric hindrance to the displacement reaction. The reaction was also performed, without success, on unprotected alcohol 102 (Entry 12). Proton transfer is a potential problem here.

Table 2.1 Alkylations of glycine enolates.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Glycine</th>
<th>Electrophile</th>
<th>Conditions</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>76a</td>
<td>CH₂=CH₂CH₂Br</td>
<td>CsOH.H₂O, CH₂Cl₂, -78→-60 °C, 22 h</td>
<td>95%</td>
</tr>
<tr>
<td>2</td>
<td>76b</td>
<td>CH₂=CH₂CH₂Br</td>
<td>CsOH.H₂O, CH₂Cl₂, -78→-60 °C, 22 h</td>
<td>85%</td>
</tr>
<tr>
<td>3</td>
<td>76a</td>
<td>CH₃(CH₂)₃I</td>
<td>CsOH.H₂O, CH₂Cl₂, -78→-60 °C, 22 h</td>
<td>54%</td>
</tr>
<tr>
<td>4</td>
<td>76b</td>
<td>CH₃(CH₂)₃I</td>
<td>CsOH.H₂O, CH₂Cl₂, -78→-60 °C, 22 h</td>
<td>53%</td>
</tr>
<tr>
<td>5</td>
<td>76a</td>
<td>CH₃(CH₂)₃I</td>
<td>50 % aq. KOH, H₂O, PhMe, RT, 24 h</td>
<td>50%</td>
</tr>
<tr>
<td>6</td>
<td>76b</td>
<td>CH₃(CH₂)₃I</td>
<td>50 % aq. KOH, H₂O, PhMe, RT, 24 h</td>
<td>46%</td>
</tr>
<tr>
<td>7</td>
<td>76b</td>
<td>CH₃(CH₂)₃I</td>
<td>50 % aq. KOH, CH₂Cl₂-PhMe (7:1), 24 h</td>
<td>34%</td>
</tr>
<tr>
<td>8</td>
<td>76a</td>
<td>103b</td>
<td>CsOH.H₂O, CH₂Cl₂, -78→-60 °C, 22 h</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>76a</td>
<td>104b</td>
<td>CsOH.H₂O, CH₂Cl₂, -78→-60 °C, 22 h</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>76a</td>
<td>104a</td>
<td>CsOH.H₂O, CH₂Cl₂, -78→-60 °C, 22 h</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>76a</td>
<td>104a</td>
<td>CsOH.H₂O, CH₂Cl₂, RT, 22 h</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>76b</td>
<td>102</td>
<td>CsOH.H₂O, CH₂Cl₂, -78→-60 °C, 22 h</td>
<td>-</td>
</tr>
</tbody>
</table>
Collectively, the results described show that activated alkyl halides (e.g., allyl bromide in Table 2.1 and benzylic bromides in Schemes 2.9 and 2.10) are the best electrophiles for these reactions. The need to engage a five-fold excess of electrophile in these alkylations is not widely appreciated and is a drawback when synthetically valuable electrophiles are involved. Even then, the use of excess electrophile didn’t afford our desired product. Also, the neighboring silyl ether provides severe steric hindrance to the displacement reaction.

We employed commercially available methyl 4-bromobut-2-enoate (107) as the electrophile and successful asymmetric alkylation of the glycine imine 76b was achieved as shown in Scheme 2.14.

Scheme 2.14 Alkylation of glycine imine with an allylic bromide.

While looking for a suitable method for diastereoselective epoxidation, or conjugate addition of an O-nucleophilic species, we became aware of another approach that might deliver the 1,3-syn amino alcohol in a more reliable fashion. This will be the subject of Chapter 3.

2.8 Experimental Section

2.8.1 General Methods

All reactions were performed under a dry nitrogen atmosphere unless otherwise noted. Reagents were obtained from commercial sources and used directly; exceptions are noted. Diisopropylethylamine, 2,4,6-collidine, pyridine, triethylamine and 2,6-lutidine were dried and distilled from CaH₂ and stored over KOH pellets. Methanol was distilled from Mg turnings and stored over 4 Å molecular sieves. Flash chromatography was performed using silica gel (32-63 µ) from Dynamic Absorbents Inc. Reactions were followed by TLC on precoated silica plates.
(200 µm, F-254 from Dynamic Adsorbents Inc.). The compounds were visualized by UV fluorescence or by staining with phosphomolybdic acid (PMA), cerium-ammonium-molybdate (CAM), ninhydrin, potassium permanganate, or 2,4-dinitrophenylhydrazine (2,4-DNP) stains. HPLC was performed on Waters 600E multisolver delivery system (Waters 2487 dual λ absorbance detector). Optical rotations were recorded on JASCO P-2000 digital polarimeter. NMR spectra were recorded on a Bruker AV-400-liquid spectrometer. Proton NMR data is reported in ppm downfield from TMS as an internal standard. High resolution mass spectra were recorded using either an Agilent 6210 time-of-flight MS, or a Hitachi MS-8000 3DQ LC-ion trap mass spectrometer with electrospray ionization.

2.8.2 Procedures

(BnOOC)COOBn

(S)-Dibenzyl 2-hydroxysuccinate (92)

Benzyl alcohol (1.50 mL, 1.61 g, 14.90 mmol, 2.00 equiv.) and p-toluenesulfonic acid monohydrate (14 mg, 0.074 mmol, 0.01 equiv.) were added to a solution of D-malic acid (91) (1.00 g, 7.46 mmol, 1.00 equiv.) in dry toluene (12 mL). The reaction mixture was heated under reflux with a Dean-Stark apparatus to effect the azeotropic removal of water. When no more water appeared in the distillate, the mixture was allowed to cool to rt and washed with saturated aqueous NaHCO₃ (25 mL) and brine (25 mL). The organic layer was dried over MgSO₄, filtered and evaporated. The residue was purified by flash column chromatography on silica gel with Hex:EtOAc (4:1) as eluent to give the dibenzyl ester 92 as a colorless oil (80%). Rₜ 0.42 (4:1 Hex-EtOAc). [α]D²⁵ +42.98 (c 2.5, CHCl₃). ¹H NMR (CDCl₃, 400 MHz) δ 2.80 (dd, J = 16.4, 6.1 Hz, 1H), 2.86 (dd, J = 16.4, 4.7 Hz, 1H), 3.41 (br s, 1H), 4.52 (dd, J = 5.9, 4.8 Hz, 1H), 5.06 (s, 2H), 5.13 (s, 2H), 7.24-7.33 (m, 10H); ¹³C NMR (CDCl₃, 100 MHz) δ 38.8, 66.8, 67.5, 67.6, 128.3, 128.4, 128.5, 128.6, 128.7, 135.2, 135.6, 170.3, 173.2. HRMS (ESI) calcd for C₁₈H₁₉O₅ (M+H)+ 315.1227, obsd 315.1220.
Borane-dimethylsulfide complex (175 µL, 0.350 mmol, 2.0 M solution in THF, 1.10 equiv.) was added dropwise over 15 min to a solution of (S)-dibenzyl-2-hydroxsuccinate (92) (100 mg, 0.318 mmol, 1.00 equiv.) in dry THF (1 mL) at 0 °C under N₂. After 1 h of stirring, sodium borohydride (0.6 mg, 0.016 mmol, 0.05 equiv.) was added and the mixture was stirred overnight at rt. The mixture was quenched by the addition of methanol (1.5 mL) and stirring continued for 30 min. The solvent was evaporated and the residue purified by column chromatography on silica gel (1:1 Hex-EtOAc) to obtain diol 93 as a colorless oil (41 mg, 61%).

\[ \text{Rf} 0.31 \text{ (1:1 Hex-EtOAc). [\alpha]_D^{25} +18.84 (c 1, CHCl}_3)\]

\[ ^1\text{H NMR (CDCl}_3, 400 MHz) \delta 2.49 (dd, J = 16.3, 4.3 Hz, 1H) 2.55 (dd, J = 16.3, 8.4 Hz, 1H), 3.35 (br s, 2H) 3.48 (dd, J = 11.4, 6.4 Hz, 1H) 3.62 (dd, J = 11.4, 3.5 Hz, 1H), 4.08-4.17 (m, 1H), 5.13 (s, 1H), 7.30-7.35 (m, 5H); ^13\text{C NMR (CDCl}_3, 100 MHz) \delta 37.8, 65.7, 66.7, 68.6, 128.3, 128.4, 128.6, 135.6, 172.3. \]

HRMS (ESI) calcd for C₁₁H₁₄NaO₄ (M+Na)\(^+\): 233.0784, obsd 233.0793.

To a solution of diol 93 (200.0 mg, 0.95 mmol, 1.00 equiv.) in dry CH₂Cl₂ (2.5 mL) was added successively \(^n\)Bu₂SnO (7.0 mg, 0.03 mmol, 0.03 equiv.), a solution of Et₃N (135 µL, 98.4 mg, 0.97 mmol, 1.02 equiv.) in dry CH₂Cl₂ (2.5 mL) and p-toluenesulfonyl chloride (190.7 mg, 0.97 mmol, 1.02 equiv.) at rt. The mixture was stirred for 6 h, then treated with brine (10 mL). The aqueous layer was extracted with CH₂Cl₂ (4 x 15 mL). The combined organic layers were dried with anhydrous MgSO₄, filtered and concentrated. The residue was purified by silica gel column chromatography (2:1 Hex-EtOAc) to obtained tosylated product 94 (222 mg, 64%), \[ \text{Rf} 0.43 \text{ (1:1 Hex-EtOAc). [\alpha]_D^{25} +7.34 (c 1.5, CHCl}_3)\]

\[ ^1\text{H NMR (CDCl}_3, 400 MHz) \delta 2.00 (br s, 1H), 2.45 (s, 3H), 2.53 (dd, J = 16.6, 4.8 Hz, 1H), 2.62 (dd, J = 16.7, 7.5 Hz, 1H), 4.04 (d, J = 8.3 Hz, 2H), 4.25-4.28 (m, 1 H), 5.14 (s, 2H), 7.26-7.39 (m, 7H), 7.78 (d, J = 8.3 Hz, 2H); ^13\text{C NMR} \]

\[ \]
(CDCl₃, 100 MHz) δ 21.7, 37.4, 66.0, 66.9, 71.9, 128.0, 128.2, 128.5, 128.7, 130.0, 132.5, 135.3, 145.2, 171.4. HRMS (ESI) calcd for C₁₈H₂₁O₆S (M+H)+ 365.1053, obsd 365.1043.

(S)-Benzy1 3-hydroxy-4-iodobutanoate (102)

Sodium iodide (42 mg, 0.274 mmol, 2.0 equiv.) was added to a solution of (S)-Benzy1 3-hydroxy-4-(tosyloxy)butanoate (94) (50 mg, 0.137 mmol, 1.0 equiv.) in acetone (5 mL). The mixture was stirred and heated at reflux overnight. Saturated aqueous NaHCO₃ (4 mL) was added and the mixture extracted with diethyl ether (3 x 10 mL). The combined organic layers were washed with brine (5 mL), dried over MgSO₄, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 4:1) afforded a brownish yellow oil 102 (24 mg, 55%). Rf 0.46 (2:1 Hex-EtOAc). [α]D²⁵ +12.31 (c 1, CHCl₃). ¹H NMR (CDCl₃, 400 MHz) δ 2.00 (br s, 1H), 2.60 (dd, J = 15.4, 7.1 Hz, 1H), 2.73 (dd, J = 15.4, 4.9 Hz, 1H), 3.21-3.30 (m, 2H), 4.05 (app. pent, J = 5.5 Hz, 1H), 5.09 (d, J = 12.3 Hz, 1H), 5.14 (d, J = 12.3 Hz, 1H), 7.25-7.35 (m, 5H); ¹³C NMR (CDCl₃, 100 MHz) δ 9.0, 37.8, 66.7, 128.3, 128.4, 128.6, 135.6, 172.3. HRMS (ESI) calcd for C₁₁H₁₄IO (M+H)+ 320.9817, obsd 320.9821.

(S)-Benzy1 3-[(tert-butyldimethylsilyl)oxy]-4-(tosyloxy)butanoate (103a)

2,6-Lutidine (178 µL, 163 mg, 1.5 mmol, 3.0 equiv.) was added dropwise to a solution of (S)-benzy1 3-hydroxy-4-(tosyloxy)butanoate (94) (185 mg, 0.5 mmol, 1.0 equiv.) in dry CH₂Cl₂ (7.5 mL) under N₂ at rt. The mixture was stirred for 30 min before the dropwise addition of TBDMSOTf (140 µL, 161 mg, 0.6 mmol, 1.2 equiv.). The mixture was stirred overnight, the solvent evaporated and the residue purified by silica gel chromatography, eluting with 2:1→1:1 (Hex-EtOAc), to give TBS ether 103a (206 mg, 85%); Rf 0.38 (1:1 Hex-EtOAc). [α]D²⁵ +8.01 (c 1, CHCl₃). ¹H NMR (CDCl₃, 400 MHz) δ 0.02 (s, 3H), 0.03 (s, 3H), 0.81 (s, 9H), 2.45 (s, 3H), 2.49 (dd, J = 15.4, 6.9 Hz, 1H), 2.56 (dd, J = 15.4, 5.2 Hz, 1H), 3.93-4.00 (m, 2H), 4.3-4.35 (m, 1H),
5.06 (d, J = 12.3 Hz, 1H), 5.12 (d, J = 12.3 Hz, 1H), 7.32–7.38 (m, 5H), 7.77–7.79 (m, 4H), \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\) -5.1, -4.7, 17.9, 21.6, 25.6, 39.5, 66.5, 67.2, 72.3, 128.0, 128.2, 128.3, 128.6, 129.9, 132.8, 135.6, 144.9, 170.3. HRMS (ESI) calcd for C\(_{24}\)H\(_{35}\)O\(_6\)SSi (M+H)\(^+\) 479.1918, obsd 479.1925.

\((S)-\text{Benzyl 3-[(\text{tert-butyldiphenylsilyl})oxy]-4-(tosyloxy)butanoate (103b)}\)

2,6-Lutidine (168 \(\mu\)L, 155 mg, 1.44 mmol, 3.5 equiv.) was added dropwise to a solution of \((S)-\text{benzyl 3-hydroxy-4-(tosyloxy)butanoate (94)}\) (150 mg, 0.41 mmol, 1.0 equiv.) in dry CH\(_2\)Cl\(_2\) (7.5 mL) under N\(_2\) at rt. The mixture was stirred for 30 min before the dropwise addition of TBDPSOTf (320 \(\mu\)L, 320 mg, 0.82 mmol, 2.0 equiv.). The mixture was stirred overnight, the solvent evaporated and the residue purified by silica gel chromatography, eluting with 5:1 (Hex-EtOAc) to yield TBDPS ether \(103b\) (141 mg, 57%). \(R_f\) 0.62 (1:1 Hex-EtOAc). \([\alpha]_D^{25}\) +5.76 (c 0.75, CHCl\(_3\)). \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\) 0.98 (s, 9H), 2.41 (s, 3H), 2.54 (d, \(J = 5.9\) Hz, 2H), 3.89-3.98 (m, 2H), 4.23-4.27 (m, 1H), 4.90 (d, \(J = 12.3\) Hz, 1H), 4.99 (d, \(J = 12.3\) Hz, 1H), 7.21-7.53 (m, 10H), 7.54-7.59 (m, 5H); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\) 19.3, 21.8, 26.9, 39.0, 66.6, 67.9, 71.8, 127.8, 128.1, 128.4, 128.7, 129.9, 130.1, 132.9, 133.0, 133.1, 135.0, 135.7, 135.9, 136.0, 144.9, 170.2. HRMS (ESI) calcd for C\(_{34}\)H\(_{39}\)O\(_6\)SSi (M+H)\(^+\) 603.2231, obsd 603.2212.

\((S)-\text{Benzyl 3-[(\text{tert-butyldimethylsilyl})oxy]-4-iodobutanoate (104a)}\)

Sodium iodide (392 mg, 2.08 mmol, 8.0 equiv.) was added to a solution of \((S)-\text{benzyl 3-[(\text{tert-butyldimethylsilyl})oxy]-4-(tosyloxy)butanoate 103a}\) (125 mg, 0.26 mmol, 1.0 equiv.) in acetone (5 mL). The mixture was stirred and heated at reflux overnight. Saturated aqueous NaHCO\(_3\) (10 mL) was added and the mixture extracted with diethyl ether (5 x 15 mL). The combined organic layers were washed with brine (20 mL), dried over MgSO\(_4\), filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 5:1) afforded as a brownish
yellow oil 104a (88 mg, 78%). $R_f$ 0.58 (2:1 Hex-EtOAc). $[\alpha]_D^{25} +10.01$ (c 1, CHCl$_3$). $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 0.04 (s, 3H), 0.11 (s, 3H), 0.87 (s, 9H), 2.60 (dd, $J = 15.4$, 7.1 Hz, 1H), 2.73 (dd, $J = 15.4$, 4.9 Hz, 1H), 3.21-3.30 (m, 2H), 4.05 (app. pent, $J = 5.5$ Hz, 1H), 5.09 (d, $J = 12.3$ Hz, 1H), 5.14 (d, $J = 12.3$ Hz, 1H), 7.25-7.35 (m, 5H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ -4.9, -4.5, 12.8, 17.9, 25.7, 42.6, 66.5, 68.4, 128.3, 128.4, 128.6, 135.7, 170.7. HRMS (ESI) calcd for C$_{17}$H$_{28}$O$_3$Si (M+H)$^+$ 435.0847, obsd 435.0831.

(S)-Benzyl 3-[(tert-butyldiphenylsilyl)oxy]-4-iodobutanoate (104b)

Sodium iodide (169 mg, 1.13 mmol, 8.0 equiv.) was added to a solution of (S)-benzyl 3-[(tert-butyldiphenylsilyl)oxy]-4-(tosyloxy)butanoate (103b) (85 mg, 0.14 mmol, 1.0 equiv.) in acetone (3.5 mL). The mixture was stirred and heated at reflux overnight. Saturated aqueous NaHCO$_3$ (7 mL) was added and the mixture extracted with diethyl ether (5 x 10 mL). The combined organic layers were washed with brine (15 mL), dried over MgSO$_4$, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 5:1) afforded 104b as a yellow oil (50 mg, 64%). $R_f$ 0.58 (2:1 Hex-EtOAc). $[\alpha]_D^{25} +5.01$ (c 1, CHCl$_3$); $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 1.05 (s, 9H), 2.65 (dd, $J = 15.7$, 6.1 Hz, 1H), 2.75 (dd, $J = 15.7$, 5.9 Hz, 1H), 3.20 (d, $J = 4.6$ Hz, 2H), 3.92-3.98 (m, 1H), 5.65 (d, $J = 12.4$ Hz, 1H), 5.04 (d, $J = 12.4$ Hz, 1H), 7.25-7.45 (m, 10H), 7.62-7.69 (m, 5H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ -0.0, 13.4, 19.3, 26.9, 42.2, 66.4, 68.7, 127.7, 127.8, 128.2, 128.5, 129.9, 130.0, 133.2, 135.8, 135.9, 170.4. HRMS (ESI) calcd for C$_{27}$H$_{32}$O$_3$Si (M+H)$^+$ 559.1160, obsd 559.1153.

Ethyl 2-[(diphenylmethylene)amino]pent-4-enoate (Table 2.1, Entry 1)

Allyl bromide (144 µL, 199.6 mg, 1.65 mmol, 5.0 equiv.) was added dropwise to a mixture of ethylglycinate benzophenone imine (76a) (88.2 mg, 0.330 mmol, 1.0 equiv.), O(9)-
allyl-N-9-anthracenylmethylcinchonidium bromide (86) (20.0 mg, 0.033 mmol, 0.1 equiv.) and CsOH.H₂O (554.2 mg, 3.300 mmol, 10.0 equiv.) in CH₂Cl₂ (3 mL) at -78 °C. The mixture was warmed to -60 °C and stirred vigorously for 24 h. The suspension was diluted with ether (30 mL), washed with water (2 x 10 mL), brine (20 mL), dried over MgSO₄, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 4:1) afforded ethyl 2-[(diphenylmethylene)amino]pent-4-enoate as a yellow colored oil (82 mg, 80%). Rᵣ 0.39 (1:1 Hex-EtOAc). [α]ᵣ²⁵ +9.81 (c 1, CHCl₃); ¹H NMR (CDCl₃, 400 MHz) δ 1.25 (t, J = 7.1 Hz, 3H), 2.61 (dd, J = 14.0, 7.5 Hz, 1H), 2.68 (dd, J = 13.0, 7.0 Hz, 1H), 4.10-4.22 (m, 3H), 5.01 (d, J = 10.8 Hz, 1H), 5.06 (dd, J = 16.5, 1.38 Hz, 1H), 5.63-5.74 (m , 1H), 7.16-7.46 (m, 7H), 7.48-7.57 (m, 3H); ¹³C NMR (CDCl₃, 100 MHz) δ 14.2, 38.1, 60.9, 65.3, 117.5, 127.9, 128.0, 128.3, 128.5, 128.8, 132.4, 134.4, 139.6, 170.5, 171.8. HRMS (ESI) calcd for C₂₀H₂₂NO₂ (M+H)+ 308.1620, obsd 308.1614.

Allyl bromide (74 µL, 102.2 mg, 0.845 mmol, 5.0 equiv.) was added dropwise to a mixture of tert-butylglycinate benzophenone imine (76b) (50.0 mg, 0.170 mmol, 1.0 equiv.), O(9)-allyl-N-9-anthracenylmethylcinchonidium bromide (86) (10.2 mg, 0.017 mmol, 0.1 equiv.) and CsOH.H₂O (283.8 mg, 1.700 mmol, 10.0 equiv.) in CH₂Cl₂ (3 mL) at -78 °C. The mixture was warmed to -60 °C and stirred vigorously for 24 h. The suspension was diluted with ether (30 mL), washed with water (2 x 10 mL), brine (20 mL), dried over MgSO₄, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 4:1) afforded tert-butyl 2-[(diphenylmethylene)amino]pent-4-enoate as a yellow oil (48 mg, 85%, 92% e.e.). The enantiomeric excess was determined by HPLC analysis using Chiralcel OD-H column (5% iPrOH-Hex, 0.5 mL/min); major enantiomer tᵣ = 8.7 min and minor enantiomer tᵣ = 17.7 min;
Detection 254 nm. \( R_f \) 0.40 (1:1 Hex-EtOAc). \([\alpha]_D^{25}\) +8.21 (c 1, CHCl\(_3\)); \(^1\)H NMR (CDCl\(_3\), 400 MHz) \( \delta \) 1.44 (s, 9H), 2.59 (dd, \( J = \)14.0, 7.5 Hz, 1H), 2.67 (dd, \( J = \)14.0, 6.6 Hz, 1H), 4.00 (dd, \( J = \)7.4, 5.4 Hz, 1H), 5.0 (d, \( J = \)10.1 Hz, 1H), 5.06 (d, \( J = \)17.1 Hz, 1H), 5.67-5.78 (m, 1H), 7.16-7.56 (m, 10 H); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \( \delta \) 28.1, 38.2, 65.9, 81.0, 117.2, 128.0, 128.3, 128.4, 128.5, 128.8, 130.1, 130.2, 132.4, 134.8, 136.7, 139.8, 170.1, 170.9. HRMS (ESI) calcd for C\(_{22}\)H\(_{26}\)NO\(_2\) (M+H)\(^+\) 336.1958, obsd 336.1966.

