Abstract

Latta III, Paul Richard. SYNTHESIS OF DIENE AND DIENOPHILE SUBSTRATES FOR *IN-VITRO* SELECTION OF RNA-HETERO-DIELS-ALDER CATALYSTS (Under the direction of Bruce E. Eaton)

The hetero-Diels-Alder reaction provides a general and facile entry for the synthesis of six-membered heterocyclic structures, which serve as precursors for a host of biologically active compounds. Although there are a wide variety of heterodienes and heterodienophiles available for synthetic applications, our research focuses on the cycloaddition of activated 1,3-butadienes with an activated aromatic aldehyde dienophile and an unactivated aliphatic aldehyde dienophile. Cycloadditions of this type provide access to an important class of compounds known as dihydropyrans, which are useful intermediates for the preparation of carbohydrates and many other biologically active compounds. Synthesis of pyrans by HDA cycloaddition has been limited to the reaction of either highly electron rich dienes (e.g. Danishefsky's diene) with a broad range of aldehydes or highly activated aldehydes (e.g. glyoxylates) with dienes of varying activity. Currently there are few examples of cycloadditions between unactivated aldehyde dienophiles and dienes that are less activated than Danishefsky's diene. Also, the catalysts currently available for this transformation provide access to only one of two possible regioisomers and provide a single diastereomer almost exclusively. If efficient

catalysts for the uncommon HDA regio- and stereoisomers are attainable, then we believe that *in vitro* selection of these catalysts from a pool of 10^{14} random RNA sequences will provide invaluable clues as to structural properties necessary for such catalysts. To this end, we have prepared novel dienes and dienophiles suitable for *in vitro* selection of new RNA biocatalysts capable of assembling novel heterocyclic products via a hetero-Diels-Alder cycloaddition.

Synthesis of Diene and Dienophile Substrates for *In-Vitro* Selection of RNA-Hetero-Diels-Alder Catalysts

by

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Approved By:

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Jonathan S. Lindsey Bruce M. Novak

Dedication

This work is dedicated to Dr. Robert Soeder, Dr. Claire Olander, and Dr. Don Olander of Appalachian State University.

Biography

The author was born on February 11, 1974 in Raleigh, North Carolina. He has one sister, JoAnne. At the age of four Rich moved to Lizard Lick, North Carolina, where he grew up. Rich graduated from East Wake High School, and although he received an appointment to West Point Military Academy, Rich chose to attend Appalachian State University on a football scholarship. He earned a bachelors degree in Chemistry with a Certified Chemist Concentration in 1997. After teaching organic chemistry labs for a year at ASU Rich began his graduate education at North Carolina State University.

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BINOL	1-1'-binapthol	DMSO	dimethylsulfoxide
BOC	*tert*-butoxycarbonyl	DNA	deoxyribonucleic Acid
bs	broad singlet	EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
Bu	butyl	ee	enantiomeric excess
Cbz	carboxybenzyloxy	eq	equivalent
CL	cleavable linker	EWG	electron withdrawing group
d	doublet	FMO	frontier molecular orbital
δ	chemical shift	HDA	hetero Diels-Alder
DA	Diels-Alder	HOBt	1-hydroxybenzotriazole
DCC	dicyclohexylcarbodiimide	HOMO	highest occupied molecular orbital
dd	doublet of doublets	LA	Lewis acid
DMAP	dimethylaminopyridine	LUMO	lowest unoccupied molecular orbital
DMF	dimethylformamide	m	multiplet

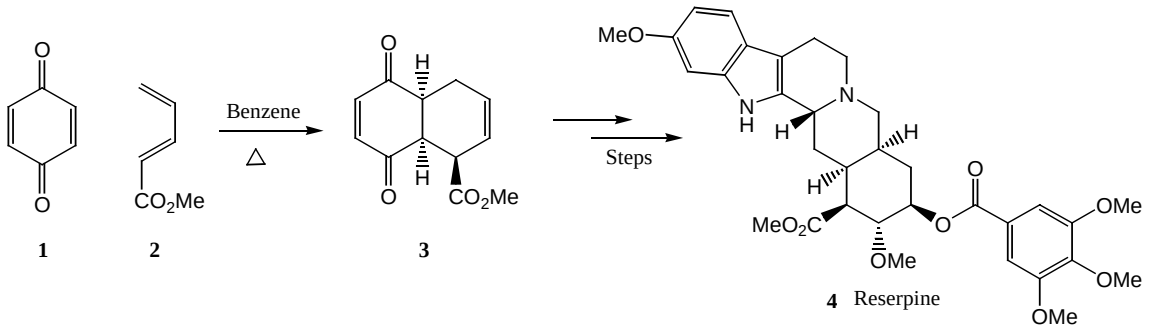
Me	methyl	s	singlet
N	normality	S	sinister (left)
NMR	nuclear magnetic resonance	t	triplet
p	pentet	TBDMS	*tert*-butyldimethyl silyl
PCC	pyridinium chlorochromate	TEA	triethylamine
PCR	polymerase chain reaction	Tf	triflate
PEG	polyethylene glycol	TFA	trifluoroacetic acid
Ph	phenyl	THF	tetrahydrofuran
R	rectus (right)	TLC	thin layer chromatography
R_f	retention factor	TMS	trimethylsilyl
RNA	ribonucleic acid	TS	transition state
RT	room temperature		

Chapter 1: Introduction and Background

1.1 The Diels-Alder Reaction

The Diels-Alder (DA) reaction is a powerful transformation that creates two new carbon-carbon bonds and up to four stereocenters simultaneously.[1] Since its discovery in 1928 by Otto Diels and Kurt Alder, the DA cycloaddition has been one of the most valuable and researched reactions in organic chemistry.[2] While the importance of the DA reaction toward natural product synthesis was realized early on, the transformation was not prominent in any total syntheses prior to that of cantharidin by Stork et al. in 1951.[3] Since the pioneering work of Woodward and his research group during the 1950's on cholesterol[4] and reserpine[5] (Scheme 1.1) a new school of thought has emerged regarding the synthesis of complex molecules. Along the way, the DA reaction has expanded its scope to include intramolecular DA cycloadditions, pressure accelerated reactions, hetero-Diels-Alder (HDA) cycloadditions, and Lewis acid (LA) accelerated reactions. Recently, Eaton et al. reported the *in vitro* selection of the first RNA Diels-Alderase, which is capable of catalyzing the all-carbon DA cycloaddition.[6] Current research is focused on controlling the regio- and stereochemical outcome of the DA and HDA cycloadditions.[7-13] Although the carbocycle DA reaction has been the main focus of research to date, the HDA reaction has received significant interest during the past two decades and will be the focus of our research.

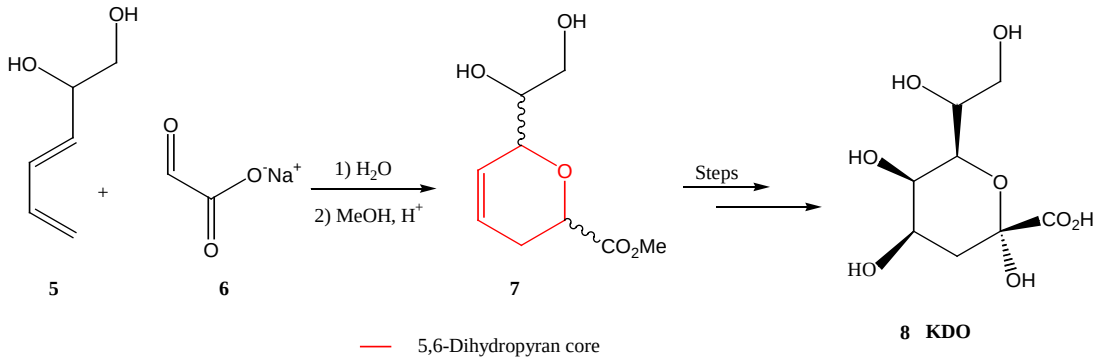
Scheme 1.1: Application of the Diels-Alder reaction in the total synthesis of reserpine.



1.2 The HDA Reaction: Mechanism and Stereochemistry

The hetero-Diels-Alder reaction provides a general and facile entry for the synthesis of six-membered heterocyclic structures, which serve as precursors for a host of biologically active compounds.[7-9] For example, the cycloaddition between a 1,3-diene and a dienophile bearing a single oxygen atom (i.e. activated aldehyde or activated ketone) will produce a 5,6-dihydropyran ring-system, which is a useful intermediate for the preparation of carbohydrates such as **KDO** (Scheme 1.2)[9] and many other natural products.[7,10]

Scheme 1.2: Application of the hetero-Diels-Alder cycloaddition to the synthesis of 3-deoxy-D-manno-2-octulosonic acid (KDO).



In order to understand the driving force behind the development of RNA HDA catalysts and the limitations of current state-of-the-art HDA Lewis acid catalysts, an examination of the mechanistic aspects and the concept of Lewis acid (LA) activation will be discussed. A majority of cycloaddition reactions present in the literature can be classified into two types of (π2s + π4s) cycloadditions, the normal and the inverse electron demand HDA reactions, as defined by the relative energies of the frontier molecular orbitals (FMO) of the diene and dienophile.[11,12] The normal electron demand HDA reaction is a $HOMO_{diene}$-$LUMO_{dienophile}$ interaction between an electron-rich diene and electron-deficient dienophile. The inverse electron demand HDA reaction is primarily controlled by a $LUMO_{diene}$-$HOMO_{dienophile}$ interaction between an electron-rich dienophile and an electron-deficient diene. In a typical normal electron demand reaction (Figure 1.1), the effect of an electron-withdrawing group (EWG) is to lower the energy of the $LUMO_{dienophile}$, thereby increasing the oribital mixing with the $HOMO_{diene}$. Alternatively, electron-donating groups will increase the energy of the $HOMO_{diene}$, once again enhancing the orbital mixing of the $HOMO_{diene}$-$LUMO_{dienophile}$ pair.[13]

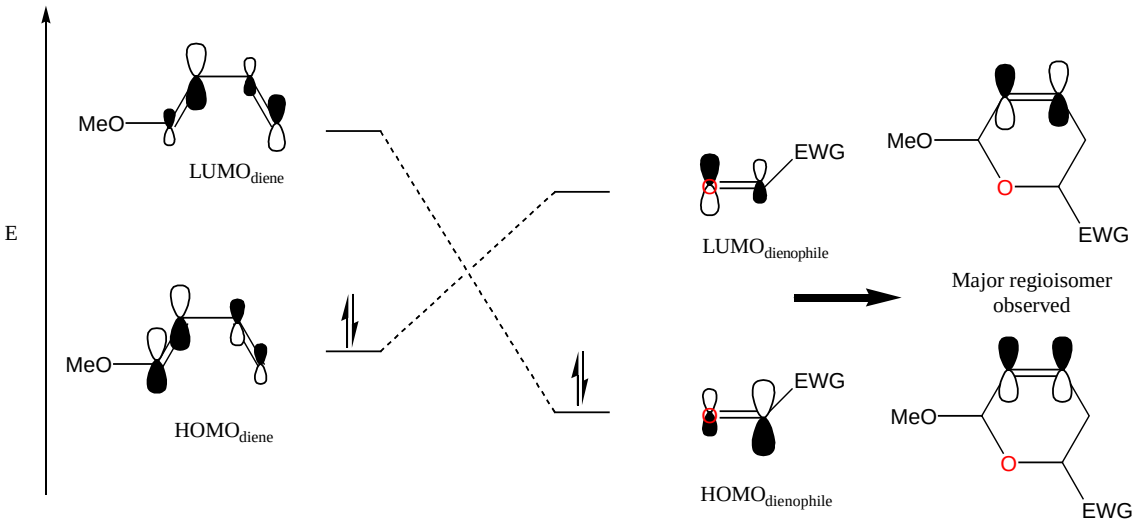


Figure 1.1: Orbital interactions of the HOMO$_{diene}$-LUMO$_{dienophile}$ in a normal electron demand reaction.

Another aspect of the Diels-Alder reaction that can be rationalized by FMO theory is the regiochemistry observed with unsymmetric dienes and/or dienophiles. Figure 1.1 shows a HDA cycloaddition for which there are two possible regioisomers (see Figure 1.2). Figure 1.1 indicates that regioisomer A in Figure 1.2 is the major isomer. The product distribution is predicted by the size of the orbital coefficients on the diene/dienophile pair. In general, the major regioisomer will result from the diene/dienophile orientation that provides the more favorable overlap of the π orbitals (i.e. large lobe of diene pairs with large lobe of the dienophile).[14]

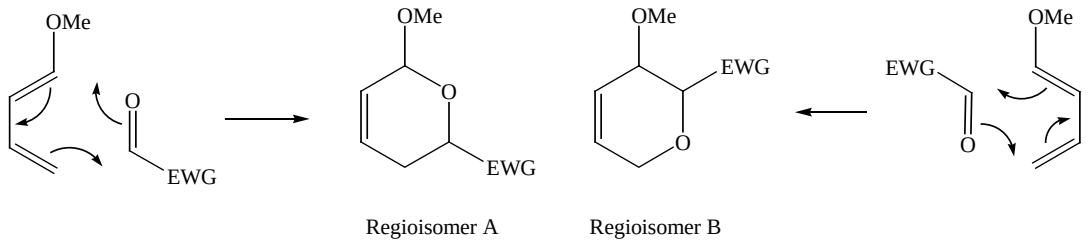
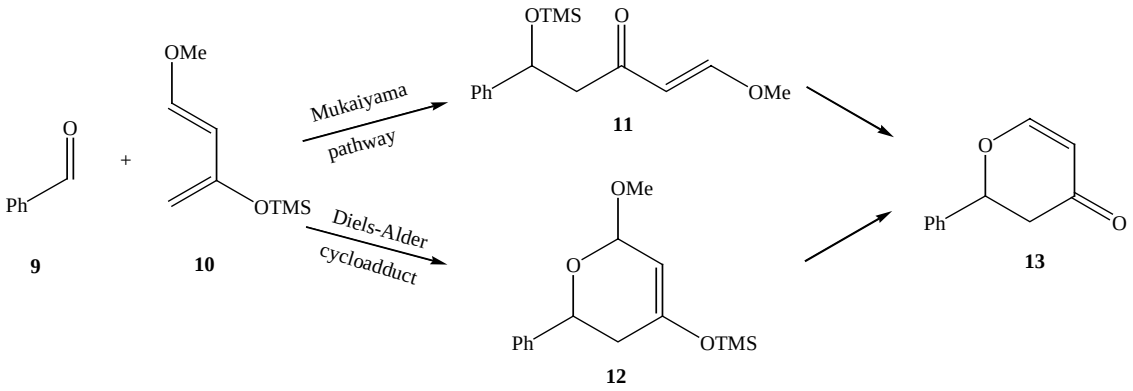


Figure 1.2: Two possible regioisomers of the hetero-Diels-Alder cycloaddition between an aldehyde dienophile and an unsymmetrical diene.

