Synthesis of Unnatural Alkaloid-Type Compounds from Terpenoid-Derived Natural Products by Nitrogen Insertion via Aminocyclization

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http://hdl.handle.net/2144/13406
Boston University
Honors Thesis:
Synthesis of Unnatural Alkaloid-Type Compounds from Terpenoid-Derived Natural Products by Nitrogen Insertion via Aminocyclization

Submitted in partial fulfillment of the requirement for Honors in Chemistry: Biochemistry, CH 401, 402

April 26th 2013

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Acknowledgements

I am very thankful for having the opportunity to participate in a research lab. I would like to thank the Undergraduate Research Opportunities Program (UROP) at Boston University for awarding me two semesters of FROG grants, and UROP and the Mason Fund for a travel grant to present this project at the ACS National Meeting in New Orleans. More importantly, I am grateful to those who have helped me with my research at Boston University. I am thankful that Dr. John Snyder provided me the opportunity to work in his organic research group. I would like to thank Dr. Seann Mulcahy for being my lab mentor for my first year in the research lab. Finally, I would like to express my gratitude to members of Snyder Group, who provided assistance and insight to me throughout the project. In the research lab, I have learned many important skills which will be useful in my future as a chemist as well as life lessons. The experiments in the lab taught me that success requires many failures beforehand.
Abstract

The synthesis of unnatural alkaloid-type compounds from natural products using an oxidative alkene cleavage, reductive aminocyclization sequence was explored. The natural product cholesterol, as the initial model, was treated with ozone to cleave the 5, 6-alkene, forming the B-ring seco ketoaldehyde. Various reaction conditions were then tested with 4-methoxybenzylamine as primary amine source to optimize the reductive aminocyclization reaction. It was found that running this reaction in THF at 60°C for one hour in the microwave provided a yield of 70%. Using this protocol, the reaction of cholesterol derived ketoaldehyde with various other primary amines gave mixed results.
Introduction

Alkaloids are a group of natural products containing at least one basic nitrogen atom in their structures, which often possess multiple stereocenters as well as interesting polycyclic skeletons. Insertion of a nitrogen atom into a hydrocarbon skeleton increases the polarity of the organic compound and brings potential hydrogen bonding with other proteins with its lone pair, which allows alkaloids to have potent biological effects. Lycopodine (Figure 1), isolated from Lycopodium clavatum spores, was found to trigger apoptosis in prostate cancer cells\(^1\). The indolealkaloids aspidospermine (Figure 1) possesses adrenergic blocking activities\(^2\).

Terpenoids are another large class of natural products biosynthetically assembled from five-carbon isoprene (Figure 2) units. The chemical structures of terpenoids vary greatly in terms of functional groups as well as the carbon skeleton. Betulin, luteone, and callicarpenal (Figure 2) are few examples in this diverse chemical class.

Typically, natural products exist in nature only have one specific diastereomeric, enantiomerically pure form, which can be a synthetic challenge in an organic chemistry laboratory. Therefore, we are interested in preparing a small library of synthetic, unnatural
alkaloids from non-polar terpenoids in a minimum number of steps. Our goal was to convert relatively abundant, terpenoid natural products into readily reactive scaffolds, which could then react with different amine sources, thereby incorporating a nitrogen into the structure of the natural product and create a small library of unnatural alkaloids for biological testing. The insertion of nitrogen into a non-polar terpenoid increases the ClogP of the structure, providing better bioavailability in cells. Furthermore, due to the original of their natural starting material, the synthetic alkaloids would contain complex chemical structures, which diversify a library collection of purely synthetic organic compounds. When the reductive aminocyclization with the formation of new stereocenters, it was hoped that the stereochemistry of the original natural product scaffold would exert high levels of stereocontrol.

Reductive amination is a classic organic chemistry reaction to form $\sigma$-bond between a carbonyl (aldehyde or ketone) and an amine in the presence of a reducing agent. A Brønsted or Lewis acid is often added to catalyze the reaction by activating the carbonyl equivalent. Reductive aminocyclization is a double reductive amination between a dione substrate and primary amine. The end product of reductive aminocyclization is a tertiary amine from the formation of two new carbon-nitrogen bonds. There are many literature references for this reaction. Hudlicky and coworkers prepared the hexahydroazepine core of natural product (-)- and (+)-balanol by reductive aminocyclization (Scheme 1). This synthetic approach took advantage of the reactive and unhindered aldehyde centers, which are readily to react with benzyl amine to

