Abietic acid in the synthesis of unnatural alkaloids

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Honors Thesis:
Abietic Acid in the Synthesis of Unnatural Alkaloids

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Abstract

The synthesis of unnatural alkaloid-type products from abietic acid was explored. In order to synthesize these products the protection of the carboxylic acid was achieved followed by a selective dihydroxylation of the C-ring was followed by oxidative diol cleavage. Using the dione yielded a reductive aminocyclization followed. This reaction was first explored using 4-methoxybenzylamine and after optimization the alkaloid-type product was synthesized in a 34% yield. Using the optimized conditions a small library of analogues were synthesized utilizing different primary amines.
Introduction

Natural products have represented a rich resource for drug development. The early pharmaceutical industry was dependent upon isolated natural products as drugs, and indeed perhaps the most influential drug, penicillin, is a natural product. Even with the growth of HTS and combinatorial chemistry starting in the late 1980’s, natural products were still found to be particularly promising screening hits. In the period between 1981 and 2006, of the 1184 FDA approved New Chemical Entities (NCEs) 52% were based upon natural product scaffolds\(^1\).

As the pharmaceutical industry has continued to grow, the focus on natural products has decreased. Currently, the number of natural product-based drugs in the pipeline is relatively consistent with past numbers, but in the future this number is anticipated to drop\(^2\). Many of the larger pharmaceutical companies have either significantly downsized or completely eliminated their natural products investigation teams. The major reason for this is economics. The cost of bringing a natural product lead to market is high compared to synthetic drugs. There are several causes for this. One significant reason is that an adequate and environmentally responsible method of collecting or synthesizing the natural product is required. Due to the high complexity of most natural products, the synthetic tractability is extremely low and is non-trivial\(^3\). The low synthetic tractability of many natural products also makes it more challenging to obtain a valid SAR. This makes it more challenging to obtain a valid mechanism of action and binding mode\(^2\). Also, the readily obtained natural products have already been largely tapped; therefore, any novel natural product targets will be difficult to obtain in sufficient quantities to be a marketable drug. For these reasons, the drug
development community has moved further and further away from their roots in natural products chemistry.

The powerful techniques being developed today focus on screening large numbers of molecules, ultra-high throughput screening (UHTPS) and DNA encoded libraries (DELs), and covering chemical space in the most efficient way possible.\textsuperscript{4,5} While these methods are extremely powerful, they typically leave a large area of chemical space unexplored. Natural products tend to violate one of the dogmas of the pharmaceutical industry, $\text{MW} \leq 500$, as Lipinski noted in his original work.\textsuperscript{6} Fully half of the natural product drugs approved during the period discussed above were well beyond this limit. One reason for this characteristic is that biologically active natural products have evolved over millennia for specific biological reasons. As a consequence, their bioavailability is usually quite high. This allows access to a larger degree of scaffold diversity and complexity.

Natural products can also be exploited as starting materials for synthesis; with a twist on simple natural products, unnatural derivatives can be synthesized. This is the field of natural product remodeling. A natural product with a high level of defined stereochemistry allows access to a larger degree of scaffold diversity and complexity. In the subsets of natural product scaffolds remodeled, none of these have been alkaloids. Nitrogen heterocycles represent perhaps the largest group of synthetic targets in the pharmaceutical industry,\textsuperscript{2} and many can be considered unnatural alkaloids.

The approach investigated in this work involves the modification of readily available natural products in order to yield alkaloid-like molecules for screening through the incorporation of
nitrogen into the carbon scaffold. Such a strategy is distinct from simple derivitizations which merely decorate an existing scaffold with a nitrogen-containing substituent. This approach has been used in the past on terpenoids such as betulin\textsuperscript{7} and fumagillol\textsuperscript{8} resulting in a number of synthetic analogues. Starting with a natural product with a structurally complex scaffold allows for the synthesis of relatively complex molecules in a minimal number of steps.

The specific focus of this project was the natural product abietic acid. This diterpene is readily available as the major component of pine resin, and is relatively inexpensive commercially. The particular challenge associated with using this as the starting point is the already high lipophilicity of the substrate, cLogP of 5.01. Therefore, a major goal of the synthesis was to increase structural diversity while also increasing the polarity of the molecule. The solution determined to balance both synthetic tractability and structural diversity was to synthesize a small library of alkaloid-like derivatives of abietic acid. Alkaloids are basic, nitrogen-containing natural products, and as such, the impact of nitrogen on the ADME properties of a product is two-fold.

The nitrogen heteroatom increases polarity, and the ability of a basic nitrogen to be protonated at neutral pH drastically increases aqueous
solubility, and therefore the bioavailability. Along with the favorable ADME properties, nitrogen atoms are commonly found to participate actively through hydrogen bonding in target binding. A large number of currently marketed drugs are classified as alkaloids. The goal of this research was to incorporate nitrogen into abietic acid through an alkene cleavage/reductive aminocyclization sequence.

