Chemoenzymatic Synthesis of Amaryllidaceae Alkaloids and Their C-I Analogues. Symmetry Based Approach to Total Synthesis of Thebaine.

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ABSTRACT

Described herein is the chemoenzymatic total synthesis of several Amaryllidaceae constituents and their unnatural C-1 analogues. A new approach to pancratistatin and related compounds will be discussed along with the completed total synthesis of 7-deoxypancratistatin and trans-dihydrolycoricidine. Evaluation of all new C-1 analogues as cancer cell growth inhibitory agents is described.

The enzymatic oxidation of dibromobenzenes by Escherichia coli JM 109 (pDTG601) is presented along with conversion of their metabolites to (-)-conduritol E. Investigation into the steric and functional factors governing the enzymatic dihydroxylation of various benzoates by the same organism is also discussed. The synthetic utility of these metabolites is demonstrated through their conversion to pseudo-sugars, aminocyclitols, and complex bicyclic ring systems.

The current work on the total synthesis of some morphine alkaloids is also presented. Highlighted will be the synthesis of several model systems related to the efficient total synthesis of thebaine.
ACKNOWLEDGEMENTS

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<table>
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<th>Description</th>
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<tbody>
<tr>
<td>Ac</td>
<td>Acetyl</td>
</tr>
<tr>
<td>AIBN</td>
<td>2,2’-Azob(isobutynitrile)</td>
</tr>
<tr>
<td>Bn</td>
<td>Benzyl</td>
</tr>
<tr>
<td>Bz</td>
<td>Benzyol</td>
</tr>
<tr>
<td>CDI</td>
<td>Carbonyl diimidazole</td>
</tr>
<tr>
<td>COSY</td>
<td>(proton) Correlated spectroscopy</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-Diazabicyclo[5.4.0]undec-7-ene</td>
</tr>
<tr>
<td>DCC</td>
<td>Dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DDQ</td>
<td>2,3-Dichloro-5,6-dicyano-1,4-benzoquinone</td>
</tr>
<tr>
<td>DIAD</td>
<td>Diisopropyl azodicarboxylate</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-(Dimethylamino)pyridine</td>
</tr>
<tr>
<td>DME</td>
<td>1,2-Dimethoxyethane</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
<tr>
<td>DMP</td>
<td>2,2-Dimethoxypropane</td>
</tr>
<tr>
<td>DMS</td>
<td>Dimethylsulfide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>HRMS</td>
<td>High resolution mass spectrometry</td>
</tr>
<tr>
<td>IBX</td>
<td>2-Iodoxybenzoic acid</td>
</tr>
<tr>
<td>IMDA</td>
<td>Intramolecular Diels-Alder (reaction)</td>
</tr>
<tr>
<td>Imid</td>
<td>Imidazole</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>KHMDS</td>
<td>Potassium hexamethyldisilazide</td>
</tr>
<tr>
<td>LAH</td>
<td>Lithium aluminum hydride</td>
</tr>
<tr>
<td>m-CPBA</td>
<td>m-Chloroperoxybenzoic acid</td>
</tr>
<tr>
<td>MOM</td>
<td>Methoxymethyl</td>
</tr>
<tr>
<td>mp</td>
<td>Melting point</td>
</tr>
<tr>
<td>Ms</td>
<td>Mesyl (methanesulfonyl)</td>
</tr>
<tr>
<td>NADPH</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NMO</td>
<td>N-methylmorpholine-N-oxide</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance (spectroscopy)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>nOe</td>
<td>Nuclear Overhauser effect</td>
</tr>
<tr>
<td>PAD</td>
<td>Potassium azodicarboxylate</td>
</tr>
<tr>
<td>PCC</td>
<td>Pyridinium chlorochromate</td>
</tr>
<tr>
<td>Py</td>
<td>Pyridine</td>
</tr>
<tr>
<td>SMEAH</td>
<td>bis(2-methoxyethoxy)aluminum hydride</td>
</tr>
<tr>
<td>TBAF</td>
<td>Tetrabutylammonium fluoride</td>
</tr>
<tr>
<td>TBS</td>
<td>t-Butyldimethylsilyl</td>
</tr>
<tr>
<td>Tf</td>
<td>Triflyl (trifluoromethanesulfonyl)</td>
</tr>
<tr>
<td>TDO</td>
<td>Toluene dioxygenase</td>
</tr>
<tr>
<td>TEA</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>THS</td>
<td>Thexyl (dimethyl-(2,3-dimethyl-2-butyl)silyl)</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>Trimethylsilyl</td>
</tr>
<tr>
<td>Ts</td>
<td>Tosyl (p-toluenesulfonyl)</td>
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I. Introduction

The ideal synthetic target for any organic chemist is one that incorporates both structural complexity and prospective utility. Intricate molecules provide interesting synthetic problems for chemists to tackle in addition to encouraging the development of new methods. The envisioned utility of a chosen target provides synthetic organic chemists with real-world relevance with which to ground their intellectual pursuits. One group of natural products which fulfill both criteria is the *Amaryllidaceae* constituents. The synthesis of these biologically active compounds and their unnatural analogues has a rich history in the Hudlicky group. Our current strategy relies on construction of the highly oxygenated C ring of 7-deoxypancratistatin (2) and *trans*-dihydrolycoricidine (3) from chiral *cis*-cyclohexadienediols. We arrive at these building blocks through an enzymatic dihydroxylation of substituted benzenes (Figure 1).

![Figure 1. General strategy for synthesis of *Amaryllidaceae* constituents.](image)

The biooxidation is high yielding and allows for the subsequent construction of the isocarbostyril framework in an enantioselective fashion while at the same time
reducing the environmental impact of the synthesis. Natural supply of the most active congeners is often lacking, so the development of a truly efficient synthesis is crucial to their further advancement as candidates for the treatment of cancer.

The flexibility of these synthetic designs has the added benefit of allowing for ready synthesis of unnatural analogues of the targeted Amaryllidaceae constituents. This work represents an important contribution given the remarkable activity associated with many of these natural targets and their sometimes less than desirable bioavailability characteristics. The synthesis and biological evaluation of a variety of C-1 analogues has been pursued in parallel with total synthetic efforts and constitutes a significant portion of this manuscript.

In addition to their application in the synthesis of Amaryllidaceae constituents we also set out to further explore the utility of new cis-cyclohexadienediols. We desired to investigate various dibromobenzenes as substrates for the above mentioned enzymatic dihydroxylation with the hope of producing new and synthetically useful materials. In the same vein, we also endeavored to establish the scope and limitations of the chemoenzymatic transformation of benzoate esters. This work was undertaken at the time for its intended use in the synthesis of cyclitols and the antiviral agent Oseltamivir (Tamiflu).

Another group of natural products that present interesting synthetic challenges is the opiates. The design and execution of a synthesis genuinely adaptable to large-scale preparation of morphine alkaloids has been a long standing goal in the Hudlicky group. This has led to the development of a number
of approaches and total synthesis efforts. Given the increasing importance of thebaine in recent years we have elected to pursue its total synthesis through a cascade Diels-Alder route (Figure 2). Initial investigation of this strategy utilized thiophene as a diene partner in an intramolecular cycloadditions but recent reports by Boger and Bodwell have inspired us to incorporate nitrogen-containing heterocycles as the diene component. The results of our efforts in this endeavor are presented as the third component of this manuscript.

Figure 2. Retrosynthetic analysis of thebaine.

A unifying theme in the work of the Hudlicky group is the use of chemoenzymatic processes to generate chiral building blocks useful in synthesis. As enzymatic oxidation of arenes is a component in the majority of the work contained here, the next section begins with a review of this key dihydroxylation reaction.
II. Historical

II-1 Aromatic Dioxygenases

II-1.1 History of Microbial Oxidation of Aromatics

While the use of various microorganisms in the production of foodstuffs predates recorded history, the first use of biocatalysis is credited to Brown. While this early work demonstrated that biocatalysis could be used to effect chemical transformations systematic study of microbial metabolism of hydrocarbons did not begin until the early 20th century. Degradation of aromatics by bacteria has been described as early as 1908 when the bacterium *Bacillus hexacarbavorum* was shown to grow on toluene and benzene. Five years later a report by Söhngen described a bacterium that tolerated various concentrations of benzene. The first isolated product of these fermentations was shown to be catechol. In this study the gram-negative *Pseudomonas aeruginosa* was shown to grow in the presence of both benzene and catechol. A long debated question of the possible intermediacy of phenol in the oxidation of benzene was put to rest when Marr and Stone demonstrated that these bacteria did not metabolize phenol effectively. With their proposed intermediate, 3,5-cyclohexadiene-1,2-diol, being described as “unavailable” they nonetheless reasoned its existence along with the presence of the required dehydrogenase by analogy with mammalian systems such as the 1,2-dihydonaphthalene-1,2-diol isolated following rat metabolism of naphthalene.

The continued study of bacteria-mediated arene metabolism led to the 1968 seminal paper by Gibson in which he described a strain of *Pseudomonas*
*putida* that utilized toluene and benzene as a carbon source.\textsuperscript{10} It was demonstrated that benzene and toluene were oxidized at equal rates and that one mole of oxygen was taken up per mole of substrate en route to the formation of catechol. The possibility of an epoxidation as the first step in the pathway was discounted when it was shown that trans-benzene glycol was not effectively metabolized by cells that were shown to rapidly transformed the cis isomer (*cis*-cyclohexa-3,5-diene-1,2-diol). Gibson eventually proposed a mechanism for the transformation of benzene to catechol that involved dioxobicycle \textbf{10} (Figure 3).

![Figure 3. Proposed pathway for catechol formation from benzene.](image)

In an effort to provide stronger evidence for intermediate \textbf{10} Gibson began work on the oxidation of more diversely substituted aromatics.

**II-1.2 On the Mechanism of Arene Oxidation by Oxido-reductase Enzymes**

In the fall of 1968 Gibson reported the oxidation of halogenated benzenes and *p*-chlorotoluene by the soil bacteria *P. putida*.\textsuperscript{11} Gibson was able to show that the halogenated benzenes were metabolized at decreasing rates with increase of halogen substituent size. In each case the halogenated catechols were isolated. In the case of the *p*-chlorotoluene (13) a mixture of compounds was isolated (Scheme 1). The initial biotransformation of 13 yielded a mixture of catechol 15
and at that time unknown 2,3-dihydroarenediol 14. Treatment of diol 14 with 2 N HCl gave rapid conversion to phenols 16 and 17. This observation combined with a variety of spectroscopic methods led Gibson to propose the correct structure of 4-chloro-2,3-dihydroxy-1-methylcyclohexa-4,6-diene (14). Isolation of the first cis-2,3-dihydroarenediol derived from toluene was reported less than a year later through the action of the mutant *P. putida* 39/D. 12

![Chemical structure diagram](image)

**Scheme 1.** Proposed pathway for the metabolism of *p*-chlorotoluene by toluene-grown cells of *P. Putida*.

The absolute stereochemistry of (+)-cis-2,3-dihydroxy-1-methylcyclohexa-4,6-diene was later assigned by its conversion to (R)-(−)-2-methyladipic acid. 13 In this proof the stereocenters of the cis-diol were destroyed leaving assignment to be inferred from the assumed face-selectivity of a hydrogenation step. It was not until 15 years later that unambiguous assignment of absolute configuration would be demonstrated in the synthesis of PGE₂α. 14
The diverse pathways by which oxo-reductase enzymes process aromatic substrates have been a subject of study since the mid 1930s. The first dihydrodiol derived from aromatics to be isolated was trans-1,2-dihydroxy-1,2-dihydroanthracene, the product of cytochrome P-450 epoxidation of anthracene. In 1962 Boyland and Booth demonstrated that only one atom of $^{18}O_2$ was incorporated in the dihydroxylation of benzene (18), (Scheme 2). In a similar experiment, Gibson demonstrated that both atoms of $^{18}O_2$ are incorporated in the cis-dihydroxylation of naphthalene (23).

![Scheme 2. Isotope labeling experiments in the oxidation of arenes.](image)

In the oxidation of arenes by eukaryotic organisms the aromatic substrates are converted to catechols through their epoxide intermediates. Arene oxides formed in this manner either undergo rearrangement or trans-opening by various nucleophiles. In the case of prokaryotic organisms, such as soil bacteria, dioxygenase enzymes introduce two oxygen atoms. Intermediates such as 24 then usually undergo re-aromatization through the action of catechol dehydrogenases, a pathway later established by Gibson in his characterization of mutant *P. putida* strains.
The identification and characterization of the mutant *P. putida* 39/D was a major breakthrough in the development of arene *cis*-diol research.\(^\text{12}\) The blocked mutant lacked the ability to further metabolize the intermediate dihydroarene diols and so allowed for their accumulation and isolation. Extensive study of this and other mutant strains led to the identification of the nucleotide sequence of the genes responsible for coding the toluene catabolic pathway.\(^\text{18}\) The elaborate electron transport chain enabling this metabolism was also identified. These genes were actively expressed in *E. coli* hosts creating a variety of organisms which reproduce either part of or the entire catabolic pathway (Scheme 3).

Scheme 3. Metabolism of toluene by soil bacteria and recombinant strains of *E. coli*

The recombinant organism JM109(pDTG601) was created to over-express the first part of the pathway allowing for efficient production of *cis*-dihydrodiols.\(^\text{18}\) The recombinant organism JM109(pDTG602) was also created allowing for the chemoenzymatic synthesis of catechols from either arenes or the isolated *cis*-dihydrodiols 26. A third organism JM109(pDTG603) was also
created which expressed 1,2-catechol dehydrogenase and metabolized toluene to 2-hydroxy-6-oxo-2,4-heptadienoate (28). To this day, the least understood aspect of the pathway remains the remarkable dihydroxylation catalyzed by the toluene dioxygenase enzyme (TDO). Several mechanisms have been proposed\textsuperscript{17,19,20} and despite reporting of the X-ray crystal structure of naphthalene dioxygenase,\textsuperscript{21} a related enzyme, the exact mechanism remains elusive.

Even without the availability of exact mechanistic details of the key dihydroxylation these biocatalysts have become readily available tools in synthetic organic chemistry.\textsuperscript{22} Whole-cell fermentation protocols facilitate these transformations without the need to account for the complex electron-transport chains and cofactors associated with isolated oxidoreductase enzymes. The use of recombinant \textit{E. coli} in these transformations provides several advantages over the same transformations done using the wild or mutant strains. Protocols for use of blocked mutants require toluene or chlorobenzene as inducers for protein synthesis. The complication of having the inducer also be a substrate is avoided in the recombinant organism where the sugar analogue, $\beta$-isopropylthiogalactopyranoside (IPTG) is used to induce enzyme production. Also recombinant cells contain multiple copies of the plasmid allowing for higher concentrations of the desired enzyme and greater space/time yields of diols.

In the little over forty years since Gibson’s isolation of the first stable \textit{cis}-cyclohexadienediol the metabolic pathway has been mapped, the recombinant organisms engineered, and more than 400 substrates of toluene dioxygenase and related enzymes have been identified. With such a large substrate library it is
surprising that only a fraction of these richly functionalized compounds have been exploited in synthesis. Listings of known metabolites and their synthetic applications have been extensively reviewed.\textsuperscript{23} The broad number of metabolites with varying substitution patterns has allowed for the development of an empirical model for the regio- and stereoselectivity of the oxidation (Figure 4).\textsuperscript{24}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure4.png}
\caption{Model for regio- and stereoselectivity of the oxidation of single ring aromatics.}
\end{figure}

In the model, oxidation proceeds as shown according to the orientation of the ring substitution. It is proposed that the smaller group of disubstituted, single ring aromatics is more easily accommodated into the active site to provide oxidation products as shown. This predictive model has helped in the incorporation of new cis-dihydrodiols into synthetic schemes, in some cases before the diols are even confirmed metabolites.
II-1.3 Diversity of Known Dihydroarenediols and Their Utility in Synthesis

The first application of these cis-dihydrodiol metabolites in synthesis was the preparation of polyphenylene using benzene cis-dihydrodiol 11 in 1983. In this seminal publication researchers at ICI exploited the diene functionality in a radical polymerization reaction. Elimination of the protected diols followed to provide polyphenylene fibers and films. It is important to note that the diol functionality was destroyed in this process reforming the arene. The first true exploitation of the diol functionality would have to wait until academic interest in the same meso diol (11) was initiated by Ley with his synthesis of (±)-pinitol (44). In Ley’s synthesis, the diols were retained and utilized in their protected form to facially direct epoxidation of the diene (Figure 5). Selective opening of the resulting vinyl epoxide with methanol set the stage for the introduction of the final oxygenation with OsO₄. The use of the diol functionality to direct further transformations and the steric and electronic differentiation of the olefins would prove to be standard strategies in the application of these cis-dienediols in synthesis.

![Figure 5. Ley’s synthesis of (±)-pinitol.](image)
Oxidative Cleavage of Arene cis-Diols

The first enantioselective synthesis that incorporated arene cis-diols was the formal synthesis of PGE$_{2\alpha}$ (32) by Hudlicky$^{14}$ The use of the chiral cis-diol (30) derived from enzymatic oxidation of toluene differentiated this synthesis from the previous work by Ley. It is also noteworthy for its adaptation of oxidative cleavage protocols to the dihydroarene diol framework (Figure 6).

![Figure 6. Hudlicky's formal synthesis of PGE$_{2\alpha}$.]

The *Pseudomonas putida* mutant strain 39/D was used to transform toluene to its corresponding cis-diol in a yield of 3 g/L of culture. The diol was protected as its acetonide prior to oxidative cleavage of both olefins with ozone. The resulting ketoaldehyde underwent intramolecular aldol condensation to provide enone 31, a formal intermediate in the synthesis of prostiglandins.$^{27}$ The formal synthesis was completed in four steps, a substantial improvement upon reported synthesis at the time.$^{23a}$

Use of Arene cis-Diols as Cycloaddition Partners

Other classes of transformations that have been applied to cis-dienediols include cycloadditions. The ability of these diol intermediates to behave as the
diene component in Diels-Alder reactions was first demonstrated by Gibson. The first application of Diels-Alder reactions to cis-dihydrodiol metabolites in total synthesis was Hudlicky’s preparation of Condramine A-1 (36) (Figure 7).

**Figure 7.** Hudlicky’s synthesis of Condramine A-1.

Chloro- or bromo-dienediol 33 or 34 were protected as their acetonides prior to facial and regioselective [4+2] cycloadditions with nitrosyl dienophiles to yield 35. Reductive cleavage of the N-O bond with sodium amalgam also resulted in dehalogenation with retention of stereochemistry at the three asymmetric positions. The synthesis of conduramine A-1 (36) was completed in five steps from bromo- or chlorobenzene with complete regio- and stereocontrol of the Diels-Alder reaction being achieved by exploiting key functionality of the cis-dienediols 33 and 34.

**Use of Arene cis-Diols as Cross-Coupling Partners**

In addition to Diels-Alder reactions these diols have also been employed in a variety of coupling reactions en route to natural products. In their synthesis of platencin (40), the Banwell group combined an intramolecular cycloaddition
with a cross-coupling protocol to provide an intermediate synthesized by Nicolaou (Figure 8).  

![Diagram](image)

**Figure 8.** Banwell's formal synthesis of Platencin.

Diol 37 was derived from the enzymatic oxidation of iodobenzene by recombinant organisms expressing TDO. The tethered dieneophile 38 was generated using a Negishi cross-coupling reaction. This was followed by a facially selective intramolecular Diels-Alder reaction to provide adduct 39, a formal intermediate in the synthesis of platencin (40).

**Application of Rearrangements to the Arene cis-Diol Framework**

In addition to coupling reactions, the *cis*-dienediols have been transformed using various transpositions and sigmatropic rearrangements. One noteworthy example is provided by Micalizio in the total synthesis of phorbasin C (44), (Figure 9). The starting material was *cis*-dihydroarenediol 34 derived from oxidation of bromobenzene by TDO. Dihydroxylation of the distal olefin was followed by radical debromination yielding 41. The key step relied upon a reductive cross-coupling of diol 41 with TMS-propyne. The alkylative transposition of the remaining olefin proceeds through a metalo-[3,3]
rearrangement (42) to give the 1,4-diene 43. This new diene was then taken on to
(+)-phorbacin C (44).

![Chemical structure and reaction scheme]

**Figure 9.** Micalizio's use of metalo-[3,3] rearrangement in the synthesis of (+)-phorbacin C.

The examples highlighted above represent only a fraction of the known metabolites and their synthetic applications. In the time since Gibson's original disclosure, over 400 metabolites of TDO or related enzymes have been reported. These metabolites and their use as synthetic intermediates have been extensively reviewed. More recently *cis*-dihydroarendiols have been used in the synthesis of codiene, various montanine alkaloids, and oseltamivir (Tamiflu). The *cis*-dihydriodiols derived from 4-chloroquinolines have also been used in the synthesis of chiral 2,2'-and 4,4'-bipyridyl ligands for use in asymmetric transition-metal catalysis.

When considering the design and execution of future synthesis using *cis*-dihydroarendiols, it is helpful to study previous synthetic efforts. Many of the
targets generated to date contain highly oxygenated cores or amino-cyclitol moieties. As epoxide or aziridine scaffolds are readily accessible from the cis-dihydrodiol framework, their coupling with various nucleophiles is also well established. One group of natural products which contain a highly oxygenated core that can efficiently be constructed using this motif is the *Amaryllidaceae* alkaloids. These structurally complex constituents have a rich history both in their use as medicinal agents and in the efforts applied toward their synthesis.

**II-2 *Amaryllidaceae* alkaloids**

The application of several plants of the *Amaryllidaceae* family to the treatment of illness related to cancer has been known since antiquity. The first recorded treatments specifically designed for cancerous conditions were prescribed by Hippokrates of Cos (ca. B.C. 460-370). This “Father of medicine” and founder of the school of medicine bearing his name recommended application of narcissus oil (most likely from *Narcissus poeticus* L.) for the treatment of tumors of the uterus. Topical treatments prepared from *Narcissus poeticus* L. and *Narcissus pseudonarcissus* were prescribed by Gaius Plinius Secundus (A.D. 23-79). Hippokrates’ treatment for uterine cancer was continued by Soranos of Ephesos, physician and prominent gynaecologist, who taught in Rome and Alexandria under Trajan and Hadrian (98–138 A.D.). In fact, historical use of plants from over 30 members of the *Amaryllidaceae* family in remedies for cancer is reported well into the 19th and 20th century, at which point individual congeners began to be isolated and tested for anti-tumor activity.
The first alkaloid to be isolated from an *Amaryllidaceae* plant was lycorine (45) in 1877. As lycorine is the major constituent of *N. poeticus* L., it was suspected that it was also responsible for the therapeutic benefits of these plants in herbal treatments of cancer. This theory was seemingly confirmed in 1958 with the disclosure of lycorine’s antitumor activity. Since that time interest has intensified and more than 100 structurally diverse constituents from a variety of *Amaryllidaceae* plant species have been isolated.

![Image of Amaryllidaceae constituents](image)

**Figure 10.** Representative *Amaryllidaceae* constituents.

The most active congeners have proven to be the isocarbostyril grouping, represented by select members above (Figure 10). Narciclasine (46) and lycoricidine (47) were isolated from the bulbs of *Lycoris radiate* and were reported to have potent cytotoxic activities. In 1984 Pettit and coworkers isolated isocarbostyril 1 from the bulbs of Hawaiian *Hymenocalis littoralis* and
named it pancratistatin. A pattern of isolation of 7-hydroxy and 7-deoxy pairs of isocarbostyril congeners began to emerge with the isolation of 7-deoxypancratistatin (2) in 1989. The last pair to be isolated was trans-dihydronarciclasine (48) and trans-dihydrolycoricidine (3) (7-deoxy-trans-dihydronarciclasine).

The interesting biological activity of these congeners and their structural complexity has made them appealing targets for synthetic chemists. The total synthesis of lycorine (45), pancratistatin (1), 7-deoxypancratistatin (2), narciclasine (46), lycoricidine (47), trans-dihydronarciclasine (48), and trans-dihydrolycoricidine (3) have been completed and made the subject of a number of excellent reviews.

II-2.1 Biosynthesis of *Amaryllidaceae* alkaloids

The biosynthesis of the *Amaryllidaceae* alkaloids can vary widely according the type of ring subgroup being produced. In the majority of cases a common intermediate in the biosynthesis of these molecules has been shown to be O-methylnorbelladine (49), (Figure 11). The core of this central intermediate, imine 50, is formed from a condensation reaction between protocatechuic aldehyde (51) and tyramine (52) which are derived from phenylalanine (53) and tyrosine (54) respectively.
Two main pathways are proposed for formation of the various constituents comprising the Amaryllidaceae alkaloids. The first consists of para-ortho oxidative coupling of O-methylnorbelladine (49) to give intermediate 55 which then undergoes conjugate attack of the amine to form norpluvine (56), a known precursor to lycorine (45), (Figure 12). The second pathway begins with a para-para oxidative coupling yielding intermediate 57, which undergoes intramolecular Michael addition to form noroxomaritidine (58). Conversion of 58 to narcicasine (46) was elucidated in a series of experiments using $^{14}$C and $^3$H labeled O-methylnorbelladine (49) and 11-hydroxyvittatine (59). The transition from 59 to narcicasine (46) is hypothesized to proceed through a retro-Prins reaction followed by oxidation steps.
The true biosynthesis of pancratistatin has yet to be reported, but the two pathways described above account for the general skeleton of many of the most biologically active congeners of this family.

II-2.2 Biological Activity and Pharmacophore Studies

Members of the Amaryllidaceae alkaloid family have been recognized for generations for their utility as folk medicines, particularly with regards to the treatment of illness related to cancer. It was not until 1958 that the antitumor properties of lycorine (1) were formally reported.\(^6^0\) In addition to its strong activity against murine P-388 lymphoectic leukemia lycorine (1) has also been shown to possess antiviral activity.\(^6^1\) The antitumor activity of narciclasine (46) was reported following its screening against the mouse Sarcoma 180 cell line.\(^6^2\) The murine P-388 lymphocytic leukemia, cell line has been used as the initial screening bioassay for many of the most potent Amaryllidaceae alkaloids. Pancratistatin (1), 7-deoxypancratistatin (2), narciclasine (46), lycoricidine (47),
trans-dihydronarciclasine (48), and trans-dihydrolycoricidine (3) have registered GI\textsubscript{50} values as low as 0.01\% \mu g/mL.\textsuperscript{63,64,65,66} These isocarbostyril-type congeners were also tested against the NCI (National Cancer Institute) 60 human cancer cell line \textit{in vitro} screen and were shown to be most effective against the melanoma subgroup.\textsuperscript{67} Strong activity was also documented within the other subpanels (NCS lung, colon, brain, and renal), but activities varied by as much as 1000 fold between different cells lines in each group.

The mechanism of action for these strongly anti-cancer agents has been studied with narciclasine as a model. In initial studies by Carrasco it was shown that narciclasine inhibited poly U-directed incorporation of \textsuperscript{14}C labeled phenylalanine in rabbit reticulocyte ribosomes.\textsuperscript{68} Narciclasine was also shown to inhibit association of [\textsuperscript{14}C]trichodermin (60) and [\textsuperscript{3}H]anisomycin (61), two known inhibitors of protein synthesis by eukaryotic ribosomes (Figure 13).

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{fig13.png}
\caption{Known 60S ribosomal subunit binders trichodermin (60) and anisomycin (61).}
\end{figure}

As 60 and 61 are known to shut down protein biosynthesis by binding to the 60S ribosome subunit, experiments were conducted using the mutant \textit{Saccharomyces cerevisiae} TR\textsubscript{1} strain. This mutant is resistant to 60 and 61 through alteration of the peptidyl transferase center of the 60S ribosomal subunit.\textsuperscript{69,70} Narciclasine (46)
was shown to be ineffective in suppressing protein biosynthesis in the resistant mutant leading to assignment of the 60S eukaryotic ribosome subunit as the location of narciclasine’s activity. While similar experiments have not been conducted with pancratistatin (1) and other congeners, it can be argued that a similar mode of action exists for these compounds.

Specific studies investigating the mechanism of action of pancratistatin have recently been reported. Selective apoptosis in the Jurkat cell leukemia model vs normal nucleated blood cells was induced by pancratistatin. Pandey postulated that the selectivity was because of pancratistatin’s ability to differentiate between mitochondria from cancer cells and those of healthy cells. This approach is currently being explored in more complicated systems. It is unclear whether this new mode of action represents the primary source of activity for these congeners or if the previously established protein synthesis inhibition predominates. It is highly possible that the two modes of action are complimentary, combining for strong biological activity and selectivity in initiation of apoptosis in cancer cells.

Initial attempts to determine the cytotoxic pharmacophore of the Amaryllidaceae alkaloids relied on comparison of isocarbostyrils in the natural series and/or analogs derived from semisynthesis. In general, the constituents containing C-7 hydroxylation are more potent by an order of magnitude or more. Dihydronarciclasine has been generated in both its cis- and trans- form with the trans- (trans B/C ring juncture) displaying significantly more potent activity. Analogs containing the amide functionality of the B ring as opposed
to the semisynthetic amine are more active by up to two orders of magnitude.\textsuperscript{64} With these studies a general view of the pharmacophore of this \textit{Amaryllidaceae} isocarbostyril subgroup begins to take shape (Figure 14).

\textbf{Figure 14.} Pancratistatin as a model for required pharmacophore in the \textit{Amaryllidaceae} alkaloids as proposed by Pettit.

Modification of the A ring has included replacement of the dioxymethylene moiety to form singly oxygenated derivatives of 7-deoxypancratistatin,\textsuperscript{76} indole mimics,\textsuperscript{77} and bis-silylated analogues,\textsuperscript{78} all displaying significantly reduced or no activity. Ring B modifications have yielded various seco-analogues,\textsuperscript{79} lactones\textsuperscript{80} and the semisynthetic amines mentioned above. All have been shown to be inactive. The necessity of the trans B/C ring juncture has also been demonstrated by the C10b-epimer of 7-deoxypancratistatin prepared by Hudlicky.\textsuperscript{81} This \textit{cis}-fused analogue proved completely inactive in screens against cancer cell panels. Possibilities for ring C modifications are numerous because of its rich substitution pattern. A host of C ring analogues, bearing a variety of hydroxylation patterns, have been synthesized to date.\textsuperscript{82,76} These studies have demonstrated the necessity of having at least three
of the four hydroxyl groups present in ring C. Of these oxygenated positions, it has been shown that the 2, 3, and 4 hydroxylation patterns are most important for strong biological activity. Most recently, reports detailing the synthesis of several B,C-seco-analogues have appeared. These derivatives failed to provide substantial activity, but again confirmed the importance of intact B and C rings for strong antitumor response.

In the course of their semi-synthesis of pancratistatin, Pettit and coworkers generated a C-1 benzoyl derivative of pancratistatin that displayed remarkably potent activity. In an effort to improve the aqueous solubility of various Amaryllidaceae constituents, the Pettit group has synthesized 7- O-phosphate prodrug analogues of 1, 3,4- O-cyclic phosphate analogues of 1, 3, 46-48 and a C-1 benzoyl analog. These phosphate prodrugs are currently being evaluated on a preclinical level.

Synthetic efforts to generate meaningful analogues have provided much needed information concerning the minimal structure requirements detailed above. With continued development in this field reliant upon supplies of relatively scarce natural congeners, synthetic efforts will no doubt persist in this area of research.

II-2.3 Selected Syntheses of Amaryllidaceae Alkaloids

Pancratistatin

The first total synthesis of pancratistatin was reported by Danishefsky and Lee in 1989. The synthesis begins with pyrogallol 62 as the precursor to the A
ring of pancratistatin (Scheme 4). Further functionalization provided amide 63, which contained all of the 14 skeletal carbons in pancratistatin. Iodolactonization to form 64 was achieved only after cleavage of the silyl-ether and stannylation of the phenol.

Reaction conditions: (a) HC(OEt)_3, Amberlyst-15, C_6H_5, 86%; (b) NaH, Et_3NCOCl, THF, then MeOH, TsOH, rt 86%; (c) K_2CO_3, CH_2Br_2, CuO, DMF, 70%; (d) s-BuLi, TMEDA, THF, 58%; (e) TBSCI, imidazole, CH_2Cl_2, 86%; (f) s-BuLi, TMEDA, THF, 70%; (g) allylmagnesium bromide, Et_2O, 92%; (h) (i) CH_3SO_2Cl, Et_3N, CH_2Cl_2; (ii) DBU, 54%; (i) 1-(benzenesulfonyl)-2-nitroethene, CHCl_3, 96%; Bu_3SnH, AIBN, PhCH_3, 72%; (j) TBAF, THF, 79%; (k) (i) (Bu_3Sn)O, C_6H_5CH_3, (ii) I_2, THF, 67%; (l) BnBr, Ag_2O, DMF, 85%; (m) OsO_4, NMO, CH_2Cl_2, THF, H_2O, 90%; (n) DBU, C_6H_6, 88%; (o) 2-acetoxysobutyl bromide, CH_3CN, 88%; (p) OsO_4, NMO, CH_2Cl_2, THF, H_2O, 88%; (q) Bu_2SnO, C_6H_5CH_3, 4-methoxybenzyl bromide, n-Bu_4NI, (r) BnBr, Ag_2O, DMF, 95%; (s) DDQ, CH_2Cl_2, H_2O, 75%; (t) Zn, AcOH, H_2O, CH_2Cl_2, 81%; (u) NaH, CCl_3CN, THF, 74%; (v) 100-105 °C, 0.05-0.1 mm Hg, 56%; (w) OsO_4, NMO, THF, H_2O, 75%; (x) K_2CO_3, MeOH, CH_2Cl_2, DCC, 82%; (y) H_2, Pd(OH)_2, EtOAc, 90%.

Scheme 4. Danishefsky’s synthesis of pancratistatin.
The needed regio-selective formation of imidate 68 necessitated a lengthy series of manipulations to achieve but the required Overman rearrangement provided amino moiety 69 in fair yield. Dihydroxylation was followed by rearrangement of the lactone to the phenanthredone core. With the final hydrogenation, the racemic synthesis of pancratistatin (2) was completed in 26 steps and in an approximate overall yield of 0.13%.

Scheme 5. Hudlicky's synthesis of pancratistatin.

The first asymmetric total synthesis of pancratistatin was reported in 1995 by Hudlicky. Chirality in this synthesis was derived from the microbial dihydroxylatoin of bromobenzene which provided cis-diol 34. Aziridination of the acetonide of 34 using Yamada's iodonium ylide followed by radical
debromination provided vinyl aziridine 70 (Scheme 5). Trans-diaxial opening of the aziridine with the higher order cuprate of 74 coupled the A and C rings. At this stage all attempts at a transamidation reaction failed and additional functional group transformations were required (71-73). The remaining C-ring oxygenation was installed by opening of epoxide 73. Additionally it was discovered that in the course of this final step cleavage of the benzyl ether, cleavage of the the Boc group, debenzylation, and formation of the lactam all occurred to provide pancratistatin (1) in a single operation.

