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"Echinacea" and "Spilanthes" are popular over-the-counter herbal supplements derived from raw tissue or tissue extracts from plants such as *Echinacea purpurea* and *Spilanthes acmella*, respectively. Several chemical constituents have been identified as possible candidates for these plants' supposed medicinal activities, and include the "alkylamides," of which dienyl and diynyl analogs are prominent members. Isolation of these compounds from the plants is often difficult and results in poor yields. The goal of our research was to develop an encompassing synthesis scheme for dienyl and diynyl alkylamides (Figure 1) addressing consistent functional groups, using a Wittig reaction, a Horner-Wadsworth-Emmons reaction and a Sonogashira reaction. The total syntheses for these compounds have been reported, but are rather lengthy procedures. We have developed a novel synthesis for a Spilanthol derivative which involves only three reactions and based on the Sonogashira coupling reaction for introduction of the diynyl structure.

Figure 1. General Diynyl and Dienyl Alkylamide Structures

A CONVERGENT AND MODULAR SYNTHESIS OF DIENYL AND DIYNYL ISOBUTYLAMIDE NATURAL PRODUCTS FROM *ECHINACEA AND SPILANTHES* HERBAL MEDICINES

By

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Approved by

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Committee Chair

APPROVAL PAGE

This thesis has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro.

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Date of Final Oral Examination

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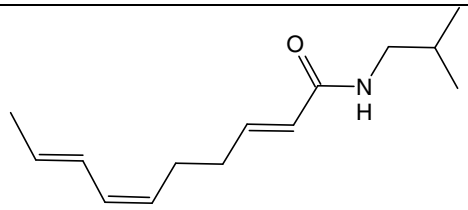
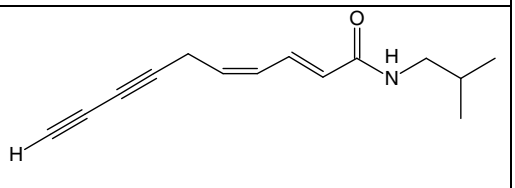
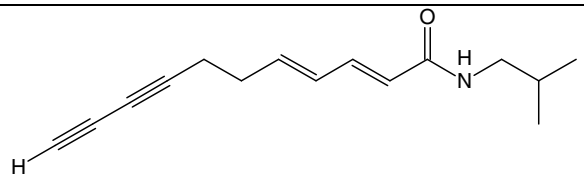
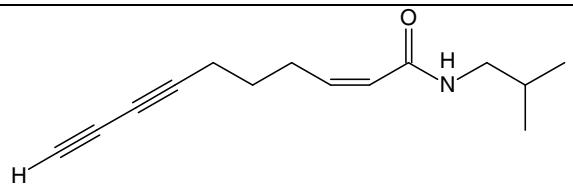
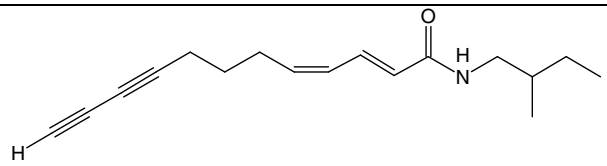
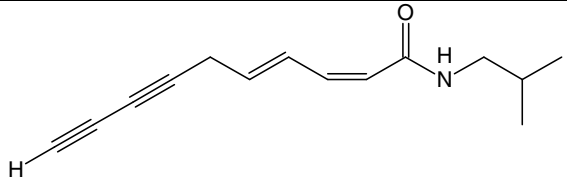
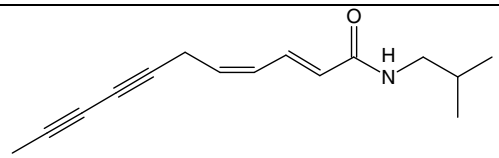
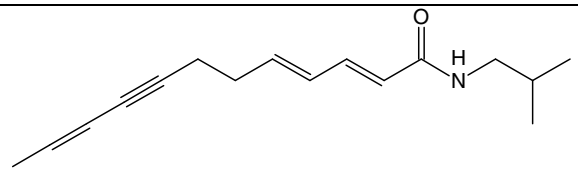
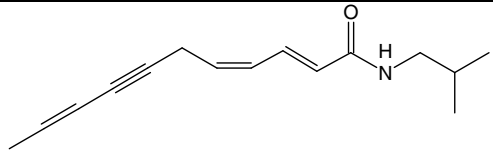
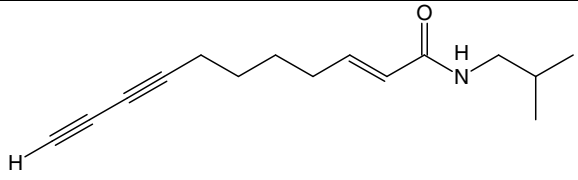
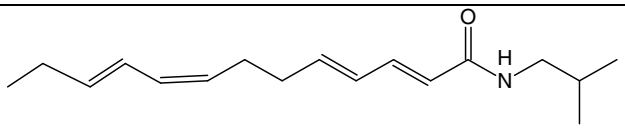
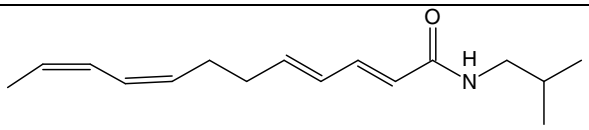
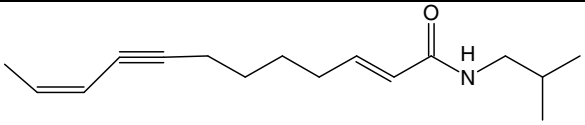
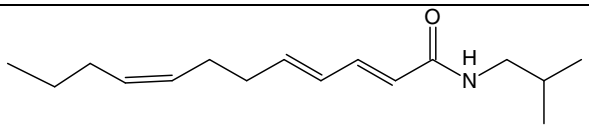
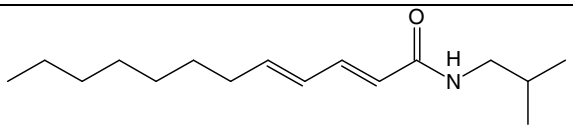
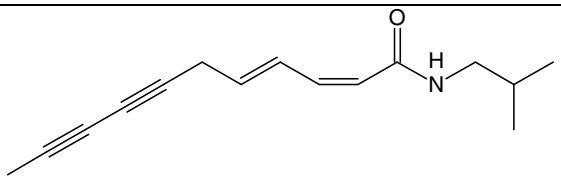
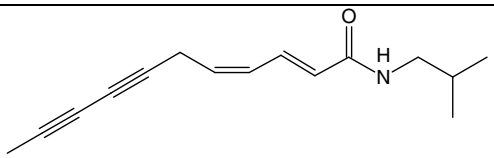
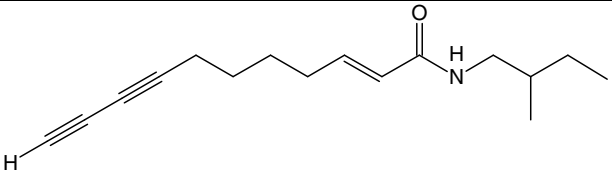
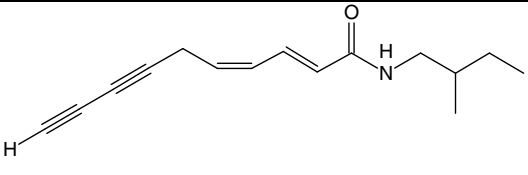
CHAPTER I

INTRODUCTION

Echinacea and *Spilanthes* have served as herbal remedies for the treatment and prevention of various inflammations and diseases. *Echinacea* has primarily been associated with treatments for the common cold and respiratory infections.[1] *Spilanthes*, also known as the toothache plant, has been used for the relief of ailments such as toothaches, stomatitis and throat inflammations.[2] As an herbal supplement, *Echinacea* currently earns in excess of 23 million dollars a year, second only to garlic, in the US market.[3] The extracts of *Echinacea* and *Spilanthes* contain compounds that are known to possess immunomodulatory and anti inflammatory properties.[2, 4, 5] Among these is a class of lipophilic molecules known as alkamides or alkylamides (Figure 1[6]).

Deca-2E,6Z,8E-trienoic acid isobutylamide	
Undeca-2E,4Z-diene-8,10-diynoic acid isobutylamide	

*Undeca-2E-ene-8,10-diynoic acid isobutylamide	
*Undeca-2Z-ene-8,10-diynoic acid isobutylamide	
Undeca-2E,4Z-diene-8,10-diynoic acid 2-methylbutylamide	
Dodeca-2Z,4E-diene-8,10-diynoic acid isobutylamide	
Dodeca-2E,4Z-diene-8,10-diynoic acid isobutylamide	
Dodeca-2E,4E,10E-triene-8-ynoic acid isobutylamide	
Dodeca-2E,4Z,10Z-triene-8-ynoic acid isobutylamide	
*Dodeca-2E-ene-8,10-diynoic acid isobutylamide	

*Dodeca-2E,4E,8Z,10E-tetraenoic acid isobutylamide	
*Dodeca-2E,4E, 8Z,10Z-tetraenoic acid isobutylamide (is this Echinacein?)	
Dodeca-2*E*,10*Z*-dien-8-ynoic acid isobutylamides	
Dodeca-2E,4E,8Z-trienoic acid isobutylamide	
*Dodeca-2E,4E-dienoic acid isobutylamide	
Dodeca-2Z,4E-diene-8,10-diynoic acid isobutylamide	
Dodeca-2E,4Z-diene-8,10-diynoic acid isobutylamide	
Dodeca-2E-ene-8,10-diynoic acid 2-methylbutyl amide	
Dodeca-2E,4Z-diene-8,10-diynoic acid 2-methylbutylamide	

