A Rhodium Catalyzed 1,3-Dipolar Cycloaddition Utilized Toward the Synthesis of Azaphorbol

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ABSTRACT

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A Rhodium Catalyzed 1,3-Dipolar Cycloaddition Utilized Toward the Synthesis of Azaphorbol

Thesis Advisor: Dr. Mark C. McMills

Synthetic chemistry, especially natural product synthesis, requires reactions that form complex products out of relatively simple starting materials. The metal catalyzed 1,3-dipolar cycloaddition is one such reaction. In this reaction, the metal will undergo several different reactions (diazo decomposition, cycloaddition) to form oxygen bridged heterocycles.

The use of this type of reaction can generate the six and seven membered rings that make up part of the phorbol core. Phorbols are a class of natural products that belong to the tigliane diterpene family. They are protein kinase C (PKC) activators and as such, have a wide variety of biological activity. Activity of interest includes: tumor promotion, HIV inhibition, and antileukemic activity. Very few nitrogen derivatives of phorbol are found in the literature, but the few that have been identified display an array of biological activities. Using a rhodium catalyzed 1,3-dipolar cycloaddition to form the core structure of azaphorbol will allow the development of alternative analogs to help define the necessary structural elements for biological activity.

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

ACN: Acetonitrile

ADP: Adenosine diphosphate

ATP: Adenosine triphosphate

DAG: Diacylglycerol

DBU: 1,8-diazobicyclo[5.4.0]undec-7-ene

DCC: N,N-dicyclohexylcarbodiimide

DCM: dichloromethane

DMAP: 4-dimethylaminopyridine

DMP: Dess-Martin Periodinane

EA: Ethyl Acetate

Et: ethyl

Et3N: trimethylamine

Hex: Hexanes

HOMO: highest occupied molecular orbital

LUMO: lowest unoccupied molecular orbital

NaH: Sodium Hydride

p-ABSA: para-acetamidobenzenesulfonyl azide

PCC: pyridinium chlorochromate

p-DBSA: para-dodecylbenzenesulfonyl azide

PKC: Protein Kinase C

PMA: Phosphomolybdic Acid

$Rh_2(OAc)_4$: dirhodium(II) tetraacetate

$Rh_2(pfm)_4$: rhodium(II)perfluorobutyramide

RT: Room temperature

TBSCl: tert-butyldimethylsilyl chloride

TFA: trifluoroacetic acid

THF: tetrahydrofuran

TLC: thin layer chromatography

TPA: 4-β-13-O-tetradecanolyphorbol-13-acetate

CHAPTER 1: INTRODUCTION

1.1 Overview

Some natural products were found to be therapeutically effective and became the basis for pharmacology as we know it today. Drugs such as morphine are derived from opium which was first extracted from poppy seeds. Some antibiotics are biological agents discovered as metabolites from bacteria and other microorganisms. Humans initially treated ailments and injuries with plant and animal extracts, which then evolved to producing novel, synthetically viable drugs while maintaining a strong interest in natural products. However, many of today's drugs are natural products that have been prepared synthetically, due to a lack of availability and/or modified to increase their activity and specificity. It has been estimated that 50-70% of existing drugs used are either derived from natural products or are a natural product themselves[1]. As such, when looking for novel drugs to use therapeutically, natural products offer a unique starting point in search of novel biological activity.

Natural products are often structurally complex and generally require multiple steps to complete a total synthesis. Obtaining samples of the same natural product from remote locations can be difficult and expensive, and in some cases the extraction does not lead to enough of the natural product to be useful. The product itself can also be unstable to oxygen and/or water and difficult to isolate. Products that come from deep sea organisms have an added complication with the difficulty of retrieval of the organisms. Synthetic chemistry allows the natural product to be synthesized from readily available

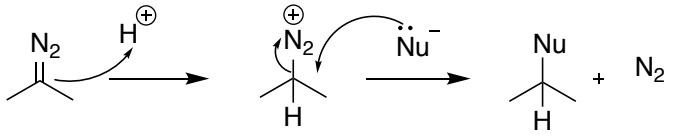
starting materials, but also requires cost minimization, high yielding reactions with sufficient purity.

The diazo-decomposition reaction is one of very few reactions that afford multiple access to creating carbon-carbon bonds from a single precursor, a technique that is highly valued in synthetic organic chemistry. The diazo-group of the α-diazocarbonyl itself contains two nitrogen atoms bonded linearly to the alpha carbon of a carbonyl group. The release of molecular nitrogen that occurs during a diazo-decomposition reaction forms a carbene or carbenoid species and provides for the chemical reactivity found[2]. Carbenes are electronically neutral carbon species, covalently bonded to either a single divalent group or two univalent groups[3]. The decomposition reaction is one method used to provide a precursor to [3+2]-cycloaddition reactions, where five- to seven-membered rings can be formed.

CHAPTER 2: BACKGROUND OF DIAZO COMPOUNDS AND RHODIUM CATALYZED CYCLOADDITIONS

2.1 Diazo-transfer Reagents

The diazo-group provides a broad range of reactivity, which make them useful in synthetic chemistry and in chemical biology. Early diazo-reagents, such as diazomethane or other diazoalkanes and their precursors are toxic and far too reactive for use in chemical biology. The elevated basicity of the group leads to the ready protonation of the alpha carbon of the diazo group which leads to the production of a diazonium ion that readily eliminates to form nitrogen gas [4]



Scheme 1: Diazo protonation and following S_N2 reaction

This limitation necessitated the development of "stabilized" diazo-compounds. In compounds of this type, electron density produced at the alpha carbon is delocalized to adjacent electron withdrawing functional group which decreases the overall basicity. This allows this class of compounds to be readily used in chemical biology and other synthetic processes. Diazo-compounds are considered to be precursors to carbenoids that can react with alkenes and alkynes in the presence of a catalyst. These compounds are strong nucleophiles due to increased electron density resulting from anionic nature of the carbon alpha to the diazo-moiety during cycloadditions with electron deficient dipolarophiles.

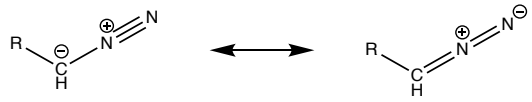
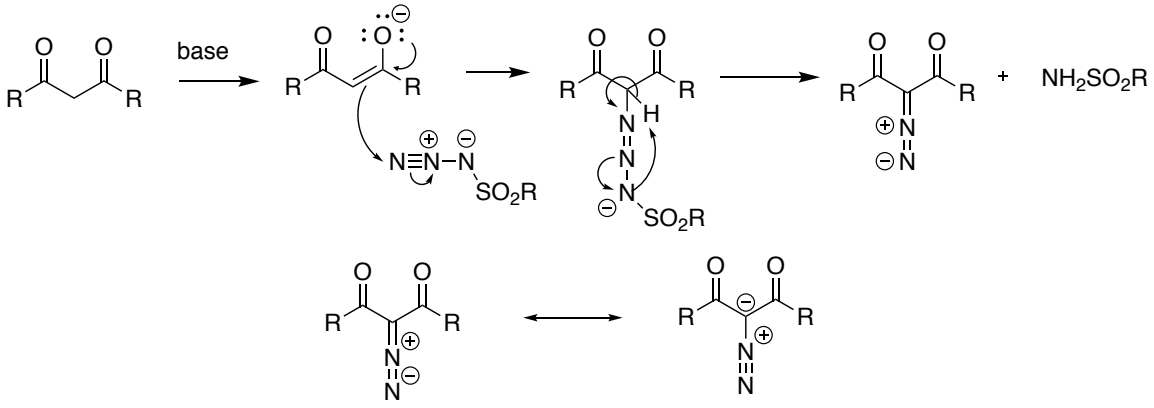


Figure 1: Resonance forms of a diazo group

Common methods used to prepare diazo-compounds include diazo-transfer reactions, diazotization[5], hydrazone decomposition[6] or hydrazone oxidation,[7] along with others. Diazo-transfer reagents can be used when the pK_a of the proton of the acceptor carbon is sufficiently acidic to be removed by a mild, organic base. The delocalization of electrons that helps to stabilize the compound after the proton removal, will also stabilize the diazo functional group once it is attached.

The basic diazo transfer mechanism is shown below in scheme 2. An activated methylene compound, typically a 1,3-β-dicarbonyl group, is treated with base, forming the enolate ion. The enolate attacks the electrophilic nitrogen atom of a diazo-transfer reagent like p-ABSA. Proton transfer then gives the α-diazocarbonyl substrate and a water soluble sulfonamide byproduct[8].



Scheme 2: Diazo-transfer mechanism and resulting resonance structure of a β-dicarbonyl

The stability of the diazo group determines the overall reactivity of the diazo-functional groups in Figure 2, especially those that react with a transition metal catalyst to provide a reactive carbenoid for additional reactions[9]. Electron withdrawing groups bonded at a carbon adjoining the diazo-containing carbon, (or alpha to the diazo group) act to stabilize the diazo functional group, which in turn makes the substrate more stable and generally easier to prepare than less stable substrates. The most stable diazo group is one found between β-1,3-dicarbonyls due to the resonance stabilization the structure affords.

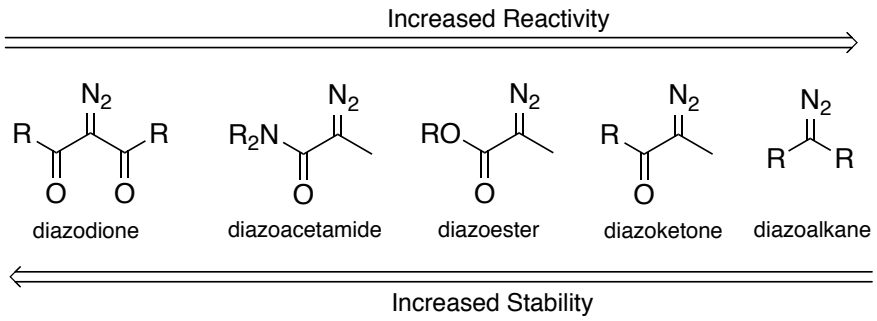


Figure 2: Reactivity trends of diazo substrates

The most stable diazo-scaffold is the diazodione, due to the delocalization of electrons through the two carbonyls and the carbonyl oxygens, so the electrons and therefore the charge are spread out and not localized on a single atom. The ester analogs of diazo compounds are more stable than the ketone for the same reason. Diazoacetamides are more stable than diazo-esters, but less stable than the diketo groups. In general, the more delocalized the electrons, the greater the stability of the diazo compound. Diazo compounds follow the general trend where reactivity and stability are inversely proportional. Because of this stability trend, the diazo-decomposition reactions of diazomalonates and diazoacetamides require higher temperature or more reactive conditions than diazoacetates, which can undergo decomposition below room temperature, but provide higher selectivity[10].

