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Synthesis and Biosynthesis of Polyketide Natural Products

Atahualpa Pinto

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Abstract

Traditionally separate disciplines of a large and broad chemical spectrum, synthetic organic chemistry and biochemistry have found in the last two decades a fertile common ground in the area pertaining to the biosynthesis of natural products. Both disciplines remain indispensable in providing unique solutions on numerous questions populating the field. Our contributions to this interdisciplinary pursuit have been confined to the biosynthesis of polyketides, a therapeutically and structurally diverse class of natural products, where we employed both synthetic chemistry and biochemical techniques to validate complex metabolic processes. One such example pertained to the uncertainty surrounding the regiochemistry of dehydration and cyclization in the biosynthetic pathway of the marine polyketide spiculoic acid A. The molecule's key intramolecular cyclization was proposed to occur through a linear chain containing an abnormally dehydrated polyene system. We synthesized a putative advanced polyketide intermediate and tested its viability to undergo a mild chemical transformation to spiculoic acid A. In addition, we applied a synthetic and biochemical approach to elucidate the biosynthetic details of thioesterase-catalyzed macrocyclizations in polyketide natural products. The outcome of the enzyme's activity is divided between hydrolysis and macrocyclization and limited information is currently available pertaining to its selectivity. We synthesized four enantioenriched NAC-thioester analogs and assayed them with overexpressed DEBS TE, the canonical thioesterase of polyketide natural products, to gain insight into the effect key structural and stereochemical elements of the polyketide substrate have in the macrocyclization selectivity and regioselectivity of the enzyme.
SYNTHESIS AND BIOSYNTHESIS OF POLYKETIDE NATURAL PRODUCTS

By

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DISSERTATION
Submitted in partial fulfillment of the requirements for the
Doctor of Philosophy in Chemistry in the
Graduate School of Syracuse University

December 2011
For my sisters
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<td>Acyltransferase</td>
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<tr>
<td>ATP</td>
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<td>LB</td>
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<tr>
<td>LC/MS</td>
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Chapter 1. Synthetic chemistry, biochemistry and the biosynthetic discovery: an essential feedback loop at the interface of chemistry and biology

"I can no longer, so to speak, hold my chemical water and must tell you that I can make urea without needing a kidney, whether of man or dog; the ammonium salt of cyanic acid is urea."

Excerpt from Friedrich Wöhler's letter to his mentor Jöns Jacob Berzelius
22 February 1828

1.1. Introduction

The development of modern synthetic chemistry, through both rationally designed and serendipitous discoveries, has been inexorably linked to the understanding of the chemical processes of organisms. This connection however was not born of a natural progression but of the separate growth of the fields of organic chemistry and biochemistry from pioneering works of 19th century scientists such as Friedrich Wöhler and Eduard Buchner. Their discoveries regarding the synthesis of urea and cell-free fermentations, respectively, had profound repercussions to the established view of vitalism and its élan vital so championed by the likes of Berzelius and Pasteur.

It was these momentous scientific developments that set the tone and laid the foundation for research endeavors in the 20th century to gradually develop free from the confines of doctrine. For the chemical and biochemical disciplines this path would allow for a gradual yet certain establishment of their interconnectedness, as exemplified by the eventual work by Hans Krebs and Kurt Henseleit to identify the metabolic origins and mechanism of the urea cycle.

The maturation of the synthetic chemistry discipline owes to the isolation and characterization of compounds of therapeutic value and/or as commodity chemicals from both prokaryote and eukaryote natural sources. Limited access to sufficient quantities of
compound from their biological sources, and the associated difficulties in providing sustainable growing and culturing conditions in the laboratory have historically been the motivation for the development of synthetic routes and scale up methodologies for structure determination, research and industrial production. Nevertheless the tacit underlying motivation amongst synthetic chemists has been the remarkable creative potential provided by the enormous structural diversity found within the various subclasses of natural products.

Recent developments in the understanding of metabolic pathways and their relationship with the biosynthesis of natural products has opened new avenues for exploration by the synthetic chemist. This chapter will review some notable strategies and contributions synthetic chemists have had on the advancement of the biosynthetic field with a particular focus on the formulation and testing of biosynthetic hypotheses and the development of biosynthetic precursors for the in depth study of individual enzymatic mechanisms.

1.2. Synthesis and the biosynthetic study of secondary metabolites

Whereas the isolation, analysis and characterization of the biosynthetic machinery of natural products is the ideal path to take to decipher their assembly, realistically the process is time consuming and complex endeavor. Given this, the unavailability of a natural product's biosynthetic gene cluster has not hampered the attuned chemist from proposing reasonable steps to rationalize its origins. It is the purpose of this section to highlight the complementary role synthetic experiments and/or chemical hypotheses have had on the understanding of natural product biosynthesis.
1.2.1. Alkaloids

Alkaloids are a large, medicinally significant class of nitrogen-containing natural products. Generally viewed as the products of eukaryotic organisms, plants in particular, these compounds are widespread in Nature. Their biosynthesis is known to involve diverse pathways, unique building blocks, and a plethora of strategies\(^9\), each tailored to produce exquisitely complex structures (Figure 1.1). The origin of nitrogen incorporation in these compounds is a consequence of their amino acid origins or from a PLP-mediated transamination.\(^10\)

1.2.1.1. Daphniphyllum alkaloids

![Figure 1.1. Structural diversity of the alkaloid class of natural products.](image1)

**Figure 1.1.** Structural diversity of the alkaloid class of natural products.

**Figure 1.2.** Daphniphyllum alkaloids.

![Figure 1.2. Daphniphyllum alkaloids.](image2)
Daphniphyllum alkaloids\(^{11-17}\) are structurally related compounds isolated from the plant family Daphniphyllaceae (Figure 1.2). Suzuki et al. were first to scrutinize the origins of their carbon skeleton by incorporation experiments of racemic \([2-^{14}C]\)-mevalonic acid.\(^{18}\) They proposed from observed scintillation measurements that daphniphylline and its congeners likely share triterpene squalene as a biosynthetic precursor. Beyond this however they were unable to provide a satisfactory hypothesis to explain the steps leading to the formation of its characteristic polycyclic scaffold.

This challenge was seized 15 years later by the Heathcock laboratory.\(^{19-23}\) With the initial intent to design an efficient synthetic route to daphniphyllum alkaloids, they sought inspiration from the elegant simplicity of Nature. They reasoned a pathway from squalene consisting of a combination of biosynthetically preceded and chemically intuitive steps (Figure 1.3). They hypothesized a dioxidation of squalene to key dialdehyde \(1.1\), which would undergo the formation of azadiene \(1.2\) by amination with common cofactor pyridoxamine phosphate. The azadiene intermediate's conjugate cyclization to the dihydropyran-containing compound \(1.5\) is preceded by strategic imine/enamine tautomerizations and azadiene deconjugation, proposed to occur with an amino acid residue localized in the enzyme's active site, to yield the reactive and nucleophilic enamine species \(1.4\).
FIGURE 1.3. Proposed biosynthetic hypothesis of daphniphyllum alkaloids.

Known to synthetic chemists as effective nucleophiles in conjugate addition reactions\textsuperscript{24}, compounds such as enamine 1.4 may undergo conversion to 1.5, after which an aminal hydrolysis and rearrangement of the bicycle leads to key azadiene 1.7. This bicyclic intermediate is proposed to undergo a regio- and stereoselective cyclization cascade to form the pentacyclic secodaphniphylline core. The sequence consists of an
aza Diels-Alder followed by an aza Prins carbocyclization and generation, by proton transfer, of isopropenyl-containing 1.9, which they proposed to be the precursor to other structural motifs in the compound class. The biosynthetic hypothesis informed and served as a blueprint for the design of a synthetic strategy. Their approach was simplified by the employment of dihydrosqualene aldehyde 1.10 as a starting material (Figure 1.4). The decision would permit direct access to a reactive enamine, eliminating the intermediate tautomerization steps and setting the stage for the ensuing intramolecular cyclization cascade. Noteworthy was their chance discovery of the contrasting effects an alkylamine and ammonia showed in the mechanism leading to the structure of the isopropenyl side chain. Whereas with ammonia a 1,5 proton transfer resulted in the expected isopropenyl side chain of 1.11, methyl amine however resulted in the isolation of product containing a saturated isopropyl moiety 1.12, suggesting a 1,5-hydride shift mechanism to be operative.

**Figure 1.4.** Synthesis of seco-daphniphylline alkaloids 1.11 and 1.12.

Heathcock's hypothesis remains biosynthetically unverified as the genes responsible for daphniphyllum biosynthesis have not yet been isolated and characterized, yet the
sound mechanistic insight and elegance in design that resulted from these studies will be hard to ignore and will likely remain as the point of reference for future analyses.

1.2.2. Terpenoids

Terpenoids are an enormous and highly varied family of natural products that have traditionally been associated with multicellular organisms including plants and animals. These compounds are not only of great interest to researchers for their physiologically active properties but for the tremendous complexity produced from the cationic rearrangement of simple dimethyl allyl pyrophosphate (DMAPP) and its regioisomer isopentenyl pyrophosphate (IPP). In animals these key metabolites are the products of the mevalonic acid pathway (Figure 1.5).

**Figure 1.5.** Mevalonic acid pathway and the biosynthesis of terpenoid natural products.

1.2.2.1. Furanocembranoids

An exceptional example of the use of chemical techniques to initiate the rationalization of a natural product's biosynthetic origins is that of the conversion of bipinnatin J and intricarene by the Trauner laboratory. The work perfectly illustrates
the "screening hypothesis" concept,\textsuperscript{27-31} developed to explain the underpinnings of why and how organisms generate enormous structural diversity and complexity through choice modifications of key metabolites. The strategy is proposed to yield a few reactive intermediates that, either enzymatically or spontaneously, undergo drastic restructuring of their scaffold to lead to increased production of structurally diverse congeners (Figure 1.6).

\textbf{FIGURE 1.6.} The furanocembranoid terpenoids.

Bipinnatin J \textbf{(1.13)} and intricarene \textbf{(1.14)} are examples of a large family of structurally related macrocyclic diterpenoids isolated from gorgonian corals of the genus \textit{Pseudopterogorgia}. The representative furanocembranoid macrocycle, exemplified by \textbf{1.13}, owes its origins to an enzyme-catalyzed stereoselective cationic intramolecular cyclization of geranylgeranyl diphosphate, followed by a putative series of P450-dependent oxygenations of the carbon skeleton (Figure 1.7).\textsuperscript{32, 33}
However, the rearrangement of the tricyclic furanocembranoid archetype to a molecule of such complexity as intricarene, which contains a trispiropentacyclic core remained an elusive, unexplored topic. That is until the elegant work of Trauner and co-workers showed their relationship to be the product of an Achmatowicz reaction$^{34}$-[3+2] dipolar cycloaddition$^{35}$ cascade (Figure 1.8).

To test their hypothesis they subjected their synthetic sample of 1.13$^{26}$ to a regioselective epoxidation of the furan with $m$CPBA mediated by the adjacent furfuryl alcohol to produce hydroxypyranone 1.15. Acetylation to 1.16 followed by elimination grants access to oxidopyrillium dipole 1.17 which, by heating to 150 °C, initiates the expected [3+2] dipolar cycloaddition thus granting access to 1.14 in 26% yield. The high
temperatures necessary to effect cyclization suggest an enzyme must be involved to regulate the energetics of the reaction.

### 1.2.3. Polyketides

![Polyketide natural products](image)

**Figure 1.9.** Polyketide natural products are complex and structurally diverse.

Polyketide natural products are a structurally diverse group of highly oxygenated secondary metabolites (Figure 1.9) biosynthesized by a complex collection of enzymes known as a polyketide synthase (PKS). Several varieties of PKSs have been identified, however, the standard biosynthetic model of assembly is that exemplified by a modular type I polyketide synthase (Figure 1.10) operative in the assembly of macrolide antibiotics and polyether biotoxins to name a few. The process takes place by iterative thio-Claisen condensations of malonyl-CoA and/or its derivatives in a manner analogous to fatty acid biosynthesis to produce β-keto thioester intermediates. The basic enzymatic mechanism of modular PKSs involves three indisspensable catalytic domains arranged into repetitive units or modules: ketosynthase (KS), acyl transferase
(AT) and acyl carrier protein (ACP); the number of modules present in the PKS dictates the length of the carbon chain of the end product. In addition, the modules may also possess tailoring domains arranged in various configurations that facilitate access to β-hydroxy units (ketoreductase – KR); conjugated E alkenes (dehydratase – DH); and fully saturated products (enoyl reductase – ER). Release from the enzyme complex is mediated by a terminal thioesterase domain (TE) that, depending on the intra- or intermolecular availability of a nucleophile, may yield macrolactones or seco-acids respectively.

**FIGURE 1.10.** PKS chain elongation of a malonyl-CoA derivative displaying the consensus mechanism for the essential KS-AT-ACP catalytic triad.
1.2.3.1. Polyether biotoxins

The biosynthetic hypotheses discussed thus far have provided a glimpse of chemically reasonable pathways that have gone without independent corroboration from molecular genetics and/or enzymology experiments. However, when controversies occur among experimentally supported hypotheses, the best and only solution in discriminating between these is the characterization of the natural product's biosynthetic pathway. This scenario unfolded with two proposals of polyether biosynthesis: the Cane-Celmer-Westley\textsuperscript{40} and Townsend-Basak-McDonald\textsuperscript{41} hypotheses.

The Cane-Celmer-Westley hypothesis (Figure 1.11-A) was proposed to explain the biosynthesis of polyether biotoxins such as monensin A (1.18). It attempted to rationalize the molecule's origins as the product of an elegant intramolecular epoxide ring opening cascade set in motion by a P450 monoxygenation of putative $E,E,E$-premonensin polyketide intermediate (1.19). Key to its recognition as a viable and compelling biosynthetic step is its application in the facile synthesis of complex polyether natural products in accordance with Baldwin's ring-closure rules.\textsuperscript{42} Conversely, the Townsend-Basak-McDonald hypothesis (Figure 1.11-B) predicts the polyether scaffold to form by sequential intramolecular syn-oxidative additions of a metal-oxo species owing to a [2+2] metallaoxetane-reductive elimination mechanism. By this logic, the stereochemistry of intermediate 1.18 was recognized to necessitate the hemiketal of key polyketide intermediate $Z,Z,Z$-premonensin (1.20). McDonald and coworkers explored and confirmed the potential of their proposed step to the synthesis of 1.18 by employing PCC as the oxidant of short $Z,Z$-hydroxydienes.\textsuperscript{43}
Refutal of one of these hypotheses required the isolation, sequence and expression of a polyether gene cluster and the piecemeal spectroscopic analysis of multiple intermediates in the pathway. Work by Leadlay and coworkers\textsuperscript{44} provided the first evidence of the stereochemical nature of premonensin's triene system. They generated a mutant of the monensin biosynthetic gene cluster by fusing an erythromycin thioesterase domain downstream of module 4. In this manner, they effectively suppressed monensin production, yet isolated and characterized a truncated metabolite containing an olefin with an (E)-stereochemistry. Further molecular genetic studies of various mutants of the monensin biosynthetic cluster confirmed the accumulation of an $E,E,E$-premomensin.
derivative in the pathway, effectively confirming the Cane-Celmer-Westley hypothesis as the key model in the biosynthesis of polyether natural products.

1.3. Precursor and analog design for enzymology of polyketide natural products

Another key area in the field of biosynthesis where synthetic chemists have made profound contributions has been that of enzymology and the understanding of the molecular underpinnings of biosynthetic pathways. These studies deal with available biosynthetic enzymes and attempt to answer questions as varied as those regarding substrate specificity, regio- and stereochemical characteristics of products, enzyme kinetics, substrate-enzyme complex inhibitors and the development of novel compounds. The high point of the application of the experimental possibilities has been reached with the enormous polyketide class of natural products.

1.3.1. Precursor directed biosynthetic studies

As was succinctly described by Boddy and coworkers: "precursor-directed biosynthesis provides a powerful method to introduce non-native starting materials into biosynthetic pathways.".45 Aside from the obvious goal of this concept which is to chemoenzymatically generate a diverse set of chemical congeners with novel therapeutic profiles, a fascinating consequence is the exploration and elucidation of the specificity and selectivity of steps in individual pathways towards non-native structural motifs.

1.3.1.1. Precursor-directed biosynthesis of erythromycin analogs

One of the first examples of precursor-directed biosynthesis of polyketide natural products was designed by the Khosla group in 1997.46 Employing the canonical erythromycin PKS pathway, they generated a null mutant by deleting the ketosynthase
domain of module 1 (KS1), rendering the pathway dependent on the exogenous supply of synthetic substrates (Figure 1.12).  

**Figure 1.12.** Precursor directed biosynthesis of erythromycin analogs.

The study served to underline the capacity of the pathway to accept and produce interesting analogs, and moreover to reveal the tolerance of downstream enzymatic steps to marked steric and structural differences in the substrate.

### 1.3.2. Domain-level studies in PKS pathways

The enzymology of polyketide biosynthetic domains has been fundamental in creating a normative understanding and model of their mechanistic underpinnings. The advent of sequencing technology and heterologous expression systems have had a tremendous impact on the researchers availability and investigation of biosynthetic genes and their products. Chemical synthesis is an excellent tool in the elucidation of these processes and interactions.

#### 1.3.2.1. Decarboxylative chain termination in curacin A biosynthesis

The terminal step in the biosynthesis of polyketide natural products is catalyzed by a thioesterase domain (TE). These catalytic domains mediate the cleavage of the thioester bond of the upstream ACP-bound mature polyketide and concomitantly produce either a macrocyclic or hydrolytic product. This model mechanism is not applicable when analyzing the anticancer cyanobacterial metabolite curacin A (1.21) as it is characterized
by a terminal olefin moiety in place of the canonical seco-acid (Figure 1.13). The molecule's gene cluster was isolated and sequenced by Gerwick and coworkers to reveal a termination module containing a putative sulfotransferase domain (ST) among the ACP and TE domains.48

**Figure 1.13.** CurM, the termination module of the curacin A PKS, contains a sulfotransferase domain (ST) flanked by an ACP and a TE domain

From analysis of the predicted mature polyketide the ST was hypothesized to act on an ACP-bound β-hydroxythioester intermediate. The precise sequence of events leading to formation of 1.21 however, remained unclear. To determine ST domain's role and its timing within the termination of the mature polyketide leading to 1.21, Sherman and coworkers synthesized a simple ACP-bound heptaketide analog (1.22) containing the requisite β-hydroxythioester moiety (Figure 1.14).49 Individual and combined biochemical analyses of 1.22 with recombinant ST and TE domains ratified the sequence of events to 1.23. The ACP-bound β-hydroxythioester polyketide is shown to be sulfated prior to the action of the thioesterase domain which catalyzes a hydrolysis reaction and mediates the acid's decarboxylative β-elimination to yield 1.23.
FIGURE 1.14. Substrate studies of curacin A's sulfotransferase (ST) and thioesterase domains.

These results demonstrated an unprecedented mechanism of polyketide termination by decarboxylative elimination of a $\beta$-sulfate containing advanced intermediate and represents a potential new and useful tool for the inclusion of terminal olefins in engineered polyketide pathways.

1.3.2.2. High resolution structural studies of the pikromycin thioesterase domain

Synthetic chemistry has been fundamental in the elucidation of key structural substrate-enzyme interactions and the mechanistic underpinnings of catalysis. To gain structural information that would guide their desired rational engineering of a PKS thioesterase domain, Fecik and coworkers designed a non-hydrolysable pentaketide phosphonate-based inhibitor structurally analogous to the mature heptaketide native to the pikromycin biosynthetic pathway (Figure 1.15).

FIGURE 1.15. Synthesis of phosphonate 1.25, an irreversible inhibitor of the pikromycin thioesterase domain.
The structural characteristics of the enzyme that stabilize the substrate in the channel remained unclear. Initially thought to be due to various hydrophilic residues lining the channel, Fecik et al. obtained an X-ray crystal structure of co-crystallized 1.25-PIKS TE (Figure 1.16) where they observed that the co-crystallized phosphonate inhibitor made few specific contacts with the channel, suggestive of hydrophobic interactions being key for the stabilization of the substrate-enzyme intermediate. Furthermore, a weak barrier of water molecules in the channel's exit was suggested to be the underlying cause of the enzyme's macrocyclization mechanism, as it was observed to direct the substrate's $O$-nucleophile towards the enzyme active site.

**Figure 1.16.** Crystal structure of phosphonate 1.25 (circled) bound to the active site of the pikromycin thioesterase domain.

### 1.4. Conclusion

Natural product biosynthetic studies have experienced enormous growth in the last 20 years by the swift technological advances and invaluable contributions of the synthetic chemistry and biochemistry disciplines. These, have been employed effectively in the development and testing of biosynthetic hypotheses, and as probes of the underlying
mechanistic underpinnings of enzymatic processes. Presented herein are our efforts to showcase the seamless relationship the chemical sciences, whether synthetic chemistry or biochemistry, have had in comprehending the biosynthetic origins of polyketide natural product spiculoic acid A, a prospective antitumor therapeutic, in addition to the substrate specificity and mechanism of the DEBS-TE, the thioesterase involved in the cyclization of macrolide polyketide erythromycin.
1.5. References


CHAPTER 2. Investigating the cyclization of an alternate biomimetically-inspired linear intermediate of spiculoic acid A

2.1. Introduction

The Diels-Alder reaction, discovered by Otto Diels and Kurt Alder in the early 20th century,¹ has become a powerful tool in the synthetic chemist's collection of reactions. Characterized by the coupling of a conjugated diene and an olefin (dienophile), the reaction's value resides in its ability to predictably yield potentially complex cyclohexene-containing products with superb regio- and stereochemical control.

![Diels-Alder Reaction Diagram](image)

Since its discovery, the Diels-Alder reaction underwent a series of theoretical breakthroughs which laid down the sophisticated mechanistic foundation necessary for its application in numerous natural product syntheses.² Formally a pericyclic [4+2] reaction, the Diels-Alder is typified by a cyclic transition state where, in a single concerted step, three \( \pi \) bonds are broken concomitantly forming two \( \sigma \) bonds and a \( \pi \) bond (Figure 2.1-A). The key orbital interactions involving the reaction's components are their highest occupied and lowest unoccupied molecular orbitals (HOMO and LUMO respectively) as predicted by frontier molecular orbital theory (FMO)³ and symmetry conservation considerations (Figure 2.1-B).⁴, ⁵ If the HOMO-LUMO energy gap decreases, the reactivity of the system increases. This phenomenon can be modulated by inclusion of polarizing functionalities (electron donating and withdrawing) on one or both components of the reaction.

Perturbation of the diene-dienophile by inclusion of electron rich or poor substituents changes the energetic landscape of the reaction as regio- and stereoselective pathways to product become available (Figure 2.1-C). Fortunately an analysis of stabilizing electronic effects in the transition state effectively eliminates the majority of these putative pathways. On account of the reaction being concerted and stereospecific, only two major pathways are operative in explaining the reaction's diastereoselection. Formation of the kinetically favored \( \text{endo} \) transition state is rationalized by invoking secondary orbital bonding interactions between the reaction components. The thermodynamically controlled product, obtained from an \( \text{exo} \) transition state, may be also isolated owing to favorable steric interactions of reactants. Of note is also the marked effect the polarizing substituents have on the orbital coefficients of the reaction's
components and thus its regiochemical outcome. This effect creates geometric asymmetries in the key frontier molecular orbitals which affect the synchronicity of bond formation at the transition state.\textsuperscript{6} The favored transition state will then be that which arises from complementing orbital coefficients of the diene-dienophile pair.

The flow of electrons determines how the Diels Alder reaction is classified. A normal-electron-demand Diels-Alder is characterized by a reaction between an electron-rich diene and an electron-poor dienophile and, as expected, the HOMO of the diene and the LUMO of the dienophile are its key frontier molecular orbital interactors (Figure 2.1-B1 and C1). The opposite polarization is also applicable and the reaction is known as an inverse-electron-demand Diels-Alder reaction\textsuperscript{7,8} (Figure 2.1-B2 and C2).

Shown in Figure 2.2, are a few relevant cases where a Diels-Alder cycloaddition figures prominently as a key transformation in the synthetic route to a natural product's framework. Moreover they are examples of intramolecular Diels-Alder reactions (IMDA), a common synthetic cyclization strategy increasingly associated with the biosynthetic origins of various secondary metabolites.\textsuperscript{9-12}
2.1.1. The enzymatic Diels-Alder in the biosynthesis of polyketide natural products

Examples abound in secondary metabolism of intramolecular cyclizations reminiscent of a Diels-Alder reaction\textsuperscript{13-19} (Figure 2.3). The isolation of cyclic natural products containing the familiar scaffold set in motion the intense search for the enzymatic step believed to be involved in each individual pathway. The few results thus far have been subject to intense debate. In vitro assays with enzyme preparations (crude or otherwise) and NAC-thioester intermediates of polyketide synthase (PKS) systems such as the macrophomate and lovastatin synthases, have shown product mixtures consistent with a stepwise mechanism of catalysis.\textsuperscript{11,20}
FIGURE 2.3. Polyketide natural products with cyclic elements derived from a putative Diels-Alder reaction.

Most recently, a study on the cyclization of the polyketide spinosyn A led by Liu et al. successfully cloned and isolated gene product SpnF, a putative Diels-Alderase that catalyzes an exclusive transannular cyclization of 2.7 in vitro (Figure 2.4) and produced a single \( \text{trans} \)-hydrindane diastereomer (2.8).\(^{21}\)

FIGURE 2.4. SpnF is the first example of a putative Diels-Alderase that exclusively yields a single diastereomer.

The clear implication of this discovery is that a concerted reaction mechanism may be taking place. Carefully crafted mechanistic studies and high resolution structural data will be necessary to verify the fundamental mechanism of action of this enzyme. If indeed the mechanism is found to be pericyclic, the often-quoted enzyme catalysis paradigm of stabilization of the transition state by electrostatic interactions in the active site\(^{22}\) may likely undergo a period of intense debate.
2.1.2. *trans*-Hydrindane polyketides: the product of a Diels-Alderase?

The *trans*-hydrindane (bicyclo[4.3.0]nonane) contained in some polyketide natural products is a rare scaffold biosynthesized by diverse microbial species isolated from evolutionarily distinct populations (Figure 2.5).

![Figure 2.5. *trans*-Hydrindane polyketides.](image)

Largely regarded to be the product of an enzyme-catalyzed Diels-Alder cycloaddition\(^\text{11}\), mechanistic insight on its origin remains inconclusive and an active source of speculation and debate\(^\text{20, 25-29}\). Although the biosynthetic gene clusters of the ionophore antibiotic indanomycin has been isolated,\(^\text{30-32}\) its study has yet to shed light on the chemistry involved in *trans*-hydrindane formation from the putative linear polyketide precursor.

An important example of this class of scaffold is found in spiculoic acid A (2.11, Figure 2.6), a natural product isolated from tissue extracts of the Caribbean sponge *Plakortis angulospiculatus*; it possesses modest activity as a chemotherapeutic\(^\text{33}\). The molecule is characterized by a highly congested *trans*-hydrindane framework, displaying six stereocenters, of which two are adjacent, strained quaternary centers.
The biosynthetic origin of the compound’s elaborate carbocyclic framework is likely due to an intramolecular Diels-Alder (IMDA) cycloaddition of a linear polyketide product\textsuperscript{33}. The nature of this enzyme-bound linear chain remains a subject of debate since its biosynthetic gene cluster has yet to be isolated, sequenced and characterized; the complexity of both the sponge microbiome and the concomitant metagenomic mining studies the likely culprit.

2.1.3. Spiculoic acid A is putatively biosynthesized by a type I PKS

The linear chain of \textbf{2.11} is hypothesized\textsuperscript{33} to be the product of a type I PKS chain extension of phenylacetyl-CoA starting unit\textsuperscript{34-36} by a sequential condensation of ethyl and methyl malonyl-CoA extender units (Figure 2.7).
The timing of the cyclization leading to spiculoic acid A remains unknown. This step is thought to be mediated by a putative Diels-Alderase\textsuperscript{37-41} enzyme, following the enzymatic hydrolysis of the polyketide intermediate. However, examples of intramolecular cyclizations\textsuperscript{42, 43} of PKS-bound intermediates poses the question of whether this step may take place while bound to the PKS enzyme. In this case, the lower energy LUMO of the conjugated thioester found in the enzyme-bound intermediate would be more reactive in a Diels-Alder reaction than the seco-acid of the hydrolyzed product (Figure 2.8).
FIGURE 2.8. Spiculoic acid A IMDA cyclization may take place during polyketide chain extension.

The double bond regiochemistry of the enzyme-bound linear polyketide remains an issue of considerable interest. The dehydration mechanism of DH domains\textsuperscript{44-46} in type I polyketides and fatty acids is known to consistently produce double bonds in conjugation with the acyl-enzyme intermediate. If this logic is applied to the biosynthesis of the acyclic intermediate of 2.11, an additional regiosomerization of the triene olefins would be necessary to form a diene conformation reactive towards a Diels-Alder reaction. Despite this, Huang et al have justified the feasibility of the intermediate's intramolecular cyclization through a sequence of abnormal $\beta,\gamma$ dehydrations during the biosynthesis of 2.11 (Figure 2.9-A). The $\text{HOMO}_{\text{diene}}-\text{LUMO}_{\text{dienophile}}$ energy gap of the mature enzyme-bound intermediate suggest a cyclization at milder, physiologically relevant conditions would require a Diels-Alderase\textsuperscript{47-49}.
Consistent with this view have been the elevated temperature employed to effect the IMDA in the synthetic studies and total syntheses of 2.11\textsuperscript{50-53} (Figure 2.10). Baldwin et al. showed that under the reaction conditions selected to install the molecule's dienophile (100 °C), their advanced intermediate spontaneously undergoes an IMDA. Matsumura et al. successfully isolated an IMDA-capable aldehyde intermediate however, it was slow to react at elevated temperatures (70 °C). These studies suggest a couple of interpretations: either the \textit{HOMO}_{dienen}-\textit{LUMO}_{dienophile} energy gap\textsuperscript{54} of the intermediate is too large to allow for a spontaneous IMDA and/or the energy of activation of the IMDA is exceedingly high given the entropic and enthalpic cost of organizing the sterically crowded intermediate into a reactive conformation.
FIGURE 2.10. Synthetic strategies employed for the formation of spiculolic acid A’s trans-hydrindane framework.50

We propose an appealing alternative biosynthetic hypothesis where the sequence of DH-mediated dehydrations occurs normally as shown in Figure 2.9-B. As a consequence, we expect the intermediate to remain unreactive towards the IMDA. The olefins defining the conjugated diene in the system would have to undergo a regioisomerization step which we predict to be facilitated by an acid/base residue either contained in the putative cyclase or in the active site of a non-canonical PKS domain (Figure 2.11).55-58
FIGURE 2.11. An intermediate regioisomerization step may take place prior to cyclization by a putative cyclase.

The prospect of a mild, spontaneous IMDA cyclization may be directly facilitated by the favorable electronics of the extended enol or enolate intermediate. The increased electron density of the diene provided by the extended conjugation of the enol moiety will decrease the $\text{HOMO}_{\text{dieno}} - \text{LUMO}_{\text{dienophile}}$ energy gap$^{54}$ of the system.

Guided by our biosynthetic rationale and understanding of polyketide pathways, herein we describe our efforts in the development and synthesis of a novel biomimetic acyclic polyketide intermediate of 2.11 and its ability to undergo an IMDA under a variety of reaction conditions.
2.2. RESULTS AND DISCUSSION

2.2.1. Computational analysis

To predict the favored stereochemical outcome of the linear intermediate of 2.11 under IMDA reaction conditions, we employed gas-phase \textit{ab initio} density functional theory (DFT) computational methods at the B3LYP/6-31G(d,p) level of theory. We envisioned the results would provide insight into the equilibrium conformation energies of putative key intermediates and stereoisomers populating the potential energy surface of the reaction coordinate to 2.11. Unlike the single concerted step to product shown in Figure 2.12-A, we anticipated the path from linear intermediate 2.18 to cyclic 2.11 to go through a transient cyclic enol such as 2.19, which in route to product would require an equilibration step to set the molecule’s stereochemistry at C6 (Figure 2.12-B).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure_2.12.png}
\caption{Steps involved in the conversion of linear polyketide chains to 2.11. A) IMDA reaction of putative intermediate 2.17 directly yields 2.11. B) IMDA reaction of 2.18 may produce an enol intermediate 2.19 that after equilibration may yield product 2.11.}
\end{figure}

In addition, the outcome of the IMDA reaction will be biased by the \textit{endo} vs. \textit{exo} selectivity and facial approach of the molecule's dienophile. Synthetic precedence\textsuperscript{50, 51, 53} suggests that the cyclization will take place preferentially through an \textit{endo} transition state produced by a \(\beta\)-face approach of the dienophile on the diene. Assuming our acyclic
analog 2.39 (see Scheme 2.4) displays similar *endo* selectivity, we hypothesize that the facial approach will be dictated by the energy profile of the reaction's transition state conformation. The congested structure of the cyclic enol stereoisomers, suggests a late transition state. Thus, we expect key steric interactions present in the products to reflect key steric interactions in the transition state.\(^{59}\)

We have modeled and optimized the *endo* cyclic enol products of the IMDA reaction of 2.18 (Figure 2.13) and the products from their C6 tautomerization. The energies of the putative products are shown relative to the energy of spiculoic acid A. Unexpectedly 2.21 was lower in energy by 2.4 kcal/mol relative to 2.19, implying the potential presence of a cyclase to guide the stereochemical outcome of cyclization. Likewise, this result may be a reflection of the molecules’ relaxation to its equilibrium pseudo-chair conformation from the more strained boat conformation required for the IMDA cyclization, necessitating a closer inspection by direct computation of their transition state energies. Analysis of the structures resulting from tautomerization of C6 show the *anti* epimer 2.11 to be 9.2 kcal/mol lower than the *syn* epimer 2.20, likely due to the absence of the 1,3 diaxial interaction between the alkyl groups at C4 and C6. On the contrary, the epimers originating from 2.21 show a great degree of strain from 1,3-diaxial interactions between the C2 and C4 ethyl side chains. Noteworthy from these separate processes is the evident interrelatedness of all four diastereomeric products through an additional tautomerization step at C4 to yield enantiomers. Encouragingly, the lowest energy C4 and C6 *anti* product, which act as a thermodynamic sink, corresponds with the relative stereochemical configuration of 2.11.
FIGURE 2.13. Cyclic endo products from the IMDA cycloaddition of 2.18. Dienophile-diene facial selectivity has the potential of producing two diastereomers which are expected to undergo an enol-keto tautomerization to products.
2.2.2 Retrosyntheses

2.2.2.1. Strategy I

Our initial strategy was centered on synthesizing 2.11 by an enantioselective route that would emulate our hypothetic biosynthetic pathway of PKS-mediated linear chain extension. The corresponding retrosynthetic analysis is summarized in Scheme 2.1.

