

Chemoenzymatic Synthesis of Amaryllidaceae Alkaloids and Their C-1 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 (-)-condurol 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.

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LIST OF ABBREVIATIONS

Ac	Acetyl
AIBN	2,2'-Azob(isobutyronitrile)
Bn	Benzyl
Bz	Benzoyl
CDI	Carbonyl diimidazole
COSY	(proton) Correlated spectroscopy
DBU	1,8-Diazobicyclo[5.4.0]undec-7-ene
DCC	dicyclohexylcarbodiimide
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DIAD	Diisopropyl azodicarboxylate
DMAP	4-(Dimethylamino)pyridine
DME	1,2-Dimethoxyethane
DMF	<i>N,N</i> -Dimethylformamide
DMP	2,2-Dimethoxypropane
DMS	Dimethylsulfide
DMSO	Dimethylsulfoxide
HRMS	High resolution mass spectrometry
IBX	2-Iodoxybenzoic acid
IMDA	Intramolecular Diels-Alder (reaction)
Imid	Imidazole
IR	Infrared
KHMDS	Potassium hexamethyldisilazide
LAH	Lithium aluminum hydride
<i>m</i> -CPBA	<i>m</i> -Chloroperoxybenzoic acid
MOM	methoxymethyl
mp	melting point
Ms	mesyl (methanesulfonyl)
NADPH	nicotinamide adenine dinucleotide phosphate
NMO	<i>N</i> -methylmorpholine- <i>N</i> -oxide
NMR	Nuclear magnetic resonance (spectroscopy)

nOe	Nuclear Overhauser effect
PAD	Potassium azodicarboxylate
PCC	Pyridinium chlorochromate
Py	Pyridine
SMEAH	bis(2-methoxyethoxy)aluminum hydride
TBAF	Tetrabutylammonium fluoride
TBS	t-Butyldimethylsilyl
Tf	Triflyl (trifluoromethanesulfonyl)
TDO	Toluene dioxygenase
TEA	Triethylamine
THF	Tetrahydrofuran
THS	Thexyl (dimethyl-(2,3-dimethyl-2-butyl)silyl)
TLC	Thin layer chromatography
TMS	Trimethylsilyl
Ts	Tosyl (<i>p</i> -toluenesulfonyl)

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).

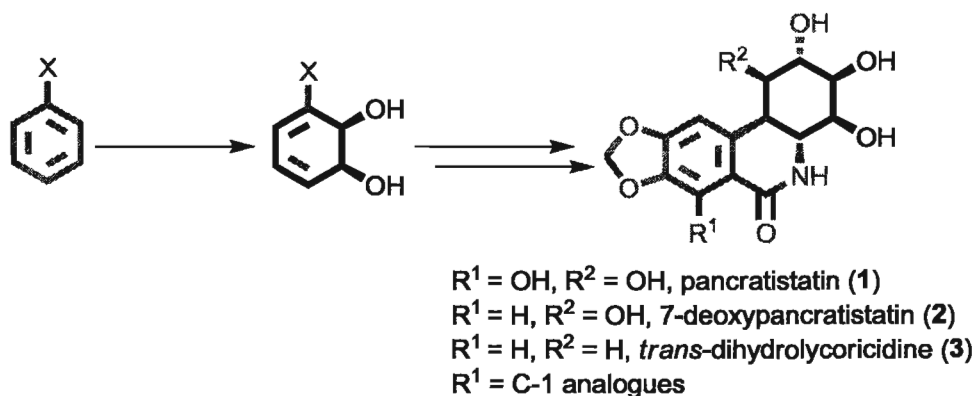


Figure 1. General strategy for synthesis of *Amaryllidaceae* constituents.

The biooxidation is high yielding and allows for the subsequent construction of the isocarbostryl 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.¹ 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 Boger² and Bodwell³ 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.

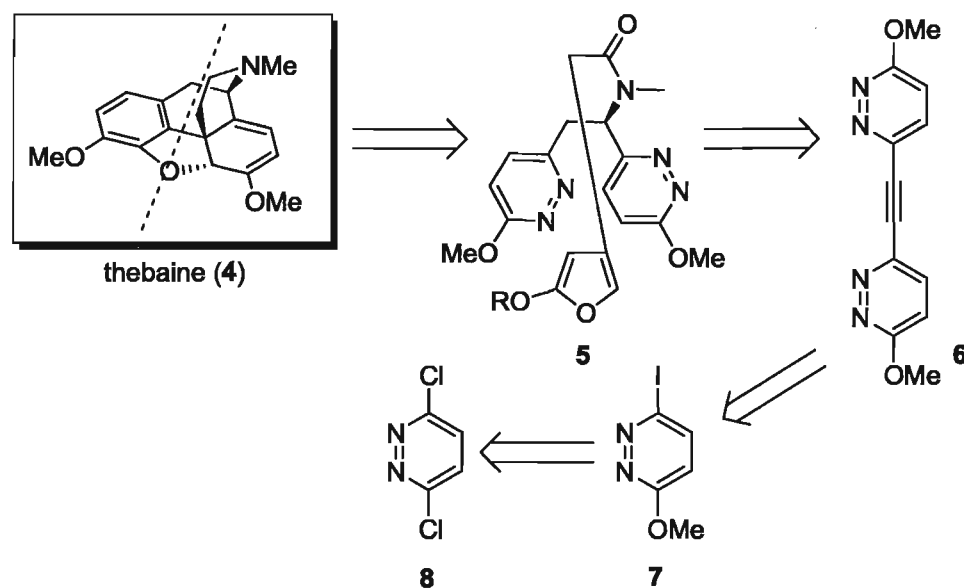


Figure 2. Retrosynthetic analysis of thebaine.

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.⁴ 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.⁵ 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.⁶ Five years later a report by Söhngen described a bacterium that tolerated various concentrations of benzene.⁷ The first isolated product of these fermentations was shown to be catechol.⁸ 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.⁹ 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-dihydronaphthalene-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.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 dioxobicyclic **10** (Figure 3).

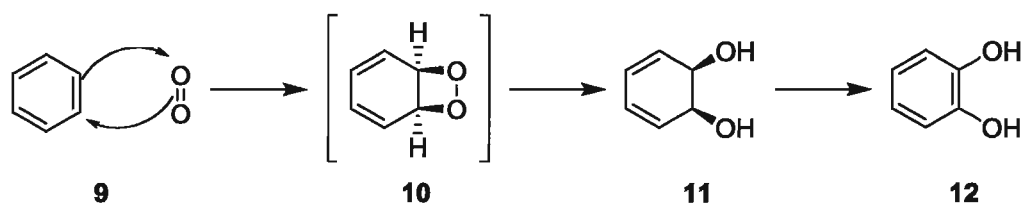


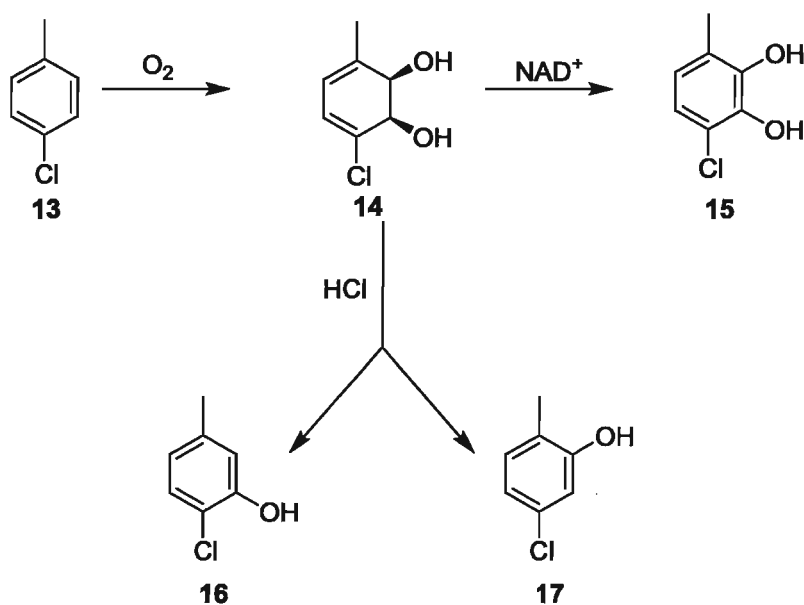
Figure 3. Proposed pathway for catechol formation from benzene.

In an effort to provide stronger evidence for intermediate **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*.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**

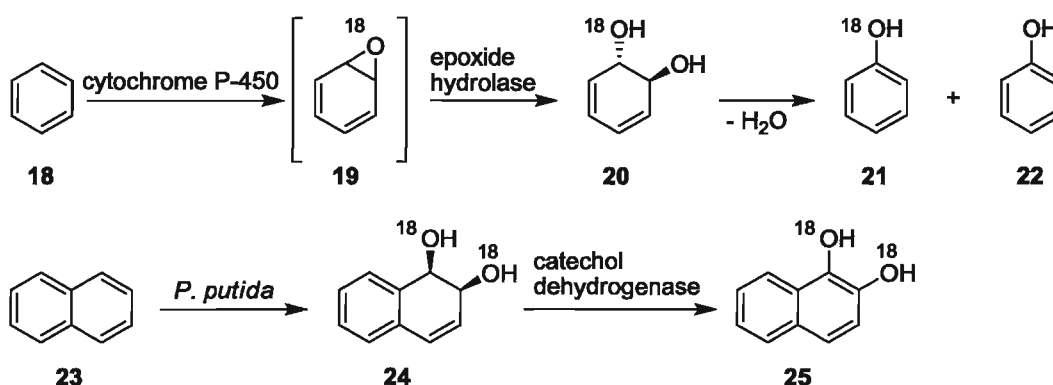
and at that time unknown 2,3-dihydroarenediol **14**. Treatment of diol **14** with 2 N HCl gave rapid conversion to phenols **16** and **17**. This observation combined with a variety of spectroscopic methods led Gibson to propose the correct structure of 4-chloro-2,3-dihydroxy-1-methylcyclohexa-4,6-diene (**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



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 $PGE_{2\alpha}$.¹⁴

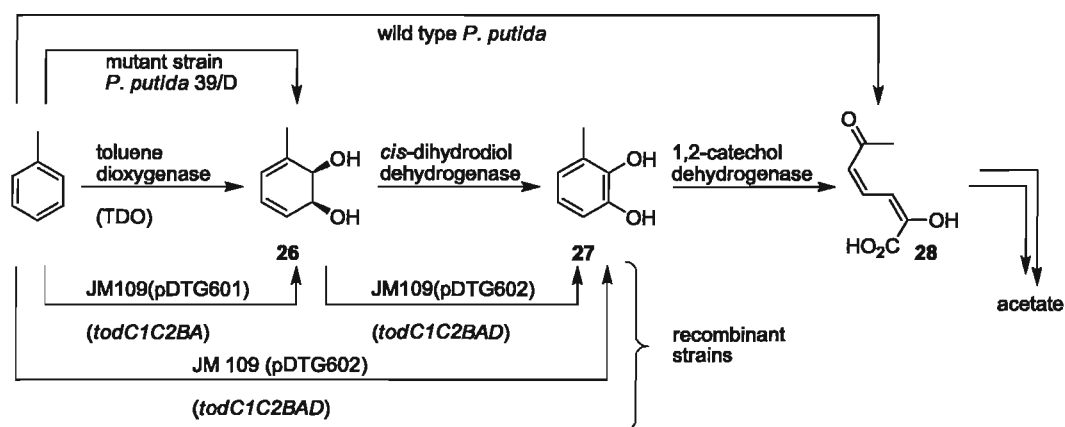
The diverse pathways by which oxo-reductase enzymes process aromatic substrates have been a subject of study since the mid 1930s. The first dihydrodiol derived from aromatics to be isolated was *trans*-1,2-dihydroxy-1,2-dihydroanthracene, the product of cytochrome P-450 epoxidation of anthracene.¹⁵ In 1962 Boyland and Booth demonstrated that only one atom of ¹⁸O₂ was incorporated in the dihydroxylation of benzene (**18**), (Scheme 2).¹⁶ In a similar experiment, Gibson demonstrated that both atoms of ¹⁸O₂ are incorporated in the *cis*-dihydroxylation of naphthalene (**23**).¹⁷



Scheme 2. Isotope labeling experiments in the oxidation of arenes.

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.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.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.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^{17,19,20} and despite reporting of the X-ray crystal structure of naphthalene dioxygenase,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.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 *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, β -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 *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.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).²⁴

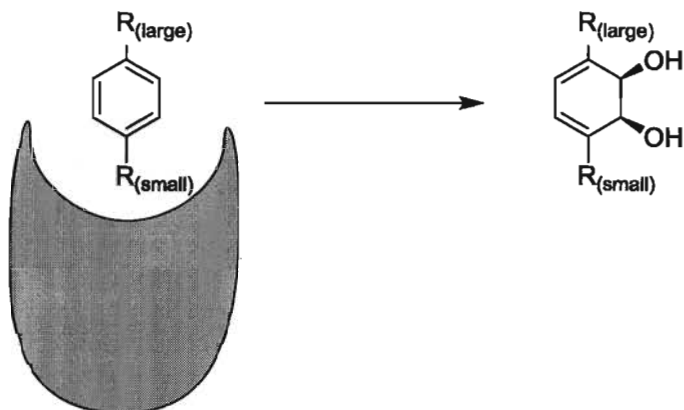


Figure 4. Model for regio- and stereoselectivity of the oxidation of single ring aromatics.

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 1983.²⁵ 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 (\pm)-pinitol (**44**).²⁶ 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.

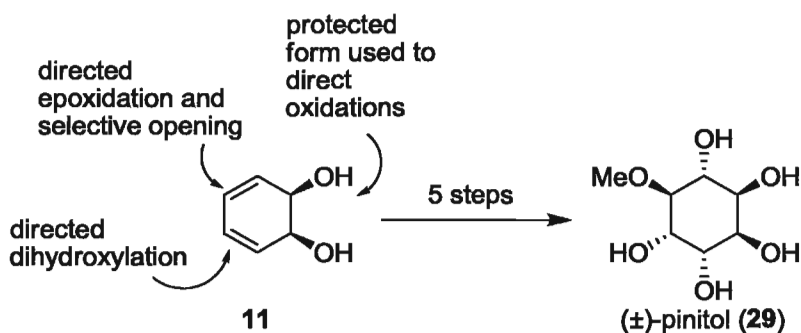


Figure 5. Ley's synthesis of (\pm)-pinitol.

Oxidative Cleavage of Arene *cis*-Diols

The first enantioselective synthesis that incorporated arene *cis*-diols was the formal synthesis of PGE_{2α} (**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).

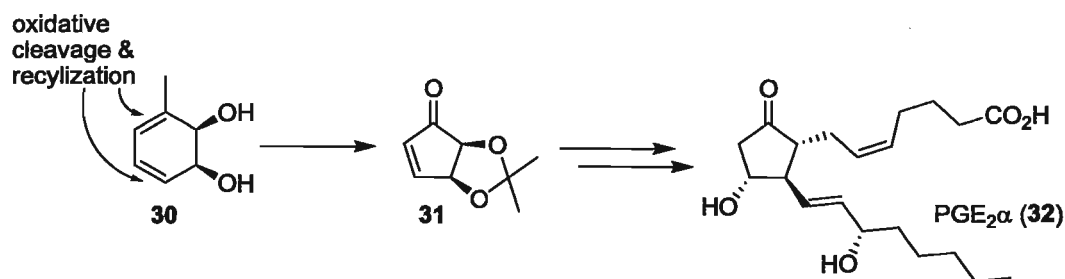


Figure 6. Hudlicky's formal synthesis of PGE_{2α}.

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.¹² The first application of Diels-Alder reactions to *cis*-dihydrodiol metabolites in total synthesis was Hudlicky's preparation of Conduramine A-1 (**36**) (Figure 7).

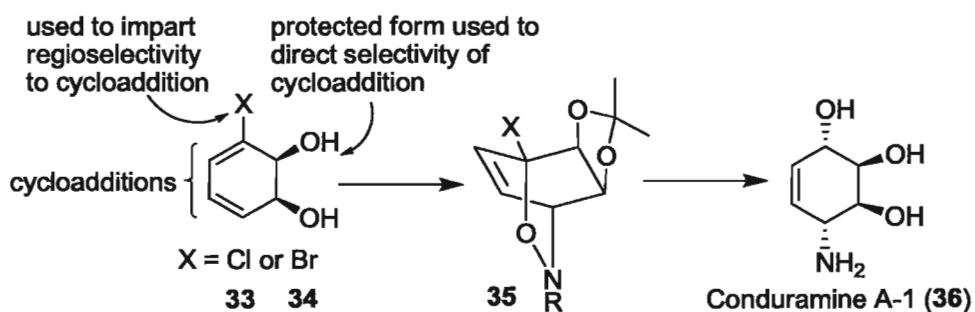


Figure 7. Hudlicky's synthesis of Conduramine A-1.

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**)²⁸, the Banwell group combined an intramolecular cycloaddition

with a cross-coupling protocol to provide an intermediate synthesized by Nicolaou (Figure 8).²⁹

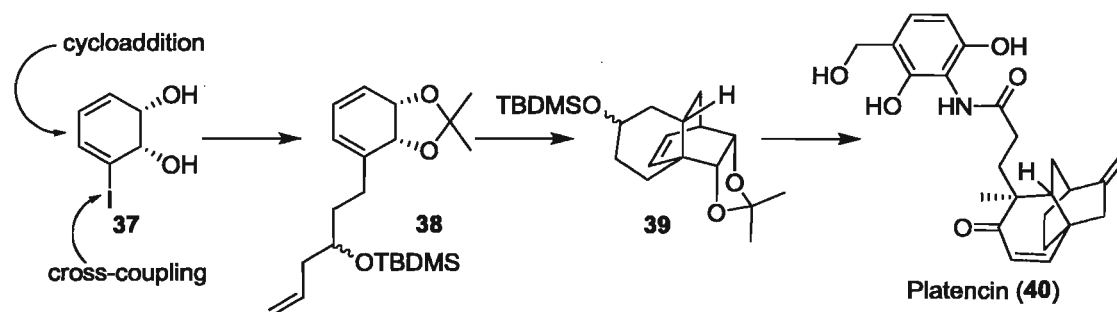


Figure 8. Banwell's formal synthesis of Platencin.

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.³⁰ 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 phorbacin C (**44**), (Figure 9).³¹ 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**).

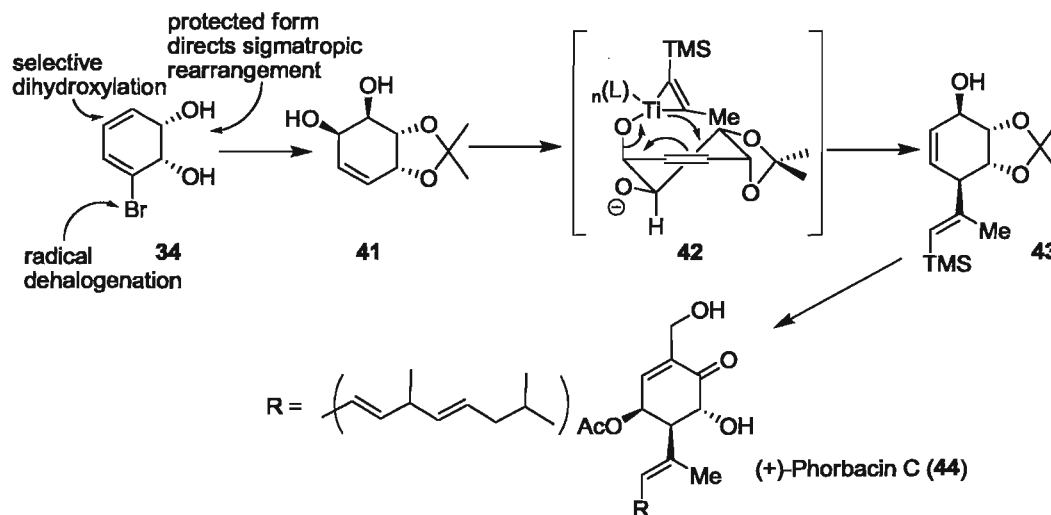


Figure 9. Micalizio's use of metalo-[3,3] rearrangement in the synthesis of (+)-phorbacin C.

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.²³ More recently *cis*-dihydroarenediols have been used in the synthesis of codiene³², various montanine alkaloids,³³ and oseltamivir (Tamiflu).³⁴ The *cis*-dihydrodiols 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.³⁵

When considering the design and execution of future synthesis using *cis*-dihydroarenediols, 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.³⁶ The first recorded treatments specifically designed for cancerous conditions were prescribed by Hippocrates of Cos (ca. B.C. 460-370).³⁷ 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).³⁸ Hippocrates’ 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.).³⁷ 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 1877.³⁹ 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.⁴⁰ Since that time interest has intensified and more than 100 structurally diverse constituents from a variety of *Amaryllidaceae* plant species have been isolated.

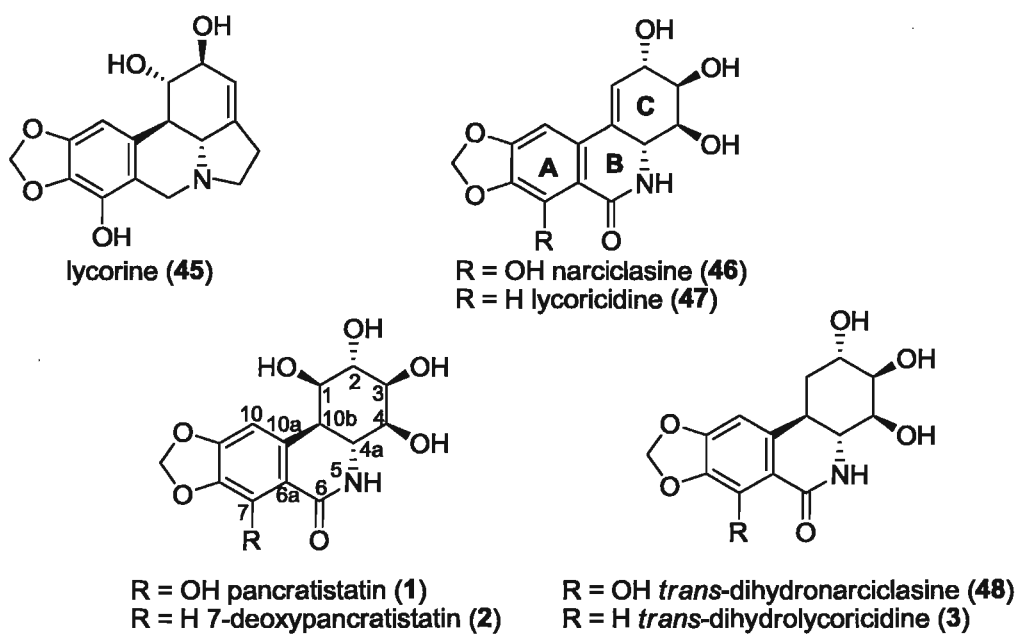


Figure 10. Representative *Amaryllidaceae* constituents.

The most active congeners have proven to be the isocarbostryl 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.⁴¹ In 1984 Pettit and coworkers isolated isocarbostryl 1 from the bulbs of Hawaiian *Hymenocallis littoralis*⁴² and

named it pancratistatin.⁴³ A pattern of isolation of 7-hydroxy and 7-deoxy pairs of isocarbostryl congeners began to emerge with the isolation of 7-deoxypancratistatin (**2**) in 1989.⁴⁴ The last pair to be isolated was *trans*-dihydronarciclasine (**48**) and *trans*-dihydrolycoricidine (**3**) (7-deoxy-*trans*-dihydronarciclasine).^{45,46}

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**)⁴⁷, pancratistatin (**1**)⁴⁸, 7-deoxypancratistatin (**2**)⁴⁹, narciclasine (**46**)⁵⁰, lycoricidine (**47**)⁵¹, *trans*-dihydronarciclasine (**48**)⁵², and *trans*-dihydrolycoricidine (**3**)⁵³ have been completed and made the subject of a number of excellent reviews.⁵⁴

II-2.1 Biosynthesis of *Amaryllidaceae* alkaloids

The biosynthesis of the *Amaryllidaceae* alkaloids can vary widely according to the type of ring subgroup being produced.^{55,56} 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.

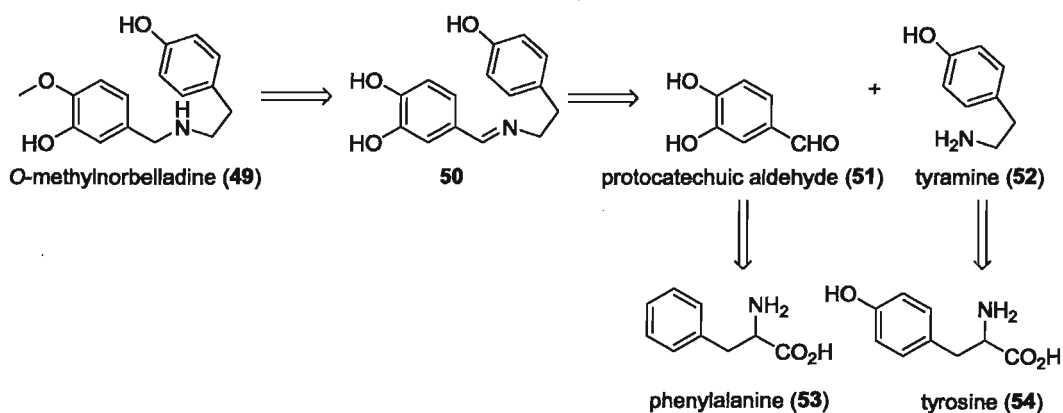


Figure 11. Biosynthesis of *O*-methylnorbelladine (49)

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),⁵⁷ a known precursor to lycorine (45), (Figure 12).⁵⁸ 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 narciclasine (46) was elucidated in a series of experiments using ¹⁴C and ³H labeled *O*-methylnorbelladine (49) and 11-hydroxyvittatine (59).^{58,59} The transition from 59 to narciclasine (46) is hypothesized to proceed through a *retro*-Prins reaction followed by oxidation steps.⁵⁶

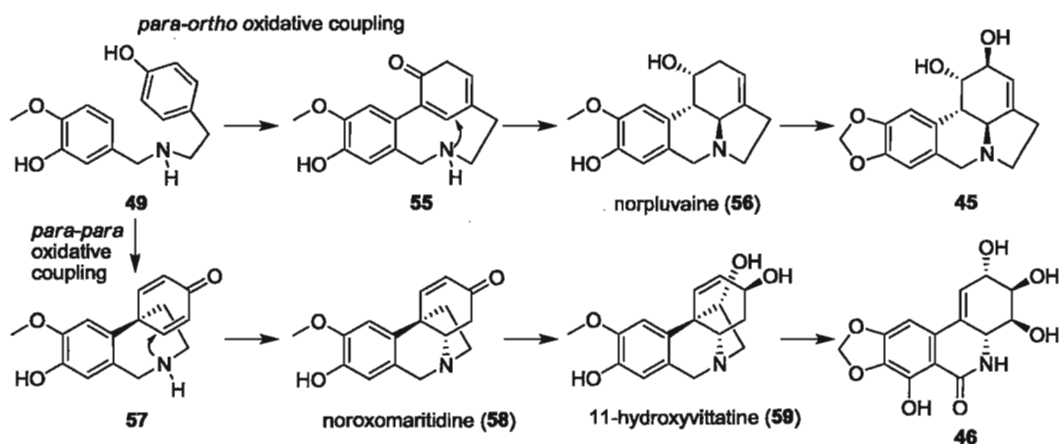


Figure 12. Biosynthesis of *Amaryllidaceae* alkaloids.

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.⁶⁰ In addition to its strong activity against murine P-388 lymphocytic leukemia lycorine (**1**) has also been shown to possess antiviral activity.⁶¹ The antitumor activity of narciclasine (**46**) was reported following its screening against the mouse Sarcoma 180 cell line.⁶² 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-dihydranarciclasine (**48**), and *trans*-dihydrolycoricidine (**3**) have registered GI₅₀ values as low as 0.01% $\mu\text{g/mL}$.^{63,64,65,66} These isocarbostyryl-type congeners were also tested against the NCI (National Cancer Institute) 60 human cancer cell line *in vitro* screen and were shown to be most effective against the melanoma subgroup.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 ¹⁴C labeled phenylalanine in rabbit reticulocyte ribosomes.⁶⁸ Narciclasine was also shown to inhibit association of [¹⁴C]trichodermin (**60**) and [³H]anisomycin (**61**), two known inhibitors of protein synthesis by eukaryotic ribosomes (Figure 13).

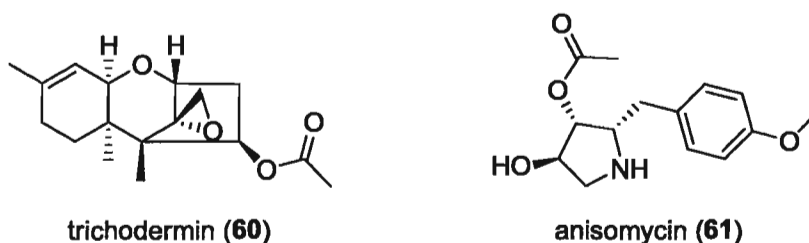


Figure 13. Known 60S ribosomal subunit binders trichodermin (**60**) and anisomycin (**61**).

As **60** and **61** are known to shut down protein biosynthesis by binding to the 60S ribosome subunit, experiments were conducted using the mutant *Saccharomyces cerevistae* TR₁ strain. This mutant is resistant to **60** and **61** through alteration of the peptidyl transferase center of the 60S ribosomal subunit.^{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.⁷¹ Pandey postulated that the selectivity was because of pancratistatin's ability to differentiate between mitochondria from cancer cells and those of healthy cells.⁷² This approach is currently being explored in more complicated systems.⁷³ 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 isocarbostryls 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.^{64,74} Dihydronearciclasine has been generated in both its *cis*- and *trans*- form with the *trans*- (*trans* B/C ring juncture) displaying significantly more potent activity.⁷⁵ 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.⁶⁴ With these studies a general view of the pharmacophore of this *Amaryllidaceae* isocarbostryril subgroup begins to take shape (Figure 14).

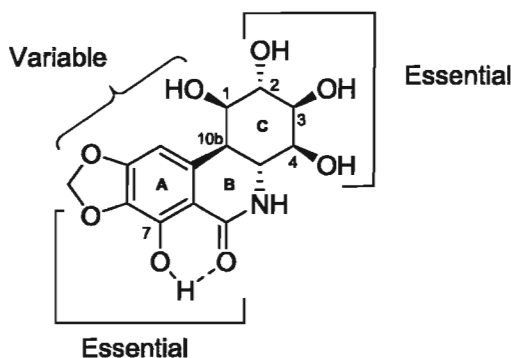


Figure 14. Pancratistatin as a model for required pharmacophore in the *Amaryllidaceae* alkaloids as proposed by Pettit.

Modification of the A ring has included replacement of the dioxymethylene moiety to form singly oxygenated derivatives of 7-deoxypancratistatin,⁷⁶ indole mimics,⁷⁷ and bis-silylated analogues,⁷⁸ all displaying significantly reduced or no activity. Ring B modifications have yielded various seco-analogues,⁷⁹ lactones⁸⁰ 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.⁸¹ This *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.^{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.^{83,84} 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.^{48h} In an effort to improve the aqueous solubility of various *Amaryllidaceae* constituents, the Pettit group has synthesized 7-*O*-phosphate prodrug analogues of **1**,⁸⁵ 3,4-*O*-cyclic phosphate analogues of **1**, **3**, **46-48** and a C-1 benzoyl analog.⁸⁶ These phosphate prodrugs are currently being evaluated on a preclinical level.

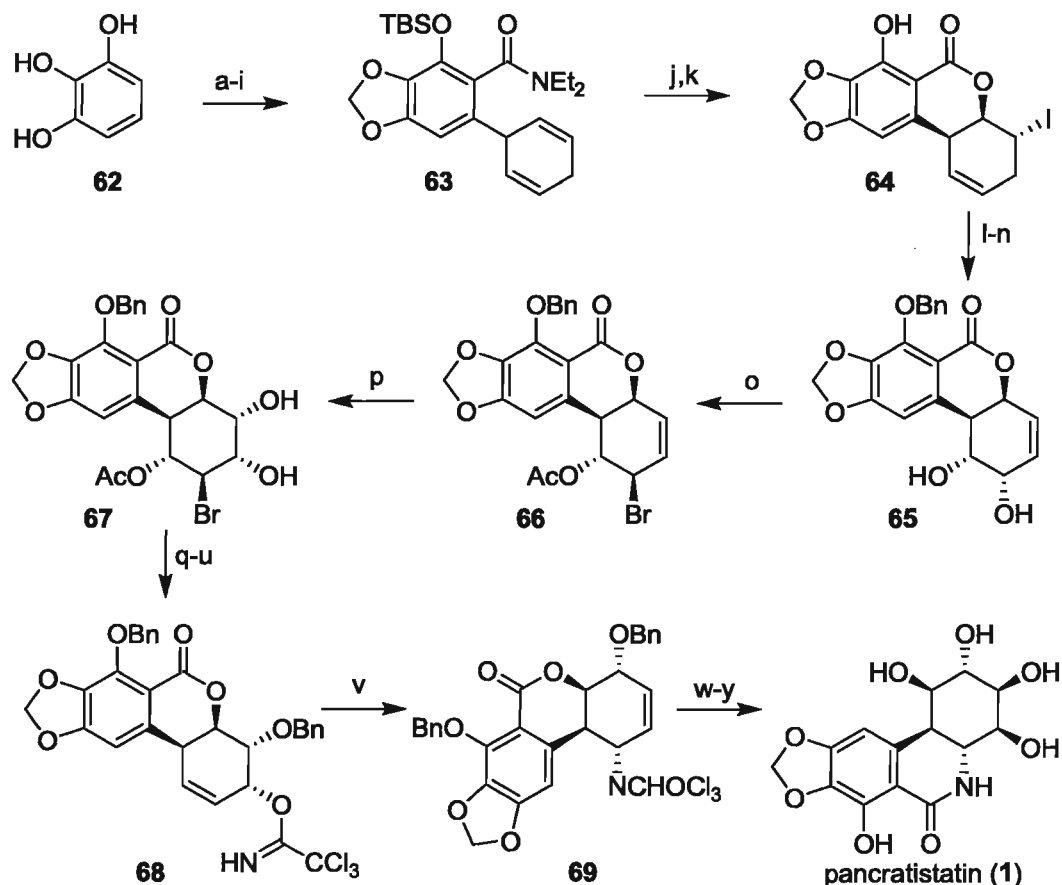
Synthetic efforts to generate meaningful analogues have provided much needed information concerning the minimal structure requirements detailed above. With continued development in this field reliant upon supplies of relatively scarce natural congeners, synthetic efforts will no doubt persist in this 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.^{48a} The synthesis begins with pyrogallol **62** as the precursor to the A

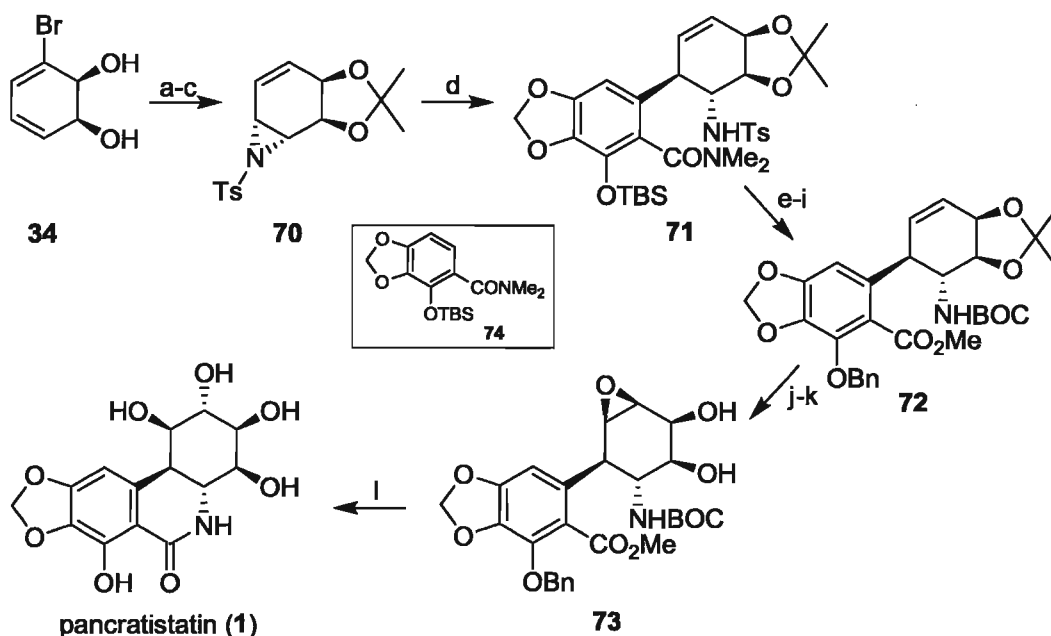
ring of pancratistatin (Scheme 4). Further functionalization provided amide **63**, which contained all of the 14 skeletal carbons in pancratistatin. Iodolactonization to form **64** was achieved only after cleavage of the silyl-ether and stannylation of the phenol.



Reaction conditions: (a) $\text{HC}(\text{OEt})_3$, Amberlyst-15, C_6H_6 , 86%; (b) NaH , Et_2NCOCl , THF, then MeOH, TsOH, rt 86%; (c) K_2CO_3 , CH_2Br_2 , CuO, DMF, 70%; (d) *s*-BuLi, TMEDA, THF, 58%; (e) TBSCl, imidazole, CH_2Cl_2 , 86%; (f) *s*-BuLi, TMEDA, THF, 70%; (g) allylmagnesium bromide, Et_2O , 92%; (h) (i) $\text{CH}_3\text{SO}_2\text{Cl}$, 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) $(\text{Bu}_3\text{Sn})_2\text{O}$, $\text{C}_6\text{H}_5\text{CH}_3$, (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-acetoxyisobutyryl bromide, CH_3CN , 88%; (p) OsO_4 , NMO, CH_2Cl_2 , THF, H_2O , 88%; (q) Bu_2SnO , $\text{C}_6\text{H}_5\text{CH}_3$, 4-methoxybenzyl bromide, *n*-Bu₄Ni, (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 , $\text{Pd}(\text{OH})_2$, 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%.



Reaction conditions: (a) DMP, *p*-TsOH, acetone; (b) PhINTs, Cu(acac)₂, CH₃CN, 27% (over 2 steps), (c) Bu₃SnH, AIBN, THF, reflux, 78%; (d) (i) **74**, *s*-BuLi, TMEDA, THF, (ii) CuCN, (iii) **70**, BF₃·Et₂O, 49%; (e) *s*-BuLi, (BOC)₂O, THF, 68%; (f) Na / anthracene, DME, 62%; (g) morpholine-SMEA, THF, 72%; (h) BnBr, K₂CO₃, DMF, 83%; (i) NaClO₂, KH₂PO₄, 2-methyl-2-butene, *t*-BuOH, H₂O, CH₂N₂, 98%; (j) HOAc, THF, H₂O, 73%; (k) *t*-BuOOH, VO(acac)₂, C₆H₆, 53%; (l) C₆H₅COONa, H₂O, 100 °C, 51%.

Scheme 5. Hudlicky's synthesis of pancratistatin.

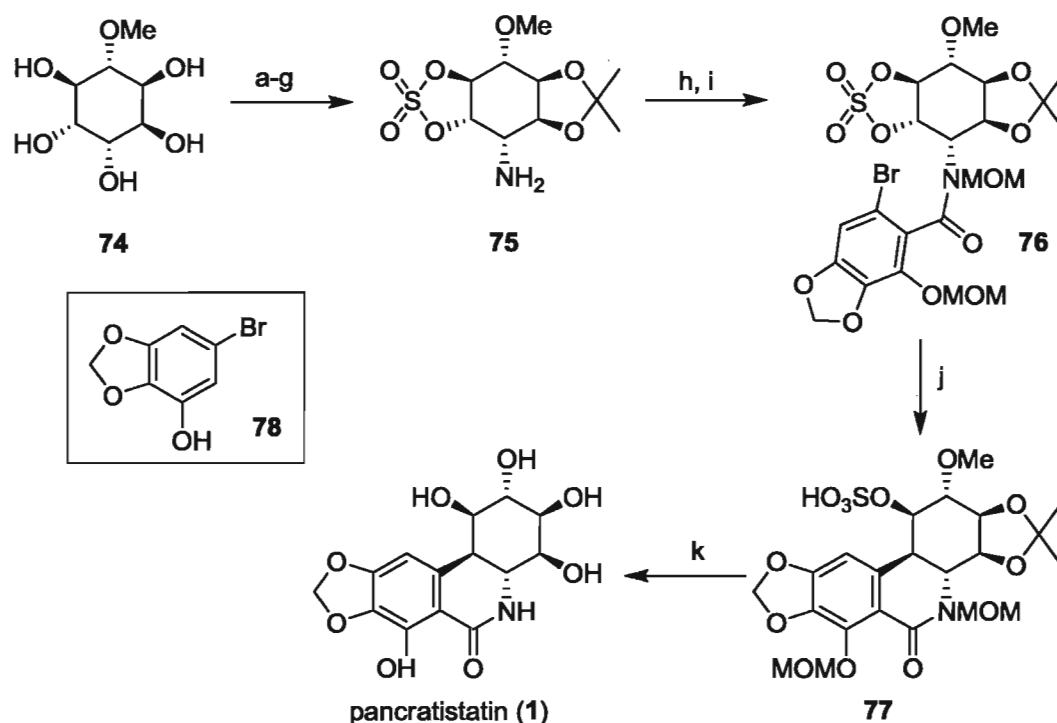
The first asymmetric total synthesis of pancratistatin was reported in 1995 by Hudlicky.^{48b} Chirality in this synthesis was derived from the microbial dihydroxylation of bromobenzene which provided *cis*-diol **34**. Aziridination of the acetone of **34** using Yamada's iodonium ylide followed by 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.^{48j} The starting material for this asymmetric synthesis is D-pinitol (**74**), an expensive starting material (\$145/gram)⁸⁷ 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 phenanthridone **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**).

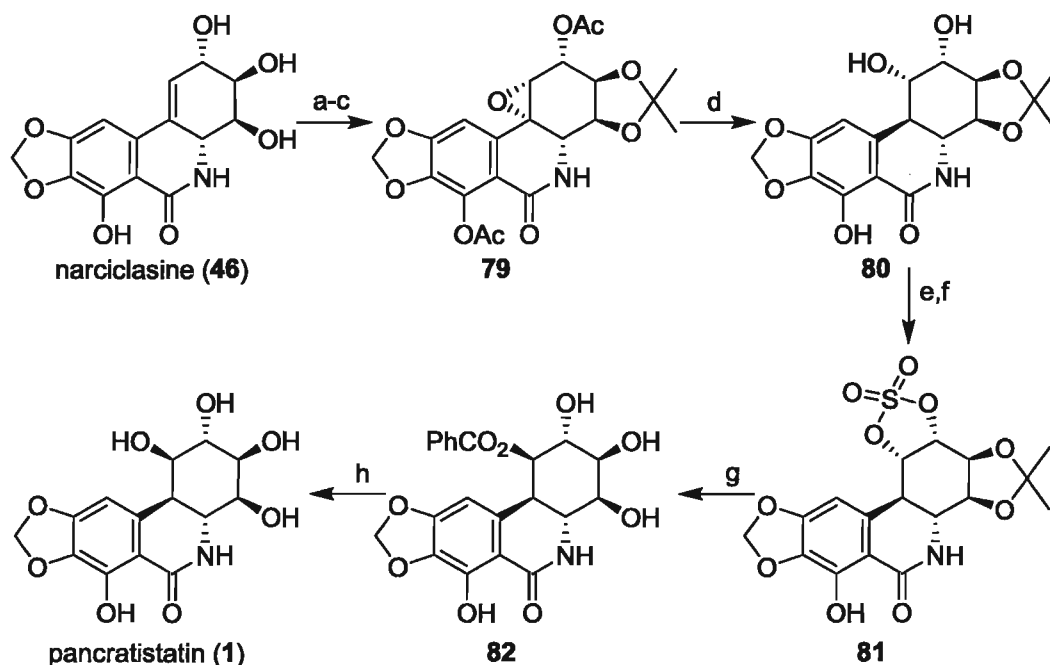


Reaction conditions: (a) TIPDSCl₂, imidazole, DMAP, CH₂Cl₂, 94%; (b) (MeO)₂C(CH₃)₂, *p*-TsOH, 81%; (c) (i) PPh₃, DEAD, CH₃SO₃H, CH₂Cl₂, 0 °C to rt; (ii) NaN₃, DMF, 60 °C, 72%; (d) TBAF, THF, 0 °C to rt, 100%; (e) SOCl₂, Et₃N, CH₂Cl₂, 0 °C; (f) NaIO₄, RuCl₃, aq. CH₃CN, 87% over two steps; (g) PPh₃, aq THF, 0 °C to rt, 94%; (h) **78**, MgBr₂·OEt₂, COCl₂, ether, 0 °C, **75**, 64%; (i) K₂CO₃, MOMCl, DMF, rt, 84%; (j) *t*-BuLi, CeCl₃, ultrasound, THF, -78 °C to rt, 72%; (k) (i) BBr₃, CH₂Cl₂, -78 to 0 °C, 1 h; (ii) MeOH, **77** 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).^{48h} The free hydroxyl groups were protected as their acetonide and acetates before epoxidation

with *m*CPBA 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%.



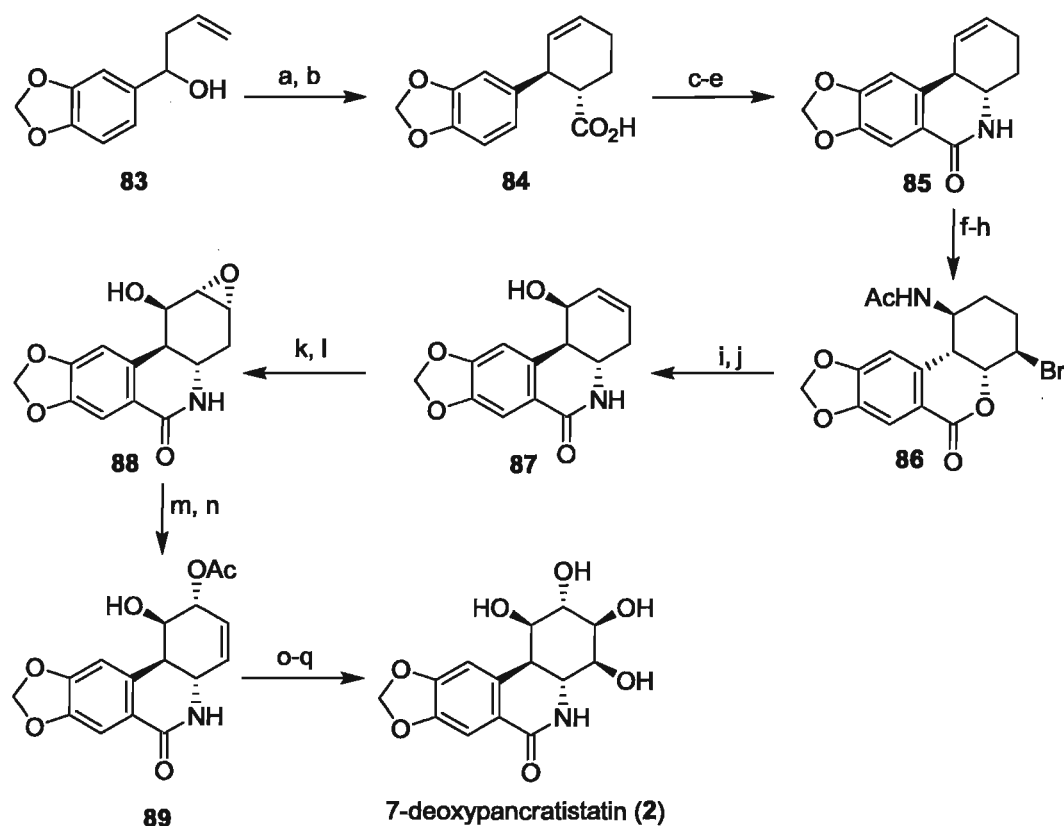
Reaction conditions: (a) DMF, DMP, *p*-TSOH, 97%; (b) Ac_2O , pyr, 81%; (c) *m*CPBA, phosphate buffer, CH_2Cl_2 , H_2O , 52%; (d) (i) H_2 , Pd/C 10%, (ii) K_2CO_3 , CH_3OH , H_2O , 28%; (e) SOCl_2 , Et_3N , THF; (f) $\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$, NaIO_4 , CH_3CN , CCl_4 , H_2O , 45% (over 2 steps); (g) (i) PhCO_2H , CsCO_3 , DMF, (ii) THF, H_2O , H_2SO_4 , 74%; (h) K_2CO_3 , CH_3OH , 75%.

Scheme 7. Pettit's relay synthesis of pancratistatin.