Diazomethane and ethyldiazoacetate (EDA) are two reagents that are synthetically useful for the addition of diazo groups, however, both are explosive and their precursors tend to be highly toxic. Diazomethane has been used for years, both as a diazo-transfer reagent and as a simple methylating reagent, and is one of the most hazardous diazo reagents due to the highly reactive nature of the compound. Typically,

biological nucleophiles are easily methylated, resulting in the possibility of mutations. Trimethylsilyl-diazomethane has been used in place of diazomethane due to the increased stability it affords[11]. While TMS-diazomethane is commercially available, it is expensive and has limited purity.

Diazo transfer reagents have evolved from simple diazoalkanes to substituted azides such as tosyl azide **2** developed by Doering and DePuy in 1953[12]. Unfortunately, tosyl azide was found to be one of the most hazardous reagents to handle during a study by Merck scientists, who developed a number of large scale diazo-transfer reagents[13]. As various azide reagents were developed, it was found that a difficult separation occurred between the side product of the reaction and the resulting diazo-product. At the time of the Merck study, German law provided that pure tosyl azide be classified as a high explosive, and that a number of accidents had been reported. Mesyl azide **1** became a second-generation transfer reagent, being used based on the ease of which the sulfonamide byproduct can be removed. However, it requires the use of triflic anhydride for synthesis, which is extremely expensive and cost prohibitive for larger scale reactions. It is also a severe methylating agent, leading to various cancers.

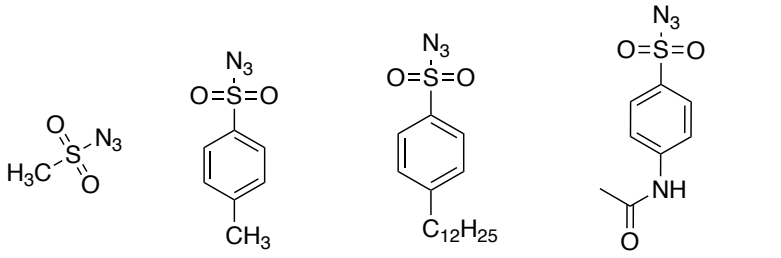
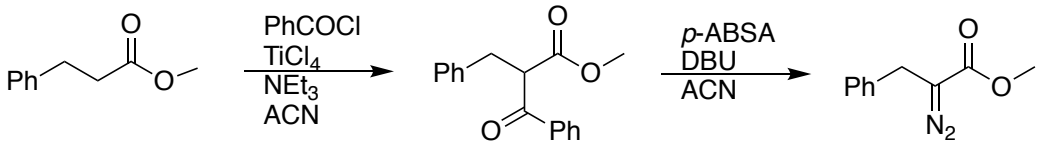


Figure 3: Diazo Transfer Reagents

p-ABSA **4** has been shown to be a safer alternative to tosyl azide **2** because of the low amount of energy released during decomposition. The side product produced during transfer is a liquid which allows for easy isolation from the crystalline diazo products.[14]

Direct diazo transfer is not always accessible from a desired starting material, especially from mono-stabilized substrates such as diazomethyl ketones and alpha-diazocarboxylates. In some cases, these compounds must first be activated through the introduction of additional electron withdrawing groups to help stabilize the compound. Under certain methodology, this additional electron withdrawing group will be removed during the subsequent diazo-transfer reaction. Taber et. al. developed a method that uses a $TiCl_4$ mediated benzoylation reaction (addition of a benzolate group to the alpha carbon of a carbonyl) to allow for the synthesis of alpha-diazocarbonyls and their corresponding alpha-diazocarboxylate compounds from starting materials that do not contain additional stabilization.[15]



Scheme 3: Taber et. al. diazo transfer to an activated carboxylate

2.2 Rhodium Catalysts

Carbenoid intermediates are generated through transition metal catalyzed reactions. A number of transition and non-transition metals can be used including copper, cobalt, iron, palladium, rhodium, ruthenium, gold, osmium, iridium, nickel, silver and others. Rhodium (II) catalysts have been found more effective and are generally preferred

due to their effectivity and the availability of different ligands that can be coordinated to it. Assorted catalyst ligands allow for contrasting selectivity and reactivity, making Rhodium (II) catalysts among the most widely used in organic synthesis[16].

Rhodium (II) carboxylates contain bridging ligands in four equatorial sites on each rhodium atom, containing D_{4h} symmetry. This leaves two axial coordination sites open to bond with exogenous ligands or solvent. These positions are typically taken up by solvent in the absence of diazocarbonyl species.

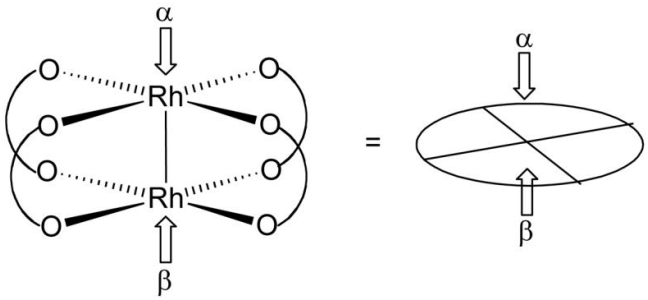


Figure 4: General structure of rhodium (II) complexes[17]

The carbenoid precursor is formed by the diazo-containing carbon attack of the open coordination site (Figure 4). This occurs with the concomitant loss of nitrogen gas (N_2). This generates a metal stabilized carbene(oid).

Although many structural motifs are available, two types of rhodium (II) catalysts commonly used are rhodium(II) carboxylates and rhodium(II) carboxamidates (Figure 6). Rhodium (II) complexes of this type are diamagnetic and a d^7 electron configuration complex. They are generally stable at room temperature and to air oxidation without being hygroscopic, which makes them much easier to use than other, more reactive catalysts like copper (I) catalysts[18].

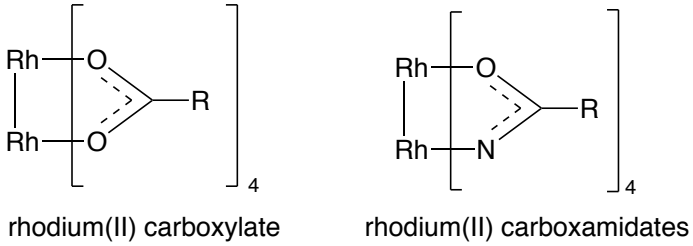


Figure 5: General structures of rhodium (II) complexes

Rhodium (II) tetraacetate ($Rh_2(OAc)_4$) is one of the most widely used of the rhodium catalysts. It is the electronic midpoint between electron donating and electron withdrawing ligands. It was identified as an efficient catalyst for diazo-decomposition type reactions by Teyssié in 1973[19]. The catalyst structure has a look similar to that of a lantern when looking down the metal-metal bond with an electron deficient center and electron rich outer shell[20] (Figure 7). The acetate ligands easily exchange with alternate ligands such as amidates, sulfates, or phosphates.

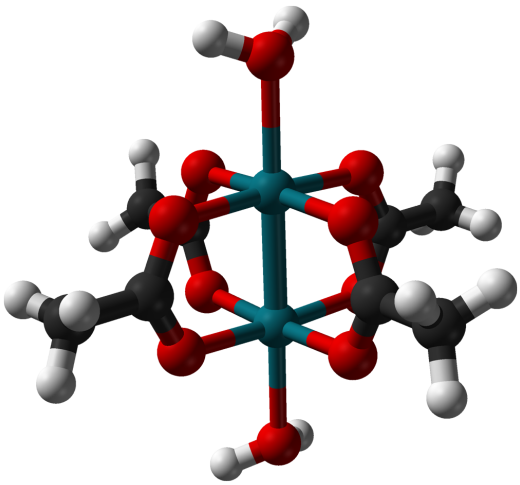


Figure 6: Rhodium (II) tetraacetate dihydrate crystal structure[21] (water is bonded to the open coordination sites)

Rhodium carboxylates participate in a process where electrons of the transition metal d orbitals are transferred to the ligand π* antibonding orbital, called π-back bonding or π-back donation. This results in a conformation where two of the ligands are eclipsed by the carbene (Figure 8). When rhodium is bonded to π-back bonding ligands, the length of the Rh-Rh bond and the Rh-C bond both significantly shorten. The Rh-Rh bond length will vary depending on the ligand identity. The electronic character of the ligand and the π-acceptor/σ-donor capabilities of the carbenoid carbon in part determine the amount of back-bonding present in a rhodium carboxylate[22]. Both effects determine how much electron density the carbene can accept in the Rh- π* orbital and donate electron density into the Rh- σ* orbital.

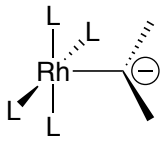


Figure 7: Eclipsed conformation resulting from π back bonding

The reactivity and selectivity of rhodium (II) catalysts is determined by the electronic nature of the bound ligands. When the ligand has greater electron donation, the catalyst will be less reactive but more selective. If the ligand is more electron withdrawing, the carbenoid intermediate will bond to the substrate in an earlier transition state which both increases the reactivity and decreases the selectivity. Rhodium perfluorobutyrate, $Rh_2(pfb)_4$ (Figure 9) is a reactive rhodium catalyst due to the electron withdrawing nature of the perfluorobutyrate ligands. It also contains several C-F electron-withdrawing bonds, which make the rhodium (II) complex more electrophilic and contribute to its reactivity[23].

