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Synthesis of Dipeptide Aldehyde Fragment of Janolusimide

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By

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CAPSTONE THESIS

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Abstract

Janolusimide A, and its analogue Janolusimide B, are natural marine tripeptide toxins that act as cholinergic agents and are thus effective antifeedants. Their neurotoxic activity is shown to be antagonized by atropine suggesting an inhibition interaction with acetylcholine receptors and is consequently a potential pesticide. This research project consists of developing conditions for the synthesis of the dipeptide aldehyde fragment in Janolusimide using a Weinreb amide reduction approach. Previous syntheses of Janolusimide required an expensive organoborane catalyst and several synthetic steps. Our process via the Weinreb amide is likely to produce the desired compound in a shorter synthetic process. Progress on this project is still ongoing through optimizing reduction conditions for the Weinreb amide. Future use of the finished product can range from advancement of its enantiomers and their chemical effects to use as a pesticide.

Acknowledgements

I would like to acknowledge my research advisors, Dr. T. Nicholas Jones and Dr. Kate Graham, for mentoring me and allowing me to be apart of their research group. I would like to extend special thanks to Alex Patton for being an amazing lab partner and sharing in the insanity of this project. Thanks to my past research colleagues Adrian Demeritte and Andy McCrea for their contribution to this research. I would like to acknowledge the NSF FoCuS program for giving me the chance to attend CSB/SJU and the ability develop my scientific career. I would like to thank the CSB/SJU Undergraduate Research Program and Chemistry Department for providing the opportunity to conduct this research. Likewise, I would like to send thanks to the CSB/SJU Undergraduate Research Grant Fund for the funding to conduct this research.

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Introduction

Janolusimide A and, its N-methyl analog, Janolusimide B (fig. 1) are natural lipophilic linear tripeptide marine toxins found in Bugula flabellate (bryozoan moss animals) and Janolus cristatus (nudibranch soft bodied mollusks), their predators.[1] Janolusimide and several unusual peptides endowed with interesting biological activities have been isolated from marine organisms; Janolusimide B being an optically active yellow oil. Some peptides have been synthesized both for their interesting biological activity and in order to prove (or disprove) structure and stereochemistry.[2, 3]

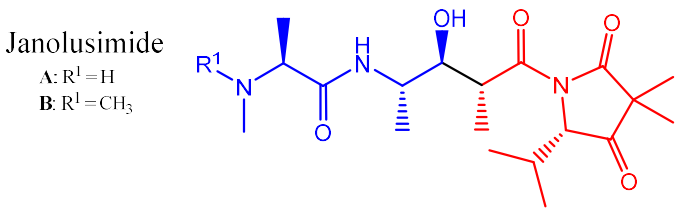


Figure 1: Janolusimide A & B

Applications

These natural products, such as Epiantillatoxin, Mirabazole C and the Janolusimide's, act as cholinergic agents and thus are effective antifeedants for rodents and other predators. Moreover, the Janolusimides' neurotoxicity, which is documented in their ability as a denfense mechanism in byozoans, is shown to be antagonized by atropine. This suggests an interaction with acetylcholine receptors; hence, making them prime targets for total synthesis.[4]

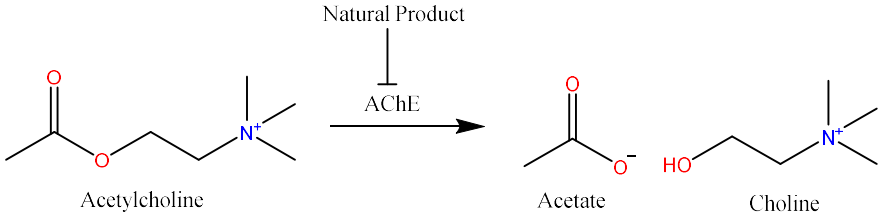


Figure 2: Natural Product cholinergic modulation

As an anticholinergic agent, the Janolusimides would be able to exert their neurotoxic activity through the inhibition of cholinesterase activities - acetylcholinesterase, AChE (fig. 2), and pseudo cholinesterase, BChE - and, consequentially, the cholinergic neurotransmitter system.[5] These enzymes are modulators of the cholinergic signaling in its cyclic pathway. In it, acetylcholine (ACh) is made from choline and acetyl CoA. Normally, after interaction with a synaptic vesicle ACh binds to the cholinergic receptor in the pre-synaptic cell. In the synaptic cleft ACh is rapidly broken down by the enzyme acetylcholinesterase. It is then recycled into the body to restart the process. AChE catalyses the degradation of acetylcholine (ACh) and is required to terminate cholinergic signalling in neuronal synapses.[6] This in turn affects intracellular responses

driven by both nicotinic and muscarinic receptors. When the Janolusimides inhibit AChE, ACh is not broken down. This allows for the build up and reuptake of ACh into the body; which later promotes muscle spasms in organism. In this way the Janolusimide's may cause alteration of all functions of the cholinergic neurotransmission system, and of other neurotransmitters, whose release is regulated by the pre-synaptic ACh receptors.[7]

Natural marine toxins have the ability for a potential biological synthesis as a pesticide; specifically, a biopesticide. As biopesticides, the Janolusimides would be readily biodegradable synthetic insecticides. Due to them being natural products from an organism, they would not persist in the environment due to microbes. Terrestrial microbes - similar to aquatic mocrobes - have adapted to live in the thin films of water that may cover the surface of plants and soil. The microbial communities of marine sediments and terrestrial soils share similar adaptations. Hence, microbial degradation of the Janolusimides would occur either by biological activity of soil microorganisms or under normal atmospheric conditions.[8, 9]

Previous Syntheses

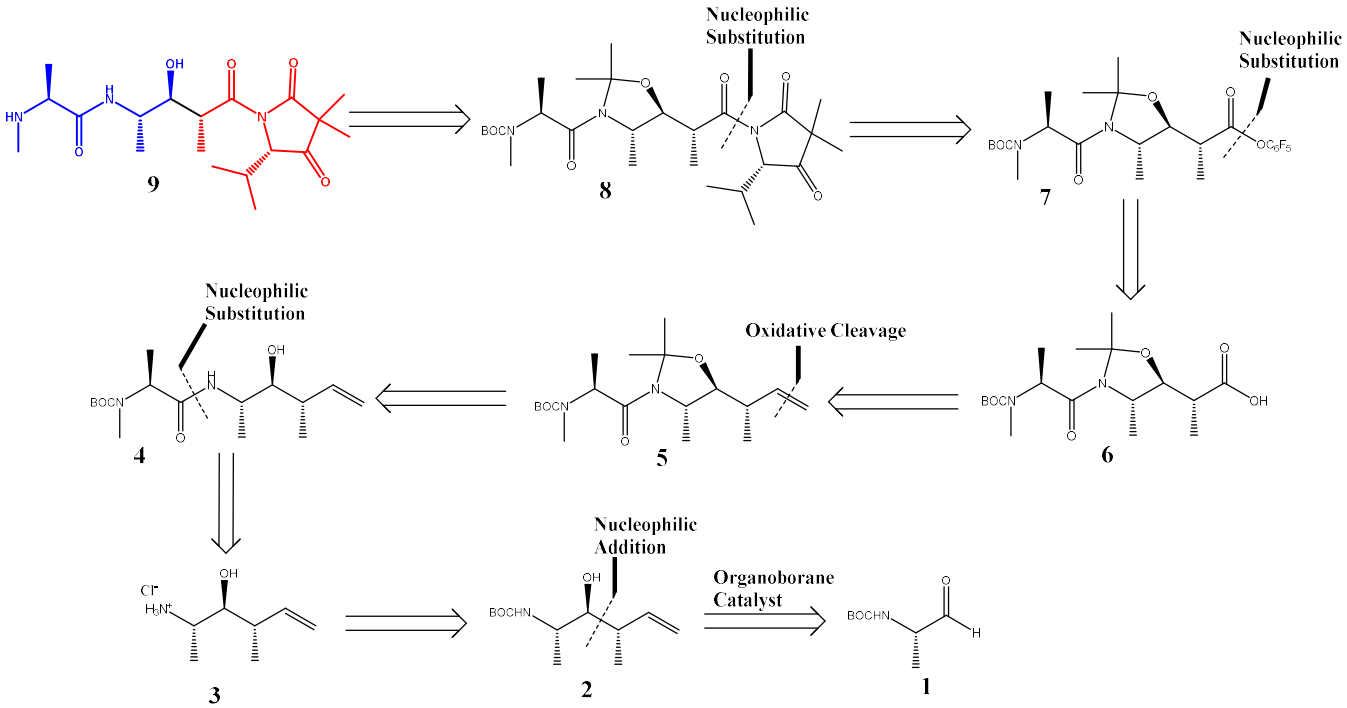


Figure 3: Retrosynthetic Scheme for Sodana and Spinella Synthesis of Janolusimide A