The shortest total synthesis of pancratistatin to date (12 steps) is that of Li and coworkers. The starting material for this asymmetric synthesis is D-pinitol (74), an expensive starting material ($145/gram) but one containing all necessary oxygenation of the final C-ring (Scheme 6). Several protection and deprotection steps in addition to installation of the C4a nitrogen of pancratistatin were required to arrive at cyclic-sulfate 75. The A-ring fragment 78 was introduced by a phosgene mediated coupling with amine 75. Protection of the phenol and amide with MOMCl provided cyclization precursor 76. The crux of the synthesis was the intramolecular opening of this cyclic-sulfate to close the B-ring and complete the skeleton. Various conditions were attempted by the authors to effect this transformation but initial trials with aryllithium or arylmagnesium bromide provided complex mixtures of products. It was not until generation of the organocerium that meaningful yields of phenathridone 77 were observed. Interestingly, this reaction was later optimized to include ultrasonication during
the generation of the organocerium intermediate to provide 77 in good yield. Subsequent deprotection occurred uneventfully to provide pancratistatin (1).

![Chemical structures and reaction conditions]

Reaction conditions: (a) TIPDSCI2, imidazole, DMAP, CH2Cl2, 94%; (b) (MeO)2C(CH3)2, p-TsOH, 81%; (c) (i) PPh3, DEAD, CH3SO3H, CH2Cl2, 0 °C to rt; (ii) NaN3, DMF, 60 °C, 72%; (d) TBAF, THF, 0 °C to rt, 100%; (e) SOCl2, Et3N, CH2Cl2, 0 °C; (f) NaIO4, RuCl3, aq. CH3CN, 87% over two steps; (g) PPh3, aq THF, 0 °C to rt, 94%; (h) 78, MgBr2·OEt2, COCl2, ether, 0 °C, 75, 64%; (i) K2CO3, MOMCl, DMF, rt, 64%; (j) t-BuLi, CeCl3, ultrasound, THF, -78 °C to rt, 72%; (k) (i) BBr3, CH2Cl2, -78 to 0 °C, 1 h; (ii) MeOH, 78 to 0 °C, 2 h, 52%.

**Scheme 6.** Li's synthesis of pancratistatin.

The synthesis of medicinally important targets by relay from more readily available natural products is commonly accepted in the pharmaceutical industry. The greater availability of narciclasine (46) in the bulbs of *Amaryllidaceae* plants compared with that of pancratistatin prompted Pettit to explore synthesis of pancratistatin (1) using narciclasine (46) as a precursor (Scheme 7). The free hydroxyl groups were protected as their acetonide and acetates before epoxidation
with mCPBA to provide derivative 79. Hydrogenation of the epoxide was followed by removal of the acetate protecting groups to provide a mixture of compounds from which 80 was isolated in 28% yield. Formation of the cyclic sulfate 81, opening with benzoate, and deprotection provided pancratistatin (1) in 10 steps and an overall yield of 3.6%.

7-Deoxypancratistatin

The first preparation of 7-deoxypancratistatin occurred prior to its isolation from natural sources and was reported by Ohta as an intermediate in the synthesis of lycoricidine (Scheme 8).\(^{49a}\) Installation of the C-ring carbons proceeded through a Diels-Alder cycloaddition between the diene derived from precursor 83 and ethyl
acrylate. Hydrolysis of the intermediate ester provided acid 84, which was converted to an isocyanate before formation of lactam 85 under Lewis acid catalysis. The lactam-lactone transformation occurred through a protection, hydrolysis, and bromolactonization sequence to give bromide 86. Elimination of the bromide was followed by a lactone-lactam transform, protection, and oxidation to epoxide 88. Further elaboration to allylic acetate 89 allowed for installation of the final C-ring hydroxylation via OsO4. Removal of the acetate provided 7-deoxypancratistatin (2).

**Scheme 8. Ohta and Kimoto's synthesis of 7-deoxypancratistatin.**

Reaction conditions: (a) ethyl acrylate, p-TsOH, 180 °C, 56%; (b) NaOEt, EtOH, reflux, then H2O, reflux, 74%; (c) CICO2Et, Et3N, acetone, H2O; (d) NaN3, H2O, then C6H5CH3, reflux; (e) BF3·Et2O, 89% (over 3 steps); (f) Ac2O, pyr, reflux, 83%; (g) 1 N KOH, CH3OH, 68%; (h) NBS, THF, 96%; (i) DBU, pyr, reflux, 98%; (j) 20% NaOH, EtOH, 90 °C, 90%; (k) 2,3-dihydropyrane, p-TsOH, reflux, 75%; (l) MCPBA, CHCl3, 85%; (m) diphenyldiselenide, EtOH, NaBH4, reflux, then H2O2, 63%; (n) Ac2O, pyr, 97%; (o) p-TsOH, AcOH, MeOH, reflux, 59%; (p) OsO4, pyr, 87%; (q) 1 N KOH, CH3OH, reflux, 49%.
The first asymmetric total synthesis of 7-deoxypancratistatin was also completed in the course of a total synthesis of lycoricidine.\textsuperscript{49c} Chirality in the synthesis was derived from glucose, which was used to make nitro olefin 90 (Scheme 9). Introduction of the A-ring was accomplished by metal-halogen exchange of the appropriate bromobenzoate and Michael addition to the nitro olefin to afford furanose derivative 91. The acetonide was removed and the lactone 92 formed upon treating with base. Reduction of the nitro group and cleavage of the benzyl ether was followed by lacone-lactam rearrangement to give 7-deoxypancratistatin(2).

\begin{center}
\includegraphics[width=\textwidth]{s9.png}
\end{center}

\textit{Reaction conditions:} (a) (i) isopropyl (6-bromo-3,4-(methylenedioxy)benzoate, n-BuLi, THF, -110 °C; (ii) 90, 77%; (b) (i) HOAc, H₂O, reflux; (ii) K₂CO₃, CH₃OH, 34%; (c) H₂, Pd/C, CH₃OH, 77%; (d) K₂CO₃, CH₃OH, reflux, 72%.

\textbf{Scheme 9.} Paulsen and Stubbe’s synthesis of 7-deoxypancratistatin.
Hudlicky continued exploration of chemoenzymatic synthesis of these constituents in his asymmetric total synthesis of 7-deoxypancratistatin. As in the previous synthesis of pancratistatin, vinyl aziridine 70 was opened with a cyanocuprate to couple the A and C rings (Scheme 10). The tosyl moiety of intermediate 94 was replaced with methyl carbamate before the removal of the acetonide and the directed epoxidation that provided diol 95. The replacement of the N-tosyl protecting group with methyl carbamate was needed in order to avoid the difficulties experienced in closure of the B ring in Hudlicky’s first generation synthesis of pancratistatin (discussed previously). Stereoselective opening of the epoxide and protection of all free alcohols as their acetates provided cyclization precursor 96. The B ring was closed using Bischler-Napieralki conditions reported by Banwell, completing the core ring structure 97 with all necessary chirals centers set. Final deprotection occurred without incident to give 7-deoxypancratistain (2).
A recent total synthesis of 7-deoxypancratistatin was accomplished by Padwa utilizing a Stille coupling/intramolecular Diels–Alder cascade (Scheme 11). Furan-2-yl carbamic acid tert-butyl ester was coupled with A-ring fragment 98 via its carbamate anion to give amidofuran 99. BOC protection on the nitrogen was exchanged for a p-methoxybenzyl group. Stille coupling between the newly formed iodo derivative and methyl 2-tri-n-butyl stannylacrylate was followed by a spontaneous intramolecular [4+2]-cycloadDITION to furnish cycloadduct 100 in good yield over the two step cascade.
Dihydroxylation of the olefin was followed by acetonide protection, and final cleavage of the oxo-bridge under Lewis acid catalysis gave alcohol 101. Multiple functional group manipulations were required to arrive at olefin 102 which was converted to 7-deoxypancratistatin (2) by dihydroxylation, formation of the cyclic sulfate, opening with benzoate, and deprotection steps.
Trans-dihydrolycoricidine

Synthetic trans-dihydrolycoricidine was prepared by Gabrielsen and Pettit in 1992. In this synthesis the more readily available lycoricidine (47) was converted to its dihydro analog through catalytic hydrogenation with Adams' catalyst (Scheme 12). The mixture of cis and trans-dihydrolycoricidine 103 was only separable by first protection as its triacetate and then column chromatography. Cleavage of the acetate groups under basic conditions yielded synthetic trans-dihydrolycoricidine (3), which was screened in a variety of antiviral assays.

Scheme 13. Chida’s synthesis of trans-dihydrolycoricidine.

Chida’s asymmetric synthesis of trans-dihydrolycoricidine (3) was completed by protocols developed in his previously reported total synthesis of lycoricidine (47).\textsuperscript{51e,53b} Glucose derived diol 104 was first protected as its MOM ether before formation of the vinyl ether and a Ferrier rearrangement to give ketone 105. The hydroxyl moiety was eliminated giving the enone. Luche reduction of the ketone and protection of the resulting alcohol afforded 106,
which underwent azide reduction and coupling with the A-ring fragment. The nitrogen of cyclization precursor 107 was protected and the B ring was closed using an intramolecular Heck reaction. The Heck product 108 marked a point of divergence in the synthesis. This material was used as a common intermediate in the total synthesis of 7-deoxypancratistatin (2) and lycoricidine (47). Hydrogenation of 108 gave the trans isomer 109 as the sole product in 87% yield. This transformation was remarkably selective when compared to other hydrogenation reactions involving narciclasine and its analogues. In the process of hydrogenation the O-MPM group was also cleaved and the resulting C-2 hydroxyl was inverted via displacement of its triflate. Deprotection occurred uneventfully to provide trans-dihydrolycoricidine (3).

Ogasawara and Iwabuchi introduced chirality into their synthesis of trans-dihydrolycoricidine by use of cyclohexanoid building block 110, generated from the free diol by a lipase-mediated desymmetrization. This intermediate was converted to iodoenone 111 before the coupling with the aromatic A-ring via the Stille protocol (Scheme 14). Reduction of the enone gave 112. A Mitsunobu reaction using azide gave exclusively the SNi product which was convert to carbamate 113 over several steps. Cleavage of the tetrahydrofuran and elimination of bromine was mediated by Zn. Protection of the resulting alcohol allowed for a facile retro Diels-Alder reaction to provide cyclohexene 114. The TBS group was exchanged for the bulkier naphthoyl and a Bischler-Napieralski reaction closed ring B of the skeleton (115). Cyclic sulfate 116 was formed using standard conditions and was opened selectively at the more hindered C-3 position
by assistance of the naphthoyl group. Acidic hydrolysis and subsequent treatment with NaOMe provided \textit{trans}-dihydrolycoricidine (3) in 21 steps and 3.2\% overall yield from 110.

Scheme 14. Ogasawara and Iwabuchi’s synthesis of \textit{trans}-dihydrolycoricidine.
The selected approaches to *Amaryllidaceae* constituents presented above represents only a small portion of the work published on the synthesis of these complex natural products. Despite this large body of synthetic work the attainment of a truly efficient synthesis has yet to be realized. Another category of natural products that suffer a similar lack of efficient synthetic supply is the opiates. Those compounds related to morphine have a rich history that predates even that of the *Amaryllidaceae* constituents.

II-3 Morphine Alkaloids

The use of opium and morphine alkaloids predates even recorded history. Evidence for the cultivation of poppies in the 6th millennium B.C., most likely for their seeds, has recently been uncovered at Neolithic archeological sites in what are modern day Spain, France, and Germany.\(^88,89\) It is often difficult to identify instances of poppy cultivation and use from early written records because of ambiguity in individual accounts. Historians generally agree that the Sumerians, living in what is today Iraq, not only cultivated poppies but isolated opium from their seed capsules at the end of the third millennium B.C.\(^90\) The plant they cultivated was *Papaver Somniferum* but to the Sumerians it was known as “hul gil” the “joy plant.”\(^90\) The *Ebers Papyrus*, one of the oldest known medical texts, makes a mention around 1500 B.C. of opium as an additive in a concoction used to quiet the excessive crying of children. The text even goes as far as to make the guarantee that “the crying will stop at once” if the treatment, which includes paste made from flies, is administered on four successive days. Use of opium in
religious rituals as a pain killer and as a sleep inducing agent is well recorded through much of Greek and Roman history.91

In the eighth century A.D. opium was introduced to India and China by Arab traders and quickly became popular.92 Opium use as we know it today then migrated west, back into Europe between the tenth and thirteenth century. By the sixteenth century the problems of addiction and tolerance development were well known and were recorded in text throughout Europe.90

In the mid eighteenth century, amid growing trade deficits with China, the British Empire began the massive importation of opium from India to China. Even after a ban on opium smoking by the Yangzheng Emperor, the British smugglers increased opium exports from 15 tons in 1730 to over 75 tons in 1773.93 By the 1820s more than 900 tons of Indian opium was flooding into China every year. The tension of this situation resulted in two conflicts collectively known as the Opium Wars. The defeat of China in both conflicts resulted in almost complete destruction of Chinese sovereignty and subjugation of the Qin Dynasty by Britain and France. The anti imperialist (western) sentiment bred from this led to future conflicts and the eventual fall of dynastic China in 1911.

While the East India Company was tightening its grip on the opium trade, work was ongoing to isolate the components responsible for the strong biological activity of opium. Friedrich Wilhelm Sertürner isolated the alkaloid morphine (117) from raw opium in 1805.94 He named the compound after Morpheus, the Greek God of Dreams. Morphine (117) accounts for 10-16% of
the mass of raw opium in addition to the alkaloids codeine (118) 1-3%, papaverine (119) 0.8-1%, thebaine (4) 0.5-2%, and narcotine (120) 1-7% (Figure 15).

![Chemical structures of morphine (117), thebaine (4), codeine (118), papaverin (119), and narcotine (120).](image)

**Figure 15.** Naturally occurring morphine alkaloids.

The process by which opiates are extracted from poppy plants begins with harvesting of the raw opium latex. This is done by scoring the ripening seed pod with a sharp blade, traditionally in the afternoon and at a time to avoid rain, wind, or dew. In many opium producing regions a special 4-bladed tool called a *nishtar* is used to score the pod in intervals (3 times over 3 days). Raw latex dries to a sticky resin and is usually collected the following morning. Illicit drug traders often process the raw opium into morphine base in field processing labs. The morphine base is pressed into bricks and dried in the sun. This crude product can be smoked directly but is often converted to heroin.
Sertürner’s initial isolation of morphine in 1805 occurred only with his knowledge of its basic character. More than forty years would pass before the correct empirical formula \((C_{17}H_{19}NO_3)\) was established by Laurent. The most important work in the elucidation of morphine’s oxygenation is credited to Wright. It is interesting to note that in the course of his work Wright was the first to synthesize diacetylmorphine or heroin. It would be more than twenty years later before heroin would be “discovered” by chemists at Bayer and marketed as an over-the-counter, non-addictive substitute for morphine.

The presence of an oxygenated phenanthrene was established by numerous degradation studies by Greichten, Hofmann, and Pschorr. The correct structure of morphine was proposed in 1925 by Robinson and confirmed in 1952 with the first total synthesis of morphine by Gates. With the emergence of X-ray diffraction analysis around that time, the relative configuration of morphine was established by MacKay and Hodgkin. The story of the isolation, structure elucidation, and eventual synthesis of morphine is one that spans more than 150 years and has been made the subject of several reviews. It is an accomplishment that is to be both admired for its sound science and also its representation of a classic subdiscipline in organic chemistry, structure determination by degradation, which has all but disappeared in the modern day. It is interesting to imagine what might be accomplished if those same researchers, so trained in the academic rigor of structure proof by chemical degradation, were given access to modern equipment and spectroscopic techniques.
If any group of natural products could be said to possess the power to affect events on an international scale, it would undoubtedly be the opiates. The sheer scale of opium production and proceeds from its sale are staggering. In 2007 alone raw opium production was estimated at 8,200 tons and proceeds from illicit sales totaled more than $760 billion. This figure alone is a testament to the influence of the opium trade at a global level. Opium production occurs in many locations around the world, but is centered in India, Turkey, Tasmania, Pakistan, and Afghanistan. The raw opium harvested in these regions primarily contains morphine, thebaine, codeine, papaverin, and narcotine. While some of the more unstable countries, such as Afghanistan, are responsible primarily for illicit production of opiates, world supply for morphine alkaloids and their semisynthetic derivatives is still deepened upon natural isolation. This means that access to reliable supply for medical purposes is subject to environmental change, various pathogens, and geopolitical upheaval. One way to assure steady supply is the development of a truly practical synthesis route. Since the first synthesis of morphine by Gates in 1952 more than 30 total syntheses of morphine alkaloids have been reported. The attainment of a truly efficient synthesis, capable of competing with natural production, has remained elusive.

Starting in the first quarter of the 20th century, thebaine has increasingly gained notoriety as an important semisynthetic intermediate. Oxycodone (121), oxymorphone (123), naloxone (125), naltrexone (126), etorphine (124), and buprenorphine (127) are all manufactured through semisynthesis from thebaine (Figure 16). In addition to use as anelgesics (oxycodone, oxymorphone), these
synthetic opiates have a range of applications from the treatment of alcohol dependency (naltrexone) to use as elephant and rhino tranquilizers (etorphine).

![Chemical structures of synthetic opiates](image)

**Figure 16.** Semisynthetic opioids.

### II-3.1 Biosynthesis of morphine alkaloids

The importance of the morphine alkaloids has also placed great value on elucidation of their biosynthesis. The mechanistic pathway by which these benzyllisoquinoline alkaloids are synthesized by nature is fairly well understood and documented.\(^{105,106}\) The efficiency of nature is made obvious with the realization that all but one carbon atom of the morphine alkaloid skeleton is derived solely from the amino acid L-tyrosine (128) shown in Figure 17. Through a series of enzymatic transformation the L-tyrosine is converted to dopamine.
(129) and 4-hydroxyphenylacetaldehyde (130), the precursors to the C and A rings respectively.

![Chemical structures]

Figure 17. Biosynthesis of (R)-reticuline (134).

The formation of (S)-norcoclaurine (131) is accomplished by an asymmetric Pictet-Spengler condensation between 129 and 130. This is followed by N-methylation and further oxidation to (S)-3’-hydroxy-N-methylcoclaurine (132). Further methylation of each catechol provides (S)-reticuline (133), the precursor to more than 2500 benzylisoquinoline alkaloids. Conversion of 133 to its enantiomer (R)-reticuline (134) is accomplished via reduction of its Schiff base.
The most radical transformation in the biosynthesis of these molecules is undoubtedly the C-12/C-13 oxidative phenolic coupling to form salutaridine (135) (Figure 18). This reaction is catalyzed by salutaridine synthase, a NADPH-dependent cytochrome enzyme. In this case, P-450 enzyme behaves as an oxidase, as opposed to a monooxygenase, an atypical role compared with other alkaloid biosynthetic pathways. The ketone of salutaridine (135) is reduced to (7S)-salutaridinol which is subsequently converted to its acetate 137.
Displacement of the acetate by the phenolic hydroxyl in an $S_{N}2'$ fashion gives thebaine (4). Demethylation of the C-6 enol ether provides neopinone (138) and subsequently codeinone (139). Reduction of the C-6 ketone gives codeine (118), which is demethylated to give morphine (117).

The 19-step biosynthetic pathway by which plants make morphine and other congeners is considered to be well understood. Nevertheless alternative biosynthetic pathways and natural sources of morphine alkaloids have been reported. The synthesis of morphine (117) from thebaine (4) with oripavine (140) as an intermediate has been proposed (Figure 19). The existence of the poppy mutant thebaine oripavine poppy 1 ($top1$), which accumulates oripavine (140) and thebaine (4) but not codeine (118) and morphine (117) lends credence to this theory. It was speculated that mutation of the enzyme responsible for demethylation of thebaine (C-6 oxygen) is responsible for the arrest of the morphine biosynthetic pathway. The cultivation of this same mutant has also provided a means of producing oripavine (140) and thebaine (4) in a cost effective manner. This in turn has increased the availability of many medicinally important semi-synthetic opioids.

Figure 19. Alternative biosynthesis of morphine.
II-3.2 Selected Syntheses of thebaine and its use in semi-synthesis

Few classes of synthetic targets have the combination of medicinal utility and structural complexity present in morphine alkaloids. Congeners such as morphine and codeine have enjoyed wide recognition both within the synthetic community and among the general public. While thebaine does not enjoy the celebrity status of its cousins it nonetheless has enjoyed considerable attention from synthetic chemists. Its lack of useful medicinal activity, but availability as a natural congener has contributed to its use in a number of semisynthetic routes to more desirable alkaloids. This concept of turning trash into treasure is not new and was first applied to thebaine in Freund and Speyer's semisynthesis of oxycodone. The importance of thebaine as a semisynthetic intermediate has fueled enthusiasm for its total synthesis as a means to supplement or replace natural supply. With no fully synthetic, commercial route currently in existence for morphine and its congeners this goal has yet to be met. The following section summarizes several syntheses of thebaine followed by an overview of several semisyntheses of morphine congeners which feature thebaine as a key intermediate.

The first synthetic preparation of thebaine (4) by any means was disclosed by Rapoport in 1956. Dihydrothebaine (141) was obtained by enol ether formation of dihydrocodeinone and was treated with methyl hypobromite to give bromoacetal 142 (Scheme 15). Elimination of the bromide and hydrolysis yielded thebaine (4).
The first total synthesis of thebaine was published in 1975 by Schwartz (Scheme 16). Protection of (±)-N-norreticuline (144) as its trifluoroacetate was followed by oxidative para-ortho coupling to give (±)-N-trifluoroacetylnorsalutaridine (145). Replacement of the trifluoroacetate moiety with an ethyl carbamate and then reduction provided alcohol 146 which was converted to (±)-thebaine under acidic conditions.

Reaction conditions: (a) trifluoroacetic anhydride, K$_2$CO$_3$, CH$_2$Cl$_2$, rt; (b) thallium tris(trifluoro)acetate, CH$_2$Cl$_2$, -78 °C, 11%; (c) K$_2$CO$_3$, MeOH, rt, 86%; (d) EtOCOCl, Et$_3$N, CHCl$_3$, rt; (e) LAH, THF, reflux, 81% (3 steps); (f) 1 N HCl, rt

\section*{Scheme 15. Rapoport's synthesis of thebaine.}

\section*{Scheme 16. Schwartz's synthesis of thebaine.}
While the majority of synthetic routes to thebaine utilize other opiates as semisynthetic intermediates, work in the area of total synthesis is ongoing. Most recently a total synthesis of thebaine (4) has been reported by Stork, who took advantage of an intramolecular cycloaddition between a benzofuran and a tethered diene (Scheme 17). The benzofuran cycloaddition precursor 151 was constructed over a number of steps starting from iodoisovanillin (147). Most notably, the formation of benzofuran 149 was accomplished by intramolecular Heck coupling of iodide 148. The ketal was removed and the aldehyde chain extended under Wittig conditions to give 150. Homologation of aldehyde 150 using an alkoxyalkenylzirconocene was mediated by silver triflate. The [4+2] cycloaddition of 151 required somewhat harsh conditions but proceeded to give phenanthrofuran 152 in good yield. Cleavage of the silyl ether, oxidation to the C-9 ketone, and conversion of the methyl ester to its enol ether gave intermediate 153. The C-9 ketone was reduced and converted to its mesylate prior to introduction of methylamine via reductive amination. The resulting basic nitrogen smoothly displaced the mesylate to give (+)-condeine methyl ether (154) which had been previously converted to thebaine by Rapoport. 112e
As mentioned previously, thebaine (4) is a common starting material for the semisynthesis of more active and more commercially valuable opioids.
Oxycodone (121) is a synthetic opiate that possesses much of the analgesic properties of morphine (117) and is used in the treatment of medium to severe pain.\textsuperscript{113} Oxycodone was first synthesized from thebaine in 1916 by a two step process (Scheme 18).\textsuperscript{111} The C-ring of thebaine (4) is first oxidized to oxycodone (121) by treatment with acidic hydrogen peroxide. A subsequent hydrogenation gives the 14-hydroxylated, oxycodone (121). Interest in this commonly prescribed analgesic has prompted optimization of this net transformation by a number of academic and industrial researchers.\textsuperscript{114}

The analgesic and antitussive (cough suppressant) hydrocodone (122) is a semisynthetic opioid derived from either codeine (118)\textsuperscript{115} or thebaine (4).\textsuperscript{116} The synthesis, starting from thebaine (4), begins with diimide reduction of the C-8/C-14 olefin. The intermediate enol ether 156 is then subjected to hydrolysis to provide hydrocodone (122) (Scheme 19).

A variety of opioid receptor antagonists have been developed for the treatment of opiate dependency and overdose. The semisynthetic opioids
naltrexone (126) and naloxone (125) are two such antagonists which are produced commercially from thebaine (4).\textsuperscript{117}

![Scheme 19: Synthesis of hydrocodone from thebaine.](image)

Thebaine is converted to oxycodone (121) which is a common intermediate for both opioids (Scheme 20). Demethylation of the nitrogen is followed by alkylation with the appropriate chain to provide intermediates 157 or 158. These derivatives then undergo 3-\textit{O}-demethylation to provide antagonists naltrexone (126) and naloxone (125).
Scheme 20. Synthesis of naltrexone and naloxone from thebaine.

The preceding chapter has described previous contributions to the areas of enzymatic dihydroxylation of arenes, study of *Amaryllidaceae* constituents, and preparation of opiates related to thebaine. It is hoped that this will serve to frame our recent work in these areas and provide emphasis for the relevance of the presented research. As our progress in the synthesis of *Amaryllidaceae* constituent is built upon the chemoenzymatic oxidation of substituted benzenes, our work entailing TDO mediated oxidation of arenes is discussed first.
III. Discussion

III-1 Introduction

The development of methods to enhance brevity, selectivity, and efficiency in synthetic processes is one of the cornerstones of modern organic chemistry. Despite continued developments in catalysis, the prowess of enzymes in all three of the aforementioned areas remains unmatched. The availability of enzymatic methods/reactions which have direct equivalents in traditional organic chemistry is still lacking and this has contributed greatly to the slow adoption of these methods. Problems also exist with enzymatic reactions being “too specific” and lacking the generality needed for widespread acceptance. As part of our research in the direct application of enzymatic methods to total synthesis we have continually pursued exploration of the limits of the chiral dihydroxylation of arenes using various whole-cell fermentation processes. Our recent work in this area will be discussed. Highlighted will be the recent investigation of chemoenzymatic oxidation of various dibromobenzenes and their application to the total synthesis of (-)-conduritol E (162) (Figure 20). This will be followed by a discussion of our investigations into the steric factors governing the oxidation of benzoates by the TDO enzyme. The utility of these previously unidentified metabolites will be demonstrated by their application in the synthesis of pseudosugars, aminocyclitols, and various bicyclic ring systems.
Figure 20. Enzymatic oxidation of substituted benzenes in the synthesis of cyclitols.

The *Amaryllidaceae* constituents have garnered considerable attention from the scientific community for both their structural complexity and their intriguing biological activities. Several of the most important members of this family, pancratistatin (1), 7-deoxypancratistatin (2), narciclasine (46), and trans-dihydrolycoricidine (3) are available only in minute quantities. The future of these constituents as potential treatments for cancer is dependent on adequate supply. There have been many elegant approaches to the synthesis of these constituents over the years but few, if any, approach the brevity or efficiency required to advance these compounds as therapeutics.

In addition to the issue of supply, the above mentioned *Amaryllidaceae* constituents suffer from poor solubility profiles. While this issue has been partially addressed through the synthesis of various phosphate prodrug analogues, the generation of these derivatives is still dependent on supply of the natural product. A major portion of this discussion will focus on our efforts to both
provide general methods for the synthesis of the isocarbostyril skeleton and synthetic routes to novel C-1 analogues of 7-deoxypancratistatin. The total synthesis of 7-deoxypancratistatin (2) and trans-dihydrolycoricidine (3) were achieved from a common C-1 aldehyde analogue of 7-deoxypancratistatin (166) (Figure 21). This C-1 aldehyde was also used to synthesize several C-1 derivatives (167 - 170). These new analogues were evaluated for their efficacy against a number of human and nonhuman cancer cell lines providing strong evidence of the positive influence of C-1 derivatization on the activity profiles of these compounds.

![Chemoenzymatic synthesis of 7-deoxypancratistatin (2), trans-dihydrolycoricidine (3) and various C-1 analogues of 7-deoxypancratistatin (167-170).](image)

**Figure 21.** Chemoenzymatic synthesis of 7-deoxypancratistatin (2), trans-dihydrolycoricidine (3) and various C-1 analogues of 7-deoxypancratistatin (167-170).
In the last section of this discussion an approach to the synthesis of morphine alkaloids is presented. This double Diels-Alder approach is reliant on the latent symmetry contained in morphine alkaloids and is intended as a general entry to the synthesis of many medicinally vital opioids. A detailed investigation of various model systems will be discussed in addition to progress toward the total synthesis of thebaine (4) (Figure 22).

Figure 22. Proposed model system and the total synthesis of thebaine.

III-2 Dihydroxylation of o- and m-Dibromobenzene and Total Synthesis of (-)-Conduritol E

The use of whole-cell fermentation techniques in the production of cis-dienediols and the application of these metabolites to total synthesis has proven to be a powerful combination. As part of our ongoing research in this area we have become interested in the discovery of new substrates of the toluene
dioxygenase enzyme. Focus has been placed on elucidation of new metabolites that provide data on the mechanism of oxidation, materials suitable for total synthesis, or both. As disubstituted aromatics (ortho, meta, and para) account for some of the most richly functionalized compounds accepted by TDO we elected to investigate dibromobenzenes as possible substrates.

In general, m-substituted aromatics give lower yields of the corresponding diol compared their ortho and para counterparts. One exception to this is m-dibromobenzene, which is transformed to diene diol (174) in yields of up to 4 g/L fermentation broth.\(^{118}\) This provided access to the diol (174) in sufficient enough quantity to allow for its use in the total synthesis of narciclasine (46).\(^{50c}\) In order to complete the series, an investigation of the enzymatic oxidation of the remaining two isomers (ortho and para) was undertaken.\(^{119}\) p-Dibromobenzene (175) was converted to its corresponding dienediol (176) in 55 mg/L on a shakeflask scale (Figure 23). While the resulting diol is a meso compound, it is theoretically possible to incorporate it into asymmetric synthesis via subsequent desymmetrization steps.
Figure 23. Symmetry of dibromobenzenes and the metabolism to diols.

\( o \)-Dibromobenzene (159) was converted to the corresponding dienediol 161 by enzymatic oxidation with recombinant E. coli JM 109 (pDTG601). The scale of the reaction was increased from 0.5 L to 15 L to give an eventual yield of 4.1 g/L of the diol. This oxidation occurred selectively to provide only the 1,2-regioisomer as opposed to the meso 3,4-hydroxyl regioisomer. The absolute configuration of diol 161 was established by its conversion to (-)-conduritol E (162) (Scheme 21).\(^{120}\) The optical purity of (-)-conduritol E (162) was compared to a literature value and found to be within 3% error.\(^{120b}\)
The enantiomeric excess of diol 161 was also determined by its conversion to Mosher ester derived from monoprotected diol 182, (Scheme 22). This Mosher derivative 183 was previously prepared from homochiral and racemic alcohols 182. Examination of the $^{19}$F NMR spectrum showed a single peak at -72.8 ppm, indicating enantioselectivity $\geq 95\%$ in the original biooxidation of o-dibromobenzene.
III-3 Dihydroxylation of Benzoate Esters

The further exploration of new diols amenable to synthesis led us to investigate the processing of various benzoates by the toluene dioxygenase enzyme. Initial investigation into the precedent for dihydroxylation of benzoate esters revealed a single example of enzymatic oxidation of methyl benzoate (160) by *Pseudomonas putida* wild strains NCIB 1176 and NCIB 11680 or *Pseudomonas putida* UV4. In this report diol 163 was only partially characterized (¹H-NMR, MS, optical rotation), and so a more thorough investigation of the steric and functional limits for the processing of these benzoate esters by TDO was undertaken (Figure 24).
Figure 24. Enzymatic dihydroxylation of benzoate esters.

