Greener Methods Towards the Synthesis of Stachybotrin D, a Potential Anti-HIV Drug

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Abstract

Stachybotrin D is a secondary metabolite from a sponge-derived fungus with potential applications as an anti-HIV drug. Human immunodeficiency virus (HIV) affects 36.9 million people worldwide and is prone to developing drug resistance. While there are currently several approved HIV therapies, the tendency for mutation requires that we constantly find new anti-HIV drugs, especially those with different binding abilities. Stachybotrin D has several structural features that may promote different binding properties than the drugs currently on the market. This is important with the increase of drug resistance to current drugs used in existing therapeutic cocktails for HIV. Since fungi are unsustainable drug sources, this research focuses on the application of sustainable resources and greener techniques towards the chemical synthesis of Stachybotrin D. Retrosynthetic analysis performed on Stachybotrin D revealed the molecule can be synthesized from three segments. This research is focused on the synthesis of one segment composed of a bicyclic ring fused to a furanyl ring as well as its stereochemically specific substituents. To obtain the desired product, the synthesis incorporated a Robinson annulation using 2-methylcyclohexane-1,3-dione and ethyl vinyl ketone followed by protecting the saturated carbonyl, methylation at the α-position on the unsaturated carbonyl carbon, reducing the methylated carbonyl to an alcohol, and removing the protecting group from the carbonyl. However, after examining methods for methylating the alpha position on the unsaturated carbonyl carbon, the synthesis was revised to synthesis isopropyl vinyl ketone and using it in place of ethyl vinyl ketone in the Robinson annulation in order to add the methyl group in a greener way.

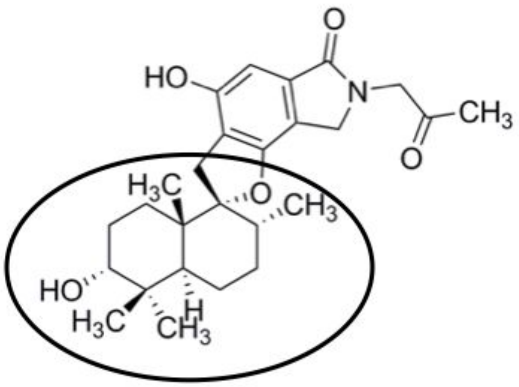


Figure 1: The structure of Stachybotrin D where the circle is highlighting the bicyclic moiety on which this research focuses.

Current Treatment of HIV

The human immunodeficiency virus (HIV) is a health crisis that affects 36.9 million people worldwide.[1] It is a disease that can be transmitted through sexual contact, sharing injection needles, transfusions, and from mother to child during pregnancy, birth, or breastfeeding.[2] According to United Nations Program on HIV/AIDS (UNAIDS), over 940,000 people died from acquired immunodeficiency syndrome (AIDS) related diseases (ex. tuberculosis, pneumonia, and fungal infections) in just the year 2017 alone.[3] There is no current widely applicable cure for HIV, but there are medications that can improve the quality of life of HIV patients.

HIV attacks the body by targeting CD4 cells which are important for immune responses.[4] With less CD4 cells, the body has diminished ability to fight infections. Even mild infections could be serious to fatal for people with HIV. The consequence is that the person is immunocompromised, from which this virus gets its name.

To develop treatments for HIV, it is important to first understand the mechanism of how HIV attacks CD4 cells. HIV carries the reverse transcriptase enzyme and HIV RNA inside a protein core called a capsid. Around the capsid of HIV is the HIV envelope. The glycoproteins on the HIV envelope bind to the receptors on the surface of the CD4 cell and the HIV envelope fuses with the CD4 to allow the HIV capsid into the cell. In order to infect the CD4 cell, HIV must convert its RNA into DNA using its reverse transcriptase enzyme. The viral DNA, then, can be inserted into the DNA of the CD4 cell. The integrated DNA will code for the replication of HIV and the machinery in the host cell carries out the instructions of the integrated DNA. Once HIV is replicated many times, the host cell will lyse and release HIV so that it can spread and attack other CD4 cells which proliferates the infection.[4] If an anti-HIV drug can stop HIV from being replicated and released, the individual's symptoms could be alleviated since the infection is not proliferating.

There are many HIV drugs that are currently on the market to stop the process of HIV infecting cells and replicating as described above. These drugs impact different stages within the process. For example, fusion inhibitors prevent HIV from being able to enter the CD4 cell.[4] An example of an FDA approved fusion inhibitor is Enfuvirtide (Fuzeon).[5] While nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors, prevent the transcription and replication of the viral RNA.[6] Protease inhibitors blocks HIV protease which is an enzyme needed for HIV to make copies of itself. Some FDA approved protease inhibitors are Atazanavir (Reyataz), Darunavir (Prezista), and Fosamprenavir (Lexiva) among others. Nucleoside reverse transcriptase inhibitors blocks HIV reverse transcriptase, thereby preventing HIV from translating viral RNA to DNA and making

copies of itself. Some FDA approved nucleoside reverse transcriptase inhibitors are Abacavir (Ziagen), Emtricitabine (Emtriva), and Lamivudine (Epivir) among others. Non-nucleoside reverse transcriptase inhibitors bind to and alters the HIV reverse transcriptase, thereby preventing HIV from translating viral RNA to DNA and making copies of itself.[6] There are currently five FDA approved non-nucleoside reverse transcriptase inhibitors: Nevirapine (Vitamune), Doravirine (Pifelto), Efavirenz (Sustiva), Etravirine (Intelence), and Rilpivirine (Edurant).[5] The molecular structure of these compounds can be seen in Figure 2.

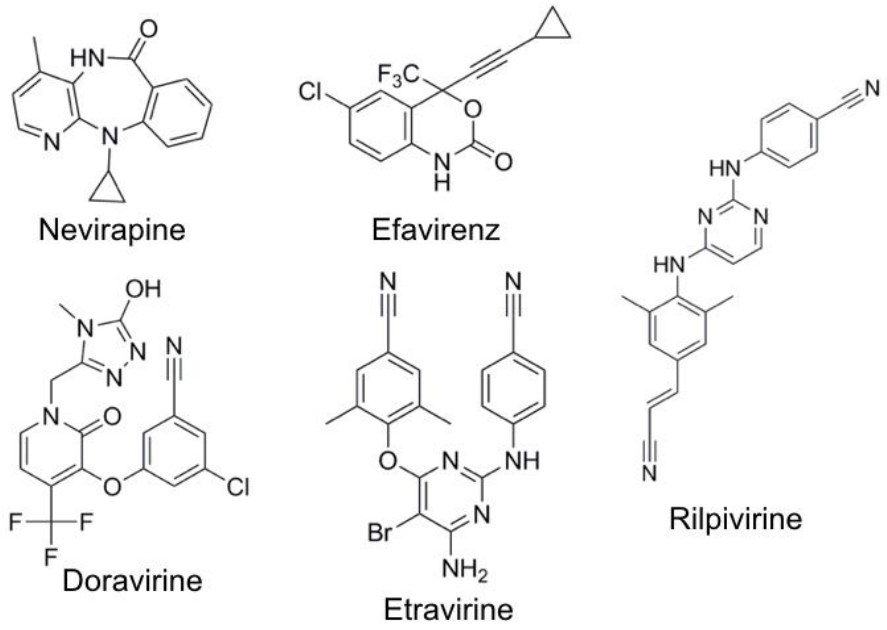


Figure 2: The molecule structure of five non-nucleoside reverse transcriptase inhibitor drugs that are currently on the market for treating HIV.[5]

However, one of the reasons that HIV affects so many people is that the virus can develop resistance to HIV drugs making it harder to treat. As HIV is replicated in the body, the virus can change form or mutates. HIV drugs may not stop the spread of mutated HIV since the

mutated virus is different structurally and/or functionally from the wild type virus. If a mutated virus survives the HIV drugs, then it will replicate so that more mutated, drug-resistant HIV is present in the body. This drug-resistant HIV can be transmitted from one person to another.[7] According to the World Health Organization (WHO), 6 of the 11 countries surveyed in Africa, Asia, and Latin America in 2017 showed that over 10% of the individuals starting antiretroviral treatment had resistance to some of the most widely used HIV drugs.[8] The WHO suggests that countries that reach over a 10% resistance threshold reevaluate their HIV treatment programs.[8]

One way to lower the chance of drug resistance is to take HIV drugs in combination which is known as highly active antiretroviral therapy (HAART). HAART is usually a combination of three antiretroviral drugs that have different mechanism of stopping HIV proliferation.[9] The idea behind HAART is that if a mutation in HIV is able to evade one HIV drug, it should not be able to evade the other two drugs. Thus, the mutated virus is prohibited from replicating and the HIV is kept in check. For HAART to work effectively, medication adherence or taking medications exactly as prescribed every day is important.[7]

Additionally, since the early 2000s, the United States has started to perform drug resistance testing before treatment to determine which drugs will best prevent the proliferation of HIV in that specific individual.[10] In addition to individualized treatment plans, drug resistance testing can give statistics about transmitted drug resistance. Kaiser Permanente Northern California clinic performed a genotypic drug resistance test of 4253 antiretroviral-naïve patients between 2003 and 2015 and found that just under 14% of their patients had transmitted drug resistance.[10] The drugs that had little to no resistance functioned as protease inhibitors or integrase inhibitors while drugs that interacted with the reverse transcriptase had the highest

level of resistance.[10] Resistance to non-nucleoside reverse transcriptase inhibitors was found for 7.2% of the patients.[10] Additionally, the resistance to non-nucleoside reverse transcriptase inhibitors increased by 11% per year.[10]

This study indicates that more research should be done on non-nucleoside reverse transcriptase inhibitors. One way to counteract resistance to non-nucleoside reverse transcriptase inhibitors is to find new compounds that display anti-HIV characteristics, especially those with novel or different structural features than those in current drugs. Stachybotrin D is a promising compound that has anti-HIV properties. It was found to be a novel non-nucleoside reverse transcriptase inhibitor of type 1 HIV and five non-nucleoside resistant strains.[11] The mechanism of Stachybotrin D was determined by performing time of addition assays. Stachybotrin D had a 50% failure (FT_{50}) at a time of 12.66 hours on the time of addition assay. This fell in between the FT_{50} for a nucleoside reverse transcriptase inhibitor and an integrase inhibitor that were tested and was about the same as the non-nucleoside reverse transcriptase inhibitor, Efavirenz. The time of addition assay lead the researchers to believe the mechanism of Stachybotrin D was to inhibit reverse transcriptase. To confirm this the researchers tested the effect of Stachybotrin D on reverse transcriptase activity and found that it inhibited reverse transcriptase RNA-dependent DNA polymerase activity in a dose-dependent manner.

Stachybotrin D is a secondary metabolite that was extracted from a sponge-derived fungi named *Stachybotrys chartarum* MXH-X73.[11] Secondary metabolites are compounds that are produced by organism for a variety of reasons, one of which is defense against pathogens in the environment. Examples of secondary metabolites are steroids, waxes, and caffeine.[12] Some

secondary metabolites also have anti-cancer, anti-fungal, anti-diabetic, and immunosuppressant compounds.[13]

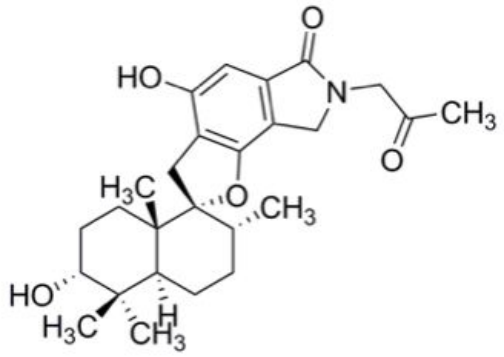


Figure 3: The molecular structure of Stachybotrin D

Stachybotrin D is a promising compound because the structure, shown in Figure 3, is quite different from the non-nucleoside reverse transcriptase drugs that are currently on the market, shown in Figure 2. The differences in structural features may allow it to bind to the reverse transcriptase enzyme in a different way than previous anti-HIV compounds. The structure of Stachybotrin D can also be varied to improve it's activity and even screen for other biological activity. As seen in Figure 3, Stachybotrin D has no cyclopropyl groups or alkynyl features and only possesses non-aromatic nitrogens which were distinguished characteristics of the current non-nucleoside reverse transcriptase inhibitors: Nevirapine, Doravirine, Efavirenz, Etravirine, and Rilpivirine. A similar functionality however is the amide, which is also present in some of the other drugs and could be a potential area of interest on Stachybotrin D that may be significant to its effect. Thus, Stachybotrin D is an interesting compound to further examine as an HIV drug.

As mentioned above, Stachybotrin D is a secondary metabolite from a fungus and unfortunately fungi are not sustainable sources of secondary metabolites. Mass extraction of a secondary metabolite from a fungus could cause ecological damage. An example of this is Pacific yew tree, as environmentalist thought that it may go extinct because scientists were using the bark of the tree as a cancer treatment.[14] Additionally, growing the fungus is not an option because secondary metabolites typically form in response to specific aspects of an environment.[12] Thus, metabolites are often very difficult to produce in an artificial setting. For these reasons, the best way to produce Stachybotrin D is through laboratory synthesis.

