Approaches to the Synthesis of the Natural Products, Azaphorbol and Frondosin B, via Diazo Decomposition Reactions

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Alicia J. Frantz

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This dissertation titled
Approaches to the Synthesis of the Natural Products, Azaphorbol and Frondosin B, via
Diazo Decomposition Reactions

by

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ABSTRACT

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Approaches to the Synthesis of the Natural Products, Azaphorbol and Frondosin B, via Diazo Decomposition Reactions

Director of Dissertation: Mark C. McMills

The use of carbonyl ylides as reactive intermediates has numerous potential applications in the field of natural product synthesis. These highly reactive intermediates can be formed from the metal catalyzed diazo-decomposition reactions. They can undergo several subsequent reactions, including the 1,3-dipolar cycloaddition reaction, resulting in bridged oxygen heterocycles.

Use of 1,3-dipolar cycloaddition reactions can be used to generate the seven and six membered rings of the bicycle[5.4.0]undecene core of various natural products. The natural product phorbol belongs to the tigliane diterpene family and is a protein kinase C (PKC) activator. Of the compounds belonging to the phorbol family, many show biological activity (e.g., tumor promotion, HIV inhibition, and antileukemic activity). Nitrogen containing derivatives of phorbol (azaphorbol) are relatively rare in the literature. An efficient route to the azaphorbol core will enable the generation of analogs to be investigated for biological activity. Another natural product containing the bicycle[5.4.0]core is frondosin B. Frondosin B also shows biological activity by the inhibition of interleukin-8 (IL-8) and PKC inhibition. Generation of the core of these natural products is attempted via the rhodium(II) catalyzed 1,3-dipolar cycloaddition reaction.
We have also devised a synthetic route to the unsubstituted cyclic diazoacetamides, a precursor to the 1,3-dipolar cycloaddition reaction, successfully. These diazo-moieties can play a role in late stage synthesis and the discovery of a facile, high yielding route is highly valuable.
DEDICATION

For the little scientists in my life: Ella, Dominic, Brenna, and Eli. Thanks for helping me keep it all in perspective.
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<td>acetonitrile</td>
</tr>
<tr>
<td>Anti-Ig</td>
<td>antiimmunoglobulin antibodies</td>
</tr>
<tr>
<td>ATP</td>
<td>adenine triphosphate</td>
</tr>
<tr>
<td>Boc$_2$O</td>
<td>di-$\text{tert}$-butyl dicarbonate</td>
</tr>
<tr>
<td>CPE</td>
<td>cytopathic effects</td>
</tr>
<tr>
<td>DAG</td>
<td>diacylglycerol</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-diazabicyclo[5.4.0]undec-7-ene</td>
</tr>
<tr>
<td>DCC</td>
<td>N,N-dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DCE</td>
<td>1,2-dichloroethane</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DEAD</td>
<td>diethyl azodicarboxylate</td>
</tr>
<tr>
<td>DIBAL</td>
<td>diisobutyaluminum hydride</td>
</tr>
<tr>
<td>DMAD</td>
<td>dimethyl acetylenedicarboxylate</td>
</tr>
<tr>
<td>DMAP</td>
<td>dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>$N, N$-dimethylformamide</td>
</tr>
<tr>
<td>DMP</td>
<td>Dess-Martin periodinane</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>ED$_{50}$</td>
<td>median effective dose</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
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</tr>
<tr>
<td>EDG</td>
<td>electron donating group</td>
</tr>
<tr>
<td>ER</td>
<td>endoplasmic reticulum</td>
</tr>
<tr>
<td>Et</td>
<td>ethyl</td>
</tr>
<tr>
<td>Et&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>diethyl ether</td>
</tr>
<tr>
<td>Et&lt;sub&gt;3&lt;/sub&gt;N</td>
<td>triethylamine</td>
</tr>
<tr>
<td>EWG</td>
<td>electron withdrawing group</td>
</tr>
<tr>
<td>HAART</td>
<td>highly active antiretroviral treatment</td>
</tr>
<tr>
<td>HOMO</td>
<td>highest occupied molecular orbital</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>half maximal inhibitory concentration</td>
</tr>
<tr>
<td>IL-8</td>
<td>interleukin-8</td>
</tr>
<tr>
<td>IP&lt;sub&gt;3&lt;/sub&gt;</td>
<td>inositol triphosphate</td>
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<td>KHMDS</td>
<td>potassium bis(trimethylsilyl)amide</td>
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<tr>
<td>LAH</td>
<td>lithium aluminum hydride</td>
</tr>
<tr>
<td>LDA</td>
<td>lithium diisopropyl amide</td>
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<tr>
<td>Li(t-BuO)&lt;sub&gt;3&lt;/sub&gt;AlH</td>
<td>tri-&lt;i&gt;tert&lt;/i&gt;-butoxylithium aluminum hydride</td>
</tr>
<tr>
<td>LUMO</td>
<td>lowest unoccupied molecular orbital</td>
</tr>
<tr>
<td>&lt;i&gt;m&lt;/i&gt;CPBA</td>
<td>&lt;i&gt;meta&lt;/i&gt;-Chloroperoxybenzoic acid</td>
</tr>
<tr>
<td>MeOH</td>
<td>methanol</td>
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<tr>
<td>n-BuLi</td>
<td>n-butyllithium</td>
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<tr>
<td>p-ABSA</td>
<td>&lt;i&gt;para&lt;/i&gt;-acetamidobenzencesulfonfyl azide</td>
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<td>PCC</td>
<td>pyridinium chlorochromate</td>
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<tr>
<td>p-DBSA</td>
<td>&lt;i&gt;para&lt;/i&gt;-dodecylbenzenesulfonfyl azide</td>
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PIP\textsubscript{2}  \hspace{1cm} \text{phosphatidylinositol bispophate}  
PKC \hspace{1cm} \text{protein kinase C}  
pTsOH \hspace{1cm} \text{para-toluenesulfonic acid}  
RACK \hspace{1cm} \text{receptor for activated C-kinase}  
Rh\textsubscript{2}(acam)\textsubscript{4} \hspace{1cm} \text{dirhodium(II) tetraacetamide}  
Rh\textsubscript{2}(OAc)\textsubscript{4} \hspace{1cm} \text{dirhodium(II) tetraacetate}  
Rh\textsubscript{2}(OHex)\textsubscript{4} \hspace{1cm} \text{dirhodium(II) tetrahexanoate}  
Rh\textsubscript{2}(pfb)\textsubscript{4} \hspace{1cm} \text{dirhodium(II)tetrakis(perfluorobutyrate)}  
RT \hspace{1cm} \text{room temperature}  
SOCl\textsubscript{2} \hspace{1cm} \text{thionyl chloride}  
TBAF \hspace{1cm} \text{tert-butylammonium fluoride}  
TBDPSCI \hspace{1cm} \text{tert-butyldiphenylsilyl chloride}  
TBSCI \hspace{1cm} \text{tert-butyldimethylsilyl chloride}  
TBSOTf \hspace{1cm} \text{tert-butyldimethylsilyl trifluoromethane}  
t-BuLi \hspace{1cm} \text{tert-butyllithium}  
TFA \hspace{1cm} \text{trifluoroacetic acid}  
THF \hspace{1cm} \text{tetrahydrofuran}  
TiCl\textsubscript{4} \hspace{1cm} \text{titanium(IV) chloride}  
TLC \hspace{1cm} \text{thin layer chromatography}  
TMEDA \hspace{1cm} \text{tetramethylethylenediamine}  
TMS \hspace{1cm} \text{trimethylsilane}  
TMSCl \hspace{1cm} \text{trimethylsilyl chloride}
TPA  4-\(\beta\)-13-\(O\)-tetradecanoylphorbol-13-acetate
CHAPTER 1: INTRODUCTION

1.1 Overview

The origins of modern organic synthesis can be traced back to 1828 in Germany with the preparation of urea, a naturally occurring organic compound, from ammonium cyanate.\(^1\) General organic synthesis can be divided into several main areas of research, including total synthesis (e.g., natural product synthesis), semi-synthesis, and methodology development.\(^2\) For the foundation of natural product synthesis, several goals included 1) to unambiguously establish the structure and 2) generation of an adequate amount of material for use in further chemical or biological studies. It was not until after World War II that organic chemistry and natural product synthesis experienced an incredible growth period. In addition to structure elucidation, natural product synthesis was used for myriad reasons including the production of material for biological testing when sufficient amounts were not available from natural sources, or as a means to develop new synthetic methodology in the pursuit of the natural product.\(^3\) Prior to completing a new synthesis, the organic chemist will plan a number of strategies and/or contingencies for perfecting either known reactions or invent novel reactions to accomplish a total synthesis as proof of concept for the methodology.

Discovering innovative reactions that enable chemists to construct highly complex scaffolds or molecules from comparatively simple reagents in a facile manner is especially desirable and important in organic synthesis. Often, the need to develop efficient and selective synthetic pathways stems from the high cost of procuring a compound from its natural source. It may be difficult to obtain natural products derived
from plants or marine organisms, owing to the challenge of physically collecting samples. Obtaining samples from remote locations often require permission for collection and shipment of materials, which can be hard to obtain. Those compounds derived from marine organisms may have the added challenge of requiring expensive procurement from the ocean. Minimizing the cost of a synthesis will require reactions that provide high yields, minimize the step count, and provide high regioselectivity and stereoselectivity.

One reaction type that demonstrates many of the positive aspects that are necessary to use in a successful synthesis is the diazo-decomposition reaction. The diazo-decomposition reaction is highly effective for the formation of carbon-carbon bonds, a synthesis transformation highly valued in organic synthesis. The diazo group itself consists of two nitrogen atoms bonded linearly together α to the carbonyl moiety. The diazo group provides a mechanistic pathway to generate a reactive carbenoid that mimics the reactivity of a non-stable carbene while, at the same time, is more stable than the carbene, leading to greater synthetic use. The generation occurs through a metal catalyzed decomposition of the diazo-intermediate dinitrogen followed by the loss of N₂ leading to the formation of a metallocarbenoid intermediate. The intermediate is useful in a number of different reactions types. One such reaction type is a pericyclic reaction; in this case a 1,3-dipolar cycloaddition. With the cycloaddition reaction, it is possible to generate highly functionalized five – seven membered rings, containing several contiguous stereocenters. A general example of the 1,3-dipolar cycloaddition is shown in Scheme 1,
where the creation of the 1,3-dipole 1 is shown, followed by the intramolecular cycloaddition to produce this bicycle[5.4.0]decanone ring system 2.\textsuperscript{6}

![Scheme 1: Intramolecular 1,3-cycloaddition reaction.](image)

This reaction has been utilized in several total syntheses, including pseudoularic acids, along with the alkaloids (±) lycopodine, dehydrovindorosin and compounds from the amaryidaceae family.\textsuperscript{5} The cycloaddition reaction has also been found to be extremely useful in the synthesis of phorbol, the naturally occurring carbon based compound. Phorbol displays a wide array of biological activities, making it an important target for total synthesis and analog preparation. Phorbol belongs to the tigliane family of natural products, all of which consist of a 5/7/6/3-tetracyclic ring system as the core of the compounds.\textsuperscript{7} Utilizing a dipolar cycloaddition, both the 7- and 6-membered rings can be formed simultaneously by this pericyclic reaction. The efficiency of this preparation rivals that of a Diels-Alder reaction in the overall formation of multiple rings and stereocenters. The pursuit of a phorbol analog via the 1,3-dipolar cycloaddition, along with the structurally similar Frondosin B, will be the focus of this dissertation.
CHAPTER 2: BACKGROUND OF DIAZO COMPOUNDS

2.1 Formation of Diazo Compounds

Diazo-decomposition reactions, followed by the resultant reactions such as cycloaddition or carbon-carbon (C-C) or carbon-heterocyclic (C-X) insertions, are utilized in various types of natural product synthesis and drug discovery. Reactions that involve transition metal complexes, such as the diazo-decomposition reactions, are a valuable method for rapidly building polyfunctionalized scaffolds often present in biologically active compounds. The process of installing a diazo functional group on a substrate, also known as diazotization, was discovered by Peter Griess in 1858. He found, and was later confirmed by other chemists, that diazotization of aromatic amines occurs with nitrous acid in acidic medium at 0°C (Scheme 2). This process became the basis for the Griess test for detection of nitrite.

\[
{\text{ArNH}}_2 + 2\text{HX} + \text{NaNO}_2 \quad \longrightarrow \quad \text{ArN}_2^+\text{X}^- + \text{NaX} + 2\text{H}_2\text{O}
\]

\[
X = \text{Cl}^-, \text{Br}^-, \text{NO}_3^-, \text{HSO}_4^-
\]

Scheme 2: Generalized Griess diazotization reaction.

When first gaining popularity as a synthetic method in organic synthesis, the only method known for formation of the diazo group involved compounds that already comprised a nitrogen atom on the impending diazo-carbon. Regitz reported various reactions utilizing this type of scaffold in ensuing reactions; the first example of this process comprises the reaction of an amine and nitric acid (Scheme 3). Upon protonation
and subsequent loss of water, the diazo moiety is formed. The second example from the Regitz group utilizes an oxime and chloramine in what is commonly called the Forster reaction. \(^{11}\) Unfortunately, this method does not always produce the desired product; the intermediates formed from primary aliphatic amines are unstable, converting rapidly to the carbon cation upon loss of N\(_2\) to yield substitution, elimination, or rearrangement products. Curtius first employed this method for the preparation of ethyl diazoacetate in 1883, using a method similar to the Griess method.

\[
\text{NH}_2 \xrightarrow{\text{HNO}_3, -2\text{H}_2\text{O}} \text{N}_2
\]

\[
\text{NOH} \xrightarrow{\text{NH}_2\text{Cl, -H}_2\text{O, -HCl}} \text{N}_2
\]

Scheme 3: Preparation of diazo moieties from nitrogen containing precursors containing a nitrogen atom bound to the future carbon of the diazo carbon.

Alternatively, examples are known to have both nitrogen atoms of the subsequent diazo species present, prior to diazo-formation. In Scheme 4, \(N\)-acyl-\(N\)-nitrosoalkylamines are deacylated resulting in formation of the diazo moiety. \(^{10}\)

\[
\text{N}^+\text{O}^\ominus \xrightarrow{\ominus\text{OH, -H}_2\text{O, -RCOO}^\ominus} \text{N}_2
\]

Scheme 4: Deacylation of \(N\)-acyl-\(N\)-nitrosoalkylamines.
In the Bamford-Stevens reaction\(^{12}\), tosyl hydrazones 3 are treated with a strong base (i.e., NaOMe) to form a diazo intermediate that is stable enough to be isolated (Scheme 5). Once the diazo group has been formed, the succeeding reaction is strongly influence by the choice of solvent. In proic solvents, loss of \(N_2\) results in the formation of a solvent stabilized carbocation, while in aprotic solvents, loss of \(N_2\) results in the formation of a carbene intermediate followed by a hydride shift, both reactions ultimately producing an alkene.

**Scheme 5: Bamford-Stevens reaction mechanism.**

### 2.2 Diazo-Transfer Reagents

Historically, diazo-containing substrates could only be prepared using the techniques described above (i.e., choosing substrates already containing one or both of the impending diazo-nitrogen atoms). This lack of general methodology to easily produce
a diazo-substrate drastically reduces the scope of diazo-decomposition reactions. In order to make this methodology available for many types of substrates, new procedures continually need to be developed. The design and synthesis of reagents containing an azide along with a good leaving group were developed as “diazo-transfer reagents”, so named because of their ability to transfer a diazo moiety to a desired substrate in exchange for H$_2$. The diazo-transfer reagents have dramatically increased the utility for this chemistry. A number of different diazo-transfer reagents have been developed (Figure 1). They vary in structure, reactivity, and most importantly, safety. The process of diazo-transfer involves the anionic attack on a reagent containing a $[\text{N}_2]^+$ moiety and a leaving group (Scheme 6).$^{13,14}$

![Figure 1: Diazo transfer reagents.](image-url)
Scheme 6: Generalized diazo transfer mechanism.\textsuperscript{14}

Diazo-transfer reagents that have found success in transfer reactions with activated methylene compounds (i.e., β-ketoesters) include \textit{p}-toluene sulfonyl azide (tosyl azide) \textsuperscript{5}, though purification issues have led to the popularity of other similar diazo-transfer reagents.\textsuperscript{15–17} Tosyl azide \textsuperscript{5} is hazardous because of its high specific heat of decomposition and high shock sensitivity.\textsuperscript{13} The reagent works well with β-ketoesters and formyl ketones to form different diazocarbonyl species.\textsuperscript{18} Methanesulfonyl azide (mesyl azide)\textsuperscript{19} \textsuperscript{4} is, in general, a superior transfer reagent because the sulfonamide byproduct is easily removed by washing with 10\% NaOH (aq).\textsuperscript{18} Additionally, a safe diazo-transfer reagent is \textit{p}-dodecylbenzenesulfonyl azide (\textit{p}-DBSA) \textsuperscript{6}. Owing to its low specific heat of decomposition, high initiation temperature and extremely high impact sensitivity, the dodecyl derivative has found extensive use as a transfer reagent.\textsuperscript{13} This reagent is valuable for preparation of crystalline diazo compounds, while generating noncrystalline sulfonamide byproducts.\textsuperscript{20} However, the high cost of this reagent and lower reactivity prevents its widespread use.
The most widely used commercially available diazo-transfer reagent, \(p\)-acetamidobenzenesulfonyl azide \((p\text{-}ABSA)\) 7 is generally viewed as the best general use transfer reagents. Though the reaction times are often longer than those of other diazo transfer reagents, its level of safety and ease of use, including facile product separation, make it one of the most useful diazo-transfer reagents that has been developed.\(^{13}\)

A more detailed representation of the diazo-transfer mechanism is shown in Scheme 7. An activated methylene compound 8 is treated with a base forming enolate ion 9, which attacks the electrophilic nitrogen atom of the diazo-transfer reagent, followed by proton transfer resulting in the formation of the diazo-containing substrate 10 and the sulfonamide byproduct 11.