All of the required benzoate esters were either commercially available or prepared by standard procedures. Each benzoate (entries 1-8, Table 1) was screened on small scale in Fernach shake flasks with cells harvested from a 15 L fermentor. In cases where benzoates were substrates the resulting metabolites were isolated and characterized prior to preparative fermentation (15 L scale). Diols 163, 191, 196, and 197 were isolated in approximately 1 g/L. The slightly larger or more branched benzoates, n-Pr and i-Pr, were shown to be poor substrates, while n-Bu and t-Bu benzoates provided no detectable quantity of metabolites.
Table 1. Dihydroxylation of benzoate esters.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate (benzoate)</th>
<th>Product</th>
<th>Conversion (%)</th>
<th>Yield (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Me</td>
<td>163</td>
<td>95</td>
<td>1.30</td>
</tr>
<tr>
<td>2</td>
<td>Et</td>
<td>191</td>
<td>56</td>
<td>0.80</td>
</tr>
<tr>
<td>3</td>
<td>n-Pr</td>
<td>192</td>
<td>78</td>
<td>0.07</td>
</tr>
<tr>
<td>4</td>
<td>i-Pr</td>
<td>193</td>
<td>41</td>
<td>0.05</td>
</tr>
<tr>
<td>5</td>
<td>n-Bu</td>
<td>194</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>t-Bu</td>
<td>195</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>allyl</td>
<td>196</td>
<td>89</td>
<td>0.60</td>
</tr>
<tr>
<td>8</td>
<td>propargyl</td>
<td>197</td>
<td>91</td>
<td>1.0</td>
</tr>
</tbody>
</table>

The stability of the isolated diols proved to be quite remarkable when compared with their more labile Cl, Br, I, Me cousins. This stability allowed for purification of the diols by crystallization or chromatography and even permitted their combustion analysis. The absolute configuration of the new diols was established by stereochemical proof (Scheme 23). Diol 191 underwent PAD reduction to give vinyl ester 199. The structure of 199 was matched by chemical synthesis performed by Bradford Sullivan. Vinyl ester 199 was generated from diol 34 in 4 steps which included a carbonylation of vinyl bromide 202. The remaining diols (163, 192-193, 196-197) were saturated at the site of the olefin prior to transesterification to ethyl ester 199. In the case of allyl and propargyl diols 196
and 197 the additional unsaturation was reduced with excess PAD to provide vinyl ester 200, a compound previously converted and matched to ester 199. The conversion of all diols to intermediate 199 allowed for assignment of absolute stereochemistry as identical to that of well established diol 34.

\[
\begin{align*}
\text{CO}_2R & \quad \text{OH} & \quad \text{OH} \\
163 & \quad R = \text{Me} & \\
191 & \quad R = \text{Et} & \\
192 & \quad R = \text{n-Pr} & \\
193 & \quad R = \text{i-Pr} & \\
196 & \quad R = \text{allyl} & \\
197 & \quad R = \text{propargyl} & \\
198 & \quad R = \text{Me} & \\
199 & \quad R = \text{Et} & \\
200 & \quad R = \text{n-Pr} & \\
201 & \quad R = \text{i-Pr} & \\
198 & \quad R = \text{Me} & \\
199 & \quad R = \text{Et} & \\
200 & \quad R = \text{n-Pr} & \\
201 & \quad R = \text{i-Pr} & \\
34 & \quad R = \text{Me} & \\
202 & \quad R = \text{Et} &
\end{align*}
\]

Reaction conditions: (a) potassium azodicarboxylate, AcOH, MeOH, 0 °C; (b) H₂SO₄, EtOH, rt; (c) 2,2-DMP, p-TsOH, acetone, rt; (d) (i) t-BuLi, Et₂O, -78 °C, (ii) CO₂, -78 °C - rt; (e) EtOH, EDC, DMAP, CH₂Cl₂, 0 °C

**Scheme 23.** Conversion of diene carboxylates to ester 199 and proof of absolute configuration.

### III-3.1 Synthesis of Pseudo-sugars, Amino Cyclitols, and Bicyclic Ring Systems

The substitution of these new diols provides ready access to various pseudo-sugars. To demonstrate this utility, carba-α-L-galactopyranose (164) was synthesized in five steps starting with diol 163 (Scheme 24). Facial control of the dihydroxylation of the distal olefin was achieved by protection of the diol as its
acetonide prior to treatment with OsO₄. The isolation of the free diol from this oxidation was confounded by its polarity and decomposition during purification. Protection of the crude diols as their acetates allowed for isolation of intermediate 203 in good yield over the three steps. The acrylate 203 was reduced under hydrogenation conditions to provide 204 and 205 as a mixture of diastereomers (4:1). Separation of these isomers by chromatography allowed for reduction of ester 204 to give triol 206, an intermediate in the synthesis of carba-α-L-galactopyranose (164). Protected carbasugar 206 was identical in every respect to the previously prepared material, save for a slightly higher optical rotation ([α]D²⁰⁻⁵⁷.⁰⁸ vs. [α]D⁻⁴⁷).

\[
\begin{align*}
\text{163} & \xrightarrow{a} \text{203} \quad \text{b} \quad \text{c} \\
\text{203} & \xrightarrow{\text{4:1}} \text{204} + \text{205} \\
\text{205} & \xrightarrow{\text{206}} \text{carba-α-L-galactopyranose (164)}
\end{align*}
\]

Reaction conditions (a) (i) 2,2-DMP, acetone, p-TsOH, rt, (ii) OsO₄, NMO, acetone/H₂O, rt, (iii) Ac₂O, pyridine, rt, 70%; (b) H₂ 60 psi, Rh/Al₂O₃, EtOH, 69% of 206; (c) LAH, THF, reflux, 86%

**Scheme 24.** Synthesis of carba-α-L-galactopyranose (164).
In an effort to explore the synthetic possibilities afforded by these diols their utility in a number of cycloaddition reactions was investigated (Scheme 25.). The facile dimerization of acetonides derived from diol 34 is well known and documented. We first sought to explore the tendency of these new substrates to undergo a similar cycloaddition with the hope that it would likewise proceed with high regio- and stereoselectivity. The acetonides 207 and 208 derived from diols 191 and 197 smoothly underwent an analogous [4 + 2] cycloaddition when maintained at room temperature for prolonged periods of time (7 days) giving dimers 209 and 210, respectively. This same dimerization could be accelerated by heating a concentrated solution of each acetonide in toluene (6 hours). In each case, the cycloadducts were formed by an apparent endo-approach of the dieneophile to the less hindered face of both cycloaddition partners. The dimers 209 and 210 were formed as single diastereomers whose structures were confirmed by NOESY spectroscopy. The dimerization of the free diol 191 was also explored at a variety of temperatures and concentrations. Heating of the free diol at rt or 60 °C provided no change in composition, even after prolonged reaction times (3 days). On the other hand, heating the same diol 191 at 110 °C for 12 hours prompted the loss of water and the formation of the corresponding phenol.
Acetonide 207 was also reacted with electron poor dieneophile dimethylacetylenedicarboxylate (DMAD). The cycloaddition between electron poor diene 207 and DMAD afforded mixtures of cycloaddition product 211 and dimer 209 (2:1 ratio).

Finally, these dienes were used in a series of inverse electron demand Diels-Alder reactions with an in situ-generated acetyl nitroso dieneophile. The cycloaddition proceeded with complete regio- and stereoselectivity to provide dihydrooxazine 212. The labile N-O bond in this highly functionalized
intermediate was cleaved to provide hydroxyl ester 213, a potential precursor to amino pseudo-sugars and aminocyclitols. Most recently this intermediate has been used in the total synthesis of the antiviral oseltamivir (214).\textsuperscript{34b}

### III-4 Intramolecular Aziridine Opening Approach to the Amaryllidaceae Alkaloids

#### III-4.1 Synthesis of C-1 Analogues of 7-Deoxypancratistatin

The efforts of the Hudlicky group to provide viable synthetic routes to Amaryllidaceae constituents and their unnatural analogues have spanned almost 20 years. Our current work represents the 8\textsuperscript{th} generation approach to the efficient synthesis of these congeners. In this case, the ready access to a specifically chosen class of derivatives was a driving force in the development of the current generation. It was envisioned that an aldehyde such as 166, which contains all of the structural elements needed for strong activity, would provide an ideal common intermediate for not only the total synthesis of several Amaryllidaceae constituents, but also a variety of C-1 analogues (Figure 25). The oxidative cleavage and recyclization of a functionalized phenanthrene 215 suggested itself as an ideal transformed to attain the desired core configuration with the required C-1 aldehyde.
**Figure 25.** Retrosynthetic analysis for the synthesis of *Amaryllidaceae* alkaloids and their C-1 analogues.

Phenanthrene 215 could, in turn, be attained from an intramolecular aziridine opening and cyclization of a tethered olefin such as 216. This transformation is second in importance only to the selective opening of the oxirane 217. The enzymatic dihydroxylation of bromobenzene would provide the diol 34 inclusive of all the functionality necessary for the synthesis of epoxy-aziridine 217.

The synthesis of the key aldehyde intermediate 166 began with the enzymatic dihydroxylation of bromobenzene with *E. coli* JM109(pDTG601) to provide diol 34 (Scheme 26). The protection of the diol as its acetonide 218 allowed for facial-selective aziridination via Yamada-Evans protocol to give 219. This *N*-tosyl aziridine was matched to that known previously in the synthesis of *Amaryllidaceae* constituents. The removal of the bromide proceeded under radical conditions to provide vinyl-*N*-tosylaziridine 70.
Epoxidation of the olefin was accomplished by treatment with *m*-CPBA in refluxing DCE. The epoxides 217 and 220 were obtained in good yield (96%) but as a 3:1 mixture of diastereomers. Improvement of this ratio under a variety of reaction conditions proved elusive but it was discovered that higher reaction temperatures and shorter reaction times provided optimal yields of the desired compounds. Successive recrystallizations from isopropyl alcohol allowed for enrichment of the ratio up to 10:1 (217:220), but in general two recrystallizations provided a workable ratio of 7:1. These enriched mixtures of epoxyaziridines were added to the alane derived from acetylene 222. The synthesis of 222 from...
piperonal (224) followed procedures established by Corey and Overman (Scheme 27). Piperonal (224) was allowed to react with carbon tetrabromide in the presence of triphenylphosphine to give dibromide 225 which upon treatment with n-BuLi provided the desired alkyne 222 in good overall yield.

![Reaction scheme]

**Scheme 27.** Synthesis of acetylene 222.

The addition of epoxyaziridines 217 and 220 to the alane derivative of 222 produced intermediate alcohol 221 that was immediately protected as its silyl ether 223. The cumulative yield for this two-step sequence was eventually optimized to 77%, but initial attempts at the epoxide opening provided the desired product in poor yield (10-20%). This troubling bottleneck prompted further investigation which revealed a rather strict set of reaction conditions necessary for attainment of reproducible yields. Stringent control of temperature, both in the formation of the alane and in the addition of the epoxide, was found to be crucial. Deviation from this protocol led to decreased yields attributed to either the incomplete formation of the alane or various side reactions of the epoxyaziridine. Predominant side products included those derived from the nucleophilic opening of the aziridine and/or epoxide with chloride anion as well as opening of the aziridine with the alkyne (structures are not shown). It was also discovered that
for complete conversion of the epoxy aziridine two equivalents of the alane were required. Fewer equivalents of the alane resulted in incomplete conversion of starting material while more equivalents yielded increased production of the byproducts mentioned above.

The reduction of alkyne 223 to its cis-alkene 216 was investigated using a variety of the catalytic hydrogenation and borane-based conditions (Table 2). The majority of hydrogenation conditions attempted proved ineffective for the selective reduction of the alkyne to the desired cis-alkene 216. Poisoning of Lindlar’s catalyst with quinoline allowed for chemoselective reduction of the alkyne in excellent yield. An alternate reduction protocol utilizing borane dimethylsulfide complex also provided selective reduction of the alkyne, albeit in lower yields (76%).

**Table 2. Hydrogenation of alkyne 223.**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Time (h)</th>
<th>Ratio of 223:226:216</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lindlar’s cat. 20 mol%, MeOH, 40 psi H₂</td>
<td>24 h</td>
<td>1 : 3 : 2</td>
</tr>
<tr>
<td>Lindlar’s cat. 20 mol%, EtOAc, 40 psi H₂</td>
<td>24 h</td>
<td>1 : 4 : 1</td>
</tr>
<tr>
<td>Lindlar’s cat. 20 mol%, EtOH, 40 psi H₂</td>
<td>24 h</td>
<td>1 : 2.5 : 1</td>
</tr>
<tr>
<td>Lindlar’s cat. 40 mol%, MeOH, 1 atm H₂</td>
<td>3.5 h</td>
<td>0 : 1 : 3</td>
</tr>
<tr>
<td>Lindlar’s cat. 40 mol%, MeOH, 1 atm H₂</td>
<td>3 h</td>
<td>0 : 0 : 1</td>
</tr>
<tr>
<td>quinoline (20 mol%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pd/C 40 mol%, MeOH, 1 atm H₂</td>
<td>3 h</td>
<td>0 : 1 : 0</td>
</tr>
<tr>
<td>BH₃/SMᵉ₂, cyclohexene, 0 °C</td>
<td>3.5 h</td>
<td>0 : 0 : 1</td>
</tr>
</tbody>
</table>
The key precursor 216 for the intramolecular aziridine opening and cyclization was adsorbed onto rigorously dried silica and heated at 120 °C for 24 hours to provide phenanthrene 215 (Scheme 28). This reaction is performed in the solid phase with an approximate mass ratio of 5:1 (dry silica : substrate). Purification of the reaction products was conveniently accomplished by introduction of the dry silica mixture onto a prepared silica column followed by elution.

Scheme 28. Intramolecular aziridine opening and synthesis of C-1 aldehyde by oxidative phenanthrene cleavage.
In order to attain the proper phenanthridone core an oxidative cleavage of olefin 215 was required. Several methods for the efficient cleavage of the phenanthrene were examined in an attempt to efficiently generate aldehyde 166 or similar phenanthridone species.

Initial osmylation of 215 produced cis-diol 227 as a mixture with over-oxidized ketoalcohol 228. The crude mixture was reduced completely to cis-diol 227 before the oxidative cleavage with periodate to provide dialdehyde 229 in 83% over the 3 steps. The use of excess equivalents of co-oxidant (NMO) in the osmylation step allowed for isolation of hydroxyketone 228 as the sole product in 89% yield. The complete assignment of stereochemistry in 228 was determined by extensive NMR analysis of its acetate 232 by Dr. Ion Ghiviriga at the University of Florida (Figure 26). The hydroxyketone 228 was likewise converted to dialdehyde 229 following a similar reduction and oxidative cleavage procedure. Dialdehyde 229 was observed in crude form as a complex mixture of alcohol 231 and atropisomers. It is interesting to note that first attempts at the oxidative cleavage of diol 227, which incorporated ethanol as a cosolvent, resulted in formation of ether 230. This material was converted quantitatively to alcohol 231 under acidic, aqueous conditions. Oxidation of 231 provided the desired phenanthridone 166, a fully protected C-1 aldehyde analogue of 7-deoxypancratistatin, in 69% overall yield from ketone 228.
3.78

OH

Figure 26. Full assignment of NMR chemical shifts for the acetate of keto alcohol 232.

In addition to the protocols described above, a more direct route to dialdehyde 229 and phenanthridone 166 was investigated using direct ozonolysis of olefin 215 (Scheme 29). Treatment of the alkene with ozone followed by reductive workup allowed isolation of products similar to those obtained previously in the cleavage of diol 227. Treatment of this mixture with IBX provided the desired C-1 aldehyde 166 in 76% over the two steps. This procedure was a considerable improvement over the previous oxidative cleavage protocol resulting in an increase in yield of 15% and elimination of two operations. In addition, the oxidative cleavage of hydroxyl ketone 228 was investigated using sodium periodate under a variety of acidic and basic conditions. Unfortunately, all reactions failed to provide the desired acid 233 or methyl ester 234. In the end, the ideal procedure for the oxidative cleavage for olefin 215 was found to be
direct ozonolysis to alcohol 231 and oxidation to C-1 aldehyde 166. This protocol provides the desired phenanthridone in the most efficient and fewest steps.

Reaction conditions: (a) i) \text{O}_3, \text{CH}_2\text{Cl}_2, \text{Sudan Red}, -78^\circ\text{C}, \text{ii) Me}_2\text{S}, -78^\circ\text{C} - \text{rt}; (b) IBX, DMF, rt, 76\% (over 2 steps); (c) OsO_4, NMO, CH_2Cl_2/H_2O, rt, 89\%

**Scheme 29.** Ozonolysis route to aldehyde 166 and proposed oxidative cleavage of hydroxyketone 228.

The synthesis of C-1 analogues of 7-deoxypancratistatin proceeded from aldehyde intermediate 166 (Scheme 30). Oxidation of aldehyde 166 with \textit{m}-CPBA cleanly provided the acid at the C-1 positions. The acid 235 was converted to its methyl ester 236. This transformation provided both access to a second C-1 ester derivative and easier handling of the otherwise polar acid. Reductive cleavage of the \textit{N}-tosyl protecting group gave phenanthridone 237, deprotection of which under acidic conditions provided the fully hydroxylated C-1 ester 169. Saponification with lithium hydroxide gave the corresponding C-1 carboxylic acid derivative of 7-deoxypancratistatin 168.
Reaction conditions: (a) m-CPBA, Na$_2$HPO$_4$, CH$_2$Cl$_2$, 40 °C, 85%; (b) CH$_2$N$_2$, Et$_2$O, 0 °C; (c) Na/naphthalene, DME, -50 °C, 58%; (d) 3% HCl in MeOH, rt, 69%; (e) LiOH, MeOH, rt, 95%

Scheme 30. Conversion of C-1 aldehyde 166 to C-1 ester 169 and acid 178.

An alternative procedure for the synthesis of methyl ester 236 through ozonolysis was also envisioned (Scheme 31). A procedure for the cleavage of olefins to diesters reported by Marshall appeared particularly attractive for this transformation. The direct cleavage of alkene 215 by treatment with ozone in the presence of methoxide provided an intermediate containing a monoester and an aldehyde after only ten minutes. This material was exposed to the same reaction conditions under prolonged reaction time (1.25 hours) to give ester 236 in 6% yield. It is likely that oxidative cleavage occurs quickly resulting in the observed monoester but that prolonged reaction time is required to form and subsequently oxidize the hemiacetal from the aldehyde. The low yield of this
transformation was attributed to the instability of other functionality under prolonged treatment with ozone.

\[
\begin{array}{c}
\text{Reaction conditions: (a) } \text{O}_3, \text{NaOH, MeOH, CH}_2\text{Cl}_2, \text{-78 °C, 6%}
\end{array}
\]

**Scheme 31.** Alternative oxidative cleavage of olefin 215.

The C-1 hydroxymethyl and acetoxyethyl analogues were synthesized using a similarly straightforward protocol. The aldehyde 166 was reduced with sodium borohydride to give alcohol 238 in good yield (Scheme 32). Control of temperature was important for selective reduction of the aldehyde in the presence of the activated phenanthridone. Reactions performed above 0 °C resulted in reduction of the benzamide moiety. The alcohol 238 was converted to its acetate prior to reductive detosylation. Intermediate phenanthridone 240 was not isolated but instead converted directly to alcohol 241 via cleavage of the silyl ether by treatment with TBAF.
Reaction conditions: (a) NaBH₄, dioxane, EtOH, 0 °C, 85%; (b) Ac₂O, pyridine, DMAP, CH₂Cl₂, 81%, (c) Na/naphthalene, DME, -78 °C; (d) TBAF, THF, 0 °C, 74% (2 steps); (e) (i) K₂CO₃, MeOH, H₂O, (ii) HCl, H₂O, 75%; (f) 3% HCl in MeOH, rt, 45%

Scheme 32. Conversion of C-1 aldehyde 166 to alcohol 167 and acetoxyethyl derivative 170.

Hydrolysis of the C-1 acetoxyethyl derivative 241 with K₂CO₃ was followed by acidification and cleavage of the acetonide to furnish the fully hydroxylated derivative 167. The C-1 acetate analogue 170 was provided by controlled hydrolysis of the acetonide with 3% HCl in methanol.

Alternative routes to alcohol 238 were investigated. Since the alkane 226 could be readily formed from hydrogenation of alkyne 223, it was reasoned that
selective oxidation at the benzylic position following ring closure would give ketone 243 (Scheme 33). Formation of lactone 245 through oxidative ring expansion would be followed by cleavage and rearrangement to provide efficient synthesis of alcohol 238.

![Chemical structures](image)

**Reaction conditions:** (a) silica gel, 120 °C, 24 h, 69%; (b) PCC, CH₂Cl₂, rt, 11 h, 243:244 (46%:23%); (c) m-CPBA, Na₂HPO₄, CH₂Cl₂, 40 °C, 22%

**Scheme 33.** Alternative approaches to alcohol 238.

Intramolecular ring closure and aziridine opening of 226 was achieved under solvent free conditions as described previously¹²⁸ to give 242. The resulting cyclic alkane was treated with PCC in dry methylene chloride producing ketone
243 along with fully aromatic compound 244 as a minor product. Ketone 243 was subjected to Baeyer-Villiger oxidation conditions in the hopes of generating lactone 245, but instead ester 246 was isolated in only moderate yield. The identity of 246 was confirmed by transterification in basic methanol followed by a positive test for phenol by ferric chloride.

In addition to the C-1 oxygenated derivatives mentioned above, we also desired to generate a series of analogues bearing an amine at the C-1 position. In theory, this would allow for further conversion to various amine salts that would confer greater water solubility. Our intention was to use the C-1 aldehyde functional handle for this transformation (Scheme 34).

**Scheme 34.** Attempted synthesis of C-1 amines.

Reaction conditions: (a) Na/naphthalene, DME, -78 °C, 78%
Table 3. Attempted reductive amination reactions.

<table>
<thead>
<tr>
<th>Amine</th>
<th>Conditions</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNEt₂</td>
<td>NaBH₄, AcOH,</td>
<td>reduction of amide and aldehyde</td>
</tr>
<tr>
<td></td>
<td>MeOH, rt</td>
<td></td>
</tr>
<tr>
<td>HNEt₂</td>
<td>NaBH₃CN, AcOH,</td>
<td>no reaction at rt, reduction of aldehyde</td>
</tr>
<tr>
<td></td>
<td>MeOH, rt - 50 °C</td>
<td>and cleavage of acetonide at 50 °C</td>
</tr>
<tr>
<td>HNMe₂·HCl</td>
<td>NaBH₃CN, MeOH, rt-</td>
<td>no reaction, decomposition at 50 °C</td>
</tr>
<tr>
<td></td>
<td>50 °C</td>
<td></td>
</tr>
<tr>
<td>MeNH₂</td>
<td>NaBH₃CN, AcOH,</td>
<td>reduction of aldehyde, slow cleavage of acetonide</td>
</tr>
<tr>
<td></td>
<td>MeOH, rt</td>
<td>(15 h)</td>
</tr>
<tr>
<td>MeNH₂·HCl</td>
<td>NaBH₃CN, MeOH, rt</td>
<td>no reaction</td>
</tr>
<tr>
<td>MeNH₂</td>
<td>NaBH₃CN, TiCl₄, rt</td>
<td>reduction of amide and aldehyde (249, 52%)</td>
</tr>
</tbody>
</table>

Reductive amination was seen as the most straightforward route to the desired amines and so was pursued first. A number of protocols were examined for the reductive amination of aldehyde 166 in the hopes of installing the desired amine unit (Table 3). In early attempts the use of excess sodium borohydride resulted in reduction of both the phenanthridone and aldehyde moieties with no incorporation of amine (249). Attempts to cleave the N-tosyl group with Na/naphthaline resulted in epimerization at the C-1 position (248). Use of sodium cyanoborohydride resulted in improved stability of the phenanthridone but failed to provide the desired reductive amination using a variety of amines. Reactions at elevated temperature with NaBH₃CN and acetic acid led to reduction of the aldehyde and/or cleavage of the acetonide protecting group. Treatment of aldehyde 166 with methyl amine in the presence of TiCl₄ followed by sodium cyanoborohydride likewise failed to produce the methylamine. The fully reduced product 249 was isolated, albeit in moderate yield. It is thought that steric factors
play a defining role in the stability of the C-1 aldehyde under the conditions that were employed.

In addition to attempts at reductive amination, installation of C-1 amine functionality was pursued through direct displacement of C-1 mesylate 250. Alcohol 238, derived from selective reduction of aldehyde 166, was converted to the corresponding mesylate in good yield (Scheme 35). Treatment of mesylate 250 with an excess of methylamine at 55 °C allowed for smooth displacement of the mesylate, but also transamidation to amine 251. As observed previously, the activated phenanthridone generates problems in further functionalization of these C-1 analogues. Future work will focus on either transformations on the free phenanthridone or functionalization of the C-1 position prior to closure of the B ring.

![Scheme 35. Displacement of C-1 mesylate and synthesis of amide 251.](image)

Reaction conditions: (a) MsCl, Et₃N, CH₂Cl₂, 0 °C, 88%; (b) MeNH₂, THF, 55 °C (sealed tube), 41%.

**III-4.2 Total Synthesis of trans-Dihydrolycoricidine**

In the course of exploring routes toward the total synthesis of 7-deoxypancratistatin, it was recognized that the *Amaryllidaceae* constituent *trans*-dihydrolycoricidine (3) could readily be accessed from our common aldehyde...
intermediate 166. Formation of decarbonylated species 252 was attained by treatment of 166 with Wilkinson's reagent at 130 °C (Scheme 36). Subsequent detosylation gave phenanthridone 253 in good yield (74%) over the two steps. Acid catalyzed hydrolysis of the acetonide and silyl ether yielded natural congener trans-dihydrolycoricidine (3) in 71% yield from 253.

![Chemical structure](image)

Reaction conditions: (a) RhCl(Ph₃P)₃, toluene, 130 °C; (b) Na/naphthalene, DME, -78 °C, 74% (2 steps); (c) 3% HCl in MeOH, rt, 71%

**Scheme 36.** Synthesis of trans-dihydrolycoricidine (3).

### III-4.3 Formal Total Synthesis of 7-Deoxypancratistatin

Initial design for a general synthetic approach to the *Amaryllidaceae* constituents included completion of the total synthesis of 7-deoxypancratistatin by a Baeyer-Villiger type transformation to give formate 254, a protected form of the natural product (Figure 27).

85
The proposed transformation was based on a small number of reports which describe Baeyer-Villiger oxidation of cyclohexane carboxyaldehydes to their corresponding formates.\textsuperscript{134} In general, aliphatic aldehydes give acids when treated under Baeyer-Villiger conditions.\textsuperscript{135} In our hands, treatment of aldehyde 166 under a series of conditions known to promote the desired transformation either failed to promote reaction or cleanly provided C-1 carboxylic acid derivative 235, an intermediate described above in the synthesis of ester 236. The alternative Baeyer-Villiger substrate such as methyl ketone 257 was thought to provide a greater electronic differentiation between the two possible alkyl migrations (Scheme 37). It was expected that the more electron rich secondary center would migrate to produce a C-1 acetate protected version of 7-deoxypancratistatin. The synthesis of the required methyl ketone 257 began with addition of methyl Grignard to aldehyde 166. It is interesting to note that other nucleophilic sources of a methyl moiety, such as MeLi, failed to provide the desired alcohol. Up to 12 equivalents of MeMgI were required for total conversion of starting material. The crude alcohol 256 was then directly oxidized with Collins reagent to provide methyl ketone 257 in excellent yield.
Methyl ketone 257 proved unreactive to basic or buffered reactions with peracids but upon treatment with m-CPBA, under acid catalysis, clean conversion to lactone 261 and free diol 260 was observed. In the case of product 261, definitive assignment of the structure was only realized after IR spectroscopy showed a very characteristic C=O lactone stretch at 1788 cm$^{-1}$. Formation of this product results from transesterification of the C-1 methyl ester formed in the Baeyer-Villiger oxidation by C-3 hydroxyl. It was unclear whether the cleavage of the acetonide precedes the formation of the C-1 methyl ester but treatment of
diol 260 under identical reaction conditions provided clean conversion to lactone 261. It was thought that the steric bulk of the functionality present in the C ring of 257 was inhibiting migration of the electronically preferred secondary center. It was thought that replacement of the bulky sily ether and acetonide in 257 with acetates might lessen steric congestion around the C-1 position. Triacetate 258 was generated from ketone 257. However, Baeyer-Villiger oxidation of this triacetate provided methyl ester 259 as the sole product in 85% yield. At this point approaches toward 7-deoxypancratistatin via Baeyer-Villiger oxidation were discontinued in favor of other plans for a formal synthesis.

In 1942, Hunsdiecker reported the conversion of the silver salts of carboxylic acids to the corresponding alkyl bromides of one less carbon.136 It was thought that if alkyl bromide 262 could be formed from the readily available acid 255, cleavage of the silyl ether at the C-2 position would allow for formation of epoxide 263 (Scheme 38). Similar epoxides have been shown to undergo trans-diaxial opening with oxygen nucleophiles to yield properly configured diols at the C-1/C-2 position. Initially we attempted Hunsdiecker transformation using the Cristol-Firth modification in which the, often difficult to prepare, silver salt is avoided.137 Acid 255 was treated with excess red HgO and one equivalent of bromine in refluxing carbon tetrachloride to provide compound 262 in poor yield and as a 3:1 mixture of isomers. Repetition of this reaction proved problematic and continued to give yields in the range of 5-15%. In an effort to provide synthetically useful quantities of bromide 262, more traditional Hunsdiecker conditions were investigated. The formation of the required silver salt from the
carboxylic acid is often the most difficult step in these reactions, a generalization that proved true in this case. A variety of conditions were attempted to form silver salt 264 (Table 4). Use of sodium ethoxide as base proved to be most effective in promoting silver-salt formation. Unfortunately, treatment of the silver salt 264 with bromine in CCl$_4$ failed to provide bromide 262 and resulted in recovery of $\geq 80\%$ of the acid 255 (based on silver salt) in each case.

Reaction conditions: (a) (i) HgO (red), CCl$_4$, (ii) Br$_2$, reflux, 15%; (b) Br$_2$, CCl$_4$, reflux

Scheme 38. Hunsdiecker conditions employed toward the synthesis of 263.
Table 4. Conditions attempted for formation of silver salt 264.

<table>
<thead>
<tr>
<th>Conditions for salt formation</th>
<th>Notes about salt</th>
<th>Result of Hunsdiecker</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtOH, AgNO₃</td>
<td>Fine white solid, 25 % yield</td>
<td>Recovery of starting material</td>
</tr>
<tr>
<td>EtOH, i) NaOH, ii) AgNO₃</td>
<td>Black solid, 42 %</td>
<td>No reaction, recovery of starting material</td>
</tr>
<tr>
<td>EtOH, i) NaOEt, ii) AgNO₃</td>
<td>Black solid, 68%</td>
<td>No reaction, recovery of starting material</td>
</tr>
</tbody>
</table>

Given the success of the facile C-1 decarbonylation protocol used in the total synthesis of *trans*-dihydrolycoricidine (3), we thought to employ a similar transformation toward the total synthesis of 7-deoxypancratistatin. Our goal was to generate an α,β-unsaturated aldehyde such as 266 that upon decarbonylation and epoxidation would provide epoxide 267 and subsequently 7-deoxypancratistatin (Scheme 39).

![Scheme 39. Attempted synthesis of epoxide 267 through C-2 hydroxyl elimination.](image)

Reaction conditions: (a) TBAF, THF, rt, 22%; (b) (i) TsCl, pyridine, DMAP, CH₂Cl₂, rt. (ii) various bases
Cleavage of the silyl ether in 166 generated hydroxyl-aldehyde 265, albeit in modest yield. The alcohol was converted to its tosylate, but treatment with a variety of bases (pyridine, DBU, n-BuLi, LDA) at a variety of temperatures ( -78 °C – 100 °C) failed to provide the desired transformation. In most cases the starting O-tosylate was recovered but in the case of treatment with neat DBU at 80 °C, total decomposition was observed.

Inspired by reports of Nicolaou’s taxol synthesis\(^{138}\) and/or Grieco’s synthesis of compactin,\(^{139}\) we sought to generate olefin 270 by either thermal elimination of formaldehyde from oxetane 269 or Grob fragmentation of mesylate 268, (Scheme 40). In each case, mesylate 268 served as a common intermediate for both syntheses. Its preparation began with alcohol 238, which was protected as its acetate 239 (Scheme 41).

Scheme 40. Attempted olefin formation by Grob fragmentation or through elimination oxetane 269.
Cleavage of the C-2 silyl ether was followed by mesylation of the resulting alcohol to give 272. When mesylate 272 was treated with basic methanol at 0 °C clean conversion to hemiacetal 273 was observed in less than ten minutes. Careful work-up allowed for isolation of this material. Resubmission of this intermediate to the same reaction conditions provided alcohol 268 in good yield.

As mentioned previously, it was envisioned that olefin 270 could be generated from mesylate 268 by both formation and thermal elimination of oxetane 269 or by Grob fragmentation of alcohol 268. A variety of conditions were investigated to effect the desired transformation and are summarized in Table 5. Mesylate 268 proved unreactive to treatment with stoichiometric amounts of base at various temperatures. Even treatment with large excesses of
base and at elevated temperatures proved unfruitful. Last, the upper limits of the reaction were probed by treatment of alcohol 268 with excess KH at 180 °C providing considerable decomposition along with recovery of a minor portion of starting material.

Table 5. Attempted Grob fragmentations and oxetane formation.

<table>
<thead>
<tr>
<th>Base</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH (1.5 eq)</td>
<td>Toluene/THF</td>
<td>0 °C - rt - 120 °C</td>
<td>Recovery of starting material 83%</td>
</tr>
<tr>
<td>KH (12 eq)</td>
<td>Toluene/THF</td>
<td>150 °C (sealed tube)</td>
<td>Recovery of starting material 74%</td>
</tr>
<tr>
<td>KH (10 eq)</td>
<td>DME with 18-crown-6</td>
<td>Rt - 120 °C (sealed tube)</td>
<td>Starting material 58%</td>
</tr>
<tr>
<td>KH (10 eq)</td>
<td>o-xylene</td>
<td>180 °C (sealed tube)</td>
<td>Starting material 34%</td>
</tr>
</tbody>
</table>

Another possibility considered for the formation of olefin 270 was the decarboxylation and elimination of a tosylate such as 275 (Scheme 42). The C-2 silyl ether in 236 was cleaved to give alcohol 274. Upon treatment with TsCl in CH₂Cl₂ the unexpected formation of α,β-unsaturated ester 276 was observed. This product results from abstraction of the alpha proton of the ester and elimination of the intermediate tosylate. This elimination, under such mild conditions, came as a surprise given the difficulty in elimination of the C-2 hydroxyl of aldehyde 265 (previously mentioned).
An improved synthesis of tosylate 275 was devised in order to avoid ester 274 and formation of elimination product 276. Cleavage of the silyl ether 235 proceeded in modest yield to provide hydroxyl acid 277. Despite little precedent for the transformation, alcohol 277 was converted to tosylate 278 by treatment with two equivalence of methyl lithium followed by TsCl. Decarboxylation by treatment with base under elevated temperature failed to generate olefin 270.

An alternative to the elimination of tosylate 275 is decarbonylation and dehydration of hydroxyacid 277 using DMF dimethyl acetal. This transformation, developed by Hara,140 was successfully employed by Wender in his synthesis of (−)-retigeranic acid.141 The substrate for Wender's transformation was acyclic, nevertheless we endeavored to produce olefin 270 by this method. Acid 277 was treated with DMF dimethyl acetal in refluxing chloroform but instead of olefin 270, mixed acetal 279 was formed. The methyl ester in this product is the results
of liberation of methanol during formation of the mixed anhydride followed by esterification of the acid.

\[
\begin{align*}
\text{235} & \xrightarrow{a} \text{277} & \xrightarrow{b} \text{275} \\
\text{279} & \xrightarrow{c} \text{270} \\
\end{align*}
\]

Reaction conditions: (a) TBAF, THF, 50 °C, 44%; (b) MeLi, TsCl, THF, 35%; (c) KHMDS, toluene, 120 °C; (d) DMF dimethyl acetal, CHCl₃, reflux; 58%

Scheme 43. Additional approaches to olefin 270 through acid 277.

