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SYNTHESIS OF NOVEL QUATERNARY ALPHA-AMINO ACIDS AND 2-IMIDAZOLINES DERIVED FROM OXAZOL-5(4H)-ONES AND EVALUATION OF THEIR PROTEASOMAL INHIBITION ACTIVITY

presented by

ROBERT ADAM MOSEY

has been accepted towards fulfillment
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Doctoral degree in Chemistry

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By

Robert Adam Mosey

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Chemistry

2010

ABSTRACT

SYNTHESIS OF NOVEL QUATERNARY ALPHA-AMINO ACIDS AND 2-IMIDAZOLINES DERIVED FROM OXAZOL-5(4H)-ONES AND EVALUATION OF THEIR PROTEASOMAL INHIBITION ACTIVITY

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The research presented in this dissertation was focused towards the synthesis of densely functionalized amino acid derivatives and heterocycles derived from alkylation products of oxazol-5(4H)-ones. The opening chapter introduces oxazol-5(4H)-ones and describes their reactivity and synthetic utility for the construction of pharmaceutically relevant compound libraries. The remaining chapters describe the development of an oxazol-5(4H)-one alkylation and reduction protocol for the synthesis of quaternary α-amino acid derivatives and the subsequent use of quaternary α-amino acid derivatives towards the synthesis of proteasome inhibitors.

The first study in this dissertation describes the diastereoselective synthesis of quaternary α-amino acid derivatives by means of a protocol involving sequential oxazol-5(4H)-one alkylation and hydride reduction. Previous studies in our lab demonstrated that oxazol-5(4H)-ones participate in ene-type alkylation reactions with enol ethers. The racemic reaction was revisited in our current studies, wherein solvents were optimized and Brønsted acids were used to promote diastereoselectivity. Hydride reduction of the oxazol-5(4H)-one alkylation products was then performed, resulting in the stereoselective formation of highly substituted *tert*-alkyl amino hydroxy carboxylic acids. Mechanistic

investigation also revealed that the ene-type oxazol-5(4H)-one alkylation reaction likely occurs through a stepwise process.

The second study in this dissertation details the use of quaternary α-amino acid derivatives prepared from oxazol-5(4H)-one alkylation products towards the synthesis of proteasome inhibitors and proteasome inhibitor precursors. A quaternary α-amino acid derivative was first utilized to construct an advanced intermediate used in the synthesis of salinosporamide A. A very similar quaternary α-amino acid derivative was later used for the generation of a 2-imidazoline scaffold. Further elaboration of the 2-imidazoline resulted in the culmination of a 2-imidazoline bearing a β-lactone functionality. 2-Imidazolines prepared in this chapter were then evaluated for their ability of inhibit proteolytic activity of the mammalian 20S proteasome.

The final study in this dissertation describes annulation reactions arising from underutilized reactivity of 2-imidazolines. It was discovered that 2-imidazolines underwent cyclization reactions with β-hydroxy carboxylic acids when treated with BOP-Cl and triethylamine to afford molecules containing a unique bicyclic oxazinone/imidazolidine ensemble. The reaction has been explored and has revealed that N-Boc derivatives of the α-amino acids serine and threonine may be used in the reaction to generate products diastereoselectively. Muticyclic adducts prepared in this chapter were then evaluated for their ability of inhibit proteolytic activity of the mammalian 20S proteasome.

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LIST OF SYMBOLS or ABBREVIATIONS

4-PPY – 4-(1-Pyrrolidino)pyridine

BOP-Cl – Bis(2-oxo-3-oxazolidinyl)phosphonic chloride

CSA – Camphor Sulfonic Acid

DBU – 1,8-Diazabicyclo[5.4.0]undec-7-ene

DCE – 1,2-Dichloroethane

DCM – Dichloromethane

DEPT – Distortionless Enhancement by Polarization Transfer

DMAP – 4-Dimethylaminopyridine

DMF – N,N-Dimethylformamide

DMSO – Dimethylsulfoxide

DOS – Diversity Oriented Synthesis

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

EtOAc – Ethyl Acetate

EWG – Electron-withdrawing group

FDA – Food and Drug Administration

GCMS – Gas Chromatography - Mass Spectrometry

HPLC – High Performance Liquid Chromatography

HRMS – High Resolution Mass Spectrometry

HTS – High Throughput Screening

IBS – Imidazoline binding site

IC_{50} – Half maximal inhibitory concentration

IκB – Inhibitor of NF-κB

IR – Infrared

LDA – Lithium diisopropylamide

LHMDS – Lithium bis(trimethylsilyl)amide

MS – Mass Spectrometry

MsCl – Methanesulfonyl chloride

nOe – Nuclear Overhauser effect

NF-κB – Nuclear Factor-KappaB

NMR – Nuclear Magnetic Resonance

Nu – Nucleophile

PMB – *para*-Methoxybenzyl

rt – Room temperature

SAR – Structure Activity Relationship

SDS – Sodium dodecyl sulfate

TEA – Triethylamine

TFA – Trifluoroacetic acid

TFAA – Trifluoroacetic anhydride

THF – Tetrahydrofuran

TLC – Thin layer chromatography

TMS-Cl – Chlorotrimethylsilane

TOS – Target Oriented Synthesis

Tris – Tris(hydroxymethyl)aminomethane

UV – Ultraviolet

CHAPTER I

OXAZOL-5(4H)-ONES AS PLURIPOTENT SCAFFOLDS FOR THE SYNTHESES OF DIVERSE LIBRARIES OF COMPOUNDS

A. Introduction to drug discovery.

Modern disease treatment has resulted in increases in both longevity and quality of human life. During the course of human history, disease treatment has had a synergistic relationship with drug discovery. Early drug discovery was honed in early civilizations through crude experimentation, as components of plants, fungi, minerals, and animals were administered to sick patients with varying degrees of success. However, advancements in biological and physical sciences have permitted the amendment of more sophisticated techniques to the sciences of drug discovery and disease treatment. Future scientific developments are vital in order to confront challenges associated with emerging and evolving diseases, some of which undoubtedly will pose a legitimate threat to the stability and livelihood of modern civilizations.

The goal of modern drug discovery is to study the pathology of diseases and to apply appropriate methods for their prevention or treatment. Due to recent scientific breakthroughs, such as the milestone completion of the human genome project, many potential therapeutic targets have been identified which were previously unknown. As such, researchers have become better enabled to investigate and cure a host of diseases. However, it is noteworthy that while the number of known drug targets has increased, the number of new drugs approved by the FDA has steadily decreased.[1]

Pharmaceutical sciences have adopted the dogma of drug discovery and actively pursue molecular candidates that modulate desired biological responses. The process of drug discovery can be roughly divided into two parts: 1) the identification of a suitable biological target for disease treatment and 2) the identification and construction of a small molecule to act on that target. The identification of a biological target is a challenging undertaking in itself as the pathology of a given disease often encompasses numerous biological pathways, some of which are important for additional biological functions. The search for an appropriate small molecule to interact with the chosen biological target is an equally daunting task, especially considering that the number of molecules predicted to have drug-like properties is estimated to exceed the number of atoms occupying the planet ($>10^{62}$ as compared to 10^{51}).[1] Moreover, most molecules are biologically active in that they elicit some response when introduced to a biological system.[1] As such, the usual focus of pharmaceutical chemistry is to pursue molecular candidates which elicit a selective response when introduced to a biological system. Drugs with a selective mode of action or a distinct biological target are valued in pharmaceutical research since unwanted side effects often stem from undesirable and usually unknown biological interactions. Recent studies have identified a significant number of undesirable biological interactions of known drugs, bringing to light new opportunities for the prediction and prevention of such interactions.[2] Nonetheless, notable exceptions exist and some nonselective drugs have proven to be of great therapeutic importance. For example, many mood disorders including depression and

schizophrenia are considered to be polygenic in origin, and such disorders are often treated successfully through the administration of drugs which affect numerous biological targets.[3]

Screening large libraries of small molecules to identify selective interactions with known receptors, enzymes, and other biological targets or biological pathways of interest is a routine practice in modern drug discovery.[4] Recent synthetic advances have allowed for rapid and efficient production of compound libraries comprising a high degree of complexity. These collections of compounds are populated in part by natural products and molecules that have been inspired by nature.[5,6] Such compound libraries are often screened against numerous biological targets by a method known as high throughput screening (HTS) to identify "hits", or compounds that show some level of desired biological activity.[7] Once "hits" are identified, researchers often construct additional compound libraries to identify structural features important for biological activity. These structure-activity relationship (SAR) studies are useful for the development of lead compounds and drug candidates. Unfortunately, this method may fail to direct researchers towards identifying drug candidates if initial screens using known compound libraries do not produce appropriate "hits".

Fragment-based drug design is an emerging strategy used to identify structural features within a small molecule which are required for biological activity.[8,9] This approach utilizes a screen of low molecular weight compounds to evaluate for weak binding (mM range) to biological targets of interest. The screen often identifies numerous small molecules which interact with the target,

and these compounds are considered as fragments which may combined to produce a potential drug candidate. This strategy is considered to be in its infancy and has yet to produce any marketed drugs.[10] However, it is viewed favorably as a useful tool for enabling researchers with a reasonable starting point in their search for drug candidates.

Once researchers have determined a course of focus for their drug development studies, they often rely on common strategies for the rapid production of compound libraries. Two frequently-used strategies are target-oriented synthesis (TOS) and combinatorial synthesis. TOS is described as the synthesis of a predetermined or predesigned molecular target. These target molecules are often complex and may be synthesized from simpler fragments. Researchers utilizing TOS routinely plan their syntheses *via* retrosynthetic analyses to establish the building blocks, as well as the order of their assemblage, required for the construction of molecular targets of interest. Strategically planned synthetic routes are useful for the synthesis of desired targets and often become amended for the construction of structurally related analogs.

Combinatorial synthesis is often used in combination with TOS for the construction of libraries comprised of structurally similar compounds. Combinatorial synthesis is often described as parallel synthesis, since numerous compounds can be prepared in parallel from the same reaction through the variation of reagents. This strategy is common especially in academic research in order to investigate the scope of chemical reactions. However, while

combinatorial synthesis is useful for the rapid production of compound libraries, the method is criticized by some for being too focused in terms of populating chemical space. Chemical space is described as the theoretical landscape in which all potential molecules exist, and molecules in this space are arranged according to physical and electronic chemical descriptors. Compounds prepared in parallel using combinatorial synthesis often vary only slightly in terms of their skeletal and stereochemical makeup and are considered to populate similar chemical space, as is depicted in Figure I-1. Needless to say, there is much to learn about the relationship between chemical space and so-called biological space. Therefore, accessing different regions of chemical space is considered to be an advantageous strategy for the discovery of pharmaceutically relevant molecules.

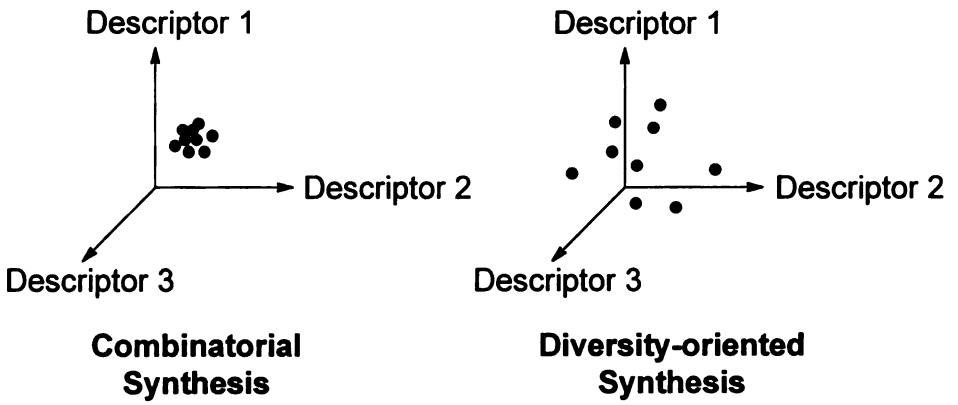


Figure I-1. Population of chemical space by chemical libraries prepared *via* combinatorial synthesis and diversity-oriented synthesis.

One method used for the construction of compound collections in which molecules differ greatly in their population of chemical space has been termed diversity-oriented synthesis (DOS).[11,12] DOS involves utilizing similar starting

scaffolds in orthogonal reactions to build a diverse set of compounds, which undergo further derivatizations to generate molecules with increasing complexity and diversity.[12] DOS is undoubtedly more successful than TOS at populating a variety of regions of chemical space, as is depicted in Figure I-1. However, the level of effectiveness by which an appropriate drug candidate can be discovered by this approach is in question, as the new population of constructed compounds is diluted into the large vastness of chemical space. Consequently, in consideration of the time and resources needed for the construction of compound libraries, there is arguably an equal, if not reduced, probability of producing a compound suitable for drug development utilizing DOS when compared to TOS.

Drug discovery has played a significant role in the development of modern synthetic chemistry. The isolation and characterization of complex natural products has often inspired researchers to pursue new chemical methods for their preparation.[13] Major synthetic advancements, notably in asymmetric synthesis, have thus been required in order to emulate the amazing level of molecular complexity found in natural products.[14,15] New methods of chemical synthesis developed to construct products found in nature have in turn been utilized in the construction of novel pharmaceuticals. The perpetual cycle of discovery and advancement has had far reaching effects that have been felt even in academia.

Discoveries involving synthetic reactions and methodologies have long been and continue to be a hallmark of chemical education. Additionally, synthetic and medicinal chemistry programs carried out at the academic level are

becoming increasingly more involved in drug discovery science. Therefore, it is not uncommon for academic researchers to construct compound libraries according to new methodologies with the explicit aim of obtaining drug-like compounds. Such academic research currently ongoing in the Tepe lab involves routine construction and screening of compound libraries derived from oxazolones in order to elucidate molecular candidates which exhibit anti-cancer properties.

B. Introduction to oxazol-5(4H)-ones.

Oxazolones are recognized for their utility as versatile scaffolds in organic synthesis.[16] Five isomeric forms of oxazolones exist, each of which exhibits unique chemical reactivity (Figure I-2).[17] Oxazol-5(4H)-ones, also called "azlactones", have been extensively utilized in the Tepe lab for the preparation of diverse libraries of small molecules of heterocycles and amino acids.[18]

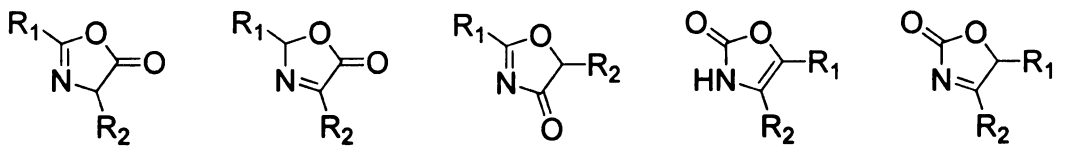
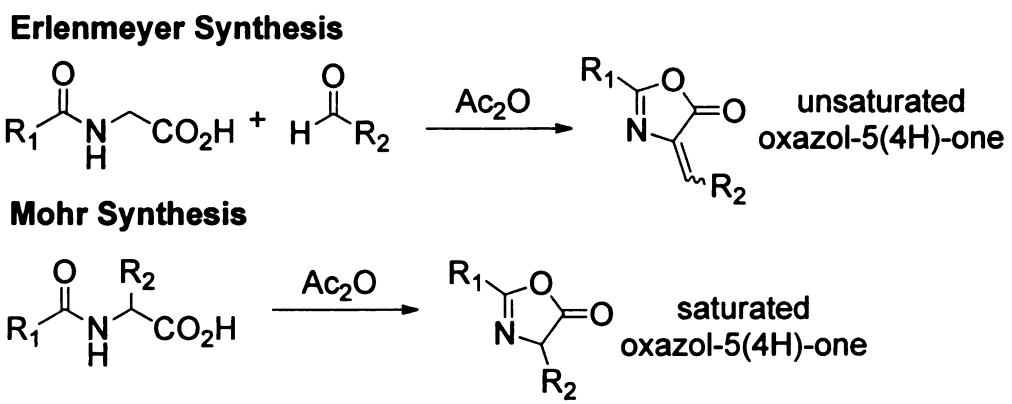


Figure I-2. The five isomeric forms of oxazolones.

Oxazol-5(4H)-ones are cyclic esters of N-acyl α-amino acids and are generally classified as being either saturated or unsaturated (Scheme I-1). The first unsaturated oxazol-5(4H)-one was synthesized by Plöchl more than

a century ago in 1883 *via* condensation of benzaldehyde with hippuric acid in the presence of acetic anhydride.[19] Unsaturated oxazol-5(4H)-ones were prepared in a similar fashion by Erlenmeyer, who established their correct structure in 1900 and named them "azlactones" (Scheme I-1).[20] Almost a decade after Erlenmeyer's discovery, Mohr and co-workers sucessfully prepared the first saturated oxazol-5(4H)-ones by treating N-acyl amino acids with acetic anhydride (Scheme I-1).[21-23] The chemistry of oxazol-5(4H)-ones then remained fairly unexplored until the 1940's, at which time the structure of penicillin was incorrectly thought to be an oxazol-5(4H)-one.[24] Due to the high interest of penicillin at the time, many research groups focused on the reactivity of oxazol-5(4H)-ones. The structure of penicillin was eventually corrected to contain a β-lactam ring system instead of an oxazol-5(4H)-one ring, but the information obtained from these studies was important for the advancement of chemistry involving the oxazol-5(4H)-one scaffold.



Scheme I-1. Erlenmeyer and Mohr syntheses of oxazol-5(4H)-ones.

A variety methods appear in the literature for naming oxazol-5(4H)-ones.[20,25] The ring is generally numbered according to the Hantzsch-Widman

rules giving priority to the oxygen atom and numbering the ring in the direction of the nitrogen atom as shown in Figure I-3.[25] One method of naming refers to the oxazol-5(4H)-one substrate as an amino acid derivative.[20] For example, the oxazol-5(4H)-one derived from N-benzoyl alanine would be refered to as benzoyl alanine azlactone. A second method for naming oxazol-5(4H)-ones describes the substrate as a dihydrooxazole.[25] The oxazol-5(4H)-one derived from N-benzoyl alanine would then be referred to as 5-keto-2-phenyl-4-methyl-4,5-dihydrooxazole. A third system, which will be used throughout this disertation, assigns a parent name of oxazol-5(4H)-one and numbers substituents about the ring in accordance with the Hantzsch-Widman rules. Using this system the oxazolone prepared from N-benzoyl alanine would be described as 2-phenyl-4-methyl-5(4H)-oxazolone.

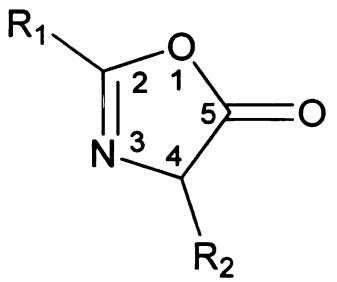
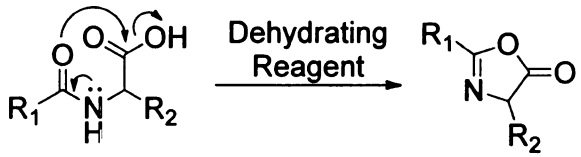


Figure I-3. Numbering of the oxazol-5(4H)-one system in accordance with the Hantzsch-Widman rules.

C. Synthesis and general reactivity of oxazol-5(4H)-ones.

Numerous examples exist for the preparation of oxazol-5(4H)-ones. Modern syntheses of oxazol-5(4H)-ones often involve mild reaction conditions, wherein N-acyl-α-amino acids are treated with dehydrating reagents (Scheme I-2). N-acyl-α-amino acids used in these reactions are readily prepared under

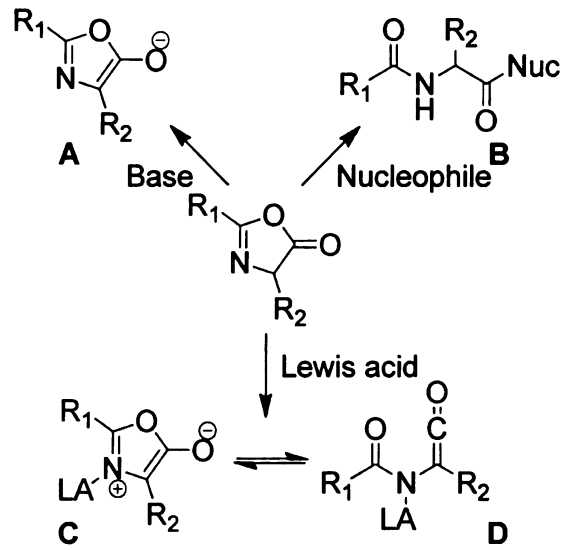
Schotten-Baumann conditions using α-amino acids or from the N-acylation of α-amino esters followed by hydrolysis.[26] Since an abundance of natural and unnatural α-amino acids are commerically available, researchers have easy access to a wide variety of oxazol-5(4H)-ones. Traditionally, cyclodehydration reactions of N-acyl-α-amino acids were performed in refluxing acetic acid, conditions that often lead to reduced product purities and yields as well as difficulties with product isolation. However, these issues are generally avoided in modern preparations of oxazol-5(4H)-ones through the use of more reactive dehydrating reagents, such as activated anhydrides[26,27] and carbodiimides.[16]



Scheme I-2. Cyclodehydrative formation of oxazol-5(4H)-ones.

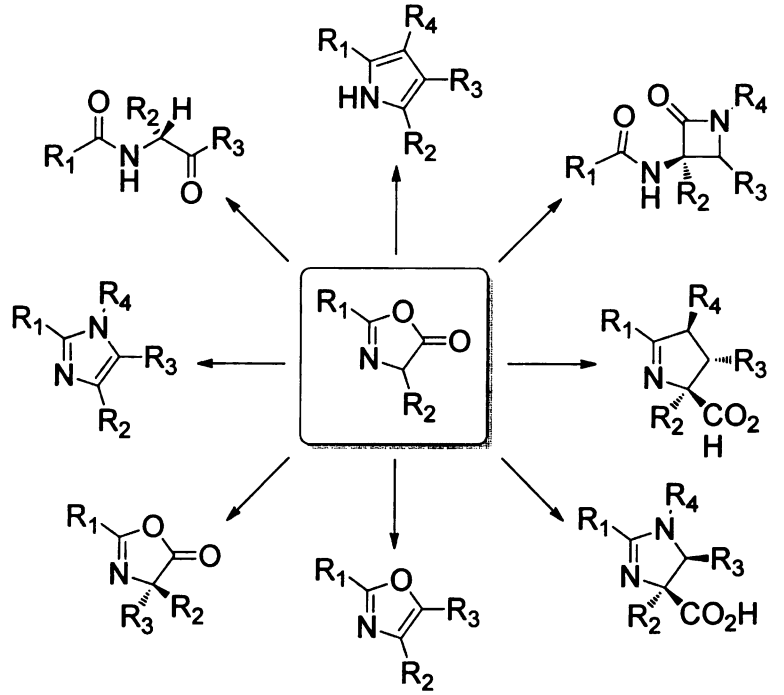
The oxazol-5(4H)-one ring system contains numerous reactive sites allowing for a wide variety of transformations (Scheme I-3). The acidic nature of the proton(s) found at the C4 position of the oxazol-5(4H)-one scaffold enables the formation of an oxazole enolate **A**,[28,29] which can react with a range of electrophiles (Scheme I-3). Alternatively, the oxazol-5(4H)-one ring can be opened readily by a nucleophilic attack at the carbonyl carbon to generate various protected amino acid derivatives (**B**, Scheme I-3). Additionaly, treatment of oxazol-5(4H)-ones with Lewis acids results in the formation of a mesionic 1,3-dipole **C** (also known as a münchnone) or of a

reactive ketene intermediate **D** (Scheme I-3), both of which undergo cycloaddition reactions to yield novel heterocyclic compounds.[30]



Scheme I-3. Reactivity of oxazol-5(4H)-ones.

The diverse reactivity of oxazol-5(4H)-ones makes them excellent substrates for use in the synthesis of a variety of compounds (Scheme I-4).[31] Highly substituted heterocyclic scaffolds can be directly accessed from oxazol-5(4H)-ones with relative ease and in a stereoselective manner. Natural and unnatural amino acids, as well as related derivatives, are also easily constructed in enantiopure form by using oxazol-5(4H)-one intermediates. The focus of the remainder of this chapter will be to describe some of the synthetic applications of oxazol-5(4H)-ones used towards the construction of biologically interesting scaffolds.



Scheme I-4. Scaffolds available from oxazol-5(4H)-ones.

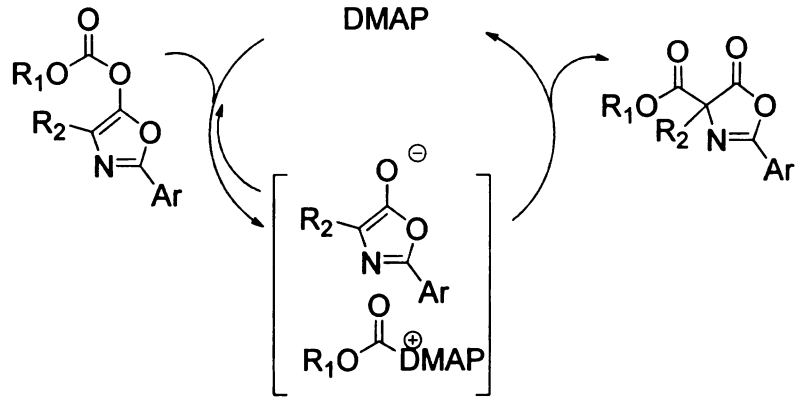
D. Nucleophilic reactions of oxazol-5(4H)-ones.

Oxazol-5(4H)-ones are often recognized for the diverse transformations they undergo due to their nucleophilic character. Oxazol-5(4H)-one α-protons, or those at C4, have a relatively high acidity compared to α-amino acids, and deprotonation results in the formation of an aromatic oxazole (**A**, Scheme 1-3).[28,32] Oxazol-5(4H)-one deprotonation leads to cyclic enolates which are less sterically encumbered than related acylic α-amino acids and display high reactivity towards electrophiles. One consequence of facile enolate formation is that optically active oxazol-5(4H)-ones undergo rapid racemization.[28] Thus, treatment of oxazol-5(4H)-ones with various electrophiles under basic conditions is a practical method used for the preparation of various racemic

quaternary α-amino acid progenitors. Advancements in recent years have surfaced to address the lack of stereoselectivity in these reactions, often through the employment of modern methods of organocatalysis.

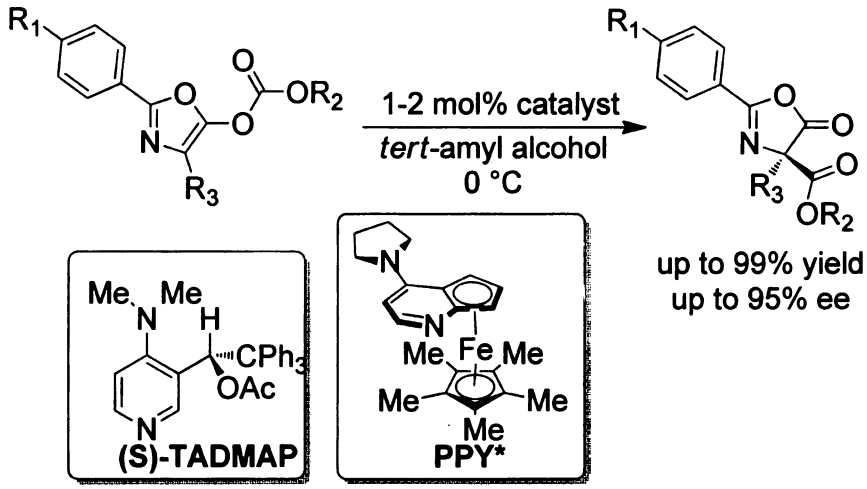
1. Acylation reactions.

Early experiments by Steglich and coworkers demonstrated the utility of oxazol-5(4H)-ones as nucleophiles. In 1970, Steglich and co-workers described the synthesis of C4-acyl quaternary oxazol-5(4H)-ones by means of a rearrangement of O-acylated oxazoles (Scheme I-5).[33] The authors discovered that treatment of the O-acylated oxazoles with nucleophilic organocatalysts such as 4-dimethylaminopyridine (DMAP) readily promoted a rearrangement (now referred to as the Steglich rearrangement) to afford quaternary oxazol-5(4H)-one products. The generally accepted mechanism involves reversible DMAP-adduct formation from the reaction of DMAP with O-acylated oxazole, followed by acylation, which appears to be irreversible due to the stability of the oxazol-5(4H)-one products (Scheme I-5).[34]



Scheme I-5. Steglich rearrangment of O-acyl oxazoles.

Since the initial discovery of the reaction, several chiral organocatalysts have been used substoichiometrically in Steglich rearrangements to generate enantiomerically enriched quaternary oxazol-5(4H)-ones.[34-42] The first asymmetric acyl migration of oxazol-5(4H)-ones was reported in 1998 by Fu and co-workers, whose studies involved the use of the chiral DMAP derivative PPY* (Scheme I-6).[34,35] Vedejs and co-workers demonstrated a similar asymmetric acyl migration in which their DMAP derivative, TADMAP, was used as an organocatalyst in the rearrangement (Scheme I-6).[36,40] Both groups reported obtaining quaternary oxazol-5(4H)-ones in good yields with a high degree of enantioselectivity.

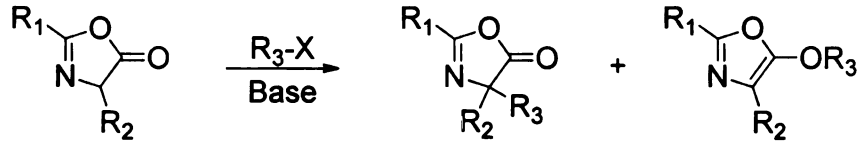


Scheme I-6. Organocatalyzed asymmetric Steglich reactions.

2. Alkylation reactions.

Steglich and co-workers also investigated alkylation reactions of oxazol-5(4H)-ones.[43] In their studies, oxazol-5(4H)-ones were suspended in polar aprotic solutions and treated with Hünig's base in the presence of highly reactive electrophiles to afford quaternary oxazol-5(4H)-ones in moderate to good yields

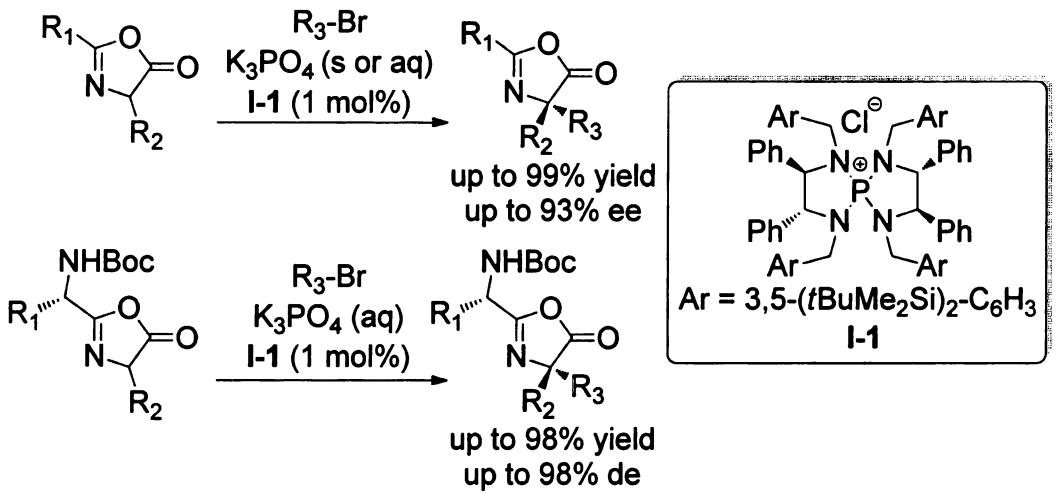
(Scheme I-7). The reaction yields both quaternary oxazol-5(4H)-ones and O-alkyl oxazoles, products which arise from respective C- and O-alkylation reactions of the ambident enolate intermediate. The issue of chemoselectivity in the alkylation of such an ambident nucleophile has since been addressed by utilizing the hard-soft properties of each of the two nucleophilic atoms. The enolate oxygen is considered a hard base and it reacts with hard electrophiles, whereas the softer carbon is expected to react with softer electrophiles. Therefore, O-alkylation is expected to be more S_N1-like when compared to C-alkylation.[44] Optimizations in reaction conditions, including the use of protic or nonpolar solvents and the use of soft electrophiles, such as alkyl halides, have thus been constructive for promoting the formation of C-alkylation products.



Scheme I-7. Alkylation of oxazol-5(4H)-ones under basic conditions.

Recent developments have resulted in the preparation of enantiomerically enriched quaternary oxazol-5(4H)-ones *via* alkylation reactions. Ooi and coworkers have recently reported the use of a chiral tetraaminophosphonium phase transfer catalyst **I-1** in biphasic mixtures to invoke enantioselectivity in alkylation reactions of oxazol-5(4H)-ones (Scheme I-8).[45,46] The authors demonstrated the use of biphasic reaction mixtures that were either liquid-liquid (cyclopentyl methyl ether - saturated K_3PO_4 aqueous solution)[45] or liquid-solid

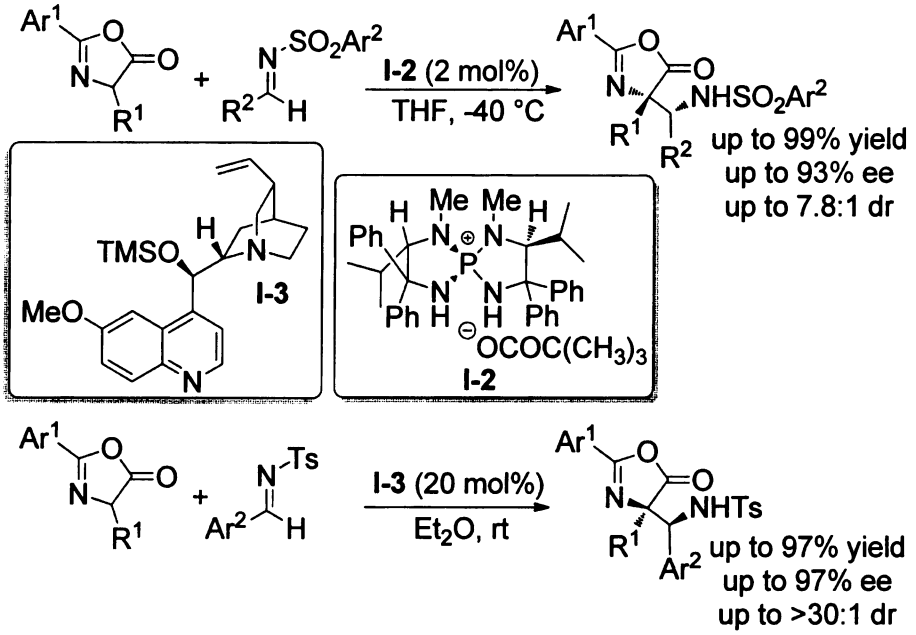
(*tert*-butyl methyl ether – ground solid K_3PO_4)[46] in composition, both of which were found to promote the generation of quaternary products in high yield and enantiomeric excess. Furthermore, excellent diastereoselectivity was observed in alkylation reactions of oxazol-5(4H)-ones prepared from dipeptides, tripeptides, and tetrapeptides (Scheme I-8).[45]



Scheme I-8. Selective oxazol-5(4H)-one alkylation *via* phase transfer catalysis.

Studies aimed towards the development of new oxazol-5(4H)-one alkylation reactions have also revealed methods for the stereoselective generation of Mannich-type products.[47,48] Such Mannich products are easily converted into α-disubstituted α,β-diamino acids,[49] which are valued as synthetic precursors to molecules of pharmaceutical interest.[50] All reported stereoselective Mannich-type alkylation reactions of oxazol-5(4H)-ones have involved addition to electron deficient imines in the presence of a chiral organocatalyst. Chiral tetraaminophosphonium carboxylate catalysts (e.g. **I-2**) developed in Dr. Ooi's lab effectively promote this transformation to afford alkylation products in

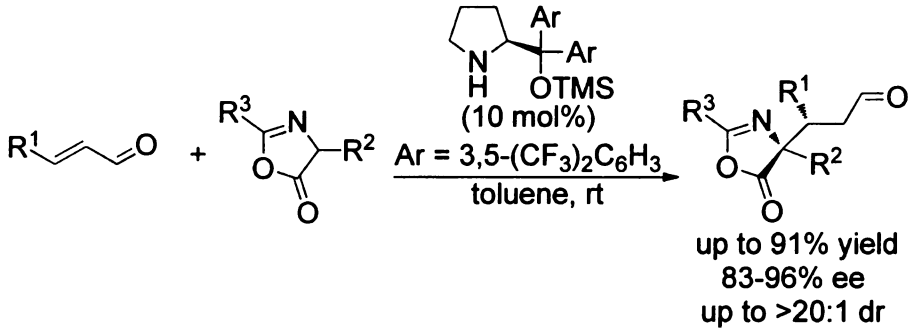
excellent yields and stereoselectivities (Scheme I-9).[47] An oxazol-5(4H)-one enolate is initially generated in the reaction *via* deprotonation by the weakly basic pivalate anion and becomes coordinated to the chiral phosphonium salt through a defined hydrogen-bonding network. Stereoselective alkylation to the imine electrophile then proceeds, followed by protonation of the resultant nitrogen anion to regenerate catalytic pivalate.[47] The use of small-molecule hydrogen-bond donors, such as catalysts **I-2,** has become increasingly common in reactions involving asymmetric organocatalysis.[51] Cinchona alkaloids are one such family of asymmetric H-bond donors that have shown utility in stereoselective Mannich-type reactions of oxazol-5(4H)-ones.[52] Recently, Wang and co-workers reported an asymmetric cinchona alkaloid-catalyzed Mannich reaction which allows access to enantiomers opposite those prepared *via* Ooi's protocol (Scheme I-9).[48] The reaction involves deprotonation of the oxazol-5(4H)-one by the basic quinuclidine nitrogen of alkaloid **I-3**, and the resultant enolate and ammonium species establish a chiral H-bonding complex, leading to the observed stereoselective alkylation.



Scheme I-9. Selective Mannich alkylation reactions of oxazol-5(4H)-ones.

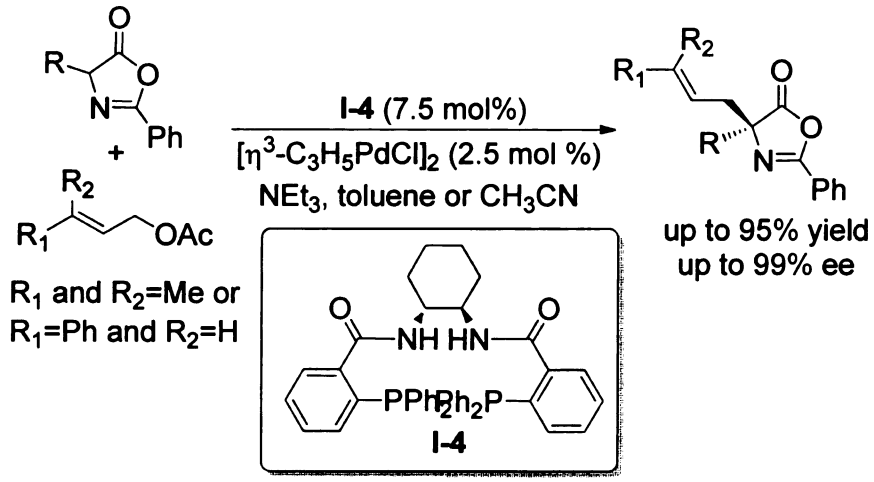
Oxazol-5(4H)-one alkylation chemistry has recently been extended to include stereoselective Michael addition reactions.[53,54] In 2008, Jørgensen and co-workers demonstrated that the addition of racemic oxazol-5(4H)-ones to α,β-unsaturated aldehydes in the presence of chiral organocatalyst resulted in the formation of quaternary substrates with two new stereogenic centers (Scheme I-10).[53] The authors reported complete control of the new chiral center created from the α,β-unsaturated aldehyde portion and good control of the stereocenter formed on the oxazol-5(4H)-one, with product diastereomeric ratios ranging from 2:1 to greater than 20:1 and enantiomeric excesses ranging between 83 and 96%. The reaction was found to tolerate various aldehydes as well as oxazol-5(4H)-ones bearing a wide variety of substituents, leading to products containing

alkyl, benzyl, and aryl groups at all positions. In a similar manner, organocatalyzed stereoselective additions of oxazol-5(4H)-ones to nitroalkenes have been performed, and regioselectivity has been obtained in the reactions to give rise to either C2 or C4 alkylation products.[55,56]



Scheme I-10. Organocatalytic Michael addition of oxazol-5(4H)-ones.

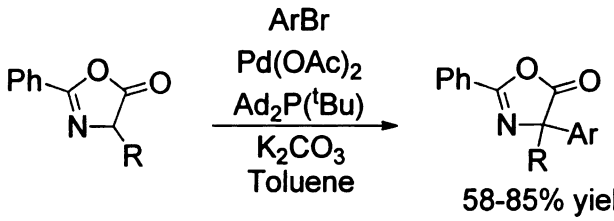
Quaternary oxazol-5(4H)-ones have also been prepared through the installation of allylic substituents. Trost and co-workers have developed several methods to construct quaternary oxazol-5(4H)-ones *via* stereoselective allylic alkylation reactions.[57-61] In one such preparation, the authors treated oxazol-5(4H)-ones with allylic acetates under conditions of palladium catalysis with a chiral diamine ligand **I-4** (Scheme I-11).[58] The reaction was demonstrated using racemic oxazol-5(4H)-ones bearing C4 alkyl substituents to give high yields of prenylation and cinnamylation products (up to 95% yield) with excellent enantiomeric excess (up to 99% ee).[58] An alternative stereoselective palladium-catalyzed allylic alkylation reaction protocol has since been reported by Kawatsura and co-workers for the preparation of similar products in good yields.[62]



Scheme I-11. Pd-catalyzed asymmetric allylic alkylation of oxazol-5(4H)-ones.

3. Arylation reactions.

The synthesis of quaternary oxazol-5(4H)-ones *via* the installation of aryl substitutents has become a useful means for the preparation of unique nonproteinogenic quaternary α-amino acids.[63-65] In 2003, Hartwig and co-workers demonstrated the first palladium-catalyzed arylation reaction of oxazol-5(4H)-ones (Scheme I-12).[64] The reaction involves the coupling of an sp^2 carbon of an aryl bromides with the enolate of an oxazol-5(4H)-one. The catalyst system for this reaction consists of $Pd(OAc)_2$ and the sterically hindered electron rich ligand $Ad_2P(t\text{-Bu})$ (Scheme I-12). This protocol was useful for the generation of quaternary substrates derived from various oxazol-5(4H)-ones and aryl bromides, with the highest yielding reactions resulting from the use of electron rich or electron neutral aryl bromides (75-85% vs ~60% for electron poor aryl bromides).



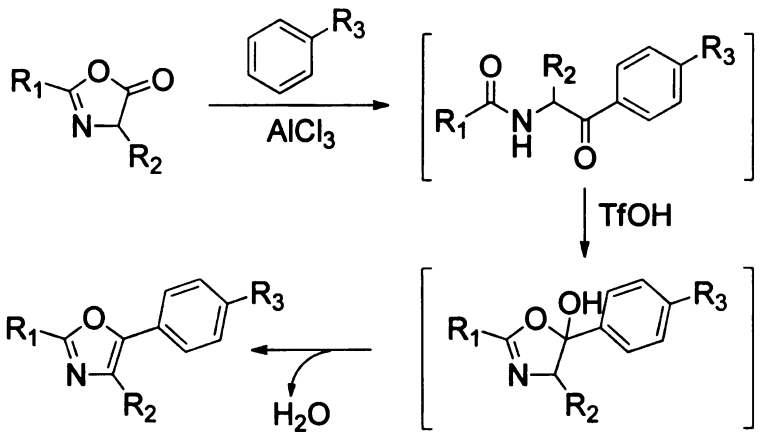
Scheme I-12. Arylation reaction of oxazol-5(4H)-ones.

E. Electrophilic reactions of oxazol-5(4H)-ones

The oxazol-5(4H)-one scaffold contains a highly electrophilic carbonyl center that reacts with an assortment of nucleophiles. Exposure of oxazol-5(4H)-ones to nucleophiles such as alcohols, amines, hydrides, and even arenes (under Friedel-Crafts conditions) has resulted in the construction of α-amino acid derivatives and heterocyclic compounds (Scheme I-3, **B**).[66,67] The electrophilic character of oxazol-5(4H)-ones has also made them ideally suited for use in medically-relevant biopolymers;[68] such oxazol-5(4H)-one-containing polymers have been shown to be useful for the prevention or promotion of mammalian cell adhesion and bacterial biofilm formation.[69,70]

A variety of heterocyclic scaffolds have been prepared from electrophilic reactions of oxazol-5(4H)-ones. In an effort to develop a new route to highly substituted oxazoles, Manasi Keni in the Tepe lab devised a one pot Friedel-Crafts / Robinson-Gabriel synthesis for the production of 2,4,5-trisubstituted oxazoles from oxazol-5(4H)-ones.[71] Key to this protocol is the ability of oxazol-5(4)-ones to undergo Friedel-Crafts alkylation reactions with arenes to produce 2-acylamino ketones,[71-74] substrates which are known to undergo Robinson-Gabriel cyclodehydration reactions to afford oxazoles. The two sequential

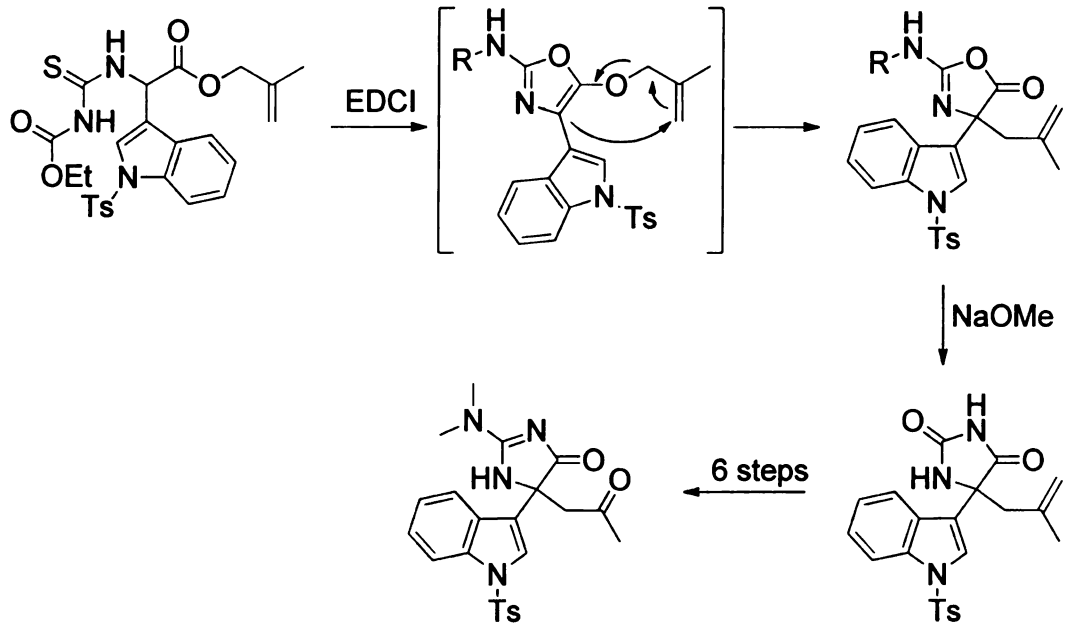
reaction steps were combined in a one-pot synthesis using aluminum chloride and trifluoromethanesulfonic acid, resulting in the culmination of oxazoles directly from oxazol-5(4H)-ones (Scheme I-13).[71] The reaction works well for a wide variety of oxazol-5(4H)-one substrates containing both aromatic and alkyl substituents. In agreement with known Friedel-Crafts reactions, the reaction works best with either electron neutral or electron rich arenes, whereas electron deficient arenes provide little or no product formation. It is believed that the oxazol-5(4H)-one is first activated by the aluminum chloride and a Friedel-Crafts acylation ensues. The resulting 2-acylaminoketone becomes protonated by the trifluoromethanesulfonic acid and then dehydrative cyclization proceeds to yield the corresponding oxazole.



Scheme I-13. Synthesis of oxazoles from oxazol-5(4H)-ones.

