Stereoselective Synthesis of an Omuralide Analogue Through a Lewis Acid Catalyzed Reaction of an Aldehyde and an Oxazole

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Stereoselective Synthesis of an Omuralide Analogue Through a Lewis Acid
Catalyzed Reaction of an Aldehyde and an Oxazole

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A Thesis Submitted in Partial Fulfillment of the Requirement for the Bachelor of Arts Degree with Honors in Chemistry at Wellesley College
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There are a lot of thank yous to be said, for my four years at Wellesley and two and a half years of working in the Haines lab. First, thank you to Elizabeth Regan, whom I first shadowed when deciding whether I wanted to do organic chemistry research, and whose work ethic and research have been a major inspiration in my own thesis research. Thank you to all the other members of the Haines lab, past and present. Thank you to the Ott Research group at T.U. Braunschweig, for letting me explore another aspect of organic chemistry research and making me feel welcome in Germany. Thank you to Wellesley Cross Country and Track and Field, for being my family away from home and for all the long runs that keep me sane. Thank you to my non-runner friends for sticking around when I disappear under mounds of schoolwork, and for pulling me out of it from time to time. Thank you to my family for being the best cheering squad in existence. Thank you to my thesis committee, Professor Verschoor, who has the most amazing cookies and life advice; Professor Moyer, who has great comments and hard-hitting questions; and Professor Menkiti, who inspires me to be an ethical person and hopefully one day a good doctor. A huge thank you to Flick Coleman, the ‘Gaussian guru’, for showing me some of the complexities of computational chemistry. Finally, a huge thank you to Professor Haines, who has been the most amazing mentor, as he encouraged me to go abroad and to try new things at Wellesley. I never expected to be a chemistry major, but with his support I have grown to love the subject, and am a better student, scientist, researcher, and person for it.
Abstract

Lactacystin, or more specifically, its biologically active form, omuralide, is a known inhibitor of cell cycle progression in a number of cell lines. However, omuralide is also an inhibitor of the proteasome, an essential protease within eukaryotic cells. Because of the complexity of the available syntheses, development of selective analogs of omuralide has progressed slowly. Our research seeks to develop efficient and highly stereoselective syntheses of omuralide analogs via the metal catalyzed reaction of a chiral aldehyde with a protected oxazole. This catalysis has been accomplished with high stereoselectivity using a simple aluminum catalyst but the efficiency of this reaction has not been high. Results from a variety of additional catalysts will be discussed.

Biological Introduction

Chlamydia trachomatis is the cause of the most prevalent sexually transmitted disease in the United States: Chlamydia. This obligate intracellular bacteria can infect both the urogenital tract and ocular epithelia.\(^1\) For females with urogenital tract infections, this can lead to a host of problems, including pelvic inflammatory diseases, ectopic pregnancy, and infertility. Blindness often results from cases of ocular infection left untreated. This is unfortunately still a frequent occurrence, particularly in impoverished areas of the world. Chlamydia is currently the leading cause of preventable blindness worldwide.\(^2\)
The pathogenic mechanism of *C. trachomatis* infection is still debated. One explanation of the mechanism is that the infectious form of Chlamydia, called elementary bodies, attach and enter epithelial cells through the cellular membrane. Once inside, they turn into replicative reticulate bodies, which create and reside in parasitic vacuoles inside the cell. Reticulate bodies change back to elementary bodies and go on to infect other cells once they have released the necessary enzymes and proteins to control host cellular processes. The number of elementary bodies produced is at a maximum two days post infection, however, many infections are asymptomatic for long periods of time past the two-day point.\(^3\) This, combined with the fact that one infected cell can produce several hundred new bacteria, are likely the reasons the disease is so persistent. It remains unclear how exactly chlamydial organisms invade the epithelial cells they infect or how these organisms communicate with their host cells.\(^7\)

The Chlamydia bacteria secrete chlamydial protease/proteasome-like activity factor (CPAF), into eukaryotic host cells (fig. 1).\(^9\) Proteases are the major enzymes of protein degradation in the cytosol and nucleus, and CPAF performs this function. It is, however, sometimes classified as a “proteasome-like activity factor” instead of a protease because its form and mechanism are different from many other proteases. Once in the host cells, CPAF degrades several host transcription factors and proteins responsible for a range of cellular functions, including inflammation and DNA damage control.\(^10\) Introducing CPAF to uninfected human cells replicates many of the symptoms of the disease, implicating it as a major factor in the generation of infection symptoms.\(^12\)
**Figure 1:** Proposed mechanism of CPAF function. If CPAF is inhibited with an anti-CPAF peptide, as shown on the right of this figure, the parasitic vacuole/inclusion becomes unstable and breaks down (Jorgensen et al., 2011).8