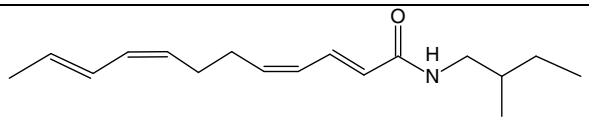
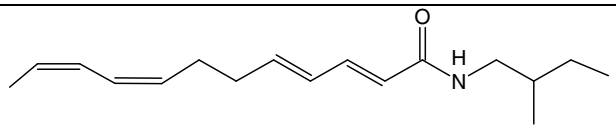
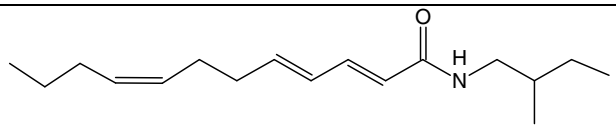
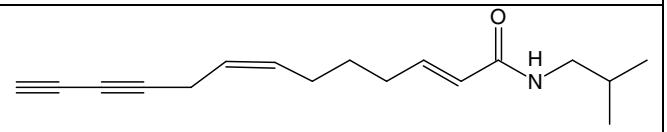
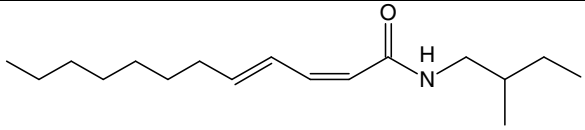
Dodeca-2E,4Z,8Z,10E-tetraenoic acid 2-methylbutylamide	
Dodeca-2E,4E,8Z,10Z-tetraenoic acid 2-methylbutylamide	
Dodeca-2E,4E,8Z-trienoic acid 2-methylbutylamide	
Trideca-2Z-7E-diene-10,12-diynoic acid isobutylamide	
Dodeca-2Z-4E-dienoic acid 2-methylbutylamide	

Figure 1. Alkylamides found in the extracts of *Echinacea* and *Spilanthes.*

Known immune and anti-inflammatory effects of alkylamides include the inhibition of 5-lipoxygenase and cyclooxygenase.[7, 8] Alkylamides upregulated the production of tumor necrosis factor α mRNA and inhibited the production of NO in macrophages upon treatment of lipopolysaccharide.[9, 10] In mice, alkylamides have increased the phagocytic activity of macrophages upon oral administration.[11] Recently alkylamides have demonstrated selectivity for CB2 receptors, by activity similar to that of cannabinoids,[12, 13] and have inhibited the production of interleukin-2 production by human Jurkat T cells.[14] Alkylamides found in *Spilanthes* have demonstrated diuretic properties in rats.[15]

Still, there remains considerable debate as to the medical effectiveness of alkylamides and their parent plants among the medical community.[16] A possible source of this debate stems from the lack of standards in how *Echinacea* and *Spilanthes*, or their extracts, are obtained and used, which leads to a corresponding inconsistency of data in medical trials.[1]

Alkylamides are obtained from the different parts of the *Echinacea* and *Spilanthes* plants and require a variety of extraction methods. For example, researchers have shown that concentrations of alkylamides differ among parts of the Echinacea plant; the roots usually contain higher levels than the aerial parts.[17] Methods of removal involve some or all of the following: cutting, compression, crushing, bruising and drying. The order or combination of these methods often determines the extracted concentrations of alkylamides. For example, bruising Echinacea does not increase (vs. undamaged plant tissue) alkylamide concentration unless it is immediately followed by cutting of the plant.[18] Yet, if the extraction technique is effective, several other factors can influence alkylamide concentration. Latitude[18], growing season[19], wild-grown vs. greenhouse, and transplanting[20] are all factors that influence Echinacea's alkylamides. Finally, some plants will only yield an average of .59 mg of alkylamide per gram of *Echniacea* plant.[21]

Goal of Research

Dr. Nadja Cech's lab at UNCG is investigating the activities of several *Echinacea* and *Spilanthes* alkylamides. The goal of our project was to synthesize members of the alkylamide group to aid Dr. Cech's research. Spilanthol is considered to be one of the most potent alkylamides found in *Spilanthes acmella* and our goal was to develop a synthesis for Spilanthol and derivatives along with the diyne alkylamides. Synthesizing bioactive compounds found in plants, like the alkylamides, would provide facile methods of obtaining these compounds at a lower cost and with greater consistency. Syntheses of alkylamides found in both *Echinacea* and *Spilanthes* have been documented[13, 22-25] however, we offer a novel and convergent model for an alkylamide synthesis. Furthermore, the development of a synthesis for these compounds could aid in the development of novel and potentially bioactive analogs.

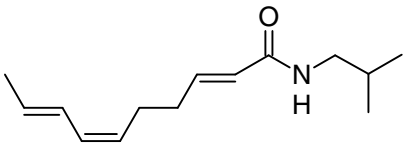


Figure 2. The structure of Spilanthol found in a variety of *Spilanthes* plants.

Alkylamide Synthesis Strategy

In the early stages of developing a synthesis for the *Echinacea* and *Spilanthes* alkylamides, a simple inspection of the alkylamides yielded some consistent moieties.[4] A summary of the structures (Figure 3) includes a couple of terminal alkynes or alkenes linked in tandem, via a varied number of saturated carbons or olefins, to an *N*-isobutyl amide group. Therefore, with these functional groups in mind, we need reactions that 1) form olefin and *sp* hybridized carbon-carbon bonds, 2) are versatile with respect to variety and size within the starting materials and finally, 3) are multifaceted, meaning the reaction must not only link certain moieties to the compound but form needed functional groups in doing so (Figure 4a,b).

Figure 3. The general structures of alkylamides found in *Echinacea* and *Spilanthes* extracts.[2, 26]

We began with a retrosynthetic analysis of each model alkylamide. Immediately apparent is the need of a Wittig type reaction both in the synthesis of the terminal alkenes in olefinic alkylamides and in the installation of the α, β-unsaturated isobutyl amide required for both compounds. The greatest area of variation among the alkylamides in Figure 1 is found within the terminal end, opposite the α, β-unsaturated isobutyl amide. A traditional Wittig reaction would satisfy the terminal olefin moiety as well as allow for

the selection of conditions which offer different stereocontrol, which will ultimately afford the development of analogous compounds with varied olefin geometries. The olefin nearest the carbonyl will be installed using a derivative of the traditional Wittig reaction, a Horner-Wadsworth-Emmons reaction because little variation is needed in this location. With the olefin requirements satisfied, we needed a reaction that would couple two terminal alkynes for the diyne alkylamide. Several transition-metal-catalyzed reactions are available to facilitate the coupling of unsaturated carbons. We specifically selected the palladium-catalyzed reaction known as the Sonogashira reaction for its utility in dealing with acetylenes.

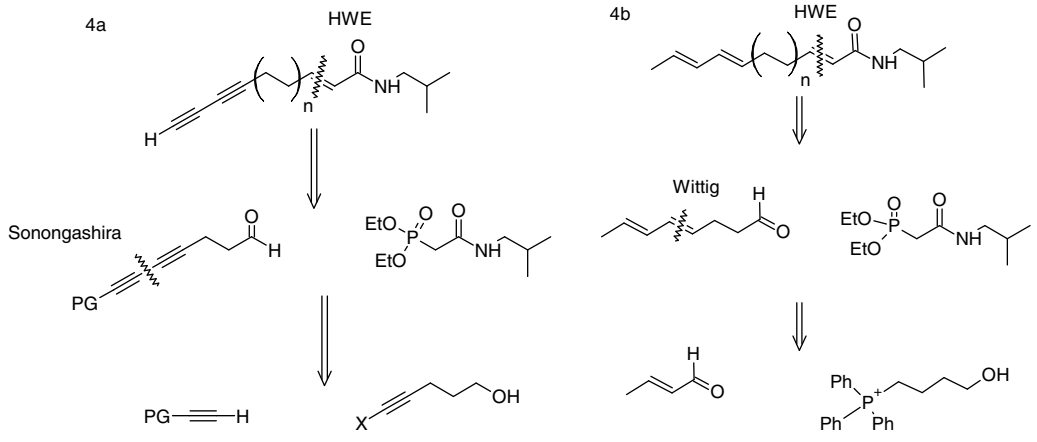


Figure 4. The retrosynthesis schemes for the alkylamides.

Retrosynthesis Diyne Scheme 4a. A general retrosynthesis scheme indicating the use of the primary reactions. The palladium coupling joining the alkynes and the Horner Wadsworth Emmons reaction forming the α, β-unsaturated isobutyl amide.

Retrosynthesis Diene Scheme 4b. A general retrosynthesis scheme demonstrating the application of the Wittig reaction to form one of the olefins of the terminal alkenes and the Horner Wadsworth Emmons reaction forming the α, β-unsaturated isobutyl amide.

Synthesis Schemes

Figures 5 and 7 are a summary of the synthetic approach we took to obtain the desired alkylamides. Figure 5 outlines the pathway for the diyne alkylamides. The pathway followed for the olefinic alkylamide is shown in Figure 7. Figure 6 is the synthesis of the *N*-isobutylamide functional group required for both alkylamide varieties.

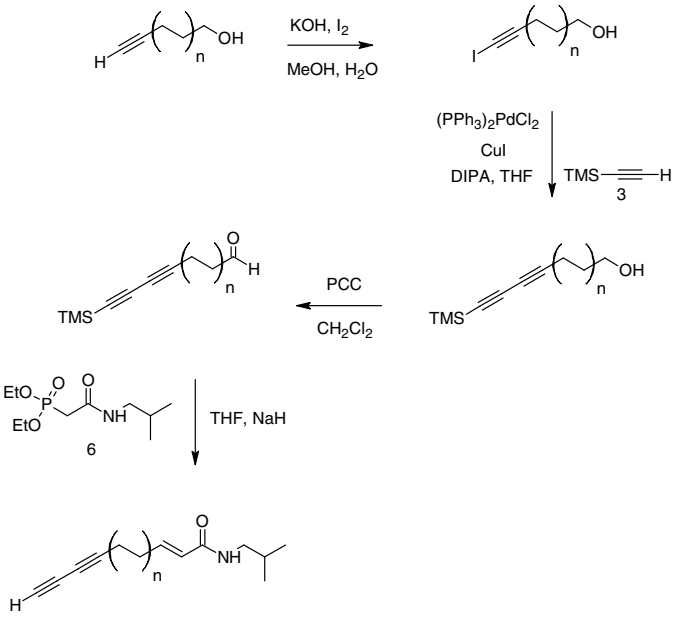


Figure 5. Synthesis Scheme 1, diyne alkylamides.