**Scheme 2.1.** Strategy I: Chiral retrosynthetic strategy to 2.11.

Esterification and disconnection between C2-C10 and C3-C7 of 1 reveals chiral tetraene 2.46, the route’s key linear target. Upon the retrosynthetic reduction and silylation of the secondary alcohol at C5, the disassembly of compound 2.46 can begin by cleavage at the C2-C3 olefin. The step reveals chiral β-hydroxy aldehyde 2.45; related in the synthetic sense by the employment of a Horner-Wadsworth-Emmons olefination. The rationale of this methodology lies in our desire to obtain an intermediate that, by control of the oxidation state at C5, would enable us to test various IMDA conditions to efficiently access the scaffold of 2.11. The *syn* relationship between the C4 and C5 substituents in 2.45 leads our analysis to β-hydroxy intermediate 2.44, in which either an Evans or Crimmins asymmetric aldol methodology\(^{60, \; 61}\) has been employed to obtain
selectivity. Disconnection between C4-C5 of 2.44 yields key trienal 2.43. This intermediate may be disassembled by an oxidoreduction sequence of the conjugated aldehyde to the carboxylic acid oxidation state, followed by an olefin cleavage. This sequence revealed commercially available phenylacetaldehyde (2.24), an analog of spiculoic acid A’s biosynthetic starter unit62. In the forward sense, the aldehydes may be olefinated by reaction with stabilized phosphorus ylides.

Regrettably, at the crux of our strategy, the Evans aldol reaction failed to provide reproducible results. In addition, by switching to a Crimmins methodology we found a proclivity of 2.43 to enolize under the reaction conditions, which prevented us entry to the chiral β-hydroxy target in the manner proposed. The results, which will be discussed in detail in Section 2.2.3.1, forced us to re-evaluate our synthetic strategy.

2.2.2.2. Strategy II

Given the successful diastereoselective synthesis of 2.1150, and the setbacks experienced while implementing our initial strategy, the design was simplified to include construction of rac-2.46 (Scheme 2.2). We envisioned 2.11 to arise directly from the acid-catalyzed deprotection of ketal intermediate 2.54. It was hoped that the ketalization of the C5 ketone would permit the isolation and spectroscopic characterization of the IMDA precursor. In keeping with the linear theme of Strategy I, disconnection of 2.54 at the C2-C3 olefin exposes aldehyde 2.53 accessible by oxidation of the alcohol derived from reduction of the C3 ester of 2.50. Formation of the C4-C5 bond in ester 2.50 was designed to arise from a Reformatsky addition63 of an α-bromoester on aldehyde 2.43, the common link amongst strategies.
During this synthesis, ketalization of the C5 ketone that resulted from the oxidization of 2.50 proved difficult, as described in section Section 2.2.3.2. Despite a series of attempts to circumvent this obstacle, the chemistry remained uncooperative and forced us again to redesign the route.

2.2.2.3. Strategy III

A convergent approach was chosen that would incorporate and recycle some elements of our prior designs. As per Scheme 2.3, retrosynthetic bond cleavage of the C6-C7 trisubstituted olefin in rac-2.46 yields dienal 2.30 and the novel phosphonate 2.59 which may be crafted from the addition of commercial diethyl ethylphosphonate (2.31) to the acyl chloride of acid 2.57. Disassembly of this fragment can be accomplished by cleavage of the C2-C3 olefin and modification of the oxidation state of C5. This acid is likely to be accessible from a Wittig olefination, C5 desylilation of the hydroxyester intermediate and the Jones oxidation of β-hydroxyaldehyde 2.34, an intermediate suggestive of commercially available diethyl ethylmalonate (2.31).
Scheme 2.3. Strategy III: Convergent and achiral retrosynthetic strategy to 2.11.

The synthesis of fragment 2.59 was smoothly carried out from 2.34, however, we discovered that under a number of conditions (Masamune-Roush\textsuperscript{68}, KOH, NaH and pyridine/heat) and temperature profiles, the fragment rapidly decomposed, as detailed in section Section 2.2.3.3. Based on these results, we once again redesigned our synthesis to follow a similar strategy while avoiding the unstable phosphonate coupling partner.

2.2.2.4. Strategy IV

Our final route relied on the use of an aldol coupling to access a similar olefination product as envisioned in the prior strategy. This route also exploited the symmetry element of malonate 2.31 (Scheme 2.4). The strategy's most salient feature, however, was the incorporation of the C6-C7 double bond of fragment 2.39 by a Lewis acid-mediated aldol condensation-dehydration sequence of aldehyde 2.30 and enone 2.37, in
which stereocontrol of the C5 hydroxyl group was not necessary.

**Scheme 2.4.** Strategy IV: Redesigned convergent and achiral retrosynthetic strategy to 2.11.

The C5-C6 bond can be formed from a Grignard alkylation of conjugated Weinreb amide 2.36, in turn formed from a nucleophilic displacement of the conjugated ester produced by the olefination of protected β-hydroxyaldehyde 2.34 (Section 2.2.3.4). This sequence would provide us with our most advanced intermediate, and enable us to test our Diels-Alder hypothesis.

### 2.2.3. Syntheses of 2.39, the putative polyketide intermediate of spiculoic acid A

#### 2.2.3.1. Strategy I

Scheme 2.5 outlines the synthesis of aldehyde 2.43 starting with commercially available phenylacetaldehyde (2.24).
SCHEME 2.5. Synthesis of aldehyde 2.43.$^a$

![SCHEME 2.5. Synthesis of aldehyde 2.43.](image)

$^a$ Reagents and conditions: (a) 2.40, toluene, reflux, overnight; (b) DIBAL-H, CH$_2$Cl$_2$, 0°C (or toluene, -78°C), 1hr; (c) IBX, DMSO, r.t., 1.5hrs; (d) 2.47, benzene, reflux, overnight.

The sequence began with the olefination of aldehyde 2.24 with known ylide 2.40$^{69, 70}$ in refluxing toluene. The resulting conjugated ester 2.25 was obtained in 77% yield with excellent $E$ selectivity ($E/Z = 17:1$ by $^1$H NMR). The stereoselectivity of this step has been discussed in the literature; the authors rationalize the result through analysis of the oxaphosphetane dissociation step.$^{69}$ The build-up of double bond character in the transition state is stabilized to a greater extent by conjugation with the carbonyl's π orbital, the expected scenario when the oxaphosphetane's bulkiest groups are anti with respect to each other. Reduction of ester 2.25 by dropwise addition of DIBAL-H carried out in an ice-cold solution of DCM furnished alcohol 2.26 in 100% yield. Oxidation of alcohol 2.26 to aldehyde 2.27 was attempted using a variety of methods including PCC$^{71}$, TCCA/TEMPO$^{72}$, manganese dioxide$^{73}$ and IBX$^{74}$. Aldehyde 2.27 (97% yield) was obtained by oxidation with a solution of IBX in DMSO at room temperature (r.t.). To limit decomposition$^{63}$, aldehyde 2.27 was immediately olefinated with ylide 2.40 in refluxing benzene to yield a 1:2 mixture of chromatographically inseparable dienyl esters 2.28 and 2.28a respectively in 33% yield (Figure 2.14), as well as unreacted starting
material 2.27 (22% recovery).

Ester mixture 2.28 and 2.28a was reduced with DIBAL-H at 0°C in DCM to furnish chromatographically inseparable alcohol isomers 2.29 and 2.29a as a 1:1 ratio (52% yield). Oxidation of this mixture with IBX in DMSO at room temperature to aldehydes 2.30 and 2.30a (1:1 ratio, 93% yield), followed by immediate olefination with commercially available ylide 2.47 provided trienyl esters 2.41 and 2.41a as a 1:1 mixture (89% yield). The terminal carbonyl in 2.41 and 2.41a was reduced with DIBAL-H at -78°C in toluene to alcohols 2.42 and 2.42a (1:1 ratio, 87% yield), which in turn were oxidized with IBX in DMSO to aldehydes 2.43 and 2.43a (1:1 ratio, 81% yield). Notable among compounds 2.28-2.30 and 2.41-2.43 was the presence of a double bond pattern analogous to that of dienyl ester mixture 2.28 and 2.28a. Although the increased length of the polyene chain would suggest an increased number of regioisomers in solution, NMR data confirmed the mixtures consisted only of two isomers (Figure 2.15).

The C4-C5 coupling and chain extension proved to be problematic when an asymmetric methodology was attempted (Scheme 2.6).
SCHEME 2.6. Asymmetric methodologies employed in forming the C4-C5 bond with aldehyde 2.43.\textsuperscript{a}

Initially, an Evans chiral auxiliary (2.48) was employed and, to our delight, it furnished alcohol 2.44 in 62% yield. Unfortunately, after alcohol silylation, the amide proved to be unreactive towards cleavage under hydrolysis, aminolysis, and reduction conditions. Attempts to scale up the chiral aldol for further analysis proved challenging, as the aldol reaction was highly irreproducible, leading us to examine the Crimmins\textsuperscript{61} (2.49) chiral aldol reaction. Regrettably, under the reaction conditions, 2.43 remained unreactive, allowing for its full recovery upon quenching. We believe the combination of a highly oxophilic Lewis acid (TiCl\textsubscript{4}) and an enolization-prone aldehyde such as 2.43 quickly led to suppression of the C5 electrophilic center prior to its reaction with the preformed chiral enolate of 2.49.

\textsuperscript{a} Reagents and conditions: (a) 2.48, nBu\textsubscript{2}BOTf, iPr\textsubscript{2}NEt, CH\textsubscript{2}Cl\textsubscript{2}, -78°C; (b) TBSOTf, 2,6-lutidine, THF, 0°C; (c) Auxiliary cleavage – DIBAL-H; HN(Me)OMe; LiOH; (d) 2.49, TiCl\textsubscript{4}, (-)-Sparteine, CH\textsubscript{2}Cl\textsubscript{2}, -78°C.
2.2.3.2. Strategy II

We shifted our focus towards synthesizing the linear chain in a non-selective manner (Scheme 2.7).

**SCHEME 2.7.** Synthetic studies towards the C3 chain extension of aldehyde 2.43.

A Reformatsky reaction\textsuperscript{75} coupled ethyl 2-bromobutyrate (2.55) and aldehyde 2.43 to effectively yield syn and anti β-hydroxy esters 2.50\textsubscript{a/b}, and their respective olefin regioisomers, in a combined 78% yield (45% and 33% yield respectively). The expected loss of the C5 stereocenter by oxidation at a later stage allowed us to bypass the assignment of the products’ relative stereochemistry. We anticipated that a simple series of protecting group manipulations and modifications in the oxidation state of key carbon
atoms on 2.50a/b would permit access to rac-2.46. Yet the molecule remained unreactive upon the multiple attempts to reach our goal. Our initial plan was to protect C5 at the keto oxidation state, a move that would subsequently allow us to extend the chain at C3. Oxidation of 2.50a with IBX followed by ethylene glycol ketalization under acidic and refluxing conditions (Scheme 2.7-A) failed to yield product. Access to the C3 electrophile was again denied after silylation of 2.50a and 2.50b (Scheme 2.7-B). As with TBS-protected 2.44, and likely due to steric crowding, the molecule proved unreactive toward reduction with DIBAL-H or LiBH4, hydrolysis with KOH, or amidation with the Weinreb amine. Treatment with LAH effectively reduced C3, but also removed the alcohol's TBS protection.

A secondary protecting group sequence employed the reduced products of β-hydroxy esters 2.50a/b with LAH: 1,3 diols 2.51a/b (Scheme 2.7-C1). The sequence, attempted on diol 2.51a, involved pivalate acylation and silylation of the primary and secondary hydroxyl groups, respectively, furnishing differentially dprotected intermediate 2.52a in a combined 32% yield. If a selective pivalate deprotection was successful, oxidation of C3 and olefination would effectively lead to the protected dihydro-2.52 and to the route’s completion. Several pivalate deprotection methodologies involving hydrolysis, nucleophilic attack, and reduction were attempted, and the moiety was either unreactive, or a concomitant removal of the TBS group was observed.

As a last resort, and by taking into consideration the differential electrophilicity of aldehydes and ketones, we hoped that double oxidation of diols 2.51a/b followed by an olefination reaction might lead to target rac-2.46 (Scheme 2.7-C2). Oxidation of diol 13a with 2 equivalents of IBX quickly led to formation of the enone, but left the primary
alcohol untouched. Addition of an extra 2 equivalents of IBX led to substrate decomposition.

2.2.3.3. Strategy III

The route was redesigned to include the synthetic convergence by a HWE olefination of the previously employed mixture of aldehydes 2.30/2.30a and phosphonate 2.59 (Scheme 2.8). Synthesis of 2.59 began with the hydride reduction of commercially available diethyl ethylmalonate (2.31) in refluxing THF to produce 2.32 in 82% yield. Desymmetrization and monoprotection of diol 2.32 was necessary to extend the molecule’s carbon skeleton. We employed a methodology developed by our lab where diol 2.32 and TBSCI were dissolved in a binary solvent system comprised of hexanes and acetonitrile, and vigorously stirred overnight to furnish monoprotected diol 2.33 (56% yield). Oxidation of alcohol 2.33 under Swern conditions\(^\text{76}\) furnished aldehyde 2.34 in 64% yield, which was easily converted via a Wittig reaction and deprotection to ester 2.56 in 39% yield over two steps.

**SCHEME 2.8.** Synthesis of carboxylic acid 2.57.\(^a\)

\[^a\] Reagents and conditions: (a) LAH, THF, r.t. to reflux, 36 h, 82%; (b) TBDPSCI, 1:4 MeCN-Hexanes, \(\text{Et}_3\text{N}, \text{r.t.}, \ 72 \text{ h}, \ 56\%\); (c) (COCl)\(_2\), DMSO, \(\text{Et}_3\text{N}, -60 \text{ to } -78^\circ\text{C}\) to r.t., 5 h, 64%; (d) 32, benzene, reflux, overnight; 10%HCl/MeOH, r.t., 3 h, 39% over two steps; (e) \(\text{K}_2\text{Cr}_2\text{O}_7\), \(\text{H}_2\text{SO}_4\), Acetone, 0°C, 2.5 h, 27%; (f) 32, benzene, reflux, overnight, 74%.

Jones oxidation\(^\text{77}\) of alcohol 2.56 to converted it to carboxylic acid 2.57 in 27% yield.
Initially, several attempts to access phosphonate 2.59 were met with inconsistent results and side reactions. However, careful study of the reaction conditions of alkyl phosphonate addition and the later substitution of the electrophile in the reaction to ester 2.35 resulted in phosphonate 2.61 in good yield (Scheme 2.9).

**SCHEME 2.9.** Synthesis of advanced intermediates to employ for the olefination of 2.30.\(^a\)

\[^a\] Reagents and conditions: (a) 2.60, \(n\)-BuLi, THF, \(-78^\circ\text{C}\) to \(-40^\circ\text{C}\), 3 h, 60%.

As was previously touched upon, the conditions employed for the HWE reaction failed to yield the expected linear chain. It was observed that the phosphonate, under the reaction conditions and in the absence of an electrophile, produced the same uncharacterized mixture (by \(^1\)H NMR) as that found for the HWE reaction, suggesting decomposition of the phosphonate.
Heathcock et al. used a similar approach and phosphonate 2.66 towards the synthesis of the halichlorine family of natural products.\textsuperscript{64} They reported difficulty with the HWE olefination; their best yield (27\%) was achieved under the Masamune-Roush conditions. Without additional insight into the basis of their results, they replaced the phosphonate 2.66 with the phosphorane equivalent 2.67 (Figure 2.16) and found an increase in yield (66\%).

![Figure 2.16. Key olefination strategies in the synthesis of pinnaic acid.](image)

We attempted a similar approach by the basic hydrolysis of ester 2.35; conversion to the pivaloyl mixed anhydride and nucleophilic addition of ethylenetriphenylphosphorane. The latter step proved ineffective in the synthesis of our system, precipitating our decision to adjust our route.

2.2.3.4. Strategy IV

Our fourth and final strategy was conceived to circumvent our setback in strategy 3 and retain the simplicity of the route (Scheme 2.10). The feature of our scheme is the Lewis acid-mediated aldol reaction between dienal 2.30 and ketone 2.37. We converted ester 2.35 to Weinreb amide 2.36 by a nucleophilic addition of the deprotonated Wenreib amine in THF (61\%). The product was treated with ethyl Grignard to smoothly furnish ketone 2.37 in 62\% and unreacted 2.36. With both key fragments in hand, we proceeded to couple them using an aldol methodology. Generation of the enolate was effected by
complexation of 2.37 at -78°C in DCM with TiCl₄, followed by deprotonation of the activated intermediate with Hunig's base. Dienal 2.30 was diluted in DCM and slowly added by syringe pump overnight to produce the β-hydroxyketone adduct, which was treated with MsCl/Et₃N in THF to form the C6-C7 olefin, and immediately followed by deprotection of the C1 silyl ether with TBAF in THF to isolate the stable hydroxyketone tetraene 2.38 as a mixture of diastereomers (6% over three steps).

**Scheme 2.10. Synthesis of 2.39.**

![Scheme 2.10](image)

*a Reagents and conditions: (a) i-PrMgCl, HCl·HN(OMe)Me, THF, -20°C, 2 h, 61%; (b) EtMgBr, THF, 0°C, 2 h, 62%; (c) TiCl₄, (i-Pr)₂NEt, CH₂Cl₂, -78°C, overnight; MsCl, Et₃N, 0°C, overnight; TBAF, THF, r.t., overnight, 6% over three steps; (d) (COCl)₂, DMSO, Et₃N, -78°C, overnight, 100%.

The end game towards the synthesis of 2.11 began with the oxidation of alcohol mixture 2.38 using the Swern conditions which furnished tetraene aldehyde 2.39 as a mixture of diastereomers. Gratifyingly, the C3-C4 double bond from conjugation with the C5 ketone to conjugation with the newly formed C1 aldehyde was unambiguously confirmed by ¹H NMR; its signal was a singlet at 9.38 ppm.

To test the viability of aldehyde 2.39 as a hypothetical intermediate in the biosynthesis of 2.11, we explored reaction conditions that we predicted would promote double bond regioisomerization (Scheme 2.11). We considered MacMillan's catalyst⁷⁸ an attractive method to mimic an enzyme's strategy for dienophile activation at mild
temperature conditions. With the addition of stoichiometric amount of a Brønsted acid (p-TSOH) in CDCl₃, we expected proton transfer and the concomitant enolization to lead to product. The ¹H NMR showed full conversion to the iminium ion upon treatment with the catalyst. After five days and slow heating from room temperature to reflux, the aldehyde slowly decomposed. To introduce greater polarization of the dienophile and increase the reactivity of the acyclic intermediate, we opted to employ MeAlCl₂, a strong Lewis acid common in Diels-Alder reactions. The acyclic intermediate 2.39 was treated with the Lewis acid at -78°C in DCM and was slowly warmed up to room temperature overnight. After aqueous workup, we were surprised to discover the intact, unreacted starting material.

**Scheme 2.11.** Conditions attempted for the IMDA cyclization of aldehyde 2.39 to 2.11. The molecule’s equilibrium will be dominated by the presence of its Z or E enolate tautomers. Only the E enolate will be reactive towards an IMDA. Formation of the bicyclic IMDA adduct depends on accessing the conjugated E enol, as this conformation will favor the intramolecular approach of the reactive moieties.

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a Reagents and conditions: (1) p-TSOH, MacMillan’s catalyst, r.t. to reflux, 5 d; (2) MeAlCl₂, CH₂Cl₂, -78°C to r.t., overnight; (3) H₂O, d-DMSO, 80°C, 5 d.

Lastly, we considered the inclusion of water in the reaction as it is known to cause an acceleration by promoting a minimization of the substrate's surface area exposed to the solvent, and consequently the enforcement of self-stabilizing hydrophobic interactions.
The substrate was dissolved in $d$-DMSO and 10% H$_2$O, and heated to 80°C for five days. The use of water as a co-solvent was favored over its deuterated counterpart to prevent proton exchange and better follow formation of precededented bicyclic aldehyde 2.69 by $^1$H NMR. However, intermediate 2.39 remained unreactive under the reaction conditions; no enolization or IMDA was observed.

2.3. Conclusions

Through our synthesis of linear intermediate 2.39, we set to rationalize the biosynthesis of the polyketide chain and its relationship with the trans-hydrindane scaffold of spiculoic acid A. We were concerned that the initial biosynthetic hypothesis of spiculoic acid A did not adequately address the double bond regiochemistry in the nascent polyketide. Consequently, we developed an alternative hypothesis, and set to confirm it by the synthesis of a linear polyketide polyene consistent with the canon of PKS dehydration, with the belief it might under mild, enzyme free conditions, grant access to the spiculoic acid A framework. Our convergent route was effective in generating the full carbon skeleton of our linear putative biomimetic intermediate. However, we were unable to coax the linear precursor to cyclize under either mild or harsh reaction conditions.

2.3.1. A spontaneous cycloaddition reaction is not operative

Our hypothesis for formation of the trans-hydrindane scaffold under mild reaction conditions did not prove correct. We have rationalized our results by analyzing the orbital coefficients of the reactive centers in our linear enol precursor to spiculoic acid A. We expect coefficient overlap of the reactants at the transition state to lead to the
preferred route to products\textsuperscript{7}. For our analysis we designed, modeled and optimized two simplified cyclic analogs of our enol intermediate, each representing an individual component of the IMDA reaction. The A semi-empirical extended Hückel method was employed to generate the frontier molecular orbitals of each analog. Our goal was to extract from the frontier orbitals calculated a qualitative description of the influence the polarizing groups would have on the coefficients and, consequently, on the asynchronicity of the reaction\textsuperscript{7}. Our results clearly show the incompatibility of the analog's orbital coefficients (Figure 2.17). Although we did not observe enolization experimentally, we believe our analysis is sufficiently broad to extend to the cycloaddition of \((E)\text{-2.68}\).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{model_analogs.png}
\caption{Modeled analogs employed for the orbital coefficient analysis of the IMDA of linear precursor \((E)\text{-2.68}\) in route to spiculoic acid A.}
\end{figure}

In addition, the stability of \textbf{2.39} under the various reaction conditions employed was surprising. The absence of a mixture of aldehyde regioisomers in solution suggests a high barrier for enolization, likely due to a buildup of 1,3-allylic strain. Accordingly, we set to understand the energy landscape and key structural characteristics of the steps involved in the conversion of \textbf{2.39} to \textbf{2.70}, the compounds involved in the reaction coordinate of a putative regioisomerization step. The results of our \textit{ab initio} computational study are summarized in Figure 2.18.
FIGURE 2.18. *Ab initio* analysis of regioisomerization of linear precursor 2.39. The C10 proton (blue) is coplanar to the C8-C9 and C11-C12 olefins, which results in its decreased acidity and likely prevents the regioisomerization of the molecule's olefin system.

In the optimized, lowest energy structure of 2.39, the coplanarity of the C10 proton to the C8-C9 and C11-C12 olefins's π system would minimize its acidity and clearly prevent the molecule's regioisomerization to a conformation favorable towards a Diels-Alder
cycloaddition. Moreover, the high equilibrium energy difference between (Z)-2.68 (+10.8 kcal/mol) and 2.39 may explain the reason for the latter's stability in solution. Interestingly, the (E)-2.68 necessary for an IMDA failed to converge in our attempts to find its energy minimum. The structure appeared to have been caught oscillating between two energy minima, a telling result that strengthens the likelihood the structure is not viable, at least in the gas phase.

The tacit biosynthetic implications of our analyses are two-fold: a non-canonical enzymatic regioisomerization step is indispensable to produce a viable compound for an IMDA, and likely a protein is involved in the cyclization of spiculoic acid A.

2.3.2. A non-canonical domain is likely responsible for the olefin regioisomerization

The general mechanism of incorporation of double bonds in the biosynthesis of polyketides is shown in Figure 2.19. The mechanism of dehydration proceeds by an E1cb syn elimination promoted by the catalytic histidine contained in the enzyme's signature HX₃GX₃P motif. The acidic pro-Şα-proton is transferred to the general base histidine, forming an intermediate thioester enolate which promptly undergoes elimination of the β-hydronium ion, a step that ensures that conjugation with the ACP-thioester is preordained.⁸⁷,⁸⁸

**FIGURE 2.19.** Canonical mechanism of dehydration in PKS DH domains.
Given this, an unusual non-canonical step must take place to regioisomerize the olefins of our linear polyketide on its way to spiculoic acid A. Biosynthetic gene clusters have recently been identified of a novel class of polyketide natural products containing uncharacteristic domains capable of effecting a plethora of non-canonical structural modifications during the assembly of the linear chain.\(^5^5,^5^8\) For example, modules 7 and 9 of the rhizoxin D's biosynthetic pathway (Figure 2.20) contain abnormal DH domains that isomerize the \(\alpha,\beta\)-olefins to a \(\beta,\gamma\) conformation by entirely different mechanisms\(^5^5\), a model we predict to be also present during the assembly of spiculoic acid A.

**Figure 2.20.** Modules 7 and 9 from the rhizoxin D biosynthetic pathway.
2.4. References


2.5. Experimental section

2.5.1. General methods

Reactions were carried out under an argon atmosphere with dry solvents and oven-dried glassware under anhydrous conditions unless specified otherwise. All reactions were carried out under an inert argon atmosphere with dry tetrahydrofuran, diethyl ether, and DCM solvents by passing them through activated alumina columns. Toluene, benzene, and triethylamine were freshly distilled over calcium hydride. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Ethyl 2-(triphenylphosphoranylidene)butyrate (2.40), and 2-iodoxybenzoic acid (IBX) were prepared by literature methods.1, 2 Yields refer to chromatographically and spectroscopically (1H NMR) homogeneous materials.

Reactions were monitored by analytical thin-layer chromatography (TLC) and carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as a visualizing agent and/or ceric ammonium molybdate (CAM), p-anisaldehyde (PA), and potassium permanganate (KMnO₄) staining solutions. Flash column chromatography was performed with E. Merck silica gel (60, particle size 0.040-0.063 mm). Preparative thin-layer chromatography (PTLC) separations were carried out on 0.25 mm E. Merck silica gel plates (60F-254).

1H NMR, 13C NMR, COSY, HSQC, HMQC and HMBC spectra were recorded on the Bruker DPX-300 or AMX-300, AMX-400, DRX-500, and Varian Inova 500 spectrometers and calibrated using residual undeuterated solvent as an internal reference. Data are reported as follows: chemical shifts in ppm (δ); multiplicities are indicated by the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, dd
= doublet of doublets, dt = doublet of triplets, td = triplet of doublets, ddd = doublet of doublet of doublets, m = multiplet, b = broad; coupling constants in Hz ($J$). Infrared spectra (IR) were obtained on NaCl discs on a Nicolet IR200 series FT-IR spectrometer. Low resolution atmospheric pressure chemical ionization (APCI) mass spectra were obtained on a Shimadzu LCMS-2010A quadrupole ion trap mass spectrometer. HRMS were obtained on a Kratos Analytical Concept instrument (University of Ottawa Mass Spectrum Centre).

2.5.2. Experimental procedures

2-Ethyl-4-phenyl-but-2-enoic acid ethyl ester (2.25)$^a$

Phenylacetaldehyde (2.24, +90% purity) (2.87 mL, 23.0 mmol) and ylide 2.40 (10.6 g, 28.2 mmol, 1.1 equiv) were dissolved in toluene (20 mL) and gently refluxed for 10 h. The reaction mixture was purified directly and without work-up by silica gel chromatography (3% EtOAc-hexanes) affording ester 2.25 (5.02 g, 23.0 mmol, 100%) as a yellow oil.

Analytical data for 2.25

- **M.W.:** 218.29
- **TLC ($R_f$):** 0.62 (20% EtOAc-hexanes)
- **$^1$H NMR:** (300 MHz, CDCl$_3$)
  - 7.30 (m, 5 H, PhH), 6.95 (t, $J = 7.5$ Hz, 1 H, H3), 4.25 (q, $J = 7.2$ Hz, 2 H, H13), 3.56 (d, $J = 7.5$ Hz, 2 H, H4), 2.50 (q, $J = 7.5$ Hz, 2 H, H11), 1.32
(t, J = 7.2 Hz, 3 H, H14), 1.14 (t, J = 7.5 Hz, 3 H, H12)

$^{13}$C NMR: (75 MHz, CDCl$_3$)
168.1 (C(1)), 140.0 (C(Ph)), 137.6 (C(2)), 135.1 (C(3)), 129.0 (C(Ph)),
128.9 (C(Ph)), 126.8 (C(Ph)), 60.8 (C(13)), 34.9 (C(4)), 20.5 (C(11)), 14.6
(C(14)), 14.4 (C(12))

LRMS: (APCI) m/z calcd for C$_{14}$H$_{19}$O$_2$ [(M+H)$^+$] 219.13, found 219.00

Reference: Tanaka, T.; Hiramatsu, K.; Kobayashi, Y.; Ohno, H., Chemo- and
stereoselectivity in titanium-mediated regioselective ring-opening reaction
of epoxides at the more substituted carbon. Tetrahedron 2005, 61 (28),
6726-6742

$^a$ Ester 3 was identical to its previously reported spectroscopic values

2-Ethyl-4-phenyl-but-2-en-1-ol (2.26)$^a$

Ester 2.25 (4.09 g, 18.7 mmol) was dissolved in dry dichloromethane (DCM, 20 mL)
and cooled to 0 °C while vigorously stirring under argon. Diisobutylaluminum hydride
(DIBAL-H, 1.0 M in toluene, 37.5 mL, 37.5 mmol, 2.0 equiv) was added dropwise over 5
min. The mixture was slowly warmed-up to room temperature and stirred for 1 h. Upon
completion (as per TLC) the reaction was cooled to 0°C and quenched by the slow,
stepwise addition of water (1.52 mL), aqueous 15% NaOH solution (1.52 mL), and water
(3.8 mL). The white, gel-like precipitate was filtered and the mother liquor extracted
with ethyl acetate (EtOAc, 3 × 100 mL). The organic layers were combined and washed
with brine (150 mL), dried over Na$_2$SO$_4$ and filtered. The solvent was removed in vacuo
and the residue was purified by silica gel chromatography (10% EtOAc-hexanes)
affording alcohol 2.26 (3.30 g, 100%) as a colorless oil.
Analytical data for 2.26

M.W.: 176.25

TLC ($R_f$): 0.22 (20% EtOAc-hexanes)

$^1$H NMR: (300 MHz, CDCl$_3$)
7.30 (m, 5 H, PhH), 5.60 (t, $J = 7.5$ Hz, 1 H, H3), 4.11 (d, $J = 0.9$ Hz, 2 H, H1), 3.48 (d, $J = 7.5$ Hz, 2 H, H4), 2.28 (q, $J = 7.5$ Hz, 2 H, H11), 1.13 (t, $J = 7.5$ Hz, 3 H, H12)

$^{13}$C NMR: (75 MHz, CDCl$_3$)
141.7 (C(2)), 141.2 (C(Ph)), 128.7 (C(Ph)), 128.5 (C(Ph)), 124.6 (C(Ph)), 66.0 (C(1)), 33.8 (C(4)), 21.3 (C(11)), 13.5 (C(12))

LRMS: (APCI) m/z calcd for C$_{12}$H$_{15}$ [(M+H)$^+$-H$_2$O] 159.12, found 159.00


$^a$ Alcohol 2.26 was identical to its previously reported spectroscopic values.

**2-Ethyl-4-phenyl-but-2-enal (2.27)$^a$**

![Chemical structure of 2.26 and 2.27]

Alcohol 2.26 (1.54 g, 8.68 mmol) and iodoxybenzoic acid (IBX, 4.87 g, 17.4 mmol, 2.0 equiv) were dissolved in dimethylsulfoxide (DMSO, 22 mL) by vigorously stirring at room temperature for 1 h 20 min. Upon completion, the mixture was purified without work-up by silica gel chromatography (10% EtOAc-hexanes) affording aldehyde 2.27 (1.46 g, 8.38 mmol, 97%) as a colorless oil.

Analytical data for 2.27

M.W.: 174.10
**TLC** \((R_f)\) : 0.60 (20% EtOAc-hexanes)

**IR:** (Film)
2970, 1691, 1493, 1454, 1376, 1224, 1065, 796, 745, 699, 519

**\(^1\)H NMR:** (300 MHz, CDCl\(_3\))
9.41 (s, 1 H, H1), 7.29 (m, 5 H, PhH), 6.57 (t, \(J = 7.5\) Hz, 1 H, H3), 3.71 (d, \(J = 7.5\) Hz, 2 H, H4), 2.40 (q, \(J = 7.5\) Hz, 2 H, H11), 1.05 (t, \(J = 7.5\) Hz, 3 H, H12)

**\(^{13}\)C NMR:** (75 MHz, CDCl\(_3\))
195.1 (C(1)), 152.1 (C(2)), 145.5 (C(3)), 138.4 (C(Ph)), 129.0 (C(Ph)), 128.6 (C(Ph)), 126.9 (C(Ph)), 35.0 (C(4)), 17.5 (C(11)), 13.6 (C(12))

**LRMS:** (APCI) m/z calcd for C\(_{12}\)H\(_{16}\)O \([\text{M+H}]^+\) 175.12, found 175.00


\(^a\) Aldehyde 2.27 was identical to its previously reported spectroscopic values.