Given previous difficulties in the generation of C-1/C-2 olefin 270 we chose to pursue generation of this material using a simplified precursor. Phenanthridone 253 was converted to its free alcohol 280. Three routes for conversion of this alcohol to olefin 282 were pursued. The first entailed conversion of the alcohol to its mesyl derivative 281. Unfortunately, all attempts at an E₂ type elimination failed to give the desired olefin. Treatment of alcohol 280 with nucleophile-free Mitsunobu conditions also failed to elicit the needed dehydration. Finally, given the Hudlicky group's recent work with the Burgess reagent and its derivatives¹⁴² we attempted this dehydration using freshly prepared Burgess reagent. Treatment of alcohol 280 with Burgess salt provided
sulfamidate 283 as the only isolated product. It was thought that reformation of
the sulfamidate anion by treatment with base would allow for completion of the
dehydration reaction. To our dismay treatment of this intermediate with
potassium hydride in refluxing dimethoxy ethane did not result in the formation of
the desired alkene 282.

Scheme 44. Further attempts at C-2 hydroxyl elimination and formation of olefin
282.

It was recognized that the much sought-after olefins 270 and 282 were
strikingly similar to an intermediate (285, Scheme 45.) prepared in Padwa’s
synthesis of 7-deoxypancratistatin.\textsuperscript{49k} This intermediate became our new target
and was prepared starting from silyl ether 253 (Scheme 45). \textit{N}-Protection of the
phenanthridone with \textit{p}-methoxybenzyl bromide was followed by cleavage of the
silyl ether to yield alcohol 284. Chugaev elimination of the C-2 alcohol provided olefin 285, a formal intermediate in the synthesis of 7-deoxypancratistatin.

\[
\begin{align*}
\text{OTBS} & \quad \text{OH} \\
0 & \quad \text{O} \\
\text{NPMB} & \quad \text{OH} \\
\text{NH} & \quad \text{O} \\
253 & \quad 284 & \quad 285
\end{align*}
\]

mp 172-174 °C, lit. 171-173 °C

reaction conditions: (a) (i) NaH, PMBBr, DMF, 0 °C - rt, (ii) TBAF, THF, 0 °C, 64% (2 steps); (b) NaH, CS\(_2\), Mel, xylenes, 165 °C, 35%

Scheme 45. Formal synthesis of 7-deoxypancratistatin.

III-4.4 Biological Evaluation of Intermediates

In addition to the formal synthesis of 7-deoxypancratistatin (2) and total synthesis of trans-dihydrolycoricidine (3), our synthetic strategy has provided access to four new C-1 analogues of 7-deoxypancratistatin. These new Amaryllidaceae constituents were screened *in vitro* for their antitumor activities through collaboration with the Pandey group at the University of Windsor, ON and the Kornienko group at the Newmexico Institute of Mining and Technology. In the course of preliminary screening, it was discovered that C-1 acid 168 and C-1 methyl ester 169 were inactive. In contrast, the one carbon homologated C-1
alcohol 167 and its acetate 170 displayed significant activities and were promptly tested for anticancer activity against a panel of human cancer cells. These two analogues were also evaluated for their ability to selectively induce apoptosis in human leukemia and neuroblastoma cells compared to normal human blood cells.

The antiproliferative activities of alcohol 167 and acetate 170 are displayed in Table 6 along with the activities of several related natural and unnatural Amaryllidaceae constituents. Compounds 167 and 170 are compared directly to their related parent compound while the potencies of C-1 benzoyl analogues 286 and 287, prepared by Pettit, are provided to highlight further viability of derivitization at the C-1 position. These two analogues in particular are among the most active derivatives ever tested. The two novel analogues 167 and 170 display anticancer activity that is lower than the naturally occurring pancratistatin and narciclasine by an order of magnitude or more. When compared to their direct parent compound, 7-deoxypancratistatin (2), the analogues are shown to possess potency equal to or greater than the natural constituent. This result stands as further vindication of the idea of C-1 derivatization since previous modification of any other portions of these compounds has universally failed to produce potencies comparable to the natural products. The failure of compounds 167 and 170 to match the activity of the more potent congeners pancratistatin (1) and narciclasine (46) is consistent with the drop in potency associated with the absence of the 7-hydroxyl moiety in the natural series.
 Except for our work and the initial investigations of Pettit, C-1 analogues have not been explored to determine factors contributing to the increased activity of these derivatives. From these preliminary investigations it appears that the trend is for relatively lipophilic C-1 substitutions to display greater potency. This is demonstrated by the increased activity of the 167 and 170 compared with the more polar acid 168. The C-1 methyl ester 169 most likely displays poor activity because of its facile conversion to acid 168 under physiological conditions. These results match nicely with the increased potencies associated with the more lipophilic C-1 benzoyl analogue 286. The precise mode by which the benzoyl and acetyl substitutions are affecting the pharmacophore is not known. A more detailed examination of C-1 substitution is required to determine if these moieties are actually participating in binding or are behaving as prodrugs and promoting greater cell penetration before being cleaved.

**Table 6. Human Cancer Cell Line and Murine P-388 Lyphocytic Inhibitory Activities.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Leukemia P388</th>
<th>Pancreas BxPC-3</th>
<th>Breast MCF-7</th>
<th>CNS SF-268</th>
<th>Lung NSC NCI-H460</th>
<th>Colon KM20L2</th>
<th>Prostate DU-145</th>
<th>Leukemia Jurkat</th>
<th>Neuroblastoma Shsy5y</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.039</td>
<td>0.028</td>
<td>0.032</td>
<td>0.017</td>
<td>0.048</td>
<td>0.062</td>
<td>0.016</td>
<td>0.163</td>
<td>0.163</td>
</tr>
<tr>
<td>1</td>
<td>0.44</td>
<td></td>
<td></td>
<td></td>
<td>0.29</td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>0.001</td>
<td>0.026</td>
<td>0.019</td>
<td>0.021</td>
<td>0.032</td>
<td>0.021</td>
<td>0.011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.029</td>
<td>0.046</td>
<td>0.034</td>
<td>0.059</td>
<td>0.043</td>
<td>0.051</td>
<td>0.040</td>
<td></td>
<td></td>
</tr>
<tr>
<td>286</td>
<td>0.0016</td>
<td>0.0019</td>
<td>0.00031</td>
<td>0.00055</td>
<td>0.0001</td>
<td>0.00037</td>
<td>0.00021</td>
<td></td>
<td></td>
</tr>
<tr>
<td>287</td>
<td>0.061</td>
<td>0.25</td>
<td>0.041</td>
<td>0.17</td>
<td>0.029</td>
<td>0.13</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>167</td>
<td>0.19</td>
<td>0.65</td>
<td></td>
<td>0.09</td>
<td>0.26</td>
<td>1.615</td>
<td>1.615</td>
<td></td>
<td></td>
</tr>
<tr>
<td>170</td>
<td>0.11</td>
<td>0.29</td>
<td></td>
<td>0.11</td>
<td>0.37</td>
<td>0.183</td>
<td>0.183</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

99
The C-1 hydroxymethyl analogue 167 and acetoxyethyl analogue 170 were also evaluated for their ability to induce apoptosis in Jurkat and SH-SY5Y cells. The cells were treated for periods up to 72 hours with compound 167 and 170. The apoptotic effect was observed through Hoechst staining (Figure 28). The results from Hoechst staining were confirmed by Annexin-V binding by treatment of the cell lines with 167 and 170 with 0.5 µM samples for 48 hours (Figure 29). In these experiments, cells with brightly stained and condensed nuclei are considered apoptotic.
Figure 28. Evaluation of apoptosis induced by 167 and 170 after 72 hours with Hoechst staining.

Figure 29. Evaluation of apoptosis induced by 167 and 170 after 48 hours and Annexin-V binding.

The ED50 (50% of cells were apoptotic) for alcohol 167 was determined to be 1 μM in Jurkat cells (Figure 30). This same compound was found to be
effective at a concentration of 10 μM against the SH-SY5Y cell line. As in the case of the previously mentioned cell line screen, the acetate 170 displayed stronger activity in these experiments. The ED50 for 170 was 0.5 μM for both the Jurkat and SH-SY5Y cell lines (Figure 31).

**Figure 30.** Apoptosis induced by 167 after 72 hours and determination of ED50.

**Figure 31.** Apoptosis induced by 170 after 72 hours and determination of ED50.
Both alcohol 167 and acetate 170 were found to be effective against these cell lines but 170 in particular displayed potency remarkably similar to the natural congener pancratistatin (1). This result warrants attention because, as described previously, pancratistatin (containing 7-hydroxyl) is traditionally more potent than 7-deoxypancratistatin (2) by an order of magnitude or more. Derivative 170 appears to circumvent this deficiency of 7-deoxypancratistatin by matching the activity of the more potent pancratistatin. Given that C-1 substitution is the only modification present, it can be additionally inferred that the acetate moiety is responsible for the positive addition to the potency over the parent 7-deoxypancratistatin.

Selective induction of apoptosis is a trait recently associated with pancratistatin. In order to examine the potential of acetate 170 as a selective apoptosis inducer it was screened against normal human fibroblast (NHF) and peripheral mono-nucleated blood cells (PMBC). The results of these screens are shown in Figure 33. Evaluation of these experiments revealed no visible apoptotic morphology in either the NHFs or PMBC non-cancerous cell lines. The lack of apoptosis induction in healthy cells indicates that acetate 170 possesses the ability to selectively kill cancerous cells that had previously only been observed in pancratistatin (1).
Figure 32. Screening of 170 against non-cancerous human cells with Hoechst staining.

III-5 Latent Symmetry, Diels-Alder Approach to Thebaine

The use of heteroaromatic cycloadditions has enjoyed widespread application in synthesis. One class of heteroaromatics which has found particular use in recent years is disubstituted pyridazines. In many cases these heterocycles, while aromatic, can behave as dienes. They have the added advantage of allowing for extrusion of nitrogen following initial [4 + 2] cycloaddition via a retro Diels-Alder process. This provides interesting synthetic options given the ability to essentially regenerate the diene after a Diels-Alder
reaction. These reports and others have inspired us to develop new synthetic route to thebaine (4) that takes advantage of the pseudo-$C_2$ symmetry present in the target opioid. The key step relies on the ability of these pyridazines’s behavior as dienes to allow for formation of the complete thebaine pentacyclic core in a cascade sequence involving two intramolecular Diels-Alder reactions (Figure 33).

**Figure 33.** Retrosynthetic analysis of latent symmetry approach to thebaine (4).

Important in this strategy is an intramolecular Diels-Alder (IMDA) reaction between two suitably substituted pyridazines and the appropriate furan-based dieneophile. The proposed cycloaddition of a compound such as 5 would provide control in the formation of the key C-13 quaternary center in thebaine (4). Cyclization precursor 5 could theoretically be accessed from desymmetrization of bis(pyridazines) 6, which would be available in a short number of operations from commercial 3,6-dichloropyridazine (8).
As the synthesis relied on the first IMDA reaction occurring between our proposed furan derivative and a tethered pyridazines, we elected to investigate this reaction using a model system. Since it was uncertain that a fully aromatic furanyl system would behave as a dieneophile, two models were envisioned. The first would consist of a furan moiety 290 linked to a simplified pyridazine through an amine or amide linker. Model system 293 could be constructed from furan 290, derived from furanal (288) (Figure 34). The second model would consist of a tethered dihydrofuran 294, accessible from ester 292. In both cases, the proposed dieneophiles would be linked to pyridazine 291 either by reductive amination of their amine derivatives or by amide formation with an amino-pyridazine unit derived from 291.

![Chemical structures](image)

**Figure 34.** Proposed model systems for the synthesis of thebaine (4).
The synthesis of ketopyridazines began with 3,6-dichloropyridazine (8) which was converted to its diiodo derivative 295 (Scheme 46). Displacement of iodide with methoxide yielded 3-iodo-6-methoxypyridazine (7). To our delight, iodide 7 smoothly coupled with TMS acetylene under Sonogashira conditions giving alkyne 296. The synthesis of this intermediate was important as it could also serve as an intermediate in the planned total synthesis. Generation of the desired ketone from this intermediate was achieved by an oxymercuration of the alkyne under strongly acidic conditions. In our hands, direct formation of ketone 291 was found to be problematic because of the persistence of mercuric adduct 297. Conditions suitable to provide the free ketone under acid hydrolysis were subsequently strong enough to provide hydroxypyridazine 299. We elected to first remove mercury with standard reductive conditions. These conditions also reduced the ketone but oxidation of the alcohol 298 with Collins reagent gave ketopyridazine 294 in modest yield over the three steps (61%).
With pyridazines in hand, we began generation of the furanyl tethers (Scheme 47). Furanal 288 was oxidized to a mixture of furanones 300 and 301 in a 1:2 ratio. Quantitative conversion of this mixture to single furanone 301 was achieved by treatment with triethylamine at room temperature. Reaction of furanone 301 with ethyl diazoacetate furnished pyrazole 302 in moderate yield. Elimination of nitrogen under thermal conditions led to ester 303. Synthesis of furan 290 with the appropriate ether moiety at the 1-position was investigated (Table 7). The conversion of furanone such as 303 to the corresponding 5-methoxy furans has been reported (entry 1), but in our hands only exhaustive alkylation at the 4-position was observed. Formation of the silyl ether was
thought to be more facile, but TBSCI proved to be poorly reactive in this case.

The more activated triflate did provide the desire furan 305 in good yield (67%).

![Scheme 47. Synthesis of furan tether 290.]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dimethylsulfate, Et₃N, CH₂Cl₂, rt</td>
<td>![304] 67% 305</td>
</tr>
<tr>
<td>2</td>
<td>TBSCI, Et₃N, CH₂Cl₂, 0 °C - rt</td>
<td>No reaction</td>
</tr>
<tr>
<td>3</td>
<td>TBSOTf, Et₃N, CH₂Cl₂, rt</td>
<td>![TBSO] 67%, 305</td>
</tr>
</tbody>
</table>

Table 7 Formation of furan tether.
The synthesis of the dihydrofuran tether proceeded through first
cyclopropanation of dihydrofuran 289 with ethyl diazoacetate to give ester 306
(Scheme 48). Cleavage of the cyclopropane moiety with copper bronze in
refluxing acetonitrile provided the desired dihydrofuran 292.

![Chemical structure diagram]

**Reaction conditions:** (a) ethyl diazoacetate, (Rh(OAc)\(_2\))\(_2\), rt, 85%; (b) Cu bronze,
CH\(_3\)CN, reflux, 54%

**Scheme 48.** Synthesis of dihydrofuran 292.

In addition to our investigations of the model systems described above, we
elected to pursue synthesis of the bis(pyridazines) 6 required for the total
synthesis. 3-Iodo-6-methoxypyridazine (7) was converted to acetylene 307 by
first Sonogashira coupling with TMS-acetylene followed by treatment with TBAF
(Scheme 49). This material was then converted to bis(pyridazines) 6 by a second
Sonogashira coupling with another equivalent of pyridazine 7. Since the desired
acetylene 6 is symmetrical, an obvious improvement of this three step procedure
would be to perform the two coupling reactions in a single pot using acetylene
gas. Such reactions are not known for pyridazines, but are well known for other
aromatic systems. Using the conditions reported by Li and coworkers,\(^{147}\) we were
able to generate acetylene 6 in a single step from iodopyridazine 7.
At this time it became apparent that the ease with which acetylene 6 could be synthesized obviated the need to deal with a separate model system. The most direct route for the introduction of methyl amine functionality would be a hydroamination reaction between methyl amine and acetylene 6. The Doye hydroamination catalyst 312 was chosen for initial investigation of this reaction because it could be readily prepared and had recently been demonstrated to effect hydroaminations with small amines. The required catalyst was synthesized by treatment of indene (311) with 4 equiv of methyllithium and titanium tetrachloride (Scheme 50).
With both the catalyst $312$ and acetylene $6$ in hand, we pursued the direct hydroamination reaction. Fortunately, treatment of $6$ with titanocene $312$ in the presence of methyl amine provided evidence for the desired transformation. Initially the imine $308$ was identified from a crude reaction mixture and subsequently reduced to provide amine $309$. Later this two-step procedure was combined into a single pot to provide amine $309$ in good yield.

Scheme 50. Synthesis of hydroamination catalyst $312$.

Scheme 51. Sonogashira coupling of TMS acetylene with chloropyridazine $313$.
The use of chloropyridazine 8 in various Sonagashira coupling reactions was also investigated as a means of reducing step count and improving synthetic efficiency. Chloropyridazine 8 was converted to methoxy derivative 313. Sonogashira coupling of 313 with TMS acetylene provided 296. Unfortunately attempts at a double Sonagashira coupling were unsuccessful and resulted in isolation of the starting pyridazine 313.

\[ \text{Reaction conditions: (a) LiOH, MeOH, 50 °C; (b) oxalyl chloride, DMF; (c) DMAP, Et}_3\text{N, 309; (d) 309, toluene, 150 °C; (e) CDI, 309, CH}_2\text{Cl}_2, \text{ reflux; (f) toluene, 150 °C} \]

Scheme 52. Tethering of dihydrofuran 292 to amine 309.

The coupling of amine 309 to dihydrofuran 292 was attempted in several stages and with several derivatives of the ester moiety of 292 (Scheme 52). At first, a simple displacement reaction was attempted with the hopes of forming the more stable amide. The two partners proved unreactive toward one another at lower temperatures, but heating to 150 °C in toluene (sealed tube) provided 317. The formation of olefin 317 presumably occurs through Hofmann type elimination of the amine. Treatment of acid 314, derived from 292, with
carbonyldiimidazole (CDI) also gave the olefin 317 as the only isolated product. Eventually tethered product 316 was generated by coupling of amine 309 with acid chloride 315. The first attempted IMDA reaction was performed in a sealed tube in toluene at 150 °C and provided only elimination of the amide tether to give olefin 309.

While these initial investigations toward the total synthesis of thebaine by latent symmetry approach have proven unsuccessful, work to effect the first of the Diels-Alder reactions is ongoing. The information garnered through the synthesis of these models will prove invaluable in future attempts to refine the route and complete the total synthesis.
IV. Conclusions and Future Work

The *Amaryllidaceae* constituents 7-deoxypancratistatin (2) and *trans*-dihydrolycoricidine (3) are isocarbostyril natural products which possess strong anticancer activity. In the course of the presented study, their total syntheses were completed using chemoenzymatic methods. The synthesis of four new C-1 analogs of 7-deoxypancratistatin was accomplished with two of the new analogues (acetate 170 and alcohol 167) displaying significant activity against a panel of human cancer cell lines. Through this work the use of C-1 derivatization as a means of augmenting the potency of these congeners has been established. Application of this concept to other members of the isocarbostyril family, especially C-1 derivatives of pancratistatin, is ongoing.

In addition to the abovementioned syntheses, chemoenzymatic methods have been explored at a more fundamental level in the course of this work. Various dibromobenzenes and benzoates were identified as new substrates for the TDO enzyme. The absolute stereochemistry of the new diols was matched by stereochemical proof and these metabolites were applied to the synthesis of (-)-conduritol E, pseudo-sugars, aminocyclitols, and complex bicyclic ring systems.

A new approach toward the synthesis of morphine alkaloids was developed and several model systems for the total synthesis of thebaine (4) were completed. Future work toward the synthesis of thebaine includes establishment of intra- or intermolecular Diels-Alder cycloadditions involving any of the described bis(pyridazines). Study and possible modification of the existing bis(pyridazine)
framework is required to remedy the problematic Hofmann elimination observed in amine 309.
V. Experimental Section

V-1 General Experimental Details

All non-hydrolytic reactions were carried out under an argon atmosphere. Glassware used for moisture-sensitive reactions was flame-dried under vacuum and subsequently purged with argon. THF was distilled from potassium/benzophenone. Methylene chloride and acetonitrile were distilled from calcium hydride. Electrochemistry supplies and apparatus were purchased from EG&G Princeton Applied Research. Flash column chromatography was performed using Kieselgel 60 (230-400 mesh). Analytical thin-layer chromatography was performed using silica gel 60-F254 plates. Melting points were measured on a Thomas-Hoover melting point apparatus and are reported uncorrected. IR spectra were obtained on a Perkin-Elmer FT-IR 1600 Series Spectrum One instrument and were recorded as neat samples. $^1$H and $^{13}$C NMR spectra were obtained on either a 300-MHz or 600 MHz Bruker instrument. Data are reported as (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad); coupling constants(s) in Hz, integration. Specific rotation measurements are given in deg cm$^3$ g$^{-1}$ dm$^{-1}$ and were recorded on a Perkin-Elmer 341 Polarimeter. Ultraviolet spectroscopy was performed using a Perkin-Elmer 8452 A diode array spectrophotometer. Large-scale fermentation was performed in a 15-L B. Braun Biostat C-15 fermentor. All biological media was purchased through Sigma-Aldrich Canada. Combustion analyses were performed by Atlantic Microlabs, Norcross, Georgia, USA.
**General Experimental Procedures for Biotransformations**

Small-scale fermentation with *E. coli* JM 109 (pDTG601)

**Growth of colonies.** Agar plates consisted of bactotryptone (10 g L\(^{-1}\)), yeast extract (5 g L\(^{-1}\)), NaCl (5 g L\(^{-1}\)), agar (30 g L\(^{-1}\)) and ampicillin (100 mg L\(^{-1}\)). *E. coli* JM 109 pDTG601 cells were streaked onto a plate and were incubated at 35 \(^{\circ}\)C for 12-24 h. A single bacterial colony was selected for the preculture preparations described in the following section.

**Preparation of preculture.** Luria Bertani (LB) liquid medium consisted of bactotryptone (10 g L\(^{-1}\)), yeast extract (5 g L\(^{-1}\)), NaCl (5 g L\(^{-1}\)) and ampicillin (100 mg L\(^{-1}\)). The preculture medium (3 mL) was inoculated with a single colony of *E. coli* JM 109 (pDTG601) and the resulting inoculum was grown at 35 \(^{\circ}\)C on an orbital shaker (200 rpm) for 6 h.

**Fernbach flask preparation.** LB liquid medium consisted of bactotryptone (10 g L\(^{-1}\)), yeast extract (5 g L\(^{-1}\)), NaCl (5 g L\(^{-1}\)), glucose (5 g L\(^{-1}\)) and ampicillin (100 mg L\(^{-1}\)). 500 mL of LB medium was inoculated with 1 mL of *E. coli* JM 109 (pDTG601) preculture medium. This inoculum was grown at 35 \(^{\circ}\)C on an orbital shaker (180 rpm) for 5 h. A chemical inducer, isopropyl-1-thio-\(\beta\)-D-galactopyranoside (IPTG) (10 mg L\(^{-1}\)), was added via sterile filter and the cells were grown for additional 7 h at 35 \(^{\circ}\)C on an orbital shaker (200 rpm).
Substrate Addition. The cells were separated from the supernatant by centrifugation at 7000 rpm for 15 min and the supernatant was decanted. The cell pellet was re-suspended in 500 mL of 0.1 M phosphate buffer consisting of KH₂PO₄ (6.8 g L⁻¹), K₂HPO₄ (8.7 g/L) and glucose (2 g L⁻¹). The aromatic substrate (400 mg L⁻¹) was added as a solution in isopropyl alcohol. Product formation was monitored by thin-layer chromatography (hexane-ethyl acetate, 1:1).

Product Isolation. After 5 h of incubation with substrate the pH of the culture medium was adjusted with 6 M NaOH to 8.5, and a cell pellet was obtained by centrifugation at 7000 rpm and 4 °C for 20 min. The supernatant liquid was extracted with acid-free ethyl acetate (prepared by stirring with a saturated solution of Na₂CO₃) and separation of the organic from the aqueous layer. The extract was dried over anhydrous MgSO₄, filtered and the solvent was removed under reduced pressure. The crude material was purified by crystallization or flash column chromatography (silica gel deactivated with 10% distilled water) immediately after concentration of the solvent in order to minimize decomposition of the unstable dienediols.

Large-scale fermentations were carried out in a 15-L (8-L working volume) B. Braun Fermentor according to a published procedure.¹¹⁸
Extraction of Products. Dienediols obtained from large-scale (8-L fermentation) were extracted from the aqueous fermentation broth into ethyl acetate either by standard manual extraction or by continuous extraction. The dienediols derived from small-scale fermentations (<1-L) were extracted manually. Progress of either manual or continuous extraction was monitored by thin layer chromatographic analysis of the aqueous layer.

General Procedure for the Activation of Silica Gel. Silica gel (1.5 g, 230-400 mesh) was poured onto a sintered glass Büchner funnel and washed with reagent grade THF (2 x 15 mL) and diethyl ether (2 x 15 mL). The silica gel was transferred to a round-bottomed flask and heated externally at 140 °C under vacuum (1 mm Hg) for 24 h.

General procedure for PAD reduction of diols. To a stirring solution of diene (2.5 mmol) and potassium azodicarboxylate (PAD) (7.5 to 15.0 mmol) in MeOH (4 mL), glacial acetic acid was added (17.5 to 37.5 eq.) dropwise at -15 °C. The reaction was allowed to warm to room temperature slowly over 14 h, then quenched by the addition of Na₂CO₃ (7 to 15 mL) and concentrated under reduced pressure and extracted with ethyl acetate (5 x 5 mL). The combined organic layers were washed with brine (1 x 7 mL), dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was recrystallized from ethyl acetate/hexanes.
General procedure for the transesterification of PAD-reduced diols. A solution of PAD-reduced diol (0.79 mmol), conc. H$_2$SO$_4$ (5 drops) in dry ethanol (10 mL) was refluxed for 4 days. The mixture was concentrated, diluted in saturated NaHCO$_3$ (2 mL) and extracted with ethyl acetate (3 x 20 mL). The organic extracts were combined, dried over MgSO$_4$, filtered, concentrated, and purified by flash chromatography (eluant 1:1 ethyl acetate/hexanes) and recrystallized from ethyl acetate/hexanes.

General procedure for formation of acetonide (ketalization) of diols with 2,2-dimethoxypropane. To a stirring solution of diol (0.832 mmol) and 2,2-dimethoxypropane (0.71 mL, 5.83 mmol) in acetone (1 mL) was added a catalytic amount of $p$-TsOH. The reaction was allowed to stir at room temperature for 2 h, then it was diluted with ethyl acetate (5 mL) and washed with saturated NaHCO$_3$ (3 x 2 mL). The organic layer was washed with brine (1 x 3 mL) then dried with Na$_2$SO$_4$. The crude material was purified via flash column chromatography with a solvent gradient of 2:1 hexanes-ethyl acetate.
V-2 Detailed Experimental Procedures

(1S,2S)-3,4-Dibromocyclohexa-3,5-diene-1,2-diol (161).

The biooxidation of o-dibromobenzene was performed according to the general procedure for large-scale fermentation. o-Dibromobenzene (159) (60 g) was added dropwise over 45 min to a 15-L fermentor containing a growing culture of *E. coli* JM 109 (pDTG601). After stirring the media for an additional 1 h, the cell broth was separated from the cells by centrifugation. The broth was extracted with 3 L of ethyl acetate using a rotary evaporator-driven continuous extractor over a three-day period. The combined organic layers were washed twice with approximately 10% by volume saturated sodium carbonate solution to remove any phenolic residue. The organic extracts of the fermentation broth were concentrated *in vacuo* and the dienediol precipitated by addition of pentane. Recrystallization from ethyl acetate/pentane provided the title compound as a white solid (32.8 g, 4.1 g/L): mp 144-146 °C (from ethyl acetate-pentane); $[\alpha]_D^{22} +104$, (c 0.15, diethyl ether); $R_f$ 0.36 (hexanes/ethyl acetate, 1:1); IR (film) ν 3175, 1621 cm$^{-1}$; $^1$H NMR (300 MHz, acetone-$d_6$) δ: 6.03 (dd, $J = 9.9$, 2.1 Hz, 1H), 6.00 (dd, $J = 6.6$, 2.4 Hz, 1H), 4.62 (d, $J = 6.9$ Hz, 1H), 4.55 (m, 1H), 4.28 (m, 2H); $^{13}$C NMR (75 MHz, acetone $d_6$) δ: 133.4, 126.5, 126.2, 120.6, 74.2, 69.0; HRMS-EI calcd for C$_6$H$_6$BrO$_2$ (M$^+$): 267.8731, found: 267.8733; Anal. calcd for C$_6$H$_6$BrO$_2$; C, 26.70; H, 2.24; Found C, 27.04; H, 2.32.

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Dienediol 161 (3.0 g, 11mmol, 1 equiv) was transferred to a 100 mL round-bottomed flask and suspended in 5 mL acetone and 15 mL of 2,2-DMP. A few crystals of pTsOH were added and the reaction mixture was stirred at rt for 3 h. The reaction was quenched with 12 mL of 10% aq sodium hydroxide solution, and the acetone was removed under reduced pressure. The residue was diluted with ethyl acetate, and the layers were separated. The aqueous layer was then extracted with several portions of ethyl acetate. The combined organic layers were washed with brine and dried over anhydrous MgSO₄. The solvent was removed under vacuum to provide a light oil (3.21 g, 95%), which was essentially pure. An analytical sample was obtained after chromatography over 10% deactivated silica gel (hexanes:ethyl acetate, 8:1), affording the title compound as a clear and colorless oil. $[\alpha]_D^{22} +101$ (c 0.75, CHCl₃); $R_f$ 0.50 (50% ethyl acetate in hexanes); IR (film) $\nu$ 2988, 2933, 2896, 1634 cm⁻¹; $^1$H NMR (300 MHz, CDCl₃) $\delta$ 6.14 (d, $J = 9.9$ Hz, 1H), 5.93 (dd, $J = 9.9$, 3.6 Hz, 1H), 4.82-4.72 (m, 2H), 1.45 (s, 6H); $^{13}$C NMR (75 MHz, CDCl₃) $\delta$ 128.7, 125.6, 123.8, 120.3, 106.9, 77.3, 71.3, 26.6, 25.0; HRMS-EI Calcd for C₉H₁₀Br₂O₂, 307.9047; Found, 307.9037.
(3aS,4R,5S,7aS)-2,2-Dimethyl-6,7-dibromo-4,5-dihydroxybenzo[1,3]dioxole (178).

The protected dienediol 177 (3.1 g, 10 mmol, 1 equiv) was suspended in 60 mL of an 8:1 (by volume) mixture of acetone/water. N-Methylmorpholine-N-oxide (2.34 g, 20 mmol, 2 equiv) was added followed by 4 crystals of osmium tetraoxide. The reaction mixture darkened slightly and was stirred for 18 h until consumption of starting material was complete as evidenced by TLC analysis. The reaction mixture was quenched by addition of 5 mL saturated aq. sodium bisulfite, followed by a further addition of 2 g solid sodium bisulfite, and the pH of the mixture was adjusted to approximately 2 by addition of conc HCl. The mixture was stirred for 15 min and the acetone was removed under reduced pressure. The remaining aqueous portion was extracted repeatedly with ethyl acetate (3 x 60 mL), the combined organic extracts were washed sequentially with 1 N HCl, 20% aq solution of KOH, and brine before being dried over anhydrous MgSO₄. The organic solution was filtered through a short column of silica gel and the solvent evaporated to furnish 2.4 g of a white crystalline solid (71%) which required no further purification for the subsequent reaction. An analytical sample was obtained from a portion of the solid, recrystallized from ethyl acetate/pentane. mp 155-156 °C; [α]D 22° +5.47 (c 0.75, MeOH); Rf 0.3 (50% ethyl acetate in hexanes); IR (KBr) v 3435, 3360, 2994, 2905, 1606 cm⁻¹; ¹H NMR (300...
MHz, CDCl$_3$) $\delta$ 4.77 (dd, $J = 5.4$, 1.2 Hz, 1H), 4.50-4.45 (m, 2H), 4.34 (m, 1H), 2.68 (bs, 2H), 1.43 (s, 6H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 127.6, 125.6, 77.6, 75.3, 70.9, 69.1, 27.6, 26.1; HRMS-EI calcd for C$_9$H$_9$O$_4$Br, 326.8867; found, 326.8863; Anal Calcd for C$_9$H$_{12}$Br$_2$O$_4$: C, 31.42; H, 3.52, Found: C, 31.55; H, 3.78.

\[\text{-OH} \quad \text{HO} \quad \text{-OH}\]

\[\text{-OH}\]

(-)-Conduritol E (162).

Dibromide 178 (0.50 g, 1.4 mmol, 1 equiv) was dissolved in 50 mL distilled THF and transferred to a flame-dried 100 mL round-bottomed flask equipped with a reflux condenser. The solution was degassed in an ultrasound bath and under positive argon pressure for 10 min. Azo(bisisobutyronitrile) (23 mg, 0.14 mmol, 0.1 equiv) was added, and the solution heated to steady reflux (83 °C external temp). At this time, tributyltin hydride (1.0 mL, 3.36 mmol, 2 equiv) was added in a single portion. Reflux was maintained for 1.5 h until complete consumption of starting material was evidenced by TLC analysis. The reaction mixture was cooled and potassium fluoride (2 g) was added. The resulting precipitate was filtered and the filtrate concentrated under reduced pressure. The residue was purified by flash column chromatography, eluting with 4:1 hexanes/ethyl acetate to 100% ethyl acetate to provide 170 mg (64%) of the de-brominated material. The solid was dissolved in 5 mL of methanol and to this solution was added 2 mL of a 3% (by volume) solution of conc HCl in methanol and the resulting solution
stirred for 40 h after which time the solvent was removed under reduced pressure to provide a white solid. The solid was purified by flash column chromatography (4:1 chloroform/methanol) to give (-)-conduritol E as a white crystalline solid (78 mg, 81%). mp 194-195 °C (EtOH) (lit\textsuperscript{120b} mp 193 °C); $[\alpha]_D^{20}$ -285 (c 1.0, H\textsubscript{2}O), lit\textsuperscript{120b} $[\alpha]_D^{20}$ -294 (c 1.0, H\textsubscript{2}O); $R_f$ 0.18 (chloroform-methanol, 4:1); IR (film) v 3434, 1634 cm\textsuperscript{-1}; $^1$H NMR (300 MHz, MeOD) $\delta$ 5.79 (m, 2H), 4.27 (s, 2H), 3.93 (m, 2H); $^{13}$C NMR (75 MHz, MeOD) $\delta$ 130.7, 70.9, 67.6; HRMS-EI Calcd for C\textsubscript{6}H\textsubscript{10}O\textsubscript{4}, 146.0579; Found, 146.0577.