Stachybotrin D comes from a family of compounds with the name Stachybotrys. Some of the other compounds in the Stachybotrys family have been synthesized. For example, (+)-Stachyflin, Figure 4 right, was a derivative of the Stachybotrys family that was synthesized from (+)-5-methyl-Wieland-Miescher ketone due to its anti-influenza properties.[15] Additionally, Stachybotrin C, Figure 4 left, was synthesized from 3,5-dihydroxybenzoic acid because it is a neuroprotective compound.[16]

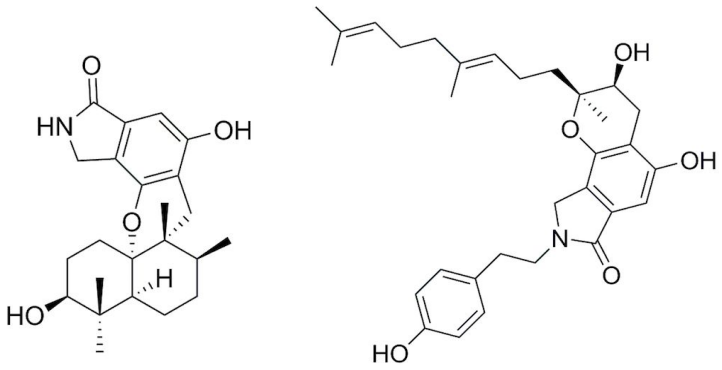


Figure 4: The molecular structures of (+)-Stachyflin (right) and Stachybotrin C (left).

As Stachybotrin D is a relatively large and complex structure, the first step in the synthesis was to break the the molecule into three segments to make it easier to synthesize. The three segments of the structure of Stachybotrin D are shown in Figure 6 below. The segment that this proposed research will be focusing on is segment C. Segment C is a bicyclic moiety that can be found in subsects of other structures and has been synthesised in the past. This research focuses on synthesizing this segment with the correct stereochemistry while integrating principles of green chemistry into the reaction.

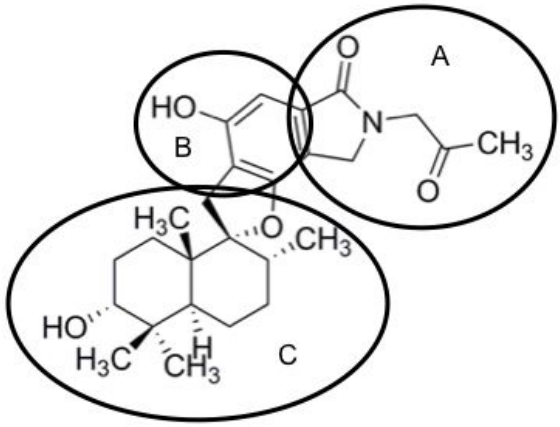


Figure 6: The structure of Stachybotrin D broken down into segments to simplify the synthesis process. Segment C, the bicyclic moiety, is focused on in this research.

Stereochemistry in Synthesis

The substituents on the bicyclic moiety of segment C need to be added with the correct stereochemistry because changes in the stereochemistry on a compound can change the properties of the compound. A wedge on a compound indicates that the substituent is pointing out of the plane while a dash indicates that a substituent is pointing behind the plane.[17] If these

directions are switched in drugs, the drugs are not able to bind to the protein receptor correctly. One example of this is Ibuprofin, where only one particular structure can relieve pain.[18]

In some cases, drugs with the incorrect stereochemistry can cause major problems such as the case with Thalidomide, a drug taken for morning sickness among other things (R enantiomer). Thalidomide can convert between both possible configurations or enantiomers. This made it hard to produce just the R enantiomer in a stable manner, so the drug was manufactured in a racemic mixture. The other configuration of this drug, (S)-thalidomide, caused mutations leading to birth defects and death.[19] Upon discovering the detrimental effects of (S)-thalidomide, the way that drugs are tested has changed. Thalidomide was tested in mice before it was released, but mice showed less sensitivity to the S enantiomer. Now drug screening involves new drugs being tested in several species and *in vitro* testing before it is given to a human to prevent something like this from happening again.[19] Thalidomide is still on the market as it also treats skin lesions and cancer, however, regulations are very strict for people taking the drug. People taking thalidomide must receive a packet of patient education material, sign a consent form, use two forms of contraception and frequent pregnancy testing for females, and use condoms for males. Additionally, safer analogs of Thalidomide, such as Lenalidomide and Pomalidomide, have been produced.[20] The examples of Ibuprofin and Thalidomide prove that it is important for Stachybotrin D to be synthesized with the correct stereochemistry.

Green Chemistry in Synthesis

Green chemistry is the idea of performing sustainable reactions that maximize resource efficiency, eliminate and minimize hazards and pollution, and design systems holistically using

life cycle thinking.[21] Green chemistry is mostly associated with the twelve principles proposed by Paul Anastas and John C. Warner in 1998.The twelve principles of green chemistry are as follows: prevent waste, atom economy, less hazardous chemical synthesis, designing safer chemicals, safer solvents and auxiliaries, design for energy efficiency, use of renewable feedstocks, reduce derivatives, catalysis, design for degradation, real time analysis for pollution prevention, and inherently safer chemistry for accident prevention.[22] Using less dangerous chemicals is an important aspect both for the safety of the scientists using the chemicals and the safety of the animals and plants after the chemicals are disposed. To improve the 'greenness' of any reaction, chemists try to adjust reaction conditions or reagents to align with one or more principles. It is notable to incorporate multiple principles, however there is a balance between effectiveness of the reaction and reduction of hazards or waste.

In experiments, implementing green chemistry can take many forms. One could replace the solvent methanol with ethanol because ethanol comes from corn which is renewable and has slightly less toxic metabolites (e.g. ethanal vs. methanal) or using ethyl acetate in place of diethyl ether which is potentially explosive. Additionally, 2-methyltetrahydrofuran could be substituted for dichloromethane which is a suspected carcinogen. Often reactions are heated to overcome the activation energy or drive the reaction towards completion. Using a microwave reactor instead of refluxing a reaction on a hot plate or in a heating mantle for extended periods of time may be considered energy efficient. Depending on the length of time needed to heat a reaction, the energy draw from a microwave reaction in a short time, could be offset by the energy associated with long term heating. Though indirect, heating requires energy from the grid which is often in many municipalities coal. The microwave reactors may also reduce reaction times, may promote

a specific product selectivity, and can provide more uniform heating. Additionally, reducing derivatives and utilizing most of the atoms used in the reaction (atom economy) improves the efficiency of the reaction and hence, reduces waste. The pharmaceutical industry strives to implement green chemistry by seeking ways to develop medicines with less harmful side effects and employing processes that produce less toxic waste. An example of this is the synthesis of sitagliptin (Figure 7), a treatment for type 2 diabetes, by Merck and Codexis that uses an enzymatic process that reduces waste, improves yield and safety, and eliminates the need for a metal catalyst.[23] A green synthesis of Simvastatin, a statin drug for high cholesterol, was developed by Professor Yi Tang from the University of California. Professor Yi Tang used an engineered enzyme and a low-cost feedstock instead of the traditional multistep reaction that used large quantities of hazardous reagents and generated large amounts of toxic waste. His synthesis plan was optimized by Codexis and reduced the hazards and waste, was cost-effective, and met the needs of customers.[23] The goal of this research is to create a synthesis scheme of Stachybotrin D that could be used by pharmaceutical companies in the future. Since pharmaceutical companies are shifting towards implementing sustainable chemistry, it is important that this synthesis of Stachybotrin D utilizes the principles of green chemistry.

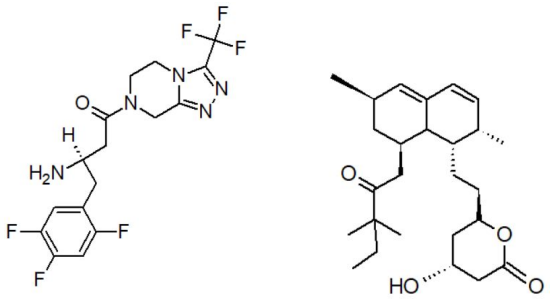


Figure 7: Sitaglipti and Simvastatin respectively

Previous Work on the Bicyclic Moiety of Stachybotrin D

One article that synthesized the bicyclic moiety present like that on Stachybotrin D suggests a method that starts with the (+)-Wieland-Miescher ketone (**2**), as shown in Figure 8. An acetal protecting group is added to the ketone to prevent the carbonyl group farther away from the double bond (**a**) from reacting with the reagents. Next, a methylene group is added to the alkene (**c** on compound 3). Then, the conjugated carbonyl (**b**) is rearranged and the phenylsulfide (SPh) is substituted to make a CH_3. In this process a hydrogen is added behind the molecule (**d**). During the intermediate stage were the conjugated carbonyl is rearranged, an alkyl group (RX) is added to the structure at the same position (**c** on compound 4) that the methyl is substituted for the phenylsulfide.[24] To complete the synthesis of the bicyclic moiety present in Stachybotrin D, a methyl group must be added at position **e** on compound 6, the carbonyl (**b**) must be reduced to an alcohol (dashed OH), and the protecting group (**2a**) must be removed. The synthesis described above is depicted below in Figure 8.

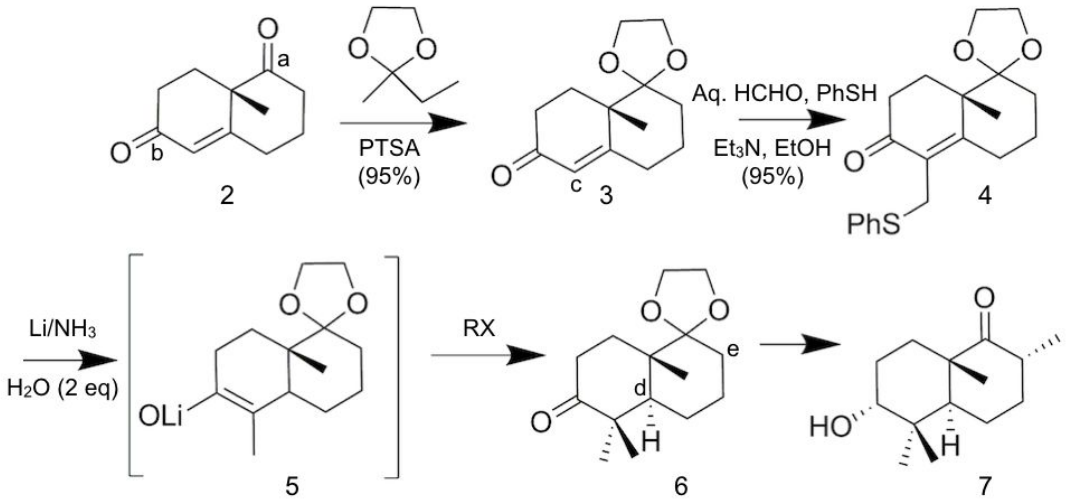


Figure 8: Synthesis from the literature for the stereochemistry of the bicyclic ring in Stachybotrin D

The synthesis depicted by Figure 8 has some hazardous components that are not in line with the principles of green chemistry. One problem with the synthesis is the use of formaldehyde (CH_2O) to add the alkyl substituent to compound 3 in Figure 8. Formaldehyde is carcinogenic and thus could be improved to better meet the green principle of less hazardous chemical synthesis.[25] Another problem with this synthesis is the use of lithium metal to add the second methyl group to compound 5 in Figure 8. Lithium metal is flammable and highly corrosive making it a difficult chemical to handle.[26] The addition of the second methyl group could also be improved to be a less hazardous chemical synthesis.

Synthesis Scheme for Stachybotrin D

The reactions in Figure 8 ares not very green so this research focuses on a new synthesis method that uses stereoselective reactions and more green principles. In order to create a synthetic plan, a retrosynthesis of the bicyclic moiety was developed. This backwards design helps us develop ideas for the synthesis which can be supported with details in the literature. The retrosynthesis of the bicyclic moiety of Stachybotrin D is shown in Figure 9 below.