The electrophilic nature of oxazol-5(4H)-ones has also been utilized in natural product synthesis. In 2008, Dr. Christopher Hupp in the Tepe lab completed the first total synthesis of a marine alkaloid isolated from the marine tunicate *Dendrodoa grossularia*.[75] His synthesis featured a Claisen oxazole

rearrangement to first prepare a quaternary oxazol-5(4H)-one (Scheme I-14). Methanolysis with NaOMe was used to open the electrophilic oxazol-5(4H)-one ring, followed by subsequent rearrangement to furnish a hydantoin ring. Six steps were then required to complete the synthesis of the natural product.[75,76] The methodology developed by Dr. Hupp was also utilized to prepare analogs of the natural product which were later evaluated for their biological activity.[76,77]



Scheme I-14. Use of an oxazol-5(4H)-one in the synthesis of an indole alkaloid isolated from *Dendrodoa grossularia*.

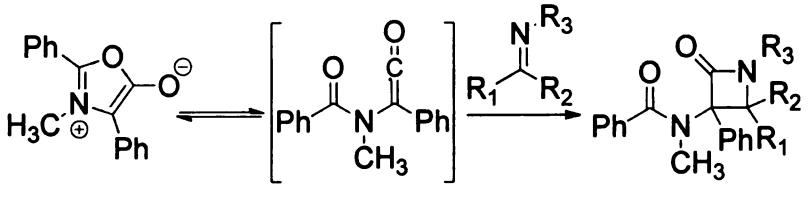
F. Cycloaddition reactions of oxazol-5(4H)-ones

Cycloaddition reactions of oxazol-5(4H)-ones are useful in the generation of an assortment of heterocycles.[31] The ability of oxazol-5(4H)-ones to undergo cycloaddition reactions arises from their reactive azomethine ylide and amidoketene tautomers (**C** and **D** respectively, Scheme I-3), which are known to react with dipolarophiles. Azomethine ylides are zwitterionic species comprising

a C-N-C fragment in which 4π electrons are delocalized across 3 p-orbitals. These unstable dipolar species are generally prepared *in situ* by various means, such as deprotonation of imine derivatives or thermolysis of aziridines and 4-oxazolines.[78-80] Ketenes are also reactive species which generally need to be generated *in situ*, often through treatment of an acid chloride with a tertiary amine base. Ketenes react readily with nucleophiles to generate carboxylic acid derivatives and are also known participants in polymerization reactions.[81,82]

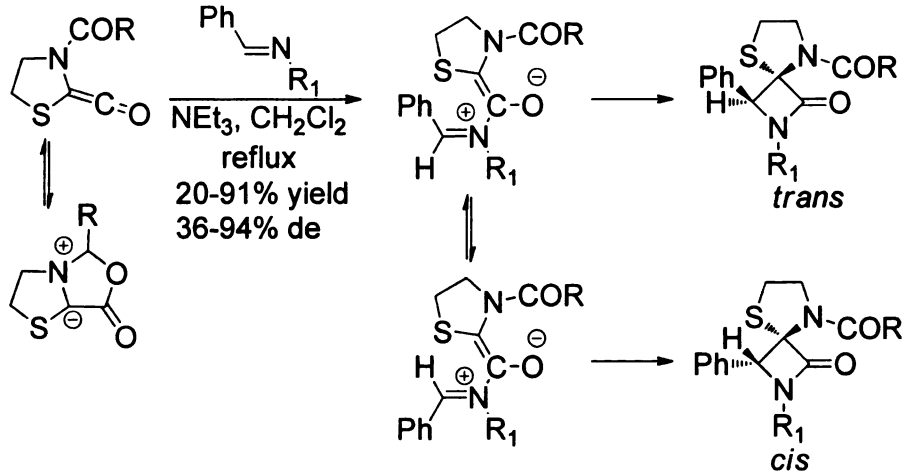
1. [2+2] cycloaddition reactions.

The cycloaddition reaction of ketenes with imines, known as the Staudinger reaction,[83] is a method used to prepare β-lactams, structural features common to several families of antibiotics.[84] Asymmetric variants of this reaction are known, and β-lactams produced in the reactions are valued synthetically for their utility as pharmacores.[85-87] Likewise, the β-lactam scaffold is accessible *via* the Staudinger reaction of imines with oxazol-5(4H)-ones, as was first demonstrated in 1971 by Huisgen and coworkers.[88] In their studies, the authors generated ketenes *in situ* by means of mesionic oxazol-5(4H)-one formation (Scheme I-15). The ketenes were treated with imines and thermal [2+2] cycloaddition reactions proceeded to afford β-lactams.



Scheme I-15. Proposed mechanism of β-lactam formation.

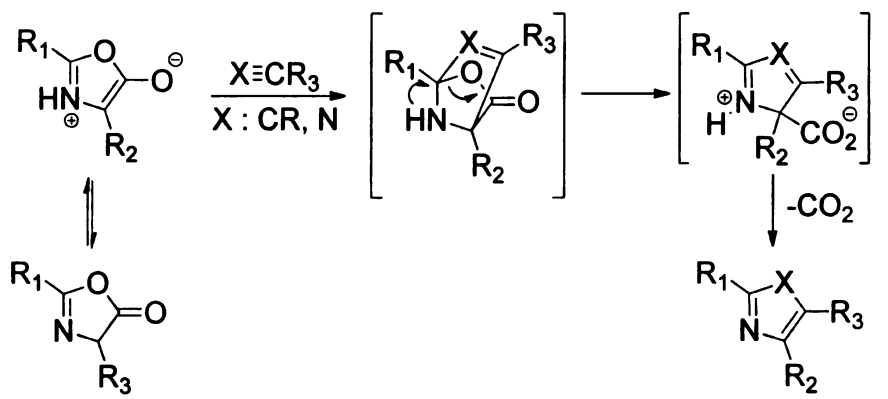
Diastereoselective β-lactam formation was later demonstrated by Cremonesi and coworkers, who treated bicyclic oxazol-5(4H)-ones with imines under basic conditions (Scheme I-16).[89] The authors illustrated good control of diastereoselectivity through manipulation of the imine N-substituent. The reaction provided mainly the *cis*-product (with respect to the sulfur and phenyl groups) when performed with imines containing electron withdrawing moieties. Conversely, high yields of *trans* β-lactams could be selectively formed when imines contained electron donating groups.[89] Both products are proposed to arise from initial attack of the imine to the least-hindered side of the ketene, as shown in Scheme I-16. Electron donating groups stabilize the initial iminium intermediate to afford the *trans* products after the [2+2] cycloaddition. However, the iminium intermediate is destabilized by electron withdrawing groups, allowing the intermediate to undergo double bond isomerization before ring formation occurs, thus giving rise to the thermodynamically favored *cis* products.[89]



Scheme I-16. β-lactam formation from bicyclic münchnones.

2. [3+2] cycloaddition reactions.

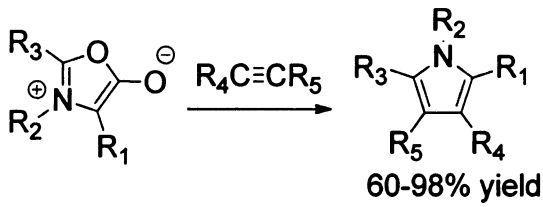
There are numerous literature examples wherein oxazol-5(4H)-ones participate in [3+2] cycloaddition reactions with dipolarophiles to give rise to 5-membered nitrogen heterocycles.[31,78,90] Both aromatic and nonaromatic ring systems are available *via* the reaction, and the product outcome is dependent upon the dipolarophile used as well as reaction conditions. It is presumed that equilibrium amounts of mesionic oxazol-5(4H)-ones react with dipolarophiles to initially form bicyclic intermediates (Scheme I-17).[91] Decarboxylation then proceeds and aromatization ensues to afford the heterocyclic products. In this way, pyrroles and imidazoles are prepared from alkynes and nitriles, respectively (Scheme I-17).



Scheme I-17. 1,3-dipolar cycloaddition reactions of oxazol-5(4H)-ones.

Pyrroles were first prepared *via* cycloadditions of oxazol-5(4H)-ones by Huisgen, Gotthardt, and Bayer in 1964.[92,93] The authors reportedly generated oxazol-5(4H)-ones *in situ* and treated them with alkynes at various

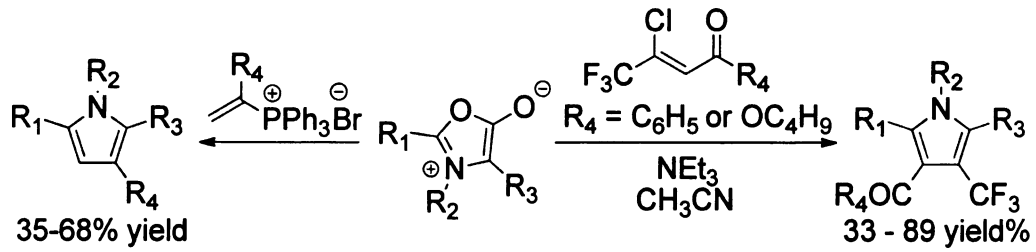
temperatures to afford pyrroles in good yields. Using a variety of oxazol-5(4H)-ones and alkynes, pyrroles were prepared with substituent diversity at every atom of the heterocyclic scaffold (Scheme I-18).[92-94] This procedure has recently been amended to include *in situ* N-alkylation through the addition of 2,6-di-tert-butylpyridine and a highly reactive alkylating reagent.[95] In this way, N-alkyl pyrroles are produced from nitrogen-unsubstituted N-acyl amino acids.



Scheme I-18. Pyrrole formation from oxazol-5(4H)-ones and alkynes.

Similarly, pyrroles have been synthesized from mesionic oxazol-5(4H)-ones *via* reaction with alkyne equivalents. One such synthesis has been reported using vinyl phosphonium salts as alkyne equivalents in cycloaddition reactions with münchnones.[96] The reaction conditions involving heat (refluxing THF/DMF solvent mixture) or sonication promote the cycloaddition, after which decarboxylation and PPh_3 elimination occur to afford the anticipated products regioselectively in moderate yields (Scheme I-19). The high regioselectivity in the reaction has been attributed to the strong electrostatic forces between the phosphonium and carbonyl groups.[96] The preparation of pyrroles has also been reported *via* the reaction of münchnones with vinyl-chlorinated alkenes (Scheme I-19).[97] This reaction was performed

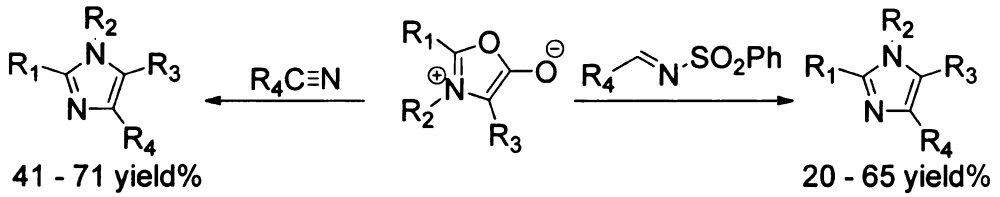
using α, β-unsaturated esters or ketones to regioselectively form the desired pyrrole products in good yields. Placement of the chlorine substitutent on the alkene is key to product formation, as its elimination after the decarboxylation event gives rise to the aromatic products.[97]



Scheme I-19. Pyrroles derived from oxazol-5(4H)-ones and alkyne equivalents.

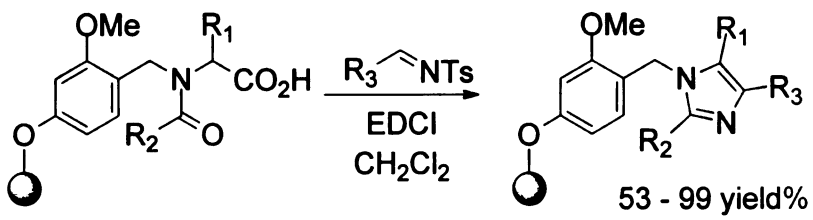
Oxazol-5(4H)-ones are also known to participate in [3+2] cycloaddition reactions with nitriles and nitrile equivalents to generate imidazoles. Shortly after reporting their pyrrole synthesis,[94] Huisgen and coworkers reported a similar decarboxylative cycloaddition reaction wherein imidazole formation was observed when münchnones were treated with electron-deficient nitriles (Scheme I-20).[98] The authors performed the reaction under a variety of conditions to afford imidazoles with electron withdrawing substituents in moderate yields. Consonni and coworkers prepared imidazoles through an analagous reaction utilizing nitrile equivalents.[99] The authors first generated oxazol-5(4H)-ones *in situ* and treated them with N-(phenylmethylene)benzenesulphonamides (Scheme I-20). The resulting bicyclic intermediates consequently decomposed, releasing CO_2 and benzenesulphinic acid, to form imidazole products. Pivotal to their approach

was the incorporation of the N-phenylsulphonyl moiety to act as a good leaving group, and its expulsion permitted aromatization to yield the imidazole products.



Scheme I-20. Imidazole syntheses from oxazol-5(4H)-ones.

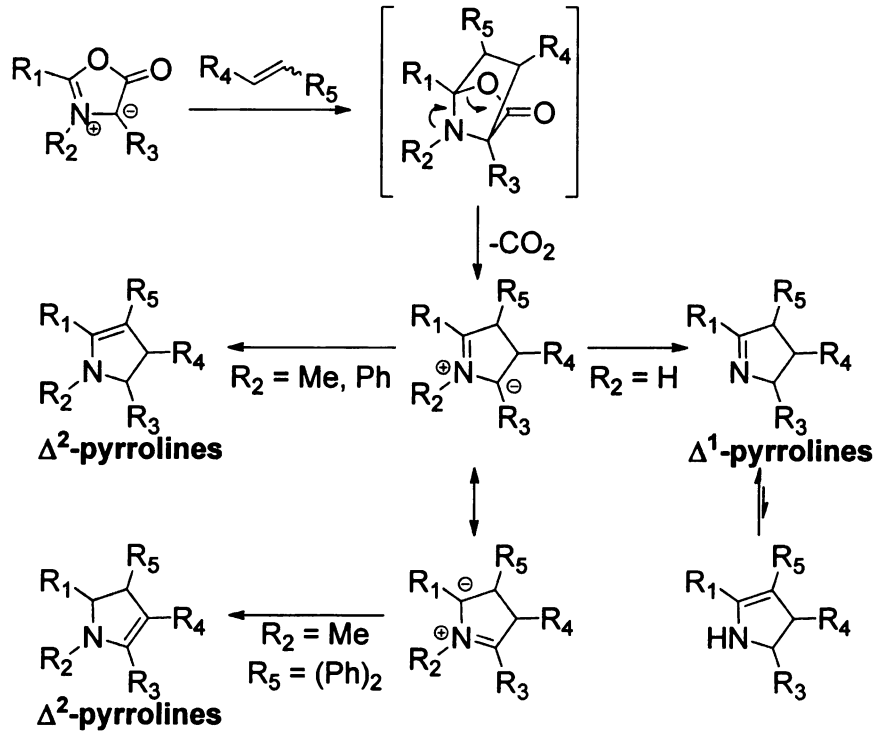
Formation of imidazole products by the aforementioned methods often occur in low yields due to competing reactions of oxazol-5(4H)-ones, such as self-condensation, a process resulting in dimerized products.[91] Bilodeau and coworkers have demonstrated repression of self-condensation reactions of oxazol-5(4H)-ones through the incorporation of solid-phase support.[100] Their studies involved the *in situ* formation of münchnones through dehydration of resin-bound amino acids followed by 1,3-dipolar cycloaddition reactions with N-tosyl imines to give polymer-bound imidazoles (Scheme I-21). Liberation from the resin by heating in acetic acid at 100 °C for 2 hours then afforded the desired imidazoles in high yields.



Scheme I-21. Solid-support synthesis of imidazoles.

Preparations of nonaromatic pyrrolines *via* cycloaddition reactions involving oxazol-5(4H)-ones have also been reported. Gotthardt, Huisgen, and Schaefer investigated the formation of both Δ^1-pyrrolines and Δ^2-pyrrolines from the reaction of mesionic oxazol-5(4H)-ones with electron deficient olefins (Scheme I-22).[101-103] For the construction of Δ^1-pyrrolines, the authors utilized N-unsubstituted oxazol-5(4H)-ones and gently heated them in xylenes in the presence of alkenes (Scheme I-22).[101,103] The intermediate bicyclic adducts formed from the 1,3-dipolar cycloaddition then underwent successive decarboxylation and protonation to afford Δ^1-pyrrolines. Δ^1-pyrrolines contain an imine which is susceptible to imine-enamine tautomerization, resulting in observed inversion of stereochemistry of the R_5 substituent. (Scheme I-22).[101,103] Exploitation of the equilibrium which exists between the two tautomers enabled the authors to also explore the formation of Δ^2-pyrrolines.[102] The use of N-substituted oxazol-5(4H)-ones in similar cylcoaddions reaction with alkenes thus gave rise to Δ^2-pyrrolines *via* zwitterionic intermediates formed after decarboxylation (Scheme I-22). The zwitterionic intermediates are proposed to undergo prototropic isomerization to yield the more energetically favorable enamine tautomer.[102] Such zwitterionic intermediates are trapped as byclic adducts when an additional alkene equivalent is added to the reaction mixture *via* a subsequent [3+2] cycloaddition.[102] The authors also demonstrated olefin regiocontrol through quaternization of one of the pyrroline carbons.[102] For example, use of 1,1-diphenylethylene as a dipolarophile in the reaction generates a zwitterionic

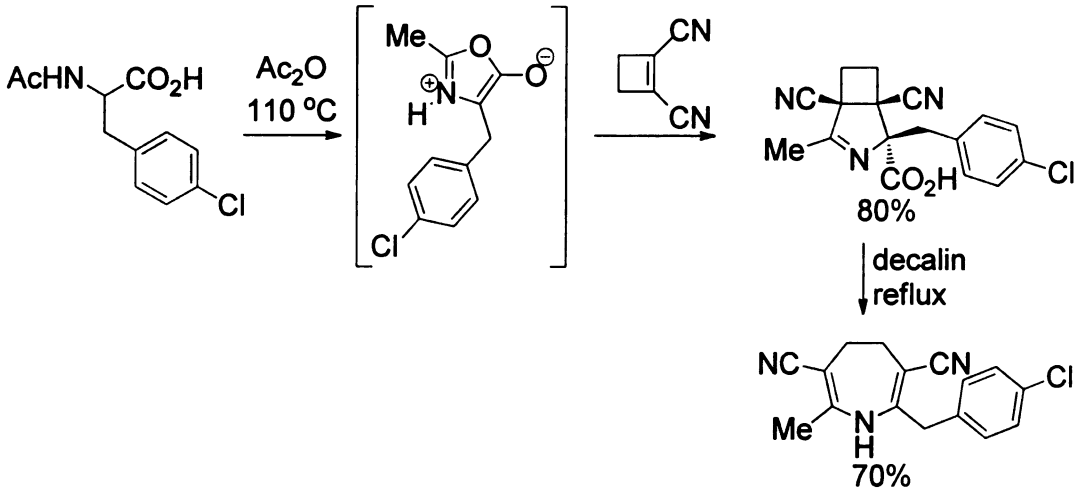
intermediate that can only undergo the aforementioned isomerization *via* one resonance contributer in order for product formation to occur (Scheme I-22).



Scheme I-22. Synthesis of Δ^1- and Δ^2-pyrrolines from oxazol-5(4H)-ones.

Diastereoselective preparations of Δ^1-pyrrolines from oxazol-5(4H)-ones have since been reported.[104-106] In 1989, Maryanoff and co-workers demonstrated the *exo*-selective formation of a Δ^1-pyrroline cycloadduct from the exposure of an *in situ*-generated mesionic oxazol-5(4H)-one to an electron deficient alkene (Scheme I-23).[104] The reaction, which was conducted in refluxing acetic anhydride, selectively afforded the Δ^1-pyrroline product in 80% yield along with some dihydropyrazine (18%). The dihydopyrazine is derived from the Δ^1-pyrroline *via* successive decarboxylation and ring expansion, and

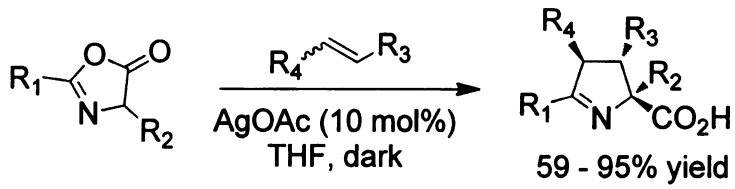
heating the Δ^1-pyrroline in refluxing decalin affords the 7-membered heterocycle in 70% yield.[104,107]



Scheme I-23. *Exo* selective synthesis of Δ^1-pyrrolines from oxazol-5(4)-ones.

A more recent example of Δ^1-pyrroline synthesis *via* an *exo*-selective cycloaddition of oxazol-5(4H)-ones with alkenes was demonstrated by Dr. Mahesh Peddibhotla in the Tepe group (Scheme I-24).[106] The reaction involved generation of mesionic oxazol-5(4H)-ones through exposure to Lewis acids. In contrast to analogous cycloadditions which occurred under harsh reaction conditions, the Lewis acid-mediated cycloadditions were carried out at room temperature. Thus, treatment of oxazol-5(4H)-ones with 10 mol % silver acetate and electron-deficient alkenes gave rise to Δ^1-pyrrolines in good yields. The *exo*-selective reaction provided pyrrolines with R_2 and R_3 substituents *syn* to each other. Moreover, the relationship between the R_3 and R_4 substituents was *trans* in the collected products, regardless of the

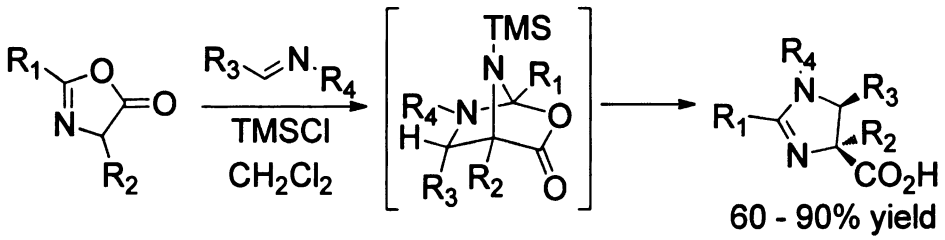
starting alkene geometry. This *trans* relationship is proposed to arise from isomerization *via* imine-enamine tautomerization to give the more thermodynamically stable product.[106] Toste and co-workers have since reported an analogous reaction which utilizes their chiral gold catalyst (S)-Cy-SEGPHOS(AuOBz)$_2$ to furnish Δ^1-pyrrolines enantioselectively from oxazol-5(4H)-ones.[108]



Scheme I-24. Diasteroselective synthesis of Δ^1-pyrrolines.

Lewis acid-mediated cylcoadditions of oxazol-5(4H)-ones have also been utilized for the preparation of 2-imidazolines.[109-111] Dr. Mahesh Peddibhotla of the Tepe lab demonstrated that treatment of oxazol-5(4H)-ones with imines and TMS-Cl in refluxing dichloromethane diastereoselectively afforded 2-imidazolines containing four point diversity (Scheme I-25).[109] Various oxazol-5(4H)-ones and imines are tolerated in the reaction, giving rise to 2-imidazolines containing alkyl, acyl, aryl, and heterocyclic substituents.[110] The major products in this reaction were predominantly *trans* (with respect to R_2 and R_3) when R_1 was aryl, and it was steric interactions which were postulated to direct the stereochemical outcome of the reaction[110]. The reaction proceeds through a cycloaddition wherein the *endo* cycloadduct is formed; it is proposed that steric interaction of the bulky silyl group with the

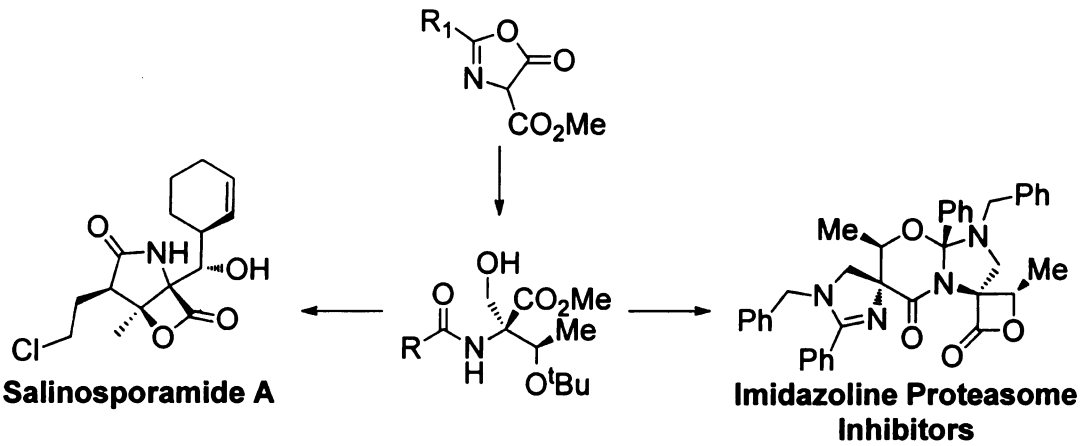
imine R_3 substituent disfavors the *exo* approach.[110] Additional studies by Dr. Vasudah Sharma of the Tepe group later demonstrated complete reversal or degradation of diastereoselectivity in the reaction through variation of substituents about the oxazol-5(4H)-one ring.[111]



Scheme I-25. Diastereoselective synthesis of 2-imidazolines *via* a cycloaddition reaction of oxazol-5(4H)-ones.

G. Current studies.

The following chapters of this dissertation describe reactions which take advantage of the nucleophilic and electrophilic properties of oxazol-5(4H)-ones for the preparation of nonproteinogenic amino acids (Scheme I-26). Such amino acids prepared in these studies have proven to be valuable as building blocks towards the construction of the natural product proteasome inhibitor salinosporamide A.[112] Furthermore, non-natural amino acids prepared from oxazol-5(4H)-ones in the Tepe lab have been utilized to synthesize heterocycles of interest, most notably 2-imidazolines, for use in our proteasome inhibition studies.



Scheme I-26. Nonproteinogenic amino acids available from oxazol-5(4H)-ones for use towards the construction of proteasome inhibitors.

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CHAPTER II

DIASTEREOSELECTIVE SYNTHESIS OF *TERT*-ALKYL AMINO HYDROXY CARBOXYLIC ESTERS *VIA* AN INTERMOLECULAR ALKYLATION REACTION OF OXAZOL-5(4H)-ONES AND ENOL ETHERS

A. Introduction to quaternary α-amino acids.

The diversity of applications for which non-proteinogenic amino acids are used make them valuable substrates within the fields of synthetic organic, bioorganic and medicinal chemistry.[1,2] One such group of amino acids that has received considerable attention from the scientific community as of late are the quaternary α-amino acids (or α,α-disubstituted α-amino acids) (Figure II-1).[3-10] The presence of the additional alkyl substituent at the α-carbon of quaternary amino acids generally results in changes in biophysical properties as compared to the naturally occurring nonquaternary α-amino acid counterparts. When incorporated into peptides, for example, the additional substituent often helps to sterically constrain the free rotation of the residue's side chain leading to unique folding.[1,8,10] Peptides containing quaternary α-amino acids also generally exhibit increased hydrophobicity, as well as an increased stability towards both chemical[11,12] and metabolic[13,14] decomposition. These unique physical properties make them intriguing tools for the design and study of peptides and proteins. Additionally, naturally-occurring quaternary α-amino acids and derivatives are found within the structures of many heterocyclic molecules that exhibit interesting biological activities, making them attractive synthetic targets.[7,15]

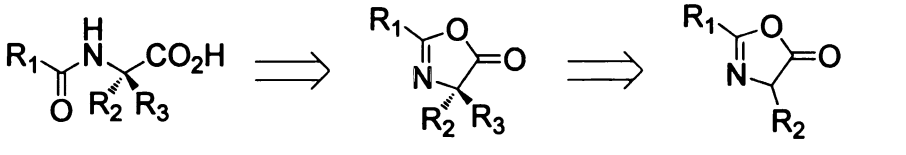


α-amino acid quaternary α-amino acid

Figure II-1. General structures of both α-amino and quaternary α-amino acids.

B. Syntheses of quaternary α-amino acids using oxazol-5(4H)-ones.

The importance of quaternary α-amino acids in synthetic and biological studies has prompted interest in the development of new and efficient methods for their construction.[3-7,9,15] Classical methods for the synthesis of quaternary α-amino acids include the alkylation of α-amino esters protected as imines[16] and the Strecker reaction[17] of ketimines. An alternative approach is to prepare quaternary α-amino acids from the oxazol-5(4H)-one scaffold.[18-21] Quaternary substituted α-amino acid derivatives can be directly accessed from the nucleophilic ring opening of quaternary substituted oxazol-5(4H)-ones, substrates which have been generated using a variety of methods (Scheme II-1).[19]



Quaternary α-Amino Acid Derivative

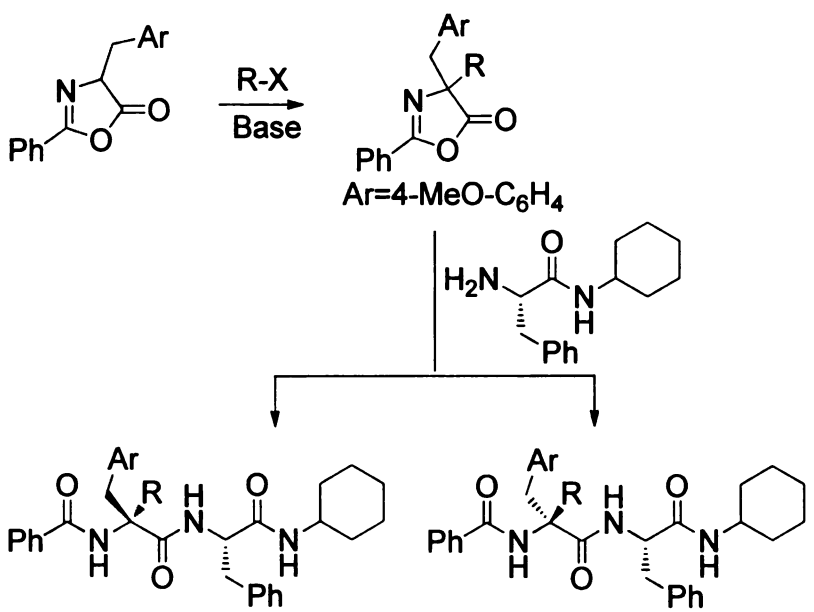
Quaternary Oxazol-5(4H)-one

Oxazol-5(4H)-one

Scheme II-1. Synthesis of quaternary α-amino acids from oxazol-5(4H)-ones.

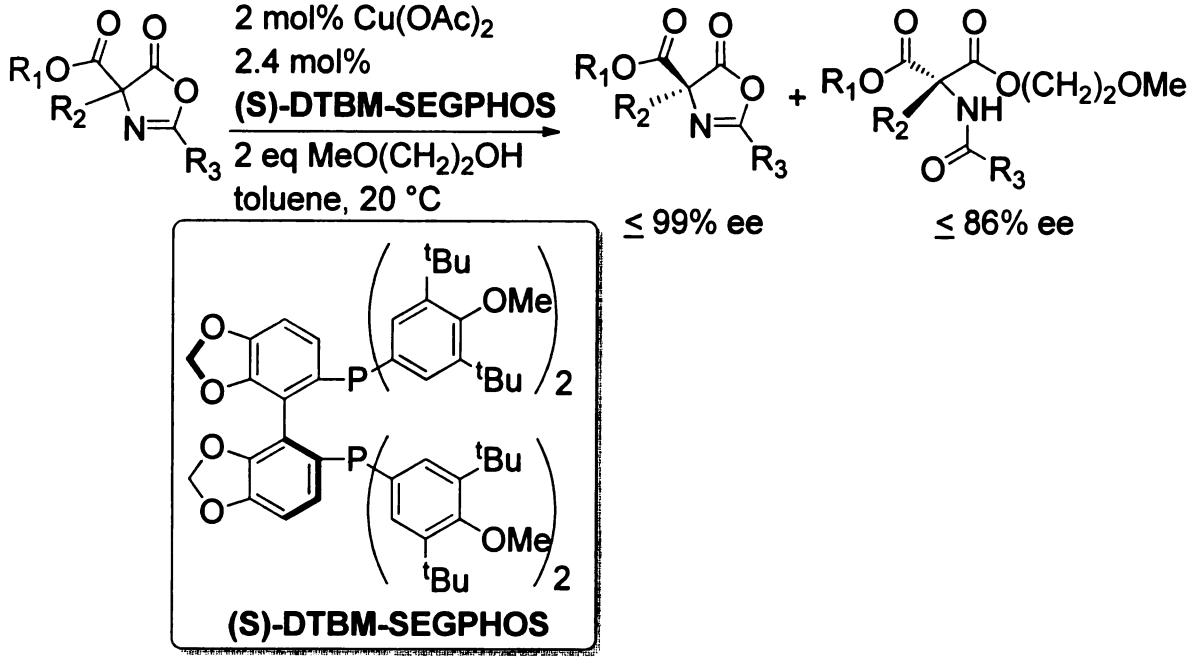
Oxazol-5(4H)-one alkylation has been used strategically in the synthesis of enantiomerically pure quaternary α-amino acid derivatives. Various methods used for the generation of quaternary oxazol-5(4H)-ones were described in

Chapter I, including alkylation reactions. Traditional alkylation reactions, like those reported by Steglich, resulted in the formation of racemic quaternary oxazol-5(4H)-ones. One method for the resolution of these racemic mixtures involves nucleophilic ring opening of the oxazol-5(4H)-one scaffold by chiral nucleophiles, thus producing separable diastereomeric mixtures (Scheme II-2).[22-26] This technique has been successfully applied by Obrecht and co-workers for the preparation of enantiomerically pure quaternary tyrosine derivatives.[25] Their strategy involved nucleophilic ring opening of racemic quaternary oxazol-5(4H)-ones with chiral amino acid derivatives followed by separation of the resultant diastereomers *via* silica gel chromatography (Scheme II-2). Selective cleavage of the newly formed amide bond then afforded enantiomerically pure N-benzoyl protected quaternary α-amino acids and/or esters.[26]



Scheme II-2. Resolution of racemic quaternary α-amino acid derivatives.

The kinetic resolution of quaternary oxazol-5(4H)-ones is another means for the preparation of chiral quaternary α-amino acid derivatives. Extensive studies have demonstrated that nonquaternary oxazol-5(4H)-ones readily undergo both small molecule- and enzyme-catalyzed dynamic kinetic resolutions to afford high yields of enantiomerically pure α-amino acids and esters.[27-32] Recently, Tsuji and co-workers demonstrated the first transition-metal catalyzed kinetic resolution of quaternary oxazol-5(4H)-ones (Scheme II-3).[33,34] Exposure of quaternary oxazol-5(4H)-ones to a catalytic amount of copper in combination with the chiral phosphine ligand **(S)-DTBM-SEGPHOS** activates quaternary oxazol-5(4H)-ones towards methanolysis. Coordination of the oxazol-5(4H)-one to the copper-ligand complex generates a chiral environment wherein nucleophilic attack can occur at the reactive carbonyl carbon for only one of the enantiomers. Thus, one oxazol-5(4H)-one enantiomer undergoes nucleophilic ring opening to yield the corresponding quaternary amino ester derivative, whereas the opposing enantiomer remains unreacted.



Scheme II-3. Kinetic resolution of racemic quaternary oxazol-5(4H)-ones.

C. Introduction to *tert*-alkyl hydroxy carboxylic acids.

Representative of quaternary α-amino acid derivatives are *tert*-alkyl amino hydroxy carboxylic acids, densely functionalized quaternary carbon centers surrounded by a combination of alkyl, amino, carboxylic, and hydroxyl substituents.[7,15] These structural motifs are present in numerous microorganism metabolites including the sphingofungins, lactacystins, altemicidines, oxazolomycins and many others (Figure II-2).[7,15] The diverse and potent biological activity of these metabolites has stimulated many researchers to pursue their total syntheses[15,35-39] and in-depth biological evaluations.[40-45]

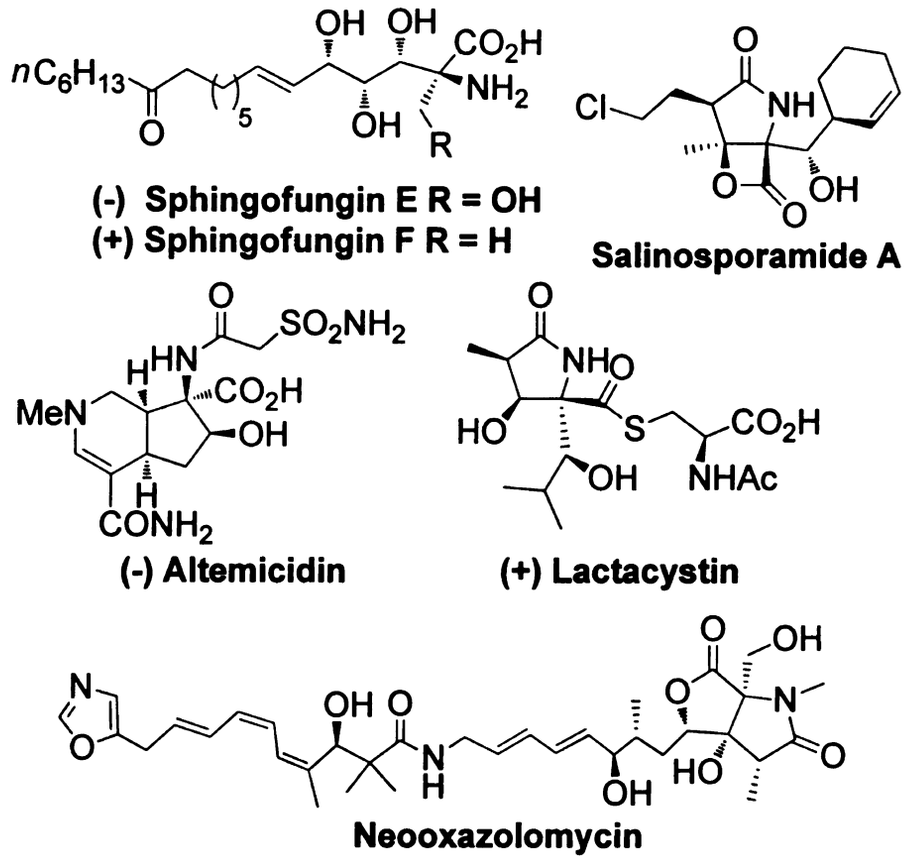


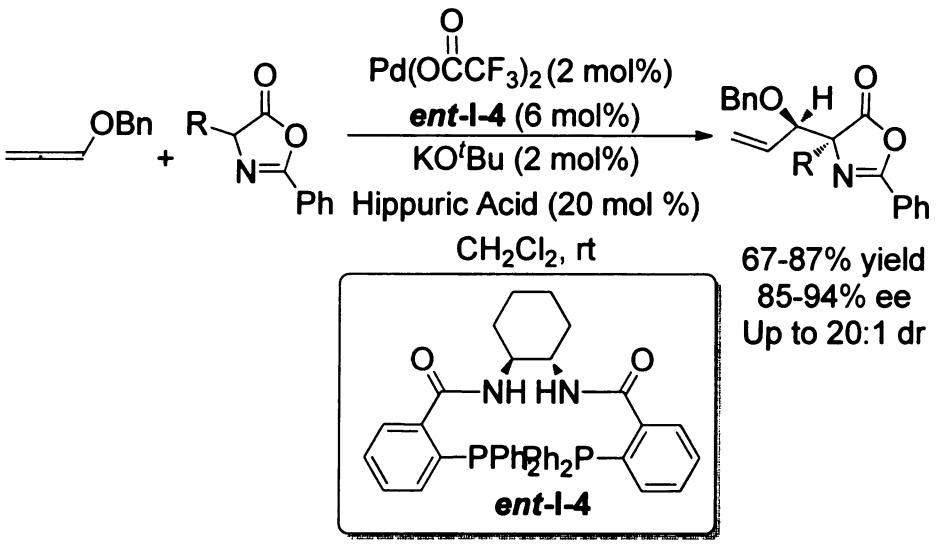
Figure II-2. Naturally occurring *tert*-alkyl amino hydroxy carboxylic acids.

D. Synthesis of *tert*-alkyl hydroxy carboxylic acids from oxazol-5(4H)-ones.

Construction of the *tert*-alkyl amino hydroxy carboxylic acid moiety found within the aforementioned biological targets of interest has proven to be a significant synthetic challenge and has been the focus of many research groups.[7,15] Approaches targeted towards the synthesis of the quaternary center in these molecules have differed greatly due to the complexity of the target molecules and a lack of general approaches available for the preparation of the core scaffold. Some of these approaches will be discussed in Chapter III as methods for the synthesis of such a quaternary center present in the marine metabolite salinosporamide A.

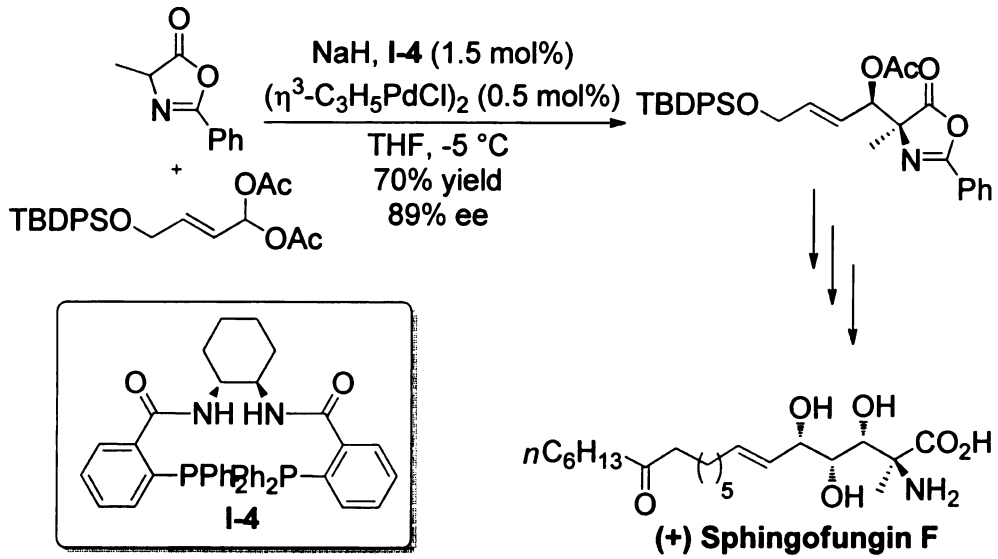
Quaternary oxazol-5(4H)-ones have been prepared using a variety of methods in which functionalities have been installed to make them suitable precursors to *tert*-alkyl amino hydroxy carboxylic acids.[19] Several of these syntheses were described in Chapter I, including those in which the Steglich rearrangement was used for the preparation of quaternary oxazol-5(4H)-ones bearing C4 esters. Such quaternary oxazol-5(4H)-ones are susceptible to hydride reduction, which results in ring decomposition to reveal amide and alcohol functionalities. In this way, appropriately substituted quaternary oxazol-5(4H)-ones are transformed into the corresponding *tert*-alkyl amino hydroxy carboxylic acids.[46]

Quaternary oxazol-5(4H)-ones suitable for the construction of *tert*-alkyl amino hydroxy carboxylic acids have also been generated through the installation of allylic substituents. The Trost group has reported various conditions used for this type of transformation. One such report describes the regioselective and enantioselective preparation of quaternary oxazol-5(4H)-ones under conditions of palladium catalysis using chiral diamine ligand ***ent*-I-4** (Scheme II-4).[47] The authors treated oxazol-5(4H)-ones with electron rich alkoxy-substituted allenes to promote selective nucleophilic addition to the substituted allene terminus, resulting in the culmination of quaternary products in good yields and selectivity.[48]



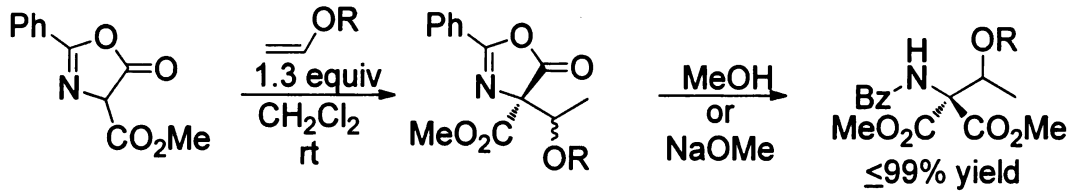
Scheme II-4. Palladium-catalyzed alkylation of oxazol-5(4H)-ones with allenes.

In another report by the Trost group, an allylation reaction of oxazol-5(4H)-ones was utilized to complete the total synthesis of the fungal metabolite sphingofungin F.[49,50] Using chemistry developed in their labs,[51] the authors treated 2-phenyl-4-methyl-5(4H)-oxazolone with a gem-diacetate-substituted alkene under conditions of palladium catalysis with chiral diamine ligand **I-4** (Scheme II-5). The resultant quaternary oxazol-5(4H)-one then underwent methanolysis to afford a quaternary α-amino acid derivative. The alkylation reaction was reported to give the quaternary oxazol-5(4H)-one in 70% yield with 89% ee. Several synthetic manipulations were then required to prepare the natural product, which was formed in just 15 linear steps with an impressive 17% overall yield.[49,50]



Scheme II-5. Trost's synthesis of sphingofungin F.

Recently, racemic quaternary oxazol-5(4H)-ones have been prepared *via* an intermolecular ene-type alkylation reaction of oxazol-5(4H)ones and enol ethers (Scheme II-6).[52] Developed by Dr. Jason S. Fisk in the Tepe lab, this reaction takes advantage of the strong enol character of the oxazol-5(4H)-one ring. Oxazol-5(4H)-ones containing substituents that help stabilize the substrate's enol tautomer appear to best facilitate the reaction. For instance, oxazol-5(4H)-ones bearing acyl groups in the C4 position quantitatively provide products in minutes at room temperature. However, the use of oxazol-5(4H)-ones with C4 aryl substituents require higher temperatures for product formation. Moreover, C4 alkyl-substituted oxazol-5(4H)-ones do not yield any alkylation products under the reported reaction conditions. Methanolysis of the quaternary oxazol-5(4H)-ones then affords quaternary malonate derivatives in excellent yields.

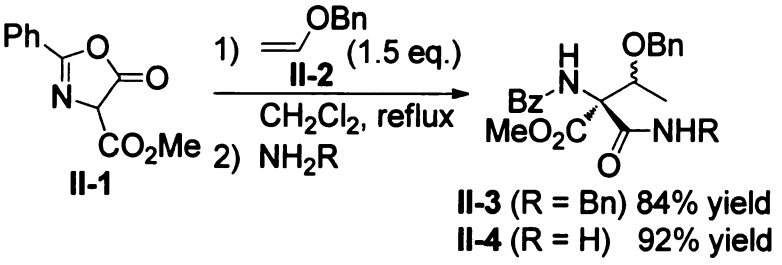


Scheme II-6. Intermolecular alkylation reaction of oxazol-5(4H)-ones with enol ethers and subsequent methanolysis.

E. Syntheses of quaternary α-amino acid derivatives *via* nucleophilic ring-opening reactions of quaternary oxazol-5(4H)-ones.

The oxazol-5(4H)-one ene-type alkylation reaction discovered by Dr. Fisk enabled access to numerous quaternary amino acid derivatives which we planned to use in the synthesis of natural products. As discussed in Chapter I, oxazol-5(4H)-ones react with a variety of nucleophiles, making them appropriately suited for the synthesis of α-amino acid derivatives. In an effort to desymmetrize the malonate products and effectively apply our alkylation chemistry towards molecular targets of interest, we decided to exploit the electrophilic nature of oxazol-5(4H)-ones.

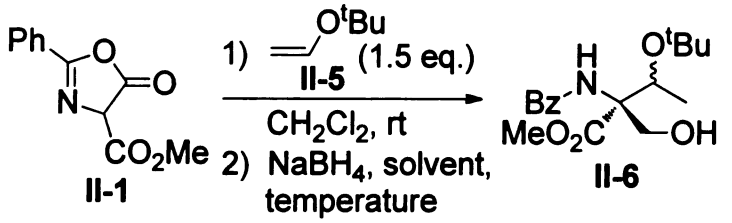
Initially, we investigated the formation of diamide products from ring-opening reactions of quaternary oxazol-5(4H)-ones derived from our alkylation reaction. We anticipated such products to be available from the addition of amines to the same reaction flask containing quaternary oxazol-5(4H)-ones once the alkylation reactions were complete, as could be monitored by TLC (Scheme II-7). Indeed, treatment of 2-phenyl-4-carbmethoxy-5(4H)-oxazolone **II-1** with benzyl vinyl ether **II-2** in refluxing CH_2Cl_2, followed by addition of either benzylamine or ammonia to the reaction pot afford diamides **II-3** and **II-4** in 84% and 92% yields respectively.