Iodobutane (34 µL, 54.5 mg, 0.296 mmol, 1.2 equiv.) was added dropwise to a mixture of ethylglycinate benzophenone imine (76a) (66.2 mg, 0.250 mmol, 1.0 equiv.), O(9)-allyl-N-9-anthracenylmethylcinchonidium bromide (86) (15.0 mg, 0.025 mmol, 0.1 equiv.) and 50% aqueous KOH (0.5 mL) in toluene (3 mL) at rt. The mixture was stirred vigorously for 24 h and extracted with EtOAc (4 \( \times \) 15 mL). The combined organic layers were washed with brine (20 mL), dried over MgSO\(_4\), filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 4:1) afforded ethyl 2-[(diphenylmethylene)amino]hexanoate as a yellow oil (40 mg, 50%). \( R_f \) 0.43 (2:1 Hex-EtOAc). \([\alpha]_D^{25}\) +20.50 (c 1, CHCl\(_3\)); \(^1\)H NMR (CDCl\(_3\), 400 MHz) \( \delta \) 0.83 (t, \( J = \)7.4 Hz, 3H), 1.17-136 (m, 7H), 1.89 (dd, \( J = \)15.0, 7.5 Hz, 2H), 4.04 (t, \( J = \)6.6 Hz, 1H), 4.16 (t, \( J = \)7.0 Hz, 2H), 7.25-7.61 (m, 6H), 7.79-7.81 (m, 4H); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \( \delta \) 13.8, 14.2, 19.3, 28.3, 32.9, 66.9, 127.9, 128.0, 128.3, 128.5, 128.8, 130.0, 130.2, 132.4, 137.6, 169.9, 170.2. HRMS (ESI) calcd for C\(_{21}\)H\(_{26}\)NO\(_2\) (M+H)\(^+\) 324.1960, obsd 324.1953.

![Ethyl 2-[(diphenylmethylene)amino]hexanoate (Table 2.1, Entry 3)](image)

![tert-Butyl 2-[(diphenylmethylene)amino]hexanoate (Table 2.1, Entry 4)](image)
Iodobutane (97 µL, 155.5 mg, 0.845 mmol, 5.0 equiv.) was added dropwise to a mixture of tert-butylglycinate benzophenone imine (76b) (50.0 mg, 0.170 mmol, 1.0 equiv.), O(9)-allyl-N-9-anthracenylmethylcinchonidium bromide (86) (10.2 mg, 0.017 mmol, 0.1 equiv.) and CsOH·H₂O (283.8 mg, 1.700 mmol, 10.0 equiv.) in CH₂Cl₂ (3 mL) at -78 °C. The mixture was warmed to -60 °C and stirred vigorously for 24 h. The suspension was diluted with ether (30 mL), washed with water (2 x 10 mL), brine (20 mL), dried over MgSO₄, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 4:1) afforded tert-butyl 2-[(diphenylmethylene)amino]hexanoate as a yellow oil (38 mg, 51%). R₉ 0.44 (1:1 Hex-EtOAc). [α]₀²⁵ +15.01 (c 1, CHCl₃); ¹H NMR (CDCl₃, 400 MHz) δ 0.92 (t, J = 7.4 Hz, 3H), 1.37-1.43 (m, 2H), 1.46 (s, 9H), 1.80 (pent, J = 7.1 Hz, 2H), 3.19 (t, J = 7.0 Hz, 2H), 4.12 (s, 1H), 7.17-7.41 (m, 10H); ¹³C NMR (CDCl₃, 100 MHz) δ 6.8, 12.9, 23.6, 28.1, 35.5, 56.4, 81.0, 127.7, 128.0, 128.6, 128.7, 130.3, 136.2, 139.4, 169.8, 171.4. HRMS (ESI) calcd for C₂₃H₃₁NO₂ (M+H)⁺ 352.2271, obsd 352.2282.

(S,E)-6-tert-butyl-1-methyl-5-[(diphenylmethylene)amino]hex-2-enedioate (108)

(E)-Methyl-4-bromobut-2-enoate (107) (151 mg, 0.845 mmol, 5.0 equiv.) was added dropwise to a mixture of tert-butylglycinate benzophenone imine (76b) (50 mg, 0.170 mmol, 1.0 equiv.), O(9)-allyl-N-9-anthracenylmethylcinchonidium bromide (86) (11 mg, 0.017 mmol, 0.1 equiv.) and CsOH·H₂O (2834 mg, 1.700 mmol, 10.0 equiv.) in CH₂Cl₂ (5 mL) at -78 °C. The mixture was warmed to -60 °C and stirred vigorously for 24 h. The suspension was diluted with ether (30 mL), washed with water (2 x 10 mL), brine (20 mL), dried over MgSO₄, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 5:1) afforded compound 108 as a yellow oil (60 mg, 89%). R₉ 0.30 (4:1 Hex-EtOAc). [α]₀²⁵ +39.24 (c 1, CHCl₃); ¹H NMR (CDCl₃, 400 MHz) δ 1.46 (s, 9H), 2.59 (dd, J =14.0, 7.5 Hz, 1H), 2.67 (dd, J = 14.0, 6.6 Hz, 1H), 3.73 (s,
3H), 4.00 (dd, J = 7.4, 5.4 Hz, 1H), 5.0 (d, J = 10.1 Hz, 1H), 5.06 (d, J = 17.1 Hz, 1H), 5.67-5.78 (m, 1H), 7.16-7.56 (m, 10 H); $^1$H NMR (CDCl$_3$, 100 MHz) δ 28.1, 38.2, 65.9, 81.0, 117.2, 128.0, 128.3, 128.4, 128.5, 128.8, 130.1, 130.2, 132.4, 134.8, 136.7, 139.8, 168.8, 170.1, 170.9.

HRMS (ESI) calcd for C$_{24}$H$_{28}$NO$_4$ (M+H)$^+$ 394.2610, obsd 394.2614.
2.8.3 Spectra

Compound 92 (Scheme 2.11) - $^1$H NMR spectrum

SY-01-(S)-dibenzyl-2-hydroxysuccinate in CDCl3 at 400 MHz
Compound 92 (Scheme 2.11) - $^{13}$C NMR spectrum

SY-01-(S)-dibenzy1-2-hydroxysuccinate in CDCl3 at 100 MHz
Compound 93 (Scheme 2.11) - $^1$H NMR spectrum

SY-01-084 in CDCl$_3$ at 400 MHz
Compound 93 (Scheme 2.11) - $^{13}$C NMR spectrum

SY-01-084 in CDCl$_3$ at 100 MHz
Compound 94 (Scheme 2.13) - $^1$H NMR spectrum

SY-01-85 in CDCl$_3$ at 400 MHz

\[
\text{BnOOC} \xrightarrow{\text{O}} \text{OTs}
\]

\[
\begin{array}{c}
\text{2.06} \\
\text{7.00} \\
\text{2.28} \\
\text{1.21} \\
\text{2.49} \\
\text{2.42} \\
\text{3.83} \\
\text{1.58}
\end{array}
\]

ppm
Compound 94 (Scheme 2.13) - $^{13}$C NMR spectrum

SY–01–85 in CDCl3 at 100 MHz
Compound 102 (Scheme 2.13) - $^1$H NMR spectrum

SY-01-137 in CDCl$_3$ at 400 MHz
Compound 102 (Scheme 2.13) - $^{13}$C NMR spectrum

SY-01-137 in CDCl$_3$ at 100 MHz
Compound 103a (Scheme 2.13) - $^1$H NMR spectrum

SY-01-103 in CDCl3 at 400 MHz
Compound **103a** (Scheme 2.13) - $^{13}$C NMR spectrum

SY-01-103 in CDCl₃ at 100 MHz

![Carbon-13 NMR spectrum of compound 103a](image-url)
Compound 103b (Scheme 2.13) – $^1$H NMR spectrum

SY-01-91 in CDCl$_3$ at 400 MHz
Compound 103b (Scheme 2.13) - $^{13}$C NMR spectrum

SY-01-91 in CDCl$_3$ at 100 MHz
Compound 104a (Scheme 2.13) – $^1$H NMR spectrum

SY-01-104 in CDCl3 at 400 MHz

BnOOC
\[\text{OTBS}\]
Compound **104a** (Scheme 2.13) - $^{13}$C NMR spectrum

SY-01-104 in CDCl$_3$ at 100 MHz

```
BnOOC

OTBS
```
Compound 104b (Scheme 2.13) – $^1$H NMR spectrum

SY-01-96 in CDCl$_3$ at 400 MHz
Compound 104b (Scheme 2.13) – $^{13}$C NMR spectrum
Table 2.1, Entry 1 – $^1$H NMR spectrum

SY-01-126 in CDCl3 at 400 MHz
Table 2.1, Entry 1 $^{13}$C NMR spectrum

SY-01-126 in CDCl$_3$ at 100 MHz
Table 2.1, Entry 2 – $^1$H NMR spectrum

SY–01–125 in CDCl$_3$ at 400 MHz

Ph$_2$N–COO'Bu

8.0  7.5  7.0  6.5  6.0  5.5  5.0  4.5  4.0  3.5  3.0  2.5  2.0  1.5  1.0  0.5  ppm
Table 2.1, Entry 2 – $^{13}$C NMR spectrum

SY-01-125 in CDCl$_3$ at 100 MHz
Table 2.1, Entry 3 – $^1$H NMR spectrum

SY-01-112 in CDCl$_3$ at 400MHz

![NMR spectrum graph](image-url)
Table 2.1, Entry 3 – $^{13}$C NMR spectrum

SY-01-112 in CDCl3 at 100 MHz
Table 2.1, Entry 4 – $^1$H NMR spectrum

SY-01-118 in CDCl3 at 400MHz
Table 2.1, Entry 4 – $^{13}$C NMR spectrum

SY-01-118 in CDCl₃ at 100 MHz
Compound 108 (Scheme 2.14) – $^1$H NMR spectrum

SY-01-147 in CDCl$_3$ at 400 MHz

[Diagram of Compound 108 with NMR spectrum]
Compound 108 (Scheme 2.14) – $^{13}$C NMR spectrum

SY-01-147 in CDCl$_3$ at 100 MHz
CHAPTER 3: AN AMINE CONJUGATE ADDITION APPROACH TO AHAD

3.1 Conjugate Addition Reactions to Afford β-Amino Acids

Conjugate addition reactions are well known in organic chemistry. Most familiar is the conjugate addition of organometallic reagents to α,β-unsaturated carbonyl compounds for assembling structurally complex organic molecules.\textsuperscript{49} For example, addition of the functionalized organocuprate reagent obtained from iodide 110 to the chiral cyclopentenone 111 occurred in a \textit{trans}-selective fashion to give intermediate compound 112 (Scheme 3.1) which was used for the synthesis of prostaglandin $E_2$.\textsuperscript{50,51,52}

\[
\begin{align*}
1. & \text{BuLi, Et}_{2}O, -95 \, ^\circ\text{C} \\
2. & \text{Cu, } ^{11} \text{Bu}_{2}P, \text{ THF, } -78 \, ^\circ\text{C} \\
3. & \text{O} \\
\end{align*}
\]

Scheme 3.1 Preparation of intermediate for the synthesis of prostaglandin $E_2$ through conjugate addition.

Over the last two decades, there has been an increasing interest in functionalized β-amino acids and their derivatives\textsuperscript{53} due to their biologically important properties and their occurrence in natural products (Figure 3.1). β-Alanine (113) is a β-amino acid that occurs in the western hemisphere of theonellamide C (1) (Figure 1.1, Chapter 1). (3\textit{R})-β-Leucine\textsuperscript{54} (114) exists in equilibrium with (2\textit{S})-α-leucine and their interconversion is catalyzed by leucine-2,3-aminomutase.\textsuperscript{55} β-Arginine is found in the natural dipeptide antibiotic TAN 1057 (115).\textsuperscript{56} \textit{N}-Acetyl-β-phenylalanine (116) is a substrate for a bacterial tRNA(Phe) peptidyltransferase.\textsuperscript{57} β-Amino-α-phenylpropionic acid is present in the antibiotic betacine (117) which displays a broad spectrum of antibacterial activity\textsuperscript{58} and its derivatives show important neurophysiological control properties.\textsuperscript{59}
Figure 3.1 β-Amino acids (highlighted in red where they are part of a natural product).

A potentially straightforward method for the synthesis of β-amino acids is the conjugate addition of nitrogen nucleophiles to α,β-unsaturated carboxylic acid derivatives (Scheme 3.2).

![Scheme 3.2 General retrosynthesis of conjugate amine addition.](image)

This reaction is also known as an aza-Michael reaction wherein there is 1,4-addition of a nitrogen nucleophile to the β-carbon vinlogously attached to an electron-withdrawing group.60 There are two major approaches to achieve asymmetric induction: by using chiral auxiliaries, typically amines (Scheme 3.3-A), or enantiomerically pure starting materials, e.g. a chiral acceptor (Scheme 3.3-B)

![Scheme 3.3 Examples of the aza-Michael reaction.](image)
The first example of an enantioselective conjugate amine addition was reported by Jørgensen in 1996. They used a titanium BINOL catalyst 125 to catalyze the addition of benzyloxyamine (BnONH$_2$) to N-acloyxazolidinone 123. N-Substituted hydroxylamines are more nucleophilic than O-substituted hydroxylamines which are useful precursors for β-amino acids. High conversions were obtained, but enantioselectivity was moderate (up to 42% e.e.) (Scheme 3.4)

Scheme 3.4 Enantioselective conjugate amine addition using BINOL catalyst.

Sibi and co-workers have made major contributions to the field of amine conjugate additions. They developed a highly enantioselective protocol for the conjugate addition of O-benzylhydroxylamine to 3,5-dimethylpyrazole-derived enamide 126 using catalytic amounts of a chiral Lewis acid prepared from magnesium bromide diethyl etherate and a bisoxazoline 129. β-Amino acid derivatives, e.g., 127, were synthesized in good chemical yields and high e.e. The high e.e.'s obtained were accounted by use of Lewis acid along with catalyst (Scheme 3.5).

Scheme 3.5 Conjugate addition using a chiral catalyst and Lewis acid.

The use of azide as a nucleophile in conjugate addition reactions is also well known. Jacobsen et al. demonstrated the conjugate addition of hydrazoic acid (HN$_3$) to α,β-unsaturated imides (e.g. 130) using a chiral aluminium(III)salicylaldimine complex catalyst 132 (Scheme 3.6).
Scheme 3.6 Conjugate addition using azide as nucleophile.

Miller and co-workers developed milder reaction conditions for azidation under metal-free conditions, in which the azide was generated from a mixture of trimethylsilyl azide (TMSN₃) and pivalic acid in toluene.66,66b, 67 β-Amino acid derivative 134 was obtained in 92% e.e. by conjugate addition of azide to enamide 133 mediated by β-turn tripeptide 135 (Scheme 3.7).

Scheme 3.7 Preparation of β-azido derivative.

3.2 MacMillan Conjugate Addition

MacMillan and coworkers have developed organocatalysts to achieve a highly chemo- and stereoselective amine conjugate addition that is founded upon a rationally designed N-centered nucleophile, as depicted in Scheme 3.8.68,69,70

Scheme 3.8 Iminium catalyzed amination requires selective amine participation.

They showed that the rate determining step for conjugate addition reactions involving carbon and hydrido nucleophiles is the reversible formation of iminium ions from chiral amine
catalysts and α,β-unsaturated aldehydes. Moreover, the MacMillan Group illustrated a design plan for the enantioselective conjugate amination using iminium catalysis.

In their design, they stipulated that the amine required to function as a 1,4-addition nucleophile must not participate in iminium activation; this presents a racemic pathway via TS-II (Scheme 3.8). Moreover, the catalyst, a chiral secondary amine, must perform as an iminium catalyst (giving rise to TS-I) but not compete as a nucleophile. The reaction should be kinetically controlled, whereby irreversible loss of the nucleophile’s proton (Nu-H) must accompany the stereodefining heteroatom addition step. More importantly, this deprotonation step removes the possibility of an equilibrium-controlled process involving a reversible N-addition step, a thermodynamic pathway that would demand the formation of product mixture. N-Silyloxycarbamates were deemed to be the best candidates for these requirements and act as the nucleophilic component on the basis that the N−O functionality enhances nucleophilicity at the nitrogen center via the α-effect (Figure 3.2)\(^7\) (estimated pK\(_a\) of NH in a silyloxycarbamate is 9.0 whereas pK\(_a\) of regular carbamate is nearly 13).

![Figure 3.2 Carbamate nucleophilicity enhanced by the α-effect.](image)

They successfully applied the organocatalytic amine addition to various substrates. For example, exposure of 2-hexenal 137 to the asymmetric amination conditions followed by oxidation provided the corresponding β-amino acid 138 with excellent enantioselectivity. The cleavage of the N-O bond was accomplished under mildly reducing conditions (Zn/AcOH) (Scheme 3.9).\(^7\)
Scheme 3.9 Two-step synthesis of a β-amino acid.

They also showed that the amino-oxy moiety can be strategically exploited to generate 1,3-amino alcohols (Scheme 3.10). Hexenal amination was followed by in situ Wittig homologation to afford the unsaturated ester 143 in a single step (Scheme 3.10).

Scheme 3.10 Enantioselective synthesis of a 1,3-amino alcohol.

The amino enoate 143 was exposed to a fluoride ion source which then enabled both silyl group removal and intramolecular oxy-Michael addition to afford isoxazolidine 144 with excellent yield and diastereosecontrol (99% yield, 10:1 syn/anti). The N–O bond was reduced with samarium diiodide (SmI₂) to afford the 1,3-amino alcohol 145 in high yield (70% yield over three steps).³³

3.3 Application of the MacMillan Strategy to Ahad

According to Scheme 3.11, a new retrosynthesis was designed for Ahad, based on MacMillan’s enantioselective organocatalytic amine conjugate addition. The γ-hydroxy group would be installed intramolecularly; exposure of 147 to fluoride ion would generate an oxyanion that would add in a conjugate fashion to the α,β-unsaturated ester. Wittig homologation of aldehyde 148 would afford the α,β-unsaturated ester 147. Conjugate addition of Cbz-NHOTBS (136) to a chiral iminium ion generated from aldehyde 149 would occur diastereoselectively to
generate the first stereocenter of Ahad. The stereocenter of the chiral auxiliary/catalyst is only transient.

Scheme 3.11 Second generation retrosynthesis of Ahad.

The benzyl tert-butyldimethylsilyloxy carbamate 136a and benzyl tert-butyldiphenylsilyloxy carbamate 136b were prepared, according to the literature,70 from commercially available benzyl hydroxycarbamate (150) (Scheme 3.12).

Scheme 3.12 Synthesis of silyloxy carbamate nucleophiles.

α,β-Unsaturated aldehyde 149 was synthesized according to Scheme 3.13. Diethyl(benzyloxycarbonylmethyl)phosphonate 152 was prepared by hydrolysis of the carboxylate ester of triethylphosphonoacetate (151) and conversion to the benzyl ester. Compound 154 was prepared via a Horner-Emmons reaction in a biphasic reaction mixture of cyclohexane and an aqueous solution of glyoxal dimethyl acetal (153).74,75 Hydrolysis of the dimethyl acetal in compound 154 was attempted with various reagents: 10 mole percent p-toluenesulfonic acid in acetone,74,75 10 mole percent iodine in acetone76 and 25 percent aqueous acetic acid.77 The best result was obtained with HCl in acetone78 which led to the formation of aldehyde 149 (Scheme 3.13).
In order to develop some experience of the enantioselective organocatalytic amine conjugate addition, we repeated a known example from the literature. We investigated the exposure of crotonaldehyde (155) to benzyl tert-butyldimethylsilyloxycarbamate (136a) in the presence of the (R,R)-imidazolidinone catalyst 140 with p-toluenesulfonic acid (Scheme 3.14). This provided the β-amino aldehyde product 156 that was subjected to Wittig homologation to afford the unsaturated ester 157. Although we recognized that we would need the (S,S)-catalyst to give the (2S,4R) stereochemistry of Ahad, we began investigations with the (R,R)-catalyst 140 because it was on-hand.

Scheme 3.14 Enantioselective organocatalytic conjugate amination.

MacMillan’s 2006 communication stated that variation in the carbamate (Boc, Cbz, Fmoc) and trialkysilyl (TBS, TBDPS) groups is possible. Therefore, we tried the conjugate addition of benzyl tert-butyldimethylsilyloxycarbamate 136a to aldehyde 149 under their standard reactions conditions. Unfortunately, no product 148a was isolated (Scheme 3.15).
Scheme 3.15 Attempted synthesis of β-amino aldehyde 148a.

While the scope for variation in reaction components was emphasized in their paper, we note with interest that the conjugate addition of tert-butyl-(tert-butyldiphenylsilyloxy)carbamate (141b) with methyl 3-formoylacrylate (158) was reported in the presence of a stronger acid co-catalyst (trifluoroacetic acid, TFA) (Scheme 3.16). A more hindered/stable silyloxycarbamate and elevated reaction temperature afforded compound 159. This example is particularly relevant to our similarly electron-deficient electrophile.

Scheme 3.16 Conjugate amine addition to electron deficient electrophile.