Scheme 7: Diazo-transfer mechanism.

The overall stability of the diazo-containing substrate greatly affects the reactivity of all subsequent reactions involving the transition metal catalyst (Figure 2).\(^{13}\) The presence of electron withdrawing groups that are \(\alpha\) (at a carbon adjacent to the diazo group) to the diazo-moiety helps stabilize the substrate. Substrates stabilized by electron withdrawing groups in this manner are easier to prepare than their less stable counterparts. Substrates which include electron donating groups \(\alpha\) to the diazo-moiety are more reactive, and are usually more difficult to prepare. Compounds in which carbonyl
groups flank the diazo moiety are the most stable. This stabilization is due to the
delocalization of electrons through resonance (Scheme 8).

Figure 2: Reactivity and selectivity trends of diazo substrates.

Compounds containing one flanking carbonyl group are less stable than two
because the negative charge can only be delocalized with one oxygen atom. Diazo
compounds with esters are generally more stable than their ketone counterparts.
Diazoacetaamides are more stable than diazoesters, but less stable than dicarbonyl
groups. Like all substrates, the stability and reactivity have an inverse relationship.
Because of this trend, diazoacetoacetates and diazomalonates require higher reaction
temperatures, while diazoacetates can undergo diazo decomposition at or below room
temperature.
Scheme 8: Resonance stabilization of diazo-dicarbonyl compounds.

2.3 Carbenes and Carbenoids

Carbenes can be synthetically useful intermediates in organic synthesis as a method for various carbon-carbon (C-C) and carbon-heteroatom (C-X) bond formation. Chemists have experimented with carbenes since the 1950s. The term “carbene” was coined by Doering, Winstein, and Woodward in 1951 to describe a highly reactive divalent carbon intermediate, in which a carbon is covalently linked to two adjacent groups and also contains two nonbonding electrons. Electrons with antiparallel spins (i.e., the singlet state) are characterized by the unshared pair of electrons residing in the $\sigma$ orbital leaving an empty $p$ orbital. Electrons with parallel spins (i.e., the triplet state) have one electron in the $\sigma$ orbital and one electron in the $p$ orbital (Figure 3) and acts as a diradical species. The singlet carbene can be either electrophilic or nucleophilic in character, depending on the electron withdrawing or donating ability of groups attached to the carbene carbon. Substituents that display electron donating characteristics (e.g., amines) cause the carbene to be nucleophilic in nature. $\pi$-Donation of the lone pair of electrons on the substituent help stabilize the empty orbital of the carbene. On the other hand, substituents less able to participate in resonance stabilization (e.g., alkyl groups) cause the carbene to be more electrophilic, and therefore more reactive.
This neutral, six-electron carbon species can be problematic to generate, requiring harsh conditions that may include high heat, light or use of a strong base (e.g., \(n\text{BuLi}\)). Unfortunately, simple non-stabilized carbenes are highly reactive and display low selectively, drastically limiting their usefulness in organic synthesis. There are three distinct classes of divalent carbons, including free carbenes, stable metal carbenes, and reactive transition metal carbene complexes (Scheme 4). Free carbenes can be generated through several methods, including the photolysis of diazoalkanes, ketenes, diazirines, arylcyclopropanes, or oxiranes. The treatment of alkyl halides with strong bases, such as \(\text{CHCl}_3\) treated with \(\text{NaOH}\) for example, affords dichlorocarbene. Because of the problems associated with free carbenes, including a lack of selective and high reactivity, decomposition reactions involving transition metal complexes were explored as an alternative. It was found that the overarching problems associated with free carbenes could be significantly reduced utilizing various transition metals.
The “carbenoid” is a term coined to describe the functional group reactively similar to the carbene, but is stabilized by the addition of a transition metal. This stability is caused by the donating of $\sigma$-electrons of the filled lone pair orbital of the carbene atom to an empty metal $d$-orbital and the $\pi$-back-bonding of a filled metal $d$-orbital to the empty $p$ orbital on the carbon (Figure 5).

The carbenoid species is structurally related to singlet carbenes having similar reactivity and is produced from the decomposition of diazo-compounds in the presence of a late stage transition metal. Carbenoid intermediates are more stable than simple carbene intermediates and therefore easier to generate and show greater usefulness in organic synthesis than carbenes. Interaction with the transition metal lowers the reactivity
of the carbene while increasing selectivity. The d-orbital electrons associated with the transition metal reduce the electron deficiency of the carbenoid carbon through π-back-bonding. The generation of carbenoid intermediates via a transition metal has been explored in depth. Transition metals such as copper, rhodium, ruthenium, and palladium have been utilized in these reactions to form what is known as Fischer carbenoids (Figure 4). Fischer carbenoids occur in the singlet state and are electrophilic in nature at the carbenoid carbon. These reactive intermediates are more synthetically useful than the stable carbenoid intermediates (e.g., those derived from tungsten, chromium, molybdenum, and iron). These late transition metals form reactive species that are highly valuable in organic synthesis.

2.4 Rhodium Catalysts

The carbenoid substrate is generated through catalytic addition of a late stage transition metal such as copper, cobalt, iron, palladium, rhodium, ruthenium, gold, osmium, iridium, nickel, or silver to a diazo-containing substrate. The most effective catalyst for diazo-decomposition reactions are those prepared with rhodium(II). A preference for rhodium(II) arises owing to the ease of ligand substitution that can occur to provide a wide array of catalysts with different selectivity and reactivity. Two specific types of rhodium catalysts have been utilized for this reaction, rhodium(II) carboxylates and rhodium(II) carboxamidates (Figure 6), in which the ligands can be tailored to change the reactivity, diastereoselectivity, and enantioselectivity of the reaction. The carbenoid complex is formed from the attack of a diazo containing carbon on the open
coordination site of the metal catalyst.\cite{29} This addition is followed by the loss of N$_2$ forming the metal stabilized carbene.

Rhodium(II) carboxylates are $D_{4h}$ symmetric compounds with bridging ligands in four equatorial sites on each rhodium atom. Two remaining axial sites are vacant coordination sites, usually occupied by solvent in its native state (Figure 7). Several examples of carboxylate ligand catalyst are shown in Figure 8.

![Figure 6: General structure of rhodium(II) carboxylates and rhodium(II) carboxamidates.](image)

![Figure 7: General structure of a rhodium(II) catalyst, indicating the open coordination site for carbene binding.](image)
Rhodium(II) carboxylates are a $d^7$ complex and diamagnetic, which is attributed to the metal-metal bond. This class of catalysts are air-stable, making them easier to handle than the more active copper(I) catalysts previously used. All rhodium(II) carboxylates participate in back-bonding, a process in which the electrons in the $d$-orbital of the metal are transferred to a ligand’s $\pi^*$ antibonding orbital. Back-bonding results in a conformation in which the carbene eclipses two of the carboxylate ligands (Figure 9).

Figure 8: Rhodium(II) carboxylate examples.

Figure 9: Eclipsed conformation of carbene and carboxylate ligands resulting from $\pi$-back bonding.
One of the most widely used rhodium(II) carboxylate catalyst, rhodium(II) tetraacetate (Rh2(OAc)4) 12 (Figure 10) was first prepared and characterized in the 1960s. In 1973, Teyssié found it to be an exceptionally efficient catalyst for a number of different diazo-decomposition reaction types. The structure has a circular shape, resembling a “paddlewheel”. Looking down the Rh-Rh bond, the structure resembles a lantern with an electron deficient center and electron rich circumference. The acetate ligands can be easily manipulated to other carboxylates, amidates, sulfates, or phosphates by a simple ligand exchange or direct synthesis from RhCl₃ under acidic conditions.

![Figure 10: Rhodium(II) tetraacetate (12).](image)

Rhodium(II) tetratriphenylacetate (Rh₂tpa₄) 13 is an extremely efficient catalyst for C-H insertion reactions with α-diazo-β-keto esters and various C-H bonds. The catalyst is characterized by the steric bulk of the ligands, which affords high selectivity and regiocontrol. In the example shown in Scheme 9, Ikegami and co-workers demonstrate the intramolecular C-H insertions to produce corresponding bicyclic products. Here, formation of the linear five-membered ring product is the only one
observed. The steric bulk of the bridging ligands prevents the formation of the spirocyclic compound.

Scheme 9: Intramolecular C-H insertion reaction via Rh$_2$tpa$_4$.

The bond length between the two rhodium atoms of the catalyst can serve as a measure of reactivity of a particular complex. Predicted values range from 2.37-2.44 Å. When the measured bond length decreases, the reactivity decreases. This phenomenon can be witnessed with strongly electron-donating ligands, such as the ones found in carboxamidates.

Evidence of π-back-bonding was discovered by measuring the Rh-Rh (of the catalyst) and Rh-C (of the carbenoid) bond lengths. In the presence of π-back-bonding ligands, the Rh-Rh and Rh-C bond lengths both shorten. The Rh-Rh bond length greatly varies depending on the presence and identity of axial ligands and bridging groups associated with the rhodium atoms. In several examples, different bridging ligands of rhodium(II) catalysts are compared (Figure 11). In all cases, the axial ligand is H$_2$O. In carbenoid complexes, crystal-field theory suggests π-back-bonding is possible between the LUMO of the carbene and the filled metal π* orbital, that arise from the antibonding overlap of the two Rh d$_{xz}$ (or Rh d$_{yz}$) orbitals. The degree to which back-bonding is
present is influenced by the electronic nature of the ligands (carboxylate vs. carboxamidate) and the π-acceptor and σ-donor capabilities of the carbenoid carbon. These competing effects influence the ability of the carbene to accept electron density from the Rh- \( \pi^* \) orbital and donate electron density into the Rh- \( \sigma^* \) orbital.

<table>
<thead>
<tr>
<th>t-BuCO₂</th>
<th>HCO₂</th>
<th>AcO</th>
<th>CF₃CO₂</th>
<th>H₂PO₄</th>
<th>AcS</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.37</td>
<td>2.38</td>
<td>2.39</td>
<td>2.41</td>
<td>2.49</td>
<td>2.55</td>
</tr>
</tbody>
</table>

Figure 11: Differences in Rh-Rh bond length with varying bridging ligands.²⁸

Doyle proposed a carbenoid intermediate without π-back-bonding in which the Rh-C possess a formal single bond based on molecular modeling studies of chiral rhodium-carboxamidate complexes.³⁶ In this model, the electrophilic carbon resembles a carbocation, rather than an alkylidene carbon of a metal carbenoid-complex.

Ligand substitution of the carboxylates bound to rhodium(II) effect the electrophilicity of the carbene and control of reaction selectivity.³⁶,³⁷ This effect was first recognized by Drago by comparing association complexes of rhodium(II) butyrate (Bu) and perfluorobutyrate (pfb) with Lewis bases (e.g., pyridine, acetonitrile) and attributed this phenomenon to the π-backbonding of the rhodium(II) carboxylates into π-acceptor ligands.³⁸,³⁹

Rhodium(II) carboxamidates are characterized by a bridge structure featuring two oxygen atoms and two nitrogen atoms bound to each rhodium, in which the two nitrogen atoms are adjacent to each other. They exhibit \( C_2 \) symmetry. Several examples are shown.
in Figure 12. The carboxamidates show lower reactivity along with greater selectivity than the corresponding carboxylate catalysts.\textsuperscript{13} The high selectivity is attributed to the electron donating nature of the bridging acetamidate ligand. $\text{Rh}_2(\text{acam})_4$ \textbf{17} was first prepared by Bear in 1986.\textsuperscript{40} Rhodium(II) caprolactam \textbf{18} is especially effective for cyclopropanation reactions.\textsuperscript{37}

\begin{center}
\textbf{Rhodium(II) carboxamidates}
\end{center}

\begin{center}
\begin{align*}
\text{Rh}_2(\text{acam})_4 & (\textbf{17}) \\
\text{Rh}_2(\text{cap})_4 & (\textbf{18}) \\
\text{Rh}_2(\text{tfacam})_4 & (\textbf{19})
\end{align*}
\end{center}

Figure 12: Rhodium(II) carboxamidate examples.

Doyle and co-workers developed several chiral rhodium(II) carboxamidates using ligands such as 2-oxopyrrolidine, 2-oxazolidinone, $N$-acylimidazolidin-2-one, and 2-azetidinone.\textsuperscript{41,42}

By decreasing the electron withdrawing power of the ligands, the reactivity is decreased, but selectivity is increased (Figure 13).\textsuperscript{36} With increased electron withdrawal by the ligands, the carbenoid intermediate generated undergoes bond formation with the substrate through an earlier transition state, reducing the selectivity. $\text{Rh}_2(\text{pfb})_4$ \textbf{14} is the most reactive catalyst shown, due to the electron withdrawing nature of the bridging
ligands. The presence of multiple electronegative C-F bonds makes the catalyst more electrophilic, and therefore more reactive. At the other end of the reactivity spectrum, \( \text{Rh}_2(\text{S-DOSP})_4 \) 20 is the most selective catalyst listed. The electron donating ability of the bridging atoms causes the catalyst to be less electrophilic, and therefore more selective.

![Increased Selectivity and Reactivity](image)

Figure 13: Trends in selectivity and reactivity of rhodium (II) carboxylates.

To demonstrate this reactivity trend, in the scheme below, the example provides two possible C-H insertion sites, one being a CH\(_3\) (1\(^{\circ}\)) or a C-H insertion at a C\(_3\)H (3\(^{\circ}\)). Generation of the rhodium carbenoid with \( \text{Rh}_2(\text{pfb})_4 \) 14, the most electron withdrawing catalyst tested, provided 21:22 in a chemical ratio of 39:61 showing a preference of 3\(^{\circ}\):1\(^{\circ}\) in the observed insertion. Use of a catalyst with less electron withdrawing ability than the \( \text{Rh}_2(\text{pfb})_4 \) 14, the preference is reversed. Testing electron donating ligands such as \( \text{Rh}_2(\text{OAc})_4 \) 12 and \( \text{Rh}_2(\text{acam})_4 \) 17 found the observed ratio for 3\(^{\circ}\):1\(^{\circ}\) to be 90:10 and 99:1, respectively. That result is a 13-fold increase in reactivity for tertiary to primary (beyond
a statistical preference) for the acetate ligand and a 150-fold difference for acetamide (Scheme 10).\textsuperscript{36}

![Scheme 10: Reaction diazo compound comparing tertiary and primary insertion preference.](image)

McKervey and co-workers developed rhodium(II) catalysts that possess chiral carboxylate ligands. They were the first to refer to the catalyst as “homochiral” so named because the ligands, not the rhodium(II) center, display asymmetry.\textsuperscript{43} Using catalyst \textbf{23} (Z = H, t-Bu) (Figure 14), high enantiocontrol is achieved for intermolecular\textsuperscript{44–46} and intramolecular\textsuperscript{47} cyclopropanation and C-H insertion reactions in methodology developed by McKervey, Davies, Corey, and Doyle. Other chiral catalysts developed by Ikekami and Hashimoto have been successfully applied to C-H insertion and aromatic substitution reactions.\textsuperscript{48–50}
Davies used rhodium(II) $\text{(S)-N-}[p-(\text{dodecylphenyl})\text{sulfonyl}]\text{prolinate}$ to catalyze asymmetric Si-H insertion reactions forming benzylsilanes and allylsilanes in 77-95% ee (Scheme 11). These reactions are run at low temperatures and in nonpolar solvents with high levels of success.

Scheme 11: Davies’ use of chiral rhodium(II) catalysts for asymmetric Si-H insertion reactions.
2.5 Diazo Decomposition Reactions

Once created, the diazo-substrate can participate in a multitude of reactions including carbon and heteroatom insertion reactions, coupling, cyclization, and ylide formation. In an early example of insertion reactions, Yates used copper to prepare the copper carbenoid, while using phenol, thiolphenol, and aniline as nucleophiles in the X-H insertion reaction (Scheme 12).\(^\text{52}\)

\[
\begin{align*}
\text{R} & \text{C}=\text{N} \quad \xrightarrow{\text{Cu}} \quad \text{R} \quad \text{C}=\text{N} \\
\text{Nu} & \quad \text{Nu} \\
\text{Nu} = & \text{PhOH, PhSH, PhNH}_2
\end{align*}
\]

Scheme 12: Yates’ copper catalyzed decomposition of diazoketones.

The first example of rhodium carbenoid generation \textit{via} diazo decomposition was published in 1973 (Scheme 13).\(^\text{33}\) Testing various rhodium complexes such as Rh\(_2\)(OAc)\(_4\), RhCl\(_3\) \cdot H\(_2\)O, and RhCl(PPh\(_3\))\(_3\), Reimlinger and co-workers experimented with an insertion reaction with hydroxylic bonds with great success. Rh\(_2\)(OAc)\(_4\) was found overwhelmingly to be the most efficient catalyst, even at very low molar concentration, providing excellent yields of product at room temperature.
A few years later, Hubert and co-workers reported using rhodium(II) carboxylates as catalysts for carbenoid formation followed by the cyclopropanation of olefins in excellent yields.\textsuperscript{34} In 1980, Noels and co-workers surveyed a number of catalysts that are proficient in metal-carbenoid reaction.\textsuperscript{53} Copper triflate had been used previously with mixed results. Investigating alternative group 8 metals found more effective candidates for use as catalysts, including those containing rhodium(II) metals. Cyclopropanation of various dienes and trienes made use of this metal-carbenoid reaction (Scheme 14). Noels compared rhodium(II), palladium(II), and copper(II) catalysts and found differences in the resulting regiochemistry of the reaction and discovered rhodium(II) was normally more efficient than either palladium or copper in both product yield and selectivity.\textsuperscript{54} High levels of regioselectivity were due to the low reactivity of the halogenated double bond resulting from the deactivating effect of the chlorine atoms.

