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Honeybees, *Apis mellifera*, are domesticated to produce honey and pollinate crops. Honeybees play a significant role in the environment and food security. However, honeybee colonies are in decline because of anthropogenic stressors such as their natural habitat being destroyed, pesticide exposure, and being exposed to various parasites and diseases. These are anthropogenic stressors because they originate from the activity of humans. Arguably the greatest threat to the survival of western honeybees is the ectoparasitic mite, *Varroa destructor*. The *Varroa* parasite puts a physical strain on the honeybees by feeding on the fat body of the brood which adversely influences the wellbeing of the honeybees. It is important to note that the *Varroa* parasite, must invade the honeybee brood cell for it to reproduce. Disrupting the olfactory senses of the *Varroa* mite we prevent them from reaching the brood cell; this is where their reproduction happens. The parasites that attach themselves to the honeybees can be stopped from reproducing through hygienic behavior. Hygienic behavior is the identifying, uncapping, and eliminating of the unhealthy brood from the honey bee hive. The hygienic behavior displayed by the honeybees is triggered by a chemical signal emitted by the brood. Honeybees produce compounds such as unsaturated fats and hydrocarbons for communication. The objective of the proposed research is to synthesize the $Z_{10}C_{31}$ hydrocarbon due to a recent analysis that suggest that the $Z_{10}C_{31}$ isomer is the major isomer and may be more biologically active than the current Z_8C_{31} hydrocarbon being used to induce hygienic behavior. We also were interested in synthesizing the 5-(2′-methoxyethyl)-cyclopent-2-en-1-butoxyl diether that has been reported to disrupt the olfactory senses of the *Varroa* mite.

SYNTHESIS OF *Z*-MONOALKENE AND A DIETHER COMPOUND.

by

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Approved by

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CHAPTER I: BEHAVIOR OF HONEY BEES

I.1 Honeybee Importance

Honey bees (*Apis mellifera*) are considerable contributors to the ecological health of the environment. [1] They contribute to the ecological health of the environment through crop pollination. [7] Honey bees have social, cooperative, navigational, and perseverance characteristics as well as remarkable learning and memory abilities, which make them important models for conducting studies.[10] There are many threats to the wellbeing of honey bees, including anthropogenic stressors. Anthropogenic relates stressors caused by human activity. One anthropogenic stressor is the destruction of their natural habitat and the presence of toxins. The main threat to the wellbeing of honey bees is the ectoparasitic mite *Varroa destructor*.

I.2 *Varroa* Destructor

Varroa reproduction begins when the parasite invades the cell that the larvae is in before it is capped. Once the cell is capped the *Varroa* parasite arises and feeds on the fat body of the brood. [16] *Varroa* vector various honeybee pathogens that can develop into harmful diseases. The sucking of the hemolymph is unsafe to honey bee wellbeing and frequently prompts decline in protein levels as well as reduction in body weight. [17] *Varroa* parasites do more than just cause physical alterations to honey bees. [18] *Varroa* vector many viruses to honeybees, but the most detrimental virus *Varroa* vector to honey bees is Deformed Wing Virus (DWV), which is a single stranded RNA virus. DWV causes distortion in the wings of the honeybee, a shortened abdomen, diminished weight, discoloration, and sudden passing in honey bees. [19,20]

I.3 Olfactory Senses

For *Varroa* to infect honey bees it has to locate and parasitize a brood cell. They do so by utilizing their olfactory senses. Olfactory senses of the *Varroa* mite are how they differentiate the nurse honeybees from the drone honeybees. Honey bee recognition is important to the *Varroa* mite because the nurse honey bee is how the *Varroa* mite would reach the brood cell while the nurse honey bee feeds the larvae. *Varroa* are not able to reproduce outside of the honey bee colony. The forager honey bee leaves the colony to pollinate crops and if *Varroa* attaches themselves to a forager honey bee it will not be able reproduce. This makes the synthesis of the 5-(2′-methoxyethyl)-cyclopent-2-en-1-butoxyl diether compound of great interest because it disrupts the olfactory senses of the *Varroa* mite thus improving honey bee wellbeing.

I.4 Hygienic Behavior

To eliminate the parasites and related viral infections, honey bees utilize what is known as hygienic behavior. Hygienic behavior happens when honey bees identify, uncap, and remove the infected brood from the hive. [5] Hygienic behavior is a known social behavior of worker honey bees to reduce the spread of parasites and diseases in the colony. Nazzi et al. put infected live parasites into brood cells and affirmed the discoveries that honey bees have the ability for perceiving invaded cells and removing them.[28] The pentadecene showed significantly enhanced removal rate of unhealthy broods in comparison to other hydrocarbons used. These finding are important because it provides data that hydrocarbons are responsible for the hygienic behavior displayed by honey bees, and being able to synthesize these compound will allow for the determination of which particular hydrocarbons are the most biologically active through various field test, pentadecene being one in particular shown to have a direct effect on hygienic behavior. Hygienic behavior is not taught or learned, but rather it has a hereditary premise and is a heritable trait.

To improve honey bee wellbeing, beekeepers can breed hygienic honey bees which can recognize and remove the broods that are infected by *Varroa*. It is important to note that there is natural variation in the hygienic abilities of honey bee colonies meaning not all honeybee colonies exhibit this behavior well and can differ from colony to colony. Beekeepers can test for hygienic behavior using a liquid nitrogen test, in which brood is frozen and the ability of adult bees to remove the dead brood is quantified. The process of selecting hygienic colonies using the liquid nitrogen test is time consuming for beekeepers and is not specific for the testing against honeybee parasites and pathogens. These shortcomings led to the development of a new biological assay uses pheromones to measure the hygienic behavior of honeybee colonies. This new assay is known as the unhealthy brood odor (UBO) test. Compared to the liquid nitrogen test the UBO test is a better indicator of whether a honeybee responses hygienically to parasites and diseases. [21] The UBO test works by applying synthetic pheromones to the wax cap of a brood cell. The synthetic pheromones applied to the brood cell are hydrocarbons that induce hygienic behavior. Early utilization of the UBO test has shown that if a colony can uncap greater than 60% of cells that are treated with the synthetic pheromones within two hours of application, then that colony can be considered as hygienic. [21] This is advantageous in comparison to the liquid nitrogen test in that the UBO test is better at predicting *Varroa* resistance.

I.5 Previous Work and Hydrocarbons

Previously identified and synthesized active compounds are Z_6C_{15}, Z_8C_{17}, Z_8C_{31}, and $Z_{10}C_{33}$. Mixture of these compounds were used to test hygiene response associated and how it could serve as an improved tool for predicting colony level *Varroa* resistance. [2,31,40] The synthetic alkene proposed here will be tested for the activity in evoking hygienic behaviors against the already known compounds used to determine which is more biologically active in honey bees.