CPAF is a serine protease, one of a very common class of peptide cleaving molecules that use the amino acid serine as part of a catalytic triad to catalyze the breakage of the N-C bond in proteins (fig. 2). CPAF displays resistance to the often-used serine protease inhibitors N-p-Tosyl-L-phenylalanine chloromethyl ketone (TPCK) and phenylmethanesulfonyl fluoride (PMSF), however. This is likely because these drugs have large hydrophilic moieties unlike lactacystin, a molecule that displays potent anti-CPAF properties and has large hydrophobic portions. Because of its potency as an inhibitor of CPAF, it has been used to elucidate its X-ray crystallography structure (fig. 3).13
Figure 2: Catalytic properties of CPAF. A. The figure on the top left shows a crystal structure model of CPAF. Structural information such as this helps to confirm that CPAF is a Serine protease, as S499 is in the center of the catalytic site. Two CPAF molecules (in pink and blue) form an asymmetric unit. B. The catalytic triad of CPAF: S499, H105, and E558 (Adapted from Huang et. al., 2008). C. The bottom figure shows the mechanism of another serine protease, chymotrypsin, which is believed to be very similar to CPAF’s mechanism, with a catalytic triad of amino acids at its core (Blow et. al., 1969).
Individuals do not develop immunity to chlamydia once the infection is cleared. A person could easily be affected multiple times. Antimicrobial resistance is a growing problem, for Chlamydia as well as a host of other microbial diseases. Although viable antibiotics against Chlamydia already exist, it is still necessary to synthesize novel antibiotics in case traditional methods lose their efficacy. While there are few cases of antibiotic-resistant chlamydia so far, chlamydia easily develops antibiotic resistance in in-vitro studies. Already, HeLa cells infected with Chlamydia show no decrease in viability of the Chlamydia bacteria when treated with traditional antibiotics. The underlying mechanisms for this resistance are still unclear. Other sexually transmitted diseases have already developed antibiotic resistance. For example, Gonorrhea has such widespread immunity to the traditional treatment fluoroquinolone that a newer drug, cephalosporin is now preferentially prescribed. It is important to find
other options for treatment. One molecule that has been the focus of a substantial amount of research is lactacystin (fig. 4).

![Diagram of (+)-Lactacystin and Omuralide](image)

**Figure 4**: (+)-Lactacystin and omuralide

Much research has been done to discover a way of chemically inhibiting bacterial CPAF. Probable active sites of CPAF have been identified, and lactacystin, or more specifically, its active form, the β-lactone-γ-lactam omuralide, has shown much promise. Lactacystin is a natural microbial product that was isolated in 1991. Further studies showed that lactacystin inhibits the 20S proteasome in mammalian and bacterial cells (fig. 5). Lactacystin is converted to omuralide spontaneously in the extracellular medium. While lactacystin is incapable of crossing the cell membrane, omuralide can enter the cell unaided and inhibit the proteasome.
Figure 5: This is a topological model of the 20S proteasome in yeast. The 20S proteasome has been found to have similar structures in yeast, bacteria, and mammalian cells. This figure shows the surface structure of the 28 subunits of this proteasome. The proteasome has two entry points, through which substrates can enter and the product can be released (Figure from Groll et al. 1997).

Omuralide shows promise in the treatment of several diseases other than chlamydia. Recent research has shown that targeting the proteasome in cancer cells with molecules such as omuralide leads to apoptosis of the cancerous cells. Omuralide is now being proposed as a treatment for cancer, malaria, and a host of other diseases, as well as chlamydia, which are controlled by inhibition of the 20S proteasome.\(^9\)

While omuralide has been proposed as an antibiotic, it also affects the host cell proteasome, so synthesis of a similar molecule that does not affect the host cell but still inhibits bacterial CPAF is required. One proposed molecule is a phenyl-omuralide analogue in which the isopropyl functional group is replaced by a benzene ring. It has been shown that this analogue does not inhibit the host cell proteasome, most prominently in a paper by Corey and Li, in which they tested over twenty analogues of
omuralide, and showed that only two, the phenyl analogue and a ketone analogue, showed no inhibition of the bovine 20S proteasome. The effect of these analogues on CPAF is still unknown as the amount synthesized was not sufficient for further biological testing.\textsuperscript{10} An important goal is therefore to test the efficacy of these compounds in inhibiting the chlamydial protease. The Haines lab is working to synthesize an adequate amount of this analogue to evaluate its effectiveness in cellular and animal models of chlamydial infection.

**Experimental Goals and Purpose**

The synthesis of omuralide has been undertaken by several research groups. The Haines lab has been working on the synthesis of a phenyl-omuralide analogue with four specific chiral centers (fig. 6). This synthesis is modified from the procedure published by Soucy\textsuperscript{11} and is three steps shorter than the initial synthesis by E.J. Corey.\textsuperscript{10,13,14} This synthetic scheme is particularly useful because it allows for substitution of various functional groups for further biological testing.

![Phenyl analogue of omuralide](image)

**Figure 6**: Phenyl analogue of omuralide.
This paper is primarily concerned with improving the reaction between 5-methoxy-2-pmethoxyphenyloxazole with N, N-Diethyl-2-(R)-methyl-3-oxopropionamide in the presence of a Lewis acid catalyst (figure 6). Once the oxazole (compound 3 of fig. 8) and the aldehyde (compound 6 of fig. 7) intermediates are created, they are joined through a diastereoselective aldol reaction, setting up the required chirality of the chiral carbons for the rest of the synthesis. Both the requisite aldehyde, N, N-Diethyl-2-(R)-methyl-3-oxopropionamide, and oxazole, 5-methoxy-2-methoxyphenyloxazole, were created, and their synthesis is described in the following sections.

**Figure 7:** Synthesis of N,N-Diethyl-2-(R)-methyl-3-oxopropionamide.

The aldehyde (compound 6 of figure 7) is noteworthy in its one chiral center. We see that nucleophilic attack is most likely to occur at the terminal carbonyl that has the least steric hindrance. We can also predict the direction from which the attack is most
likely to happen, from the front if the methyl substituent is pointing back, also due to steric effects.

