

ABSTRACT

MOAZAMI, YASAMIN. Progress Towards the Total Synthesis of Pentacyclic Guanidinium Alkaloids. (Under the direction of Dr. Joshua Pierce).

In the recent past, the marine environment has become one of the most prolific sources of chemical and biological diversity. One such example of a marine natural product is the recently isolated apoptosis-inducing pentacyclic guanidinium alkaloid, monanchocidin A. The intricacy and novelty observed in the structure of the pentacyclic guanidinium alkaloids such as the monanchocidin family, coupled with the wide range of biological activities exhibited by these molecules, have attracted significant attention from the scientific community.

The first chapter of this dissertation describes our efforts towards the development of an approach to the pentacyclic guanidinium core of the monanchocidin family and related natural products. Our synthetic approach combines asymmetric synthesis with a biomimetic cascade to provide greatly increased efficiency and selectivity. Furthermore, our approach should allow for pinpoint modification of the molecules' complex functionality to further optimize its potent biological activities, uncover its mechanism of action and potentially develop simplified lead molecules for chemical probe development. To date, we have successfully prepared the key intermediates in our approach and efforts are underway to explore the remainder of the designed synthetic sequence to this core. Additionally, we are working towards the synthesis of simplified analogs and other less complex pentacyclic guanidinium scaffolds in this class of natural products.

The second chapter of this dissertation describes our work on the development of a mild and efficient method for the synthesis of the potentially biologically active class of heterocycles known as 2,3-dihydro-1,3-oxazin-4-ones or 1,3-oxazinones. This method

involved a silver mediated formal [4+2] cycloaddition reaction of an imine and an acylketene intermediate at room temperature. A series of electronically diverse heterocycles, possessing various substitution pattern were prepared using this method.

The final chapter of this dissertation describes our collaborative efforts with the department of biological sciences at North Carolina State University. Our efforts involved the chemical synthesis of an anti-inflammatory dialkylamide natural product, isolated from *Echinacea purpurea* and several libraries of its analogs. Through our efforts, we were able to reveal the structural requirements for biological activity, evaluated the general cytotoxicity of these compounds, and provide lead compounds for further investigation of these poorly understood molecules.

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Progress Towards the Total Synthesis of Pentacyclic Guanidinium Alkaloids

by
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DEDICATION

This work is dedicated to my husband Christopher Kuhn for his unconditional love and support in the past 5 years of my life. To my parents, Jahan and Haydeh for the many sacrifices that they have made to make me the woman that I am today. No words in the world can describe my love, respect, and admiration for you all. Thank you for believing in me and always being there!

BIOGRAPHY

Yasamin Moazami was born on July 24, 1985 in Tehran, Iran to Haydeh Mirdamadi and Jahan Moazzami. Yasamin, along side her family, moved to Charlotte, North Carolina at the age of 15 in 2001, where she finished high school and started her undergraduate studies. Yasamin completed her B.S. degree in biology and B.A. degree in chemistry with honors at the University of North Carolina at Charlotte in 2008. Yasamin became fascinated with organic chemistry while taking the organic course taught by Professor Craig Ogle. From that point, she became even more interested with the world of organic chemistry and started her undergraduate research experience. Yasamin quickly became very passionate about her research and a change of heart later, she decided to continue her studies in chemistry rather than her intended dental school plans! Upon graduation, Yasamin then earned her master's degree in organic chemistry under the supervision of Professor Ogle in 2011 and married the love of her life Chris Kuhn a week after. She then moved to Raleigh, North Carolina shortly after where she wholeheartedly committed herself to pursue her Ph.D. studies in organic chemistry under the supervision of Professor Joshua Pierce at North Carolina State University.

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LIST OF ABBREVIATIONS

Ac₂O: Acetic anhydride

AcOH: Acetic acid

AEA: *N*-arachidonylethanolamine

2-AG: 2-Arachidonoylglycerol

AgO: Silver oxide

Amt.: Amount

Ar: Aryl

Bn: Benzyl

Cat.: Catalytic

CB: Cannabinoid receptor

CH₃CN: Acetonitrile

CHCl₃: Chloroform

CH₂Cl₂: Dichloromethane

CCl₄: Carbon tetrachloride

CH₃OH: Methanol

CoF₃: Cobalt fluoride

CO: Carbon monoxide

COSY: Correlation spectroscopy

CSA: Camphor sulfonic acid

DBMP: 2,6-di-*t*-butylmethyl pyridine

DCM: Dichloromethane

DDQ: 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone

DHS: Deoxyhypusine synthase

DIAD: Diisopropyl azodicarboxylate

DIBAL-H: Diisobutylaluminium hydride

DIEA: *N,N'*-Diisopropylethylamine

DCC: *N,N'*-Dicyclohexylcarbodiimide

DMAP: *N,N'*-Dimethyl amino pyridine

DMF: Dimethylformamide

DMSO: Dimethylsulfoxide

Δ: Heat

dr: Diastereomer

EDCI: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

eIF5A: Eukaryotic translation initiation factor 5A

ELISA: Enzyme-linked immunosorbent assay

Equiv: Equivalence

Et₂O: Diethyl ether

FAAH: Fatty acid amide hydrolase

FDA: Food and drug administration

FMF: Familial Mediterranean Fever

h: Hour

HG-II: Hoveyda-Grubbs 2nd generation catalyst

HMBC: Heteronuclear multiple bond correlation

HRMS: High resolution mass spectroscopy

HSQC: Heteronuclear single quantum correlation

h ν : Light

IPA: Isopropyl alcohol

KHMDS: Potassium hexamethyldisilazide

KOH: Potassium hydroxide

LC-MS: Liquid Chromatography mass spectrometry

LDA: Lithium diisopropylamide

LHS: Lithium hexamethyldisilazide

LPS: Lipopolysaccharide

M: Molar

m-CPBA: *meta*-Chloroperoxybenzoic acid

Min: Minutes

Ms: Mesyl

MVK: Methyl vinyl ketone

μ M: Micro molar

NaH: Sodium Hydride

NE₃: Triethyl amine

NMO: *N*-Methylmorpholine *N*-oxide

NMR: Nuclear magnetic resonance

NOESY: Nuclear Overhauser effect spectroscopy

Nu: Nucleophile

Ph: Phenyl

PEP: *para*-Ethoxy phenyl

PPh₃: Triphenyl phosphine

PMP: *para*-Methoxy phenyl

P(OEt)₃: Triethyl phosphite

PPTS: Pyridinium *p*-toluenesulfonate

p-TsOH: *para*-toluene sulfonic acid

RB flask: Round bottom flask

RCM: Ring closing metathesis

ROESY: Rotating frame nuclear Overhauser effect spectroscopy

rt: Room temperature

SAR: Structure-activity relationship

SM: Starting material

T3P[®]: Propane phosphonic acid anhydride

TBAF: Tetrabutylammonium fluoride

TBDPS: *tert*-Butyldiphenylsilyl

TBS: *Tert*butyldimethylsilyl

TIPS: Triisopropylsilyl

TNF- α : Tumor necrosis factor- α

TFA: Trifluoroacetate

THF: Tetrahydrofuran

TLC: Thin layer chromatography

TPAP: Tetrapropylammonium perruthenate

VR-1: Vanilloid type-1 receptor

CHAPTER 1

General Introduction

1.1 Introduction to Natural Products and Drug Discovery

For centuries, nature has served as a rich source of diverse molecular architectures and therapeutically significant molecules. Even today, nature continues to be explored by modern medicine for biologically relevant compounds.¹ In fact, some of the most important contemporary pharmaceuticals discovered have either been isolated from nature, or inspired by the molecular framework of natural products (Figure 1.1).^{2,3}

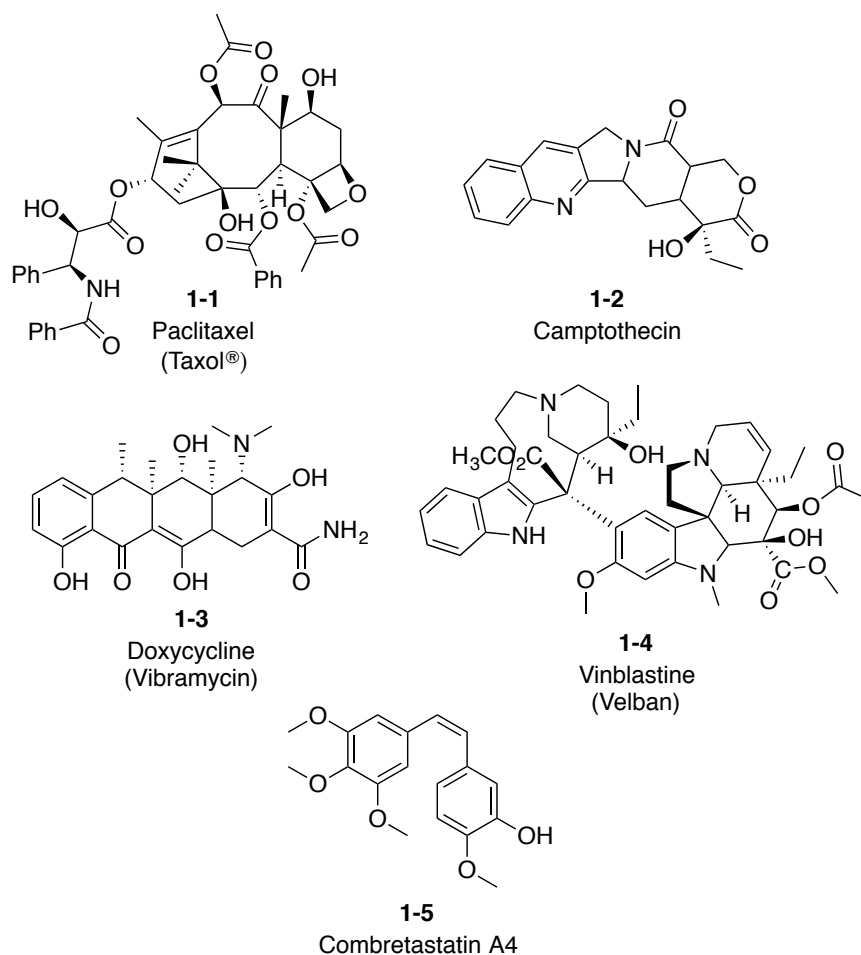


Figure 1.1: Examples of pharmaceutical agents isolated from nature.

Historically, the use of natural products as medical remedies dates back to the start of civilization.⁴ (-)-Colchicine (**1-6**, Figure 1.2), the biologically active constituent of *colchicum autumnale*, has been exploited for over 2000 years, and continues to be utilized for the treatment of the excruciating pain associated with gout.⁵ (-)-Colchicine is also approved by the Federal Drug Administration (FDA) for the treatment of several other conditions such as familial Mediterranean fever (FMF), scleroderma, and amyloidosis.^{5,6}

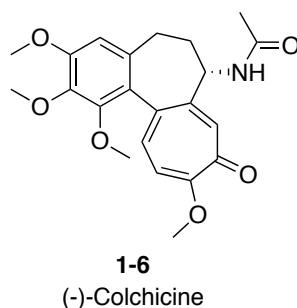


Figure 1.2: (-)-*aR,7S*-Colchicine, an apoptosis inducing natural product.

Early studies involving (-)-colchicine illustrated a broad range of physiological actions.⁷ One such study demonstrated that (-)-colchicine exerted its effects by interfering with the dynamics of the actively polymerizing tubulins, major constituents of microtubules and an integral part of mitosis. As a result of this interaction, the polymerization of the ensuing tubulin polymers is effectively inhibited. This can leave an actively dividing cell culture in mitotic arrest, and induce apoptosis.⁸ Many neoplastic conditions are devoid of the characteristic pathways regulating apoptosis.⁹ Consequently, the ability to be able to modulate and ultimately induce apoptotic pathways through the utilization of natural products such as (-)-colchicine offers an intriguing target based approach in the fight against

cancer.⁵ It should be noted that the application of (-)-colchicine as a chemotherapeutic agent in the treatment of cancer has thus far been limited due to its inherently low therapeutic index. Accordingly, a great deal of research has been performed and is ongoing to pursue alternate drugs and analogs of (-)-colchicine with broader therapeutic indices.¹⁰

Another example of an important natural product with a significant success story is the anti-cancer agent paclitaxel (**1-1**), sold under the trademark Taxol[®] (Figure 1.1). Paclitaxel was originally isolated from the bark of the plant *Taxus brevifolia*, commonly known as the pacific yew.² Over the years, paclitaxel has become one of the most celebrated and fascinating natural products, not to mention one of the top-selling anti-cancer drugs.¹¹ Initial biological studies of paclitaxel revealed remarkable anti-tumor activities against murine tumors and leukemia, which ultimately led to the elucidation of its mechanism of action.² The tremendous success achieved through the discovery and clinical use of paclitaxel initiated a worldwide search for related molecular architectures, expressing similar pro-apoptotic behavior, along with improved features. As a result, the structure of paclitaxel has undergone several modifications and refinements, which has led to a series of analogs.²

Conversely, the utilization of natural products as pharmacopoeias, or templates, is accompanied by associated challenges. Acquisition of most compounds of natural origin with significant pharmacological activities, including the taxanes and many other natural products, is usually limited to their sources. The low abundance of the compounds produced as secondary metabolites by various organisms, along with the environmental effects associated with exploiting those resources, hinder the development and commercialization of natural products as pharmaceuticals. Consequently, alternative approaches and sources of

natural products have become of interest. Having the ability to engineer scale-up reactions in a reliable and an economical manner to meet the demands of the bulk production of biologically active compounds has become a task that is taken by organic synthesis. For instance, the famous multibillion dollar anti-cancer drug Taxol[®], discussed earlier, was originally isolated from the bark of pacific yew, with the natural abundance as low as 0.01%-0.05%.¹² Currently, the issues regarding the scarcity of supply to meet the clinical demands of Taxol[®] is met through a semi-synthetic pathway, which utilizes a related metabolite isolated from the needles of the European yew in much larger quantities.¹³

Very often a natural product may not have all of the required characteristics suitable for application as a pharmaceutical agent. Indeed, a formidable hurdle faced in the area of drug discovery is the development of a natural product lead into a “drug-like” molecule.¹¹ Through the expertise offered by synthetic chemists and the knowledge gained from the total synthesis of natural products, molecules created by nature can be improved upon by rendering changes to their structures and consequently their properties, thereby resulting in analogs having desirable pharmaceutical profiles.⁴

The perception that natural products are too complex and intricate for utilization as chemical leads has resulted in decline in interest by pharmaceutical companies in drug development research based on natural product scaffolds. A tremendous amount of molecules have become available in the pharmaceutical community through the emergence of technological advancements and the use of robotics in combinatorial chemistry at a pace that could not be matched by natural product synthesis; however, this increase in sheer quantity has not translated to an increase in FDA approved pharmaceuticals, in part due to

limitations in structural diversity in the screening libraries.^{4,14} Nature continues to provide chemical and biological diversity that is not offered by typical molecules generated through combinatorial chemistry, presenting a strong argument for the pursuit of natural products in drug discovery.

1.2 Marine Natural Products

Most of the natural products used as leads in drug development research are of terrestrial origin and the wealth of biological and chemical diversity offered by the terrestrial ecosystem is undisputable. In a similar vein, over half of the biodiversity in nature is observed in marine species, with the marine environment accounting for more than 70% of the earth's surface.¹⁵ Indeed, marine life has proven to be a prolific source of structurally unique chemical entities, specific to the aquatic environmental requirements and not produced by terrestrial life.

Through many years of perpetual competition and an ever changing environment, marine inhabitants, many having sedentary lifestyles, have become equipped with an array of complex secondary metabolites as part of their defense mechanisms.¹⁶ Furthermore, the metabolites produced by marine organisms are diluted upon being released in the aquatic environment; as a result, they have to be very potent in order to have the necessary effects for survival.

Despite the enormous potential offered by marine life, this source of novel small molecules remains largely unexplored, partially due to the lack of necessary technologies required for sample collection and the difficulties in the isolation and purification of the

many metabolites obtained in a single sample.¹⁷ Nevertheless, in a matter of only a few decades of exploration, five marine natural products have become FDA approved, along with a plethora of other molecules in different phases of preclinical and clinical trials (Table 1.1).¹⁸

Table 1.1: Marine natural products: approved and in clinical trials.

Clinical Status	Compound	Marine Source	Treatment Area
Approved	Cytarabine, Ara-C	Sponge	Cancer
	Vidarabine, Ara-A	Sponge	Antiviral
	Zincotide	Cone snail	Pain
	Yondelis (ET-743)	Tunicate	Cancer
	Eribulin Mesylate (E7389)	Sponge	Cancer
Phase III	Soblidotin (TZT 1027)	Bacteria	Cancer
Phase II	Pinabulin (NPI-2358)	Fungus	Schizophrenia
	PM1004	Nudibranch	Cancer
	Plitidepsin	Tunicate	Cancer
	Elisidepsin	Mollusc	Cancer
	Pseudopterosins	Soft coral	Cancer
Phase I	Hemiasterlin (E7974)	Sponge	Cancer
	Marizomib (NPI-0052)	Bacteria	Cancer

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CHAPTER 2

Studies Towards the Synthesis of Pentacyclic Guanidinium Natural Products

2.1 Abstract

For many years the diverse molecular architectures of natural products have been a major source of inspiration for both novel reaction development and therapeutic lead molecules. The marine environment has become one of the most prolific sources of chemical and biological diversity. One such example is the recently isolated apoptosis-inducing pentacyclic guanidinium alkaloid, monanchocidin A. The complexity and novelty observed in the structure of the pentacyclic guanidinium alkaloids such as the monanchocidin family, coupled with the wide range of biological activities exhibited by these molecules have attracted significant attention from the scientific community. We are developing an approach to the pentacyclic guanidinium core of the monanchocidins and related natural products that overcome the stereochemical challenges associated with the previous syntheses of this class of natural products. We are planning to ultimately utilize these scaffolds as chemical probes in biological pathways. Our recent efforts in this area will be presented in this chapter.

2.2 Introduction and Background

2.2.1 Polycyclic Guanidinium Natural Products

Within the biodiversity offered by the marine environment, sponges have been shown to produce novel structural scaffolds. In light of this, they have become the most investigated aquatic organisms in the search for potential therapeutics.¹ An example of an unprecedented motif isolated from marine sponges is the polycyclic guanidinium ring system observed in several structurally related classes of alkaloids (Figure 2.1 and Figure 2.2).² Such natural

products have been shown to exhibit a wide range of biological activities including cytotoxicity, antifungal, antiviral, antimalarial, anti-microbial, and antiprotozoal.²

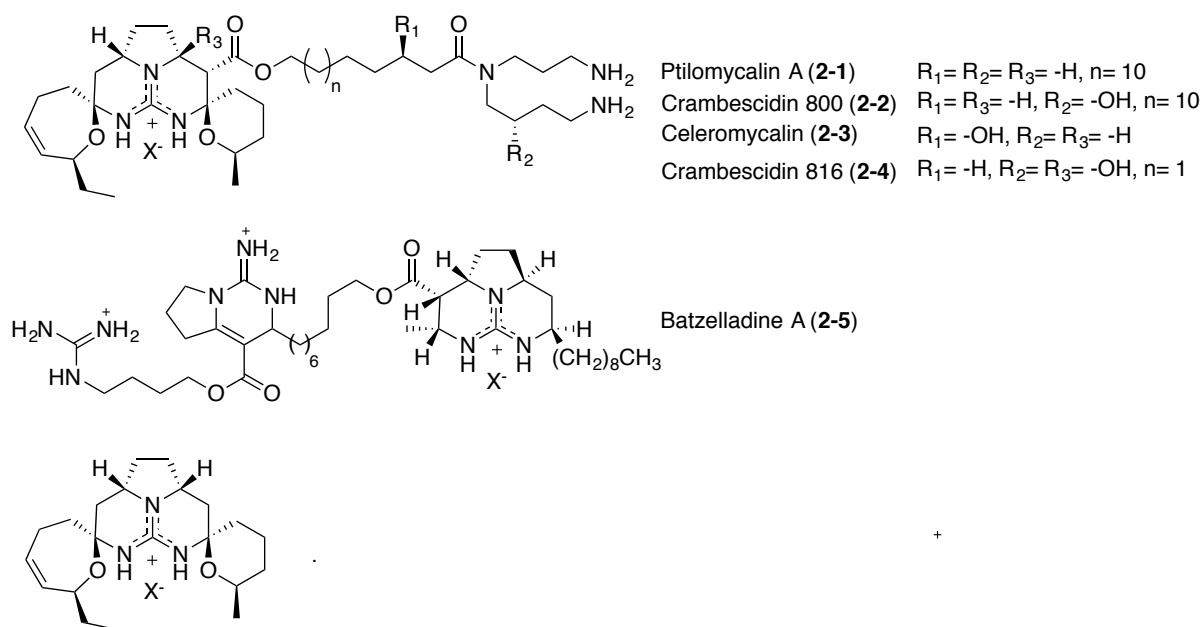


Figure 2.1: Examples of marine guanidinium alkaloids.

The monanchocidin family of natural products (Figure 2.2) are one of the most recently discovered guanidinium alkaloids isolated from the Far-Eastern sponge *Monanchora pulchra* by Makarieva et al. in 2010.³ Monanchocidin A was the first member of this family isolated in 0.15% abundance. Preliminary biological studies of monanchocidin A (**2-7**) demonstrated interesting pro-apoptotic behavior. In the following year, Makarieva and co-workers reported several other additional members (monanchocidins B-E, **2-8** to **2-11**), isolated from the same sponge in a similar type of environment.⁴

The structures of the monanchocidins were elucidated on the basis of 1H NMR, ^{13}C NMR, HMBC, HSQC, COSY, HRMS experiments and chemical transformations. The

relative stereochemistry of such structures was assigned on the basis of NOESY and ROESY experiments.^{3,4} Of particular interest from a structural point of view, the monanchocidins contain a densely functionalized and rigid guanidinium fragment (“the vessel unit”) and a novel hemiketal bearing substituted morpholinone fragment (“the anchor unit”) and the two units are linked together via a long hydrocarbon ester linkage.^{3,4} It should be noted that other structurally related pentacyclic guanidinium alkaloids² (Figure 2.1) have been isolated in the past; however, the morpholinone fragment and the ring structure in the guanidinium core (5- vs. 7-membered spiro-ring) are unique features of the monanchocidin family. Overall, the pentacyclic guanidinium natural products represent some of the most complex guanidinium alkaloids isolated to date, and pose a daunting challenge for synthetic chemistry.

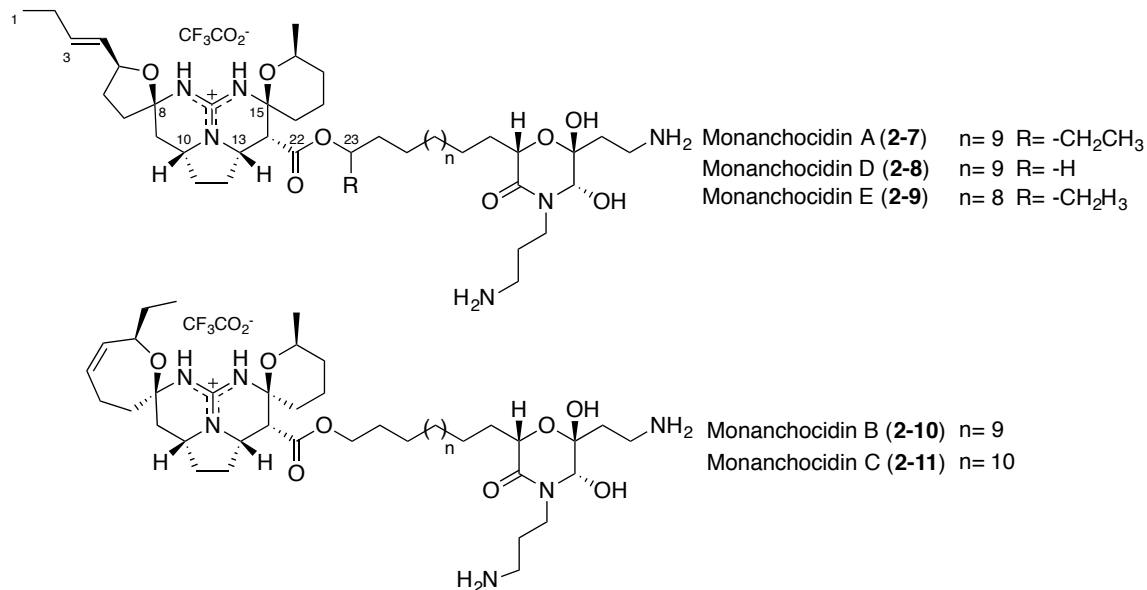


Figure 2.2: Pentacyclic guanidinium alkaloids, monanchocidin A-E.

Additionally, the monanchocidins were screened for their biological activities and have shown to have potential in the development of novel therapeutics. Monanchocidin A has demonstrated activities against human leukemia THP-1 ($IC_{50} = 5.1 \mu\text{M}$), human cervix epithelioid carcinoma HeLa ($IC_{50} = 11.8 \mu\text{M}$), and mouse epidermal JB6Cl41 ($IC_{50} = 12.3 \mu\text{M}$) cell lines. Additionally, monanchocidin A has been shown to induce early apoptosis in THP-1 cell line at $3.0 \mu\text{M}$ concentration.³ Monanchocidins B-E were also evaluated against HL-60 human leukemia cells and exhibited inhibitory activities with IC_{50} values of 540, 200, 110, 830, and 650 nM, respectively.⁴

More recently, the monanchocidins have been linked to inactivation of eukaryotic translation initiation factor (eIF5A), a protein involved in cell proliferation and extracellular matrix formation. Inactivation of this protein is a phenomenon that has been associated with the induction of cell cycle arrest. This inhibitory effect exhibited by monanchocidins is presumably the result of the morpholinone unit, specifically due to the presence of the imbedded spermidine motif in this unit, which is known for eIF5A inactivation through inhibition of deoxyhypusine synthase (DHS).^{5a,b} DHS is an enzyme responsible for the attachment of spermidine onto a lysine residue of the precursor protein to eIF5A, and thereby producing the active form of eIF5A protein. eIF5A is the only eukaryotic protein that bears this post-translational modification.^{5b} As a result, the enzyme responsible for this modification, DHS is an ideal target for small molecule therapeutics in tackling conditions such as cancer and inflammation. Moreover, as previously mentioned, other related members of the pentacyclic guanidinium family exhibit a variety of pharmacological activities

including cytotoxicity, antiviral, antifungal, anti-HIV, and more recently inhibition of melanogenesis.^{2,6}

2.2.2 Previous Synthetic Approaches Toward the Pentacyclic Guanidinium Core

The intricacy and novelty observed in the structure of the pentacyclic guanidinium alkaloids, coupled with the wide range of biological activities exhibited by these molecules, have attracted significant attention from the scientific community. Although there has been no total synthesis of any members of the monanchocidin family published to date, significant synthetic contributions have been made in this area by a number of research groups. The structurally related metabolites of the batzelladine, crambescidin, and ptilomycalin families have all been accessed through total synthesis (Figure 2-1).² The majority of the efforts aimed at the total synthesis of this class of compounds have focused on the construction of the guanidine unit, with special attention placed on stereoselectivity. A selected number of these efforts specifically related to the synthesis of ptilomycalin A are described in the following sections, with particular emphasis placed on key reactions and the overall strategies utilized.

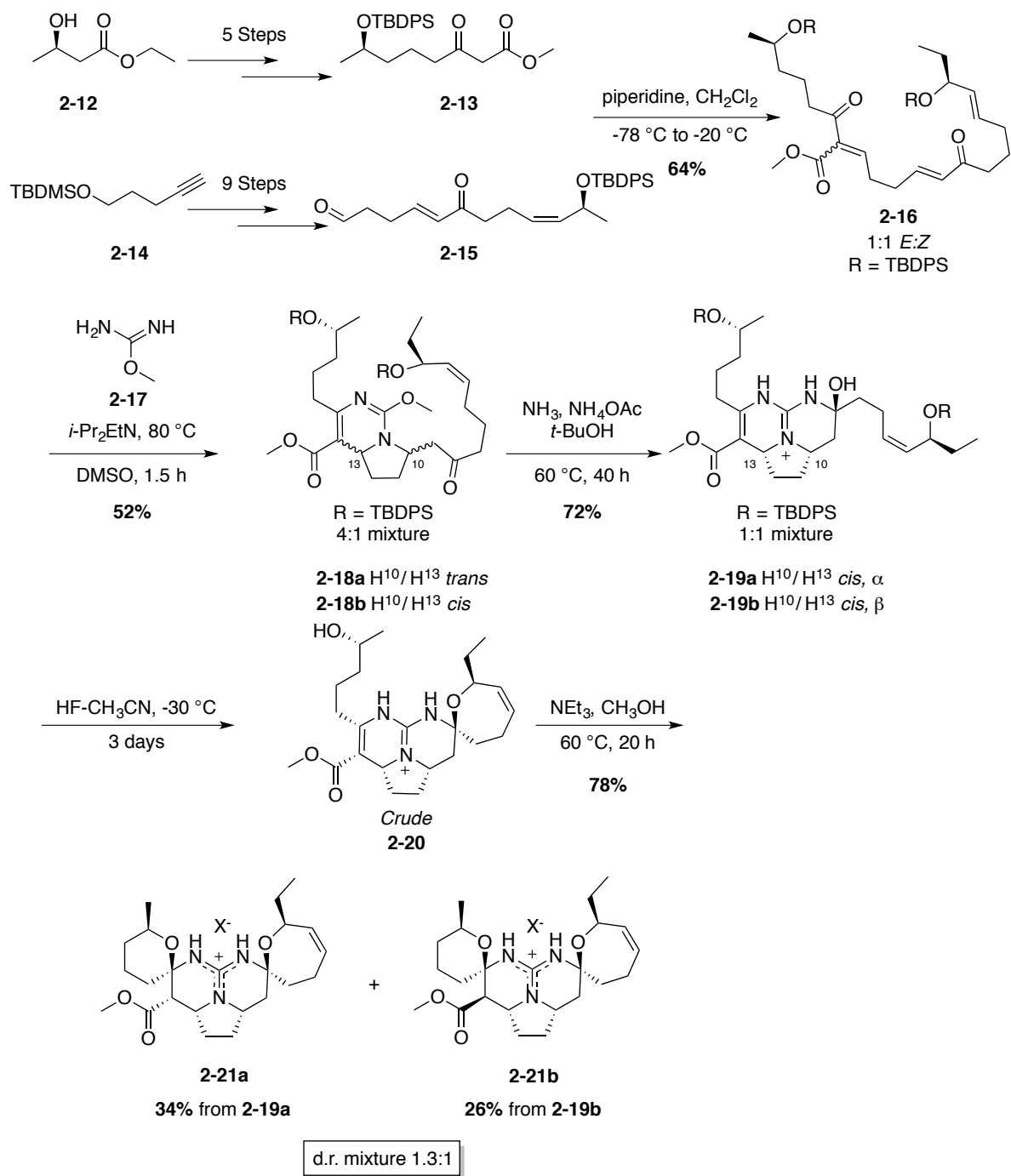
2.2.2.1 Snider and Shi's Biomimetic Approach to the Guanidinium Core

Snider and co-workers developed a synthetic route to the methyl ester of the guanidinium core of ptilomycalin A, based on a biomimetic approach. The use of a biomimetic approach was of particular interest to them based on the hypothesis that the biogenesis of the natural product follows a similar path.⁷ A conceptually similar strategy, also

utilizing a biomimetic approach was employed by Murphy and co-workers in the synthesis of ptilomycalin A and batzelladine alkaloids as well.⁸ The strategy utilized by the Snider group was based on the construction of bis-enone **2-16**, which with further protecting and functional group manipulations would allow for quick access to the pentacyclic guanidinium core (Scheme 2.1).

Compound **2-16** was in turn synthesized from the Knoevengal condensation of β -keto ester **2-13** and aldehyde **2-15**, which proved to be a problematic step, but nevertheless allowed for the preparation of **2-16** as a 1:1 mixture of stereoisomers. With this mixture in hand, efforts were directed towards the conjugate addition of *O*-methylisourea **2-17** to **2-16**, which generated a mixture of diastereomers **2-18a** and **2-18b**. Treatment of this mixture under ammonolysis conditions resulted in a 1:1 mixture of the two *cis* diastereomers **2-19a** and **2-19b**. Further deprotection of silyl ether protecting groups using a 3:7 mixture of 50% aqueous HF and acetonitrile resulted in a crude mixture containing intermediate **2-20**. Treatment of **2-20** with triethyl amine (NEt₃) and methanol generated the pentacyclic guanidinium **2-21a** and the corresponding diastereomer **2-21b**.

Overall, this synthesis was accomplished in 14 steps with a 2.7% yield starting from commercially available materials. The inability to control stereoselectivity lessens the synthetic utility of this sequence, but at the same time demonstrates the feasibility of a biomimetic approach in complex guanidinium natural product synthesis. As mentioned previously, this approach was also utilized for the construction of the tricyclic guanidinium core of another structurally related natural product batzelladine A.⁸



Scheme 2.1: Snider's biomimetic approach to the guanidinium pentacyclic core.

2.2.2.2 Overman's Enantioselective Approach to the Guanidinium Core

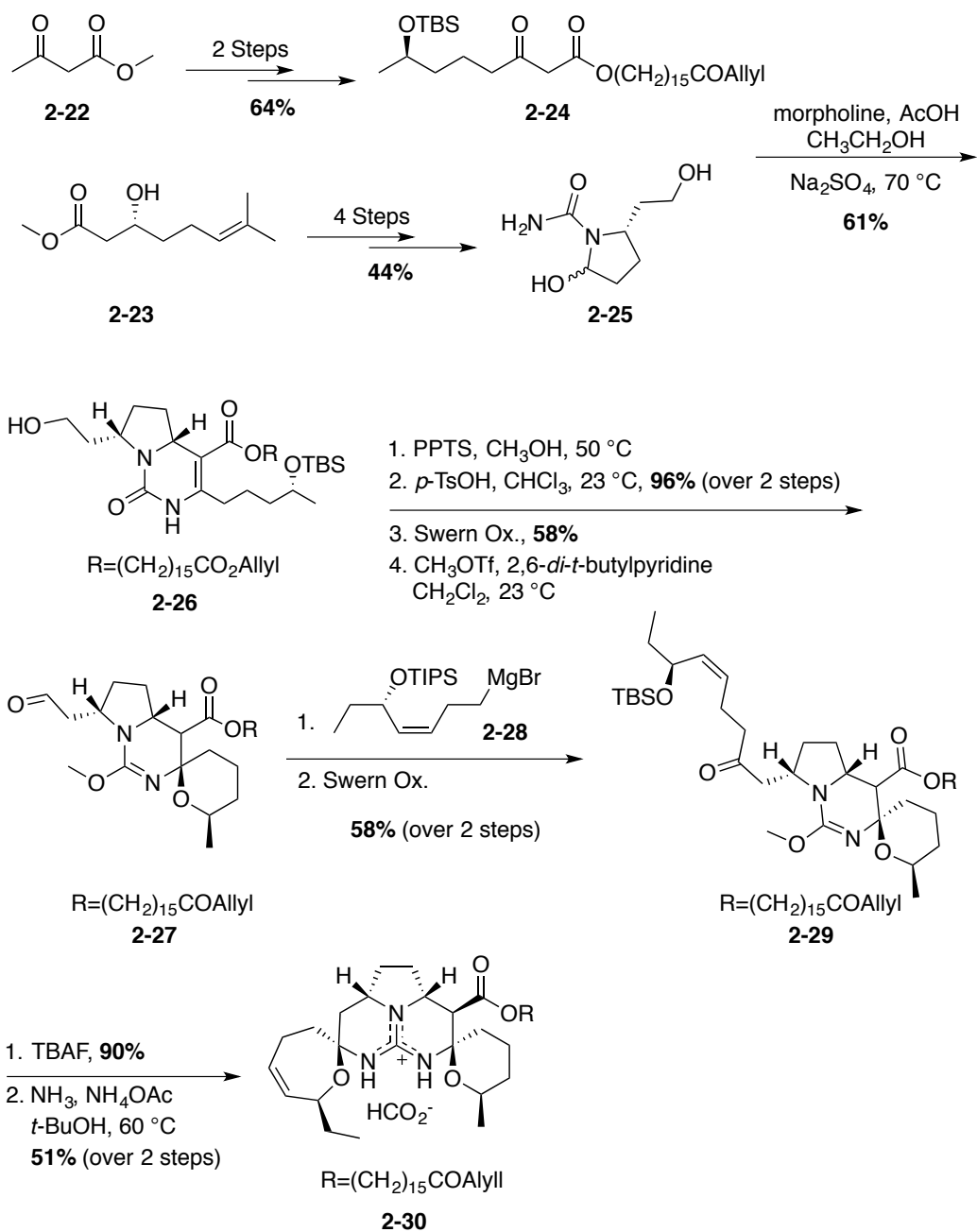
One of the first significant steps towards the asymmetric total synthesis of the guanidinium pentacyclic alkaloids was achieved through the work contributed by the Overman group, who has been one of the pioneers in this area of research. The first total synthesis of ptilomycalin A (**2-1**) was accomplished through the synthetic plan devised by the Overman group.⁹

Their strategy was based on the recognition that the *cis*-disubstituted pyrrolidine ring of ptilomycalin A (**2-1**) could be constructed through an intramolecular version of the Biginelli condensation, allowing for the stereoselective spirocyclization of the hydropyran ring (Scheme 2.2). This synthetic route not only allowed for the first enantioselective total synthesis of the natural product, but the sequence also controlled the stereochemical configuration of either *cis* or *trans* pyrrolidine via the proper choice of reaction conditions.

The synthesis of pyrimidone **2-26** was accomplished through the tethered Biginelli condensation of fragments **2-24** and **2-25** with good diastereoselectivity. β -keto ester **2-24** was prepared in two steps starting from the commercially available methyl acetoacetate in 64% overall yield. Compound **2-25**, which served as the urea and the masked aldehyde units of the Biginelli condensation was prepared in four steps from the enantiopure β -hydroxy ester **2-23** and used as a crude mixture due to difficulties in the purification of this compound.

Deprotection of the silyl ether group in pyrimidone **2-26** was followed by the conversion of the requisite intermediate to the spirocyclic compound **2-27** through several protecting group and functional group manipulations. Reaction of **2-27** with Grignard

reagent **2-28** (prepared in 5 steps), followed by the oxidation of the corresponding alcohol furnished compound **2-29**. Subsequent cleavage of the silyl ether protecting group and treatment with ammonia and ammonium acetate resulted in the formation of a single pentacyclic guanidinium core **2-30**, which was isolated as the formate salt in 7% yield in 13 linear steps. Overall, this synthesis was a landmark achievement, which allowed for the first enantioselective preparation of this core.



Scheme 2.2: Overman's enantioselective synthesis of the pentacyclic guanidinium core.

2.2.3 Project Aim and Overview

In the recent past, the field of natural product synthesis has evolved in such a way that it is no longer optimal to devise a synthetic route only to prepare a targeted compound. The concepts of atom/step economy, diverted total synthesis, and function-oriented synthesis have become important strategies in the modern synthetic world. Moreover, the emergence of fields such as chemical biology further emphasizes the importance of natural products as chemical probes and the need for a reasonable means of production of such compounds in sufficient quantities to advance biological studies.

The structural complexities and low natural abundance, along with the impressive biological activities exhibited by the polycyclic guanidinium alkaloids including the monanchocidin family, have made these compounds exciting targets for total synthesis. The challenges faced in all of the previous approaches to this class of natural products have highlighted the many difficulties associated with the synthesis of a stereochemically rich structure in an asymmetric fashion.

As is the nature of total synthesis, structurally diverse and complex molecules are often hampered with equally complex synthetic routes, accompanied by low yields. The hurdles associated with the previous syntheses of the pentacyclic guanidinium core necessitates the investigation into alternative approaches that would simplify and shorten the route to this core, and at the same time allow for structure modification to generate libraries of compounds. Additionally, little information regarding the true interaction of the pentacyclic guanidinium alkaloids with biological targets is known, mainly due to inability to access sufficient quantities of these molecules.

The underlying intent of this project is not only to engineer a sequence of steps to access the guanidinium pentacyclic skeleton, but is also to accomplish this task in a concise, asymmetric, and modular manner. To that end, we hope to devise a synthetic approach for the preparation of the guanidinium core of the monanchocidin family that would be amenable to the synthesis of other related complex and simpler natural products, in a scalable and step-economical fashion. Pursuant to this, studies concerning varying avenues of physiological and pharmacological activities and selectivities will shed light into the mechanism of action of these compounds and potentially reveal interesting chemical probes and biological pathways.

2.2.3.1 Overview of the Previous and Current Synthetic Plans

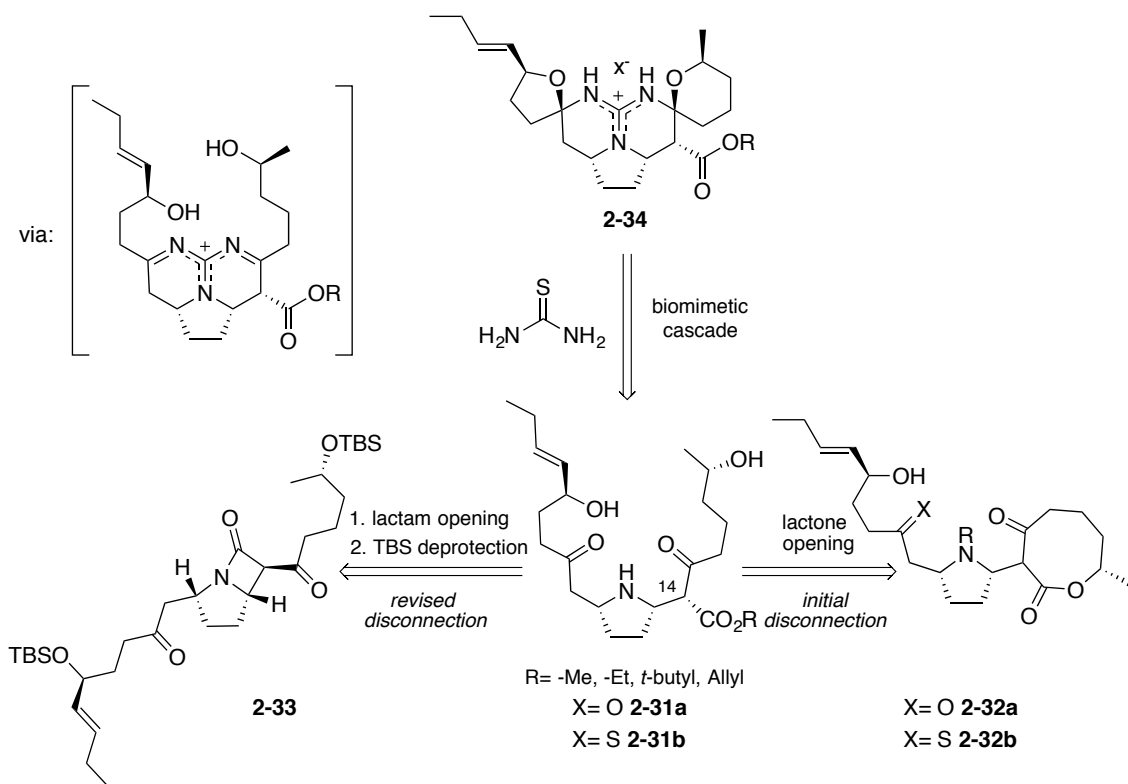
Examination of the pentacyclic guanidinium core structure of the monanchocidins, specifically monanchocidin A, reveals several synthetic challenges. One major hurdle is the preparation of the pentacyclic ring system having the correct stereochemistry at the seven stereogenic centers. Additionally, monanchocidin A possess a charged guanidine moiety and a combination of spirocyclic ring systems not observed in the previously reported guanidinium alkaloids. Notably, the presence of the ester linkage at C14 (Scheme 2.3) poses a concern regarding the stereochemistry and the issue of epimerization at this site.

Following the complete synthesis of the pentacyclic guanidinium core, further elaboration of the exposed carboxylic acid at C14 via coupling to the Eastern fragment of monanchocidin A furnishes the fully constituted natural product. As was previously

investigated by the Overman group, an allylic ester functionality appears to be the most easily cleaved functional group in the presence of the fully assembled guanidinium core.¹⁰

An overview of two distinct synthetic strategies for the synthesis of the monanchocidin A guanidinium core is presented in **Scheme 2.3**. Both synthetic approaches were designed to avoid the extensive use of protecting groups as well as enabling modifications around the pentacyclic skeleton. Both approaches consisted of the stereoselective preparation of linear precursor **2-31**, which is set to undergo a biomimetic cascade cyclization transformation (similar in concept to the approach employed by the Snider group) to install the pentacyclic guanidinium motif. The construction of several rings and stereocenters in a single operation provides a potentially expedient route, while taking advantage of the existing stereocenters to relay the stereochemistry throughout the synthesis.

Initial focus of the project was to investigate the stereoselective nucleophilic addition of an eight-membered lactone to an imine, followed by an amino metallation reaction to generate pyrrolidine **2-32**. After considerable experimentation, this strategy proved to be unsuccessful in providing the desired target intermediates, and a revision was made to enable further investigation.



Scheme 2.3: An overview of the proposed approaches to the monanchocidin A core.

Our attention was then focused on an alternative strategy which relied on the construction of β -lactam **2-33**. From a synthetic perspective, β -lactam **2-33** serves as a key intermediate in the synthesis as the thermodynamic preference for *trans*-substituents allows for the installation of the sensitive β -keto ester functional group, while preventing racemization of the acidic α -proton at that site through several synthetic steps. The rationale behind employing a β -lactam building block in our revised and current strategy was driven from the difficulties faced in the previous synthetic efforts in installing and controlling the stereochemistry at the highly epimerizable ester-bearing carbon. To enable access to

precursor **2-33**, an appropriately functionalized β -lactam was required and the details of the efforts towards the preparation of that precursor are discussed in later sections.

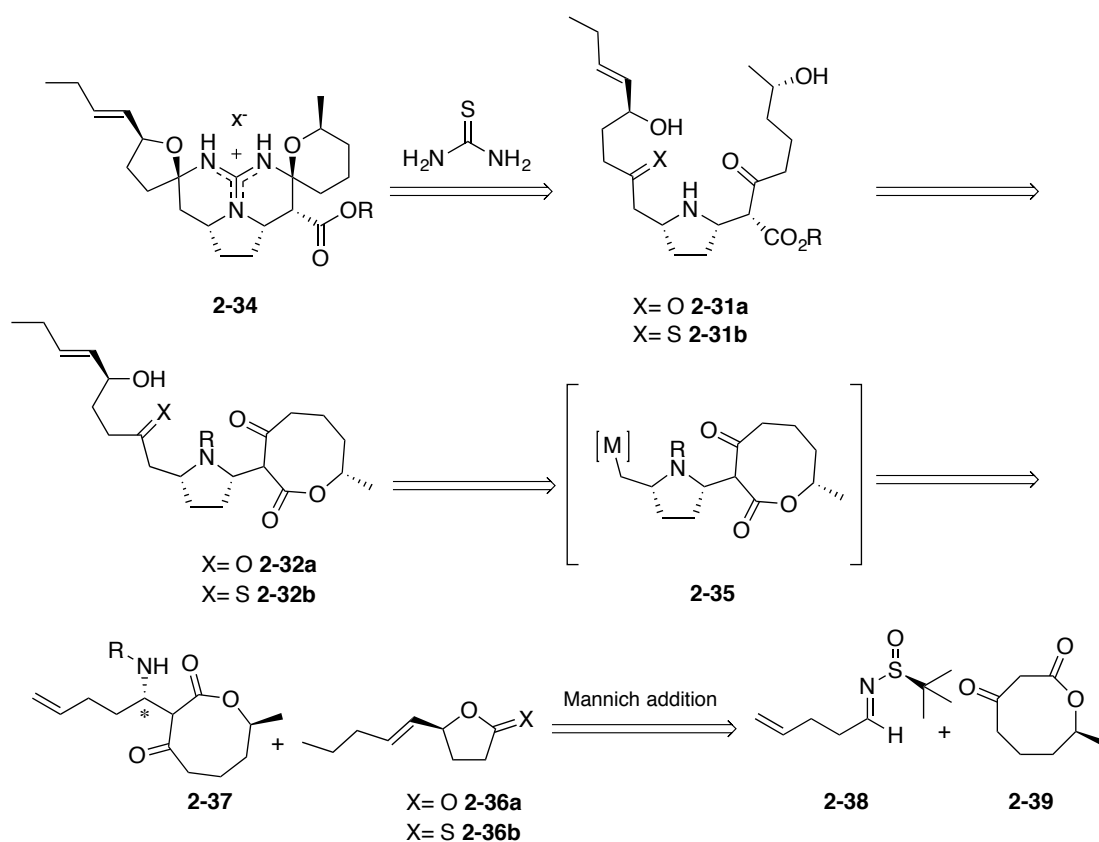
2.3 Results and Discussions

2.3.1 Initial Synthetic Plan

In accordance with the overall aim of the project, which was to develop a concise and practical approach to the guanidinium core, particular attention was placed on the stereochemical control and synthetic ease of the strategy. Initially, our efforts were directed toward the synthesis of pyrrolidine **2-32** as a means of installing the substituted appendage of the biomimetic precursor **2-31** (Scheme 2.4). It was believed that this strategy would offer an advantage by providing the appropriately substituted unit without the use of a protecting group. Further disconnection suggested that pyrrolidine **2-32** could be accessed through the Mannich addition of the enolate generated from β -keto lactone **2-39** to imine **2-38**, followed by a amino-metallation/coupling cascade, utilizing an unprecedented thiolactone **2-36** (or a lactone) coupling partner. Although the Mannich addition of ester enolates to imine functionalities is a well-established method, very few examples of 1,3-dicarbonyls specifically β -keto ester nucleophilic additions have been reported.¹¹ To the best of our knowledge, no examples of the addition of an eight-membered ring β -keto lactone enolate have been reported to date.

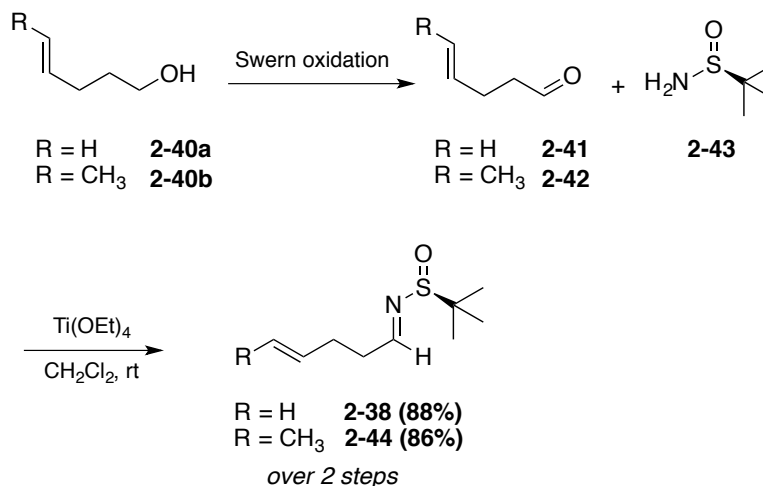
Recently, the *t*-butanesulfinyl imine functionality has emerged as a versatile chiral building block, specifically in the preparation of enantiopure amine derivatives. Incorporation of this building block in the proposed synthetic scheme was an appealing

option for a number of reasons. The enantiopure *t*-butanesulfinyl imine is readily prepared via the condensation reaction of the corresponding aliphatic or aromatic aldehyde and the commercially available chiral *t*-butanesulfinamide. The addition of organometallic carbon nucleophiles to the activated C=N bond of the *t*-butanesulfinyl imine in a Mannich addition is a very facile and practical reaction, providing high degrees of diastereoselectivity. Such reactions are proposed to proceed through a six-membered ring chair transition state.¹² To this end, the Mannich addition of the enolate of β -keto lactone **2-39** to imine **2-38** became the proposed approach in the installation of the requisite stereocenter (C*) in the targeted compound **2-37** (Scheme 2.4).