Scheme 13: Insertion into hydroxylic bonds using diazocompounds by Reimlinger.\textsuperscript{33}
Scheme 14: Cyclopropanation of dienes via transition metal catalyzed reaction of diazoesters by Noels and co-workers.\textsuperscript{53}

The mechanism of diazo decomposition was first proposed in 1952 by Yates.\textsuperscript{52} It describes the presence of a carbene carbon bound to a transition metal and is generally accepted as the correct mechanistic interpretation. The catalytic formation of the metal-carbenoid species is thought to be accomplished \textit{via} the mechanism shown in Scheme 15.\textsuperscript{55} The nucleophilic diazo-carbon 25 attacks the electrophilic metal center 26 to initiate the metal-carbene formation. Electrophilic addition of the diazo-substrate and the transition metal complex followed by the loss of dinitrogen, produces the metal-stabilized carbene 28. The electrophilic carbene is transferred to an electron-rich substrate (S\(_\text{e}^{-}\)) and the catalyst 26 is regenerated, completing the catalytic cycle. The formal metal carbene 28 has a metal-stabilized carbocation resonance form 29, which helps to explain the electrophilic nature of this intermediate.
2.6 1,3-Dipolar Cycloaddition Reactions

Cyclic ethers are present in many naturally occurring compounds, including ionophores\textsuperscript{56}, brevetoxins\textsuperscript{57}, and others (Figure 15).\textsuperscript{58–60} Efficient methods to synthesize this class of compounds is essential for developing a synthetic strategy toward a natural product or analog. Using transition metal catalyzed 1,3-dipolar cycloaddition reactions of diazocarbonyls provides one such route.\textsuperscript{61} The first example dates back to the 1890s, when Buchner and von Pechmann reported that ethyl diazoacetate and diazomethane reacted \textit{via} cycloaddition across carbon-carbon multiple bonds.\textsuperscript{62,63} Because the products formed from this reaction are often considerably more complex than the reactants, it has gained popularity in total synthesis.
These reactions require a 1,3-dipolar species, commonly identified as ylides. Their existence first suggested in 1965\textsuperscript{64}, ylides are formally neutral reactive intermediates containing formally charged anionic and cationic sites within the same structure. Usually the anionic moiety is generated from the carbene carbon, while the cation can be formed from nitrogen, oxygen, or sulfur acting as a Lewis base.\textsuperscript{65} Two such examples of ylides are found in Figure 16, a carbonyl ylide 30 and an azomethine ylide 31.
Once formed, these 1,3-dipolar species can undergo various types of reactions, including 1,3-dipolar cycloaddition, [2,3]-sigmatropic rearrangement reactions of allyl-substituted ylide intermediates, β-hydride elimination and a [1,2]-insertion reaction, or Stevens rearrangement. This reaction involves the insertion of a diazocarbonyl carbon with a tertiary amine to produce an ammonium ylide, which then rearranges via a [1,2]-sigmatropic shift (Scheme 16).\textsuperscript{66}

\begin{center}
\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure16.png}
\caption{General structures of carbonyl and azomethine ylides.}
\end{figure}
\end{center}

\begin{center}
\begin{figure}
\centering
\includegraphics[width=\textwidth]{scheme16.png}
\caption{Formation of cyclic amine via Stevens rearrangement.}
\end{figure}
\end{center}

In an interesting example of the utility of the ylide intermediates, West developed an approach to polycyclic ethers in which different catalysts can produce both the [2,3]-sigmatropic rearrangement product and the C-H insertion product (Scheme 17).\textsuperscript{67} When a copper catalyst is employed (i.e., Cu(tfacac)\textsubscript{2} or Cu(hfacac)\textsubscript{2}), the product of a [2,3]-sigmatropic shift is observed in high yield, with Cu(tfacac)\textsubscript{2} yielding a diastereomeric
mixture ratio of 93:2. The ratio decreases to 72:14 with Cu(hfacac)$_2$ is used. Using the catalyst Rh$_2$(OAc)$_4$ the preference favors the insertion product.

Scheme 17: West’s approach to polycyclic ethers via oxonium ylides.$^{68}$

Carbonyl ylides can also be used to participate in intramolecular cycloaddition reactions to generate epoxides. These epoxides are in equilibrium, and often are seen as precursors to carbonyl ylides, and form substituted ethers via concerted rearrangements and internal proton transfers (Figure 17).$^5$
The reaction of ylides commences with the diazo-compound reacting with the transition metal catalyst to form an electrophilic carbene complex. Addition of the nucleophile induces the dissociation of the catalytic active metal species and ylide.\textsuperscript{55}

Ylides can be used as the dipolar intermediate in a dipolar cycloaddition reaction. Intramolecular carbonyl ylide formation, followed by cycloaddition, was first described by Ibata and co-workers in 1986 (Scheme 18).\textsuperscript{69,70} Once formed, the carbonyl ylide \textsuperscript{35} can be trapped by a variety of dipolarophiles, including alkenes \textsuperscript{36} or alkynes \textsuperscript{37}, in a 1,3-dipolar cycloaddition reactions, similar to Diels-Alder reaction ([3\pi+2\pi] vs. [4\pi +2\pi]), that lead to oxacycle formation (Scheme 19).\textsuperscript{13} Cycloaddition reactions of this variety involve 2\pi electrons of the dipolarophile and 4 electrons of the dipole (a pair of \pi electrons and a pair of nonbonding electrons from the heteroatom of the 1,3-dipole) in a concerted manner to form a five-membered heterocyclic compounds.
Scheme 18: Ibata’s cycloaddition chemistry with carbonyl ylides.

Scheme 19: General 1,3-dipolar cycloaddition mechanism via a carbonyl ylide.

In the intramolecular cycloaddition reaction, the formation of five- and six-membered rings is favored, but examples of seven-membered rings are also preceded. Trapping the ylide depends on both the substrate structure and presence of alternative competing intramolecular pathways. This method has been utilized in the synthesis of several sesquiterpenes, including (±)-illudin M and pterosin.
H 41, I 40, and Z 39\textsuperscript{74,75} (Scheme 20). Utilizing an intermolecular pathway and an azomethine ylide, spirotryprostatin B 43 was also prepared in this manner (Scheme 21)\textsuperscript{76}.

Scheme 20: Retrosynthesis of pterosin H, I, and Z, and illudin M, both formed through 1,3-dipolar cycloaddition.
Scheme 21: Synthesis of spirotryprostatin B via 1,3-dipolar cycloaddition reaction with an azomethine ylide.

Similar to the Diels-Alder reaction, the dipolar cycloaddition can proceed either through an endo or exo transition state. The diastereoselectivity is influenced by both secondary orbital interactions (similar to those found in the Diels-Alder reaction) and the repulsive steric interaction. \(^{61}\) In the reaction between N-methyl nitrene 44 and the dipolarophile acrylonitrile 45 there is observed a preference for the trans product 46 over the cis (77%: 23%). \(^{77}\) The preference can be explained by the favorable secondary orbital interaction in the endo transition state (Figure 18).
Substituents bound to the dipolarophile can greatly affect selectivity and reactivity. Electron withdrawing groups (EWG) (i.e., electrophilic dipolarophiles) have lower Lowest Unoccupied Molecular Orbital (LUMO) values and normally favor an orbital interaction of the LUMO of the dipolarophile and the Highest Occupied Molecular Orbital (HOMO) of the dipole. Conversely, electron donating groups (EDG) (i.e., nucleophilic dipolarophiles) have higher HOMO values and favor interaction between the LUMO of the dipole and the HOMO of the dipolarophile.  

1,3-Dipolar cycloadditions are highly regioselective, as seen in Scheme 22. The reaction between phenyl azide 47 and methyl acrylate 48 forms mainly 1-phenyl-4-carboxylic ester 49 caused from interaction between the HOMO of the dipole and the LUMO of the dipolarophile. Conversely, the reaction between phenyl azide 47 and 1-
hexene 50 mainly produces 1-phenyl-5-butyltriazole 51 due to the interaction between the LUMO of the dipole and the HOMO of the dipolarophile.  

Scheme 22: Example of regioselectivity of 1,3-dipolar cycloadditions.

2.7 Phorbol Esters

Phorbol belongs to the family of tigliane diterpenes, found in several plants including *Croton tiglium* L, *Sapium indicum*, and *Jatropha curcas*. Once extracted, the naturally occurring phorbol esters are unstable and prone to degradation (e.g., susceptible to oxidation, hydrolysis, transesterification, and epimerization) making the efficient synthesis of these compounds especially important. Phorbol esters are polycyclic compounds containing two hydroxyl groups which are esterified to form fatty acids. Biologically active members of the phorbol family are amphiphilic, having both hydrophilic (affinity for water/polar molecules) and lipophilic (affinity for fat/nonpolar
molecules) properties, and have a tendency to bind to phospholipid membrane receptors without significant interaction with the hydrocarbon core.\textsuperscript{81,83} The binding to these receptors leads to initial membrane effects including modification in cell receptor activities, altered cell adhesion, inhibition of binding of epidermal growth factor to cell surface receptors, and alterations in membranes (including increased membrane fluidity, changed cell surface morphology and cellular adhesion).

Tigliane diterpenes have a core scaffold that are comprised of four linearly fused rings (designated A-D), encompassing a 5-membered ring A, which is stereochemically linked in trans conformation, fused to a 7-member ring B. The 6-member ring C is cis fused to the 3-member ring D. The related daphnane ring system differs only in bond scission of ring D, providing an isopropyl group, rather than the cyclopropane (Figure 19).\textsuperscript{81,84}

![Tigliane and Daphnane Ring Systems](image)

Figure 19: General structure of tigliane and daphnane ring systems.

Phorbol esters are known to activate protein kinase C (PKC) isozymes, which are involved in a number of growth factor-dependent cellular responses.\textsuperscript{85} Protein kinases include all enzymes of the human body that catalyze phosphorylation (or the transfer of a
phosphate group) of a specific substrate from a high energy molecule (e.g., adenosine triphosphate (ATP)). There are more than 518 different protein kinases in the human body and they are divided into different families depending on the selectivity for those substrates. Amino acids serine, threonine, and tyrosine all contain hydroxyl groups available for phosphorylation. Serine/threonine kinases will phosphorylate a serine or threonine amino acid, while tyrosine kinases will phosphorylate a tyrosine amino acid. Phorbol esters will intervene in the phosphorylation process, thus altering the phosphorylation of some cellular targets.

The family of PKCs are serine/threonine kinases involved in the induction of cell differentiation, regulation of apoptosis, and inhibition of tumor invasion and other physiological processes. PKC’s carboxylic-terminal catalytic site contains an ATP-binding site, while the regulatory domain at the amino terminus available for phorbol binding. Molecular modeling of bound phorbol ester/PKC complexes suggests the molecular basis of action is due to the amphiphilic properties of phorbol. The hydrophilic moiety binds to PKC through a network of hydrogen bonding through the oxygen atoms at the 20-, 4- and 3-positions with three residues of PKC (Figure 20). The hydrophobic ester chain at C-12 helps to keep the complex in the cell membrane where PKC is activated. It is thought that phorbol ester converts PKC into a constitutively active form that is irreversibly inserted into the membrane.
The normal activation process of PKC arises from the rapid degradation of phosphatidylinositol bisphosphate (PIP$_2$) to inositol triphosphate (IP$_3$) and diacylglycerol (DAG) by anti-immunoglobulin antibodies (anti-Ig). DAG remains in the cell membrane and activates PKC. PIP$_2$ degradation is usually associated with an increase in Ca$^{2+}$ concentration. This increase is mediated by the formation of IP$_3$, which upon formation, enters the cytoplasm and activates IP$_3$ receptors on smooth endoplasmic reticulum (ER) which opens Ca$^{2+}$ channels. After activation, PKC is translocated to the plasma membrane by RACK (receptor for activated C-kinase) proteins, where it participates in various other signal transduction pathways.

Phorbol esters function as analogues of the endogenous activator, DAG. As such, the two compounds competitively bind at the same sites. DAGs and phorbol esters regulate PKC by the same mechanism, with the major difference between the two compounds being the strength of interaction. DAGs are metabolized within minutes, while phorbol esters have a much longer life in the cell. Additionally, when the enzyme is activated by DAG it is rapidly hydrolyzed, unlike phorbol esters which bind irreversibly with the active form in the cell membrane. PKC has a 250-fold increase in affinity for...
phorbol esters over DAG. Because phorbol esters are better pharmacological agents than DAG, they are considered useful tools for identifying the physiological systems in which PKC is involved.  

Given the role in which PKC plays in the signal transduction pathway, activators of PKC are being explored as potential targets for the pharmaceutical inhibition of cancer, cardiovascular disease, renal disease, immunosuppression, and autoimmune disease. Phorbol and its derivatives have been used extensively in medical research due to their wide range of biological effects. For example, the most active phorbol ester, 4-β-13-O-tetradecanolyphorbol-13-acetate (TPA), is a tumor promoting compound, making it a valuable tool for oncologic studies (Figure 21). The cocarcinogenic properties of phorbol esters are due to its interaction with PKC, which regulates the signaling pathways to induce gene transcription, leading to the induction of cell proliferation, thus amplifying the efficacy of known carcinogens. TPA (and other tumor promoting phorbol esters) suppress the release of inositol phosphates by inhibiting the hydrolysis of PIP2 preventing the formation of IP3 and the mobilization of Ca²⁺ from the ER.

TPA was assayed for HIV-1 inhibition, having an IC_{100} = 0.48 ng/mL, but also activated PKC (100% activated at 10 ng/mL). Another phorbol derivative, phorbol 12-acetate-13-decanoate (isophorbol) 53, showed anti-HIV-1 induced cytopathic effects (CPE; the structural changes in host cells caused by viral infection) activity with an IC_{100} of 7.6 ng/mL without also activating PKC. It also did not influence the cell growth or viability of the MT-4 cells at this concentration. The two compounds’ structure-activity
relationships were evaluated for the inhibition of HIV-1-induced CPE on MT-4 cells and their activation of PKC. A series of derivatives were prepared and assayed, with the following conclusions: (1) compound derivatives with trans-fused A/B configuration had higher activity, (2) acetylation of hydroxyl group at C-20, methylation of the alcohol at C-4, or reduction of carbonyl group at C-3 significantly reduced the inhibition of CPE and activation of PKC and (3) isophorbol sees a significant reduction in inhibition depending on either an increase or decrease in the length of the fatty acid chain. Based on those results, further study of phorbol derivatives with anti-HIV (and without activating PKC) should be pursued. The phorbol ester prostratin activates latent HIV, which in combination with highly active antiretroviral therapy (HAART) could possibly eliminate HIV reservoirs of nonreplicating virus and eradicate HIV.  

![Figure 21: The phorbol esters 4β-12-O-tetradecanoylphorbol-13-acetate (TPA) and prostratin.](image)
TPA 52 has a dramatic effect on cell differentiation. Weinstein reported that while it does not involve covalent bonding to cellular DNA, it does mimic the effects of transformation, a process in which foreign DNA is introduced to the cell.\textsuperscript{92} These effects include alteration in membrane morphology, increased saturation density, altered cell surface fucose glycopeptides, increased transport of deoxyglucose and increased levels of plasminogen activator and ornithine decarboxylase.

Varying the functional groups of phorbol derivatives shows marked difference in activity. While exploring uses for phorbol esters in anticancer therapies, Wada found that modifying the hydrophobicity of the 12-ester chain greatly affects the stability of the phorbol ester-PKC membrane complex (Figure 22).\textsuperscript{88} Because PKC activation requires interaction with the cell membrane, modifying the 12-ester chain to a hydrophilic moiety and decreasing the affinity of phorbol-PKC complex and the cell membrane, the new derivative becomes a competitive inhibitor of PKC activation by an agonist (e.g., TPA 52).
Krauter found that both the oxygen at C-20 and the O-acyl group at C-13 were necessary to the tumor-promoting bioactivities of phorbol esters. They also found that reduction of the oxygen carbonyl at C-3 to the alcohol did not affect the bioactivity.

It has been reported that some derivatives of phorbol esters show significant anticancer activity. This somewhat surprising deviation from the cocarcinogenic properties of many phorbol esters is most likely due to PKC’s role in many diverse metabolic pathways, cellular localization, phosphorylation, interaction with other signal molecules, and accessibility to different substrates. Several compounds derived from 12-deoxyphorbol demonstrated antileukemic activity with ED$_{50}$ values in the range of 0.4 – 3.4 µg/mL in a P-388 lymphocytic leukemia system in vitro (Figure 23). 55, containing a long acyl chain at C-13 and a free hydroxyl group at C-20, is especially potent. When assayed for cytotoxicity against Ramos B cell lines it had an IC$_{50}$ value of 0.0051

Figure 22: Wada’s modification of 12-ester chain from a hydrophobic moiety to a hydrophilic moiety.
µg/mL. 99 However, when the oxygen atom at C-20 is an aldehyde, the compounds show little to no cytotoxicity toward several human cancer cell lines. In a somewhat unique phorbol derivative 58, isolated from *Euphorbia fischeriana*, a hydroxyl group is present at C-3 (Figure 24). 100 This compound showed no cytotoxicity against the human cancer cell lines, MDA-MB-231 and HepG2 and against one human immortalized cell line. Collectively, these results suggest the importance of the long saturated aliphatic chain at C-13, a carbonyl at C-3 and a free hydroxyl at C-20 to the activity of the derivatives.

