The Novel Synthesis of 3,5-disubstituted Δ^2-isoxazoline Through the Utilization of Lead (II) Acetate and Nickel (II) Chloride in the Metal-Mediated Cyclization

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1. Introduction

Type 2 Diabetes is an ever-growing threat in the United States with more than 29.1 million diagnosed cases and approximately 8.1 million undiagnosed cases (CDC). It is expected that 84 million Americans will be affected by Type II Diabetes in the coming years (CDC). Current treatment consists of using the prescribed drug, metformin. However, it has been shown that metformin can cause serious cases of lactic acidosis (Mayo Clinic). Lactic acidosis causes the buildup of lactic acid within the blood stream which effectively lowers the body's pH and can be life-threatening if left untreated (Misbin). A new treatment has been developed using glucose-derived spiro-isoxazolines (Goyard). This drug works by targeting and inhibiting glycogen phosphorylase which is an enzyme that depolymerizes glycogen into glucose (Goyard). When this enzyme is inhibited by the drug, it will create glucose at slower rates thereby reducing the blood sugar concentration (Goyard).

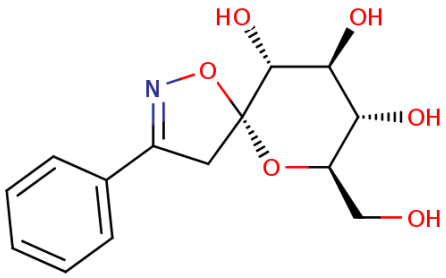

Figure 1: The chemical structure of glucose-derived spiro-isoxazolines. It has many implications to the treatment of Type II Diabetes.

An isoxazole ring structure is an important part of glucose-derived spiro-isoxazolines (Figure 1) as it has been implicated as a precursor to a therapeutic agent in the treatment of Type II Diabetes (Goyard). However, using current methods in the synthesis of isoxazoline, the drug is rendered nearly unaffordable by the high costs of production. Another implication of isoxazoline

is its use in a drug called ISO-1. ISO-1 inhibits Macrophage Migratory Inhibition Factor (MIF) which plays a role in both types of diabetes but especially in Type I Diabetes (Mosher & Norman) (Sánchez-Zamora, Yuriko I.). MIF is a pro-inflammatory cytokine which acts as an alert to the immune system (Sánchez-Zamora, Yuriko I., & Miriam Rodriguez-Sosa). When the immune system is activated by MIF, Beta cells in the pancreas are destroyed through apoptosis. As Beta cells produce the insulin to regulate blood sugar levels, Type I Diabetes occurs when they are destroyed. (Sánchez-Zamora, Yuriko I.,). Because ISO-1 can inhibit MIF, Beta cells will survive and produce insulin to regulate glucose levels in the blood. This would lead to the effective treatment of Type I Diabetes.

The main focus in conducting this research is to carry out an attempt on gaining higher yields in a more cost-efficient manner when synthesizing 3,5-disubstituted Δ^2-isoxazoline (Figure 2). 3,5-disubstituted Δ^2-isoxazoline is an important part of both glucose-derived spiro isoxazolines and ISO-I as their most basic structures are adjacent to 3,5-disubstituted Δ^2-isoxazoline.

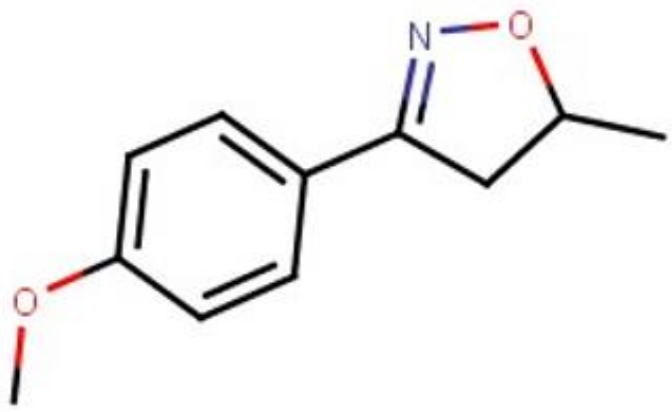

Figure 2: Distinguishing ring of 3,5-disubstituted Δ^2-isoxazoline which is the target product of our experiments. Note that isoxazoline is specifically characterized by the closed ring structure consisting of a nitrogen bonded to an oxygen.

From working in this field of study, it is expected that novel methods of synthesizing 3,5-disubstituted Δ^2-isoxazoline can be identified. By using new materials in the cyclization process, the price of 3,5-disubstituted Δ^2-isoxazoline has great potential to be reduced. This would benefit a large portion of society as it would lead to an overall price drop in the cost of diabetic treatment.

In essence, the research consists of three distinct purposes. The first goal is to successfully synthesize 3,5-disubstituted Δ^2-isoxazoline using Palladium (II) Chloride in the metal mediated cyclization reaction. It is hypothesized that using palladium in the final cyclization reaction will successfully synthesize 3,5-disubstituted Δ^2-isoxazoline in acceptable yields. The second part of this project is to conduct research on using Nickel (II) Chloride in place of Palladium (II) Chloride during the end reaction. It is hypothesized that using nickel chloride in place of palladium will indeed successfully synthesize 3,5-disubstituted Δ^2-isoxazoline in acceptable yields but also in a much more cost-efficient manner. The third part of this research is to conduct novel research on using Lead (II) Acetate in the cyclization reaction. As a cyclization reaction has never been conducted using Lead (II) Acetate, the results may vary in terms of yield and purity. However, it is hypothesized that Lead (II) Acetate will produce identifiable amounts of 3,5-disubstituted Δ^2-isoxazoline.