Scheme 2.4: Initial retrosynthetic analysis of pentacyclic guanidinium core.

The synthesis of *t*-butanesulfinyl imine was achieved via a two-step process, starting with the Swern oxidation of the commercially available 4-penten-1-ol (or 4-hexen-1-ol) (**2-40**) to obtain the requisite aldehyde **2-41** (or **2-42**). Condensation of the corresponding aldehyde and (*S*)-*t*-butanesulfinamide (**2-43**) afforded the desired product **2-38** (or **2-44**) in excellent yields (Scheme 2.5).



Scheme 2.5: Synthesis of *t*-butanesulfinyl imine **2-38** (or **2-44**).

The eight-membered lactone **2-39** was strategically designed to serve as the masked form of the linear precursor **2-45** (Figure 2.3) and thereby eliminate the need for protecting groups. To this end, ring-closing metathesis (RCM) became the key reaction and the synthesis began with efforts directed at the preparation of the requisite precursors leading to this reaction.

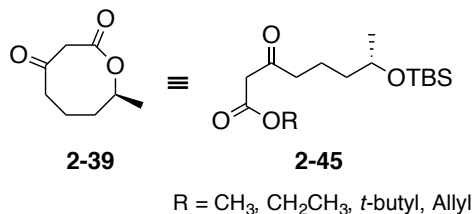
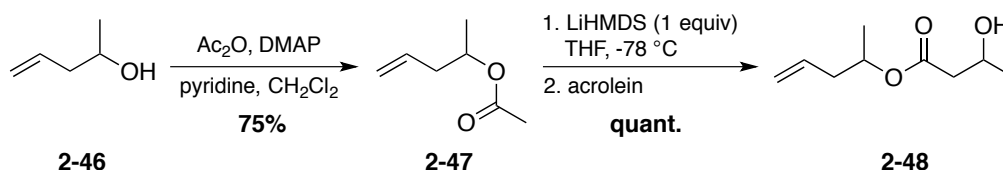


Figure 2.3: Linear equivalent of the lactone **2-39**.

To this end, following examination of various approaches, the RCM precursor **2-48** was prepared in the racemic form for the purpose of exploratory work (Scheme 2.6). With this substrate in hand, our attention was focused on investigation into the cyclization step.

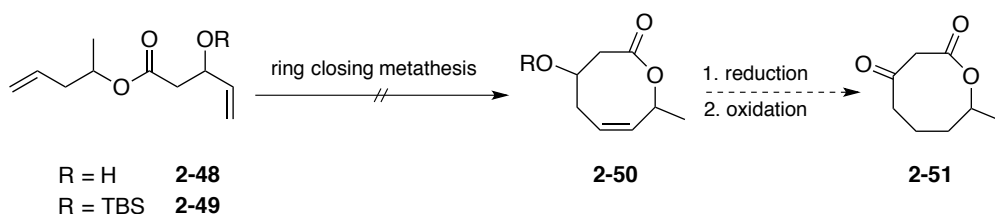


Scheme 2.6: Preparation of the RCM precursor **2-48**.

Various metathesis catalysts such as Grubbs' 1st generation, 2nd generation and Hoveyda-Grubbs' 2nd generation catalyst in the presence and absence of additives under high dilution conditions were utilized. Unfortunately, all attempts at this transformation resulted in the recovery of the intact starting material (Table 2.1). With the hope of promoting the RCM reactions, stoichiometric amounts of titanium (IV) isopropoxide were incorporated into the reaction conditions; however, no success was achieved. The Lewis acidic titanium additive was presumed to chelate the polar electron rich group such as the free hydroxyl functionality and prevent the potential coordination of such groups to the ruthenium center.

Subsequent attempts to modify the structure of the substrate by protecting the hydroxyl moiety as a silyl ether also proved to not be a successful metathesis substrate. A selected number of trials of the RCM reactions are shown in **Table 2.1**. In a few cases, trace amounts of intermolecular dimerization product were observed as well.

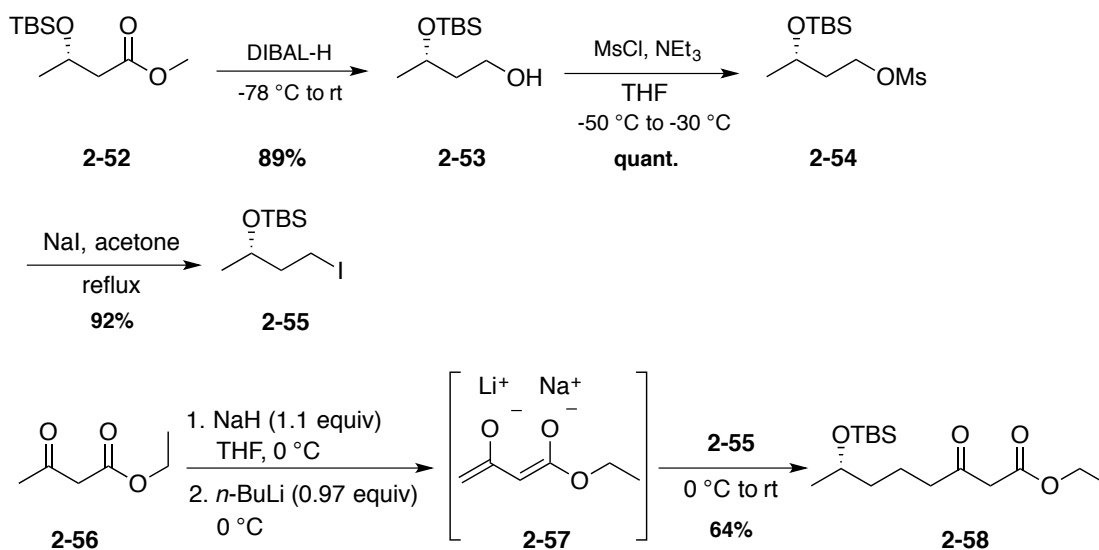
Table 2.1: Attempts at the RCM reaction.



Entry	Substrate	Catalyst	Concentration	Additives	Solvent	Temperature	Time	Result
1	2-48	Grubbs' I (15 mol%)	0.002 M	n/a	toluene	reflux	12 h	recovered SM
2	2-49	Grubbs' I (15-20 mol%)	0.005 M	n/a	CH ₂ Cl ₂	reflux	12 h	SM and trace amt. of dimer
3	2-48	Grubbs' II (20 mol%)	0.001 M	Ti(O <i>i</i> Pr) ₄ (10 mol%)	CH ₂ Cl ₂	reflux	24 h	recovered SM
4	2-48	Grubbs' II (20 mol%)	0.001 M	Ti(O <i>i</i> Pr) ₄ (1.0 equiv)	CH ₂ Cl ₂	reflux	24 h	recovered SM
5	2-49	Hoveyda-Grubbs II (15 mol%)	0.001 M	n/a	toluene	reflux	24 h	recovered SM
6	2-49	Grubbs' II (20 mol%)	0.001 M	Ti(O <i>i</i> Pr) ₄ (10 mol%)	CH ₂ Cl ₂	reflux	24 h	SM and trace amt. of dimer

The lack of reactivity in attempted RCM reactions may be attributed to a number of factors. One of the very challenging tasks in chemical synthesis is the construction of medium sized rings, specifically eight-membered rings due to the high degree of transannular strain and enthalpic factors associated with this ring size.¹³ Moreover, entropic factors related

to the probability of the end-to-end encounter are usually outweighed by the enthalpic factors associated with the strain of the eight-membered lactones. Additionally, the conformational predisposition taken by the starting material is also of significant importance. The presence of polar substituents such as the oxygen group of the hydroxyl and the ester moiety, and the proximity of these substituents to the reacting alkene centers may also provide an explanation as to the lack of reactivity.¹⁴ Due to the difficulties encountered in the synthesis of the eight-membered ring, a sequence adapted from previously reported work by Overman et al. was used in the preparation of the linear Mannich addition precursor **2-58** (Scheme 2.7).¹⁰

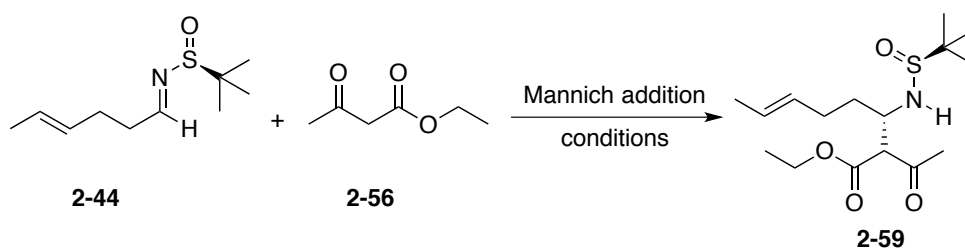


Scheme 2.7: Synthetic approach for the preparation of the linear precursor **2-58**.

In parallel to these studies, a model system employing the commercially available ethyl acetoacetate and *t*-butanesulfinyl imine **2-44** was explored in order to assess the feasibility and stereochemical outcome of the Mannich-enolate reaction. A number of

conditions were surveyed and the optimum reaction conditions were found to employ NaI or NaHCO₃ as the base under solvent-free conditions (Table 2.2).¹⁵ Examination of the ¹H NMR spectra of these reactions revealed that the nucleophilic additions were essentially non-selective. This was suggestive of a post-addition epimerization as there are accounts of highly diastereoselective addition of ester enolates to imines in the literature.¹⁶ This outcome was due to the basic nature of the Mannich reaction conditions, albeit very mild in this case, coupled with the very acidic nature of the α -proton of the β -keto ester.

Table 2.2: Model system employed in the Mannich addition reaction.



Entry	Base	Solvent	Temperature	Time	Result
1	NEt ₃	CH ₃ CN	0 °C to rt	12 h	1:1 mixture of diastereomers
2	NaH	THF	0 °C to rt	12 h	1:1 mixture of diastereomers
3	NaI	neat	rt	48 h	1:1 mixture of diastereomers
4	NaHCO ₃	neat	rt	48 h	1:1 mixture of diastereomers

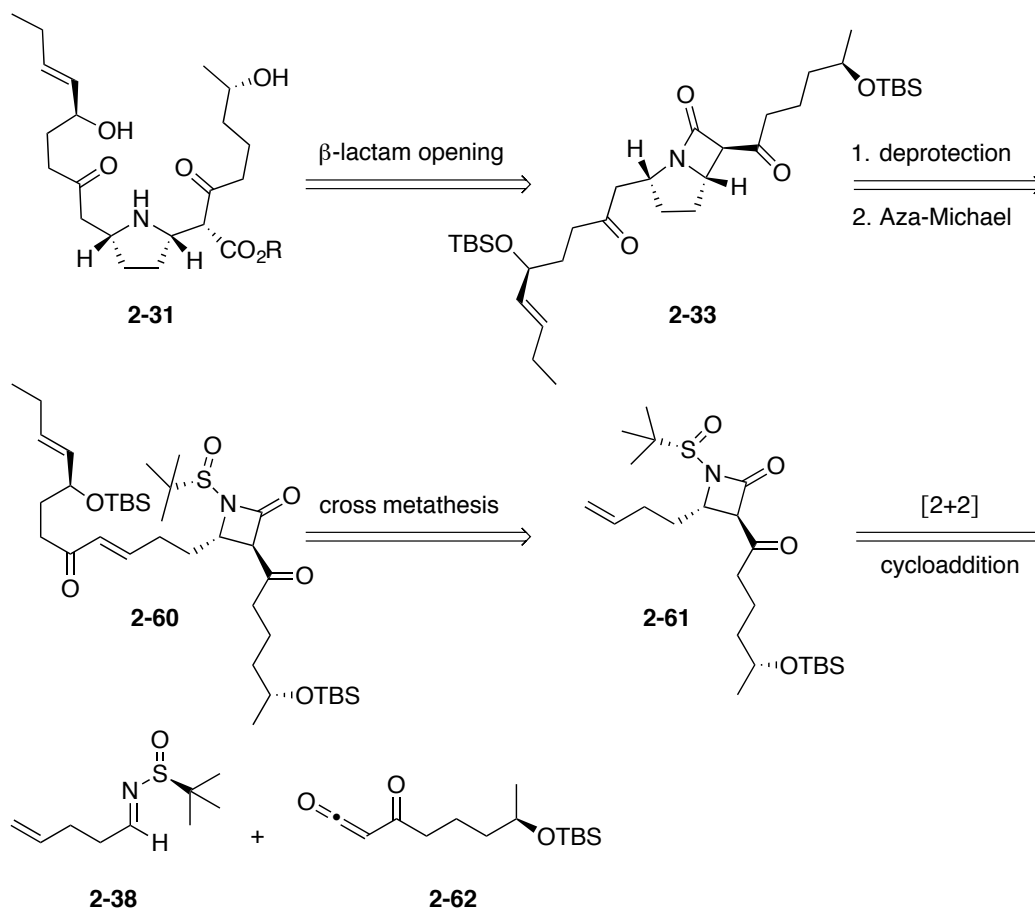
• Diastereoselectivity was determined based on the ¹H NMR analysis

At the onset of the project, the novelty of the application of an eight-membered ring lactone as a means of protecting group-free strategy and the presumed synthetic ease of this approach appeared to be a very enticing option; however, during the course of our

investigation, we have come to appreciate the challenges associated with the synthesis of the required eight-membered ring. The lack of reactivity in the RCM reactions, along with the lack of stereoselectivity of the Mannich addition reaction in the linear model system further emphasized the need for a modification in our synthetic strategy.

2.3.2 Revised Synthetic Plan: Initial Studies into β -lactam Preparation

An alternative approach to the targeted intermediate **2-31** was devised based on the strategic manipulation of β -lactam **2-33** to provide a more facile synthetic sequence (Scheme 2.8). β -Lactam **2-33** serves as a key intermediate in the synthesis as the preference for *trans*-substituents allows for the installation of the sensitive β -keto ester functional group and the possibility to carry it through several synthetic steps. The inability to control the stereochemistry at the ester-bearing site has plagued even the most efficient synthetic approaches to date.^{2,6} Furthermore, the electrophilicity of the bicyclic β -lactam intermediate **2-33** provides an internal activation for coupling to the necessary alcohol side chain to assemble the natural products since epimerization and/or decarboxylation of the corresponding ester/carboxylic acid is problematic. Additionally, the ring opening of intermediate **2-33** is anticipated to serve as a stereo-controlled means of installing the *cis*-pyrrolidine functionality while providing a reactive intermediate for side chain introduction. To this end, the [2+2] cycloaddition reaction of imine **2-38** and acylketene **2-62** became the initial subject of investigation.

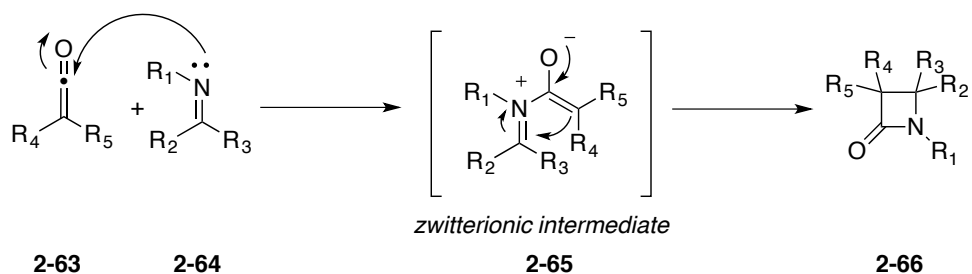


Scheme 2.8: The revised retrosynthetic analysis of the pentacyclic guanidinium core.

2.3.2.1 A Brief Overview of the β -Lactam Scaffold

The ubiquity of the β -lactam scaffold in a number of important antibiotics and other biologically relevant pharmaceuticals has made this chemical architecture the subject of interest in the field of organic synthesis. Testament to this is the number of synthetic methods that exist in the literature to prepare β -lactams.¹⁸ Among these various methods, the Staudinger [2+2] cycloaddition is one of the most utilized and practical routes to β -lactams. The Staudinger cycloaddition developed by Hermann Staudinger in 1907, involves the

nucleophilic addition of an imine **2-64** to a ketene **2-63**, which generates the zwitterion intermediate **2-65** (Scheme 2.9). Subsequent cyclization of the zwitterion intermediate furnishes the desired β -lactam. The imine functionality is readily accessible via the direct condensation of the appropriate aldehyde and amine. The ketene intermediate is typically generated *in situ* via the dehydrohalogenation of the requisite acid chloride using a tertiary amine base or the activation of the corresponding carboxylic acid by activating reagents. The mechanism of the Staudinger cycloaddition has been the subject of much debate; however, evidence from computational and experimental studies substantiates a step-wise mechanism rather than a concerted mechanism.¹⁸



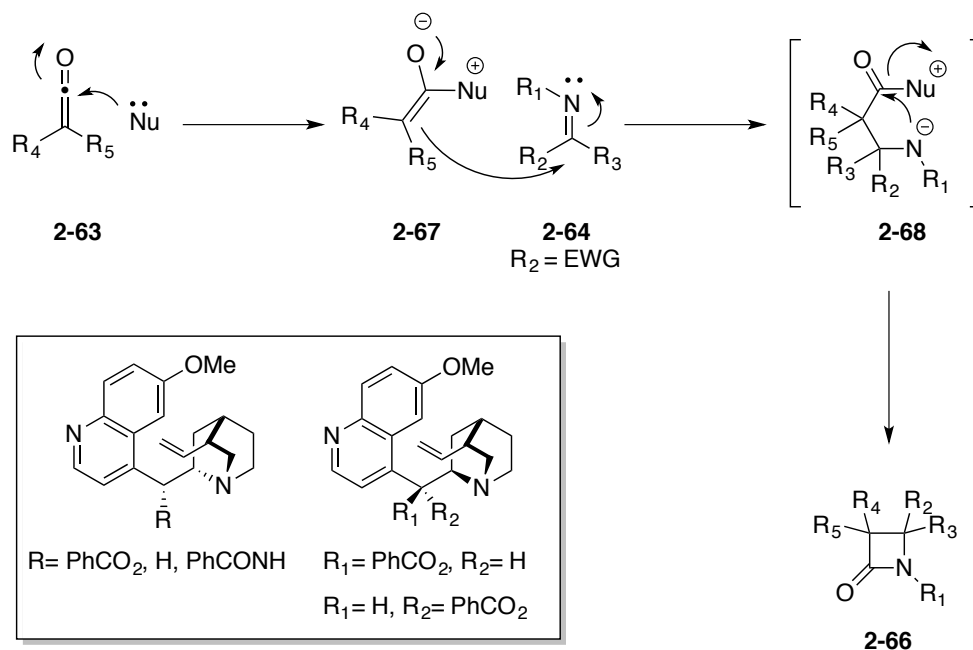
Scheme 2.9: Staudinger [2+2] cycloaddition of imines and ketenes.

Generally, the zwitterionic intermediate is regarded as a 4 π -electron system, and as such it is thought to undergo conrotatory electrocyclic ring-closure. However, the origin of diastereoselectivity in the reaction of monosubstituted ketenes and aldimines usually provides a rather unpredictable *cis/trans* ratio of products. A tremendous amount of effort has been dedicated to the understanding of the factors influencing the stereochemical outcome of the Staudinger cycloaddition. In summary, the rate of the ring closure and the

competing imine isomerization in the zwitterionic intermediate tend to have profound effects on the outcome. To this end, the reaction of electron-rich ketenes with electron-poor substituted imines typically results in the formation of *cis*- β -lactams. On the contrary, reaction of ketenes bearing electron-withdrawing groups with electron-rich imines results in the formation of *trans*- β -lactams. Another pivotal factor in the stereochemical outcome of the Staudinger reaction is the temperature parameter, which is often utilized in tuning the diastereoselectivity of such reactions; however, solvent, additives, and ketene preparation methods have very little influence on the stereochemical outcome of this reaction.¹⁸

Other synthetic methods developed and implemented in the preparation of β -lactams include the Kinugasa reaction¹⁹ and various metal mediated ester-enolate addition reactions, collectively referred to as Gilman-Speeter reactions.²⁰ Efforts by a number of research groups have contributed to the extension of the aforementioned methods, including the Staudinger cycloaddition, to their asymmetric variants. The efforts made by Lectka and co-workers represent one of the most noteworthy asymmetric modifications of the Staudinger cycloaddition reaction.²¹ One of the features of the Staudinger cycloaddition is the lack of requirement for a catalyst, even at low temperature, due to the very low energy of activation associated with the nature of this reaction. However, in order to render this reaction asymmetric, the classical Staudinger cycloaddition pathway was bypassed by the addition of a nucleophilic catalyst in the work executed by the Leckta group. This modification resulted in the inversion of polarity of the reacting substrates. As such, ketene **2-63** was converted to enolate **2-67** via the addition of a tertiary amine as a nucleophilic catalyst. Subsequent addition of enolate **2-67** to an electrophilic imine **2-64**, generated the targeted β -lactam **2-66**

(Scheme 2.10). The chiral nucleophiles investigated by the Lectka group included the cinchona alkaloid derivatives, which provided a high degree of diastereoselectivity and enantioselectivity (Scheme 2.10).²² It should be pointed out that, the mechanistic pathway followed by this reaction is the result of the inversion of the polarity in the reacting substrates and it is distinct from that of the Staudinger cycloaddition. As a result, this reaction is not to be referred to as a Staudinger cycloaddition.

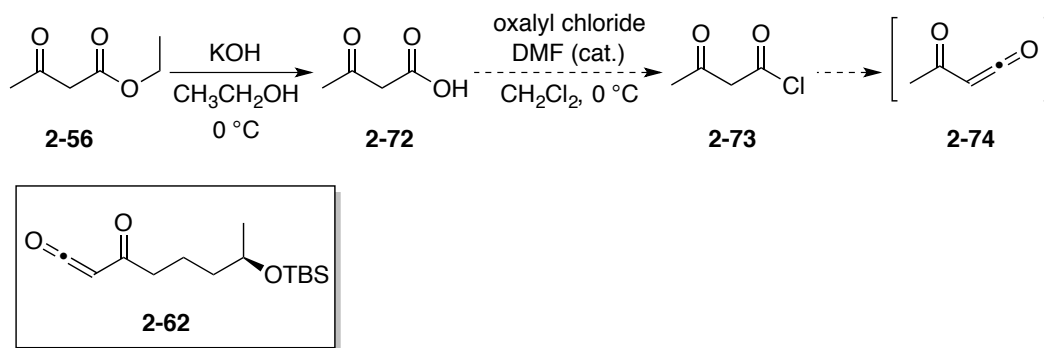


Scheme 2.10: Lectka's modification of the Staudinger [2+2] cycloaddition.

2.3.3.2 Cycloaddition Efforts to Disubstituted *trans* β -lactams

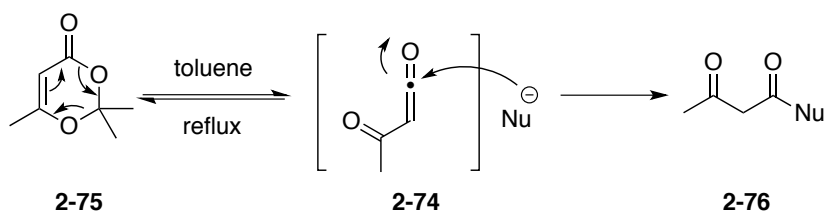
In light of the general disconnection of β -lactams by [2+2] cycloaddition, our early investigations into the preparation of acylketene **2-62** (Scheme 2.8) rested on a model system utilizing the simple acylketene **2-74**. Initial efforts to generate this intermediate relied on the

preparation of **2-73** from saponification and the subsequent chlorination of the corresponding β -keto carboxylic acid **2-72**. Various attempts at this transformation utilizing the commercially available ethyl acetoacetate **2-56** proved to be challenging, presumably due to the corresponding decarboxylation reaction (Scheme 2.11).



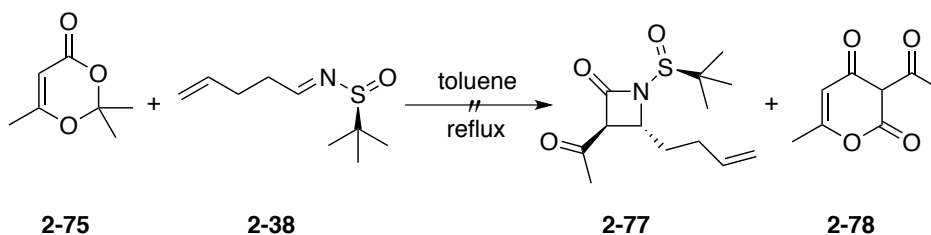
Scheme 2.11: Model studies on the preparation of acylketene **2-62**.

In addition to dehydrohalogenation of acid chlorides, ketenes can also be prepared through routes such as thermolytic and photolytic reactions.²³ As literature precedent suggests, the commercially available and inexpensive 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one, “dioxinone” (**2-75**) undergoes thermal decomposition in a pseudo retro Diels-Alder reaction at temperatures above 100 °C to generate the acylketene intermediate **2-74**, which is typically intercepted by a nucleophile (Scheme 2.12). Functionalization of dioxinone **2-75** at the vinylic methyl position is also possible through the use of a strong base such as lithium diisopropyl amide (LDA), followed by the subsequent alkylation or the vinylogous aldol reaction.²⁴



Scheme 2.12: Dioxinone **2-75** as an acylketene precursor.

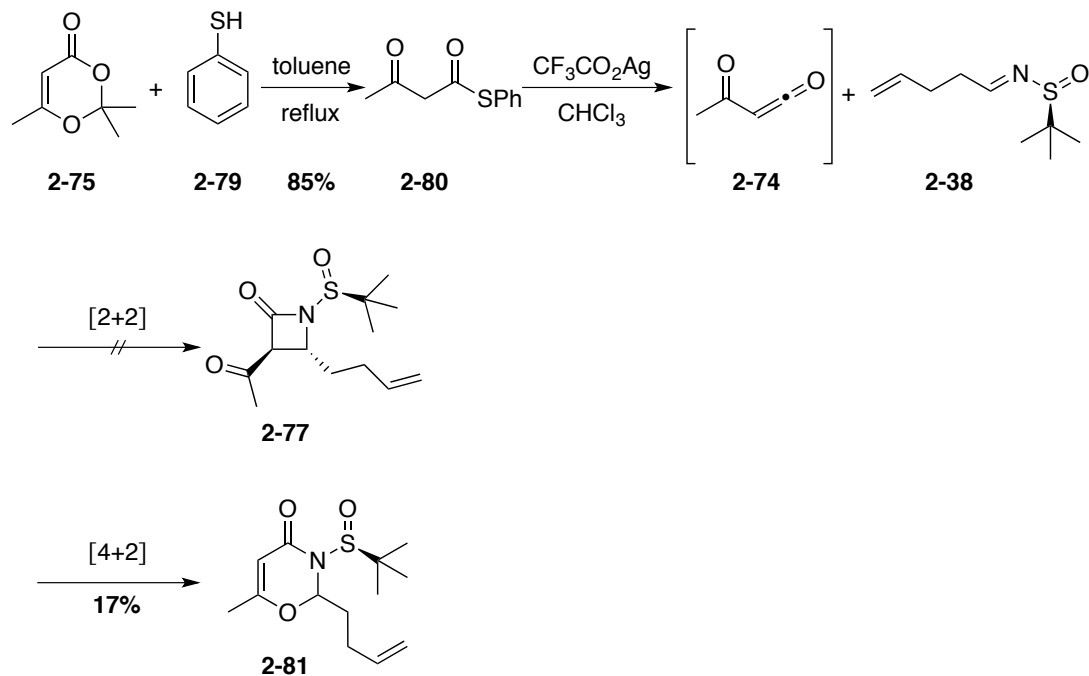
In light of its synthetic utility and applicability, dioxinone **2-75** as an acylketene source was explored in the corresponding [2+2] cycloaddition reaction. Unfortunately, the reaction of **2-75** with **2-38** in toluene or xylene at reflux resulted in no β -lactam formation (Scheme 2.13). Analysis of the crude ¹H NMR of the reaction mixture was indicative of compound **2-78**, which is the product of acylketene dimerization. It should be noted that various unidentified peaks in the crude ¹H NMR were also observed, possibly products of imine decomposition.



Scheme 2.13: Model studies of dioxinone **2-75** in the [2+2] cycloaddition.

In a work published by Hoyer and co-workers, the intermediacy of acylketenes, generated via silver activation of phenyl thioacetoacetate (**2-80**) at room temperature was reported.²⁵ The possibility of using a thiophilic metal to generate an acyl ketene at room

temperature, followed by the subsequent [2+2] cycloaddition with an imine to furnish the targeted β -lactam **2-77** was a potential alternative to the high reaction temperatures explored previously. To this end, the reactivity of phenyl thioacetate (**2-80**) with imine **2-38** in the presence of silver trifluoroacetate in chloroform at room temperature was explored (Scheme 2.14).



Scheme 2.14: [4+2] Cycloaddition reaction: 1,3-oxazinone formation.

The product of that reaction following purification and isolation was determined to be 1,3-oxazinone **2-81**, which arises from [4+2] cycloaddition reaction. From a mechanistic standpoint, acylketenes behave as 4π components in [4+2] cycloaddition reactions and are rarely observed to partake in [2+2] cycloadditions.²⁶ In light of their reactivity mode, they have been considered as suitable intermediates in inverse electron hetero-Diels Alder

reactions with carbonyl compounds (aldehydes and ketones), aldimines, cyanates, and isocyanates.²⁶ Nevertheless, 1,3-oxazinones are an important class of biologically relevant heterocycles. The scope of this method was expanded to a wide range of electronically diverse heterocycles possessing various substitution patterns. The details of this published work are discussed in chapter 3.²⁷

After devoting a considerable amount of effort into the synthesis of *trans*- β -lactams using cycloaddition approaches, it became evident that our designed route clearly suffered from issues regarding substitution at C3 position with electron withdrawing groups. Despite the extensive list of methods for the preparation of β -lactams, very little precedent exists for the synthesis of acyl bearing β -lactams at the C3 position.²⁸ In fact, a survey of literature reveals that the acyl group is most commonly masked and unveiled following the cyclization step.²⁹ As a result, our focus was shifted on delineating a strategy to include a less functionalized system earlier in the synthesis that would allow for further functionalization at the C3 position following the β -lactam ring formation.

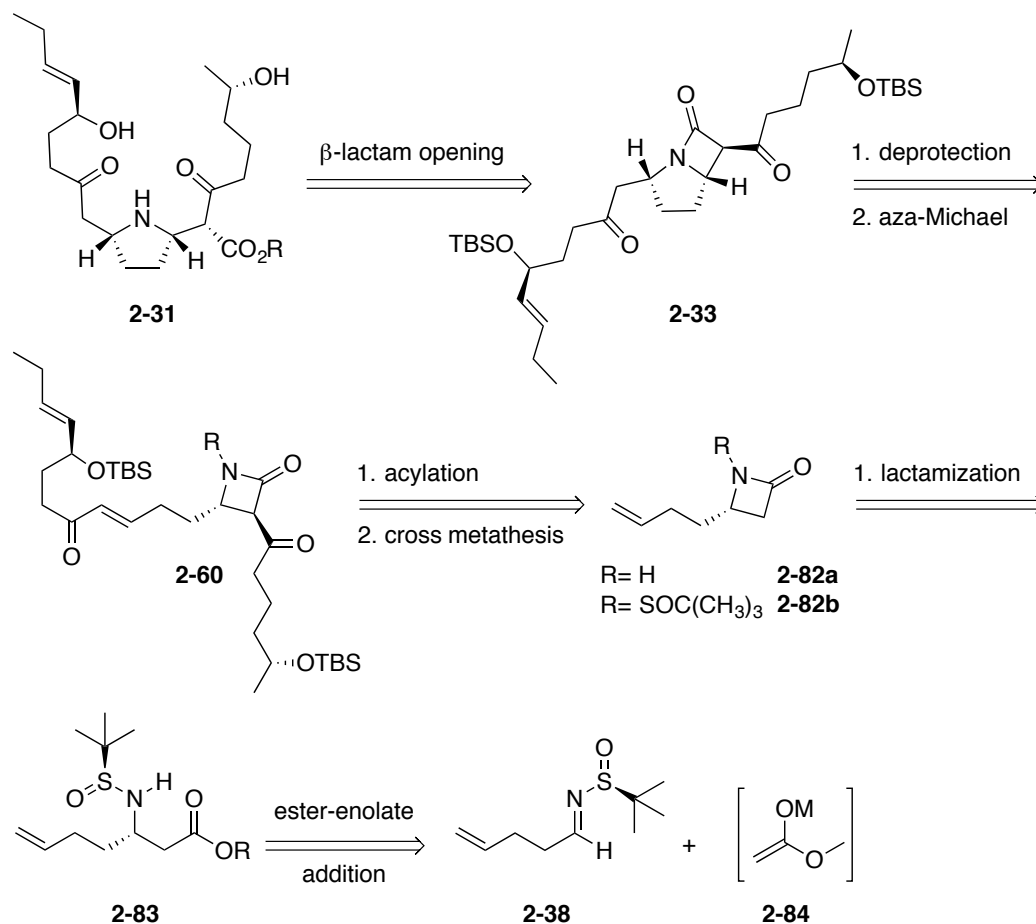
2.3.3 Generation II Revised Synthetic Plan

2.3.3.1 Cyclization Efforts to β -lactam Synthesis

To explore the potential of β -lactams, we began to evaluate methods to prepare monosubstituted β -lactams **2-82** (Scheme 2.15). This simplified β -lactam, devoid of the C3 substitution, was thought to exhibit a good balance of stability and reactivity, a combination that would allow for the introduction of the requisite acyl side chain through deprotonation with a strong base such as LDA, followed by treatment with an appropriate electrophile to

introduce the required acyl side chain.³⁰ Moreover, this method can be applied for further functionalization of unsubstituted *N*-H β -lactam via dianionic intermediate. Such reactions are known to produce *trans*- β -lactams as the major product, whereas most other utilized methods would result in the *cis* configuration. Further functionalization of the fully constituted *trans*- β -lactam was envisaged through a cross-metathesis reaction, followed by an intramolecular aza-Michael cyclization to afford the bicyclic β -lactam **2-33** (Scheme 2.15). Our initial efforts towards the preparation of β -lactam **2-82** began with studies into cyclization of β -amino ester **2-83**.

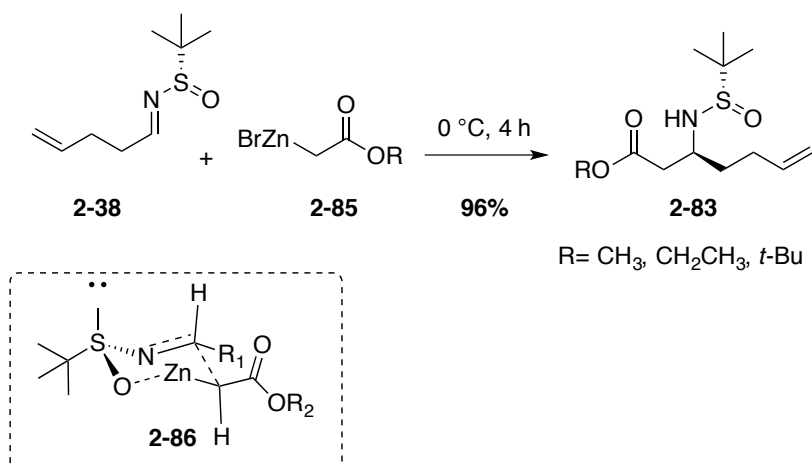
An obvious disconnection of **2-83** led to the imine **2-38** and the ester enolate intermediate **2-84** as reacting partners. First, the presence of the *t*-butanesulfinyl moiety would provide the desired stereoselectivity by directing the approach of the enolate **2-84**, thereby providing an asymmetric route for the preparation of **2-83**. Additionally, the preparation of imine **2-38** was previously achieved in a short and high yielding synthetic sequence utilizing inexpensive starting materials (Scheme 2.5). Finally, *t*-butanesulfinyl groups are removed with relative ease under acidic conditions.



Scheme 2.15: An alternative approach to the proposed retrosynthetic analysis.

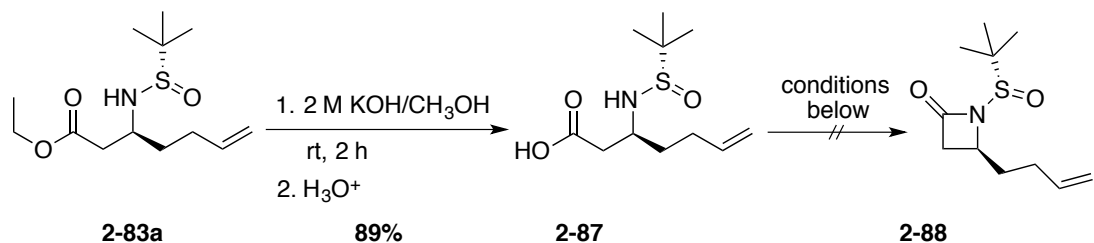
Following the protocol developed by Poon et al., the Reformatsky reagent prepared from commercially available alkyl bromoacetate (R= -CH₃, -CH₂CH₃, *t*-Bu) and activated zinc was employed in the addition to imine **2-38** to furnish the desired product **2-83** with excellent diastereoselectivity (> 99:1) and excellent yield on a multi-gram scale without the need for purification.³¹ The high degree of diastereoselectivity in this reaction can be rationalized through the closed six-membered transition state **2-86** shown in **Scheme 2.16**. Having successfully developed a rapid and efficient synthesis with complete stereochemical

control, our attention was directed to a number of approaches for cyclization to generate β -lactams.



Scheme 2.16: Preparation of β -amino ester **2-83**.

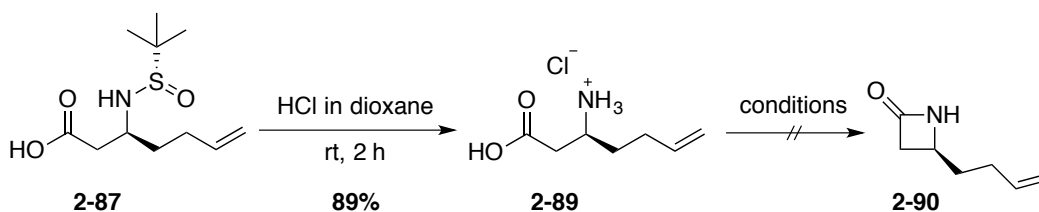
To this end, β -amino ester **2-90** was converted to the corresponding β -amino carboxylic acid **2-93** using a 2M methanolic solution of KOH in excellent yield. Acid **2-93** was in turn subjected to a number of coupling conditions and their variants (Table 2.3). Disappointingly, all attempts resulted in recovery of unreacted starting material and no desired product formation.

Table 2.3: Attempted cyclization conditions on β -amino carboxylic acid **2-87**.

Entry	Reagent(s)	Solvent	Temperature	Time	Results
1	Oxalyl chloride (1.1 equiv) DBMP (1.1 equiv)	THF	0 °C to rt	12 h	SM only
2	DCC (1.8 equiv)	THF	rt	24 h	SM only
3	MsCl (1.1 equiv) NaHCO ₃ (5 equiv)	CH ₃ CN	reflux	12 h	SM only
4	Mukaiyama's reagent (1.5 equiv) NEt ₃ (1.5 equiv)	CH ₃ CN	reflux	2 h	SM only
6	Mukaiyama's reagent (1.5 equiv) NEt ₃ (1.5 equiv)	CH ₂ Cl ₂	reflux	2 h	SM only

- DBMP = 2,6-di-*t*-butyl-4-methylpyridine
- DBMP = *N,N*-dicyclohexylcarbodiimide

In light of the complete lack of reactivity at the attempted cyclization conditions above, the *t*-butanesulfinyl group of **2-83** was removed to expose the ammonium hydrochloride salt in compound **2-89**. Following this a few cyclodehydration reactions were attempted, and all proved to be ineffective, returning the intact starting material (Table 2.4). The underlying reasons for this lack of reactivity are not clear, but it could be explained through the lack of substitution at the α -positions, which would increase the likelihood of cyclization since the reacting termini would be in close proximity through the Thorpe-Ingold effect.

Table 2.4: Attempted cyclization conditions on β -amino carboxylic acid **2-89**.

Entry	Reagent(s)	Solvent	Temperature	Time	Results
1	EDCI (1.1 equiv) DMAP (1.1 equiv) DIEA (3.0 equiv)	CH ₂ Cl ₂	0 °C to rt	12 h	SM
2	PPh ₃ /CCl ₄ NEt ₃	CH ₃ CN	80 °C	6 h	SM

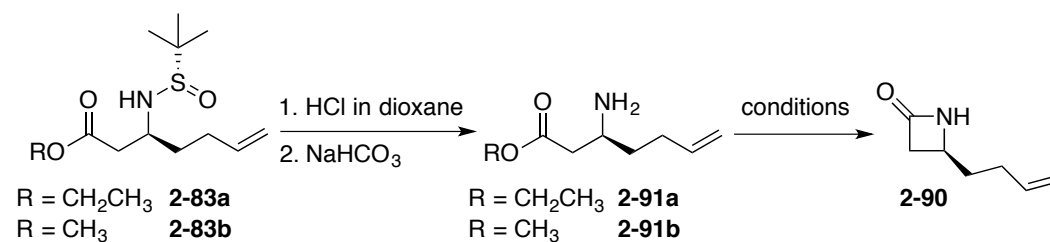
- DIEA = *N,N*-diisopropylethylamine
- EDCI = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
- DMAP = *N,N*-dimethylamino pyridine

Next, our attention was focused on conditions to cyclize substrate **2-91**, obtained from acid deprotection of the chiral auxiliary group in β -amino ester **2-83** to expose the free amino moiety. This approach involved the deprotonation of the amino group using an organometallic reagent, followed by the subsequent ring closure to provide the desired β -lactam. A number of Grignard reagents including *t*-butylmagnesium chloride, ethylmagnesium bromide, and phenylmagnesium bromide have been utilized for this purpose in the literature, although the majority of these reactions were achieved in modest yields.³² One of the advantages of using a Grignard reagent is the lack of tendency for α -deprotonation of the ester, thereby eliminating the possibility of a retro-Michael reaction.³³

Initially, a considerable amount of effort was dedicated towards the Grignard mediated cyclization reaction of **2-91a** (Table 2.5); however, all attempts resulted in the

recovery of the starting material, even in the presence of 10 equivalents of the Grignard reagent. The formation of unidentified byproducts was also observed in some of the attempted reactions. Additionally, other strong bases such as lithium bis(trimethylsilyl)amide (LiHMDS) and NaH did not afford the desired β -lactam product (entries 3 and 4, Table 2.5).

Table 2.5: Attempted cyclization conditions on β -amino carboxylic acid **2-91**.



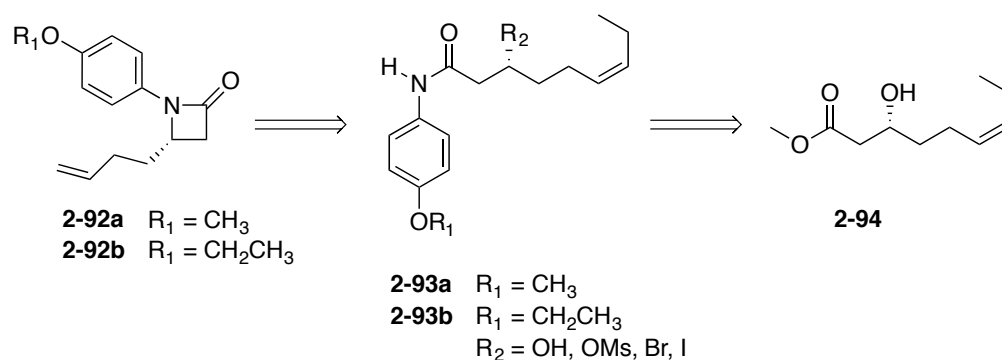
Entry	Substrate	Reagent(s)	Solvent	Temperature /Time	Results
1	2-91a	EtMgBr (3 equiv)	THF	0 °C / 3 h rt / overnight	SM
2	2-91a	1. TMSCl (3 equiv) NEt ₃ (3 equiv) 2. <i>t</i> -BuMgCl (10 equiv)	CH ₂ Cl ₂	0 °C to rt overnight	SM
3	2-91a	1. LiHMDS (1.1 equiv) 2. NH ₄ Cl (sat.)	THF	-78 °C / 4 h	SM
4	2-91a	1. NaH (1.2 equiv) 2. NH ₄ Cl (sat.)	THF	0 °C to rt overnight	SM
5	2-91b	EtMgBr (4 equiv)	THF	0 °C / 3 h rt / overnight	12% of β -lactam
6	2-91b	EtMgBr (3 equiv)	THF	rt / overnight	17% of β -lactam
7	2-91b	EtMgBr (3 equiv)	THF	3 h / 0 °C rt / overnight	14% of β -lactam (performed at 1 M)

• All reactions were performed at 0.1 M concentration, unless otherwise noted

After several attempts at the cyclization of substrate **2-91a**, the ethyl ester was replaced by the methyl ester (**2-91b**) and the Grignard mediated cyclization was tested once more (Table 2.5). Gratifyingly, following purification of the reaction, 12% of the desired β -lactam was isolated (entry 5, Table 2.5). This result was encouraging, although a yield of 12% was far from ideal and did not represent a useful synthesis of the targeted β -lactam. Moreover, all attempts at optimization (not shown) failed to improve the yield of the reaction to higher than 17%. A plausible explanation for the increased reactivity of the methyl ester could be provided on the grounds of the steric hindrance associated with the ethyl ester moiety in compound **2-91a**, which in turn impeded the cyclization reaction.

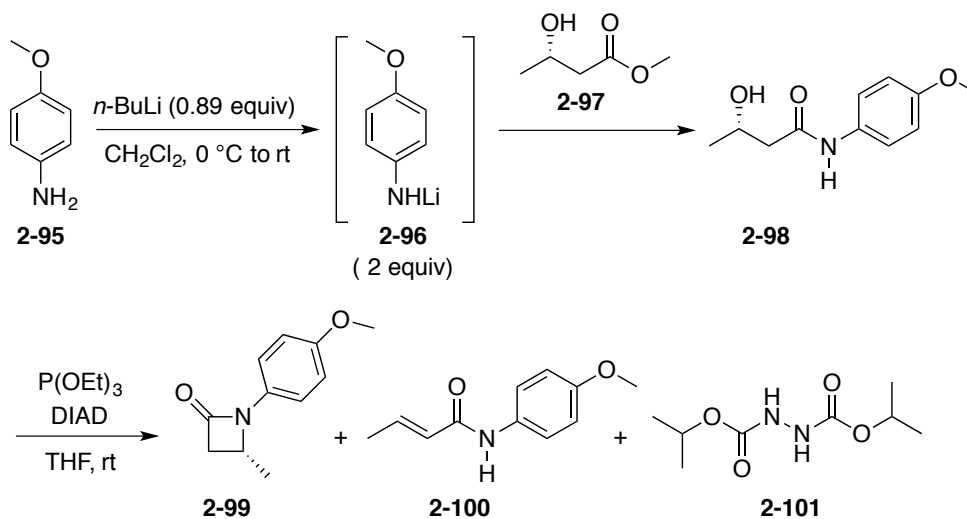
2.3.4 Generation III Revised Synthetic Plan

With various options at amino ester cyclization explored at this point, we next considered a completely different disconnection in preparation of *N*-*p*-methoxyphenyl- β -lactam **2-92**, which led to the asymmetric approach outlined in **Scheme 2.17**.



Scheme 2.17: An alternative approach to the targeted β -lactam intermediate.

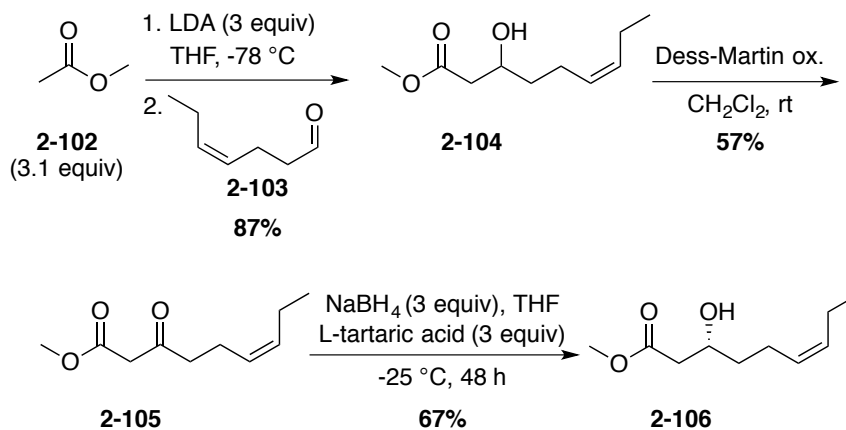
Initially, the viability of this approach was tested through a model system employing the commercially available enantiopure 3-hydroxy butyrate **2-97**. Conversion of **2-97** to 3-hydroxy butanamide **2-87** was accomplished via treatment with intermediate **2-96**, generated from the reaction of anisidine (**2-95**) with *n*-butyllithium (Scheme 2.18). After exploring Mitsunobu cyclization conditions (PPh₃/DIAD, PPh₃/DEAD), the desired β -lactam **2-99** was obtained using triethyl phosphite (P(OEt)₃) and diisopropyl azodicarboxylate (DIAD).³⁴ The major cause of concern in this reaction was the competing elimination reaction leading to the α,β -unsaturated amide **2-100**, which was avoided by employing P(OEt)₃/DIAD in the cyclization step. Although promising, the yield of the reaction remained ambiguous due to difficulties in complete removal of byproduct **2-101** generated from DIAD.



Scheme 2.18: Mitsunobu cyclization for the preparation of model β -lactam **2-99**.

Following this success, efforts were focused on preparation of the requisite starting material en route to β -lactam **2-92**. After much exploration, we were able to devise a

stereoselective and high-yielding route to access β -lactam **2-92** on multi-gram scale. This synthetic sequence initiated with preparation of β -hydroxy ester **2-104** from the addition of the enolate of methyl acetate **2-101** to the commercially available *cis*-heptenal **2-103** in excellent yield (Scheme 2.19). Compound **2-104** was carried through as a racemic mixture to test the feasibility and to further explore the designed synthetic route. It should be noted the desired β -hydroxy ester can also be accessed asymmetrically via the stereoselective reduction of the corresponding β -keto esters **2-105**, obtained from oxidation of the racemic β -hydroxy ester **2-104**. We are currently exploring a protocol which employs a complex generated from the inexpensive reagents NaBH_4 and L-(+)-tartaric acid in reduction to the appropriate enantiomer (Scheme 2.19).³⁶



Scheme 2.19: Racemic and asymmetric preparation of β -hydroxy esters.

This protocol is unique to esters that are functionalized at the α - or β - position with substituents capable of chelation such as ethers, esters, and amides (Figure 2.4). Additionally, the use of D-(-)-tartaric acid results in reduction to the other enantiomer, suggesting that chirality can be transferred from tartaric acid.³⁶ It should also be noted that

enzymatic reduction protocols can also be utilized in selective reduction of β -keto esters and β -diketones. One of the most frequently utilized enzymatic reagents in such reductions is the Baker's yeast (*Saccharomyce cerevisiae*). Sih et al. have reported a dependence on the size of the ester moiety and the stereochemical outcome of the reduction. In Sih's studies, reduction of smaller ester groups such as ethyl acetoacetate predominantly resulted in formation of the (*S*)-alcohol, whereas the longer chain ester groups afforded the (*R*)-alcohol.³⁷

Alternatively, this reduction can be accomplished using catalytic asymmetric hydrogenation protocols such as the Noyori asymmetric hydrogenation.³⁸ In such a case, the reaction can be terminated before reaching completion in order to avoid the reduction of the olefin moiety. The starting material can in turn be recovered and subjected to hydrogenation conditions once more.