In Scheme 1, two target product types are shown. Due to the highly conserved synthetic scheme for each product type, these two product types were developed in parallel. The final step in the synthesis of both product types is a reductive aminocyclization. This reaction has been largely underrepresented in the literature to date. The process uses the standard conditions for a simple reductive amination, however, instead of using a simple ketone or aldehyde as the substrate, a dione is used. When starting with a primary amine the reaction proceeds through two successive reductive aminations in order to yield cyclic tertiary amines. There are several isolated examples of this type of chemistry being utilized. Scheme 2 shows the work done by Hudlicky and coworkers in the synthesis of the hexahydropyrazine core of (+) and (−)-balanol. Their approach is remarkably analogous to the required transformation to reach our products. The cleavage of the six membered ring in order to yield the required dione is followed directly by the reductive aminocyclization to give a fair yield over the two steps, 64%. There are two major differences between this chemistry and the chemistry proposed for abietic acid. The first is that
the Hudlicky system is largely unhindered and therefore the reduction should proceed more smoothly. Second, both carbonyls participating in the reaction in Hudlicky’s work were aldehydes. This has two implications. First, the more reactive aldehyde should react more efficiently to form the desired substrate and, more importantly, there was no new stereogenic center being formed. In the case of both product classes being targeted in our research, a new chiral center is formed from the keto-aldehyde precursor to the aminocyclization. This adds a layer of structural complexity at the cost of potential issues with diastereoselectivity of the reduction.

**Scheme 3.** Enantioselective synthesis of indolizidine alkaloids. Another approach reported in the literature to yield aminocyclization products is shown in Scheme 3. This approach utilized Pd/C as a hydrogenation catalyst, which also accomplished deprotection of the CBZ group. This procedure depends upon the reduction of the iminium ion by the catalyst to occur preferentially to the reduction of the ketones. In order to ensure this selective reactivity, the reaction was carried out without increased pressure, which is usually required for the hydrogenation of ketones. The tethered amine also facilitates the reductive amination. The authors report that the first reduction is entirely stereoselective, due to the preexisting stereogenic center, and the second reduction results in a 5:1 diastereomeric ratio. It should be noted that a loss of yield in this reaction was attributed to the increase volatility of the product resulting in loss during work up.\(^{10}\)
Comparing these procedures, the process employed by the Hudlicky group in the synthesis of balanol was adopted by us as the starting point for reaction optimization. There are several reasons for this approach being chosen. First, the diones derived from abietic acid, Scheme 4, in our work both contain an aldehyde, which are more reactive than the ketone used in Scheme 3. In addition, the dione, 4, contains a double bond which would be reduced under the hydrogenation conditions.

My research focused on the synthesis of alkaloid-type products 5 from abietic acid. The reaction sequence followed is shown in Scheme 4 above. The first step involves the methylation of the abietic acid to form the abietic methyl ester. This step was then followed by the stereoselective dihydroxlation of the C ring alkene in order to form diol 3, converted by diol cleavage to form the dione precursor, 4, for the aminocyclization. Using this dione as a starting material, a small library was constructed by incorporating different amines into the scaffold. The majority of the work done on this project focused upon the optimization of the aminocyclization chemistry used to synthesize these final products, which proved challenging.
Scheme 4. Overall reaction scheme to yield type 5 analogues

1. $\text{Me}_2\text{SO}_4, \text{K}_2\text{CO}_3, \text{K}_2\text{O}_2\text{SO}_4 \text{ (6 h) } 94\%$

2. $\text{K}_2\text{O}_2\text{SO}_4 \text{ (48 h) } 66\%$

3. $\text{NMO, pyr. (48 h) } 99\%$

4. $\text{NaIO}_4 \text{ H}_2\text{O/ EtOH (6 h) }$

5a. $\text{H}_2\text{N} - \text{O}\text{Me}$

5b. $\text{H}_2\text{N} - \text{O}\text{Me}$

5c. $\text{H}_2\text{N} - \text{O}\text{Me}$

R-NH$_2$
Results and Discussion:

Synthesis of the methyl ester of abietic acid.

Scheme 5. Methylation of abietic acid.