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

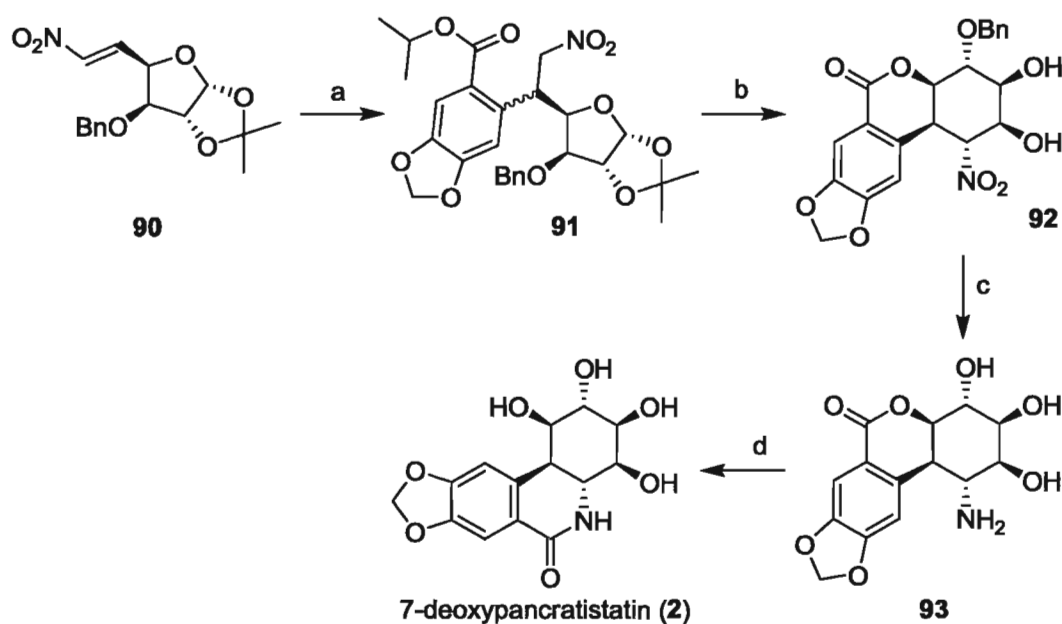
acrylate. Hydrolysis of the intermediate ester provided acid **84**, which was converted to an isocyanate before formation of lactam **85** under Lewis acid catalysis. The lactam-lactone transformation occurred through a protection, hydrolysis, and bromolactonization sequence to give bromide **86**. 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**).



Reaction conditions: (a) ethyl acrylate, *p*-TsOH, 180 °C, 56%; (b) NaOEt, EtOH, reflux, then H₂O, reflux, 74%; (c) ClCO₂Et, 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-dihydropryran, *p*-TsOH, reflux, 75%; (l) *m*CPBA, 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%.

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

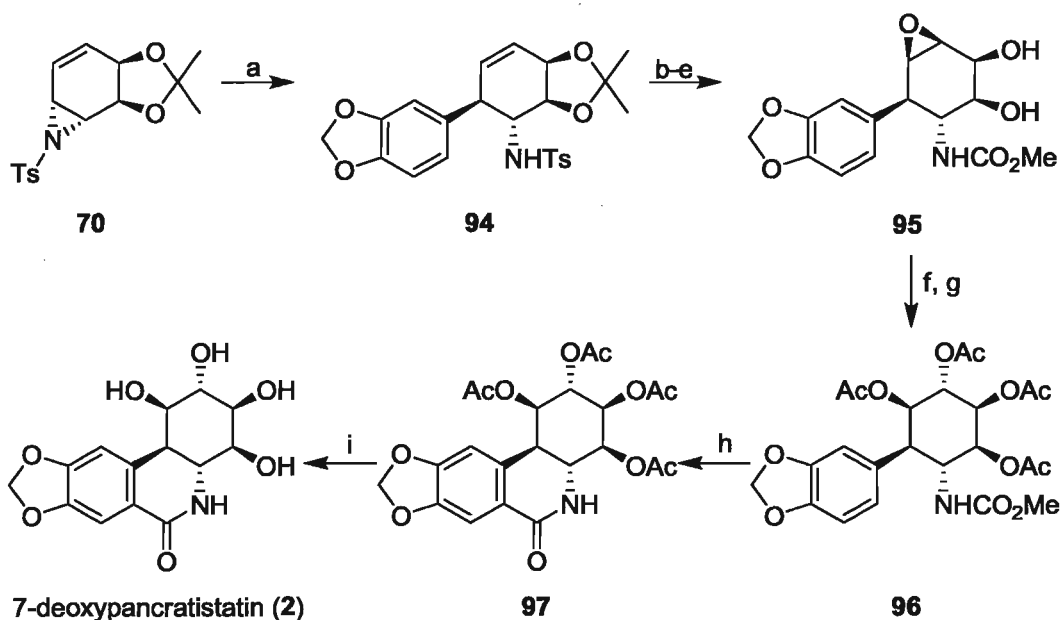
The first asymmetric total synthesis of 7-deoxypancratistatin was also completed in the course of a total synthesis of lycoricidine.^{49c} Chirality in the synthesis was derived from glucose, which was used to make nitro olefin **90** (Scheme 9). Introduction of the A-ring was accomplished by metal-halogen 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 lactone-lactam rearrangement to give 7-deoxypancratistatin(**2**).



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%.

Scheme 9. Paulsen and Stubbe's synthesis of 7-deoxypancratistatin.

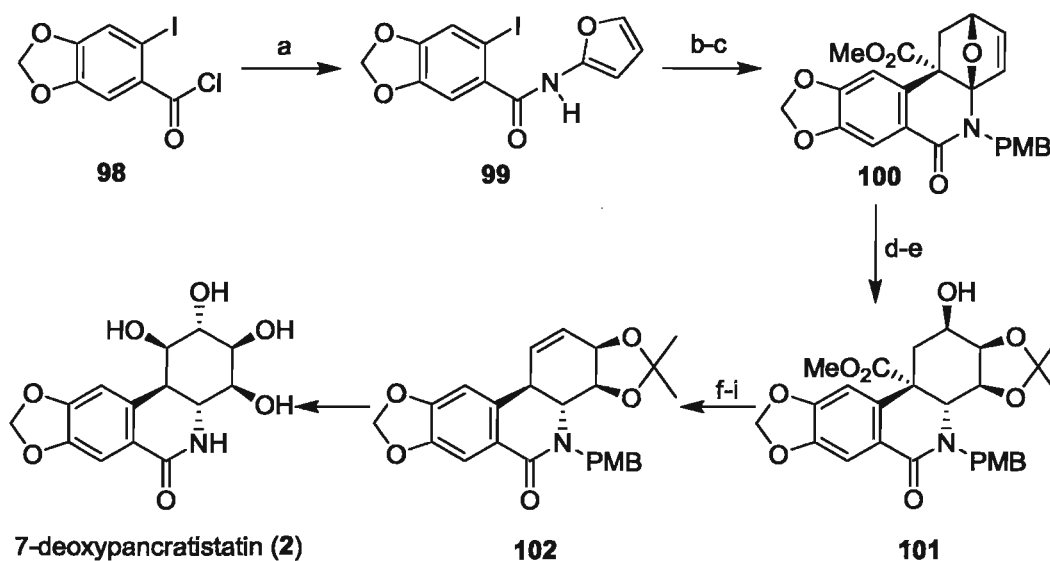
Hudlicky continued exploration of chemoenzymatic synthesis of these constituents in his asymmetric total synthesis of 7-deoxypancratistatin. As in the previous synthesis of pancratistatin, vinyl aziridine **70** was opened with a cyanocuprate to couple the A and C rings (Scheme 10). The tosyl moiety 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 chiral centers set. Final deprotection occurred without incident to give 7-deoxypancratistatin (**2**).



Reaction conditions: (a) (i) 4-bromo-1,2-(methylenedioxy) benzene, *n*-BuLi, THF, -78 °C, (ii) CuCN, (iii) **70**, BF₃·Et₂O, 32%; (b) *s*-BuLi, THF, (BOC)₂O, 76%; (c) Na/anthracene, DME, -78 °C; 69%; (d) AcOH, THF, H₂O, 65 °C, 94%; (e) *t*-BuOOH, VO(acac)₂, C₆H₆, 85%; (f) H₂O, BzONa, 100 °C, 82%; (g) Ac₂O, DMP, pyr, 84%; (h) Tf₂O, DMAP, CH₂Cl₂, 5 °C, 61%; (i) NaOCH₃, CH₃OH, 72%.

Scheme 10. Hudlicky's synthesis of 7-deoxypancratistatin.

A recent total synthesis of 7-deoxypancratistatin was accomplished by Padwa utilizing a Stille coupling/intramolecular Diels–Alder cascade (Scheme 11). Furan-2-ylcarbamic 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.



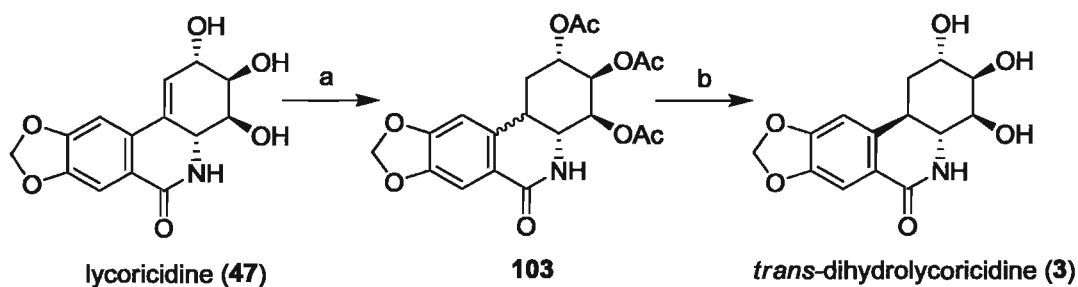
Reaction Conditions: (a) (i) furan-2-ylcarbamic acid 2-*tert*-butyl ester, *n*-BuLi, **98**, THF, 0 °C, (ii) Mg(ClO₄)₂, CH₃CN, 50 °C, 75%; (b) NaH, 4-methoxybenzyl chloride, DMF, 0 °C, 83%; (c) LiCl, Pd(PPh₃)₄, CuCl, 2-(tri-*n*-butylstannyl)acrylate, DMSO, -78 - 60 °C, 82%; (d) (i) 4-methylmorpholine-*N*-oxide, OsO₄, rt, CH₃CN/H₂O, (ii) 2,2-dimethoxypropane, pyridinium *p*-toluenesulfonate, DMF, rt, 80%; (e) (i) Zn(BH₄)₂, TMSOTf -78 °C - rt, CH₂Cl₂, (ii) TBAF, rt, THF, 74%; (f) NaH, benzyl bromide, 0 °C - rt, DMF, 91%; (g) (i) LiOH, reflux, THF/MeOH/H₂O, (ii) (COCl)₂, DMF, CH₂Cl₂, (iii) Zn(BH₄)₂, 0 °C - rt, CH₂Cl₂, (iv) molecular sieves, NMO, TPAP, rt, CH₂Cl₂, 77%; (h) RHCl(PPh₃)₃, reflux, benzonitrile, 63%; (i) (i) H₂, Pd(OH)₂, rt, EtOAc, (ii) NaH, CS₂, MeI, 0 °C - rt, (iii) reflux, *o*-dichlorobenzene, 85%; (j) NMO, OsO₄, rt, acetone, 68%; (k) (i) Et₃N, SOCl₂, 0 °C - rt, CH₂Cl₂, (ii) RuCl₃·3H₂O, NaIO₄, CH₃CN/H₂O, 82%; (l) (i) benzoic acid, Cs₂CO₃, DMF, (ii) 40% H₂SO₄, 80 °C, THF, 75%; (m) (i) NaOMe, 25 °C, THF, (ii) Pd(OH)₂, H₂, rt, THF, 80%

Scheme 11. Padwa's synthesis of 7-deoxypancratistatin.

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.

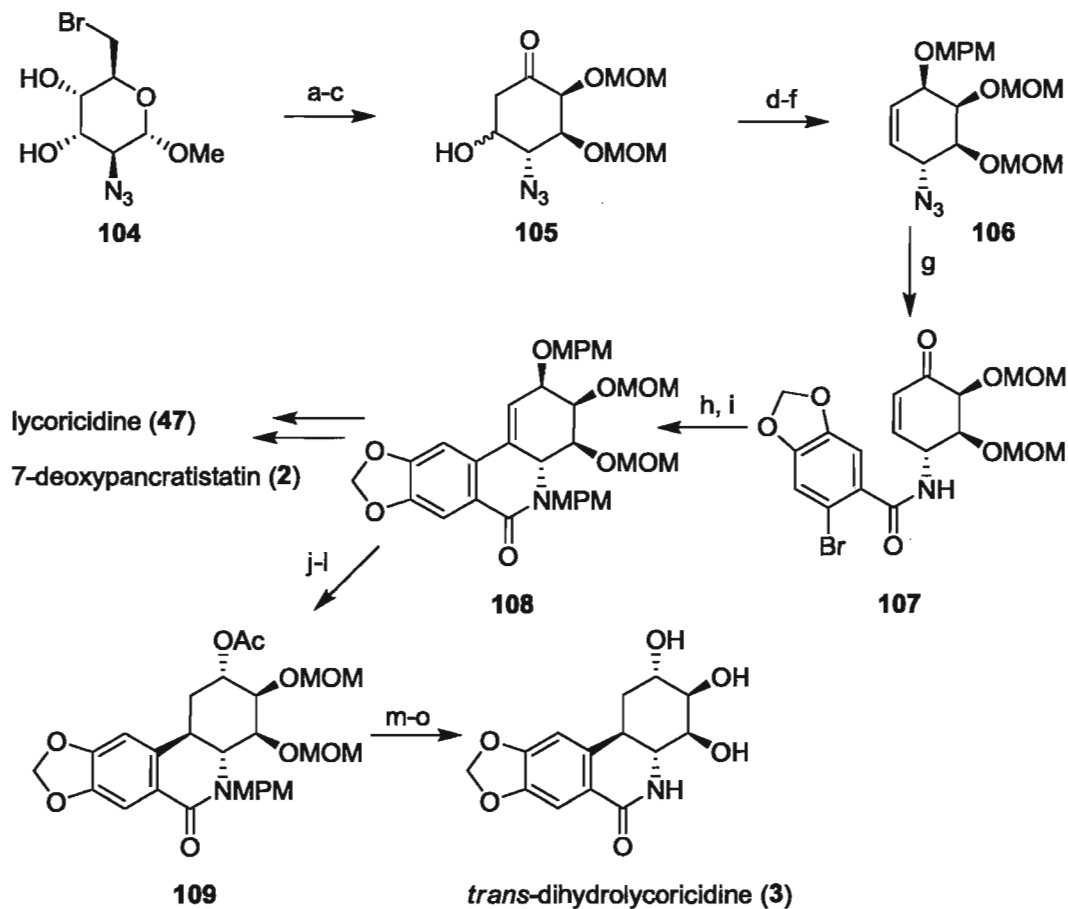
***Trans*-dihydrolycoricidine**

Synthetic *trans*-dihydrolycoricidine was prepared by Gabrielsen and Pettit in 1992.^{53f} 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.^{53f}



Reaction Conditions: (a) (i) PtO₂, rt, MeOH, (ii) Ac₂O, pyridine, rt, 10%; (b) Ba(OH)₂, rt, MeOH, 83%

Scheme 12. Gabrielsen's relay synthesis of *trans*-dihydrolycoricidine.



Reaction conditions: (a) MOMCl, *i*-Pr₂NEt, CH₂Cl₂, 87%; (b) DBU, C₆H₅CH₃, 73%; (c) Hg(OCOCF₃)₂, acetone, H₂O; (d) MsCl, Et₃N, CH₂Cl₂, 63%; (e) NaBH₄, CeCl₃·7H₂O, CH₃OH, 86%; (f) NaH, MPMCl, DMF, 69%; (g) (i) LAH, Et₂O, (ii) 6-bromopiperonylic acid, (EtO)₂P(O)CN, Et₃N, DMF, 89%; (h) NaH, MPMCl, DMF, quant.; (i) Pd(OAc)₂, 1,2-bis(diphenylphosphino)ethane, TIOAc, DMF, 68%; (j) H₂, 5% Pd/C, EtOH/EtOAc, rt, 87%; (k) Tf₂O, pyridine, 0 °C, CH₂Cl₂; (l) KOAc, 18-crown-6, benzene, rt, 81%; (m) (i) 1 N HCl, THF, 50 °C, (ii) Ac₂O, pyridine, rt, 93%; (n) TFA/CHCl₃, rt, 85%; (o) NaOMe, MeOH, 0 °C, 83%.

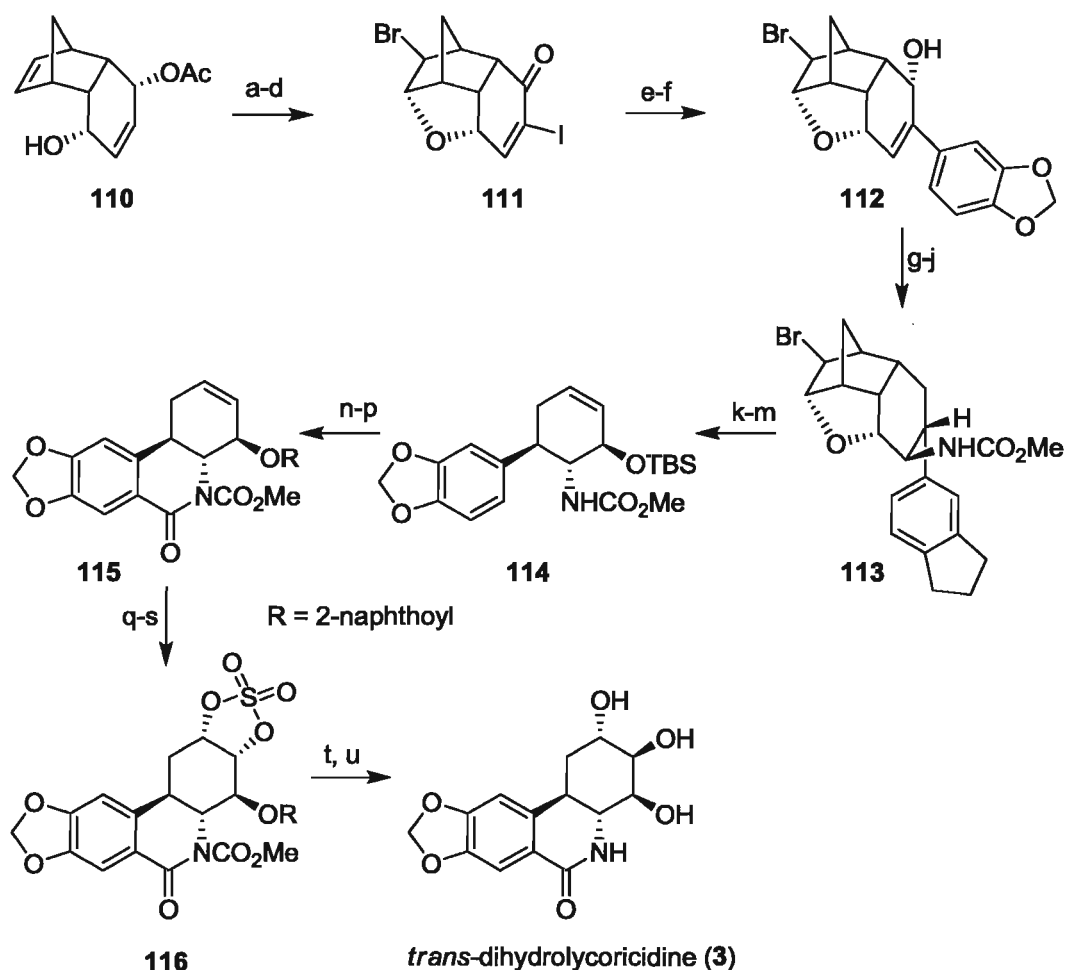
Scheme 13. Chida's synthesis of *trans*-dihydrolycoricidine.

Chida's asymmetric synthesis of *trans*-dihydrolycoricidine (3) was completed by protocols developed in his previously reported total synthesis of lycoricidine (47).^{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.^{53c} 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 S_N2' product which was converted 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 *trans*-dihydrolycoricidine (**3**) in 21 steps and 3.2% overall yield from **110**.



Reaction conditions: (a) NBS, CH₂Cl₂, -30 °C, 62%; (b) K₂CO₃, MeOH, rt, 99%; (c) PDC, CH₂Cl₂, rt, 96%; (d) I₂, pyridine/CCl₄, rt; (e) (3,4-methylenedioxyphenyl)tributylstannane, PdCl₂(PPh₃)₂, Ph₃As, CuCl, THF, rt, 63%; (f) DIBAL, 2,6-di-*tert*-butyl-4-methylphenol, CH₂Cl₂, -78 °C, 86%; (g) DPPA, PPh₃, DIAD, rt, 76%; (h) H₂, 10% Pd/C, EtOAc, rt, 95%; (i) ClCO₂Me, 1 N NaOH, THF, rt, 95%; (j) H₂, 50% Pd(OH)₂/C, MeOH, rt, 92%; (k) Zn, MeOH/AcOH/THF, reflux, 94%; (l) TBSOTf, Et₃N, CH₂Cl₂, -78 °C, 95%; (m) NaHCO₃, Ph₂O, reflux, 87%; (n) TBAF, THF, 97%; (o) 2-naphthoyl chloride, pyridine, DMAP, rt, 97%; (p) Tf₂O, DMAP, CH₂Cl₂, 0 °C, 84%; (q) OsO₄, NMO, THF/H₂O, 92%; (r) SOCl₂, pyridine, -30 - -20 °C; (s) RuCl₃·H₂O, NaIO₄, CCl₄/MeCN, rt, 52%; (t) (i) pyridine, reflux, (ii) 2N H₂SO₄/CHCl₃, 70 °C, 64%; (u) NaOMe, MeOH, rt, 84%.

Scheme 14. Ogasawara and Iwabuchi's synthesis of *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 Qing 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).

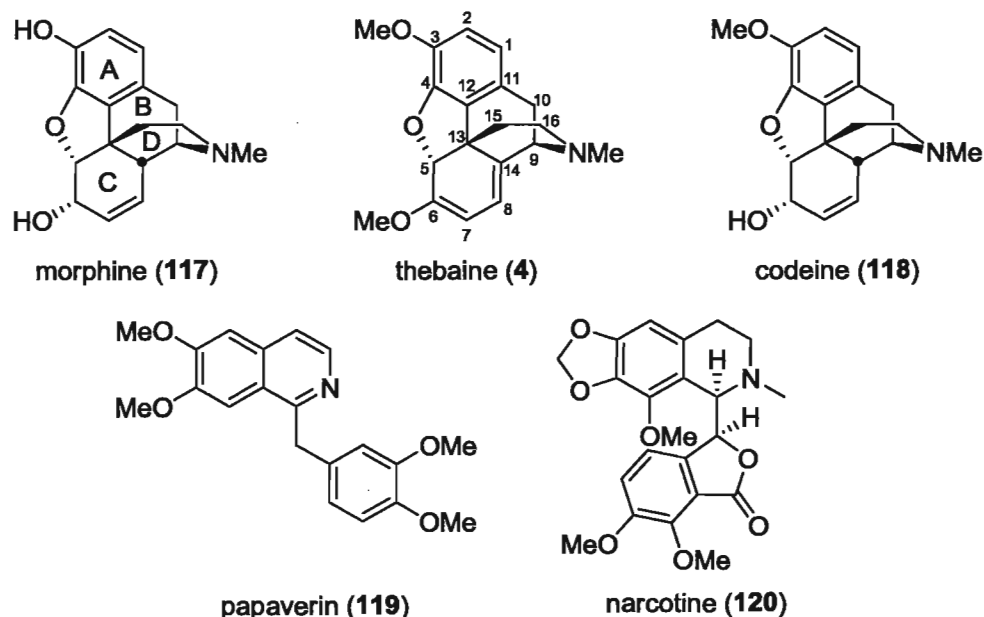


Figure 15. Naturally occurring morphine alkaloids.

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.⁹⁴ More than forty years would pass before the correct empirical formula ($C_{17}H_{19}NO_3$) was established by Laurent.⁹⁵ The most important work in the elucidation of morphine's oxygenation is credited to Wright.⁹⁶ 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.⁹⁷ The correct structure of morphine was proposed in 1925 by Robinson⁹⁸ and confirmed in 1952 with the first total synthesis of morphine by Gates.⁹⁹ With the emergence of X-ray diffraction analysis around that time, the relative configuration of morphine was established by MacKay and Hodgkin.¹⁰⁰ 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.¹⁰¹ 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.¹⁰² 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.¹⁰³ 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.¹⁰⁴ 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 analgesics (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).

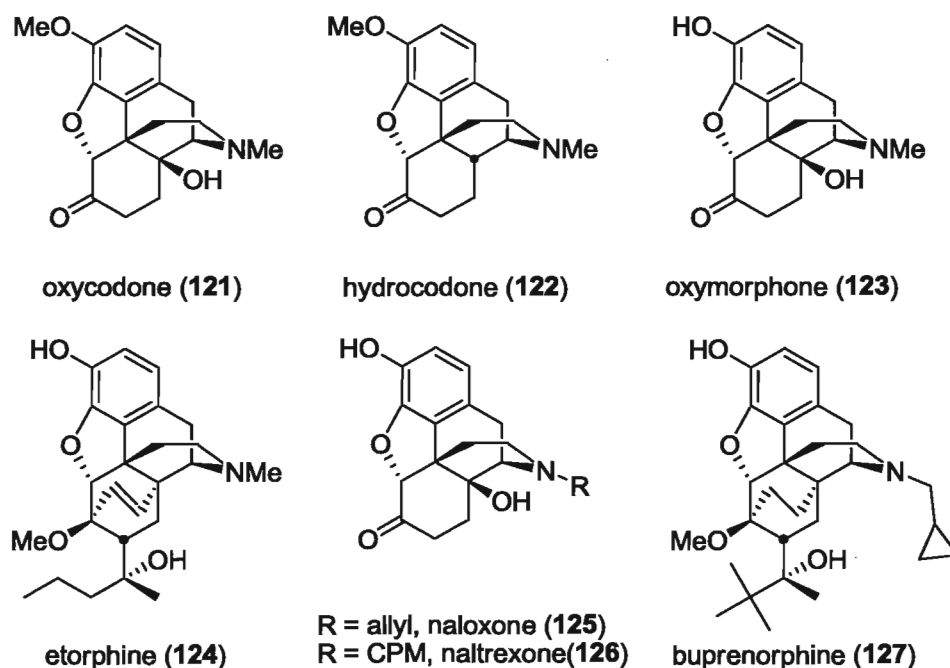


Figure 16. Semisynthetic opioids.

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 benzylisoquinoline 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.

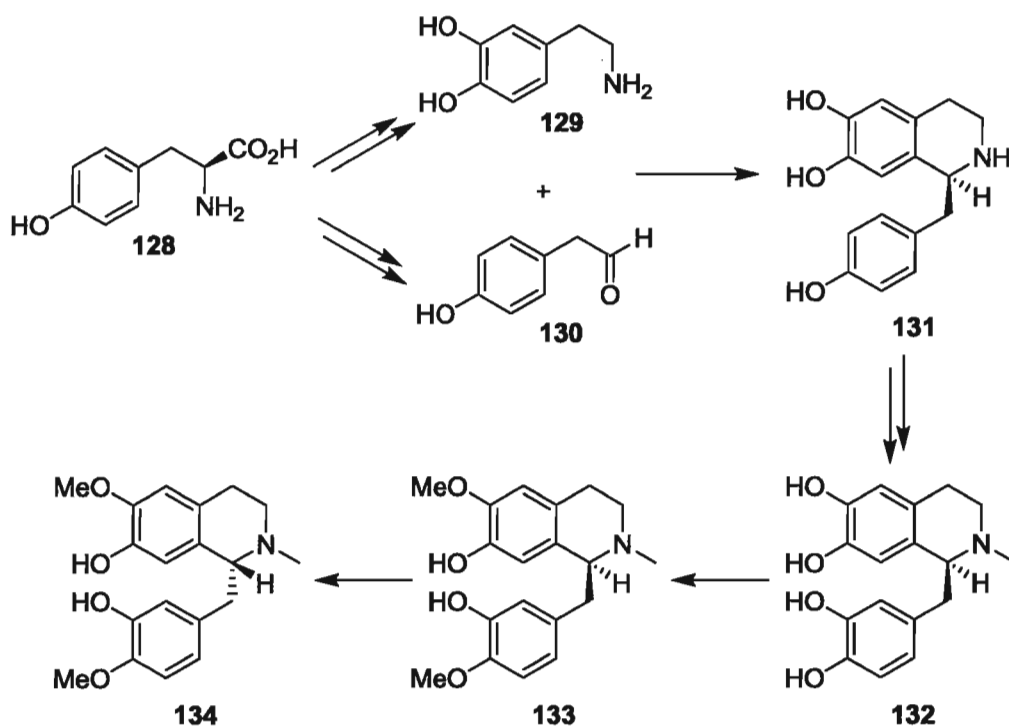


Figure 17. Biosynthesis of (*R*)-reticuline (**134**).

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 benzyloisoquinoline alkaloids. Conversion of **133** to its enantiomer (*R*)-reticuline (**134**) is accomplished *via* reduction of its Schiff base.

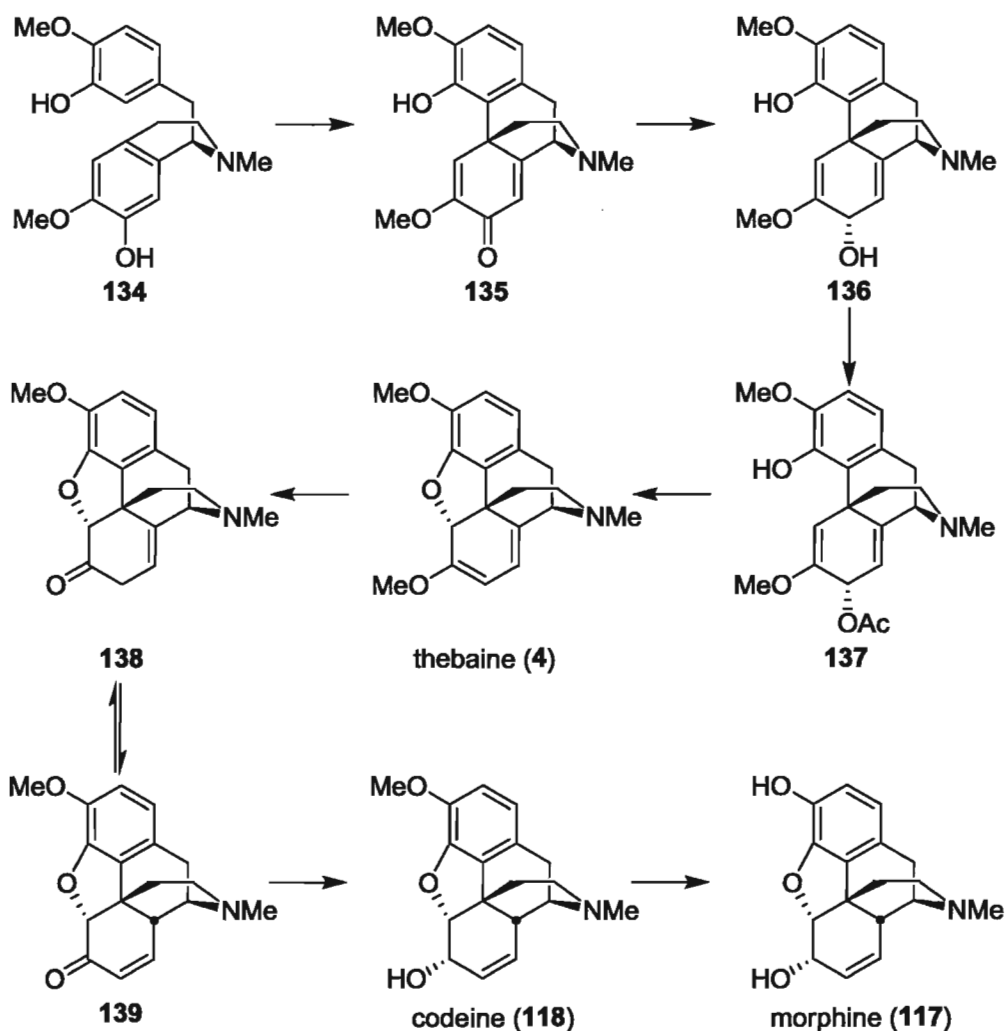


Figure 18. Biosynthesis of thebaine, codeine, and morphine.

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.¹⁰⁷ 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 (7*S*)-salutaridinol which is subsequently converted to its acetate 137.

Displacement of the acetate by the phenolic hydroxyl in an S_N2' 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.¹⁰⁵ 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).¹⁰⁸ 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.¹⁰⁹ 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.

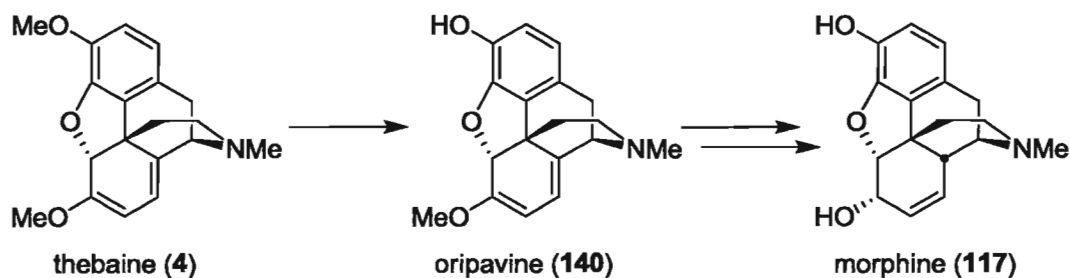
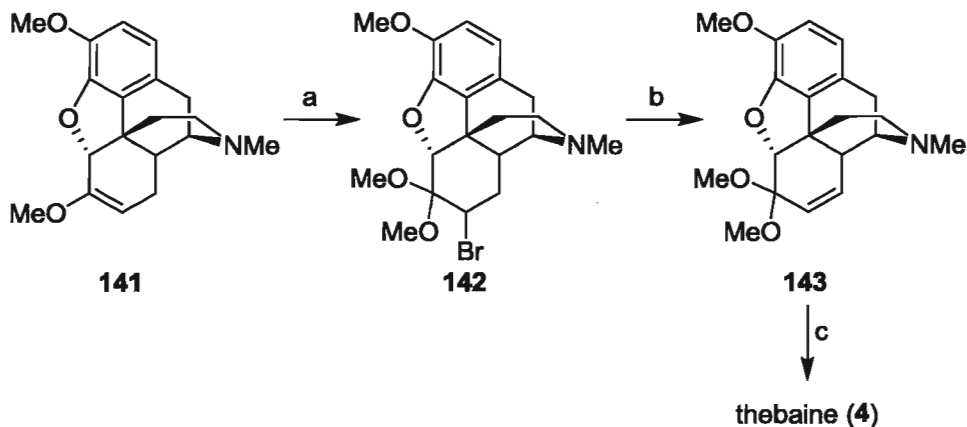


Figure 19. Alternative biosynthesis of morphine.

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.¹¹⁰ 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.¹¹¹ The importance of thebaine as a semisynthetic intermediate¹¹¹ has fueled enthusiasm for its total synthesis¹¹² 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.

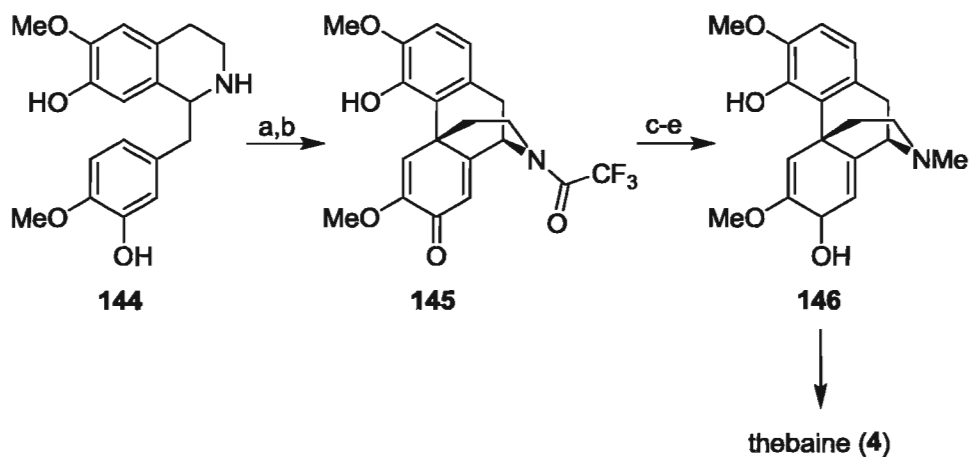
The first synthetic preparation of thebaine (**4**) by any means was disclosed by Rapoport in 1956.^{112a} Dihydrothebaine (**141**) was obtained by enol ether formation of dihydrocodeinone and was treated with methyl hypobromite to give bromoacetal **142** (Scheme 15). Elimination of the bromide and hydrolysis yielded thebaine (**4**).



Reaction Conditions: (a) CH_3OBr , MeOH , 15°C , 62%; (b) $\text{KO}t\text{-Am}$, $\text{HO}t\text{-Am}$, 102°C , 80%; (c) $p\text{-TSA}$, CH_2Cl_2 , H_2O , rt , 40%

Scheme 15. Rapoport's synthesis of thebaine.

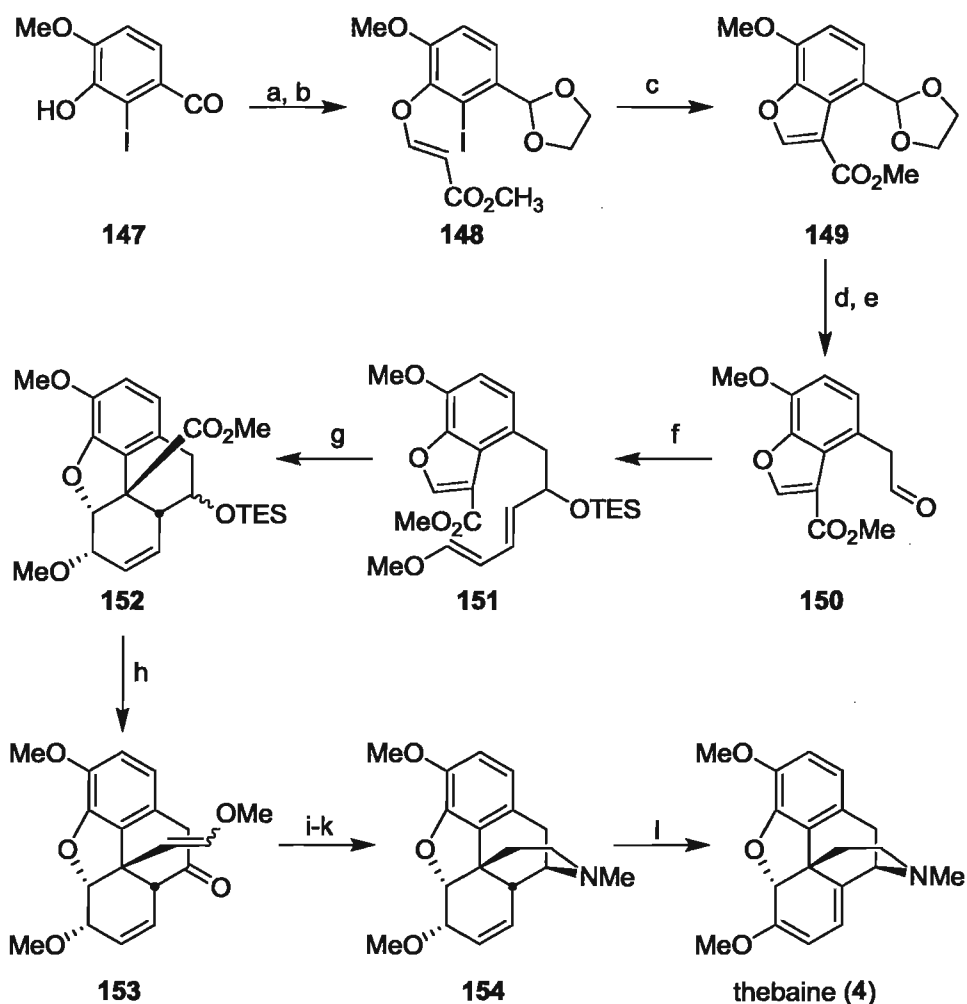
The first total synthesis of thebaine was published in 1975 by Schwartz (Scheme 16).^{112f} Protection of (\pm)-*N*-norreticuline (**144**) as its trifluoroacetate was followed by oxidative *para-ortho* coupling to give (\pm)-*N*-trifluoroacetylnorsalutaridine (**145**). Replacement of the trifluoroacetate moiety with an ethyl carbamate and then reduction provided alcohol **146** which was converted to (\pm)-thebaine under acidic conditions.



Reaction conditions: (a) trifluoroacetic anhydride, K_2CO_3 , CH_2Cl_2 , rt ; (b) thallium tris(trifluoro)acetate, CH_2Cl_2 , -78°C , 11%; (c) K_2CO_3 , MeOH , rt , 86%; (d) EtOCOCI , Et_3N , CHCl_3 , rt ; (e) LAH , THF , reflux, 81% (3 steps); (f) 1 N HCl , rt

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).¹¹²ⁱ 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 phenanthrofurans **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}

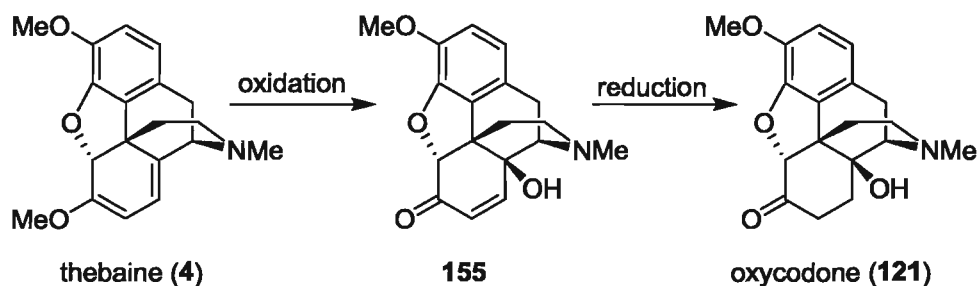


Reaction conditions: (a) HO(CH₂)₃OH, *p*-TsOH, toluene, 85%; (b) methyl propiolate, Et₃N, THF, 89%; (c) Pd(OAc)₂, Ph₃P, NaOAc, *n*-Bu₄NCl, DMF, 125 °C, 84%; (d) HCl, THF, 98%; (e) (i) Ph₃PCH₂OCH₃Cl, KHMDS, THF, (ii) HCl, THF, 95%; (f) (i) 4-methoxy-3-butene-1-yne, ZrCp₂(H)Cl, AgOTf, CH₂Cl₂, (ii) TESCl, imidazole, 95%; (g) decalin, Et₃N, 240 °C, 24 h, 69%; (h) (i) Super hydride, THF, (ii) Dess-Martin, CH₂Cl₂, (iii) Ph₃PCH₂OCH₃Cl, KHMDS, CH₂Cl₂-THF, (iv) TBAF, THF, (v) Dess-Martin periodane, CH₂Cl₂, 0 °C, 51%; (i) (i) L-Selectride, THF, 0 °C, (ii) MsCl, Et₃N, CH₂Cl₂, 0 °C, 34%; (j) (i) HCl, THF, (ii) MeNH₂ · HCl, Et₃N, Ti(OiPr)₄, MeOH, (iii) NaBH₄, 80% (k) K₂CO₃, benzene, 75 °C, 24 h. (l) Reference 113e

Scheme 17. Stork's synthesis of thebaine.

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.¹¹³ Oxycodone was first synthesized from thebaine in 1916 by a two step process (Scheme 18).¹¹¹ The C-ring of thebaine (**4**) is first oxidized to oxycodoneinone **155** 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.¹¹⁴

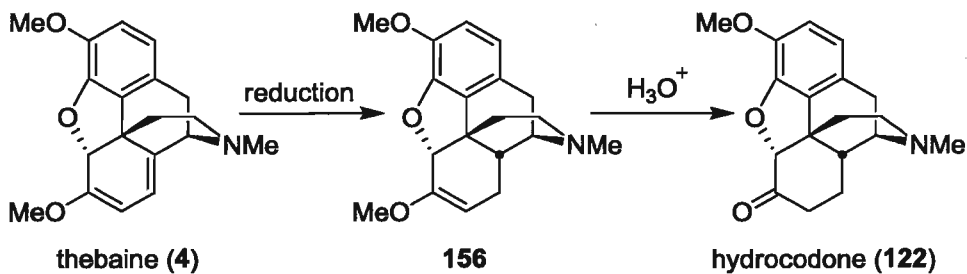


Scheme 18. Synthesis of oxycodone from thebaine.

The analgesic and antitussive (cough suppressant) hydrocodone (**122**) is a semisynthetic opioid derived from either codeine (**118**)¹¹⁵ or thebaine (**4**).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).

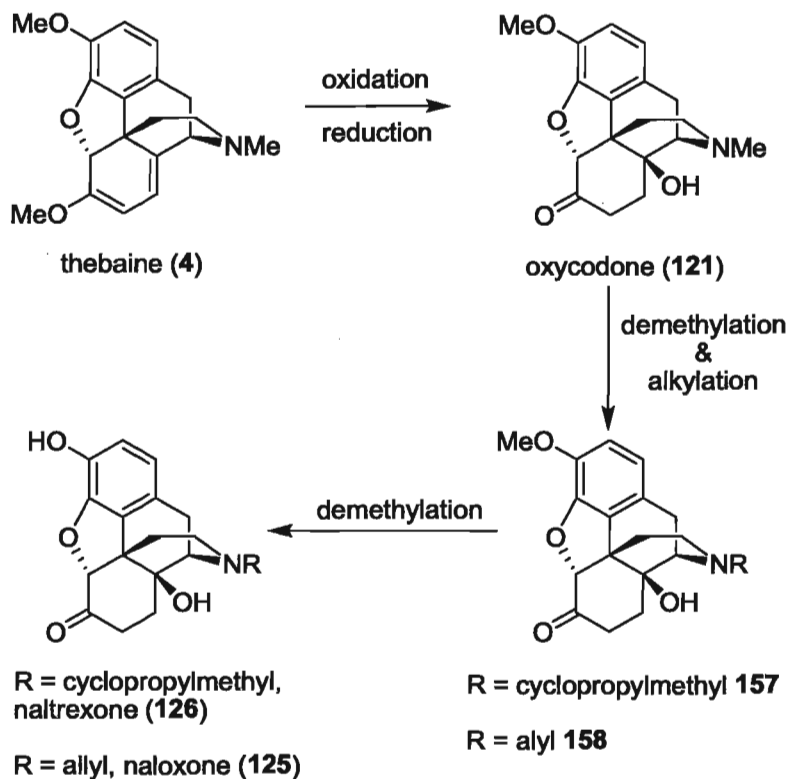
A variety of opioid receptor antagonists have been developed for the treatment of opiate dependency and overdose. The semisynthetic opioids

naltrexone (**126**) and naloxone (**125**) are two such antagonists which are produced commercially from thebaine (**4**).¹¹⁷



Scheme 19. Synthesis of hydrocodone from thebaine.

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-*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 pseudo-sugars, aminocyclitols, and various bicyclic ring systems.

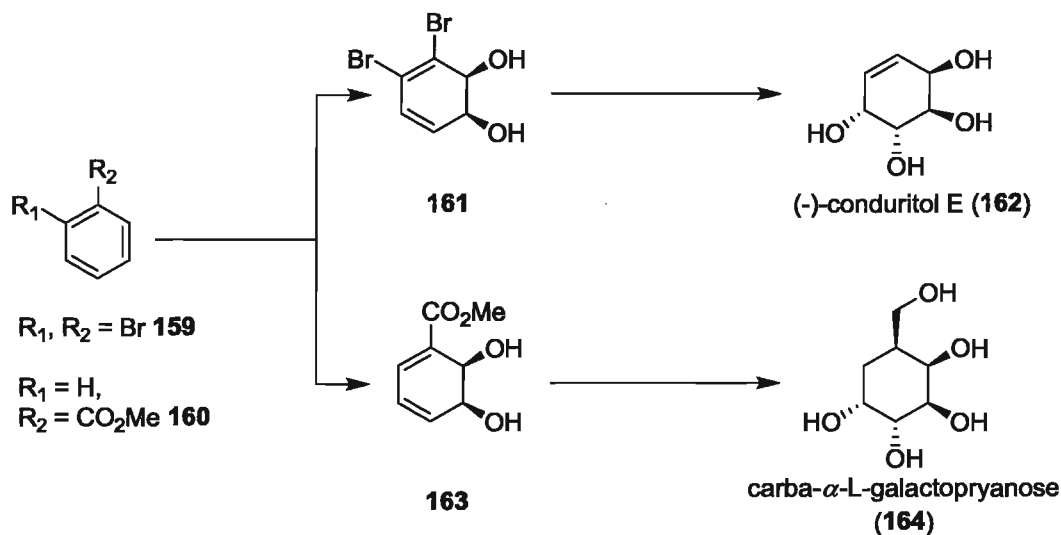


Figure 20. Enzymatic oxidation of substituted benzenes in the synthesis of cyclitols.