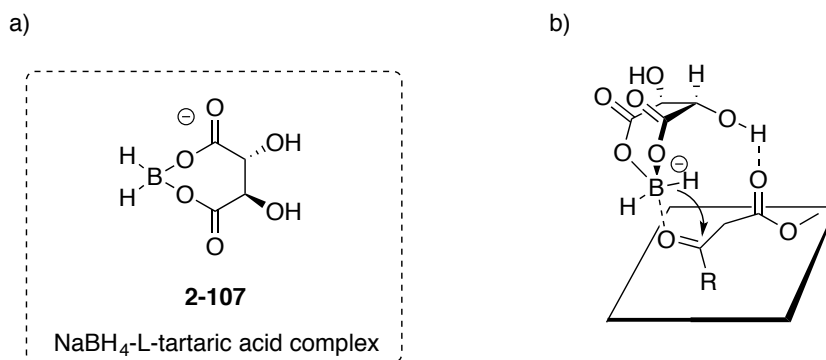
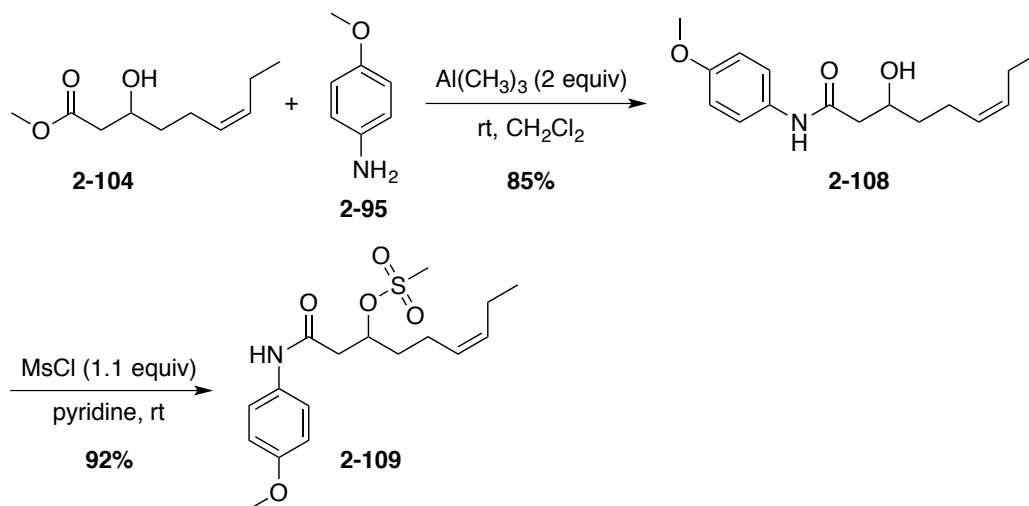


Figure 2.4: a) Chiral NaBH_4 -L-tartaric acid complex. b) Asymmetric reduction of β -keto esters.

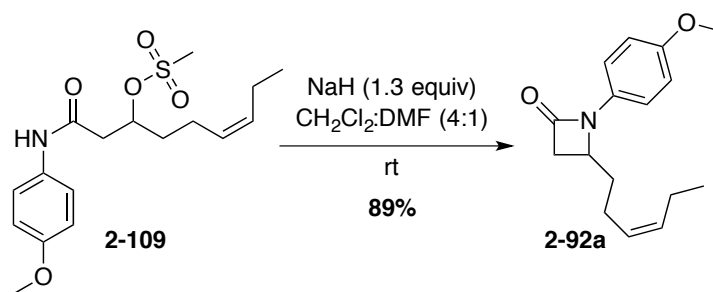
With ample quantities of β -hydroxy ester **2-104** in hand, efforts were focused on conversion to the β -hydroxy amide **2-108** using a trimethyl aluminum coupling protocol. The

secondary alcohol moiety of the β -hydroxy amide **2-108** was then mesylated to provide compound **2-109** on gram-scale in excellent yield (Scheme 2.20). Due to difficulties faced in removal of the byproduct generated from DIAD in the intramolecular Mitsunobu cyclization (Scheme 2.18), an alternative cyclization condition was utilized at this point.



Scheme 2.20: Further functionalization of β -hydroxy ester **2-104**.

Having the target of a stereoselective and high-yielding preparation of β -lactam building blocks, we explored a seldom-employed method of nucleophilic displacement for β -lactam synthesis (Scheme 2.21).³⁹ To this end, compound **2-109** was subjected to a base mediated intramolecular cyclization using NaH in a mixture of CH_2Cl_2 :DMF (4:1) as the solvent system to provide the desired β -lactam with inversion of stereochemistry in 91% yield following optimization. β -Lactams that lack α -substitution are unusual building blocks and the synthetic route outlined here provides a stereoselective and scalable approach to this class of compounds. With a reliable route to the desired β -lactam, attention was focused on further functionalization of this intermediate.



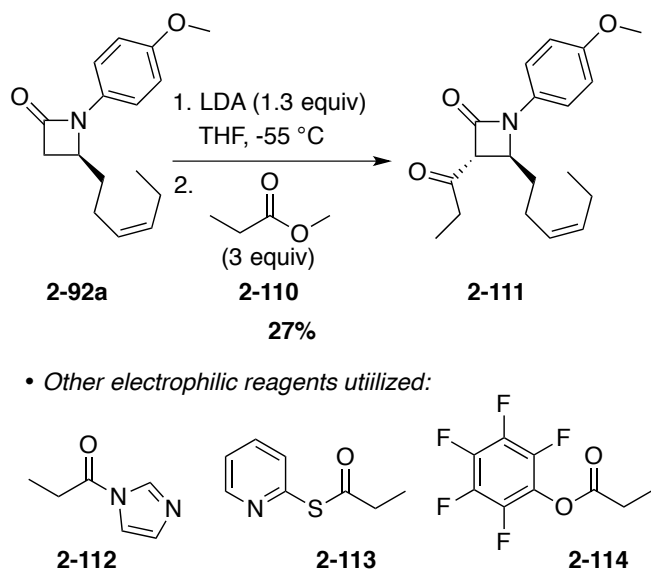
Scheme 2.21: Cyclization of **2-109** to β -lactam **2-92a**.

2.3.5 Further Functionalization of the β -Lactam Intermediate

Based on literature precedent, further functionalization of β -lactam **2-92a** at the C3 position can be achieved through deprotonation with LDA, followed by treatment with various electrophiles.³⁰ As mentioned previously, such reactions have the preference to produce the thermodynamically favorable *trans*- β -lactams, due to the approach of the electrophile from the less hindered face of the enolate.

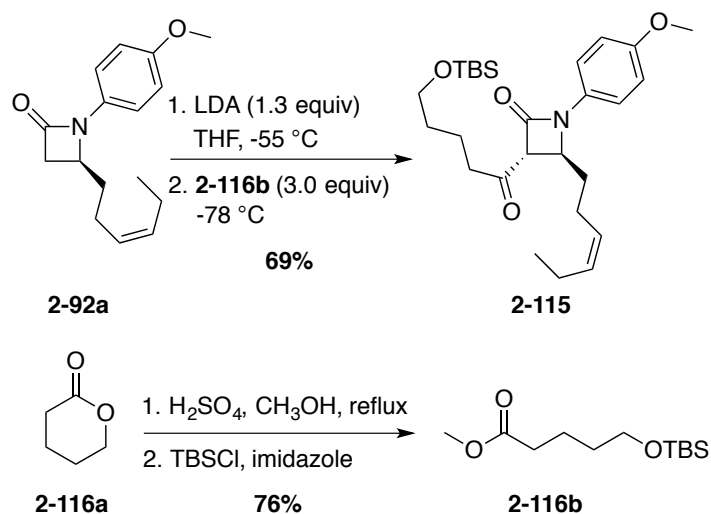
As such, we investigated deprotonation of β -lactam **2-92a** using LDA, followed by treatment with the commercially available methyl propionate **2-110** (Scheme 2.22). Initially, numerous efforts were focused on optimizing this transformation; however, those attempts predominantly resulted in recovery of unreacted starting material. In addition to varying several parameters such as temperature, time, and equivalences of LDA, we investigated other electrophiles as well (Scheme 2.22). Unfortunately, despite the many attempts at this transformation, only the starting material was recovered in the cases of other electrophilic reagents. Overall, the deprotonation followed by reaction with methyl propionate proved to

be very inconsistent and provided the desired product **2-111** in low yields only in a handful of trials out of the many attempted.



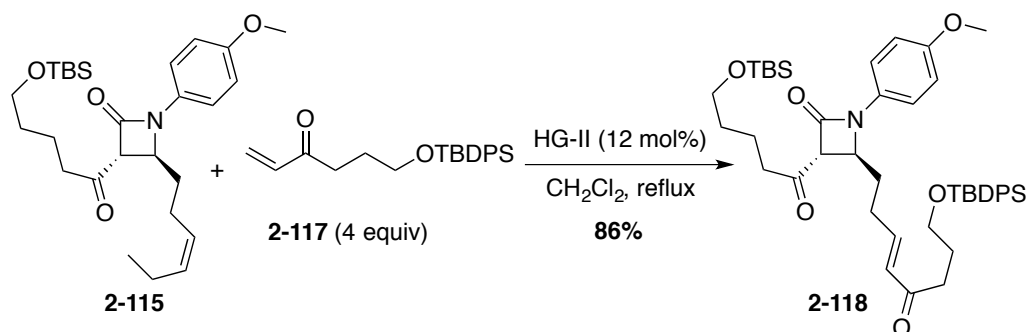
Scheme 2.22: Acylation studies on β -lactam **2-92a**.

Following this result, the possibility of acylation with ester **2-116b** prepared using the sequence depicted in **Scheme 2.23**, was explored. We were pleased to isolate the desired functionalized *trans*- β -lactam **2-115** in 44% yield. The yield of the reaction was further increased to 69% following optimization. Although this is a simplified substrate, it will serve to demonstrate the viability of this approach in retaining the ester stereocenter under the proposed conditions.



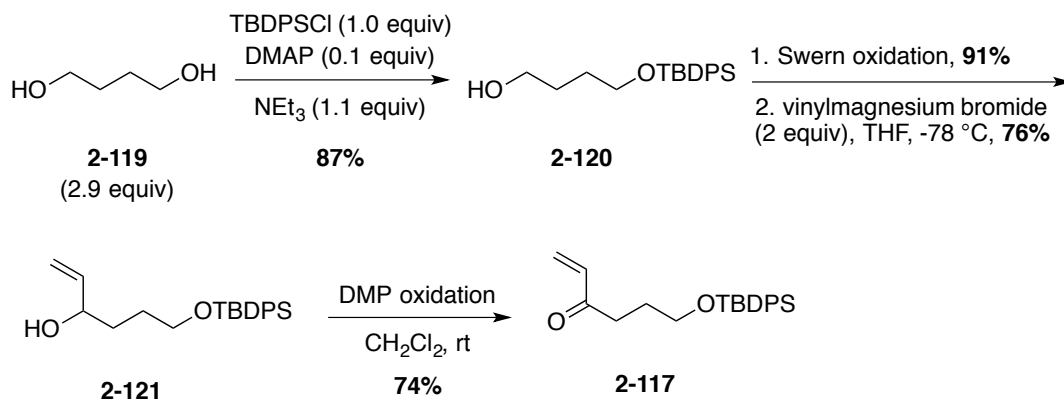
Scheme 2.23: Further acylation studies on β -lactam **2-92a**.

The reason for the lack of reactivity and inconsistency in the majority of attempted trials using the simple methyl propionate (or its equivalences) is not fully clear. Nevertheless, with the construction of β -lactam **2-115** completed, further elaboration of the terminal olefin was accomplished using a cross metathesis reaction catalyzed by Hoveyda-Grubbs 2nd generation catalyst (HG-II) with enone **2-117** to provide the desired product in 86% yield following optimization (Scheme 2.24). It should be noted that the we were able to recover the excess unreacted enone following purification in most attempted trials.



Scheme 2.24: Further functionalization of β -lactam **2-115** using cross metathesis.

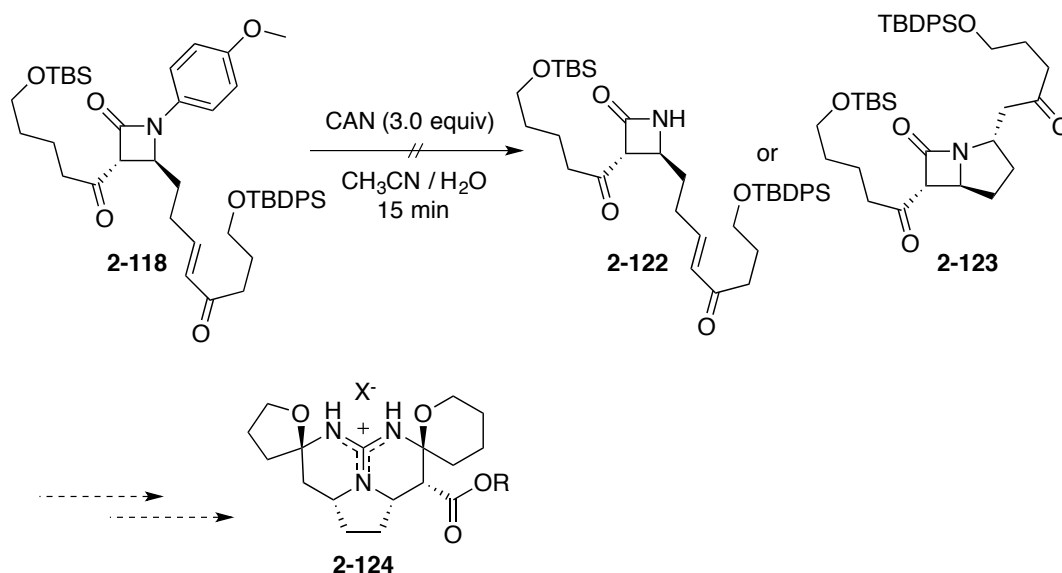
The enone **2-117** was in turn prepared using a short synthetic sequence starting with the mono-protection of diol **2-119**, followed by the oxidation of the free alcohol under Swern oxidation conditions. Treatment of the requisite aldehyde with vinyl magnesium bromide provided intermediate **2-121**. Compound **2-121** was further oxidized to the targeted enone **2-117** under Dess-Martin periodinane (DMP) oxidation conditions (Scheme 2.25).



Scheme 2.25: Synthetic sequence for the preparation of enone **2-117**.

With the key β -lactam intermediate **2-118** in hand, efforts were focused on removal of the *p*-methoxyphenyl (PMP) nitrogen protecting group to obtain the deprotected β -lactam **2-**

122 or the product of the subsequent aza-Michael cyclization, compound **2-123**. Such deprotections on β -lactams are typically accomplished under oxidative conditions using excess equivalences of the one-electron oxidant ceric ammonium nitrate (CAN) in an aqueous acetonitrile solution.⁴⁰ However, these conditions have drawbacks such as toxicity, extremely laborious work-up protocols, inconsistencies, and mediocre to low yields. Additionally, none of the reported protocols are performed on functionalized substrates such as compound **2-118**. Several substrates including compound **2-118** with varying degrees of complexity were subjected to these deprotection conditions. Unfortunately, all resulted in extremely low yields or unidentifiable byproducts, possibly due to decomposition. Alternative oxidizing reagents such as 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), argentic oxide (AgO) in acidic medium⁴¹, and CoF_3 ⁴² were also utilized for this purpose; however, none provided the desired product.

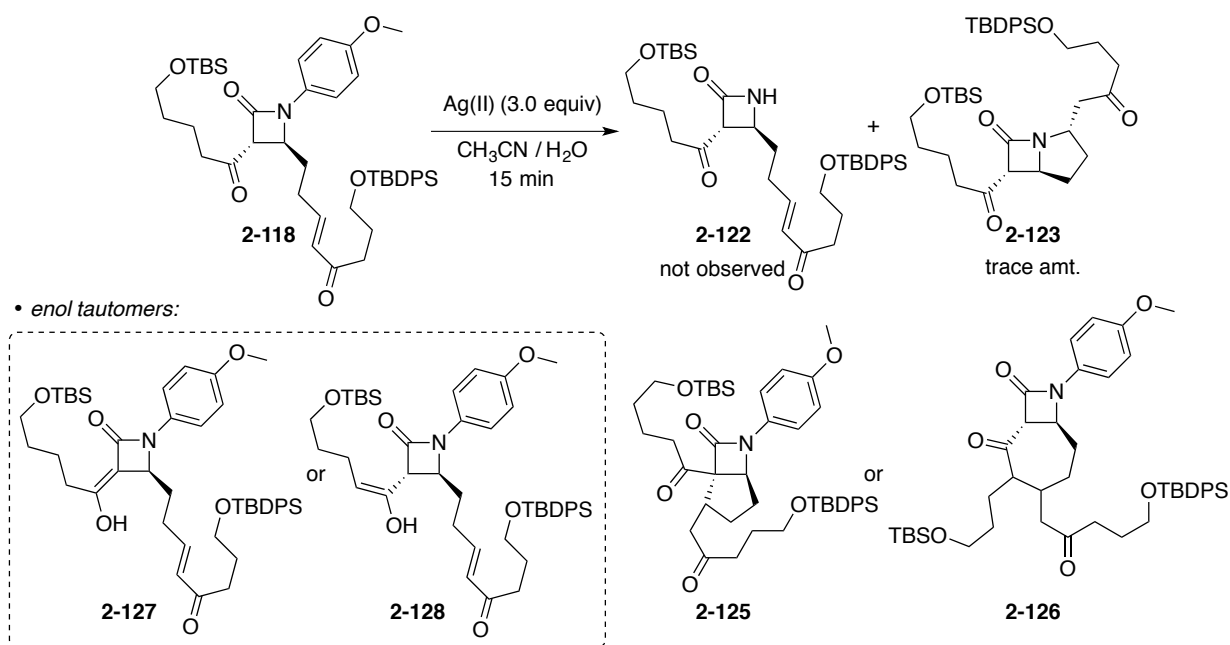


Scheme 2.26: *N*-Dearylation of β -lactam intermediate **2-118** using CAN.

We then investigated a more effective oxidant tetrapyridinesilver(II) peroxydisulfate ($[\text{Ag}(\text{Py})_4]\text{S}_2\text{O}_8$), which has also been reported for the *N*-dearylation of β -lactams.⁴³ This air stable silver(II)-persulfate complex was readily prepared from an aqueous solution of silver nitrate, pyridine, and potassium peroxydisulfate. Such a complex is referred to as a twin oxidant/co-oxidant system, as it contains Ag(II) as an oxidant and persulfate as a co-oxidant and functions in a similar manner as CAN, producing the same *p*-benzoquinone byproduct.

The deprotection of β -lactam **2-118** using Ag(II)-persulfate complex was attempted, and following purification a minor amount of a compound which appears to be the desired bicyclic β -lactam **2-123** was isolated. Further exploration of the major product isolated from this reaction following extensive 1D and 2D NMR analysis was indicative of compounds **2-125** or **2-126**, arising from the respective enol intermediates **2-127** or **2-128** undergoing intramolecular Michael addition (Scheme 2.27).

It should be noted that this reactivity was observed on β -lactam **2-118** over time upon storage, even at $-30\text{ }^\circ\text{C}$. β -keto amide functionalities behave much like their β -keto ester counterparts, which exist as the enol tautomer in significant percentage at room temperature. To circumvent the issues regarding the inherent reactivity of β -keto amides in the enol tautomer, a less reactive form of the functionalized β -lactam building block was sought after. The details of those studies are discussed in the following section.



Scheme 2.27: *N*-Dearylation of β -lactam intermediate **2-118** using a Ag(II)-persulfate complex.

In recognition of the difficulties faced in removal of the PMP group, we investigated the dearylation reaction of other less functionalized substrates possessing a different substitution pattern and number of methoxy moieties with the hopes of a more facile deprotection (Figure 2.5). In all cases, the substrates were prepared using the previously developed synthetic sequence utilized in preparation of *N*-PMP β -lactam **2-91a**. Much to our disappointment, all proved to be inferior alternatives to the PMP group.

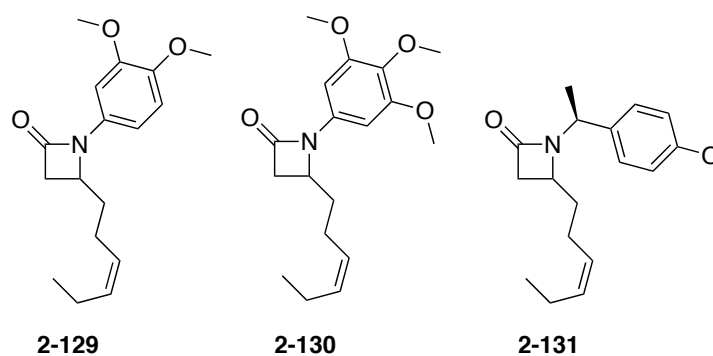


Figure 2.5: Other β -lactam substrates utilized in Ag(II)-persulfate complex deprotection.

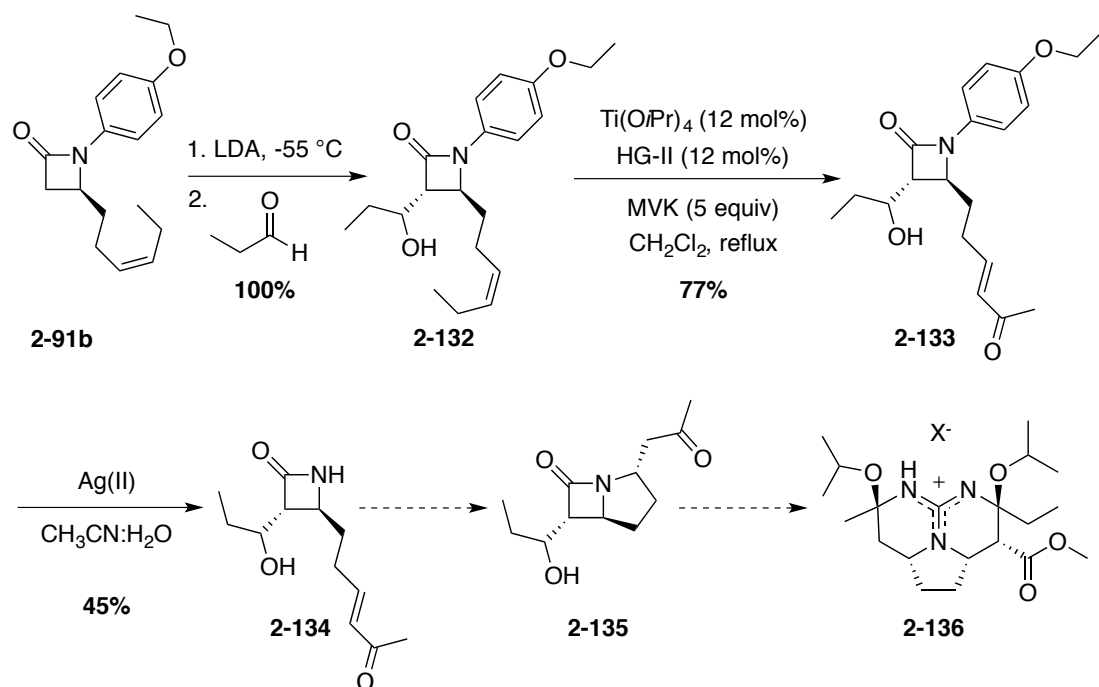
2.3.6 Alternative Approach to Further Functionalization of β -lactams

Following the limited success in utilizing acylated β -lactam building blocks and the hurdles faced in deprotection of various *N*-aryl β -lactam substrates, an alternative plan was investigated. We were prompted to modify the oxidation state of the side chain at C3 position of the requisite β -lactam, in order to prevent the undesired side reactions. To this end, β -lactam **2-133** became the new target intermediate and was readily accessed through the previously established protocol applied to the synthesis of the acylated β -lactam **2-115** (Scheme 2.28).

The synthesis of **2-133** began by smooth alkylation of β -lactam **2-92b** to generate β -lactam **2-132** in quantitative yield. As discussed previously, the deprotonation, followed by the subsequent alkylation of β -lactams typically proceeds in a stereoselective manner, generating the preferred *trans* disubstituted product due to the approach of the electrophile to the sterically less hindered face of the enolate. The presence of the additional stereocenter at the hydroxyl bearing carbon in **2-132** resulted in formation of a mixture of diastereomers;

however, further oxidation of the secondary alcohol to the corresponding ketone resulted in a NMR spectrum identical to that of the acylated β -lactam intermediates, further reassuring that the alkylation was indeed stereoselective.

It should be noted that the PMP group was replaced by a *para*-ethoxy phenyl group (PEP), presumably due to a more facile removal of this moiety using Ag(II)-persulfate complex as observed in a literature report.⁴³ β -Lactam **2-132** was further functionalized at the olefin with methyl vinyl ketone (MVK) under cross metathesis conditions using Hoveyda-Grubbs 2nd generation catalyst to provide the desired product in good yields. With the desired intermediate **2-133** in hand, efforts were focused on removal of the PEP group using the previously utilized Ag(II)-persulfate complex. Much to our surprise, we isolated the dearylated and non-cyclized β -lactam **2-134** in place of the expected intermediate **2-135**, arising from the intramolecular aza-Michael cyclization (Scheme 2.28). Efforts are ongoing to optimize the yield of the Ag(II)-mediated deprotection to obtain sufficient quantities of β -lactam **2-134**. Furthermore, various bases and Lewis acids will be screened for their ability to facilitate the intramolecular aza-Michael addition of the weakly nucleophilic nitrogen nucleophile to obtain the bicyclic β -lactam **2-135**. Finally, building on literature precedent, the guanidine moiety will be introduced at the next and final stage of the synthesis following the ring opening of **2-135** to provide target analog **2-136**.⁷

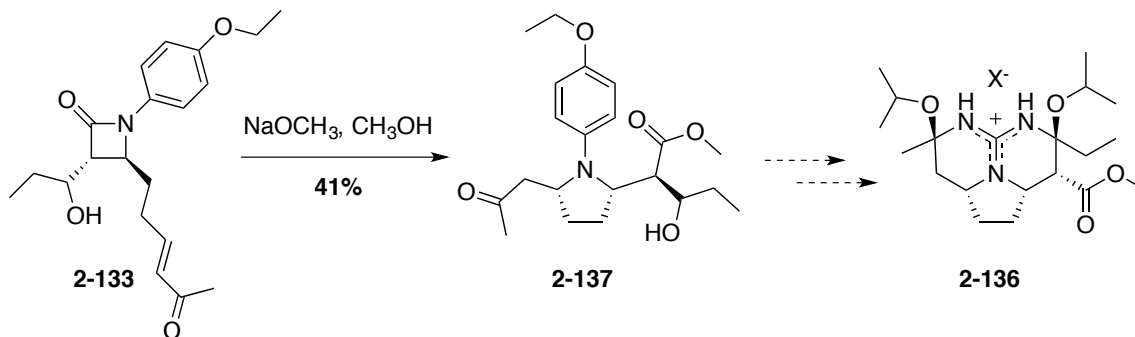


Scheme 2.28: Alternative route to β -lactam functionalization.

The intramolecular aza-Michael reaction has attracted a significant amount of interest in the past few years, mainly due to its utility in preparation of pharmacologically important heterocycles such as pyrrolidine and piperidine scaffolds. The scope of this reaction has been extended to the use of various nitrogen nucleophiles and a plethora of Michael acceptors under catalyzed and non-catalyzed conditions.⁴⁴ While there are reports on the enantioselective intramolecular aza-Michael reaction, very little work has been published on the diastereoselective variant of this reaction.⁴⁵ More importantly, there are reports of weakly nucleophilic amines such as carbamates and sulfonamides tethered to α,β -unsaturated ketones participating in intramolecular aza-Michael reactions.^{44,46} The success of a weakly

nucleophilic nitrogen in such reports is encouraging, given the electronic similarities of this model to that of a β -lactam unit.

Alternatively, β -lactam **2-132** can be deprotected first and then subjected to cross metathesis conditions for further functionalization of the olefin, followed by possible concomitant aza-Michael cyclization to the bicyclic system under those conditions. We have also attempted the ring opening reaction of β -lactam **2-133** under basic (or acidic) conditions to obtain the *N*-aryl pyrrolidine **2-137**. We are currently investigating the optimum order of reactions to the target analog **2-136**, in order to conserve the stereochemical integrity of the acidic α -proton of the β -keto ester produced once the oxidation state of the alcohol side chain is increased in intermediate **2-137** (Scheme 2.29).

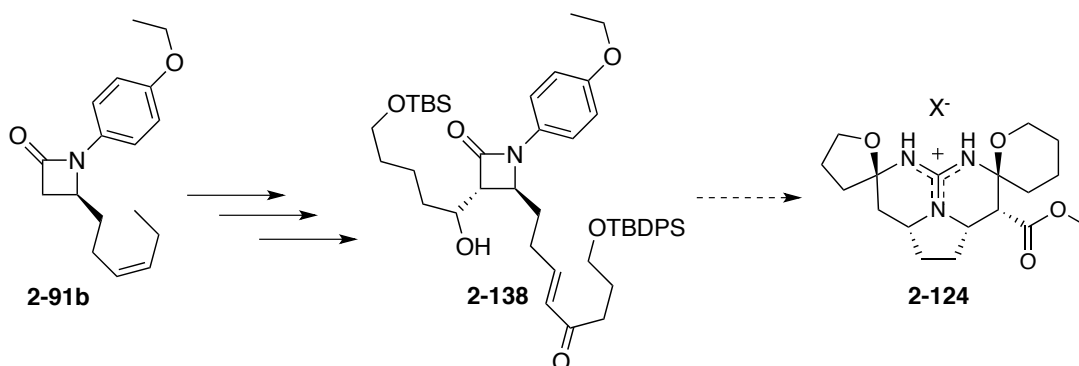


Scheme 2.29: An additional alternative route to β -lactam functionalization.

With the aim to further probe diversity, we are hoping to extend our synthetic sequences to simplified analogs of the monanchocidin family, and in general other pentacyclic guanidinium alkaloids. Studies by the Overman group and others have focused on analog preparation, specifically on ptilomycalin A; however, most of those efforts were directed towards modifications at C14 ester linkage and none on the guanidinium core

itself.^{9,10} As discussed earlier, the guanidinium core is a crucial component in the biological behavior of these molecules.

Pursuant to this information, analogs varying in the guanidinium core are highly desirable and valuable in order to fully evaluate the SAR profiles of this class of compounds and potentially contribute to our understanding of the possible mechanism of action of these molecules. Once viable solutions to our pending transformations are developed in preparation of analog **2-136** (Scheme 2.28 and Scheme 2.29), we are planning to apply our synthetic strategy to the preparation of other more elaborate analogs such as **2-124** (Scheme 2.30).



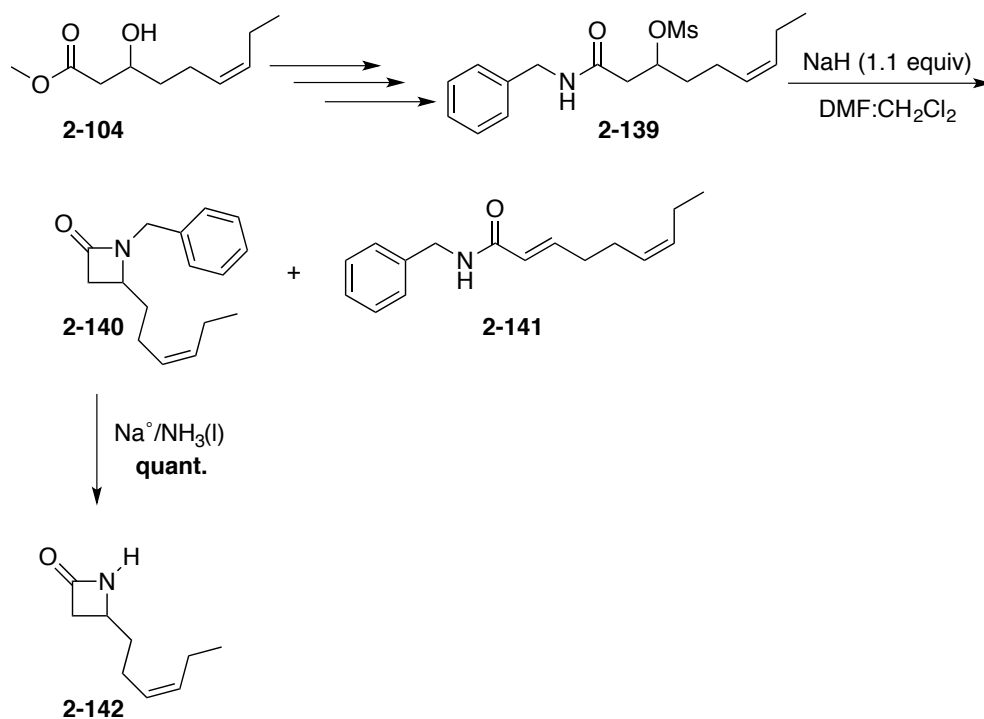
Scheme 2.30: Synthetic sequence to pentacyclic guanidinium analog **2-124**.

2.3.7 Other Attempted Approaches

In parallel to the studies discussed and in recognition of the difficulties associated with *N*-deacylation of β -lactams, an alternative nitrogen protecting group was also investigated. Based on literature precedent, *N*-benzyl protected β -lactams are readily deprotected under dissolving metal reduction conditions.⁴⁷ In light of this precedent and the seemingly compatible nature of this transformation with the designed substrate in our

synthetic sequence, we embarked on the synthesis of β -lactam **2-140** with the intentions of providing a facile route to the deprotected β -lactam.

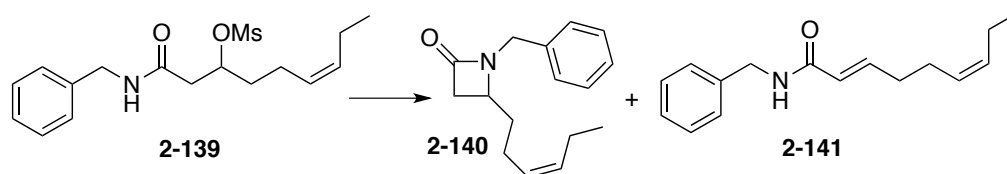
To this end, the synthesis of β -lactams **2-140** began by using the already established protocol utilized in the synthesis of *N*-aryl β -lactam substrates. We were able to gain access to intermediate **2-139** efficiently in yields similar to previously observed yields in preparation of *N*-aryl substrates. Following treatment of **2-139** with NaH in the intramolecular cyclization reaction, a 1:1 mixture of the desired β -lactam **2-140** and enamide **2-141** were obtained. Nevertheless, β -lactam **2-140** was isolated in the pure form following multiple purifications.



Scheme 2.31: Synthesis and deprotection of *N*-benzyl β -lactam **2-140**.

With **2-140** in hand, the viability of the dissolving metal reduction employing sodium metal in liquid ammonia for removal of the benzyl group was tested. Much to our delight, the debenzylation proceeded smoothly to furnish the desired β -lactam **2-142** in quantitative yield. Following this result, we embarked on conditions that would prevent the competing side reaction to form enamide **2-141** in β -lactam preparation (Table 2.6). Unfortunately, only enamide **2-141** was obtained as the sole product when K_2CO_3 in acetone and Cs_2CO_3 in acetonitrile were used.

Table 2.6: Other attempted conditions at β -lactam **2-140** synthesis.

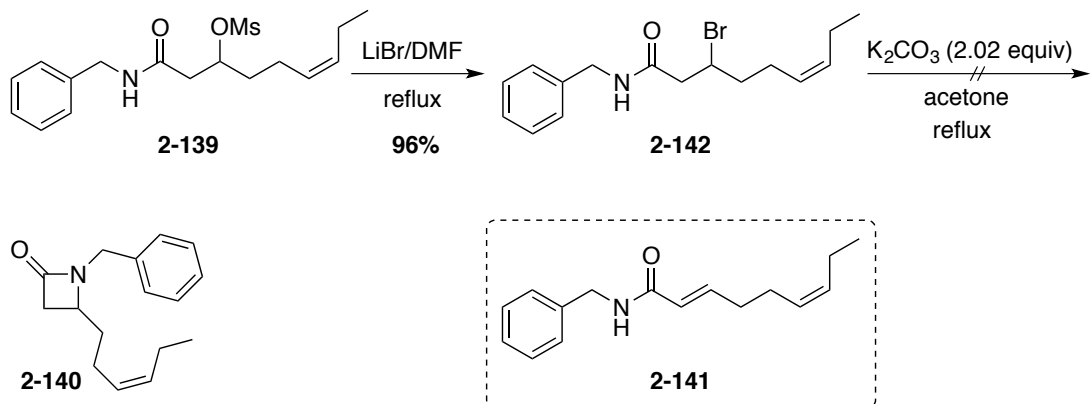


Entry	Reagent(s)	Solvent	Temperature / time	Results /Remarks
1	Cs_2CO_3 (1.2 equiv)	CH_3CN	rt / overnight	2-141
2	K_2CO_3 (2.02 equiv)	acetone	reflux / overnight	2-141

• All reactions were performed at 0.1 M concentration, unless otherwise noted

In an effort to halt or delay the elimination of the mesyl leaving group to allow for cyclization to occur, the bromide substrate was prepared following treatment of **2-139** with LiBr in DMF (Scheme 2.32). Additionally, the bromide intermediate **2-142** is more similar in structure to the substrate scope utilized in the reported literature precedent; however, the presence of an additional substituent at the α -position completely mitigated the issue of

elimination in their case. Upon treatment of **2-142** with K_2CO_3 in refluxing acetone, enamide **2-141** was obtained as the sole product. Future studies will be focused on the efficient synthesis of **2-140**, followed by exploration into further reactivity of this building block in the following alkylation and functionalization steps.



Scheme 2.32: Synthesis of *N*-benzyl β -lactam **2-140**.

2.3.8 Progress Towards the Total Synthesis of Crambescidin 359 and Analogs

2.3.8.1 Introduction to Crambescidin 359

As discussed earlier, the complexity of the pentacyclic guanidinium alkaloids has been the driving force in a number of method design projects. Despite the broad range of potent biological activities exhibited by this class of natural products, very little is known about their SAR profile. Minale and co-workers, who investigated the biological activities of a number of pentacyclic guanidinium alkaloids including ptilomycalin A, proposed that the guanidinium core, colloquially referred to as the vessel, was responsible for much of the biological activity.⁴⁹ One of the underlying reasons for the limited understanding into the

biology behind these molecules is the lack of success in removal of the C14 side chain for further studies of the guanidinium core. Exposure of ptilomycalin A to hydrolysis (acid or base), metal hydride reduction, hydrogenation, and oxidation conditions have all resulted in the degradation of the pentacyclic structure.⁵⁰

The unique structure of crambescidin 359 (**2-143**) as the first member of the pentacyclic guanidinium alkaloids isolated that is devoid of the C14 ester linkage, along with the poorly defined biological data regarding this class of molecules has made this core the subject of total synthesis by a number of research groups.^{50,51} Crambescidin 359 was isolated by Braekman and co-workers from the marine sponge *Monanchom unguiculata* in 2000.⁵²

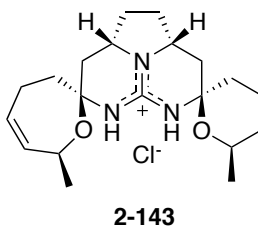


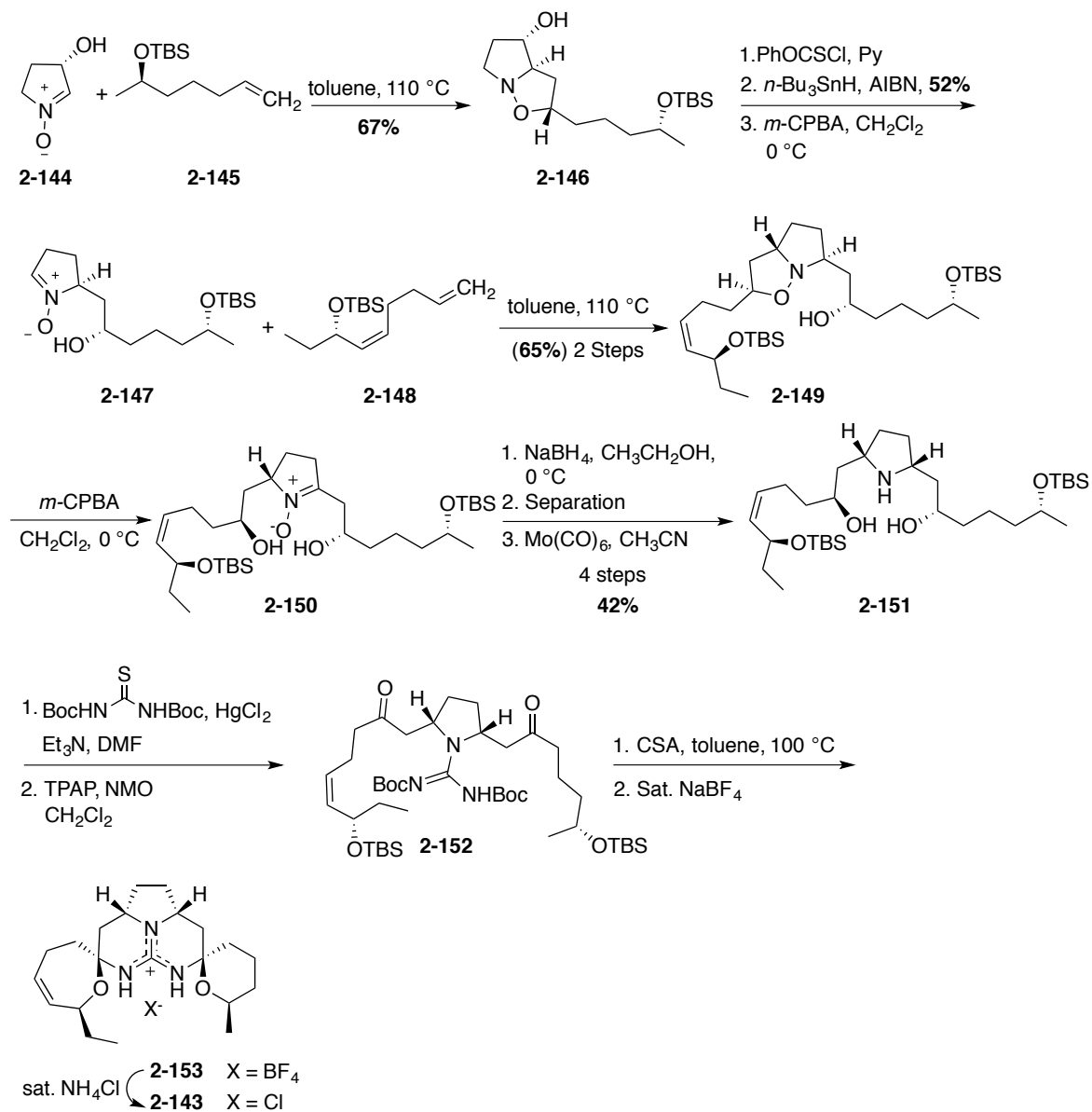
Figure 2.6: Structure of crambescidin 359 (**2-143**).

The first total synthesis of crambescidin 359 (**2-143**) was accomplished by Nagasawa and co-workers in 12 linear steps with a 3.8% overall yield.⁵¹ Overman and co-workers were able to gain access to crambescidin 359 (**2-143**) following spontaneous decarboxylation of the acid core, which was synthesized in 29 steps and 2.8% overall yield starting with commercially available starting material.⁵⁰ Inspired by the work of Nagasawa, Murphy and co-workers have also developed a convergent and potentially biomimetic synthesis of **2-143** with an 18% overall yield.⁵³

2.3.8.2 Nagasawa's 1,3-Dipolar Cycloaddition Approach

The approach developed by the Nagasawa group for the synthesis of crambescidin 359 (**2-143**) was a completely distinct strategy utilized in synthesis of the pentacyclic guanidinium core of this class of natural products (Scheme 2.33).⁵¹ Their synthetic strategy relied on the stereoselective preparation of a *cis*-2,5 disubstituted pyrrolidine ring **2-151** through successive 1,3-dipolar cycloaddition reactions. Their synthesis commenced with the stereoselective 1,3-dipolar cycloaddition reaction of the optically active nitron **2-144** and olefin **2-145** to generate the isoxazolidine **2-146**. The free secondary alcohol moiety was then removed in 2 steps through thiocarbonation, and reduction with *n*-Bu₃SnH. Oxidation of the resultant isoxazolidine using *m*-CBPA provided the requisite nitron **2-147**, required for the second 1,3-dipolar cycloaddition reaction. The 1,3-dipolar cycloaddition proceeded in a regioselective and stereoselective manner from the less sterically hindered face of the nitron to furnish isoxazoline **2-149**. Cleavage of the isoxazolidine using *m*-CPBA, followed by reduction of the corresponding nitron provided 2,5-*cis* pyrrolidine **2-151** with 7:1 diastereoselection. The condensation of pyrrolidine **2-151** with bis-*N*-Boc thiourea, followed by the oxidation of the diols resulted in formation of compound **2-152**. Subsequent deprotection of the silyl ether and Boc protecting groups proceeded smoothly; however, the following *N,O*-acetalization proved to be problematic. Nevertheless, optimized conditions for the orthogonal deprotection of the protecting groups followed by the concomitant cyclization using CSA in toluene at 100 °C furnished the final natural product as the camphorsulfonate salt. The camphorsulfonate anion was further exchanged for the chloride salt by treatment with a saturated ammonium chloride solution. Overall, the total synthesis

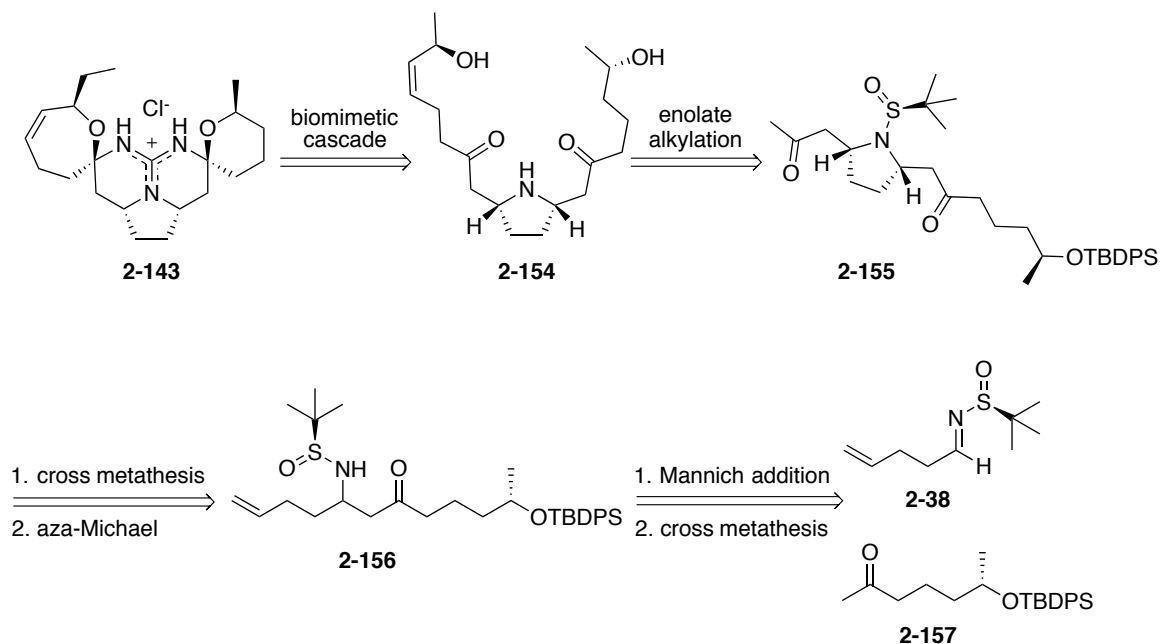
of **2-143** was accomplished in 12 linear steps, starting from the optically active nitron **2-144** in 3.8% yield. This synthetic route was initially developed for the synthesis of crambescidin 359, but was later extended to the synthesis of batzelladine A, which possess the *anti*-configuration of the pyrrolidine ring and the ester linkage at C14.



Scheme 2.33: Nagasawa's total synthesis of crambescidin 359 (**2-143**).

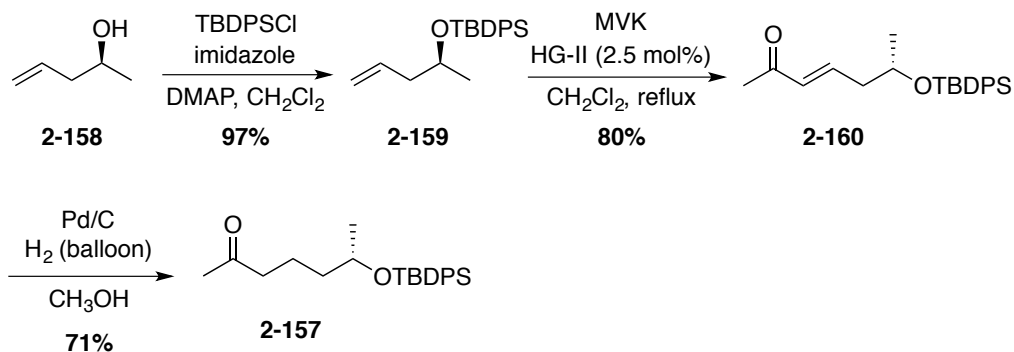
2.3.8.3 Our Approach toward Crambescidin 359

In light of the successes accomplished by the previous total syntheses, we have devised a simplified and short synthetic sequence for the preparation of crambescidin 359 (**2-143**, Scheme 2.34). Our synthetic plan began with a Mannich addition to provide β -amino ketone **2-156**. Cross metathesis of **2-156** with MVK, and subsequent cyclization would provide pyrrolidine **2-155** as a single *cis*-diastereomer. It should be noted that the *trans*-isomer can be obtained under alternate conditions for the preparation of analogs and other pentacyclic guanidinium natural products. Further functionalization of **2-155** to install the appropriate side chain, followed by treatment with guanidine or a guanidine derivative, and deprotection of the silyl ether groups is envisioned to result in the formation of the fully constituted crambescidin 359.



Scheme 2.34: Our approach to crambescidin 359 (**2-143**).

We initiated our efforts toward **2-143** with the preparation of ketone **2-157**. Silyl protection of the commercially available alcohol **2-158**, followed by cross metathesis reaction with MVK and reduction of the alkene using hydrogenation conditions furnished the target ketone **2-157** in excellent yield on gram scale (Scheme 2.35).