Scheme II-7. Synthesis of diamides from oxazol-5(4H)-ones.

We were also interested in utilizing our alkylation chemistry in the synthesis of *tert*-alkyl amino hydroxy carboxylic esters, which we anticipated could be used to construct natural products and natural product analogs (Figure II-2). We predicted that such compounds should be accessible *via* hydride reduction[46,52-55] of quaternary oxazol-5(4H)-ones obtained from our alkylation reaction. To our delight, sodium borohydride reduction of the quaternary adduct prepared from the treatment of **II-1** with *tert*-butyl vinyl ether **II-5** proceeded smoothly to give quaternary amino acid derivative **II-6**. Moreover, the mixture of diastereomers generated in reaction were found to be separable *via* silica gel chromatography (Table II-1). Optimal results were obtained in the reduction when a solvent mixture composed of THF and water (2:1) was used (Table II-1, condition 3). An organic solvent such as THF was required for solubility of the oxazol-5(4H)-ones, and water as a co-solvent was found to increase the reaction rate and yield, presumably by increasing the solubility of sodium borohydride (Table II-1, conditions 1-3). Carboxylic acid products arising from hydrolysis of quaternary oxazol-5(4H)-ones were observed in the reaction when water was

used as a co-solvent. However, these undesired products were minimized by cooling the reaction to 0 °C.



Entry	Solvent	Temperature (°C)	% Yield
1	THF	25	65
2	H_2O	25	Low
3	THF:H_2O (2:1)	0	91

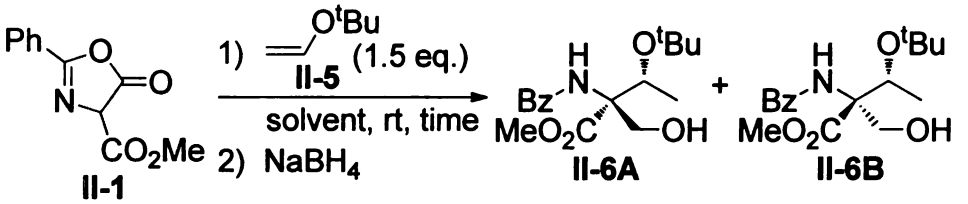
Table II-1. Optimization of $NaBH_4$ reduction of quaternary oxazol-5(4H)-ones.

F. Optimization of reaction diastereoselectivity.

The aforementioned alkylation reaction developed in our lab was used to generate quaternary oxazol-5(4H)-ones, albeit with little or no product diastereoselectivity.[52] In an effort to make this synthetic approach more broadly applicable, we investigated several reaction conditions in an attempt to improve the stereoselectivity of the reaction.[56]

We initiated our optimization studies by first screening a variety of solvents in the alkylation reaction of oxazol-5(4H)-one **II-1** and *tert*-butyl vinyl ether **II-5**. The majority of solvents used in the alkylation reaction provided little if any diastereoselectivity, as was determined *via* ^{1}H-NMR analysis of the reaction mixtures after $NaBH_4$ reduction. Reaction rates increased when polar solvents were used, and products were completely formed in CH_2Cl_2 in one hour (Table II-

2, entry 2). However, reaction times increased when solvents bearing lone pairs were used, which can be attributed to hydrogen bond basicity (Table II-2, entries 1 and 3).[57,58] Finally, the use of a nonpolar solvent such as benzene greatly decreased the reaction rate, yet an increase in stereoselectivity (d.r. = 67:33) was observed (Table II-2, entry 4). It is noteworthy that oxazol-5(4H)-one **II-1** appears to be only sparingly soluble in benzene, and it is proposed that poor solubility contributes to the increase in reaction time when using benzene as a solvent.

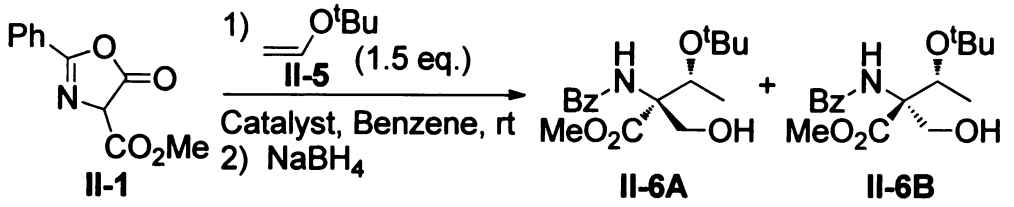


Entry	Solvent	Time (h)	6A : 6B	% Yield
1	CH_3CN	3	50 : 50	90
2	CH_2Cl_2	1	55 : 45	91
3	THF	19	52 : 48	90
4	Benzene	36	67 : 33	90

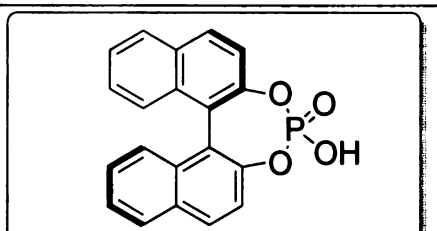
Table II-2. Solvent screening to optimize selectivity in the alkylation reaction.

In an effort to increase the rate of the reaction while maintaining selectivity, the use of protic catalysis was next investigated. Through screening a variety of acids, we found that a substoichiometric amount of diphenyl phosphate improved both the reaction rate and diastereoselectivity (Table II-3, entry 3). Brønsted acids less acidic than diphenyl phosphate also improved the

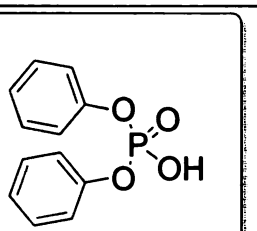
diastereoselectivity of the reaction, but did little for increasing the reaction rate (Table II-3, entry 1). The use of more acidic Brønsted acids resulted in lower yields of the desired product, presumably due to enol ether decomposition (Table II-3, entries 4 and 5). Enol ether decomposition was notably problematic when TFA was used in the reaction, as **II-1** was not fully consumed even after 48 hours, as observed by TLC (Table II-3, entry 5). Chiral Brønsted acid (R)-(-)-1,1'-binaphthyl-2,2'-diyl hydrogenphosphate provided the highest diastereoselectivity (79:21) of the catalysts investigated, but provided little observable enantioselectivity (2% ee) as determined by chiral HPLC (Table II-3, entry 3).[59] Several other phosphoric acids prepared by Dr. Fisk were screened in the reaction,[59] and they were found to provide diastereomeric ratios similar to those obtained through the use of commercially available diphenyl phosphate. Additionally, several Lewis acids were screened resulting in little or no product formation and low diastereoselectivity (Table II-3, entries 6-10). The significant rate enhancement found when using Brønsted acids and not other Lewis acids is suggestive of the catalyst protonating the enol ether to form an oxonium ion.[60,61] The relative stereochemistry of the major diastereomer **II-6A** obtained using conditions in Tables II-2 and II-3 has been established unambiguously *via* X-ray crystal structure (Figure II-3).



Entry	Catalyst	Time (hr)	A:B	% Yield
1	3,5-Dinitrobenzoic acid	16	70 : 30	90
2	Diphenyl phosphate	3	75 : 25	90
3	(R)-(-)-1,1'-binaphthyl-2,2' -diyl hydrogenphosphate	13	79 : 21	90
4	CSA	1	74 : 26	83
5	TFA	48	67 : 33	81
6	$Ti(O^iPr)_4$	48	67 : 33	61
7	$Yb(OTf)_3$	48	-- : --	0
8	TMSOTf	48	-- : --	0
9	$Zn(OTf)_2$	48	52 : 48	24
10	$Cu(OTf)_2$	48	-- : --	0



(R)-(-)-1,1'-binaphthyl-2,2' -diyl hydrogenphosphate



Diphenyl Phosphate

Table II-3. Effect of Brønsted and Lewis acids on reaction diastereoselectivity.

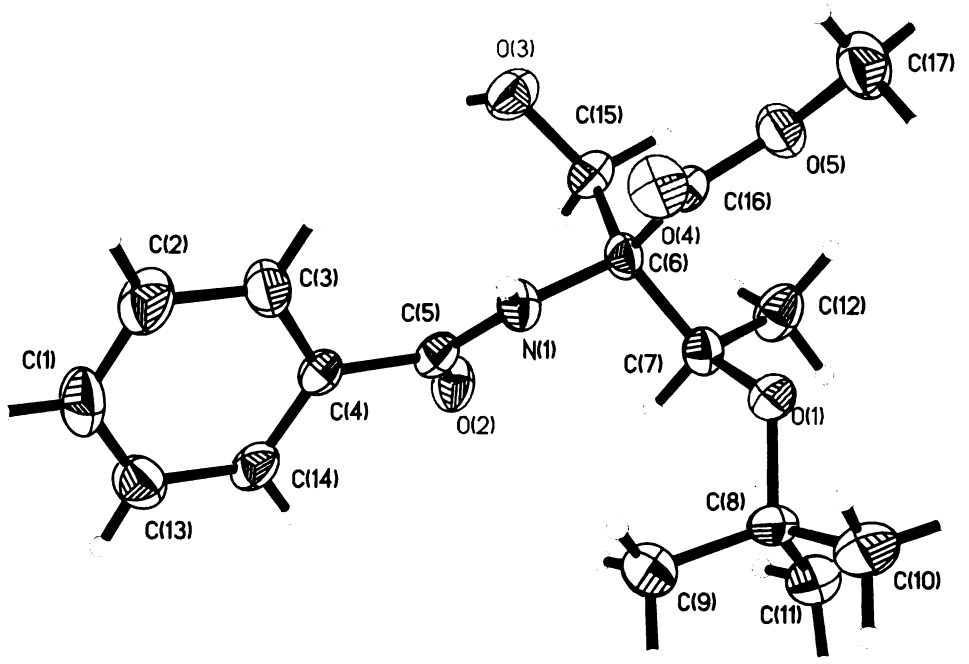
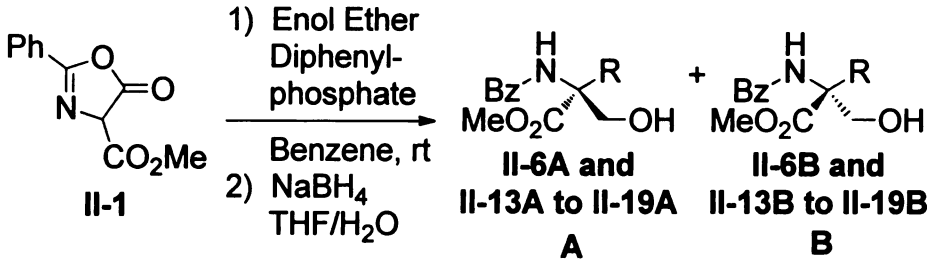


Figure II-3. X-Ray crystal structure representation of **II-6A**.

G. Scope of alkylation/reduction reaction of oxazol-5(4H)-ones.

Utilizing conditions optimized for diastereoselectivity, the scope of the reaction was further explored. Exposure of 2-phenyl-4-carbmethoxy-5(4H)-oxazolone **II-1** to various vinyl enol ethers in the presence of 10 mol % of diphenyl phosphate resulted in high yields of the desired products after sodium borohydride reduction (Table II-4). The *tert*-butyl and benzyl protecting groups yielded better diastereoselectivity than the ethyl protecting group (Table II-4, entries 1-3), indicating that enol ethers containing more sterically demanding oxygen protecting groups generally produce better diastereoselectivities. The less reactive higher substituted enol ethers gave lower yields and required the use of

heat (Table II-4, entries 4-6) to produce the desired products in good yields. Both butoxyethyne **II-11** and 2-methoxypropene **II-12** (Table II-4, entries 7 and 8, respectively) also provided reasonable yields of products when the reactions were performed in dichloromethane. Dichloromethane was used as the solvent for the formation of **II-18** and **II-19** to increase the rates of the reactions, being that the products contained only one chiral center apiece. Furthermore, reduction of the intermediate derived from the alkylation reaction of **II-11** needed to be performed in a 2:1 ratio of THF and EtOH at -41 °C. Hydrolysis or esterification products were predominantly observed when water or ethanol, respectively, were used as a cosolvent with THF at 0 °C. However, reducing the temperature for the reduction reaction from 0 °C to -41 °C resulted in an increase in product yield (67% yield at -41 °C versus 20% yield at 0 °C).



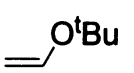
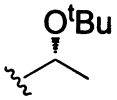
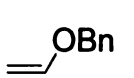
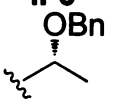
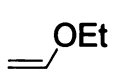
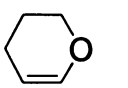
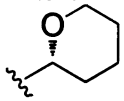
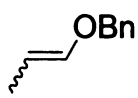
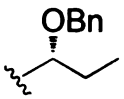
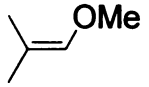
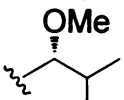
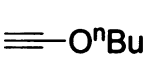
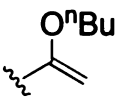
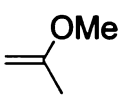
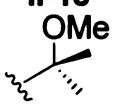
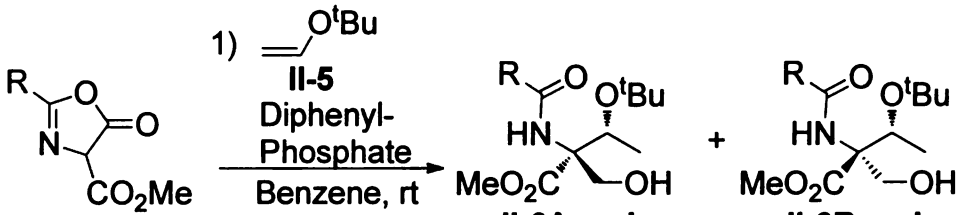
Entry	Substrate	R	Temp (°C)	A:B	% Yield
1	 **II-5**	 **II-6**	25	75 : 25	90
2	 **II-2**	 **II-13**	25	75 : 25	77
3	 **II-7**	 **II-14**	25	67 : 33	85
4	 **II-8**	 **II-15**	50	38 : 62	57
5	 (5:1 trans to cis) **II-9**	 **II-16**	50	69 : 31	48
6	 **II-10**	 **II-17**	50	60 : 40	33
7	 **II-11**	 **II-18**	25	-- : --	67
8	 **II-12**	 **II-19**	25	-- : --	62

Table II-4. Alkylation/reduction reaction performed with various enol ethers.

The role of the 2-position of the oxazol-5(4H)-one scaffold was also investigated for its effect on reactivity and selectivity (Table II-5). Several oxazol-5(4H)-ones with different substituents in the 2-position were prepared and were reacted with *tert*-butyl vinyl ether **II-5** in the presence of diphenyl phosphate. Oxazol-5(4H)-ones with aryl substituents gave high yields and erosion of diastereoselectivity was observed as electron deficiency increased (Table II-5, entries 1-3). Selectivity was all but lost when oxazol-5(4H)-ones containing alkyl substituents were used (Table II-5, entries 4 and 5). The relative stereochemistry of the diastereomer **II-26B** from the reaction of oxazol-5(4H)-one **II-22** with **II-5** was ascertained by X-ray analysis (Figure II-4).



II-1 and II-20 to II-23 | **II-6A and II-24A to II-27A** (A) | **II-6B and II-24B to II-27B** (B)

Entry	R	A:B	% Yield
1	Ph **II-1**	75 : 25 **II-6A : II-6B**	90
2	4-MeO-Ph **II-20**	74 : 26 **II-24A : II-24B**	88
3	4-CF_3-Ph **II-21**	67 : 33 **II-25A : II-25B**	81
4	Et **II-22**	43 : 57 **II-26A : II-26B**	50
5	Bn **II-23**	52 : 48 **II-27A : II-27B**	58

Table II-5. Alkylation/reduction reaction with various oxazol-5(4H)-ones.

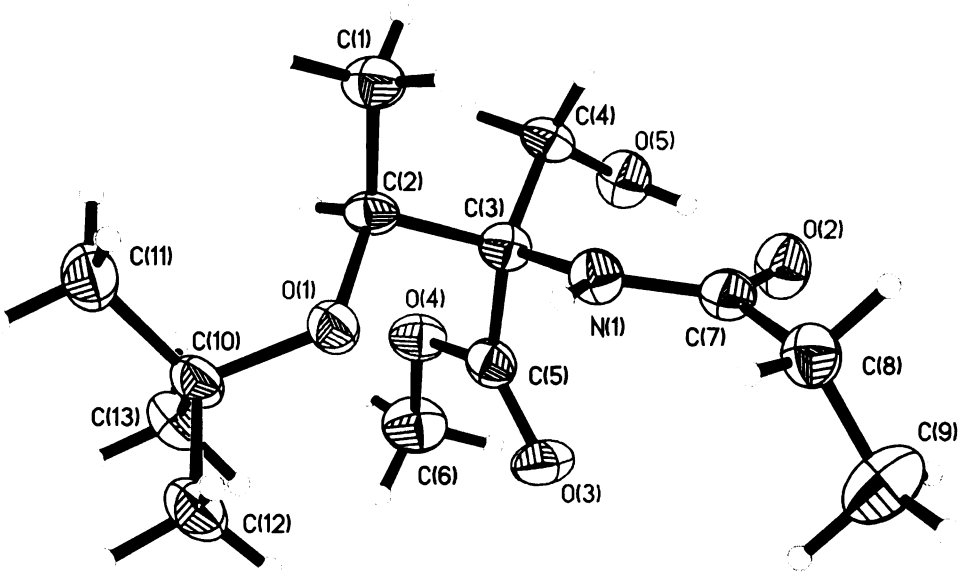
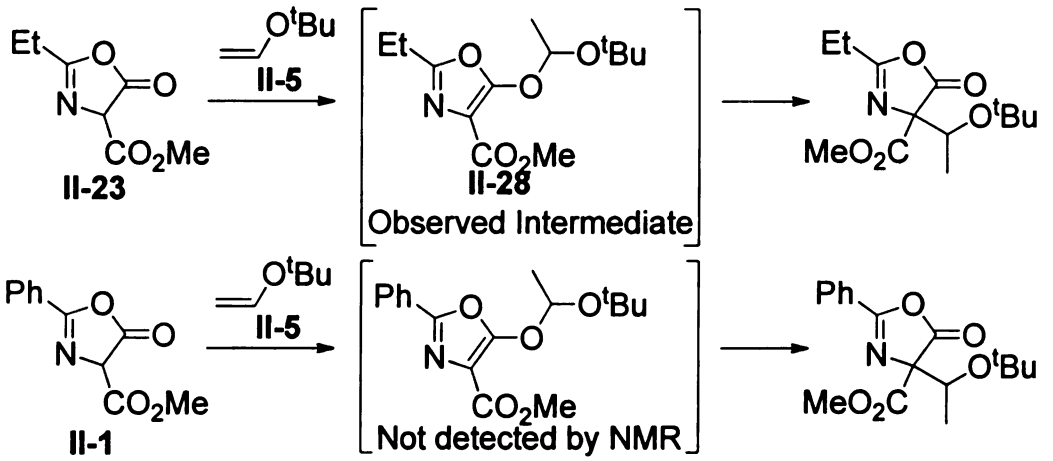


Figure II-4. X-Ray crystal structure of **II-26B**.

H. Mechanistic investigation.

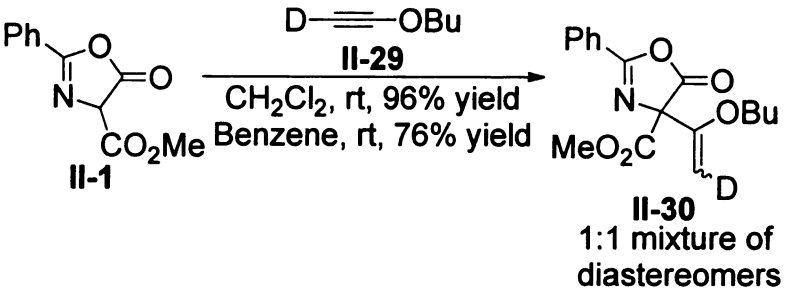
The decrease in both stereoselectivity and yield in the reactions involving 2-alkyl-oxazol-5(4H)-ones as compared to those involving 2-aryl-oxazol-5(4H)-ones may potentially be explained by a difference in mechanism. To investigate this phenomenon, we treated both 2-ethyl-4-carbmethoxy-5(4H)-oxazolone **II-22** and 2-phenyl-4-carbmethoxy-5(4H)-oxazolone **II-1** with *tert*-butyl vinyl ether **II-5** in the absence of catalyst (Scheme II-8). Analysis of the crude reaction mixtures revealed an O-alkylated oxazole intermediate **II-28** in the reaction involving **II-22**, while the reaction of **II-1** produced only C-alkylated product. Upon standing, we observed the conversion of the O-alkylated intermediate to the C-alkylated

product along with some degradation of the enol ether component. This observation infers that these ene-type reactions might proceed through an O to C migration,[46,62,63] although this was never observed for any of the 2-aryl substituted oxazol-5(4H)-ones.



Scheme II-8. Observation of O-alkylation intermediate in the alkyation reaction.

To provide additional insight into the nature of the mechanism, we conducted a deuterium study wherein oxazol-5(4H)-one **II-1** was treated with deuterium labeled alkoxy alkyne **II-29** (Scheme II-9). The reaction resulted in a 1:1 mixture of diastereomers, indicating that these alkylation reactions may also proceed *via* a step-wise mechanism. Furthermore, treatment of **II-30** with excess **II-1** did not result in a loss of deuterium incorporation, demonstrating that the acidic oxazol-5(4H)-one itself does not promote degradation of reaction diastereoslectivity by isomerizing the enol ether generated in **II-30**. The results of these deuterium-labeling studies are in agreement with studies previously performed by Dr. Fisk, which also suggested the possibility of a stepwise mechanism.



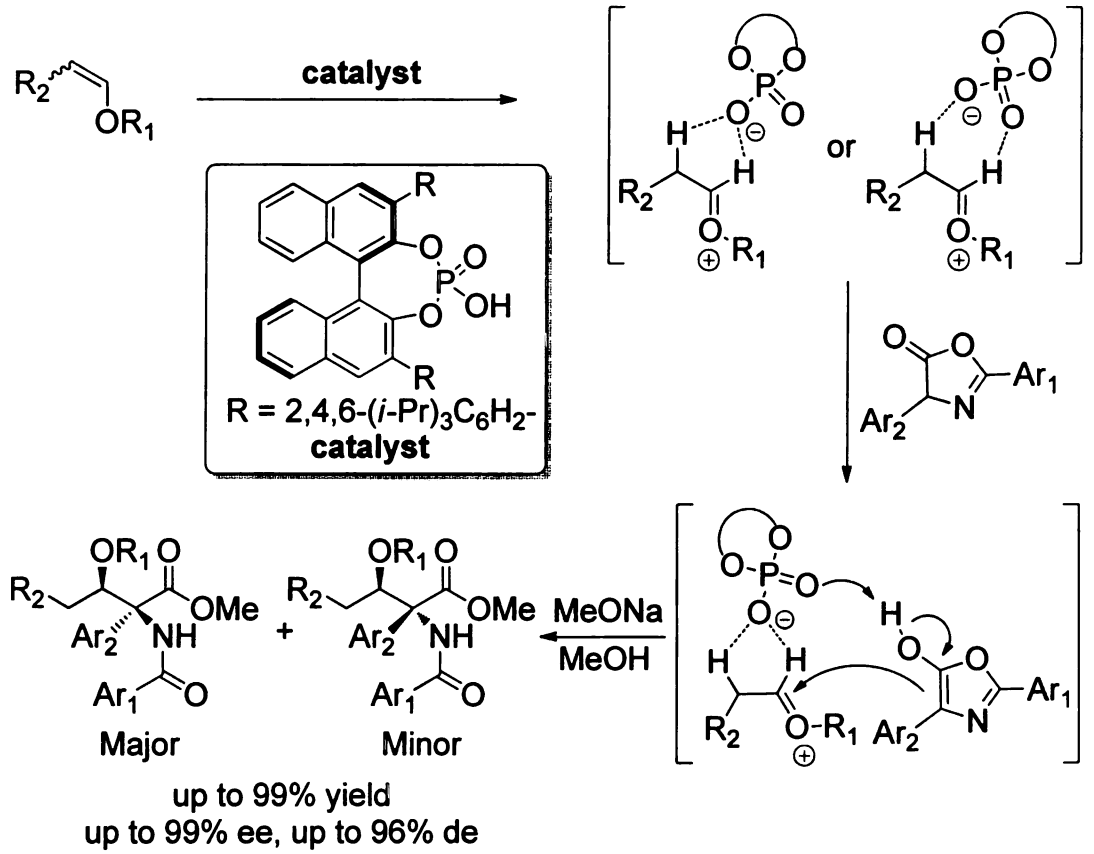
Scheme II-9. Oxazol-5(4H)-one alkylation using a deuterated alkoxy alkyne.

Supported by the significant rate enhancement found using only Brønsted acids, we hypothesize that the acidic proton of the oxazol-5(4H)-one[64] in the absence of catalyst, or in this case the catalyst itself, protonates the enol ether, forming an oxonium ion. However, coordination of the catalyst to the oxazolone substrate, as typically seen with oxazol-5(4H)-ones, cannot be dismissed.[19,65] Therefore, it is likely that the mechanistic nature of these alkylation reactions is highly dependent on both the oxazol-5(4H)-one and enol ether being used in the reaction.

I. Enantioselective ene-type alkylation reaction of oxazol-5-(4H)-ones.

More recently, an asymmetric version of the intermolecular ene-type alkylation reaction of oxazol-5(4H)-ones and enol ethers has been reported by Terada and co-workers.[66] Their work involves enol ether protonation by a chiral Brønsted acid catalyst to generate a reactive oxocarbenium ion which is proposed to form a hydrogen-bonding complex with the chiral conjugate base (Scheme II-10). Steric interactions between the resultant bulky chiral complex and the nucleophilic oxazol-5(4H)-one promote stereoselective alkylation that can only

occur when the reagents adopt appropriate orientations. Thus, treatment of C2,C4-diaryl oxazol-5(4H)-ones with *tert*-butyl vinyl ether and a chiral phosphoric acid followed by subsequent methanolysis afforded quaternary α-amino acid derivatives in good yields with excellent diastereoselectivities and enantioselectivities.[66]



Scheme II-10. Terada's enantioselective quaternary α-amino acid synthesis.

The enantioselective reaction reported by Terada and co-workers is an excellent extension of work which was previously completed in the Tepe lab. Undoubtedly, future studies involving alkylation reactions of oxazol-5(4H)-ones performed in the Tepe lab will make use of chiral Brønsted acids, such as those

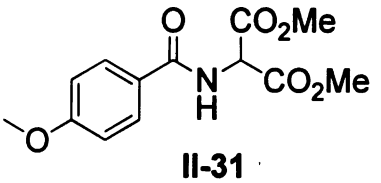
utilized by Terada, for the stereoselective preparation of densely functionalized amino acid derivatives.

J. Experimental.

1. General information. Reactions were carried out in flame-dried glassware under nitrogen atmosphere. All reactions were magnetically stirred and monitored by TLC with 0.25 μm pre-coated silica gel plates using either UV light or iodine to visualize the compounds. Column chromatography was carried out on Silica Gel 60 (230-400 mesh) supplied by EM Science. Yields refer to chromatographically and spectroscopically pure compounds unless otherwise noted. Infrared spectra were recorded on a Nicolet IR/42 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Plus-500 spectrometer. Chemical shifts are reported relative to the residual peaks of the solvent. The following abbreviations are used to denote the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, and m = multiplet. Diastereomeric ratios were determined using the integration values obtained from ^{1}H NMR. Gas chromatography / low resolution mass spectra were recorded on a Hewlet-Packard 5890 Series II gas chromatograph connected to a TRIO-1 EI mass spectrometer. HRMS were obtained at the Mass Spectrometry Facility of Michigan State University with a JEOL JMS HX-110 mass spectrometer. Elemental analysis data were obtained on a Perkin Elmer 2400 Series II CHNS/O analyzer. Melting points were obtained using an Electrothermal® capillary melting point apparatus and are uncorrected.

2. Materials. Reagents and solvents were purchased from commercial suppliers and used without further purification. Anhydrous methylene chloride, benzene, acetonitrile, and tetrahydrofuran were dispensed from a delivery system which passes the solvents through a column packed with dry neutral alumina. Ethyl vinyl ether **II-7**, *tert*-butyl vinyl ether **II-5**, 2-methoxypropene **II-12**, benzyl alcohol, mercuric acetate, trifluoroacetic anhydride, sodium borohydride, benzylamine, diphenyl phosphate, camphor sulfonic acid, trifluoroacetic acid, and 3,5-dinitrobenzoic acid were all purchased from Sigma Aldrich, checked for purity, and used without further purification.

3. Synthesis and characterization of oxazol-5-(4H)-one precursors. Oxazol-5-(4H)-ones used in these studies were prepared in three steps starting from commercially available dimethylaminomalonate HCl. The steps consist of amidation of dimethylaminomalonate HCl, followed by monoester hydrolysis and finally dehydration to give the desired oxazol-5-(4H)-one products. Additionally, carboxylic acids 2-benzamido-3-methoxy-3-oxopropanoic acid[67] and 2-(methoxycarbonyl)-2-(propionamido)acetic acid, the starting materials used to synthesize **II-1** and **II-22**, respectively, were prepared and characterized by Dr. Jason S. Fisk. For further details regarding either the synthesis or characterization of 2-benzamido-3-methoxy-3-oxopropanoic acid or 2-(methoxycarbonyl)-2-(propionamido)acetic acid, please see the Ph.D. dissertation of Jason S. Fisk.

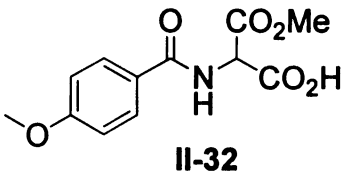


II-31

Figure II-5. Dimethyl 2-(4-methoxybenzamido)malonate (**II-31**).

Dimethyl 2-(4-methoxybenzamido)malonate (II-31).[68] A suspension of dimethylaminomalonate HCl (6.0435 g, 32.9 mmol) and TEA (4.60 mL, 32.9 mmol) in CH_2Cl_2 (200 mL) stirred at room temperature for 45 minutes before being cooled to 0 °C in an ice bath. The reaction was successively treated with 4-methoxybenzoic acid (5.0259 g, 33.1 mmol), DMAP (0.4060 g, 3.32 mmol), and DCC (6.9428 g, 33.6 mmol), and the reaction stirred overnight while warming to room temperature. The reaction was filtered, and the filtrate was concentrated and subsequently diluted in THF to form a precipitate. The solid was filtered, and the filtrate was again concentrated. This step was repeated until no additional precipitate was formed upon dilution of the concentrated filtrate in THF. The concentrated filtrate was then diluted in CH_2Cl_2 and was washed successively with 5% aqueous HCl solution, saturated $NaHCO_3$ solution, and brine before being dried (Na_2SO_4) and concentrated. Purification *via* crystallization from EtOAc and hexanes afforded 7.3 g of the title compound[68] (79% yield) as a fluffy crystalline solid (m.p. = 100 – 101 °C). ^{1}H NMR (500 MHz) ($CDCl_3$): δ 3.827 and 3.831 (2s, 9 H), 5.38 (d, J = 6.9 Hz, 1 H), 6.92 (d, J = 8.9 Hz, 2 H), 7.09 (d, J = 6.8 Hz, 1 H), 7.80 (d, J = 9.0 Hz, 2 H). ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 53.4, 55.3, 56.4, 113.7, 125.0, 129.1, 162.7, 166.2, 166.9. IR (neat): 3327 cm^{-1}, 1746 cm^{-1},

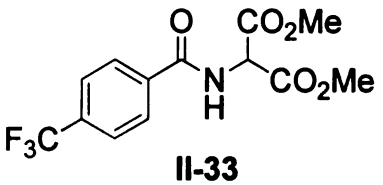
1610 cm^{-1}, 1505 cm^{-1}, 1254 cm^{-1}, 1180 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{13}H_{16}NO_6$ [M+H], 282.0978; found, 282.0983.



II-32

Figure II-6. 3-methoxy-2-(4-methoxybenzamido)-3-oxopropanoic acid (**II-32**).

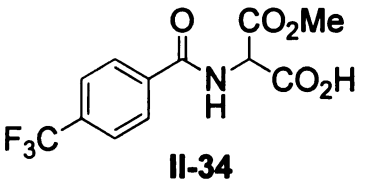
3-methoxy-2-(4-methoxybenzamido)-3-oxopropanoic acid (II-32). To a stirring solution of **II-31** (2.0280 g, 7.21 mmol) in MeOH (60 mL) cooled to 0 °C in an ice bath was added dropwise a solution of LiOH monohydrate (0.3034 g, 7.23 mmol) in H_2O (60 mL). Once all LiOH had been added, the reaction warmed to room temperature and stirred under N_2 atmosphere for the requisite amount of time, as monitored by TLC. The reaction was concentrated under vacuum to remove most of the alcohol, and the remaining solution was acidified with concentrated HCl. The aqueous layer was extracted with EtOAC (4 x 40 mL), and the combined organic fractions were washed with brine before being dried (Na_2SO_4) and concentrated under vacuum. Purification *via* crystallization from EtOAc and hexanes afforded 1.6233 g of the title compound (84% yield) as a crystalline solid (m.p. = 106 – 107 °C). ^1H NMR (500 MHz) ($(CD_3)_2SO$): δ 3.73 (s, 3 H), 3.81 (s, 3 H), 5.27 (d, J = 7.6 Hz, 1 H), 7.01 (d, J = 8.7 Hz, 2 H), 7.92 (d, J = 8.6 Hz, 2 H), 9.00 (d, J = 7.6 Hz, 1 H), 13.46 (broad s, 1 H). ^{13}C NMR (125 MHz) ($(CD_3)_2SO$) δ: 52.6, 55.4, 56.6, 113.6, 125.3, 129.7, 162.1, 165.9, 167.8, 167.9. IR (neat): 3432 cm^{-1}, 3347 cm^{-1}, 1723 cm^{-1}, 1608 cm^{-1}, 1504 cm^{-1}, 1250 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{12}H_{14}NO_6$ [M+H], 268.0821; found, 268.0826.



II-33

Figure II-7. Dimethyl 2-(4-(trifluoromethyl)benzamido)malonate (**II-33**).

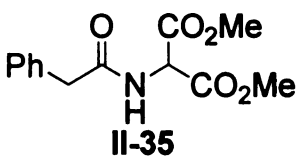
Dimethyl 2-(4-(trifluoromethyl)benzamido)malonate (II-33).[68] Following the procedure used for the synthesis of **II-31**, a 0 °C suspension of dimethylaminomalonate HCl (2.0198 g, 11.0 mmol) and TEA (1.60 mL, 11.5 mmol) in CH_2Cl_2 (140 mL) was successively treated with 4-(trifluoromethyl)benzoic acid (2.0918 g, 11.0 mmol), DMAP (0.1371 g, 1.12 mmol), and DCC (2.2899 g, 11.1 mmol). Purification *via* column chromatography on silica gel (6% ether / 94% CH_2Cl_2) afforded 3.0165 g of the title compound (86% yield) as a white solid (m.p. = 132 – 134 °C). ^{1}H NMR (500 MHz) ($CDCl_3$): δ 3.48 (s, 6 H), 5.02 (d, J = 6.9 Hz, 1 H), 6.88 (d, J = 6.7 Hz, 1 H), 7.34 (d, J = 8.2 Hz, 2 H), 7.58 (d, J = 8.1 Hz, 2 H). ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 53.6, 56.5, 123.5 (q, J = 273 Hz), 125.7 (q, J = 4 Hz), 127.8, 133.8 (q, J = 33 Hz), 136.1, 165.6, 166.5. IR (neat): 3316 cm^{-1}, 1743 cm^{-1}, 1639 cm^{-1}, 1530 cm^{-1}, 1330 cm^{-1}, 1165 cm^{-1}, 1129 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{13}H_{13}F_3NO_5$ [M+H], 320.0746; found, 320.0751.



II-34

Figure II-8. 3-methoxy-3-oxo-2-(4-(trifluoromethyl)benzamido)propanoic acid (**II-34**).

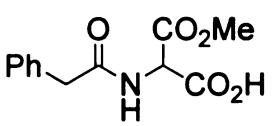
3-methoxy-3-oxo-2-(4-(trifluoromethyl)benzamido)propanoic acid (II-34). Following the procedure used for the synthesis of **II-32**, a solution of **II-33** (1.5862 g, 4.97 mmol) in MeOH (60 mL) was treated with a solution of LiOH monohydrate (0.2084 g, 4.97 mmol) in H_2O (60 mL). Purification *via* column chromatography on silica gel (50% EtOAc / 50% hexanes → EtOAc → 10% MeOH / 90% EtOAc) followed by recrystallization from hot benzene afforded 0.9357 g of the title compound (62% yield) as a white solid (m.p. = 145 – 146 °C). ^{1}H NMR (500 MHz) ($(CD_3)_2SO$): δ 3.73 (s, 3 H), 5.27 (d, J = 7.6 Hz, 1 H), 7.86 (d, J = 8.1 Hz, 2 H), 8.10 (d, J = 8.1 Hz, 2 H), 9.48 (d, J = 7.5 Hz, 1 H), 13.58 (broad s, 1 H). ^{13}C NMR (125 MHz) ($(CD_3)_2SO$) δ: 52.7, 56.6, 123.9 (q, J = 273 Hz), 125.4 (q, J = 4 Hz), 127.1, 128.6, 131.6 (q, J = 32 Hz), 165.4, 167.36, 167.44. IR (neat): 3432 cm^{-1}, 3347 cm^{-1}, 3304 cm^{-1}, 1718 cm^{-1}, 1626 cm^{-1}, 1530 cm^{-1}, 1330 cm^{-1}, 1275 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{12}H_{11}F_3NO_5$ [M+H], 306.0589; found, 306.0601.



II-35

Figure II-9. Dimethyl 2-(2-phenylacetamido)malonate (**II-35**).

Dimethyl 2-(2-phenylacetamido)malonate (II-35).[67] To a stirring suspension of dimethyl aminomalonate HCl (1.4085 g, 7.67 mmol) and TEA (2.15 mL, 15.4 mmol) in CH_2Cl_2 (150 mL), cooled to 0 °C in an ice bath, was added phenylacetyl chloride (1.65 mL, 12.5 mmol) and the reaction stirred overnight while warming to room temperature. The reaction was washed successively with saturated $NaHCO_3$ solution (x2), 5% HCl solution (x2), and brine, before being dried (Na_2SO_4) and concentrated under vacuum. Purification *via* crystallization from EtOAc and hexanes afforded 1.3628 g of the title compound (70% yield) as a white fine crystalline solid (m.p. = 85 – 86 °C). ^1H NMR (500 MHz) ($CDCl_3$): δ 3.66 (s, 2 H), 3.79 (s, 6 H), 5.19 (d, J = 6.9 Hz, 1 H), 6.45 (d, J = 6.0 Hz, 1 H), 7.26 – 7.40 (m, 5 H). ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 43.1, 53.4, 56.2, 127.5, 129.0, 129.4, 134.0, 166.5, 170.6. IR (neat): 3316 cm^{-1}, 1755 cm^{-1}, 1743 cm^{-1}, 1645 cm^{-1}, 1530 cm^{-1}, 1183 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{13}H_{16}NO_5$ [M+H], 266.1028; found, 266.1030.



II-36

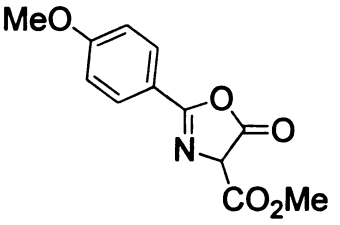
Figure II-10. 3-methoxy-3-oxo-2-(2-phenylacetamido)propanoic acid (**II-36**).

3-methoxy-3-oxo-2-(2-phenylacetamido)propanoic acid (II-36).[67] Following the procedure used for the synthesis of **II-32**, a solution of **II-35** (1.0080 g, 3.80 mmol) in MeOH (50 mL) was treated with a solution of LiOH monohydrate (0.1602 g, 3.82 mmol) in H_2O (50 mL). Purification *via* crystallization from hot EtOAc and hexanes afforded 0.7843 g of the title compound (79% yield) as a

white crystalline solid (m.p. = 132 – 133 °C). ^{1}H NMR (500 MHz) ($(CD_3)_2SO$): δ 3.57 (s, 2 H), 3.68 (s, 3 H), 5.01 (d, J = 7.4 Hz, 1 H), 7.18 – 7.30 (m, 5 H), 8.87 (d, J = 7.4 Hz, 1 H), 13.54 (broad s, 1 H). ^{13}C NMR (125 MHz) ($(CD_3)_2SO$) δ: 41.3, 52.6, 56.3, 126.4, 128.2, 129.0, 135.9, 167.47, 167.50, 170.4. IR (neat): 3341 cm^{-1}, 3286 cm^{-1}, 1755 cm^{-1}, 1627 cm^{-1}, 1530 cm^{-1}, 1232 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{12}H_{14}NO_5$ [M+H], 252.0872; found, 252.0873.

4. General procedure for synthesis of oxazol-5(4H)-ones. Oxazol-5(4H)-ones were synthesized using a known literature procedure.[69,70] A suspension of carboxylic acid in anhydrous diethyl ether was treated with TFAA (2.2 eq) dropwise and then stirred for 1.5 hours. The reaction was then cooled to 0°C and water (1.1 eq) was added dropwise, after which the ice bath was removed and the reaction stirred for an additional 1.5 hours. The reaction was cooled in a freezer for 30 minutes causing the oxazol-5(4H)-one product to precipitate out of solution which was collected via filtration and washed with cold diethyl ether. The oxazol-5(4H)-ones were then analyzed and used without further purification. On occasion full characterization of oxazol-5(4H)-ones could not be completed due to high moisture sensitivity of the compounds. Additionally for certain oxazol-5(4H)-ones carbon peaks may be hidden underneath the deuterated solvent peaks. Using this general procedure **II-1** was prepared and characterized by Dr. Jason S. Fisk. For further details regarding either the synthesis or characterization of **II-1**, please see the Ph.D. dissertation of Jason

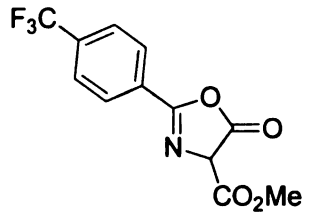
S. Fisk or the supporting information of the publication in which this compound was described.[56]



II-20

Figure II-11. 2-(4-methoxyphenyl)-4-carbmethoxy-5(4H)-oxazolone (**II-20**).

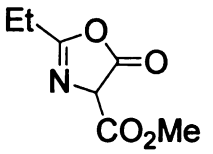
2-(4-methoxyphenyl)-4-carbmethoxy-5(4H)-oxazolone (II-20). Using the general procedure, TFAA (0.68 g, 3.2 mmol) was added to a suspension of **II-32** (0.40 g, 1.5 mmol) in diethyl ether (10 mL), and water (0.03 g, 2 mmol) was added once the reaction had been cooled. Collection of the precipitate afforded 0.30 g of title compound (m.p. = 181 – 183 °C, 79% yield). The title compound was used immediately without further purification in the preparation of **II-24**. ^{1}H NMR (500 MHz) (2:1 $CDCl_3$:C_5D_5N): δ 3.74 (s, 3H), 3.90 (s, 3H), 6.98 – 7.02 (m, 2H), 8.08 - 8.12 (m, 2H), 9.26 (s, 1H). ^{13}C NMR + **DEPT** (125 MHz) (2:1 $CDCl_3$:C_5D_5N): δ 48.1 (–**C**H_3), 53.1 (–**C**H_3), 112.3 (aromatic –**C**H), 119.7 (quaternary –**C**), 124.9 (aromatic –**C**H), 144.4 (quaternary –**C**), 158.4 (quaternary -**C**), 163.4 (quaternary –**C**), 166.2 (quaternary –**C**). IR (neat): 1779 cm^{-1}, 1632 cm^{-1}, 1514 cm^{-1}, 1267 cm^{-1}, 1113 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{12}H_{12}NO_5$ [M+H], 250.0714; found, 250.0715.



II-21

Figure II-12. 2-(4-trifluoromethylphenyl)-4-carbmethoxy-5(4H)-oxazolone (**II-21**).

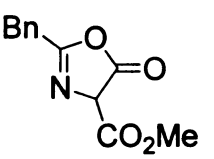
2-(4-trifluoromethylphenyl)-4-carbmethoxy-5(4H)-oxazolone (II-21). Using the general procedure, TFAA (0.71 g, 3.4 mmol) was added to a suspension of **II-34** (0.47 g, 1.6 mmol) in diethyl ether (10 mL), and water (0.03 g, 2 mmol) was added once the reaction had been cooled. Collection of the precipitate afforded 0.44 g of the title compound (solid, m.p. = 192 – 194 °C, 97% yield). The title compound was used immediately without further purification in the preparation of **II-25**. ^{1}H NMR (500 MHz) (2:1 $CDCl_3$:C_5D_5N): δ 3.90 (s, 3H), 7.66 (d, J = 8.2 Hz, 2H), 8.19 (d, J = 8.1 Hz, 2H), 13.40 (broad s, 1H). ^{13}C NMR + **DEPT** (125 MHz) (2:1 $CDCl_3$:C_5D_5N): δ 48.4 (–**C**H$_3$), 98.5 (quaternary –**C**), 122.7 (quaternary –**C**F$_3$, q, J = 272 Hz), 123.1 (aromatic –**C**H), 123.6 (aromatic –**C**H, q, J = 4 Hz), 127.0 (quaternary –**C**, q, J = 32 Hz), 130.8 (quaternary –**C**), 142.5 (quaternary –**C**), 163.9 (quaternary –**C**), 167.1 (quaternary –**C**). IR (neat): 1796 cm^{-1}, 1763 cm^{-1}, 1644 cm^{-1}, 1613 cm^{-1}, 1337 cm^{-1}, 1200 cm^{-1}, 1121 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{12}H_9F_3NO_4$ [M+H], 288.0485; found, 288.0484.



II-22

Figure II-13. 2-ethyl-4-carbmethoxy-5(4H)-oxazolone (**II-22**).

2-ethyl-4-carbmethoxy-5(4H)-oxazolone (II-22). Using the general procedure, TFAA (0.38 g, 1.80 mmol) was added to a suspension of 2-(methoxycarbonyl)-2-(propionamido)acetic acid (0.15 g, 0.80 mmol) in diethyl ether (3 mL), and water (16.5 mg, 0.92 mmol) was added once the reaction had been cooled. Collection of the precipitate afforded 0.11 g of the title compound (80% yield). The title compound is moisture sensitive and fairly unstable only allowing for partial characterization. The title compound was used immediately without further purification in the preparation of **II-26**. ^{1}H NMR (500 MHz) (2:1 $CDCl_3$:C_5D_5N): δ 1.24 (t, J = 7.6 Hz, 3 H), 2.67 (q, J = 7.6 Hz, 2 H), 3.82 (s, 3 H), 15.83 (s, 1 H). ^{13}C NMR + **DEPT** (125 MHz) (2:1 $CDCl_3$:C_5D_5N): δ 8.5 (–**C**H_3), 19.8 (–**C**H_2), 48.0 (–**C**H_3), 161.8 (quaternary –**C**). IR (KBr pellet): 3040 cm^{-1}, 1789 cm^{-1}, 1629 cm^{-1}, 1495 cm^{-1}, 1269 cm^{-1}.



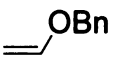
II-23

Figure II-14. 2-benzyl-4-carbmethoxy-5(4H)-oxazolone (**II-23**).

2-benzyl-4-carbmethoxy-5(4H)-oxazolone (II-23). Using the general procedure, TFAA (0.32 g, 3.45 mmol) was added to a suspension of **II-36** (0.39

g, 1.6 mmol) in ether (6 mL), and water (0.03 g, 2 mmol) was added once the reaction had been cooled. Collection of the precipitate afforded 0.28 g of the title compound (75% yield). The title compound is moisture sensitive and fairly unstable only allowing for partial characterization. The title compound was used immediately without further purification in the preparation of **II-27**. ^{1}H NMR (500 MHz) (2:1 $CDCl_3$:C_5D_5N): δ 3.82 (s, 3H), 4.00 (s, 2H), 7.18–7.22 (m, 1H), 7.24-7.40 (m, 2H), 7.39-7.41 (m, 2H), 10.65 (broad s, 1H). ^{13}C NMR (125 MHz) ($(CD_3)_2SO$): δ 32.8, 50.2, 127.6, 128.1, 128.8, 128.9, 129.0. IR (neat): 1784 cm^{-1}, 1657 cm^{-1}, 1501 cm^{-1}, 1456 cm^{-1}, 1391 cm^{-1}, 1206 cm^{-1}.