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 isocarbostyryl 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.

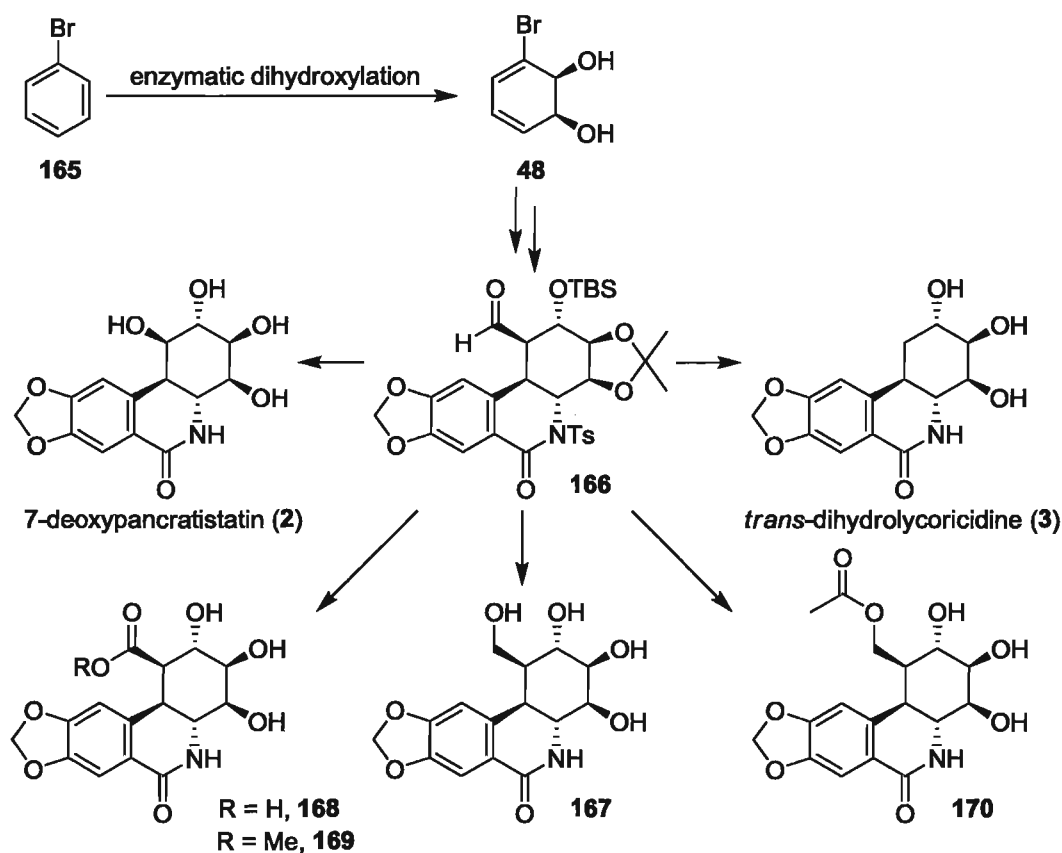


Figure 21. Chemoenzymatic synthesis of 7-deoxypancratistatin (**2**), *trans*-dihydrolycoricidine (**3**) and various C-1 analogues of 7-deoxypancratistatin (**167**-**170**).

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).

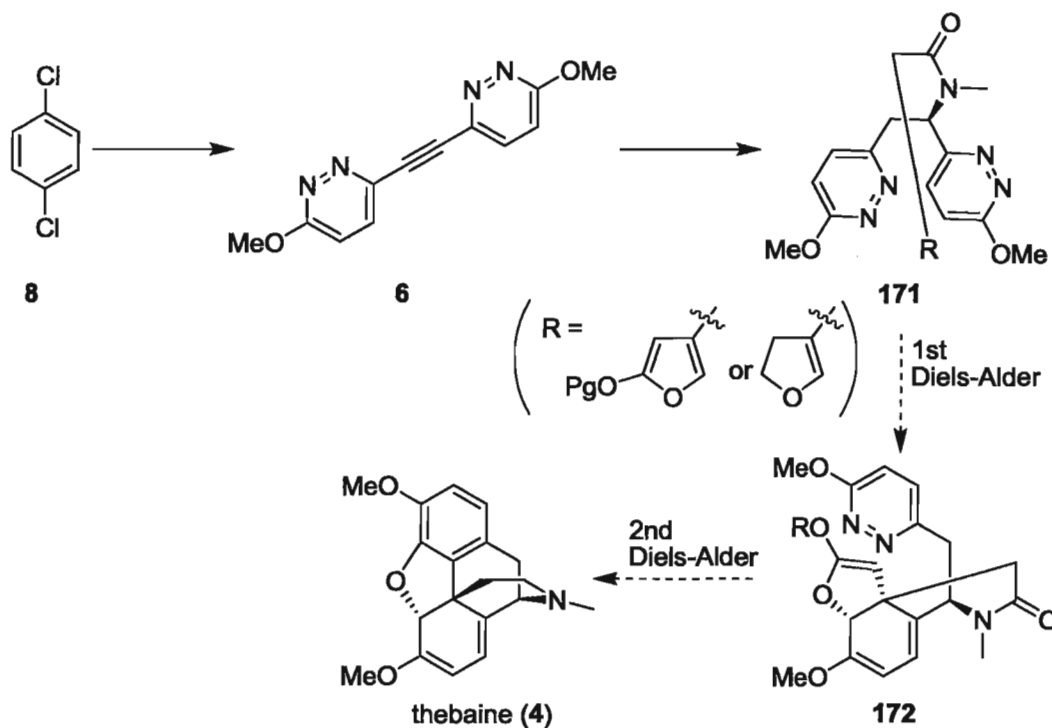


Figure 22. Proposed model system and the total synthesis of thebaine.

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.²³ 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.

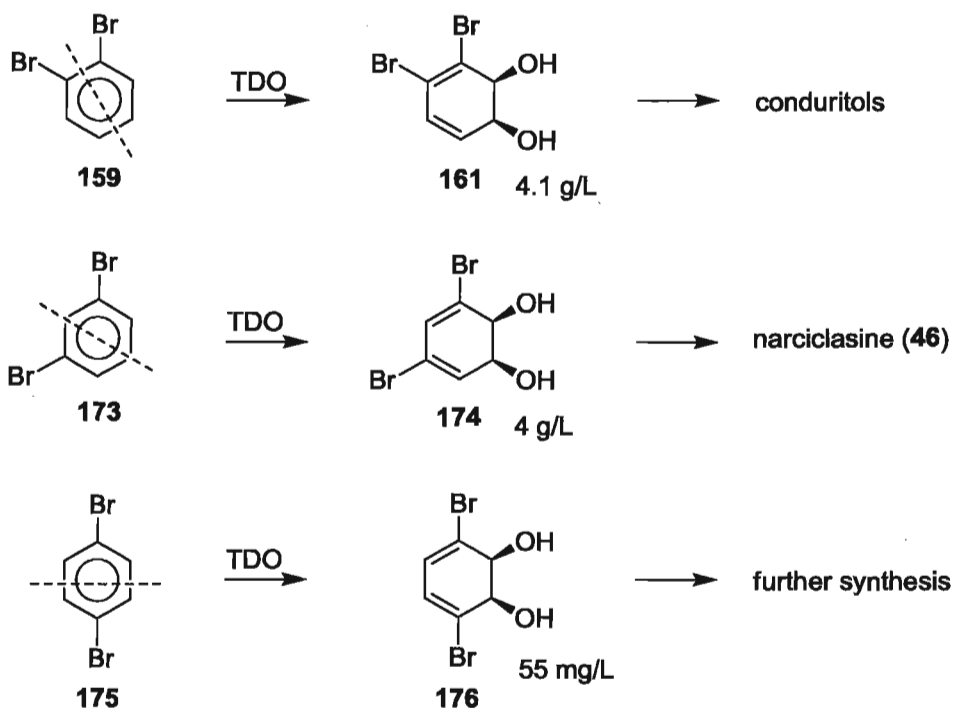
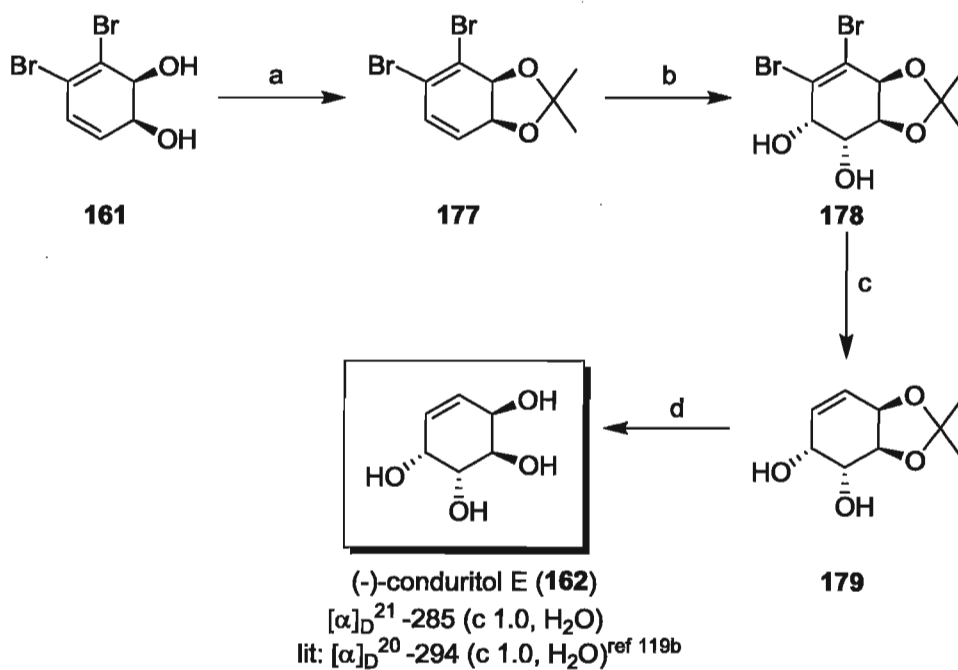


Figure 23. Symmetry of dibromobenzenes and the metabolism to diols.

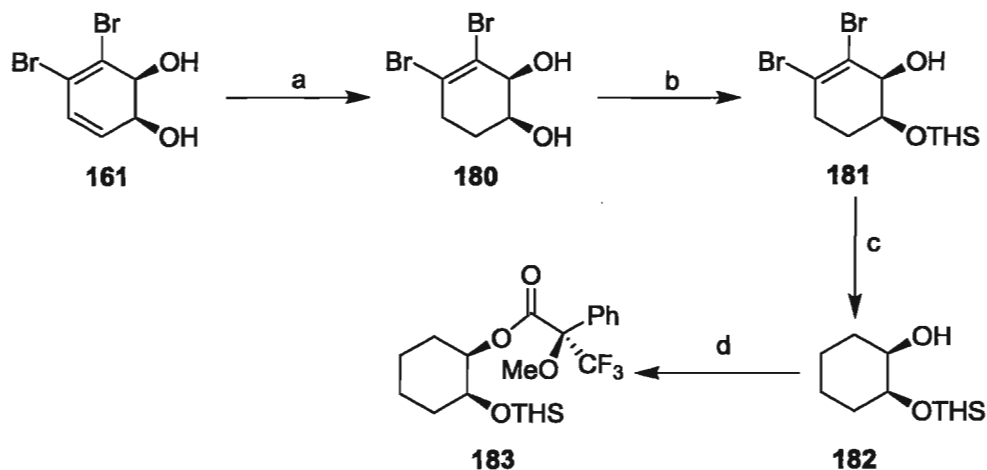
o-Dibromobenzene (**159**) was converted to the corresponding dienediol **161** by enzymatic oxidation with recombinant *E. coli* JM 109 (pDTG601). The scale of the reaction was increased from 0.5 L to 15 L to give an eventual yield of 4.1 g/L of the diol. This oxidation occurred selectively to provide 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}



Reaction conditions: (a) 2,2-DMP, *p*TsOH, rt, 95%; (b) OsO₄, NMO, acetone-H₂O, rt, 71%; (c) *n*Bu₃SnH, AIBN, THF, reflux, 64%; (d) 3% conc HCl, MeOH, rt, 81%

Scheme 21. Synthesis of (-)-conduritol E (162).

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**.¹²¹ Examination of the ¹⁹F NMR spectrum showed a single peak at -72.8 ppm, indicating enantioselectivity $\geq 95\%$ in the original biooxidation of *o*-dibromobenzene.



Reaction conditions: (a) potassium azodicarboxylate, AcOH, MeOH, 0 °C, 95%; (b) THSCL, imidazole, DCM, 0 °C, 86%; (c) H₂, PtO₂, Et₃N, rt, 52%; (d) Mosher's acid chloride, Et₃N, DCM

Scheme 22. Synthesis of Mosher ester **183**.

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.¹²² 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* UV4.¹²³ 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).

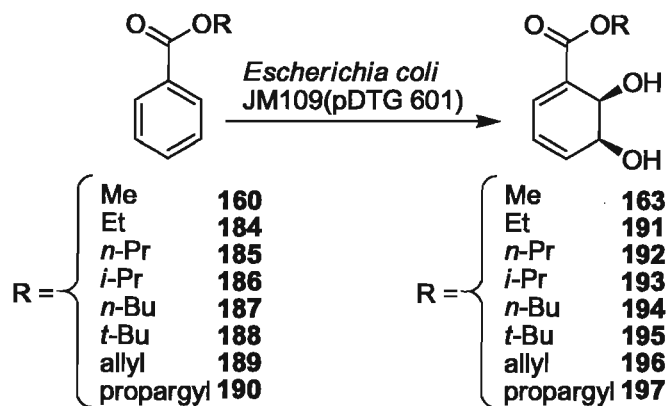


Figure 24. Enzymatic dihydroxylation of benzoate esters.

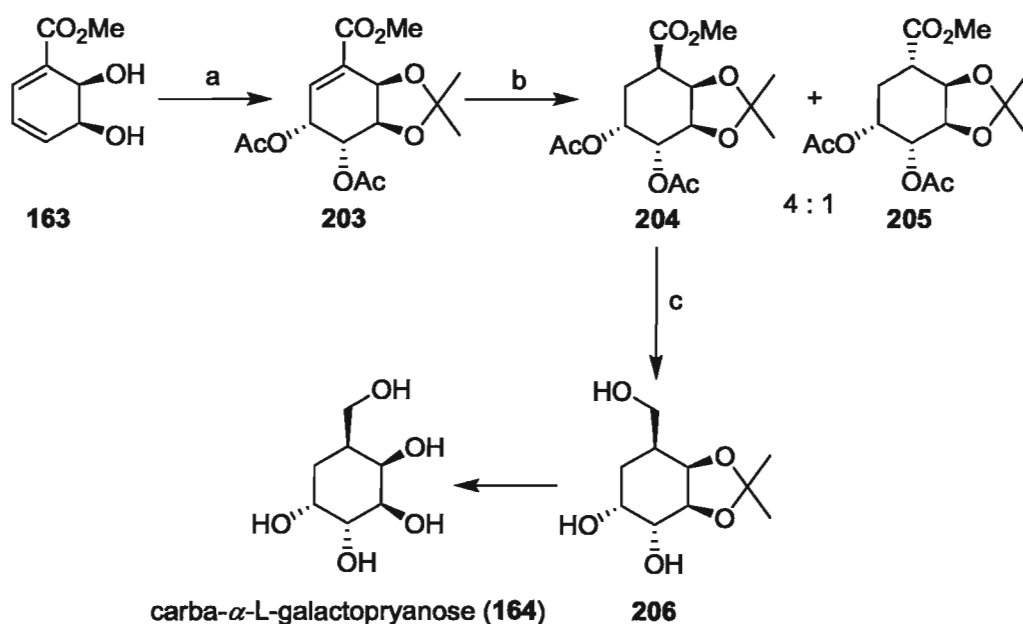
All of the required benzoate esters were either commercially available or prepared by standard procedures.¹²⁴ Each benzoate (entries 1-8, Table 1) was screened on small scale in Fernach shake flasks with cells harvested from a 15 L fermentor. In cases where benzoates were substrates the resulting metabolites 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.

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

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.¹²² 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**

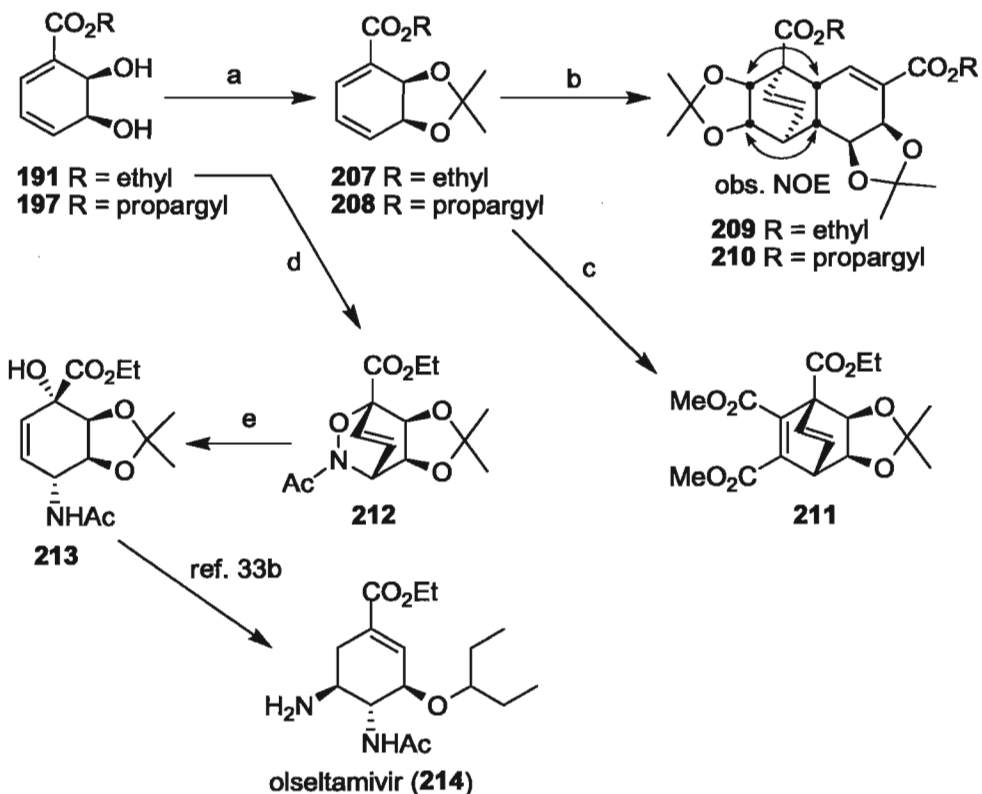
acetonide prior to treatment with OsO₄. The isolation of the free diol from this oxidation was confounded by its polarity and decomposition during purification. Protection of the crude diols as their acetates allowed for isolation of intermediate **203** in good yield over the three steps. The acrylate **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**).¹²⁵ Protected carbasugar **206** was identical in every respect to the previously prepared material, save for a slightly higher optical rotation ($[\alpha]_D^{20}$ -57.08 vs. $[\alpha]_D$ -47).



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.¹²⁶ 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.



Reaction conditions: (a) 2,2-DMP, *p*-TsOH, acetone, R = ethyl 92%, R = propargyl 63%; (b) rt, neat, 7 d, R = ethyl, 93%, R = propargyl 82%; (c) DMAD, toluene, 57%; (d) (i) 2,2-DMP, *p*-TsOH, (ii) AcNHOH, NaIO₄, MeOH, rt, 70% (over 2 steps); (e) Mo(CO)₆, CH₃CN/H₂O, reflux, 75%

Scheme 25. Application of Diels-Alder reactions to benzoate-derived diols.

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**.¹²⁷ 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**).^{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 8th 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.

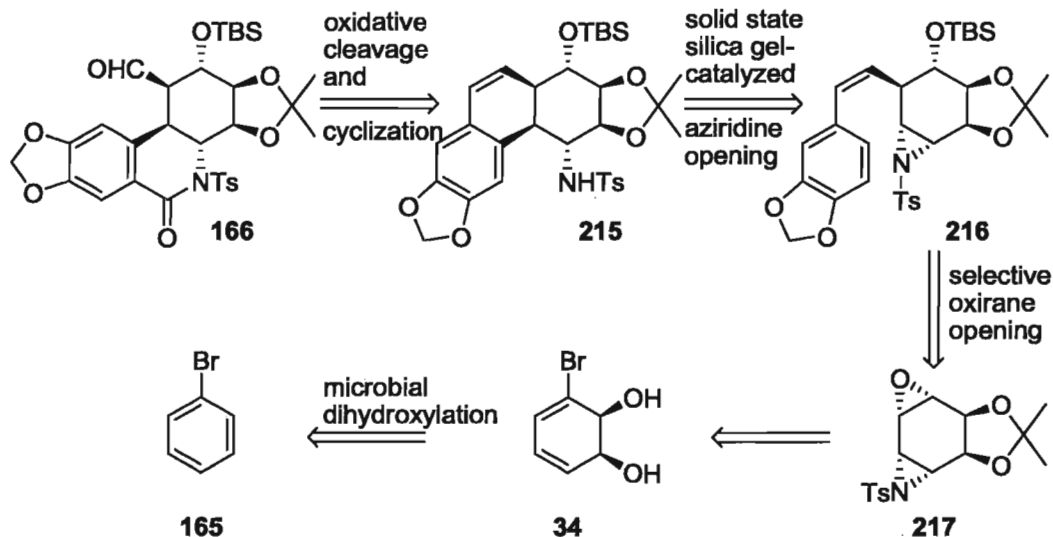
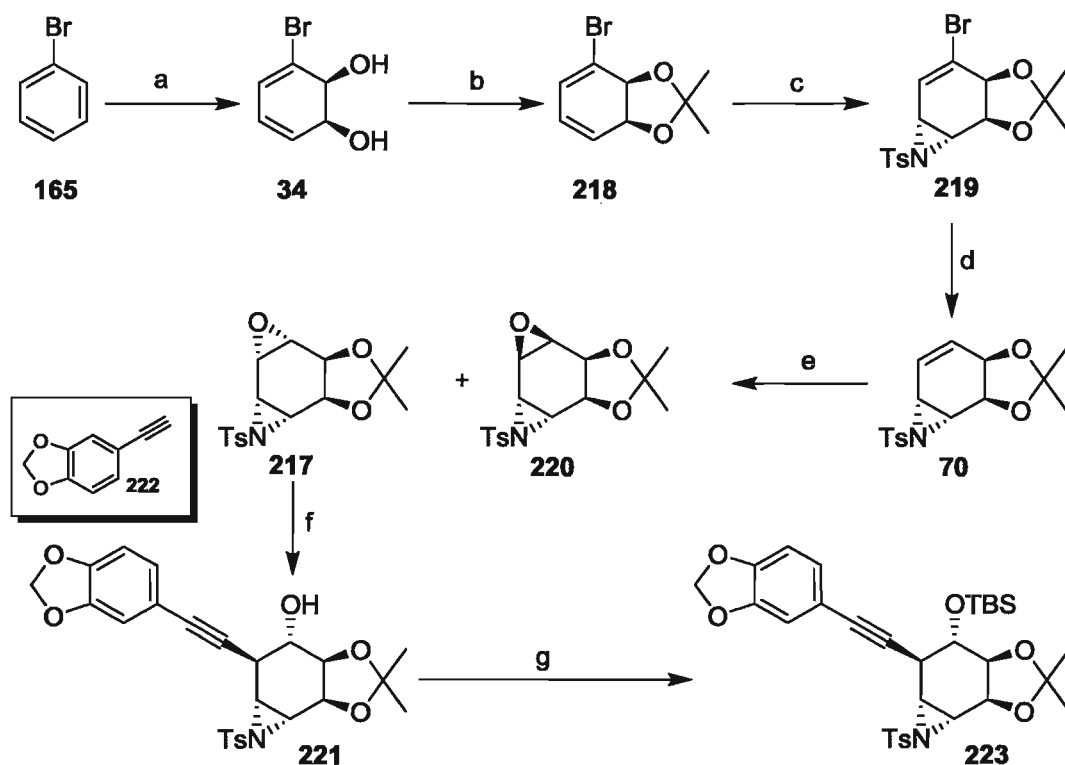


Figure 25. Retrosynthetic analysis for the synthesis of *Amaryllidaceae* alkaloids and their C-1 analogues.

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).¹²⁸ The protection of the diol as its acetonide **218** allowed for facial-selective aziridination *via* Yamada-Evans protocol to give **219**.¹²⁹ 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**.

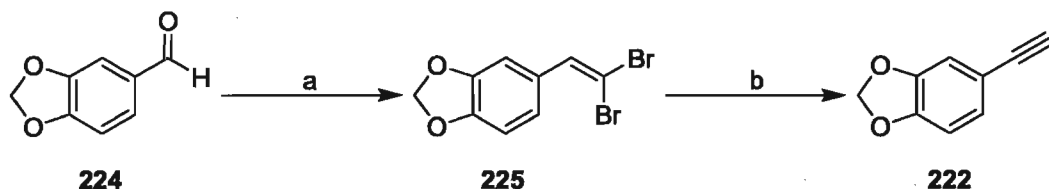


Reaction conditions: (a) *E. coli* JM109 (pDTG601A), 20 g/L; (b) 2,2-DMP, *p*-TsOH, acetone; (c) PhINTS, Cu(acac)₂, acetonitrile, 0 °C - rt; (d) *n*-Bu₃SnH, THF, AIBN, reflux, 76%; (e) *m*-CPBA, DCE, reflux, 96%; (f) **222**, *n*-BuLi, Me₂AlCl, toluene, -50 to -20 °C; (g) TBSOTf, Et₃N, CH₂Cl₂, -78 °C, 77% (2 steps)

Scheme 26. Synthesis of epoxyaziridine (**217**) and selective oxirane opening.

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 Corey¹³⁰ and Overman (Scheme 27).¹³¹ 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 conditions: (a) CBr_4 , Ph_3P , CH_2Cl_2 ; (b) *n*-BuLi, THF, $-78\text{ }^\circ\text{C}$, 75% (over 2 steps)
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

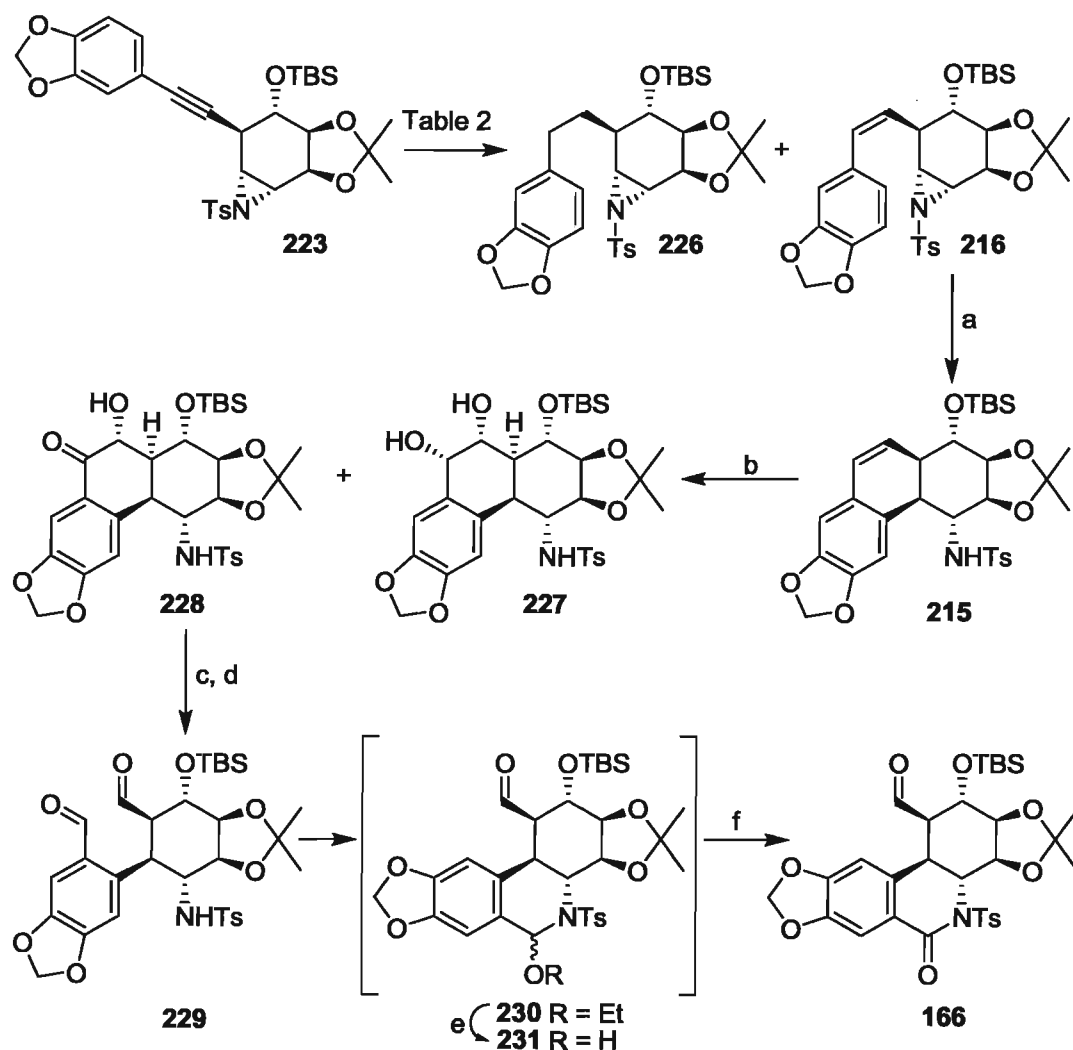
for complete conversion of the epoxy aziridine two equivalents of the alane were required. Fewer equivalents of the alane resulted in incomplete conversion of starting material while more equivalents yielded increased production of the byproducts mentioned above.

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**.

Conditions	Time (h)	Ratio of 223:226:216
Lindlar's cat. 20 mol%, MeOH, 40 psi H ₂	24 h	1 : 3 : 2
Lindlar's cat. 20 mol%, EtOAc, 40 psi H ₂	24 h	1 : 4 : 1
Lindlar's cat. 20 mol%, EtOH, 40 psi H ₂	24 h	1 : 2.5 : 1
Lindlar's cat. 40 mol%, MeOH, 1 atm H ₂	3.5 h	0 : 1 : 3
Lindlar's cat. 40 mol%, MeOH, 1 atm H ₂ quinoline (20 mol%)	3 h	0 : 0 : 1
Pd/C 40 mol%, MeOH, 1 atm H ₂	3 h	0 : 1 : 0
BH ₃ /SMe ₂ , cyclohexene, 0 °C	3.5 h	0 : 0 : 1

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).¹³² 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.



Reaction conditions: (a) silica gel, 120 °C, 24 h, 74%; (b) OsO₄, NMO, CH₂Cl₂/H₂O, rt, 89% (**228**); (c) NaBH₄, dioxane/EtOH, 0 °C; (d) NaIO₄, dioxane/H₂O, rt; (e) THF/H₂O, TsOH, rt, 2 h (f) IBX, DMF, rt, 61% (over 3 steps)

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**.

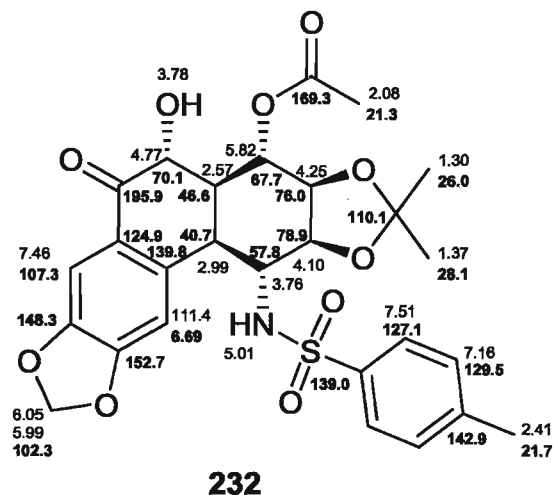
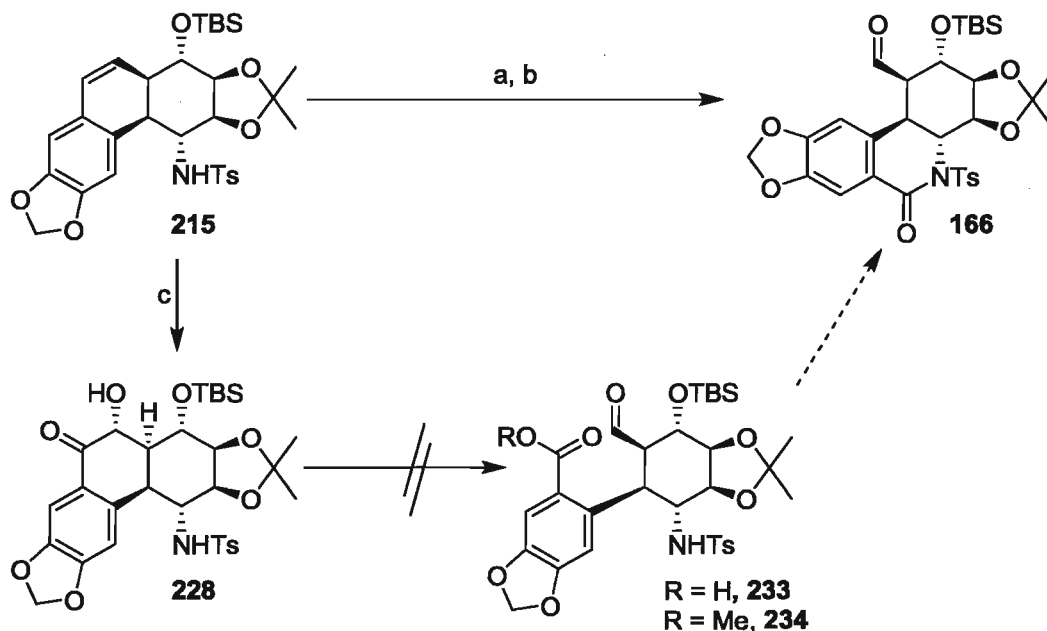


Figure 26. Full assignment of NMR chemical shifts for the acetate of keto alcohol **232**.

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

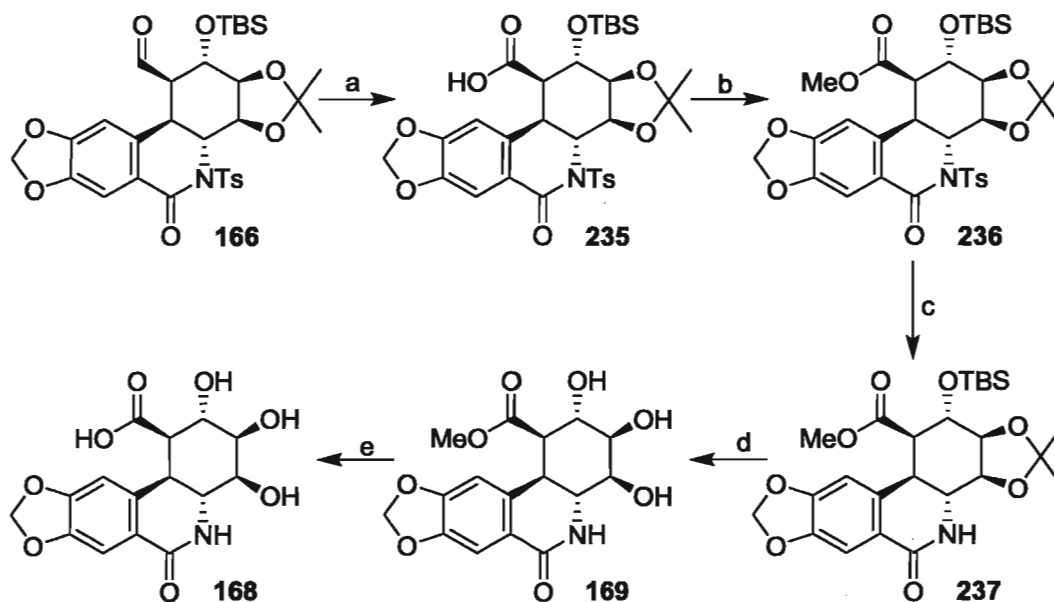
direct ozonolysis to alcohol **231** and oxidation to C-1 aldehyde **166**. This protocol provides the desired phenanthridone in the most efficient and fewest steps.



Reaction conditions: (a) i) O₃, CH₂Cl₂, Sudan Red, -78 °C, ii) Me₂S, -78 °C - rt; (b) IBX, DMF, rt, 76% (over 2 steps); (c) OsO₄, NMO, CH₂Cl₂/H₂O, 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 *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 *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**.

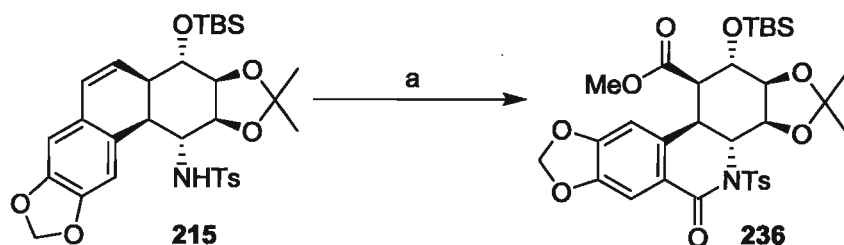


Reaction conditions: (a) *m*-CPBA, Na₂HPO₄, CH₂Cl₂, 40 °C, 85%; (b) CH₂N₂, Et₂O, 0 °C; (c) Na/naphthalene, DME, -50 °C, 58%; (d) 3% HCl in MeOH, rt, 69%; (e) LiOH, MeOH, rt, 95%

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.¹³³ 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

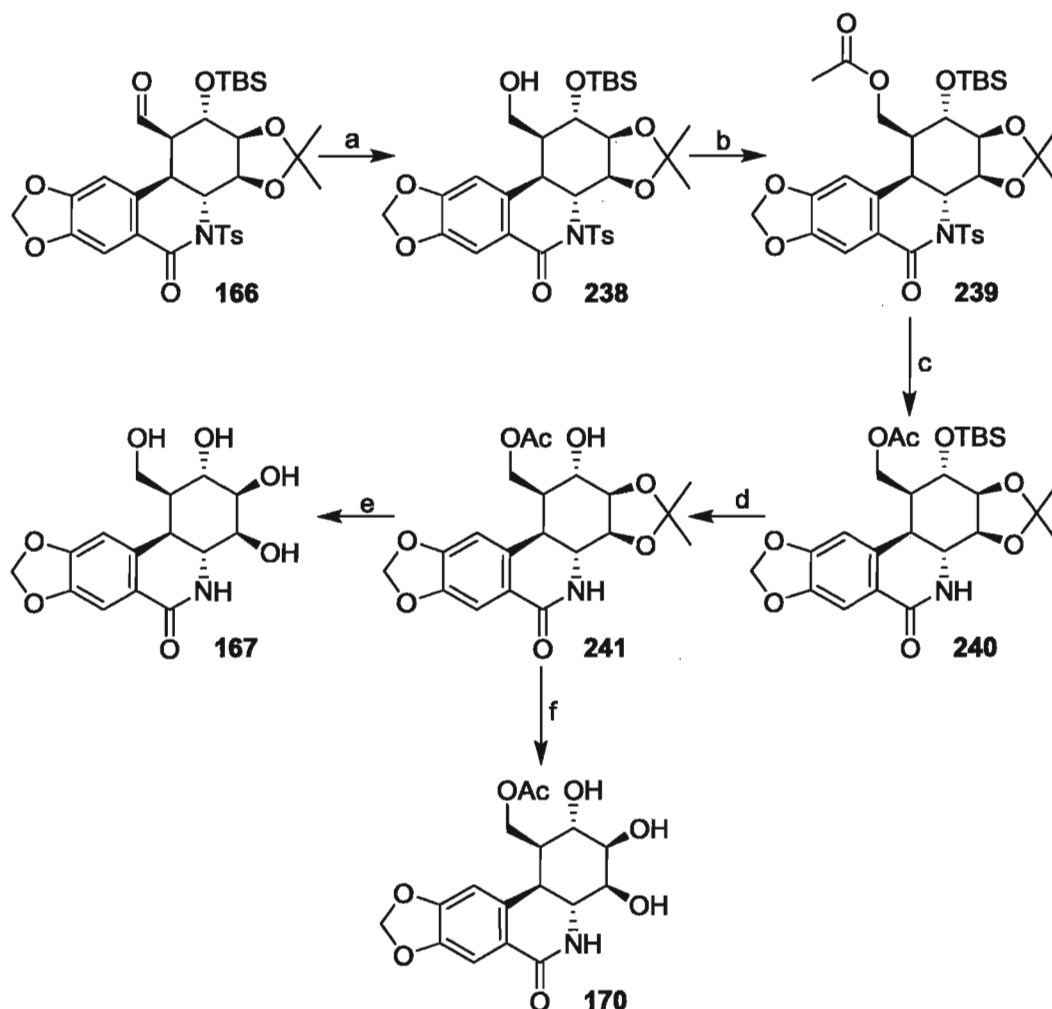
transformation was attributed to the instability of other functionality under prolonged treatment with ozone.



Reaction conditions: (a) O₃, NaOH, MeOH, CH₂Cl₂, -78 °C, 6%

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

The C-1 hydroxymethyl and acetoxymethyl 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.



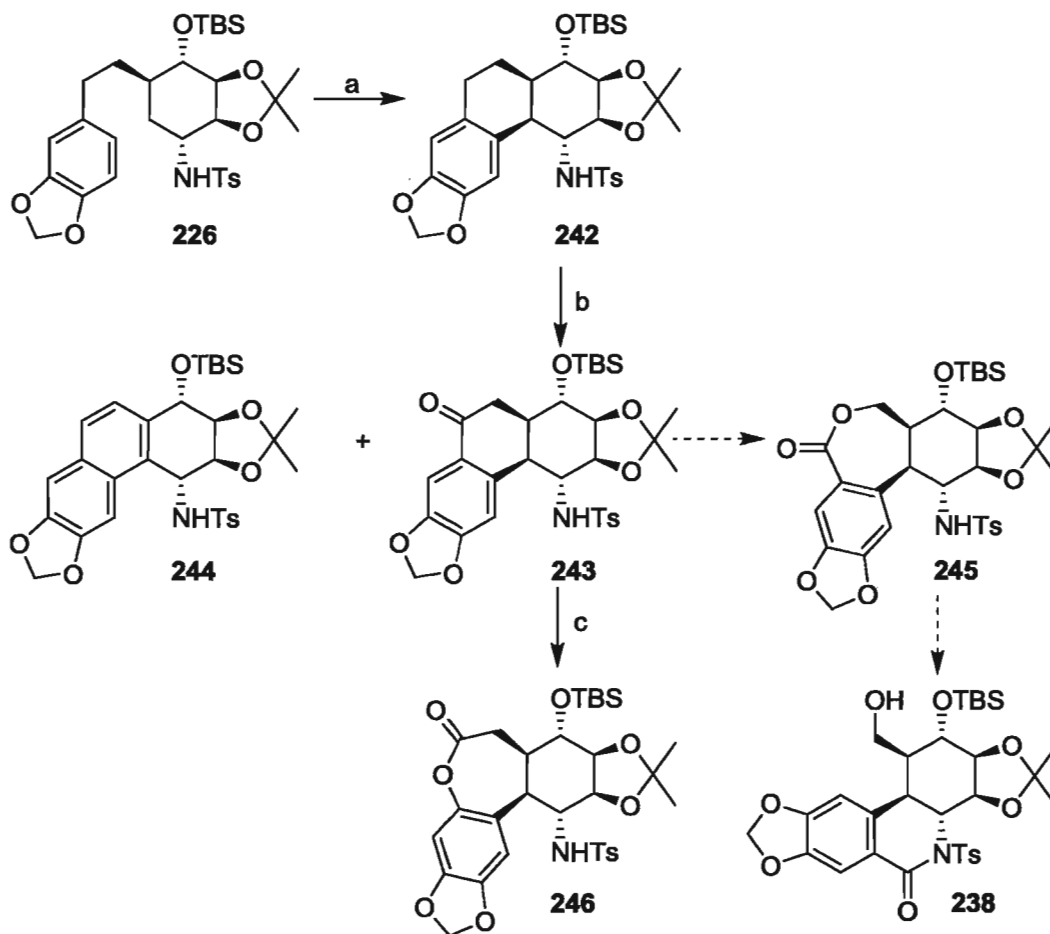
Reaction conditions: (a) NaBH_4 , dioxane, EtOH, 0°C , 85%; (b) Ac_2O , pyridine, DMAP, CH_2Cl_2 , 81%; (c) Na/naphthalene, DME, -78°C ; (d) TBAF, THF, 0°C , 74% (2 steps); (e) (i) K_2CO_3 , MeOH, H_2O , (ii) HCl, H_2O , 75%; (f) 3% HCl in MeOH, rt, 45%

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

Hydrolysis of the C-1 acetoxymethyl derivative **241** with K_2CO_3 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**.



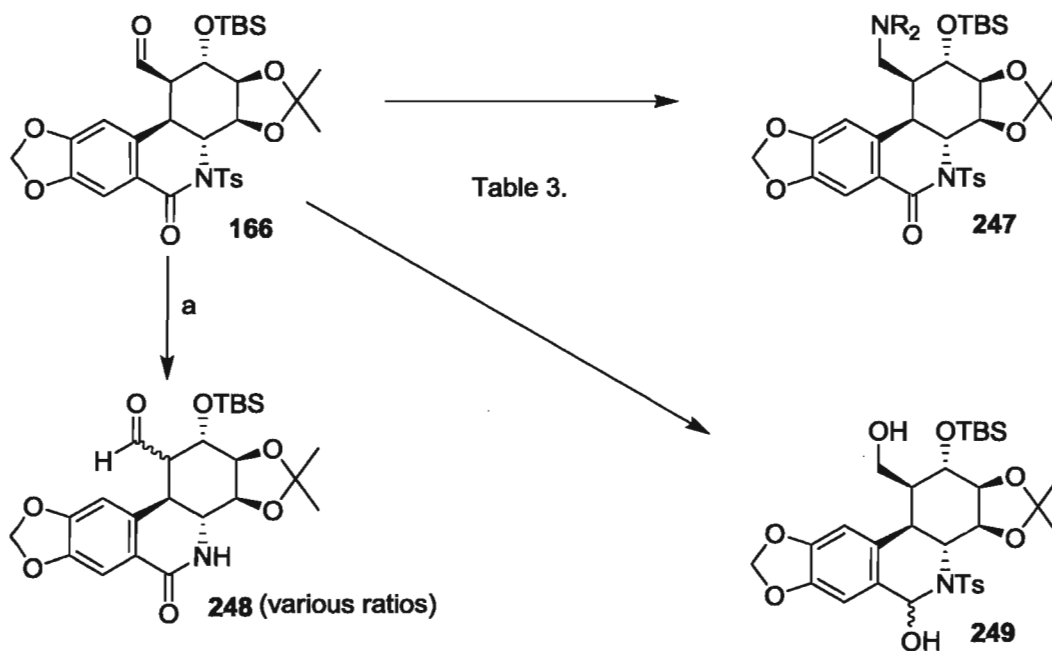
Reaction conditions: (a) silica gel, 120 °C, 24 h, 69%; (b) PCC, CH₂Cl₂, rt, 11 h, **243:244** (46%:23%); (c) *m*-CPBA, Na₂HPO₄, CH₂Cl₂, 40 °C, 22%

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 transesterification 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).



Reaction conditions: (a) Na/naphthalene, DME, -78 °C, 78%

Scheme 34. Attempted synthesis of C-1 amines.

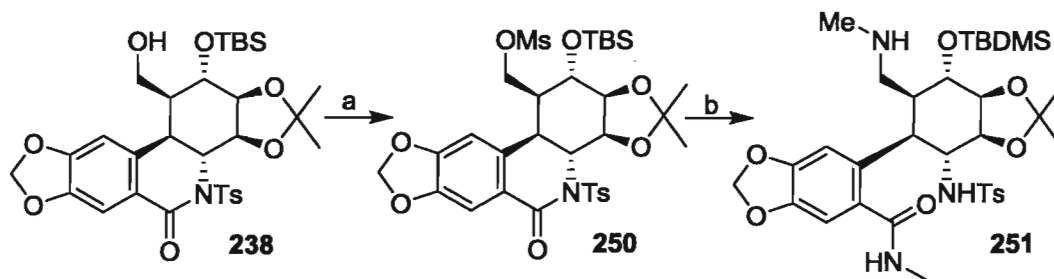
Table 3. Attempted reductive amination reactions.

Amine	Conditions	Result
HNEt ₂	NaBH ₄ , AcOH, MeOH, rt	reduction of amide and aldehyde
HNEt ₂	NaBH ₃ CN, AcOH, MeOH, rt – 50 °C	no reaction at rt, reduction of aldehyde and cleavage of acetonide at 50 °C
HNMe ₂ HCl	NaBH ₃ CN, MeOH, rt – 50 °C	no reaction, decomposition at 50 °C
MeNH ₂	NaBH ₃ CN, AcOH, MeOH, rt	reduction of aldehyde, slow cleavage of acetonide (15 h)
MeNH ₂ HCl	NaBH ₃ CN, MeOH, rt	no reaction
MeNH ₂	NaBH ₃ CN, TiCl ₄ , rt	reduction of amide and aldehyde (249 , 52%)

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/naphthalene 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.