![Scheme 1: Oxidative cleavage followed by reductive aminocyclization to generate the hexhydroazepine core of balanol.](image_url)
generate a tertiary amine. In this example, stereocontrol was not an issue since no new stereocenters were formed.

\[ \text{Scheme 2: Generation of 2-N-pyrrolidine derivatives using reductive amination ring closure} \]

Reductive aminocyclization was also used to generate a library collection, as reported in the literature. Derivatives of 2-N-pyrrolidine were prepared using reductive aminocyclization by Jenkins lab \(^5\)(Scheme 2). Using acetic acid as a catalyst, diones of sugar derivative were converted to pyrrolidine derivatives with a wide range of yields. It was noted that the reductive amination ring closure for the diketone substrate had poor diastereomeric selectivity. It only had a 2:1 diastereomer ratio, favoring the \(\beta\)-methyl at C9. This poor stereocontrol can be attributed to the acyclic ketone, where the closest stereocenter is located at the \(\beta\) position to the carbonyl. As a result, the reducing agent did not have a strong stereoselectivity to reduce the iminium ion intermediate of one side over the other.

For this project, cholesterol was selected as the starting material for unnatural alkaloid library collection (Scheme 3). The dione substrate of cholesterol was prepared upon ozonolysis using a modification of literature procedure \(^6\). The isolated ketoaldehyde

\[ \text{Scheme 3: Retrosynthesis of unnatural alkaloid library collection using cholesterol as starting material and reaction optimization for reductive aminocyclization} \]
then underwent reductive aminocyclization to generate a tertiary amine. Reaction optimization for reductive aminocyclization was performed and different types of amine sources were attempted with mixed result.

Further diversification of unnatural alkaloid was performed with propargyl amine derived substrate. 1,2,3-Triazole analogues were synthesized (Scheme 4) using a literature procedure of the Click chemistry\textsuperscript{7} of Azide-alkyne Huisgen cycloaddition\textsuperscript{8}, with \textit{in situ} generation of the organic azide. 1,2,3-Triazoles can be found in several pharmaceutical drugs. Tazobactam, an active ingredient in ZOSYN\textsuperscript{®}, is used in combination with piperacillin to increase the antibiotic activities against species with beta-lactamase expression\textsuperscript{9}. The \textit{in situ} generation of azide and subsequent Click reaction offered an effective method to synthesize a small library of 1,2,3-triazoles, further diversifying the small library collection of unnatural alkaloid.

\textit{Figure 3: Tazobactam, an active ingredient in ZOSYN\textsuperscript{®}, patented by Wyeth}

\textit{Scheme 4: Library synthesis of 1,2,3-triazole using in situ azide generation and click chemistry}
Results and Discussion

Preparation of Dione Derivative 2 of Cholesterol: The preparation of the B-ring seco ketoaldehyde 2 was accomplished by ozonolysis of cholesterol (1) (Scheme 4). Surprisingly, the dangerous ozonide intermediate was isolated when following the literature procedure\textsuperscript{6} using metallic zinc as a workup due to a solubility problem. When dimethyl sulfide was used as an alternative to break up the ozonide, a yield of 80\% was obtained, which was relatively high given the harsh nature of ozone.

Scheme 5: Preparation of dione from cholesterol using ozone

Optimization of Reductive Aminocyclization Using 4-Methoxybenzylamine: The reductive aminocyclization was first performed in dichloromethane (DCM) with acetic acid as catalyst, and sodium cyanoborohydride in 40°C for 16 hours. The reaction yield was only 19\%. Then a small screening of solvent and reducing agent suggested that THF performed slightly better as a reaction solvent with a 24\% yield. The reaction was then placed in a microwave at 40°C for 1 hour in the presence of zinc chloride, but no cyclized product was observed. Only the first reductive amination, non-cyclized reaction product (Figure 4) was observed. The temperature was then increased to 60°C.