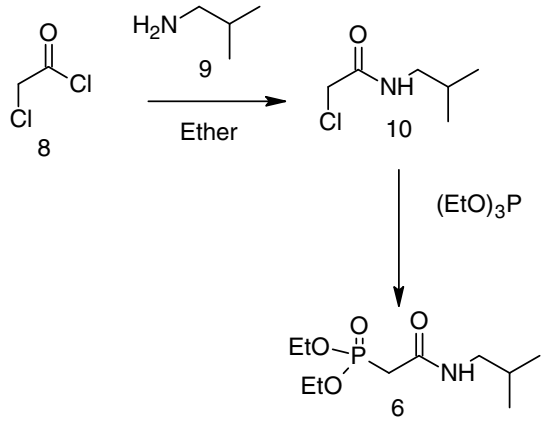


Figure 6. Synthesis Scheme 2, HWE Reagent for Model Alkylamide.[27]

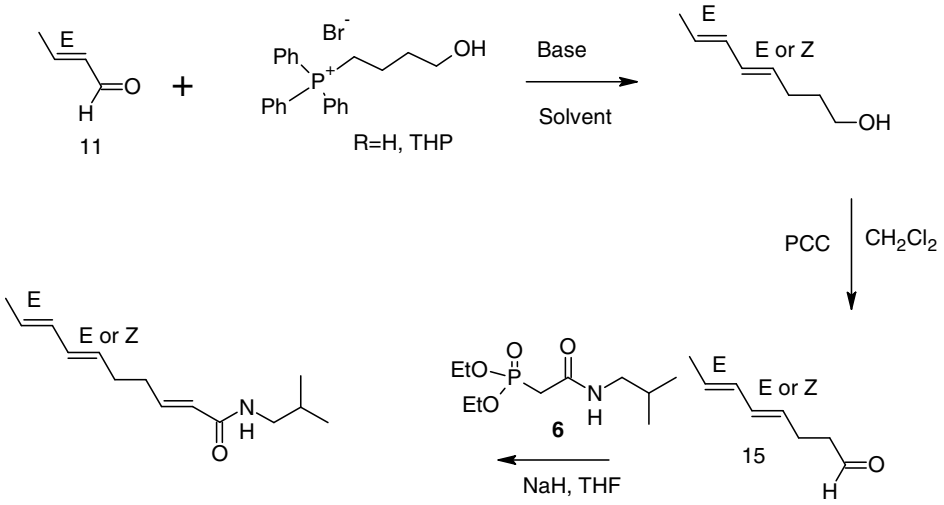


Figure 7. Synthesis scheme 3, spilanthol and analogues.

CHAPTER II

REACTION LITERATURE REVIEW

The Wittig Reaction

The Wittig olefination was first documented in 1919 by Staudinger[28], however the reaction's namesake was not coined until decades later when Georg Wittig realized the significance of the reaction and changed the science of synthesis forever. In 1953 Wittig published a paper[29] demonstrating the ability to selectively synthesize a carbon-carbon double bond, using a phosphorane ylide, a reaction that would eventually lead to his Nobel Prize. Not long after, Bergelson[30, 31] demonstrated that one could control the stereochemistry of the newly synthesized olefin through the manipulation of reaction conditions, further cementing the Wittig reaction as a powerful tool available to organic chemistry.

The Wittig reaction requires a nucleophilic ylide derived from a phosphorane, and an electophilic carbonyl, either an aldehyde or ketone. An explanation of the mechanism for this type of reaction has led to decades of debate and research. The mechanistic knowledge is expected to lead to ultimate control over the stereochemistry of the products, which would eliminate some of the trial and error that can surround the use of the Wittig reaction. There are three proposed intermediates (Figure 8) that the Wittig reaction is thought to follow. The first mechanism originally proposed by Wittig[29]

proceeds through the formation of a 1,2 oxaphosphetane ring(III). The second proposal involves a zwitterionic betaine intermediate(I), later supported by Wittig.[32, 33] Recently, a third intermediate has been suggested which involves a formation of spin-pair diradicals(II).[34, 35]

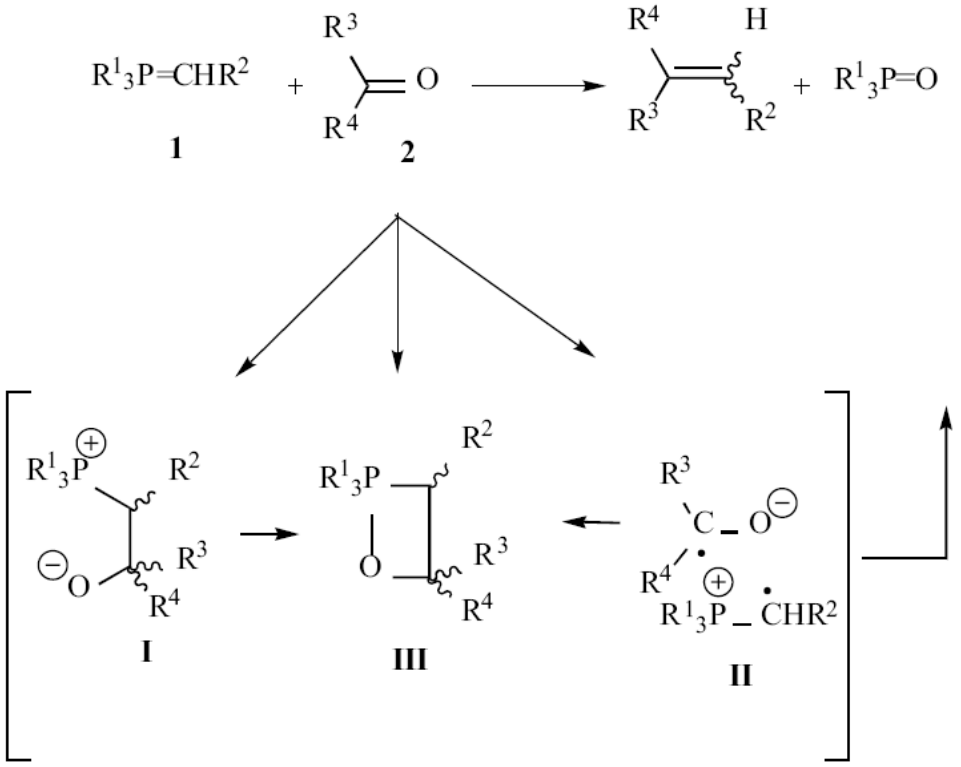


Figure 8. The proposed intermediates of the Wittig Reaction.[36]

The only definite proof of any proposed intermediate first came in 1973 when the Vedejs group[37] observed through ^{31}P NMR spectroscopy the presence of the 1,2 oxaphosphetane intermediate. However, the only oxaphosphetane rings that have been observed and isolated involve unconjugated (nonstabilized) ylides.[38] Oxaphosphetanes in

reactions involving semiconjugated (semistabilized) and heavily conjugated (stabilized) ylides have not been observed which adds to the difficulty in identifying the mechanism.[38]

An explanation for the difficulty in identifying the mechanism is that the conditions may determine the mechanistic action and the intermediates involved. For instance the betaine complex may form in the presence of the lithium cations to form the *E* isomer[38, 39] which leads to the oxaphosphetane ring. On the other hand only the oxaphosphetane may be the observed intermediate in the "lithium salt" free conditions to form the Z isomer.[39] Because only the oxaphosphetane has truly been isolated and quantified,[38] it is thought to participate in all Wittig reactions[38] therefore the discussion of the Wittig reaction will center around this intermediate. However, it should be noted that the betaine has gained some popularity based on some noted phenomena in the literature. Lithium cation adducts with betaine complexes have been observed along with trapped betaines at low temperature and in the presence of HBr. However, the energy calculations of betaines are less favored, 20 kcal/mol, in energy than oxaphosphetanes.[38]

As the research of Bergelson showed, practically every aspect of the Wittig reaction influences the stereocontrol, including temperature, base and corresponding counter cation, solvent, phosphorous functional groups and the presence of certain anionic groups on the ylide. These conditions have been systematically investigated[38] through a series of reactions[40], in an effort to determine the effect and extent each has in the stereochemical outcome of the Wittig reaction.

The presence of the lithium cation has the most noticeable effecton stereochemistry. Using nonstabilized ylides the preferred stereochemistry of the products is the *Z* configuration[38] however, when using two equivalents[40] of base with a lithium cation, better known as "lithium salt" conditions, a stereochemical drift[38] takes place and there is an increase in the *E* isomer. Although other conditions do not exhibit as dramatic of an affect as demonstrated by the "lithium salt" phenomenon, there are additional noteworthy drifting affects. Thermodynamic control causes an increase in formation of the *Z* isomer[38, 41, 42] with a decrease in temperature. Protic solvents, such as alcohols, increase the formation of *E* isomers.[38] The general structure and reactivity of the ylides is also related to the product chemistry. As mentioned earlier with the oxaphosphetane rings, Wittig ylides have been classified into three categories: stabilized, semistabilized and nonstabilized. These classifications are based on the general reactivity of the ylide which is related to the degree of conjugation[38]. Stabilized ylides are heavily conjugated and yield the *E* stereochemistry. The semistabilized are less conjugated than the stabilized and tend to be unbiased with respect to stereochemistry. The nonstabilized ylides are completely devoid of conjugation and favor the formation of the *Z* olefin.