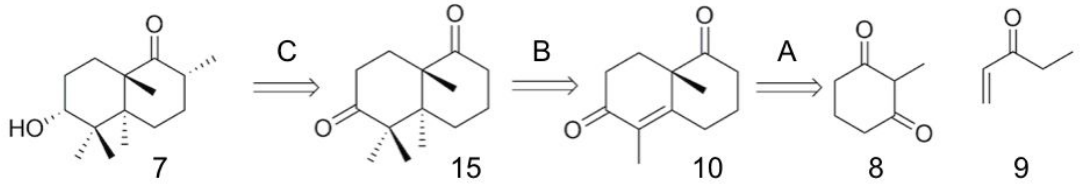


Figure 9: Retrosynthetic analysis of the bicyclic moiety (**7**) of Stachybotrin D

As seen in Figure 9, the desired product is compound 7, the bicyclic moiety of Stachybotrin D. A more simplified version of compound 7 is compound 11. To get the compound 7, compound 11 must undergo a reaction labeled C which includes a reduction of the carbonyl on the left side of the compound and a methylation at the alpha position of the carbonyl at the top of the compound. To get to compound 11 from compound 10, the α,β-unsaturated ketone must be methylated (reaction B). To get to compound 10 from compounds 8 and 9, a Robinson annulation can be performed (reaction A). Compounds 8 and 9, or ethyl vinyl ketone and 2-methyl-1,3-cyclohexanedione, respectively are both readily available for purchase so they are the two starting materials for this reaction.

Using the retrosynthesis, the forward synthesis plan is designed in more detail and incorporates steps such as protecting the saturated carbonyl so that the reagents used for the methylation of the α,β-unsaturated ketone will not react with the saturated carbonyl. Thus, the forward synthesis is as follows: a Robinson annulation, protection of a carbonyl, methylation of an alpha-beta unsaturated ketone, reduction of the ketone, deprotection of the carbonyl, and a stereoselective methylation of the newly deprotected ketone. This reaction progression is shown in the research scheme in Figure 10 and described in more detail below.

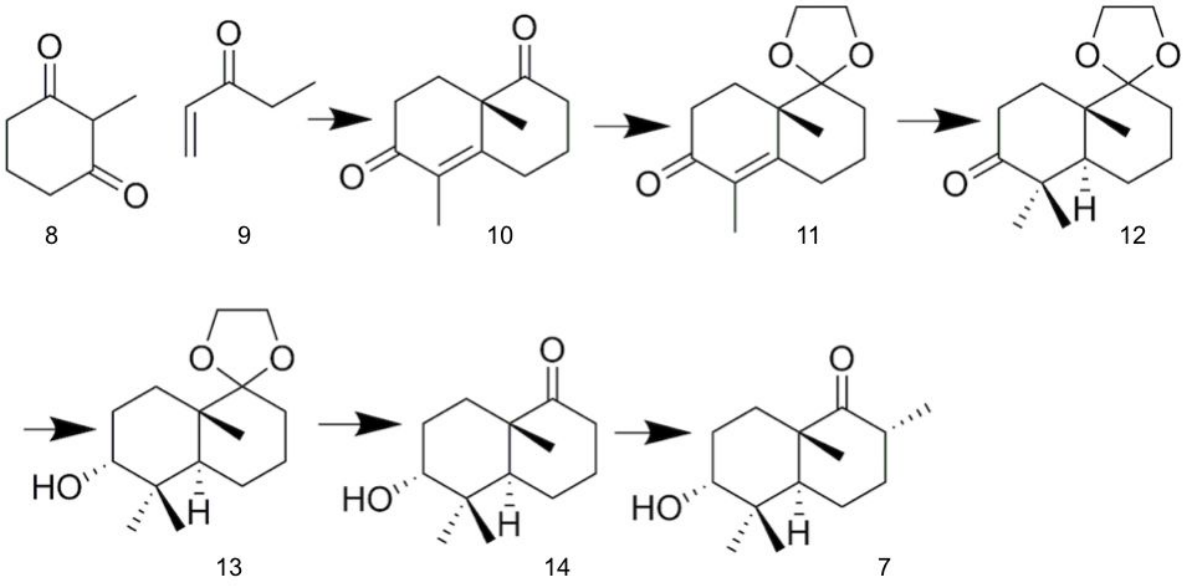


Figure 10: The forward synthesis of the bicyclic moiety of Stachybotrin D (**7**).

In place of the addition of the alkyl group with formaldehyde (figure 8) performed in the previous work above, this research used a Robinson annulation. With the starting materials 2-methyl-1,3-cyclohexanedione (**9**) and ethyl vinyl ketone (**8**), the Robinson annulation formed the product (**10**) with a methyl group at the same position the alkyl group would have been added in the past work (figure 8).[27] The Robinson annulation is shown as the first step in Figure 10. Robinson annulations are considered green reactions because they have a high atom economy and are a less hazardous chemical synthesis than the alkylation in the previous reaction. The next step in this research plan was to add another methyl group on compound 10 to the ***α,β***-unsaturated ketone position. Since the reagents used to perform a methylation in this position tend not to be regioselective, a protecting group was used on the carbonyl at the top of the structure since it is far away from the desired site of reaction. The protecting group can be

selective for the carbonyl on top since that carbonyl is expected to be more reactive than the unsaturated carbonyl on the left side of the molecule. The protection of compound 10 via acyl nucleophilic addition results in compound 11. The rest of the proposed plan was to methylate compound 11 at the α,β-unsaturated ketone position to get compound 12. The unprotected carbonyl in compound 12 was planned to be stereoselectively reduced to an alcohol in the dashed position to get compound 13. To obtain compound 14, the protecting group was planned to be removed from compound 13. Lastly, the alpha position of the newly unprotected carbonyl could have then been stereoselectively methylated to form the bicyclic moiety, compound 7.

The method used for the Robinson annulation was based off an article by Burns et al. and is described in the experimental section below.[28] The Robinson annulation works by joining two reagents to form a single compound called a Robinson adduct which is then reduced via Aldol condensation to form a bicyclic ring.

Two literature sources were compared for the adding the protecting group. The first method used p-toluenesulfonic acid, ethylene glycol, 4Å Molecular Sieves, and was stirred at ambient temperatures overnight.[29] The second method used D-camphorsulfonic acid, ethylene acetal of 2 butanone, ethylene glycol, and is heated for 36 hour.[30] These methods are described in more detail in the experimental section below. The first method appears to be more green as it does not need to be heated and it is stirred for less time. Additionally, the first method does not use ethylene acetal of 2-butanone which is highly flammable.[31] Thus, the first method using p-toluenesulfonic acid was preferred and performed in the laboratory.

Finding literature that would methylate compound 11 at the α,β-unsaturated ketone position was challenging. Most literature uses lithium and ammonium to add this methyl group [24,

[29, 30] however, lithium is reactive, extremely flammable, and corrosive making it a poor reagent in a green synthesis.[26] An alternative that was examined was using a palladium catalyst as described in the paper by Murahashi et al.[32] however, this method still seemed like it could be improved because the reaction occurred in multiple steps and had a relatively low percent yield. After more consideration for this step in the reaction, a new method was proposed. This method would employ a new set of reactants in the Robinson annulation for the first step. One of these new reactants needed to be synthesized due its cost. The newly proposed retrosynthesis, which implements the change in Robinson Annulation reactants, is shown in Figure 11 below.

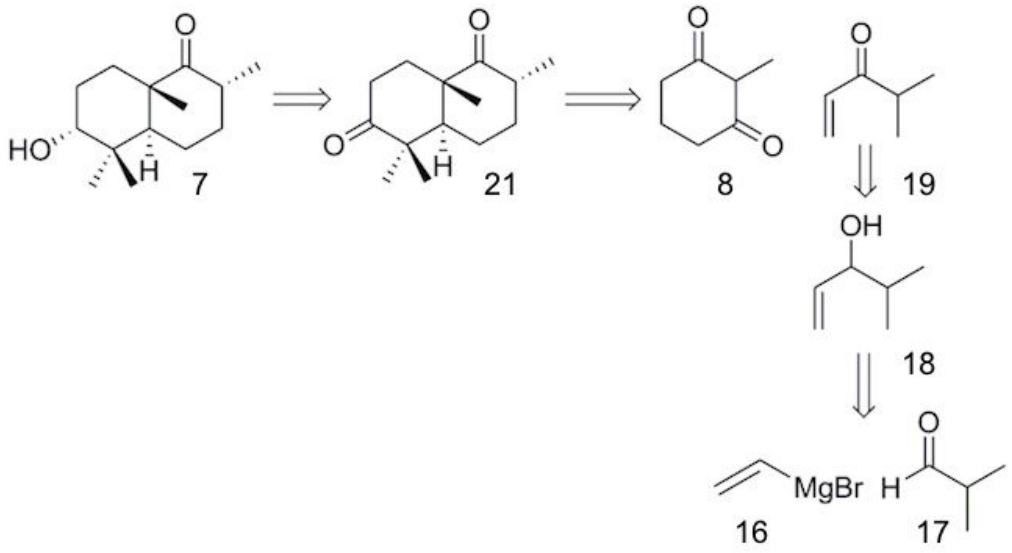


Figure 11: New retrosynthetic analysis of the bicyclic moiety of Stachybotrin D

As shown in Figure 11, compounds 7 and 8 are the same in this retrosynthesis as they are in the previous retrosynthesis shown in Figure 9. However, the starting material for the Robinson annulation which was ethyl vinyl ketone (**9**) in the original retrosynthesis (Figure 9) has been

replaced with isopropyl vinyl ketone (**19**) in this retrosynthesis. This should add two methyl groups to the ***α,β***-unsaturated ketone position and bypass the formation of compound 10 and reaction B in the original retrosynthesis. Unfortunately, isopropyl vinyl ketone (**19**) is very expensive to purchase, so this research has elected to synthesis it. Isopropyl vinyl ketone (**19**) can be produced by oxidizing isopropyl vinyl alcohol (**18**). To synthesis isopropyl vinyl alcohol (**18**), a Grignard reaction can be performed using bromo(vinyl)magnesium (**16**) and 2-methylpropanal (**17**). Bromo(vinyl)magnesium (**16**) and 2-methylpropanal (**17**) are both readily available for purchase at a more reasonable price, so they are the two starting materials for this retrosynthesis. The new forward synthesis plan is shown below.

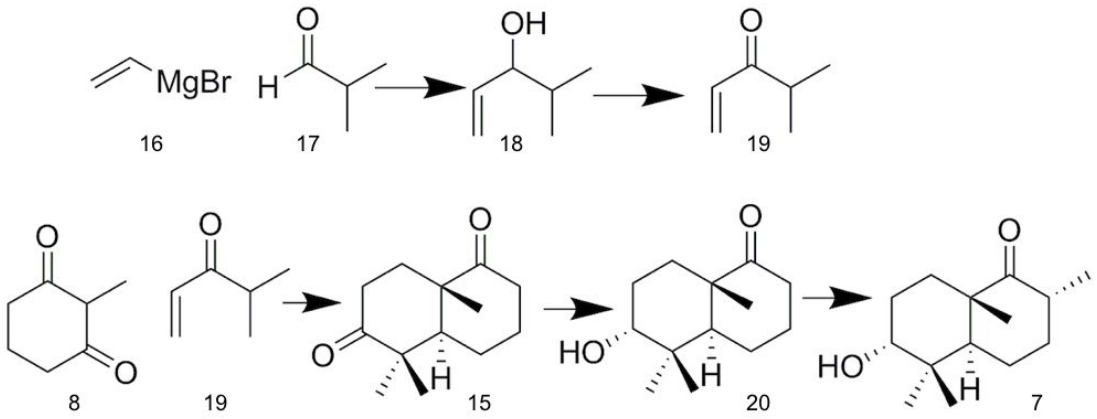


Figure 12: New forward synthesis for the bicyclic moiety of Stachybotrin D

The new forward synthesis starts with a Grignard reaction using bromo(vinyl)magnesium (**16**) and 2-methylpropanal (**17**) in tetrahydrofuran (THF) at 0˚C.[33] The Grignard reaction will form the isopropyl vinyl alcohol (**18**) which can be oxidized to isopropyl vinyl ketone (**19**) using ozone and sodium chloride.[34] The isopropyl vinyl ketone can be used in a Robinson annulation

with 2-methyl-1,3-cyclohexanedione (**9**) to produce compound 15. Then, the carbonyl on the left side of compound 11 should be stereoselectively reduced to produce compound 20. This reduction could use sodium borohydride [30] and a chiral auxiliary could be used to make the reduction stereoselective. Lastly, the alpha position of the remaining ketone in compound 20 should be stereoselectively methylated to form the bicyclic moiety of Stachybotrin D (compound 7). This can be done by transforming the carbonyl to an imine using phenylalanine and methylating it with methyl iodide. The products of these reactions were characterized using NMR, GC-MS, and IR.

The method for the grignard reaction was adapted from a lab procedure written by the Missouri University of Science and Technology [35]. The purpose of Grignard reactions are to create a carbon-carbon bond through an organometallic intermediate called a grignard reagent.[36] A grignard reagent is composed of an alkyl group bonded to magnesium metal and a halogen. The magnesium in a grignard reagent causes the carbon in the grignard reagent to be carbanionic in character and allows it to act as a nucleophile. Since the carbon in a carbonyl group has a partially positive charge, the carbon in a carbonyl group is a common place for the alkyl group of a Grignard reagent to attack. This reaction forms a carbon-carbon bond.[35]

Grignard reagent can also react with water to form alkanes so for a Grignard reaction to occur as planned, the reaction must be kept very dry.[37] For this reason, the reaction described in the experimental section below has a glassware set-up that helps to prevent water from enter the reaction vial and interacting with the reagents.