Hydrocarbons can be described as any organic compound that contains only carbon and hydrogen atoms. An unsaturated hydrocarbon contains double or triple bonds for example an alkene or an alkyne. The physical properties of unsaturated hydrocarbons are nonpolar and moderately insoluble in water. The intermolecular forces present are van der Waals, which are the weakest of all intermolecular forces. In honey bees, hydrocarbon alkenes are created in oenocytes.[27] Oenocytes is a specific ectodermic cell found in the abdomen of insects that makes very long chain fatty acids that then produce pheromones.[28,29]

The objectives for this project are to successfully synthesize the $Z_{10}C_{31}$ alkene and the 5-(2′-methoxyethyl)-cyclopent-2-en-1-butoxyl diether. The hopes for these two compounds is that they will be useful promoters of honey bee wellbeing. The alkene in the affect of being a major compound for inducing hygienic behavior and the cyclopentene in the affect of preventing *Varroa* from reaching the brood cell and reproducing. These are two different ways in which honey bee wellbeing can be improved; the alkene synthesis, which is affecting the honey bee and the cyclopentene synthesis, which is affecting the *Varroa* mite.

CHAPTER II: SYNTHESIS OF Z-MONOALKENE HYDROCARBON

II.1 $Z_{10}C_{31}$

The first part of the project was to synthesize 500mg of the required $Z_{10}C_{31}$ alkene due to a new analysis that suggest that the Z_{10} isomer is the major C_{31} isomer present on honey bee brood rather than the *Z*-isomer currently being used (Figure 1). The goal of the project was achieved using two separate synthetic routes; one being a two-step reaction synthesis composed of an alkylation followed by a hydrogenation, (Figures 2) and the other being a DMP oxidation reaction followed by a Wittig reaction. (Figure 9.)

Figure 1. Previously Identified Compounds

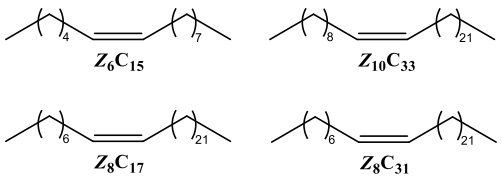
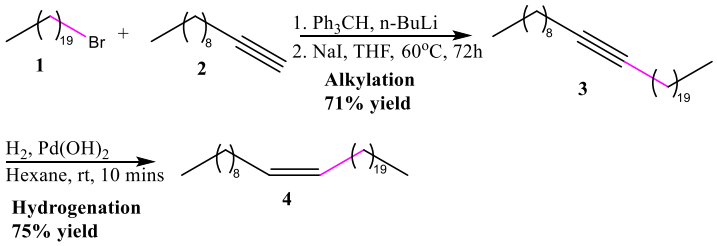


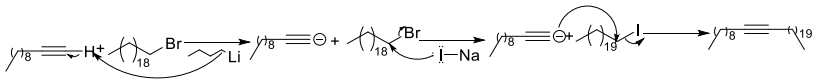
Figure 2. Two Step Synthesis: Alkylation and Hydrogenation



To achieve this desired alkyne product, 10-hentricosyne, starting materials 1-undecyne, and 1-bromoeicosane, were used. The first step in the proposed mechanism of this synthetic route is for the n-BuLi to deprotonate the terminal proton on 1-undecyne. That then gives the carbon on 1-undecyne a negative charge. Next the sodium iodide, NaI, substitutes for the bromine, Br, in 1-bromoeicosane. Lastly, the carbanion of that was formed on the 1-undecyne, now a good

nucleophile, attacks the electrophilic carbon of 1-bromoeicosane which results in the new C-C bond at carbon 10 yielding the product 10-hentricosyne.

Figure 3. Reaction Mechanism of Alkylation



The alkylation reaction produced yields as high as 71%. The reaction was monitored using GC. Proton NMR was used to verify the compound 10-hentricosyne

The second step in the synthesis of the *Z*-isomer of C_{31} is the hydrogenation reaction. In this reaction the starting material alkyne **(3)** was initially added to a suspension of Lindlar's catalyst (Figure 4). Lindlar's catalyst is a poisoned reduction catalyst typically used to reduce alkynes to cis-alkenes without over reduction to the alkane. The reduction was monitored by GC and it was observed that only a 2% conversion to alkene was seen and 98% was still the starting material alkyne (Figure 7). This led to changing our catalyst to palladium hydroxide, which is typically used to reduce alkynes to alkanes. The thought process behind changing to a stronger catalyst was to see if we could observe any reduction to our starting material (Figure 5). Keeping the same reaction conditions; it was observed that no alkyne was present. All the starting material had been converted to the corresponding alkane **(5)**. Knowing that the reduction must go from alkyne to alkene then to alkane it became evident that a shorter reaction time could yield the desired alkene using the palladium hydroxide The reaction was then performed using palladium hydroxide for four hours (figure 8) and showed only a 20 conversion to the desired product. Through reaction optimization (Table 1) it was determined that the best reaction conditions for the hydrogenation using palladium hydroxide was to stop the reaction after ten minutes. Allowing the reaction to go for ten minutes resulted in the observation of 10% of the alkyne remaining unreduced and 90%

conversion to the desired $Z_{10}C_{31}$ long carbon chain alkene (Figure 9) **(4)**. (Figure 6) Shows a GC of the three possible products retention times while doing the hydrogenation.

Figure 4. Hydrogenation Reaction

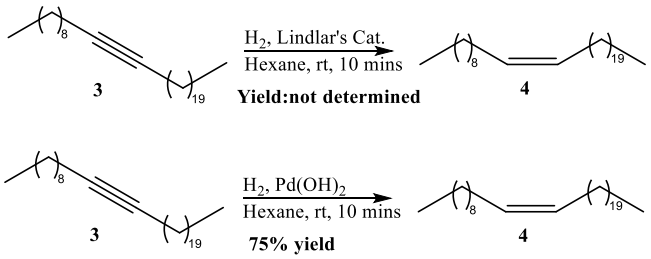


Figure 5. GC Retention Times for Corresponding Hydrocarbons

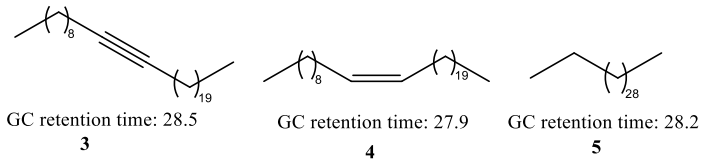
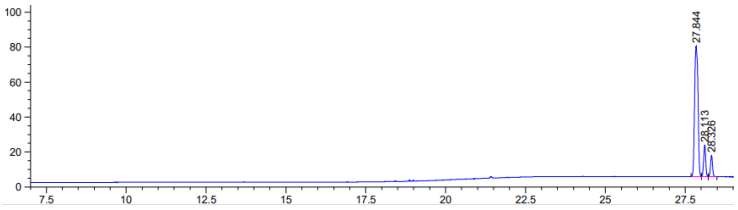
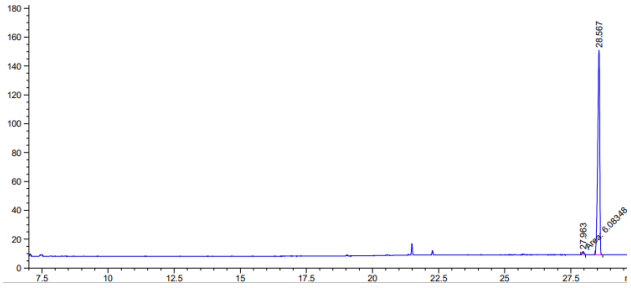


