
The Synthesis of Mycobactin Analogs and Heterocyclic Scaffolds From Acylnitroso Hetero-Diels-Alder Cycloadducts

Brian Scott Bodnar

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THE SYNTHESIS OF MYCOBACTIN ANALOGS AND HETEROCYCLIC
SCAFFOLDS FROM ACYLNITROSO HETERO-DIELS-ALDER CYCLOADDUCTS

VOLUME I

A Dissertation

Submitted to the Graduate School
of the University of Notre Dame
in Partial Fulfillment of the Requirements
for the Degree of

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by

Brian Scott Bodnar

Marvin J. Miller, Director

Graduate Program in Chemistry and Biochemistry

Notre Dame, Indiana

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Abstract

By

Brian Scott Bodnar

The focus for this dissertation involved two main projects that complement the known chemistry of acylnitroso hetero-Diels-Alder (HDA) reactions. The first project involved the synthesis of novel mycobactin analogs from nitroso HDA cycloadducts, and also included the discovery and study of a new synthesis of imidazoles. The second main project involved developing new ways to use acylnitroso HDA cycloadducts to synthesize biologically useful heterocyclic molecules.

In chapter 2, the rationale behind the synthesis of mycobactin analogs was described. In chapter 3, the synthesis of mycobactin analog fragments is described from amino acids and acylnitroso HDA cycloadducts. Chapter 4 described the assembly of mycobactin analog fragments into fully elaborated and deprotected 1,2-diol-containing mycobactin analogs. General strategies toward the synthesis of α -hydroxy carboxylate-

containing mycobactin analogs were also presented as well as interesting results from biological assays on final molecules and intermediates.

In chapter 5, the discovery of a new method for synthesizing imidazole analogs of mycobactin fragments from azides and 2-amidoacrylates was described. The initial reaction, optimization of reaction conditions, and a limited study of reaction scope was presented as well as the results of biological assays performed on selected intermediates. This discovery provided a new method for synthesizing imidazole-containing mycobactin analogs as well as a general method for preparing 1-substituted- and 1,2-disubstituted-imidazole-4-carboxylates in three steps from serine, carboxylic acids, and azides.

Chapter 6 described the development of the addition of azides to acylnitroso HDA cycloadducts and the effect of alkene strain on reactivity. The scope of the reaction was investigated as well as conversion of the triazoline products to aziridines. The significance of this research as it related to the synthesis of aziridine- and triazoline-containing 5'-norcarbocyclic nucleosides and bioconjugation chemistry was also described.

In chapter 7, other ways of utilizing the acylnitroso HDA reaction were investigated. Preliminary results on addition of diazoalkanes to cycloadducts, Brønsted acid-catalyzed opening of cycloadducts, acylnitroso [2+2+2] homo-Diels-Alder reactions, and acylnitroso cycloadditions to indole *ortho*-quinodimethanes were presented. A discussion of their significance toward the synthesis of biologically useful molecules was also included.

For my wife, Katie

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ABBREVIATIONS

| | |
|--------------------|-------------------------------------|
| [α] | specific rotation (deg•mL)/(g•dm) |
| ABH | 2-azabicyclo[2.2.]hept-5-en-3-one |
| Ac | Acetyl |
| Ad | 1-adamantyl |
| AMP | adenosine 5'-monophosphate |
| ATP | adenosine 5'-triphosphate |
| BCG | bacilli Calmette-Guérin |
| BMIM ⁺ | 1-butyl-3-methylimidazolium |
| Bn | benzyl |
| Boc | <i>tert</i> -butoxycarbonyl |
| Boc ₂ O | di- <i>tert</i> -butoxy dicarbonate |
| Br | broad (spectra) |
| Bu | butyl |
| °C | degrees Celsius |
| cat | catalytic |
| Cbz | benzyloxycarbonyl |
| COSY | correlation spectroscopy |
| mCPBA | <i>meta</i> -chloroperbenzoic acid |

| | |
|-------------------|---|
| δ | chemical shift in parts per million |
| Δ | heat/thermal or change |
| d..... | doublet |
| DAST | diethylaminosulfur trifluoride |
| DBAD | di- <i>tert</i> -butylazo dicarboxylate |
| DBU | 1,8-diazabicyclo[5.4.0]undec-7-ene |
| DCC | <i>N,N'</i> -dicyclohexylcarbodiimide |
| DCU | <i>N,N'</i> -dicyclohexylurea |
| decomp..... | decomposition |
| DIPEA..... | diisopropylethylamine |
| DMAP | 4-dimethylaminopyridine |
| DME..... | 1,2-dimethoxyethane |
| DMF..... | <i>N,N</i> -dimethylformamide |
| DMSO | dimethyl sulfoxide |
| DNA..... | deoxyribonucleic acid |
| DOTS | directly observed therapy short-course |
| E^+ | electrophile |
| EDC..... | 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide |
| Et..... | ethyl |
| EtOH | ethanol |
| eq or equiv | equivalents |
| FT..... | fourier transform |
| FVP | flash vacuum pyrolysis |

| | |
|------------------------|---|
| GAS..... | glycerol-alanine-salts medium |
| GAST | low-iron GAS medium with added Tween 80 |
| h..... | hours |
| HDA..... | hetero-Diels-Alder |
| HETCOR..... | heteronuclear correlation experiment |
| HMBC..... | heteronuclear multiple bond coherence |
| HOAc | acetic acid |
| HOBt..... | 1-hydroxybenzotriazole |
| HPLC | high performance liquid chromatography |
| HRMS | high resolution mass spectroscopy |
| Hz..... | hertz |
| IC ₅₀ | concentration required for 50% inhibition |
| IR..... | infrared spectroscopy |
| IREP | iron-regulated envelope proteins |
| kcal..... | kilocalorie |
| LA | Lewis acid |
| LC/MS..... | liquid chromatography/mass spectroscopy |
| 5-LO..... | 5-lipoxygenase |
| LORA..... | low-oxygen recovery assay |
| m | multiplet (spectra) |
| μ | micro |
| M..... | molar |
| MABA..... | microplate alamar blue assay |

| | |
|---------------|--|
| MCA | mycothio-S-conjugate amidase |
| MDR-TB | multi-drug resistant tuberculosis |
| Me | methyl |
| MeOH | methanol |
| MHz | megahertz |
| MIC | minimum inhibitory concentration |
| min | minute |
| mL | milliliter |
| μ M | micromolar |
| mM | millimolar |
| MO | molecular orbital |
| mol | mole |
| mmol | millimole |
| mp | melting point |
| MS | mass spectroscopy <i>or</i> molecular sieves |
| m/z | mass-to-charge ratio (mass spectroscopy) |
| NADPH | reduced form of nicotinamide adenine dinucleotide phostphate |
| NHS | <i>N</i> -hydroxysuccinimide |
| NIH | National Institute of Health |
| NMO | <i>N</i> -methyldmorpholine- <i>N</i> -oxide |
| NMR | nuclear magnetic resonance (spectroscopy) |
| 2-NPP | (2-nitrophenyl)prolyl |
| Nuc | nucleophile |

| | |
|---------------------|--|
| [O] | oxidation |
| OBHA | <i>O</i> -benzylhydroxylamine |
| PEG | polyethylene glycol |
| Ph | phenyl |
| PPA | polyphosphoric acid |
| Pr | propyl |
| PS-PPh ₃ | polymer-supported triphenylphosphine |
| R _f | retention factor (for thin layer chromatography) |
| ROESY | rotating-frame Overhauser effect spectroscopy |
| rt or RT | room temperature |
| R _t | retention time (liquid chromatography) |
| SAR | structure-activity relationship |
| TB | tuberculosis |
| TBAF | tetrabutylammonium fluoride |
| TBS or TBDMS | <i>tert</i> -butyldimethylsilyl |
| TEMPO | 2,2,6,6-tetramethylpiperidin- <i>N</i> -oxide |
| THF | tetrahydrofuran |
| Tf | trifluoromethanesulfonyl |
| TFA | trifluoroacetic acid |
| Tfeoc | 2,2,2-trifluoroethoxycarbonyl |
| TfOH | trifluoromethanesulfonic acid |
| TLC | thin layer chromatography |
| TMS | trimethylsilyl |

| | |
|----------------------|--|
| TPP..... | tetraphenylporphyrin |
| Tr..... | trityl |
| ^s Tr..... | “super”-trityl |
| Ts..... | <i>p</i> -toluenesulfonyl (tosyl) |
| <i>p</i> TsOH..... | <i>p</i> -toluenesulfonic acid |
| VSMC | vascular smooth muscle cells |
| WHO | World Health Organization |
| XDR-TB..... | extremely (or extensively) drug-resistant tuberculosis |

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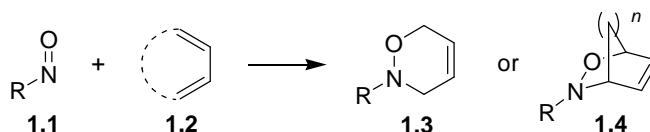
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CHAPTER 1:
APPLICATIONS OF THE ACYLNITROSO HETERO-DIELS-ALDER REACTION IN
ORGANIC SYNTHESIS

1.1 Introductory remarks

The nitroso hetero-Diels-Alder (HDA) reaction provides access to 3,6-dihydro-1,2-oxazines (**1.3** or **1.4**) from nitroso compounds **1.1** and dienes **1.2** (Scheme 1.1). The use of the nitroso HDA reaction in a number of synthetic efforts has been reviewed¹⁻⁷ and illustrates the utility of this powerful method.

This dissertation will detail the exploration of two main projects that complement the known chemistry of nitroso HDA reactions. The first project involved the synthesis of mycobactin analogs from bicyclic nitroso HDA cycloadducts **1.4** and will be described in detail in chapters 2-4. The second project involved the investigation of new reactivity of bicyclic cycloadducts **1.4** and will be described in detail in chapters 6-7. In order to place the work presented in this dissertation within the proper context, this chapter will detail the rich chemistry surrounding HDA reactions of acylnitroso compounds and nitrosoformate esters.



Scheme 1.1. The nitroso hetero-Diels-Alder reaction

1.2 Nitroso compounds

The nitroso functional group has been intensely studied since the first synthesis of nitrosobenzene by Baeyer more than one hundred years ago.⁸ An early report found that nitroso compounds could add to activated methylene groups to form azomethine compounds (the Ehrlich-Sachs reaction),⁹ and since this discovery, the nitroso group has been found to participate in nitroso aldol reactions,¹⁰⁻¹³ ene reactions,¹⁴ hetero-Diels-Alder reactions, and other fundamental organic processes.¹

1.2.1 C-Nitroso compounds and simple nitroso compounds

The simplest nitroso compound, nitroxyl or hyponitrous acid (HNO), has seen limited use in cycloaddition reactions due to its high propensity to dimerize with loss of H₂O to form nitrous oxide.^{15, 16} In contrast, C-nitroso compounds have been used extensively as dienophiles in cycloaddition reactions (Figure 1.1).^{3, 10, 17} Cyanonitroso **1.5**,¹⁷ arylnitroso **1.9**, pyridylnitroso **1.10**,¹⁸ α -halonitroso **1.6a**,¹⁹⁻²¹ α -acetoxynitroso **1.6b**,^{22, 23} vinylnitroso **1.7**, iminonitroso **1.8**,²⁴ acylnitroso **1.11**,¹⁷ and nitrosoformate ester **1.12**²⁵ compounds are all commonly used in HDA transformations.

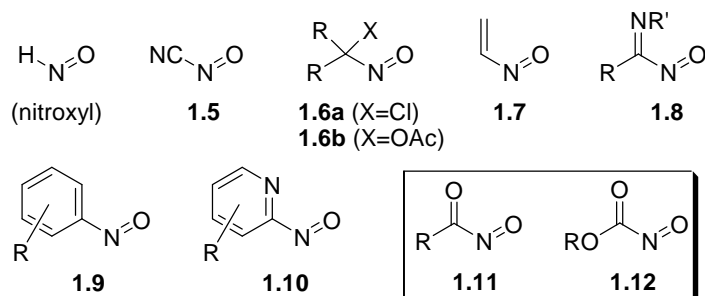


Figure 1.1. Examples of C-nitroso compounds

Arylnitroso compounds **1.9** were among the first discovered and are stable reagents that react slowly with dienes in [4+2] cycloaddition reactions.³ Electron-withdrawing groups on the aromatic ring greatly accelerated the reaction.¹⁷ Similar effects were observed for nitrosoalkane compounds that were substituted at the α -position such as chloronitroso species **1.6a**¹⁹⁻²¹ and acetoxynitroso species **1.6b**.^{22, 23} The most reactive nitroso compounds include those directly connected to an electron-withdrawing group, and nitroso compounds **1.5**, **1.8**, **1.11**, and **1.12** are among the most reactive nitroso dienophiles in HDA reactions.

1.2.2 Heteroatom-nitroso compounds

Compounds where the nitroso group is directly connected to a heteroatom possessing a free electron pair are much less reactive than C-nitroso compounds toward dienes because of resonance stabilization of the nitroso moiety (Figure 1.2). Consequently, HDA reactions of heteroatom-nitroso compounds are studied much less compared to their C-nitroso counterparts.

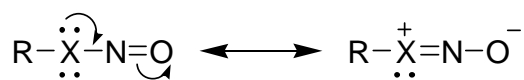


Figure 1.2. Resonance stabilization of X-N=O compounds

Deactivation of the dienophilic character of nitroso compounds through resonance stabilization (Figure 1.2) can be overcome if no lone pairs of electrons are available for π -donation. Some noteworthy examples that make use of this concept include *P*-nitroso phosphine oxides **1.13**²⁶⁻²⁸ and *S*-nitroso sulfonyl compounds **1.14** (Figure 1.3).²⁹ *N*-

nitroso compounds **1.15** were found to be unreactive toward dienes,³⁰ even though the presence of the sulfonyl group should diminish the effect of lone pair stabilization.

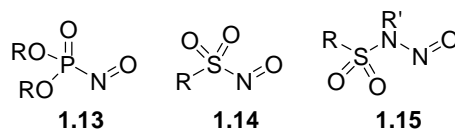


Figure 1.3. Examples of heteroatom-nitroso compounds

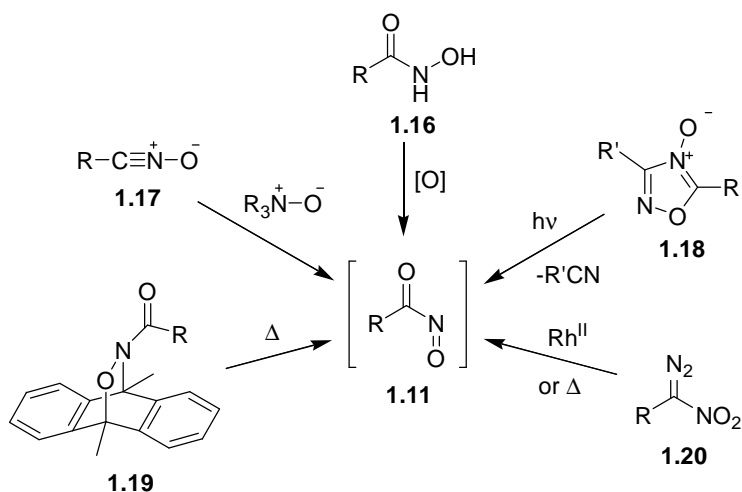
1.3 Acylnitroso compounds

Acylnitroso species **1.11** and nitrosoformate esters **1.12**, which will herein be referred to collectively as “acylnitroso species” or “acylnitroso compounds”, are among the most reactive nitroso dienophiles. First proposed as transient intermediates in the oxidation of hydroxamic acids,³¹ the only early evidence of the existence of acylnitroso species were products resulting from nucleophilic attack at the acylnitroso carbonyl¹⁷ and [4+2] cycloaddition reactions.³²

1.3.1 Preparation of acylnitroso compounds

Since acylnitroso compounds **1.11** are extremely reactive species, they are prepared and used *in situ* in chemical reactions (Scheme 1.2). By far the most common method for preparing acylnitroso compounds **1.11** is through oxidation of the corresponding hydroxamic acid **1.16**.³¹ Generation of acylnitroso compounds **1.11** in this manner has been realized using a multitude of conditions that include, but are not limited to, the use of periodate, Swern oxidation,³³ lead and silver oxide,³⁴ and Dess-Martin periodinane.³⁵ There are also a number of methods that reportedly generate acylnitroso

compounds **1.11** from hydroxamic acids **1.16** by transition metal-catalyzed oxidations with hydrogen peroxide as the stoichiometric oxidant.³⁶⁻⁴⁰ A thorough study of metal catalysts that perform this transformation has been reported.⁴¹



Scheme 1.2. Synthetic routes to acylnitroso species

Other methods commonly used to prepare acylnitroso species **1.11** include oxidation of nitrile oxides **1.17**,⁴² cycloreversion from 9,10-dimethylantracene adducts **1.19**,⁴³ photochemical generation from 1,2,4-oxadiazole-4-oxides **1.18**,⁴⁴ and rearrangement of nitrocarbenes generated from diazo compounds **1.20**.⁴⁵

1.3.2 Structure and reactivity of acylnitroso compounds

Although acylnitroso compounds have been studied for well over 50 years, relatively little is known about their structure. Acylnitroso species were first detected spectroscopically in the gas phase in 1991 by neutralization-reionization mass spectrometry⁴⁶ and then in solution in 2003 by time-resolved infrared spectroscopy.⁴⁷ It

is estimated that the lifetime of the acylnitroso species at infinite dilution in organic solution is on the order of 1 ms.⁴⁷

Acylnitroso compounds **1.11** can exist in either an *s-cis* (**1.11-cis**) or *s-trans* (**1.11-trans**) conformation along the carbonyl-nitrogen bond (Figure 1.4). Based on data reported in the literature, the preference for a given acylnitroso species **1.11** to exist as either conformer must be calculated on a case by case basis. Additionally, the preference for either conformer is not necessarily minor, and reported energy differences between the *s-cis* and *s-trans* conformers of various acylnitroso species have spanned from about 0-2 kcal mol⁻¹ to nearly 15 kcal mol⁻¹.⁴⁸⁻⁵³

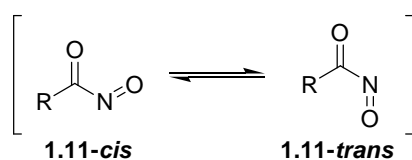
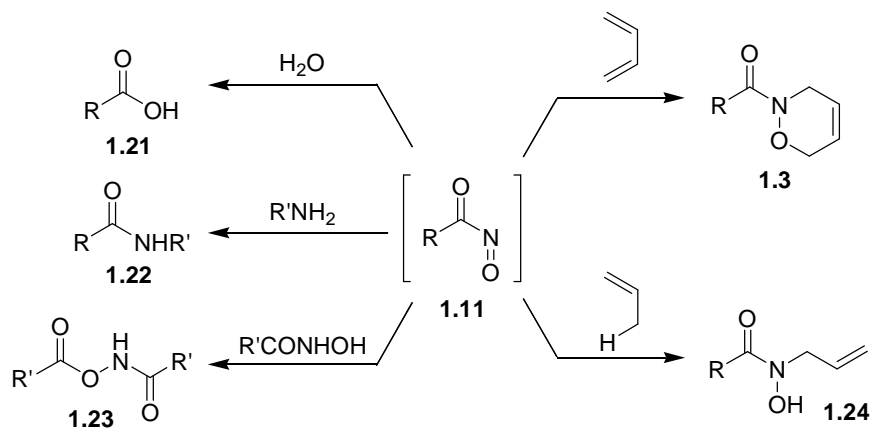


Figure 1.4. *s-cis* and *s-trans* isomers of acylnitroso compounds

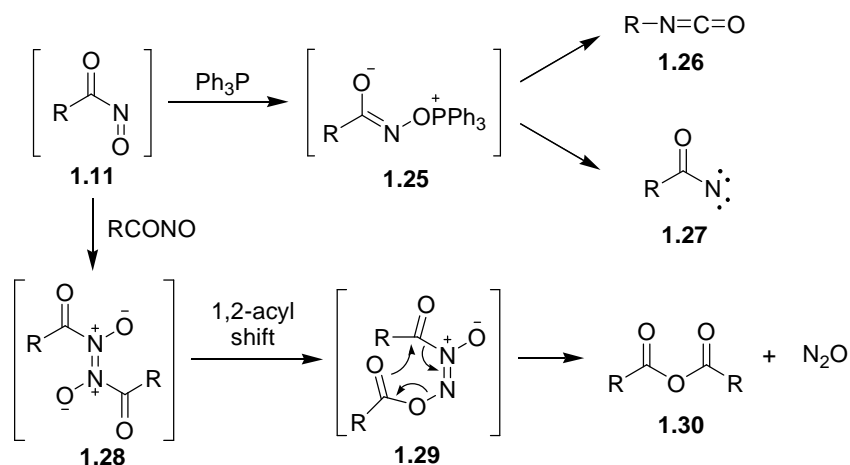
In addition to participating in HDA reactions with dienes to provide *N*-acyl-3,6-dihydro-1,2-oxazines **1.3** and in ene reactions to provide *N*-allyl hydroxamates **1.24**, acylnitroso compounds **1.11** also undergo a number of other transformations (Scheme 1.3). The high stretching frequency of the carbonyl of acylnitroso compounds **1.11** (1735 cm⁻¹)⁴⁷ reflects their susceptibility to nucleophilic attack at the acylnitroso carbonyl. In the presence of nucleophiles such as water, amines, and hydroxamic acids, the corresponding carboxylic acids **1.21**, amides **1.22**, and *O*-acylhydroxamates **1.23** are obtained, respectively.¹⁷



Scheme 1.3. Reactions of acylnitroso compounds

One of the earliest reactions observed with acylnitroso compounds **1.11** was their tendency to be deoxygenated by phosphines to yield isocyanates **1.26** through the phosphonium intermediate **1.25** (Scheme 1.4).⁵⁴ Interestingly, nitrosoformate esters **1.11** (R=alkoxy) yield products arising from the generation of the acylnitrene species **1.27**, due to the unfavorable migratory aptitude of the alkoxy substituent from phosphonium intermediate **1.25**.²⁵

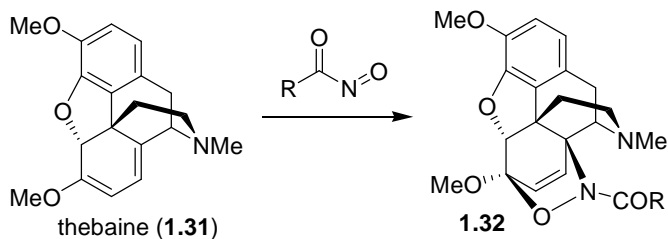
Acylnitroso compounds **1.11** have also been known to generate symmetrical anhydrides **1.30** and nitrous oxide in the absence of other reactants. Presumably, this process proceeds through nitroso dimer **1.28** which undergoes a 1,2-acyl shift to compound **1.29** followed by an intramolecular cyclization event.⁴³



Scheme 1.4. Other reactions of acylnitroso compounds

1.4 The acylnitroso hetero Diels-Alder reaction

The most common use of nitroso compounds has involved their ability to participate in [4+2] cycloaddition reactions. The first nitroso HDA reactions using aryl- and alkylnitroso compounds were reported by Wichterle⁵⁵ and Arbuzov⁵⁶ in 1947 and 1948, respectively. One of the earliest examples of a HDA reaction using an acylnitroso compound was reported by Kirby in 1973 where acylnitroso compounds were generated in the presence of thebaine (**1.31**) to afford cycloadducts **1.32** selectively in high yield (Scheme 1.5).³¹



Scheme 1.5. Cycloaddition reported by Kirby *et al.*

The remarkable selectivity observed in acylnitroso HDA reactions provides access to 3,6-dihydro-1,2-oxazines and ultimately 1,4-aminoalcohols. This section will document efforts toward the study of mechanism, selectivity, and asymmetric variants of the acylnitroso HDA reaction.

1.5 Mechanism

The mechanism of the acylnitroso HDA reaction has been studied computationally by Houk^{57, 58} and was found to proceed in a concerted fashion through a highly asynchronous transition state (Figure 1.5). In the calculated transition state, the C-N bond distance is shorter than the C-O bond, whereas in the product, the situation is reversed. Additionally, the authors found that the *endo* transition state, **1.33-endo**, was preferred over the *exo* transition state, **1.33-exo**, by 8.6 kcal mol⁻¹ based on RB3LYP/6-31G**//RB3LYP/6-31G* theory.⁵⁸ The n- π repulsion exhibited by the nitrogen lone pair, termed the “exo lone pair effect”,^{59, 60} is responsible for this strong preference for the placement of the substituent on the nitrogen in an *endo* position.

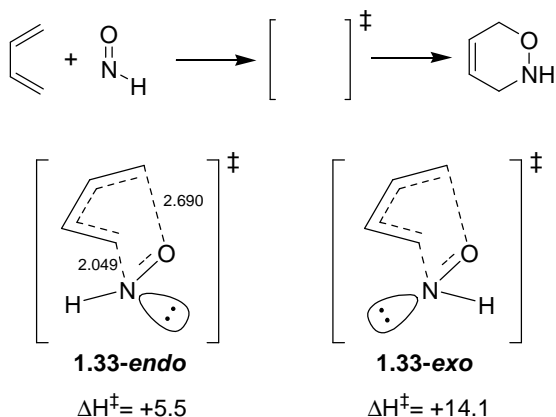
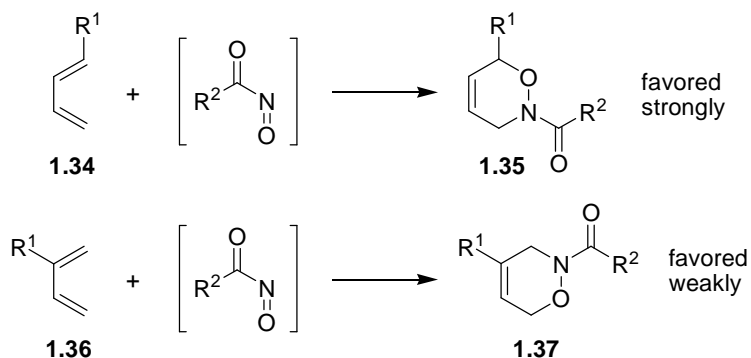


Figure 1.5. Computed energies for nitroso HDA transition states

The combined preference for placement of the substituent on the nitrogen in an *endo* position with the shorter C-N bond distance in the transition state can explain the high regio- and stereoselectivities observed for acylnitroso HDA reactions.

1.6 Regioselectivity

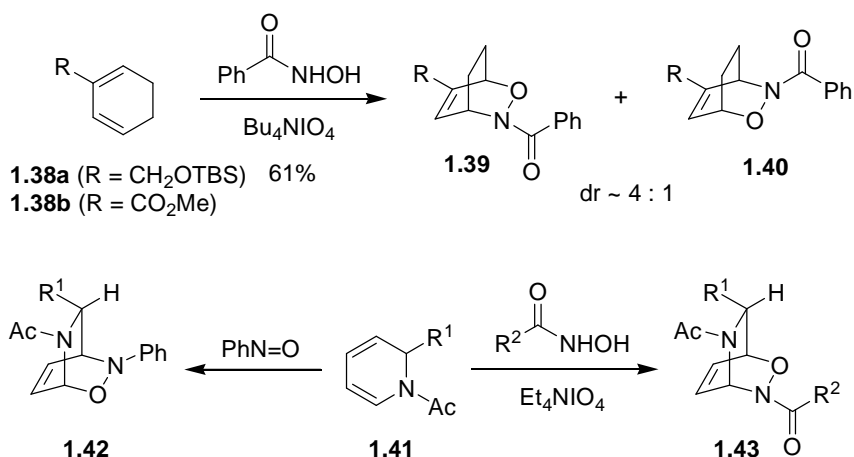
The regioselectivity of intermolecular acylnitroso HDA reactions has been studied experimentally^{7, 61} as well as through the use of computational methods.⁵⁸ Most unsymmetrical dienes add to nitroso compounds regioselectively according to the general scheme below (Scheme 1.6). 1-Substituted 1,3-dienes **1.34** provide oxazines **1.35** (termed the “proximal” product in the literature) with high selectivity, whereas 2-substituted 1,3-dienes **1.36** provide the oxazines **1.37** (termed the “distal” product in the literature) with moderate selectivity. Regioselectivity in nitroso HDA reactions is rationalized based on frontier MO theory, and dienes with substituents that are strongly electron-donating or electron-withdrawing provide cycloadducts with higher regioselectivity than dienes with substituents that are only weakly electron-donating or electron-withdrawing.⁵⁸ It should also be noted that in most cases solvent polarity has been shown to have little effect on regioselectivity in intermolecular nitroso HDA reactions.⁶¹



Scheme 1.6. General regioselectivity observed for unsymmetrical dienes

Similar trends for regioselectivity are observed for substituted cyclic dienes in acylnitroso HDA reactions (Scheme 1.7). When benzohydroxamic acid was oxidized in the presence of substituted cyclohexadienes **1.38a** and **1.38b**, cycloadducts **1.39** and **1.40** were obtained with moderate regioselectivity.⁶¹

In most cases, arylnitroso and acylnitroso species yield products with the same regioselectivity, however in a few cases the selectivities are reversed. For example, opposite regioselectivities were observed when *N*-acyl-1,2-dihydropyridines **1.41** were reacted with arylnitroso and acylnitroso compounds. Arylnitroso compounds afforded adducts **1.42**,^{62, 63} while acylnitroso compounds resulted in cycloadducts **1.43**.⁶³ Reasoning for this observed difference in regioselectivity has not been proposed.



Scheme 1.7. Examples of regioselectivity in nitroso Diels-Alder reactions

1.7 Stereoselectivity

There are a number of reviews detailing the use of asymmetric nitroso HDA reactions in organic synthesis.^{2, 3, 5, 6, 49} Methods for performing asymmetric nitroso HDA reactions include the use of chiral nitroso dienophiles, chiral dienes, and with mixed success, the use of chiral catalysis. All three of these general approaches toward asymmetric nitroso HDA reactions will be described briefly in the following sections.

1.7.1 Asymmetric induction using chiral acylnitroso dienophiles

The use of chiral acylnitroso dienophiles, specifically as chiral auxiliaries, is the most common method for inducing chirality in acylnitroso HDA reactions. A variety of chiral acylnitroso species **1.44** have appeared in the literature that have been found to offer the 1,3-cyclohexadiene adduct **1.45** with excellent diastereoselectivity (Figure 1.6).

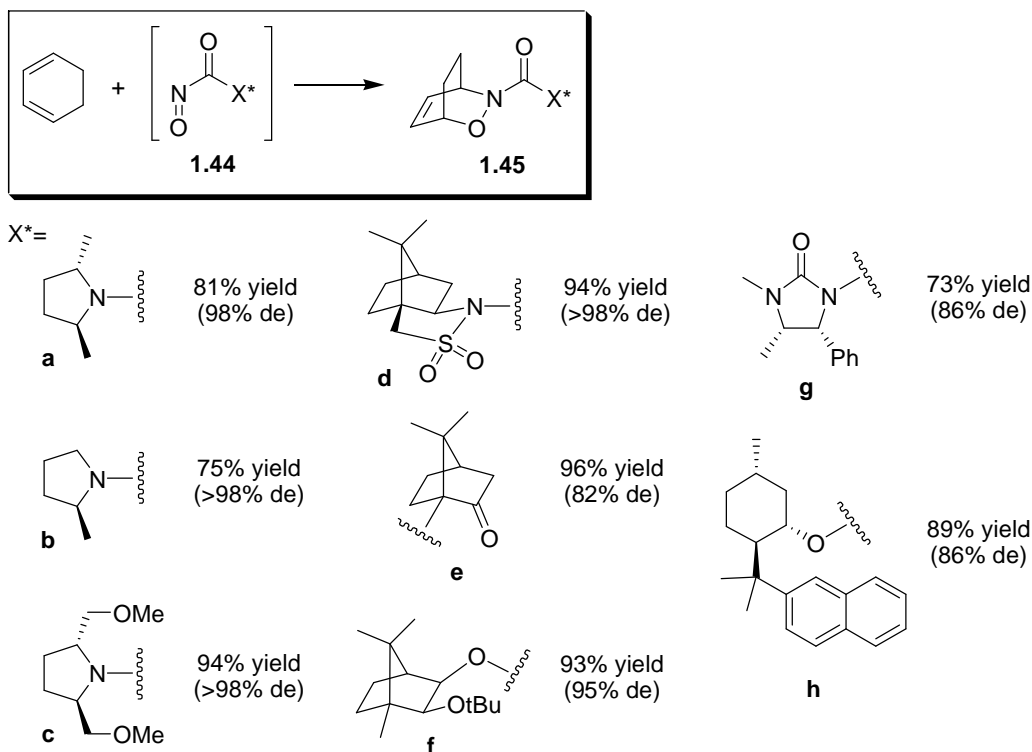


Figure 1.6. Examples of chiral acylnitroso species

All acylnitroso species **1.44** were prepared in situ by oxidation from the corresponding hydroxamic acid. Substituted pyrrolidines **1.44a-c** offered cycloadducts **1.45** in high diastereomeric excesses.⁴⁸ Additionally, a variety of camphor derivatives **1.44d-f** have also been reported.^{30, 33, 50} Other auxiliaries include imidazolidin-2-one **1.44g**⁵¹ and compound **1.44h**,⁶⁴ derived from menthol.

Chiral α -substituted acylnitroso compounds that undergo asymmetric HDA reactions have included nitroso species **1.46**, derived from α -amino acids,^{3, 65, 66} and nitroso species **1.47**, derived from mandelic acid (Figure 1.7).^{52, 53, 67-70} These auxiliaries benefit from relatively simple preparation from readily available sources of chirality.

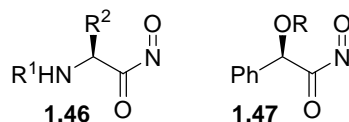


Figure 1.7. Other chiral acylnitroso species

1.7.2 Asymmetric induction using chiral dienes

The use of both chiral cyclic and acyclic dienes in diastereoselective acylnitroso HDA reactions has been reported in the literature. Generally, using chiral acyclic dienes yields cycloadducts in lower diastereomeric excess than does the use of chiral acylnitroso dienophiles. This is probably a result of the asynchronous transition state of the nitroso HDA reaction, where the nitrogen substituent is hypothesized to be closer in proximity to the diene than the oxygen lone pairs. This, in turn, places the chiral moiety of 1-substituted acyclic dienes spatially distant from the bulk of the incoming nitroso dienophile. Nevertheless, chiral acyclic dienes have been successfully used in asymmetric nitroso HDA reactions and include chiral *N*-dienyl lactams **1.48**,^{71, 72} pseudoephedrine-derived oxazolidines **1.49**,⁷³ and chiral 1-sulfinyl dienes **1.50** (Figure 1.8).^{74, 75}

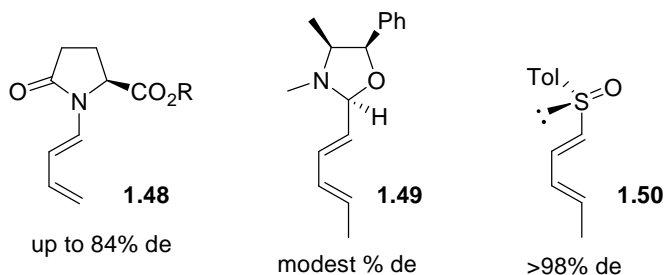
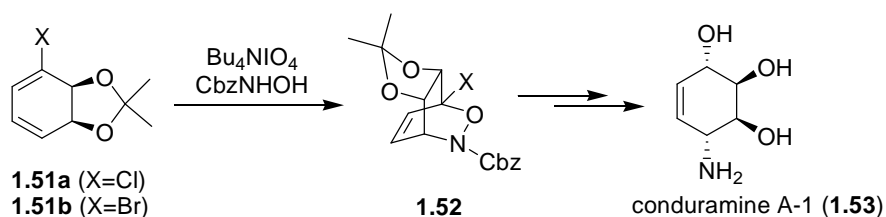


Figure 1.8. Examples of chiral acyclic dienes

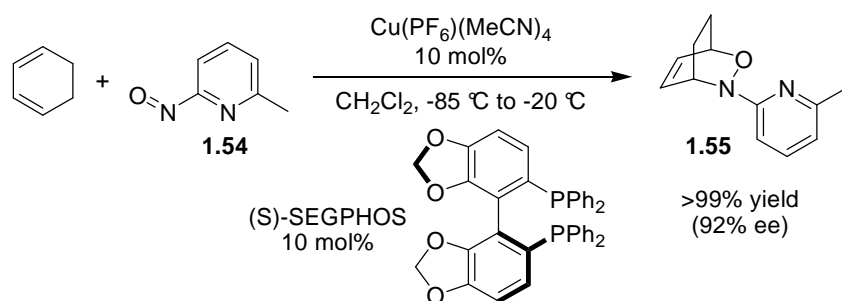
Compared to chiral acyclic dienes, chiral cyclic dienes often yield cycloadducts with excellent diastereoselectivity. Hudlicky has reported the use of chiral dienes **1.51a** and **1.51b**, obtained by microbial oxidation of halobenzenes, in asymmetric nitroso HDA reactions (Scheme 1.8).⁷⁶ Cycloadducts **1.52** were obtained in high yields with complete diastereo- and regioselectivity. Conversion of cycloadducts **1.52** to conduramine A-1 (**1.53**) was also described.



Scheme 1.8. Use of chiral diene for acylnitroso cycloaddition

1.7.3 Catalytic asymmetric nitroso HDA reactions

For many years, attempts at developing a catalytic asymmetric nitroso HDA reaction resulted in only extremely low ee values (ca. 15%).⁷⁷ It was not until 2004, when Yamamoto published an asymmetric nitroso HDA reaction with pyridylnitroso species **1.54**, that an effective catalytic asymmetric nitroso HDA reaction was realized (Scheme 1.9).⁷⁸ This ground-breaking discovery should prove very useful for the synthesis of enantiomerically pure oxazines **1.55**, however a similar method for the acylnitroso HDA reaction is still lacking.

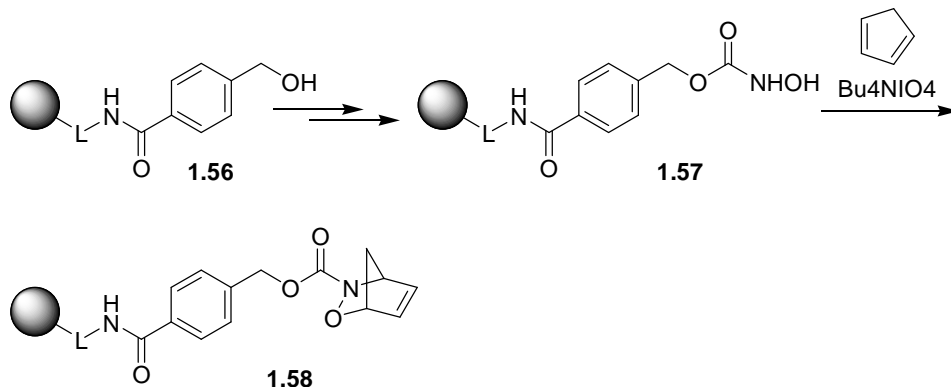


Scheme 1.9. Catalytic asymmetric pyridylnitroso cycloaddition

The difficulties faced in the development of a catalytic asymmetric method for the acylnitroso HDA reaction have included an extremely facile background reaction and the susceptibility of acylnitroso species to dimerize. These problems plagued the study of aryl- and heteroaryl nitroso species for some time before Yamamoto's discovery;⁷⁷ however, acylnitroso compounds are more reactive than aryl- or heteroarylnitroso species and react as rapid or more rapidly without catalysts than as a Lewis acid-bound acylnitroso species.^{36, 38} A better understanding of the metal coordination chemistry of acylnitroso species will be essential for the development of a catalytic asymmetric acylnitroso HDA reaction.

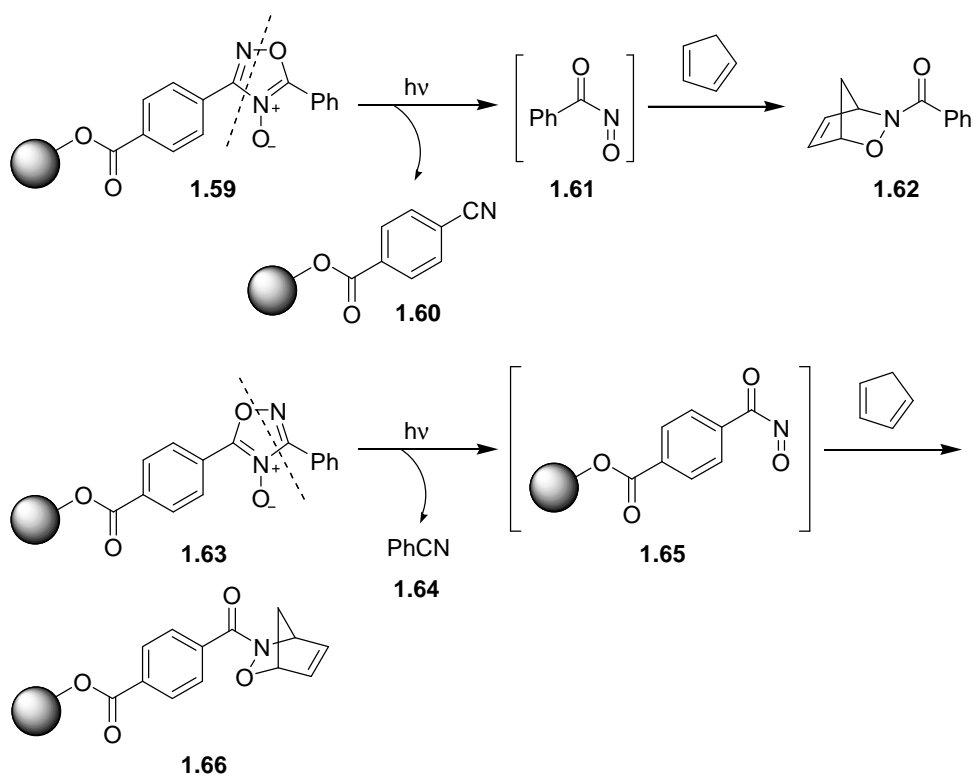
1.8 The acylnitroso hetero Diels-Alder reaction on solid-phase

Although acylnitroso HDA reactions have been widely used in organic synthesis in solution, there have only been a few accounts of performing acylnitroso HDA reactions on a solid-support. One example reported by Krchnak⁷⁹⁻⁸¹ utilized solid-supported hydroxamic acids **1.57** derived from alcohols **1.56** on Wang resin (Scheme 1.10). Hydroxamic acids **1.57** were oxidized using tetrabutylammonium periodate in the presence of dienes to yield cycloadducts **1.58**.



Scheme 1.10. Example of acylnitroso Diels-Alder on solid phase

Other solid-supported nitroso HDA reactions reported by Quadrelli include the generation of acylnitroso compounds from solid-supported nitrile oxides⁸² and the photochemical generation of acylnitroso compounds from solid-supported 1,2,4-oxadiazole-4-oxides **1.59** and **1.63** (Scheme 1.11).⁸³ Upon irradiation, compounds **1.59** generated the solid-supported nitrile **1.60** and acylnitroso compound **1.61** that was trapped *in situ* with cyclopentadiene to afford cycloadduct **1.62**. Alternatively, 1,2,4-oxadiazole-4-oxide **1.63** was irradiated and provided benzonitrile (**1.64**) and solid-supported acylnitroso compound **1.65** that was subsequently trapped by dienes to yield cycloadducts such as compound **1.66**.

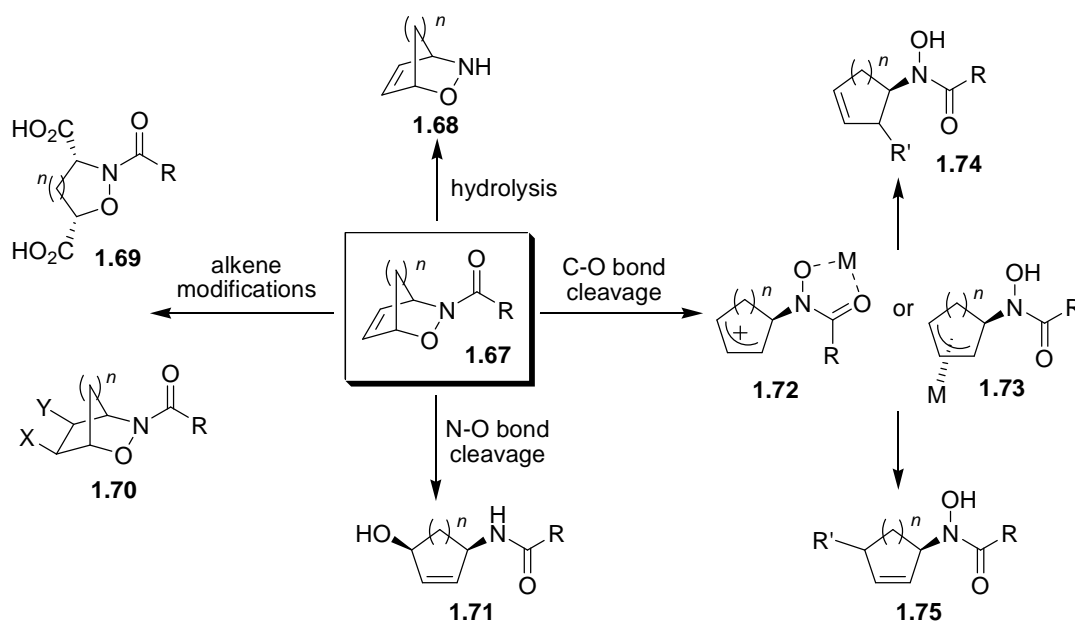


Scheme 1.11. Another example of acylnitroso Diels-Alder on solid phase

1.9 Chemistry of 3,6-dihydro-1,2-oxazines

Most of the utility of the acylnitroso HDA reaction in organic syntheses stems from the rich chemistry of their cycloaddition products, 3,6-dihydro-1,2-oxazines **1.3** and **1.4**. Oxazines **1.3** and **1.4** contain allylic oxygen and nitrogen substituents, an N-O bond, a Weinreb amide,⁸⁴ and an alkene. The rapid construction of such a wide variety of functional groups in one molecule allows access to a number of molecular scaffolds from simple bicyclic cycloadducts **1.67** (Scheme 1.12). Consequently, structural modification of cycloadducts **1.67** fall under one of four main areas: N-Acyl bond cleavage to yield oxazines **1.68**, N-O bond cleavage to yield amino alcohols **1.71**, C-O bond cleavage to yield compounds **1.72-1.75**, and alkene modification to compounds **1.69** and/or **1.70**.

Additionally, both monocyclic oxazines **1.3** and bicyclic oxazines **1.67** have demonstrated the ability to undergo a number of rearrangements and other chemical reactions.



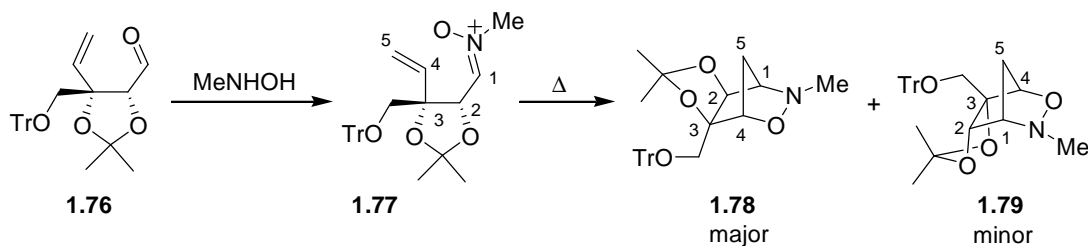
Scheme 1.12. General chemical modifications of bicyclic 3,6-dihydro-1,2-oxazines

The carbonyl of cycloadducts **1.67** is susceptible to hydrolysis under relatively mild conditions (when R = alkyl or aryl).⁸⁵ This provides the basis for removing many of the chiral auxiliaries previously described in section 1.4.3.1. The following section details various transformations of cycloadducts **1.67** commonly utilized in synthetic organic applications. Although most of the methodology has been developed using bicyclic oxazines **1.67**, much of the chemistry presented here is also applicable to monocyclic oxazines **1.4**.

1.9.1 Alternative routes to 3,6-dihydro-1,2-oxazines

While the nitroso HDA reaction is an excellent method for preparing 3,6-dihydro-1,2-oxazines, alternative methods for preparing these heterocyclic systems exist and have been reviewed.^{86, 87} Methods of preparing monocyclic 1,2-oxazines have included using alkene⁸⁸ and enyne^{89, 90} ring-closing metathesis reactions, the addition of nitrones to methoxy allenes⁹¹ and activated cyclopropanes,^{92, 93} and the use of nitroso aldol reactions.⁹⁴

An example of an alternative synthesis of bicyclic 1,2-oxazine systems **1.4** involved an intramolecular nitron [3+2] cycloaddition (Scheme 1.13).⁹⁵ The aldehyde **1.76**, derived from *L*-arabinose, was converted to nitron **1.77** which cyclized selectively to yield oxazines **1.78** and **1.79**.

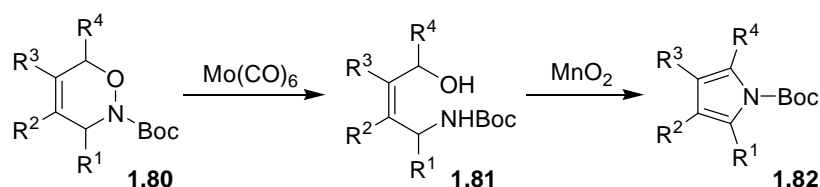


Scheme 1.13. [3+2] cycloaddition route to 3,6-dihydro-1,2-oxazines

1.9.2 N-O bond cleavage

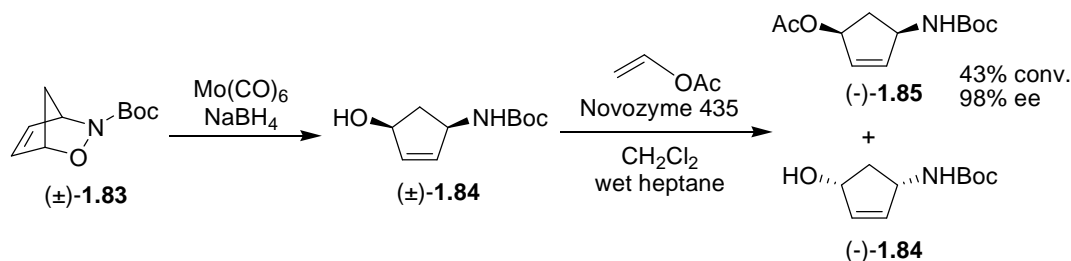
Reductive N-O bond cleavage is one of the most widely utilized methods for derivatizing 1,2-oxazines. Common reagents that facilitate N-O bond cleavage include molybdenum hexacarbonyl ($\text{Mo}(\text{CO})_6$),^{96, 97} zinc in acetic acid, catalytic hydrogenation, and samarium diiodide.^{98, 99} Other methods of N-O bond reduction include the use of photochemical¹⁰⁰ and enzymatic¹⁰¹ processes.

N-O bond reduction of monocyclic 1,2-oxazines **1.80** yielded 1,4-aminoalcohols **1.81**, which have been cyclized using manganese dioxide and provided access to pyrroles **1.82** (Scheme 1.14).¹⁰²



Scheme 1.14. Pyrrole synthesis using N-O bond reduction

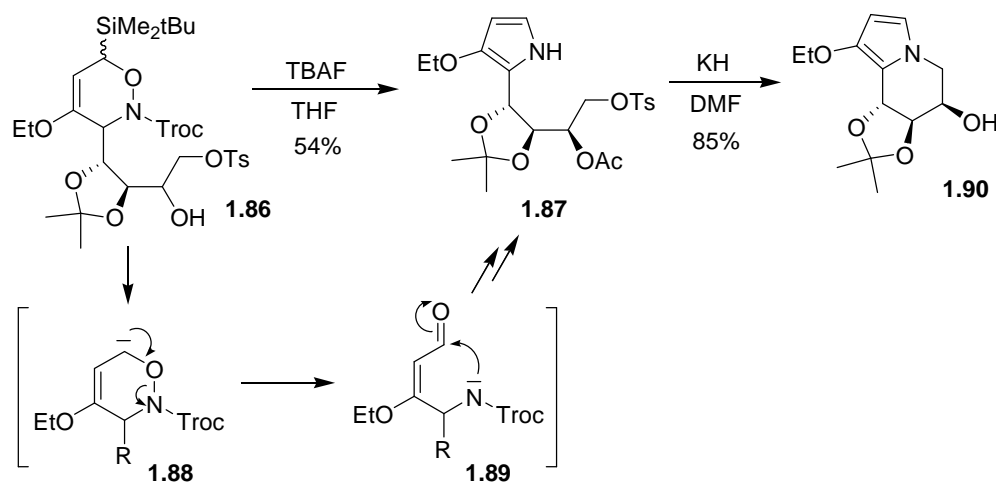
Treatment of racemic cycloadduct (\pm)-**1.83** with molybdenum hexacarbonyl yielded the aminocyclopentenol (\pm)-**1.84** (Scheme 1.15). The Miller group has developed a kinetic enzymatic resolution method that yielded enantiomerically pure acetate (-)-**1.85** and aminocyclopentenol (-)-**1.84** using an immobilized lipase from *Candida antarctica*.¹⁰³ Acetate (-)-**1.85** has been an important intermediate in the synthesis of 5'-norcarbocyclic nucleosides, which will be covered in section 1.6.1.



Scheme 1.15. Enzymatic resolution of a racemic alcohol

Other methods of N-O bond reduction have included eliminative ring-opening reactions similar to that reported by Grierson (Scheme 1.16).¹⁰⁴ 1,2-Oxazine **1.86** was

treated with TBAF to provide pyrrole **1.87**. The anion intermediate **1.88** generated by treatment with fluoride yielded the aldehyde intermediate **1.89** that subsequently underwent dehydrative cyclization to afford the pyrrole **1.87**. Intramolecular cyclization to the pyrrolo-castanospermine product **1.90** was effected using KH in DMF. This reaction sequence was similar to that reported for the base-catalyzed decomposition of dialkyl peroxides (the Kornblum DeLaMare rearrangement),¹⁰⁵ and has also been reported for other monocyclic oxazine systems **1.3**.¹⁰⁶

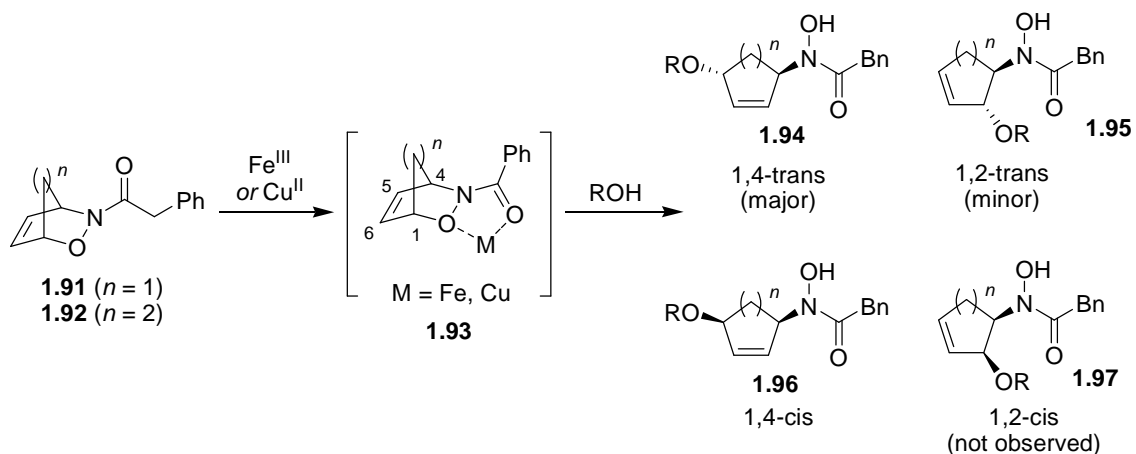


Scheme 1.16. Alternative method of N-O bond cleavage

1.9.3 C-O bond cleavage

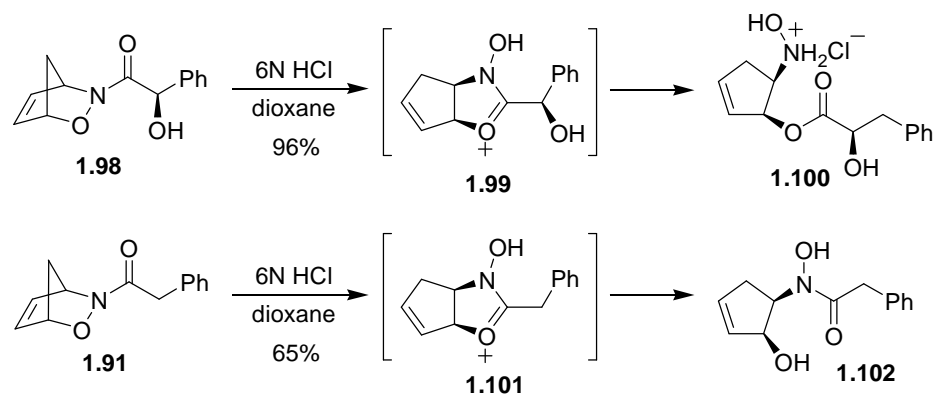
The Miller group discovered that Lewis acids could mediate C-O bond breakage of cycloadducts **1.91** and **1.92** in the presence of alcoholic solvents to afford hydroxamates **1.94-1.96** (Scheme 1.17).^{107, 108} Presumably, this transformation proceeded by coordination of the Lewis acid to the hydroxamate portion of the oxazine system through a structure similar to complex **1.93**. The reaction was found to be

moderately selective for the 1,4-*trans* hydroxamate **1.94**; however, 1,2-*cis* hydroxamate **1.97** was not observed.



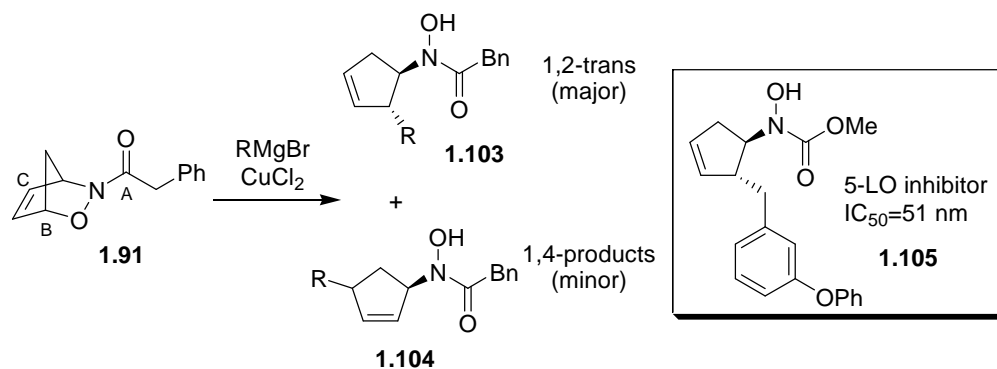
Scheme 1.17. Lewis acid-mediated C-O bond cleavage

The C-O bond has also been cleaved in the presence of Brønsted acids to yield products arising from intramolecular cyclizations (Scheme 1.18). For example, Procter reported that when cycloadduct **1.98**, derived from mandelic acid, was treated with aqueous HCl in dioxane, hydroxylamine **1.100** was obtained.^{109, 110} Interestingly, when cycloadduct **1.91** was treated under the same conditions, hydroxamate **1.102** was obtained.¹⁰⁷ It would appear that both reactions proceeded through the bicyclic intermediates **1.99** and **1.101**, respectively; however, no explanation was given for the different products arising from hydrolysis.