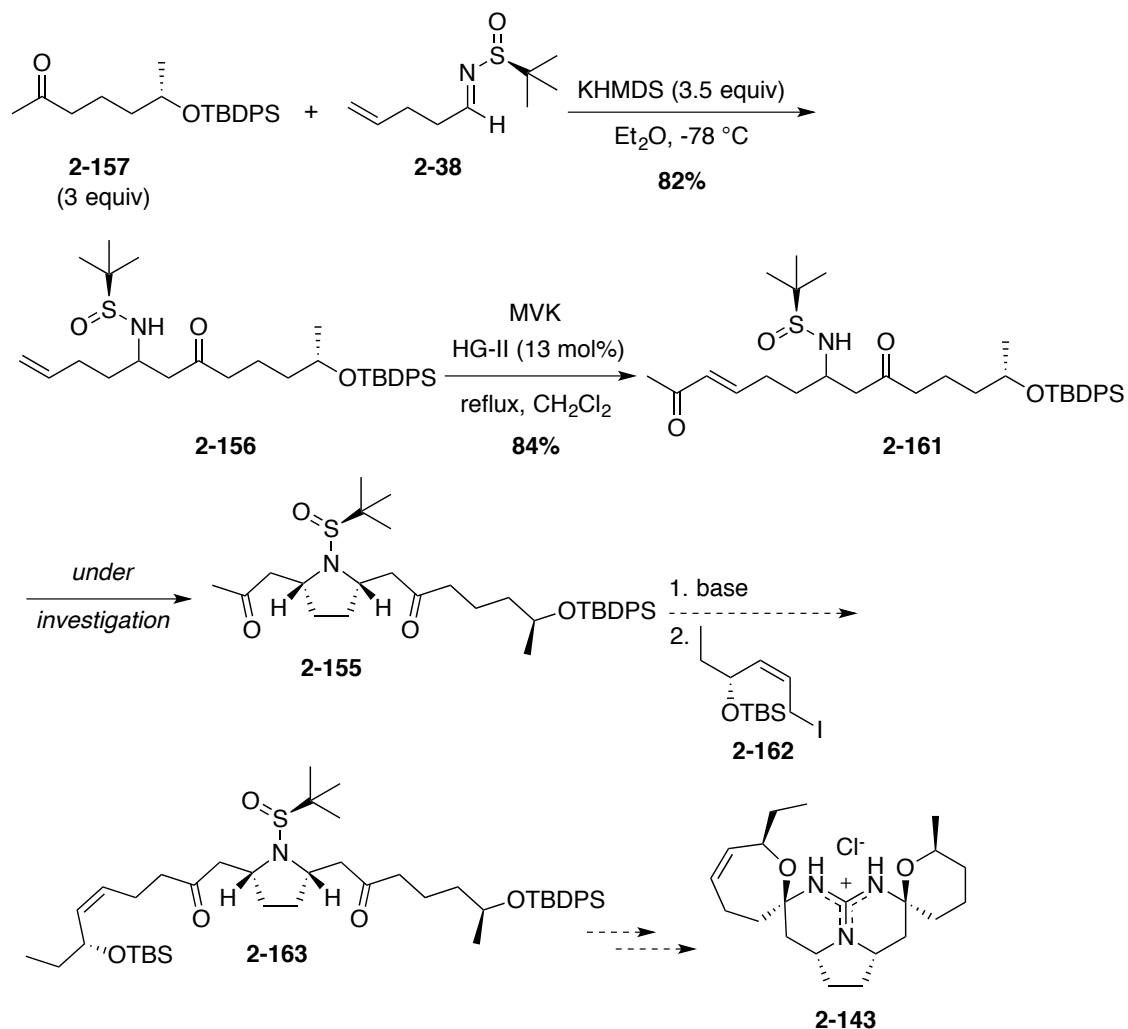
Scheme 2.35: Preparation of ketone **2-157**.

Diastereoselective Mannich addition of ketone **2-157** to imine **2-38** (see Scheme 2.5 for preparation) under optimized conditions utilizing potassium bis(trimethylsilyl)amide (KHMDS) as a base in anhydrous diethyl ether provided the desired β -amino ketone **2-156** in 82% yield (Scheme 2.36). β -Amino ketone **2-156** was further functionalized under cross metathesis conditions with MVK using Hoveyda-Grubbs 2nd generation catalyst to install the appropriate Michael acceptor group.

Cyclization of intermediate **2-161** using various bases, Lewis acids, and thermal conditions to pyrrolidine **2-155** is a subject of ongoing investigation. Following formation of pyrrolidine **2-155**, further functionalization through generation of the kinetic enolate, followed by alkylation with iodide **2-162** (prepared using literature precedent)⁵⁴ is envisioned

to install the requisite side chain for the natural product. The removal of the chiral auxiliary group of pyrrolidine **2-163** under acidic conditions at room temperature would furnish the deprotected pyrrolidine intermediate.

Installation of the guanidine moiety and conversion to the pentacyclic guanidinium core would provide the fully constituted natural product in one step. We are currently pursuing the pyrrolidine **2-155** formation on test scale, and working to optimize and scale up. Pyrrolidine **2-155** represents a key intermediate in our synthesis as it can be directly processed to a number of analogs for the biomimetic cascade cyclization to provide a number of natural product analogs. We propose that this synthetic sequence would prove to be a short, rapid and simple route utilizing commercially available starting materials, which would allow for the generation of the crambescidin 325 (**2-143**) in 10 linear steps.



Scheme 2.36: Synthetic efforts towards crambescidin 359 (**2-143**).

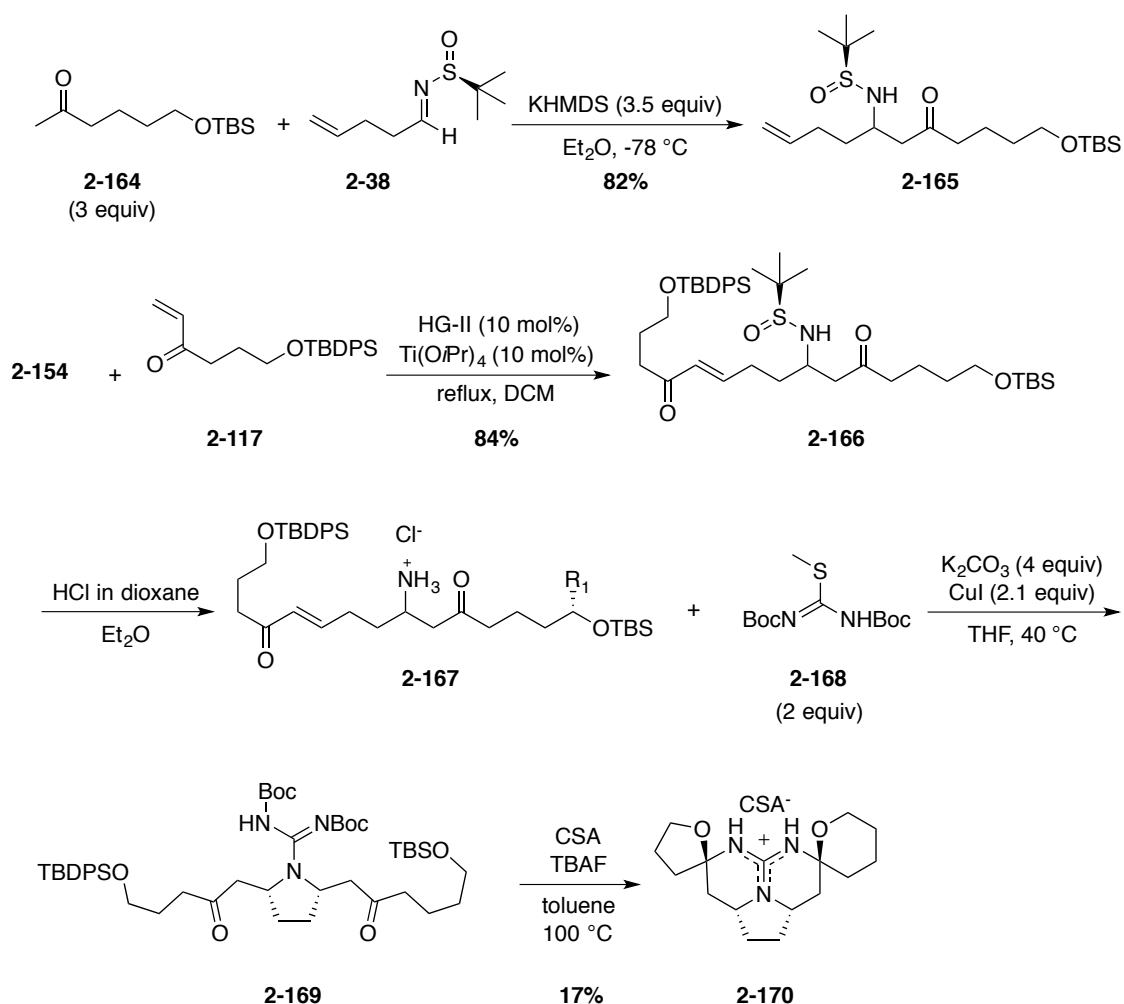
In parallel efforts, we devised a short and simplified synthetic sequence to a crambescidin 359 (**2-142**) analog (Scheme 2.37). This work has served to prepare linear precursor **2-166**, which in turn would allow us to explore the biomimetic cascade transformation designed above in the synthesis of crambescidin 359 (**2-143**), while providing material for the synthesis of analog **2-170**.

Following the previously developed protocol used en route to crambescidin 359 (**2-143**), the synthesis of **2-170** began with the diastereoselective Mannich addition of the enolate of the simplified ketone **2-164** to imine **2-38** to furnish β -amino ketone **2-165** in excellent yield with excellent diastereoselectivity. The corresponding β -amino ketone **2-165** was then further functionalized with enone **2-117** (see Scheme 2.25 for synthesis) to provide intermediate **2-166**. Acid mediated cleavage of the chiral auxiliary group using a solution of anhydrous hydrochloric acid in dioxane furnished the ammonium salt intermediate **2-167**, which was directly used in the next step.

It should be noted that the removal of this auxiliary proved to be challenging at this stage due to instability of the silyl protecting groups of the primary alcohols, specifically the *tert*-butyl dimethyl silyl group (TBS) under acidic conditions, which at times resulted in a mixture of compounds. Similarly, the use of bulkier silyl ether protecting groups such as triisopropyl silyl ether (TIPS), and *tert*-butyldiphenyl silyl ether (TBDPS) on the primary alcohol also resulted in formation of mixtures due to cleavage of such groups under acidic conditions.

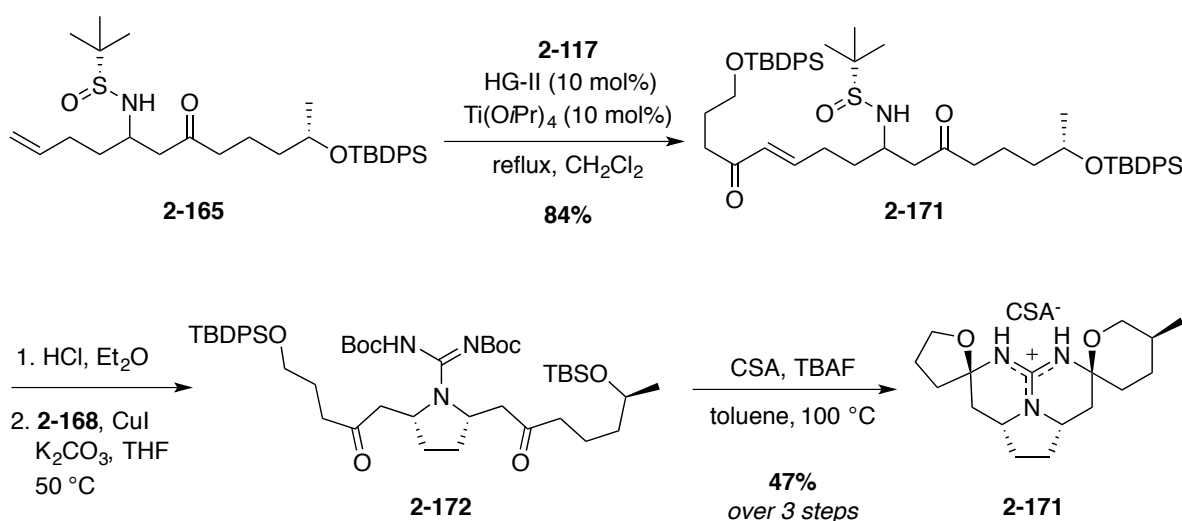
The crude intermediate **2-167** was then subjected to a copper(I)-mediated intermolecular amination of *S*-methylisothiourea using a reported synthetic protocol.⁵⁵ This mild and operationally simple protocol avoids the use of the very toxic HgCl₂ salt that are commonly utilized in such transformations. Moreover, the utility of this mild guanylation procedure in our synthetic scheme was further demonstrated through the use of excess base (NEt₃), which in turn facilitated the intramolecular aza-Michael cyclization to generate the corresponding pyrrolidine through neutralization of the ammonium salt intermediate **2-167**

all in one pot. Unfortunately, we have not been able to isolate sufficient quantities of intermediate **2-169** following purification, presumably due to the instability of this intermediate to chromatography conditions, or more likely the presence of other byproducts generated in the acid mediated deprotection step. Nevertheless, treatment of intermediate **2-169** in the pure form or as the crude mixture with the biomimetic cascade cyclization conditions (CSA, TBAF, toluene, 100 °C) resulted in the global deprotection of all protecting groups and cyclization to a product with the desired mass.



Scheme 2.37: Preparation of pentacyclic guanidinium analog **2-170**.

To further probe the viability of this approach in preparation of pentacyclic guanidinium core, the use of secondary silyl protected ether in place of the primary TBS protected silyl ether was explored (Scheme 2.39). Intermediate **2-171** was prepared using the already established synthetic protocol in similar yield. Deprotection of intermediate **2-171** under acidic conditions allowed for a cleaner reaction. This was followed by installation of the protected guanidine moiety to furnish intermediate **2-172**. Compound **2-172** was directly subjected to heating under acidic conditions which resulted in global deprotection and cyclization to provide the pentacyclic guanidinium analog **2-171**. We are currently focused on optimizing this sequence to gain access to **2-171** in pure form.



Scheme 2.38: Preparation of pentacyclic guanidinium analog **2-171**.

2.4 Conclusions

During the course of our studies, the pentacyclic guanidinium core of the proapoptotic monanchocidin family of natural products was disconnected in a number of ways and many different approaches and their revisions were evaluated. Our initial studies focused on the preparation of an elusive eight-membered β -keto lactone using a ring closing metathesis reaction. Unfortunately, all attempts at preparing this compound were unsuccessful.

As a result, our focus was evolved to a different strategy, which relied on the preparation of a β -lactam building block to overcome the stereochemical challenges which have plagued even the most efficient previous approaches. In our initial studies, a number of reacting substrates generated through many revisions to our synthetic sequence were exploited. However, most attempted reactions were unsuccessful. After extensive exploration, we were able to devise a stereoselective, high yielding, and an expedient synthetic sequence for the preparation of *N*-aryl substituted β -lactam units using a seldom-employed method of nucleophilic displacement. With ample quantities of the desired β -lactam unit in hand, we were able to further explore and optimize the subsequent functionalization of the C3 position using deprotonation, followed by acylation or alkylation. Moreover, further functionalization of the alkene moiety using the appropriate side chain were accomplished in excellent yields following optimization. We are currently exploring the various possibilities of deprotection conditions of the β -lactam building block, followed by the subsequent intramolecular aza-Michael cyclization.

To date, substantial progress has been made in preparation of several key intermediates en route to the guanidinium core and the utility of the pentacycle-forming biomimetic cascade has been successfully demonstrated using model system studies.

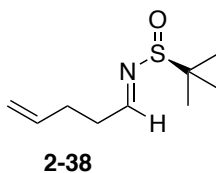
We have also devised a short and flexible synthetic sequence for the preparation of pentacyclic guanidinium natural products devoid of the ester linkage at the C14 positions such as crambescidin 359. We have successfully prepared gram quantities of the key intermediate in our synthesis. We are currently exploring and optimizing further functionalization of this key intermediate. Efforts in this area have also enabled the preparation of simplified analogs. Our work using the simplified analog system assures us that this transformation will proceed to yield the target product, while providing compounds for structure-activity studies.

Overall, our synthetic route combines asymmetric synthesis with biomimetic cascades to provide greatly increased efficiency and selectivity. Furthermore, our approach should allow for pinpoint modification of the molecules' complex functionality to further optimize its potent biological activity, uncover its mechanism of action, and potentially develop simplified lead molecules for chemical probe development.

2.5 Experimental Section

General considerations: THF and CH₂Cl₂ were purified using an alumina filtration system. Ethyl magnesium bromide, thiophenol, diisopropyl amine, δ -valerolactone, Ti(OEt)₄, *cis*-heptenal, anilines, 4M solution of hydrochloric acid in dioxane, acrolein, DIBAL-H, anhydrous DMSO and anhydrous DMF were purchased from Sigma-Aldrich and Fisher Scientific and were used without further purification. Reactions were monitored by TLC analysis (EM Science pre-coated silica gel 60 F₂₅₄ plates, 250 μ m layer thickness) and visualization was accomplished with a 254 nm UV light and by staining with a KMnO₄ solution (1.5 g of KMnO₄, 10 g of K₂CO₃, and 1.25 mL of a 10% NaOH solution in 200 mL of water). Reactions were also monitored by LC-MS (Shimadzu LC-MS 2020 with Kinetex 2.6 μ m C18 50 x 2.10 mm). Flash chromatography on SiO₂ was used to purify the crude reaction mixtures and performed on a Biotage Isolera utilizing Biotage cartridges and linear gradients. Melting points were determined using a Thomas Hoover Capillary Melting Point Apparatus. Infrared spectra were determined on a Jasco FT/IR-4100 spectrometer. ¹H, ¹³C spectra were obtained on a Varian Mercury-VX 300, a Varian Mercury-VX 400, or a Varian Mercury-Plus 300 instrument in CDCl₃ unless otherwise noted. Chemical shifts were reported in parts per million with the residual solvent peak used as an internal standard (CDCl₃ = 7.26 ppm for ¹H and 77.23 ppm for ¹³C). ¹H NMR spectra were run at 300 or 400 MHz and are tabulated as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, dt = doublet of triplet, ddd = doublet of doublet of doublet, qd = quartet of doublets), number of protons, and coupling constant(s). ¹³C NMR spectra were run at 100 MHz using a proton-decoupled pulse sequence with a d₁ of

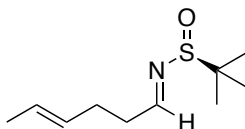
1 second unless otherwise noted, and are tabulated by observed peak. High-resolution mass spectra were obtained on a Thermo Fisher Scientific, Exactive Plus mass spectrometer using Heated Electrospray Ionization (HESI).



(*S,E*)-2-Methyl-*N*-(pent-4-en-1-ylidene)propane-2-sulfonamide (2-38).⁵⁶ **General**

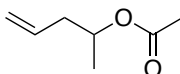
Protocol A. A solution of oxalyl chloride (3.09 g, 24.4 mmol) in CH₂Cl₂ (44.0 mL) in a round bottom flask, equipped with a Claisen adaptor and an addition funnel connected to a nitrogen line was cooled to -78 °C. Anhydrous DMSO (3.81 g, 48.8 mmol) was added to this solution in a drop-wise manner and the mixture was stirred for 20 min at -78 °C, followed by the drop-wise addition of 4-penten-1-ol (1.50 g, 17.4 mmol). The mixture was stirred for an additional 45 min, followed by the addition of NEt₃ (7.93 g, 78.4 mmol). The suspension was allowed to warm to rt gradually, quenched with sat. aq. NH₄Cl (30.0 mL). The layers were separated, and the aqueous layer was extracted with CH₂Cl₂. The combined organic layers were washed with brine, and dried (MgSO₄). The crude (*E*)-4-hexenal mixture was filtered into a round bottom flask equipped with a magnetic stir bar and placed under an atmosphere of nitrogen. The flask was charged with (*S*)-*tert*-butanesulfonamide (1.15 g, 17.4 mmol), followed by the addition of Ti(OEt)₄ (6.26 g, 22.0 mmol), and the mixture was stirred at rt overnight. The reaction was then poured into a rapidly stirring solution of brine, filtered through Celite®, extracted with EtOAc, washed with brine, dried (MgSO₄), and concentrated

under reduced pressure to afford the crude product as a brown oil. The residue was purified by flash column chromatography on SiO₂ (hexanes:EtOAc, 0 to 100%) to provide 1.56 g (88%) of **2-83** as a yellow oil: ¹H NMR (300 MHz, CDCl₃) δ 8.08 (t, 1 H, *J* = 4.5 Hz), 5.89-5.77 (m, 1 H), 5.10-5.04 (m, 2 H), 2.63 (m, 2 H), 2.40 (m, 2 H), 1.19 (s, 9 H); ESIMS *m/z* 229 [M+MeCN]⁺.



2-44

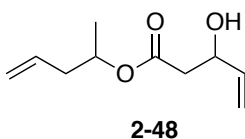
(*S,E*)-2-Methyl-*N*-(pent-4-en-1-ylidene)propane-2-sulfinamide (2-44). According to General Protocol A, *trans*-hex-4-ene-1-ol (2.00 g, 19.2 mmol) afforded 2.75 g (86%) of **2-44** as a yellow oil following purification on SiO₂ (hexanes:EtOAc, 0 to 100%): ¹H NMR (300 MHz, CDCl₃) δ 8.05 (t, 1 H, *J* = 4.5 Hz), 5.47-5.44 (m, 2 H), 2.55 (m, 2 H), 2.29 (q, 2 H, *J* = 7.2, 6.6 Hz), 1.64 (d, 3 H, *J* = 4.5 Hz), 1.19 (s, 9 H); ESIMS *m/z* 243 [M+MeCN]⁺.



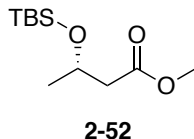
2-47

Pent-4-en-2-yl 3-hydroxypent-4-enoate (2-47).⁵⁷ A round bottom flask equipped with a magnetic stir bar was charged with 4-penten-2-ol (500 mg, 5.57 mmol), DMAP (17.0 mg, 0.0250 mmol), pyridine (11.0 mg, 0.140 mmol) and CH₂Cl₂ (50.0 mL). Acetic anhydride (1.31 g, 12.8 mmol) was added and the mixture was stirred for 2 h. The progression of the reaction was monitored using TLC (20:80 EtOAc:hexanes). Upon completion, the reaction was quenched with sat. aq. NH₄Cl (10.0 mL), the layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried

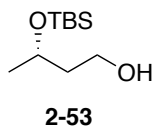
(Na₂SO₄), and concentrated under reduced pressure. The crude colorless oil, which contained CH₂Cl₂, was further purified by vacuum distillation using a dry ice/acetone cold bath to afford 530 mg (74%) of **2-47** as a colorless oil: ¹H NMR (300 MHz, CDCl₃) δ 5.81-5.65 (m, 1 H), 5.10-5.02 (m, 2 H), 4.98-4.89 (m, 1 H), 2.38-2.252 (m, 2 H), 1.98 (s, 3 H), 1.20 (d, 3 H, *J* = 6.6 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 170.3, 133.5, 117.4, 69.8, 40.1, 21.1, 19.2.



Pent-4-en-2-yl 3-hydroxy-4-enoate (2-48).⁵⁸ A flame-dried 250-mL round bottom flask equipped with a Claisen adaptor and a stir bar was charged with a solution of LiHMDS (1.00 M in THF, 10.1 mmol) under a stream of nitrogen. The flask was cooled to -78 °C and compound **2-47** (1.18 g, 9.11 mmol) was added in a drop-wise manner. The mixture was stirred at -78 °C for 1 h, followed by the rapid addition of a solution of acrolein (0.511 g, 9.11 mmol) in THF (46.0 mL). The mixture was stirred for 10 min and quenched with sat. aq. NH₄Cl, and extracted with Et₂O. The combined organic extracts were washed with brine, washed with water, dried (MgSO₄), and concentrated under reduced pressure to afford 1.42 g (85%) of **2-48** as a yellow oil which was used without further purification: ¹H NMR (300 MHz, CDCl₃) δ 5.92-5.66 (m, 1 H), 5.35-5.0 (m, 5 H), 4.61-4.45 (m, 1 H), 2.56-2.53 (m, 2 H), 2.35-2.25 (m, 2 H), 2.17 (s, 1 H), 1.24 (d, 3 H, *J* = 6.0 Hz); ESIMS *m/z* 185 [M+H]⁺.

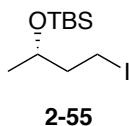


(S)-Methyl 3-((tert-butyldimethylsilyl)oxy)butanoate (2-52).⁵⁹ To a solution of methyl (*S*)-(+)-hydroxybutyrate (50.0 mg, 4.23 mmol) in CH₂Cl₂ (100.0 mL), imidazole (1.73 g, 25.4 mmol), and TBSCl (1.95 g, 12.7 mmol) were added at 0 °C. The reaction was stirred for 10 min at 0 °C, and allowed to warm to rt overnight. The mixture was quenched with sat. aq. NH₄Cl, extracted with CH₂Cl₂, washed with brine, dried (Na₂SO₄), concentrated under reduced pressure to provide the crude product as a yellow oil. The residue was purified using flash chromatography (hexanes:EtOAc, 0 to 10%) to provide 0.98 g (94%) of **2-52** as a colorless oil: ¹H NMR (400 MHz, CDCl₃) δ 4.22-4.30 (m, 1 H), 3.65 (s, 3 H), 2.47 (dd, 1 H, *J* = 14.7, 7.6 Hz), 2.36 (dd, 1 H, *J* = 14.4, 5.3 Hz), 1.18 (d, 3 H, *J* = 6.0 Hz), 0.85 (s, 9 H), 0.05 (s, 3 H), 0.03 (s, 3 H). ¹³C NMR (100 MHz, CDCl₃) δ 172.1, 65.7, 51.4, 44.6, 25.7, 23.9, 17.9, -4.5, -5.2.



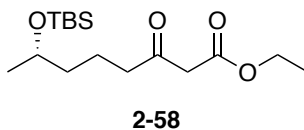
(S)-3-((tert-Butyldimethylsilyl)oxy)butan-1-ol (2-53).⁵⁹ To a stirred solution of compound **2-52** (4.23 g, 18.2 mmol) in CH₂Cl₂ (15.0 mL) was added DIBAL-H (1M in hexane, 19.0 mL, 19.0 mmol) at -78 °C in a drop-wise manner. The mixture was stirred for 30 min at -78 °C, and then for 1.5 h at -20 °C. The reaction mixture was allowed to reach -10 °C, followed by addition of methanol (55.0 mL) and sat. aq. potassium sodium tartrate (220.0 mL). The mixture was vigorously stirred overnight until both phases became clear. The organic layer

was separated and the aqueous layer was extracted with CH₂Cl₂. The combined organic layers were washed with brine, washed with water, dried (MgSO₄), and concentrated under reduced pressure to afford 3.63 g (98%) of **2-53** as a colorless oil: R_f (hexanes/ethyl acetate, 8:2) 0.37; ¹H NMR (400 MHz, CDCl₃) δ 4.14-4.06 (m, 1 H), 3.87-3.78 (m, 2 H), 1.82-1.58 (m, 2 H), 1.20 (d, 3 H, *J* = 6.0 Hz), 0.88 (s, 9 H), 0.09 (s, 3H), 0.08 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 68.5, 60.4, 40.4, 25.8, 23.4, 17.9, -4.4, -5.0.

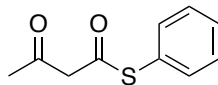


(S)-tert-Butyl((4-iodobutan-2-yl)oxy)dimethylsilane (2-55).^{51,59} To a solution of alcohol **2-53** (92.0 mg, 4.50 mmol) and NEt₃ (2.05g, 20.3 mmol) in CH₂Cl₂ (40.0 mL) was added methanesulfonyl chloride (88.0 mg, 7.65 mmol) in a drop-wise manner at -50 °C. The mixture was allowed to reach -30 °C and stirred for an additional 2 h at this temperature, then quenched with sat. aq. NaHCO₃ (30.0 mL), extracted with CH₂Cl₂, washed with sat. aq. NaHCO₃, dried (Na₂SO₄), and concentrated under reduced pressure to provide the crude mesylate as a yellow oil. The crude mesylate was then dissolved in acetone (45.0 mL), followed by the addition of NaI (3.71 g, 24.8 mmol). The mixture was heated to reflux for 5 h. After cooling, the resulting suspension was filtered and the solid was thoroughly washed with Et₂O. The combined organic layers were washed with 10% sodium bisulfite, brine, and water consecutively, dried (MgSO₄), and concentrated under reduced pressure to afford the crude **2-55** as a light yellow oil. The oil was triturated with hexane (3x 30.0 mL) and the combined triturates were concentrated under reduced pressure to provide 1.30 g of **2-55** (92%) as a yellow oil: R_f (hexanes/ethyl acetate, 9:1) 0.71; ¹H NMR (400 MHz, CDCl₃) δ

3.90-3.84 (m, 1 H), 3.27-3.02 (m, 2 H), 1.97-1.87 (m, 2 H), 1.16 (d, 3 H, $J = 6.1$ Hz), 0.89 (s, 9 H), 0.09 (s, 3H), 0.08 (s, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 68.5, 43.2, 25.8, 23.5, 17.9, 3.5, -4.2, -5.6.

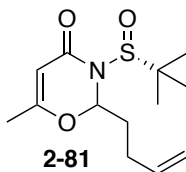


(S)-Ethyl-7-((tert-butyldimethylsilyl)oxy)-3-oxooctanoate (2-58).⁵² To a suspension of NaH (0.124 g, 3.11 mmol, 60% dispersion in mineral oil) in THF (3.00 mL) was added ethyl acetoacetate (0.368 g, 3.83 mmol) in a drop-wise manner at 0 °C. The resulting mixture was stirred for 10 min, followed by the addition of *n*-butyllithium (1.29 mL, 2.83 mmol, 2.2 M in hexane) at 0 °C. The resulting orange solution was stirred at 0 °C for an additional 10 min, followed by the addition of iodide **2-55** (0.978 g, 3.11 mmol) at 0 °C. The mixture was stirred 0 °C for 10 min and then allowed to warm to rt, cooled to 0 °C again and quenched with 3.5 M HCl, extracted with Et_2O , washed with 5% aqueous $\text{Na}_2\text{S}_2\text{O}_3$, washed with 30% NaHCO_3 , washed with brine, and dried (MgSO_4). The filtrate was concentrated under reduced pressure and purified using flash chromatography on SiO_2 (hexanes:EtOAc, 0 to 20%) to yield 0.674 g (75%) **2-58** as a yellow oil: R_f (hexanes/ethyl acetate, 80:20) 0.56; ^1H NMR (400 MHz, CDCl_3) δ 4.97 (s, 0.01 H, enol), 4.22 (q, 2 H, $J = 4.2$ Hz), 3.81-3.75 (m, 1 H), 3.42 (s, 2 H), 2.54 (t, 2 H, $J = 7.5$ Hz), 2.19 (t, 0.2 H, $J = 7.5$ Hz, enol), 1.71-1.60 (m, 2 H), 1.43-1.33 (m, 2 H), 1.28 (t, 3 H, $J = 1.3$ Hz), 1.09 (d, 3 H, $J = 6.1$ Hz), 0.89 (s, 9 H), 0.02 (s, 3 H), 0.04 (s, 6 H); ^{13}C NMR (100 MHz, CDCl_3) δ 202.5, 178.6 (enol), 172.7 (enol), 167.1, 89.0 (enol), 68.1, 61.1, 59.8 (enol), 49.1, 43.2, 38.8, 34.9, 25.8, 23.4, 22.3 (enol), 19.6, 18.0, 14.2 (enol), 14.1, 3.39 (enol), -4.45, -4.80.



2-80

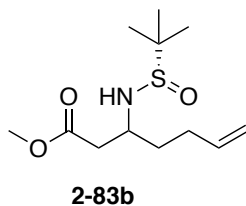
Phenyl thioacetoacetate (2-80).⁵³ A solution of thiophenol (736 mg, 6.68 mmol) and 2,2,6-trimethyl-4H-1,3-dioxin-4-one (1.00 g, 6.68 mmol) in toluene (15.0 mL) was heated to reflux for 3 h. The reaction was then cooled to rt and concentrated under reduced pressure to afford a dark brown residue. The residue was purified using flash chromatography on SiO₂ (hexanes:EtOAc, 0 to 30%) to provide 1.11 g (86%) of **2-80** as a light orange oil: R_f (hexanes/EtOAc, 90:10) 0.26; ¹H NMR (400 MHz, CDCl₃) δ 7.45 (m, 1.02 H, enol), 7.44 (s, 5 H), 5.49 (s, 0.46 H, enol), 3.76 (s, 2 H, enol), 2.29 (s, 3 H), 1.96 (s, 2 H, enol); ¹³C NMR (100 MHz, CDCl₃) δ 199.8, 174.9, 135.2, 134.6, 130.1 (enol), 129.8 (enol), 129.6, 129.4, 127.1, 58.0, 30.6, 21.4 (enol).



2-81

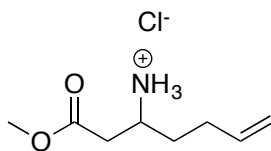
2-(But-3-en-1-yl)-3-((S)-tert-butylsulfinyl)-6-methyl-2H-1,3-oxazin-4(3H)-one (2-81). To a solution of imine **2-38** (50.0 mg, 0.272 mmol) in CH₂Cl₂ (4.70 mL) was added thioester **2-80** (0.158 g, 0.815 mmol) and silver trifluoroacetate (0.186 g, 0.978 mmol) under an argon atmosphere at rt. The mixture was stirred at rt for 5 h, diluted with CH₂Cl₂, filtered through a pad of Celite[®], and concentrated under reduced pressure to afford the crude product as a brown oil. The crude product was purified using flash chromatography on SiO₂ (hexanes:EtOAc, 0 to 100%) to afford 12.5 mg (17%) of **2-81** as a 2:1 mixture of diastereomers: ¹H NMR (400 MHz, CDCl₃) δ 5.83-5.72 (m, 2.5 H, major and minor), dd

(5.66, 0.50 H, $J = 10.2, 4.1$ Hz, minor), 5.30-5.29 (m, 1 H, major), 5.28-5.27 (m, 0.50 H, minor), 5.11-4.98 (m, 3.0 H, major and minor), 2.68-2.58 (m, 1 H, major), 2.45-2.35 (m, 0.50 H, minor), 2.22-2.13 (m, 2 H, major), 2.12-2.07 (m, 1.5 H, minor), 2.00 (s, 1.50 H, minor), 1.97 (s, 1 H, major), 1.77-1.68 (m, 0.50 H, minor), 1.61-1.52 (m, 1 H, major), 1.23 (s, 4.5 H, minor), 1.22 (s, 9 H, major); ^{13}C NMR (100 MHz, CDCl_3) δ 167.3, 166.3 (minor), 163.8 (minor), 162.8, 136.6 (minor), 136.2, 116.7, 116.0 (minor), 100.0 (minor), 99.2 (major), 83.0 (minor), 78.8, 61.7 (minor), 59.5, 32.3 (minor), 31.6, 30.1, 29.4 (minor), 22.8, 22.1 (minor), 20.3, 20.0 (minor); IR (neat) 2974, 1719, 1666, 1400, 1358, 1152, 811 cm^{-1} ; ESIMS m/z 313 $[\text{M}+\text{MeCN}]^+$; HRMS m/z calculated for $\text{C}_{13}\text{H}_{21}\text{NO}_3\text{SNa}$ $[\text{M}+\text{Na}]^+$ 294.1134, found 294.1123.



Methyl-3-(((S)-tert-butylsulfinyl)amino)hept-6-enoate (2-83b).³¹ A flamed dried 25-mL round bottom flask equipped with a magnetic stir bar and a Claisen adaptor and a reflux condenser was charged with a mixture of freshly activated Zn dust (1.39 g, 21.2 mmol) and Cu(I)Cl (0.210 g, 2.12 mmol), which were ground in a mortar and pestle. The two solids were stirred under a slow stream of nitrogen while the flask was heated with a flame. The flask was allowed to cool to rt, followed by the addition of THF (5.30 mL) to produce a dark slurry. The resulting reaction mixture was heated to reflux and stirred vigorously for 30 min. Ethyl bromoacetate (0.885 g, 5.30 mmol) in dry THF (2.00 mL) was added drop-wise. Once the addition was completed, the reaction mixture was stirred for an additional 30 min at rt,

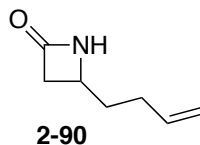
then at 50 °C for 30 min. The mixture was then cooled to 0 °C, followed by the drop-wise addition of a solution of imine **2-38** (42.7 mg, 2.12 mmol) in THF (3.00 mL). The mixture was stirred for an additional 4 h at 0 °C, and filtered through a pad of Celite[®]. The milky white mixture was washed with 0.25 M aq. citric acid solution, washed with sat. aq. NaHCO₃, washed with brine, dried (Na₂SO₄), and concentrated under reduced pressure to provide 61.4 mg (93%) of **2-83b** as a colorless oil, which was used without further purification: ¹H NMR (400 MHz, CDCl₃) δ 5.49-5.30 (m, 2 H), 4.15 (d, 1 H, *J* = 3.0 Hz), 4.14 (q, 2 H, *J* = 7.2 Hz), 3.53 (s, 3 H), 2.82-2.77 (dd, 1 H, *J* = 16.1, 5.2 Hz), 2.60-2.54 (dd, 1 H, *J* = 16.1, 5.2 Hz), 2.14-1.94 (m, 2 H), 1.63-1.40 (m, 2H), 1.63 (d, 3 H, *J* = 6.6 Hz), 1.26 (t, 3 H, *J* = 4.2 Hz), 1.22 (s, 9 H); ¹³C NMR δ 172.7, 130.1, 126.4, 60.8, 53.5, 40.7, 35.5, 29.2, 22.9, 18.2, 14.4; IR (neat) 3440, 3077, 2870, 1737, 1641 cm⁻¹; ESIMS *m/z* 290 [M+H]⁺.



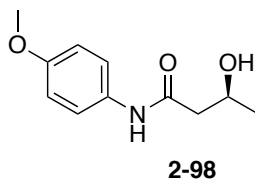
2-91b

(S)-Methyl-3-ammoniohept-6-enoate chloride (2-91b).⁵⁶ To a solution of compound **2-83b** (0.143 g, 0.518 mmol) in methanol (5.00 mL) was added hydrochloric acid in dioxane (4 M, 0.26 mL, 1.04 mmol). The mixture was stirred at rt for 3 h and then concentrated under reduced pressure, and the residue was washed with Et₂O to remove the impurities. The residue was further evaporated to afford 0.884 g (66%) of **2-91b** a yellow waxy solid: ¹H NMR (400 MHz, CDCl₃) δ 8.39 (br s, 3 H), 5.78-5.76 (m, 1 H), 5.15-5.02 (m, 1 H), 3.75 (s, 3 H), 3.71-3.59 (m, 1 H), 2.94-2.76 (m, 2 H), 2.26-2.23 (m, 2 H), 2.14-2.05 (m, 1 H), 1.85-1.76

(m, 1H); ESIMS m/z 159 $[M+H]^+$.

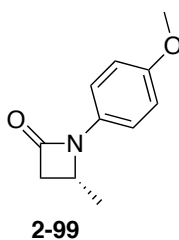


(S)-4-(But-3-en-1-yl)azetidin-2-one (2-90). To a THF (0.60 mL) solution of compound **2-91b** (0.100 g, 0.436 mmol) was added a 2.6 M THF solution of ethylmagnesium bromide (0.671 mL, 1.75 mmol) at 0 °C under an argon atmosphere. The mixture was stirred for 3 h at 0 °C, and followed using TLC (50:50 EtOAc:hexanes). After 3 h, the reaction was quenched with a solution of sat. aq. NH_4Cl , extracted with EtOAc, washed with brine, dried (MgSO_4), concentrated under reduced pressure to afford the crude product as a brown oil. The residue was purified using flash chromatography on Si_2O (hexanes:EtOAc, 0 to 100%) to provide 7.89 mg (14%) of **2-90** as a light yellow oil: ^1H NMR (400 MHz, CDCl_3) δ 6.14 (brs, 1 H), 5.84-5.74 (m, 1 H), 5.06-4.99 (m, 2 H), 3.65-3.60 (m, 1 H), 3.08-3.03 (ddd, 1 H, $J = 14.8, 5.0, 2.2$ Hz), 2.60-2.56 (ddd, 1 H, $J = 14.8, 2.4, 1.3$ Hz), 2.14-1.94 (m, 2 H), 1.79-1.70 (m, 2 H); ^{13}C NMR (100 MHz, CDCl_3) δ 168.8, 130.4, 126.9, 56.9, 48.7, 44.3, 35.9, 30.3, 24.9, 18.6; IR (neat) 3254, 2961, 2933, 1753, 1382, 667 cm^{-1} . Compound is unstable to LCMS analysis.



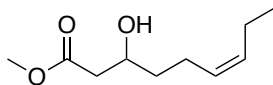
(S)-3-Hydroxy-N-(4-methoxyphenyl)butanamide (2-98).⁶⁰ A solution of *n*-butyllithium (1.84 M in hexane, 8.09 mmol, 4.40 mL) was added to *p*-anisidine (1.03 g, 8.28 mmol) in

THF (1.60 mL) at -78 °C and was stirred at this temperature for 20 min before warming to rt gradually. After 10 min, the solution was cooled to -78 °C, followed by the addition of methyl (*S*)-(+)-3-hydroxy butyrate (0.449 g, 3.76 mmol) in THF (1.60 mL). The mixture was stirred at -78 °C for 40 min, and quenched with sat. aq. NH₄Cl (6.50 mL). The mixture was allowed to warm to rt, followed by the addition of EtOAc. The aqueous phase was extracted with EtOAc, and the combined organic layers were washed with 10% aqueous hydrochloric acid until TLC showed the absence of *p*-anisidine. The mixture was further washed with brine, dried (MgSO₄), and concentrated under reduced pressure to provide the crude product. The residue was purified using flash chromatography on Si₂O (hexanes:EtOAc, 1 to 100%) to afford a brown solid which was further recrystallized from a mixture of hexanes and EtOAc to afford 0.57 g (57%) of **2-98** as a off-white solid: ¹H NMR (300 MHz, CDCl₃) δ 7.59 (brs, 1 H), 7.39 (d, 2 H, *J* = 9.0 Hz), 6.85 (d, 2 H, *J* = 9.0 Hz), 4.29 (m, 1 H), 3.79 (s, 3 H), 3.32 (d, 1 H, *J* = 2.9 Hz), 2.51-2.46 (m, 2 H), 1.28 (d, 3 H, *J* = 6.0 Hz); ESIMS *m/z* 210 [M+H]⁺.



(*R*)-1-(4-Methoxyphenyl)-4-methylazetidin-2-one (2-99).⁶¹ To a solution of **2-98** (0.0736 g, 0.348 mmol) in THF (5.70 mL) at 0 °C was added DIAD (96.4 mg, 0.453 mmol) and P(OEt)₃ (88.6 mg, 0.522 mmol). The mixture was stirred overnight and quenched with sat. aq. NH₄Cl (1.50 mL), concentrated under reduced pressure and the residue was purified

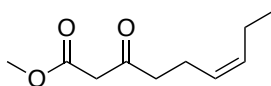
using flash chromatography on Si₂O (hexanes:EtOAc, 10 to 100%): ¹H NMR (400 MHz, CDCl₃) δ 7.32 (d, 2 H, *J* = 9.1 Hz), 6.86 (d, 2 H, *J* = 9.1 Hz), 4.07-4.14 (m, 1 H), 3.74 (s, 3 H), 3.21-3.16 (dd, 1 H, *J* = 14.9, 5.3 Hz), 2.65-2.60 (dd, 1 H, *J* = 14.9, 2.4 Hz), 2.14-1.94 (m, 1 H), 1.45 (d, 3 H, *J* = 6.1 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 163.7, 155.7, 130.1, 126.9, 118.0, 114.2, 55.26, 47.0, 43.6.



2-104

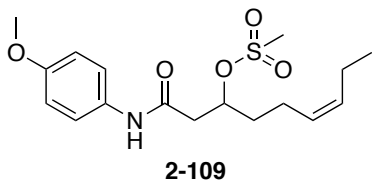
Methyl-(*Z*)-3-hydroxynon-6-enoate (2-104). A solution of *n*-butyllithium (28.8 mmol, 11.6 mL, 2.47 M in hexanes) was added to a solution of diisopropylamine (3.22 g, 31.8 mmol) in THF (80.0 mL) at -78 °C and the mixture was stirred for 15 min. To this light yellow solution was added methyl acetate (2.29 g, 30.8 mmol) in a drop-wise manner at -78 °C and the mixture was stirred for an additional 20 min, followed by the addition of *cis*-4-heptenal (1.20 g, 10.3 mmol) in THF (1.40 mL). The reaction was stirred for 2 h and quenched with sat. aq. NH₄Cl, and concentrated under reduced pressure to remove THF. The residue was then extracted with Et₂O, washed with brine, dried (Na₂SO₄), and concentrated under reduced pressure to provide the crude product as a yellow oil. The residue was purified by flash chromatography on SiO₂ (hexanes:EtOAc, 0 to 40%) to yield 1.64 g (86%) of **2-104** as a yellow oil: R_f (hexanes/EtOAc, 80:20) 0.22; ¹H NMR (300 MHz, CDCl₃) δ 5.45-5.27 (m, 2 H), 4.18-3.96 (m, 1 H), 3.71 (s, 3 H), 2.86 (brs, 1 H), 2.55-2.38 (m, 2 H), 2.20-2.12 (m, 2 H), 2.10-2.00 (m, 2 H), 2.20-2.12 (m, 2 H), 1.65-1.40 (m, 2 H), 0.95 (t, 3 H, *J* = 7.5 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 173.6, 132.8, 128.2, 67.7, 52.0, 41.3, 36.6, 23.4, 20.7, 14.5; IR

(neat) 3444, 2967, 1737, 1303, 877 cm^{-1} ; ESIMS m/z 187 $[\text{M}+\text{H}]^+$; HRMS m/z calculated for $\text{C}_{10}\text{H}_{19}\text{NO}_3$ $[\text{M}+\text{H}]^+$ 187.1329, found 187.1327.



2-105

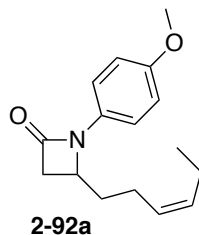
Methyl-(Z)-3-oxonon-6-enoate (2-105). To a solution of **2-104** (1.63 g, 8.70 mmol) in anhydrous CH_2Cl_2 (44.0 mL) was added Dess-Martin periodinane (4.06 g, 9.57 mmol) at 0 $^\circ\text{C}$. The mixture was stirred at 0 $^\circ\text{C}$, and followed using TLC (20:80 EtOAc:hexanes). Upon completion, the mixture was quenched with sat. aq. NaHCO_3 and sat. aq. Na_2SO_4 and stirred for an additional 40 min. The mixture was extracted with CH_2Cl_2 , washed with brine, dried (MgSO_4), and concentrated under reduced pressure to afford the crude product as a mixture of colorless oil and solid. The residue was purified using flash column chromatography on SiO_2 (hexanes:EtOAc, 0 to 100%) to provide 920 mg (57%) of **2-105** as a colorless oil: R_f (hexanes/EtOAc, 80:20) 0.44; ^1H NMR (400 MHz, CDCl_3) δ 5.45-5.23 (m, 2 H), 3.74 (s, 3 H), 3.45 (s, 2 H), 2.59 (t, 3 H, $J = 7.4$ Hz), 2.33 (m, 2 H), 2.08-2.00 (m, 2 H), 0.96 (t, 3 H, $J = 7.5$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 202.3, 167.7, 133.1, 126.6, 52.3, 49.1, 43.0, 21.3, 20.5, 14.2; IR (neat) 3003, 2963, 1725, 1748, 1323, 1248 cm^{-1} ; HRMS m/z calculated for $\text{C}_{10}\text{H}_{17}\text{O}_3$ $[\text{M}+\text{H}]^+$ 185.1172, found 185.1167.



(Z)-1-((4-Methoxyphenyl)amino)-1-oxonon-6-en-3-yl-methanesulfonate (2-109). General

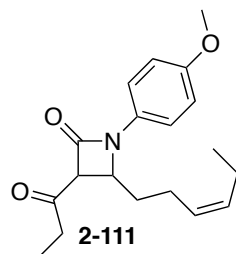
protocol B. To a solution of anisidine (2.58 g, 20.7 mmol) in CH₂Cl₂ (80.0 mL) at 0 °C was added 2 M Al(CH₃)₃ solution in hexanes (20.8 mmol, 10.3 mL). The mixture was stirred for 30 min at 0 °C, and allowed to warm to rt over 1 h, and stirred for an additional 15 min at rt. A solution of **2-104** (1.93 g, 10.3 mmol) in CH₂Cl₂ (10.0 mL) was then added in a drop-wise manner at 0 °C and the mixture was allowed to warm to rt overnight. The mixture was cooled to 0 °C and quenched by slow addition of water, and acidified to pH 6 at 0 °C. The aqueous layer was then extracted with CH₂Cl₂ and the combined organic layers were washed with 10% HCl to remove excess *p*-anisidine, washed with brine, dried (Na₂SO₄), and concentrated under reduced pressure to afford the crude as a brown residue. The residue was triturated with hexanes to afford 2.56 g (89%) of β-hydroxy amide **2-108** as a silver colored solid. The solid (2.02 g, 7.21 mmol) was dissolved in in pyridine (7.20 mL) and cooled to 0 °C, followed by the addition of MsCl (0.996 g, 8.65 mmol) in a drop-wise manner. The mixture was stirred at 0 °C for 1 h and allowed to warm to rt gradually. Upon completion, pyridine was removed under reduced pressure and the residue was purified using flash column chromatography on SiO₂ (hexanes:EtOAc, 12 to 98%) to provide 2.52 g (98%) of **2-109** as a brown oil: ¹H NMR (400 MHz, CDCl₃) δ 7.47 (brs, 1 H), 7.41 (d, 2 H, *J* = 9.0 Hz), 6.86 (d, 2 H, *J* = 8.9 Hz), 5.47-5.30 (m, 2 H), 5.14-5.10 (m, 1 H), 3.79 (s, 3 H), 3.03 (s, 3 H),

2.89-2.66 (m, 2 H), 2.19 (q, 2 H, $J = 7.4$ Hz), 2.07-2.02 (m, 2 H), 1.97-1.89 (m, 2 H), 0.96 (t, 3 H, $J = 7.5$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 167.5, 156.0, 134.5, 133.2, 131.2, 126.9, 121.9, 114.2, 80.8, 55.5, 42.1, 39.9, 38.2, 35.1, 22.8, 20.7, 14.3; IR (neat) 3250, 2931, 2959, 1661, 1536, 1338 cm^{-1} ; ESIMS m/z 356 $[\text{M}+\text{H}]^+$; HRMS m/z calculated for $\text{C}_{17}\text{H}_{26}\text{NO}_5\text{S}$ $[\text{M}+\text{H}]^+$ 356.1562, found 356.1514.



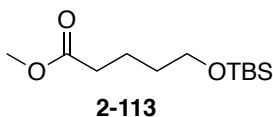
(Z)-4-(Hex-3-en-1-yl)-1-(4-methoxyphenyl)azetidin-2-one (2-92a). General protocol C. A solution of **2-109** (2.78 g, 7.74 mmol) in anhydrous CH_2Cl_2 :DMF (4:1) (7.70 mL) was added to a suspension of NaH (0.403 g, 10.1 mmol, 60% dispersion in mineral oil) in CH_2Cl_2 :DMF (4:1) (10.1 mL) at rt using an addition funnel over a period of 30 min. The mixture was stirred and monitored using TLC (50:50 EtOAc:hexanes). Upon completion (3 h), the CH_2Cl_2 was removed under reduced pressure and the mixture was dissolved in EtOAc, washed with water and brine, dried (MgSO_4), concentrated under reduced pressure to provide the crude product as a brown residue. The residue was purified using flash column chromatography on SiO_2 (hexanes:EtOAc, 0 to 100%) to provide 1.79 g (89%) of **2-92a** as a yellow oil: R_f (hexanes/EtOAc, 50:50) 0.29; ^1H NMR (300 MHz, CDCl_3) δ 7.30 (d, 2 H, $J = 9.1$ Hz), 6.87 (d, 2 H, $J = 9.1$ Hz), 5.47-5.26 (m, 2 H), 4.07-3.99 (m, 1 H), 3.78 (s, 3 H), 3.16 (dd, 1 H, $J = 15.0, 5.3$ Hz), 2.75 (dd, 1 H, $J = 15.0, 2.4$ Hz), 2.15-2.12 (m, 2 H), 2.08-1.97 (m, 2 H), 1.69-1.56 (m, 2 H), 0.96 (t, 3 H, $J = 7.5$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 164.1,

156.1, 133.3, 131.3, 127.2, 118.7, 114.6, 55.7, 55.6, 51.1, 42.4, 32.3, 22.8, 20.7, 14.4; IR (neat) 2963, 1740, 1512, 1382, 1244, 1039 cm^{-1} ; HRMS m/z calculated for $\text{C}_{16}\text{H}_{22}\text{NO}_2$ $[\text{M}+\text{H}]^+$ 260.1645, found 260.1638.