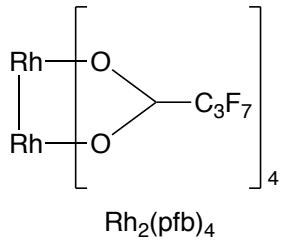
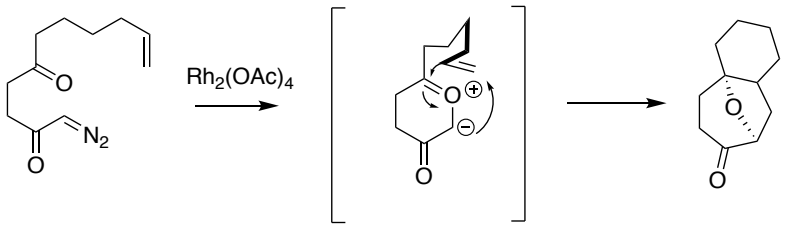


Figure 8: $Rh_2(pfb)_4$ structure

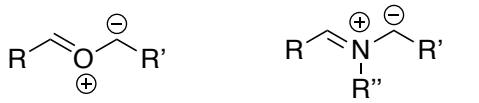
2.3 Ylides and 1,3-Dipolar Cycloaddition Reactions

Cyclic ethers are a common organic motif for a number of natural products. The development of efficient methods to synthesize these types of compounds are of paramount importance in natural product chemistry. The utilization of a transition metal catalyzed 1,3-dipolar cycloaddition reaction can provide entry into this class of natural products. In general, cycloaddition reactions allow for the formation of highly functionalized cyclic structures containing a number of stereocenters, which is a reaction type that is extremely useful in synthetic chemistry.[24] The products of cycloaddition tend to be much more complex than the reactants used in this type of reaction. A general example of the 1,3-dipolar cycloaddition used in this case is shown below in scheme 4.



Scheme 4: An example of a [1,3]-dipolar cycloaddition

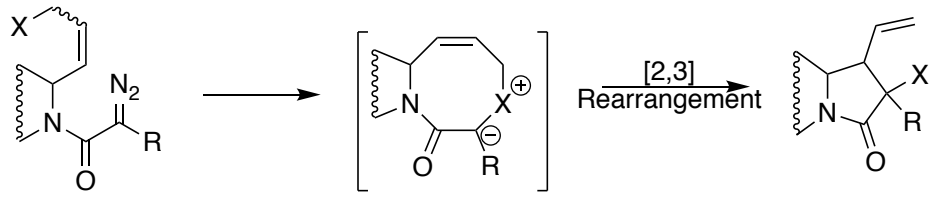
1,3-dipolar species, commonly called ylides, are a necessary to the cycloaddition reactions. Ylides are formally neutral compounds that contain a formal cation and a formal anion in the same structure. The anion is typically formed from the carbene carbon, where the cation is usually formed from oxygen, nitrogen, or sulfur atoms that act as a Lewis base. Examples are shown below in Figure 9.



Carbonyl Ylide Azomethine Ylide

Figure 9: Examples of Ylides

Once the ylide is created, it can undergo several different types of reactions depending on the substitution present. These reactions are: 1,3-dipolar cycloaddition, [2,3]-sigmatropic rearrangement reactions of allyl- substituted ylide intermediates, β-hydride elimination and a [1,2]-insertion reaction (also known as a Stevens rearrangement). A [2,3]-sigmatropic rearrangement involves a heteroatom (X) interacting with an α-diazocarbonyl moiety. The heteroatom is attacked by the heteroatom lone pair of electrons to produce the ylide. The ylide then rearranges *via* a [2,3]- sigmatropic shift (Scheme 3).



Scheme 5: McMills group example of a [2,3] sigmatropic rearrangement to form a cyclic amine[25]

Simple acyclic carbonyl ylides can also be used in an intramolecular cycloaddition process to form epoxides. The epoxide is typically part of an equilibrium process, but epoxides have also been specifically generated from ylides. These are often seen as precursors to the ylide formation, as the epoxide is in equilibrium[26]. Additionally, they can be used to form substituted ethers through a concerted rearrangement and subsequent internal proton transfer. Figure 10 shows the formation of an epoxide (left), heterocycle (right) and substituted ether (bottom).

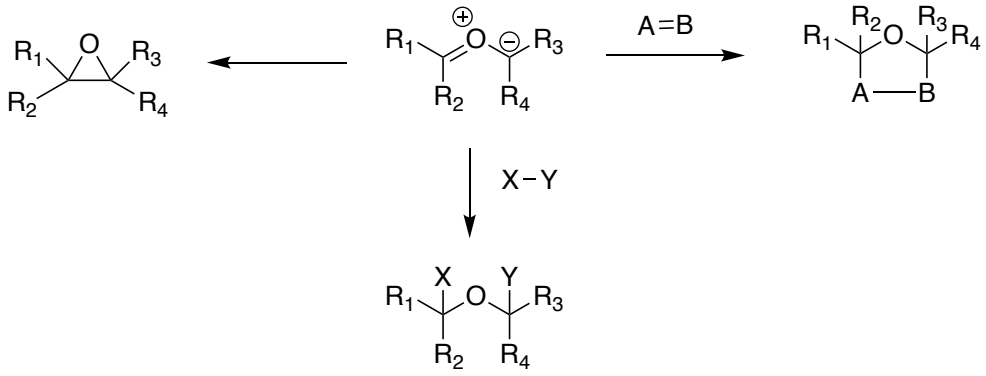
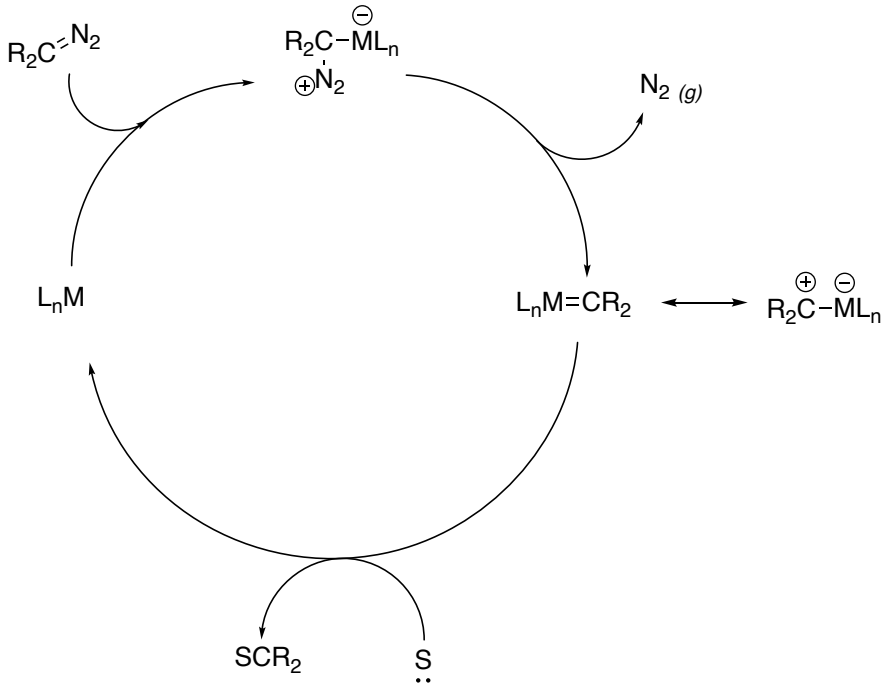


Figure 10: Reactions of carbonyl ylides

Ylide reactions are initiated through the decomposition of the α-diazocarbonyl species; once formed, reaction with a transition metal catalyst results in formation of an

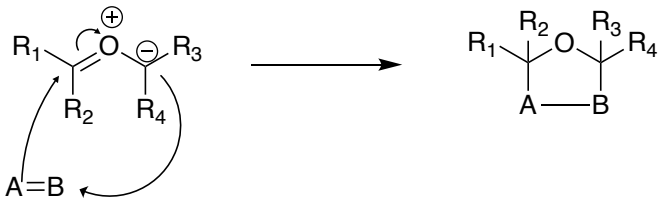
electrophilic carbene complex. Subsequently, the diazo group dissociates, releasing nitrogen gas, and forming a metal-carbenoid species. The reactive carbene intermediate is stabilized by the transition metal catalyst. The carbene is then transferred from the transition metal to an acceptor through the addition of a nucleophile or Lewis base (S) to the reaction. This causes the metal ligand complex to dissociate and generates the ylide intermediate.[27]



Scheme 6: Catalytic cycle of transition metal catalyzed diazodecomposition

Ylides are often used as the dipolar intermediate in 1,3-dipolar cycloaddition reactions. Once the ylide is formed, it can be trapped by an alkene or alkyne dipolarophile. The cycloaddition reaction between a dipolar intermediate and a dipolarophile resembles a neutral Diels-Alder cycloaddition reaction[28] ([3π+2π] vs. [4π +2π]), resulting in the formation of an oxacycle) (Scheme 4). This reaction involves 2π

electrons of the dipolarophile and a pair of π electrons as well as a pair of nonbonding electrons from the heteroatom of the 1,3-dipole which form a 5-member heterocyclic compound. In general, the intramolecular reaction will form 5 and 6 membered cycles are the favored product, however, 7-membered rings are also generated.



Scheme 7: General mechanism of 1,3-dipolar cycloaddition via ylide intermediate

Like the Diels-Alder reaction, the transition state of the dipolar cycloaddition can be either endo or exo. Both secondary orbital interactions (similar to those in a Diels-Alder reaction) and repulsive steric interactions influence the diastereoselectivity of the reaction. In the reaction between dipolarophile acrylonitrile and dipole N-methyl nitrene, there was marked preference for the trans product (77% trans: 23% cis). This can be attributed to the favorable secondary orbital interaction of the endo transition state (Figure 11).