2,4-Diethyl-6-phenyl-hexa-2,4-dienoic acid ethyl ester (2.28)
2,4-Diethyl-6-phenyl-hexa-2,5-dienoic acid ethyl ester (2.28a)

![Diagram](image)

Aldehyde 2.27 (1.46 g, 8.38 mmol) and ylide 2.40 (5.04 g, 13.4 mmol, 1.6 equiv) were dissolved in benzene (21 mL), and the pale yellow solution was refluxed for 20 h. Purification was performed directly without work-up by silica gel chromatography (4% EtOAc-hexanes) affording an inseparable mixture of ester isomers 2.28 and 2.28a (400 mg, 1.47 mmol, 37%) as well as unreacted starting material (470 mg, 2.7 mmol, 32%) that was re-subjected to the above conditions.
Analytical data for **2.28**

M.W.: 272.38

**TLC** ($R_f$): 0.51 (10% EtOAc-hexanes).

**IR:** (Film)
3027, 2968, 2934, 2875, 1711, 1640, 1494, 1236, 1128, 746, 698

$^1$H **NMR:** (500 MHz, CDCl$_3$)

7.38-7.34 (m, 5 H, PhH), 7.27 (s, 1 H, H3), 5.82 (t, $J = 7.5$ Hz, 1 H, H5),
4.45-4.35 (q, $J = 7$ Hz, 2 H, H17), 3.67 (d, $J = 7.5$ Hz, 2 H, H6), 2.61 (q, $J = 7.5$ Hz, 2 H, H15), 2.46 (q, $J = 7.5$ Hz, 2 H, H13), 1.46 (t, $J = 7$ Hz, 3 H, H18),
1.23-1.17 (m, 6 H, H14 & H16)

$^{13}$C **NMR:** (125 MHz, CDCl$_3$)

168.62 (C(1)), 141.27 (C(3)), 140.68 (C(4)), 138.88 (C(Ph)), 136.07 (C(2)),
129.88 (C(5)), 129.36 (C(Ph)), 128.70 (C(Ph)), 126.26 (C(Ph)), 60.61
(C(17)), 34.33 (C(6)), 23.72 (C(13)), 21.17 (C(15)), 14.49 (C(18)), 14.06
(C(16)), 13.57 (C(14))

HRMS: (EI) m/z calcd for C$_{18}$H$_{24}$O$_2$ [(M)$^+$] 272.1776, found 272.1770

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Analytical data for **2.28a**

M.W.: 272.38

**TLC** ($R_f$): 0.51 (10% EtOAc-hexanes).

**IR:** (Film)
3027, 2968, 2934, 2875, 1711, 1640, 1494, 1236, 1128, 746, 698

$^1$H **NMR:** (500 MHz, CDCl$_3$)

7.47-7.44 (m, 5 H, PhH), 6.78 (d, $J = 10$ Hz, 1 H, H3), 6.53 (d, $J = 16$ Hz, 1 H, H6),
6.25 (dd, $J = 15.5$, 7.5 Hz, 1 H, H5), 4.44-4.36 (q, $J = 7$ Hz, 2 H, H17),
3.30 (m, 1 H, H4), 2.54 (q, $J = 7.5$ Hz, 2 H, H13), 1.80-1.67 (m, 2 H, H15),
1.46 (t, $J = 7$ Hz 3 H, H18), 1.23-1.17 (m, 3 H, H14), 1.10 (t, $J = 7.5$
Hz, 3 H, H16)

$^{13}$C **NMR:** (125 MHz, CDCl$_3$)

168.48 (C(1)), 143.26 (C(3)), 135.24 (C(Ph)), 134.02 (C(2)), 131.67 (C(5)),
130.20 (C(6)), 128.62 (C(Ph)), 128.46 (C(Ph)), 128.26 (C(Ph)), 60.88
(C(17)), 44.17 (C(4)), 28.43 (C(15)), 21.00 (C(13)), 14.49 (C(18)), 14.73
(C(14)), 12.03 (C(16))

HRMS: (EI) m/z calcd for C$_{18}$H$_{24}$O$_2$ [(M)$^+$] 272.1776, found 272.1770
Esters 2.28 and 2.28a (1.35 g, 4.96 mmol) were dissolved in toluene (25 mL) and cooled in an acetone-dry ice bath to -78 °C under argon. DIBAL-H (1.0 M in toluene, 19.8 mL, 19.8 mmol, 4.0 equiv) was added by syringe over 15 min. The solution was warmed up slowly to room temperature and stirred for 1 h. Quenched the mixture by the slow, stepwise addition of water (0.79 mL), aqueous 15% NaOH solution (0.79 mL), and water (1.98 mL). The gel-like precipitate was filtered and the mother liquor extracted with ethyl acetate (EtOAc, 2 × 20 mL), washed with brine (20 mL), dried over Na₂SO₄ and filtered. The solvent was removed in vacuo and the residue purified by silica gel chromatography (10% EtOAc-hexanes) affording an inseparable mixture of alcohols 2.29 and 2.29a (1.14 g, 100%) as a colorless oil.

Analytical data for 2.29

M.W.: 230.35

TLC (Rf): 0.39 (30% EtOAc-hexanes).

IR: (Film)
3315, 3026, 2964, 2873, 1494, 1454, 1029, 744, 695

¹H NMR: (500 MHz, CDCl₃)
7.20-7.00 (m, 5 H, PhH), 5.74 (s, 1 H, H3), 5.30 (t, J = 7.5 Hz, 1 H, H5), 3.95 (s, 2 H, H1), 3.31 (d, J = 7.5 Hz, 2 H, H6), 2.17-1.99 (m, 4 H, H13 & H15), 0.98-0.84 (m, 6 H, H14 & H16)

¹³C NMR: (125 MHz, CDCl₃)
142.03 (C(4)), 141.43 (C(2)), 139.13 (C(Ph)), 128.75 (C(Ph)), 128.45 (C(Ph)), 127.65 (C(3)), 126.17 (C(5)), 125.99 (C(Ph)), 66.62 (C(1)), 34.22 (C(6)), 24.23 (C(15)), 21.98 (C(13)), 13.71 (C(14)), 13.52 (C(16))
HRMS: (EI) m/z calcd for C_{16}H_{22}O [(M^+)] 230.1671, found 230.1665

Analytical data for 2.29a

**M.W.:** 230.35

**TLC (R_f):** 0.39 (30% EtOAc-hexanes)

**IR:** (Film)
3315, 3026, 2964, 2873, 1494, 1454, 1029, 744, 695

**^1^H NMR:** (500 MHz, CDCl$_3$)
7.20-7.00 (m, 5 H, PhH), 6.20 (d, $J = 16$ Hz, 1 H, H6), 5.95 (dd, $J = 16, 7.5$ Hz, 1 H, H5), 5.14 (d, $J = 9.5$ Hz, 1 H, H3), 3.93 (s, 2 H, H1), 2.90 (m, 1 H, H4), 2.17-1.99 (m, 2 H, H13), 1.50-1.20 (m, 2 H, H15), 0.98-0.84 (m, 3 H, H14), 0.77 (t, $J = 7.5$ Hz, 3 H, H16)

**^1^C NMR:** (125 MHz, CDCl$_3$)
141.11 (C(2)), 137.90 (C(Ph)), 133.76 (C(5)), 128.91 (C(6)), 128.61 (C(Ph)), 128.35 (C(Ph)), 128.20 (C(3)), 126.61 (C(Ph)), 66.71 (C(1)), 43.03 (C(4)), 28.87 (C(15)), 21.53 (C(13)), 13.39 (C(14)), 12.02 (C(16))

HRMS: (EI) m/z calcd for C_{16}H_{22}O [(M^+)] 230.1671, found 230.1665

2.4-Diethyl-6-phenyl-hexa-2,4-dienal (2.30)
2.4-Diethyl-6-phenyl-hexa-2,5-dienal (2.30a)

Alcohols 2.29 and 2.29a (790 mg, 3.43 mmol) and IBX (1.92 g, 6.86 mmol, 2.0 equiv) were dissolved in DMSO (9 mL) by vigorously stirring at room temperature for 1 h and 20 min. Purified the mixture directly and without prior work-up by silica gel chromatography (10% EtOAc-hexanes) affording inseparable aldehydes 2.30 and 2.30a (760 mg, 3.33 mmol, 97%) as a colorless oil.
Analytical data for **2.30**

**M.W.:** 228.33

**TLC (**$R_f$**):** 0.67 (30% EtOAc-hexanes)

**IR:** (Film)
3027, 2968, 2875, 2711, 1685, 1613, 1454, 1377, 1189, 1060, 967, 910, 793, 747, 697

$^1$H NMR: (500 MHz, CDCl$_3$)
9.40 (s, 1H, H1), 7.39-7.23 (m, 5 H, PhH), 6.72 (s, 1 H, H3), 5.94 (t, $J = 7.5$ Hz, 1 H, H5), 3.59 (d, $J = 7.5$ Hz, 2 H, H6), 2.48-2.37 (m, 4 H, H13 & H15), 1.12-1.03 (m, 6 H, H14 & H16)

$^{13}$C NMR: (125 MHz, CDCl$_3$)
195.26 (C(1)), 152.98 (C(3)), 145.03 (C(4)), 143.51 (C(2)), 139.02 (C(7)), 134.53 (C(5)), 128.89 (C(Ph)), 127.71 (C(Ph)), 126.38 (C(Ph)), 34.59 (C(6)), 23.25 (C(15)), 18.35 (C(13)), 13.92 (C(14)), 13.68 (C(16))

**HRMS:** (EI) m/z calcd for C$_{16}$H$_{20}$O [(M$^+$)] 228.1514, found 228.1497

Analytical data for **2.30a**

**M.W.:** 228.33

**TLC (**$R_f$**):** 0.67 (30% EtOAc-hexanes)

**IR:** (Film)
3027, 2968, 2875, 2711, 1685, 1613, 1454, 1377, 1189, 1060, 967, 910, 793, 747, 697

$^1$H NMR: (500 MHz, CDCl$_3$)
9.44 (s, 1 H, H1), 7.39-7.23 (m, 5 H, PhH), 6.45 (d, $J = 16$ Hz 1 H, H6), 6.33 (d, $J = 10$ Hz, 1 H, H3), 6.13 (dd, $J = 16$, 7.5 Hz, 1 H, H5), 3.36 (m, 1 H, H4), 2.36-2.31 (m, 2 H, H13), 1.74-1.61 (m, 2 H, H15), 1.12-1.03 (m, 3 H, H14), 0.98 (t, $J = 7.5$ Hz, 3H, H16)

$^{13}$C NMR: (125 MHz, CDCl$_3$)
196.04 (C(1)), 155.54 (C(3)), 152.08 (C(2)), 137.30 (C(7)), 130.97 (C(6)), 130.62 (C(5)), 128.81 (C(Ph)), 128.54 (C(Ph)), 126.53 (C(Ph)), 44.30 (C(4)), 28.33 (C(15)), 17.84 (C(13)), 13.84 (C(14)), 11.92 (C(16))

**HRMS:** (EI) m/z calcd for C$_{16}$H$_{20}$O [(M$^+$)] 228.1514, found 228.1497
4,6-Diethyl-2-methyl-8-phenyl-octa-2,4,6-trienoic acid ethyl ester (2.41)
4,6-Diethyl-2-methyl-8-phenyl-octa-2,4,7-trienoic acid ethyl ester (2.41a)

Aldehydes 2.30 and 2.30a (760 mg, 3.33 mmol) and ylide 2.47 (3.02 g, 8.33 mmol, 2.5 equiv) were dissolved in benzene (20 mL) and refluxed overnight. The mixture was directly purified without work-up by silica gel chromatography (4% EtOAc-hexanes) affording a mixture of inseparable esters 2.41 and 2.41a (880 mg, 2.82 mmol, 85%) as a pale yellow oil.

**Analytical data for 2.41**

- **M.W.:** 312.45
- **TLC ($R_f$):** 0.70 (20% EtOAc-hexanes)
- **IR:**
  - (Film)
  - 3061, 3026, 2964, 2874, 1713, 1631, 1453, 1367, 1248, 1114, 1031, 964, 912, 746, 696
- **$^1$H NMR:**
  - (500 MHz, CDCl$_3$)
  - 7.20-7.00 (m, 5 H, PhH), 6.96 (s, 1 H, H3), 5.72 (s, 1H, H5), 5.36 (t, $J = 7.5$ Hz, 1 H, H7), 4.04 (q, $J = 7.5$ Hz, 2 H, H20), 3.33 (d, $J = 7.5$ Hz, 2 H, H8), 2.10-2.05 (m, 4 H, H16 & H18), 1.83 (d, $J = 1.5$ Hz, 3 H, H15), 1.15 (t, $J = 7.5$ Hz, 3 H, H21), 0.88-0.76 (m, 6 H, H17 & H19)
- **$^{13}$C NMR:**
  - (125 MHz, CDCl$_3$)
  - 169.13 (C1)), 142.27 (C3), 142.05 (C4), 139.19 (C6), 138.35 (C(Ph)) 134.29 (C5), 131.55 (C2), 128.52 (C(Ph)), 127.25 (C7), 126.26 (C(Ph)), 60.81 (C20), 34.40 (C8), 24.31 (C16), 24.04 (C18), 14.53 (C21), 14.34 (C15), 14.18 (C17), 13.46 (C19)
- **LRMS:** (APCI) m/z calcd for C$_{21}$H$_{29}$O$_2$ [(M+H)$^+$] 313.22, found 313.05

**Analytical data for 2.41a**
M.W.: 312.45

TLC ($R_f$): 0.70 (20% EtOAc-hexanes)

IR: (Film)
3061, 3026, 2964, 2874, 1713, 1631, 1453, 1367, 1248, 1114, 1031, 964, 912, 746, 696

$^1$H NMR: (500 MHz, CDCl$_3$)
7.20-7.00 (m, 5 H, PhH), 6.96 (s, 1 H, H3), 6.20 (d, $J = 16$ Hz, 1 H, H8),
5.94 (dd, $J = 16$, 7.5 Hz, 1 H, H7), 5.18 (d, $J = 10$ Hz, 1 H, H5), 4.04 (q, $J$
= 7.5 Hz, 2 H, H20), 2.96 (m, 1 H, H6), 2.15 (q, $J = 7.5$ Hz, 2 H, H16), 1.82
(d, $J = 1.5$ Hz, 3 H, H15), 1.47-1.30 (m, 2 H, H18), 1.15 (t, $J = 7.5$ Hz, 3 H, H21), 0.88-0.76 (m, 6H, H17 & H19)

$^{13}$C NMR: (125 MHz, CDCl$_3$)
169.06 (C(1)), 141.87 (C(3)), 141.25 (C(4)), 137.86 (C(Ph)), 135.29 (C(5)),
133.16 (C(7)), 131.38 (C(2)), 129.35 (C(8)), 128.97 (C(Ph)), 128.70
(C(Ph)), 126.14 (C(Ph)), 60.81 (C(20)), 43.67 (C(6)), 28.90 (C(18)), 24.76
(C(16)), 14.53 (C(21)), 14.29 (C(15)), 13.69 (C(17)), 12.14 (C(19))

LRMS: (APCI) m/z calcd for C$_{21}$H$_{29}$O$_2$ [(M+H)$^+$] 313.22, found 313.05

4,6-Diethyl-2-methyl-8-phenyl-octa-2,4,6-trien-1-ol (2.42)
4,6-Diethyl-2-methyl-8-phenyl-octa-2,4,7-trien-1-ol (2.42a)

Esters 2.41 and 2.41a (880 mg, 2.82 mmol) were dissolved by vigorously stirring in
toluene (5 mL) and cooled in an acetone-dry ice bath to -78 °C under argon. DIBAL-H
(1.0 M in toluene, 26.3 mL, 26.3 mmol, 9.0 equiv) was added dropwise over 15 min. The
reaction was warmed-up to room temperature and stirred an additional 2.5 h. The
reaction was quenched by the slow, stepwise addition of water (0.6 mL), aqueous 15%
NaOH solution (0.6 mL), and water (1.5 mL). The precipitate was filtered and the
mother liquor washed with brine (15 mL), dried over Na$_2$SO$_4$ and filtered. The solvent
was removed in vacuo and the residue purified by silica gel chromatography (10% EtOAc-hexanes) affording alcohols 2.42 and 2.42a (662 mg, 2.45 mmol, 87%) as a colorless oil.

**Analytical data for 2.42**

**M.W.:** 270.41  
**TLC (Rf):** 0.22 (20% EtOAc-hexanes)  
**IR:** (Film) 3323, 3025, 2963, 1494, 1452, 1376, 1070, 963, 908, 745, 695  
**¹H NMR:** (500 MHz, CDCl₃)  
7.20-7.00 (m, 5 H, PhH), 5.70 (s, 1 H, H3), 5.57 (s, 1 H, H5), 5.32 (t, J = 7.5 Hz, 1 H, H7), 3.91 (s, 2 H, H1), 3.32 (d, J = 7.5 Hz, 2 H, H8), 2.11-1.98 (m, 4 H, H16 & H18), 1.65 (d, J = 1.5 Hz, 3 H, H15), 0.89-0.77 (m, 6 H, H17 & H19)  
**¹³C NMR:** (125 MHz, CDCl₃)  
139.63 (C(6)), 139.51 (C(4)), 138.53 (C(2)), 136.44 (C(Ph)), 130.76 (C(5)), 128.55 (C(Ph)), 127.73 (C(3)), 126.13 (C(Ph)), 125.93 (C(7)), 125.80 (C(Ph)), 69.10 (C(1)), 34.28 (C(8)), 25.10 (C(18)), 24.49 (C(16)), 15.48 (C(15)), 13.98 (C(19)), 13.44 (C(17))  
**LRMS:** (APCI) m/z calcd for C₁₉H₂₅ [(M+H)⁺-H₂O] 253.20, found 253.05

**Analytical data for 2.42a**

**M.W.:** 270.41  
**TLC (Rf):** 0.22 (20% EtOAc-hexanes)  
**IR:** (Film) 3323, 3025, 2963, 1494, 1452, 1376, 1070, 963, 908, 745, 695  
**¹H NMR:** (500 MHz, CDCl₃)  
7.20-7.00 (m, 5 H, PhH), 6.21 (d, J = 16 Hz, 1 H, H8), 5.99 (dd, J = 16, 7 Hz, 1 H, H7), 5.75 (s, 1 H, H3), 4.99 (d, J = 7 Hz, 1 H, H5), 3.91 (s, 2 H, H1), 2.94 (m, 1 H, H6), 2.11-1.98 (m, 2 H, H16), 1.67 (d, J = 1.5 Hz, 3 H, H15), 1.50-1.35 (m, 2 H, H18), 0.89-0.77 (m, 6 H, H17 & H19)  
**¹³C NMR:** (125 MHz, CDCl₃)
1441.48 (C(4)), 137.99 (C(2)), 135.93 (C(Ph)), 133.95 (C(7)), 131.10 (C(5)), 128.74 (C(8)), 126.55 (C(Ph)), 127.44 (C(Ph)), 126.97 (C(Ph)), 69.10 (C(1)), 43.47 (C(6)), 28.97 (C(18)), 24.39 (C(16)), 15.48 (C(15)), 13.39 (C(17)), 12.10 (C(19))

LRMS: (APCI) m/z calcd for C_{19}H_{25} [(M+H)^{+}-H_2O] 253.20, found 253.05
Calcd: 271.21
Found: 253.05 (C_{12}H_{15}^{+}(M-18)^{+})

4,6-Diethyl-2-methyl-8-phenyl-octa-2,4,6-trienal (2.43)
4,6-Diethyl-2-methyl-8-phenyl-octa-2,4,7-trienal (2.43a)

Alcohols 2.42 and 2.42a (662 mg, 2.45 mmol) and IBX (1.37 g, 4.90 mmol, 2.0 equiv) were dissolved in DMSO (22 mL) and vigorously stirred at room temperature for 45 min. The mixture was purified directly and without prior work-up by silica gel chromatography (10% EtOAc-hexanes) affording an inseparable mixture of aldehydes 2.43 and 2.43a (536 mg, 2.0 mmol, 82%) as a yellow oil.

Analytical data for 2.43

M.W.: 268.39
TLC ($R_f$): 0.59 (20% EtOAc-hexanes)
IR: (Film)
3341, 2963, 2710, 1686, 1494, 1378, 1198, 1014, 912, 746

$^1$H NMR: (500 MHz, CDCl$_3$)
9.27 (s, 1 H, H1), 7.20-6.99 (m, 5 H, PhH), 6.58 (s, 1 H, H3), 5.99 (s, 1 H, H5), 5.44 (t, $J=7.5$ Hz, 1 H, H7), 3.34 (d, $J=7.5$ Hz, 2 H, H8), 2.29 (q, $J=7.5$ Hz, 2 H, H18), 2.12 (q, $J=7.5$ Hz, 2 H, H16), 1.77 (d, $J=1.5$ Hz, 3 H, H15), 0.93-0.79 (m, 6 H, H17 & H19)

$^{13}$C NMR: (125 MHz, CDCl$_3$)
195.99 (C(1)), 153.95 (C(3)), 139.19 (C(2)), 138.91 (C(6)), 138.62 (C(4)), 138.05 (C(5)), 137.02 (C(Ph)), 128.70 (C(Ph)), 128.49 (C(7) & (C(Ph))), 126.26 (C(Ph)), 34.43 (C(8)), 24.20 (C(16) & C(18)), 14.48 (C(17)), 13.50 (C(19)), 11.06 (C(15))

LRMS: (APCI) m/z calcd for C_{19}H_{25}O [(M+H)^+] 269.19, found 269.05

Analytical data for 2.43a

M.W.: 268.39

TLC (R_f): 0.59 (20% EtOAc-hexanes)

IR: (Film) 3341, 2963, 2710, 1686, 1494, 1378, 1198, 1014, 912, 746

^1^H NMR: (500 MHz, CDCl_3)
9.28 (s, 1 H, H1), 7.39-7.23 (m, 5 H, PhH), 6.59 (s, 1 H, H3), 6.22 (d, J = 16 Hz 1 H, H8), 5.94 (dd, J = 16, 7.5 Hz, 1 H, H7), 5.47 (d, J = 9.5 Hz, 1 H, H5), 3.02 (m, 1 H, H6), 2.21 (q, J = 7.5 Hz, 2 H, H16), 1.78 (d, J = 1.5 Hz, 3 H, H15), 1.48-1.36 (m, 2 H, H18), 0.93-0.79 (m, 6 H, H17 & H19)

^13^C NMR: (125 MHz, CDCl_3)
196.00 (C(1)), 153.58 (C(3)), 140.91 (C(2)), 139.73 (C(5)), 137.64 (C(Ph)), 137.49 (C(4)), 132.38 (C(7)), 129.85 (C(8)), 128.70 (C(Ph)), 128.49 (C(7) & (C(Ph))), 126.26 (C(Ph)), 43.91 (C(6)), 28.81 (C(18)), 23.53 (C(16)), 13.99 (C(17)), 12.06 (C(19)), 10.96 (C(15))

LRMS: (APCI) m/z calcd for C_{19}H_{25}O [(M+H)^+] 269.19, found 269.05

(4E,6E,8E)-Ethyl 2,6,8-triethyl-3-hydroxy-4-methyl-10-phenyldeca-4,6,8-trienoate (2.44)

To a well stirred solution of aldehyde 2.43 and 2.43a (107 mg, 0.40 mmol) in benzene (0.40 mL), zinc metal (29 mg, 0.44 mmol, 1.1 equiv) and 2-3 crystals of iodine
were added. Ethyl 2-bromobutyrate (2.55, 62 μL, 1.05 equiv) was added over 5 minutes and heated mixture to reflux. After 10 minutes, dark green solution had turned ochre. Continued to reflux for a total of 3 hours and solution was then cooled to room temperature. Once cool, quenched reaction with 10% aqueous H₂SO₄ (1 mL) and extracted with EtOAc (3 x 1 mL), dried organic phase over Na₂SO₄ and filtered. Evaporated solvent in vacuo and purified residue by silica gel chromatography (10% EtOAc-hexanes) affording alcohols 2.50 (68 mg, 0.18 mmol, 45%) and 2.50a (51 mg, 0.13 mmol, 33%) as pale yellow oils.

Analytical data for 2.50 (two regioisomers)

M.W.: 384.27

TLC (Rf): 0.33 (20% EtOAc-hexanes)

IR: (Film)
3462, 2965, 2930, 1731, 1375, 1262, 1179, 1031, 746, 696

¹H NMR: (500 MHz, CDCl₃) - Regioisomer I
7.18-6.97 (m, 5 H), 5.76 (s, 1 H), 5.49 (s, 1 H), 5.26 (t, 1 H), 4.07 (d, J = 6.5 Hz, 1 H), 4.03-3.92 (m, 2 H), 3.30 (d, J = 7.5 Hz, 2H), 2.40 (m, 1 H), 2.40 (m, 1 H), 2.06-1.93 (m, 2 H), 1.60 (s, 3 H), 1.56-1.27 (m, 4 H), 1.11-1.01 (m, 3 H), 0.85-0.66 (m, 9 H)

¹H NMR: (500 MHz, CDCl₃) - Regioisomer II
7.18-6.97 (m, 5 H), 6.18 (dd, J = 15.5, 7.5 Hz, 1 H), 5.94 (m, 1 H), 5.74 (s, 1 H), 4.91 (d, J = 9.5 Hz, 1 H), 4.07 (d, J = 6.5 Hz, 1 H), 4.03-3.92 (m, 2 H), 2.90 (quintet, J = 8.0 Hz, 1 H), 2.40 (m, 1 H), 2.06-1.93 (m, 2 H), 1.61 (s, 3 H), 1.56-1.27 (m, 4 H), 1.11-1.01 (m, 3 H), 0.85-0.66 (m, 9 H)

Analytical data for 2.50a (two regioisomers)

M.W.: 384.27

TLC (Rf): 0.25 (20% EtOAc-hexanes)

IR: (Film)
3461, 2964, 2931, 2874, 1733, 1459, 1376, 1267, 1178, 1028, 746, 695
$^1$H NMR: (500 MHz, CDCl$_3$) – Regioisomer I

$^1$H NMR of Regioisomer I is inconclusive.

$^1$H NMR: (500 MHz, CDCl$_3$) – Regioisomer II

7.17-7.00 (m, 5 H), 6.19 (dd, $J = 16, 4.5$ Hz, 1 H), 5.94 (ddd, $J = 16, 4.5, 2.0$ Hz, 1 H), 5.72 (s, 1 H), 4.93 (d, $J = 9.5$ Hz, 1 H), 4.00 (m, 3 H), 2.90 (quintet, $J = 8.0$ Hz, 1 H), 2.42 (m, 1 H), 2.08-1.93 (m, 2 H), 1.58 (s, 3 H), 1.46-1.28 (m, 4 H), 1.12-1.07 (m, 3 H), 0.84-0.69 (m, 9 H)

2-(Hydroxymethyl)butanol (2.32)

Diethyl ethylmalonate 2.31 (941 mg, 5.00 mmol) was added dropwise to a solution of lithium aluminum hydride (LAH, 285 mg, 7.53 mmol, 30.0 equiv) vigorously stirred in tetrahydrofuran (THF, 50 mL) at 0°C. The resulting reaction was warmed up to r.t. and stirred overnight. To bring the reaction to completion, solution was refluxed for an additional 24 h. After cooling the mixture, it was quenched with 0.4 ml H$_2$O, followed by 0.4 mL 15% NaOH(aq) and lastly 1.2 mL H$_2$O. Precipitated aluminum salts were filtered and the oil was purified by silica gel chromatography (30% EtOAc-hexanes) affording diol 2.32 (427 mg, 4.10 mmol, 82%) as a clear oil.

Analytical data for 2.32$^a$

M.W.: 104.15

TLC ($R_f$): 0.20 (40% Acetone-hexanes)

$^1$H NMR: (400 MHz, CDCl$_3$)

3.80 (dd, $J = 12, 4$ Hz, 2 H, H1’ & H3’), 3.64 (dd, $J = 12, 8$ Hz, 2 H, H1” & H3”), 1.66 (m, 1 H, H2), 1.44 (bs, 2 H, OH), 1.27 (quintet, $J = 8$ Hz, 2 H, H4), 0.93 (t, $J = 8$ Hz, 3 H, H5)

$^{13}$C NMR: (100 MHz, CDCl$_3$)

66.49 (C(1)), C(3)), 43.69 (C(2)), 20.61 (C(4)), 11.74 (C(5))

Diol **2.32** was identical to its previously reported spectroscopic values.

### 2-((tert-Butyldiphenylsilyloxy)methyl)butan-1-ol (2.33)

![Diagram of 2-((tert-Butyldiphenylsilyloxy)methyl)butan-1-ol](image)

Diol **2.32** (521 mg, 5.00 mmol) was dissolved in a 1:4 biphasic solution of acetonitrile and hexanes (10 mL and 40 mL respectively), to which triethylamine (Et$_3$N, 0.84 mL, 1.2 equiv) and finally tert-butyldiphenylsilyl chloride (TBDPSCl, 1.3 mL, 5.0 mmol, 1.0 equiv) were added. Stirred at r.t. for 72 h, quenched with a saturated NH$_4$Cl solution, and extracted with EtOAc (3 x 20 mL), dried organic phase over Na$_2$SO$_4$ and filtered. Solvent was evaporated in vacuo and the residual oil purified by silica gel chromatography (5% EtOAc-hexanes) affording monoprotected diol **2.33** (962 mg, 2.81 mmol, 56%) as a colorless oils.

### Analytical data for **2.33**

- **M.W.**: 342.55
- **TLC** ($R_f$): 0.38 (20% EtOAc-hexanes)
- **$^1$H NMR**: (400 MHz, CDCl$_3$)
  - 7.67-7.64 (m, 4 H, PhH), 7.45-7.36 (m, 6 H, PhH), 3.84-3.60 (m, 4 H, H1 & H3), 1.73-1.64 (dd, $J = 4.4$, 2 Hz, 1 H, OH), 1.31-1.18 (m, 2 H, H4), 1.04 (s, 9 H, H6), 0.83 (t, $J = 7.6$ Hz, 3 H, H5)
- **$^{13}$C NMR**: (100 MHz, CDCl$_3$)
135.61 (2 C, Ph), 135.60 (2 C, Ph'), 133.10 (C(Ph)), 133.08 (C(Ph')), 129.83 (2 C, Ph, Ph'), 127.79 (2 C, Ph), 127.73 (2 C, Ph'), 67.22 (C(1)), 65.93 (C(3)), 43.94 (C(2)), 26.86 (3 C, C(6)), 20.55 (C(4)), 19.15 (C(7)), 11.69 (C(5))


*2.33* was identical to its previously reported spectroscopic values.

### 2-((tert-Butyldiphenylsilyloxy)methyl)butanal (2.34)

In a round bottomed flask (r.b.f.) oxalyl chloride ((COCl)\(_2\), 0.27 mL, 3.1 mmol, 3.5 equiv) was dissolved in dichloromethane (5.8 mL) and cooled to -60°C. Dimethylsulfoxide (0.31 mL, 4.4 mmol, 5.0 equiv) was added dropwise at this temperature, and immediately upon addition gas generation was observed. The solution was stirred for an additional 30 min at -60°C, at which point the temperature was lowered to -78°C. A precooled (-78°C) solution of monoprotected diol *2.33* (300 mg, 0.88 mmol) in DCM (3 mL) was added dropwise by cannula and the white and opaque mixture was stirred for 1 h. Triethylamine (1.22 mL, 8.8 mmol, 10 equiv) was added dropwise and the thick slurry was slowly warmed to r.t. after which distilled water (3mL) was added to quench. Extracted with EtOAc (3 x 10 mL), dried organic phase over Na\(_2\)SO\(_4\) and filtered. After evaporation of solvent the oil was purified by silica gel chromatography (5% EtOAc-hexanes) affording aldehyde *2.34* (192 mg, 0.56 mmol, 64%) as a clear oil.

**Analytical data for 2.34**

M.W.: 340.53
**TLC** \((R_f)\): 0.64 (20% EtOAc-hexanes)

**\(^1\)H NMR:** (400 MHz, CDCl\(_3\))

9.71 (d, \(J = 2.4\) Hz, 1 H, H3), 7.63-7.61 (m, 4 H, PhH), 7.42-7.24 (m, 6 H, PhH), 3.86 (m, 2 H, H1), 2.34 (m, 1 H, H2), 1.74 (m, 1 H, H4\('\)), 1.46 (m, 1 H, H4\('\)), 1.01 (s, 9 H, H6), 0.86 (t, \(J = 7.2\) Hz, 3 H, H5)

**\(^{13}\)C NMR:** (100 MHz, CDCl\(_3\))

204.6 (C(3)), 135.59 (4 C, Ph, Ph'), 133.19 (C(Ph)), 133.17 (C(Ph')), 129.81 (2 C, Ph, Ph'), 127.76 (4 C, Ph, Ph'), 62.32 (C(1)), 55.85 (C(2)), 26.76 (3 C, C(6)), 19.24 (C(7)), 18.51 (C(4)), 11.43 (C(5))


\(^a\) Aldehyde 2.34 was identical to its previously reported spectroscopic values.

\((E)\)-Ethyl 4-\((\text{tert}-\text{butyldiphenylsilyloxy})\text{methyl}\)-2-ethylhex-2-enoate (2.35)

Aldehyde 2.34 (670 mg, 1.96 mmol) and ylide 2.40 (1.5 g, 4.0 mmol, 2.0 equiv) were dissolved in benzene (20 mL, 0.1M) and stirred vigorously while it refluxed for 24 h. The mixture was cooled to r.t. and the solvent evaporated in vacuo. The mixture was filtered without work-up through a silica gel plug (100% hexanes), and the residual oil was purified by column chromatography (10% EtOAc-hexanes) furnishing ester 2.35 (635 mg, 1.45 mmol, 74%) as a clear oil.

**Analytical data for 2.35**

M.W.: 438.67

**TLC** \((R_f)\): 0.65 (20% EtOAc-hexanes)

**\(^1\)H NMR:** (400 MHz, CDCl\(_3\))
7.81-7.61 (m, 4 H, PhH), 7.43-7.33 (m, 6 H, PhH), 6.51 (d, $J = 10.5$ Hz, 1 H, H3), 4.18 (m, 2 H, H10), 3.55 (dq, $J = 9.9$, 3.7 Hz, 2 H, H1), 2.52 (m, 1 H, H2), 2.25 (m, 2 H, H6), 1.67 (m, 1 H, H8'), 1.27 (m, 1 H, H8''), 1.27 (t, $J = 7.1$ Hz, 3 H, H11), 1.01 (s, 9 H, H12), 0.94 (t, $J = 7.4$ Hz, 3 H, H7), 0.83 (t, $J = 7.4$ Hz, 3 H, H9)

$^{13}$C NMR: (100 MHz, CDCl$_3$)
167.83 (C(5)), 143.35 (C(3)), 135.65 (2 C, Ph), 135.63 (2 C, Ph'), 135.30 (C4), 133.66 (C(Ph)), 133.63 (C(Ph')), 129.62 (C(Ph)), 129.61 (C(Ph')), 129.63 (4 C, Ph, Ph'), 66.58 (C(1)), 60.29 (C(10)), 43.13 (C(2)), 26.80 (3 C, C(12)), 24.11 (C(8)), 20.43 (C(6)), 19.26 (C(13)), 14.30 (C(11)), 14.29 (C(7)), 11.85 (C(9))

HRMS: (EI) m/z calcd for C$_{23}$H$_{29}$O$_3$Si [(M-C$_4$H$_9$)$_+^+$] 381.1880, found 381.1855

*(E)-Ethyl 2-ethyl-4-(hydroxymethyl)hex-2-enoate (2.56)*

![Diagram of compound 2.56](image)

Ester 2.35 (327 mg, 0.96 mmol) was treated with a 10% HCl/MeOH (5 mL) solution and stirred at r.t. for 3 h. The solution was slowly quenched with a saturated sodium bicarbonate solution, diluted with H$_2$O and EtOAc and extracted thrice with EtOAc. The organic layers were combined, dried with Na$_2$SO$_4$ and, after evaporation, the oil was purified by column chromatography (20% EtOAc-hexanes) furnishing alcohol 2.56 (67 mg, 0.60 mmol, 39%) as a clear oil.