It is generally accepted that many all-carbon DA reactions proceed through a concerted non-synchronous transition state.[15] However, due to the inherent asymmetry of the heterodienophile (or heterodiene) and often highly polarized nature of the carbon-heteroatom bond, two mechanistic pathways have been observed for HDA reactions. The two pathways depicted in Scheme 1.3 are described as (1) a traditional DA cycloaddition (concerted) and (2) formation of the HDA-like adduct by a Mukaiyama-aldol pathway (stepwise). The latter typically occurs in the presence of a Lewis acid catalyst.[12] Few mechanistic studies have been performed on Lewis acid catalyzed HDA reactions with aldehyde dienophiles. However, Danishefsky et al. concluded that the reaction of benzaldehyde with trans-1-methoxy-3-(trimethylsiloxy)-1,3,-dimethyl-1,3-butadiene can switch between the concerted and stepwise mechanism depending on the Lewis acid catalyst used.[16]

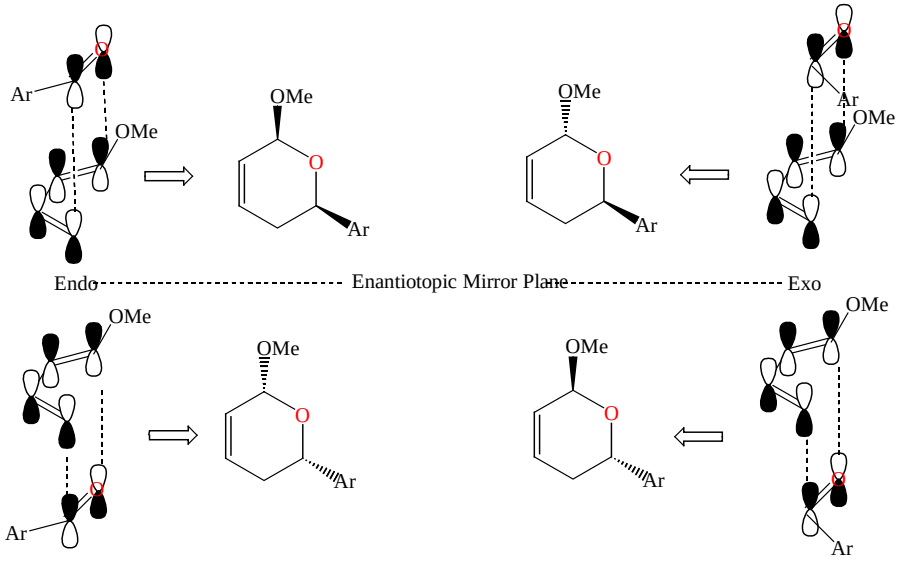
Scheme 1.3: Two mechanistic pathways observed for the Lewis acid catalyzed reaction of an unactivated dienophile with Danishefsky's diene.[13]



The hetero-Diels-Alder reaction involving aldehyde dienophiles has the potential to create three new stereocenters. The new stereocenters formed from this cycloaddition result from either of two possible reaction topologies for successful reaction of the diene-dienophile π-systems. One such topology involves a diene/dienophile interaction in which the electron-withdrawing group of the dienophile is positioned under the conjugated π-system of the diene in an approach often referred to as the endo transition state (Scheme 1.4). In an alternative approach, the exo transition state results from a diene/dienophile interaction with the EWG of the dienophile positioned externally to the diene π-system.[13] The majority of HDA cycloadditions occur via an endo transition state, which accounts for the inherent diastereoselectivity (endo/exo selectivity) of HDA reactions in a relative sense.[12,13] The endo preference has been rationalized on the basis of secondary orbital overlap (Figure 1.3).[17] In the endo transition state the favorable interaction between secondary orbitals (π orbitals not converted to sp^3 orbitals) lowers the energy of the endo transition state relative to the exo transition state providing the means by which the energetically less favorable (i.e. kinetic) product can preferentially form.

Although generally used to enhance reaction rates and facial selectivity, Lewis acid activation has been shown to increase the endo/exo ratio as a result of beneficial enhancement of the secondary orbital interaction.

Scheme 1.4: Schematic representation of the four possible stereoisomers from a HDA cycloaddition.



For dienes and dienophiles containing no other stereocenters, there are two enantiotopic endo and exo transition states leading to four stereoisomers overall (Scheme 1.4). The enantioselectivity of the HDA reaction is governed by the facial, *si* or *re*, approach of the diene with respect to dienophile (Figure 1.4, bottom).[18] The use of a chiral Lewis acid catalyst is one means of accomplishing facial selectivity in the HDA reaction (Figure 1.4, top), thereby enhancing the enantioselectivity of the HDA

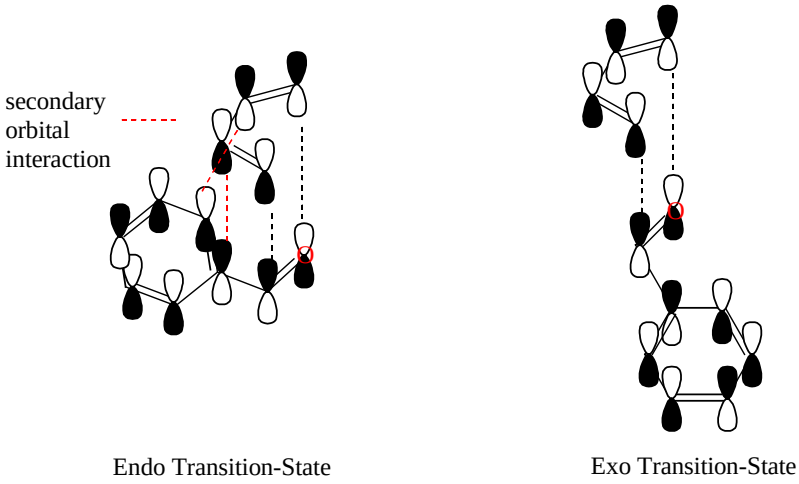


Figure 1.3: Secondary orbital interactions help to stabilize the more sterically hindered Endo TS.[15]

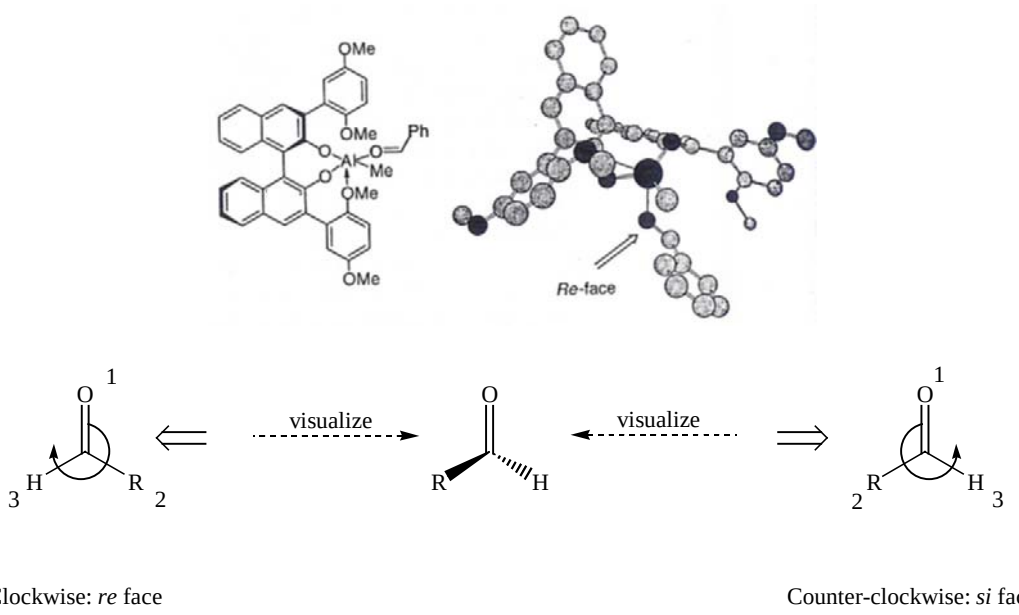


Figure 1.4: Top: Use of a chiral Lewis acid to block the *si* face of the dienophile.**13** Bottom: Schematic for visualizing the *re* and *si* face of a carbonyl dienophile based on the Cahn-Ingold-Prelog system.[18]

cycloaddition. When additional chiral centers are present in the diene or dienophile, facial selectivity will result in enhanced diastereoselectivity of the cycloadducts. It should be noted that the energy differences driving the formation of different regio- and stereoisomers are typically small and that activation by Lewis acids can alter the regio- and stereochemical course of the HDA cycloaddition. This is of great value, synthetically, as Lewis acid activation can generate products that would otherwise not be observed in a simple, thermally induced reaction.[14,19]

1.3 Lewis Acid Activation and Control of Stereochemistry

Hetero-Diels-Alder cycloadditions have been reported for a wide range of hetero-dienes and dienophiles (Figure 1.5).[20] However, due to the specific goals of this project the remainder of our work will focus on the HDA cycloaddition of activated 1,3-butadienes and aldehyde dienophiles.

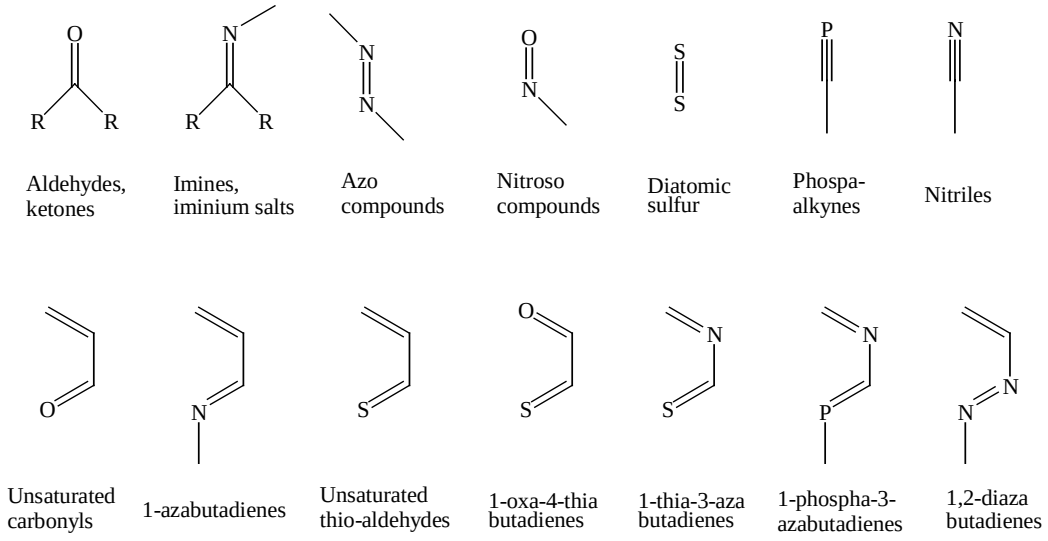


Figure 1.5: Examples of hetero-dienophile (top) and hetero-diene (bottom) substrates that have been used in HDA cycloadditions.[20]

The basic concept of Lewis acid activation in the HDA reaction is to utilize oxygen lone pair electrons of the aldehyde functionality for coordination to a Lewis acid (LA). Coordination of the oxygen lone pair to the Lewis acid changes the FMO's of the aldehyde dienophile, and for the normal electron demand reaction a decrease in the $LUMO_{dienophile}$ and $HOMO_{diene}$ energies is observed. Analogous to the incorporation of EWG's on the dienophile, the energy decrease observed upon Lewis acid activation allows for a more favorable interaction with the electron-rich diene (Figure 1.6, right side),[13-15] thereby increasing the reaction rate. For the inverse electron demand, coordination of the LA to the heteroatom of the 1,3-diene leads to a decrease in energy of the $LUMO_{diene}$ and $HOMO_{dienophile}$ and a more favorable diene-dienophile interaction is observed.