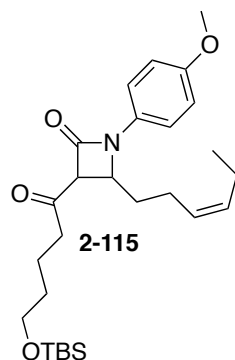


(Z)-4-(Hex-3-en-1-yl)-1-(4-methoxyphenyl)-3-propionylazetidin-2-one (2-111). To a solution of diisopropyl amine (31.2 mg, 0.307 mmol) in THF (0.80 mL) at 0 °C was added a solution of *n*-butyllithium (0.285 mmol, 0.16 mL, 1.77 M in hexanes) under an atmosphere of nitrogen. The mixture was stirred at 0 °C for 30 min (light yellow) and was cooled to -78 °C, followed by the addition of **2-92a** (57.5 mg, 0.220 mmol) in THF (1.00 mL) in a drop-wise manner. The reaction was stirred 40 min (bright yellow), followed by the addition of methyl propionate (58.0 mg, 0.658 mmol). The mixture was stirred for another 30 min and quenched with sat. aq. NH_4Cl , and allowed to warm to rt gradually. The mixture was concentrated under reduced pressure to remove THF, and was extracted with EtOAc. The combined organic layers were washed with brine, dried (MgSO_4), and concentrated under reduced pressure. The residue was purified using flash column chromatography on SiO_2 (hexanes:EtOAc, 5 to 100%) to provide 18.7 mg (27%) of **2-111** as a yellow oil: R_f (hexanes/EtOAc, 80:20) 0.41; ^1H NMR (300 MHz, CDCl_3) δ 7.27 (d, 2 H, $J = 9.0$ Hz), 6.87 (d, 2 H, $J = 8.9$ Hz), 5.45-5.38 (m, 2 H), 4.47 (d, 1 H, $J = 9.6$ Hz), 3.96 (d, 1 H, $J = 2.3$ Hz), 2.86-2.73 (m, 1 H), 2.65-2.51 (m, 1 H), 2.18-2.09 (m, 2 H), 1.97 (q, 2 H, $J = 7.4$ Hz), 1.70-

1.57 (m, 2 H), 1.10 (t, 3 H, $J = 7.5$ Hz), 0.93 (t, 3 H, $J = 7.5$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 202.7, 162.8, 156.6, 133.6, 130.6, 127.1, 119.4, 114.6, 66.8, 55.7, 53.2, 36.3, 31.3, 22.9, 20.7, 14.4, 7.4; IR (neat) 3486, 2855, 1744, 1729, 1095 cm^{-1} ; ESIMS m/z 357 $[\text{M}+\text{H}]^+$; HRMS m/z calculated for $\text{C}_{19}\text{H}_{25}\text{NO}_3\text{Na}$ $[\text{M}+\text{Na}]^+$ 338.1727, found 338.1715.

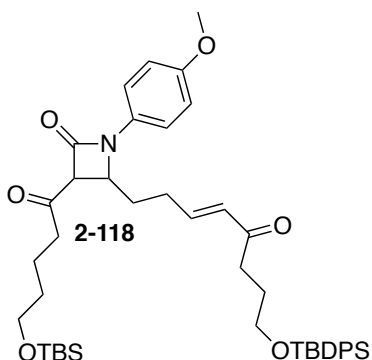


Methyl-5-((*tert*-butyldimethylsilyloxy)pentanoate (2-116b).⁶² To a solution of δ -valerolactone (3.00 g, 22.5 mmol) in CH_3OH (220 mL) was added concentrated H_2SO_4 (11 drops) and the mixture was heated at reflux for 5 h. The mixture was cooled to rt and neutralized with solid NaHCO_3 (1.85 g) at 0 $^\circ\text{C}$. The mixture was stirred for 10 min and was cooled at -30 $^\circ\text{C}$ for 2 h. The cold mixture was filtered and the solvent was removed under reduced pressure. The residue was then dissolved in CH_2Cl_2 (250 mL) and cooled to 0 $^\circ\text{C}$. TBSCl (3.46 g, 22.5 mmol) and imidazole (4.10 g, 426 mmol) were added consecutively and the mixture was stirred at rt overnight. The mixture was then diluted with H_2O , the layers were separated, and the organic layer was washed with water, washed sat. aq. CuSO_4 , washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified using flash column chromatography on SiO_2 (petroleum ether: Et_2O , 0 to 1%) to provide 4.21 g (76%) of **2-116b** as a colorless oil: ^1H NMR (300 MHz, CDCl_3) δ 3.66 (s, 3 H), 3.61 (t, 2 H, $J = 6.2$ Hz), 2.33 (t, 2 H, $J = 7.4$ Hz), 1.73-1.62 (m, 2 H), 1.58-1.51 (m, 2 H), 0.88 (s, 9 H), 0.03 (s, 6 H); ^{13}C NMR (100 MHz, CDCl_3) δ 174.3, 62.7, 51.2, 33.8, 32.1, 26.0, 21.4, 18.3, -5.4.



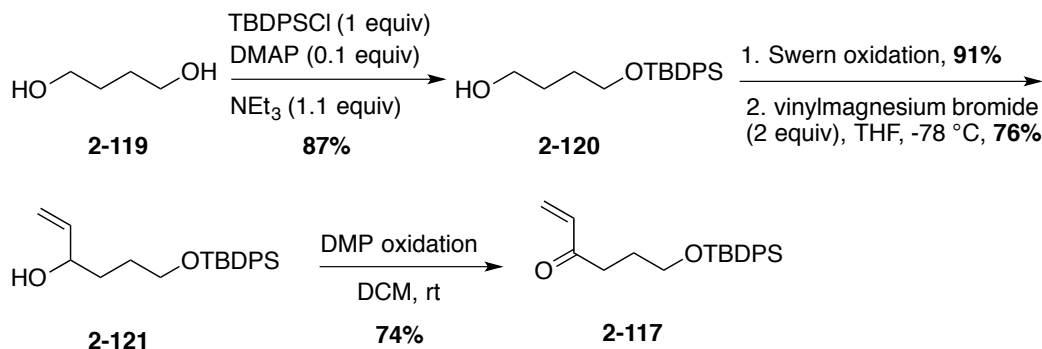
(Z)-3-(5-((*tert*-Butyldimethylsilyloxy)pentanoyl)-4-(hex-3-en-1-yl)-1-(4methoxyphenyl)azetidin-2-one (2-115). **General protocol D.** To a solution of diisopropyl amine (42.4 mg, 0.417 mmol) in THF (1.00 mL) at 0 °C was added a solution of *n*-butyllithium (0.378 mmol, 0.18 mL, 2.11 M in hexanes) under an atmosphere of nitrogen. The mixture was stirred at 0 °C for 15 min and was cooled to -55 °C, followed by the addition of **2-92a** (68.3 mg, 0.261 mmol in THF (0.30 mL) in a drop-wise manner. The reaction was stirred for 30 min (bright yellow), and cooled to -55 °C. Ester **2-113** (0.257 mg, 1.04 mmol) was then added, the mixture was stirred for another 30 min, quenched with sat. aq. NH₄Cl, and allowed to warm to rt gradually. The mixture was concentrated under reduced pressure to remove THF, and was extracted with EtOAc. The combined organic layers were washed with brine, dried (MgSO₄), and concentrated under reduced pressure. The residue was purified using flash column chromatography on SiO₂ (hexanes:EtOAc, 5 to 100%) to provide 85.8 mg (69%) of **2-115** as a yellow oil: ¹H NMR (300 MHz, CDCl₃) δ 7.27 (d, 2 H, *J* = 8.9 Hz), 6.87 (d, 2 H, *J* = 8.9 Hz), 5.45-5.23 (m, 2 H), 4.48-4.42 (m, 1 H), 3.96 (d, 1 H, *J* = 2.4 Hz), 3.78 (s, 3 H), 3.61 (t, 3 H, *J* = 6.2 Hz), 2.82-2.55 (m, 2 H), 2.18-2.06 (m, 2 H), 1.98-1.87 (m, 2 H), 1.72-1.48 (m, 6 H), 0.93 (t, 3 H, *J* = 7.5 Hz), 0.88 (s, 9 H), 0.04 (s, 6 H); ¹³C NMR (100 MHz,

CDCl₃) δ 202.0, 159.8, 156.6, 133.5, 130.5, 127.0, 119.0, 114.6, 67.1, 62.9, 55.7, 53.1, 42.6, 32.2, 31.2, 26.1, 22.9, 20.7, 19.7, 18.5, 14.4, -5.14; IR (neat) 3484, 2856, 1744, 1713, 1512, 1098, 774 cm⁻¹; ESIMS m/z 474 [M+H]⁺; HRMS m/z calculated for C₂₇H₄₃NSO₄SiNa [M+Na]⁺ 496.2854, found 496.2838.

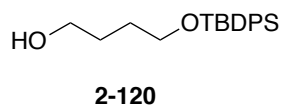


(E)-3-(5-((*tert*-Butyldimethylsilyl)oxy)pentanoyl)-4-(8-((*tert*-butyldiphenylsilyl)oxy)-5-oxooct-3-en-1-yl)-1-(4-methoxyphenyl)azetidin-2-one (2-118). General protocol E. To a solution of **2-115** in degassed CH₂Cl₂ (0.70 mL) was added enone **2-117** (94.8 mg, 0.261 mmol), Hoveyda-Grubbs 2nd generation catalyst (5.20 mg, 8.05 μ mol) under an argon atmosphere. The mixture was stirred at reflux for 48 h. Upon completion, the mixture was concentrated under reduced pressure to afford the crude as a black oil. The residue was purified using flash column chromatography on SiO₂ (hexanes:EtOAc, 2 to 100%) to provide 38.7 mg (77%) of **2-118** as a black oil: ¹H NMR (300 MHz, CDCl₃) δ 7.65-7.62 (m, 4 H), 7.42-7.36 (m, 6 H), 7.27 (d, 2 H, J = 8.9 Hz), 6.88 (d, 2 H, J = 9.1 Hz), 6.76 (dt, 1 H, J = 16.2, 6.7), 6.11 (d, 1 H, J = 16.9 Hz), 4.52-4.47 (m, 1 H), 3.91 (d, 1 H, J = 6.6 Hz), 3.78 (s, 3 H), 3.70 (t, 3 H, J = 6.0 Hz), 3.61 (t, 3 H, J = 6.2 Hz), 2.65-2.60 (m, 4 H), 2.31-2.24 (m, 2 H), 1.98-1.78 (m, 2 H), 1.70-1.47 (m, 6 H), 1.03 (s, 9 H), 0.88 (s, 9 H), 0.04 (s, 6 H); ¹³C NMR

(100 MHz, CDCl₃) δ 210.9, 200.0, 159.5, 156.7, 144.1, 135.7, 133.9, 131.2, 130.3, 129.8, 127.8, 119.0, 114.7, 66.9, 63.2, 62.9, 55.7, 52.7, 45.5, 37.1, 32.3, 29.7, 28.0, 27.1, 27.0, 26.1, 19.7, 19.4, 18.5, -5.12; IR (neat) 2948, 2935, 2856, 1761, 1697, 1516, 1102 cm⁻¹; ESIMS *m/z* 770 [M+H]⁺; HRMS *m/z* calculated for C₄₅H₆₄NSO₆Si₂ [M+H]⁺ 770.4267, found 770.4258.

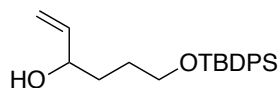


Compound **2-117** was prepared in four steps from the commercially available alcohol **2-119** as described below:



4-((*tert*-Butyldiphenylsilyloxy)butan-1-ol (2-120).⁶³ To a solution of TBDPSCl (1.5 g, 5.35 mmol) and NEt₃ (0.595 g, 5.88 mmol) in CH₂Cl₂ (41.0 mL) was added DMAP (66.0 mg, 0.535 mmol) and 1,4-butanediol (1.41 g, 15.5 mmol) consecutively. The mixture was stirred overnight and quenched with sat. aq. NH₄Cl (33.0 mL), extracted with CH₂Cl₂, dried (MgSO₄), and concentrated under reduced pressure to afford the crude product as a colorless viscous oil. The residue was purified using flash column chromatography on SiO₂ (hexanes:EtOAc, 1 to 80%) to provide 1.53 g (87%) of **2-120** as a colorless oil: R_f

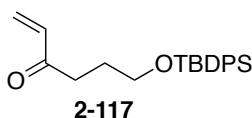
(hexanes/EtOAc, 80:20) 0.19; ^1H NMR (300 MHz, CDCl_3) δ 7.69-7.66 (m, 4 H), 7.45-7.26 (m, 6 H), 3.72-3.65 (m, 4 H), 1.73-1.63 (m, 4 H), 1.06 (s, 9 H); ESIMS m/z 330 $[\text{M}+\text{H}]^+$.



2-121

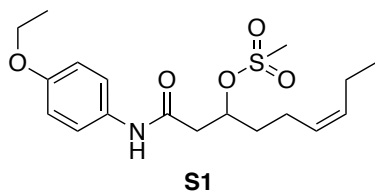
Methyl-5-((*tert*-butyldimethylsilyl)oxy)pentanoate (2-121).^{64,65} To a solution of oxalyl chloride (1.85 g, 14.25 mmol) in anhydrous CH_2Cl_2 (71.0 mL) was added anhydrous DMSO (2.24 g, 28.5 mmol) in CH_2Cl_2 (2.9 mL) at -78 °C and the mixture was stirred for 30 min. A solution of **2-120** (2.34 g, 7.12 mmol) in CH_2Cl_2 (17.0 mL) was added drop-wise and stirring was continued at -78 °C for 45 min, followed by the addition of Et_3N (3.60 g, 35.6 mmol). The reaction was stirred for an additional 20 min at -78 °C, and then for another 30 min at 0 °C, and was then quenched with water (395 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 . The combined organic layers were dried (Na_2SO_4), concentrated under reduced pressure to afford the crude as a colorless oil. The residue was re-dissolved in anhydrous Et_2O and cooled at -30 °C for 3 h. The precipitate was filtered off, and the filtrate was concentrated under reduced pressure and azeotropically co-evaporated with benzene three times to provide 2.12 g (91%) of the corresponding aldehyde as a colorless oil, which was used directly in the next step. A solution of the aldehyde (2.12 g, 6.49 mmol) in dry THF (7.20 mL) was added drop-wise to a 1.0 M solution of vinylmagnesium bromide (8.44 mmol, 8.44 mL) in THF over 15 min at -78 °C. The solution was stirred for 5 min and warmed to 0 °C, and stirred for an additional 15 min. The reaction was then warmed to rt gradually, followed by the addition of 2-propanol (0.50 mL), Et_2O

(20.0 mL) and sat. aq. NH₄Cl (20.0 mL) consecutively. The layers were separated, and the aqueous layer was extracted with Et₂O. The combined organic layers were washed with brine, dried (Na₂SO₄), and concentrated under reduced pressure to yield the crude product. The residue was purified using flash column chromatography on SiO₂ (hexanes:EtOAc, 1 to 40%) to provide 1.75 g (76%) of **2-121** as a colorless oil: R_f (hexanes/EtOAc, 80:20) 0.35; ¹H NMR (300 MHz, CDCl₃) δ 7.70-7.65 (m, 4 H), 7.45-7.34 (m, 6 H), 5.90-5.82 (m, 1 H), 5.23 (dt, 1 H, *J* = 17.2, 1.5 Hz), 5.11 (dt, 1 H, *J* = 10.4, 1.4 Hz), 4.16-4.11 (m, 1 H), 3.66 (t, 2 H, *J* = 5.4 Hz), 1.67-1.64 (m, 4 H), 1.04 (s, 9 H).

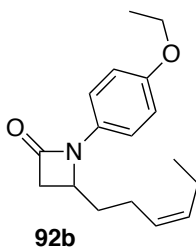


6-((*tert*-Butyldiphenylsilyl)oxy)hex-1-en-3-one (2-117). To a solution of **2-121** (0.51 g, 1.42 mmol) in anhydrous CH₂Cl₂ (7.10 mL) was added Dess-Martin periodinane (0.784 g, 0.185 mmol) at 0 °C and stirred for 5 min. The cold bath was removed, and the reaction was stirred for 1 h and monitored using TLC. Upon completion, the reaction was quenched with sat. aq. NaHCO₃ (2.00 mL) and sat. aq. Na₂S₂O₃ (2.00 mL) and stirred for 40 min. The layers were separated, and the aqueous layer was extracted with CH₂Cl₂, washed with brine, dried (MgSO₄), and concentrated under reduced pressure to afford the crude product as a mixture of yellow oil and solid. The residue was purified using flash column chromatography on SiO₂ (hexanes:EtOAc, 2 to 100%) to provide 371 mg (74%) of **2-117** as a colorless oil: R_f (hexanes/EtOAc, 80:20) 0.58; ¹H NMR (400 MHz, CDCl₃) δ 7.65-7.62 (m, 4 H), 7.66-7.61 (m, 4 H), 7.44-7.36 (m, 6 H), 6.35 (dd, 1 H, *J* = 17.7, 10.3 Hz), 6.21 (dd, 1 H, *J* = 17.6, 1.4 Hz), 5.82 (dd, 1 H, *J* = 10.2, 1.5 Hz), 3.69 (t, 3 H, *J* = 6.1 Hz), 2.71 (t, 3 H, *J* = 7.4 Hz), 1.91-

1.85 (m, 2 H), 1.04 (s, 9 H); ^{13}C NMR (100 MHz, CDCl_3) δ 201.0, 136.8, 135.8, 134.0, 129.8, 127.9, 63.2, 36.2, 27.2, 19.4.

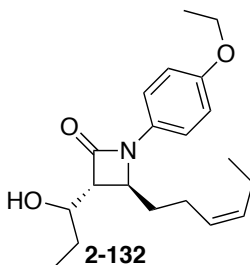


(Z)-1-((4-Ethoxyphenyl)amino)-1-oxonon-6-en-3-yl methanesulfonate (S1). According to general protocol B, **S1** was obtained in 95% yield (0.342 g) as a brown oil following purification by chromatography on SiO_2 (hexanes:EtOAc, 12 to 100%): R_f (hexanes/EtOAc, 50:50) 0.61; ^1H NMR (400 MHz, CDCl_3) δ 7.39 (d, 2 H, $J = 9.0$ Hz), 6.85 (d, 2 H, $J = 6.9$ Hz), 5.47-5.28 (m, 2 H), 5.13-5.08 (m, 1 H), 4.00 (q, 2 H, $J = 7.00$ Hz), 3.03 (s, 3 H), 2.80 (dd, 1 H, $J = 15.0, 4.4$ Hz), 2.69 (dd, 1 H, $J = 15.0, 7.2$ Hz), 2.20-2.16 (m, 2 H), 2.06-2.00 (m, 2 H), 1.99-1.89 (m, 2 H), 1.40 (t, 3 H, $J = 7.0$ Hz), 0.96 (t, 3 H, $J = 7.5$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 167.1, 156.1, 133.4, 130.6, 126.9, 122.0, 115.0, 80.5, 53.9, 42.5, 38.4, 35.1, 22.9, 20.8, 15.0, 14.4; IR (neat) 3365, 3314, 2931, 1658, 1599, 917 cm^{-1} ; ESIMS m/z 370 $[\text{M}+\text{H}]^+$; HRMS m/z calculated for $\text{C}_{18}\text{H}_{28}\text{NO}_5\text{S}$ $[\text{M}+\text{H}]^+$ 370.1683, found 370.1676.



(Z)-1-(4-Ethoxyphenyl)-4-(hex-3-en-1-yl)azetidin-2-one (92b). According to general protocol C, **S1** (3.42 g, 9.18 mmol) and NaH (0.476 g, 12.5 mmol) afforded 2.27 g (91%) of **92b** as a yellow oil following purification by chromatography on SiO_2 (hexanes:EtOAc, 0 to

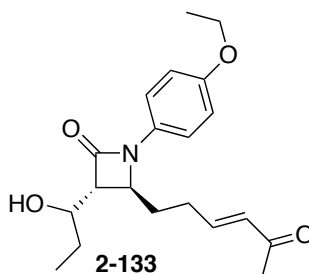
70%): R_f (hexanes/EtOAc, 50:50) 0.31; $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.29 (d, 2 H, $J = 8.9$ Hz), 6.86 (d, 2 H, $J = 9.0$ Hz), 5.41-5.27 (m, 2 H), 4.01-3.97 (m, 1 H), 3.78 (s, 3 H), 3.15 (dd, 1 H, $J = 15.0, 5.2$ Hz), 2.74 (dd, 1 H, $J = 14.9, 2.4$ Hz), 2.18-2.10 (m, 2 H), 2.07-1.96 (m, 2 H), 1.67-1.54 (m, 2 H), 1.40 (t, 3 H, $J = 7.0$ Hz), 0.96 (t, 3 H, $J = 7.5$ Hz); $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 164.1, 155.5, 133.2, 131.2, 127.2, 118.7, 115.2, 63.9, 51.2, 42.2, 32.3, 22.9, 20.8, 15.0, 14.5; IR (neat) 2965, 2924, 1745, 1510, 1385, 1236, 1046 cm^{-1} ; HRMS m/z calculated for $\text{C}_{17}\text{H}_{24}\text{NO}_2$ $[\text{M}+\text{H}]^+$ 274.1802, found 274.1797.



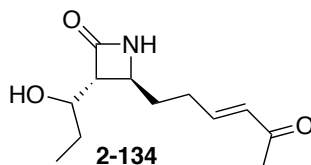
(Z)-1-(4-Ethoxyphenyl)-4-(hex-3-en-1-yl)-3-(1-hydroxypropyl)azetidin-2-one (2-132).

According to general protocol D, diisopropyl amine (0.124 g, 1.22 mmol), *n*-butyllithium (1.13 mmol, 0.53 mL, 2.11 M in hexanes), **92b** (0.239 g, 0.867 mmol), and propionaldehyde (0.403 g, 6.94 mmol) afforded 0.287 g (100%) of **2-132** as a yellow oil following purification by chromatography on SiO_2 (hexanes:EtOAc, 12 to 100%): R_f (hexanes/EtOAc, 50:50) 0.48; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.28 (d, 2 H, $J = 9.0$ Hz), 6.86 (d, 2 H, $J = 9.1$ Hz), 5.46-5.30 (m, 2 H), 4.00 (q, 2 H, $J = 7.00$ Hz), 3.94-3.91 (m, 1 H), 3.88-3.82 (m, 1 H), 3.02 (dd, 1 H, $J = 6.2, 2.2$ Hz), 2.14-2.03 (m, 2 H), 2.01-1.98 (m, 2 H), 1.71-1.67 (m, 2 H), 1.40 (t, 3 H, $J = 7.0$ Hz), 1.04 (t, 3 H, $J = 7.4$ Hz), 0.96 (t, 3 H, $J = 7.5$ Hz); $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 165.5, 155.7, 133.3, 130.7, 127.3, 118.9, 115.2, 72.0, 63.9, 60.3, 54.7, 31.9, 28.0, 22.9, 20.8,

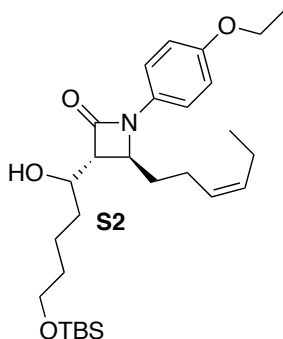
15.0, 14.4, 10.5; IR (neat) 3428, 2967, 1725, 1512, 1240, 972, 834 cm^{-1} ; ESIMS m/z 332 $[\text{M}+\text{H}]^+$; HRMS m/z calculated for $\text{C}_{20}\text{H}_{30}\text{NO}_3$ $[\text{M}+\text{H}]^+$ 332.2220, found 332.2206.



(E)-1-(4-Ethoxyphenyl)-3-(1-hydroxypropyl)-4-(5-oxohex-3-en-1-yl)azetidin-2-one (2-133). According to general protocol E, **2-132** (0.270 g, 0.815 mmol), MVK (0.288 g, 4.07 mmol), $\text{Ti}(\text{O}i\text{Pr})_4$ (23.6 mg, 0.0815 mmol) and Hoveyda-Grubbs 2nd generation catalyst (7.55 mg, 0.117 mmol) afforded 0.217 g (77%) of **2-133** as a black oil following purification by chromatography on SiO_2 (hexanes:EtOAc, 2 to 100%): R_f (hexanes/EtOAc, 50:50) 0.14; ^1H NMR (400 MHz, CDCl_3) δ 7.26 (d, 2 H, $J = 8.9$ Hz), 6.86 (d, 2 H, $J = 9.0$ Hz), 6.76 (dt, 1 H, $J = 15.8, 6.6$), 6.09 (d, 1 H, $J = 15.9$ Hz), 4.12-4.10 (m, 1 H), 3.99 (q, 2 H, $J = 6.9$ Hz), 3.88-3.79 (m, 1 H), 3.00 (ddd, 1 H, $J = 14.8, 5.7, 1.9$ Hz), 2.32-2.30 (m, 2 H), 2.21 (s, 3 H), 1.87-1.75 (m, 2 H), 1.69-1.66 (m, 2 H), 1.39 (t, 3 H, $J = 7.0$ Hz), 1.02 (t, 3 H, $J = 7.0$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 198.4, 165.1, 155.8, 146.0, 131.9, 130.5, 118.9, 115.2, 71.6, 70.7, 63.9, 60.2, 54.1, 30.1, 28.5, 27.9, 27.3, 15.0, 10.3; IR (neat) 3412, 2920, 1965, 1725, 1508, 1236 cm^{-1} ; ESIMS m/z 346 $[\text{M}+\text{H}]^+$; HRMS m/z calculated for $\text{C}_{17}\text{H}_{24}\text{NO}_2\text{Na}$ $[\text{M}+\text{Na}]^+$ 368.1832, found 368.1836.

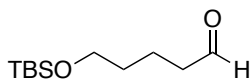


(E)-1-(4-Ethoxyphenyl)-3-(1-hydroxypropyl)-4-(5-oxohex-3-en-1-yl)azetidin-2-one (2-134). To a solution of **2-133** (12.0 mg, 0.0362 mmol) in CH₃CN–H₂O (3:1) (0.50 mL) was added [Ag(Py)₄]S₂O₈ (67.0 mg, 0.109 mmol) at rt and the mixture was stirred for 20 min. The mixture was diluted with H₂O (0.50 mL), extracted with EtOAc (6x). The combined organic layers were washed with 10% aqueous NaHCO₃ (1.20 mL), and the aqueous layer was extracted with EtOAc (2x). The combined organic layers were washed successively with 10% NaHSO₃ (1.20 mL), brine, dried (Na₂SO₄) and concentrated under reduced pressure to afford 3.7 mg (45%) of **2-134** as a yellow oil: ¹H NMR (400 MHz, CDCl₃) δ 6.81-6.71 (m, 2 H), 6.12 (d, 1 H, *J* = 15.6), 5.99 (brs, 1 H), 3.83-3.82 (m, 1 H), 3.57 (td, 1 H, *J* = 6.8, 2.4 Hz), 2.90 (dd, 2 H, *J* = 6.4, 2.0 Hz), 2.33-2.36 (m, 2 H), 2.25 (s, 3 H), 1.87-1.82 (m, 2 H), 1.66-1.60 (m, 2 H), 1.01 (t, 3 H, *J* = 7.4 Hz); ESIMS *m/z* 226 [M+H]⁺.



(3R,4S)-3-((R)-5-((tert-Butyldimethylsilyl)oxy)-1-hydroxypentyl)-1-(4-ethoxyphenyl)-4-((Z)-hex-3-en-1-yl)azetidin-2-one (S2). According to general protocol D, diisopropyl amine (0.114 g, 1.12 mmol), *n*-butyllithium (1.04 mmol, 0.49 mL, 2.11 M in hexanes), **2-91b**

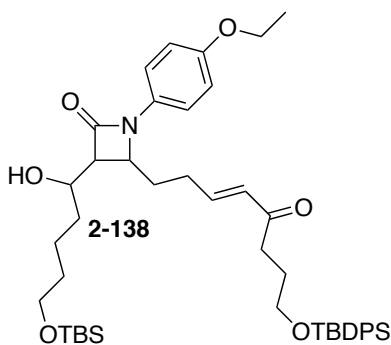
(0.220 g, 0.797 mmol), and aldehyde **S3** (1.04 g, 3.19 mmol) afforded 0.285 g (73%) of **S2** as a yellow oil following purification by chromatography on SiO₂ (hexanes:EtOAc, 12 to 100%): R_f (hexanes/EtOAc, 80:20) 0.32; ¹H NMR (400 MHz, CDCl₃) δ 7.28 (d, 2 H, *J* = 9.1 Hz), 6.86 (d, 2 H, *J* = 9.0 Hz), 5.40-5.32 (m, 2 H), 4.00 (q, 2 H, *J* = 6.9 Hz), 3.96-3.92 (m, 1 H), 3.64-3.61 (m, 3 H), 3.00 (dd, 1 H, *J* = 6.0, 2.1 Hz), 2.15-2.11 (m, 2 H), 2.02-1.99 (m, 2 H), 1.72-1.68 (m, 4 H), 1.57-1.54 (m, 6 H), 1.40 (t, 3 H, *J* = 7.0 Hz), 0.94 (t, 3 H, *J* = 7.0 Hz), 0.89 (s, 9 H), 0.05 (s, 6 H); ¹³C NMR (100 MHz, CDCl₃) δ 165.5, 155.7, 133.3, 127.6, 127.3, 119.0, 118.9, 115.2, 70.6, 69.3, 64.0, 63.3, 60.6, 54.7, 35.5, 32.7, 31.9, 26.2, 23.0, 22.9, 22.3, 20.8, 18.6, 15.0, 14.4, -5.1; IR (neat) 3377, 2927, 2371, 1732, 1512, 1382, 1098 cm⁻¹; ESIMS *m/z* 490 [M+H]⁺; HRMS *m/z* calculated for C₂₈H₄₈NO₄Si [M+H]⁺ 490.3347, found 490.3344.



S3

5-((*tert*-Butyldimethylsilyl)oxy)pentanal (S3).⁶⁴ To a solution of 1,5-pentanediol (1.50 g, 14.1 mmol) and TBSCl (2.53 g, 16.3 mmol) in CH₂Cl₂ (28.0 mL) was added NEt₃ (2.50 g, 24.7 mmol) at rt. The mixture was stirred at rt overnight, and was diluted with CH₂Cl₂, washed with sat. aq. NH₄Cl, dried (MgSO₄), and concentrated under reduced pressure to afford 2.25 g (73%) of the mono-protected alcohol which was used directly. To a solution of oxalyl chloride (1.80 g, 13.7 mmol) at -78 °C in anhydrous CH₂Cl₂ (71.0 mL) was added anhydrous DMSO (2.09 g, 26.6 mmol) in CH₂Cl₂ (2.90 mL) and the mixture was stirred for 30 min at -78 °C. A solution of the mono-protected alcohol (2.25 g, 6.54 mmol) in CH₂Cl₂ (17.0 mL) was added drop-wise and stirring was continued at -78 °C for 45 min, followed by

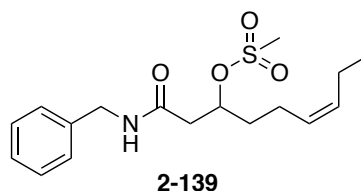
the addition of NEt₃ (3.48 g, 34.4 mmol). The reaction was stirred for an additional 20 min at -78 °C, and then for another 30 min at 0 °C, and was then quenched with water (395 mL). The layers were separated, and the aqueous layer was extracted with CH₂Cl₂. The combined organic layers were dried (Na₂SO₄), and concentrated under reduced pressure to afford the crude as a colorless oil. The residue was re-dissolved in anhydrous Et₂O and cooled at -30 °C for 3 h. The precipitate was filtered off, and the filtrate was concentrated under reduced pressure and azeotropically co-evaporated with benzene three times to provide 1.74 g (78%) of **S3** as a colorless oil: R_f (hexanes/EtOAc, 80:20) 0.32; ¹H NMR (300 MHz, CDCl₃) δ 9.77 (t, 1 H, *J* = 1.7 Hz), 3.62 (t, 2 H, *J* = 6.1 Hz), 2.46 (td, 2 H, *J* = 7.2, 1.7 Hz), 1.75-1.65 (m, 2 H), 1.59-1.50 (m, 2 H), 0.89 (s, 9 H), 0.04 (s, 6 H).



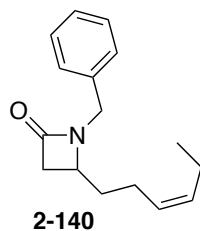
(*E*)-3-(5-((*tert*-Butyldimethylsilyl)oxy)-1-hydroxypentyl)-4-(8-((*tert*-butyldiphenylsilyl)oxy)-5-oxooct-3-en-1-yl)-1-(4-ethoxyphenyl) azetidin-2-one (2-138).

According to general protocol E, **S2** (0.617 g, 1.26 mmol), **2-117** (1.83 g, 5.04 mmol), Ti(O*i*Pr)₄ (54.8 mg, 0.189 mmol) and Hoveyda-Grubbs 2nd generation catalyst (0.127 g, 0.197 mmol) afforded 0.560 g (57%) of **2-138** as a black oil following purification by chromatography on SiO₂ (hexanes:EtOAc, 2 to 100%): ¹H NMR (400 MHz, CDCl₃) δ 7.65-7.62 (m, 4 H), 7.41-7.25 (m, 8 H), 6.86 (d, 2 H, *J* = 9.0 Hz), 6.76 (dt, 1 H, *J* = 14.9, 8.3 Hz),

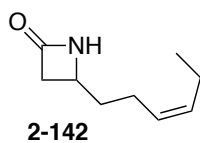
6.11 (d, 1 H, $J = 16.0$ Hz), 4.12-4.10 (m, 4 H), 3.68 (t, 3 H, $J = 6.0$ Hz), 3.66-3.63 (m, 2 H), 2.98 (dd, 1 H, $J = 17.7, 5.0$ Hz), 2.62 (t, 2 H, $J = 6.8$ Hz), 2.30-2.23 (m, 2 H), 2.23-2.17 (m, 2 H), 1.70-1.58 (m, 6 H), 1.39 (t, 3 H, $J = 6.9$), 1.03 (s, 9 H), 0.89 (s, 9 H), 0.05 (s, 6 H); ^{13}C NMR (100 MHz, CDCl_3) δ 200.1, 168.3, 165.0, 144.7, 135.7, 133.9, 129.8, 127.8, 118.9, 115.3, 70.3, 63.9, 63.2, 60.5, 54.1, 36.9, 32.3, 27.2, 27.0, 26.1, 22.3, 19.4, 18.6, 15.0, -5.00; IR (neat) 3448, 2942, 2927, 1729, 1516, 1243, 708 cm^{-1} ; ESIMS m/z 786 $[\text{M}+\text{H}]^+$; HRMS m/z calculated for $\text{C}_{46}\text{H}_{68}\text{NO}_6\text{Si}$ $[\text{M}+\text{H}]^+$ 786.4580, found 786.4573.



(Z)-1-(Benzylamino)-1-oxonon-6-en-3-yl methanesulfonate (2-139). According to general protocol B, **2-139** was obtained in 85% (0.420 mg) as a yellow oil following purification by chromatography on SiO_2 (hexanes:EtOAc, 12 to 100%): R_f (hexanes/EtOAc, 50:50) 0.46; ^1H NMR (400 MHz, CDCl_3) δ 7.38-7.26 (m, 5 H), 6.05 (brs, 1 H), 5.47-5.30 (m, 2 H), 5.12-5.03 (m, 1 H), 4.44 (dd, 1 H, $J = 5.6, 1.6$ Hz), 4.33 (d, 1 H, $J = 6.1$ Hz), 2.91 (s, 3 H), 2.63-2.58 (m, 2 H), 2.17-2.08 (m, 2 H), 2.03-2.01 (m, 2 H), 1.99-1.82 (m, 2 H), 0.96 (t, 3 H, $J = 7.5$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 168.8, 138.0, 133.4, 129.1, 128.0, 127.9, 127.0, 80.2, 47.4, 43.9, 41.6, 41.3, 38.1, 35.1, 22.9, 20.8, 14.4; IR (neat) 3314, 2935, 2964, 1654, 1453, 1342, 1173 cm^{-1} ; ESIMS m/z 340 $[\text{M}+\text{H}]^+$; HRMS m/z calculated for $\text{C}_{17}\text{H}_{26}\text{NO}_4\text{S}$ $[\text{M}+\text{H}]^+$ 340.1577, found 340.1575; mp = 58-61 $^\circ\text{C}$.

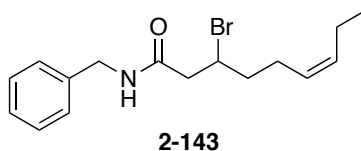


(Z)-1-Benzyl-4-(hex-3-en-1-yl)azetidin-2-one (2-140). According to general protocol C, **2-139** (0.420 g, 1.23 mmol) and NaH (68.7 mg, 1.72 mmol) afforded 31.0 mg (10%) of **2-140** as a colorless oil following purification by chromatography on SiO₂ (hexanes:EtOAc, 12 to 100%) followed by purification using prep-TLC [hexanes:EtOAc, 60:40]: R_f (hexanes/EtOAc, 50:50) 0.58; ¹H NMR (400 MHz, CDCl₃) δ 7.36-7.25 (m, 5 H), 5.40-5.16 (m, 2 H), 4.57 (d, 2 H, *J* = 15.4 Hz), 4.15 (d, 2 H, *J* = 15.4 Hz), 3.50-3.45 (m, 1 H), 3.01 (dd, 1 H, *J* = 14.6, 4.5 Hz), 2.62 (dd, 1 H, *J* = 14.6, 2.1 Hz), 2.01-1.91 (m, 4 H), 1.80-1.77 (m, 1 H), 1.46-1.37 (m, 1 H), 0.93 (t, 3 H, *J* = 7.5 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 167.4, 136.4, 133.0, 129.0, 128.3, 127.8, 51.5, 44.9, 43.0, 33.0, 30.2, 23.4, 20.7, 14.5; IR (neat) 3303, 2959, 2924, 1744, 1682, 1389, 712 cm⁻¹; ESIMS *m/z* 285 [M+MeCN]⁺; HRMS *m/z* calculated for C₁₆H₂₂NO [M+H]⁺ 244.1696, found 244.1689.

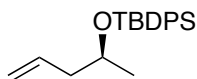


(Z)-4-(Hex-3-en-1-yl)azetidin-2-one (2-142).⁶⁶ To a solution of condensed anhydrous ammonia was added a solution of **2-140** (27.2 mg, 0.111 mmol) in anhydrous THF (0.40 mL) at -78 °C. To this solution was added small pieces of sodium until the royal blue color persisted. The mixture was stirred at -78 °C for an additional hour, and was quenched with a sat. aq. NH₄Cl (the reaction cleared up and the septum was removed to release pressure). The

ammonia was boiled off as the reaction was warmed gradually. The mixture was diluted with EtOAc, washed with brine, dried (MgSO₄), and concentrated under reduced pressure to afford 17.0 mg (100%) of the crude **2-142** as a yellow oil: R_f (hexanes/EtOAc, 50:50) 0.16; ¹H NMR (300 MHz, CDCl₃) δ 6.16 (brs, 1 H), 5.46-5.25 (m, 2 H), 3.62 (qd, 1 H, *J* = 6.8, 2.4 Hz), 3.05 (ddd, 1 H, *J* = 14.8, 4.9, 2.1 Hz), 2.58 (dd, 1 H, *J* = 14.8, 1.3 Hz), 2.11-1.97 (m, 4 H), 1.71-1.54 (m, 2 H), 0.95 (t, 3 H, *J* = 7.5 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 168.2, 133.2, 127.4, 48.2, 43.8, 35.5, 29.9, 24.3, 20.7, 14.5; IR (neat) 3257, 2959, 2931, 1752, 1382, 664 cm⁻¹; HRMS *m/z* calculated for C₉H₁₆NO [M+H]⁺ 154.1226, found 154.1225.

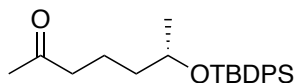


(Z)-N-Benzyl-3-bromonon-6-enamide (2-143). To a solution of **2-139** (0.105 g, 0.310 mmol) in acetone (3.10 mL) was added LiBr (81.5 mg, 0.929 mmol) and the mixture was heated to reflux overnight. Upon cooling, the solvent was removed under reduced pressure to afford crude **2-143**. The residue was purified using flash column chromatography on SiO₂ (hexanes:EtOAc, 15 to 100%) to provide 732 mg (73%) of **2-143** as a colorless oil: ¹H NMR (400 MHz, CDCl₃) δ 7.34-7.25 (m, 5 H), 6.16 (brs, 1 H), 5.46-5.24 (m, 2 H), 2.80 (dd, 1 H, *J* = 14.8, 4.5 Hz), 2.69 (dd, 1 H, *J* = 14.8, 9.0 Hz), 2.27-2.23 (m, 1 H), 2.10-2.03 (m, 2 H), 1.87 (q, 2 H, *J* = 7.5, 7.1 Hz), 0.96 (t, 3 H, *J* = 7.5 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 169.6, 138.1, 133.6, 128.9, 128.0, 127.7, 126.9, 51.6, 46.8, 43.9, 39.0, 25.4, 20.8, 14.5; IR (neat) 3293, 2967, 1658, 1551, 1433, 735 cm⁻¹; ESIMS *m/z* 367 [M+MeCN]⁺; HRMS *m/z* calculated for C₁₆H₂₃BrNO [M+H]⁺ 324.0958, found 324.0958.



2-157

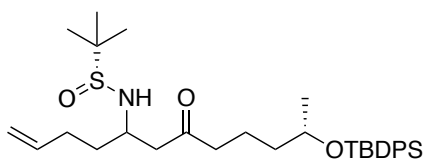
(S)-tert-Butyl(pent-4-en-2-yloxy)diphenylsilane (2-157). To a stirred solution of (*R*)-(-)-4-penten-2-ol (1.00 g, 11.0 mmol) in CH₂Cl₂ (110 mL) was added imidazole (1.52 g, 22.1 mmol) at 0 °C and the mixture was stirred for 30 min. After 30 min, TBDPSCl (3.71 g, 13.2 mmol) was added and the reaction was stirred overnight. Upon completion, the reaction was quenched with sat. aq. NH₄Cl, and the aqueous layer was extracted with CH₂Cl₂, washed with brine, dried (Na₂SO₄), and concentrated under reduced pressure to afford the crude product as a colorless oil. The residue was purified using flash column chromatography on SiO₂ (hexanes:EtOAc, 0 to 20%) to provide 3.47 g (97%) of **2-157** as a colorless oil: R_f (hexanes/EtOAc, 90:10) 0.81; ¹H NMR (300 MHz, CDCl₃) δ 7.70-7.67 (m, 4 H), 7.45-7.34 (m, 6 H), 5.81 (m, 1 H), 4.99-4.93 (m, 2 H), 3.92-3.86 (m, 1 H), 2.19-2.17 (m, 1 H), 1.06 (d, 3 H, *J* = 6.00 Hz), 1.05 (s, 6 H); ¹³C NMR (100 MHz, CDCl₃) δ 136.1, 129.7, 127.7, 117.0, 69.4, 44.1, 27.2, 23.0, 19.5.



2-155

(S)-6-((tert-Butyldiphenylsilyl)oxy)heptan-2-one (2-155). To a solution of **2-157** (0.65 g, 2.00 mmol) in anhydrous CH₂Cl₂ (20.0 mL) was added MVK (1.26 g, 18.0 mmol) and Hoveyda-Grubbs 2nd generation catalyst (75.0 mg, 0.119 mmol) under an argon atmosphere. The reaction was stirred at reflux overnight. Upon completion, the solvent was removed under reduced pressure to afford the crude product as a brown oil. The crude was dissolved

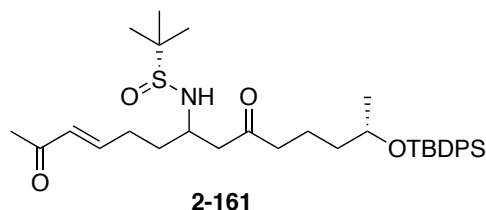
in methanol and the mixture was degassed using an argon balloon. To this mixture was added Pd/C (21.3 mg, 0.20 mmol) and the heterogeneous mixture was further degassed using an argon balloon. The argon balloon was replaced with a hydrogen balloon, and the mixture was saturated with hydrogen gas, and stirred under a hydrogen atmosphere overnight. The solid was filtered through Celite®, and washed with methanol. The filtrate was concentrated under reduced pressure and the residue was purified using flash column chromatography on SiO₂ (hexanes:EtOAc, 15 to 100%) to provide 523 mg (71%) of **2-155** as a colorless oil: R_f (hexanes/EtOAc, 90:10) 0.61; ¹H NMR (400 MHz, CDCl₃) δ 7.69-7.65 (m, 4 H), 7.44-7.34 (m, 6 H), 3.87-3.79 (m, 1H), 2.28 (t, 2 H, *J* = 7.3 Hz), 2.06 (s, 3 H), 1.57-1.52 (m, 2 H), 1.46-1.39 (m, 2 H), 1.06 (d, 3 H, *J* = 6.0 Hz), 1.04 (s, 9 H).



2-156

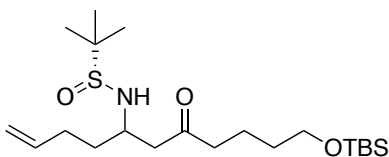
(*R*)-*N*-((11*S*)-11-((*tert*-Butyldiphenylsilyl)oxy)-7-oxododec-1-en-5-yl)-2-methylpropane-2-sulfonamide (2-156**). **General protocol E.** A flame-dried flask cooled under a stream of argon was charged with Et₂O (25.0 mL), and cooled to -78 °C. Solid KHMDS (2.00 g, 10.0 mmol) was added and the mixture was stirred for 5 min at -78 °C. Ketone **2-155** (3.58 g, 9.71 mmol) in Et₂O (9.00 mL) was added drop-wise and the mixture was stirred at -78 °C for 1 h. A solution of imine **2-38** (0.606 g, 3.24 mmol) in Et₂O (1.00 mL) was added to the reaction and stirred at -78 °C for 3 h. The reaction was quenched with a solution of sat. aq. NH₄Cl at -78 °C and gradually warmed to rt. The mixture was transferred to a separatory**

funnel and the layers were separated. The aqueous layer was extracted with EtOAc and the combined organic layers were washed with brine, dried (Na₂SO₄), and concentrated under reduced pressure to afford the crude product as a single diastereomer. The residue was purified using flash column chromatography on SiO₂ (hexanes:EtOAc, 15 to 100%) to provide 1.44 g (82%) of **2-156** as a yellow oil: R_f (hexanes/EtOAc, 50:50) 0.37; ¹H NMR (400 MHz, CDCl₃) δ 7.70-7.65 (m, 4 H), 7.44-7.34 (m, 6 H), 5.82-5.71 (m, 1 H), 5.30-4.95 (m, 2 H), 4.05 (d, 1 H, *J* = 7.8 Hz), 3.84-3.79 (m, 1 H), 3.56-3.45 (m, 1 H), 2.82 (dd, 1 H, *J* = 17.4, 5.8 Hz), 2.68 (dd, 1 H, *J* = 17.5, 4.4 Hz), 2.26 (t, 2 H, *J* = 7.6 Hz), 2.21-2.06 (m, 2 H), 1.76-1.66 (m, 2 H), 1.60-1.37 (m, 6 H), 1.20 (s, 9 H), 1.05 (d, 3 H, *J* = 6.1 Hz), 1.03 (s, 9 H); ¹³C NMR (100 MHz, CDCl₃) δ 210.5, 137.8, 136.0, 134.8, 134.5, 129.6, 129.6, 127.7, 127.5, 115.4, 69.3, 60.5, 56.0, 53.4, 48.0, 43.9, 38.8, 34.8, 30.5, 27.2, 23.2, 22.8, 19.4, 19.3; IR (neat) 3294, 2959, 2931, 1713, 1429, 1052, 1106, 708 cm⁻¹; ESIMS *m/z* 556 [M+H]⁺; HRMS *m/z* calculated for C₃₂H₅₀NO₃SSi₂ [M+H]⁺ 556.3275, found 556.3260.



(R)-N-((13S,E)-13-((tert-Butyldiphenylsilyl)oxy)-2,9-dioxotetradec-3-en-7-yl)-2-methylpropane-2-sulfonamide (2-161). According to general protocol E, **2-156** (0.716 g, 1.29 mmol), MVK (0.451 g, 6.44 mmol), Ti(OiPr)₄ (45.3 mg, 0.155 mmol) and Hoveyda-Grubbs 2nd generation catalyst (0.100 g, 0.115 mmol) afforded 0.647 g (84%) of **2-161** as a black oil following purification by chromatography on SiO₂ (hexanes:EtOAc, 12 to 100%):

^1H NMR (400 MHz, CDCl_3) δ 7.68-7.59 (m, 4 H), 7.42-7.26 (m, 6 H), 6.76 (m, 1 H), 6.07 (d, 1 H, $J = 17.2$ Hz), 4.17-4.03 (m, 2 H), 3.88-3.78 (m, 1 H), 3.52-3.41 (m, 1 H), 2.86 (dd, 1 H, $J = 17.9, 6.3$ Hz), 2.70 (dd, 1 H, $J = 17.8, 4.6$ Hz), 2.45-2.33 (m, 2 H), 2.24 (s, 3 H), 1.87-1.79 (m, 2 H), 1.70-1.66 (m, 2 H), 1.66-1.47 (m, 6 H), 1.24 (s, 9 H), 1.04 (s, 9 H); ^{13}C NMR (100 MHz, CDCl_3) δ 210.1, 198.2, 170.9, 146.9, 135.7, 134.5, 134.2, 131.6, 129.4, 129.3, 127.4, 127.3, 69.0, 55.9, 53.2, 47.9, 43.5, 38.5, 33.8, 29.1, 26.9, 26.8, 23.0, 22.6, 19.1, 19.0, 14.12; IR (neat) 3246, 2924, 2959, 1627, 1674, 1362, 1055, 747 cm^{-1} ; ESIMS m/z 598 $[\text{M}+\text{H}]^+$; HRMS m/z calculated for $\text{C}_{34}\text{H}_{52}\text{NO}_4\text{SSi}$ $[\text{M}+\text{H}]^+$ 598.3381, found 598.3361.