**Analytical data for 2.56**

M.W.: 200.27

TLC ($R_f$): 0.23 (20% EtOAc-hexanes)

$^1$H NMR: (400 MHz, CDCl$_3$)
6.42 (d, $J = 10.4$ Hz, 1 H, H3), 4.18 (q, $J = 7.2$ Hz, 2 H, H10), 3.61 (dd, $J = 5.6$, 5.2 Hz, 1 H, H1''), 3.49 (dd, $J = 7.6$, 2.8 Hz, 1 H, H1''), 2.55 (m, 1 H, H2), 2.34 (dq, $J = 7.2$, 3.2 Hz, 2 H, H6), 1.55 (m, 2 H, H8' & OH), 1.28 (m, 1 H, H8''), 1.28 (t, $J = 7.2$ Hz, 3 H, H11), 1.01 (t, $J = 7.2$ Hz, 3 H, H7), 0.87 (t, $J = 7.2$ Hz, 3 H, H9)
\[ \text{\(^{13}\)C NMR: (100 MHz, CDCl}_3 \) } \\
167.6 (C(5)), 142.4 (C(3)), 136.8 (C(4)), 65.8 (C(1)), 60.5 (C(10)), 43.4 (C(2)), 24.0 (C(8)), 20.5 (C(6)), 14.3 (C(11)), 14.2 (C(7)), 11.8 (C(9)) \\

\( (E)-4-(\text{Ethoxycarbonyl})-2\text{-ethylhex-3-enoic acid (2.57)} \)

Alcohol 2.56 (67 mg, 0.33 mmol) was pre-dissolved in an acidic solvent system of aqueous 10% H\(_2\)SO\(_4\) (0.3 mL) and acetone (3 mL) while stirring at 0°C under an argon atmosphere. Prior to addition, the Jones reagent was freshly prepared by vigorously mixing in a vial potassium dichromate (294.8 mg, 1 mmol), conc. H\(_2\)SO\(_4\) (0.2 mL) followed by distilled water (1 mL). The bright orange mixture (0.43 mL, 0.36 mmol, 1.1 equiv) was slowly syringed into the alcohol solution and stirred for 2.5 h at which time the chromium's oxidation state change was evidenced by a concomitant color change from bright orange to olive green. Quenching was accomplished by addition of a saturated solution of NaHCO\(_3\) (4 mL) and dilution with EtOAc (1 mL). The aqueous layer was acidified by an aqueous 0.1M HCl solution until the pH reached ~2.0. After extraction with EtOAc (3x5 mL), drying of organic layers with Na\(_2\)SO\(_4\) and evaporation solvent in vacuo, the oil was purified by column chromatography (20% EtOAc-hexanes) isolating acid 2.57 (19 mg, 0.089 mmol, 27%).

**Analytical data for 2.57**

- **M.W.:** 214.26
- **TLC \((R_f)\):** 0.23 (20% EtOAc-hexanes)
- **\(^1\)H NMR:** (400 MHz, CDCl\(_3\))
1H NMR: (400 MHz, CDCl3)

(E)-Diethyl 6-(tert-butyldiphenylsilyloxy)methyl)-4-ethyl-3-oxooct-4-en-2-ylphosphonate (2.61)

Triethyl ethylphosphonate 2.60 (100 µL, 0.62 mmol, 2.5 equiv) was dissolved in THF (1.5 mL) and cooled to -78°C while stirring under argon. After 10 min n-BuLi (250 µL, 0.63 mmol, 2.5 equiv) was added and warmed up to -40°C over 1.5 hrs. Ester 2.35 (109 mg, 0.25 mmol) dissolved in THF (1 mL) was cooled to -40°C and transferred by cannula to the phosphonate-containing solution. Stirred overnight. Quenched with HCl (1 mL, 10%), diluted with water (0.5 mL) and extracted with EtOAc (3 x 2 mL). Dried with MgSO4 and evaporated solvent in vacuo. The viscous oil was purified by column chromatography (20% EtOAc-hexanes to 100% EtOAc) isolating inseparable mixture (a 1.4:1 ratio) of phosphonate diastereomers 2.61 (84 mg, 0.15 mmol, 60%).

Analytical data for 2.61

**M.W.:** 558.76

**TLC (Rf):** 0.09 (20% EtOAc-hexanes)
7.64-7.59 (m, 4 H), 7.42-7.33 (m, 6 H), 6.41 (d, \(J = 10.1\) Hz, 1 H), 6.29 (d, \(J = 10.1\) Hz, 1 H), 4.07 (m, 4 H), 3.83 (m, 1 H), 3.60 (m, 2 H), 2.62 (m, 1 H), 2.26 (m, 1 H), 1.74 (m, 1 H), 1.31 (m, 1 H), 1.15 (t, \(J = 7.1\) Hz, 3 H), 1.03 (s, 9 H), 1.00 (s, 9 H), 0.85 (m, 6 H)

\(^{13}\)C NMR: (100 MHz, CDCl\(_3\))


\((E)-4-((\text{tert-Butyldiphenylsilyloxy})\text{methyl})-2\text{-ethyl-N-methoxy-N-methylhex-2-enamide (2.36)}\)

Ester 2.35 (343 mg, 0.78 mmol) and N,O-dimethylhydroxylamine hydrochloride (117 mg, 1.2 mmol, 1.5 equiv) were stirred vigorously in THF (4 mL, 0.2M) and cooled under argon to -20°C. \(i\)-PrMgCl (1.2 mL, 2.4 mmol, 3.0 equiv) was added over 10 minutes and warmed up to 0°C. After 2 hrs, quenched with NH\(_4\)Cl (2 mL) and extracted with EtOAc (2 x 5 mL). Dried with MgSO\(_4\) and after evaporation of solvent in vacuo the crude was purified by column chromatography (20% EtOAc-hexanes) isolating amide 2.36 (214.6 mg, 0.47 mmol, 61%) while also recovering unreacted ester 2.35 (130 mg, 0.30 mmol).

Analytical data for 2.36

M.W.: 453.69

TLC \((R_f)\): 0.21 (20% EtOAc-hexanes)

\(^1\)H NMR: (300 MHz, CDCl\(_3\))
7.64-7.62 (m, 4 H, PhH), 7.43-7.32 (m, 6 H, PhH), 5.48 (d, J = 10.1 Hz, 1 H, H3), 3.58 (s, 3 H, H10), 3.54 (d, J = 6.2 Hz, 2 H, H1), 3.20 (s, 3 H, H11), 2.46 (m, 1 H, H2), 2.67 (dq, J = 7.6, 0.9 Hz, 2 H, H6), 1.69 (m, 1 H, H8'), 1.23 (m, 1 H, H8''), 1.03 (s, 9 H, H12), 0.94 (t, J = 7.5 Hz, 3 H, H7), 0.85 (t, J = 7.4 Hz, 3 H, H9)

$^{13}$C NMR: (75 MHz, CDCl$_3$)
171.96 (C(5)), 138.33 (C(3)), 135.59 (2 C, Ph), 135.57 (2 C, Ph'), 133.73 (C(4)), 133.68 (C(Ph)), 133.62 (C(Ph')), 129.60 (C(Ph)), 129.58 (C(Ph')), 127.61 (4 C, Ph, Ph'), 66.70 (C(1)), 60.82 (C(10)), 42.30 (C(2)), 34.13 (C(11)), 26.84 (3 C, C(12)), 24.30 (C(8)), 21.80 (C(6)), 19.23 (C(13)), 13.21 (C(7)), 11.73 (C(9))

HRMS: (EI) m/z calcd for C$_{23}$H$_{30}$NO$_3$Si [(M-C$_4$H$_9$)$_+^+$] 396.1989, found 396.2013

$(E)$-$6$-((tert-Butyldiphenylsilyloxy)methyl)-4-ethyloct-4-en-3-one (2.37)

Amide 2.36 (80 mg, 0.18 mmol) was dissolved by stirring in THF (1.8 mL, 0.1M) cooled under argon to 0°C. After 10 min, EtMgBr (0.14 mL, 0.42 mmol, 3.0 equiv) were added and the solution stirred for another 2 hrs at 0°C. Quenched with NH$_4$Cl (0.5 mL), diluted with water (0.3 mL) and extracted with EtOAc (3 x 2 mL). Dried with MgSO$_4$ and after evaporation of solvent in vacuo the crude was purified by column chromatography (20% EtOAc-hexanes) isolating ketone 2.37 (47 mg, 0.11 mmol, 62%) while also recovering unreacted amide 2.36 (20 mg, 0.044 mmol).

Analytical data for 2.37

M.W.: 422.67
TLC ($R_f$): 0.72 (20% EtOAc-hexanes)
$^1$H NMR: (400 MHz, CDCl$_3$)
7.64-7.60 (m, 4 H, PhH), 7.43-7.33 (m, 6 H, PhH), 6.33 (d, J = 10.2 Hz, 1 H, H3), 3.63 (m, 2 H, H1), 2.60 (m, 3 H, H2, H10), 2.25 (dq, J = 7.4, 1.1 Hz, 2 H, H6), 1.68 (m, 1 H, H8'), 1.23 (m, 1 H, H8''), 1.07 (t, J = 7.3 Hz, 3 H, H11), 1.01 (s, 9 H, H12), 0.88 (t, J = 7.4 Hz, 3 H, H7), 0.84 (t, J = 7.5 Hz, 3 H, H9)

$^{13}$C NMR: (100 MHz, CDCl$_3$)
202.36 (C(5)), 144.11 (C(4)), 143.36 (C(3)), 135.62 (2 C, Ph), 135.58 (2 C, Ph'), 133.56 (2 C, Ph, Ph'), 129.71 (C(Ph)), 129.68 (C(Ph')), 127.67 (4 C, Ph, Ph'), 66.59 (C(1)), 43.28 (C(2)), 30.60 (C(10)), 26.81 (3 C, C(12)), 24.27 (C(8)), 19.38 (C(6)), 19.27 (C(13)), 14.25 (C(7)), 11.85 (C(9)), 8.94 (C(11))

HRMS: (EI) m/z calcd for C$_{23}$H$_{29}$O$_2$Si [(M-C$_4$H$_9$)$_3$] 365.1931, found 365.1925

(4E,7E,9E,12E)-5,9,11-Triethyl-3-(hydroxymethyl)-7-methyl-13-phenyltrideca-4,7,9,12-tetraen-6-one (2.38)

Aldol. Ketone 2.37 (386 mg, 0.92 mmol) was dissolved in DCM (15 mL, 0.03M) and cooled under argon to -78°C. After stirring for 20 min, to the clear solution, titanium tetrachloride (TiCl$_4$, 121 µL, 1.1 mmol, 1.2 equiv) was added dropwise over 15 min. The light yellow solution was stirred for an additional 30 min at -78°C at which time N,N-diisopropylethylamine (DIPEA, 192 µL, 1.1 mmol, 1.2 equiv) was added, immediately showing enolate formation by the solution's notable change of coloration to a dark red/purple hue. Stirred the solution for 1 hr at -78°C, after which a solution of aldehyde 2.30/2.30a (158 mg, 0.69 mmol, 0.75 equiv) dissolved in DCM (20 mL) was added by syringe pump (0.7 mL/hr) overnight. Quenched with MeOH (10 mL), and slowly warmed up to r.t. The solution was diluted with water (10 mL) and extracted with EtOAc (3 x 10 mL). Dried the organic phase with MgSO$_4$, evaporated solvent in vacuo, and partially purified the crude oil by column chromatography (5% EtOAc-hexanes) to obtain
β-hydroxy ketone (272 mg) which was mesylated without further purification.

**Mesylation-Elimination/Deprotection.** Crude β-hydroxy ketone (243 mg, ~0.37 mmol), DMAP (114 mg, 0.93 mmol, 2.5 equiv) were dissolved in DCM and cooled at -20°C while stirring under argon. Slowly added TEA (780 µL, 5.6 mmol, 15 equiv) followed by methanesulfonyl chloride (MsCl, 147 µL, 1.9 mmol, 5.0 equiv). The solution was slowly warmed up to r.t. and stirred overnight. Quenched with a saturated solution of NaHCO₃ (5 mL), diluted with H₂O (10 mL), and extracted with EtOAc (3 x 10 mL). Dried the organic phase with MgSO₄, evaporated solvent in vacuo, and partially purified the crude oil by column chromatography (5% EtOAc-hexanes). The oil obtained was deprotected by addition of TBAF (2 mL, 2.0 mmol, 5.5 equiv) to a solution in THF (10 mL) while stirring at r.t. under argon overnight. Quenched by addition of H₂O and extracted with EtOAc (3 x 5 mL). The organic phase was dried with MgSO₄, evaporated solvent in vacuo, and purified the crude oil by column chromatography (10% EtOAc-hexanes) to furnish alcohol 2.38 (20 mg, 0.051 mmol, 6% over three steps) and its uncharacterized, minor diastereomer.

**Analytical data for 2.38**

**M.W.:** 394.59

**TLC (Rₜ):** 0.28 (20% EtOAc-hexanes)

**¹H NMR:** (500 MHz, CDCl₃)

7.32-7.15 (m, 5 H, PhH), 6.65 (s, 1 H, H7), 6.36 (d, J = 15.8 Hz, 1 H, H12), 6.09 (ddd, J = 15.9, 7.4, 2.6 Hz, 1 H, H11), 5.66 (d, J = 10.3 Hz, 1 H, H3), 5.38 (dd, J = 9.6, 2.2 Hz, 1 H, H9), 3.60 (dd, J = 10.6, 5.6 Hz, 1 H), 3.48 (dd, J = 10.9, 7.5 Hz, 1 H), 3.11 (p, J = 7.6 Hz, 1 H, H10), 2.61 (m, 1 H, H2), 2.42 (m, 3 H, H15, OH), 2.22 (m, 2 H, H18) 2.00 (t, J = 1.1 Hz, 3 H, H17), 1.63-1.45 (m, 3 H, H13, H20''), 1.25 (m, 1 H, H20''), 1.04-0.80 (m, 12 H)

**¹³C NMR:** (125 MHz, CDCl₃)
201.98 (C(5)), 144.69 (C(8)), 143.03 (C(7)), 139.70 (C(6)), 139.68 (C(3)), 137.56 (C(4)), 135.24 (C(9)), 135.11 (C(Ph)), 132.84 (C(11)), 129.15 (C(12)), 128.48 (2 C, Ph), 127.05 (C(Ph)), 126.01 (2 C, Ph), 65.95 (C(1)), 43.62 (C(10)), 43.23 (C(2)), 28.68 (C(13)), 24.28 (C(18)), 24.11 (C(20)), 21.22 (C(15)), 14.31 (C(17)), 13.51 (C(19)), 13.48 (C(16)), 11.91 (C(21)), 11.88 (C(14))

HRMS: (EI) m/z calcd for C_{19}H_{23}O [(M-C_{8}H_{15}O)^+] 267.1740, found 267.1743

(2E,6E,8E,11E)-2,4,8,10-Tetraethyl-6-methyl-5-oxo-12-phenyldodeca-2,6,8,11-tetraenal (2.39)

Oxalyl chloride ((COCl)$_2$, 16 µL, 0.18 mmol, 3.5 equiv) was dissolved in dichloromethane (2.0 mL) and cooled to -78°C. Dimethylsulfoxide (17 µL, 0.26 mmol, 5.0 equiv) was added dropwise and the solution stirred for an additional 30 min at -78°C. A precooled (-78°C) solution of alcohol 2.38 (20 mg, 0.052 mmol) in DCM (0.5 mL) was added by cannula and the white and opaque mixture was stirred for 1 h. Triethylamine (73 µL, 0.52 mmol, 10 equiv) was added and the thick slurry was slowly warmed to r.t. overnight. H$_2$O (2 mL) was added to quench, followed by extraction with EtOAc (3 x 5 mL). The organic phase was dried over MgSO$_4$ and filtered. After evaporation in vacuo the oil was purified by column chromatography (20% EtOAc-hexanes) affording a mixture of aldehyde 2.39, and uncharacterized stereoisomers (20 mg, 0.052 mmol, 100%).

Analytical data for 2.39

M.W.: 392.57

TLC ($R_f$): 0.67 (20% EtOAc-hexanes)
$^1$H NMR: (500 MHz, CDCl$_3$)
9.38 (s, 1 H, H1), 7.17-7.35 (m, 5 H, PhH), 6.97 (d, $J$ = 1.1 Hz, 1 H, H7),
6.46 (d, $J$ = 10.5 Hz, 1 H, H3), 6.37 (dd, $J$ = 16.2, 3.3 Hz, 1 H, H12), 6.08
(dd, $J$ = 15.9, 7.4 Hz, 1 H, H11), 5.40 (d, $J$ = 9.8 Hz, 1 H, H9), 4.29 (m, 1
H, H4), 3.13 (bp, $J$ = 7.7 Hz, 1 H, H10), 2.28 (m, 4 H, H15, H20), 1.95 (d,
$J$ = 0.9 Hz, 3 H, H17), 1.66-1.46 (m, 2 H), 1.25 (m, 2 H), 1.01-0.80 (m, 12
H)

2.5.3. References

1. Frigerio, M.; Santagostino, M.; Sputore, S., A User-Friendly Entry to 2-

2. House, H. O.; Rasmusson, G. H., Stereoselective Synthesis of $\alpha$-Substituted $\alpha$,\$\beta$-
2.5.4. Selected 1D and 2D NMR spectra
Regioisomer 1 + Regioisomer 2

$2.50 + 2.50a$
CHAPTER 3. Regiochemistry and stereochemistry of thioesterase catalyzed macrocyclizations in polyketides

3.1. Introduction

The development of intramolecular cyclization techniques for the synthesis of natural products has been a lively area of research.\(^1\) The great complexity and variety of these structures have served as inspiration for the development of a plethora of methodologies. These large structures are rife with synthetic challenges owing to case-specific thermodynamic considerations of size, entropy and strain of the macrocycle and its acyclic counterpart. Historically, a large portion of the methodologies developed has been for the synthesis of macrolactone-containing natural products. The approaches taken have commonly been classified by the mode of activation of the alcohol or acid involved in the reaction. Both strategies involve chemoselective reactions that polarize the targeted moiety and consequently increase their reactivity. Some common acid activation methodologies involve formation of mixed anhydrides\(^2\) (3.1), thioesters\(^3\) (3.2), and \textit{O}-acylisoureas\(^4\) (3.3) (Figure 3.1-A). Although less prevalent, alcohol activation methodologies are also employed, and they generally involve its conversion to leaving groups such as oxyphosphonium ions\(^5\) (3.4), bromides\(^6\) (3.5), and methanesulfonates\(^7\) (3.6) (Figure 3.1-B).
FIGURE 3.1. Macrolactones can be synthesized by numerous (A) acid activation and (B) alcohol activation methods.

Regiochemical selectivity, however, remains at best an elusive proposition to attain synthetically. The common approach is to include extra atom-inefficient steps to protect and chemoselectively deprotect the requisite functionality prior to macrocyclizing, a process that lengthens routes and minimizes yields. Few compounds in the literature with multiple unprotected groups undergo regioselective macrocyclizations. Enzymatic processes may provide a viable alternative towards this important objective, in particular the thioesterase domains of polyketide biosynthesis.

3.1.1. Macrolide polyketides

Macrolide polyketides (Figure 3.2), are an economically important class of natural products that have found widespread applications as therapeutic agents. Molecules such as spiramycin and pimaricin have been clinically relevant in the treatment and prevention of common human and animal microbial ailments. Others, such as the epothilone family of natural products and their synthetic analogs, represent a class of
novel anticancer agents that exhibit greater effectiveness and lower toxicity than the common taxane diterpenoids employed in therapy\textsuperscript{17-25}.

**Figure 3.2.** Macrolide polyketides share a common biosynthetic origin which is capable of generating molecules of great structural diversity and assorted therapeutic applications.

Macrolides are biosynthesized by type I modular polyketide synthase (PKS) pathways.\textsuperscript{2-8} Erythromycin, the canonical macrolide system, and its core precursor 6-deoxyerythronolide B (6dEB, 3.7), derive from a biosynthetic gene cluster comprised of three large open reading frames each coding for the homodimeric megaproteins DEBS1, DEBS2 and DEBS3.\textsuperscript{26} Each megaprotein includes separate modules with multiple catalytic domains that mediate the stepwise thio-Claisen condensations of acetate equivalents, and reductively tailor the newly formed keto functional group (Figure 3.3).
3.1.2. Thioesterases catalyze regioselective macrocyclizations

A fundamental step in the biosynthesis of erythromycin, and of other macrolides by extension, is that catalyzed by the DEBS thioesterase domain (TE).\textsuperscript{27-29} The enzyme assures substrate turnover from the PKS, delivering it in its bioactive conformation. Its crystal structure\textsuperscript{27} exhibits a two-fold symmetric homodimer configuration with a molecular mass of 66kDa and a tertiary structure characterized by an $\alpha,\beta$-hydrolase fold (Figure 3.4).\textsuperscript{30} The active site of the DEBS TE is contained within a hydrophobic substrate channel that effectively traverses the monomer, a feature that is in contrast to the homologous fatty acid thioesterases (FA TEs)\textsuperscript{31}. The presence of a substrate channel was shown to not be an isolated example, but a conserved element of PKS TEs, and has
been seen in structures of the picromycin\textsuperscript{32} and curacin\textsuperscript{33} thioesterases. The function of the substrate channel is a topic of debate; a widely viewed hypothesis\textsuperscript{27} predicts the channel-substrate interaction to possess directionality, that is the transfer of the mature polyketide from its ACP-bound state to the TE active site takes place at one end of the channel, while exit of the macrocyclic or hydrolytic product occurs at the opposite end. Computational docking studies\textsuperscript{27} support this hypothesis by pointing to an Arg-rich site in the DEBS-TE in close proximity to the substrate channel that presumably serves as a region of non-covalent interaction with DEBS ACP\textsuperscript{6}.

![Figure 3.4](image_url)

\textbf{FIGURE 3.4.} DEBS TE crystal structure (PDB ID: 1KEZ). (A) Secondary structure representation of the homodimer. (B) Van der Waals space-filling model. The substrate channel traversing the enzyme is conspicuous. (C) Topographical map of DEBS TE. The connectivity of the enzyme's $\alpha$-helices (red rods) and $\beta$-strands (yellow arrows) is shown. The catalytic residue (red dots) and their approximate location is also shown.

The DEBS TE active site is comprised of the conserved catalytic triad Ser-142, Asp-169, His-259.\textsuperscript{34} In a classic charge-relay mechanism (Figure 3.5), key residue Ser-142 in the active site is deprotonated to generate a strong $O$-nucleophile that attacks the incoming $S$-acyl-ACP, cleaving the thioester bond.\textsuperscript{35} In vivo, this new $O$-acyl-TE
intermediate exclusively undergoes a regioselective intramolecular cyclization. However, under in vitro conditions hydrolysis is also routinely observed.

**Figure 3.5.** Canonical mechanism of the TE domain. Common among α/β hydrolase family, it involves a charge relay mechanism. The active site serine is deprotonated in the loading step, and a new O-acyl-enzyme intermediate is formed upon its attack on the upstream S-acyl-ACP. The conformation of the substrate in the channel may lead to either a regioselective intramolecular macrocyclization or intermolecular attack by a molecule of water to yield a seco-acid. The latter has been observed as an occurrence of in vitro experiments.

### 3.1.3. The factors influencing thioesterase regioselectivity remain unclear

Very little is known about the underlying factors that determine the regioselectivity of macrocyclization of thioesterase domains. The DEBS TE has shown to be remarkably tolerant of a broad range of substrate lengths by ably generating 6, 8, 12, 14 and 16 member rings (Figure 3.6).36-40

**Figure 3.6.** DEBS TE competently loads a wide range of linear chain lengths.

Results from site-directed mutagenesis studies support enzyme-substrate hydrophobic interactions as the main stabilizing forces of the substrate in the channel,41 likely inducing
the substrate into a favorable conformation towards a regioselective intramolecular macrocyclization, while shielding it from hydrolysis by exogenous water molecules. A weak hydrophilic barrier at the PICS TE channel’s exit consisting of bulk water and a glutamine residue has been proposed to explain the substrate’s bent arrangement in the channel and the proximity of the proper O-nucleophile to the active site.32

A recent study of serine hydrolases and acyltransferases42, both belonging to the α,β-fold class, correlated differences in the type of β-turn present between helix A and B in their respective active sites to hydrolysis or esterification activity. Depending on the configuration of this element, Kazlauskas et al. propose it can either increase the nucleophilicity of ordered water in the active site by hydrogen bonding interactions, or make way for a nucleophile to gain access to the O-acyl enzyme intermediate. This turn is, however, substantially different in macrolide-forming TEs, generally possessing multiple additional residues, dramatically limiting the predictability of TE activity based on sequence data.

No systematic study has been yet conducted with a full length substrate where the molecular basis of regioselectivity of polyketide TE macrocyclizations can be rigorously investigated. The stereochemical complexity of 6dEB (3.7), its native substrate, combined with its challenging multistep synthesis,43-47 has been an impediment to this goal. An approach involving a systematic modification of the native substrate, resulting in several structural variants for the examination of their interaction with the enzyme, would be out of the question because of its intricacy and inefficiency. Furthermore, a thioester such as 3.10 would be susceptible to multiple side reactions that would impede their utility in such a study (Figure 3.7). For example, the δ-hydroxy ketone structure in
**3.10** is known to undergo hemiketalization in aqueous buffers. In addition, formation of a δ-lactone would be a highly favorable step, yielding structure **3.11**. These examples serve to highlight the important role the binding cavity has in stabilizing the intermediates in the enzyme.

**FIGURE 3.7.** Putative side reactions of NAC-6dEB substrate **3.10** meant to illustrate the difficulties associated with its use as a probe for thioesterase macracyclization. Under thermodynamic control, hemiketalization (structures **3.8** and **3.9**) would likely take place. In addition, formation of a δ-lactone would be kinetically favored to yield structure **3.11**.

### 3.1.4. Discovery of a full length peptidyl substrate analog

Our lab recently reported the ability of wild-type, heterologously expressed DEBS TE to hydrolyze short thioester-containing substrates with greater catalytic efficiency than their polyketide analogs (Figure 3.8).
Informed by this discovery, we recognized the potential of the 7-aminoheptanoate NAC-thioester (3.15) as an element in a convergent synthesis of a linear analog of 6dEB (3.7, Figure 3.9). The other half of our envisioned analog, a phenylhexanoic acid moiety (3.14), was designed to be quickly prepared by simple chemical transformations.

Among the salient features in the design of the substrate is the inclusion of a phenyl chromophore on the backbone. The DEBS TE has shown to be amenable to this structural element, and its presence is expected to simplify the analysis of UV traces obtained by LC/MS. Retaining the native substrate's 1,3 diol would be key in providing answers to the DEBS TE regiochemistry of cyclization: the presence of both nucleophiles may yield 12 and 14-membered macrolactones. Furthermore, assuming the analog's effective macrocyclization, enantioselective methodologies applied to the synthetic route
would aid in correlating stereo- and regioselectivity of the enzyme. Lastly, the compound would be the first example of a polyketide TE mediated cyclization of a non-native substrate.

Initial work with the racemic and diastereoisomeric mixture of isomers, substrate 3.13 showed considerable potential.\(^5\) Upon treatment of DEBS TE with NAC-thioester 3.13, we were pleased to observe a mixture of products (Figure 3.10) by LCMS analysis, including two peaks that suggested either the presence of macrolactone regioisomers 3.16 and 3.17 or that of cyclic diastereoisomers.

\[\text{Effectively interpreting these results required us to look into the individual contributions of all stereoisomers to the observed product distribution. For this purpose, a stereocontrolled synthetic route was designed and a suitable hypothesis was formulated to guide and inform our discoveries. We have based the latter on a stereochemical pattern observed on numerous substrates ably cyclized by a DEBS TE. We believe that}\]

\[\text{\textbf{FIGURE 3.10.} Treatment of isolated DEBS TE with analog 3.13 generated a mixture of products (as monitored by LCMS).}\]
the regiochemical outcome of TE-mediated macrocyclization is predetermined by the substrate's terminal $O$-nucleophile of $R$ absolute stereochemistry (Figure 3.11).

**FIGURE 3.11.** We propose that the absolute stereochemistry of the terminal $O$-nucleophile (highlighted in red) determines the outcome of the TE-catalyzed macrolactonization.

Herein we present: (1) the total synthesis of four enantioenriched substrates; (2) the total synthesis of authentic macrolactone standards; (3) results of our enzymatic assays; and (4) a brief synopsis of our future efforts towards this project.

### 3.2. Results and discussion

#### 3.2.1. Retrosyntheses

**3.2.1.1. Enantioenriched NAC thioesters substrates**

To evaluate the contribution each discrete stereoisomer of 3.13 made to the product mixture observed upon treatment with the DEBS TE (Figure 10), we developed a general stereoselective route that would allow us to efficiently synthesize them. The salient features of this strategy are summarized in Scheme 3.1.
Our analysis begins with thioester 3.29, one of four stereoisomers necessary for our enzymatic assays. Embedded in the design is the inclusion of a NAC-thioester as it is a convenient and broadly employed methodology for mimicking CoA and ACP-bound thioesters, enabling the incorporation of substrates onto polyketide and fatty acid synthases. Disconnection of 3.29 at the amide bond (shown), reveals the NAC-thioester of methyl-7-aminoheptanoate 3.15, a compound obtained from coupling of commercially available HSNAC and seco-acid 3.33. The chiral dihydroxyacid coupling partner of amine 3.15 can be obtained from terminal olefin 3.25 through oxidative cleavage of the latter. Establishment of the homoallylic stereocenter can be accomplished from a regioselective vinyl cuprate addition to chiral epoxide 3.22. The latter is a product of a Jacobsen's salen(Co)-catalyzed hydrolytic kinetic resolution (HKR) of the epoxide mixture derived from mCPBA epoxidation of the chiral olefin 3.21. Disconnection of this intermediate, as shown, reveals commercially available phenylacetaldehyde 3.20 as a
suitable starting material, and clearly suggests an asymmetric allylation methodology, such as the Brown allylboration,\textsuperscript{54} to set the first stereocenter of the route.

\textbf{3.2.1.2. 12 and 14-membered standards}

Full spectroscopic characterization of the products formed in each enzymatic reaction would be possible only if a set of standards was also synthesized. Toward this goal, we designed a route adapted from the stereoselective methodology employed in the synthesis of our NAC-thioester substrates. The standard's retrosynthetic analysis was simplified since only diastereopurity (not enantiopurity) was needed (Scheme 3.2). Two racemic, diastereomERICALLY pure 1,3-diols (\textit{syn} and \textit{anti}) are at the centerpiece of our design. Cleavage of \textit{syn} macroolactones \textit{syn-3.42} and \textit{syn-3.43} reveals diprotected methyl ester \textit{syn-3.41}, their common intermediate. We predicted that the absence of obvious conformational bias and large number of degrees of freedom of these linear molecules would provide access to both ring sizes in about equal amounts.\textsuperscript{55} Macrolactonization studies with substrates containing multiple unprotected nucleophiles have shown selectivity dependent on the conformation of the chains and energies of the transition state. The enthalpy differences between 12- and 14-membered rings\textsuperscript{56} suggest of a slight preference toward formation of the 14-membered ring, however, the difference is sufficiently small to lead us to believe a mixture of products may be observed. Disconnection at the bond shown yields intermediates \textit{syn-3.40} and 3.34, both easily accessed from simple precursors and chemical steps. In the forward sense oxidative cleavage of the terminal olefin of \textit{syn-3.40} is expected to procure the carboxylic acid coupling partner of 3.34 which, in turn, can be assembled by protecting group manipulations of 7-aminoheptanoic acid (3.35). Our preliminary studies indicated that
the separation of the diastereomers of olefin 3.38 would not be possible without prior removal of the TBS protecting group. Removal afforded only limited success with the various chromatographic separations performed. The decision was taken to incorporate a preparative HPLC step to cleanly separate the mixture. At this juncture, olefin 3.38 would be accessible even more readily than homochiral analog 3.25 (Scheme 3.1), starting from phenylacetaldehyde (3.20), using non-selective allylation, and omitting HKR of the intermediate epoxide 3.37.

**Scheme 3.2.** Retrosynthesis of the racemic 12 and 14-membered macrolactone standards syn-3.42 and syn-3.43. For simplicity, only the route of the syn-standards is shown.
3.2.2. Syntheses

3.2.2.1. Enantioenriched NAC thioester substrates

The synthesis of our four enantioenriched analogs was accomplished by two complementary routes in under nine linear steps. Our enantioselective route was initiated by an asymmetric allylation of phenylacetaldehyde (3.20). 3.20 was treated separately with both optical isomers of allyldiisopinocampheylborane (Ipc2BAlyl)\(^{54}\) in an Et\(_2\)O/N\(_2(l)\) bath at \(-100^\circ\text{C}\) for 30 minutes (Scheme 3.3) furnishing homoallylic alcohols 3.21 and ent-3.21 in 63% and 44% yield respectively. Epoxidation of each enantiomer with a buffered solution of \(m\)CPBA produced a 1:1 mixture of diastereomers, as confirmed by \(^1\)H NMR. These were independently silylated to produce two inseparable mixtures, each consisting of epoxides 3.22-3.23, and ent-3.22-ent-3.23.