Previous syntheses of Janolusimide A (fig. 3) have been executed using an expensive chiral organoborane catalyst over thirteen steps with only a 0.8% total yield.[10] Sodana and Spinella were able to start from a N-BOC-alaninal (**1**) and, using an expensive organoborane catalyst, formed a homoallylic alcohol (**2**). Deprotection occurred to form the aminoalcohol (**3**). This alcohol was coupled to another N-BOC-alaninal and oxidized using a Ruthenium catalyst to form the carboxylic acid (**6**). This acid was transformed into the activated pentafluorophenyl ester (**7**).

Attachment of the heterocyle and dipeptide fragments consisted of nucleophilic substitution (**8**) of the anionic heterocycle to the end carbonyl of the dipeptide aldehyde component; which the Janolusimide A (**9**) product was generated from. Stereochemistry of the stereogenic center in the ring was attained by synthesis from N-carboxybenzyl-L-valine. Whereas, stereoisomers of the dipeptide aldehyde were obtain through a synthetic strategy based on Brown crotylboration of N-BOC-alaninal.[11]

New Approach

Examination of Janolusimide A's structure reveals a potential aldol coupling by detachment via retrosynthetic cleavage of the C-C bond of the alpha carbon near the lactam and carbon on the hydroxyl group (fig. 4). These syntheses failed to effectively take use of the inherent lactam in the structural formula (fig. 1) for stereochemical selectivity, instead of merely attaching it to the dipeptide chain. Since this synthesis was possible for previous researchers, the intention was to synthesize the Janolusimides as well, with key differences. Instead of a nucleophilic substitution detachment an aldol disconnection was proposed. This reaction would take advantage of the one existing chiral center on the heterocycle and the two on the dipeptide aldehyde. By using this stereochemical preference, would set the two new stereocenters from the 3 existing ones; gaining the desired diasteromer of the Janolusimides. To effectively influence the stereochemistry of the aldol reaction, aldol chiral auxiliary based models were analyzed.

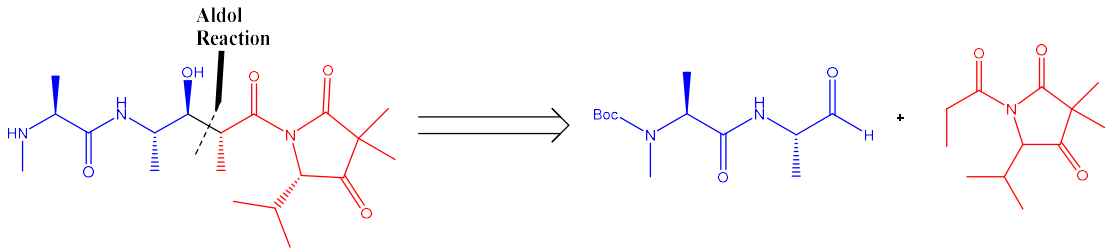


Figure 4: Retrosynthetic Analysis of Janolusimide A

Overtime, the stereoselectivity of the selective aldol reactions have been studied. Through combination of two carbonyl compounds an enolate ion, and subsequentially, a new β-hydroxy carbonyl compound is formed (fig. 5). Although, this structural motif contains a stereogenic center, it lacks stereoselective control. Models have been introduced in order to control the centers depending on the desired relative and absolute configuration.[10]

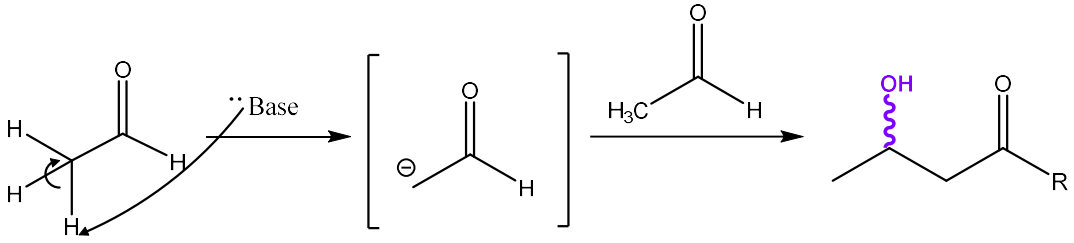


Figure 5: General Aldol Reaction

Early on, Zimmerman and Traxler proposed a model using metal enolates. The aldol reaction proceeds via a closed chair-like transition state for kinetic selectivity due to the unexpected stereochemical outcomes of the Ivanov and Reformatsky reactions.[12] Not only was the reaction thermodynamically favored, but it also held for a variety of enolates both E and Z (fig. 6). However, stereochemical outcome of the model may be unpredictable because only few metals, such as boron and lithium, reliably follow the Zimmerman-Traxler model.[12]

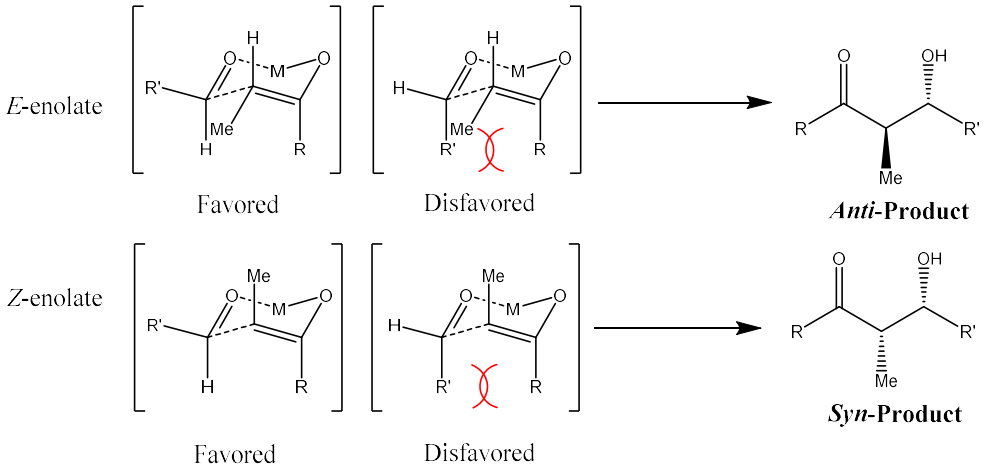


Figure 6: Zimmerman-Traxler Enolate Model

Whereas, the Evan's oxazolidinone chiral auxiliary described a chelate organization that allowed for highly diastereoselective alkylations of imide enolates.[13] The Evans acyl oxazolidinone method works to temporarily create a chiral enolate by affixing a chiral auxiliary, oxazolidinone, for better stereoselectivity. Through a diastereoselective reaction the pre-existing chirality of the auxiliary is then transferred to the aldol adduct. Therefore, after removal of the auxiliary, the desired aldol stereoisomer is formed.[14, 15]

The anti-selective aldol addition using the magnesium halide-catalyzed protocol of Evans seemed the most promising.[16] Evans anti-aldol approach utilizes sterics and electronics to influence oxazolidinones in the presence of magnesium halide salts to influence stereochemistry of an Aldol reaction. Janolusimides' heterocycle structures are similar in nature to the oxazolidinones, both sterically and electronically, thus providing an attractive synthetic approach.