5. Synthesis of enol ethers and alkynyl ethers. The following enol ethers used in these studies were purchased and used as received: **II-5** (*tert*-butyl vinyl ether), **II-7** (ethyl vinyl ether), **II-8** (3,4-dihydro-(2H)-pyran), and **II-12** (2-methoxypropene). Vinyl ethers 1-((prop-1-enyloxy)methyl)benzene (**II-9**)[71,72] and 1-methoxy-2-methylprop-1-ene (**II-10**),[73] as well as alkynyl ethers 1-butoxyethyne (**II-11**)[74,75] and 2-deutero-1-butoxyethyne (**II-30**) were prepared by Dr. Jason S. Fisk according to literature procedures. For further details regarding either the synthesis or characterization of these compounds, please see the Ph.D. dissertation of Jason S. Fisk or the supporting information of the publication in which the compounds were described.[56] The remaining enol ether used in these studies, benzyl vinyl ether **II-2**, was prepared as follows:

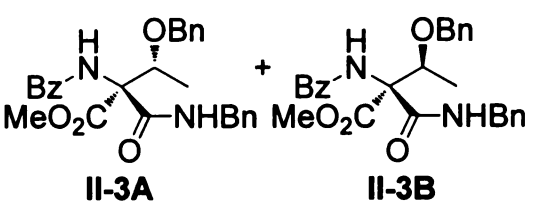


II-2

Figure II-15. Benzyl vinyl ether (**II-2**).

Benzyl vinyl ether (II-2). This compound was prepared according to a literature procedure.[76,77] A solution of benzyl alcohol (5.0 mL, 48 mmol) and $Hg(OAc)_2$ (0.557 g, 1.75 mmol) in ethyl vinyl ether (45.0 mL, 470 mmol) was prepared and stirred under reflux for 16 hours before an additional portion of $Hg(OAc)_2$ (0.264 g, 0.828 mmol) was added. The reaction stirred under reflux for an additional 8 hours, after which it was concentrated *in vacuo*. The resulting crude oil was eluted through a silica gel plug with hexanes, and the resultant eluent was concentrated under vacuum to give the product as an oil. The spectral data matches that previously reported in the literature.[77] ^{1}H NMR (500 MHz) ($CDCl_3$): δ 4.01 (dd, J_1 = 2.1 Hz, J_2 = 6.8 Hz, 1 H), 4.24 (dd, J_1 = 2.1 Hz, J_2 = 14.4 Hz, 1 H), 4.67 (s, 2 H), 6.49 (dd, J_1 = 6.8 Hz, J_2 = 14.3 Hz, 1 H), 7.19 – 7.33 (m, 5 H). ^{13}C NMR (125 MHz) ($CDCl_3$): δ 70.0, 87.3, 127.5, 127.9, 128.5, 136.8, 151.6.

6. Synthesis of diamides.

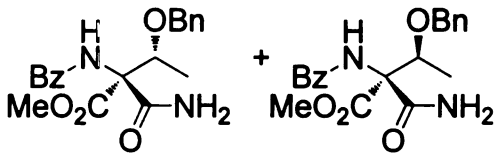


II-3A **II-3B**

Figure II-16. Methyl 2-benzamido-2-(benzylcarbamoyl)-3-(benzyloxy)butanoate (**II-3**).

Methyl 2-benzamido-2-(benzylcarbamoyl)-3-(benzyloxy)butanoate (II-3). A suspension of **II-2** (1.72 g, 9.10 mmol) and **II-1** (1.0 g, 4.6 mmol) in CH_2Cl_2 (125 mL) stirred under reflux for 21 hours, after which the reaction was concentrated to about half volume *in vacuo*. Benzylamine (1.80 mL, 16.5 mmol) was added and the reaction stirred at room temperature under nitrogen atmosphere for 7 hours. The reaction was concentrated under vacuum and the resulting mixture was purified *via* column chromatography on silica gel (nonlinear gradient from 20% EtOAc / 80% hexanes to 40% EtOAc / 60% hexanes) to afford 1.76 g (84% yield) of the title compound as a mixture of diastereomers. The relative stereochemistry of each diastereomer was determined through analysis of the ^{1}H-NMR spectrum obtained from aminolysis (with benzylamine) of a 3 : 1 mixture of quaternary oxazol-5(4H)-one diastereomers obtained from the diphenyl phospate-catalyzed reaction of **II-1** with **II-2**. **II-3A** (solid, m.p. = 98 – 100 °C): ^{1}H NMR (500 MHz) ($CDCl_3$): δ 1.23 (d, J = 6.4 Hz, 3 H), 3.75 (s, 3 H), 4.41 (dd, J_1 = 5.5 Hz, J_2 = 14.9 Hz, 1 H), 4.52 (dd, J_1 = 6.0 Hz, J_2 = 14.9 Hz, 1 H), 4.69 (d, J = 10.8 Hz, 1 H), 4.87 – 4.92 (m, 2 H), 7.17 – 7.20 (m, 2 H), 7.25 – 7.33 (m, 8 H), 7.44 (t, J = 7.6 Hz, 2 H), 7.52 (t, J = 7.4 Hz, 1 H), 7.77 (t, J = 5.7 Hz, 1 H), 7.89 (d, J = 7.1 Hz, 2 H), 8.18 (s, 1 H). ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 15.2, 43.9, 52.6, 67.4, 72.9, 74.9, 127.0, 127.1, 127.2, 127.5, 127.7, 128.2, 128.3, 131.7, 133.1, 137.1, 137.7, 165.3, 166.0, 168.4. IR (KBr pellet): 3358 cm^{-1}, 3312 cm^{-1}, 1725 cm^{-1}, 1649 cm^{-1}, 1551 cm^{-1}, 1532 cm^{-1}, 1252 cm^{-1}. MS (GCMS): *m/z* calcd for $C_{27}H_{28}N_2O_5$ [M^+], 460.20; found, 460.3. Anal. Calcd. For $C_{27}H_{28}N_2O_5$: C, 70.42; H, 6.13; N, 6.08. Found: C, 70.40; H, 6.21; N, 6.06. **II-3B** (solid, m.p. =

148 - 149 °C): ^{1}H NMR (500 MHz) ($CDCl_3$): δ 1.43 (d, J = 6.3 Hz, 3 H), 3.74 (s, 3 H), 4.42 (d, J = 11.7 Hz, 1 H), 4.45 (q, J = 6.3 Hz, 1 H), 4.49 (dd, J_1 = 5.6 Hz, J_2 = 15.0 Hz, 1 H), 4.55 (dd, J_1 = 5.8 Hz, J_2 = 14.9 Hz, 1 H), 4.67 (d, J = 11.7 Hz, 1 H), 7.24 – 7.37 (m, 11 H), 7.44 (t, J = 7.6 Hz, 2 H), 7.53 (t, J = 7.4 Hz, 1 H), 7.81 – 7.84 (m, 2 H), 7.89 (t, J = 5.6 Hz, 1 H). ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 15.2, 43.8, 53.2, 69.9, 71.5, 77.6, 127.22, 127.23, 127.5, 127.6, 127.8, 128.40, 128.45, 128.49, 131.9, 133.4, 137.4, 137.9, 165.3, 167.5, 170.6. IR (KBr pellet): 3330 cm^{-1}, 3276 cm^{-1}, 1730 cm^{-1}, 1661 cm^{-1}, 1645 cm^{-1}, 1532 cm^{-1}, 1235 cm^{-1}, 1132 cm^{-1}. MS (GCMS): *m/z* calcd for $C_{27}H_{28}N_2O_5$ [M^+], 460.20; found, 460.3. Anal. Calcd. For $C_{27}H_{28}N_2O_5$: C, 70.42; H, 6.13; N, 6.08. Found: C, 70.13; H, 6.19; N, 6.06.



II-4A **II-4B**

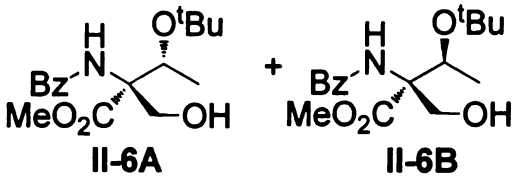
Figure II-17. Methyl 2-benzamido-3-(benzyloxy)-2-carbamoylbutanoate (**II-4**).

Methyl 2-benzamido-3-(benzyloxy)-2-carbamoylbutanoate (II-4). A suspension of **II-2** (0.449 g, 2.38 mmol) and **II-1** (0.400 g, 1.82 mmol) in CH_2Cl_2 (100 mL) stirred under reflux for 24 hours, after which the reaction was concentrated to approximately 25 mL *in vacuo*. The reaction was cooled to -78 °C and excess liquid NH_3 was added. The reaction stirred overnight as it slowly warmed to room temperature. The reaction was concentrated under vacuum and the resulting mixture was purified *via* column chromatography on silica gel (55%

EtOAc / 45% hexanes) to afford 0.620 g (92% yield) of the title compound as a mixture of diastereomers. The relative stereochemistry of each diastereomer was determined through analysis of the ^{1}H-NMR spectrum obtained from aminolysis (with ammonia) of a 3 : 1 mixture of quaternary oxazol-5(4H)-one diastereomers obtained from the diphenyl phosphate-catalyzed reaction of **II-1** with **II-2**. **II-4A** (solid, m.p. = 64 – 66 °C): ^{1}H NMR (500 MHz) ($CDCl_3$): δ 1.24 (d, J = 6.4 Hz, 3 H), 3.78 (s, 3 H), 4.71 (d, J = 10.7 Hz, 1 H), 4.84 (q, J = 6.4 Hz, 1 H), 4.89 (d, J = 10.7 Hz, 1 H), 5.72 (broad s, 1 H), 7.27 – 7.56 (m, 9 H), 7.82 – 7.85 (m, 2 H), 7.94 (s, 1 H). ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 15.3, 53.0, 67.9, 73.3, 74.9, 127.2, 128.0, 128.2, 128.5, 128.6, 132.0, 133.3, 137.9, 166.4, 167.6, 168.4. IR (KBr pellet): 3357 cm^{-1}, 3295 cm^{-1}, 3170 cm^{-1}, 1722 cm^{-1}, 1691 cm^{-1}, 1660 cm^{-1},1543 cm^{-1}, 1250 cm^{-1}, 1129 cm^{-1}. MS (GCMS): *m/z* calcd for $C_{20}H_{23}N_2O_5$ [M + H], 371.16; found: 371.2. Anal. Calcd. For $C_{20}H_{22}N_2O_5$: C, 64.85; H, 5.99; N, 7.56; found: C, 64.56; H, 5.79; N, 7.67. **II-4B** (solid, m.p. = 102 - 104 °C): ^{1}H NMR (500 MHz) ($CDCl_3$): δ 1.45 (d, J = 6.3 Hz, 3 H), 3.77 (s, 3 H), 4.40 (q, J = 6.3 Hz, 1 H), 4.47 (d, J = 11.7 Hz, 1 H), 4.70 (d, J = 11.7 Hz, 1 H), 5.58 (broad s, 1 H), 7.12 (s, 1 H), 7.30 – 7.56 (m, 8 H), 7.81 – 7.84 (m, 2 H). ^{13}C NMR (125 MHz) ($CDCl_3$) δ: 15.1, 53.2, 69.8, 71.5, 77.4, 127.2, 127.6, 127.7, 128.3, 128.4, 131.8, 133.3, 137.4, 167.4, 167.6, 170.5. IR (KBr pellet): 3399 cm^{-1}, 3267 cm^{-1}, 3214 cm^{-1}, 1728 cm^{-1}, 1677 cm^{-1}, 1657 cm^{-1},1529 cm^{-1}, 1292 cm^{-1}. MS (GCMS): *m/z* calcd for $C_{20}H_{23}N_2O_5$ [M + H], 371.16; found, 371.4. Anal. Calcd. For $C_{20}H_{22}N_2O_5$: C, 64.85; H, 5.99; N, 7.56. Found: C, 64.47; H, 5.74; N, 7.72.

7. General procedure for the synthesis of *tert*-alkyl amino hydroxy carboxylic esters. To a stirring suspension of oxazol-5(4H)-one (0.5 mmol) in 20 mL of solvent were successively added enol ether (0.75 mmol) and diphenyl phosphate (0.05 mmol), and the reaction stirred at room temperature under nitrogen atmosphere for the requisite amount of time as monitored by TLC. The reaction was washed successively with saturated $NaHCO_3$ solution and brine before being dried over $MgSO_4$ and concentrated *in vacuo*. The crude reaction was diluted in THF and cooled to 0 °C before $NaBH_4$ (1 mmol) and cold H_2O were added. The reaction stirred at 0 °C until complete by TLC. Saturated NH_4Cl solution was added and the organic layer was extracted with CH_2Cl_2 (x3). The combined organic extractions were dried ($MgSO_4$) and concentrated. The crude reaction mixtures were purified via column chromatography on silica gel (diethyl ether / CH_2Cl_2).

Using the general procedure, *tert*-alkyl amino hydroxy carboxylic esters **II-15**, **II-16**, and **II-17** were all prepared and fully characterized by Jason S. Fisk. Additionally, oxazol-5(4H)-one products **II-28** and **II-30** were prepared and characterized by Jason S. Fisk. For further details regarding either the synthesis or characterization of these compounds, please see the Ph.D. dissertation of Jason S. Fisk or the supporting information of the publication these compounds were described.[56]

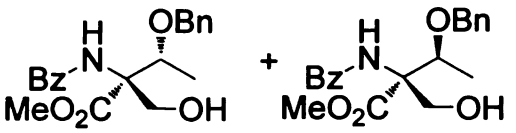


II-6A **II-6B**

Figure II-18. Methyl-2-benzamido-3-*tert*-butoxy-2-(hydroxymethyl)butanoate (**II-6**).

Methyl-2-benzamido-3-*tert*-butoxy-2-(hydroxymethyl)butanoate (II-6). Using the general procedure, a suspension of **II-1** (0.10 g, 0.46 mmol), **II-5** (69.1 mg, 0.69 mmol), and diphenyl phosphate (11.5 mg, 0.046 mmol) in anhydrous benzene (20 mL) was stirred at room temperature for 3 hours. After washings, the crude reaction intermediate was diluted in 3 mL THF and cooled to 0 °C before $NaBH_4$ (34.8 mg, 0.92 mmol) and water (1.5 mL) were added. Purification *via* silica gel chromatography (7% ether / 93% CH_2Cl_2) afforded 0.14 g of the title compound (90% yield) as a 3 : 1 ratio of diastereomers. **II-6A** (solid, m.p. = 83 – 85 °C.): ^{1}H NMR (500 MHz) ($CDCl_3$): δ 1.12 (s, 9H), 1.30 (d, J = 6.3 Hz, 3H), 3.79 (s, 3H), 3.95 (dd, J_1 = 7.2 Hz, J_2 = 11.6 Hz, 1H), 4.26 (dd, J_1 = 6.3 Hz, J_2 = 11.7 Hz, 1H), 4.37 (q, J = 6.3 Hz, 1H), 4.57 (t, J = 6.7 Hz, 1H (O-H)), 7.41–7.46 (m, 3H), 7.50 (t, J = 7.4 Hz, 1H), 7.79 (d, J = 7.7 Hz, 2H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$) δ: 17.9 (–**C**H_3), 28.6 (–**C**H_3), 52.6 (–**C**H_3), 64.1 (–**C**H_2), 69.8 (–**C**H), 72.1 (quaternary –**C**), 75.2 (quaternary –**C**), 127.1 (aromatic –**C**H), 128.7 (aromatic –**C**H), 131.8 (aromatic –**C**H), 134.6 (aromatic quaternary –**C**), 169.4 (quaternary –**C**), 171.3 (quaternary –**C**). IR (neat): 3412 cm^{-1}, 3320 cm^{-}1, 1736 cm^{-1}, 1653 cm^{-1}, 1522 cm^{-1}, 1487 cm^{-1}, 1234 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{17}H_{26}NO_5$ [M+H], 324.1811; found, 324.1823. Anal. Calcd. For $C_{17}H_{25}NO_5$: C,

63.14; H, 7.79; N, 4.33. Found: C, 63.04; H, 7.73; N, 4.55. **II-6B** (solid, m.p. = 76 – 79 °C.): ^{1}H NMR (500 MHz) ($CDCl_3$): δ 1.13 (d, J = 6.2 Hz, 3H), 1.16 (s, 9H), 3.74 (s, 3H), 3.83 (t, J = 11.8 Hz, 1H), 3.95 (dd, J_1 = 2.8 Hz, J_2 = 11.8 Hz, 1H), 4.27 (q, J = 6.2 Hz, 1H), 5.71 (dd, J_1 = 2.8 Hz, J_2 = 11.6 Hz, 1H (O-H)), 7.26 (broad s, 1H), 7.42 – 7.54 (m, 3H), 7.79 (dd, J_1 = 3.2 Hz, J_2 = 5.3 Hz, 2H). ^{13}C NMR (125 MHz) + **DEPT** ($CDCl_3$) δ: 19.1 (–**C**H_3), 28.7 (–**C**H_3), 52.7 (–**C**H_3), 64.6 (–**C**H_2), 68.6 (–**C**H), 70.0 (quaternary –**C**), 74.8 (quaternary –**C**), 127.0 (aromatic –**C**H), 128.6 (aromatic –**C**H), 131.8 (aromatic –**C**H), 134.2 (aromatic quaternary –**C**), 167.7 (quaternary –**C**), 171.2 (quaternary –**C**). IR (neat): 3405 cm^{-1}, 3584 – 3156 cm^{-1}, 1752 cm^{-1}, 1669 cm^{-1}, 1520 cm^{-1}, 1487 cm^{-1}, 1115 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{17}H_{26}NO_5$ [M+H], 324.1811; found, 324.1801.



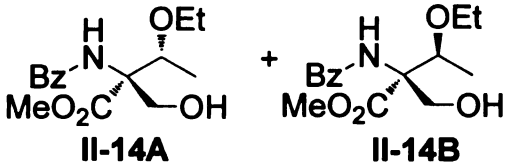
II-13A **II-13B**

Figure II-19. Methyl-2-benzamido-3-benzyloxy-2-(hydroxymethyl)butanoate (**II-13**).

Methyl-2-benzamido-3-benzyloxy-2-(hydroxymethyl)butanoate (II-13): Using the general procedure, a suspension of **II-1** (0.21 g, 0.94 mmol), **II-2** (0.19 g, 1.4 mmol), and diphenyl phosphate (24.1 mg, 0.096 mmol) in anhydrous benzene (40 mL) was stirred at room temperature for 4 hours. After washings, the crude reaction intermediate was diluted in 6 mL THF and cooled to 0 °C before $NaBH_4$ (76.4 mg, 2.0 mmol) and water (3 mL) were added. Purification *via* silica gel chromatography (17% ether / 83% CH_2Cl_2) afforded 0.26 g of the title compound

(77% yield) as a 3 : 1 ratio of diastereomers. **II-13A** (solid, m.p. = 96 – 98 °C.): ^{1}H NMR (500 MHz) ($CDCl_3$): δ 1.41 (d, J = 6.4 Hz, 3H), 3.84 (s, 3H), 4.05 (dd, J_1 = 6.7 Hz, J_2 = 11.8 Hz, 1H), 4.27 - 4.35 (m, 2H), 4.45 (d, J = 11.7 Hz, 1H), 4.49 (t, J = 6.9 Hz, 1H), 4.68 (d, J = 11.7 Hz, 1H), 7.26 – 7.29 (m, 5H), 7.39 (s, 1H), 7.41–7.45 (m, 2H), 7.51 – 7.55 (m, 1H), 7.72 – 7.76 (m, 2H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$) δ: 14.9 (–**C**H_3), 52.8 (–**C**H_3), 64.3 (–**C**H_2), 69.3 (quaternary –**C**), 71.7 (–**C**H_2), 75.6 (–**C**H), 127.1 (aromatic –**C**H), 127.76 (aromatic –**C**H), 127.81 (aromatic –**C**H), 128.4 (aromatic –**C**H), 128.6 (aromatic –**C**H), 131.8 (aromatic –**C**H), 134.0 (aromatic quaternary –**C**), 137.6 (aromatic quaternary –**C**), 167.7 (quaternary –**C**), 170.7 (quaternary –**C**). IR (neat): 3415 cm^{-1}, 1734 cm^{-1}, 1653 cm^{-1}, 1520 cm^{-1}, 1485 cm^{-1}, 1240 cm^{-1}. MS (GCMS): *m/z* calcd for $C_{20}H_{23}NO_5$ [M^+], 357.16; found, 358.2. Anal. Calcd. For $C_{20}H_{23}NO_5$: C, 67.21; H, 6.49; N, 3.92. Found: C, 66.96; H, 6.53; N, 4.09. **II-13B** (oil): ^{1}H NMR (500 MHz) ($CDCl_3$): δ 1.22 (d, J = 6.3 Hz, 3H), 3.71 (s, 3H), 3.84 (dd, J_1 = 7.8 Hz, J_2 = 15.4 Hz, 1H), 4.04 (dd, J_1 = 3.3 Hz, J_2 = 11.9 Hz, 1H), 4.19 (q, J = 6.3 Hz, 1H), 4.42 (d, J = 11.8 Hz, 1H), 4.63 (d, J = 11.8 Hz, 1H), 5.42 (dd, J_1 = 3.3 Hz, J_2 = 11.3 Hz, 1H), 7.05 (broad s, 1H), 7.24–7.54 (m, 8H), 7.76 - 7.79 (m, 2H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$) δ: 13.9 (–**C**H_3), 52.7 (–**C**H_3), 63.9 (–**C**H_2), 71.0 (–**C**H_2), 71.4 (quaternary –**C**), 76.6 (–**C**H), 127.1 (aromatic –**C**H), 127.8 (aromatic –**C**H), 128.0 (aromatic –**C**H), 128.5 (aromatic –**C**H), 128.7 (aromatic –**C**H), 131.9 (aromatic –**C**H), 134.4 (aromatic quaternary –**C**), 137.3 (aromatic quaternary –**C**), 169.4 (quaternary –**C**), 171.1 (quaternary –**C**). IR (neat): 3412 cm^{-1}, 3328 cm^{-1},

1749 cm^{-1}, 1658 cm^{-1}, 1520 cm^{-1}, 1487 cm^{-1}, 1069 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{20}H_{24}NO_5$ [M+H], 358.1652; found, 358.1654.

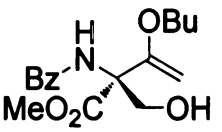


II-14A **II-14B**

Figure II-20. Methyl-2-benzamido-3-ethoxy-2-(hydroxymethyl)butanoate (**II-14**).

Methyl-2-benzamido-3-ethoxy-2-(hydroxymethyl)butanoate (II-14). Using the general procedure, a suspension of **II-1** (0.10 g, 0.46 mmol), **II-7** (0.09 mL, 0.9 mmol), and diphenyl phosphate (11.3 mg, 0.045 mmol) in anhydrous benzene (20 mL) was stirred at room temperature for 3 hours. After washings, the crude reaction intermediate was diluted in 2.5 mL THF and cooled to 0 °C before $NaBH_4$ (34.7 mg, 0.92 mmol) and water (1.25 mL) were added. Purification *via* silica gel chromatography (10% ether / 90% CH_2Cl_2) afforded 0.12 g of the title compound (85% yield) as a 2 : 1 ratio of diastereomers. **II-14A** (solid, m.p. = 89 - 91 °C) ^{1}H NMR (500 MHz) ($CDCl_3$): δ 1.13 (t, J = 7.0 Hz, 3H), 1.30 (d, J = 6.4 Hz, 3H), 3.40 (dq, J_1 = 7.0 Hz, J_2 = 9.5 Hz, 1H), 3.65 (dq, J_1 = 7.0 Hz, J_2 = 9.5 Hz, 1H), 3.79 (s, 3H), 3.99 (dd, J_1 = 7.2 Hz, J_2 = 11.7 Hz, 1H), 4.14 (q, J = 6.4 Hz, 1H), 4.24 (dd, J_1 = 6.0 Hz, J_2 = 11.8 Hz, 1H), 4.49 (t, J = 6.8 Hz, 1H), 7.34 (s, 1H), 7.42 – 7.46 (m, 2H), 7.49 – 7.54 (m, 1H), 7.77 – 7.81 (m, 2H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$) δ: 15.1 (–CH_3), 15.4 (–CH_3), 52.8 (–CH_3), 64.5 (–CH_2), 65.4 (–CH_2), 69.0 (quaternary –C), 75.7 (–CH), 127.1 (aromatic –CH), 128.7 (aromatic –CH), 131.9 (aromatic –CH), 134.1 (aromatic quaternary –C), 167.9 (quaternary –C), 170.9 (quaternary –C). IR (KBr pellet): 3413 cm^{-1}, 3352 cm^{-1},

1728 cm^{-1}, 1644 cm^{-1}, 1522 cm^{-1}, 1489 cm^{-1}, 1244 cm^{-1}, 1067 cm^{-1}. MS (GCMS): *m/z* calcd for $C_{15}H_{21}NO_5$ [M^+], 295.14; found, 295.9. Anal. Calcd. For $C_{15}H_{21}NO_5$: C, 61.00; H, 7.17; N, 4.74. Found: C, 61.12; H, 7.19; N, 4.91. **II-14B** (oil): ^1H NMR (500 MHz) ($CDCl_3$): δ 1.11 – 1.16 (m, 6H), 3.38 (dq, J_1 = 7.0 Hz, J_2 = 9.5 Hz, 1H), 3.63 (dq, J_1 = 7.0, J_2 = 9.5 Hz, 1H), 3.78 (s, 3H), 3.82 (dt, J_1 = 1.0 Hz, J_2 = 11.8 Hz, 1H), 4.00 (dd, J_1 = 3.1 Hz, J_2 = 11.9 Hz, 1H), 4.07 (q, J = 6.3 Hz, 1H), 5.51 (dd, J_1 = 3.1 Hz, J_2 = 11.4 Hz, 1H), 7.02 (broad s, 1H), 7.45 (tt, J_1 = 1.4 Hz, J_2 = 6.7 Hz, 2H), 7.50–7.55 (m, 1H), 7.78–7.81 (m, 2H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$) δ: 14.01 (–CH_3), 15.3 (–CH_3), 52.8 (–CH_3), 63.9 (–CH_2), 64.7 (–CH_2), 71.4 (quaternary –C), 76.9 (–CH), 127.1 (aromatic –CH), 128.7 (aromatic –CH), 131.9 (aromatic –CH), 134.5 (aromatic quaternary –C), 169.5 (quaternary –C), 171.3 (quaternary –C). IR (neat): 3414 cm^{-1}, 3327 cm^{-1}, 1752 cm^{-1}, 1661 cm^{-1}, 1522 cm^{-1}, 1074 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{15}H_{22}NO_5$ [M+H], 296.1498; found, 296.1496.

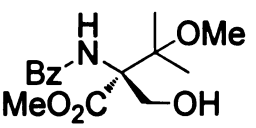


II-18

Figure II-21. Methyl-2-(benzamido)-3-butoxy-2-(hydroxymethyl)but-3-enoate (**II-18**).

Methyl-2-(benzamido)-3-butoxy-2-(hydroxymethyl)but-3-enoate (II-18). Using the general procedure, a suspension of **II-1** (0.11 g, 0.49 mmol) and **II-11** (72.7 mg, 0.74 mmol) in anhydrous dichloromethane (20 mL) was stirred at room temperature overnight. After concentration, the crude reaction intermediate was

diluted in THF (3 mL) and cooled to -41 °C before anhydrous EtOH (1.5 mL) and $NaBH_4$ (92.7 mg, 2.45 mmol) were added. Purification *via* silica gel chromatography (15% ether / 85% CH_2Cl_2) afforded 0.11 g of the title compound (67% yield) as an oil. ^1H NMR (500 MHz) ($CDCl_3$): δ 0.90 (t, J = 7.4 Hz, 3 H), 1.33 – 1.41 (m, 2 H), 1.60 – 1.68 (m, 2 H), 3.40 (dd, J_1 = 5.1 Hz, J_2 = 8.7 Hz, 1 H), 3.70 – 3.76 (m, 2 H), 3.80 (s, 3 H), 4.15 (dd, J_1 = 8.7 Hz, J_2 = 11.3 Hz, 1 H), 4.26 (d, J = 3.7 Hz, 1 H), 4.32 (d, J = 3.7 Hz, 1 H), 4.47 (dd, J_1 = 4.9 Hz, J_2 = 11.4 Hz, 1 H), 7.33 (broad s, 1 H), 7.41 – 7.46 (m, 2 H), 7.49 – 7.54 (m, 1 H), 7.77 – 7.81 (m, 2 H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$): δ 13.7 (–**C**H_3), 19.2 (–**C**H_2), 30.6 (–**C**H_2), 53.3 (–**C**H_3), 64.1 (–**C**H_2), 67.9 (–**C**H_2), 84.6 (–**C**H_2), 127.1 (aromatic –**C**H), 128.7 (aromatic –**C**H), 131.9 (aromatic –**C**H), 134.0 (aromatic quaternary –**C**), 156.6 (quaternary –**C**), 167.2 (quaternary –**C**), 170.8 (quaternary –**C**); IR (neat): 3575-3125 cm^{-1}, 3416 cm^{-1}, 1742 cm^{-1}, 1651 cm^{-1}, 1287 cm^{-1}, 1225 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{17}H_{24}NO_5$ [M+H], 322.1656; found, 322.1654.

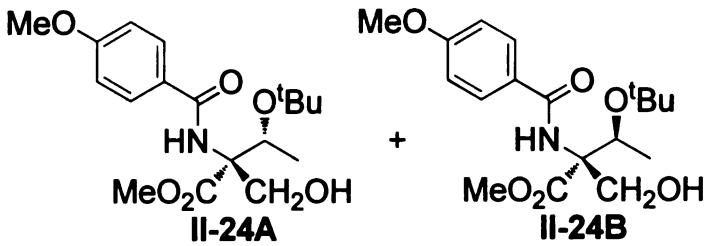


II-19

Figure II-22. Methyl-2-(benzamido)-2-(hydroxymethyl)-3-methoxy-3-methylbutanoate (**II-19**).

Methyl-2-(benzamido)-2-(hydroxymethyl)-3-methoxy-3-methylbutanoate (II-19). Using the general procedure, a suspension of **II-1** (0.10 g, 0.46 mmol) and **II-12** (0.051 g, 0.70 mmol) in anhydrous benzene (20 mL) was stirred at room

temperature overnight. After washings, the crude reaction intermediate was diluted in THF (3 mL) and cooled to 0 °C before $NaBH_4$ (0.15 g, 4.1 mmol) and water (0.1 mL) were added. Purification *via* silica gel chromatography (20% ether / 80% CH_2Cl_2) afforded 0.084 g of the title compound (62% yield) as an oil. ^1H NMR (500 MHz) ($CDCl_3$) δ: 1.28 (s, 3 H), 1.41 (s, 3 H), 3.22 (s, 3 H), 3.76 (s, 3 H), 4.15 (dd, J_1 = 9.9 Hz, J_2 = 12.0 Hz, 1 H), 4.24 (dd, J_1 = 4.3 Hz, J_2 = 12.0 Hz, 1 H), 4.81 (dd, J_1 = 4.3 Hz, J_2 = 9.8 Hz, 1 H), 7.38 (broad s, 1 H), 7.41 – 7.46 (m, 2 H), 7.48 - 7.53 (m, 1 H), 7.77 – 7.80 (m, 2 H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$) δ: 20.3 (–**C**H_3), 20.9 (–**C**H_3), 49.2 (–**C**H_3), 52.6 (–**C**H_3), 62.9 (–**C**H_2), 72.5 (quaternary –**C**), 79.6 (quaternary –**C**), 127.1 (aromatic –**C**H), 128.6 (aromatic –**C**H), 131.7 (aromatic –**C**H), 134.7 (aromatic quaternary –**C**), 168.4 (quaternary –**C**), 170.5 (quaternary –**C**). IR (neat): 3407 cm^{-1}, 3299 cm^{-1}, 1742 cm^{-1}, 1659 cm^{-1}, 1514 cm^{-1}, 1485 cm^{-1}, 1233 cm^{-1}, 1055 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{15}H_{22}NO_5$ [M+H], 296.1498; found, 296.1496.



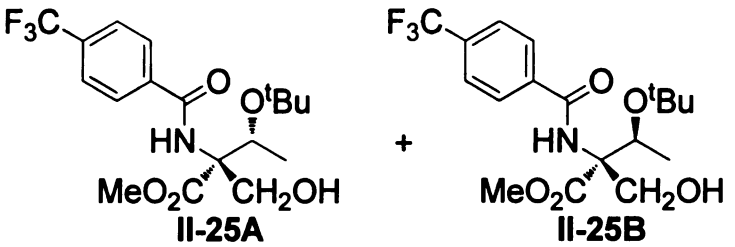
II-24A **II-24B**

Figure II-23. Methyl-2-(4-methoxybenzamido)-3-*tert*-butoxy-2-(hydroxymethyl)butanoate (**II-24**).

Methyl-2-(4-methoxybenzamido)-3-*tert*-butoxy-2-(hydroxymethyl)butanoate (II-24). Using the general procedure, a suspension of **II-20** (0.14 g, 0.55 mmol), **II-5** (0.084 g, 0.84 mmol), and diphenyl phosphate (14.3 mg, 0.057 mmol) in

anhydrous benzene (20 mL) was stirred at room temperature overnight. After washings, the crude reaction intermediate was diluted in THF (3 mL) and cooled to 0 °C before $NaBH_4$ (46.0 mg, 1.2 mmol) and water (1.5 mL) were added. Purification *via* silica gel chromatography (10% ether / 90% CH_2Cl_2) afforded 0.17 g of the title compound (88% yield) as a 2.9 : 1 ratio of diastereomers. **II-24A** (solid, m.p. = 109 - 111): ^{1}H NMR (500 MHz) ($CDCl_3$): δ 1.11 (s, 9H), 1.30 (d, J = 6.3 Hz, 3 H), 3.79 (s, 3 H), 3.83 (s, 3 H), 3.94 (dd, J_1 = 6.9 Hz, J_2 = 11.7 Hz, 1 H), 4.25 (dd, J_1 = 6.5 Hz, J_2 = 11.7 Hz, 1 H), 4.36 (q, J = 6.3 Hz, 1 H), 4.72 (t, J = 6.7 Hz, 1 H), 6.90 – 6.94 (m, 2 H), 7.36 (broad s, 1 H), 7.73 – 7.78 (m, 2 H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$) δ: 19.1 (–**C**H_3), 28.7 (–**C**H_3), 52.7 (–**C**H_3), 55.4 (–**C**H_3), 64.8 (–**C**H_2), 68.7 (–**C**H), 70.0 (quaternary –**C**), 74.8 (quaternary –**C**), 113.8 (aromatic –**C**H), 126.4 (aromatic quaternary –**C**), 128.9 (aromatic –**C**H), 162.4 (aromatic quaternary –**C**), 167.3 (quaternary –**C**), 171.3 (quaternary –**C**). IR (neat): 3600 – 3245 cm^{-1}, 3416 cm^{-1}, 1738 cm^{-1}, 1653 cm^{-1}, 1609 cm^{-1}, 1499 cm^{-1}, 1258 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{18}H_{28}NO_6$ [M + H], 354.1917; found, 354.1914. **II-24B** (solid, m.p. = 115 - 117): ^{1}H NMR (500 MHz) ($CDCl_3$): δ 1.13 (d, J = 6.2 Hz, 3 H), 1.16 (s, 9 H), 3.74 (s, 3 H), 3.80 – 3.86 (m, 4 H), 3.93 (dd, J_1 = 2.7 Hz, J_2 = 11.8 Hz, 1 H), 4.26 (q, J = 6.2 Hz, 1 H), 5.82 (dd, J_1 = 2.7 Hz, J_2 = 11.6 Hz, 1 H), 6.92 – 6.96 (m, 2 H), 7.18 (broad s, 1 H), 7.74 – 7.78 (m, 2 H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$) δ: 17.9 (–**C**H_3), 28.6 (–**C**H_3), 52.6 (–**C**H_3), 55.4 (–**C**H_3), 64.2 (–**C**H_2), 69.8 (–**C**H), 72.0 (quaternary –**C**), 75.1 (quaternary –**C**), 113.9 (aromatic –**C**H), 126.8 (aromatic quaternary –**C**), 128.9 (aromatic –**C**H), 162.5 (aromatic quaternary –**C**), 168.9 (quaternary –**C**),

171.5 (quaternary –**C**). IR (neat): 3411 cm^{-1}, 3295 cm^{-1}, 1752 cm^{-1}, 1653 cm^{-1}, 1607 cm^{-1}, 1499 cm^{-1}, 1258 cm^{-1}, 1115 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{18}H_{28}NO_6$ [M+H], 354.1917; found, 354.1915.

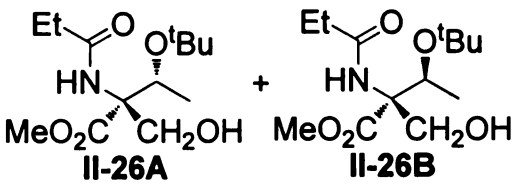


II-25A **II-25B**

Figure II-24. Methyl-2-(4-(trifluoromethyl)benzamido)-3-*tert*-butoxy-2-(hydroxymethyl) butanoate (**II-25**).

Methyl-2-(4-(trifluoromethyl)benzamido)-3-*tert*-butoxy-2-(hydroxymethyl) butanoate (II-25). Using the general procedure, a suspension of **II-21** (0.13 g, 0.45 mmol), **II-5** (0.069 g, 0.68 mmol), and diphenyl phosphate (11.4 mg, 0.046 mmol) in anhydrous benzene (20 mL) was stirred at room temperature overnight. After washings, the crude reaction intermediate was diluted in THF (2 mL) and cooled to 0 °C before $NaBH_4$ (38.4 mg, 1.0 mmol) and water (1 mL) were added. Purification *via* silica gel chromatography (10% ether / 90% CH_2Cl_2) afforded 0.14 g of the title compound (81% yield), separated as a 2 : 1 ratio of diastereomers. **II-25A** (oil): ^{1}H NMR (500 MHz) ($CDCl_3$): δ 1.12 (s, 9 H), 1.29 (d, J = 6.3 Hz, 3 H), 3.79 (s, 3 H), 3.92 – 3.99 (m, 1 H), 4.23 – 4.30 (m, 2 H), 4.37 (q, J = 6.3 Hz, 1 H), 7.43 (s, 1 H), 7.67 – 7.71 (m, 2 H), 7.87 – 7.91 (m, 2 H). ^{13}C **NMR + DEPT** (125 MHz) ($CDCl_3$) δ: 19.1 (–**C**H_3), 28.7 (–**C**H_3), 52.8 (–**C**H_3), 64.2 (–**C**H_2), 68.6 (–**C**H), 70.0 (quaternary –**C**), 74.9 (quaternary –**C**), 123.6 (quaternary –**C**F_3, q, J = 273 Hz), 125.7 (aromatic –**C**H, q, J = 4 Hz), 127.5

(aromatic –CH), 133.5 (aromatic quaternary –C, q, J = 33 Hz), 137.6 (aromatic quaternary –C), 166.3 (quaternary –C), 171.1 (quaternary –C). IR (neat): 3447 cm^{-1}, 3407 cm^{-1}, 1740 cm^{-1}, 1664 cm^{-1}, 1653 cm^{-1}, 1528 cm^{-1}, 1327 cm^{-1}, 1129 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{18}H_{25}F_3NO_5$ [M + H], 392.1685; found, 392.1687. **II-25B** (oil): ^1H NMR (500 MHz) ($CDCl_3$): δ 1.14 (d, J = 6.2 Hz, 3 H), 1.16 (s, 9 H), 3.75 (s, 3 H), 3.84 (dt, J_1 = 1.0 Hz, J_2 = 11.8 Hz, 1 H), 3.97 (dd, J_1 = 2.9 Hz, J_2 = 11.9 Hz, 1 H), 4.28 (q, J = 6.2 Hz, 1 H), 5.45 (dd, J_1 = 2.9 Hz, J_2 = 11.6 Hz, 1 H), 7.27 (s, 1 H), 7.71 – 7.74 (m, 2 H), 7.88 – 7.92 (m, 2 H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$) δ: 17.9 (–CH_3), 28.6 (–CH_3), 52.7 (–CH_3), 63.9 (–CH_2), 69.7 (–CH), 72.3 (quaternary –C), 75.4 (quaternary –C), 123.6 (quaternary –CF_3, q, J = 273 Hz), 125.8 (aromatic –CH, q, J = 4 Hz), 127.6 (aromatic –CH), 133.5 (aromatic quaternary –C, q, J = 33 Hz), 138.0 (aromatic quaternary –C), 168.1 (quaternary –C), 171.1 (quaternary –C). IR (neat): 3600 – 3250 cm^{-1}, 3401 cm^{-1}, 1754 cm^{-1}, 1667 cm^{-1}, 1530 cm^{-1}, 1327 cm^{-1}, 1121 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{18}H_{25}F_3NO_5$ [M+H], 392.1685; found, 392.1687.

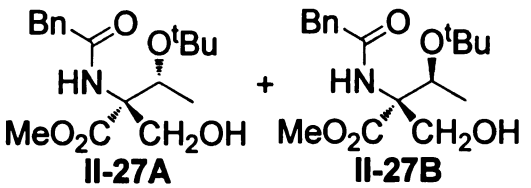


II-26A **II-26B**

Figure II-25. Methyl-3-*tert*-butoxy-2-(hydroxymethyl)-2-(propionamido)butanoate (**II-26**).

Methyl-3-*tert*-butoxy-2-(hydroxymethyl)-2-(propionamido)butanoate (II-26). Using the general procedure, a suspension of **II-22** (0.17 g, 1.0 mmol), **II-5** (0.15 g, 1.5 mmol), and diphenyl phosphate (23.8 mg, 0.095 mmol) in anhydrous

benzene (40 mL) was stirred at room temperature for 3 hours. After washings, the crude reaction intermediate was diluted in THF (6 mL) and cooled to 0 °C before $NaBH_4$ (79.7 mg, 2.1 mmol) and water (3 mL) were added. Purification *via* silica gel chromatography (10% ether / 90% CH_2Cl_2) afforded 0.14 g of the title compound (50% yield) as a 1 : 1.3 ratio of diastereomers. **II-26A** (oil): ^1H NMR (500 MHz) ($CDCl_3$): δ 1.05 – 1.10 (m, 12 H), 1.17 (d, J = 6.3 Hz, 3 H), 2.19 – 2.30 (m, 2 H), 3.68 (s, 3H), 3.79 (dd, J_1 = 6.8 Hz, J_2 = 11.8 Hz, 1 H), 4.06 (dd, J_1 = 6.0 Hz, J_2 = 11.6 Hz, 1 H), 4.15 (q, J = 6.3 Hz, 1 H), 4.67 (t, J = 6.5 Hz, 1 H), 6.60 (broad s, 1 H). ^{13}C NMR (125 MHz) + **DEPT** ($CDCl_3$) δ: 9.8 (–**C**H_3), 17.7 (–**C**H_3), 28.6 (–**C**H_3), 30.4 (–**C**H_2), 52.5 (–**C**H_3), 64.2 (–**C**H_2), 69.6 (–**C**H), 71.8 (quaternary –**C**), 75.0 (quaternary –**C**), 171.3 (quaternary –**C**), 176.2 (quaternary –**C**). IR (neat): 3413 cm^{-1}, 3328 cm^{-1}, 1742 cm^{-1}, 1655 cm^{-1}, 1516 cm^{-1}, 1235 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{13}H_{26}NO_5$ [M+H], 276.1811; found, 276.1810. **II-26B** (solid, m.p. = 63 – 64 °C): ^1H NMR (500 MHz) ($CDCl_3$): δ 1.05 (d, J = 6.2 Hz, 3 H), 1.12 (s, 9 H), 1.19 (t, J = 7.6 Hz, 3 H), 2.32-2.41 (m, 2H), 3.67 – 3.75 (m, 4 H), 3.79 (dd, J_1 = 2.4 Hz, J_2 = 11.7 Hz, 1 H), 4.16 (q, J = 6.2 Hz, 1 H), 5.65 (d, J = 11.4 Hz, 1 H), 6.43 (s, 1 H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$) δ: 9.4 (–**C**H_3), 18.8 (–**C**H_3), 28.5 (–**C**H_3), 30.0 (–**C**H_2), 52.5 (–**C**H_3), 64.6 (–**C**H_2), 68.7 (–**C**H), 69.7 (quaternary –**C**), 74.4 (quaternary –**C**), 170.9 (quaternary –**C**), 174.4 (quaternary –**C**). IR (KBr pellet): 3360 cm^{-1}, 3305 cm^{-1}, 1755 cm^{-1}, 1659 cm^{-1}, 1541 cm^{-1}, 1273 cm^{-1}, 1115 cm^{-1}. MS (GCMS): *m/z* calcd for $C_{11}H_{22}NO_3$ [M – CO_2Me], 216.16; found, 216.0. Anal. Calcd. For $C_{13}H_{25}NO_5$: C, 56.71; H, 9.15; N, 5.09. Found: C, 56.96; H, 9.12; N, 5.06.



II-27A **II-27B**

Figure II-26. Methyl-2-(2-phenylacetamido)-3-*tert*-butoxy-2-(hydroxymethyl)butanoate (**II-27**).

Methyl-2-(2-phenylacetamido)-3-*tert*-butoxy-2-(hydroxymethyl)butanoate (II-27). Using the general procedure, a suspension of **II-23** (0.24 g, 1.0 mmol), **II-5** (0.16 g, 1.6 mmol), and diphenyl phosphate (27.0 mg, 0.11 mmol) in anhydrous benzene (40 mL) was stirred at room temperature overnight. After washings, the crude reaction intermediate was diluted in THF (6 mL) and cooled to 0 °C before $NaBH_4$ (84.8 mg, 2.2 mmol) and water (3 mL) were added. Purification *via* silica gel chromatography (10% ether / 90% CH_2Cl_2) afforded 0.20 g of the title compound (58% yield) as a 1.1 : 1 ratio of diastereomers. **II-27A** (oil): ^{1}H NMR (500 MHz) ($CDCl_3$): δ 1.02 (s, 9 H), 1.06 (d, J = 6.3 Hz, 3 H), 3.60 (d, J = 5.7 Hz, 2 H), 3.69 (s, 3 H), 3.82 (dd, J_1 = 7.9 Hz, J_2 = 11.7 Hz, 1 H), 4.03 – 4.11 (m, 2 H), 4.43 (dd, J_1 = 5.7 Hz, J_2 = 7.7 Hz, 1 H), 6.53 (broad s, 1 H), 7.25 – 7.36 (m, 5 H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$) δ: 18.8 (–**C**H$_3$), 28.5 (–**C**H$_3$), 44.2 (–**C**H$_2$), 52.5 (–**C**H$_3$), 64.8 (–**C**H$_2$), 68.6 (–**C**H), 69.6 (quaternary –**C**), 74.5 (quaternary –**C**), 127.4 (aromatic –**C**H), 128.9 (aromatic –**C**H), 129.5 (aromatic –**C**H), 134.4 (aromatic quaternary –**C**), 170.8 (quaternary –**C**), 172.0 (quaternary –**C**). IR (neat): 3600 – 3470 cm^{-1}, 3386 cm^{-1}, 1740 cm^{-1}, 1653 cm^{-1}, 1509 cm^{-1}, 1498 cm^{-1}, 1235 cm^{-1}, 1078 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{18}H_{28}NO_5$ [M + H], 338.1967; found, 338.1967. **II-27B** (oil): ^{1}H NMR (500 MHz) ($CDCl_3$): δ 0.87 (d, J

= 6.2 Hz, 3 H), 0.91 (s, 9 H), 3.63 – 3.71 (m, 6 H), 3.74 (dd, J_1 = 2.9 Hz, J_2 = 11.8 Hz, 1 H), 4.02 (q, J = 6.1 Hz, 1 H), 5.55 (dd, J_1 = 2.9 Hz, J_2 = 11.5 Hz, 1 H), 6.46 (broad s, 1 H), 7.24 – 7.36 (m, 5 H). ^{13}C **NMR + DEPT** (125 MHz) ($CDCl_3$) δ: 17.4 (–**C**H_3), 28.3 (–**C**H_3), 44.3 (–**C**H_2), 52.4 (–**C**H_3), 63.8 (–**C**H_2), 69.4 (–**C**H), 71.8 (quaternary –**C**), 74.7 (quaternary –**C**), 127.4 (aromatic –**C**H), 129.0 (aromatic –**C**H), 129.7 (aromatic –**C**H), 134.4 (aromatic quaternary –**C**), 171.0 (quaternary –**C**), 173.5 (quaternary –**C**). IR (neat): 3366 cm^{-1}, 3306 cm^{-1}, 1754 cm^{-1}, 1665 cm^{-1}, 1522 cm^{-1}, 1281 cm^{-1}, 1063 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{18}H_{28}NO_5$ [M+H], 338.1967; found, 338.1967.