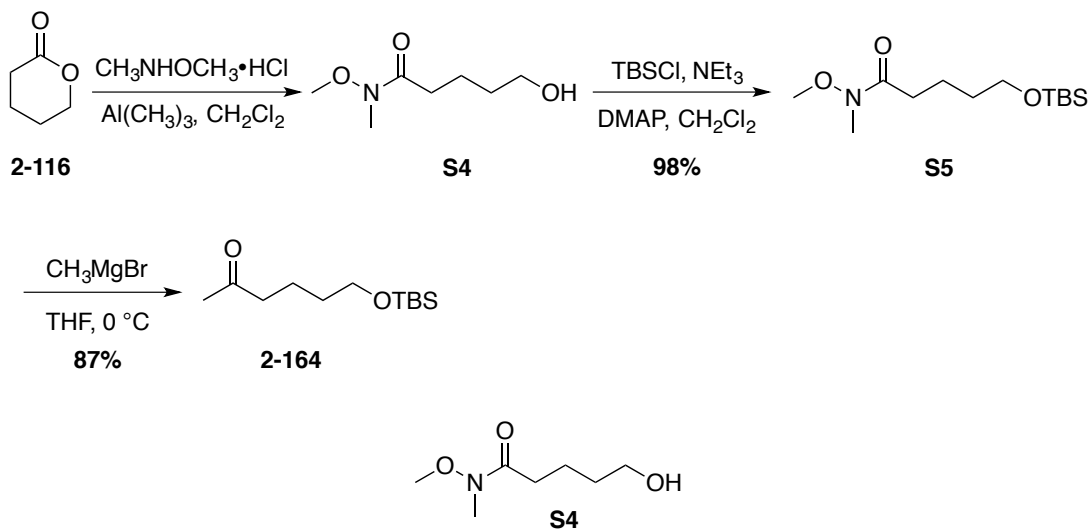


2-165

(*R*)-*N*-(11-((*tert*-Butyldimethylsilyl)oxy)-7-oxoundec-1-en-5-yl)-2-methylpropane-2-sulfonamide (2-165). According to general protocol E, **2-38** (0.98 g, 5.23 mmol), ketone **2-164** (3.62 g, 15.7 mmol), and KHMDS (3.85 g, 18.3 mmol) afforded 1.79 g (84%) of **2-165** as a yellow oil following purification by chromatography on SiO_2 (hexanes:EtOAc, 12 to 100%): R_f (hexanes/EtOAc, 50:50) 0.37; ^1H NMR (400 MHz, CDCl_3) δ 5.82-5.71 (m, 1 H), 5.03-4.97 (m, 2 H), 4.07 (d, 1 H, $J = 9.7$ Hz), 3.59 (t, 2 H, $J = 6.3$ Hz), 3.56-3.48 (m, 1 H), 2.89 (dd, 1 H, $J = 17.7, 5.7$ Hz), 2.76 (dd, 1 H, $J = 17.7, 4.3$ Hz), 2.42 (t, 2 H, $J = 7.3$ Hz), 2.23-2.03 (m, 2 H), 1.78-1.68 (m, 2 H), 1.67-1.63 (m, 2 H), 1.57-1.47 (m, 2 H), 1.21 (s, 9 H), 0.88 (s, 9 H), 0.04 (s, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 210.6, 137.8, 115.4, 62.9, 56.0, 53.4, 48.1, 44.0, 34.8, 32.3, 30.3, 26.1, 22.8, 20.1, 18.4, -5.2; IR (neat) 3275, 3216, 2856,

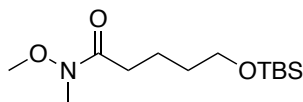
1717, 1460, 1059 cm^{-1} ; ESIMS m/z 418 $[\text{M}+\text{H}]^+$; HRMS m/z calculated for $\text{C}_{21}\text{H}_{43}\text{NO}_3\text{SSi}$ $[\text{M}+\text{H}]^+$ 418.2806, found 418.2795.

Compound **2-164** was prepared in three steps from the commercially available δ -valerolactone **2-116** as described below:



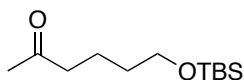
5-Hydroxy-*N*-methoxy-*N*-methylpentanamide (S4). A 2.0 M solution of $\text{Al}(\text{CH}_3)_3$ in toluene (11.2 mL, 22.5 mmol) was added drop-wise to a solution of *N,O*-dimethylhydroxylamine hydrochloride (2.24 g, 22.5 mmol) in CH_2Cl_2 (80.0 mL) at $0\text{ }^\circ\text{C}$ in a very slow drop-wise manner and the mixture was stirred at $0\text{ }^\circ\text{C}$ for 20 min. δ -Valerolactone (2.00 g, 14.5 mmol) was added drop-wise and stirring was continued at $0\text{ }^\circ\text{C}$ for 20 min, followed by the addition of CH_2Cl_2 (40.0 mL). The reaction was then quenched with slow addition of a solution of 0.1 M aqueous solution of HCl (aq.) (3.00 mL) and stirring was continued at $0\text{ }^\circ\text{C}$ for 1 h. MgSO_4 (1.20 g) was added, the mixture was stirred for 10 min at rt, filtered through Celite® and concentrated under reduced pressure to provide 2.42 g

(100%) of **S4** as a colorless oil: R_f (hexanes/EtOAc, 80:20) 0.41; $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 3.68 (s, 3 H), 3.64 (t, 2 H, $J = 6.2$ Hz), 3.18 (s, 3 H), 2.47 (t, 2 H, $J = 7.0$ Hz), 1.79-1.69 (m, 2 H), 1.66-1.56 (m, 2 H); ESIMS m/z 162 $[\text{M}+\text{H}]^+$.



S5

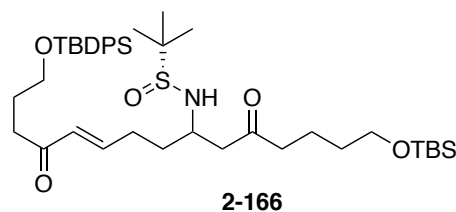
5-((*tert*-Butyldimethylsilyloxy)-*N*-methoxy-*N*-methylpentanamide (S5). To a solution of **S4** (3.01 g, 14.0 mmol) in CH_2Cl_2 (27.0 mL) was added *t*-butyl dimethylchlorosilane (2.80 g, 18.2 mmol), NEt_3 (1.84 g, 18.2 mmol), and DMAP (86.4 mg, 0.700 mmol) successively at rt. The reaction was stirred for 4 h and was quenched with sat. aq. NH_4Cl , extracted with CH_2Cl_2 , washed with brine, dried (MgSO_4), and concentrated under reduced pressure to afford the crude as a colorless oil. The residue was purified using flash column chromatography on SiO_2 (hexanes:EtOAc, 5 to 40%) to provide 3.77 g (98%) of **S5** as a colorless oil: R_f (hexanes/EtOAc, 80:20) 0.41; $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 3.67 (s, 3 H), 3.63 (t, 2 H, $J = 6.2$ Hz), 3.17 (s, 3 H), 2.44 (t, 2 H, $J = 7.5$ Hz), 1.73-1.49 (m, 4 H), 0.88 (s, 9 H), 0.04 (s, 6 H); ESIMS m/z 276 $[\text{M}+\text{H}]^+$.



2-164

6-((*tert*-Butyldimethylsilyloxy)hexan-2-one (2-164). A 3M solution of methyl magnesium bromide (16.2 mmol, 5.40 mL) in Et_2O was added to a solution of Weinreb amide **S5** (1.70 g, 4.63 mmol) in THF (46.0 mL) at 0°C . The mixture was stirred at 0°C for 4 h, and monitored using TLC. Upon completion, the mixture was quenched with sat. aq. NH_4Cl , and the

mixture was then extracted with Et₂O, washed with brine, dried (MgSO₄), and concentrated under reduced pressure to afford the crude product as a colorless oil. The residue was purified using flash column chromatography on SiO₂ (hexanes:EtOAc, 0 to 20%) to provide 0.929 g (87%) of **2-164** as a colorless oil: R_f (hexanes/EtOAc, 90:10) 0.31; ¹H NMR (300 MHz, CDCl₃) δ 3.61 (t, 2 H, *J* = 6.2 Hz), 2.45 (t, 2 H, *J* = 7.3 Hz), 2.13 (s, 3 H), 1.68-1.47 (m, 4 H), 0.88 (s, 9 H), 0.04 (s, 6 H); ¹³C NMR (100 MHz, CDCl₃) δ 209.4, 63.0, 43.7, 32.4, 30.1, 26.2, 20.5, 18.6, -5.1; ESIMS *m/z* 231 [M+H]⁺.



(*R*)-*N*-((*E*)-2,2,3,3,22,22-Hexamethyl-9,16-dioxo-21,21-diphenyl-4,20-dioxa-3,21-disilatricos-14-en-11-yl)-2-methylpropane-2-sulfonamide (2-166). According to general protocol E, **2-165** (0.249 g, 0.597 mmol), enone **2-117** (0.736 g, 2.09 mmol), Ti(O*i*Pr)₄ (24.2 mg, 0.0836 mmol) and Hoveyda-Grubbs 2nd generation catalyst (69.4 mg, 0.107 mmol) afforded 0.371 g (84%) of **2-166** as a black oil following purification by chromatography on SiO₂ (hexanes:EtOAc, 5 to 100%): R_f (hexanes/EtOAc, 90:10) 0.35; ¹H NMR (300 MHz, CDCl₃) δ 7.66-7.63 (m, 4 H), 7.42-7.35 (m, 6 H), 6.78 (dt, 1 H, *J* = 16.0, 8.0 Hz), 6.09 (d, 1 H, *J* = 15.9 Hz), 4.11 (d, 1 H, *J* = 15.9 Hz), 3.69 (t, 3 H, *J* = 6.1 Hz), 3.59 (t, 3 H, *J* = 6.2 Hz), 3.55-3.45 (m, 1 H), 2.99 (dd, 1 H, *J* = 17.7, 5.7 Hz), 2.76 (dd, 1 H, *J* = 18.3, 4.0 Hz), 2.65 (t, 2 H, *J* = 7.3 Hz), 2.42 (t, 3 H, *J* = 7.2 Hz), 1.88-1.84 (m, 2 H), 1.63-1.56 (m, 4 H), 1.52-1.43 (m, 2 H), 1.21 (s, 9 H), 1.04 (s, 9 H), 0.87 (s, 9 H), 0.03 (s, 6 H); ¹³C NMR (100 MHz,

CDCl₃) δ 210.6, 200.4, 145.9, 135.7, 134.0, 131.0, 129.8, 127.8, 63.3, 62.9, 56.2, 53.7, 48.1, 43.8, 36.8, 34.2, 32.3, 29.4, 27.2, 26.2, 22.9, 20.2, 19.4, 18.5, -5.11; IR (neat) 3263, 2860, 2931, 1708, 1453, 1102, 696 cm⁻¹; ESIMS *m/z* 764 [M+Na]⁺; HRMS *m/z* calculated for C₄₁H₆₇NO₅SSi₂ [M+H]⁺ 742.4351, found 742.4327.

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CHAPTER 3

Synthesis of 2,3-Dihydro-1,3-oxazin-4-ones via a Mild Formal [4+2]

Cycloaddition of Acylketenes with Aldimines

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3.1 Abstract

A mild and efficient silver-mediated synthesis of 2,3-dihydro-1,3-oxazin-4-ones through a formal [4+2] cycloaddition reaction of aldimines and acylketene intermediates is described. A variety of functionalized and potentially biologically relevant heterocycles have been accessed in moderate to high yields through this process.

3.2 Introduction and Background

3.2.1 2,3-Dihydro-1,3-oxazin-4-ones and Biological Significance

Oxazines, oxazinones, and related derivatives are found in a number of biologically relevant molecules possessing activity as antimicrobial¹, anti-inflammatory² and antinociceptive³ agents (Figure 3.1a). One of the examples of oxazinone-containing pharmaceuticals is the clinically utilized benzodiazepine derivative ketazolam **3-2**, prescribed for the treatment of anxiety (Figure 3.1b).⁴ Additionally, this family of heterocycles has found utility in preparation of biologically active natural and synthetic compounds.⁵

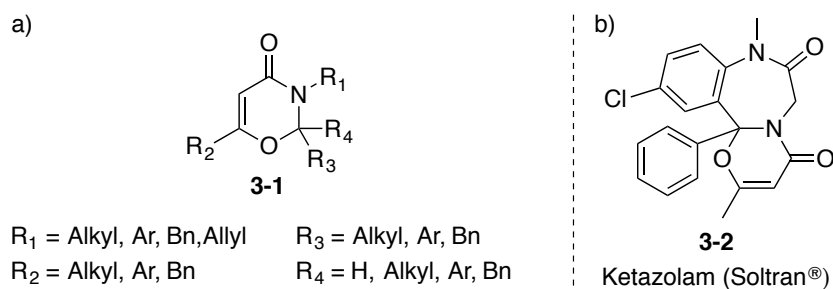
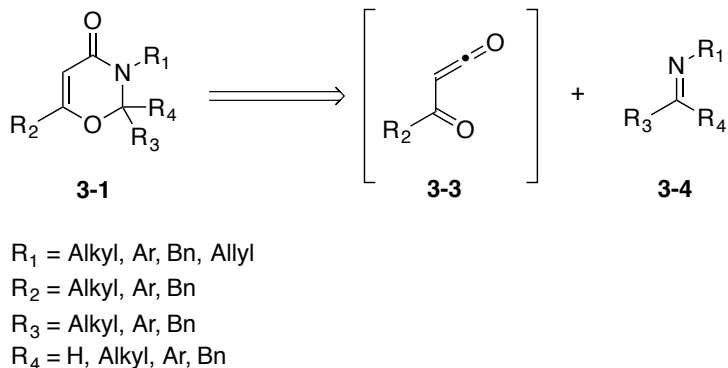


Figure 3.1: a) Structure of 2,3-dihydro-1,3-oxazin-4-one **3-1**. b) Structure of ketazolam **3-2**.

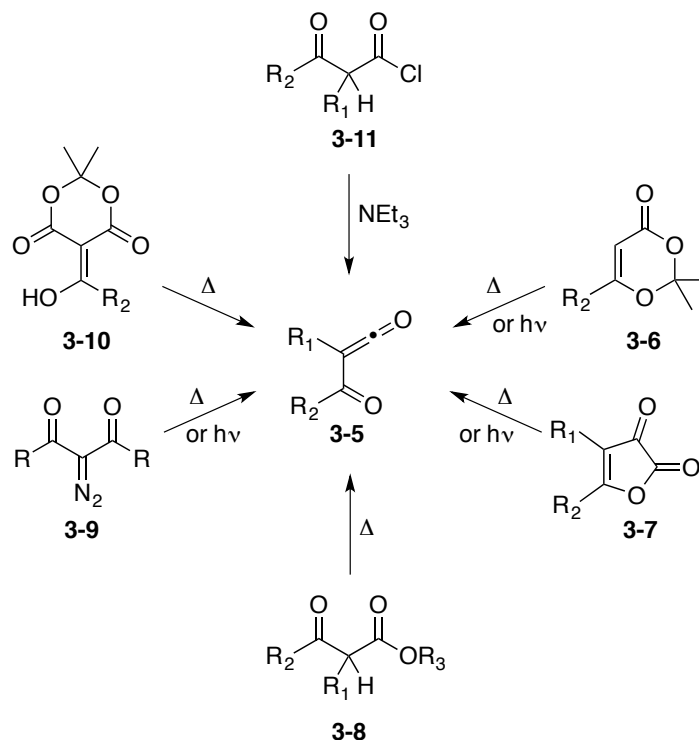
3.2.2 Synthesis of 2,3-dihydro-1,3-oxazin-4-ones

To date, there have been only a limited number of approaches for the preparation of 2,3-dihydro-1,3-oxazin-4-ones or 1,3-oxazinones and their derivatives. Traditionally, 1,3-oxazin-4-ones (**3-1**) are prepared through the reaction of aldimines **3-4** and acylketene surrogates under thermal or photoirradiation conditions (Scheme 3.1).



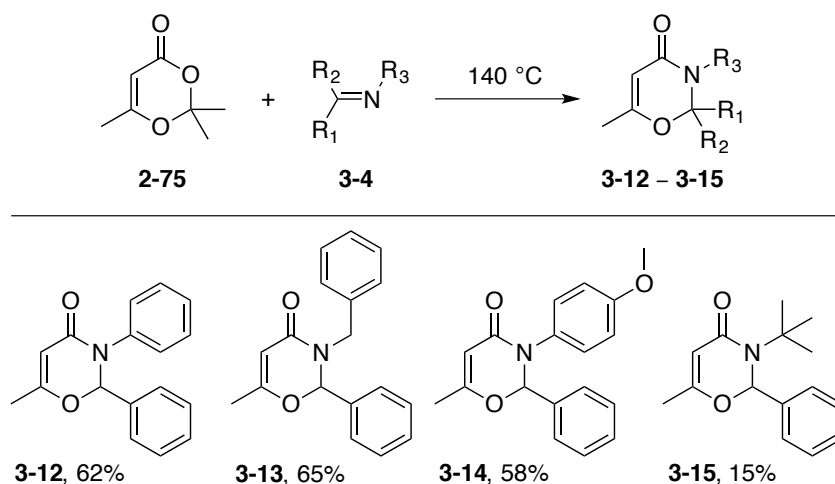
Scheme 3.1: Synthesis of 2,3-dihydro-1,3-oxazin-4-one via aldimines and acylketenes.

Acylketenes represent one of the most reactive intermediates in organic chemistry, and as such are generated *in situ* under thermal or photoirradiation conditions from reagents such as diketene⁶, dioxinone (**3-6**)⁷, furan-2,3-dione (**3-7**)⁸, diazo- β -dicarbonyl (**3-9**)⁹, acyl Meldrum's acid derivatives (**3-10**)¹⁰, and β -keto acids and esters (**3-11** and **3-8**)^{11,12} (Scheme 3.2). The acylketene intermediate generated in turn undergoes cycloaddition reaction with a wide variety of aldehydes, ketones, alkenes, alkynes, and imines to afford six membered ring structures reminiscent of Diels-Alder and hetero Diels-Alder products.¹³



Scheme 3.2: Selected examples of acylketene surrogates.

One of the most extensively utilized sources of acylketenes in oxazinone formation is the commercially available diketene-acetone adduct, 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one, “dioxinone” (**3-6**, Scheme 3.2). Dioxinone **3-6** undergoes thermal decomposition in a pseudo retro Diels-Alder manner at temperatures above 100 °C to generate acylketenes.^{7,14} The first use of dioxinone in a cycloaddition reaction with aldimines was reported in 1982 by Kato et al. A selected example of the prepared oxazinones using this reagent as the acylketene source are shown in **Scheme 3.3**.⁷

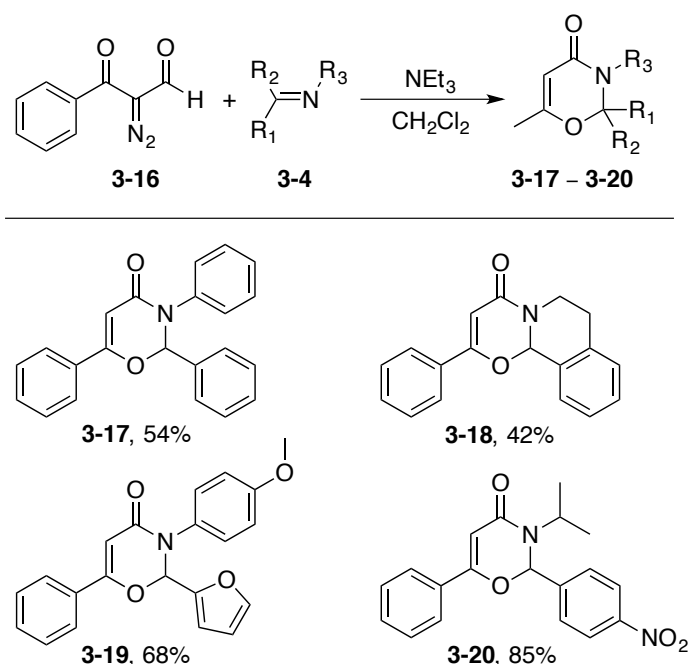


Scheme 3.3: Selected examples of oxazinone synthesis from dioxinone **2-75**.

While the use of this readily available reagent allows access to oxazinones, the requirement for higher temperature has hampered the utility and applicability of this reagent in the synthesis of more elaborate and complex scaffolds. Furthermore, the thermal generation of acylketenes may also lead to alternate reaction pathways that are not accessible at lower temperature, and as a result lead to formation of byproducts. Indeed, such high temperature reactions have been reported to be low-yielding and accompanied by formation of byproducts such as β -lactams.⁷

As mentioned earlier, one other source of acylketene intermediates and an important class of molecules in organic chemistry is the α -diazo- β -dicarbonyl compounds and the utility of this class of molecules in oxazinone formation has been reported.⁹ Xu et al. has previously demonstrated that 2-diazo-3-oxoalkanal compounds, upon extrusion of nitrogen, undergo Wolff rearrangement to generate acylketenes as intermediates under photoirradiation conditions. The generated acylketene intermediates were in turn treated with imines to

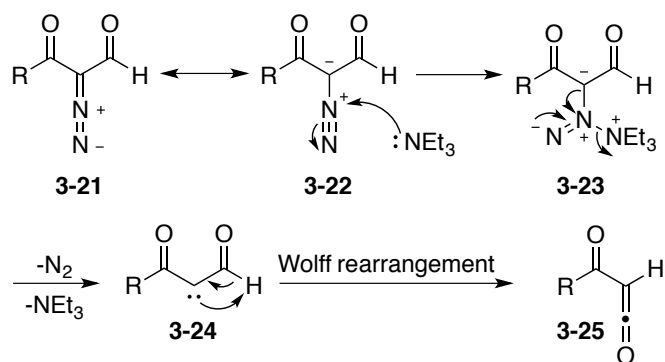
provide oxazinones in moderate yields. Such reactions typically suffer from low yields and high temperatures under thermal conditions or decreased reactivity under photoirradiation conditions. Based on literature precedent, following thorough screening of various organic and inorganic bases, Xu et al. were able to increase the rate of the reaction under fairly mild conditions via the addition of catalytic amounts of NEt_3 .⁹ Using their optimized conditions, a variety of imines were employed to prepare a series of oxazinones in satisfactory yields (Scheme 3.4).



Scheme 3.4: Selected examples of oxazinone synthesis from 2-diazo-3-oxoalkanal **3-16**.

The proposed mechanism by which the ketene intermediate is generated is depicted in **Scheme 3.5**. The authors have postulated that the amine catalyst initially attacks the nitrogen of the azo group bearing the positive charge, leading to the zwitterionic intermediate **3-23**.

This intermediate, upon loss of nitrogen and the amine, further undergoes an electron transfer reaction to form the diacylcarbene **3-24**. This carbene intermediate **3-24**, following the Wolff rearrangement, furnishes **3-25** which then participates in hetero Diels-Alder reaction to provide the desired oxazinones. Although this work offers a mild approach for acylketene generation and the subsequent cycloaddition to the targeted oxazinone, the complete lack of reactivity of α -diazo- β -diketones limits the substrate scope of this transformation.

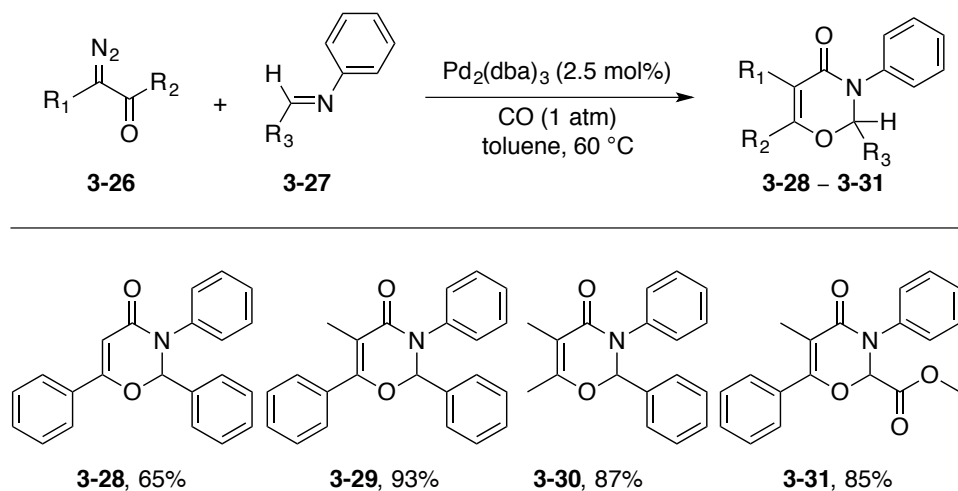


Scheme 3.5: Proposed mechanism for the reaction of α -diazo- β -dicarbonyls with imines under amine catalysis.

Overall, the modest yields of the reported cycloaddition reactions of acylketenes and aldimines coupled with the toxicity, complexity, and the inherent high reactivity of such reagents, limit the utility of many of these reported approaches. In addition to acylketene cycloaddition approaches, several alternate strategies for the preparation of 1,3-oxazin-4-ones and their derivatives have been developed.

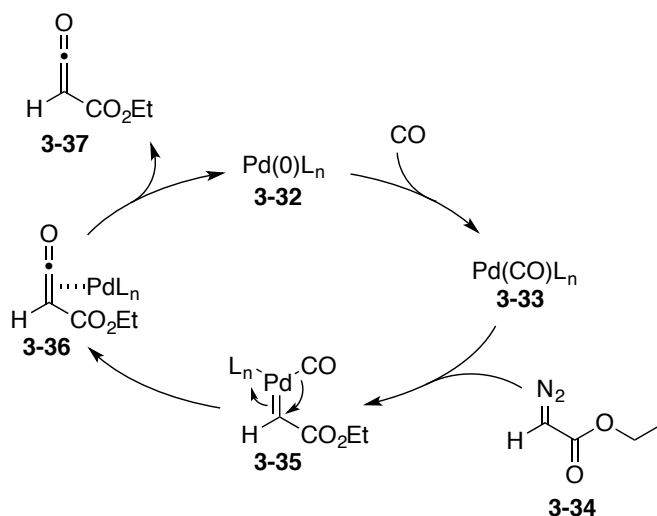
Recently, a mild Pd-catalyzed carbonylation reaction of α -diazo- β -dicarbonyl compounds (**3-26**) was utilized to generate a small library of 1,3-oxazin-4-ones (Scheme 3.6).¹⁵ In this report, the utility of catalytic carbonylation of metal-carbenes to generate

ketenes and acylketenes under thermal conditions at atmospheric pressure of CO has been demonstrated. The acylketene intermediate was further reacted with an imine in a formal [4+2] cycloaddition reaction to furnish the oxazinone derivatives.



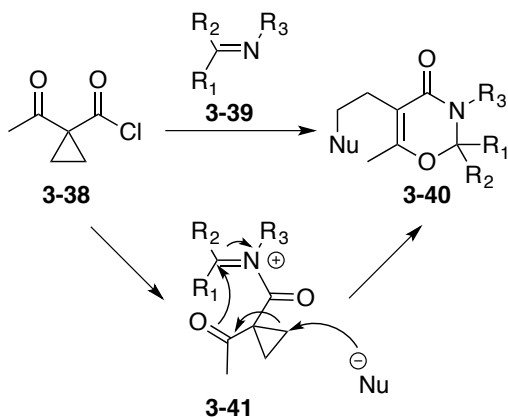
Scheme 3.6: Selected examples of substrate scope for Pd-catalyzed carbonylation.

The mechanism of this reaction proceeds through the formation of the CO-complexed Pd species **3-33**, which is the active catalyst in this system. This is followed by formation of the Pd-carbene complex **3-35**, which occurs through the decomposition of diazo compounds **3-34**, induced by the active Pd catalyst. Carbonylation of intermediate **3-35** leads to the ketene intermediate **3-36** or **3-37**, which can further react with an imine in a cycloaddition reaction. This is different than the case of carbonylation of a metal-carbon bond which results in formation of an acyl-metal species.



Scheme 3.7: Proposed mechanism of the Pd-catalyzed carbonylation of α -diazo- β -dicarbonyl.

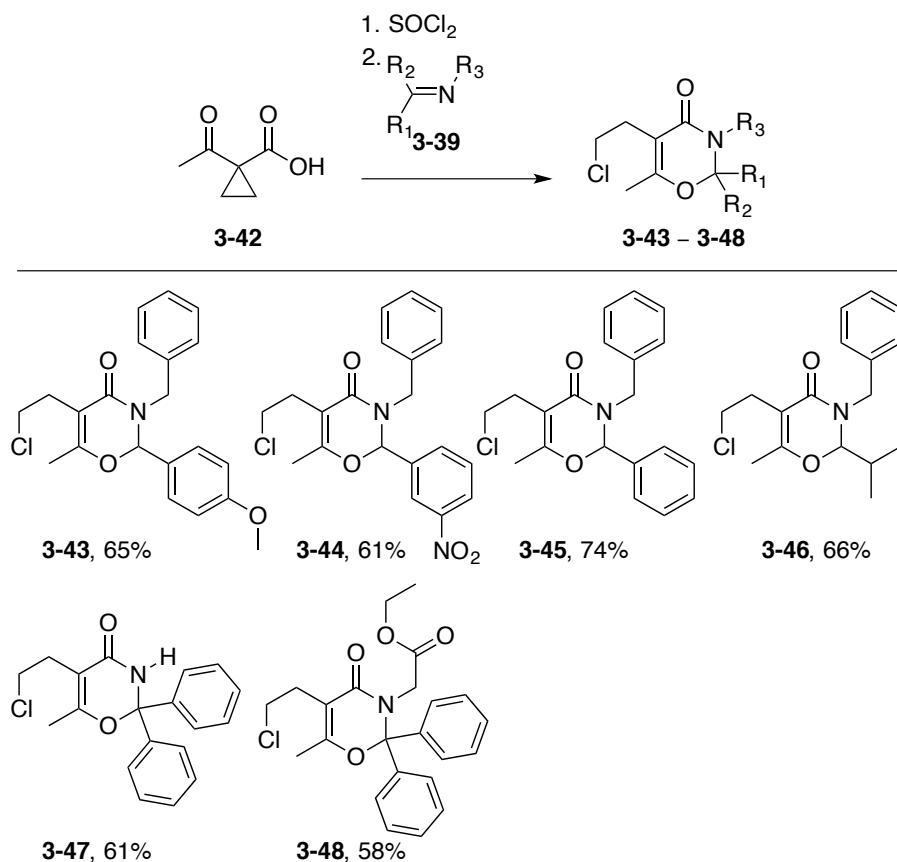
In a more recent report Hawkins et al. have demonstrated the conversion of a series of cyclopropyl carboxylic acid and imine derivatives to 1,3-oxazin-4-ones under metal free conditions.¹⁶ Building on the abundant literature precedent on the nucleophilic opening of doubly activated cyclopropanes, the authors sought to investigate the nucleophilic ring opening of the activated *N*-acyl iminium cyclopropane **3-41** (Scheme 3.8). Traditionally, the intermolecular ring opening of activated cyclopropane adducts requires harsher conditions such as elevated temperatures, high pressure, or the use of an organometallic catalyst.¹⁷



Scheme 3.8: Synthetic strategy to access oxazinones under metal-free conditions.

In this work, the authors postulated that the electrophilic cyclopropane moiety in intermediate **3-41** could undergo a homologous conjugate addition with an appropriate nucleophile to generate an enolate. The resultant enolate could in turn attack the carbon of the iminium to provide the targeted oxazinone **3-40**.

Using this approach, a series of structurally diverse oxazinones under atmospheric pressure and temperature in moderate yields were prepared. As is evident, a variety of electronically diverse aromatic imines as well as alkyl imines were tolerated in this transformation. Further scope of this approach was demonstrated by the reaction of the acid chloride with the corresponding benzophenone imine and diphenyliminoglycinate imine to provide products **3-47** and **3-48** in moderate yields. Despite the various approaches to these bioactive scaffolds, there remains a need for a mild and selective preparation of 1,3-oxazin-4-ones from readily available starting materials.



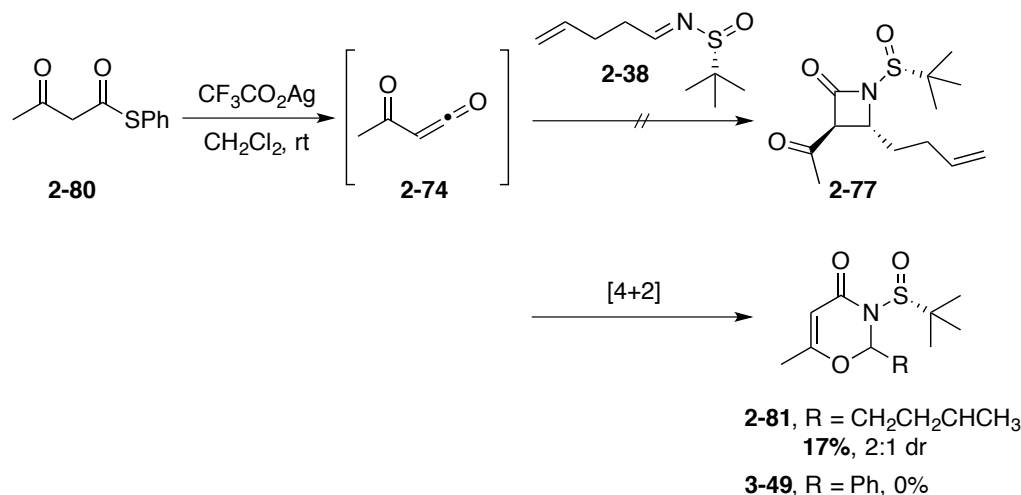
Scheme 3.9: Selected examples of substrate scope of metal-free oxazinone formation.

3.3 Results and Discussions

3.3.1 Development of a [4+2] Cycloaddition of Acylketenes and Aldimines

While exploring the reactivity of acylketene intermediates (generated at room temperature) towards [2+2] cycloaddition reaction with imine **2-38** to form β -lactam **2-77** (refer to section 2.3.3.2), we observed the formation of an unknown product, which after careful analysis was determined to be oxazinone **2-81**. Compound **2-81** is the product of [4+2] cycloaddition, which is the preferred mode of reactivity of acylketenes. Encouraged by this result, we became intrigued with the possibility of employing a thiophilic metal to

generate an acylketene intermediate at room temperature, followed by the subsequent reaction with an aldimine to furnish a 1,3-oxazin-4-one as a plausible solution to the elevated temperatures utilized in most previous approaches to this class of heterocycles.



Scheme 3.10: Initial studies on reactivity of acylketene **2-74** in cycloaddition reactions

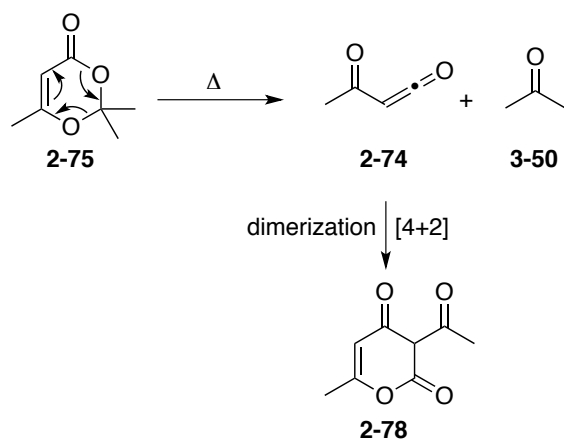
Following our initial efforts, we obtained 1,3-oxazin-4-one **2-81** in 17% yield; however, the low diastereoselectivity and the inability to improve these initial low yields did not allow for further development of this reaction using that substrate. Furthermore, the alternative aromatic *N-tert*-butanesulfinyl imine proved to be completely inactive in the cycloaddition reaction to furnish oxazinone **3-49**, even after prolonged reaction times (Scheme 3.10).

With these results in hand, it became clear that a more electron-rich imine was required to intercept the acylketene intermediate for a successful cycloaddition. Encouraged by our preliminary findings and in continuation of our interest in aza-heterocyclic compounds as biologically relevant and useful synthetic building blocks, we became

interested in further investigating the reactivity of acylketenes towards aldimines in this transformation.

3.3.2 Acylketenes From Thioacetoacetate-Electrophilic Activation

As mentioned earlier, acylketenes are valuable building blocks in cycloaddition reactions as well as highly reactive acylating reagents for a variety of nucleophiles. Acylketenes are generally derived from one of the reagents discussed earlier (Scheme 3.1) under predominantly photoirradiation or thermal conditions. To date, pyrolysis of dioxinone **2-75** to acylketene **2-74** and acetone is one of the most versatile and convenient methods of acylketene preparation (Scheme 3.11); however, this transformation occurs at elevated temperatures and requires a trapping agent that is more reactive than the acetone byproduct, which can also undergo cycloaddition with the resultant acylketene to furnish the dioxinone starting material. In the absence of an appropriate nucleophile or a trapping reagents, acylketenes dimerize via [4+2] cycloaddition to form the stable dehydroacetic acid byproduct **2-78**.¹³



Scheme 3.11: Dioxinone as a source of acylketene and its dimerization.

Furthermore, acylketenes react with a variety of alcohol, sulfur, and amine nucleophiles to generate β -keto esters, β -keto thioesters, and β -keto amides in both intermolecular and intramolecular manner.¹³ Further functionalization of dioxinone **2-75** at the vinylic methyl position is also possible through the use of a strong base such as LDA, followed by subsequent alkylation or vinylogous aldol reaction.¹⁸ The utility of acylketenes has also been showcased in the total synthesis of complex natural products through macrolactonization transformations. Over the years, the intramolecular trapping of acylketene intermediates has become one of the most versatile methods of medium sized lactone formation.¹⁹ Despite their versatility, the use of acylketenes has its inherent limitations as well, specifically at higher temperatures which is most often required for their preparation.

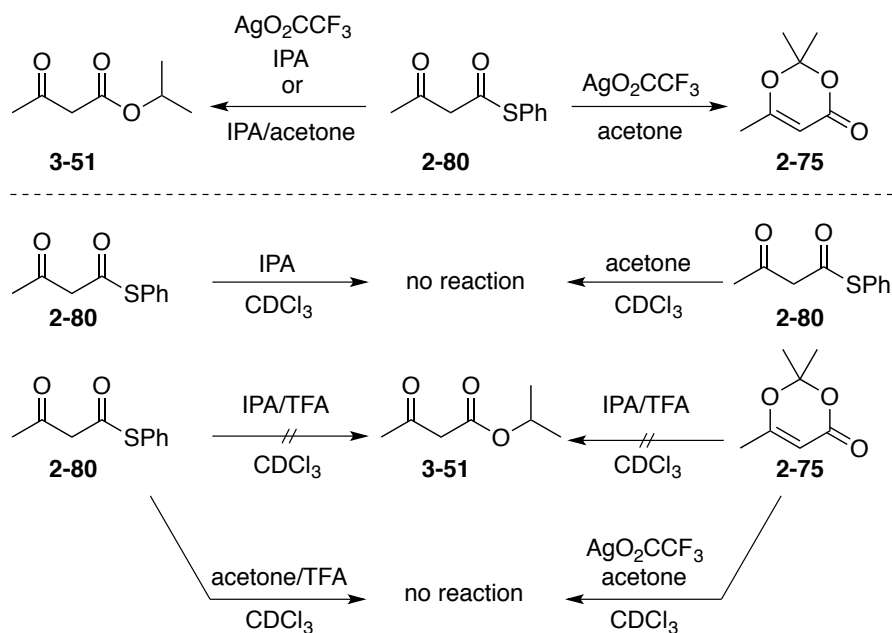
Hoye et al. has recently reported a mild preparation of acylketenes via silver activation of phenyl thioacetate **2-80** at room temperature.²⁰ The formation of acylketenes from β -keto thioesters was first suggested by Douglas et al. in their studies on the

alkaline hydrolysis of S-acetoacetyl-CoA in 1980.²¹ Building on this precedent and the studies reported by the groups of Kishi²² and Ley²³, where silver(I) and copper(I) salts were utilized in the intramolecular activation of β -keto thioesters in the context of macrolactonization, silver(I) salt was chosen as a starting point for the activation of phenyl thioacetoacetate **2-80**.

In their studies, the Hoye group observed the formation of dioxinone **2-75** upon treatment of phenyl thioacetoacetate **2-80** with acetone in the presence of silver trifluoroacetate, consistent with the reactivity pattern of acylketenes towards ketones. Hoye also observed the formation of β -keto ester **3-51** when **2-80** was treated with acetone or acetone/isopropyl alcohol (IPA) mixture in the presence of silver(I) salt, also typical of acylketene reactivity. Indeed, acylketenes are known to be trapped orders of magnitude faster by alcohols to form β -keto esters than ketones to generate dioxinones.²⁴ This was further demonstrated in studies where β -keto ester **3-51** was the only product formed when IPA was present in the reaction medium (IPA:acetone, ~1:1). Additionally, no “silver mirror” or thiophenol formation in the reaction mixture was observed, suggesting that Ag(0) was not produced and the thiophenol was actually captured by silver to produce the insoluble solid AgSPh (Scheme 3.12).

Control experiments were also performed in order to establish the necessity of silver(I) salt as an activator in the reaction. Phenyl thioacetoacetate **2-80** is completely unreactive in the presence of only IPA in CDCl₃ and only acetone in CDCl₃. Moreover, the formation of dioxinone **2-75** from phenyl thioacetoacetate **2-80** is not an acid-catalyzed reaction, since **2-80** is completely unreactive when treated with acetone and TFA. The

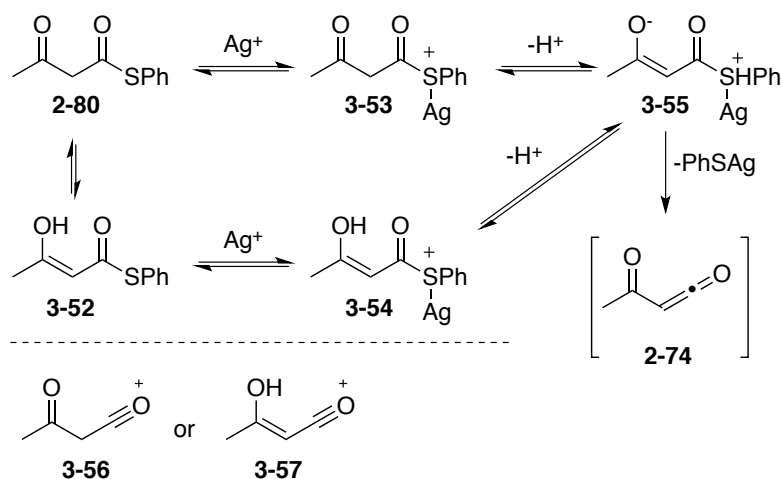
formation of **3-51** from phenyl thioacetoacetate **2-80** is also not acid-catalyzed, since no esterification product was observed in the presence of IPA and TFA only (Scheme 3.12). Collectively, it can be deduced that dioxinone **2-75** is stable to the reaction conditions (acidic or neutral), and phenyl thioacetoacetate **2-80** is also stable to neutral and acidic conditions.



Scheme 3.12: Intermediacy of acylketene in phenyl thioacetoacetate activation.

A proposed mechanism by which the acylketene is generated is depicted in **Scheme 3.13**. The electrophilic activation of the thioester **2-80** (or the enol **3-52**) by silver(I) ion leads to formation of cationic intermediate **3-53** (or **3-52**). Proton loss from the cationic intermediate pair gives rise to zwitterion **3-55**, which could directly lead to the acylketene intermediate **2-74**. Alternatively, the formation of acylketene could be explained through the high energy acylium ion intermediates **3-56** or **3-57**, generated through the loss of PhSAg

from either cationion intermediate **3-53** or **3-54**, respectively. A simple proton loss from either intermediate then results in formation of acylketene **2-74**.



Scheme 3.13: Proposed mechanism for silver(I) activation of phenyl thioacetoacetate **2-80**.

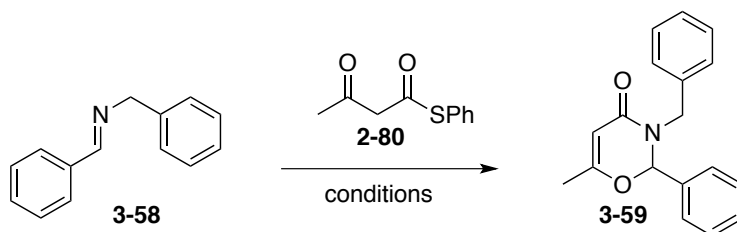
3.3.3 Exploration into Aldimine Reactivity and Optimization

Aldimines bearing electron-rich alkyl groups on nitrogen are common substrates for [2+2] Staudinger cycloaddition reactions with most ketenes,²⁵ but are known to undergo [4+2] cycloaddition reactions with acylketenes.⁶⁻¹² To this end, aldimine **3-58**, prepared via condensation reaction of benzaldehyde and benzylamine served as a model system in our studies. Aldimine **3-58** was then reacted with the *in situ* generated acylketene derived from **2-80** (entry 1, Table 3.1) using CH_2Cl_2 as the solvent and silver trifluoroacetate as an activator. To our delight, 1,3-oxazin-4-one **3-59** was isolated in 58% yield under these conditions and this result served as a starting point for reaction optimization.

Next, a series of solvents (entry 2 – 4, Table 3.1) and activators (entry 5 – 7, Table 3.1) were screened. The use of more polar solvents such as THF, CHCl_3 and the aromatic

solvents such as benzene resulted in much lower yields of the desired product. Ultimately, the use of CH₂Cl₂ provided the highest yield of the product and proved to be the optimum solvent for this transformation.

Table 3.1: Optimization of acylketene cycloaddition with imine **3-58**.



Entry	Conditions ^a	Yield (%) ^b
1	2-80 (2 equiv), AgO ₂ CCF ₃ (2 equiv), CH ₂ Cl ₂ , 2 h	58
2	2-80 (2 equiv), AgO ₂ CCF ₃ (2 equiv), THF, 2 h	28
3	2-80 (2 equiv), AgO ₂ CCF ₃ (2 equiv), CHCl ₃ , 2 h	36
4	2-80 (2 equiv), AgO ₂ CCF ₃ (2 equiv), benzene, 2 h	22
5	2-80 (2 equiv), CuOTf (2 equiv), CH ₂ Cl ₂ , 72 h	0
6	2-80 (2 equiv), CuBr ₂ (2 equiv), CH ₂ Cl ₂ , 18 h	0
7	2-80 (2 equiv), Ag ₃ O ₄ P (2 equiv), CH ₂ Cl ₂ , 48 h ^c	0
8	2-80 (2 equiv), AgO ₂ CCF ₃ (2.75 equiv), CH ₂ Cl ₂ , 2 h	77
9	2-80 (2 equiv), AgO ₂ CCF ₃ (3.1 equiv), CH ₂ Cl ₂ , 2 h	90

^a Reactions performed on a 10.0 mg scale.

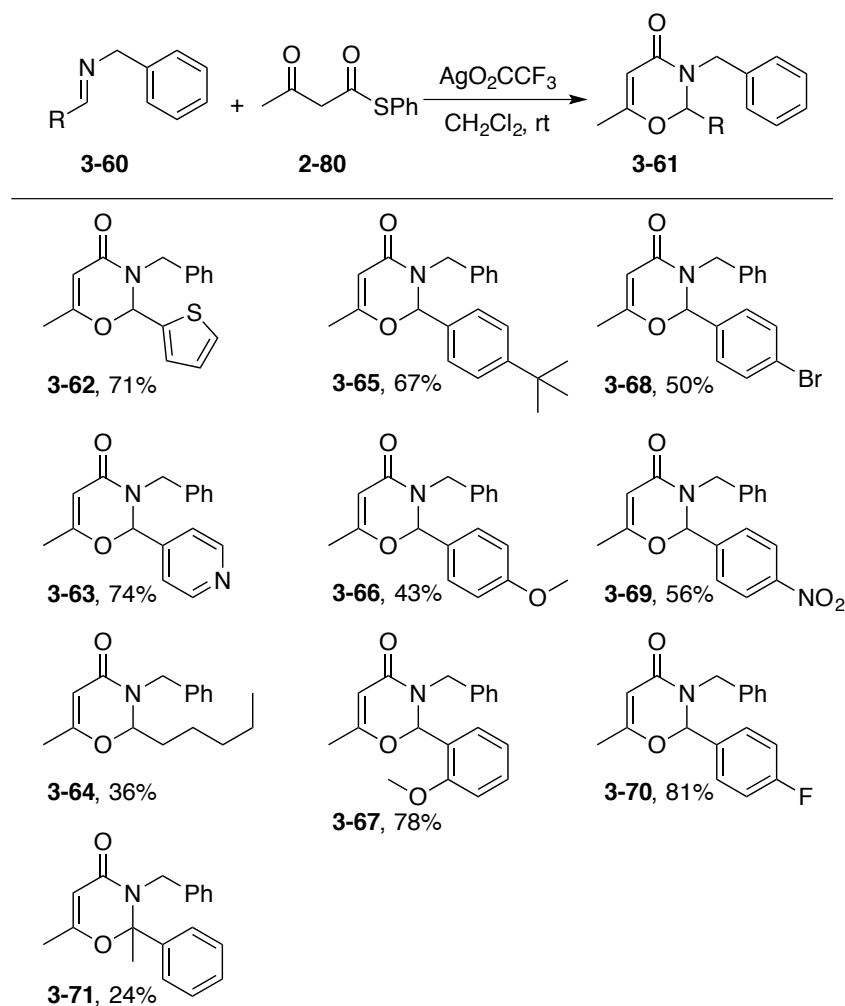
^b Isolated yields.

^c Ag₃O₄P = trisilver phosphate.

Furthermore, the use of other activators such as CuOTf (entry 5, Table 3.1), CuBr₂ (entry 6, Table 3.1), and Ag₃O₄P (entry 7, Table 3.1) in place of AgO₂CCF₃ resulted in no product formation. It is worth mentioning that the formation of **3-59**, followed by its decomposition before the complete consumption of the starting imine **3-58** was observed when CuBr₂ was used as an activator

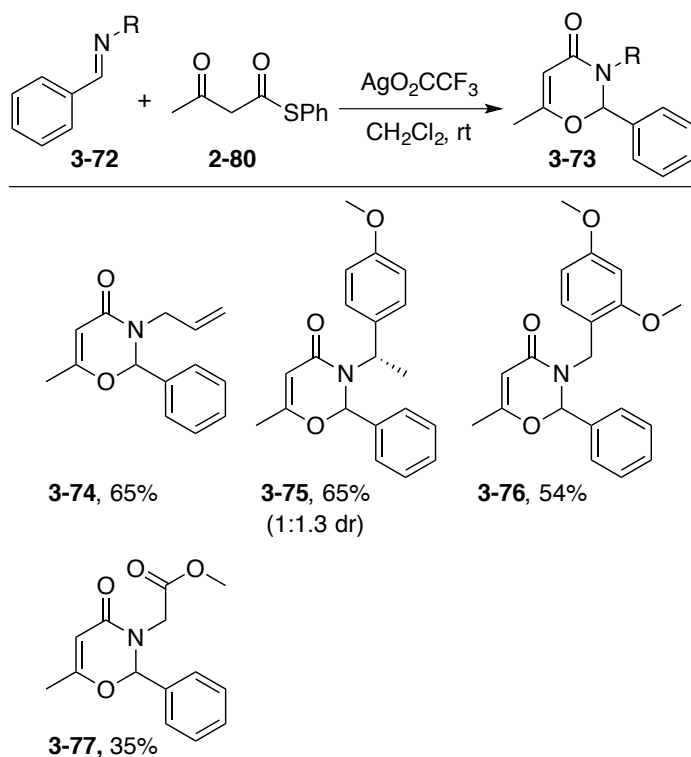
Following screening of several parameters, the original combination of CH₂Cl₂ and silver trifluoroacetate proved most effective. The yield of the product was further improved by increasing the amount of thioester **2-80** and silver salt (entry 8 – 9, Table 3.1). Ultimately, the combination of thioester **2-80** (3 equiv) and silver trifluoroacetate (3.1 equiv) in CH₂Cl₂ afforded the desired product **3-59** in 90% yield in less than 2 hours (entry 9, Table 3.1). It is important to highlight that no β-lactam byproducts were observed for any of the conditions screened (in contrast to mixtures that can be obtained under thermal conditions).⁷

With a high yielding method for 1,3-oxazin-4-one synthesis in hand, we evaluated the substrate scope for this transformation (Scheme 3.14). Heteroaromatic aldimines (**3-62** and **3-63**), electron-rich aromatic aldimines (**3-65** to **3-67**) and electron-poor aldimines (**3-68** to **3-70**) all performed well in the cycloaddition reaction providing yields from 43 to 90%. Moreover, the heteroaromatic products (**3-62** and **3-63**) and substrates possessing aryl halides (**3-68** and **3-70**) are important building blocks from a synthetic point of view due to the possibility of further functionalization. The electronically rich but sterically demanding *o*-methoxy substituted imine also underwent the cycloaddition smoothly to generate oxazinone **3-67**. In addition to aldimines, ketimines also underwent the desired cycloaddition to generate quaternary 1,3-oxazin-4-one **3-71**, albeit in 24% unoptimized yield. Unfortunately, enolizable aldimines leading to product **3-64** did not perform well in our reaction, likely due to their instability under the reaction conditions (< 40% yield).