The next step of the new synthesis scheme is an oxidation of the Grignard product. There are many ways to perform an oxidation. Some reagents that could be used for an oxidation are

potassium dichromate or potassium permanganate. However, potassium dichromate is carcinogenic [38] so it is not a green reagent. Potassium permanganate may seem like more of a green reagent because it is used to remove sulfur from drinking water however, very little to no potassium permanganate makes it into the drinking water as it can be harmful to the skin.[39] Additionally, potassium permanganate decomposes into potassium manganate, manganese dioxide, and oxygen gas.

$2\ KMnO_4 \rightarrow K_2MnO_4 + MnO_2 + O_2$ Decomposition of Potassium Manganate

The oxygen gas is used for the oxidation reaction.[40] However, the side product, manganese dioxide is acutely toxic upon inhalation.[41] Thus, a more green alternative to potassium permanganate can be implemented. A greener method that was published in a paper by Schulze et al. used oxone ($KHSO_5$) as an oxidizing agent. Oxone is considered greener because it is inexpensive, stable, easy to transport, and nontoxic. The method also used a small amount of NaCl as a catalyst.[34] Unfortunately, upon further examination of the paper, it was found that these reagents do not work on a starting material that contain a double bond such as the one this research is trying to oxidize. This is likely due to the possible oxidation of the alkene to the epoxide. Another well-known oxidant is sodium hypochlorite (NaOCl). NaOCl is a promising oxidant because it is nonexplosive and inexpensive. After the oxidation, the waste is harmless and nontoxic since it is just NaCl. However, at concentrations above 8-13%, NaOCl is unstable. Additionally NaOCl has a high pH and may need to be lower to speed up the rate of the reaction. An article by Kirihara et al. suggested using sodium hypochlorite pentahydrate crystals

($NaOCl{\cdot}5H_2O$) instead of NaOCl and found that $NaOCl{\cdot}5H_2O$ was a stronger oxidant, had higher volumetric efficiency, reduced wastewater by ⅛, more stability, and did not require titration.[42]

Experimental Results and Discussion

All reagents purchased through Sigma Aldrich, Fisher or Oakwood Chemicals. Scientific, All NMR were obtained using JEOL 400 MHz instrument in $CDCl_3$ except as noted. IR obtained on Thermo S10. All GC-MS were obtained on ThermoScientific Trace 1310 and analyzed after dilution in ethyl acetate. Thin Layer Chromatography (TLC) analysis done on silica gel plates with fluorescent indicator and visualized by short wave UV lamp (254 nm) and stained with potassium permanganate when the compounds are not UV active. Column chromatography are conducted using high-purity grade (Merck Grade 9385), pore size 60 Å, 230-400 mesh particle size silica gel.

Robinson Annulation with 2-methyl-1,3-cyclohexanedione and ethyl vinyl ketone

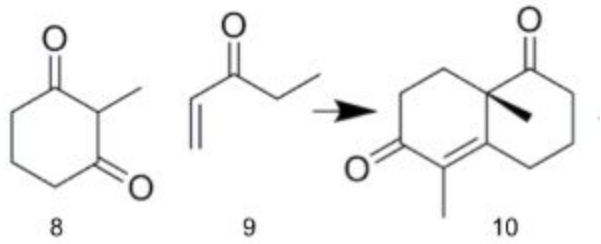


Figure 13: Robinson annulation using 2-methyl-1,3-cyclohexanedione (**9**) and ethyl vinyl ketone (**8**)

Michael Adduct - 2-methyl-2-(3-oxopentyl)cyclohexane-1,3-dione: 2.417mL of DABCO and 1.825 mL ethyl vinyl ketone (**8**) were added in one portion to a stirred suspension of 2.5641g 2-methyl-1,3-cyclohexanedione (**9**) in 25 mL ethyl acetate at room temperature. This mixture was stirred at room temperature for around 20 hours. Then, mixture was cooled in an ice bath, 25 mL of 3 M HCl(aq) was added, and this was stirred in the ice bath for 10 minutes. The mixture was extracted with diethyl ether and the combined organic extracts were dried with magnesium sulfate and evaporated under reduced pressure to give the crude Michael adduct as a yellow oil.[28] Aldol Adduct - *5,8a-dimethyl-3,4,8,8a-tetrahydronaphthalene-1,6(2H,7H)-dione*, **10**: 2.23mL Et_3N and 2.7147g benzoic acid were added to a stirred solution of the 1.6251g crude Michael adduct in 30 mL xylene at room temperature. The resulting solution was stirred and heated at reflux for around 20 hours using a Dean-Stark trap. The reaction mixture was allowed to cool to room temperature and then 25 mL of saturated $NaHCO_3$(aq) was added and the two layers were separated. The aqueous layer was extracted with using diethyl ether and the combined organic layers were dried using magnesium sulfate and evaporated under reduced pressure to give the crude product (**10**).[28] Purification by column chromatography on silica with hexanes and a solvent mixture of 1:1 hexanes to ethyl acetate as eluent gave a yellow oil.. ^{13}C NMR (300 MHz, $CDCl_3$) δ: 212.404 (A), 198.044 (B), 158.607 (C), 133.615 (D), 130.852 (xylenes), 130.154 (xylenes), 128.510 (xylenes), 50.753 (E), 37.779-21.574 (F), 11.402 (G). The spectra for this NMR can be seen as Spectra 1 in the appendix. DEPT 135 (300 MHz, $CDCl_3$) δ: 130.852 (xylenes), 130.154 (xylenes), 128.510 (xylenes), 37.779-21.574 (F), 23.458 (F*), 11.402 (G). The spectra for this NMR can be seen as Spectra 2 in the appendix. ^1H NMR (300 MHz, $CDCl_3$) δ:8.053 (Benzoic Acid, 0.81), 7.546 (Xylenes, 0.38), 7.417 (Xylenes, 0.81), 7.255 ($CDCl_3$,

23.77m), 2.824 (A, 1.00), 2.632 (B. 1.03), 2.426 (C, 4.28), 2.026 (D, 3.21), 1.752 (E, 4.06), 1.364 (F, 3.14), 1.098 (Hexanes, 0.81). The spectra for this NMR can be seen as Spectra 3 in the appendix. These NMR confirmed that the Robinson Annulation product was formed. Unfortunately, upon calculating the percent yield, the reaction only had a purified yield of 7%. A contributing factor to this lower yield was that separation was not complete. Some of the Robinson Annulation product remained in a mixture with the side product of the Robinson Annulation reaction. In the future, experimenting with different solvent ratios could improve the percent purified yield. IR 2930.79, 1709.11 (C=O), 1662.26 (C=O), 1603.39 (C=C), 1450.10, 1377.14, 1358.65, 1313.84, 1237.20, 1172.95, 1094.18, 1070.47, 1008.12, 886.11, 783.53, 713.61, 647.23, 628.53cm^{-1}. The spectra for this IR can be seen as Spectra 4 in the appendix.

Selective protection of the saturated carbonyl (leave the α, β- unsaturated carbonyl unprotected)

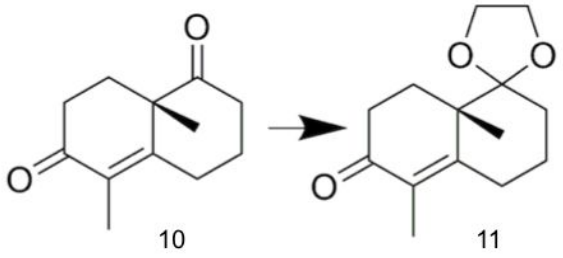


Figure 14: Protection of Robinson annulation product (**10**)

Method 1 (P-toluenesulfonic acid)

5',8'a-dimethyl-3',4',8',8'a-tetrahydro-2'H-spiro[1,3-dioxolane-2,1'-naphthalen]-6'(7'H)-one, **11**. The product of the Robinson annulation from 2-methyl-1,3-cyclohexanedione and ethyl vinyl ketone (**10**) (0.3287g), p-toluenesulfonic acid (0.3101g), ethylene glycol (20 mL), and a couple

molecules sieves were stirred at room temperature overnight. The reaction mixture was then poured into a solution of crushed ice and saturated $NaHCO_3$ and extracted three times with ethyl acetate. The combined organic extracts were washed with brine and dried with magnesium sulfate. The solvent was removed in vacuo to afford the protected compound (**11**) as a yellow oil. The spectral data in the paper by Jung et al. gave IR (neat) 2950, 2878, 1734, 1665, 1181, 1092, 1036 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 3.87–3.98 (m, 4H), 2.70 (d, 1H), 1.53–2.65 (m, 9H), 1.75 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 198.8, 160.3, 130.1, 112.8, 65.3, 65.1, 45.3, 33.7, 29.7, 26.5, 26.4, 21.4, 20.9, 11.4.[29] GC-MS was run and it seemed to suggest that some of the protected product was formed, but there was starting material present. The gas chromatogram and mass spectrums of this reaction are shown in the appendix (Spectra 5-7). The mass spectrum for the protected product (Spectra 7) seemed to have a strange fragmentation pattern. Because of the interesting GC-MS results and to confirm the data, an NMR was run on the protected product. The carbon NMR of the crude protected product (Spectra 8) indicates that both the starting material (indicated on spectra by uppercase letters) and protected product (indicated on spectra by lowercase letters) are present. ^{13}C NMR (300 MHz, $CDCl_3$) δ:212.175 (A), 198.924 (b), 197.805 (B), 171.160 (Ethyl acetate), 160.653 (c), 158.568 (C), 130.632 (D), 129.915 (d), 112.677 (a), 65.276 (h), 65.008 (h), 63.593 (Ethylene glycol), 63.125 (Ethylene glycol), 60.342 (Ethyl acetate), 50.638 (E), 45.256 (e), 37.311-20.761 (F&f), 14.107 (Ethyl acetate), 11.344 (G), 11.220 (g). This NMR confirmed that the starting material and protected product were both present. To separate these compounds, a silica column was run. The first column used 2:1 hexanes to ethyl acetate and was not nonpolar enough to separate these compounds. The next column started with 9:1 hexanes to ethyl acetate to separate the

compounds and then 4:1 hexanes to ethyl acetate was used to push the compounds through the column. This solvent ratio was able to separate the two products and an NMR of the two spots from the column were taken (Spectra 9-11). ^{13}C NMR (300 MHz, $CDCl_3$) δ:198.561 (b), 160.242 (c), 129.973 (d), 112.677 (a), 65.324 (h), 65.027 (h), 45.246 (e), 33.649-20.800 (f), 11.411 (g). DEPT 135 (300 MHz, $CDCl_3$) δ: 65.324 (h), 65.027 (h), 33.649-21.392 (f), 20.800 (f*), 11.411 (g). ^{1}H NMR (300 MHz, $CDCl_3$) δ:3.814 (a, 4.09), 2.549 (b, 1.11), 2.290-2.020 (c, 4.88), 1.659 (d, 0.73), 1.622 (e, 2.85), 1.522 (f, 3.05), 1.186 (g, 3.00). Unfortunately, the purified yield for this reaction was only 10%, which was partially due to the product not being fully purified by the column. An even larger contributing factor is that much of the product remained unreacted. Potential ways to improve the reaction for the future could be to leave the reaction running for a longer amount of time or heating the reaction slightly. More work should be done to optimize this reaction in the future. If it cannot be optimized successfully, protecting method 2, as listed below, could be used to protect the carbonyl. Protecting method 2 is not as green as protecting method 1 though, so being able to optimize this method would be ideal.

Method 2 (D-camphorsulfonic acid)

5',8'a-dimethyl-3',4',8',8'a-tetrahydro-2'H-spiro[1,3-dioxolane-2,1'-naphthalen]-6'(7'H)-one, **11**.

A solution of the product of the Robinson annulation from 2-methyl-1,3-cyclohexanedione and ethyl vinyl ketone (**10**) (4.7 mmol) and D-camphorsulfonic acid (0.36 mmol) in ethylene acetal of 2-butanone (5mL) and ethylene glycol (3mL) should be heated at 40°C for 36 hours under an atmosphere of nitrogen. After cooling in an ice bath, the resulting solution is to be poured into aqueous $NaHCO_3$, and the product extracted with diethyl ether. The combined extracts then are

washed with water, brine, and dried over anhydrous sodium sulfate. Evaporation of the solvent followed by purification by column chromatography of the residue should provide the protected compound (**11**). The spectra data in the paper by Hagiwara et al. gave ^{1}H NMR (60 MHz) 61.30 (s,3H), 1.75 (8,3H), 1.3-1.9 (m,10H), 3.92 (s,4H) for the protected compound.[30] This method would only be performed if the first protecting method can not be optimized to favor the product.