Figure 4: non-cyclized product of cholesterol derivative
Table 1: Optimization of Reductive Aminocyclization using 4-methoxybenzylamine

<table>
<thead>
<tr>
<th>Reducing Agent (4 eq)</th>
<th>Acid catalyst (1 eq)</th>
<th>Reaction condition</th>
<th>Solvent</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCNBH₃</td>
<td>AcOH</td>
<td>16h, 40°C</td>
<td>DCM</td>
<td>19% yield</td>
</tr>
<tr>
<td>NaCNBH₃</td>
<td>AcOH</td>
<td>16h, 40°C</td>
<td>DCE</td>
<td>Non-cyclized product</td>
</tr>
<tr>
<td>NaCNBH₃</td>
<td>AcOH</td>
<td>16h, 40°C</td>
<td>THF</td>
<td>45% yield</td>
</tr>
<tr>
<td>NaB(OAc)₂ H₂O</td>
<td>AcOH</td>
<td>16h, 40°C</td>
<td>DCM</td>
<td>Trace Product</td>
</tr>
<tr>
<td>NaCNBH₃</td>
<td>ZnCl₂</td>
<td>1h, 40°C microwave</td>
<td>THF</td>
<td>Non-cyclized product</td>
</tr>
<tr>
<td>NaCNBH₃</td>
<td>ZnCl₂</td>
<td>1h, 60°C Microwave</td>
<td>THF</td>
<td>70% yield</td>
</tr>
<tr>
<td>NaCNBH₃</td>
<td>ZnCl₂</td>
<td>5min, 100°C Microwave</td>
<td>PhCl</td>
<td>M-18 as major product</td>
</tr>
<tr>
<td>NaCNBH₃</td>
<td>Zr(OiPr)₄</td>
<td>1h, 60°C Microwave</td>
<td>THF</td>
<td>mixture of diastereomeric cyclization products</td>
</tr>
<tr>
<td>NaCNBH₃</td>
<td>Ti(OiPr)₄</td>
<td>1h, 60°C Microwave</td>
<td>THF</td>
<td>mixture of diastereomeric cyclization products</td>
</tr>
<tr>
<td>NaCNBH₃</td>
<td>LiClO₄</td>
<td>1h, 60°C Microwave</td>
<td>THF</td>
<td>Non-cyclized product</td>
</tr>
<tr>
<td>NaCNBH₃</td>
<td>Zr(OiPr)₄</td>
<td>1h, 60°C Microwave</td>
<td>MeOH</td>
<td>M-16 as major product</td>
</tr>
</tbody>
</table>

and a 70% yield of the desired product was observed as a single diastereomer. Solvent change to chlorobenzene and methanol did not yield the desired product. Lewis acid titanium (IV) isopropoxide and zirconium (IV) isopropoxide did not have diastereomer control at the ketone carbon, though both resulted in the desired aminocyclization. As a result, the optimized reaction (Scheme 6) for 4-methoxybenzylamine was used for incorporation of other primary amines into the cholesterol.

Scheme 6: Optimized reaction condition for reductive aminocyclization using 4-methoxybenzylamine
skeleton structure.

**Preparation of Propargyl Derivative of Unnatural Alkaloid 3b**: Using the optimized reaction conditions, propargyl amine was incorporated into the cholesterol-derived ketoaldehyde 2 (Scheme 7). Cyclization product 3b was isolated in 43% yield as a single diastereomer. The stereochemistry of the new stereocenter was determined using coupling constant of the proton, with proton assignments accomplished with a gCOSY spectrum. The H-5 hydrogen could be easily assigned from the spin system delineated in the gCOSY beginning from H3 –H2–H4–H5. Based on the coupling constants of 12.5 and 4.0Hz, proton H5 at the new stereocenter is therefore axial, showing trans diaxial and axial/equatorial couplings with the C4 methylene protons. The generation of the axial proton can be justified by the steric effect at the iminium ion. The axial methyl group blocked the approach of the sodium cyanoborohydride from top part of the molecule. As a result, the hydride preferred to approach from the bottom part of the molecule and generated an axial proton. In general, axial approach is typically favored in borohydride educations of cyclohexanones, and this generality also holds in the case of this iminium ion reduction.
Preparation of 1,2,3-Triazole Derivatives 4a, b: The preparation of 1,2,3-triazole compounds can be achieved by the 1,3-dipolar addition of an organic azide with a dipolarophile, such as the alkyne in substrate 3b in the presence of copper (I) species. The *in situ* generation of organic azide from an inorganic azide and a primary alkyl bromide increases the safety of the reaction as the potentially dangerous organic azide is never isolated. Furthermore, the reaction is economic and green, as the reagents are simple compounds and have little environmental impact. Following the procedure of Sharpless and his colleagues, the triazoles 4a and 4b were prepared from their respective benzylic bromides in modest yield. The reaction yields for both reactions were relatively low, as the conditions were not optimized for the specific substrate. Nonetheless, these two reactions demonstrated that diversification of the aminocyclization products into triazoles is feasible.