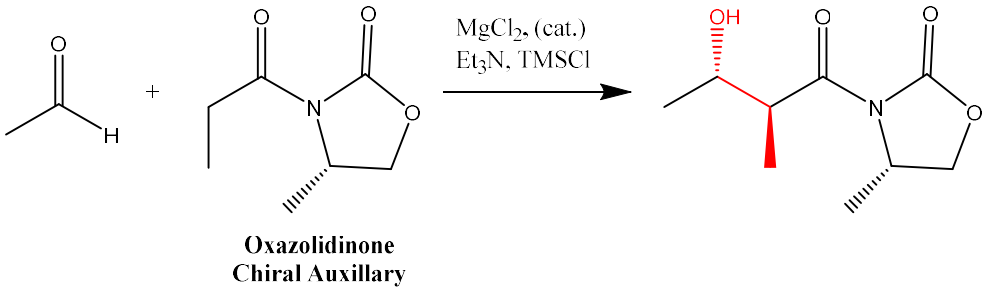


Figure 7: Evans' anti-Aldol Approach

This reaction has shown to have good yields and selectivities. Effectiveness of the anti-aldol reaction would need to be determine via diastereoselective alkylation, and cleavage of an Evans oxazolidinone chiral auxiliary. In preparation for such reaction, acylation of the chiral auxiliary is required.[17]

The source of chirality in the oxazolidinone (fig. 7) is an amino alcohol, which is usually commercially available and cheap, or can be synthesized from the reduction of an amino acid.[18] The implications of this procedure are essential in the enantioselective aldol reaction, using chiral metal complexes to form enolate adducts. The acylated oxazolidinone scaffold controls additions with high facial selectivity (fig. 8).

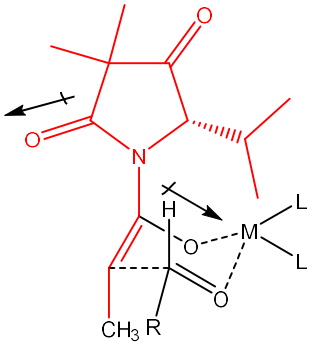


Figure 8: Steroselective Control Using Oxazolidinon Chiral Auxilary

By taking advantage of the chiral lactam to guide steroselectivity, a total synthesis of Janolusimide can be generated. Consequently, the goal of this research is to create a shorter synthesis by utilizing the chiral lactam through a magnesium catalyzed Evans anti-Aldol reaction, by combining a Dipeptide Aldehyde fragment and a Valine Derived Chiral Lactam fragment.[19]

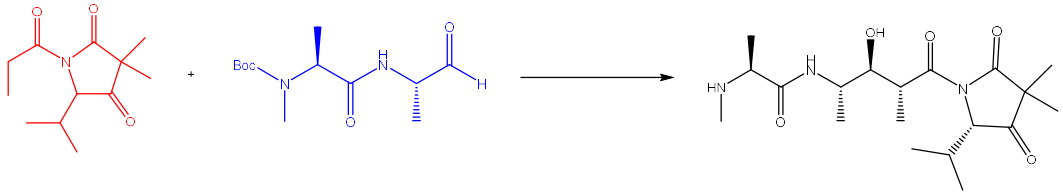


Figure 9: General Reaction Scheme for Fragment Formation

Due to the longevity of the synthesized chiral lactam, a motif aldol (fig. 10), resembling the neurotoxin was created. With a similar structure and an expected similar reactivity to that of Janolusimide A & B, synthesis of this aldol will provide much information on the selectivity of reactions prior to the total synthesis of Janolusimide A & B. Synthesis of this compound required the chiral motif (S)-(+)-4-Isopropyl-3-propionyl-2-oxazolidinone and an acyclic dipeptide aldehyde fragment.

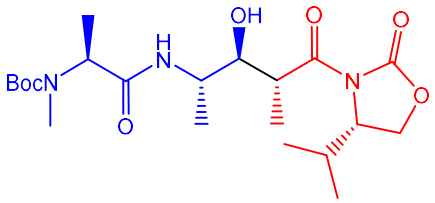


Figure 10: Motif Aldol

The reagents used in the synthesis would be inexpensive compared to the expensive organoborane catalyst used in previous synthesis. The efficiency of the synthesis would also be greater due to the different disconnection of the fragments, allowing for better chiral auxiliary. Due to being previously synthesized the heterocycle, was not the focus of this study (fig. 11).

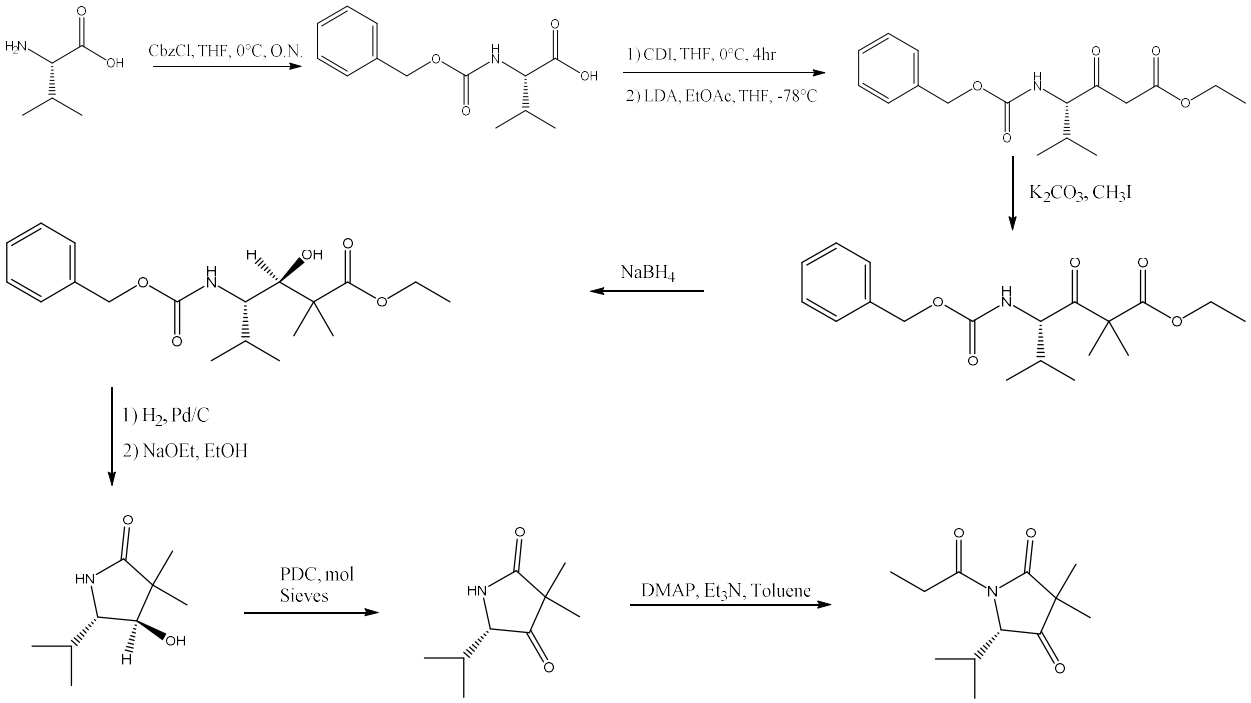


Figure 11: Synthesis of Heterocycle Fragment

The acyclic dipeptide aldehyde fragment of Janolusimide A & B, with small alterations in procedure, could be used in the synthesis of many other natural products with dipeptide fragments.[3, 20] Production of the acyclic dipeptide fragment could be carried out using, N-(tert-Butoxycarbonyl)-L-alanine N′-methoxy-N′-methylamide, and the method described by Morwick *et al*. with slight modifications.