**SCHEME 3.3.** Synthesis of enantioenriched epoxides 3.22, 3.23, ent-3.22 and ent-3.23.\(^a\)

\(^a\) Reagents and conditions: (a) (-)-Ipc\(_2\)BAlyl, Et\(_2\)O, -100°C, 30 min, 63%; (b) (+)-Ipc\(_2\)BAlyl, Et\(_2\)O, -100°C, 30 min, 44%; (c) \(m\)-CPBA, NaHCO\(_3\), CH\(_2\)Cl\(_2\), r.t., 12hr; TBSCl, Imidazole, DMF, r.t., 24hr; (\(R,R\))-Salen, H\(_2\)O, THF, 16hr, 39% over three steps; (d) \(m\)-CPBA, NaHCO\(_3\), CH\(_2\)Cl\(_2\), r.t., 12hr; TBSCl, Imidazole, DMF, r.t., 24hr; (\(S,S\))-Salen, H\(_2\)O, THF, 16hr, 51% over three steps; (e) Bu\(_2\)SnO, TsCl, CH\(_2\)Cl\(_2\), 4hr; NaH, THF, overnight, 100% over two steps; (f) Bu\(_2\)SnO, TsCl, CH\(_2\)Cl\(_2\), 4hr; NaH, overnight, THF, 58% over two steps.

All four epoxide stereoisomers were obtained by using the Jacobsen hydrolytic kinetic
resolution. This methodology diastereoselectively hydrolyzed epoxides 3.23 and ent-3.23, and yielded enantioenriched epoxides 3.22 and ent-3.22 (50%, 38% yield respectively), and triols 3.24 and ent-3.24 (42%, 36% yield respectively). The triols were easily recycled into epoxides 3.23 and ent-3.23 by selective monosulfonylation\(^\text{57}\) followed by intramolecular nucleophilic displacement\(^\text{58}\) in 100% and 58% yields respectively (over two steps). This straightforward route provided access to our four key diastereo- and enantiopure epoxides.

With all four epoxide isomers in hand, we selected 3.22 to examine the steps leading to all four NAC-thioester substrates (Scheme 3.4). Vinylation of 3.22 proceeded smoothly (85%) to produce olefin 3.25 in 85% yield. The final steps to our key acid began by the silylation of alcohol 3.25, which was immediately followed by the oxidative cleavage of its terminal olefin with a buffered KMnO\(_4\)-NaIO\(_4\) mixture. This step, after workup and aqueous extraction, gratifyingly yielded the expected acid as confirmed by \(^1\)H NMR of the crude product mixture. The key acid's coupling partner, NAC-aminothioester 3.12, was synthesized from commercially available 3.33 (87%). Regrettably, standard deprotection conditions of 3.12’s terminal amino group (20% TFA in CH\(_2\)Cl\(_2\)) led, upon quenching, to substrate decomposition which forced us to modify our approach to the synthesis.
SCHEME 3.4. First attempt towards the synthesis of substrate 3.29. Failure to cleanly deprotect 3.12 hindered our progress.

To solve this problem, we examined a longer route relying on the amino acid methyl ester 3.34 as our coupling substrate (Scheme 3.5). 3.34 was synthesized by esterification of 7-aminoheptanoic acid (3.35) with TMSCl/MeOH (100% yield)\(^5\). Protection was followed by KMnO\(_4\)/NaIO\(_4\) cleavage of olefin 3.25. The crude acid was immediately treated with EDC and coupled with 3.34 to yield bis-silylated substrate 3.31 in 28% yield over three steps. Hydrolysis with LiOH in MeOH/THF/H\(_2\)O, followed by EDC coupling with HSNAC yielded thioester 3.27 in 53% yield over two steps. Deprotection in an acetonitrile solution of HF/Pyridine completed the synthesis of enantioenriched substrate 3.29 in 35% yield.
SCHEME 3.5. End game for the synthesis of NAC enantioenriched substrate 3.29.4

![Scheme 3.5](image)

Reagents and conditions: (a) TMSCl, MeOH, r.t., overnight, 100%; (b) TBSCI, Imidazole, DMF, r.t., overnight; KMnO₄, NaIO₄, 1:1 t-BuOH-buffer (pH 7), r.t., 1 h; 3.34, EDC, DMAP, CH₂Cl₂, r.t. overnight, 28% over three steps; (c) LiOH, MeOH/H₂O/THF, r.t., 6 h; HSNAC, EDC, DMAP, CH₂Cl₂, r.t., overnight, 53% over two steps; (d) HF, Pyridine, MeCN, r.t., 5 hrs, 35%.

To shorten our synthesis and improve our overall yield, we explored the literature for methodologies to remove Boc protecting groups of amines in the presence of thioesters. We came across the work of Kokotos et al.60 that reported an efficient removal of this group by treatment with an anhydrous HCl/Dioxane solution. Upon treatment of our protected amine 3.12 with this solution, we were pleased to observe clean and quantitative deprotection to amine 3.15. The synthesis of the remaining substrates is presented in Scheme 3.6; it differs only from our first attempt in the use of amine 3.15 in the convergent step of our route.
SCHEME 3.6. Synthesis of the remaining enantioenriched substrates.¹

Reagents and conditions: a) VinylMgBr, CuI, THF, -78°C, 85%; (b) 3.12, HCl/Dioxane, r.t., 45 min; c) TBSCl, imidazole, DMF, r.t., overnight; KMnO₄, NaIO₄, 1:1 t-BuOH-buffer (pH 7), r.t., 1 h; 3.15, EDC, DMAP, CH₂Cl₂, r.t. overnight; HSNAC, EDC, DMAP, CH₂Cl₂, r.t., overnight; (d) HF, Pyridine, MeCN, r.t., 5hrs.

3.2.2.2. 12 and 14-membered standards

In a manner analogous to our enantiopure NAC-thioester substrates, the route to our racemic diastereomerically pure macrolactone standards featured a short sequence of transformations from phenylacetaldehyde (3.20). As shown in Scheme 3.7, the synthesis commences with the addition of allylmagnesium bromide to 3.20, cleanly converting it to racemic homoallylic alcohol rac-3.21. Treatment of olefin rac-3.21 with mCPBA⁶¹-⁶³ furnished epoxide 3.36 (77% yield) as evidenced by the loss of the signature olefinic
NMR signals between 5-6 ppm. Predictably, the NMR integration ratios point towards the formation of a 1:1 mixture of diastereomers.

**Scheme 3.7.** Synthesis of macrolactone standards.

*Reagents and conditions:* (a) AllylMgBr, Et₂O, 0°C, 30 min, 100%; (b) mCPBA, CH₂Cl₂, r.t., 12hr, 77%; (c) TBSCI, Imidazole, DMF, r.t., 24hr, 100%; (d) VinylMgBr, Cul, THF, -20°C, 35%; (e) PTSA, EtOH, r.t., overnight, 60%; (f) TBSCI, Imidazole, DMF, r.t., overnight; (g) KMnO₄, NaIO₄, 1:1 t-BuOH-buffer (pH 7), r.t., 1 h; (h) LiOH, THF/H₂O; HF/MeCN; (i) (PyS)₂, PPh₃; Toluene, 80°C.
The regioselective vinylation of epoxide \textbf{3.36} required the protection of the alcohol moiety. This was accomplished with TBSCI\textsuperscript{64} to produce epoxide \textbf{3.37} in quantitative yield. Vinylation\textsuperscript{65} of \textbf{3.37} proceeded smoothly to generate \textbf{3.38} as a 1:1 mixture of homoallylic alcohols in 35\% yield. Attempts to separate \textbf{3.38} into its diastereomeric components by reverse-phase PHPLC were initially unsuccessful. From many test runs, a compromise was reached, requiring the removal of all protective groups from the mixture by treatment with \textit{p}-TsOH, to furnish the more polar 1,3-diol \textbf{3.39} in 60\% yield. We cleanly separated both diastereomers of the compound mixture by silica gel chromatography; however, we determined that a scale-up of the diol mixture would benefit from purification by reverse-phase preparative high pressure liquid chromatography (PHPLC). The mixture was found to separate well under the conditions stated, providing pure diols \textit{syn-3.39} and \textit{anti-3.39}, both in 32\% yield. Their stereochemistry was unambiguously established by $^{13}$C NMR from their respective \textit{syn-} and \textit{anti-}acetonides (Scheme 3.8).\textsuperscript{66} Treatment of each diol with a solution of \textit{p}-TsOH in acetone provided \textit{syn-3.44} (83\% yield) and \textit{anti-3.44} (33\% yield).

\begin{scheme}
Rychnovsky's acetonide methodology was used to determine the relative stereochemistry of racemic 1,3-diols separated by PHPLC.\textsuperscript{a}

\textit{Reagents and conditions:} (a) Acetone, PTSA, r.t., overnight.

With the carbodiimide coupling of our synthetic intermediates in mind, we treated both \textit{anti-3.39} and \textit{syn-3.39} to a sequence comprised of a silylation with TBSCI under...
standard conditions, followed by treatment with a buffered solution of potassium permanganate in tert-butanol, and lastly, EDC coupling with free amine to provide full length chain esters and syn-3.41 in 32% and 36% yields, respectively (Scheme 3.7). The endgame route to our macrocycle standards was initiated by the basic hydrolysis of each anti-3.41 and syn-3.41 ester. The crude acids were individually desilylated in a solution of HF/MeCN/pyridine, and after extraction with EtOAc, the crude dihydroxyacid products were macrocyclized using the Corey-Nicolaou conditions by treatment with 2,2'-dipyridyl disulfide and triphenylphosphine in THF to form reactive 2-pyridinethioesters. These were slowly added by syringe pump to hot toluene (80 °C) to effect the high dilution conditions necessary to minimize intermolecular reactivity. The reactions were monitored by LC/MS and after no more than 10 days the products anti-3.42, anti-3.43, syn-3.42, syn-3.43 were purified by PHPLC and isolated (2%, 0.1%, 18%, 7% respectively).

### 3.2.3. Enzymatic assay of enantioenriched NAC-thioester substrates

To determine the outcome of the TE-catalyzed macrolactonization in vitro, we set up four simultaneous enzyme assays with recombinant DEBS TE and each of our enantiopure NAC-thioester substrates. Wild type DEBS TE (213 μM) employed in our assays was expressed and purified as previously described. Our four enzymatic reactions, each with a different enantiopure standard, were prepared by incubating 5 μM DEBS TE with 2.5 mM substrate in 50 mM phosphate buffer (pH 7.4) at 20°C with DMSO (5% v/v) as a solubility additive. After 18 hrs, the reaction was diluted 4-fold and analyzed for product formation by LCMS ESI (Figure 3.12).
Figure 3.12. LC traces and product distribution from enzymatic assays with wild type DEBS TE and enantioenriched NAC substrates.
The results from syn NAC-substrates 3.29 and ent-3.29 are fully consistent with our initial stereochemical working hypothesis. The enzyme competently and predominantly cyclizes substrate 3.29. Comparison of the retention time from the assay's macrocyclic product and the syn-3.42 standard confirms that DEBS TE regioselectively generates the 14-membered ring macrolactone product from 3.29. Substrate ent-3.29 is not converted to a macrocycle under the assay conditions.

However, our assay results for the anti NAC-substrates 3.30 and ent-3.30 did not fully agree with our working hypothesis. While substrate ent-3.30 underwent hydrolysis exclusively, consistent with our hypothesis, unexpectedly 3.30 also produced a seco-acid. Because macrocyclization possesses a late transition state geometry, we expect the macrocyclic product of 3.30 to be highly analogous to the reaction's transition state, and thus bind tightly in the active site. This will favor restoration of the acyl-enzyme intermediate. Subsequent hydrolysis will rapidly release the seco-acid of 3.30 from the site. This hypothesis is supported by an earlier study\textsuperscript{68} where the epothilone macrolactone was competently loaded on to the Epo TE and hydrolyzed to form the seco acid. We thus set to examine if this mode of reactivity was operative within the DEBS TE.

3.2.4. Hydrolysis of macrolactone standard 3.42

To determine if the DEBS TE hydrolyzes the 14-membered macrolactone (R,S)-3.42, we treated DEBS TE with authentic standard anti-3.42. We followed the reaction progress at various times by monitoring the occurrence of seco-acid by analytical HPLC (Figure 3.13). Hydrolysis was not observed even under reaction conditions and incubation times identical to those used for macrocyclization formation. This result
unambiguously confirms the DEBS TE’s inability to open the macrocycle. Furthermore, it is clear that the DEBS TE does not macrocyclize substrate 3.30.

**Figure 3.13.** Results of the enzymatic hydrolysis assay of macrolactone standard 3.42 by the DEBS TE.
(A) Standard *anti-seco-3.41*; (B) Hydrolysis assay at 180 min; (C) negative control with no enzyme.

3.3. Conclusions

Our systematic study of our substrate analogs and their relationship with the DEBS TE provides a glimpse of the importance absolute stereochemistry has on the selectivity of macrocyclization. The effect these small changes have on the substrate-enzyme interactions show them to be sufficiently significant to change their product distribution. Our initial hypothesis stated that the absolute stereochemistry of the nucleophilic alcohol
needed to be in the $R$ configuration for macrocyclization. This hypothesis was based on the observation that all isolated macrocyclic products from the DEBS TE have nucleophilic alcohols of the $R$ configuration. The inability of the DEBS TE to macrocyclize substrate 3.30 which possesses the $R$ configuration on the terminal nucleophilic alcohol, demonstrates that absolute configuration is not sufficient to predict the ability of the DEBS TE to macrocyclize.

### 3.3.1. Absolute stereochemistry is a key factor driving enzyme specificity

It is clear that our initial stereochemical hypothesis is insufficient in explaining the macrocyclization selectivity of the DEBS TE. The hydrolysis of substrate 3.30, was surprising, as it possesses an absolute and relative stereochemistry at C11 and C13 analogous to the native substrate. This implies that additional interactions between the enzyme and the native substrate not captured in our analog are required for the proper positioning of the nucleophile to effect macrocyclization. To understand the precise enzyme-substrate interactions necessary for macrocyclization, high-resolution structural analysis of the acyl-enzyme intermediate will be required. Ongoing work in the laboratory is engaged in the synthesis of non-hydrolysable phosphonate-based acyl-enzyme intermediates of substrates 3.29 and 3.30. This system is expected to provide contrasting detailed enzyme-substrate interactions between a macrocyclization-competent substrate and a substrate that undergoes hydrolysis exclusively.

Our data demonstrates that substrate 3.30 does not model all the native features of the 6dEB substrate. Previous work hypothesized the importance of the C9 carbonyl to effect the bent conformation of the native substrate. In addition, our hypothesis underlines the importance of the absolute stereochemistry of the substrate's terminal $O$-nucleophile to
effect macrocyclization. Both these features are likely complementary, yet insufficient in explaining the macrocyclization specificity of the DEBS TE. Previous examples show that methyl side chains close to the O-nucleophiles are highly conserved.\textsuperscript{36–40} Due to the hydrophobic nature of the substrate-enzyme interactions, these methyl side chains may play an important role in dictating the conformation of the substrate in the enzyme active site, and thus its reactivity. Ongoing work in our lab is focusing on introducing these key side chains into our substrates, to evaluate if they are sufficient to make substrate \textsuperscript{3.30} macrocyclization competent. The ultimate goal of this effort is to identify substrate functional groups that are necessary and sufficient to predict the macrocyclization activity with the DEBS TE.

3.3.2. Applications of DEBS TE in synthesis and biosynthesis

The DEBS TE has been used as a critical feature in numerous engineered PKS pathways to produce macrocycles. However, for each of these published success stories there are countless examples of engineered chimeric systems which did not produce macrocyclic products. Our systematic examination of the DEBS TE macrocyclization activity suggests that its exquisite and as yet unpredictable substrate specificity may limit its utility as an enzyme in engineered systems for macrolactone production. However, because of its reliable hydrolytic activity, it is ideally suited for applications where a carboxylic acid metabolite is desired. For example, a recent study relied on engineered PKS enzymes terminating in the DEBS TE to hydrolyze key metabolite intermediates for characterization of the double bond geometry in the monensin A pathway, which ultimately confirmed the Cane-Celmer-Westley mechanism of polyether biosynthesis.\textsuperscript{69}
Broadly substrate-tolerant macrocyclizing TEs may have an enormous impact on the synthesis of macrolactone natural products and their analogs. Current methodologies employed in the macrocyclization of natural products remain limited in their scope and selectivity. Our studies underline this by demonstrating how the Corey-Nicolaou macrocyclization produced an equimolar mixture of 12 and 14 membered ring macrolactone regioisomers. A clear advantage of the thioesterase can be seen in our highly regioselective macrocyclization of substrate 3.29, which produced exclusively the 14 membered ring macrolactone. While this enhanced regioselectivity is enticing, in the case of the DEBS TE its unpredictable substrate specificity is a serious liability to chemoenzymatic macrocyclization approaches. A highly regioselective thioesterase would be ideal in examples where macrolactonization steps have been problematic, such as Woodward's synthesis of erythronolide A,70 and White's synthesis of polycavernoside A.8 A thioesterase with broad substrate tolerance and high regioselectivity would be ideal for the development of chemoenzymatic syntheses of macrocyclic natural products.

Broadly substrate-tolerant TEs would be of great benefit to both engineered PKS pathways and chemoenzymatic syntheses of macrocyclic natural products. Two approaches can be devised to find them. The first approach involves screening known native TEs from a variety of PKS pathways in the hope of finding one with broad substrate tolerance. The polyfunctionality of polyketide natural products such as 6dEB have likely influenced TEs to retain stringent substrate selectivity, likely rendering this approach ineffective. The second approach involves protein engineering of a known TE to relax its substrate tolerance. As protein engineering based on high-resolution
structural data has been reliably used to modify substrate specificity\textsuperscript{71,72}, this approach is perhaps best suited to producing broadly substrate tolerant TEs for multiple applications.
3.4. References


3.5. Experimental section

3.5.1. General methods

Reactions were carried out under an argon atmosphere with dry solvents and oven-dried glassware under anhydrous conditions unless specified otherwise. All reactions were carried out under an inert argon atmosphere with dry tetrahydrofuran, diethyl ether, and DCM solvents by passing them through activated alumina columns. Toluene, benzene, and triethylamine were freshly distilled over calcium hydride. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Yields refer to chromatographically and spectroscopically (\(^1\)H NMR) homogeneous materials.

Reactions were monitored by analytical thin-layer chromatography (TLC) and carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as a visualizing agent and/or ceric ammonium molybdate (CAM), \(p\)-anisaldehyde (PA), and potassium permanganate (KMnO\(_4\)) staining solutions. Flash column chromatography was performed with E. Merck silica gel (60, particle size 0.040-0.063 mm). Preparative thin-layer chromatography (PTLC) separations were carried out on 0.25 mm E. Merck silica gel plates (60F-254).

\(^1\)H NMR, \(^{13}\)C NMR, COSY, HSQC, HMQC and HMBC spectra were recorded on the Bruker DPX-300 or AMX-300, AMX-400, DRX-500, and Varian Inova 500 spectrometers and calibrated using residual undeuterated solvent as an internal reference. Data are reported as follows: chemical shifts in ppm (\(\delta\)); multiplicities are indicated by the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, dd = doublet of doublets, dt = doublet of triplets, td = triplet of doublets, ddd = doublet of
doublet of doublets, m = multiplet, b = broad; coupling constants in Hz ($J$).

Analytical capillary gas chromatography (GC) analyses were obtained from an Agilent 6890 fitted with a flame ionization detector (He carrier gas, 3 mL/min). The column used was a J&W Scientific chiral Cyclosil B column (30m x 0.25mm x 0.25µm). The injector temperature was 250°C, the detector temperature was 320°C. Retention times ($t_R$) and integrated ratios were obtained from the Agilent Technologies GC Chemstation software. The temperature program used for the analytical GC was as follows: initial temperature = 110°C; ramp = 0.2°C/min; final temperature = 120°C.

Analytical high pressure liquid chromatography (HPLC) was performed on an Agilent 1100 series HPLC-DAD system equipped with a reversed-phase analytical scale Gemini 250 × 21.2 mm column, particle size 10 µm. Preparative high pressure liquid chromatography (PHPLC) was carried out on an Agilent 1200 series system comprising a reversed-phase Gemini Axia 250 × 21.2 mm column, particle size 10 µm (Phenomenex Inc., Torrance, CA). All ESI-LC-MS/MS analyses were collected on an API2000 LC/MS/MS System (Applied Biosystems) equipped with a turbo-ion spray ESI probe interfaced with a Prominence UFLC (Shimadzu) equipped with a reverse phase BDS Hypersil C18 100 × 2.1 mm column, particle size 3 µm (Thermo Scientific). HPLC/LCMS UV absorption was monitored at 210 nm. HRMS were obtained on a Kratos Analytical Concept instrument (University of Ottawa Mass Spectrum Centre).

*E. coli* BL21(DE3) was grown in sterile LB media supplemented with ampicillin using standard protocols. IPTG was purchased from Sigma-Aldrich, nickel-nitrotriacetic acid resin (Ni-NTA) from QIAGEN, while antibiotics and media/buffer components were purchased from Fisher Scientific. Bacterial concentration (OD$_{600}$) was monitored with a
GENESYS 20 spectrophotometer (Thermo Electron Corporation) and 1 mL cuvettes (BrandTech).

3.5.2. Experimental procedures

3.5.2.1. Macrolactone standards

1-Phenylpent-4-en-2-ol (rac-3.21)

Phenylacetaldehyde (3.20, +90% purity) (1.12 mL, 10.0 mmol) was dissolved in diethyl ether (Et₂O, 100 mL, 0.1 M) and cooled to 0°C while vigorously stirring under an argon atmosphere. After 20 minutes, allylmagnesium bromide (10.5 mL, 10.5 mmol, 1.05 equiv) was added dropwise over 5 min. The reaction was monitored by TLC and after 30 min. it was quenched with an aqueous saturated solution of NH₄Cl (50 mL). Diluted mixture with EtOAc (50 mL) and extracted aqueous layer with EtOAc (4x30 mL). The combined EtOAc fractions were washed brine (2x100 mL) and subsequently dried over anhydrous Na₂SO₄. The slurry was filtered through a plug of cotton and the filtrate evaporated in vacuo. Crude oil was purified by silica gel chromatography (20% EtOAc-hexanes) affording homoallylic alcohol rac-3.21 (1.62 g, 10.0 mmol, 100%) as a clear oil.

Analytical data for rac-3.21²

M.W.: 162.23
TLC (Rₓ): 0.38 (20% EtOAc-hexanes)
¹H NMR: (300 MHz, CDCl₃)
7.38-7.24 (m, 5 H, PhH), 5.88 (m, 1 H, H2), 5.21 (m, 1 H, H1'), 5.16 (m, 1 H, H1''), 3.90 (m, 1 H, H4), 2.80 (m, 2 H, H5), 2.32 (m, 2 H, H3), 1.94 (bs, 1 H, OH)

$^{13}$C NMR: (75 MHz, CDCl$_3$)
138.59 (C(Ph)), 134.87 (C(2)), 129.56 (2 C, C(Ph)), 126.56 (C(Ph)), 118.10 (C(1)), 71.83 (C(4)), 43.41 (C(5)), 41.28 (C(3))


$^a$ Alcohol *rac-3.21* was identical to its previously reported spectroscopic values

**1-Oxiranyl-3-phenyl-propan-2-ol (3.36)**

[Diagram]

Alcohol *rac-3.21* (1.62 g, 10.0 mmol) and *m*CPBA (3.20 g, 13 mmol, 1.3 eq) were dissolved in dry dichloromethane (DCM, 100 mL) and stirred under an argon atmosphere at room temperature. After 12 h., the reaction was quenched with an aqueous saturated solution of NaHCO$_3$ followed by extraction with EtOAc (4x60 mL). The organic fractions were combined and extracted in addition with NaHCO$_3$ (3x50 mL) and repeatedly washed with brine (6x30 mL). The organic layer was dried over Na$_2$SO$_4$ and filtered, after which the solvent was evaporated in vacuo. The residual oil was purified by silica gel chromatography (200% EtOAc-hexanes) affording the 1:1 diastereomeric mixture of epoxides 3.36 (1.38 g, 77%) as a colorless oil.

Analytical data for 3.36 (*syn & anti* mixture)$^a$

- **M.W.**: 178.23
- **TLC ($R_f$)**: 0.27 (20% EtOAc-hexanes)
- **$^1$H NMR**: (300 MHz, CDCl$_3$)
7.36-7.22 (m, 10 H, PhH), 4.08 (m, 2 H, H4), 3.19-3.10 (m, 2 H, H2), 2.89-2.72 (m, 6 H, H5, H1'), 2.61 (dd, J = 2.8, 2.0 Hz, 1 H, H1''), 2.51 (dd, J = 2.8, 2.0 Hz, 1 H, H1''), 2.17 (d, J = 3.1 Hz, 1 H, OH), 2.14 (d, J = 3.1 Hz, 1 H, OH), 1.90 (m, 2 H, H3), 1.64 (m, 2 H, H3)

$^{13}$C NMR: (75 MHz, CDCl$_3$)
138.16 (2 C, C(Ph)), 129.64 (2 C, C(Ph)), 129.62 (2 C, C(Ph)), 128.80 (4 C, C(Ph)), 126.81 (2 C, C(Ph)), 71.37 (C(4)), 70.37(C(4)), 50.53 (C(1)), 50.30 (C(1)), 47.16 (C(2)), 46.76 (C(2)), 44.33 (C(5)), 44.11 (C(5)), 39.10 (C(3)), 38.88 (C(3))

(1-Benzyl-2-oxiranylethoxy)-tert-butyldimethylsilane (3.37)

Epoxide 3.36 (3.34 g, 18.9 mmol), tert-butyldimethylchlorosilane (4.28 g, 28.4 mmol, 1.5 eq) and imidazole (9.39 g, 32.1 mmol, 1.7 equiv) were dissolved in dimethylformamide (DMF, 200 mL, 0.1 M) while stirring at room temperature for 1 h 20 min. Quenched with NH$_4$Cl (100 mL) and extracted with EtOAc (4x100 mL). Washed combined organic fractions with brine (6x60 mL) and dried with Na$_2$SO$_4$, followed by the evaporation of the solvent in vacuo. Purified residual oil by silica gel chromatography (5% EtOAc-hexanes) affording an inseparable 1:1 (syn/anti) mixture of silylated epoxide diastereomers 3.37 (5.53 g, 18.9 mmol, 100%) as a colorless oil.

Analytical data for 3.37 (syn & anti mixture)

M.W.: 292.49

TLC ($R_f$): 0.71 (20% EtOAc-hexanes)

$^{1}$H NMR: (300 MHz, CDCl$_3$)
7.28-7.17 (m, 10 H, PhH), 4.13-4.04 (m, 2 H, H4), 3.14-3.12 (m, 1 H, H2), 3.05-3.03 (m, 1 H, H2), 2.89-2.75 (m, 6 H, H5, H1'), 2.48-2.42 (m, 2 H, H1''), 1.70 (t, J = 5.5 Hz, 2 H, H3), 1.65-1.58 (m, 2 H, H3), 0.89 (s, 9 H, H9), 0.87 (s, 9 H, H9), 0.05 (s, 3 H, H6), -0.02 (s, 3 H, H6), -0.10 (s, 3 H,
H7), -0.19 (s, 3 H, H7)

$^{13}$C NMR: (75 MHz, CDCl$_3$)  
139.09 (C(Ph)), 138.68 (C(Ph)), 130.01 (2 C, C(Ph))), 129.96 (2 C, C(Ph))), 128.44 (2 C, C(Ph))), 128.41 (2 C, C(Ph))), 126.47 (C(Ph)), 126.41 (C(Ph)), 72.22 (C(4)), 71.63 (C(4)), 49.98 (C(2)), 49.57 (C(2)), 47.95 (C(1)), 46.90 (C(1)), 44.99 (C(5)), 44.21 (C(5)), 40.31 (C(2)), 40.03 (C(2)), 26.06 (3 C, C(9)), 26.03 (3 C, C(9)), 18.25 (2 C, C(8)), -4.61 (C(6)), -4.67 (2 C, C(6), C(7)), -4.85 (C(7))

HRMS: (EI) m/z calcd for C$_{13}$H$_{19}$O$_2$Si [(M-C$_4$H$_9$)$^+$] 235.1149, found 235.0973

**6-(tert-Butyldimethylsilyloxy)-7-phenylhept-1-en-4-ol (3.38)**

![Chemical Structure](image)

A heterogeneous solution in THF (200 mL) of CuI (3.9 g, 21 mmol, 1 equiv) was cooled to -20°C over 30 min., under an argon atmosphere. To this suspension, vinylmagnesium bromide (63 mL, 63 mmol, 3 equiv) was added and stirred for an additional 30 min., producing a brown solution. Epoxide 3.37 (6.0 g, 21 mmol) was dissolved in THF (100 mL) and added by canula over 20 min., and stirred vigorously for 2.5 hrs. Quenching with NH$_4$Cl (50 mL), immediately produced a brown precipitate, which was filtered and the organic layer extracted with excess water. Organic extract was washed with brine (2x50 mL), dried over Na$_2$SO$_4$ and the solvent evaporated in vacuo. Purification of the residual oil was accomplished by silica gel chromatography (10% EtOAc-hexanes) affording an inseparable mixture of alcohol diastereomers 3.38 (2.3 g, 7.2 mmol, 35%) as a yellow oil.

**Analytical data for 3.38 (syn & anti mixture)**

M.W.: 320.54
TLC ($R_f$): 0.45 (20% EtOAc-hexanes)

$^1$H NMR: (300 MHz, CDCl$_3$)
7.28-7.12 (m, 10 H, PhH), 5.86-5.67 (m, 2 H, H2), 5.09-5.01 (m, 4 H, H1), 4.19 (m, 1 H, H6), 4.03 (m, 2 H, H4, H6), 3.77 (m, 1 H, H4), 3.33 (s, 1 H, OH), 2.94-2.76 (m, 4 H, H7), 2.23-2.15 (m, 4 H, H3), 1.63-1.55 (m, 4 H, H5), 0.88 (s, 9 H, H11), 0.86 (s, 9 H, H11), 0.08 (s, 3 H, H8), 0.03 (s, 3 H, H8), -0.01 (s, 3 H, H9), -0.16 (s, 3 H, H9)

$^{13}$C NMR: (75 MHz, CDCl$_3$)
138.60 (C(Ph)), 138.29 (C(Ph)), 134.88 (C(2)), 134.74 (C(2)), 129.56 (2 C, (C(Ph)), 129.55 (2 C, (C(Ph)), 128.31 (2 C, (C(Ph)), 128.27 (2 C, (C(Ph)), 126.28 (C(Ph)), 126.27 (C(Ph)), 117.50 (C(1)), 117.34 (C(1)), 73.94 (C(6)), 73.08 (C(6)), 69.75 (C(4)), 67.69 (C(4)), 44.84 (C(7)), 43.00 (C(7)), 42.42 (C(5)), 42.33 (C(5)), 42.08 (C(3)), 40.76 (C(3)), 25.82 (6 C, (C(11)), 17.92 (2 C, C(10)), 4.37 (C(8)), -4.73 (C(8)), -4.97 (C(8)), -5.11 (C(9))

HRMS: (EI) m/z calcd for C$_{15}$H$_{23}$O$_2$Si [(M-C$_4$H$_9$)$_+^+$] 263.1462, found 263.1445

1-Phenylhept-6-ene-2,4-diols (syn-3.39/anti-3.39)

A solution in EtOH (10 mL) of monoprotected diol 3.38 (1.3 g, 4.2 mmol) and PTSA (5.6 g, 29 mmol, 7 equiv) was stirred under argon for 4 hrs. After checking by TLC, the reaction was quenched with a saturated NaHCO$_3$ solution (5 mL) and extracted with EtOAc (4 x 10 mL). Washed the organic extract with brine (2 x 20 mL), dried over MgSO$_4$ and the solvent was evaporated in vacuo. Purification by silica gel chromatography (20% EtOAc-hexanes to 100% EtOAc gradient) furnished the racemic diastereomeric mixture of diols 3.39 (520 mg, 2.5 mmol, 60%).

To rapidly and efficiently separate, HPLC conditions were optimized on a reversed phase analytical scale Gemini 250 × 21.2 mm column, particle size 10 µm by applying water with methanol, acetonitrile and their 50:50 combination as organic modifiers using
an Agilent 1100 series HPLC-DAD system. The final method with optimal binary linear gradient conditions was transferred and upscaled on an Agilent 1200 series preparative HPLC system comprising a binary pump (flow rate range 5-100 mL/min), an autosampler with a 2 mL loop, a diode array detector with a flow cell (path length 3 mm and maximum pressure limit 120 bar) and a fraction collector (40 mL collection tubes). Preparative scale separations were performed on a reverse-phase Gemini Axia 250 × 21.2 mm column, particle size 10 µm (Phenomenex Inc., Torrance, CA) using a linear gradient of 30-100% acetonitrile in 20 min at a flow rate of 31.5 mL/min at room temperature. Fractions were collected, combined, and the solvent evaporated in vacuo to yield anti-3.39 (163 mg, 0.79 mmol, 32%) as a crystalline solid and syn-3.39 (160 mg, 0.78 mmol, 32%) as a pale yellow oil.

**Analytical data for anti-3.39**

M.W.: 206.13

TLC (Rf): 0.67 (100% EtOAc)

$^1$H NMR: (400 MHz, CDCl$_3$)
7.32-7.19 (m, 5 H, PhH), 5.83-5.74 (m, 1 H, H2), 5.14-5.10 (m, 2 H, H1), 4.17 (p, $J = 7.4$ Hz, 1 H, H6), 4.02 (p, $J = 6.8$ Hz, 1 H, H4), 2.78 (m, 2 H, H7), 2.25 (m, 2 H, H3), 2.25 (bs, 2 H, OH), 1.68 (dd, $J = 6.1$, 5.4 Hz, 2 H, H5)

$^{13}$C NMR: (100 MHz, CDCl$_3$)
138.27 (C(Ph)), 134.63 (C(2)), 129.38 (2 C, C(Ph)), 128.65 (2 C, C(Ph)), 126.59 (C(Ph)), 118.29 (C(1)), 70.11 (C(6)), 68.11 (C(4)), 44.07 (C(7)), 42.03 (C(5)), 41.56 (C(3))

HRMS: (EI) m/z calcd for C$_6$H$_{11}$O$_2$ [(M-C$_7$H$_7$)$^+$] 115.0754, found 115.0772

**Analytical data for syn-3.39**

M.W.: 206.13

TLC (Rf): 0.71 (100% EtOAc)
**H NMR:** (400 MHz, CDCl₃)
7.32-7.19 (m, 5 H, PhH), 5.84-5.73 (m, 1 H, H2), 5.14-5.08 (m, 2 H, H1), 4.05 (m, 1 H, H6), 3.87 (m, 1 H, H4), 2.89 (bs, 1 H, OH), 2.76 (d, J = 6.5 Hz, 2 H, H7), 2.22 (m, 2 H, H3), 1.69 (dt, J = 14.4, 2.3 Hz, 1 H, H5'), 1.54 (m, 1 H, H5'')

**13C NMR:** (100 MHz, CDCl₃)
137.97 (C(Ph)), 134.34 (C(2)), 129.46 (2 C, C(Ph)), 128.63 (2 C, C(Ph)), 126.60 (C(Ph)), 118.30 (C(1)), 73.72 (C(6)), 71.66 (C(4)), 44.52 (C(7)), 42.41 (C(5)), 41.82 (C(3))

**HRMS:** (EI) m/z calcd for C₆H₁₁O₂ [(M-C₇H₇⁺] 115.0754, found 115.0768

**anti-4-Allyl-6-benzyl-2,2-dimethyl-1,3-dioxane (anti-3.44)**

In an acetone solution (0.1 mL, 1.4 mmol, 50 equiv), PTSA (1.6 mg, 0.008 mmol, 0.3 equiv) and **anti-3.39** (5.5 mg, 0.027 mmol) were stirred overnight under an argon atmosphere. Quenched with TEA (5.0 µL 0.04 mmol, 1.3 equiv) and after evaporating solvent in vacuo, purified by flash chromatography (20% EtOAc-hexanes) affording acetonide **anti-3.44** (2.2 mg, 0.0089 mmol, 33%) as a clear oil.