The Horner Wadsworth Emmons Reaction

Since the discovery of the Wittig reaction several alternatives to the traditional phosphorane ylide have emerged. Phosphonates (Horner-Wadworth-Emmons reaction), phosphonamides (the Corey reaction) and phosphine oxides (Horner reaction).[36] The

Horner-Wadsworth-Emmons (HWE) reaction was popularized in 1961 by William Wadsworth and William Emmons.[43] Credit for the discovery of the HWE phosphonate named reaction is shared by Wadsworth, Emmons and Horner. Horner's group was one of the first to use a phosphonate ylide although[38] they dealt mostly with phosphine oxides. A phosphonate reagent has several advantages over the phosphorane including a lower cost, milder reaction conditions, increased reactivity towards ketones and aldehydes and the ionic phosphonate product is water soluble enhancing the ease in product extraction.[43] The mechanism for the mode of action is very similar to that of the phosphorane in that a ring intermediate is thought to form, however the products greatly favor the *trans* product. This is ideal for our synthesis and the main reason the HWE reaction was selected for installation of the α, β-unsaturated isobutyl amide.[38, 43]

The Sonogashira Reaction

The Sonogashira reaction is one of many transition metal coupling reactions, however, it is particularly useful when dealing with acetylenes.[44] It uses bis(triphenylphosphine)palladium dichloride and copper iodide as catalysts making this a cocatalyzed reaction. These conditions were published by Kenkichi Sonogashira in 1975, the same year Cassar[45] and Heck[46] independently published similar palladium catalyzed reactions but without the CuI cocatalyst. The cocatalytic conditions of the Sonogashira reaction allow the reaction to be run at room temperature conditions and yield better results with acetylenes than the Cassar and Heck reactions.[45-47] As with the Wittig

reaction, the true mechanism of the Sonogashira reaction is still not fully understood, however an accepted theory is shown in Figure 9.[47]

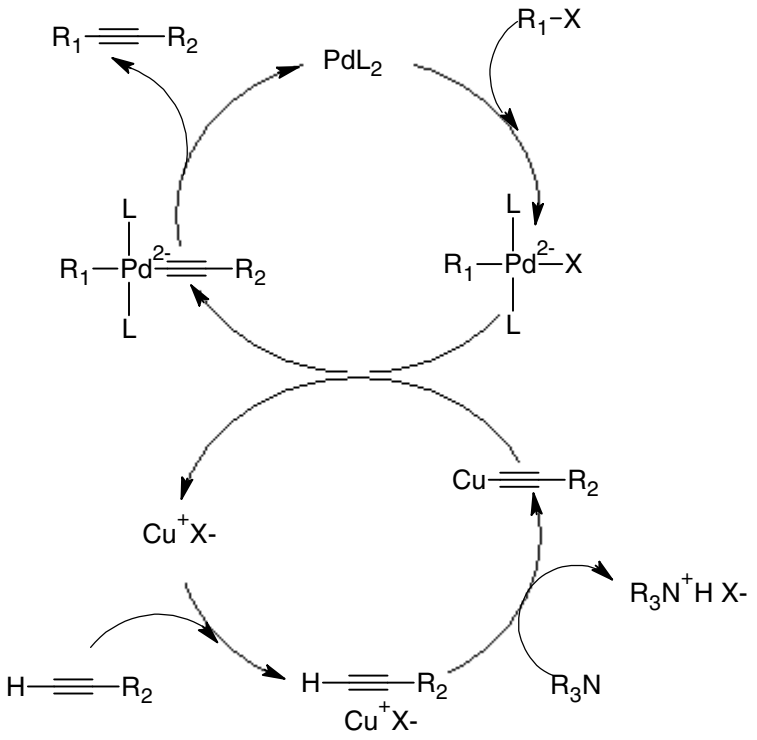


Figure 9. A proposed mechanism for the Sonogashira palladium and copper catalyzed coupling reaction.

The coupling is thought to occur through cycles of palladium oxidation and reduction. The reaction begins with a palladium complex at an oxidation state of zero where an alkyl or vinyl halogen is complexed with the palladium complex through oxidative insertion. The next step, which is poorly understood, involves the CuI. The CuI forms a π-complex with the alkyne, which lowers the pKa of the terminal hydrogen. A base is able to deprotonate the terminal acetylene hydrogen, and the Cu-acetylid intersects the palladium cycle where the palladium displaces the copper by transmetalation. The

product is formed through a removal of the Pd complex via a *trans/cis* isomerization followed by a reductive elimination.[47]

CHAPTER III

EXPERIMENTAL PROCEEDURES

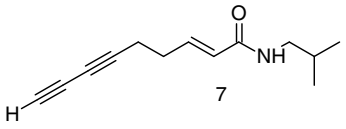
Equipment

Equipment used: 500 MHz Joel FT-NMR, Mass Spectrometer, 2001 Thermofinnigan LCQ Advantage Electrospray Ionization Mass Spectrometer, Flash Chromatography (200-400 mesh of silica), Thin Layer Chromatography.

Solvents were dried by alumina purification, and chemicals were used as supplied from Aldrich and Acros.

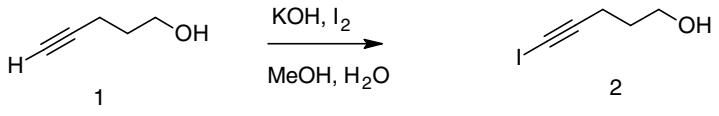
Diynyl Alkylamides Synthesized

(E)-N-isobutylnona-2-en-6,8-diynamide **1**[27, 48-50]



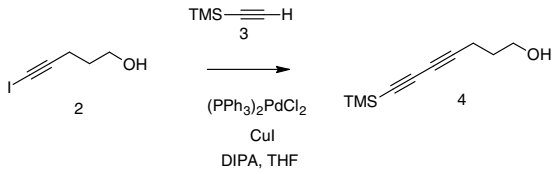
This synthesis was performed without prior knowledge of a patent[51] containing the identical synthesis for alkylamide **7**. However, this synthesis was followed for additional alkylamides and the synthesis of this alkylamide was still relevant to other analogs synthesized.

5-iodopent-4-yn-1-ol **2**[48]



Solid KOH (8.4 g, .150 mol) was dissolved in 12 mL of water and cooled to room temperature. The alkyne alcohol **1** (5.06 g, .06 mol) was dissolved in methanol and stirred. The KOH solution was slowly added and stirring continued for 10 min. Iodine (16.76 g, .066 mol) was added and the reaction was stirred for 3 hours. The product was extracted using ethyl ether (3*50 mL) and concentrated *in vacuo* to leave a yellow oil. The yellow oil was dissolved in methylene chloride, washed with saturated brine (50 mL) and dried with sodium sulfate and concentrated *in vacuo* to yield the crude alkynyl iodide **2**. The product was purified by flash chromatography on silica gel using 60% hexane in ethyl acetate (8.8 g, 70%). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ 7.25 (s, 1 H), 3.67 (t, J = 2.85 Hz, 2 H), 2.50 (s, 1 H), 2.45 (t, J = 6.90 Hz, 2 H), 1.76-1.68 (m, 2 H).

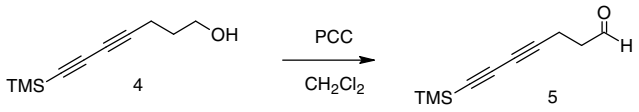
7-(trimethylsilyl)hepta-4,6-diyn-1-ol **4**[50]



The diyne moiety was formed via a palladium-catalyzed Sonogashira coupling reaction. Alkynyl iodide **2** (.199 g, .00095 mol) was dissolved in dry THF (6.9 mL) under argon. The TMS acetylene **3** (.111 g, .0014 mol), copper iodide (.006 g, .00003 mol), and dichlorobis(triphenylphosphine)-palladium (II) (.021 g, .00003 mol) were added turning the solution from a yellow to a dark orange with a precipitate. Diisopropyl amine (DIPA) (.173 g, .0171 mol) was added slowly and reaction was stirred for 1.5

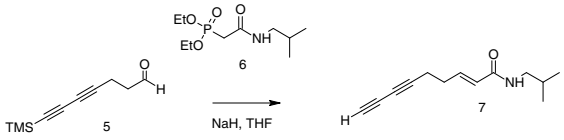
hours. After 1.5 hours, the reaction was diluted with ether, washed with 1 M hydrochloric acid, water, and saturated brine. The ether layer was dried with magnesium sulfate and evaporated to yield the diyne **4**. Product was purified by flash chromatography on silica gel using 60% hexane in ethyl acetate (.06 g, 35%). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ 7.25 (s, H), 3.74 (t, J = 5.70 Hz, 2 H), 2.41 (t, J = 6.85 Hz, 2 H), 1.81-1.73 (m, 2H), 1.56 (s, 1 H), 0.17 (s, 9H).

7-(trimethylsilyl)hepta-4,6-diynal **5**[52]



Pyridinium chlorochromate (PCC) (.3228 g, .0015 mol) was slowly added to the alcohol (.180 g, .001 mol) dissolved in methylene chloride (6 mL). The reaction was stirred at room temp for 3.5 hours to yield the aldehyde **5**. Product was purified by flash chromatography on silica gel using 85% hexane in ethyl acetate solution (.08 g, 45 %). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ 9.76 (s, 1 H), 7.25 (s, 1 H), 2.70 (t, 2 H), 2.58 (t, 2 H), 0.16 (s, 9 H).

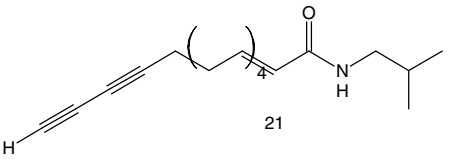
(E)-N-isobutylnona-2-en-6,8-diynamide **7**[27]



The sodium hydride (.036 g, .0015 mol) was suspended in dry THF (4.2 mL) forming a white slurry. The slurry was cooled in an ice bath for 15 minutes. The Horner Wadsworth Emmons reagent **6** (.136 g, .0005 mol) was added to the slurry and stirred for 30 minutes in the ice bath. After 30 min, the aldehyde **5** (.080 g, .00045 mol) was added

and raised to room temp where the reaction stirred for 1 hour. The product was removed and purified by flash chromatography on silica gel using a 60% solution of hexanes in ethyl acetate to yield **7** (70 mg, 77%). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ 7.25 (s, 1 H), 5.83 (d, J = 15.5 Hz, 2 H), 3.15 (t, J = 6.9 Hz, 2 H), 2.42 (d, 2 H), 2.04 (s, 1 H), 1.98 (s, 1 H), 1.80 (m, 1 H), 1.59 (s, 1 H), 1.27 (m, 2 H), 0.92 (d, 6 H).