The usage of palladium in the metal mediated cyclization reaction has been proven to succeed through an analysis of its chemical properties. Palladium contains empty valence d-orbitals which attract extra electrons to the double bond structure within the previously

synthesized oxime (Mikesell, Joshua, Michael D. Mosher). This removes the hydrogen from the oxime group and the extra electrons form a bond between from nitrogen to oxygen and from oxygen to a carbon group as seen in Figure 3.

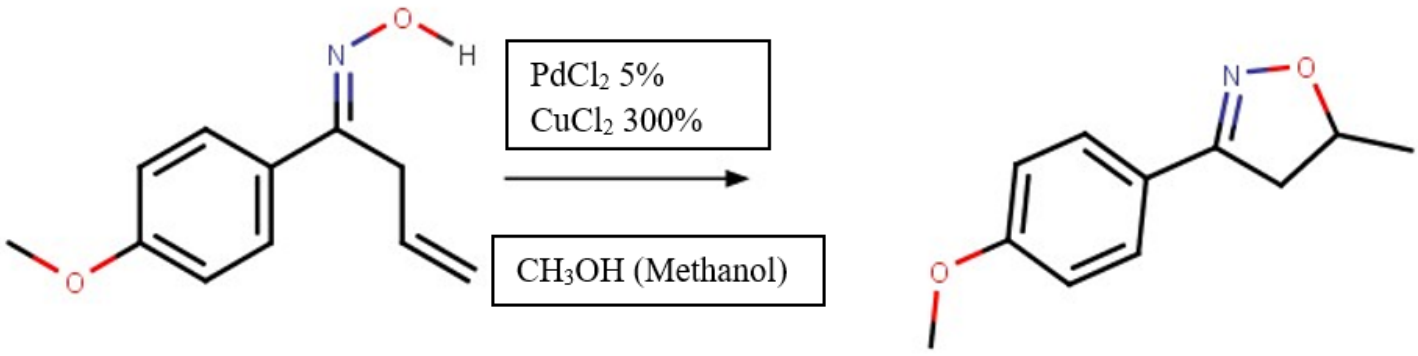

Figure 3: The oxime group is reconfigured when palladium (PdCl2) is introduced to the previously synthesized oxime group. *Mosher, (2016).*

Nickel chloride is hypothesized to work because it is in the same family as palladium on the periodic table. This means that it also has an empty valence d-orbital to attract electrons. Nickel chloride would be far superior to palladium if proven to work due to the fact that it is substantially cheaper than palladium per ounce. Currently palladium costs approximately 950 dollars per ounce while nickel chloride costs just 2.75 dollars an ounce. The usage of nickel chloride in the synthesis of isoxazoline would be tremendously beneficial to decreasing the cost of diabetic treatment.

Lead (II) Acetate is hypothesized to work because successful research has been conducted using Mercuric Acetate. Mercuric Acetate has many similar properties to Lead (II) Acetate in terms of molecular mass and period on the periodic table. Due to these similarities, it

is possible that the same experimental set up that was used for Mercuric Acetate will also apply in the cyclization of Lead (II) Acetate.

Previous research has been conducted on using palladium in the final cyclization reaction. There has also been research conducted upon using zinc in the cyclization reaction. While the zinc is cheaper per ounce than the palladium, the 12% yields were not high enough to provide justification to deviate from using palladium (Das, Prasanta, and Ashton T. Hamme). The goal of this research is to improve upon the aforementioned methods by identifying a different metal to use in this end reaction to successfully synthesize 3,5-disubstituted Δ^2-isoxazoline at a decreased price.

2. Methodology

1. Grignard Reaction for synthesis of 1-(4-Methoxyphenyl)-3-buten-1-ol (ROH)

This reaction required the P-Anisaldehyde to act as a starting material, tetrahydrofuran (THF) was obtained for use as a solvent, and Allylmagnesium chloride was used as a reagent in this reaction. The experimental equipment that is needed for this reaction is a hotplate with an internal magnetic stir system, a Teflon coated stir rod, two syringes, two 100 ml round bottom flasks. A separation flask, rotary evaporation machine, filter paper, and a funnel were needed. Thin Layer Chromatography materials (plates and flask) and an NMR machine were also needed for the separation and verification processes of this reaction.

1.1 Grignard Reaction Preparation

To prepare the reagents of this reaction, P-Anisaldehyde was measured to 0.4468 ml and Allylmagnesium chloride was measured to 6.239 ml. Tetrahydrofuran (THF) was measured to approximately 17 ml.

1.2 Synthesis of 1-(4-Methoxyphenyl)-3-buten-1-ol

P-Anisaldehyde (0.4468 ml) was added into the 100 ml round bottom flask via syringe and the stir rod was subsequently engaged. This round bottom flask was then sealed with a rubber plug and argon gas was circulated into the flask via a small hole in the plug. Argon is an inert gas meaning that it does not react with other substances and in this case, it was used to protect the reaction from the impure atmosphere.