**Analytical data for anti-3.44**

- **M.W.:** 246.34
- **TLC (Rf):** 0.78 (20% EtOAc-hexanes)

**H NMR:** (300 MHz, CDCl₃)
7.29-7.16 (m, 5 H, PhH), 5.82-5.69 (m, 1 H, H2), 5.08-4.99 (m, 2 H, H1), 4.04 (m, 1 H, H6), 3.86 (m, 1 H, H4), 2.88 (dd, J = 14.0, 7.0 Hz, 1 H, H7'), 2.65 (dd, J = 14.0, 6.4 Hz, 1 H, H7''), 2.28-2.08 (m, 2 H, H3), 1.70-1.48 (m, 2 H, H5), 1.35 (s, 3 H, H8(eq)), 1.29 (s, 3 H, H9(ax))

**13C NMR:** (75 MHz, CDCl₃)
138.34 (C(Ph)), 134.43 (C(2)), 129.18 (2 C, C(Ph)), 128.16 (2 C, C(Ph)), 126.14 (C(Ph)), 116.78 (C(1)), 100.34 (C(10)), 67.43 (C(6)), 66.24 (C(4)), 46.83 (C(7)), 42.41 (C(5)), 41.82 (C(3))
42.08 (C(7)), 40.07 (C(5)), 37.51 (C(3)), 24.84 (2 C, C(8), C(9))

**HRMS:** (EI) m/z calcd for $C_{15}H_{19}O_2 [(M-CH_3)^+]$ 231.1380, found 231.1388

**syn-4- Allyl-6-benzyl-2,2-dimethyl-1,3-dioxane (syn-3.44)**

In an acetone solution (0.1 mL, 1.4 mmol, 50 equiv), PTSA (1.0 mg, 0.005 mmol, 0.2 equiv) and **syn-3.39** (5.5 mg, 0.027 mmol) were stirred overnight under an argon atmosphere. Quenched with TEA (5.0 µL 0.04 mmol, 1.3 equiv) and after evaporating solvent in vacuo, purified by flash chromatography (20% EtOAc-hexanes) affording acetonide **syn-3.44** (5.5 mg, 0.022 mmol, 83%) as a clear oil.

**Analytical data for syn-3.44**

- **M.W.:** 246.34
- **TLC ($R_f$):** 0.78 (20% EtOAc-hexanes)
- **$^1$H NMR:** (300 MHz, CDCl$_3$)
  - 7.30-7.17 (m, 5 H, PhH), 5.80-5.68 (m, 1 H, H2), 5.07-4.98 (m, 2 H, H1), 4.00 (m, 1 H, H6), 3.78 (m, 1 H, H4), 2.90 (dd, $J = 13.4, 5.8$ Hz, 1 H, H7'), 2.60 (dd, $J = 13.4, 7.4$ Hz, 1 H, H7''), 2.30-2.08 (m, 2 H, H3), 1.44-1.40 (m, 1 H, H5'), 1.41 (s, 3 H, H8(eq)), 1.40 (s, 3 H, H9(ax)), 1.17 (m, 1 H, H5'')
- **$^{13}$C NMR:** (75 MHz, CDCl$_3$)
  - 137.93 (C(Ph)), 134.19 (C(2)), 129.42 (2 C, C(Ph)), 128.22 (2 C, C(Ph)), 126.22 (C(Ph)), 116.96 (C(1)), 98.57 (C(10)), 70.06 (C(6)), 68.63 (C(4)), 43.02 (C(7)), 40.77 (C(5)), 35.82 (C(3)), 30.17 (C(8)), 19.80 (C(9))
- **HRMS:** (EI) m/z calcd for $C_{15}H_{19}O_2 [(M-CH_3)^+]$ 231.1380, found 231.1375
**anti-5-allyl-7-benzyl-2,2,3,3,9,9,10,10-octamethyl-4,8-dioxo-3,9-disilaundecane (anti-3.40)**

Diol **anti-3.39** (155 mg, 0.75 mmol) was dissolved by stirring in anhydrous DMF (3.8 mL, 0.2M) at r.t. under an argon atmosphere. Imidazole (116 mg, 1.7 mmol, 2.2 equiv) was added followed by TBSCl (256 mg, 1.7 mmol, 2.2 equiv) and the reaction was allowed to proceed overnight. After checking by TLC for completion, extracted product with hexanes (4x2 mL), dried over MgSO₄ and the solvent was evaporated in vacuo. Purification of the residual oil was accomplished by silica gel chromatography (10% EtOAc-hexanes) affording bis-silylated diol **anti-3.40** (326 mg, 0.75 mmol, 100%) as a clear and viscous oil.

**Analytical data for anti-3.40**

- **M.W.:** 434.30
- **TLC (Rf):** 0.71 (20% EtOAc-hexanes)
- **¹H NMR:** (400 MHz, CDCl₃)
  7.25-7.13 (m, 5 H, PhH), 5.82-5.72 (m, 1 H, H2), 5.05-5.00 (m, 2 H, H1), 3.92 (p, J = 6.4 Hz, 1 H, H6), 3.80 (p, J = 6.3 Hz, 1 H, H4), 2.72 (dddd, J = 6.4, 3.4 Hz, 2 H, H7), 2.21 (m, 2 H, H3), 1.57 (m, 2 H, H5), 0.82 (s, 9 H, H11), 0.81 (s, 9 H, H15), -0.01 (s, 3 H, H8), -0.02 (s, 3 H, H12), -0.04 (s, 3 H, H9), -0.17 (s, 3 H, H13)
- **¹³C NMR:** (100 MHz, CDCl₃)
  139.01 (C(Ph)), 135.08 (C(2)), 129.70 (2 C, C(Ph)), 128.13 (2 C, C(Ph)), 126.05 (C(Ph)), 116.90 (C(1)), 71.58 (C(6)), 69.93 (C(4)), 44.92 (C(5)), 44.67 (C(7)), 42.45 (C(3)), 25.91 (3 C, C(11)), 25.88 (3 C, C(15)), 18.07 (C(10)), 18.05 (C(14)), -3.99 (C(8)), -4.28 (C(12)), -4.44 (2 C, C(9), C(13))
- **HRMS:** (EI) m/z calcd for C₂₁H₃₇O₂Si₂ [(M-C₄H₉)⁺] 377.2327, found 377.2306
**syn-5-allyl-7-benzyl-2,2,3,3,9,9,10,10-octamethyl-4,8-dioxo-3,9-disilaundecane (syn-3.40)**

Diol **syn-3.39** (157 mg, 0.76 mmol) was dissolved by stirring in anhydrous DMF (3.8 mL, 0.2M) at r.t. under an argon atmosphere. Imidazole (116 mg, 1.7 mmol, 2.2 equiv) was added followed by TBSCl (256 mg, 1.7 mmol, 2.2 equiv) and the reaction was allowed to proceed overnight. After checking by TLC for completion, extracted product with hexanes (4x2 mL), dried over MgSO₄ and the solvent was evaporated in vacuo. Purification of the residual oil was accomplished by silica gel chromatography (10% EtOAc-hexanes) affording bis-silylated diol **syn-3.40** (330 mg, 0.76 mmol, 100%) as a clear and viscous oil.

**Analytical data for syn-3.40**

M.W.: 434.30

**TLC (R)**: 0.71 (20% EtOAc-hexanes)

**¹H NMR**: (400 MHz, CDCl₃)

- 7.25-7.13 (m, 5 H, PhH), 5.78-5.67 (m, 1 H, H2), 4.98-4.92 (m, 2 H, H1), 3.89 (p, J = 6.5 Hz, 1 H, H6), 3.84 (p, J = 6.2 Hz, 1 H, H4), 2.75 (dd, J = 13.3, 5.6 Hz, 1 H, H7'), 2.66 (dd, J = 13.3, 6.8 Hz, 1 H, H7''), 2.16 (m, 2 H, H3), 1.59 (m, 2 H, H5), 0.87 (s, 9 H, H11), 0.82 (s, 9 H, H15), 0.05 (s, 3 H, H8), 0.04 (s, 3 H, H12), -0.07 (s, 3 H, H9), -0.21 (s, 3 H, H13)

**¹³C NMR**: (100 MHz, CDCl₃)

- 139.04 (C(Ph)), 134.85 (C(1)), 129.86 (2 C, C(Ph)), 128.07 (2 C, C(Ph)), 126.03 (C(Ph)), 116.98 (C(2)), 70.88 (C(6)), 69.16 (C(4)), 44.45 (C(5)), 44.17 (C(7)), 41.91 (C(3)), 25.91 (6 C, C(11), C(15)), 18.09 (C(10)), 18.03 (C(14)), -4.21 (C(8)), -4.38 (C(12)), -4.71 (C(9)), -4.79 (C(13))

**HRMS**: (EI) m/z calcd for C₂¹H₃₇O₂Si₂ [(M-C₄H₉)⁺] 377.2327, found 377.2318
Methyl 7-aminohexanoate hydrochloride (3.34)

7-Aminohexanoic acid (3.35) (150 mg, 1.03 mmol) was dissolved in MeOH (1.0 mL, 24.7 mmol, 24 equiv) at r.t. under argon. To the solution, trimethylsilyl chloride (TMSCl, 0.13 mL, 1.03 mmol, 1.0 equiv) was added and stirred overnight. The solvent was evaporated in vacuo yielding crude methyl ester 3.34 (202 mg, 1.03 mmol, 100%) as a pale green solid.

Analytical data for 3.34

M.W.: 195.69

$^1$H NMR: (300 MHz, D$_2$O)
3.55 (s, 3 H, H8), 2.85 (bt, $J = 7.5$ Hz, 2 H, H7), 2.26 (t, $J = 7.5$ Hz, m, 2 H, H2), 1.48 (m, 4 H, H3, H6), 01.22 (m, 4 H, H4, H5)

$^{13}$C NMR: (75 MHz, D$_2$O)
177.60 (C(1)), 51.99 (C(8)), 39.31 (C(7)), 33.44 (C(2)), 27.51 (C(6)), 26.40 (C(4)), 25.09 (C(5)), 23.88 (C(3))


$^a$ Aminoester 3.34 was identical to its previously reported spectroscopic values

Methyl 7-anti-3,5-bis(tert-butyldimethylsilyloxy)-6-phenylhexanamido)heptanoate (anti-3.41)
In a flask, NaIO$_4$ (780 mg, 1.4 mmol, 8.0 equiv) and KMnO$_4$ (34 mg, 0.22 mmol, 1.2 equiv) were dissolved in pH 7.24 phosphate buffer (1.0 mL, 0.05M) and stirred vigorously at r.t. Olefin *anti*-3.40 (80 mg, 0.18 mmol) was dissolved in $t$-BuOH (1.8 mL) while stirring. After 15 min, the dark purple permanganate solution was transferred by Pasteur pipette to the olefin solution and stirred overnight. Two buffer washes (0.4 mL) were employed to quantitatively transfer the oxidant mixture. Upon contact, the new heterogeneous solution changed coloration to a pale brown/purple. Quenched with a saturated solution of sodium thiosulfate (1.0 mL), stirred for 30 min and extracted with EtOAc (4x1.5 mL). The organic extract was washed with brine (2x2 mL), dried over MgSO$_4$ and the solvent evaporated in vacuo. The isolated crude oil (42 mg) was dissolved in DCM (1.9 mL, 0.05M) and, while stirring under argon, added: EDC (27 mg, 0.14 mmol, 1.5 equiv), DMAP (1.1 mg, 0.009 mmol, 0.1 equiv) and amine 3.34 (22 mg, 0.11 mmol, 1.2 equiv). After 5 min, TEA was added (31 μL, 0.22 mmol, 2.4 equiv) and the solution was stirred overnight. Quenched with NH$_4$Cl (1.0 mL) and extracted with EtOAc (5x2 mL). Organic extracts were washed with brine (2x5 mL) and dried over MgSO$_4$. After solvent evaporation, the oil obtained was purified by flash column chromatography (30% EtOAc-hexanes) furnishing amide *anti*-3.41 (34 mg, 0.058 mmol, 32% over two steps) as an amber oil.

**Analytical data for *anti*-3.41**

- **M.W.:** 593.99
- **TLC ($R_f$):** 0.26 (20% EtOAc-hexanes)
- **$^1$H NMR:** (400 MHz, CDCl$_3$)
7.25-7.13 (m, 5 H, PhH), 6.33 (bt, J = 5.5 Hz, 1 H, NH), 4.04 (m, 1 H, H3), 3.87 (p, J = 6.3 Hz, 1 H, H5), 3.64 (s, 3 H, H14), 3.26-3.07 (m, 2 H, H7), 2.70 (d, J = 6.4 Hz, 2 H, H6), 2.50 (dd, J = 15.1, 4.0 Hz, 1 H, H2'), 2.28 (m, 3 H, H12, H2'), 1.68 (m, 2 H, H4), 1.58 (m, 2 H, H11), 1.41 (m, 2 H, H8), 1.28 (m, 4 H, H9, H10), 0.82 (s, 9 H, H22), 0.81 (s, 9 H, H18), 0.00 (s, 6 H, H15, H19), -0.03 (s, 3 H, H16), -0.18 (s, 3 H, H20)

\[ ^{13}C \text{NMR:} \] (100 MHz, CDCl₃)
174.08 (C(13)), 170.55 (C(1)), 138.55 (C(Ph)), 129.71 (2 C, C(Ph)), 128.17 (2 C, C(Ph)), 126.19 (C(Ph)), 71.30 (C(5)), 67.64 (C(3)), 51.44 (C(14)), 44.33 (C(4)), 44.15 (C(6)), 44.14 (C(2)), 39.14 (C(7)), 33.96 (C(12)), 29.36 (C(8)), 28.76 (C(9)), 26.68 (C(10)), 25.88 (3 C, C(18)), 25.74 (3 C, C(22)), 24.80 (C(11)), 17.98 (C(17)), 17.85 (C(21)), -4.52 (C(15)), -4.56 (C(19)), -4.60 (C(18)), -4.68 (C(20))

HRMS: (EI) m/z calc'd for C₂₈H₅₀NO₅Si₂ [(M-C₄H₉)⁺] 536.3222, found 536.3251

Methyl 7-syn-3,5-bis(tert-butyldimethylsilyloxy)-6-phenylhexanamido)heptanoate (syn-3.41)

In a flask, NaIO₄ (780 mg, 1.4 mmol, 8.0 equiv) and KMnO₄ (34 mg, 0.22 mmol, 1.2 equiv) were dissolved in pH 7.24 phosphate buffer (1.0 mL, 0.05M) and stirred vigorously at r.t. Olefin syn-3.40 (80 mg, 0.18 mmol) was dissolved in t-BuOH (1.8 mL) while stirring. After 15 min, the dark purple permanganate solution was transferred by Pasteur pipette to the olefin solution and stirred overnight. Two buffer washes (0.4 mL) were employed to quantitatively transfer the oxidant mixture. Upon contact, the new heterogeneous solution changed coloration to pale brown. Quenched with a saturated solution of sodium thiosulfate (1.0 mL), stirred for 30 min and extracted with EtOAc (4x1.5 mL). The organic extract was washed with brine (2x2 mL), dried over MgSO₄ and the solvent evaporated in vacuo. The isolated crude oil (51 mg) was dissolved in
DCM (2.2 mL, 0.05M) and added while stirring under argon: EDC (33 mg, 0.17 mmol, 1.5 equiv), DMAP (1.2 mg, 0.01 mmol, 0.1 equiv) and amine 3.34 (25 mg, 0.13 mmol, 1.2 equiv). After 5 min, TEA was added (36 μL, 0.26 mmol, 2.4 equiv) and the solution was stirred overnight. Quenched with NH₄Cl (1.0 mL) and extracted with EtOAc (5 x 2 mL). Organic extracts were washed with brine (2 x 5 mL) and dried over MgSO₄. After solvent evaporation, the oil obtained was purified by flash column chromatography (30% EtOAc-hexanes) furnishing amide syn-3.41 (34 mg, 0.065 mmol, 36% over two steps) as an amber oil.

Analytical data for syn-3.41

M.W.: 593.99

TLC (Rf): 0.24 (20% EtOAc-hexanes)

1H NMR: (400 MHz, CDCl₃)
7.24-7.10 (m, 5 H, PhH), 6.28 (bt, J = 5.4 Hz, 1 H, NH), 4.17 (m, 1 H, H3), 3.93 (m, 1 H, H5), 3.63 (s, 3 H, H14), 3.21-3.00 (m, 2 H, H7), 2.76 (dd, J = 13.5, 6.3 Hz, 1 H, H6'), 2.67 (dd, J = 13.4, 6.7 Hz, 1 H, H6''), 2.43 (dd, J = 14.9, 4.3 Hz, 1 H, H2'), 2.26 (t, J = 7.5 Hz, 2 H, H12), 2.20 (dd, J = 14.9, 5.2 Hz, 1 H, H2''), 1.60 (m, 4 H, H4, H11), 1.35 (m, 2 H, H8), 1.25 (m, 4 H, H9, H10), 0.85 (s, 9 H, H22), 0.84 (s, 9 H, H18), 0.05 (s, 3 H, H15), 0.04 (s, 3 H, H19), -0.01 (s, 3 H, H16), -0.13 (s, 3 H, H20)

13C NMR: (100 MHz, CDCl₃)
174.09 (C(13)), 170.62 (C(1)), 138.42 (C(Ph)), 129.66 (2 C, C(Ph)), 128.19 (2 C, C(Ph)), 126.17 (C(Ph)), 70.54 (C(5)), 67.17 (C(3)), 51.45 (C(14)), 44.41 (C(6)), 43.58 (C(4)), 43.45 (C(2)), 39.09 (C(7)), 33.97 (C(12)), 29.35 (C(11)), 28.75 (C(9)), 26.65 (C(10)), 25.90 (3 C, C(18)), 25.81 (3 C, C(22)), 24.80 (C(10)), 18.00 (C(17)), 17.89 (C(21)), -4.57 (3 C, C(15), C(16), C(19)), -4.76 (C(20))

HRMS: (EI) m/z calcd for C_{28}H_{50}NO_{5}Si_{2} [(M-C₄H₉)^+] 536.3222, found 536.3256
General procedure for the deprotection and Corey-Nicolaou macrocyclization of bis-silylated diols \textit{anti}-3.41 and \textit{syn}-3.41

**Hydrolysis.** The methyl ester and LiOH (10 equiv) are dissolved and vigorously stirred in a 5:1 solution of MeOH:H$_2$O (0.02M) at r.t. for 6 hr. Solvent was evaporated off and the crude was acidified to a pH of 3 units by the dropwise addition of 1M HCl. Extracted with EtOAc (4x0.25 volumes), dried with brine and MgSO$_4$, and evaporated in vacuo. **Silyl deprotection.** In an Eppendorf tube the crude acid was dissolved in MeCN (0.1 M) and vortexed for 10 sec to homogeneity. To this solution, pyridine (10 equiv) and hydrofluoric acid (HF, 50 equiv) were added and, following mixing by vortexing 10 sec, the mixture was left to react 6 hr. The mixture was diluted with H$_2$O, extracted with EtOAc (5 x 0.5 volumes) and the organic phase was dried with MgSO$_4$ and evaporated in vacuo. **Macrolactonization.** The crude hydroxyacid, 2,2'-dithiopyridine (DTP, 1.5 equiv), and PPh$_3$ (1.5 equiv) were dissolved in THF (0.01 M) and stirred at r.t. for 5.5 hr. The bright yellow solution was quantitatively transferred and diluted into a syringe by portionwise addition of toluene (~0.005 M). The solution was transferred dropwise by syringe pump (0.6 mL/hr) into a round bottom flask fitted with a reflux condenser containing toluene (~0.0005 M) heated to 85°C under an argon atmosphere. The reaction was stirred for 5 to 8 days while monitoring for completion by LC/MS, at which time the solvent was evaporated in vacuo and the crude partially purified by chromatographic techniques (PTLC and/or FCC) employing a 5%MeOH/EtOAc solvent system. The presence of macrocycle in the eluted fractions was checked by LC/MS. After pooling the fractions of interest and evaporating the solvent in vacuo, purified by PHPLC on a reverse phase Eclipse XDB-C18 150 × 4.6 mm column, particle size 5 µm (Agilent
Technologies, Inc., Santa Clara, CA) using a linear gradient of 0-40% 95% acetonitrile:water in 30 min at a flow rate of 2.2 mL/min at 40°C.

anti-2-Benzyl-4-hydroxy-1-oxa-7-azacyclotetradecane-6,14-dione (anti-3.42)
anti-2-(2-Hydroxy-3-phenylpropyl)-1-oxa-5-azacyclododecane-4,12-dione (anti-3.43)

Hydrolysis. Methyl ester anti-3.41 (117 mg, 0.20 mmol) was treated with LiOH (84 mg, 2.0 mmol, 10 equiv) and dissolved in MeOH:H₂O (12 mL, 0.02 M) at r.t. Upon acidification of aqueous layer, extracted with EtOAc (4x3 mL) and dried with brine (1 x 1 mL) and MgSO₄. Filtered and evaporated solvent in vacuo. Silyl deprotection. The isolated crude oil (~114 mg) was dissolved in MeCN (2 mL) and vortexed to homogeneity (10-20 sec). Pyridine (145 μL, 2.0 mmol, 10 equiv) followed by HF (174 μL, 10 mmol, 50 equiv) were added and after vortexing thoroughly, the reaction was left undisturbed for 6 hr. Diluted with H₂O (1.5 mL) and extracted with EtOAc (5x1 mL). The organic layers were combined, dried with MgSO₄ and, after evaporation in vacuo, a crude white solid was isolated (~60 mg). Macrolactonization. Crude hydroxyacid (3.5 mg, 0.010 mmol) was dissolved in THF (0.8 mL, 0.01 M) followed by DTP (3.3 mg, 0.015 mmol, 1.5 equiv) and PPh₃ (3.9 mg, 0.015 mmol, 1.5 equiv). After 5.5 hr, diluted with toluene (5 mL, 0.002 M) and added via syringe pump (0.6 mL/hr) to hot toluene. After 5 days at 86°C, solvent was evaporated and the crude was purified by PHPLC. Two fractions were collected and lyophilized to yield 1) spectroscopically pure anti-3.42
(0.5 mg, 2 μmol, 2% after three steps) and 2) a crude mixture of **anti-3.42** and **anti-3.43** (0.05 mg, 0.15 μmol, 0.1% after three steps) as white solids. The latter mixture was repurified by LC/MS and regrettably its yield was insufficient for full spectroscopic characterization.

**Analytical data for anti-3.42**

**M.W.:** 333.42

**TLC (R_f):** 0.23 (100% EtOAc)

**LCMS:** t_R, 17.24 min

**^1H NMR:** (500 MHz, CDCl_3)

7.28-7.15 (m, 5 H, PhH), 6.15 (bd, J = 4.4 Hz, 1 H, NH), 5.21 (dq, J = 8.3, 5.6 Hz, 1 H, H12), 3.83 (bs, 2 H, H10, OH), 3.52 (m, 1 H, H7”), 3.02 (m, 1 H, H7”), 2.95 (dd, J = 14.3, 8.4 Hz, 1 H, H13’), 2.88 (dd, J = 14.2, 5.4 Hz, 1 H, H13”), 2.44 (m, 2 H, H9), 2.25 (m, 2 H, H2), 1.95 (m, 1 H, H11’), 1.79 (m, 1H, H3’), 1.75 (m, 1 H, H11”), 1.59 (1 H, H3”), 1.53 (2 H, H6), 1.37 (m, 2 H, H4’, H5’), 1.30 (m, 1 H, H4”), 1.19 (m, 1 H, H5”)

Note: Protons on C3, C4, C5, C6 are covered by H_2O peak and solvent. Peaks were assigned from COSY and HSQC correlation spectra

**^13C NMR:** (75 MHz, CDCl_3)

174.62 (C(1)), 171.80 (C(8)), 136.91 (C(Ph)), 129.11 (2 C, C(Ph)), 128.53 (2 C, C(Ph)), 126.78 (C(Ph)), 72.11 (C(12)), 66.29 (C(10)), 41.94 (C(9)), 40.98 (C(11)), 40.42 (C(13)), 39.11 (C(7)), 34.24 (C(2)), 29.12 (C(4)), 27.35 (C(6)), 25.59 (C(5)), 23.20 (C(3))

**HRMS:** (EI) m/z calcd for C_{19}H_{27}NO_4 [(M)+] 333.1940, found 333.1951

**Analytical data for anti-3.43**

**M.W.:** 333.42

**TLC (R_f):** 0.23 (100% EtOAc)

**LCMS:** t_R, 16.54 min

**^1H NMR:** (500 MHz, CDCl_3)

7.28-7.15 (m, 5 H, PhH), 5.50 (m, 1 H, H10), 5.30 (bd, J = 9.0 Hz, 1 H, NH), 3.73 (m, 2 H, H12)
Peaks picked from mixture of products are unambiguously from anti-3.43. Insufficient quantities of anti-3.43 limited our spectroscopic analysis.

**LRMS:** (ESI) m/z calcd for C_{19}H_{28}NO_{4} [(M+H)^+] 334, found 334

**syn-2-Benzyl-4-hydroxy-1-oxa-7-azacyclotetradecane-6,14-dione (syn-3.43)**  
**syn-2-(2-Hydroxy-3-phenylpropyl)-1-oxa-5-azacyclododecane-4,12-dione (syn-3.43)**

**Hydrolysis.** Methyl ester syn-3.41 (99 mg, 0.17 mmol) was treated with LiOH (71 mg, 1.7 mmol, 10 equiv) and dissolved in MeOH:H_{2}O (12 mL, 0.014 M) at r.t. Upon acidification of aqueous layer, extracted with EtOAc (4 x 3 mL) and dried with brine (1 x 1 mL) and MgSO_{4}. Filtered and evaporated solvent in vacuo. **Silyl deprotection.** The isolated crude oil (~94 mg) was dissolved in MeCN (1.6 mL) and vortexed to homogeneity (10-20 sec). Pyridine (116 μL, 2.0 mmol, 10 equiv) followed by HF (140 μL, 10 mmol, 50 equiv) were added and after vortexing thoroughly, the reaction was left undisturbed for 6 hr. Diluted with H_{2}O (1.5 mL) and extracted with EtOAc (5 x 1 mL). The organic layers were combined, dried with MgSO_{4} and, after evaporation in vacuo, a crude white solid was isolated (~50 mg). **Macrolactonization.** Crude hydroxyacid (30 mg, 0.085 mmol) was dissolved in THF (0.85 mL, 0.1 M) followed by DTP (29 mg, 0.13 mmol, 1.5 equiv) and PPh_{3} (34 mg, 0.13 mmol, 1.5 equiv). After 5.5 hr, diluted with toluene (10 mL, ~0.009 M) and added via syringe pump (0.6 mL/hr) to hot toluene. After 10 days at 85°C, solvent was evaporated and the crude was partially purified by FCC (5%MeOH/EtOAc); the macrolactone-containing fractions were combined and the crude obtained (~10 mg) was purified by PHPLC. Two fractions were collected and
lyophilized to yield 1) spectroscopically pure *syn*-3.42 (5.0 mg, 15 μmol, 18% after three steps) and 2) *syn*-3.43 (2.0 mg, 6 μmol, 7% after three steps) as white solids.

**Analytical data for *syn*-3.42**

- **M.W.:** 333.42
- **TLC (R_f):** 0.46 (100% EtOAc)
- **LCMS:** t_R, 17.28 min
- **1H NMR:** (500 MHz, CDCl_3)
  - 7.28-7.14 (m, 5 H, PhH), 5.90 (bd, J = 8.4 Hz, 1 H, NH), 5.24 (m, 1 H, H12), 3.80 (m, 1 H, H10), 3.66 (m, 1 H, H7'), 2.88 (m, 3 H, H7'', H13), 2.56 (dd, J = 13.7, 2.2 Hz, 1 H, H9'), 2.24 (m, 2 H, H2), 2.10 (dd, J = 13.7, 7.7 Hz, 1 H, H9''), 1.95 (m, 1 H, H1'H'), 1.76 (m, 2 H, H3', H11''), 1.52 (m, 2 H, H3'', H5'), 1.43 (m, 2 H, H5'', H6'), 1.23 (m, 3 H, H4, H6'')
- **13C NMR:** (75 MHz, CDCl_3)
  - 174.96 (C(1)), 172.49 (C(8)), 136.85 (C(Ph)), 129.25 (2 C, C(Ph)), 128.45 (2 C, C(Ph)), 126.73 (C(Ph)), 71.60 (C(12)), 67.38 (C(10)), 42.44 (C(9)), 41.57 (C(13)), 40.13 (C(11)), 39.51 (C(7)), 34.06 (C(2)), 28.83 (C(6)), 26.59 (C(5)), 26.05 (C(4)), 22.37 (C(3))
- **HRMS:** (EI) m/z calcd for C_{19}H_{27}NO_4 [(M)^+] 333.1940, found 333.1947

**Analytical data for *syn*-3.43**

- **M.W.:** 333.42
- **TLC (R_f):** 0.46 (100% EtOAc)
- **LCMS:** t_R, 15.55 min
- **1H NMR:** (500 MHz, CDCl_3)
  - 7.31-7.18 (m, 5 H, PhH), 5.52 (m, 1 H, H10), 5.36 (bd, J = 6.9 Hz, 1 H, NH), 3.91 (m, 1 H, H12), 3.67 (m, 1 H, H7'), 3.06 (dd, J = 13.4, 2.7 Hz, 1 H, H7''), 2.89 (dd, J = 13.4, 4.4 Hz, 1 H, H13'), 2.65 (dd, J = 13.5, 8.5 Hz, 1 H, H13''), 2.55 (dd, J = 13.5, 2.9 Hz, 1 H, H9'), 2.37 (m, 2 H, H2', H9''), 2.27 (m, 1 H, H2''), 1.83 (m, 3 H, H3', H11), 1.58 (m, 4 H, H3'', H4, H6'), 1.29 (m, 3 H, H5, H6'')
- **13C NMR:** (75 MHz, CDCl_3)
  - 173.35 (C(1)), 169.55 (C(2)), 137.84 (C(Ph)), 129.41 (2 C, C(Ph)), 128.64 (2 C, C(Ph)), 126.65 (C(Ph)), 69.86 (2 C, C(10), C(12)), 44.07 (C(13)), 41.26 (C(9)), 39.84 (C(11))
42.91 (C(9)), 41.41 (C(11)), 37.37 (C(7)), 33.51 (C(2)), 25.81 (C(4)), 24.63 (C(5)), 23.99 (C(6)), 23.63 (C(3))

**HRMS:** (EI) m/z calcd for C$_{19}$H$_{27}$NO$_4$ [(M)$^+$] 333.1940, found 333.1932

3.5.2.2. Enantioenriched NAC thioesters

**General procedure for the Brown allylation of phenylacetaldehyde (3.20)**

In a round bottomed flask (r.b.f.), Ipc$_2$BAlyl (5 mL, 1M in pentanes) was cooled to -100°C in a Et$_2$O/N$_2$(l) bath. To this, a solution of phenylacetaldehyde in 10 mL Et$_2$O was slowly cannulated over 5 min. After 30 min and warming to -78°C, quenched with 2 mL MeOH and slowly allowed mixture to warm up to r.t., at which point 2 mL of a 15% NaOH solution was added followed by 4 mL of 30% H$_2$O$_2$. This mixture was equipped with a water condenser and refluxed for 30 min. Purification was accomplished by extracting with EtOAc (3x15 mL), and drying with brine and Na$_2$SO$_4$. The organic fractions were combined and solvent evaporated in vacuo. The residual clear oil was purified by flash column chromatography (50% DCM-Hexanes).

(S)-1-Phenylpent-4-en-2-ol (3.21)

The procedure outlined above was employed in the conversion of aldehyde 3.20 to homoallylic alcohol 3.21 (460 mg, 2.8 mmol, 63%, 90% ee) with (-)-Ipc$_2$BAlyl.

**Analytical data for 3.21**

- **M.W.:** 162.23
- **TLC ($R_f$)$_2$** 0.38 (20% EtOAc-hexanes)
**$^1$H NMR:** (300 MHz, CDCl$_3$)
7.33-7.19 (m, 5 H, PhH), 5.81 (m, 1 H, H2), 5.16 (m, 1 H, H1'), 5.12 (t, $J = 1.2$ Hz, 1 H, H1''), 3.86 (m, 1 H, H4), 2.75 (m, 2 H, H5), 2.29 (m, 2 H, H3), 1.65 (d, $J = 10$ Hz, 1 H, OH)

**$^{13}$C NMR:** (75 MHz, CDCl$_3$)
138.43 (C(Ph)), 134.73 (C(2)), 129.46 (2 C, C(Ph)), 128.56 (2 C, C(Ph)), 126.51 (C(Ph)), 118.14 (C(1)), 71.72 (C(4)), 43.32 (C(5)), 41.21 (C(3))

**GC:** $t_R$, 41.45 min (95%)


a Alcohol 3.21 was identical to its previously reported spectroscopic values

(R)-1-Phenylpent-4-en-2-ol (ent-3.21)

The general procedure previously outlined was employed in the conversion of aldehyde 3.20 to homoallylic alcohol *ent*-3.21 (320 mg, 1.97 mmol, 44%, 90% ee) with (+)-Ipc$_2$BAlyl.