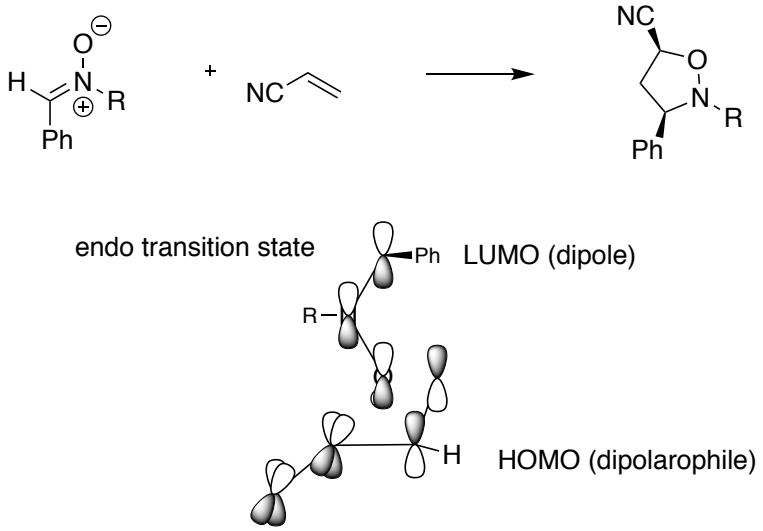
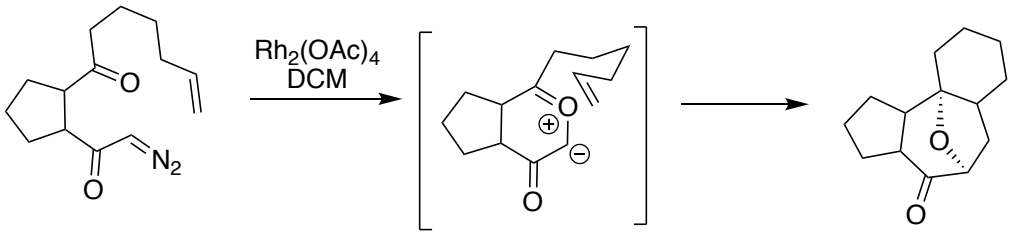


Figure 11: Endo transition state of the reaction between the N-methyl nitrone ylide and acrylonitrile

The genesis for the synthesis of an azaphorbol began with the carbocyclic intramolecular 1,3-dipolar cycloaddition using tethered alkenes, side chains of a certain length, (in the case of azaphorbol 6 carbons) the stereoselectivity is attributed to the tethered alkene forming a chair-like, exo transition state. This was used by the McMills group during their synthesis of phorbol in 1994 (Scheme 8).[29]



Scheme 8: McMills group synthesis of phorbol

The substituents bonded to the dipolarophile can also influence the selectivity and reactivity of the reaction. Electron withdrawing groups (electrophilic dipolarophiles) have

a lowest unoccupied molecular orbital (LUMO) value that will favor the interaction of dipolarophile LUMO and the highest occupied molecular orbital (HOMO) of the dipole. Electron donating groups have higher HOMO's and favor the interaction between the HOMO of the dipolarophile and the LUMO of the dipole.

2.4 Phorbol Esters

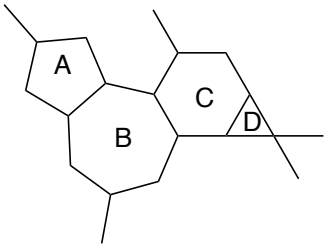


Figure 12: General structure and ring labeling of tigliane compounds

Phorbols are members of the tigliane diterpenes, a class are molecules that are tricyclic in nature, containing multiple hydroxyl groups that can be esterified to provide fatty acid-like side chains for the molecule. They are found in several plants including *Croton tiglium L*, *Sapium indicum*, and *Jatropha curcas*. The bioactive phorbol esters result from the esterification of two hydroxyl groups on neighboring carbons to prepare fatty acid-like side chains with long hydrocarbon tails. Tigliane diterpenes have a core scaffold that contains four linearly fused rings encompassing a 5-membered ring A, which is trans disposed to the adjoining fused 7-member ring B. The 6-member C ring is fused to the 3-member ring D.

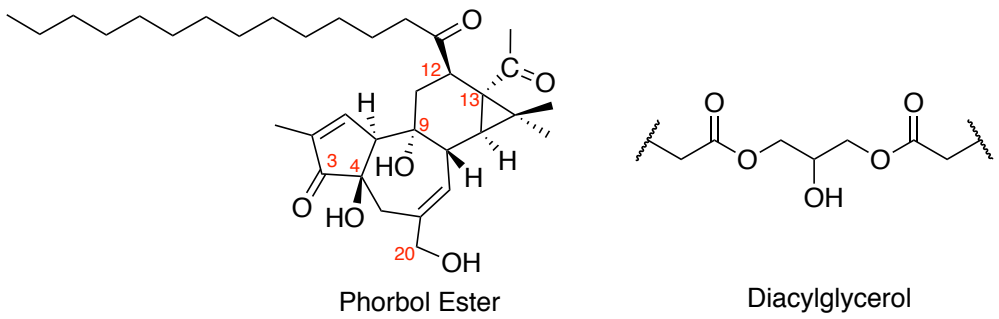


Figure 13: Phorbol ester and diacylglycerol

This class of molecules has been found to display a wide variety of biological activities. Biologically active members of the phorbol family are amphiphilic, having both hydrophilic (affinity for water/polar molecules) and hydrophobic (affinity for fat/nonpolar molecules) properties, as well as the ability to bind to phospholipid membrane receptors with little interaction with the hydrocarbon core. They have been found to be tumor promoters as well as having anti-tumor properties[30]. Tumor promoting activity can be used to help study the mechanism and pathways of carcinogenesis, which is useful in the study of cancers and their pharmacological basis. Some of the anti-tumor properties that make this class novel drug-like molecules, provide a new target or pathway for cancer drugs. Activity is thought to be due to its binding of protein kinase C (PKC). PKC is normally activated by diacylglycerol (DAG), the endogenous ligand, which has some structural similarities to the phorbol ester. Wender et. al. published a study showing that the hydroxyls/esters at C9, C12, and C13 (Figure 13) correspond to a bioactive conformation of diacylglycerol, and that the hydroxyl groups at C4, C9, C20 actually correspond better to the same conformation of DAG. In the low energy conformation of DAG, the central carbons and oxygens of both the R and S enantiomers of DAG overlay specified oxygens of phorbol.[31] However, phorbol esters are stronger

binders and thus stronger activators of PKC than the endogenous substrate, DAG, which is rapidly hydrolyzed after interacting with PKC.

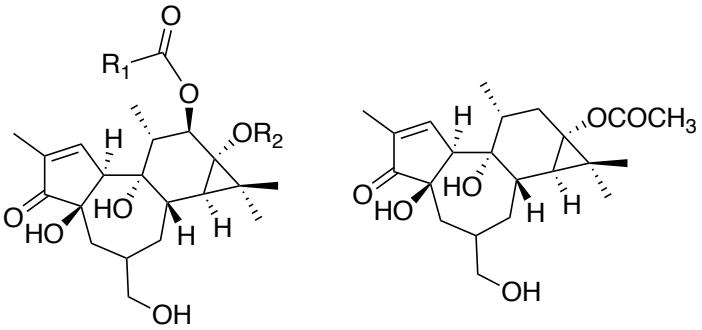
Protein Kinase C displays less specificity than other protein kinases, but typically phosphorylates serine and threonine residues in basic protein sequences. PKC has phosphorylation, ATPase, and phosphatase activity, with phosphatase activity usually in the presence of large amounts of ADP. The kinase itself typically uses the energy provided through the dephosphorylation of ATP through its ATPase activity to phosphorylate many substrates like inositol triphosphate (IP3), that can then act downstream in a signaling cascade.[32] Through these activities, PKC is involved in receptor desensitization, modulation of membrane structure events, immune response modulation, cell growth regulation, learning, memory, and other functions.

Phorbol esters and diacylglycerol recruit PKC to the membrane and act as hydrophobic anchors for the enzyme in the membrane, causing an increase in the membrane affinity of PKC. It has been proposed that phorbol esters convert PKC into its constitutive form, meaning that once activated by a phorbol ester, PKC will remain active until the enzyme is degraded. Phorbol ester binding alters the surface hydrophobicity of the hydrophilic ligand groove of PKC and promotes membrane interaction without conformational change of the enzyme. This leads to the enzyme being irreversibly inserted into the cell membrane, which then hyper-activates PKC and leads to cell proliferation and the tumor promoting cellular activity of phorbol esters.

This class of compounds is co-carcinogenic, while on their own are not carcinogenic. But, when cells are exposed to phorbol esters and a sub-onset dosage of the

carcinogen together, the phorbol ester will amplify the effects of the carcinogen. Longer incubations of phorbol esters in PKC can lead to down regulation of the kinase and apoptotic activity.

Tetradodecanoyl phorbol acetate (TPA) (Figure 14) has been assayed for HIV-1 inhibition and was found to have an IC_{100} = 0.48 ng/mL. It was also found to activate 100% of PKC at a level of 10 ng/mL. Another phorbol derivative, phorbol 12- acetate-13-decanoate commonly known as isophorbol, was also found to produce anti-HIV cytopathic effects with an IC_{100} of 7.6 ng/mL without activating PKC. A structure-activity relationship study was done on these two compounds, looking at their inhibition of HIV-1 and cytopathic effects, as well as the activation of PKC. Multiple derivatives were prepared and assayed. It was concluded that a) compounds with a trans-fused A/B ring juncture had higher bioactivity, b) acetylation of the C20 hydroxyl group, methylation of the C4 alcohol or reduction of C3 carbonyl group, all significantly reduced the inhibition of CPE and activation of PKC, and c) increasing or decreasing the length of the fatty acid chain of isophorbol lead to a corresponding reduction of PKC inhibition. Another phorbol analog, prostratin, has been shown to activate latent HIV, providing a method which could potentially eliminate HIV in combination with highly active antiretroviral therapy.



TPA
$R_1 = (CH_2)_{12}CH_3$
$R_2 = Ac$
isophorbol
$R_1 = Ac$
$R_2 = (CH_2)_8CH_3$

Prostratin

Figure 14: Phorbol esters 4β-12-O-tetradecanoylphorbol-13-acetate (TPA) and prostratin

CHAPTER 3: AZOPHORBOL-SYNTHETIC APPROACHES

3.1 Overview

Synthetic Groups chose phorbol esters as a synthetic target due to their wide range of biological activities. The activity of this class of compounds at PKC is due to their structural similarities to the endogenous tumor promoter DAG. PKC is involved in several different regulation and other biological pathways. The synthesis of a nitrogen substituted analog, 4-Azaphorbol, was chosen due to the dearth of nitrogen analogs currently found in literature and the promising biological activity of similar structural motifs, including aconitine and cephalotaxine.