![Figure 23: Several examples of 12-deoxyphorbol derivates showing antileukemic activity.](image)

Figure 23: Several examples of 12-deoxyphorbol derivates showing antileukemic activity.

![Figure 24: Phorbol Ester derivative.](image)

Figure 24: Phorbol Ester derivative.

There are relatively few examples of nitrogen containing phorbol analogs (azaphorbol, 59) in the literature (Figure 25). The alkaloid cephalotaxine 60, comprised
of a 5-7-6 ring system, exhibits antileukemic activity, suggesting the incorporation of a nitrogen atom into the ring system could present interesting biological activity.\textsuperscript{84,101}

![Structure of azaphorbol and cephalotaxine](image)

Figure 25: General structure of azaphorbol and cephalotaxine.

The related 5-7-6 daphnane skeleton has also exhibited biological activity. For example, gnidilatin \textbf{61} possesses antileukemic properties and resiniferatoxin \textbf{62} possesses analgesic properties (Figure 26). Aconitine also contains the 5-7-6 tricyclic core and has cardiotonic and sedative properties. It has been noted that because of the biological activity of both the daphnane skeleton and the nitrogen containing phorbols, the synthesis of aza-analogues would be beneficial.\textsuperscript{84}
Figure 26: Structures with daphnane skeleton that exhibit biological activity.

There have been several approaches to the phorbol core (Figure 27), including the first synthesis by Wender, using a oxidopyrylium alkene [5 + 2] cycloaddition reaction to generate the oxabicyclo[3.2.1]octane framework. McMills and Dauben both utilized a 1,3-dipolar cycloaddition reaction to create the bicycle[5,4,0]undecene core. Then, in 2001, Cha utilized a [4+3] oxyallyl cycloaddition reaction and subsequent intramolecular Heck reaction for the construction of the B and C ring system.\textsuperscript{102}
Wender’s synthesis in 1989, consisted of 52 steps resulting in a 0.16% chemical yield.\(^{103}\) He began by developing a synthetic route to the polycyclic phorbol skeletal structure that is a general precursor to phorbol esters, daphnane families, and the related ingenane families (Scheme 23).\(^{104}\) The synthesis commenced with a hetero-Diels-Alder reaction between 2-methoxybutadiene 63 and ethyl glyoxalate 64 to yield the pyran in 60% chemical yield. 65 was treated with LDA and 1-bromo-2,4-pentadiene to introduce a diene functionality. The resulting compound was reduced with LAH and the resulting alcohol protected with benzyl bromide to produce ether 66 (82%, 3 steps).
m-CPBA was used for selective oxidation to afford the epimeric alcohols at C-10. Swern oxidation produced the keto ketal 67. The crossed aldol condensation between acetaldehyde and enolate 67 was more difficult than anticipated. Addition of lithium bromide to the enolate solution gave the desired aldol product. The alcohol was dehydrated by sulfonylation, then eliminated with DBU to produce trienone 68 as a single isomer. Heating of the Diels-Alder precursor 68 produced the cycloadduct 70. Wender attributed the exo-selectivity to the steric congestion between the diene and the methoxy group, which arise in the endo transition state 69 (52% yield, 4 steps).

The installation of the A and D rings was executed next. 71 was produced via an olefination and hydrolysis sequence (91% yield), followed by a hetero-Diels-Alder reaction with the ketene acetal of ethyl acetate to give a single ortholactone. Sterically and stereoelectronically controlled protonation occurred to provide the keto ester 72 (72% yield, 2 steps). The D ring was constructed utilizing Seyferth’s reagent. The addition proceeded exclusively to the less hindered, convex face of the C ring, producing the gem-dibromocyclopropane 73 (92% yield). The kinetically controlled addition of cyanide to the sterically less hindered face of the ketone produced 74 (72% yield).

Reduction with DIBAL afforded both nitrile and ester functional groups. Swern oxidation produced the dialdehyde, which subsequently underwent base-catalyzed intramolecular aldol condensation (31.5%, 3 steps). 76 was produced through the sequential DIBAL reduction, higher order cuprate substitution on halide, and allylic transposition (73% yield, 4 steps).
Benzoyl protection of alcohol 76, followed by deprotection of the benzyl ether with ZnI$_2$ afforded the primary alcohol (64% yield, 2 steps). The alcohol was converted to the iodide 77 and then treated with $t$-butyllithium, resulting in the cleavage of the ether bridge at C-6, providing the tetracyclic compound 78 (51% yield, 3 steps). The alcohol was protected and the allylic position of the olefin of the B-ring was oxidized via SeO$_2$ produced allylic alcohol 79. Chloride 80 was produced by treatment of the alcohol with thionyl chloride in the presence of the acid scavenger, propylene oxide. 80 was treated with silver acetate and potassium acetate-TMEDA complex (50% yield, 4 steps). Hydrolysis of the protecting groups provided final compound 82, completing the first synthesis of the phorbol skeleton including introduction of stereochemistry.
Scheme 23: Wender’s synthesis of the phorbol skeleton.

Shortly after the Wender synthesis, Dauben and McMills simultaneously reported employing an 1,3-dipolar cycloaddition to form the B and C ring system via a rhodium-mediated metallocarbenoid species. McMills’s synthesis of the phorbol skeleton
began with the addition of the Grignard reagent derived from 6-bromo-1-hexene to cyclopentene-1-carboxaldehyde 83. The resulting alcohol was oxidized using Swern conditions to afford the enone 84. Nitrile 85 was produced via conjugate addition of cyanide, which was subjected to basic hydrolysis to afford acid 86. 86 was converted to the acid chloride by treatment with oxalyl chloride followed by immediate treatment with diazomethane to afford the diazoketone 87. Treatment with a catalytic amount of Rh₂(OAc)₄ produced the transient ylide 88, which participated in 1,3-dipolar cycloaddition to form the tetracyclic ether 89.

Scheme 24: McMills’ synthesis of the phorbol core.
2.8 Frondosin B

The frondosin family of natural products was first isolated in 1997 from the Micronesian sponge *Dysidea frondosa*. This family of sesquiterpene derivatives contain a scaffold based on a bicycle[5.4.0]undecene core. Frondosins B-E are an annulated hydroquinone derivative and frondosin A is a substituted dihydroquinone derivative (Figure 28). Like many compounds isolated from marine sponges, the frondosins were found to be biologically active. It was found that frondosin inhibited binding of interleukin-8 (IL-8) to its native receptor and inhibited PKC with an IC₅₀ = 4.8 μM. PKC inhibition is achieved through blocking of the catalytic site. Assays of various natural products that showed PKC inhibition include staurosporine (IC₅₀ = 2.7 nM), (Z)-axinohydantoin (IC₅₀ = 9 μM), and debromo-Z-axinohydantion (IC₅₀ = 22 μM). Kinase inhibitors of these general types are predicted to be widely explored in the pharmaceutical industry, with more than 130 kinase inhibitors reported to be in either phase I or phase II clinic trials as of 2011.
IL-8 is a cytokine, a small protein used in cell signaling, as well as a chemoattractant, inducing the recruitment and activation of neutrophils, and inducing phagocytosis. As a chemoattractant, IL-8 attracts and stimulates leukocytes at the site of inflammation, a major source of cartilage-degrading enzymes. It is also believed to play a role in pathogenesis of respiratory tract diseases. It is produced by human airway smooth-muscle cells and when produced, the airway is narrowed, making breathing more difficult. IL-8 is also linked to involvement in autoimmune disease including psoriasis and rheumatoid arthritis.

All members of the frondosin family show IL-8 antagonist activity, which inhibits the binding of the cytokine to its receptors, CXCR1 and CXCR2. These receptors are
found on neutrophils, basophils, lymphocytes, monocytes, keratinocytes, endothelial cells, as well as a variety of tumor cells. Because of this activity, the frodosins have been explored as potential leads for the treatment of inflammatory diseases. Also, elevated IL-8 levels are correlated to angiogenesis, tumor progression and metastasis in several cancers, including lung cancer, leading researchers to consider the frondosins as possible leads in various oncology studies. It is known that chemokines (including IL-8) function in a variety of biological processes, including HIV-1 infection by stimulating HIV-1 replication and monocyte adherence and attracting T cells, as well as its primary function as a chemoattractant for neutrophils. Therefore, it is not surprising that elevated levels of IL-8 have been found in serum and lungs of HIV-infection patients. When assayed for biological activity, it was discovered that frondosins A and D also showed anti-HIV properties at the low micromolar level. It has been revealed that blocking the actions of IL-8 with small molecule inhibitors of the IL-8 receptors also dramatically reduced HIV-1 replication.

There are several total syntheses of frondosin B, varying in synthetic design, number of steps, and total synthetic yield (Scheme 25). Both Danishefsky and Trauner used a similar benzofuran disconnection (C10-C11) in their approaches. Ovaska’s approach relied on a 5-exo-dig cyclization-Claisen rearrangement of optically active homopropargylic allyl alcohol. In an extremely efficient synthesis, MacMillian used an enantioselective conjugate addition of extended aldehydes to activated aryl systems. The Wright group also published an asymmetric synthesis of (+)- and (-)-frondosin B and (+)-frondosin A. Their synthesis included a diastereoselective [4 + 3]
Diels Alder cycloaddition between the tetrabromocyclopropene dienophile and an annulated furan diene to produce the B ring. There are also several racemic syntheses published by Mehta, Flynn, and Winne.\textsuperscript{122–124}

Scheme 25: Various retrosyntheses of frondosin B.

The first asymmetric total synthesis of frondosin B was carried out by the Danishefsky group in 2001.\textsuperscript{118} They were able to confirm the structure and assign the \( R \) configuration to C8. Beginning with the known methyl-7-hydroxy-5-(\( E \))-heptanoate 95,
epoxidation under Sharpless conditions in the presence of (+)-diisopropyl-L-tartrate afforded (-)-methyl (5S,6S)-5,6-epoxy-7-hydroxyheptanoate \textbf{96} with 84% enantiomeric excess (\textit{ee}) (Scheme 26). The epoxide was treated at low temperatures with an excess of AlMe$_3$ to provide methyl diol \textbf{97} (>95% \textit{ee}). This reaction established the only stereogenic center (C8) in the final compound. The resulting diol was cleaved with periodate to afford crude 5-\textit{(R)}-methyl-6-oxohexanoate and was converted to the alkyne \textbf{98} when treated with potassium dimethyl(methyl)-phosphonate (Gilbert reagent). The synthesis of the benzofuran framework was carried out using a Sonogashira coupling reaction between iodophenol and alkyne \textbf{98}. The ester \textbf{99} was converted to the corresponding acyl chloride and through an intramolecular Friedel-Crafts acylation, yielded ketone \textbf{100}. 
Scheme 26: Danishesky’s synthesis of (+)-frondosin B.

When subjected to normal aldol conditions between the ketone 100 with acetone, epimerization at the C8 center was observed, likely due to the presence of excess base after the formation of the enolate. Using Mukaiyama aldol reaction conditions to generate the silyl enolether \textit{in situ}, furnished tertiary alcohol 101 without epimerization. Dehydration with PdCl$_2$(MeCN)$_2$ in benzene of the mesylate afforded the olefin.
Methylation of the ketone was accomplished using Tebbe’s reagent buffered with pyridine yielding 102.

With the diene in hand, Diels-Alder reactions conditions were attempted. Unfortunately, reactions with maleic anhydride provided only low yields (26%) even after two days at elevated temperatures. While attempting a Lewis-acid promoted Diels-Alder reaction, olefin isomerization was observed. Excess nitroethylene was used as an effective ethylene equivalent in the presence of di-tert-butyl pyridine at 80°C followed by the removal of the nitro group by radical reduction to afford 103. Final deprotection of the aryl methyl ether with sodium ethanethiolate afforded (+)-frondosin B 91. This eighteen step synthesis resulted in a 0.8% chemical yield. The final compound was found to have an optical rotation of $[\alpha]_D = +15.2$. Pure (+)-frondosin has an optical rotation of $[\alpha]_D = +18.5$ and it was concluded the naturally occurring product exists as the $R$ enantiomer.

The Trauner group published the second total synthesis in 2002, utilizing a Heck reaction as part of a 20 step synthesis with an overall yield of 7.3%. They assigned the absolute configuration at C8 as $S$. They explained the discrepancy by incorrectly hypothesizing that a double-inversion epoxide opening with AlMe$_3$ in the Danishefsky sequence caused inversion at the C8 stereocenter. Though Trauner’s synthesis also contained a similar epoxide opening with AlMe$_3$, he claimed their synthesis did not go through the same inversion process. They rationalized this hypothesis by comparing proposed transition states in both their synthesis and that of Danishefsky. It was postulated that Danishefsky’s intermediate participated in a five membered transition state 104,
leading to inversion (Scheme 27). Trauner speculated that their compound would not participate in a similar transition state due to the unfavorable size of the four-membered ring intermediate. MacMillan duplicated both Danishefsky’s and Trauner’s syntheses and suggested that the inversion was actually occurring in Trauner’s syntheses. MacMillan hypothesized that Trauner’s inversion of stereochemistry at the C8 center occurred during the intramolecular Heck reaction. The stereocenter at C8 was destroyed and subsequent protonation of the enol ether generated the \( S \)-configuration. Wright also confirmed this conclusion and suggested the inversion occurred during the installation of the geminal dimethyl group.\(^{121}\)

![Scheme 27: Trauner’s rationale for inversion of stereochemistry in Danishefsky’s synthesis of (+)-frondosin B.](image)

(-)-Frondosin B was synthesized by Ovaska in 2011, which utilized a 5-\textit{exo-dig} cyclization-Claisen rearrangement as the key synthetic step to construct the bicyclic 6-7 core (Scheme 28).\(^{120}\) The synthesis commenced with a Sonogashira coupling reaction between alkyne 105 and 2-bromo-1,4-dimethoxybenzene. The primary alcohol was oxidized under Swern conditions to afford the aldehyde 106. Geminal dimethyl cyclohexyllithium was generated \textit{in situ} from the vinyl iodide 107 via lithium-halogen
exchange and an addition reaction. The allylic alcohol formed was oxidized to the prochiral ketone 109 and subsequently asymmetrically reduced to the secondary alcohol 110 using CBS (98% ee). 110 was treated with catalytic MeLi and heated under microwave irradiation to yield the 6-7-fused bicyclic compound 112 (95% ee) through a 5-exo-dig cyclization, then underwent the thermal [3,3] Claisen rearrangement. The methyl group at C8 was introduced through a stereoselective enolate addition to MeI under kinetic conditions (97% ee). The benzofuran ring was closed through a Lewis acid induced cyclization, furnishing the entire tetracyclic scaffold. The final step required treatment with catalytic TsOH in benzene at reflux to achieve the isomerization of the trisubstituted double bond, affording (-)-Frondosin B with the optical rotation of $[\alpha]_D = -17.3$. These results also confirmed Danishefsky’s assignment of the $R$ configuration of the naturally occurring compound.
Scheme 28: Ovaska synthesis of (-)-frondosin B.
The MacMillan group published their total synthesis in 2010, relying on a ring closure via π-allyl Friedel-Craft cyclization (Scheme 29). The synthesis began with the conjugate addition of boronic acid and crotonaldehyde. The presence of a co-catalyst (dichloroacetic acid, DCA) afforded with high stereoselectivity (93% ee). The resulting aldehyde was treated with vinylithium, prepared through a Shapiro reaction with hydrazone. The hydrazone itself was easily generated through the condensation of commercially available 2,2-dimethylcyclohexanone and trisylhydrazide (trisyl = 2,4,6-triisopropylbenzene). The allylic alcohol was generated in good chemical yield. BBr$_3$ was used to initiate the Friedel-Crafts alkylation and also elegantly achieved the final demethylation. Their entire synthesis consisted of only three steps and proceeded in a 50% overall yield.

Scheme 29: MacMillan synthesis of (+)-frondosin B.
CHAPTER 3: AZAPHORBOL: SYNTHETIC APPROACHES

3.1 Background

The phorbol esters were chosen as a synthetic target because of their wide array of bioactivities found in this family of tigliane diterpenes.\textsuperscript{81,125,126} They are mostly known for their tumor promoting activity via local similarities to the endogenous tumor promoter, DAG, an activator of PKC, used in the regulation of several different signal transduction pathways.\textsuperscript{81} The decision to synthesize 4-azaphorbol was made, in part, due to the lack of nitrogen containing analogues found in the literature. The few that have been synthesized, including aconitine and cephalotaxine, have shown promising biological activity.\textsuperscript{84}

3.2 Synthetic Strategy

We envisioned utilizing a dipolar cycloaddition in the synthesis of the tigliane core of 4-azaphorbol. Once we complete a synthesis of the structural core, a variety of analogues can be prepared and tested for biological activity. The basic bicycle[5.4.0]undecane core can be constructed via a diazo decomposition reaction to form the 1,3-dipolar intermediate followed by a cycloaddition reaction to simultaneously form the six and seven membered rings of 119. The diazo-precursor can be synthesized in several steps from the commercially available D- or L-proline. A retrosynthesis can be seen in Scheme 30. A disconnection was envisioned between C9 and C8 and between C7 and C6 to produce the ylide 120. These bonds can be formed through rhodium(II) catalyzed 1,3-dipolar cycloaddition. The ylide 120 can be generated through the diazo-decomposition reaction of the diazo-dicarbonyl 121. Ultimately, compound 121 can be
generated through a synthetic series involving a Grignard addition for the olefin tether and an acylation to attach the dicarbonyl moiety.