![Figure 8: Synthesis of 5-methoxy-2-methoxyphenyloxazole.](image)

From examining the resonance in 5-methoxy-2-methoxyphenyloxazole, it can be seen that the secondary carbon adjacent to the nitrogen has the most nucleophilicity due to the electron-donating methoxy groups on each end of the oxazole. This is the carbon that acts as the nucleophile in the complexation reaction between the oxazole and aldehyde (fig. 9).

![Figure 9: Electron pushing diagram of 5-methoxy-2-methoxyphenyloxazole.](image)
In another synthesis published by Francois Soucy’s research group, the trivalent Lewis acid dimethyl aluminum chloride was used to catalyze the reaction between the aldehyde and a deprotonated oxazoline. This Lewis acid complexes to the aliphatic aldehyde and oxazoline, holding them in place and giving a new complex with specific chirality. This is an example of controlling stereochemistry, but not the activation needed since a deprotonated nucleophile is already sufficiently activated by deprotonation by a strong base. Dr. David Evans’ research group also used an aluminum-based catalyst (shown below in fig. 10) to add benzaldehyde to the oxazole, but this reactivity was restricted to aromatic aldehydes.\(^{15}\)

![Figure 10: The complex binaphthyl aluminum catalyst, \((R)-2,2'-\text{bis}(3,5-\text{Di-terr}-\text{butyl}-2\text{-hydroxylbenzylideneamino})-1,1'-\text{binaphthyl aluminum chloride synthesized by Evans et al.}\(^{15}\) The red line represents a covalent bond between the two carbons it connects. The catalyst is activated by loss of the chloride upon addition of AgSbF\(_6\).]

The Haines lab has also had some success with the dimethyl aluminum chloride catalyst in a reaction between 5-methoxy-2-methoxyphenyloxazole and N, N-Diethyl-2-(R)-methyl-3-oxopropionamide. The yield of this reaction was only twenty percent, however. This reaction combines the work of Soucy and Evans in a new reaction where the aldehyde does not have to be aromatic. When this reaction was done previously, only
one stereoisomer was isolated, and we can assume it was the desired stereoisomer since the attack by the oxazole is favored from the less sterically hindered direction.

Dimethyl aluminum chloride is unfortunately particularly flammable when in the presence of oxygen, and even when working in a nitrogen environment, it often ignites in the syringe transition from the storage container (which is under nitrogen) to the reaction vessel (which is also under nitrogen). It also produces product in low yield in part due to the methyl’s ability to act as a nucleophile instead of the oxazole. It is important therefore to find a more stable and less nucleophilic catalyst that is still capable of complexation to both carbonyls of our aldehyde. Such a catalyst makes the carbon of the aldehyde that bonds to the oxazole more electrophilic. This catalyst should also move the two carbonyls on the aldehyde into the correct spatial parameters for addition to the oxazole.

This thesis contains some of the attempts to find another Lewis acid catalyst that will activate aldehydes with no benzyl substituent. From previous studies conducted by the Haines lab and other groups, we have narrowed our approach to Lewis acid catalysts containing three metals: aluminum, zinc, and copper. This paper describes experiments with several simple catalysts. Those tested so far are zinc chloride, dimethyl aluminum chloride, and diethyl aluminum chloride.

Finally, the molecules of interest (shown in fig. 11) were created with Gaussview, and several semi-empirical and Hartree-Fock calculations were run on the complexation reactions. The goal was to accurately model the effect of the dimethyl aluminum
chloride catalyst and to elucidate which enantiomer is formed during the complexation reaction. The results for these calculations are presented in this paper as well.

![Molecular models of 5-methoxy-2-pmethoxyphenyloxazole (left) and N, N-Diethyl-2-(R)-methyl-3-oxopropionamide (right) created in Gaussview.](image)

**Figure 11:** Molecular models of 5-methoxy-2-pmethoxyphenyloxazole (left) and N, N-Diethyl-2-(R)-methyl-3-oxopropionamide (right) created in Gaussview.

**Results and Discussion**

*Total synthesis of aldehyde, N,N-Diethyl-2-(R)-methyl-3-oxopropionamide*

First, methyl 2(R)-3-hydroxy-2-methylpropionate was reacted with benzyl 2,2,2-trichloroacetimidate in a nucleophilic substitution reaction. Triflic acid, $\text{CF}_3\text{SO}_3\text{H}$, is a very strong acid that protonates the nitrogen on the methylpropionate, making it a better leaving group, promoting attack at the benzylic carbon and the leaving of the entire amide functional group (see Fig. 12).
**Figure 12:** Mechanism of N,N-Diethyl-2-(R)-methyl-3-oxopropionamide formation.

In the next step of the synthesis, lithium hydroxide is used as a strong nucleophile. The hydroxyl attacks the carbon at the C=O double bond in a base catalyzed ester hydrolysis (fig. 13).