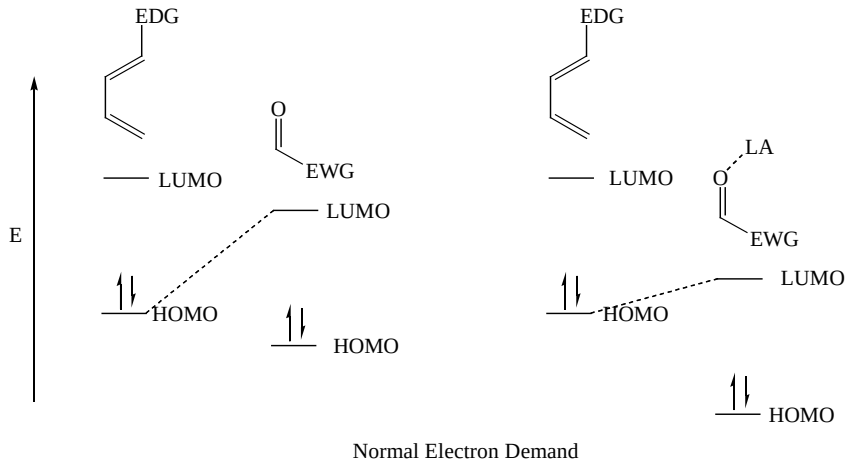


Figure 1.6: Effect of Lewis acid (LA) activation on the relative HOMO-LUMO energies in the normal electron demand cycloadddition.[13]

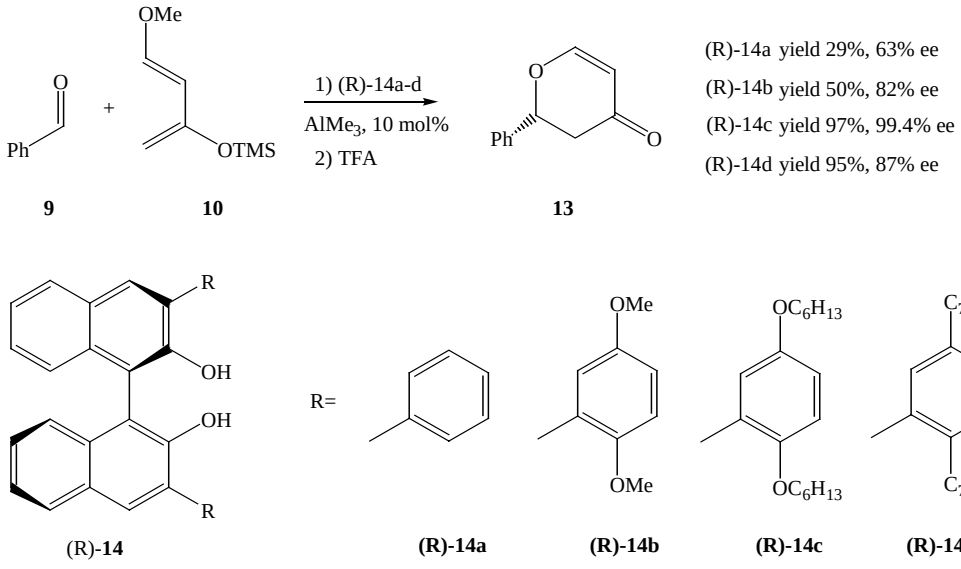
In recent years research has moved toward the development of chiral Lewis acids for use in the asymmetric synthesis of 6-membered heterocycles. In general, only

activated carbonyl compounds such as glyoxylates; ketomalonate; 1,2,3-triketones; and related compounds are active enough to react with dienes bearing electron-donating groups. The use of Lewis acids, and particularly the application of chiral Lewis acid catalysts, however, has provided new opportunities for stereoselective HDA cycloadditions. A number of main group metals (aluminum and boron), transition metals (copper, gallium, tin, titanium, and chromium), and some lanthanide elements (europium and ytterbium) are oxophilic metals that have been widely used in combination with chiral ligands containing oxygen, nitrogen, or phosphorus as the coordinating atom. [21-24]

When a chiral Lewis acid is designed for a reaction, many parameters must be taken into account. The substrate should posses certain reactivity and should be able to coordinate to a metal. Furthermore, the structural properties of the metal-ligand complex, as well as the overall Lewis acidity of the metal, need to be carefully considered. For example, among the most commonly used chiral ligands for complexing aluminum (III) are the 1,1'-binaphthols (BINOL). When designing catalysts around BINOL-Al^{III} complexes, problems may occur. Due to the high oxophilicity of aluminum, aggregation of the catalyst can occur, leading to an inactive species. Designing ligands in such a way as to prevent aggregation has become a topic of current interest and includes the introduction of sterically demanding groups around the oxygen atoms, attachment of monomeric catalyst to a polymer, and development of rigid polymeric chiral catalysts.[13] In fact, fine-tuning of the metal center to attain just the right balance of Lewis acidity and the ability to coordinate has occupied the research interest of prominent chemists for years.[23,25-27]

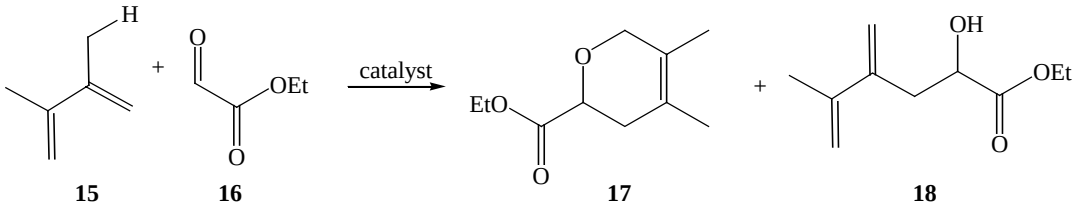
Jorgensen et al. have recently reported the development of highly enantioselective HDA catalysts based on the chiral BINOL-Al^{III} system.[28] The reaction of benzaldehyde and Danishefsky's diene was catalyzed by various (R)-BINOL-Al-Me analogs (Scheme 1.5). Prior to the work of Jorgensen, the best optical yield of this reaction had been reported as 95% ee (enantiomeric excess), but with a modest yield of 55% (Scheme 1.5, R = -Si(3,5-xylyl)$_3$).[29] Jorgensen attributed the unusually high enantioselectivity of this reaction to two factors. First, like other chiral catalysts, the C_2 symmetric (R)-BINOL catalysts efficiently blocked one face of the aldehyde; in this particular case it was the *si* face (Figure 1.4, top). Second, hypercoordination of a single oxygen of one aryl substituent (**(R)-14 b-c**, Scheme 1.5) could further expose the *re* face of the dienophile, thereby enhancing the selectivity of the BINOL-Al-Me catalyst.[28]

Scheme 1.5: Yield and % ee for the HDA cycloaddition between benzaldehyde and Danishefsky's diene in the presence of various (R)- BINOL-Al^{III} catalysts.[13,28]



More interesting, however, is the observation that the cycloaddition of benzaldehyde with Danishefsky's diene will proceed through different pathways depending on the catalyst employed. The Mukaiyama aldol pathway has been observed with catalysts based on titanium and boron complexes, while the Diels-Alder pathway has been positively identified for reactions catalyzed by europium, chromium, and zinc based catalysts.[19] The reaction pathway for aluminum based compounds has yet to be established, but theoretical calculations by Jorgensen et al. favor the Mukaiyama pathway.[13] It is worth mentioning that when the diene possesses allylic protons, as in the cycloaddition of ethyl glyoxylate with 2,3-dimethyl-1,3-butadiene (Scheme 1.6), two mechanistic pathways have been observed. In the reaction catalyzed by BINOL-$TiCl_2$, the ene-pathway dominated, providing **18** as the major product. However, changing the metal content of the catalyst to aluminum produced the HDA cycloadduct as the major product, once again showing how sensitive HDA reactions are to the metal-ligand complex.

Scheme 1.6: Relative product distribution of 2,3-dimethyl-1,3-butadiene and ethyl glyoxylate in the presence of titanium and aluminum based BINOL catalyst.



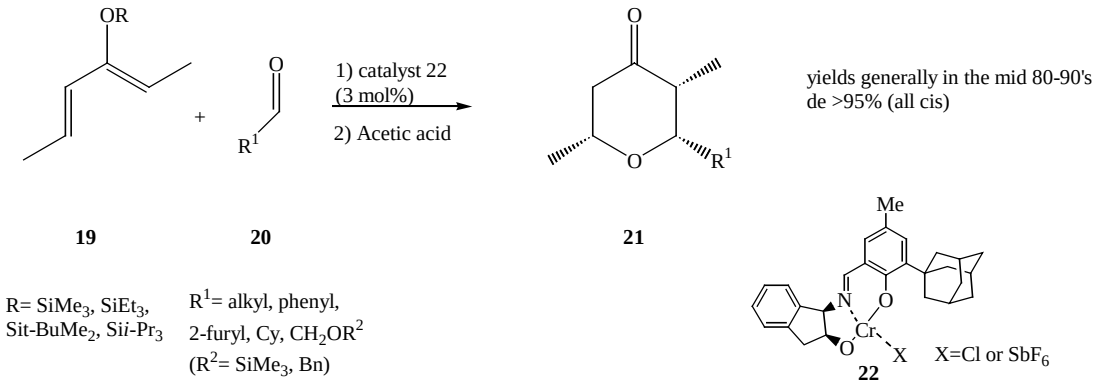
15 **16** **17** **18**

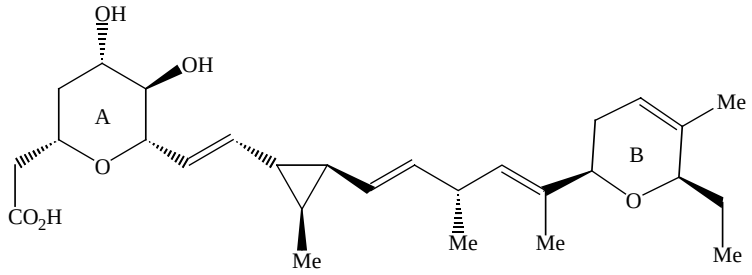
catalyst	Product distrtibution	
	17	**18**
BINOL-TiX_2	1	9
BINOL-AlMe	7	1

In studies, Jacobsen et al. have shown the chiral salen-chromium(III) complexes to be efficient and stereoselective HDA catalysts with a broad range of aldehyde dienophiles and Danishefsky's diene.[23] Although enantioselectivity in excess of 90% ee was achieved in only one example, several of the cycloadducts could be recrystallized to enantiomeric purity. Mechanistic studies heavily favor the [4 + 2] cycloaddition pathway. More recently Jacobsen and his group have introduced the chiral tridentate Schiff base chromium (III) complexes. These catalysts are highly enantio- and diastereoselective with regard to unactivated aldehydes of both aliphatic and aromatic nature (Scheme 1.7).[30] A recent novel application of this asymmetric catalyst to solve a long-standing synthetic problem is found in the total synthesis of (+)-ambruticin (Figure 1.7).[31] The salen-type catalyst (**22**, Scheme 1.7) and its enantiomer smoothly directed the asymmetric synthesis of two pyran rings with opposite facial selectivity in exceptional yield (> 64%) and exceedingly high enantiomeric excess (> 97%). This catalyst system presents the first known method of inducing asymmetry in HDA reactions of unactivated carbonyl compounds and dienes that are less reactive than the Danishefsky-type which

bear two oxygen substituents. In another first, the chiral Schiff base Cr ^{III} complex catalyzed the inverse electron demand cycloaddition of the first α,β-unsaturated aldehyde, not bearing a β-keto group, with an electron-rich vinyl ether dienophile.[32]

Scheme 1.7: Jacobsen's chiral Schiff base Cr^{III} catalysts achieve extremely high de and ee with aromatic and aliphatic aldehydes and dienes of less reactivity than Danishefsky's diene.[30]





(+)-ambruticin

23

Figure 1.7: Total synthesis of (+)-ambruticin by Jacobsen et al., in which two pyran rings (A and B) with opposite facial selectivity were synthesized using a single chiral Lewis acid and its enantiomer.[31]

Although the catalysts of Jorgensen and Jacobsen provide access to pyrans with good efficiency and in some cases exceptional stereoselectivity, they present several limitations. Jorgensen's catalysts have only been shown to work with Danishefsky's diene, which provides the dihydropyranone cycloadduct instead of the dihydropyran cycloadduct. While both dihydropyranones and dihydropyrans can be manipulated to give glycals, the use of Danishefsky's diene provides stereocontrol of only one of three possible stereocenters formed during HDA cycloadditions. Jacobsen's chiral Schiff base Cr^{III} catalyst provides excellent stereoselectivity for all three stereocenters, but provides near perfect selectivity for only the endo product in all cases. Also, to the best of our knowledge, no Lewis acid catalyst has been shown to provide access to the second regioisomer (regioisomer B, Figure 1.2). If the HDA cycloaddition of 1,3-butadienes and aldehyde dienophiles is to become a general synthetic method for pyran and glycal synthesis, then new catalysts must be developed which allow access to more of the possible regio- and stereoisomers of this cycloaddition.

Chapter 2: Results and Discussion

2.1 Introduction

Before describing our work on this project, it is necessary to discuss the process of *in vitro* selection and its benefits. Perhaps the most enabling property of RNA as a catalytic platform is the ability to iteratively select and amplify individual molecules with a desired property from a beginning pool of $> 10^{14}$ random RNA sequences. It seems

logical that if it is possible to attain HDA catalysts that provide the minor stereoisomer and unobserved regioisomer for the cycloaddition of activated dienes and aldehyde dienophiles, then selection of these catalysts from a library of > 10^{14} random RNA sequences would be a far more efficient process than designing such catalysts by traditional methods. Indeed, this is our belief and the focus of research by others in our group.

The *in vitro* selection cycle shown in Figure 2.1 will be applied to the dienes and dienophiles synthesized in this project. The selection process will use RNA containing 5-(carboxamide-4-pyridylmethyl)-uridine triphosphate and selected main group metal ions (Na^+, K^+, Mg^{2+}, and Ca^{2+}) and transition metal ions (Cu(II), Mn(II), Co(II), Ni(II), Fe(II), and Zn(II)). The pyridine modification in combination with the metal ions has been shown to create a unique metal binding site capable of Lewis acid catalysis in other systems.[6] In the first round of selection, a library of > 10^{14} random RNA sequences that have been covalently linked to a diene substrate through a polyethylene glycol (PEG) linker containing a cleavage (CL) site, will be incubated with an aldehyde dienophile covalently bound to a biotin anchor group. The RNAs that successfully catalyze the HDA reaction will be bound to the biotin anchor group. These RNA species will be separated from the unreacted RNA species by a streptavidin partitioning step. Incorporation of a cleavage site within the linker allows for successful isolation of RNAs that catalyze the HDA reaction from the RNAs that have reacted with the dienophile, but not at the attached diene. The RNAs that exhibit the desired catalytic activity are then reverse transcribed to DNA and amplified by polymerase chain reaction (PCR). The

DNA will be transcribed to RNA and ligated to the DNA-PEG-CL-diene moiety, providing a catalytically enriched pool of RNAses for a subsequent round of selection. This cycle will be repeated until the most catalytically active RNA HDAses dominate the pool.