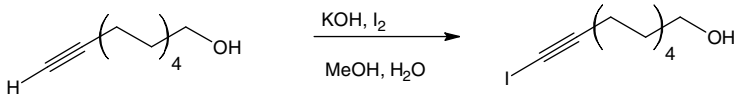
(2E)-N-isobutylpentadec-2-ene-12,14-diynamide **21**



21

The general synthesis scheme that was followed for the following two compounds was modeled by compound **7**

11-iodoundec-10-yn-1-ol **18**

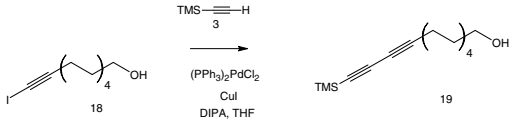


17 18

Solid KOH (1.4g, 25mmol) was dissolved in 2 mL of water and cooled to room temperature. The alkyne alcohol **17** (1.68g, 10mmol) was dissolved in methanol (10mL) and stirred. The KOH solution was slowly added and stirring continued for 10 min. Iodine (2.79g, 11mmol) was added and the reaction was stirred for 3 hours. The product was extracted using ethyl ether (3*50 mL) and concentrated *in vacuo* to leave a yellow oil. The yellow oil was dissolved in methylene chloride, washed with saturated brine (50 mL), dried with sodium sulfate and concentrated *in vacuo* to yield the crude alkynyl iodide. The product was purified by flash chromatography on silica gel using 60%

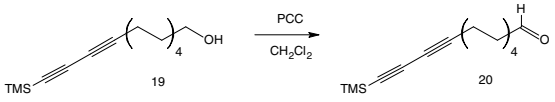
hexane in ethyl acetate **18** (3.2g, quantitative). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ 7.22 (s, 1 H), 3.55 (t, 2 H), 2.27 (t, 2 H), 2.10 (t, 2 H), 2.04 (s, 1 H), 1.49-1.22 (m, 10 H).

13-(trimethylsilyl)trideca-10,12-diyn-1-ol **19**



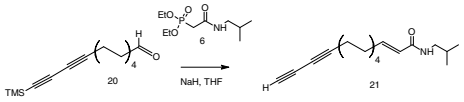
The Iodide **18** (3.2g, 10.9mmol) was dissolved in dry THF (76.8mL) under argon. The TMS acetylene **3**(1.28g, 13.1mmol), copper iodide (63mg, 0.33mmol), and dichlorobis(triphenylphosphine)-palladium (II) (231mg, 0.33mmol) were added, turning the solution from a yellow to a dark orange with a precipitate. DIPA (1.99g, 19.6mmol) was added slowly and reaction was stirred for 1.5 hours. After 1.5 hours, the reaction was diluted with ether, washed with 1 M hydrochloric acid, water, and saturated brine. The ether layer was dried with magnesium sulfate and evaporated to yield the diyne. Product was purified by flash chromatography on silica gel using 60% hexane in ethyl acetate to yield **19** (1.86g, 40%). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ 7.22 (s, 1 H), 3.56 (t, 2 H), 2.20 (t, 2 H), 2.12 (t, 2 H), 1.81 (s, 1 H), 1.47 (m, 6 H), 1.23 (m, 6 H), 0.121 (s, 9 H).

13-(trimethylsilyl)trideca-10,12-diynal **20**



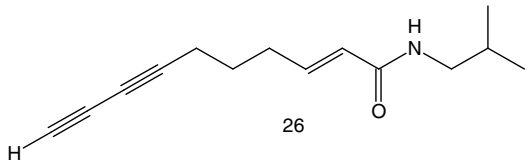
The alcohol **19** (1.9g, 7.9mmol) was dissolved in methylene chloride (43.1mL) and stirred. PCC (2.3g, 10.7mmol) was added to the solution and stirred at room temperature for 1.5 hrs. The crude reaction was purified with no workup by flash chromatography on silica using 80% hexanes in ethyl acetate to yield the product **20** (2g, quantitative yield). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ 9.70 (s, 1 H), 7.22 (s, 1 H), 2.36, (t, 2

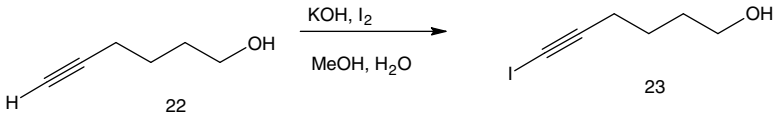
H), 2.28 (t, 2 H), 2.21 (t, 2 H), 2.12 (t, 2 H), 1.90 (t, 1 H), 1.55 (m, 2 H), 1.45 (m, 2 H), 1.24 (m, 4 H), 0.1214 (s, 9 H).

(2E)-N-isobutylpentadec-2-ene 12,14-diynamide **21** 

Sodium hydride (65mg, 2.74mmol) was dissolved in 1mL of THF and stirred forming a white slurry. The HWE reagent **6** (250 mg, 1mmol) was added, dropwise, evolving H_2 gas. A solution of the aldehyde **20** (220mg, 0.83mmol) in 7.7 mL of THF was added dropwise and stirred for 36hrs. The reaction was quenched following the usual workup and purified by flash chromatography on silica using 50% hexanes in ethyl acetate to yield the product **21** (190mg, 80%). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ 7.20 (s, 1 H), 6.75 (t, J = 14.9 Hz, 1 H), 5.72 (s, J = 14.9 Hz, 1 H), 5.65 (s, 1 H), 3.06 (t, 2H), 2.20 (t, 2 H), 2.09 (t, 2 H), 1.86 (s, 2 H), 1.70 (m, 1 H), 1.50-1.20 (m, 8 H), 0.85 (d, 6 H).

The required 36 hours to complete the Horner-Wadsworth-Emmons reaction could be attributed to a decrease in solubility of the reactants in THF.

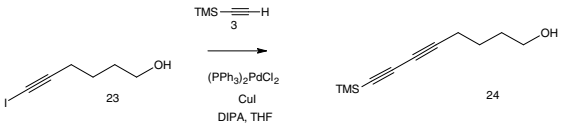
(2E)-N-isobutyldec-2-ene-7,9-diynamide **26** 

6-iodohex-5-yn-1-ol **23** 

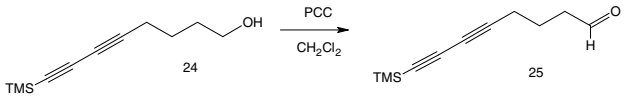
Solid KOH (8.4g, 150mmol) was dissolved in 12 mL of water and cooled to room temperature. The alkyne alcohol **23** (5.89g, 60mmol) was dissolved in methanol (60mL)

and stirred. The KOH solution was slowly added and stirring continued for 10 min. The I_2 (16.76g, 66mmol) was added and the reaction was stirred for 3 hours. The product was extracted using ethyl ether (3*50 mL) and concentrated *in vacuo* to leave a yellow oil. The yellow oil was dissolved in methylene chloride, washed with saturated brine (50 mL), dried with sodium sulfate and concentrated *in vacuo* to yield the crude alkynyl iodide. The product **23** was purified by flash chromatography on silica gel using 60% hexane in ethyl acetate (15.46g, quantitiative).). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ 7.24 (s, 1 H), 3.59 (t, 2 H), 2.41 (t, 1 H), 2.34 (t, 2 H), 1.53 (m, 4 H).

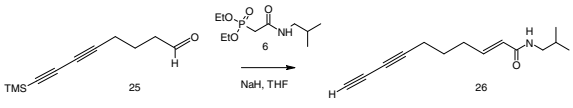
6-(trimethylsilyl)trideca-5,7-diyn-1-ol **24**



The iodide **23** (1.60g, 7.1mmol) was dissolved in dry THF (50mL) under argon. The TMS acetylene **3**(836mg, 8.52mmol), copper iodide (42mg, 0.2mmol), and dichlorobis(triphenylphosphine)-palladium (II) (147mg, 0.2mmol) were added, turning the solution from a yellow to a dark orange with a precipitate. DIPA (1.3g, 12.8mmol) was added slowly and reaction was stirred for 1.5 hours. After 1.5 hours, the reaction was diluted with ether, washed with 1 M hydrochloric acid, water, and saturated brine. The organics were combined, dried with magnesium sulfate and concentrated to yield the diyne. Product **24** was purified by flash chromatography on silica gel using 60% hexane in ethyl acetate (940mg, 66%). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ 7.23 (s, 1 H), 5.83 (d, J = 15.5 Hz, 2 H), 3.15 (t, J = 6.9 Hz, 2 H), 2.42 (d, 2 H), 2.04 (s, 1 H), 1.98 (s, 1 H), 1.80 (m, 1 H), 1.59 (s, 1 H), 1.27 (m, 2 H), 0.92 (d, 6 H).

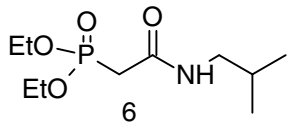
8-(trimethylsilyl)hexa-5,7-diynal **25** 

The alcohol **24** (810mg, 4.1mmol) was dissolved in methylene chloride (24.6mL) and stirred. PCC (1.33g, 6.2mmol) was added to the solution and stirred at room temperature for 1.5 hrs. The crude product was purified directly by flash chromatography on silica using 85% hexanes in ethyl acetate to yield the product **25** (490mg, 61%) ^{1}H-NMR ($CDCl_3$, 500 MHz), δ 9.75 (s, 1 H), 7.22 (s, 1 H), 5.25 (d, 2 H), 2.25 (t, 2 H), 2.30 (t, 2 H), 1.80 (m, 2 H), 0.12 (s, 9 H).