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CHAPTER III
UTILIZATION OF OXAZOL-5(4H)-ONES TOWARDS THE SYNTHESIS OF PROTEASOME INHIBITORS

A. Introduction to the 26S proteasome.

The synthesis and degradation of cellular proteins are integral processes involved in maintaining biological homeostasis. Protein degradation, or proteolysis, plays an essential role in many basic biological processes including cell cycle control, cell differentiation, and apoptosis.[1] Proteolysis is also a cellular method used to regulate protein quality control, whereby misfolded, mutant, and damaged proteins can be removed from the cellular environment.[2] The sites of proteolysis are compartmentalized within cells to prevent unregulated protein hydrolysis, a process that would prove to be deleterious. In eukaryotic cells, proteolysis occurs either inside the lysosome, by action of the enclosed proteases, or in the proteasome.[3] However, it is commonly recognized that the majority of intracellular proteolysis in all eukaryotic cells occurs within the proteasome.[4]

The 26S proteasome is a large 2.5 MDa complex consisting of a core 20S particle, also known as the 20S proteasome, sandwiched between two 19S regulatory complexes, (Figure III-1).[5-8] Protein hydrolysis occurs within the cavity of the 20S particle, a cylindrical structure comprised of four superimposed rings (Figure III-1).[9] The two outer α-rings are each made up of seven different α-subunits which surround a narrow entry channel into the 20S proteasome, and the two inner β-rings each consist of seven β-subunits.[7] Three of the seven β-

subunits (β1, β2, and β5) in each of the two β-rings are catalytically active, and proteins are hydrolyzed at these active sites *via* interaction with nucleophilic N-terminal threonine residues.[10] The three different active sites of protein hydrolysis are often described based on their preference towards protein substrates. The β1, β2, and β5 subunits exhibit specificity towards acidic, basic, and hydrophobic residues respectively, such that they are said to have respective caspase-like, trypsin-like, and chymotrypsin-like activities.[6] However, studies have demonstrated that the active sites within the proteasome are not entirely specific with relation to the proteins they hydrolyze.[11]

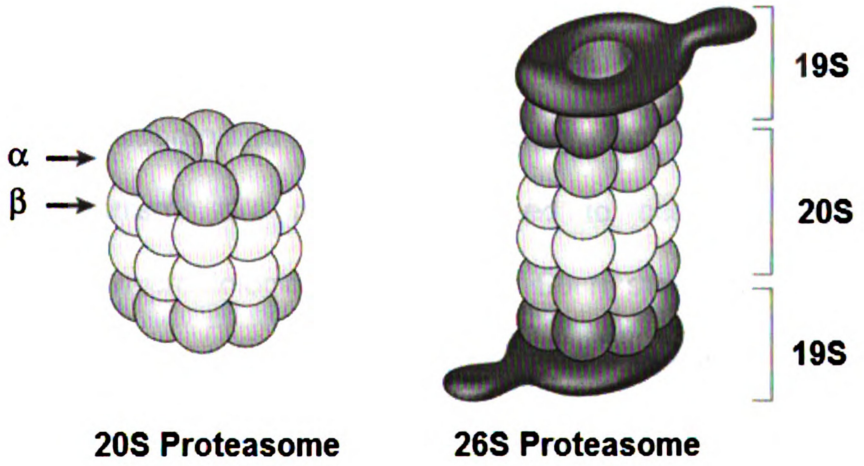


Figure III-1. Representation of the 20S and 26S mammalian proteasomes.[12]

Protein degradation occurs selectively in the cytosol and nucleus of eukaryotic cells by means of the ubiquitin-proteasome pathway. Proteins become selected or "tagged" for degradation by the proteasome by means of poly-ubiquitination, an enzymatic process by which a chain of ubiquitin molecules becomes covalently bound to the substrate.[13,14] Poly-ubiquitinated peptides are

recognized by the 19S regulatory complex and are shuttled into the 20S proteasome. The 19S regulatory complex is critical for this process, and its involvement includes binding poly-ubiquinated proteins, cleaving the poly-ubiquitin chain, unfolding the substrate, and translocating it into the narrow entry channel of the outer α ring of the 20S proteasome.[6,15,16] Protein hydrolysis then occurs with the assistance of water molecules at active sites within the β rings to generate peptide fragments. The fragments may then undergo further proteolytic events until oligopeptides are generated which are sufficiently small enough, usually three to 22 amino acid residues in length, to diffuse through the proteasomal entry channels.[8,17]

B. The 26S proteasome and the NF-κB pathway.

Multicellular organisms are routinely exposed to chemical, environmental, mechanical, and microbiological stresses.[18] Such stresses are managed in organisms at the cellular level by inducible gene regulation. Inducible gene regulators, such as the nucleic transcription factor NF-κB, are present within cells and react to cellular stresses by transcribing a variety of genes as required.[19]

The NF-κB pathway has been a longstanding target of interest in the scientific community. The NF-κB pathway is implicated in cellular responses such as inflammation and apoptosis,[20,21] and it is one of the many biological pathways in eukaryotic cells regulated through the ubiquitin-proteasome pathway.[22] Under normal cellular conditions, NF-κB is located within the cytoplasm and is complexed to the IκB protein (Figure III-2). However, cellular

stimulation by an agonist, such as chemotherapy, radiation, viruses, antigens, or oxidants causes a cascade of events in which IκB undergoes successive phosphorylation, poly-ubiquitination, and then proteolysis by the 26S proteasome.[12] Following the degradation of IκB, newly liberated NF-κB translocates to the nucleus where a transcription event occurs, resulting in the inhibition of the cellular event known as apoptosis, which is essentially programmed cell death.

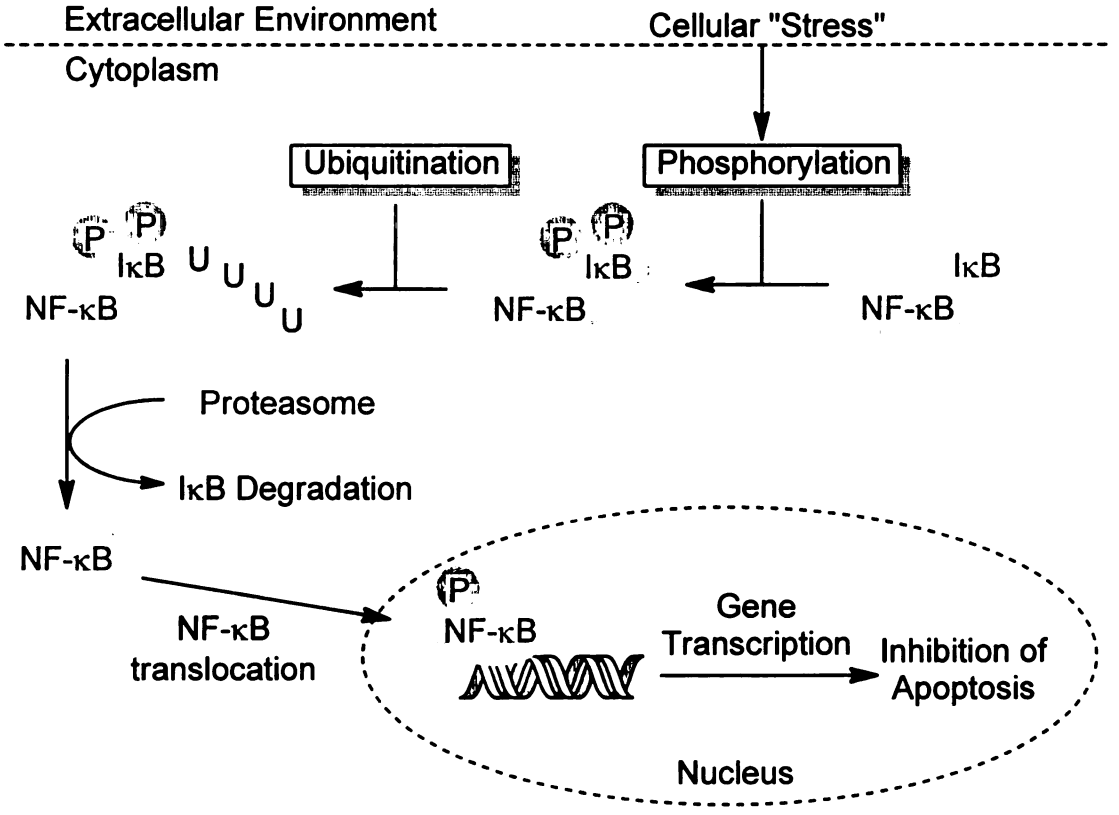


Figure III-2. The role of the proteasome in the NF-κB pathway.

Recent discoveries concerning the NF-κB pathway have resulted in a better understanding of diseases and their treatments, notably in the area of anticancer research.[18,21,23-26] Treatments traditionally used in anticancer therapy,

such as chemotherapy and radiation, trigger the aforementioned cascade of events that lead to release of NF-κB and subsequent transcription of genes responsible for anti-apoptosis[27-30] (Figure III-2). By this mechanism, cancerous cells survive anticancer treatments and are allowed to continue their rampant replication. As noted above, NF-κB cannot translocate to the nucleus and trigger the anti-apoptotic pathway until it is released from its complex with IκB. Since the proteasome is responsible for the degradation of the NF-κB/IκB complex, it has become an attractive target in anticancer therapy for sequestering NF-κB-mediated anti-apoptosis. Indeed, proteasome inhibition has emerged as a useful means of impeding cancerous cell survival, so much so that proteasome inhibitors have made their way into clinical use for anticancer treatment.[31] In 2003, the proteasome inhibitor bortezomib, also known as Velcade®, was approved by the FDA for the treatment of multiple myeloma, and in 2005 it was approved for the treatment of non-Hodgkin lymphoma.[32-34] Since then, other small molecule proteasome inhibitors, such as carfilzomib and the natural marine metabolite salinosporamide A, have since entered clinical studies for use as anticancer agents.[35-40]

C. Proteasome inhibition by small molecules.

Inhibition of the proteasome has been an intense focus of research in recent years. A wealth of information regarding proteasomal structure and activity has been attained from many of these studies through the use of small molecule proteasome inhibitors.[7,9,10,41-46] Numerous proteasome inhibitors are currently

known, having been both isolated from natural sources and prepared synthetically (Figure III-3). A number of these small molecules that inhibit the proteasome also inhibit certain cellular proteases. Additionally, some proteasome inhibitors exhibit little selectivity towards the different active sites of the proteasome. As such, many synthetic proteasome inhibitors have been designed to demonstrate excellent specificity towards the proteasome and even to specific active sites.[9,17,47] For example, vinyl sulfone Ac-APnLL-VS (Figure III-3) exhibits potent and specific inhibition of the caspase-like active site of the 20S proteasome.[9] Additionally, the demand for new clinical candidates for use in disease treatment has required the preparation of small molecule proteasome inhibitors which exhibit reduced toxicity.

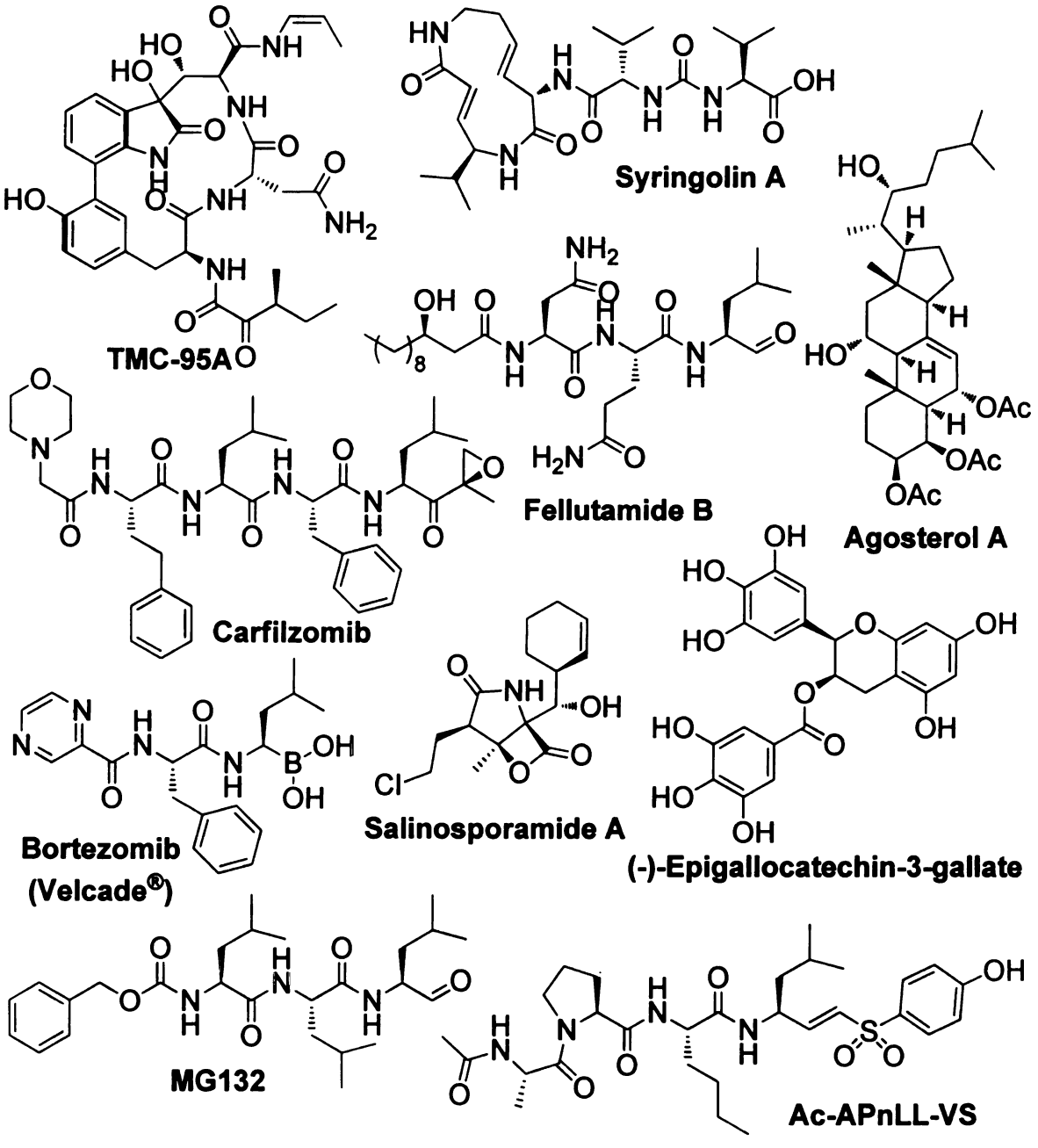
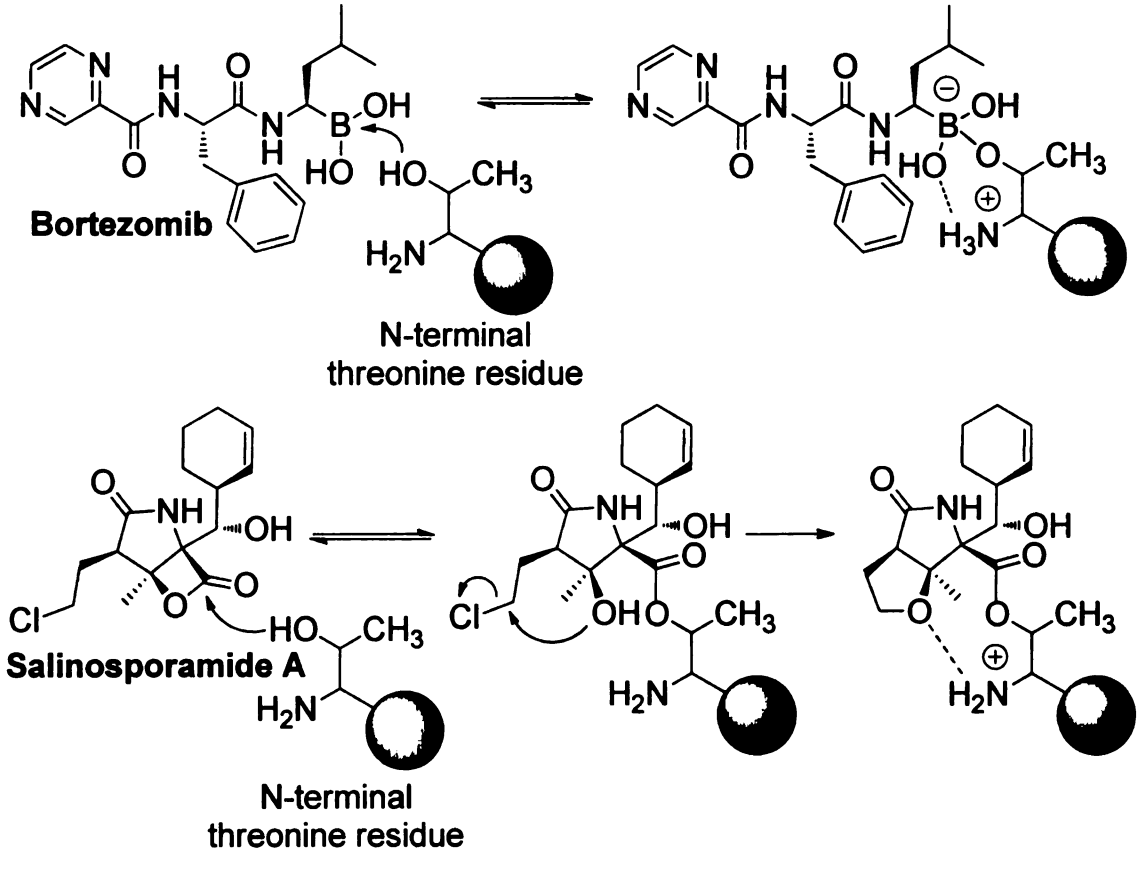


Figure III-3. Natural and synthetic proteasome inhibitors.

As was previously discussed, each protolytically active site within the 20S proteasome contains an N-terminal threonine residue which acts to catalytically cleave polypeptide amide bonds. Inhibitors of the 20S proteasome generally contain electrophilic centers to which the N-terminal threonine residues

covalently bond (Scheme III-1).[9,17,48] Thus, irreversible or slowly reversible bond formation to small molecule inhibitors inactivates the proteasome towards further proteolysis due to occupation of an active site. Proteolytic functionality of the active site can only be reestablished once the covalent linkage as been abolished. Several electrophilic functionalities exist within small molecules which make them ideally suited for proteasome inhibition, including aldehydes, epoxy ketones, β-lactones, esters, α,β-unsaturated amides, vinyl sulfones, and boronic esters (Figure III-3). Of these functionalities, only boronic esters and vinyl sulphones are representative of totally synthetic classes of proteasome inhibitors.[9,49]



Scheme III-1. Mechanism of proteasomal inhibition by bortezomib and salinosporamide A.

However, a few compounds lacking the aforementioned electrophilic functionalities are known to inhibit proteolytic activity.[47,50-54] One such molecule, TMC-95A, has been shown to competitively inhibit all active sites of the 20S proteasome through the formation of H-bonding networks .[53-56] Disruption of such noncovalent interactions and subsequent restoration of proteolytic activity has been performed *via* dialysis, thereby demonstrating reversible inhibition by TMC-95A.[53,54] The discovery of this unique mode of proteasome inhibition has energized researchers to prepare analogs of the natural product.[50]

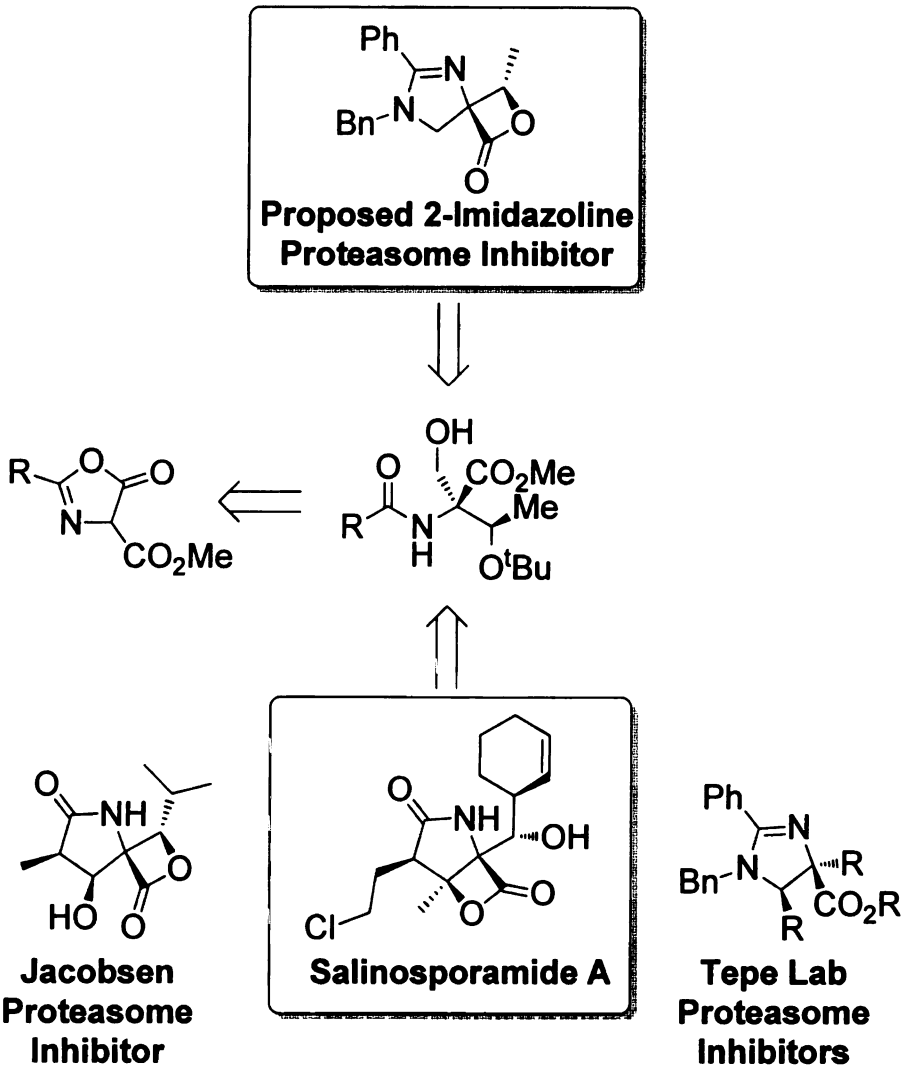
Certain molecules can also inhibit proteolytic activity through allosteric interactions. Inhibition of the 20S proteasome and the 26S proteasome has been recently demonstrated through treatment with porcine bone-marrow derived 39-amino acid peptide PR39 and related synthetic analogs.[57] These polypeptides, in which proline and arginine residues are abundant, cause gross changes in proteasomal shape, as has been observed *via* atomic force microscopy.[57] The degree to which these peptides inhibit proteolysis has been shown to vary with changes in pH, suggesting that the allosteric changes are promoted by strong ionic and H-bonding interactions.[57]

Recently, studies in the Tepe lab have demonstrated inhibition of the chymotrypsin-like activity of the 20S proteasome *via* treatment with appropriately substituted 2-imidazolines. The evaluated 2-imidazolines do not appear to covalently bind to the active site N-terminal threonine residues. Instead, it is currently proposed that the 2-imidazolines inhibit the proteasome allosterically. To the best of our knowledge, no small molecules have ever been reported to

inhibit the proteasome allosterically. This interesting discovery has prompted our investigation into the synthesis of small libraries of novel 2-imidazolines to better understand their mode of proteasome inhibition.

The 2-imidazolines prepared and evaluated in our lab have demonstrated non-competitive inhibition of the chymotryspin-like active site of the 20S proteasome at low micromolar concentrations. Conversely, competitive inhibition is observed when the proteasome is treated with small molecule inhibitors comprised of pyrrolidinone and electrophilic β-lactone moieties, such as salinosporamide A, omuralide, and a novel spiro-β-lactone compound prepared in Jacobsen's lab (Scheme III-2).[58] Moreover, these small molecules inhibit the chymotryspin-like active site at low nanomolar concentrations. All thus-far evaluated 2-imidazolines prepared in our lab (Scheme III-2) have lacked an electrophilic functionality, the likes of which are known to covalently adhere to proteasomal active site threonine residues. Therefore, we became interested in determining the effect that β-lactone incorporation into a 2-imidazoline might have on the potency and mode of proteasome inhibition. We sought, then, to synthesize a 2-imidazoline bearing a spiro-β-lactone, which we anticipated could be derived from a *tert*-alkyl amino hydroxy carboxylic ester prepared through oxazol-5(4H)-one alkylation chemistry developed in the Tepe lab (Scheme III-2).[59] In addition, we envisioned that salinosporamide A could also be constructed from a similar *tert*-alkyl amino hydroxy carboxylic ester starting material (Scheme III-2). The remainder of Chapter III will describe our endeavors towards 1) the racemic formal total synthesis of salinosporamide A and 2) the

synthesis and evaluation of the proposed β-lactone-containing 2-imidazoline proteasome inhibitor.



Scheme III-2. Strategy for the synthesis of salinosporamide A and a novel β-lactone-containing 2-imidazoline.

D. Introduction to salinosporamide A.

Numerous biologically active compounds have been isolated from marine sources, resulting in the development of new therapeutic agents.[60-62] In 2003, efforts by Fenical and co-workers to discover new marine natural products resulted in the cultivation and characterization of salinosporamide A from a strain

of seawater-requiring actinomycete bacteria (Figure III-4).[63,64] Salinosporamide A has been shown to inhibit the 26S proteasome, and is currently being used in clinical trials for anticancer therapy.[6,33,34] The structure of salinosporamide A comprises a bicylic core, composed of a pyrrolidinone and a β-lactone. These structural features are critical for the biological activity of natural product[64,65] and are common to the biologically active terrestrial microbial products omuralide[66] and cinnabaramide A[67] (Figure III-4). Many of the structural features necessary for activity in compounds of this class have been identified through structure-activity relationships,[68] synthesis of congeners (e.g. **III-1 – III-3**, Figure III-4),[58,68-78] and X-ray crystallography.[42,43] The complex molecular structure combined with the potent biological activity has made salinosporamide A an attractive synthetic target; consequently, several groups have reported total syntheses of the marine metabolite.[79-85] Even though many elegant syntheses have been reported of this intriguing class of proteasome inhibitors, its potential clinical relevance warrants the development of new synthetic approaches that may provide access to additional candidates.

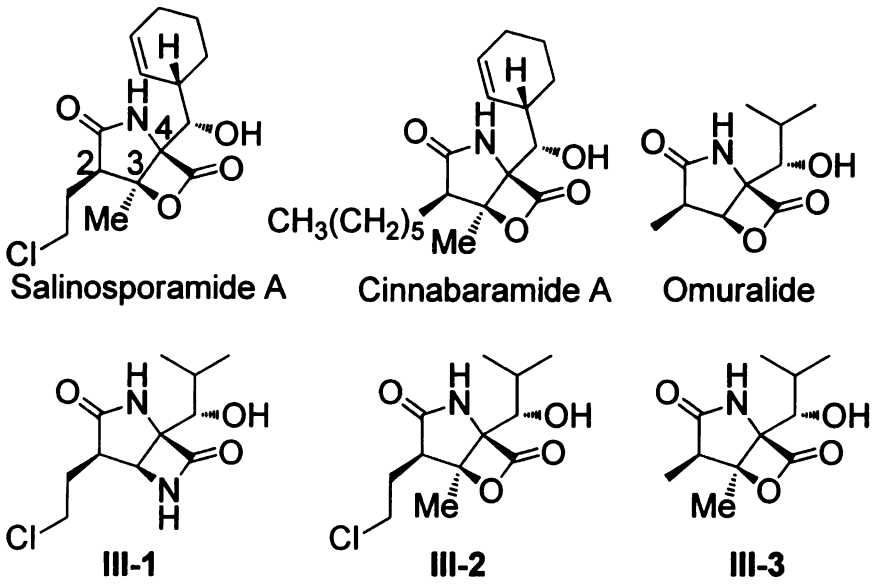


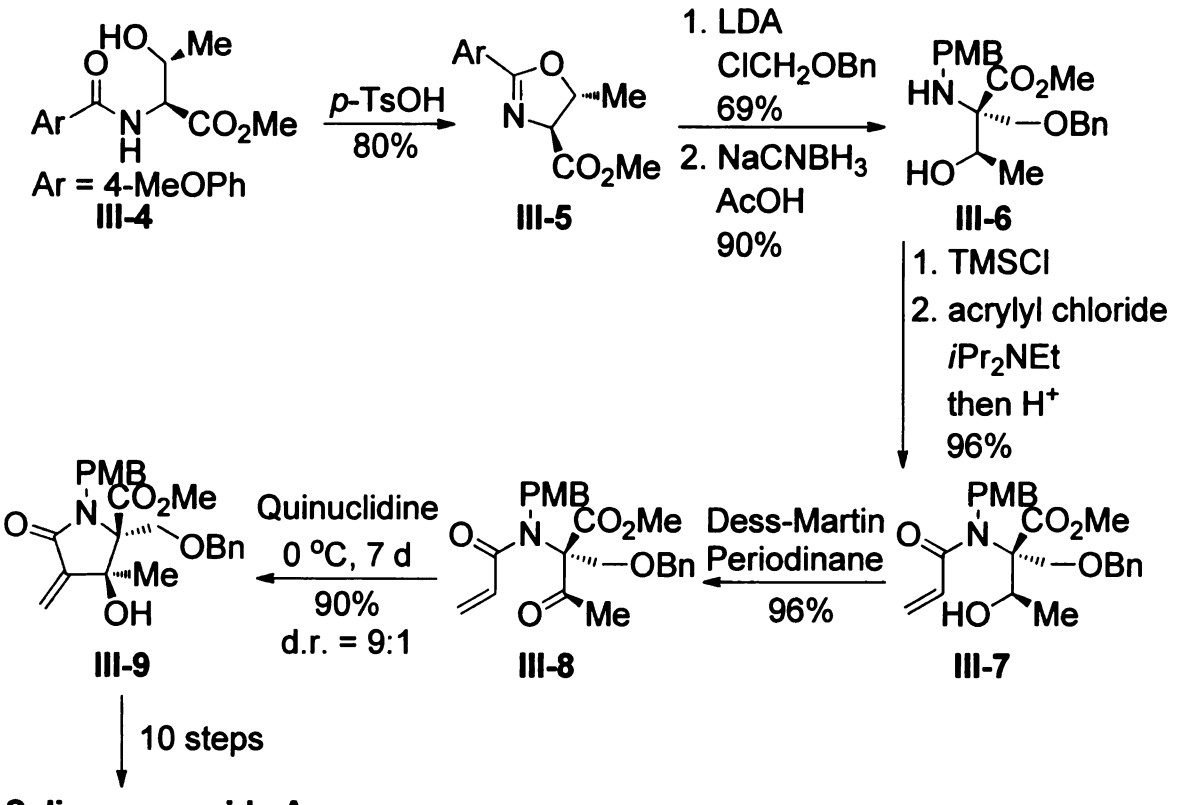
Figure III-4. Natural pyrrolidinone proteasome inhibitors and related congeners.

E. Syntheses of salinosporamide A.

Several structural features are present in salinosporamide A which have made it an attractive and challenging synthetic target. The natural product contains five contiguous stereogenic centers, two of which are quaternary, which bisect a bicyclic pyrrolidinone/β-lactone ensemble and extend through a cyclohexenyl substituent. Also featured is a chloroethyl substituent at C2 on the pyrrolidinone ring (numbering shown in Figure III-4), which has been shown to contribute to the potency of the marine metabolite as a proteasome inhibitor.[68,86,87] The quaternary stereocenter located at C4 of the pyrrolidinone ring is a *tert*-alkyl hydroxy carboxylic ester, a quaternary α-amino acid subtype which was described in Chapter II.

Salinosporamide A was first synthesized in 2004 in the labs of E. J. Corey.[84] The authors prepared the natural product in 17 linear steps beginning

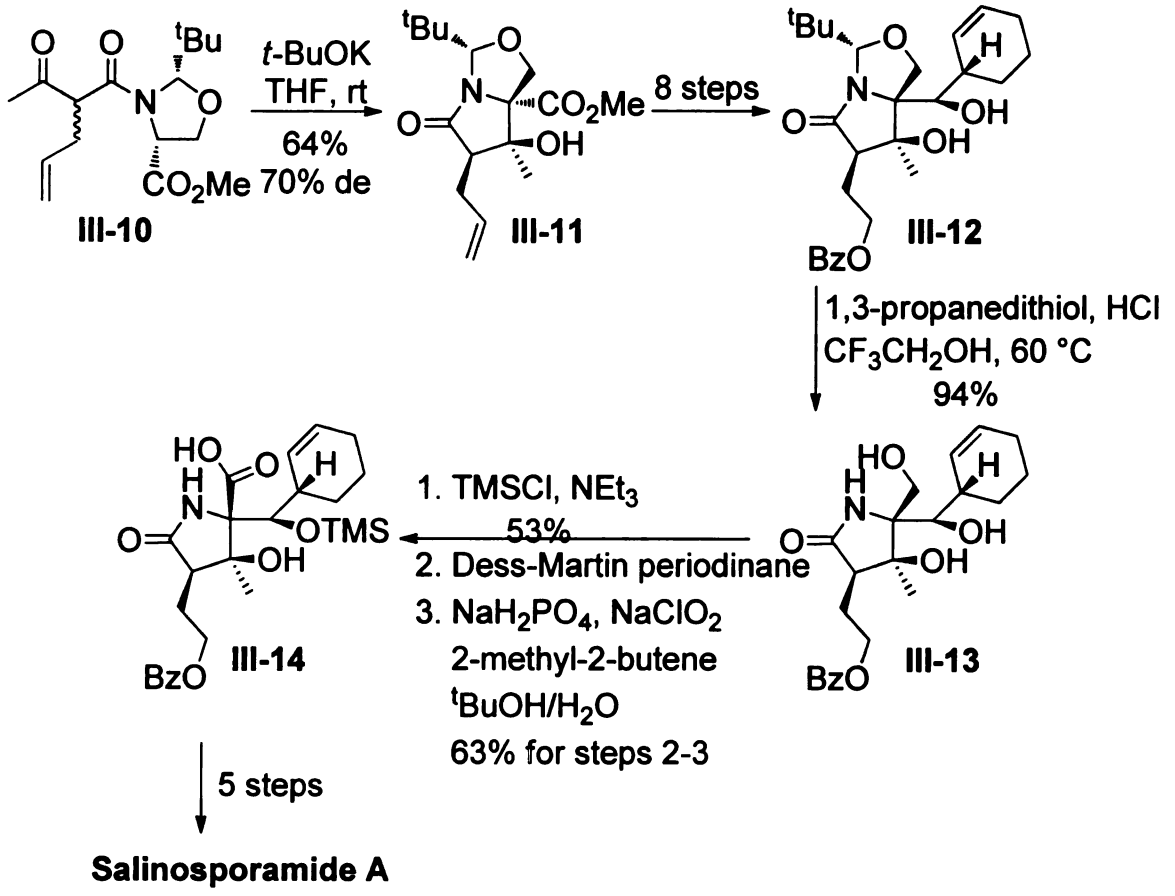
from (S)-N-anisoyl threonine methyl ester **III-4** (Scheme III-3). Key to their synthetic strategy was early construction of the *tert*-alkyl amino hydroxy carboxylic acid center, followed by successive formation of the pyrrolidinone and β-lactone rings. The authors synthesized the *tert*-alkyl amino hydroxy carboxylic acid center present in salinosporamide A from an 2-oxazoline intermediate.[84] 2-oxazolines are known to undergo a variety of transformations and have been used to construct heterocycles and amino acid derivatives.[88-90] Moreover, 2-oxazolines have previously been used to construct the *tert*-alkyl amino hydroxy carboxylic acid centers found in omuralide and lactacystin.[91,92] 2-oxazoline **III-5** was first prepared through cyclodehydration of **III-4** with *p*-TsOH (Scheme III-3).[84] Selective installation of the desired ether-functionalized methylene substituent was then performed *via* treatment of **III-5** with LDA and chloromethyl benzyl ether, followed by acidic hydride reduction of the resultant quaternary 2-oxazoline to afford amino alcohol **III-6**. Successive N-acylation and alcohol oxidation reactions gave rise to keto amide **III-8**, which was utilized to construct the desired pyrrolidinone ring through a diastereoselective Baylis-Hillman-aldol reaction (d.r. = 9:1) to yield **III-9**. The authors later reported the construction of a structurally similar pyrrolidinone ring in high diastereoselectivity (d.r. = >99:1) through an unprecedented use of the Kulinkovich reagent.[73,74]



Scheme III-3. Corey's synthesis of the pyrrolidinone ring of salinosporamide A.

The *tert*-alkyl amino hydroxy carboxylic ester stereocenter present in salinosporamide A has since been synthesized from oxazolidines, the saturated analogs of 2-oxazolines. Total syntheses reported independently by the Potts and Danishefsky groups described the use of oxazolidine ring systems as templates for the asymmetric construction of the highly functionalized stereocenter.[79,81] The total synthesis reported by Potts and co-workers involved early preparation and use of N-acyl oxazolidine **III-10**. (Scheme III-4).[81] Exposure of **III-10** to basic conditions was performed to promote intramolecular Aldol condensation, thereby establishing the C2, C3, and C4 stereocenters present in pyrrolidinone intermediate **III-11**. In later synthetic steps, decomposition of the oxazolidine ring was accomplished under acidic conditions,

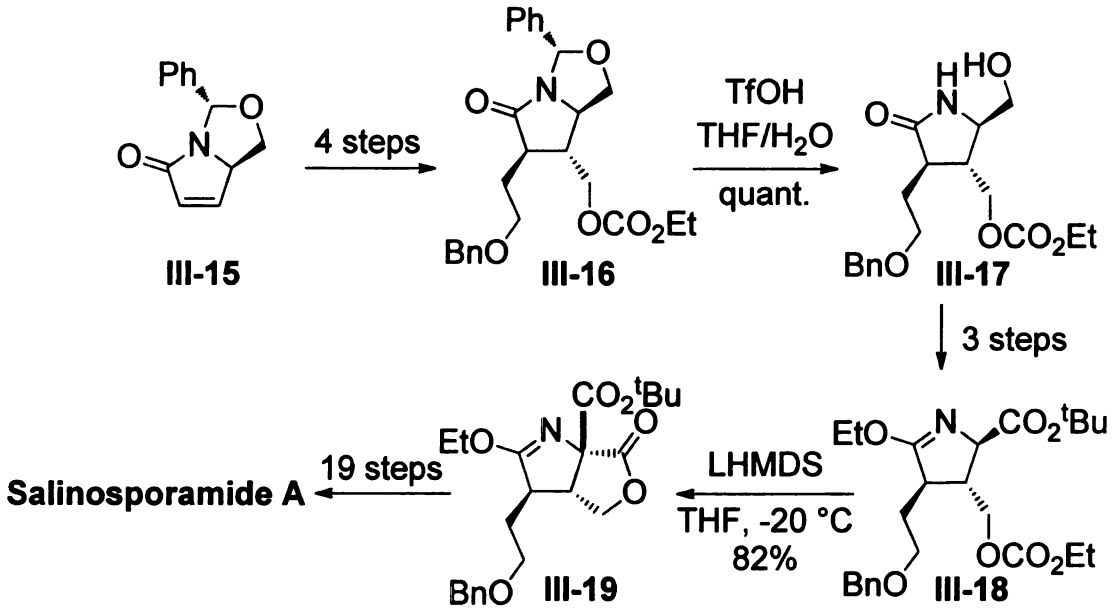
and the resultant alcohol **III-13** was oxidized to yield chiral quaternary amino acid-containing **III-14**.[81]



Scheme III-4. Quaternary α-amino acid center preparation *via* an oxazolidine.

Danishefsky and co-workers prepared the quaternary amino acid center by an alternate means in their total synthesis, instead utilizing bicyclic oxazolidine **III-15** in which the pyrrolidinone framework was already present (Scheme III-5).[79] Similar to the Potts total synthesis, their synthesis also involved acidic oxazolidine ring cleavage and subsequent oxidation to form the carbonyl functionality present in **III-18.** However, this sequence was performed early and prior to quaternization of the amino acid center. Imidate protection of the lactam

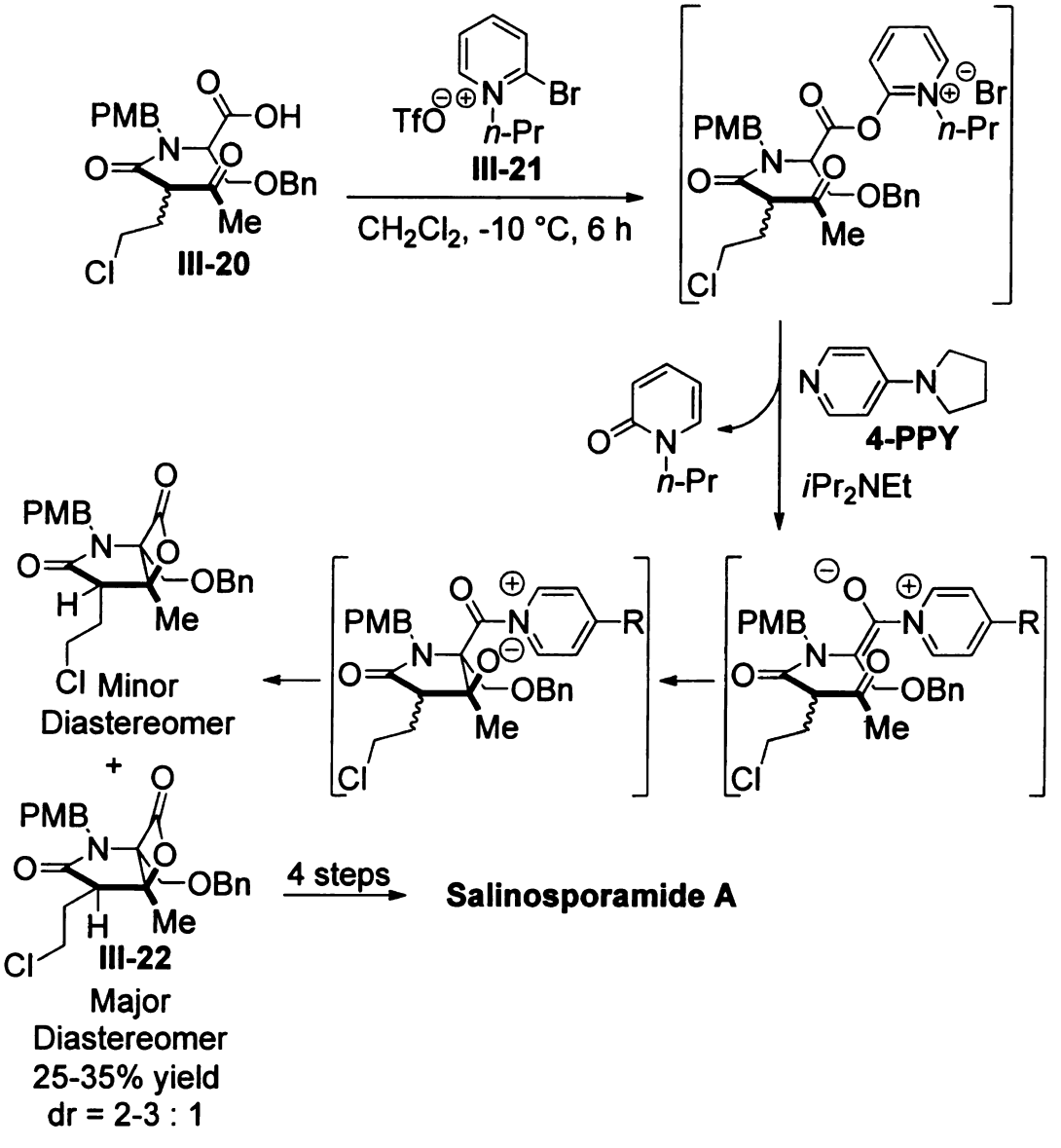
present in **III-18** followed by *cis*-fused bicyclic ring formation *via* lactonization under basic conditions furnished the anticipated quaternary center in bicyclic adduct **III-19**. An additional 19 synthetic steps were then required to complete the total synthesis.[79]



Scheme III-5. The Danishefsky synthesis of salinosporamide A.

Another common approach for the synthesis of the quaternary α-amino acid center in salinosporamide A is the construction of the pyrrolidinone ring *via* C3-C4 bond formation.[82,83,85] The Romo group reported one such total synthesis of the natural product wherein the fused pyrrolidinone/β-lactam ring system was generated *via* bis-cyclization.[82] The authors initially prepared β-ketoamide **III-20** in 4 steps from commercially available O-benzyl-L-serine. Treatment of **III-20** with modified Mukaiyama reagent **III-21**, organocatalyst **4-PPY**, and Hünig's base afforded bicyclic adduct **III-22** as a mixture of diastereomers (Scheme III-6). The authors propose that the bis-cyclization occurs in a stepwise fashion, whereby an

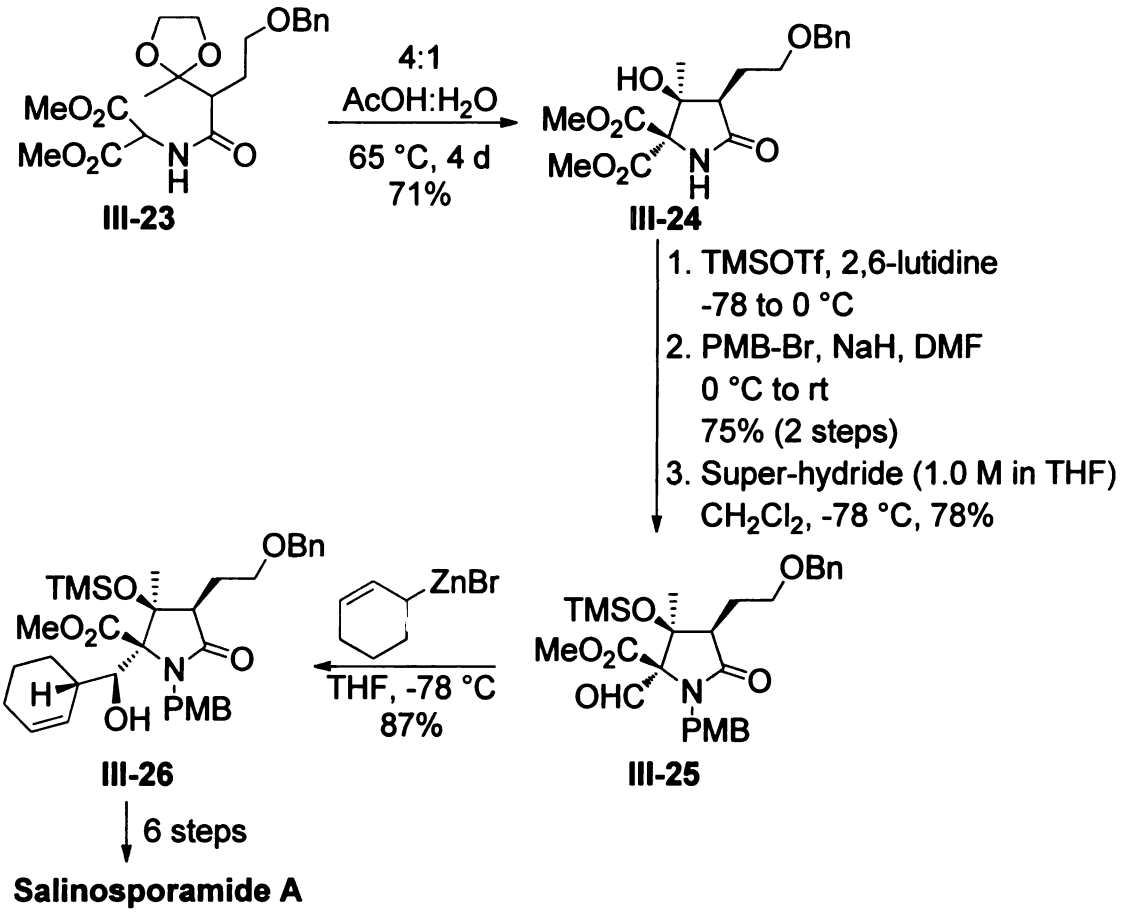
intramolecular aldol reaction first occurs through an ammonium enolate to form the pyrrolidinone ring followed by lactonization of the resultant alkoxide intermediate.[82] In this way, the authors prepared an advanced racemic intermediate containing the fused ring ensemble with an intact chloroethyl substituent, all with the correct relative stereochemistry. The synthesis then concluded through removal of the benzyl protecting groups and installation of the cyclohexenyl substituent using Corey's protocol.[84] The Romo synthesis is quite remarkable in that the labile β-lactone and halogen functionalities are installed in early synthetic stages. In fact, Romo's synthesis is the only reported total synthesis of salinosporamide A which grossly strays from Corey's original protocol involving successive β-lactone formation and halogen installation during the final synthetic steps.[84]



Scheme III-6. Pyrrolidinone/β-lactone formation *via* bis-cyclization.