Abietic acid is an extremely well characterized compound and significant structural modifications have been reported ranging back to 1969. A common first step for any synthesis that plans to conserve the carboxylic acid moiety is methyl ester formation. This may seem counter intuitive considering that in terms of both increasing water solubility and maximizing potential bioavailability, the carboxylic acid would be preferable to the methyl ester. A recent study on the bioactivity of abietane derivatives against HeLa shows better bioactivity for the ester than the carboxylic acid, 3.6 μM vs 14.9 μM CC<sub>50</sub>.<sup>11</sup>

The main reason why the ester is the preferred starting point of an overwhelming majority of synthetic schemes is the chromatographic properties of carboxylic acid derivatives in general and these compound in particular. When run on a silica gel column, carboxylic acids tend to either streak, making separation problematic, or to stick to the silica to such a large extend that a significant amount of yield is compromised, although using a drop of acetic acid as a component of the mobile phase can help alleviate this latter issue. Another factor in the
decision to methylate as the first step is the tolerance of many methylation procedures. The abietic acid that is commercially available is technical grade, 85% purity. Methyl ester formation followed by purification helps “clean up” the abietic acid.

Dimethyl sulfate was chosen as the methylation reagent due to the reported high yields. The other main reported methylation reagent used for this transformation is diazomethane, which displays mildly higher yield at the cost of more preparation time and larger safety concerns.

**Dihydroxylation of the abietic methyl ester.**

![Scheme 6. Dihydroxylation of the C-ring alkene of abietic acid methyl ester](image)

The dihydroxylation of the methyl ester of abietic acid is a well characterized reaction. When considering the osmylation of compound 2 there are two possible sites of reactivity: the alkenes in the B and C rings, respectively. At first it may not appear that there should be overwhelming selectivity for either alkene; however, there is a large body of work that indicates that the reaction is highly regioselective for dihydroxlation of the C ring. Also, a possible concern for this process would be the dihydroxylation of both alkenes. In order to test this, several reactions were left for various periods of time after the reaction had proceeded it’s normal course. There was no observed dihydroxylation of the B ring.
The startling degree of regioselectivity of this reaction has been prescribed to several factors. The first is the lesser steric demands of the reactive alkene. Although both of the alkenes appear to be relatively hindered, the ring fusion center of, C9, the B/C rings is the more hindered of the two positions. Also, when considering the electronics of the reaction, the C ring would be expected to be more electron rich due to the ability of the isopropyl to line up with the π-cloud, allowing for greater sigma-donation.

Also, there is the question of the diastereoselectivity of the dihydroxylation, which is important in the preparation of the alkaloid-type 6 products, which retain the diol functionality. The major product of the dihydroxylation, formed in a 3:1 ratio, using the procedure shown in Scheme 5, was separated and completely characterized by NMR. The structural assignment as the β,β-diol was made via an NOE between H17 and the equatorial proton of H12 centered around 1.72 ppm. The equatorial assignment for H12 was made via coupling constants (J = 13.5, 3.3, 3.3 Hz). Only one large gem-coupling (J = 13.5 Hz) was observed for this ddd, the other couplings were assigned as eq/eq vicinal coupling (J = 3.3 Hz), and eq/ax coupling (J = 3.3 Hz). The other proton on this carbon, identified via HSQC, displays two large coupling constants (J = 13.5, 13.1, 3.3 Hz), typical for an axial hydrogen with one gem-coupling and one vicinal trans-diaxial coupling, and confirming these assignments of the C-12 methylene hydrogens.

Given that the modified C-ring is in a chair-like orientation, this equatorial proton will be facing down. Therefore, observing the NOE between the isopropyl methyl and the equatorial proton of H12 confirms that the β,β-diol is the major product. It should be noted that no other constructive NOEs were found due to the spin quenching of the hydroxyl groups.
The standard dihydroxylation procedure achieves a 2:1 diastereomeric ratio determined via NMR. Isolated yields tend to produce a 3:1 diastereomeric ratio. This is presumably due to more favorable chromatographic properties of the major diastereomer. In an attempt to yield a more favorable diastereomeric ratio, both commercially available AD-Mix dihydroxylation reagents were screened. The hope was that one of the AD-Mix reagents would prove complimentary to the preexisting stereochemical bias. The results of this screen are shown below in Table 1. All reactions were run in 2:1 \textsuperscript{1}BuOH:H\textsubscript{2}O for 64 hours. The potassium osmate reaction used N-methylmorpholine N-oxide (NMO) as the stoichiometric reoxidant and pyridine was used as the amine ligand.

**Figure 2.** NOE observed between H17 and H12.
Table 1. Screen of dihydroxylation procedures\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Method</th>
<th>Yield $\beta$</th>
<th>Yield $\alpha$</th>
<th>RSM$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD-mix $\alpha$</td>
<td>19%</td>
<td>11%</td>
<td>58%</td>
</tr>
<tr>
<td>AD-mix $\beta$</td>
<td>0%</td>
<td>16%</td>
<td>84%</td>
</tr>
<tr>
<td>Standard</td>
<td>45%</td>
<td>14%</td>
<td>0%</td>
</tr>
</tbody>
</table>

\textsuperscript{a} A 100 mg sample of product 2 was dissolved in 0.84 mL $^t$BuOH. For the AD-Mix reactions 44 mg samples of the mixes were added. For the standard conditions a 55 mg sample of NMO and a 0.01 mL sample of pyridine were added followed by 2.3 mg of potassium osmate. All reactions were diluted with 0.42 mL of water then heated to 80°C for 64 hours.