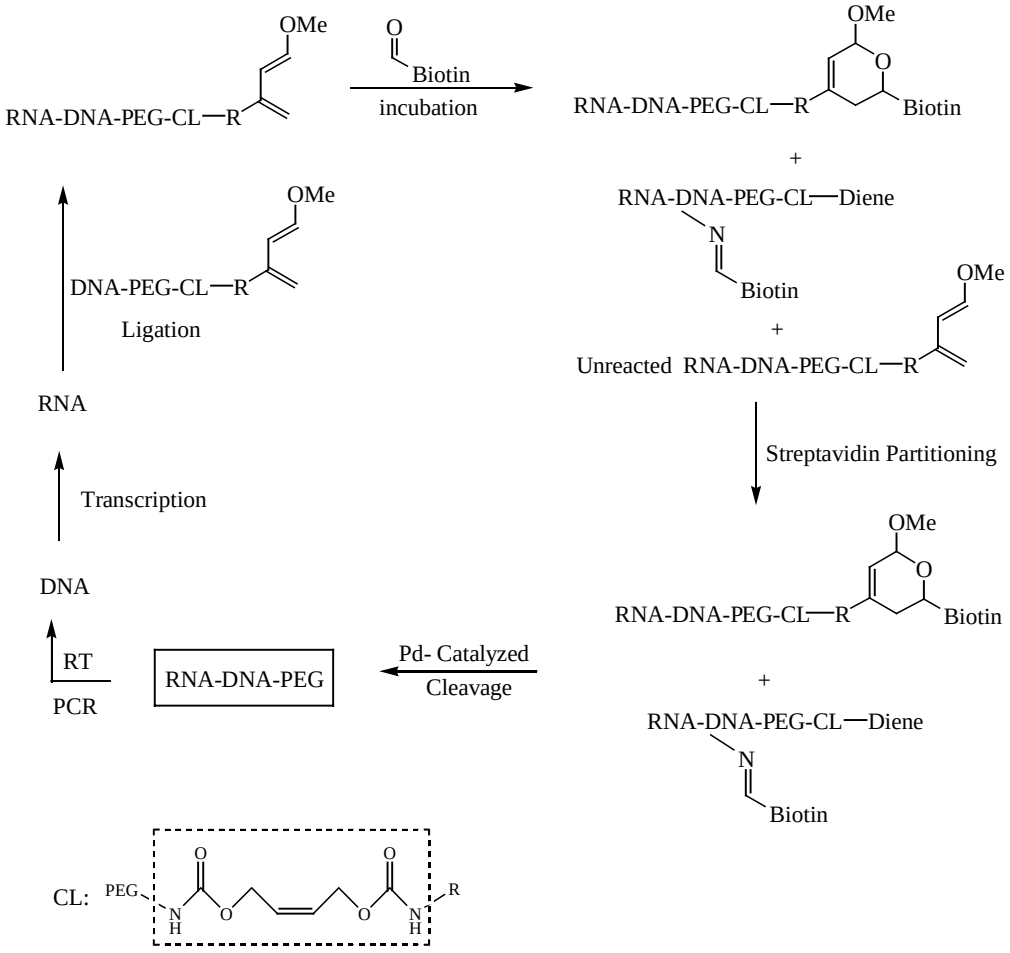


Figure 2.1: General outline of *in vitro* selection (SELEX) cycle.

It is the goal of our research to show that *in vitro* selection can produce HDAses which rival the current state-of-the-art catalysts of Jacobsen. Therefore, when choosing

dienes and dienophiles for the selection cycles we chose to use diene/dienophile pairs that were similar in reactivity to the ones Jacobsen used in his chiral tridentate Schiff base Cr^{III} research. Also, it is necessary to use compounds that would form cycloadditions under thermal, non-Lewis acid catalyzed, aqueous conditions. It is critical that the diene/dienophile pairs not only react under these conditions, but that they form the cycloadduct at a rate slow enough as not to produce too much "background noise" when compared to the RNA-catalyzed HDA reaction. It is also necessary that the diene contain a functional group (-OH, -NHR) suitable for attachment to the RNA via a cleavable linker. Given these requirements in combination with what is currently known in the literature regarding HDA reactions in aqueous media, the diene and dienophiles shown in Figure 2.2 were chosen for the *in vitro* selection of RNA HDAases.

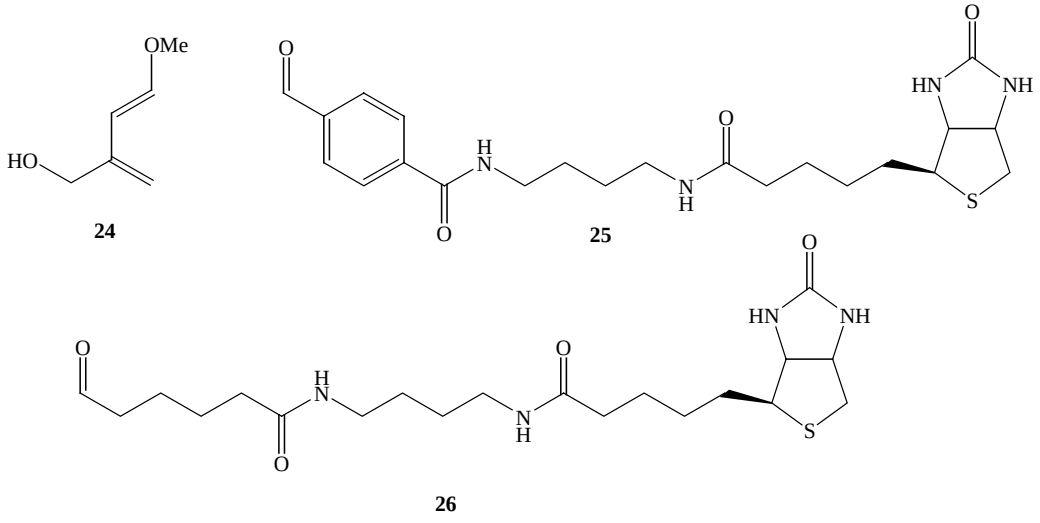
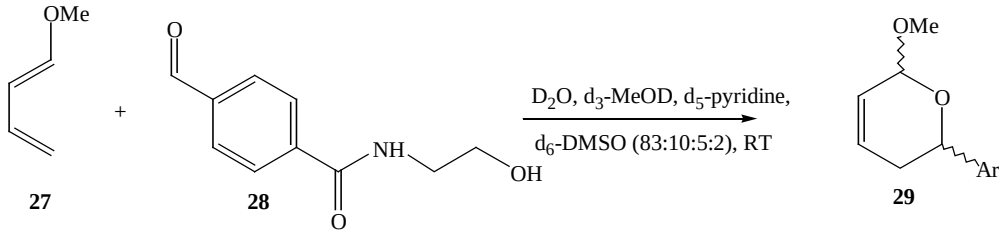


Figure 2.2: Target diene (24), aromatic dienophile (25) and aliphatic dienophile (26) for *in vitro* selection of RNA HDA catalysts.

2.2 Synthesis and Evaluation of a Model Dienophile

Before engaging in the arduous task of synthesizing the diene and dienophiles, a simple model system was used to test the reactivity of benzaldehyde-type dienophiles under aqueous conditions. Dienophile **28** (Scheme 2.1) was synthesized from 4-formylbenzoic acid (**30,** Scheme 2.2) and TBDMS-protected ethanolamine (**31)** under typical dicyclohexylcarbodiimide (DCC) conditions.[33] The crude oil was purified by silica gel chromatography to give amide **32** as a white solid. Cleavage of the t-butyldimethylsilyl (TBDMS) protecting group was accomplished by stirring **32** in a THF/H_2O/AcOH (1:1:3) solution for 15 minutes at room temperature. Removal of the solvents *in vacuo* and column chromatography afforded dienophile **28** as a colorless oil. It is worth mentioning that direct amidation of 4-formyl benzoic acid with ethanolamine was unsuccessful. It is believed that dienophile **28**, which is highly soluble in water, was retained by the aqueous layer under standard work-up conditions.

Scheme 2.1: Thermal HDA cycloaddition for use as a model study.

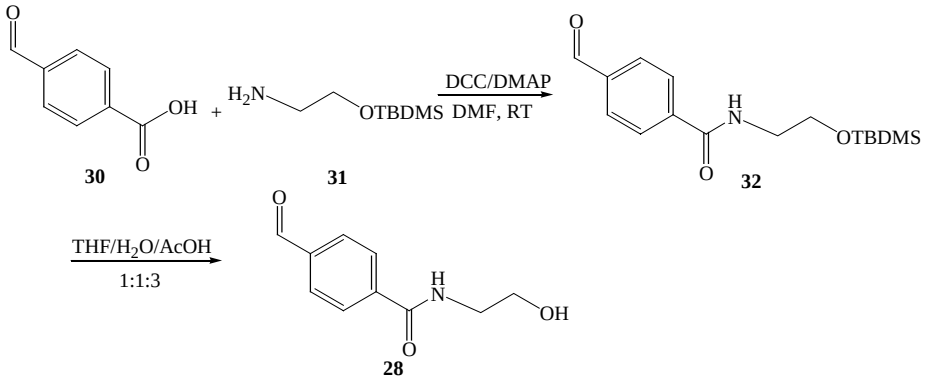


27 **28** **29**

The model HDA cycloaddition was set up in a J-young tube with a diene-dienophile concentration of 0.1 M in a D_2O, d_3-MeOD, d_5-Pyridine, d_6-DMSO

(83:10:5:2) solvent mixture. The reaction was monitored by ^{1}H NMR and it was determined that the thermal HDA reaction has a half-life of approximately half a year at RT. With a half-life of this magnitude, thermal HDA reactions of this type should provide for an undetectable background rate during the course of the *in vitro* selection cycles.

Scheme 2.2: Synthesis of a model aromatic dienophile for thermal HDA cycloaddition.

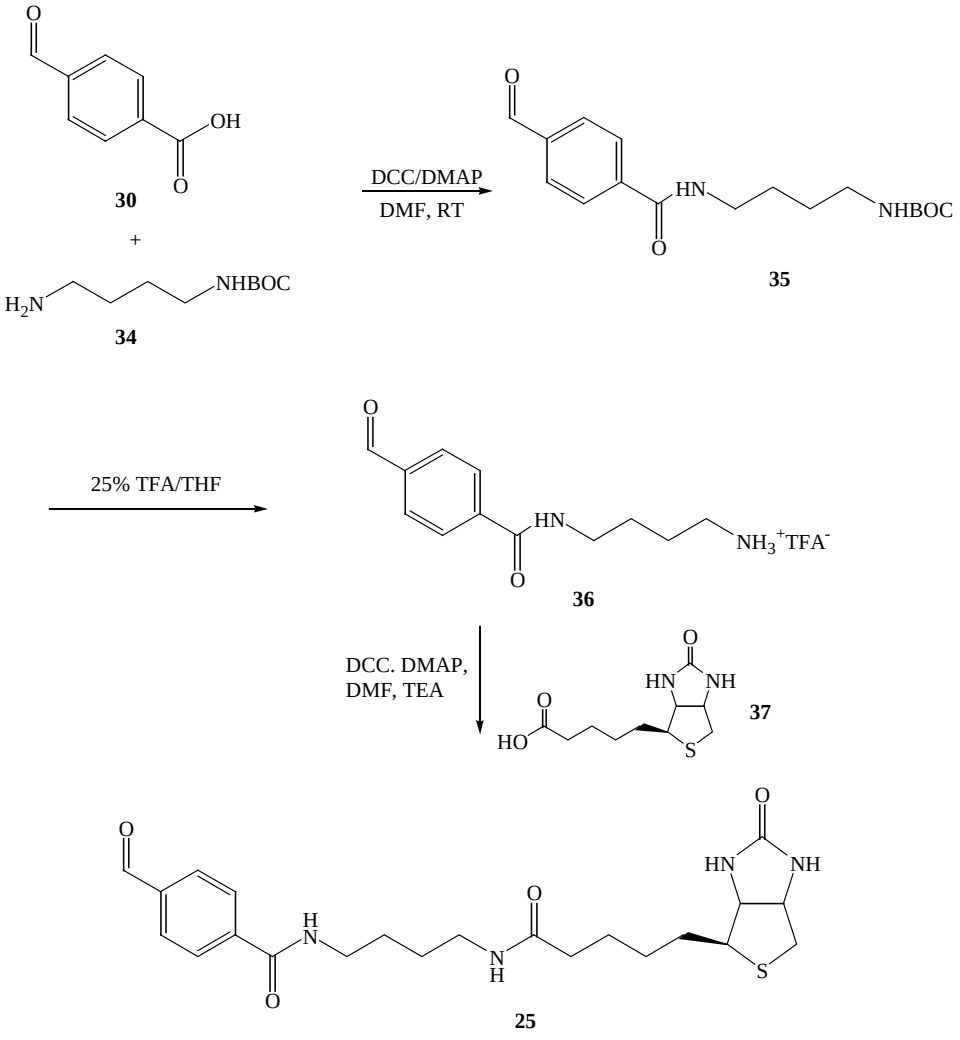


2.3 Synthesis of the Aromatic Dienophile

In a synthesis closely related to that of the model dienophile, an aromatic dienophile (**25,** Scheme 2.3) was synthesized. Starting with 4-formyl benzoic acid, amide **35** was prepared by DCC activation and subsequent condensation with N-t-butoxy-carbonyl-1,4-diaminobutane. The crude amide was purified on silica to afford **35** as a white solid. The t-butoxycarbonyl (BOC) protecting group was cleaved in the presence of a 25% trifluoroacetic acid (TFA)/tetrahydrofuran (THF) mixture and the solvent was removed *in vacuo* to provide **36** in quantitative yield. No imine/iminium salt formation was observed during this step. (+)-Biotin (**37**, Scheme 2.3) was coupled to the TFA salt

through standard DCC methodology in the presence of triethylamine (TEA). The resulting solid was purified on silica to provide compound **25**.