*Scheme 8: Azide-alkyne Huisgen cycloaddition with propargyl derivative 3b*
Future Works

Future work will focus on expanding upon the synthetic protocols established in this work to build the targeted libraries of unnatural alkaloids from terpenoid natural products like cholesterol. Considering that the B-ring seco ketoaldehyde 2 can be prepared in one step in good yield from cholesterol, optimization of the reductive aminocyclization with other primary amines can be performed to obtain a more diverse novel alkaloid collection. Incorporation of amino acid derivatives, such as serine ethyl ester and tryptophan methyl ester, can further increase the polarity of the product. The scope of reductive aminocyclization can also be explored with aniline derivatives.

The incorporation of nitrogen can also be applied to other terpenoid systems. Pimaric acid, which can be found in pine trees, can be converted to a trione substrate by oxidation of termination olefin and oxidative cleavage of the substituted alkene (Scheme 9). The two aldehydes can then be joined with different primary amines in a reductive aminocyclization to generate a small library collection of unnatural alkaloids. The remaining ketone can then be modified further to diversify the library collection.

Scheme 9: Creation of alkaloid library with pimaric acid
**Experimental**

**General Methods:** Reactions were carried out in anhydrous solvents (unless noted) under argon atmosphere and performed with oven-dried glassware. Commercially available reagents (Sigma-Aldrich and Alfa Aesar) and solvents (Pharmco, unless noted) were used without further purification. THF was obtained from a still to ensure dryness. Microwave reactions were performed in CEM microwave reactor. NMR spectra were recorded in deuterated chloroform (Cambridge Isotope Laboratories Inc.) solutions on Varian NMR (70.50kG or 117.42kG) for the proton and 2-D spectra. Purification by flash chromatography was performed on silica gel (Sorbent Technologies, 60Å, 230x400 mesh).

**Dione of cholesterol 2 Synthesis:** This compound was synthesized as generally described in Detection of Cholesterol Ozonation Products. 1g of cholesterol 1 was dissolved in 100ml of 9:1 chloroform: methanol mixture in a round bottom. Solution was then cooled to -78°C using isopropanol/dry ice bath and ozonized for 10 minutes. Solution was then purged with nitrogen and 1mL of dimethyl sulfide was added. Solution was stirred at room temperature for 3 hours. The solution was then diluted with 50mL of dichloromethane and washed with water (3 x 100mL). The organic layer was then dried with sodium sulfate and evaporated to dryness in vacuo. The residue was purified using silica-gel chromatography [ethyl acetate: petroleum ether (2:3)] to give a white solid (870mg, 80%). 1H NMR (400MHz, CDCl₃) δ 9.533 (s, 1H, CHO), 4.388 (m, 1H, H-3), 3.000 (dd, J=14.0, 4.0 Hz, 1H, H-4e), 0.927 (s, 3H, CH₃-19), 0.827 (d, J=6.8 Hz, 3H, CH₃-21), 0.782 (d, J=6.8 Hz, 3H, CH₃), 0.778 (d, J=6.8 Hz, 3H, CH₃), 0.603 (s, 3H, CH₃-18);

**4-Methoxybenzylamine Cholesterol Derivative 3a Synthesis:** Substrate 2 (20mg) and 12mg of sodium cyanoborohydride were added to a 10mL microwave reaction vessel equipped with a stir bar. Anhydrous THF (2.2mL) was added to the vessel along with 4Å molecular sieves. Stock solution (0.3M) of zinc chloride in THF (0.16mL, 1 eq.) and 8µl of 4-methoxybenzylamine (8.4mg, 1.28 eq.) were added to the solution using disposable syringe and microsyringe, respectively. Reaction was then subjected to 60°C in a microwave for an hour. Solution was then diluted with 10mL of dichloromethane and washed with 6mL of water and 1mL of saturated aqueous sodium carbonate solution. The organic layer was then dried with sodium sulfate and evaporated to dryness in vacuo. The residue was purified using silica-gel chromatograph [ethyl acetate: petroleum ether, (1:9) to (3:2)]. A white solid was isolated after the fractions with product were evaporated to dryness (17.4mg, 70%). 1H NMR (400MHz, CDCl₃) δ 7.23 (d, J = 7.6Hz, 1H), 6.83 (d, J = 7.6Hz, 1H), 4.00 (d, J=13.6Hz, 1H), 3.80 (s, 3H), 3.56 (br. dd, J=1H), 3.47 (d, J = 13.6Hz, 1H), 2.86(d, J = 12.0Hz, 1H), 0.68 (s, 3H).