\textsuperscript{b} Residual Starting Material

Table 1 shows that using the AD-Mix reagents had significant effects on the rate of the reaction. In both cases, a large amount of starting material was recovered despite allowing each to react for another 16 hours beyond typical conditions. This suggests that the use of the bulky amine ligands slowed the reactions significantly. This has been previously observed with other highly hindered alkenes. The most startling result was the complete reversal in selectivity observed when AD-Mix $\beta$ was used. There was none of the $\beta$-diol product observed either via NMR. Despite this increase in diastereoselectivity, the extremely low yield and high reaction time required made it unsuitable for further use.
Periodate Cleavage of the Diol Mixtures.

Scheme 7. Periodate cleavage of the diol mixture.

The classic reagent used for the cleavage of cis-diols is sodium meta-periodate and as such this was the first reagent tried. It was found that this reaction proceeded very cleanly to give nearly quantitative yield, 99% of keto-aldehyde 4. A mixture of both diastereomers of diol was used, as the stereogenic centers of the diol are destroyed in the reaction. The one lesson learned in this reaction has been the intolerance of product 4 to column chromatography, which is not especially surprising for a dione. Even when run through a short plug of silica gel, a large loss of mass, approximately 30%, was observed.

Ensuring purity of the starting diol proved to be a more effective method to obtain pure keto-aldehyde 4. The one caveat is that the cleanness of the reaction seems highly dependent upon the quality of the meta-periodate used. As the meta-periodate ages, side products begin to appear and reaction time increases.
Direductive Aminocyclization.

Scheme 8. Microwave conditions initially used for aminocyclization to yield type 6 products. The original trials done in order to achieve the reductive aminocyclization were developed in large part by two other members of the Snyder group, Cody Schwarzer and Howard Szeto. Cody Schwarzer’s work focused on the synthesis of the type 6 products, Scheme 8, while Howard Szeto was doing similar chemistry using cholesterol as the starting material, Scheme 9. The standard procedures from their work were applied to 4.

Scheme 9. Reduction condition used to convert the cholesterol derived dione to the aminocyclization product.

Using this as the starting point, dione 4 was subjected to these conditions using 4-methoxybenzylamine (PMB amine). The PMB amine was used as the initial amine due to its ease of monitoring by both TLC and NMR. Also, the use of PMB amine allows for possible deprotection through hydrogenation or other means, thereby allowing for further diversification.
An exhaustive screen was done by Howard Szeto in order to determine the best Lewis catalyst for the aminocyclization, and zinc chloride gave the best yields. The protocols called for the use of microwave irradiation to effect the aminocyclization. This catalyst system was applied to keto-aldehyde 4 in the synthesis of the type 5 products; however, extremely poor yields, 22%, were observed.

Scheme 10. Microwave conditions initially used for aminocyclization.

![Scheme 10](image)

The ring closure step forms a new stereocenter. The formation of product 5a results in only one isolable diastereomer, though trace amount of another diastereomer may be seen on crude NMRs, but this has not been confirmed. The favored face of hydride addition is not obvious when looking at the structure. In order to help elucidate the structure of the favored product, an NMR study is being conducted. The difficulty with such a study is that there are no conclusive NOEs. We hope to make the stereochemical assignment through the coupling constant of H13 and the protons of carbon 12. Unfortunately, the conformations of 7-membered rings are extremely difficult to predict. Therefore, in order to obtain proper dihedral angles, a geometry optimization using Gaussian 5.03 must be done.
In order to optimize the aminocyclization for keto-aldehyde 4, zinc triflate was examined as a catalyst. Zinc triflate is a stable solid and therefore, the quality of this reagent was not in question. The change in the counter ion should in theory have very little effect on the catalytic activity, assuming comparable solubility. Once this change in catalyst system was made, a relative increase in yield was seen, to 33%. This was promising and suggested two possibilities. The first possibility is that the zinc catalyst was indeed the active catalyst and the higher quality reagent was responsible for the increase in yield. A second possibility was that residual triflic acid was the actual active catalytic species.

In order to test this supposition, the catalyst system was switched once again to using a drop of glacial acetic acid. Triflic acid could have been used, however, such a catalyst system would be extremely harsh, since triflic acid’s pKa is -12. Acetic acid is perhaps the most classic catalyst used in reductive aminations, and therefore was the obvious choice for an acid catalyst. Using acetic acid, the yield was once again observed to be 33%. This result suggested that the residual acid was indeed acting as the catalyst when using zinc triflate. With this information, a permanent switch to the acetic acid catalyst system was made. Further investigation also proved that the synthesis of type 6 compound was most efficiently carried out using acetic acid as the catalyst.