Scheme 2.3: Synthesis of an aromatic aldehyde dienophile.



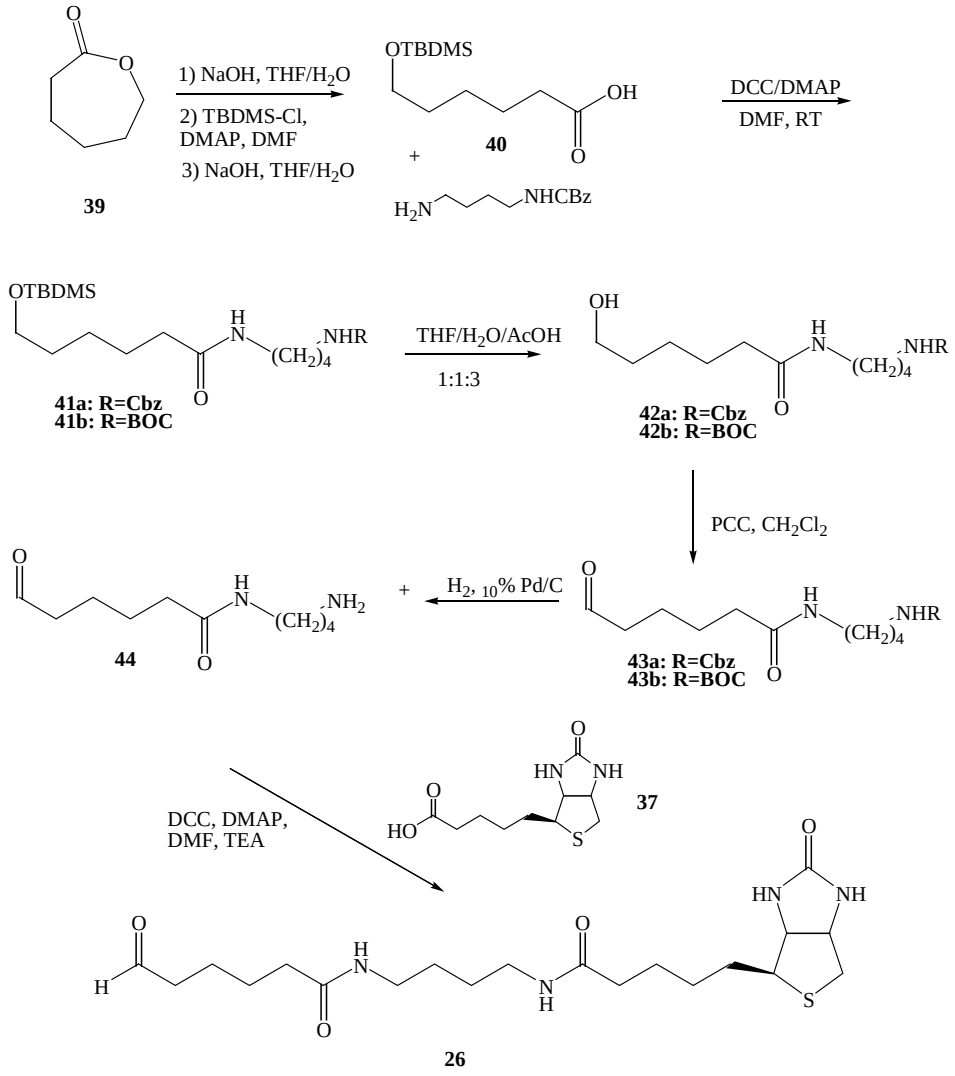
2.4 Synthesis of the Aliphatic Dienophile

As outlined in Scheme 2.4, synthesis of the aliphatic aldehyde dienophile started with the saponification of ε-caprolactone (**39)** to yield the disodium salt of 6-hydroxyhexanoic acid. After removal of the solvent *in vacuo*, the salt was resuspended in DMF and allowed to react with TBDMS-Cl (tertbutyldimethylsilyl chloride) and DMAP (4-dimethylaminopyridine) to form the silyl ether-silyl ester intermediate. Chemoselective hydrolysis of the silyl-ester was accomplished by addition of NaOH (3eq) in THF/H_2O (4:1) to afford **40**.[34] N-carbobenzyloxy-1,4-diaminobutane was attached to **40** by DCC activation to provide **41.** Cleavage of the silyl protecting group was accomplished by stirring **41** in a THF/H_2O/AcOH (1:1:3) solution and proceeded smoothly to afford **42** in near quantitative yield. Oxidation of the alcohol to aldehyde **43** was achieved by PCC oxidation in dichloromethane (DCM).[35] The crude aldehyde was subjected to palladium (10% Pd/C) catalyzed hydrolysis to cleave the carbobenzyloxy protecting group. On a small scale (< 5 mg) the reaction proceeded according to the literature and the crude amine was coupled to biotin by carbodiimide activation to provide dienophile **26**. However, attempts to scale-up the reaction sequence to provide usable amounts of dienophile **26** were unsuccessful. Each time the hydrogenolysis of **43** resulted in disappearance of the aldehyde functional group as verified by ^{1}H NMR.

It was assumed that under these conditions the longer time required for hydrogenolysis of the larger reaction was responsible for the reduction of the carbonyl group either directly or indirectly via imine formation. Attempts to suppress imine formation by addition of aqueous acid (HCl or AcOH) to the hydrogenolysis solution

were unsuccessful. To circumvent this problem a t-butoxy carbonyl protecting group was used for the amine. This protecting group was successfully used to synthesize the aromatic aldehyde, **25.** **I**nitial attempts to use this protecting group in the aliphatic dienophile synthesis proved unsuccessful due to our inability to successfully monitor the reactions with standard chromatography techniques (TLC with staining). Since we had well-defined purification procedures for compounds **41a**-**43a** we were able to successfully implement these purification steps with compounds **41b**-**42b**. Compound **43b** was not purified and characterized before the conclusion of our work on this project.

Scheme 2.4: Synthesis of an aliphatic aldehyde dienophile.

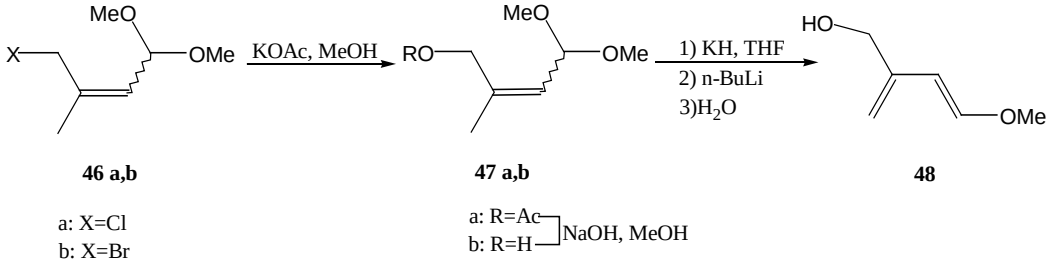


2.5 Synthesis of 1,3-disubstituted-1,3-butadienes

Initial attempts to synthesize trans-3-hydroxymethyl-1-methoxy-1,3-butadiene, **24**, proved to be more synthetically challenging than first anticipated. The initial route

(Scheme 2.5) was outlined in an article by Duhamel et al.[36] In this report, only a schematic drawing of the experimental conditions was provided, and the only mention of the starting chloroacetal (**46a**, Scheme 2.5) was that it was an industrial intermediate provided by Rhone-Poulenc Industries. Initial attempts to synthesize the chloroacetal by means of a Wittig reaction were unsuccessful. However, an alternative route to **46b** was available in the literature (Scheme 2.6).[37]

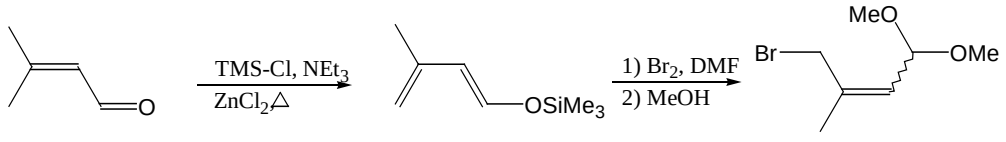
Scheme 2.5: Synthesis of 3-hydroxymethyl-1-methoxy-1,3-butadiene.[37]



Senecialaldehyde (**49**, Scheme 2.6) was converted to **50** in the presence of chlorotrimethylsilane (TMS-Cl) and zinc chloride in refluxing triethylamine (TEA). Addition of bromine to the TMS-ether followed by subsequent elimination of TMS-Br led to the formation of a bromoaldehyde intermediate, which was converted to dimethoxyacetal **46b** by addition of anhydrous methanol. Initial interpretation of Duhamel's synthetic scheme was to perform the transformation from the chloroacetal to the hydroxyacetal (**46a → 47b**, Scheme 2.5) in a one pot, sequential reaction. However, it was soon realized that isolation of acetate **47a**, followed by saponification to **47b** was the intended route. A stable sample of **47b** was never maintained for more than a few

hours, even after purification by chromatography and storage at –20°C. In most cases, ^{1}H NMR showed the presence of a complex mixture of which at least one compound contained an aldehyde proton. In order to rule out the possibility of an acetal/aldehyde mixture, a sample of the crude **47b** was subjected to conditions favorable for acetal formation in related systems.[38] No apparent change in ^{1}H NMR was visualized. It was hypothesized that the close proximity of the hydroxyl group to the acetal could stabilize an intermediate in which loss of methanol would lead to a stable heterocycle (Figure 2.3).

Scheme 2.6: Synthesis of 4-Bromo-1,1-dimethoxy-3-methyl-but-2-ene.[37]



49 **50** **46 b**

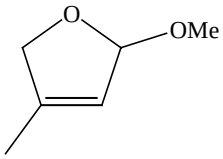
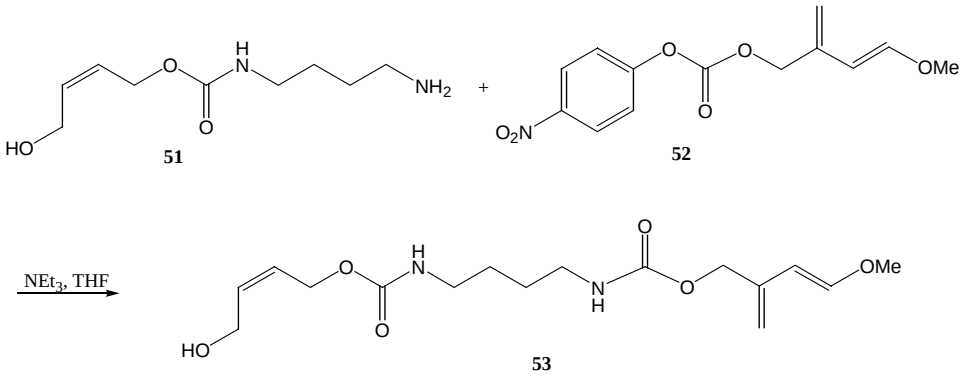


Figure 2.3: Proposed cyclic intermediate.

The original intent of the hydroxy functional group was to provide a synthetic handle for the incorporation of a cleavable linker to the diene through a dicarbamate spacer (**53,** Scheme 2.7). Not to be discouraged by the previous results, we decided to remove the dicarbamate spacer and couple the cleavable linker directly to the diene (**55**,

Figure 2.4). Maddaluno et al. have recently reported the successful formation of **56** from the double elimination of a dimer formed from **46a** in the presence of sodium hydroxide.[39] Realizing the functional similarity around the allylic ether in Maddaluno's dimer (**56**) and our proposed diene (**55**), it was anticipated that this transformation could be achieved by employing Maddaluno's procedure. Compound **54** was synthesized by the dropwise addition of **46b** to a solution of 2-butene-1,4-diol (5 eq) and NaH (1 eq) in anhydrous THF.[40] After aqueous work-up, the acetal was subjected to the conditions reported by Maddaluno in his double elimination reaction to provide **55**.[39] ^{1}H NMR provided evidence that the desired diene was present (allylic methylene protons of the diene were visible) but only as a minor component of a complex mixture. It has been noted that dienes of this type can rapidly decompose, even when stored at –20 ^{o}C.

Scheme 2.7: Addition of cleavable linker (2-buten-1,4-diol functionality) to target diene.



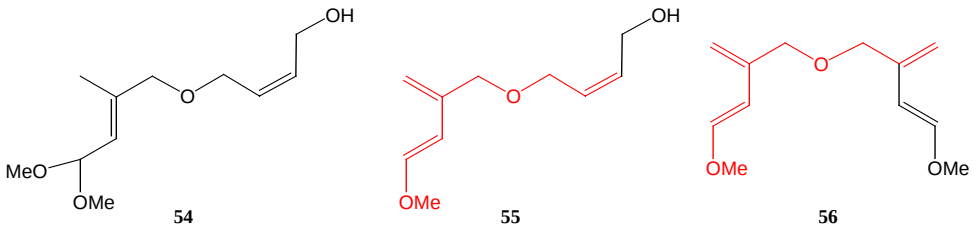


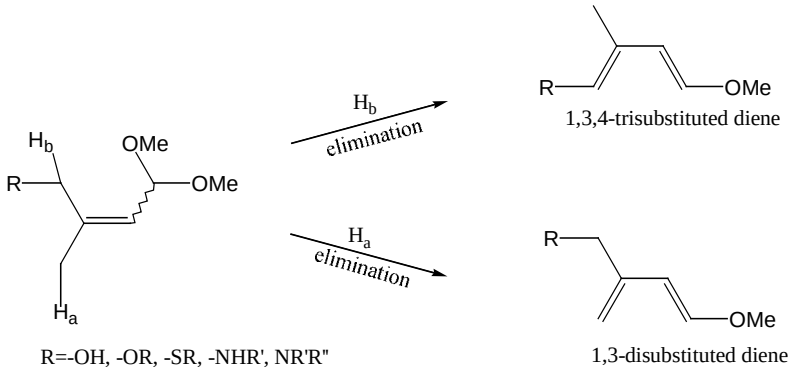
Figure 2.4: Structural similarities between proposed diene and Maddaluno 's diene dimer. Corresponding fragments are highlighted in red.