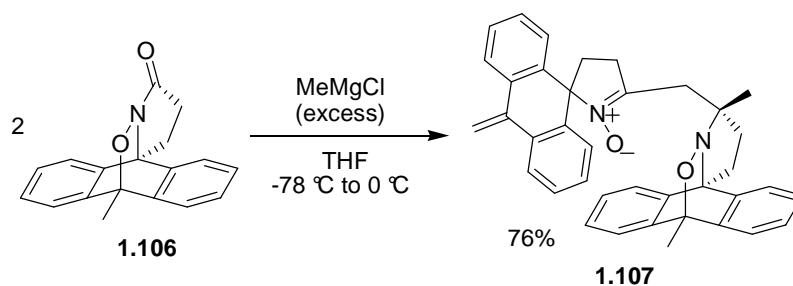
Scheme 1.18. Brønsted acid-mediated C-O bond cleavage

Treatment of cycloadduct **1.91** with Grignard reagents in the presence of Cu^{II} resulted in the selective formation of hydroxamates **1.103** arising from attack at the “C” position and minor amounts of hydroxamates **1.104** arising from attack at the “B” position (Scheme 1.19).¹¹¹ Similar reactivity was observed when bicyclic cycloadducts **1.67** were treated with dialkylzinc reagents in the presence of copper catalysts.^{112, 113} Even though attack at the carbonyl was expected based on studies by Keck,⁸⁵ no products arising from attack at position “A” were observed. Again, this probably illustrates the weakening of the C-O bond that arises from metal coordinated species such as complex **1.93**. This method was applied toward the synthesis of hydroxamate **1.105**, a potent 5-lipoxygenase inhibitor.¹¹¹



Scheme 1.19. C-O bond cleavage using Grignard reagents

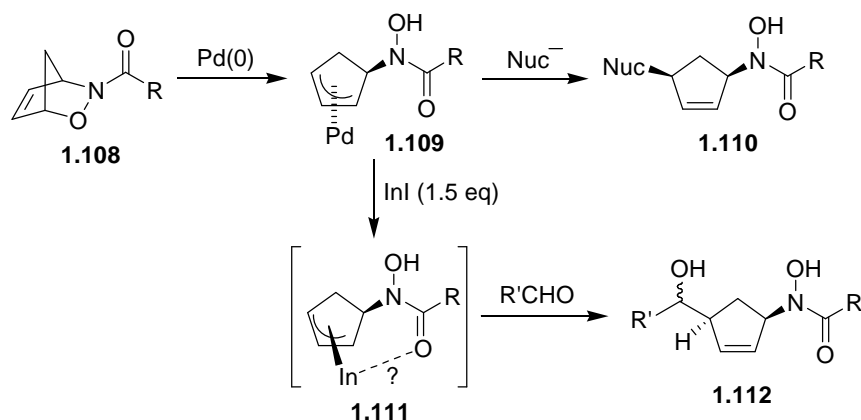
Other unexpected reactions have been reported when acylnitroso HDA cycloadducts were treated with Grignard reagents. When 9,10-dimethylantracene adduct **1.106** was treated with excess MeMgCl in THF, the unusual dimeric nitron compound **1.107** was obtained in 76% yield (Scheme 1.20).¹¹⁴ The authors have proposed a possible mechanistic explanation for this result, however the details concerning the formation of compound **1.107** are still not clear.



Scheme 1.20. Unusual reaction with a Grignard reagent

Upon treatment with Pd(0), cycloadducts **1.108** yielded π -allyl species **1.109** that were trapped with nucleophiles and provided 1,4-*cis* cyclopentenes **1.110** selectively (Scheme 1.21).^{107, 115} Reductive transmetalation of π -allyl species **1.109** with In(I)

yielded indium complex **1.111** that was trapped with reactive aldehydes and ketones and yielded 1,4-*cis* cyclopentenones **1.112**.¹¹⁶ The exact nature of the structure of indium complex **1.111** is not fully understood at this time.

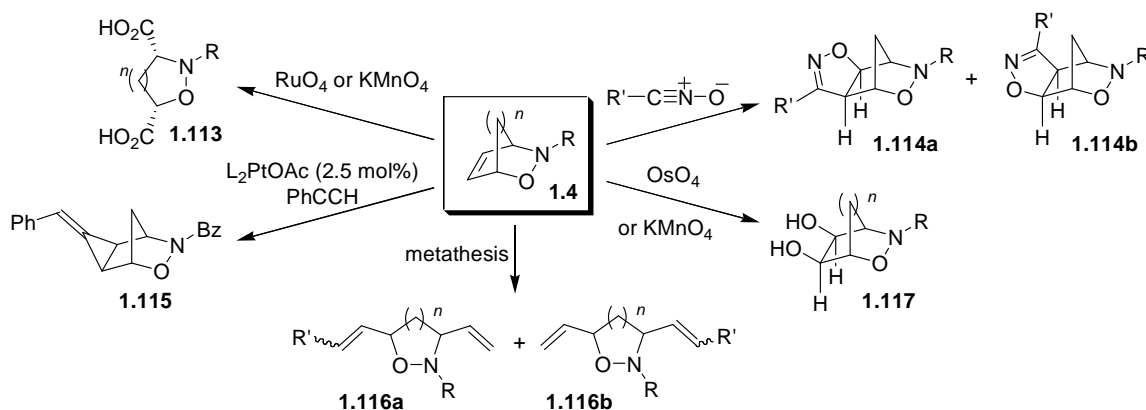


Scheme 1.21. C-O bond cleavage using Pd/In chemistry

1.9.4 Alkene cleavage and modification

Compared to other functionality in bicyclic oxazines **1.4**, relatively little effort has been concentrated on modifying the alkene portion of the 3,6-dihydro-1,2-oxazine system. Accordingly, the strained nature of the 2-oxa-3-aza-bicyclo[2.2.1]hept-5-ene system has been under-utilized for its potential to promote selective functionalization of the alkene system. Only a handful of transformations have been made to the alkene moiety in bicyclic oxazines **1.4** (Scheme 1.22). Oxidative cleavage of cycloadducts yielded diacid compounds **1.113**. Additions to the alkene have included the [3+2] cycloaddition of nitrile oxides to form compounds **1.114a** and **1.114b**,^{117, 118} dihydroxylation to yield diols **1.117**,¹¹⁹⁻¹²¹ and alkylidenecyclopropanation to yield oxazine **1.115**.¹²² Other studies have shown the alkene of bicyclic oxazines **1.4** to be

suitable for ring-opening cross metathesis reactions, and yielded in structures **1.116a** and **1.116b**.^{123, 124}



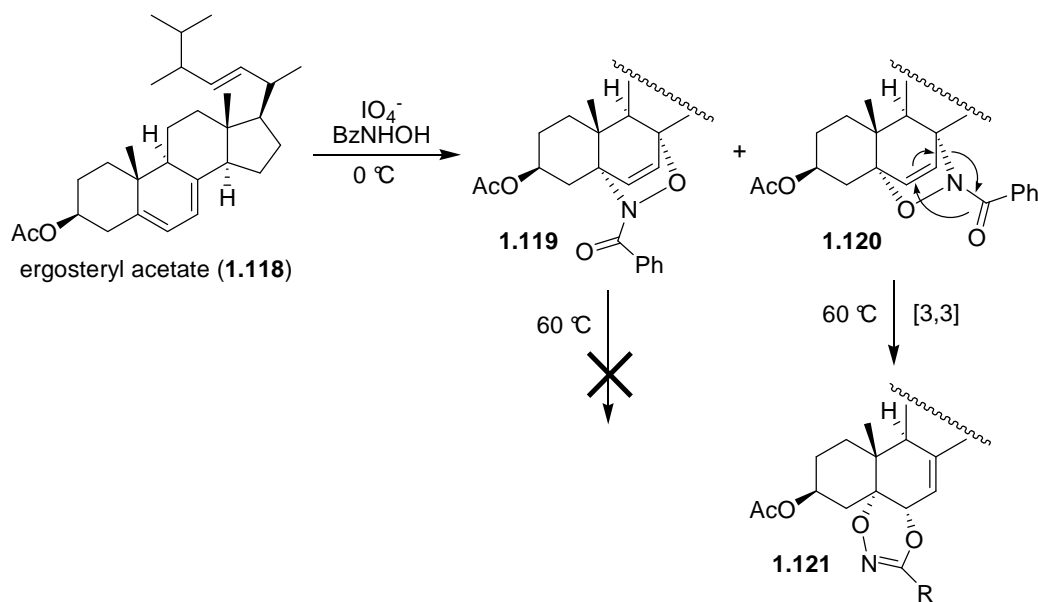
Scheme 1.22. Examples of alkene modification/cleavage of bicyclic adducts

Additions to the alkene of bicyclic oxazine **1.4** have often proceeded with high facial selectivity, but not with high regioselectivity. Consequently, dihydroisoxazoles **1.114** and **1.115** were obtained as a 1:1 mixture.

1.9.5 Other chemistry and rearrangements

In addition to the chemistry outlined above, acylnitroso HDA cycloadducts **1.3** and **1.4** have participated in a number of other unusual and mechanistically interesting transformations.^{17, 125} Kirby reported that when ergosteryl acetate (**1.118**) was treated with acylnitroso compounds in refluxing benzene, cycloadduct **1.119** was obtained along with the unusual dihydrodioxazine **1.121** (Scheme 1.23).¹²⁶ When the reaction was repeated at 0 °C, the regioisomeric cycloadducts **1.119** and **1.120** were obtained, however upon heating to reflux, cycloadduct **1.120** was transformed into the dioxazine compound

1.121 through a [3,3]sigmatropic rearrangement. Oxazine **1.119** did not undergo the rearrangement, which was explained by steric crowding of the dioxazine product.



Scheme 1.23. [3,3] rearrangement of an ergosteryl cycloadduct

1.10 Synthetic applications of intermolecular acylnitroso hetero Diels-Alder reactions

The utility of the intermolecular acylnitroso HDA reaction in organic syntheses is reflected by the wide variety of molecules that are accessible using this chemistry. The following section will outline various classes of molecules that have been synthesized using acylnitroso HDA methodology.

1.10.1 Carbocyclic nucleosides

Carbocyclic nucleosides, in which the furanose oxygen of the nucleoside is replaced by a methylene unit, have received attention for their use as antiviral agents.¹²⁷⁻

¹³¹ Aristeromycin (**1.123**) is the direct carbocyclic nucleoside analog of adenosine

(**1.122**) and has demonstrated potent antiviral properties linked to the inhibition of AdoHcy hydrolase (Figure 1.9).¹³² The synthesis and study of carbocyclic nucleosides has been an important area of therapeutic research, and many methods have been developed that allow access to this class of molecules.

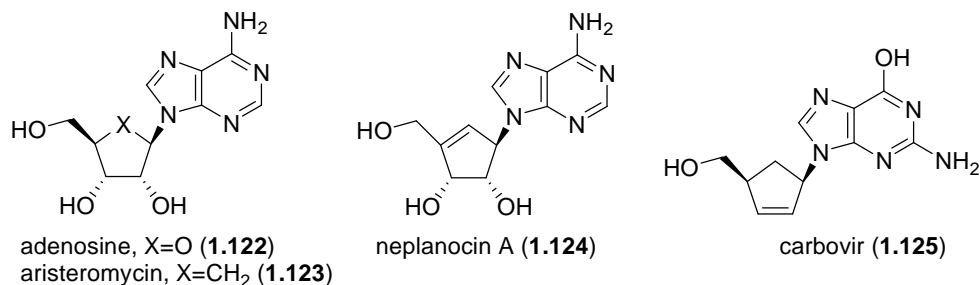
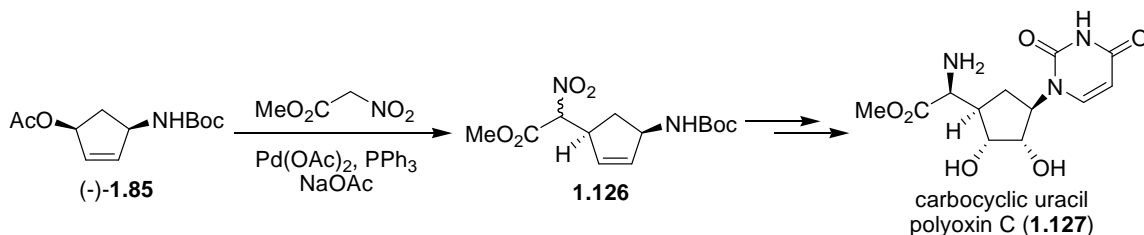


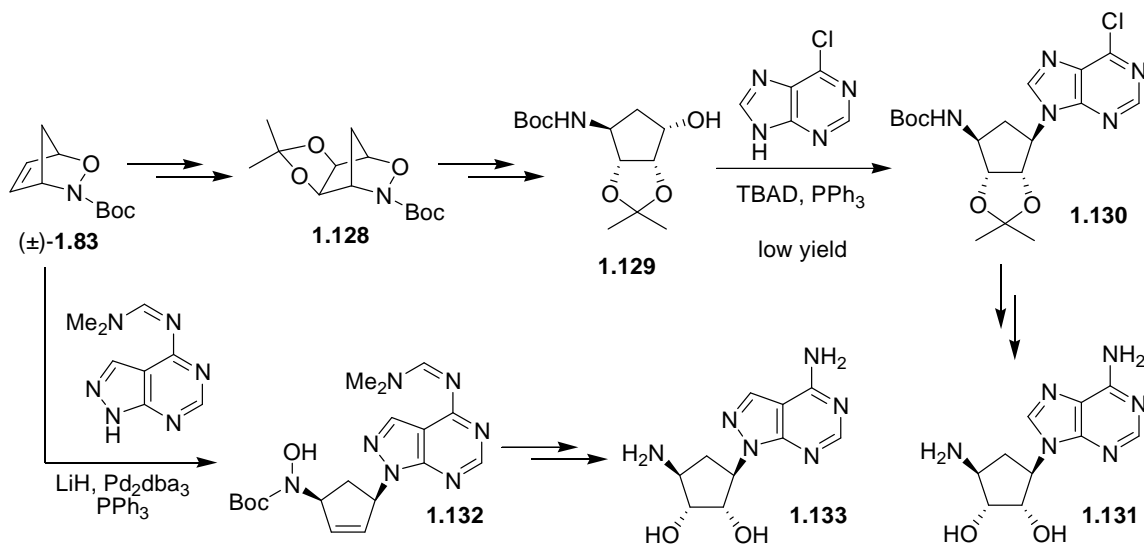
Figure 1.9. Representative carbocyclic nucleosides

The Miller group has published a number of papers regarding the use of acylnitroso HDA methodology to construct carbocyclic nucleoside analogs.^{65, 133-137} Enantiomerically pure acetate (-)-**1.85**,¹⁰³ obtained from the kinetic enzymatic resolution process described earlier, was used to synthesize carbocyclic uracil polyoxin C (**1.127**) and its epimer through the intermediate **1.126** (Scheme 1.24).^{135, 138} The opposite enantiomer of acetate (-)-**1.185** was used to synthesize the carbocyclic fragment of nucleoside Q.¹³⁹



Scheme 1.24. Synthesis of carbocyclic uracil polyoxin C

Cowart has also published a method for synthesizing azacarbocyclic nucleoside analogs such as compounds **1.133** and **1.131** from cycloadduct **1.83** (Scheme 1.25).¹²¹ From acetonide **1.128**, N-O bond reduction followed by inversion of the alcohol group through an oxidation/reduction sequence yielded alcohol **1.129**. The nucleoside base was installed using Mitsunobu conditions and yielded compound **1.130** which was ultimately transformed into analog **1.131**. This method suffered from low yields for the Mitsunobu reaction, so an alternative strategy was employed that made use of Pd- π -allyl chemistry to install the base directly from cycloadduct **1.83** and yielded hydroxamate **1.132**. Reduction of the hydroxamate followed by dihydroxylation and deprotection provided an efficient route to analog **1.133**.

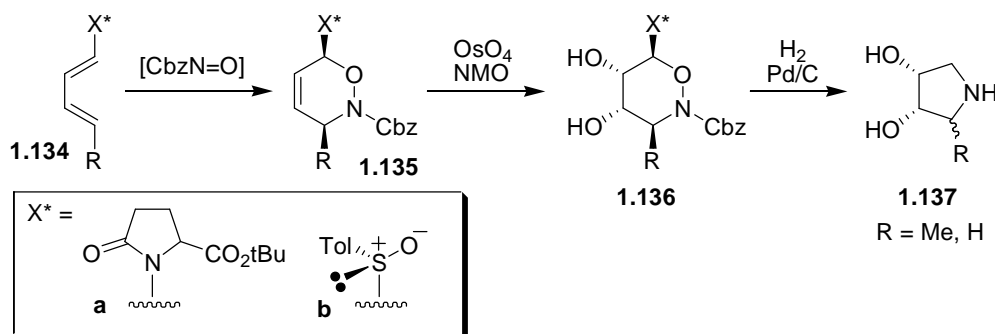


Scheme 1.25. Carbocyclic azacarbocyclic nucleoside analogs

1.10.2 Azasugars

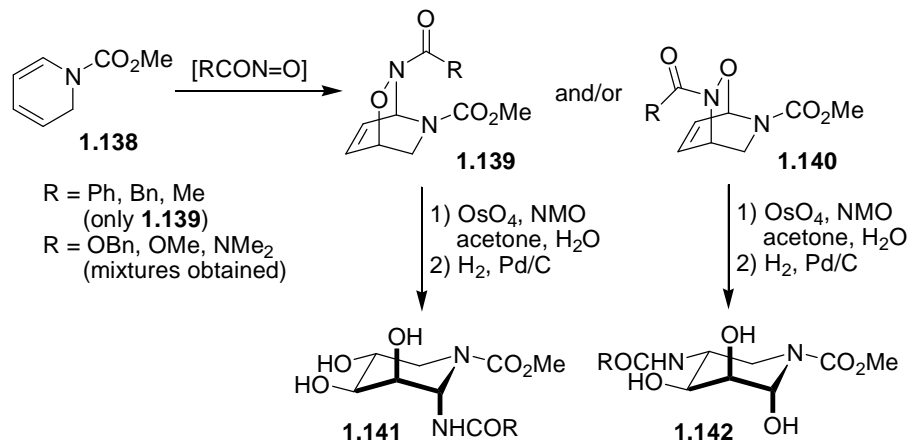
The nitroso HDA reaction allows the construction of 3,6-dihydro-1,2-oxazine rings that possesses the required substitution pattern for the synthesis of many azasugars. The use of the nitroso HDA reaction for azasugar synthesis has been reviewed,^{4, 49} and allows access to both pyrrolidine and piperidine sugar analogs.

Acyclic dienes **1.134a**^{71, 140-142} and **1.135b**⁷⁴ have been used toward the synthesis of pyrrolidine-based sugar derivatives (Scheme 1.26). Cycloadducts **1.135** have been obtained in high yield and diastereoselectivity. Dihydroxylation afforded diol **1.136** with excellent facial selectivity, and N-O bond reduction followed by intramolecular condensation provided access to pyrrolidines **1.137**.



Scheme 1.26. Synthetic route to pyrrolidines

Piperidine-based sugar derivatives have been synthesized utilizing an acylnitroso HDA reaction with 1,2-dihydropyridines **1.138** (Scheme 1.27).⁴ While nitrosoformate esters yielded mixtures of cycloadducts **1.139** and **1.140**, the use of acylnitroso species derived from carboxylic acids selectively yielded cycloadduct **1.139**. Facially selective dihydroxylation followed by catalytic hydrogenation yielded the azasugar derivatives **1.141** and **1.142**.



Scheme 1.27. Route to azasugars from 1,2-dihydropyridines

1.10.3 Tropane and related alkaloids

Ever since the first landmark synthesis of tropinone (**1.143**) by Robinson in 1917,¹⁴³ the tropane alkaloids have continued to elicit the interest of synthetic organic chemists (Figure 1.10). Various members of the tropane alkaloids include nortropanes (**1.144**), homotropanes (**1.145**), scopine (**1.146**), and polyhydroxylated nortropanes such as calystegines (**1.147**).

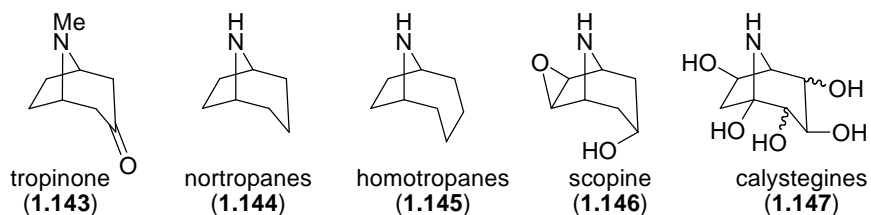
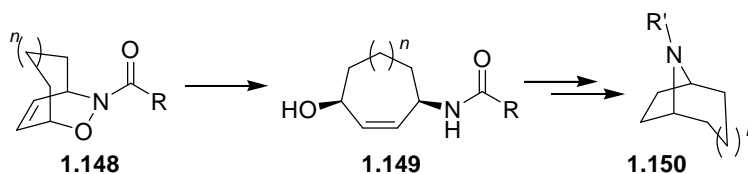


Figure 1.10. Structures of the tropane alkaloid family

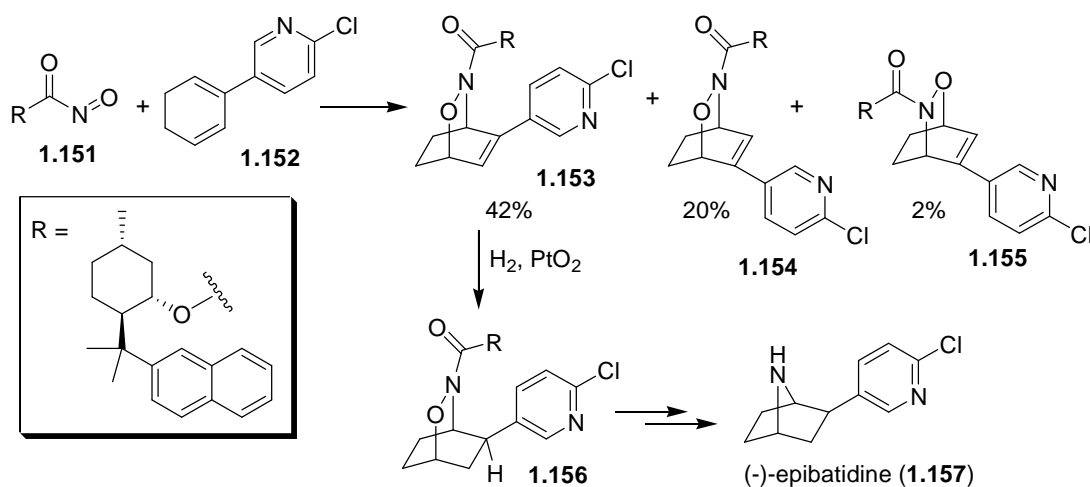
A number of acylnitroso HDA approaches to the tropane alkaloids have been reported, and all follow the same general scheme first outlined by Kibayashi (Scheme

1.28).¹⁴⁴ N-O bond reduction of cycloadducts **1.148** provided the aminoalcohols **1.149**. An intramolecular cyclization yielded the aza-bridged tropane system **1.150**.



Scheme 1.28. General synthetic route to the tropane alkaloids

This general approach to tropanes has been extended to the enantioselective total synthesis of (-)-epibatidine (**1.157**) (Scheme 1.29).^{64, 145} Chiral nitrosoformate ester **1.151** was generated in the presence of diene **1.152** and yielded the three cycloadducts **1.153**, **1.154**, and **1.155** with moderate selectivity. Cycloadduct **1.153** was used to complete the synthesis of (-)-epibatidine (**1.157**) through intermediate **1.156**.



Scheme 1.29. Total synthesis of (-)-epibatidine

Other groups have utilized similar approaches toward the synthesis of members of the tropane family such as the nortropanes (**1.144**),¹⁴⁶ homotropanes (**1.145**),¹⁴⁷⁻¹⁴⁹ scopine (**1.146**) and pseudoscopine,¹⁵⁰ and polyhydroxylated nortropanes **1.147**.^{151, 152}

1.10.4 Amaryllidacea alkaloids and related structures

Alkaloids from plants in the *Amaryllidacea* family have been used in the treatment of cancer.¹⁵³ Members of this family of alkaloids include lycorine (**1.158**), pancratistatin (**1.159**), deoxypancratistatin (**1.160**), narciclasine (**1.161**), and lycoricidine (**1.162**) (Figure 1.11).

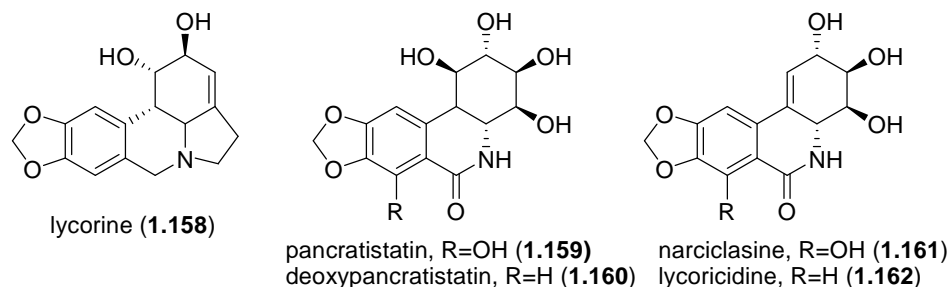
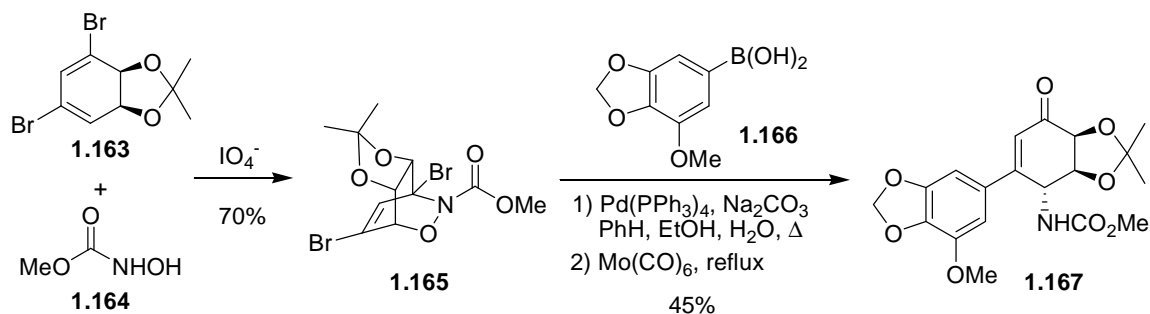


Figure 1.11. Structures of the amaryllidacea alkaloids

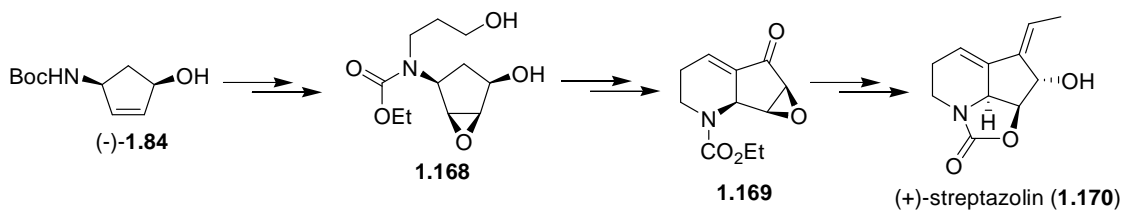
Acylnitroso HDA reactions to substituted 1,3-cyclohexadienes have been used in the synthesis of the amaryllidacea alkaloids. The Hudlicky group has published synthetic routes to narciclasine (**1.161**).^{76, 154, 155} Nitrosoformate ester **1.164** was oxidized in the presence of the chiral diene **1.163** and yielded cycloadduct **1.165** (Scheme 1.30).¹⁵⁴ A one pot Suzuki-Miyaura reaction followed by N-O bond reduction yielded the key intermediate **1.167** that was further elaborated to furnish narciclasine (**1.161**). Other

routes to the amaryllidacea alkaloids and their core structure have been reported that utilize acylnitroso HDA reactions through a similar methodology.^{33, 155-159}



Scheme 1.30. Synthetic route to narciclasine

The total synthesis of the related fused polycyclic piperidine-containing alkaloid (+)-streptazolin (**1.170**) has also been reported by the Miller group (Scheme 1.31).^{160, 161} Chiral cyclopentenol (-)-**1.184** was converted to intermediate **1.168** that furnished compound **1.169** using an intramolecular aldol condensation. Selective installment of the Z-alkene was realized using a silicon-tethered ring-closing metathesis strategy¹⁶¹ and ultimately provided (+)-streptazolin (**1.170**).



Scheme 1.31. Synthetic route to (+)-streptazolin

1.10.5 Amino acid analogs and other biologically important molecules

The acylnitroso HDA reaction has provided access to a number of novel amino acid analogs and other biologically important molecules. The Miller group has reported the synthesis of a variety of therapeutically relevant molecules. A number of amino acid analogs have been synthesized that are structurally similar to antibacterial diacid compounds **1.171**¹⁶² through the oxidative cleavage of nitroso HDA cycloadducts **1.4** (Figure 1.12).^{67, 163-167} Other syntheses reported by the Miller group have included the preparation of biologically active agents such as BCX-1812 (**1.172**), LY354740 analogs **1.173**, 5-lipoxygenase inhibitors **1.105**,¹¹¹ phosphodiesterase inhibitors,¹⁶⁸⁻¹⁷⁰ and the conformationally restricted substrate analog of siderophore biosynthesis **1.174**.¹⁷¹

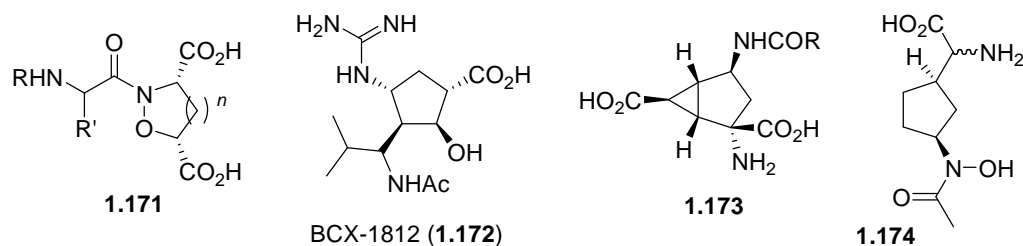
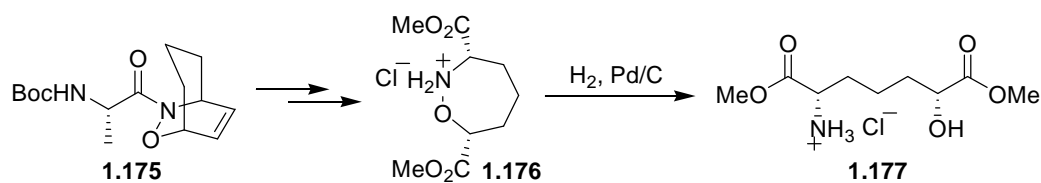


Figure 1.12. Representative biologically active structures

Other amino acid analogs have been synthesized from 1,3-cycloheptadiene-derived cycloadduct **1.175** (Scheme 1.32).¹⁷² Oxidative cleavage of the alkene afforded diester **1.176**, and N-O bond reduction provided *meso*-diaminopimelic acid (DAP) analogs such as compound **1.177**.



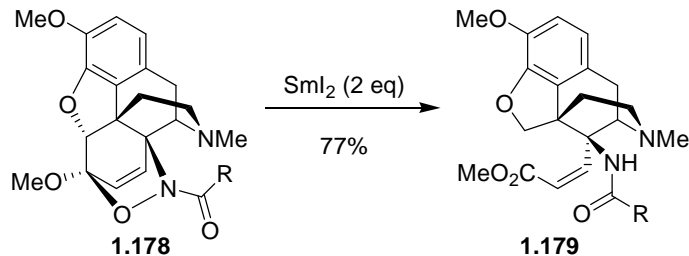
Scheme 1.32. Synthesis of meso-DAP analogs

1.10.6 Natural product derivatization

Ever since Kirby reported acylnitroso HDA reactions with thebaine,³¹ the acylnitroso HDA reaction has been used as a method for synthesizing natural product derivatives. The benefits of using nitroso HDA reactions for this purpose include the often exquisite stereo- and regioselectivity of the cycloaddition as well as the rich chemistry of their products.

A number of efforts have used the nitroso HDA reaction to provide access to steroids and novel analogs and derivatives.^{25, 126, 173-175} The Miller group has recently disclosed a strategy for natural product derivatization that exclusively utilizes nitroso cycloaddition chemistry to prepare analogs of natural products from a variety of molecular classes.¹⁷⁶

Thebaine has provided an interesting look into the chemistry that can be used to offer structurally novel derivatives of natural products in a few steps using the chemistry of nitroso HDA cycloadducts. Kirby has reported a number of unusual reactions that use acylnitroso cycloadducts of thebaine.^{17, 177} In a recent example, Sheldrake has reported the selective opening of the thebaine skeleton from cycloadducts **1.178** using samarium diiodide (Scheme 1.33).¹⁷⁸ Similar to other reactions of acylnitroso HDA cycloadducts,¹⁷⁹ samarium diiodide facilitated N-O and C-C bond cleavage in one pot and provided the novel derivative **1.179**.



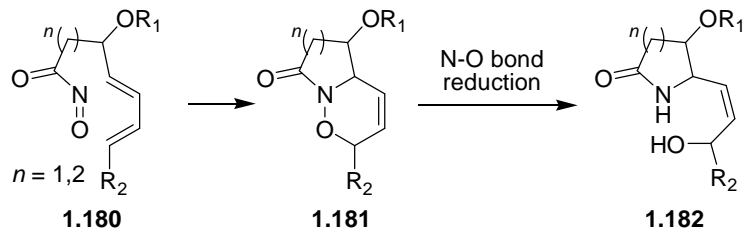
Scheme 1.33. Thebaine analogs from an unexpected ring cleavage

1.11 Synthetic applications of intramolecular acylnitroso hetero Diels-Alder reactions

Intramolecular nitroso HDA reactions have been used in the synthesis of natural products, alkaloids, and other biologically important molecules. Although intermolecular nitroso HDA reactions are often regioselective, tethering the acylnitroso group to the reacting diene affords oxazines regiospecifically and often diastereoselectively. This section will survey the use of intramolecular nitroso HDA reactions in the synthesis of a variety of alkaloid classes and will again emphasize the utility of the nitroso HDA reaction as a method to construct complex structural systems.

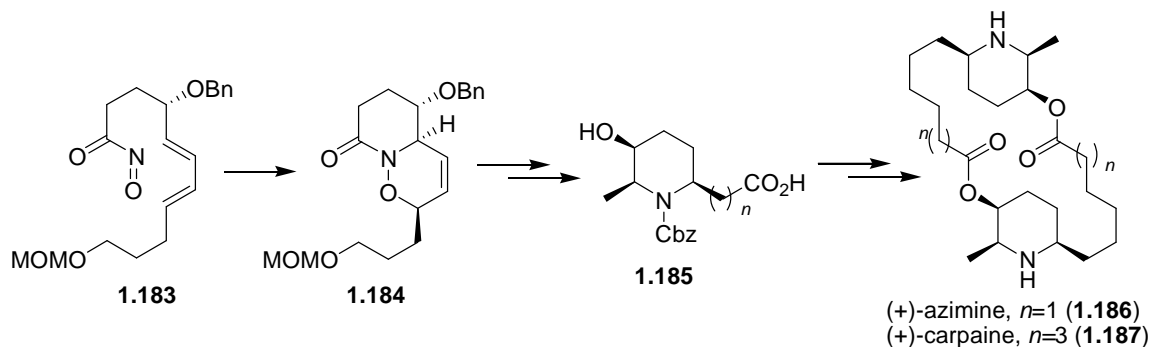
1.11.1 Monocyclic alkaloids

The simplest alkaloids that have been synthesized utilizing intramolecular acylnitroso HDA reactions are the monocyclic alkaloids. The synthesis of compounds **1.182** from acylnitroso species **1.180** represents a general method that often closely resembles the initial steps in the synthesis of monocyclic as well as polycyclic alkaloid systems (Scheme 1.34). Preliminary studies in this area by Keck¹⁸⁰ and Kibayashi¹⁸¹⁻¹⁸³ provided the necessary methodology required for more elaborate structures.



Scheme 1.34. General route to monocyclic alkaloids

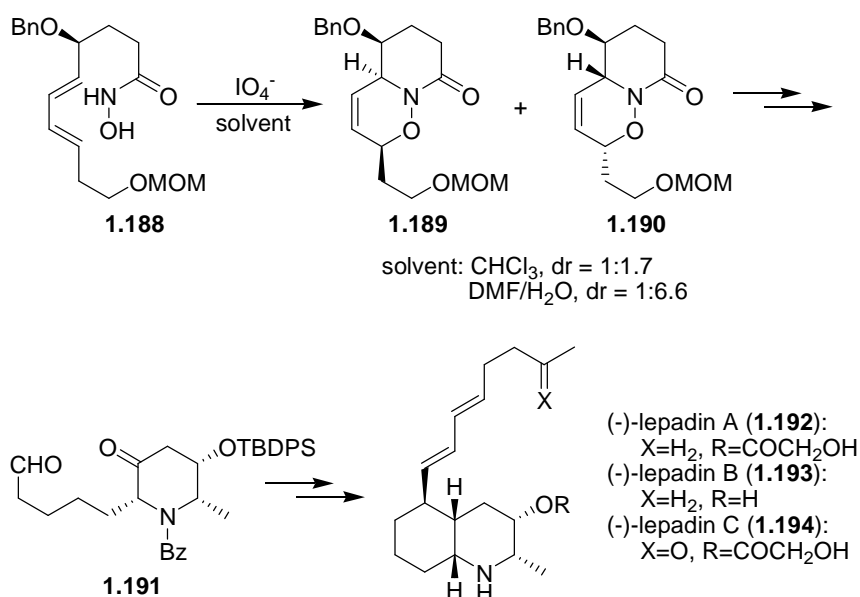
Recently, Kibayashi published the enantioselective total synthesis of (+)-azimine (**1.186**) and (+)-carpaine (**1.187**) that highlighted the use of the intramolecular acylnitroso HDA reaction (Scheme 1.35).¹⁸⁴ Acylnitroso compound **1.183** underwent a spontaneous, stereoselective HDA reaction and formed oxazine **1.184**, which, over a number of synthetic steps, was transformed into the monomeric key intermediate **1.185**. Dimerization of the monomeric unit **1.185** through the formation of the two ester bonds yielded the aforementioned natural products **1.186** and **1.187**.



Scheme 1.35. Synthesis of (+)-azimine and (+)-carpaine

1.11.2 Decahydroquinoline alkaloids

Decahydroquinoline alkaloids have been synthesized using similar methodology to monocyclic alkaloids. The synthesis of (-)-lepadins A (**1.192**), B (**1.193**), and C (**1.194**) was reported in 2001 (Scheme 1.36).^{185, 186} The synthesis of the lepadin family also illustrated an important difference between the intra- and intermolecular acylnitroso HDA reactions regarding the effect of solvent polarity on reaction selectivity. Selectivity in intermolecular nitroso HDA reactions has most often been insensitive to solvent polarity; however, the use of aqueous media for intramolecular acylnitroso HDA reactions has resulted in significant enhancement in diastereoselectivity.¹⁸⁷ Thus, when hydroxamic acid **1.188** was oxidized in aqueous solvent mixtures, cycloadduct **1.190** was formed more selectively over cycloadduct **1.189** than when the reaction was performed in traditional nonpolar solvents. Cycloadduct **1.190** was transformed into the monocyclic intermediate **1.191**, which was further transformed into the (-)-lepadins **1.192-1.194**.



Scheme 1.36. Total synthesis of (-)-lepadin A, B, and C

Using similar chemistry, Kibayashi has also reported the synthesis of the pumiliotoxin alkaloids.¹⁸⁷⁻¹⁹⁰

1.11.3 Indolizidine and pyrrolizidine alkaloids

Pyrrolizidine and indolizidine alkaloids have been isolated from a wide variety of natural sources and have demonstrated interesting biological properties.¹⁹¹ Representative compounds for this class of alkaloids that have been synthesized using an intramolecular acylnitroso HDA strategy include swainsonine (**1.195**) and its derivatives,^{192, 193} fascicularin (**1.196**) and lepadiformine (**1.197**),¹⁹⁴ and other indolizidine alkaloids (Figure 1.13).^{180, 182, 192, 195-202}

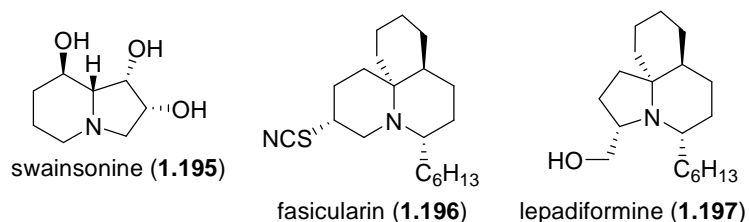
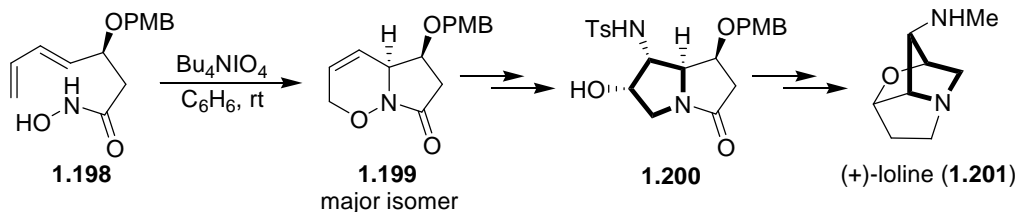


Figure 1.13. Representative indolizidine and pyrrolizidine alkaloids

The synthesis of a particularly interesting member of the pyrrolizidine class of alkaloids, (+)-loline (**1.201**) was reported using an intramolecular acylnitroso HDA strategy (Scheme 1.37).^{203, 204} Hydroxamic acid **1.198** was oxidized to yield the oxazine **1.199**. Subsequent modifications yielded the intermediate **1.200** which was converted to (+)-loline **1.201**.



Scheme 1.37. Total synthesis of (+)-loline

1.11.4 Bridged oxazinolactams using type II intramolecular cycloadditions

The vast majority of intramolecular acylnitroso HDA reactions have involved the use of dienes tethered at the 1-position. “Type II” intramolecular acylnitroso HDA reactions are tethered at the 2-position of the diene (Figure 1.14), and provide access to bridged oxazinolactam compounds.

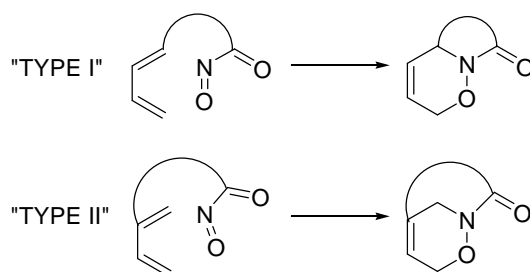
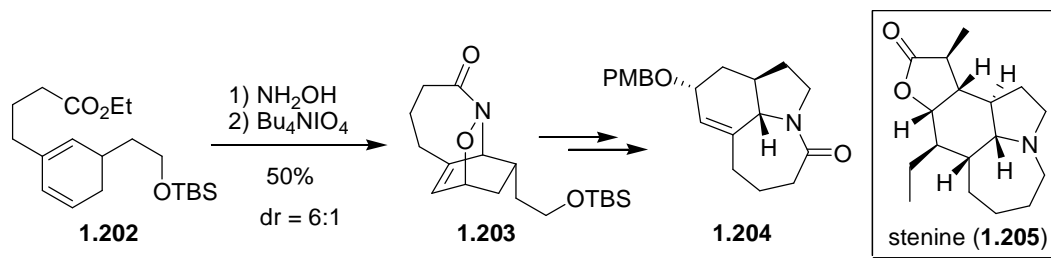


Figure 1.14. Type I vs. Type II intramolecular HDA reactions

Recently, the synthesis of the tricyclic core of the alkaloid stenine (**1.205**) has been reported using a type II intramolecular acylnitroso HDA reaction (Scheme 1.38).²⁰⁵ Ethyl ester **1.202** was converted to a hydroxamic acid and upon oxidation yielded the tricyclic structure **1.203**. Subsequent modification lead to advanced intermediate **1.204**.



Scheme 1.38. Recent example of a type II acylnitroso cycloaddition

Other examples of the use of type II intramolecular acylnitroso HDA reactions in synthetic applications have been reported by the Shea group,²⁰⁶⁻²⁰⁹ and have demonstrated the potential of these often overlooked variations of the more typical type I intramolecular acylnitroso HDA reactions.

1.12 Summary

The acylnitroso HDA reaction is a valuable tool for the synthetic organic chemist since it allows for the rapid, selective construction of a the versatile 3,6-dihydro-1,2-oxazine system. This chapter has described how the chemistry of the oxazine ring has been utilized in the synthesis of a wide array of biologically active molecules and natural products from functionalization of the *N*-acyl, *N*-O, C-O, and C=C bonds of nitroso HDA cycloadducts. The following chapters of this dissertation will describe 1) the synthesis of novel mycobactin analogs through the use of acylnitroso HDA methodology (chapters 2-4), 2) the discovery of a new method for constructing imidazoles from amino acids (chapter 5), 3) the development of new chemistry that capitalizes on the strained nature of bicyclic acylnitroso HDA cycloadducts (chapter 6), and 4) the investigation of new directions and future research regarding the use of acylnitroso HDA reactions (chapter 7).

CHAPTER 2:

DESIGN OF NEW MYCOBACTIN ANALOGS FROM ACYLNITROSO CYCLOADDUCTS

2.1 Introductory remarks

The following three chapters of this dissertation describe an application of the acylnitroso HDA reaction toward the synthesis of novel mycobactin analogs. The chemistry described in the preceding chapter as well as new synthetic strategies were employed in the synthesis of the target mycobactin analogs.

This chapter describes the origin of this project and how the target mycobactin analogs will help to provide a better understanding of the biological activity demonstrated by mycobacterial siderophores.

2.2 Tuberculosis: a global health problem

Mycobacterium tuberculosis is the pathogen responsible for the deadly disease tuberculosis (TB), which causes nearly 2 million deaths per year worldwide. TB is the leading cause of death worldwide from a single pathogen, claiming more lives each year than all other tropical diseases combined.^{210, 211} Someone in the world is newly infected with TB bacilli every second and overall one-third of the world's population is currently infected with TB.²¹² For most that are infected, TB exists in a resting or "latent" stage, and only five to ten percent of people infected will become sick or develop the disease

during their lifetime. For those who have HIV or who have otherwise compromised immune systems, active infections are much more likely to develop. A weakened immune system combined with TB infection is especially deadly, and as a result, TB is the leading cause of death for those who are HIV-positive.^{210, 212-214}

TB has long been thought of as a disease that only affects the underdeveloped nations of the world; however, the emergence of an alarming number of strains resistant to first-line, second-line, and even last resort drug therapies has resulted in a number of efforts to combat a growing worldwide TB epidemic.

2.2.1 Difficulties associated with TB drug therapies

M. tuberculosis is an intracellular human pathogen that colonizes in the alveolar sacs in the lungs. Mycobacteria are gram-positive bacteria that are covered with an unusually thick cell wall consisting largely of mycolic acids **2.1** (Figure 2.1)²¹⁵ which contain alkyl chains from 60-90 carbon atoms long.²¹⁶ This unusually tough cell wall allows the *M. tuberculosis* to survive phagocytosis by the host macrophages and results in a high level of resistance to host defense mechanisms and conventional drugs.²¹⁷ *M. tuberculosis* is therefore effectively walled-off inside the host phagosome where it forms a lesion, called a “tubercule”, that is the pathogen’s namesake.²¹⁴⁻²¹⁷

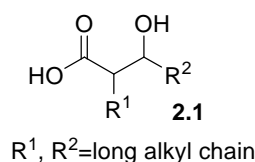


Figure 2.1. Mycolic acid general structure

Due to this unusual cell wall structure and the extreme difficulties associated with effective transport of drugs to the pathogen, conventional antibiotics are ineffective in the treatment of TB.²¹⁸ There is a vaccine available for the treatment of TB, the bacille Calmette-Guérin (BCG) vaccine, that protects against severe forms of childhood tuberculosis; however, immunity to TB weakens in adolescence and does not offer protection in adults.²¹⁷ In the 1940s and 1950s, a number of drugs were discovered that demonstrated clinical anti-TB activity. Currently there exist a variety of “first-line” and “second-line” drugs used to treat the disease (Figure 2.2). First-line anti-TB drugs include rifampin (**2.7**), isoniazid (**2.3**), pyrazinamide (**2.4**), and ethambutol (**2.6**). Second-line drugs include the fluoroquinolones such as ciprofloxacin (**2.10**) and ofloxacin (**2.13**), injectable agents such as the aminoglycosides streptomycin (**2.12**), kanamycin (**2.8**), and amikamycin (**2.9**), oral bacteriostatic agents such as ethionamide (**2.5**), cycloserine (**2.11**), and other drugs such as *p*-aminosalicylic acid (**2.2**).²¹⁹

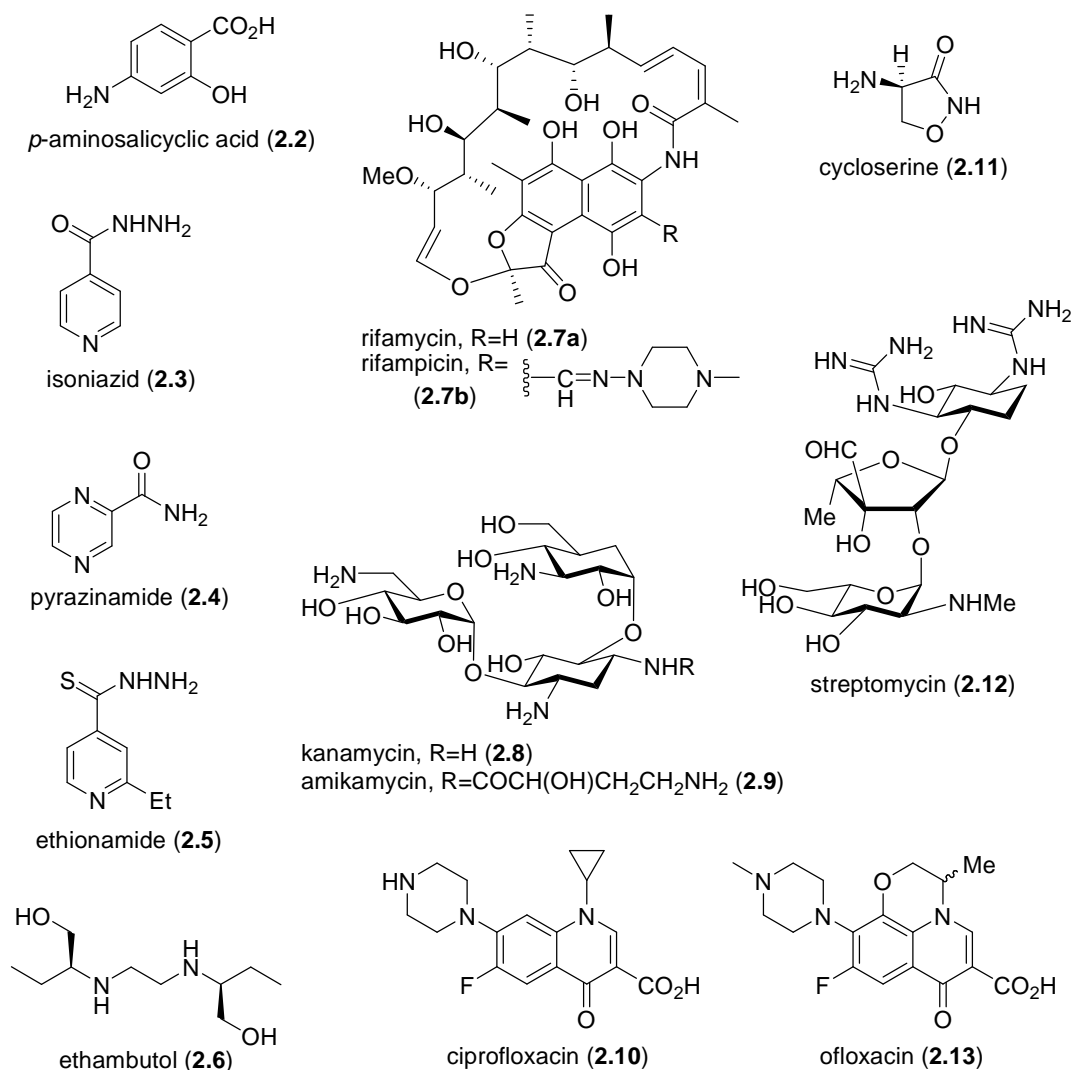


Figure 2.2. Structures of first- and second-line anti-TB drugs

2.2.2 The growing problem of drug resistance

Although there a number of anti-TB drugs are in use that treat infection, the emergence of strains resistant to first- and second-line anti-TB drugs is of growing concern, especially since tuberculosis is quick to develop resistance to antibiotics. In 1944, the first anti-TB drug, streptomycin (**2.12**), was isolated and reported to have antimycobacterial activity.²²⁰ Resistance to the antibiotic was reported soon thereafter.²²¹ Astonishingly, the first person to be treated with streptomycin at the Mayo Clinic in 1944

developed resistance to the drug!²¹⁹ The rate of spontaneous mutation resulting in resistance to antibiotics in *M. tuberculosis* is at a level that makes treatment of this disease with single-drug therapies ineffective.^{218, 222} As a result, the current treatment for TB as specified by the World Health Organization (WHO) is tailored to combat the development of resistance using a variety of different first-line drugs.

The current method of treatment for TB, Directly Observed Therapy Short-course (DOTS), is a multi-disciplinary program that involves a daily treatment with isoniazid (2.3), rifampin (2.7), pyrazinamide (2.4), and ethambutol (2.6) for 2 months followed by 4 months of daily doses of isoniazid (2.3) and rifampin (2.7) with constant monitoring by clinicians.^{212, 218, 223} Unfortunately due to inconsistent or partial treatment, patient noncompliance with regular drug treatments (patients will stop taking the drugs when they “feel better”), prescription errors by doctors or clinicians, and unreliable drug supplies, resistance has developed and is a problem.^{218, 222}

Strains that are resistant to a single drug have been documented in every country surveyed, and multi-drug resistant tuberculosis (MDR-TB), strains that are resistant to multiple first-line drugs, have emerged.^{218, 222} Earlier worldwide statistics reported that one in every ten new infections is resistant to at least one anti-TB drug.²¹⁰

Recent data suggests that the problem of resistance is growing even more. Extensively (or extremely) drug-resistant TB (XDR-TB) does not respond to known anti-TB drugs and is especially lethal.^{219, 224, 225} XDR-TB is defined as being resistant to first-line drugs rifampin (2.7) and isoniazid (2.3) plus fluoroquinones and at least one of the injectable drugs capreomycin, kanamycin (2.8), or amikamycin (2.9).²¹⁹ In a rural district in KwaZulu Natal, South Africa, of the 536 patients with TB, 221 had MDR-TB and 53

of those were defined as XDR-TB. Of the 53 infected with XDR-TB, only one survived the infection.²²⁴ XDR-TB has been identified all over the world and from a broad sampling of nearly 18,000 TB isolates between 2000 and 2004, 20% were identified as MDR-TB strains and 2% were XDR-TB strains.²²⁴

2.2.3 Current strategies and the development of new drugs

The growing trends in anti-TB drug resistance imply that the implementation of the DOTS program is not enough. The WHO's new Stop TB Strategy defines specific objectives and components directed toward halting and beginning to reverse the incidence of TB by 2015. A vital part of that plan will be to foster research in the development of new classes of drugs to combat MDR-TB and XDR-TB as well as the HIV/TB problem.²²⁶⁻²²⁸

For the first time in decades, there are nearly 30 drugs in development for tuberculosis that target a variety of areas such as cell growth, carbon uptake, cell maintenance, cell wall biosynthesis, ATP depletion and pH imbalance, protein and lipid synthesis, and DNA replication and transcription (Figure 2.3).^{211, 223, 229}

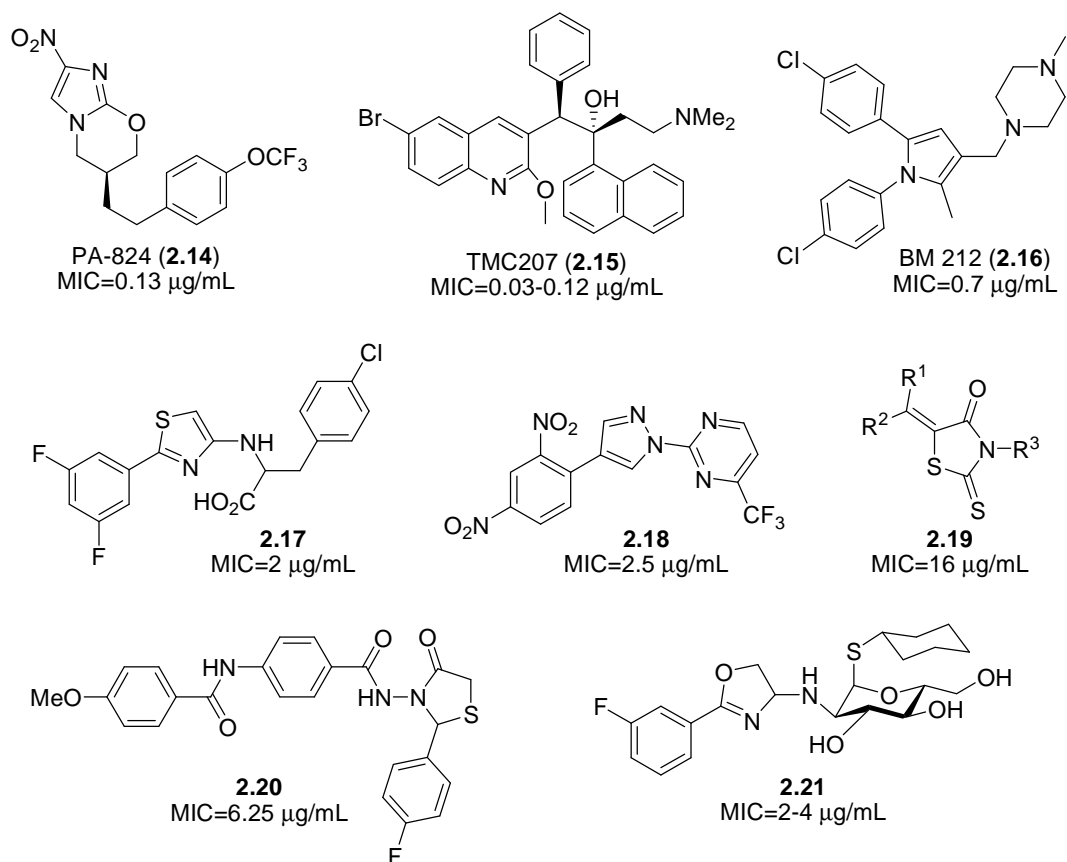


Figure 2.3. Representative anti-TB drugs in development

The development of new drugs has been aided by the determination of the complete genome sequence for *M. tuberculosis*,²³⁰⁻²³⁴ as well as the isolation of a number of antimycobacterial natural products.^{235, 236} PA-824 (**2.14**),²³⁷ thought to interfere with mycobacterial cell wall synthesis, as well as the diarylquinoline compound TMC207 (**2.15**),²³⁸ found to inhibit an ATP synthase proton pump, are two of the more exciting compounds in clinical trials that have demonstrated exceptional activity against TB. Other anti-TB compounds in development include the pyrrole compound **2.16**,^{239, 240} UDP-galactopyranose synthesis inhibitor **2.17**,²⁴¹ mycolic acid biosynthesis inhibitor

2.18,²⁴² dTDP-rhamnose biosynthesis inhibitor **2.19**,²⁴³ peptidoglycan biosynthesis inhibitor **2.20**,²⁴⁴ and mycothio-S-conjugate amidase (MCA) inhibitor **2.21**.²⁴¹

Recently, compounds **2.23a** and **2.23b** were reported as demonstrating anti-TB activity (Figure 2.4).²⁴⁵⁻²⁴⁷ Compounds **2.23a** and **2.23b** are substrate analogs of salicyl-AMP (**2.22**) and inhibit the first step in the synthesis of mycobacterial siderophores, the mycobactins and carboxymycobactins. *p*-Aminosalicylic acid (**2.2**) has also been thought to inhibit the first step in mycobacterial siderophore biosynthesis.^{248, 249} The inhibition of siderophore synthesis and disruption of mycobacterial iron transport represent attractive targets for the development of new anti-TB drugs. The next sections of this chapter will detail mycobacterial iron transport mechanisms and the therapeutic potential of mycobactin analogs synthesized using acylnitroso HDA chemistry.

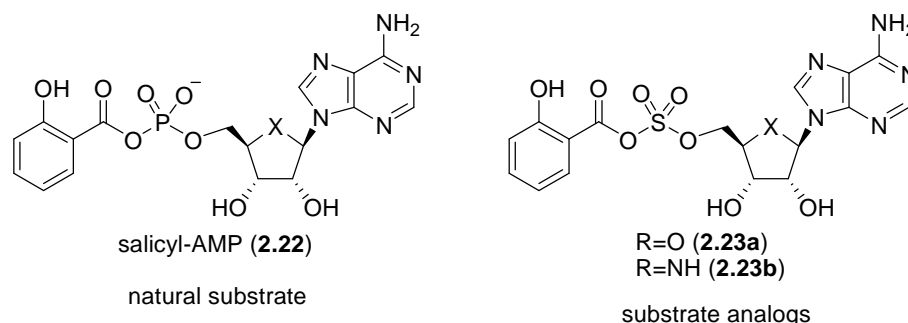


Figure 2.4. Salicyl-AMP analogs as anti-TB inhibitors

2.3 Iron acquisition in mycobacteria

2.3.1 Biological importance of iron

Iron is an essential nutrient for virtually all forms of life that is involved in crucial biological processes such as respiration, energy production, and DNA synthesis. The

ability of an organism to acquire iron is therefore an essential aspect for its survival, and this includes mammals and other vertebrates as well as simple microorganisms such as mycobacteria.