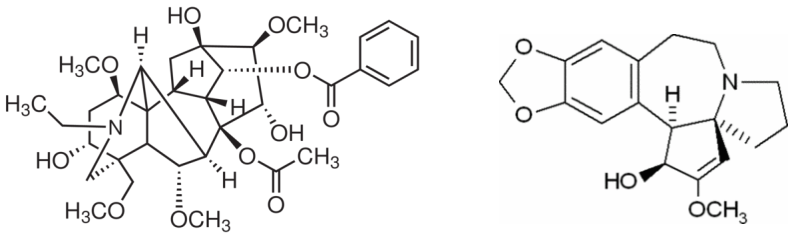
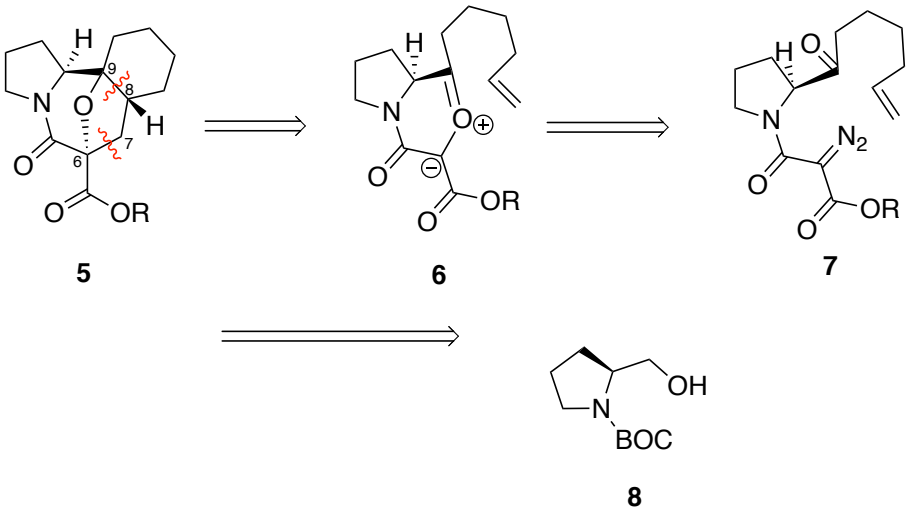


Figure 15: Structures of aconitine (left) and cephalotaxine (right)

3.2 Synthetic Strategy

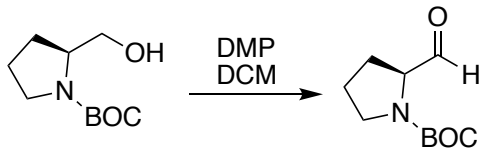
It was determined through a thorough retrosynthetic analysis of an azaphorbol analog that a [1,3]-dipolar cycloaddition could provide tigliane core of 4-azaphorbol. Once a synthesis of the structural core is completed, a variety of analogs can be prepared and tested for biological activity and structure/activity relationships. The basic azabicyclo[5.4.0]undecane core can be formed through a diazodecomposition reaction that generates the 1,3-dipolar intermediate followed by an intramolecular cycloaddition reaction forming rings B and C, while using a proline A-ring core as the anchor for synthesis. The diazo-precursor can be synthesized in several steps beginning with commercially available D- or L-proline. Our basic retrosynthetic analysis is seen in Scheme 6. A rhodium(II) catalyzed 1,3-dipolar cycloaddition can be used to form carbon bonds C9-C8, and C7-C6. The ylide can be generated through the diazo- decomposition reaction of the α-diazodicarbonyl **7**. Ultimately, the precursor to the diazo-decomposition can be generated through a series of reactions involving a Grignard addition for the olefin tether and an acylation to attach the dicarbonyl group.



Scheme 9: Retrosynthesis of Azaphorbol

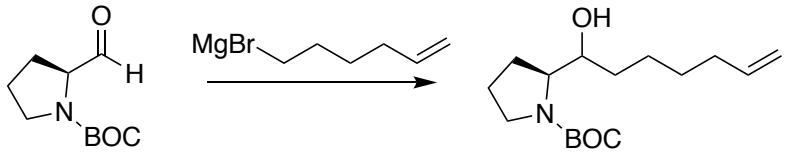
3.3 Synthesis of Azaphorbol

We began the synthesis by preparing BOC-prolinol from L-proline. After finding the BOC-prolinol to be commercially available, it was determined that the purchase of this material was less expensive than the overall synthesis from proline. Dess-Martin Periodinane (DMP) was chosen over other oxidants, due to its mild reaction conditions and ease of product purification. The oxidation of BOC-prolinol was performed in DCM at room temperature and resulted in an 80% yield of a carbonyl-containing precursor to further reaction *via* Grignard addition.



Scheme 10: Oxidation of BOC-prolinol

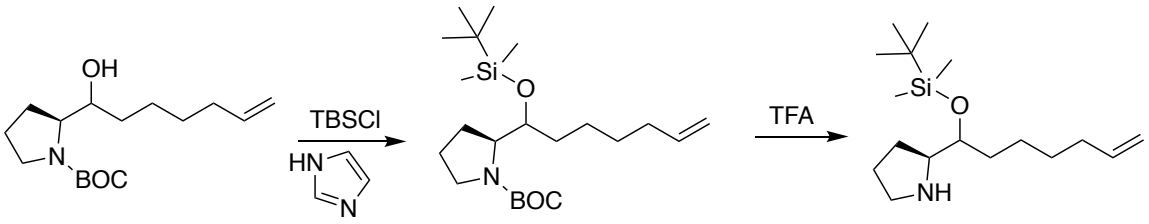
Commercially available 6-bromo-1-hexene was used as the precursor to Grignard reagent formation, forming the Grignard upon addition of magnesium metal at reflux in THF. The aldehyde was then added dropwise to the cooled Grignard reagent to afford a racemic mixture of secondary alcohols as the 1,2-addition product. This was confirmed through the ^{1}H NMR and the presence of two new alkene resonances at δ5.66 ppm and δ4.83 ppm as well as the loss of the aldehyde doublet at δ9.36 ppm.



Scheme 11: BOC-Prolinal Grignard Reaction

The resulting alcohol from the Grignard reaction was protected (tert-butyl dimethylsilyl chloride (TBS-Cl)) as its silyl ether due to presumed interference of the hydroxyl O-H bond in subsequent reactions in the synthetic sequence. This reduces any meddlesome reactivity of the hydroxyl group to allow later reactions such as the strategic N-amidoester bond formation to occur. In order for the N-substitution of the proline nitrogen to be successful later in the synthesis, it was determined that nitrogen of the proline moiety needed to be deprotected. The deprotection was carried out using a strong acid, trifluoroacetic acid (TFA). ^{1}H-NMR was used to detect the loss of the N-protecting BOC group. The BOC protecting group t-butyl singlet peak no longer appeared in the ^{1}H NMR (Figure 15) after a rapid reaction (15-30 min). This corresponded to the loss of the 9 equivalent protons of the BOC protecting t-butyl group. Purification *via* flash column chromatography proved difficult due to interaction of the basic amine with the acidic

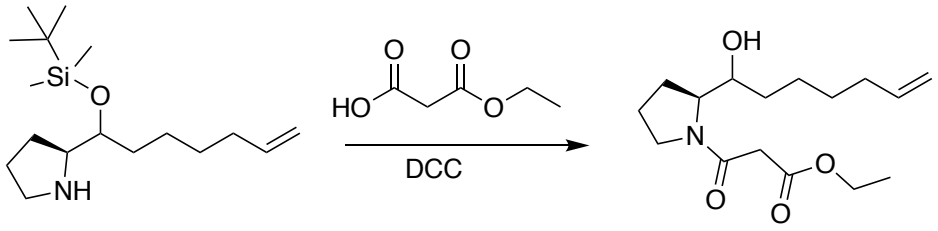
silica gel, causing band widening and thus low yields. Band widening increases the total time it takes for a product to elute from the column. It increases the potential for elution overlap with other compounds in the column and decreases the column efficiency. This leads to a decrease in the total amount of isolated product. The proton NMR of the crude product was compared with that of the purified product and it was determined that the crude reaction product was of sufficient purity to continue without further purification. This allowed for higher overall yields for the reaction.



Scheme 12: TBS protection and BOC deprotection

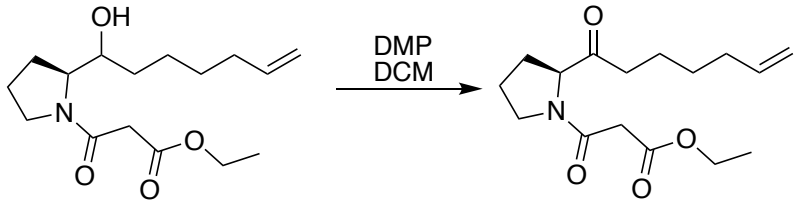
Amide formation of the proline secondary amine with ethyl malonyl chloride proved to be a difficult reaction. It was attempted with various bases such as diisopropylethyl amine (Hünig's base), NaH, and triethyl amine, all which proved either inconsistent or ineffective. The same reaction was attempted adding additional catalyst, DMAP. This provided a product, however the ^{1}H NMR showed peaks at δ4.02, δ3.12, and δ0 ppm, (Figure 16) which were inconsistent with the expected product. Originally, the resonances were thought to correspond to ethyl acetate or other solvent, but after leaving on high vacuum overnight to remove any excess solvent, the peaks remained. It was suspected that the peaks instead, corresponded to ethyl malonate or some reaction by-product associated with it. The two functional groups are similar in structure. This product was taken on to the next oxidation step, despite the impurity. An amidation

coupling reaction with mono-ethyl malonic acid and DCC, as the coupling agent, was also attempted, and while a 40% chemical yield was obtained, the yield was inconsistent when the reaction was repeated. Both reactions, with the mono-acid chloride or the malonate itself resulted in an unexpected deprotection of the silyl-protected alcohol as well as the expected amidation of the amine.



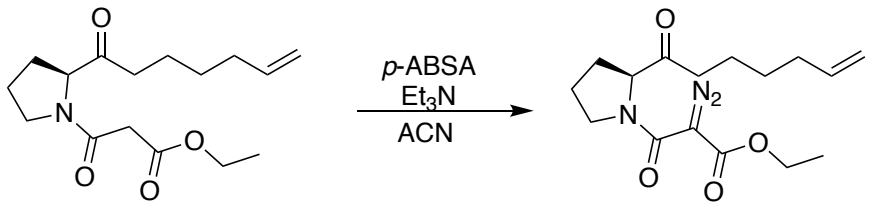
Scheme 13: N-Amidation and silyl ether deprotection

The hydroxyl group at C9 of the β-ester-amide can either be oxidized to the ketone or alternatively, the β-amidoester can be diazotized. Our original experiment involved the diazotization of the β-amidoester. However, while the diazo-transfer was successful, every subsequent oxidation attempt was unsuccessful. This was not totally unexpected, as diazo groups are prone to oxidation under many oxidative conditions. To correct the issues found in this approach, the diazo-transfer reaction was postponed until after the alcohol oxidation. Fortunately, the alcohol oxidized smoothly to the C9 ketone using Dess-Martin Periodinane (DMP).