Figure 6. GC Chromatograph of Hydrocarbon Retention Times



Peak #	RetTime [min]	Sig	Type	Area [pA*s]	Height [pA]	Area %
1	27.844	1	BV	550.65247	74.73457	80.55946
2	28.113	1	VV	80.14294	18.23116	11.72477
3	28.326	1	VB	52.74002	12.19535	7.71577

Figure 7. GC Result from Hydrogenation using Lindlar's Catalyst

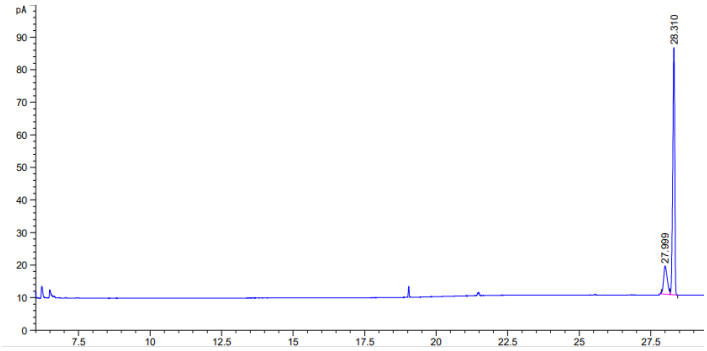


Peak #	RetTime [min]	Sig	Type	Area [pA*s]	Height [pA]	Area %
1	27.963	1	MM	6.08348	1.67245	0.80116
2	28.567	1	BB	753.24823	141.97842	99.19884

Table 1. Hydrogenation Reaction Optimization

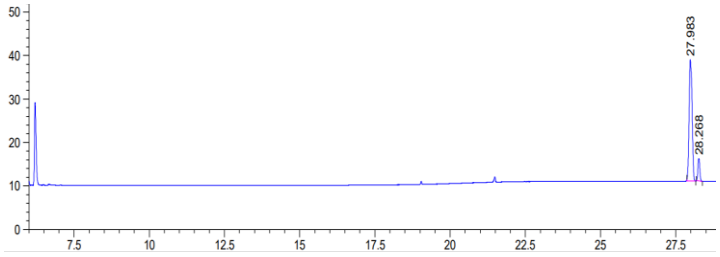
Catalyst	Reaction Time	Conversion (alkyne:alkene:alkane)
Lindlar's Catalyst	24 hours	98:2:0
$Pd(OH)_2$	24 hours	0:0:100
$Pd(OH)_2$	4 hours	0:17:83
$Pd(OH)_2$	30mins	0:45:55
$Pd(OH)_2$	10 mins	0:90:10

Figure 8. GC of Hydrogenation Reaction check at 4 hours using $Pd(OH)_2$



Peak #	RetTime [min]	Sig	Type	Area [pA*s]	Height [pA]	Area %
1	27.999	1	BV	76.42214	8.66715	17.35383
2	28.310	1	VB	363.95404	75.96648	82.64617

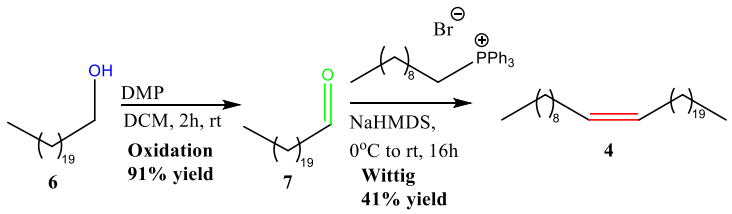
Figure 9. GC of Hydrogenation Reaction check at 10mins using $Pd(OH)_2$



Peak #	RetTime [min]	Sig	Type	Area [pA*s]	Height [pA]	Area %
1	27.983	1	BB	193.91476	27.95604	89.54969
2	28.268	1	BB	22.62956	5.16010	10.45031

The other synthetic route taken to achieve the $Z_{10}C_{31}$ compound was the use of oxidizing the corresponding alcohol to an aldehyde followed by a Wittig olefination reaction. (Figure 10.)

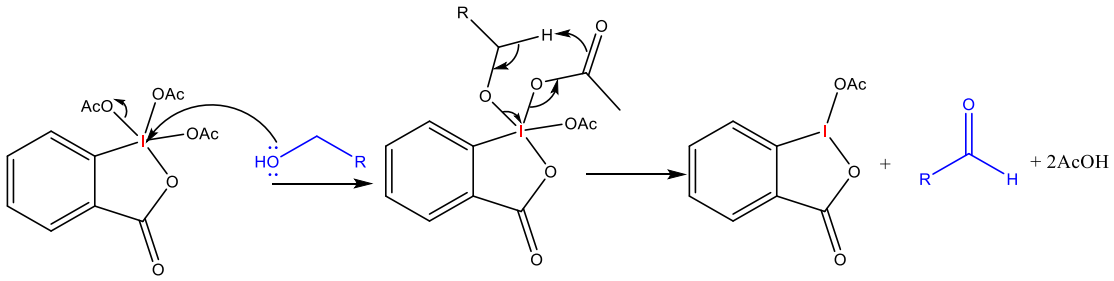
Figure 10. Two Step Synthesis: Oxidation Reaction and Wittig Olefination



The first step in the oxidation reaction (Figure 10) is utilizing a hypervalent iodine compound, Dess Martin periodinane, to oxidize an alcohol group into an aldehyde. The resulting aldehyde will then be used as the starting material for synthesizing the $Z_{10}C_{31}$ compound.

The oxidation mechanism proposed is shown in (Figure 11). First, the alcohol coordinates to the iodine center. This leads to the release of one of the acetate ligands and the intermediate diacetoxyalkoxyperiodinane, is formed. The second acetate ligand then removes the α-hydrogen. The carbonyl compound then leaves the iodine center and kicks off another acetate ligand from the iodine center. An aldehyde compound is formed as a product with acetic acid and iodinane as byproducts.

Figure 11. Mechanism of Oxidation Reaction using Dess-Martin Periodinane



To prepare the starting aldehyde for the hydrocarbon *Z*-alkene, an alcohol group such as heneciosanol (**6**) was dissolved in dichloromethane and DMP was added while the solution was placed in an ice bath. The reaction was then stirred for two hours and quenched with sodium bicarbonate and sodium thiosulfate and allowed to stir for 10 minutes. An aqueous extraction was performed and concentrated in vacuo to yield heneciosanal (**7**) with yields as high as 91%. The

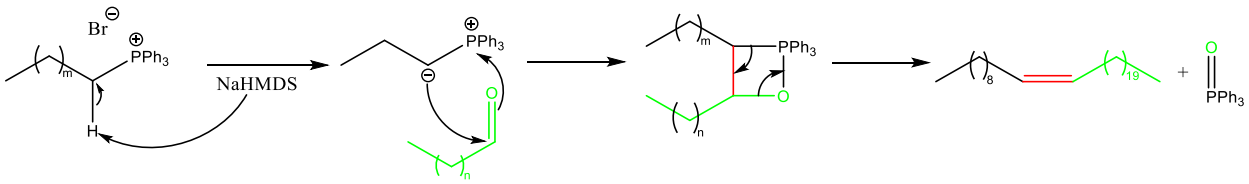
reaction was monitored with TLC and GC. Proton NMR was performed to verify the compound with known spectral data.