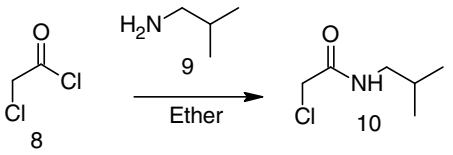
(2E)-N-isobutyldec-2-ene-7,9-diynamide **26** 

Sodium hydride (134mg, 5.73mmol) was dissolved in 5mL of THF and stirred forming a white slurry. The HWE reagent **6** (520mg, 2.1mmol) was added, dropwise, evolving H_2 gas. A solution of the aldehyde **25** (340mg, 1.74mmol) in 11.2 mL of THF was added dropwise and stirred for 1.5hrs. The reaction was quenched following the usual workup and purified by flash chromatography on silica using 60% hexanes in ethyl acetate to yield the product **26** (30mg, 7%) ^{1}H-NMR ($CDCl_3$, 500 MHz), δ 7.19 (s, 1 H), 6.73 (dt, J = 14.9 Hz, 1 H), 5.75 (ds, J = 14.9 Hz, 1 H), 5.42 (s, 1 H), 3.09 (t, 2 H), 2.26-1.47 (m, 7 H), 1.18 (s, 1 H), 0.79 (m, 6 H).

Diethyl N-*Isobutylcarbamoylmethylphosphonate* .[27]



N-Isobutychloroacetamide **10**[27]

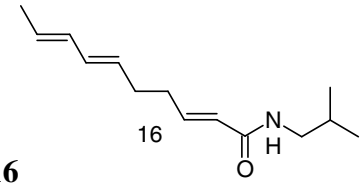


Isobutylamine **9** (29.196 g, .399 mol) was mixed in anhydrous ether (50 mL) and cooled to 0 °C. The chloroacetyl chloride **8** (28.4 g, .253 mol) was added and the mixture was warmed to room temperature for 24 hours. A white solid formed and was removed through vacuum filtration. The filtrate was dissolved in choloroform and washed with ether to yield **10** (34.54 g, 52%).

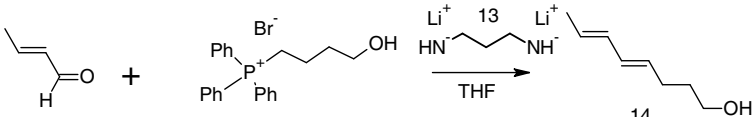
Diethyl N-*Isobutylcarbamoylmethylphosphonate* **6**[27]

N-isobutylchloroacetamide **10** (8.41g, .0608 mol) was slowly added to triethyl phosphite (10.0 g, .060 mol) at 110 °C. The temperature was maintained and reaction was refluxed for 30 minutes. The low boiling point material was removed through distillation at approximately 1.0 mmHg to yield the phosphonate **6** (10.03 g, 60 %). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ 7.25 (s, 1 H), 4.17-4.08 (m, 4 H), 3.10-3.05 (t, 2 H), 2.82 (d, J = 20.6 Hz, 2 H), 2.14 (s, 2 H), 2.02 (s,), 1.82-1.71 (m,), 1.33-1.29 (t, 6H), 0.92-0.89 (d, 6H).

Dienyl Alkylamides Synthesized



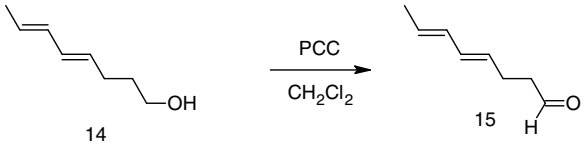
(2E,6E,8E)-N-isobutyldeca-2,6,8-trienamide **16**



(4E, 6E)-octa-4,6-dien-1-ol **14**

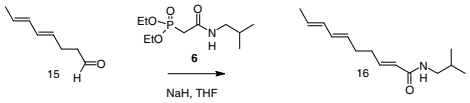
The *Spilanthes* derivative alkylamide synthesis began with a Wittig reaction involving crotonaldehyde (0.9 g, .0125 mol) and the Wittig phosphorane salt **12** (5 g, .0125 mol). The nonstabilized Wittig ylide was generated by stirring the Wittig reagent in the dilithium salt of 1,3-diaminopropane **13** [53, 54] (25mL) at 0°C, turning the solution bright red. The lithium salt of 1,3-diaminopropane **13** was prepared[53] by dissolving lithium wire (0.350 mg, 0.051 mol) in 1,3-diaminopropane (25ml) at 75°C until the blue color faded, the volume was then doubled with tetrahydrofuran (25ml). The aldehyde **11** was added dropwise to the reaction and the progress was monitored by TLC. After 1.5 hours the reaction was poured over ether in a separatory funnel and quenched with 1M hydrochloric acid. The organic layers were combined, dried with $MgSO_4$ and concentrated. The crude material was purified with flash chromatography on silica using 60 % hexanes in ethyl acetate to yield the product **14** (140 mg, 8.9%). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ 7.20 (s, 1 H), 6.27 (dd, J = 14.9, 1 H), 6.00-5.89 (dm, J = 14.9, 1 H), 5.69-5.59(dm, J = 14.9, 1 H), 5.57-5.45 (dt, J = 14.9, 1 H), 5.22 (dt, J = 14.9). 3.59 (q, 2 H), 2.20 (m, 2), 1.70-1.50 (m, 5).

(4E, 6E)-octa-4,6-dienal **15**



The entire yield of the previous product **14** (140 mg, 0.001 mol) was used to form the aldehyde through the oxidation of the alcohol. The procedure was followed according to the aforementioned reaction conditions of compound **5**. The aldehyde was purified directly from the crude reaction mixture by flash chromatography on silica using 80% hexanes in ethyl acetate to yield the aldehyde **15** (0.09 g, 64%). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ 9.70 (s, 1 H), 7.20 (s, 1 H), 6.27 (dd, J = 14.9, 1 H), 6.00-5.89 (dm, J = 14.9, 1 H), 5.69-5.59(dm, J = 14.9, 1 H), 5.57-5.45 (dt, J = 14.9, 1 H), 5.22 (dt, J = 14.9). 2.44 (m, 2 H), 2.36 (m, 2), 1.70-0.70 (m, 4).

(2E, 6E, 8E)-N-isobutyldeca-2,6,8-trienamide **16**



The addition of isobutyl amide group followed the same conditions as mentioned in the synthesis of **7**. Again, the entire yield **15** (0.09g, 0.71 mmol) of the previous step afforded, was used in the Horner-Wadsworth-Emmons reaction. The product was poured over ether and washed with 1M HCl. The organic layers were combined, dried with $MgSO_4$, and concentrated. The crude product was purified with flash chromatography on silica, using 50% hexanes in ethyl acetate to yield **16** (0.060g, 27%).

The spilanthol derivative **16** ^{1}HNMR spectrum was very faint, therefore the stereochemistry was determined based on the coupling of **14** and **15** from ^{1}HNMR. The sample was analyzed using reversed phase HPLC coupled to electrospray ionization mass

spectrometry (ESI-MS). The MS and MS-MS spectra, Figures 10 and 11, respectively were compared against a Spilanthol standard (Figure 12) extracted from *Spilanthes acmella*[55] and a positive correlation was observed with the fragmentation patterns of the derivative and standard. Also, a MALDI-TOF MS-MS analysis of the sample provided a similar parent weight of 222 and fragmentation pattern similar to the ESI-MS spectrum. Based on the similarity in fragmentation pattern between Spilanthol and the analogue, the stereochemistry cannot be elucidated from an MS-MS spectrum.

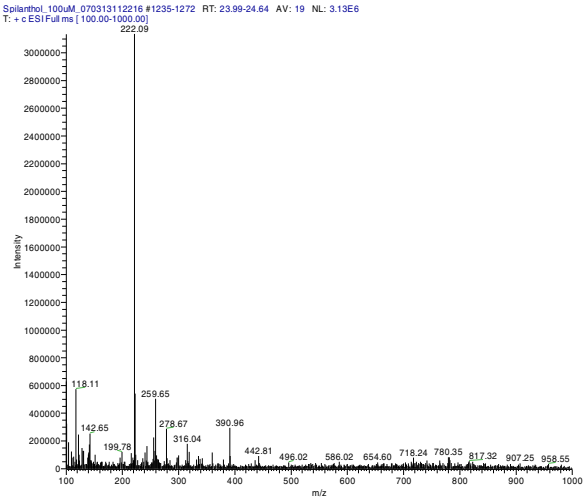


Figure 10. The MS Spectrum of the spilanthol derivative.