The acquisition of iron is made difficult due to the low solubility of Fe(III) in aqueous environments at neutral pH ranges. At pH 7.4 in the absence of chelating ligands, the total amount of soluble iron (defined as $\text{Fe}^{3+}_{(aq)} + \text{Fe}(\text{OH})^{2+}_{(aq)} + \text{Fe}(\text{OH})_2^{+}_{(aq)}$) is in the range of 10^{-9} to 10^{-10} M.^{248, 250, 251} Solubility of iron increases by 10^{-3} M for every unit decrease in pH so that at a pH of 5, the concentration of soluble iron is 10^{-3} M.²⁴⁸ For bacteria and other microbes that can grow in highly acidic conditions, iron acquisition is therefore not often a problem. Mycobacteria reside in the phagosome of macrophages, where the pH is between 6.1 and 6.5,²⁵² and at this pH range, the concentration of free Fe(III) would be only 1-10 ng/mL.²⁴⁸

Iron acquisition for mycobacteria is made even more difficult due to the host's natural tendency to limit available iron upon infection. In humans, most of the available iron is associated and sequestered by iron-containing heme proteins, the iron transport proteins transferrin and lactotransferrin, and the iron storage protein ferritin.^{248, 253} Upon infection with a pathogen, humans and other mammals will attempt to limit the amount of available iron even further by restricting the amount of iron circulating in the body in the form of transferrin and also by restricting the assimilation of dietary iron.²⁴⁸ Through the use of these strict iron-withholding measures, the free serum concentration of soluble Fe(III) is as low as 10^{-24} M.²⁵⁴

In order to combat the host iron-withholding mechanisms and satisfy the organism's nutritional iron requirements, microorganisms such as mycobacteria have

developed sophisticated iron acquisition systems that ensure a constant supply of iron is made available to the microbe. Three general mechanisms are used by microorganisms to acquire iron: 1) use of iron-chelating molecules known as siderophores, 2) low-affinity Fe(III) transport directly from host iron sources, and 3) ferric ion (Fe(III)^{3+}) reduction to the much more soluble ferrous ion (Fe(II)^{2+}) before transport (Figure 2.5).^{250, 254, 255}

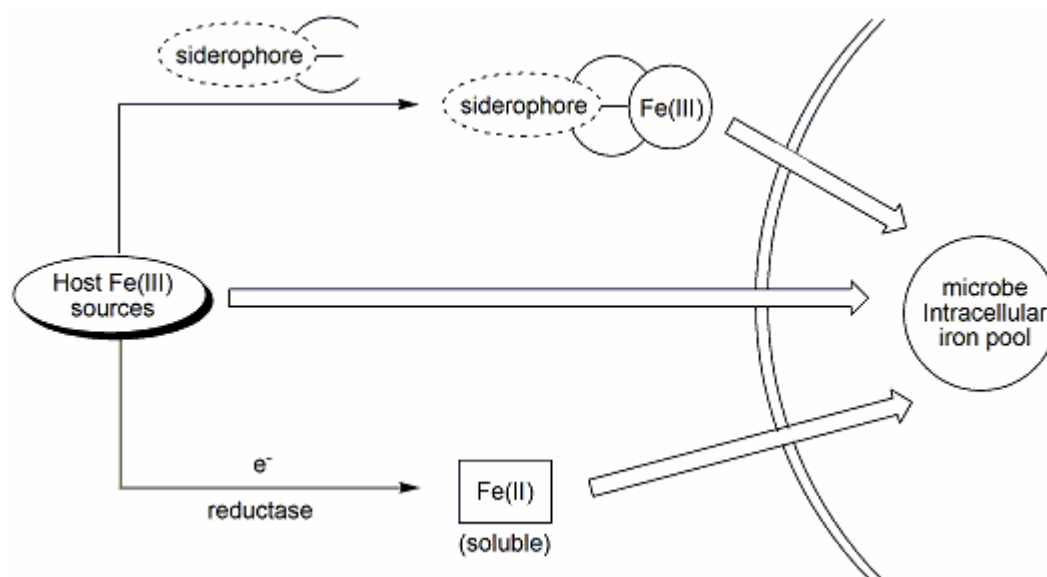


Figure 2.5. Mechanisms of microbial iron transport

2.3.2 Siderophores

The most common mechanism of iron acquisition in microbes is through the production of siderophores, or low molecular weight iron-specific chelating molecules. Siderophore-mediated iron transport is operational in a number of microorganisms, and a number of reviews have been published that cover this topic.^{251, 254, 256-263} Siderophores are exceptionally strong iron-binding molecules, often exhibiting iron-binding constants ranging from 10^{30} to as high as 10^{52} . This high binding constant is a result of the

structure of many siderophores, which often utilize three bidentate iron-binding ligands to form stable 1:1 octahedral complexes with iron(III). Most siderophores use either hydroxamic acids, catechols, or α -hydroxy carboxylates as iron-binding ligands with a few exceptions. General structures of some siderophores will be discussed here.

Many siderophores use one iron-binding ligand exclusively for binding (Figure 2.6). Siderophores that have been isolated that utilize only hydroxamates as iron-binding ligands include compounds such as ferrichrome (2.24), ferricrocin (2.25), and deferrioxamine B (2.26).²⁵⁶ Representative siderophores that utilize only catechols for iron-binding include enterobactin (2.27),²⁶⁴ and the related structures fluvibactin (2.28) and agrobactin (2.29).²⁵⁶

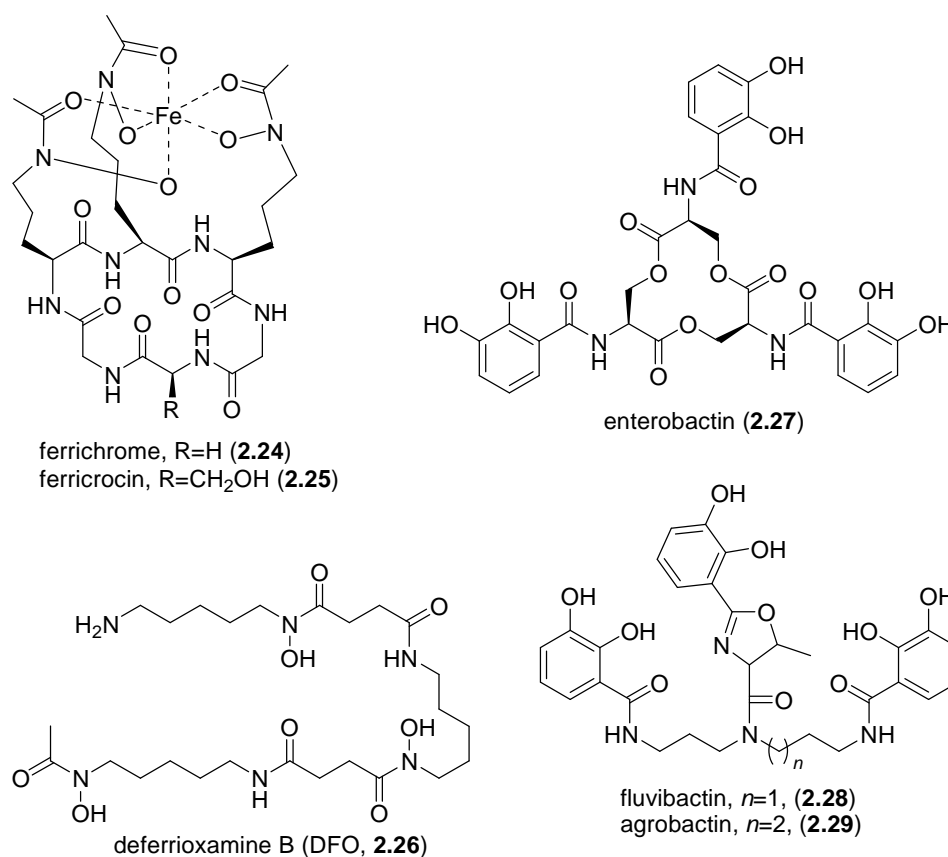


Figure 2.6. Representative siderophores that utilize one Fe-binding ligand

A far greater number of siderophores have been isolated that use more than one type of ligand to bind iron (Figure 2.7). Many of these mixed-ligand siderophores belong to the citrate-based siderophore family and use α -hydroxy carboxylate ligands in addition to hydroxamates or other ligands. Citrate-based siderophores include aerobactin (**2.31**),²⁶⁵ nannochelin (**2.32**),^{266, 267} staphyloferrin A (**2.33**),²⁶⁸ schizokinen (**2.35**),^{269, 270} acinetoferrin (**2.36**),^{215, 271-273} and the unusual siderophore rhizoferrin (**2.30**) that utilizes two α -hydroxy carboxylate ligands in addition to two monodentate carboxylate ligands for iron-binding in a 1:1 iron-siderophore complex.²⁷⁴ The siderophore pseudobactin (**2.34**) utilizes all three types of iron-binding groups: a catechol, an α -hydroxy carboxylate, and a hydroxamate.²⁷⁵

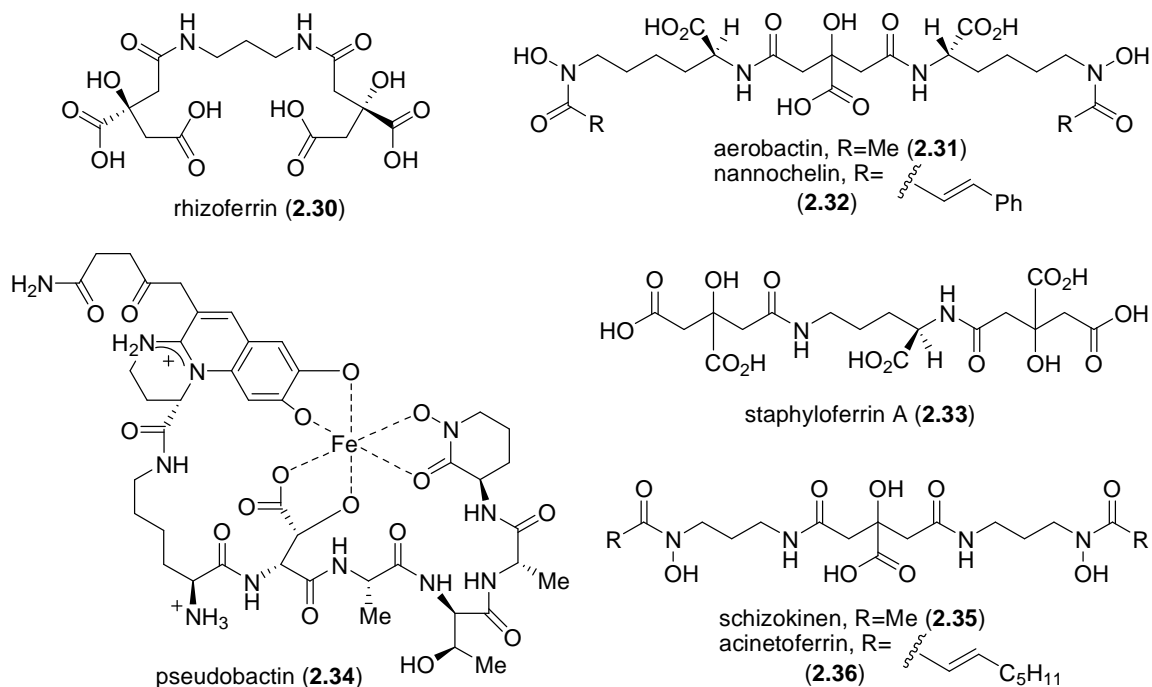


Figure 2.7. Representative mixed-ligand siderophores

2.3.3 Mycobacterial siderophores

Mycobacteria produce lipid-soluble, mixed-ligand siderophores named mycobactins. The first mycobactin was isolated from *M. johnei* as an aluminum complex in 1949,²⁷⁶ and as a metal-free compound in 1953.²⁷⁷ Mycobactins have since been isolated from nearly every strain of mycobacteria. The review of mycobactins published by Snow in 1970²⁷⁸ remains the most comprehensive source of information on mycobactin structure, characterization, and isolation.

All mycobacteria produce mycobactins that are structurally different from one another, but possess the same structural core (Figure 2.8). All mycobactins share the same group of iron binding ligands: a 2-hydroxyphenyl oxazoline, a central lysine-derived hydroxamate, and a cyclic lysine-derived hydroxamate. Although the structure of mycobactins varies by species, they can be grouped into two different classes, the P-type mycobactins and M-type mycobactins, which differ from one another predominantly based on the placement of the long alkyl chain that imparts lipid solubility to the mycobactin. P-type mycobactins, such as mycobactin P (**2.37**) isolated from *M. phlei*, mycobactin S (**2.38**) isolated from *M. smegmatis*, and mycobactin T (**2.39**) isolated from *M. tuberculosis*, contain a long alkyl chain at the central hydroxamate ligand portion of the molecule. The M-type mycobactins, such as mycobactin M (**2.40**) isolated from *M. marinum* and mycobactin N (**2.41**) isolated from *M. neoaurum*, contain a long alkyl chain in the central 3-hydroxy acid portion of mycobactin backbone.²⁷⁸

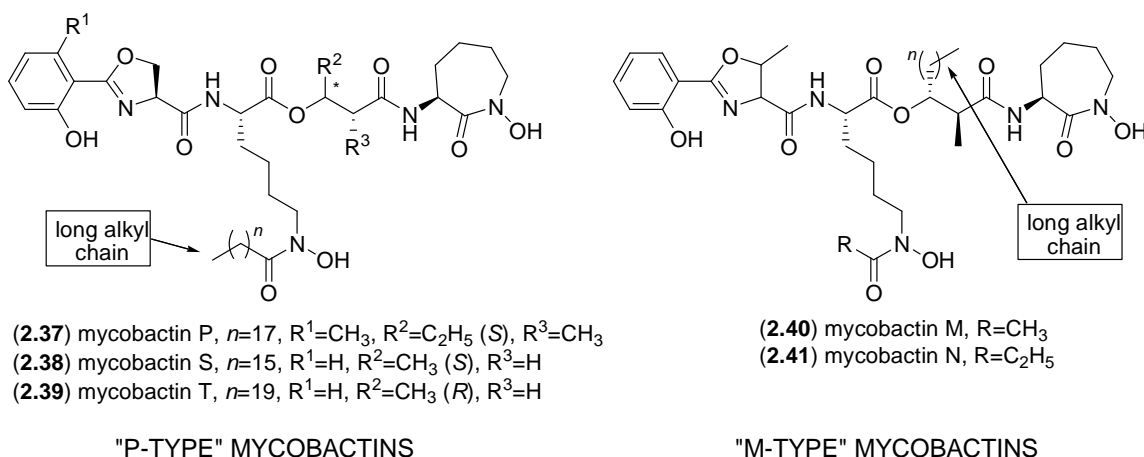


Figure 2.8. Structures of water-insoluble mycobacterial siderophore

Mycobactins are produced by nonribosomal peptide synthesis, and in 1998 the 10-gene Mbt gene cluster was identified as being responsible for mycobactin synthesis in *M. tuberculosis*.²³⁰ Proteins encoded that are involved in mycobactin biosynthesis include polyketide synthetases, peptide synthetases, isochorismate synthase (for the production of salicylic acid), and lysine-*N*-oxygenase. The importance of mycobactins to the growth of mycobacteria has been demonstrated in a recent study of a mutant of *M. tuberculosis* lacking the MbtB gene, involved in one of the first steps in mycobactin synthesis. The mutant demonstrated a considerably decreased ability to grow in human macrophages.²⁷⁹

In addition to the water-insoluble/lipid-soluble mycobactins, mycobacteria also produce a variety of water-soluble extracellular siderophores (Figure 2.9). In older literature, all extracellular mycobacterial siderophores were termed "exochelins"; however, it became apparent that there exist two distinct classes of extracellular mycobacterial siderophores that differ greatly in regard to chemical structure.

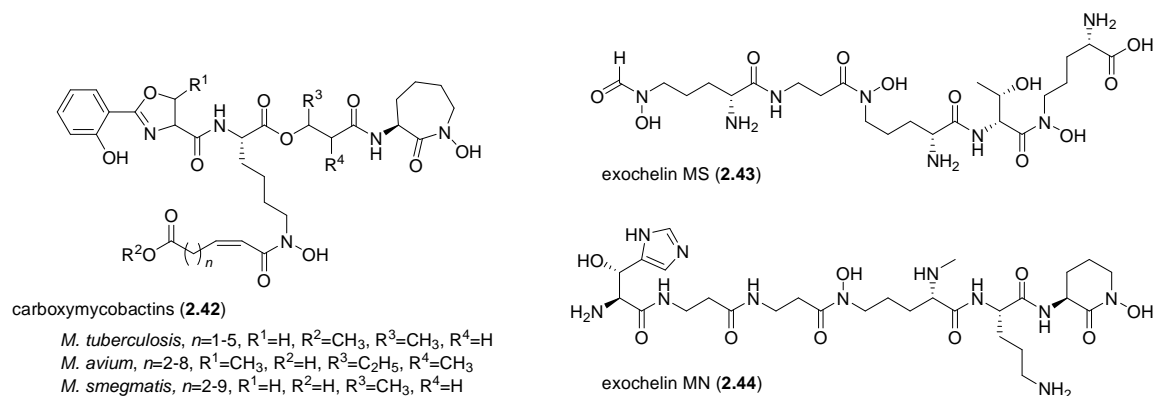


Figure 2.9. Structures of water-soluble mycobacterial siderophores

The chloroform- and water-soluble siderophores, carboxymycobactins **2.42**, have been isolated primarily from pathogenic mycobacteria²⁸⁰⁻²⁸² and were found to contain the same basic structure as the P-type mycobactins. Carboxymycobactins **2.42** differ from the P-type mycobactins **2.37-2.39** by the fact that the long alkyl chain on the central lysine-derived hydroxamate terminate in a carboxylic acid or a methyl ester (the methyl ester may be an artifact of isolation).^{281, 282} These molecules were termed “carboxymycobactins” to reflect their structural similarities to the mycobactins, although the name “exochelin” is still used occasionally in the literature.

The nonpathogenic (saprophytic) mycobacteria, *M. smegmatis* and *M. neoaurum*, produce water-soluble peptide-based siderophores **2.43** and **2.44**, respectively.²⁸³⁻²⁸⁷ In addition to possessing a completely different core structure, the exochelins also demonstrate different mechanisms of iron transport. The role of mycobacterial siderophores in iron transport will be described in the next section.

2.3.4 Use of siderophores in mycobacterial iron transport

As described in section 2.2.1 above, *M. tuberculosis*, like all mycobacteria, contain an unusually thick cell wall that is responsible for most of the difficulties associated with drug treatments and for host defense immunities; however, the thick cell wall also complicates the acquisition of nutrients by the mycobacteria from extracellular sources. Consequently, mycobacteria have developed a complex iron acquisition system that utilizes both the extracellular carboxymycobactins and exochelins as well as intracellular mycobactins (Figure 2.10).

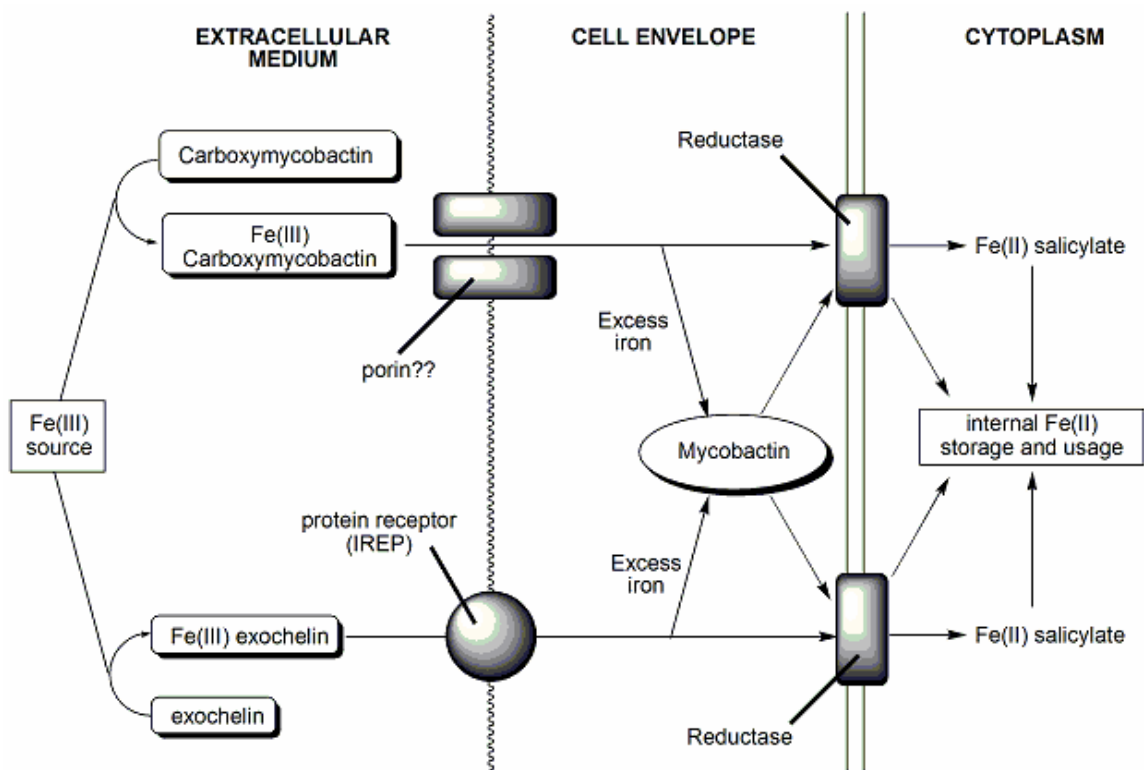


Figure 2.10. Iron acquisition in mycobacteria

Both carboxymycobactins and exochelins have demonstrated the ability to acquire Fe(III) from extracellular transferrin and intracellular macrophage iron pools,²⁸⁸⁻²⁹⁰

although the acquisition of iron from intracellular iron sources was much slower (days versus hours).²⁸⁹

The uptake of Fe(III)-carboxymycobactins and Fe(III)-exochelins operate through different mechanisms in mycobacteria. Uptake of Fe(III)-carboxymycobactins is thought to occur through an energy-independent process such as porin-mediated diffusion, while uptake of Fe(III)-exochelins has been shown to require ATP and is thought to involve IREPs, iron-regulated envelope proteins.^{248, 291} Release of Fe(III) from mycobacterial siderophores occurs through the NADPH-dependent ferric mycobactin reductase and is incorporated into iron storage proteins in mycobacteria such as bacterioferritin.²⁴⁸

The role of mycobactins in mycobacterial iron transport is still not clear, even though it has been demonstrated that mycobactins facilitate and are essential for the growth of mycobacteria.²⁷⁸ Mycobactins are found within the cell envelope of mycobacteria and are located next to, but not embedded in, the cytoplasmic membrane.²⁹² Ratledge has proposed mycobactins can serve as a temporary storage of Fe(III) in the mycobacterial cell envelope that allows for better control of the mycobacteria over cellular iron concentration when extracellular concentrations of iron change.²⁴⁸ Although mycobactins and carboxymycobactins bind iron very strongly (binding constant on the order of 10^{30}),²⁴⁸ Crumbliss has demonstrated that iron exchange between siderophores may be an operative, but often overlooked, aspect of microbial iron transport.^{251, 284, 293}

2.4 Biological activity of mycobactins and analogs

Mycobacterial siderophores play a vital role in mycobacterial iron acquisition; however, their biological activity has demonstrated their potential as anti-TB agents.

Somewhat puzzling is the activity of the closely related analogous siderophores from *Nocardia* and other actinomycetes, which demonstrate potent cytotoxicity against a number of tumor cell lines. The biological activity of natural and synthetic mycobactins, carboxymycobactins, and other actinomycete siderophores will be covered in the following section.

2.4.1 Natural mycobactins and synthetic analogs

Since the first isolation of mycobactins by Snow in the 1950s, mycobactins produced by one species have demonstrated the ability to affect the growth of another species of mycobacteria. Snow first observed that while many mycobactins promoted the growth of *M. paratuberculosis*, mycobactins M (**2.41**) and N (**2.42**) showed an unusual growth-depressing effect on *M. paratuberculosis*, *M. kansasii*, and *M. tuberculosis* at high concentrations (Figure 2.11).²⁷⁸ Later studies demonstrated that mycobactin A, the siderophore naturally produced by *M. aurum*, promoted the growth of *M. aurum* at concentrations up to 5 μ M, while heterologous siderophores mycobactins J and S had an inhibitory effect on the growth of *M. aurum* at concentrations higher than 5 μ M.²⁹⁴

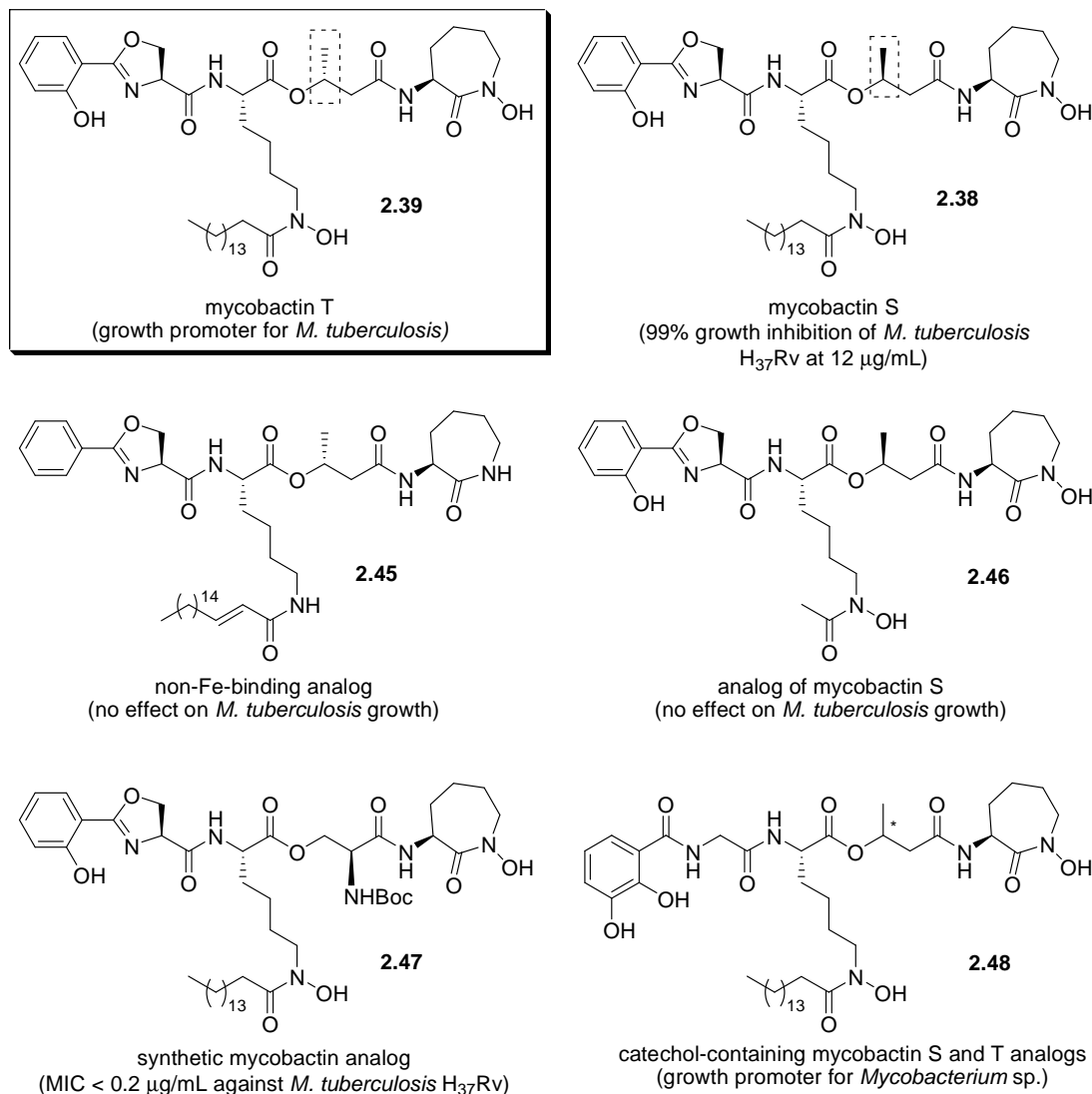


Figure 2.11. Natural and synthetic mycobactins and mycobactin analogs

The ability of synthetic mycobactins to inhibit the growth of *M. tuberculosis* was also explored. The first synthetic mycobactin analog synthesized, compound **2.45**, lacked Fe-binding groups and had no effect on the growth of *M. tuberculosis*.²⁹⁵ The same result was observed for the mycobactin S analog **2.46**.²⁹⁶ Astoundingly, synthetic mycobactin S (**2.38**), naturally produced by *M. smegmatis* and differing from the *M. tuberculosis*-produced mycobactin T (**2.39**) by only one stereocenter, demonstrated 99% growth

inhibition of *M. tuberculosis* H₃₇Rv at 12 µg/mL!²⁹⁷ Another synthetic mycobactin analog, compound **2.47**, showed significant anti-TB activity as well and was found to inhibit the growth of *M. tuberculosis* H₃₇Rv with an MIC of < 2 µg/mL.²⁹⁸ The replacement of the 2-hydroxyphenyl-oxazoline ligand with a catechol ligand provided mycobactin S and T analogs **2.48**, which promoted the growth of a variety of mycobacterial strains.²⁹⁹

2.4.2 Carboxymycobactins and analogs

Since Snow isolated and reported activity on the ability of mycobactins to affect the growth of mycobacteria before the isolation of carboxymycobactins,²⁷⁸ very little information is available on antimycobacterial properties of carboxymycobactins or analogs. One study, however, reported the solid-phase synthesis of carboxymycobactin T7 and analogs (**2.49**), which only displayed moderate growth inhibition of *M. avium* at high concentrations that diminished after 2 weeks (Figure 2.12).³⁰⁰ Horwitz has reported a number of studies on “exochelin 772SM” (**2.50**), a carboxymycobactin from *M. tuberculosis*, which was found to possess interesting biological activity. Carboxymycobactin **2.50** was found to reversibly inhibit the growth of human vascular smooth muscle cells (VSMC) *in vitro* with no apparent cytotoxicity, suggesting a possible use as a therapeutic agent to limit restenosis following angioplasty.³⁰¹ Additionally, carboxymycobactin **2.50** demonstrated the ability to kill T47D-YB and MCF-7 human breast cancer cells by inducing apoptosis, but only reversibly arrested the growth of normal human mammary epithelial cells with no apparent cytotoxicity.³⁰² Both of these studies found that another water-soluble chelator, DFO (**2.26**) required a

10-fold excess to have a similar effect and demonstrated cytotoxicity at those levels.³⁰¹,
³⁰² Subsequent studies indicate that the mode of action of carboxymycobactin **2.50** in human breast cancer cell lines may be due to inhibition of the iron-containing enzyme ribonucleotide reductase.³⁰³

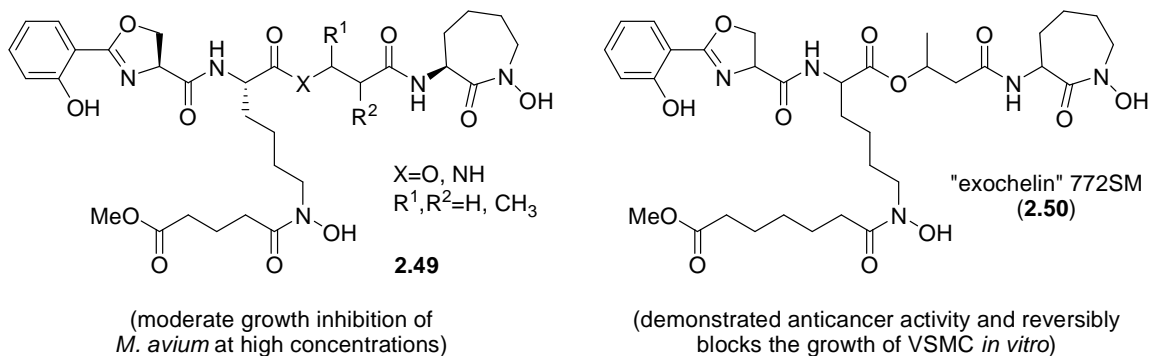


Figure 2.12. Reported biological activity of synthetic carboxymycobactins

2.4.3 Siderophores from other actinomycetes

The biological activity exhibited by carboxymycobactin **2.50** is unusual when compared to the biological activities observed for mycobactins and synthetic analogs (section 2.4.1); however, similar anticancer activity has been reported for the siderophores isolated from other actinomycete species (Figure 2.13).

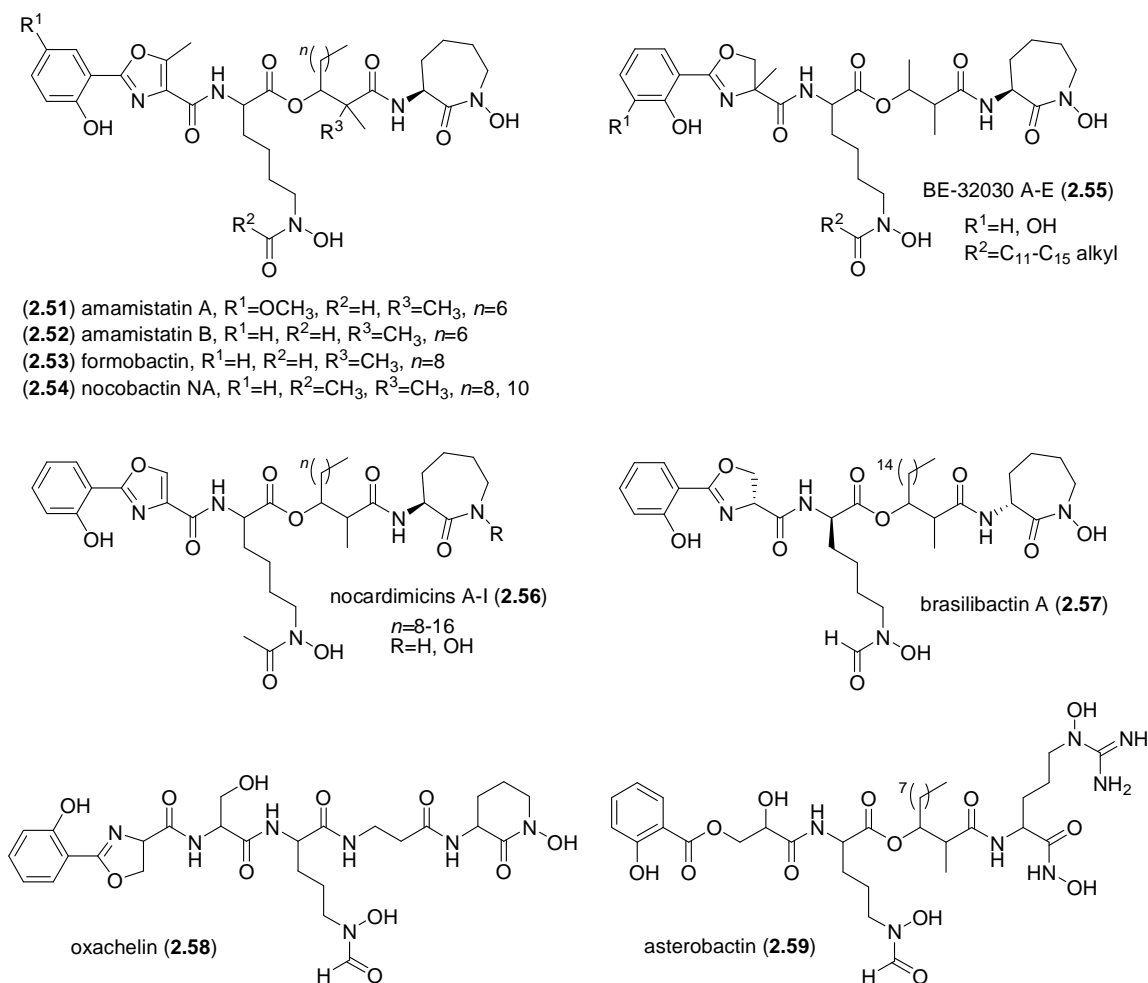


Figure 2.13. Biologically active siderophores from other actinomycetes

Amamistatins A (**2.51**)³⁰⁴ and B (**2.52**),^{305, 306} isolated from *Nocardia asteroides*, demonstrated cytotoxicity to P388 mouse lymphocytic leukemia cells (IC_{50} 15 and 16 nM, respectively), and amamistatin A (**2.51**) was found to have anti-proliferative effects against MCF-7 breast, A549 lung, and MKN45 stomach cancer cell lines (IC_{50} 0.48, 0.56, 0.24 μM , respectively).^{307, 308} The structurally related formobactin (**2.53**)³⁰⁹ and nocobactin NA (**2.54**)³¹⁰ were also isolated from *Nocardia asteroides*, but were not tested for anticancer activity. Asterobactin (**2.59**), another siderophore isolated from *Nocardia*

asteroides, does not possess the same structural core of *Nocardia* siderophores, but displayed potent anticancer activity across a variety of cell lines.³¹¹

BE-32030 compounds A-E (**2.55**), isolated from *Nocardia* sp. A32030, demonstrated growth inhibition against P388 mouse leukemia as well as three human tumor cell lines.³¹² The nocardimicin series A-I (**2.56**) was isolated from another strain of *Nocardia*, and exhibited inhibition of the muscarinic M3 receptor.^{313, 314} Brasilibactin A (**2.57**),³¹⁵ isolated from *N. brasiliensis*, exhibited cytotoxicity against mouse leukemia and human epidermoid carcinoma KB cells (IC₅₀ 0.02 and 0.04 µg/mL, respectively).³¹⁶ Oxachelin (**2.58**), isolated from *Streptomyces* sp. GW9/1258, exhibited antifungal activity, but did not display activity against tumor cell lines.³¹⁷

The siderophores isolated from *Nocardia* display structural characteristics that are unmistakably similar to the M-type mycobactins, mycobactins M (**2.40**) and N (**2.41**). The total synthesis of amamistatin B (**2.52**) and analogs as well as their biological activity was recently reported; however, amamistatin B (**2.52**) and analogs only demonstrated moderate growth inhibition against *M. tuberculosis* at high concentrations.³⁰⁶ Unpublished work by the Miller group has also demonstrated that the anticancer activity of mycobactin analog **2.47** was negligible.

2.5 Mycobactin analogs from acylnitroso cycloadducts

Based on the interesting and seemingly conflicting biological activity observed for mycobactins and carboxymycobactin analogs and the related siderophores from *Nocardia*, the Miller group has been interested in probing this class of molecules through the synthesis and biological testing of novel mycobactin analogs. The following section

will describe the design of new mycobactin analogs through the use of an acylnitroso HDA synthetic strategy. The full synthetic details of this project will be described in chapters 3 and 4.

2.5.1 Design of target mycobactin analogs

In an effort to better understand the structure-activity relationships (SAR) surrounding the mycobactin core structure, the Miller group has reported the synthesis of a number of mycobactin analogs^{257, 296-299} and has recently reported the synthesis of the related siderophore, amamistatin B (**2.52**) and analogs.^{305, 306} Through the isolation of naturally occurring mycobactins and using synthetic methods from the Miller group and a number of other synthetic research groups, we are now able to access structural variations along a considerable portion of the mycobactin core structure (Figure 2.14).

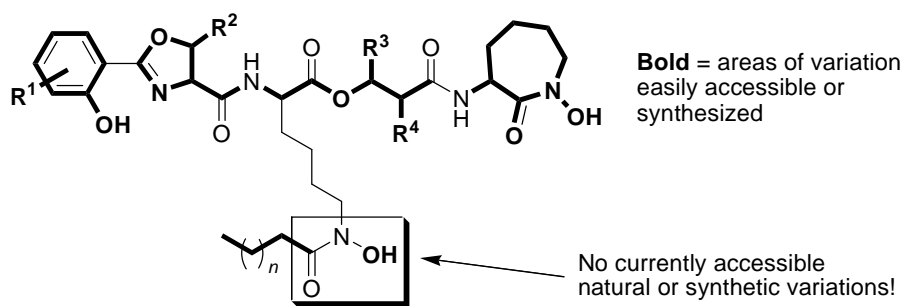


Figure 2.14. Accessible mycobactin analogs

An area of the mycobactin structure that has been only partially analyzed is the modification of the central iron-binding hydroxamate ligand. While non-iron binding amide analogs of the central iron-binding hydroxamate are readily accessible (analog

2.45),²⁹⁵ synthetic strategies that investigate replacement of the central iron-binding group with alternative binding functionalities have not yet been explored.

We have reasoned that using an acylnitroso HDA synthetic strategy, we would be able to access mycobactin analogs that replace the central iron-binding ligand with either an α -hydroxy carboxylate ligand (analogs **2.60a** and **2.60b**), or a weaker metal-binding group, a 1,2-diol functionality (analogs **2.61a** and **2.61b**) (Figure 2.15).

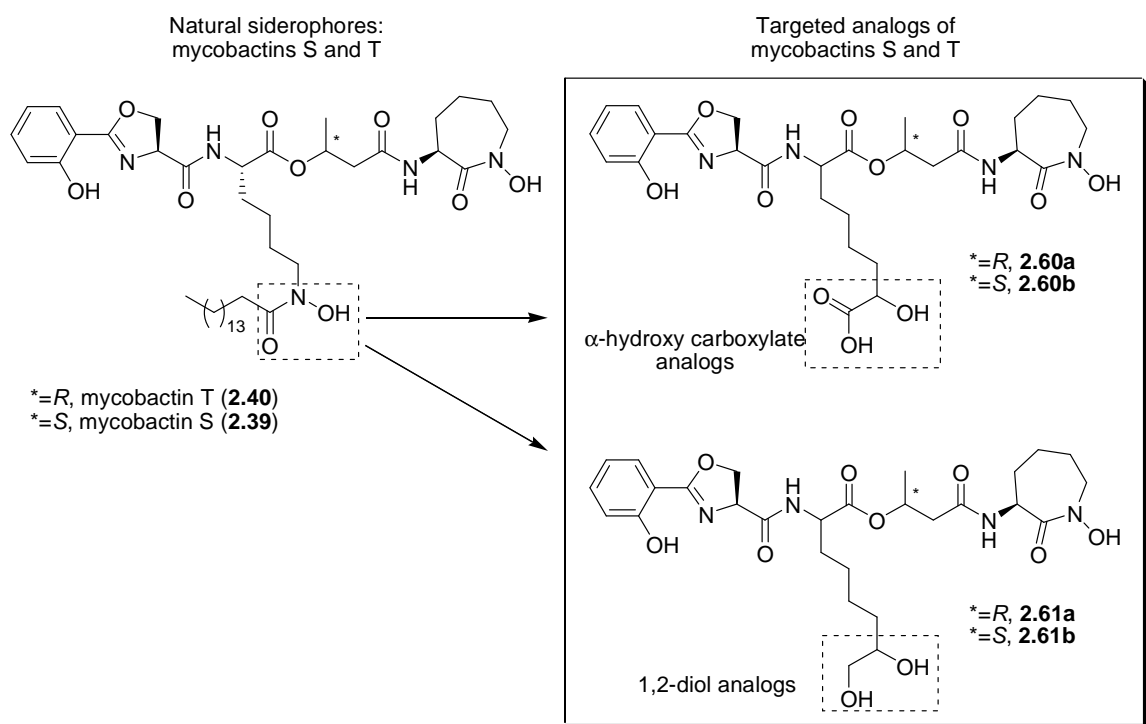
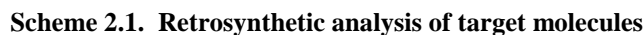


Figure 2.15. Synthetic mycobactin analog targets

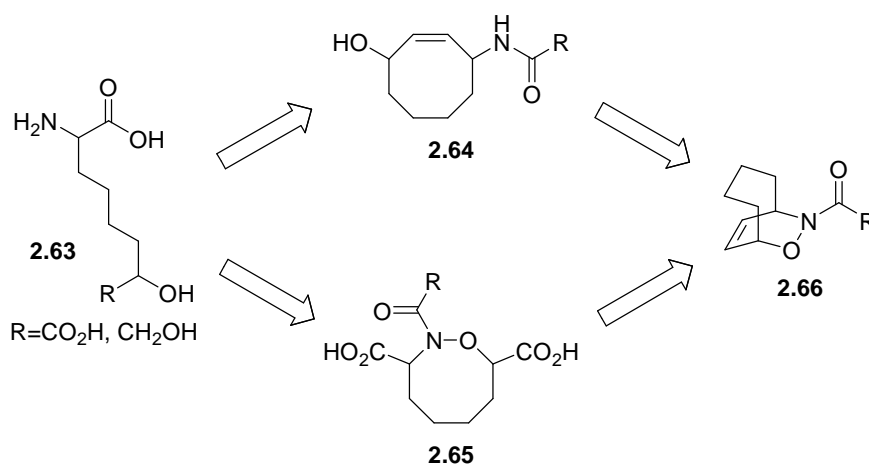
α -Hydroxy carboxylate groups are ubiquitous iron-binding groups that are found in numerous other siderophores (see section 2.3.2), and the 1,2-diol moiety, although not found in many naturally occurring siderophores, is also a weak iron-binding group. Additionally, the four analogs **2.60a**, **2.60b**, **2.61a** and **2.61b**, would be expected to have

The four targeted analogs **2.60a**, **2.60b**, **2.61a**, and **2.61b** were of interest due to the differences in activity exhibited by the naturally occurring mycobactins T (**2.39**) and S (**2.38**), where the reversal of the configuration of one stereocenter from *R* to *S* resulted in a complete reversal of biological activity against *M. tuberculosis* (section 2.4.1).

The retrosynthetic strategy toward the synthesis of the target mycobactin analogs **2.60** and **2.61** was similar to the synthesis of other mycobactin compounds carried out by the Miller group (Scheme 2.1). Disconnection of the central ester and amide bonds of the target compounds **2.60** and **2.61** provided three fragments A (**2.62**), B (**2.63**) and C (**2.64**).



Fragment A (**2.62**) was accessible ultimately from *L*-serine benzyl ester and methyl salicylate. Fragment C (**2.64**) was accessible from the commercially available *R*- or *S*-3-hydroxybutyrate and Cbz-protected *L*-lysine. Retrosynthetic analysis of fragment B (**2.63**) lead us to the acylnitroso HDA cycloadduct **2.66** derived from 1,3-cyclooctadiene (Scheme 2.2).



Scheme 2.2. Retrosynthetic analysis of fragment B

There were two distinct pathways to fragment B (**2.63**) from cycloadduct **2.66**. One pathway resulted from N-O bond reduction to the amino alcohol intermediate **2.64**, wherein the final synthetic step involved cleavage of the olefin, and the other pathway resulted from oxidative cleavage of the olefin of cycloadduct **2.66**, wherein the final synthetic step involved N-O bond reduction. Our synthetic plan was to explore both possible pathways in an attempt to arrive at the most efficient route to fragment B (**2.63**).

In the synthetic direction, fragments A (**2.62**), B (**2.63**), and C (**2.64**) were synthesized separately followed by fragment assembly to arrive at the targeted analogs **2.60a**, **2.60b**, **2.61a**, and **2.61b**.

2.6 Summary of project goals

Overall, the goal of this project was to provide the target molecules in order to better understand the SAR surrounding the mycobactin core structure as it pertains to anti-TB and anticancer activity. The specific aims of this project were as follows:

- Provide an efficient route to synthetic fragments A, B, and C as well as develop synthetic methods for fragment assembly
- Synthesize the α -hydroxy carboxylate analogs of mycobactins S and T, compounds **2.60a** and **2.60b**
- Synthesize the 1,2-diol analogs of mycobactins S and T, compounds **2.61a** and **2.61b**
- Assay all intermediates and target compounds for biological activity against *M. tuberculosis*, human prostate (PC-3) and breast (MCF-7) cell lines

The following two chapters will detail the synthetic efforts toward these specific aims and will also describe the biological activity of the intermediates and target compounds. Chapter 3 will detail the chemistry surrounding the synthesis of fragments A, B, and C. Chapter 4 will detail the chemistry surrounding the assembly of fragments A, B, and C and efforts toward the synthesis of target compounds. Biological activity of compounds and intermediates will also be described in chapter 4.

CHAPTER 3:
SYNTHESIS AND STUDY OF MYCOBACTIN ANALOG FRAGMENTS

3.1 Introductory remarks

The following chapter will detail the synthesis of fragments A (**2.62**), B (**2.63**), and C (**2.64**) (Figure 3.1). Section 3.2 will also describe the synthesis of fragment A analogs based on interesting an unexpected biological activity of an intermediate in the synthesis of fragment A (**2.62**). All biological data for fragments and analogs will be detailed at the end of chapter 4. The synthesis of fragment C (**2.64**) is straightforward and will be covered in this chapter in section 3.6.

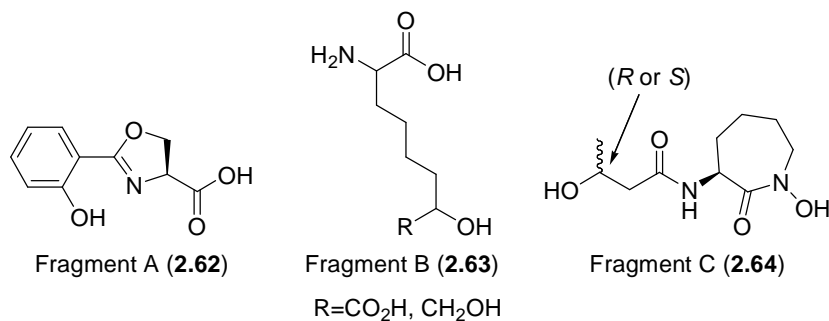
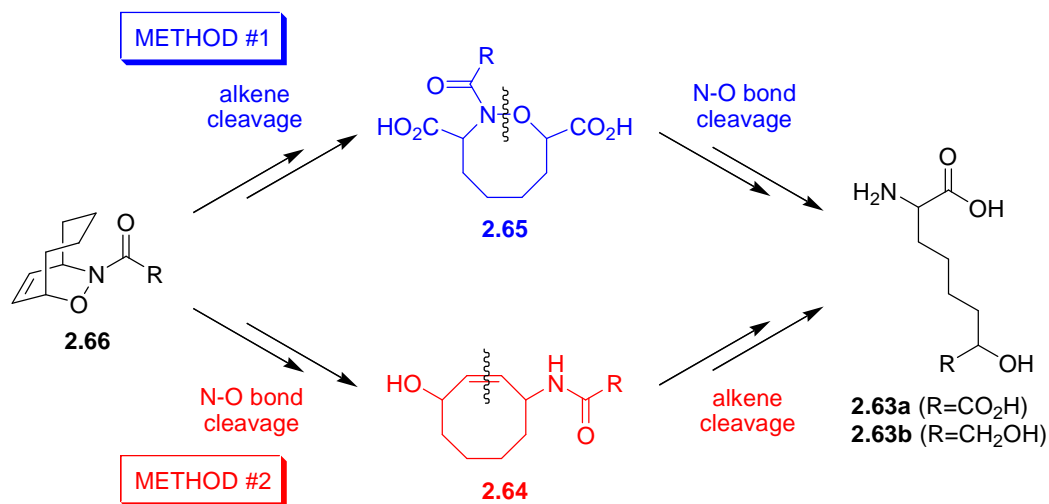


Figure 3.1. Fragments A, B, and C

Two synthetic routes to fragment B (**2.63a** and **2.63b**) have been explored (Scheme 3.1). Method #1 involved alkene cleavage of cycloadduct **2.66** as the initial important transformation followed by N-O cleavage of the intermediate **2.65**. Method #2

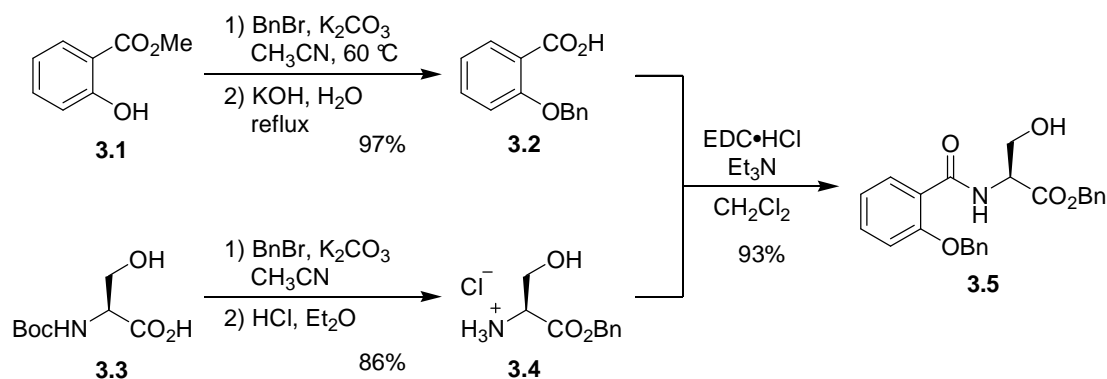
involved N-O bond reduction of cycloadduct **2.66** as the initial important transformation followed by alkene cleavage of the intermediate 1,4-amino alcohol **2.64**. Each route has been detailed separately in sections 3.4 and 3.5, respectively.



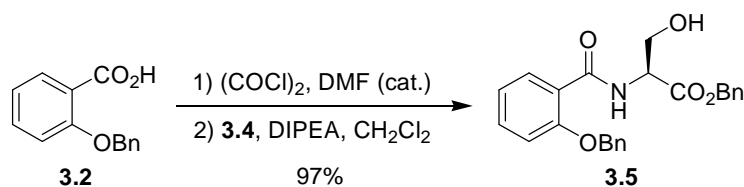
Scheme 3.1. Two methods used to synthesize fragment B

3.2 Synthesis of fragment A

Fragment A (**2.62**) was derived from methyl salicylate (**3.1**) and protected serine **3.3** (Scheme 3.2). The most efficient route to fragment A and analogs followed a strategy that was similar to those reported for the synthesis of other salicylate-based siderophores.^{296, 297, 304, 305} Methyl salicylate (**3.1**) was converted to the protected salicylic acid **3.2** in two steps in high overall yield. Amine **3.4** was prepared from Boc-protected serine **3.3** in two steps in equally high yield. Coupling of serine **3.4** to salicylate **3.2** was effected using a water soluble carbodiimide to cleanly yield amide **3.5** in excellent yield. Alternatively, amide **3.5** was synthesized by first converting salicylate **3.2** to an acid chloride using oxalyl chloride and catalytic DMF followed by *N*-acylation (Scheme 3.3).



Scheme 3.2. Synthesis of amide from methyl salicylate and *L*-serine

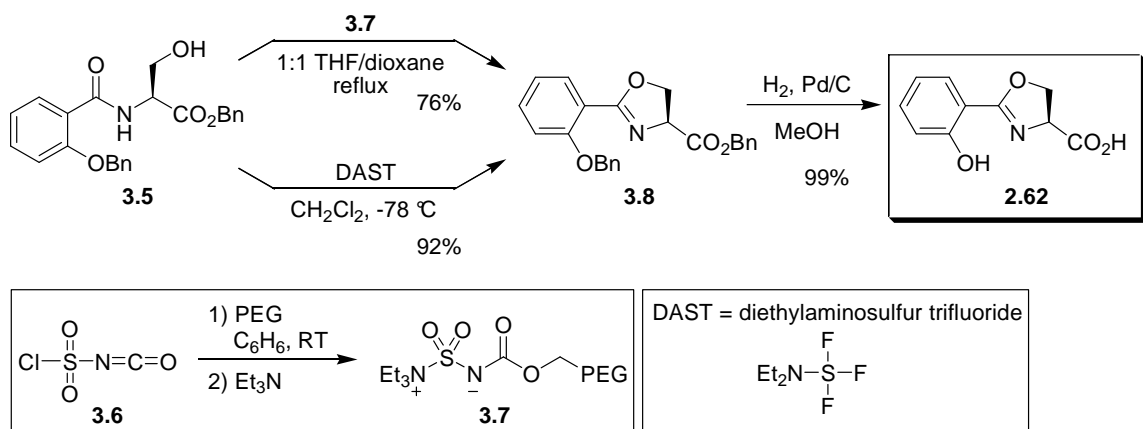


Scheme 3.3. Alternative amide formation

The cyclization of compounds similar to amide **3.5** to oxazolines has been reported in the literature using a variety of reagents such as thionyl chloride,²⁹⁶ and Burgess's reagent.³¹⁸ We found that cyclization of amide **3.5** to oxazoline **3.8** was easily performed using PEG-supported Burgess's reagent **3.7** (Scheme 3.4).³¹⁹ Although yields of oxazoline **3.8** were satisfactory, the conditions appeared to be unnecessarily harsh and resulted in mixtures that required careful purification. Additionally, although PEG-supported Burgess's reagent **3.7** is prepared in one step from chlorosulfonyl isocyanate (**3.6**), this reagent decomposes readily and is difficult to handle.

A milder, higher-yielding alternative to PEG-supported Burgess's reagent was the use of diethylaminosulfur trifluoride (DAST) as reported by the Wipf group.³²⁰ The use of DAST allowed oxazoline **3.8** to be obtained in high yield and was sufficiently clean

enough to allow the purification of oxazoline **3.8** by recrystallization. Hydrogenolysis of the benzyl ether and ester afforded fragment A (**2.62**).



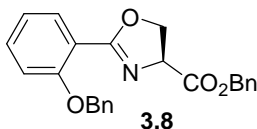
Scheme 3.4. Completion of fragment A synthesis

Through the use of this synthetic route, fragment A (**2.62**) was obtained in 5 steps from methyl salicylate in 70% overall yield. More importantly, all intermediates could be purified through recrystallization alone and did not require chromatographic purification.

3.3 Fragment A analogs as small-molecule anti-TB compounds

The Miller group has previously found oxazoline **3.8**, a synthetic intermediate toward the synthesis of mycobactin S,²⁹⁷ to have growth inhibitory activity against *M. tuberculosis*.^{321, 322} Subsequently, oxazoline **3.8** was synthesized and tested for biological activity against various mycobacterial species, and to our surprise, appeared to be selective for *M. tuberculosis* (Table 3.1).

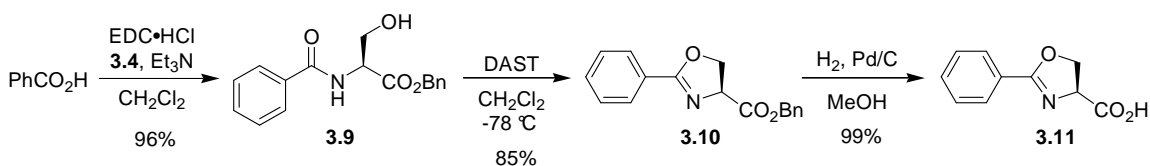
TABLE 3.1

ANTI-TB ACTIVITY OF INTERMEDIATE **3.8**

| <i>Mycobacterium</i> sp. | MIC (μM) |
|---|----------|
| <i>M. tuberculosis</i> H ₃₇ Rv | 7-12 |
| <i>M. smegmatis</i> | >250 |
| <i>M. vaccae</i> | >250 |
| <i>M. fortuitum</i> | >250 |
| <i>M. avium</i> | >32 |

3.3.1 Fragment A analogs: modifying stereochemistry and substitution

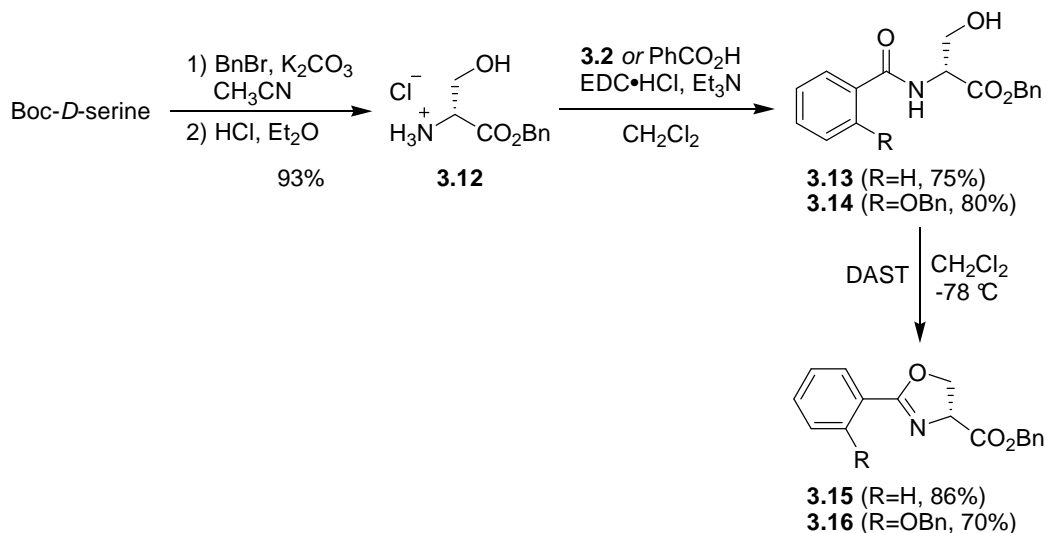
An analog of fragment A, compound **3.11**, was synthesized from benzoic acid as a non-Fe-binding analog following a similar route to the one outlined above (Scheme 3.5). Amide **3.9** was prepared through carbodiimide-mediated coupling with serine ester **3.4**, and dehydrative cyclization to oxazoline **3.10** proceeded through the use of DAST. Hydrogenolysis afforded the analog **3.11**.