A major setback in the synthesis of our 1,3-disubstituted-1,3-butadienes has been the isolation of pure acetals (**54** and **47b**) for use in the elimination step (Scheme 2.8). Starting with crude acetal samples and due to the competitive nature of the elimination step we obtained complex mixtures from which neither the 1,3-disubstituted nor the 1,3,4-trisubstituted diene could be isolated in pure form. In fact, isolation of one diene over the other is not of particular importance, as we can select HDA catalysts for both dienes simultaneously. However, we prefer to use the 1,3-disubstituted dienes for their decreased reactivity compared to the 1,3,4-trisubstituted dienes, as this provides a bigger hurdle for currently available HDA Lewis acid catalysts.

Previous research utilizing this general scheme (Scheme 2.5) to synthesize 1,3-disubstituted and 1,3,4-trisubstituted dienes has provided mixed results depending on the nature of the allylic heteroatom. In general, acetals containing allylic ether and sulfide functionalities (Scheme 2.8, R= -OR',-SR') produce more of the thermodynamically stable 1,3,4-trisubstituted-1,3-butadienes. When the acetal contains either a hydroxy, secondary amine, or tertiary amine functionality (Scheme 2.8, R= -OH, -NHR', or –NR'R'' respectively) greater regioselectivity can be obtained in the elimination step, in

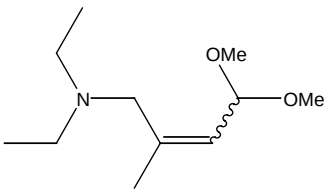
many cases producing the kinetic 1,3-disubstituted-1,3-butadienes as the only product.[39-41] Hoping to capitalize on this observation, we changed the allylic substituent of the acetal to include a tertiary amine. Not only have acetals of this type been shown to produce more of the 1,3-disubstituted dienes, but the tertiary amine should provide internal buffering against decomposition of the aminoacetal (**57**) by trace acids prior to the elimination step.

Scheme 2.8: Two possible elimination pathways of unsaturated acetals in the presence of a strong base.

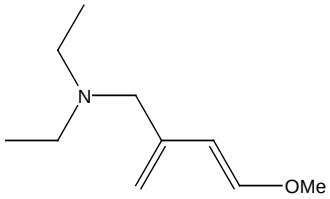


In a model study, bromoacetal **46b** was refluxed in diethylamine. After aqueous work-up, the solvents were removed to afford the aminoacetal (**57**, Figure 2.5) in near quantitative yield with no further purification necessary. Treatment of **57** with t-butyllithium (2 eq) in anhydrous THF (-78 → RT) provided **58** after aqueous work-up and chromatography on silica in the presence of 1% TEA. In order to provide a synthetic handle for incorporation of a cleavable linker, acetal **46b** was allowed to react with neat butanolamine at room temperature. The addition of butanolamine generated an enormous

amount of heat that led to the formation of a brownish-red residue. It was evident from ^{1}H NMR that the intense heat generated from the reaction led to the decomposition of **46b** and/or the formation of various side reactions. To control the heat generated during the dropwise addition of the acetal to the amine, the reaction was repeated at -10 ^{o}C. Once mixed, the solution was allowed to warm to room temperature and stirred overnight. The desired aminoacetal **59** was never isolated. Perhaps a better solvent choice or increasing the concentration of the reactants would allow for a successful transformation. It is worth noting that initial attempts to react diethyl amine with bromoacetal **46b** using THF as the solvent were unsuccessful as well. Not until the reactants were mixed neat were we able to isolate the aminoacetal **57**. It was assumed that the increased nucleophilicity of butanolamine would overcome the need for a neat reaction, but it seems the best recourse for a successful reaction would be to monomethylate the nitrogen of butanolamine and allow the reaction with **46b** to occur under neat conditions.



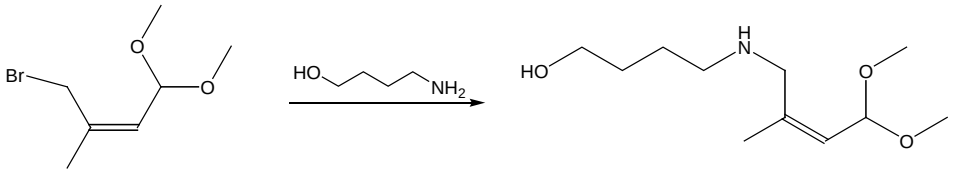
57



58

Figure 2.5: Desired targets for a model study on the formation of dienes containing the cleavable linker tethered through a tertiary amine.

Scheme 2.9: Synthesis of an amino acetal with a synthetic handle suitable for attachment to a cleavable linker.



46b **59**

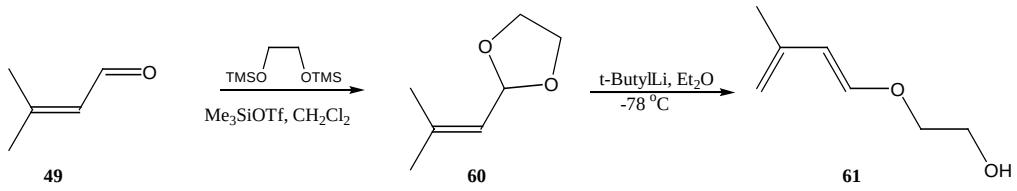
As work progressed on the development of 1,3-disubstituted-1,3-dienes with the core structure of diene **24**, we began to develop a second type of 1,3-disubstituted-1,3-diene. The goals were to simplify the synthetic steps required to make our dienes, retain the overall activity of the new diene with respect to diene **24** and to retain the ability to explore various steric and electronic parameters influencing cycloaddition rates and the selection of successful RNA HDA catalysts. Building on our previous experience with diene synthesis, it was determined that the most efficient strategy to simplify the synthesis of our dienes was to correct the problems present with our existing methodology. Our current method for synthesizing dienes was plagued by three recurring problems: (1) instability of the acetal following substitution of the halogen with a heteroatom, (2) incorporation of a side chain capable of tethering to the RNA via a cleavable linker, and (3) a tendency to favor the 1,3,4-trisubstituted diene during the elimination step. It is likely that using a cyclic acetal would increase the stability of the acetals prior to the elimination step. Also, there are several examples in the literature reporting the successful substitution of a halogenated cyclic acetal similar to **46a** and **46b**.[42] In addition to stabilizing the acetal, the cyclic acetal provides a suitable "point of attachment" for a cleavable linker after the elimination step has occurred. (Figure 2.6).

Figure 2.6: Elimination of cyclic acetal to generate a free hydroxyl group.

With suitable solutions for enhancing acetal stability and incorporating a "point of attachment" for the cleavable linker, we turned our focus to the competitive nature of the elimination step (Scheme 2.11). Drawing from our experience, we saw only two methods for increasing the yield of the 1,3-disubstituted-1,3-dienes over the 1,3,4-trisubstituted-1,3-dienes: (1) eliminate the possibility of competition between the allylic protons by creating a symmetrical proton environment or (2) increase the reactivity of H_a (scheme 2.11) with respect to H_b. Using senecialaldehyde as our starting point, we were able to create a cyclic acetal with no preference for either proton during the elimination step of the sequence. Senecialaldehyde (**49**, Scheme 2.10) was allowed to react with 1,2-((trimethylsiloxy)ethane in the presence of trimethylsilyl-trifluoromethanesulfonate under an inert atmosphere.[43] The reaction was quenched by the addition of pyridine, poured over $NaHCO_3$, and extracted with dichloromethane. The solution was dried over $MgSO_4$, concentrated in vacuo, and purified by chromatography on silica gel to provide acetal **60**. The Acetal was dissolved in dry ether at –78 ^oC and t-BuLi was added dropwise. After warming to room temperature, the reaction was quenched by the addition of absolute methanol. $NaHCO_3$ was added and the mixture was extracted with ether. The

organic layer was dried over $MgSO_4$, concentrated in vacuo and purified on silica to provide a compound **61** as a yellowish oil.

Scheme 2.10: Synthesis of an activated diene from a cyclic acetal



49 **60** **61**

By utilizing a cyclic acetal we were able to shorten the number of steps to make our diene(s) and provide a synthetic handle for attachment to the RNA. Although we did not demonstrate the formation of dienes from cyclic acetals with allylic heteroatoms, several examples exist in the literature to support the transformation. The choice of senecialaldehyde allowed us to eliminate the formation of the two dienes during the elimination step. However, once a heteroatom is re-introduced into the acetal prior to the elimination step, we expect that cyclic acetals will be subject to the same competitive pathways (**Scheme 2.8**) as the dimethoxy acetals. Whether or not the cyclic acetal will be more stable remains to be determined, but data from similar systems suggests that they will be more robust compounds. The use of a cyclic acetal has provided us with a more efficient synthetic route to make dienes suitable for *in vitro* selection of HDA catalysts. Although we changed the location on the diene backbone to attach the RNA, we were able to retain the overall reactivity of the diene in an HDA cycloaddition. This will allow

us to evaluate what role, if any, the point of attachment performs during the *in vitro* selection of RNA HDA catalysts.

2.6 Conclusion

In order to further demonstrate the "catalytic" potential of RNA, we have designed several dienes and dienophiles suitable for the *in vitro* selection of HDA RNAses. We have also developed a synthetic methodology for diene synthesis that will allow us to produce a series of functionally diverse dienes. This will enable our group to investigate what effect sterics, electronics, and the site of RNA attachment will have on the *in vitro* selection of RNA HDAses. Utilizing the dienes and dienophiles synthesized in this project, our group hopes to isolate RNA HDAses that rival the catalytic potential of the current "state of the art" HDA catalyst of Jorgensen.

Chapter 3: Experimental

4-Formyl-N-(2-hydroxyethyl)-benzamide (28) To a clean, dry, 25 mL pear-shaped flask was added **32** (42.6 mg, 0.138 mmol) and 5 mL of AcOH:H_2O:THF (3:1:1). The solution was stirred overnight at room temperature. The solvent was removed *in vacuo* to afford 28 as a colorless oil (28.0 mg, quantitative). ^{1}H NMR (300 MHz, CD_3OD) δ 10.55 (s, 1H), 7.99 (s, 4H), 7.05 (dd, 1H, *J* = 6 Hz), 3.71 (dd, 2H, *J* = 4.5 Hz), 3.50 (m, 2H, *J* = 4.5 Hz). ^{13}C NMR δ 191.9, 167.7, 139.5, 138.4, 61.9, 43.1.

N-(2-Hydroxyethyl)-4-(6-methoxy-3,6-dihydro-2H-pyran-2-yl)-benzamide N-(2-Hydroxyethyl)-4-(6-methoxy-3,6-dihydro-2H-pyran-2-yl)-benzamide (29). To a clean, dry J-Young tube was added **28** (26.7 mg, 0.138 mmol) and 1 mL of D_2O:d_3-MeOD:d_5-pyridine:d_6-DMSO (88:10:5:2). To this was added 1-methoxy-1,3-butadiene (28 μL, 2 eq), and the J-Young tube was sealed. The mixture was allowed to react at room temperature with occasional mixing of the contents. ^{1}H NMR was used to monitor the reaction for the formation of 2 allylic protons at 2.16 ppm. A half-life of approximately one-half year was estimated from time studies.

2-(tert-Butyldimethylsilyloxy)-ethylamine (31). To a solution of *t*-butyldimethylsilyl-chloride (2.2413 g, 14.7 mmol) in 30 mL of dry CH_2Cl_2 was added NEt_3 (2.2 mL, 14.2 mmol), DMAP (5.2 mg, catalytic) and ethanolamine (0.8 mL, 13.3 mmol). 20 mL of dry CH_2Cl_2 was added to the mixture, and the reaction was stirred

overnight at room temperature. Water (100 mL) was added to the reaction, and the mixture was stirred vigorously for 10 minutes. The organic layer was separated and washed with H_2O (100 mL) and brine (450 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* to yield **31** as a colorless oil (2.0012g, 86%). The R_f of **34** matched the literature reported value of 0.22 (CH_2Cl_2/MeOH, 10:1). No NMR was recorded.

***N*-[2-(tert-Butyldimethylsilyloxy)-ethyl]-4-formyl-benzamide (32).** To a clean, dry, 25 mL pear-shaped flask was added 4-formyl benzoic acid (500.2 mg, 3.33 mmol), DCC (825.6 mg, 4.00 mmol) and TEA (1 mL, 2 eq) in 10 mL of CH_2Cl_2/DMF (1:1) at 0 ^oC. To this was added HOBt (501.3 mg, 3.66 mmoles), and the mixture stirred under argon for 1 hour at 0 ^oC. Amine **31** (595.1 mg, 3.66 mmol) and DMAP (79.2 mg, 0.649 mmol) were added to the reaction mixture and, the mixture was allowed to stir for 18 hours under argon. The mixture was placed in a freezer at –20 ^oC for 30 minutes, and a white solid was removed by gravity filtration. The solvents were removed *in vacuo*, and the residual oil was dissolved in 50 mL of EtOAc. The mixture was washed with 3% acetic acid (2 x 50 mL), brine (50 mL), saturated $NaHCO_3$ (2 x 50 mL), and brine (50 mL). The organic layer was dried over $MgSO_4$, and the solvent was removed to yield a yellow oil. The crude oil was purified by chromatography on silica (32 – 63 μm, CH_2Cl_2/EtOAc 9:1) to provide **32** as a white solid (692.3 mg, 68%). ^1H NMR (300 MHz, $CDCl_3$) δ 10.06 (s, 1H), 7.93 (m, 4H, *J* = 5.2 Hz), 6.61 (s, 1H), 3.80 (t, 2H, *J* = 4.1 Hz), 3.60 (dd, 2H, *J* = 4.1 Hz), 0.91 (s, 9H), 0.09 (s, 6H). ^{13}C NMR 191.7, 171.8, 166.5, 140.1, 138.4, 130.1, 127.7, 61.8, 42.4, 26.1, 18.5, –5.1.