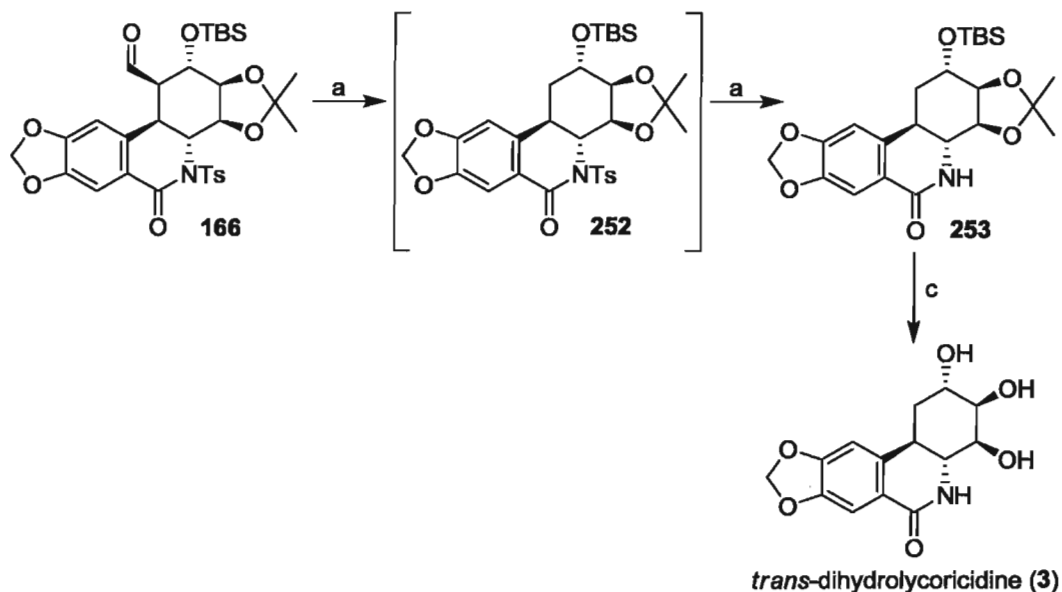
Reaction conditions: (a) MsCl, Et₃N, CH₂Cl₂, 0 °C, 88%; (b) MeNH₂, THF, 55 °C (sealed tube), 41%.

Scheme 35. Displacement of C-1 mesylate and synthesis of amide **251**.

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**.



Reaction conditions: (a) $\text{RhCl}(\text{Ph}_3\text{P})_3$, 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).

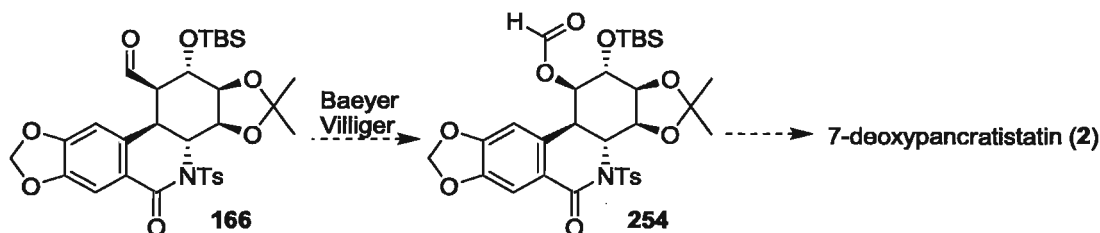
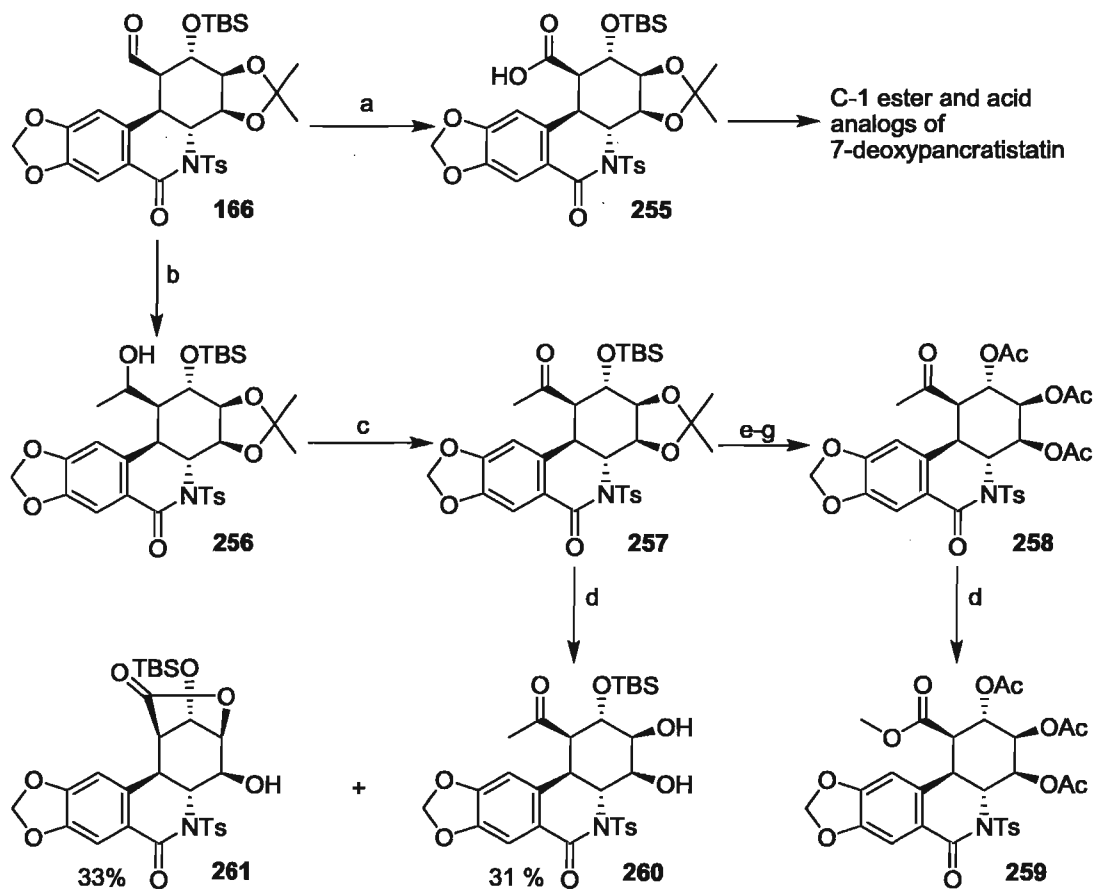


Figure 27. Baeyer-Villiger reaction in the synthesis of 7-deoxypancratistatin (**2**).

The proposed transformation was based on a small number of reports which describe Baeyer-Villiger oxidation of cyclohexane carboxyaldehydes to their corresponding formates.¹³⁴ In general, aliphatic aldehydes give acids when treated under Baeyer-Villiger conditions.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.



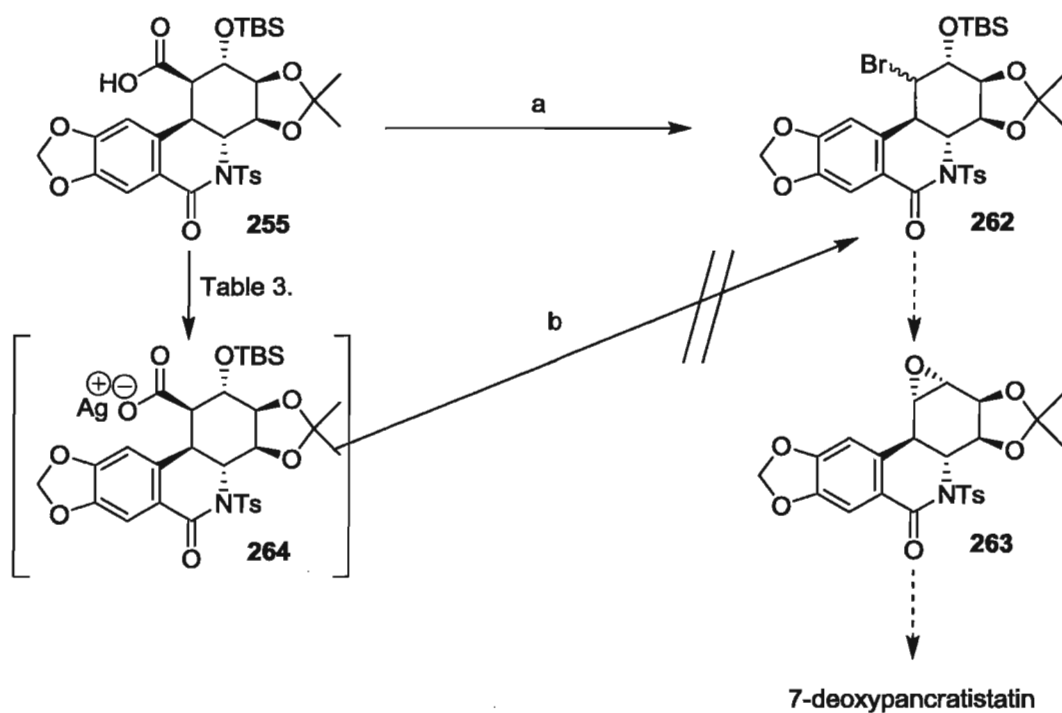
Scheme 37. Investigation of Baeyer Villiger oxidation for C-1 functionalization.

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 silyl 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.

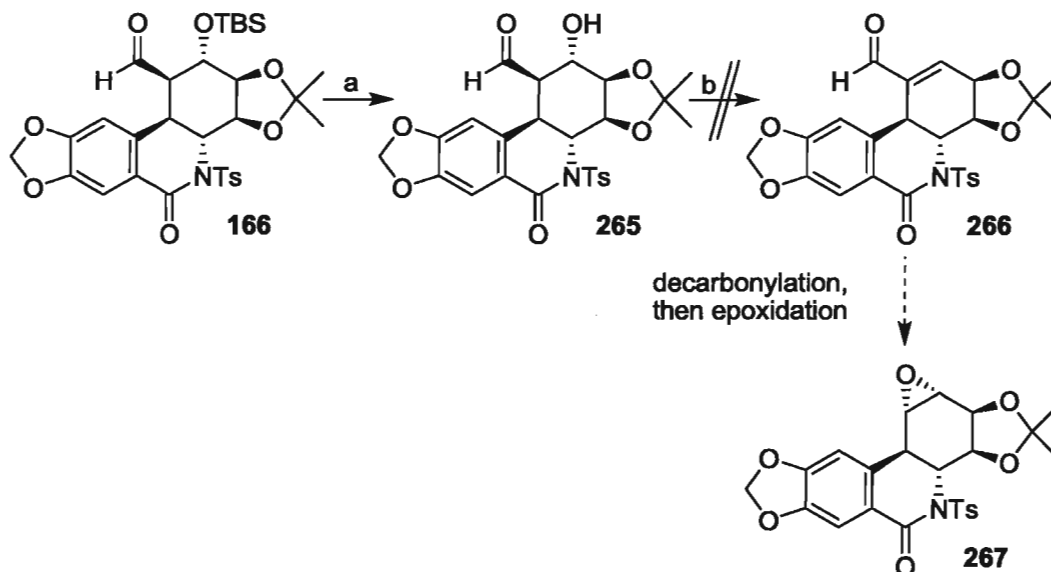


Reaction conditions: (a) (i) HgO (red), CCl_4 , (ii) Br_2 , reflux, 15%; (b) Br_2 , CCl_4 , reflux
Scheme 38. Hunsdiecker conditions employed toward the synthesis of **263**.

Table 4. Conditions attempted for formation of silver salt **264**.

Conditions for salt formation	Notes about salt	Result of Hunsdiecker
EtOH, AgNO ₃	Fine white solid, 25 % yield	Recovery of starting material
EtOH, i) NaOH, ii) AgNO ₃	Black solid, 42 %	No reaction, recovery of starting material
EtOH, i) NaOEt, ii) AgNO ₃	Black solid, 68%	No reaction, recovery of starting material

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).

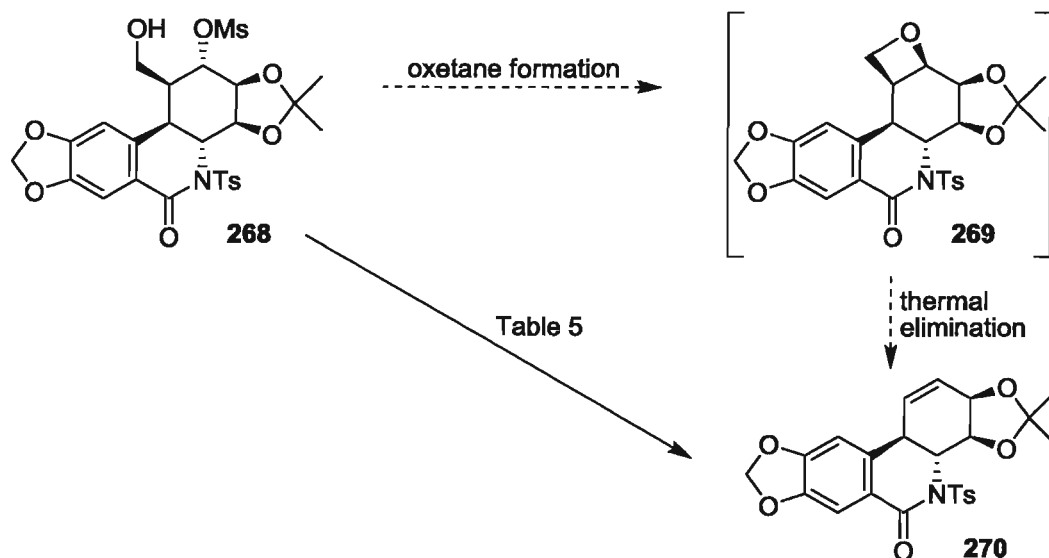


Reaction conditions: (a) TBAF, THF, rt, 22%; (b) (i) TsCl, pyridine, DMAP, CH₂Cl₂, rt. (ii) various bases

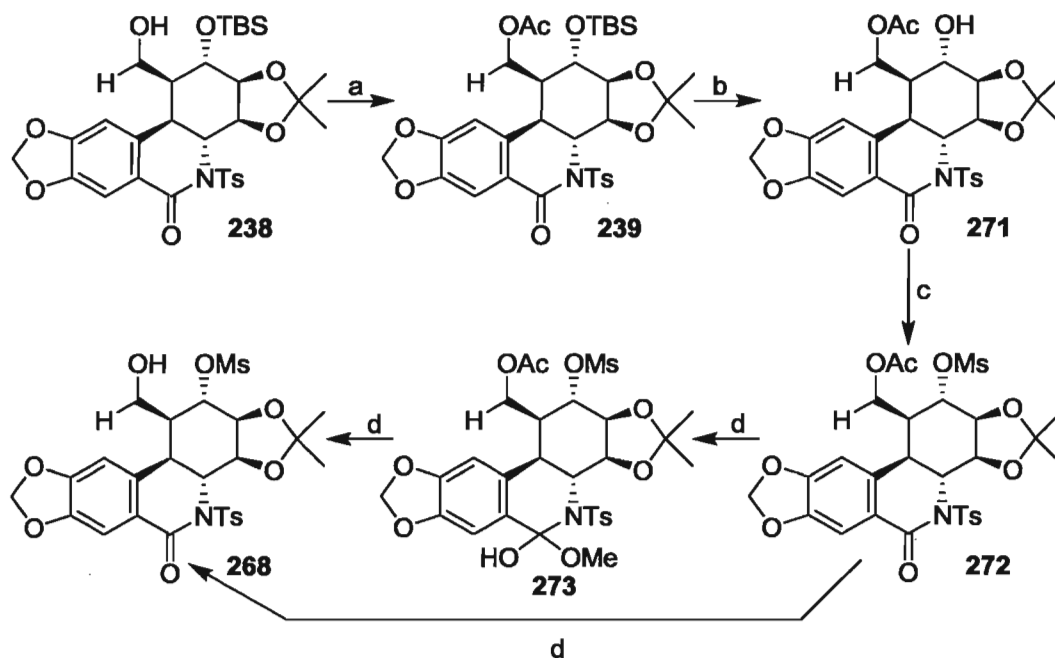
Scheme 39. Attempted synthesis of epoxide **267** through C-2 hydroxyl elimination.

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 synthesis¹³⁸ 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**.



Reaction conditions: (a) Ac_2O , DMAP, pyridine, CH_2Cl_2 , rt, 81%; (b) TBAF, THF, $0\text{ }^\circ\text{C}$, 84%; (c) MsCl , Et_3N , CH_2Cl_2 , $0\text{ }^\circ\text{C}$, 96%; (d) K_2CO_3 , $\text{MeOH}/\text{H}_2\text{O}$, $0\text{ }^\circ\text{C}$ - rt, 85%

Scheme 41. Synthesis of mesylate **268**.

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 $0\text{ }^\circ\text{C}$ 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.

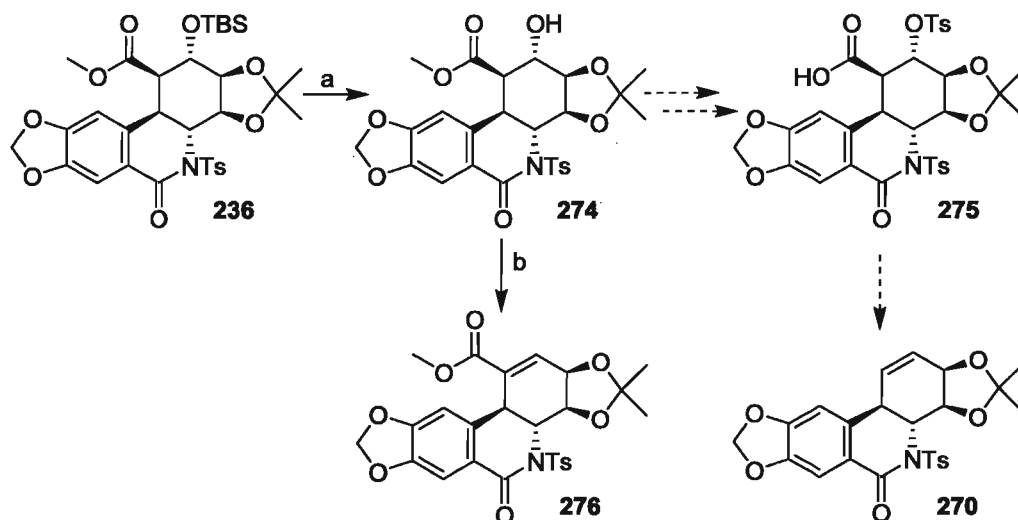
As mentioned previously, it was envisioned that olefin **270** could be generated from mesylate **268** by both formation and thermal elimination of oxetane **269** or by Grob fragmentation of alcohol **268**. A variety of conditions were investigated to effect the desired transformation and are summarized 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.

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

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).



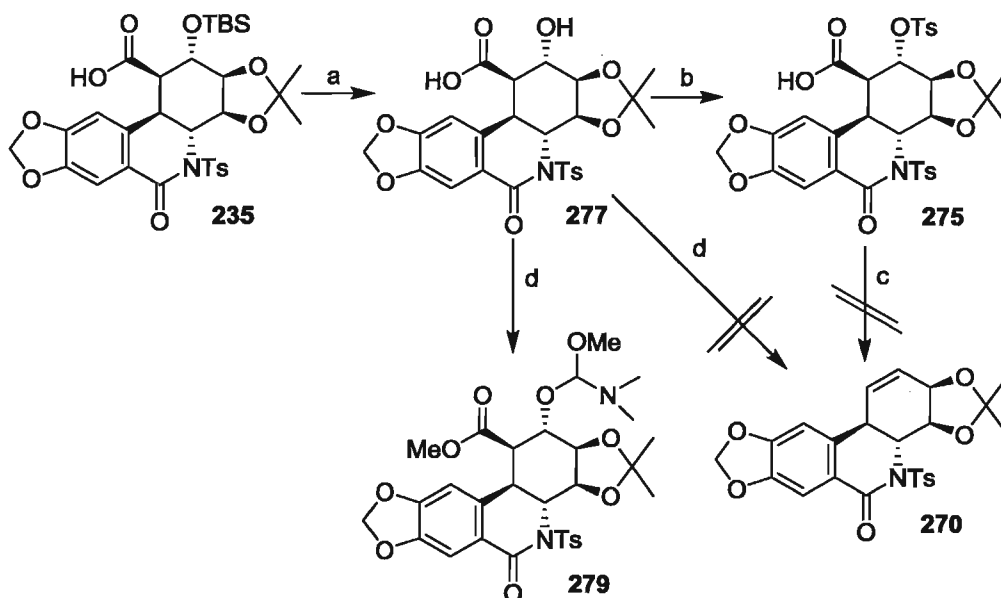
Reaction conditions: (a) TBAF, THF, rt, 82%; (b) TsCl, pyridine, DMAP, CH₂Cl₂, rt, 28% with 41% recovery of starting material.

Scheme 42. Approaches toward olefin **270** by decarboxylation.

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.

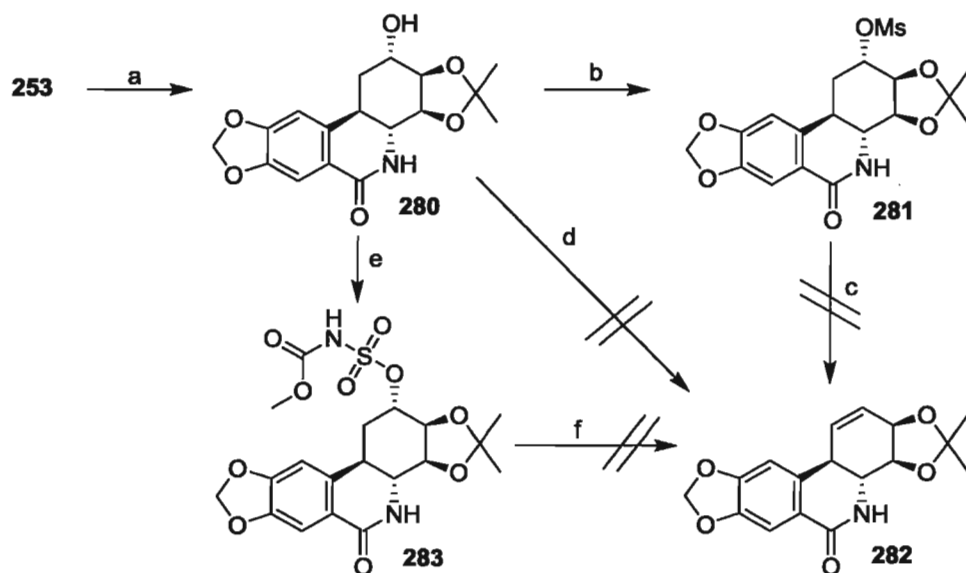


Reaction conditions: (a) TBAF, THF, 50 °C, 44%; (b) MeLi, TsCl, THF, 35%; (c) KHMDS, toluene, 120 °C; (d) DMF dimethyl acetal, CHCl_3 , reflux; 58%

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 E_2 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**.

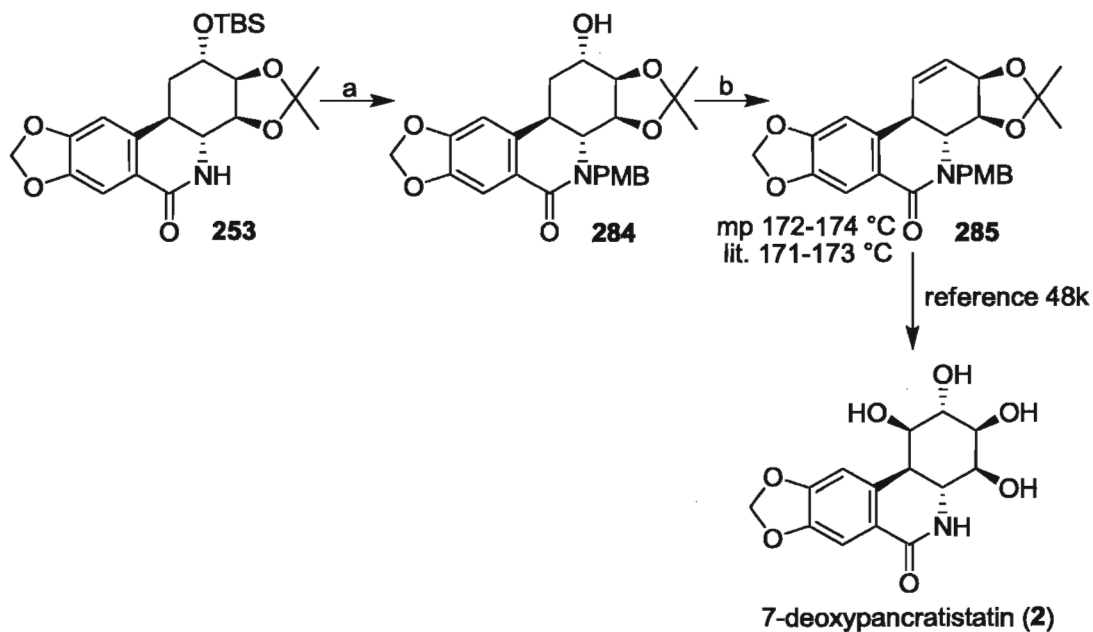


Reaction conditions: (a) TBAF, THF, 0 °C; (b) MsCl, pyridine, CH₂Cl₂, rt, 84% (2 steps); (c) pyridine, 100 °C; (d) PPh₃, DIAD, dioxane, rt - reflux; (e) Burgess reagent, THF, rt - reflux, 42%; (f) KH, DME, reflux

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.^{49k} This intermediate became our new target and was prepared starting from silyl ether **253** (Scheme 45). *N*-Protection of the phenanthridone with *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.



Reaction conditions: (a) (i) NaH, PMBBBr, DMF, 0 °C - rt, (ii) TBAF, THF, 0 °C, 64% (2 steps); (b) NaH, CS₂, MeI, 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

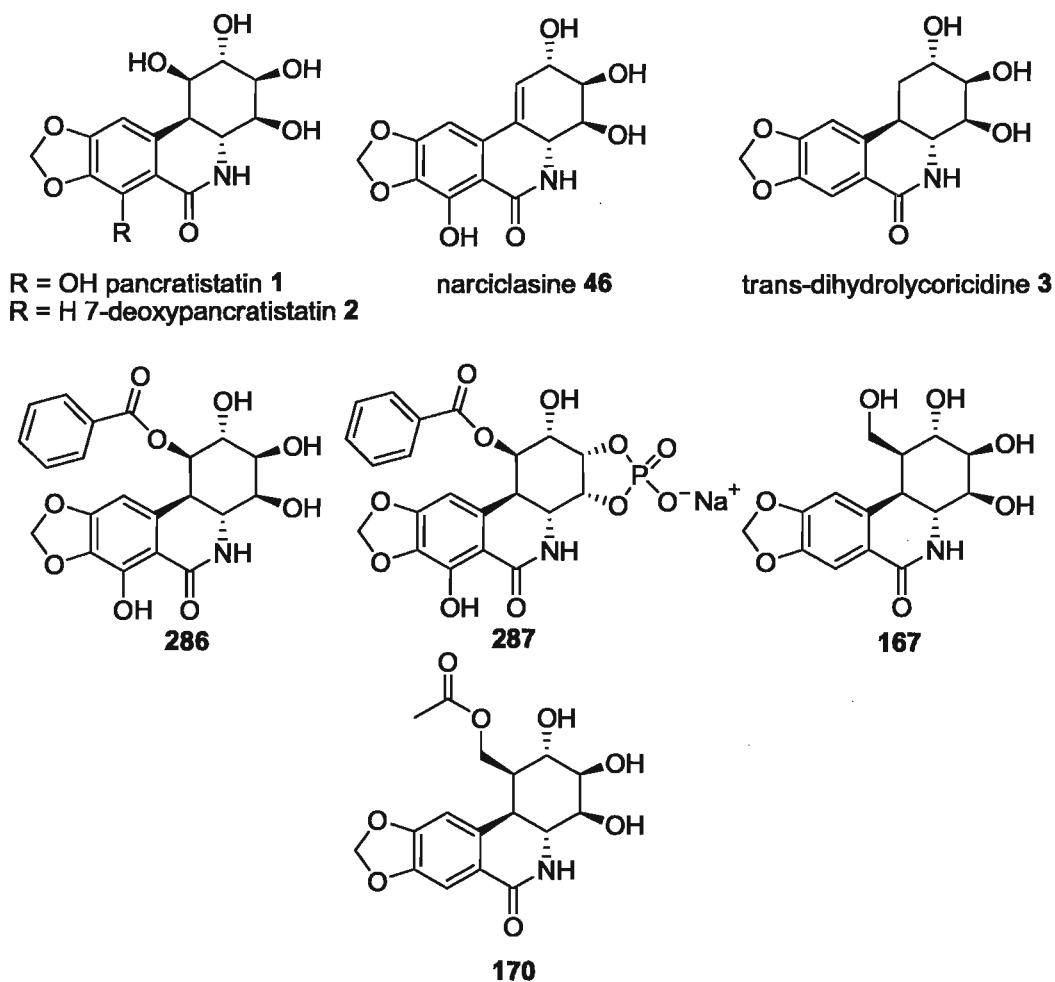
alcohol **167** and its acetate **170** displayed significant activities and were promptly tested for anticancer activity against a panel of human cancer cells. These two analogues were also evaluated for their ability to selectively induce apoptosis in human leukemia and neuroblastoma cells compared 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 derivitization 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 Lymphocytic Inhibitory Activities.

P388 Murine Lymphocytic Leukemia and Human Cancer Cell Results [$\mu\text{g/mL}$] (ED 50)									
Compound	leukemia P388	pancreas BxPC-3	breast MCF-7	CNS SF-268	lung-NSC NCI-H460	colon KM20L2	prostate DU-145	leukemia Jurkat	neuroblastoma Shsy5y
1	0.039	0.028	0.032	0.017	0.048	0.062	0.016	0.163	0.163
1	0.44	–	–	–	0.29	0.22	–	–	–
46	0.001	0.026	0.019	0.021	0.032	0.021	0.011	–	–
3	0.029	0.046	0.034	0.059	0.043	0.051	0.040	–	–
286	0.0016	0.0019	0.00031	0.00055	0.0001	0.00037	0.00021		
287	0.061	0.25	0.041	0.17	0.029	0.13	0.13		
167		0.19	0.65		0.09		0.26	1.615	1.615
170		0.11	0.29		0.11		0.37	0.183	0.183



The C-1 hydroxymethyl analogue **167** and acetoxymethyl 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.

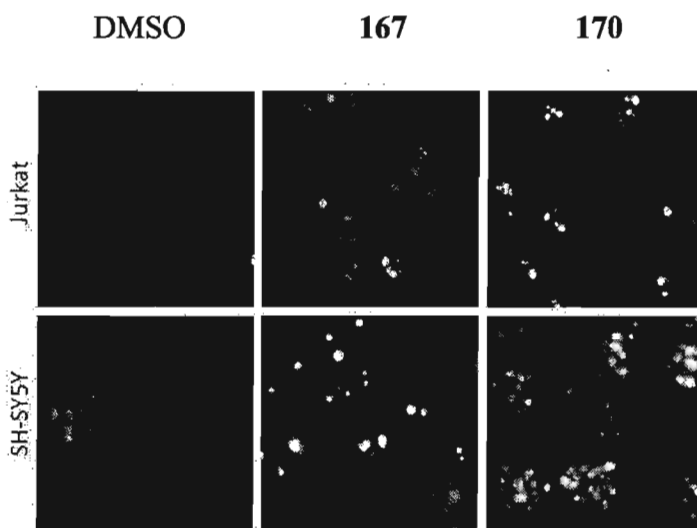


Figure 28. Evaluation of apoptosis induced by **167** and **170** after 72 hours with Hoechst staining.

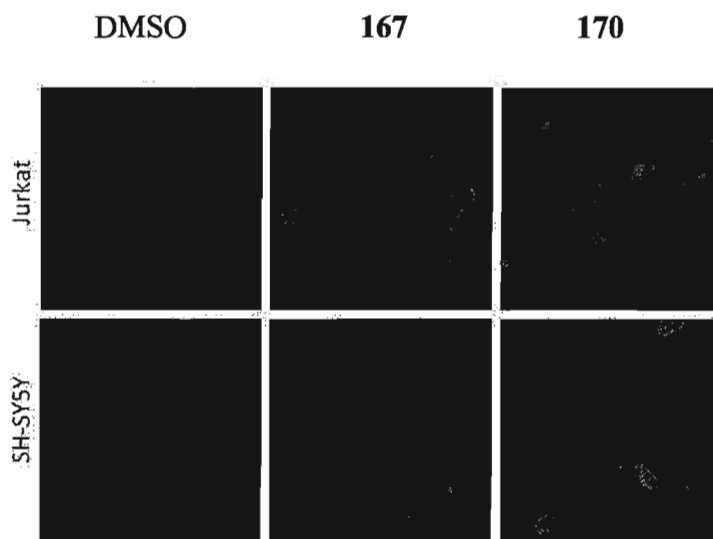


Figure 29. Evaluation of apoptosis induced by **167** and **170** after 48 hours and Annexin-V binding.

The ED50 (50% of cells were apoptotic) for alcohol **167** was determined to be 1 μ M 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).

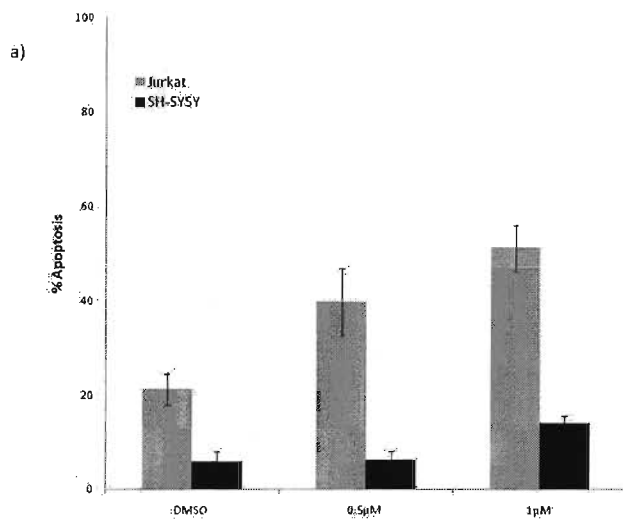


Figure 30. Apoptosis induced by **167** after 72 hours and determination of ED50.

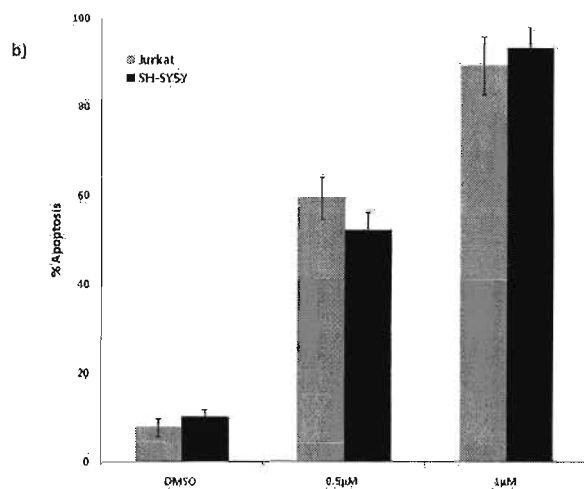


Figure 31. Apoptosis induced by **170** after 72 hours and determination of ED50.

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**).

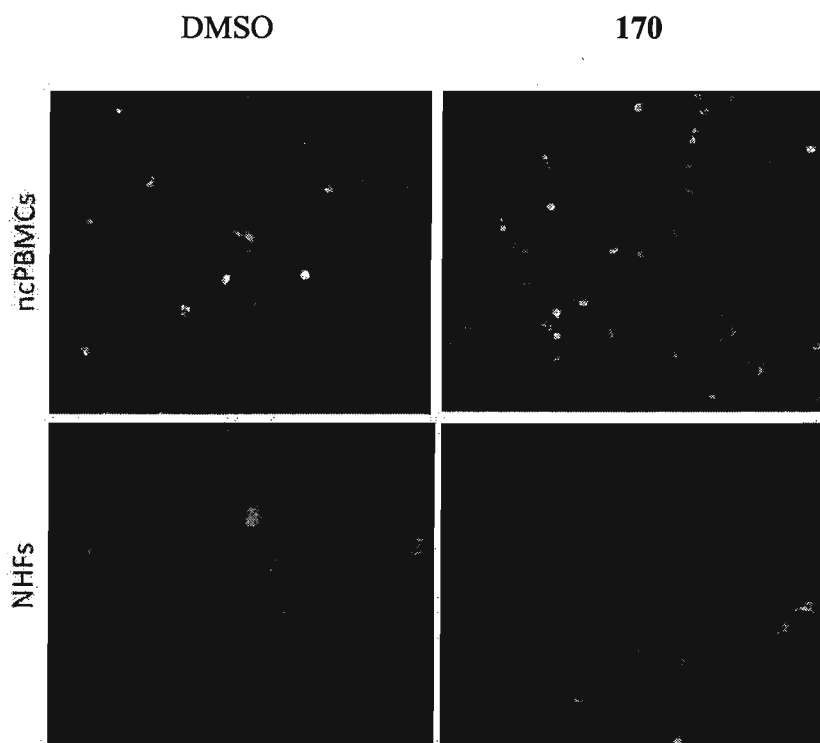


Figure 32. Screening of 170 against non-cancerous human cells with Hoechst staining.

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

The use of heteroaromatic cycloadditions has enjoyed widespread application in synthesis.¹⁴³ One class of heteroaromatics which has found particular use in recent years is disubstituted pyridazines.^{2,3,144} 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).

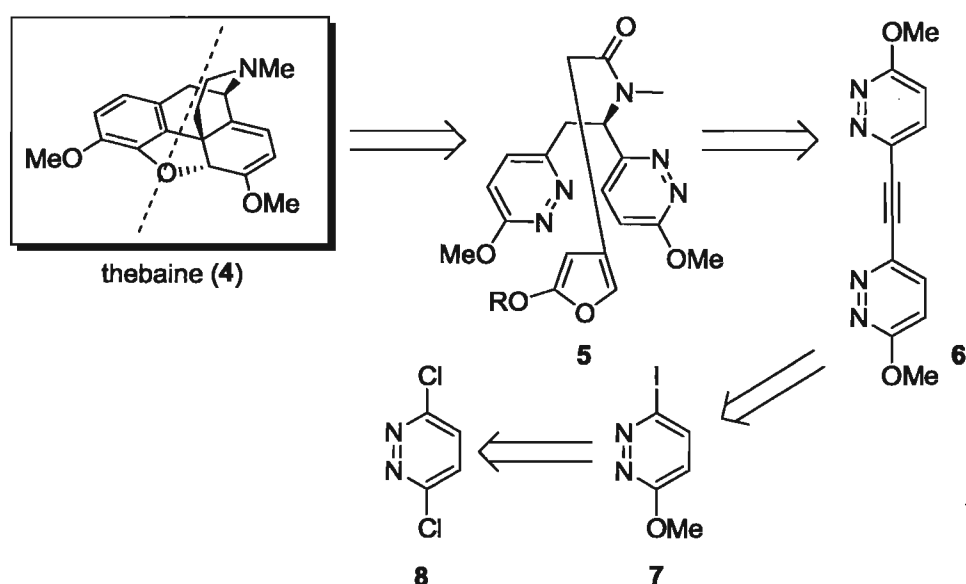


Figure 33. Retrosynthetic analysis of latent symmetry approach to thebaine (**4**).

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**.

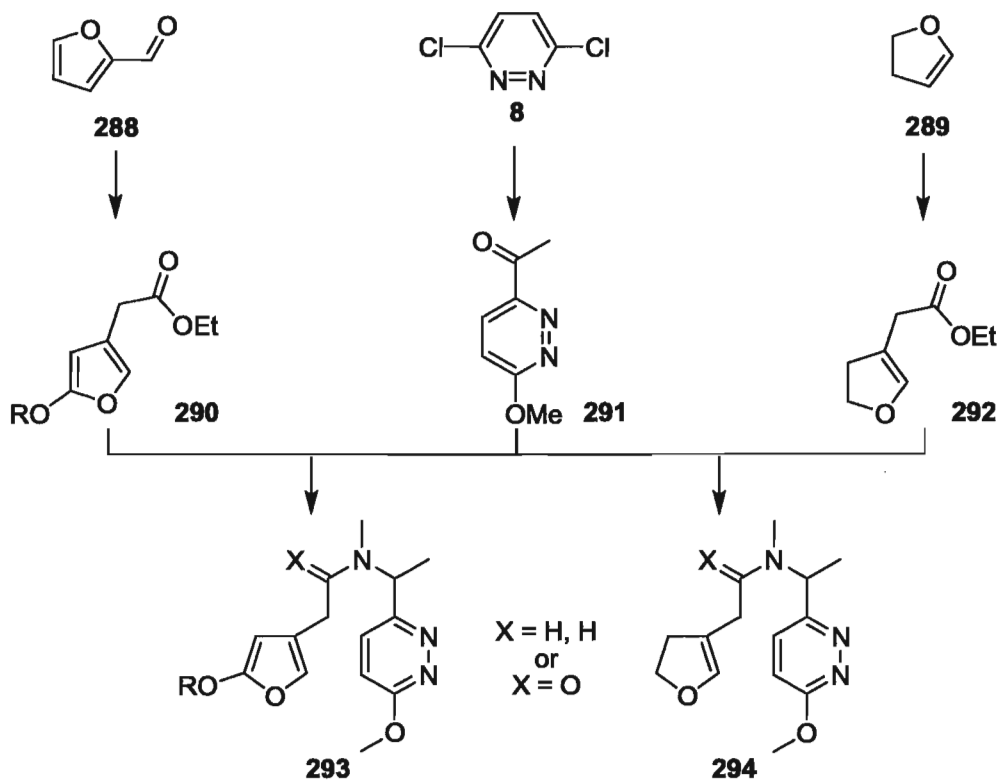
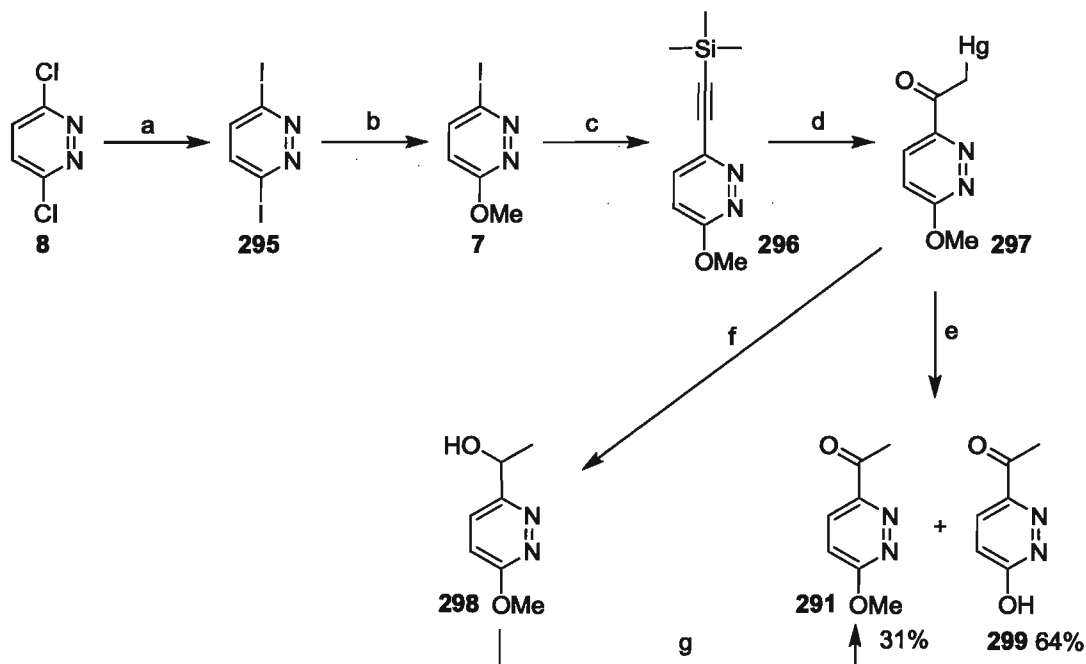


Figure 34. Proposed model systems for the synthesis of thebaine (**4**).

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%).



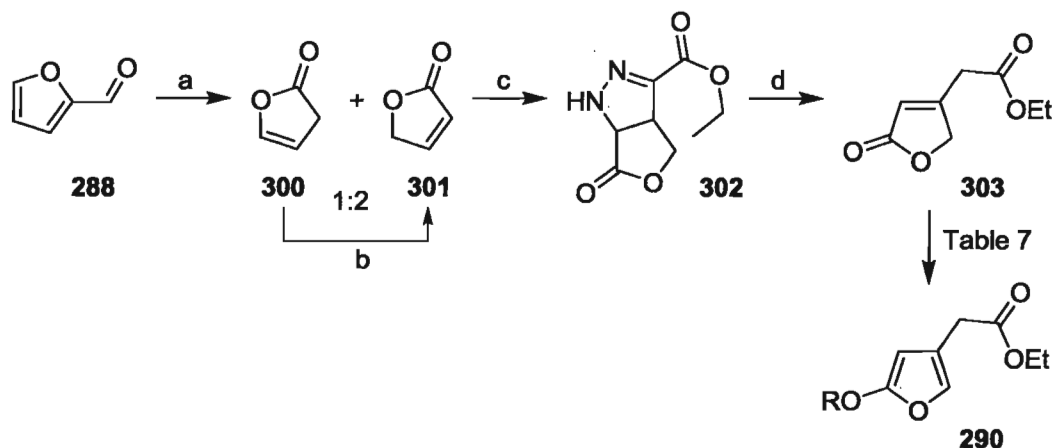
Reaction conditions: (a) KI, HI, acetone, reflux, 75%; (b) K_2CO_3 , MeOH, reflux, 52%; (c) TMS acetylene, CuI, $PdCl_2(PPh_3)_2$, Et_3N , THF, rt, 84%; (d) $HgSO_4$, H_2SO_4 , THF/ H_2O , 58%; (e) 20% H_2SO_4 in H_2O /acetone, reflux; (f) $NaBH_4$, EtOH, rt; (g) Collins' reagent, CH_2Cl_2 , 61% (2 steps)

Scheme 46. Synthesis of pyridazine **291**.

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.¹⁴⁵ 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),¹⁴⁶ 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 TBSCl proved to be poorly reactive in this case.

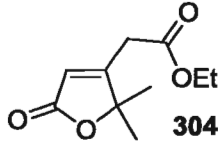
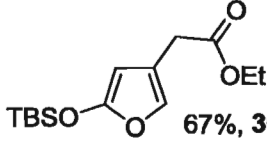
The more activated triflate did provide the desire furan **305** in good yield (67%).



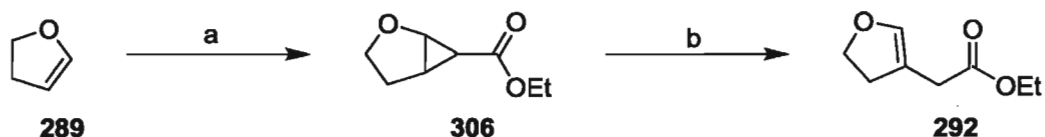
Reaction conditions: (a) 2-(dimethylamino)ethanol, formic acid, H_2O_2 , Na_2SO_4 , CH_2Cl_2 ; (b) Et_3N , 78% (2 steps); (c) ethyldiazoacetate, dioxane, $95\text{ }^\circ\text{C}$, 48%; (d) dioxane, $150\text{ }^\circ\text{C}$ (sealed tube), 89%.

Scheme 47. Synthesis of furan tether **290**.

Table 7 Formation of furan tether.