![Structure of (-)-conduritol E](image)

\textit{(1S,2S)-3,4-Dibromo-cyclohexa-3-ene-1,2-diol (180)}.

diol 161 (0.384g, 1.47 mmol, 1 equiv) was dissolved in 6 mL MeOH, and the round-bottomed flask containing the solution was subsequently placed into an ice/NaCl bath. Potassium azodicarboxylate (0.93g, 4.3 mmol, 3 equiv) was added in two portions to the methanolic solution. Acetic acid (0.85 mL, 13 mmol, 9 equiv) in 2 mL MeOH was added dropwise over 40 min. The reaction flask was allowed to warm to room temperature overnight (15 h). The reaction was quenched by adding 2 mL saturated Na\textsubscript{2}CO\textsubscript{3} solution and stirring for 20 min. Methanol was removed under reduced pressure and the residue diluted with 10 mL EtOAc. The layers were separated and the aqueous phase was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine, dried over MgSO\textsubscript{4}, and treated with activated charcoal. Filtration and subsequent
concentration of the filtrate under reduced pressure afforded 180 as a white crystalline solid (0.367 g, 95%). mp 175-176 °C (ethyl acetate/hexane); [α]D 21 = -50.4 (c 0.75, MeOH); Rf 0.23 (Hex: EtOAc, 1:1); IR (KBr pellet) ν 3246, 1626 cm⁻¹; ¹H NMR (300 MHz, acetone d₆) δ 4.61 (d, J = 6 Hz, 1H), 4.26 (s, 1H), 3.96-3.84 (m, 2H), 2.79-2.51 (m, 2H), 2.05-1.92 (m, 1H), 1.85-1.73 (m, 1H); ¹³C NMR (75 MHz, acetone d₆) δ 127.1, 124.9, 73.5, 68.25, 35.2, 26.9; HRMS-EI Calcd for C₆H₈Br₂O₂, 271.8872; Found, 271.8871; Anal calcd for C₆H₈Br₂O₂; C, 26.50; H, 2.97; Found C, 27.34; H, 3.16

(1S,6S)-2,3-dibromo-6-((2,3-dimethylbutan-2-yl)dimethylsilyloxy)cyclohex-2-enol (181).

A 5 mL round-bottomed flask was charged with diol 180 (200 mg, 0.74 mmol, 1 equiv), imidazole (65 mg, 0.96 mmol, 1.3 equiv) and 1 mL anhydrous DMF. The flask was cooled externally to -30 °C then the xylyldimethylsilyl chloride (0.15 mL, 0.78 mmol, 1.1 equiv) was added. The mixture was stirred at -30 °C for 1 h then placed in a freezer (-18 °C) for 21 h. The mixture was allowed to warm to room temperature and diluted with 50 mL ether, washed with distilled H₂O (10 mL), brine, and then dried over MgSO₄. After filtration, the filtrate was concentrated under reduced pressure. The crude silyl ether was purified by flash column chromatography (pentane: Et₂O, 10:1) to give 181 as a clear and colorless oil (0.26 g, 86%). [α]D 21 = -41.1 (c 0.75, MeOH); Rf 0.23 (pentane: Et₂O, 10:1); IR
(film) ν 3547, 2958, 2868, 1628 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 4.26-4.17 (m, 1H), 4.03-3.93 (m, 1H), 2.84 (d, J = 4.0, 1H), 2.77-2.64 (m, 1H), 2.63-2.48 (m, 1H), 2.10-1.90 (m, 1H), 1.77-1.57 (m, 2H), 0.95-0.84 (m, 13H), 0.17 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 130.8, 127.8, 122.9, 74.1, 73.6, 69.6, 35.1, 34.1, 27.2, 24.8, 20.2, 20.0, 18.5, 18.4; HRMS-EI Calcd for C₁₄H₂₆Br₂O₂Si, 328.9032; Found, 328.9026; Anal calcd for C₁₄H₂₆Br₂O₂Si: C, 40.59; H, 6.33; Found C, 40.96; H, 6.36.

![Chemical structure](image)

**1R, 2S)-2-[(thexyldimethylsilyl)oxy]cyclohexan-1-ol (182).**

A flask containing a magnetic stirring bar was charged with dibromide 181 (0.219 g, 0.53 mmol, 1 equiv), triethylamine (0.5 mL, 3.5 mmol, 7 equiv), platinum oxide (Adams catalyst, 24 mg, 0.11 mmol, 0.2 equiv) and 0.5 mL MeOH. The reaction flask was evacuated, flushed with hydrogen via a filled balloon (1 atm), and stirred until total consumption of starting material was observed by TLC (6 h). The crude mixture was filtered through a short plug of Celite and the solvent was removed under reduced pressure. The crude residue was purified by flash column chromatography (pentane: diethyl ether, 10:1) to give the title compound as a clear and colorless oil (71 mg, 52%) having spectral data matching that of previously reported. [α]D²³ + 3.4 (c 1.0, CHCl₃); lit:¹²¹α [α]D²³ + 3.3 (c 1.0, CHCl₃).
Mosher ester derivative 183.

Alcohol 182 (20 mg, 0.076 mmol, 1 equiv) was transferred to a flame-dried round-bottomed flask containing a magnetic stirring bar under an argon atmosphere. Anhydrous triethylamine (17 μL) was added followed by DMAP (4.8 mg, 0.038 mmol, 0.5 equiv). (R)-(−)-α-Methoxy-α-(trifluoromethyl)phenylacetic acid chloride (23 μL, 0.11 mmol, 1.5 equiv) was added dropwise. Within minutes, a white precipitate was observed. The reaction was stirred overnight. The reaction mixture was then diluted with 5 mL methylene chloride, transferred to a separatory funnel and washed with 5 mL of a saturated solution of sodium bicarbonate. The layers were separated and the organic layer dried (MgSO₄) and the solvent evaporated to provide the ester as a crude oil. The ester was purified by flash column chromatography (pentane: diethyl ether, 10:1) to afford the ester as a clear and colorless oil (20 mg, 57%). Rf 0.46 (pentane: Et₂O, 10:1); IR (film) 2948, 2867, 1745, 1463, 1450, 1379, 1263, 1169 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ: 7.60 (m, 2 H), 7.40 (m, 3 H), 5.12 (m, 1H) 3.80 (m, 1H), 3.55 (m, 3H), 1.75-1.55 (m, 7H), 1.5-1.2 (m, 2H) 0.88 (dd, J = 6.8, 5.2 Hz, 6H), 0.81 (d, J = 4.1 Hz, 6H), 0 (s, 3H), -0.10 (s, 3H) ¹⁹F NMR (188 MHz, CDCl₃) δ: -72.4 ppm. Lit¹²¹a ¹⁹F NMR (188 MHz, CDCl₃) δ: -72.8 ppm.
6-Carboxymethyl-(1S,2S)-1,2-dihydroxycyclohexa-3,5-diene (163).

See General Experimental Procedures for Biotransformations: Large-scale fermentations. (12.92 g, 19.2%, 75.8% based on recovered starting material) pale yellow oil; \( R_f \) 0.33 (1:2 hexanes/ethyl acetate); \([\alpha]_D^{23}\) +71.3 (c 1.6, CHCl₃); IR (film) \( \nu \) 3412, 2098, 1690, 1639, 1291, 820, 772 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl₃) \( \delta \) 7.05 (d, \( J = 5.3 \) Hz, 1H), 6.17 (dd, \( J = 10.3, 0.6 \) Hz, 1H), 6.05 (qd, \( J = 5.1, 2.2 \) Hz, 1H), 4.50-4.56 (m, 1H), 4.41-4.50 (m, 1H), 3.77 (s, 3H), 3.52-3.67 (m, 2H) ppm; \(^{13}\)C NMR (75 MHz, CDCl₃) \( \delta \) 167.5, 138.6, 134.3, 128.4, 122.6, 69.5, 64.8, 52.1 ppm; MS (EI) \( m/z \) (%): 170(M⁺, 33), 152(61), 139(22), 138(96), 136(71), 121(100), 110(95), 109(66), 105(23), 93(42), 92(22) 82(57), 81(56), 65(59), 53(49), 51(22); HRMS calcd for C₈H₁₀O₄ 170.0579, found 170.0580.

6-Carboxymethyl-(1S,2S)-1,2-dihydroxycyclohexa-3,5-diene (191).

See General Experimental Procedures for Biotransformations: Large-scale fermentations. (8.06 g, 43.4%, 45.6% based on recovered starting material) Colorless crystals, mp 48 °C (ethyl acetate/hexanes); \( R_f \) 0.31 (1:2 hexanes/ethyl acetate); \([\alpha]_D^{23}\) +54.7 (c 3.8, CHCl₃); IR (film) \( \nu \) 3385, 2981, 2934, 1700, 1280, 1243, 1104, 1068, 825, 771 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl₃) \( \delta \) 7.04 (d, \( J = 5.3 \) Hz, 1H), 6.15 (dt, \( J = 1.1, 9.4 \) Hz, 1H), 6.03 (dq, \( J = 2.3, 9.2 \) Hz, 1H), 4.49-4.55
(m, 1H), 4.40-4.48 (m, 1H), 4.22 (q, J = 7.0 Hz, 2H), 3.65-3.78 (m, 2H), 1.28 (t, J = 7.2 Hz, 3H) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$) δ 167.1, 138.7, 134.1, 128.7, 122.5, 69.8, 64.5, 60.9, 14.2 ppm; MS (EI) m/z (%): 184(M$^+$, 9), 166(20), 138(26), 122(33), 121(52), 105(100), 77(39), 51(21), 45(20); HRMS calcd for C$_9$H$_{12}$O$_4$ 184.0736, found 184.0731; Anal. calcd: C 58.69, H 6.57, found C 58.77, H 6.60.

6-Carboxypropyl-(1S,2S)-1,2-dihydroxycyclohexa-3,5-diene (192).

See General Experimental Procedures for Biotransformations: Large-scale fermentations. (711 mg, 5.8%, 65.0% based on recovered starting material) waxy solid; Rf 0.15 (1:1 ethyl acetate/hexanes); [$\alpha$]$_D^{22}$ +58.8 (c 1.1, CHCl$_3$); IR (film) ν 3398, 2968, 1700, 1280, 1240 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) δ 7.08 (d, J = 5.4 Hz, 1H), 6.20 (dd, J = 9.5, 2.5 Hz, 1H), 6.09 (ddd, J = 9.5, 5.4, 2.2 Hz, 1H), 4.58 (d, J = 6.3 Hz, 1H), 4.48 (ddd, J = 6.3, 2.5, 2.2 Hz, 1H), 4.16 (t, J = 6.7 Hz, 2H), 3.40 (bs, 2H), 1.72 (qt, J = 7.4, 6.7 Hz, 2H), 0.98 (t, J = 7.4 Hz, 3H) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$) δ 167.1, 138.4, 133.9, 128.7, 122.6, 69.4, 66.6, 64.8, 22.0, 10.4 ppm; MS (EI) m/z (%): 198 (M$^+$, 18), 180(22), 138(100), 121(81), 110 (54), 105 (77); HRMS calcd for C$_9$H$_{12}$O$_4$ 198.0892, found 198.0892.

6-Carboxyisopropyl-(1S,2S)-1,2-dihydroxycyclohexa-3,5-diene (193).
See General Experimental Procedures for Biotransformations: Large-scale fermentations. (488 mg, 4.1%, 34.0% based on recovered starting material) colourless crystals; mp 83-85 °C (ethyl acetate/hexane); \( R_f = 0.31 \) (6:4 ethyl acetate/hexane); \([\alpha]_D^{22} +64.70 \) (c 1.1, CHCl₃); IR (KBr) v 3274, 2981, 1698, 1263, 1241 cm⁻¹; \(^1\)H NMR (300 MHz, CDCl₃) \( \delta \) 7.05 (dd, \( J = 5.5, 1.0, 0.5 \) Hz, 1H), 6.20 (ddt, \( J = 9.6, 2.7, 0.9 \) Hz, 1H), 6.08 (dd, \( J = 9.6, J = 5.5, 2.2 \) Hz, 1H), 5.12 (hept, \( J = 6.3 \) Hz, 1H), 4.58 (dd, \( J = 6.4, 0.5 \) Hz, 1H), 4.48 (br m, 1H), 3.60-3.25 (br s, 2H), 1.30 (d, \( J = 6.3 \) Hz, 6H) ppm; \(^{13}\)C NMR (75 MHz, CDCl₃) \( \delta \) 166.5, 138.2, 133.6, 128.9, 122.63, 99.4 69.2, 68.5, 64.9, 21.8 ppm; MS (EI) \( m/z \) (%): 198(M⁺, 19), 180(16), 156(14), 138(100); HRMS (EI) calcld for C₁₀H₁₄O₄: 198.08921, found: 198.08896. Anal. Calcd. for C₁₀H₁₄O₄: C, 60.59; H, 7.12. Found: C, 60.68; H, 7.19.

![Chemical Structure](image)

**6-Carboxyallyl-(1S,2S)-1,2-dihydroxycyclohexa-3,5-diene (196).**

See General Experimental Procedures for Biotransformations: Large-scale fermentations. (5.79 g, 52.0%, 73.6% based on recovered starting material) colourless crystals; mp 48-50 °C (ethyl acetate/hexane); \( R_f = 0.23 \) (1:1 ethyl acetate/hexane); \([\alpha]_D^{22} +72.54 \) (c 1.6, CHCl₃); IR (KBr) v 3394, 1704, 1273, 1238 cm⁻¹; \(^1\)H NMR (300 MHz, CDCl₃) \( \delta \) 7.12 (d, \( J = 5.5 \) Hz, 1H), 6.23 (ddt, \( J = 9.6, 2.7, 1.0 \) Hz, 1H), 6.11 (ddd, \( J = 9.5, 5.5, 2.2 \) Hz, 1H), 5.98 (ddt, \( J = 17.2, 10.4, 5.7 \) Hz, 1H), 5.37 (dt, \( J = 17.2, 1.5 \) Hz, 1H), 5.28 (dt, 10.4, 1.3 Hz, 1H), 4.72 (dd, \( J = \)
5.7, 1.5, 1.3 Hz, 1H), 4.61 (br s, 1H), 4.50 (br s, 1H), 3.25 (d, $J = 3.8$ Hz, 1H), 3.18 (brd, $J = 7.3$ Hz, 1H) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 166.6, 138.8, 134.4, 131.9, 128.4, 122.4, 118.3, 69.6, 65.5, 64.5 ppm; MS (EI) $m/z$ (%): 196(M$^+$, 20), 178(18), 138(80), 121(95), 41(100). HRMS (EI) calcd for C$_{10}$H$_{12}$O$_4$: $m/z$ 196.07356, found: 196.07364. Anal. Calcd. for C$_{10}$H$_{12}$O$_4$ + 1/8 H$_2$O: C, 60.52; H, 6.22. Found: C, 60.52; H, 6.26.

6-Carboxyisopropyl-(1S,2S)-1,2-dihydroxycyclohex-3-ene (197).

See General Experimental Procedures for Biotransformations: Large-scale fermentations. (9.20g, 69.1%, 75.8% based on recovered starting material) colourless crystals; mp 70-72 °C (ethyl acetate/hexane); $R_f = 0.31$ (6:4 ethyl acetate/hexane); [$\alpha$]$_D^{22} +88.20$ (c 1.6, CHCl$_3$); IR (KBr) v 3385, 3291, 1707, 1270, 1234 cm$^{-1}$; $^1$H NMR (300 MHz, acetone-$d_6$) $\delta$ 7.01 (dd, $J = 5.3$, $J = 1.1$ Hz, 1H), 6.16 (dq, $J = 9.5$, $J = 1.4$ Hz, 1H), 6.09 (ddd, $J = 9.5$, $J = 5.3$, $J = 2.2$ Hz, 1H), 4.86 (dd, $J = 15.8$, $J = 2.5$ Hz, 1H), 4.80 (dd, $J = 15.8$, $J = 2.5$ Hz, 1H), 4.50-4.23 (m, 2H), 4.10 (d, $J = 7.4$ Hz, 1H), 3.96 (d, $J = 5.0$ Hz, 1H), 3.06 (t, $J = 2.5$ Hz, 1H) ppm; $^{13}$C NMR (75 MHz, acetone-$d_6$) $\delta$ 167.2, 142.5, 136.3, 131.1, 123.5, 80.0, 77.3, 72.5, 65.5, 53.4 ppm; MS (EI) $m/z$ (%): 194(M$^+$, 7%), 176(28), 138(47), 121(100); HRMS (EI) calcd for C$_{10}$H$_{10}$O$_4$: $m/z$ 194.0579, found: 194.0581; Anal. Calcd. for C$_{10}$H$_{10}$O$_4$: C, 61.85; H, 5.19. Found: C, 62.08; H, 5.18.
6-Carboxymethyl-(1S,2S)-1,2-dihydroxycyclohex-3-ene (198).

See General procedure for PAD reduction of diols. (848 mg, 84%), colorless oil; 
\( RF 0.29 \) (1:1 ethyl acetate/hexanes); \([\alpha]_D^{23} -52.9 \) (c 2.9, CHCl₃); IR (film) ν 3411, 2998, 2951, 2668, 1703, 1648, 1437, 1370, 1254, 1210, 1160, 1108, 1073, 1041, 995, 963, 920, 878, 852, 757 670 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl₃) δ 7.02 (dd, \( J = 4.4, J = 3.3 \) Hz, 1H), 4.46 (t, \( J = 3.1\) Hz, 1H), 4.05 (s, 1H), 3.70 (m, 4H), 3.52 (s, OH), 2.42-2.30 (m, 1H), 2.21-2.09 (m, 1H), 1.18-1.61 (m, 1H) ppm; \(^{13}\)C NMR (75 MHz, CDCl₃) δ 161.2, 143.3, 130.4, 68.5, 64.8, 51.8, 24.7, 24.4 ppm; MS (EI) \( m/z \) (%): 172(M⁺, 3), 129(11), 128(100), 97(70), 68(30); HRMS calcd for C₆H₁₂O₄ 172.0731, found 172.0730.

6-Carboxyethyl-(1S,2S)-1,2-dihydroxycyclohex-3-ene (199).

See General procedure for PAD reduction of diols. (378 mg, 84%), colorless crystals; mp 91-92 °C (ethyl acetate/hexanes); \( RF 0.29 \) (1:1 ethyl acetate/hexanes); \([\alpha]_D^{23} -53.3 \) (c 1.6, CHCl₃); IR (film) ν 3400, 2981, 2937, 2909, 1731, 1647, 1372, 1251, 1105, 1073, 993, 921, 878, 761, 670 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl₃) δ 7.09 (t, \( J = 4.1\) Hz, 1H), 4.50 (d, \( J = 3.8\) Hz, 1H), 4.22 (q, \( J = 7.2\) Hz, 2H), 3.81-3.93 (m, 1H), 3.51 (s, 1H), 2.69 (s, 1H), 2.37-2.50 (m, 1H), 1.79-1.94 (m, 1H), 1.66-1.75 (m, 1H), 1.29 (t, \( J = 7.2\) Hz, 3H) ppm; \(^{13}\)C NMR (75 MHz,
CDCl$_3$ $\delta$ 167.1, 143.1, 130.1, 67.7, 65.7, 60.9, 25.0, 23.9, 14.2 ppm; MS (El) $m/z$ (%): 186(M$^+$, 1), 143(12), 142(90), 105(12), 97(16), 96(100), 68(38), 67(10), 41(12); HRMS calcd for C$_9$H$_{14}$O$_4$ 186.0892, found 186.0892; Anal. calcd: C 58.05; H 7.58. found C 57.97; H 7.51.

6-Carboxypropyl-(1S,2S)-1,2-dihydroxycyclohex-3-ene (200).

See General procedure for PAD reduction of diols. (276 mg, 91%) colourless crystals; mp 73-75 $^\circ$C; $R_f$ 0.15 (1:1 ethyl acetate/hexanes); $[\alpha]_D^{20}$ -50.85 (c 1.5, CHCl$_3$); IR (film) v 3406, 2966, 2934, 2876, 1707, 1648, 1247 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.14 (t, $J = 3.4$ Hz, 1H), 4.55 (d, $J = 3.4$ Hz, 1H), 4.16 (t, $J = 6.6$ Hz, 2H), 3.92 (br s, 1H), 3.93 (m, 1H), 3.28 (br s, 1H), 3.56-2.54 (m, 1H), 2.31-216 (m, 1H), 1.98-1.85 (m, 1H), 1.81-1.69 (m, 1H), 1.73 (qt, $J = 7.4$, $J = 6.6$ Hz, 2H), 1.00 (t, $J = 7.4$ Hz, 1H) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 167.0, 143.0, 130.5, 68.3, 66.4, 65.1, 24.53, 24.49, 21.9, 10.4 ppm; MS (El) $m/z$ (%): 200(M$^+$, 3), 156(M$^+$-C$_2$H$_4$O, 68), 141(17), 114(80), 96(100); HRMS (El) calcd for C$_{10}$H$_{16}$O$_4$: $m/z$ 200.1048; found: 200.1052. Anal. Calcd. for C$_{10}$H$_{16}$O$_4$: C, 59.98; H, 8.05. Found: C, 59.87; H, 8.04.

6-Carboxyisopropyl-(1S,2S)-1,2-dihydroxycyclohex-3-ene (201).
See General procedure for PAD reduction of diols. (203 mg, 56%) pale yellow oil; $R_f = 0.31$ (6:4 ethyl acetate/hexane); $[\alpha]_D^{20} = -44.74$ (c 1.3, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.08 (t, $J = 3.9$ Hz, 1H), 5.11 (hept, $J = 6.3$ Hz, 1H), 4.51 (br s, 1H), 4.48 (br m, 1H), 3.94-3.84 (m, 1H), 3.59 (d, $J = 2.6$ Hz, 1H), 2.7 (d, $J = 5.7$ Hz, 1H), 2.52-2.38 (m, 1H), 2.67-2.12 (m, 1H), 1.95-1.81 (m, 1H), 1.77-1.66 (m, 1H), 1.29 (d, $J = 6.3$ Hz, 3H), 1.28 (d, $J = 6.3$ Hz, 3H) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 166.6, 142.7, 130.4, 68.4, 67.7, 65.7, 25.1, 23.8, 21.9, 21.8 ppm; IR (film) $\nu$ 3403, 2980, 1706, 1260 cm$^{-1}$; MS (EI) m/z (%): 156(M$^+$-C$_2$H$_4$O, 50), 141(18), 114(100), 96(94); HRMS (EI) calcd for C$_{16}$H$_{16}$O$_4$: m/z 200.1049; found: 200.1054.

(3aR,4R,5R,7aR)-7-(methoxycarbonyl)-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[dl][1,3]dioxole-4,5-diyldiacetate (203).

To a solution of diol 163 (300 mg, 1.76 mmol) in 2,2-dimethoxypropane (2 mL) and acetone (1 mL) was added p-toluenesulfonic acid (catalytic amount) at room temperature. After complete consumption of starting material (TLC analysis), the solution was diluted with ethyl acetate (60 mL), washed with saturated NaHCO$_3$ (3 x 5 mL), dried over Na$_2$SO$_4$, filtered, and concentrated. The crude acetonide was dissolved in a mixture of acetone/H$_2$O (5:1, 12 mL) and N-methylmorpholine-N-oxide (309 mg, 2.64 mmol) was added, followed by a single crystal of OsO$_4$. The solution was stirred until total consumption of starting
material (15 h) as monitored by TLC analysis. The reaction mixture was concentrated to dryness under reduced pressure, suspended in DCM (10 mL), and cooled in an ice bath. To the stirring suspension was added triethylamine (1.1 mL, 8 mmol), Ac₂O (0.60 mL, 6.2 mmol), and DMAP (43 mg, 0.35 mmol). The reaction was stirred overnight (12 h) before being quenched with NaHCO₃ (5 mL). The layers were separated and the aqueous phase was extracted with DCM (3 x 20 mL). The organic layers were combined and dried over Na₂SO₄, filtered, and concentrated. The crude material was purified via flash column chromatography with a solvent gradient of 5:1 – 3:1 hexanes-ethyl acetate. Yield: 401 mg, 69%, colourless oil; Rf 0.65 (1:1 hexanes/ethyl acetate); [α]D²⁰ -67.54 (c 2.5, EtOH); IR (film): v 2989, 2954, 2938, 1754, 1726, 1661, 1437, 1372, 1303, 1234, 1219 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 6.83 (dd, J = 3.0, 1.1 Hz, 1H), 5.72 (t, J = 3.7 Hz, 1H), 5.53 (ddd, J = 5.3, 3.8, 1.1 Hz, 1H), 5.07 (dd, J = 5.8, 0.8 Hz, 1H), 4.43 (dd, J = 5.6, 5.3 Hz, 1H), 3.84 (s, 1H), 2.08 (s, 3H), 2.07 (s, 3H), 1.41 (s, 6H), ppm; ¹³C NMR (75 MHz, CDCl₃) δ 169.9, 169.7, 165.4, 136.7, 131.4, 110.2, 72.9, 70.3, 69.6, 66.3, 52.3, 27.4, 25.7, 20.8, 20.7 ppm; MS (El) m/z (%): 313(M⁺-CH₃, 52), 211(19), 169(36), 168(12), 137(11), 85(43), 83(58), 47(12), 43(100); HRMS (M⁺-CH₃) calcd for C₁₄H₁₇O₈ 313.0923, found 313.0919.

\begin{figure}
\centering
\includegraphics[width=0.2\textwidth]{structure.png}
\caption{(3aR,4R,5R,7R,7aR)-7-(methoxycarbonyl)-2,2-dimethylhexahydrobenzo[d][1,3]dioxole-4,5-diyl diacetate (204).}
\end{figure}
To a solution of ester 203 (40 mg, 0.122 mmol) in ethanol (3 mL) was added 5% Rh/Al₂O₃ (40 mg). The reaction was stirred under an atmosphere of H₂ (60 psi, 24 h). The reaction was filtered through Celite by elution with EtOH and concentrated. The crude material was purified via flash column chromatography with a solvent gradient of 5:1 hexanes-ethyl acetate. Yield: 28 mg, 70%, colorless oil; Rf 0.60 (1:1 hexanes/ethyl acetate); [α]D²⁰ -71.56 (c 0.93, CHCl₃); IR (film): ν 2976, 2954, 2938, 1750, 1734, 1732, 14337, 1372, 11240, 1221, 1195 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) δ 5.40 (m, 1H), 4.93 (dd, J = 7.9, 2.6 Hz, 1H), 4.69 (t, J = 4.9 Hz, 1H), 4.23 (dd, J = 8.0, 5.0 Hz, 1H), 3.78 (s, 3H), 3.13 (dt, J = 12.8, 4.5 Hz, 1H), 2.21 (dt, J = 14.9, 12.9 Hz, 1H), 2.12-2.06 (m, 4H), 1.52 (s, 3H), 1.38 (s, 3H) ppm; ¹³C NMR (150 MHz, CDCl₃) δ 171.4, 170.3, 169.7, 109.9, 75.5, 73.9, 7.1, 68.7, 52.2, 38.11, 27.9, 26.2, 24.9, 20.9 ppm; MS (EI) m/z (%): 313 (M⁺-CH₃, 13), 241(15), 171(23), 153(47), 43(100); HRMS (M⁺-CH₃) calcd for C₁₄H₁₉O₈ 315.1082, found 315.1082.

(3aS,4R,5R,7S,7aR)-7-(hydroxymethyl)-2,2-dimethylhexahydrobenzo[d][1,3]dioxole-4,5-diol (206)

LAH (26 mg, 0.73 mmol) was added to a solution of ester 204 (40 mg, 0.121 mmol) in dry THF (2 mL). The reaction mixture was brought to reflux and stirred for 4 hours, allowed to cool to room temperature, and quenched with a mixture of THF/H₂O. The reaction was filtered, dried over Na₂SO₄, filtered again, and
concentrated. The crude material was purified via flash column chromatography with a solvent gradient of 5:1 hexanes-ethyl acetate. Yield: 23 mg, 86%, colorless oil; \( R'f_0 \) 0.14 (9:1 chloroform/methanol); \([\alpha]_D^{20} -57.08 \) (c 0.70, MeOH); \(^1\)H NMR (600 MHz, acetone-\(d_6\)) \( \delta \) 4.30 (t, \( J = 4.4, 1H \)), 4.01 (dd, \( J = 6.9, 5.5 \) Hz, 1H), 4.00-3.94 (m, 2H), 3.70-3.60 (m, 2H), 3.58-3.48 (m, 3H), 2.44-2.37 (m, 1H), 1.75 (dt, \( J = 13.0, 4.6 \) Hz, 1H), 1.48 (dt, \( J = 6.6, 2.8 \) Hz, 1H), 1.40 (s, 3H), 1.28 (s, 3H) ppm; \(^{13}\)C NMR (150 MHz, CDCl\(_3\)) \( \delta \) 107.8, 79.4, 74.6, 73.7, 68.9, 63.58, 34.2, 28.3, 27.7, 25.7 ppm; MS (EI) \( m/z \) (\%) 203 (M\(^+\)-CH\(_3\), 100), 204(10), 203(100), 125(19), 107(11), 100(13), 97(13), 95(21), 83(32), 79(33), 73(14), 71(11), 70(14), 69(20), 67(16), 60(17), 59(55), 57(18), 55(18), 43(53), 41(22); HRMS (M\(^+\)-CH\(_3\)) calcd for C\(_9\)H\(_{15}\)O\(_5\) 203.0933, found 203.0926.

(3aR,7aS)-Ethyl-3a,7a-dihydro-2,2-dimethylbenzo[d][1,3]dioxole-4-carboxylate (207).

(173 mg, 92%) colorless oil; \( R'f_0 \) 0.56 (1:1 hexanes/ethyl acetate); \([\alpha]_D^{23} +74.6 \) (c 4.02, CHCl\(_3\)); IR (film): v 3018, 2987, 2936, 1712, 1651, 1425, 1380, 1259, 1155, 1031, 917, 856, 697, 667, 512 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 7.06 (dd, \( J = 5.3, 3.1 \) Hz, 1H), 4.84 (d, \( J = 5.7 \) Hz, 1H), 4.28-4.41 (m, 1H), 4.07-4.26 (m, 2H), 2.21-2.45 (m, 1H), 1.99-2.16 (m, 1H), 1.86-1.99 (m, 1H), 1.58-1.72 (m, 1H), 1.33 (d, \( J = 10.2 \) Hz, 6H), 1.24 (t, \( J = 7.2 \) Hz, 3H) ppm; \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \( \delta \) 166.2, 142.3, 130.0, 108.5, 72.6, 70.4, 60.5, 27.8, 26.2, 25.1, 20.9, 14.2 ppm; MS
(3aR,7aS)-Prop-2-ynyl-3a,7a-dihydro-2,2-dimethylbenzo[\textit{d}][1,3]dioxole-4-carboxylate (208).

(764 mg, 63%) colorless oil; \( R_f = 0.54 \) (2:8 ethyl acetate/hexane); \([\alpha]_D^{22} +112.70 \) (c 1.3, CHCl₃); IR (KBr) \( \nu \) 2987, 2935, 1718, 1030 cm⁻¹; \(^1\)H NMR (300 MHz, CDCl₃) \( \delta \) 7.22 (dd, \( J = 5.3, 1.1 \) Hz, 1H), 6.19-6.09 (series of m, 2H), 4.95 (d, \( J = 8.4 \) Hz, 1H), 4.89 (dd, \( J = 8.4, J = 2.4 \) Hz, 1H), 4.84 (dd, \( J = 2.5, J = 1.0 \) Hz, 2H), 2.50 (t, \( J = 2.5 \) Hz, 1H), 1.47 (s, 3H), 1.41 (s, 3H) ppm; \(^{13}\)C NMR (75 MHz, CDCl₃) \( \delta \) 165.3, 134.8, 134.4, 125.5, 121.2, 105.7, 77.7, 74.9, 71.8, 68.0, 52.3, 26.7, 25.0 ppm; MS (EI) \( m/z \) (%): 219(\( \text{M}^+\text{-Me}, 42 \)), 177(41), 163(17), 121(83), 43(100). HRMS (EI) calcd for C\textsubscript{12}H\textsubscript{11}O\textsubscript{4}: \( m/z \) 219.0657, found: 219.0659. Anal. Calcd. for C\textsubscript{13}H\textsubscript{14}O\textsubscript{4}: C, 66.66; H, 6.02. Found: C, 66.68; H, 6.08.
(1S,2R,3S,4S,4aS,5S,6R,8aR)-1,2,3,4,4a,5,6,8a-octahydro-2,3,5,6-tetrahydroxy-\textit{O},\textit{O}-diisopropylyden-1,4-ethenonaphthalene-1,7-bis(ethyldicarboxylate) (209).