In order to acquire the tetrahydrofuran, a syringe was used to draw out 17 ml. This amount of tetrahydrofuran was introduced into the same round bottom flask and was immediately mixed to form a solution with p-Anisaldehyde.

After introducing the Tetrahydrofuran, Allylmagnesium chloride was drawn out of its respective sealed flask. This had to be performed using a new syringe to pierce a septum that was covering the flask. This Allylmagnesium chloride was then slowly added to the existing solution over a period of 20 minutes. This had to be done to avoid a violent reaction which could lead to an explosion due to the buildup of pressure within the round bottom flask.

Following the complete introduction of Allylmagnesium chloride, the solution was left to react for another 30 minutes. Both the stir rod and argon gas were actively engaged through the entirety of this reaction. At the end of this reaction, the 1-(4-Methoxyphenyl)-3-buten-1-ol was formed although it needed to be further purified through an extensive separation process.

1.2.1 Separation

After the solution has fully reacted, the 1-(4-Methoxyphenyl)-3-buten-1-ol from the round botton flask was poured into a separation funnel that had its drain closed. Then ethyl acetate and deionized water were poured into the separatory funnel until an organic layer formed on top and an aqueous

layer formed underneath it. The solution filled 75% of the entire separatory funnel with the organic layer occupying approximately half an inch. Once this solution was settled, a stopper was placed on top of the separatory funnel. Then the separatory funnel was tilted towards the back of the fume hood and lightly shaken for 2 to 3 seconds. The drain was briefly opened to release pressure and then immediately closed. This process was then repeated once more and the separatory funnel was placed back to its original position. Next, the bottom aqueous layer was drained from the separatory funnel into a 500 ml Erlenmeyer flask. This left only top layer which consisted of ethyl acetate and the organic portion of the 1-(4-Methoxyphenyl)-3-buten-1-ol. Deionized water was then added into the separatory funnel to regain the original volume of solution. The stopper was placed back on top of the separatory funnel and both the shaking and draining processes were repeated again. Once the organic layer was all that was left in the separation flask after two initial separations, it was poured into a clean 250 ml beaker. Following this, the aqueous contents of the Erlenmeyer flask were poured back into the separatory funnel to undergo another further separation. A new, clean Erlenmeyer flask was then placed under the funnel to prepare for the second stage of separation. The aforementioned separation process was then performed once but preferably twice to ensure that only the organic layer consisting of ethyl acetate and 1-(4-Methoxyphenyl)-3-buten-1-ol was left.

As it could not be ensured that there was no deionized water left with the 1-(4-Methoxyphenyl)-3-buten-1-ol and ethyl acetate mixture, another gravity filter to remove the water had to be performed. The 1-(4-Methoxyphenyl)-3-buten-1-ol and ethyl acetate mixture was transferred into a small Erlenmeyer flask. Then, magnesium sulfate was added into the flask. Enough was added until the mixture looked between translucent and opaque. The next step in this filter process was to remove the magnesium sulfate from the 1-(4-Methoxyphenyl)-3-buten-1-ol

and ethyl acetate. The mixture from the Erlenmeyer flask was poured through a funnel that had been lined with filter paper. This allowed the1-(4-Methoxyphenyl)-3-buten-1-ol and ethyl acetate to drip through into a round bottom flask that was placed below the funnel. This process removed all water from the product which was ready to enter its final separation by means of a rotary evaporator.

The rotary evaporator heated the solution to 77°C and which caused the ethyl acetate to boil and evaporate. The evaporated ethyl acetate was then condensed by the rotary evaporator and dripped into a collection flask where it was disposed of. This left 0.8965 ml of the purified 1-(4-Methoxyphenyl)-3-buten-1-ol in the 100 ml round bottom flask.

1.2.2 Grignard Verification

Using Thin Layer Chromatography (TLC) the composition of the 1-(4-Methoxyphenyl)-3-buten-1-ol was observed in a general manner. To do this, a TLC plate and TLC flask were acquired. The TLC plate is composed of a thinly spread and highly polar silica gel. This causes non-polar substances to move up the plates and polar substances to be stationary due to the fact that they form bonds that they form with the silica gel. These differences in polarity provide a rough purity test.

A more specific and precise verification of this substance's purity was through the usage of proton Nuclear Magnetic Resonance (NMR) imaging. An NMR tube was prepared before the NMR could be used. The tube consisted of a small amount of the 1-(4-Methoxyphenyl)-3-buten-1-ol and chloroform-d ($CDCl_3$). To make this, an open pipette was lowered into the 1-(4-Methoxyphenyl)-3-buten-1-ol so that a small amount was transferred to the tip of the pipette. This pipette was then inserted into an NMR tube. Finally, chloroform-d was injected through the pipette

and into the tube so that approximately an inch of the tube was occupied by the solution. This tube was placed into the NMR machine which was controlled by a computer. In approximately 30 seconds, the NMR machine scanned the substance 8 times and provided a graph that showed distinct peaks which were indicative of the chemicals that a pure sample of 1-(4-Methoxyphenyl)-3-buten-1-ol consists of. This allowed for the precise and accurate confirmation of the fact that the synthesized 1-(4-Methoxyphenyl)-3-buten-1-ol was pure enough to continue to the subsequent reaction.