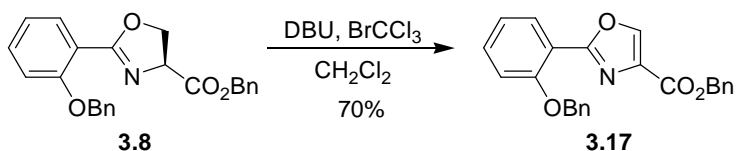
Scheme 3.5. Non-chelating fragment A analog

Through the use *D*-serine instead of *L*-serine, fragment A analogs **3.15** and **3.16** were synthesized (Scheme 3.6). Starting from Boc-protected *D*-serine, serine benzyl ester **3.12** was synthesized in excellent yield. Amide coupling with either benzoic acid or

salicylate **3.2** afforded the amides **3.13** or **3.14**, respectively in high yield. Cyclization with DAST followed yielded fragment A analogs **3.15** and **3.16**. Additionally, oxazoline **3.8** was oxidized using DBU and bromotrichloromethane³⁰⁵ to the oxazole compound **3.17** (Scheme 3.7).



Scheme 3.6. Fragment A from *D*-serine



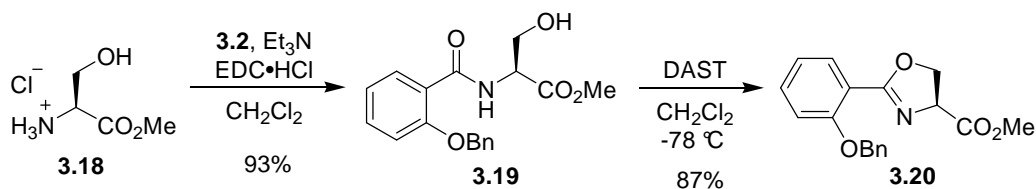
Scheme 3.7. Synthesis of an oxazole

3.3.2 Fragment A-based hydroxamates and hydroxamic acids

A number of hydroxamate and hydroxamic acid analogs of fragment A were synthesized with the dual purpose of expanding the structure-activity relationship (SAR) around the biologically active oxazoline intermediate **3.8** as well as providing substrates

for use in acylnitroso HDA reactions. The synthesis of hydroxamates and hydroxamic acids will be described here, and HDA reactions will be described in chapter 4.

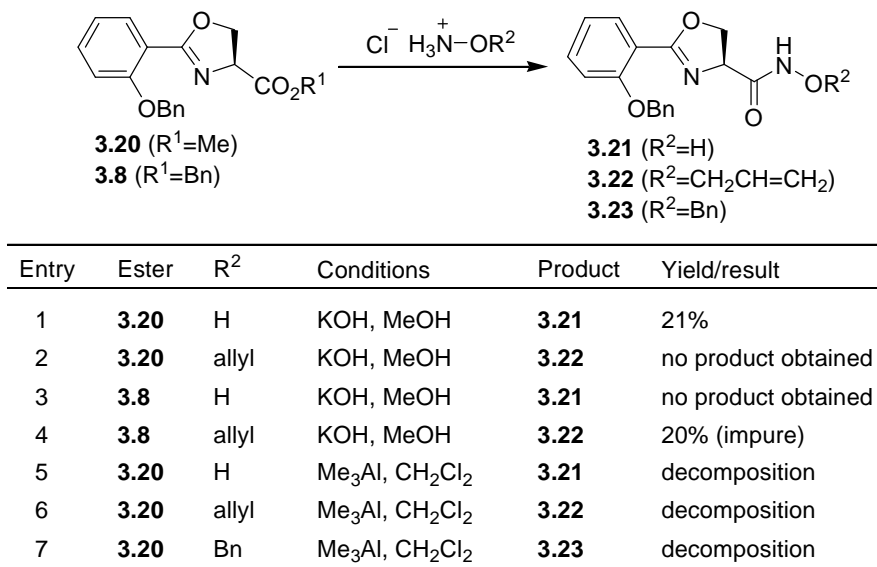
We hypothesized that methyl esters would provide a useful starting material for hydroxamate synthesis, and methyl ester **3.20** was prepared from serine methyl ester **3.18** in two steps in excellent yield (Scheme 3.8).



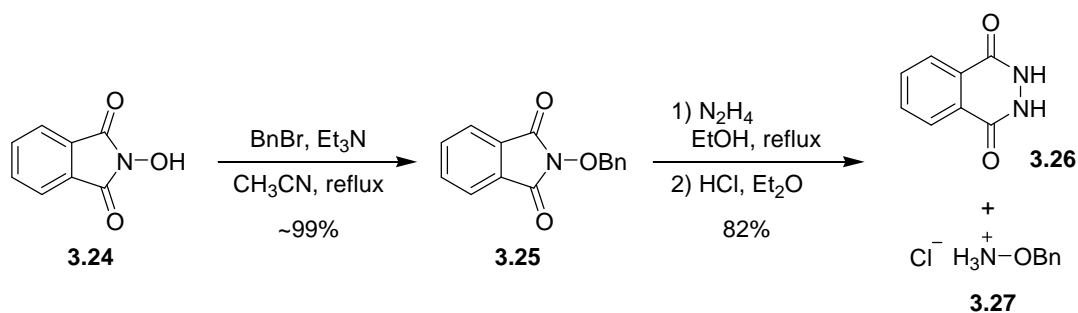
Scheme 3.8. Synthesis of methyl ester compound

The synthesis of hydroxamates **3.21-3.23** from esters **3.20** and **3.8** was studied using two methods (Table 3.2). Following published procedures,^{323, 324} *O*-benzylhydroxylamine hydrochloride (OBHA•HCl, **3.27**) was prepared from *N*-hydroxyphthalimide (**3.24**) in two steps (Scheme 3.9). *O*-allylhydroxylamine hydrochloride as well as hydroxylamine hydrochloride and OBHA•HCl (**3.27**) were used for the synthesis of hydroxamates **3.21-3.23** (Table 3.2).

TABLE 3.2
SYNTHESIS OF HYDROXAMATES FROM ESTERS



Weinreb³²⁵ and others^{326, 327} reported that hydroxamates can be obtained from methyl esters using trimethylaluminum-complexes of hydroxylamines; however, when these conditions were attempted on ester **3.20**, only decomposition and complex mixtures were observed (Table 3.2, entries 5-7). Although low-yielding, the use of methanolic potassium hydroxide allowed for the isolation of reasonably pure hydroxamic acid **3.21** and impure *O*-allyl hydroxamate **3.22** (Table 3.2, entries 1-4). The reason why no product was obtained in entries 2 and 3 (Table 3.2) was not clear.



Scheme 3.9. Synthesis of *O*-benzylhydroxylamine hydrochloride

The use of carboxylic acids **2.62** and **3.11** rather than esters **3.20** and **3.8** for the synthesis of hydroxamates was more successful (Table 3.3). While the use of *N*-hydroxysuccinimide (NHS) and dicyclohexylcarbodiimide (DCC) produced a complex mixture, using water-soluble carbodiimide as an activating agent for acid **2.62** yielded the desired *O*-benzyl hydroxamate **3.28** (Table 3.3, entries 1 and 2). Higher yields were obtained when the reaction was performed in aqueous conditions with careful control of the apparent pH of the mixture rather than when the reaction was performed in an organic solvent (Table 3.3, entries 2 and 3).³²⁸ The use of carboxylic acid **3.11**, derived from benzoic acid, yielded the hydroxamate **3.29** in somewhat lower yield (Table 3.3, entry 3), which was attributed to the low solubility of carboxylic acid **3.11** in the aqueous solvent mixture.

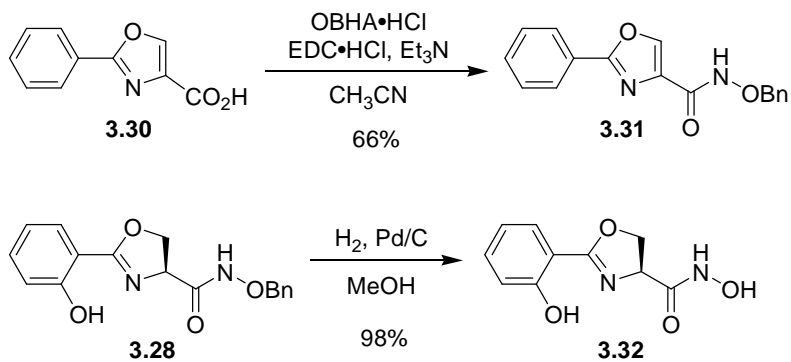
Other hydroxamates and hydroxamic acids were prepared using similar methods. Since the oxazole **3.30** was not soluble at all in THF/H₂O mixtures, acetonitrile was used as the solvent when treated with EDC•HCl and OBHA•HCl and provided hydroxamate **3.31** in moderate yield (Scheme 3.10). Hydrogenolysis of the benzyl hydroxamate **3.28** cleanly provided the hydroxamic acid **3.32** in excellent yield.

TABLE 3.3

SYNTHESIS OF HYDROXAMATES FROM CARBOXYLIC ACIDS

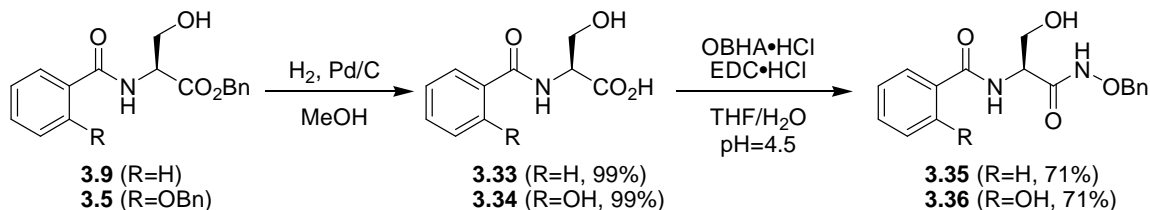
2.62 (R=OH)
 3.11 (R=H)
 $\xrightarrow{\text{OBHA}\cdot\text{HCl}}$
 3.28 (R=OH)
 3.29 (R=H)

| Entry | Acid | Conditions | Product | Yield/result |
|-------|-------------|--|-------------|-----------------|
| 1 | 2.62 | i. NHS, DCC; ii. NaHCO ₃ | 3.28 | complex mixture |
| 2 | 2.62 | EDC·HCl, Et ₃ N, CH ₃ CN | 3.28 | 48% |
| 3 | 2.62 | EDC·HCl, pH=4.5, THF/H ₂ O | 3.28 | 83% |
| 4 | 3.11 | EDC·HCl, pH=4.5, THF/H ₂ O | 3.29 | 39% |



Scheme 3.10. Synthesis of other hydroxamates and hydroxamic acids

Hydroxamates of un-cyclized analogs of fragment A were also prepared. Hydrogenolysis of compounds **3.9** and **3.5** afforded the carboxylic acids **3.33** and **3.34** (Scheme 3.11). Aqueous coupling with OBHA•HCl mediated by EDC•HCl provided the hydroxamates **3.35** and **3.36**.

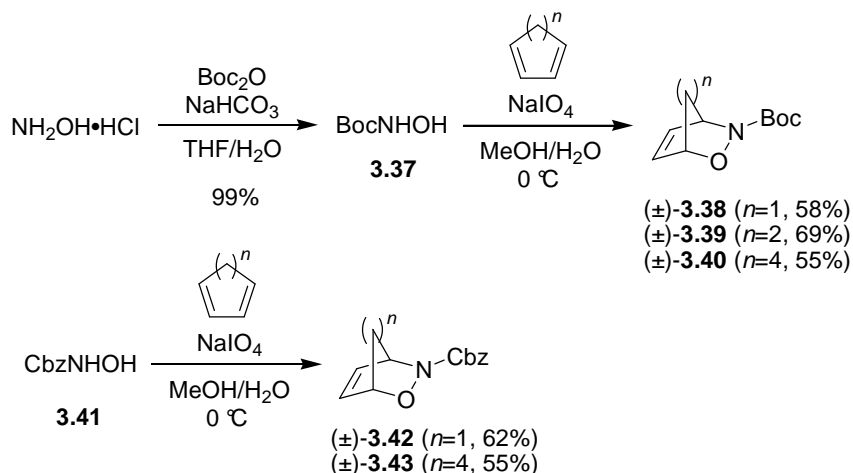


Scheme 3.11. Synthesis of hydroxamates of hydroxyl amide intermediates

3.4 Synthesis of fragment B using method #1

3.4.1 Nitroso-hetero Diels-Alder reactions

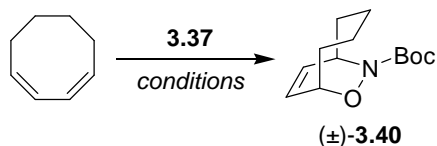
The synthesis of fragment B compounds **2.63a** and **2.63b** began with a nitroso HDA reaction. A variety of cycloadducts **3.38-3.43** were prepared in order to investigate conditions for subsequent alkene- and N-O bond-cleavage reactions (Scheme 3.12). BocNHOH (**3.37**) was prepared from hydroxylamine hydrochloride in one step in excellent yield and was treated with periodate in the presence of cyclopentadiene, 1,3-cyclohexadiene, and 1,3-cyclooctadiene to yield cycloadducts **3.38**, **3.39**, and **3.40**, respectively. Hydroxamate **3.41** was similarly treated with periodate in the presence of cyclopentadiene and 1,3-cyclooctadiene to yield cycloadducts **3.42** and **3.43**.



Scheme 3.12. Preparation of cycloadducts

A variety of conditions were studied for the cycloaddition reactions with *cis,cis*-1,3-cyclooctadiene in an attempt to increase the yield of cycloadduct **3.40**, the direct precursor to fragment B compounds **2.63** (Table 3.4). Typically the reaction was performed by adding an aqueous solution of sodium periodate to a mixture of the diene and *N*-hydroxycarbamate **3.37** in methanol and water (Table 3.4, entry 1); however, a considerable decrease in the yield of cycloadduct **3.40** was observed when a solution of *N*-hydroxycarbamate **3.37** was added to a mixture of sodium periodate and the diene in MeOH/H₂O (“reverse addition”, Table 3.4, entries 1-2). Interestingly, when this same order of addition was performed using Bu₄NIO₄ in chloroform instead of NaIO₄ in methanol/water, a lower yield of cycloadduct **3.40** was not observed (Table 3.4, entry 4).

TABLE 3.4
OPTIMIZATION OF HETERO-DIELS-ALDER REACTIONS WITH 1,3-
CYCLOOCTADIENE



| Entry | Oxidant | Conditions | Yield |
|----------------|---|---|-------------------|
| 1 | NaIO ₄ | MeOH/H ₂ O, 0 °C-rt | 55% |
| 2 | NaIO ₄ | MeOH/H ₂ O, 0 °C-rt ^a | 29% |
| 3 | NaIO ₄ | "on H ₂ O", 0 °C-rt | N.R. ^b |
| 4 | Bu ₄ NIO ₄ | CHCl ₃ , 0 °C-rt | 54% |
| 5 | NaIO ₄ -SiO ₂ | CH ₂ Cl ₂ , rt | 35% |
| 6 | CuCl (15 mol%) tBuOOH (1 eq.) | CH ₂ Cl ₂ , rt | 33% ^c |
| 7 ^d | FeCl ₃ (3 mol%) H ₂ O ₂ (8 eq) H ₂ N-CH ₂ -CH ₂ -NH ₂ (15 mol%) | CH ₂ Cl ₂ , rt | decomp. |

NOTE: (a) "reverse addition" – **3.37** was added to diene and oxidant; (b) N.R.=no reaction; (c) product was contaminated with Cu salts; (d) used **3.41** instead of **3.37**; intended product was cycloadduct **3.43** instead of **3.40**.

Sharpless and others have reported that cycloaddition reactions in particular can benefit from reacting "on water" compared to reacting in solution;^{329, 330} however, when the reaction using NaIO₄ was performed "on water", no cycloadduct **3.40** was obtained (Table 3.4, entry 3).

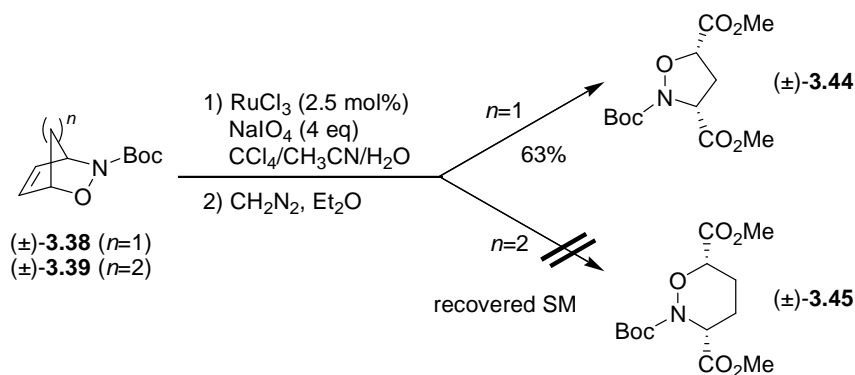
Periodate supported on silica^{331, 332} was identified as a suitable replacement for periodate in solution; however, cycloadduct **3.40** was only obtained in low yield (Table 3.4, entry 5). Similarly, unfavorable results were observed when metal-based oxidants⁴¹

were used in the cycloaddition reaction, resulting in either decomposition or low yields of cycloadduct **3.40** (Table 3.4, entries 6-7).

While cycloadditions with cyclopentadiene and cyclohexadiene were observed to be exceptionally rapid, cycloadditions with 1,3-cyclooctadiene were comparatively sluggish, and required higher temperatures to proceed. As an example, when **3.37** was oxidized using Swern-Moffatt³³³ conditions (i. oxalyl chloride/DMSO; ii. Et₃N) at -78 °C in the presence of *cis,cis*-1,3-cyclooctadiene, only a low yield of cycloadduct **3.40** was obtained, presumably due to the slow rate of the HDA reaction compared to decomposition of the nitroso species at such low temperatures. The highest yields of cycloadduct **3.40** were recorded when the reaction was performed using NaIO₄ in a methanol/water mixture at room temperature, and lower temperatures often resulted in a lower yield of cycloadduct **3.40**.

3.4.2 Alkene cleavage reactions of cycloadducts

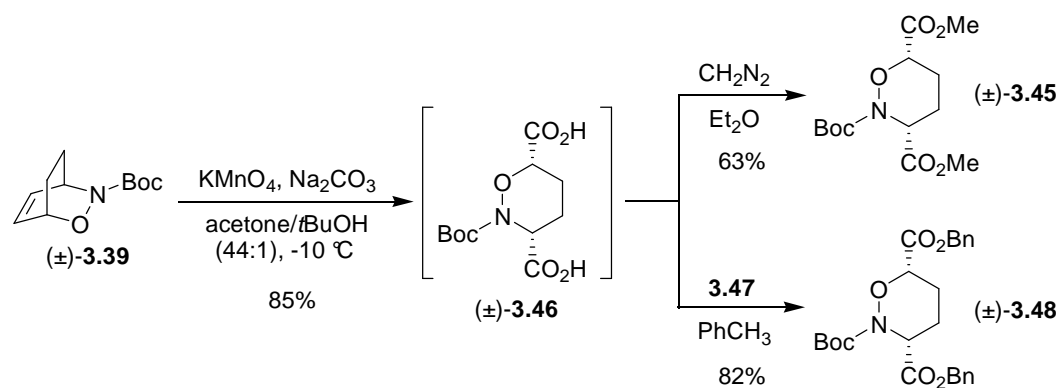
Oxidative cleavage reactions were investigated using cyclopentadiene- and cyclohexadiene-derived cycloadducts **3.38** and **3.39**. Olefin cleavage of cycloadducts **3.38** and **3.39** was attempted using ruthenium tetroxide followed by treatment of the crude mixture with excess diazomethane in order to obtain diester compounds **3.44** and **3.45** (Scheme 3.13).



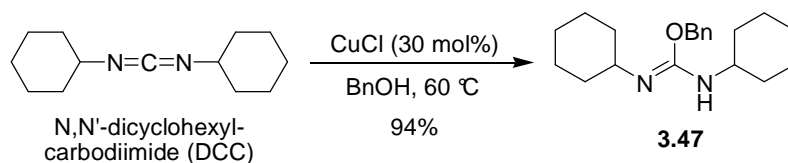
Scheme 3.13. Oxidative cleavage of cycloadducts

Cycloadduct **3.38** yielded the diester compound **3.44** in good yield; however, the alkene of cycloadduct **3.39** was resistant to oxidative cleavage under these conditions. Initially, we hypothesized that the difference in reactivity may be due to less ring-strain inherent in the bicyclo[2.2.2]-system than the bicyclo[2.2.1]-system; however, perhaps a more plausible reason for the lack of reactivity might have been due to inexperience with the chemistry in the author's hands. Fortunately, potassium permanganate effected oxidative cleavage of the alkene of cycloadduct **3.39** and yielded the crude diacid **3.46** (Scheme 3.14). Diacid **3.46** was treated with excess diazomethane to afford dimethyl ester **3.45** or with excess *O*-benzyl-*N,N'*-dicyclohexyl-isourea (**3.47**) to afford dibenzyl ester **3.48**. Isourea compound **3.47** was prepared according to published procedure (Scheme 3.15).

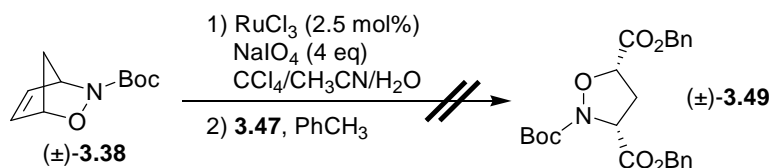
When cycloadduct **3.38** was treated with ruthenium tetroxide followed by an excess of isourea **3.47**, dibenzyl ester compound **3.49** was not isolated and a complex mixture was observed. Since benzyl esterification using isourea **3.47** often required heating to proceed, it is possible that the diacid intermediate decomposed under the reaction conditions.



Scheme 3.14. Oxidative cleavage of cyclohexadiene-derived cycloadduct



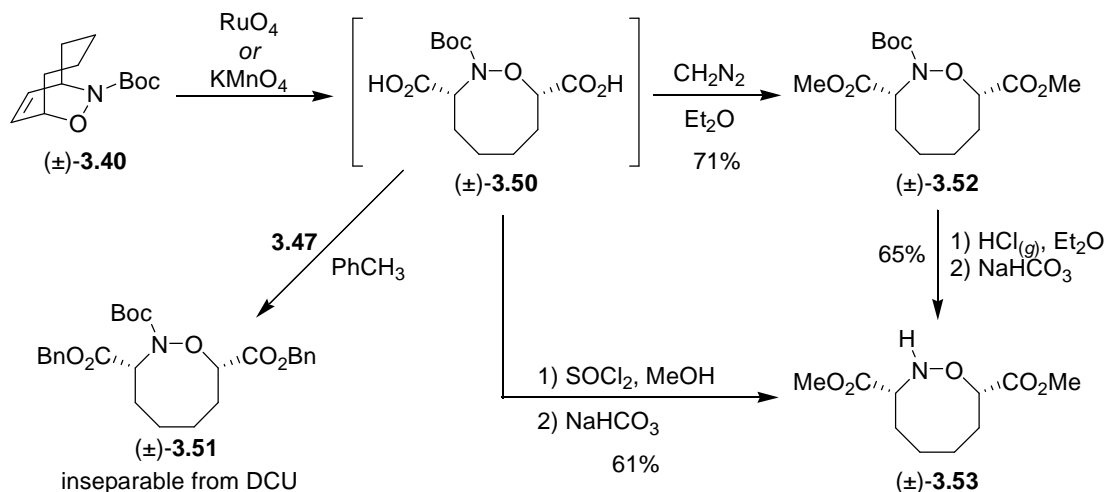
Scheme 3.15. Preparation of *O*-benzyl isourea compound



Scheme 3.16. Attempted preparation of dibenzyl ester compound

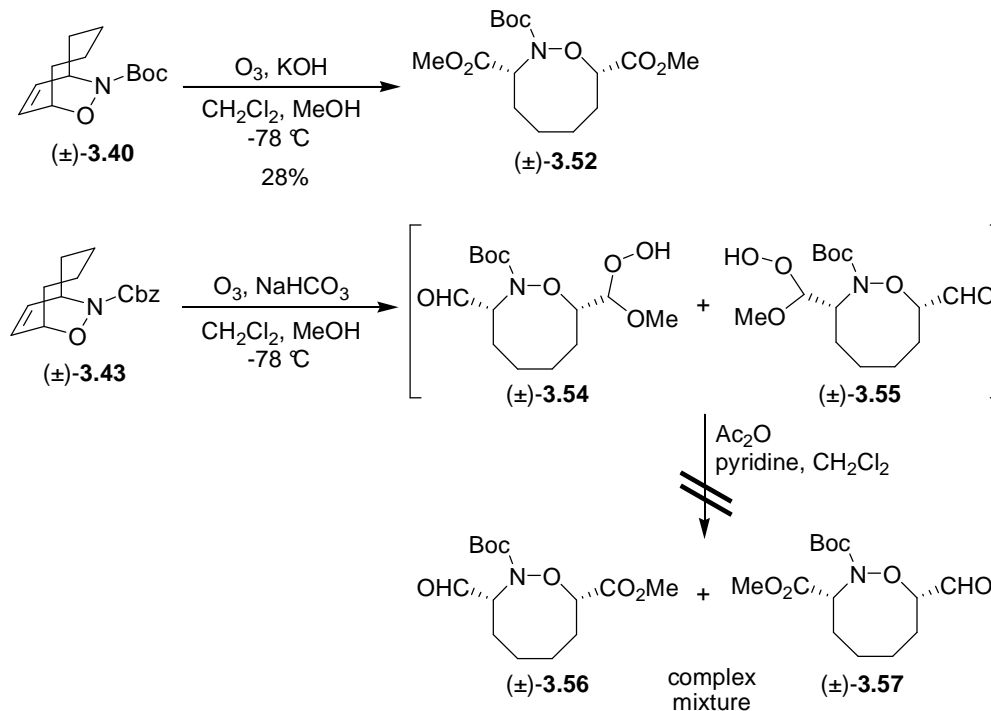
With an understanding of the oxidative cleavage reactions of cycloadducts **3.38** and **3.39**, oxidative cleavage reactions of cycloadduct **3.40** were investigated using ruthenium tetroxide and KMnO_4 (Scheme 3.17). Diacid intermediate **3.50** was prepared using either ruthenium tetroxide or KMnO_4 . Treatment of diacid **3.50** with excess isourea **3.47** yielded dibenzyl ester compound **3.51**, but separation of ester **3.51** from *N,N'*-dicyclohexylurea (DCU) was difficult. Cyclic hydroxylamine **3.53** could be

obtained by treatment of diacid **3.50** with excess diazomethane followed by deprotection of compound **3.52**, but was more easily prepared in one-step from diacid **3.50** by treatment with thionyl chloride in methanol.



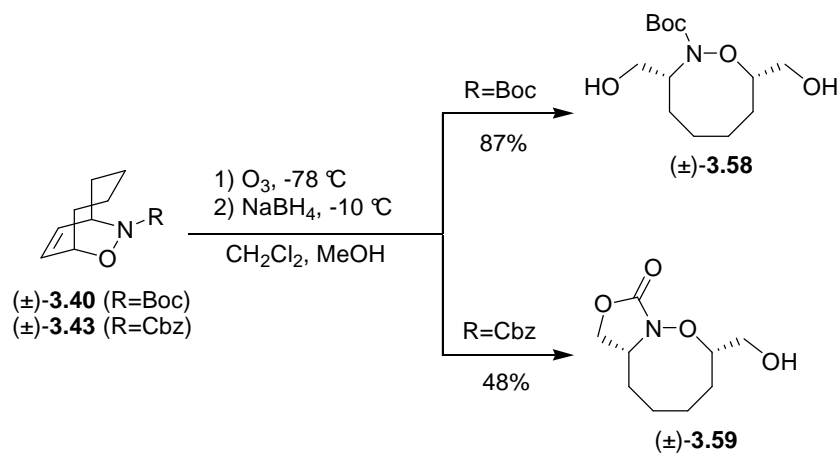
Scheme 3.17. Oxidative cleavage reactions of cyclooctadiene cycloadduct

Olefin cleavage using ozonolysis was also studied for cyclooctadiene-derived cycloadducts. Marshall has reported direct preparation of diester compounds from alkenes by ozonolysis using potassium hydroxide in alcoholic solvents.³³⁴ Using this method, diester compound **3.52** was obtained in low yield from cycloadduct **3.40** (Scheme 3.18). We were also interested in methods that might differentiate the two ester groups of diester compound **3.52**. Treatment of cycloadduct **3.43** with excess ozone and bicarbonate in CH_2Cl_2 /methanol was anticipated to yield the two peroxide compounds **3.54** and **3.55**. We hoped that treatment of the mixture with acetic anhydride and pyridine would afford compounds **3.56** and **3.57**; however, only a complex mixture was observed.



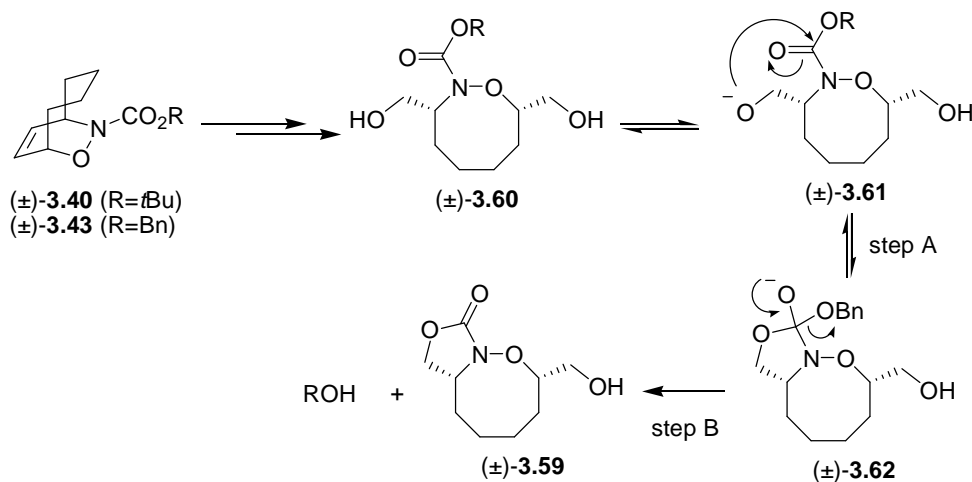
Scheme 3.18. Alkene cleavage using ozonolysis

A fortuitous discovery occurred when cycloadducts **3.40** and **3.43** were treated with excess ozone followed by reduction of the ozonide intermediate with sodium borohydride (Scheme 3.19). As expected, the diol compound **3.58** was obtained when cycloadduct **3.40** was treated with ozone followed by reduction with NaBH_4 ; however, when cycloadduct **3.43** was treated with the same conditions, the bicyclic hydroxamate **3.59** was recovered in 48% yield along with benzyl alcohol!



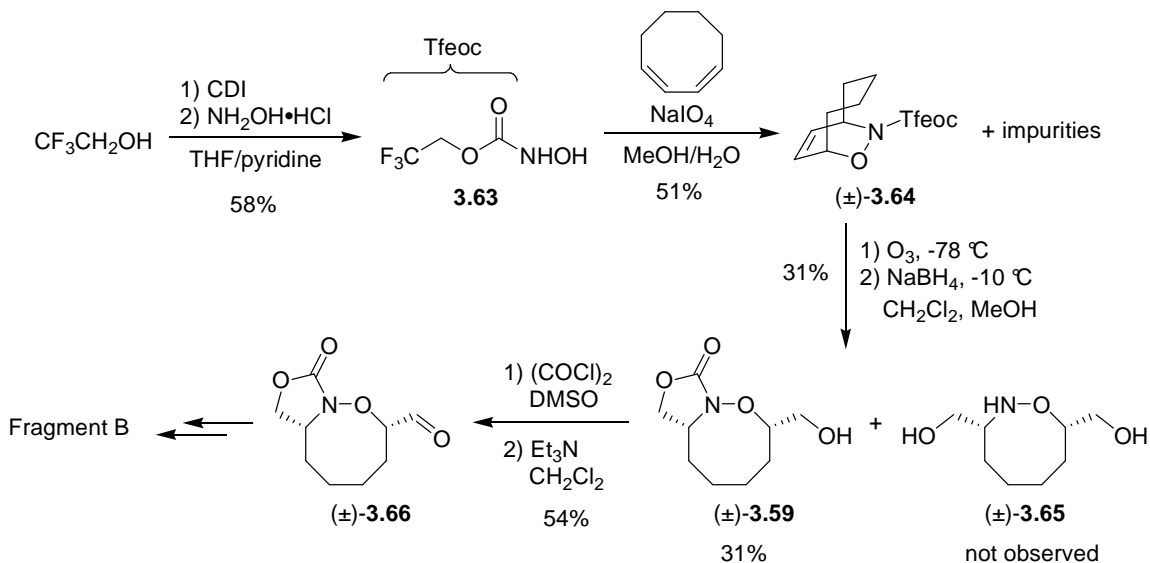
Scheme 3.19. Unexpected formation of a cyclic hydroxamate

Presumably, both cycloadducts **3.40** and **3.43** provide the diol compounds **3.60** in the course of the reaction (Scheme 3.20). Under the basic conditions, alkoxide intermediate **3.61** could attack the *N*-alkoxycarbamate carbonyl to yield the tetrahedral intermediate **3.62**. Breakdown of the tetrahedral intermediate should yield hydroxamate **3.59** along with 1 equivalent of alcohol ROH.



Scheme 3.20. Proposed mechanism for hydroxamate formation

In order to probe this reaction further, cycloadduct **3.64** was prepared (Scheme 3.21). We reasoned that the trifluoroethyl group, being more strongly electron-withdrawing than a benzyl group, might result in a higher yield of hydroxamate **3.59**. The trifluoroethylcarbamate **3.63** was prepared from 2,2,2-trifluoroethanol and successfully underwent cycloaddition with cyclooctadiene when treated with periodate to yield cycloadduct **3.64**, although a clean sample of adduct **3.64** was never obtained. Ozonolysis of an impure sample of cycloadduct **3.64** followed by treatment with NaBH₄ yielded the desired hydroxamate **3.59**, but in low yield. Oxidation of alcohol **3.59** to aldehyde **3.66** was accomplished using Swern-Moffatt oxidation.



Scheme 3.21. Investigation of hydroxamate-formation

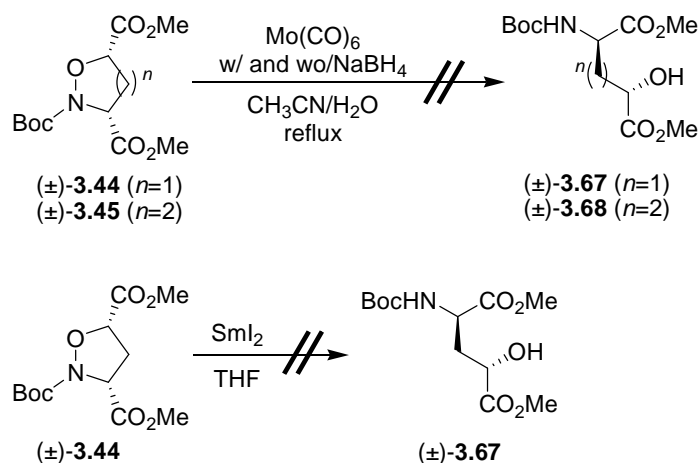
It is unclear why a lower yield of hydroxamate **3.59** was obtained in this reaction; however, if the strongly electron-withdrawing 2,2,2-trifluoroethoxy group increased the electrophilicity of the carbamate carbonyl, it would be likely that this would also increase

the likelihood of the carbamate being removed by treatment with NaBH₄. As a result, compound **3.65** would be expected, however only a low yield of hydroxamate **3.59** was isolated from the reaction mixture.

Although we envisioned the use of hydroxamate **3.59** as a substrate for the synthesis of fragment B, α -amino acid analogs, and other biologically interesting molecules, time did not permit additional investigation of this methodology.

3.4.3 N-O bond reduction of intermediates

N-O bond reduction of diester compounds **3.44** and **3.45** was unsuccessful using either Mo(CO)₆^{96, 97} or freshly prepared samarium diiodide (Scheme 3.22). In all cases, complex mixtures were observed and the desired α -hydroxy esters **3.67** and **3.68** were not isolated.



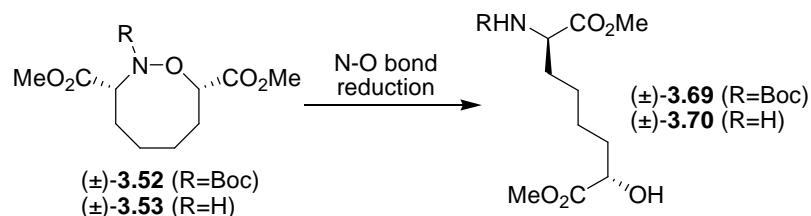
Scheme 3.22. Attempted N-O bond reductions

A more thorough study of N-O bond reduction conditions was performed on the cyclic hydroxylamine compounds **3.52** and **3.53** (Table 3.5). A variety of conditions has

been reported to reduce N-O bonds in the literature, including the use of hydrogenolysis, $\text{NiCl}_2/\text{NaBH}_4$,³³⁵ molybdenum hexacarbonyl,^{96, 97} samarium diiodide,^{98, 99, 179} zinc/acetic acid, and sodium-mercury amalgam.³³⁶ When compounds **3.52** and **3.53** were treated with most of these conditions decomposition and complex reaction mixtures resulted; however, we were pleased to find that α -hydroxy ester **3.69** was obtained in a modest 39% yield upon treatment of hydroxamate **3.52** with samarium diiodide (Table 3.5, entry 4). All attempts to optimize the reaction conditions only resulted in lower yields of compound **3.69**.

TABLE 3.5

INVESTIGATION OF N-O BOND REDUCTIONS OF DIESTER INTERMEDIATES



| Entry | R | Reduction conditions | Yield/result |
|-------|-----|--|----------------------------------|
| 1 | Boc | i. TFA, Et ₃ SiH, CH ₂ Cl ₂ ii. H ₂ (up to 3 bar), Pd/C, MeOH | complex mixture |
| 2 | Boc | Mo(CO) ₆ , CH ₃ CN/H ₂ O, reflux | recovered 3.52 |
| 3 | Boc | Zn, HOAc, Δ | recovered 3.52 + mixture |
| 4 | Boc | Sml ₂ , THF | 22-39% yield of (±)- 3.69 |
| 5 | Boc | Na-Hg, Na ₂ HPO ₄ , THF/MeOH | complex mixture |
| 6 | H | H ₂ (up to 3 bar), Pd/C, MeOH, HOAc | complex mixture |
| 7 | H | H ₂ (up to 3 bar), Pd/C, MeOH, HCl | complex mixture |
| 8 | H | H ₂ , Pd/C, MeOH, HCl, Δ | complex mixture |
| 9 | H | H ₂ (up to 3 bar), Pt ₂ O, MeOH, HCl | complex mixture |
| 10 | H | Mo(CO) ₆ , CH ₃ CN/H ₂ O, reflux | complex mixture |
| 11 | H | Zn, HOAc, Δ | recovered 3.53 + mixture |
| 12 | H | Sml ₂ , THF | complex mixture |
| 13 | H | Na-Hg, Na ₂ HPO ₄ , THF/MeOH | complex mixture |

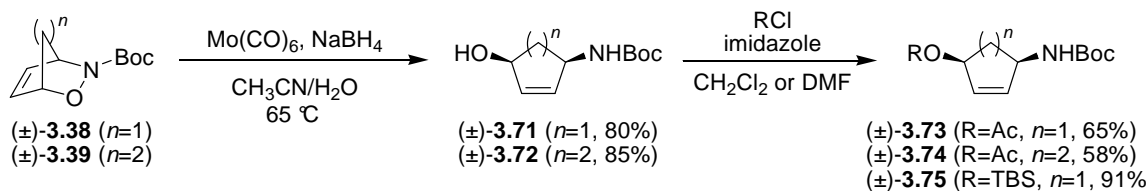
3.5 Synthesis of fragment B using method #2

Through the use of method #1, the only protected fragment B compound accessible was α-hydroxy ester **3.70**. This method was abandoned shortly thereafter due to the success of the alternative strategy, method #2. Using method #2, protected forms of both α-hydroxy carboxylate compound **2.63a** and 1,2-diol compound **2.63b** were

prepared in excellent yields from cycloadduct **3.40**. Additionally, enantiomerically-enriched compounds could be accessed through the development of a kinetic enzymatic resolution process. Details of these procedures will be described in the following section.

3.5.1 N-O bond reduction of cycloadducts

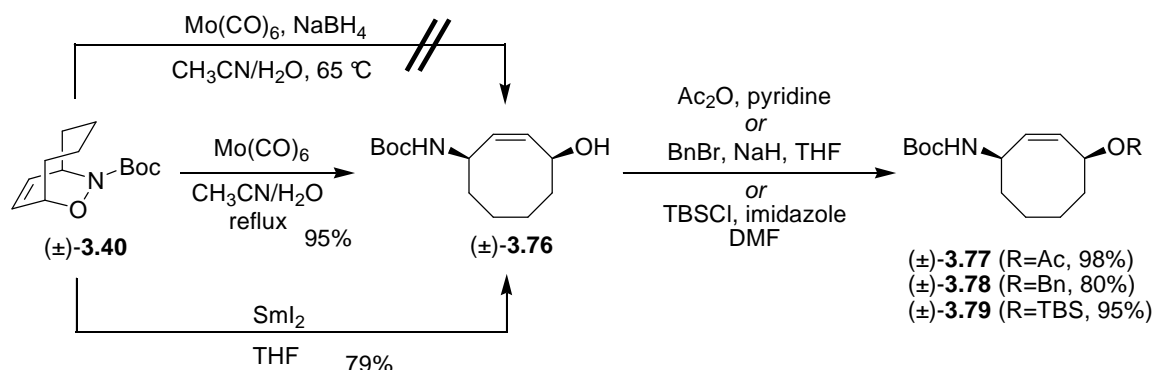
Similar to the strategies employed using method #1, the investigation of chemical techniques and procedures was initially explored using the cyclopentadiene- and cyclohexadiene-derived cycloadducts **3.38** and **3.39**. The N-O bond of cycloadducts **3.38** and **3.39** was reduced using molybdenum hexacarbonyl and cleanly yielded 1,4-aminoalcohols **3.71** and **3.72** (Scheme 3.23). Typically, N-O bond reductions using Mo(CO)_6 are performed with 0.7-1.0 equivalents of the reagent;^{96, 97} however, as reported previously in the Miller group,¹³⁵ only 30 mol% of Mo(CO)_6 was required for the reaction to proceed when excess NaBH_4 was added. A qualitative survey of a variety of other inorganic reducing agents ($\text{Na}_2\text{S}_2\text{O}_3$, Na_2SO_3 , $\text{Na}_2\text{S}_2\text{O}_5$, NaHSO_3 , Na ascorbate, Na oxalate) revealed that the use of sodium sulfite and sodium oxalate were suitable replacements for sodium borohydride. Protection of alcohols **3.71** and **3.72** yielded compounds **3.73-3.75**.



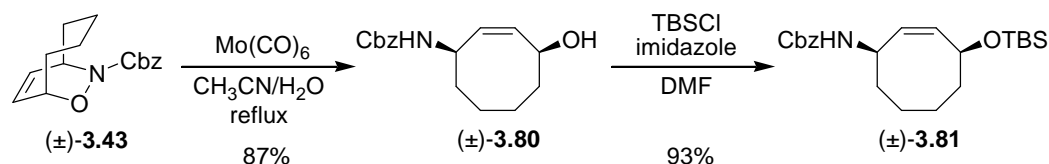
Scheme 3.23. Investigation of N-O bond reductions

The use of sub-stoichiometric molybdenum hexacarbonyl and sodium borohydride to reduce the N-O bond of the cyclooctadiene-derived cycloadduct **3.40** was unsuccessful (Scheme 3.24). One full equivalent of $\text{Mo}(\text{CO})_6$ and higher temperatures were required before cycloadduct **3.40** was completely consumed in the reaction, which offered 1,4-aminocyclooctenol **3.76** in excellent yield. Alternatively, samarium diiodide was used to effect the same transformation, albeit in lower yield. Protection of alcohol **3.76** yielded compounds **3.77-3.79** without incident.

The same sequence was successful when performed on Cbz-protected cycloadduct **3.43**, which offered alcohols **3.80** and **3.81** in excellent yields (Scheme 3.25).



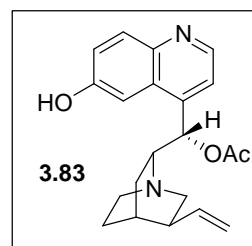
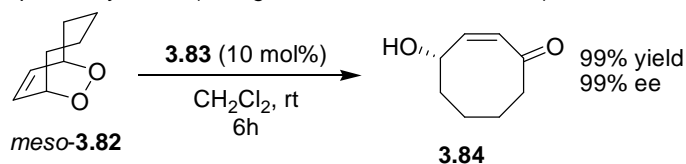
Scheme 3.24. N-O bond reduction of cyclooctadiene-derived cycloadduct



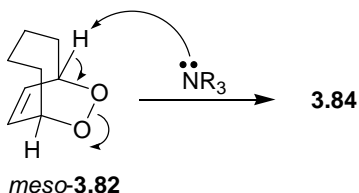
Scheme 3.25. N-O bond reduction of Cbz-protected cycloadduct

As described in chapter 1, eliminative ring-opening reactions have also been used to effect N-O bond cleavage of acylnitroso HDA cycloadducts.^{104, 106} Recently, Toste has reported³³⁷ the desymmetrization of *meso*-bicyclic endoperoxide **3.82** to γ -hydroxyenone **3.84** via a chiral base-catalyzed Kornblum DeLaMare rearrangement (Scheme 3.26).¹⁰⁵ Tertiary amine **3.83** provided γ -hydroxyenone **3.84** in excellent yield and enantiomeric excess.

Reported by Toste (*J. Org. Chem.* **2006**, 128, 12658):

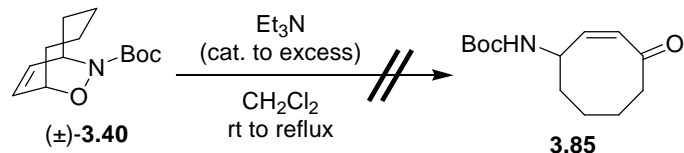


Proposed mechanism:



Scheme 3.26. Kornblum-DeLaMare Rearrangement reported by Toste

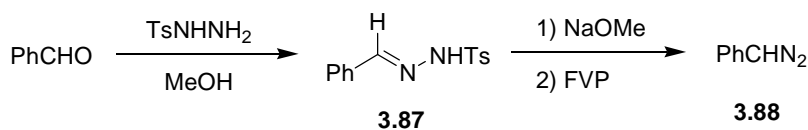
Through analogy, we hypothesized that cycloadduct **3.40** could undergo a similar ring-opening process to provide γ -aminoenone **3.85** using tertiary amine catalysts; however, when cycloadduct **3.40** was treated with triethylamine in CH_2Cl_2 , no reaction was observed (Scheme 3.27). The addition of excess amine or heating of the reaction mixture had no observable effect on the reaction. According to eliminative N-O bond cleavage reactions of monocyclic oxazines as reported by Kefalas¹⁰⁴ and Desai,¹⁰⁶ stronger bases may be needed in order to obtain compound **3.85**.



Scheme 3.27. Failed attempt at the Kornblum-DeLaMare rearrangement

3.5.2 Oxidative cleavage reactions: synthesis of α -hydroxy carboxylates

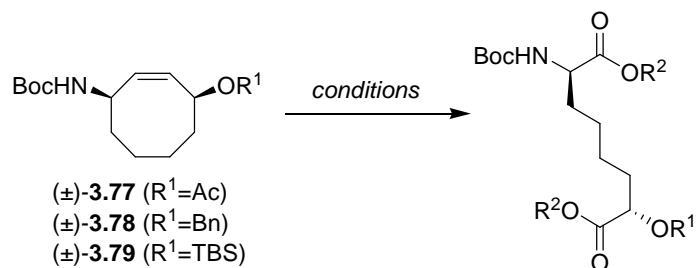
A variety of conditions were examined for the oxidative cleavage of the olefin of protected 1,4-aminocyclooctenols **3.77-3.79** (Table 3.6). Oxidation conditions used included permanganate (Table 3.6, entries 1-4), ruthenium tetroxide (Table 3.6, entries 5-9), and $\text{OsO}_4/\text{Oxone}$ ³³⁸ with and without added sodium bicarbonate (Table 3.6, entries 10-13). The crude diacid intermediates were subsequently esterified to methyl esters using excess diazomethane (Table 3.6, entries 1, 4, 5, 7, and 9) or esterified to benzyl esters using phenyldiazomethane (**3.88**) (Table 3.6, entries 2 and 6) or benzyl bromide and potassium carbonate (Table 3.6, entry 3). Phenyldiazomethane (**3.88**) was prepared by flash vacuum pyrolysis (FVP) from hydrazone **3.87** (Scheme 3.28).³³⁹



Scheme 3.28. Preparation of phenyldiazomethane

TABLE 3.6

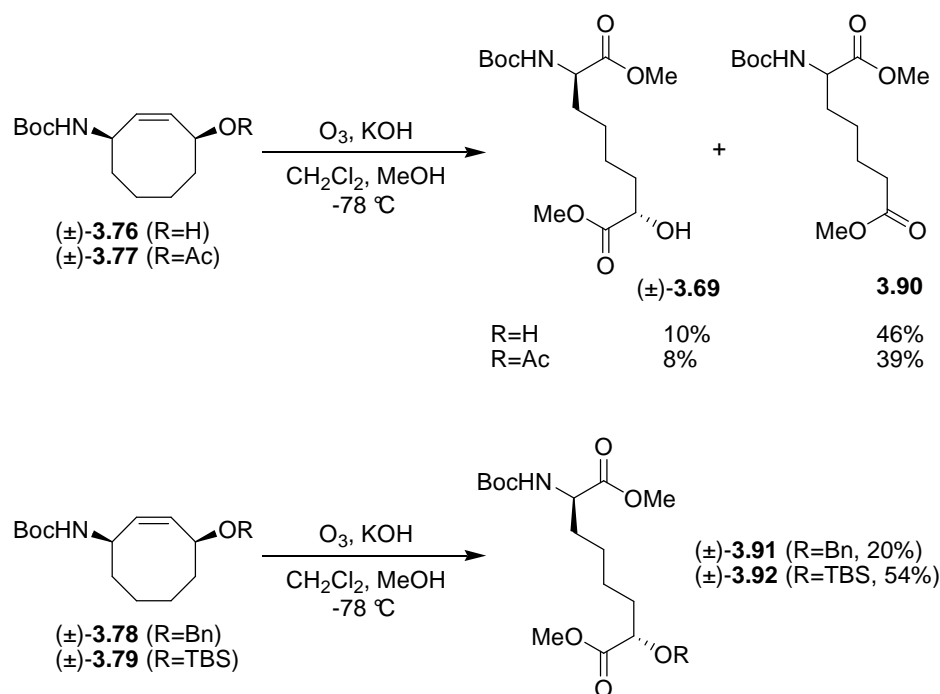
INVESTIGATION OF OXIDATIVE CLEAVAGE CONDITIONS FOR PROTECTED
1,4-AMINOALCOHOLS



| Entry | Alkene | Conditions ^a | R ¹ | R ² | Product (yield)/result |
|-------|---------------------|---|----------------|----------------|--------------------------------------|
| 1 | $(\pm)\text{-3.77}$ | i. KMnO ₄ ; ii. CH ₂ N ₂ | Ac | Me | $(\pm)\text{-3.86}$ (33%) |
| 2 | $(\pm)\text{-3.77}$ | i. KMnO ₄ ; ii. PhCHN ₂ | Ac | Bn | complex mixture |
| 3 | $(\pm)\text{-3.77}$ | i. KMnO ₄ ; ii. BnBr, K ₂ CO ₃ | Ac | Bn | complex mixture |
| 4 | $(\pm)\text{-3.79}$ | i. KMnO ₄ ; ii. CH ₂ N ₂ | TBS | Me | 100% recovery of $(\pm)\text{-3.79}$ |
| 5 | $(\pm)\text{-3.77}$ | i. RuO ₄ ; ii. CH ₂ N ₂ | Ac | Me | $(\pm)\text{-3.86}$ (75%) |
| 6 | $(\pm)\text{-3.77}$ | i. RuO ₄ ; ii. PhCHN ₂ | Ac | Bn | complex mixture |
| 7 | $(\pm)\text{-3.78}$ | i. RuO ₄ ; ii. CH ₂ N ₂ | Bn | Me | complex mixture |
| 8 | $(\pm)\text{-3.78}$ | i. RuO ₄ ; ii. isourea 3.47 | Bn | Bn | inseparable from DCU |
| 9 | $(\pm)\text{-3.79}$ | i. RuO ₄ ; ii. CH ₂ N ₂ | TBS | Me | complex mixture |
| 10 | $(\pm)\text{-3.77}$ | OsO ₄ , Oxone | Ac | H | complex mixture |
| 11 | $(\pm)\text{-3.79}$ | OsO ₄ , Oxone | TBS | H | complex mixture |
| 12 | $(\pm)\text{-3.77}$ | OsO ₄ , Oxone, NaHCO ₃ | Ac | H | complex mixture |
| 13 | $(\pm)\text{-3.79}$ | OsO ₄ , Oxone, NaHCO ₃ | TBS | H | complex mixture |

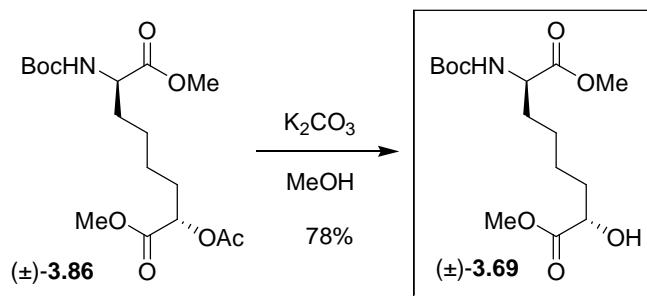
NOTE: (a) RuO₄ = RuCl₃ (2.5 mol%), NaIO₄ (4 eq); KMnO₄ = KMnO₄ (2.5 eq), Na₂CO₃ (5 eq).

has reported decarboxylation to occur with other protected allylic alcohol substrates under these conditions.³³⁴



Scheme 3.30. One-step synthesis of diester compounds

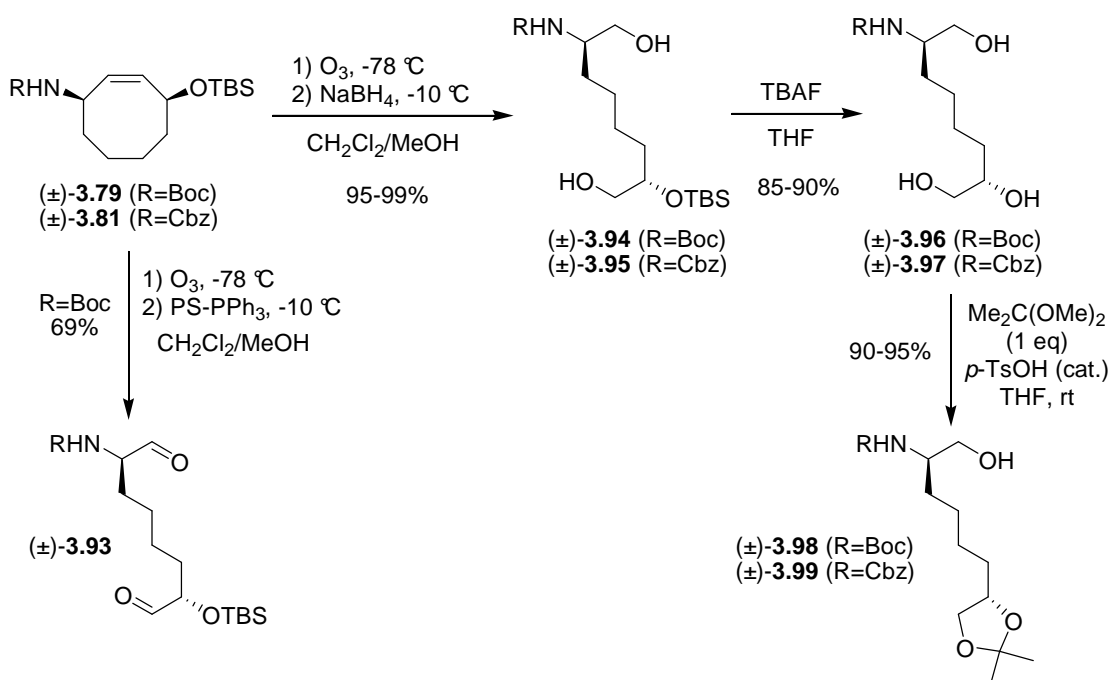
When benzyl ether **3.78** and silyl ether **3.79** were treated with the same conditions, the ester compounds **3.91** and **3.92** were recovered in low to moderate yield (Scheme 3.39). Deprotection of acetate **3.86** with potassium carbonate in methanol yielded α -hydroxy ester **3.69** (Scheme 3.31). Compared to the synthesis of α -hydroxy ester **3.69** using method #1, this sequence afforded α -hydroxy ester **3.69** in higher overall yield from cycloadduct **3.40** (55% for method #2 vs. 28% for method #1).



Scheme 3.31. Synthesis of an α -hydroxy ester compound

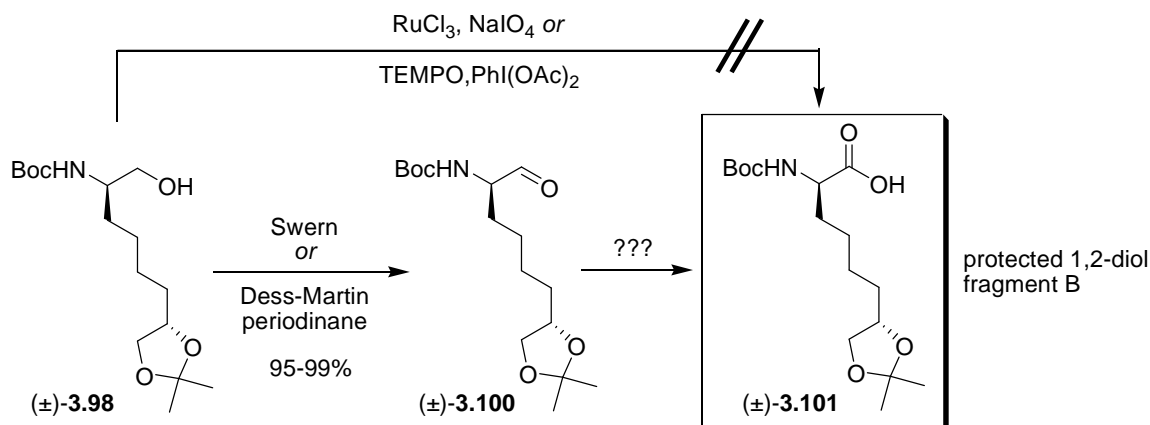
3.5.3 Alkene cleavage reactions: synthesis of 1,2-diol compounds

Due to the successful use of ozonolysis to cleave the alkene of protected 1,4-aminocyclooctenol compounds **3.78** and **3.79** to yield diester compounds **3.91** and **3.92**, we examined the use of ozonolysis to synthesize other fragment B compounds. Protected 1,4-aminocyclooctenol **3.79** was subjected to ozonolysis followed by treatment with polymer-supported triphenylphosphine (PS-PPh₃) to yield dialdehyde **3.93** (Scheme 3.32). Ozonolysis of alkenes **3.79** and **3.81** followed by subsequent reduction of the ozonide intermediate with NaBH₄ offered the diol compounds **3.94** and **3.95** in excellent yield. Silyl deprotection was effected with tetrabutylammonium fluoride and yielded the triol compounds **3.96** and **3.97**. Treatment of triols **3.96** and **3.97** with excess 2,2-dimethoxypropane and catalytic *p*-toluenesulfonic acid resulted in complex mixtures of acetal products; however, when only one equivalent of 2,2-dimethoxypropane was used in the reaction, acetonides **3.98** and **3.99** were obtained in excellent yields.



Scheme 3.32. Ozonolysis reactions on alkene intermediates

From acetonide **3.98**, we hypothesized that 1,2-diol-containing fragment B compound **3.101** could be obtained through direct oxidation or through initial oxidation to an aldehyde intermediate, compound **3.100** (Scheme 3.33). Direct oxidation to carboxylic acid **3.101** from acetonide **3.98** was tested using both ruthenium tetroxide and TEMPO/PhI(OAc)₂. While carboxylic acid **3.101** was observed, the reactions did not proceed cleanly and difficulties were encountered during purification that resulted in product decomposition. We then examined conditions for oxidizing aldehyde **3.100**, prepared easily from acetonide **3.98** using Swern-Moffatt oxidation³³³ or Dess-Martin periodinane,³⁴¹ to the desired carboxylic acid **3.101**.