3.3 Synthesis of Azaphorbol

Our first effort in developing a synthetic route to the bicycle\[5.3.0\]decane scaffold structure of 4-azaphorbol centered on the use of a proline derivative to serve as the template for the synthesis, while providing a nitrogenous base embedded in the core. Using commercially available L-proline 123, the synthesis commenced with the protection of the nitrogen as the carbamate derived BOC-protecting group, affording a yellow oil in a 69% yield (Scheme 31).\(^{127}\) The BOC-protected L-proline 122 was treated with borane methyl sulfide, reducing the carboxylic acid to the primary alcohol 124 in an excellent yield.\(^{128}\)
Several different oxidative conditions were attempted to prepare the aldehyde 125, including PCC oxidation\textsuperscript{128}, Swern oxidation\textsuperscript{129}, and Parikh-Doering oxidation\textsuperscript{130}, all of which proved unsuccessful (Scheme 32). Ultimately, Dess-Martin Periodinane (DMP)\textsuperscript{127} provided the aldehyde 125, that served as a precursor to Grignard addition (Table 1).

Scheme 32: Oxidation conditions for the synthesis of BOC-prolinal.
Table 1: Oxidation conditions for conversion to BOC-prolinal.

<table>
<thead>
<tr>
<th>entry</th>
<th>conditions</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PCC</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>DMSO, SO$_3$ py, Et$_3$N</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>(COCl)$_2$, DMSO, Et$_3$N</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>DMP</td>
<td>80</td>
</tr>
</tbody>
</table>

Commercially available 6-bromo-1-hexene was utilized to form a Grignard reagent through addition of magnesium at reflux in THF. The dropwise addition of the aldehyde to the hexenyl Grignard reagent afforded the 1,2-addition product as the racemic secondary alcohol $\text{126}$. $\text{126}$ was further oxidized using standard DMP oxidation conditions, producing the ketone $\text{127}$ in 71% yield. Deprotection of the amine was accomplished using trifluoroacetic acid (TFA): DCM (1:1) with excellent yield (96%).
Scheme 33: Synthesis of (S)-1-pyrrolidin-2-yl)hept-6-en-1-one.

Preparation of the diazo-containing side chain needed for dipole formation proved more difficult than anticipated. Initially, we decided to install the diazo-moiety to the α-carbon of the 1,3-dicarbonyl of ethyl malonyl chloride 129 prior to addition of the side chain to the amine (Scheme 34). We tried different diazo-transfer reagents (i.e., p-ABSA and 4-dodecylbenzenesulfonyl azide) and the base deprotonation (i.e., DBU and NaH) utilized for Table 2. Unfortunately, no product was recovered in any attempt.

Scheme 34: Unsuccessful diazo-transfer reaction to prepare the 2-diazo- ethyl malonyl chloride.
Table 2: Unsuccessful reaction conditions for diazo-transfer reaction to 129.

<table>
<thead>
<tr>
<th>entry</th>
<th>diazo-transfer reagent</th>
<th>base</th>
<th>solvent</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pABSA</td>
<td>DBU</td>
<td>ACN</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>pABSA</td>
<td>NaH</td>
<td>ACN</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>pDBSA</td>
<td>NaH</td>
<td>ACN</td>
<td>0</td>
</tr>
</tbody>
</table>

It was decided to postpone introduction of the diazo moiety until a later step in the synthesis. We then directed our efforts to adding the oxopropionate side chain (used to ultimately generate the dipole prior to cycloaddition) to the deprotected L-proline derivative 128. Again, we experienced complications. Acylation with acid chloride 129 was attempted at 0°C, while stirring overnight, but no product was isolated. We surmised that the hydrogens of the α-carbon of the β-dicarbonyl were too acidic for the reaction to occur. To eliminate the acidity problem, oxidation of the secondary alcohol 126 was postponed to a later step in the synthesis.

Instead of following the oxidation to the ketone 127, it was decided to protect the secondary alcohol as the tert-butyl dimethylsilyl ether. Towards that end, alcohol 126 was protected with tert-butyl dimethyl silyl chloride (TBS-Cl) (Scheme 35). 133 It was our vision that the reduced reactivity at this center (protected 2° alcohol versus ketone) would enable the amine to be acylated. With two differentially protected groups, selective deprotection of the amine 131 was first attempted using the Lewis acid, aluminum chloride. 134 Unfortunately, the TBS ether was deprotected, while leaving the BOC group
intact, as indicated by $^1$H-NMR. TFA was selected as the next reagent to attempt the selective deprotection\textsuperscript{135}. The reaction was successful, with the mono-deprotected product \textbf{132} forming rapidly (i.e., within 15 minutes). The disappearance of the t-butyl peak of the Boc protecting group (at 1.35 ppm on the $^1$H NMR while retaining peaks at 0.86 and 0.01 ppm of the t-butyl and dimethyl groups of the TBS) confirms the expected product. Purification \textit{via} flash column chromatography proved difficult due to band widening from the interaction of the basic amine with the acidic silica gel, causing low yields. The crude product $^1$H-NMR was compared with the purified $^1$H-NMR and the crude product was deemed adequately pure to move forward without further purification. This allowed for much better overall yields for the reaction.

Scheme 35: Preparation of secondary amine.

Acylation with ethyl malonyl chloride was next attempted. Different reaction conditions were chosen including using different bases: NaH and Hünig’s base\textsuperscript{136} and
Et$_3$N and without a base$^{137}$ (Scheme 36). Deprotonating of the amine 132 using NaH was unsuccessful. Regardless of reaction times, excess of base and/or ethyl malonyl chloride 129, only starting material remained. Similar results occurred with Et$_3$N, again provided no product. Reaction of the TBS protected amine 132 with Hünig’s base produced the acylated product 133 and unexpectedly loss of the silyl ether from the alcohol (Table 3). This side reaction could be formed from the exogenous HCl formed as a byproduct of the acylation reaction.

Scheme 36: Acylation conditions to produce the dicarbonyl side chain.

Table 3: Conditions for acylation of amine 132.

<table>
<thead>
<tr>
<th>entry</th>
<th>base</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n/a</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Et$_3$N</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>NaH</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>DIPEA</td>
<td>36</td>
</tr>
</tbody>
</table>

In order to generate the precursor of the 1,3-dicycloaddition reaction, the diazo moiety must be installed at the α-carbon of the dicarbonyl and the secondary alcohol
must be oxidized to the ketone. Several different pathways can be envisioned. We choose to attempt to install the diazo-moiety first, then oxidize the 2° alcohol. Diazotransfer was accomplished with p-ABSA and Et$_3$N in ACN (Scheme 37). We were concerned that the base would also deprotonate the alcohol and cause side reactions, but IR confirmed the hydroxyl group was intact and proved the transfer of the diazo-moiety was successful, revealing the C=N=N at 2129 cm$^{-1}$ as well. The $^1$H-NMR confirmed the transfer due to the loss of the methylene peak.

![Scheme 37: Diazo-transfer to dicarbonyl compound.](image)

Oxidation of the secondary alcohol 134 was attempted using DMP, PCC, and PDC (Scheme 38). Each separate oxidation reaction provided no appreciable product; despite attempting extended reaction times (i.e., >3 days), utilizing excess reagent, and elevated reaction temperatures, only starting material remained (Table 4).
Scheme 38: Attempted oxidation of diazo-dicarbonyl.

Table 4: Conditions for the oxidation of the secondary alcohol 134.

<table>
<thead>
<tr>
<th>entry</th>
<th>conditions</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DMP</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>PCC, NaOAc</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>PDC</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>PCC, NaOAc, Δ</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>PDC, Δ</td>
<td>0</td>
</tr>
</tbody>
</table>

Owing to the difficulties found with the diazo moiety present, we chose to attempt oxidation prior to the diazo-transfer reaction (Scheme 39). Several conditions were tested, including DMP, PCC, and PDC (Table 5). In all cases, only starting material remained. We determined that the conditions were too mild to oxidize the somewhat sterically hindered secondary alcohol and attempted the reaction with a stronger oxidant. Under Jones oxidation conditions\(^{139}\) (CrO₃, H₂SO₄, H₂O) we succeed in preparing the ketone from the 2° alcohol. IR confirmed the loss of the alcohol peak and introduction of a new carbonyl peak at 1751 cm\(^{-1}\).
Scheme 39: Oxidation of secondary alcohol 133.

Table 5: Oxidation conditions for synthesis of ketone 135.

<table>
<thead>
<tr>
<th>entry</th>
<th>conditions</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DMP</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>PDC</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>PDC, Δ</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>CrO₃, H₂SO₄, H₂O</td>
<td>41</td>
</tr>
</tbody>
</table>

Installation of the diazo-moiety was attempted using p-ABSA and Et₃N in ACN (Scheme 40). Preliminary analysis of the isolated product leads us to believe the N₂ was successfully added to the substrate based on ¹H NMR (loss of methylene peak). Cyclization to generate the phorbol core was catalyzed with 4 mol% Rh₂(OAc)₄ in refluxing toluene (Scheme 41). Initial data suggested the formation of an unexpected product instead of the desired cyclized product.
Two possible products could have formed in place of the desired product. Literature examples lead us to believe that Rh$_2$(OAc)$_4$ caused the formation of an epoxide derivative or participate in a 1,4-sigmatropic shift (Figure 29). These compounds would help explain the presence of peaks in the olefinic region of the $^1$H NMR. Substituting Rh$_2$(OAc)$_4$ with a more electron withdrawing catalyst (e.g., Rh$_2$(pfb)$_4$, Rh$_2$(tfa)$_4$, Rh$_2$(pfm)$_4$) could lead to the generation of the desired product.$^{140}$

Scheme 41: Rh$_2$(OAc)$_4$ catalyzed 1,3-dipolar cycloaddition to generate the phorbol core.
3.4 Summary

Preliminary data suggest the formation of the epoxide derivative or the product of the 1,4-sigmatropic shift. The use of a catalyst with highly electron withdrawing ligands would likely generate desired product. To this end, additional material is currently being synthesized to attempt the cyclization. Future work on this synthesis will be carried on in our lab, including the cleavage of the ether bond at C-6 with $\text{SmI}_2$ to yield the secondary alcohol and will be assayed for biological activity.
CHAPTER 4: FRONDOSIN B: SYNTHETIC APPROACHES

4.1 Background

The frondosin family of natural products, characterized by their bicycle[5.4.0]-undecane ring system, inhibit the binding of IL-8, a chemoattractant known for its participation in inflammatory disorders, including psoriasis, gout, asthma, and rheumatoid arthritis.\textsuperscript{118,142} As IL-8 receptor antagonists, these compounds have the potential to suppress autoimmune hyperactivity.\textsuperscript{142} This family of natural products is an ideal candidate for synthesis by our group due to the structural similarities to the phorbols. They contain the same tigliane core as the phorbol esters and similar methodology could be employed.

4.2 Synthetic Strategy

Similar to the phorbol esters, the family of frondosins also contains the tigliane core. We envisioned utilizing a similar 1,3-dipolar cycloaddition to build the seven and six membered rings simultaneously of Frondosin B. The diazo-precursor is to be synthesized \textit{via} a Suzuki reaction. The two precursors to be coupled are synthesized through several steps starting from the commercially available compounds, glutaric acid monomethyl ester chloride and 1-(5-methoxy-2-benzofuranyl)ethanone (Scheme 42).
4.3 Towards the Synthesis of Frondosin B

In our synthetic pursuit of a synthetic approach of Frondosin B, we commenced with the preparation of the halide to be used as part of the Suzuki reaction. Glutaric acid monomethyl ester chloride 138 was chosen as one starting material for the synthesis. We first attempted to convert the acid chloride 138 directly to the enone 139 in a variant of a Friedel Crafts-type reaction using aluminum chloride and dry ethylene (Scheme 43).\textsuperscript{143} The reaction was carried out in DCE at 35°C. Despite numerous attempts, no product was isolated from any of the reactions.
Scheme 43: Attempted synthesis of enone 139 via ethylene in a Friedel-Crafts reaction.

We next turned our efforts to forming enone 139 via a Grignard addition to the acid chloride. Direct conversion of the acid chloride to the enone was attempted using methodology developed by the Zhou group. Zhou found that several different types of alkyl Grignard reagents could be added to various acid chlorides successfully. We attempted the reaction using vinyl Grignard and copper(I) iodide in THF to prepare enone 139 (Scheme 44). The reaction was initially carried out at -78°C and was slowly allowed to warm to room temperature. Despite numerous attempts to control the internal temperature of the reaction, we were unable to isolate any product. We also attempted to run the reaction colder than the usual -78°C for the duration of the reaction. Following the reaction progress by TLC appeared to produce several new reaction products. Each new product spot was separated by flash column chromatography and analyzed via ¹H-NMR, but no peaks were found in the olefinic region of the spectra, indicative of product formation.
With a Grignard reaction still in mind as the principle method of preparing enone 139, we chose Grignard addition to aldehyde 140, rather than reaction with the acid chloride (Scheme 44). Functionally, this adds an additional oxidation step, but will likely produce the intermediate needed. We attempted to prepare the aldehyde from the acid chloride 138 in one step using a bulky reducing agent, LiAlH(O-t-Bu)_3 in ethanol at -78°C. The reaction was monitored by TLC, but the starting material spot did not produce any change in Rf after the reducing agent was added. Several additional attempts were made, including monitoring the temperature of the reaction closely for a longer period of time. The longer reaction period provided a complex mixture of inseparable products without the ester/aldehyde being formed. The reaction was repeated using NaBH₄, along with pyridine as a borane scavenger to prevent over reduction of the starting material to the ester/alcohol or the diol. After work up, the proton NMR showed a similar complex mixture from the reaction. Separation was attempted via flash column chromatography, but multiple attempts proved unsuccessful.
Despite the difficulties in preparing enone 139, we began the synthesis of the benzofuran boronic acid derivative. Bromination of commercially available benzofuran 141 was achieved using Br$_2$ in chloroform generating the bromobenzofuran 142 in excellent chemical yield. Preparation of the boronic acid 144 needed for a Suzuki coupling reaction involved a two step sequence (Scheme 45). First, the ketone 142 was protected using ethylene glycol and p-TsOH as the acid source. The crude product 143 was used without further purification for conversion to the boronic acid 144. Triisopropylborate and n-butyllithium were used for the conversion, but the yields were too low to continue.

Scheme 45: Synthesis of boronic acid.
Due to the need to change strategy toward the coupling intermediates, we have chosen to continue the project later, while developing strategies toward the enone and alternatives and a more robust synthesis of the benzofuran coupling partner. However, we have devised a total synthesis and the current iteration will be described here. Continuing the pursuit of halide, once the aldehyde is successfully produced the ethylene portion can be introduced via the Grignard reaction, using a vinyl Grignard reagent. The subsequent secondary alcohol can be oxidized using Jones reagent to produce the vinyl ketone (Scheme 44). The α,β-unsaturated ketone can then be transformed to vinylcyclopropane. Cp₂ZrCl₂ is used to form a zirconocene(ethylene) complex, which forms a oxazirconacyclopentane intermediate. Use of a Lewis acid promotes the ring contraction and formation of the vinylcyclopropane compound over the alcohol. The literature precedent shows promising results with α,β-enones containing terminal double bonds. This newly installed cyclopropane ring will ultimately become the source of the gem-dimethyl functionalization required for Frondosin B. Alternatively, analogs retaining the cyclopropane may provide interesting complementary compounds with differing bioactivity. Ester will then be converted to the carboxylic acid via basic hydrolysis using LiOH, followed by treatment with thionyl chloride to produce the acid chloride (Scheme 46). Once the two intermediate compounds have been prepared, the Suzuki reaction can be attempted.
Scheme 46: Proposed synthesis of acid chloride portion of Suzuki reaction.

Using Suzuki methodology developed by Haddach, the coupling reaction can be completed using our two coupling partners, along with Pd(PPh₃)₄ and Cs₂CO₃ in toluene at 100°C. The Suzuki cross coupled reaction is expected to proceed to provide a precursor to diazotization 147 (Scheme 47). Using Davies conditions (i.e., p-ABSA and DBU), the diazo can be installed alpha to the ketone. Complications may arise from the diazo-transfer reaction due to the presence of two ketones with α-hydrogens with similar pKas. By using the commercially available dicarbonyl derivative of the benzofuran, the diazo-transfer reaction will install the diazo-moiety in the correct position. With the diazocarbonyl intermediate 148 in hand, the compound can undergo diazo-decomposition under rhodium(II) catalysis to initially form the rhodium-carbene complex. Once the dipole has formed, an intramolecular 1,3-dipolar cycloaddition should occur, simultaneously forming both the six- and seven-membered rings while retaining the cyclic ether 150.
Once the Frondosin core has been completed, the cyclopropane ring can be reductively cleaved to afford the gem-dimethyl using a number of different conditions including H$_2$ and PtO$_2$ in acetic acid (Scheme 48).$^{151}$ It has been previously shown by Padwa$^{141}$ and subsequently by the McMills’ group that the ether bridge can be cleaved using SmI$_2$ through the intermediacy of a samarium enolate.$^{106}$ The alcohol can be eliminated via a dehydration reaction using a proton source and heat. Literature precedence can be found for this procedure using acetic anhydride and potassium.
acetate. An alternate method involves acetylation, followed by K$_2$CO$_3$ and alumina elimination at elevated temperatures. The ketone 152 is susceptible to Wittig olefination to prepare the exocyclic olefin, followed by a reduction to afford a racemic mixture of the methyl group using LiAlH$_4$. It is also possible to attempt an asymmetric hydrogenation of the olefin using the catalyst Ru(S)-BINAP(OAc)$_2$ and H$_2$. The methoxy ether group can be converted to the alcohol following the reaction conditions found in MacMillan’s synthesis of Frondosin B (i.e., BBr$_3$ in DCM).