**Propargyl Amine Cholesterol Derivative 3b Synthesis:** Substrate 2 (20mg) and 12mg of sodium cyanoborohydride were added to a 10mL microwave reaction vessel equipped with a stir bar. Anhydrous THF (2.2mL) was added to the vessel along with 4Å molecular sieves. Stock solution (0.3M) of zinc chloride in THF (0.16mL, 1 eq.) and 4µl of propargyl amine (3.43mg,
1.3 eq.) were added to the solution using disposable syringe and microsyringe, respectively. Reaction was then subjected to 60°C in a microwave for an hour. Solution was diluted with 10mL of dichloromethane and washed with 6mL of water and 1mL of saturated aqueous sodium carbonate solution. The organic layer was then dried with sodium sulfate and evaporated to dryness in vacuo. The residue was purified using silica-gel chromatograph [ethyl acetate: petroleum ether, (1:9) to (3:2)]. A white solid was isolated after the fractions with product were evaporated to dryness (9.2mg, 44%). 1H NMR (500MHz, CDCl3) δ 3.62 (br. d, J =17.5Hz, 1H), 3.51-3.55 (m, 1H), 3.38 (br. d, J=17.5Hz, 1H), 3.00(dd, J=10.5, 6.5Hz, 1H), 2.77 (dd, J=12.5, 4.0Hz, 1H), 2.47 (ddd, J = 12.5, 11.6, 6.5Hz, 1H), 2.41 (dddd, J= 12.0, 4.0Hz, 4.0Hz, 2.5Hz, 1H), 2.19 (br.s, 1H), 0.89 (d, J = 6.5Hz, 3H), 0.87 (d, J=2.5Hz, 3H), 0.85 (d, J=2.0Hz, 3H), 0.83 (s, 3H), 0.66 (s, 3H).

Triazole Derivative of 3, 5-dimethoxybenzyl 4a Synthesis: Substrate 3b (10mg), 2mg (1.36eq.) sodium azide, and 8mg (1.53eq.) of 3, 5-dimethoxybenzyl bromide were added to a reaction vial equipped with stir bar. A stock solution of 3mg copper (II) sulfate and 5mg of (+)-sodium ascorbate with 3mL of 1:1 tert-butanol: water were made and 0.3mL of the solution was added to the reaction mixture. Reaction was then heated to 70°C for 16 hours and diluted with 10mL of dichloromethane. It was then washed with 6mL of water and the organic phase was dried with sodium sulfate. The dried layer was then evaporated to dryness in vacuo and product was purified using silica-gel chromatograph [ethyl acetate in petroleum ether: 10% to 100%, methanol in dichloromethane: 5% to 10%]. Brown foam was isolated from the concentration of fractions (4mg, 28%).

Triazole Derivative of 2, 6-difluorobenzyl 4b Synthesis: Substrate 3b (10mg), 2mg (1.36eq.) sodium azide, and 6mg (1.28eq.) of 2, 6-difluorobenzyl bromide were added to a reaction vial equipped with stir bar. A stock solution of 3mg copper (II) sulfate and 5mg of (+)-sodium ascorbate with 3mL of 1:1 tert-butanol: water were made and 0.3mL of the solution was added to the reaction mixture. Reaction was then heated to 70°C for 16 hours and diluted with 10mL of dichloromethane. It was then washed with 6mL of water and the organic phase was dried with sodium sulfate. The dried layer was then evaporated to dryness in vacuo and product was purified using silica-gel chromatograph [ethyl acetate in petroleum ether: 10% to 100%, methanol in dichloromethane: 5% to 10%]. Yellow foam was isolated from the concentration of fractions (5mg, 36%).
References


Appendix

1: 4-Methoxybenzylamine Cholesterol Derivative 3a ¹H NMR Spectrum
2: 4-Methoxybenzylamine Cholesterol Derivative 3a ¹H NMR Spectrum (2-5ppm)
3: Propargyl Amine Cholesterol Derivative 3b ¹H NMR Spectrum
4: Propargyl Amine Cholesterol Derivative 3b ¹H NMR Spectrum (2-4ppm)
5: Propargyl Amine Cholesterol Derivative 3b gCOSY
6: Propargyl Amine Cholesterol Derivative 3b gCOSY (2-4ppm)