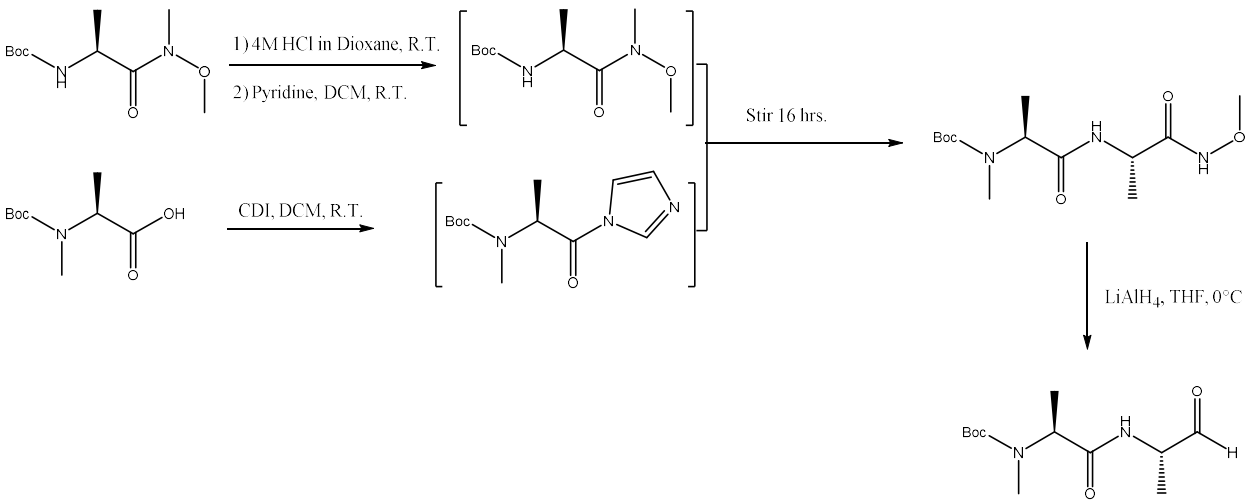


Figure 12: New Reaction Scheme for Synthesis of Dipeptide

Although, before trying to synthesize the Dipeptide Aldehyde, a practice reaction is currently being carried out in order to create a similar structure, using the same conditions.[21] This reaction is being carried out due to the high cost of the BOC-N-Me-Ala-OH, starting material, needed to run the actual synthesis. The model reaction gives a handling on the unpredictability of the different stages of the dipeptide synthesis, such as the hydride reduction.

Formation of the Weinreb Amide is the first step in this reaction. Examination of the amide lead to a retrosynthetic cleavage of the amide bond forming benzoyl chloride and N, O-dimethylhydroylamine (fig. 13).

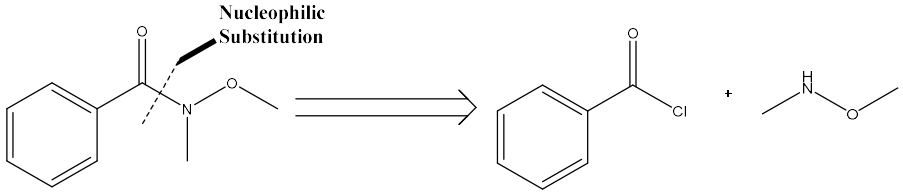
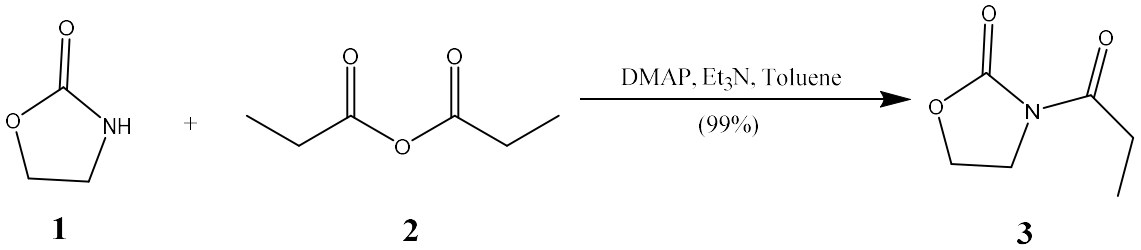


Figure 13: Retrosynthetic Analysis of Weinreb Amide

Experimental

IR spectra was recorded using a Nicolet 360 FT-IR spectrophotometer. While, ^{1}H and ^{13}C-NMR spectra were recorded and analysed using a Joel 400 MHz NMR spectrophotometer with $CDCl_3$ as a solvent. The products from each step were identified by comparison to the IR/ NMR spectra of starting materials. All reactions were carried out in oven-dried glassware in an inert atmosphere. Solvents and reagents used were manufactured by Sigma Aldrich, Alfa Aesar, Spectrum and TCI America.

Acylation



1 **2** **3**

Figure 14: Reaction Scheme for Acylation of 2-oxazolindinone

The acylation was done using the method described by *Smith et al*.[18] with slight modifications. To an oven-dried flask, fitted with a Claisen adapter and a reflux condenser, was added 2-oxazolidinone (0.49 g, 5.64 mmol) and catalytic DMAP (0.014 g, 0.122 mmol). This flask was capped and purged with nitrogen. Anhydrous toluene (10 mL) and triethylamine (1.3 mL, 9.25 mmols) were added to the reaction via syringe. Propionic anhydride (1.4 mL, 11.3 mmols) was added via syringe over 2 minutes. This new solution was heated to reflux (110ºC). Once at reflux the solution was allowed to stir for another 30 minutes. Deionized water (10 mL) was added to the reaction flask and the mixture was heated at to reflux for an additional 10 minutes. The flask was removed from heat and allowed to cool. The solution was diluted with diethyl ether (7.5 mL). The organic layer was separated and washed with 2M HCl, 2M NaOH and brine, then dried with anhydrous sodium sulfate. This yielded a yellowish oil.

3-propionyl-2-oxazolidinone **3**: 0.48g; 99% yield; yellow oil; IR (ATR, diamond) 3180.29; 1643.04; 1274.64; 1096.12 cm^{-1}; ^{13}C-NMR (100MHz, $CDCl_3$) δ 171.6, 152.1, 62.7, 44.2, 32.9, 11.4 ; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.27 (2H, t), 4.02 (2H, t), 2.33 (2H, q), 1.28 (3H, t).

Heterocycle Fragment

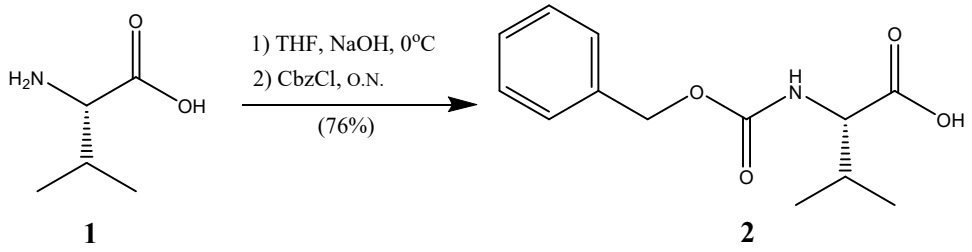


Figure 15: Reaction Scheme for Synthesis of Cbz-L-valine

A solution of L-valine (0.88g, 7.5 mmol) was placed in an oven-dried flask, capped and purged with nitrogen. and Tetrahydrofuran (15 mL) was added and the solution was placed in an ice bath, cooling it to 0°C. 1.25 mL NaOH (15 mL) was then added to the reaction flask. Benzyl chloroformate (1.16 mL in 7.5 mL THF) was added slowly dropwise over 2 minutes via a glass syringe. The reaction was stirred for two hours before the ice bath was removed. The solution was allowed to warm to room temperature overnight. The reaction was then quenched with deionized water (12.5 mL). The addition of 82 drops of 6M HCl yielded a product with a pH of 3. The aqueous layer was extracted using ethyl acetate, then the combined organic phases were washed with brine in a separatory funnel. The resulting liquid was dried using anhydrous sodium sulfate and evaporated in vaccuo.

Carbobenzyloxy-L-valine **2**: 0.72 g; 76% yield; clear liquid; IR (ATR, diamond) 3184.94, 2965.92, 1734.92, 16.88.40, 1454.58, 1238.67, 1043.78 cm^{-1}; ^{13}C-NMR (100MHz, $CDCl_3$) δ 172.4, 156.6, 137.5, 128.8, 128.4, 127.0, 65.2, 60.5, 31.1, 19.1, 17.5 ; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.38-7.32 (5H, elongated m), 5.09 (2H, s), 4.58 (1H, d), 1.84 (1H, m), 0.95 (3H, d), 0.88 (3H, d).