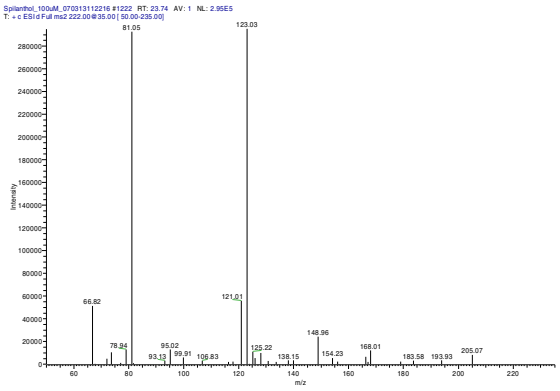


Figure 11. The MS-MS spectrum of the spilanthol derivative

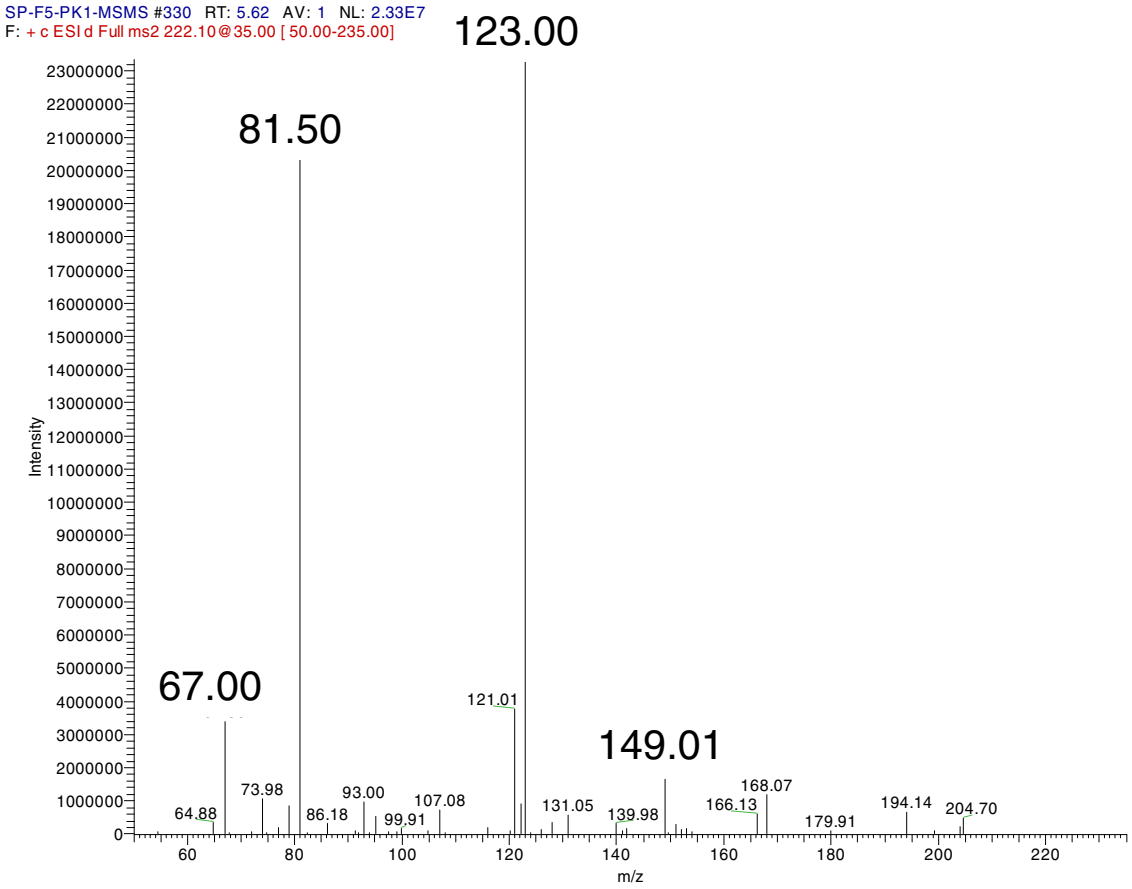
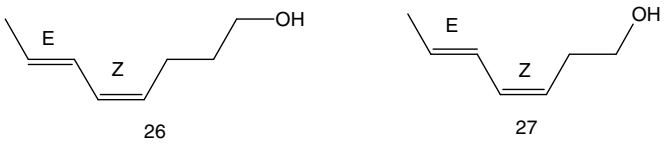


Figure 12. Isolated Spilanthol MS MS from an extract of *Spilanthes acmella.*[55]

Attempted Methods of Product Development

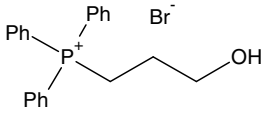


The desired EZ stereochemistry 26 27

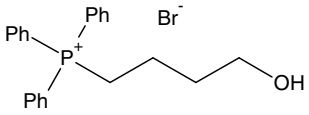
Several experiments were performed in an attempt to duplicate the *E, Z* stereochemistry shown above. All began with either a four or three carbon Wittig reagent. The three carbon Wittig reagent was an attempt at a model scheme but was later

substituted with the four carbon Wittig reactant. Below are varieties of synthetic pathways that involved one or both of the Wittig reagents, however, the focus will remain on the four carbon reagent due to its requirement for the synthesis of Spilanthol. Originally, the alcohol groups were left unprotected but these turned out to be very difficult to remove from the reaction mixture and monitor by TLC. Therefore, the reader will notice the oxido groups were protected with tetrahydropyran (THP). However, the results were often inconclusive. Ultimately, the inability to duplicate the *E, Z* stereochemistry is what prevented the synthesis of the true spilanthol.

The wittig reagents



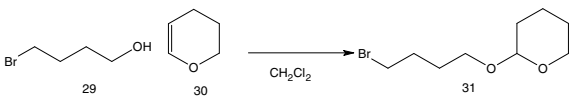
28



12

Alternative conditions[56, 57] for the synthesis of both unprotected **12, 28** and protected Wittig reagent **32** were to reflux in acetonitrile in the presence of potassium carbonate. These particular conditions were said[57] to prevent the side product synthesis of a bisphosphonium salt $Ph_3^+(CH_2)_nP^+Ph_3\ 2Br^-$. However, similar results were observed for both conditions.

2-(4-bromobutoxy)tetrahydro-2H-pyran **31**

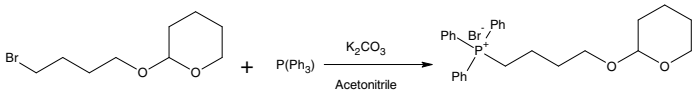


29 30 31

The protected wittig **31** was synthesized to alleviate any interactions the unprotected hydroxyl may have with the reaction solvents DMSO and DMF. The alcohol **29** (1.59g, 0.011 mol) was presented to an excess of dihydropyran **30** (1.3 mL) and stirred in methylene chloride for four hours. The reaction was washed with water (10mL) and

the product was extracted with methylene chloride (2*10mL). The organics were combined, washed with brine, dried with $MgSO_4$ and concentrated. The crude product was purified by flash chromatography on silica using 90 % hexanes in ether to yield **31** (4.30g, quantitative yield).). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ 7.26 (s, 1 H), 485 (t, 2 H), 4.50 (t, 2 H), 3.46 (m, 2 H), 3.33 (m, 2 H), 2.00-1.42 (m, 9 H).

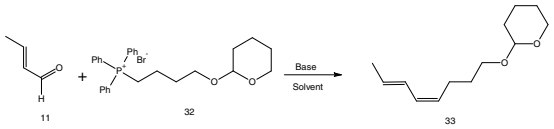
The protected wittig reagent **32**



31 32

The protected bromobutanol **31** (10.09g, 43mmol), triphenylphosphine (11.30g, 43 mmol) and potassium carbonate (5.94 were dissolved in acetonitrile. The mixture was stirred and refluxed overnight. The potassium carbonate was filtered off and the solvent was removed *in vacuo* to yield the product **32** (7.25g, 34%)

2-[(4Z, 6E)-octa-4,6-dien-1-yloxy]tetrahydro-2H-pyran **33**

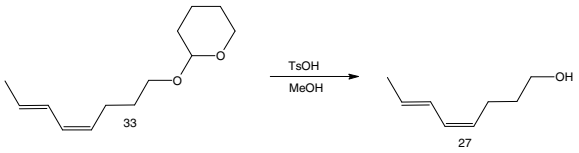


11 32 33

A solution of the protected Wittig reagent **32** (1.00g, 0.02mol) in DMF (4.67mL) was added to a slurry of sodium hydride(0.124g, 0.52mol) in DMF(2.67mL) and stirred at room temperature, turning the reaction orange. The aldehyde **11** was added dropwise and stirred at room temperature for 2.5 hours. The reaction was poured over ether and quenched with sodium bicarbonate and potassium carbonate. The organic extracts were combined, dried with $MgSO_4$, and concentrated. The crude solution was purified with flash chromatography on silica using 85% hexanes in ethyl acetate to yield **33** (200 mg, 12%).

Alternative reaction conditions involved DMSO as a solvent[58]. The same proportions, workup and purifications were followed to yield the product **33** (20 mg, 5%).

Additional reaction solvents used in an attempt to form product were ethyl ether and tetrahydrofuran neither of which yielded the desired product **33**.



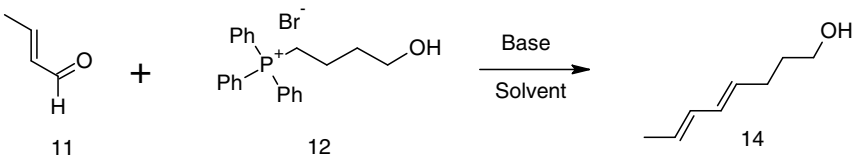
(4Z,6E)-octa-4,6-dien-1-ol **27**

Several of the reactions may have yielded the desired product, however, in the attempts to deprotect the alcohol, all product was lost.

Deprotection Reaction[59]. The THP alcohol **33** (200mg, 1.02 mmol) was dissolved in methanol (3.4 mL). The catalyst TsOH (19.9mg, 0.102) was added and stirred at room temperature for 2 hours. The desired product **27** was not found.

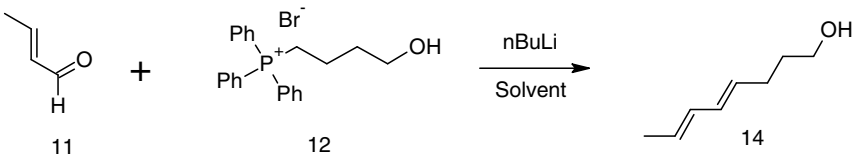
The Wittig Reactions Involving the Unprotected Alcohol

The synthesis of spilanthol using a Wittig reagent with an unprotected γ alcohol is virtually impossible (see discussion). However, these attributes made the synthesis of a *E, E* spilanthol derivative ideal. The following are the failed conditions producing the E isomer during the initial coupling of the alkenes.



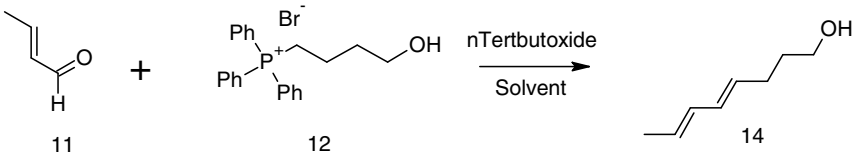
11 12 14

The unprotected four carbon Wittig reagent **12** (500 mg, 1.2 mmol) was dissolved in THF (5mL) and the temperature was decreased to -80°C. nBuLi[38] (2.48mL, 3.72mmol) was added dropwise and stirred for 30 min. The aldehyde **11** (270 mg, 3.6 mmol) was added dropwise and stirred for 4 hours at -80°C. The workup called to pour the reaction over ether and quench with water.[38] The organic extracts were combined, dried with $MgSO_4$ and concentrated. The desired product was sought by flash chromatography using 60% hexanes in ethyl acetate. The desired product was not located.