1.3 Ketone Synthesis

This reaction required 1.88 grams of pyridinium chlorochromate (PCC), 0.8965 ml of 1-(4-Methoxyphenyl)-3-buten-1-ol, and 9.4 grams of that was prepared in the previous Grignard reaction. These were used as the reagents for this particular synthesis. Additional equipment required for this section was a 100 ml round bottom flask and a vacuum separation device.

1.4 Preparation for the synthesis of desired Ketone

This synthesis began with adding 1.88 grams of PCC and 9.4 grams of Magnesium sulfate to 18 ml of dichloromethane in a 100 ml round bottom flask that was clamped above a hot plate. In this case, the hot plate was used to power the stir bar that was also in the round bottom. Then, 0.8965 ml of 1-(4-Methoxyphenyl)-3-buten-1-ol was added into the round bottom flask. The container that had previously contained 1-(4-Methoxyphenyl)-3-buten-1-ol was rinsed twice with dichloromethane and poured into the round bottom flask. This was to ensure a complete transfer of material between flasks. The Teflon coated stir bar was turned to spin at level 6 and the solution was left to react for 1 hour at room temperature. Once this reaction had been completed, the ketone was synthesized but needed separation to remove the dichloromethane solvent.

1.4.1 Separation of Ketone

To separate the ketone, a vacuum enhanced separator was used. Essentially, a beaker with a drain on the bottom was tightly sealed into a 500 ml Erlenmeyer flask. The flask was connected to a vacuum pump with a rubber hose. An approximate amount of florisil powder was poured into the beaker. The ketone and waste was poured on top of the florisil powder. After the ketone was poured into the filter, ethyl acetate was poured on top and used to carry the pure ketone through the filter with it. The vacuum helped further accelerate this process by pulling the ketone and ethyl acetate through the florisil while the larger waste particles became trapped in the fine powder. This left a solution made up of ethyl acetate and ketone in the 500 ml Erlenmeyer flask. The contents of the Erlenmeyer flask were transferred into a 250 ml round bottom flask which was then attached to the rotary evaporator. Here, the ethyl acetate was evaporated, leaving only the ketone in the flask.

1.4.2 Ketone purity verification

Like the Grignard reaction, Thin Layer Chromotography was used to roughly judge the purity of the previously synthesized ketone. After this, an NMR tube containing the ketone was prepared and the substance was further analyzed for purity on the NMR machine.

1.5 Oxime Synthesis

This reaction required a 100 ml round bottom flask, Teflon coated stir bar, hot plate, 8.028 grams of sodium acetate, 3.0907 grams of hydroxylamine hydrochloride, ethanol, and deionized water. The ketone synthesized in the previous reaction is also needed. Thin Layer Chromatography materials, NMR tubes, and an NMR machine were needed in verifying the purity of the synthesized

oxime. A rotary evaporator, separation flask, ethyl acetate, and a 500 ml Erlenmeyer flask were needed in the separation to further purify the oxime.

1.5.1 Oxime synthesis preparation

The ketone was first added into the round bottom flask with the Teflon coated stir bar in it. Then 3.0907 grams of hydroxylamine hydrochloride was added and 8.028 grams of sodium acetate was added immediately after. The ketone and hydroxylamine hydrochloride acted as the reagents in this synthesis while the sodium acetate helped stabilize the reaction. After they were added, 12 ml of ethanol and 12 ml of deionized water were added to act as solvents in the reaction. The stir bar was set to 6 and the mixture was left to react overnight. The products from this reaction were put through the previously described separation process once again. Then a rotary evaporator boiled out the ethyl acetate from the solution which left the final oxime product.

1.5.2 Oxime purity verification

Like before, a Thin Layer Chromatography test was performed using a TLC beaker along with a TLC plate. Once it was deemed that there was indeed a new substance formed, NMR testing was used to further verify purity and chemical content of the oxime.

1.6 Metal Mediated Cyclization Leading to the Synthesis of 3,5-disubstituted Δ^2-isoxazoline

Nickel (II) chloride, Palladium (II) chloride, and Copper chloride were acquired for use in this cyclization. Methanol was also acquired. Additional equipment used in this section was an analytical balance, 25 ml beakers, metal scoops, two 100 ml round bottom flasks, two hot plates, two Teflon coated stir bars, and a funnel.

1.6.1 Preparation of Chemicals

Nickel (II) chloride was massed to 0.0043 grams on an analytical balance. This was kept in a beaker. Following this, copper chloride was massed to 2.666 grams and kept in another beaker. One of the 100 ml round bottom flasks was clamped above the hot plate and had a stir bar inserted into it. 10 ml of methanol were added into the flask and the stir bar was set to 6. The 2.666 grams of copper chloride was added into the flask and this was quickly followed by the introduction of Nickel (II) chloride into the mixture. The beakers that previously held the Nickel (II) chloride and copper chloride were then rinsed with methanol and poured into the round bottom flask.

After the set up of the Nickel (II) chloride and copper chloride reaction was completed, 0.0048 grams of Palladium (II) chloride was massed and kept in a clean beaker. Following this, copper chloride was massed to 0.2170 grams and kept in another clean beaker. The other 100 ml round bottom flask was clamped above the hot plate and another stir bar was inserted. Like before, 10 ml of methanol were added into the round bottom flask and the stir bar was set to 6. The 2.170 grams of copper chloride was added into the flask which was quickly followed by adding the palladium (II) chloride into the flask. Methanol was used to rinse any leftover material from the beakers into the round bottom flask. These reactions were loosely covered and left to react overnight.