Grignard Reaction

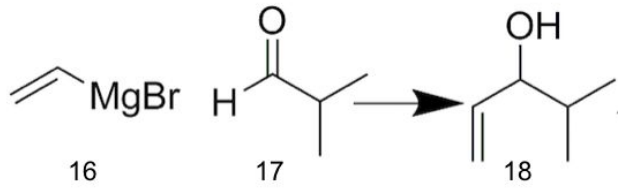


Figure 15: Grignard reaction with bromo(vinyl)magnesium (**16**) and 2-methylpropanal (**17**) to form 4-methylpent-1-en-3-ol (**18**)

4-methylpent-1-en-3-ol, **18**: The glassware, 5 mL conical vial, two 3 mL conical vials, caps and septas for the vials, a Claisen head, a jacketed condenser, a drying tube, a metal spatula, a large teflon stir vane, and a 2 mL glass syringe, was thoroughly cleaned and dried in the oven to remove any trace of water. The glassware was set up according to Figure 14. A cotton plug and drying agent were inserted into the drying tube to help prevent water from entering the system.

Figure 16: Set-up of the glassware for the Grignard Reaction

According to the set-up in Figure 16, the stir vane was placed into the 5 mL conical vial and the conical vials rested in an ice bath. The claisen head was attached to the conical vial. On the opening above the conical vial, a septum and screw cap covered the opening. Once the glassware was set up reagents were added via syringe to this opening of the Claisen head. The other opening was attached to a jacketed condenser with water flowing in the bottom and out the top of the condenser. To the top of the condenser, the drying tube with the cotton plug and drying agent was added. The glassware had to be set up quickly after it was taken from the oven to prevent any water from getting on the inside of the glassware.

After the glassware is set up, the reagents were added. First, 1.6 mL of bromo(vinyl)magnesium (**16**) in THF was pushed via syringe into the conical vial. Then 0.4 mL of 2-methylpropanal (**17**) was added dropwise to the stirring bromo(vinyl)magnesium. The reaction was left to run for about 20 minutes. Then, 1 mL of 6M HCl was added dropwise to the

solution to neutralize the reaction mixture and induce hydrolysis. After the reaction was neutralized, the work-up could began. First, a microscale liquid-liquid extraction was done used ether. After the organic layer was extracted, it was washed with about 1 mL of sodium bicarbonate and about 1 mL of sodium chloride. Then, the organic layer was dried with sodium sulfate and evaporated under reduced pressure to give the crude product.[35] The NMR for the Grignard reaction is located in the appendix (Spectra 12). The results were messy and in need of purification, however, it does seem that the Grignard product was formed as indicated by the following peaks on the carbon NMR. ^{13}C NMR (300 MHz, $CDCl_3$) δ: 139.457 (A), 115.632 (B), 78.316 (C), 33.582 (D), 18.170 (E), 17.912 (F). The other peaks on the carbon NMR were starting material and other unknown impurities. Since the carbon NMR was messy, the proton NMR was not analyzed. Some possible origins of the unknown impurities are the glassware used in the reactions, the vial used for storing the product, and the NMR tube in which the product was characterized. Another possible origin was the starting material, however an NMR of the 2-methylpropanal was taken and found to be pure. The reaction was performed again with closer attention on the cleanliness of the glassware used in the reaction, as well as the storage and characterization of the Grignard product. The ^{13}C NMR again showed that it had many peaks in the alkane region indicating that the Grignard product was impure. Thus, preparation TLC was performed to purify the Grignard product. Prep TLC was used in place of a column because only a small amount of the product was formed. The TLC was run in 4:1 hexanes to ethyl acetate and was stained with potassium permanganate because the products did not show up under UV light. An IR was taken of the purified Grignard product and is shown in Spectra 13 of the appendix. IR 3450.00 (A), 2967.50 (B), 1709.94 (C), 1469.43 (D), 1362.25-647.27 (alkyl groups) cm^{-1}.

Unfortunately, the IR indicated that some of the starting aldehyde (**17**) was present in the purified sample in addition to the Grignard product (**18**). This may be due to the similarity of polarity in the two compounds making them difficult to separate using TLC. NMR was not able to be taken to confirm the findings of the IR and to quantify the amount of the starting material versus the amount of product because the NMR was down. Thus, the only conclusion that can be drawn from the data is that the separation was not entirely successfully and different solvent conditions as well as separation methods should be examined in the future to isolate the Grignard product. The predicted NMR of the purified Grignard product is shown as Spectra 14 and 15 in the appendix and should have the following peaks ^{13}C NMR (300 MHz, $CDCl_3$) δ:136.2, 115.8, 78.2, 33.8, 19.1. ^{1}H NMR (300 MHz, $CDCl_3$) δ:5.89 (m), 5.79 (s), 5.29 (d), 5.28 (d), 4.08 (m), 1.73 (m), 0.88 (d).

Conclusion

In conclusion, this research focused on creating and testing a synthesis scheme to create the bicyclic moiety of Stachybotrin D. Several of the major synthetic steps such as the Robinson Annulation, protecting reaction, and Grignard reaction were completed successfully. Unfortunately, percent yields were lower than anticipated in many of these reactions. Some of this has to do with human error associated in experimentation while other error can be contributed to experimental conditions. One example of a problem in experimental conditions is that the lab where the research occurred is below typical room temperature. Since the temperature was about 5˚C lower than room temperature, the percent yield of the Michael Adduct in the Robinson Annulation and the protecting reaction may have been negatively

impacted since they both occurred at room temperature. Another source of error could be in the separation methods. It seems in all the reactions performed, some of the product was lost in the separation step. Thus, maybe new solvent systems or separation should be evaluated for any future work.

Future work that could be performed for this project includes finishing the steps in the the new synthesis scheme to complete the synthesis of the bicyclic moiety. This would include oxidizing the Grignard product, using the oxidized Grignard product in the Robinson annulation to form the base of the bicyclic moiety, and then reducing the carbonyl at the bottom left of the structure and methylating the alpha position of the carbonyl at the top right of the structure. Additionally, the reactions that were performed could be optimized for the best percent yield obtainable by modifying experimental conditions such as room temperature and separation techniques that could be leading to error. Once the reactions to form the bicyclic moiety are completed and optimized, work can begin on attaching the bicyclic moiety to the other parts of the Stachybotrin D molecule. Once a green synthesis of Stachybotrin D is complete, structure-activity relationship tests can be performed on the compound to optimize the potential drug's effectiveness against HIV. After these steps are completed, the optimized drug could be ready to under clinical trials and FDA approval. There is certainly a lot more work that is required before Stachybotrin D is market-ready as an anti-HIV drug however, this research has helped to contribute to the process.

References

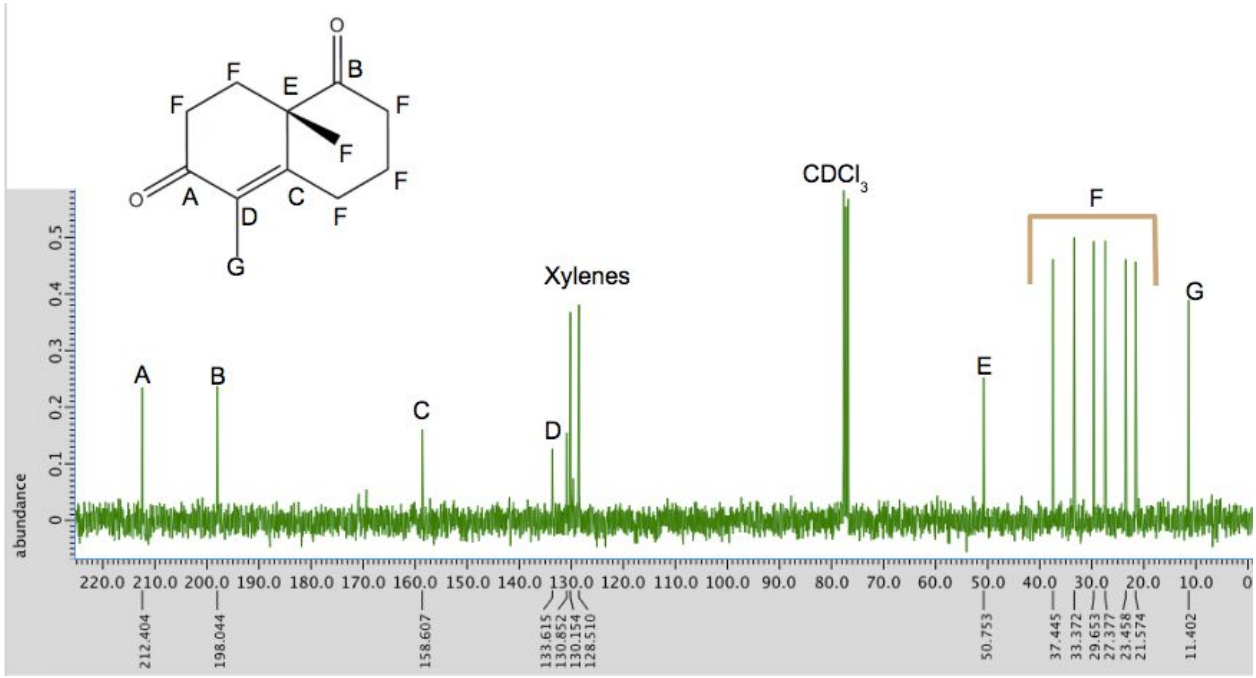
[1] Global Statistics. https://www.aids.gov/hiv-aids-basics/hiv-aids-101/global-statistics/ (accessed Jan 31, 2019).
[2] About HIV/AIDS https://www.cdc.gov/hiv/basics/whatishiv.html (accessed Mar 24, 2017).

[3] Global HIV & AIDS statistics - 2018 fact sheet. http://www.unaids.org/en/resources/fact-sheet (accessed Aug 31, 2018).
[4] The HIV Life Cycle Understanding HIV/AIDS. https://aidsinfo.nih.gov/understanding-hiv-aids/fact-sheets/19/73/the-hiv-life-cycle (accessed Feb 11, 2019).
[5] New Drug Targets for HIV. https://academic.oup.com/cid/article/41/Supplement_1/S96/402603 (accessed Feb 11, 2019).
[6] FDA-Approved HIV Medicines Understanding HIV/AIDS. https://aidsinfo.nih.gov/understanding-hiv-aids/fact-sheets/21/58/fda-approved-hiv-medicines (accessed Feb 11, 2019).
[7] Drug Resistance Understanding HIV/AIDS. https://aidsinfo.nih.gov/understanding-hiv-aids/fact-sheets/21/56/drug-resistance (accessed Feb 11, 2019).
[8] WHO urges action against HIV drug resistance threat. https://www.who.int/news-room/detail/20-07-2017-who-urges-action-against-hiv-drug-resistance-threat (accessed Feb 11, 2019).
[9] Clavel, F.; Hance, A. *New England Journal of Medicine* **2004**, 350 (10), 1023-1035.
[10] Transmitted drug resistance now less likely to harm HIV treatment options, US study finds. http://www.aidsmap.com/Transmitted-drug-resistance-now-less-likely-to-harm-HIV-treatment-options-US-study-finds/page/3293665/ (accessed Feb 11, 2019).
[11] Ma, X.; Li, L.; Zhu, T.; Ba, M.; Li, G.; Gu, Q.; Guo, Y.; Li, D. *Journal of Natural Products* **2013**, *76* (12), 2298–2306.
[12] Plant Secondary Compounds. http://www2.nau.edu/~gaud/bio300w/secpd.htm (accessed Apr 8, 2017).
[13] Sakurai, J.; Kikuchi, T.; Takahashi, O.; Wantanabe, K.; Katoh, T. *Journal of Organic Chemistry* **2011**, 2011 (16) 2948–2957.
[14] Demand for Yew Tree Concerns Environmentalists. http://www.nytimes.com/1987/05/03/us/demand-for-yew-tree-concerns-environmentalists.html (accessed Apr 8, 2017).
[15] Sakurai, J.; Kikuchi, T.; Takahashi, O.; Wantanabe, K.; Katoh, T. *Journal of Organic Chemistry* **2011**, 2011 (16) 2948–2957.
[16] Jacolot, M.; Jean, M.; Tumma, N.; Bondon, A.; Chandrasekhar, S.; Weghe, P. V. D. *The Journal of Organic Chemistry* **2013**, *78* (14), 7169–7175.
[17] Klein, D. R. *Organic Chemistry: WileyPLUS Student Package, 2nd Edition*; John Wiley & Sons, **2014**, 293-306.
[18] Winter, A. Stereochemistry tutorial: Biological Importance of Stereochemistry http://www.chemhelper.com/biostereo.html (accessed Mar 24, 2017).
[19] Vargesson, N. *Birth Defects Research Part C: Embryo Today: Reviews* **2015**, *105* (2), 140–156.
[20] Thalidomide: Research advances in cancer and more. https://www.mayoclinic.org/diseases-conditions/cancer/in-depth/thalidomide/art-20046534 (accessed Feb 11, 2019).
[21] Design Principles. https://www.acs.org/content/acs/en/greenchemistry/principles.html (accessed Feb 11, 2019).