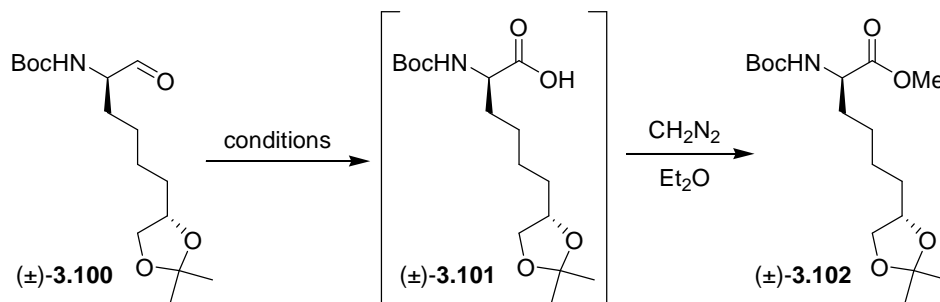


Scheme 3.33. Strategies toward the synthesis of 1,2-diol 3.101

A number of conditions were investigated to oxidize aldehyde **3.100** to acid **3.101** (Table 3.7). Due to the difficulties previously encountered during the attempted purification of carboxylic acid **3.101**, crude reaction mixtures were treated with excess diazomethane to obtain a yield of methyl ester **3.102**. Sodium chlorite effectively yielded methyl ester **3.102** (Table 3.7, entries 1-2). The use of a chlorine scavenger (2-methyl-2-butene, Table 3.7, entry 2)^{342, 343} yielded appreciably more ester **3.102** than when no scavenger was added Table 3.7, entry 1).³⁴⁴ Using oxone,³⁴⁵ only decomposition was observed (Table 3.7, entry 3). A low yield of ester **3.102** was observed using Ag_2O , generated *in situ* using AgNO_3/KOH ^{346, 347} (Table 3.7, entry 5). Potassium permanganate with added phase transfer agent benzyltriethylammonium chloride³⁴⁸ offered the highest yield of ester **3.102**. Additionally, the oxidation was very clean and offered nearly pure carboxylic acid **3.101** without purification (Table 3.7, entry 6).

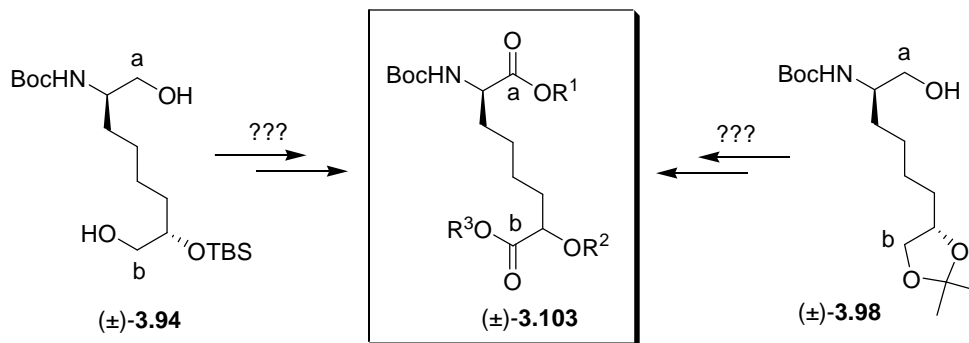
TABLE 3.7

INVESTIGATION OF REACTION CONDITIONS FOR THE OXIDATION OF AN
ALDEHYDE



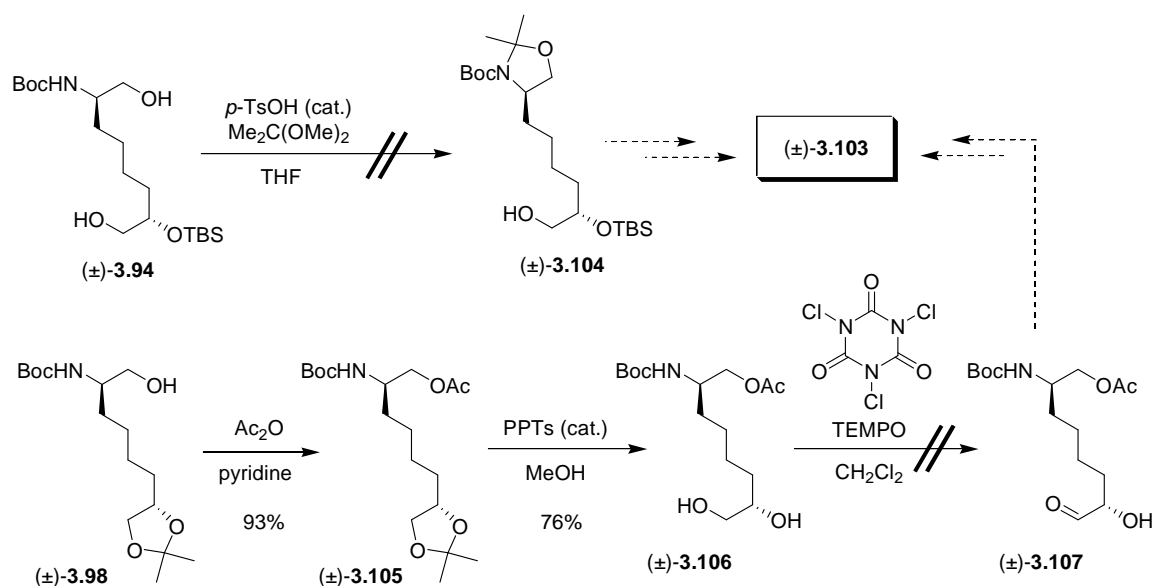
| Entry | Oxidation Conditions | Yield [(±)-3.102] |
|-------|---|-------------------------------------|
| 1 | NaClO ₂ , H ₂ O ₂ tBuOH, H ₂ O | not determined |
| 2 | NaClO ₂ , NaH ₂ PO ₄ 2-methyl-2-butene tBuOH, H ₂ O | 44% |
| 3 | Oxone, DMF | complex mixture |
| 4 | KMnO ₄ , BnNEt ₃ Cl MeOH, H ₂ O | 72% (91% yield of 3.101) |
| 5 | AgNO ₃ , KOH EtOH, H ₂ O | 24% |

Two strategies were also examined toward the synthesis of orthogonally-protected α -hydroxy carboxylate compound **3.103** (Scheme 3.34). An important aspect of these two strategies from either silyl ether **3.94** or acetonide **3.98** was the ability to differentiate between the two ester groups *a* and *b*.



Scheme 3.34. Strategies toward α -hydroxy carboxylate 3.103

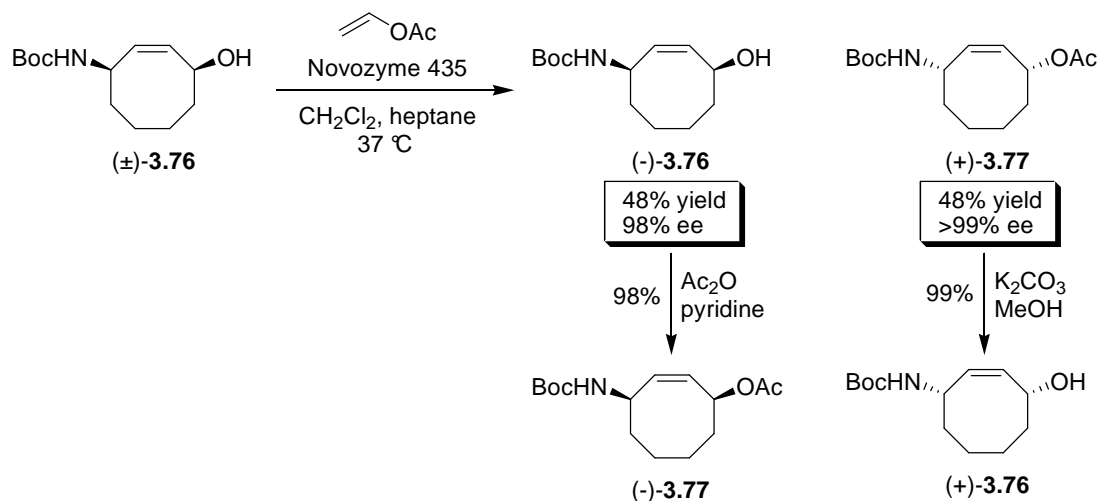
Formation of acetonide **3.104** from diol **3.94** failed under all attempted conditions (Scheme 3.35). Oxidation of alcohol **3.104** followed by a deprotection-oxidation-protection sequence should have yielded compound **3.103**. Alcohol **3.98** was protected as acetate **3.105**, and the acetonide group was cleanly removed to provide diol **3.106**. Giacomelli *et al* has reported the use of a TEMPO oxidation using stoichiometric trichloroisocyanuric acid to selectively oxidize the primary hydroxyl of a 1,2-diol group.³⁴⁹ When these conditions were used on diol **3.106**, only complex mixtures were obtained.



Scheme 3.35. Toward the synthesis of α -hydroxy carboxylate 3.103

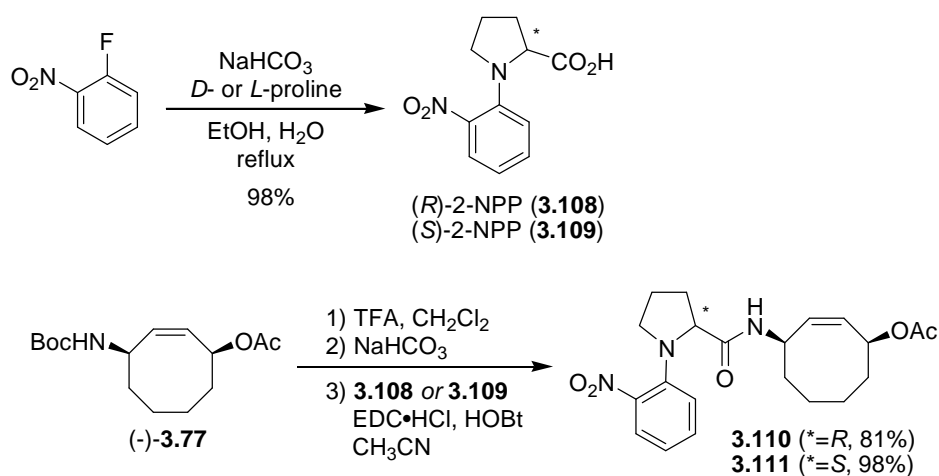
3.5.4 Synthesis of enantiomerically pure 1,2-diol compounds fragment B

The Miller group has reported the synthesis of enantiomerically pure 1,4-aminocyclopentenols using a kinetic enzymatic resolution.^{103, 137, 138, 168} We envisioned the use of a kinetic enzymatic resolution process as a means to generate enantiomerically pure fragment B analogs from 1,4-aminocyclooctenol **3.76**. To our delight, when alcohol **3.76** was treated with vinylacetate and immobilized *Candida antarctica* B lipase (Novozyme 435) in a mixture of dichloromethane and wet heptane at 37 °C, the enantiomerically-enriched alcohol (-)-**3.76** and acetate (+)-**3.77** were obtained with excellent yield and optical purity (Scheme 3.36). Alcohol (-)-**3.76** was easily transformed into acetate (-)-**3.77**, and acetate (+)-**3.77** was easily transformed into alcohol (+)-**3.76**. The enantiomeric excess was calculated based on analysis of the Mosher ester of alcohols (+)-**3.76** and (-)-**3.76** as well as racemic alcohol (±)-**3.76**.



Scheme 3.36. Kinetic enzymatic resolution of 1,4-aminocyclooctenol

While the Mosher ester analysis allowed for the determination of enantiomeric purity, this did not allow us to determine the absolute configuration. Based on a recent report by Choi *et al.*,³⁵⁰ absolute configuration of primary amines with an adjacent chiral center could be determined by analysis of their *N*-(2-nitrophenyl)prolyl (2-NPP) amides. Based on reported success of this method on structures similar to compounds **3.77**, we decided to use this method to determine absolute configuration. Nitrophenylprolines **3.108** and **3.109** were prepared according to literature procedures (Scheme 3.37).³⁵⁰ Compound **3.77** was converted to the *R*- and *S*-2-NPP amides **3.110** and **3.111** through deprotection and EDC-mediated amide coupling.



Scheme 3.37. Synthesis of *N*-(2-nitrophenyl)proline (2-NPP) amides

^1H NMR and COSY NMR were used to assign the chemical shifts of all protons in 2-NPP amides **3.110** and **3.111** (Figure 3.2). Following the model for determination of absolute chemistry of α -chiral amines as reported by Choi,³⁵⁰ the absolute configuration for the 2-NPP amides **3.110** and **3.111** was determined as $1R,4S$ based on the pattern of $\Delta\delta^{RS}$ values (Figure 3.3). The distribution and magnitude of $\Delta\delta^{RS}$ values is in agreement with reported values for similar structures. The consistency and reliability of the use of 2-NPP amides as a method for determining absolute configuration was reported to be a consequence of intramolecular hydrogen bonding.

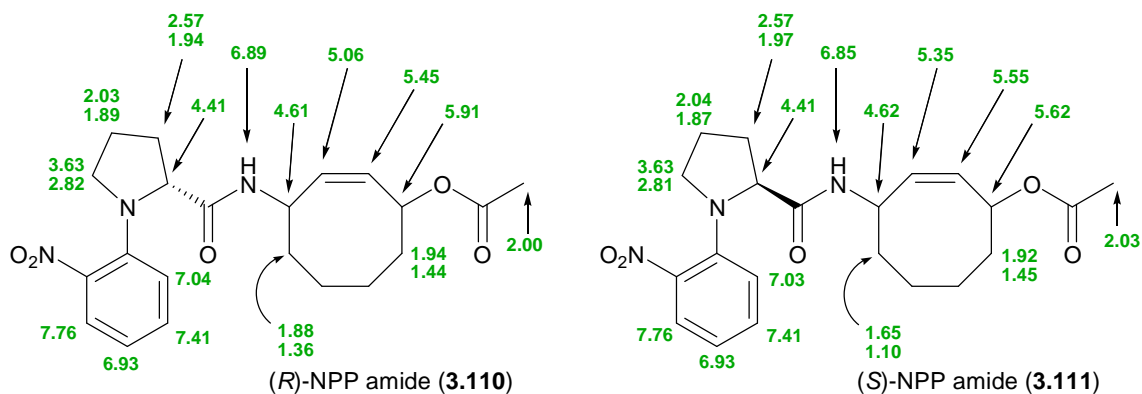


Figure 3.2. Chemical shifts (δ) of all protons in NPP amides **3.110** and **3.111**

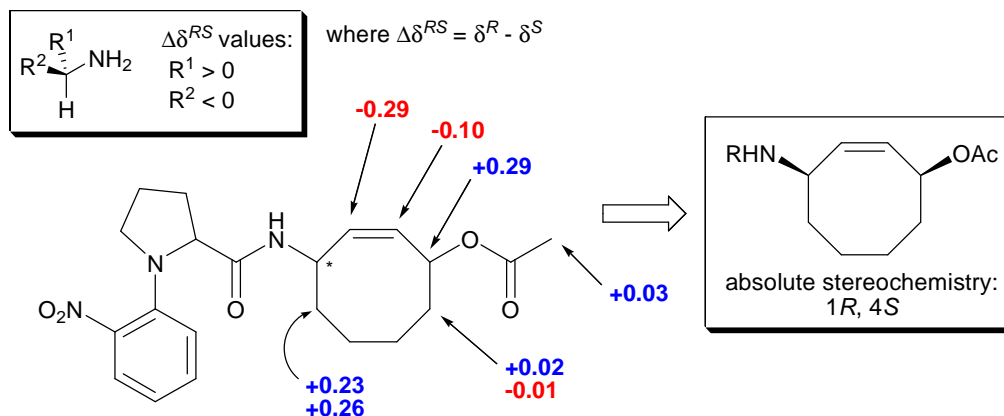
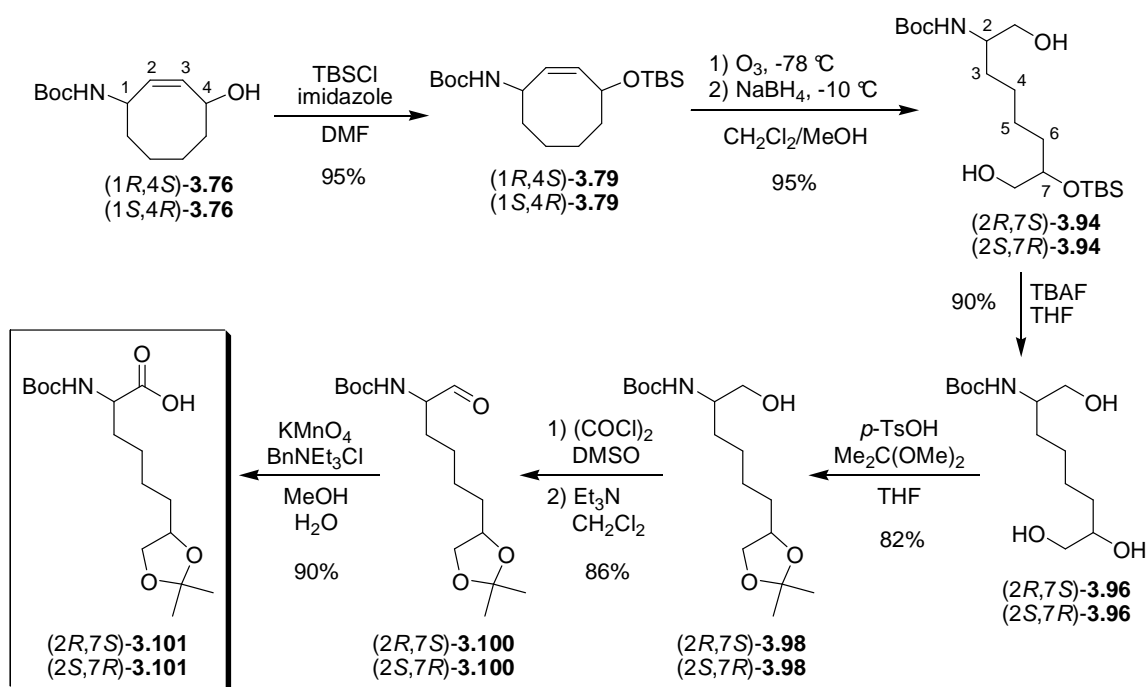


Figure 3.3. Absolute stereochemistry of NPP amides **3.110** and **3.111**

Following the method reported in section 3.5.3, the enantiomerically pure alcohols (+)-**3.76** and (-)-**3.76** were used to prepare both enantiomers of acetamide-containing fragment B compounds **3.101** (Scheme 3.38). Alcohols **3.76** were converted to silyl ethers **3.79** in excellent yield. Ozonolysis and reduction with sodium borohydride yielded diols **3.94**, which were deprotected to yield triols **3.96**. Acetamide protection followed by the optimized two-stage oxidation sequence afforded compounds **3.101**. No epimerization was observed from careful analysis of ^{13}C NMR spectra for all compounds.

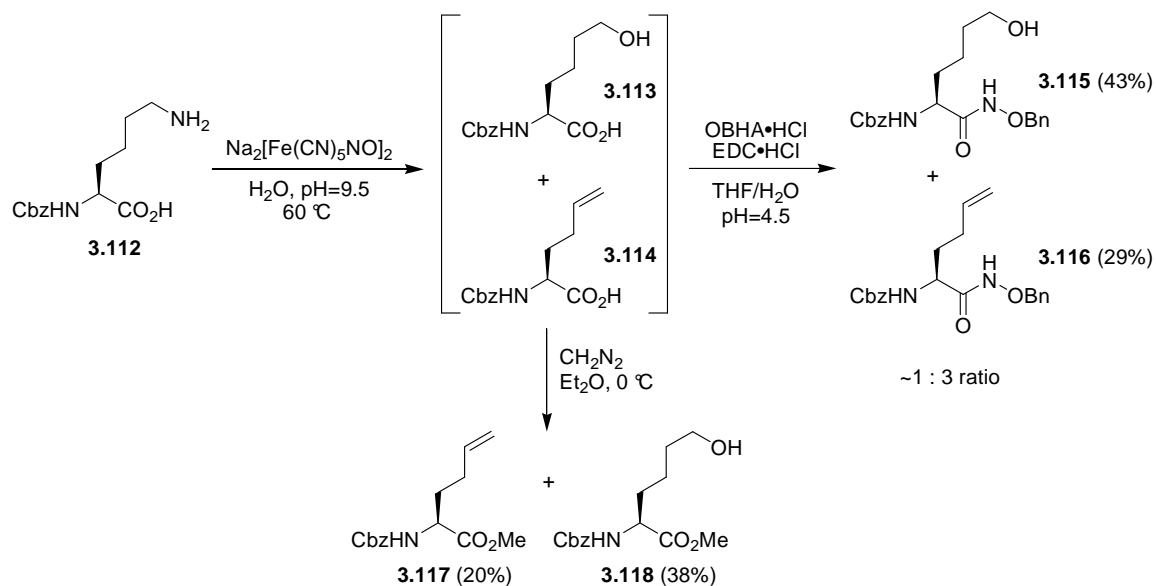


Scheme 3.38. Synthesis of enantiomerically pure fragment B compounds

3.6 Synthesis of fragment C

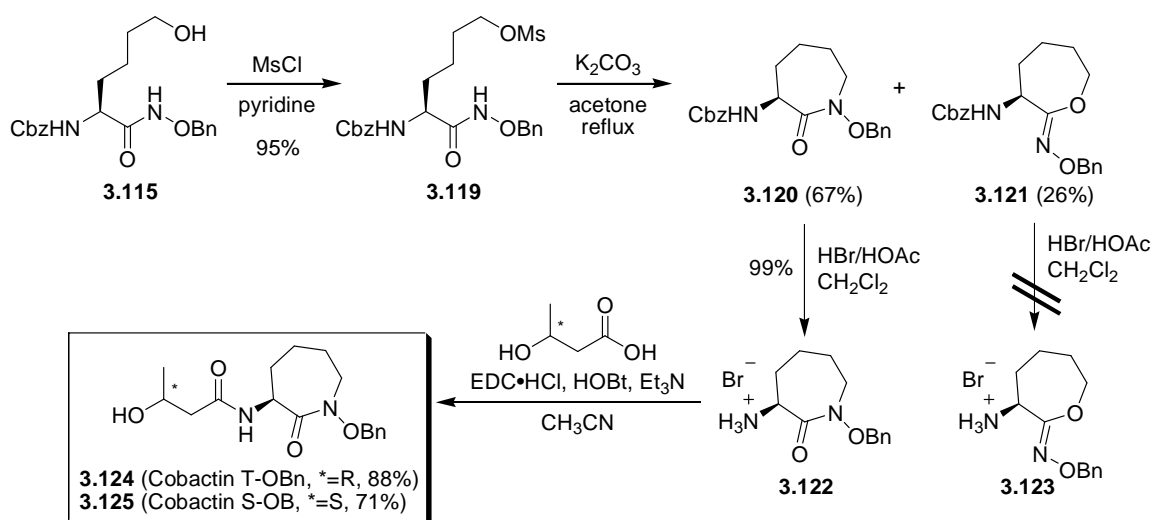
Fragment C, often termed the “cobactin” fragment in mycobactin literature, is derived from lysine and 3-hydroxybutyrate. The following synthesis is largely based upon earlier reported syntheses by Miller,²⁹⁶⁻²⁹⁹ and the recently reported synthesis of the related siderophore, Amamistatin B.³⁰⁶ For the initial step of the reaction sequence, the diazotization of protected *L*-lysine **3.112** was performed using nitroferricyanide according to the procedure reported by Baldwin³⁵¹ (Scheme 3.39). Alkene **3.114** was produced in the reaction in addition to hydroxynorleucine **3.113**, arising from subsequent dehydration of compound **3.113**. The crude mixture was treated with *O*-benzylhydroxylamine hydrochloride and EDC•HCl under aqueous conditions to yield the

two hydroxamates **3.115** and **3.116**. Alternatively, the crude mixture was treated with excess diazomethane to yield methyl esters **3.117** and **3.118**.



Scheme 3.39. Diazotization of lysine

Alcohol **3.115** was converted to mesylate **3.119**, and cyclization to hydroxamate **3.120** and hydroximate **3.121** was effected by potassium carbonate in refluxing acetone (Scheme 3.40). While deprotection of hydroxamate **3.120** proceeded without incident to yield the amine **3.122** as an HBr salt, deprotection of hydroximate **3.121** resulted in considerable decomposition. Coupling of amine **3.122** with either *R*- or *S*-3-hydroxybutyrate yielded benzyl-protected cobactin T (**3.124**) or benzyl-protected cobactin S (**3.125**), respectively.

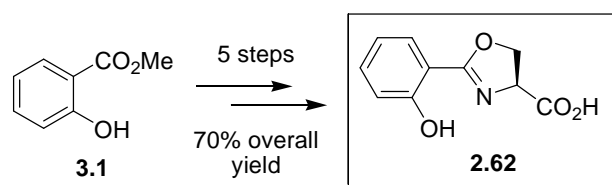


Scheme 3.40. Synthesis of protected cobactins S and T (fragment C)

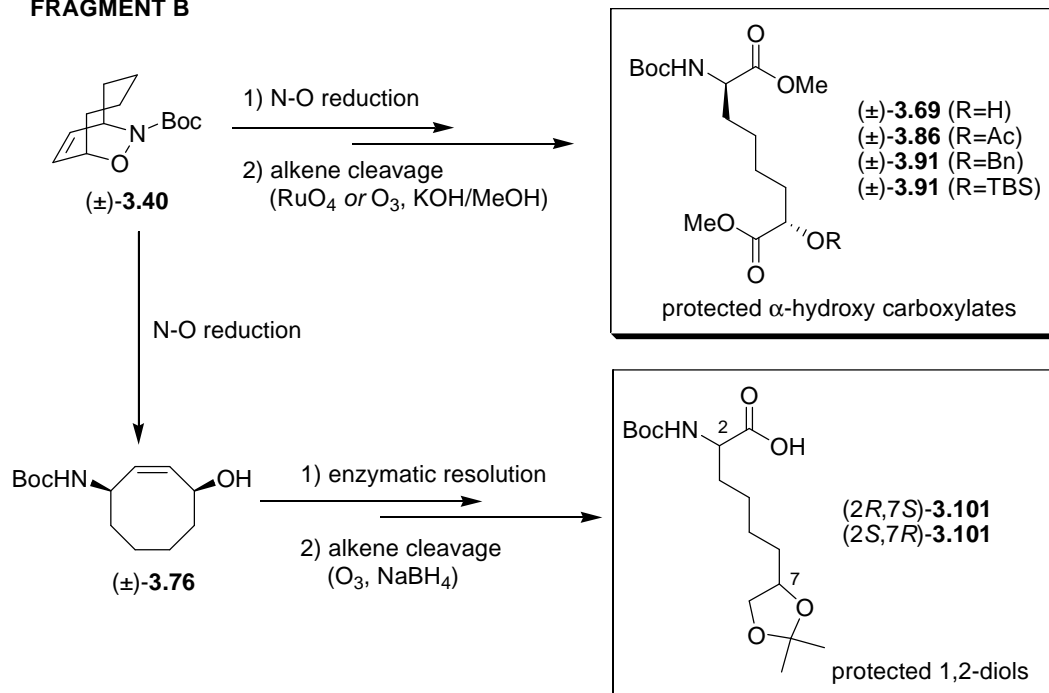
3.7 Summary of fragment synthesis

This chapter described the synthesis of mycobactin fragments and analogs. Fragments A (**2.62**) and C (cobactins **3.124** and **3.125**) were synthesized in a few steps from methyl salicylate (**3.1**) and lysine **3.112**, respectively (Scheme 3.41). Both α -hydroxy carboxylate compounds (**3.69**, **3.86**, **3.91**, and **3.92**) and 1,2-diol compounds **3.101** were prepared from acylnitroso cycloadduct **3.40**. Using a kinetic enzymatic resolution process, racemic alcohol **3.76** was used to prepare enantiomerically pure 1,2-diols **3.101**. The assembly of all fragments and the synthesis of 1,2-diol- and α -hydroxy carboxylate-containing mycobactin analogs as well as biological activity will be covered in chapter 4.

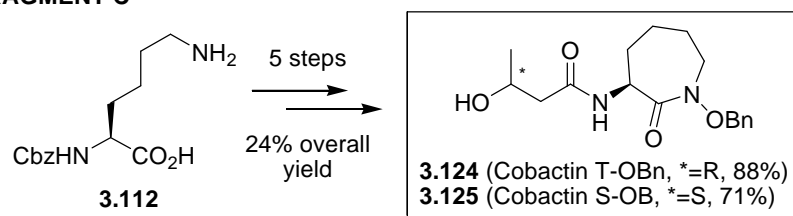
FRAGMENT A



FRAGMENT B



FRAGMENT C

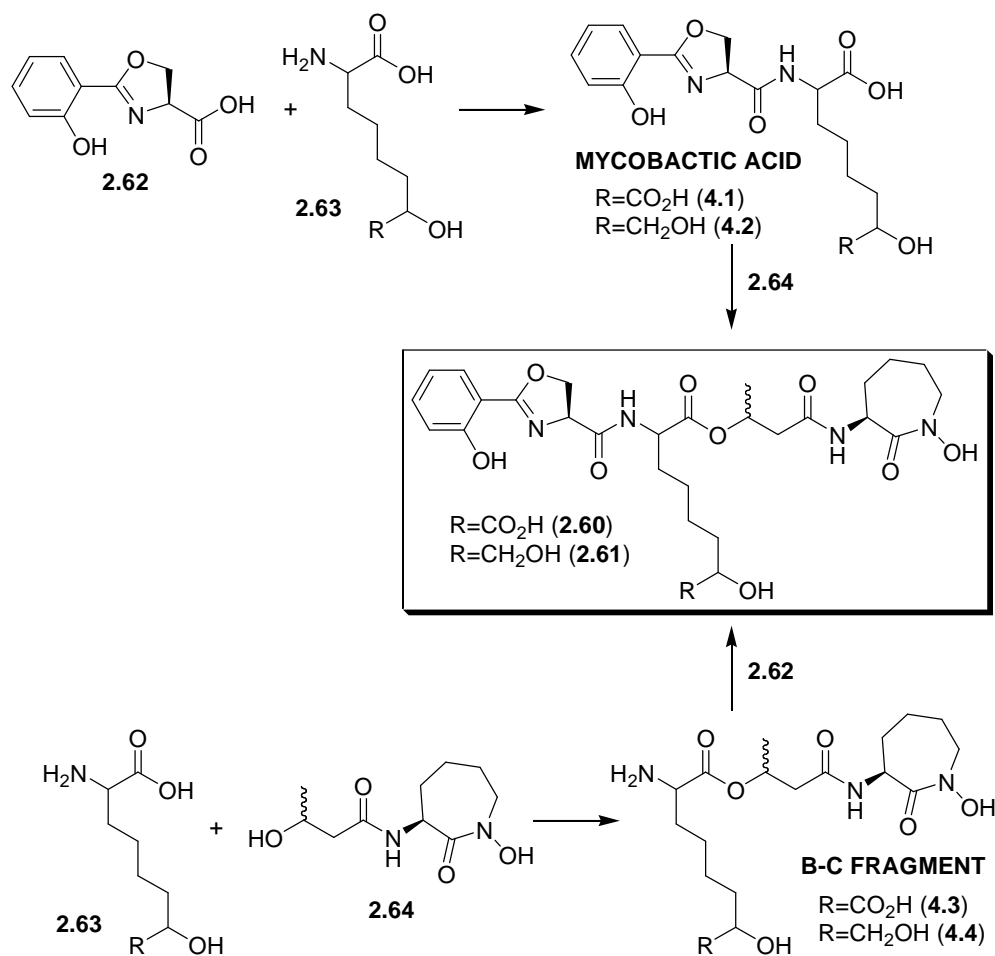


Scheme 3.41. Summary of fragment A, B, and C syntheses

CHAPTER 4:
ASSEMBLY OF MYCOBACTIN FRAGMENTS AND SYNTHESIS OF
MYCOBACTIN ANALOGS

4.1 Introductory remarks

Two strategies for the assembly of fragments A (**2.62**), B (**2.63**), and C (**2.64**) were examined toward the synthesis of α -hydroxy carboxylate mycobactin analogs **2.60** and 1,2-diol mycobactin analogs **2.61** (Scheme 4.1). The first strategy involved coupling fragments A (**2.62**) and B (**2.63**) to form mycobactic acid analogs **4.1** and **4.2** followed by esterification with fragment C (**2.64**) to yield analogs **2.60** and **2.61**. The second strategy utilized an esterification of fragments B (**2.63**) and C (**2.64**) to form B-C fragments **4.3** and **4.4** followed by amide coupling with fragment A (**2.62**) to yield analogs **2.60** and **2.61**. Both strategies were examined during the course of this project. Progress toward the synthesis of all mycobactin analogs will be described in this chapter. Other areas that will be detailed in this chapter include an investigation of an alternative strategy toward mycobactin analogs, recommendations for future studies, and a report on the anti-TB and anticancer activity of all tested intermediates and mycobactin analogs.



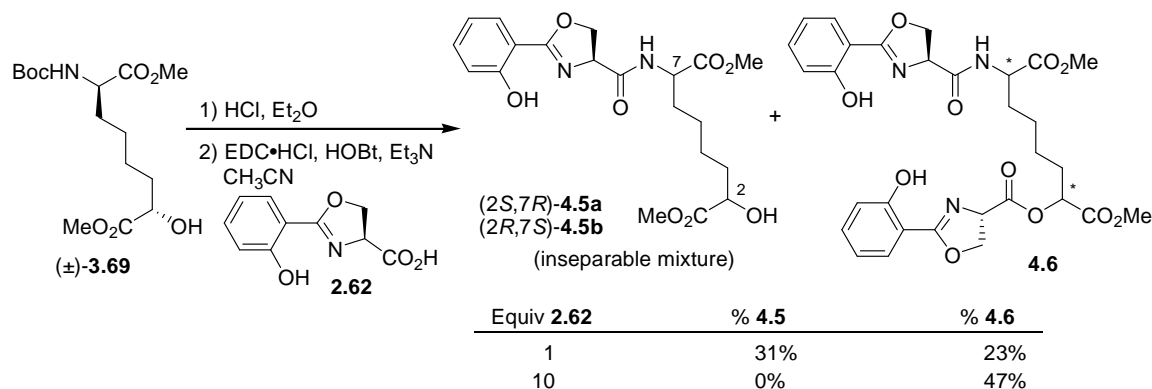
Scheme 4.1. Two strategies toward fragment assembly

4.2 Synthesis of mycobactic acid analogs: assembly of A and B fragments

4.2.1 Direct coupling of fragments A and B

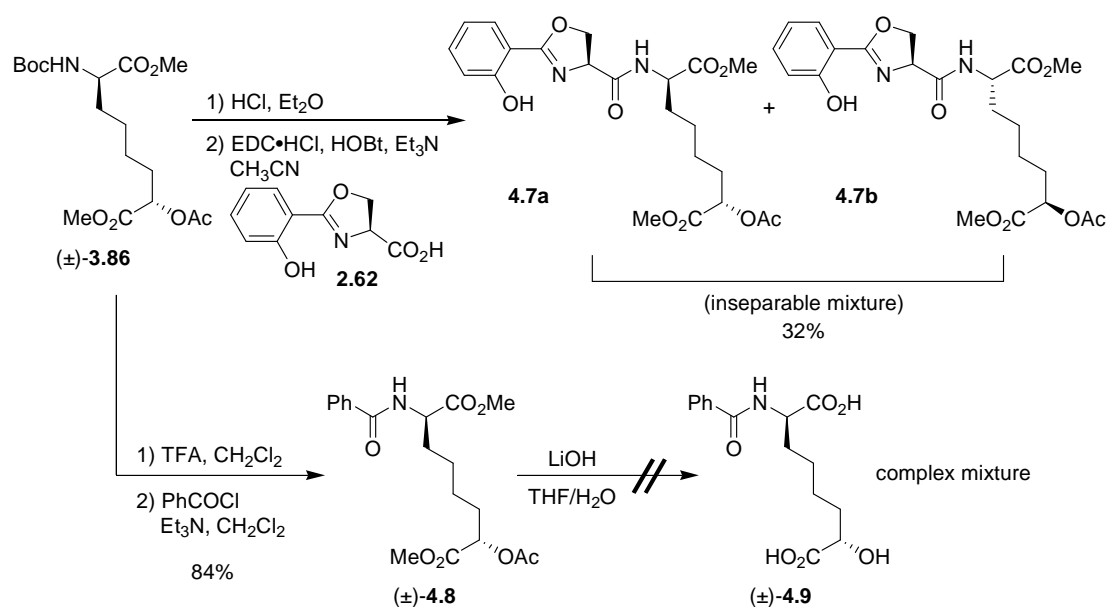
A-B fragments, termed “mycobactic acids” in the literature,^{257, 296-299, 306} were prepared through the direct coupling of fragment A (**2.62**) with either α -hydroxy carboxylate fragment B compounds (**3.69** and **3.86**) or 1,2-diol fragment B compound **3.99**. Initially, the synthesis of α -hydroxy carboxylate-containing mycobactic acids was investigated. The Boc-protecting group of α -hydroxy ester **3.96** was removed using HCl

in ether followed by EDC-mediated coupling with oxazoline **2.62** (Scheme 4.2). A mixture of amides **4.5a** and **4.5b** was obtained along with the diacylated compound **4.6**. Using an excess of oxazoline **2.62**, only diacylated compound **4.6** was obtained in moderate yield.



Scheme 4.2. Synthesis of α -hydroxy carboxylate mycobactic acid analogs

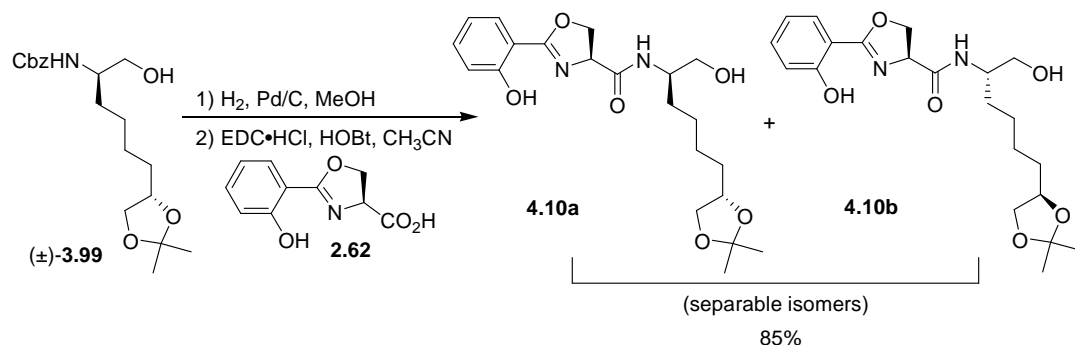
To avoid the isolation of the diacylation product **4.6**, α -acetoxy ester **3.86** was treated under the same conditions (Scheme 4.3). A low yield of mycobactic acid analogs **4.7a** and **4.7b** was obtained as an inseparable mixture of diastereomers. Compound **3.86** was also coupled to benzoic acid and provided diester compound **4.8** in good yield.



Scheme 4.3. Synthesis of α -acetoxy carboxylate mycobactic acid analogs

The attempted global deprotection of compound **4.8** by hydrolysis was unsuccessful. Consequently, compounds **4.5a**, **4.5b**, **4.7a**, and **4.7b** were left protected until conditions for deprotection were optimized.

Protected 1,2-diol compound **3.99** was deprotected by hydrogenolysis and the free amine was subsequently coupled to oxazoline **2.62** using a carbodiimide-mediated coupling reaction (Scheme 4.4). Mycobactic acid analogs **4.10a** and **4.10b** were obtained in excellent yield as a separable mixture of diastereomers.



Scheme 4.4. Synthesis of protected 1,2-diol mycobactic acid analogs

4.2.2 Mycobactic acid analogs from lysine

While the focus of this project was the synthesis of α -hydroxy carboxylate- and 1,2-diol-containing mycobactin analogs, other analogs were also envisioned. We hypothesized that fragment B analogs **4.12** and **4.13** would be easily synthesized from *L*-lysine and provide access to alternative mycobactic acid analogs (Figure 4.1).

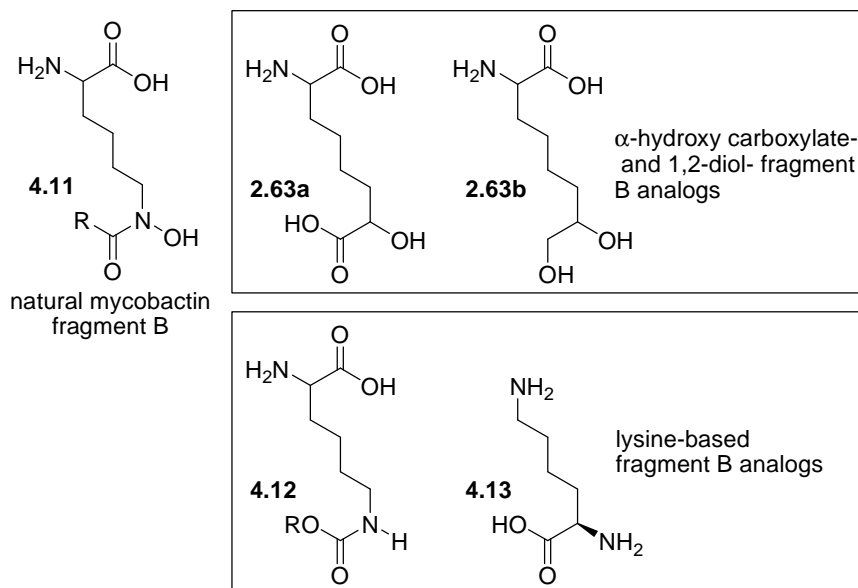
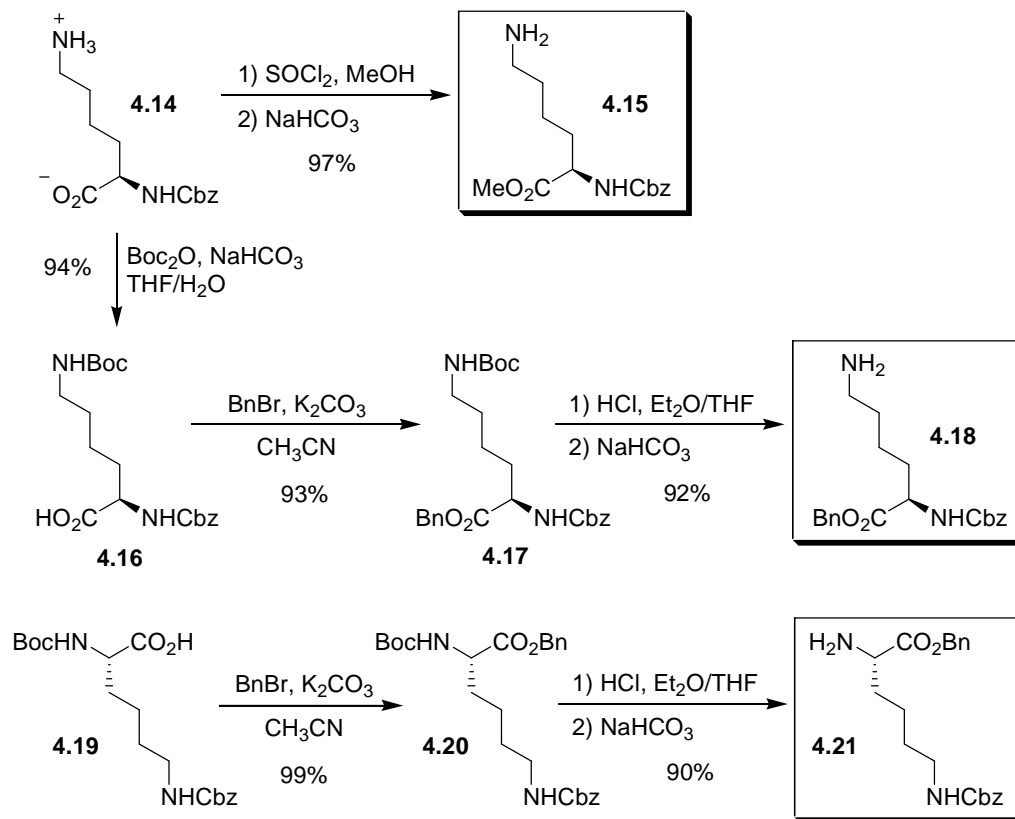


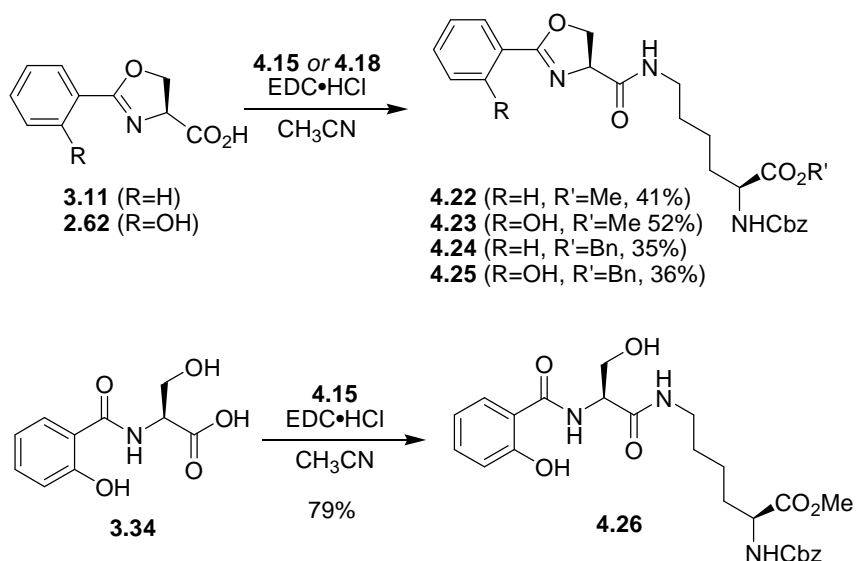
Figure 4.1. Lysine-derived analogs of fragment B

Lysine-derived fragment B analogs **4.15**, **4.18**, and **4.21** were readily synthesized from protected lysine derivatives **4.14** and **4.19** (Scheme 4.5). Cbz-protected lysine **4.14** was treated with thionyl chloride in methanol, which upon neutralization yielded amine **4.15**. Protection of Cbz-lysine **4.14** yielded the orthogonally-protected lysine **4.16** that was transformed into benzyl ester **4.17**. Deprotection and neutralization of lysine **4.17** yielded amine **4.18**. Amine **4.21** was prepared similarly from the orthogonally protected lysine **4.19** through the benzyl ester intermediate **4.20** in excellent yield.

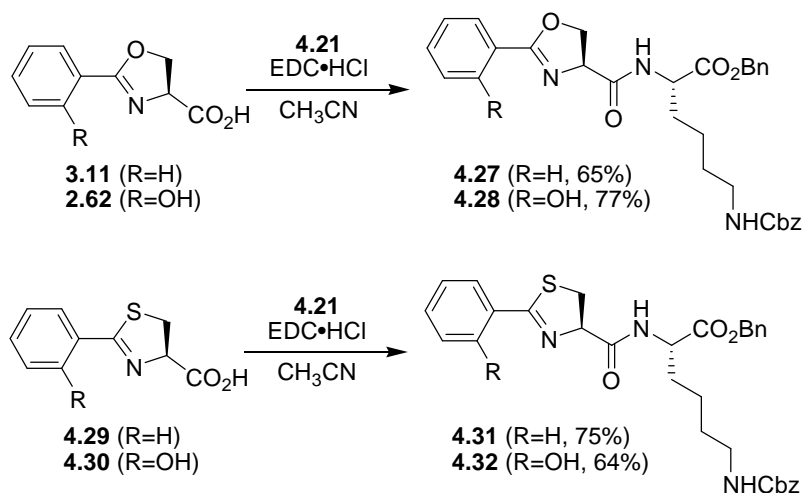


Scheme 4.5. Synthesis of lysine-derived fragment B analogs

With lysine-derived amines **4.15** and **4.18** in hand, EDC-mediated coupling reactions with carboxylic acids **3.11**, **2.62**, or **3.34** yielded the amides **4.22-4.26** in moderate to excellent yields (Scheme 4.6). Similarly, amine **4.21** was coupled to oxazoline carboxylates **3.11** and **2.62** as well as thiazoline carboxylates **4.29** and **4.30** and provided amides **4.27**, **4.28**, **4.31**, and **4.32** (Scheme 4.7).

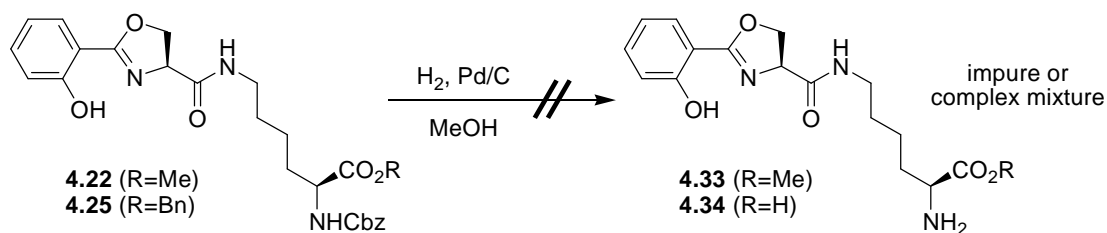


Scheme 4.6. Synthesis of lysine-derived mycobactin analogs



Scheme 4.7. Synthesis of more lysine-derived mycobactin analogs

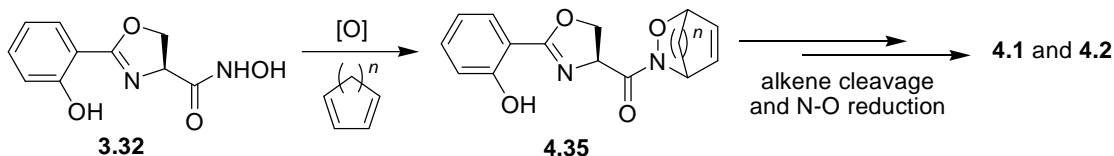
While the synthesis of mycobactin analogs using amines **4.15**, **4.18**, and **4.21** was not attempted, we wanted to prepare α -amino ester **4.33** and α -amino acid **4.34** for purposes of biological evaluation (Scheme 4.8). Hydrogenolysis of **4.22** and **4.25** appeared to proceed to completion; however, compounds **4.33** and **4.34** were obtained along with complex mixtures of products.



Scheme 4.8. Failed deprotections of lysine-derived mycobactin acid analogs

4.2.3 Alternative strategies toward A-B fragments

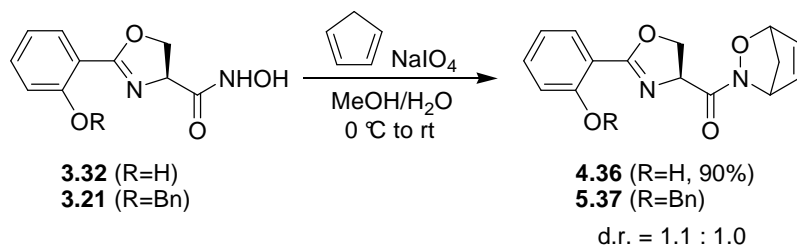
While the strategies toward mycobactin analogs **2.60** and **2.61** outlined in Scheme 4.1 are straightforward and were ultimately successful, we also examined alternative strategies toward the synthesis of mycobactin analogs. One strategy initially investigated was to perform the acylnitroso cycloaddition using hydroxamate **3.32** and “build-in” fragment B from cycloadducts **4.35** (Scheme 4.9). Efforts toward the investigation of this strategy are described in this section.



Scheme 4.9. An alternative strategy toward mycobactin acid analogs

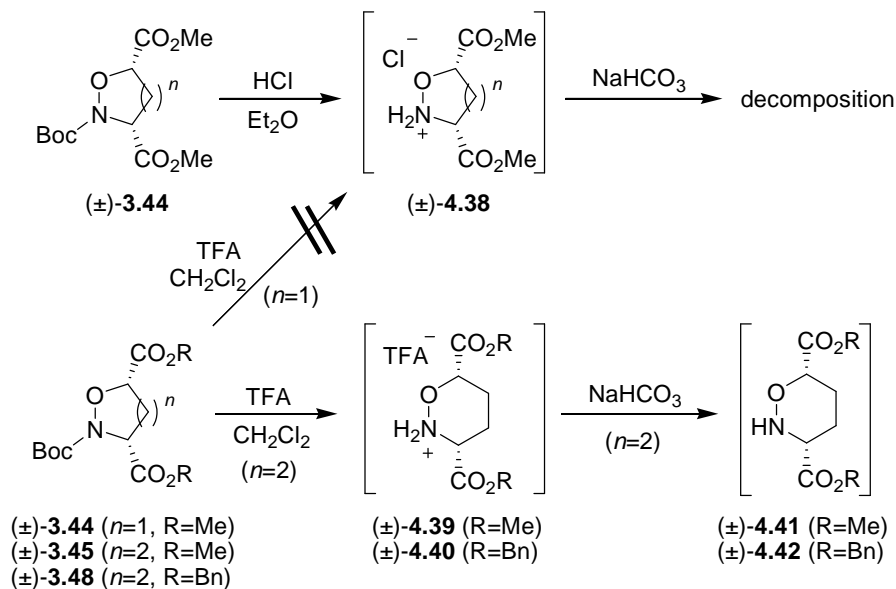
The acylnitroso HDA reactions of hydroxamates **3.32** and **3.21** were studied using cyclopentadiene (Scheme 4.10). We were pleased to find that treatment of hydroxamates **3.32** and **3.21** with sodium periodate in the presence of cyclopentadiene provided cycloadducts **4.36**, and **4.37** in excellent yield. Amino acid-derived acylnitroso species have been used as dienophiles in diastereoselective nitroso HDA reactions;^{3, 65, 66}

however, no diastereoselectivity was observed for either acylnitroso species derived from hydroxamates **3.32** or **3.21**.



Scheme 4.10. Acylnitroso HDA reactions of fragment A hydroxamates

Based on unpublished results from the Miller group, we hypothesized that the phenol functional group of cycloadducts **4.36** was potentially problematic for oxidative cleavage reactions. To avoid these potential problems, coupling reactions using diester compounds **3.44**, **3.45**, and **3.48** were investigated (Scheme 4.11). Deprotection of compounds **3.45** and **3.48** with TFA yielded hydroxylamine salts **4.39** and **4.40**. When hydroxylamine **3.44** was treated with the same conditions, decomposition was observed; however, deprotection of compound **3.44** was effected using HCl gas to provide the hydrochloride salt **4.38**. Storage of amine salts **4.38-4.40** resulted in decomposition, therefore amine salts **4.39** and **4.40** were treated with sodium bicarbonate to yield the free amines **4.41** and **4.42**, which were isolated as meta-stable compounds. Treatment of hydrochloride salt **4.38** with bicarbonate resulted in decomposition.

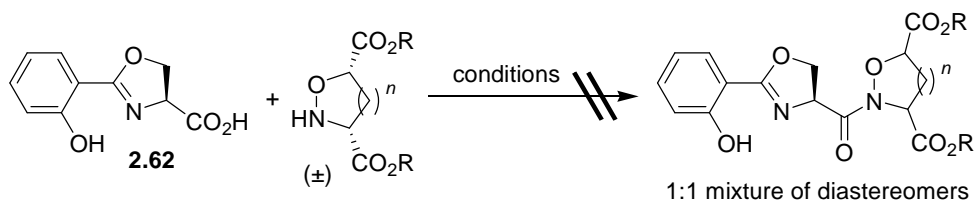


Scheme 4.11. Deprotection of hydroxylamine fragments

Reaction conditions for coupling cyclic hydroxylamines **4.38**, **4.41**, and **4.42** to oxazoline carboxylate **2.62** were examined (Table 4.1). The use of EDC in acetonitrile did not facilitate amide bond formation and resulted in either no reaction or decomposition (Table 4.1, entries 1, 3, and 5). EDC-mediated coupling in aqueous conditions was only effective for hydroxylamine salt **4.38** and provided hydroxamate **4.43** in low yield (Table 4.1, entry 2).

TABLE 4.1

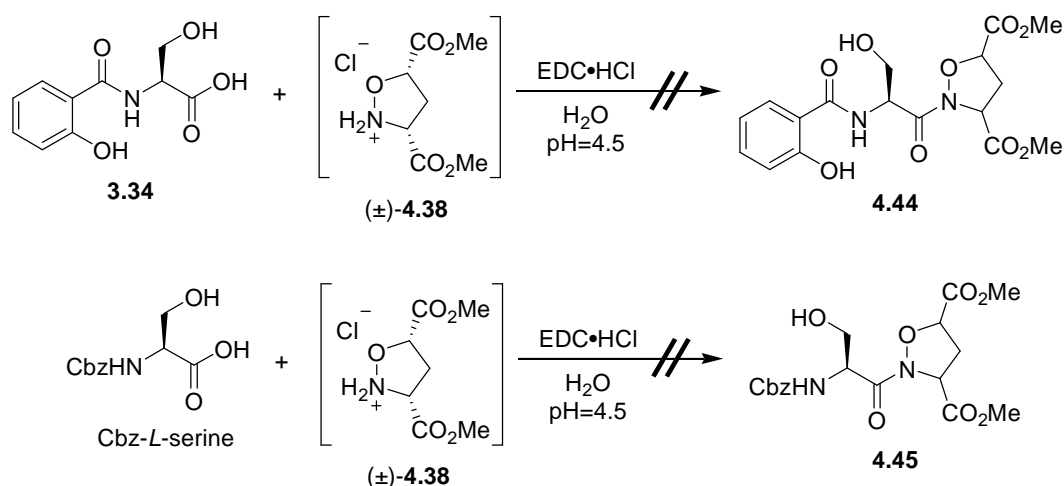
ATTEMPTED COUPLING REACTIONS WITH CYCLIC HYDROXYLAMINES



| Entry | <i>n</i> | R | amine form | conditions | Product (yield)/Result |
|-------|----------|----|------------|--|--------------------------|
| 1 | 1 | Me | HCl salt | EDC•HCl, Et ₃ N, CH ₃ CN | complex mixture |
| 2 | 1 | Me | HCl salt | EDC•HCl, H ₂ O, pH=4.5 | 4.43 ^a |
| 3 | 2 | Me | free base | EDC•HCl, Et ₃ N, CH ₃ CN | complex mixture |
| 4 | 2 | Me | free base | EDC•HCl, H ₂ O, pH=4.5 | N.R. ^b |
| 5 | 2 | Bn | free base | EDC•HCl, Et ₃ N, CH ₃ CN | N.R. ^b |
| 6 | 2 | Bn | free base | EDC•HCl, H ₂ O, pH=4.5 | N.R. ^b |

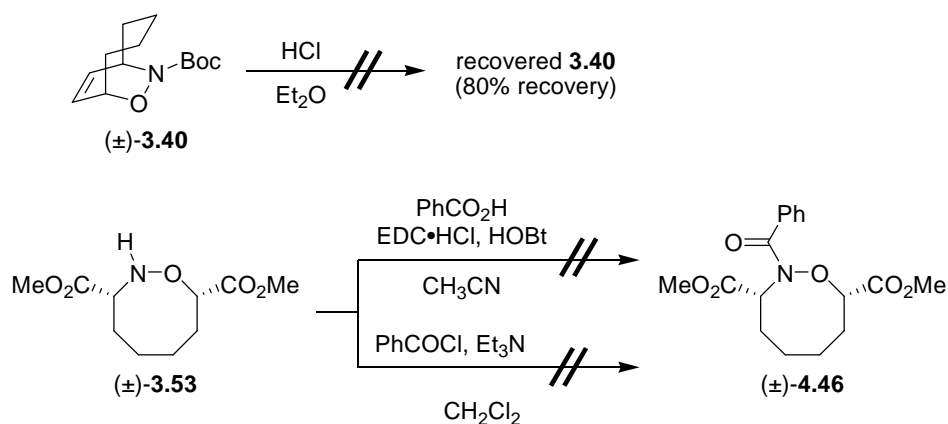
NOTE: (a) Desired product was detected by nominal MS. Purification afforded 20% yield after purification; however, compound was impure by NMR. (b) N.R.=no reaction observed.

The use of aqueous EDC-mediated coupling conditions for amine salt **4.38** was also examined for other carboxylic acids (Scheme 4.12). Carboxylic acid **3.34** failed to provide compound **4.44** when subjected to EDC and amine **4.38**. As a point of comparison, Cbz-*L*-serine was treated with the same conditions and also failed to provide the desired hydroxamate product **4.45**.



Scheme 4.12. Failed coupling reactions with *N*-acylated serine derivatives

The use of similar strategies was explored with cyclooctadiene cycloadduct **3.40** and diester compound **3.53** (Scheme 4.13). Surprisingly, the Boc-group of cycloadduct **3.40** was resistant to deprotection using standard conditions and 80% of compound **3.40** was recovered after treatment with excess HCl in ether. Similarly, cyclic hydroxylamine **3.53** resisted *N*-acylation with benzyl chloride or with EDC-mediated coupling with benzoic acid.