The second step for the synthesis of the *Z*-alkene uses a Wittig reaction (Figure 10 step 2). In this reaction, the aldehyde group of the hydrocarbon chain is reacted with decyl triphenyl phosphonium bromide. Ylide is produced as a result of deprotonation with sodium bis(trimethylsilyl)amide (NaHMDS) to the alkyl phosphonium bromide. The Wittig reaction produces the *Z*-alkene as a product and triphenylphosphine oxide as a byproduct. To purify the synthesized product, column chromatography was performed with 100% hexane.

The Wittig mechanism, shown in Figure 12, involves a nucleophilic attack of the negatively charged carbon atom of the ylide on the carbonyl group to obtain oxaphosphatane. Next, a bond is formed between an electrophilic carbon atom of the ylide and carbonyl carbon atom and phosphorus bonds to an oxygen atom. The phosphorous intermediate immediately decomposes to yield the alkene and triphenylphosphine oxide. It is a concerted step because bonds break and form simultaneously. The driving force of the Wittig reaction is the formation of a very strong phosphorus-oxygen π bond.

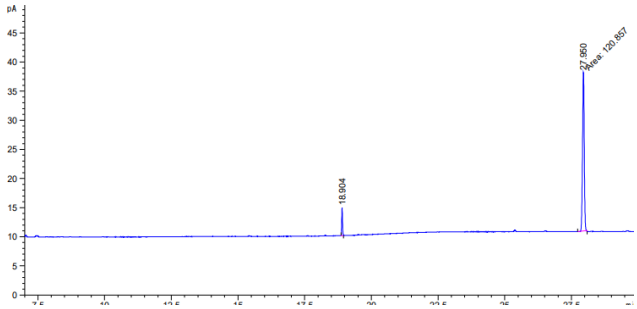
To synthesize **(4)**, 1 equivalence of (decyl)-triphenyl phosphonium bromide was dissolved in THF, followed by the addition of 1 equivalence of NaHMDS, and finally 1 equivalence of heneciosanal was added dropwise while placing the solution in an ice bath. The reaction was stirred overnight while allowing the temperature to rise to room temperature. Once the reaction was completed, an aqueous separation was done, and the mixture was concentrated in vacuo to yield $Z_{10}C_{31}$ with a yield as high as 41% (Figure 11). The resulting compound was a white solid and obtained with purity greater than 95% verified through GC. Proton NMR and carbon NMR were performed to verify the compound.

Figure 12. Wittig Olefination Mechanism



Initially, the synthesis of the alkenes was performed using 1 equivalence of aldehyde starting materials, 2 equivalence of NaHMDS and 2 equivalence of alkyl-triphenyl phosphonium bromides. The reactions were monitored by GC and silica gel column chromatography was used to purify the products. GC was obtained for some of the fractions which showed two peaks at different retention times (Figure 13). The peak at 27.9 min correlated to the desired compound, $Z_{10}C_{31}$, while the peak at 18.9 min correlated to the formation of a dimerized byproduct. The byproduct that was formed during the reaction caused challenges in the purification of the compounds because when the products were purified through silica gel column chromatography, the byproducts eluted along with the desired products. Many fractions that contained the desired product also contained the byproduct.

Figure 13. GC Result to Show Purity of Desired Compound from Wittig Reaction

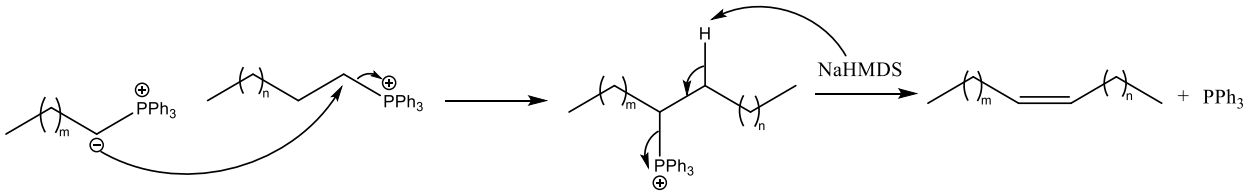


Peak #	RetTime [min]	Sig	Type	Area [pA*s]	Height [pA]	Area %
1	18.904	1	BB	10.03064	4.81313	7.66355
2	27.950	1	MM	120.85702	27.23181	92.33645

This finding of an unexpected dimerized by product led us to do a mechanistic study of the Wittig olefination to determine how the byproduct was being formed with hopes that understanding its mechanistic formation would allow for the improvement of the reaction to possibly eliminate its formation all altogether.

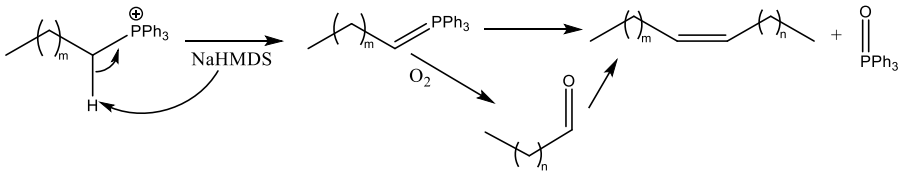
To study the mechanism of the Wittig olefination the reaction experiments were run in the presence of the corresponding aldehyde, heneicosanal, along with control experiments. The control experiments were run under the same conditions as the Wittig olefination, but without the addition of heneicosanal. The initial hypothesis for the formation of the byproduct was that any excess triphenyl phosphonium bromide in the reaction mixture was reacting with itself to produce the dimer byproduct along with triphenyl phosphine (Figure 15). After a series of experiments and controls were run it was concluded that even without the presence of the aldehyde the formation of the dimerized byproduct is still observed.

Figure 14. Proposed Mechanism for Dimerization of Decyl-Triphenyl Phosphonium Bromide



TLC and GC was used to monitor and visualize the products being formed in the mechanistic study. The results showed that triphenyl phosphine was not observed as a byproduct in the control experiments, but there was presence of triphenyl phosphonium oxide. This led to the conclusion that the triphenyl phosphonium bromide was not reacting with itself, but going through an autooxidation where O_2 was acting as an oxidizing agent to produce the dimer along with the triphenyl phosphonium oxide (Figure 15) that was also seen in the reaction when the corresponding aldehyde was used. [30]

Figure 15. Autooxidation of Dimerization of Decyl-Triphenyl Phosphonium Bromide



II.2 Conclusion

The goal of the research was to synthesize approximately 500 mg of $Z_{10}C_{31}$ alkene. The route we decided to use for completing the synthesis of the $Z_{10}C_{31}$ alkene was the alkylation followed by the hydrogenation. We were able to synthesize approximately 650mg of compound using the hydrogenation reaction. This route was better than the Wittig route in terms of purification, time efficiency, and cost. Purification was difficult in the Wittig reaction due to the

presence of the dimerized by product that co-eluded with our desired product. The compound will be tested for its ability to trigger hygienic behavior in the honey bees and determine if the Z_{10} isomer is more active than the Z_8 isomer being used currently in the UBO test. The results from the field test of inducing hygienic behavior has the potential to improve honey bee health by facilitating selective breeding *Varroa* resistant honey bees.