Therefore, we switched from the tert-butyldimethylsilyl group (TBS), to the tert-butyldiphenylsilyl (TBDPS) protecting group for our hydroxylamine nucleophile. We presume that the TBS group is less stable than the TBDPS protecting group in the presence of a strong acid (TFA). The conjugate addition of compound 136b to compound 149 was performed using the TFA salt of (R,R) chiral amine catalyst 140 (Scheme 3.17). This afforded compound 148b in 31% yield. These addition reactions were impossible to follow by TLC because the intermediate iminium species was hydrolyzed on silica gel but progress can be monitored by $^1$H NMR (Figure 3.3). The product of conjugate addition showed the disappearance of olefinic H1 and H2 and appearance of Hα and Hβ on sp$^3$ C. Wittig reaction of aldehyde 148b with methyl-(triphenylphosphoranylidene)-acetate (142) afforded unsaturated ester 147b (Scheme 3.17).
In terms of the mechanism of the reaction, the lone pair on the nitrogen attacks the carbonyl carbon of the aldehyde to form the complex 160. Protonation of the aminal facilitates the elimination of water. The intermediate iminium ion species 162 is planar and the amine nucleophile will come from the opposite face of the tBu and Bn groups flanking the iminium ion nitrogen to give the desired product after hydrolysis (Scheme 3.18).
Scheme 3.18 Mechanism of stereoselective conjugate addition.

Treatment of compound 147b with tetra-n-butylammonium fluoride (TBAF) in the presence of acetic acid removed the TBDPS ether but did not give the cyclized product (2R,4S)-163 (Scheme 3.19). The low yield of the conjugate addition product and subsequent difficulties in achieving the intramolecular oxy-Michael addition to afford the isoxazolidine led us to investigate other organocatalysts which are commercially available and may meet our requirements.

Scheme 3.19 Attempted synthesis of isoxazolidine.

In 2007, Cordova et al.\textsuperscript{79} used a chiral pyrrolidine-based catalyst 165 for these types of conjugate addition reactions. They reported that simple proline-derived compounds catalyze highly enantioselective conjugate additions of protected N-silyloxycarbamates to α,β-unsaturated aldehydes. The reaction gives access to the β-amino aldehydes in good yields and 82–99% e.e. Reactions were conveniently performed at room temperature (Scheme 3.20).

Scheme 3.20 Enantioselective conjugate amine addition using a pyrrolidine-based catalyst.
Therefore, we investigated the conjugate addition of CbzNHOTBS \(136a\) to \(\alpha,\beta\)-unsaturated aldehyde \(149\) with catalyst \(165\). We obtained our first desired stereocenter as \(2S\). This catalyst gave us a better yield of the conjugate addition product \(148a\) compared with the MacMillan catalyst \(140\). Compound \(148a\) was then subjected to a Wittig reaction to obtain \(147a\) (Scheme 3.21).

![Scheme 3.21 Synthesis of \(\alpha,\beta\)-unsaturated ester \(147a\).](image)

Treatment of compound \(147a\) with fluoride ion facilitated silyl group removal with concomitant, intramolecular oxy-Michael addition to afford isooxazolidine \((2S,4R)-163\). Initially, we attempted the reaction with 1.5 equivalents each of tetrabutylammonium fluoride solution and acetic acid, but no product was observed. Switching to tetrabutylammonium fluoride solution containing 5 percent water gave a 65% yield of the product. Compound \((2S,4R)-163\) was then subjected to hydrogenolysis\(^{80}\) to afford unprotected Ahad \(167\) (Scheme 3.22).

![Scheme 3.22 Synthesis of unprotected Ahad.](image)

The next challenge was to find the right combination of protecting groups for Ahad that would be compatible with the synthesis of the western hemisphere of theonellamide C. Chapter 4 will address this issue of protecting group strategy and will reveal that the \(\delta\)-COOMe, introduced in the Wittig reaction, was incompatible with other protecting groups on Ahad.
3.4 Experimental Section

3.4.1 General Methods

The general methods are as described in Chapter 2.

3.4.2 Procedures

Benzyl (tert-butyldimethylsilyl)oxycarbamate (136a)

Triethylamine (249 µL, 181 mg, 1.79 mmol, 1.2 equiv.) was added to a solution of benzyl-N-hydroxycarbamate (250 mg, 1.49 mmol, 1.0 equiv.) in dry CH₂Cl₂ (5 mL) under N₂. The mixture was cooled to 0 °C and TBSCI (270 mg, 1.79 mmol, 1.2 equiv.) was added. The reaction mixture was warmed to rt and stirred overnight. The mixture was washed with water (5 mL) and brine (5 mL) and the organic layer was dried over MgSO₄, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 4:1→1:2) afforded 136a as a clear oil (321 mg, 77%).

\[ R_f 0.66 \ (2:1 \ \text{Hex}-\text{EtOAc}) \]

\[ ^{1}H \ \text{NMR (CDCl}_3, 400 \ \text{MHz}) \delta 0.15 \ (s, 6H), 0.94 \ (s, 9H), 5.16 \ (s, 2H), 7.02 \ (s, 1H), 7.30-7.35 \ (m, 4H); \]

\[ ^{13}C \ \text{NMR (CDCl}_3, 100 \ \text{MHz}) \delta -5.8, 18.0, 25.9, 67.6, 128.3, 128.4, 128.5, 135.7, 158.6. \]

HRMS (ESI) calcd for C₁₄H₂₄NO₃Si (M+H)+ 282.1520, obsd 282.1516.

Benzyl tert-butyldiphenylsilyloxycarbamate (136b)

Triethylamine (0.83 mL, 0.605 g, 5.98 mmol, 2.0 equiv.) was added to a solution of benzyl-N-hydroxycarbamate (0.500 g, 2.99 mmol, 1.0 equiv.) in dry CH₂Cl₂ (15 mL) under N₂. The mixture was cooled to 0 °C and TBDPSCI (1.55 mL, 1.644 g, 5.98 mmol, 2.0 equiv.) was added. The reaction mixture was warmed to rt and stirred overnight. The mixture was washed with water (30 mL) and brine (25 mL) and the organic layer was dried over MgSO₄, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 8:1→5:1) afforded as 136b a
clear oil (910 mg, 75%). \( R_f \) 0.39 (2:1 Hex-EtOAc). \( ^1H \) NMR (CDCl\textsubscript{3}, 400 MHz) \( \delta \) 1.12 (s, 9H), 5.07 (s, 2H), 6.97 (s, 1H), 7.24-7.45 (m, 11H), 7.70-7.73 (m, 4H); \( ^{13}C \) NMR (CDCl\textsubscript{3}, 100 MHz) \( \delta \) 19.1, 26.9, 67.6, 127.7, 127.9, 128.4, 128.6, 129.7, 130.3, 131.7, 134.9, 135.6, 135.8, 157.9. HRMS (ESI) calcd for C\textsubscript{24}H\textsubscript{27}N\textsubscript{4}O\textsubscript{5}Si (M+Na\textsuperscript{+}) \( 428.1652 \), obsd 428.1643.

Benzyl 2-(diethoxyphosphoryl)acetate (152)

Triethylphosphonoacetate (151) (500 µL, 560 mg, 2.49 mmol, 1.00 equiv.) was dissolved in 1M NaOH (1 mL, 2.49 mmol, 1.0 equiv.) and stirred at 60 °C overnight. The ethanol formed in the reaction was evaporated and aqueous layer was acidified to pH 2 with 10% KHSO\textsubscript{4}. The aqueous layer was extracted with CH\textsubscript{2}Cl\textsubscript{2} (4 x 15 mL) and dried over MgSO\textsubscript{4}, filtered and concentrated to give the carboxylic acid (373 mg) which was dissolved in dry MeOH (5 mL). Cesium carbonate (310 mg, 0.95 mmol, 0.50 equiv.) was added and the mixture stirred for 2 h under N\textsubscript{2}. The mixture was concentrated, dissolved in DMF (5 mL) and cooled to 0 °C. Benzyl bromide (274 µL, 391 mg, 2.28 mmol, 1.20 equiv.) was added, the mixture warmed to rt, stirred overnight, diluted with EtOAc (10 mL), washed with water (15 mL) and brine (15 mL). The organic layer was dried over MgSO\textsubscript{4}, filtered and concentrated. Flash chromatography on silica gel (EtOAc-Hex 1:1→2:1) afforded 152 as a colorless oil (326 mg, 60%). \( R_f \) 0.53 (2:1 EtOAc-Hex). \( ^1H \) NMR (CDCl\textsubscript{3}, 400 MHz) \( \delta \) 1.29 (t, \( J = 7.1 \) Hz, 6H), 2.99 (d, \( ^3J_{\text{H-P}} = 21.5 \) Hz, 2H), 4.09-4.16 (m, 4H), 5.18 (s, 2H), 7.30-7.40 (m, 5H); \( ^{13}C \) NMR (CDCl\textsubscript{3}, 100 MHz) \( \delta \) 16.3 (\( ^3J = 16.3 \) Hz), 34.4 (\( ^1J_{\text{C-P}} = 134.2 \) Hz), 62.7 (\( ^2J = 6.3 \) Hz), 67.3, 128.3, 128.4, 128.5, 135.4, 165.6. HRMS (ESI, -ve) calcd for C\textsubscript{13}H\textsubscript{18}O\textsubscript{5}P (M-\textsuperscript{H}) * 285.0897, obsd 285.0897.

(2E)-Benzyl 4,4-dimethoxybut-2-enoate (154)

Cesium carbonate (2.20 g, 6.75 mmol, 1.5 equiv.) was added to a solution of benzyl 2-(diethoxyphosphoryl)acetate 152 (1.28 g, 4.5 mmol, 1.0 equiv.) in cyclohexane (20 mL). The
suspension was heated to 65 °C and then 2,2-dimethoxyacetaldehyde (153) (15.6 mL of a 60 wt.% solution in H₂O, 937 mg, 9.0 mmol, 2.0 equiv.) was added. The biphasic mixture became clear pale yellow and stirred for 2 h at 65 °C. Saturated aqueous NH₄Cl (25 mL) was added and the mixture was extracted with EtOAc (4 x 25 mL). The combined organic layers were washed with brine (25 mL), dried over MgSO₄, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 2:1) afforded compound 154 as a colorless oil (947 mg, 89%). Rf 0.56 (1:1 Hex-EtOAc). ¹H NMR (CDCl₃, 400 MHz) δ 3.32 (s, 6H), 4.94 (d, J = 3.0 Hz, 1H), 5.19 (s, 2H), 6.19 (d, J = 15.9 Hz, 1H), 6.81 (dd, J = 15.8, 3.9 Hz, 1H), 7.30-7.37 (m, 5H); ¹³C NMR (CDCl₃, 100 MHz) δ 52.8, 66.4, 100.5, 124.2, 128.3, 128.6, 135.8, 143.2, 165.6. HRMS (ESI) calcd for C₁₃H₁₇O₄ (M+H)+ 237.1160, obsd 237.1153.

(2E)-Benzyl 4-oxobut-2-enoate (149)

A solution of (2E)-benzyl 4,4-dimethoxybut-2-enoate 154 (150 mg, 0.635 mmol) in acetone (30 mL) was treated with 2M HCl (0.3 mL) and stirred at rt for 2 d. The acetone was evaporated and residue was treated with water (5 mL) and extracted with CHCl₃ (3 x 15 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 2:1) afforded aldehyde 149 as a colorless oil (90 mg, 75%). Rf 0.46 (1:1 Hex-EtOAc). ¹H NMR (CDCl₃, 400 MHz) δ 5.26 (s, 2H), 6.74 (d, J = 15.9 Hz, 1H), 6.98 (dd, J = 15.9, 7.6 Hz, 1H), 7.32-7.39 (m, 5H), 9.72 (d, J = 7.6 Hz, 1H); ¹³C NMR (CDCl₃, 100 MHz) δ 67.5, 128.5, 128.7, 135.0, 140.0, 140.6, 164.7, 192.43. HRMS (ESI) calcd for C₁₁H₁₁O₃ (M+H)+ 191.0712, obsd 191.0719.

(R)-Benzyl-2-[(benzyloxy carbonyl)(tert-butyldiphenylsilyloxy)amino]-4-oxobutanoate (148b)
A solution of \((2R,5R)-2\text{-tert\text{-}butyl}-5\text{-benzyl}\text{-}3\text{-methylimidazolidine}\text{-}4\text{-one} (140)\) (12.8 mg, 0.052 mmol, 0.2 equiv.) in CHCl\(_3\) (0.75 mL) was treated with trifluoroacetic acid (4.0 µL, 5.9 mg, 0.052 mmol, 0.2 equiv.) and stirred for 10 min at 4 °C. The aldehyde 149 (50.0 mg, 0.260 mmol, 1.0 equiv.) was added, followed by the addition of the silyloxy carbamate nucleophile 136b (105.0 mg, 0.26 mmol, 1.0 equiv.) in one portion, stirred for 48 h at 4 °C and the reaction mixture was then filtered through a plug of silica, eluted with Et\(_2\)O then concentrated. Purification by silica gel chromatography (Hex-EtOAc 5:1) provided compound 148b as a clear oil (35 mg, 31%). \(R\) \(\text{f} \) 0.32 (4:1 Hex-EtOAc). \([\alpha]_D^{25} +32.72\) (c 0.8, CHCl\(_3\)); \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta 1.04\) (s, 9H), 2.50 (dd, \(J = 17.9, 5.2\) Hz, 1H), 3.11 (ddd, \(J = 17.9, 8.0, 1.1\) Hz, 1H), 4.80 (d, \(J = 12.0\) Hz, 1H), 4.88 (d, \(J = 12.0\) Hz, 1H), 4.98 (d, \(J = 12.4\) Hz, 1H), 5.02-5.05 (m, 2H) 7.05 (dd, \(J = 7.8, 1.4\) Hz, 2H), 7.22-7.42 (m, 14H), 7.57 (d, \(J = 7.5\) Hz, 4H), 9.42 (s, 1H); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta 19.5, 27.0, 42.8, 60.3, 67.3, 68.3, 127.5, 127.6, 128.1, 128.2, 128.3, 128.4, 128.5, 128.7, 130.1, 130.2, 131.7, 132.0, 135.1, 135.2, 136.3, 158.7, 168.9, 197.9. HRMS (ESI) calcd for C\(_{35}\)H\(_{38}\)N\(_2\)O\(_6\)Si (M+H)\(^+\) 596.2712, obsd 596.2719.

A solution of \((R\)-benzyl 2-[(benzyloxy carbonyl)(\text{tert\text{-}butyldiphenylsilyloxy})amino]hex-2-enedioate (147b)\)

A solution of \((R\)-benzyl 2-[(benzyloxy carbonyl)(\text{tert\text{-}butyldiphenylsilyloxy})amino]4-oxobutanoate 148b\) (20.0 mg, 0.034 mmol, 1.0 equiv.) in chloroform (1 mL) was treated with methyl (triphenylphosphorylidine)acetate (142) (16.8 mg, 0.050 mmol, 1.5 equiv.) at 0 °C and allowed to warm to rt. The reaction mixture was stirred for 20 h, concentrated to a viscous oil, and the triphenylphosphine oxide byproduct was precipitated by the addition of Et\(_2\)O. The solid was removed by filtration and the filtrate concentrated. Flash chromatography on silica gel (Hex-EtOAc 8:1→4:1) afforded an \(\alpha,\beta\)-unsaturated ester 147b as a colorless oil (18 mg, 82%). \(R\) \(\text{f} \) 0.31 (4:1 Hex-EtOAc). \([\alpha]_D^{25} +38.12\) (c 0.6, CHCl\(_3\)); \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta 1.01\) (s, 9H), 2.68-2.84 (m, 2H), 3.69 (s, 3H), 4.59 (dd, \(J = 8.4, 6.3\) Hz, 1H), 4.71 (d, \(J = 12.0\) Hz, 1H), 4.82
(d, $J = 12.0$ Hz, 1H), 4.86 (d, $J = 12.4$ Hz, 1H), 5.04 (d, $J = 12.4$ Hz, 1H), 5.65 (d, $J = 15.7$ Hz, 1H), 6.64 (dt, $J = 22.8$, 7.2 Hz, 1H), 7.02 (d, $J = 6.6$ Hz, 2H), 7.12-7.42 (m, 15H), 7.56 (t, $J = 8.0$ Hz, 3H); $^{13}$C NMR (CDCl$_3$, 100 MHz) δ 19.5, 26.9, 31.4, 51.4, 64.3, 67.1, 68.2, 123.5, 127.4, 127.5, 128.2, 128.3, 128.5, 128.7, 129.9, 130.1, 131.8, 132.3, 135.2, 135.3, 136.2, 136.3, 144.1, 158.9, 166.4, 168.9. HRMS (ESI) calcd for C$_{38}$H$_{42}$N$_7$Si (M+H)$^+$ 652.2725, obsd 652.2712.

$\text{(S)}$-benzyl-2-[[{\text{benzyloxy} carbonyl}][\text{(tert-butyldimethylsilyl)}oxy]amino]-4-oxobutanoate ($148a$)

A solution of benzyl (tert-butyldimethylsilyl)oxycarbamate $136a$ (207.0 mg, 0.736 mmol, 1.4 equiv.) in chloroform (1 mL) was treated with (S)-2-{diphenyl[(trimethylsilyl)oxy]methyl}pyrrolidine ($165$) (34.0 mg, 0.105 mmol, 0.2 equiv) and acetic acid (9 µL, 9.4 mg, 0.105 mmol, 0.2 equiv.) and stirred for 10 min at rt. Benzyl 4-oxo-buty-2-enoate ($149$) (100.0 mg, 0.525 mmol, 1.0 equiv.) was added and the reaction mixture was stirred for 3.5 h at rt, concentrated and the residue was subjected to silica gel chromatography (Hex-EtOAc 5:1) and provided compound $148a$ as a colorless oil (239 mg, 69%). $R_f$ 0.30 (2:1 Hex-EtOAc). $[\alpha]_D^{25}$ -13.5 (c 0.9, CHCl$_3$). $^1$H NMR (CDCl$_3$, 400 MHz) δ 0.08 (s, 3H), 0.12 (s, 3H), 0.87 (s, 9H), 2.87 (dd, $J = 17.7$, 5.8 Hz, 1H), 3.26 (dd, $J = 17.7$, 5.7 Hz, 1H), 5.07-5.18 (m, 5H), 7.26-7.40 (m, 10H), 9.81 (s, 1H); $^{13}$C NMR (CDCl$_3$, 100 MHz) δ -4.8, -4.7, 18.1, 25.8, 42.7, 60.1, 67.5, 68.6, 128.1, 128.3, 128.5, 128.5, 128.6, 135.3, 159.2, 169.1, 198.2. HRMS (ESI) calcd for C$_{25}$H$_{34}$NO$_6$Si (M+H)$^+$ 472.215, obsd 472.2139.

$\text{(S,4E)}$-6-benzyl-1-methyl-5-[[{\text{benzyloxy} carbonyl}][\text{(tert-butyldimethylsilyl)}oxy]amino]hex-2-enedioate ($147a$)

A solution of (S)-benzyl 2-[[{\text{benzyloxy} carbonyl}][\text{(tert-butyldimethylsilyl)}oxy]amino]-4-oxobutanoate ($148a$) (137.0 mg, 0.290 mmol, 1.0 equiv.) in chloroform (2.5 mL) was treated
with methyl (triphenylphosphorylidine)acetate (142) (164.0 mg, 0.435 mmol, 1.5 equiv.) at 0 °C and allowed to warm to rt. The reaction mixture was stirred for 1.5 h and concentrated to a viscous oil. Flash chromatography on silica gel (Hex-EtOAc 5:1) afforded the α,β-unsaturated ester as colorless oil 147a (140 mg, 91%). $R_f$ 0.30 (2:1 Hex-EtOAc); $[\alpha]_{D}^{25}$ -41.2 (c 0.4, CHCl₃).

$^1$H NMR (CDCl₃, 400 MHz) δ 0.07 (s, 3H), 0.09 (s, 3H), 0.85 (s, 9H), 2.78-2.93 (m, 2H), 3.73 (s, 3H), 4.64 (dd, $J$ = 8.9, 6.1 Hz, 1H), 5.07 (d, $J$ = 12.0 Hz, 1H), 5.10 (d, $J$ = 12.2 Hz, 1H), 5.12 (d, $J$ = 12.0 Hz, 1H), 5.16 (d, $J$ = 12.0 Hz, 1H), 5.94 (d, $J$ = 15.7 Hz, 1H), 6.97 (dt, $J$ = 15.7, 7.0 Hz, 1H), 7.26-7.40 (m, 10H); $^{13}$C NMR (CDCl₃, 100 MHz) δ -4.8, -4.7, 18.1, 25.8, 31.1, 51.5, 64.2, 67.2, 68.5, 123.6, 128.2, 128.3, 128.4, 128.5, 128.5, 128.6, 135.2, 144.3, 168.8. HRMS (ESI) calcd for C$_{29}$H$_{38}$NO$_7$Si (M+H)$^+$ 528.2412, obsd 528.2420.