Scheme 48: Continued proposed synthesis toward Frondosin B.

4.4 Summary

We have attempted a synthesis toward Frondosin B with limited success up to this stage. Low chemical yields and failed reactions have lead to the decision to continue on this synthetic path at a later stage. Once the two coupling partners are generated, the
synthesis described above will be attempted the total synthesis of Frondosin B based on a 1,3-dipolar cycloaddition reaction.
CHAPTER 5: SYNTHESIS OF CYCLIC UNSUBSTITUTED DIAZOACETAMIDES

5.1 Background

The late stage preparation of diazoacetamides can be a difficult step in any synthetic endeavor. Because use of diazo-groups in synthesis can lead to highly complex ring systems that are present in many biologically active systems, it was determined that finding a facile synthetic route to these moieties was of great importance. Diazo-groups are popular precursors to carbenes and carbenoids, which subsequently undergo ylide formation, cycloaddition, cyclopropanation, and C-H insertion reactions.\textsuperscript{157}

Diazoacetamides have been present in the literature as far back as the early 1900’s.\textsuperscript{158–161}

The diazoacetamides were first characterized by Curtius, et. al., but were not tested for biological activity until 1966 by Baldini and Brambilla.\textsuperscript{162} Bioassay included various diazoacetamides synthesized by Curtius, along with others. Diazoacetamide analogues were found to display antitumor properties against Ehrlich ascots carcinoma when tested in mice, and markedly extended the life expectancy of the tumor-bearing mice.

There are various reports in the literature of diazoacetamides analogue synthesis. In one such paper, Challis and colleagues were able to introduce a diazo moiety on peptide ethyl esters. Diazopeptides are compounds of interest, exhibiting both antibacterial properties and tumor suppression.\textsuperscript{163} The diazopeptides were synthesized using liquid N\textsubscript{2}O\textsubscript{4} at -40\textdegree C. Triethylamine was used to keep the reaction mixture neutral, since diazopeptides are easily cleaved under acidic conditions, while the sodium sulfate was added to absorb water formed during the reaction. Despite optimizing conditions, the
yield was moderate at best, providing an overall 40-50% chemical yield. Two examples are shown below (Scheme 49).

Scheme 49: Challis synthesis of diazopeptides.

Non-cyclic diazoacetamides have been prepared previously, using a succinimidyl diazoacetate (Figure 30) as the diazo transfer reagent. This reagent is a highly stable crystalline compound, and can be stored at room temperature without fear of decomposition. A similar approach was envisioned for the synthesis of cyclic diazoacetamides (Scheme 50).

Figure 30 Succinimidyl diazoacetate 155.
5.2 Synthesis of Cyclic Diazoacetamides

The succinimidyldiazo transfer reagent 155 was synthesized via a reaction between p-toluenesulfonylhydrazide and glyoxylic acid under acidic conditions.\textsuperscript{165} Glyoxylic acid p-toluenesulfonylhydrazone 156 was then coupled with hydroxysuccinimide using dicyclohexylcarbodiimide (DCC) to produce succinimydial diazoacetate 155 in 46\% yield (Scheme 51).\textsuperscript{166}

With the diazo-transfer reagent in hand, three nitrogen heterocycles were chosen as substrates: azetidine, pyrrolidine, and piperidine. The diazo moiety was transferred under basic conditions with moderate yields in all cases (156-158) (40-59\%). (Scheme 52).\textsuperscript{167}
Another synthetic pathway was explored to synthesize various diazoacetamides using \( N,N' \)-ditosylhydrazine 161 as the diazo-transfer reagent. This reagent was synthesized in one step using tosyl chloride 159 and tosylhydrazine 160 in an excellent yield (96%) (Scheme 53).168

The substrate upon which the diazo moiety was installed was synthesized from bromoacetyl bromide and the appropriate amine.157 The acylation proceeded smoothly to afford the \( \alpha \)-bromoacetamide 163-165 in moderate to good yields. The diazo transfer was carried out with 161 and 1,1,3,3-tetramethylguanidine (TMG) to afford the diazoacetamides (156-158) in moderate yields (Scheme 54). TMG allowed for
convenient non-aqueous work up by the removal of TMG-\(p\)-toluenesulfinat salt via filtration.

Scheme 54: Alternative synthesis of diazoacetamides.

\[
\begin{align*}
\text{Br} & \quad \text{Br} & \quad \text{Br} \\
\text{O} & \quad \text{Br} & \quad \text{N} \\
\text{N} & \quad \text{H} & \quad \text{N} \\
\text{K}_3\text{PO}_4 & \quad \text{DCM} & \quad \text{THF} \\
\text{TMG} & \quad -10^\circ\text{C} - \text{RT} & \quad -10^\circ\text{C} - \text{RT} \\
n = 1 & \quad \text{(163) 37\%} & \quad n = 1 & \quad \text{(156) 37\%} \\
n = 2 & \quad \text{(164) 62\%} & \quad n = 2 & \quad \text{(157) 44\%} \\
n = 3 & \quad \text{(165) 90\%} & \quad n = 3 & \quad \text{(158) 46\%}
\end{align*}
\]

5.3 Summary

We explored two methods for creating cyclic unsubstituted diazoacetamides. The first method utilizes succinimidyldiazoacetate as the diazo-transfer reagent and generated all three ring sizes with moderate yields. The second method involved the intermediate \(\alpha\)-bromoacetamide and using \(N,N'\)-ditosylhydrazine as the diazo-transfer reagent to afford all three ring sizes. Future work includes increasing the chemical yield of these reactions, especially with the azetidine ring, for use in future syntheses.
CHAPTER 6: EXPERIMENTAL

6.1 General Experimental

6.1.1 General materials and methods

All reactions were carried out under an argon atmosphere employing anhydrous conditions unless otherwise noted. Dichloroethane (DCE) and acetonitrile (ACN) were dried over CaH\(_2\) and then distilled. Dichloromethane (DCM) and tetrahydrofuran (THF) were dried using Solv-Tek Inc. column purification/drying system, which uses low-pressure nitrogen or argon gas to force solvents through various filter materials to remove moisture and impurities from solvents. Reagents purchased from commercial sources were used without further purification unless noted otherwise. Analytical TLC was performed on 0.25 mm silica gel (MF254) plates purchased from EMD Chemicals, Inc. UV light, potassium permanganate solution (1.5 g KMnO\(_4\), 10 g K\(_2\)CO\(_3\), 1.25 mL 10% NaOH in 200 mL H\(_2\)O), and ninhydrin (1.5 g ninhydrin, 100 mL n-butanol, 3 mL acetic acid) were used as visualizing reagents. Flash column chromatography was carried out using Merck silica 60 (230-400 mesh). \(^1\)H NMR and \(^13\)C NMR spectra were recorded at 300 and 500 MHz on both a Bruker AVANCE-300 and Bruker ASCEND-500 spectrometer. Chemical shifts (\(\delta\)) are quoted in parts per million (PPM) downfield from tetramethylsilane (TMS). Multiplicities are abbreviated as follows: s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; m, multiplet or overlap of non-equivalent resonances; br, broad. Infrared spectra were obtained on a Shimadzu FTIR-8400 spectrometer as neat oils. Elemental analyses were performed by Midwest Microlab, LLC., Indianapolis, IN.
Melting point (mp) were recorded on a capillary melting point apparatus and are uncorrected.

6.2 Synthesis of Cyclic Unsubstituted Diazoacetamides

![Glyoxylic acid p-toluenesulfonylhydrazone (156).](image)

Glyoxylic acid p-toluenesulfonylhydrazone (156).

A solution of glyoxylic acid (50% w/w) (3.97 g, 26.8 mmol) in 27.0 mL of H₂O was placed in a 125 mL Erlenmeyer flask and warmed on a steam bath to approximately 60°C. The solution was treated with a warm (~30°C) solution of p-tosylhydrazide (5.0 g, 34.9 mmol) in 2.5 M HCl (14 mL). The resulting mixture was heated on a steam bath with continuous stirring until all hydrazone solidified. The reaction mixture was cooled to room temperature and allowed to stand in the refrigerator overnight. The crude compound was filtered and was washed with copious amounts of cold water, and the filter cake was transferred to a watch glass and dried on the benchtop for 2 days. The crude product was dissolved in boiling ethyl acetate (50.0 mL), filtered to remove any insoluble material, then carbon tetrachloride (100 mL) was added and the solution cooled to room temperature. After approximately 1 hour, a white solid formed and the mixture was stored in the refrigerator overnight. The recrystallized product was filtered using a cold solution of ethyl acetate:carbon tetrachloride (1:2). The white solid was transferred to a watch glass and let dry in room temperature for a period of 2 days. White solid (2.355 g, 37%). ¹H NMR (500 MHz, CDCl₃) δ 12.27 (br.s., 1H), 7.71-7.69 (m, 1H), 7.54-
7.53 (m, 1H), 7.44-7.42 (m, 1H), 7.37-7.35 (m, 1H), 7.17 (s, 1H), 5.32 (br s, 1H), 2.38 (m, 3H); IR (NaCl disc) 3477, 3002, 2912, 1665, 1438, 1410, 1311, 1043, 957, 700, 668 cm⁻¹.

succinimidyldiazoacetate (155).

To a 50 mL round bottom flask containing a solution of N-hydroxysuccimide (250 mg, 2.17 mmol) and glyoxylic acid tosylhydrazone (519 mg, 2.17 mmol) in ice cold dioxane (3.0 mL) was added a solution of DCC (1.79 g, 4.34 mmol) in dioxane (4.0 mL), dropwise. The solution was warmed to room temperature and stirred overnight. The reaction mixture turned yellow followed by the formation of a precipitate. The reaction mixture was filtered and the white precipitate was identified as dicyclohexylurea (DCU). The yellow filtrate was concentrated under reduced pressure. The crude product was purified by flash chromatography (100% DCM to 5% MeOH in DCM) to produce a yellow solid (182 mg, 46%). \(^1\)H NMR (500 MHz, CDCl₃) δ 3.73 (s, 1H), 2.87 (s, 4H); \(^1^3\)C NMR (500 MHz, CDCl₃) δ 207.0, 169.3, 67.1, 30.9. IR (NaCl disc) 3153, 2983, 2252, 1744, 1712, 1472, 1378, 1225, 1097, 910, 737, 653 cm⁻¹;
1-(azetidin-1-yl)-2-diazoethanone (156). \textsuperscript{157,167}

**Method A:** To a 25 mL round bottom flask containing an ice cooled solution containing azetidine HCl (31.0 mg, 0.327 mmol) and Et$_3$N (0.05 mL, 0.409 mmol) in DCM (1.0 mL) was added succinimidyl diazoacetate (50.0 mg, 0.273 mmol) in DCM (1.0 mL), dropwise. The reaction mixture stirred for 30 minutes at 0°C and then warmed to room temperature. The orange solution stirred overnight at room temperature and then was concentrated under reduced pressure. The crude product was purified via flash chromatography (50% hexane: ethyl acetate to 100% ethyl acetate) to yield a brown oil (19.0 mg, 56% yield).

**Method B:** To a 25 mL round bottom flask containing α-bromoacetamide (0.200 g, 1.123 mmol) were added N,N’-ditosylhydrazine (0.620 g, 2.246 mmol), and THF (3.0 mL). The suspension was cooled to -10°C. 1,1,3,3-tetramethylguanidine (0.71 mL, 5.617 mmol) in THF (1.0 mL) was added in small aliquots while maintaining the internal temperature of 0°C. The cooling bath was removed and the yellow reaction mixture was allowed to warm to room temperature. A white precipitate formed, while bubbles were observed evolving from the solution. Within 10 minutes, the gas evolution ceased. After 1 hour, the suspension was concentrated under reduced pressure, keeping the temperature of the solution below 25°C. The precipitate was diluted with Et$_2$O, filtered and the filter cake was rinsed with Et$_2$O until white. The yellow filtrate was transferred to a round bottom flask, a spatula tip amount of silica gel was added. The suspension stirred for 5 minutes,
then filtered through a pad of silica gel. Et₂O was used to wash the filter cake. The filtrate was concentrated \textit{in vacuo} to afford a yellow oil. The crude product was purified \textit{via} flash chromatography (90% Et₂O in petroleum ether) to afford a yellow oil (64 mg, 46%).; \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) \(\delta\) 4.55 (s, 1H), 3.97 (t, \(J=6.55\), 4H), 2.26 (quin, \(J=7.75\), 2H). ; \textsuperscript{13}C NMR (500 MHz, CDCl\textsubscript{3}) \(\delta\) 166.1, 44.5, 30.9, 15.7; IR (NaCl disc) 3153, 3228, 2252, 2109, 1708, 1614, 1474, 1382, 1095, 910, 734, 651 cm\textsuperscript{-1}; Anal. Calcd. For C\textsubscript{5}H\textsubscript{7}N\textsubscript{3}O: C, 47.99; H, 5.64; N, 33.58. Found: C, 47.98; H, 5.48; N, 33.35; mp 54-56.3°C.

\[ \text{N} \bigg| \begin{array}{c} \text{O} \\ \text{H} \end{array} \bigg| \text{N}_2 \]

\textbf{2-diazo-1-(pyrrolidin-1-yl)ethanone (157).} \textsuperscript{157,167}

Method A: To a 25 mL round bottom flask containing an ice cooled solution of pyrrolidine (0.02 mL, 0.196 mmol) and Et\textsubscript{3}N (0.03 mL, 0.245 mmol) in DCM (0.5 mL) was added a solution of succinimidyl diazoacetate (30.0 mg, 0.163 mmol) in DCM (0.6 mL), dropwise. The reaction mixture stirred for 30 minutes at 0°C and then warmed to room temperature. The orange solution was purified \textit{via} flash chromatography (50% hexane: ethyl acetate to 30% hexane: ethyl acetate) to yield a yellow oil (12.0 mg, 59%).

Method B: To a 25 mL round bottom flask containing \(\alpha\)-bromoacetamide (0.162 g, 0.843 mmol) were added \(N,N'\)-ditosylhydrazine (0.466 g, 1.687 mmol) and THF (1.5 mL). The suspension was cooled to -10°C. 1,1,3,3-tetramethylguanidine (0.53 mL, 4.215 mmol) in THF (1.0 mL) was added in small aliquots while maintaining the internal temperature of 0°C. The cooling bath was removed and the yellow reaction mixture was allowed to
warm to room temperature. A white precipitate formed and bubbles were witnessed and within 10 minutes gas evolution ceased. After 1 hour, the suspension was concentrated under reduced pressure, keeping the temperature below 25°C. The precipitate was diluted with Et₂O and filtered. The filter cake was rinsed with Et₂O until white. The yellow filtrate was transferred to a round bottom flask and a spatula tip full of silica gel was added. The suspension stirred for 5 minutes and was then filtered through a pad of silica gel. Et₂O was used to wash the filter cake. The filtrate was concentrated under reduced pressure to afford a yellow oil. The crude product was purified via flash chromatography (90% Et₂O in petroleum ether) to afford a yellow oil (51 mg, 44%). ¹H NMR (500 MHz, CDCl₃) δ 4.78 (s, 1H), 3.50 (br s, 2H), 3.18 (br s, 2H), 1.92 (br s, 2H), 1.85 (br s, 2H); ¹³C NMR (500 MHz, CDCl₃) δ 163.9, 46.6, 46.1, 45.8, 25.9, 24.6; IR (NaCl disc) 3155, 2981, 2252, 2109, 1603, 1431, 1384, 910, 734, 651 cm⁻¹. Spectral results match literature values.¹⁵⁷

![Chemical Structure](image)

**2-diazo-1-(piperidin-1-yl)ethanone (158).**¹⁵⁷,¹⁶⁷

Method A: To a 25 mL round bottom flask containing an ice cooled solution of piperidine (0.02 mL, 0.196 mmol) and Et₃N (0.034 mL, 0.245 mmol) in DCM (0.5 mL) was added a solution of succinimidyld diazoacetate (30.0 mg, 0.163 mmol) in DCM (0.6 mL), dropwise. The reaction mixture stirred for 30 minutes at 0°C and then warmed to room temperature. The orange solution stirred for 1.5 hours and was then concentrated
under reduced pressure. The crude product was purified via flash chromatography (50% hexane: ethyl acetate to 30% hexane: ethyl acetate) to yield a yellow oil (10.0 mg, 40%).

Method B: To a 25 mL round bottom flask containing α-bromoacetamide (0.100 g, 0.485 mmol) were added N,N'-ditosylhydrazine (0.268 g, 0.97 mmol), and THF (1.5 mL). The suspension was cooled to -10°C. 1,1,3,3-tetramethylguanidine (0.30 mL, 2.425 mmol) in THF (1.0 mL) was added in small aliquots while maintaining the internal temperature of 0°C. The cooling bath was removed and the yellow reaction mixture was allowed to warm to room temperature. A white precipitate formed and bubbles were observed. Within 10 minutes gas evolution ceased. After 1 hour, the suspension was concentrated under reduced pressure, keeping the temperature below 25°C. The precipitate was diluted with Et₂O and filtered. The filter cake was rinsed with Et₂O until white. The yellow filtrate was transferred to a round bottom flask and a spatula tip full of silica gel was added. The suspension stirred for 5 minutes and was filtered through a pad of silica gel. Et₂O was used to wash the filter cake. The filtrate was concentrated under reduced pressure to afford a yellow oil. The crude product was purified via flash column chromatography (90% Et₂O in petroleum ether) to afford a yellow oil (48 mg, 65%).