The synthesis of the C4 stereocenter of salinosporamide A has also been performed through desymmetrization of malonate-containing pyrrolidinones.[83,85] The Pattenden group first utilized this approach in their total synthesis of the marine metabolite (Scheme III-7). The authors prepared and treated amido malonate **III-23** with acetic acid to perform an *in situ* deprotection of an acetal-protected ketone and subsequent diastereoselective aldol cyclization to form

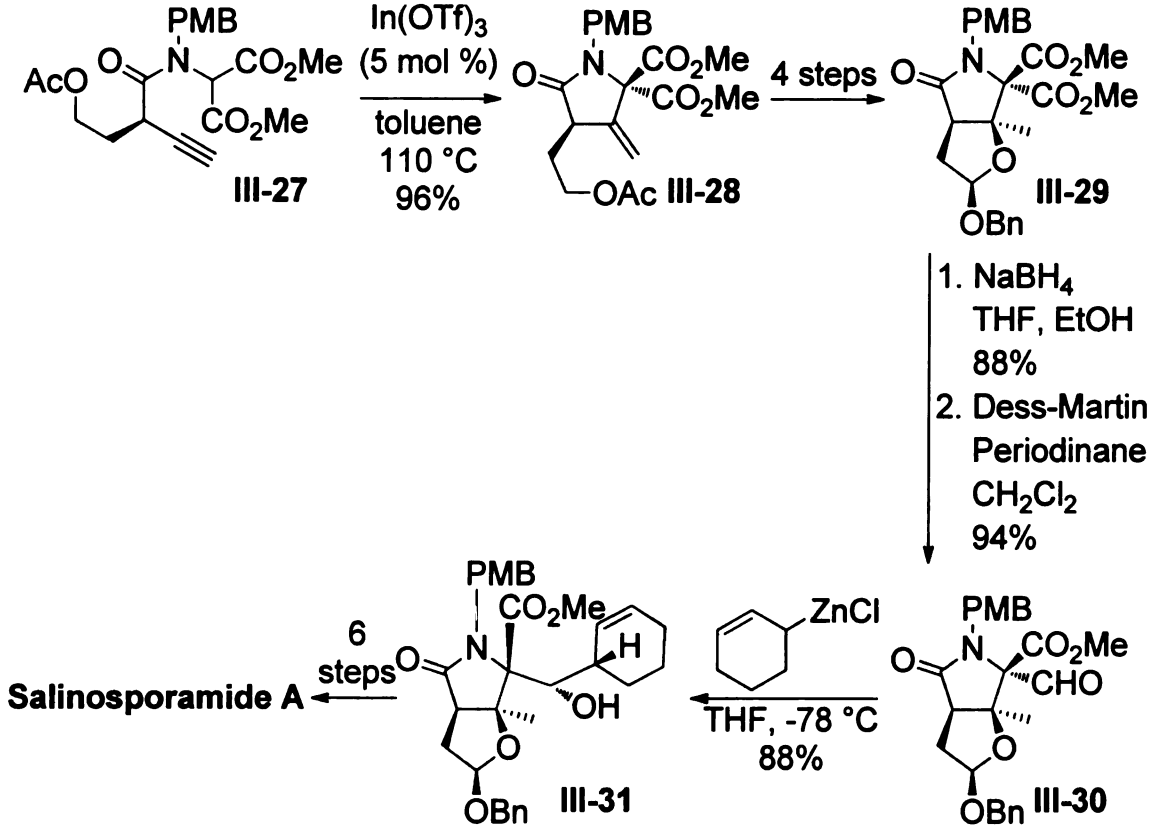
pyrrolidinone **III-24**. Next, protection of the alcohol and amide functionalities present in **III-24** preceded regioselective ester reduction to afford aldehyde **III-25**. Installation of the cyclohexenyl substituent according to Corey's protocol[84] then furnished **III-26** with the desired functionalities positioned about the C4 stereocenter.



Scheme III-7. Pattenden synthesis of salinosporamide A.

Desymmetrization of a malonate intermediate was also featured in the total synthesis of salinosporamide A reported by Hatakeyama and coworkers.[85] The authors utilized an intramolecular Conia-ene reaction to construct the pyrrolidinone scaffold in **III-28**, and in later steps, substrate-controlled selective

ester reduction and subsequent oxidation afforded aldehydic ester **III-30** (Scheme III-8). Only seven more steps, including cyclohexenyl installation *via* Corey's protocol,[84] were then required to complete their total synthesis.

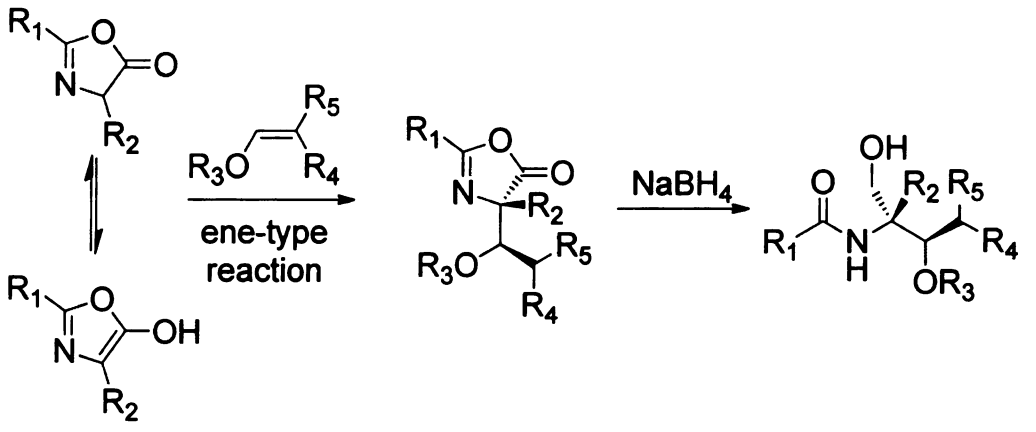


Scheme III-8. Hatakeyama synthesis of salinosporamide A.

F. Use of oxazol-5(4H)-ones towards the synthesis of salinosporamide A.

In Chapter II the diastereoselective synthesis of *tert*-alkyl amino hydroxy carboxylic esters by means of a sequential alkylation reaction of oxazol-5(4H)-ones with enol ethers followed by hydride reduction was described (Scheme III-9).[59] The functionalities surrounding the quaternary stereocenter formed in the reaction are represented at the C4 center in salinosporamide A. To the best of

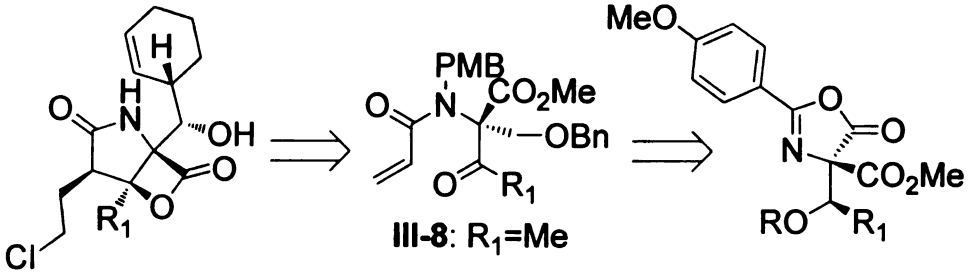
our knowledge, this center had never been constructed *via* an oxazol-5(4H)-one intermediate.



Scheme III-9. Intermolecular alkylation/reduction reaction of oxazol-5(4H)-ones.

As previously discussed, the total synthesis of salinosporamide A by Corey and co-workers involves the construction of pivotal intermediate **III-8**, which undergoes cyclization *via* a Baylis-Hillman-aldol reaction to give the core pyrrolidinone scaffold (Scheme III-10).[84] Moreover, recent formal syntheses of salinosporamide A have been reported in which molecules structurally analogous to **III-8** have been prepared for use in pyrrolidinone formation.[75,76] We anticipated that this key intermediate in Corey's synthesis should also be readily accessible from our chemistry after a few functional group modifications. Due to the skeletal diversity afforded by the use of various enol ethers in our oxazol-5(4H)-one alkylation reaction (Table II-4), this could potentially serve as a strategy for the synthesis of C3 analogues of salinosporamide A (Scheme III-10). We sought to prepare Corey's intermediate **III-8**,[74,84] then, in order to

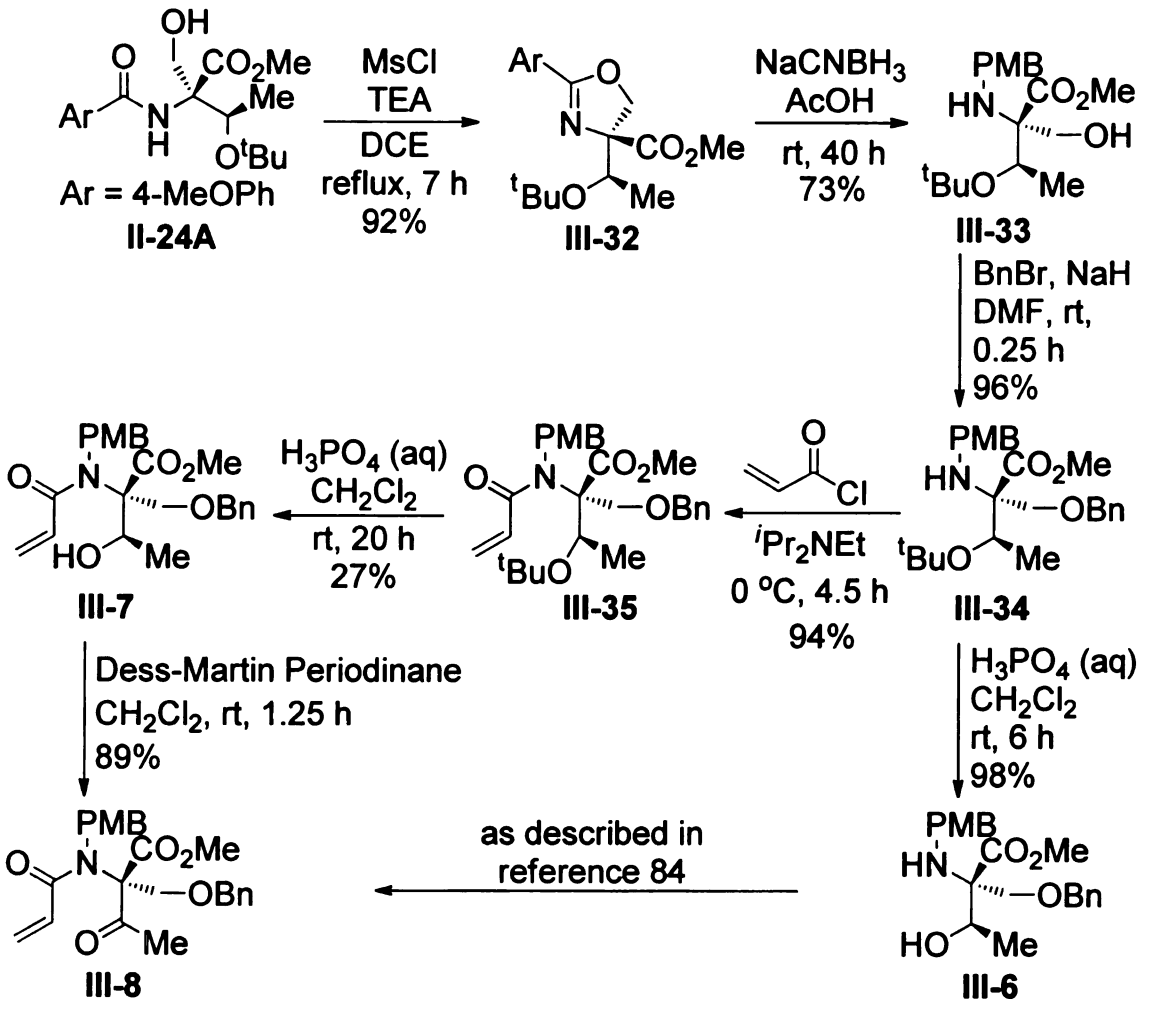
demonstrate the utility of quaternary centers produced *via* the oxazol-5(4H)-one template for the synthesis of salinosporamide A and related congeners.



Scheme III-10. Strategy for the synthesis of Corey intermediate **III-8**.

The starting material for our synthesis of **III-8** was obtained through our alkylation/reduction reaction protocol to yield amino hydroxy carboxylic ester **II-24** as a 3:1 mixture of diastereomers in 88% yield (Table **II-5**).[59] For the synthesis of **III-8,** only the major diastereomer **II-24A** was used, as indicated in Scheme III-10. However, it should be noted that the mixture of diastereomers could be used to complete the racemic synthesis of salinosporamide A due to the destruction of the second stereocenter during the oxidation of alcohols **III-7** or **III-6** (Scheme III-11).[93] The synthesis of **III-8** initiated with the reduction of the amide functionality present in *tert*-alkyl amino hydroxy carboxylic ester **II-24A**. This reduction was performed smoothly *via* cyclodehydration under basic conditions with MsCl followed by oxazoline reduction with sodium cyanoborohydride in acetic acid to afford amino-alcohol **III-33** (Scheme III-11). This transformation accomplished two goals in our synthesis: 1) the reduction of a stable amide in the presence of an ester, a feat we found to be unsuccessful under a variety of attempted conditions, and 2) the protection the amine as a PMB amine. The resultant

primary alcohol was treated under basic conditions with benzyl bromide to give benzyl ether **III-34** in 96% yield without any N-alkylation, an observation in agreement with similar alkylation reactions of 1,2-amino alcohols.[94] Acylation of the secondary amine with acrylyl chloride under Corey's conditions[84] then gave amide **III-35** in 94% yield. Unfortunately, deprotection of the *tert*-butyl ether in **III-35** using acidic conditions proved to be problematic. The reaction was often messy and low yielding, as treatment with TFA or aqueous phosphoric acid gave 25% and 27% yields respectively of alcohol **III-7** as a single diastereomer. Finally, oxidation of the free alcohol in **III-7** was performed using the reported procedure[84] to cleanly afford racemic ketone **III-8**, the spectroscopic data of which matches that of the known compound.



Scheme III-11. Racemic synthesis of Corey intermediate **III-8**.

The disappointing yields obtained from the deprotection of the *tert*-butyl ether prompted investigation into its removal at an earlier stage of the synthesis. The original synthesis reported by Corey involved access to **III-8** through modification of alcohol **III-6**. We anticipated **III-6** to also be accessible in our synthesis through amendment of our synthetic route. Thus, deprotection of the *tert*-butyl ether in compound **III-34** was performed by treatment with aqueous phosphoric acid[95] to afford secondary alcohol **III-6** in near quantitative yield, thereby granting access to desired compound **III-8**.

This racemic synthesis of **III-8** was completed in just 6 steps with a 15% overall yield starting from **II-24A**. However, racemic **III-6** was derived from **III-24A** in just 4 steps with an overall yield of 63%. In comparison, Corey's original route featured a six step synthesis starting from (S)-threonine methyl ester to construct **III-6** in 33% yield.[84,96] While our racemic synthesis of **III-8** (and **III-6**) appears no more efficient than Corey's original protocol, it demonstrates a useful synthetic application of highly functionalized molecules derived from oxazol-5(4H)-one alkylation products.

G. Introduction to 2-imidazolines.

2-imidazolines have gained recognition for their ability to affect numerous biological processes and for their utility as useful synthetic building blocks (Figure IV-5). In biological systems, several 2-imidazolines have high affinity for imidazoline binding sites (IBS), also known as imidazoline receptors. These IBS are of interest to researchers for their role in hypertension, blood pressure regulation, opioid dependence and tolerance, and neurological disorders.[97] Studies have also indicated the possible application of 2-imidazolines as antihyperglycemic,[98] anti-inflammatory,[99] antihypertensive,[100] and antihypercholesterolemic[101] agents. Additionally, some highly substituted 2-imidazolines known as Nutlins have recently been found to bind to the MDM2 protein, thereby liberating p53 to suppress tumor growth in human cancer.[102] Certain 2-imidazolines have also been found to sensitize leukemia T cells towards chemotherapeutic agents.[103,104] Furthermore, 2-imidazolines have been

used as building blocks for the construction pharmaceutically relevant molecules such as azapenams, dioxocyclams, and diazapinones.[105,106]

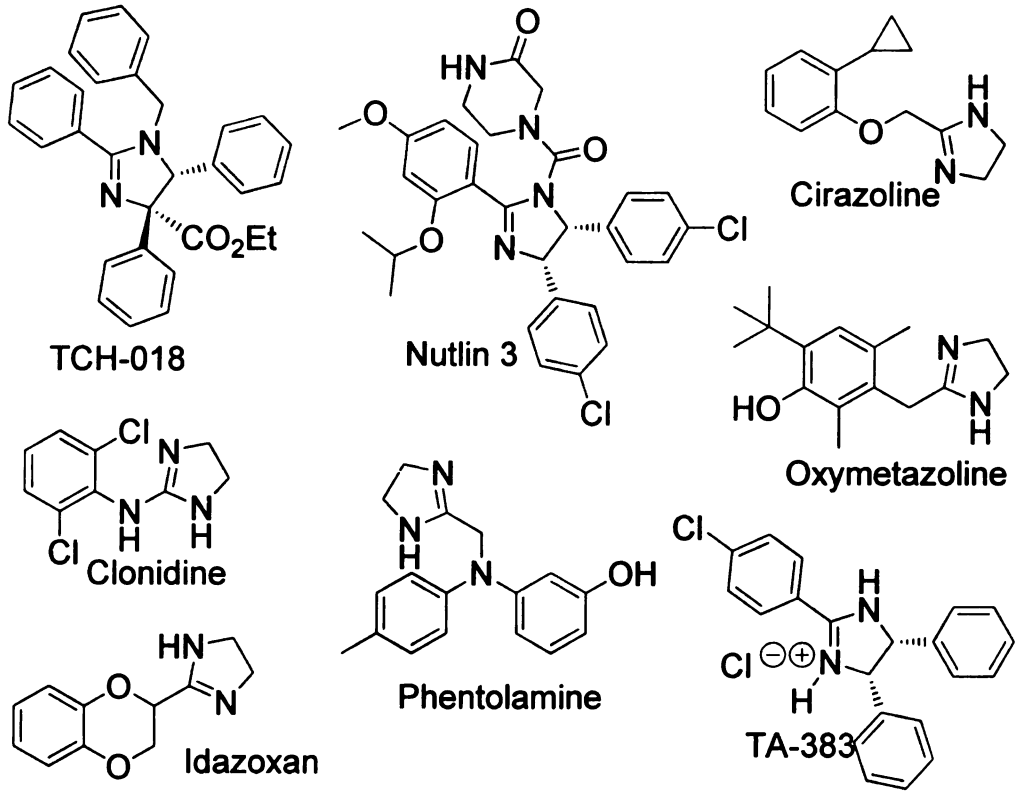
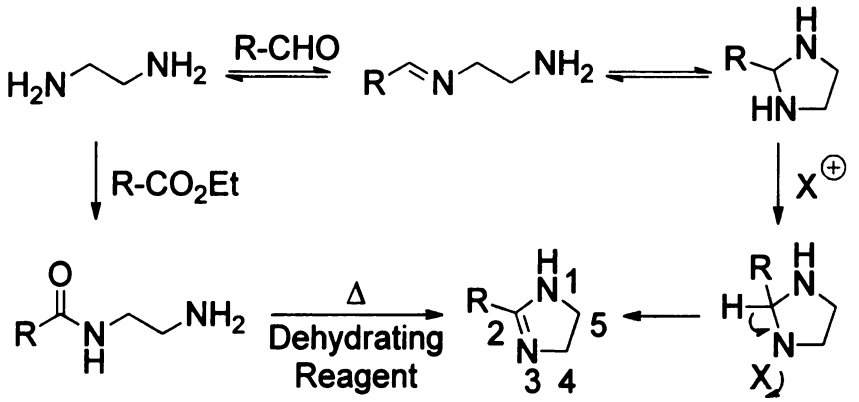


Figure III-5. Structures of some biologically active 2-imidazolines.

H. Syntheses of 2-imidazolines.

A variety of methods appear in the literature for the preparation of 2-imidazolines.[107-109] Many early syntheses of the scaffold involve dehydrative cyclization of monoacyl ethylenediamine derivatives. For example, Hill and Aspinall demonstrated that 2-imidazolines were available from the treatment of esters with ethylenediamine. The authors heated several esters with ethylenediamine to afford 2-alkyl- and 2-aryl-2-imidazolines in good yields (2-imidazoline numbering shown in Scheme IV-12).[110,111] The reaction involves initial monoamide formation followed by intramolecular cyclodehydration.[112,113]

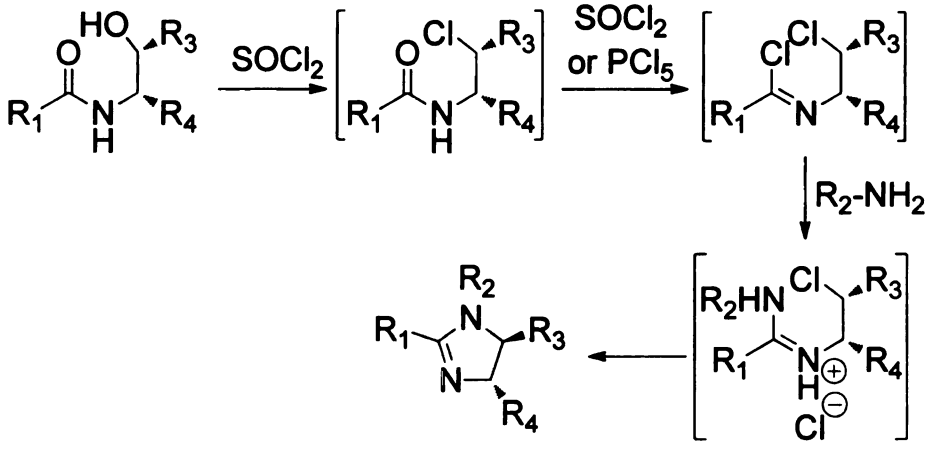
Amides prepared from alkyl esters, such as acetate esters, could be isolated by distillation and further converted to the desired 2-imidazolines by heating to high temperatures in the presence of a dehydrating agent. However, the more reactive amides prepared from aryl esters could not be isolated. Instead, substrate dehydration occurred during attempts to purify the amino amides by distillation, and the resultant 2-imidazolines were instead isolated.



Scheme III-12. Synthesis of 2-imidazolines derived from diamines.

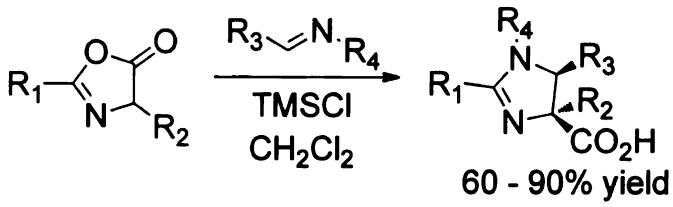
2-imidazolines have also been conveniently generated from diamines and aldehydes. Such reactions require the use of an oxidant, such as NBS,[114] *t*BuOCl,[115] or I_2[116,117] in order to generate desired 2-imidazolines (Scheme IV-12). Initial intermolecular dehydration occurs to generate a 2-imidazolidine intermediate[114] followed by subsequent oxidation *via* treatment with an electrophilic halogenating reagent to afford 2-imidazoline products. The protocol involving the addition of an oxidant are attractive in that they are routinely performed at room temperature, which allows for the preparation of heat-sensitive compounds.

Alternatively, 2-imidazolines have been generated from hydroxyl-substituted amides.[118] Successive treatment of appropriately substituted β-hydroxy amides with $SOCl_2$ and primary amines gives rise to 2-imidazolines, which were observed to proceed *via* an imidoyl chloride intermediate (Scheme IV-13).[118] This reaction tolerates various aliphatic and aromatic amides, although aryl amides exhibited greater reactivity. Consequently, less reactive aliphatic amides required treatment with PCl_5 in addition to $SOCl_2$ to induce imidoyl chloride formation and subsequent 2-imidazoline formation.



Scheme III-13. Synthesis of 2-imidazolines derived from β-hydroxy amides.

The Tepe lab has also developed a method for the preparation of 2-imidazolines, by which oxazol-5(4H)-ones undergo Lewis acid-mediated cycloaddition reactions with imines, as was described in Chapter I (Scheme IV-14). In this way, numerous 2-imidazolines have been prepared and evaluated for their biological activity.[22,103,104,119]

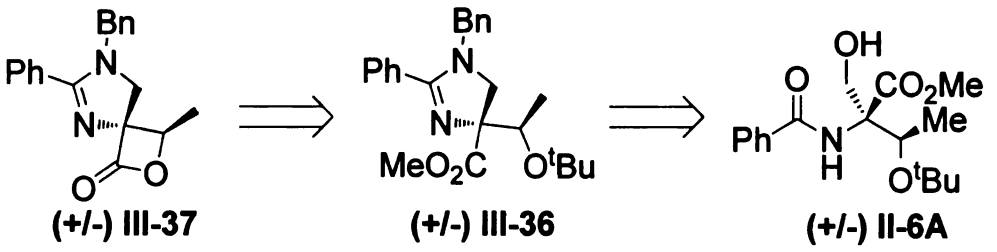


Scheme III-14. Synthesis of 2-imidazolines derived from oxazol-5(4H)-ones.

I. Synthesis of 2-imidazoline proteasome inhibitors.

Structure activity relationship studies are currently underway in our laboratories to gain understanding into the mode of proteasome inhibition by 2-imidazolines. We have become particularly interested in the synthesis of a 2-imidazoline bearing a β-lactone functionality, such as **III-37** (Scheme III-15). Unfortunately, such a derivative was deemed to be unattainable by our current methodology involving cycloaddition reactions of oxazol-5(4H)-ones (Scheme III-14). Thus, we required a new strategy for the synthesis of 2-imidazolines comprising the carbon framework present in desired 2-imidazoline **III-37**.

Apparent structural similarities are noticeable in proposed 2-imidazoline **III-37** and salinosporamide A. As such, it was envisioned that 2-imidazoline **III-37** and salinosporamide A could be constructed from similar staring materials. Our reported formal total synthesis of salinosporamide A involved modifications to *tert*-alkyl amino hydroxy carboxylic ester **II-24A** prepared from the alkylation reaction of oxazol-5(4H)-ones and enol ethers (Scheme III-11).[93] Therefore, we anticipated preparation of 2-imidazoline **III-37** to successfully arise through the use of structurally similar starting material **II-6A** (Scheme III-15).

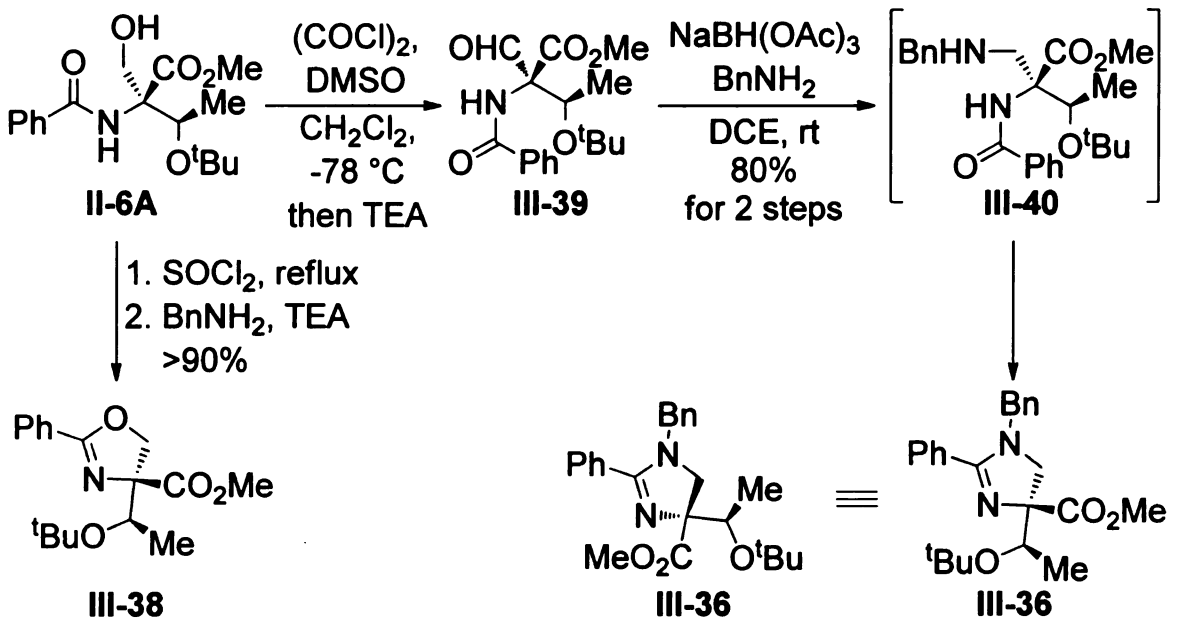


(+/-) III-37 (+/-) III-36 (+/-) II-6A

Scheme III-15. Strategy for the synthesis of a C4-spiro- β-lactone 2-imidazoline.

Our proposed synthesis for the formation of **III-37** involved initial generation of the 2-imidazoline scaffold, followed by functional group modification and subsequent β-lactone installation. Previous studies have demonstrated that hydroxyl-substituted amides can be converted to the corresponding 2-imidazolines by means of dehydration with $SOCl_2$ or PCl_5 (Scheme IV-12).[118] However, various attempts to perform the cyclodehydration reaction according to the published procedure were unsuccessful, and the only product obtained in the reaction when **II-6A** was used was 2-oxazoline **III-38** (Scheme IV-16). Alternatively, we believed 2-imidazolines of interest to be attainable *via* the corresponding amino amides, as was observed in the work of Hill and Aspinall (Scheme IV-12).[111] Thus, **II-6A** was submitted to Swern or Dess-Martin oxidation conditions immediately followed by reductive amination with $NaBH(OAc)_3$ and benzylamine to give rise to 2-imidazoline **III-36** (Scheme IV-16). The structure of **III-36** has been confirmed *via* X-ray analysis, as is shown in Figure IV-6. Only a minor amount of the intermediate amine **III-40** was recovered from the reaction, and it was observed to undergo cyclodehydration to form 2-imidazoline **III-36** upon standing at room temperature for one week. A Dess-Martin oxidation/reductive amination reaction sequence also generates the desired 2-

imidazoline when performed in a single pot, albeit in a lower yield (64% for the synthesis of **III-36**).



Scheme IV-16. Synthesis of 2-imidazolines *via* an oxidation/reductive amination protocol.

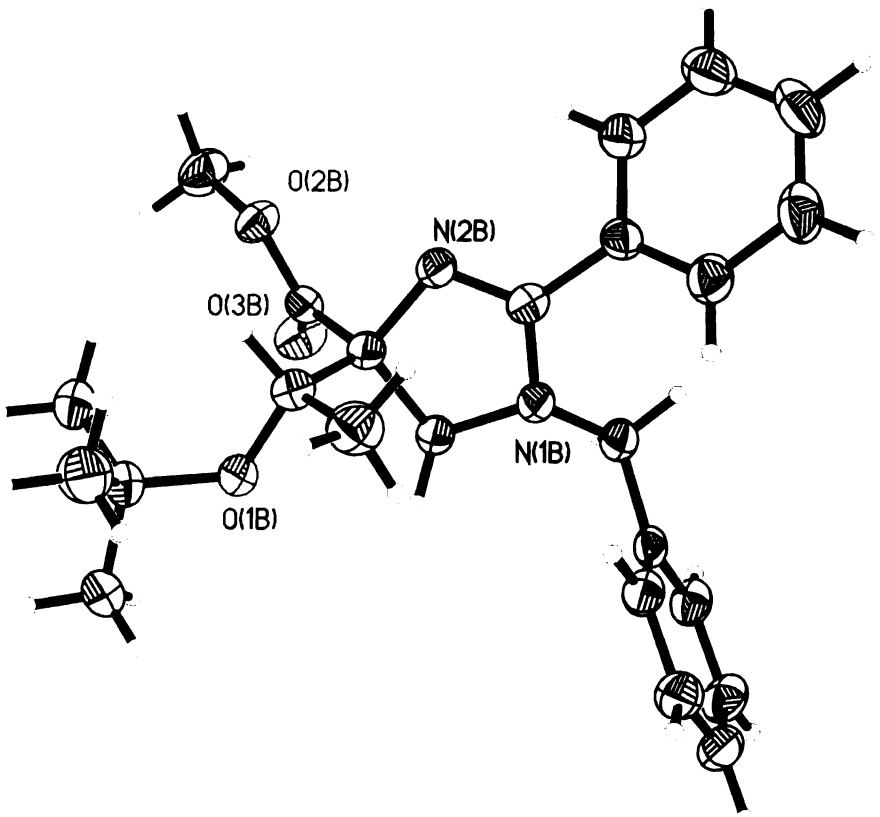
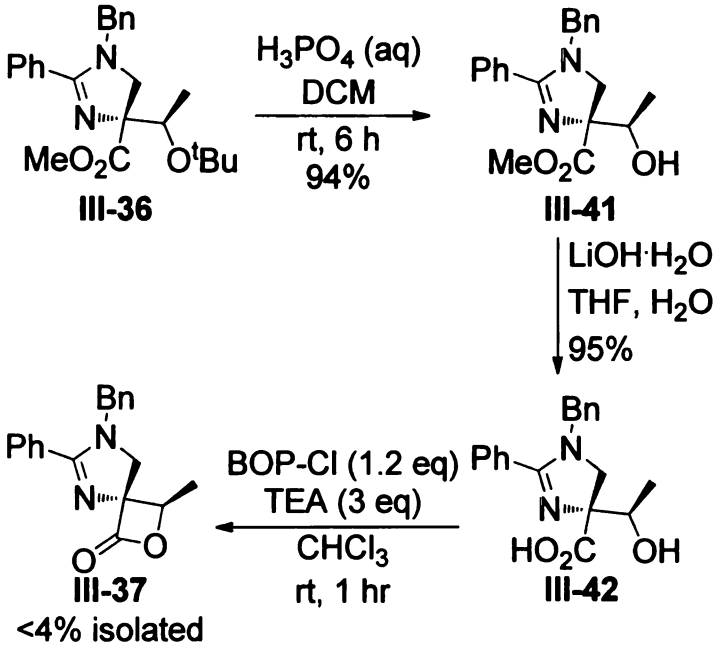


Figure III-6. X-ray crystal structure of **III-36**.

With 2-imidazoline **III-36** in hand, we next focused our attention to the synthesis of target 2-imidazoline **II-37**. Our synthetic strategy was to unveil the alcohol and carboxylic acid functionalities present in **III-36**, available from the ether and ester substituents respectively, and to use the reactive handles to install the β-lactone funtionality (Scheme IV-17). Towards this end, **III-36** was modified through *tert*-butyl ether deprotection with aqueous phosphoric acid to afford **III-41** in 94% yield, followed by hydrolysis in 95% yield to give carboxylic acid **III-42** (Scheme IV-17). Treatment of **III-42** with bis(2-oxo-3-

oxazolidinyl)phosphonic chloride (BOP-Cl) and triethylamine then resulted in the generation of the desired β-lactone **III-37**. Purification of **III-37** proved to be problematic, though, due to its ease of hydrolysis, and only poor yields of impure product were ever recovered. The labile nature of **III-37** appears to parallel the reported instability of β-lactone-containing salinosporamide A at physiological pH.[65,70,120] However, from the reaction mixture containing **III-37** was cleanly isolated an unexpected product which was found to inhibit chymotrypsin-like activity of the 20S proteasome at low micromolar concentrations. This unexpected product and the chemistry associated with its synthesis will be discussed in Chapter IV of this dissertation.



Scheme IV-17. Synthesis of **III-37**.

J. Evaluation of proteasome inhibition.

The 2-imidazolines prepared in this chapter were tested for their ability to inhibit the chymotryptic-like activity of the 20S proteasome in an *in vitro* assay. The results of the assay are shown in Table III-1. It was discovered that **III-36**, which contains the bulky hydrophobic *tert*-butyl ether, inhibits chymotrypsin-like activity at low micromolar levels (IC_{50} = 7.5 μM). In comparison, the most potent 2-imidazolines thus far evaluated in our lab have exhibited IC_{50} values in the range of 1 – 2 μM. However, removal of the *tert*-butyl group appears to markedly reduce potency, such that >20 μM of either **III-41** or **III-42** is necessary to inhibit 50% of proteolytic activity. Finally, a mixture containing **III-37** was found to inhibit chymotrypic activity at a low micromolar concentration (IC_{50} = 1.94 μM). However, this mixture was comprised of **III-37** and **IV-1** (1 : 3 ratio as determined by ^{1}H-NMR), and **IV-1** alone was later found to be more potent than the mixture (IC_{50} = 1.41 μM for **IV-1**). Therefore, the extent to which **III-37** inhibits chymotryptic-like activity of the proteasome is currently unknown.

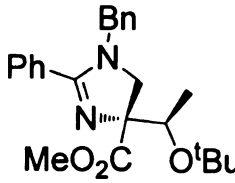
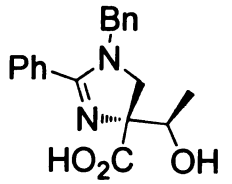
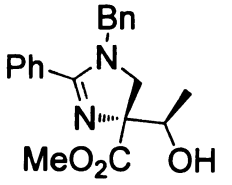
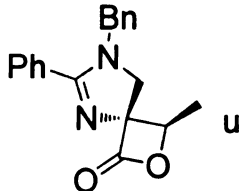
Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
 **III-36**	7.45	 **III-42**	>20
 **III-41**	>20	 **III-37**	unknown

Table III-1. Inhibition of chymotrypsin-like activity of the 20S proteasome by 2-imidazolines.

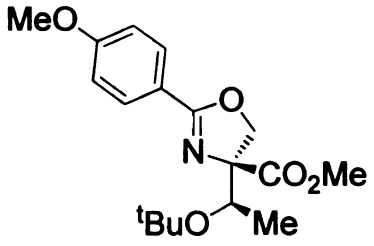
K. Experimental.

1. General Information. Reactions were carried out in flame-dried glassware under nitrogen atmosphere. All reactions were magnetically stirred and monitored by TLC with 0.25 μm pre-coated silica gel plates using either UV light or iodine to visualize the compounds. Column chromatography was carried out on Silica Gel 60 (230-400 mesh) supplied by EM Science. Yields refer to chromatographically and spectroscopically pure compounds unless otherwise noted. Infrared spectra were recorded on a Nicolet IR/42 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Plus-500 spectrometer. Chemical shifts are reported relative to the residue peaks of the solvent ($CDCl_3$: 7.27 ppm for ^{1}H and 77.0 ppm for ^{13}C). The following abbreviations are used to denote the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, and m = multiplet. HRMS were obtained at the Mass Spectrometry

Facility of Michigan State University with a JEOL JMS HX-110 mass spectrometer. Melting points were obtained using an Electrothermal® capillary melting point apparatus and are uncorrected.

2. Materials. Reagents and solvents were purchased from commercial suppliers and used without further purification. Anhydrous methylene chloride was dispensed from a delivery system which passes the solvents through a column packed with dry neutral alumina. Anhydrous chloroform and triethylamine were distilled from calcium hydride and stored over activated molecular sieves prior to use. The synthesis of **II-6A** and **II-24A** were described in Chapter II of this dissertation.

3. Synthesis and Characterization.

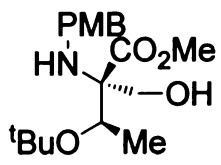


III-32

Figure III-7. DL-(*R*)-Methyl 4-((*R*)-1-(*tert*-butoxy)ethyl)-2-(4-methoxyphenyl)-4,5-dihydrooxazole-4-carboxylate (**III-32**).

DL-(*R*)-Methyl 4-((*R*)-1-(*tert*-butoxy)ethyl)-2-(4-methoxyphenyl)-4,5-dihydrooxazole-4-carboxylate (III-32). A 0 °C solution of **II-24A** (0.12 g, 0.34 mmol) and triethylamine (0.37 g, 3.6 mmol) in 1,2-dichloroethane (15 mL) was treated with methanesulfonyl chloride (0.12 g, 1.0 mmol), and the reaction stirred

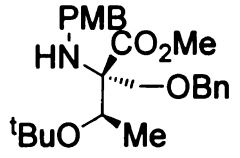
under nitrogen atmosphere while it warmed to room temperature. The reaction was then refluxed until complete, as observed by TLC (about 7 hours). After cooling to room temperature the reaction was diluted in CH_2Cl_2 (15 mL) and was made acidic by the addition of 30% aqueous HCl solution. The organic layer was collected and the aqueous layer was extracted with CH_2Cl_2 (2 x 30 mL). The combined organic extracts were washed successively with saturated $NaHCO_3$ solution and brine before being dried ($MgSO_4$) and concentrated under vacuum. Purification *via* silica gel chromatography (5% ether / 95% CH_2Cl_2) afforded 0.11 g of the title compound (96% yield) as an oil. ^{1}H NMR (500 MHz) ($CDCl_3$): δ 1.03 (d, J = 6.2 Hz, 3 H), 1.08 (s, 9 H), 3.73 (s, 3 H), 3.76 (s, 3 H), 4.43 (q, J = 6.2 Hz, 1 H), 4.53 (d, J = 9.5 Hz, 1 H), 4.90 (d, J = 9.5 Hz, 1 H), 6.83 (d, J = 8.9 Hz, 2 H), 7.86 (d, J = 8.9 Hz, 2 H). ^{13}C NMR + DEPT (125 MHz) ($CDCl_3$): δ 17.0 (–CH_3), 28.5 (–CH_3), 52.3 (–CH_3), 55.1 (–CH_3), 69.2 (–CH_2), 70.0 (–CH), 73.9 (quaternary C), 82.2 (quaternary C), 113.4 (aromatic –CH), 119.4 (quaternary –C), 130.2 (aromatic –CH), 162.2 (quaternary –C), 164.6 (quaternary –C), 172.4 (quaternary –C). IR (neat): 2978 cm^{-1}, 1740 cm^{-1}, 1638 cm^{-1}, 1611 cm^{-1}, 1514 cm^{-1}, 1260 cm^{-1}, 1082 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{18}H_{26}NO_5$ [M+H], 336.1805; found, 336.1820.



III-33

Figure III-8. DL-(*2R, 3R*)-Methyl 3-*tert*-butoxy-2-(hydroxymethyl)-2-((4-methoxybenzyl)amino)butanoate (**III-33**).

DL-(*2R*, *3R*)-Methyl 3-*tert*-butoxy-2-(hydroxymethyl)-2-((4-methoxybenzyl)amino)butanoate (III-33). A solution of **III-32** (0.29 g, 0.86 mmol) in glacial acetic acid (4 mL) was treated with excess $NaCNBH_3$ (0.21 g, 3.3 mmol) and the reaction stirred at room temperature for 2 days with more $NaCNBH_3$ (0.10 g, 1.6 mmol) added after 24 hours. The reaction was diluted with H_2O (10 mL) and the pH was adjusted to pH = 7 with the slow addition of solid Na_2CO_3. The reaction was extracted with EtOAc (3 x 25 mL) and the combined organic extracts were dried ($MgSO_4$) and concentrated under vacuum. Purification *via* silica gel chromatography (9% ether / 91% CH_2Cl_2) afforded 0.21 g of the title compound (73% yield) as a white solid (m.p. = 41 – 43 °C). ^{1}H NMR (500 MHz) ($CDCl_3$): δ 1.19 (s, 9 H), 1.20 (d, J = 6.4 Hz, 3 H), 2.15 – 2.33 (broad s, 1 H), 2.95 – 3.10 (broad s, 1 H), 3.58 (d, J = 12.4 Hz, 1 H), 3.65 (d, J = 12.3 Hz, 1 H), 3.77 (s, 3 H), 3.78 – 3.83 (m, 4 H), 3.86 (d, J = 11.6 Hz, 1 H), 4.01 (q, J = 6.3 Hz, 1 H), 6.87 (d, J = 8.6 Hz, 2 H), 7.27 (d, J = 8.4 Hz, 2 H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$): δ 18.3 (–**C**H_3), 28.7 (–**C**H_3), 46.9 (–**C**H_2), 51.6 (–**C**H_3), 55.1 (–**C**H_3), 60.6 (–**C**H_2), 69.5 (quaternary –**C**), 69.8 (–**C**H), 74.5 (quaternary –**C**), 113.8 (aromatic –**C**H), 129.0 (aromatic –**C**H), 132.4 (quaternary –**C**), 158.6 (quaternary –**C**), 173.4 (quaternary –**C**). IR (KBr pellet): 3391 cm^{-1}, 3337 cm^{-1}, 2973 cm^{-1}, 1721 cm^{-1}, 1617 cm^{-1}, 1516 cm^{-1}, 1240 cm^{-1}, 1044 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{18}H_{30}NO_5$ [M+H], 340.2118; found, 340.2127.

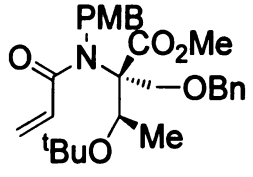


III-34

Figure III-9. DL-(*2R*, *3R*)-Methyl 2-((benzyloxy)methyl)-3-*tert*-butoxy-2-((4-methoxybenzyl)amino)butanoate (**III-34**).

DL-(*2R*, *3R*)-Methyl 2-((benzyloxy)methyl)-3-*tert*-butoxy-2-((4-methoxybenzyl)amino)butanoate (III-34). A solution of **III-33** (114.8 mg, 0.34 mmol) in DMF (2.5 mL) was treated successively with NaH (60% in mineral oil, 42.1 mg, 1.1 mmol) and benzyl bromide (64.8 mg, 0.38 mmol), and the reaction stirred under nitrogen atmosphere until complete by TLC (about 15 minutes). The reaction was carefully quenched by the addition of saturated NH_4Cl solution (5 mL) and then diluted in EtOAc (10 mL). The organic layer was collected and the reaction was extracted with EtOAc (2 x 20 mL). The combined organic extracts were washed with 10% LiCl solution (2 x 50 mL) before being dried (Na_2SO_4) and concentrated under vacuum. Purification *via* silica gel chromatography (2.5% ether / 97.5% CH_2Cl_2) afforded 0.14 g of the title compound (96% yield) as an oil. ^1H NMR (500 MHz) ($CDCl_3$): δ 1.16 (s, 9 H), 1.20 (d, J = 6.2 Hz, 3 H), 2.11 – 2.21 (broad s, 1 H), 3.64 (d, J = 12.1 Hz, 1 H), 3.72 (s, 3H), 3.78 (d, J = 9.8 Hz, 1 H), 3.80 (s, 3 H), 3.87 (d, J = 12.1 Hz, 1 H), 3.93 (d, J = 9.7 Hz, 1 H), 4.04 (q, J = 6.2 Hz, 1 H), 4.56 (s, 2 H), 6.85 (d, J = 8.6 Hz, 2 H), 7.26 – 7.40 (m, 7 H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$): δ 18.7 (–**C**H_3), 28.8 (–**C**H_3), 47.6 (–**C**H_2), 51.8 (–**C**H_3), 55.3 (–**C**H_3), 69.7 (quaternary –**C**), 70.0 (–**C**H_2), 71.3 (–**C**H), 73.4 (–**C**H_2), 73.9 (quaternary –**C**), 113.6 (aromatic –

CH), 127.5 (aromatic –CH), 127.6 (aromatic –CH), 128.3 (aromatic –CH), 129.4 (aromatic –CH), 133.7 (aromatic quaternary –C), 138.4 (aromatic quaternary –C), 158.4 (aromatic quaternary –C), 173.9 (quaternary –C). IR (neat): 3355 cm^{-1}, 2977 cm^{-1}, 1740 cm^{-1}, 1513 cm^{-1}, 1456 cm^{-1}, 1248 cm^{-1}, 1192 cm^{-1}, 1094 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{25}H_{36}NO_5$ [M+H], 430.2588; found, 430.2569.

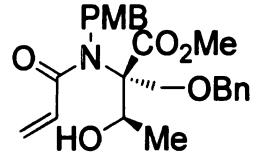


III-35

Figure III-10. DL-(*2R*, *3R*)-Methyl 2-((benzyloxy)methyl)-3-*tert*-butoxy-2-(N-(4-methoxybenzyl)acrylamido)butanoate (**III-35**).