A solution of benzyl (tert-butyldimethylsilyl)oxycarbamate (136a) (305.00 mg, 1.05 mmol, 1.4 equiv.) in chloroform (1 mL) was treated with (S)-2-{diphenyl[(trimethylsilyl)oxy]methyl}pyrrolidine (165) (50.35 mg, 0.15 mmol, 0.2 equiv), followed by the addition of acetic acid (10 µL, 9.28 mg, 0.15 mmol, 0.2 equiv.) and stirred for 10 min at rt. Benzyl 4-oxo-but-2-enoate (149) (147.20 mg, 0.77 mmol, 1.0 equiv.) was added and the reaction mixture was stirred for 3.5 h at rt. Upon completion (determined by TLC), methyl (triphenylphosphorylidine)acetate (142) (357.00 mg, 0.75 mmol, 1.5 equiv.) was added in one portion. The reaction mixture was stirred for 2.5 h and then concentrated to a viscous oil. Flash chromatography on silica gel (Hex-EtOAc 5:1) afforded the α,β-unsaturated ester as colorless oil 147a (280 mg, 71%). $R_f$ 0.30 (2:1 Hex-EtOAc). NMR data was identical to that obtained via the two-step procedure.
To a solution of (2S,5E)-6-benzyl 1-methyl 5-{{(benzyloxy)carbonyl}{(tert-butyl(dimethyl)silyl)oxy}amino}hex-2-enedioate (147a) (105 mg, 0.198 mmol, 1.0 equiv.) in CHCl₃ (1 mL) was added acetic acid (17 µL, 17 mg, 0.297 mmol, 1.5 equiv.). The mixture was cooled to 0 °C and TBAF (297 µL, 1 M in THF with 5% H₂O, 0.297 mmol, 1.5 equiv.) was added. The reaction was allowed to stir at 0 °C for 4 d. Reaction was monitored by TLC and upon completion, the reaction mixture was concentrated and applied directly to a flash column, eluting with 4:1→2:1 (Hex-EtOAc), to obtain (3S,5R)-dibenzy 1-(2-methoxy-2-oxoethyl)isoxazolidine-2,3-dicarboxylate 163b as a colorless oil (50 mg, 65%); Rf 0.23 (2:1 Hex-EtOAc). [α]D²⁵ -33.9 (c 0.5, CHCl₃). ¹H NMR (CDCl₃, 400 MHz) δ 2.23 (ddt, J = 12.8, 7.9, 4.8 Hz, 1H), 2.60 (dd, J = 16.4, 7.8 Hz, 1H), 2.83-2.93 (m, 2H), 3.68 (s, 3H), 4.35 (app. p, J = 7.1 Hz, 1H), 4.88 (dd, J = 9.5, 5.7 Hz, 1H), 5.19 (d, J = 2.4 Hz, 2 H), 5.21 (s, 2H), 7.33-7.36 (m, 10H); ¹³C NMR (CDCl₃, 100 MHz) δ 36.9, 37.9, 52.0, 60.6, 67.4, 68.4, 128.1, 128.2, 128.4, 128.5, 128.6, 135.2, 135.5, 156.9, 170.0. HRMS (ESI) calcd for C₂₂H₂₄NO₇ (M+H)⁺ 414.1547, obsd 414.1548.
3.4.3 Spectra

Compound 136a (Scheme 3.12) - $^1$H NMR spectrum

SY-01-159 in CDCl$_3$ at 400 MHz
Compound 136a (Scheme 3.12) - $^{13}$C NMR spectrum

SY-01-159 in CDCl$_3$ at 100 MHz
Compound 136b (Scheme 3.12) - $^1$H NMR spectrum

SY-02-70 in CDCl3 at 400 MHz
Compound 136b (Scheme 3.12) – $^{13}$C NMR spectrum

SY-02-70 in CDCl$_3$ at 100 MHz
Compound 152 (Scheme 3.13) - $^1$H NMR spectrum

SY-01-165 in CDCl3 at 400 MHz

\[
\text{BnOOC} \rightleftharpoons \text{P(OEt)}_2
\]
Compound 152 (Scheme 3.13) - $^{13}$C NMR spectrum

SY-01-165 in CDCl$_3$ at 100 MHz
Compound 154 (Scheme 3.13) - $^1$H NMR spectrum

SY-01-177 in CDCl$_3$ at 400 MHz
Compound 154 (Scheme 3.13) - $^{13}$C NMR spectrum

SY-01-177 in CDCl3 at 100 MHz
Compound 149 (Scheme 3.13) - $^1$H NMR spectrum

SY-02-13 in CDCl3 at 400 MHz
Compound **149** (Scheme 3.13) - $^{13}$C NMR spectrum

SY-02-13 in CDCl$_3$ at 100 MHz
Compound 148b (Scheme 3.17) - $^1$H NMR spectrum

SY-02-73 in CDCl3 at 400 MHz

Cbz $\text{N}^{\text{OTBDPS}}$

BnOOCC$^\text{---}$C

$\delta$ (ppm): 3.48, 11.59, 1.51, 4.05, 0.82, 0.86, 9.11
Compound 148b (Scheme 3.17) - $^{13}$C NMR spectrum

SY–02–73 in CDCl₃ at 100 MHz
Compound **147b** (Scheme 3.17) - $^1$H NMR spectrum

SY-02-80 in CDCl$_3$ at 400 MHz
Compound 147b (Scheme 3.17) – $^{13}$C NMR spectrum

SY-02–80 in CDCl3 at 100 MHz

[Diagram of Compound 147b structure]
Compound 148a (Scheme 3.21) - $^1$H NMR spectrum

SY–03–17 in CDCl3 at 400 MHz
Compound 148a (Scheme 3.21) – $^{13}$C NMR spectrum

SY–03–17 in CDCl3 at 100 MHz
Compound 147a (Scheme 3.21) - $^1$H NMR spectrum

SY-03-11 in CDCl3 at 400 MHz
Compound 147a (Scheme 3.21) - $^{13}$C NMR spectrum

SY-03-11 in CDCl3 at 100 MHz
Compound 163 (Scheme 3.22) - $^1$H NMR spectrum

SY-03-81 in CDCl3 at 400 MHz
Compound 163 (Scheme 3.22) - $^{13}$C NMR spectrum

SY-03-81 in CDCl3 at 100 MHz
4.1 The Protecting Group Challenge

A major challenge in the synthesis of the Ahad building block was selection of the right combination of protecting groups that would be compatible with the total synthesis of theonellamide C. The proposed assembly of the western hemisphere is illustrated in Scheme 4.1. The amino terminus of tetrapeptide 52 will first be coupled to the D-Ala carboxylic acid of the τ-histidinoalanine residue 53. Following appropriate deprotections, the free δ-amino of the Ahad building block 170 will be coupled with the L-His primary amine of 169, thereby leading to cyclization precursor 49. Our goal is to deblock the C-terminal allyl group and the N-terminal allyloxycarbonyl group simultaneously with Pd(0), and then cyclize to obtain the western hemisphere of theonellamide C (Scheme 4.1).

Scheme 4.1 Proposed assembly of the western hemisphere of theonellamide C.
A successful protecting group strategy for the Ahad building block requires (Figure 4.1):

1. Nα-protection as an allyloxy carbonyl (Alloc) carbamate will enable deprotection by Pd(0) concomitantly with the allyl ester as a prelude to cyclization.
2. The Cα-COOH is not part of the macrocycle and therefore requires “permanent” protection. Our intent is for all side chain protecting groups to be fluoride-labile.
3. The Oγ-OH (side chain) also requires permanent protection, viz., fluoride lability.
4. The Cδ-COOH protecting group should be removed under conditions where all other protecting groups are inert, to enable conjugation to τ-HAL.

![Figure 4.1 Schematic representation of protecting group requirements for Ahad residue.](image)

These challenges prompted us to use α-amino adipic acid (α-AAA) as a model system. The difference between Ahad and α-amino adipic acid (Figure 4.2) is that the former is γ-hydroxylated; the latter appears in other congeners of the theonellamides. Moreover, α-AAA is commercially available and we can work initially with racemic α-AAA (US$64.20/g) and penultimately L-α-AAA (US$171.50/g).

![Figure 4.2 Structure of amino adipic acid and Ahad.](image)

We investigated the acetol ester (Ac*) and the 2-(trimethylsilyl)ethoxymethyl (SEM) ester as candidates for protecting the α-carboxylic acid. Both these protecting groups have been found to be stable to hydrogenolytic and acidolytic conditions normally used in peptide
synthesis. Fluoride ion treatment has been demonstrated to cleave acetol and SEM esters (Figure 4.3), compatible with a global side chain deprotection of the TBS ether in the final stages of theonellamide synthesis.

![Figure 4.3 Structure of acetol and SEM ester protecting groups.](image)

**4.2 Acetol Esters**

Kundu introduced the use of acetol esters to peptide synthesis in 1992. He synthesized several N-protected amino acid acetol esters with acetol using DCC/DMAP and showed that the acetol esters were stable to acidolytic (HCl/dioxane, TFA/CH₂Cl₂) and hydrogenolytic (H₂, Pd-C) conditions routinely used in peptide synthesis for the removal of Boc and Cbz groups respectively. The deprotection of an acetol ester can be carried out using tetrabutylammonium fluoride solution in tetrahydrofuran (THF) at room temperature (Scheme 4.2).

![Scheme 4.2 Synthesis and deprotection of acetol esters.](image)

Acetol esters have not seen much utilization. An exception was in 2001, when Rebek used an acetol ester in the synthesis of the marine cyclopeptide dendroamide C (Scheme 4.3) and differentiated three acids (benzyl, tert-butyl and acetol) from each other. First, the acetol ester 177 was prepared by removing the benzyl ester from 176 via hydrogenolysis and re-protecting the free acid with acetol using the Yamaguchi method. After removal of the trichloroethyl (TCE) ester, compound 178 was then incorporated into protected linear tripeptide 180. Deblocking of the trichloroethyl ester afforded the free acid and deprotection of the N-
terminus under acidic conditions afforded the linear precursor. Cyclization was performed in DMF with PyBOP activation in the presence of diisopropylethylamine, yielding the orthogonally protected triester 181 in 24% yield from protected linear trimer 180. The acetol ester was removed using TBAF to obtain free acid 182 (Scheme 4.3), without affecting the benzyl and tert-butyl esters.

Scheme 4.3 Use of acetol ester by Rebek et al. in dendroamide C synthesis.

4.3 SEM Esters

Joullié et al. used the SEM ester as a protecting group in their total synthesis of didemnins A, B, and C.84 The synthesis of a fragment of didemnin B is illustrated in Scheme 4.4. The acid of Cbz-\(N\)-methyl-\(D\)-leucine-OH (183) was protected as its 2-(trimethylsilyl)ethyl ester via a carbodiimide-mediated coupling with trimethylsilylethanol. After removal of the Cbz group by hydrogenolysis, coupling of the \(N\)-methyl-\(D\)-leucine trimethylsilylethyl ester 184 and the protected lactyl proline acid 185 was achieved by premixing bis(2-oxo-3-
oxazolidinyl)phosphonic chloride (BOP-CI) and triethylamine with the acid 185, and adding a solution of the secondary amine 184 and base to the solution of activated species. Simultaneous removal of the two silyl groups was effected with tetrabutylammonium fluoride in DMF to afford compound 187 (Scheme 4.4).

Scheme 4.4 SEM ester in the synthesis of didemnins A, B and C.

4.4 Synthesis of α-AAA-α-Esters

4.4.1 Precedents for N-Protection

Herin and coworkers protected (+)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (188) as its allyloxycarbamate. They used two equivalents of allyl chloroformate in the reaction.

Scheme 4.5 Protection of amino group as an Alloc carbamate.

Later on we came across a procedure by Hachisako et al. where they protected the L-2-amino adipic acid (-)-(64) as its benzyloxycarbamate (Scheme 4.6).

Scheme 4.6 Cbz protection of α-AAA.
Initially we followed the procedure by Herin and coworkers for protection of the primary amine in (±)-2-aminoadipic acid (±)-64 as its allyloxycarbamate, but product was obtained in low yield. We got a mixture of two species, one was more polar and corresponded to the product 192 and the other compound was less polar and we suspected that the cyclic anhydride 193 was forming with the use of two equivalents of allylchloroformate (Scheme 4.7).

Scheme 4.7 Cyclic anhydride formation in preparation of an Nα-Alloc carbamate.

Our next attempt at Alloc-protection of (±)-64 was by analogy to the procedure for the synthesis of Nα-Cbz adipic acid by Hachisako with slight modifications. The reaction time was changed from two hours to four hours at 0 °C and after the work-up, the aqueous layer was extracted thoroughly several times with ethyl acetate to afford compound (±)-192 in good yield (Scheme 4.8).

Scheme 4.8 Protection of amino group as an Alloc carbamate.

Baldwin and coworkers have demonstrated that it is possible to selectively esterify the α-carboxylic acid of α-amino adipic acid (Scheme 4.9), since its pKa is about one unit lower than the δ-carboxylic acid.87
The synthesis of the acetol ester is illustrated in Scheme 4.10. Regioselective esterification of the α-acid in compound 192 was attempted by forming the mono-cesium salt and then reacting with chloroacetone.

The product mixture containing compound 195 was subjected, without any purification, to methyl ester formation using an excess of trimethylsilyldiazomethane. The overall yield, after purification by silica gel chromatography, was low because of poor regioselectivity during formation of the first ester and the lack of purification following the previous two steps. The major product obtained after purification on silica gel chromatography was in fact the dimethyl ester 197, not the desired compound 196 (Scheme 4.11).

The SEM group was investigated in an analogous series of reactions to the acetol protecting group (Scheme 4.12).
Despite the poor overall yields, compounds 196 and 200 were available in reasonable quantities to explore chemoselective liberation of δ-COOH.

4.5 Liberation of δ-COOH from Protected α-AAA Derivatives

Nicolaou and coworkers had reported a mild and selective method for the hydrolysis of methyl esters with trimethyltin hydroxide. They showed that the methyl ester is hydrolyzed preferentially, even in the presence of ethyl and isopropyl esters, in good yield, for both aromatic and aliphatic systems (Scheme 4.13).

We therefore hoped that these reaction conditions would be selective in our α-AAA derivatives in which the α-COOH ester is a substituted ethyl ester in each case. We thus attempted to hydrolyze the methyl ester in the presence of the acetol ester protecting group. We
tried several small-scale reactions, varying the reaction temperature and the stoichiometry of trimethyltin hydroxide; but the desired product was invariably obtained in very low yield (Scheme 4.14).

\[
\begin{align*}
\text{Scheme 4.14 Mixture of products after hydrolysis of methyl ester in compound 196.}
\end{align*}
\]

The \(^1\)H NMR spectrum of the product mixture showed retention of the methyl ester functionality. Analysis of the reaction mixture by LCMS showed that four species were present (Table 4.1). The major peak corresponded to acetol deprotection (compound 206). The other minor peaks corresponded to starting material 196, the product of methyl ester hydrolysis 195, and fully deprotected diacid 192. The distribution of compounds is summarized in Table 4.1.

Likewise, treatment of compound 200 with trimethyltin hydroxide led to cleavage of the SEM ester in preference to the methyl ester (Scheme 4.15). Analysis of the product mixture by LCMS showed a major peak corresponding to SEM ester deprotection (compound 206). The other minor peaks corresponded to starting material 200, desired product 198, and fully deprotected diacid 192 (Table 4.1).

\[
\begin{align*}
\text{Scheme 4.15 Mixture of products obtained after treatment of diester (±)-200 with trimethyltin hydroxide.}
\end{align*}
\]
Table 4.1 Composition of product mixture, following hydrolysis by trimethyltin hydroxide, as determined by LCMS.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ac*</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fully protected starting material</td>
<td>196 (11.5%)</td>
<td>200 (2.7%)</td>
</tr>
<tr>
<td>δ-COOMe cleavage</td>
<td>195 (12.5%)</td>
<td>198 (18.1%)</td>
</tr>
<tr>
<td>α-COOR cleavage</td>
<td>206 (69.5%)</td>
<td>206 (77.7%)</td>
</tr>
<tr>
<td>Diacid (double cleavage)</td>
<td>192 (6.5%)</td>
<td>192 (1.6%)</td>
</tr>
</tbody>
</table>

In summary, both SEM and acetol esters were cleaved in preference to methyl esters upon hydrolysis using trimethyltin hydroxide. After consideration of this problem and assessing potential substitutes for the methyl ester, we decided to pursue a tert-butyl ester. The δ-tert-butyl ester ought to be removed under acidic conditions which should not affect the other protecting groups of the Ahad building block.

Using racemic α-AAA, the synthesis of the building block was repeated with a tert-butyl ester for racemic adipic acid. Compound 192 was protected as its α-acetol ester and then subjected to isobutylene under acidic conditions to form 6-tert-butyl 1-(2-oxopropyl)-2-[(allyoxy)carbonyl]amino]hexanedioate (207) (Scheme 4.16).

Scheme 4.16 Synthesis of α-AAA with δ-COO' Bu ester.

As expected, the cleavage of the tert-butyl ester was effected using TFA without any harm to the acetol or Alloc protecting groups (Scheme 4.17). Chemoselective deprotection of
the acetol ester was also demonstrated using TBAF. Conversely, we observed some cleavage of the SEM ester under acidic conditions in the reaction; therefore, we gave preference to the acetol ester.

Scheme 4.17 Orthogonal deprotection of acetol and tert-butyl esters.

*L*-2-Aminoadipic acid was subjected to these series of reactions to obtain α-tert-butyl-δ-(2-oxopropyl)-*L*-N-allyloxycarbonyl-2-amino adipate.

### 4.6 Synthesis of Ahad Building Block

Following these model studies, the next task was to make the Ahad precursor with a δ-tert-butyl ester instead of a methyl ester as described in Chapter 3. As shown in Scheme 4.18, the analogous reactions were performed with the tert-butyl ester protecting group being introduced using the commercially available phosphorane during the Wittig reaction to produce 209. At this time we measured the enantiomeric purity of compound 209 by chiral HPLC and obtained 87.4% e.e. Treatment of compound 209 with tetrabutylammonium fluoride solution with 5% water in tetrahydrofuran facilitated silyl group removal but didn’t cyclize to give compound 210. Increasing the temperature from 0 °C to room temperature had no impact. We added cesium carbonate to the reaction mixture after two days to make the solution more basic. Addition of cesium carbonate did facilitate the cyclization but the yield was very low.

We treated compound 209 with tetrabutylammonium fluoride solution in 1M THF with acetic acid at room temperature and obtained the major syn isoaxazolidine 210 in good yield with a 3.8:1.0 d.r. (Scheme 4.18). The major diastereomer was confirmed to have syn relative
stereochemistry by a NOESY experiment which showed a cross peak between α and γ protons (5.6% nOe). The syn diastereomer could be further purified by normal phase HPLC, although in practice the minor diastereomer was gradually removed during chromatography following the next few reaction steps.

Scheme 4.18 Synthesis of isooxazolidine precursor to Ahad.

While MacMillan and co-workers had employed samarium diiodide (Chapter 3, Section 3.2) to reductively cleave the N-O bond in a compound analogous to 210 (shown in a box in Scheme 4.19), Sibi and coworkers showed that exposure to standard hydrogenolytic conditions also effectively cleaves N-O bonds (Scheme 4.19).

Scheme 4.19 Precedents for cleavage of N-O bond.

We anticipated that exposure of 210 to standard hydrogenolysis conditions would simultaneously remove the Cbz and benzyl ester protecting groups, along with cleavage of the N-O bond. Hydrogenolysis was performed to give compound 213 ready for introduction of a suitable suite of protecting groups. The free amine was protected as its allyloxycarbamate by analogy to the procedure for racemic α-AAA. Esterification of the α-acid was attempted by the addition of half an equivalent of cesium carbonate, followed by chloroacetone. The major
product isolated from the reaction mixture was lactone 216 (Scheme 4.20). We also attempted coupling of the α-acid to acetol in the presence of activating agents, for instance under Yamaguchi conditions (precedent shown in a box in Scheme 4.20). The only product isolated from various reaction conditions was the lactone 216. Activated acyl species, including the acetol ester, were susceptible to nucleophilic attack by the γ-OH, mandating that we protect the secondary alcohol before the carboxylic acid.

Scheme 4.20 Attempted synthesis of Ahad.

As we neared the completion of our Ahad synthesis we sought an approach to correlate our amino acid with that obtained from degradation of the theonellamides. In 1989, Matsunaga et al. deduced the structure of Ahad with the aid of NMR studies as described in Chapter 2, Section 2.1.

They treated a 1:2 mixture of triethylammonium salts of 2-amino-4-hydroxyadipic acids with 2-(tert-butoxycarbonyloxyimino)-2-phenylacetonitrile (BocON) followed by methylation and normal phase HPLC separation afforded methylated Boc-lactone 58b. They found out that the minor trans-lactone that has (2S,4R) stereochemistry is the native constituent of theonellamide C.
Scheme 4.21 Lactone formation reported by Matsunaga et al.

We compared the NMR data of Matsunaga’s lactone 58b with the undesired product isolated from α-acid esterification of Ahad precursor (Scheme 4.20). Nuclear magnetic resonance data for compound 216 showed resemblance to that of the (2S,4R) lactone 58b. Moreover lactone 216 afforded further confirmation of the (2S,4R) stereochemistry since the Hβ protons gave rise to a multiplet at 2.4-2.8 ppm, consistent with a trans-1,4-disubstituted γ-lactone (Table 4.2).

Table 4.2. NMR data of lactones 58b and 216

<table>
<thead>
<tr>
<th></th>
<th>Matsunaga et al.</th>
<th>Yadav and Taylor</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ 5.01 (m, 1NH, 1Hα)</td>
<td>δ 5.22-5.30 (m, 1NH, 1Hα)</td>
<td></td>
</tr>
<tr>
<td>δ 4.38 (ddd, J = 6.4, 9.8, 9.8 Hz, 1Hγ)</td>
<td>δ 4.48 (dd, J = 16.1, 9.3 Hz, 1Hγ)</td>
<td></td>
</tr>
<tr>
<td>δ 2.73 (m, 2Hδ)</td>
<td>δ 2.38-2.79 (m, 2Hδ &amp; 1Hβ)</td>
<td></td>
</tr>
<tr>
<td>δ 2.50 (m, 2Hβ)</td>
<td>δ 2.14-2.17 (m, 1Hβ)</td>
<td></td>
</tr>
</tbody>
</table>

Indeed, Matsunaga et al. stated that the C-4 hydroxyl group might have been epimerized through a backside attack of the carboxyl group during lactonization. Moreover, equilibration to the more stable cis-2,4-disubstituted γ-lactone may have enhanced the amount of (2S,4S) diastereomer. We also observed the same results during the lactone formation of (2S,4R) compound 167 (Scheme 4.22). The NMR experiments showed that we also obtained the more stable (2S,4S) cis-lactone 58a.
Scheme 4.22 Preparation of γ-lactone 58a by Yadav and Taylor.

Lajoie and coworkers had shown that a free alcohol can be masked with TBSCI in the presence of a free acid. They protected the alcohol of the Cbz-protected serine 220 as a TBS ether with TBSCI (Scheme 4.23).

Scheme 4.23 Protection of alcohol as TBS ether.

We attempted the protection of the alcohol in compound 214 with stoichiometric quantities of TBSCI and 2,6-lutidine. Carbodiimide-mediated coupling of the resulting crude acid with acetal gave rise to compound 222. Chemoselective cleavage of the tert-butyl ester with tert-butyldimethylsilyl triflate was carried out in readiness for coupling to the L-His amino group of τ-HAL in the western hemisphere of theonellamide C (Scheme 4.24).

Scheme 4.24 Synthesis of orthogonally protected Ahad.

4.7 Experimental Section

4.7.1 General Methods

The general methods are as described in Chapter 2.
4.7.2 Procedures

\[
\begin{align*}
\text{HOOC} & \quad \text{NHAlloc} & \quad \text{COOH} \\
\end{align*}
\]

2-(Allyloxy carbonylamino) adipic acid (192)

(±)-α-Amino adipic acid (64) (250 mg, 1.55 mmol, 1.0 equiv.) was added to a solution of sodium hydroxide (223 mg, 5.60 mmol, 3.6 equiv.) in water (12.5 mL) at 0 °C. Allyl chloroformate (247 µL, 281 mg, 2.35 mmol, 1.5 equiv.) was added dropwise over 30 min and stirring continued for 4 h in an ice bath. The mixture was washed with diethyl ether (20 mL). The aqueous layer was acidified to pH 2 by the addition of conc. HCl and extracted with EtOAc (4 x 20 mL). The combined extracts were dried over MgSO\(_4\), filtered and concentrated to give (±)-2-(Allyloxy carbonylamino) adipic acid (192) (350 mg, 92%). \(R\)\(_f\) 0.29 (6:4:1 CHCl\(_3\)-MeOH-H\(_2\)O).