Entry	Conditions	Result
1	Dimethylsulfate, Et_3N , CH_2Cl_2 , rt	 304
2	TBSCl, Et_3N , CH_2Cl_2 , $0\text{ }^\circ\text{C}$ - rt	No reaction
3	TBSOTf, Et_3N , CH_2Cl_2 , rt	 67%, 305

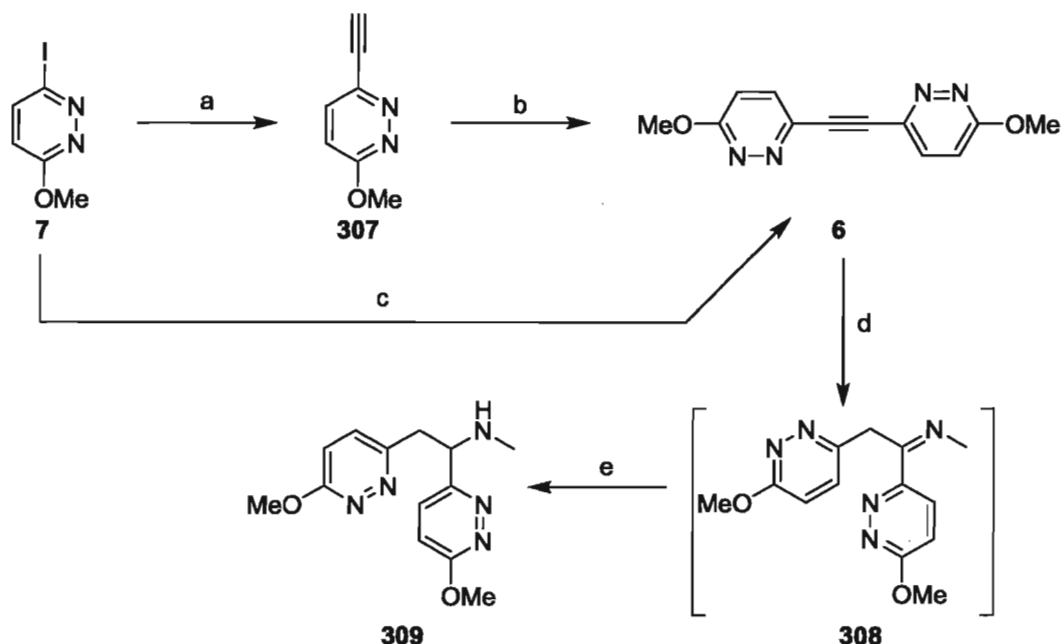
The synthesis of the dihydrofuran tether proceeded through first cyclopropanation of dihydrofuran **289** with ethyl diazoacetate to give ester **306** (Scheme 48). Cleavage of the cyclopropane moiety with copper bronze in refluxing acetonitrile provided the desired dihydrofuran **292**.



Reaction conditions: (a) ethyldiazoacetate, $(\text{Rh}(\text{OAc})_2)_2$, rt, 85%; (b) Cu bronze, CH_3CN , reflux, 54%

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

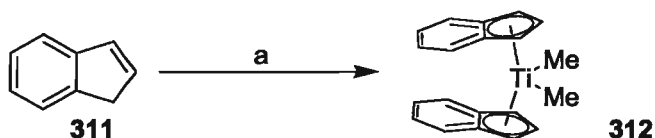
In addition to our investigations of the model systems described above, we elected to pursue synthesis of the bis(pyridazines) **6** required for the total synthesis. 3-Iodo-6-methoxypyridazine (**7**) was converted to acetylene **307** by first Sonogashira coupling with TMS-acetylene followed by treatment 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**.



Reaction conditions: (a) (i) TMS acetylene, CuI, PdCl₂(Ph₃)₂, Et₃N, THF, rt, (ii) TBAF, THF, rt, 69%; (b) **7**, CuI, PdCl₂(Ph₃)₂, Et₃N, rt, 71%; (c) acetylene, Pd(OAc)₂, PPh₃, CH₃CN/H₂O, Et₃N, CuI, 78%; (d) MeNH₂, Ind₂TiMe₂, toluene, 110 °C; (e) NaBH₃CN, MeOH, ZnCl₂, 68%, (2 steps)

Scheme 49. Hydroamination of bis(pyridazines) **6**.

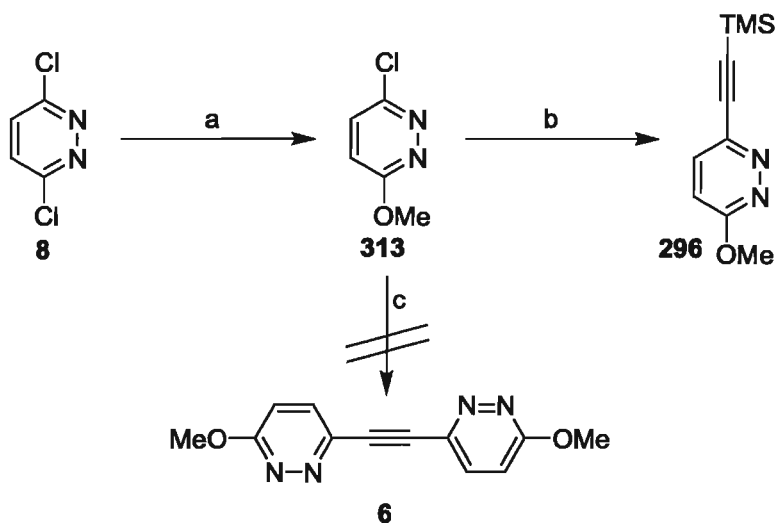
At this time it became apparent that the ease with which acetylene **6** could be synthesized obviated the need to deal with a separate model system. The most direct route for the introduction of methyl amine functionality would be a hydroamination reaction between methyl amine and acetylene **6**. The Doye hydroamination catalyst **312**¹⁴⁸ 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.¹⁴⁹ The required catalyst was synthesized by treatment of indene (**311**) with 4 equiv of methyllithium and titanium tetrachloride (Scheme 50).



Reaction conditions: (a) MeLi, TiCl₄

Scheme 50. Synthesis of hydroamination catalyst **312**.

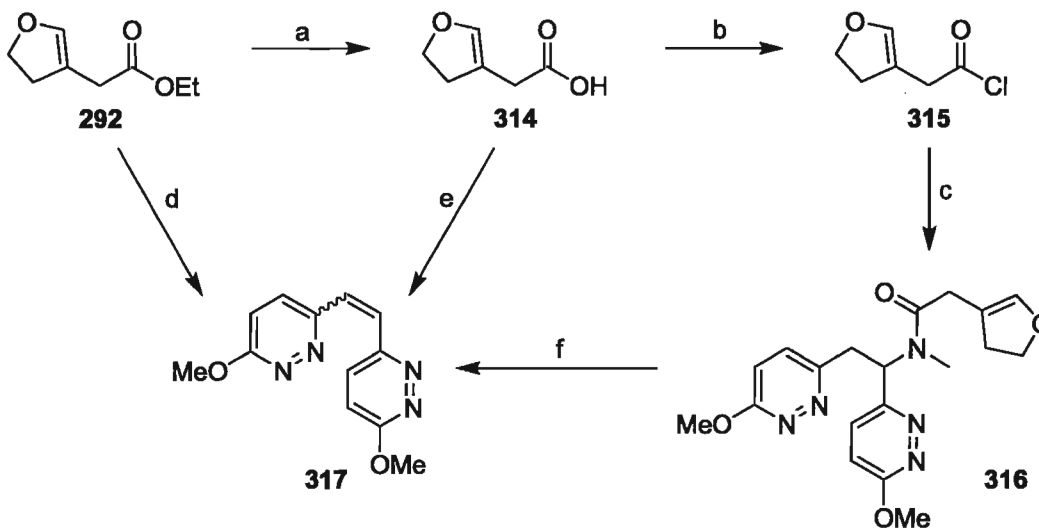
With both the catalyst **312** and acetylene **6** in hand, we pursued the direct hydroamination reaction. Fortunately, treatment of **6** with titanocene **312** in the presence of methyl amine provided evidence for the desired transformation. Initially the imine **308** was identified from a crude 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.



Reaction conditions: (a) K₂CO₃, MeOH, reflux, 63%; (b) TMS acetylene, CuI, PdCl₂(Ph₃)₂, Et₃N, THF, rt, 56% (c) acetylene, Pd(OAc)₂, PPh₃, CH₃CN/H₂O, Et₃N, CuI

Scheme 51. Sonogashira coupling of TMS acetylene with chloropyridazine **313**.

The use of chloropyridazine **8** in various Sonogashira 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 Sonogashira coupling were unsuccessful and resulted in isolation of the starting pyridazine **313**.



Reaction conditions: (a) LiOH, MeOH, 50 °C; (b) oxalyl chloride, DMF; (c) DMAP, Et₃N, **309**; (d) **309**, toluene, 150 °C; (e) CDI, **309**, CH₂Cl₂, 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 isocarbostryl 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 isocarbostryl 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. ¹H and ¹³C 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 cm³ 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 °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 °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 °C on an orbital shaker (180 rpm) for 5 h. A chemical inducer, isopropyl-1-thio-β-D-galactopyranoside (IPTG) (10 mg L⁻¹), was added via sterile filter and the cells were grown for additional 7 h at 35 °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 KH_2PO_4 (6.8 g L^{-1}), K_2HPO_4 (8.7 g/ L^{-1}) 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 Na_2CO_3) and separation of the organic from the aqueous layer. The extract was dried over anhydrous MgSO_4 , 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.

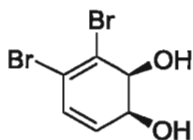
General Procedure for the Activation of Silica Gel. Silica gel (1.5 g, 230-400 mesh) was poured onto a sintered glass Büchner funnel and washed with reagent grade THF (2 x 15 mL) and diethyl ether (2 x 15 mL). The silica gel was transferred to a round-bottomed flask and heated externally at 140 °C under 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. H₂SO₄ (5 drops) in dry ethanol (10 mL) was refluxed for 4 days. The mixture was concentrated, diluted in saturated NaHCO₃ (2 mL) and extracted with ethyl acetate (3 x 20 mL). The organic extracts were combined, dried over MgSO₄, filtered, concentrated, and purified by flash chromatography (eluant 1:1 ethyl acetate/hexanes) and recrystallized from ethyl acetate/hexanes.

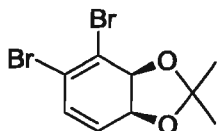
General procedure for formation of acetonide (ketalization) of diols with 2,2-dimethoxypropane. To a stirring solution of diol (0.832 mmol) and 2,2-dimethoxypropane (0.71 mL, 5.83 mmol) in acetone (1 mL) was added a catalytic amount of *p*-TsOH. The reaction was allowed to stir at room temperature for 2 h, then it was diluted with ethyl acetate (5 mL) and washed with saturated NaHCO₃ (3 x 2 mL). The organic layer was washed with brine (1 x 3 mL) then dried with Na₂SO₄. The crude material was purified via flash column chromatography with a solvent gradient of 2:1 hexanes-ethyl acetate.

V-2 Detailed Experimental Procedures



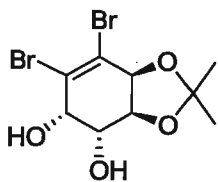
(1*S*,2*S*)-3,4-Dibromocyclohexa-3,5-diene-1,2-diol (161).

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} ; ^1H 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 C₆H₆BrO₂ (M⁺): 267.8731, found: 267.8733; Anal. calcd for C₆H₆BrO₂; C, 26.70; H, 2.24; Found C, 27.04; H, 2.32.



(3a*S*,7a*S*)-2,2-Dimethyl-4,5-dibromo-4,6-benzo[1,3]dioxole (177).

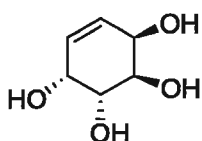
Dienediol **161** (3.0 g, 11mmol, 1 equiv) was transferred to a 100 mL round-bottomed flask and suspended in 5 mL acetone and 15 mL of 2,2-DMP. A few crystals of *p*TsOH were added and the reaction mixture was stirred at rt for 3 h. The reaction was quenched with 12 mL of 10% aq sodium hydroxide solution, 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); *Rf* 0.50 (50% ethyl acetate in hexanes); IR (film) ν 2988, 2933, 2896, 1634 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 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); ¹³C NMR (75 MHz, CDCl₃) δ 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.



(3a*S*,4*R*,5*S*,7a*S*)-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 tetroxide. 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; $[\alpha]_D^{22} +5.47$ (c 0.75, MeOH); *R*_f 0.3 (50% ethyl acetate in hexanes); IR (KBr) ν 3435, 3360, 2994, 2905, 1606 cm⁻¹; ¹H NMR (300

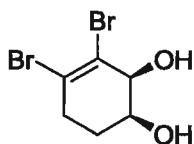
MHz, CDCl₃) δ 4.77 (dd, $J = 5.4, 1.2$ Hz, 1H), 4.50-4.45 (m, 2H), 4.34 (m, 1H), 2.68 (bs, 2H), 1.43 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 127.6, 125.6, 77.6, 75.3, 70.9, 69.1, 27.6, 26.1; HRMS-EI calcd for C₈H₉O₄Br, 326.8867; found, 326.8863; Anal Calcd for C₉H₁₂Br₂O₄: C, 31.42; H, 3.52, Found: C, 31.55; H, 3.78.



(-)-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(bisobutyronitrile) (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

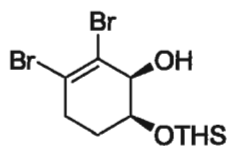
stirred for 40 h after which time the solvent was removed under reduced pressure to provide a white solid. The solid was purified by flash column chromatography (4:1 chloroform/methanol) to give (-)-conduritol E as a white crystalline solid (78 mg, 81%). mp 194-195 °C (EtOH) (lit^{120b} mp 193 °C); $[\alpha]_D^{20}$ -285 (c 1.0, H₂O), lit^{120b} $[\alpha]_D^{20}$ -294 (c 1.0, H₂O); *R_f* 0.18 (chloroform-methanol, 4:1); IR (film) ν 3434, 1634 cm⁻¹; ¹H NMR (300 MHz, MeOD) δ 5.79 (m, 2H), 4.27 (s, 2H), 3.93 (m, 2H); ¹³C NMR (75 MHz, MeOD) δ 130.7, 70.9, 67.6; HRMS-EI Calcd for C₆H₁₀O₄, 146.0579; Found, 146.0577.



(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 Na₂CO₃ 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 MgSO₄, and treated with activated charcoal. Filtration and subsequent

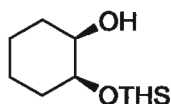
concentration of the filtrate under reduced pressure afforded **180** as a white crystalline solid (0.367g, 95%). mp 175-176 °C (ethyl acetate/hexane); $[\alpha]_D^{21}$ -50.4 (c 0.75, MeOH); *R_f* 0.23 (Hex: EtOAc, 1:1); IR (KBr pellet) ν 3246, 1626 cm^{-1} ; ^1H NMR (300 MHz, acetone d_6) δ 4.61 (d, $J = 6$ 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); ^{13}C NMR (75 MHz, acetone d_6) δ 127.1, 124.9, 73.5, 68.25, 35.2, 26.9; HRMS-EI Calcd for $\text{C}_6\text{H}_8\text{Br}_2\text{O}_2$, 271.8872; Found, 271.8871; Anal calcd for $\text{C}_6\text{H}_8\text{Br}_2\text{O}_2$; 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).

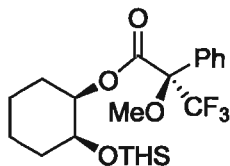
A 5 mL round-bottomed flask was charged with diol **180** (200 mg, 0.74 mmol, 1 equiv), imidazole (65 mg, 0.96 mmol, 1.3 equiv) and 1 mL anhydrous DMF. The flask was cooled externally to -30 °C then hexyldimethylsilyl chloride (0.15 mL, 0.78 mmol, 1.1 equiv) was added. The mixture was stirred at -30 °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 H_2O (10 mL), brine, and then dried over MgSO_4 . After filtration, the filtrate was concentrated under reduced pressure. The crude silyl ether was purified by flash column chromatography (pentane: Et_2O , 10:1) to give **181** as a clear and colorless oil (0.26 g, 86%). $[\alpha]_D^{21}$ -41.1 (c 0.75, MeOH); *R_f* 0.23 (pentane: Et_2O , 10:1); IR

(film) ν 3547, 2958, 2868, 1628 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) 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); ^{13}C NMR (75 MHz, CDCl_3) 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 $\text{C}_{14}\text{H}_{26}\text{Br}_2\text{O}_2\text{Si}$, 328.9032; Found, 328.9026; Anal calcd for $\text{C}_{14}\text{H}_{26}\text{Br}_2\text{O}_2\text{Si}$; C, 40.59; H, 6.33; Found C, 40.96; H, 6.36.



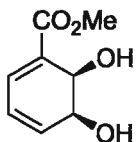
(1R, 2S)-2-[(hexyldimethylsilyl)oxy]cyclohexan-1-ol (182).

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. $[\alpha]_{\text{D}}^{23} + 3.4$ (c 1.0, CHCl_3); lit:^{121a} $[\alpha]_{\text{D}}^{23} + 3.3$ (c 1.0, CHCl_3).



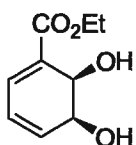
Mosher ester derivative 183.

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 μ L) was added followed by DMAP (4.8 mg, 0.038 mmol, 0.5 equiv). (*R*)-(-)- α -Methoxy- α -(trifluoromethylphenyl)acetic acid chloride (23 μ L, 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 (MgSO_4) 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%). *R*_f 0.46 (pentane: Et_2O , 10:1); IR (film) 2948, 2867, 1745, 1463, 1450, 1379, 1263, 1169 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): 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) ^{19}F NMR (188 MHz, CDCl_3) δ : -72.4 ppm. Lit^{121a} ^{19}F NMR (188 MHz, CDCl_3) δ : -72.8 ppm.



6-Carboxymethyl-(1*S*,2*S*)-1,2-dihydroxycyclohexa-3,5-diene (163).

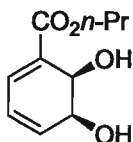
See General Experimental Procedures for Biotransformations: Large-scale fermentations. (12.92 g, 19.2%, 75.8% based on recovered starting material) pale yellow oil; *R_f* 0.33 (1:2 hexanes/ethyl acetate); $[\alpha]_D^{23} +71.3$ (*c* 1.6, CHCl₃); IR (film) ν 3412, 2098, 1690, 1639, 1291, 820, 772 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 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; ¹³C NMR (75 MHz, CDCl₃) δ 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-(1*S*,2*S*)-1,2-dihydroxycyclohexa-3,5-diene (191).

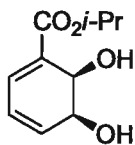
See General Experimental Procedures for Biotransformations: Large-scale fermentations. (8.06 g, 43.4%, 45.6% based on recovered starting material) Colorless crystals, mp 48 °C (ethyl acetate/hexanes); *R_f* 0.31 (1:2 hexanes/ethyl acetate); $[\alpha]_D^{23} +54.7$ (*c* 3.8, CHCl₃); IR (film) ν 3385, 2981, 2934, 1700, 1280, 1243, 1104, 1068, 825, 771 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 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 $\text{C}_9\text{H}_{12}\text{O}_4$ 184.0736, found 184.0731; Anal. calcd: C 58.69, H 6.57, found C 58.77, H 6.60.



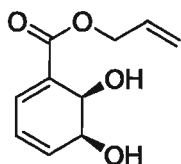
6-Carboxypropyl-(1S,2S)-1,2-dihydroxycyclohexa-3,5-diene (192).

See General Experimental Procedures for Biotransformations: Large-scale fermentations. (711 mg, 5.8%, 65.0% based on recovered starting material) waxy solid; R_f 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} ; ^1H 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 $\text{C}_9\text{H}_{12}\text{O}_4$ 198.0892, found 198.0892.



6-Carboxyisopropyl-(1S,2S)-1,2-dihydroxycyclohexa-3,5-diene (193).

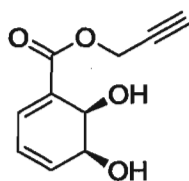
See General Experimental Procedures for Biotransformations: Large-scale fermentations. (488 mg, 4.1%, 34.0% based on recovered starting material) colourless crystals; mp 83-85 °C (ethyl acetate/hexane); $R_f = 0.31$ (6:4 ethyl acetate/hexane); $[\alpha]_D^{22} +64.70$ (c 1.1, CHCl_3); IR (KBr) ν 3274, 2981, 1698, 1263, 1241 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 7.05 (ddd, $J = 5.5, 1.0, 0.5$ Hz, 1H), 6.20 (ddt, $J = 9.6, 2.7, 0.9$ Hz, 1H), 6.08 (ddd, $J = 9.6, J = 5.5, 2.2$ 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, CDCl_3) δ 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) calcd for $\text{C}_{10}\text{H}_{14}\text{O}_4$: 198.08921, found: 198.08896. *Anal.* Calcd. for $\text{C}_{10}\text{H}_{14}\text{O}_4$: C, 60.59; H, 7.12. Found: C, 60.68; H, 7.19.



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

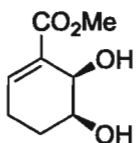
See General Experimental Procedures for Biotransformations: Large-scale fermentations. (5.79 g, 52.0%, 73.6% based on recovered starting material) colourless crystals; mp 48-50 °C (ethyl acetate/hexane); $R_f = 0.23$ (1:1 ethyl acetate/hexane); $[\alpha]_D^{22} +72.54$ (c 1.6, CHCl_3); IR (KBr) ν 3394, 1704, 1273, 1238 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 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 (ddd, $J =$

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) δ 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 $\text{C}_{10}\text{H}_{12}\text{O}_4$: m/z 196.07356, found: 196.07364. Anal. Calcd. for $\text{C}_{10}\text{H}_{12}\text{O}_4 + 1/8 \text{H}_2\text{O}$: C, 60.52; H, 6.22. Found: C, 60.52; H, 6.26.



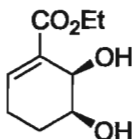
6-Carboxyisopropyl-(1S,2S)-1,2-dihydroxycyclohex-3-ene (197).

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) ν 3385, 3291, 1707, 1270, 1234 cm^{-1} ; ^1H NMR (300 MHz, acetone- d_6) δ 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) δ 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 $\text{C}_{10}\text{H}_{10}\text{O}_4$: m/z 194.0579, found: 194.0581; Anal. Calcd. for $\text{C}_{10}\text{H}_{10}\text{O}_4$: C, 61.85; H, 5.19. Found: C, 62.08; H, 5.18.



6-Carboxymethyl-(1*S*,2*S*)-1,2-dihydroxycyclohex-3-ene (198).

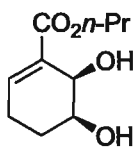
See General procedure for PAD reduction of diols. (848 mg, 84%.) colorless oil; *R_f* 0.29 (1:1 ethyl acetate/hexanes); $[\alpha]_D^{23}$ -52.9 (*c* 2.9, CHCl₃); IR (film) ν 3411, 2998, 2951, 2668, 1703, 1648, 1437, 1370, 1254, 1210, 1160, 1108, 1073, 1041, 995, 963, 920, 878, 852, 757 670 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.02 (dd, *J* = 4.4, *J* = 3.3 Hz, 1H), 4.46 (d, *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; ¹³C 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 C₈H₁₂O₄ 172.0731, found 172.0730.



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

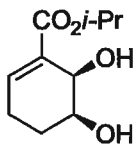
See General procedure for PAD reduction of diols. (378 mg, 84%.) colorless crystals; mp 91-92 °C (ethyl acetate/hexanes); *R_f* 0.29 (1:1 ethyl acetate/hexanes); $[\alpha]_D^{23}$ -53.3 (*c* 1.6, CHCl₃); IR (film) ν 3400, 2981, 2937, 2909, 1731, 1647, 1372, 1251, 1105, 1073, 993, 921, 878, 761, 670 cm⁻¹; ¹H 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; ¹³C NMR (75 MHz,

CDCl₃) δ 167.1, 143.1, 130.1, 67.7, 65.7, 60.9, 25.0, 23.9, 14.2 ppm; MS (EI) *m/z* (%): 186(M⁺, 1), 143(12), 142(90), 105(12), 97(16), 96(100), 68(38), 67(10), 41(12); HRMS calcd for C₉H₁₄O₄ 186.0892, found 186.0892; Anal. calcd: C 58.05; H 7.58. found C 57.97; H 7.51.



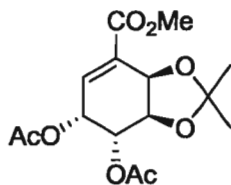
6-Carboxypropyl-(1*S*,2*S*)-1,2-dihydroxycyclohex-3-ene (200).

See General procedure for PAD reduction of diols. (276 mg, 91%) colourless crystals; mp 73-75 °C; *R_f* 0.15 (1:1 ethyl acetate/hexanes); [α]_D²⁰ -50.85 (*c* 1.5, CHCl₃); IR (film) ν 3406, 2966, 2934, 2876, 1707, 1648, 1247 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 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), 2.31-2.16 (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; ¹³C NMR (75 MHz, CDCl₃) δ 167.0, 143.0, 130.5, 68.3, 66.4, 65.1, 24.53, 24.49, 21.9, 10.4 ppm; MS (EI) *m/z* (%): 200(M⁺, 3), 156(M⁺-C₂H₄O, 68), 141(17), 114(80), 96(100); HRMS (EI) calcd for C₁₀H₁₆O₄: *m/z* 200.1048; found: 200.1052. Anal. Calcd. for C₁₀H₁₆O₄: C, 59.98; H, 8.05. Found: C, 59.87; H, 8.04.



6-Carboxyisopropyl-(1*S*,2*S*)-1,2-dihydroxycyclohex-3-ene (201).

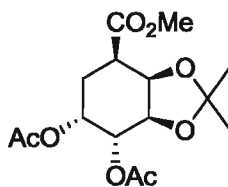
See General procedure for PAD reduction of diols. (203 mg, 56%) pale yellow oil; $R_f = 0.31$ (6:4 ethyl acetate/hexane); $[\alpha]_D^{20} -44.74$ (c 1.3, CHCl_3); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.08 (t, $J = 3.9$ Hz, 1H), 5.11 (hept, $J = 6.3$ Hz, 1H), 4.51 (br s, 1H), 4.48 (br m, 1H), 3.94-3.84 (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; $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 166.6, 142.7, 130.4, 68.4, 67.7, 65.7, 25.1, 23.8, 21.9, 21.8 ppm; IR (film) ν 3403, 2980, 1706, 1260 cm^{-1} ; MS (EI) m/z (%): 156($\text{M}^+ - \text{C}_2\text{H}_4\text{O}$, 50), 141(18), 114(100), 96(94); HRMS (EI) calcd for $\text{C}_{10}\text{H}_{16}\text{O}_4$: m/z 200.1049; found: 200.1054.



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

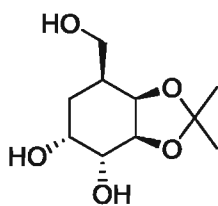
To a solution of diol **163** (300 mg, 1.76 mmol) in 2,2-dimethoxypropane (2 mL) and acetone (1 mL) was added *p*-toluenesulfonic acid (catalytic amount) at room temperature. After complete consumption of starting material (TLC analysis), the solution was diluted with ethyl acetate (60 mL), washed with saturated NaHCO_3 (3 x 5 mL), dried over Na_2SO_4 , filtered, and concentrated. The crude acetone was dissolved in a mixture of acetone/ H_2O (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; *R*_f 0.65 (1:1 hexanes/ethyl acetate); [α]_D²⁰ -67.54 (*c* 2.5, EtOH); IR (film): ν 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 (EI) *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.



(3aR,4R,5R,7R,7aR)-7-(methoxycarbonyl)-2,2-dimethylhexahydrobenzo[d][1,3]dioxole-4,5-diyl diacetate (204).

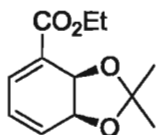
To a solution of ester **203** (40 mg, 0.122 mmol) in ethanol (3 mL) was added 5% Rh/Al₂O₃ (40 mg). The reaction was stirred under an atmosphere of H₂ (60 psi, 24 h). The reaction was filtered through Celite by elution with EtOH and concentrated. The crude material was purified via flash column chromatography with a solvent gradient of 5:1 hexanes-ethyl acetate. Yield: 28 mg, 70%, colorless oil; *R_f* 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 315.1080, found 315.1082.



(3a*S*,4*R*,5*R*,7*S*,7a*R*)-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

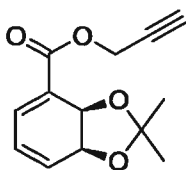
concentrated. The crude material was purified via flash column chromatography with a solvent gradient of 5:1 hexanes-ethyl acetate. Yield: 23 mg, 86%, colorless oil; *R_f* 0.14 (9:1 chloroform/methanol); $[\alpha]_{\text{D}}^{20}$ -57.08 (*c* 0.70, MeOH); ^1H NMR (600 MHz, acetone-*d*₆) δ 4.30 (t, *J* = 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, CDCl₃) δ 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^+ -CH₃, 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^+ -CH₃) calcd for C₉H₁₅O₅ 203.0933, found 203.0926.



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

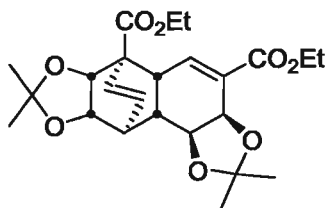
(173 mg, 92%) colorless oil; *R_f* 0.56 (1:1 hexanes/ethyl acetate); $[\alpha]_{\text{D}}^{23}$ +74.6 (*c* 4.02, CHCl₃); IR (film): ν 3018, 2987, 2936, 1712, 1651, 1425, 1380, 1259, 1155, 1031, 917, 856, 697, 667, 512 cm⁻¹; ^1H NMR (300 MHz, CDCl₃) δ 7.06 (dd, *J* = 5.3, 3.1 Hz, 1H), 4.84 (d, *J* = 5.7 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, CDCl₃) δ 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

(EI) m/z (%): 226 (M^+-CH_3), 211(77), 181(15), 169(17), 123(100), 105(17), 95(13), 83(11), 79(76), 67(14), 59(10), 55(11), 43(82), 41(14); HRMS (M^+-CH_3) calcd for $C_{12}H_{16}O_4$ 211.0970, found 211.0969; Anal. calcd: C 64.27; H 7.19. Found C 64.52; H 7.08.



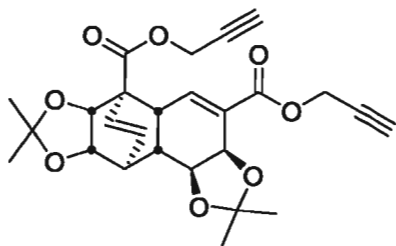
(3aR,7aS)-Prop-2-ynyl-3a,7a-dihydro-2,2-dimethylbenzo[d][1,3]dioxole-4-carboxylate (208).

(764 mg, 63%) colorless oil; R_f = 0.54 (2:8 ethyl acetate/hexane); $[\alpha]_D^{22}$ +112.70 (c 1.3, $CHCl_3$); IR (KBr) ν 2987, 2935, 1718, 1030 cm^{-1} ; 1H NMR (300 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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(M^+-Me , 42), 177(41), 163(17), 121(83), 43(100). HRMS (EI) calcd for $C_{12}H_{11}O_4$: m/z 219.0657, found: 219.0659. Anal. Calcd. for $C_{13}H_{14}O_4$: C, 66.66; H, 6.02. Found: C, 66.68; H, 6.08.



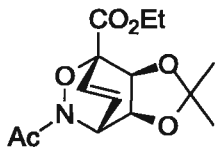
(1*S*,2*R*,3*S*,4*S*,4*aS*,5*S*,6*R*,8*aR*)-1,2,3,4,4*a*,5,6,8*a*-octahydro-2,3,5,6-tetrahydro-*O,O*-diisopropyliden-1,4-ethenonaphthalene-1,7-bis(ethyl dicarboxylate) (209**).**

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, CHCl₃). IR (KBr) ν 2984, 2938, 1722, 1262, 1221, 1071 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) δ 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 (1 H, 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. ¹³C NMR (150 MHz, CDCl₃) δ 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 C₂₄H₃₂O₈: *m/z* 448.20972, found: 448.20863.



(1*S*,2*R*,3*S*,4*S*,4*aS*,5*S*,6*R*,8*aR*)-1,2,3,4,4*a*,5,6,8*a*-octahydro-2,3,5,6-tetrahydro-*O,O*-diisopropyliden-1,4-ethenonaphthalene-1,7-dipropargyldicarboxylate (210**).**

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, CHCl_3). $^1\text{H NMR}$ (600 MHz, CDCl_3) 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; $^{13}\text{C NMR}$ (150 MHz, CDCl_3) 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($\text{M}^+ - \text{Me}$, 26), 395(32), 297(15), 121(31), 100(60), 85(22), 43(100). HRMS (EI) calcd for $\text{C}_{24}\text{H}_{32}\text{O}_8$: m/z 453.15494, found: 453.15470. *Anal.* Calcd. for $\text{C}_{26}\text{H}_{28}\text{O}_8$: C, 66.66; H, 6.02. Found: C, 66.61; H, 6.16.

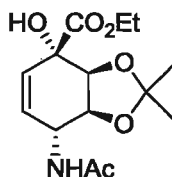


(1R,2S,6S,7S)-9-Acetyl-4,4-dimethyl-3,5,8-trioxa-9-azatricyclo[5.2.2.0(2,6)]undec-10-ene-7-carboxylic acid ethyl ester (212).

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.

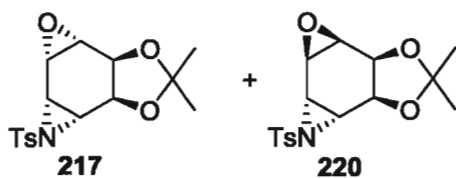
NaIO₄ (5.80 g, 27.1 mmol) was added to the reaction flask prior to the addition of a solution of acetohydroxamic acid (2.03 g, 27.1 mmol) in MeOH (25 mL) dropwise over 5 minutes. The resulting solution was stirred at room temperature for 16 h, quenched by the slow addition of sat. NaHSO₃ (10 mL) and 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); *R*_f 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 calcd for $C_{14}H_{19}NO_6$ 297.1212, found 297.1215.



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

To a stirred solution of **212** (955 mg, 3.21 mmol) in 15:1 $CH_3CN:H_2O$ (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; R_f 0.20 (ethyl acetate); mp 97-99 °C (hexanes-ethyl acetate); $[\alpha]_D^{23}$ -94.3 (c 0.79, $CHCl_3$); IR (film) ν 3433, 2094, 1644, 1271, 1217, 1060 cm^{-1} ; 1H NMR (600 MHz, $CDCl_3$) δ 6.25 (d, J = 8.7 Hz, 1H), 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; ^{13}C NMR (150 MHz, $CDCl_3$) 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^+-CH_3), 199(99), 153(38), 125(36), 96(37), 86(61), 84(100), 83(47), 43(90); HRMS calcd for $C_{13}H_{18}NO_6$ 284.1130, found 284.1137.



(3a*S*,4*R*,5*S*,5a*S*,6a*S*,6b*S*)-2,2-dimethyl-7-[(4-methylphenyl)sulfonyl]hexahydro-4,5-epiminooxireno[e][1,3]benzodioxole (217),

(3a*S*,4*R*,5*S*,5a*R*,6a*R*,6b*S*)-2,2-dimethyl-7-[(4-methylphenyl)sulfonyl]hexahydro-4,5-epiminooxireno[e][1,3]benzodioxole (220).

A solution of *m*-CPBA (10.5 g, 46.7 mmol) in 1,2-dichloroethane (150 mL) was dried over Na₂SO₄ 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 NaHSO₃ (2 x 100 mL), carbonate (2 x 100 mL), and brine (35 mL). The organic phase was dried over Na₂SO₄, 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**

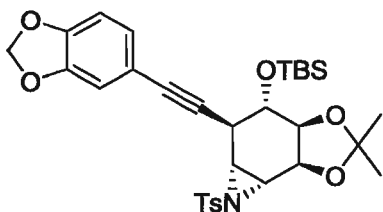
Epoxide **217**

Rf 0.43 (hexanes/ethyl acetate, 2:1); mp 115-116 °C (isopropanol); $[\alpha]_D^{21}$ - 79.36 (*c* 1.0, CHCl₃); IR (film) ν 2988, 2936, 1597, 1383, 1373, 1329, 1251, 1218, 1158, 1091, 1055 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ : 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); ¹³C NMR (75 MHz, CDCl₃) δ : 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 C₁₆H₁₉NO₅S (M⁺-15): 322.0749, Found: 322.0744; Anal. calcd for C₁₆H₁₉NO₅S 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) ν 2985, 2929, 1602, 1384, 1366, 1324, 1245, 1222, 1163, 1153, 1041, 1001 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) δ : 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); ¹³C NMR (150 MHz, CDCl₃) δ : 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 C₁₆H₁₉NO₅S

(M⁺-15): 322.0749, Found: 322.0753; Anal. calcd for C₁₆H₁₉NO₅S C, 56.96; H, 5.68; found C, 56.23; H, 5.61.



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

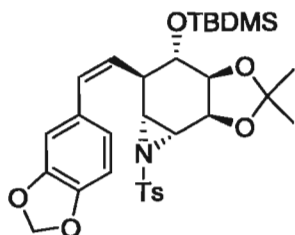
To a solution of acetylene **222** (0.43 g, 2.96 mmol) in 8 mL dry toluene at -50 °C was added 1.46 mL of a solution of *n*BuLi in hexanes (2.01 M, 2.96 mmol) over 5 min. The solution was stirred for 15 minutes before 2.97 mL of a solution of Me₂AlCl (1.0M in CH₂Cl₂, 2.96 mmol) was added dropwise over 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 **(3a*S*,4*R*,5*R*,6*R*,7*S*,7a*R*)-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; $[\alpha]_D^{22}$ -113.05 (*c* 0.5, CHCl₃); *R*_f 0.30 (hexanes:ethyl acetate, 2:1); IR (film) ν 3491, 2988, 1163 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) 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; ¹³C NMR (75 MHz, CDCl₃) 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 M⁺) calcd for C₂₅H₂₅NO₇S 484.1430, found 484.1428.

The free alcohol intermediate **221** (0.8 g, crude) was dissolved in 20 mL of CH₂Cl₂ 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 CH₂Cl₂ (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; $[\alpha]_D^{24} +57.7$ (c 0.5, CHCl_3); R_f 0.49 (hexanes:ethyl acetate, 2:1); IR (film) ν 2953, 2929, 2892, 2856, 1599, 1490 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ : 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) δ : 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 $\text{C}_{30}\text{H}_{36}\text{NO}_7\text{SSi}$: 540.1481; Found, 540.1487; Anal. calcd for $\text{C}_{31}\text{H}_{39}\text{NO}_7\text{SSi}$ C, 62.28; H, 6.58; found C, 62.22; H, 6.73.



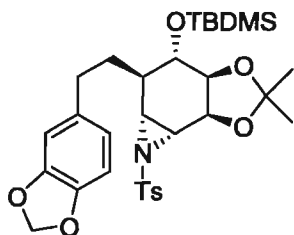
(3a*S*,4*R*,5*R*,6*R*,7*S*,7a*S*)-6-[(*Z*)-2-(1,3-benzodioxol-5-yl)vinyl]-7-[[*tert*-butyl(dimethyl)silyl]oxy]-2,2-dimethyl-8-[(4-methylphenyl)sulfonyl]hexahydro-4,5-epimino-1,3-benzodioxole (216).

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%); $[\alpha]_D^{23}$ -26.14 (c 1.0, CHCl₃; *R*_f 0.35 (hexanes:ethyl acetate, 4:1); IR (film) ν 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,

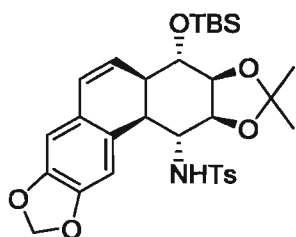
25.51, 21.7, 18.1, -4.3, -4.7 ppm; HRMS-EI Calcd for C₃₁H₄₁NO₇SSi: 599.2373; Found, 599.2376; Anal. calcd for C₃₁H₄₁NO₇SSi C, 62.28; H, 6.58; found C, 61.30; H, 6.63



(3a*S*,4*R*,5*R*,6*R*,7*S*,7a*S*)-6-[2-(1,3-benzodioxol-5-yl)ethyl]-7-[[*tert*-butyl(dimethyl)silyl]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 H₂ before being placed under H₂ 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 _D²³] -47.2 (c 1.8, CHCl₃) *R*_f 0.35 (hexanes/ethyl acetate, 2:1); IR (film) ν 2986, 2954, 2929, 2891, 2857, 1598, 1504, 1489 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) 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,

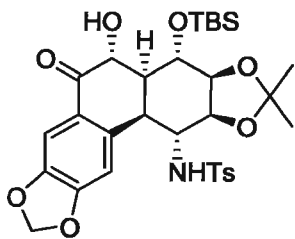
1H), 3.43 (m, 1H), 3.18 (d, $J = 6$ Hz, 1H), 2.84 (m, 1H), 2.60 (m, 1H), 2.45 (s, 3H), 2.40 (m, 1H), 2.05 (m, 1H), 1.68 (s, 3H), 1.34 (s, 3H), 0.83 (s, 9H), 0.06 (s, 3H), 0.03 (s, 3H); ^{13}C NMR (250 MHz, CDCl_3) δ : 146.6, 145.7, 144.8, 135.1, 134.9, 129.8, 128.0, 127.9, 121.2, 109.2, 108.9, 108.1, 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 $\text{C}_{31}\text{H}_{43}\text{NO}_7\text{SSi}$: 601.2530; Found, 601.2534.



***N*-[*(1R,2aS,4aS,5S,5aR,12bR)*-5-(*tert*-Butyldimethylsilyloxy)-3,3-dimethyl-1,2a,4a,5,5a,12b-hexahydrophenanthro[2,3-*d*][1,3]dioxol-1-yl]4-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%; $[\alpha]_{\text{D}}^{23}$ -123.7 (c 1.0, CHCl_3); R_f 0.35 (hexanes/ethyl acetate, 2:1); IR (film) ν 3268, 2929, 2887, 2857,

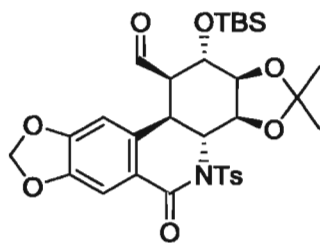
1598,1503, 1485 cm^{-1} ; ^1H NMR (600 MHz, CDCl_3) δ : 7.43 (d, $J = 7.0$ Hz, 2H), 7.13 (d, $J = 7.2$ Hz, 2H), 6.49 (s, 2H), 6.34 (d, $J = 8.1$ Hz, 1H), 5.95 (s, 1H), 5.86 (s, 1H), 5.76 (d, $J = 8.0$ Hz, 1H), 4.51 (d, $J = 7.2$ Hz, 1H), 4.28 (m, 1H), 4.11 (m, 1H), 3.99 (m, 1H), 3.79 (m, 1H), 2.82 (m, 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) δ : 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 $\text{C}_{31}\text{H}_{41}\text{NO}_7\text{SSi}$: 599.2373; Found, 599.2370; Anal. calcd for $\text{C}_{31}\text{H}_{41}\text{NO}_7\text{SSi}$ C, 62.07; H, 6.89; found C, 62.16; H, 6.94.



***N*-[(1R,2aS,4aS,5S,5aS,12bR)-5-(*tert*-Butyl-dimethyl-silanyloxy)-6-hydroxy-3,3-dimethyl-7-oxo-1,2a,4a,5,5a,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) ν 3478, 3263, 2929, 2857, 1670, 1614, 1504, 1482, 1444, 1386, 1330, 1252, 1218, 1156, 1075, 1039 cm^{-1} ; ^1H NMR (600 MHz, CDCl_3) δ : 7.54 (d, $J = 7.8$ Hz, 2H), 7.49, (s, 1H), 7.18 (d, $J = 7.8$ Hz, 2H), 6.70 (s, 1H), 6.07 (s, 1H), 6.00 (s, 1H), 4.79 (d, $J = 8.7$ Hz, 1H), 4.71 (m, 2H), 4.19 (m, 1H), 4.08 (m, 1H), 3.74 (m, 2H), 3.08 (dd, $J = 10.2$ Hz, 1.8 Hz, 1H), 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 NMR (150 MHz, CDCl_3) δ : 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 ppm; HRMS-EI Calcd for $\text{C}_{27}\text{H}_{32}\text{NO}_9\text{SSi}$ ($\text{M}^+ - 57$): 574.1567, Found: 574.1572



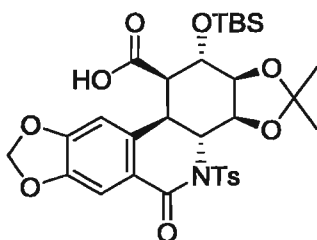
(3aS,3bR,10bR,11R,12S,12aS)-12-(*tert*-Butyl-dimethyl-silanyloxy)-2,2-dimethyl-5-oxo-4-(toluene-4-sulfonyl)-3a,3b,4,5,10b,11,12,12a-octahydro-1,3,7,9-tetraoxa-4-aza-dicyclopenta[*a,h*]phenanthrene-11-carbaldehyde (166).

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; *R_f* 0.31 (hexanes:ethyl acetate, 4:1); mp >200 °C,

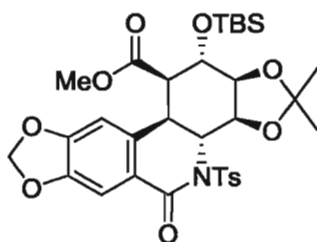
recrystallized from hexanes/ethyl acetate 4:1; $[\alpha]_D^{21} + 31.67$ (c 0.5, CHCl_3); IR (film) ν 2929, 2857, 1725, 1689, 1619, 1505, 1484, 1386, 1361, 1287, 1255, 1220, 1172, 1077, 1036 cm^{-1} ; ^1H NMR (600 MHz, CDCl_3) δ : 9.49 (s, 1H), 8.3 (d, $J = 8.2$ Hz, 2H), 7.58 (s, 1H), 7.33 (d, $J = 8.2$ 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, CDCl_3) δ : 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 $\text{C}_{30}\text{H}_{36}\text{NO}_9\text{SSi}$ ($M^+ - 15$): 614.1879, Found: 614.1870; Anal. calcd for $\text{C}_{31}\text{H}_{39}\text{NO}_9\text{SSi}$ C, 59.12; H, 6.24; found C, 59.31; H, 6.29.



(3a*S*,3b*R*,10b*R*,11*R*,12*S*,12a*S*)-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

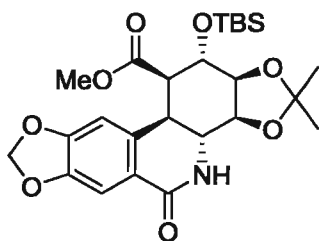
reaction flask was sealed and heated at 40 °C overnight. The reaction mixture was diluted with methylene chloride (80 mL) and washed sequentially with saturated aqueous sodium bisulfite (10 mL), sodium bicarbonate (10 mL), and dried over sodium sulfate. The organic phase was filtered and concentrated in vacuo to provide carboxylic acid **235** as a white crystalline solid (0.125 g, 85%) that was used without further purification. *R_f* 0.1 (hexanes/ethyl acetate, 1:1); mp >200 °C (chloroform/ether); $[\alpha]_D^{22}$ - 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



(3a*S*,3b*R*,10b*R*,11*R*,12*S*,12a*S*)-12-(*tert*-Butyl-dimethyl-silanyloxy)-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 (30mL) 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) ν 2986, 2953, 2931, 2896, 2858, 1739, 1692, 1620, 1598, 1505, 1485, 1361, 1289, 1264, 1173 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ : 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; ¹³C NMR (75 MHz, CDCl₃) δ : 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,

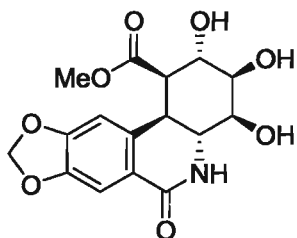
102.0, 72.9, 68.2, 65.2, 51.9, 48.1, 35.9, 27.5, 26.8, 25.7, 21.6, 18.0, -4.8, -4.9 ppm; HRMS-EI Calcd for C₂₈H₃₂NO₁₀SSi (M⁺-57): 602.1516, Found: 602.1516



(3a*S*,3b*R*,10b*R*,11*R*,12*S*,12a*S*)-12-(*tert*-Butyl-dimethyl-silyloxy)-2,2-dimethyl-5-oxo-3a,3b,4,5,10b,11,12,12a-octahydro-1,3,7,9-tetraoxa-4-azadicyclopenta[*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 CH₂Cl₂ (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; *R*_f 0.28 (hexanes:ethyl acetate, 1:1); [α]_D²² -14.51 (*c* 0.50, CHCl₃); IR (film) ν₃₃₂₀, 2952, 2930, 2895, 2857, 1743, 1669, 1619, 1504, 1484, 1460, 1385, 1369, 1321, 1288, 1260, 1222 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) δ: 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

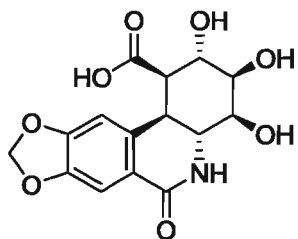
(s, 1H), 1.40 (s, 3H), 1.37 (s, 3H), 0.92 (s, 9H), 0.21 (s, 3H), 0.20 (s, 3H) ppm; ^{13}C NMR (150 MHz, CDCl_3) δ :169.6, 165.4, 151.4, 146.6, 135.4, 122.6, 110.5, 108.6, 103.3, 101.7, 69.2, 53.1, 51.9, 45.9, 33.4, 27.6, 26.5, 25.7, 17.9, -4.9, -5.0 ppm; HRMS-EI Calcd for $\text{C}_{25}\text{H}_{35}\text{NO}_8\text{Si}$ (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-j]phenanthridine-1-carboxylic acid methyl ester (169).