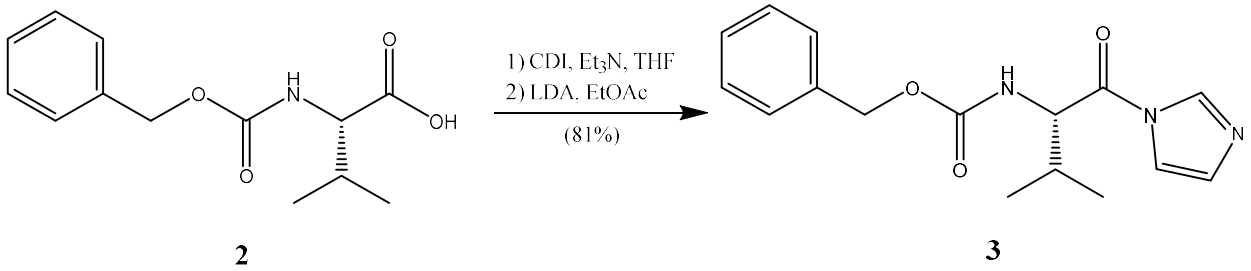


Figure 16: Reaction Scheme for β-ketoester Formation

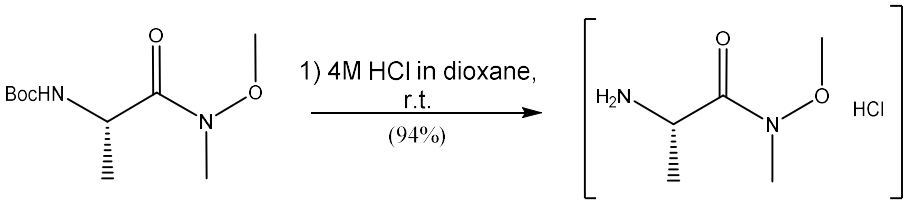
Synthesis of the β-ketoester was done using the method described by *Jouin et al.*[22] with slight modifications. Carbonyldiimidazole (0.175 g, 1.08 mmol) was added to an oven-dried flask along with **2**, prepared above, (0.245 g, 0.975 mmol). This mixture was purged with nitrogen for 5 minutes and anhydrous tetrahydrofuran (5 mL) was added. Anhydrous triethylamine (0.007 mL) was added to the reaction flask and the new solution was allowed to stir for 2 hours to react. A

separate oven-dried flask was flushed with nitrogen and capped with a septum. In it, lithium diisopropylamide (0.18 mL) and anhydrous tetrahydrofuran (2 mL) was added. To the LDA solution, ethyl actate (1 mL) was slowly added over a minute to allow for full deprotonation. and stirred for 5 minutes. A double-ended cannula was used to transfer the LDA-Ethyl acetate anion mixture to the reaction flask containing the CDI activated Cbz-valine. This new solution was allowed to sit for 20 minutes to react. The final solution was washed with 2M HCl, 2M NaOH, brine and dried with anhydrous sodium sulfate. Evaporation of solvent yield a clear liquid, **3**.

benzyl (S)-(1-(1H-imidazol-1-yl)-3-methyl-1-oxobutan-2-yl) carbamate, **3**: 0.23 g; 81% yield; clear liquid; IR (ATR, diamond) 3352.41, 3168.35, 2934.74, 1721.39, 1682.78, 1539.61, 1493.31, 1274.28 cm^{-1}; ^{13}C-NMR (100MHz, $CDCl_3$) δ 177.7, 156.8, 136.6, 134.3, 129.1, 128.5, 127.9, 127.4, 120.4, 68.0, 68.7, 31.4, 19.5; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.98 (1H, s), 7.61 (1H, d), 7.32 (1H, d), 7.29-7.25 (5H, elongated m), 7.06 (1H, d), 5.70 (1H, s), 5.05 (1H, s), 4.09 (1H, d), 3.01 (1H, m), 0.94 (3H, d), 0.89 (3H, d).

Acyclic Dipeptide Aldehyde Fragment

Production of the acyclic dipeptide fragment was done using the method described by *Morwick et al*.[21] with slight modifications.



4 **5**

Figure 17: Reaction Scheme for Removal of Protecting Group

N-(*tert*-Butoxycarbonyl)-L-alanine *N*′-methoxy-*N*′-methylamide, **4**, (1 g, 4.3 mmol) was dissolved in 4M HCl in dioxane (10 mL, 40 mmol) and the resultant mixture was stirred at room temperature for 2 hours. Solvent was removed with vacuum leaving a hydrochloride salt, **5**, as a thick waxy substance.

Hydrochloride salt **5**: 0.84 g; 94% yield; thick clear oil; ^{13}C-NMR (100MHz, $CDCl_3$) δ 170.1, 63.5, 46.3, 32.6, 17.1; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.47 (2H, s), 4.62 (1H, m), 3.81 (3H, s), 3.23 (3H, s), 1.64 (3H, d).

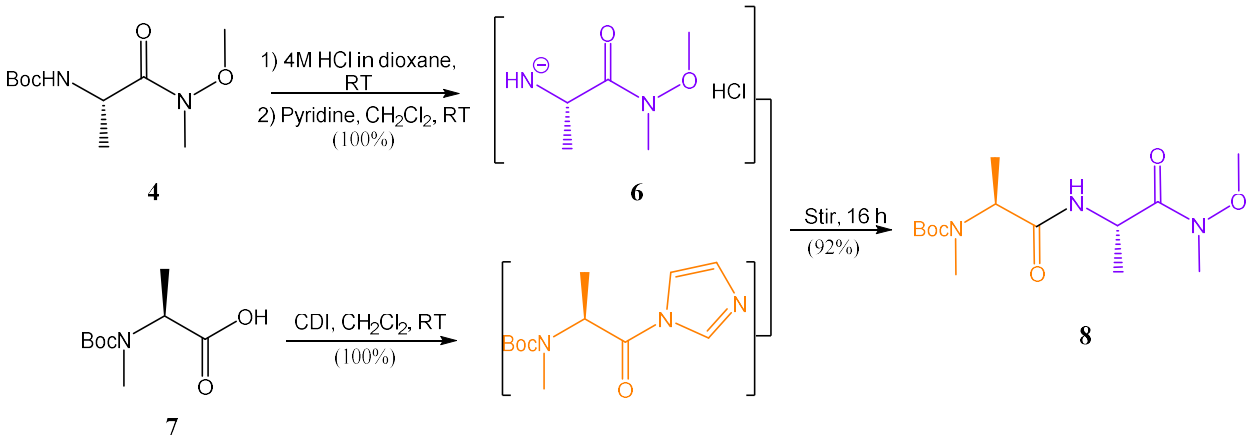


Figure 18: Reaction Scheme for Synthesis of Dipeptide

The resulting amine hydrochloride salt, **5**, (0.125 g, 0.74 mmol) was dispersed in dichloromethane (0.065 mL) and pyridine (0.1 mL, 1.2 mmol) was added and the resulting mixture was stirred at room temperature for 1 hour. In a separate flask BOC-N-Me-Ala-OH, **7**, (0.18g, 0.88 mmol) was reacted with carbonyldiimidazole (0.18 g, 1.11 mmol) in dichloromethane (0.065 mL) for 1 hour to produce the necessary acyl imidazole. After 1 hour, the amino Weinreb amide reaction of **6** was added to the acyl imidazole reaction via cannula and the resulting combination was stirred at room temperature for 16 hours (fig. 4). The reaction mixture was diluted with 20 mL of water and the organic layer was separated and washed with 1M HCl (9 mL), saturated aqueous $NaHCO_3$ (9 mL), and brine (9 mL) then dried with Na_2SO_4. Evaporation of solvent yielded a clear viscous oil **8**.

Acyclic Dipeptide **8**: 0.261 g; 89% yield; clear oil; ^{13}C-NMR (100MHz, $CDCl_3$) δ 169.26, 62.72, 55.35, 46.66, 27. 20, 17.94; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.82 (1H, s), 5.03 (1H, m), 4.46 (1H, m), 3.69 (3H, s), 3.22 (1H, s), 2.64 (1H, m), 1.46 (9H, s), 1.35 (3H, d), 1.30 (3H, d).