Scheme 14: Oxidation to secondary ketone

Despite the additional keto group present at C9, the acidity of the β-amidoester group is orders of magnitude more acidic than any other α-hydrogens. After the oxidation, the diazo-transfer reaction was accomplished easily using Davies reagent, p-ABSA in acetonitrile stirring overnight. The p-ABSA by-product is easily separated from the diazo product due to its insolubility in acetonitrile. This was confirmed through ^{1}H NMR, seen from the loss of the singlet that corresponds to the 2-methylene hydrogens of the β-amidoester at δ1.93 ppm (Figure 17).



Scheme 15: Diazo transfer reaction

Previously our group had attempted the 1,3-dipolar cycloaddition using rhodium (II) tetraacetate. This did not produce the desired cycloadduct however, and it was determined that a more reactive rhodium (II) catalyst was needed to produce the desired product. Rhodium (II) perfluorobutyramide was identified as having highly electron withdrawing ligands which increases the oxophilicity of the reaction and the reactivity of

the catalyst itself. Keaney and Wood synthesized this catalyst in 2005 from rhodium (II) tetraacetate utilizing microwave irradiation to decrease the labor intensity of previously reported synthesis.[33] A reaction was attempted with the diazo product from the previous reaction and rhodium (II) perfluorobutyramide. However, after 24 hours at room temperature and 24 hours under reflux, the ^{1}H NMR was almost identical to the starting material. This is believed to be due to the small amount of both starting material (10 mg, 0.0311 mmol) and catalyst (0.7 mg, 0.00156 mmol) and potential impurities in the starting material.

To complete this synthesis, our lab will work to bring more material forward so that there is sufficient material to obtain more spectroscopic data and insure purity. More of the rhodium (II) perfluorobutyramide catalyst will be synthesized, and the 1,3-dipolar cycloaddition will be carried out on a larger scale than was attempted in this thesis. Once the cycloaddition has been completed, the product will be tested for biological activity. If this proves promising, the method described can be used to prepare derivatives of the azaphorbol for structure-activity studies.

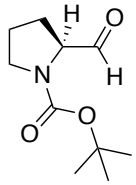
CHAPTER 4: EXPERIMENTAL

4.1 General Experimental

All reactions were carried out under an argon or nitrogen (N_2) atmosphere employing anhydrous conditions unless otherwise noted. Acetonitrile (ACN) was dried over molecular sieves. Dichloromethane (DCM) and tetrahydrofuran (THF) were dried using a Solv-Tek Inc. column purification/drying system, which uses low-pressure nitrogen or argon gas to force solvents through various filter to remove moisture and

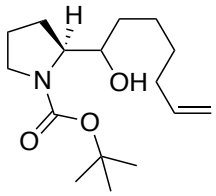
impurities. Reagents purchased from commercial sources were used without further purification unless otherwise noted. Analytical TLC was performed on 0.25 mm silica gel (MF254) plates purchased from EMD Chemicals, Inc. UV light, potassium permanganate solution (1.5 g $KMnO_4$, 10 g K_2CO_3, 1.25 mL 10% NaOH in 200 mL H_2O), and phosphomolybdic acid solution (10 g phosphomolybdic acid, 100 mL ethanol) were used as visualizing reagents. Flash column chromatography was carried out using Merck silica 60 (230-400 mesh). ^1H NMR and ^{13}C NMR spectra were recorded at 300- and 500MHz on both a Bruker AVANCE-300 and Bruker ASCEND-500 spectrometer respectively. Chemical shifts (δ) 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 or solutions, using NaCl plates.

4.1.1 General Materials and Methods



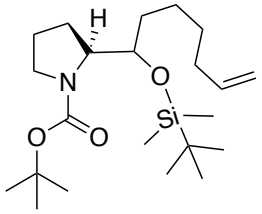
(S)-tert-butyl-2-formylpyrrolidine-1-carboxylate: To a dry round bottom flask containing (*S*)-*tert*-butyl-2-(hydroxymethyl)pyrrolidine-1-carboxylate (5.0 g, 24.8 mmol) in DCM (25 mL) at 0°C was added Dess-Martin periodinane (DMP) (11.593 g, 27.3 mmol). The resulting suspension was stirred at room temperature until completion by

TLC. The reaction was then quenched with both saturated $Na_2S_2O_3$ (aq) and saturated $NaHCO_3$ (aq), and stirred vigorously until clear. The aqueous layer was extracted with EA (x2). The combined organic layers were washed with saturated $NaHCO_3$ (aq) and brine. The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The crude mixture was purified *via* flash column chromatography (20% ethyl acetate: hexane) to afford a clear oil (3.92 g, 80% yield). ^{1}H NMR (500 MHz $CDCl_3$) δ9.361 (s, 1H) δ4.048 (t, 0.4H) δ3.970 (q, 2H) δ3.892 (t, 0.6H) δ3.3930 (m, 2H) δ1.9685 (m, 1H) δ1.8865 (s, 3H) δ1.802 (m, 1H) δ1.7215 (t, 2H) δ1.5063 (m, 1H) δ1.2942 (d, 9H) δ1.096 (t, 3H). Additional data available in the dissertation of Dr. Alicia Frantz.[34]

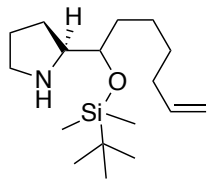


(*S*)-*tert*-butyl-2-(1-hydroxyhept-6-enyl)pyrrolidine-1-carboxylate: To a 3-necked round bottom flask containing Mg turnings (0.57 g, 23.7 mmol), fitted with a condenser and a dropping funnel, was added 6-bromo-1-hexene (3.2 mL, 23.7 mmol) in dry THF (15 mL), dropwise. After approximately 25% of the 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 and no Mg turnings remained in the mixture. The reaction mixture was then cooled to 0°C and (*S*)-*tert*-butyl-2-formylpyrrolidine-1-carboxylate (0.392 g, 19.7 mmol) in dry THF (15 mL) was added dropwise. The reaction mixture was stirred at 0°C for 2 h, until the reaction was complete by TLC. Upon completion, the reaction was

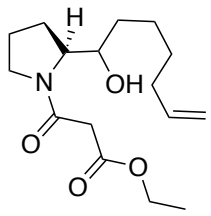
quenched with saturated NH_4Cl (aq) and extracted with ethyl acetate (3x). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. The crude material was purified *via* flash column chromatography (1:1 hexane: ethyl acetate) to afford a yellow oil (4.54 g, 81.5% yield). ^1H NMR (500 MHz $CDCl_3$) 5.65 (m, 1H) δ 4.82 (dd, 2H) δ4.05-3.76 (m, 2H) δ3.39-3.31 (m, 2H) δ3.14 (m, 1H) δ 1.91-1.89 (m, 2H) δ1.81-1.57 (m, 3H) δ1.45 (s, 2H) δ1.31 (s, 9H) 1.27-1.19 (m, 6H) δ 1.11 (t, 1H). Additional data available in the dissertation of Dr. Alicia Frantz.



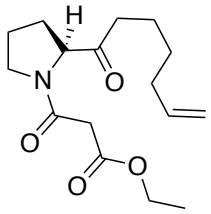
(*S*)-*tert*-butyl-2-(1-(*tert*-butyldimethylsilyloxy)hept-6-enyl)pyrrolidine-1-carboxylate: To a solution containing (*S*)-*tert*-butyl-2-(1-hydroxyhept-6-enyl)pyrrolidine-1-carboxylate (4.04 g, 14.2 mmol) and imidazole (2.42 g, 35.6 mmol) in dry DCM (50 mL) at 0°C was added TBS-Cl (3.22 g, 21.3 mmol). The reaction mixture was warmed to room temperature and stirred for 21 h, until the reaction was determined to be complete by TLC. The reaction mixture was diluted with DCM (50 mL), washed with sat. $NaHCO_3$ (aq) and with brine. The organic layer was dried over Na_2SO_4 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). ^1H NMR (500 MHz $CDCl_3$) δ5.81 (m, 1H) δ4.95 (dd., 2H) δ4.12-3.26 (m, 2H) δ2.04 (m, 2H) δ1.89-1.71 (m, 1H) δ1.61 (s, 3H) δ1.51-1.42 (m, 4H) δ1.26 (s, 9H) δ1.14 (s.1H) δ0.91 (s, 9H) δ0.88 (s, 3H) 0.09 (s, 3H). Additional data available in the dissertation of Dr. Alicia Frantz.



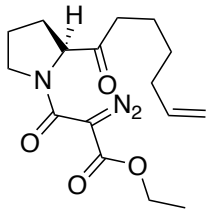
***(S)*-2-(-1(*tert*-butyldimethylsilyloxy)hept-6-enyl)pyrrolidine:** To a solution of (*S*)-*tert*-butyl-2-(1-(*tert*-butyldimethylsilyloxy)hept-6-enyl)pyrrolidine-1-carboxylate (0.50 g, 1.25 mmol) in dry DCM (7.0 mL) under argon at 0ºC was added TFA (7.0 mL) dropwise. The reaction mixture stirred at 0ºC 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 $NaHCO_3$ (aq). The organic layer was washed with brine, dried ($MgSO_4$), and concentrated *in vacuo* to yield a yellow oil (227 mg, 61% yield). ^{1}H NMR (500 MHz $CDCl_3$) δ5.704 (m.1H) δ4.898 (dd, 2H) δ4.041-3.207 (m, 2H) δ1.968 (m, 3H) δ1.796-1.706 (m, 2H) δ1.419 (s, 6H) δ1.318-1.162 (m, 6H) δ0.827 (s, 9H) δ0.011 (s, 3H) ^{13}C NMR (500 MHz, $CDCl_3$) δ138.461, 114.681, 91.849, 77. 275, 77.021, 72.876, 71.324, 65.552, 61.522, 53.417, 37.669, 33.439, 28.846, 25.647, 22.772, 17.983. Additional data available in the dissertation of Dr. Alicia Frantz.