Neat 207 (400 mg, 1.78 mmol) was maintained for 7 d at rt, purified by flash-chromatography (eluant 3:7 ethyl acetate/hexane) to yield 209 (372 mg, 93%) as a clear and colorless oil; $\alpha_{D}^{22} +62.90$ (c 1.3, CHCl$_3$). IR (KBr) $\nu$ 2984, 2938, 1722, 1262, 1221, 1071 cm$^{-1}$; $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 6.44 (d, $J=3.7$ Hz, 1H), 6.39 (d, $J=8.5$ Hz, 1H), 6.05 (dd, $J=8.5$, 6.3 Hz, 1H), 4.61 (dd, $J=7.2$, 1.2 Hz, 1H), 4.59 (d, $J=4.9$ Hz, 1H), 4.43 (ddd, $J=7.2$, $J=3.4$, 0.5 Hz, 1H), 4.36 (qd, $J=7.1$, 1.7 Hz, 2H), 4.28-4.18 (m, 2H), 4.17 (dd, $J=4.9$, 2.5 Hz, 1H), 3.02 (1H, m), 2.95 (ddd, $J=9.2$, 3.7, 1.3 Hz, 1H), 2.34 (ddd, $J=9.2$, 1.3, 1.2 Hz, 1H), 1.37 (s, 3H), 1.36 (t, $J=7.1$ Hz, 3H), 1.31 (s, 3H), 1.30 (s, 3H), 1.28 (t, $J=7.1$ Hz, 3H), 1.28 (s, 3H) ppm. $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$ 171.5, 165.8, 136.4, 131.4, 130.2, 128.8, 109.7, 108.2, 80.7, 78.3, 76.9, 69.2, 61.4, 60.7, 53.9, 40.4, 38.6, 34.9, 28.1, 26.5, 25.3, 25.1, 14.20, 14.15 ppm;; MS (EI) $m/z$ (%): 433(M$^+$-Me, 6), 390(8), 375(7), 345(4), 100(17), 61(21), 43(100). HRMS (EI) calcd for C$_{24}$H$_{32}$O$_8$: $m/z$ 448.20972, found: 448.20863.
A solution of 208 (390 mg, 1.66 mmol) in toluene (1 mL) was maintained at 110 °C for 6 hours, purified by flash-chromatography (eluant 3:7 ethyl acetate/hexane) to afford 210 as a foamy solid (321 mg, 82%); mp 43-45 °C (ethyl acetate/hexanes). \( R_f = 0.32 \) (3:7 ethyl acetate/hexane); \([\alpha]_D^{22} +53.03\) (c 1.2, CHCl₃). \(^1\)H NMR (600 MHz, CDCl₃) δ 6.58 (d, \( J = 3.8 \) Hz, 1H), 6.40 (d, \( J = 8.4 \) Hz, 1H), 6.08 (dd, \( J = 8.4, 6.4 \) Hz, 1H), 4.96 (dd, \( J = 15.5, 2.4 \) Hz, 1H), 4.83 (dd, \( J = 15.5, 2.4 \) Hz, 1H), 4.82 (dd, \( J = 15.6, 2.4 \) Hz, 1H), 4.72 (dd, \( J = 15.6, 2.4 \) Hz, 1H), 4.64 (d, \( J = 7.2 \) Hz, 1H), 4.59 (d, \( J = 4.9 \) Hz, 1H), 4.45 (dd, \( J = 7.2, 3.5 \) Hz, 1H), 4.61 (dd, \( J = 4.9, 2.2 \) Hz, 1H), 3.04 (m, 1H), 3.03 (dd, \( J = 9.1, 3.5 \) Hz, 1H), 2.64 (t, \( J = 2.4 \) Hz, 3H), 2.48 (t, \( J = 2.4 \) Hz, 3H), 2.7 (d, \( J = 9.1 \) Hz, 1H), 1.37 (s, 3H), 1.309 (s, 3H), 1.303 (s, 3H), 1.29 (s, 3H) ppm; \(^{13}\)C NMR (150 MHz, CDCl₃) δ 170.7, 164.9, 137.3, 131.0, 129.6, 129.1, 109.9, 108.3, 80.5, 78.2, 77.6, 77.1, 76.8, 75.7, 75.0, 69.0, 53.8, 52.9, 52.3, 40.4, 38.8, 34.6, 28.1, 26.5, 25.3, 25.1 ppm; IR (KBr) ν 2987, 2938, 1728, 1243, 1217, 1071 cm\(^{-1}\); MS (EI) \( m/z \) (%): 453(M⁺-Me, 26), 395(32), 297(15), 121(31), 100(60), 85(22), 43(100). HRMS (EI) calcd for C\(_{24}\)H\(_{32}\)O\(_8\): \( m/z \) 453.15494, found: 453.15470. \textit{Anal.} Calcd. for C\(_{26}\)H\(_{38}\)O\(_8\): C, 66.66; H, 6.02. Found: C, 66.61; H, 6.16.
(1S,4S,5S,6R)-1-Ethyl-2,3-dimethyl-5,6-dihydroxy-O,O-isopropyldenbicyclo[2.2.2]octa-2,7-diene-1,2,3-tricarboxylate (211).

A solution of 207 (400 mg, 1.78 mmol), dimethylacetylenedicarboxylate (DMAD) (380 mg, 2.68 mmol) in toluene (1.0 mL) was stirred at rt for 72 hours. The resulting mixture was purified by flash-chromatography (eluant ethyl acetate/hexane in gradient from 2:8 to 3:7) to afford an oil that was purified by crystallization from ethyl acetate/hexane to provide 211 as colorless crystals (210 mg, 57%); mp 70-71 °C (ethyl acetate/hexanes). \( R_f = 0.35 \) (3:7 ethyl acetate/hexane); \([\alpha]_D^{22} +21.75 \) (c 2.0, CHCl₃). \(^1\)H NMR (300 MHz, CDCl₃) \( \delta \) 6.76 (ddd, \( J = 7.3, J = 1.3, J = 0.9 \) Hz, 1H), 6.44 (dq, \( J = 7.3, 6.0, 0.7 \) Hz, 1H), 4.71 (dd, \( J = 6.9, 0.9 \) Hz, 1H), 4.49 (ddd, \( J = 6.9, 3.6, 0.7 \) Hz, 1H), 4.37 (ddd, \( J = 6.0, 3.6, J = 1.3 \) Hz, 1H), 4.33 (qd, \( J = 7.2, 1.7 \) Hz, 2H), 3.77 (s, 6H), 1.34 (t, \( J = 7.2 \) Hz, 3H), 1.34 (s, 3H), 1.28 (s, 3H) ppm; \(^{13}\)C NMR (75 MHz, CDCl₃) \( \delta \) 169.5, 165.5, 164.1, 144.8, 137.8, 131.4, 130.4, 114.3, 81.8, 78.3, 62.0, 60.0, 52.48, 52.39, 43.7, 25.70, 25.67, 14.0 ppm; IR (KBr) ν 2989, 2953, 1748, 1724, 1261 cm⁻¹; MS (El) \( m/z \) (%): 351(M⁺-Me, 6), 207(21), 221(62), 175(88), 100(100). HRMS (EI) calcd for C₁₇H₁₉O₈: \( m/z \) 351.10799, found: 351.10811. Anal. Calcd. for C₁₇H₁₉O₈: C, 59.01; H, 6.05. Found: C, 59.16; H, 6.08.
(1R,2S,6S,7S)-9-Acetyl-4,4-dimethyl-3,5,8-trioxa-9-aza-tricyclo[5.2.2.02,6]undec-10-ene-7-carboxylic acid ethyl ester (212).

To a stirring solution of diol 191 (5.0 g, 27 mmol) in 2,2-dimethoxypropane (80 mL) was added p-toluenesulfonic acid (catalytic amount) at room temperature. After complete consumption of starting material (TLC analysis), the solution was cooled 0 °C before the addition of H2O (10 mL). On a preparative scale the intermediate acetonide 207 was not isolated.

NaIO4 (5.80 g, 27.1 mmol) was added to the reaction flask prior to the addition of a solution of acetohydroxamic acid (2.03 g, 27.1 mmol) in MeOH (25 mL) dropwise over 5 minutes. The resulting solution was stirred at room temperature for 16 h, quenched by the slow addition of sat. NaHSO3 (10 mL) and extracted into Et2O (3 x 100 mL). The combined organic layers were washed with brine (2 x 30 mL) and dried over Na2SO4. The crude material was purified via flash column chromatography with a solvent system of 1:4 (hexanes/ethyl acetate) to yield 212 as a white solid (5.65 g, 70% over 2 steps); Rf 0.33 (3:7 hexanes/ethyl acetate); mp 89-90 °C (hexanes/ethyl acetate); [α]D23 -18.0 (c 0.54, CHCl3); IR (film) ν 3466, 2938, 2987, 1747, 1684, 1620, 1372, 1275, 1086 cm⁻¹; 1H NMR (600 MHz, CDCl3) δ 6.57-6.65 (m, 2H), 5.47-5.52 (m, 1H), 4.71 (d, J = 6.8 Hz, 1H), 4.56 (dd, J = 4.7, 6.6 Hz, 1H), 4.38 (q, J = 7.2 Hz, 2H), 2.01 (s, 3H), 1.38 (t, J = 7.2 Hz, 3H), 1.32 (s, 3H), 1.30 (s, 3H) ppm; 13C NMR (150 MHz, CDCl3) δ 173.9, 166.6, 132.4, 128.4, 111.7, 79.2, 76.1, 72.8, 62.7, 50.0, 25.6, 25.4, 21.7,
14.1 ppm; MS (EI) m/z (%): 297(M⁺), 124(52), 105(35), 100(32), 96(30),
43(100); HRMS calc'd for C₁₄H₁₉NO₆ 297.1212, found 297.1215.

7-Acetylamino-4-hydroxy-2,2-dimethyl-3a,4,7,7a-tetrahydro-
benzo[1,3]dioxole-4-carboxylic acid ethyl ester (213)

To a stirred solution of 212 (955 mg, 3.21 mmol) in 15:1 CH₃CN:H₂O (10 mL)
was added molybdenum hexacarbonyl (848 mg, 3.21 mmol) at room temperature.
The reaction was brought to reflux for 3 h before being allowed to cool to room
temperature. The reaction was concentrated and filtered through a plug of celite.
The crude material was purified via flash column chromatography with a solvent
system of 1:9 (hexanes/ethyl acetate) to yield 213 (720 mg, 75%) as a white solid;
Rf 0.20 (ethyl acetate); mp 97-99 °C (hexanes-ethyl acetate); [α]D²³ -94.3 (c 0.79,
CHCl₃); IR (film) ν 3433, 2094, 1644, 1271, 1217, 1060 cm⁻¹; ¹H NMR (600
MHz, CDCl₃) δ 6.25 (d, J = 8.7 Hz, 1NH), 5.98 (dd, J = 3.8, 9.8 Hz, 1H), 5.94
(dd, J = 0.9, 9.9 Hz, 1H), 4.77-4.81 (m, 1H), 4.37 (t, J = 8.3 Hz, 1H), 4.34 (dd, J =
4.3, 7.7 Hz, 1H), 4.22-4.29 (m, 2H), 4.12 (s, 1OH), 1.99 (s, 3H), 1.35 (s, 3H),
1.32 (t, J = 7.4 Hz, 3H), 1.28 (s, 3H) ppm; ¹³C NMR (150 MHz, CDCl₃) δ 172.7,
170.0, 132.9, 129.6, 109.3, 81.0, 76.3, 74.5, 62.8, 48.8, 26.2, 24.2, 23.5, 14.0
ppm; MS (EI) m/z (%): 284 (M⁺-CH₃), 199(99), 153(38), 125(36), 96(37), 86(61),
84(100), 83(47), 43(90); HRMS calc'd for C₁₃H₁₈NO₆ 284.1130, found 284.1137.
A solution of \( m \)-CPBA (10.5 g, 46.7 mmol) in 1,2-dichloroethane (150 mL) was dried over \( \text{Na}_2\text{SO}_4 \) and filtered into a flask containing aziridine \( 70^{64} \) (5.0 g, 16 mmol). The resulting solution was heated to reflux until total consumption of starting material (6 hours). The reaction was allowed to cool to room temperature at which time \( m \)-chlorobenzoic acid crystallized out as a white solid. The reaction was filtered and concentrated. The residue was taken up EtOAc (350 mL) and washed sequentially with NaHSO\(_3\) (2 x 100 mL), carbonate (2 x 100 mL), and brine (35 mL). The organic phase was dried over \( \text{Na}_2\text{SO}_4 \), filtered, and concentrated to yield a 2.89:1 mixture of epoxides 217 and 220 (5.04 g, 96 %) as a slightly yellow solid. The epoxide could be used without further purification or enriched in favor of the major isomer by successive recrystallizations from isopropanol. A small sample of the mixture taken and the two epoxides were separated by flash column chromatography (10% deactivated silica gel,
hexanes/ethyl acetate 7:1 – 4:1) to provide analytical samples of epoxides 217 and 220.

Epoxide 217

*Rf 0.43 (hexanes/ethyl acetate, 2:1); mp 115-116 °C (isopropanol); \([\alpha]_D^{21} - 79.36\) (c 1.0, CHCl₃); IR (film) v2988, 2936, 1597, 1383, 1373, 1329, 1251, 1218, 1158, 1091, 1055 cm⁻¹; \(^1\)H NMR (300 MHz, CDCl₃) \(\delta\): 7.90 (d, \(J = 8.3\) Hz, 2H), 7.36 (d, \(J = 8.3\) Hz, 2H), 4.4 (d, \(J = 6.2\) Hz, 1H), 4.26 (d, \(J = 6.0\) Hz, 1H), 3.53 (d, \(J = 3.7\) Hz, 1H), 3.38 (dd, \(J = 6.8\) Hz, 3.8 Hz, 1H), 3.13 (dd, \(J = 3.5\) Hz, 0.9 Hz, 1H), 3.04 (dd, \(J = 6.8\) Hz, 1.1 Hz, 1H), 2.46 (s, 3H), 1.46 (s, 3H), 1.36 (s, 3H); \(^13\)C NMR (75 MHz, CDCl₃) \(\delta\): 144.9, 134.5, 129.9, 128.0, 110.2, 70.8, 69.8, 50.1, 46.7, 37.3, 35.7, 27.4, 25.2, 21.7; HRMS-EI Calcd for \(\text{C}_16\text{H}_{19}\text{NO}_5\text{S}\) (M⁺-15): 322.0749, Found: 322.0744; Anal. calcd for \(\text{C}_16\text{H}_{19}\text{NO}_5\text{S}\) C, 56.96; H, 5.68; found C, 56.23; H, 5.61.

Epoxide 220

*Rf 0.40 (hexanes/ethyl acetate, 2:1); mp 179-180 °C (isopropanol); \([\alpha]_D^{21} - 62.82\) (c 1.0, CHCl₃); IR (film) v2985, 2929, 1602, 1384, 1366, 1324, 1245, 1222, 1163, 1153, 1041, 1001 cm⁻¹; \(^1\)H NMR (600 MHz, CDCl₃) \(\delta\): 7.83 (d, \(J = 8.1\) Hz, 2H), 7.39 (d, \(J = 8.1\) Hz, 2H), 4.40 (d, \(J = 6.3\) Hz, 1H), 4.31 (dd, \(J = 6.2\) Hz, 2.3 Hz, 1H), 3.58 (dd, \(J = 3.9\) Hz, 2.4 Hz, 1H), 3.44 (dd, \(J = 6.7\) Hz, 2.0 Hz, 1H), 3.27 (d, \(J = 3.5\) Hz, 1H), 3.18 (t, \(J = 6.8\) Hz, 1H), 2.48 (s, 3H), 1.54 (s, 3H), 1.35 (s, 3H); \(^13\)C NMR (150 MHz, CDCl₃) \(\delta\): 145.3, 133.7, 130.0, 128.1, 111.1, 71.9, 69.9, 52.4, 25.0, 39.3, 38.6, 26.6, 25.9, 21.7; HRMS-EI Calcd for \(\text{C}_16\text{H}_{19}\text{NO}_5\text{S}\)
(M⁺-15): 322.0749, Found: 322.0753; Anal. calcd for C₁₆H₁₉NO₅S C, 56.96; H, 5.68; found C, 56.23; H, 5.61.

(3aS,4R,5R,6R,7S,7aS)-6-(1,3-benzodioxol-5-ylethynyl)-7-{{tert-butyldimethyl)silyl]oxy}-2,2-dimethyl-8-[(4-methylphenyl)sulfonyl]hexahydro-4,5-epimino-1,3-benzodioxole (223).

To a solution of acetylene 222 (0.43 g, 2.96 mmol) in 8 mL dry toluene at -50 °C was added 1.46 mL of a solution of nBuLi in hexanes (2.01 M, 2.96 mmol) over 5 min. The solution was stirred for 15 minutes before 2.97 mL of a solution of Me₂AlCl (1.0 M in CH₂Cl₂, 2.96 mmol) was added dropwise over 10 min. The reaction flask was kept at -50 °C for 0.5 h and then moved to an ice bath and stirred an additional 0.5 h before being allowed to warm to room temperature and stir for 0.5 h. The reaction flask was then cooled to -20 °C and 8 mL of a solution of epoxide 217:220 (7:1 mixture of isomers, 0.5 g, 1.48 mmol) in toluene was added dropwise over 20 min before being allowed to slowly warm to room temperature over 5 h. The reaction was cooled in an ice bath and quenched with 1 M HCl (1 ml). Ethyl acetate (50 mL) was added and the layers were separated. The aqueous phase was extracted with EtOAc (3 x 50 mL) and the combined organic layers dried over Na₂SO₄. Concentration under reduced pressure gave
0.883 g of crude alcohol intermediate which was immediately subjected to protection protocol. A small sample for characterization was purified by flash column chromatography (hexanes/ethyl acetate, 7:1 to 4:1) afforded alcohol (3aS,4R,5R,6R,7S,7aR)-6-(1,3-benzodioxol-5-ylethynyl)-2,2-dimethyl-8-[(4-methylphenyl)sulfonyl]hexahydro-4,5-epimino-1,3-benzodioxol-7-ol (221) as a clear and colorless oil; [α]$_D^{22}$ -113.05 (c 0.5, CHCl$_3$); Rf 0.30 (hexanes:ethyl acetate, 2:1); IR (film) v 3491, 2988, 1163 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) δ: 7.78 (d, J = 8.1 Hz, 2H), 7.38 (d, J = 8.1 Hz, 2H), 6.91 (dd, J = 8.2 Hz, 1.8 Hz 1H), 6.83 (d, J = 1.5 Hz, 1H), 6.73 (d, J = 7.9 Hz, 1H), 5.97 (s, 2H), 4.47 (d, J = 6.4 Hz, 1H), 4.22 (dd, J = 6.1, 4.4 Hz, 1H), 3.98 (m, 1H), 3.40 (d, J = 6.4 Hz, 1H), 3.24 (m, 2H), 3.06 (d, J = 9.6 Hz, 1H), 2.47 (s, 3H), 1.49 (s, 3H), 1.32 (s, 3H) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$) δ: 148.1, 147.5, 145.7, 134.2, 130.4, 128.1, 126.4, 116.2, 111.8, 110.3, 108.6, 101.5, 84.2, 83.8, 75.4, 70.1, 68.7, 42.3, 40.5, 31.1, 27.4, 25.2, 21.9 ppm; HRMS (FAB $^+$) calcd for C$_{25}$H$_{25}$N$_2$O$_7$S 484.1430, found 484.1428.

The free alcohol intermediate 221 (0.8 g, crude) was dissolved in 20 mL of CH$_2$Cl$_2$ and triethylamine (0.484 mL, 3.48 mmol) was added. The reaction flask was cooled to -78 °C and t-butyldimethylsilyltriflate (0.457 mL, 0.1.99 mmol) was added dropwise to the stirring solution. After stirring for 30 minutes at -78 °C the reaction was quenched with water (20 ml) and the two phases separated. The aqueous phase was extracted with CH$_2$Cl$_2$ (3 x 50 mL) and the combined organic solution was washed sequentially with 5% citric acid (2 mL) and brine (2 mL) before drying over sodium sulfate. The solvent was removed under reduced
pressure and the residue was purified by flash column chromatography (hexane/ethyl acetate, 9:1-2:1) affording 223 (0.677 g, 77% over 2 steps) as a colorless oil; $\left[\alpha\right]_{D}^{24} +57.7$ (c 0.5, CHCl$_3$); $R_f$ 0.49 (hexanes:ethyl acetate, 2:1); IR (film) $\nu$ 2953, 2929, 2892, 2856, 1599, 1490 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 7.83 (d, $J = 8.1$ Hz, 2H), 7.38 (d, $J = 8.1$ Hz, 2H), 6.94 (d, $J = 8.1$ Hz, 1H), 6.84 (s, 1H), 6.77 (d, $J = 8.1$ Hz, 1H), 5.99 (s, 2H), 4.45 (d, $J = 5.1$ Hz, 1H), 3.83 (m, 2H), 3.26 (m, 2H), 2.84 (d, $J = 7.5$ Hz), 2.47 (s, 3H), 1.52 (s, 3H), 1.35 (s, 3H), 0.87 (s, 9H), 0.11 (s, 6H) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$: 147.8, 147.3, 134.7, 129.8, 127.9, 126.1, 111.6, 109.7, 108.4, 101.3, 86.3, 83.5, 71.7, 43.2, 39.53, 34.58, 27.9, 25.8, 25.79, 25.7, 21.7, 18.12, -4.4, -4.7 ppm; HRMS-EI Calcd for C$_{30}$H$_{38}$NO$_3$Si: 540.1481; Found, 540.1487; Anal. calcd for C$_{31}$H$_{39}$NO$_3$Si C, 62.28; H, 6.58; found C, 62.22; H, 6.73.

(3aS,4R,5R,6R,7S,7aS)-6-[(Z)-2-(1,3-benzodioxol-5-yl)vinyl]-7-[(tert-butyl(dimethyl)silyl]oxy]-2,2-dimethyl-8-[(4-methylphenyl)sulfonyl]hexahydro-4,5-epimino-1,3-benzodioxole (216).

A) Alkyne 223 (500 mg, 0.837 mmol) was taken up in 45 mL MeOH and quinoline (34 mg, 0.168 mmol) was added. Lindlar's catalyst (35 mg, 0.335 mmol) added. The reaction mixture was purged with aspirator vacuum and
flushed with H₂ before being placed under H₂ using a balloon, and stirred for 3 h. The reaction mixture was filtered through a short plug a Celite and the solvent was removed under reduced pressure. The crude residue was purified by flash column chromatography (hexanes/ethyl acetate, 4:1) to give the title compound as a clear and colorless oil (480 mg, 95%)

B) To a 1.0 M solution of BH₃·THF complex (2.5 mL, 2.5 mmol) was added cyclohexene (0.484 mL, 4.77 mmol) at 0 °C. After 10 m a heavy precipitate was formed. The reaction mixture was kept at 0 °C for 1 h before acetylene derivative 223 (0.356 mg, 0.596 mmol) in 4.5 mL of THF was added. The reaction mixture was stirred at 0 °C until total consumption of starting material (2 h, TLC) before being quenched with 1 mL HOAc. EtOAc (60 mL) was added and the reaction mixture was washed with saturated aq. NaHCO₃ (2 x 15 mL), H₂O (2 x 15 mL), and brine (10 mL) before drying over sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (hexanes/ethyl acetate, 8:1) affording 0.271 g of 216 (76%);

[α]₂³⁺ =-26.14 (c 1.0, CHCl₃; Rf 0.35 (hexanes:ethyl acetate, 4:1); IR (film) v 2986, 2930, 2894, 2856, 1598, 1489 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ: 7.78 (d, J = 8.1 Hz, 2H), 7.29 (d, J = 8.1 Hz, 2H), 6.65 (m, 3H), 6.51 (d, J = 11.7 Hz, 1H), 5.97 (s, 2H), 5.54 (t, J = 11.3 Hz, 1H), 4.43 (d, J = 6, 1H), 3.85 (t, J = 6.3, 1H), 3.61 (t, J = 7.2 Hz), 3.18 (d, J = 6.6, 1H), 2.91 (m, 2H), 2.44 (s, 3H), 1.52 (s, 3H), 1.33 (s, 3H), 0.79 (s, 9H), 0.02 (s, 3H), -0.04 (s, 3H) ppm; ¹³C NMR (150 MHz, CDCl₃) δ: 147.5, 146.6, 144.6, 134.7, 132.0, 129.8, 129.7, 128.5, 122.5, 109.35, 109.0, 108.1, 100.9, 83.2, 78.0, 72.6, 71.8, 43.7, 39.9, 30.1, 27.8, 25.8, 25.79,
25.51, 21.7, 18.1, -4.3, -4.7 ppm; HRMS-EI Calcd for C$_{31}$H$_{41}$NO$_{7}$Si: 599.2373; Found, 599.2376; Anal. calcd for C$_{31}$H$_{41}$NO$_{7}$Si C, 62.28; H, 6.58; found C, 61.30; H, 6.63

(3aS,4R,5R,6R,7S,7aS)-6-[2-(1,3-benzodioxol-5-yl)ethyl]-7-{[tert-
butyldimethylsilyl]oxy}-2,2-dimethyl-8-[(4-
methylphenyl)sulfonyl]hexahydro-4,5-epimino-1,3-benzodioxole
(226).

Alkyne 223 (100 mg, 0.168 mmol) was taken up in 10 mL MeOH and Lindlar's catalyst (35 mg, 0.335 mmol) added. The reaction mixture was purged with aspirator vacuum and flushed with H$_2$ before being placed under H$_2$ using a balloon, and stirred for 24 hours. The reaction mixture was filtered through a short plug a celite and the solvent was removed under reduced pressure. The crude residue was purified by flash column chromatography (hexanes:ethyl acetate, 4:1) to give the title compound as a clear and colorless oil (95 mg, 94%); [α]$^2$$_D$ -47.2 (c 1.8, CHCl$_3$) Rf 0.35 (hexanes/ethyl acetate, 2:1); IR (film) ν 2986, 2954, 2929, 2891, 2857,1598,1504,1489 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) δ: 7.87 (d, $J$ = 7 Hz, 2H), 7.37 (d, $J$ = 7 Hz, 2H), 6.70 (d, $J$ = 6.5 Hz, 1H), 6.55 (s, 1H), 6.51 (d, $J$ = 7 Hz, 1H), 5.93 (s, 2H), 4.41 (d, $J$ = 5 Hz, 1H), 3.79 (t, $J$ = 5 Hz,
1H), 3.43 (m, 1H), 3.18 (d, J = 6 Hz, 1H), 2.84 (m, 1H), 2.60 (m, 1H), 2.45 (s, 3H), 2.40 (m, 1H), 2.05 (m, 1H), 1.68 (s, 3H), 1.34 (s, 3H), 0.83 (s, 9H), 0.06 (s, 3H), 0.03 (s, 3H); 13C NMR (250 MHz, CDCl3) δ: 146.6, 145.7, 144.8, 135.1, 134.9, 129.8, 128.0, 127.9, 121.2, 109.2, 108.9, 108.1, 100.8, 78.6, 72.5, 71.9, 43.2, 39.9, 39.5, 32.9, 32.7, 27.8, 25.9, 25.8, 25.5, 21.7, 18.2, -4.0, -4.8; HRMS-EI Calcd for C31H43NO7SSi: 601.2530; Found, 601.2534.

\[ N-[(1R,2aS,4aS,5S,5aR,12bR)-5-(\text{tert-Butyldimethylsilyloxy})-3,3\text{-dimethyl-1,2a,4a,5a,12b-hexahydrophenanthro[2,3-}d][1,3]\text{dioxol-1-yl}]4\text{-methylbenzenesulfonamide (215).} \]

A flame-dried 25-mL flask was charged with olefin 216 (336 mg, 0.561 mmol) and silica gel (1.5 g) which has been activated by heating under vacuum at 120 °C overnight. The starting materials were suspended in 10 mL freshly distilled methylene chloride and the solvent removed under reduced pressure. The silica gel supporting the adsorbed reactants was heated externally at 120 °C under nitrogen atmosphere for 24 h, after which time the silica gel was loaded onto flash silica gel column and eluted with hexanes/ethyl acetate (8:1 – 5:1) to give olefin 215 as a clear and colorless oil. Yield 249 mg, 74%; [α]D23 -123.7 (c 1.0, CHCl3); Rf 0.35 (hexanes/ethyl acetate, 2:1); IR (film) ν 3268, 2929, 2887, 2857,
1598, 1503, 1485 cm\(^{-1}\); \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\): 7.43 (d, \(J = 7.0\) Hz, 2H), 7.13 (d, \(J = 7.2\) Hz, 2H), 6.49 (s, 2H), 6.34 (d, \(J = 8.1\) Hz, 1H), 5.95 (s, 1H), 5.86 (s, 1H), 5.76 (d, \(J = 8.0\) Hz, 1H), 4.51 (d, \(J = 7.2\) Hz, 1H), 4.28 (m, 1H), 4.11 (m, 1H), 3.99 (m, 1H), 3.79 (m, 1H), 2.82 (m, 1H), 2.62 (dd, \(J = 11.1\) Hz, 5.4 Hz, 1H), 2.40 (s, 3H), 1.43 (s, 3H), 1.33 (s, 3H), 0.89 (s, 9H), 0.11 (s, 3H), 0.07 (s, 3H) ppm; \(^{13}\)C NMR (150 MHz, CDCl\(_3\)) \(\delta\): 146.7, 145.9, 142.1, 138.9, 128.9, 128.6, 127.7, 126.8, 126.3, 126.2, 110.4, 109.2, 107.0, 79.0, 78.3, 70.3, 54.1, 42.5, 41.5, 38.9, 27.8, 26.3, 25.7, 25.3, 22.7, 21.5, 18.0, -5.0, -5.0 ppm; HRMS-EI Calcd for C\(_{31}\)H\(_{41}\)NO\(_7\)SSi: 599.2373; Found, 599.2370; Anal. calcd for C\(_{31}\)H\(_{41}\)NO\(_7\)SSi C, 62.07; H, 6.89; found C, 62.16; H, 6.94.

\[\text{N-[(1R,2aS,4aS,5S,5aS,12bR)-5-(tert-Butyl-dimethyl-silanyloxy)-6-hydroxy-3,3-dimethyl-7-oxo-1,2a,4a,5a,6,7,12b-octahydro-phenanthro[2,3-d][1,3]dioxol-1-yl]4-methyl-benzenesulfonamide (228).}\]

To a solution of olefin 215 (0.240 mg, 0.4 mmol) in methylene chloride (10 mL) was added 4-methylmorpholine-\(N\)-oxide (58 mg, 0.48 mmol). The reaction mixture was allowed to stir for 10 minutes before the introduction of a single crystal of osmium tetroxide and two drops of water. The reaction was stirred until total consumption of starting material (10 h) before being quenched with a
saturated solution of saturated sodium bisulfite (6 mL). The two layers were separated and the aqueous phase was extracted with ethyl acetate (3 x 30 mL). The organic phase was dried over sodium sulfate, filtered, and concentrated to provide hydroxyketone 228 as a white crystalline solid (0.227 g, 89%) that was used without further purification; \( R_f 0.42 \) (hexanes:ethyl acetate, 1:1); mp >200 °C (hexane/ethyl acetate); IR (film) \( \nu 3478, 3263, 2929, 2857, 1670, 1614, 1504, 1482, 1444, 1386, 1330, 1252, 1218, 1156, 1075, 1039 \text{ cm}^{-1} ; {^1}H \text{ NMR (600 MHz, CDCl}_3) \delta: 7.54 (d, \( J = 7.8 \text{ Hz}, 2\)H), 7.49, (s, 1H), 7.18 (d, \( J = 7.8 \text{ Hz}, 2\)H), 6.70 (s, 1H), 6.07 (s, 1H), 6.00 (s, 1H), 4.79 (d, \( J = 8.7 \text{ Hz}, 1\)H), 4.71 (m, 2H), 4.19 (m, 1H), 4.08 (m, 1H), 3.74 (m, 2H), 3.08 (dd, \( J = 10.2 \text{ Hz}, 1.8 \text{ Hz}, 1\)H), 2.45 (m, 1H), 2.41 (s, 3H), 1.36 (s, 3H), 1.31 (s, 3H), 0.87 (s, 9H), 0.12 (s, 3H), 0.07 (s, 3H) ppm; \( ^{13}C \text{ NMR (150 MHz, CDCl}_3) \delta: 196.6, 152.5, 147.9, 142.6, 140.5, 138.9, 129.1, 126.9, 124.7, 111.2, 109.6, 106.9, 102.1, 78.9, 78.7, 70.3, 65.9, 57.9, 49.4, 39.7, 27.9, 25.7, 21.5, 17.95, -5.1 \text{ ppm}; \text{HRMS-EI Calcd for C}_{27}H_{32}NO_{9}S_{2}Si (M^{+}-57): 574.1567, \text{Found: 574.1572}

(3aS,3bR,10bR,11R,12S,12aS)-12-(tert-Butyl-dimethyl-silyloxy)-2,2-dimethyl-5-oxo-4-(toluene-4-sulfonyl)-3a,3b,4,5,10b,11,12,12a-octahydro-1,3,7,9-tetraoxa-4-aza-dicyclopenta[a,h]phenanthrene-11-carbaldehyde (166).

To a 10 mL round-bottomed flask was added hydroxyl ketone 228 (0.4 g, 0.6
mmol) and 6 mL of a 1:1 mixture of ethanol/dioxane. The reaction flask was cooled externally in an ice bath and NaBH₄ (24 mg, 0.63 mmol) was added in one portion. The reaction was removed from the bath and allowed to warm to room temperature over 1 h. The reaction was quenched with 1 N HCl (4 mL) and separated. The aqueous phase was extracted with CH₂Cl₂ (3 x 20 mL) and the organic phase combined before drying over sodium sulfate. The crude mixture was concentrated in a 25 mL round bottomed flask and taken up in dioxane (8 mL). A stirring bar was added and the reaction was stirred while sodium periodate (0.332, 1.5 mmol) was added. The flask was covered to exclude light and H₂O (15 drops) added. The reaction was stirred until total consumption of starting material (23h) as monitored by TLC. The reaction was quenched with H₂O (10 mL) and separated. The aqueous phase was extracted with CH₂Cl₂ (3 x 50 mL) and the combined organic phases dried over sodium sulfate.

Concentration provided hemi-aminal 231.

To a solution of hemi-aminal 231 (394 mg, 0.620 mmol) in N,N-Dimethylformamide (3 mL) was added 2-Iodoxybenzoic acid (520 mg, 1.86 mmol). After total consumption of starting material (by TLC), the reaction mixture was diluted with diethyl ether (200 mL) and washed sequentially with saturated aqueous sodium bisulfite (10 mL), sodium bicarbonate (3 x 10 mL), H₂O (10 x 1mL), and brine (10 mL). The organic phase was dried over magnesium sulfate, filtered and concentrated. The final product 166 was isolated by column chromatography (hexanes/ethyl acetate, 4:1). Yield: 225 mg, 61%, over 3 steps, white solid; Rf 0.31 (hexanes:ethyl acetate, 4:1); mp >200 °C,
recrystallized from hexanes/ethyl acetate 4:1; \([\alpha]_D^{21} + 31.67\) (c 0.5, CHCl₃); IR (film) \(\nu\) 2929, 2857, 1725, 1689, 1505, 1484, 1386, 1361, 1287, 1255, 1220, 1172, 1077, 1036 cm\(^{-1}\); \(^1\)H NMR (600 MHz, CDCl₃) \(\delta\): 9.49 (s, 1H), 8.3 (d, \(J\) = 8.2 Hz, 2H), 7.58 (s, 1H), 7.33 (d, \(J\) = 8.2 Hz, 2H), 7.28 (s, 1H), 6.55 (s, 1H), 6.04 (d, \(J\) = 5 Hz, 2H), 5.81 (dd, \(J\) = 8.4 Hz, 5.2 Hz, 1H), 4.79 (m, 1H), 4.50 (dd, \(J\) = 12.7 Hz, 8.4 Hz, 1H), 4.27 (dd, \(J\) = 5.2 Hz, 2.7 Hz, 1H), 3.83 (dd, \(J\) = 12.6, 4.0 Hz, 1H), 3.31 (m, 1H), 2.45 (s, 3H), 1.42 (s, 3H), 1.32 (s, 1H), 0.99 (s, 9H), 0.26 (s, 3H), 0.25 (s, 3H) ppm; \(^{13}\)C NMR (150 MHz, CDCl₃) \(\delta\): 196.2, 166.0, 153.0, 147.1, 143.9, 138.8, 137.0, 128.9, 128.8, 110.1, 109.4, 104.2, 102.2, 72.4, 66.6, 65.5, 55.6, 35.4, 31.0, 27.9, 26.9, 25.7, 22.7, 21.7, 18.1, 14.2, -4.7, -4.9 ppm; HRMS-EI Calcd for C\(_{30}\)H\(_{36}\)N\(_{9}\)S\(_{2}\)Si (M\(^+\)-15): 614.1879, Found: 614.1870; Anal. calcd for C\(_{31}\)H\(_{39}\)N\(_{9}\)S\(_{2}\)Si C, 59.12; H, 6.24; found C, 59.31; H, 6.29.