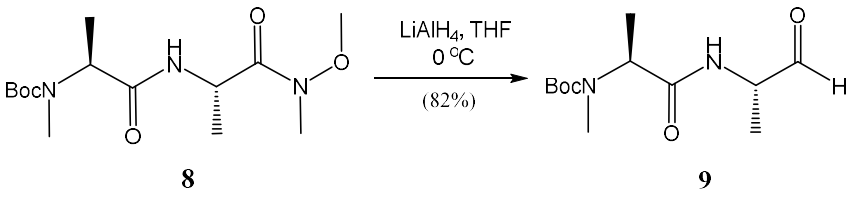


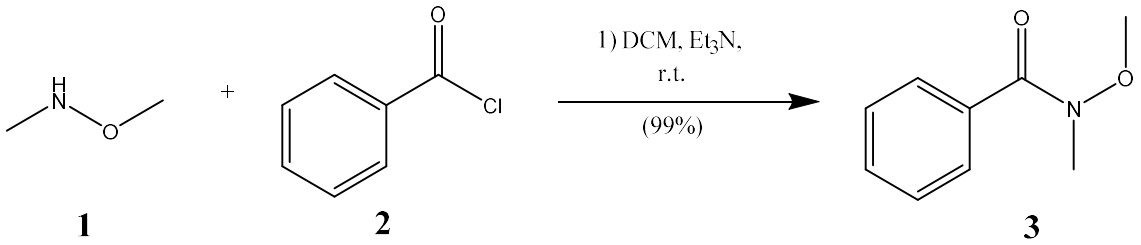
Figure 19: Reaction Scheme for Hydride Reduction

Lithium aluminium hydride (0.02 g, 0.49 mmol) was weighed into an oven-dried flask, sealed with a septum and flushed with nitrogen. Anhydrous tetrahydrofuran (2 mL) was added via syringe and the resultant solution was cooled to -78°C via an acetone and dry ice bath. **3**, prepared above, (0.15 g, 0.47 mmol) was dissolved in anhydrous tetrahydrofuran (2 mL) in a separate round bottom flask and added dropwise via cannula to the $LiAlH_4$ solution. The cooling bath was

removed and replaced with an ice bath at 0°C. This resulting solution was allowed to react for 20 minutes then cooled again to -78°C. An aqueous solution of potassium bisulfate (0.14 g, 2.35 mmol) was added quickly. The mixture was allowed to warm to room temperature and extracted twice with diethyl ether. The combined extracts were washed with brine and dried with sodium sulfate. Evaporation of solvents yielded a clear oil **9**.

Acyclic Dipeptide Aldehyde **9**: 0.217 g; 64% yield; clear oil; ^{13}C-NMR (100MHz, $CDCl_3$) δ 201.73, 171.42, 80.26, 55.49, 30.71, 27.34, 14.97, 13.30; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.64 (1H, s), 6.64 (1H, s), 4.83 (1H, m), 4.28 (1H, m), 2.64 (3H, s), 1.48 (9H, s), 1.34 (3H, d), 1.30 (3H, d).

Model Reaction

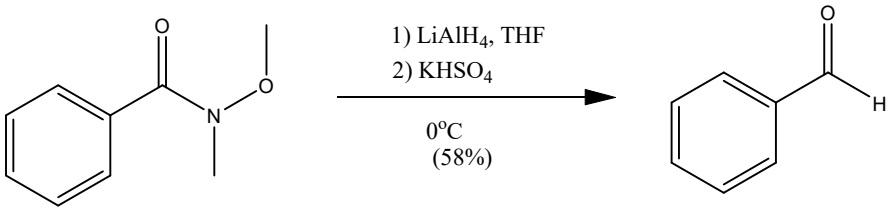


1 **2** **3**

Figure 20: Reaction Scheme for Synthesis of Weinreb Amide

Production of the Weinreb amide was done using the method described by *Kerr et al*.[23] with slight modifications. N, O-Dimethylhydroxylamine, **1**, (0.49 g, 5 mmol) and dichloromethane (12.5 mL) was added to an oven-dried flask and stirred. This solution was placed in an ice bath and cooled to 0°C. Once at 0°C, triethylamine (1.4 mL, 10 mmol) was added slowly to the stirring solution and allowed to sit for 5 minutes. Benzoyl chloride, **2**, (0.58 mL, 5 mmol) was added dropwise to the stirring solution. Once added, the mixture was removed from the ice bath and allowed to reach room temperature. This stirred for roughly 3 hours. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (7.5 mL). The two layers were separated, and the organic phase was washed with 1M HCl and brine. The resulting solution was dried using anhydrous magnesium sulfate, filtered and concentrated using a rotary evaporator; yielding a brownish oil, **3**.

N-methoxy-*N*-methylbenzamide **3**: 0.51 g; 99% yield; brownish oil; IR (ATR, diamond) 2935.26, 1636.42, 1377.41, 1212.59 cm^{-1}; ^{13}C-NMR (100MHz, $CDCl_3$) δ 169.9, 134.1, 130.5, 128.1, 128.0, 61.0, 33.8; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.61 (2H, d), 7.38-7.31 (3H, elongated m), 3.54 (3H, s), 3.35 (3H, s).



3 **4**

Figure 21: Reaction Scheme for Hydride reduction of Weinreb Amide

Hydride reduction of the Weinreb amide was done using the method described by *Morwick et al*.[21] with slight modifications. Lithium aluminium hydride (0.02 g, 0.5 mmol) was weighed into an oven-dried flask, sealed with a septum and flushed with nitrogen. Anhydrous tetrahydrofuran (1 mL) was added via syringe and the resultant solution was cooled to -78°C via an acetone and dry ice bath. **3**, prepared above, (0.08 g, 0.48 mmol) was dissolved in anhydrous tetrahydrofuran (1 mL) in a separate round bottom flask and added dropwise via cannula to the $LiAlH_4$ solution. The cooling bath was removed and replaced with an ice bath at 0°C. This resulting solution was allowed to react for 20 minutes then cooled again to -78°C. An aqueous solution of potassium bisulfate (0.14 g, 2.35 mmol) was added quickly. The mixture was allowed to warm to room temperature and extracted twice with diethyl ether (5 mL). The combined extracts were washed with brine and dried with sodium sulfate. Evaporation of solvents yielded a clear liquid **4**.

Benzaldehyde **4**: 0.053 g; 58% yield; pale colourless liquid; IR (ATR, diamond) 3086.42, 2850.75, 1703.38, 1597.21 cm^{-1}; ^{13}C-NMR (100MHz, $CDCl_3$) δ 195.4, 136.7, 135.2, 128.9, 127.6; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.82 (1H, s), 7.78 (2H, d), 7.56 (1H, t), 7.43 (2H, t).

Results and Discussion

Acylation

The first reaction carried out was the acylation of 2-oxazolidinone as a mimic in order to get a handle on the Evans chiral auxilary. Although the synthesis of the Janolusimides can be done with 2-oxazolidinone, it would produce adverse effects, such as attaining both wedge and dash stereochemistry, instead of just dash, in the product. In figure 13, **3** being formed is an effective mimic for the (S)-4-isopropyl-3-propionyloxazolidin-2-one being used in the formation of Janolusimide. Hence, **3**, is synthesized to test the aldol reaction and show the coupling between the dipeptide aldehyde and heterocycle (fig. 9). Due to the reactions' accessibility it is a great way to start towards the goal of producing Janolusimide.

DMAP was used to deprotonate the nitrogen in **2**. This accelerates the acylation, doubling it, so that the reaction goes faster. After adding deionized water and refluxing, for high yield, the solution was allowed to cool to room temperature. For dilution, diethyl ether was used since it is volatile and therefore easier to rotovap. When drying the organic layer, sodium sulfate was used instead of magnesium sulfate. This is because $MgSO_4$ will activate the carbonyl, due to its 2^+ charge, allowing another compound to attack it. The ^{1}H-NMR shift of the N-CH_2 peak from 3.43 ppm to 4.02 ppm proves the conversion of starting material to product. Yields on this experiment were improved, 99%, compared to those indicated by *Smith et al*.[18]

Heterocycle Fragment

Synthesis of **2** was expressed through the appearance of the benzene ring at 7.38-7.32 ppm in the ^{1}H-NMR. Likewise, product formation was proven through the presence of the CH_2 peak at 5.09 ppm in the ^{1}H-NMR. Along with compatible peaks in the ^{13}C-NMR, these ^{1}H-NMR further indicated product formation. Yields on this experiment compared well likened to those indicated by *Jouin et al*.[22]

Acyclic Dipeptide Aldehyde Fragment

The Weinreb amide is formed though deprotonation of nitrogen and coupling it to the BOC protected alanine acid. In the reaction, 4M HCl in dioxane was used in an effort to deprotonate **4**. Deprotection was indicated by the phase change of the original starting solid to a thick waxy oil and further confirmed by the disappearance of the BOC protecting group peak at 1.58 ppm in the 1H-NMR for **5**. The complete disappearance of the protecting group peak indicated 100% conversion of starting material to product.