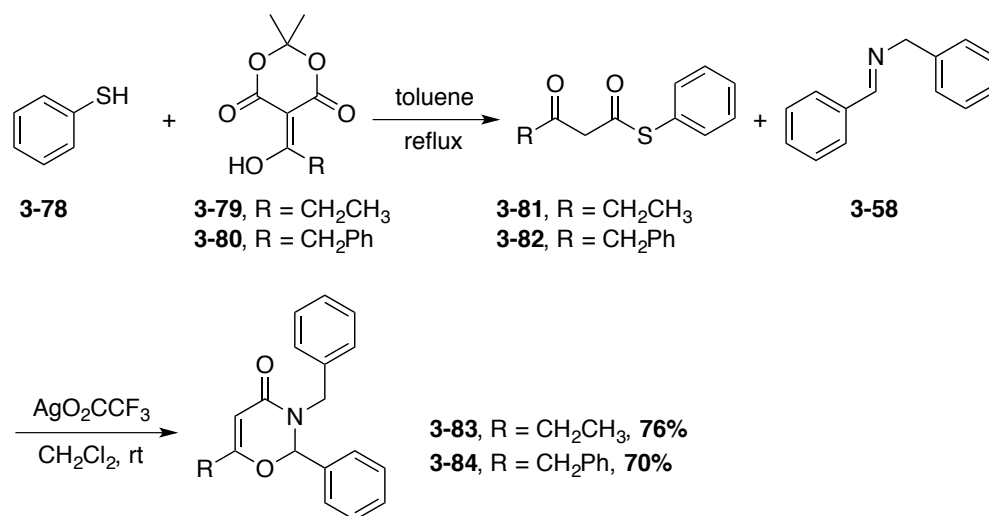
Scheme 3.14: Substrate scope for aldimines utilized in 1,3-oxazin-4-one synthesis.

Variation of the substituents on the nitrogen was also well tolerated (Scheme 3.15) providing allyl (**3-74**), substituted benzyl (**3-75** and **3-76**) and ester (**3-77**) functionalized heterocycles in modest to high yields. The corresponding aldimine **3-72** was prepared using standard protocols from the requisite aldehyde and the amine.



Scheme 3.15: Substrate scope for aldimines utilized in 1,3-oxazin-4-one synthesis.

Finally, variants of thioester **2-80** were employed in the cycloaddition to provide substitution of the methyl group, yielding ethyl **3-83** and benzyl **3-84** derivatives in high yields. The synthetic route to the requisite thioesters is depicted in **Scheme 3.16**. Acylation of Meldrum's acid using the corresponding acyl chloride, followed by the heating and trapping of the resultant functionalized acylketenes with thiophenol **3-78** provided the targeted β -keto thioesters **3-81** and **3-82** in 74% and 74% yields.



Scheme 3.16: Substrate scope for β -keto thioesters utilized in 1,3-oxazin-4-one synthesis.

All of the isolated compounds were purified by chromatography on SiO₂ without significant decomposition; however, extended treatment of the 1,3-oxazin-4-ones to SiO₂ or aqueous acid led to decomposition. The oxazinones are stable to storage at room temperature with the exception of electron rich substrates **3-66** to **3-67** which slowly decomposed upon storage.

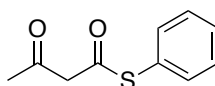
3.4 Conclusions

In conclusion, we have developed a facile and mild approach for the synthesis of 1,3-oxazin-4-ones via a silver(I)-mediated [4+2] cycloaddition reaction of aldimines and acylketenes (generated from β -keto thioesters). The requisite starting materials utilized in this reaction are readily accessed from commercially available reagents. Furthermore, we have demonstrated the utility and functional group compatibility of this transformation through the rapid synthesis of a wide range of electronically diverse scaffolds with various substitution patterns in moderate to high yields. Studies on the biological activity of these heterocycles and their application as building blocks towards more complex structures are underway and will be reported in due course.

3.5 Experimental Section

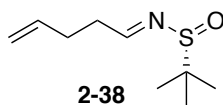
General considerations: All reactions were performed under an argon atmosphere and all glassware was dried in an oven at 134 °C for 2 h prior to use, unless otherwise noted. Silver trifluoroacetate, 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one, Meldrum's acid, benzylamine and aldehyde derivatives were purchased from Sigma-Aldrich and Fisher Scientific and were used without further purification. Reactions were monitored by TLC analysis (EM Science pre-coated silica gel 60 F₂₅₄ plates, 250 μm layer thickness) and visualization was accomplished with a 254 nm UV light and by staining with a KMnO₄ solution (1.5 g of KMnO₄, 10 g of K₂CO₃, and 1.25 mL of a 10% NaOH solution in 200 mL of water). Reactions were also monitored by LC-MS (Shimadzu LC-MS 2020 with Kinetex 2.6 μm C18 50 x 2.10 mm). Flash chromatography on SiO₂ was used to purify the crude reaction mixtures and performed on a Biotage Isolera utilizing Biotage cartridges and linear gradients. ¹H and ¹³C NMR spectra were obtained on a Varian Mercury-VX 300, a Varian Mercury-VX 400, or a Varian Mercury-Plus 300 instrument in CDCl₃ unless otherwise noted. Chemical shifts were reported in parts per million with the residual solvent peak used as an internal standard (CDCl₃ = 7.26 ppm for ¹H, CDCl₃ = 77.23 ppm for ¹³C). ¹H NMR spectra were run at 300 or 400 MHz and are tabulated as follows: chemical shift, multiplicity (s = singlet, d = doublet, m = multiplet, dd = doublet of doublet, dt = doublet of triplet, td = triplet of doublet, tt = triplet of triplet, ddd = doublet of doublet of doublet), number of protons, and coupling constant(s). ¹³C NMR spectra were run at 100 MHz using a proton-decoupled pulse sequence with a d₁ of 1 second unless otherwise noted, and are tabulated by observed peak. Infrared spectra were determined on a Jasco FT/IR-4100 spectrometer. High-resolution mass

spectra were obtained on a Thermo Fisher Scientific, Exactive Plus mass spectrometer using Heated Electrospray Ionization (HESI). Melting points were determined using a Thomas Hoover Capillary Melting Point Apparatus.



2-80

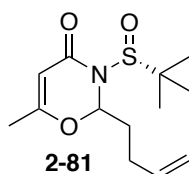
Phenyl thioacetoacetate (2-80). This compound was prepared according to modification of a literature procedure.²⁶ A solution of thiophenol (736 mg, 6.68 mmol) and 2,2,6-trimethyl-4H-1,3-dioxin-4-one (1.00 g, 6.68 mmol) in toluene (15.0 mL) was heated to reflux for 3 h. The reaction was then cooled to rt and concentrated under reduced pressure to afford a dark brown residue. The crude residue was purified using flash chromatography on SiO₂ (hexanes:EtOAc, 0 to 30%) to provide 1.11 g (86%) of **2-80** as a light orange oil: R_f (hexanes/EtOAc, 90:10) 0.26; ¹H NMR (400 MHz, CDCl₃) δ 7.45 (m, 1.02 H, enol), 7.44 (s, 5 H), 5.49 (s, 0.46 H, enol), 3.76 (s, 2 H), enol), 2.29 (s, 3 H), 1.96 (s, 2 H, enol); ¹³C NMR (100 MHz, CDCl₃) δ 199.8, 174.9, 135.2, 134.6, 130.1 (enol), 129.8 (enol), 129.6, 129.4, 127.1, 58.0, 30.6, 21.4 (enol).



2-38

(*R,E*)-2-Methyl-*N*-(pent-4-en-1-ylidene)propane-2-sulfonamide (2-38). This compound was prepared according to modification of literature procedure.²⁷ A round bottom flask equipped with a Claisen adaptor and an addition funnel was charged with a solution of oxalyl

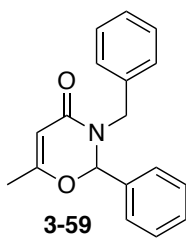
chloride (3.09 g, 24.4 mmol) in CH₂Cl₂ (44.0 mL) under a stream of argon at -78 °C. Anhydrous DMSO (3.81 g, 48.8 mmol) was added to this solution in a drop-wise manner and the mixture was stirred for 20 min at -78 °C, followed by the drop-wise addition of 4-penten-1-ol (1.50 g, 17.4 mmol). The mixture was stirred for an additional 45 min, and NEt₃ (7.93 g, 78.4 mmol) was added. The suspension was allowed to warm to rt gradually, quenched with sat. aq. NH₄Cl (30.0 mL) and extracted with CH₂Cl₂. The organic layer was separated, washed with brine and water, and dried (MgSO₄). The crude (*E*)-4-hexenal mixture was filtered into a RB flask equipped with a magnetic stir bar and placed under an atmosphere of argon. The flask was charged with (*R*)-*t*-butanesulfinamide (1.76 g, 14.5 mmol), and Ti(OEt)₄ (6.62 g, 29.0 mmol). The mixture was stirred at rt overnight, and poured into a rapidly stirring solution of brine, filtered, extracted with EtOAc, and dried (MgSO₄). The crude mixture was concentrated under reduced pressure and purified by flash column chromatography on SiO₂ (hexanes:EtOAc, 1 to 100%) to provide 1.56 g (88%) of **2-38** as a yellow oil: ¹H NMR (300 MHz, CDCl₃) δ 8.08 (t, 1 H, *J* = 4.5 Hz), 5.89-5.77 (m, 1 H), 5.10-5.04 (m, 2 H), 2.63 (m, 2 H), 2.40 (m, 2 H), 1.19 (s, 9 H); ESIMS *m/z* 229 [M+MeCN]⁺.



2-(But-3-en-1-yl)-3-((*R*)-*tert*-butylsulfinyl)-6-methyl-2,3-dihydro-4*H*-1,3-oxazin-4-one

(2-81). To a solution of imine **2-38** (50.0 mg, 0.272 mmol) in CH₂Cl₂ (4.70 mL) was added thioester **2-81** (0.158 g, 0.815 mmol) and silver trifluoroacetate (0.186 g, 0.978 mmol) under an argon atmosphere at rt. The mixture was stirred at rt for 5 h, diluted with CH₂Cl₂, filtered

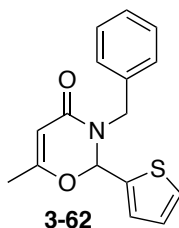
through a pad of Celite[®], and concentrated under reduced pressure to afford the crude as a brown oil. The crude residue was purified using flash chromatography on SiO₂ (hexanes:EtOAc, 0 to 100%) to afford 12.5 mg (17%) of **2-81** as a 2:1 mixture of diastereomers: ¹H NMR (400 MHz, CDCl₃) δ 5.83-5.72 (m, 2.5 H, major and minor), dd (5.66, 0.50 H, *J* = 10.2, 4.1 Hz, minor), 5.30-5.29 (m, 1 H, major), 5.28-5.27 (m, 0.50 H, minor), 5.11-4.98 (m, 3.0 H, major and minor), 2.68-2.58 (m, 1 H, major), 2.45-2.35 (m, 0.50 H, minor), 2.22-2.13 (m, 2 H, major), 2.12-2.07 (m, 1.5 H, minor), 2.00 (s, 1.50 H, minor), 1.97 (s, 1 H, major), 1.77-1.68 (m, 0.50 H, minor), 1.61-1.52 (m, 1 H, major), 1.23 (s, 4.5 H, minor), 1.22 (s, 9 H, major); ¹³C NMR (100 MHz, CDCl₃) δ 167.3, 166.3 (minor), 163.8 (minor), 162.8, 136.6 (minor), 136.2, 116.7, 116.0 (minor), 100.0 (minor), 99.2 (major), 83.0 (minor), 78.8, 61.7 (minor), 59.5, 32.3 (minor), 31.6, 30.1, 29.4 (minor), 22.8, 22.1 (minor), 20.3, 20.0 (minor); IR (neat) 2974, 1719, 1666, 1400, 1358, 1152, 811 cm⁻¹; ESIMS *m/z* 313 [M+MeCN]⁺; HRMS *m/z* calculated for C₁₃H₂₁NO₃SNa [M+Na]⁺ 294.1134, found 294.1123.



3-Benzyl-6-methyl-2-phenyl-2,3-dihydro-4H-1,3-oxazin-4-one (3-59). General protocol

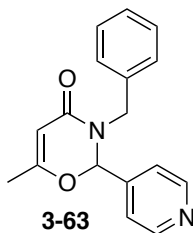
A. To a solution of benzaldehyde (70.0 mg, 0.656 mmol) in anhydrous CH₂Cl₂ (6.50 mL) was added benzylamine (71.0 mg, 0.656 mmol) and MgSO₄. The heterogeneous mixture was stirred overnight, filtered and concentrated under reduced pressure to afford the benzaldimine

as a yellow oil. The benzaldimine was transferred to an oven-dried round bottom flask in anhydrous CH₂Cl₂ (13.0 mL) under an argon atmosphere. The flask was then charged with thioester **2-80** (0.119 g, 1.96 mmol) and silver trifluoroacetate (0.449 g, 2.03 mmol) at rt. The mixture was stirred for 1.5 h, diluted with CH₂Cl₂, filtered through a pad of Celite[®] and concentrated under reduced pressure to afford the crude as a brown oil. The residue was purified using flash column chromatography on SiO₂ (hexanes:EtOAc, 0 to 100%) to provide 0.167 g (91%) of **3-59** as a yellow oil: R_f (hexanes/EtOAc, 50:50) 0.59; ¹H NMR (300 MHz, CDCl₃) δ 7.40-7.28 (m, 8 H), 7.24-7.12 (m, 2 H), 6.08 (s, 1 H), 5.37 (d, 1 H, *J* = 15.5 Hz), 5.32 (s, 1 H), 3.80 (d, 1 H, *J* = 15.5 Hz), 1.87 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 165.2, 163.9, 136.9, 135.6, 129.9, 128.8, 128.0, 127.7, 127.6, 100.1, 87.8, 46.5, 19.9; IR (neat) 3065, 1670, 1430, 1354, 1252, 696 cm⁻¹; ESIMS *m/z* 321 [M+MeCN]⁺; HRMS *m/z* calculated for C₁₈H₁₇NO₂ [M+H]⁺ 280.1332, found 280.13303.

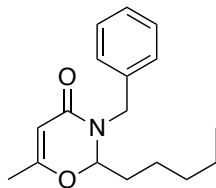


3-Benzyl-6-methyl-2-(thiophen-2-yl)-2,3-dihydro-4H-1,3-oxazin-4-one (3-62). According to general protocol A, 2-thiophenecarboxaldehyde (30.0 mg, 0.262 mmol), benzylamine (28.4 mg, 0.262 mmol), thioester **2-80** (99.2 mg, 0.786 mmol) and silver trifluoroacetate (0.180 g, 0.813 mmol) in anhydrous CH₂Cl₂ (5.20 mL) stirred for 3 h afforded 53.1 mg (71%) of **3-62** as a yellow oil after purification by chromatography on SiO₂ (hexanes:EtOAc, 0 to 100%): R_f (hexanes/EtOAc, 50:50) 0.55; ¹H NMR (300 MHz, CDCl₃) δ 7.36-7.31 (m, 4

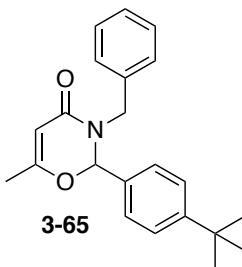
H), 7.25-7.22 (m, 2 H), 7.00-6.95 (m, 2H), 6.31 (s, 1 H), 5.42 (d, 1 H, $J = 15.6$ Hz), 5.43 (s, 1 H), 3.89 (d, 1 H, $J = 15.4$ Hz), 1.92 (d, 3 H, $J = 0.85$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 165.3, 163.6, 146.2, 139.1, 136.3, 129.0, 128.1, 128.1, 128.0, 127.6, 126.8, 99.7, 84.0, 46.7, 20.0; IR (neat) 3068, 1670, 1605, 1449, 1418, 1183, 1138, 712 cm^{-1} ; ESIMS m/z 327 $[\text{M}+\text{MeCN}]^+$; HRMS m/z calculated for $\text{C}_{16}\text{H}_{15}\text{NSO}_2$ $[\text{M}+\text{H}]^+$ 286.0896, found 286.0896.



3-Benzyl-6-methyl-2-(pyridin-4-yl)-2,3-dihydro-4H-1,3-oxazin-4-one (3-63). According to general protocol A, 4-pyridinecarboxaldehyde (40.0 mg, 0.366 mmol), benzylamine (39.6 mg, 0.366 mmol), thioester **2-80** (0.119 g, 1.10 mmol) and silver trifluoroacetate (0.251 g, 1.13 mmol) in anhydrous CH_2Cl_2 (5.20 mL) stirred for 3 h afforded 75.7 mg (74%) of **3-63** as a brown oil after purification by chromatography on SiO_2 (CH_2Cl_2 :MeOH, 0 to 10%): R_f (CH_2Cl_2 /MeOH, 95:5) 0.43; ^1H NMR (300 MHz, CDCl_3) δ 8.61 (br d, 2 H, $J = 4.8$ Hz), 7.34-7.26 (m, 7 H), 6.05 (s, 1 H), 5.40 (d, 1 H, $J = 15.5$ Hz), 5.29 (s, 1 H), 3.91 (d, 1 H, $J = 15.4$ Hz), 1.86 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 165.0, 162.3, 151.4, 146.7, 135.8, 129.3, 128.5, 128.2, 123.7, 101.2, 85.4, 47.8, 19.9; IR (neat) 3027, 1667, 1600, 1362, 1036, 730, 507 cm^{-1} ; ESIMS m/z 322 $[\text{M}+\text{MeCN}]^+$; HRMS m/z calculated for $\text{C}_{17}\text{H}_{16}\text{N}_2\text{O}_2$ $[\text{M}+\text{H}]^+$ 281.1285, found 281.1278.

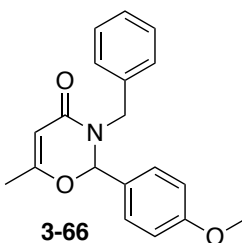


3-Benzyl-6-methyl-2-pentyl-2,3-dihydro-4H-1,3-oxazin-4-one (3-64). According to general protocol A, hexenal (20.0 mg, 0.120 mmol), benzylamine (12.9 mg, 0.120 mmol), thioester **2-80** (69.7 mg, 0.359 mmol) and silver trifluoroacetate (81.9 mg, 0.371 mmol) in anhydrous CH₂Cl₂ (2.50 mL) stirred for 3 h afforded 27.0 mg (36%) of **3-64** as a colorless oil after purification by chromatography on SiO₂ (hexanes:EtOAc, 0 to 100%): R_f (hexanes/EtOAc, 50:50) 0.48; ¹H NMR (300 MHz, CDCl₃) δ 7.39-7.23 (m, 5 H), 5.27 (s, 1 H), 5.11 (d, 1 H, *J* = 15.5 Hz), 4.11 (d, 1 H, *J* = 15.7 Hz), 1.93 (s, 3 H), 1.63-1.55 (m, 2 H), 1.29-1.17 (m, 6 H), 0.86 (t, 3 H, *J* = 7.30); ¹³C NMR (100 MHz, CDCl₃) δ 165.4, 163.0, 137.5, 129.2, 127.7, 126.2, 99.5, 88.1, 46.0, 31.4, 30.9, 24.7, 19.9, 19.7, 14.1; IR (neat) 2959, 1667, 1457, 1393, 1027, 701 cm⁻¹; ESIMS *m/z* 274 [M+H]⁺; HRMS *m/z* calculated for C₁₇H₂₄NO₂ [M+H]⁺ 274.1802, found 274.1802.



3-Benzyl-2-(4-(tert-butyl)phenyl)-6-methyl-2,3-dihydro-4H-1,3-oxazin-4-one (3-65). According to general protocol A, 4-*t*-butylbenzaldehyde (20.0 mg, 0.120 mmol), benzylamine (12.9 mg, 0.120 mmol), thioester **2-80** (69.7 mg, 0.359 mmol) and silver

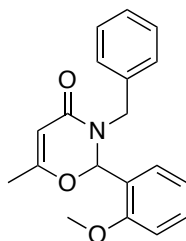
trifluoroacetate (81.9 mg, 0.371 mmol) in anhydrous CH₂Cl₂ (2.50 mL) stirred for 3 h afforded 27.0 mg (67%) of **3-65** as a colorless oil after purification by chromatography on SiO₂ (hexanes:EtOAc, 0 to 100%): R_f (hexanes/EtOAc, 50:50) 0.56; ¹H NMR (300 MHz, CDCl₃) δ 7.37 (d, 2 H, *J* = 8.3 Hz), 7.27-7.21 (m, 5 H), 7.13-7.10 (m, 2 H), 6.06 (s, 1 H), 5.36 (s, 1 H), 5.33 (d, 1 H, *J* = 15.9 Hz), 3.82 (d, 1 H, *J* = 15.4 Hz), 1.88 (s, 3 H), 1.32 (s, 9 H); ¹³C NMR (100 MHz, CDCl₃) δ 165.4, 164.0, 153.1, 137.0, 132.5, 129.9, 128.7, 128.0, 127.6, 127.3, 125.7, 99.9, 87.9, 46.4, 35.0, 31.4, 19.9; IR (neat) 2958, 1674, 1457, 1430, 1005, 819, 727, 696 cm⁻¹; ESIMS *m/z* 377 [M+MeCN]⁺; HRMS *m/z* calculated for C₂₂H₂₅NO₂ [M+H]⁺ 336.1958, found 336.1956.



3-Benzyl-2-(4-methoxyphenyl)-6-methyl-2,3-dihydro-4H-1,3-oxazin-4-one (3-66).

General protocol B. To a solution of 4-methoxybenzaldehyde (25.6 mg, 0.186 mmol) in anhydrous EtOH (1.90 mL) was added benzylamine (20.1 mg, 0.186 mmol) and anhydrous MgSO₄ under a nitrogen atmosphere. The mixture was refluxed for 24 h, and then concentrated *in vacuo* to afford the crude imine as a yellow oil. The imine was transferred to an oven-dried round bottom flask in anhydrous CH₂Cl₂ (3.70 mL) under an argon atmosphere. The flask was then charged with thioester **2-80** (0.103 g, 0.558 mmol) and silver trifluoroacetate (0.127 g, 0.577 mmol) at rt. The mixture was stirred for 3 h, diluted with CH₂Cl₂, filtered through a pad of Celite[®] and concentrated under reduced pressure to

afford the crude as a yellow oil. The residue was purified using flash column chromatography on SiO₂ (hexanes:EtOAc, 0 to 100%) to provide 24.7 mg (43%) of **3-66** as a colorless oil: R_f (hexanes/EtOAc, 50:50) 0.39; ¹H NMR (400 MHz, CDCl₃) δ 7.29-7.21 (m, 5 H), 7.13-7.11 (m, 2 H), 6.86 (d, 2 H, *J* = 8.7 Hz), 6.03 (s, 1 H), 5.32 (s, 1 H), 5.31 (d, 1 H, *J* = 13.1 Hz), 3.82 (s, 3 H), 3.78 (d, 1 H, *J* = 15.5 Hz), 1.87 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 165.0, 163.9, 160.7, 137.1, 129.1, 128.7, 128.0, 114.1, 100.1, 87.9, 55.6, 46.2, 19.9; IR (neat) 2962, 1719, 1662, 1605, 1514, 1255, 1179, 819, 727 cm⁻¹; ESIMS *m/z* 351 [M+MeCN]⁺; HRMS *m/z* calculated for C₁₉H₁₉NO₃ [M+H]⁺ 310.1438, found 310.1439.

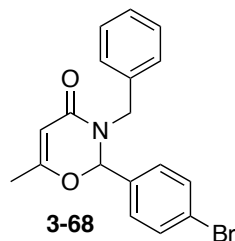


4-67

3-Benzyl-2-(2-methoxyphenyl)-6-methyl-2,3-dihydro-4H-1,3-oxazin-4-one (3-67).

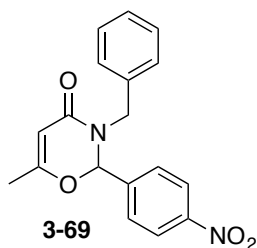
According to general protocol B, 2-methoxybenzaldehyde (26.5 mg, 0.195 mmol), benzylamine (20.9 mg, 0.195 mmol), thioester **2-80** (0.114 g, 0.585 mmol) and silver trifluoroacetate (0.133 g, 0.604 mmol) in anhydrous CH₂Cl₂ (4.00 mL) stirred for 3 h afforded 47.2 mg (78%) of **3-67** as a light yellow oil after purification by chromatography on SiO₂ (hexanes:EtOAc, 0 to 100%): R_f (hexanes/EtOAc, 50:50) 0.47; ¹H NMR (300 MHz, CDCl₃) δ 7.41-7.28 (m, 2 H), 7.23-7.20 (m, 3 H), 7.03-6.86 (m, 4 H), 6.51 (s, 1 H), 5.46 (s, 1 H), 4.99 (d, 1 H, *J* = 15.2 Hz), 3.91 (d, 1 H, *J* = 15.5 Hz), 3.69 (s, 3 H), 1.92 (s, 3 H); ¹³C NMR (75 MHz, CDCl₃) δ 166.9, 157.8, 137.1, 131.5, 128.8, 128.5, 127.8, 127.4, 120.6,

111.1, 99.0, 83.6, 55.6, 46.0, 19.9; IR (neat) 2921, 1674, 1598, 1418, 1252, 1031, 936 cm^{-1} ; ESIMS m/z 351 $[\text{M}+\text{MeCN}]^+$; HRMS m/z calculated for $\text{C}_{19}\text{H}_{19}\text{NO}_3$ $[\text{M}+\text{H}]^+$ 310.1438, found 310.1437.

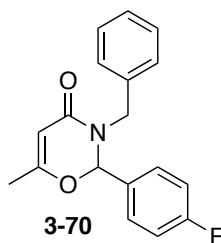


3-Benzyl-2-(4-bromophenyl)-6-methyl-2,3-dihydro-4H-1,3-oxazin-4-one (3-68).

According to general protocol A, 4-bromobenzaldehyde (27.0 mg, 0.146 mmol), benzylamine (15.6 mg, 0.146 mmol), thioester **2-80** (84.4 mg, 0.437 mmol) and silver trifluoroacetate (99.7 mg, 0.451 mmol) in anhydrous CH_2Cl_2 (3.0 mL) stirred for 3 h afforded 26.3 mg (50%) of **3-68** as a white solid after purification by chromatography on SiO_2 (hexanes:EtOAc, 0 to 100%): R_f (hexanes/EtOAc, 50:50) 0.65; ^1H NMR (400 MHz, CDCl_3) δ 7.48 (d, 2 H, $J = 8.5$ Hz), 7.32-7.23 (m, 3 H), 7.18-7.13 (m, 4 H), 6.03 (s, 1 H), 5.34 (d, 1 H, $J = 15.4$ Hz), 5.31 (s, 1 H), 3.81 (d, 1 H, $J = 15.4$ Hz), 1.86 (s, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 164.8, 163.4, 136.7, 134.9, 131.9, 129.2, 128.9, 128.0, 127.8, 124.0, 100.4, 87.2, 46.5, 19.9; IR (neat) 3034, 1670, 1449, 1408, 1354, 1009, 731, 506 cm^{-1} ; ESIMS m/z 358, 360 $[\text{M}+\text{H}]^+$; HRMS m/z calculated for $\text{C}_{18}\text{H}_{16}\text{BrNO}_2$ $[\text{M}+\text{H}]^+$ 358.0437, found 358.0434; mp = 86-89 $^\circ\text{C}$.

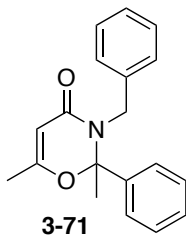


3-Benzyl-6-methyl-2-(4-nitrophenyl)-2,3-dihydro-4H-1,3-oxazin-4-one (3-69). According to general protocol A, 4-nitrobenzaldehyde (15.0 mg, 0.0983 mmol), benzylamine (10.6 mg, 98.3 μ mol), thioester **2-80** (57.3 mg, 0.295 mmol) and silver trifluoroacetate (67.3 mg, 0.305 mmol) in anhydrous CH_2Cl_2 (2.00 mL) stirred for 3 h afforded 15.9 mg (50%) of **3-69** as a yellow oil after purification by chromatography on SiO_2 (hexanes:EtOAc, 0 to 100%): R_f (hexanes/EtOAc, 50:50) 0.39; ^1H NMR (300 MHz, CDCl_3) δ 8.19 (d, 2 H, $J = 8.8$ Hz), 7.47 (d, 2 H, $J = 8.8$ Hz), 7.30-7.26 (m, 3 H), 7.18-7.15 (m, 2 H), 6.16 (s, 1 H), 5.34 (s, 1 H), 5.35 (d, 1 H, $J = 15.3$ Hz), 3.94 (d, 1 H, $J = 15.4$ Hz), 1.87 (d, 3 H, $J = 0.69$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 164.4, 163.0, 148.9, 143.0, 136.3, 129.0, 128.4, 128.1, 124.0, 100.7, 86.6, 47.1, 19.9; IR (neat) 2920, 1761, 1658, 1529, 1195, 1009, 696 cm^{-1} ; ESIMS m/z 366 $[\text{M}+\text{MeCN}]^+$; HRMS m/z calculated for $\text{C}_{18}\text{H}_{16}\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$ 325.1183, found 325.1184.



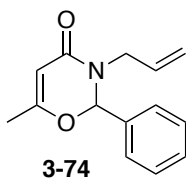
3-Benzyl-2-(4-fluorophenyl)-6-methyl-2,3-dihydro-4H-1,3-oxazin-4-one (3-70). According to general protocol A, 4-fluorobenzaldehyde (25.4 mg, 0.205 mmol), benzylamine (22.0 mg, 0.205 mmol), thioester **2-80** (0.119 g, 0.615 mmol) and silver trifluoroacetate

(0.140 g, 0.635 mmol) in anhydrous CH₂Cl₂ (5.00 mL) stirred for 2 h afforded 98.0 mg (81%) of **3-70** as a yellow oil after purification by chromatography on SiO₂ (hexanes:EtOAc, 0 to 100%): R_f (hexanes/EtOAc, 50:50) 0.55; ¹H NMR (400 MHz, CDCl₃) δ 7.28-7.26 (m, 5 H), 7.11 (d, 2 H, *J* = 6.8 Hz), 7.03 (t, 2 H, *J* = 8.5 Hz), 6.06 (s, 1 H), 5.35 (s, 1 H), 5.30 (d, 1 H, *J* = 15.4 Hz), 3.82 (d, 1 H, *J* = 15.4 Hz), 1.87 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 165.2, 164.8, 163.8, 136.8, 131.5, 129.6, 129.5, 128.8, 128.0, 127.7, 115.9, 115.7, 100.2, 87.4, 46.5, 19.9; IR (neat) 2365, 1674, 1510, 1408, 1232, 815 cm⁻¹; ESIMS *m/z* 339 [M+MeCN]⁺; HRMS *m/z* calculated for C₁₈H₁₆FNO₂ [M+H]⁺ 298.1238, found 298.1242.



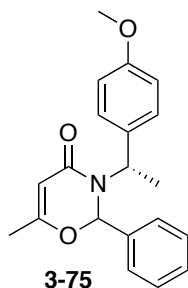
3-Benzyl-2,6-dimethyl-2-phenyl-2,3-dihydro-4H-1,3-oxazin-4-one (3-71).²⁸ A mixture of acetophenone (20.0 mg, 0.163 mmol), benzylamine (17.7 mg, 0.163 mmol) and *p*-TsOH (3.20 mg, 0.0163 mmol) in toluene (1.60 mL) was stirred at reflux under nitrogen for 3 h. The mixture was concentrated under reduced pressure to afford the crude imine. The imine was transferred to an oven-dried round bottom flask in anhydrous CH₂Cl₂ (13.0 mL) under an argon atmosphere. The flask was then charged with thioester **2-80** (0.103 g, 0.489 mmol) and silver trifluoroacetate (0.112 g, 0.506 mmol) at rt. The mixture was stirred for 3 h, diluted with CH₂Cl₂, filtered through a pad of Celite[®] and concentrated under reduced pressure to afford the crude as a yellow oil. The residue was purified using flash column chromatography on SiO₂ (hexanes:EtOAc, 0 to 100%) to provide 11.1 mg (23%) of **3-71** as a

white solid: R_f (hexanes/EtOAc, 50:50) 0.65; ^1H NMR (400 MHz, CDCl_3) δ 7.37-7.29 (m, 10 H), 5.59 (d, 1 H, $J = 16.1$ Hz), 5.25 (s, 3 H), 4.08 (d, 1 H, $J = 16.1$ Hz), 1.87 (s, 3 H), 1.79 (s, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 164.7, 164.0, 142.1, 139.2, 129.0, 128.7, 128.6, 127.1, 127.0, 125.8, 100.7, 94.7, 46.1, 27.6, 20.1; IR (neat) 2925, 1658, 1631, 1404, 1358, 974, 696 cm^{-1} ; ESIMS m/z 317 $[\text{M}+\text{Na}]^+$; HRMS m/z calculated for $\text{C}_{19}\text{H}_{20}\text{NO}_2$ $[\text{M}+\text{H}]^+$ 294.1489, found 294.1488; mp = 144-146 $^\circ\text{C}$.



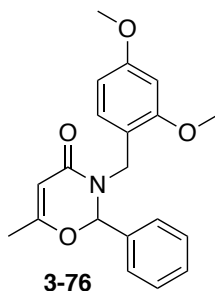
3-Allyl-6-methyl-2-phenyl-2,3-dihydro-4H-1,3-oxazin-4-one (3-74).²⁹ A mixture of benzaldehyde (30.9 mg, 0.291 mmol), allylamine (17.4 mg, 0.306 mmol), and MgSO_4 in anhydrous CH_2Cl_2 (0.5 mL) was refluxed for 1 h. The mixture was cooled to rt, filtered, and concentrated under reduced pressure to afford the crude imine as a yellow oil. The imine was transferred to an oven-dried round bottom flask in dichloromethane (6.0 mL) under an argon atmosphere. The flask was then charged with thioester **2-80** (0.103 g, 0.872 mmol) and silver trifluoroacetate (0.199 g, 0.901 mmol) at rt. The mixture was stirred for 2 h, diluted with CH_2Cl_2 , filtered through a pad of Celite[®] and concentrated under reduced pressure to afford crude as a yellow oil. The residue was purified using flash column chromatography on SiO_2 (hexanes:EtOAc, 0 to 100%) to provide 42.0 mg (63%) of **3-74** as a light yellow oil: R_f (hexanes/EtOAc, 50:50) 0.52; ^1H NMR (400 MHz, CDCl_3) δ 7.40 (m, 5 H), 6.15 (s, 1 H), 5.79-5.66 (m, 1 H), 5.27 (s, 1 H), 5.16-5.07 (m, 2 H), 4.61 (1H, ddt, $J = 15.9, 3.9, 1.6$ Hz), 3.33 (dd, 1 H, $J = 15.9, 6.7$ Hz), 1.87 (s, 3 H); ^{13}C NMR (100 MHz,

CDCl₃) δ 165.1, 163.6, 135.8, 132.7, 129.9, 128.8, 127.5, 117.4, 100.3, 87.9, 45.6, 19.8; IR (neat) 2962, 1670, 1426, 1202, 799, 723 cm⁻¹; ESIMS *m/z* 270 [M+MeCN]⁺; HRMS *m/z* calculated for C₁₄H₁₅NO₂ [M+H]⁺ 230.1176, found 230.1174.



3-((S)-1-(4-Methoxyphenyl)ethyl)-6-methyl-2-phenyl-2,3-dihydro-4H-1,3-oxazin-4-one (3-75). According to general protocol A, benzaldehyde (18.0 mg, 0.170 mmol), (S)-(-)-1-(4-methoxyphenyl)ethylamine (25.7 mg, 0.170 mmol), thioester **2-80** (65.9 mg, 0.510 mmol) and silver trifluoroacetate (0.116 g, 0.527 mmol) in anhydrous CH₂Cl₂ (5.00 mL) stirred for 2 h afforded 35.6 mg (65%) of **3-75** (1:1.3 mixture of diastereomers) as a off-white oil after purification by chromatography on SiO₂ (hexanes:EtOAc, 0 to 100%): R_f (hexanes/EtOAc, 50:50) 0.48; ¹H NMR (400 MHz, CDCl₃) δ 7.39-7.34 (m, 5.05 H, major and minor), 7.28-7.22 (m, 2.24 H, major and minor), 7.18-7.06 (m, 5.09 H, major and minor), 6.90 (d, 2 H, *J* = 8.8 Hz, major), 6.67 (d, 1.54 H, *J* = 8.8 Hz, minor), 6.22 (s, 0.77 H, minor), 6.03-5.96 (m, 2 H, major and minor), 5.82 (q, 1 H, *J* = 7.35 Hz, major), 5.26 (d, 1 H, *J* = 0.89 Hz), 5.26 (d, 0.77 H, *J* = 0.91 Hz, minor), 3.82 (s, 3 H, major), 3.69 (s, 2.31 H, minor), 1.76 (d, 2.31 H, *J* = 0.88 Hz, minor), 1.71 (d, 3 H, *J* = 0.88 Hz, major), 1.66 (d, 2.31 H, *J* = 7.11 Hz, minor), 1.36 (d, 3 H, *J* = 7.2 Hz, major); ¹³C NMR (100 MHz, CDCl₃) δ 163.5, 163.7 (minor), 163.1 (minor), 163.0, 159.2, 159.1 (minor), 138.2, 137.3 (minor), 133.0, 131.4 (minor), 129.5,

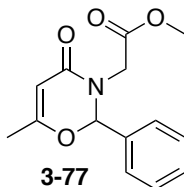
129.3 (minor), 128.7, 128.5 (minor), 128.5, 127.9 (minor), 127.5, 127.2, 114.2 (minor), 113.8, 101.9 (minor), 101.1, 84.7 (minor), 84.3, 55.5 (minor), 55.4, 51.2, 49.8 (minor), 20.0 (minor), 18.2, 17.9 (minor); IR (neat) 2921, 1654, 1609, 1453, 1248, 1035, 692 cm^{-1} ; ESIMS m/z 365 $[\text{M}+\text{H}]^+$; HRMS m/z calculated for $\text{C}_{20}\text{H}_{21}\text{NO}_3$ $[\text{M}+\text{H}]^+$ 324.1594, found 324.1592.



3-(2,4-Dimethoxybenzyl)-6-methyl-2-phenyl-2,3-dihydro-4H-1,3-oxazin-4-one (3-76).

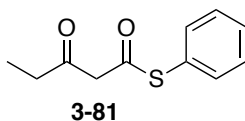
According to general protocol B, to a solution of benzaldehyde (24.1 mg, 0.227 mmol) in anhydrous EtOH (4.50 mL) was added 2,4-dimethoxybenzylamine (38.0 mg, 0.227 mmol) and anhydrous MgSO_4 under a nitrogen atmosphere. The mixture was refluxed for 24 h, and then concentrated under reduced pressure to afford the crude imine as a yellow oil. The imine was transferred to an oven-dried round bottom flask in CH_2Cl_2 (4.40 mL) under an argon atmosphere. The flask was then charged with thioester **2-80** (0.103 g, 0.682 mmol) and silver trifluoroacetate (0.156 g, 0.704 mmol) at rt. The mixture was stirred for 3 h, diluted with CH_2Cl_2 , filtered through a pad of Celite[®] and concentrated under reduced pressure to afford the crude as a yellow oil. The residue was purified using flash column chromatography on SiO_2 (hexanes:EtOAc, 0 to 100%) to provide 42.0 mg (54%) of **3-76** as a colorless oil: R_f (hexanes/EtOAc, 50:50) 0.42; ^1H NMR (400 MHz, CDCl_3) δ 7.33-7.26 (m, 5 H), 6.45 (d, 1 H, $J = 10.1$ Hz), 6.34 (s, 1 H), 6.25 (s, 1 H), 5.24 (s, 1 H), 5.05 (d, 1 H, $J =$

15.1 Hz), 4.09 (d, 1 H, $J = 15.1$ Hz), 3.78 (s, 3 H), 3.60 (s, 3 H), 1.80 (s, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 164.2, 163.5, 160.6, 158.6, 136.9, 131.0, 129.3, 128.5, 127.1, 117.6, 104.4, 100.5, 98.5, 87.9, 55.6, 55.3, 42.0, 19.9; IR (neat) 2921, 1674, 1602, 1442, 1407, 1179, 1354, 697 cm^{-1} ; ESIMS m/z 340 $[\text{M}+\text{H}]^+$; HRMS m/z calculated for $\text{C}_{20}\text{H}_{21}\text{NO}_4$ $[\text{M}+\text{H}]^+$ 340.1543, found 340.1537.



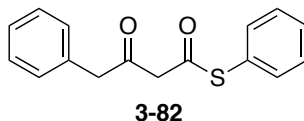
Methyl-2-(6-methyl-4-oxo-2-phenyl-2H-1,3-oxazin-3(4H)-yl)acetate (3-77).³⁰ To a suspension of glycine methyl ester hydrochloride (28.8 mg, 0.225 mmol) and MgSO_4 (36.2 mg, 0.300 mmol) in CH_2Cl_2 (2.00 mL) was added Et_3N (26.6 mg, 0.263 mmol). The mixture was stirred at rt for 1 h, followed by the addition of benzaldehyde (20.0 mg, 0.188 mmol). The mixture was stirred at rt overnight, and then the resulting precipitate was removed by filtration. The filtrate was washed once with water, the aqueous phase was extracted with CH_2Cl_2 (x1) and the combined organic layers were washed with brine (x3), dried over (MgSO_4) and concentrated under reduced pressure to provide 26.8 mg (81%) the imine as a yellow oil. The imine (26.8 mg, 0.151 mmol) was transferred to an oven-dried round bottom flask in anhydrous CH_2Cl_2 (4.40 mL) under an argon atmosphere. The flask was then charged with thioester **2-80** (0.104 g, 0.537 mmol) and silver trifluoroacetate (0.128 g, 0.581 mmol) at rt. The mixture was stirred for 3 h, diluted with CH_2Cl_2 , filtered through a pad of Celite[®] and concentrated under reduced pressure to afford the crude as a yellow oil. The residue was purified using flash column chromatography on SiO_2 (hexanes:EtOAc, 0 to

100%) to provide 14.0 mg (35%) of **3-77** as a colorless oil: R_f (hexanes/EtOAc, 50:50) 0.35; ^1H NMR (400 MHz, CDCl_3) δ 7.50-7.42 (m, 5 H), 6.33 (s, 1 H), 5.40 (s, 1 H), 4.49 (d, 1 H, $J = 17.7$ Hz), 3.64 (s, 3 H), 3.23 (d, 1 H, $J = 17.8$ Hz), 2.00 (s, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 170.1, 167.8, 165.0, 134.2, 130.7, 129.2, 128.2, 99.8, 90.2, 52.4, 44.1, 19.7; IR (neat) 2954, 1749, 1662, 1418, 1206, 1005, 696 cm^{-1} ; ESIMS m/z 303 $[\text{M}+\text{MeCN}]^+$; HRMS m/z calculated for $\text{C}_{14}\text{H}_{15}\text{NO}_4$ $[\text{M}+\text{H}]^+$ 262.1074, found 262.1076.



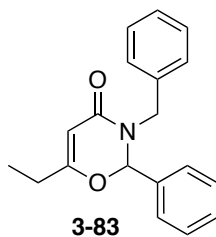
S-Phenyl 3-oxopentanethioate (3-81). To a solution of Meldrum's acid (0.500 g, 3.47 mmol) in anhydrous CH_2Cl_2 (4.30 mL) at 0 °C under an argon atmosphere was added pyridine drop-wise. The mixture was stirred for 5 min and propionyl chloride (0.353 g, 3.82 mmol) was added neat. The bright orange mixture was stirred at 0 °C for 1 h, and then for an additional 1 h at rt. The mixture was then poured into a 2 M hydrochloric acid solution (4.00 mL). The layers were separated and the organic layer was washed with 2 M hydrochloric acid (6.00 ml), washed with water, dried (MgSO_4) and concentrated under reduced pressure to afford the crude acyl Meldrum's acid as a brown oil.³¹ A solution of the crude acyl Meldrum's acid and thiophenol (0.306 g, 2.78 mmol) in toluene (25.0 mL) was heated to reflux for 4 h in an Erlenmeyer flask. The mixture was then cooled to rt, and concentrated under reduced pressure to afford a dark brown residue. The residue was purified using flash column chromatography on SiO_2 (hexanes:EtOAc, 0 to 50%) to provide 0.48 g (74%) of **3-81** as a colorless oil: R_f (hexanes/EtOAc, 80:20) 0.59; ^1H NMR (400 MHz, CDCl_3) δ 7.45 (m, 1.02 H, enol), 7.44 (s, 5 H), 5.50 (s, 0.46 H, enol), 3.75 (s, 2 H), 2.60 (q, 2 H, $J = 7.2$ Hz),

2.24 (q, 1 H, $J = 7.6$ Hz, enol), 1.14 (t, 1.3 H, $J = 7.6$ Hz, enol), 1.08 (t, 3 H, $J = 7.2$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 202.5, 190.9, 179.4 (enol), 135.2 (enol), 134.6, 130.1 (enol), 129.8 (enol), 129.6, 129.4 (enol), 127.2, 97.5 (enol), 56.9, 36.8, 28.4 (enol), 10.6 (enol), 7.7; IR (neat) 2974, 1723, 1688, 1663, 1099, 978, 750 cm^{-1} ; HRMS m/z calculated for $\text{C}_{11}\text{H}_{12}\text{SO}_2$ $[\text{M}+\text{H}]^+$ 209.0631, found 209.0629.

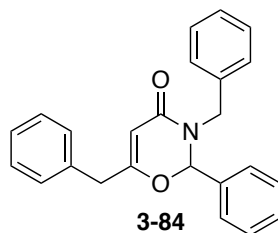


S-Phenyl-3-oxo-4-phenylbutanethioate (3-82). To a solution of Meldrum's acid (0.500 g, 3.47 mmol) and pyridine (0.549 g, 6.94 mmol) in anhydrous CH_2Cl_2 (7.00 mL) at 0 °C was added phenylacetyl chloride (0.536 g, 3.47 mmol) drop-wise. The solution was stirred for 30 min at 0 °C, and warmed to rt gradually, and stirred overnight. The reaction mixture was washed with 10% aqueous HCl (x2), washed with water, dried (MgSO_4), and concentrated under reduced pressure to afford the crude acyl Meldrum's acid as a brown oil.³² A solution of the crude acyl Meldrum's acid and thiophenol (0.382 g, 3.47 mmol) in toluene (3.00 mL) was heated to reflux for 4 h. The mixture was then cooled to rt, and concentrated under reduced pressure to afford a dark brown residue. The residue was purified using flash column chromatography on SiO_2 (hexanes:EtOAc, 0 to 100%) to provide 0.514 g (74%) of **3-82** as a yellow oil: R_f (hexanes/EtOAc, 80:20) 0.59 R_f (hexanes/EtOAc, 80:20) 0.50; ^1H NMR (300 MHz, CDCl_3) δ 7.45 (m, 5 H), 7.36-7.32 (m, 2 H), 7.23-7.20 (m, 2 H), 5.41 (s, 0.85 H, enol), 3.85 (s, 2 H), 3.76 (s, 2 H), 3.51 (s, 1.57 H, enol); ^{13}C NMR (100 MHz, CDCl_3) δ 199.7, 193.7, 190.8, 176.2, 135.2, 134.7, 133.2, 130.1, 129.9, 129.6, 129.5, 129.4, 128.9, 127.7, 99.2

(enol), 56.0, 50.4, 41.6 (enol); IR (neat) 3027, 1727, 1695, 1616, 1580, 1071, 845, 745, 702 cm^{-1} ; HRMS m/z calculated for $\text{C}_{16}\text{H}_{14}\text{SO}_2$ $[\text{M}+\text{H}]^+$ 271.0784, found 271.0783.



3-Benzyl-6-ethyl-2-phenyl-2,3-dihydro-4H-1,3-oxazin-4-one (3-83). According to general protocol A, benzaldehyde (27.0 mg, 0.146 mmol), benzylamine (15.6 mg, 0.146 mmol), thioester **3-81** (84.4 mg, 0.437 mmol) and silver trifluoroacetate (99.7 mg, 0.451 mmol) in anhydrous CH_2Cl_2 (3.00 mL) stirred for 3 h afforded 32.5 mg (76%) of **3-83** as a colorless oil after purification by chromatography on SiO_2 (hexanes:EtOAc, 0 to 100%): R_f (hexanes/EtOAc, 50:50) 0.53; ^1H NMR (400 MHz, CDCl_3) δ 7.41-7.34 (m, 2 H), 7.31-7.25 (m, 6 H), 7.17-7.12 (m, 2 H), 6.10 (s, 1 H), 5.39 (d, 1 H, $J = 15.4$ Hz), 5.38 (s, 1 H), 3.80 (d, 1 H, $J = 15.5$ Hz), 2.13 (qd, 2 H, $J = 7.6, 3.3$ Hz), 0.99 (s, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 169.9, 164.4, 136.8, 135.4, 129.9, 128.8, 128.7, 128.0, 127.7, 127.6, 98.2, 87.7, 46.6, 26.7, 10.3; IR (neat) 2985, 2938, 1671, 1454, 1024, 1177, 700 cm^{-1} ; ESIMS m/z 335 $[\text{M}+\text{MeCN}]^+$; HRMS m/z calculated for $\text{C}_{19}\text{H}_{19}\text{NO}_2$ $[\text{M}+\text{H}]^+$ 294.1489, found 294.1484.



3,6-Dienzyl-2-phenyl-2,3-dihydro-4H-1,3-oxazin-4-one (3-84). According to general protocol A, benzaldehyde (15.0 mg, 0.141 mmol), benzylamine (15.2 mg, 0.141 mmol), thioester **3-82** (0.114 mg, 0.422 mmol) and silver trifluoroacetate (96.3 mg, 0.436 mmol) in anhydrous CH₂Cl₂ (2.30 mL) stirred for 2.5 h afforded 35.1 mg (70%) of **3-84** as a yellow oil after purification by chromatography on SiO₂ (hexanes:EtOAc, 0 to 100%): R_f (hexanes/ethyl acetate, 50:50) 0.64; ¹H NMR (300 MHz, CDCl₃) δ 7.38-7.13 (m, 13 H), 7.02-6.99 (m, 2 H), 6.09 (s, 1 H), 5.40 (d, 1 H, *J* = 15.1 Hz), 5.38 (s, 1 H), 3.80 (d, 1 H, *J* = 15.4 Hz), 3.42 (d, 2 H, *J* = 1.8 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 167.1, 164.4, 136.4, 134.7, 134.7, 129.8, 129.2, 128.9, 128.7, 128.6, 128.0, 127.9, 127.4, 127.2, 100.0, 87.7, 46.8, 40.0; IR (neat) 3031, 1658, 1426, 1385, 731, 700 cm⁻¹; ESIMS *m/z* 397 [M+MeCN]⁺; HRMS *m/z* calculated for C₂₄H₂₁NO₂ [M+H]⁺ 356.1645, found 356.1643.