Considering that the zinc catalyst system developed for cholesterol was not as effective as acetic acid for the type 5 compounds, the necessity of microwave reactivity was revisited. The major drawback in using the microwave is the limited scalability of such reactions. Specifically pertaining to the cholesterol series, a reduction in yield was observed when going beyond a 50
mg scale. Furthermore, a large amount of variability has been observed in the effectiveness of the microwave reactor utilized for these reactions. Therefore, the reaction was attempted using conventional heating methods.

A 10 mg scale pilot reaction was run and monitored via LCMS. Using catalytic acetic acid the product was observed via UPLC-MS, [M+1]=454.1. Given this positive result, attempts to optimize this chemistry by changing several parameters were undertaken. It was discovered that for the best results, the reaction needs to be anhydrous initially, presumably because the imminium ion formation is slowed considerably with water present, and water itself is formed in the course of the reaction. Therefore, all glassware used in this reaction was flame dried under high vacuum and then put under an argon atmosphere. Still-dried THF has to be used for the best results.

Three variables subsequently studied were the concentration of the starting material, the equivalency of the PMB amine, and finally the amount of acid catalyst used. These reactions were carried out on 25 mg scale, and were monitored via UPLC-MS. The first variable tested was concentration. Using 4 equivalents of the PMB amine, the concentration of 4 was varied between 1.43 mM and 0.18 mM. No significant concentration dependence was observed. Given that concentration did not seem to affect reactivity unduly, a median concentration of 0.5 mM was chosen for the remaining experiments.

<table>
<thead>
<tr>
<th>Concentration of 4 (mM)</th>
<th>Reaction Time(^a,b)</th>
</tr>
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<tbody>
<tr>
<td>0.18</td>
<td>15h</td>
</tr>
<tr>
<td>0.36</td>
<td>15h</td>
</tr>
<tr>
<td>0.72</td>
<td>15h</td>
</tr>
<tr>
<td>1.43</td>
<td>15h</td>
</tr>
</tbody>
</table>

\(^a\) Reaction was attempted using conventional heating methods.
\(^b\) UPLC-MS was used to monitor the reaction.
The next variable considered was the equivalency of the primary amine. The UPLC data showed that the reaction was slowed considerably when less than two equivalents of amine were used. If more than 2 equivalents were used, there was no discernable effect on the reaction rate. Also, earlier studies in our group had shown that excessive amine considerably complicated purification. The choice was therefore made to used 2 equiv. of amine, thereby balancing reaction rate and ease of purification.

| Table 3. Results of the amine equivalency optimization trial |
|-----------------|-----------------|
| Equiv. PMB-NH₂  | Reaction Time<sup>a,b</sup> |
| 1               | >21h             |
| 1.5             | >21h             |
| 2               | 15h              |
| 4               | 15h              |

<sup>a</sup>Reactions were considered complete when product 4, [M+1] = 349.1, was no longer observed.

<sup>b</sup>Reaction was checked for completeness at 3h intervals.

Lastly, the amount of acetic acid was optimized for the reaction. The major comparison being made here was between the use of sub-stoichiometric, 0.2 equivalents, versus super-stoichiometric, 2 equivalents, acetic acid. There is literature supporting the use of sub- and super-stoichiometric acetic acid in reductive amination. In our hands, a large rate enhancement was observed when the reaction is carried out with super-stoichiometric acetic acid (4 hours versus 15). No change in yield was observed. Therefore, super-stoichiometric acetic acid was determined to be superior. A fourth obvious variable to be examined would have been reaction temperature. However, given the sufficient reactivity at room temperature, it was determined that this was not necessary.
Given all of these optimization studies, it was hoped that an increase in yield would have result; however, the yield remained moderate at best, 34%. The aspects of the reaction that was optimized, was the rate (4 fold increase). In a final attempt to optimize the yield, a change in reducing agent was examined. When triacetoxyborohydride was used, no discernable effect was seen, 34% yield was obtained.

The aminocyclization products tend to have considerable contamination and were generally difficult to purify. In an attempt to alleviate this problem, a large number of solvent systems were screened for extraction and chromatographic efficacy. Several lessons were learned. The first and most obvious was that polar solvents such as methanol and acetone were extremely unsuitable for use in purification. When purification was attempted with either of these modifiers as part of the mobile phase for silica gel chromatography, severe streaking was observed. The most efficacious polar modifier found was ethyl acetate. The most effective non-polar component of the eluent was found to be toluene.

A number of other things were done in an attempt to optimize the purification conditions. Alkaloid purification is regarded as extremely difficult using silica gel, and as such a number of specialized techniques can be cited. The technique found to be most suited to this purification was to wash the column with triethylamine (TEA). A wash with the TEA neutralizes the silanols and typically allows for better purification.