**Figure 13:** Mechanism for the synthesis of (2R)-3-Benzzyloxy-2-methylpropionic acid.

According to the original synthesis, the next step uses the peptide coupling reagent N,N,N',N'-Tetramethyl-O-(benzotriazol-1-yl)uronium tetrafluoroborate (TBTU) and diisopropylethylamine, but this synthesis was modified to use hydroxybenzotriazole (HOBT) and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), which used in conjunction, activate the carboxyl and promote the formation of amide bonds. EDC acts as the first electrophile/leaving group, and then HOBT replaces the EDC since it is a better electrophile than EDC. The basic diethyl amine is in excess, so it deprotonates the carboxylic acid, but will only add once there is a good leaving group (HOBT not OH) (Fig. 14).
Next, a catalytic hydrogenation reaction was performed. In the presence of a metal catalyst (in this case Pd(OH)$_2$ on carbon), the hydrogen-hydrogen bond breaks and each hydrogen attaches to the surface of the metal catalyst. The aromatic benzene is also drawn to the surface of the metal catalyst, and hydrogens are transferred from the metal surface to the oxygen and benzylic carbon, breaking the bond between them. The benzylic bond is the weakest, so even if the hydrogen complexes to the pi bonds in the ring, they do not break as easily, and the benzylic bond is ultimately broken (fig. 15).

**Figure 14:** Mechanism for the synthesis of 3-Benzylxoy-N,N-diethyl-2-(R)-methylpropionamide.
In the final step of the synthesis, Dess-Martin periodinane, a highly sensitive and expensive catalyst that selectively oxidizes alcohols to aldehydes or ketones, and minimizes the possibility of further oxidation to carboxylic acids, is used. This creates the final product, N,N-Diethyl-2-(R)-methyl-3-oxopropionamide. The hydroxyl oxygen attacks the iodine (either in an SN1 or SN2 type mechanism), pushing off an acetate leaving group. Then, through internal attack by the carbonyl oxygen and subsequent rearrangement, a second acetate group leaves and the aldehyde is formed (fig. 16).
Figure 16: Mechanism of the Dess-Martin Oxidation reaction.

Synthesis of 5-methoxy-2-methoxyphenlyoxazole.

In the first step of this synthesis, glycine methyl ester hydrochloride was reacted with methoxybenzoyl chloride in the presence of the basic triethyl amine. This resulted in a simple substitution reaction, with the nucleophilic nitrogen attacking at the carbonyl carbon (Figure 17).

Figure 17: Mechanism of formation of N-4-methoxybenzoylglycine methyl ester.

Phosphorus pentoxide, which is a dehydrating agent, bonds to the most negative oxygen of the amide carbonyl, making the oxygen a good leaving group, and then through elimination, the oxygen carbon bond forms and the oxygen-phosphorous compound leaves, completing the favorable five-membered ring and forming 5-methoxy-2-methoxyphenlyoxazole (fig. 18).
Figure 18: Mechanism of ring closure reaction in the formation of 5-methoxy-2-p-methoxyphenyloxazole.

Reaction of 5-methoxy-2-pmethoxyphenyloxazole with N, N-diethyl-2-(R)-methyl-3-oxopropionamide in the presence of a zinc chloride catalyst.

Two attempts at the zinc chloride catalyzed reaction of 5-methoxy-2-p-methoxyphenyloxazole with N, N-diethyl-2-(R)-methyl-3-oxopropionamide were made. If the reaction proceeds as planned, the zinc of zinc chloride will attack the carbonyl of the aldehyde and amide, holding them in place and withdrawing electron density. The carbon is then susceptible to nucleophilic attack by the carbon on the oxazole that is most negative because of resonance. The oxazole attacks at the less sterically hindered carbonyl carbon. In the first attempt, the predominant product was identified as a hydrolyzed byproduct.
In the first attempt at this reaction, the predominant product was identified as a hydrolyzed byproduct. Once water was added, the zinc chloride could act as a Lewis acid, and the zinc could complex to nitrogen. Then, the hydrogen adds to the nitrogen and the hydroxyl group adds to the carbon. The potential hydrolyzed product is shown in the figure below (fig. 19).

![Figure 19: Proposed by-product of oxazole synthesis due to presence of water.]

When the reaction was run the second time, proton NMR was taken of the impure mixture, and key new peaks in the range of 5-6 ppm were observed. This is good primary evidence of some complexation reaction occurring (Fig. 20). However, the separation of the reaction mixture was not a success, and no products were isolated.

![Figure 20: Complexation reaction as catalyzed by zinc chloride.](https://via.placeholder.com/150)
Reaction of 5-methoxy-2-pmethoxyphenyloxazole with N, N-Diethyl-2-(R)-methyl-3-oxopropionamide in the presence of a diethyl aluminum chloride catalyst

In this reaction, diethyl aluminum chloride, which acts as a Lewis acid, will attack the carbonyl of the aldehyde and the carbonyl of the amide, holding them in place and withdrawing electron density. This makes the carbon susceptible to nucleophilic attack by the carbon on the oxazole that is most negative because of resonance. The oxazole is nucleophilic because of the electron donating methoxy groups, and it attacks at the less sterically hindered carbonyl carbon. An NMR peak indicative of an aldehyde/oxazole product in low yield was isolated (appendix 11), as well as a peak indicative of an aldehyde/hydrolyzed oxazole product (appendix 12).