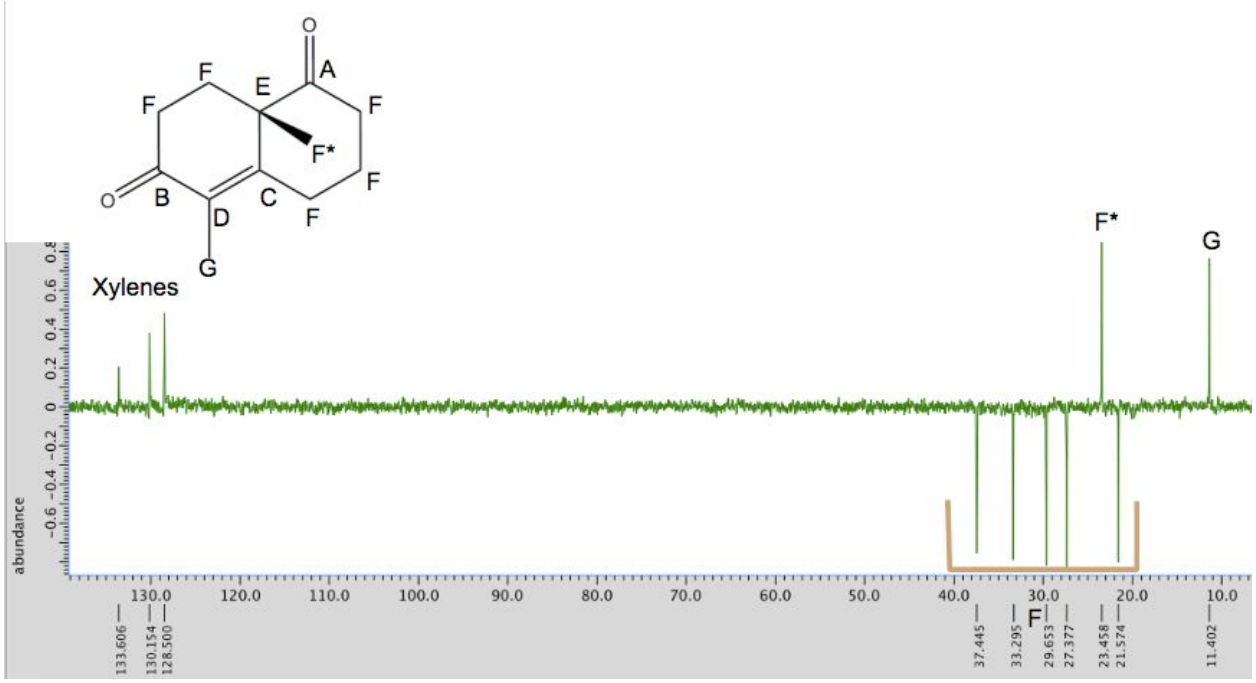
[22] 12 Principles of Green Chemistry. https://www.acs.org/content/acs/en/greenchemistry/principles/12-principles-of-green-chemistry.html (accessed Feb 11, 2019).
[23] Green Chemistry Examples. https://www.acs.org/content/acs/en/greenchemistry/what-is-green-chemistry/examples.html (accessed Feb 11, 2019).
[24] Smith, A. B.; Mewshaw, R. *The Journal of Organic Chemistry* **1984**, *49* (20), 3685–3689.
[25] Formaldehyde 37% solution MSDS http://www.sciencelab.com/msds.php?msdsId=9924095 (accessed May 3, 2017).
[26] Lithium MSDS http://www.sciencelab.com/msds.php?msdsId=9927559 (accessed Mar 9, 2018).
[27] Lazarski, K. E.; Rich, A. A.; Mascarenhas, C. M. *Journal of Chemical Education* **2008**, *85* (11), 1531-1534.
[28] Burns, D. J.; Mommer, S.; O'Brien, P.; Taylor, R. J. K.; Whitwood, A. C.; Hachisu, S. *Organic Letters* **2013**, *15* (2), 394–397.
[29] Jung, M. E.; Duclos, B. A. *Tetrahedron* **2006**, *62* (40), 9321–9334.
[30] Hagiwara, H.; Uda, H. *The Journal of Organic Chemistry* **1988**, *53* (10), 2308–2311.
[31] 2-Butanone. https://www.chemicalbook.com/ChemicalProductProperty_EN_CB4854386.htm (accessed Feb 11, 2019).
[32] Murahashi, S.; Makabe, Y.; Kunita, K. *The Journal of Organic Chemistry* **1988**, *53* (19), 4489-4495.
[33] Greeves, N.; Lee, W.-M.; Barkley, J. V. *Tetrahedron Letters* **1997**, *38* (36), 6453–6456.
[34] Schulze, A.; Pagona, G.; Giannis, A. *ChemInform* **2006**, *37* (35), 1147–1156.
[35] Experiment 3: Grignard Reaction. http://web.mst.edu/~tbone/Subjects/TBone/chem228/grignard.pdf (accessed Apr 4, 2019).
[36] Hass, M. http://organicchem.org/oc2web/lab/exp/grig/grigbg.html (accessed Mar 15, 2019).
[37] An Introduction to Grignard Reagents. https://www.chemguide.co.uk/organicprops/haloalkanes/grignard.html (accessed Apr 4, 2019).
[38] Potassium Dichromate. https://www.fishersci.com/msds?productName=AC197760050 (accessed Apr 4, 2019).
[39] Is the potassium permanganate used to restore oxidizing power of greensand water filters toxic or harmful? https://www.freedrinkingwater.com/water_quality/quality1/47-08-potassium-permanganate-used-in-greensand-filters-toxic.htm (accessed Apr 4, 2019).
[40] Libretexts. Oxidation of Alkenes with Potassium Manganate. https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Supplemental_Modules_(Organic_Chemistry)/Alkenes/Reactivity_of_Alkenes/Oxidation_of_Alkenes_with_Potassium_Manganate (accessed Apr 4, 2019).
[41] Manganese Dioxide. https://beta-static.fishersci.com/content/dam/fishersci/en_US/documents/programs/education/regulatory-documents/sds/chemicals/chemicals-m/S25420.pdf (accessed Apr 4, 2019)
[42] Kirihara, M.; Okada, T.; Sugiyama, Y.; Akiyoshi, M.; Matsunaga, T.; Kimura, Y. *Organic Process Research & Development* **2017**, *21* (12), 1925–1937.

Appendix

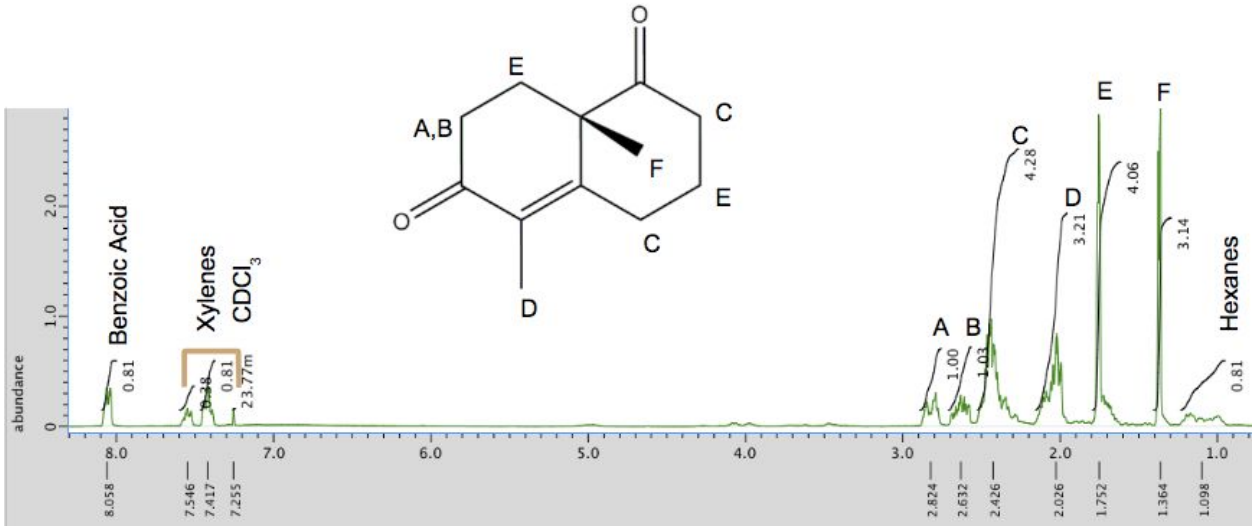
Spectra 1: ^{13}CNMR of the purified product from the Robinson Annulation using 2-methyl-1,3-cyclohexanedione and ethyl vinyl ketone



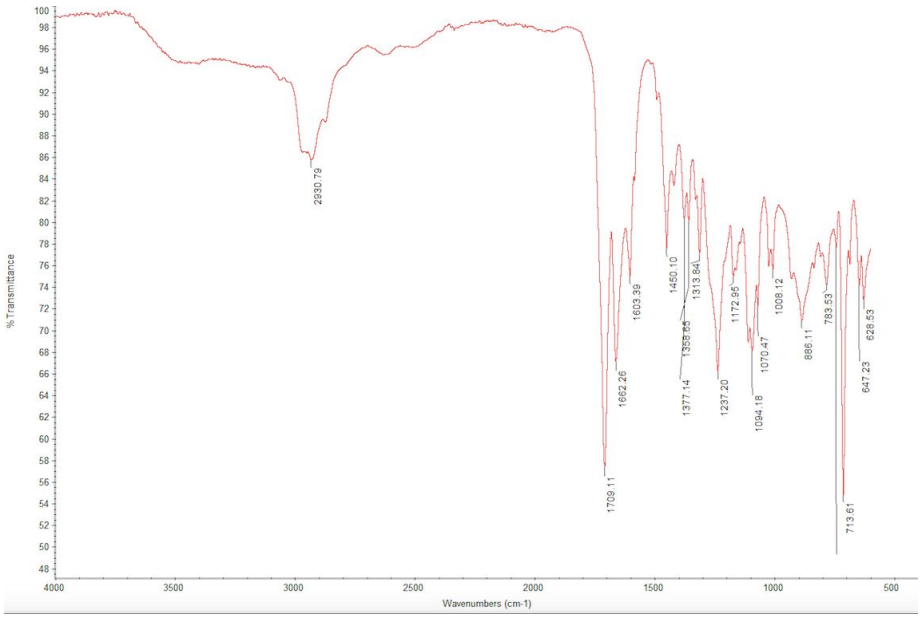
Spectra 2: DEPT 135 of the purified product from the Robinson Annulation using 2-methyl-1,3-cyclohexanedione and ethyl vinyl ketone



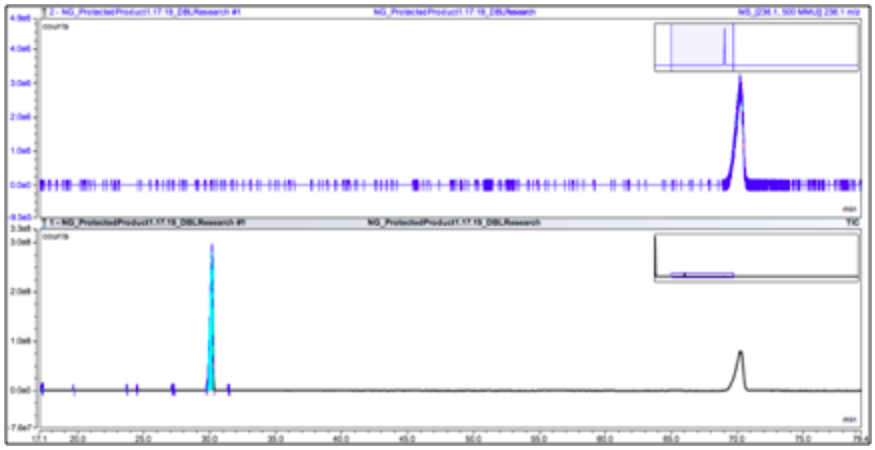
Spectra 3: ^{1}H NMR of the purified product from the Robinson Annulation using 2-methyl-1,3-cyclohexanedione and ethyl vinyl ketone