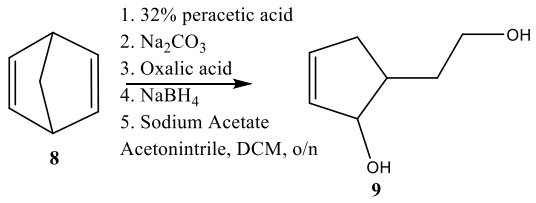
CHAPTER III: DISRUPTING THE SENSING ABILITY OF *VARROA*

III.1 Synthesis of 5-(2′-methoxyethyl)-cyclopent-2-en-1-butoxyl diether

Being able to selectively breed for hygienic honey bees using the *Z*-isomers is important and significant to controlling and removing the mite before the colony can be greatly affected, but it is also possible to improve the honeybee health by targeting *Varroa* directly. Synthesizing the 5-(2′-methoxyethyl)-cyclopent-2-en-1-butoxyl diether compound allows us to disrupt the olfactory senses of the *Varroa* mite. This promotes honey bee wellbeing by not allowing *Varroa* the ability to locate and parasitize the brood cell. It begins with synthesizing the starting material 5-(2-hydroxyethyl)cyclopent-2-en-1-ol. Once the starting material diol was synthesized the desired 5-(2′-methoxyethyl)-cyclopent-2-en-1-butoxyl diether was recovered after a four-step synthesis. It begins with an alcohol protection, followed by an alkylation of the remaining free alcohol, then a deprotection of the previously protected alcohol, and lastly a methylation at the primary alcohol position.

The first route we used to synthesize the starting material diol **(9)** was a one pot synthesis (Figure 16.) that allowed for us to use 2,5-norbornadiene **(8)** to be converted directly to **(9)** in one step. **(8)** is extremely moisture sensitive and required that the reaction be done in a glovebox to ensure all materials and reagents were kept inert under argon gas.

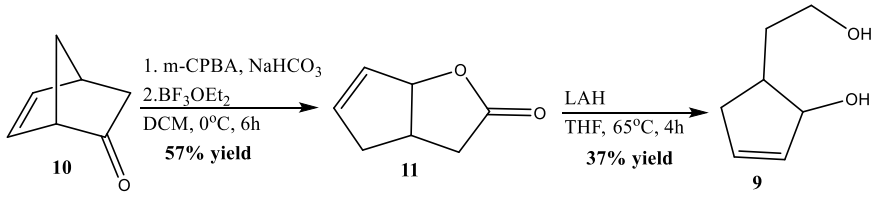
Figure 16. One Pot Synthesis of 5-(2-hydroxyethyl)cyclopent-2-en-1-ol



The proposed mechanism for the reaction is hypothesized to make use of an epoxidation followed by a Meinwald Rearrangement. The reaction was monitored using TLC and GC. Proton NMR was performed to verify the compound was matched with known spectral data.

The purification of this reaction proved to be extremely challenging. One reason for the this would be since **(9)** is so polar it is very difficult to purify using column chromatography. This reaction also yielded many unwanted by-products that caused the isolation of compound **(9)** to be extremely difficult. The reaction did result in the recovery of **(9)**, but the yields were drastically low, so the synthesis of the diol using the one pot synthetic route was abandoned. This led to utilizing synthetic route B: a two-step reaction process that was composed of a Baeyer-Villiger oxidation followed by a lithium aluminum hydride, $LiAlH_4$, reduction to yield **(9)** (Figure 17).

Figure 17. Two-Step Synthesis: Baeyer-Villiger Oxidation and LAH Reduction



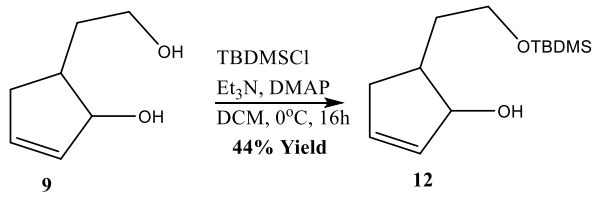
The first step is the Baeyer Villiger Oxidation followed by a rearrangement using the Boron Trifluoride etherate. This type of oxidation is often used to form an ester from a ketone, or in our case form a lactone from a cyclic ketone. The Baeyer Villiger oxidation did pose a concern because of a competing reaction where you could oxidize at the alkene rather than adjacent to the

carbonyl which would result in an undesired epoxidation. Realizing that there were two possible reactions that could take place it was important for us to see the alkene protons when taking proton NMR to verify we has synthesis the correct compound. The reaction was monitored by TLC.

Once the desired lactone, **(10)** was recovered and purified using column chromatography a $LiAlH_4$ reduction was performed on the compound. $LiAlH_4$ is often used to reduce carboxylic acid derivatives to primary and secondary alcohols. $LiAlH_4$ is extremely reactive with water so extra precaution was taken to ensure that no water was exposed to the $LiAlH_4$. The reduction reaction was performed at reflux in order to push the reaction forward. After performing the $LiAlH_4$ reduction the reaction mixture was concentrated in vacuo and a crude proton NMR was obtained. The data obtained from the crude proton NMR showed that column chromatography was not needed to further purify **(9)**. Proton NMR was used to verify the structure of **(9)** and matched with known spectral data.

After verifying we had synthesized **(9)** we were able to being the synthesis toward **(15)**. To begin, we performed an alcohol protection (Figure 18). This was performed with tert-butyldimethylsilyl chloride as our protecting group. Protecting the primary alcohol before performing an alkylation is important when there are two alcohol groups present. In our case there is a primary and a secondary alcohol present. A primary alcohol has less steric bulk around it in comparison to a secondary alcohol, so the alkylating agent being used would likely attack at the primary alcohol position since it easier to access than that of the secondary alcohol.

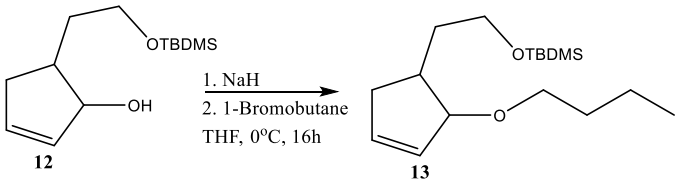
Figure 18. Primary Alcohol Protection



An aqueous work up was performed, and the reaction mixture was concentrated in vacuo. Reaction was seen to produce by-products of the diprotected diol, monoprotected diol at the secondary alcohol, and monoprotection of the primary alcohol **(12)**. The mixture was purified through column chromatography and monitored using TLC. Reaction produced yields as high as 44% of **(12)** and was carried through to the alkylation at the secondary alcohol position.