\(^1\)H NMR (CD\(_3\)OD, 400 MHz) \(\delta\) 1.67-1.74 (m, 3H), 1.87-1.90 (m, 1H), 2.33 (t, \(J = 6.8\) Hz, 2H), 4.14 (dd, \(J = 8.0, 4.4\) Hz, 1H), 4.54 (d, \(J = 5.3\) Hz, 2H), 4.97 (br s, 1H), 5.18 (dd, \(J = 10.5, 1.4\) Hz, 1H), 5.31 (dd, \(J = 17.2, 1.5\) Hz, 1H), 5.93 (ddt, \(J = 15.9, 10.6, 5.3\) Hz, 1H); \(^{13}\)C NMR (CD\(_3\)OD, 100 MHz) \(\delta\) 19.4, 29.1, 31.2, 52.0, 63.5, 114.5, 131.3, 155.6, 172.7, 174.0. HRMS (ESI) calcd for C\(_{10}\)H\(_{15}\)NNaO\(_6\) (M+Na\(^+\)) 268.0792, obsd 268.0786.

(\(L\))-N-Allyloxy carbonyl-2-amino-adipic acid was prepared using \(L\)-2-amino adipic acid (100 mg) according to the same procedure to give (\(-\))-193 (130 mg, 89%). \([\alpha]_D^{25}\) -7.82 (c 1.0, MeOH).

\[
\begin{align*}
\text{O} & \quad \text{O} & \quad \text{COOMe} \\
\end{align*}
\]

2-(Allyloxy carbonylamino)-6-methyl-1-(2-oxopropyl)-adipate (196)

Cesium carbonate (166 mg, 0.51 mmol, 0.5 equiv.) was added to a solution of (±)-N-allyloxy carbonyl-2-amino-adipic acid (192) (250 mg, 1.02 mmol, 1.0 equiv.) in dry MeOH (1 mL). The mixture was stirred for 2 h under N\(_2\) at rt. The mixture was concentrated, dissolved in DMF (2 mL) and cooled to 0 °C. Chloroacetone (97 µL, 113 mg, 1.22 mmol, 1.2 equiv.) was added,
the mixture warmed to rt, stirred overnight, diluted with H₂O (15 mL) and extracted with EtOAc (3 x 25 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated to give the mixture containing acid 5-H₂O (15 mL) and extracted with EtOAc (3 x 25 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated to give the mixture containing acid 5-[(allyloxy)carbonyl]amino]-6-oxo-6-(2-oxopropoxy)hexanoic acid as a colorless oil (300 mg) that was dissolved in acetonitrile (3 mL) and methanol (300 µL). Trimethylsilyldiazomethane (498 µL, 2 M solution in Et₂O, 0.99 mmol, 1.1 equiv.) added dropwise and stirred for 2.5 h at rt under N₂. The mixture was concentrated and applied to a flash column. Elution with a gradient of 3:1→1:1 (Hex-EtOAc) gave 2-(Allyloxy)carbonylamino)-6-methyl-1-(2-oxopropyl)-adipate (196) as a colorless oil (64 mg, 20%). Rf 0.40 (2:1 EtOAc-Hex). ¹H NMR (CDCl₃, 400 MHz) δ 1.73-1.82 (m, 3H), 1.97-2.02 (m, 1H), 2.15 (s, 3H), 2.37 (t, J = 6.4 Hz, 2H), 3.66 (s, 3H), 4.42-4.46 (m, 1H), 4.56 (d, J = 5.5 Hz, 2H), 4.63 (d, J = 16.8 Hz, 1H), 4.88 (d, J = 16.8 Hz, 1H), 5.21 (dd, J = 10.4, 1.3 Hz, 2H), 5.28-5.35 (m, 2H), 5.90 (ddt, J = 16.1, 10.5, 5.5 Hz, 1H); ¹³C NMR (CDCl₃, 100 MHz) δ 20.5, 26.0, 31.7, 33.3, 51.6, 53.5, 65.9, 68.7, 117.9, 132.5, 155.8, 171.7, 173.5, 200.6. HRMS (ESI) calcd for C₁₄H₂₁NNaO₇ (M+Na⁺) 338.1216, obsd 338.1220.

![2-(Allyloxy)carbonylamino)-6-methyl-1-(2-trimethylsilyl-ethoxy-methyl)-adipate (200)](image.png)

Cesium carbonate (166 mg, 0.51 mmol, 0.5 equiv.) was added to a solution of (±)-N-allyloxy carbonyl-2-amino-adipic acid (192) (250 mg, 1.02 mmol, 1.0 equiv.) in dry MeOH (1 mL). The mixture was stirred for 2 h under N₂ at rt. The mixture was concentrated, the residue dissolved in DMF (2 mL) and cooled to 0 °C. 2-(Trimethylsilyl)ethoxymethyl chloride (217 µL, 204 mg, 1.22 mmol, 1.2 equiv.) was added, the mixture warmed to rt, stirred overnight, diluted with H₂O (15 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated to give a mixture containing acid 5-[(allyloxy)carbonyl]amino]-6-oxo-6-[(2-(trimethylsilyl)ethoxy)methoxy]hexanoic acid as a
colorless oil (370 mg) that was dissolved in acetonitrile (4 mL) and methanol (400 µL). Trimethylsilyldiazomethane (541 µL, 2 M solution in Et₂O, 1.08 mmol, 1.1 equiv.) added dropwise and the mixture stirred for 2.5 h at rt under N₂. The mixture was concentrated and applied to a flash column. Elution with a gradient of 3:1→1:1 (Hex-EtOAc) gave 2-(Allyloxy carbonylamino)-6-methyl-1-(2-trimethylsilyl-ethoxy-methyl)-adipate (200) as a colorless oil (99 mg, 25%). Rᵣ 0.49 (2:1 EtOAc-Hex). ¹H NMR (CDCl₃, 400 MHz) δ 0.01 (s, 9H) 0.95 (app.t, J = 8.4 Hz, 2H), 1.68-1.73 (m, 3H), 1.87-1.92 (m, 1H), 2.31-2.38 (m, 2H), 3.65 (s, 3H), 3.66-3.73 (m, 2H), 4.35-4.38 (m, 1H), 4.56 (d, J = 5.4 Hz, 2H), 5.20 (dd, J = 10.4, 1.3 Hz, 2H), 5.26-5.36 (m, 3H), 5.90 (ddt, J = 16.1, 10.6, 5.4 Hz, 1H); ¹³C NMR (CDCl₃, 100 MHz) δ -1.5, 18.0, 20.4, 20.6, 31.8, 33.3, 33.5, 51.6, 52.4, 53.5, 53.6, 65.8, 67.9, 68.2, 88.9, 89.9, 117.8, 132.6, 155.8, 171.9, 172.7, 173.3. HRMS (ESI) calcd for C₁₇H₃₁NNaO₇Si (M+Na)⁺ 412.1762, obsd 412.1748.

Cesium carbonate (332 mg, 1.02 mmol, 0.5 equiv.) was added to a solution of (±)-N-allyloxycarbonyl-2-amino-adipic acid (192) (500 mg, 2.04 mmol, 1.0 equiv.) in dry MeOH (4 mL). The mixture stirred for 2 h under N₂ at rt. The mixture was concentrated, dissolved in DMF (5 mL) and cooled to 0 °C. Chloroacetone (194 µL, 226 mg, 2.44 mmol, 1.2 equiv.) was added, the mixture warmed to rt, stirred overnight, diluted with H₂O (30 mL) and extracted with EtOAc (3 x 40 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated to give acid 5-[[allyloxy]carbonyl]amino]-6-oxo-6-(2-oxopropoxy)hexanoic acid as a colorless oil (500 mg) that was dissolved in CH₂Cl₂ (10 mL) and added p-TsOH (663 mg, 3.49 mmol, 2.1 equiv.). The reaction mixture was added to condensed isobutylene (10 mL) and stirred for 3d at rt. The mixture was diluted with 20 mL CH₂Cl₂ and added sat. sol. of NaHCO₃ (25 mL), extracted the organic layer. The remaining aqueous layer was washed with CH₂Cl₂ (2 x 30 mL).
The combined organic layers were dried over MgSO$_4$, filtered, concentrated and applied to a flash column. Elution with a gradient of 3:1→1:1 (Hex-EtOAc) gave 2-(allyloxy carbonylamino)-6-(tert-butyl)-1-(2-oxo-propyl)-adipate (207) as a colorless oil (198 mg, 34%). $R_f$ 0.32 (2:1 EtOAc-Hex). $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 1.41 (s, 9H), 1.64-1.79 (m, 2H), 1.91-2.02 (m, 2H), 2.14 (s, 3H), 2.25 (t, $J$ = 6.9 Hz, 2H), 4.42 (dd, $J$ = 12.4, 7.4 Hz, 1H), 4.55 (d, $J$ = 5.4 Hz, 2H), 4.62 (d, $J$ = 16.8 Hz, 1H), 4.75 (d, $J$ = 16.8 Hz, 1H), 5.19 (dd, $J$ = 10.4, 1.2 Hz, 1H), 5.26-5.39 (m, 1H), 5.89 (ddt, $J$ = 16.2, 10.6, 5.6 Hz, 1H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 20.7, 26.0, 28.1, 31.6, 34.7, 53.4, 65.9, 68.7, 80.4, 117.8, 132.6, 155.8, 171.8, 172.4. HRMS (ESI) calcd for C$_{17}$H$_{28}$NO$_7$ (M+H)$^+$ 358.1820, obsd 358.1826.

(L)-N-allyloxy carbonyl-2-amino-adipic acid (50 mg) was subjected to the same procedure to give $\alpha$-tert-Butyl $\delta$-(2-oxopropyl)-(L)-N-allyloxy carbonyl-2-amino adipate (207) (20 mg, 30%). $[\alpha]_D^{25}$ -7.75 ($c$ 1.0, MeOH).

To a solution of 2-(allyloxy carbonylamino)-6-(tert-butyl)-1-(2-oxo-propyl)-adipate (207) (25 mg, 0.069 mmol, 1.0 equiv.) in dry CH$_2$Cl$_2$ (2 mL) was added trifluoroacetic acid (2 mL) at 0 ºC and allowed to stir at 0 ºC for 1 h. The reaction mixture was warmed to rt and stirred overnight, concentrated and applied to a flash column. Elution with a gradient of 3:1→4:1 (EtOAc-Hex) gave 2-(Allyloxy carbonylamino)-1-(2-oxopropyl)-adipic acid (195) (15 mg, 65 %). $R_f$ 0.23 (3:1 EtOAc-Hex). $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 1.20-1.29 (m, 2H), 1.78-1.84 (m, 2H), 2.17 (s, 3H), 2.43 (t, $J$ = 7.1 Hz, 2H), 4.43-4.50 (m, 1H), 4.58 (d, $J$ = 5.0 Hz, 2H), 4.65 (d, $J$ = 16.8 Hz, 1H), 4.80 (d, $J$ = 16.8 Hz, 1H), 5.22 (dd, $J$ = 10.4, 1.1 Hz, 1H), 5.29 (d, $J$ = 2.6 Hz, 1H), 5.34 (s, 1H), 5.91 (ddt, $J$ = 16.2, 10.7, 5.5 Hz, 1H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 20.4, 26.0, 31.7, 32.9, 53.4, 66.0, 68.7, 118.0, 132.5, 155.9, 171.6, 176.6. HRMS (ESI) calcd for C$_{13}$H$_{19}$NNaO$_7$ (M+Na)$^+$ 324.1054, obsd 324.1054.
To a solution of α-tert-butyl δ-(2-oxopropyl) N-allyloxycarbonyl-2-aminoadipate (207) (20 mg, 0.056 mmol, 1.0 equiv.) in dry THF (1 mL) was added TBAF (111 µL, 1 M in THF, 0.112 mmol, 2.0 equiv.) at 0 °C and allowed to stir at 0 °C for 1 h. The reaction mixture was warmed to rt and stirred overnight, concentrated and applied to a flash column. Elution with a gradient of 3:1→4:1 (EtOAc-Hex) gave 2-(Allyloxycarbonylamino)-6-(tert-butyl)adipate (208) (11 mg, 68 %). 

\[ R_f 0.25 \ (2:1 \text{ EtOAc-Hex}). \]

\[ ^1H \text{ NMR (CDCl}_3, 400 MHz) \delta 1.41 \ (s, 9H), 1.64-1.79 \ (m, 2H), 1.91-2.02 \ (m, 2H), 2.25 \ (t, J = 6.9 Hz, 2H), 4.42 \ (dd, J = 12.4, 7.4 Hz, 1H), 4.55 \ (d, J = 5.4 Hz, 2H), 5.19 \ (dd, J = 10.4, 1.2 Hz, 1H), 5.26-5.39 \ (m, 1H), 5.89 \ (ddt, J = 16.2, 10.6, 5.6 Hz, 1H); ^{13}C \text{ NMR (CDCl}_3, 100 MHz) \delta 20.7, 28.1, 31.6, 34.7, 53.4, 65.9, 80.4, 117.8, 132.6, 155.8, 171.8, 172.4. \]

\[ \text{HRMS (ESI) calcd for C}_{14}H_{23}NNaO_6 (M+Na)^+ 324.2020, obsd 324.2026.} \]

A solution of benzyl (tert-butyldimethylsilyl)oxycarbamate (136a) (250.0 mg, 0.88 mmol, 1.4 equiv.) in chloroform (1 mL) was treated with (S)-2-{diphenyl[(trimethylsilyl)oxy]methyl}pyrrolidine (165) (41.0 mg, 0.12 mmol, 0.2 equiv), followed by the addition of acetic acid (7 µL, 7.5 mg, 0.12 mmol, 0.2 equiv.) and stirred for 10 min at rt. Benzyl 4-oxo-but-2-enooate (149) (121.0 mg, 0.63 mmol, 1.0 equiv.) was added and the reaction mixture was stirred for 3.5 h at rt. Upon completion (determined by TLC), tert-butyl-2-(triphenylphosphoranylidene)acetate (237.0 mg, 0.63 mmol, 1.0 equiv.) was added in one portion. The reaction mixture was stirred for 1.5 h and then concentrated to a viscous oil. Flash chromatography on silica gel (Hex-EtOAc 12:1) afforded the α,β-unsaturated ester as colorless oil 209 (354 mg, 70%, 87.4% e.e.). The enantiomeric excess was determined by HPLC analysis.
using Chiralcel OD-H column (5% iPrOH-Hex, 0.5 mL/min); major enantiomer $t_r = 20.1$ min and minor enantiomer $t_r = 36.4$ min; Detection 218 nm. $R_f$ 0.30 (2:1 Hex-EtOAc). $[\alpha]_D^{25} -37.2$ (c 0.5, CHCl$_3$). $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 0.08 (s, 3H), 0.10 (s, 3H), 0.85 (s, 9H), 1.47 (s, 9H), 2.76-2.91 (m, 2H), 4.57 (dd, $J = 8.7, 6.2$ Hz, 1H), 5.06-5.18 (m, 4H), 5.84 (d, $J = 15.6$ Hz, 1H), 6.86 (dt, $J = 7.0, 15.6$ Hz, 1H), 7.26-7.36 (m, 10H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ -4.8, -4.7, 18.1, 25.8, 28.1, 31.2, 64.5, 67.2, 68.4, 80.3, 125.8, 128.1, 128.2, 128.4, 128.5, 128.5, 128.6, 135.3, 142.6, 159.1, 165.4, 168.9. HRMS (ESI) calcd for C$_{31}$H$_{44}$NO$_7$Si (M+H)$^+$ 570.2412, obsd 570.2420.

To a solution of (2S,4E)-1-benzyl-6-tert-butyl-2-[[((benzyloxy)carbonyl){{(tertbutyldimethylsilyl)oxy}amino}hex-2-enedioate (209) (500.0 mg, 0.88 mmol, 1.0 equiv.) in CH$_2$Cl$_2$ (3 mL) was added acetic acid (0.078 mL, 78.9 mg, 1.32 mmol, 1.5 equiv.). The mixture was cooled to 0 °C and TBAF (1.3 mL, 1 M in THF, 1.32 mmol, 1.5 equiv.) was added and allowed to stir at 0 °C for 30 min. The reaction mixture was warmed to rt and stirred for 2 days. Reaction was monitored by TLC and upon completion, the reaction mixture was concentrated and applied directly to a flash column, eluting with 4:1 (Hex-EtOAc), to obtain (2S,4R)-dibenzyl 2-[(tert-butoxy)-oxoethyl]isoxazolidine-2,3-dicarboxylate (210) (344 mg, 55%). $R_f$ 0.25 (2:1 Hex-EtOAc). $[\alpha]_D^{25} -23.25$ (c 0.5, CHCl$_3$). $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 1.42 (s, 9H), 2.20 (ddt, $J = 12.7, 9.1, 5.9$ Hz, 1 H), 2.48 (dd, $J = 16.0, 8.0$ Hz, 1H), 2.77-2.88 (m, 2H), 4.24-4.31 (m, 1H), 4.86 (dd, $J = 9.6, 5.9$ Hz, 1H), 5.16 (d, $J = 4.9$ Hz, 1 H), 5.18 (d, $J = 2.0$ Hz, 1 H), 5.19 (d, $J = 1.0$ Hz, 1 H), 5.22 (d, $J = 4.0$ Hz, 1 H), 7.26-7.37 (m, 10H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 28.0, 38.0, 38.4, 60.6, 67.4, 68.4, 81.5, 128.2, 128.3, 128.4, 128.5, 128.6, 135.2, 135.5, 168.8, 170.1. HRMS (ESI) calcd for C$_{25}$H$_{30}$NO$_7$ (M+H)$^+$ 456.5547, obsd 456.5548.
(3S,5R)-3-(Allyloxycarbonylamino)-5-[(tert-butoxycarbonyl)-methyl]-2-oxotetrahydrofuran (216)

(2S,4R)-2-Amino-6-tert-butyl-4-hydroxyadipic acid (213): (3S,5R)-benzy-5-[(tert-butoxycarbonyl)methyl]-isoxazolidine-2-carboxylate (210) (100 mg, 0.219 mmol, 1.0 equiv.) was dissolved in dry MeOH (2 mL) and 10 % Pd-C (93 mg, 0.879 mmol, 4.0 equiv.) was added. The suspension was stirred at rt overnight under an atmosphere of H₂. The reaction mixture was filtered through Celite® and the filtrate concentrated to give the α-amino-carboxylic acid 213 as a colorless oil (40 mg). Rf 0.56 (6:4:1 CHCl₃-MeOH-H₂O).

(2S,4R)-2-(Allyloxycarbonylamino)-6-tert-butyl-4-hydroxy-adipic acid (214): The amino acid was dissolved in a solution of sodium hydroxide (32 mg, 0.788 mmol, 3.6 equiv.) in water (2 mL) at 0 °C and followed by dropwise addition of allyl chloroformate (28 µL, 32 mg, 0.263 mmol, 1.2 equiv.) at 0 °C. The mixture was stirred an additional 1 h at 0 °C and then warmed to RT and stirred overnight. The reaction mixture was washed with diethyl ether (5 mL) to remove unreacted allylchloroformate. The aqueous layer was cooled to 0 °C and then acidified to ~pH 4 by the dropwise addition of 0.5 M HCl. The aqueous layer was extracted with EtOAc (5x10 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated to obtain 6-tert-butyl-N-allyloxycarbonyl-(2S,4R)-2-amino-4-hydroxy-α-oxohexanoic acid (214). Rf 0.54 (9:1 CH₂Cl₂-MeOH)

(3S,5R)-3-(Allyloxycarbonylamino)-5-[(tert-butoxycarbonyl)-methyl]-2-oxotetrahydrofuran (216): Cesium carbonate (36 mg, 0.109 mmol, 0.5 equiv.) was added to a solution of acid 214 in dry MeOH (4 mL). The mixture stirred for 2 h under N₂ at rt. The mixture was concentrated, dissolved in DMF (5 mL) and cooled to 0 °C. Chloroacetone (21 µL, 24 mg, 0.263 mmol, 1.2 equiv.) was added, the mixture warmed to rt, stirred overnight, diluted with H₂O (5 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried over MgSO₄, filtered
and concentrated to give a colorless oil that was dissolved in CH$_2$Cl$_2$ (2mL) and added 2,6-
Lutidine (254 µL, 235 mg, 2.190 mmol, 10.0 equiv.) and TBSOTf (402 µL, 463 mg, 1.752 mmol,
8.0 equiv.). Reaction mixture was stirred and concentrated to remove the CH$_2$Cl$_2$. Water (2 mL)
was added to the reaction mixture which was then cooled to 0 °C and acidified to ~pH 4 by the
dropwise addition of 0.5 M HCl. The acidic solution was then extracted with EtOAc (4 X 10 mL).
The combined organic layers were dried over MgSO$_4$, filtered and then concentrated to afford a
viscous oil. Flash chromatography on silica gel (Hex-EtOAc 3:1) afforded the compound 216 as
colorless oil (57 mg, 45% over 4 steps). $R_f$ 0.54 (1:1 Hex-EtOAc). [α]$^D_{25}$ -8.47 (c 2.5, CHCl$_3$). $^1$H
NMR (CDCl$_3$, 400 MHz) δ 1.45 (s, 9H), 2.14-2.17 (m, 1H), 2.38-2.79 (m, 3H), 4.48 (dd, $J$ = 16.1,
9.3 Hz, 1H), 4.58 (d, $J$ = 5.3 Hz, 2H), 4.98 (dd, $J$ = 16.1, 9.3 Hz, 1H), 5.22 (app. d, $J$ = 10.4 Hz,
1H), 5.30 (app. d, $J$ = 16.4 Hz, 1H), 5.46 (d, $J$ = 5.7 Hz, 1H), 5.90 (ddt, $J$ = 16.4, 10.8, 5.6 Hz,
1H); $^{13}$C NMR (CDCl$_3$, 100 MHz) δ 28.0, 33.6, 40.6, 49.5, 66.2, 74.2, 81.9, 118.2, 132.3, 155.9,
168.5, 174.6. HRMS (ESI) calcd for C$_{14}$H$_{21}$NNaO$_6$ (M+Na)$^+$ 322.1261, obsd 322.1270.