N-BOC-diaminobutane (34). To a clean, dry, 250 mL round-bottomed flask was added 1,4-butanediamine (2.25 mL, 20.0 mmol) in 100 mL of dry CH_2Cl_2 at 0 ^oC. BOC_2-O (0.9755 g, 4.4 mmol) was dissolved in 50 mL of dry CH_2Cl_2 at 0 ^oC and dropwise added to the diamine solution. The reaction was allowed to warm to room temperature and stir for 3 hours. A white precipitate was collected by vacuum filtration, and the filtrate was concentrated *in vacuo*. The oily residue was dissolved in 50 mL of EtOAc and washed with brine (2 x 15 mL). The aqueous layers were combined and extracted with EtOAc (2 x 30 mL). The organic layers were combined and dried over $MgSO_4$. The solvent was removed *in vacuo* to afford **34** as a colorless oil (3.1905 g, 85%). R_f (0.45, MeO/NH_4OH 9:1). ^1H NMR (300 MHz, CD_3OD) δ 4.68 (bs, 1H), 3.11 (q, 2H), 2.69 (t, 2H), 1.47 (m, 13 H).

N-BOC-[4-(4-Formylbenzoylamino)-butyl]-butylamine (35). To a clean, dry, 25 mL pear-shaped flask was added EDC (356.0 mg, 1.68 mmol), TEA (260 μL, 1.90 mmol) and 4-formylbenzoic acid (251.3 mg, 1.67 mmol). The solution was stirred for 4 hours at 0 ^oC. (4-aminobutyl)carbamic acid-1,1-dimethylethylester (340 mg, 1.06 mmol) was dissolved in 2 mL of dry CH_2Cl_2 and dropwise added to the 4-formylbenzoic acid mixture. The solution was stirred overnight, diluted to 100 mL with CH_2Cl_2 and washed with saturated $NaHCO_3$ (50 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 25 mL) and the organic layers were combined and washed with water (30 mL), 5 % HCl (30 mL), water (30 mL), and brine (30 mL). The organic layer was dried over $MgSO_4$ and concentrated to give a yellowish oil that was purified on silica (Hexanes/EtOAc, 1:2,

0.1% TEA) to afford **35** as a white solid (154.5 mg, 29%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (s, 1H), 7.91 (m, 4H, *J* = 6.3 Hz), 6.91 (bs, 1H), 4.71 (bs, 1H), 3.49 (t, 2H, *J* = 6.3 Hz), 3.15 (m, 2H, *J* = 6.3 Hz), 1.65 (dd, 2H, *J* = 6.0 Hz), 1.60 (dd, 2H, *J* = 6.0Hz), 1.41 (s, 9H). ^{13}C NMR δ 191.9,

166.7, 156.6, 140.1, 138.2, 130.0, 128.0, 79.7, 40.1, 40.0, 28.6, 28.2, 26.2. HRMS (FAB) [M+1] Anal. Calcd. For $C_{17}H_{24}N_2O_4$: 321.1814. Found 321.1817.

4-(4-formylbenzoylamino)-butyl-ammonium-trifluoroacetate (36). To a clean, dry, round-bottomed flask was added **35** (42.3 mg, 0.132 mmol) and 10 mL of 25% TFA/THF. The solution was stirred overnight at room temperature, and the solvent was removed *in vacuo* to provide **36** as a white solid (44.1 mg, quantitative). ^{1}H NMR (300 MHz, CD_3OD) δ 9.77 (s, 1H), 7.73 (dd, 4H, *J* = 8.1 Hz), 7.45 (dd, 1H, *J* = 8.1 Hz), 3.22 (t, 2H, *J* = 6.0 Hz), 2.80 (t, 2H, *J* = 6.0 Hz), 1.85 (s, 2H), 1.50 (s, 2H).

4-Formyl-N-{4-[5-(2-oxohexahydrothieno[3,4-d]imidazol-4-yl)-pentanoylamino]-butyl}-benzamide (25). To a clean, dry, 10 mL pear-shaped flask was added NHS-biotin (50.3 mg, 0.130 mmol) in 1 mL of anhydrous DMF. In a separate flask **36** (44.1 mg, 0.138 mmol) was dissolved in 1 mL of anhydrous DMF, and to this was added TEA (35 μL, 0.260 mmol). The solution stirred for 5 minutes. The contents of the flask containing **36** in a DMF/TEA solution were transferred to the flask containing NHS-biotin by cannulation, and the reaction was stirred at room temperature overnight. The solvents were removed *in vacuo* and the resulting residue was dissolved in 2 mL of

MeOH. Unreacted biotin was removed by filtration and 1 g of silica (63 – 200 μm) was added to the filtrate. The solvent was removed *in vacuo* and the silica was loaded onto the Biotage chromatography system. The product was eluted from the silica using a gradient of CH_2Cl_2/MeOH (20:1 → 18:1) to afford **25** as a white powder (27.5 mg, 47%). ^{1}H NMR (400MHz, $CDCl_3$) δ 9.93 (s, 1H), 7.86 (d, 2H, J = 8.4 Hz), 7.83 (d, 2H, J = 8.4 Hz), 4.36 (dd, 1H, J_1 = 5.0 Hz, J_2 = 8.0 Hz), 4.16 (dd, 1H, J_1 = 5.0 Hz, J_2 = 8.0 Hz), 3.30 (dt, 2H, J = 6.6 Hz), 3.27 (s, 1H), 3.23 (dd, 1H, J = 1.6 Hz), 3.10 (t, 2H , J = 6.6 Hz), 3.02 (m, 1H, J = 6.6 Hz), 2.77 (dd, 1H, J_1 = 5.2 Hz, J_2 = 12.8 Hz), 2.57 (d, 1H, J = 12.8 Hz), 2.07 (t, 2H, J = 6.8 Hz), 1.52 (m, 10H), 1.32 (dt, 2H, J = 8Hz). ^{13}C NMR δ 192.4, 129.8, 94.6, 77.6, 77.3, 77.0, 62.0, 60.2, 55.6, 40.4, 39.8, 38.9, 35.8, 28.4, 28.1, 26.8, 26.5, 25.6. HRMS (FAB) [M+1] Anal. Calcd. For $C_{22}H_{30}N_4O_4S$: 447.2066. Found 447.2044.

6-(tert-Butyldimethylsilyloxy)-hexanoic acid (40). To a clean, dry, 50 mL round-bottomed flask was added ε-caprolactone (2 mL, 18 mmol) and NaOH (0.8013 g, 20 mmol) in 20 mL of THF/H_2O (4:1). The mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure to give the disodium salt of 6-hydroxyhexanoic acid (2.9602 g). The salt was suspended in anhydrous DMF (5 mL). TMS-Cl (4.7712 g, 41.0 mmol) and DMAP (6.5946 g, 54 mmol) were added to the suspension and stirred overnight at room temperature. NaOH (1.6 g, 234 mmol) was dissolved in 20 mL of THF/H_2O (4:1), and the mixture was added to the disodium salt/TMS-Cl solution and stirred overnight. The reaction was acidified to pH 4 with 1N

HCl and extracted with EtOAc (3 x 75 mL). The organic layers were dried over $MgSO_4$ and concentrated *in vacuo* to provide a pale yellow oil. The crude product was purified over silica using an ether/petroleum ether gradient (0% → 20%) to provide **40** as a colorless oil (2.394 g, 54%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.35 (bs, 1H), 3.60 (t, 2H, *J* = 7.0 Hz), 2.34 (t, 2H, *J* = 7.0), 1.64 (tt, 2H, *J* = 7.0 Hz), 1.48 (tt, 2H, *J* = 7.0 Hz), 1.38 (tt, 2H, *J* = 7.0 Hz). ^{13}C NMR δ 180.4, 63.1, 34.3, 32.6, 26.2, 25.5, 24.7, 18.6, -5.1.

N-CBz-{4-[6-(tert-Butyldimethylsilyl-)-hexanoylamino]-butylamine (41a). To a clean, dry 10 mL pear-shaped flask was added **40** (50.2 mg, 0.205 mmol), DCC (50.7 mg, 0.249 mmol), and 2 mL of anhydrous DMF. The reaction was cooled to 0 ^{o}C for 20 minutes with stirring under Argon. HOBt (31.2 mg, 0.221 mmol) was added, and the reaction stirred at 0 ^{o}C for 20 minutes. Compound **34** (38.9 mg, 0.226 mmol) and DMAP (5.0 mg, 0.011 mmol) were added, and the reaction was stirred overnight at room temperature under argon. The sample was diluted with EtOAc (50 mL) and extracted with 5% citric acid (3 x 25 mL), brine (25 mL), saturated $NaHCO_3$ (3 x 25 mL), and brine (25 mL). The organic layer was dried over $MgSO_4$ and the solvent removed *in vacuo*. The crude solid was purified on silica (63 – 200 μm) using a hexane/EtOAc gradient (6:1 → 1:1) to afford **41a** as a white solid (69.2 mg, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.75 (bs, 1H), 5.41 (bs, 1H), 5.08 (s, 2H), 3.59 (t, 2H, *J* = 6.6 Hz), 3.21 (m, 4H, *J* = 7.0 Hz), 2.14 (t, 2H, *J* = 6.6 Hz), 1.60 (tt, 2H, *J* = 7.0 Hz), 1.51 (t, 6H, *J* = 6.6 Hz), 1.34 (tt, 2H, *J* = 7.0 Hz), 0.89 (s, 9H), 0.03 (s, 6H). ^{13}C NMR δ 173.3, 156.7, 136.8, 128.7, 128.3, 105.8, 66.9, 63.2, 40.8, 39.2, 37.0, 32.8, 27.7, 27.0, 26.2, 25.8,

18.6, –5.1. HRMS (FAB) [M+1] Anal. Calcd. For $C_{24}H_{42}N_2O_4Si$: 451.2992. Found 451.2993.

N-*t*-butoxycarbonyl-{4-[6-(tert-Butyldimethylsilyloxy)-hexanoylamino]-butylamine (**41b).** To a clean, dry 50 mL pear-shaped flask was added **40** (1.004 g, 4.06 mmol), EDC 934.1 mg, 4.87 mmol), and 16 mL of anhydrous DCM. The reaction was cooled to 0 ^oC for 20 minutes with stirring under Argon. HOBt (658.0 mg, 4.87 mmol) was added, and the reaction stirred at 0 ^oC for 20 minutes. Compound **34** (917.0 mg, 4.67 mmol) and DMAP (25.1 mg, 0.20 mmol) were added, and the reaction was stirred overnight at room temperature under argon. The sample was diluted with EtOAc (50 mL) and extracted with 5% citric acid (3 x 25 mL), brine (25 mL), saturated $NaHCO_3$ (3 x 25 mL), and brine (25 mL). The organic layer was dried over $MgSO_4$ and the solvent removed *in vacuo*. The crude solid was purified on silica (63 – 200 μm) using a hexane/EtOAc gradient (6:1 → 1:1) to afford **41b** (1.1894 g, 65%). ^1H NMR (300 MHz, $CDCl_3$) δ 6.19 (bs, 1H), 4.84 (bs, 1H), 3.51 (t, 2H, *J* = 8.8 Hz), 3.17 (q, 2H, *J* = 7.6 Hz), 3.05 (q, 2H, *J* = 7.6 Hz), 2.10 (t, 2H, *J* = 8.8 Hz), 1.54 (tt, 2H, *J* = 9.6 Hz), 1.43 (tt, 6H, *J* = 9.6 Hz), 1.36 (s, 9H), 1.26 (m, 2H, *J* = 9.6 Hz), 0.81 (s, 9H), 0.03 (s, 6H). ^{13}C NMR δ 173.3, 156.3, 79.3, 63.3, 40.4, 39.3, 37.0, 32.9, 28.7, 27.9, 27.0, 26.3, 26.0, 25.9, 18.6, –4.9. HRMS (FAB) [M+1] Anal. Calcd. For $C_{21}H_{44}N_2O_4Si$: 417.3149. Found 417.3139.

N-CBz-[4-(6-Hydroxyhexanoylamino)-butylamine (**42a).** To a clean, dry 25 mL round-bottomed flask was added **41a** (61.0 mg, 0.135 mmol) and 5 mL of

THF/H_2O/AcOH (1:1:3). The reaction was stirred at room temperature overnight, and the solvent was removed *in vacuo* to afford **42a** as a white solid (45.1 mg, quantitative). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.75 (bs, 1H), 5.41 (bs, 1H), 5.08 (s, 2H), 3.59 (t, 2H, *J* = 6.6 Hz), 3.21 (m, 4H, *J* = 7.0 Hz), 2.14 (t, 2H, *J* = 6.6 Hz), 1.60 (tt, 2H, *J* = 7.0 Hz), 1.51 (t, 6H, *J* = 6.6 Hz), 1.34 (tt, 2H, *J* = 7.0 Hz). ^{13}C NMR 173.3, 156.7, 136.8, 128.3, 105.8, 66.9, 63.2, 40.8, 39.2, 37.0, 32.8, 27.7, 26.2, 25.8.