DL-(*2R*, *3R*)-Methyl 2-((benzyloxy)methyl)-3-*tert*-butoxy-2-(N-(4-methoxybenzyl)acrylamido)butanoate (III-35). A 0 °C solution of **III-34** (112.9 mg, 0.26 mmol) and diisopropylethylamine (51.9 mg, 0.40 mmol) in CH_2Cl_2 (1 mL) was treated with acryloyl chloride (35.8 mg, 0.40 mmol) and the reaction stirred under nitrogen atmosphere at 0 °C for 4.5 hours. The reaction was diluted in CH_2Cl_2 and was washed successively with 5% HCl solution, saturated $NaHCO_3$ solution, and brine before being dried (Na_2SO_4) and concentrated under vacuum. Purification *via* silica gel chromatography (20% EtOAc / 80% hexanes) afforded 119.0 mg of the title compound (94% yield) as an oil. ^1H NMR (500 MHz) ($CDCl_3$): δ 1.22 (s, 9 H), 1.34 (d, J = 6.2 Hz, 3 H), 3.54 (d, J = 7.2 Hz, 1 H), 3.73 (s, 3 H), 3.84 (s, 3 H), 3.99 (d, J = 7.2 Hz, 1 H), 4.21 (d, J = 11.8 Hz, 1 H), 4.35 (d, J = 11.8 Hz, 1 H), 4.55 (q, J = 6.2 Hz, 1 H), 4.67 (d, J = 19.0 Hz, 1 H), 4.92 (d, J = 19.0 Hz, 1 H), 5.59 (dd, J_1 = 4.8 Hz, J_2 = 7.6 Hz, 1 H), 6.33 – 6.37

(m, 2 H), 6.91 (d, J = 8.7 Hz, 2 H), 7.10 – 7.14 (m, 2 H), 7.22 – 7.29 (m, 5 H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$): δ 20.0 (–CH_3), 29.0 (–CH_3), 48.8 (–CH_2), 51.8 (–CH_3), 55.3 (–CH_3), 69.5 (quaternary –C), 70.3 (–CH), 70.6 (–CH_2), 73.0 (–CH_2), 74.2 (quaternary –C), 114.1 (aromatic –CH), 126.7 (–CH), 127.3 (–CH), 127.4 (–CH), 128.1 (–CH), 128.6 (–CH), 129.0 (–CH_2), 131.0 (aromatic quaternary –C), 137.5 (aromatic quaternary –C), 158.6 (aromatic quaternary –C), 167.6 (quaternary –C), 170.3 (quaternary –C). IR (neat): 2975 cm^{-1}, 1750 cm^{-1}, 1653 cm^{-1}, 1512 cm^{-1}, 1248 cm^{-1}, 1188 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{28}H_{38}NO_6$ [M+H], 484.2694; found, 484.2716.

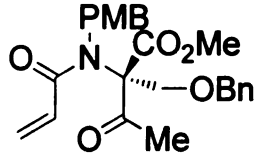


III-7

Figure III-11. DL-(*2R*, *3R*)-Methyl 2-((benzyloxy)methyl)-3-hydroxy-2-(N-(4-methoxybenzyl)acrylamido)butanoate (**III-7**).

DL-(*2R*, *3R*)-Methyl 2-((benzyloxy)methyl)-3-hydroxy-2-(N-(4-methoxybenzyl)acrylamido)butanoate (III-7). A solution of **III-35** (21.6 mg, 0.045 mmol) in CH_2Cl_2 (0.5 mL) was treated with aqueous phosphoric acid (85%, 0.03 mL, 0.4 mmol) and the reaction stirred at room temperature for 20 hours. The reaction was quenched with saturated $NaHCO_3$ (5 mL) solution and diluted in EtOAc (10 mL), and the organic layer was collected. The aqueous layer was extracted with more EtOAc (2 x 15 mL) and the combined organic extracts were dried ($MgSO_4$) and concentrated under vacuum. Purification *via* silica gel chromatography (40% EtOAc / 60% hexanes) afforded 5.2 mg of the title

compound[84] (27% yield) as an oil. ^{1}H NMR (500 MHz) ($CDCl_3$): δ 1.23 (d, J = 6.5 Hz, 3 H), 3.16 (d, J = 7.3 Hz, 1 H), 3.80 (s, 3 H), 3.81 (s, 3 H), 3.83 (d, J = 10.3 Hz, 1 H), 4.00 (d, J = 10.3 Hz, 1 H), 4.38 (s, 2 H), 4.72 (p, J = 6.7 Hz, 1 H), 4.84 (s, 2 H), 5.60 (t, J = 6.2 Hz, 1 H), 6.35 (d, J = 6.0 Hz, 2 H), 6.87 (d, J = 8.6 Hz, 2 H), 7.18 (d, J = 6.6 Hz, 2 H), 7.24 – 7.33 (m, 5 H). ^{13}C NMR (125 MHz) ($CDCl_3$): δ 18.6, 48.7, 52.3, 55.3, 67.4, 69.5, 70.2, 73.5, 114.0, 126.9, 127.5, 127.7, 128.2, 128.4, 129.5, 130.8, 137.5, 158.6, 168.6, 171.7.

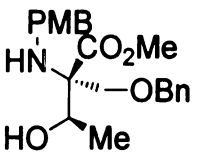


III-8

Figure III-12. DL-(*R*)-Methyl 2-((benzyloxy)methyl)-2-(N-(4-methoxybenzyl)acrylamido)-3-oxobutanoate (**III-8**).

DL-(*R*)-Methyl 2-((benzyloxy)methyl)-2-(N-(4-methoxybenzyl)acrylamido)-3-oxobutanoate (III-8). This reaction was performed according to the known procedure.[84] To a stirring solution of **III-7** (14.3 mg, 0.033 mmol) in CH_2Cl_2 (0.5 mL) was added Dess-Martin periodinane (17.1 mg, 0.040 mmol) and the reaction stirred at room temperature for 1.25 hours. The reaction was quenched with a mixture of saturated aqueous $NaHCO_3$ and $Na_2S_2O_3$ solutions (1 : 1, 3 mL) and extracted with EtOAC (3 x 5 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated under vacuum. Purification *via* silica gel chromatography (35% EtOAc / 65% hexanes) afforded 12.6 mg of the title compound (89% yield) as an oil. The spectral data matches that previously reported in the literature.[84] ^{1}H NMR (500 MHz) ($CDCl_3$): δ 2.45 (s, 3 H), 3.78 –

3.82 (m, 5 H), 3.83 (s, 3 H), 4.31 (m, 2 H), 4.79 (d, J = 18.4 Hz, 1 H), 4.94 (d, J = 18.5 Hz, 1 H), 5.68 (dd, J_1 = 4.5 Hz, J_2 = 7.7 Hz, 1 H), 6.38 – 6.42 (m, 2 H), 6.92 (d, J = 8.6 Hz, 2 H), 7.11 – 7.15 (m, 2 H), 7.26 – 7.31 (m, 3 H), 7.34 (d, J = 8.5 Hz, 2 H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$): δ 28.0 (–**C**H_3), 48.8 (–**C**H_2), 52.9 (–**C**H_3), 55.3 (–**C**H_3), 70.5 (–**C**H_2), 73.7 (–**C**H_2), 77.3 (quaternary –**C**), 114.1 (aromatic –**C**H), 127.1 (aromatic –**C**H), 127.5 (aromatic –**C**H), 127.9 (aromatic –**C**H), 128.4 (aromatic –**C**H), 130.2 (–**C**H_2), 130.4 (aromatic quaternary –**C**), 136.7 (aromatic quaternary –**C**), 158.8 (aromatic quaternary –**C**), 168.4 (quaternary –**C**), 169.1 (quaternary –**C**), 198.0 (quaternary –**C**).

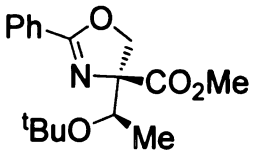


III-6

Figure III-13. DL-(*2R*, *3R*)-Methyl 2-((benzyloxy)methyl)-3-hydroxy-2-((4-methoxybenzyl)amino)butanoate (**III-6**).

DL-(*2R*, *3R*)-Methyl 2-((benzyloxy)methyl)-3-hydroxy-2-((4-methoxybenzyl)amino)butanoate (III-6). A solution of **III-34** (40.0 mg, 0.093 mmol) in CH_2Cl_2 (1 mL) was treated with aqueous phosphoric acid (85%, 0.04 mL, 0.6 mmol) and the reaction stirred at room temperature for 6 hours. The reaction was quenched with saturated $NaHCO_3$ (5 mL) solution and diluted in EtOAc (10 mL), and the organic layer was collected. The aqueous layer was extracted with more EtOAc (2 x 15 mL) and the combined organic extracts were dried ($MgSO_4$) and concentrated under vacuum to cleanly afford 34.0 mg of the title compound (98% yield) as an oil without any further purification needed. The

spectral data matches that previously reported in the literature.[84] ^{1}H NMR (500 MHz) ($CDCl_3$): δ 1.16 (d, J = 6.4 Hz, 3 H), 2.06 (broad s, 1 H), 3.37 (broad s, 1 H), 3.65 (d, J = 11.9 Hz, 1 H), 3.72 (d, J = 11.9 Hz, 1 H), 3.78 (s, 3 H), 3.82 (s, 3H), 3.83 (d, J = 9.8 Hz, 1 H), 3.88 (d, J = 9.8 Hz, 1 H), 4.00 (q, J = 6.4 Hz, 1 H), 4.56 (d, J = 2.3 Hz, 2 H), 6.88 (d, J = 8.5 Hz, 2 H), 7.24 – 7.40 (m, 7 H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$): δ 17.9 (–**C**H_3), 47.4 (–**C**H_2), 52.0 (–**C**H_3), 55.3 (–**C**H_3), 69.3 (quaternary –**C**), 69.7 (–**C**H), 70.5 (–**C**H_2), 73.5 (–**C**H_2), 113.8 (aromatic –**C**H), 127.7 (aromatic –**C**H), 127.8 (aromatic –**C**H), 128.4 (aromatic –**C**H), 129.4 (aromatic –**C**H), 132.4 (quaternary –**C**), 137.6 (quaternary –**C**), 158.7 (quaternary –**C**), 173.2 (quaternary –**C**). IR (neat): 3357 cm^{-1}, 2926 cm^{-1}, 1734 cm^{-1}, 1514 cm^{-1}, 1248 cm^{-1}, 1100 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{21}H_{28}NO_5$ [M+H], 374.1962; found, 374.1972.

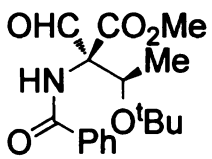


III-38

Figure III-14. DL-(R)-methyl 4-((R)-1-*tert*-butoxyethyl)-2-phenyl-4,5-dihydrooxazole-4-carboxylate (**III-38**).

DL-(R)-methyl 4-((R)-1-*tert*-butoxyethyl)-2-phenyl-4,5-dihydrooxazole-4-carboxylate (III-38). **III-38** has been prepared in our lab by various methods, and the optimized procedure is as follows: A 0 °C solution of **II-6A** (1.24 g, 3.85 mmol) and triethylamine (5.4 mL, 39 mmol) in anhydrous CH_2Cl_2 (125 mL) was treated with methanesulfonyl chloride (0.90 mL, 12 mmol), and the reaction stirred under nitrogen atmosphere while it warmed to room temperature. The

reaction was then refluxed until complete, as observed by TLC (about 20 hours). The reaction was neutralized with 30% aqueous HCl solution and was extracted with CH_2Cl_2 (3 x 150 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated under vacuum. Purification via silica gel chromatography (7% ether / 93% CH_2Cl_2) afforded 1.17 g of the title compound (solid, m.p. = 68 – 69 °C, 99% yield). ^1H NMR (500 MHz) ($CDCl_3$): δ 1.04 (d, J = 6.2 Hz, 3 H), 1.09 (s, 9 H), 3.74 (s, 3 H), 4.45 (q, J = 6.2 Hz, 1 H), 4.57 (d, J = 9.5 Hz, 1 H), 4.93 (d, J = 9.5 Hz, 1 H), 7.33 (t, J = 7.6 Hz, 2 H), 7.42 (t, J = 7.4 Hz, 1 H), 7.90 – 7.94 (m, 2 H). ^{13}C NMR (125 MHz) ($CDCl_3$): δ 17.0, 28.5, 52.3, 69.4, 69.9, 73.9, 82.3, 127.0, 128.1, 128.4, 131.5, 164.8, 172.2. IR (neat): 2978 cm^{-1}, 1742 cm^{-1}, 1640 cm^{-1}, 1264 cm^{-1}, 1086 cm^{-1}, 1069 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{17}H_{24}NO_4$ [M+H], 306.1705; found, 306.1703.

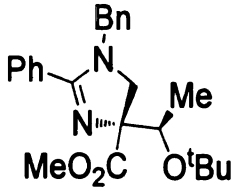


III-39

Figure III-15. DL-(2S,3R)-methyl 2-benzamido-3-*tert*-butoxy-2-formylbutanoate (**III-39**).

DL-(2S,3R)-methyl 2-benzamido-3-*tert*-butoxy-2-formylbutanoate (III-39). DMSO (0.38 mL, 5.3 mmol) was added dropwise to a stirring solution of oxalyl chloride (0.23 mL, 2.6 mmol) in CH_2Cl_2 (4 mL), cooled to -78 °C, and the reaction stirred under N_2 atmosphere for 30 minutes. A solution of **II-6A** (0.5619 g, 1.7 mmol) in CH_2Cl_2 (3 mL) was added dropwise and the reaction stirred at -78 °C for 45 minutes. TEA (0.64 mL, 4.6 mmol) was then added and the reaction stirred at

-78 °C for 5 minutes before warming to room temperature. The reaction was diluted with H_2O (8 mL) and extracted with CH_2Cl_2 (4 x 15 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated to an oil, which was used immediately in the next step. However, purification of the crude reaction *via* silica gel chromatography (25% EtOAc / 75% hexanes) affords **III-39** as an oil. ^{1}H NMR (500 MHz) ($CDCl_3$): δ 1.19 (s, 9 H), 1.28 (d, J = 6.2 Hz, 3 H), 3.79 (s, 3 H), 4.41 (q, J = 6.2 Hz, 1 H), 7.04 (s, 1 H), 7.43 (t, J = 7.5 Hz, 2 H), 7.51 (t, J = 7.5 Hz, 1 H), 7.80 (d, J = 7.1 Hz, 2 H), 9.89 (s, 1 H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$): δ 19.2 (–**C**H$_3$), 28.6 (–**C**H$_3$), 52.7 (–**C**H$_3$), 68.9 (–**C**H), 72.5 (quaternary –**C**), 75.4 (quaternary –**C**), 127.1 (aromatic –**C**H), 128.5 (aromatic –**C**H), 132.0 (aromatic –**C**H), 132.9 (aromatic quaternary –**C**), 167.5 (quaternary –**C**), 167.9 (quaternary –**C**), 194.2 (quaternary –**C**). IR (neat): 3337 cm^{-1}, 2880 cm^{-1}, 1730 cm^{-1}, 1653 cm^{-1}, 1260 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{17}H_{24}NO_5$ [M+H], 322.1654; found, 322.1663.

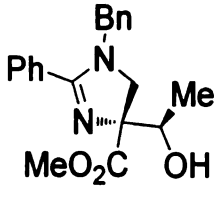


III-36

Figure III-16. DL-(R)-methyl 1-benzyl-4-((R)-1-(*tert*-butoxy)ethyl)-2-phenyl-4,5-dihydro-1H-imidazole-4-carboxylate (**III-36**).

DL-(R)-methyl 1-benzyl-4-((R)-1-(*tert*-butoxy)ethyl)-2-phenyl-4,5-dihydro-1H-imidazole-4-carboxylate (III-36). To a stirring solution of crude **III-39** in 1,2-dichloroethane (12 mL) were added benzylamine (0.24 mL, 2.2 mmol) and

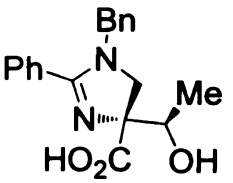
$NaBH(OAc)_3$ (0.5102 g, 2.4 mmol), and the reaction stirred at room temperature overnight. The reaction was quenched with $NaHCO_3$ (25 mL) and extracted with CH_2Cl_2 (3 x 30 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated, and purification *via* silica gel chromatography (50% EtOAc / 50% hexanes → 80% EtOAc / 20% hexanes) was performed to give 0.5524 g of the title compound (80% yield) as an off-white solid (m.p. = 96 – 99 °C). ^1H NMR (500 MHz) ($CDCl_3$): δ 1.11 – 1.15 (m, 12 H), 3.60 (d, J = 11.0 Hz, 1 H), 3.78 (s, 3 H), 4.09 (d, J = 11.0 Hz, 1 H), 4.30 (d, J = 15.8 Hz, 1 H), 4.40 (d, J = 15.8 Hz, 1 H), 4.47 (q, J = 6.1 Hz, 1 H), 7.25 – 7.32 (m, 3 H), 7.35 – 7.45 (m, 5 H), 7.56 – 7.60 (m, 2 H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$): δ 17.3 (–CH_3), 28.8 (–CH_3), 51.6 (–CH_2), 52.2 (–CH_3), 52.3 (–CH_2), 70.5 (–CH), 73.7 (quaternary –C), 80.4 (quaternary –C), 127.0 (aromatic –CH), 127.4 (aromatic –CH), 128.39 (aromatic –CH), 128.40 (aromatic –CH), 128.7 (aromatic –CH), 130.2 (aromatic –CH), 130.4 (aromatic quaternary –C), 137.4 (aromatic quaternary –C), 166.1 (quaternary –C), 173.7 (quaternary –C). IR (KBr pellet): 2978 cm^{-1}, 1728 cm^{-1}, 1593 cm^{-1}, 1260 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{24}H_{30}N_2O_3$ [M+H], 395.2335; found, 395.2339. Anal. Calcd. For $C_{24}H_{31}N_2O_3$: C, 73.07; H, 7.66; N, 7.10. Found: C, 72.96; H, 7.77; N, 7.17.



III-41

Figure III-17. DL-(R)-methyl 1-benzyl-4-((R)-1-hydroxyethyl)-2-phenyl-4,5-dihydro-1H-imidazole-4-carboxylate (**III-41**).

DL-(R)-methyl 1-benzyl-4-((R)-1-hydroxyethyl)-2-phenyl-4,5-dihydro-1H-imidazole-4-carboxylate (III-41). A solution of **III-36** (0.5524 g, 1.4 mmol) in CH_2Cl_2 (8.0 mL) was treated with aqueous phosphoric acid (85%, 0.49 mL, 8.4 mmol) and the reaction stirred at room temperature for 6 hours. The reaction was quenched with saturated $NaHCO_3$ solution (25 mL) and diluted in CH_2Cl_2 (15 mL), and the organic layer was collected. The aqueous layer was then extracted with CH_2Cl_2 (3 x 40 mL), and the combined organic extracts were dried ($MgSO_4$) and concentrated under vacuum to cleanly afford 0.4467 g of the title compound (84% yield) as a white solid (m.p. = 132 – 134 °C) without any further purification needed. ^{1}H NMR (500 MHz) ($CDCl_3$): δ 1.18 (d, J = 6.4 Hz, 3 H), 2.64 (broad s, 1 H), 3.59 (d, J = 10.6 Hz, 1 H), 3.77 – 3.81 (m, 4 H), 4.23 (q, J = 6.4 Hz, 1 H), 4.35 (d, J = 2.7 Hz, 2 H), 7.21 – 7.48 (m, 8 H), 7.59 – 7.62 (m, 2 H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$): δ 17.0 (–**C**H_3), 52.0 (–**C**H_2), 52.5 (–**C**H_3), 53.8 (–**C**H_2), 70.8 (–**C**H), 79.1 (quaternary –**C**), 127.0 (aromatic –**C**H), 127.6 (aromatic –**C**H), 128.4 (aromatic –**C**H), 128.6 (aromatic –**C**H), 128.8 (aromatic –**C**H), 130.1 (aromatic quaternary –**C**), 130.4 (aromatic –**C**H), 136.9 (aromatic quaternary –**C**), 167.4 (quaternary –**C**), 174.3 (quaternary –**C**). IR (neat): 3202 cm^{-1}, 2960 cm^{-1}, 1735 cm^{-1}, 1560 cm^{-1}, 1257 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{20}H_{23}N_2O_3$ [M+H], 339.1709; found, 339.1717.

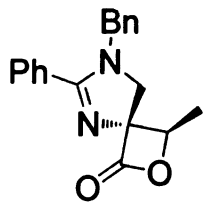


III-42

Figure III-18. DL-(R)-1-benzyl-4-((R)-1-hydroxyethyl)-2-phenyl-4,5-dihydro-1H-imidazole-4-carboxylic acid (**III-42**).

DL-(R)-1-benzyl-4-((R)-1-hydroxyethyl)-2-phenyl-4,5-dihydro-1H-imidazole-4-carboxylic acid (III-42). To a stirring solution of **III-41** (27.5 mg, 0.081 mmol) in THF (0.5 mL) and H_2O (0.3 mL) was added $LiOH{\cdot}H_2O$ (4.3 mg, 0.10 mmol) and the reaction stirred at room temperature for 45 minutes. The reaction was made acidic with concentrated HCl (about 3 drops) and concentrated under vacuum to give a white residue, which was shown to be free of organic contaminants by ^{1}H-NMR. Removal of the inorganic salts was performed by dilution of the residue in *i*PrOH and $CHCl_3$ (1 : 9, 10 mL) followed by filtration of the resultant suspension and concentration of the filtrate to give 25.0 mg of the product (95%) as a white solid (m.p. = 138 – 142 °C). ^{1}H NMR (500 MHz) (CD_3OD): δ 1.27 (d, J = 6.5 Hz, 3 H), 4.08 (q, J = 6.5 Hz, 1 H), 4.23 (dd, J_1 = 12.1 Hz, J_2 = 15.4 Hz, 2 H), 4.64 (d, J = 15.8 Hz, 1 H), 4.75 (d, J = 15.7 Hz, 1 H), 7.26 (d, J = 15.7 Hz, 2 H), 7.33 – 7.41 (m, 3 H), 7.67 (t, J = 7.8 Hz, 2 H), 7.75 – 7.79 (m, 3 H). ^{13}C NMR + **DEPT** (125 MHz) (CD_3OD): δ 17.9 (–**C**H_3), 51.8 (–**C**H_2), 56.8 (–**C**H_2), 70.4 (–**C**H), 73.7 (quaternary –**C**), 123.7 (aromatic quaternary –**C**), 128.7 (aromatic –**C**H), 129.8 (aromatic –**C**H), 129.8 (aromatic –**C**H), 130.3 (aromatic –**C**H), 130.8 (aromatic –**C**H), 134.6 (aromatic quaternary –**C**), 134.9 (aromatic –**C**H), 168.4 (quaternary –

C), 172.2 (quaternary –C). IR (neat): 3218 cm^{-1}, 2915 cm^{-1}, 1732 cm^{-1}, 1595 cm^{-1}, 1556 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{19}H_{21}N_2O_3$ [M+H], 325.1552; found, 325.1555.



III-37

Figure III-19. DL-(3R,4R)-7-benzyl-3-methyl-6-phenyl-2-oxa-5,7-diazaspiro[3.4]oct-5-en-1-one (**III-37**).

DL-(3R,4R)-7-benzyl-3-methyl-6-phenyl-2-oxa-5,7-diazaspiro[3.4]oct-5-en-1-one (III-37). To a stirring suspension of **III-42** (0.1185 g, 0.37 mmol) in $CHCl_3$ (2.8 mL) were successively added TEA (0.15 mL, 1.1 mmol) and BOP-Cl (0.1167 g, 0.46 mmol), and the reaction mixture stirred vigorously at room temperature under N_2 atmosphere for 1.5 hours. The reaction was then diluted with CH_2Cl_2 (7 mL) and washed with ice cold H_2O/brine mixture (5:1, 15 mL). The organic layer was collected and the aqueous layer was extracted with CH_2Cl_2 (2 x 15 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to a white residue. Purification of the residue *via* silica gel chromatography (2% TEA / 35% EtOAc / 63% hexanes on silica gel pretreated with TEA) afforded 30.1 mg of a 3 : 1 mixture containing the title compound (<4%) and **IV-1**. There appeared to be a considerable amount of overlap between NMR peaks of **III-37** and **IV-1**, especially in the aromatic regions, such that only partial NMR data could be extracted for **III-37**. ^1H NMR (500 MHz) ($CDCl_3$): δ 1.43 (d, J = 6.4 Hz, 3 H), 3.62

(d, J = 11.5 Hz, 1 H), 3.77 (d, J = 11.5 Hz, 1 H), 4.38 (d, J = 15.9 Hz, 1 H), 4.45 (d, J = 15.8 Hz, 1 H), 4.91 (q, J = 6.6 Hz, 1 H). ^{13}C NMR (150 MHz) ($CDCl_3$): 15.5, 50.7, 80.4, 84.5, 126.9, 127.9, 128.7, 129.0, 129.3, 130.9, 136.4, 168.8, 171.8.

4. Procedure for proteasome inhibition assay. The 2-imidazolines and oxazinone/imidazolidine adducts prepared in this chapter were tested for their ability to inhibit the chymotryptic-like activity of the 20S proteasome in an *in vitro* assay using purified human recombinant 20S proteasome (Boston Biochem, Cambridge, Ma.) and the fluorogenic peptide substrate Suc-LLVY-AMC (Boston Biochem, Cambridge, Ma.). All proteasome inhibition assays were performed by Teri L. Lansdell according to a modification of known procedures.[70,121]

The modified procedure is as follows: An activated enzyme solution was first prepared through addition of 20S proteasome to a reaction buffer (50 mM Tris-HCl, pH 7.5, 0.03% SDS) to give a final concentration of 1 nM. To each well of a black, clear bottom 96-well plate (Corning, Inc.) was added 100 µL of the activated enzyme solution. Compounds being evaluated were diluted in neat DMSO and 1 µL of test agent or vehicle (DMSO) was added to each well of the 96-well plate; compounds were evaluated at 20, 10, 5, 2.5, 1.3, and 0.6 µM final concentrations. Enough chymotryptic substrate Suc-LLVY-AMC was then added to each well to give a final concentration of 100 µM. The rate of peptide hydrolysis (V_{max}) was determined using a Spectramax M5e (Molecular Devices,

Sunnyvale, Ca.) at 37° C over 30 minutes (λ_{ex} 380, λ_{em} 440). The initial linear portions of the curves were used to calculate the IC_{50} values.

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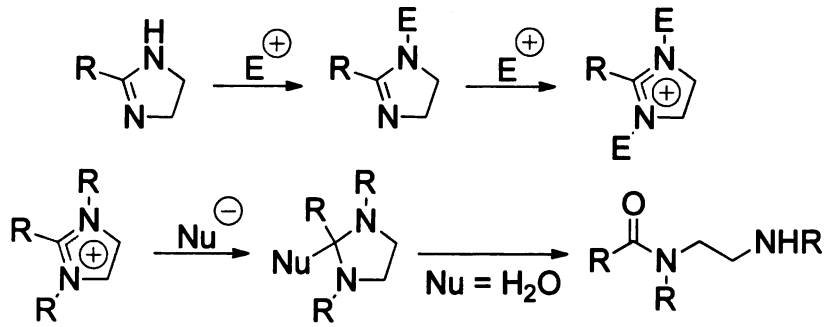
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CHAPTER IV

SYNTHESIS OF NOVEL MULTICYCLIC OXAZINONE/IMIDAZOLIDINE ADDUCTS FROM AN ANNULATION REACTION OF 2-IMIDAZOLINES

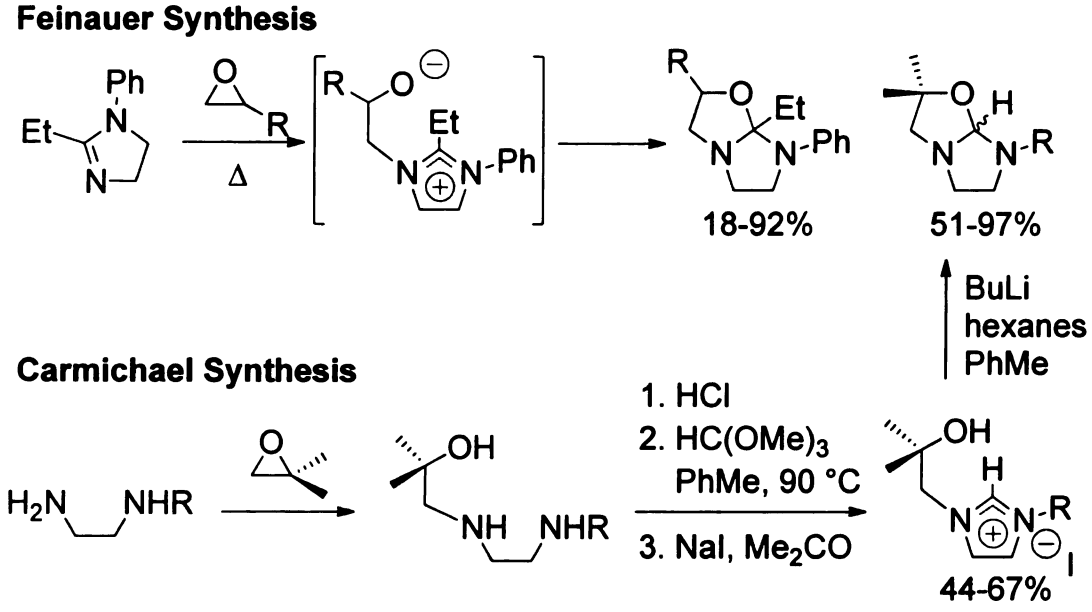
A. Reactivity of 2-imidazolines.

The reactivity of 2-imidazolines arises from the basic amidine functionality present within the heterocyclic scaffold.[1,2] Both amidine nitrogen atoms react with acids and electrophiles to generate a variety of substituted 2-imidazolines, including those obtained from N-alkylation, N-acylation, and N-sulfonylation reactions (Scheme IV-1).[1] Substitution of both nitrogen atoms results in the formation of 2-imidazolinium salts, many of which are isolable and stable. 2-imidazolinium salts differ in reactivity from the precursor 2-imidazolines and are susceptible to nucleophilic attack at the C2 or amidinium carbon (Scheme IV-1). Thus exposure of 2-imidazolinium salts to nucleophiles such as alcohols, amines, hydrides, malonate derivatives, and Grignard reagents results in the formation of a wide variety of imidazolidine adducts.[3-28] Such imidazolidines have been used as organocatalysts[26-28] as well as synthetic precursors to N-heterocyclic carbenes.[23-25] Also characteristic of 2-imidazolines is their ability to undergo hydrolysis when heated in basic or acidic aqueous solutions to give ring-opened acyclic β-aminoamides (Scheme IV-1).[1,29]



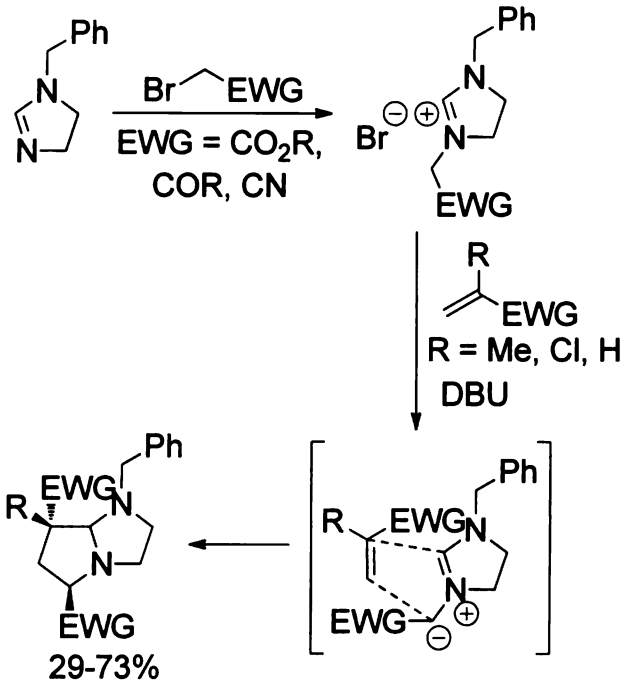
Scheme IV-1. Reactivity of 2-imidazolines and 2-imidazolinium salts.

Utilizing the combination of nucleophilic and electrophilic characteristics of 2-imidazolines in single-pot reactions has led to a number of interesting transformations. Molecules bearing both nucleophilic and electrophilic properties react with 2-imidazolines to generate novel annulation products. The earliest such reaction was reported in 1966 by Dr. Feinauer and involved heating epoxides in the presence of 2-imidazolines to form bicyclic products (Scheme IV-2).[4] The reaction likely proceeds through stepwise ring opening of the epoxide by the amidine nitrogen followed by annulation *via* nucleophilic attack of the resultant alkoxide to furnish the oxazolidine ring. Carmichael and co-workers have recently synthesized a related imidazolidine-oxazolidine ring system (Scheme IV-2). Their synthesis initiated with monoalkylation of ethylenediamine with an epoxide, followed by imidazolinium ring formation. The resultant hydroxy imidazolinium salt was then treated with BuLi to furnish the bicyclic adduct through alkoxide attack onto the amidinium carbon (Scheme IV-2).[25]



Scheme IV-2. Bicyclic adducts derived from 2-imidazolines and epoxides.

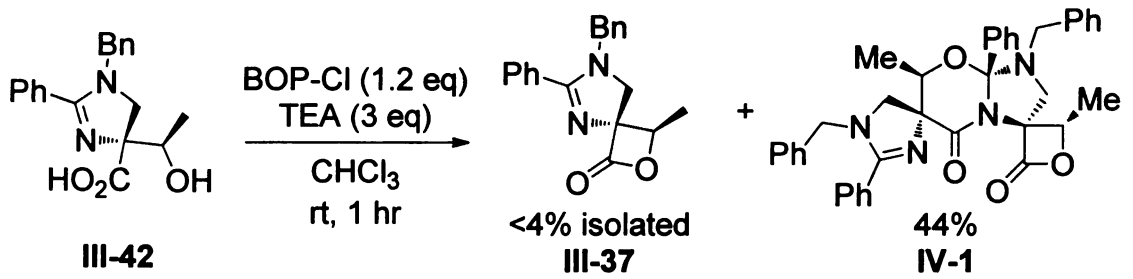
2-imidazolines have also been shown to be progenitors of azomethine ylides that can be used in cycloaddition reactions. Jones and co-workers have demonstrated that N-alkylation with an appropriate alkyl halide generates 2-imidazolinium salts.[19-22] These particular 2-imidazolinium salts react as azomethine ylides in cycloaddition reactions when treated with a base and electron deficient alkenes, resulting in the formation of bicyclic adducts (Scheme IV-3). The authors' strategy relies on N-alkylation of 2-imidazolines with alkyl halides bearing electron-withdrawing groups, such that azomethine ylides may later be generated for the formation of annulation products.



Scheme IV-3. Reaction of azomethine ylides generated from 2-imidazolines.

B. Synthesis of multicyclic imidazolidine/oxazinone adducts.

As previously described in Chapter III of this dissertation, the synthesis of 2-imidazoline **III-37** from **III-42** proceeded in low yields, and the desired product was isolated along with an unexpected product. X-ray crystal analysis revealed the structure of this unexpected product to be tetracyclic imidazoline **IV-1** (Scheme IV-4 and Figure IV-1). **IV-1** was formed as the major product in the reaction (in 44% yield), and interestingly it was found to inhibit the chymotrypsin-like activity of the 20S proteasome at low micromolar concentrations.

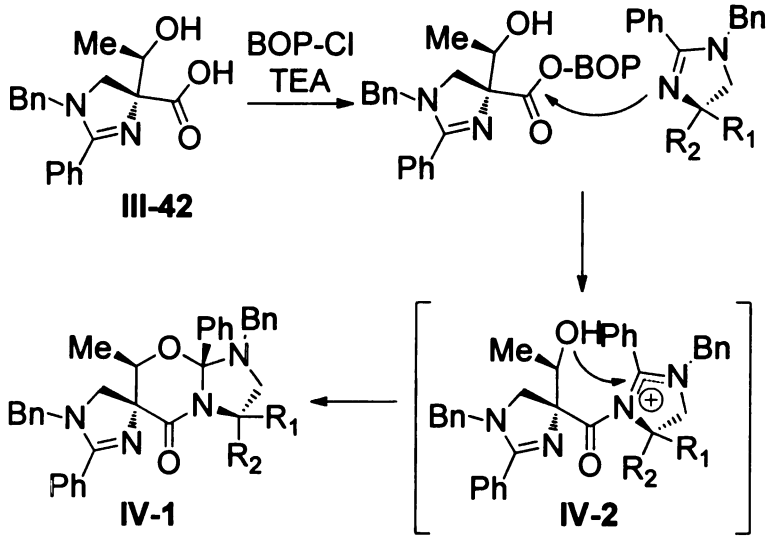


Scheme IV-4. Synthesis of 2-imidazolines comprising a β-lactone.

The potency exhibited by **IV-1** as an inhibitor of the chymotrypsin-like activity of the 20S proteasome was found to rival that of most of the 2-imidazolines thus far evaluated in our lab. Naturally, we were interested in determining the extent to which certain structural components present in **IV-1** contributed to its efficacy as a proteasome inhibitor. The three components comprising **IV-1** which were speculated to contribute most to the biological activity were the amidine, the β-lactone, and the fused bicyclic oxazinone/imidazolidine ensemble. Of these components, the unique fused bicyclic oxazinone/imidazolidine ensemble is arguably the most visually striking. Little is known about the reactivity or general biological profile of this scaffold which has received scarce literature exposure.[30,31] We decided, then, to pursue the construction of analogs of **IV-1** as a means of investigating the novel scaffold and its potential role in biological processes.

Initially, we focused our attention towards the formation of **IV-1**, as we anticipated similar reactions would give rise to structurally related analogs. The oxazinone ring in multicyclic adduct **IV-1** is believed to arise from stepwise intermolecular amide formation followed by annulation. Treatment of carboxylic

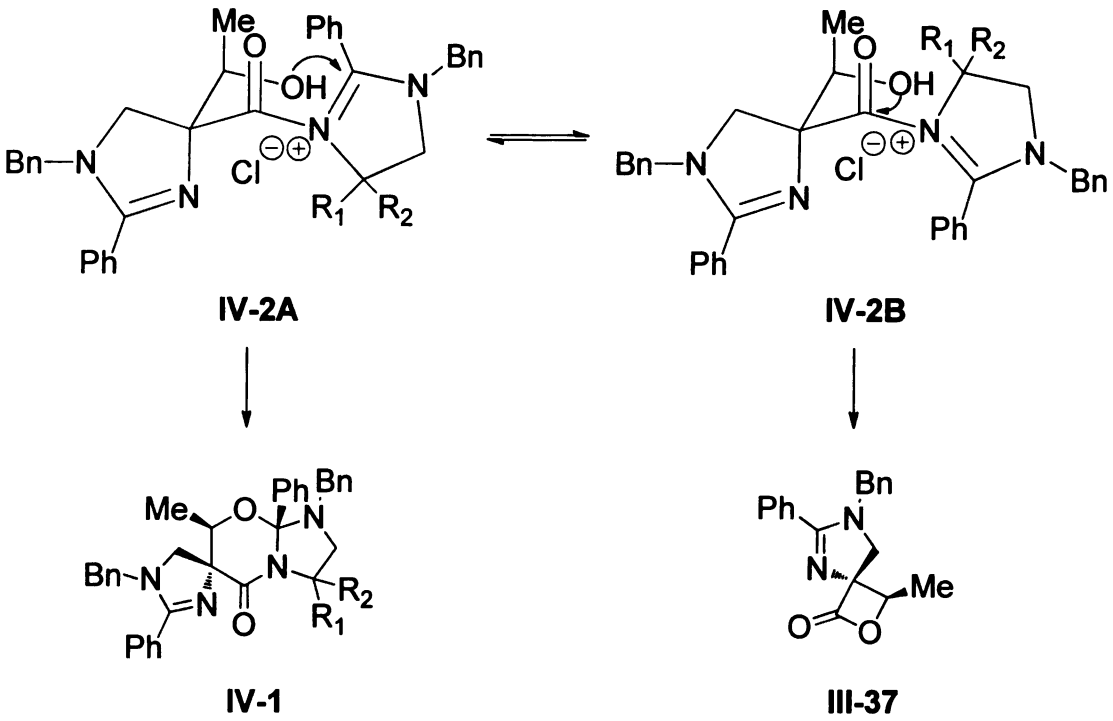
acid **III-42** with TEA and BOPCl initially generates a mixed BOP-anhydride or acid chloride intermediate (Scheme IV-5). Intermolecular amide formation is then believed to occur through attack of the nucleophilic amidine onto the carboxylic acid derivative to give rise to **IV-2**. Formation of the C-N bond would result in an enhancement of electrophilicity at C2, such that annulation should proceed to afford the oxazinone ring present in **IV-1**.



Scheme IV-5. Oxazinone formation from stepwise amidation and annulation.

Novel heterocyclic adduct **IV-1** was isolated as a single diastereomer, a finding that warranted further investigation into the mechanism of its formation. Interestingly, only heterodimer **IV-1** was observed in the reaction (the relative stereochemistry is depicted in Figure IV-1), which arises from the interaction of one enantiomer of **III-42** with the opposite enantiomer. One possibility for the observed selectivity involves initial β-lactone formation, followed by intermolecular attack of the labile lactone by **III-42** (or **III-37**) for the formation of **IV-1**. However, when the reaction progress was monitored *via* kinetic ^{1}H-NMR

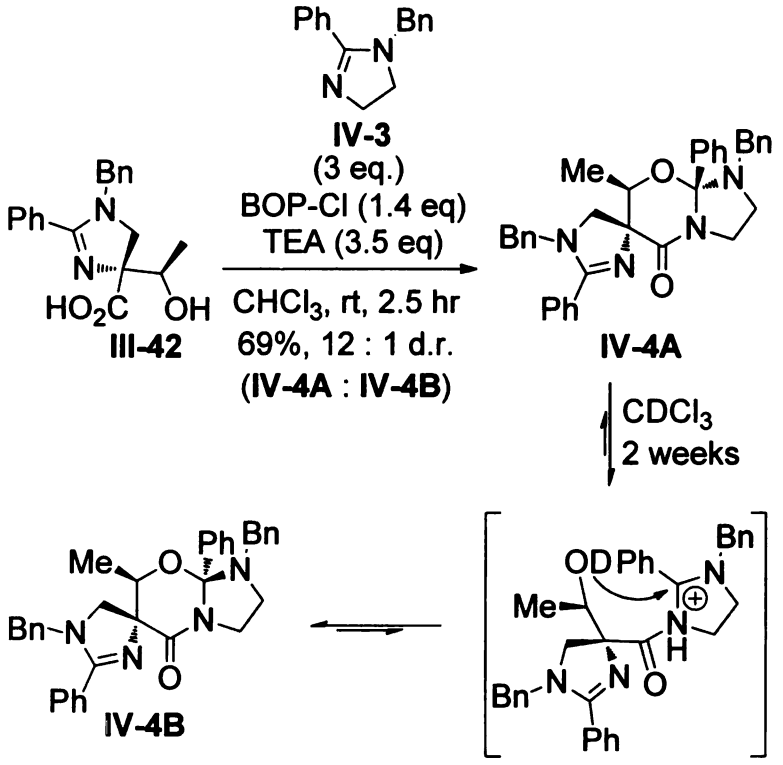
studies it was observed that 2-imidazolines **III-37** and **IV-1** accumulated at a similar rate and in overall equimolar amounts, suggesting that **III-37** is likely not consumed for the formation of **IV-1**. In light of similar rates of formation of **III-37** and **IV-1**, it is envisioned that the 2-imidazoline approaches the activated carbonyl from either face, leading to two interconvertible rotamer-like intermediates (**IV-2A** and **IV-2B**, Scheme IV-6). **IV-2A** is envisioned to undergo facile ring formation *via* a chair transition state to generate the 6-membered oxazinone ring. However, analogous oxazinone ring formation from intermediate **IV-2B** is less favorable, as ring formation would arise by means of a boat or twist-boat transition state. Instead, it is proposed that the alcohol of **IV-2B** intercepts the imidazolinoyl amide carbonyl to form β-lactone **III-37**. However, β-lactone formation from intermediate **IV-2A** is also plausible, and therefore cannot be ruled out. Alternatively, it is possible that **IV-2A** and **IV-2B** are preorganized into chair conformations mediated by H-bonding between the activated carbonyl oxygen and the nucleophilic alcohol.[32] Such preorganized chair conformations are predicted to be thermodynamically favorable, albeit oxazinone ring formation from these conformations is anticipated to proceed through a twist-boat transition state. Finally, the β-lactone ring present in **IV-1** is believed to originate from intramolecular attack of the alcohol onto the BOP-activated carbonyl, although it has not been determined whether this lactone ring forms before or after intermolecular adduct formation.



Scheme IV-6. Proposed mechanism of selective oxazinone ring formation.

The mechanism proposed for the formation of **IV-1** suggested that **III-42** should react with various 2-imidazolines under similar reaction conditions. Treatment of an *in situ*-generated mixed anhydride or acid chloride derived from **III-42** with different 2-imidazolines was anticipated to result in the formation of adducts varying by substitution about the resultant imidazolidine ring. Our hypothesis was tested by treating **III-42** with **IV-3** under basic conditions at room temperature (Scheme IV-7). The desired product obtained from the use of 2-imidazoline **IV-3** was expected to contain all structural features present in **IV-1** except the β-lactone moiety. Thus, the reaction would also to serve to probe the extent to which the β-lactone contributed to the potency of **IV-1** as a proteasome inhibitor. Three equivalents of **IV-3** were used in the annulation reaction to favor

the formation of the desired muticyclic adduct relative to **III-37** and **IV-1**. To our delight, the reaction afforded 69% yield of **IV-4** as a 12 : 1 ratio of diastereomers, along with minor amounts of **III-37** and **IV-1**. The proposed relative stereochemistry in each of the newly formed chiral centers in **IV-4A** and **IV-4B** was determined *via* nOe analysis, as shown in Figure IV-2. Major diastereomer **IV-4A** is proposed to arise from a transition state analogous to that for the formation of **IV-1**. Additionally, diastereomer **IV-4A** was discovered to undergo epimerization upon standing in $CDCl_3$ for 2 weeks, thereby affording primarily diastereomer **IV-4B**. The epimerization is believed to occur due to the trace acid present in $CDCl_3$, which promotes ring opening of the oxazinone ring *via* protonation of the ether oxygen (Scheme IV-7). An similar observation has been reported by Carmichael and coworkers, who treated their bicyclic ring system (Scheme IV-2) with $CDCl_3$, resulting in deuterium incorporation, presumably through a ring-opened intermediate.[25]



Scheme IV-7. Synthesis of oxazinone derivatives **IV-4A** and **IV-4B**.

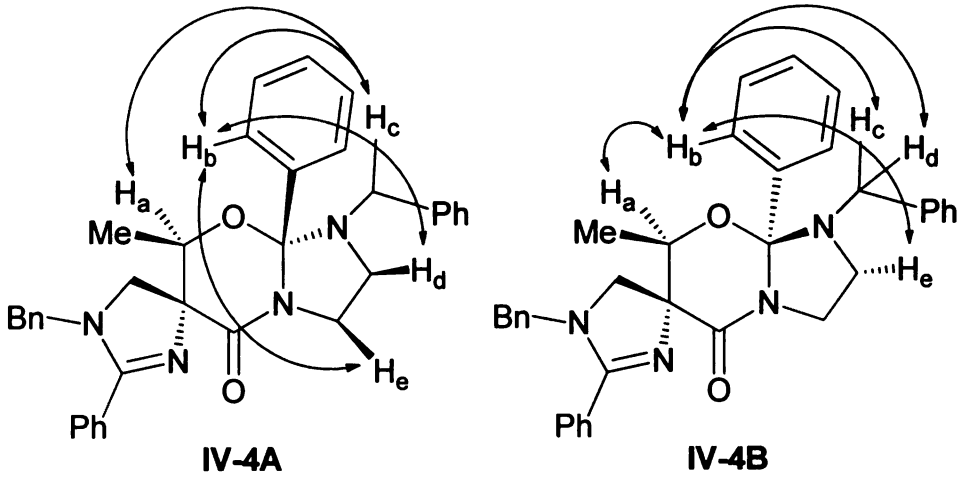
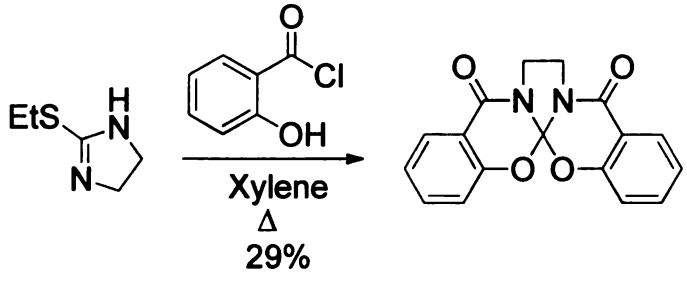


Figure IV-2. Major nOe correlations for **IV-4A** and **IV-4B**.