Even with this optimized solvent system, the separation remained poor, though improved. In order to further improve the purification preparatory, thin layer chromatography was utilized. The advantage of prep TLC is that much higher RFs can be used in order to achieve separation.
Using this technique, it was possible for the first time to easily and reliably obtain pure product. The major drawback of this method is the limited scale, usually maximizing at 100 mg of product per plate. Although this could prove problematic in the future, the current reaction scale never exceeded 100 mg, so prep TLC was adequate.

Given the optimized reaction procedure and purification protocols, the project moved ahead using other primary amines to create a small library of compounds for screening. The second amine used in the aminocyclization was 3,4,5-trimethoxybenzylamine. This cyclization, as expected due to the extreme structural similarity to PMB amine, proceeded in a similar manner as the synthesis of product 5a. A reduced yield was observed for this product, 17%.

The third amine incorporated into the scaffold was 2-furfuryl amine, producing 5c. The reaction proceeded under standard conditions, however, in the NMR spectrum of the crude product, a 2:1 diastereomeric ratio of major to minor products was observed. The purification of these diastereomers was attempted via preparatory TLC; however, the major and minor products have nearly identical RF values and the separation proved impossible.

An attempt was subsequently made to improve this diastereomeric ratio through the use of a bulky reducing reagent. There is remarkably little literature discussing this
topic; however, there a single is precedent for using 9-cyano-9-borobicyclo(3.3.1)nonane (9-BBNCN) in reductive aminations, Scheme 11. The work done in our group using this reagent has shown modest diastereoselectivity improvement when reducing cyclohexyl imminium ions, 0.64:0.36 equatorial attack to axial attack, compared to a ratio of 0.32:0.68 when using sodium cyanoborohydride.

The first reaction attempted using this reagent was done to synthesize product 5a, as this chemistry was the best optimized. The conditions used in this reaction were identical to the standard procedure, except that 9-BBNCN replaced the sodium cyanoborohydride. When these conditions were used it was found that the use of 9-BBNCN resulted in the reduction of aldehyde of 4, though not the ketone. In order to address this issue, a stepwise one pot method was attempted. The amine was added to the dione along with the acetic acid catalyst. The 9-BBNCN was only added after all of 4 had reacted to produce the imine, observable via TLC and the bright yellow color of the solution. Using this strategy, the first reduction preceded smoothly, producing the primary amine (Figure 4); however, even after 48 hours, only a trace of cyclized product 5a was observed. This was puzzling because using the standard procedures with cyanoborohydride, the initial amination product 5d is usually converted to the cyclized product before it can be detected. In this case 5d could not be pushed forward with 9-BBNCN, even after moderate heating, 24 hours 40°C.

Scheme 11. Reductive amination using 9-BBNCN
A possible reason for this is that the more nucleophillic secondary amine formed after the first addition was displacing the cyano ligand of the 9-BBNCN. The amine as the borane complex 7b was then blocked from reacting with the ketone, and therefore no cyclized product could be formed. In order to test this theory, the reductive amination of 4-tertbutil-cyclohexanone was attempted using the 9-BBNCN with morpholine (Scheme 12). When the 9-BBNCN was added to the reaction mixture, a precipitate formed immediately and reduction did not occur. The precipitate is most probably 9-morpholine-9-borobicyclo(3.3.1)nonane. Similar amine-borane complexes have been reported by the Brown group.13 When characterization of this species was attempted, however, it was found that the solubility was too low, even in DMSO and pyridine, to obtain conclusive NMR spectra. Due to the problems discussed above, the pursuit of 9-BBNCN as a possible reducing agent for our chemistry was not further pursued.
Future Work

The research presented in this thesis has demonstrated the strategy of using readily available natural products, with alkenes in the core structure, to prepare alkaloid-type unnatural products, incorporating an alkene cleavage/reductive aminocyclization strategy. Our results show that this chemistry is feasible and can result in interesting scaffolds. Scaffolds 5a, 5b, and 5c have all been obtained, albeit in only modest yields, and submitted for biological screening through the CMLD. The diversity and size of this library will be expanded in the future. As part of this desire to increase diversity, a strategy for the diastereoselective synthesis of product 5c will also be pursued. It is likely that the diastereoselectivity of the reaction will be problematic with other amines tried in the future, and therefore, in order to synthesize the large possible library having a procedure capable of addressing this problem is important.