N-BOC-[4-(6-Hydroxyhexanoylamino)-butylamine (42b). To a clean, dry, 25 mL round-bottomed flask was added **41b** (0.7009 g, 16.8 mmol) and 105 mL of THF/H_2O/AcOH (1:1:3). The reaction was stirred at room temperature overnight, and the solvent was removed *in vacuo* to afford **42b** as a white solid (0.5092 g, quantitative). ^{1}H NMR (300 MHz, CD_3OD) δ 3.54 (t, 2H, *J* = 6.6Hz), 3.16 (t, 2H, *J* = 6.6 Hz), 3.03 (t, 2H, *J* = 6.6 Hz), 2.18 (t, 2H, *J* = 7.2 Hz), 1.62 (tt, 2H, *J* = 6.6 Hz), 1.54 (tt, 2H, *J* = 6.6 Hz), 1.48 (m, 4H, *J* = 3.2Hz), 1.42 (s, 9H), 1.37 (dt, 2H, J_1 = 7.2 Hz, J_2 = 3.2 Hz). ^{13}C NMR δ 176.2, 158.6, 79.9, 62.8, 41.1, 40.1, 37.2, 33.4, 28.9, 28.5, 27.8, 27.0, 26.7.
HRMS (FAB) [M+1] Anal. Calcd. For $C_{15}H_{30}N_2O_4$: 303.2284. Found 303.2283.

N-Cbz-[4-(6-Oxohexanoylamino)-butylamine (**43a).** To a clean, dry, 25 mL round-bottomed flask was added the **42a** (27mg, 0.08 mmol) and 5 mL dry DCM. To this was added PCC (40mg) and stirred at RT for 3.5 hours. The sample was filtered through a silica plug (3x) to remove the PCC. The sample was then purified on the Biotage (20:1 DCM:MeOH) to afford **43a** as a white solid (10 mg, 37%). ^{1}H NMR (400MHz, $CDCl_3$) δ9.75 (s, 1H), δ7.35 (s, 5H), 5.75 (s, 1H), 4.94 (s, 1H), 3.26 (d, 2H, *J* = 6Hz), 3.21 (d, 2H, *J* = 6Hz), 2.47 (q, 2H, *J* = 6Hz), 2.18 (t, 2H, *J* = 6Hz), 1.64 (m, 4H, *J* =

4Hz), 1.53 (4H, s). ^{13}C NMR δ 202.52, 172.6, 156.7, 136.7, 128.75, 128.52, 128.25, 66.89, 43.84, 40.80, 39.29, 39.53, 27.72, 26.91, 25.25, 21.75. HRMS (FAB) [M+1] Anal. Calcd. For $C_{18}H_{26}N_2O_4$: 335.1971. Found 335.1978.

4-Bromo-1,1-dimethoxy-3-methyl-2-butene (**46b).** To a clean, dry, 25 mL round-bottom flask was added 1-trimethylsiloxy-3-methyl-1,3-butadiene (860mg, 5.52 mmoles), anhydrous DMF (5.5 mL) and cooled to –20^{o}C. After 10 minutes of stirring under argon, the solution was warmed to room temperature and 5.5 mL of anhydrous MeOH was added to the reaction. After 4 hours of stirring at room temperature the reaction was poured over saturated $NaHCO_3$ (30 mL) and extracted with ether (3x60 mL). The organic layers were combined and dried over $MgSO_4$ to produce a light yellow oil, which was purified on silica using a gradient system of hexane/ethyl acetate (20:1 → 14:1) to afford **46b** as a colorless oil (571 mg, 54% yield). ^{1}H NMR $CDCl_3$ (300 MHz) Z isomer, 1.87 (d, 3H,*J* = 6.3 Hz), 3.31 (s, 6H), 3.94 (s, 2H), 5.01 (d, 1H, *J* = 6.3 Hz), 5.63 (d, 1H, *J* = 6.3 Hz); E isomer, 1.91 (, d, 3H, *J* = 1.4 Hz), 3.31 (s, 6H), 4.02 (s, 2H), 5.05 (d, 1H, *J* = 5.6 Hz), 5.41 (d, 1H, *J* = 5.6 Hz).

4,4-dimethoxy-2-methyl-but-2-enyl acetate(**47a).** To a clean, dry, 25 mL round-bottom flask was added **46b** (2.989 g, 14.2 mmole), KOAc (1.983 g, 15.6 mmoles), and anhydrous MeOH (20 mL). The solution was heated to reflux and stirred for 20 hours under argon. After cooling to room temperature the solution was poured over brine (30 mL) and extracted with ether (3x 60 mL). The organic fractions were collected, dried

over $MgSO_4$ and concentrated to produce a yellow residue (3.1025 g). The residue was purified on silica using a hexanes/EtOAc gradient (25:1 → 16:1) to provide **47a** as colorless oil (2.015 g, 75% yield). ^{1}H NMR $CDCl_3$ (300 MHz) Z isomer, 1.64 (s, 3H), 1.97 (d, 3H, *J* = 1 Hz), 3.18 (d, 6H, *J* = 1 Hz), 4.37 (s, 2H), 4.95 (d,1H, *J* = 6.3 Hz), 5.41 (d, 1H, J = 6.3 Hz); E isomer, 1.69 (s, 3H), 1.84 (d, 3H, *J* = 1 Hz), 3.20 (d, 6H, *J* = 1 Hz), 4.54 (s, 2H), 4.99 (d, 1 H, *J* = 6.0 Hz), 5.33 (d, 1H, *J* = 6.0 Hz).

4,4-Dimethoxy-2-methyl-2-buten-1-ol (**47b).** To a clean, dry, 50 mL round-bottom flask was added **47a** (0.251 g, 1.33 mmoles), KOH (0.0752 g, 1.49 mmoles) and 2 mL of THF/water (4:1). MeOH (0.5 mL) was added to the mixture and the solution was stirred for 3.5 hours at room temperature, then poured over of brine (30 mL). The aqueous mixture was extracted with ether (3x 60 mL) and dried over $MgSO_4$. The crude oil was purified on silica using a hexanes/EtOAc gradient (6:1 → 1:1) to produce **47b** as a light yellow oil (100 mg, 52%). ^{1}H NMR CD_3CN (300 MHz, CD_3CN) Z isomer, 1.68 (s, 3H), 3.00-3.20 (bs, 1H), 3.27 (s, 6H), 3.91 (s, 2H), 5.06 (d, 1H, *J* = 6.0 Hz), 5.42 (d, 1H, *J* = 6.0 Hz); E isomer, 1.80 (s, 3H), 3.23 (s, 6H), 4.08 (s, 2H), 5.06 (d, 1H, *J* = 6.0 Hz), 5.28 (d, 1H, *J* = 6.0 Hz).

3-Methyl-2-butenal (**49).** To a clean, dry, 100 mL round-bottomed flask was added PCC (3.8842 g, 18.00 mmol) in anhydrous CH_2Cl_2 (40 mL) at 0 ^{o}C. 3-Methyl-2-buten-1-ol (1.2102 g, 13.60 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL) and dropwise added to the PCC solution at 0 ^{o}C. The reaction was stirred for 3 hours at room

temperature followed by a bulb-to-bulb distillation. The solvent was evaporated without vacuum to afford **49** as a colorless oil. (1.0219 g, 88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.96 (s, 1H), 5.88 (t, 1H, *J* = 1 Hz), 2.19 (d, 3H, *J* = 1 Hz), 1.99 (d, 3H, *J* = 1 Hz). ^{13}C NMR δ 191.0, 160.7, 128.1, 27.2, 18.9.

1-(Trimethylsiloxy)-3-methyl-1,3-butadiene (**50).** To a clean, dry, 25 mL round-bottom flask was added NEt_3 (3.4 mL, 24.42 mmole) and zinc chloride (32.1 mg, 0.20 mmole). The solution was stirred for 10 minutes at room temperature. Senecialdehyde (2 mL, 23.80 mmole) and diethyl ether (4 mL) were added to the reaction mixture, followed by the dropwise addition of TMS-Cl (3.4-mL, 24.00 mmole). The reaction mixture was heated to reflux and stirred for 24 hours under an argon atmosphere. Pentane (16 mL) was added to the reaction and a white pricipate formed. The precipitate was removed by vacuum filtration and washed with pentane. The filtrate was concentrated *in vacuo* to give a yellowish oil that was purified by vacuum distillation to give **50** as colorless oil (2.58 g, 76%). ^{1}H NMR (300 MHz, C_6D_6) E isomer, 0.08 (s, 9H), 1.72 (s, 3H), 4.75 (s,1H), 4.85 (s, 1H), 6.09 (d, 1H, *J* = 11.4 Hz), 6.56 (d, 1H, *J* = 11.4 Hz); Z isomer 0.00 (s, 9H), 1.56 (s, 3H), 4.90 (s, 1H), 5.08 (d, 1H, *J* = .2 Hz), 6.19 (s, 1H), 6.03 (d, 1H, *J* = 6.2 Hz).

(4,4-Dimethoxy-2-methyl-but-2-enyl)-diethylamine (57). To a clean, dry, 10 mL round-bottomed flask containing NEt_2H (2 mL, 19.30 mmol) was dropwise added neat **46b** (0.5012 g, 2.47 mmol). The solution was refluxed overnight. The precipitate

was filtered off, and the filtrate was diluted with Et_2O (10 mL). The mixture was washed with water (2 x 2 mL) and dried over $MgSO_4$ to afford **57** (0.4575 g, 92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.31 (d, 1H, *J* = 1.0 Hz), 4.97 (d, 1H, *J* = 1.0 Hz), 3.21 (s, 6H), 2.82 (s, 2H), 2.36 (q, 4H, *J* = 6 Hz), 1.638 (d, 3H, *J* = 1.0 Hz), 0.89 (t, 6H, *J* = 6 Hz).

Diethyl-(4-methoxy-2-methylene-but-3-enyl)-amine (**58).** To a clean, dry, 10 mL pear-shaped flask was added **57** (0.1001 g, 0.50 mmol) in THF (2 mL). The mixture was cooled to –78 ^{o}C under argon and *t*-butyllithium (585 μL, 1.7 M) was dropwise added. The reaction was warmed to room temperature and stirred for 4 hours. The sample was diluted with ether (20 mL), washed with brine (2 x 5 mL), and the organic layer was dried over $MgSO_4$. Purification on silica (63 – 200 μM) using a gradient of Hexane/EtOAc (20:1 → 6:1) in the presence of TEA (1%) afforded **58** as a colorless oil (0.0762, 90%). ^{1}H NMR (300 MHz, CD_2Cl_2) δ 6.06 (d, 1H, *J* = 13.3 Hz), δ 5.53 (d, 1H, *J* = 13.3 Hz), 3.10 (s, 2H), 2.46 (q, 4H, *J* = 7.2 Hz), 1.02 (t, 6H, *J* = 7.2 Hz). ^{13}C NMR δ 149.8, 147.1, 112.1, 106.1, 57.5, 56.2, 46.7, 11.5.

2-(2-Methylpropenyl)-[1,3]dioxolane (**60).** To a clean, dry 50 mL round-bottomed flask was added anhydrous DCM (12.5 mL) and trimethylsilyl-trifluoromethanesulfonate (23μL, 0.13 mmol). The solution was cooled to –78 ^{o}C under argon and 1,2-bis(trimethylsiloxy)ethane (3.8 mL, 15.50 mmole) was added followed by the addition of senecialaldehyde (1.25 mL, 12.9 mmol). The reaction was stirred for 3 hours at –78 ^{o}C and quenched by the addition of pyridine (250 mL, 3.20 mmol). The

reaction mixture was poured onto saturated $NaHCO_3$ (20 mL) and extracted with DCM (3 x 20 mL). The organic layers were combined and dried over a mixture of $K_2CO_3/MgSO_4$ (1:1). The solution was concentrated and the residue was purified on silica (63 – 200 μm) using EtOAc/Hexanes (10:1) as the solvent system to afford **60** as a colorless oil (1.2501 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.45 (d, 1H, *J* = 8 Hz), 5.22 (d, 1H, *J* = 8 Hz), 3.98 (t, 2H, *J* = 6.4 Hz), 3.85 (t, 2H, *J* = 6.4 Hz), 1.75 (d, 6H, *J* = 1.2 Hz). ^{13}C NMR δ 141.5, 121.6, 100.6, 65.1 (2C), 26.1, 18.6.

2-(3-Methyl-buta-1,3-dienyloxy)-ethanol (**61).** To a clean, dry, 10 mL pear-shaped flask was added anhydrous ether (4 mL) and 60 (0.0500 g, 0.39 mmol). The flask was cooled to –78 ^{o}C under an argon atmosphere and *t*-butyllithium (45 μL, 1.7 M) was dropwise added. The reaction was stirred for 30 minutes at room temperature and quenched with absolute MeOH (1 mL). Saturated $NaHCO_3$ (10 mL) was added to the flask, and the mixture was extracted with ether (2 x 50 mL). The combined organics were dried over $K_2CO_3/MgSO_4$ (1:1) and concentrated *in vacuo* to afford a light yellow oil. The crude oil was purified on silica (63 – 200 μm) using a hexanes/EtOAc gradient (18:1 → 5:1) to afford **61** as a colorless oil (8 mg, 16%). ^{1}H NMR (400 MHz, C_6D_6) δ 6.37 (1H, d, *J* = 12 Hz), 5.68 (1H, d, *J* = 12 Hz), 4.82 (2H, q, *J* = 1 Hz), 3.31 (2H, t, *J* = 4.4 Hz), 3.26 (2H, t, *J* = 4.4 Hz), 1.65 (3H, t, *J* = 1 Hz), 1.28 (1H, bs). ^{13}C NMR δ 148.11, 139.6, 112.0, 109.9, 71.0, 61.2, 19.0. HRMS (FAB) [M+1] Anal. Calcd. For $C_7H_{12}O_2$: 129.0916. Found 129.0907.

Chapter 4: References

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