Scheme 4.13. Attempted coupling of cyclooctadiene-derived hydroxylamines

The surprising amount of resistance exhibited by hydroxylamines **4.38**, **4.41**, **4.42**, and **3.53** to *N*-acylation may be attributed to the steric effect of the neighboring carboxylate group (Figure 4.2). The carboxylate group is expected to be spatially most distant from the hydroxylamine group in compound **4.38**, and closest to the hydroxylamine group in compound **3.53**. If reactivity is based upon a steric effect, this might explain the observed increase in reactivity of the hydroxylamine compounds. This effect may also explain the resistance to N-O bond reduction along with the unusual stability observed for compound **3.53**.

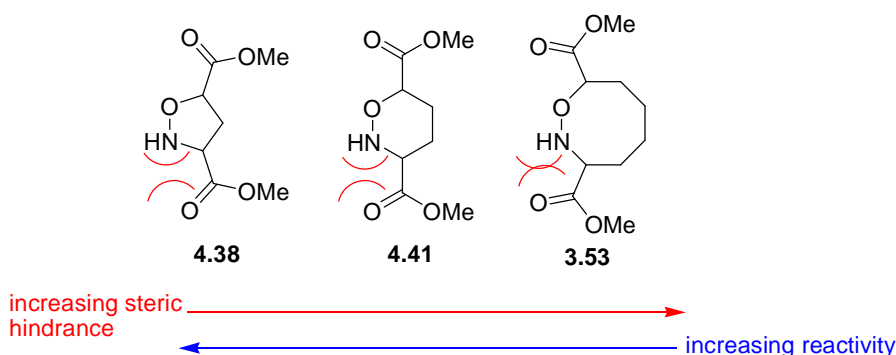


Figure 4.2. Reasoning for decreasing reactivity of diester compounds

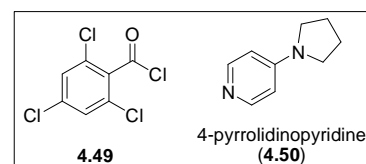
4.3 Preparation of mycobactin analogs

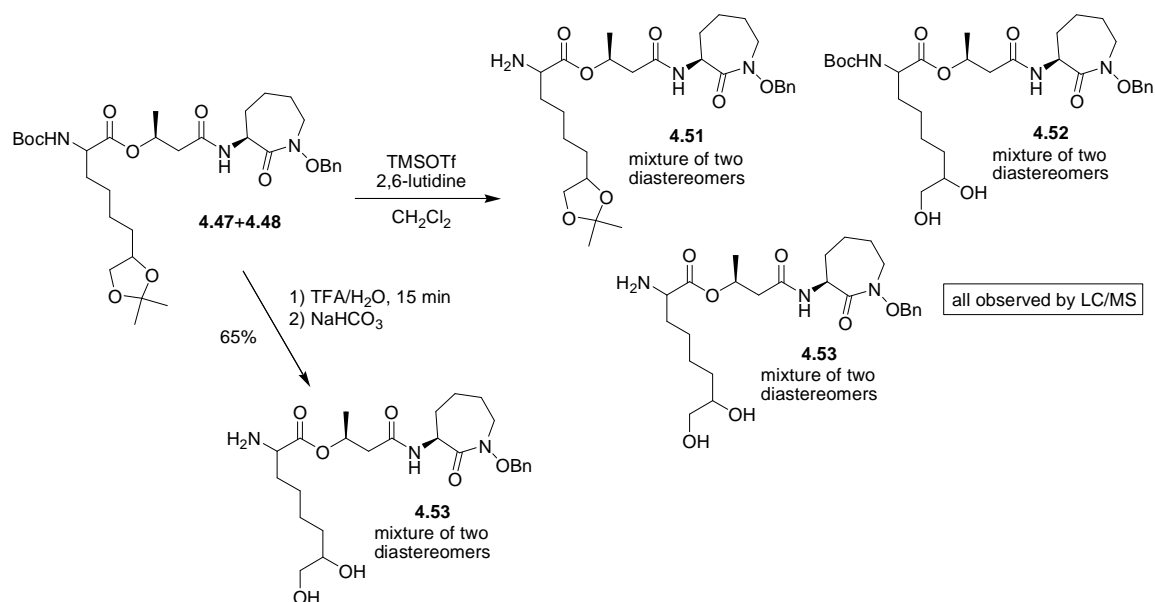
4.3.1 Synthesis of 1,2-diol-based mycobactin S analogs

1,2-Diol-based mycobactin analogs **2.61** were prepared by forming the central ester bond first followed by amide coupling with fragment A (**2.62**). Various esterification conditions were investigated for preparing B-C fragments **4.4** (Table 4.2). All conditions tested used 1 equivalent each of racemic carboxylic acid **3.101** and

protected cobactin S fragment **3.125** to yield the two diastereomers **4.47** and **4.48**. Esterification using 2,4,6-trichlorobenzoyl chloride (**4.49**) and triethylamine (conditions developed by Yamaguchi)³⁵² provided the two esters **4.47** and **4.48** in 54% yield (Table 4.2, entry 1). Equally effective was the use of 2 equivalents of water-soluble carbodiimide and 30 mol% of 4-pyrrolidinopyridine (**4.50**)³⁵³ (Table 4.2, entry 4); however, the use of less EDC and **4.50** gave esters **4.47** and **4.48** in lower yield (Table 4.2, entry 3). The central ester of mycobactin analogs has been synthesized by Miller using a Mitsunobu esterification method.²⁹⁶ Although the use of this method did yield the desired ester products, the reaction was not very clean and difficult to purify (Table 4.2, entry 2). Optimally, the esters **4.47** and **4.48** were synthesized using an excess of water-soluble carbodiimide and either 1 equivalent of DMAP^{354, 355} or a mixture of DMAP and 4-pyrrolidinopyridine (**4.50**) (Table 4.2, entries 5 and 6).

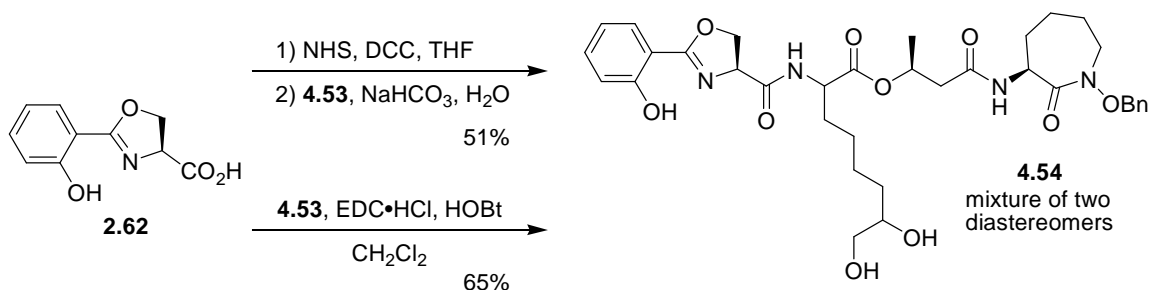
TABLE 4.2





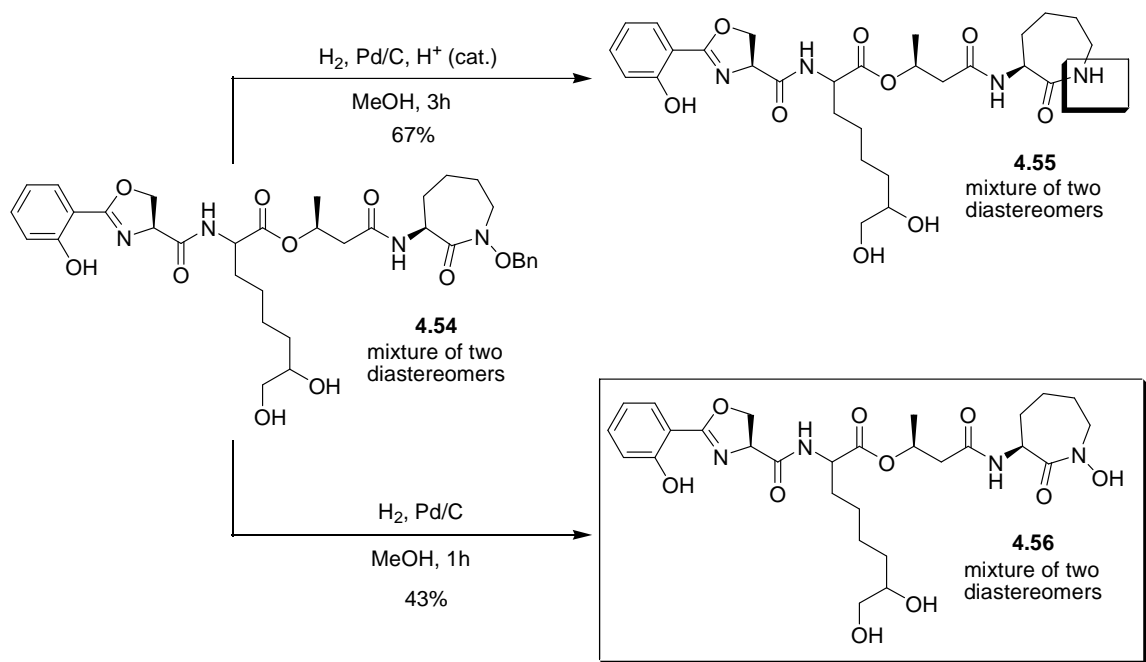
Scheme 4.14. Acidic deprotections of B-C fragments **4.47** and **4.48**

Compound **4.53** was used directly without purification for the subsequent carbodiimide-mediated coupling with oxazoline carboxylate **2.62** (Scheme 4.15). The use of DCC and NHS as activating agents for the peptide ligation provided the desired amide **4.54**; however, even after purification the product was contaminated with a substantial amount of the DCC by-product, dicyclohexylurea (DCU). Preferably, the peptide ligation was effected using water-soluble carbodiimide and HOBt to provide amide **4.54** in higher yield.



Scheme 4.15. Synthesis of mycobactin core structure

The removal of the benzyl group of compound **4.54** by hydrogenolysis was assumed to be relatively straight-forward; however, a fair amount of optimization was required before arriving at conditions that provided the desired compound **4.56** (Scheme 4.16). Compound **4.54** was stirred with 15 wt% of Pd/C (15 wt/%) in a hydrogen atmosphere, but the starting compound **4.54** was recovered unchanged from the reaction after 8 h. With the addition of a catalytic amount of HCl or HOAc, compound **4.54** was completely consumed in 3 h. Upon further investigation, we realized that the N-O bond had reduced under the acidic conditions and provided the amide compounds **4.55** rather than the desired hydroxamate **4.56**.

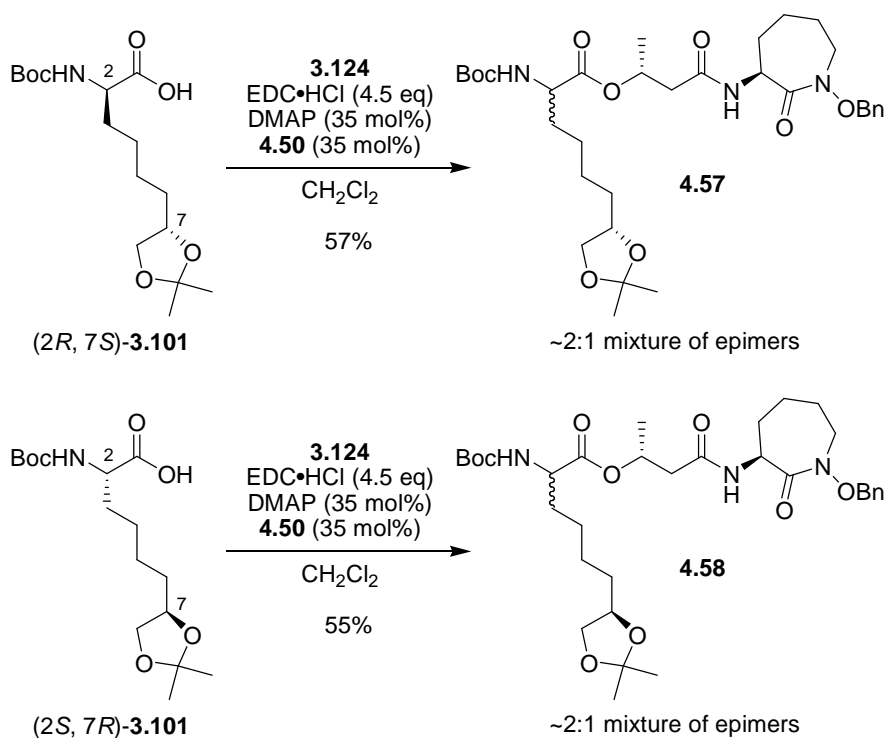


Scheme 4.16. Synthesis of 1,2-diol analogs of mycobactin S

Increasing the amount of Pd/C catalyst to 30 wt% rather than 15 wt% allowed for the clean conversion of compound **4.54** to the desired hydroxamate **4.56** in under 60 minutes; however, the amide by-product **4.55** was also obtained. We were prepared to purify compound **4.56** using preparatory HPLC; however, upon attempted dissolution of the reaction mixture in acetonitrile, white solid remained un-dissolved. Separate analyses of the filtrate and the solid revealed that the solid compound was composed entirely of the desired, pure hydroxamate compound **4.56**! This serendipitous discovery allowed for the purification of the final compound **4.56** by trituration with acetonitrile.

4.3.2 Synthesis of 1,2-diol-based mycobactin T analogs

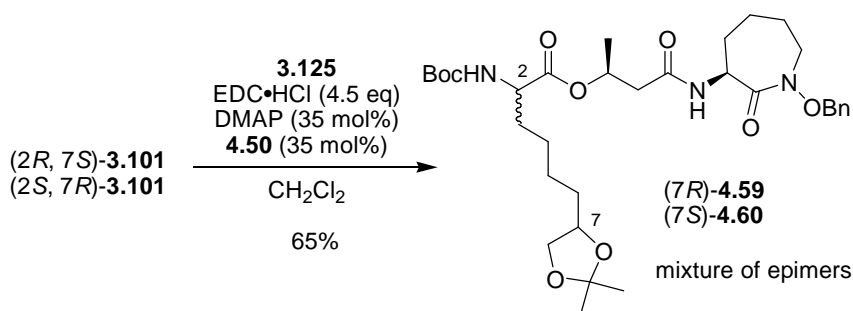
While the target 1,2-diol mycobactin S analog **4.56** was prepared as a mixture of two diastereomers using racemic B fragment **3.101**, we sought to prepare stereochemically pure 1,2-diol mycobactin T analogs from the pure enantiomers of **3.101**. Using the same procedures as outlined above, enantiomerically pure B fragments **3.101** were coupled to the protected cobactin T compound **3.124** (Scheme 4.17).



Scheme 4.17. Esterification of enantiomerically pure B fragments

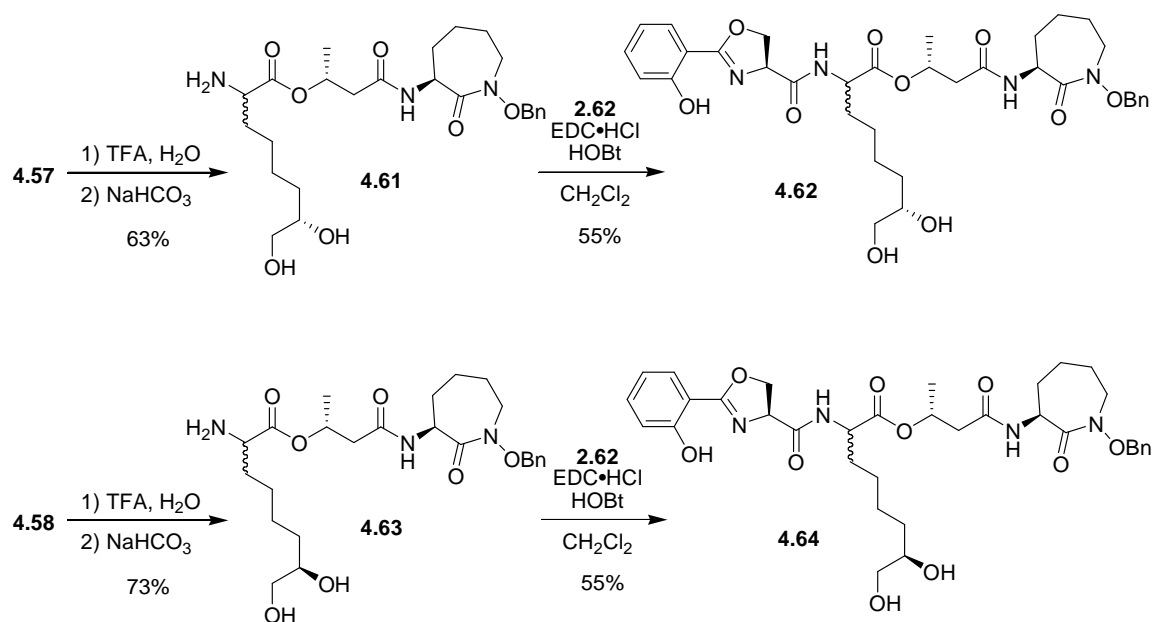
Close inspection of the ^{13}C NMR spectra of esters **4.57** and **4.58** revealed the presence of additional signals in the spectra. Analysis of COSY and HETCOR spectra for esters **4.57** and **4.58** indicated epimerization had occurred at the stereocenter adjacent to the ester carbonyl. This result was deduced by comparing the ^{13}C NMR spectra of **4.57** and **4.58** with esters **4.47** and **4.48**. Based on $\Delta\delta$ values for the epimers of **4.57** and **4.58** as well as the tendency of carbodiimide-mediated coupling reactions to lead to epimerization, we hypothesized that epimerization occurred at C^2 . This epimerization was not observed in the ^{13}C NMR spectrum for the mixture of **4.47** and **4.48**. Careful analysis of cobactins **3.124** and **3.125** as well as the enantiopure and racemic forms of B fragments **3.101** did not show evidence of epimerization occurring prior to esterification. In order to compare directly to the NMR spectrum for the mixture of esters **4.47** and **4.48**,

enantiopure B fragments **3.101** were subjected to the esterification conditions using protected cobactin S compound **3.125** to yield esters **4.59** and **4.60** (Scheme 4.18). As before, the ^{13}C NMR spectra of **4.59** and **4.60** were compared to the mixture of **4.47** and **4.48**. The signals of the epimeric compounds in **4.59** and **4.60** did not overlap with the mixture of esters **4.47** and **4.48**. The site of epimerization was again hypothesized to be C².



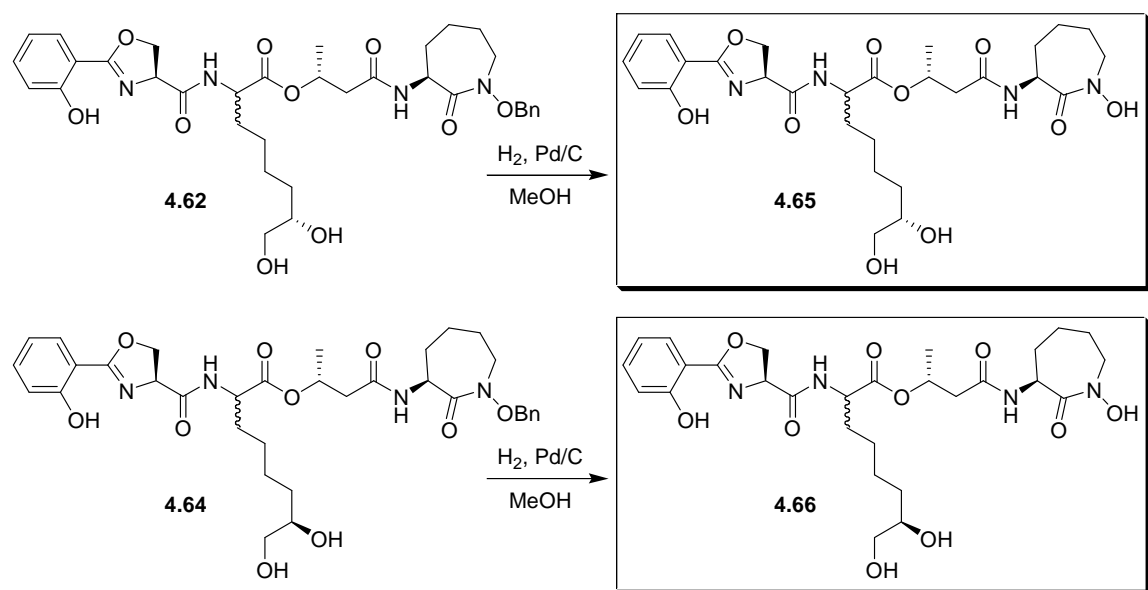
Scheme 4.18. Esterification using cobactin S fragment

Esters **4.57** and **4.58** were treated with aqueous TFA and neutralized after 15 minutes in order to provide compounds **4.61** and **4.63** in excellent yield (Scheme 4.19). Water-soluble carbodiimide-mediated coupling with oxazoline carboxylate **2.62** in the presence of HOBt yielded amides **4.62** and **4.64**.



Scheme 4.19. Assembly of A, B, and C fragments of mycobactin T analogs

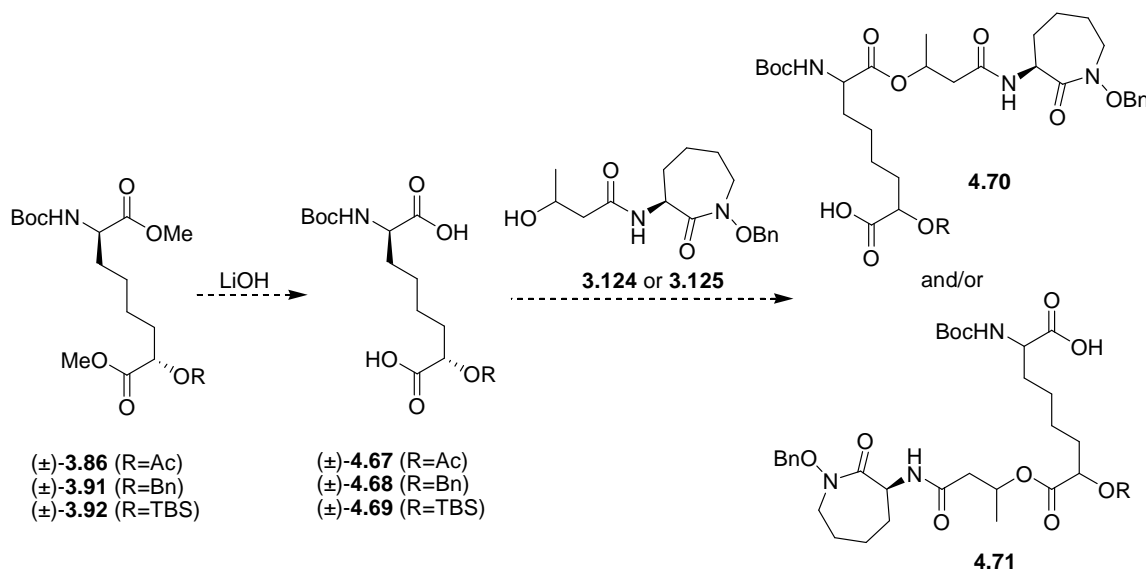
The final deprotection step in the synthesis of 1,2-diol-based mycobactin T analogs was carried out using the same method developed for the synthesis of mycobactin S analog **4.56**. Hydrogenolysis of compounds **4.62** and **4.64** provided hydroxamates **4.65** and **4.66** (Scheme 4.20).



Scheme 4.20. Synthesis of 1,2-diol analogs of mycobactin T

4.3.3 Proposed routes to α -hydroxy carboxylate analogs

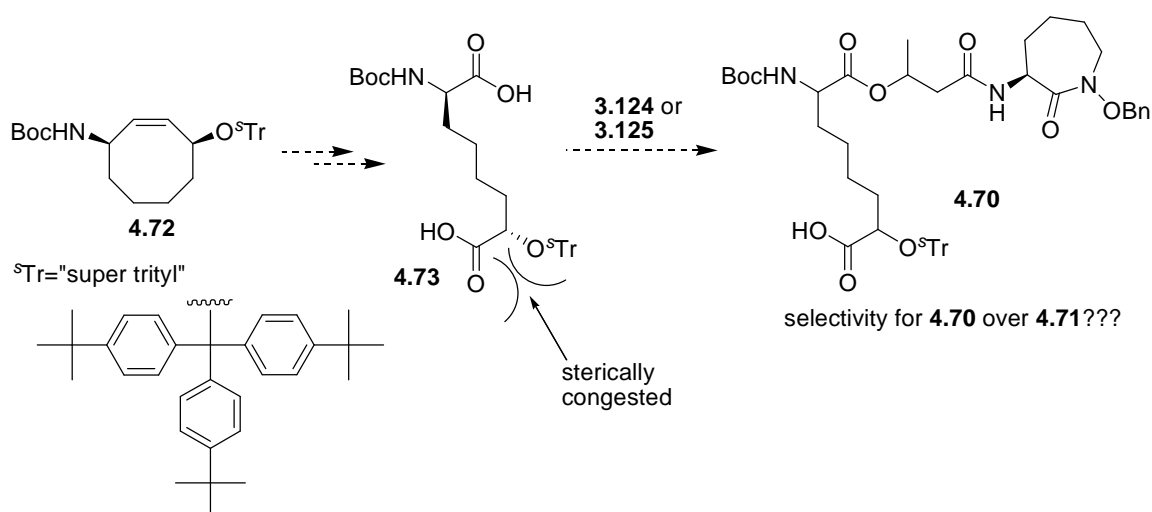
At this time, α -hydroxy carboxylate mycobactin analogs **2.60** have not been synthesized. Direct coupling of α -hydroxy carboxylate-containing B fragments **2.63** or mycobactinic acid analogs **4.1** to protected cobactins **3.124** or **3.125** presents would be problematic and unselective (Scheme 4.21). Protected α -hydroxy esters **3.86**, **3.91**, and **3.92** could be converted to the unprotected compounds **4.67-4.69**; however, esterification with cobactins **3.124** or **3.125** would provide mixtures of the esters **4.70** and **4.71**.



Scheme 4.21. The problem of differentiating carboxylic acid groups

While it is possible that the neighboring α -hydroxy, alkoxy, or acetoxy group would provide some role in providing selectivity for **4.70** over **4.71**, a larger group may be necessary to increase this selectivity. One strategy may involve the use of the tris(4-

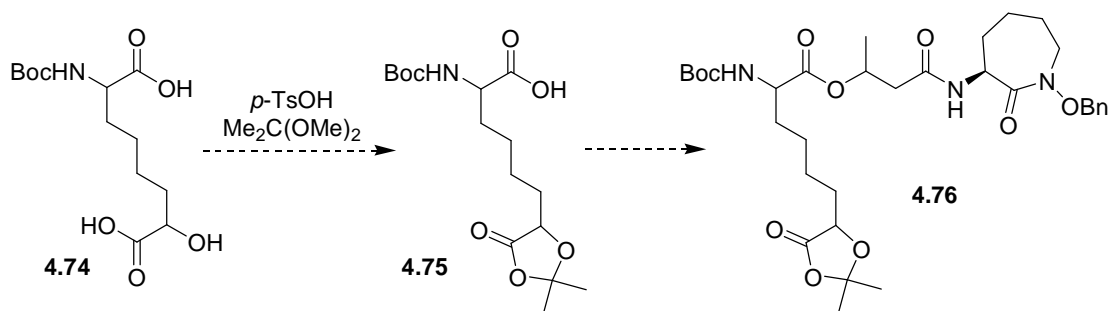
tert-butylphenyl)methyl group, or “supertrityl” (^sTr) group, as a protecting group for the alcohol as in compound **4.72** (Scheme 4.22). Designed initially by Stoddart³⁵⁷ as a cap for assembling rotaxanes, the supertrityl group has subsequently been used to differentiate the functionalization of primary hydroxyl groups of cyclodextrins.³⁵⁸ The bulky supertrityl group should therefore sterically hinder esterification at the adjacent carboxylic acid to selective provide ester **4.70**.



Scheme 4.22. Strategy for blocking esterification based on sterics

Alternatively, esterification of the α -hydroxy carboxylate group could be blocked through the preparation of a 1,3-dioxolan-4-one (Scheme 4.23). The synthesis of 1,3-dioxolan-4-ones from α -hydroxy carboxylates has been documented in the literature,³⁵⁹⁻³⁶¹ and application of this method to compound **4.74** will yield 1,3-dioxolan-4-one **4.75**. Esterification with cobactins **3.124** or **3.125** as outlined above (section 4.3.2) should provide ester **4.76** and ultimately α -hydroxy carboxylate mycobactin analogs **2.60**.

Other methods toward α -hydroxy carboxylate analogs may also involve continued investigation of compounds that present differentially protected hydroxyl or carboxylate groups such bicyclic hydroxamate **3.59** or diol **3.106** (described in chapter 3). Unfortunately, time constraints did not allow for a full investigation into the potential of these intermediates toward the synthesis of α -hydroxy carboxylate analogs of mycobactins (**2.60**).



Scheme 4.23. Strategy for blocking esterification based on protecting groups

4.4 Biological activity of compounds

All fragments, analogs, and synthetic intermediates were tested for growth inhibitory activity against *M. tuberculosis* strain H₃₇Rv (American Type Culture Collection, Rockville, MD) using the Microplate Alamar Blue Assay (MABA)^{362, 363} following incubation for one week in glycerol-alanine-salts medium (GAS) as well as in GAS medium without added iron and with added Tween 80 (GAST).²⁷⁹ Additionally, activity against non-replicating *M. tuberculosis* was determined using the low oxygen recovery assay (LORA)³⁶⁴ by exposing low oxygen-adapted *M. tuberculosis* containing a luciferase gene to test compounds under anerobic conditions for 10 days. After 28 h of

recovery in air, the luciferase signal was measured and MIC values were reported. General toxicity of compounds was assessed using a standard VERO cell assay.

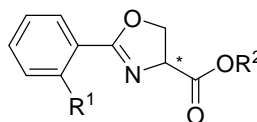
Many compounds were also tested against prostate and breast cancer lines, PC-3 and MCF-7 cells, respectively. Most compounds tested were inactive in all assays; however, a number demonstrated moderate to high potency in assays and are represented in this section.

4.4.1 Mycobactin fragments and analogs

As expected based on earlier tests of oxazoline **3.8** (see chapter 3), fragment A analogs demonstrated growth inhibitory activity against *M. tuberculosis* (Table 4.3). A large number of other fragment A analogs with structures similar to **3.8** have been synthesized by other members of the Miller group but are not reported here. Clear trends have not emerged from the limited SAR data surrounding this class of molecules. Data from collaborators has indicated that oxazoline **3.8** affects genes associated with mycobactin production in *M. tuberculosis*.

TABLE 4.3

BIOLOGICAL ACTIVITY OF FRAGMENT A ANALOGS



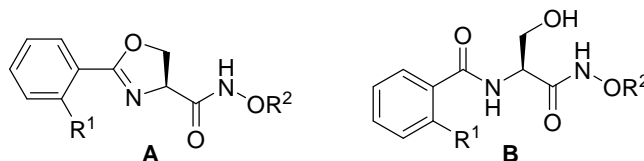
| Entry | Compound | R ¹ | R ² | * | anti-TB (MIC values) | | | PC-3 | tox. (IC ₅₀) |
|-------|-------------|----------------|----------------|---|----------------------|-----------|-----------|---------------------------|--------------------------|
| | | | | | GAS (μM) | GAST (μM) | LORA (μM) | % inhibition 20 μM (96 h) | VERO (μM) |
| 1 | 3.8 | OBn | Bn | S | 7.4 | 7.2 | 50.2 | 0% | >128 |
| 2 | 3.16 | OBn | Bn | R | 3.1 | 1.3 | >128 | ND | >128 |
| 3 | 3.20 | OBn | Me | S | 118 | >128 | ND | 0% | ND |
| 4 | 3.10 | H | Bn | S | 5.3 | 3.8 | 63.0 | 0% | >128 |
| 5 | 3.15 | H | Bn | R | 2.0 | 1.0 | 58.8 | ND | >128 |
| 6 | 3.11 | H | H | S | >128 | >128 | >128 | ND | ND |
| 7 | 2.62 | OH | H | S | >128 | >128 | ND | 0% | ND |

NOTE: ND = not determined. MIC values are reported at 90% relative to controls with no added test compound.

Hydroxamate analogs of fragment A (syntheses reported in chapter 3) were inactive against *M. tuberculosis*, but surprisingly demonstrated anticancer activity against PC-3 cells (Table 4.4). Compound **3.28** demonstrated the highest potency against PC-3 cells with an estimated IC₅₀ value of 11 μM (Table 4.4, entry 1). Analog **3.36**, which lacks the oxazoline ring, was inactive in the anticancer assay (Table 4.4, entry 2). Additionally, hydroxamic acid **3.32** was far less potent than benzyl hydroxamate **3.28** (Table 4.4, entries 1 and 3). Finally, hydroxamate **3.28** also demonstrated antifungal activity against a variety of strains relevant to the agrochemical industry (data not shown/provided).

TABLE 4.4

BIOLOGICAL ACTIVITY OF HYDROXAMATE FRAGMENT A ANALOGS



| Entry | Compound | Structure | R ¹ | R ² | anti-TB (MIC values) | | | PC-3 | |
|-------|-------------|-----------|----------------|----------------|----------------------|-----------|-----------|---------------------------|-----------------------|
| | | | | | GAS (μM) | GAST (μM) | LORA (μM) | % inhibition 20 μM (96 h) | IC ₅₀ (μM) |
| 1 | 3.28 | A | OH | Bn | 127 | >128 | >128 | 94% | 11 |
| 2 | 3.36 | B | OH | Bn | ND | ND | ND | 0% | ND |
| 3 | 3.32 | A | OH | H | >128 | >128 | >128 | 20% | ND |
| 4 | 3.29 | A | H | Bn | >128 | >128 | >128 | 2% | ND |
| 5 | 3.35 | B | H | Bn | ND | ND | ND | 0% | ND |

NOTE: ND = not determined. MIC values are reported at 90% relative to controls with no added test compound. IC₅₀ values are estimated.

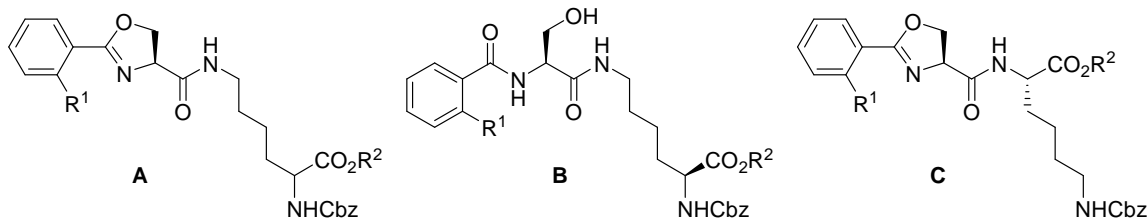
Analogues of fragments B and C were found to be generally inactive against *M. tuberculosis* and PC-3 cancer cell lines.

4.4.2 Assembled fragments and analogs

Lysine-derived mycobactric acid analogs surprisingly demonstrated moderate anticancer activity (Table 4.5), and were found not to demonstrate growth inhibitory activity against *M. tuberculosis*. The mode of action for these molecules was not studied; however, continued study of this class of molecules as possible anticancer agents is greatly encouraged.

TABLE 4.5

BIOLOGICAL ACTIVITY OF LYSINE-DERIVED MYCOBACTIC ACID ANALOGS



| Entry | Compound | Structure | R ¹ | R ² | PC-3 | |
|-------|----------|-----------|----------------|----------------|-----------------------------------|--------------------------------|
| | | | | | % inhibition 20 μ M (96 h) | IC ₅₀ (μ M) |
| 1 | 4.23 | A | OH | Me | 93% | 8 |
| 2 | 4.22 | A | H | Me | 76% | 37 |
| 3 | 4.25 | A | OH | Bn | 92% | 15 |
| 4 | 4.24 | A | H | Bn | 93% | 17 |
| 5 | 4.26 | B | OH | Me | 19% | ND |
| 6 | 4.28 | C | OH | Bn | 93% | 2.5 |
| 7 | 4.27 | C | H | Bn | 82% | 15 |

NOTE: ND = not determined. IC₅₀ values are estimated.

Mycobactic acid analogs **4.5** and **4.6** did not demonstrate anti-TB activity; however, compound **4.6** showed moderate growth inhibitory activity against MCF-7 cells (Figure 4.3). At the time this dissertation was submitted, the biological activity of most mycobactin analogs had not been received; however, compounds **4.54**, **4.62**, and **4.56** were found to be inactive against PC-3 and MCF-7 cancer cell lines.

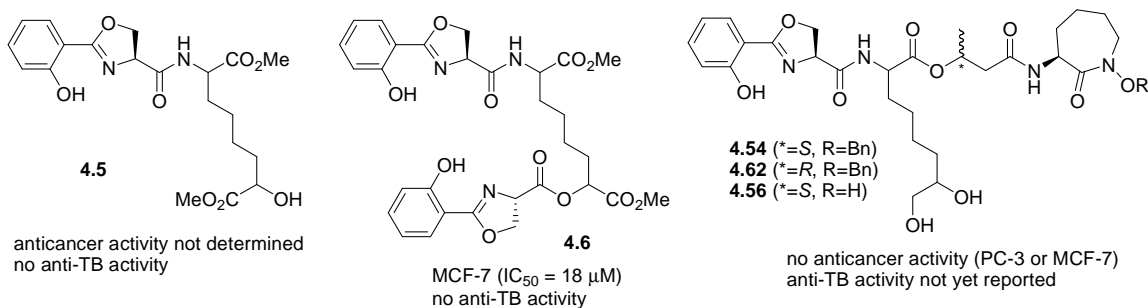
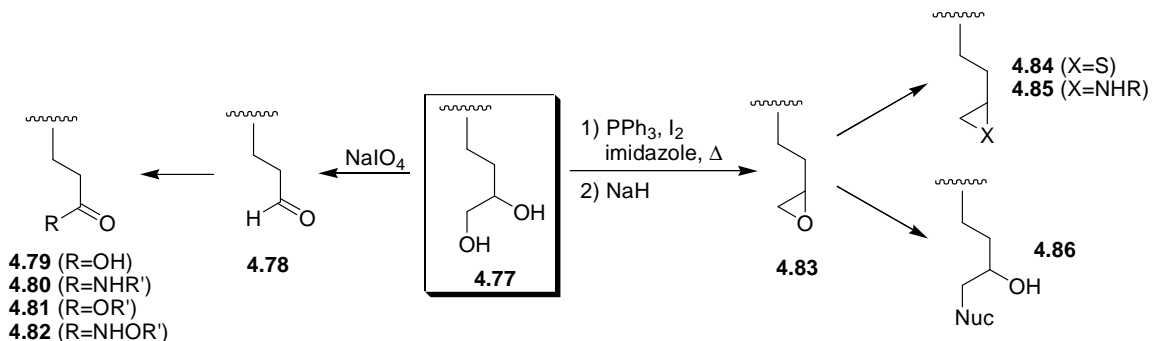


Figure 4.3. Biological activity of other assembled mycobactin analogs

4.5 Conclusions and recommendations for further study

The synthesis of 1,2-diol-containing analogs of mycobactin S (**4.56**) and mycobactin T (**4.65** and **4.66**) has been reported using acylnitroso HDA chemistry. Additionally, strategies for the synthesis of α -hydroxy carboxylate analogs of mycobactins S and T (**2.60**) have been proposed.

Additionally, 1,2-diol compounds **4.77** provide access to a number of other potential metal-binding compounds (Scheme 4.24). Diol cleavage with periodate would yield aldehyde **4.78**, which could be subsequently converted into compounds **4.79-4.82**. Based on a recent report by Mandal *et al.*,³⁶⁵ 1,2-diol **4.77** could be converted into epoxide **4.83**, which provides access to compounds **4.84-4.86**.



Scheme 4.24. Proposed route to other analogs from 1,2-diol compounds

The strategies reported in this chapter can be used to synthesize other mycobactin analogs, and should aid in the development of structure-activity-relationships (SAR) surrounding the mycobactin structure.

CHAPTER 5:
A NOVEL SYNTHESIS OF IMIDAZOLES FROM AZIDES AND 2-
AMIDOACRYLATES

5.1 Introductory remarks

In the previous chapters, the synthesis of mycobactin analogs from acylnitroso cycloadducts was described. Recall from chapter 3 that we previously discovered that a synthetic intermediate toward the synthesis of fragment A, oxazoline **3.8**, demonstrated impressive growth inhibitory activity against *M. tuberculosis* H₃₇Rv *in vitro* (Figure 5.1). Subsequently, the Miller group has focused efforts toward the synthesis of analogs of oxazoline **3.8**, some of which have been described in chapter 3.

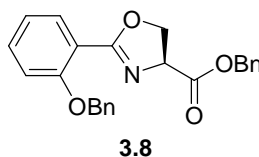
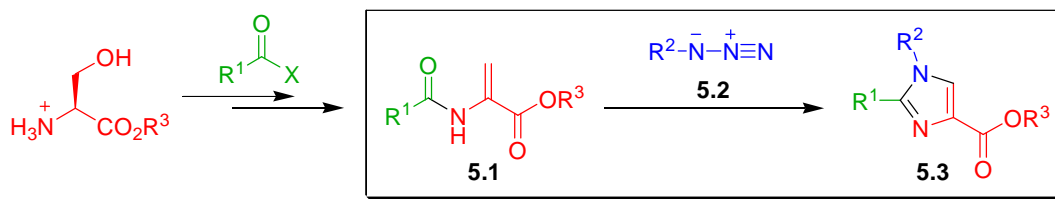


Figure 5.1. Synthetic intermediate with anti-TB activity

This chapter will detail the discovery and investigation of a novel synthesis of imidazoles **5.3** from 2-amidoacrylates **5.1** and azides **5.2** during an attempt to synthesize analogs of oxazoline **3.8** (Scheme 5.1).



Scheme 5.1. Novel imidazole synthesis from azides and 2-amidoacrylates

5.2 Design of project

5.2.1 Azides as a tool for generating chemical diversity

Azides are valuable intermediates in organic chemistry that facilitate a wide array of chemical transformations. A number of reviews and books have been published that describe the range of azide structure, properties, synthesis, and reactivity.³⁶⁶⁻³⁷² The reactivity of azides is best explained if one considers the polar mesomeric resonance structures **A-C** of the azide functional group (Figure 5.2). Structures **A** and **C** explain the nucleophilic character of the proximal nitrogen (nitrogen “a”), and structures **A** and **C** clearly illustrate the electrophilic character of the distal nitrogen of the azide group (nitrogen “c”). In some cases, nitrogen “a” can also react as an electrophile; however, most azide chemistry is explained based on this general reactivity pattern.³⁶⁷

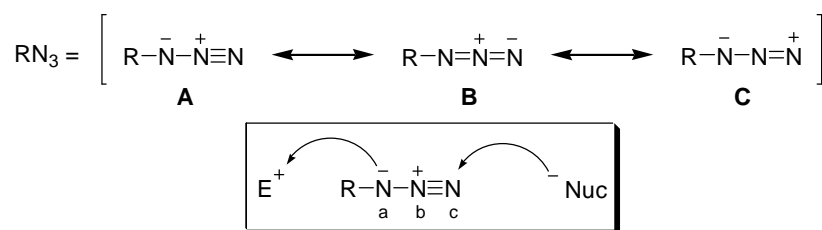
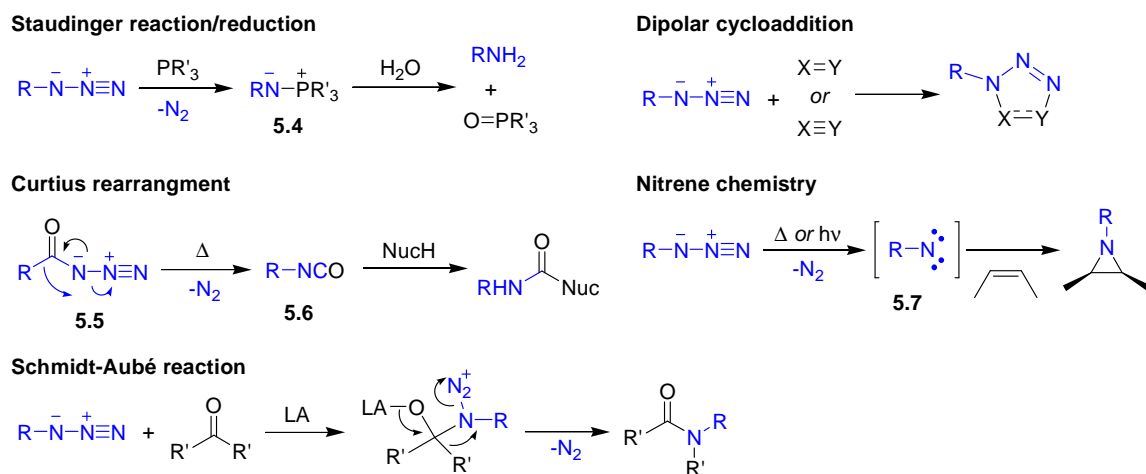


Figure 5.2. Structure and general reactivity trends for organic azides

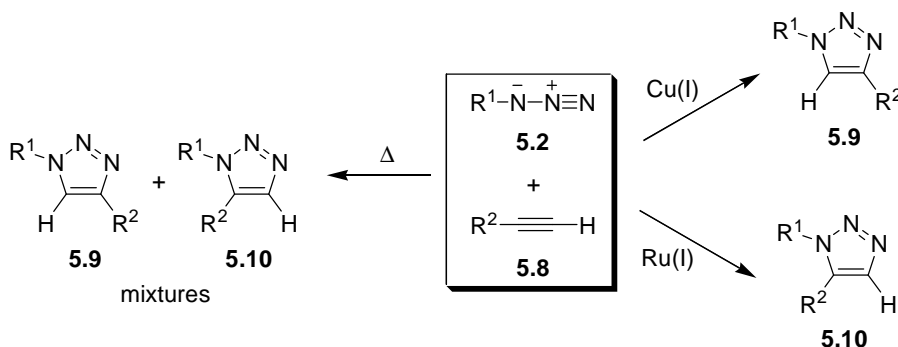
As stated above, azides participate in a number of important organic transformations (Scheme 5.2). Azides react with phosphines to form iminophosphoranes **5.4** (the Staudinger reaction),³⁶⁷ that can be hydrolyzed with water to form amines, participate in aza-Wittig reactions, and facilitate the formation of peptide bonds (Staudinger ligation). Acyl azides **5.5** rearrange thermally with concomitant loss of nitrogen to form isocyanates **5.6** (Curtius rearrangement), and is a useful transformation in a number of syntheses. Azides also participate in a number of other interesting rearrangements, a topic which has been excellently covered by Murphy.³⁶⁶ Azides are also precursors to nitrenes **5.7**,^{369, 371-375} which can be generated thermally, by photolysis, or using metal complexes (metallonitrenoids).³⁷⁵⁻³⁸⁸



Scheme 5.2. Common reactions of organic azides

Most recently, great attention has been given to dipolar cycloaddition chemistry using azides, especially azide cycloadditions to alkynes **5.8** to generate triazoles (the Huisgen cycloaddition, Scheme 5.3).³⁸⁹ Thermally, mixtures of 1,4-triazoles **5.9** and 1,5-triazoles **5.10** are obtained at high temperatures, typically in low yield.³⁸⁹ Sharpless has

described the use of Cu(I) salts to effect the addition of azides **5.2** and alkynes **5.8** to form 1,4-triazoles **5.9** selectively under very mild conditions.³⁹⁰⁻³⁹² Later work by Sharpless described the reversal of this selectivity to form 1,5-triazoles **5.10** using Ru(I) complexes.³⁹³

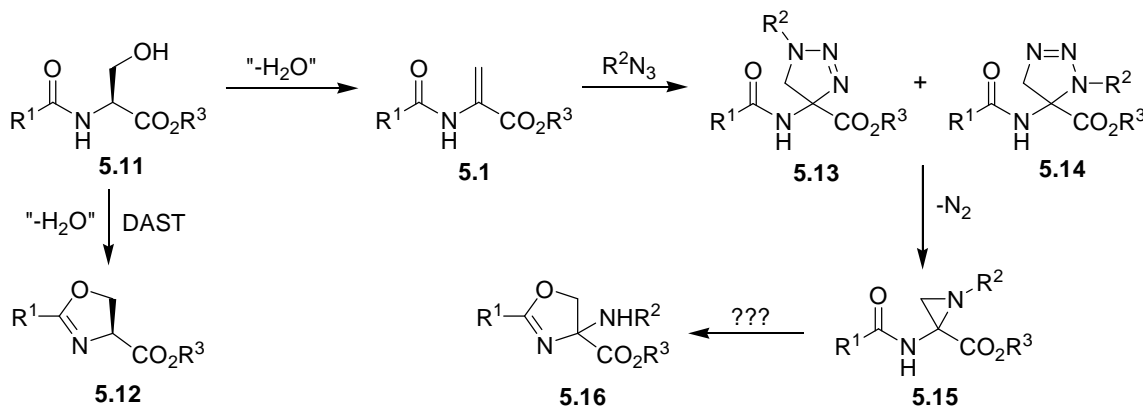


Scheme 5.3. Proposed route to fragment A analogs using azide cycloadditions

This mild, specific, and high yielding transformation has been the “poster child” of the concept of “Click Chemistry”,^{394, 395} a term coined by Sharpless for describing reactions that are “modular, wide in scope, give very high yields, generate only inoffensive byproducts that can be removed by nonchromatographic methods, and be stereospecific (but not necessarily enantioselective).”³⁹⁴ Consequently, the copper-catalyzed Huisgen cycloaddition reaction has been used in a number of applications and is a perfect method for reliably connecting molecules under mild conditions.³⁹⁶⁻⁴¹⁸ We have developed a similar cycloaddition reaction that may be useful for this purpose through azide additions to acylnitroso cycloadducts.⁴¹⁹ This topic and its potential use in this area will be covered in chapter 6.

5.2.2 Proposed strategy to mycobactin fragment A analogs using azide chemistry

As stated above and covered in chapter 3, oxazoline **3.8** and similar structures demonstrated impressive anti-TB activity *in vitro*, and we have been interested in synthesizing other fragment A analogs as potential small-molecule anti-TB drugs. Typically, amides **5.11** were transformed into oxazolines **5.12** to generate other oxazoline and oxazole analogs of compound **3.8** (Scheme 5.4). We realized that through dehydration to the dehydro-alanine derivative, 2-amidoacrylate **5.1**, a [3+2] dipolar cycloaddition using azides could provide triazolines **5.13** and **5.14** as well as aziridines **5.15**. Additionally, we hypothesized that aziridines **5.15** might potentially provide access to the interesting (but probably unstable) oxazolines **5.16**. Since azide additions to 2-amidoacrylates **5.1** were not reported in the literature, we were interested to learn about the reactivity of these systems.



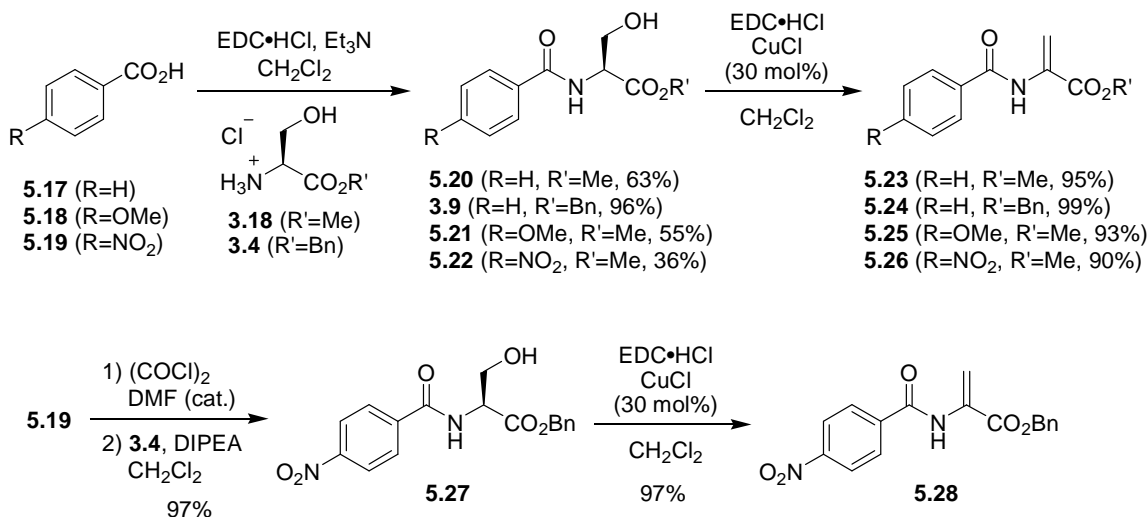
Scheme 5.4. Proposed synthesis of fragment A analogs using azide chemistry

As will be described in this chapter, although azide additions to 2-amidoacrylates **5.1** did not provide compounds **5.13-5.15** after isolation, a new synthesis of imidazoles **5.3** was discovered that provided imidazole analogs of oxazoline **3.8**.

5.3 Synthesis of starting materials

5.3.1 Synthesis of 2-amidoacrylates

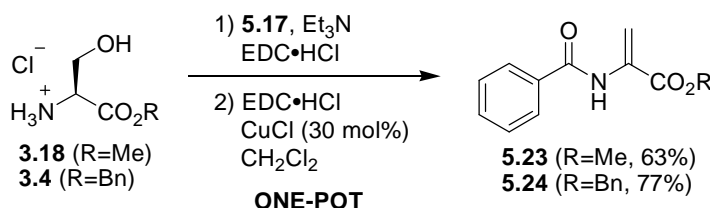
Various 2-amidoacrylates and derivatives were synthesized from serine or threonine esters in two steps. Serine esters **3.18** and **3.4** were *N*-acylated with benzoic acid (**5.17**) and substituted benzoic acids **5.18** and **5.19** and provided amides **5.20**, **3.9**, **5.21**, and **5.22** (Scheme 5.5). Copper-mediated dehydration using water-soluble carbodiimide⁴²⁰ effected the conversion of amides **5.20**, **3.9**, **5.21**, and **5.22** to 2-amidoacrylates **5.23-5.26** in excellent yield. Alternatively, the *N*-acylation reaction was carried out using an acid-chloride strategy, which was used to form amide **5.27** in excellent yield. Dehydration of amide **5.27** provided 2-amidoacrylate **5.28**.



Scheme 5.5. Synthesis of 2-amidoacrylates

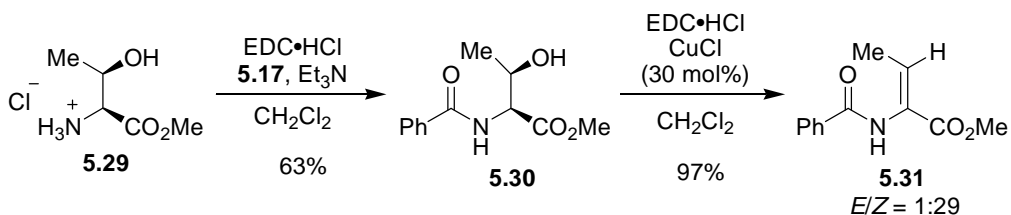
While the strategy described above was effective and reliable, we found the two-step procedure could be carried out in a two-stage, one-pot synthesis from serine esters

3.18 and **3.4** (Scheme 5.6). Treatment of the amine with benzoic acid (**5.17**), triethylamine, and EDC followed by treatment with excess EDC and CuCl provided the 2-amidoacrylates **5.23** and **5.24**.



Scheme 5.6. One-pot synthesis of 2-amidoacrylates from serine esters

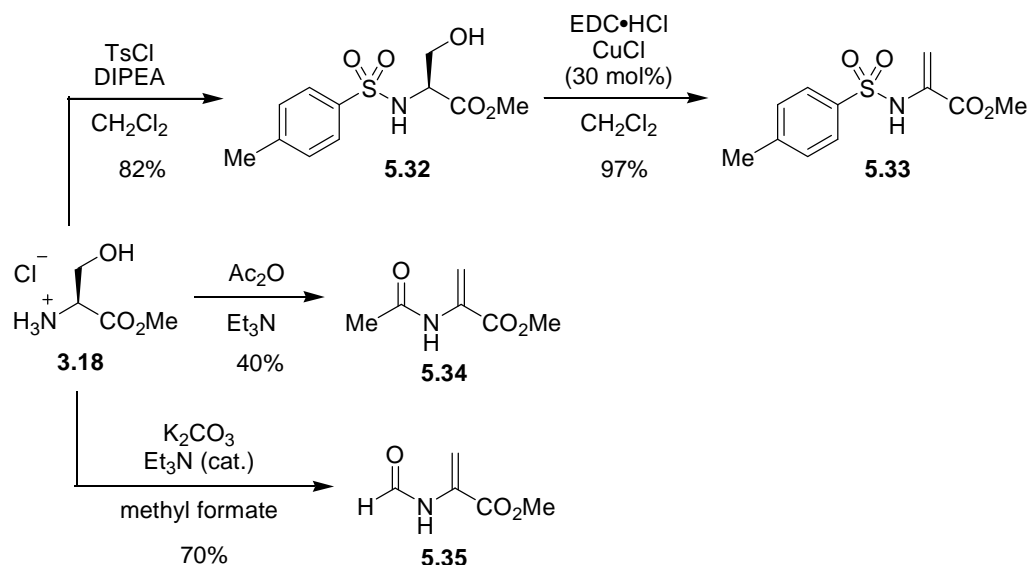
The use of threonine methyl ester **5.29** provided the *N*-benzoylated compound **5.30** (Scheme 5.7). Dehydration using the standard protocol provided 2-amidoacrylate **5.31** in excellent yield as a 29:1 ratio in favor of the *Z*-alkene as determined using ^1H NMR.⁴²¹ The selective formation of the *anti*-elimination product, the *Z*-isomer, indicated the mechanism of the dehydration reaction probably does not proceed through a *syn*-elimination process.



Scheme 5.7. Synthesis of 2-amidoacrylates from threonine methyl ester

Other 2-substituted acrylates were synthesized using serine methyl ester **3.18** (Scheme 5.8). *N*-tosylation provided sulfonamide **5.32**, which upon dehydration afforded

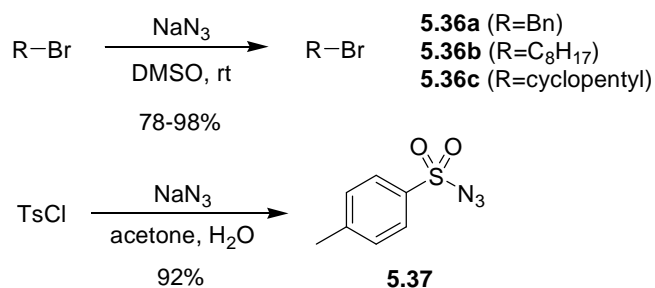
the 2-sulfonamidoacrylate **5.33** in excellent yield. Following reported literature procedures, 2-acetamidoacrylate **5.34**⁴²² and 2-formamidoacrylate **5.35**⁴²³ were prepared in one-pot from serine ester **3.18**.



Scheme 5.8. Synthesis of other acrylates from serine methyl ester

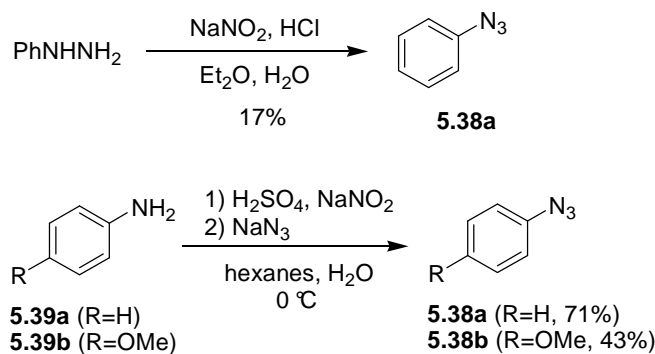
5.3.2 Synthesis of azides

Since the use of organic azides will also be described in the next chapter (chapter 6), the synthesis of all azides used throughout this dissertation will be presented herein. There are a variety of methods available for the synthesis of azides.^{367-369, 371} Secondary and primary alkyl azides **5.36a-c** were synthesized through displacement of the corresponding bromide following the procedure outlined by Alvarez (Scheme 5.9).⁴²⁴ Halogen displacement similarly provided tosyl azide (**5.37**).⁴²⁵



Scheme 5.9. Synthesis of azides by displacement of halides

Following the *Organic Synthesis* procedure for the preparation of phenyl azide (**5.38a**),⁴²⁶ only 17% yield of the desired product was obtained (Scheme 5.10). Aryl azides **5.38a** and **5.38b** were prepared more easily from the corresponding anilines **5.39a** and **5.39b** through a diazonium ion intermediate.