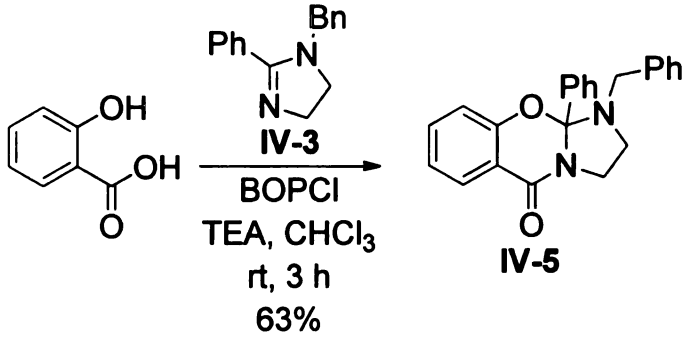
The formation of **IV-4** under our reaction conditions prompted our investigation into the use of other reactive β-hydroxy carboxylic acid derivatives.

Exhaustive literature searches have revealed only one incidence of an annulation reaction between a β-hydroxy carboxylic acid derivative and a 2-imidazoline. The lone example was reported by Kollenz and co-workers, who demonstrated that 2-thioethyl-2-imidazoline reacts with two equivalents of salicyloyl chloride when heated in xylene to give a symmetrical multicyclic adduct (Scheme IV-8).[30] In agreement with Kollenz's results, we have observed that a bicyclic adduct **IV-5** is available from the treatment of salicyloyl chloride, generated *in situ* from salicylic acid and BOP-Cl, with **IV-3** under basic conditions (Scheme IV-8).

Kollenz reaction



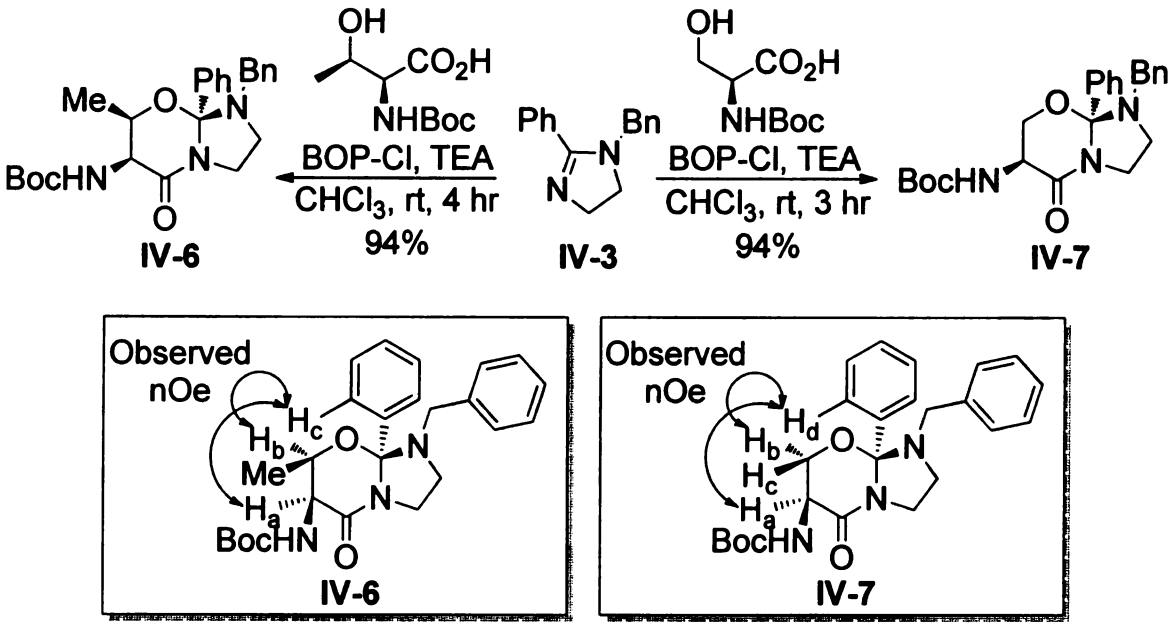
Our Observation



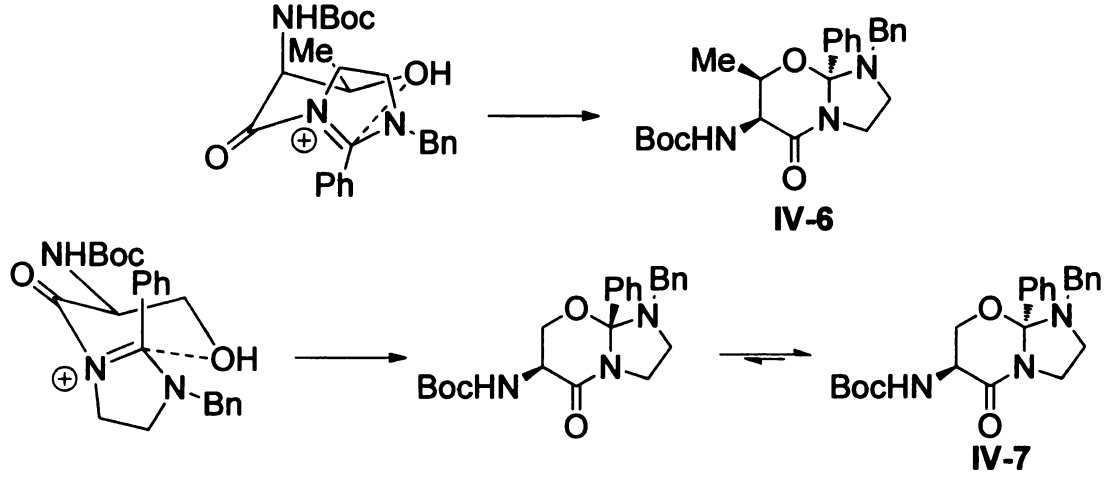
Scheme IV-8. Oxazinone/imidazolidine adducts derived from salicylic acid.

We proposed that oxazinone annulation products should also be available from 2-imidazolines and simple β-hydroxy carboxylic acids. We decided to test our hypothesis using the simple amino acid derivative N-Boc-L-threonine in the

reaction with 2-imidazoline **IV-3.** As anticipated, cyclization proceeded smoothly to afford **IV-6** in 94% yield as a single enantiomer (Scheme IV-9). The stereochemistry of the new stereocenter was determined *via* nOe analysis and was revealed be opposite of that observed in **IV-1**. It is believed that the annulation proceeds through a chair transition state, the conformation of which allows the sterically demanding methyl group to occupy an equatorial position. Such a transition state favors the stereochemistry observed in **IV-6** (Scheme IV-10). The results obtained from the stereoselective formation of **IV-6** encouraged us to also investigate the use of N-Boc-L-serine in the annulation reaction with **IV-3.** As anticipated, the annulation reaction proceeded smoothly to afford bicyclic adduct **IV-7** as a single enantiomer in 94% yield (Scheme IV-9). Interestingly, nOe analysis revealed that the stereochemistry of the new stereogenic was *trans* with respect to the carbamate. This annulation reaction is proposed to proceed through a chair transition state, wherein the carbamate occupies an equatorial position, to give the sterically strained *cis* disubstituted oxazinone ring (Scheme IV-10). Subsequent rapid epimerization of the new stereocenter, as was observed in the epimerization of **IV-4**, would then result in a reduction of steric strain to give thermodynamically favored epimer **IV-6**. Moreover, further epimerization of **IV-6** or **IV-7** has not been observed, even after allowing each compound to stand in $CDCl_3$ at room temperature for two weeks.



Scheme IV-9. Bicyclic oxazinone adducts arising from amino acid derivatives.



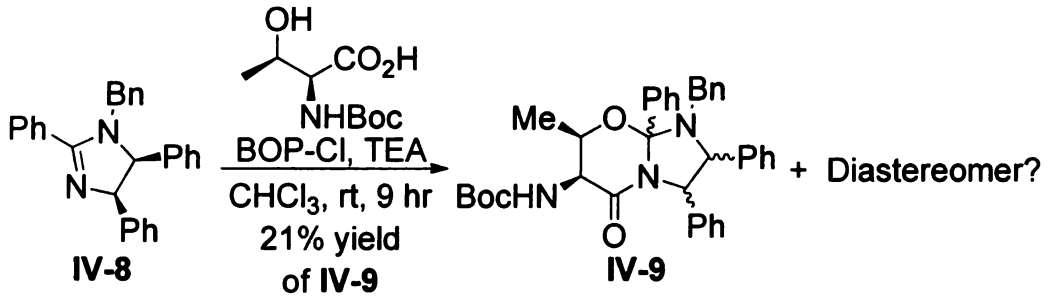
Scheme IV-10. Proposed origin of selectivity in the formation of **IV-6** and **IV-7**.

C. Towards Chiral Resolution of Racemic 2-Imidazolines

Several studies in the Tepe lab have involved the synthesis of 2-imidazolines for use in pharmaceutically relevant assays.[33-35] The majority of these compounds have been prepared by means of a diastereoselective Lewis-acid catalyzed

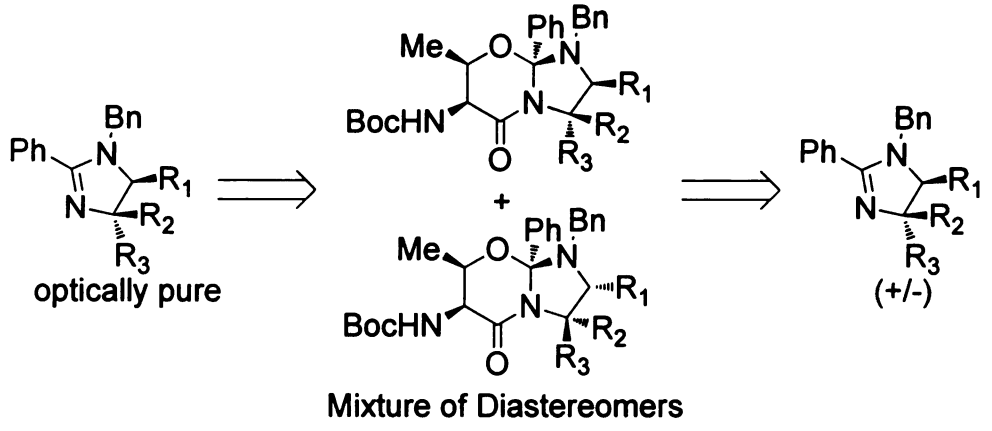
dipolar cycloaddition reaction of oxazol-5(4H)-ones and imines, as was described in Chapter I of this dissertation.[36-38] This chemistry allows for the rapid production of a variety of racemic 2-imidazolines. However, ongoing studies in our lab have required the use of enantiopure materials, and chiral HPLC has been utilized to accommodate our needs. Unfortunately, our studies sometimes require the use of multigram amounts of enantiopure 2-imidazolines, and such large amounts have been difficult to obtain using our current methods. Our lab has been interested, then, in developing chemical methods which might help us to procure enantioenriched or enantiopure 2-imidazolines.

Recent developments in our lab have demonstrated stereoselective adduct formation from an annulation reaction of 2-imidazolines. As was previously discussed, only a single diastereomer was observed to arise from the annulation reaction of either N-Boc-L-threonine or N-Boc-L-serine with **IV-3**. Interestingly, preliminary studies indicate that the use of racemic *cis*-4,5-diphenyl 2-imidazoline **IV-8** in the reaction with N-Boc-L-threonine results in the formation of two products comprising a bicyclic oxazinone/imidazolidine ensemble (Scheme IV-11). The two products were formed in nearly equal amounts in the unoptimized reaction and are presumed to be diastereomers. Unfortunately, due to difficulties encountered during purification, only one product was cleanly isolated, the stereochemistry of which has not been unambiguously established (**IV-9**, Scheme IV-11).



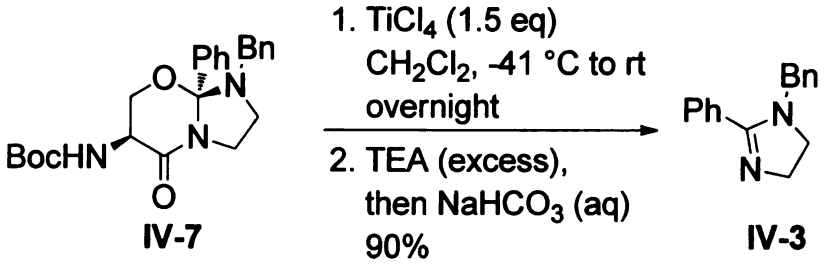
Scheme IV-11. Synthesis of **IV-9** derived from **IV-8**.

We were encouraged by the results from the above reaction, and we believed that such a reaction might be useful for the chiral resolution of racemic 2-imidazolines prepared in our lab. It is anticipated that racemic 2-imidazolines of interest in our studies should undergo annulation reactions to afford separable optically active oxazinone/imidazolidine adducts. Adducts prepared through chemistry have been shown to be sensitive to acid and have been observed to undergo epimerization, presumably through a ring-opened intermediate (Scheme IV-7). This observation has prompted our investigation into acid-mediated decomposition reactions of oxazinone/imidazolidine adducts as a means of regenerating 2-imidazolines. It is envisioned that an annulation/decomposition sequence would serve as a means of chiral resolution for racemic mixtures of 2-imidazolines prepared in our lab, thereby obviating the need to separate enantiomers *via* our current method involving chiral HPLC.



Scheme IV-12. Strategy for the resolution of 2-imidazoline enantiomers.

To this end, oxazinone/imidazolidine adduct decomposition has been successfully demonstrated. Decomposition of **IV-6** was initially attempted through treatment with Brønsted acids in MeOH. Such reactions proceeded to fully consume all starting material, but no 2-imidazoline was ever recovered in the reactions, presumably due to 2-imidazoline hydrolysis.[1,29] Oxazinone adduct decomposition was then attempted *via* treatment with Lewis acids in a non-nucleophilic solvent; similar conditions have been effective for the Lewis acid-mediated cleavage of N-acyl hemiaminal ethers.[39-41] Thus, treatment of **IV-6** with one equivalent of $TiCl_4$ in anhydrous CH_2Cl_2 followed by a water workup afforded a 2.4 : 1 ratio of **IV-6** to **IV-3** with an unknown contaminant. Furthermore, the analogous decomposition of **IV-7** has been optimized to cleanly yield **IV-3** in greater than 90% yield (Scheme IV-13).



Scheme IV-13. $TiCl_4$-mediated decomposition of **IV-7**.

Further studies involving the annulation reaction of 2-imidazolines with β-hydroxy carboxylic acid derivatives are underway in our labs. A major aim of these studies is to utilize our newly discovered chemistry for the resolution of 2-imidazolines prepared racemically by our chemistry. In addition, the annulation reaction may be applied to the formation of other fused hetereocycles, such as those derived from 2-oxazolines. We anticipate that the fused bicyclic heterocycles prepared from our chemistry will be useful as building blocks in synthesis. Moreover, compounds prepared from this chemistry may potentially demonstrate activity against thus far unevaluated biological targets. However, in order for anticipated applications to come to fruition, an increased knowledge concerning the relationship between such compounds and biological activity is necessary, and our continuing efforts in this area will hopefully bring such a relationship to light.

D. Evaluation of proteasome inhibition.

The oxazinone/imidazolidine adducts prepared in this chapter were tested for their ability to inhibit the chymotryptic-like activity of the 20S proteasome in an *in*

vitro assay. The results of the assay are shown in Table IV-1. β-lactone-containing multicyclic 2-imidazoline **IV-1** was found to inhibit chymotrypsin-like activity at low micromolar levels. Multicyclic 2-imidazolines **IV-4A** and **IV-4B** both inhibited proteolytic activity at lower concentrations than **IV-1**, indicating that the β-lactone likely doesn't significantly contribute to the biological activity of **IV-1**. Moreover, the parent 2-imidazolines from which **IV-4A** and **IV-4B** were derived (**III-42** and **IV-3**) were both found to be inactive as inhibitors (IC_{50} = >20 μM).[42] Finally, >10 μM of fused heterocycle adducts **IV-5** – **IV-7**, all of which lack 2-imidazolines, was required to inhibit chymotrypsin-like activity. It is noteworthy that multiheterocyclic adducts **IV-1** and **IV-4** inhibited proteolytic activity to a greater extent than bicyclic adducts **IV-5** – **IV-7**, as the results suggest that the amidine portion of the 2-imidazoline may likely be important for inhibition of proteolytic activity.

Multicyclic adducts **IV-1** and **IV-4** have demonstrated inhibition of the chymotryptic-like activity of the 20S proteasome at low micromolar levels, yet it is unlikely that either of these compounds will become lead compounds in our studies due to their lengthy preparation. However, the synthesis and evaluation of such compounds has given us insight into structural features which may be important for inhibition of the 20S proteasome. Furthermore, the chemistry used for the preparation of bicyclic oxazinone/imidazolidine adducts has demonstrated to us the ease by which 2-imidazolines may undergo transformations stemming from their underutilized reactivity. Such reactivity will be considered in the planning and execution of reactions involving 2-imidazolines in our future studies.

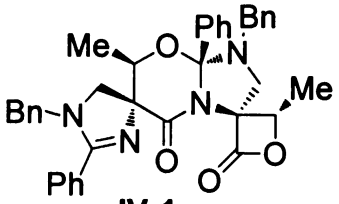
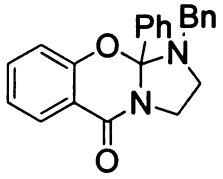
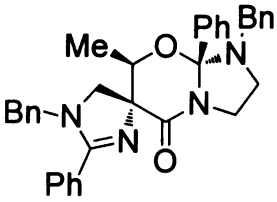
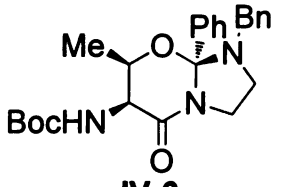
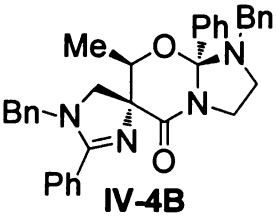
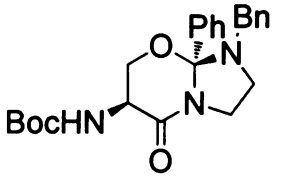
Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
 **IV-1**	1.41	 **IV-5**	15.9
 **IV-4A**	0.74	 **IV-6**	15.9
 **IV-4B**	1.24	 **IV-7**	12.8

Table IV-1. Inhibition of chymotrypsin-like activity of the 20S proteasome by oxazinone/imidazolidine adducts.

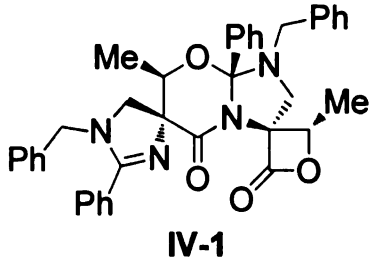
E. Experimental.

1. General information. Reactions were carried out in flame-dried glassware under nitrogen atmosphere. All reactions were magnetically stirred and monitored by TLC with 0.25 μm pre-coated silica gel plates using either UV light or iodine to visualize the compounds. Column chromatography was carried out on Silica Gel 60 (230-400 mesh) supplied by EM Science. Yields refer to chromatographically and spectroscopically pure compounds unless otherwise noted. Infrared spectra were recorded on a Nicolet IR/42 spectrometer. Optical rotations were recorded on a Perkin Elmer 341 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on either a Varian Unity Plus-500 spectrometer or a

Varian Inova-600 spectrometer. Chemical shifts are reported relative to the residue peaks of the solvent. The following abbreviations are used to denote the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, and m = multiplet. HRMS were obtained at the Mass Spectrometry Facility of Michigan State University with a JEOL JMS HX-110 mass spectrometer. Melting points were obtained using an Electrothermal® capillary melting point apparatus and are uncorrected.

2. Materials. Reagents and solvents were purchased from commercial suppliers and used without further purification. Anhydrous methylene chloride was dispensed from a delivery system which passes the solvents through a column packed with dry neutral alumina. Anhydrous chloroform, N,N-dimethylformamide, and triethylamine were distilled from calcium hydride and stored over activated molecular sieves prior to use. N-Boc-L-threonine,[43] N-Boc-L-serine,[44] and **IV-8**[34] were synthesized according to literature preparations. The synthesis of **III-42** was described in Chapter III of this dissertation.

3. Synthesis and Characterization.

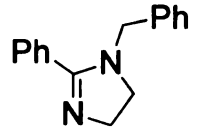


IV-1

Figure IV-3. DL-(**IV-1**).

DL-(IV-1). To a stirring suspension of **III-42** (0.1185 g, 0.37 mmol) in $CHCl_3$ (2.8 mL) were successively added TEA (0.15 mL, 1.1 mmol) and BOP-Cl (0.1167 g, 0.46 mmol), and the reaction mixture stirred vigorously at room temperature under N_2 atmosphere for 1.5 hours. The reaction was then diluted with CH_2Cl_2 (7 mL) and washed with ice cold H_2O/brine mixture (5:1, 15 mL). The organic layer was collected and the aqueous layer was extracted with CH_2Cl_2 (2 x 15 mL). The organic layer was collected and the aqueous layer was extracted with CH_2Cl_2 (2 x 15 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to a white residue. Purification of the residue *via* silica gel chromatography (2% TEA / 35% EtOAc / 63% hexanes on silica gel pretreated with TEA) afforded 48.8 mg of the title compound (44%) as a film. Recrystallization of the product from benzene and petroleum ether afforded the product as clear crystals (m.p. = 204 - 206 °C). ^{1}H NMR (500 MHz) ($CDCl_3$): δ 1.24 (d, J = 6.3 Hz, 3H), 1.30 (d, J = 6.5 Hz, 3H), 2.50 (d, J = 12.0 Hz, 1H), 3.06 (d, J = 10.8 Hz, 1H), 3.19 (d, J = 12.0 Hz, 1H), 3.37 (d, J = 10.8 Hz, 1H), 3.55 (d, J = 12.9 Hz, 1H), 4.07 (d, J = 12.9 Hz, 1H), 4.17 (d, J = 16.0 Hz, 1H), 4.37 (d, J = 16.0 Hz, 1H), 4.75 (q, J = 6.3 Hz, 1H), 5.44 (q, J = 6.5 Hz, 1H), 7.11 (d, J = 7.3 Hz, 2H), 7.25 – 7.47 (m, 16 H), 7.65 – 7.69 (m, 2 H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$): δ 14.0 (–**C**H_3), 15.3 (–**C**H_3), 47.1 (–**C**H_2), 51.2 (–**C**H_2), 52.1 (–**C**H_2), 52.2 (–**C**H_2), 72.0 (quaternary –**C**), 72.4 (–**C**H), 73.7 (quaternary –**C**), 75.8 (–**C**H), 103.2 (quaternary –**C**), 125.3 (aromatic –**C**H), 126.9 (aromatic –**C**H), 127.4 (aromatic –**C**H), 127.4 (aromatic –**C**H), 128.3 (aromatic –**C**H), 128.5 (aromatic –**C**H), 128.5 (aromatic –**C**H), 128.6 (aromatic –**C**H), 128.6 (aromatic –**C**H), 128.7 (aromatic –**C**H), 129.0 (aromatic –**C**H), 130.1

(aromatic quaternary –C), 130.5 (aromatic –CH), 136.9 (aromatic quaternary –C), 137.4 (aromatic quaternary –C), 139.6 (aromatic quaternary –C), 168.49 (quaternary –C), 168.50 (quaternary –C), 171.8 (quaternary –C). IR (neat): 2928 cm^{-1}, 1834 cm^{-1}, 1694 cm^{-1}, 1384 cm^{-1}, 1087 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{38}H_{37}N_4O_4$ [M+H], 613.2815; found, 613.2816.



IV-3

Figure IV-4. 1-benzyl-2-phenyl-2-imidazoline (**IV-3**).

1-benzyl-2-phenyl-2-imidazoline (IV-3). According to modifications to a known procedure,[45] a stirring suspension of NaH (69.9 mg, 2.9 mmmol) in DMF (4 mL), cooled to 0 °C in an ice bath, was treated dropwise with a solution of 2-phenyl-2-imidazoline (0.3040 g, 2.1 mmol) in DMF (4 mL). After stirring at 0 °C for 25 minutes, benzyl bromide (0.30 mL, 2.5 mmol) was added and the reaction stirred overnight under an atmosphere of N_2 while warming to room temperature. The reaction was poured over H_2O (40 mL) and extracted with $CHCl_3$ (4 x 30 mL). The pooled organic extracts were washed successively with 10% aqueous LiCl solution (3 x 100 mL) and brine before being dried (Na_2SO_4) and concentrated under vacuum. Purification of the crude product *via* silica gel chromatography (8% TEA / 92% EtOAc) was performed to give 0.3040 g of the title compound (62% yield) as an off-white solid (m.p. = 65 - 68 °C) The spectral data matches that previously reported in the literature.[46] ^1H NMR (500 MHz) ($CDCl_3$): δ 3.36 (t, J = 9.9 Hz, 2 H), 3.90 (t, J = 9.9 Hz, 2 H), 4.26 (s, 2 H), 7.23 – 7.27 (m, 3 H), 7.31

– 7.39 (m, 5 H), 7.59 – 7.63 (m, 2 H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$): δ 50.7 (–CH_2), 52.8 (–CH_2), 53.2 (–CH_2), 126.8 (aromatic –CH), 126.9 (aromatic –CH), 127.8 (aromatic –CH), 128.1 (aromatic –CH), 128.3 (aromatic –CH), 129.5 (aromatic –CH), 131.1 (aromatic quaternary –C), 137.7 (aromatic quaternary –C), 167.0 (quaternary –C). HRMS (FAB): *m/z* calcd for $C_{16}H_{17}N_2$ [M+H], 237.1392; found, 237.1404.

4. General procedure for the synthesis of multicyclic oxazinone adducts. The general procedure used for the formation of oxazinone adducts **IV-4** – **IV-7** and **IV-9** is as follows: To a stirring 0.2 M solution of 2-imidazoline in $CHCl_3$ were added TEA (≥3 equivalents) and β-hydroxy carboxylic acid (1.1 equivalent). It should be noted that an excess of 2-imidazoline (≥1.1 equivalents) relative to the amount of β-hydroxy carboxylic acid can also be used to achieve similar product yields. The reaction mixture was treated with BOP-Cl (1.4 equivalents) and stirred at room temperature under nitrogen atmosphere for the requisite amount of time, as monitored by TLC. The reaction was diluted in CH_2Cl_2 (10X reaction volume) and washed with saturated $NaHCO_3$/NaCl mixture (10:1 ratio of saturated aqueous solutions, volume equal to organic layer). The organic layer was collected and the aqueous layer was extracted with more CH_2Cl_2 (x2). The pooled organic extracts were dried (Na_2SO_4) and concentrated. The crude reaction mixtures were purified *via* column chromatography on silica gel.

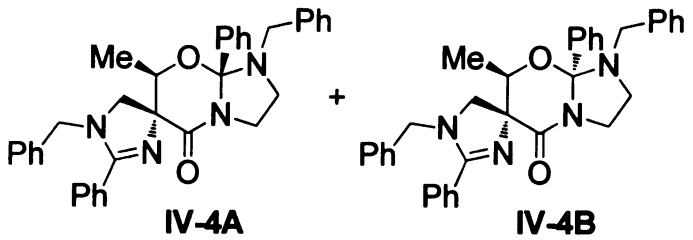
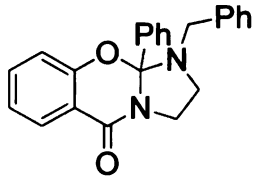


Figure IV-5. IV-4A + IV-4B.

(IV-4A + IV-4B). Following the general procedure, BOP-Cl (75.2 mg, 0.30 mmol) was added to a stirring solution of **III-42** (67.7 mg, 0.21 mmol), **IV-3** (149.9 mg, 0.63 mmol), and TEA (0.10 mL, 0.72 mmol) in $CHCl_3$ (1.0 mL), and the reaction stirred at room temperature for 2.5 hours. Purification *via* silica gel chromatography (2% TEA / 16% hexanes / 82% EtOAc on silica gel pretreated with TEA) was performed to afford 78.2 mg of the title compound (oil, 69% yield) as a 12 : 1 mixture of diastereomers. The mixture of diastereomers was allowed to stand in $CDCl_3$ for two weeks, after which it was observed that all of **IV-4A** had epimerized to **IV-4B**. **IV-4A**: ^{1}H NMR (500 MHz) ($CDCl_3$): δ ^{1}H NMR (500 MHz) ($CDCl_3$): δ 1.26 (d, J = 6.4 Hz, 3 H), 2.35 (dd, J_1 = 8.8 Hz, J_2 = 16.5 Hz, 1 H), 3.00 (d, J = 13.2 Hz, 1 H), 3.03 – 3.10 (m, 2 H), 3.52 (d, J = 10.7 Hz, 1 H), 3.57 (ddd, J_1 = 3.3 Hz, J_2 = 8.7 Hz, J_3 = 11.7 Hz, 1 H), 4.10 (d, J = 13.2 Hz, 1 H), 4.13 – 4.19 (m, 2 H), 4.40 (d, J = 16.2 Hz, 1 H), 4.71 (q, J = 6.4 Hz, 1 H), 7.16 (d, J = 7.1 Hz, 2 H), 7.20 – 7.44 (m, 16 H), 7.67 – 7.70 (m, 2 H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$): δ 14.0, 42.9, 44.8, 52.1, 52.3, 52.7, 69.9, 73.6, 101.1, 125.7, 126.7, 127.0, 127.3, 127.9, 128.1, 128.24, 128.25, 128.3, 128.5, 128.7, 130.3, 137.2, 138.4, 138.8, 167.9, 172.4. IR (neat): 1681 cm^{-1}, 1592 cm^{-1}, 1388 cm^{-1}, 1232 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{35}H_{35}N_4O_2$ [M+H], 543.2760; found,

543.2774. **IV-4B**: 1.23 (d, J = 6.4 Hz, 3 H), 2.34 (q, J = 9.2 Hz, 1 H), 2.93 – 3.01 (m, 2 H), 3.51 – 3.59 (m, 2 H), 3.84 (d, J = 10.2 Hz, 1 H), 3.90 (d, J = 12.9 Hz, 1 H), 4.01 (dt, J_1 = 8.8 Hz, J_2 = 11.8 Hz, 1 H), 4.17 (d, J = 15.7 Hz, 1 H), 4.22 (q, J = 6.5 Hz, 1 H), 4.61 (d, J = 15.8 Hz, 1 H), 7.14 – 7.44 (m, 18 H), 7.62 (d, J = 6.8 Hz, 2 H). ^{13}C NMR (150 MHz) ($CDCl_3$): δ 14.3, 43.0, 44.6, 52.4, 52.8, 55.3, 71.8, 74.0, 103.2, 126.7, 127.0, 127.1, 127.5, 128.2, 128.25, 128.28, 128.5, 128.70, 128.73, 128.8, 130.3, 130.4, 137.39, 137.42, 137.8, 168.9, 172.2. IR (neat): 3060 cm^{-1}, 2836 cm^{-1}, 1666 cm^{-1}, 1406 cm^{-1}, 1216 cm^{-1}, 1074 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{35}H_{35}N_4O_2$ [M+H], 543.2760; found, 543.2769.

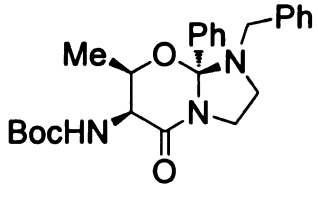


IV-5

Figure IV-6. 1-benzyl-10a-phenyl-2,3-dihydro-1H-benzo[e]imidazo[2,1-b][1,3]oxazin-5(10aH)-one (**IV-5**).

1-benzyl-10a-phenyl-2,3-dihydro-1H-benzo[e]imidazo[2,1-b][1,3]oxazin-5(10aH)-one (IV-5). Following the general procedure, BOP-Cl (73.5 mg, 0.29 mmol) was added to a stirring solution of **IV-3** (47.4 mg, 0.20 mmol), salicylic acid (31.0 mg, 0.22 mmol), and TEA (0.090 mL, 0.64 mmol) in $CHCl_3$ (1.0 mL), and the reaction stirred at room temperature for 3 hours. Purification *via* silica gel chromatography (50% EtOAc / 46% hexanes / 4% TEA) was performed to afford 45.3 mg of the title compound (63% yield) as a white solid (m.p. = 141 – 144 °C). ^{1}H NMR (500 MHz) ($CDCl_3$): δ 2.93 – 2.99 (m, 1 H), 3.14 (dt, J_1 = 6.7 Hz, J_2 = 7.7 Hz, 1 H), 3.81 (d, J = 13.8 Hz, 1 H), 3.91 (dt, J_1 = 7.0 Hz, J_2 = 10.6 Hz, 1 H), 3.97

(d, J = 13.8 Hz, 1 H), 4.21 – 4.28 (m, 1 H), 6.99 (dt, J_1 = 1.0 Hz, J_2 = 7.6 Hz, 1 H), 7.12 (dd, J_1 = 0.7 Hz, J_2 = 8.2 Hz, 1 H), 7.15 – 7.20 (m, 2 H), 7.22 – 7.33 (m, 6 H), 7.40 – 7.50 (m, 3 H), 7.79 (dd, J_1 = 1.7 Hz, J_2 = 7.7 Hz, 1 H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$): δ 45.2 (–CH_2), 47.0 (–CH_2), 50.1 (–CH_2), 104.2 (quaternary –C), 117.1 (aromatic –CH), 119.5 (aromatic quaternary –C), 122.3 (aromatic –CH), 127.27 (aromatic –CH), 127.32 (aromatic –CH), 128.0 (aromatic –CH), 128.1 (aromatic –CH), 128.3 (aromatic –CH), 128.4 (aromatic –CH), 128.7 (aromatic –CH), 134.2 (aromatic –CH), 137.8 (aromatic quaternary –C), 138.9 (aromatic quaternary –C), 155.8 (aromatic quaternary –C), 161.4 (quaternary –C). IR (neat): 3040 cm^{-1}, 2842 cm^{-1}, 1671 cm^{-1}, 1613 cm^{-1}, 1471 cm^{-1}, 1412 cm^{-1}, 1247 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{23}H_{21}N_2O_2$ [M+H], 357.1603; found, 357.1613.

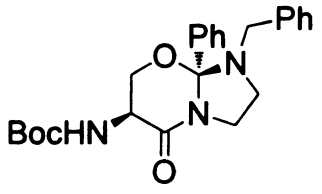


IV-6

Figure IV-7. *tert*-butyl ((6S,7R,8aS)-1-benzyl-7-methyl-5-oxo-8a-phenylhexahydro-1H-imidazo[2,1-b][1,3]oxazin-6-yl)carbamate (**IV-6**).

***tert*-butyl ((6S,7R,8aS)-1-benzyl-7-methyl-5-oxo-8a-phenylhexahydro-1H-imidazo[2,1-b][1,3]oxazin-6-yl)carbamate (IV-6)**. Following the general procedure, BOP-Cl (0.2742 g, 1.08 mmol) was added to a stirring solution of **IV-3** (0.1813 g, 0.77 mmol), N-Boc-L-threonine (0.1872 g, 0.85 mmol), and TEA (0.37 mL, 2.7 mmol) in $CHCl_3$ (3.9 mL), and the reaction stirred at room temperature for 4 hours. Purification *via* silica gel chromatography (60% EtOAc / 36%

hexanes / 4% TEA) was performed to afford 0.3170 g of the title compound (94% yield) as a fluffy white solid (m.p. = 85 – 87 °C) , which appeared as a 4:1 ratio of rotamers by ^{1}H-NMR, $[\alpha]^{20}_D$ = +27.0 (*c* 1.0, CH_2Cl_2). The rotamers were resolved at 14 °C such that splitting was observed for individual proton signals of the minor rotamer, whereas heating the sample to 41 °C resulted in a loss of splitting and convergence of some signals. ^{1}H NMR (500 MHz, 14 °C) ($CDCl_3$): δ 1.11 (d, J = 6.3 Hz, 3 H), 1.34 and 1.39 (2s, 9 H), 2.81 – 2.98 (m, 2 H), 3.52 – 3.63 (m, 2 H), 3.80 and 3.88 (2d, $J_{(3.80)}$ = 14.0 Hz, $J_{(3.88)}$ = 14.4 Hz, 1 H), 4.07 – 4.17 (m, 1 H), 4.25 (ddd, J_1 = 10.4 Hz, J_2 = 6.1 Hz, J_3 = 1.4 Hz, 1 H), 4.69 and 4.83 (2p, $J_{(4.69)}$ = 6.5 Hz, $J_{(4.83)}$ = 6.4 Hz, 1 H), 5.35 and 5.64 (2d, $J_{(5.35)}$ = 4.8 Hz, $J_{(5.64)}$ = 5.4 Hz, 1 H), 7.06 (d, J = 6.8 Hz, 2 H), 7.16 – 7.25 (m, 3 H), 7.36 – 7.46 (m, 3 H), 7.57 (d, J = 7.3 Hz, 2 H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$): δ 17.0 (–**C**H$_3$), 28.2 (–**C**H$_3$), 45.3 (–**C**H$_2$), 46.4 (–**C**H$_2$), 49.3 (–**C**H$_2$), 51.7 (–**C**H), 69.5 (–**C**H), 79.7 (quaternary –**C**), 101.9 (quaternary –**C**), 126.7 (aromatic –**C**H), 127.0 (aromatic –**C**H), 128.1 (aromatic –**C**H), 128.2 (aromatic –**C**H), 128.5 (aromatic –**C**H), 129.0 (aromatic –**C**H), 138.4 (aromatic quaternary –**C**), 140.0 (aromatic quaternary –**C**), 154.9 (quaternary –**C**), 166.8 (quaternary –**C**). IR (neat): 3413 cm^{-1}, 3322 cm^{-1}, 2976 cm^{-1}, 1718 cm^{-1}, 1686 cm^{-1}, 1427 cm^{-1}, 1165 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{25}H_{32}N_3O_4$ [M+H], 438.2393; found, 438.2409.

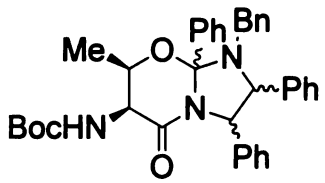


IV-7

Figure IV-8. *tert*-butyl ((6S,8aS)-1-benzyl-5-oxo-8a-phenylhexahydro-1H-imidazo[2,1-b][1,3]oxazin-6-yl)carbamate (**IV-7**).

***tert*-butyl ((6S,8aS)-1-benzyl-5-oxo-8a-phenylhexahydro-1H-imidazo[2,1-b][1,3]oxazin-6-yl)carbamate (IV-7)**. Following the general procedure, BOP-Cl (46.0 mg, 0.18 mmol) was added to a solution of N-Boc-L-serine (26.5 mg, 0.13 mmol), **IV-3** (37.1 mg, 0.16 mmol), and TEA (0.060 mL, 0.43 mmol) in $CHCl_3$ (0.60 mL). Purification *via* silica gel chromatography (50% EtOAc / 46% hexanes / 3% TEA) was performed to give 51.3 mg of the title compound (94% yield) as a as an off-white solid (m.p. = 134 – 136 °C), which appeared as a 5:1 ratio of rotamers by ^{1}H-NMR, $[\alpha]^{20}_D$ = +26.0 (*c* 1.0, CH_2Cl_2). The rotamers were resolved at 5 °C such that splitting was observed for individual proton signals of the minor rotamer, whereas heating the sample to 40 °C resulted in a loss of splitting and convergence of some signals. ^{1}H NMR at 5 °C (500 MHz, 5 °C) ($CDCl_3$): δ 1.32 and 1.39 (2s, 9H), 2.82 – 2.88 and 2.91 – 2.98 (2m, 2H), 3.49 – 3.69 and 3.77 – 3.82 (2m, 4H), 3.87 – 3.93 and 3.95 – 4.02 (2m, 1 H), 4.19 – 4.29 (m, 1 H), 4.45 and 4.59 (2t, $J_{(4.45)}$ = 8.7 Hz, $J_{(4.59)}$ = 9.2 Hz, 1 H), 5.31 and 5.58 (s and d, $J_{(5.58)}$ = 5.4 Hz, 1 H), 7.05 – 7.09 (m, 2 H), 7.17 – 7.26 (m, 3 H), 7.38 – 7.47 (m, 3 H), 7.48 – 7.56 (m, 2 H). ^{13}C NMR + **DEPT** (125 MHz) ($CDCl_3$): δ 28.2 (–**C**H$_3$), 44.8 (–**C**H$_2$), 46.4 (–**C**H$_2$), 48.2 (–**C**H), 49.8 (–**C**H$_2$), 66.1 (–**C**H$_2$), 79.9 (quaternary –**C**),

102.9 (quaternary –C), 126.6 (aromatic –CH), 127.1 (aromatic –CH), 128.19 (aromatic –CH), 128.23 (aromatic –CH), 128.7 (aromatic –CH), 129.2 (aromatic –CH), 138.1 (aromatic quaternary –C), 138.5 (aromatic quaternary –C), 155.3 (quaternary –C), 167.3 (quaternary –C). IR (neat): 3331 cm^{-1}, 2973 cm^{-1}, 1711 cm^{-1}, 1681 cm^{-1}, 1428 cm^{-1}, 1169 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{24}H_{30}N_3O_4$ [M+H], 424.2236; found, 424.2237.



IV-9

Figure IV-9. IV-9.

(IV-9). Following the general procedure, BOP-Cl (37.2 mg, 0.15 mmol) was added to a stirring solution of **IV-8** (39.8 mg, 0.10 mmol), N-Boc-L-threonine (25.3 mg, 0.12 mmol), and TEA (0.050 mL, 0.36 mmol) in $CHCl_3$ (3.9 mL), and the reaction stirred at room temperature for 9 hours. Purification *via* silica gel chromatography (10% EtOAc / 86% hexanes / 4% TEA) was performed to afford 12.5 mg of the title compound (21% yield) as a thin film, which appeared as a 3:1 ratio of rotamers by ^{1}H-NMR, $[\alpha]^{20}_D$ = -0.5 (*c* 0.6, CH_2Cl_2). ^{1}H NMR (600 MHz) ($CDCl_3$): δ 1.18 (d, J = 6.2 Hz, 3 H), 1.26 and 1.26 (2s, 9H), 3.66 (d, J = 15.8 Hz, 1 H), 4.26 (d, J = 15.9 Hz, 1 H), 4.28 – 4.37 (m, 1 H), 4.67 – 4.73 and 4.81 – 4.87 (2m, 1 H), 4.91 (d, J = 7.9 Hz, 1 H), 5.31 and 5.58 (s and d, $J_{(5.58)}$ = 5.6 Hz, 1 H), 5.50 (d, J = 7.9 Hz, 1 H), 6.65 (d, J = 7.3 Hz, 2 H), 6.86 – 7.00 (m, 8 H), 7.06 – 7.15 (m, 3 H), 7.20 – 7.23 (m, 2 H), 7.31 – 7.37 (3 H), 7.77 (d, J = 7.5 Hz, 2 H).

^{13}C NMR (150 MHz) ($CDCl_3$): δ 17.1, 28.3, 48.5, 52.1, 65.6, 69.6, 70.0, 79.7, 103.9, 125.9, 127.1, 127.3, 127.39, 127.41, 127.45, 127.6, 128.2, 128.65, 128.72, 129.2, 129.5, 135.0, 137.4, 138.6, 139.7, 154.9, 169.2. IR (neat): 3415 cm^{-1}, 3320 cm^{-1}, 1718 cm^{-1}, 1690 cm^{-1}, 1404 cm^{-1}, 1171 cm^{-1}. HRMS (FAB): *m/z* calcd for $C_{37}H_{40}N_3O_4$ [M+H], 590.3019; found, 590.3029.

5. Decomposition of oxazinone/imidazolidine adducts.

To a stirring solution of **IV-7** (41.1 mg, 0.0970 mmol) in CH_2Cl_2 (3.0 mL), cooled to -41 °C in a CH_3CN / CO_2 (s) ice bath, was added dropwise a 1 M solution of $TiCl_4$ in CH_2Cl_2 (0.15 mL, 0.15 mmol). The reaction stirred at -41 °C for 30 minutes and was then allowed to warm to room temperature while stirring overnight. The reaction was then quenched through the successive addition of TEA (0.5 mL) and aqueous $NaHCO_3$ solution (5 mL). The reaction was extracted with EtOAc (3 x 15 mL) and the pooled organic extracts were dried (Na_2SO_4) and concentrated to yield a crude product that contained only **IV-3**. Purification *via* silica gel chromatography (8% TEA / 92% EtOAc) was performed to give 20.5 mg of **IV-3** (90% yield).

6. Procedure for proteasome inhibition assay. The 2-imidazolines and oxazinone/imidazolidine adducts prepared in this chapter were tested for their ability to inhibit the chymotryptic-like activity of the 20S proteasome in an *in vitro* assay using purified human recombinant 20S proteasome (Boston Biochem, Cambridge, Ma.) and the fluorogenic peptide substrate Suc-LLVY-AMC (Boston

Biochem, Cambridge, Ma.). All proteasome inhibition assays were performed by Teri L. Lansdell according to a modification of known procedures.[47,48]

The modified procedure is as follows: An activated enzyme solution was first prepared through addition of 20S proteasome to a reaction buffer (50 mM Tris-HCl, pH 7.5, 0.03% SDS) to give a final concentration of 1 nM. To each well of a black, clear bottom 96-well plate (Corning, Inc.) was added 100 µL of the activated enzyme solution. Compounds being evaluated were diluted in neat DMSO and 1 µL of test agent or vehicle (DMSO) was added to each well of the 96-well plate; compounds were evaluated at 20, 10, 5, 2.5, 1.3, and 0.6 µM final concentrations. Enough chymotryptic substrate Suc-LLVY-AMC was then added to each well to give a final concentration of 100 µM. The rate of peptide hydrolysis (V_{max}) was determined using a Spectramax M5e (Molecular Devices, Sunnyvale, Ca.) at 37° C over 30 minutes (λ_{ex} 380, λ_{em} 440). The initial linear portions of the curves were used to calculate the IC_{50} values.

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CHAPTER V
CONCLUSION

The studies described in Chapters II – IV of this dissertation follow a linear path that spans from oxazol-5(4H)-one alkylation to 2-imidazoline synthesis to 2-imidazoline annulation, with each chapter building upon the results obtained from the prior one. Such a progression in these studies may be viewed as a utilization of DOS. As detailed in Chapter II, initial studies involved the development of a protocol by which quaternary α-amino acid derivatives are diastereoselectively synthesized from oxazol-5-(4H)-ones. This protocol allowed for the formation of densely functionalized compounds containing reactive handles which were found to be useful towards the synthesis of proteasome inhibitors, as was described in Chapter III. In addition, the quaternary α-amino acid derivatives described in Chapter II allowed access to 2-oxazoline and 2-imidazoline heterocyclic scaffolds, the syntheses of which were described in Chapter III. Finally, the surprising results observed during the synthesis of 2-imidazoline proteasome inhibitors prompted our investigation into the development of 2-imidazoline annulation chemistry, as was described in Chapter IV.

Future studies in the Tepe lab will undoubtedly utilize the oxazol-5(4H)-one scaffold for the preparation of biologically active heterocyclic products. Studies described in this dissertation in which oxazol-5(4H)-ones were used primarily involved alkylation and ring-opening *via* hydride reduction. The products derived from our alkylation/reduction protocol were of use towards the synthesis of the marine metabolite salinosporamide A, and it is anticipated that

this chemistry may also be useful for the construction of other densely functionalized natural products. Future SAR studies involving 2-imidazolines in the Tepe lab are also anticipated and are expected to play a role in better understanding proteasome inhibition as a means of anti-cancer treatment. The chemistry developed for the construction of 2-imidazoline proteasome inhibitors, as detailed in Chapter III, is expected to play a role in our future SAR studies by granting access to 2-imidazolines not available by our previous chemical methods. Moreover, it anticipated that the annulation reaction described in Chapter IV may be utilized for the chiral resolution of 2-imidazolines. In conclusion, it is envisioned that upcoming studies will prompt the development of new chemistry in the Tepe lab, such that scores of compounds with desired biological profiles may ultimately be prepared.

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