The alkylation is the second step in the synthetic pathway in synthesizing **(15)** (Figure 19). The alkylation uses 1-bromobutane to add to the free alcohol. Initially the reaction was done with adding **(12)** to a suspension of potassium hydride and THF at 0 ^{o}C, but after monitoring reaction it was seen that at 0 ^{o}C the potassium hydride, the base, was not able to fully deprotonate the starting material, so the alkylation was not taking place. The reaction was attempted again, but this time with **(12)** being added to a suspension of sodium hydride in THF at room temperature. When using the sodium hydride, we increased the equivalence to be twice as much as the potassium hydride. This greatly improved the reaction taking place as the sodium hydride was then able to fully deprotonate the free alcohol to allow for the alkylation to take place **(13)**. The purification process has proven to be extremely difficult using column chromatography. Yields have been in the range of 5%-23%. Proton NMR was used to verify **(13)** and matched with known spectral data. Since yields were rather low crude material containing compound (**13**) was used then used to complete the synthesis.

Figure 19. Alkylation of Secondary Alcohol



After alkylating at the free alcohol a deprotection was done (Figure 20) to remove the TBDMS protecting group freeing our primary alcohol **(14)** so a methylation can be done (Figure 21) to achieve the final desired compound **(15)**.

Figure 20. Alcohol Deprotection

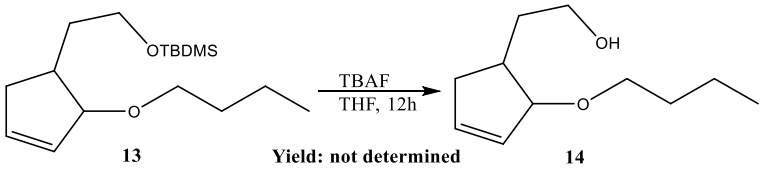
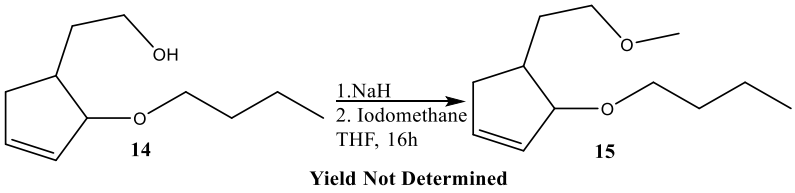


Figure 21. Alcohol Methylation



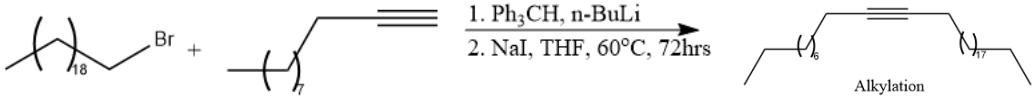
Currently we have made about 10mg of the desired diether **(15).** With the best reaction conditions worked out the process of synthesizing more should be rather easy. Another student has begun the reaction sequence to prepare more of the diether **(15)**. Future work for this project would be to synthesize more of compound **(15)**. The project could also go in the direction of synthesizing all four of the stereoisomers of compound **(15)** and determining which of stereoisomers causes the disruption of the olfactory senses of the *Varroa* mite since we have synthesized a racemic mixture.

CHAPTER IV: EXPERIMENTAL PROCEDURES AND METHODS

IV.1 General Information

All reactions were performed with dried solvents in an oven dried glassware under an argon atmosphere. Solvents and reagents were obtained from commercial sources and used without additional purification. Purification was performed using flash grade silica gel (particle size: 40-63 μm, 230 × 400 mesh). The reactions were monitored by TLC using silica G F254 precoated plates. The 1H and 13C NMR spectra were recorded on JOEL 400 MHz spectrometer. The solvent used to record the NMR was CDCl3 at room temperature.

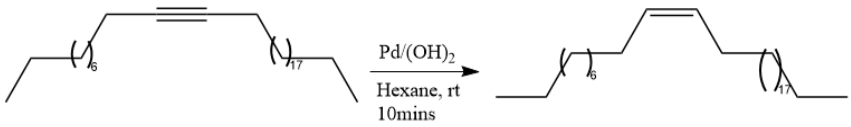
III.2 (3) Synthesis



A dry 200 ml Schlenk flask was charged with 85 ml of dry THF and 1-undecyne (4.92mL, 5.54 mmol), and a pinch of Ph_3CH as an indicator. The solution was stirred and cooled in an ice bath, and BuLi (2.5 M in hexanes, 2.53ml, 27.39 mmol) was added. NaI (0.083 g, 0.554 mmol) was then added, followed by a solution of eicosane bromide (4.5 g, 5.54 mmol) in 6ml THF, added dropwise over 1 h. When the addition was complete the mixture was heated to 60°C and stirred for 3 days. After 3 days the mixture was then allowed to cool to room temperature and quenched with saturated NH_4Cl, and extracted with hexane. The organic layer was washed with brine, dried with $MgSO_4$, and concentrated. The residue was taken up in 70 ml hexane, and eluted through a 60 ml sintered glass funnel loaded with silica gel, rinsing well with hexane, The filtrate was concentrated, and the residue was dissolved in 80 ml hot acetone. The solution was chilled

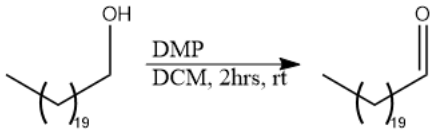
overnight to 4 °C, and then filtered cold with suction, rinsing with cold acetone, yielding the 10-hentricosyne (3.89g, 71% yield) as fluffy white crystals. (3.89g, 71% yield)

III.3 (4) Synthesis



A 200 ml round bottom flask was charged with pyridine (0.36 ml), and Pd/C (100 mg), in 42.0 ml normal hexane. The flask was flushed with Ar, then flushed with hydrogen gas, without stirring. The flask was then connected to a large balloon filled with hydrogen, stirring was started, and a solution of the alkyne (1.2g, 2.77 mmol) in 18.0 ml normal hexane was added by syringe. Stirring was continued for 10mins. The mixture was then filtered through Celite, and the filtrate was stirred for 30mins with 1M HCl to remove the pyridine. After separation of the layers, the hexane solution was washed again with 1 M HCl, and brine, dried over Na_2SO_4, and concentrated. The resulting semisolid was taken up in 20 ml of hot acetone, then cooled to 4°C overnight. The resulting mixture was filtered cold, rinsing with 20 mL of cold acetone, and the resulting white crystals were dissolved to in 100% hexane and purified using silica gel column chromatography. The alkene was recovered as white crystals (900mg, 75% yield).

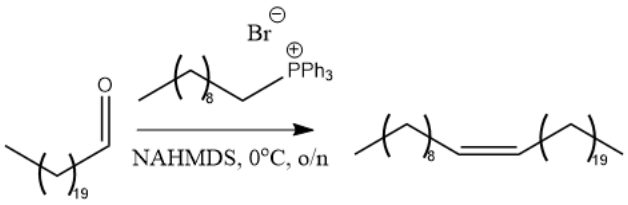
III.4 (7) Synthesis



An oven dried 250 mL round bottom flask was obtained and equipped with a magnetic stir bar and argon balloon. Heneicosanal (240 mg, 0.768 mmol,) was added to the flask along with

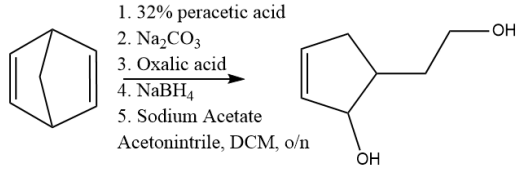
dry dichloromethane (17 mL). Dichloromethane was obtained from the solvent purification system in Dr. Croatt's lab. The mixture was stirred at room temperature until the compound was dissolved. Dess martin periodinane, DMP, (543 mg, 1.54 mmol) was added to the flask in the ice bath and stirred for 2 hrs. The mixture was then stirred with saturated Sodium bicarbonate and Sodium thiosulfate (1:1 v/v; 20 mL) for 10 minutes. The mixture was then extracted with ethyl acetate (3*15 mL). The organic layers were combined, dried with $MgSO_4$, filtered, and concentrated *in vacuo.* The mixture was purified by silica gel column chromatography (5% ethyl acetate/hexane) to obtain the compound (240 mg, 91% yield) as a white solid.