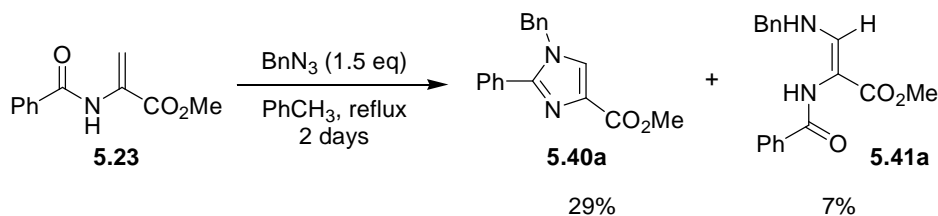
Scheme 5.10. Synthesis of aryl azides

5.4 Preliminary studies and investigation of a new reaction

5.4.1 Discovery of a new method for the synthesis of imidazoles

When 2-amidoacrylate **5.23** was heated with benzyl azide at reflux in toluene for 2 days, imidazole **5.40a** was obtained as the main product of the reaction mixture

(Scheme 5.11). Additionally, the main byproduct of the reaction was assigned as enaminone **5.41a**. The structure of the imidazole **5.40a** was confirmed using x-ray crystallography (Figure 5.3).



Scheme 5.11. Initial result of azide addition to 2-amidoacrylate 5.23

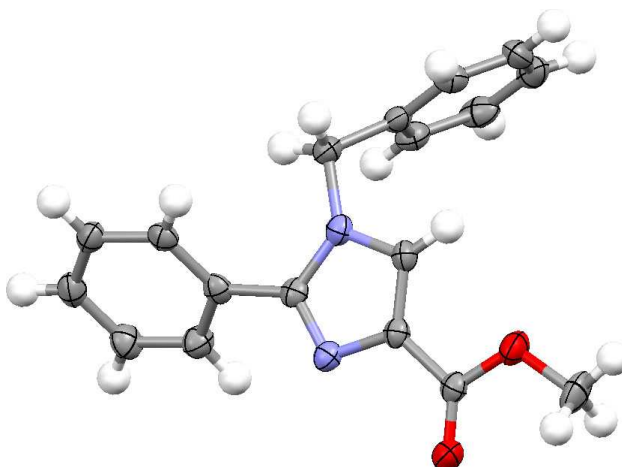


Figure 5.3. X-ray structure for imidazole 5.40a

5.4.2 Structure of the by-product

As was the case for imidazole **5.40a**, the structure and alkene geometry of enaminone **5.41a** was confirmed using x-ray crystallography (Figure 5.4). Close examination of the bond lengths revealed that the C(11)-N(2) bond length (1.336 Å) was significantly shorter than C(8)-N(1) or C(12)-N(2), which were 1.425 Å and 1.456 Å, respectively. This indicated significant delocalization of the N(2) lone pair into the alkene, and very little delocalization of the amide nitrogen into the alkene, as would be expected for the enamine structure.

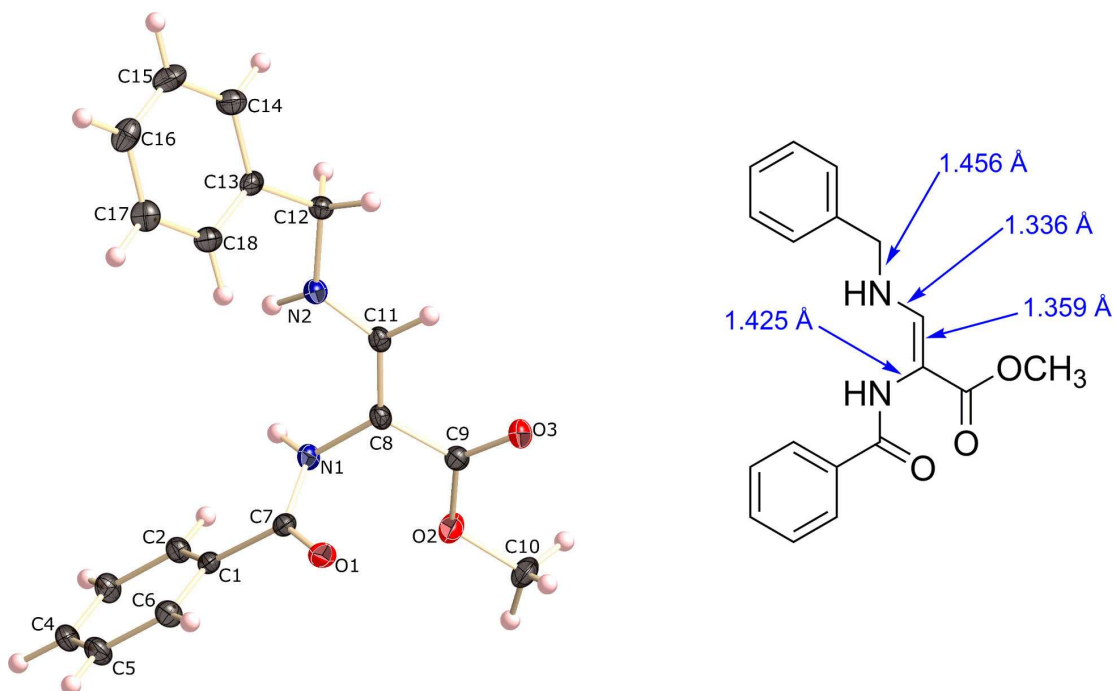


Figure 5.4. X-ray structure of byproduct with selected bond lengths shown

This delocalization is represented by the two tautomeric forms, enamine **5.41a** and imine **5.42a** (Figure 5.5). Further evidence of the equilibrium between tautomers **5.41a** and **5.42a** in solution is supported by the ^1H NMR data, which clearly illustrated the exchangeability of the tautomeric proton.

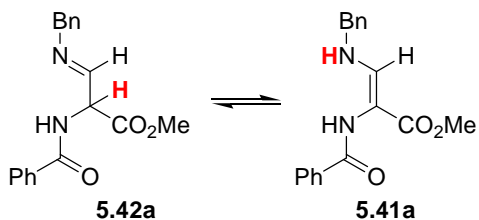


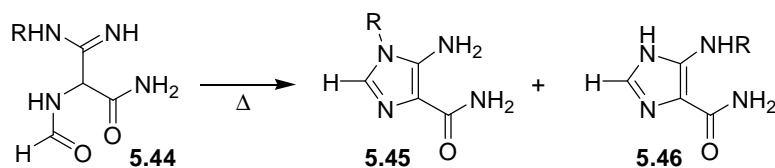
Figure 5.5. Representation of byproduct structure

5.5 Similar reactions reported in the literature

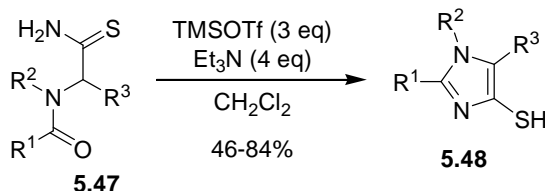
Based on similar imidazole syntheses reported in the literature, we hypothesized that the 2-amido-3-aminoacrylate byproduct **5.41** is an intermediate in the synthesis of imidazole **5.40**. Reports by other groups using compounds similar to 2-amido-3-aminoacrylate **5.41** that support this hypothesis are presented in this section, and formed the basis for a proposed mechanism of the transformation that will be presented in the following section (section 5.6).

In the late 1950s and early 1960s, Shaw described the synthesis of aminoimidazoles **5.45** and **5.46** from formamidoamidines **5.44** (Scheme 5.12).⁴²⁷⁻⁴²⁹ Yields reported for imidazoles **5.45** and **5.46** were variable, and sometimes selective for either isomer depending on the reaction conditions. Similar work was reported by Hopkins *et al*,⁴³⁰ where thionamide **5.47** provided imidazolethiols **5.48** in moderate to good yield.

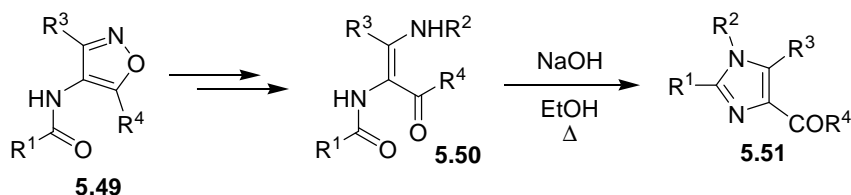
Shaw *et al*



Hopkins *et al*



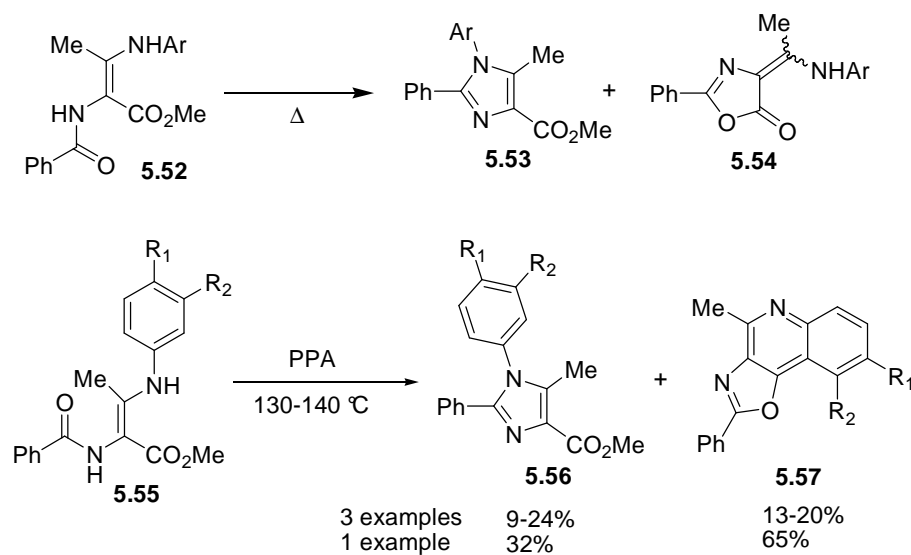
Reiter *et al*



Scheme 5.12. Literature reports of imidazole formation from 2-amido-3-aminoacrylates

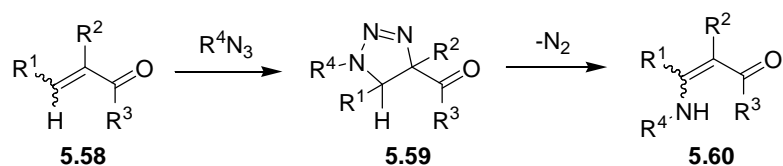
Reiter has published a number of articles on the conversion of isoxazoles **5.49** to imidazoles **5.51** in a few steps through the intermediacy of 2-amido-3-aminoacrylate **5.50** (Scheme 5.12).^{431, 432} Excellent yields of imidazoles **5.51** were reported under these conditions.

Particularly interesting were reports by Svete and Stanovik⁴³³⁻⁴³⁷ that highlighted the transformation of 2-amido-3-arylaminoacrylates **5.52** and **5.55** to imidazoles **5.53** and **5.56** (Scheme 5.13).^{434, 436} The authors reported that competing condensation of the amide carbonyl oxygen and the ester group yielded variable amounts of compounds **5.54** and the interesting heterocycle **5.57**.



Scheme 5.13. Research by Svete and Stanovik *et al*

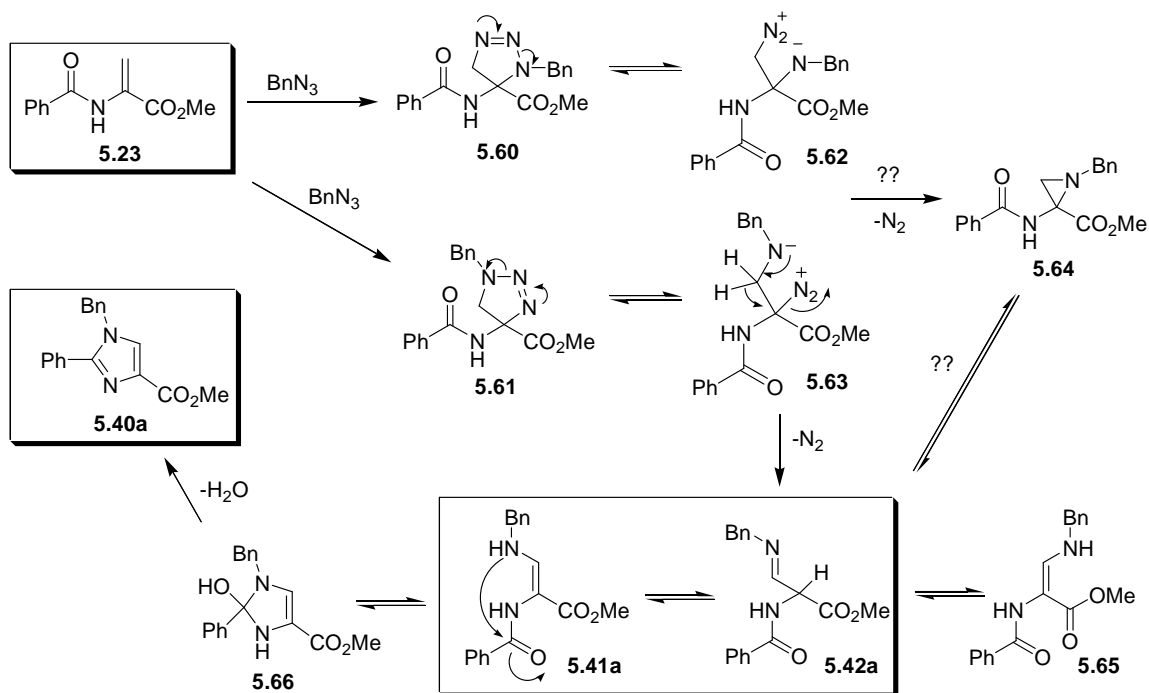
Other additions of azides to acrylates and other α,β -unsaturated carbonyl compounds **5.58** have been reported in the literature (Scheme 5.14).⁴³⁸⁻⁴⁴³ A common feature observed for all of these reactions was excellent regioselectivity for triazolines **5.59**, which often upon loss of nitrogen and rearrangement, yielded vinylogous amides **5.60**. While there are numerous examples of intra-^{438, 439, 441} and intermolecular⁴⁴⁰⁻⁴⁴² additions of azides to compounds **5.58** as well as a number of examples of cyclizations of 2-amido-3-aminoacrylates and related structures to form imidazoles, to our knowledge, no reports have described a one-pot procedure to form imidazoles **5.3** from 2-amidoacrylates **5.1**.



Scheme 5.14. Addition of azides to α,β -unsaturated carbonyl compounds

5.6 Proposed reaction mechanism

Based on similar reactions reported in the literature (see section 5.5) and the chemistry of organic azides, we proposed a mechanism that explained the formation of byproduct **5.41a** and imidazole **5.40a** (Scheme 5.15). Dipolar cycloaddition of benzyl azide to 2-amidoacrylate **5.23** should afford the regioisomeric triazolines **5.60** and **5.61**, although literature precedent from Aubé⁴⁴³ and others³⁶⁷ indicated that the formation of triazoline **5.60** would be greatly favored over the regioisomeric triazoline **5.61**. Thermal decomposition of triazolines **5.60** and **5.61** would provide compounds **5.62** and **5.63**, which, upon extrusion of nitrogen, would yield either aziridine **5.64** or imine **5.42a**. Tautomerization of imine **5.42a** would provide *E*-enamine **5.65** or the observed *Z*-enamine byproduct **5.41a**, which upon intramolecular cyclization would provide imidazole **5.40a** after dehydration of tetrahedral intermediate **5.66**.

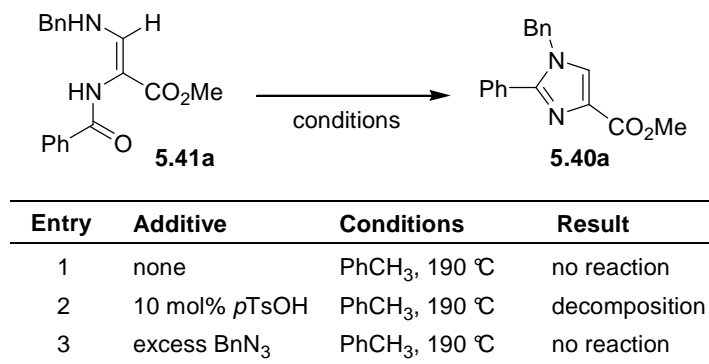


Scheme 5.15. Proposed mechanism of imidazole formation

At this time, the formation of imine **5.42a** from breakdown of an aziridine intermediate **5.64** cannot be excluded. We also considered the possibility of a nitrene mechanistic pathway. Although nitrenes have been generated thermally from alkyl azides, typically temperatures in excess of 150-170 °C are required.^{369, 374, 375} Additionally, the nitrene generated from benzyl azide would be extremely unstable and nitrenes with adjacent C-H bonds are known to undergo especially rapid rearrangement to yield imines.^{369, 374, 375}

Based on the proposed mechanism, we expected byproduct **5.41a** to provide imidazole **5.40a**, since the *Z*-enamine **5.41a** appeared to be an intermediate toward the production of imidazole **5.40a**. We were surprised to find that when enamine **5.41a** was subjected to the reaction conditions, no imidazole **5.40a** was observed by ¹H NMR (Table 5.1, entry 1). Added benzyl azide did not facilitate the transformation or decomposition of enamine **5.41a** (Table 5.1, entry 3). The addition of catalytic *p*-toluenesulfonic acid resulted in decomposition of enamine **5.41a** and again no imidazole **5.40a** formation was observed (Table 5.1, entry 2). Although literature precedent indicates that enamine byproduct **5.41a** can cyclize to imidazole **5.40a**, our results indicated that enamine **5.41a** must not be an intermediate in the mechanism as proposed.

TABLE 5.1
CONVERSION OF BYPRODUCT TO AN IMIDAZOLE

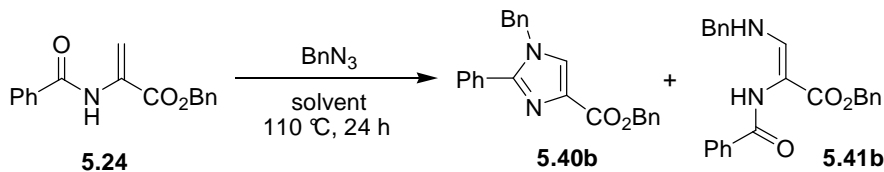


5.7 Optimization of the reaction

5.7.1 Solvent

In an effort to optimize reaction conditions, the effect of solvent on imidazole formation was studied (Table 5.2). 2-amidoacrylate **5.24** was treated with benzyl azide either acetonitrile, toluene, nitromethane, or dimethylformamide at 110 °C for 24 h and the crude reaction mixtures were analyzed by ¹H NMR spectroscopy. Toluene and nitromethane appeared to be the most suitable solvents for the reaction, with the use of nitromethane as the solvent providing slightly more imidazole **5.40b** than the reaction in toluene (Table 5.2, entries 2 and 3). Both of these solvents were chosen for further optimization studies.

TABLE 5.2
EFFECT OF SOLVENT ON IMIDAZOLE FORMATION



| Entry | Solvent | Conversion ^a | Yield of 5.40b ^b |
|-------|---------------------------------|-------------------------|-----------------------------|
| 1 | CH ₃ CN | 18% | 11% |
| 2 | PhCH ₃ | 57% | 41% |
| 3 | CH ₃ NO ₂ | 75% | 50% |
| 4 | DMF | 34% | 33% |

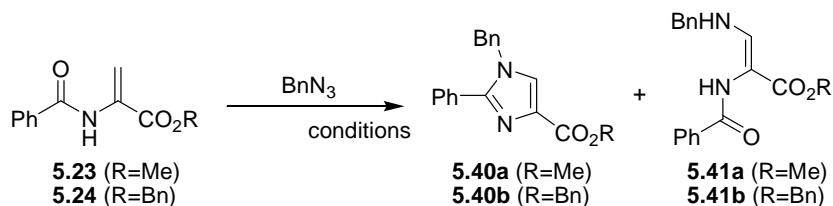
NOTE: (a) determined by ¹H NMR. (b) determined by ¹H NMR using 1,2-dichloroethane as an internal standard

5.7.2 Reaction conditions

The effect of temperature, azide equivalents, time, and additives on the yield of imidazoles **5.40a** and **5.40b** and 2-amido-3-aminoacrylate byproducts **5.41a** and **5.41b** were studied (Table 5.3). It is clear that temperature has a pivotal role in the reaction, since only trace amounts of imidazoles **5.40a** and **5.40b** were observed at temperatures lower than 100 °C and the yield of imidazoles **5.40a** and **5.40b** increased with regard to reaction temperature. Likewise, we found the time of the reaction could be shortened from 2-4 days to 18 h if the temperature of the reaction was increased from refluxing toluene to 190 °C in a sealed tube (Table 5.3, entries 4 and 9). Increasing the equivalents of benzyl azide appeared to be slightly detrimental to the yield of imidazole **5.40b** obtained, and yielded more of the byproduct **5.41b** (Table 5.3, entries 9 and 11). Optimal yield of imidazoles **5.40** were obtained when the reactants were heated in toluene in a sealed tube at 190 °C overnight (Table 5.3, entries 9 and 10).

TABLE 5.3

INVESTIGATION OF REACTION CONDITIONS FOR THE ADDITION OF
BENZYL AZIDE TO 2-AMIDOACRYLATES

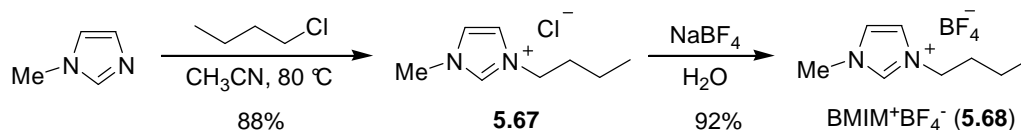


| Entry | R | BnN ₃ (equiv) | Solvent | Temp (°C) | Time | Additive(s) | Result/yield (%) | |
|-------|----|-----------------------------|--|------------------|--------|--|----------------------------|-----------------|
| | | | | | | | 5.40a/5.40b | 5.41a/5.41b |
| 1 | Me | 1.5 | CHCl ₃ | 60 | 5 days | none | incomplete reaction | |
| 2 | Me | 1.5 | PhCH ₃ | 110 | 2 days | none | 29 | 7 |
| 3 | Me | 1.5 | PhCH ₃ | 110 | 4 days | none | 33 | 4 |
| 4 | Bn | 1.5 | PhCH ₃ | 110 | 4 days | none | 39 | 10 |
| 5 | Bn | 1.5 | PhCH ₃ | 110 | 4 days | 4Å MS | 39 | 19 |
| 6 | Bn | 1.5 | PhCH ₃ | 110 | 4 days | MgBr ₂ •OEt ₂ (20 mol%) | decomposition ^a | |
| 7 | Me | 1.3 | xylenes | 150 | 26 h | none | 52 | 13 |
| 8 | Bn | 1.5 | PhCH ₃ | 140-170 (MW) | 4 h | none | incomplete reaction | |
| 9 | Bn | 1.5 | PhCH ₃ | 190 ^b | 15 h | none | 63 | 18 |
| 10 | Me | 1.5 | PhCH ₃ | 190 ^b | 15 h | none | 54 | 27 |
| 11 | Bn | 3.0 | PhCH ₃ | 190 ^b | 15 h | none | 54 | 25 |
| 12 | Bn | 3.0 | CH ₃ NO ₂ | 190 ^b | 15 h | none | 40 | ND ^c |
| 13 | Bn | 1.5 | PhCH ₃ | 190 ^b | 15 h | <i>p</i> TsOH (10 mol%) | 50 | 3 |
| 14 | Bn | 1.5 | BMIM ⁺ BF ₄ ⁻ | rt-110 | 4 days | none | incomplete reaction | |
| 15 | Me | 1.5 | CHCl ₃ / MeOH (1:1) | reflux | 2 days | none | incomplete reaction | |

NOTE: (a) isolated mostly benzamide (PhCONH₂) from the reaction as the major decomposition product. (b) reaction was performed in a sealed tube and heated in an oil bath. (c) ND = yield not determined.

Since cycloadditions and many other organic reactions have benefited from being performed in ionic liquids,⁴⁴⁴ we also attempted the transformation in BMIM⁺BF₄⁻ (**5.68**),

which was prepared from 1-methylimidazole following a literature procedure (Scheme 5.16).⁴⁴⁵ Using **5.68** as the solvent or as a co-solvent mixed with toluene for increased solubility was detrimental to the progress of the reaction (Table 5.3, entry 14). A similar detrimental effect was observed when mixtures of alcoholic solvents were used such as chloroform/methanol mixtures (Table 5.3, entry 15).



Scheme 5.16. Preparation of an ionic liquid

We explored the possibility of accelerating the reaction by the addition of Lewis acids to the reaction mixture. From a panel of common Lewis acids, $\text{MgBr}_2 \cdot \text{OEt}_2$ was identified as promising based on a qualitative study. Unlike the qualitative study, the addition of $\text{MgBr}_2 \cdot \text{OEt}_2$ to the reaction appeared to promote decomposition of the starting 2-amidoacrylate **5.24** and we isolated benzamide (PhCONH_2) as a major decomposition product (Table 5.3, entry 6). The addition of catalytic *p*-toluenesulfonic acid did not have an effect on the yield of imidazole **5.40**, but resulted in lower yields of 2-amido-3-aminoacrylate **5.41** (Table 5.3, entry 13).

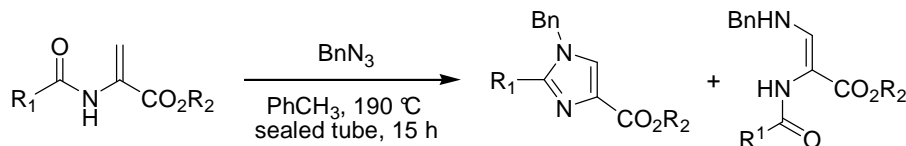
5.8 Scope of the reaction: preliminary results

5.8.1 Effect of modifying the acrylate

We have begun to investigate the scope of the reaction based on modification of the acrylate starting material (Table 5.4). Benzyl azide additions were carried out using the optimized conditions from Table 5.3 for acrylates **5.23-5.26**, **5.28**, **5.34**, and **5.35**. Substitution of the phenyl ring of acrylate **5.23** with the electron-withdrawing nitro group (acrylate **5.26**) resulted in a great increase in the yield of the imidazole product (Table 5.4, entries 2 and 4). Conversely, substitution of the phenyl ring with the electron-donating methoxy group (acrylate **5.25**) resulted in a complex mixture of products (Table 5.4, entry 5). It is tempting to hypothesize that this is a result of decreased electrophilicity of the amide carbonyl, but that hypothesis cannot be fully supported without further study. The 2-formamidoacrylate **5.35** and 2-acetamidoacrylate **5.34** provided good yield of imidazole products **5.75** and **5.73**, respectively (Table 5.4, entries 6 and 7).

TABLE 5.4

SCOPE OF IMIDAZOLE SYNTHESIS: MODIFICATION OF THE ACRYLATE
STARTING MATERIAL

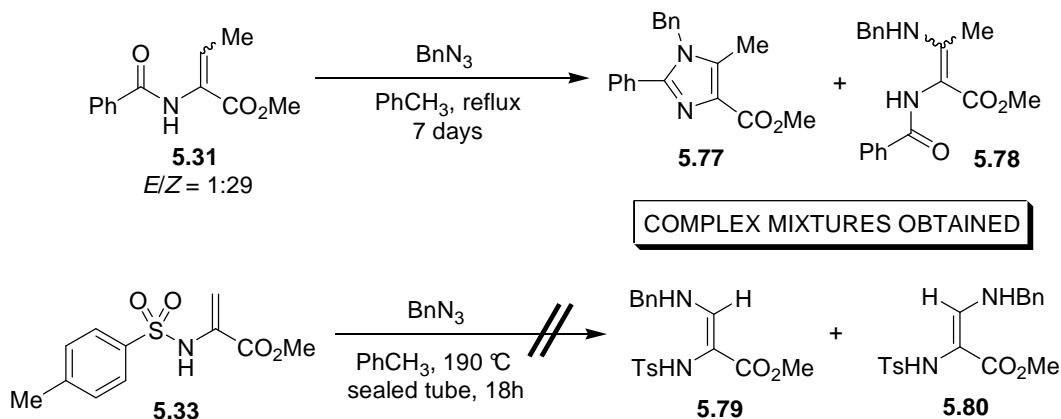


| Entry | Acrylate | R ₁ | R ₂ | Imidazole (%yield) | Byproduct (%yield) |
|-------|-------------|---|----------------|-------------------------------|--------------------------------|
| 1 | 5.24 | Ph | Bn | 5.40b (63) | 5.41b (18) ^a |
| 2 | 5.23 | Ph | Me | 5.40a (54) | 5.41a (27) ^b |
| 3 | 5.28 | <i>p</i> -NO ₂ C ₆ H ₄ | Bn | 5.69b (63) | 5.70b (8) ^c |
| 4 | 5.26 | <i>p</i> -NO ₂ C ₆ H ₄ | Me | 5.69a (-) ^d | 5.70b (-) ^d |
| 5 | 5.25 | <i>p</i> -OMeC ₆ H ₄ | Me | 5.71 (-) ^d | 5.72 (-) ^d |
| 6 | 5.34 | Me | Me | 5.73 (74) | 5.74 (14) ^c |
| 7 | 5.35 | H | Me | 5.75 (71) | 5.76 (21) ^c |

NOTE: (a) Only one isomer detected. (b) Isolated as an 8:1 mixture of isomers. (c) Yield is approximate; the compound was isolated as a mixture with impurities. (d) Complex mixture obtained.

Additionally, we also wanted to explore the possibility of forming imidazole **5.77** from the threonine-derived acrylate **5.31** (Scheme 5.17). Treatment of acrylate **5.31** with benzyl azide in refluxing toluene resulted in a slow consumption of acrylate **5.31**; however, a complex mixture was obtained. While there is evidence that imidazole **5.77** and 2-amido-3-aminoacrylate **5.78** were formed in the reaction, we could not obtain these compounds pure from the reaction mixture. We also wanted to explore the possibility of using 2-sulfonamidoacrylate **5.33** to “trap” the enamine byproducts **5.79** and **5.80**, since

these compounds would not be able to undergo cyclodehydration to imidazole products; however, a complex mixture of products was obtained.

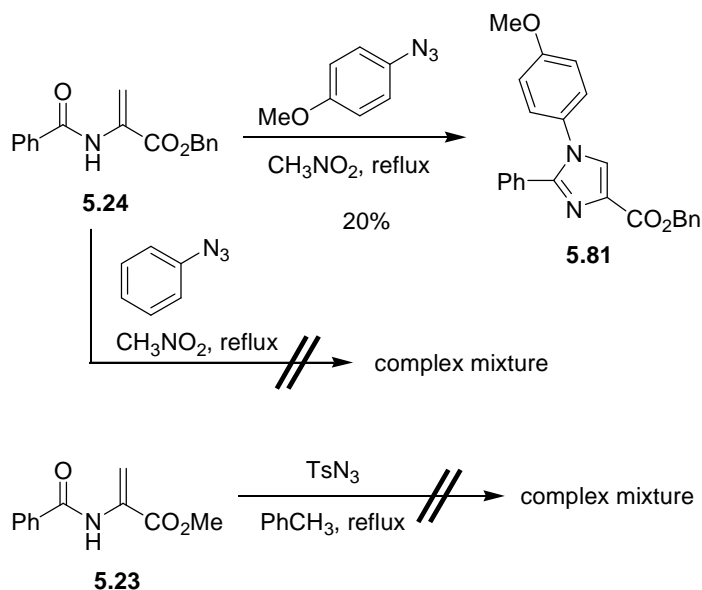


Scheme 5.17. Azide additions to other acrylates

While the imidazole reaction does appear to have some generality with respect to the acrylate, additional studies will need to be performed to accurately describe the full scope and limitations of this chemistry.

5.8.2 Effect of modifying the azide

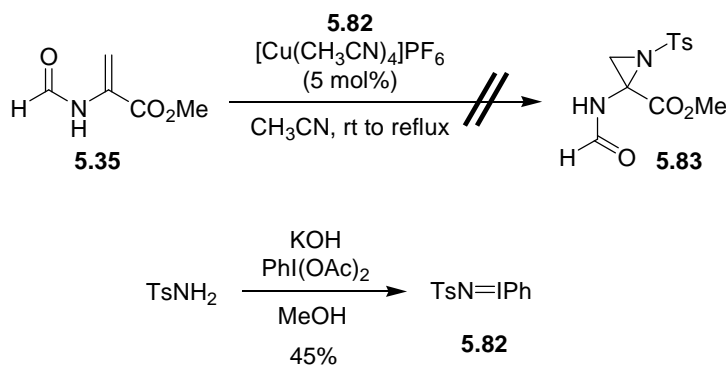
Acrylate **5.24** was subjected to the aryl azides phenyl azide (**5.38a**) and *p*-methoxyphenyl azide (**5.38b**) in nitromethane at reflux in an effort to ascertain the applicability of this chemistry toward the use of aryl azides (Scheme 5.18). When *p*-methoxyphenyl azide was used, we were able to obtain imidazole **5.81** in low yield; however, the use of phenyl azide under the same conditions resulted in only a complex mixture. Similarly, treatment of acrylate **5.23** with tosyl azide (**5.37**) in refluxing toluene also led to a complex mixture of products.



Scheme 5.18. Addition of other azides to acrylates

5.9 Aziridination of 2-amidoacrylates

In order to ascertain whether aziridines **5.64** are intermediates in the proposed mechanism, an alternate route to aziridine compounds was investigated. 2-Formamidoacrylate **5.35** was subjected to *N*-tosyliminoiodinane **5.82**³⁷⁸ and a copper(I) catalyst (Scheme 5.19).³⁸¹ After stirring at room temperature, no reaction was observed except for the generation of *p*-toluenesulfonamide from decomposition of iminoiodinane **5.82**. Even after heating for 4 days, acrylate **5.35** was recovered relatively unchanged from the reaction mixture.



Scheme 5.19. Attempted aziridination of 2-formamidoacrylate **5.35**

5.10 Imidazole from oxazolines

We hypothesized that imidazole **5.40b** would be obtained from oxazolines **3.10**. When benzyl azide and oxazoline **3.10** were heated to reflux in toluene for 7 days, no reaction was observed (Table 5.5, entry 1); however, when a catalytic amount of acetic acid was added to the reaction mixture, imidazole **5.40b** as well as acrylate **5.24** were observed within one hour. From this result, we hypothesized the addition of lewis acids and brønsted acids to a mixture of oxazoline **3.10** and benzyl azide would provide imidazole **5.40b**. *p*-Toluenesulfonic acid was able to effect the transformation of oxazoline **3.10** in toluene and provided imidazole **5.40b** in 49% yield (Table 5.5, entry 3); however, when the reaction was performed in acetonitrile with or without added *p*-toluenesulfonic acid, no reaction was observed (Table 5.5, entries 4 and 5). It is also interesting to note that although a catalytic amount of acetic acid promoted the conversion of oxazoline **3.10** to imidazole **5.40b**, when heated in a 4:1 mixture of toluene and acetic acid, the rate of the reaction was much slower (Table 5.5, entries 2 and 6). This result, combined with the lack of reactivity in acetonitrile, seems to be an effect of solvent polarity. Finally, a panel of lewis acids were investigated in a qualitative study

for potential use in catalyzing the transformation of oxazoline **3.10** to imidazole **5.40b**. Again, $\text{MgBr}_2 \cdot \text{OEt}_2$ yielded the best results of the qualitative study and was added to the reaction in refluxing toluene, and once again the decomposition product, benzamide, was isolated as the major component of the reaction mixture (Table 5.5, entries 7 and 8).

TABLE 5.5
ONE-POT FORMATION OF IMIDAZOLES FROM OXAZOLINES

c1ccccc1C2=NC(C(=O)OCC2)c3ccccc3
 $\xrightarrow[\text{conditions}]{\text{BnN}_3 (1.5 \text{ eq})}$
c1ccccc1c2nc(C(=O)OCC)c(Cn3ccccc3)c23

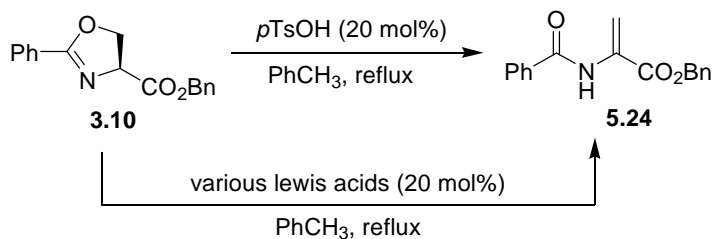
3.10 **5.40b**

| Entry | Conditions | Additive | Result |
|-------|--|--|-----------------------------|
| 1 | PhCH_3 , reflux | none | N.R. ^a |
| 2 | PhCH_3 , reflux | HOAc (cat.) | 5.40b observed |
| 3 | PhCH_3 , reflux | <i>p</i> -TsOH (20 mol%) | 5.40b (49% yield) |
| 4 | CH_3CN , reflux | none | N.R. ^a |
| 5 | CH_3CN , reflux | <i>p</i> -TsOH (20 mol%) | N.R. ^a |
| 6 | 4:1 $\text{PhCH}_3/\text{HOAc}$, reflux | none | trace 5.40b observed |
| 7 | PhCH_3 , reflux | $\text{MgBr}_2 \cdot \text{OEt}_2$ (20 mol%) | trace 5.40b observed |
| 8 | PhCH_3 , 90-140 °C ^b | $\text{MgBr}_2 \cdot \text{OEt}_2$ (20 mol%) | decomposition ^c |

NOTE: (a) N.R. = no reaction (oxazoline **3.10** unchanged). (b) reaction was performed in a sealed tube. (c) the major decomposition product isolated was benzamide (PhCONH_2).

Since 2-amidoacrylate **5.24** was observed in the acid-catalyzed conversion of oxazoline **3.10** to imidazole **5.40b**, it appeared that acrylate **5.24** was an intermediate along this pathway. To test this hypothesis, oxazoline **3.10** was treated with catalytic *p*-toluenesulfonic acid in refluxing toluene (Scheme 5.20). Within an hour acrylate **5.24**

was observed, and after a few hours, oxazoline **3.10** was completely consumed. Additionally, this transformation was also catalyzed by lewis acids.



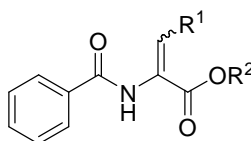
Scheme 5.20. Acrylate formation from oxazolines under acidic conditions

5.11 Biological activity of imidazoles and acrylates

Selected compounds reported in this chapter were assayed for biological activity against *M. tuberculosis* and PC-3 and MCF-7 cancer cell lines using the same assays described in chapter 4.

While all imidazole compounds tested were inactive against *M. tuberculosis* and cancer cells, we were surprised to find that acrylates **5.23**, **5.24**, and **5.31** demonstrated moderate anticancer activity against PC-3 cell lines (Table 5.6). We have not hypothesized the mode of action of acrylates **5.23**, **5.24**, and **5.31**; however, general cytotoxicity cannot be ruled out at this time.

TABLE 5.6
BIOLOGICAL ACTIVITY OF 2-AMIDOACRYLATES



| Entry | Compound | R ¹ | R ² | PC-3 | |
|-------|-------------|----------------|----------------|-----------------------------------|--------------------------------|
| | | | | % inhibition 20 μ M (96 h) | IC ₅₀ (μ M) |
| 1 | 5.23 | H | Me | 57% | 13 ^a |
| 2 | 5.24 | H | Bn | 60% | 15 |
| 3 | 5.31 | Me | Me | 14% | ND |

NOTE: (a) MCF-7 (IC₅₀ = 8 μ M).

5.12 Summary and recommendations for further study

A one-pot synthesis of imidazoles **5.3** has been discovered from the addition of azides to 2-amidoacrylates **5.1**. 2-Amidoacrylates **5.1** are easily synthesized in two steps from serine esters, and this synthetic method represents an efficient synthesis of imidazole-4-carboxylates **5.3**. Preliminary results suggest that this method may be general for many acrylates **5.1**, aryl azides and sulfonyl azides are not well-tolerated.

CHAPTER 6:

ADDITION OF AZIDES TO ACYLNITROSO CYCLOADDUCTS

6.1 Introductory remarks

The utility of acylnitroso cycloadducts **1.67** in organic synthesis has been described in detail in chapter 1, and a new application of acylnitroso chemistry was described in chapters 2-4 through the synthesis of mycobactin analogs. While extensive chemistry has been developed around functionalizing the N-O and C-O bonds of cycloadducts **1.67**, modifications of the olefin have been mainly limited to epoxidation,¹⁵⁰ dihydroxylation,¹⁹² and oxidative cleavage.^{162, 446}

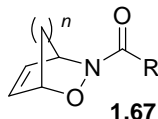
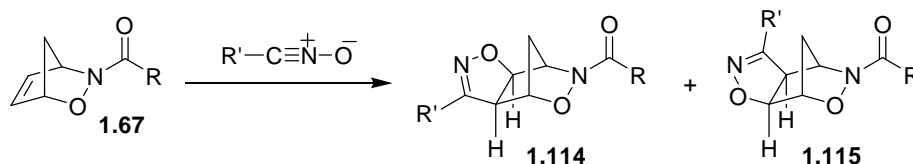


Figure 6.1. Acylnitroso cycloadducts

In an effort to expand upon the versatility of cycloadducts **1.67**, we were interested in selective functionalization of the olefin to produce new structural features. Specifically, we realized that the strained nature of cycloadducts **1.67** would allow for functionalization of the alkene that would normally be difficult on unstrained species. We were encouraged by a recent report by Quadrelli *et al* highlighting the addition of nitrile oxides to **1.67** (Scheme 6.1).¹¹⁷ This chapter will describe studies regarding the

reactivity of **1.67** with azides to form triazolines and their subsequent transformation to aziridines.



Scheme 6.1. Nitrile oxide additions to cycloadducts by Quadrelli *et al*

6.2 Design of project and the synthesis of novel norcarbocyclic nucleoside analogs

Intermolecular [3+2] cycloaddition reactions of azides to strained bicyclic alkenes are well-documented in the literature.^{367, 447-453} Examples include additions to norbornene, 2-azabicyclo[2.2.1]hept-5-en-3-one (ABH, **6.1**) to afford 2'-3'-epimino-carbocyclic nucleosides,⁴⁵⁴⁻⁴⁵⁶ 2,3-diazabicyclo[2.2.1]hept-5-enes **6.2** to afford 1,4-dihydropyridines,⁴⁵⁷ as well as many other strained systems.^{453, 458} However, the addition of azides to bicyclic oxazines such as **3.38-3.40** has not been disclosed in the literature. Since we wished to specifically probe the role of olefin strain on reactivity with azides, we chose to subject the olefins of **3.38-3.40** as well as protected amino alcohols **3.73**, **3.74**, and **3.77** to reactions with various azides (Figure 6.2).

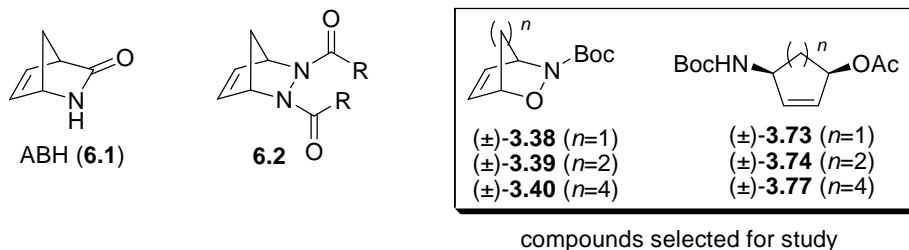
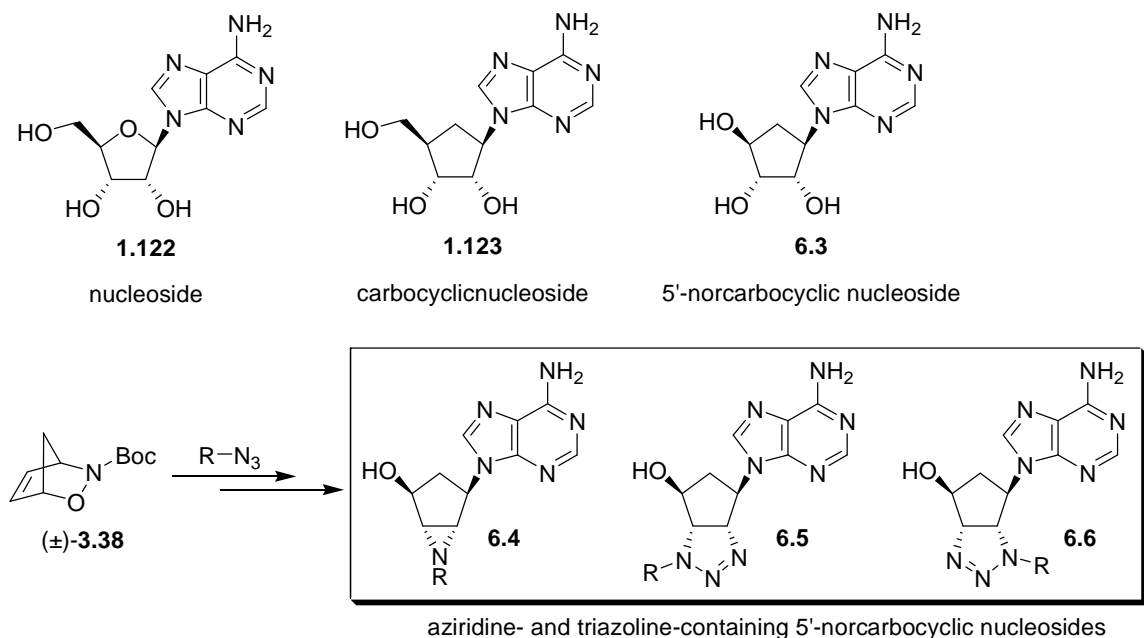


Figure 6.2. Compounds selected for study in azide-alkene cycloadditions

Carbocyclic nucleoside analogs represent a rich class of biologically active molecules that display anticancer, antiviral, and in some cases, antibacterial activity. Most naturally occurring nucleosides such as adenosine (**1.122**) contain a furanose core structure that is replaced with a carbocyclic core as in the case with the naturally occurring carbocyclic nucleoside, aristeromycin (**1.123**) (Scheme 6.2). 5'-Norcarbocyclic nucleosides **6.3** are analogs of carbocyclic nucleosides that replace the hydroxyl methyl substituent with a hydroxyl substituent, and are accessible using acylnitroso HDA chemistry.



Scheme 6.2. Potential application of azide dipolar cycloaddition chemistry

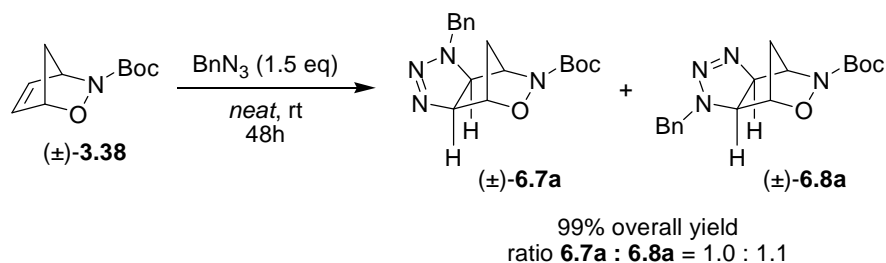
One potential application of the addition of azides to acylnitroso cycloadduct **3.38** is toward the synthesis of aziridine- and triazoline-containing 5'-norcarbocyclic

nucleoside analogs **6.4-6.6**. Ishikura has published the synthesis of aziridine-containing carbocyclic nucleosides from the addition of tosyl azide to ABH (**6.1**), and much of the methodology used for the synthesis of compounds **6.4-6.6** would follow work by Ishikura and by Miller. The following chapter will detail the efforts toward understanding and optimizing the addition of azides to acylnitroso cycloadducts **1.67** as well as reactivity of the resultant triazoline- and aziridine-containing molecules.

6.3 Addition of azides to acylnitroso cycloadducts

6.3.1 Initial studies

We were very pleased to find that when **3.38** was treated with benzyl azide,⁴²⁴ the regioisomeric *exo*-triazolines **6.7a** and **6.8a** were obtained in quantitative yield after stirring for 2 days neat at room temperature (Scheme 6.3). Similar reactions of 2-azabicyclo[2.2.1]hept-5-en-3-ones (**6.1**) reportedly required high pressure for the cycloaddition reaction to occur.⁴⁵⁶ The *exo*-specificity of the reaction is in agreement with what has been reported in the literature in related reactions of bicyclo[2.2.1]hept-5-ene-2,3-dicarboximides,⁴⁵⁹ ABH (**6.1**) and derivatives,⁴⁶⁰ 7-oxabicyclo[2.2.1]hept-5-en-2-yl derivatives,^{461, 462} and **6.2**.⁴⁶²



Scheme 6.3. Addition of benzyl azide to acylnitroso cycloadduct

The stereo- and regiochemistry of triazolines **6.7a** and **6.8a** was assigned based on their single-crystal x-ray crystal structures, which clearly demonstrates the *exo-syn* and *exo-anti* relationships of **6.7a** and **6.8a**, respectively (Figure 6.3).

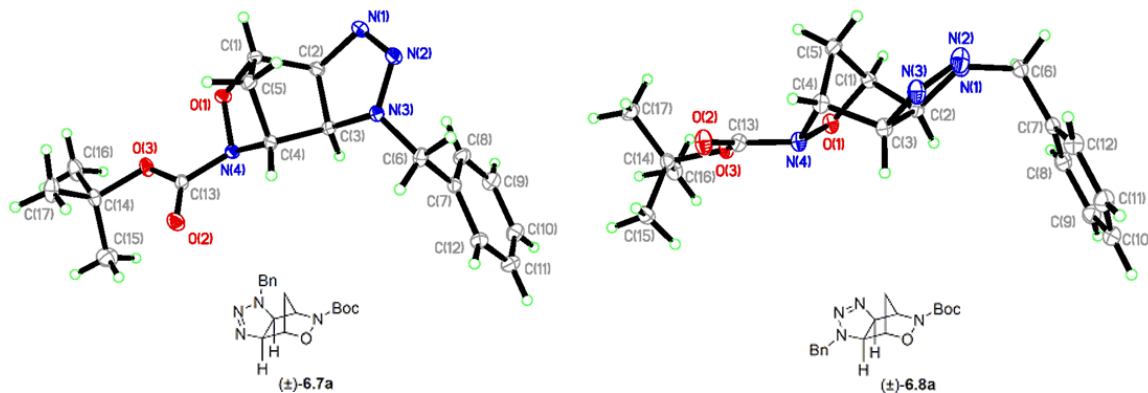
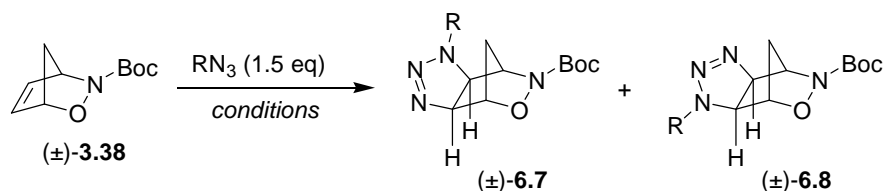


Figure 6.3. X-ray crystal structures of triazolines **6.7a** and **6.8a**

6.3.2 Effect of different organic azides on the reaction

In an effort to ascertain the limitations of this chemistry, we treated alkene **3.38** with various organic azides under a number of reaction conditions (Table 6.1). The reaction proceeded equally well refluxing in toluene or chloroform (Table 6.1, entries 4 and 8); however, it was sluggish when stirred in solution at room temperature (Table 6.1, entry 2). The steric bulk of the azide had little effect on either the regioselectivity or yield of the reaction, with primary, secondary, tertiary, and aryl azides affording triazolines **6.7** and **6.8** in excellent yield (Table 6.1, entries 8-12).

TABLE 6.1
REACTIONS OF CYCLOADDUCT 3.38 WITH VARIOUS AZIDES



| Entry | R | conditions ^a | products ^b | yield ^c |
|-------|-----------------|-------------------------|-----------------------|--------------------|
| 1 | Bn | A | 6.7a/6.8a | 99% |
| 2 | Bn | B | 6.7a/6.8a | 99% |
| 3 | Ad ^d | B | 6.7b/6.8b | 95% |
| 4 | Bn | C | 6.7a/6.8a | 88% |
| 5 | <i>n</i> -octyl | C | 6.7c/6.8c | 99% |
| 6 | cyclopentyl | C | 6.7d/6.8d | 97% |
| 7 | Ph | C | 6.7e/6.8e | 97% |
| 8 | Bn | D | 6.7a/6.8a | 88% |
| 9 | <i>n</i> -octyl | D | 6.7c/6.8c | 81% |
| 10 | cyclopentyl | D | 6.7d/6.8d | 86% |
| 11 | Ad ^d | D | 6.7b/6.8b | 85% |
| 12 | Ph | D | 6.7e/6.8e | 99% |

NOTE: (a) A = neat, rt, 2 days; B = CHCl₃, rt, 4 weeks; C = CHCl₃, reflux, 3 days; D = PhCH₃, reflux, 4 h. (b) ratio of **6.7:6.8** = 1.0:1.1 in all cases as determined by ¹H NMR. (c) isolated yield. (d) Ad = 1-adamantyl.

The structures of **6.7b-e** and **6.8b-e** were assigned using 2D-NMR experiments. The *exo*-stereochemistry was confirmed from the observed ⁴*J*-coupling (“W-coupling”) of H² and H³ with H^{5'}, but not with H⁵ (Figure 6.4). The position of the Boc group relative to the R group was confirmed by HMBC experiments based on the observed ³*J*-coupling between H⁴ (or H¹) and the carbonyl. The relationship of H¹-H⁴ with the R group was confirmed by HMBC and ROESY experiments.

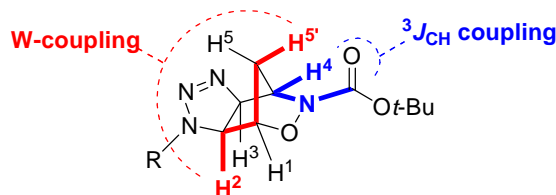
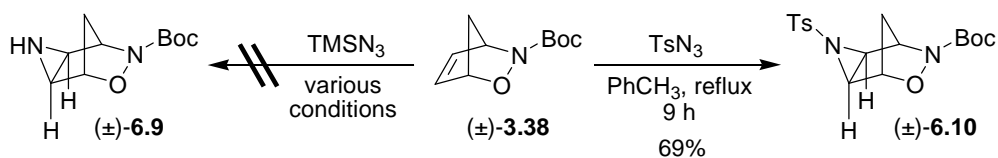


Figure 6.4. W-coupling and $^3J_{\text{CH}}$ -coupling of *exo*-triazolines **6.7** and **6.8**

6.3.3 Addition of azides and the direct synthesis of aziridines

Trimethylsilyl azide has been reported to add to norbornene systems and other bicyclic olefins to yield triazolines and/or aziridines directly.^{457, 463} Since we were interested in obtaining the unsubstituted aziridine **6.9**, **3.38** was subjected to azidotrimethylsilane (Scheme 6.4). Under a variety of reaction conditions (neat, 25-80 °C, PhCH₃, 25-110 °C), only decomposition of the alkene was observed. Tosyl azide⁴²⁵ has also been reported to afford aziridines directly,⁴⁵⁵ and upon treatment of **3.38** with tosyl azide, we were able to obtain aziridine **6.10** in good yield.

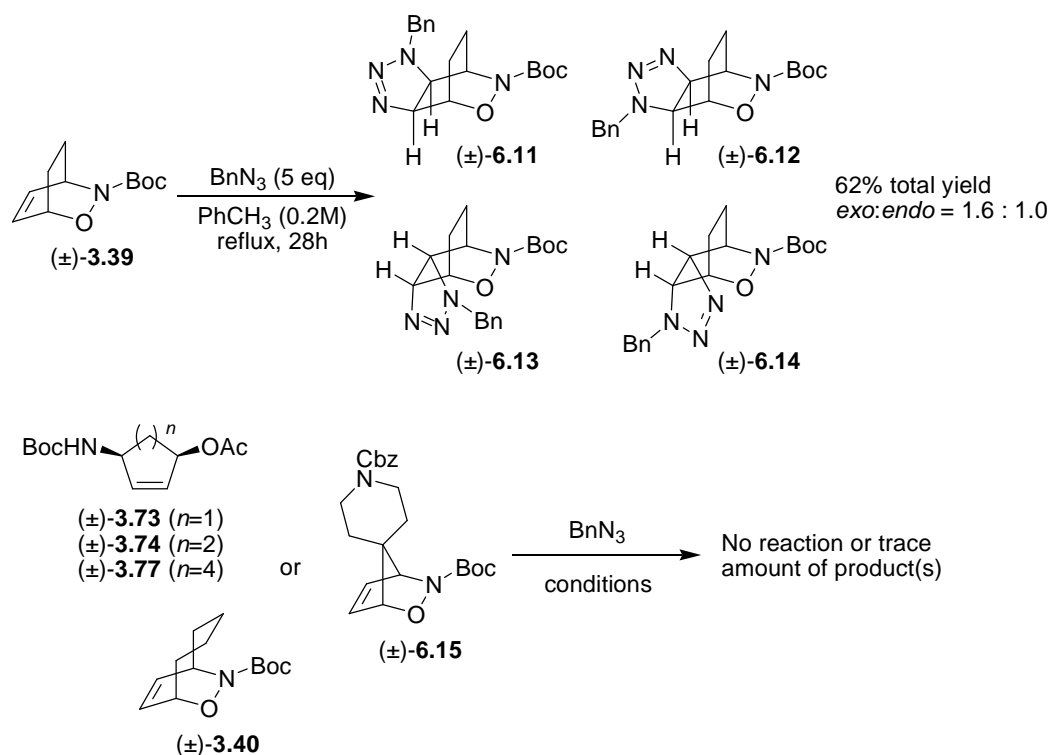


Scheme 6.4. Direct synthesis of aziridines from cycloadduct

6.3.4 Effect of ring strain on alkene reactivity

The effect of alkene strain on the formation of triazolines was also studied. When the bicyclo[2.2.2] cycloadduct **3.39** was treated with 1.4 equivalents of benzyl azide neat at room temperature, no reaction was observed. When **3.39** was treated with a large

excess of benzyl azide in refluxing toluene, however, a mixture of triazolines **6.11-6.14** was obtained in moderate yield (Scheme 6.5). In contrast to bicyclo[2.2.1] cycloadduct **3.38**, *endo*-triazolines **6.13** and **6.14** were formed along with *exo*-triazolines **6.11** and **6.12**. The influence of ring strain on reactivity was especially evident when bicyclo[2.2.4] cycloadduct **3.40** and monocyclic alkenes **3.73**, **3.74**, and **3.77** were subjected to the same conditions. When heated neat or in toluene, only trace amounts of triazoline products were observed. The sterically hindered spirocyclic oxazine **6.15** also did not yield any triazoline products, presumably due to overwhelming steric strain blocking the *exo*-attack of the azide. From dihydroxylation reactions performed by other members of the Miller group, additions to the alkene of spirocyclic oxazine **6.15** occur exclusively on the *exo*-face of the alkene even in such a sterically encumbered system.



Scheme 6.5. Addition of benzyl azide to other alkenes

Similar to triazolines **6.7** and **6.8**, *endo*- and *exo*-stereochemistry of triazolines **6.11-6.14** was determined using 2D NMR spectroscopic techniques. The presence of W-coupling between H² or H³ and H^{5'} or H^{6'}, but not H⁵ or H⁶ confirmed the *exo*-stereochemistry of triazolines **6.11** and **6.12** (Figure 6.5). ³J_{CH}-coupling between either H¹ or H⁴ and the carbonyl of the Boc group confirmed the regiochemistry as *syn* or *anti*, respectively.

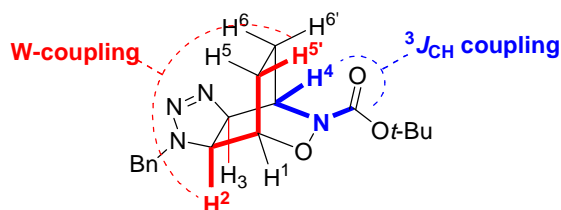


Figure 6.5. W-coupling and ³J_{CH}-coupling for triazolines **6.11** and **6.12**

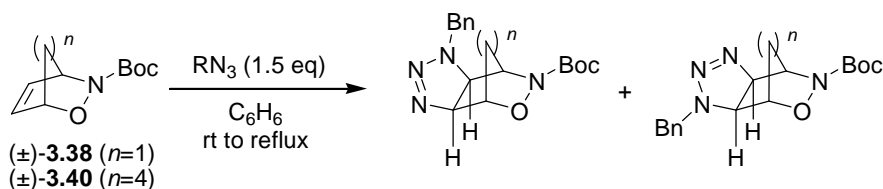
6.3.5 Investigation of Lewis acid catalysts for the reaction

Sharpless has reported additions of azides to alkynes using the ruthenium(II) catalyst **6.16**, and we tested whether this Lewis acid could promote the [3+2] dipolar cycloaddition of azides with the less activated alkenes such as cycloadduct **3.40** (Table 6.2). Cycloadducts **3.38** and **3.40** were treated with benzyl azide in benzene with and without the addition of a catalytic amount of Ru(II) catalyst **6.16** and were analyzed using ¹H NMR. Interestingly, the addition of catalyst **6.16** did not only fail to promote the dipolar cycloaddition reaction, but appeared to slow the reaction with cycloadduct **3.38** (Table 6.2, entries 1 and 2). No decomposition was observed in reactions using

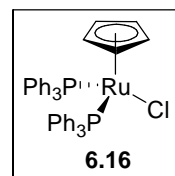
cycloadduct **3.40**, and it is possible that the Ru(II) catalyst **6.16** was responsible for the decomposition of triazoline products **6.7** and **6.8**.