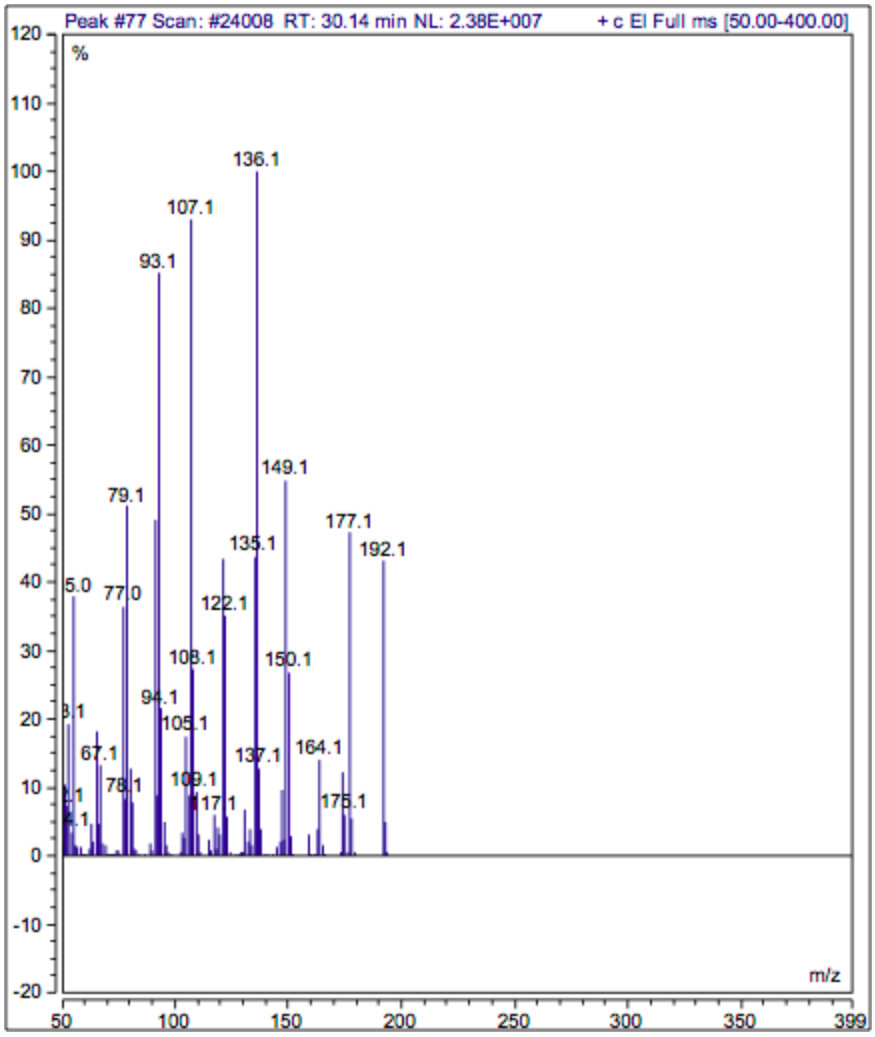
Spectra 4: IR of the purified product from the Robinson Annulation using 2-methyl-1,3-cyclohexanedione and ethyl vinyl ketone



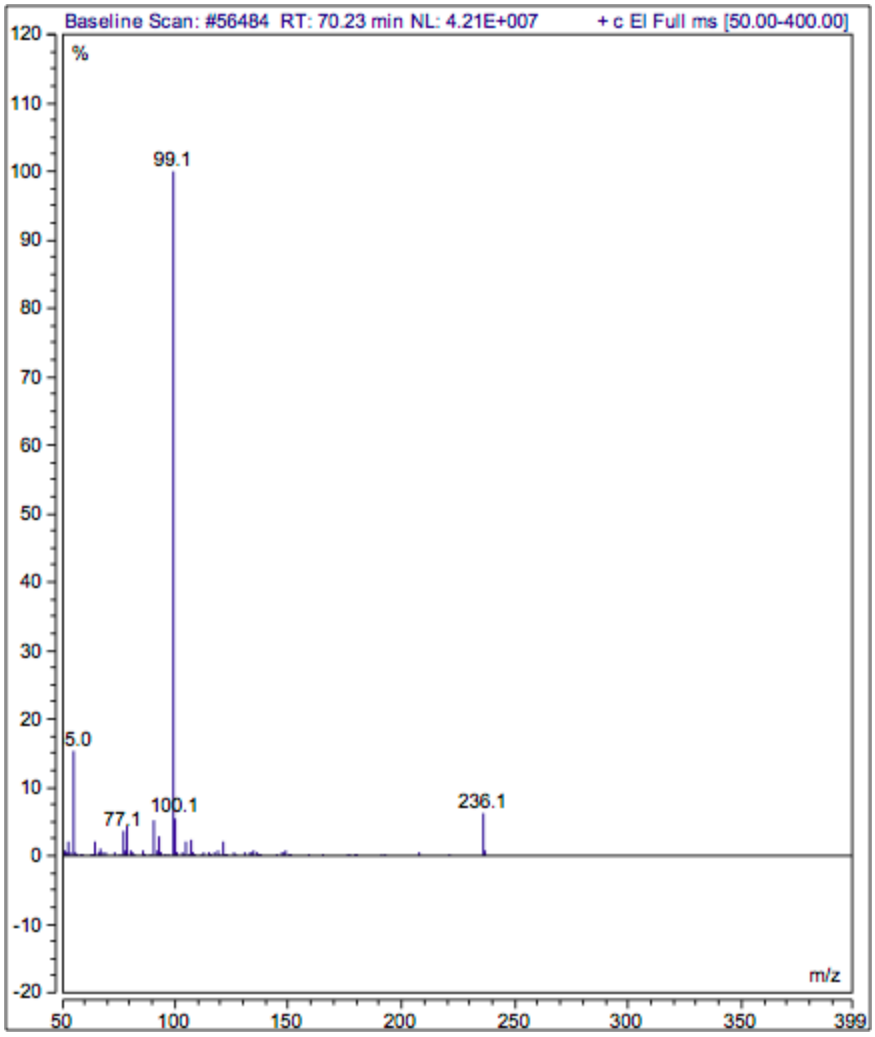
Spectra 5: Gas chromatogram of protection of Robinson Annulation product indicating that the protected product was formed but much of the unprotected Robinson Annulation product is still present



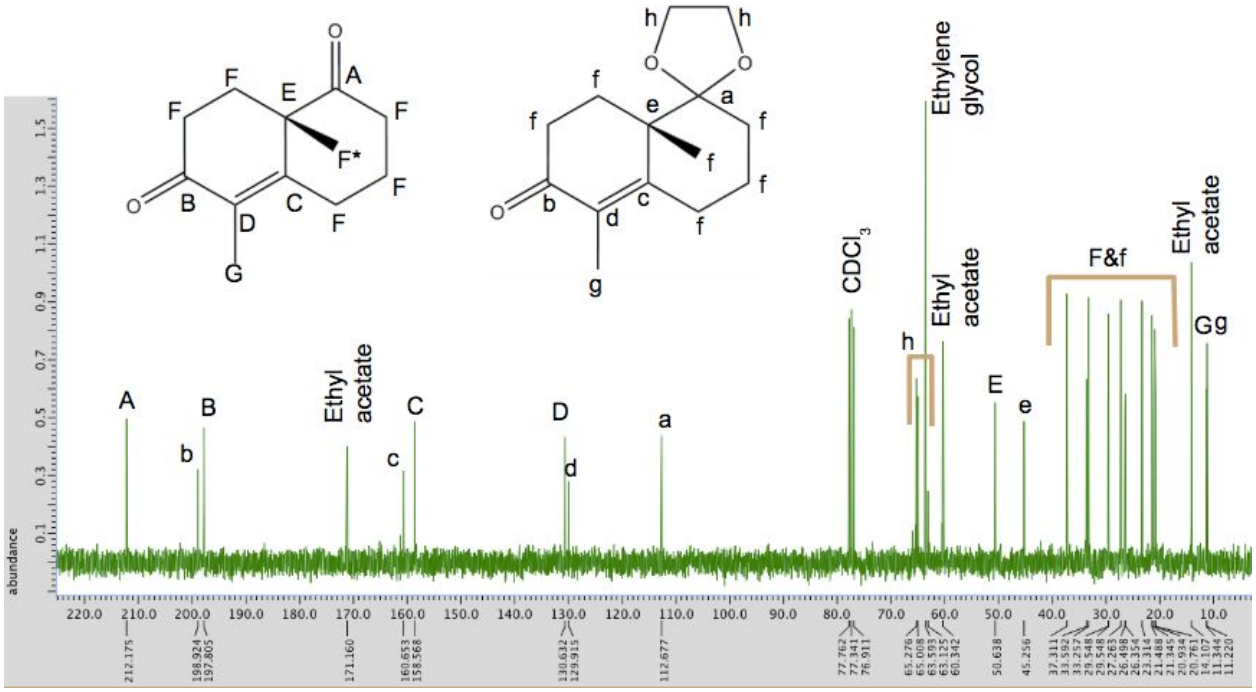
Spectra 6: Mass spectrum of the unprotected Robinson Annulation product (peak on bottom left of Spectra 5)



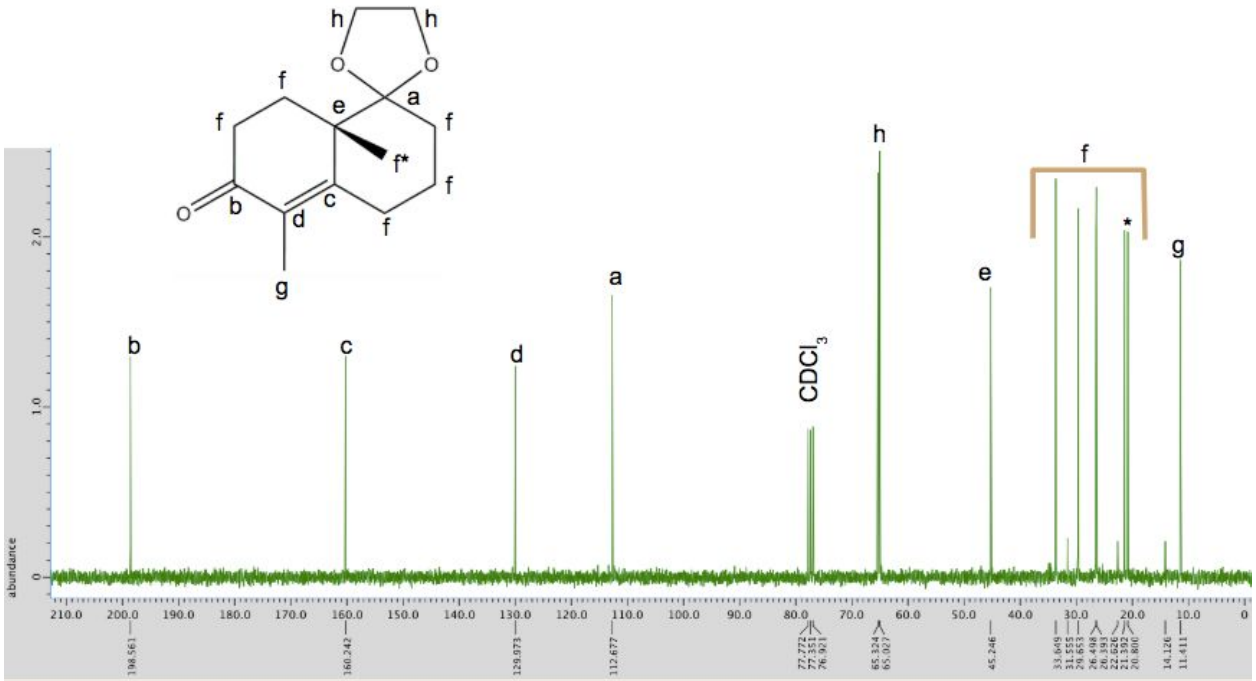
Spectra 7: Mass spectrum of the protected Robinson Annulation product (peak on top and bottom right of Spectra 5)


Baseline Scan: #56484 RT: 70.23 min NL: 4.21E+007
+ c EI Full ms [50.00-400.00]
%
99.1
5.0
77.1
100.1
236.1
m/z
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
50
100
150
200
250
300
350
399