Analytical data for *ent*-3.21$^a$

M.W.: 162.23

TLC ($R_f$): 0.38 (20% EtOAc-hexanes)

$^1$H NMR: (300 MHz, CDCl$_3$)
7.38-7.20 (m, 5 H, PhH), 5.82 (m, 1 H, H2), 5.15 (m, 1 H, H1'), 5.12 (t, $J = 1.2$ Hz, 1 H, H1''), 3.87 (m, 1 H, H4), 2.75 (m, 2 H, H5), 2.33-2.18 (m, 2 H, H3)

$^{13}$C NMR: (75 MHz, CDCl$_3$)
138.43 (C(Ph)), 134.73 (C(2)), 129.46 (2 C, C(Ph)), 128.56 (2 C, C(Ph)), 126.51 (C(Ph)), 118.14 (C(1)), 71.72 (C(4)), 43.32 (C(5)), 41.21 (C(3))

GC: $t_R$, 42.62 min (95%)

a Alcohol *ent-3.21* was identical to its previously reported spectroscopic values

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General procedure for the synthesis of enantioenriched epoxides 3.22 and *ent*-3.22

The purified chiral Brown allylation product was treated to the following sequence:

**Epoxidation.** The chiral olefin was dissolved at 0°C in DCM (0.05M conc.) along with *m*-CPBA (2.1 equiv, 77% purity) and NaHCO₃(s) (4.2 equiv) and vigorously stirred overnight and allowing the mixture to gradually warm up to r.t. Quenching was carried out by addition of 0.5 volumes of a saturated NH₄Cl solution and extraction performed with EtOAc, H₂O and copious amounts of saturated NaHCO₃ solution. Dried organic layer with Na₂SO₄ and the solvent was evaporated in vacuo. The crude oil was filtered through a plug of silica with EtOAc. **Silylation.** The crude oil was dissolved in DMF at r.t. to which imidazole and TBSCl were added sequentially. The solution was vigorously stirred for 24 hrs and subsequently quenched with NH₄Cl and diluted with EtOAc and H₂O. Extracted 8-10 times with brine and after solvent evaporation, the residual oil was filtered through a plug of silica with hexanes. **Jacobsen's hydrolytic kinetic resolution.** The catalysts employed were prepared according to a previously reported literature method¹. The epoxide and catalyst were dissolved in THF and cooled to 0°C while

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stirring in a round bottomed flask. H₂O was added and warmed up to r.t. while stirring for 16 h. The solvent was evaporated and the oil purified by flash column chromatography (100% hexanes – 50% EtOAc-hexanes).

**tert-Butyldimethyl((R)-1-((R)-oxiran-2-yl)-3-phenylpropan-2-yloxy)silane (3.22)**

**(2S,4R)-4-(tert-Butyldimethylsilyloxy)-5-phenylpentane-1,2-diol (3.24)**

![Chemical structure](image)

**Epoxidation.** General procedure outlined above was employed with homoallylic alcohol 3.21 (460 mg, 2.8 mmol), mCPBA (1.33 g, 5.9 mmol, 2.1 equiv, 77% pure), NaHCO₃ (991 mg, 11.8 mmol, 4.2 equiv) in DCM (56 mL). The reaction yielded a crude oil (273 mg, ~1.5 mmol) that was used without further purification on the next step.

**Silylation.** Crude epoxide (273 mg, ~1.5 mmol), imidazole (177 mg, 2.6 mmol, 1.7 equiv), TBSCl (347 mg, 2.3 mmol, 1.5 equiv) was stirred in DMF (30 mL). The crude oil (341 mg, ~1.2 mmol) obtained was used in the hydrolytic kinetic resolution step.

**Jacobsen's hydrolytic kinetic resolution.** Crude silylated oil (341 mg, ~1.2 mmol), R,R-Salen catalyst (692.8 mg/mmol, 17 mg, 0.024 mmol, 2 mol%), H₂O (12 μL, 0.66 mmol, 0.55 equiv), THF (1 mL). After careful purification epoxide 3.22 (175 mg, 0.60 mmol, 90% de, 21% overall) and vicinal diol 3.24 (156 mg, 0.50 mmol, 18% overall) were isolated. The three step sequence furnished 3.22 and 3.24 in a 39% overall yield.

**Analytical data for 3.22**

M.W.: 292.49  
TLC (Rf): 0.71 (20% EtOAc-hexanes)
\[ \text{H NMR: (400 MHz, CDCl}_3) \]
7.27-7.15 (m, 5 H, PhH), 4.02 (dt, \( J = 5.6, 1.2 \) Hz, 1 H, H4), 3.09 (m, 1 H, H2), 2.83 (d, \( J = 2.4 \) 2 H, H5), 2.74 (m, 1 H, H1'), 2.40 (m, 1 H, H1'"), 1.66 (t, \( J = 5.6 \) Hz, 2 H, H3), 0.83 (s, 9 H, H9), -0.05 (s, 3 H, H6), -0.22 (s, 3 H, H7)

\[ \text{C NMR: (100 MHz, CDCl}_3) \]
138.87 (C(Ph)), 129.80 (2 C, C(Ph)), 128.20 (2 C, C(Ph)), 126.20 (C(Ph)), 72.00 (C(4)), 49.38 (C(2)), 46.71 (C(1)), 43.99 (C(5)), 39.81 (C(2)), 25.82 (3 C, C(9)), 18.02 (C(8)), -4.90 (C(6), -5.07 (C(7))

HRMS: (EI) m/z calcd for C\(_{13}\)H\(_{19}\)O\(_2\)Si [(M-C\(_4\)H\(_9\))\(^+\)] 235.1149, found 235.0922

Analytical data for 3.24

M.W.: 310.49

TLC \((R_f)\): 0.14 (30% EtOAc-hexanes)

\[ \text{H NMR: (400 MHz, CDCl}_3) \]
7.28-7.13 (m, 5 H, PhH), 4.21 (m, 1 H, H4), 4.15 (m, 1 H, H2), 3.57 (m, 1 H, H1'), 3.38 (m, 1 H, H1'"), 2.93 (dd, \( J = 13.2, 6.8 \) Hz, 1 H, H5'), 2.80 (dd, \( J = 13.6, 7.2 \) Hz, 1 H, H5'"), 2.0 (m, 1 H, OH), 1.70 (m, 1H, H3'), 1.46 (m, 1H, H3'"), 0.87 (s, 9 H, H9), 0.03 (s, 3 H, H6), -0.15 (s, 3 H, H7)

\[ \text{C NMR: (100 MHz, CDCl}_3) \]
138.41 (C(Ph)), 129.54 (2 C, C(Ph)), 128.45 (2 C, C(Ph)), 126.45 (C(Ph)), 72.95 (C(4)), 68.84 (C(2)), 67.14 (C(1)), 42.90 (C(5)), 36.97 (C(2)), 25.83 (3 C, C(9)), 17.95 (C(8)), -4.97 (C(6), -5.08 (C(7))

HRMS: (EI) m/z calcd for C\(_{10}\)H\(_{23}\)O\(_3\)Si [(M-C\(_7\)H\(_7\))\(^+\)] 219.1411, found 219.1397

tert-Butyldimethyl((S)-1-((S)-oxiran-2-yl)-3-phenylpropan-2-yloxy)silane (ent-3.22)
(2R,4S)-4-(tert-Butyldimethylsilyloxy)-5-phenylpentane-1,2-diol (ent-3.24)

Epoxidation. General procedure outlined above was employed with homoallylic alcohol ent-3.21 (320 mg, 1.97 mmol), mCPBA (928 mg, 4.14 mmol, 2.1 equiv, 77%)
pure), NaHCO₃ (696 mg, 8.28 mmol, 4.2 equiv) in DCM (40 mL). The reaction yielded a crude oil (301 mg, ~1.69 mmol) that was used without further purification on the next step. **Silylation.** Crude epoxide (301 mg, ~1.69 mmol), imidazole (195 mg, 2.87 mmol, 1.7 equiv), TBSCI (383 mg, 2.54 mmol, 1.5 equiv) was stirred in DMF (34 mL). The crude oil (405 mg, ~1.38 mmol) obtained was used in the hydrolytic kinetic resolution step. **Jacobsen's hydrolytic kinetic resolution.** Crude silylated oil (405 mg, ~1.38 mmol), S,S-Salen catalyst (692.8 mg/mmol, 19 mg, 0.030 mmol, 2 mol%), H₂O (14 μL, 0.76 mmol, 0.55 equiv), THF (0.3 mL). After purification epoxide ent-3.22 (152 mg, 0.52 mmol, 90% de, 26% overall) and vicinal diol ent-3.24 (151 mg, 0.49 mmol, 25% overall) were isolated. The three step sequence furnished ent-3.22 and ent-3.24 in a 51% overall yield.

**Analytical data for ent-3.22**

M.W.: 292.49

TLC ($R_f$): 0.71 (20% EtOAc-hexanes)

$^1$H NMR: (400 MHz, CDCl₃) 7.27-7.15 (m, 5 H, PhH), 4.02 (dt, $J = 5.6, 1.2$ Hz, 1 H, H₄), 3.09 (m, 1 H, H₂), 2.83 (d, $J = 2.4$, 2 H, H₅), 2.74 (m, 1 H, H₁'), 2.40 (m, 1 H, H₁''), 1.66 (t, $J = 5.6$ Hz, 2 H, H₃), 0.83 (s, 9 H, H₉), -0.05 (s, 3 H, H₆), -0.22 (s, 3 H, H₇)

$^{13}$C NMR: (100 MHz, CDCl₃) 138.88 (C(Ph)), 129.80 (2 C, C(Ph)), 128.21 (2 C, C(Ph)), 126.20 (C(Ph)), 72.00 (C(4)), 49.38 (C(2)), 46.71 (C(1)), 43.99 (C(5)), 39.81 (C(2)), 25.82 (3 C, C(9)), 18.02 (C(8)), -4.90 (C(6)), -5.07 (C(7))

HRMS: (EI) m/z calcd for C₁₃H₁₉O₂Si [(M-C₄H₉)⁺] 235.1149, found 235.1110

**Analytical data for ent-3.24**

M.W.: 310.49

TLC ($R_f$): 0.14 (30% EtOAc-hexanes)
$^1$H NMR: (400 MHz, CDCl$_3$)
7.28-7.13 (m, 5 H, PhH), 4.21 (m, 1 H, H4), 4.15 (m, 1 H, H2), 3.57 (m, 1 H, H1'), 3.38 (m, 1 H, H1''), 2.93 (dd, $J = 13.2$, 6.8 Hz, 1 H, H5'), 2.80 (dd, $J = 13.6$, 7.2 Hz, 1 H, H5''), 2.0 (m, 1 H, OH), 1.70 (m, 1H, H3''), 1.46 (m, 1H, H3'), 0.87 (s, 9 H, H9), 0.03 (s, 3 H, H6), -0.15 (s, 3 H, H7)

$^{13}$C NMR: (100 MHz, CDCl$_3$)
138.41 (C(Ph)), 129.54 (2 C, C(Ph)), 128.45 (2 C, C(Ph)), 126.45 (C(Ph)), 72.95 (C(4)), 68.84 (C(2)), 67.13 (C(1)), 42.90 (C(5)), 36.98 (C(2)), 25.83 (3 C, C(9)), 17.95 (C(8)), -4.96 (C(6), -5.08 (C(7))

HRMS: (EI) m/z calcd for C$_{10}$H$_{23}$O$_3$Si [(M-C$_7$H$_7$)$_3$] 219.1411, found 219.1405

General procedure for the conversion of vicinal diols to epoxides 3.23 and ent-3.23

The purified vicinal diol was stirred along with dibutyltin oxide (Bu$_2$SnO, 0.2 equiv), $p$-toluene sulfonate chloride (TsCl, 1 equiv) and triethylamine (Et$_3$N, 1 equiv) in DCM (0.15M conc) at r.t. under an argon atmosphere. After 3h, filtered through a plug of cotton and evaporated solvent. The oil was redissolved in THF (0.2M conc) and added dropwise to an ice-cold suspension of sodium hydride (NaH, 2 equiv, 60% in oil) in THF (0.4M conc). Stirred overnight and quenched with NH$_4$Cl (1 mL). Extracted with EtOAc (3x2 volumes), dried organic layer with brine and Na$_2$SO$_4$ and after filtration and evaporation of the solvent, purified by flash column chromatography (100% hexanes).

tert-butyldimethyl((R)-1-((S)-oxiran-2-yl)-3-phenylpropan-2-yloxy)silane (3.23)

The above general procedure was employed with vicinal diol 3.24 (136 mg, 0.44 mmol), Bu$_2$SnO (21.7 mg, 0.087 mmol, 20 mol%), TsCl (83.9 mg, 0.44 mmol, 1.0
equiv), Et₃N (61.4 μL, 0.44 mmol, 1.0 equiv) in DCM (2.9 mL). The reaction furnished a crude oil (205 mg, ~0.44 mmol) that was redissolved in THF (3.5 mL) and added to a suspension of NaH (35.2 mg, 0.88 mmol, 2.0 equiv) in THF (1 mL). Isolated 3.23 as an amber oil (129 mg, 0.44 mmol, 90% de, 100% over two steps).

**Analytical data for 3.23**

M.W.: 292.49

TLC ($R_f$): 0.71 (20% EtOAc-hexanes)

$^1$H NMR: (400 MHz, CDCl₃)

7.27–7.13 (m, 5 H, PhH), 4.07 (m, 1 H, H4), 3.00 (m, 1 H, H2), 2.84–2.71 (m, 3 H, H5, H1'), 2.43 (m, 1 H, H1''), 1.56 (m, 2 H, H3), 0.85 (s, 9 H, H9), 0.01 (s, 3 H, H6), -0.14 (s, 3 H, H7)

$^{13}$C NMR: (100 MHz, CDCl₃)

138.47 (C(Ph)), 129.75 (2 C, C(Ph)), 128.24 (2 C, C(Ph)), 126.26 (C(Ph)), 71.41 (C(4)), 49.79 (C(2)), 47.76 (C(1)), 44.78 (C(5)), 40.09 (C(2)), 25.85 (3 C, C(9)), 18.04 (2 C, C(8)), -4.83 (C(6), -4.88 (C(7))

HRMS: (EI) m/z calcd for C₁₃H₁₉O₂Si [(M-C₄H₉)⁺] 235.1149, found 235.1057

**tert-Butyldimethyl((S)-1-((R)-oxiran-2-yl)-3-phenylpropan-2-yl)oxy)silane (ent-3.23)**

The general procedure was employed with vicinal diol ent-3.23 (151 mg, 0.49 mmol), Bu₂SnO (25 mg, 0.10 mmol, 20 mol%), TsCl (93 mg, 0.49 mmol, 1.0 equiv), Et₃N (68 μL, 0.49 mmol, 1.0 equiv) in DCM (3.3 mL). The reaction produced a crude oil that was redissolved in THF (4.0 mL) and added to a suspension of NaH (39 mg, 0.98 mmol, 2.0 equiv) in THF (1 mL). Isolated ent-3.24 as an amber oil (83 mg, 0.28 mmol, 90% de, 58% over two steps).
Analytical data for **ent-3.24**

**M.W.**: 292.49

**TLC (R<sub>f</sub>):** 0.71 (20% EtOAc-hexanes)

**<sup>1</sup>H NMR:** (400 MHz, CDCl<sub>3</sub>)
7.27-7.13 (m, 5 H, PhH), 4.06 (m, 1 H, H4), 3.00 (m, 1 H, H2), 2.80-2.73 (m, 3 H, H5, H1'), 2.43 (m, 1 H, H1''), 1.55 (m, 2 H, H3), 0.85 (s, 9 H, H9), 0.01 (s, 3 H, H6), -0.14 (s, 3 H, H7)

**<sup>13</sup>C NMR:** (75 MHz, CDCl<sub>3</sub>)
138.42 (C(Ph)), 129.71 (2 C, C(Ph)), 128.19 (2 C, C(Ph)), 126.22 (C(Ph)), 71.37 (C(4)), 49.75 (C(2)), 47.72 (C(1)), 44.73 (C(5)), 40.04 (C(2)), 25.81 (3 C, C(9)), 18.00 (C(8)), -4.87 (C(6)), -4.93 (C(7))

**HRMS:** (EI) m/z calcd for C<sub>13</sub>H<sub>19</sub>O<sub>2</sub>Si [(M-C<sub>4</sub>H<sub>9</sub>)<sup>+</sup>] 235.1149, found 235.1110

**General procedure for the vinyl cuprate opening of chiral epoxides**

A mixture of purified epoxide, copper (I) iodide (CuI, 0.1 equiv) in THF (1.0M conc) was vigorously stirred under argon at -78°C for 20 min. Vinyl magnesium bromide (VinylMgBr, 1.3 equiv) was added dropwise and the mixture allowed to slowly warm up to r.t. overnight. The dark brown solution was quenched with NH<sub>4</sub>Cl (0.5 volumes) and extracted with EtOAc (3x2 volumes). After drying the organic layer with brine (2 volumes) and Na<sub>2</sub>SO<sub>4</sub>, the mixture was filtered and the solvent evaporated. Purification was performed by flash column chromatography (10% ethyl acetate-hexanes).

**(<sup>4</sup>S,6<sup>R</sup>)-6-(tert-Butyldimethylsilyloxy)-7-phenylhept-1-en-4-ol (3.25)**

![Chemical structure](image)

The general procedure was employed with epoxide **3.22** (60.2 mg, 0.21 mmol), CuI (4.4 mg, 0.023 mmol, 0.1 equiv), THF (0.14 mL) and vinylmagnesium bromide (0.27
mL, 0.27 mmol, 1.3 equiv). After work up and purification of the crude organic extract, homoallylic alcohol 3.25 (57.2 mg, 0.18 mmol, 85%) was isolated as an amber oil.

Analytical data for 3.25

- **M.W.:** 320.54
- **TLC \((R_f)\):** 0.45 (20% EtOAc-hexanes)
- **\(^1\)H NMR:** (400 MHz, CDCl\(_3\))
  - 7.28-7.13 (m, 5 H, PhH), 5.75 (m, 1 H, H2), 5.07-5.01 (m, 2 H, H1), 4.08 (m, 1 H, H6), 3.76 (m, 1 H, H4), 2.89 (bs, 1 H, OH), 2.85 (dd, \(J = 13.2, 5.6\) Hz, 1 H, H7\(\prime\)), 2.71 (dd, \(J = 13.2, 7.6\) Hz, 1 H, H7\(\prime\)'), 2.14 (m, 2 H, H3), 1.53 (m, 2 H, H5), 0.88 (s, 9 H, H11), 0.08 (s, 3 H, H8), -0.01 (s, 3 H, H9)
- **\(^13\)C NMR:** (100 MHz, CDCl\(_3\))
  - 138.32 (C(Ph)), 134.77 (C(2)), 129.61 (2 C, (C(Ph)), 128.32 (2 C, (C(Ph)), 126.31 (C(Ph)), 117.54 (C(1)), 73.97 (C(6)), 69.79 (C(4)), 44.87 (C(7)), 42.46 (C(5)), 42.12 (C(3)), 25.87 (3 C, (C(11)), 17.96, (C(10)), -4.33 (C(8)), -4.69 (C(9))
- **HRMS:** (EI) m/z calcd for C\(_{12}\)H\(_{25}\)O\(_2\)Si [(M-C\(_7\)H\(_7\))^+] 229.1618, found 229.1514

\((4R,6R)-6-(\text{tert-Butyldimethylsilyloxy})-7\text{-phenylhept-1-en-4-ol (3.26)}\)

![Chemical structure of 3.23 to 3.26](image)

The general procedure was employed with epoxide 3.23 (35.1 mg, 0.12 mmol), CuI (2.3 mg, 0.023 mmol, 0.1 equiv), THF (0.12 mL) and vinylmagnesium bromide (0.16 mL, 0.16 mmol, 1.3 equiv). After work up and purification of the crude organic extract, homoallylic alcohol 3.26 (20 mg, 0.062 mmol, 52%) was isolated as an amber oil.

Analytical data for 3.26

- **M.W.:** 320.54
- **TLC \((R_f)\):** 0.48 (20% EtOAc-hexanes)
**1H NMR:** (400 MHz, CDCl₃)

7.25-7.07 (m, 5 H, PhH), 5.78 (m, 1 H, H2), 5.09-5.00 (m, 2 H, H1), 4.19 (m, 1 H, H6), 4.10 (m, 1 H, H4), 2.90 (dd, J = 13.2, 6.4 Hz, 1 H, H7'), 2.79 (dd, J = 13.2, 7.2 Hz, 1 H, H7''), 2.13 (m, 2 H, H3), 1.54 (m, 2 H, H5), 0.86 (s, 9 H, H11), 0.02 (s, 3 H, H8), -0.16 (s, 3 H, H9)

**13C NMR:** (100 MHz, CDCl₃)

138.64 (C(Ph)), 134.92 (C(2)), 129.59 (2 C, (C(Ph)), 128.36 (2 C, (C(Ph))), 126.33 (C(Ph)), 117.39 (C(1)), 73.12 (C(6)), 67.73 (C(4)), 43.04 (C(7)), 42.37 (C(5)), 40.78 (C(3)), 25.87 (3 C, (C(11))), 17.97 (C(10)), -4.93 (C(8)), -5.07 (C(9))

**HRMS:** (EI) m/z calcd for C₁₂H₂₅O₂Si [(M-C₇H₇)⁺] 229.1618, found 229.1617

(4R,6S)-6-(tert-Butyldimethylsilyloxy)-7-phenylhept-1-en-4-ol (ent-3.25)

![Chemical structure](image)

The general procedure was employed with epoxide ent-3.22 (152.0 mg, 0.52 mmol), CuI (10.0 mg, 0.052 mmol, 0.1 equiv), THF (0.52 mL) and vinylmagnesium bromide (0.68 mL, 0.68 mmol, 1.3 equiv). After work up and purification of the crude organic extract, homoallylic alcohol ent-3.25 (39.3 mg, 0.12 mmol, 23%) was isolated as an amber oil.

**Analytical data for ent-3.25**

M.W.: 320.54

TLC (Rf): 0.45 (20% EtOAc-hexanes)

**1H NMR:** (400 MHz, CDCl₃)

7.26-7.13 (m, 5 H, PhH), 5.75 (m, 1 H, H2), 5.08-5.02 (m, 2 H, H1), 4.08 (m, 1 H, H6), 3.76 (m, 1 H, H4), 2.89 (bs, 1 H, OH), 2.85 (dd, J = 13.2, 5.6 Hz, 1 H, H7'), 2.71 (dd, J = 13.2, 7.6 Hz, 1 H, H7''), 2.14 (m, 2 H, H3), 1.53 (m, 2 H, H5), 0.88 (s, 9 H, H11), 0.07 (s, 3 H, H8), -0.03 (s, 3 H, H9)

**13C NMR:** (100 MHz, CDCl₃)
181

138.27 (C(Ph)), 134.73 (C(2)), 129.56 (2 C, C(Ph)), 128.28 (2 C, C(Ph)),
126.27 (C(Ph)), 117.50 (C(1)), 73.95 (C(6)), 69.75 (C(4)), 44.83 (C(7)),
42.39 (C(5)), 42.07 (C(3)), 25.83 (3 C, (C(11)), 17.91 (C(10)), -4.37 (C(8)),
-4.73 (C(9))

HRMS: (EI) m/z calcd for C_{12}H_{25}O_2Si [(M-C_{7}H_{7})^+] 229.1618, found 229.1616

(4S,6S)-6-(tert-Butyldimethylsilyloxy)-7-phenylhept-1-en-4-ol (ent-3.26)

The general procedure was employed with epoxide ent-3.23 (83.0 mg, 0.28 mmol),
CuI (6.0 mg, 0.030 mmol, 0.1 equiv), THF (0.28 mL) and vinylmagnesium bromide (0.36
mL, 0.36 mmol, 1.3 equiv). After work up and purification of the crude organic extract,
homoallylic alcohol ent-3.26 (42.6 mg, 0.13 mmol, 46%) was isolated as an amber oil.

Analytical data for ent-3.26

M.W.: 320.54

TLC (R_{f}): 0.48 (20% EtOAc-hexanes)

^1H NMR: (400 MHz, CDCl_{3})
7.27-7.06 (m, 5 H, PhH), 5.78 (m, 1 H, H2), 5.09-5.02 (m, 2 H, H1), 4.19
(m, 1 H, H6), 4.09 (m, 1 H, H4), 2.90 (dd, J = 13.2, 6.4 Hz, 1 H, H7"), 2.78
(dd, J = 13.2, 7.2 Hz, 1 H, H7"'), 2.14 (m, 2 H, H3), 1.54 (m, 2 H, H5), 0.86
(s, 9 H, H11), 0.02 (s, 3 H, H8), -0.16 (s, 3 H, H9).

^13C NMR: (100 MHz, CDCl_{3})
138.64 (C(Ph)), 134.92 (C(2)), 129.59 (2 C, (C(Ph)), 128.36 (2 C, (C(Ph)),
126.33 (C(Ph)), 117.39 (C(1)), 73.12 (C(6)), 67.73 (C(4)), 43.04 (C(7)),
42.37 (C(5)), 40.78 (C(3)), 25.87 (3 C, C(11)), 17.97 (C(10)), -4.93 (C(8)),
-5.07 (C(9))

HRMS: (EI) m/z calcd for C_{12}H_{25}O_2Si [(M-C_{7}H_{7})^+] 229.1618, found 229.1604
Methyl 7-((3R,5R)-3,5-bis(tert-butyldimethylsilyloxy)-6-phenylhexanamido) heptanoate (3.31)

Homoallylic alcohol 3.25 (57.2 mg, 0.18 mmol), tert-butyldimethylchlorosilane (55.0 mg, 0.36 mmol, 2.0 equiv) and imidazole (26.0 mg, 0.38 mmol, 2.1 equiv) were dissolved in dimethylformamide (DMF, 0.42 mL, 0.4 M) while stirring at room temperature overnight. Diluted with water (1 mL) and extracted with hexanes (4x2 mL); dried with Na₂SO₄ and followed by evaporating solvent in vacuo. The crude bis-silylated olefin (45.8 mg, ~0.11 mmol) was used in the next step without purification.

In a r.b.f. sodium metaperiodate (NaIO₄, 455.0 mg, 0.84 mmol, 8.0 equiv) and potassium permanganate (KMnO₄, 18.7 mg, 0.12 mmol, 1.1 equiv) were dissolved in pH 7.0 buffer (0.5 mL, 0.05M) and stirred vigorously at r.t. The crude olefin was dissolved in $t$-BuOH (0.5 mL) while stirring. After 15 min, the dark purple permanganate solution was transferred by Pasteur pipette to the olefin solution. Two buffer washes (0.1 mL) were employed to quantitatively transfer of the oxidant. Upon contact, the mixture changed coloration to a pale brown. The solution was stirred overnight. Quenched with a saturated solution of sodium thiosulfate (0.5 mL), diluted with 1 mL of water and extracted with EtOAc (3x1 mL). The organic extract was washed with brine (2x0.5 mL), dried over Na₂SO₄ and the solvent evaporated in vacuo. The isolated crude oil was used in the next step without purification.

Dissolved crude oil in DCM (1.0 mL) and added while stirring under argon: EDC•HCl (29.9 mg, 0.16 mmol, 1.5 equiv), DMAP (1.2 mg, 0.010 mmol, 0.1 equiv) and
amine \textbf{3.34} (28.6 mg, 0.15 mmol, 1.4 equiv). After 5 min, TEA was added (21.7 \mu{}L, 0.16 mmol, 1.5 equiv) and the solution was stirred overnight. Quenched with \( \text{NH}_4\text{Cl} \) (1.0 mL) and extracted with EtOAc (3x1 mL). Organic extracts were washed with brine (2x1 mL) and dried over \( \text{Na}_2\text{SO}_4 \). Upon solvent evaporation, the oil obtained was purified by flash column chromatography (20\% EtOAc-hexanes - 50\% EtOAc) furnishing amide \textbf{3.31} (30 mg, 0.051 mmol, 28\% over three steps) as an amber oil.

\textbf{Analytical data for 3.31}

\textbf{M.W.:} 593.99

\textbf{TLC (Rf):} 0.24 (20\% EtOAc-hexanes)

\textbf{\( ^1\text{H NMR:} \)} (400 MHz, CDCl\(_3\))

- 7.25-7.11 (m, 5 H, PhH), 6.27 (t, \( J = 5.2 \) Hz, 1 H, NH), 4.18 (m, 1 H, H3), 3.93 (m, 1 H, H5), 3.64 (s, 3 H, H14), 3.16 (m, 1 H, H7'), 3.05 (m, 1 H, H7''), 2.76 (dd, \( J = 13.2, 6.4 \) Hz, 1 H, H6'), 2.69 (dd, \( J = 13.2, 6.4 \) Hz, 1 H, H6''), 2.42 (dd, \( J = 14.8, 4.4 \) Hz, 1 H, H2'), 2.27 (t, \( J = 7.6 \) Hz, 2 H, H12), 2.21 (dd, \( J = 14.8, 5.2 \) Hz, 1 H, H2''), 1.59 (m, 4 H, H11), 1.36 (m, 2 H, H8), 1.27 (m, 4 H, H10), 0.85 (s, 9 H, H22), 0.84 (s, 9 H, H18), 0.05 (s, 3 H, H19), 0.04 (s, 3 H), -0.01 (s, 3 H, H16), -0.13 (s, 3 H, H20).

\textbf{\( ^1\text{C NMR:} \)} (100 MHz, CDCl\(_3\))

- 174.12 (C(13)), 170.62 (C(1)), 138.43 (C(Ph)), 129.67 (2 C, C(Ph)), 128.20 (2 C, C(Ph)), 126.17 (C(Ph)), 70.54 (C(5)), 67.18 (C(3)), 51.47 (C(14)), 44.42 (C(6)), 43.57 (C(4)), 43.44 (C(2)), 39.10 (C(7)), 33.98 (C(12)), 29.35 (C(11)), 28.76 (C(9)), 26.66 (C(10)), 25.91 (3 C), 25.81 (3 C), 24.81 (C(10)), 18.00 (C(17)), 17.90 (C(21)), -4.57 (3 C, C(15), C(16), C(19)), -4.75 (C(20))

\textbf{7-(2-Acetamidoethythio)-7-oxoheptan-1-ammonium chloride (3.15)}

\textbf{Boc-protected aminothioester 3.12} (102 mg, 0.29 mmol) was dissolved and stirred in HCl/Dioxane (0.73 mL, 2.9 mmol, 10 equiv) at r.t. under argon. After 45 min, the
solvent was evaporated in vacuo and free amine 3.15 was isolated (82 mg, 0.29 mmol, 100%) as a white paste.

**Analytical data for 3.15**

\[ \text{M.W.:} 282.83 \]

**\[^1\] H NMR:** (300 MHz, D$_2$O)

- 3.24 (t, \( J = 6.2 \) Hz, 2 H, H3),
- 2.91 (t, \( J = 6.5 \) Hz, 2 H, H4),
- 2.85 (bt, \( J = 7.4 \) Hz, 2 H, H11),
- 2.52 (t, \( J = 7.4 \) Hz, 2 H, H6),
- 1.82 (s, 3 H, H1),
- 1.52 (m, 4 H, H7, H10),
- 1.23 (m, 4 H, H8, H9).

**\[^13\] C NMR:** (75 MHz, D$_2$O)

- 204.70 (C(5)),
- 174.18 (C(2)),
- 43.20 (C(11)),
- 39.33 (C(3)),
- 38.60 (C(4)),
- 27.92 (C(6)),
- 27.31 (C(10)),
- 26.39 (C(1)),
- 25.10 (C(7)),
- 24.91 (C(8)),
- 21.75 (C(9)).

**S-2-Acetamidoethyl 7-((3R,5R)-3,5-bis(tert-butyldimethylsilyloxy)-6-phenylhexanamido)heptanethioate (3.27)**

**Hydrolysis.** Ester 3.31 (30 mg, 0.051 mmol) and LiOH (21 mg, 0.51 mmol, 10 equiv) were dissolved in a 3:2:1 solution of MeOH:H$_2$O:THF (0.6 mL, 0.4 mL and 0.2 mL respectively) and stirred vigorously at r.t. for 6 hr. Quenched with 1M HCl (0.5 mL), and adjusted pH to approximately 3 units by adding HCl dropwise. Extracted with EtOAc (6x0.5 mL) and dried with brine (3x0.5 mL) and MgSO$_4$. **Coupling.** After evaporating the solvent in vacuo, the crude acid was dissolved in DCM (0.5 mL) and, while stirring under argon at r.t., added: EDC (14.8 mg, 0.077 mmol, 1.5 equiv), DMAP (0.6 mg, 0.005 mmol, 0.1 equiv) and HSNAC (9.0 \( \mu \)L, 0.085 mmol, 1.7 equiv). After stirring overnight, the solution was diluted with water (0.5 mL) and EtOAc (0.5 mL), and extracted with EtOAc (3x1 mL). The organic phase was washed with brine (2x1 mL) and
dried over \( \mathrm{Na}_2\mathrm{SO}_4 \). Purified crude mixture by flash column chromatography (30% Acetone-hexanes) furnishing NAC thioester 3.27 (18.4 mg, 0.027 mmol, 53% over two steps) as a clear oil.