(3aS,3bR,10bR,11R,12S,12aS)-12-(tert-Butyl-dimethyl-silyloxy)-2,2-dimethyl-5-oxo-4-(toluene-4-sulfonyl)-3a,3b,4,5,10b,11,12,12a-octahydro-1,3,7,9-tetraoxa-4-aza-dicyclopenta\([a,h]\)phenanthrene-11-carboxylic acid (235).

To a solution of aldehyde 166 (144 mg, 0.229 mmol) in dry methylene chloride (5 mL) was added sodium phosphate dibasic (81 mg, 0.57 mmol). The suspension was stirred while 3-chloroperbenzoic acid (130 mg, 0.57 mmol) was added. The
reaction flask was sealed and heated at 40 °C overnight. The reaction mixture was diluted with methylene chloride (80 mL) and washed sequentially with saturated aqueous sodium bisulfite (10 mL), sodium bicarbonate (10 mL), and dried over sodium sulfate. The organic phase was filtered and concentrated in vacuo to provide carboxylic acid 235 as a white crystalline solid (0.125 g, 85%) that was used without further purification. Rf 0.1 (hexanes/ethyl acetate, 1:1); mp >200 °C (chloroform/ether); [α]D 22° - 35.09 (c 1.25, CHCl₃); IR (KBr) ν 3246, 2930, 2891, 2857, 1710, 1688, 1619, 1505, 1484, 1361, 1240, 1220, 1172, 1078, 1033 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ: 8.29 (d, J = 8.3 Hz, 2H), 7.53 (s, 1H), 7.32 (d, J = 8.3 Hz, 2H), 7.28 (s, 1H), 6.56 (s, 1H), 6.02 (d, J = 3 Hz, 2H), 5.77 (dd, J = 8.30 Hz, J = 5.3 Hz, 1H), 4.85 (dd, J = 12.5 Hz, 8.4 Hz, 1H), 4.84 (t, J = 4.7 Hz, 1H), 4.22 (dd, J = 5.22, 2.8 Hz, 1H), 3.76 (dd, J = 12.4 Hz, 4.1 Hz, 1H), 3.38 (t, J = 3.5 Hz, 1H), 2.45 (s, 3H), 1.40 (s, 3H), 1.27 (s, 1H), 0.96 (s, 9H), 0.21 (s, 6H) ppm; ¹³C NMR (150 MHz, CDCl₃) δ: 174.3, 166.2, 152.8, 146.9, 143.8, 138.9, 137.7, 129.0, 128.8, 122.4, 109.8, 109.2, 103.4, 102.1, 72.8, 68.2, 64.9, 48.0, 35.5, 27.4, 26.9, 25.7, 21.7, 18.0, -4.9, -5.0 ppm; HRMS-EI Calcd for C₂₇H₃₀NO₁₀SSi (M⁺-57): 588.1359, Found: 588.1354; Anal. calcd for C₃₁H₃₉NO₁₀SSi C, 57.65; H, 6.09; found C, 58.01; H, 6.37
(3aS,3bR,10bR,11R,12S,12aS)-12-(tert-Butyl-dimethyl-silyloxy)-2,2-dimethyl-5-oxo-4-(toluene-4-sulfonyl)-3a,3b,4,5,10b,11,12,12a-octahydro-1,3,7,9-tetraoxa-4-aza-dicyclopenta[a,h]phenanthrene-11-carboxylic acid methyl ester (236).

To a solution of carboxylic acid 235 (45 mg, 0.069 mmol) in diethyl ether (3 mL) was added freshly prepared diazomethane solution in diethyl ether until the persistence of yellow color and total consumption of starting material (by TLC). The reaction was quenched with one drop of acetic acid followed by saturated sodium bicarbonate solution (1 mL), diluted with diethyl ether (30 mL) and washed with saturated sodium bicarbonate solution (2 x 1 mL), dried over magnesium sulfate, filtered and concentrated. The crude reaction mixture was passed through short silica plug using hexane/ethyl acetate 1:1 as eluent and concentrated to provide methyl ester 236 that was used without further purification. Yield: 38 mg, 83%, white crystalline solid; \( R_f \) 0.45 (hexanes/ethyl acetate, 1:1); mp \(>200 \) °C (hexane/ethyl acetate); \([\alpha]_D^{22} - 25.6809 \) (c 0.75, CHCl3); IR (KBr) \( \nu \) 2986, 2953, 2931, 2896, 2858, 1739, 1692, 1620, 1598, 1505, 1485, 1361, 1289, 1264, 1173 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl3) \( \delta \): 8.30 (d, \( J = 8.4 \) Hz, 2H), 7.55 (s, 1H), 7.32 (d, \( J = 8.3 \) Hz, 2H), 6.58 (s, 1H), 6.02 (s, 2H), 5.78 (dd, \( J = 8.30 \) Hz, 5.4 Hz, 1H), 4.9 (dd, \( J = 12.5 \) Hz, 8.3 Hz, 1H), 4.78 (t, \( J = 3.0 \) Hz, 1H), 4.24 (dd \( J = 5.36 \) Hz, 2.9 Hz, 1H), 3.79 (dd, \( J = 12.4 \), 4.2 Hz, 1H), 3.56 (s, 3H), 3.40 (t, \( J = 3.7 \) Hz, 1H), 2.45 (s, 3H), 1.41 (s, 3H), 1.35 (s, 1H), 0.98 (s, 9H), 0.24 (s, 3H), 0.23 (s, 3H) ppm; \(^{13}\)C NMR (75 MHz, CDCl3) \( \delta \): 169.4, 166.3, 152.8, 146.8, 143.7, 139.0, 138.2, 128.9, 128.8, 122.4, 109.8, 109.2, 103.5,
102.0, 72.9, 68.2, 65.2, 51.9, 48.1, 35.9, 27.5, 26.8, 25.7, 21.6, 18.0, -4.8, -4.9 ppm; HRMS-EI Calcd for $C_{28}H_{32}NO_{10}Si$: $602.1516$, Found: 602.1516

(3aS,3bR,10bR,11R,12S,12aS)-12-(tert-Butyl-dimethyl-silanyloxy)-2,2-dimethyl-5-oxo-3a,3b,4,5,10b,11,12,12a-octahydro-1,3,7,9-tetraoxa-4-aza dicyclopenta[a,h]phenanthrene-11-carboxylic acid methyl ester (237).

To a solution of 236 (52 mg, 0.079 mmol) in dry THF (1 mL) at -50 °C was added a 0.5 M solution of Na/naphthalene in DME until a green color persisted and total consumption of starting material was observed (by TLC). The solution was stirred for 10 minutes before the reaction was quenched with saturated aqueous ammonium chloride solution (1 mL). The reaction was warmed to room temperature and extracted with $CH_2Cl_2$ (6 x 15 mL). The combined organic phase was dried over sodium sulfate, filtered, and concentrated. The final product was isolated by column chromatography (hexanes/ethyl acetate, 5:1-2:1). Yield: 23 mg, 58%, clear and colorless oil; $Rf$ 0.28 (hexanes:ethyl acetate, 1:1); $[\alpha]D^{22} = 14.51$ ($c$ 0.50, CHCl$_3$); IR (film) v3320, 2952, 2930, 2895, 2857, 1743, 1669, 1619, 1504, 1484, 1460, 1385, 1369, 1321, 1288, 1260, 1222 cm$^{-1}$; $^1H$ NMR (600 MHz, CDCl$_3$) $\delta$: 7.62 (s, 1H), 6.56 (s, 1H), 6.02 (s, 2H), 5.96 (s, 1H), 4.86 (t, $J = 2.6$, 1H), 4.41 (dd, $J = 13.6$ Hz, 8.2 Hz, 1H), 4.18 (dd, $J = 8.25$ Hz, 4.8 Hz, 1H), 4.11 (m, 1H), 3.66 (s, 3H), 3.40 (dd, $J = 13.6$ Hz, 3.7 Hz, 1H), 3.33 (m, 1H), 2.06
(s, 1H), 1.40 (s, 3H), 1.37 (s, 3H), 0.92 (s, 9H), 0.21 (s, 3H), 0.20 (s, 3H) ppm; 
$^{13}$C NMR (150 MHz, CDCl₃) δ: 169.6, 165.4, 151.4, 146.6, 135.4, 122.6, 110.5, 108.6, 103.3, 101.7, 69.2, 53.1, 51.9, 45.9, 33.4, 27.6, 26.5, 25.7, 17.9, -4.9, -5.0 ppm; HRMS-EI Calcd for C$_{25}$H$_{35}$NO$_3$Si (M$^+$): 505.2132, Found: 505.2131

(1R,2S,3R,4S,4aR,11bR)-2,3,4-Trihydroxy-6-oxo-1,2,3,4,4a,5,6,11b-octahydro-[1,3]dioxolo[4,5-f]phenanthridine-1-carboxylic acid methyl ester (169).

To a solution of the detosylated methyl ester 237 (23 mg, 0.046 mmol) in methanol (2 mL) was added 3% HCl in methanol (0.5 mL). The reaction mixture was stirred until total consumption of starting material (3 days). The solvent was removed under reduced pressure and the residue was purified by flash column chromatography using a 30:1 to 20:1 gradient of methylene chloride/methanol as eluent to provide methyl ester 169 (11 mg, 69%) as a white crystalline solid. $R_f$ 0.06 (methylene chloride/methanol, 20:1); mp >200 °C (methylene chloride/methanol); [α]$_D^{22} + 24.53$ (c 0.25, MeOH); IR (KBr) ν 3311, 2913, 1732, 1648, 1609, 1497, 1462, 1349, 1259, 1037 cm$^{-1}$; $^1$H NMR (300 MHz, MeOD) δ: 7.33 (s, 1H), 6.59 (s, 1H), 5.93 (d, $J = 3.7$, 2H), 4.50 (t, $J = 3.12$, 1H), 4.21 (dd, $J = 13.1$ Hz, $J = 10.1$ Hz, 1H), 3.86 (m, 1H), 3.79, (dd, $J = 10.1$, $J = 3.0$, 1H), 3.51 (s, 3H), 3.39 (m, 1H), 3.29 (dd, $J = 13.1$, $J = 4.1$, 1H) ppm; $^{13}$C NMR (75 MHz, MeOD)
δ: 170.8, 166.4, 151.7, 146.4, 137.3, 121.7, 106.9, 103.7, 101.8, 72.2, 71.9, 70.9, 51.4, 50.6, 44.8, 35.4 ppm; HRMS-FAB: (m/z) (M + H)⁺: Calcd for C₁₆H₁₇NO₈: 352.1032, Found: 352.0941

(1R,2S,3R,4S,4aR,11bR)-2,3,4-Trihydroxy-6-oxo-1,2,3,4,4a,5,6,11b-octahydro-[1,3]dioxolo[4,5-j]phenanthridine-1-carboxylic acid (168).

To a solution of 169 (6 mg, 0.017 mmol) in methanol (0.5 mL) was added LiOH (1 mg, 1.5 mmol). The reaction mixture was heated at 45 °C and stirred until total consumption of starting material (2 days) as monitored by TLC. The reaction was made slightly acidic with the addition of HCl (5 drops, 1M) and concentrated to provide acid 168 (5 mg, 95%) as a white crystalline solid. mp >200 °C (methanol); Rf 0.06 (methylene chloride/methanol, 4:1); IR (KBr) ν 3412, 2920, 2115, 1641, 1505, 1471, 1409, 1462, 1363, 1267 cm⁻¹; ¹H NMR (300 MHz, MeOD) δ: 7.41 (s, 1H), 6.72 (s, 1H), 6.02 (d, J = 3.7, 2H), 4.64 (t, J = 3.12, 1H), 4.35 (dd, J = 13.1 Hz, 10.1 Hz, 1H), 3.99 (m, 1H), 3.89 (dd, J = 10.1, 3.0, 1H), 3.45 (m, 1H), 3.38 (m, 1H) ppm; ¹³C NMR (75 MHz, MeOD) δ: 172.1, 166.4, 151.7, 146.4, 137.6, 121.7, 106.8, 103.8, 101.8, 72.4, 71.9, 71.1, 51.34, 45.03, 35.4 ppm.
(3aS,3bR,10bR,11S,12S,12aS)-12-((tert-Butyl-dimethyl-silyl)oxy)-11-
hydroxymethyl-2,2-dimethyl-4-(toluene-4-sulfonyl)-3b,4,10b,11,12,12a-
hexahydro-3aH-1,3,7,9-tetraoxa-4-aza-dicyclopenta[a,h]phenanthren-5-one
(238).

To a solution of aldehyde 166 (175 mg, 0.278 mmol) in EtOH/dioxane (1:1, 5
mL) at 0°C was added NaBH₄ (3 mg, 0.08 mmol). The reaction was allowed to
warm to room temperature over 1.5 hours before being quenched with a solution
of saturated NH₄Cl (1 mL). The EtOH/dioxane mixture was removed under
reduced pressure and the aqueous residue was extracted with CH₂Cl₂ (3 x 25 mL).
The organic phases were combined, dried over sodium sulfate, filtered, and
concentrated to provide alcohol 238 which was used without further purification.
Yield: 150 mg, 85%, clear and colorless oil; \( Rf \) 0.44 (hexanes/ethyl acetate, 1:1);
\([\alpha]D^2_2\) - 47.72 (c 1.50, CHCl₃); IR (film) \( \nu \) 3547, 2986, 2932, 2586, 1692, 1616,
1594, 1508, 1481, 1360 cm⁻¹; \(^1\)H NMR (300 MHz, CDCl₃) \( \delta \): 8.28 (d, \( J = 8.3 \) Hz,
2H), 7.54 (s, 1H), 7.30 (d, \( J = 8.2 \) Hz, 2H), 6.77 (s, 1H), 6.04 (d, \( J = 1.6 \) Hz, 2H),
5.65 (dd, \( J = 8.8 \) Hz, 5.6 Hz, 1H), 4.57 (d, \( J = 1.8 \) Hz, 1H), 4.32 (d, \( J = 4.6 \) Hz,
1H), 4.16 (dd \( J = 12.8 \), 8.9 Hz, 1H), 3.78 (m 2H), 3.38 (dd \( J = 11.3 \) Hz, 3.6 Hz,
1H), 2.55 (bs, 1H), 2.43 (s, 3H), 1.96 (bs, 1H), 1.43 (s, 3H), 1.35 (s, 3H), 0.96 (s,
9H), 0.20 (s, 6H) ppm; \(^13\)C NMR (75 MHz, CDCl₃) \( \delta \): 166.4, 153.1, 147.1, 143.7,
138.9, 137.0, 129.0, 128.7, 123.2, 109.1, 108.7, 104.9, 102.1, 73.1, 67.3, 64.8,
60.0, 46.9, 37.4, 28.1, 26.3, 25.8, 21.6, 18.0, -4.8, -4.9 ppm; HRMS-EI Calcd for C_{31}H_{41}NO_{9}SSi (M+15): 616.2032, Found: 616.2032.

\{(3aS,3bR,10bR,11S,12S,12aS)-12-\{[\text{tert-butyl}(\text{dimethyl})\text{silyl}]\text{oxy}\}-2,2\text{-dimethyl}-4-[(4\text{-methylphenyl})\text{sulfonyl}]\text{-5-oxo-3a,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5-c:4',5':f]phenanthridin-11-yl}]\text{methyl acetate (239).}

To a solution of 238 (150 mg, 0.237 mmol) in dry CH_{2}Cl_{2} (10 mL) was added DMAP (1.5 mg, 0.012 mmol), followed by pyridine (0.1 mL, 1.187 mmol). Ac_{2}O (45 \mu L, 0.475 mmol) was added and the reaction was stirred for 1 h before being quenched with saturated sodium bicarbonate (5 mL). The reaction was diluted with Et_{2}O (75 mL) and separated. The aqueous layer was extracted with Et_{2}O (2 x 75 mL) and the combined organic phases were washed with H_{2}O (10 mL), brine (10 mL), dried over magnesium sulfate, filtered, and concentrated. The final product was isolated by column chromatography using 5:1 mixture of hexanes/ethyl acetate as eluent. Yield: 128 mg, 81%, clear and colorless oil; \text{Rf} 0.51 (hexanes/ethyl acetate, 1:1); \text{[\alpha]_D}^{22} = 41.081 (c 3.0, CHCl_{3}); \text{IR (film)} v 2988, 2952, 2930, 2858, 1742, 1694, 1619, 1598, 1505, 1485, 1395, 1362, 1254;
\( ^1 \text{H} \) NMR (600 MHz, CDCl\textsubscript{3}) \( \delta: \) 8.29 (d, \( J = 8.3 \) Hz, 2H), 7.54 (s, 1H), 7.31 (d, \( J = 8.2 \) Hz, 2H), 6.84 (s, 1H), 6.03 (d, \( J = 12.6 \) Hz, 2H), 5.62 (dd, \( J = 8.7 \) Hz, \( J = 5.6 \) Hz, 1H), 4.50 (s, 1H), 4.31 (d, \( J = 5.3 \) Hz, 1H), 4.18 (t, \( J = 11.1 \) Hz, 1H), 3.97 (dd, \( J = 13.0 \) Hz, \( J = 8.8 \) Hz, 1H), 3.85 (dd, \( J = 11.0 \) Hz, \( J = 3.6 \) Hz, 1H), 3.80 (dd, \( J = 13.0, J = 4.2 \) 1H), 2.7 (d, \( J = 5.2 \) Hz, 1H), 2.44 (s, 3H), 2.03 (s, 3H), 1.42 (s, 3H), 1.36 (s, 3H), 0.96 (s, 9H), 0.19 (s, 1H); \( ^{13} \text{C} \) NMR (150 MHz, CDCl\textsubscript{3}) \( \delta: \) 170.7, 166.2, 153.2, 147.3, 143.8, 138.8, 136.2, 129.1, 128.7, 123.2, 108.9, 108.8, 105.0, 102.2, 78.4, 73.0, 66.3, 64.4, 60.8, 44.0, 37.0, 28.3, 26.2, 25.8, 25.78, 25.75, 25.6, 21.6, 20.8, 18.1, -4.8, -5.0; HRMS-EI Calcd for C\textsubscript{32}H\textsubscript{40}NO\textsubscript{10}SSi (M\textsuperscript{+}-15): 658.2142, Found: 658.2152.

\( ((3aS,3bR,10bR,11S,12S,12aR)-12\text{-hydroxy-2,2-dimethyl-5-oxo-3a,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5-c:4',5'-j]phenanthridin-11-yl})\text{methyl acetate} \) (240).

To a solution of 239 (137 mg, 0.203 mmol) in dry DME (5 mL) at -78 °C was added a 0.5 M solution of Na/naphthalene in DME until a green color persisted and total consumption of starting material was observed (by TLC). The solution was stirred for 10 minutes before the reaction was quenched with saturated aqueous ammonium chloride solution (2 mL). The reaction was warmed to room
temperature, concentrated to remove DME, and extracted with CH$_2$Cl$_2$ (3 x 40 mL). The combined organic phase was dried over sodium sulfate, filtered, and concentrated. The resulting crude acetate was taken up in THF (2.5 mL) and cooled to 0 °C. TBAF (0.1 mL, 1M in THF) was added dropwise over 2 min. The reaction was stirred until total consumption of starting material was observed (TLC) before the stirring bar was removed, silica (200 mg added), and the reaction concentrated to dryness. The final product was isolated by column chromatography using 1:1 mixture of hexanes/ethyl acetate as eluent. Yield: 61 mg, 74%, white solid; mp >200 °C (ethyl acetate/hexanes); Rf 0.059 (hexanes/ethyl acetate, 1:1); [α]$_D^{22}$ = 38.301 (c 1.35, DMSO); IR (film) ν: 3303, 2982, 2922, 2901, 2853, 1734, 1655, 1652, 1612, 1483, 1459, 1364, 1246, 1235, 1215; $^1$H NMR (300 MHz, DMSO) δ: 7.76 (s, 1H), 7.35 (s, 1H), 7.03 (s, 1H), 6.09 (d, $J = 1.8$, 2H), 5.48 (d, $J = 4.2$, 1H), 4.35 (s, 1H), 4.24 (d, $J = 5.3$, 1H), 4.19 – 4.10 (m, 3H), 3.46 (dd, $J = 14.0$ Hz, 8.2 Hz, 1H), 3.21 (dd, $J = 13.9$ Hz, $J = 3.8$ Hz, 1H), 2.80 (bs, 1H), 2.02 (s, 1H), 1.39 (s, 3H), 1.31 (s, 3H); $^{13}$C NMR (75 MHz, DMSO) δ: 170.9, 163.9, 151.3, 146.7, 134.5, 124.2, 108.9, 107.6, 105.4, 102.2, 77.9, 77.2, 65.3, 61.2, 53.5, 34.7, 28.3, 26.4, 21.2; HRMS-EI Calcd for C$_{20}$H$_{23}$NO$_8$ (M$^+$): 405.1424, Found: 405.1431.
(1S,2S,3R,4S,4aR,11bR)-2,3,4-trihydroxy-6-oxo-1,2,3,4,4a,5,6,11b-octahydro-[1,3]dioxolo[4,5-j]phenanthridin-1-yl)methyl acetate (170).

To a solution of acetate 240 (21 mg, 0.052 mmol) in MeOH (1 mL) was added an HCl solution (3 % in MeOH, 3 mL). The reaction was stirred until total consumption of starting material as monitored by TLC (3 h) before being quenched to basic pH with saturated sodium bicarbonate solution. The crude reaction mixture was concentrated to dryness. The final product was isolated by column chromatography (methlene chloride/methanol, 5:1). Yield: 6 mg, 45%, white solid; mp >200 °C (methylene chloride/methanol; Rf 0.41 (methlene chloride: methanol, 5:1); [α]D22 97.32 (c 0.3, DMSO); 1H NMR (600 MHz, DMSO) δ: 7.36 (s, 1H), 7.01 (s, 1H), 6.76, (s, 1H), 6.10, (s, 2H), 5.14, (bs, 3H), 4.38 (t, J = 10.7 Hz, 1H), 4.15 – 4.10 (m, 2H), 3.84 (s, 1H), 3.70 (dd J = 9.8 Hz, 2.9 Hz, 1H), 3.50 (dd J = 13.2 Hz, 9.9 Hz, 1H), 3.27 (dd J = 13.3 Hz, J = 4.0 Hz, 1H), 2.69 (bs, 1H), 2.03 (s, 3H) ppm; 13C NMR (150 MHz, DMSO) δ: 171.0, 164.1, 151.3, 146.6, 135.3, 123.8, 107.5, 105.5, 102.2, 73.1, 71.3, 69.1, 61.9, 51.6, 36.9, 21.3 ppm; HRMS-FAB Calcd for C17H20NOS (M + 1): 366.0988, Found: 366.1088.

(1S,2S,3R,4S,4aR,11bR)-2,3,4-trihydroxy-1-(hydroxymethyl)-1,2,3,4,4a,5-hexahydro-[1,3]dioxolo[4,5-j]phenanthridin-6(11bH)-one (167).
To a solution of acetate 240 (25 mg, 0.062 mmol) at 0 °C, in MeOH (5 mL) was added K$_2$CO$_3$ (40 mg, 0.62 mmol) and H$_2$O (1 mL). The suspension was stirred until total consumption of starting material (TLC) before being quenched with HCl (4 drops, 6N). The reaction was allowed to warm to room temperature and stir (4 h). The pH of the reaction was made basic with the addition of saturated sodium bicarbonate solution and the methanol removed under reduced pressure. The resulting aqueous phase was concentrated overnight on a freeze-dryer. The salts were triturated with MeOH (5 x 5 mL) and the MeOH washes collected and concentrated. The final product was isolated by column chromatography (methylene chloride/methanol, 5:1). Yield: 15 mg, 75%, white solid; mp >200 °C methylene chloride/methanol; $R_f$ 0.20 (methlene chloride/methanol, 5:1); $\left[\alpha\right]_D^{20}$ 90.91 (c 0.25, DMSO); IR (film) ν3361, 2916, 1646, 1608, 1503, 1460, 1385, 1361, 1252; $^1$H NMR (600 MHz, DMSO) δ: 7.34 (s, 1H), 6.97 (s, 1H), 6.66, (s, 1H), 6.09, (d, $J = 0.78$, 2H), 5.04 – 4.97, (m, 3H), 4.47 (dd $J = 6.6$ Hz, 3.8 Hz, 1H), 4.19 (s, 1H), 3.89 (q, $J = 7.86$ Hz, 1H), 3.82 (s, 1H), 3.69-3.64 (m, 1H), 3.42 (dd $J = 13.2$ Hz, 9.9 Hz, 1H), 3.39 – 3.32 (m, 1H), 3.15, (dd $J = 13.3$ Hz, 4.5 Hz, 1H), 2.41 (s, 1H) ppm; $^{13}$C NMR (150 MHz, DMSO) δ: 164.2, 151.2, 146.3, 136.3, 123.7, 107.4, 105.6, 102.1, 73.3, 71.6, 69.7, 57.8, 51.8, 44.4, 37.3 ppm; HRMS-FAB Calcd for C$_{13}$H$_{18}$NO$_7$ (M + 1): 324.1085, Found: 324.1084.
N-[(1R,2aS,4aS,5S,5aR,12bR)-5-(tert-Butyl-dimethyl-silanyloxy)-3,3-
dimethyl-1,2a,4a,5a,12b-hexahydro-phenanthro[2,3-d][1,3]dioxol-5-
ylethanyl]4-methyl-benzenesulfonamide (242).

A flame-dried 10-mL flask was charged with alkane 226 (158 mg, 0.261 mmol) and silica gel which has been activate by heating under vacuum at 120 °C overnight (1.3 g). The starting materials were suspended in 5 mL freshly distilled methylene chloride and the solvent removed under reduced pressure. The silica gel supporting the absorbed reactants was heated externally at 120 °C under nitrogen atmosphere for 24 h, after which time the silica gel was loaded onto flash silica gel column and eluted with hexanes:ethyl acetate, 8:1 – 5:1 to give 109 mg (69%) of alkane 242 as a clear and colorless oil. Rf 0.35 (hexanes:ethyl acetate, 2:1); [α]_D^{23} -46.2 (c 2.8, CHCl₃); IR (film) ν 3483, 3273, 2983, 2954, 2954, 2930, 2891, 2857, 1599, 1504, 1462, 1384, 1371, 1330 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ: 7.47 (d, J = 8.3 Hz, 2H), 7.12 (d, J = 8.1 Hz, 2H), 6.45 (s, 1H), 6.44 (s, 1H), 5.90 (d, J = 1.4 Hz, 1H), 5.82 (d, J = 1.4 Hz, 1H), 4.69 (d, J = 8.9 Hz, 1H), 4.08 (m, 3H), 3.69 (q, J = 9.5 Hz, 1H), 2.86-2.61 (m, 3H), 2.40 (s, 3H), 2.22-2.01 (m, 2H), 1.73 (m, 1H), 1.44 (s, 3H), 1.31 (s, 3H), 0.87 (s, 9H), 0.10 (s, 3H), 0.04 (s, 3H) ppm; ¹³C NMR (75 MHz, CDCl₃) δ: 146.4, 145.0, 142.2, 138.9, 129.6, 128.9,
128.8, 126.7, 110.6, 109.0, 108.6, 100.6, 79.5, 79.1, 71.1, 57.2, 41.3, 39.5, 27.9, 27.7, 25.9, 25.7, 21.6, 21.4, 18.0, -4.9, -5.0 ppm; HRMS-EI Calcd for C₃₁H₄₉NO₇Si: 601.2530; Found, 601.2543; Anal. calcd for C₃₁H₄₉NO₇Si C, 61.87; H, 7.20; found C, 61.73; H, 7.29.

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N-((1R,2S,3S,4S,4aR,11bR)-4-(tert-butyldimethylsilyloxy)-3,3-dimethyl-6-oxo-1,2,3,4,4a,5,6,11b-octahydrophenanthro[3,2-d][1,3]dioxol-1-yl)-4-methylbenzenesulfonamide (243).
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Acetonide 242 (34 mg, 0.056 mmol) was taken up in dry CH₂Cl₂ (1.5 mL) and PCC (0.49 mg, 0.226 mmol) was added. The reaction was stirred until total consumption of starting material as monitored by TLC (14 h) before being diluted with diethyl ether and filtered through a short plug of celite and concentrated.

The final product was isolated by flash column chromatography (hexanes/ethyl acetate, 4:1). Yield: 16 mg, 46 %, foamy solid; [α]²³D -55.35 (c 0.06, CHCl₃); Rf 0.29 (hexanes:ethyl acetate, 2:1); IR (film) v 3460, 3266, 2984, 2953, 2929 2897, 2857, 1671, 1654, 1615, 1504, 1468, 1371, 1330, 1280 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) δ: 7.43 (d, J = 8.1 Hz, 2H), 7.36 (s, 1H), 7.13 (d, J = 8.1 Hz, 2H), 6.60 (s, 1H), 6.01 (s, 1H), 5.94 (s, 1H), 5.22 (d, J = 9.2 Hz, 1H), 4.17-4.13 (m,
2H), 4.08 (s, 1H), 3.68 (dd, J = 8.6, 8.5 Hz, 1H), 3.15 (dd, J = 18.3, 14.3 Hz, 1H), 3.0 (dd, J = 11.9, 3.6 Hz, 1H), 2.59 (dd, J = 18.4, 4.5 Hz, 1H), 2.54 (d, J = 14.8 Hz, 1H), 2.41 (s, 3H), 1.42 (s, 3H), 1.33 (s, 3H), 0.86 (s, 9H), 0.09 (s, 3H), 0.04 (s, 3H) ppm; \(^{13}\)C NMR (150 MHz, CDCl\(_3\)) \(\delta\): 194.9, 151.6, 147.6, 142.3, 140.1, 138.8, 129.1, 127.1, 126.7, 110.4, 109.3, 106.8, 101.7, 79.2, 79.1, 69.4, 57.2, 42.1, 39.6, 36.8, 27.9, 25.9, 25.7, 25.6, 25.5, 21.5, 18.0, -5.0, -5.1 ppm; HRMS-EI Calcd C\(_{27}\)H\(_{32}\)NO\(_8\)Si: 558.1618; Found, 558.1617; Anal. calcd for C\(_{31}\)H\(_{41}\)NO\(_8\)Si: C, 60.46; H, 6.71; found C, 59.41; H, 60.63.

![chemical structure]

\(N-((1R,2S,3S,4S)-4-\{[\text{tert-butyl(dimethyl)silyl}]\text{oxy}\}-3,3\text{-dimethyl}-1,2,3,4\text{-tetrahydrophenanthro}[2,3-d][1,3]\text{dioxol-1-yl})-4\text{-methylbenzenesulfonamide (244).}

Acetonide 242 (34 mg, 0.056 mmol) was taken up in dry CH\(_2\)Cl\(_2\) (1.5 mL) and PCC (0.49 mg, 0.226 mmol) was added. The reaction was stirred until total consumption of starting material as monitored by TLC (14 h) before being diluted with diethyl ether and filtered through a short plug of celite and concentrated. The final product was isolated by flash column chromatography (hexanes/ethyl acetate, 4:1). Yield: 7.7 mg, 23 %, slight yellow oil; \([\alpha]_D^{23}\) 80.07 (c 1.25, CHCl\(_3\));
Rf 0.44 (hexanes:ethyl acetate, 2:1); IR (film) ν 3666, 3024, 2954, 2929, 2858, 1621, 1598, 1503, 1470, 1411, 1381, 1334, 1214 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(δ\) 7.69 (d, \(J = 8.2\) Hz, 2H), 7.59 (d, \(J = 8.2\) Hz, 2H), 7.7.20-7.16 (m, 3H), 7.05 (s, 1H), 6.88 (s,1H), 6.41 (d, \(J = 10.7\) Hz, 1H), 6.01 (s, 1H), 5.20 (dd, \(J = 6.9\), \(J = 2.0\) Hz, 1H), 4.86 (d, \(J = 1.9\) Hz, 1H), 4.68 (dd, \(J = 6.9\), \(J = 2.0\) Hz, 1H), 2.39 (s, 3H), 1.26 (s, 3H), 0.92 (s, 6H), 0.77 (s, 3H), 0.28 (s, 3H),0.07 (s, 3H) ppm; \(^{13}\)C NMR (150 MHz, CDCl\(_3\)) \(δ\): 148.5, 147.6, 143.2, 138.3, 132.3, 131.1, 130.5, 129.4, 129.1, 127.5, 127.2, 125.9, 108.6, 104.0, 101.2, 100.1, 77.8, 77.7, 72.6, 49.5, 25.9, 25.8, 24.6, 21.5, 18.0, -4.5, -4.8 ppm; HRMS-EI Calcd for C\(_{31}\)H\(_{39}\)NO\(_7\)SSi: 597.2217; Found, 597.2209; Anal. calcd for C\(_{31}\)H\(_{39}\)NO\(_7\)SSi: C, 62.28; H, 6.58; found C, 62.15; H, 6.64

(3′aS,4′R,5′R,6′S,7′aS,7′aS)-7′-(tert-butyldimethylsilyloxy)-N,2′,2′-trimethyl-6′-((methylamino)methyl)-4′-(4-methylphenylsulfonamido)-3′a,4′,5′,6′,7′,7′a-hexahydro-5,5′-bibenzo[\(d\)][1,3]dioxole-6-carboxamide (251).