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CHAPTER 4

Synthesis and Biological Evaluation of a Series of Fatty Acid Amides From *Echinacea*

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4.1 Abstract

Alkylamides are lipophilic constituents of *Echinacea* and possess numerous biological activities. Although significant effort has been focused on the study of crude *Echinacea* extracts, very little is known regarding the activities of the individual constituents that make up these herbal treatments. Herein the structure activity relationship (SAR) of simple alkylamides found in *Echinacea* extracts with respect to their ability to decrease the production of the pro-inflammatory mediator TNF- α were explored. Our results have revealed the key structural requirements for activity and provided lead compounds for further investigation of these poorly understood molecules

4.2 Introduction and Background

4.2.1 Isolation and Pharmacological Potential of Dialkylamides

Echinacea, also known as the purple cone flower, is a perennial flowering plant indigenous to the central United States.¹ The uses of *Echinacea* as a medicinal agent dates back to over 400 years ago by the Native Americans. Historically, *Echinacea* roots and aerial parts were utilized in the treatment of various ailments ranging from sore throat, headache, toothache, swelling, and as antidotes for poisons.² Today, *Echinacea* continues to be utilized for its medicinal properties and is amongst the top selling dietary supplements in United States, with gross annual sales of 120 million dollars.³ The commercial cultivation and popularity of this natural extract as a herbal remedy has been extended to outside of

United States. *Echinacea* is one of the most frequently prescribed drugs in Germany, with annual prescription counts of over 3 million.⁴

Echinacea extracts are widely used for the treatment of cold-like symptoms due to their proposed immunomodulating capabilities.⁵ In addition to the immunological effects, they have also been associated with antiviral, antibacterial, antifungal, insecticidal, as well as anti-inflammatory properties.⁶ Despite the many biological activities, the use of *Echinacea* as a medicinal agent has been hindered, mainly due to complications in analysis of the various components' biological roles.⁷ This uncertainty in part arises from the varying constituent profile in the most commonly utilized forms of *Echinacea*, the crude extracts and the pills prepared from the powdered plant material.⁸ Most *Echinacea* preparations are manufactured from *E. purpurea*, *E. angustifolia*, and *E. pallida* species, with *E. purpurea* as the major component.⁹ However, it is very well known that the constituent make up of such products varies greatly depending on the geographical location, harvested plant material, and the method of preparation.⁷

E. purpurea is primarily composed of caffeic acid derivatives, polysaccharides, glycoproteins, alkylamides, and more recently identified, ketones. These classes of compounds possessing a wide range of polarities are presumed to be responsible for much of the biological activities exhibited by *Echinacea*.¹⁰ Of the aforementioned constituents, alkylamides or fatty acid amides are the most prevalent lipophilic compounds found in *Echinacea* extracts and the only components known to cross the intestinal barrier.¹¹ To date, about 20 alkylamides have been characterized and a selected few examples of them are depicted in **Figure 4.1**.¹²

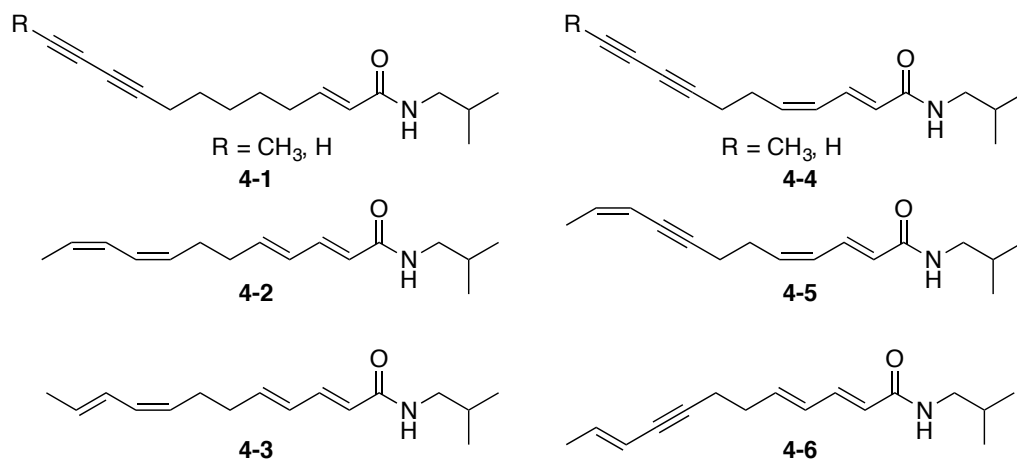


Figure 4.1: Selected examples of alkylamides isolated from *Echinacea* extracts.

Although structurally simple, fatty acid amides have diverse biological activities and are involved in signaling pathways relevant to cancer, cardiovascular disease, pain, inflammation, drug addiction, eating disorders, sleep deprivation, anxiety and depression.¹³ Additionally, significant efforts have been focused on the development of fatty acid amides as analgesic agents due to their inhibition of fatty acid amide hydrolase (FAAH), cannabinoid receptors-1 (CB₁) and vanilloid receptor-1 (VR-1).¹⁴

The diverse biological activities exhibited by alkylamides likely arises from their interaction with a number of different receptor systems including, but not limited to, the cannabinoid (CB) receptors.¹⁵ Cannabinoid receptors CB₁ and CB₂ are G protein-coupled receptors that are involved in the regulation of central nervous system and inflammatory responses, respectively. The more prevalent CB₁ receptors are expressed in high concentrations in neurons of the central and peripheral nervous system and are responsible for the psychoactive effects of cannabinoids. On the other hand, the less abundant CB₂

receptors are found in a few neurons and in a number of immune cells, particularly the inflammatory and immune-competent cells such as macrophages.¹⁶

It has previously been demonstrated that alkylamides from *Echinacea* can regulate the expression of the pro-inflammatory cytokines (cell signaling proteins) known as tumor necrosis factor- α (TNF- α), in human monocytes and macrophages *in vitro*. This regulatory activity has been further linked to interaction with the CB₂ receptors.¹⁷ Indeed, studies aimed at uncovering the mechanism of action of alkylamides regarding their immunomodulatory activities have revealed such compounds as cannabinomimetics.¹⁸ Of particular interest is the higher affinity of alkylamides for the CB receptor sites than the endogenous fatty acid ligands such as anandamide (AEA) **4-7** and 2-arachidonoylglycerol (2-AG) **4-8** (Figure 4.2). This high degree of affinity is presumably due to the structural similarities that alkylamides share with these natural fatty acid ligands.¹⁹

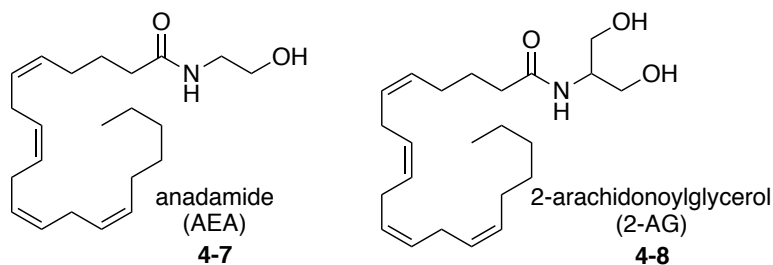


Figure 4.2: Examples of endogenous ligands of CB receptors.

Surprisingly, relatively little effort has focused on the systematic study of simple alkylamides on pro-inflammatory mediators (such as TNF- α), even though many members of this family have demonstrated anti-inflammatory effects.²⁰ This area has been further complicated by the fact that alkylamides have poorly understood and/or multiple mechanisms

of action and are known to elicit their anti-inflammatory effects via both CB-dependent and CB-independent mechanisms.¹⁸

4.2.2 Overview of Alkylamide 4-9

As mentioned earlier, recently alkylamides have generated a great deal of interest due to their ability to inhibit the production of inflammatory proteins and lipids from a variety of immune cell types such as mast cells^{21,22}, macrophages^{23,24}, T cells^{25,26}, and dendritic cells^{27,28}.

Through collaborative efforts with the department of biological sciences, we have demonstrated that the diene-containing alkylamide **4-9** (Figure 4.3), a constituent of *Echinacea purpurea*, decreases the production of pro-inflammatory mediators such as TNF- α from RAW 264.7 macrophage-like cells in a dose-dependent manner.²⁹ RAW 264.7 macrophage-like cell line is a well-established model system for such studies and was chosen due to its ability to mimic the LPS-induced inflammatory responses.

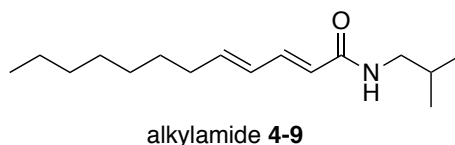
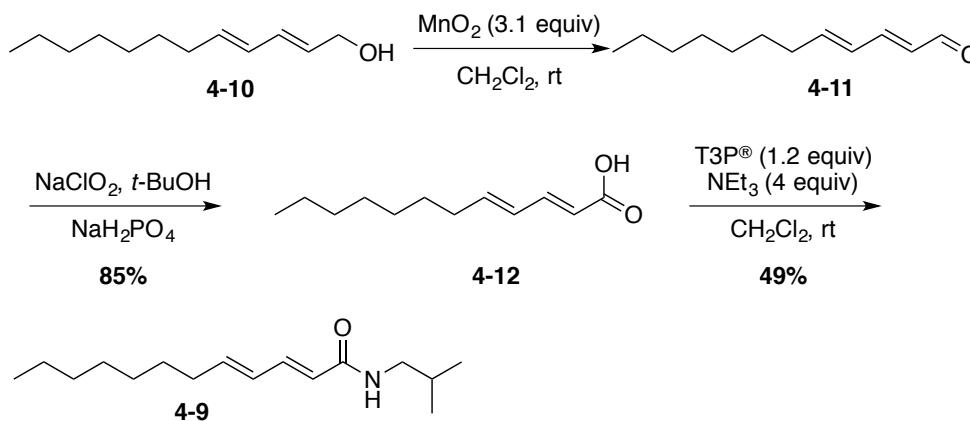


Figure 4.3: Structure of alkylamide **4-9** isolated from *Echinacea*.

As part of our investigation into the potential of dialkylamides as anti-inflammatory compounds and their underlying mechanism of action, we sought to pursue the chemical synthesis of **4-9** and a series of its analogs using a short and efficient synthetic route. To this

end, our efforts began by preparing the lead natural product as this compound was obtained in extremely small quantities from the natural sources utilized in the previously reported biological studies. It should also be noted that alkylamide **4-9** has been synthesized previously using similar synthetic routes; however, these approaches required longer reaction sequences and/or more elaborate substrates and reagents.^{11,12}

The synthesis of alkylamide **4-9** commenced by the oxidation of the commercially available alcohol **4-10** to the corresponding aldehyde **4-11** using activated MnO₂. Aldehyde **4-11** was then further oxidized to carboxylic acid **4-12** using a standard Pinnick oxidation protocol. Carboxylic acid **4-12** was then coupled to isobutyl amine using propylphosphonic anhydride (T3P[®]) as the coupling reagent to provide the target natural product in good yield (Scheme 4.1). Alkylamide **4-9** proved identical to the natural product by ¹H and ¹³C NMR analysis.



Scheme 4.1: Chemical synthesis of dialkylamide **4-9**.

4.3 Results and Discussions

4.3.1 Series I Analog Preparation and Biological Evaluation

Although alkylamide **4-9** possess intriguing biological activities, compounds with improved activity, stability, and selectivity are highly desirable. One of the main aims of this study was to design and synthesize a series of targeted derivatives that would allow for the determination of the important structural features responsible for the observed anti-inflammatory activities. The key to this approach is the ability to install pinpoint modifications along the alkylamide structure, a task that is only achievable through chemical synthesis. To this end, following the synthesis of natural product **4-9**, we sought to prepare a series of analogs to explore the role of the double bonds, alkyl chain length in the fatty acid unit and the structure of the amide head group in the observed biological activity.

The initial set of prepared analogs targeted alterations to the chain length and the the degree of unsaturation. Compound **4-13** which only bears one unsaturation, the fully saturated compound **4-14**, and a series of chain-shortened derivatives (**4-15** – **4-18**) were prepared (Figure 4.4). In all cases, the analogs were synthesized in one step from the coupling of the corresponding carboxylic acid with isobutyl amine using T3P[®] as the coupling reagent.

To evaluate the impact of these structural changes on the biological activity of **4-9**, each analog's ability to inhibit TNF- α production by LPS-stimulated RAW 264.7 cells were compared. Each compound was tested at 100 μ M in combination with 10 ng/mL of LPS and incubated for 18 hours. TNF- α concentrations in the culture supernatants were measured after the incubation period using a commercially available ELISA (eBioscience). The effects

of each compound on TNF- α production were compared to cells stimulated only with LPS. Additionally, RAW 264.7 cells were treated with each compound in the absence of LPS to determine if these molecules themselves could induce the production of TNF- α .

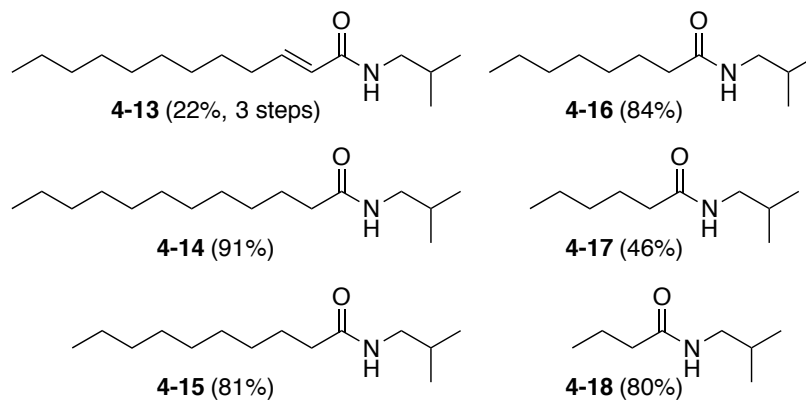


Figure 4.4: Analogs prepared to explore the impact of unsaturation and alkyl chain length. Isolated yields are shown in parenthesis.

None of the compounds were found to induce TNF- α significantly above levels from unstimulated cells (data not shown). As shown in **Figure 4.5**, compounds **4-13** and **4-14**, both significantly inhibited the production of TNF- α , with levels of suppression similar to that of **4-9**, indicating that the double bonds along the alkyl chain are not critical for inhibition of TNF- α production. Significant suppressive effects were lost with the chain shortened analogs **4-15** to **4-18** (Figure 4.5), indicating that the length of the alkyl chain is critical for determining inhibitory activity. An alkyl chain with at least 11 carbons seems to be required for this activity, in the absence of any unsaturation.

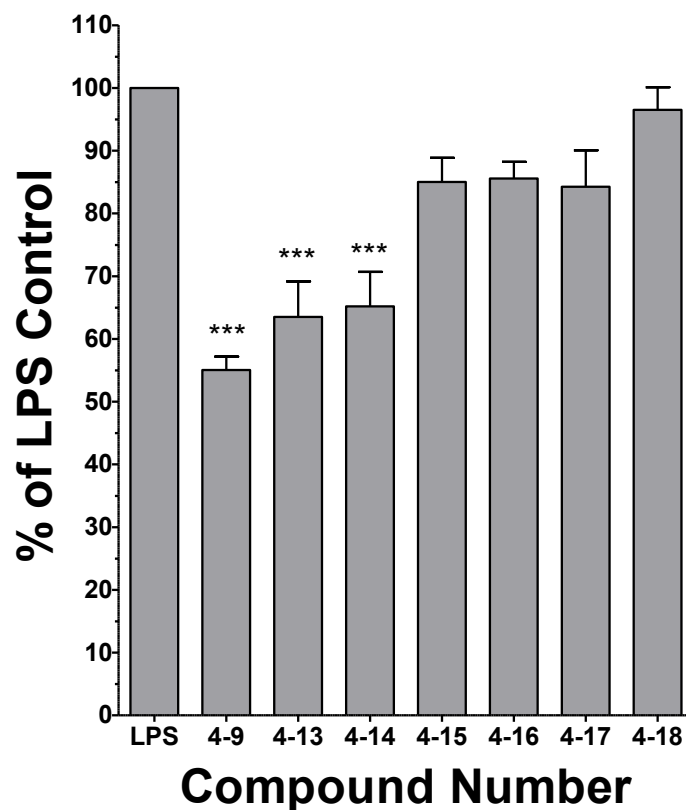


Figure 4.5: TNF- α production in the presence of alkyl chain length analogs.

4.3.2 Series II Analog Preparation and Biological Evaluation

The observations from the studies on series I analogs led to the design and synthesis of the second series of analogs (Figure 4.6). In this series, we sought to explore the impact of the amide head group on the observed activity of **4-9** and simplified derivatives. Accordingly, compounds **4-19** to **4-26** were prepared to evaluate the impact of replacing the isobutyl amide with 2-methylbutyl amide (**4-19** and **4-20**), benzyl amide (**4-21** and **4-22**), hexyl amide (**4-23**), thiazole amide isosteres (**4-24** and **4-25**) and isobutyl amine derivatives (**4-26**). This series of alkyl amide derivatives served to not only evaluate the steric

requirements of the amide head group, but also to answer key questions regarding the amide functionality itself.

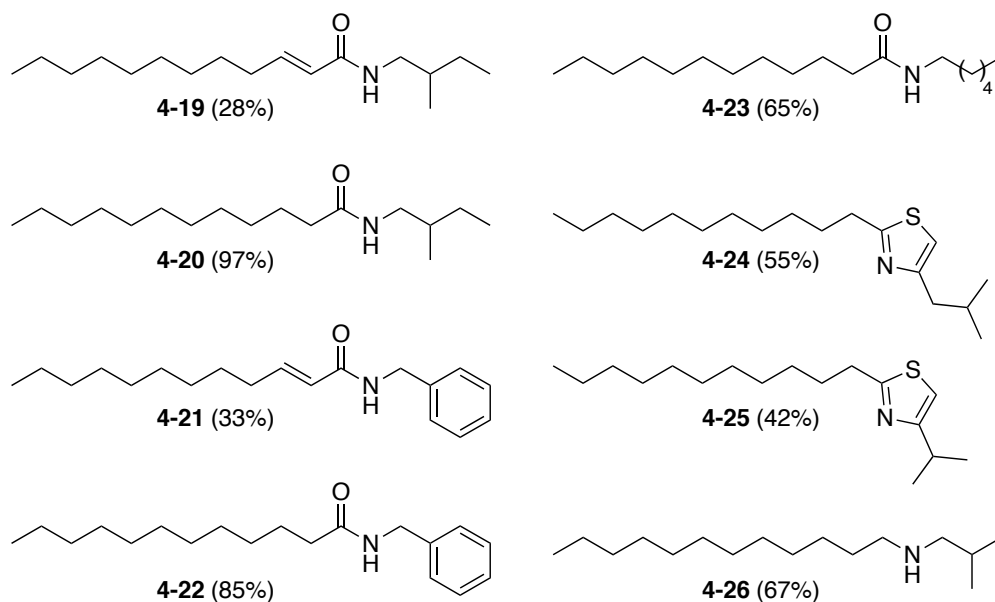


Figure 4.6: Analogs prepared to explore the impact of amide head group. Isolated yields are shown in parenthesis.

The addition of a single carbon to the isobutyl amide head group in **4-19** and **4-20** did not impair their ability to significantly inhibit TNF- α production (Figure 4.7). Replacement of the isobutyl amide group with a benzyl ring in **4-21** and **4-22** reduced the overall level of inhibition from $50.2 \pm 5.1\%$ to $29.8 \pm 5.5\%$ and $32.4 \pm 0.8\%$, respectively, although both still produced significant levels of suppression. Similarly, the addition of a 6-carbon alkyl chain on the amide nitrogen in compound **4-23** also produced significant suppression. In contrast, the two thiazole-containing compounds, **4-24** and **4-25**, did not display significant TNF- α suppressive activity, highlighting the importance of the amide functionality for the observed

activity. Interestingly, compound **4-26**, which lacks the carbonyl group present in most of these compounds, completely inhibited the production of TNF- α ($99.8 \pm 0.2\%$); however, this activity likely arises from cell death, since this analog displayed significant cytotoxic activity (see below). Overall, the presence of the isobutyl amide head group seemed to be not necessary for inhibiting TNF- α production, although there do appear to be structural constraints in this region that limit inhibitory activity.

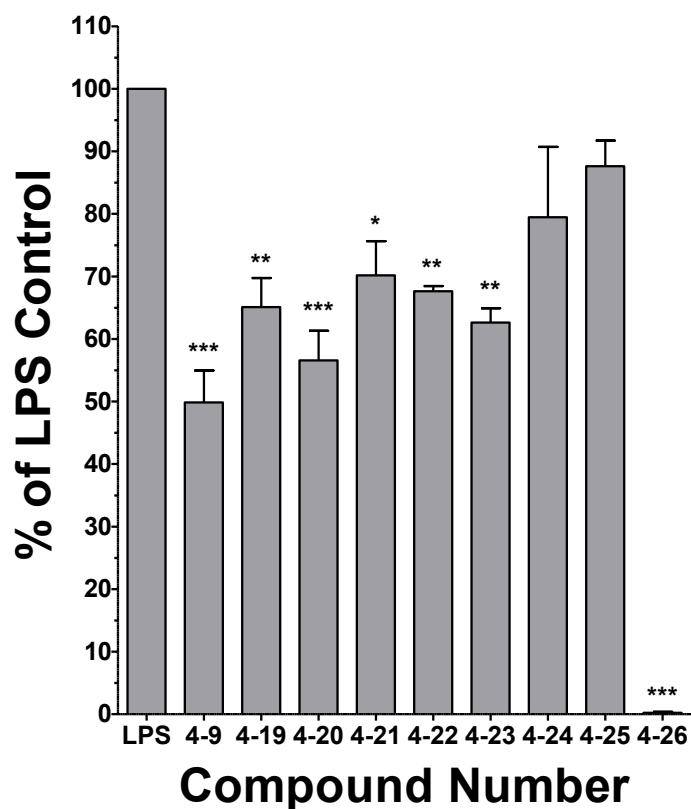


Figure 4.7: TNF- α production in the presence of amide head group analogs.

4.3.3 Cytotoxic Effects of the Prepared Alkylamide and Analogs

In parallel studies, the cytotoxic effects of each compound were tested to ensure that inhibition of TNF- α production was not due to cell death. RAW 264.7 cells were treated with each compound at 100 μ M and incubated for 18 hours before supernatants were collected and analyzed for lactate dehydrogenase (LDH) activity (Pierce LDH Cytotoxicity Assay Kit, Thermo Scientific). LDH is a cytosolic enzyme that is released from dead or dying cells. A lysis buffer was used to determine the maximal LDH release while spontaneous background release was determined by treatment with media and ethanol. While most compounds did not display significant cytotoxic effects, **4-19** and **4-26** did induce statistically significant increases in cytotoxicity (Figure 4.8). As mentioned above for amine **4-26**, the suppression of TNF- α shown in **Figure 4.8** was likely due to its significant cytotoxic effects. Other factors, including cell permeability, could play a role in the varying activities of the studied compounds and further research is warranted in order to uncover the mechanism of action of these compounds.

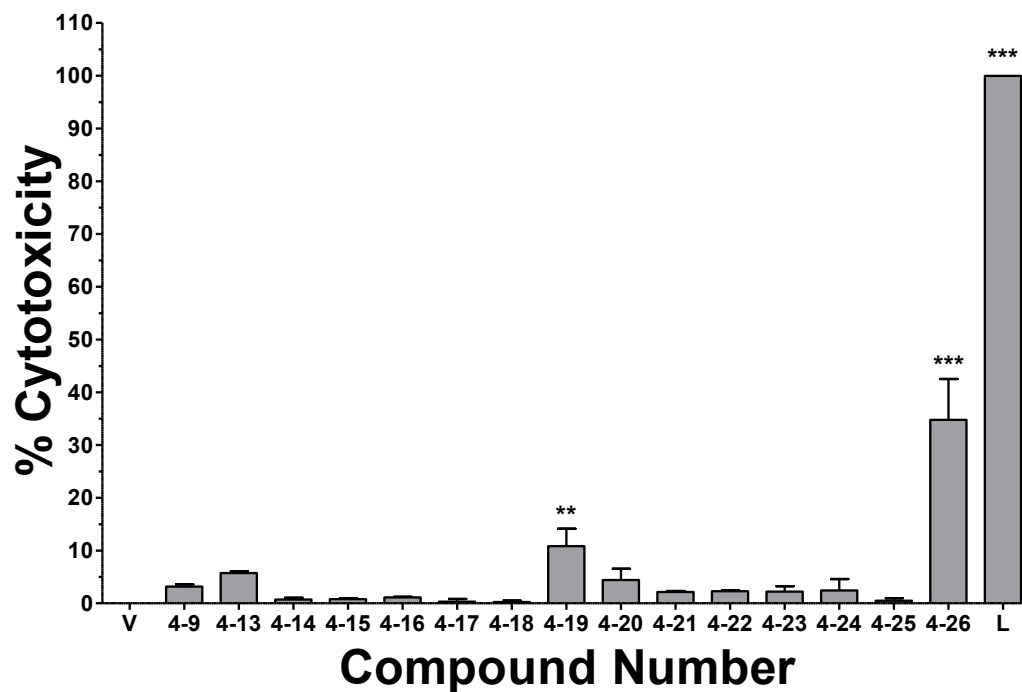


Figure 4.8: Cytotoxicity of the alkylamide structural analogs.

4.4 Conclusions

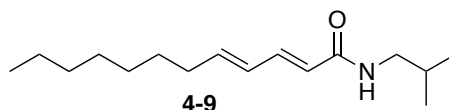
In conclusion, the structural requirements of alkylamides to inhibit production of pro-inflammatory cytokines appear to be dependent on the alkyl chain length, and while there is structural flexibility within the amide head group, certain chemical properties within this region appear to be required to avoid reducing inhibitory effects. The double bonds in the alkyl chain of the natural product do not appear to be critical for suppression of TNF- α . This observation is consistent with previous publications in which additional alkylamides produced by *Echinacea*, as well as other plant genera, which vary in the number and placement of unsaturation along the alkyl chain, are also capable of suppressing cytokine production. It is unlikely that these fatty acid amides are exerting their activity selectively on

LPS or the LPS-signaling machinery since influenza A-induced production of TNF- α is also blocked. Interestingly, removal of the carbonyl group from the fully saturated version of the natural alkylamide produced a molecule with cytotoxic activity. Further experiments to develop an understanding of this effect and to further probe the SAR of this family of alkylamides are underway and will be reported in due course.

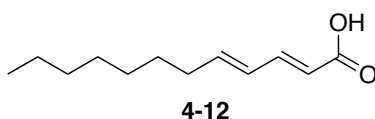
4.5 Experimental Section

General considerations: THF was purified using an alumina filtration system. (2*E*,4*E*)-dodeca-2,4-dien-1-ol, lauric acid, other carboxylic derivatives, and propylphosphonic anhydride solution (T3P[®]) were purchased from Sigma-Aldrich and Fisher Scientific and were used without further purification. Reactions were monitored by TLC analysis (EM Science pre-coated silica gel 60 F₂₅₄ plates, 250 μm layer thickness) and visualization was accomplished with a 254 nm UV light and by staining with a KMnO₄ solution (1.5 g of KMnO₄, 10 g of K₂CO₃, and 1.25 mL of a 10% NaOH solution in 200 mL of water). Reactions were also monitored by LC-MS (Shimadzu LC-MS 2020 with Kinetex 2.6 μm C18 50 x 2.10 mm). Flash chromatography on SiO₂ was used to purify the crude reaction mixtures and performed on a Biotage Isolera utilizing Biotage cartridges and linear gradients. ¹H and ¹³C NMR spectra were obtained on a Varian Mercury-VX 300, a Varian Mercury-VX 400, or a Varian Mercury-Plus 300 instrument in CDCl₃ unless otherwise noted. Chemical shifts were reported in parts per million with the residual solvent peak used as an internal standard (CDCl₃ = 7.26 ppm for ¹H, CDCl₃ = 77.23 ppm for ¹³C). ¹H NMR spectra were run at 300 or 400 MHz and are tabulated as follows: chemical shift, multiplicity (s = singlet, d = doublet, m = multiplet, dd = doublet of doublet, bs = broad singlet), number of protons, and coupling constant(s). ¹³C NMR spectra were run at 100 MHz using a proton-decoupled pulse sequence with a d₁ of 1 second unless otherwise noted, and are tabulated by observed peak. Infrared spectra were determined on a Jasco FT/IR-4100 spectrometer. High-resolution mass spectra were obtained on a Thermo Fisher Scientific, Exactive Plus mass spectrometer using

Heated Electrospray Ionization (HESI). Melting points were determined using a Thomas Hoover Capillary Melting Point Apparatus.

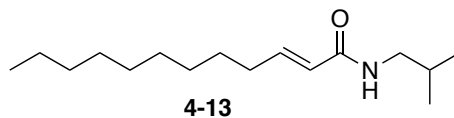


(2E,4E)-N-Isobutyldodeca-2,4-dienamide (4-9). **General protocol A.** To a solution of (2E,4E)-dodeca-2,4-dienoic acid **4-12** (0.160 g, 0.783 mmol) in anhydrous CH₂Cl₂ (8.00 mL) was added Et₃N (0.317 g, 3.13 mmol) and T3P[®] (0.598 g, 0.939 mmol) at rt and the mixture was stirred for 20 min. Isobutylamine (57.8 mg, 78.3 mmol) was added drop-wise and the mixture was stirred overnight. The solvent was removed under reduced pressure and the residue was purified by chromatography on SiO₂ (hexanes:EtOAc, 0 to 100%) to yield 97.0 mg (49%) of **4-9** as a light yellow solid: ¹H NMR (400 MHz, CDCl₃) δ 7.19 (dd, 1 H, *J* = 14.9, 9.9 Hz), 6.16-6.02 (m, 2 H), 5.75 (d, 1 H, *J* = 15.1 Hz), 5.51 (brs, 1 H), 3.16 (t, 2 H, *J* = 6.5 Hz), 2.14 (q, 2 H, *J* = 7.5, 7.1 Hz), 1.83-1.34 (m, 1 H), 1.45-1.34 (m, 2 H), 1.33-1.20 (m, 8 H), 0.92 (6 H, d, *J* = 6.7 Hz), 0.87 (3 H, *J* = 6.8 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 166.6, 143.6, 141.5, 128.3, 121.8, 47.1, 33.2, 32.0, 29.3, 28.8, 22.9, 20.3, 14.3; ESIMS *m/z* 293 [M+MeCN]⁺; HRMS *m/z* calculated for C₁₆H₃₀ON [M+H]⁺ 252.2322, found 252.2318.



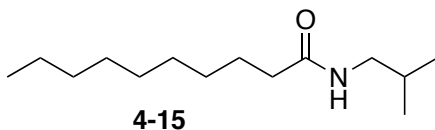
(2E,4E)-Dodeca-2,4-dienoic acid (4-12). To a solution of (2E,4E)-dodeca-2,4-dien-1-ol **4-10** (0.236 g, 1.24 mmol) in CH₂Cl₂ (4.80 mL) was added activated Mn(IV)O₂ (0.336 g, 3.86 mmol). The reaction mixture was stirred for 48 h and filtered through a pad of Celite[®]. The

filtrate was concentrated under reduced pressure to afford the crude aldehyde. To a solution of the crude aldehyde (0.181 g, 0.962 mmol) and 2-methyl-2-butene (0.675 g, 9.62 mmol) in *t*-BuOH (10.0 mL) and water (10.0 mL) at 0 °C was added a solution of sodium chlorite (0.218 g, 1.92 mmol) and monobasic sodium phosphate (0.233 g, 1.92 mmol) in water (6.40 mL). The reaction was allowed to warm to rt and stirred for 1 h. The mixture was poured into water and diluted with EtOAc. The aqueous layer was extracted with EtOAc and the combined organic layers were dried (MgSO₄), and concentrated under reduced pressure to afford 0.160g (85%) of **4-12** which was used in the subsequent step without further purification: ¹H NMR (300 MHz, CDCl₃) δ 7.39-7.26 (m, 1 H), 6.22-6.19 (m, 2 H), 5.80 (d, 1 H, *J* = 15.2 Hz), 2.23-2.13 (m, 2 H), 1.48-1.36 (m, 2 H), 1.35-1.19 (m, 10 H), 0.87 (t, 3 H, *J* = 6.9 Hz); ESIMS *m/z* 196 [M-H]⁻.

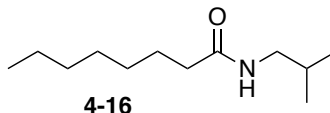


(E)-N-Isobutyldodec-2-enamide (4-13). According to general protocol A, (*E*)-dodec-2-enoic acid¹ (0.314 g, 1.57 mmol), Et₃N (0.635 g, 6.28 mmol), T3P[®] (0.999 g, 1.57 mmol) and isobutylamine (0.116, 1.57 mmol) in anhydrous CH₂Cl₂ (15.0 mL) for 16 h afforded 87.5 mg (22%) of **4-13** as a light yellow solid following purification by chromatography on SiO₂ (hexanes:EtOAc, 1 to 100%): ¹H NMR (400 MHz, CDCl₃) δ 6.83 (dt, 1 H, *J* = 15.1, 6.9 Hz), 5.75 (dt, 1 H, *J* = 15.2, 1.5 Hz), 5.45 (brs, 1 H), 3.15 (t, 2 H, *J* = 6.8 Hz), 2.19-2.13 (m, 2 H), 1.75-1.81 (m, 1H), 1.45-1.39 (m, 2H), 1.28-1.23 (m, 12 H), 0.92 (d, 6 H, *J* = 6.7 Hz), 0.87 (t, 3 H, *J* = 6.9 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 166.3, 145.1, 123.7, 47.0, 32.3, 32.1, 29.8,

29.7, 29.7, 29.5, 29.4, 28.8, 28.5, 22.9, 20.3, 14.3; ESIMS m/z 295 $[M+H]^+$; HRMS m/z calculated for $C_{16}H_{32}NO$ $[M+H]^+$ 254.2478, found 254.2472.

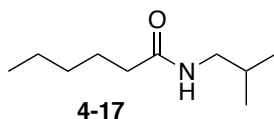


***N*-Isobutyldecanamide (4-15)**. According to general protocol A, decanoic acid (0.300 g, 1.72 mmol), Et_3N (0.698 g, 6.90 mmol), T3P[®] (1.32 g, 2.07 mmol) and isobutylamine (0.127 g, 1.72 mmol) in anhydrous CH_2Cl_2 (17.0 mL) stirred overnight afforded 0.319 g (81%) of **4-15** as a white solid following purification by chromatography on SiO_2 (hexanes:EtOAc, 5 to 100%): 1H NMR (400 MHz, $CDCl_3$) δ 5.45 (brs, 1H), 3.08 (t, 2 H, $J = 6.4$ Hz), 2.16 (t, 2 H, $J = 8.0$ Hz), 1.79-1.72 (m, 2 H), 1.64-1.60 (m, 2 H), 1.36-1.17 (m, 12 H), 0.91 (d, 6 H, $J = 6.7$ Hz), 0.87 (t, 3 H, $J = 7.2$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 47.0, 37.2, 34.1, 31.9, 31.9, 29.5, 29.2, 29.1, 28.7, 26.1, 25.0; ESIMS m/z 269 $[M+ACN]^+$; HRMS m/z calculated for $C_{14}H_{30}NO$ $[M+H]^+$ 228.2322, found 228.2316.

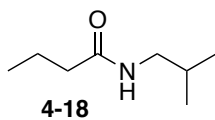


***N*-Isobutyloctanamide (4-16)**. According to general protocol A, octanoic acid (0.300 g, 2.06 mmol), Et_3N (0.317 g, 3.13 mmol), T3P[®] (0.598 g, 0.939 mmol) and isobutylamine (57.8 mg, 0.783 mmol) in anhydrous CH_2Cl_2 (20.0 mL) stirred overnight afforded 0.311 g (84%) of **4-16** as a colorless oil following purification by chromatography on SiO_2 (hexanes:EtOAc, 2 to 100%): 1H NMR (300 MHz, $CDCl_3$) δ 5.45 (brs, 1H), 3.08 (t, 2 H, $J = 6.5$ Hz), 2.16 (t, 2 H, $J = 8.1$ Hz), 1.79-1.72 (m, 2 H), 1.64-1.60 (m, 2 H), 1.36-1.17 (m, 12

H), 0.91 (d, 6 H, $J = 6.7$ Hz), 0.87 (t, 3 H, $J = 6.9$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 173.4, 94.7, 47.9, 47.0, 44.7, 37.2, 31.9, 29.5, 29.2, 28.7, 28.7, 26.1, 22.8, 20.3, 20.3, 14.3, 14.0; ESIMS m/z 241 $[\text{M}+\text{MeCN}]^+$; HRMS m/z calculated for $\text{C}_{12}\text{H}_{26}\text{ON}$ $[\text{M}+\text{H}]^+$ 200.2009, found 200.2006.

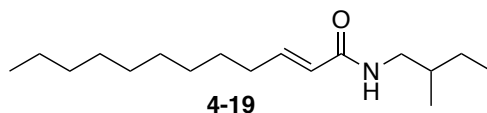


***N*-Isobutylhexanamide (4-17).** According to general protocol A, hexanoic acid (1.00 g, 8.52 mmol), Et_3N (3.96 g, 34.1 mmol), T3P[®] (6.51 g, 10.2 mmol) and isobutylamine (0.623 g, 8.52 mmol) in anhydrous CH_2Cl_2 (85.0 mL) for 16 h afforded 0.680 g (47%) of **4-17** as a light yellow oil following purification by chromatography on SiO_2 (hexanes:EtOAc, 1 to 100%): ^1H NMR (400 MHz, CDCl_3) δ 5.51 (brs, 1H), 3.07 (t, 2 H, $J = 6.0$ Hz), 2.16 (t, 2 H, $J = 7.6$ Hz), 1.80-1.70 (m, 1 H), 1.66-1.59 (m, 2 H), 1.35-1.25 (m, 4 H), 0.91-0.87 (m, 9 H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 173.3, 47.0, 37.2, 31.7, 28.8, 25.8, 22.6, 20.3, 14.2; ESIMS m/z 213 $[\text{M}+\text{MeCN}]^+$; HRMS m/z calculated for $\text{C}_{10}\text{H}_{22}\text{ON}$ $[\text{M}+\text{H}]^+$ 172.1696, found 172.1696.

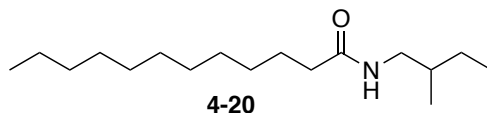


***N*-Isobutylbutyramide (4-18).** According to general protocol A, butyric acid (0.300 g, 3.40 mmol), Et_3N (1.38 g, 13.6 mmol), T3P[®] (2.60 g, 4.09 mmol) and isobutylamine (0.252 g, 3.40 mmol) in anhydrous CH_2Cl_2 (35.0 mL) for 16 h afforded 0.389 g (80%) of **4-18** as a colorless oil following purification by chromatography on SiO_2 (hexanes:EtOAc, 1 to 100%):

^1H NMR (300 MHz, CDCl_3) δ 5.56 (brs, 1H), 3.08 (t, 2 H, $J = 6.5$ Hz), 2.15 (t, 2 H, $J = 7.8$ Hz), 1.80-1.65 (m, 3 H), 0.97 (t, 3 H, $J = 7.5$ Hz), 0.91 (d, 6 H, $J = 6.7$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 173.2, 47.0, 39.1, 28.7, 20.2, 19.5, 14.0; ESIMS m/z 213 $[\text{M}+\text{MeCN}]^+$; HRMS m/z calculated for $\text{C}_8\text{H}_{19}\text{NO}$ $[\text{M}+\text{H}]^+$ 144.1383, found 144.1381

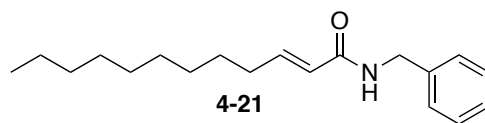


(E)-N-(2-Methylbutyl)dodec-2-enamide (4-19). According to general protocol A, (*E*)-dodec-2-enoic acid³⁰ (0.400 g, 2.00 mmol), Et_3N (0.810 g, 8.00 mmol), T3P[®] (1.53 g, 2.40 mmol) and 2-methylbutylamine (0.205 g, 2.00 mmol) in anhydrous CH_2Cl_2 (20.0 mL) for 16 h afforded 0.150 g (28%) of **4-19** as a light yellow solid following purification by chromatography on SiO_2 (hexanes:EtOAc, 1 to 100%): ^1H NMR (400 MHz, CDCl_3) δ 6.83 (dt, 1 H, $J = 15.3, 6.9$ Hz), 5.75 (dt, 1 H, $J = 15.2, 1.5$ Hz), 5.43 (brs, 1 H), 3.32-3.22 (m, 1 H), 3.18-2.98 (m, 1 H), 2.20-2.12 (m, 2H), 1.65-1.51 (m, 2 H), 1.45-1.39 (m, 4H), 1.28-1.23 (m or s, 12 H), 1.20-1.10 (m, 2 H), 0.93-0.87 (m, 9 H); ^{13}C NMR (100 MHz, CDCl_3) δ 166.3, 145.0, 123.7, 45.3, 35.2, 32.2, 32.1, 29.8, 29.7, 29.5, 29.4, 28.5, 27.2, 22.9, 17.5, 14.3, 11.5; ESIMS m/z 309 $[\text{M}+\text{MeCN}]^+$.

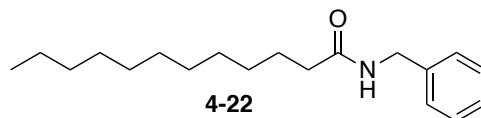


N-(2-Methylbutyl)dodecanamide (4-20). According to general protocol A, lauric acid (0.720 g, 3.53 mmol), Et_3N (1.43 g, 14.1 mmol), T3P[®] (2.70 g, 4.24 mmol) and 2-methylbutylamine (0.362 g, 3.53 mmol) in anhydrous CH_2Cl_2 (35.0 mL) stirred overnight

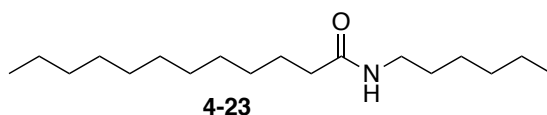
afforded 0.92 g (97%) of **4-20** as a white solid following purification by chromatography on SiO₂ (hexanes:EtOAc, 5 or 100%): ¹H NMR (400 MHz, CDCl₃) δ 5.39 (brs, 1 H), 3.24-3.18 (m, 1 H), 3.09-3.03 (m, 1 H), 2.17 (t, 2 H, *J* = 8.0 Hz), 1.62-1.51 (m, 3 H), 1.48-1.37 (m, 2 H), 1.35-1.25 (m, 12 H), 1.17-1.07 (m, 2 H), 0.93-0.85 (m, 9 H); ¹³C NMR (100MHz, CDCl₃) δ 173.3, 45.3, 38.0, 37.2, 35.2, 32.1, 29.8, 27.2, 26.1, 22.9, 17.4, 14.4, 11.5; ESIMS *m/z* 311 [M+MeCN]⁺; HRMS *m/z* calculated for C₁₇H₃₆NO [M+H]⁺ 270.2791, found 270.2785.



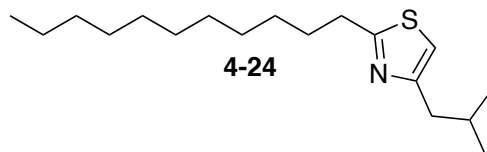
(*E*)-*N*-Benzyl dodec-2-enamide (4-21). According to general protocol A, (*E*)-dodec-2-enoic acid³⁰ (0.366 g, 1.83 mmol), Et₃N (0.20 mL, 1.83 mmol), T3P[®] (1.31 mL, 2.19 mmol) and benzylamine (0.198, 1.83 mmol) in anhydrous CH₂Cl₂ (18.3 mL) for 16 h afforded 0.173 g (33%) of **4-21** as a white solid following purification by chromatography on SiO₂ (hexanes:EtOAc, 1 to 100%): ¹H NMR (400 MHz, CDCl₃) δ 7.36–7.26 (m, 5 H), 6.88 (dt, 1 H, *J* = 15.2, 6.9 Hz), 5.77 (dt, 1 H, *J* = 15.2, 1.5 Hz), 5.71 (brs, 1 H), 4.51 (d, 2 H, *J* = 5.7 Hz), 2.17 (q, 2 H, *J* = 7.1 Hz), 1.47-1.40 (m, 2 H), 1.40-1.34 (m, 12H), 0.87 (t, 6 H, *J* = 6.8 Hz), 0.88 (t, 3 H, *J* = 8.0 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 166.3, 145.1, 123.7, 47.0, 32.3, 32.1, 29.8, 29.7, 29.7, 29.5, 29, 4, 28.8, 28.5, 22.9, 20.3, 14.3; ESIMS *m/z* 329 [M+MeCN]⁺; HRMS *m/z* calculated for C₁₉H₃₀NO [M+H]⁺ 288.2322, found 288.2317.



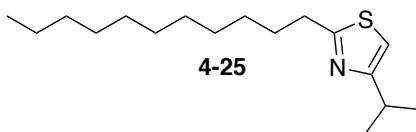
(E)-N-Benzyl dodec-2-enamide (4-22). According to general protocol A, lauric acid (0.772 g, 3.82 mmol), Et₃N (1.54 g, 15.3 mmol), T3P[®] (2.91 g, 4.58 mmol) and benzylamine (0.413 g, 3.82 mmol) in anhydrous CH₂Cl₂ (38.0 mL) for 16 h afforded 0.94 g (85%) of **4-22** as a white solid following purification by chromatography on SiO₂ (hexanes:EtOAc, 1 to 100%): ¹H NMR (400 MHz, CDCl₃) δ 5.68 (brs, 1H), 4.45 (d, 2 H, *J* = 5.5 Hz), 2.21 (t, 2 H, *J* = 7.7 Hz), 1.65 (p, 2 H, *J* = 7.5 Hz), 1.29-1.24 (m, 16 H), 0.87 (t, 3 H, *J* = 4.8 Hz); ¹³C NMR (100MHz, CDCl₃) δ 173.2, 138.6, 128.9, 128.1, 127.7, 94.5, 43.8, 37.1, 32.1, 29.8, 29.7, 29.5, 26.0, 22.9, 14.5; ESIMS *m/z* 331 [M+MeCN]⁺; HRMS *m/z* calculated for C₁₉H₃₂NO [M+H]⁺ 290.2478, found 290.2473.



N-Hexyl dodecanamide (4-23). According to general protocol A, lauric acid (0.881 g, 4.35 mmol), Et₃N (1.76g, 17.4 mmol), T3P[®] (3.23 g, 5.22 mmol) and hexylamine (0.440 g, 4.35 mmol) in anhydrous CH₂Cl₂ (43.0 mL) for 16 afforded 0.788 mg (64%) of **4-23** as a white solid after purification by chromatography on SiO₂ (hexanes:EtOAc, 1 to 100%): ¹H NMR (400 MHz, CDCl₃) δ 5.39 (brs, 1H), 3.26-2.21 (m, 2 H), 2.14 (t, 2 H, *J* = 8.0 Hz), 1.66-1.62 (m, 2 H), 1.52-1.43 (m, 2 H), 1.34-1.20 (m, 22 H), 0.89-0.86 (m, 6 H); ¹³C NMR (100 MHz, CDCl₃) δ 173.2, 39.7, 37.2, 32.1, 31.7, 29.7, 29.6, 26.8, 26.1, 22.9, 22.8, 14.4, 14.2; ESIMS *m/z* 284 [M+H]⁺; HRMS *m/z* calculated for C₁₈H₃₈ON [M+H]⁺ 284.2948, found 284.2943.

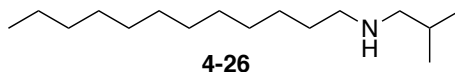


4-Isobutyl-2-undecylthiazole (4-24). General protocol B. A solution of dodecanethioamide³¹ (0.100 g, 0.457 mmol) and 1-bromo-4-methylpentan-2-one³² (83.5 mg, 0.457 mmol) in ethanol (1.00 mL) was stirred at reflux for 12 h. The solvent was removed under reduced pressure and the residue was purified by chromatography on SiO₂ (hexanes:EtOAc, 0 to 100%) to yield 0.135 g (55%) of **4-24** as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 7.11 (s, 1 H), 3.50 (t, 2 H, *J* = 8.0 Hz), 2.90 (d, 2 H, *J* = 7.2 Hz), 2.32-2.23 (m, 1 H), 1.93-1.85 (m, 2 H), 1.48-1.40 (m, 2 H), 1.36-1.17 (m, 14 H), 0.98 (d, 6 H, *J* = 6.6 Hz), 0.86 (t, 3 H, *J* = 6.8 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 177.3, 149.6, 115.3, 36.8, 32.1, 30.3, 30.1, 29.7, 29.7, 29.5, 29.5, 29.2, 29.0, 28.5, 22.9, 22.2, 14.3; ESIMS *m/z* 323 [M+MeCN]⁺; HRMS *m/z* calculated for C₁₈H₃₄SN [M+H]⁺ 296.2407, found 296.2398.



4-Isopropyl-2-undecylthiazole (4-25). According to general protocol B, a solution of dodecanethioamide³¹ (0.160 g, 0.949 mmol) and 1-bromo-3-methylbutan-2-one³³ (0.209 g, 0.949 mmol) in ethanol (1.50 mL) was stirred at reflux for 12 h. The solvent was removed under reduced pressure and the residue was purified by chromatography on SiO₂ (hexanes:EtOAc, 0 to 100%) to yield 0.112 g (55%) of **4-25** as a colorless oil. ¹H NMR (400 MHz, CDCl₃) δ 6.69 (s, 1 H), 3.10-3.02 (m, 1 H), 2.96 (t, 2 H, *J* = 7.6 Hz), 1.80-1.72 (m, 2 H), 1.40-1.32 (m, 2 H), 1.29 (d, 6 H, *J* = 6.9 Hz), 1.27-1.24 (m, 14 H), 0.87 (t, 2 H, *J* = 6.8

Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 171.2, 163.4, 109.8, 33.8, 32.1, 31.1, 30.5, 29.8, 29.7, 29.6, 29.5, 29.4, 22.9, 22.6, 14.4; ESIMS m/z 323 $[\text{M}+\text{MeCN}]^+$; HRMS m/z calculated for $\text{C}_{17}\text{H}_{32}\text{SN}$ $[\text{M}+\text{H}]^+$ 282.2250, found 282.2244.



***N*-Isobutyldodecan-1-amine (4-26)**. To a solution of lithium aluminum hydride (0.110 g, 2.75 mmol) in THF (27.0 mL) was added *N*-isobutyldodecanamide **4-14** (0.178 g, 0.688 mmol) in THF (1.00 mL) at 0 °C. The mixture was allowed to warm to rt gradually and stirred overnight. The reaction mixture was quenched using the Fieser³⁴ aqueous work up method and the precipitate was filtered through a pad of Celite[®]. The filtrate was concentrated under reduced pressure and the residue was purified using an acid-base extraction to yield 0.112 g (67%) of **4-26** as colorless oil. ^1H NMR (300 MHz, CDCl_3) δ 2.57 (t, 2 H, $J = 7.2$ Hz), 2.40 (d, 2 H, $J = 6.9$ Hz), 1.79-1.70 (m, 1 H), 1.52-1.41 (m, 2 H), 1.34-1.22 (m, 16 H), 0.90 (d, 6 H, $J = 6.6$ Hz), 0.88 (t, 3 H, $J = 7.2$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 58.4, 50.5, 32.1, 30.4, 29.9, 29.9, 29.6, 28.5, 27.7, 22.9, 20.9, 14.4; ESIMS m/z 283 $[\text{M}+\text{MeCN}]^+$; HRMS m/z calculated for $\text{C}_{16}\text{H}_{37}\text{N}$ $[\text{M}+\text{H}]^+$ 242.2842, found 242.2839.

4.6 References

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