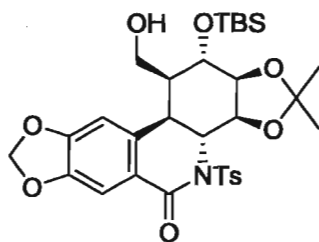
To a solution of the detosylated methyl ester **237** (23 mg, 0.046 mmol) in methanol (2 mL) was added 3% HCl in methanol (0.5 mL). The reaction mixture was stirred until total consumption of starting material (3 days). The solvent was removed under reduced pressure and the residue was purified by flash 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); $[\alpha]_{\text{D}}^{22} + 24.53$ (c 0.25, MeOH); IR (KBr) ν 3311, 2913, 1732, 1648, 1609, 1497, 1462, 1349, 1259, 1037 cm^{-1} ; ^1H 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).

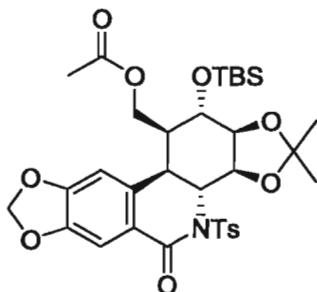
To a solution of **169** (6 mg, 0.017 mmol) in methanol (0.5 mL) was added LiOH (1 mg, 1.5 mmol). The reaction mixture was heated at 45 °C and stirred until total consumption of starting material (2 days) as monitored by TLC. The reaction was made slightly acidic with the addition of HCl (5 drops, 1M) and concentrated to provide acid **168** (5 mg, 95%) as a white crystalline solid. mp >200 °C (methanol); R_f 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.



(3a*S*,3b*R*,10b*R*,11*S*,12*S*,12a*S*)-12-(*tert*-Butyl-dimethyl-silanyloxy)-11-hydroxymethyl-2,2-dimethyl-4-(toluene-4-sulfonyl)-3b,4,10b,11,12,12a-hexahydro-3a*H*-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; ; *R_f* 0.44 (hexanes/ethyl acetate, 1:1); [α]_D²² – 47.72 (*c* 1.50, CHCl₃); IR (film) ν 3547, 2986, 2932, 2586, 1692, 1616, 1594, 1508, 1481, 1360 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ: 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; ¹³C NMR (75 MHz, CDCl₃) δ: 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,

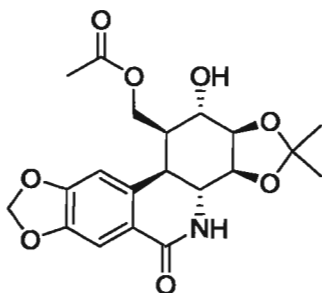
60.0, 46.9, 37.4, 28.1, 26.3, 25.8, 21.6, 18.0, -4.8, -4.9 ppm; HRMS-EI Calcd for $C_{31}H_{41}NO_9SSi$ ($M+15$): 616.2032, Found: 616.2032.



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

To a solution of **238** (150 mg, 0.237 mmol) in dry CH_2Cl_2 (10 mL) was added DMAP (1.5 mg, 0.012 mmol), followed by pyridine (0.1 mL, 1.187 mmol). Ac_2O (45 μ 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_2O (75 mL) and separated. The aqueous layer was extracted with Et_2O (2 x 75 mL) and the combined organic phases were washed with H_2O (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; R_f 0.51 (hexanes/ethyl acetate, 1:1); $[\alpha]_D^{22} - 41.081$ (c 3.0, $CHCl_3$); IR (film) ν 2988, 2952, 2930, 2858, 1742, 1694, 1619, 1598, 1505, 1485, 1395, 1362, 1254;

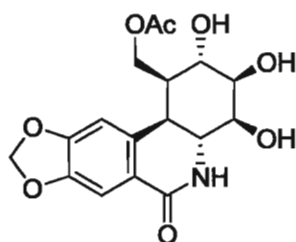
^1H NMR (600 MHz, CDCl_3) δ : 8.29 (d, $J = 8.3$ Hz, 2H), 7.54 (s, 1H), 7.31 (d, $J = 8.2$ Hz, 2H), 6.84 (s, 1H), 6.03 (d, $J = 12.6$ Hz, 2H), 5.62 (dd, $J = 8.7$ Hz, $J = 5.6$ Hz, 1H), 4.50 (s, 1H), 4.31 (d, $J = 5.3$ Hz, 1H), 4.18 (t, $J = 11.1$ Hz, 1H), 3.97 (dd, $J = 13.0$ Hz, $J = 8.8$ Hz, 1H), 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, $J = 5.2$, 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}C NMR (150 MHz, CDCl_3) δ : 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 $\text{C}_{32}\text{H}_{40}\text{NO}_{10}\text{SSi}$ ($\text{M}^+ - 15$): 658.2142, Found: 658.2152.



((3aS,3bR,10bR,11S,12S,12aR)-12-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)methyl acetate (240).

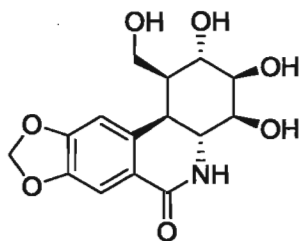
To a solution of **239** (137 mg, 0.203 mmol) in dry DME (5 mL) at -78 $^\circ\text{C}$ was added a 0.5 M solution of Na/naphthalene in DME until a green color persisted and total consumption of starting material was observed (by TLC). The solution was stirred for 10 minutes before the reaction was quenched with saturated aqueous ammonium chloride solution (2 mL). The reaction was warmed to room

temperature, concentrated to remove DME, and extracted with CH₂Cl₂ (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); *R_f* 0.059 (hexanes/ethyl acetate, 1:1); [α]_D²² – 38.301 (*c* 1.35, DMSO); IR (film) ν3303, 2982, 2922, 2901, 2853, 1734, 1655, 1652, 1612, 1483, 1459, 1364, 1246, 1235, 1215; ¹H 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); ¹³C 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 C₂₀H₂₃NO₈ (M⁺): 405.1424, Found: 405.1431.



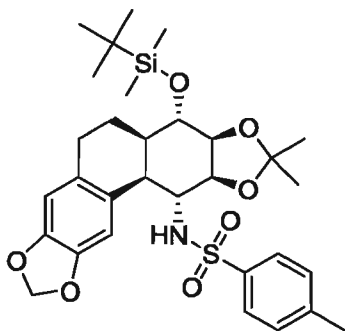
((1*S*,2*S*,3*R*,4*S*,4*aR*,11*bR*)-2,3,4-trihydroxy-6-oxo-1,2,3,4,4*a*,5,6,11*b*-octahydro-[1,3]dioxolo[4,5-*j*]phenanthridin-1-yl)methyl acetate (170).

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 (methylene chloride/methanol, 5:1). Yield: 6 mg, 45%, white solid; mp >200 °C (methylene chloride/methanol; *R_f* 0.41 (methylene 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 C₁₇H₂₀NO₈ (*M* + 1): 366.0988, Found: 366.1088.



(1*S*,2*S*,3*R*,4*S*,4*aR*,11*bR*)-2,3,4-trihydroxy-1-(hydroxymethyl)-1,2,3,4,4*a*,5-hexahydro-[1,3]dioxolo[4,5-*j*]phenanthridin-6(11*bH*)-one (167).

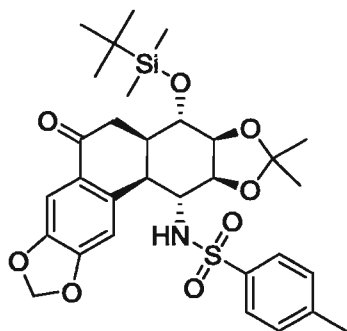
To a solution of acetate **240** (25 mg, 0.062 mmol) at 0 °C, in MeOH (5 mL) was added K₂CO₃ (40 mg, 0.62 mmol) and H₂O (1 mL). The suspension was stirred until total consumption of starting material (TLC) before being quenched with HCl (4 drops, 6N). The reaction was allowed to warm to room temperature 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 (methylene chloride/methanol, 5:1); [α]_D²² 90.91 (*c* 0.25, DMSO); IR (film) ν 3361, 2916, 1646, 1608, 1503, 1460, 1385, 1361, 1252; ¹H 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; ¹³C 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 C₁₅H₁₈NO₇ (*M* + 1): 324.1085, Found: 324.1084.



***N*-[(1*R*,2*aS*,4*aS*,5*S*,5*aR*,12*bR*)-5-(*tert*-Butyl-dimethyl-silyloxy)-3,3-dimethyl-1,2*a*,4*a*,5,5*a*,12*b*-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. *R*_f 0.35 (hexanes:ethyl acetate, 2:1); [α]_D²³ -46.2 (*c* 2.8, CHCl₃); IR (film) ν 3483, 3273, 2983, 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,

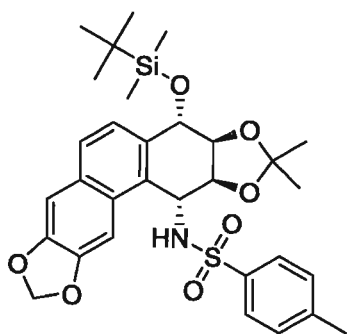
128.8, 126.7, 110.6, 109.0, 108.6, 100.6, 79.5, 79.1, 71.1, 57.2, 41.3, 39.5, 27.9, 27.7, 25.9, 25.7, 21.6, 21.4, 18.0, -4.9, -5.0 ppm; HRMS-EI Calcd for $C_{31}H_{43}NO_7SSi$: 601.2530; Found, 601.2543; Anal. calcd for $C_{31}H_{43}NO_7SSi$ C, 61.87; H, 7.20; found C, 61.73; H, 7.29.



***N*-((1*R*,2*S*,3*S*,4*S*,4*aR*,11*bR*)-4-(*tert*-butyldimethylsilyloxy)-3,3-dimethyl-6-oxo-1,2,3,4,4*a*,5,6,11*b*-octahydrophenanthro[3,2-*d*][1,3]dioxol-1-yl)-4-methylbenzenesulfonamide (243).**

Acetonide **242** (34 mg, 0.056 mmol) was taken up in dry CH_2Cl_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: 16 mg, 46 %, foamy solid; $[\alpha]_D^{23}$ -55.35 (*c* 0.06, $CHCl_3$); *R_f* 0.29 (hexanes:ethyl acetate, 2:1); IR (film) ν 3460, 3266, 2984, 2953, 2929 2897, 2857, 1671, 1654, 1615, 1504, 1468, 1371, 1330, 1280 cm^{-1} ; 1H NMR (600 MHz, $CDCl_3$) 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,

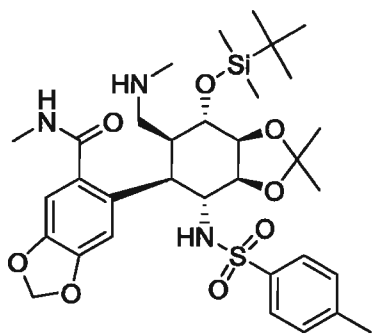
2H), 4.08 (s, 1H), 3.68 (dd, $J = 8.6, 8.5$ Hz, 1H), 3.15 (dd, $J = 18.3, 14.3$ Hz, 1H), 3.0 (dd, $J = 11.9, 3.6$ Hz, 1H), 2.59 (dd, $J = 18.4, 4.5$ Hz, 1H), 2.54 (d, $J = 14.8$ Hz, 1H), 2.41 (s, 3H), 1.42 (s, 3H), 1.33 (s, 3H), 0.86 (s, 9H), 0.09 (s, 3H), 0.04 (s, 3H) ppm; ^{13}C NMR (150 MHz, CDCl_3) δ : 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 $\text{C}_{27}\text{H}_{32}\text{NO}_8\text{SSi}$: 558.1618; Found, 558.1617; Anal. calcd for $\text{C}_{31}\text{H}_{41}\text{NO}_8\text{SSi}$: C, 60.46; H, 6.71; found C, 59.41; H, 6.63.



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

Acetonide **242** (34 mg, 0.056 mmol) was taken up in dry CH_2Cl_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; $[\alpha]_{\text{D}}^{23}$ 80.07 (c 1.25, CHCl_3);

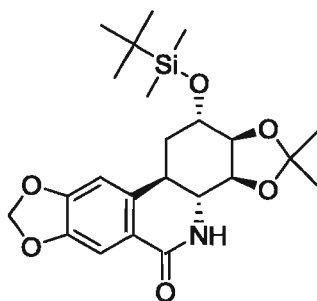
R_f 0.44 (hexanes:ethyl acetate, 2:1); IR (film) ν 3666, 3024, 2954, 2929, 2858, 1621, 1598, 1503, 1470, 1411, 1381, 1372, 1334, 1214 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ : 7.69 (d, $J = 8.2$ Hz, 2H), 7.59 (d, $J = 8.2$ Hz, 2H), 7.7.20-7.16 (m, 3H), 7.05 (s, 1H), 6.88 (s, 1H), 6.41 (d, $J = 10.7$ Hz, 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 $\text{C}_{31}\text{H}_{39}\text{NO}_7\text{SSi}$: 597.2217; Found, 597.2209; Anal. calcd for $\text{C}_{31}\text{H}_{39}\text{NO}_7\text{SSi}$: C, 62.28; H, 6.58; found C, 62.15; H, 6.64



(3'a*S*,4'*R*,5'*R*,6'*S*,7'*S*,7'a*S*)-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

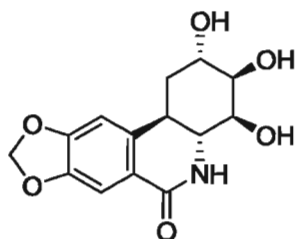
total consumption of starting material as monitored by TLC. The reaction was cooled to 0 °C and the methylamine was allowed to slowly evaporate. The reaction was transferred to a separatory funnel and diluted with EtOAc (60 mL), washed with Na₂CO₃ (2 x 2 mL), and brine (1 mL) before being dried over 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]_D²³ -63.42 (*c* 0.75, CHCl₃); *R*_f 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.



(3a*S*,3b*R*,10b*R*,12*S*,12a*S*)-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; *R*_f 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),

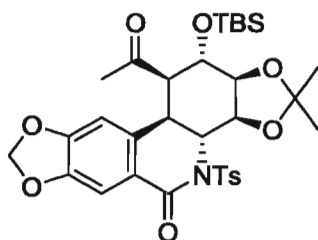
4.42 (d, $J = 2.10$, 1H), 4.18, (dd, $J = 8.3$ Hz, 4.8 Hz, 1H), 4.11 (m, 1H), 3.43 (dd, $J = 13.2$ Hz, 8.6 Hz, 1H), 3.12 (t, $J = 13.5$ Hz, 1H), 2.26 (d, $J = 13.6$ Hz, 1H), 1.77 (t, $J = 13.9$ Hz, 1H), 1.46 (s, 3H), 1.41 (s, 3H), 0.91 (s, 9H), 0.15 (s, 3H), 0.14 (s, 3H); ^{13}C NMR (150 MHz, CDCl_3) 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 $\text{C}_{23}\text{H}_{33}\text{NO}_6\text{Si}$ (M^+): 447.2077, Found: 447.2083.



7-Deoxy-trans-dihydrolycoricidine (3).

To a solution of acetone **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); R_f 0.25 (methylene chloride/methanol, 5:1); $[\alpha]_D^{25}$ 28.6 (c 0.25, DMSO), lit.¹⁵⁰ $[\alpha]_D^{25}$ 138 (c 0.96, DMSO); IR (KBr) ν 3559, 3488, 3450, 3429, 1671, 1471, 1263 cm^{-1} ; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) 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; ^{13}C

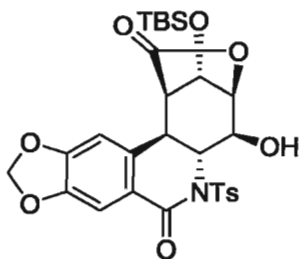
NMR (150 MHz, DMSO-*d*₆) δ : 164.7, 151.1, 146.4, 138.5, 123.7, 107.4, 104.8, 102.0, 72.1, 70.1, 69.1, 55.6, 34.7, 28.8 ppm; HRMS-EI Calcd for C₁₄H₁₆NO₆ (M⁺+1): 294.0978, Found: 294.1011.



(3*aS*,3*bR*,10*bR*,11*R*,12*S*,12*aS*)-11-Acetyl-12-(*tert*-butyl-dimethyl-silyloxy)-2,2-dimethyl-4-(toluene-4-sulfonyl)-3*b*,4,10*b*,11,12,12*a*-hexahydro-3*aH*-1,3,7,9-tetraoxa-4-aza-dicyclopenta[*a,h*]phenanthren-5-one (257)

To a solution of aldehyde **166** (200 mg, 0.318 mmol) in Et₂O (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 NH₄Cl (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 CH₂Cl₂ (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),

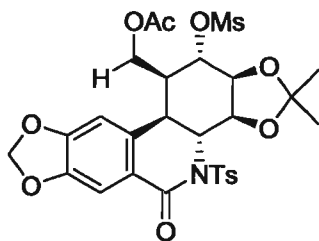
NaHCO₃ (3 mL), dried over sodium sulfate, filtered and concentrated. The final product was isolated by column chromatography (hexane/ethyl acetate, 4:1). Yield: 76 mg, 44%, clear and colorless oil; *R_f* 0.26 (hexanes:ethyl acetate, 2:1); [α]_D²² – 56.87 (*c* 4.0, CHCl₃); IR (film) ν 3002, 2975, 2861, 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 C₃₂H₄₁NO₉SSi (M⁺ 15): 628.2036, Found: 628.2036.



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

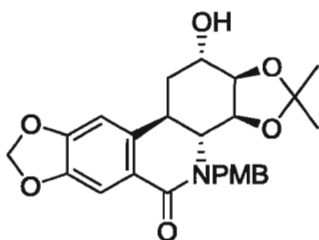
To a solution of ketone **257** (13 mg, 0.021mmol) in CH₂Cl₂ (0.5 mL) in a conical vial was added a single crystal of *p*-toluenesulfonic acid monohydrate followed

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; *R*_f 0.16 (hexanes/ethyl acetate, 2:1); IR (film) ν₃₅₀₇, 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 C₂₇H₃₀NO₉Si (M⁺-15): 572.1368, Found: 572.1389.



[(3*aS*,3*bR*,10*bR*,11*S*,12*S*,12*aS*)-2,2-dimethyl-4-[(4-methylphenyl)sulfonyl]-5-oxo-12-(sulfooxy)-3*a*,3*b*,4,5,10*b*,11,12,12*a*-octahydrobis[1,3]dioxolo[4,5-*c*:4',5'-]phenanthridin-11-yl]methyl acetate (272).

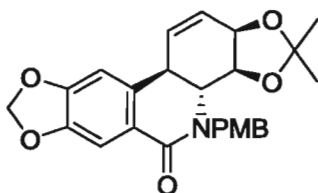
To a stirring solution of **271** (73 mg, 0.131 mmol) in dry CH₂Cl₂ (4 mL) at 0 °C was added triethylamine (0.55 μL, 0.394 mmol) followed by methanesulfonyl chloride (0.02 mL, 0.263 mmol). The reaction was stirred at 0 °C until total consumption of starting material as indicated by TLC (20 minutes). 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; *R*_f 0.27 (hexanes/ethyl acetate, 1:1); [α]_D²² 5.42 (*c* 0.75, CHCl₃); IR (film) ν 3025, 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 C₂₇H₂₈NO₁₂S₂ (M⁺-15): 622.1053, Found: 622.1057



(3a*S*,3b*R*,10b*R*,12*S*,12a*R*)-12-Hydroxy-4-(4-methoxy-benzyl)-2,2-dimethyl-3b,4,10b,11,12,12a-hexahydro-3a*H*-1,3,7,9-tetraoxa-4-azadicyclopenta[*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; *R_f* 0.41 (hexanes:ethyl acetate, 2:1); [α]_D²² + 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),

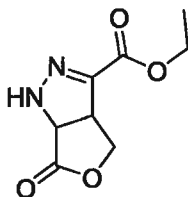
4.39 (dd, $J = 7.6, 6.2$ Hz, 1H), 4.23 (m, 1H), 4.12 (t, $J = 5.61$ Hz, 1H), 3.78 (s, 3H), 3.71 (dd, $J = 13.4, 7.9$ Hz, 1H), 3.19 (td, $J = 12.4, 5.0$ Hz, 1H), 2.36-2.28 (m, 2H), 2.01 (qd, $J = 13.6, 11.9, 4.9$ Hz, 1H), 1.387 (s, 3H), 1.34 (s, 3H) ppm; ^{13}C NMR (150 MHz, CDCl_3) δ : 165.2, 158.4, 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 $\text{C}_{25}\text{H}_{25}\text{NO}_7$ (M^+): 453.1788, Found: 453.1787.



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

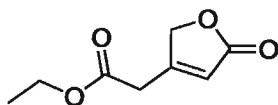
To a solution of alcohol **284** (15 mg, 0.033 mmol) in THF (1 mL) at 0 °C was added a spatula tip of NaH (60 % dispersion in mineral oil). The reaction was stirred at 0 °C for 10 min before being allowed to warm to rt and stir for 45 min. The reaction was cooled externally to 0 °C again before CS_2 (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_4Cl (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*-xylene (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; *R*_f 0.51 (hexanes:ethyl acetate, 1:1); [α]_D²² + 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), 6.05 (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 C₂₅H₂₅NO₆ (M⁺): 435.1682, Found: 435.1683



Ethyl 6-oxo-3a,4,6,6a-tetrahydro-1H-furo[3,4-c]pyrazole-3-carboxylate (302).

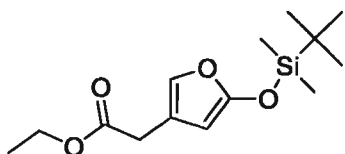
To a solution of crotonolactone **301** (1.0 g, 12 mmol) in dioxane (7 mL) was added ethyldiazoacetate (1.5 mL, 14 mmol). The reaction mixture was heated at 95 °C for 12 hours before being allowed to cool to rt and concentrated to a thick oil. Trituration with CHCl₃ provided a crude yellow solid. The final product was purified by recrystallization from Et₂O. Yield: 0.796g, 48%, off white solid, mp 132-134 °C (diethyl ether); *R_f* 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, 2H), 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, 14.2 ppm; HRMS-EI Calcd for C₈H₁₀N₂O₄ (M⁺): 198.0641, Found: 198.0638.



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

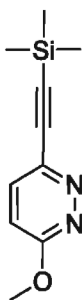
Pyrazole **302** (2.8 g, 14.13 mmol) suspended in toluene (75 mL) in a Schlenk tube equipet with a teflon stopper and a magnetic stirring bar. The tube was sealed and heated to 150 °C for 12 h. The crude reaction mixture was filtered through a silica plug and eluted with a mixture of hexane:ethyl acetate (2:1). Yield: 2.135 g, 89 %, clear and colorless oil; *R_f* 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 C₈H₁₀O₄

(M⁺): 170.0579, Found: 170.0578; Anal. calcd C₈H₁₀O₄ C, 56.47; H, 6.84; found C, 56.32; H, 6.01.



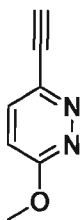
Ethyl 2-(5-(tert-butyldimethylsilyloxy)furan-3-yl)acetate (305)

Lactone **303** (980 mg, 5.79 mmol) was taken up in CH₂Cl₂ (20 mL) and cooled externally in an ice bath while NEt₃ (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 H₂O (20 mL). The two layers were separated and the aqueous phase was extracted with CH₂Cl₂ (3 x 60 mL). The organic phase was washed with a 10% solution of citric acid (2 x 10 mL), H₂O (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) ν 3127, 2932, 2860, 2744, 2715, 1743, 1625, 1573, 1524, 1472, 1392, 1366, 1287 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ : 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; ¹³C NMR (75 MHz, CDCl₃) δ : 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 C₁₄H₂₄O₄Si (M⁺): 284.1444, Found: 284.1440; Anal. calcd C₁₄H₂₄O₄Si C, 59.12; H, 8.51; found C, 58.61; H, 8.37.



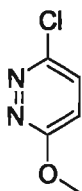
3-Methoxy-6-((trimethylsilyl)ethynyl)pyridazine (296).

To a solution of pyridazine **7** (1 g, 4 mmol) in THF (10 mL) was added triethylamine (3.55 mL, 25.53 mmol). The reaction mixture was cooled externally in an ice bath and trimethylsilylacetylene (0.662 mL, 4.68 mmol) added, followed by CuI (41 mg, 0.21 mmol) and $\text{PdCl}_2(\text{Ph}_3)_2$ (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 CH_2Cl_2 , and filtered through a silica plug (CH_2Cl_2 : Et_3N 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; R_f 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, CDCl_3) 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; ^{13}C NMR (75 MHz, CDCl_3) δ : 163.4, 143.4, 132.4, 116.4, 100.6, 98.2, 55.0, -0.4 ppm; HRMS-EI Calcd for $\text{C}_{10}\text{H}_{14}\text{N}_2\text{OSi}$ (M^+): 206.0875, Found: 206.0875; Anal. calcd $\text{C}_{10}\text{H}_{14}\text{N}_2\text{OSi}$ C, 58.21; H, 6.84; found C, 57.72; H, 6.67.



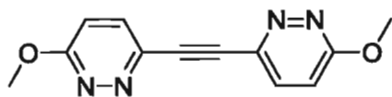
3-Ethynyl-6-methoxypyridazine (307).

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. NH_4Cl (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); R_f 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, CDCl_3) 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; ^{13}C NMR (75 MHz, CDCl_3) δ : 163.7, 142.6, 132.5, 116.6, 80.2, 79.9, 55.1 ppm; HRMS-EI Calcd for $\text{C}_7\text{H}_6\text{N}_2\text{O}$ (M^+): 134.0480, Found: 134.0482; Anal. calcd. $\text{C}_7\text{H}_6\text{N}_2\text{O}$ C, 62.68; H, 4.51; found C, 62.73; H, 4.40.



3-Chloro-6-methoxypyridazine (313)

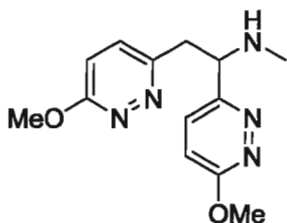
To a solution of 3,6-dichloropyridazine (**8**) (1.5 g, 10.07 mmol) in MeOH (100 mL) was added K₂CO₃ (1.67 g, 12.08 mmol). The resulting suspension was brought to reflux and heated for 11 h. The reaction was allowed to cool to rt before being concentrated to a thick slurry which was quickly added to H₂O (40 mL). The resulting white precipitate was filtered and washed with ice cold H₂O (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) ν 3272, cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ : 7.38 (d, *J* = 9.1 Hz, 1H), 6.97 (d, *J* = 9.1 Hz, 1H), 4.13 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ : 1.64, 4, 151.1, 130.7, 120.0, 55.2 ppm; HRMS-EI Calcd for C₅H₅ClN₂O (M⁺): 144.0090, Found: 144.0092; Anal. calcd C₅H₅ClN₂O 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), PPh₃ (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 NEt₃ (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 CH₂Cl₂ (300 mL), washed with H₂O (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); *R_f* 0.60 (methylene chloride:methanol, 10:1); IR (film) ν 3058, 1589, 1543, 1472, 1422, 1350, 1291 cm^{-1} ; ^1H 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 $\text{C}_{12}\text{H}_{10}\text{N}_2\text{O}_2$ (M^+): 242.0803, Found: 242.0807; Anal. calcd $\text{C}_{12}\text{H}_{10}\text{N}_2\text{O}_2$ C, 59.50; H, 4.16; found C, 59.32; H, 4.16.

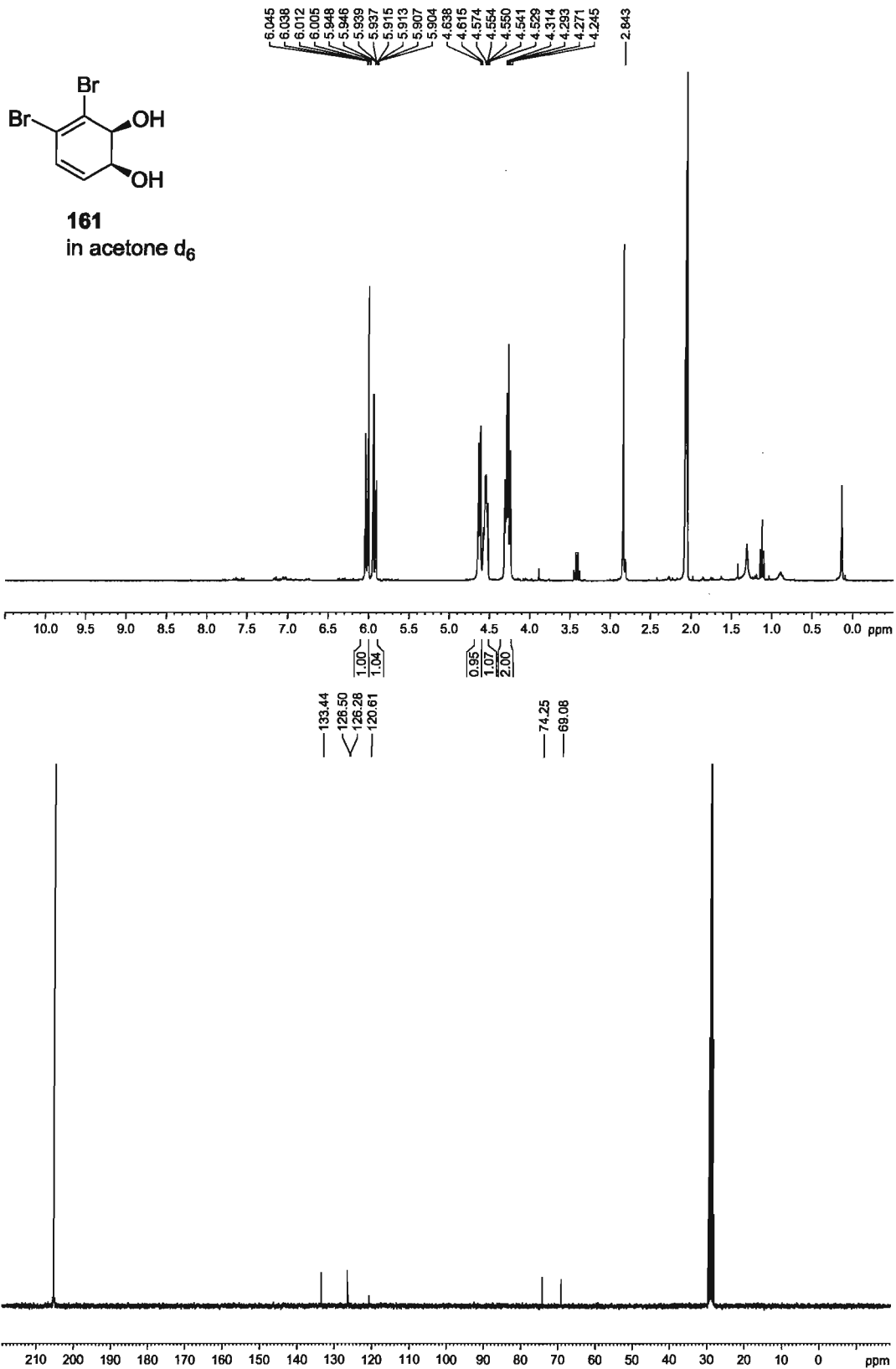


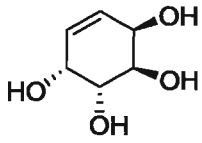
1,2-bis(6-methoxypyridazin-3-yl)-*N*-methylethanamine (309).

Pyridazine 6 (0.5 g, 2.06 mmol) in a Schlenk tube was suspended in toluene (20 mL) and cooled externally to -78 °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. $\text{Ind}_2\text{TiMe}_2$ 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 °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_3CN (127 mg, 2.06 mmol). After 1 h another portion of and NaBH_3CN (120 mg) was added and the reaction was allowed to warm to room temperature over 10 h. The reaction cooled in an

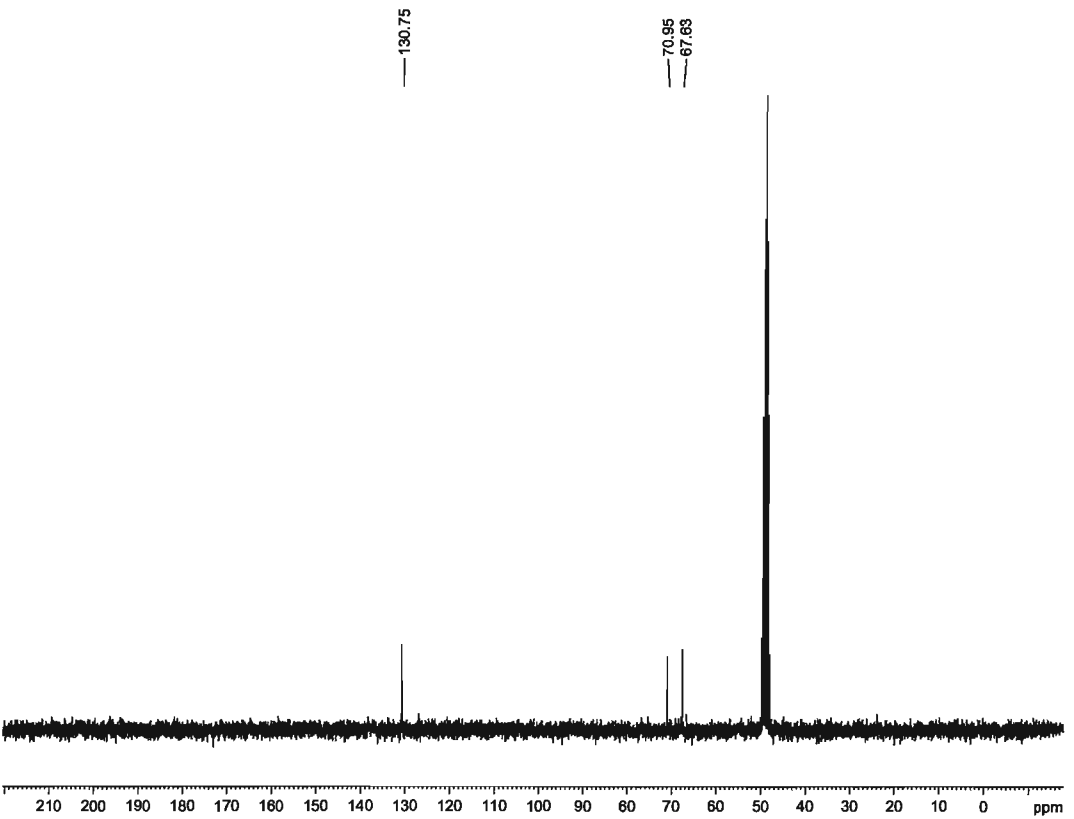
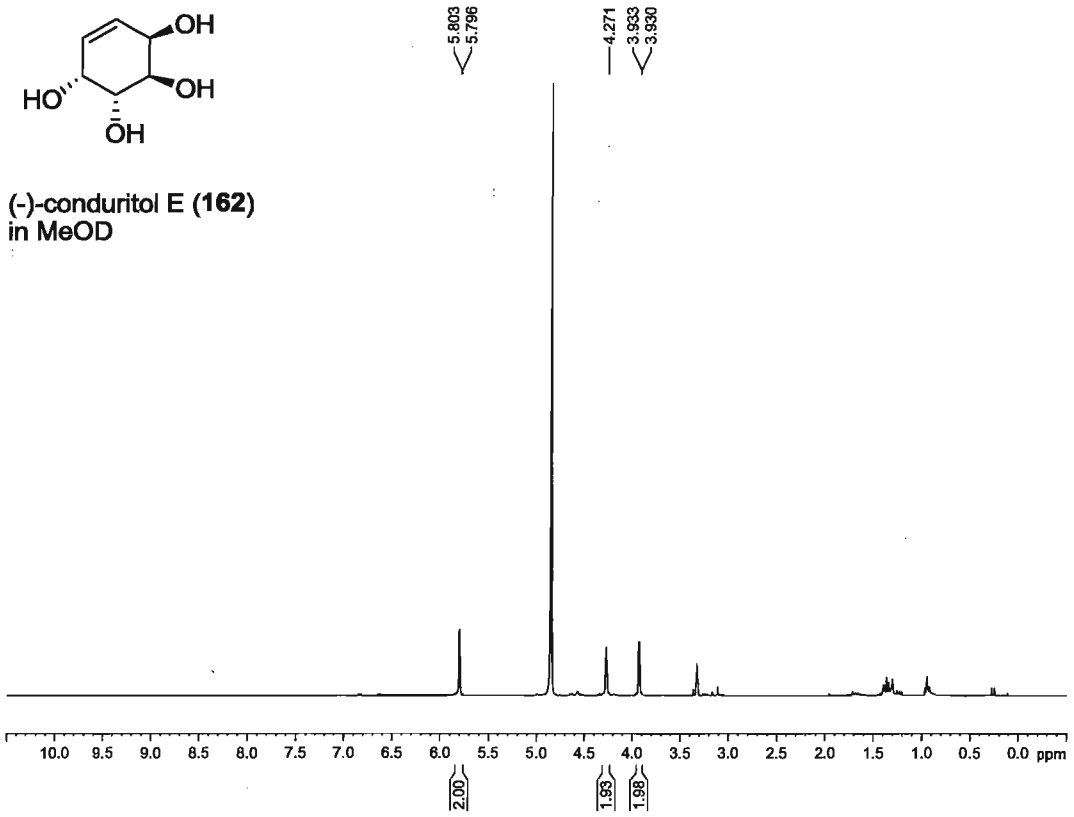
ice bath and quenched with the slow addition of sat. Na_2CO_3 solution (10 mL). The reaction mixture was diluted with CH_2Cl_2 (200 mL) and separate. The aqueous phase was extracted with CH_2Cl_2 (3 x 100 mL). The organic phase was dried over Na_2SO_4 and treated with deactivate charcoal before being 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} ; ^1H NMR (300 MHz, CDCl_3) δ : 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) δ : 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 $\text{C}_{13}\text{H}_{17}\text{N}_5\text{O}_2$ (M^+): 275.1382, Found: 275.1382; Anal. calcd $\text{C}_{13}\text{H}_{17}\text{N}_5\text{O}_2$ C, 56.71; H, 6.22; found C, 56.66; H, 6.15.

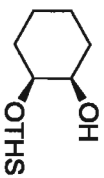
VI. Selected Spectra



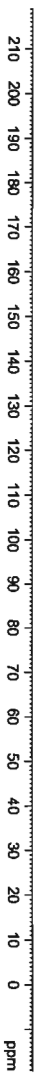
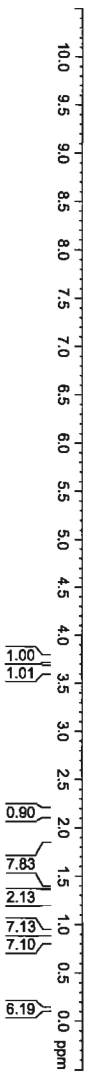
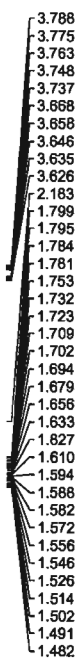


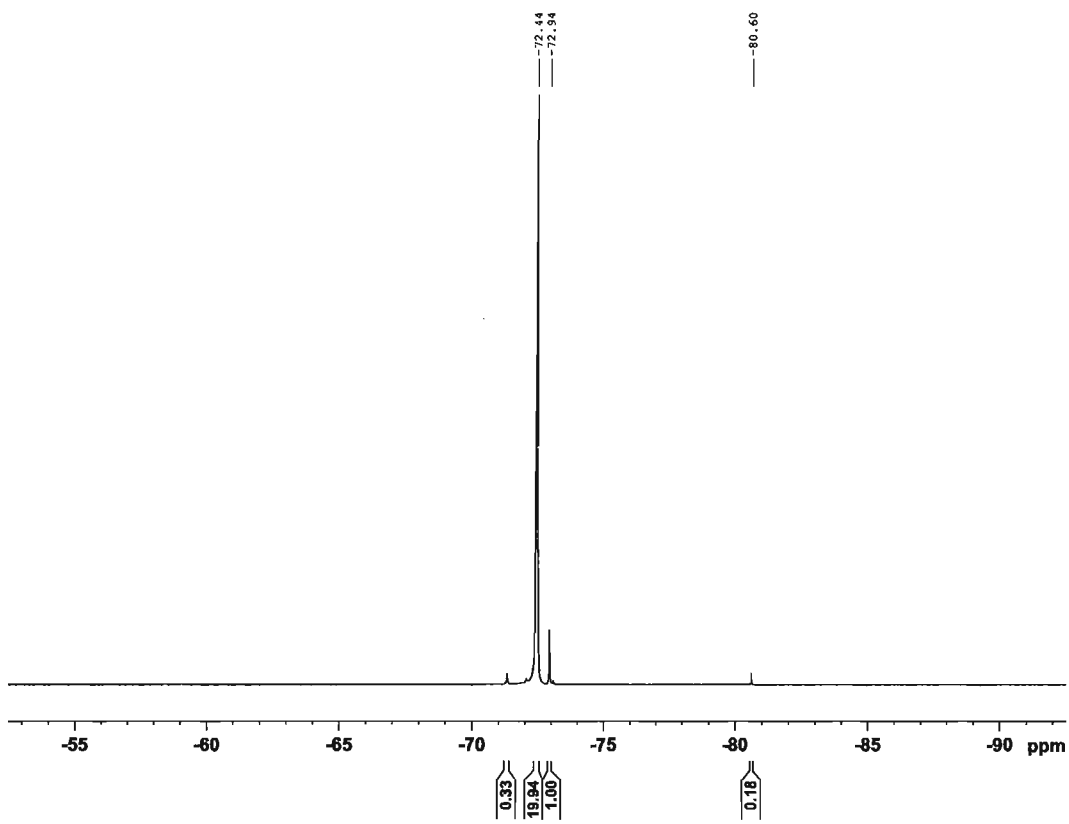
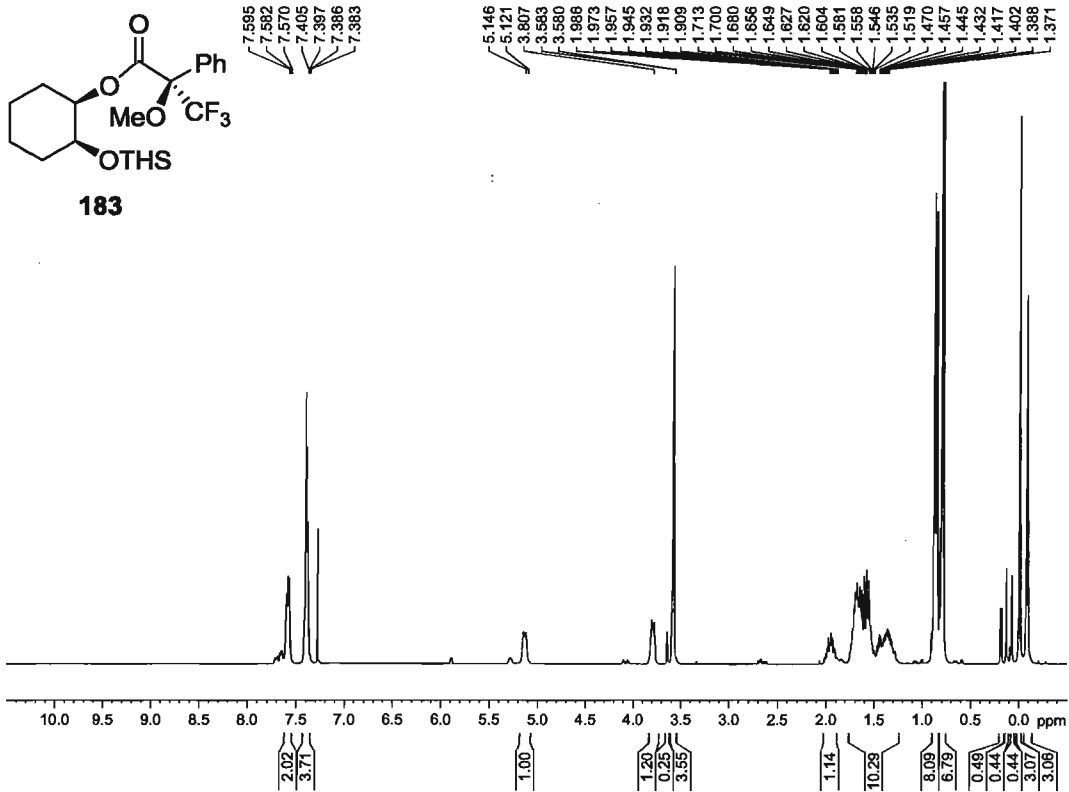
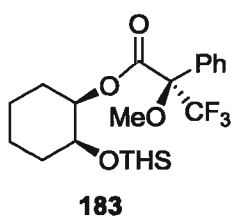
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in MeOD

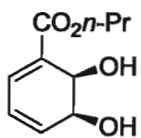




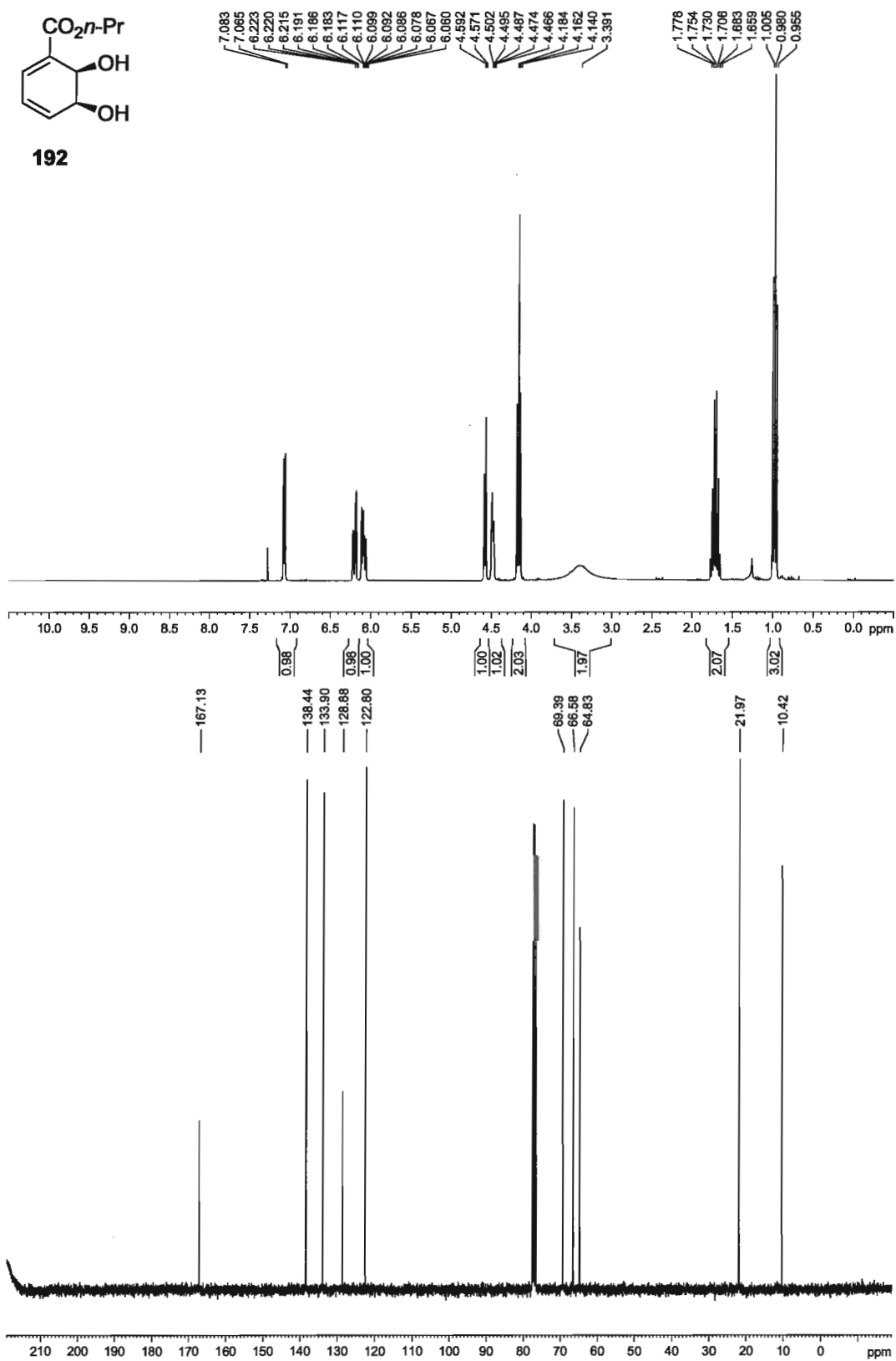
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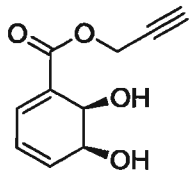