Mesylate 250 (20 mg, 0.028 mmol) was taken up in THF (1.5 mL) and cooled externally to -78 °C. MeNH\(_2\) was bubbled through until approximately 0.5 mL of methyl amine was condensed. The vessel was sealed and heated to 50 °C until
total consumption of starting material as monitored by TLC. The reaction was cooled to 0 °C and the methylamine was allowed to slowly evaporate. The reaction was transferred to a separatory funnel and diluted with EtOAc (60 mL), washed with Na₂CO₃ (2 x 2 mL), and brine (1 mL) before being dried over Na₂SO₄, filtered, and concentrated. The final product was isolated by flash column chromatography (methylene chloride/methanol, 15:1). Yield: 7.5 mg, 41 %, clear and colorless oil; [α]²³ D -63.42 (c 0.75, CHCl₃); Rf 0.17 (methylene chloride/methanol, 15:1); IR (film) ν 3416, 2956, 2930, 2895, 2857, 1631, 1550, 1506, 1487, 1384, 1327, 1250 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) δ: 7.59 (d, J = 8.3 Hz, 2H), 7.13 (d, J = 8.3 Hz, 2H), 6.98 (s, 1H), 6.74 (s, 1H), 6.70 (s, 1H), 6.03 (d, J = 1.1 Hz, 1H), 6.01 (d, J = 1.2 Hz, 1H), 4.20-4.10 (m, 3H), 3.66 (m, 1H), 3.50 (dd, J = 11.2, J = 8.0 Hz, 1H), 2.95 (d, J = 4.8 Hz, 3H), 2.88 (dd, J = 11.2, 7.1 Hz, 2H), 2.82 (d, J = 4.9 Hz, 1H), 2.38 (s, 3H), 2.33 (d, J = 10.8 Hz, 1H), 2.29 (s, 3H), 1.49 (s, 3H), 1.27 (s, 3H), 0.91 (s, 9H), 0.16 (s, 3H), 0.12 (s, 3H) ppm; ¹³C NMR (150 MHz, CDCl₃) δ: 171.4, 149.0, 146.6, 142.1, 139.3, 132.1, 128.8, 126.9, 109.5, 108.6, 107.3, 101.7, 81.2, 77.9, 71.6, 59.2, 49.8, 41.6, 35.3, 27.4, 27.1, 26.5, 25.9, 25.9, 25.3, 23.2, 21.4, 18.1, -4.3, -5.0 ppm; HRMS-EI Calcd C₃₃H₄₉N₃O₈SSi: 675.3010; Found, 675.2009.
(3aS,3bR,10bR,12S,12aS)-12-(tert-butyldimethylsilyloxy)-2,2-dimethyl-
3b,4,10b,11,12,12a-hexahydrobis[1,3]dioxolo[4,5-c:4',5'-j]phenanthridin-
5(3aH)-one (253).

To a solution of aldehyde 166 (192 mg, 0.305 mmol) in toluene (10 mL) was
added RhCl(PPh₃)₃ (424 mg, 0.458 mmol). The reaction vessel was sealed,
lowered into a pre-heated oil bath (130 °C), and stirred until total consumption of
starting material as monitored by TLC (7.5 h). The crude reaction mixture was
filtered through a silica plug using a mixture of 2:1 hexanes/ethyl acetate as eluent
and allowed to stand overnight. The resulting yellow crystals were removed by
filtration and the crude reaction mixture was concentrated under reduced pressure
and dried under vacuum. The crude was taken up in DME (8 mL) and cooled to
-60 °C. To the acetonide solution was added a 0.5 M solution of Na/naphthalene in
DME until a green color persisted and total consumption of starting material was
observed (by TLC). The solution was stirred for 10 minutes before the reaction
was quenched with saturated aqueous ammonium chloride solution (3 mL). The
reaction was warmed to room temperature, concentrated to remove DME, and
extracted with CH₂Cl₂ (3 x 50 mL). The combined organic phase was dried over
sodium sulfate, filtered, and concentrated. The final product was isolated by
column chromatography using 3:1 mixture of hexanes/ethyl acetate as eluent.
Yield: 101 mg, 74%, clear and colorless oil; Rf 0.49 (hexanes/ethyl acetate, 1:1);
[α]D²² = 20.929 (c 1.5, CHCl₃); IR (film) ν 3360, 2952, 2929, 2894, 2857, 1670,
1615, 1505, 1482, 1459, 1384, 1345, 1270, 1256, 1243, 1219; ¹H NMR (600
MHz, CDCl₃) δ: 7.59 (s, 1H), 6.77 (s, 1H), 6.28 (s, 1H) 6.03 (d, J = 5.28, 2H),
4.42 (d, J = 2.10, 1H), 4.18, (dd, J = 8.3 Hz, 4.8 Hz, 1H), 4.11 (m, 1H), 3.43 (dd, 
J = 13.2 Hz, 8.6 Hz, 1H), 3.12 (t, J = 13.5 Hz, 1H), 2.26 (d, J = 13.6 Hz, 1H),
1.77 (t, J = 13.9 Hz, 1H), 1.46 (s, 3H), 1.41 (s, 3H), 0.91 (s, 9H), 0.15 (s, 3H),
0.14 (s, 3H); 13C NMR (150 MHz, CDCl₃) δ: 165.7, 151.4, 146.7, 136.5, 122.9,
109.9, 108.4, 104.3, 101.7, 77.7, 66.9, 57.9, 31.9, 31.6, 28.3, 26.5, 25.7, 25.6,
21.1, 17.9, -4.8, -4.9 ppm; HRMS-EI Calcd for C₂₃H₃₃N₅O₆Si (M⁺): 447.2077,
Found: 447.2083.

7-Deoxy-trans-dihydrolycoricidine (3).

To a solution of acetonide 253 (13 mg, 0.29 mmol) in MeOH (1 mL) was added a
10% solution of HCl in MeOH (10 mL). The reaction was stirred until total
consumption of starting material as monitored by TLC (3 days). The final product
was isolated by flash column chromatography (chloroform/methanol, gradient
7:1-5:1). Yield: 6.1 mg, 71%, white solid; mp > 200 °C (MeOH); Rf 0.25
(methylene chloride/methanol, 5:1); [α]_D²⁸ 28.6 (c 0.25, DMSO), lit.¹⁵⁰ [α]_D²⁵ 138
(c 0.96, DMSO); IR (KBr) ν 3559, 3488, 3450, 3429, 1671, 1471, 1263 cm⁻¹; ¹H
NMR (600 MHz, DMSO-d₆) δ: 7.30 (s, 1H), 6.95 (s, 1H), 6.94 (s, 1H), 6.08 (s,
2H), 4.99 (d, J = 3.4 Hz, 1H), 4.96 (d, J = 5.9 Hz, 1H), 4.84 (d, J = 3.2 Hz, 1H),
3.89, (br s, 1H), 3.72 (br s, 2H), 3.30 (d, J = 12.3 Hz, 1H), 2.89 (td, J = 12.4, 3.6
Hz, 1H), 2.15 (dt, J = 13.0, 3.0 Hz, 1H), 1.65 (td, J = 12.9, 2.2 Hz, 1H) ppm; ¹⁳C
NMR (150 MHz, DMSO-\textit{d}6) \( \delta \): 164.7, 151.1, 146.4, 138.5, 123.7, 107.4, 104.8, 102.0, 72.1, 70.1, 69.1, 55.6, 34.7, 28.8 ppm; HRMS-EI Calcd for C\textsubscript{14}H\textsubscript{16}N\textsubscript{0}6 (M+1): 294.0978, Found: 294.1011.

(3\text{a}S,3\text{b}R,10\text{b}R,11\text{R},12\text{S},12\text{a}S)-11-Acetyl-12-(tert-butyl-dimethyl-silanyl)oxy)-2,2-dimethyl-4-(toluene-4-sulfonyl)-3\text{b},4,10\text{b},11,12,12\text{a}-hexahydro-3\text{a}H-1,3,7,9-tetraoxa-4-aza-dicyclopenta[\textit{a},\textit{h}]phenanthren-5-one (257)

To a solution of aldehyde 166 (200 mg, 0.318 mmol) in Et\textsubscript{2}O (3 mL) at 0°C was added MeMgI (423 mg, 0.382 mmol) dropwise. The reaction stirred until total consumption of starting material (2 h) as monitored by TLC. The reaction mixture was quenched with NH\textsubscript{4}Cl (3 mL). The solvent was removed under reduced pressure and the aqueous phase was extracted with EtOAc (3 x 20 mL). The organic phase was combined, dried over sodium sulfate, filtered, and concentrated. The crude mixture was concentrated and taken up in dry CH\textsubscript{2}Cl\textsubscript{2} (5 mL). A stirring bar was added and the reaction was stirred while Collins reagent (416 mg, 1.616 mmol) was added. The reaction was stirred until total consumption of starting material (15 minutes) as monitored by TLC. The reaction mixture was diluted with hexanes/ethyl acetate 2:1 (15 mL) and filtered through a silica plug. The combined organic layer was washed with 0.25 M HCl (3 mL),
NaHCO₃ (3 mL), dried over sodium sulfate, filtered and concentrated. The final product was isolated by column chromatography (hexane/ethyl acetate, 4:1).

Yield: 76 mg, 44%, clear and colorless oil; Rf 0.26 (hexanes:ethyl acetate, 2:1); [α]D² - 56.87 (c 4.0, CHCl₃); IR (film) v 3002, 2975, 2861, 2373, 1718, 1700, 1652, 1506, 1486, 1361, 1263, 1222, 1171, 1097, 1036 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ: 8.24 (d, J = 7.2 Hz, 2H), 7.58 (s, 1H), 7.35 (d, J = 8.4 Hz, 2H), 6.50 (s, 1H), 6.02 (d, J = 3.3 Hz, 2H), 5.82 (dd, J = 8.1 Hz, J = 5.4 Hz, 1H), 4.91 (m 2H), 4.29 (d, J = 2.7 Hz, 1H), 3.80 (dd J = 12.3, J = 3.9 Hz, 1H), 3.47 (m 1H), 2.48 (s, 3H), 2.22 (s, 3H), 1.44 (s, 3H), 1.34 (s, 3H), 1.01 (s, 9H), 0.31 (s, 6H) ppm; ¹³C NMR (150 MHz, CDCl₃) δ: 202.5, 166.4, 152.7, 146.6, 143.6, 139.0, 138.5, 128.9, 128.7, 123.2, 110.1, 109.2, 103.3, 101.9, 73.0, 67.2, 65.2, 56.8, 36.1, 29.3, 27.3, 26.9, 25.7, 21.6, 18.0, -4.6, -4.9 ppm; HRMS-EI Calcd for C₃₂H₄₅NO₉SSi (M⁺-15): 628.2036, Found: 628.2036.

(1R,4S,5S,5aR,12bR,13S)-13-[[tert-butyl(dimethyl)silyl]oxy]-5-hydroxy-6-[(4-methylphenyl)sulfonyl]-1,4,5,5a,6,12b-hexahydro-1,4-methano[1,3]dioxolo[4,5-g]oxepino[4,5-c]isoquinoline-2,7-dione (261).

To a solution of ketone 257 (13 mg, 0.021mmol) in CH₂Cl₂ (0.5 mL) in a conical vial was added a single crystal of p-toluenesulfonic acid monohydrate followed
by m-CPBA (7 mg, 0.31 mmol). The vial was sealed and stirred overnight (10 h).
The reaction was diluted with CH₂Cl₂ (20 mL), washed with saturated NaHSO₃ (2 mL), and NaHCO₃ (2 x 1.5 mL). The organic phase was dried over sodium sulfate, filtered, and concentrated. The final product was isolated by column chromatography using a gradient of 3:1 to 1:1 mixture of hexanes: ethyl acetate as eluent. Yield: 4 mg, 33%, clear oil; Rf 0.16 (hexanes/ethyl acetate, 2:1); IR (film) v3507, 2935, 2929, 1788, 1685, 1617, 1505, 1485, 1360, 1275; ¹H NMR (600 MHz, CDCl₃) δ: 7.99 (d, J = 7.7 Hz, 2H), 7.42 (s, 1H), 7.33 (d, J = 7.8 Hz, 2H), 6.96 (s, 1H), 6.06 (s, 2H), 5.45 (m, 1H), 4.81 (d, J = 5.4, 1H), 4.74 (t, J = 5.1 Hz, 1H), 4.12 (t, J = 10.2 Hz, 1H), 3.83 (d, J = 11.7 Hz, 1H), 3.2 (d, J = 6.7 Hz, 1H), 3.27 (s, 1H), 2.45 (s, 3H), 1.02 (s, 9H), 0.27 (s, 3H), 0.23 (s, 3H); ¹³C NMR (150 MHz, CDCl₃) δ: 171.2, 164.7, 152.7, 147.7, 144.4, 138.5, 135.0, 129.5, 127.7, 122.2, 109.6, 104.9, 102.3, 96.1, 82.8, 69.3, 69.1, 64.0, 42.9, 36.4, 25.7, 21.7, 17.9, 1.0, -0.8; HRMS-EI Calcd for C₉₇H₉₀NO₉Si (M⁺-15): 572.1368, Found: 572.1389.

[(3aS,3bR,10bR,11S,12S,12aS)-2,2-dimethyl-4-[(4-methylphenyl)sulfonyl]-5-oxo-12-(sulfooxy)-3a,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5-c;4',5'-j]phenanthridin-11-yl]methyl acetate (272).
To a stirring solution of 271 (73 mg, 0.131 mmol) in dry CH2Cl2 (4 mL) at 0 °C was added triethylamine (0.55 μL, 0.394 mmol) followed by methanesulfonyl chloride (0.02 mL, 0.263 mmol). The reaction was stirred at 0 °C until total consumption of starting material as indicated by TLC (20 minutes). The reaction was diluted with Et2O (100 mL), washed sequentially with H2O (10 mL), saturated sodium bicarbonate (5 mL), H2O (3 x 1 mL), and brine (5 mL). The ether layer was dried over magnesium sulfate, filter, and concentrated to provide mesylate 277 which was used without further purification. Yield: 80 mg, 96%, clear oil; RF 0.27 (hexanes/ethyl acetate, 1:1); [α]D22 5.42 (c 0.75, CHCl3); IR (film) ν3025, 2988, 2929, 1741, 1691, 1618, 1597, 1505, 1485, 1435, 1358, 1255; 1H NMR (600 MHz, CDCl3) δ: 8.22 (d, J = 8.3 Hz, 2H), 7.50 (s, 1H), 7.33 (d, J = 8.2 Hz, 2H), 6.89 (s, 1H), 6.08 (dd, J = 5.1 Hz, J = 1.1 Hz, 2H), 5.58 (dd, J = 7.7 Hz, J = 5.9 Hz, 1H), 5.36 (d, J = 3.5, 1H), 4.52 (t, J = 4.9 Hz, 1H), 4.15 (dd, J = 10.9 Hz, J = 10.9 Hz, 1H), 4.12 (dd, J = 13.0 Hz, J = 7.9 Hz, 1H), 4.00 (dd, J = 11.3 Hz, 3.8 Hz, 1H), 3.70 (dd, J = 12.9, 4.8, 1H), 3.21 (s, 3H), 3.11 (dd, J = 3.7, 3.7, 1H), 2.45 (s, 3H), 2.08 (s, 3H), 1.46 (s, 3H), 1.42 (s, 3H); 13C NMR (150 MHz, CDCl3) δ:170.78, 165.38, 153.36, 147.69, 144.23, 138.20, 133.86, 128.93, 128.91, 122.92, 110.06, 108.88, 105.20, 102.38, 75.85, 75.69, 73.89, 62.32, 60.30, 40.93, 37.08, 31.55, 27.96, 25.89, 21.66, 20.78 ; HRMS-EI Calcd for C27H28N012S2(M+15): 622.1053, Found: 622.1057
(3S,3bR,10bR,12S,12aR)-12-Hydroxy-4-(4-methoxy-benzyl)-2,2-dimethyl-3b,4,10b,11,12,12a-hexahydro-3aH-1,3,7,9-tetraoxa-4-aza-dicyclopenta[a,h]phenanthren-5-one (284).

To a solution of acetonide 253 (21 mg, 0.047 mmol) in DMF (0.24 mL) at 0 °C was added NaH (spatula tip, 60 % dispersion in mineral oil). The reaction was stirred at 0 °C for 10 min and then at rt for 20 min. The reaction was cooled in ice again before p-methoxy benzylbromide (11 µL, 0.07 mmol) was added and the reaction was allowed to warm to rt over 2 h. The reaction was taken up in Et₂O (5 mL) and quenched with H₂O (2 mL), transferred to a separatory funnel and diluted further with Et₂O (50 mL). The ether layer was washed with H₂O (6 x 0.5 mL), brine (1 x 1 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The crude residue was taken up in THF (1 mL) and cooled to 0 °C. TBAF (56 µL, 0.056 mmol, 1 M in THF) was added and the reaction stirred for 20 minutes. Silica (0.2 g) was added and the reaction concentrated to dryness. The final product was isolated by flash column chromatography (hexanes : ethyl acetate, gradient 2:1-1:1). Yield: 13.6 mg, 64%, slight yellow oil; Rf 0.41 (hexanes:ethyl acetate, 2:1); [α]_D^22 + 40.90 (c 0.50, CHCl₃); IR (film) ν IR (film) ν 3550, 2920, 1642, 1516, 1453 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) δ: 7.63 (s, 1H), 7.24 (d, J = 8.6 Hz, 2H), 6.82 (d, J = 8.6 Hz, 2H), 6.75 (s, 1H), 6.03, (s, 2H), 5.21 (d, J = 15.8 Hz, 1H), 4.90 (d, J = 15.5 Hz, 1H), 180
4.39 (dd, $J = 7.6, 6.2$ Hz, 1H), 4.23 (m, 1H), 4.12 (t, $J = 5.61$ Hz, 1H), 3.78 (s, 3H), 3.71 (dd, $J = 13.4, 7.9$ Hz, 1H), 3.19 (td, $J = 12.4, 5.0$ Hz, 1H), 2.36-2.28 (m, 2H), 2.01 (qd, $J = 13.6, 11.9$, 4.9 Hz, 1H), 1.387 (s, 3H), 1.34 (s, 3H) ppm; $^{13}$C NMR (150 MHz, CDCl$_3$) δ: 165.2, 158.4, 151.1, 146.8, 135.4, 131.8, 128.2, 123.1, 113.7, 109.5, 108.6, 104.2, 101.6, 78.1, 75.9, 66.6, 61.6, 61.9, 55.3, 46.3, 32.3, 21.0, 28.0, 26.1 ppm; HRMS-EI Calcd for C$_{25}$H$_{23}$NO$_7$ (M+): 453.1788, Found: 453.1787.

(3aS,3bR,10bR,12aR)-4-(4-methoxybenzyl)-2,2-dimethyl-3a,3b,4,7,8,9,10b,12a-octahydro-5H-cyclopenta[j][1,3]dioxolo[4,5-c]phenanthridin-5-one (285).$^{49k}$

To a solution of alcohol 284 (15 mg, 0.033 mmol) in THF (1 mL) at 0 °C was added a spatula tip of NaH (60 % dispersion in mineral oil). The reaction was stirred at 0 °C for 10 min before being allowed to warm to rt and stir for 45 min. The reaction was cooled externally to 0 °C again before CS$_2$ (12 μL, 0.198 mmol) was added. After stirring for 1 h at 0 °C MeI (25 μL, 0.397 mmol) was added and the reaction was allowed to warm to rt slowly over 5 h before being quenched with sat. NH$_4$Cl (2 mL). The reaction was concentrated to remove THF, the aq
residue was extracted with EtOAc (3 x 30 mL), and the organic phases combined and dried over sodium sulfate. Following filtration, the EtOAc was removed under reduced pressure, the residue taken up in o-zylene (2 mL) and heated at reflux for 21 h. The reaction was concentrated under reduced pressure at 50 °C and the crude residue loaded onto silica (100 mg). The final product was isolated by flash column chromatography (hexanes : ethyl acetate, 4:1). Yield: 5 mg, 35%, white solid; mp 172-174 °C (ethyl acetate/hexanes), lit:49k 171-173 °C; Rf 0.51 (hexanes:ethyl acetate, 1:1); [α]D + 37.48 (c 0.20, CHCl3); IR (film) ν 2920, 1647, 1511, 1451 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) δ: 7.67 (s, 1H), 7.27 (d, J = 8.6 Hz, 2H), 6.92 (s, 1H), 6.84 (d, J = 8.6 Hz, 2H), 6.34 (d, J = 10.2 Hz, 1H), 6.11 (dt, J = 10.0 Hz, 3.1 Hz, 1H), 6.15 (s, 2H), 5.43 (d, J = 15.6 Hz, 1H), 4.98 (d, J = 15.5 Hz, 1H), 4.64-4.61 (m, 1H), 4.39 (dd J = 9.3, 7.1 Hz, 1H), 3.80 (s, 3H), 3.66 (dd, J = 11.9, 9.3 Hz, 1H), 3.49 (d, J = 12.1, 1H), 1.38 (s, 3H), 1.35 (s, 3H) ppm; ¹³C NMR (150 MHz, CDCl₃) δ: 165.5, 158.3, 151.1, 146.9, 133.6, 132.6, 128.2, 127.9, 126.1, 123.7, 113.7, 109.3, 109.2, 103.8, 101.7, 74.8, 71.9, 60.8, 55.3, 45.8, 38.6, 27.6, 25.3 ppm; HRMS-EI Calcd for C₂₉H₂₄N₂O₆ (M⁺): 435.1682, Found: 435.1683

Ethyl 6-oxo-3a,4,6,6a-tetrahydro-1H-furo[3,4-c]pyrazole-3-carboxylate (302).
To a solution of crotonolactone 301 (1.0 g, 12 mmol) in dioxane (7 mL) was added ethyl diazoacetate (1.5 mL, 14 mmol). The reaction mixture was heated at 95 °C for 12 hours before being allowed to cool to rt and concentrated to a thick oil. Trituration with CHCl₃ provided a crude yellow solid. The final product was purified by recrystallization from Et₂O. Yield: 0.796 g, 48%, off white solid, mp 132-134 °C (diethyl ether); Rf 0.26 (hexanes:ethyl acetate, 1:1); ¹H NMR (300 MHz, CDCl₃) δ: 6.86 (bs, 1H), 4.77-4.60 (m, 3H), 4.37 (q, J = 8.6 Hz, 2H), 4.22 (ddd, J = 10.8 Hz, 6.8 Hz, 1.9 Hz, 1.9 Hz, 1H), 1.39 (t, J = 7.14 Hz, 3H) ppm; ¹³C NMR (75 MHz, CDCl₃) δ: 175.2, 161.5, 141.7, 70.7, 61.8, 61.2, 45.7, 45.2 ppm; HRMS-EI Calcd for C₁₀H₁₄O₄ (M⁺): 198.0641, Found: 198.0638.

Ethyl 2-(5-oxo-2,5-dihydrofuran-3-yl)acetate (303)

Pyrazole 302 (2.8 g, 14.13 mmol) suspended in toluene (75 mL) in a Schlenk tube equipet with a teflon stopper and a magnetic stirring bar. The tube was sealed and heated to 150 °C for 12 h. The crude reaction mixture was filtered through a silica plug and eluted with a mixture of hexane:ethyl acetate (2:1). Yield: 2.135 g, 89 %, clear and colorless oil; Rf 0.41 (hexanes:ethyl acetate, 1:1); IR (film) ν 3626, 3515, 3478, 3119, 2984, 2939, 1919, 1735, 1641, 1448, 1372, 1160 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ: 6.06 (m, 1H), 4.91 (m, 2H), 4.21 (q, J = 7.1 Hz, 2H), 3.52 (s, 2H), 1.29 (t, J = 7.13 Hz, 3H) ppm; ¹³C NMR (75 MHz, CDCl₃) δ: 173.1, 167.9, 161.1, 118.8, 73.0, 61.8, 34.4, 14.1 ppm; HRMS-EI Calcd for C₈H₁₀N₂O₄ (M⁺): 198.0641, Found: 198.0638.
Ethyl 2-(5-(tert-butyldimethylsilyloxy)furan-3-yl)acetate (305)

Lactone 303 (980 mg, 5.79 mmol) was taken up in CH$_2$Cl$_2$ (20 mL) and cooled externally in an ice bath while NEt$_3$ (1.21 mL, 8.69 mmol) was added. The reaction was stirred for 15 min before TBSOTf (1.66 mL, 7.24 mmol) was added dropwise over 10 min. The reaction was allowed to warm to room temperature over 2 hours before being quenched with H$_2$O (20 mL). The two layers were separated and the aqueous phase was extracted with CH$_2$Cl$_2$ (3 x 60 mL). The organic phase was washed with a 10% solution of citric acid (2 x 10 mL), H$_2$O (10 mL), dried over sodium sulfate and filtered/concentrated. The final product was isolated by flash column chromatography (hexanes/ethyl acetate, 4:1). Yield: 1.103 g, 67%, slight yellow oil; $R_f$ 0.7 (hexanes:ethyl acetate, 1:1); IR (film) $\tilde{\nu}$ 3127, 2932, 2860, 2744, 2715, 1743, 1625, 1573, 1524, 1472, 1392, 1366, 1287 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 6.77 (d, $J = 0.92$, 1H), 5.15 (d, $J = 0.92$, 1H), 4.16 (q, $J = 7.1$ Hz, 2H), 3.45 (s, 2H), 1.27 (t, $J = 7.14$ Hz, 3H), 0.97 (s, 9H), 0.24 (s, 6H) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$: 171.2, 156.8, 129.6, 118.7, 85.5, 60.7, 31.8, 25.4, 18.0, 14.2, -4.9 ppm; HRMS-EI Calcd for C$_{14}$H$_{24}$O$_4$Si (M+): 284.1444, Found: 284.1440; Anal. calcd C$_{14}$H$_{24}$O$_4$Si C, 59.12; H, 8.51; found C, 58.61; H, 8.37.
3-Methoxy-6-((trimethylsilyl)ethynyl)pyridazine (296).

To a solution of pyridazine 7 (1 g, 4 mmol) in THF (10 mL) was added triethylamine (3.55 mL, 25.53 mL). The reaction mixture was cooled externally in an ice bath and trimethylsilylacetylene (0.662 mL, 4.68 mmol) added, followed by CI (41 mg, 0.21 mmol) and PdCl₂(Ph₃)₂ (150 mg, 0.213 mmol). The reaction mixture was maintained at 0 °C for 1 hour then allowed to warm to room temperature and stir for 4 hours. The reaction was concentrated to dryness, taken up in CH₂Cl₂, and filtered through a silica plug (CH₂Cl₂:Et₃N 400:1). Silica (4g) was added and the filtrate was concentrated to dryness. The final product was isolated by flash column chromatography (hexanes : ethyl acetate, 4:1). Yield: 0.737 g, 84%, slight orange oil; RF 0.55 (hexanes:ethyl acetate, 2:1); IR (film) ν 3081, 3016, 2935, 2898, 2877, 2634, 2616, 2574, 2498, 2167, 1589, 1538, 1461, 1403, 1328, 1290, 1251, 1164, 1010 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ: 7.45 (d, J = 9.1 Hz, 1H), 6.92 (d, J = 9.3 Hz, 1H), 4.16 (s, 3H), 0.30 (s, 9H) ppm; ¹³C NMR (75 MHz, CDCl₃) δ: 163.4, 143.4, 132.4, 116.4, 100.6, 98.2, 55.0, -0.4 ppm; HRMS-EI Calcd for C₁₀H₁₄N₂OSi (M⁺): 206.0875, Found: 206.0875; Anal. calcd C₁₀H₁₄N₂OSi C, 58.21; H, 6.84; found C, 57.72; H, 6.67.
3-Ethynyl-6-methoxypyridazine (307).

To a solution of pyridazine 296 (0.262 g, 1.27 mmol) in THF (10 mL) was added TBAF (1.9 mL, 1.905 mmol). The reaction was immediately quenched with sat. NH₄Cl (5 mL) and concentrated to remove THF. The crude mixture was extracted with EtOAc (3 x 20 mL) and the organic extracts combined, washed with brine (5 mL), dried over magnesium sulfate, filtered, and concentrated. The final product was isolated by flash column chromatography (hexanes : ethyl acetate, 4:1). Yield: 0.118 g, 69%, slight yellow crystals; mp 51-53 °C (ethyl acetate/hexanes); Rf 0.27 (hexanes:ethyl acetate, 2:1); IR (film) ν 3272, 3060, 2991, 2950, 1587, 1467, 1442, 1403, 1328 cm⁻¹ ¹H NMR (300 MHz, CDCl₃) δ: 7.47 (d, J = 9.1 Hz, 1H), 6.92 (d, J = 9.1 Hz, 1H), 4.15 (s, 3H), 3.31 (s, 1H) ppm; ¹³C NMR (75 MHz, CDCl₃) δ: 163.7, 142.6, 132.5, 116.6, 80.2, 79.9, 55.1 ppm; HRMS-EI Calcd for C₇H₆N₂O (M⁺): 134.0480, Found: 134.0482; Anal. calcd. C₇H₆N₂O C, 62.68; H, 4.51; found C, 62.73; H, 4.40.

3-Chloro-6-methoxypyridazine (313)
To a solution of 3,6-dichloropyridazine (8) (1.5 g, 10.07 mmol) in MeOH (100 mL) was added K$_2$CO$_3$ (1.67 g, 12.08 mmol). The resulting suspension was brought to reflux and heated for 11 h. The reaction was allowed to cool to rt before being concentrated to a thick slurry which was quickly added to H$_2$O (40 mL). The resulting white precipitate was filtered and washed with ice cold H$_2$O (10 mL). Yield: 0.598 g, 41%, white solid; mp 88-90 °C (methanol/water); $R_f$ 0.29 (hexanes:ethyl acetate, 2:1); IR (film) v 3272, cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) δ: 7.38 (d, $J = 9.1$ Hz, 1H), 6.97 (d, $J = 9.1$ Hz, 1H), 4.13 (s, 3H; BC NMR (75 MHz, CDCl$_3$) δ: 1.64, 4.4, 151.1, 130.7, 120.0, 55.2 ppm; HRMS-EI Calcd for C$_5$H$_5$CIN$_2$O (M+): 144.0090, Found: 144.0092; Anal. calcd C, 41.54; H, 3.49; found C, 41.49; H, 3.39.

1,2-bis(6-Methoxypyridazin-3-yl)ethyne (6)

To a solution of Pd(Ac)$_2$ (119 mg, 0.53 mmol), PPh$_3$ (280 mg, 1.06 mmol), and CuI (203 mg, 1.06 mmol) in a mixture of acetonitril:water (3:1, 50 mL) was added iodopyridazine (7) (5 g, 21.28 mmol) followed by NEt$_3$ (8.87 mL, 63.82 mmol). The reaction was flushed with acetylene gas using a balloon. The reaction was stirred under an atmosphere of acetylene for 10 h before being concentrated and dried under vacuum for 6h. The black residue was taken up in CH$_2$Cl$_2$ (300 mL), washed with H$_2$O (4 x 30 mL), dried over sodium sulfate, and filtered/concentrated. The final product was isolated by recrystallizations from chloroform:ether. Yield: 2.01 g, 78%, light brown solid; decomposition point 220
°C (chloroform:ether); Rf 0.60 (methylene chloride:methanol, 10:1); IR (film) ν 3058, 1589, 1543, 1472, 1422, 1350, 1291 cm\(^{-1}\). \(^1\)H NMR (300 MHz, CDCl\(_3\) δ: 7.64 (d, \(J = 9.2\) Hz, 1H), 7.01 (d, \(J = 9.2\) Hz, 1H), 4.21 (s, 3H) ppm; \(^{13}\)C NMR (75 MHz, CDCl\(_3\) δ: 163.8, 142.8, 132.6, 116.7, 87.9, 55.2 ppm; HRMS-EI Calcd for C\(_{12}\)H\(_{10}\)N\(_2\)O\(_2\) (M+): 242.0803, Found: 242.0807; Anal. calcd C, 59.50; H, 4.16; found C, 59.32; H, 4.16.

1,2-bis(6-methoxypyridazin-3-yl)-N-methylethanamine (309).

Pyridazine 6 (0.5 g, 2.06 mmol) in a Schlenk tube was suspended in toluene (20 mL) and cooled externally to \(-78^\circ\)C. Freshly generated methylamine was condensed into the tube by bubbling through the cold solution. Approximately 5 mL of methyl amine was added in this way. Ind\(_2\)TiMe\(_2\) was added to the tube before it was sealed and allowed to warm to room temperature. The reaction was placed in a preheated oil bath at 110 °C and stirred for 4.5 h before being removed from heat and being allowed to cool to room temperature overnight. The reaction was cooled externally to \(-10^\circ\)C and argon was bubbled through until methyl amine was no longer detected by Alkacid paper. MeOH (10 mL) was added followed by ZnCl\(_2\) (0.419 g, 3.09 mmol) and NaBH\(_3\)CN (127 mg, 2.06 mmol). After 1 h another portion of and NaBH\(_3\)CN (120 mg) was added and the reaction was allowed to warm to room temperature over 10 h. The reaction cooled in an
ice bath and quenched with the slow addition of sat. Na$_2$CO$_3$ solution (10 mL). The reaction mixture was diluted with CH$_2$Cl$_2$ (200 mL) and separate. The aqueous phase was extracted with CH$_2$Cl$_2$ (3 x 100 mL). The organic phase was dried over Na$_2$SO$_4$ and treated with deactivate charcoal before being filtered and concentrated. The final product was isolated by flash column chromatography (methylene chloride:methanol, 10:1). Yield: 0.385 g, 68%, off white solid; mp 125-127 °C (chloroform/ether); $R_f$ 0.11 (methylene chloride:methanol, 10:1); IR (film) ν 3308, 3063, 2952, 2925, 2855, 2797, 1724, 1598, 1553, 1468, 1415, 1306 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 7.51 (d, $J$ = 9.0 Hz, 1H), 7.22 (d, $J$ = 9.0 Hz, 1H), 6.94 (d, $J$ = 9.0 Hz, 1H), 6.88 (d, $J$ = 9.0 Hz, 1H), 4.34 (t, $J$ = 6.9 Hz, 1H), 4.12 (s, 3H), 4.10 (s, 3H), 3.34 (d, $J$ = 4.3 Hz, 1H), 3.32 (d, $J$ = 2.8 Hz, 1H), 2.35 (s, 3H) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$: 164.7, 164.1, 159.6, 156.1, 130.4, 128.9, 117.7, 117.6, 63.5, 54.7, 54.6, 41.2, 34.3 ppm; HRMS-EI Calcd for C$_{13}$H$_{17}$N$_5$O$_2$ (M$^+$): 275.1382, Found: 275.1382; Anal. calcd C$_{13}$H$_{17}$N$_5$O$_2$ C, 56.71; H, 6.22; found C, 56.66; H, 6.15.
VI. Selected Spectra
Br~OH

U OH 161

in acetone d₆

191
197 in acetone $d_6$
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VIII. Vita

Jonathan Collins was born in Morgantown, West Virginia, USA on November 24, 1982 as one of three triplets. He and his two siblings, Steven and Jennifer, were raised by their parents Tim and Brenda. He attended Parkersburg High School in Parkersburg, WV before moving on to university studies at Allegheny College in Meadville, Pennsylvania. While at Allegheny he studied under the supervision of Professor Phillip Persichini III. After graduating with honours in 2005 he moved to St. Catharines, Ontario to begin his graduate studies under the tutelage of Professor Tomas Hudlicky at Brock University. On May 1, 2008 he was married to Dorothy Uyenaka in Jordan, Ontario. He is presently working towards completion of his PhD in chemistry. His research interests include the development of biotransformation and their application to organic synthesis and the total synthesis of natural products.