III.5 (4) Synthesis Route 2



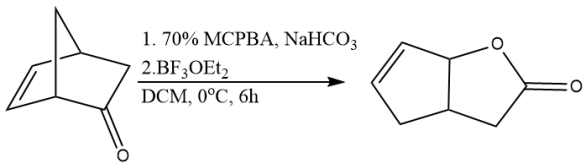
A 100 mL oven dried round bottom flask was equipped with a magnetic stir bar and argon balloon. In the flask, (1-Decyl)triphenylphosphonium bromide (78 mg, 0.161 mmol) and THF (5 mL) were added and stirred. A solution of NaHMDS (2M in THF, 0.081 mL, 0.161 mmol) was added and stirred. The solution is stirred at room temperature for 15 minutes then placed in an ice bath with brine to cool. Heneicosanal (50 mg, 0.161 mmol,) was dissolved in THF (5.0 mL) and the solution was added dropwise to the flask over 20 minutes. The mixture was warmed slowly and stirred overnight. The mixture was concentrated *in vacuo.* Silica gel column chromatography (100% hexane) was used for purification. The compound was recovered as white crystals (15mg, 30% yield)

III.6 (9) Synthesis



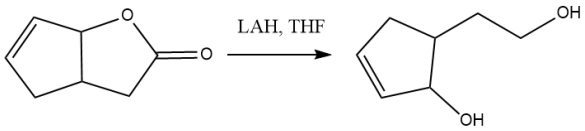
A 50 mL oven dried RBF w/ argon balloon was charged with dry DCM (9.1mL). 2,5-Norbornadiene (1.5 mL, 14.76 mmol) was then added to RBF containing dry DCM. Next, Na_2CO_3 (1.56 g, 14.76 mmol) is added to the solution and the suspension is stirred rapidly. 32% peracetic acid (1.75 g, 7.38 mmol) was then treated with sodium acetate then added to the reaction mixture *(removes any sulfuric acid present). Reaction was then stirred at rt for 4 h. After 4 h the reaction mixture is filtered and rinsed with DCM (25 mL). The solvent and excess **(7)** was then evaporated to give residue intermediate. The residue was then added to 40mL of H_2O and then to Oxalic acid (40 mg, 0.443 mmol). The reaction was then allowed to stir overnight at rt. Acetonitrile (48mL) was then added to reaction mixture. Sodium borohydride (450mg, 11.90 mmol) was then added in several portions over 10 mins. The reaction was stirred at rt for 4 h. The mixture was then concentrated to remove organic solvent. The remaining aqueous solution was saturated with NaCl (15 mL and extracted with Chloroform (20 mL*5 times). The combined organic solvent was dried over $MgSO_4$ and concentrated. Product was purified by column chromatography and collected as a colorless oil (94mg, 5% yield).

III.7 (11) Synthesis



A solution of 70% m-CPBA (2.51 g, 10.18 mmol and sodium bicarbonate (3.0 g, 32.38 mmol) in DCM (18 mL) was stirred at 0°C. Norborn-5-en-2-one (1.0 g, 9.25 mmol) in DCM (4 mL) was added dropwise to m-CPBA suspension at 0°C and allowed to stir for 6 h at 0°C. The precipitate was diluted with 5 mL DCM and filtered. The filtrate was washed with aqueous saturated sodium bicarbonate solution (15 mL). The organic layer was then concentrated to 2mL. The resulting solution was then treated with boron trifluoride etherate (0.02 mL) at 0°C and stirred for 20mins at 0°C. Saturated aqueous sodium bicarbonate solution was added to quench reaction. Organic layer was separated, and the aqueous layer was extracted with ethyl acetate (20 mL*3). Combined organic extracts were washed with brine, dried with MgSO4, and concentrated. Compounds were purified through silica gel chromatography (10% EA/Hexane). Pure compound was recovered as a colorless oil (656mg, 57% yield).

III.8 (9) Synthesis Route 2

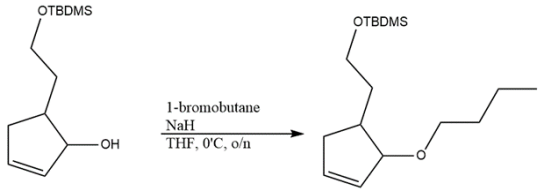


THF was added to RBF containing LAH (203 mg5.34 mmol) and stirring was begun at 0°C. THF was then added to starting material lactone (656 mg, 5.26mmol) and added dropwise to LAH slurry. The reaction is then removed from ice bath and heated to reflux (65°C) for 4 hours. After 4 h the reaction was quenched with saturated aqueous ammonium chloride solution and concentrated. The residue was diluted with water (15 mL) and extracted using ethyl acetate. Organic layer was washed with bring, dried, filtered, and concentrated. No purification was needed the as the crude was recovered as pure product (250mg, 37% yield).

III.9 (12) Synthesis

To a solution of **(9)** (100 mg, 0.780 mmol)) in DCM (10mL) was added TBDMSCl (141 mg, 0.936 mmol). After addition of TBDMSCl, triethylamine (0.13 mL, 0.936 mmol) and DMAP (9.53 mg,0.078 mmol) were added to reaction on ice. Reaction was then placed in the fridge overnight. Reaction mixture was removed from the fridge diluted with DCM and washed with DI water and brine. Organic layer was dried over MGSO4 filtered and concentrated. Silica gel column chromatography (5% EA/Hexane) was used for purification. The product was recovered as a colorless oil (56mg, 30% yield).

III.10 (13) Synthesis



The monoprotected alcohol (50 mg, 0.206 mmol) in 2 mL THF was added dropwise to suspension of NaH (55 mg, 4.12 mmol) in 13mL THF at rt. The reaction mixture was then stirred at 0°C for 30mins. 1-bromobutane was added dropwise to reaction mixture at 0°C. The reaction mixture continued to stir while in ice bath and warmed to rt overnight. An aqueous workup was performed using ethyl acetate, dried over $MgSO_4$, and concentrated. Crude mixture was purified through silica gel chromatography (2% EtOAc/Hexane) and product was recovered as a colorless oil (12mg, 24% yield).