Pd/C (10% w/w, 16 mg) was added to a solution of (2S,4R)-1-benzyl 2-tert butyl-2-(6-
methoxy-5-oxoethyl) isoxazolidine-4,5-dicarboxylate 163b (20 mg, 0.053 mmol, 1.0 equiv.) in
dry MeOH (1ml). The vessel was evacuated and then opened up to an atmosphere of hydrogen
and stirred overnight. The mixture was filtered through Celite™, washing well with MeOH (~25
mL). The filtrate was concentrated to afford acid 167, dissolved in CH$_2$Cl$_2$ (2 mL), followed by
addition of EDC (30 mg, 0.158 mmol, 3.0 equiv.) and DMAP (2 mg, 0.016 mmol, 0.3 equiv.).
Reaction mixture was stirred overnight at rt under N$_2$ atmosphere and then concentrated to
afford a viscous oil. Flash chromatography on silica gel (Hex-EtOAc 3:1) afforded lactone 58a (3
mg, 20%). $R_f$ 0.29 (2:1 Hex-EtOAc). $^1$H NMR* (CDCl$_3$, 400 MHz) δ 1.50 (s, 9H), 2.20 (ddd, $J$ =
12.6, 8.7, 5.6 Hz, 1H), 2.62 (dd, J = 16.4, 7.8 Hz, 1H), 2.81-2.86 (m, 1H), 2.90 (dd, J = 16.4, 5.6 Hz, 1H), 3.70 (s, 3H), 4.33 (app. pent., J = 7.2 Hz, 1H), 4.75 (dd, J = 9.5, 5.6 Hz, 1H); $^{13}$C NMR (CDCl$_3$, 100 MHz) δ 14.1, 28.1, 37.1, 38.0, 52.0, 61.8, 82.8, 156.3, 170.3, 170.6. HRMS (ESI) calcd for C$_{12}$H$_{18}$NO$_6$ (M-H)$^+$ 272.1140, obsd 272.1162.

* $^1$H NMR spectra of the title compound contaminated with ethyl acetate.

(2S,4R)-2-(Allyloxy carbonylamino)-6-(tert-butyl)-4-(tert-butyldimethylsilyloxy)-1-(2-oxopropyl)-adipate (222)

(2S,4R)-2-Amino-6-tert-butyl-4-hydroxy adipic acid (213): (3S,5R)-benzy-5-[(tert-butoxycarbonyl)methyl]-isooxazolidine-2-carboxylate (210) (80 mg, 0.176 mmol, 1.0 equiv.) was dissolved in dry MeOH (2 mL) and 10 % Pd-C (75 mg, 0.702 mmol, 4.0 equiv.) was added. The suspension was stirred at rt overnight under an atmosphere of H$_2$. The reaction mixture was filtered through Celite® and the filtrate concentrated to give the α-amino-carboxylic acid 213 as a colorless oil (40 mg). $R_f$ 0.56 (6:4:1 CHCl$_3$-MeOH-H$_2$O).

(2S,4R)-2-(Allyloxy carbonylamino)-6-tert-butyl-4-hydroxy-adipic acid (214): The amino acid 213 was dissolved in 10% aqueous Na$_2$CO$_3$ solution (1.5 mL) and cooled to 0 ºC in ice bath. Dioxane (1.5 mL) was added, followed by dropwise addition of allyl chloroformate (22 µL, 25 mg, 0.211 mmol, 1.2 equiv.) at 0 ºC. The mixture was stirred an additional 1 h at 0 ºC and then warmed to RT and stirred overnight. The reaction mixture was washed with diethyl ether (5 mL) to remove unreacted allylchloroformate. The aqueous layer was cooled to 0 ºC and then acidified to ~pH 4 by the dropwise addition of 0.5 M HCl. The aqueous layer was extracted with EtOAc (5x10 mL). The combined organic layers were dried over MgSO$_4$, filtered and concentrated to obtain compound 214. $R_f$ 0.54 (9:1 CH$_2$Cl$_2$-MeOH)
(2S,4R)-2-(Allyloxy carbonylamino)-6-tert-butyl-4-(tert-butyl dimethylsilyloxy)-adipic acid:

2,6-Lutidine (51 µL, 47 mg, 0.44 mmol, 2.5 equiv.) and TBSCI (66 mg, 0.44 mmol, 2.5 equiv.). were added sequentially to a solution of acid 214 in dry CH$_2$Cl$_2$ (2 mL) at rt under N$_2$. Reaction mixture was stirred and concentrated to remove the CH$_2$Cl$_2$. Water (2 mL) was added to the reaction mixture which was then cooled to 0 °C and acidified to ~pH 4 by the dropwise addition of 0.5 M HCl. The acidic solution was then extracted with EtOAc (4 X 10 mL). The combined organic layers were dried over MgSO$_4$, filtered and concentrated to give the carboxylic acid that was used directly in the next step without further purification. $R_f$ 0.61 (95:5 CH$_2$Cl$_2$-MeOH).

(2S,4R)-2-(Allyloxy carbonylamino)-6-(tert-butyl)-4-(tert-butyldimethylsilyloxy)-1-(2-oxopropyl)-adipate (222): The carboxylic acid was dissolved in dry CH$_2$Cl$_2$ (2 mL) and cooled to 0 °C under N$_2$ atmosphere. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (68 mg, 0.352 mmol, 2.0 equiv.) was added, followed by DMAP (6 mg, 0.0528 mmol, 0.3 equiv.) and acetol (36 µL, 39 mg, 0.528 mmol, 3.0 equiv.) at 0 °C. The reaction mixture was warmed to rt, stirred overnight and then concentrated to afford a viscous oil. Flash chromatography on silica gel (Hex-EtOAc 3:1) afforded the compound 222 as colorless oil (25 mg, 30% over 4 steps). $R_f$ 0.60 (1.1 Hex-EtOAc). [α]$_{D}^{25}$ -13.25 (c 0.5, CHCl$_3$). $^1$H NMR (CDCl$_3$, 400 MHz) δ 0.08 (s, 3H), 0.09 (s, 3H), 0.89 (s, 9H), 1.45 (s, 9H), 1.93-1.99 (m, 1H), 2.17 (s, 3H), 2.30-2.51 (m, 3H), 4.29 (app. pent. $J = 5.9$ Hz, 1H), 4.50 (ddd, $J = 12.4$, 7.7, 4.8 Hz, 1H), 4.57-4.78 (m, 4H), 5.20 (d, $J = 10.5$ Hz, 1H), 5.30 (d, $J = 16.0$ Hz, 1H), 5.62 (d, $J = 7.1$ Hz, 1H), 5.90 (ddt, $J = 16.0$, 10.7, 5.3 Hz, 1H); $^{13}$C NMR (CDCl$_3$, 100 MHz) δ -4.6, -4.7, 17.9, 26.1, 27.9, 28.1, 38.3, 43.1, 51.4, 65.9, 67.2, 68.7, 80.9, 117.7, 132.7, 155.9, 171.7, 173.5, 201.0. HRMS (ESI) calcd for C$_{23}$H$_{42}$NO$_8$Si (M+H)$^+$ 488.2674, obsd 488.2684.
2,6-Lutidine (107 µL, 99 mg, 0.924 mmol, 18.0 equiv.) was added dropwise to a solution of compound 222 (25 mg, 0.051 mmol, 1.0 equiv.) in dry CH₂Cl₂ (2 mL) under N₂ at rt. The mixture was cooled to 0 °C and stirred for 5 min before the dropwise addition of TBSOTf (117 µL, 135 mg, 0.510 mmol, 10.0 equiv.). Reaction mixture was warmed to rt and stirred for 6 h. A solution of potassium carbonate (8 mg, 0.061 mmol, 1.2 equiv.) in 10% H₂O:THF (1 mL) was added and reaction mixture stirred for overnight at rt. The reaction mixture was concentrated to remove the CH₂Cl₂ and then acidified with saturated aqueous NaHSO₄ to ~pH 4. Water (2 mL) was added to the reaction mixture. The acidic solution was then extracted with EtOAc (5 X 10 mL). The combined organic layers were dried over MgSO₄, filtered and then concentrated to afford a viscous oil. Flash chromatography on silica gel (Hex-EtOAc 1:2) afforded the compound 54 as colorless oil (12 mg, 54%). Rf 0.34 (1:2 Hex-EtOAc). [α]D²⁵ -10.25 (c 0.5, CHCl₃). ¹H NMR (CDCl₃, 400 MHz) δ 0.08 (s, 3H), 0.09 (s, 3H), 0.89 (s, 9H), 1.93-1.99 (m, 1H), 2.17 (s, 3H), 2.30-2.51 (m, 3H), 4.29 (app. pent. J = 5.9 Hz, 1H), 4.50 (ddd, J = 12.4, 7.7, 4.8 Hz, 1H), 4.57-4.78 (m, 4H), 5.20 (d, J = 10.5 Hz, 1H), 5.30 (d, J = 16.0 Hz, 1H), 5.62 (d, J = 7.1 Hz, 1H), 5.90 (ddt, J = 16.0, 10.7, 5.3 Hz, 1H); ¹³C NMR (CDCl₃, 100 MHz) δ -2.4, 27.5, 33.3, 43.1, 45.4, 49.4, 65.9, 71.2, 73.7, 117.7, 132.7, 155.9, 173.7, 175.1, 201.0. HRMS (ESI) calcd for C₁₉H₃₄NO₅Si (M+H)⁺ 432.2084, obsd 432.2078.
4.7.3 Spectra

Compound 192 (Scheme 4.8) - $^1$H NMR spectrum

SY-03-25 in MeOD at 400 MHz
Compound 192 (Scheme 4.8) - $^{13}$C NMR spectrum

SY–03–25 in MeOD at 100 MHz
Compound 196 (Scheme 4.11) – $^1$H NMR spectrum

SY-03-64 in CDCl$_3$ at 400 MHz
Compound 196 (Scheme 4.11) - $^{13}$C NMR spectrum

SY–03–64 in CDCl3 at 100 MHz
Compound 200 (Scheme 4.12) – $^1\text{H}$ NMR spectrum

SY-03-76 in CDCl3 at 400 MHz
Compound 200 (Scheme 4.12) - $^{13}$C NMR spectrum

SY-03-76 in CDCl$_3$ at 100 MHz
Compound 207 (Scheme 4.16) – $^1$H NMR spectrum

SY-03-85 in CDCl3 at 400 MHz
Compound 207 (Scheme 4.16) - $^{13}$C NMR spectrum

SY–03–85 in CDCl3 at 400 MHz
Compound 195 (Scheme 4.17) – $^1$H NMR spectrum

SY–03–89 in CDCl3 at 400 MHz
Compound 195 (Scheme 4.17) – $^{13}$C NMR spectrum

SY-03–89 in CDCl$_3$ at 100 MHz
Compound **208** (Scheme 4.17) – $^1$H NMR spectrum

SY–03–95 in CDCl3 at 400 MHz

![NMR Spectrum](image)
Compound 208 (Scheme 4.17) – $^{13}$C NMR spectrum

SY-03-95 in CDCl3 at 100 MHz
Compound 209 (Scheme 4.18) – $^1$H NMR spectrum

SY-03-110 in CDCl3 at 400 MHz
Compound 209 (Scheme 4.18) - $^{13}$C NMR spectrum

SY-03-110 in CDCl3 at 100 MHz
Compound 210 (Scheme 4.18) – $^1$H NMR spectrum

SY-03-125 in CDCl$_3$ at 400 MHz
Compound 210 (Scheme 4.18) - $^{13}$C NMR spectrum

SY-03-125 in CDCl3 at 100 MHz
Compound 216 (Scheme 4.20) – $^1$H NMR spectrum

SY-04–61 in CDCl3 at 400 MHz
Compound 216 (Scheme 4.20) – $^{13}$C NMR spectrum

SY-04–61 in CDCl3 at 100 MHz
Compound 58a (Scheme 4.22) – $^1$H NMR spectrum

SY-04-105 in CDCl$_3$ at 400 MHz
Compound 58a (Scheme 4.22) – $^{13}$C NMR spectrum

SY-03-105 in CDCl$_3$ at 100 MHz
Compound 222 (Scheme 4.24) – $^1$H NMR spectrum

SY–04–18 in CDCl3 at 400 MHz
Compound 222 (Scheme 4.24) – $^{13}$C NMR spectrum

SY-04-18 in CDCl3 at 100 MHz
Compound 54 (Scheme 4.24) – $^1$H NMR spectrum

SY-04-152 in CDCl$_3$ at 400 MHz
Compound 54 (Scheme 4.24) – $^{13}$C NMR spectrum

SY-01-152 in CDCl$_3$ at 100 MHz
5.1 Retrosynthesis for the Western Hemisphere of Theonellamide C

Revisiting our retrosynthetic analysis from Chapter 1, recall that our approach to the western hemisphere of theonellamide C involves major disconnections between τ-HAL/Asn, Ahad/β-Ala and τ-HAL/Ahad residues (Scheme 5.1). This leads to three building blocks: tetrapeptide 52, τ-HAL 53 and Ahad 222. In a forward sense, the initial peptide bond to be formed will be between the D-Ala acid component of τ-HAL and the amino-terminus of the tetrapeptide (I), followed by a condensation of the L-His amine component of τ-HAL with δ-acid of Ahad (II). Deallylation of the two termini will be accomplished simultaneously prior to cyclization of the amino-terminus of Ahad 222 with the carboxyl-terminus of tetrapeptide 52 (III). The site chosen for cyclization is ideal, since the β-Ala residue of the tetrapeptide is not susceptible to racemization during peptide bond formation.

Scheme 5.1 Western hemisphere of theonellamide C: Retrosynthesis.

5.2 Formation of a Dipeptide Containing erythro-HydroxyAsparagine (eHyAsn)

The synthesis of tetrapeptide 52 required the preparation of an erythro-hydroxyasparagine-phenylalanine (eHyAsn-Phe) dipeptide. Douglas Wong had made Boc-
eHyAsn-Phe-OMe (223). His approach built directly on Boger’s synthesis of the eHyAsn diastereomer.

With dipeptide 223 in-hand, we sought to assemble the western fragment of theonellamide C. The methyl ester of dipeptide 223 was hydrolyzed with lithium hydroxide (Scheme 5.2). Unfortunately, the TBS group was lost on acidification, during work-up. We therefore hydrolyzed the ester with a single equivalent of LiOH and freezedried the mixture to give the lithium salt 224. The hydrochloride salt of β-alanine allyl ester (225) was prepared according to an analogous preparation of the corresponding benzyl ester. The lithium salt 224 was coupled with 225. Poor yields led us to an alternative C-terminal protecting group that could be removed without byproducts or salt formation.

Scheme 5.2 Formation of dipeptide.

We needed more material to form the new dipeptide 234 containing a benzyl ester at the carboxyl end (Scheme 5.3). Therefore we repeated Wong’s synthesis of the dipeptide, with some modifications, to obtain useful quantities. Sharpless aminohydroxylation of the double bond in methyl p-methoxycinnamate (227) proceeded as described previously to give 228 except that 228 did not precipitate from the reaction mixture as described. Flash chromatography was required for isolation. We then inverted the configuration at the stereogenic center bearing the OH substituent to give 229 by performing a Mitsunobu reaction with p-nitrobenzoate as the nucleophile. Wong had reported tetrahydrofuran (THF) as the solvent for this reaction and subjected the crude product mixture to the cleavage of the ester. In
the current work no desired product was isolated following this protocol. We then performed the Mitsunobu reaction with toluene as solvent. The reaction was monitored with TLC which showed appearance of a less polar product. The \( p \)-nitrobenzoate ester was cleaved by azidolysis to obtain a 65% yield of inverted alcohol 229. The secondary alcohol was protected as its TBS ether using TBSCl and the methyl ester was ammonolyzed to generate the side chain amide, \( \text{viz.} \) compound 231. We swapped the Cbz group for the \textit{tert}-butyl carbamate to give 232. The oxidative cleavage of the electron-rich aromatic ring was performed to obtain the carboxylic acid that was coupled with phenylalanine benzyl ester hydrochloride (233) to give dipeptide 234 (Scheme 5.3). Detailed discussion of these reactions is given in the dissertation of Douglas Wong (Chapter 2).\(^{12}\)

Scheme 5.3 Synthesis of a dipeptide containing \textit{erythro}-hydroxyasparagine.

While performing the coupling reaction between acid 232 and amine 233 we were cognizant of side reactions with the unprotected side chain of asparagine.
5.3 Side Reactions of the Unprotected Side Chain Amide of Asparagine

Asparagine (abbreviated as Asn or N, 237 and 238) is a non-essential amino acid which is coded for by AAU and AAC in mRNA. There is one carboxamide group on the side chain with one amino and one carboxyl group on the alpha carbon atom. Hence it can be considered an amide of aspartic acid (235 and 236). Asparagine is a neutral, polar, uncharged amino acid under any biologically relevant pH conditions.

The amide group of asparagine can be slowly hydrolyzed to the carboxyl group to form aspartic acid. This conversion is related to the molecular basis of aging.

![Chemical structures of D- and L-aspartic acid and D- and L-asparagine.](image)

Figure 5.1 Chemical structures of D- and L-aspartic acid and D- and L-asparagine.

The carboxamido groups of asparagine should be suitably blocked during activation of the residues to prevent cyclization via the following two side reactions.

1. Aspartimide Formation: This is a general problem for peptides incorporating asparagine and the likelihood depends on the sequence and situation. Treatment of aspartyl/glutamyl peptides with acid or base can lead to a rearrangement through a cyclic imide intermediate and produces a mixture of two peptides (Scheme 5.4). The aspartimide is more stable than the glutamimide, and more ω-peptide (isopeptide) is formed than α-peptide. The attack of the amide nitrogen at the side chain acyl carbon is influenced by the nature of Y and the nature of the residue located at C-terminal to the susceptible residue. The reaction occurs easily when the Y is a good leaving group and less readily when unsubstituted (Y = OH). The problem is solved by ester groups that are severely hindered. Bodanszky et al. showed that the amount of imide formed decreases in the order OBzl >> OcHex > ODpn. [Dmpn = 2,4-dimethylpent-3-yl]
Scheme 5.4 Imide formation from a peptide sequence followed by generation of two peptide chains.

One way of reducing this side reaction in the case of Asn is substitution on the carboxamido group which increases the solubility of derivatives in organic solvents and eliminates the side reactions. The carboxamide group does not undergo acylation or alkylation during chain assembly.

2. Isoaspartimide formation upon activation of $\alpha$-COOH of Asn. Upon activation of the $\mathrm{C}_\alpha$ carboxylic acid, the unprotected side chain of asparagine can undergo isoaspartimide formation, followed by dehydration to give the $\beta$-cyanoalanine derivative (Scheme 5.5). This occurs prominently when coupling with phosphonium salts. The cyano group does not interfere with couplings, so the dehydrated residue is incorporated into the peptide. The dehydration is completely reversed by HF, partly reversed by TFA and very effectively suppressed for carbodiimide- or HBTU-mediated reactions by the presence of one equivalent of 1-hydroxybenzotriazole (HOBt). The role of this additive is to serve as a superior proton donor, relative to the NH of the isoaspartimide. Thus the formation of the $\mathrm{BtO}^-$ anion occurs in preference to dehydration to generate a nitrile.
5.4 Formation of Tetrapeptide

The benzyl ester of dipeptide 234 was subjected to hydrogenolysis to give acid 239 that was coupled to 225. The tripeptide was formed under conditions proven to minimize epimerization of Cα of the Phe residue. This approach now gave a satisfactory yield of tripeptide 226 (Scheme 5.6).

5.4.1 Deprotection of α-Amine

Before elongating 226 in the N-terminal direction with a suitably protected asparagine residue, it was necessary to remove the tert-butyl carbamate in the presence of the tert-butyldimethylsilyl ether (OTBS). This turned out to be challenging and several known methods were investigated.

Greshock and Funk used 10% trifluoroacetic acid (TFA) in dichloromethane to remove the Boc group from intermediate compound 240 for the synthesis of welwistatin (Scheme 5.7-A).100 Boger et al.94 achieved the deprotection of the Boc group, in the presence of TBS ethers, and trityl amides in complex peptides, with B-bromocatecholborane (BCB) (Scheme 5.7-B).101
Pearson and Cui\textsuperscript{102} used TBSOTf for the removal of a Boc group (Scheme 5.7-C). Removal of Boc via TBS-carbamatess was first introduced by Sakaitani and Ohfune.\textsuperscript{103}

Scheme 5.7 Examples of Boc group deprotection in the presence of TBS ether.

Preliminary results from attempted deprotection of tripeptide 226 by treatment of trifluoroacetic acid (TFA) gave a mixture of starting material and a compound that arises from double deprotection of both TBS and Boc groups. We used Boc-Ser(OTBS)-OMe (246) as a model system for the deprotection of Boc in presence of TBS ether. Unfortunately, when BCB was applied to both model and real systems, no desired product was isolated. The strategy of chemoselective transformation of an amino protecting group, via a tert-butyldimethylsilyl carbamate and cleaving it with TBAF worked well on a model system (246) but failed on the real system. Finally, treatment of compound 226 with an increased amount of trifluoroacetic acid in dichloromethane (1:1 TFA /CH\textsubscript{2}Cl\textsubscript{2}) resulted in amine 247 (Table 5.1, Scheme 5.8).
Table 5.1 Deprotection of N-terminus

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<thead>
<tr>
<th>Boc Protected Amine</th>
<th>Reaction Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBSO(\text{NH}^\text{Boc})(\text{OCH}_3) \text{246}</td>
<td>TBSOTf, (\text{CH}_2\text{Cl}_2)</td>
<td>Recovered starting material.</td>
</tr>
<tr>
<td>\text{246}</td>
<td>50% TFA in (\text{CH}_2\text{Cl}_2)</td>
<td>Product obtained in 45% yield after methanolysis and flash chromatography.</td>
</tr>
<tr>
<td>\text{246}</td>
<td>1 h treatment with TFA at 0 °C, reaction progress was monitored by TLC (6:4:1 (\text{CHCl}_3):MeOH:H(\text{2}O)). TFA salt of free amine was taken in to the next coupling reaction without further purification.</td>
<td></td>
</tr>
<tr>
<td>\text{226}</td>
<td>50% TFA in (\text{CH}_2\text{Cl}_2)</td>
<td>No desired product was isolated</td>
</tr>
<tr>
<td>\text{226}</td>
<td>1 h treatment with TFA at 0 °C, product was obtained in 98% yield after flash chromatography.</td>
<td></td>
</tr>
</tbody>
</table>

5.4.2. Elongation to Tetrapeptide

Formation of tetrapeptide \textbf{249} was carried out using asparagine derivative \textbf{248}, EDC and hydroxybenzotriazole (HOBt) (Scheme 5.8).