11 12 14

The four carbon unprotected Wittig reagent **12** (207 mg, 0.5 mmol) was dissolved in THF (0.9 mL). The nBuLi base (0.733mL, 1.1mmol) in THF (0.367 mL) was added and the mixture stirred for 15 min at room temperature. The reaction was then cooled to -55°C[60] and the aldehyde **11** (70 mg, 1 mmol) was added dropwise and stirred for 5 minutes before bringing solution to room temperature. The same workup and purification was followed and product could not be located.



11 12 14

The ylide **12** (207 mg, 0.5mmol) and base[60] (123mg, 1.1mmol) were dissolved in 2.5mL of THF and stirred for two hours. The aldehyde **11** (70mg, 1.0 mmol) was added dropwise and formed a solid solution after four hours. The usual work up was attempted, but no product was found.

CHAPTER IV

RESULTS AND DISCUSSION

The synthesis of the diyne compounds followed as predicted with little difficulty, however, the diene experiments, involving the wittig reaction, proved to be quite difficult and likely flawed, specifically with the *E, Z* stereochemistry **26, 27**. The spilanthol synthesis utilized a nonstabilized ylide, initially chosen because of its trended bias towards a *Z* oriented olefin[38, 39]. However, the Z isomer was never realized. Instead, the *E* isomer turned out to be the major product with this reagent in all conditions employed. According to the literature, the *E* isomer should be the least favored of the two isomers[38]. Vedejs and Snoble[37] suggested that through a cycloaddition the oxaphosphetane ring forms via orthogonal approach by the carbonyl and ylide π bonds to attain maximum overlap. This overlap requires the least hindered orientation and results in a maximally hindered cis oxaphosphetane ring which favors the formation of the Z isomer.[37, 61] Salmond et al, demonstrated the anomalous chemistry where nonstabilized ylides, with an anionic functional group, form an E isomer in traditional *Z* isomer conditions.[61] Their rationale for the chemistry was based on the Schlosser theory of an internal proton exchange (Figure 13.)[62]

Once the oxaphosphetane ring has formed through the mechanism proposed by Vedejs and Snoble[37] the ring will decompose to form the *Z* isomer. However, if an equilibrium is established between the cis ring and a deprotonated α carbon a five

centered transition state can form, allowing the α carbon to be reprotonated (Figure 13.)[61] Reprotonation will favor the less hindered trans oxaphosphetane ring and subsequently the *E* isomer[61, 62].

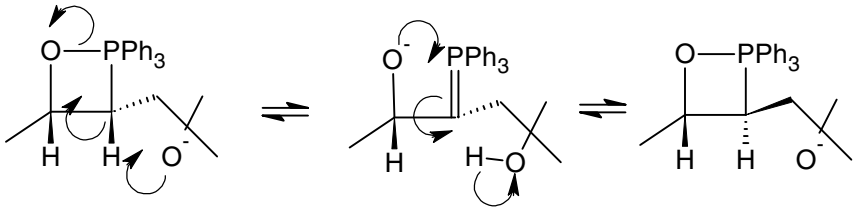


Figure 13. Schlosser's internal proton exchange.[38,61]

However, the Maryanoff group tested this theory using various ylides with anioic groups and a deuterated stereogenic carbon adjacent to the phosphorous.[38] The results were absent of a trend indicating transfer of a proton[38]. In the 1980's Maryanoff and Rietz continued to investigate the influence of anionic (carboxy or oxido) containing ylides present on the ylide sidechains and their influence on the stereochemistry. Using a variety of ylides, several conclusions were drawn. They showed that ylides with anionic sidechains definitely favored an *E* product in conditions that would normally yield a *Z* isomer, paralleling our results. In addition, they showed that the distance separating the anionic groups and the alpha carbon inversely relates to an increase in *E* stereochemistry. γ and δ oxido ylides were shown to have an almost complete reversal of stereochemistry whereas an 11 carbon separation decreases the *E* isomer significantly. Based on these results, Maryanoff proposed an alternative to the Schlosser theory. The Wittig reaction is

a reversible reaction and the rate appears to be increased with the presence of anionic groups. Furthermore, anionic group-bearing ylides demonstrate less efficiency possibly as a byproduct of an increased retro-Wittig reaction. Maryanoff suggested that a "biting back" phenomena takes place, whereby the nucleophilic anionic group attacks the phosphorous, similar to an S_N2 reaction, reversing the stereochemistry of the intermediate.

Regardless of either mechanism proposed, by Schlosser or Maryanoff, an ylide with an anionic group 2-3 carbons from the alpha carbon and the presence of a lithium cation results in the formation of the *E* isomer. Therefore, we are pleased to present the novel synthesis scheme of compound **16** (Figure 14).

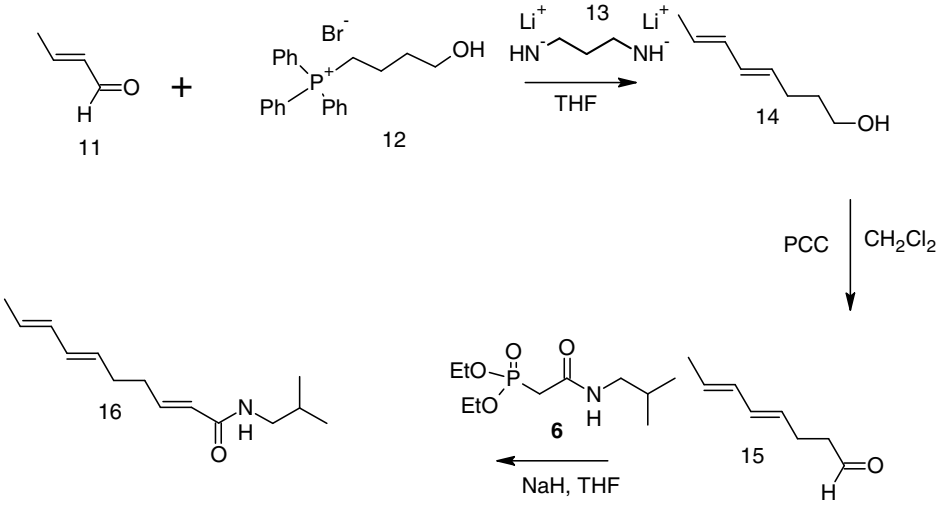


Figure 14. The successful synthesis scheme for the spilanthol analog.

Even though capabilities for analogous compounds were built into the synthesis schemes, the analogues themselves were not initially pursued. However, the synthesis of the

analogue of Spilanthol proved to be ideal based on the conditions of the reaction and the literature.

CHAPTER V

FURTHUR STUDY

Ultimately, the reactions carried in lithium salt free conditions and with a protected ylide alcohol failed to yield the desired *E* product in our synthesis. In a continued effort to still develop a novel synthesis of spilanthol we propose the following scheme (Figure 15). *Trans*-bromopropylene and compound **22** will be linked via a Sonogashira reaction. The acetylene will be reduced by a Lindlar's catalyst poisoned with quinoline to prevent complete reduction and yield the coveted *cis* isomer. The next two steps will parallel the previous reaction with the oxidation of the alcohol to an aldehyde and the HWE reaction to form the product.[63]

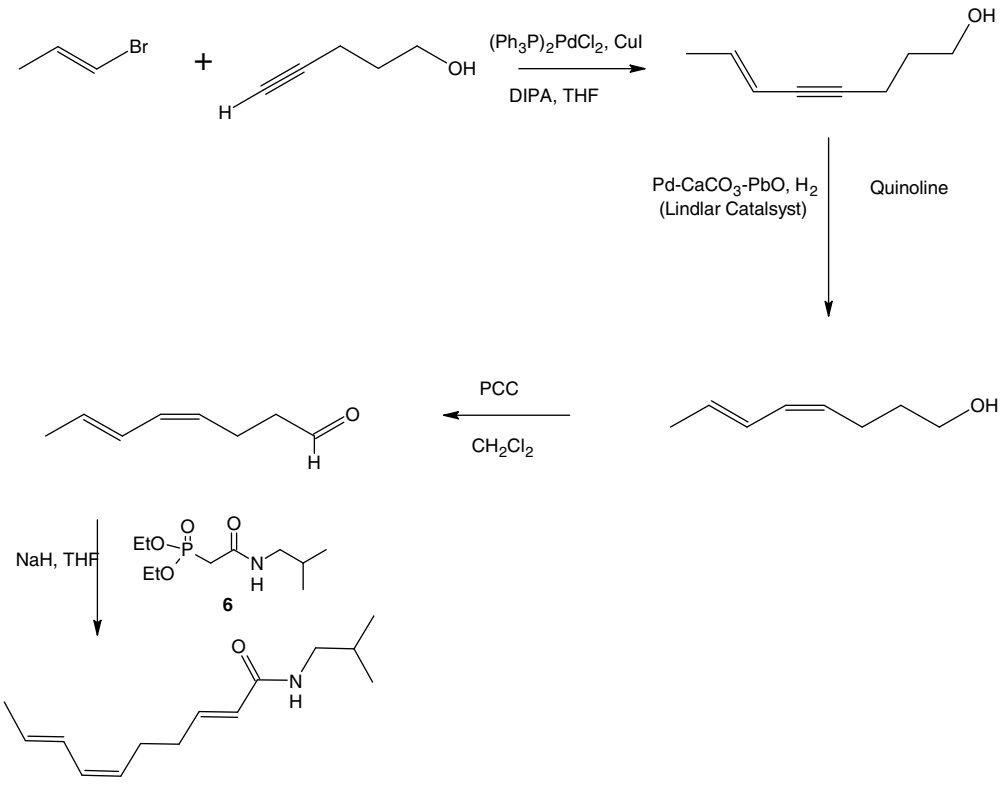


Figure 15. Proposed Spilanthol synthesis scheme for future work.

Granted this proposed synthesis scheme departs from our convergent theme, but with the removal of the Wittig reaction should alleviate some of the difficulty of the Spilanthol synthesis that surrounded our work.

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