Currently efforts in our group are underway on a parallel synthetic scheme in which abietic acid itself with the carboxylic acid functionality not protected as the ester (Scheme 12). As noted above the methyl ester and carboxylic acid display differing bioactivities and therefore the synthesis of analogues of the type 5 and 6 compounds without the methyl ester may prove fruitful.
Experimental

**General Methods:** All reagents provided were purchased through Sigma-Aldrich and Alpha Aeser and were used without further purification. The solvents used were purchased through Fischer Scientific and were used without further purification unless otherwise noted. The NMR spectra were collected in 99.8% deuterated chloroform, Cambridge Isotope Laboratories Inc. All samples were dissolved in 0.6 mL and subsequently purged with argon. The spectra were collected on Varian NMR 117.42 kG. Purification by flash chromatography was performed on silica gel (Sorbent Technologies, 60 Å, 230x400 mesh).

**Abietic acid methyl ester (2) synthesis.** The abietic acid used was provided as technical grade ≥85% and was used as is. The yield was calculated based on the assumption of 85% purity. A sample of 3.01 g of abietic acid was dissolved in 130 mL of acetone. A sample of 1.48 g (1.2 equivalents) of potassium carbonate was added. To this solution 1.25 mL (1.5 equivalents) of dimethyl sulfate were added. The reaction mixture was then heated to 40°C for 4 hours. After 4 hours the reaction mixture was cooled to room temperature and 15 mL of methanol were added to solution. After bubbling stopped the solvent was evaporated in vacuo. The crude product was dissolved in 20 mL of dichloromethane and extracted from water (3x40 mL). The combined organic phases were then dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was purified via silica gel chromatography [33% dichloromethane : petroleum ether] to give a clear viscous oil (2.55 g, 93%). $^1$H NMR (500Mhz, CDCl$_3$) ð 5.77 (brs, 1H, H-14), 5.37 (dd, J= 5.5. 2.0 Hz, 1H, H-7), 3.63 (s, 3H, H-21), 1.25 (s, 3H, H-18), 1.02 (d, J=4.5 Hz, 3H, iPr) 1.00 (d, J=4.5 Hz, 3H, iPr), 0.82 (s, 3H, H-19).

**Dihydroxylated abietic acid methyl ester (3) synthesis.** A 1.0 g sample of product 2 was dissolved in 8.4 mL of $^1$BuOH. To this a 0.55 g (1.5 equiv.) sample of N-methylmorpholine 0.1 mL (0.15 equiv.) of pyridine were added. This mixture was diluted further with 4.2 mL of H$_2$O. A 12 mg (10 mol%) sample of potassium osmate dihydrate was added with vigorous stirring. The reaction vessel was purged with argon and capped. The reaction was heated to 80°C for 48H. The reaction was removed from heat and stirred with 5 mL of saturated sodium metabisulfite for 30 minutes. The $^1$BuOH was evaporated off in vacuo and the crude product was extracted from water with dichloromethane (3x25 mL). The combined organic phases were washed with 1N H$_2$SO$_4$ and then dried over anhydrous sodium sulfate. The solvent was then removed in vacuo. The crude product was then purified using silica gel chromatography by gradient elution (90% dichloromethane: petroleum ether to 3% Ethyl acetate: dichloromethane to 8% ethyl acetate: dichloromethane to 17% ethyl acetate: dichloromethane). Product $^{3}\beta$ was collected as a pure white solid (0.613 g, 56%) and product $^{3}\alpha$ was collected as a white solid (0.140 g, 13%).
$3\beta$. $^1$H NMR (500MHz, CDCl$_3$) $\delta$ 5.88 (ddd, $J$ = 6.3, 2.3, 2, 1.8 Hz, H7), 3.96 (dd, $J$ = 7.9, 2.3 Hz, H14), 2.16 (qq, $J$ = 7.0, 7.0 Hz, H15), 2.01 (br. ddd, $J$ = 17.2, 12.3, 6.3 Hz, H6$_{ax}$), 1.89 (dd, $J$ = 12.3, 3.8 Hz, H5) 1.88-1.84 (m, H1$_{eq}$), 1.86 (d, $J$ = 7.9 Hz, 14-OH) 1.73 (ddd, $J$ = 13.5, 3.3, 3.3 Hz, H12$_{eq}$), 1.67 (brd. dd, $J$=17.2, 3.8 Hz, H6$_{eq}$), 1.59-1.51 (overlap, 3H, H11$_{eq}$, H2), 1.49 (s, 13-OH), 1.40 (dddd, $J$ = 13.1, 13.1, 13.1, 3.6 Hz, H11$_{ax}$) 1.31 (ddd, $J$ =13.5, 13.1, 3.6 Hz, H12$_{ax}$) 1.26 (s, 3H, H19) 1.16-1.08 (m, H1$_{ax}$) 0.94 (d, $J$ = 7.0 Hz, 3H, H17), 0.91 (d, $J$ = 7.0 Hz, 3H, H16), 0.87 (s, 3H, H18); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 179.1 (C20), 137.8 (C8), 120.0 (C7), 76.1 (C13), 73.2 (C14), 51.9 (C21), 51.2 (C9), 46.4 (C4), 44.6 (C5), 39.1 (C1), 36.9 (C3), 35.2 (C10), 33.0 (C15), 26.5 (C12), 25.0 (C6), 19.2 (C11), 18.0 (C2), 17.8 (C16), 17.4 (C19), 16.3 (C17), 15.5 (C18).