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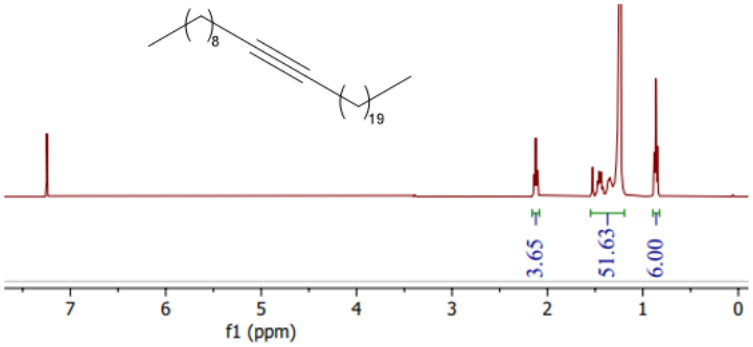
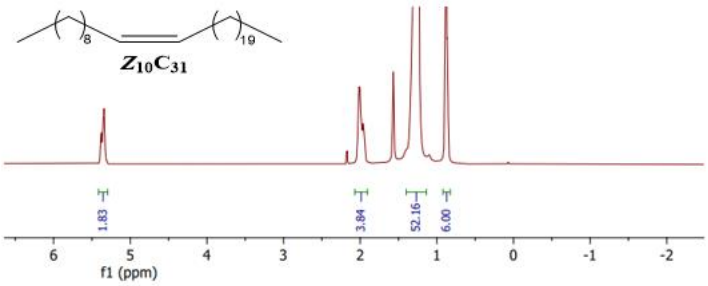
APPENDIX A: NMR SPECTRA

The 1H and 13C nuclear magnetic resonance (NMR) spectra were plotted on a JEOL ECS 400 MHz spectrometer using CDCl3 as a solvent at room temperature. The NMR chemical shifts (δ) are reported in ppm.

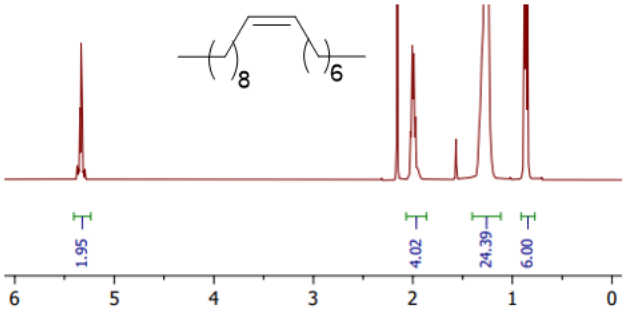
^{1}H NMR *Z*10C31 alkyne



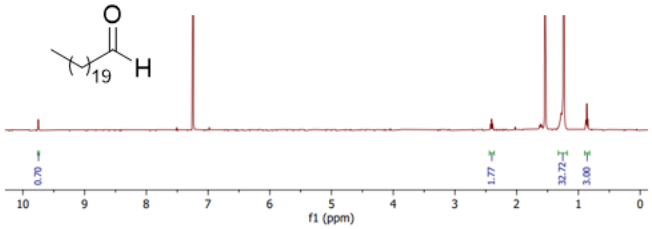
^{1}H NMR



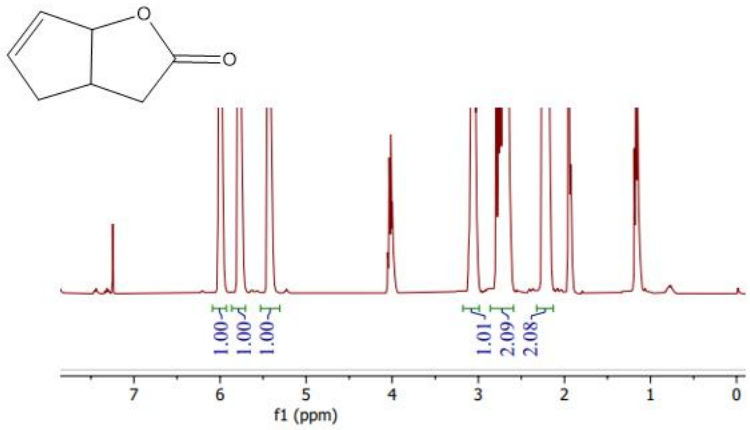
^{1}H NMR



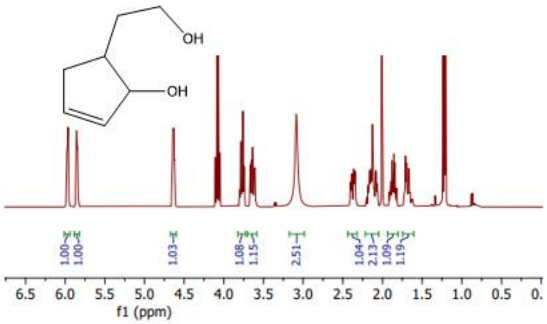
^{1}H NMR



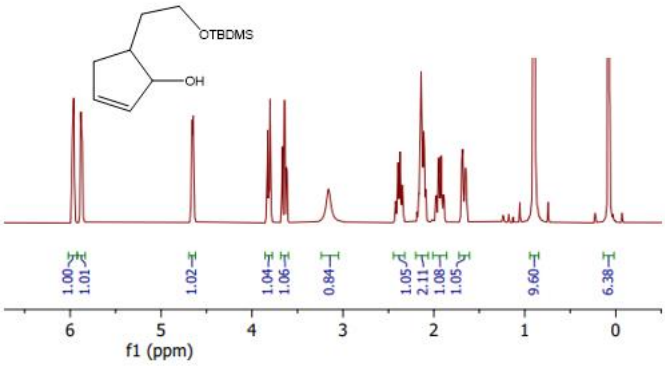
^{1}H NMR



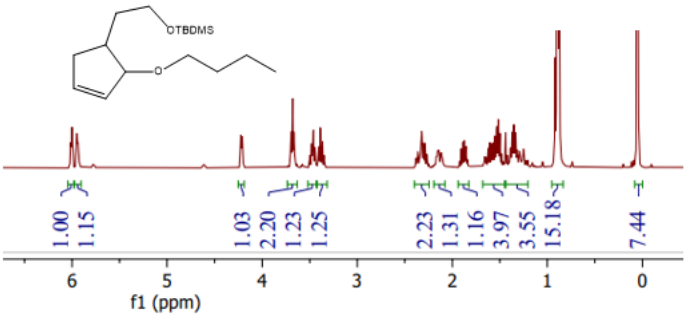
^{1}H NMR


OH
OH
1.00
1.00
1.03
1.08
1.15
2.51
1.04
2.13
1.09
1.19
6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.
f1 (ppm)

^{1}H NMR


OTBDMS
OH
1.00
1.01
1.02
1.04
1.06
0.84
1.05
2.11
1.08
1.05
9.60
6.38
6 5 4 3 2 1 0
f1 (ppm)

^{1}H NMR


OTBDMS
O
1.00
1.15
1.03
2.20
1.23
1.25
2.23
1.31
1.16
3.97
3.55
15.18
7.44
6 5 4 3 2 1 0
f1 (ppm)

APPENDIX B: GAS CHROMATOGRAPHY- MASS SPECTROMETRY

GCMS-mass spectrum were obtained using GC-MS with single quadrupole mass analyzer (QP2010S, Shimadzu equipped with electron impact ionization. The mass range of m/z 500 and unit resolution was used. Dr. Wagoner's methods were used to obtain the spectrum.

$Z_{10}C_{31}$