TABLE 6.2

EFFECT OF RU(II) CATALYST ON AZIDE CYCLOADDITION REACTIONS



| Entry | <i>n</i> | additive | result |
|-------|----------|----------------------|----------------|
| 1 | 1 | none | 99% conversion |
| 2 | 1 | 6.16 (5 mol%) | 66% conversion |
| 3 | 4 | none | no reaction |
| 4 | 4 | 6.16 (5 mol%) | no reaction |

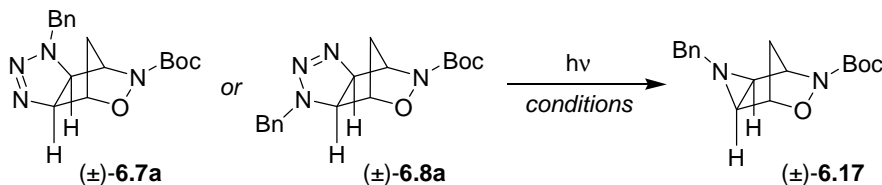


A variety of other Lewis acids were examined and did not facilitate azide cycloaddition reactions to cycloadduct **3.38**. Cycloadduct **3.38** was treated with excess (1.5 eq) benzyl azide and a stoichiometric amount (1.0 eq) of various Lewis acids (AgOTf, CuCl, Cu(OTf)₂, Fe(acac)₃, [Rh(OAc)₂]₂, In(OTf)₃, La(OTf)₃, NiCl₂, Pd(OAc)₂, Sc(OTf)₃, SnCl₂, Yb(OTf)₃, and ZnCl₂). None of the Lewis acids studied facilitated the reaction at rt in dichloromethane or 2:1 acetonitrile/H₂O, and many Lewis acids were found to promote decomposition of cycloadduct **3.38** through mechanisms outlined in chapter 1. While these results were disappointing, it is highly encouraged that additional studies with other cycloadducts (namely **3.40**), at elevated temperatures should be examined.

6.4 Reactivity of triazoline products

In the course of this study, we found that triazolines **6.7a** and **6.8a** were stable to temperatures up to 120 °C and did not decompose readily in ambient light. When irradiated below ~300 nm, **6.7a** and **6.8a** readily underwent photolytic conversion to aziridine **6.17** in 2-4 h (Table 6.3). At wavelengths higher than 300 nm, the time required for the reaction to proceed to 100% conversion increased greatly (Table 6.3, entry 5).

TABLE 6.3
PHOTOLYSIS OF TRIAZOLINES

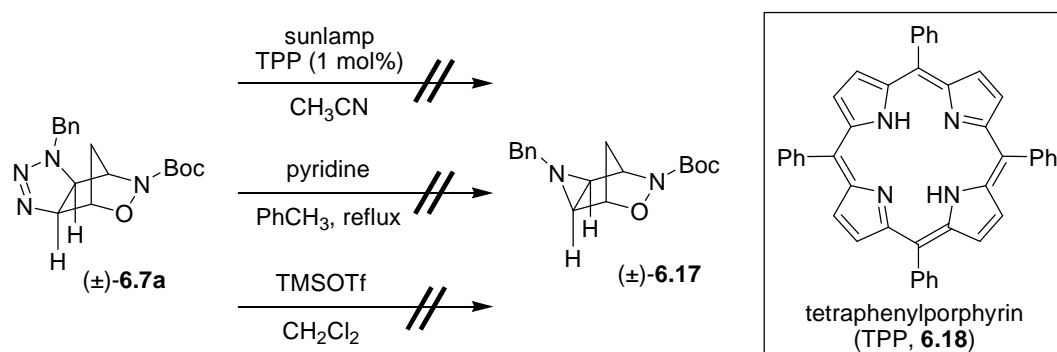


| Entry | isomer | solvent | filter | time ^a | yield |
|-------|-------------|--------------------|--------|-------------------|-------|
| 1 | 6.7a | CHCl ₃ | quartz | 1.5 h | 65% |
| 2 | 6.8a | CHCl ₃ | quartz | 3.5 h | 78% |
| 3 | 6.7a | CH ₃ CN | vycor | 2 h | 75% |
| 4 | 6.8a | CH ₃ CN | vycor | 3 h | 67% |
| 5 | 6.7a | CH ₃ CN | pyrex | 20 h | 93% |

NOTE: (a) reactions were monitored by TLC and ¹H NMR.

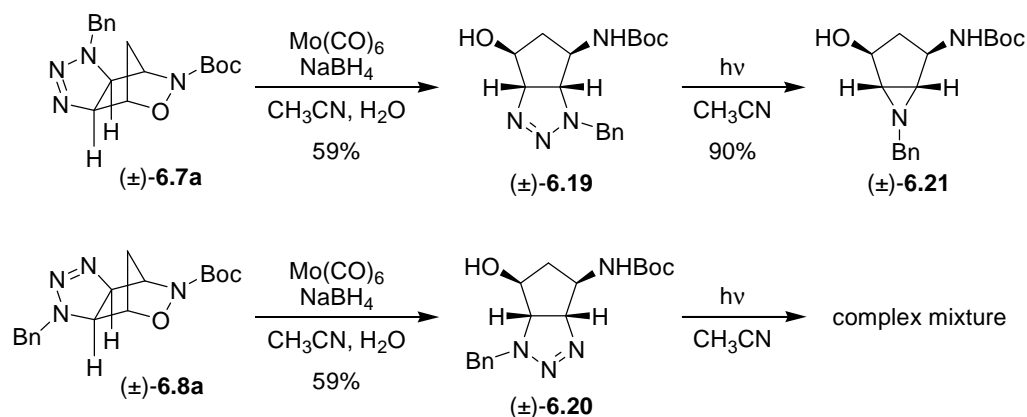
The conversion of triazoline **6.7a** to aziridine **6.17** was also attempted using alternative methods (Scheme 6.6). While triazolines were stable almost indefinitely to a sunlamp, we hypothesized that the addition of a sensitizer such as tetraphenylporphyrin (TPP, **6.18**) might facilitate the formation of aziridine **6.17** at higher wavelengths. Only triazoline **6.7a** was observed when irradiated using a sunlamp in the presence of a

catalytic amount of porphyrin **6.18** in acetonitrile. While triazolines are known to decompose to yield aziridines and imines under acidic conditions,⁴⁶⁴ the addition of up to one equivalent of TMSOTf also did not facilitate the formation of aziridine **6.17** and triazoline **6.7a** was recovered. Similarly, triazoline **6.7a** was resistant to decomposition with excess pyridine.



Scheme 6.6. Attempted alternative routes to aziridines

Surprisingly, N-O bond reduction of triazolines **6.7a** and **6.8a** proceeded cleanly to afford triazolines **6.19** and **6.20** without decomposition of the triazoline ring (Scheme 6.7). Triazoline **6.19** was cleanly transformed to aziridine **6.21** upon irradiation in acetonitrile; however, a complex mixture of products was observed when triazoline **6.20** was subjected to the same conditions. The reason for this result is unclear at this time.

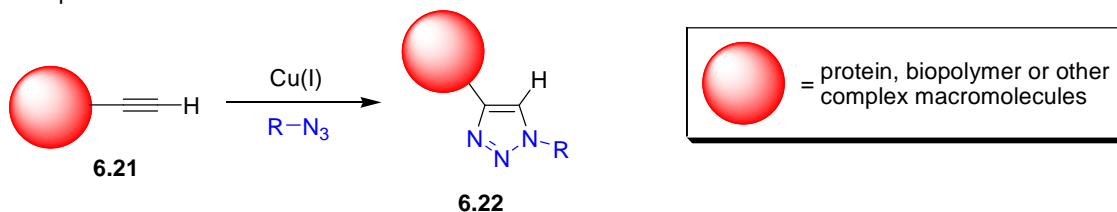


Scheme 6.7. Structural elaboration of triazolines

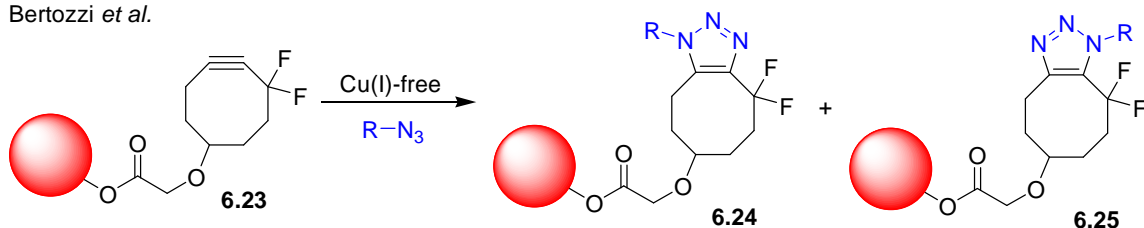
6.5 Possible application of the azide-addition reaction in bioconjugation reactions

Sharpless *et al* has developed a Cu(I)-catalyzed azide-alkyne [3+2] dipolar cycloaddition reaction (Huisgen cycloaddition) that is remarkably selective and is compatible with a variety of functional groups.³⁹⁰ The use of other metal catalysts and the mechanism of the reaction has also been reported.^{393, 465-467} Due to the reliability, complete specificity, and bio-compatibility of azides and alkynes, this method has found a number of applications in bioconjugation chemistry (Scheme 6.8).^{391, 394-397, 399-408, 410, 412, 413, 417}

Sharpless *et al.*

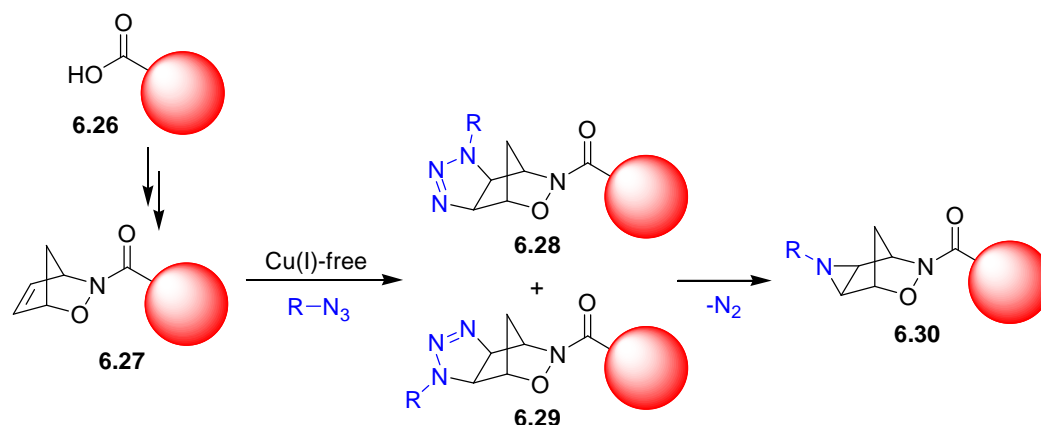


Bertozzi *et al.*



Scheme 6.8. Bioconjugation chemistry using azide-alkyne [3+2] cycloaddition chemistry

We envision the use of the azide-alkene [3+2] cycloaddition as an alternative method for bioconjugation that is complementary to the reaction developed by Bertozzi *et al.*^{468, 469} Potentially any carboxylic acid **6.26** could be converted to the acylnitroso cycloadduct **6.27**, and would provide triazolines **6.28** and **6.29** in the presence of azides (Scheme 6.9). As with the strained alkyne **6.23** developed by Bertozzi, regioselectivity is still an issue with the initial dipolar cycloadducts; however, unlike triazoles **6.24** and **6.25**, if desired, triazolines **6.28** and **6.29** can be converted to aziridines **6.30** using photolytic methods. If sulfonyl azides are used, aziridines **6.30** (where $\text{R}=\text{SO}_2\text{R}'$) can be accessed directly from cycloadducts **6.27**.



Scheme 6.9. Proposed use of azide-alkene [3+2] cycloaddition in bioconjugation chemistry

Although the application of the azide-alkene [3+2] cycloaddition has a potential application in bioconjugation chemistry, the conditions required for the reaction to proceed at a significant rate may not be compatible with biological systems or complex macromolecules. This problem of reactivity could be overcome with the development of a more reactive acylnitroso cycloadduct **6.27** through substitution with electron-withdrawing substituents.

6.6 Summary and recommendations for further study

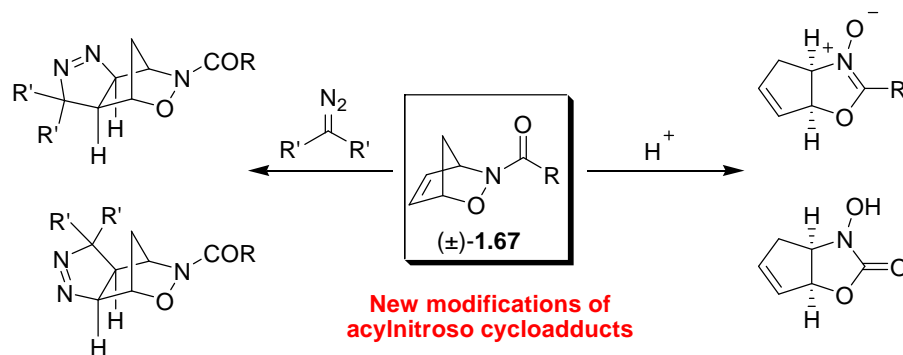
This chapter has described the study and development of the addition of azides to acylnitroso cycloadducts **1.67** for the stereoselective formation of triazolines and aziridines. This chemistry has potential applications toward the synthesis of novel aziridine- and triazoline-containing 5'-norcarbocyclic nucleoside analogs **6.4-6.6** and in bioconjugation chemistry.

CHAPTER 7:
NEW DIRECTIONS FOR ACYLNITROSO HETERO-DIELS-ALDER CHEMISTRY:
PRELIMINARY INVESTIGATIONS AND RESULTS

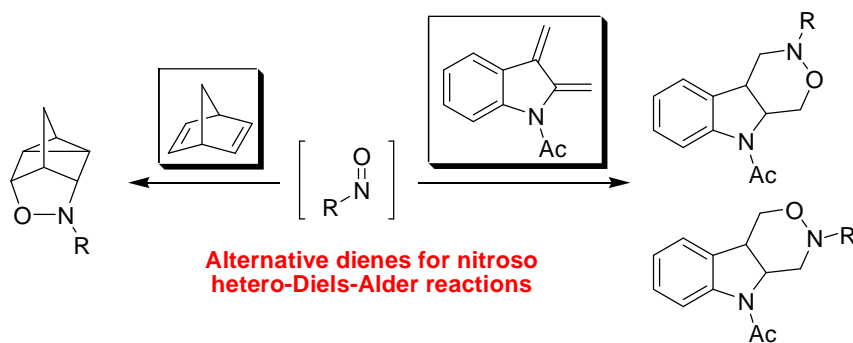
7.1 Introductory remarks

The previous chapters have highlighted the application and utility of the acylnitroso HDA reaction in organic synthesis and have concentrated on investigating new reactivity and new applications for the acylnitroso HDA reaction. This chapter will highlight preliminary results of investigations into developing new applications and reactions using acylnitroso HDA reactions. While many of these investigations are incomplete, they serve to demonstrate the chemical potential of the acylnitroso HDA reaction and will provide indications toward new directions for research in this area.

The results of four preliminary investigations will be presented here. Two of the topics, the expansion of the [3+2] dipolar cycloaddition chemistry presented in the previous chapter and the investigation of acid-promoted rearrangements, are geared toward the development of new modifications of acylnitroso cycloadducts **1.67** (Scheme 7.1). The other two topics, investigation of homo-Diels-Alder reactions with norbornadiene and synthesis of Dimebon analogs using indole *ortho*-quinodimethanes, are geared toward the investigating the reactivity of acylnitroso species with other diene systems (Scheme 7.2).



Scheme 7.1. Graphical overview of sections 2 and 3

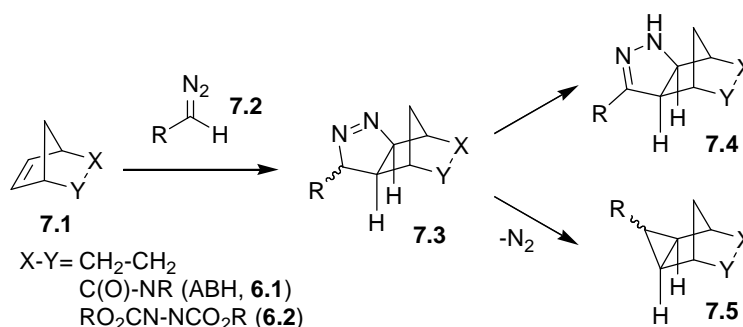


Scheme 7.2. Graphical overview of sections 4 and 5

7.2 Addition of diazoalkanes to acylnitroso cycloadducts

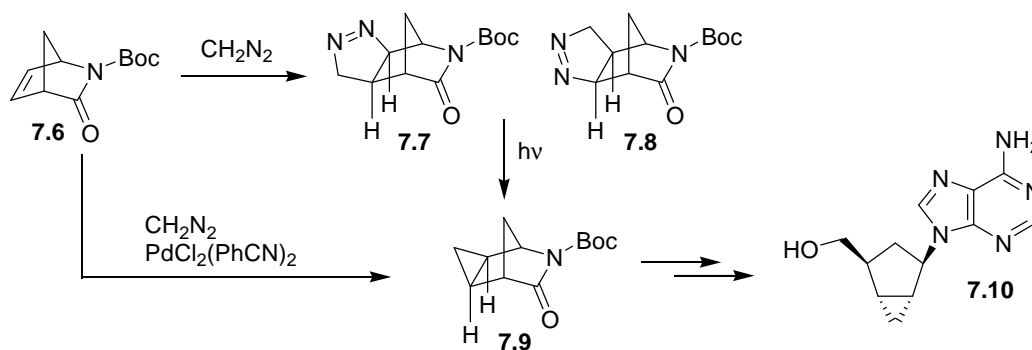
Numerous examples of intermolecular addition of diazoalkanes **7.2** to strained bicyclic alkenes **7.1** have been reported in the literature (Scheme 7.3). Examples of additions to strained bicyclo[2.2.1] systems include additions to carbocyclic norbornene systems,⁴⁷⁰⁻⁴⁷⁶ 2-azabicyclo[2.2.1]hept-5-en-one (ABH, **6.1**),^{477, 478} and 2,3-diazabicyclo[2.2.1]hept-2-enes **6.2**,⁴⁷⁹ however, to the best of our knowledge, the [3+2] cycloaddition of diazoalkane species to acylnitroso cycloadducts **1.67** has been reported. The initial dipolar cycloadducts **7.3** are obtained in the reaction; however, if diazoalkanes **7.2** are used where R is an electron-withdrawing group, often pyrazolines **7.4** are

obtained directly in reactions.^{470, 476, 480} Alternatively, 4,5-dihydro-3*H*-pyrazoles **7.3** have been decomposed to cyclopropyl compounds **7.5** using photolysis or metal-mediated chemistry.⁴⁸¹



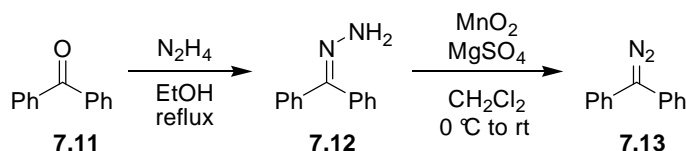
Scheme 7.3. Addition of diazoalkanes to other bicyclo[2.2.1] systems

Ishikura has used dipolar diazoalkane cycloaddition reactions to synthesize 2',3'-methano carbocyclic nucleoside **7.10** from compound **7.6** (Scheme 7.4).^{477, 478} The initial cycloadducts **7.7** and **7.8** were photolyzed to compound **7.9**. Alternatively, compound **7.9** was obtained directly using Pd(II) chemistry. Compound **7.9** was used to synthesize carbocyclic nucleoside analogs such as compound **7.10**.



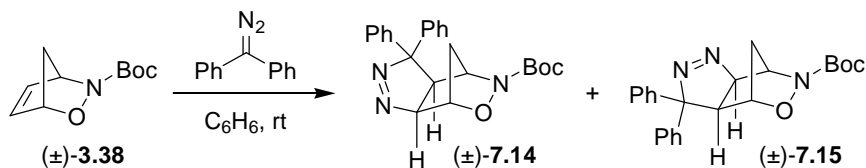
Scheme 7.4. Diazoalkane cycloaddition chemistry by Ishikura group

We wished to investigate the reactivity of cycloadducts **1.67** with diazoalkane species. Diphenyldiazomethane (**7.13**) was prepared from benzophenone (**7.11**) using a two step procedure (Scheme 7.5). Hydrazone **7.12** was easily synthesized from benzophenone (**7.11**). Oxidation of hydrazone **7.12** to diphenyldiazomethane (**7.13**) was performed using manganese dioxide.⁴⁸² A number of alternative methods have been reported in the literature,⁴⁸³ most often using HgO.^{484, 485}



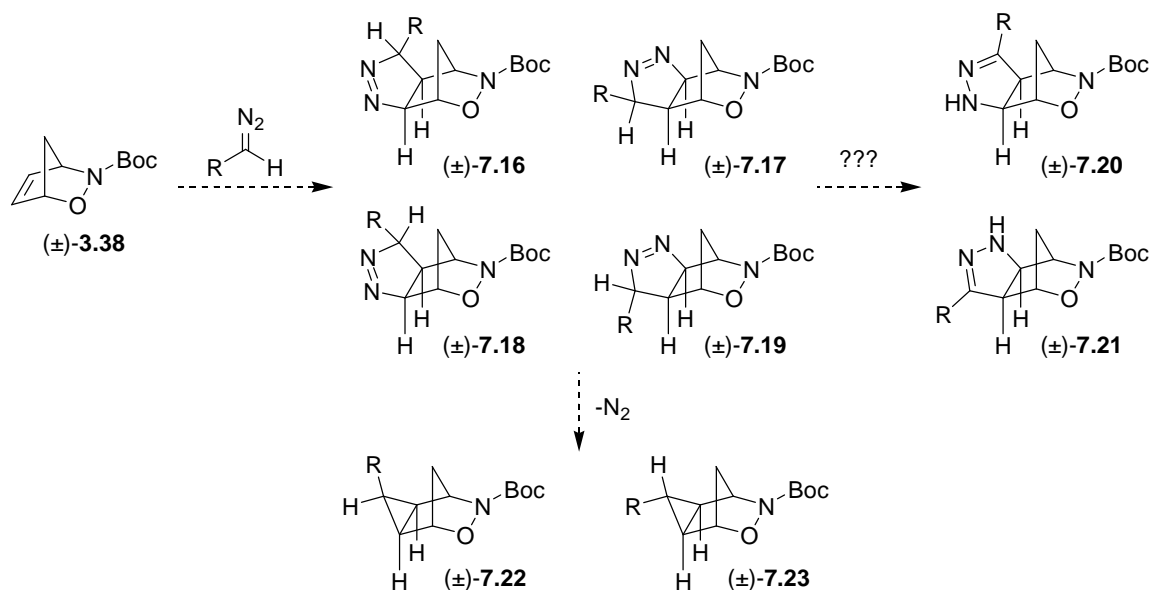
Scheme 7.5. Synthesis of diphenyldiazomethane

Cycloadduct **3.38** was stirred with the crude mixture of diphenyldiazomethane (**7.13**) in benzene at room temperature (Scheme 7.6). After a few days, consumption of cycloadduct **3.38** was observed and preliminary evidence suggested the formation of pyrazolines **7.14** and **7.15** by NMR. Unfortunately, a number of other products were identified in the mixture such as azines resulting from decomposition of diphenyldiazomethane.



Scheme 7.6. Addition of diphenyldiazomethane to an acylnitroso cycloadduct

After confirming the above results, the next stage of the investigation should be through the use of the simple diazoalkane, diazomethane (or the safer alternative, trimethylsilyldiazomethane), as well as the use of other diazoalkanes (Scheme 7.7). Based on azide addition reactions (chapter 6) and addition of diazoalkanes to other bicyclo[2.2.1] systems reported in the literature, the additions are expected to be *exo*-specific. The stereoselectivity of the diazoalkane carbon is not as apparent from the literature, and up to four initial adducts (**7.16-7.19**) would be expected. These adducts could be converted to pyrazolines **7.20** and **7.21**, or to cyclopropanes **7.22** and **7.23**.



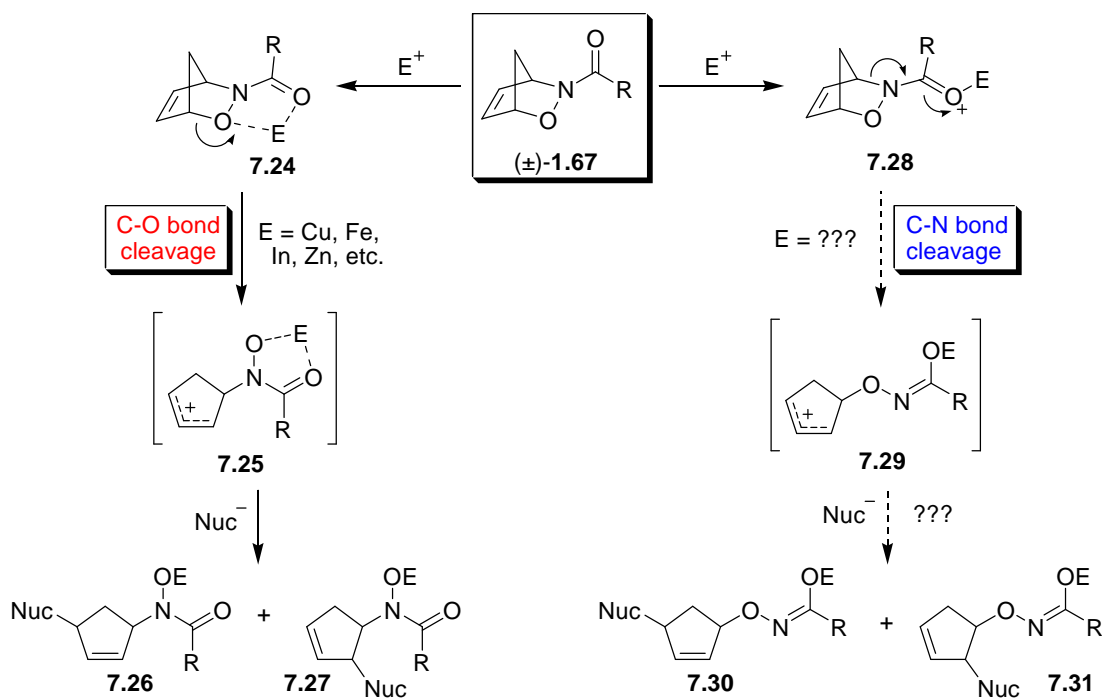
Scheme 7.7. Overview of possible applications of diazoalkane addition chemistry

While work related to this project is incomplete, preliminary evidence coupled with the success of similar reactions reported in the literature indicate the addition of diazoalkanes is an interesting avenue of study for future research in the Miller group.

7.3 Acid-catalyzed rearrangements of acylnitroso cycloadducts

7.3.1 Origin of this study

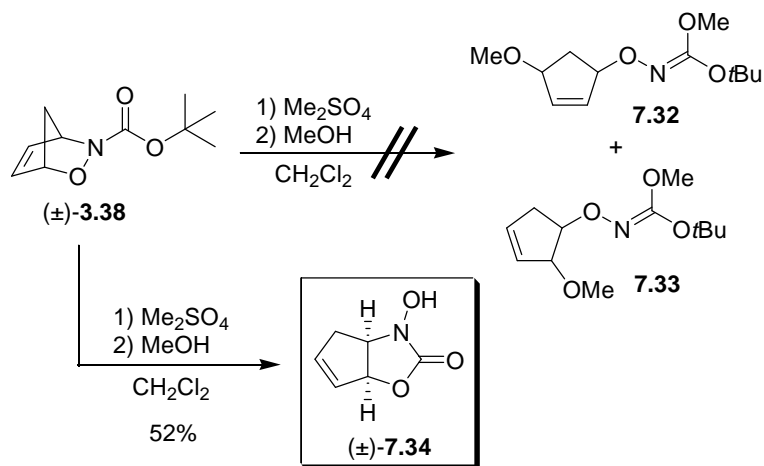
As described in chapter 1, C-O bond cleavage of acylnitroso cycloadducts **1.67** is mediated by a number of Lewis acids and other electrophiles due in part to the formation of species such as **7.24** and **7.25** (Scheme 7.8). We hypothesized that, given the correct electrophile, C-N bond cleavage could occur to yield species such as **7.29** and hydroxamate products **7.30** and **7.31**. The following section delineates an effort that originally was geared toward an attempt to realize this possible alternate reactivity pattern for cycloadducts **1.67** which yielded an unexpected, yet precedented, result.



Scheme 7.8. Outline of C-N bond-breaking strategy

7.3.2 Acid-catalyzed formation of bicyclic hydroxamates

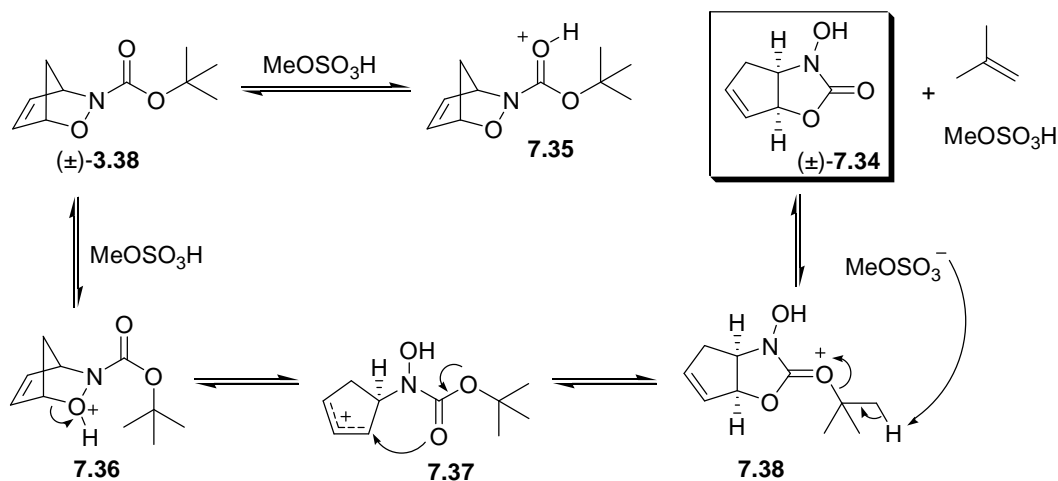
Dimethylsulfate (Me_2SO_4) has been used as a methylating agent that we hypothesized might yield hydroximates **7.32** and **7.33** upon subsequent methanolysis of the reactive intermediates (Scheme 7.9). We were surprised to find that a hydroxamate compound in agreement with structure **7.34** was formed in 52% yield!



Scheme 7.9. Attempted C-N bond cleavage reaction

This result was initially very puzzling; however, it was apparent that the dimethylsulfate used was not recently distilled and probably contained substantial amounts of monomethylsulfate (MeOSO_3H) and sulfuric acid, which could account for the formation of hydroxamate **7.34**. With this knowledge, a mechanism of the reaction has been proposed (Scheme 7.10). Protonation of cycloadduct **3.38** would occur to give species **7.35** or **7.36**. Protonated species **7.35** could result in the loss of the Boc protecting group, which would certainly lead to possible, yet unconfirmed, by-products of the reaction. C-O bond cleavage of species **7.36** would yield the allylic cation **7.37**, which upon intramolecular cyclization affords compound **7.38**. Species **7.36** might also

be able to convert to species **7.38** directly. Loss of isobutylene from compound **7.38** would yield the hydroxamate compound **7.34** and also regenerate the acid catalyst.

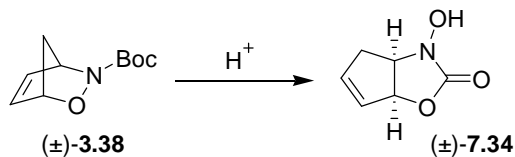


Scheme 7.10. Proposed mechanism for hydroxamate formation

This mechanism would suggest the reaction would proceed through general acid catalysis, and we proceeded to optimize the reaction conditions to yield hydroxamate **7.34** based upon this principle (Table 7.1). As expected, stronger acids yielded more hydroxamate **7.34** for the reaction. As an example, whereas 35 mol% *p*-toluenesulfonic acid offered a low yield (20%) of hydroxamate **7.34** in 2 h, only 5 mol% of triflic acid gave a higher yield (52%) of hydroxamate **7.34** in only 30 min (Table 7.1, entries 2 and 4). Optimal conditions for the reaction were at 0 °C in THF using only 1 mol% of triflic acid (Table 7.1, entry 6). The sulfonic acid-based resin, Amberlyst 15, also promoted the reaction; however, use of this resin was much slower than even 5 mol% *p*-toluenesulfonic acid (Table 7.1, entries 1 and 7).

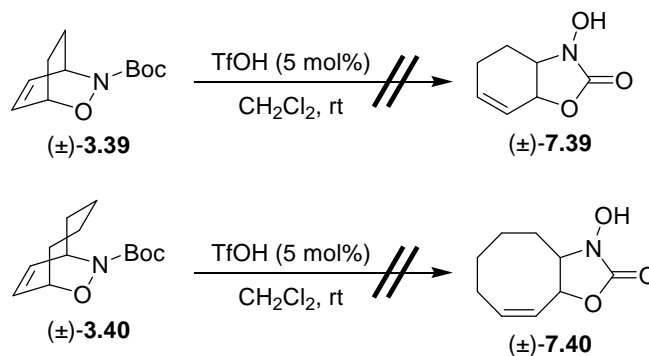
TABLE 7.1

OPTIMIZATION OF ACID-CATALYZED HYDROXAMATE FORMATION



| Entry | Acid (amount) | Conditions | Yield/Result |
|-------|-------------------------|--|-----------------------|
| 1 | <i>p</i> TsOH (5 mol%) | CH ₂ Cl ₂ , rt, 2h | incomplete rxn. |
| 2 | <i>p</i> TsOH (35 mol%) | CH ₂ Cl ₂ , rt, 4h | 20% |
| 3 | TFA (5 mol%) | CH ₂ Cl ₂ , rt, 2h | recovered 3.38 |
| 4 | TfOH (5 mol%) | CH ₂ Cl ₂ , rt, 2h | 52% |
| 5 | TfOH (5 mol%) | CH ₂ Cl ₂ , rt, 30 min | 62% |
| 6 | TfOH (1 mol%) | THF, 0 °C, 1h | 74% |
| 7 | Amberlyst 15 resin | THF, rt, 5 days | incomplete rxn. |

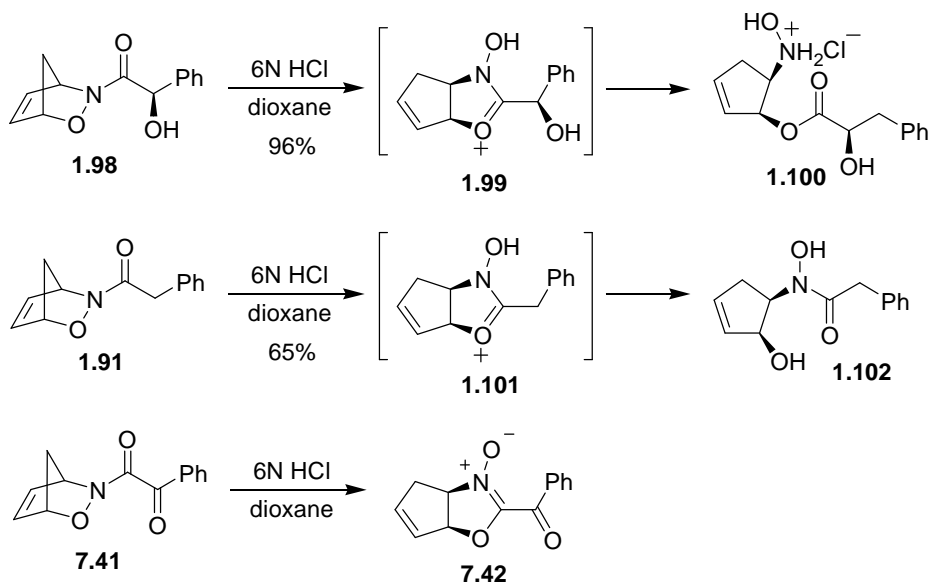
Encouraged by these results, we proceeded to investigate whether cycloadducts derived from larger cyclic dienes could also form bicyclic hydroxamate structures. Consequently, cycloadducts **3.39** and **3.40** were subjected to catalytic triflic acid in dichloromethane, but the expected hydroxamate products **7.39** and **7.40** were not observed by NMR or LC/MS (Scheme 7.11).



Scheme 7.11. Attempted hydroxamate formation with larger ring systems

7.3.3 Similar transformations reported in the literature

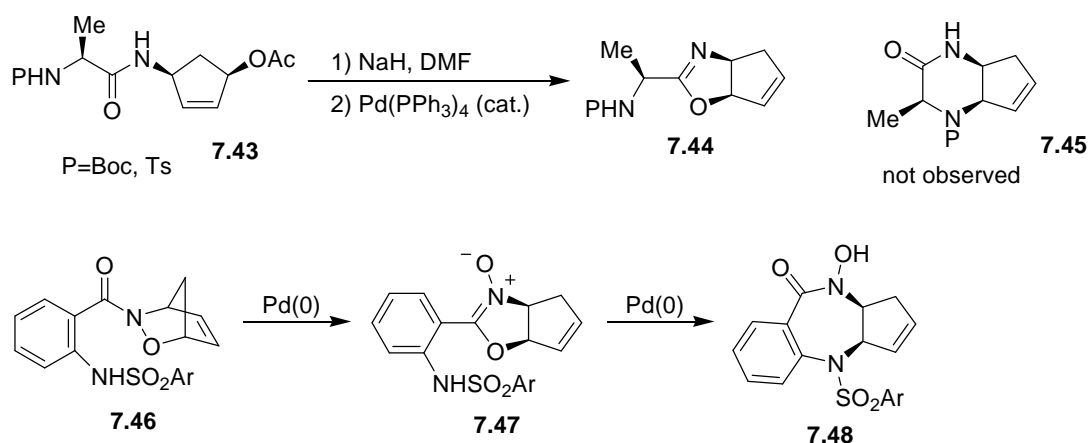
While the formation of bicyclic hydroxamate **7.34** from cycloadduct **3.38** is a novel reaction, similar transformations have been observed in the literature. Recall that in chapter 1 the formation of intermediates **1.99** and **1.101** under aqueous acidic conditions from cycloadducts **1.98** and **1.91** was highlighted (Scheme 7.12).^{107, 109, 110} Procter also described the formation of nitron **7.42** under the same conditions from cycloadduct **7.41** (yield undisclosed),¹¹⁰ which may indicate that the proposed intermediates **1.99** and **1.101** may be better represented by a nitron structure than the oxonium ion structures shown.



Scheme 7.12. Similar acid-promoted reactions reported in the literature

The Miller group has reported similar structures formed through intramolecular ring-closure reactions using Pd(0) chemistry (Scheme 7.13). When allylic acetate **7.43** was treated with Pd(0), the expected piperazine **7.45** was not observed and oxazoline **7.44**

was produced instead.¹³¹ Miller has also reported the formation of 1,4-benzodiazepines **7.48** using Pd(0) chemistry from cycloadducts **7.46**.¹¹⁵ Based on unpublished results from the Miller group, there is a clear indication that nitrones **7.47** are formed under these conditions. The characterization of nitrones **7.47** as an intermediate toward benzodiazepines **7.48** or a result of a side-reaction is still not clear at this time; however, nitrones **7.47** form benzodiazepines **7.48** with the addition of Pd(0) catalyst.



Scheme 7.13. Similar reactions using Pd(0) chemistry

These results seem to indicate that in many of the C-O bond cleavage events reported for acylnitroso cycloadducts **1.67**, intermediates such as hydroxamate **7.34** should be considered, and may have a great effect on the stereochemical outcome of the reaction.

7.3.4 Acid-promoted formation of nitrones

Based upon the formation of nitron **7.42** reported by Procter, we then explored the formation of nitrones from acylnitroso cycloadducts **7.49** and **1.91** (Table 7.2). Under

acid-catalyzed conditions, nitrones **7.50** and **7.51** were not observed and cycloadducts **7.49** and **1.91** were recovered relatively unchanged from the reaction mixture (Table 7.2, entries 1 and 2). When cycloadduct **7.49** was treated with one equivalent of triflic acid, a low yield of nitron **7.50** was recovered from the reaction mixture but cycloadduct **1.91** decomposed under the same conditions (Table 7.2, entries 3 and 4).

TABLE 7.2
NITRONE FORMATION UNDER ACIDIC CONDITIONS

$(\pm)\text{-7.49 (R=Ph)}$
 $(\pm)\text{-1.91 (R=CH}_2\text{Ph)}$

$(\pm)\text{-7.50 (R=Ph)}$
 $(\pm)\text{-7.51 (R=CH}_2\text{Ph)}$

| Entry | R | amount TfOH | Yield/Result |
|-------|--------------------|-------------|---------------|
| 1 | Ph | 5 mol% | no reaction |
| 2 | CH ₂ Ph | 5 mol% | no reaction |
| 3 | Ph | 100 mol% | 20% |
| 4 | CH ₂ Ph | 100 mol% | decomposition |

A possible explanation for why nitron **7.50** was recoverable from the reaction whereas nitron **7.51** was not recovered may be attributed to the greater stability of nitron **7.50** compared to that of **7.51** due to π -delocalization (Figure 7.1). Similarly, nitron **7.42**, reported by Procter,¹¹⁰ would benefit from π -delocalization.

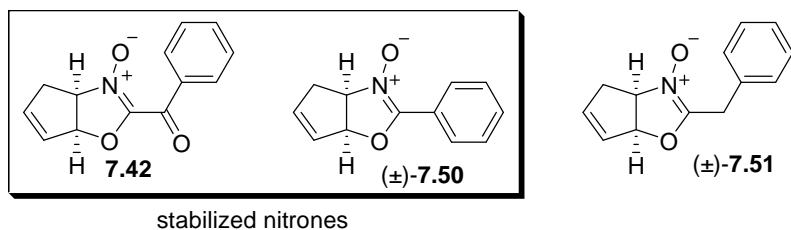
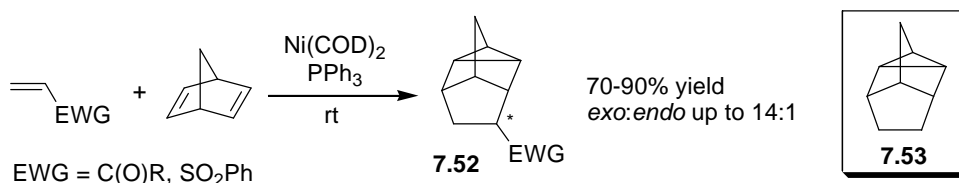


Figure 7.1. Comparing nitrone stability

7.4 Acylnitroso homo-Diels-Alder reaction with norbornadiene

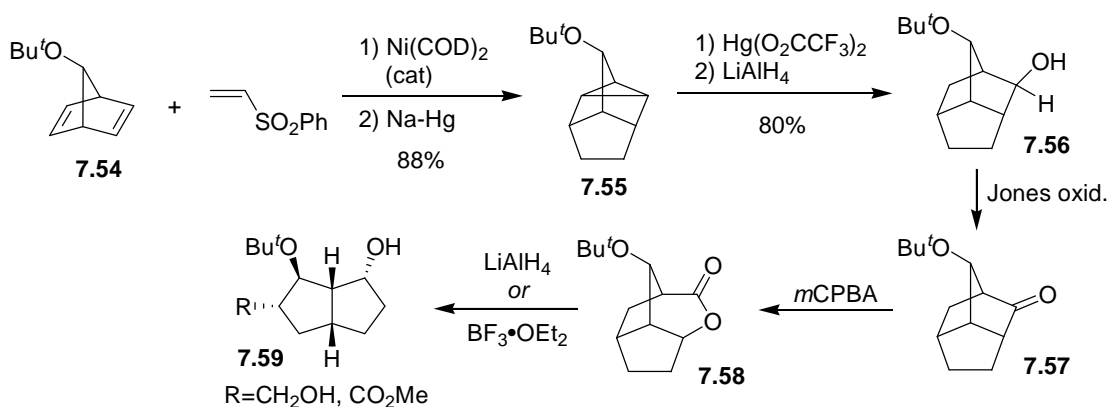
7.4.1 Homo-Diels-Alder cycloadditions in the literature

While Diels-Alder cycloadditions with conjugated 1,3-dienes have been extensively studied, Diels-Alder cycloadditions with homo-conjugated dienes (homo-Diels-Alder reaction) have not received as much attention. First discovered in the late 1950s,^{486, 487} norbornadiene was observed to undergo formal [2+2+2] cycloadditions with electron-deficient alkenes under thermal conditions.^{488, 489} Lautens has reported stereoselective homo-Diels-Alder reactions of vinyl sulfones and enones with norbornadienes catalyzed by cobalt and nickel complexes (Scheme 7.14).⁴⁸⁹⁻⁴⁹⁷ The tetracyclic homo-Diels-Alder cycloadducts **7.52** possess the core deltacyclene hydrocarbon skeleton (**7.53**). Other research groups have reported stereo- and regioselective cleavage of the cyclopropane unit of deltacyclene systems.^{498, 499}



Scheme 7.14. Representative homo-Diels-Alder cycloadditions

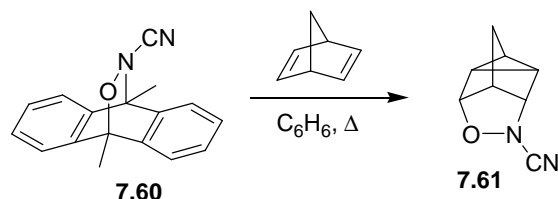
Lautens has demonstrated the application of this chemistry with the synthesis of functionalized diquinane structures **7.59** (Scheme 7.15).⁴⁹³ A formal [2+2+2] homo-Diels-Alder reaction with substituted norbornadiene **7.54** followed by reduction of the sulfone moiety provided tetracyclic compound **7.55**. Regioselective oxymercuration provided alcohol **7.56** after reduction, which was oxidized to ketone **7.57**. Baeyer-Villiger oxidation provided lactone **7.58** selectively, and ring-opening of the lactone provided diquinanes **7.59**.



Scheme 7.15. Synthesis of diquinanes reported by Lautens *et al*

Reports in the literature of formal [2+2+2] hetero Diels-Alder cycloadditions are rare; however, Kirby reported in 1981 that nitrosocyanide, generated from cycloreversion of dimethylantracene cycloadduct **7.60**, provided the homo-Diels-Alder adduct **7.61** in the presence of norbornadiene (Scheme 7.16).¹⁷³ To the best of our knowledge, this is the only reported homo-Diels-Alder cycloaddition using nitroso dienophiles. In an effort to expand the scope of the highly useful acylnitroso hetero Diels-Alder reaction, we wished

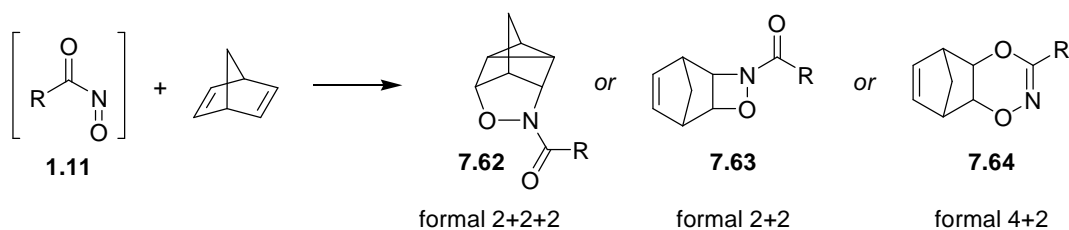
to explore a formal hetero [2+2+2] cycloaddition (homo-Diels-Alder reaction) with norbornadiene.



Scheme 7.16. Reported nitroso homo-Diels-Alder cycloaddition

7.4.2 Preliminary results of acylnitroso homo-Diels-Alder reactions

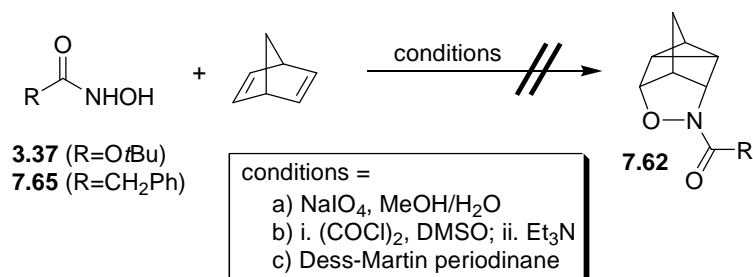
Even though Kirby *et al* only reported obtaining the formal [2+2+2] cycloadduct **7.61**,¹⁷³ we hypothesize the use of acylnitroso species **1.11** as dienophiles may yield different results (Scheme 7.17). Based upon the current understanding of acylnitroso chemistry, the homo-Diels-Alder cycloadduct **7.62** would be an expected product of the reaction; however, 1:1 adducts **7.63** and **7.64**, arising from formal [2+2] and [4+2] cycloadditions, respectively, may also be obtained.



Scheme 7.17. Possible acylnitroso cycloadducts using norbornadiene

We first attempted to form homo-Diels-Alder cycloadducts **7.62** by direct oxidation of hydroxamic acids **3.37** and **7.65** in the presence of norbornadiene (Scheme

7.18). Using a variety of oxidation methods, only complex mixtures were obtained or products resulting from decomposition of acylnitroso species were obtained.

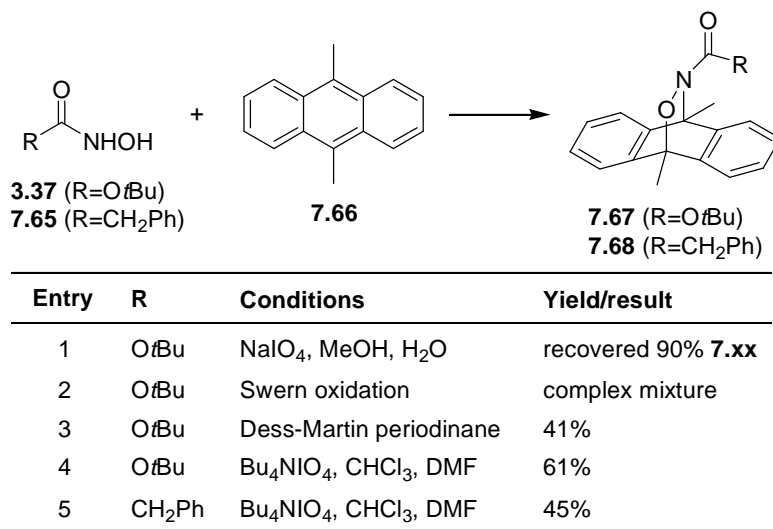


Scheme 7.18. Attempted homo-Diels-Alder reactions by direct oxidation of hydroxamates

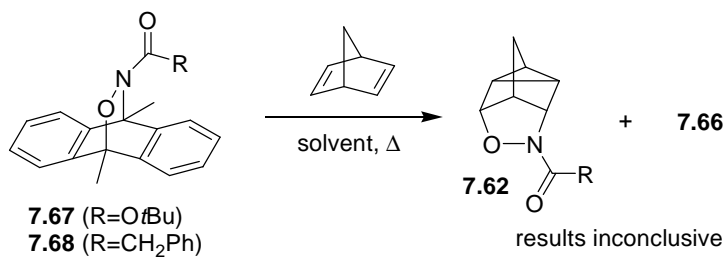
Using a similar strategy to the one employed by Kirby, we proceeded to synthesize the dimethylantracene cycloadducts **7.67** and **7.68** (Table 7.3). Hydroxamic acids **3.37** and **7.65** were oxidized in the presence of 9,10-dimethylantracene (**7.66**) to provide cycloadducts **7.67** and **7.68**. The reaction failed to yield cycloadduct **7.67** using sodium periodate in methanol/water and Swern oxidation³³³ conditions (Table 7.3, entries 1 and 2); however, oxidation using Dess-Martin periodinane^{35, 341} and tetrabutylammonium periodate in chloroform/DMF were successful (Table 7.3, entries 3 and 4). Solubility of dimethylantracene **7.66** appeared to play the largest role in the yield of cycloadducts **7.67** and **7.68**.

TABLE 7.3

PREPARATION OF 9,10-DIMETHYLANTHRACENE CYCLOADDUCTS

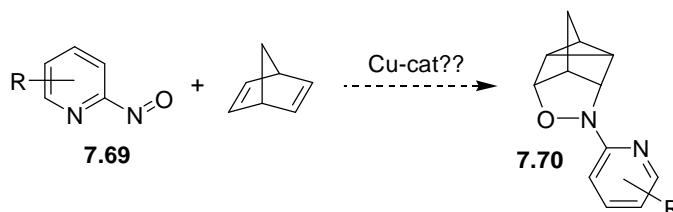


With cycloadducts **7.67** and **7.68** in hand, we proceeded to investigate thermally generating the acylnitroso species in the presence of norbornadiene (Scheme 7.19). The reactions were performed in sealed NMR tubes and monitored by ¹H NMR. At room temperature, dimethylantracene cycloadducts **7.67** and **7.68** fail to release the acylnitroso species. At temperatures above 30-40 °C, the release of 9,10-dimethylantracene (**7.66**) was observed. The formation of 1:1 adducts **7.62** cannot be confirmed at this time and will require additional studies; however, the results at this time are still encouraging.



Scheme 7.19. Attempted homo-Diels-Alder reactions by thermally-induced cycloreversion

Performing this reaction using nitrosopyridines **7.69** might be more suitable for this reaction, since cycloadditions with nitrosopyridines occur under very mild conditions (Scheme 7.20). Additionally, based on work by Yamamoto on asymmetric hetero-Diels-Alder reactions of nitrosopyridine compounds,^{2, 78} the use of copper to catalyze the reaction is an exciting possibility.

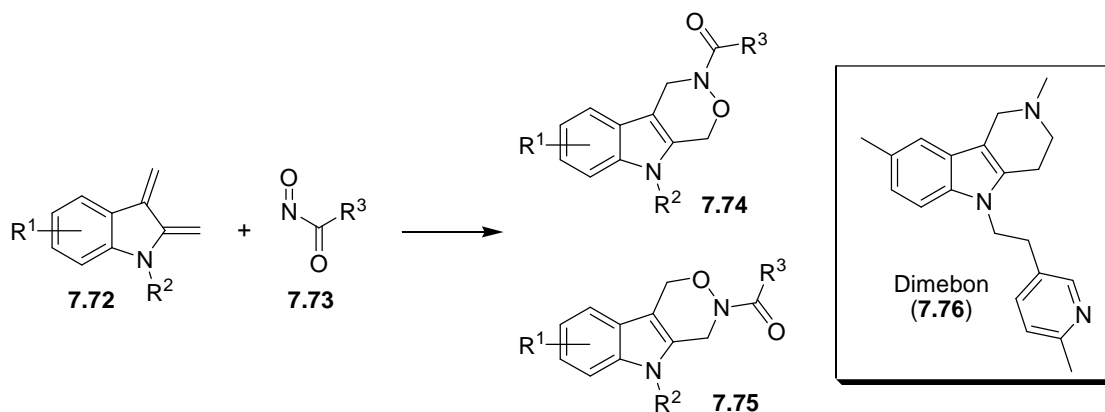


Scheme 7.20. Proposed use of nitrosopyridines for the homo-Diels-Alder reaction

7.5 Indole *ortho*-quinodimethanes as dienes in the acylnitroso HDA reaction

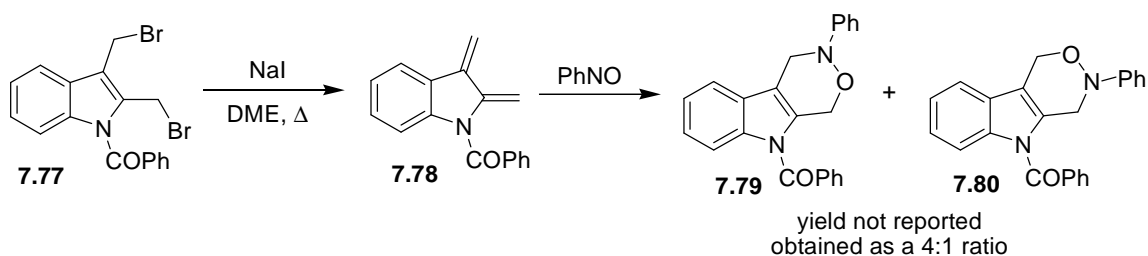
Following the example in the previous section of investigating alternative dienes for use in acylnitroso cycloaddition reactions, this section will detail a proposal for using an acylnitroso cycloaddition reaction with indole *ortho*-quinodimethanes **7.72** to yield indoles **7.74** and **7.75**, analogs of the pharmaceutically active agent, Dimebon (**7.76**) (Scheme 7.21). Previously used used for many years in Russia as an antihistamine and

sleep-aid, Dimebon (**7.76**) has recently shown promise for the treatment of Alzheimer's and Huntington's Diseases and other neurological disorders.⁵⁰⁰⁻⁵⁰⁷



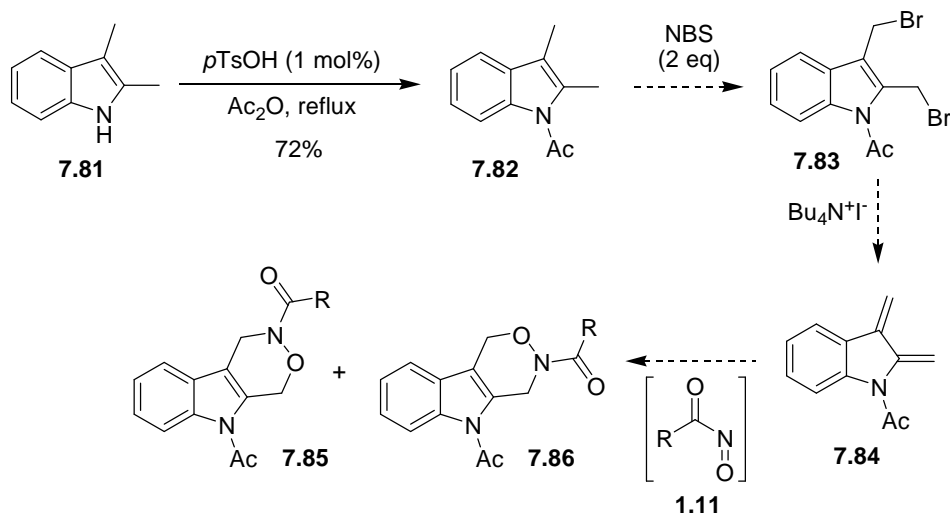
Scheme 7.21. Dimebon analogs from acylnitroso cycloadditions to indole *ortho*-quinodimethanes

A number of research groups have demonstrated the use of indole *ortho*-quinodimethanes **7.72** in cycloaddition reactions.⁵⁰⁸⁻⁵¹¹ Most notably, Dmitrienko has developed an efficient route to compounds **7.72**.^{512, 513} While a number of dienophiles have been shown to add to quinodimethanes **7.72**, there has only been one report of using a nitroso species as a dienophile. Pindur reported the addition of nitrosobenzene to indole quinodimethane **7.78** to yield an inseparable mixture of compounds **7.79** and **7.80** (Scheme 7.22).⁵⁰⁹ This chemistry was not explored further; however, this result was encouraging for the proposed reaction sequence to Dimebon analogs.



Scheme 7.22. Reported example of nitroso cycloaddition to indole quinodimethane

The proposed synthetic route to Dimebon analogs **7.85** and **7.86** is shown below (Scheme 7.23). The *N*-acetylation of 2,3-dimethylindole (**7.81**) was surprisingly problematic. A number of reaction conditions were attempted; however, all basic conditions resulted in a by-product that has not been structurally assigned. Protection of indole **7.81** under acidic conditions successfully provided the acetylated indole **7.82**.



Scheme 7.23. Progress toward Dimebon analogs

The synthesis of quinodimethane **7.84** follows the procedure outlined by Dmitrienko^{512, 513} and when generated in the presence of acylnitroso species, should provide compounds **7.86** and **7.85**.

7.6 Summary and recommendations for further study

In summary, this chapter has described short, preliminary investigations into a variety of new topics surrounding acylnitroso HDA chemistry. Due to time considerations, many of these projects have not been fully explored; however, each of these four topics would be exceptionally suitable research endeavors for future synthetic organic chemists who wish to investigate acylnitroso HDA chemistry.