$3\alpha$. $^1$H NMR (500MHz, CDCl$_3$) $\delta$ 5.69 (br. s, H7) 4.01 (br. s, H14) 3.62 (s, O-Me) 2.42 (dd, $J$ = 13.2, 2.6 Hz, H5) 1.25 (s, 3H, H19) 0.91 (d, $J$ = 2.3 Hz, 3H, iPr) 0.90 (d, $J$ = 2.3 Hz, 3H, iPr) 0.83 (s, 3H, H18).

Dione derivative of abietic acid methyl ester (4) synthesis. A 530 mg sample of $3\beta/3\alpha$ were dissolved in 8.4 mL of ethanol. This solution was further diluted with a 2.8 mL aliquot of water. A 390 mg (1.2 equiv.) sample of sodium metaperiodate was added to the reaction mixture. After a 16 hours the reaction the ethanol was evaporated in vacuo. The crude material was dissolved in 10 mL of dichloromethane and extracted from saturated sodium chloride (3x25 mL). The combined organic phases were dried over anhydrous sodium sulfate and concentrated in vacuo in order to yield a clear viscous oil (534 mg, 99%). The product was carried on without further purification. $^1$H NMR (500MHz, CDCl$_3$) $\delta$ 9.36 (s, CHO) 6.76 (ddd, $J$ = 5.1, 2.4, 2.4, H7) 3.65 (s, 3H, OMe) 3.12 (ddd, $J$ = 17.2, 10.9, 4.7, 1H) 2.65 (m, H12) 2.43 (m, H12') 2.30 (m, H15) 1.27 (s, 3H, H19) 1.11 (br. s, 3H, iPr) 1.10 (br. s, 3H, iPr) 0.83 (s, 3H, H18).

4-Methoxybenzylamine abietic methyl ester derivative (5a) synthesis. A 50 mg sample of 4 was added to a flame polished vial under argon atmosphere. This sample was then dissolved in 5 mL anhydrous THF (sodium still). A 22 μL sample of 4-Methoxybenzylamine (2.0 equiv.) was added to the solution along with 5 drops on glacial acetic acid. To this reaction mixture a 0.70 mL sample of 0.5 M NaBH$_3$CN solution (2.4 equiv.) was added. After 16 hours a 5 mL sample of saturated ammonium chloride solution was added. This was allowed to stand for 30 minutes. The organic phase was extracted from the saturated ammonium chloride with THF (4x5 mL). The combined organic phases were then washed with 1N NaOH. The organic phases were then dried over anhydrous sodium sulfate and the solvent was removed in vacuo. The residue was purified via silica gel preparatory thin layer chromatography (10% ethyl acetate: toluene) to
yield a clear viscous oil (22.4 mg, 34%). $^1$H NMR (500Mhz, CDCl$_3$) δ 7.21 (d, $J = 8.8$ Hz, H21) 6.82 (d, $J = 8.8$ Hz, H22) 5.26 (br. s, H7) 3.80 (s, 3H, OMe) 3.71 (d, $J = 13.4$ Hz, 1H) 3.68 (s, 3H, CO$_2$Me) 3.55 (br. d, $J = 14.7$ Hz, 1H) 3.52 (d, $J = 13.4$ Hz, 1H) 2.97 (d, $J = 14.7$ Hz, 1H) 2.19 (m, 1H) 2.10 (dd, $J = 12.1, 5.2$ Hz, 1H) 1.21 (s, 3H, H19) 0.95 (d, $J = 6.6$ Hz, 3H, iPr) 0.85 (d, $J = 6.6$ Hz, 3H, iPr) 0.73 (s, 3H, H18).

5b. (12.1 mg, 17%). $^1$H NMR (500Mhz, CDCl$_3$) δ 6.60 (s, 2H, H23) 5.29 (brd. s, H7) 3.84 (s, 9H, Ar-OMe) 3.74 (d, $J = 14.4$ Hz, H21) 3.66 (s, 3H, CO$_2$Me) 3.60 (d, $J = 15.8$ Hz, H14) 3.56 (d, $J = 14.4$ Hz, H21’) 3.02 (d, $J = 15.8$ Hz, H14’) 1.21 (s, 3H, H19) 1.02 (d, $J = 6.4$ Hz, iPr) 0.87 (d, $J = 6.4$ Hz, iPr) 0.74 (s, 3H, H18).
References