Formation of **8** was indicated by the reappearance of the BOC protecting group at 1.46 ppm in the ^{1}H-NMR. Additionally, shifting of the amine peak from 8.47 ppm in **5** to 6.82 ppm in **8** indicated attachment to activated carbonyl of the starting acyl imidazole. The parallel of peaks

and integrals from starting materials indicated the formation of the compound. This was further proven through correlation to the heights of ^{13}CNMR peaks.

Production of **9** was shown by the presence of an aldehyde peak at 9.64 ppm in the ^{1}H-NMR. This was synonymous by the disappearance of the methyl peaks of the Weinreb amide; which were present at 3.69 ppm and 3.22 ppm in the ^{1}H-NMR of **8**. Formation of **9** was further indicated by the presence of a peak at 201.73 ppm in the ^{13}C-NMR. The integral in the ^{1}H-NMR along with the peaks in the ^{13}C-NMR further indicated compound formation. Yields on this experiment was lower compared to that of literature (64%) indicated by *Morwick et al*.[21]. Timing and reactivity were shown to play key factors in running this experiment. When ran too long overreaction occur, forming the hydroxyl group rather than the aldehyde. Several runs of this experiment also showed degradation and fragmentation of the aldehyde peaks in the ^{1}H-NMR shortly after workup. This hydride reduction is still being optimized for future reactivity.

Model Reaction

Due to the instability and rapid degradation of the acyclic dipeptide aldehyde in the original reaction, a model reaction was devised to practice the reaction mechanisms. When first ran, benzoyl chloride starting material continued to persist in subsequent ^{1}H-NMR analysis at 8.152 ppm. Purification of the product consisted of silica gel chromatography using Ethyl acetate/Hexanes 70:30 as the mobile phase. This was consequentially ran on TLC plates to ensure purity compared to the starting materials. Further purification of unknowns consisted of a solvent exchange using the rotovap. This extensive purification process was resolved by increasing reaction stir time from 1 ½ hours to 2 hours. This increased purity and yield. When increased to 3 hours the reaction achieved a higher yield than what was first established. Formation of **3** was indicated by the shift of the methyl peaks of the Weinreb amide from 4.08 ppm and 2.96 ppm, in **2**, to 3.54 ppm and 3.35 ppm in the ^{1}H-NMR of **3**. These shifts in the ^{1}H-NMR indicated compound formation. Yields on this experiment, were similar to that of the literature by *Kerr et al.*[23]

Primarily, reaction of the Weinreb amide with $LiAlH_4$ failed to occur, resulting in spectrums containing only solvent and impurity peaks. The reaction was scaled up and potassium bisulfate concentration was increased to 5%. This reaction still failed to follow through. To test the reactivity of the hydride being used, Benzaldehyde was reduced in a subsequent reaction. This proceeded to partial synthesis, failing to react completely with the hydride, forming an intermediate instead (fig. 22). A new LAH was carried out yielding a benzyl alcohol product. To avoid such intermediates, this new LAH was used in the scaled up hydride reaction yielding benzaldehyde.

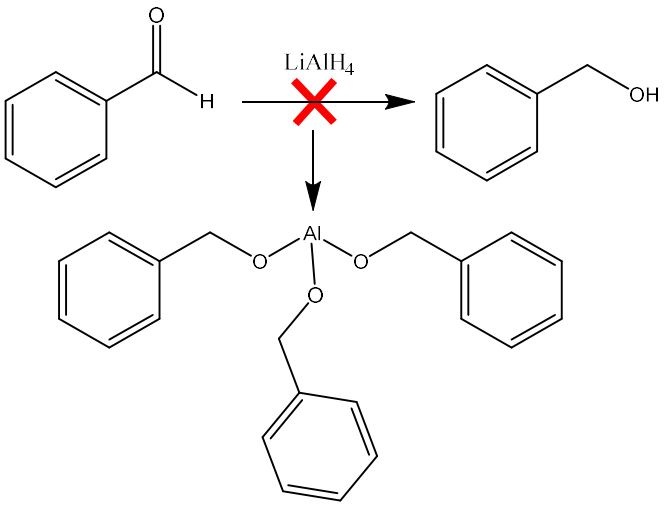


Figure 22: Reaction Scheme for Intermediate of Hydride Reaction

Production of **4** was indicated by the presence of an aldehyde peak at 9.82 ppm in the ^{1}H-NMR. This was synonymous with the disappearance of the methyl peaks of the Weinreb amide which were present at 3.54 ppm and 3.35 ppm in the ^{1}H-NMR of **3**. Aldehyde formation was further indicated by the presence of a peak at 195.4 ppm in the ^{13}C-NMR. The correlation between the peaks of the ^{1}H-NMR and the ^{13}C-NMR further indicated product formation. Yields on this experiment were lower than that of literature (58%) by *Morwick et al.*[21] Due to the time dependence of this reaction, variations in reactivity can occur. When the temperature is too high, the Weinreb amide won't form the intermediate, overreacting and instead having the $LiAlH_4$ attack again. Likewise, if the solvent used is too dry or there is water in the solution, the $LiAlH_4$ may be ripped apart and the Weinreb amide may under reduce or not react at all.

Research Proposal: Reactivity of Janolusimide A analog in a non-aldol reaction

Enolates are considered to be good nucleophiles in aldol reactions. Aldol reaction are considerd to be one of the most common and effective ways to form or cleave a carbon-carbon bond.[24] Since many compounds contain aldehyde and ketone moieties, this allows the reaction to be widely applicable when the compounds function as either enolates nucleophiles or electrophiles. However, there are drawbacks. For example, side products and the notion that aldol products are not always isolated from the reaction mixture.[25] Due to the complexity and erratic reactivity of the aldol reaction, other reactants are being considered. Instead of enolates, what if other nucleophiles were used, such as a Grignard or Gilman reagent? This new system would be able to generate a similar array of functional groups and electrostatics that may be a better fit for running dipeptide reactions and couplings.

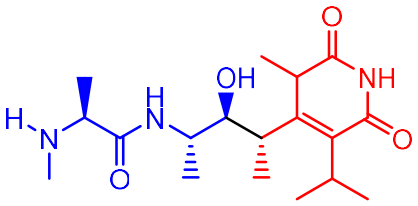


Figure 23: Analog of Janolusimide A

After development of the didpeptide aldehyde fragment and synthesis of the Janolusimides, the idea would be to vary sites at the heterocycle position; since knowledge of the dipeptide reaction would be previously known. Hence, an analog of Janolusimide A (fig. 23) was established in order to develop such a system.[1, 10] Different ring systems were developed before settling on an aryl coupling. To do this the dipeptide was kept close to its original design; however, in order to give the compound, the ability to form a handle a double bond was added. Likewise, an isopropyl containing dione ring was added, instead of the known heterocycle. This was modified with the mindset of keeping a similar electron density and structural design, although having a different reaction and disconnect. When synthesized, properties of the analog may be similar or even better than the natural product, Janolusimde A. Application of the correct technique will enable the ability to branch out from aldol reactions when forming carbon-carbon bonds to for complex compounds.

An aldol reaction would be unlikely due to the lack of the oxazolidinone-like ring. Originally depicted in Janolusimide A, it acted as a chiral auxiliary in order to guide stereoselective control during the aldol reaction. Without this component, other reactions were perused and an organometallics transformation was chosen. Specifically, a Suzuki Cross-Coupling Mechanism was decided upon due its mild reaction conditions, commercially available boronic acids and low toxicity. Thus, the hope would be to create a better Janolusimide through a non-aldol reaction.