![Figure 21: Complexation reaction as catalyzed by dimethyl aluminum chloride.](image)

Use of the ketone N,N-Diethylacetoacetamide as an aldehyde substitute:

Because the yield of the aldehyde synthesis reaction was so low, the dimethyl aluminum chloride reaction was also attempted with the commercially available N,N-Diethylacetoacetamide (Fig. 22) instead of the original aldehyde. While the central carbon is no longer chiral, and the carbonyl at which an attack would occur is more sterically hindered, it is still a good primary test of whether activation of the oxygens happens with a catalyst and complexation occurs between the two reactants. The reaction
was attempted with a dimethylaluminum chloride catalyst because it had already been proven effective with the original reactants. Although peaks with oxazole were separated (Appendix 9 and 10), the purification was likely incomplete, as no peaks containing an oxazole-ketone complex or any ketone were isolated. This compound warrants further exploration, as, although it is an imperfect match, it could be an inexpensive model compound to substitute for a very difficult compound to synthesize.

**Figure 22:** Structure of N,N-Diethylacetoacetamide.

*Gaussian calculations:*

We wished to model the complexation reaction of dimethyl aluminum to aldehyde and the attack from the oxazole to the aldehyde to have further evidence of which stereochemistry is favored in the final product. Using two models in Gaussian, semi-empirical and Hartree-Fock, attempts to quantify these reactions were made. In the first simulation (fig. 23), the dimethyl aluminum chloride catalyst can be seen complexing to the two oxygens of the aldehyde, twisting the conformation of the carbons to bring the oxygens from a staggered orientation to an orientation in which the two oxygens are in line with each other. This supports the claim that the dimethyl aluminum chloride catalyst affects the orientation of the oxygens. An optimization was run twice, first with semi-empirical parameters, which are quicker but make more assumptions, and then with
Hartree-Fock parameters, which take hours but are more accurate. Post-optimization, it could be seen that the oxygens lie parallel to each other. Additionally, before the addition of a catalyst, the O=C-C bond angle (indicated with an orange arrow in figure 23) was 121.107°, and after the addition of the catalyst it was 126.737°.

Figure 23: Semi-empirical optimization of catalytic activation using Gaussview. N, N-Diethyl-2-(R)-methyl-3-oxopropionamide is shown complexed with the dimethyl aluminum cation. The left-hand image is from before the complexation to the catalyst. The right hand image is complexation. Both molecules were optimized using semi-empirical methods.

When it was attempted to see whether dimethyl aluminum cation catalyst remained complexed to the aldehyde after the attack by the oxazole, the compounds were so large that Gaussian kept delivering error reports instead of final calculations. Further attempts should be made to quantify this reaction.

Future Research Goals:

The aldehyde used in this reaction has a short lifespan. The compound must be stringently maintained free of oxygen and water, as a problem in the past has been hydrolyzed byproducts. As such, the synthesis must be repeated frequently. It would be
useful to continue testing Lewis acid catalysts in the reaction between the aldehyde and the oxazole, but first, more aldehyde must be synthesized.

Further tests should be run on diethyl aluminum chloride to confirm initial results as well. We could modify the experimental procedure by using pure diethyl aluminum chloride instead of the diethyl aluminum chloride in hexane solution that was used for this reaction. Although the pure diethyl aluminum chloride compound still poses the flammability problem, it would be useful to try to see whether the solvent affects the reaction. If an attempt with pure diethyl aluminum chloride promotes higher yield, that would confirm our hypothesis that diethyl aluminum chloride acts as a weaker nucleophile.

Copper II catalysts are also potentially useful. A study by Evans et al. showed that Cu^{II}-bis (oxazoline) complexes show similar efficacy rates as the complex aluminum catalyst discussed above in similar reactions between oxazoles and aldehydes, to stereoselectivity of up to 95%. Such catalysts display the same bidentate complexation the reaction studied in this paper requires. The same paper also confirms the use of toluene as a solvent, as poorly coordinating solvents promote the highest rate of catalyst turnover.23

Once the optimal catalyst is selected, six more steps remain in the total synthesis. At this point, (molecule 2 of fig. 13), there are three chiral centers, two of which have the final desired chirality. The third has the right stereochemistry as well, but is directly controlled later in the synthesis. The omuralide analogue has four total desired chiral centers, the remaining two controlled by the two chiral centers that are already set.
**Conclusion**

With the conclusion of this work, we have seen that all attempts to react the aldehyde and oxazole in the presence of zinc chloride have so far been unsuccessful, and it should likely be abandoned as a potential catalyst. Diethyl aluminum chloride, however, still bears promise, and further tests should be conducted with this catalyst.
Further tests also need to be run on our ketone substitute, as it still has promise as an inexpensive model for the aldehyde. Hopefully we can test copper complexes at some point as well. Six more steps need to be achieved for total synthesis.

**Experimental**

All reagents were acquired from Sigma-Aldrich and used without further purification. All glassware and stir bars were dried overnight in an oven. Mass spectra were measured with an Agilent 6890N Network GC System coupled with an electron impact Agilent 5937 Network Mass Selective Detector. The method "RAYANNE" \( T_{\text{initial}}=325^\circ \text{C}, \text{rate}=20^\circ \text{C/sec}; \text{split \ mode \ (inlet)}; \text{mobile \ phase} = \text{He(g)}; \text{constant \ flow \ mode}, \text{flow \ rate} \ 1.0 \ \text{mL/min}; \text{injection \ volume} \ 1.0 \ \mu \text{L}, \text{pressure} \ 11.6 \ \text{psi} \) was used for GC/MS analysis. \(^1\text{H}\text{NMR and} \ ^{13}\text{C}\text{NMR spectra were recorded using Bruker} \ 300 \ \text{MHz WIN-NMR spectrometer with TMS as a standard. Flash chromatography was performed using Biotage gradient flash purification and gravity columns were prepared using silica gel from Sigma Aldrich, 70-230 mesh, 60 Å, with the slurry method of column preparation. Catalytic hydrogenation was performed with Parr Instrument Company Reaction Apparatus.}