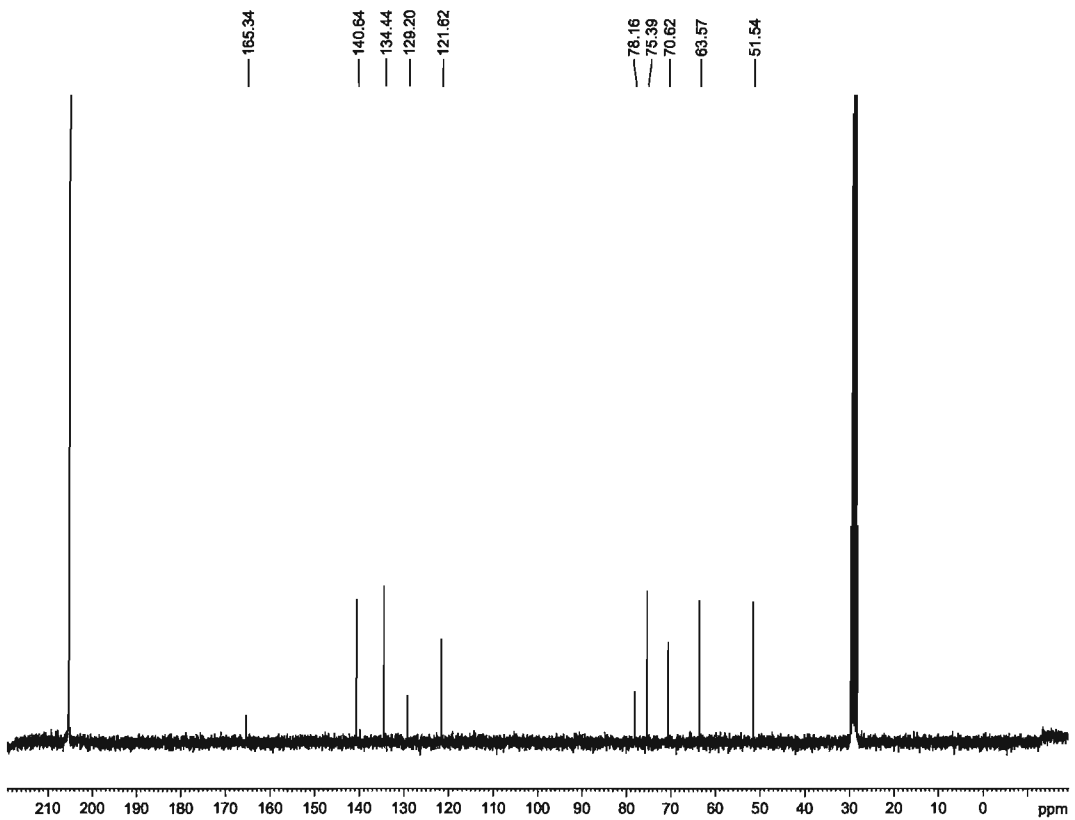
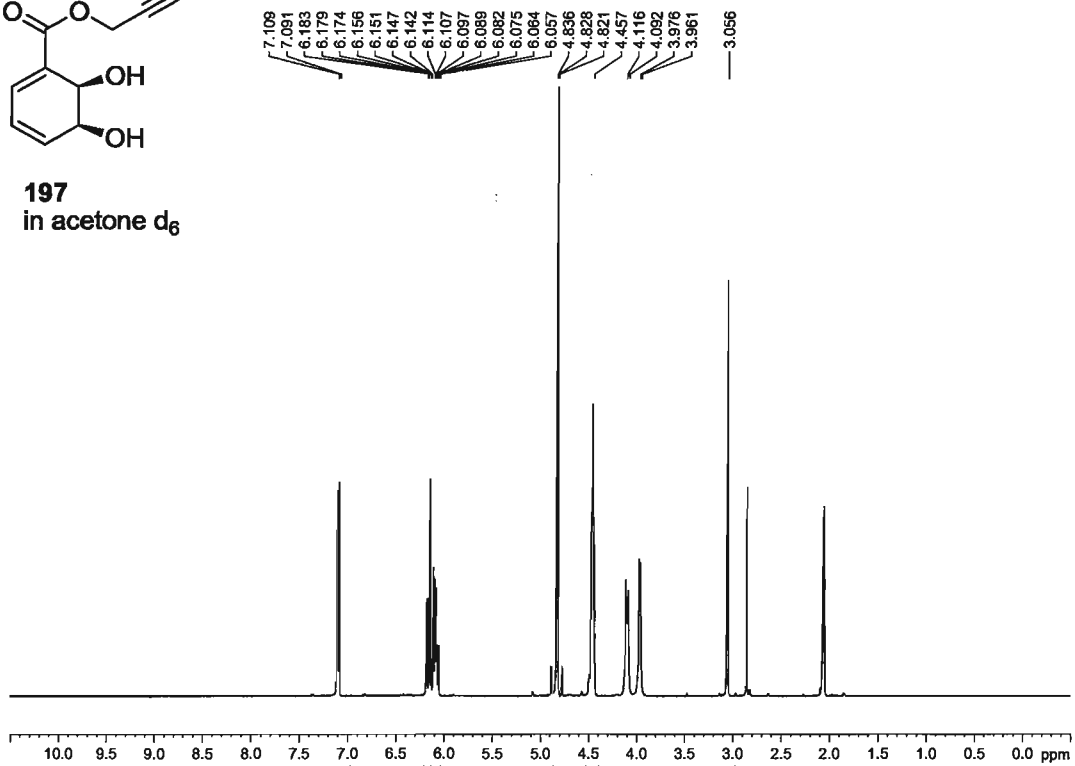


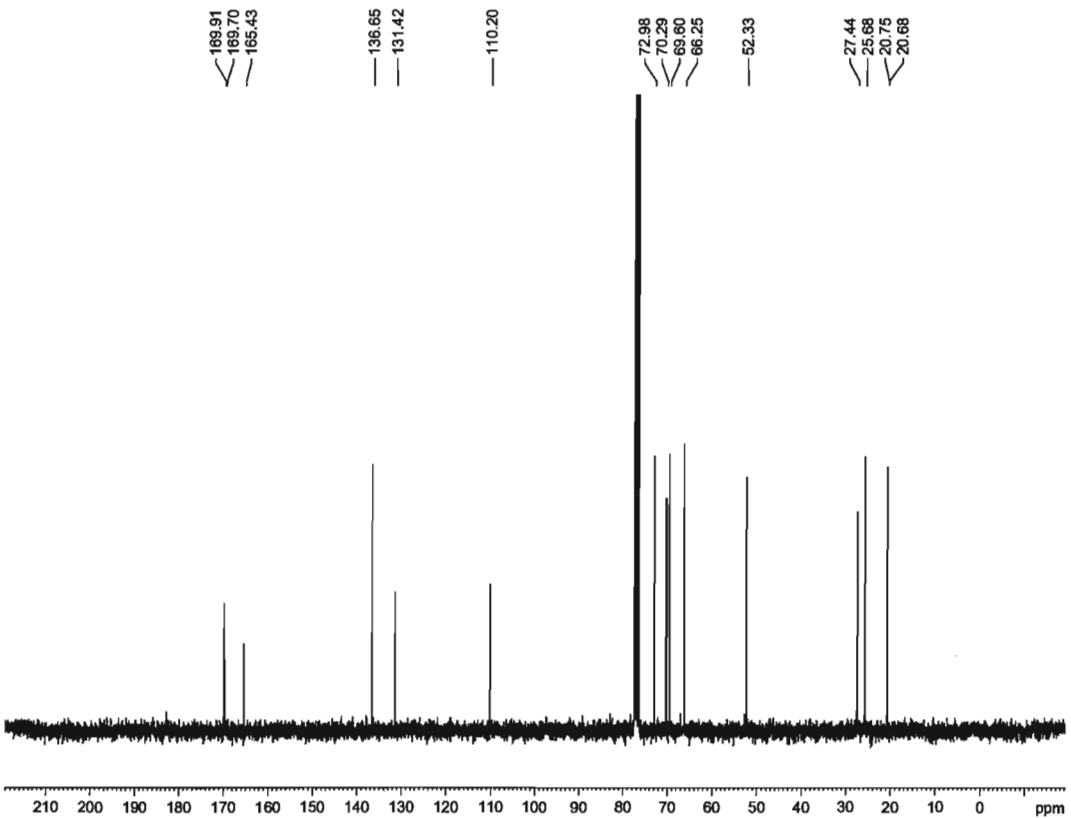
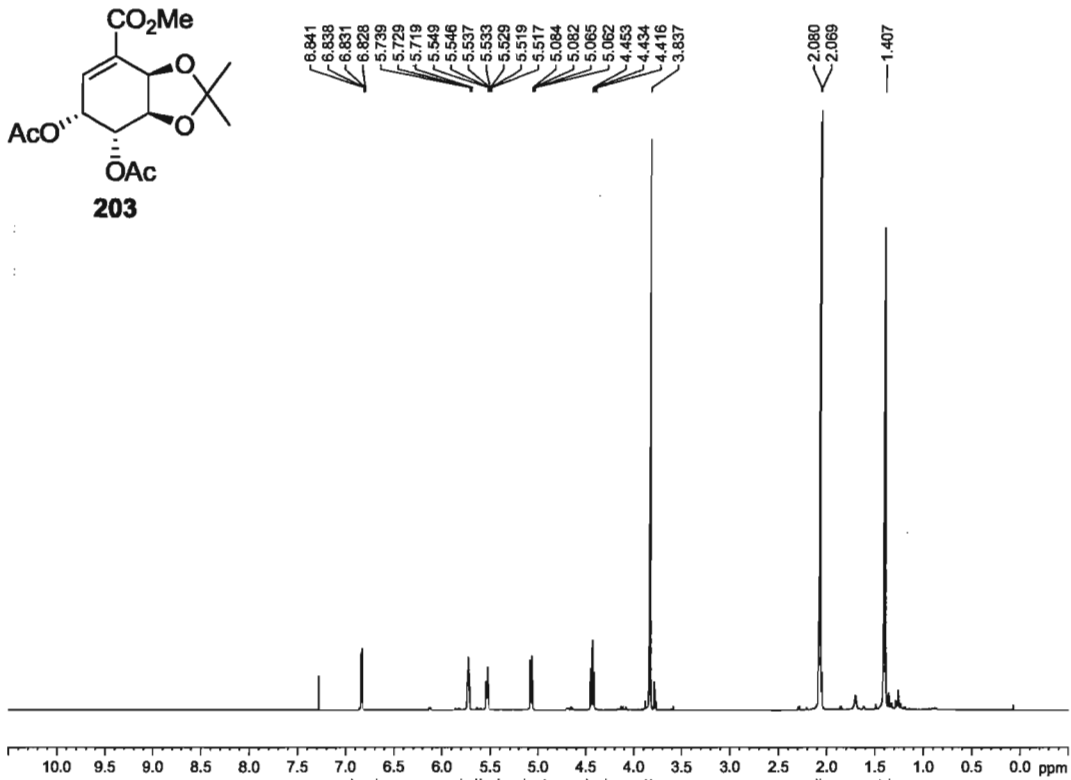
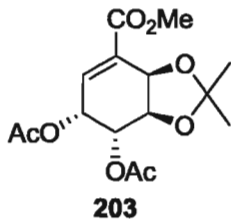
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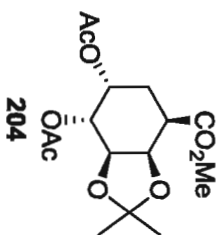




197
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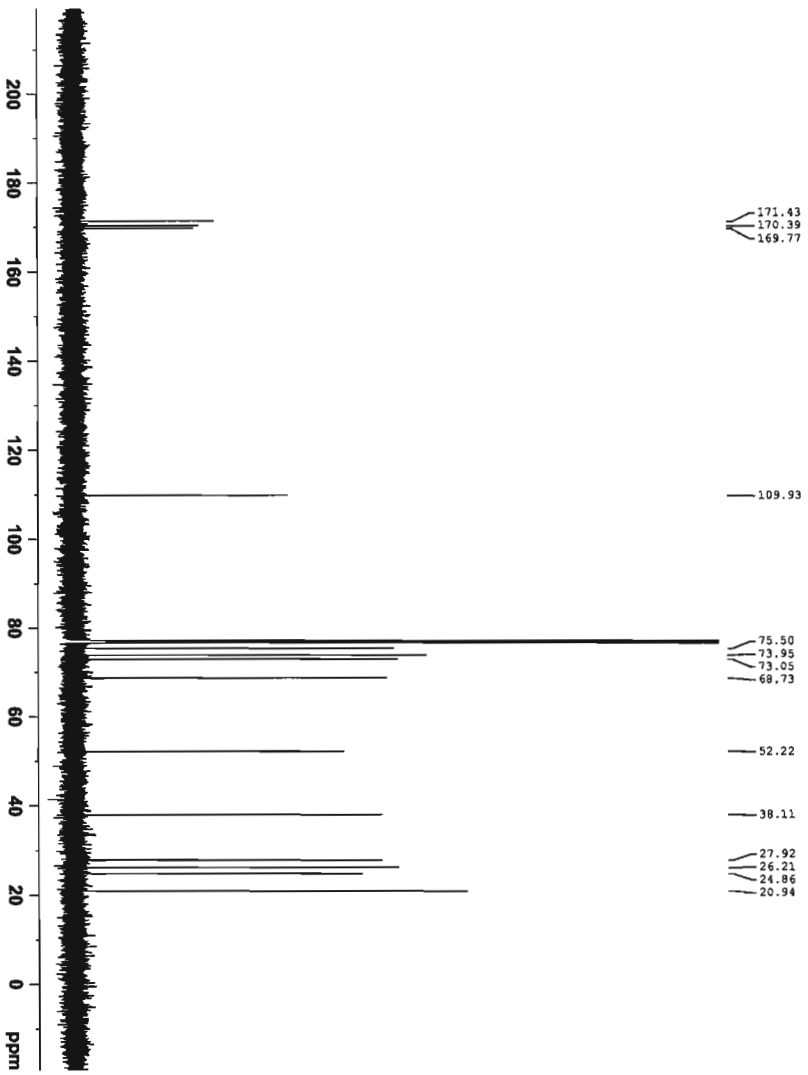
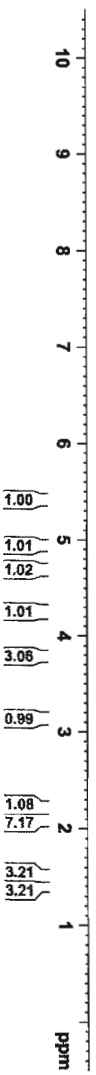




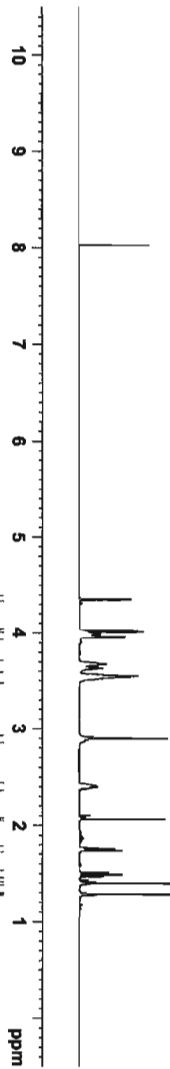
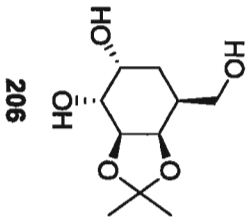


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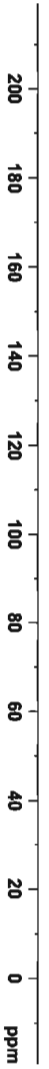


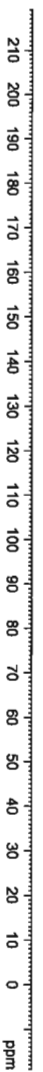
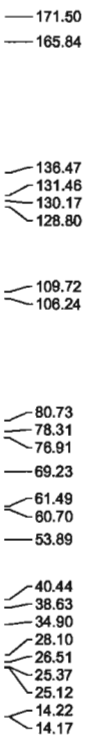
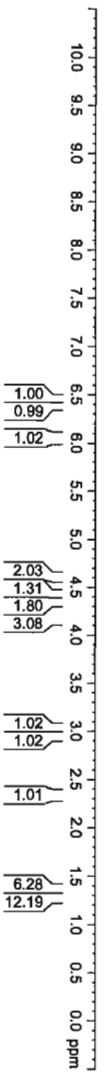
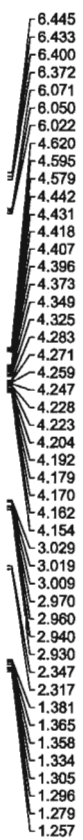
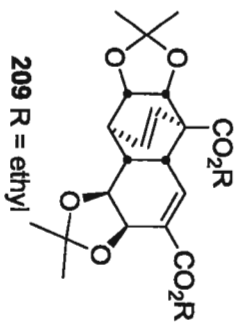
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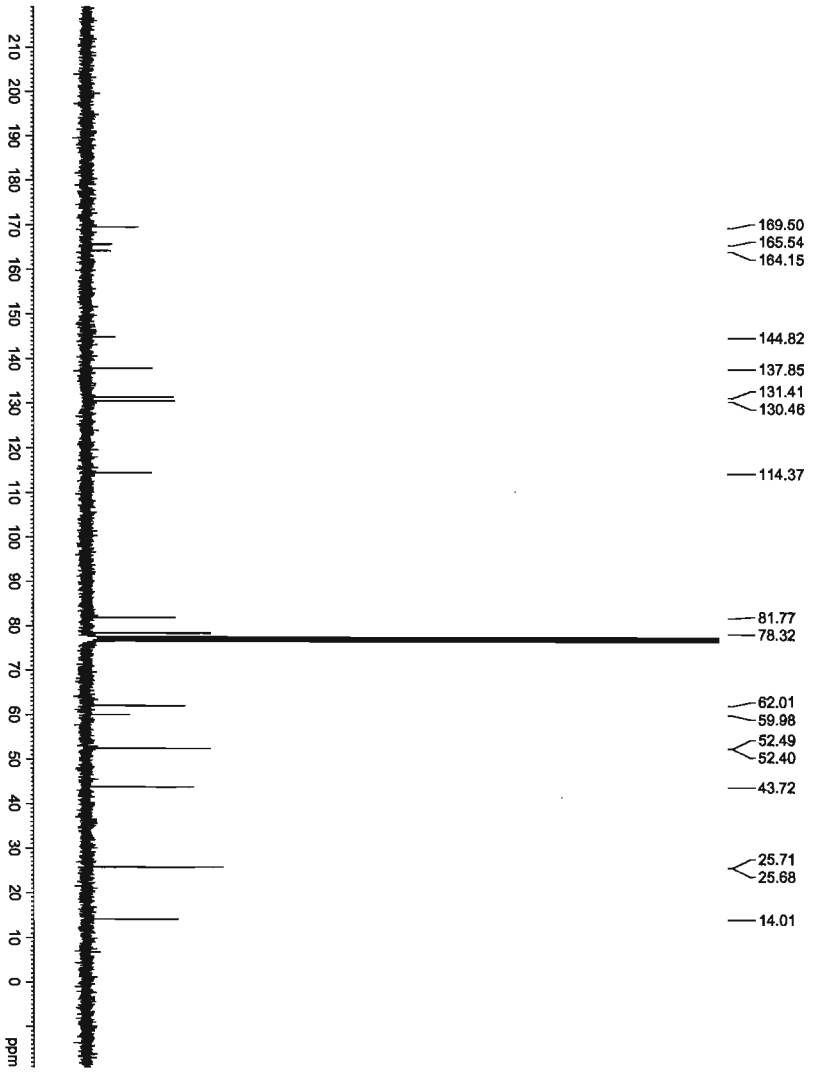
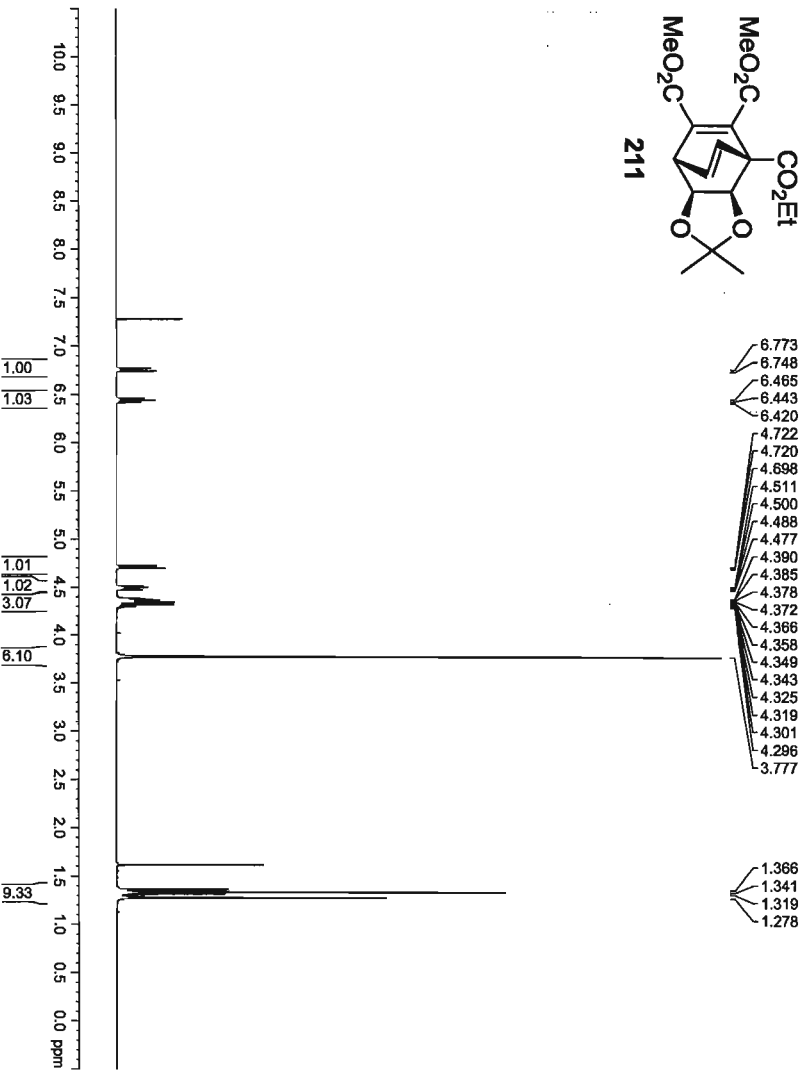
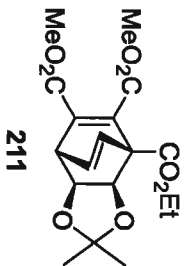


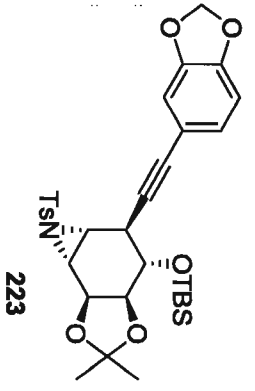
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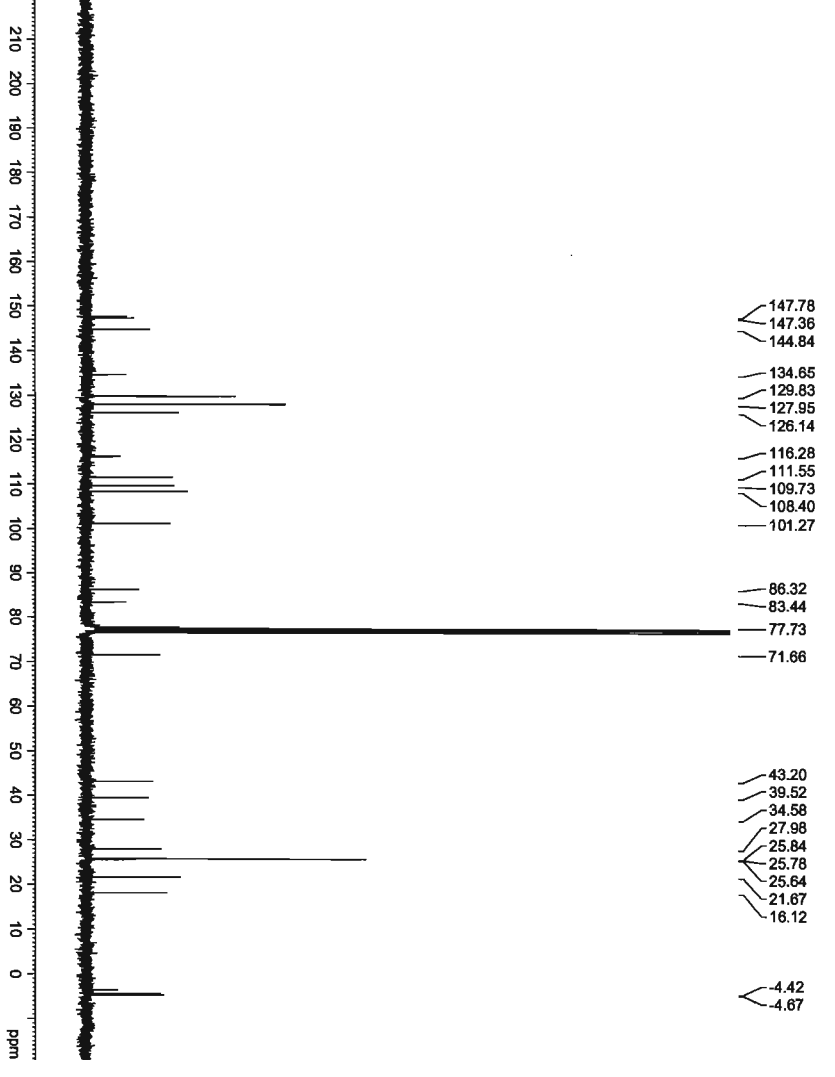
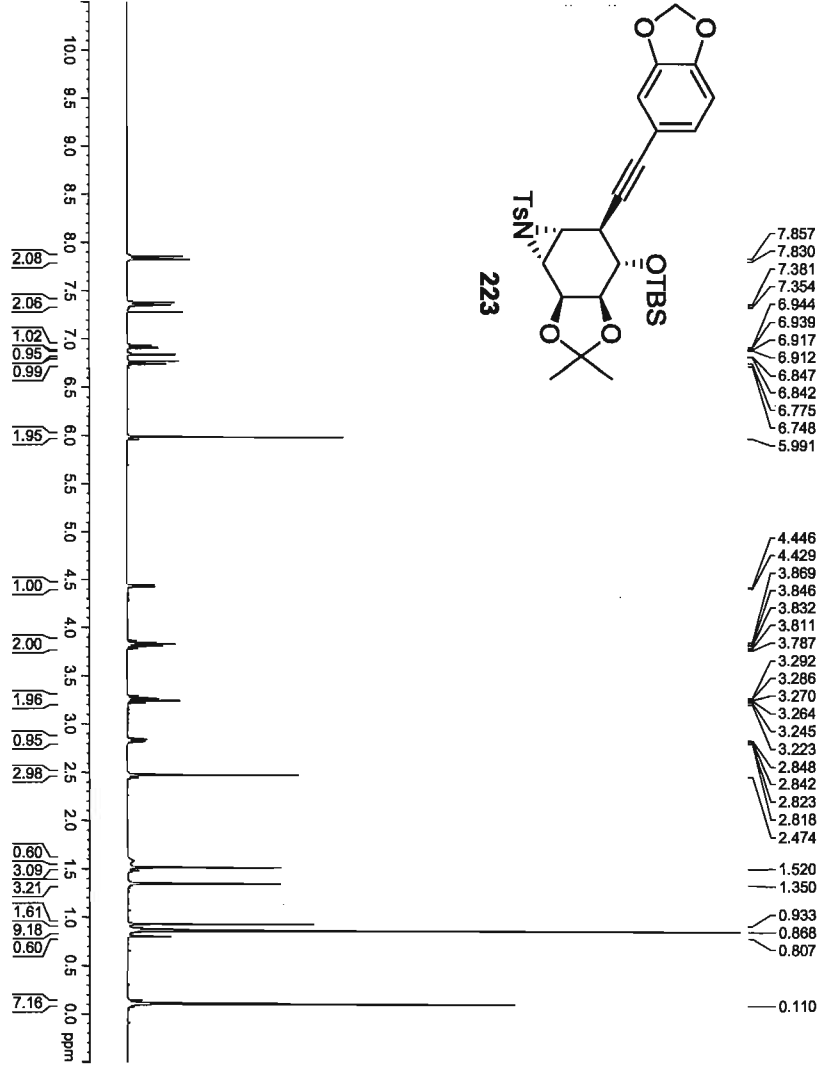


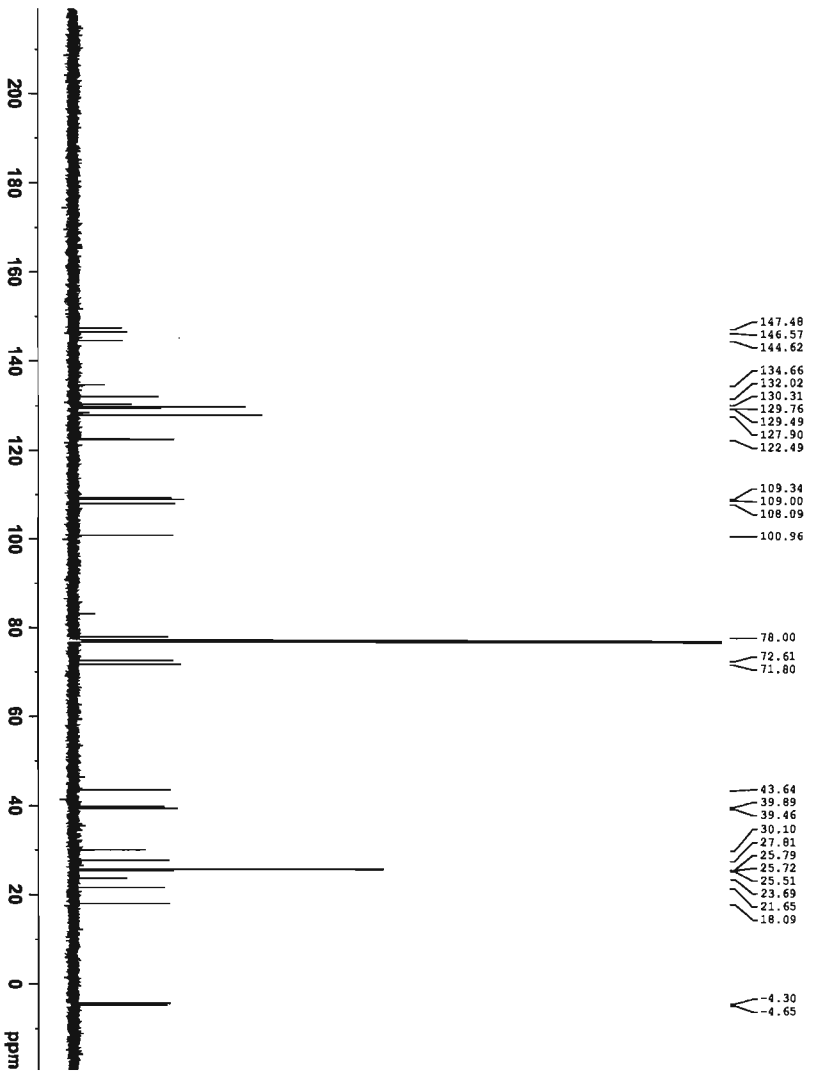
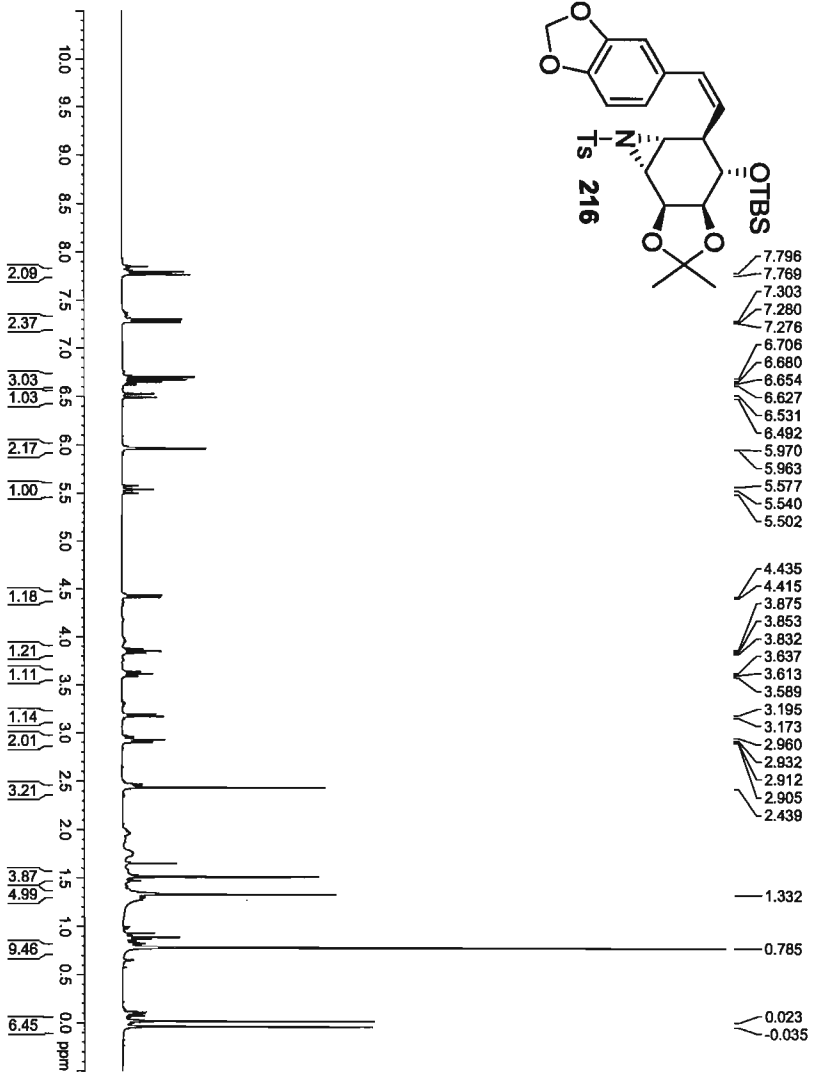
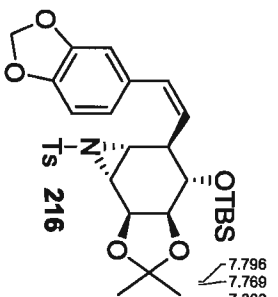


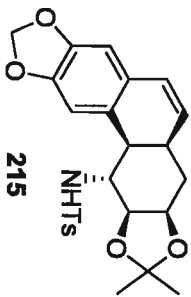




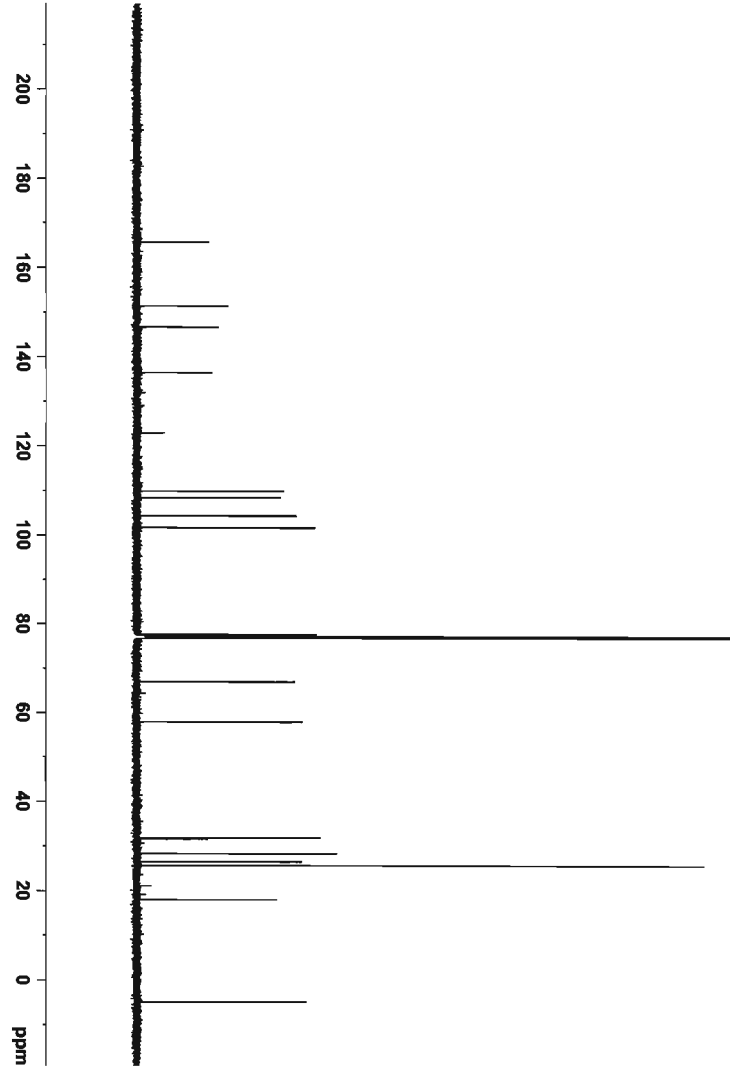
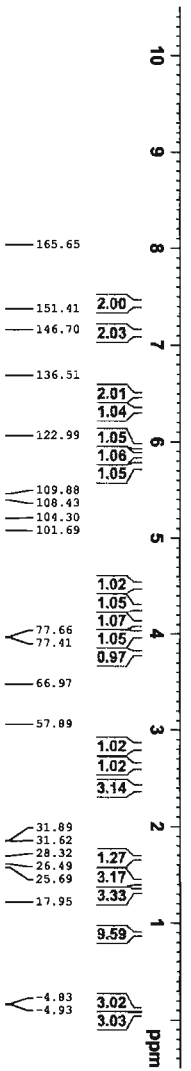
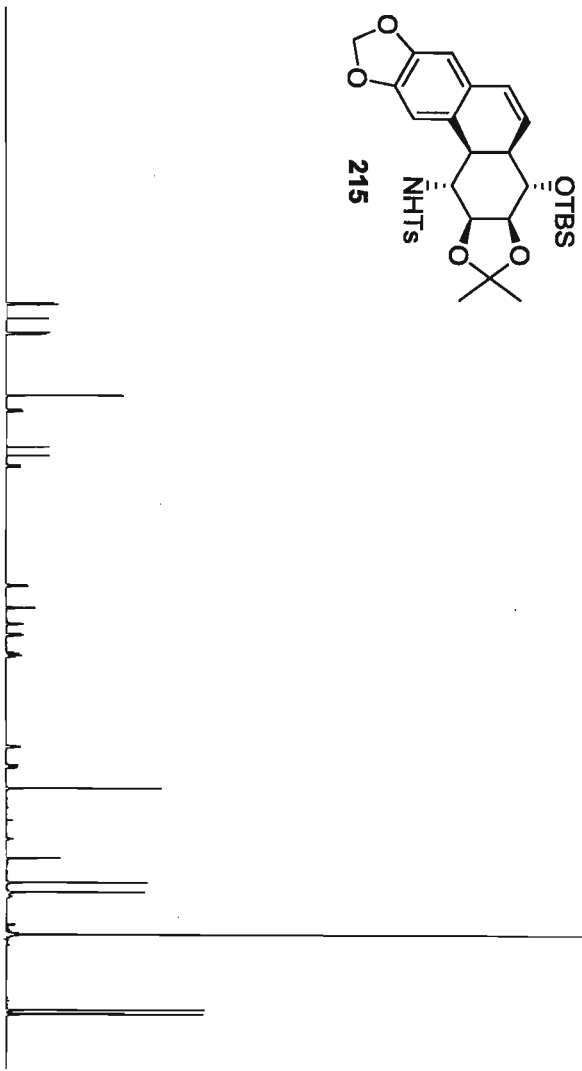
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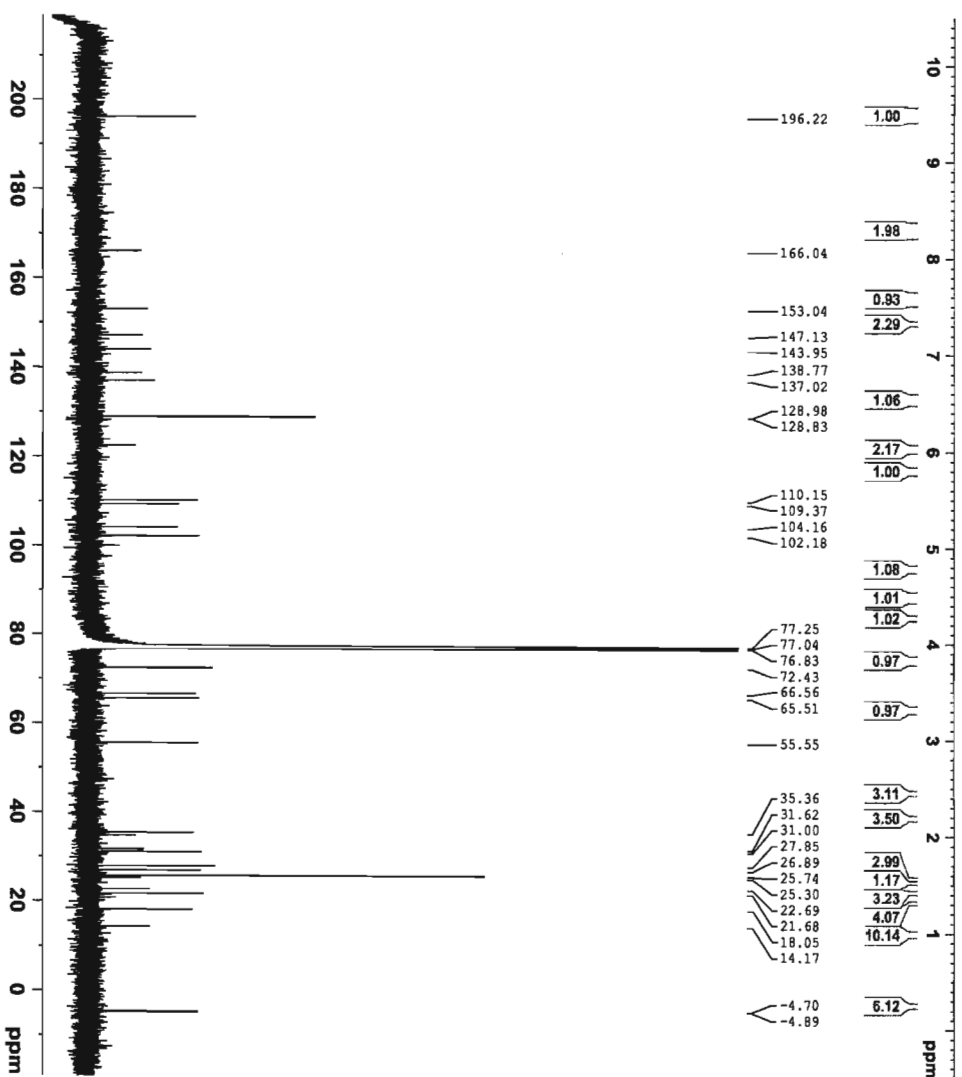
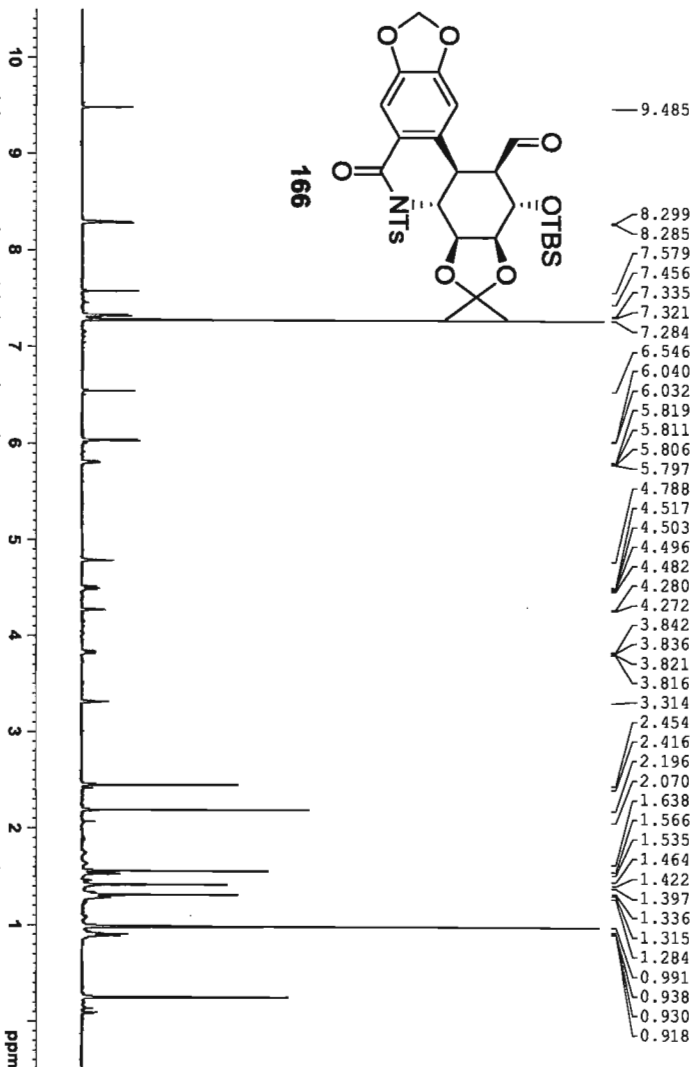
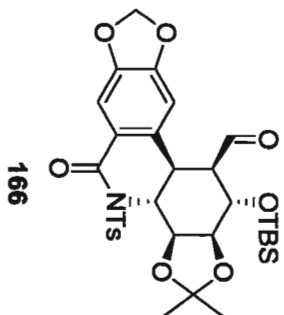


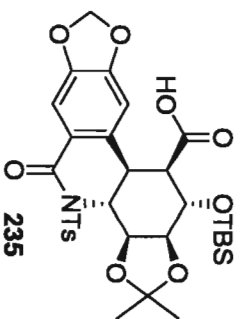




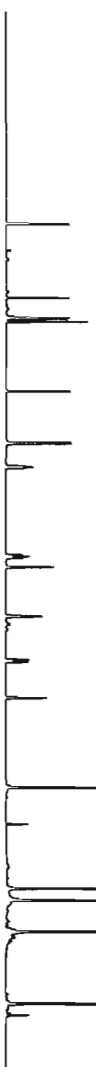
- 7.440
- 7.426
- 7.284
- 7.136
- 7.122
- 6.487
- 6.340
- 6.334
- 6.324
- 6.318
- 5.956
- 5.953
- 5.865
- 5.863
- 5.764
- 5.761
- 5.748
- 5.745
- 4.516
- 4.502
- 4.288
- 4.284
- 4.279
- 4.116
- 4.113
- 4.109
- 4.105
- 4.007
- 3.999
- 3.994
- 3.985
- 3.802
- 3.783
- 2.833
- 2.829
- 2.640
- 2.402
- 1.676
- 1.428
- 1.328
- 0.904
- 0.888
- 0.109
- 0.066





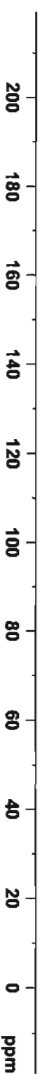


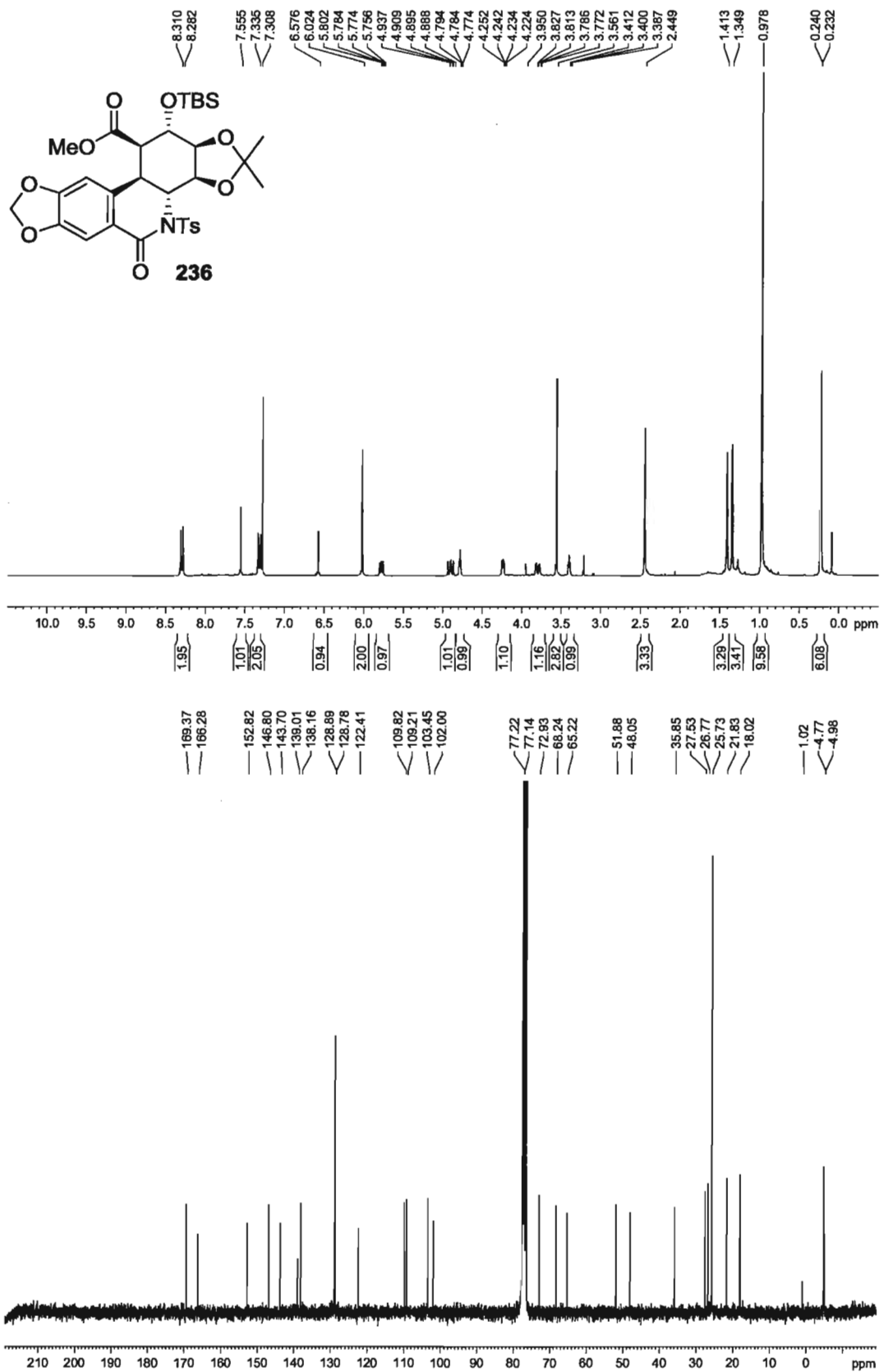
- 8.303
- 8.290
- 7.526
- 7.328
- 7.316
- 7.284
- 6.561
- 6.031
- 6.018
- 5.787
- 5.778
- 4.867
- 4.851
- 4.832
- 4.740
- 4.223
- 3.774
- 3.753
- 3.379
- 2.452
- 2.068
- 1.405
- 1.280
- 0.963
- 0.905
- 0.890
- 0.857
- 0.212
- 0.092