3. Results

The 5 NMR scans that were performed for the synthesis of 3,5-disubstituted Δ^2-isoxazoline were analyzed to determine the results. By analyzing the location of the peaks on the NMR graphs, the chemical structure of compounds can be identified. The NMR scans of the final metal mediated cyclizations are pictured below.

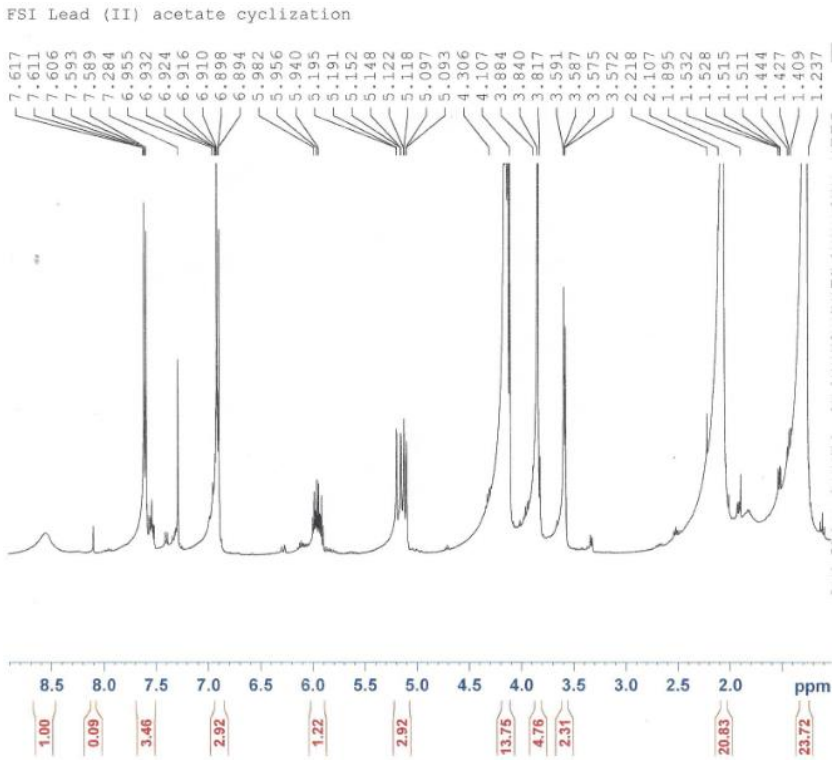

Figure 4

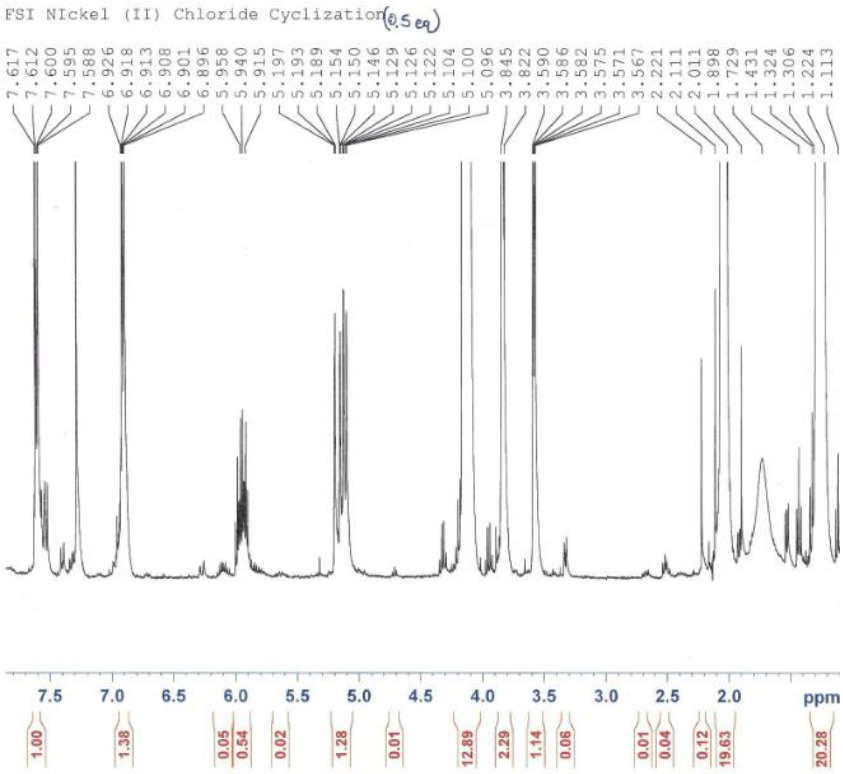

Figure 5

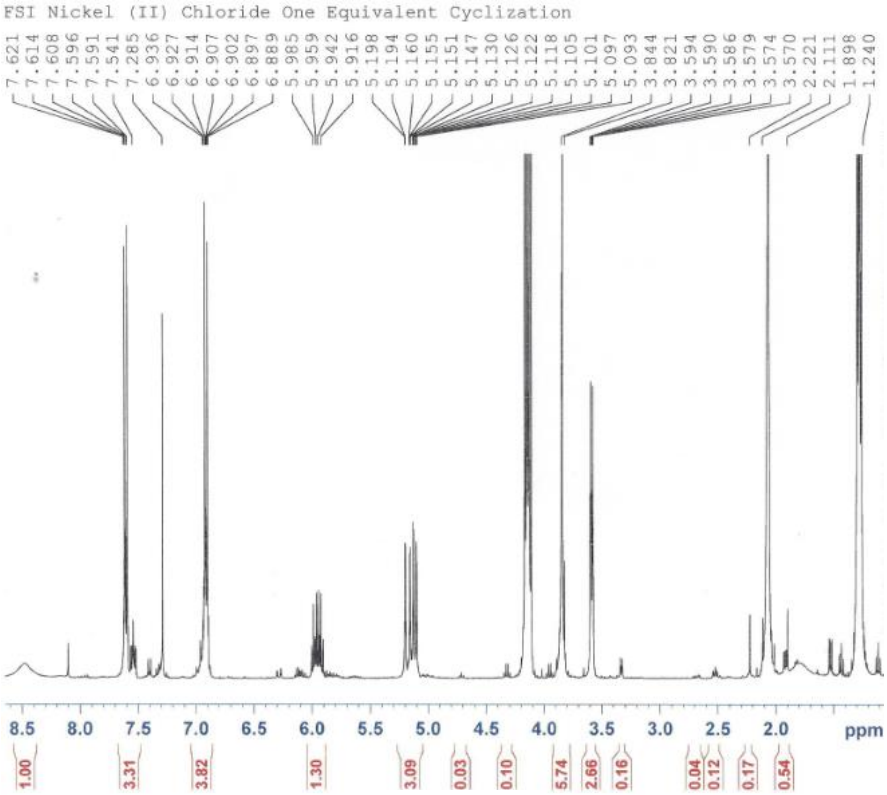

Figure 6

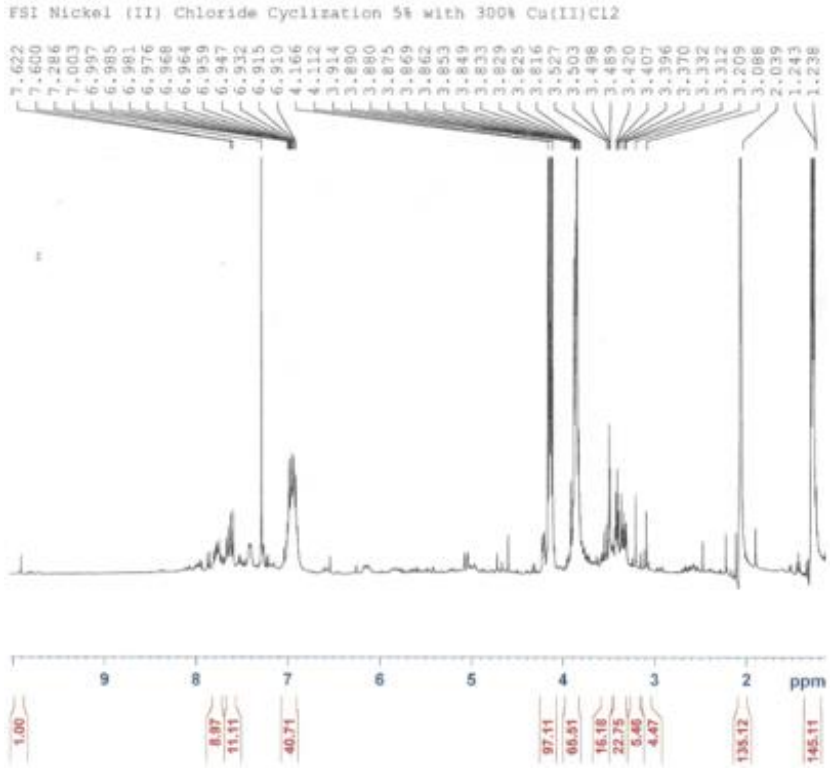

Figure 7

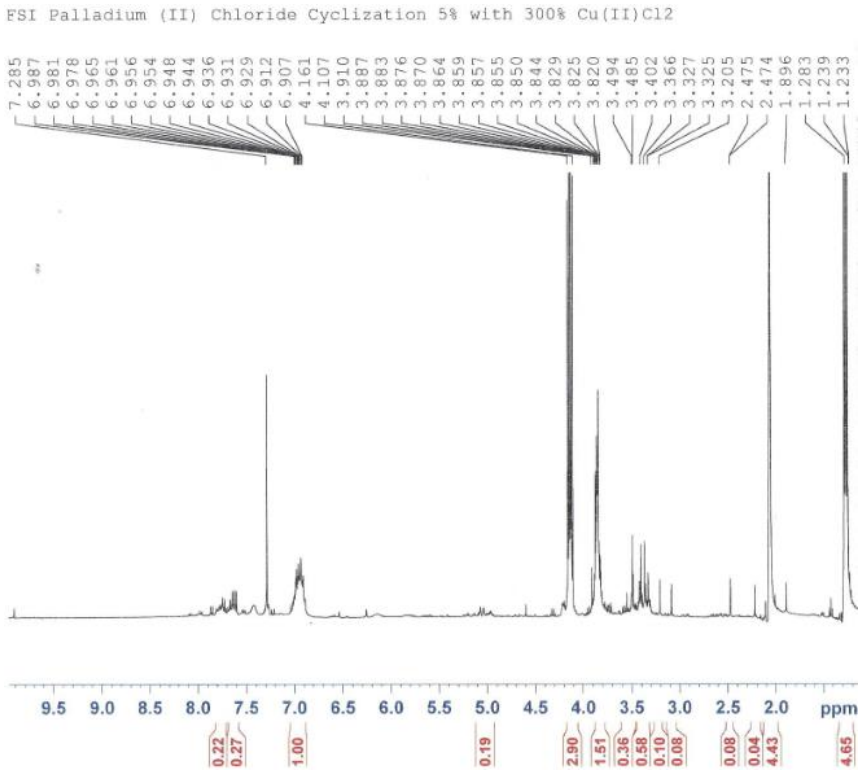

Figure 8

Figure 4 shows the result of a cyclization using Lead (II) Acetate as the metal mediator. Although the NMR scan reveals a slightly "dirty" result, traces of the 3,5-disubstituted Δ^2-isoxazoline can be seen from the peaks at 3.3 ppm and 4.7 ppm. Additionally, the hydrogen groups on the benzene ring of 3,5-disubstituted Δ^2-isoxazoline can be seen in the peaks at 7.5 and 7.0 ppm. This means that conclusive evidence is provided here showing that Lead (II) Acetate produced traces of 3,5-disubstituted Δ^2-isoxazoline when it was used as a metal mediator.

Figure 5 shows the result of the cyclization using a ½ equivalence Nickel (II) Chloride as the metal mediator. As in Figure 3, the result is slightly "dirty", but traces of 3,5-disubstituted

Δ^2-isoxazoline can still be seen due to the peaks at 3.3 ppm, 4.7 ppm, and the hydrogen group peaks at 7.5 ppm and 7.0 ppm. Again, this evidence reveals that Nickel (II) Chloride is a viable metal to use in the synthesis of 3,5-disubstituted Δ^2-isoxazoline.