Scheme 5.8 Preparation of a tetrapeptide.

5.5 Model Studies for Cyclization

Before investigating fragment condensations with valuable tetrapeptide \textbf{249} containing eHyAsn, a model de-oxy tetrapeptide \textbf{252} was prepared, substituting regular Asn for HyAsn. We
started with the formation of dipeptide 250 (Scheme 5.9) using commercially available asparagine building block 248 and phenylalanine 233 in order to rapidly assemble model tetrapeptide 250. We obtained a poor yield of the product because of the high polarity and difficulty in isolation and purification.

Scheme 5.9 Formation of a model dipeptide containing Phe and Asn.

Hence we started with Fmoc-Asn(Trt)-OH (251) in place of asparagine with an unprotected side chain, and coupled with phenylalanine benzyl ester hydrochloride (233) (Scheme 5.10). The dipeptide was then subjected to hydrogenolysis to obtain the free acid and coupled to β-Ala-OAll to form tripeptide 253. Next, deblocking of Fmoc was achieved by diethylamine and then coupled to Fmoc-Asn(Trt)-OH to form the tetrapeptide 254. It was then subjected to Fmoc cleavage with diethylamine and flash chromatography was performed to isolate the product. The trityl groups were removed with 1:1 TFA/CH₂Cl₂ to obtain free amine 255.

Scheme 5.10 Preparation of a model tetrapeptide.
In collaboration with Chyree Batton, the next step was to couple the \( \tau \)-histidinoalanine residue with the tetrapeptide assembled above. Hydrogenolysis was carried out to deblock the benzyl ester in \( \tau \)-HAL 256 to obtain 257 (Scheme 5.11). The free amine 255 was coupled to \( \tau \)-HAL derivative 257, which afforded 258. The next step is to deprotect the \( L \)-His amine of \( \tau \)-HAL using TFA to give amine 259.

Scheme 5.11 Coupling of model tetrapeptide and \( \tau \)-HAL derivative.

Proton NMR of compound 258 provided evidence for the successful coupling of the two fragments. While the 2.5-5.0 ppm region of the spectrum was complex, integration was consistent with the structure of 258 and a strong singlet at 3.8 ppm was assigned to the methyl ester. Another strong singlet appeared at 1.4 ppm for the Boc group. The allyl ester of the \( \beta \)-Ala residue gave rise to characteristic signals for the three olefinic protons in the 5-6 ppm region. An ion was observed at m/z 1065.4655, consistent with (M+H) for the molecular formula of 258 (\( C_{53}H_{85}N_{10}O_{14} \)).
In a parallel experiment, we coupled τ-HAL with of α-amino adipic acid derivative (Scheme 5.11). Removal of the Boc group from τ-HAL was carried out using TFA. The free amine was condensed with the acid of α-AAA which afforded 261. Proton NMR of compound 261 provided evidence for the successful coupling of the two fragments. A strong singlet at 3.7 ppm was assigned to the methyl ester. Another strong singlet appeared at 1.8 ppm for the CH$_3$ group of the aceto moiety. Hβs were resonated as multiplet at 2.8-3.0. Hαs were appeared in the region of 4.1-4.8 ppm. Benzyl ester protons were characteristically observed at 5.2 ppm. The allyl ester of the NHAloc group gave rise to characteristic signals for the three olefinic protons in the 5-6 ppm region. Imidazole H5 and H2 were observed at 7.2 and 6.4 ppm. Fmoc protons were observed in the aromatic region (7.3-7.4 ppm). An ion was observed at m/z 751.3841, consistent with (M+H) for the molecular formula of 261 ($C_{41}H_{45}N_{5}O_{9}$).

5.6 Precedent for Pairs of Palladium-Labile Protecting Groups

Hirschmann et al. synthesized homodetic tricyclic peptide 264 to study the conformation of the hormone somatostatin (SRIF). To execute consecutive ring closures in a controlled manner, they paired carboxyl and amine protecting groups that could be simultaneously and selectively removed. They chose Alloc/Allyl as one pair of amine/carboxy protecting group partners. Deprotection of the Alloc and Allyl group was accomplished simultaneously in 94% yield using Pd(0) as catalyst and tributyltin hydride (Bu$_3$SnH) as the allyl acceptor (Scheme 5.12). Subsequent cyclization was carried out using diphenylphosphoryl azide [DPPA, ($C_6H_5O$)$_2$P(O)N$_3$, 49]. Diphenylphosphoryl azide (49, DPPA) was also used by Tohdo et al. for ring closure of the western hemisphere of theonellamide F (Scheme 5.13). However, their protecting groups removed prior to cyclization were not allyl-based.
Scheme 5.12 Removal of Allyl and Alloc groups by Pd(0), cyclization and formation of the homodetic tricyclic peptide.

Scheme 5.13 Cyclization of the western hemisphere of theonellamide F by Tohdo et al.\textsuperscript{23}

Vernall \textit{et al.} synthesized an \textit{α}-helical mimetic 266 for the 8-12 sequence of galanin, a peptide with anti-cancer and anti-diabetic properties.\textsuperscript{105} In the final stages of their synthesis, they treated a mixture of diastereomers of 265 with Pd(0) for simultaneous deprotection of the Allyl/Alloc protecting groups and used barbituric acid as the allyl acceptor, followed by BOP-mediated cyclization to obtain cyclized product 266 as a mixture of diastereomers (Scheme 5.14).
Scheme 5.14 Synthesis of cyclic pentapeptide.

5.7 Future Work

5.7.1 Formation of Western Ring

Coupling of the free γ-acid of α-AAA building block 195 (Scheme 5.15) with the primary amine 259 should lead to cyclization precursor 267. Treatment of 267 with Pd(0) will promote simultaneous removal of the C-terminal allyl group and the N-terminal allyloxycarbonyl group to give amino acid 268.

Scheme 5.15 Formation of the western hemisphere of theonellamide C.
Cyclization will result in the western hemisphere of theonellamide, compound 172. The model system presented enables us to optimize the reaction conditions for the coupling and cyclization. Moreover, this approach will provide simplified analogs for SARs studies (Section 5.7.3).

5.7.2 Completion of Theonellamide C

The primary goal is to synthesize theonellamide C (7). In a forward sense, the western hemisphere will be constructed first followed by coupling of the D-Ala amine component of \( \tau \)-HAL with a serine residue (I) (Scheme 5.16).

![Scheme 5.16 Formation of theonellamide C.](image)

The next peptide bond to be formed (II) will be between the \( L \)-His acid component of \( \tau \)-HAL and the amino terminus of the eastern hemisphere tetrapeptide which will lead to cyclization precursor 269. The amine component of serine and acid of Aboa will undergo HATU-mediated
cyclization under high dilution conditions (III). The site chosen for cyclization is ideal, since the Aboa residue is not susceptible to racemization during peptide bond formation. Global desilylation will be achieved by treating with TBAF as shown by Benson Edagwa's work toward the synthesis of allovirodin and analogs.

5.7.3 Structure Activity Relationships (SARs) of Theonellamides

Synthesis of the western and eastern hemisphere represent penultimate goals. The plan for formation of the western hemisphere was shown earlier in Section 5.1 and the plan for synthesis of the eastern hemisphere is the subject of Douglas Wong’s dissertation. Each ring will be tested separately for antifungal activity. With the exception of the bridging τ-HAL residue, all other residues can be substituted for using commercially available building blocks to produce a bicyclic structure with a ring size consistent with the theonellamides (Figure 5.2).

![Figure 5.2](image_url)
There are two amino acids in the western hemisphere that are not commercially available, eHyAsn (purple) and Ahad (green). Synthesis of the western hemisphere by replacing one amino acid at a time with a commercially available amino acid could elucidate the importance of each modified residue (270 & 271, Figure 5.2). The modified eastern hemisphere contains commercially available allo-threonine, serine, phenylalanine, and β-phenylalanine (273, Figure 5.2). The tetrapeptide will be coupled with the τ-HAL-serine tripeptide. Each hemisphere analog will be tested for their biological activity to reveal whether or not they independently demonstrate antifungal activity. In the end, both hemispheres will be coupled together to form the bicycle.

The identification of a minimal chemical structure that will result in potent antifungal activity could be theonellamide X (274, Figure 5.3), an unnatural analog in which all the substitutions proposed above are incorporated simultaneously. Our on going synthetic efforts are necessary to probe the structure activity relationship of the theonellamides.

Figure 5.3 Chemical composition of Theonellamide X.

5.8 Experimental Section

5.8.1 General Methods

The general methods are as described in Chapter 2. NMR spectra were recorded on a Bruker AV-400 or a Bruker AV Nanobay-400 liquid spectrometer.
5.8.2 Procedures

![Chemical Structure](image)

(2S,3R)-Methyl 3-(benzyloxycarbonylamino)-2-hydroxy-3-(4-methoxyphenyl)propanoate (228)

Benzyl carbamate (9.05 g, 60.0 mmol, 2.30 equiv.) was dissolved in n-PrOH (70 mL). A solution of NaOH (2.44 g, 61.0 mmol, 2.35 equiv.) in H₂O (110 mL) was added and the resulting solution stirred for 10 min. Freshly prepared tert-butyl hypochlorite¹⁰⁷ (6.90 mL, 6.62 g, 61.0 mmol, 2.35 equiv.) was added dropwise and the resulting solution stirred for an additional 10 min. (DHQD)$_2$PHAL (0.83 g, 1.3 mmol, 5 mol %) in n-PrOH (40 mL) was added and the reaction vessel immersed in a water bath at ambient temperature and stirred for 5 min. (E)-Methyl 3-(4-methoxyphenyl)acrylate 227 (5.0 g, 26.0 mmol, 1.0 equiv.) was added as a solid all at once, followed immediately by K$_2$OsO$_2$(OH)$_4$ (0.38 g, 1.0 mmol, 4 mol %). The reaction mixture was stirred for 3 h at 0 °C. The reaction mixture was evaporated and the residue subjected to flash chromatography (Hex-EtOAc 3:1) to afford compound 228 as a colorless solid (6.35 g, 64%). $R_f$ 0.32 (Hex-EtOAc 1:1). $[\alpha]_D^{25}$ -5.9 (c 1.0, CHCl₃. $^1$H NMR (CDCl₃, 400 MHz) δ 3.18 (br s, 1H), 3.80 (s, 6H), 4.45 (s, 1H), 5.07 (dd, $J = 17.8, 12.0$ Hz, 2H), 5.21 (d, $J = 9.1$ Hz, 1H), 5.65 (d, $J = 9.3$ Hz, 1H), 6.88 (d, $J = 8.7$ Hz, 2H), 7.25-7.35 (m, 7H); $^{13}$C NMR (CDCl₃, 100 MHz) δ 53.1, 55.3, 56.0, 67.0, 73.5, 114.1, 128.0, 128.1, 128.2, 128.5, 131.0, 136.3, 155.7, 159.2, 173.3; HRMS (ESI) calcd for C$_{19}$H$_{20}$NO$_6$ (M-H)$^+$ 358.1496, obsd 358.1489.

![Chemical Structure](image)

(2R,3R)-Methyl 3-(benzyloxycarbonylamino)-2-hydroxy-3-(4-methoxyphenyl)propanoate (229)

$p$-Nitrobenzoic acid (855 mg, 5.12 mmol, 5.5 equiv.) was added to a solution of (2S,3R)-methyl 3-(benzyloxycarbonylamino)-2-hydroxy-3-(4-methoxyphenyl)propanoate 228 (500 mg, 0.93 mmol, 1.0 equiv.) in dry toluene (10 mL). Triphenylphosphine (1.43 g, 5.12 mmol, 5.5
equiv.) was added under N₂ and reaction mixture was cooled to 0 °C. Diisopropyl azodicarboxylate (1 mL, 1.04 g, 5.12 mmol, 5.5 equiv.) was slowly added via syringe. Upon completion of the addition, the ice bath was removed and the reaction mixture stirred at room temperature for 24 h. The reaction mixture was concentrated and the residue dissolved in anhydrous MeOH (10 mL). Sodium azide (302 mg, 4.65 mmol, 5.0 equiv.) was added and the mixture heated at 45 °C for 18 h. The solvent was removed under reduced pressure and the product isolated from the residue by flash chromatography (Hex-EtOAc, 3:1) to give 229 as a pale yellow solid (325 mg, 65%). Rf 0.32 (1:1 Hex-EtOAc). [α]D²⁵ -20.5 (c 1.0, CHCl₃). ¹H NMR (CDCl₃, 400 MHz) δ 2.94 (br s, 1H), 3.70 (s, 3H), 3.78 (s, 3H), 4.59 (dd, J = 6.5, 3.3 Hz, 1H), 5.11 (d, J = 12.2 Hz, 1H), 5.11 (d, J = 12.2 Hz, 1H), 5.10-5.13 (m, 1H), 5.80 (d, J = 8.4 Hz, 1H), 6.83 (d, J = 8.6 Hz, 2H), 7.18 (d, J = 12.2 Hz, 2H), 7.24-7.35 (m, 5H); ¹³C NMR (CDCl₃, 100 MHz) δ 52.7, 55.2, 56.4, 67.0, 73.1, 113.9, 128.2, 128.5, 128.6, 136.3, 155.5, 159.5, 172.3; HRMS (ESI) calcd for C₁₉H₂₀NO₆ (M-H)⁺ 358.1296, obsd 358.1295.

![Chemical Structure](image)

(2R,3R)-Methyl-3-(benzyloxycarbonylamino)-2-(tert-butyldimethylsilyloxy)-3-(4-methoxyphenyl)propanoate (230)

Imidazole (954 mg, 13.0 mmol, 15.0 equiv.) was added to a solution of (2R,3R)-methyl 3-(benzyloxycarbonylamino)-2-hydroxy-3-(4-methoxyphenyl)propanoate 229 (400 mg, 1.1 mmol, 1.0 equiv.) in dry CH₂Cl₂ (5 mL). The mixture was stirred for 10 min before addition of TBSCI (1700 mg, 11.0 mmol, 10.0 equiv.). The reaction mixture was stirred for 17 h at rt. The reaction mixture was diluted with ethyl acetate (25 mL) and concentrated. Flash chromatography on silica gel (Hex-EtOAc, 2:1) afforded the TBS ether 230 as a clear oil (448 mg, 85%). Rf 0.24 (4:1 Hex-EtOAc). [α]D²⁵ -16.8 (c 1.0, CHCl₃). ¹H NMR (CDCl₃, 400 MHz) δ 0.03 (s, 3H), 0.07 (s, 3H), 0.89 (s, 9H), 3.53 (s, 3H), 3.74 (s, 3H), 4.59 (d, J = 3.9 Hz, 1H), 5.02-5.12 (m, 3H), 5.53 (d, J = 8.0 Hz, 1H), 6.79 (d, J = 8.7 Hz, 2H), 7.22 (d, J = 7.9 Hz, 2H), 7.21-
7.32 (m, 5H); $^1$C NMR (CDCl$_3$, 100 MHz) δ -5.5, -5.1, 18.3, 25.7, 51.6, 55.2, 57.0, 66.8, 74.3, 113.7, 128.1, 128.5, 128.9, 129.6, 136.4, 155.5, 159.3, 171.4; HRMS (ESI) calcd for C$_{25}$H$_{36}$NO$_5$Si (M+H)$^+$ 474.2106, obsd 474.21

Benzyl (1$R$, 2$R$)-3-amino-2-(tert-butyldimethylsilyloxy)-1-(4-methoxyphenyl)-3-oxopropylcarbamate (231)

Ammonia gas was bubbled through a solution of (2$R$,3$R$)-methyl 3-(benzyloxycarbonylamino)-2-(tert-butyldimethylsilyloxy)-3-(4-methoxyphenyl)propanoate (230) (373 mg, 0.78 mmol) in dry MeOH (20 mL) at 0 °C. The reaction vessel was stoppered and the mixture stirred for 7 d at room temperature. The solution was cooled to 0 °C and resaturated with ammonia gas. The reaction vessel was stoppered and the mixture stirred for 7 d at room temperature. The solvent was removed under reduced pressure. Flash chromatography (Hex-EtOAc, 3:1) led to recovery of the methyl ester (72 mg, 20%) and isolation of the primary amide 231 as a colorless foam (239 mg, 66%). $R$$_f$ 0.29 (1:1 Hex-EtOAc). [α]$^2$$^5$$_D$ +3.9 (c 1.0, CHCl$_3$). $^1$H NMR (CDCl$_3$, 400 MHz) δ -0.02 (s, 3H), 0.01 (s, 3H), 0.92 (s, 9H), 3.77 (s, 3H), 4.43 (d, $J$ = 3.7 Hz, 1H), 4.95-5.08 (m, 3H), 5.61 (d, $J$ = 6.1 Hz, 1H), 6.03 (s, 1H), 6.09 (s, 1H), 6.82 (d, $J$ = 8.1 Hz, 2H), 7.23 (d, $J$ = 7.8 Hz, 2H), 7.31-7.33 (m, 5H); $^1$C NMR (CDCl$_3$, 100 MHz) δ -5.5, -5.4, 18.0, 25.7, 55.2, 57.6, 66.8, 89.9, 113.6, 128.0, 128.1, 128.4, 129.1, 129.7, 136.4, 155.5, 159.3, 171.4; HRMS (ESI) calcd for C$_{24}$H$_{35}$N$_2$O$_5$Si (M+H)$^+$ 459.2309, obsd 459.2313.

$^1$H NMR (CDCl$_3$, 400 MHz) δ -0.02 (s, 3H), 0.01 (s, 3H), 0.92 (s, 9H), 3.77 (s, 3H), 4.43 (d, $J$ = 3.7 Hz, 1H), 4.95-5.08 (m, 3H), 5.61 (d, $J$ = 6.1 Hz, 1H), 6.03 (s, 1H), 6.09 (s, 1H), 6.82 (d, $J$ = 8.1 Hz, 2H), 7.23 (d, $J$ = 7.8 Hz, 2H), 7.31-7.33 (m, 5H); $^1$C NMR (CDCl$_3$, 100 MHz) δ -5.5, -5.4, 18.0, 25.7, 55.2, 57.6, 66.8, 89.9, 113.6, 128.0, 128.1, 128.4, 129.1, 129.7, 136.4, 155.5, 159.3, 171.4; HRMS (ESI) calcd for C$_{24}$H$_{35}$N$_2$O$_5$Si (M+H)$^+$ 459.2309, obsd 459.2313.

A solution of benzyl (1$R$,2$R$)-3-amino-2-(tert-butyldimethylsilyloxy)-1-(4-methoxyphenyl)-3-oxopropylcarbamate 231 (850 mg, 2.1 mmol, 1.00 equiv.) and Boc$_2$O (443 mg, 2.3 mmol, 1.12 equiv.) in CH$_3$OH (20 mL) was treated with 10% Pd-C (45 mg). The reaction mixture was stirred...
under H$_2$ (1 atm) at 25 °C overnight. The Pd-C was removed by filtration through Celite®, and the filtrate was concentrated. Flash chromatography (Hex-EtOAc, 2:1) provided 232 as a colorless foam (745 mg, 95%). $R_f$ 0.30 (1:1 EtOAc-Hex). [$\alpha$]$^{26.5}_D$ 14.6 (c 1.0, CHCl$_3$). $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 0.04 (s, 6H), 0.93 (s, 9H), 1.40 (s, 9H), 3.76 (s, 3H), 4.40 (s, 1H), 4.91 (app s, 1H), 5.35 (app s, 1H), 6.03 (s, 1H), 6.51 (s, 1H), 6.81 (d, $J = 8.3$ Hz, 2H), 7.21 (d, $J = 8.3$ Hz, 2H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ -5.5, -5.3, 18.0, 25.7, 28.3, 55.1, 57.2, 76.0, 79.4, 113.5, 129.1, 129.9, 154.7, 159.1, 174.4. HRMS (ESI) calcd for C$_{21}$H$_{37}$N$_2$O$_5$Si (M+H)$^+$ 425.2129, obsd 425.2135.

A solution of sodium periodate (5.504 g, 26 mmol, 18.1 equiv) in water (130 mL) was added, all at once, to a solution of tert-butyl (1$R$,2$R$)-3-amino-2-(tert-butyldimethylsilyloxy)-1-(4-methoxyphenyl)-3-oxopropylcarbamate 232 (604 mg, 1.4 mmol, 1.00 equiv) in a mixture of EtOAc (16 mL) and MeCN (16 mL). The biphasic mixture was stirred by an overhead mechanical stirrer for 1 h at RT. Ruthenium trichloride trihydrate (60 mg, 0.3 mmol, 0.2 mol %) and sodium bicarbonate (464 mg, 5.5 mmol, 3.90 equiv.) were add sequentially, as solids, to give a black, biphasic mixture, that was stirred at RT overnight, during which time the mixture became orange, then yellow and eventually colorless and milky. The mixture was diluted with sat’d aq. NaHCO$_3$ (240 mL) and washed with CH$_2$Cl$_2$ (160 mL). The organic layer was back-extracted with sat’d aq. NaHCO$_3$ (240 mL). The combined aqueous layers were acidified at 0 °C with 10 % aq. HCl (~150 mL) to pH 2.5 and then extracted with EtOAc (12 X 250 mL). The combined organic layers were dried over MgSO$_4$, filtered, and concentrated to give acid (2$S$,3$R$)-4-amino-2-(tert-butoxycarbonylamino)-3-(tert-butyldimethylsilyloxy)-4-oxobutanoic acid
as a brown foam (456 mg) that was dissolved in THF (22 mL) and cooled to 0 °C under N₂.
Phenylalanine benzyl ester hydrochloride (233) (368 mg, 1.26 mmol, 1.00 equiv.) was added as a solid, followed by Et₃N (350 µL, 255 mg, 2.52 mmol, 2.00 equiv.), EDC (253 mg, 132 mmol, 1.05 equiv.) and HOBt (256 mg, 1.89 mmol, 1.50 equiv.). After 20 min the ice bath was removed, the mixture warmed to RT and stirred overnight. The mixture was concentrated and applied to a flash column in a minimum volume of CH₂Cl₂. Elution with a gradient of 2.5:1.0→1.0:2.0 (Hex-EtOAc) yielded dipeptide 234 as a colorless foam (415 mg, 50% over 2 steps). Rf 0.53 (1:1 EtOAc-Hex). [α]D²⁵ +25.7 (c 1.0, CHCl₃). ¹H NMR (CDCl₃, 400 MHz) δ 0.08 (s, 3H), 0.12 (s, 3H), 0.90 (s, 9H), 1.43 (s, 9H), 3.00-3.18 (m, 2H), 4.61-4.67 (m, 2H), 4.84 (dd, J = 13.8, 6.2 Hz, 1H), 5.07 (s, 2H), 5.47 (d, J = 6.7 Hz, 1H), 5.84 (br s, 1H), 6.46 (br s, 1H), 6.66 (d, J = 7.7 Hz, 1H), 7.04-7.16 (m, 2H), 7.18-7.28 (m, 5H), 7.33-7.35 (m, 3H); ¹³C NMR (CDCl₃, 100 MHz) δ -5.3, -5.1, 18.0, 25.6, 28.3, 37.9, 53.5, 53.5, 57.3, 67.2, 67.2, 69.6, 74.1, 80.1, 85.4, 127.1, 128.5, 128.6, 129.3, 135.1, 135.6, 154.8, 167.4, 170.8, 174.2. HRMS (ESI) calcd for C₃₁H₄₆N₃O₇Si (M+H)⁺ 600.3100, obsd 600.3118.