**Analytical data for 3.27**

- **M.W.:** 681.13
- **TLC (R\_f):** 0.33 (30% Acetone-hexanes)

**\(^1\)H NMR:** (400 MHz, CDCl\_3)

7.25-7.11 (m, 5 H, PhH), 6.30 (t, \( J = 5.6 \) Hz, 1 H, NH), 5.98 (bs, 1 H, NHAc), 4.18 (m, 1 H, H3), 3.93 (m, 1 H, H5), 3.39 (q, \( J = 6.4 \) Hz, 2 H, H15), 3.16 (m, 1 H, H7"), 3.06 (m, 1 H, H7"), 3.00 (t, \( J = 6.4 \) Hz, 2 H, H14), 2.76 (dd, \( J = 13.2, \) 6 Hz, 1 H, H6"), 2.68 (dd, \( J = 13.2, \) 6.8 Hz, 1 H, H6"), 2.53 (t, \( J = 7.2 \) Hz, 2 H, H12), 2.43 (dd, \( J = 14.8, \) 4.4 Hz, 1 H, H2"), 2.19 (dd, \( J = 14.8, \) 5.2 Hz, 1 H, H2"), 1.94 (s, 3 H, H17), 1.59 (m, 4 H, H4, H11), 1.35 (m, 2 H, H8), 1.27 (m, 4 H, H9, H10), 0.85 (s, 9 H, H25), 0.84 (s, 9 H, H21), 0.05 (s, 3 H, H22), 0.04 (s, 3 H, H18), -0.01 (s, 3 H, H23), -0.13 (s, 3 H, H19)

**\(^13\)C NMR:** (100 MHz, CDCl\_3)

199.94 (C(13)), 170.70 (C(1)), 170.31 (C(16)), 138.42 (C(Ph)), 129.67 (2 C, C(12)), 128.21 (2 C, C(12)), 126.19 (C(Ph)), 70.54 (C(5)), 67.18 (C(3)), 44.42 (C(6)), 43.94 (C(12)), 43.58 (C(4)), 43.44 (C(2)), 39.73 (C(15)), 38.95 (C(7)), 29.28 (C(8)), 28.55 (C(14)), 28.40 (C(9)), 26.50 (C(10)), 25.91 (3 C, C(21)), 25.82 (3 C, C(25)), 25.46 (C(11)), 23.20 (C(17)), 18.01 (C(20)), 17.91 (C(24)), -4.55 (2 C, C(18), C(22)), -4.57 (C(19)), -4.75 (C(23))

**General procedure for the oxidative cleavage of bis-silylated homoallylic alcohols and their carbodiimide coupling with NAC thioester 3.15**

**Silylation.** Purified monoprotected homoallylic alcohol, TBSOTf (1.1 equiv) and 2,6-lutidine (2.2 equiv) were dissolved in DCM (to a 0.1 M conc.) while stirring in an ice bath overnight. The mixture was quenched with a saturated aqueous NH\(_4\)Cl solution (1 volume) and extracted with hexanes (4x1 volumes); dried with brine and \( \mathrm{Na}_2\mathrm{SO}_4 \), and evaporated solvent in vacuo. **Oxidative cleavage.** The weighed crude bis-silylated olefin is dissolved in \( t-\)BuOH (0.05M) and stirred under argon at r.t. In a separate flask,
NaIO₄ (8.0 equiv) and KMnO₄ (1.2 equiv) were vigorously stirred in a 0.2M pH 7.0 phosphate buffer (0.05M) at r.t. After 15 min, the dark purple permanganate solution was transferred quantitatively by Pasteur pipette to the olefin-containing solution and stirred overnight. Quenched with a saturated solution of sodium thiosulfate (0.5 volumes), diluted with water and extracted thrice with EtOAc (1 volume). The organic extract was washed twice with brine, dried over Na₂SO₄ and the solvent evaporated in vacuo.

**Coupling.** The isolated crude oil was dissolved in DCM (0.1M) along EDC (1.5 equiv), DMAP (0.1 equiv) and NAC thioester 3.15 (1.2 equiv) was added while stirring under argon. After 5 min, TEA was added (1.5 equiv) and the solution stirred overnight. Quenched with NH₄Cl (0.5 volumes) and extracted thrice with EtOAc (1 volume). The organic extract was washed twice with brine and dried over Na₂SO₄. Following solvent evaporation, the crude product obtained was purified by flash column chromatography (30% acetone-hexanes).

**S-2-acetamidoethyl 7-((3S,5R)-3,5-bis(tert-butyldimethylsilyloxy)-6-phenylhexanamido)heptanethioate (3.28)**

Silylation. The general procedure was employed as described with homoallylic alcohol 3.26 (20.0 mg, 0.062 mmol), TBSOTf (30 µL, 0.13 mmol, 2.1 equiv), and 2,6-lutidine (22 µL, 0.19 mmol, 3.0 equiv) in DCM (0.62 mL, 0.1M). **Oxidative cleavage.** The bis-silylated crude was dissolved in t-BuOH (0.6 mL) and oxidatively cleaved by reaction with KMnO₄ (12.0 mg, 0.074 mmol, 1.2 equiv), NaIO₄ (270 mg, 0.5 mmol, 8.0
equiv) dissolved in pH 7.0 buffer (0.6 mL). **Coupling.** The crude acid obtained was dissolved in DCM (1.2 mL, 0.05M) and combined with NAC aminothioester 3.15 (21.0 mg, 0.074 mmol, 1.2 equiv), EDC (18.0 mg, 0.093 mmol, 1.5 equiv), DMAP (1.0 mg, 0.006 mmol, 0.1 equiv), and TEA (13 µL, 0.093 mmol, 1.5 equiv). Following purification NAC substrate 3.28 (9.0 mg, 0.013 mmol, 21% over three steps) was isolated.

### Analytical data for 3.28

- **M.W.:** 681.13
- **TLC (R_f):** 0.33 (30% Acetone-hexanes)

### 1H NMR:

*(400 MHz, CDCl₃)*

- 7.25-7.13 (m, 5 H, PhH), 6.32 (t, J = 5.2 Hz, 1 H, NH), 5.94 (bs, 1 H, NHAc), 4.06 (m, 1 H, H3), 3.89 (m, 1 H, H5), 3.40 (q, J = 6 Hz, 2 H, H15), 3.14 (m, 2 H, H7), 3.01 (t, J = 6.6 Hz, 2 H, H14), 2.70 (dd, J = 6.4, 2.4 Hz, 2 H, H6), 2.54 (t, J = 7.5 Hz, 2 H, H12), 2.48 (dd, J = 15.0, 4.0 Hz, 1 H, H2'), 2.24 (dd, J = 15.0, 5.3 Hz, 1 H, H2''), 1.94 (s, 3 H, H17), 1.61 (m, 4 H, H4, H11), 1.39 (p, J = 7 Hz, 2 H, H8), 1.25 (m, 4 H, H9, H10), 0.83 (s, 18 H, H21, H25), 0.00 (s, 6 H, H18, H22), -0.03 (s, 3 H, H23), -0.18 (s, 3 H, H19)

### 13C NMR:

*(100 MHz, CDCl₃)*

- 199.99 (C(13)), 170.65 (C(1)), 170.29 (C(16)), 138.55 (C(Ph)), 129.72 (2 C, C(13)), 128.21 (2 C, C(13)), 126.23 (C(Ph)), 71.32 (C(5)), 67.66 (C(3)), 44.30 (C(4)), 44.18 (C(6)), 44.12 (C(2)), 43.95 (C(12)), 39.70 (C(15)), 39.00 (C(7)), 29.30 (C(8)), 28.57 (C(14)), 28.42 (C(9)), 26.53 (C(10)), 25.90 (3 C, C(21)), 25.76 (3 C, C(25)), 23.23 (C(17)), 18.01 (C(20)), 17.88 (C(24)), -4.49 (C(18)), -4.54 (C(22)), -4.56 (C(19)), -4.66 (C(23))

### S-2-Acetamidoethyl 7-((3S,5S)-3,5-bis(tert-butyldimethylsilyloxy)-6-phenylhexanamido)heptanethioate (ent-3.27)

**Silylation.** The general procedure was employed as described with homoallylic alcohol ent-3.25 (39.3 mg, 0.12 mmol), TBSOTf (30 µL, 0.13 mmol, 1.1 equiv), and 2,6-
lutidine (31 µL, 0.27 mmol, 2.2 equiv) in DCM (1.2 mL, 0.1M). **Oxidative cleavage.** The bis-silylated crude (21.2 mg) was dissolved in t-BuOH (0.4 mL) and oxidatively cleaved by reaction with KMnO₄ (9.2 mg, 0.058 mmol, 1.2 equiv), NaIO₄ (209 mg, 0.39 mmol, 8.0 equiv) dissolved in pH 7.0 buffer (0.4 mL). **Coupling.** The crude acid obtained (18.3 mg) was dissolved in DCM (0.8 mL, 0.05M) and combined with NAC aminothioester 3.15 (13.9 mg, 0.049 mmol, 1.2 equiv), EDC (11.7 mg, 0.061 mmol, 1.5 equiv), DMAP (0.5 mg, 0.004 mmol, 0.1 equiv), and TEA (8.5 µL, 0.061 mmol, 1.5 equiv). Following purification NAC substrate **ent-3.27** (16.0 mg, 0.023 mmol, 19% over three steps) was isolated.

**Analytical data for ent-3.27**

M.W.: 681.13

TLC ($R_f$): 0.33 (30% Acetone-hexanes)

$^1$H NMR: (400 MHz, CDCl₃)
7.25-7.11 (m, 5 H, PhH), 6.29 (t, $J = 5.6$ Hz, 1 H, NH), 5.95 (bs, 1 H, NHAc), 4.17 (m, 1 H, H3), 3.93 (m, 1 H, H5), 3.40 (q, $J = 6.4$ Hz, 2 H, H15), 3.16 (m, 1 H, H7'), 3.06 (m, 1 H, H7''), 3.00 (t, $J = 6.4$ Hz, 2 H, H14), 2.76 (dd, $J = 13.2$, 6 Hz, 1 H, H6'), 2.68 (dd, $J = 13.2$, 6 Hz, 1 H, H6''), 2.53 (t, $J = 7.2$ Hz, 2 H, H12), 2.43 (dd, $J = 14.8$, 4.4 Hz, 1 H, H2'), 2.19 (dd, $J = 14.8$, 5.2 Hz, 1 H, H2''), 1.94 (s, 3 H, H17), 1.59 (m, 4 H, H4, H11), 1.35 (m, 2 H, H8), 1.27 (m, 4 H, H9, H10), 0.85 (s, 9 H, H25), 0.84 (s, 9 H, H21), 0.05 (s, 3 H, H22), 0.04 (s, 3 H, H18), -0.01 (s, 3 H, H23), -0.13 (s, 3 H, H19)

$^{13}$C NMR: (100 MHz, CDCl₃)
199.97 (C(13)), 170.69 (C(1)), 170.29 (C(16)), 138.42 (C(Ph)), 129.67 (2 C, C(Ph)), 128.21 (2 C, C(Ph)), 126.19 (C(Ph)), 70.54 (C(5)), 67.17 (C(3)), 44.42 (C(6)), 43.95 (C(12)), 43.57 (C(4)), 43.44 (C(2)), 39.73 (C(15)), 38.95 (C(7)), 29.28 (C(8)), 28.56 (C(14)), 28.40 (C(9)), 26.50 (C(10)), 25.91 (3 C, C(21)), 25.82 (3 C, C(25)), 25.47 (C(11)), 23.21 (C(17)), 18.01 (C(20)), 17.91 (C(24)), -4.55 (2 C, C(18), C(22)), -4.57 (C(19)), -4.75 (C(23))
Silylation. The general procedure was employed as described with homoallylic alcohol ent-3.26 (42.6 mg, 0.13 mmol), TBSOTf (34 µL, 0.15 mmol, 1.1 equiv), and 2,6-lutidine (35 µL, 0.30 mmol, 2.2 equiv) in DCM (1.3 mL, 0.1M). Oxidative cleavage. The bis-silylated crude was dissolved in t-BuOH (0.43 mL) and oxidatively cleaved by reaction with KMnO₄ (25.0 mg, 0.16 mmol, 1.2 equiv), NaIO₄ (542 mg, 1.0 mmol, 8.0 equiv) dissolved in pH 7.0 buffer (0.87 mL). Coupling. The crude acid obtained (48 mg) was dissolved in DCM (1.1 mL, 0.1M) and combined with NAC aminothioester 3.15 (37.0 mg, 0.13 mmol, 1.2 equiv), EDC (33.0 mg, 0.17 mmol, 1.5 equiv), DMAP (1.3 mg, 0.011 mmol, 0.1 equiv), and TEA (24 µL, 0.17 mmol, 1.5 equiv). Following purification NAC substrate ent-3.28 (59.5 mg, 0.088 mmol, 68% over three steps) was isolated.

Analytical data for ent-3.28

M.W.: 681.13

TLC (Rf): 0.33 (30% Acetone-hexanes)

H NMR: (400 MHz, CDCl₃)
7.24-7.12 (m, 5 H, PhH), 6.33 (t, J = 5.2 Hz, 1 H, NH), 6.03 (bs, 1 H, NHAc), 4.05 (m, 1 H, H3), 3.88 (m, 1 H, H5), 3.39 (q, J = 6 Hz, 2 H, H15), 3.13 (m, 2 H, H7), 3.00 (t, J = 6.6 Hz, 2 H, H14), 2.70 (dd, J = 6.4, 2.4 Hz, 2 H, H6), 2.53 (t, J = 7.5 Hz, 2 H, H12), 2.48 (dd, J = 15.0, 4.0 Hz, 1 H, H2'), 2.24 (dd, J = 15.0, 5.3 Hz, 1 H, H2''), 1.93 (s, 3 H, H17), 1.62 (m, 4 H, H4, H11), 1.38 (p, J = 7 Hz, 2 H, H8), 1.25 (m, 4 H, H9, H10), 0.81 (s, 18 H, H21, H25), -0.01 (s, 6 H, H18, H22), -0.05 (s, 3 H, H23), -0.18 (s, 3 H, H19)
\[ ^{13}C \text{ NMR:} \ (100 \text{ MHz, CDCl}_3) \]
\[
199.99 \ (C(13)), \ 170.65 \ (C(1)), \ 170.29 \ (C(16)), \ 138.55 \ (C(Ph)), \ 129.72 \ (2 \ C, \ C(Ph)), \ 128.21 \ (2 \ C, \ C(Ph)), \ 126.23 \ (C(Ph)), \ 71.32 \ (C(5)), \ 67.66 \ (C(3)), \\
44.30 \ (C(4)), \ 44.18 \ (C(6)), \ 44.12 \ (C(2)), \ 43.95 \ (C(12)), \ 39.70 \ (C(15)), \ 39.00 \ (C(7)), \ 29.30 \ (C(8)), \ 28.57 \ (C(14)), \ 28.42 \ (C(9)), \ 26.53 \ (C(10)), \\
25.90 \ (3 \ C, \ C(21)), \ 25.76 \ (3 \ C, \ C(25)), \ 23.23 \ (C(17)), \ 18.01 \ (C(20)), \ 17.88 \ (C(24)), \ -4.49 \ (C(18)), \ -4.54 \ (C(22)), \ -4.56 \ (C(19)), \ -4.66 \ (C(23))
\]

**General procedure for the deprotection of enantioenriched NAC thioesters**

The purified NAC substrate contained in an Eppendorf tube was dissolved in MeCN (0.1M) at r.t. To this solution pyridine (10 equiv) was added, followed by HF (50 equiv). The mixture was vortexed to homogeneity for 10 sec and the reaction was allowed to proceed without stirring for 5 hr, at which time the reaction was diluted with water (0.2 volumes) and extracted with EtOAc (5 x 0.2 volumes). The organic extracts were pooled and dried with MgSO\(_4\), and the crude purified by flash chromatography (5% MeOH-DCM).

**\textit{S-2-Acetamidoethyl 7-((3R,5R)-3,5-dihydroxy-6-phenylhexanamido)heptanethioate (3.29)}**

The general procedure was employed as described with bis-silylated NAC substrate 3.27 alcohol (16 mg, 0.023 mmol), pyridine (14 µL, 0.23 mmol, 10 equiv), and HF (99 µL, 2.3 mmol, 100 equiv) in MeCN (800 µL, 0.03M). Following purification, free diol 3.29 (3.6 mg, 0.008 mmol, 35%) was isolated.

**Analytical data for 3.29**
M.W.: 452.61

TLC ($R_f$): 0.10 (5% MeOH-DCM)

$^1$H NMR: (500 MHz, CDCl$_3$)

7.30-7.18 (m, 5 H, PhH), 6.07 (bt, $J = 5.2$ Hz, 1 H, NH), 5.88 (bs, 1 H, NHAc), 4.59 (bs, 1 H, OH), 4.19 (m, 1 H, H3), 4.10 (m, 1 H, H5), 3.40 (q, $J = 6.0$ Hz, 2 H, H6), 2.99 (t, $J = 6.4$ Hz, 2 H, H14), 2.76 (dddd, $J = 13.6$, 13.6, 7.3, 6.0 Hz, 2 H, H6), 2.55 (t, $J = 7.2$ Hz, 2 H, H12), 2.30 (m, 2 H, H2), 1.94 (s, 3 H, H17), 1.61 (m, 4 H, H4, H11), 1.47 (m, 2 H, H8), 1.31 (m, 4 H, H9, H10)

$^{13}$C NMR: (125 MHz, CDCl$_3$)

200.09 (C(13)), 171.86 (C(1)), 170.35 (C(16)), 137.82 (C(Ph)), 129.41 (2 C, C(Ph)), 128.59 (2 C, C(Ph)), 126.58 (C(Ph)), 73.43 (C(5)), 69.46 (C(3)), 44.34 (C(6)), 43.79 (C(12)), 42.86 (C(2)), 41.52 (C(4)), 39.67 (C(15)), 39.03 (C(7)), 29.10 (C(8)), 28.49 (C(14)), 28.22 (C(9)), 26.24 (C(10)), 25.38 (C(11)), 23.18 (C(17))

HRMS: (EI) m/z calcd for C$_{16}$H$_{29}$N$_2$O$_5$S [(M-C$_7$H$_7$)$^+$] 361.1792, found 361.1831

*S-2-acetamidoethyl 7-((3S,5R)-3,5-dihydroxy-6-phenylhexanamido)heptanethioate (3.30)*

The general procedure was employed as described with bis-silylated NAC substrate 3.28 alcohol (3 mg, 0.004 mmol), pyridine (3 µL, 0.04 mmol, 10 equiv), and HF (7 µL, 0.20 mmol, 50 equiv) in MeCN (40 µL, 0.1M). Following purification, free diol 3.30 (900 µg, 2 µmol, 50%) was isolated.

Analytical data for 3.30

M.W.: 452.61

TLC ($R_f$): 0.39 (10% MeOH-DCM)

$^1$H NMR: (400 MHz, CDCl$_3$)
7.32-7.18 (m, 5 H, PhH), 5.99 (bs, 1 H, NH), 5.89 (bs, 1 H, NHAc), 4.32 (m, 1 H, H3), 4.15 (m, 1 H, H5), 3.40 (q, J = 6.0 Hz, 2 H, H15), 3.22 (q, J = 6.6 Hz, 2 H, H7), 3.00 (t, J = 6.3 Hz, 2 H, H14), 2.78 (d, J = 6.6 Hz, 2 H, H6), 2.55 (t, J = 7.5 Hz, 2 H, H12), 2.34 (m, 2 H, H2), 1.94 (s, 3 H, 17), 1.66 (m, 4 H, H4, H11), 1.51 (m, 2 H, H8), 1.31 (m, 4 H, H9, H10)

$^{13}$C NMR: (100 MHz, CDCl$_3$)
200.13 (C(13)), 172.29 (C(1)), 129.34 (2 C, C(Ph)), 128.58 (2 C, C(Ph)), 126.53 (C(Ph)), 69.92 (C(5)), 66.39 (C(3)), 43.96 (C(6)), 43.78 (C(12)), 42.29 (C(2)), 41.59 (C(4)), 39.65 (C(15)), 39.03 (C(7)), 28.50 (C(14)), 26.23 (C(9)), 25.38 (C(10)), 23.21 (C(17))

HRMS: (EI) m/z calcd for C$_{19}$H$_{28}$NO$_4$ [(M-C$_4$H$_8$NOS)$^+$] 334.2013, found 334.2054

S-2-acetamidoethyl 7-((3S,5S)-3,5-dihydroxy-6-phenylhexanamido)heptanethioate (ent-3.27)

The general procedure was employed as described with bis-silylated NAC substrate
ent-3.27 alcohol (16.0 mg, 0.023 mmol), pyridine (17 µL, 0.23 mmol, 10 equiv), and HF (42 µL, 1.15 mmol, 50 equiv) in MeCN (250 µL, 0.1M). Following purification, free diol ent-3.29 (9.6 mg, 0.021 mmol, 92%) was isolated.

Analytical data for ent-3.29

M.W.: 452.61

TLC (R$_f$): 0.10 (5% MeOH-DCM)

$^1$H NMR: (400 MHz, CDCl$_3$)
7.30-7.17 (m, 5 H, PhH), 6.22 (bt, J = 5.2 Hz, 1 H, NH), 6.00 (bs, 1 H, NHAc), 4.66 (bs, 1 H, OH), 4.16 (m, 1 H, H3), 4.09 (m, 1 H, H5), 3.38 (q, J = 6.2 Hz, 2 H, H15), 3.34 (bs, 1 H, OH), 3.20 (q, J = 6.6 Hz, 2 H, H7), 2.99 (t, J = 6.4 Hz, 2 H, H14), 2.75 (ddddd, J = 13.6, 13.6, 7.3, 6.0 Hz, 2 H, H6), 2.54 (t, J = 7.2 Hz, 2 H, H12), 2.30 (m, 2 H, H2), 1.93 (s, 3 H, H17), 1.61 (m, 4 H, H4, H11), 1.47 (m, 2 H, H8), 1.31 (m, 4 H, H9, H10)
\begin{align*}
^{13}C \text{ NMR:} \quad & (100 \text{ MHz, CDCl}_3) \\
& 200.11 \text{ (C(13))}, 171.87 \text{ (C(1))}, 170.43 \text{ (C(16))}, 137.93 \text{ (C(Ph))}, 129.45 \text{ (2 C, C(Ph))}, 128.60 \text{ (2 C, C(Ph))}, 126.58 \text{ (C(Ph))}, 73.40 \text{ (C(5))}, 69.48 \text{ (C(3))}, 44.40 \text{ (C(6))}, 43.84 \text{ (C(12))}, 43.01 \text{ (C(2))}, 41.59 \text{ (C(4))}, 39.67 \text{ (C(15))}, 39.06 \text{ (C(7))}, 29.16 \text{ (C(8))}, 28.52 \text{ (C(14))}, 28.28 \text{ (C(9))}, 26.30 \text{ (C(10))}, 25.42 \text{ (C(11))}, 23.21 \text{ (C(17))}
\end{align*}

HRMS: \quad (EI) \text{ m/z calcd for C}_{16}H_{29}N_{2}O_{5}S [(\text{M-C}_7H_7)^+] \text{ 361.1792, found 361.1745}

\textit{S-2-acetamidoethyl 7-((3R,5S)-3,5-dihydroxy-6-phenylhexanamido)heptanethioate (ent-3.30)}

\begin{align*}
\text{The general procedure was employed as described with bis-silylated NAC substrate} \\
\text{ent-3.28} \text{ alcohol (22 mg, 0.032 mmol), pyridine (23 µL, 0.23 mmol, 10 equiv), and HF} \\
(58 µL, 1.6 mmol, 50 equiv) \text{ in MeCN (320 µL, 0.1M). Following purification free diol} \\
\text{ent-3.30} \text{ (5.7 mg, 0.013 mmol, 40%) was isolated.}
\end{align*}

\textbf{Analytical data for ent-3.30}

\begin{align*}
\text{M.W.:} \quad & 452.61 \\
\text{TLC (R\text{$_f$})}: \quad & 0.39 \text{ (10\% MeOH-DCM)}
\end{align*}

\begin{align*}
^{1}H \text{ NMR:} \quad & (400 \text{ MHz, CDCl}_3) \\
& 7.31-7.18 \text{ (m, 5 H, PhH)}, 6.04 \text{ (bs, 1 H, NH)}, 5.92 \text{ (bs, 1 H, NHAc)}, 4.32 \text{ (m, 1 H, H3)}, 4.15 \text{ (m, 1 H, H5)}, 3.40 \text{ (q, J = 6.0 Hz, 2 H, H15)}, 3.22 \text{ (q, J = 6.6 Hz, 2 H, H7)}, 2.99 \text{ (t, J = 6.2 Hz, 2 H, H14)}, 2.78 \text{ (d, J = 6.7 Hz, 2 H, H6)}, 2.55 \text{ (t, J = 7.3 Hz, 2 H, H12)}, 2.31 \text{ (m, 2 H, H2)}, 1.94 \text{ (s, 3 H, H17)}, 1.66 \text{ (m, 4 H, H4, H11)}, 1.48 \text{ (m, 2 H, H8)}, 1.32 \text{ (m, 4 H, H9, H10) }
\end{align*}

\begin{align*}
^{13}C \text{ NMR:} \quad & (75 \text{ MHz, CDCl}_3) \\
& 200.12 \text{ (C(13))}, 172.36 \text{ (C(1))}, 170.39 \text{ (C(16))}, 138.20 \text{ (C(Ph))}, 129.34 \text{ (2 C, C(Ph))}, 128.57 \text{ (2 C, C(Ph))}, 126.52 \text{ (C(Ph))}, 69.90 \text{ (C(5))}, 66.37 \text{ (C(3))}, 43.97 \text{ (C(6))}, 43.77 \text{ (C(12))}, 42.29 \text{ (C(2))}, 41.63 \text{ (C(4))}, 39.66 \text{ (C(15))}, 39.08 \text{ (C(7))}, 29.09 \text{ (C(8))}, 28.48 \text{ (C(14))}, 28.19 \text{ (C(9))}, 26.22 \text{ (C(10))}, 25.37 \text{ (C(11))}, 23.17 \text{ (C(17))}
\end{align*}
HRMS: (EI) m/z calcd for $C_{16}H_{29}N_{2}O_{5}S [(M-C_{7}H_{7})^+]$ 361.1792, found 361.1783

3.5.2.3. Transformation, expression and purification of wild-type DEBS TE

**Transformation.** The expression vector pRSG33 (wild-type DEBS TE)$^3$ was transformed into chemical competent *E. coli* BL21(DE3) for protein expression employing standard protocols.$^4$ Recovered cells were spread on LB+Amp plates and incubated at 37°C for 12 hr. **Expression.** *E. coli* BL21(DE3) was grown in 400 mL of standard LB medium in a shake flask supplemented with 100 μg mL$^{-1}$ of ampicillin at 37°C. At OD$_{600}$ = 0.45, protein expression was induced by addition of 1 mM IPTG, and the culture incubated at 20°C with shaking at 250 rpm for 12 h. **Purification.** Protein purification was performed at 4°C. The cells were harvested by centrifugation at 2.63x10$^3$ g for 30 min and promptly resuspended in 80 mL of lysis buffer (100 mM sodium phosphate, 300 mM NaCl, 10% (v/v) glycerol, 1 mg/mL lysozyme, 1 μg/mL pepstatin A, 1-2 μg/mL leupeptin, pH 8.0). The cells were lysed by sonication on ice and the debris was removed by centrifugation at 1.16x10$^4$ g for 45 min. The clarified lysate was gently mixed for 3 hr with 1 mL of nickel-nitritriacetic acid (Ni-NTA) resin and transferred onto a purification column. The protein was eluted with wash buffer of increasing imidazole concentrations: 20 mL x 0 mM imidazole; 20 mL x 20 mM imidazole; 2x(10 mL x 100 mM imidazole); 2x(10 mL x 250 mM imidazole). The purified protein was exchanged into dialysis buffer (100 mM Tris, 300 mM NaCl, 30% (v/v) glycerol, pH 7.43) and concentrated by centrifugation with an Amicon Ultra 5000 MWCO (Millipore) at 10°C. Upon concentration, the protein was flash-frozen in liquid nitrogen and stored at -80°C. Protein concentration was determined by the Bradford
assay (Bio-Rad). Approximately 3 mg of purified protein was obtained per L of cell culture.

3.5.2.4. Kinetic analysis of the DEBS TE

The TE-catalyzed hydrolysis of thioester substrates were monitored by observation of the formation of 5-thio-2-nitrobenzoate by reaction of released N-acetylcysteamine with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB). A typical kinetic assay mixture consists of 5 μM DEBS TE, 50 mM phosphate buffer (pH 7.37), 4 % (v/v) saturated solution of DTNB in 50 mM phosphate buffer (pH 7.37), x mM substrate (50 mM stock solutions in DMSO, where x is the variable amount) and 10 % (v/v) DMSO in total volume of 15 μL reaction. The formation of free thiol was quantified by measuring the absorption at 412 nm using a Nanodrop 2000 (Pedestal mode) from Thermo Scientific.

The reactions were monitored for 60 min at room temperature and data points were collected at 1, 3, 5, 10, 30, and 60 min intervals. Initial velocities (v) were determined by linear regression analysis of the data. All steady state kinetic assays were performed with five different concentrations of substrates (0.1, 0.2, 0.5, 2, and 5 mM) in duplicate. The kinetic parameter $k_{cat}/K_M$ was calculated from the slope of v versus [S]. Because of apparent variation in specific activity among protein preparations, a single protein preparation was used for determination of the specificity constant for all enantioenriched substrates.

Table 3.1. Calculated specificity constants ($k_{cat}/K_M$) for the reaction of enantioenriched substrates ent-3.29, ent-3.30, 3.29 and 3.30 with the DEBS TE (values represent the mean ± standard error).

<table>
<thead>
<tr>
<th>Isomer</th>
<th>$k_{cat}/K_M$ (M·s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ent-3.29</td>
<td>1.34 ± 1x10⁻²</td>
</tr>
<tr>
<td>ent-3.30</td>
<td>0.43 ± 6x10⁻²</td>
</tr>
<tr>
<td>3.30</td>
<td>0.6 ± 4x10⁻¹</td>
</tr>
<tr>
<td>3.20</td>
<td>0.3 ± 1x10⁻¹</td>
</tr>
</tbody>
</table>
3.5.2.5. Hydrolytic assay of \textit{anti-3.42} with DEBS TE

The enzymatic assays were prepared by incubating 5 μM DEBS TE in 50 mM phosphate buffer (pH 7.4) with 2.5 mM \textit{anti-3.42} macrolactone standard at 20°C. At various time points (15, 30, 60, 120, 180, 1620 min), aliquots were taken, diluted 4-fold with a 50% MeCN:H\textsubscript{2}O solution and analyzed for hydrolysis by analytical HPLC (Agilent 1100 series).

3.5.3. References


3.5.4. Selected 1D and 2D NMR spectra

rac-3.21
anti-3.40

R = TBS
\[
\begin{align*}
\text{OR} & \quad \text{OR} \\
\text{syn-3.40} & \\
R &= \text{TBS}
\end{align*}
\]
anti-3.41
R = TBS
OR
OR
O

syn: 3.41

R = TBS
HMBC
anti-3.43
HMBC
HN

OH

syn 3.43

[Chemical structure image]

[Graph showing spectral data with ppm values]
COSY

HSQC
Atahualpa (Ata) Pinto  
Department of Chemistry  
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Syracuse University  
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Date of birth: 10/14/76  
Place of birth: Caracas, Venezuela

**EDUCATION**

2011  **Ph.D. in Chemistry** – Syracuse University  
Advisor: Dr. Christopher N. Boddy  
Dissertation: *Synthesis and biosynthesis of polyketide natural products*

2006  **B.Sc. in Chemistry** – SUNY College of Environmental Science and Forestry  
Magna Cum Laude with Honors  
Concentration: Biochemistry and Natural Products  
Honors Thesis: *Progress towards the biomimetic synthesis of spiculoic acid A*

**PROFESSIONAL EXPERIENCE**

2006-2011  **Syracuse University/University of Ottawa**  
**NSF Fellow/Research Assistant – Advisor: Dr. Christopher N. Boddy**  
- Synthesis of polyketide Spiculoic Acid A by a biosynthesis inspired route. Designed the study to test our hypothesis regarding the regiochemistry of double bond incorporation and ensuing cyclization in Spiculoic Acid A biosynthesis  
- Understanding thioesterase (TE) catalyzed macrocyclization of polyketides. Carried out the first rigorous study of the role absolute stereochemistry has on the regio- and stereoselectivity in TE-catalyzed macrocyclizations using synthetic chemistry and in vitro biochemistry  
- Involved in the design, implementation and optimization of a short route towards a key Ritonavir intermediate in collaboration with the Clinton Health Access Initiative  
- Trained and supervised new undergraduate and graduate students in various aspects of the organic chemistry laboratory, with particular emphasis in theory and techniques

2006  **Syracuse University**  
**Graduate Teaching Assistant**  
- Taught, supervised and graded general and organic chemistry lab sections, each consisting of approximately 25-30 students

2005  **Syracuse University**  
**NSF Research Experience for Undergraduates – Advisor: Dr. Christopher N. Boddy**  
- Biomimetic total synthesis of Spiculoic Acid A. Carried out the preliminary studies towards the total synthesis of Spiculoic Acid A’s linear molecular framework
2004-2006  SUNY College of Environmental Science and Forestry

Undergraduate Lab Teaching Assistant
- Delivered lectures and supervised undergraduate chemistry lab sections

Research Assistant – Advisors: Dr. Arthur J. Stipanovic; Dr. José Giner; Dr. Paul Caluwe
- Provided support in the development of an algorithm capable of estimating the relative abundance of chemical components in woody materials by a method based on high resolution thermogravimetric analysis and neural networks
- Assisted in the extraction, purification and quantification of lipid and steroid content of algal samples
- Synthesized, purified and characterized functionalized derivatives of polycarboxylic acids to be employed as crosslinking additives in the manufacturing of paper products

COMPETENCIES
- Modern organic synthesis techniques
- Modern analytical techniques: NMR, HPLC, LC-MS, GC-MS, MALDI-TOF, UV/Vis, IR
- Recombinant protein expression and purification
- In vitro biochemistry
- Molecular modeling
- Scientific database searching

AWARDS
2007  NSF Predoctoral Graduate Research Fellowship
2005  SUNY ESF Honors Program
2005  SUNY ESF Hirsch Scholarship

PROFESSIONAL ORGANIZATIONS
- American Chemical Society (ACS)

PRESENTATIONS
2011  BioMolar Network Meeting; Ottawa, ON Canada
Pinto, A., Boddy, C.N. Understanding thioesterase catalyzed macrocyclization of polyketides
2008  ACS NERM; Burlington, VT
Pinto, A., Boddy, C.N. Polyketide substrate analogs: investigating the TE regiochemistry of cyclization regiochemistry of cyclization
2007  Graduate Student Symposium; Buffalo, NY
Pinto, A., Boddy, C.N. Spiculoic Acid A: understanding its biogenesis through its biomimetic total synthesis
2006  ACS NERM; Binghamton, NY
Pinto, A., Boddy, C.N. Towards the biomimetic synthesis of Spiculoic Acid A
PUBLICATIONS

- Pinto, A., Boddy, C.N. Spiculoic Acid A: Testing an Alternate Biosynthetic Hypothesis (Manuscript in preparation)
- Pinto, A., Boddy, C.N. Investigating the Regio- and Stereochemical Factors Effecting Cyclization in the DEBS TE (Manuscript in preparation)