(*S*)-ethyl-3-(2-(1-hydroxyhept-6-enyl)pyrrolidin-1-yl)-3-oxopropanoate: To a solution of *(S*)-2-(-1(*tert*-butyldimethylsilyloxy)hept-6-enyl)pyrrolidine (0.100 g, 0.336 mmol) in dry THF (10 mL) under nitrogen gas was added N,N-dicyclohexylcarbodiimide (0.104 g, 0.504 mmol). The reaction mixture was cooled to 0^{o}C and ethyl malonate (0.044 mL, 0.370 mmol) was added. The reaction mixture was allowed to warm to room temperature and stirred until complete by TLC (2 hours). The reaction mixture was filtered through a pad of celite (to remove urea by-product) and flushed using ethyl acetate. The resulting solution was washed with saturated $NaHCO_3$ (aq). The aqueous layer was extracted with ethyl acetate x3 and the combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated *in vacuo* to yield a clear oil (40 mg, 40% yield). ^{1}H NMR (500 MHz $CDCl_3$) δ5.7082 (m, 1H) δ4.922 (dd, 2H) δ4.103 (m, 2H) δ3.665-3.555 (m, 1H) δ3.1793 (s, 1H) δ 1.949 (m, 3H) δ1.722-1.636 (m, 4H) δ 1.459 (s, 1H) δ1.205-1.158 (m, 5H) δ0.819-0.7853 (m, 3H). Additional data available in the dissertation of Dr. Alicia Frantz.

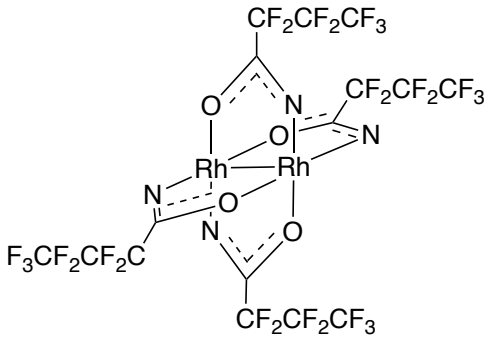


(*S*)-ethyl-2-diazo-3-(2-hept-6-enoylpyrrolidin-1-yl)-3-oxopropanoate: To a solution of (*S*)-ethyl-3-(2-(1-hydroxyhept-6-enyl)pyrrolidin-1-yl)-3-oxopropanoate (0.115 g, 0.386 mmol) in dry DCM at 0°C was added Dess-Martin periodinane (DMP) (0.197 g, 0.464 mmol). The suspension was stirred at room temperature until the reaction showed completion by TLC. Upon completion, the reaction was quenched with both saturated $Na_2S_2O_3$ (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 $NaHCO_3$ (aq), brine then dried (Na_2SO_4) and concentrated under reduced pressure. The crude mixture was purified *via* flash column chromatography (20-50% ethyl acetate: hexane) to afford a clear oil. ^{1}H NMR (500 MHz $CDCl_3$) δ5.1592 (m, 0.6H) δ4.7539 (m, 0.4H) δ4.187-4.075 (dd, 2H) δ2.2174 (t, 2H) δ1.9865 (m, 5H) δ1.5079 (m, 2H) δ1.399 (m, 2H) δ1.155 (m, 2H) δ0.774 (m, 2H) δ0.0314 (d, 1H). Additional data available in the dissertation of Dr. Alicia Frantz.



***(S)*-ethyl-2-diazo-3-(2-(1-hydroxyhept-6-enyl)pyrrolidin-1-yl)-3-oxopropanoate:** To a solution of (*S*)-ethyl-3-(2-(1-hydroxyhept-6-enyl)pyrrolidin-1-yl)-3-oxopropanoate

(0.015 g, 0.0508 mmol) in dry ACN (2.0 mL) was added Et_3N (0.01 mL, 0.0609 mmol) followed by p-ABSA(14.6 mg, 0.0609 mmol) at RT and stirred overnight, forming a white precipitate. The suspension was concentrated under reduced pressure and triturated with DCM (x2) and concentrated under reduced pressure. The crude product was purified *via* flash column chromatography (eluent: 40% ethyl acetate: hexane) to afford a yellow oil (10.9 mg, 73% yield). ^{1}H NMR (500 MHz $CDCl_3$) δ5.141 (m, 0.55H) δ4.746 (m, 0.45H) δ4.1796 (m, 2H) δ2.2103 (t, 2H) δ1.9792 (dd, 5H) δ1.5008 (m, 1H) δ1.392 (m, 3H) 1.1471 (s, 4H) 0.7697 (t, 3H). Additional data available in the dissertation of Dr. Alicia Frantz.



Rhodium perfluorobutyramide: (Keaney & Wood, 2005) To a solution of rhodium acetate (25 mg, 0.056 mmol, 1.0 equiv) in dry chlorobenzene (3 mL) in a microwave vial, was added perfluorobutyramide (120 mg, 0.56 mmol, 10.0 equiv), and Na_2CO_3 (60 mg, 0.56 mmol, 10.0 equiv). The reaction vessel was irradiated with microwave radiation (Biotage Initiator) for 30 minutes at 250°C. The reaction mixture was initially a teal color. After the 30 minute irradiation period, the vial was removed from the microwave and the reaction mixture was a purple color. The reaction mixture was then cooled to room temperature and extracted with perfluoro-n-hexane (3X). The fluorous extracts

were concentrated under reduced pressure. Excess perfluorobutyramide was removed *via* sublimation. The complex was used without further purification, but could be purified by silica gel chromatography (9:1–3:1 hexanes/ethyl acetate) to give $Rh_2(pfm)_4$ (34 mg, 53% yield) as a blue solid. ^{19}F NMR (500 MHz, CD_3CN, -81.5, -117.2, -127.4) (Figure 18).