The mechanism stems from the coupling of a vinyl halide and a heterocycle using a metal catalyst; thus, resulting in the formation of a new C-C bond. The Suzuki Cross-Coupling mechanism (fig. 24) would consist of the palladium-catalysed addition of a substituted vinyl group to an organoboron compound. The mechanism involves the oxidative addition of the halide and palladium (0) species.This compound would be activated with a sodium tert-butoxide base and the reaction speeds up. After which, the palladium performs a transmetallation which adds on the activated heterocycle to form a diorganopalladium (II) intermediate.[26] The combined compound is then reductively eliminated and the palladium (0) catalyst is regenerated.

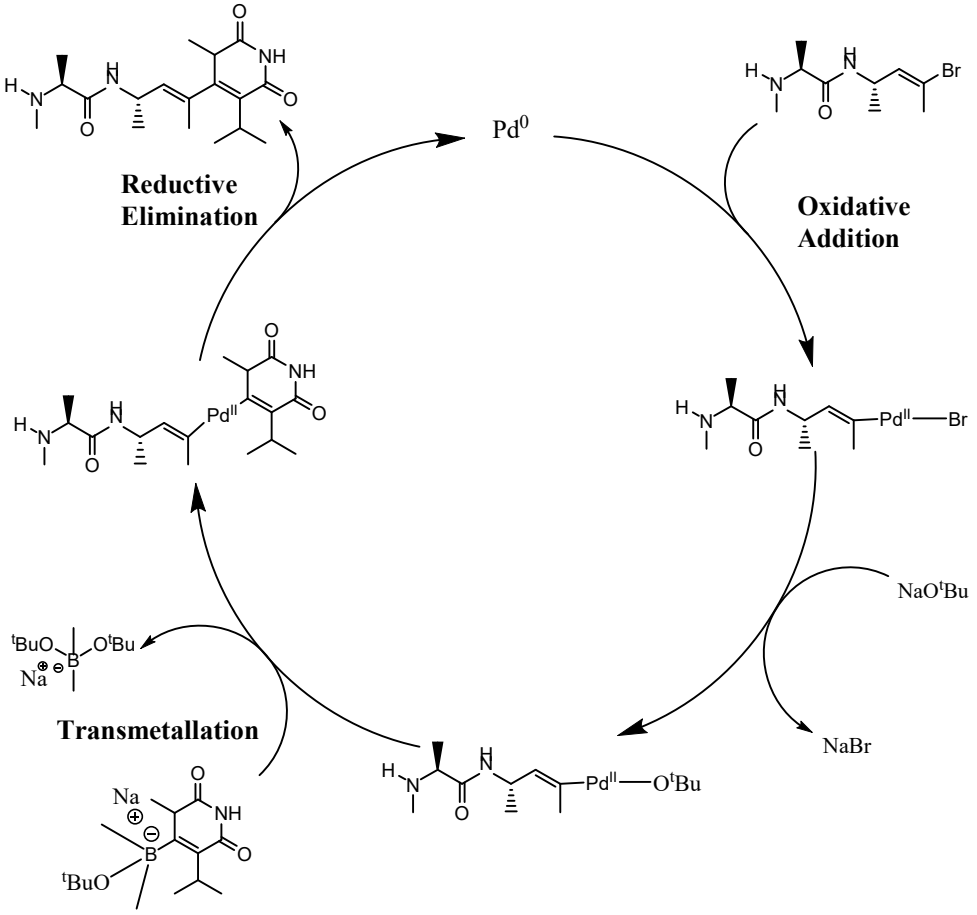


Figure 24: Analog going through Suzuki Cross-Coupling Cycle

The product would then go through a hydroboration-oxidation (fig. 25). This reaction would achieve a diastereoselective (syn) addition of the hydroxyl group and the other remaining chiral center; thereby forming the final product in this experiment. The reaction to generate this dipeptide-propenamide structure will be an effective test of the reactivity of a different nucleophile, compared to enolates, in a non-aldol, metal catalyzed reaction.

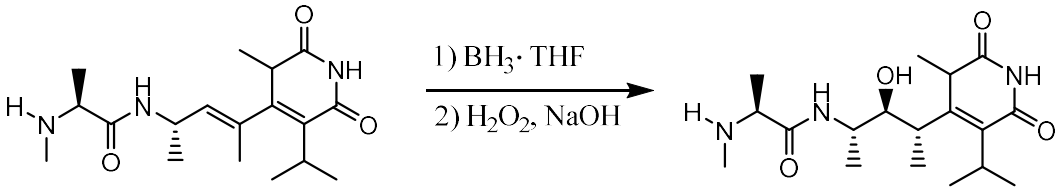


Figure 25: Hydroboration-Oxidation of Vinyl

Though a variety of conditions can be valuable tools when it comes to enhancing a Suzuki mechanism for a Janolusimide analog, there are also inherent limitations that have to be acknowledged so that they can be minimized. When dealing with reactivity, the temperature of the experiment should be monitored since overactivity can occur. Also, caution has to be taken that too much water isn't present, and products are formed correctly.

Reactivity will play a great role when it comes to limitations of the Suzuki cross- coupling. If the reaction is too dry or an especially dry solvent is used, it may promote low reactivity as the reagents may not interact efficiently with the palladium catalyst. Similarly, the reaction will depend on the thermal activation of reactants. If the temperature is too high the reagents may overreactive, failing to form appropriate intermediates and products[27]. Whereas, if the reactions temperature is too low, then the reagents will once again fail to react with the catalyst. Hence, monitoring the variance of temperature will be important in order to ensure the formation of intermediates and products correctly.[28] This monitoring can be carried out using TLC plate spotting and NMR spectroscopy analysis. Moreover, the palladium catalyst itself may pose as a limitation. This is due to the expense and lack of stability.[29]

Polarimetry is commonly used to determine stereochemistry in compounds. However, due to the numerous stereocenters in Janolusimide A and the analog, too much light would be generated so proper rotation will not occur. Hence NMR shifts and IR frequencies are used to tell the difference between the epimers.

Nuclear Magnetic Resonance (NMR) spectroscopy is based on the fact that many nuclei have spin and all nuclei are electrically charged. An energy transfer occurs, when an external magnetic field is applied, from a ground energy level to an excited energy level[30]. When the spin returns to the base level, energy is emitted at that frequency. This signal is measured and processed to obtain an NMR spectrum. An NMR tells us the relative number of protons or carbons that give rise to each signal or chemical shift.[30]

NMR spectroscopy has been known to detect a wide range of catalysts and peptides. However, problems can be caused by overlapping peaks in larger complexes. In addition to this, with the large complex the magnetization relaxes faster, which means there is less time to detect the signal. This, in turn, causes the peaks to become broader and weaker, and eventually disappear[31]. After a resonance spectrum is obtained, properties can be used to examine the chemical surroundings of the hydrogen nucleus. The different frequencies, termed chemical shifts, are expressed in ppm relative to the shifts of a standard compound.

An NMR spectrum of the peptide and catalyst will reveal all the unique nuclei of a certain type in the molecule. Nuclei that are close in space or bonded to one another can perturb each other's chemical environment, the interactions between resonance peaks can be analysed to reveal spatial relationships such as interatomic distances and bond angles.[31]

If the experiment does work, the properties of the epimer can be analyzed. If identical, then the stereocenter plays no role in the analogs and possibly Janolusimide's biological abilities.

Conclusion

The Weinreb amide has been synthesized in high yield with good purity. However, yield of the hydride reduction is too low to be considered successful. This process, with small alterations in procedure, could be used in the synthesis of many other natural products with dipeptide fragments. Although not complete, reaction of the potential aldehyde with 3-propionyl-2-oxazolidinone would generate an aldol coupling. Completion of the model reaction, presents the ability to control the reactivity and stability of the original synthesis of the acyclic dipeptide aldehyde fragment. After completion of the actual aldol synthesis we can look further into unnaturally occurring analogs of the Janolusimides for a possible better mechanism.

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