β-Alanine allyl ester hydrochloride (225)

β-Alanine (502 g, 5.6 mmol, 1.0 equiv.), allyl alcohol (3.4 mL, 2.9 g, 27.0 mmol, 4.8 equiv.) and chlorotrimethylsilane (1.2 mL, 1.03 g, 9.4 mmol, 1.6 equiv.) were heated at 100 °C for 4 h. The reaction mixture was cooled to RT and poured onto diethyl ether (115 mL) and stirred at 0 °C for 24 h. The precipitate was collected by filtration. The allyl protected β-alanine 225 was obtained as its hydrochloride salt (800 mg, 86%). m.p. 48-51 °C, Rf 0.38 (9:1 DCM-MeOH); ¹H NMR (CDCl₃, 400 MHz) δ 2.96 (t, J = 6.7 Hz, 2H), 3.38 (t, J = 6.7, 2H), 4.63 (d, J = 5.8 Hz, 2H), 5.24 (dd, J= 10.4, 1.1 Hz, 1H), 5.33 (dd, J = 17.2, 1.4), 5.86-5.96 (m, 1H), 8.2 (br, 2H); ¹³C NMR (CDCl₃, 100MHz) δ 31.3, 35.7, 65.9, 118.8, 131.7, 170.9; HRMS (ESI) calcd for C₆H₁₁NO₂ (M+H)⁺ 130.0868, obsd 130.0863.
To a solution of dipeptide benzylester (234) (100 mg, 0.16 mmol, 1.0 equiv.) in dry MeOH (6 mL) was added 10 % Pd-C (9 mg, 0.8 mmol, 0.5 equiv.). The suspension was stirred at RT overnight under H₂ gas (1 atm). The reaction mixture was filtered through Celite® to remove the Pd-C. The solution was concentrated to give the carboxylic acid as a colorless oil (85 mg) that was used directly in the next step without further purification. The intermediate carboxylic acid (85 mg, 0.166 mmol, 1.05 equiv.) was dissolved in DCM (6 mL) and cooled to 0 °C under N₂. β-Alanine allyl ester hydrochloride (225) (27 mg, 0.159 mmol, 1.00 equiv.) was added as a solid followed by 2,4,6-collidine (43 µL, 39 mg, 0.318 mmol, 2.00 equiv.) and HATU (64 mg, 0.166 mmol, 1.05 equiv.). After 20 min, the mixture was warmed to RT and stirred overnight. The mixture was concentrated and applied to a flash column in a minimum volume of DCM. Elution with a gradient of 2:1→1:2 (Hex-EtOAc) gave tripeptide 226 as a colorless oil (85 mg, 85%), R₉ 0.42 (2:1 EtOAc-Hex). [a]₀²⁵ +8.4 (c 1.0, CHCl₃). ¹H NMR (CDCl₃, 400 MHz) δ 0.13 (s, 3H), 0.15 (s, 3H), 0.91 (s, 9H), 1.43 (s, 9H), 2.35-2.52 (m, 2H), 2.96 (t, J = 6.7 Hz, 2H), 2.99 (dd, J = 13.6, 7.0 Hz, 1H), 3.18 (dd, J = 13.6, 6.1 Hz, 1H), 3.34-3.47 (m, 2H), 4.54 (d, J = 9.9 Hz, 2H), 4.50-4.64 (m, 3H), 5.25 (dd, J = 10.4, 1.3 Hz, 1H), 5.30 (dd, J = 17.2, 1.3 Hz, 1H), 5.38 br d, J = 6.3 Hz, 1H), 5.84-5.94 (m, 1H), 6.45 (br, 1H), 6.54 (br, 1H), 6.60 (br, 1H), 7.17-7.30 (m, 5H); ¹³C NMR (CDCl₃, 100MHz) δ -5.3, -5.0, 18.0, 25.7, 28.3, 33.7, 34.8, 37.8, 54.8, 57.7, 65.4, 74.0, 80.3, 118.6, 127.0, 128.7, 129.3, 131.8, 136.4, 155.0, 167.7, 170.2, 172.0, 174.0; HRMS (ESI) calcd for C₃₀H₄₆N₄O₈Si (M+H)⁺ 621.3328, obsd 621.3334.
Boc-Asn-HyAsn(OTBS)-Phe-β-Ala-OAll (249)

To a solution of tripeptide 226 (100 mg, 0.161 mmol, 1.00 equiv.) in dry CH₂Cl₂ (0.5 mL) was added TFA (0.5 mL). The reaction mixture was stirred for 1 hr at 0 °C and then concentrated to give the free amine 247 as a colorless oil (84 mg) that was used directly in the next step without further purification.

The intermediate amine 247 (84 mg, 0.161 mmol, 1.00 equiv.) was dissolved in CH₂Cl₂ (2 mL) and cooled to 0 °C under N₂. Nα-Boc-L-asparagine (248) (39 mg, 0.169 mmol, 1.05 equiv.) was added as a solid followed by diisopropylethylamine (56 µL, 42 mg, 0.322 mmol, 2.0 equiv.) and HATU (64 mg, 0.169 mmol, 1.05 equiv.). After 20 min, the mixture was warmed to RT and stirred overnight. The mixture was concentrated and applied to a flash column in a minimum volume of DCM. Elution with a gradient of 95:5→9:1 (CH₂Cl₂-MeOH) gave tetrapeptide 249 as a colorless oil (64 mg, 54%), Rᵣ 0.42 (2:1 EtOAc-Hex). [α]ₒ²⁵ +16.2 (c 1.0, MeOH).

¹H NMR (CD₃OD, 400 MHz) δ 0.13 (s, 3H), 0.15 (s, 3H), 0.91 (s, 9H), 1.43 (s, 9H), 2.36 (dd, J = 13.6, 7.0 Hz, 2H), 2.41-2.52 (m, 2H), 2.96 (t, J = 6.7 Hz, 2H), 2.99 (dd, J = 13.6, 7.0 Hz, 1H), 3.18 (dd, J = 13.6, 6.1 Hz, 1H), 3.34-3.47 (m, 2H), 4.54 (d, J = 9.9 Hz, 2H), 4.50-4.64 (m, 3H), 5.25 (dd, J = 10.4, 1.3 Hz, 1H), 5.30 (dd, J = 17.2, 1.3 Hz, 1H), 5.38 br d, J = 6.3 Hz, 1H), 5.84-5.94 (m, 1H), 7.17-7.30 (m, 5H); ¹³C NMR (CD₃OD, 100MHz) δ -5.3, -5.0, 25.7, 28.3, 30.5, 33.7, 34.8, 37.8, 38.1, 52.8, 55.7, 57.7, 65.4, 74.0, 80.3, 118.6, 127.0, 128.7, 129.3, 131.8, 136.4, 155.0, 167.7, 170.2, 172.0, 173.4, 173.5, 174.0; HRMS (ESI) calcd for C₃₄H₅₅N₆O₁₀Si (M+H)⁺ 735.3228, obsd 735.3234.
Fmoc-Asn(Trt)-Phe-β-Ala-OAll (253)

Fmoc-Asn(Trt)-Phe-OBn (252): Na-Fmoc-N-β-trityl-L-asparagine (251) (194 mg, 0.34 mmol, 1.00 equiv.) was dissolved in dry CH₂Cl₂ (2 mL) and cooled to 0 °C under N₂. Phenylalanine benzyl ester hydrochloride (100 mg, 0.34 mmol, 1.00 equiv.) was added as a solid, followed by Et₃N (95 µL, 69 mg, 0.68 mmol, 2.00 equiv.), EDC (69 mg, 0.36 mmol, 1.05 equiv.) and HOBt (69 mg, 0.51 mmol, 1.50 equiv.). After 20 min the ice bath was removed, the mixture warmed to RT and stirred overnight. The mixture was filtered through a pad of silica, washing well with 98:2 CH₂Cl₂-MeOH and concentrated to provide Fmoc-Asn(Trt)-Phe-OBn (252).

The dipeptide 252 was dissolved in dry MeOH (2 mL) and 10 % Pd-C (18 mg, 0.34 mmol, 1.0 equiv.) was added. The suspension was stirred at RT overnight under an atmosphere of H₂. The reaction mixture was filtered through Celite® and the filtrate concentrated to give the carboxylic acid as a colorless oil (85 mg) that was used directly in the next step without further purification.

The intermediate carboxylic acid (140 mg, 0.188 mmol, 1.05 equiv.) was dissolved in CH₂Cl₂ (2 mL) and cooled to 0 °C under N₂. β-Alanine allyl ester hydrochloride (225) (30 mg, 0.179 mmol, 1.00 equiv.) was added as a solid followed by 2,4,6-collidine (48 µL, 43 mg, 0.358 mmol, 2.00 equiv.) and HATU (71 mg, 0.188 mmol, 1.05 equiv.). After 20 min, the mixture was warmed to RT and stirred overnight. The mixture was concentrated and applied to a flash column in a minimum volume of CH₂Cl₂. Elution with a gradient of 98:2→95:5 CH₂Cl₂-MeOH yielded tripeptide 253 as a colorless foam (152 mg, 55% over 3 steps). $[\alpha]_D^{25}$ -5.2 (c 0.5, CHCl₃). ¹H NMR (CDCl₃, 400 MHz) δ 2.22-2.34 (m, 2H), 2.76 (dd, J = 15.3, 6.2 Hz, 1H), 2.80-3.09 (m, 3H), 3.25 (sept., J = 7.7 Hz, 2H), 4.17 (t, J = 6.8 Hz, 1H),
4.22-4.52 (m, 4H), 4.49 (d, J = 5.1 Hz, 2H), 4.56 (q, J = 6.6 Hz, 1H), 5.23 (dd, J = 10.4, 1.1 Hz, 1H), 5.28 (dd, J = 17.2, 1.4 Hz, 1H), 5.87 (ddt, J = 16.2, 10.7, 5.5 Hz, 1H), 6.25 (d, J = 6.7 Hz, 1H), 6.60 (t, J = 5.9 Hz, 1H), 6.88 (d, J = 8.2 Hz, 1H), 7.06-7.34 (m, 23H), 7.41 (t, J = 7.0 Hz, 2H), 7.56 (d, J = 6.7 Hz, 2H), 7.76 (d, J = 5.9 Hz, 2H); 13C NMR (CDCl3, 100 MHz) δ 29.7, 33.6, 34.9, 37.6, 38.2, 47.1, 51.8, 54.7, 65.2, 67.4, 70.9, 118.4, 120.0, 125.1, 126.9, 127.1, 127.2, 127.8, 128.0, 128.6, 129.2, 132.0, 136.5, 141.3, 143.7, 144.2, 170.1, 170.5, 171.6. HRMS (ESI) calcd for C53H51N4O7 (M+H)+ 855.3752, obsd 855.3752.

To a solution of tripeptide 253 (80 mg, 0.094 mmol, 1.00 equiv.) in dry acetonitrile (1 mL) was added Et2NH (1 mL). The reaction mixture was stirred at RT for 2 h and then concentrated to give the free amine as a colorless solid (80 mg) that was used directly in the next step without further purification.

The intermediate amine (59 mg, 0.094 mmol, 1.00 equiv.) was dissolved in CH2Cl2 (2 mL) and cooled to 0 °C under N2. Nα-Fmoc-N-β-trityl-L-asparagine (251) (58 mg, 0.098 mmol, 1.05 equiv.) was added as a solid followed by 2,4,6-collidine (27 µL, 25 mg, 0.207 mmol, 2.2 equiv.) and HATU (37 mg, 0.098 mmol, 1.05 equiv.). After 20 min, the mixture was warmed to RT and stirred overnight. The mixture was concentrated and applied to a flash column in a minimum volume of CH2Cl2. Elution with 98:2 (CH2Cl2-MeOH) gave tetrapeptide 254 as a colorless foam (91 mg, 64%), Rf 0.52 (9:1 CH2Cl2-MeOH). [α]D25 -18.8 (c 1.0, CHCl3). 1H NMR (CDCl3, 400 MHz) δ 2.28-2.34 (m, 1H), 2.72 (dd, J = 15.7, 6.0 Hz, 2H), 2.85 (dd, J = 15.4, 3.8 Hz, 2H), 3.00 (d, J = 5.4 Hz, 2H), 3.30 (sept., 6.4 Hz, 2H), 4.22-4.27 (m, 1H), 4.50 (d, J = 4.6Hz, 2H), 4.58 (dd, J = 11.8, 7.7 Hz, 2H), 5.22-5.27 (m, 1H), 5.32 (dd, J = 15.9, 5.7 Hz, 1H), 5.87
(ddt, $J = 16.3, 10.7, 5.4$ Hz, 1H), 6.55 (t, $J = 6.4$ Hz, 1H), 6.89 (d, $J = 7.4$ Hz, 1H), 7.03–7.30 (m, 39H), 7.56 (d, $J = 6.7$ Hz, 2H), 7.76 (d, $J = 5.9$ Hz, 2H); 8.34 (t, $J = 6.4$ Hz, 2H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 28.2, 33.6, 34.9, 37.4, 37.5, 45.5, 47.1, 48.5, 50.8, 54.8, 65.3, 70.9, 118.4, 126.9, 127.1, 127.2, 128.0, 128.5, 128.7, 128.8, 129.3, 132.0, 136.6, 142.3, 143.1, 144.1, 168.8, 169.6, 170.0, 170.2, 172.3. HRMS (ESI) calcd for $C_{76}H_{17}N_{6}O_{9}$ (M+H)$^+$ 1212.4025, obsd 1212.4006.

Boc-$\tau$-HAL-Asn-Asn-Phe-$\beta$-Ala-OAll (258)

H.Asn-Asn-Phe-$\beta$-Ala-OAll 255: To a solution of tetrapeptide 254 (20 mg, 0.016 mmol, 1.00 equiv.) in dry acetonitrile (1 mL) was added Et$_2$NH (1 mL). The reaction mixture was stirred at rt for 2 h and then concentrated to give the amine as a colorless solid (16 mg) that was dissolved in dry CH$_2$Cl$_2$ (0.3 mL) and cooled to 0 °C under N$_2$. Trifluoroacetic acid (0.3 mL) was added dropwise. The reaction mixture was stirred for 1 h at 0 °C. The mixture was concentrated three times from toluene (1 mL each time), then diluted with MeOH (3 mL) and neutralized by the addition of solid Na$_2$CO$_3$ to ~pH 7. The reaction mixture was filtered through a pad of silica gel with a gradient of 9:1→4:1 (CH$_2$Cl$_2$-MeOH) to afford tetrapeptide amine 255 (8 mg).

$\tau$-HAL-COOH (257): The $\tau$-HAL derivative 256 (11 mg, 0.016 mmol, 1.0 equiv.) was dissolved in dry MeOH (2 mL) and 10 % Pd-C (2 mg, 0.016 mmol, 1.0 equiv.) was added. The suspension was stirred at RT overnight under an atmosphere of H$_2$. The reaction mixture was filtered through Celite® and the filtrate concentrated to give the carboxylic acid 257 as a colorless oil (9 mg) that was used directly in the next step without further purification.
The intermediate amine 255 (8 mg, 0.0158 mmol, 1.00 equiv.) was dissolved in CH₂Cl₂ (1.5 mL) and cooled to 0 °C under N₂. τ-HAL-COOH 257 (9 mg, 0.016 mmol, 1.05 equiv.) was added as a solution in CH₂Cl₂ (1.5 mL) followed by collidine (2 µL, 1.8 mg, 0.322 mmol, 2.0 equiv.) and HATU (6 mg, 0.016 mmol, 1.05 equiv.). After 20 min, the mixture was warmed to rt and stirred overnight. The mixture was concentrated and applied to a flash column in a minimum volume of CH₂Cl₂. Elution with a gradient of 95:5→9:1 (CH₂Cl₂-MeOH) gave hexapeptide 258 as a colorless oil (6 mg, 36%). Rf 0.35 (9:1 CH₂Cl₂-MeOH). HRMS (ESI) calcd for C₅₃H₆₄N₁₀O₁₄ (M+H)+ 1065.4676, obsd 1065.4655.

\[
\text{ τ-HAL-N}(\text{Alloc})-\text{C}(\text{Ac}^* )-\text{AAA (261)}
\]

To a solution of τ-HAL 256 (10 mg, 0.015 mmol, 1.00 equiv.) in dry CH₂Cl₂ (0.5 mL) was added TFA (0.5 mL). The reaction mixture was stirred for 1 h at 0 °C and then concentrated three times from toluene to give the free amine 260 as a colorless oil (8.5 mg) that was used directly in the next step without further purification.

The intermediate amine 260 (8.5 mg, 0.015 mmol, 1.00 equiv.) was dissolved in CH₂Cl₂ (1.5 mL) and cooled to 0 °C under N₂. Free acid 195 (5 mg, 0.015 mmol, 1.00 equiv.) was added as a solution in CH₂Cl₂ (1.5 mL) followed by collidine (4 µL, 3.5 mg, 0.030 mmol, 2.0 equiv.) and HATU (6 mg, 0.016 mmol, 1.05 equiv.). After 20 min, the mixture was warmed to rt and stirred overnight. The mixture was concentrated and applied to a flash column in a minimum volume of CH₂Cl₂. Elution with a gradient of 95:5→9:1 (CH₂Cl₂-MeOH) gave tripeptide 261 as a colorless oil (6 mg, 54%). Rf 0.43 (95:5 CH₂Cl₂-MeOH). HRMS (ESI) calcd for C₄₁H₄₅N₅O₉ (M+H)+ 751.3852, obsd 751.3841.
5.8.3 Spectra

**Compound 228 (Scheme 5.3) – $^1$H NMR spectrum**

SY-04-140 in CDCl3 at 400 MHz
Compound **228** (Scheme 5.3) – $^{13}$C NMR spectrum

SY-04-140 in CDCl$_3$ at 100 MHz
Compound 229 (Scheme 5.3) – $^1$H NMR spectrum

SY–04–135 in CDCl₃ at 400 MHz
Compound 229 (Scheme 5.3) \(-^{13}C\) NMR spectrum

SY-04-135 in CDCl3 at 100 MHz
Compound 230 (Scheme 5.3) – $^1$H NMR spectrum

SY-04-136 in CDCl3 at 400 MHz
Compound 230 (Scheme 5.3) – $^{13}$C NMR spectrum

SY-04-136 in CDCl3 at 100 MHz
Compound 231 (Scheme 5.3) – $^1$H NMR spectrum

SY-04-139 in CDCl3 at 400 MHz
Compound 231 (Scheme 5.3) – $^{13}$C NMR spectrum

SY-04–139 in CDCl$_3$ at 100 MHz
Compound 232 (Scheme 5.3) – $^1$H NMR spectrum

SY-04-146 in CDCl$_3$ at 400 MHz
Compound 232 (Scheme 5.3) – $^{13}$C NMR spectrum

SY-04–146 in CDCl3 at 100 MHz
Compound 234 (Scheme 5.3) – $^{13}$C NMR spectrum

SY-01-129 in CDCl$_3$ at 400 MHz
Compound 234 (Scheme 5.3) – $^{13}$C NMR spectrum

SY-01-129 in CDCl$_3$ at 100 MHz
Compound 225 (Scheme 5.6) – $^1$H NMR spectrum

SY-01-40 in CDCl$_3$ at 400 MHz
Compound 225 (Scheme 5.6) – $^{13}$C NMR spectrum

SY-01-40 in CDCl3 at 100 MHz
Compound 226 (Scheme 5.6) – $^1$H NMR spectrum

SY-01–69 in CDCl3 at 400 MHz
Compound 226 (Scheme 5.6) – $^{13}$C NMR spectrum

SY-01-69 in CDCl3 at 100 MHz
Compound 249 (Scheme 5.8) – $^1$H NMR spectrum

SY-04–150 in CD3OD at 400 MHz
Compound 249 (Scheme 5.8) – $^1$H NMR spectrum

SY-04-150 in MeOD at 100 MHz
Compound 253 (Scheme 5.10) – $^1$H NMR spectrum

SY-04-99 in CDCl₃ at 400 MHz
Compound 253 (Scheme 5.10) – $^{13}$C NMR spectrum

SY-04-99 in CDCl3 at 100 MHz
Compound 254 (Scheme 5.10) – $^1$H NMR spectrum

SY-04-120 in CDCl3 at 400 MHz
Compound 254 (Scheme 5.10) – $^{13}$C NMR spectrum

SY-01-120 in CDCl$_3$ at 100 MHz
REFERENCES


APPENDIX: LETTERS OF PERMISSION

Figure 1.2 - Page 3

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Title: Interaction between the Marine Sponge Cyclic Peptide Theonellamide A and Sterols in Lipid Bilayers As Viewed by Surface Plasmon Resonance and Solid-State 2H Nuclear Magnetic Resonance

Author: Rafael Atillo Espiritu, Nobuaki Matsumori, Michio Murata, Shinichi Nishimura, Hideaki Kakeya, Shigeki Matsunaga, and Minoru Yoshida

Publication: Biochemistry
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THE VITA

Saroj Yadav was born in Gurgaon, India, to Jagadish Yadav and Sushila Yadav. She received her Bachelor of Science in Chemistry from Panjab University in May 2006. She later enrolled for Master of Science in Organic Chemistry and successfully graduated in May 2008 from Panjab University. In fall 2008, she was accepted to Graduate School Doctoral Program at Louisiana State University in the Department of Chemistry where she is currently a doctoral candidate in organic chemistry working under the direction of Dr. Carol M. Taylor. She is working on synthesis of the western hemisphere of theonellamide C. Saroj Yadav is a member of the American Chemical Society and Indian Student Association at LSU.