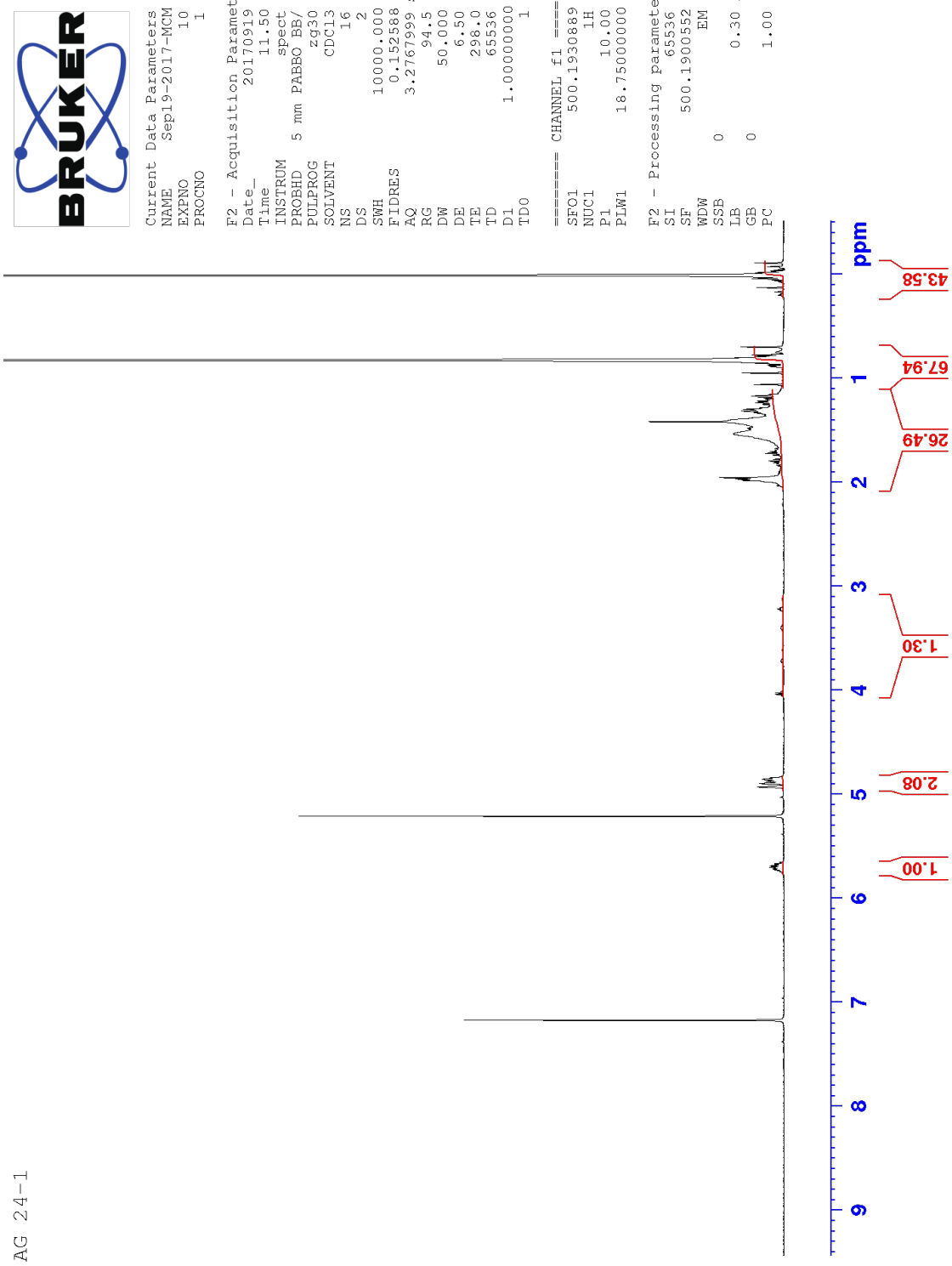


Figure 16: ^{1}H NMR of BOC deprotected substrate

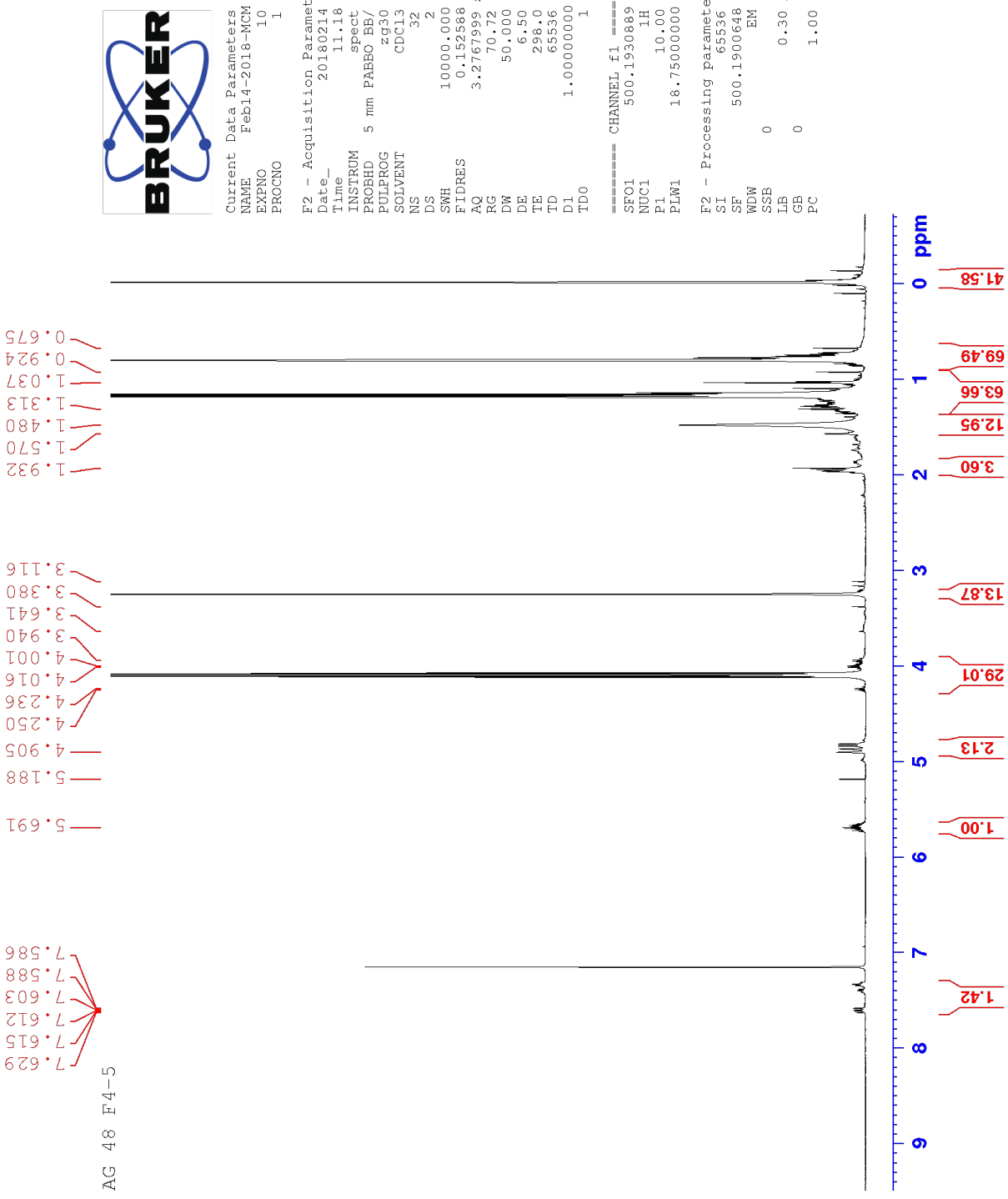


Figure 17: ^{1}H NMR of N-acetylation

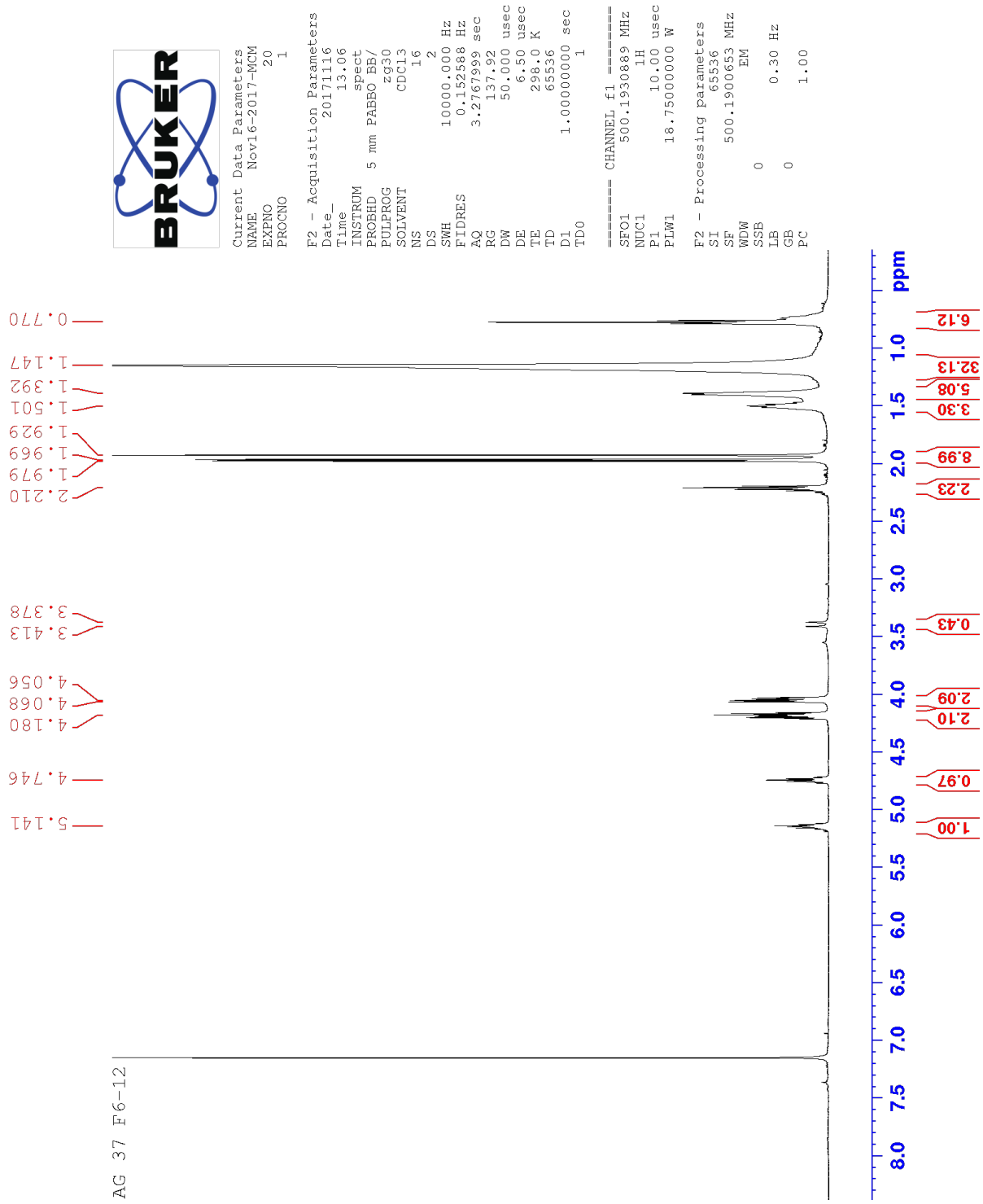


Figure 18: ^{1}H NMR of Azaphorbol precursor

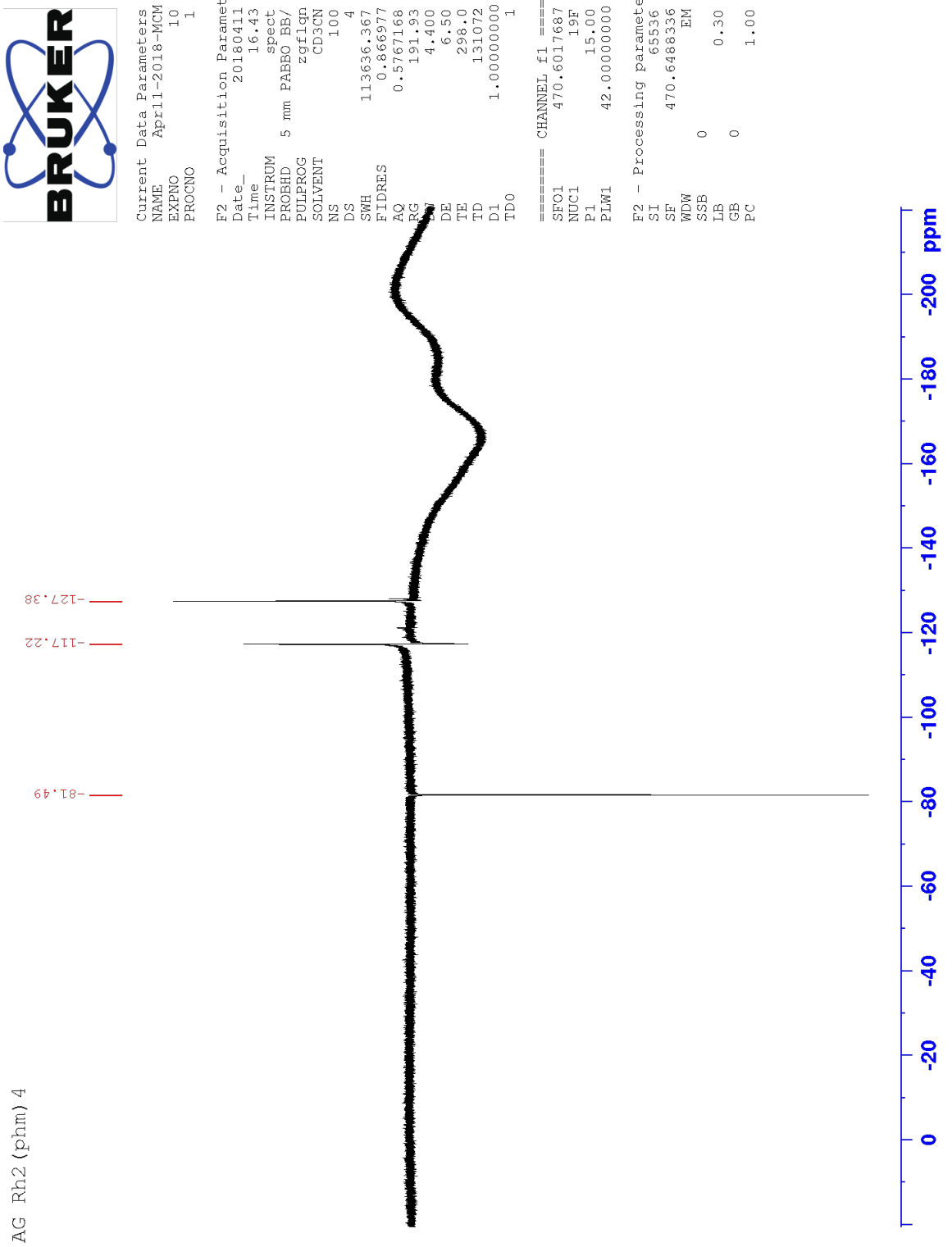


Figure 19: ^{19}F NMR of $Rh_2(pfm)_4$

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