**Preparation of Methyl 2(R)-3-benzyl-2-methylpropionate.**
Methyl (2R)-3-hydroxy-2-methylpropionate (5.16 g, 43.8 mmol) was dissolved in 100 mL of cyclohexane and 50 mL of dichloromethane. Benzyl 2,2,2-trichloroacetimidate (13.54g, 53.7 mmol) was added to the reaction mixture, followed by adding triflic acid (0.4 mL, 4.6 mmol) slowly to the reaction mixture. The reaction was stirred at room temperature for 24 hours. The suspension was then vacuum filtered and the filtrant washed well with dichloromethane. The filtrant was then added to the original filtrate. Solvents were washed with water (2x100mL), saturated sodium hydrogen carbonate (2x50 mL), and brine (2x25mL). The organic fraction was separated with a separatory funnel and placed in an Erlenmeyer flask, then dried over magnesium sulfate. The solution was filtered to remove MgSO₄, and solvent was evaporated. GC/MS of unpurified reaction showed a peak with m/z of 208 at a retention time of 7.759 minutes, similar to previous experiments (Appendix 1).

To isolate the desired product, a silica gravity column was run. All products were dissolved in 5 mL of hexanes and added to the column, and the glassware was washed two more times with 5mL of hexanes. The column was run at 0-15% EtOH in hexanes, 200 mL of each concentration, increasing 1% at a time. Column fractions of 125 mL
were drawn for a total of 23 fractions. The purified compound was 90% pure as determined by GC/MS (Appendix 2). The remaining solvent was evaporated using a rotovap, yielding 6.315 g of slightly yellow liquid (percent yield: 57%).

**Preparation of 2(R)-3-Benzyl oxy-2-methylpropionic acid**

Methyl (2R)-3-benzyl-2-methylpropionate (6.34 g, 44.94 mmol) was added to 100 mL of a 1:1 mixture of THF and water, and the mixture was cooled on ice. To this cold mixture, LiOH (1.096 g, 45.7 mmol) was added in small portions over 10 minutes, and the reaction was stirred at 4°C. After 24 hours left stirring at 4°C, NaHCO₃ (3.769 g, 44.9 mmol) and water (16mL) were added and most of the THF was removed under vacuum. The aqueous solution was washed with dichloromethane (4x20 mL) and acidified to pH 1 with 6 M HCl. The pH was tested with pH paper, and a yellow organic layer quickly formed. The acidified suspension was extracted with dichloromethane (3x40 mL). The organic fraction was dried over sodium sulfate, and the solvent was evaporated under vacuum to give 2.1 g of a nearly pure product, which was a slightly yellow oil (2.1g, 10.8 mmol, 24% yield). ¹H NMR (appendix 3) (CDCl₃) • 9.95 (1H, OH) 7.5 (s, 5H, Ph), 4.6 (s, 2H, benzylic), 3.8 (d, 2H, propionyl CH₂), 2.9 (s, 1H, propionyl CH), 1.2 (s, 3H, propionyl CH₃).
Preparation of 3-Benzyloxy-N,N-diethyl-2-(R)-methyl propionamide

3-Benzyloxy-N,N-diethyl-2-(R)-methyl propionamide (2.1 g, 10.8 mmol) was dissolved in low water CH$_3$CN (12.57 mL), and the solution was cooled on ice for 15 minutes. Diethylamine (1.32 mL, 2.30 g, 31.4 mmol), Hydroxybenzotriazole (HOBT, 1.67 g, 10.9 mmol) and Ethyl-3-(3-dimethyaminopropyl)carbodiimide (EDC, 1.70 g, 10.9 mmol) were added to the solution. The reaction was stirred on ice for two hours. Ether (14.2 mL) and water (57 mL) were added to the reaction and ether was collected. The aqueous layer was extracted with additional ether (2x14.2 mL). The combined organic layers were washed with 5% HCl (2x5 mL) and then saturated NaHCO$_3$ (10 mL). The organic layer was dried with Na$_2$SO$_4$, and the solvent was evaporated, leaving 5.05 g of product as a deep yellow oil.

To purify the resulting mixture, a 125 mL gravity column in a 1:1 mixture of EtOAc and hexanes was run. Fractions of 25 mL were collected for 375 mL total. After separation, 0.45 g of 3-Benzyloxy-N,N-diethyl-2-(R)-methyl propionamide remained, as a very light yellow oil (1.8 mmol, percent yield, 10%). $^1$H NMR (appendix 4) (CDCl$_3$), 7.3 (5H, Ph), 4.5 (m, 2H, benzyl CH$_2$), 4.3 (d, 2H, propionyl CH$_2$), 4.0 (s, 1H, propionyl CH), 3.0-3.3 (multiplet, 4H, amide CH$_2$a and CH$_2$b), 1.1 (9H, multiplet, CH$_3$-s).
Preparation of N,N-Diethyl-3-hydroxy-2(R)-methyl propionamide.