$^1$H NMR (500 MHz, CDCl₃) δ 4.96 (s, 1H), 3.32 (br s, 4H), 1.62-1.57 (m, 2H), 1.54-1.50 (m, 4H); $^{13}$C NMR (300 MHz, CDCl₃) δ 164.3, 46.3 (2C), 25.8, 24.5; IR (NaCl disc) 3153, 2943, 2860, 2252, 2109, 1596, 1444, 1380, 1223, 1097, 910, 739, 653 cm⁻¹. Spectral results match literature values.
To a 250 mL round bottom flask was added tosyl chloride (7.62 g, 40.0 mmol) and tosylhydrazide (5.0 g, 26.0 mmol) and was suspended in DCM (70.0 mL). The suspension was cooled to 0°C and a solution of pyridine (3.23 mL, 40.0 mmol) in DCM (6.0 mL) was added dropwise to the yellow reaction mixture. A white precipitate formed and the ice bath was allowed to expire. The reaction mixture stirred for 3.5 hours and was then diluted with Et₂O (40.0 mL) and the suspension was transferred to a beaker. Water (40.0 mL) and Et₂O (40.0 mL) was added in the given order and the suspension stirred for 10 minutes. It was filtered and the solid was transferred to a 250 mL round bottom flask. The white solid was broken up with a glass stirring rod and diluted with 100 mL of MeOH. The mixture refluxed for 3 hours. The solution was cooled to room temperature and filtered. The filtrate was concentrated and a white solid precipitated. The precipitate was filtered and combined with the first crop. The white solid was placed on a watch glass and air dried for a period of 2 days. White solid (6.968 g, 96%). \(^1\)H NMR (500 MHz, DMSO-d₆) \(\delta\) 9.57 (s, 2H), 7.64-7.63 (m, 4H), 7.39-7.37 (m, 4H), 2.39 (s, 6H); \(^1^3\)C NMR (500 MHz, DMSO-d₆) \(\delta\) 144.7, 134.9, 130.1, 128.1, 21.3; IR (NaCl disc) 3481, 3002, 2916, 1663, 1436, 1406, 1311, 1041, 953, 698, 668 cm\(^{-1}\). Spectral results match literature values.
1-(azetidin-1-ly)-2-bromoethanone (163).\textsuperscript{157}

To a 50 mL round bottom flask was added, in the given order, bromoacetyl bromide (0.70 mL, 8.02 mmol), DCM (8.0 mL), and K$_3$PO$_4$ (2.84 g, 13.36 mmol). The reaction mixture was cooled with an ice bath. Azetidine HCl (0.50 g, 5.34 mmol) was added portionwise. The solid amine dissolved after approximately 3 hours. The reaction mixture continued to stir overnight and was quenched with 3 mL of 1.0 M HCl, then stirred for 5 minutes. The reaction mixture, which was initially white, turned clear. Approximately 3.0 mL each of brine and water were added, then the solution was transferred to a separatory funnel. The layers were separated and the aqueous layer was extracted once with DCM. The combined organic layers were washed with both 5% NaHCO$_3$ and brine, then brine. The organic layer was dried over MgSO$_4$, filtered, and concentrated under reduced pressure. The crude oil was stored in the freezer and solidified to a white solid (350 mg, 37%). The product was carried on without further purification. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 4.24 (t, $J$=7.75, 2H), 4.06 (t, $J$=7.65, 2H), 3.61 (s, 2H) 2.31 (quin, $J$=7.85, 2H); $^{13}$C NMR (300 MHz, CDCl$_3$)$\delta$ 166.1, 51.1, 48.7, 24.0, 15.3; IR (NaCl disc) 3153, 3228, 2261, 1712, 1652, 1466, 1361, 910, 737, 653 cm$^{-1}$
2-bromo-1-(pyrrolidin-1-yl)ethanone (164).\textsuperscript{157}

To a 25 mL round bottom flask was added, in the given order, bromoacetyl bromide (0.18 mL, 2.109 mmol), DCM (1.5 mL), and K$_3$PO$_4$ (0.746 g, 3.515 mmol). The reaction mixture was cooled with an ice bath. Pyrrolidine (0.12 mL, 1.406 mmol) in DCM (2.0 mL) was added dropwise and the reaction mixture turned pink. The ice bath was allowed to expire. After 45 minutes, the reaction mixture turned orange. It was quenched with 1.0 mL of 1.0 M HCl and stirred for 5 minutes. The reaction mixture, which was initially white, turned clear. Approximately 1.0 mL each of brine and H$_2$O was added and the solution as transferred to a separatory funnel. The layers were separated and the aqueous layer was extracted once with DCM. The combined organic layers washed with both 5\% NaHCO$_3$ and brine, then brine. The organic layer was dried over MgSO$_4$, filtered, and concentrated under reduced pressure. The crude oil was stored in the freezer and solidified to a white solid (162 mg, 62\%). The product was carried on without further purification. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$3.77 (s, 2H), 3.51-3.45 (m, 4H), 1.99-1.96 (m, 2H), 1.87-1.84 (m, 2H); $^{13}$C NMR (500 MHZ, CDCl$_3$)$\delta$163.9, 46.6, 46.1, 45.8, 25.9, 24.5. IR (NaCl disc) 2981, 2879, 2252, 1639, 1453, 1378, 1097, 908, 739, 651 cm$^{-1}$ Spectral results match literature values.\textsuperscript{157}
2-bromo-1-(piperidin-1-yl)ethanone (165)\textsuperscript{157}

To a 25 mL round bottom flask was added, in the given order, bromoacetyl bromide (0.15 mL, 1.76 mmol), DCM (1.5 mL) and K\textsubscript{3}PO\textsubscript{4} (0.621 g, 2.925 mmol). The reaction mixture was cooled with an ice bath. Piperidine (0.115 mL, 1.17 mmmol) in DCM (2.0 mL) was added dropwise. The orange reaction mixture stirred for 1 hour, and was quenched with 1.0 mL of 1.0 M HCl and stirred for 5 minutes. The reaction mixture, which was initially white, turned clear. Approximately 1.0 mL of brine and water were added and the solution was transferred to a separatory funnel. The layers were separated and the aqueous layer was extracted once with DCM. The combined organic layers washed with both 5% NaHCO\textsubscript{3} and brine, then brine. The organic layer was dried over MgSO\textsubscript{4}, filtered, and concentrated under reduced pressure. The crude oil (217 mg, 90%) was stored in the freezer and solidified to a white solid. The product was carried on with further purification. \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) δ 3.84 (s, 2H), 3.55-3.53 (m, 2H), 3.43-3.41 (m, 2H), 1.64-1.63 (m, 4H), 1.56-1.52 (m, 2H); \textsuperscript{13}C NMR (500 MHz, CDCl\textsubscript{3}) δ 165.0, 47.9, 43.2, 26.2, 26.1, 25.3, 24.2; IR (NaCl disc) 3153, 2983, 2256, 1706, 1635, 1472, 1382, 910, 737, 646 cm\textsuperscript{-1}. Spectral results match literature values.\textsuperscript{157}
6.3 Synthesis of Azaphorbol

![Chemical Structure](image)

**(S)-1-(tert-butoxycarbonyl)pyrrolidine-2-carboxylic acid (122).**

A suspension of L-proline (0.300 g, 2.61 mmol) in ACN (10 mL) was added BOC anhydride (0.598 g, 2.74 mmol). The reaction mixture was cooled to 0°C followed by the addition of Et$_3$N (0.47 mL, 3.39 mmol). The ice bath was removed and the reaction mixture stirred at room temperature until the reaction reached completion by TLC by which time a clear solution had formed. The reaction mixture was washed with 1 M HCl, saturated aqueous NaHCO$_3$ and brine. The organic layer was dried (MgSO$_4$) and concentrated under reduced pressure. The crude material was purified via flash column chromatography (10% hexane: 90% ethyl acetate) to afford a yellow oil (0.387 g, 69%).

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$4.32 (br s, 1H), 3.51-3.32 (m, 3H), 2.37-2.34 (m, 1H), 1.91-1.86 (m, 3H), 1.45 (s, 9H). Spectral results match literature values.

**(S)-tert-butyl-2-(hydroxymethyl)pyrrolidine-1-carboxylate (124).**

![Chemical Structure](image)
Borane methyl sulfide complex ($\text{BH}_3\text{SMe}_2$) (1.1 mL, 12.1 mmol) was added dropwise to a solution of BOC-L-proline (2.00 g, 0.929 mmol) in dry THF (20 mL) cooled to 0°C. When gas evolution ceased, the ice bath was removed and the solution refluxed for 1 h. Upon completion of the reflux, the solution was cooled followed by the slow addition of methanol. The resulting solution was concentrated under reduced pressure. The residue was redissolved twice in 5 mL of methanol and 5 mL of toluene and then concentrated to afford an oil (0.160 g, 83.5% yield). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$3.95-3.92 (m, 1H), 3.65-3.52 (m, 2H), 3.47-3.39 (m, 1H), 3.32-3.24 (m, 1H), 2.33 (s, 1H), 2.04-1.93 (m, 1H), 1.86-1.70 (m, 2H), 1.61-1.5 (m, 1H), 1.44 (s, 9H). Spectral results match literature values.

$^{127}$

(\textit{S})-\textit{tert}-butyl-2-formylpyrrolidine-1-carboxylate (125).

To a round bottom flask containing (\textit{S})-\textit{tert}-butyl-2-(hydroxymethyl)pyrrolidine-1-carboxylate (5.0 g, 24.8 mmol) in DCM (25 mL) at 0°C was added Dess-Martin periodinane (DMP) (12.6 g, 29.8 mmol). The suspension was stirred at room temperature until the reaction showed completion by TLC. Upon completion, the reaction was quenched with both saturated Na$_2$S$_2$O$_3$ (aq) and saturated NaHCO$_3$ (aq), while being stirred vigorously until clear. The aqueous layer was extracted with EA (x2). The
combined organic layers were washed with saturated NaHCO₃ (aq) and brine. The combined organic layers were dried (MgSO₄) and concentrated under reduced pressure. The crude material was purified via flash column chromatography (20% ethyl acetate: hexane) to afford a clear oil (3.92 g, 80% yield). Mixture of rotamers: ¹H NMR (500 MHz, CDCl₃) δ 9.60 (s, 0.4H) 9.506 (s, 0.6H), 4.249-4.074 (m, 1H), 3.628-3.460 (m, 1H), 2.207-1.962 (m, 2H), 1.946-1.908 (m, 2H), 1.521 (s, 3H), 1.471 (s, 6H); ¹³C NMR (300 MHz, CDCl₃) δ 200.5, 153.9, 80.6, 65.0, 46.8, 28.4, 26.7, 23.9; IR (NaCl disc) 2977, 2931, 2885, 1735, 1681, 1396, 1365, 1257, 1164, 1118 cm⁻¹. Spectral results match literature values.

(S)-tert-buty-2-(1-hydroxyhept-6-enyl)pyrrolidine-1-carboxylate (126).

To a round bottom flask containing Mg turnings (0.57 g, 23.7 mmol) under an atmosphere of argon, fitted with a condenser was added 6-bromo-1-hexene (3.2 mL, 23.7 mmol) in dry THF (15 mL), dropwise. After approximately one 25% of the allyl bromide was added, the reaction mixture was heated to reflux, while continuously adding the remaining allyl bromide. The reaction mixture refluxed for 15 mins, at which time the Mg turnings had reacted to form the Grignard reagent. The reaction mixture was then cooled to 0°C and (S)-tert-buty-2-formylpyrrolidine-1-carboxylate (0.392 g, 19.7 mmol) in dry THF (15 mL) was added dropwise. The reaction mixture stirred at 0°C for 2 h,
until the reaction was shown to be complete by TLC. Upon completion, the reaction was quenched with saturated NH₄Cl (aq) and extracted with ethyl acetate (3x). The combined organic layers were washed with brine, dried (MgSO₄), and concentrated under reduced pressure. The crude material was purified via flash column chromatography (1:1 hexane: ethyl acetate) to afford a yellow oil (4.54 g, 81.5% yield). ¹H NMR (500 MHz, CDCl₃) δ5.78-5.70 (ddt, J = 16.9, 10.2, 6.7, 1H), 4.94-4.84 (m, 2H), 3.75-3.71 (m, 1H), 3.42-3.37 (m, 2H), 3.23-3.19 (m, 2H), 2.00-1.98 (m, 2H), 1.91-1.84 (m, 2H), 1.79-1.65 (m, 4H).

(S)-tert-butyl-2-hept-6-enoylpyrrolidine-1-carboxylate (127).

To a round bottom flask containing (S)-tert-butyl-2-(1-hydroxyhept-6-enyl)pyrrolidine-1-carboxylate (5.60 g, 19.7 mmol) in DCM (15.0 mL) at 0°C, was added DMP (12.5 g, 29.5 mmol) in one portion. The reaction mixture stirred until completion by TLC. Upon completion, the reaction was quenched with both 10% aqueous Na₂S₂O₃ and saturated aqueous NaHCO₃ stirred vigorously until clear. The layers were separated, with the aqueous layer extracted with ethyl acetate (3x). The combined organic layers were washed with saturated aqueous NaHCO₃ and brine, dried (MgSO₄), filtered, and concentrated under reduced pressure. The crude material was purified via flash column chromatography (7:2 hexane: ethyl acetate) to afford a clear oil (4.55 g, 81.5% yield) ¹H NMR (300 MHz, CDCl₃) δ5.83-5.69 (ddt, J = 16.9, 10.2, 6.7, 1H), 5.00-4.91 (m, 2H),
4.33-4.30 (m, 1H), 4.20-4.17 (m, 1H), 3.53-3.39 (m, 2H), 2.49-2.36 (m, 2H), 2.05-2.00 (m, 3H), 1.85-1.81 (m, 3H), 1.60-1.54 (m, 3H), 1.37 (s, 9H). Spectral results match literature values.  

\[(S)-1\text{-pyrrolidin-2-yl} \text{hept-6-en-1-one (128).}\]

To a round bottom flask under an atmosphere of argon was added \((S)-\text{tert-butyl-2-hept-6-enoylpyrrolidine-1-carboxylate (50.0 mg, 0.177 mmol)}\) in DCM (0.5 mL). The reaction mixture was cooled to 0°C and TFA (0.5 mL, 6.49 mmol) was added dropwise. The reaction mixture stirred at RT until the reaction was shown to be complete by TLC and was concentrated under reduced pressure to afford an oil. The oil was taken up in ethyl acetate and washed with sat. NaHCO₃ (aq) and brine, dried (MgSO₄) and concentrated. The product was not purified further. Clear oil (31 mg, 96% yield) \(\text{^1H NMR (300 MHz, CDCl₃)}\) δ5.78-5.72 (ddt, \(J = 16.9, 10.2, 6.7, 1\text{H}\)), 5.00-4.90 (m, 2H), 2.90 (t, \(J=7.5, 1\text{H}\)), 2.55-2.45 (m, 2H), 2.07-2.00 (m, 7H), 1.63-1.60 (m, 3H), 1.42-1.35 (m, 3H).
(S)-tert-butyl-2-(1-(tert-butyldimethylsilyloxy)hept-6-enyl)pyrrolidine-1-carboxylate (131).

To a solution containing (S)-tert-butyl-2-(1-hydroxyhept-6-enyl)pyrrolidine-1-carboxylate (4.04 g, 14.2 mmol) and imidazole (2.42 g, 35.6 mmol) in dry DCM (50 mL) under an atmosphere of argon at 0°C was added TBS-Cl (3.22 g, 21.3 mmol). The reaction mixture was warmed to room temperature and stirred for 21 h, until the reaction was whoen to be complete by TLC. The reaction mixture was diluted with DCM (50 mL), washed with sat. NaHCO₃ (aq) and with brine. The organic layer was dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified via flash chromatography (eluent: 10% ethyl acetate: hexane) to afford a clear oil (3.55 g, 63% yield). $^1$H NMR (500 MHz, CDCl₃) δ5.78-5.70 (m, 1H), 4.95-4.86 (m, 2H), 4.09-4.02 (m, 1H), 3.85-3.75 (m, 1H), 3.48-3.21 (m, 2H), 1.98 (q, $J$=6.9, 2H), 1.82-1.70 (m, 4H), 1.43 (s, 9H), 1.37-1.25 (m, 2H), 1.22-1.19 (m, 3H), 0.83 (s, 9H), 0.017 (s, 3H), -0.0002 (s, 3H). $^{13}$C NMR (500 MHz, CDCl₃) δ154.7, 138.9, 114.3, 79.4, 72.1, 60.8, 47.5, 33.8, 30.4, 28.7, 25.8, 25.6, 23.6, 18.0, -4.59; IR (NaCl disc) 2931, 2854, 1681, 1458, 1257, 1203, 1134, 1087 cm$^{-1}$. 
To a solution of (S)-tert-butyl-2-(1-(tert-butyldimethylsilyloxy)hept-6-enyl)pyrrolidine-1-carboxylate (0.50 g, 1.25 mmol) in dry DCM (7.0 mL) under argon at 0°C was added TFA (7.0 mL) dropwise. The reaction mixture stirred at 0°C for 15 minutes, until completion by TLC. The reaction mixture was concentrated under reduced pressure to afford a yellow oil. The oil was taken up in DCM and washed with saturated NaHCO₃ (aq). The organic layer is washed with brine, dried (MgSO₄), and concentrated in vacuo to yield a yellow oil (227 mg, 61% yield). H NMR (500 MHz, CDCl₃) δ5.709-5.629 (ddt, J = 16.8, 9.8, 6.7, 1H), 4.904-4.828 (m, 2H), 3.956-3.945 (m, 2H), 3.736 (br. s., 1H), 3.661-3.646 (m, 1H), 3.582-3.542 (m, 1H), 3.313-3.264 (m, 1H), 3.232-3.124 (m, 1H), 1.945-1.881 (m, 5H), 1.775-1.718 (m, 1H), 1.533-1.466 (m, 2H), 1.364-1.245 (m, 3H), 1.151 (br. s., 1H), 0.795 (s, 9H), 0.012 (s, 3H), -0.0001 (s, 3H); C NMR (500 MHz, CDCl₃) δ138.5, 114.6, 71.39, 61.81, 45.99, 35.11, 33.53, 28.77, 27.70, 25.79, 24.21, 24.01, 18.02, -4.02, -4.08; IR (NaCl disc) 3633, 3463, 2985, 2908, 1735, 1643, 1442, 1373, 1303, 1242, 1049 cm⁻¹.
(S)-ethyl-3-(2-(1-hydroxyhept-6-enyl)pyrrolidin-1-yl)-3-oxopropanoate (133).