Figure 6 shows the result of a cyclization using a full (1) equivalence Nickel (II) Chloride as the metal mediator. The result of this cyclization was slightly cleaner than the previous two scans. This means that there was less amounts of ethyl acetate (a reaction solvent) and chloroform-D (a solvent used in the NMR tubes) in the solution. However, while the overall result was cleaner, less amounts of 3,5-disubstituted Δ^2-isoxazoline can be seen in this particular scan. This can be distinguished due to the reduced size of the peaks at 3.3 ppm and 4.7 ppm. However, this shows that the full equivalence Nickel (II) Chloride will indeed work to produce small percentages of 3,5-disubstituted Δ^2-isoxazoline.

Figure 7 shows the result of a cyclization using a full (1) equivalence Nickel (II) Chloride as the metal mediator and Copper (II) Chloride as an oxidizing agent. This NMR was extremely "dirty" due to the disintegration of many compounds within 3,5-disubstituted Δ^2-isoxazoline. The peaks at 7.0 ppm and 7.5 ppm have been severely diminished and the peaks at 3.3 ppm and 4.7 ppm have also broken apart and turned into a forest of peaks.

Figure 8, like Figure 7, shows another failed cyclization, this time using Palladium (II) Chloride as the metal mediator and Copper (II) Chloride as an oxidizing agent. This NMR was also very "dirty", due to disintegration of compounds in the product. Again, the peaks at 7.0 ppm and 7.5 ppm have been diminished. The peak at 3.3 ppm is still distinguishable but the peak at 4.7 ppm has been reduced to a forest.

3. Discussion