3-Benzyloxy-N,N-Diethyl-2-(R)-methyl propionamide (4.85g, 19.4mmol) was dissolved in 33 mL of CH$_3$OH. Palladium hydroxide (20% on C, 0.37g) was added to a reaction flask, and the flask was connected to the hydrogenator. The reaction was placed under H$_2$ (10 PSI). After 48 hours the reaction mixture was gravity filtered and the solvent was evaporated. $^1$H NMR (appendix 5) (CDCl$_3$) * 4.5 (s, 3H, OH hydrogen and propynyl CH$_2$), 3.5 (m, 4H, ethyl CH$_2$), 3.0 (s, methine CH), 1.3 (m, 9H, methyl CH$_3$'s).

Preparation of N,N-Diethyl-2-(R)-methyl-3-oxopropionamide.

Wet CH$_2$Cl$_2$ was prepared by stirring 50 mL of CH$_2$Cl$_2$ with 2 mL of water for 48 hours. N,N-Diethyl-3-hydroxy-2-(R)-methylpropionamide (0.41 g, 2.59 mmol) was dissolved in the mixture. Dess-Martin periodinane (1.8 g) was added slowly over 8 minutes. The reaction mixture was stirred at room temperature for 1 hour. The reaction was cooled in an ice bath and quenched by the addition of a -4°C solution of Na$_2$S$_2$O$_3$ (6.72g) and NaHCO$_3$ (1.48g) dissolved in 40 mL of water. The mixture was stirred on
ice for 10 minutes, ether (50 mL) was added, and the mixture was stirred vigorously at RT for an additional 10 minutes. The organic layer was collected and the aqueous layer was washed with 1:2 CH₂Cl₂:ether (2x20mL). Organic layers were washed with brine (2x25mL) then dried over Na₂SO₄. The solvent was evaporated and the impure aldehyde was stored as a solution in CH₂Cl₂ under nitrogen at 0°C. ¹H NMR (appendix 7) (CDCl₃)
* 9.7 (1H, aldehyde H), 3-4, (m 5H, ethyl CH₂’s and CH), 1-1.5 (15H, M, propionyl CH₃a, ethyl CH₃s).

**Preparation of 5-methoxy-2-methoxyphenyloxazole through dehydrated ring closure.**

![Diagram](https://via.placeholder.com/150)

N-4-Methoxybenzoylglycine methyl ester (34.23 g) was dissolved in 310 mL of chloroform. To get rid of water, 100 mL chloroform was evaporated off with a rotovap. Phosphorus pentoxide (95g) was added, and the reaction mixture was heated to reflux. Solids formed as the reaction progressed, inhibiting the stir bar. These solids were broken up over the course of the reaction. After 41 hours, the reaction was removed from heat and placed on ice. When the reaction mixture was sufficiently cool, it was poured into a 2.6 molar solution of sodium hydroxide (89.71g in 900mL water). This was also kept on ice.
The mixture was poured into a separatory funnel, and the aqueous and organic layers were separated. The organic layer was placed in a freezer overnight, and crystals formed. The organic layer was vacuum filtered, and the solids were collected on filter paper. GC/MS in dichloromethane showed two main peaks, one 49% of the product, with an m/z of 166 and the other 41% of the product with an m/z of 205 (appendix 1). The solvent was evaporated, and then hexanes were added to the mixture. The mixture dissolved only partially in hexanes, so another GC/MS was run of the liquid, and it was found that the suspected byproduct was much more soluble in hexanes than the suspected oxazole. The solid that did not dissolve was filtered through vacuum filtration. The solid was then washed with 20% ethyl acetate in hexanes. GC/MS showed that our product was pure and had an m/z of 205 (appendix 2).

Reaction of 5-methoxy-2-pmethoxyphenyloxazole with N, N-Diethyl-2-(R)-methyl-3-oxopropionamide in the presence of a zinc chloride catalyst.
Since there was a very limited amount of aldehyde left over from earlier experimentation, the first test was run on a very small scale. From previous experimentation, it was likely that since it had been left in a freezer for several months, it would be decarboxylated. However, 'HNMR of the product showed that our product was still an aldehyde. A 1:1 molar ratio of aldehyde to oxazole was used (0.03 g of aldehyde with 0.04 g of oxazole) in 3 mL of a 1 molar solution of zinc chloride in diethyl ether. The reaction was run under nitrogen to eliminate the presence of oxygen as much as possible. The reaction was first stirred on ice and then warmed to room temperature. After 48 hours, water was added to the solution and the solution poured into a separatory funnel. The organic layer was separated, the solvent was evaporated, and an 'HNMR was taken of the remaining solid.

The HNMR unfortunately showed many peaks indicative of oxazole as the primary compound present. Flash chromatography was used to separate the products, and the column was run from 24-80% ethyl acetate in hexanes. Four peaks were separated. The first peak was identified as recovered aldehyde, the second and largest peak was identified as oxazole (appendix 15), and the third and fourth peaks did not come off of the column until it was run at 80% ethyl acetate in hexanes for several column volumes. These third and fourth peaks were identified through HNMR as likely hydrolyzed byproducts of the oxazole, due to the water that was present during the reaction (appendix 14).