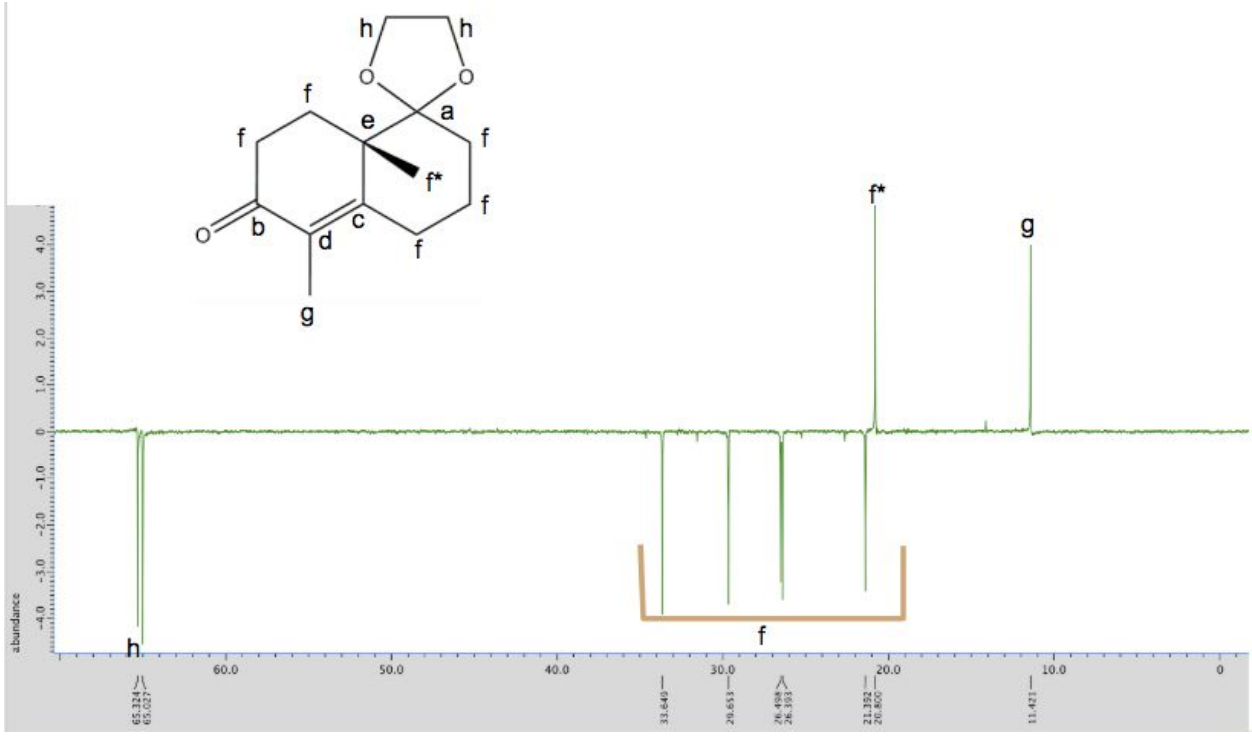
Spectra 8: ^{13}C NMR of the crude protected product from the Robinson Annulation



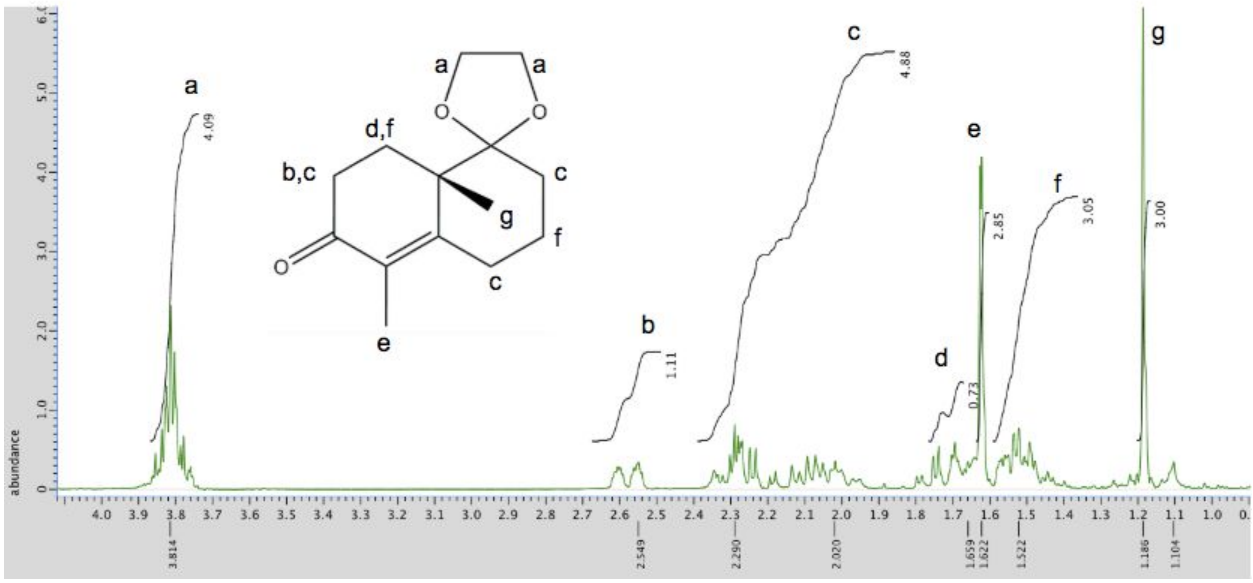
Spectra 9: ^{13}C NMR of protected product (spot 2 from the column)



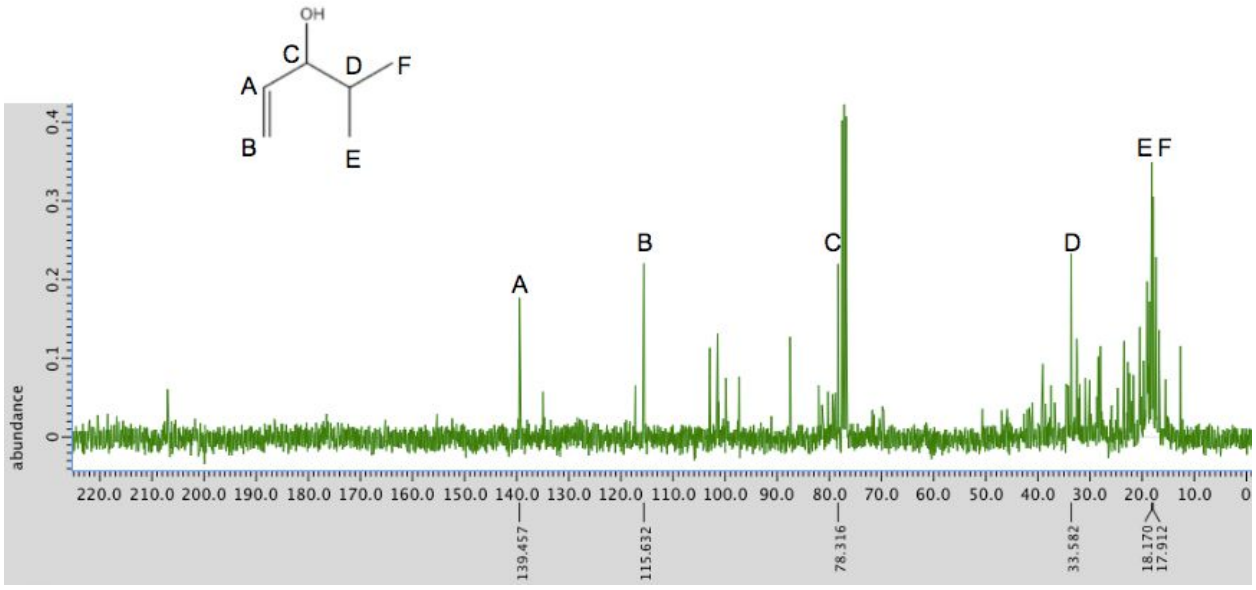
Spectra 10: DEPT 135 of protected product (spot 2 from the column)



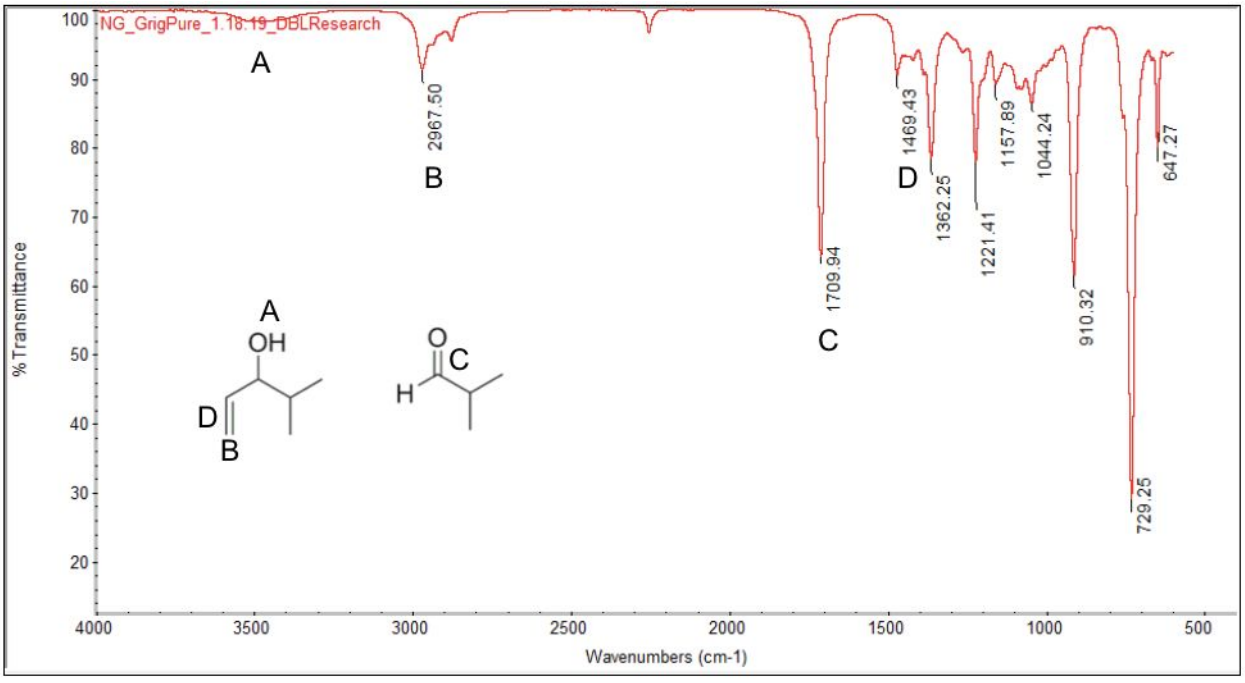
Spectra 11: ^{1}H NMR of protected product (spot 2 from the column)



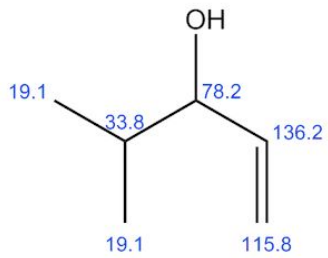
Spectra 12: ^{13}C NMR of crude Grignard reaction product



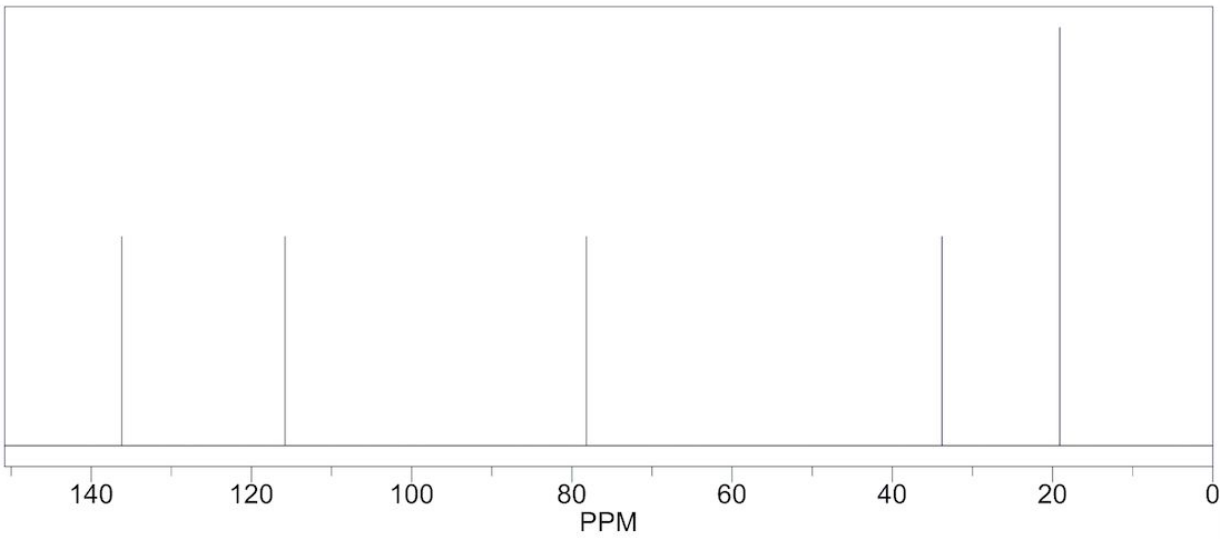
Spectra 13: IR of Purified Grignard Product



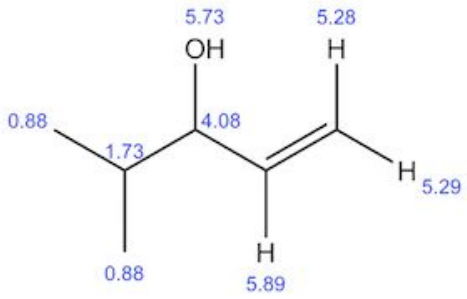
Spectra 14: Predicted ^{13}C NMR of purified Grignard reaction product



Estimation quality is indicated by color: good, medium, rough



Spectra 15: Predicted ^{1}H NMR of purified Grignard reaction product



Estimation quality is indicated by color: good, medium, rough