In regards to the cyclization using Lead (II) Acetate as the metal mediator, the NMR scan showed promising results. These results partially support the hypothesis as the 3,5-disubstituted Δ^2-isoxazoline was successfully synthesized. However, the yields were much lower than what was expected. The identifiable peaks of 3,5-disubstituted Δ^2-isoxazoline were all present although some were harder to distinguish due to the fact that the product was not particularly clean. However, as Lead (II) Acetate is a novel approach to the synthesis of 3,5-disubstituted Δ^2-isoxazoline, the final result was very acceptable. Similarly, the ½ equivalent Nickel (II) Chloride also contained small percentages of the 3,5-disubstituted Δ^2-isoxazoline. The profile of it's NMR scan was relatively similar to that of Lead (II) Acetate. The last cyclization that provided acceptable results was the full equivalence Nickel (II) Chloride. Again, this was much like the previous cyclizations aside from the cleaner overall product and slightly lower yields. These three cyclizations act as a proof-of-concept for future research as they show that they are all feasible pathways to synthesize 3,5-disubstituted Δ^2-isoxazoline. In addition, they provide a dramatically cheaper way to complete this synthesis when compared to the high price of palladium. To further optimize the yields of these three cyclizations, it would be necessary that new ratios of metal mediator to oxime be experimented with. It may also require more work in the laboratory in order to gain familiarity with lab equipment and techniques. This could reduce variables from this experiment, therefore leading to a cleaner product with higher yields.