The second time this reaction was run, a 1:1 mixture of zinc chloride to aldehyde was dissolved in anhydrous toluene, and then the mixture was placed on a rotovap where a third of the solvent was evaporated. This was in an attempt to get rid of most of the
diethyl ether to get better activation of the product. The zinc chloride in the first reaction likely remained complexed to the diethyl ether instead of to the aldehyde, which is why we did not get a high product yield. The hope was that by evaporating off most of the diethyl ether, we could displace the remaining ether with aldehyde. The reaction was once again performed under a nitrogen line. Interestingly, this time there were bubbles when the zinc chloride was added to the aldehyde. The mixture was cooled on dry ice, and oxazole was added. The mixture was left to stir for 48 hours and let warm to room temperature. TLC in 40% ethyl acetate and hexanes showed four distinct spots and product left at origin. NMR of crude product (Appendix 15) showed key new peaks in the range of 5-6 ppm. HPLC was performed, using a 25-40% ethyl acetate in hexanes solution, but only two peaks were observed during the separation. It might be useful to conduct this experiment again, since the two most interesting spots were lost during column chromatography, however the control oxazole spot (TLC was run with mixture spot and pure oxazole spot) was the largest, so it is likely that most of the product remained starting material.

**Reaction of oxazole with aldehyde in the presence of a diethyl aluminum chloride catalyst.**
5-Methoxy-2-pmethoxyphenyloxazole (0.0474 g, 0.21 mmol) was dissolved in toluene (2.5 mL). N,N-Diethyl-2-(R)-methyl-3-oxopropionamide (0.1199 g, 0.21 mmol) was dissolved in toluene (2.5 mL) as well. The aldehyde solution was placed under a nitrogen line for 3 hours, in an -84°C bath of ethyl acetate (cooling first with dry ice and then with liquid nitrogen). Then approximately 1mL of diethyl aluminum chloride was added to the reaction mixture. This mixture was stirred for 10 minutes, and then the oxazole was added. Reaction agitation was stopped after 48 hours, and the reaction was refrigerated. TLC of the mixture with oxazole as a control showed an extra spot that we hypothesized was the desired product.

To separate the compounds present, purification was done using flash chromatography, at 10% ethyl acetate in hexanes for 4 column volumes (CV), 15-50% for 4CV, and then 50-75% for 4CV. Four peaks were found, and NMR were taken of each. The second peak was identified as the product of the aldehyde and oxazole complex. Although there was an excess integration at ppm of 1.3, likely due to excess ethyl, the key protons around 4-5 that were seen in previous results exist in the correct ratio with the disubstituted benzene peaks. $^1$H NMR (appendix 12) (CDCl$_3$) * 7-8 (dd,
4H, disubstituted benzene), 4-5 (s, 2H, H’s next to carbonyl), 1.3 (m, aliphatic hydrogens). The third, although the trace was broad, was likely the complex of the aldehyde and a hydrolyzed oxazole due to excess integration in the range of 3-4 pointing to an extra OH proton (appendix 13).

**Attempt at a reaction of ketone with oxazole in the presence of a dimethyl aluminum chloride catalyst.**

Six drops of the liquid N,N-Diethylacetoacetamide were added to the reaction vessel (82 mg, 0.002 mol) and dissolved in 2.5 mL toluene. The reaction was placed stirring under nitrogen for 3 hours in a -84°C bath of ethyl acetate. Six mL (0.0075 mol) of dimethyl aluminum chloride were added to the reaction mixture, let stir, and then 47.8 mg (.002 mol) of oxazole in 2.5 mL of toluene was added to the reaction mixture. The reaction was run under nitrogen gas for 38 hours while agitating.

Purification was attempted with flash column chromatography at 20-75% ethyl acetate in hexanes for 20 column volumes. Three peaks were visible, and NMR were taken of each, but while two showed peaks indicative of oxazole (appendix 10 and 11),
none was definitively the aldehyde or complexation peak. It is likely that the separation was incomplete and other peaks were lost in the cleaning run.
Appendix 1: GC/MS of unpurified reaction of the synthesis of Methyl (2R)-3-benzyl-2-methylpropionate.
Appendix 2: GC/MS of Methyl (2R)-3-benzyl-2-methylpropionate

Appendix 3: HNMR of 2R-3-benzylcloxy-2-methylpropionic acid
Appendix 4: HNMR of 3-Benzylmethoxy-N,N-Diethyl-2-(R)-methyl propionamide

Appendix 5: HNMR of N,N-Diethyl-3-hydroxy-2(R)-methylpropionamide
Appendix 6: HNMR of N, N-Diethyl-2-(R)-methyl-3-oxopropionamide

Appendix 7: HNMR of N, N-Diethyl-2-(R)-methyl-3-oxopropionamide
Appendix 8: GC/MS of 5-methoxy-2-methoxyphenyloxazole

Appendix 9: HNMR of N,N-Diethylacetoacetamide
Appendix 10: Peak 1 of ketone dimethyl aluminum chloride catalyst purification.

Appendix 11: Peak 2 of ketone dimethyl aluminum chloride catalyst purification.
Appendix 12: Peak 2 of diethyl aluminum chloride catalyst experiment.

Appendix 13: Peak 3 of diethyl aluminum chloride catalyst experiment.
Appendix 14: Potential hydrolyzed by-product of reaction with zinc chloride catalysis.

Appendix 15: Unseparated product from second ZnCl2 Catalysis
Appendix 16: Second ZnCl2 Catalysis after purification (oxazole peak)
Works Cited


23. Huang, Z. e. a., Structural Basis for Activation and Inhibition of the Secreted Chlamydia Protease CPAF. *Cell Host Microbe* **2008**, *4*.