To a solution of (S)-2-(1(tert-butyldimethylsilyloxy)hept-6-enyl)pyrrolidine (1.5 g, 5.04 mmol) in dry DCM (20 mL) under an atmosphere of argon was added ethyl malonyl chloride (0.77 mL, 6.05 mmol) and DIPEA (1.14 mL, 6.55 mmol) at 0°C. The reaction mixture was warmed to room temperature and stirred overnight. Upon completion by TLC, the reaction mixture was cooled to 0°C and quenched with 1 N HCl. The reaction mixture was extracted with ethyl acetate (x 1). The organic layer was washed sequentially with 1 N HCl, brine, sat. NaHCO₃(aq), and brine, dried (MgSO₄) and concentrated in vacuo. The crude product was purified via flash chromatography (eluent: 1:1 hexanes: ethyl acetate) to afford an orange oil (0.551 g, 36% yield). ¹H NMR (500 MHz, CDCl₃) δ 5.80-5.75 (m, 1H), 5.02-4.83 (m, 2H), 4.20-4.18 (m, 2H), 3.54-3.52 (m, 1H), 3.45-3.43 (m, 2H), 3.40-3.34 (m, 4H), 2.06-1.95 (m, 4H), 1.94-1.86 (m, 3H), 1.42-1.32 (m, 4H), 1.28-1.25 (m, 3H). ¹³C NMR (500 MHz, CDCl₃) δ 167.8, 166.7, 138.7, 114.7, 75.9, 75.0, 61.4, 47.4, 33.5, 33.4, 31.1, 28.6, 27.3, 24.0, 22.3, 14.1. IR (NaCl disc) 3409, 3078, 2931, 2854, 1735, 1620, 1427, 1303, 1242, 1157, 1095, 1033, 995, 910, 971 cm⁻¹
(S)-ethyl-2-diazo-3-(2-(1-hydroxyhept-6-enyl)pyrrolidin-1-yl)-3-oxopropanoate (134).

To a solution of (S)-ethyl-3-(2-(1-hydroxyhept-6-enyl)pyrrolidin-1-yl)-3-oxopropanoate (0.10 g, 0.336 mmol) in dry ACN (2.0 mL) was added Et$_3$N (0.05 mL, 0.403 mmol) followed by pABSA (97.0 mg, 0.403 mmol) at RT and stirred overnight, forming a white precipitate. The suspension was concentrated under reduced pressure and triturated with DCM (x 2) and concentrated under reduced pressure. The crude product was purified via flash column chromatography (eluent: 40% ethyl acetate: hexane) to afford a yellow oil (80 mg, 73% yield). $^1$H NMR (500 MHz, CDCl$_3$) $^\delta$5.79-5.74 (m, 1H), 4.99-4.90 (m, 2H), 4.31-4.21 (m, 5H), 3.58-3.53 (m, 1H), 3.43 (br s, 1H), 2.03-2.01 (m, 4H), 1.81-1.66 (m, 2H), 1.30-1.24 (m, 9H); IR (NaCl disc) 3533, 2977, 2931, 2129, 1758, 1681, 1620, 1396, 1272, 1064, 1018, 910, 864, 756 cm$^{-1}$.

(S)-ethyl-3-(2-hept-6-enoylpyrrolidin-1-yl)-3-oxopropanoate (135).$^{139}$

The chromic oxidizing reagent was prepared by dissolving 33.5 g of CrO$_3$ in 62.5 mL of distilled H$_2$O and 29 mL of concentrated sulfuric acid was carefully added. Any salts that
precipitated during addition of sulfuric acid were dissolved by the addition of a minimal
amount of distilled H$_2$O.

To a solution of (S)-ethyl-3-(2-(1-hydroxyhept-6-enyl)pyrrolidin-1-yl)-3-oxopropanoate
(50.0 mg, 0.168 mmol) in acetone (1.0 mL) was added chromic acid reagent (5 drops)
until the orange color persists for ~20 mins. The reaction mixture was transferred to a
round bottom flask containing a stir bar and the residual green salts were rinsed with
acetone (3 mL x2) and added to the main acetone solution. Chromic acid is added to
ensure complete conversion to the ketone (2 drops) and the reaction mixture stirred
vigorously. Isopropanol (5 mL) is added slowly until excess chromic acid is destroyed.
Small portions of NaHCO$_3$ are added carefully until the suspension is pH neutral. The
suspension is filtered through a pad of celite and concentrated. The residue was taken up
in diethyl ether and transferred to a separatory funnel containing brine. The layers are
separated and the aqueous layer is extracted with diethyl ether (3x). The combined
organic layers was washed with H$_2$O, dried (MgSO$_4$) and concentrated under reduced
pressure.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$5.79-5.74 (m, 1H), 4.99-4.92 (m, 2H), 4.26-4.15 (m, 2H),
3.53-3.45 (m, 3H), 3.43 (s, 2H), 2.06-2.01 (m, 5H), 1.88-1.84 (m, 3H), 1.42-1.39 (m,
4H), 1.28-1.21 (m, 3H); $^{13}$C NMR (500 MHz, CDCl$_3$) $\delta$166.4, 165.7, 155.8, 138.5, 113.8,
73.2, 65.8, 51.83, 33.5, 31.3, 30.9, 29.7, 25.0, 24.9, 14.1; IR (NaCl disc) 2977, 2939,
1751, 1735, 1689, 1450, 12503, 1149 cm$^{-1}$. 
(S)-ethyl-2-diazo-3-(2-hept-6-enoylpyrrolidin-1-yl)-3-oxopropanoate (136).

To a solution of (S)-ethyl-3-(2-hept-6-enoylpyrrolidin-1-yl)-3-oxopropanoate (20.0 mg, 0.067 mmol) in dry ACN (1.0 mL) was added Et$_3$N (0.01 mL, 0.082 mmol) followed by pABSA (19.5 mg, 0.082 mmol) at RT and stirred overnight. The solution was concentrated under reduced pressure and triturated with DCM (x 2) and concentrated under reduced pressure. The crude product was purified via flash column chromatography (eluent: 20% ethyl acetate:hexane) to afford a clear oil (11 mg, 51% yield).

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 5.80-5.74 (m, 1H), 5.00-4.92 (m, 2H), 4.28 (q, $J$ = 7.1, 2H), 3.48-3.44 (m, 1H), 2.04-1.96 (m, 4H), 1.70-1.52 (m, 3H), 1.42-1.39 (m, 3H), 1.32-1.29 (m, 4H), 1.28-1.24 (m, 3H).
ADDENDUM

First isolated in 1955 by Corbaz, nonactin (166) is a naturally occurring macrotetrolide, produced by Streptomyces griseus (Figure 31). The tetrameric species consists of two subunits of (+)-nonactate and two subunits of (-)-nonactate. Though the molecule has sixteen stereogenic centers, the molecule is a stereogenic meso compound and therefore achiral. Nonactin is an ionophore, binding a number of cationic ions (e.g., Na+, K+, and NH4+) and is able to transport them across cell membranes, leading to antibiotic properties shown by the molecule. Its affinity for NH4+ ions lead to its widespread use in ammonia-selective electrodes. Nonactin possess antitumor activity against mammalian cell lines in vitro and against Crocker Sarcoma 180 in mice studies. Nonactin itself is toxic, uncoupling oxidative phosphorylation of mitochondria thus inhibiting the formation at ATP.

![Figure 31: Structure of nonactin and MNA.](image)

The monomer methyl ester subunit that comprises nonactin is methyl nonactate (MNA 167). It was our vision that the synthesis of a series of analogs of MNA could
potentially modulate the biological activity of the monomer and be assayed for potential therapeutic uses. One such modification is the C-H insertion reaction of the corresponding sulfamate ester derivative of MNA. The reaction of is well preceded by Du Bois et al. and provides the six-membered ring formed through γ-C-H bond amination. Oxathiazinane formation is favorable due to the elongated S-O and S-N bonds (1.58 Å) and the obtuse N-S-O angle (103°) that closely resemble the parameters of the heterocycle. These oxathiazinanones are susceptible to nucleophilic addition with primary and secondary amines, thiolates, AcO−, and N3−. Ring opening to generate the alcohol is accomplished through the addition of H2O.

Intramolecular C-H aminations involve a nitrogen-based nucleophile with an electrophilic carbon center. For example, carbamates can be converted to the corresponding oxazolidinones. The carbamate (168) undergoes oxidation to the corresponding iodoimine (169) through the addition of Phl(OAc)2 (Scheme 55). Addition of a transition metal catalyst (e.g., Rh2(OAc)4) generates the metallonitrene (170), which undergoes a C-H insertion to form the corresponding cyclic compound (171).

Scheme 55: C-H insertion pathway for preparation of oxazolidinone from corresponding carbamate.
In order to utilize the MNA scaffold for subsequent transformations, pure (+)-
MNA needed to be prepared. Fermentation of *Streptomyces griseus* of nonactin and its
homologues provides pure nonactin and a residue of higher macrotetrolide homologues.
The residue undergoes methanolysis generating a mixture of MNA and homononactate
(HMNA). Direct separation of these compounds is not possible; however, separation is
possible based on the differential reaction rates in a lipase-mediated hydrolysis (Scheme
56).^{179}

![Scheme 56: Synthesis of the methyl ester monomer, methyl nonactate (MNA).](image)

We obtained a mixture of MNA and HMNA (172) and following the procedure
reported by Priestley^{179} set about isolating MNA. To the crude mixture (5.0 g, approx.
22.6 mmol) under an atmosphere of argon was added DMAP (0.27 g, 2.26 mmol),
pyridine (5.5 mL, 67.8 mmol) and DCM (150 mL). Acetic anhydride (7.5 mL, 67.8 mmol) was added dropwise and stirred at RT overnight. Upon completion by TLC, the reaction mixture was quenched with brine (150 mL) and the layers separated. The aqueous layer was extracted with DCM (x2). The combined organic layers were washed with 10% CuSO$_4$ (aq) solution (150 mL x2) and saturated aqueous NH$_4$Cl solution (x1). The organic layer was dried over MgSO$_4$, filtered, and concentrated under reduced pressure to afford an oil (2.5 g, 42% yield) containing a mixture of the methyl and ethyl derivatives of 173.

To phosphate buffer (0.1 M potassium phosphate; pH = 7) containing Amano PS lipase (2.5 g) was added a solution of acetoxy derivatives (2.5 g) in methanol (5.0 mL). The reaction mixture was heated at 42°C for 30 h. The reaction progress was monitored by GC/MS. Upon completion, the mixture was extracted with ethyl acetate (x3). Any emulsions formed upon extraction were broken up with centrifugation. The combined organic layers were dried over MgSO$_4$, filtered, and concentrated to produce a mixture of compounds 174 and 175. Desired product 174 (0.520 g) was obtained by purification by flash column chromatography (2:1 hexane:ethyl acetate).

The acetoxy derivative 174 (0.800 g, 3.24 mmol) was dissolved in 5% H$_2$SO$_4$ in methanol (40.0 mL) and heated at reflux for 2.5 h. The reaction mixture was neutralized with aqueous saturated NaHCO$_3$ and extracted with DCM (x3) and concentrated. The crude material was purified via flash column chromatography (1:1 hexane:ethyl acetate) to afford an oil (167) (0.380 g, 54% yield).
Installation of the sulfamate ester commenced with the dropwise addition of formic acid (0.13 mL, 2.5 mmol) to neat chlorosulfonyl isocyanate (0.21 mL, 2.5 mmol) at 0°C with rapid stirring, solidifying after ~ 5 minutes (Scheme 57). ACN (1.0 mL) was added and the reaction mixture was warmed to RT and stirred overnight (Scheme 57). The reaction mixture was cooled to 0°C and a solution of MNA (167) (216 mg, 1.0 mmol) in DMA (0.8 mL) was added. Upon completion of the condensation reaction of sulfamoyl chloride, ClSO₂NH₂ with the alcohol, the reaction was quenched by the addition of H₂O. The mixture was transferred to a separatory funnel containing Et₂O and H₂O. The layers were separated and the aqueous layer was extracted several times with Et₂O. The separated organic layers were combined, washed with H₂O, dried over MgSO₄, and concentrated under reduced pressure to produce an oil. The crude product was purified via flash column chromatography (100% ethyl acetate) to afford a clear oil (176) (0.23 g, 78% yield).

![Scheme 57: Synthesis of sulfamate ester.](image)

To conduct a C-H insertion reaction, the sulfamate ester 176 previously prepared was reacted with several rhodium(II) catalysts to provide insertion at several C-H bond possibilities. To a solution of sulfamate ester 176 (92.0 mg, 0.312 mmol) in DCM (2.0
mL) were added MgO (28.9 mg, 0.72 mmol), PhI(OAc)$_2$ (110 mg, 0.343 mmol) and Rh$_2$(OAc)$_4$ (4 mol%, 5.3 mg, 0.012 mmol) (Scheme 58). The suspension was stirred at room temperature until the reaction was complete indicated by a change in the TLC for the reaction versus starting material. The reaction mixture was diluted with DCM (1.0 mL) and filtered through a pad of celite. The filter cake was washed with DCM and the filtrate was concentrated under reduced pressure. The crude product was purified via flash column chromatography (2:1 pentane:ethyl acetate) to afford a clear oil (177) (19 mg, 20% yield). The use of different rhodium(II) carboxylates were employed to try to improve the yield (Table 6), but enough material could not be synthesized to continue with nucleophilic ring opening. Chemical yield was calculated via GC/MS.

Scheme 58: C-H insertion reaction of sulfamate ester.
Table 6: Chemical yields for C-H insertion reaction with different rhodium(II) carboxylate catalysts.

<table>
<thead>
<tr>
<th>entry</th>
<th>catalyst</th>
<th>time</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rh₂(OAc)₄</td>
<td>24 h</td>
<td>20.9</td>
</tr>
<tr>
<td>2</td>
<td>Rh₂esp₂</td>
<td>16 h</td>
<td>24.6</td>
</tr>
<tr>
<td>3</td>
<td>Rh₂tpa₄</td>
<td>16 h</td>
<td>16.8</td>
</tr>
<tr>
<td>4</td>
<td>Rh₂DOSP₄</td>
<td>20 h</td>
<td>13.2</td>
</tr>
<tr>
<td>5</td>
<td>Rh₂pfb₄</td>
<td>24 h</td>
<td>8.0</td>
</tr>
</tbody>
</table>

Methyl nonactate (167). $^1$H NMR (300 MHz, CDCl$_3$)$\delta$4.12-4.09 (m, 1H), 4.00-3.97 (m, 2H), 3.66 (s, 3.66), 2.51 (quin, $J = 7.3$, 1H), 1.97-1.94 (m, 2H), 1.66-1.60 (m, 4H), 1.17 (d, $J = 6.3$, 3H), 1.10 (d, $J = 6.9$, 3H).

(R)-methyl-2-(((2S,5R)-5-( (R)-2-(sulfamoyloxy)propyl)tetrahydrofuran-2-yl)propanoate (176). $^1$H NMR (300 MHz, CDCl$_3$)$\delta$5.29 (br s, 2H), 4.92-4.79 (m, 1H), 4.14-4.01 (m, 1H), 3.94 (q, $J = 7.8$, 1H), 3.70 (s, 3H), 2.46 (quin, $J = 8.9$, 1H), 2.15-2.03 (m, 2H), 1.92-1.79 (m, 1H), 1.63-1.50 (m, 3H), 1.40 (d, $J = 6.3$, 3H), 1.12 (d, $J = 7.1$, 3H).
(177). $^1$H NMR (300 MHz, CDCl$_3$)δ5.14-5.11 (m, 1H), 4.94 (s, 1H), 4.49 (q, $J$ = 7.2, 1H), 3.69 (s, 3H), 2.69 (quin, $J$ = 8.9, 1H), 2.19-2.15 (m, 2H), 1.89-1.73 (m, 4H), 1.43 (d, $J$ = 6.3, 3H), 1.15 (d, $J$ = 7.0, 3H).
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