There are many noteworthy concepts when observing the failed cyclizations of 5% Nickel (II) Chloride combined with 300% Copper (II) Chloride and 5% Palladium (II) Chloride combined with 300% Copper (II) Chloride. In both of these cyclizations, both compounds seemed to experience a dramatic breakdown of structure. These did not support our hypothesis as they both produced no trace of 3,5-disubstituted Δ^2-isoxazoline. One explanation as to why this

occurred can be attributed to the 300% Copper (II) Chloride that was combined with the metal mediators. The Copper (II) Chloride was meant to act as an oxidizing agent which would continuously re-oxidize the Palladium (II) Chloride, thereby allowing the reaction to continue for a longer duration. If Copper (II) Chloride was not introduced into the solutions, the reaction would have run out in a matter of minutes due to the large quantity of oxime and the comparatively miniscule amount of metal. However, it seems as if the Copper (II) Chloride may have been introduced in far too large of a quantity, causing the cyclization to overreact and thereby causing the breakdown of the targeted compound. It is highly possible that the 3,5-disubstituted Δ^2-isoxazoline may have been created at some point in these cyclizations but was broken apart as it overreacted. In future experimentation, it would be beneficial to attempt these failed cyclizations with either less Copper (II) Chloride or none of it at all. This is based on the observation that the three cyclizations that succeeded in producing any amount of 3,5-disubstituted Δ^2-isoxazoline did not rely on Copper (II) Chloride. Another point of interest in future research would be to simply let the reactions with Copper (II) Chloride to react for shorter periods of time such as 12 hours compared to the 24 hours that entailed these cyclizations. The third way that these cyclizations could be improved would be to increase the amount of metal mediator (Nickel (II) Chloride or Palladium (II) Chloride) in the reactions and decrease the amount of oxime. This would lengthen the reaction significantly without Copper (II) Chloride being necessary. Obviously, the ratios and length of reactions would need to be perfected to gain yields from these cyclizations. These cyclizations may still prove to be productive pathways if further research can be conducted.

The most promising of all collected results was the cyclization using Lead (II) Acetate.

From the research and results, it is believed that the synthesis methods practiced in these experiments have feasibility in the pharmaceutical industry. The 3,5-disubstituted Δ^2-isoxazoline holds exceptional potential to be used in pharmaceutical drugs such as glucose-derived spiro-isoxazolines and in ISO-1. The isoxazole ring that is present in the synthesized 3,5-disubstituted Δ^2-isoxazoline would be most essential to the production of the aforementioned medications. The methods in this research would also allow for the cheaper production and widespread use of spiro-isoxazolines and ISO-1.

References

CDC. "CDC Newsroom." *Centers for Disease Control and Prevention*, Centers for Disease Control and Prevention, 18 July 2017, www.cdc.gov/media/releases/2017/p0718-diabetes-report.html.

Das, Prasanta, and Ashton T. Hamme. "Zinc Mediated Direct Transformation of Propargyl N-Hydroxylamines to α,β-Unsaturated Ketones and Mechanistic Insight." *Advances in Pediatrics.*, U.S. National Library of Medicine, 22 Mar. 2017, www.ncbi.nlm.nih.gov/pmc/articles/PMC5612435/.

"Metformin (Oral Route) Side Effects" , 2017." *Mayo Clinic*, Mayo Foundation for Medical Education and Research, 1 Mar. 2017, www.mayoclinic.org/drugs-supplements/metformin-oral-route/side-effects/drg-20067074.

Mikesell, Joshua, and Michael D. Mosher. "Palladium(0)-Mediated Cyclization-Coupling of β,γ-Unsaturated Oximes and Aryl Iodides." *Tetrahedron Letters*, vol. 57, no. 9, 2016, pp. 1011–1013., doi:10.1016/j.tetlet.2016.01.076.

Misbin, Robert I. "The Phantom of Lactic Acidosis Due to Metformin in Patients With Diabetes." *Diabetes Care*, American Diabetes Association, 1 July 2004, care.diabetesjournals.org/content/27/7/1791.

Mosher, Michael D, and Amber L Norman. "Enantioselectivity in the Synthesis of 3,5-Disubstituted Δ2-Isoxazolines." ScienceDirect, Elsevier, 24 Apr. 2008, www.sciencedirect.com/science/article/pii/S0040403908007983?via=ihub.

Goyard, David. "Glucose-Derived Spiro-Isoxazolines Are Anti-Hyperglycemic Agents against Type 2 Diabetes through Glycogen Phosphorylase Inhibition." *European Journal of Medicinal Chemistry*, Elsevier, 10 Dec. 2015, www.sciencedirect.com/science/article/pii/S0223523415303901.

James. "Reactions of Grignard Reagents." *Master Organic Chemistry RSS*, 2018, www.masterorganicchemistry.com/2015/12/10/reactions-of-grignard-reagents/.

Palladium(0)-mediated cyclization-coupling of β,γ-unsaturated oximes and aryl iodides [online image]. Retrieved July 1, 2018 from https://www.sciencedirect.com/science/article/pii/S0040403916300740

Sánchez-Zamora, Yuriko Itzel et al. "Altered Macrophage and Dendritic Cell Response In *Mif*−/− Mice Reveals a Role of Mif for Inflammatory-Th1 Response in Type 1 Diabetes." *Journal of Diabetes Research* 2016 (2016): 7053963. *PMC*. Web. 18 July 2018.

Structure of 3,5-disubstituted Δ^2-isoxazoline [online image]. Retrieved July 1, 2018 from https://pubchem.ncbi.nlm.nih.gov/compound/Isoxazoline#section=2D-Structure

Santos-Longhurst, Adrienne. "Type 2 Diabetes Statistics and Facts." *Healthline*, Healthline Media, 2017, www.healthline.com/health/type-2-diabetes/statistics.