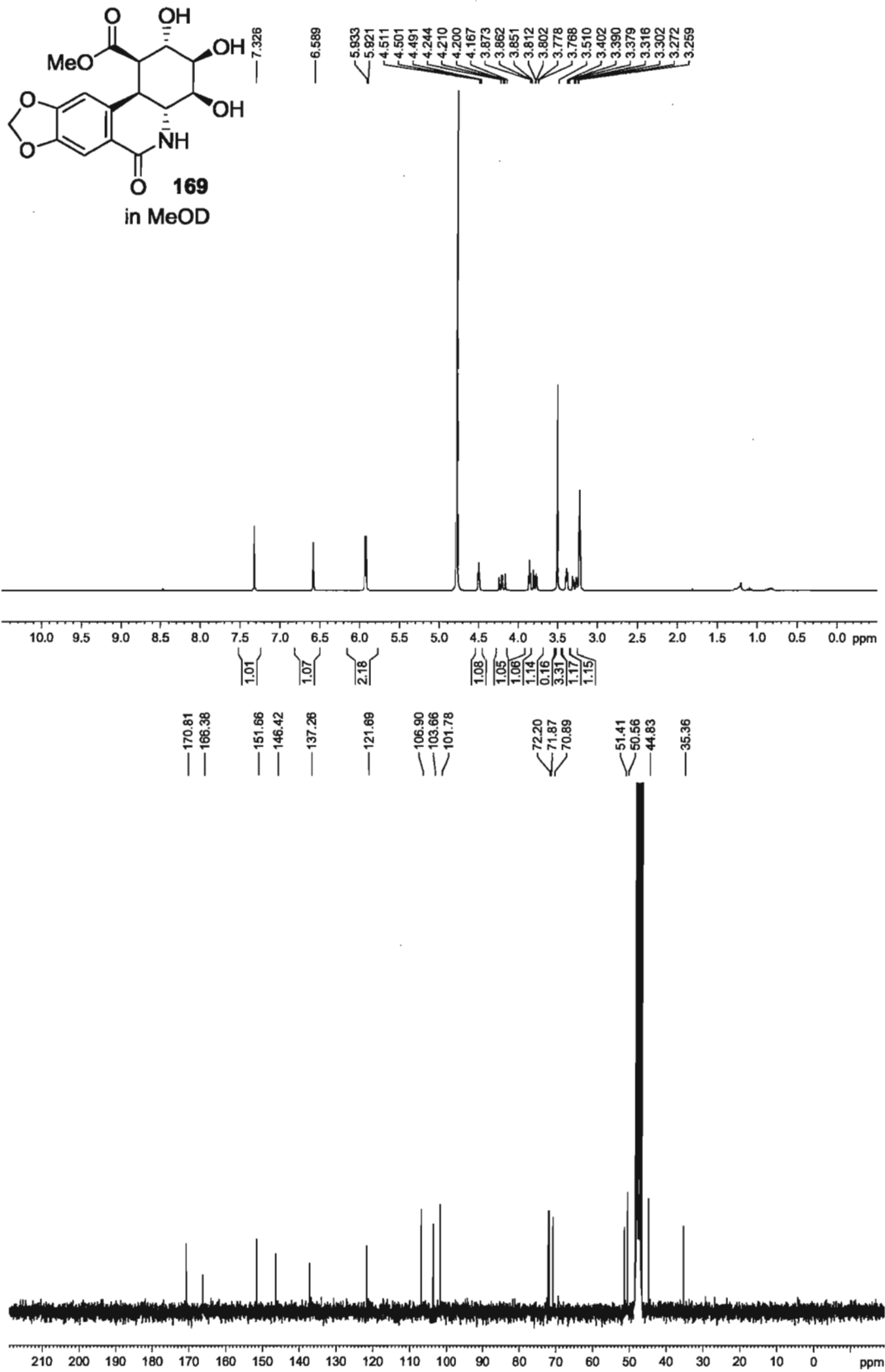


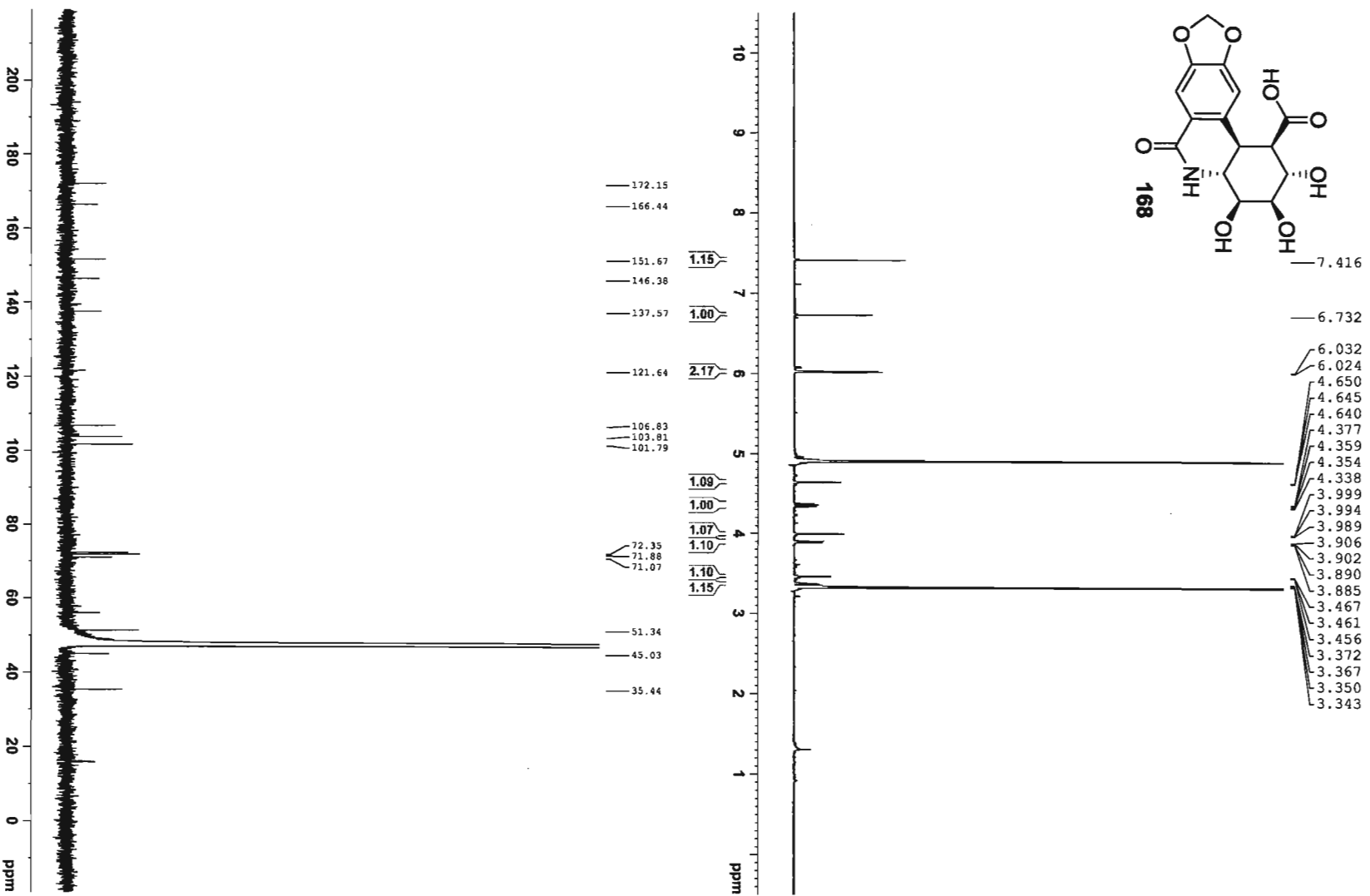
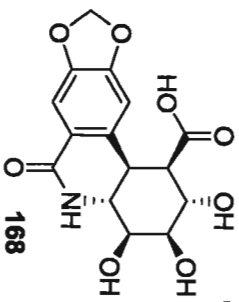
- 2.09
- 1.00
- 2.20
- 1.05
- 2.15
- 1.05
- 1.08
- 1.07
- 1.08
- 1.05
- 1.07
- 3.38
- 3.52
- 4.42
- 10.13
- 6.45

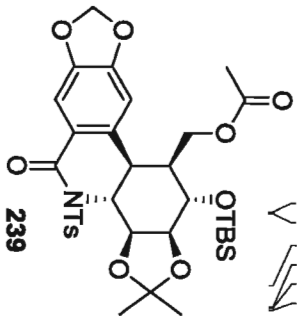
- 174.25
- 166.17
- 152.84
- 146.86
- 143.79
- 138.90
- 137.73
- 129.02
- 128.77
- 122.43
- 109.89
- 109.21
- 103.36
- 102.05
- 77.25
- 77.04
- 76.82
- 72.82
- 68.17
- 64.96
- 48.01
- 35.54
- 27.35
- 26.93
- 25.73
- 21.65
- 18.04
- 4.86
- 5.03



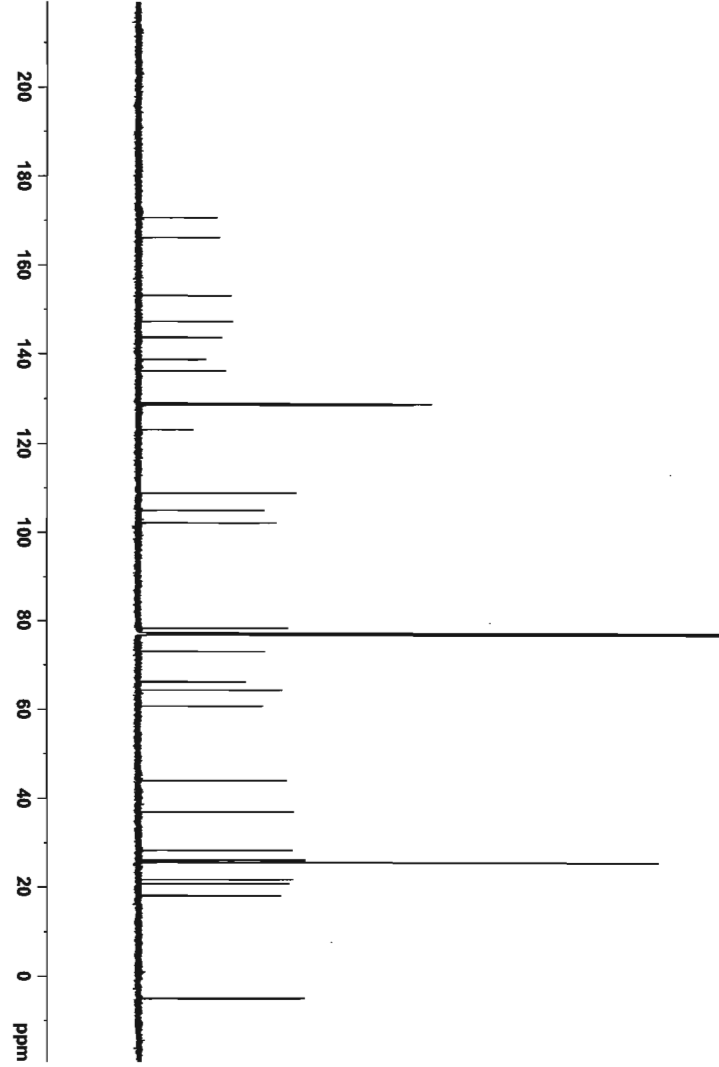
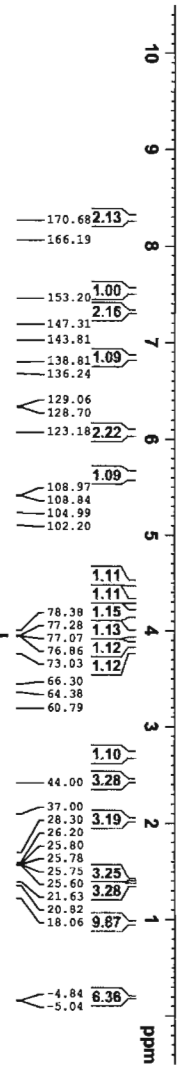
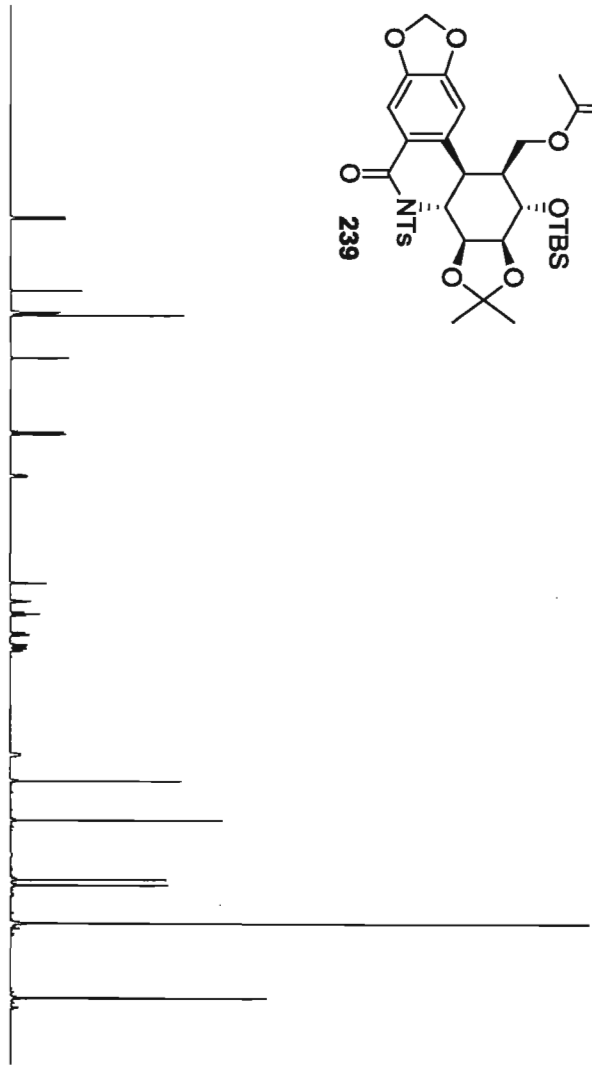


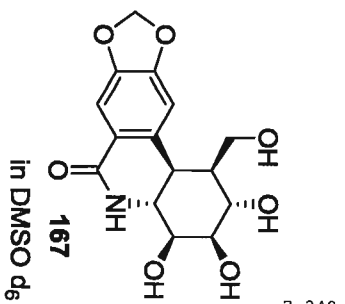




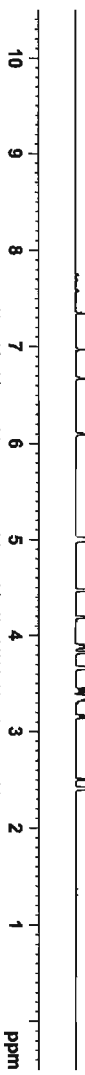


- 8.296
- 8.283
- 7.538
- 7.317
- 7.304
- 7.283
- 6.840
- 6.076
- 6.055
- 5.633
- 5.623
- 5.618
- 5.609
- 4.504
- 4.313
- 4.304
- 4.194
- 4.176
- 4.157
- 3.986
- 3.971
- 3.964
- 3.950
- 3.860
- 3.854
- 3.841
- 3.836
- 3.818
- 3.811
- 3.796
- 3.789
- 2.720
- 2.711
- 2.702
- 2.441
- 2.036
- 1.416
- 1.355
- 0.981
- 0.964
- 0.909
- 0.194
- 0.192
- 0.089

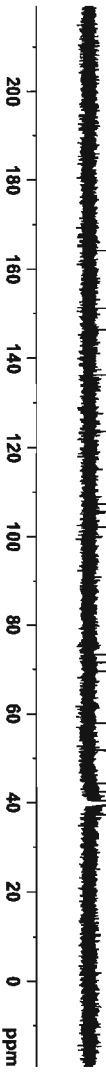


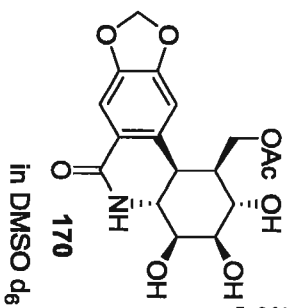


- 7.348
- 6.971
- 6.675
- 6.099
- 6.098
- 5.024
- 5.017
- 5.001
- 4.991
- 4.985
- 4.482
- 4.476
- 4.471
- 4.465
- 4.191
- 3.895
- 3.882
- 3.823
- 3.664
- 3.653
- 3.449
- 3.432
- 3.427
- 3.410
- 3.377
- 3.346
- 3.323
- 3.172
- 3.165
- 3.150
- 3.143
- 2.507
- 2.413

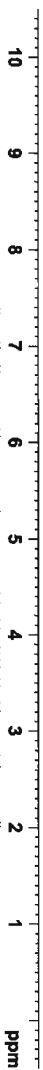


Chemical Shift (ppm)	Integration
164.18	
151.23	1.00
146.34	1.13
136.28	1.07
123.71	2.28
107.36	
105.61	
102.11	3.23
73.31	1.08
71.58	1.12
69.66	1.13
57.83	1.14
51.76	1.14
44.43	1.16
37.34	1.20
	1.15

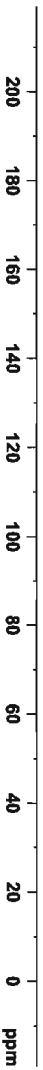


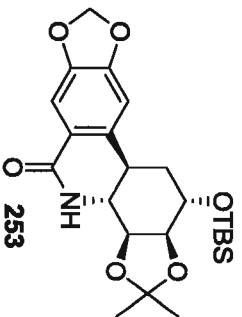


- 7.364
- 7.007
- 6.760
- 6.100
- 5.145
- 5.137
- 4.398
- 4.380
- 4.363
- 4.122
- 4.109
- 4.104
- 3.840
- 3.711
- 3.706
- 3.694
- 3.516
- 3.499
- 3.494
- 3.478
- 3.281
- 3.274
- 3.258
- 3.252
- 2.695
- 2.688
- 2.682
- 2.027



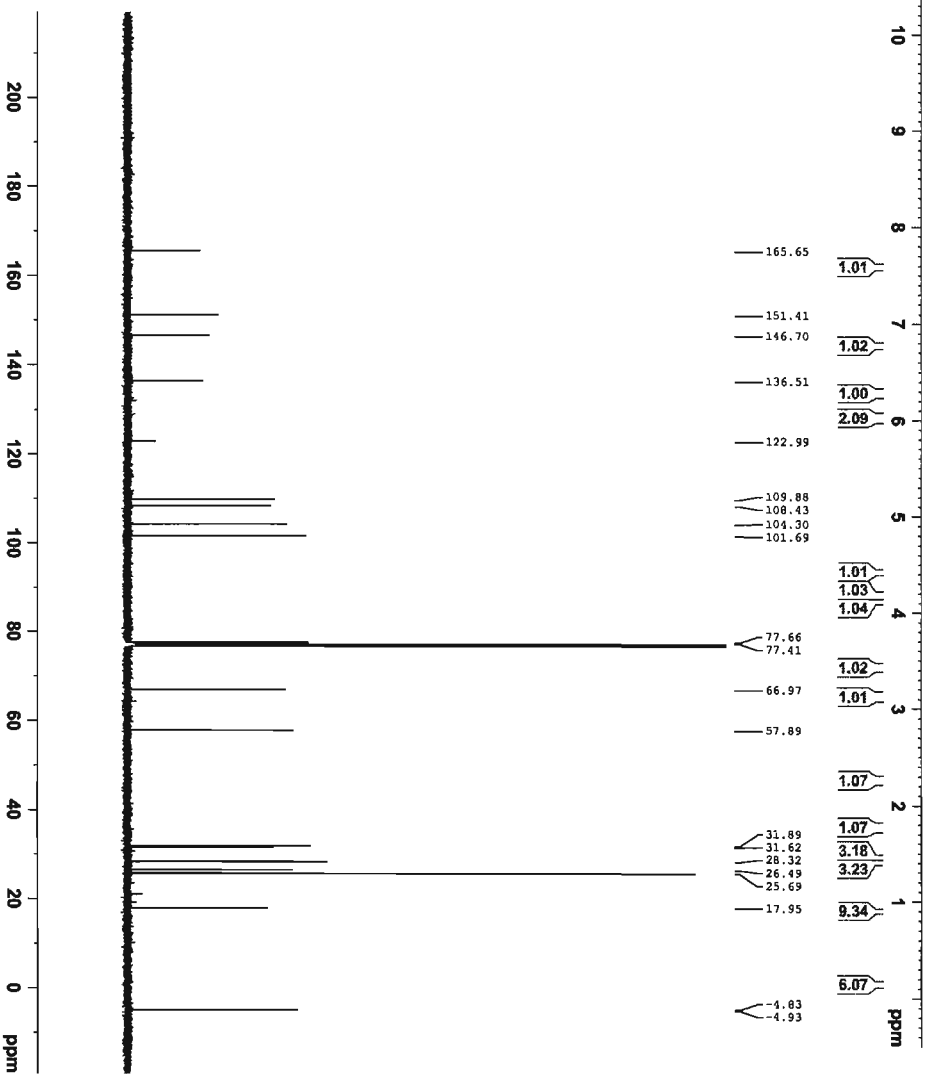
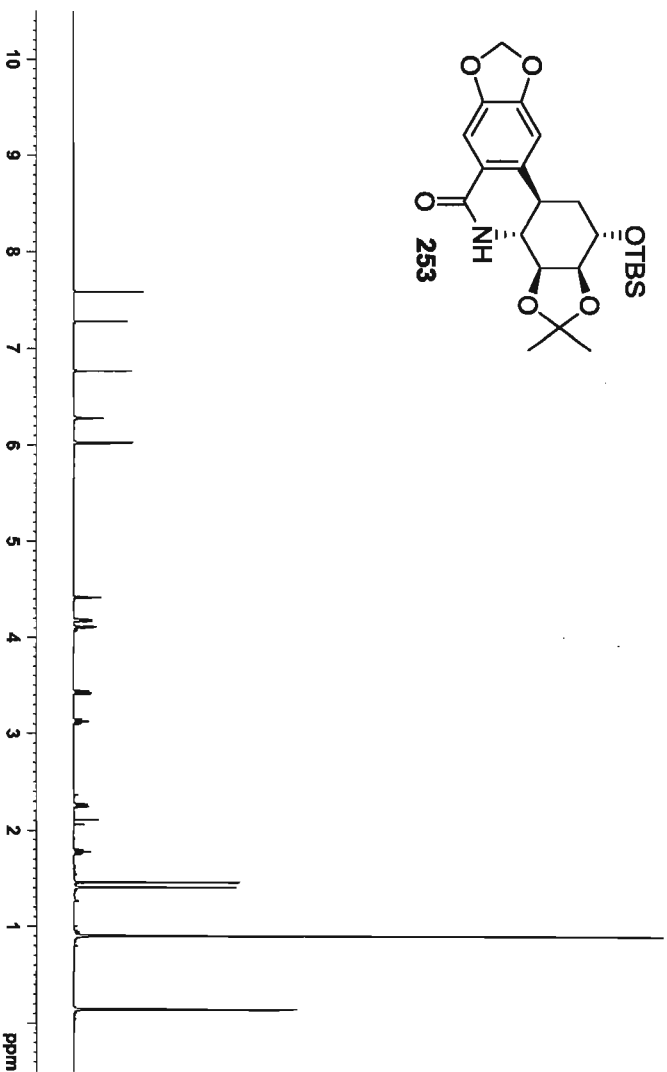
- 171.01
- 164.12
- 151.30
- 146.56
- 135.30
- 123.83
- 107.49
- 105.54
- 102.19
- 73.11
- 71.32
- 69.06
- 61.85
- 51.55
- 40.51
- 36.94
- 21.29



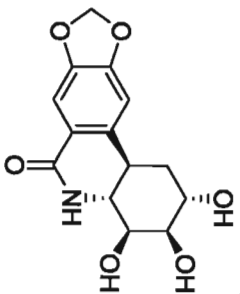


7.595
7.283
6.771
6.283
6.034
6.026

4.420
4.417
4.192
4.184
4.178
4.170
4.113
3.447
3.432
3.425
3.410
3.149
3.146
3.127
2.272
2.249
2.108
2.060
1.798
1.794
1.774
1.755
1.751
1.460
1.407
0.906
0.150
0.144

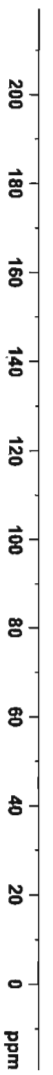
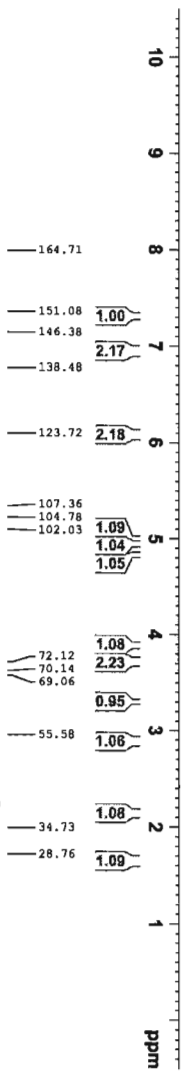


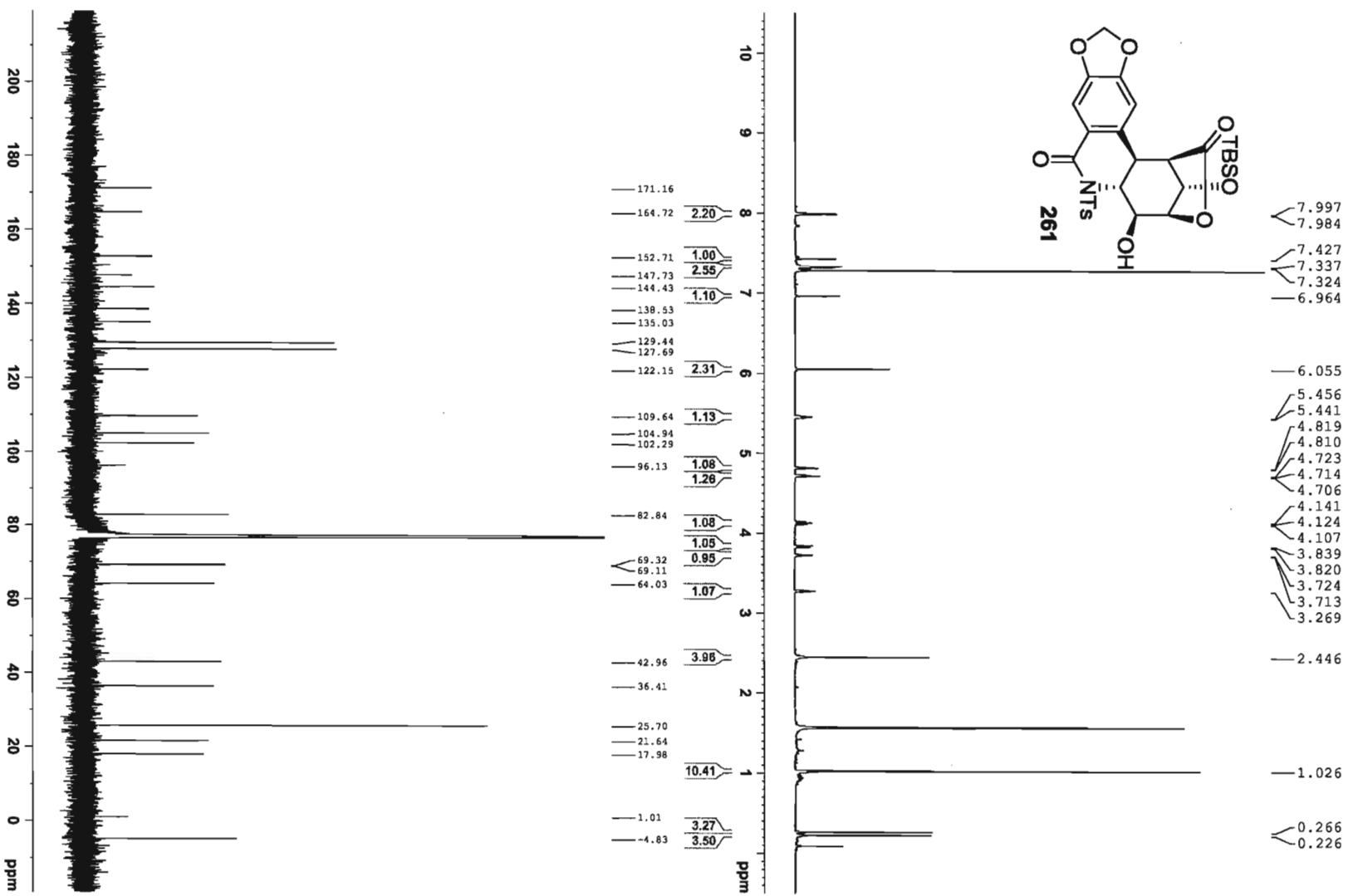
trans-dihydrolycoricidine (3)
in DMSO d₆

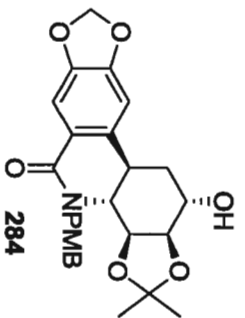


7.307
6.953
6.940

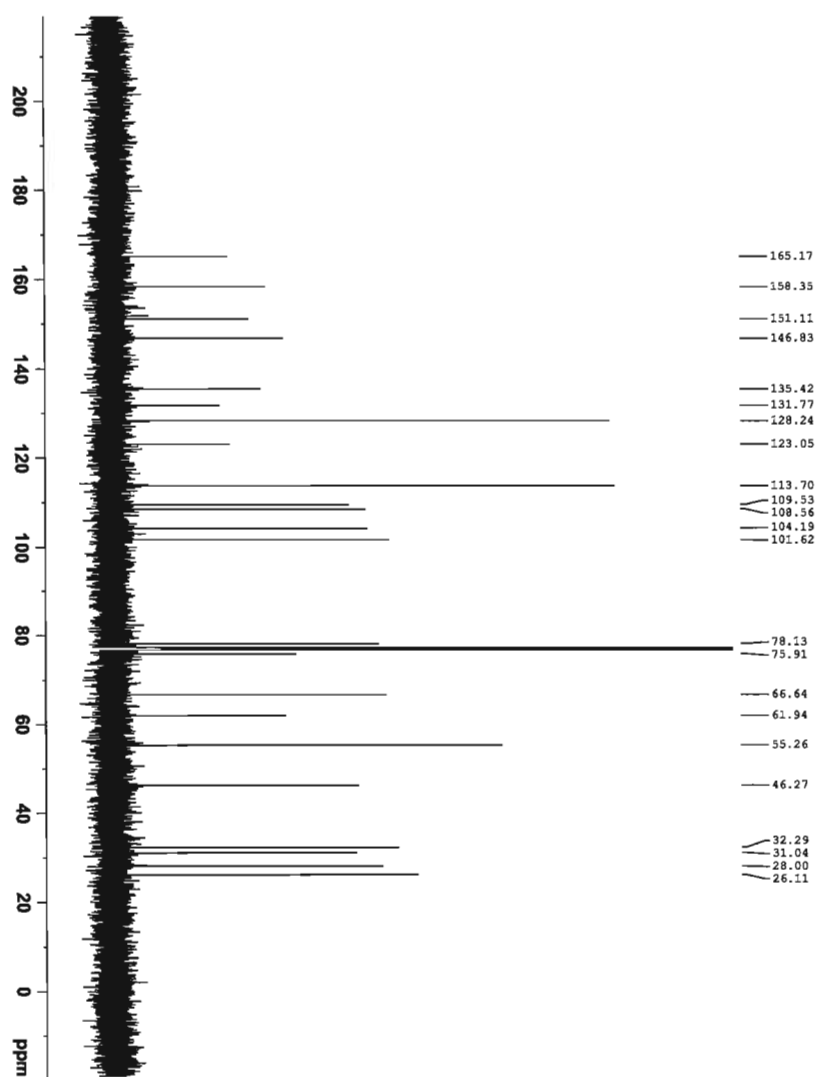
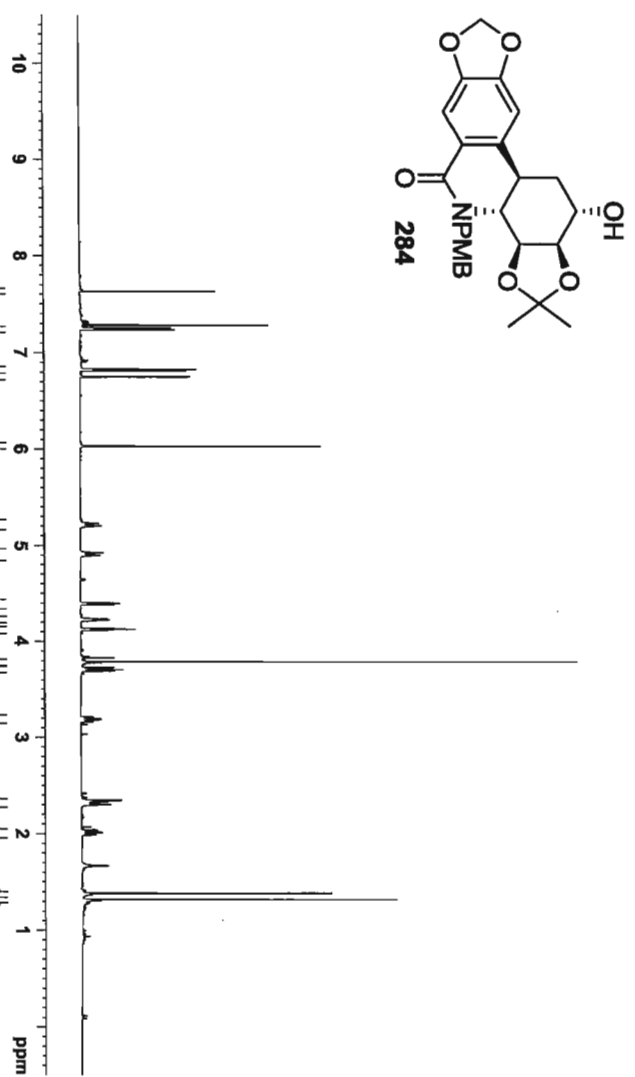
6.082
5.000
4.994
4.967
4.957
4.843
4.838
3.887
3.722
3.341
3.311
3.295
2.913
2.907
2.892
2.886
2.871
2.865
2.546
2.509
2.507
2.504
2.159
2.137
1.672
1.668
1.650
1.647
1.629
1.625

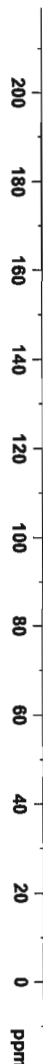
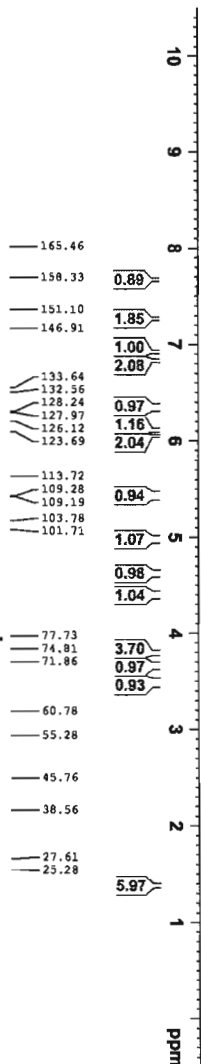
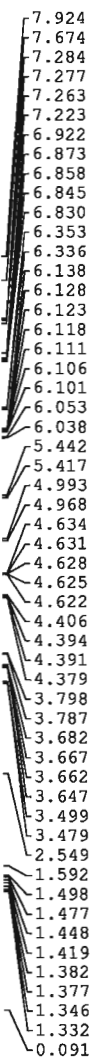
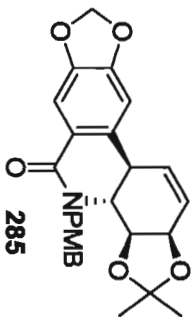


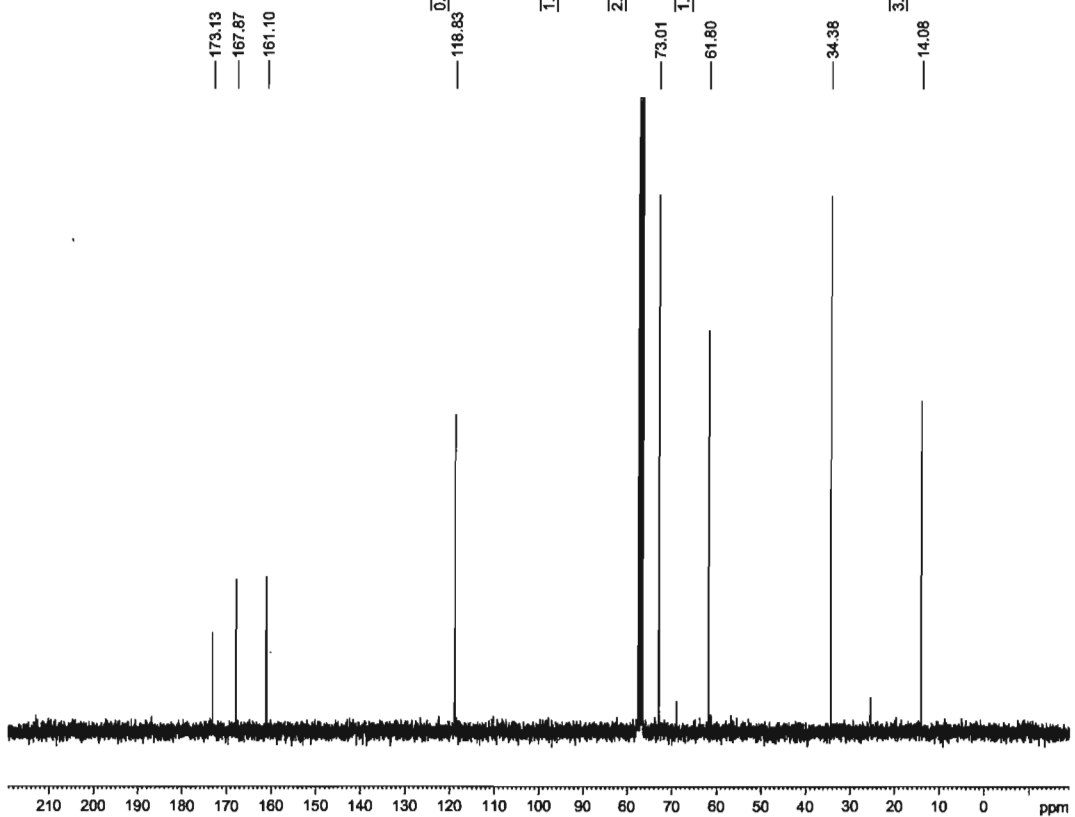
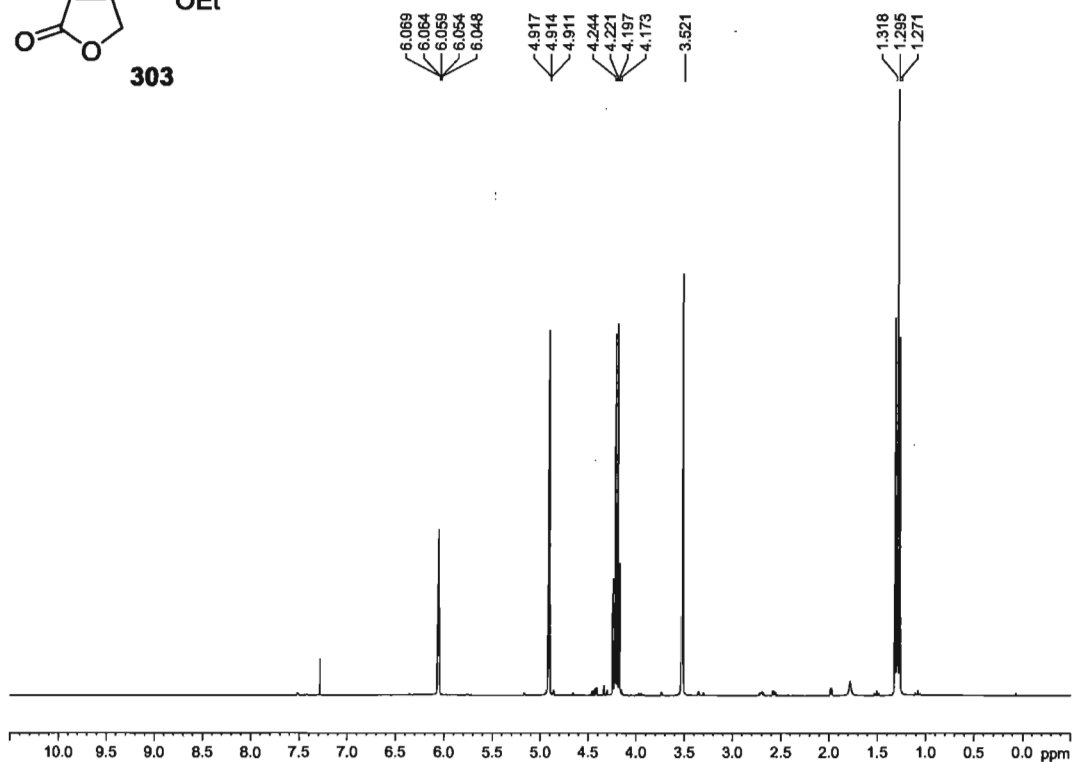
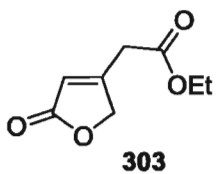


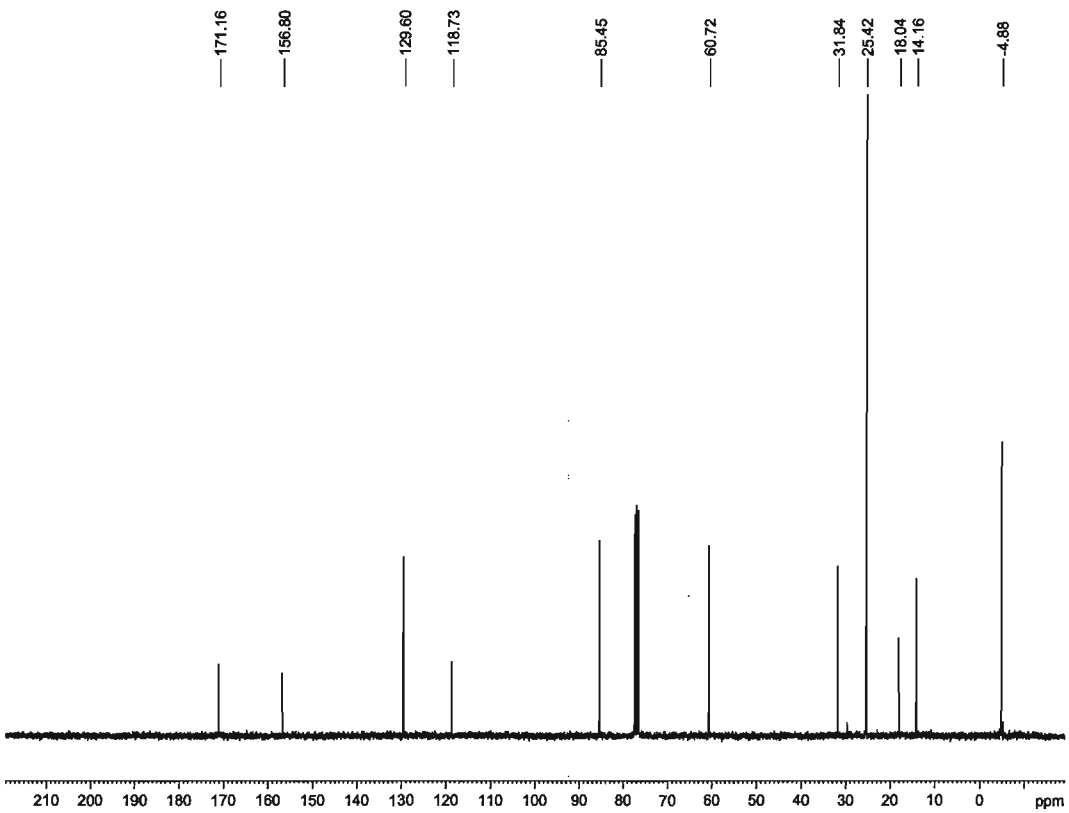
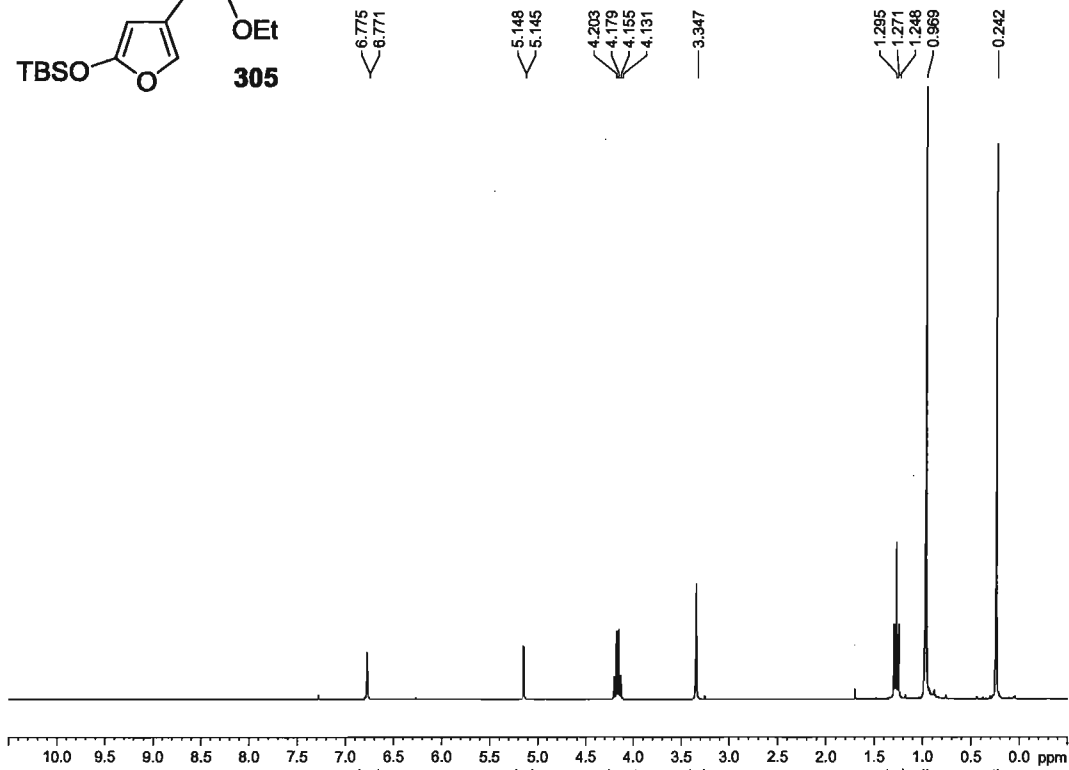
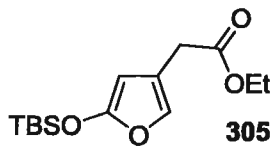


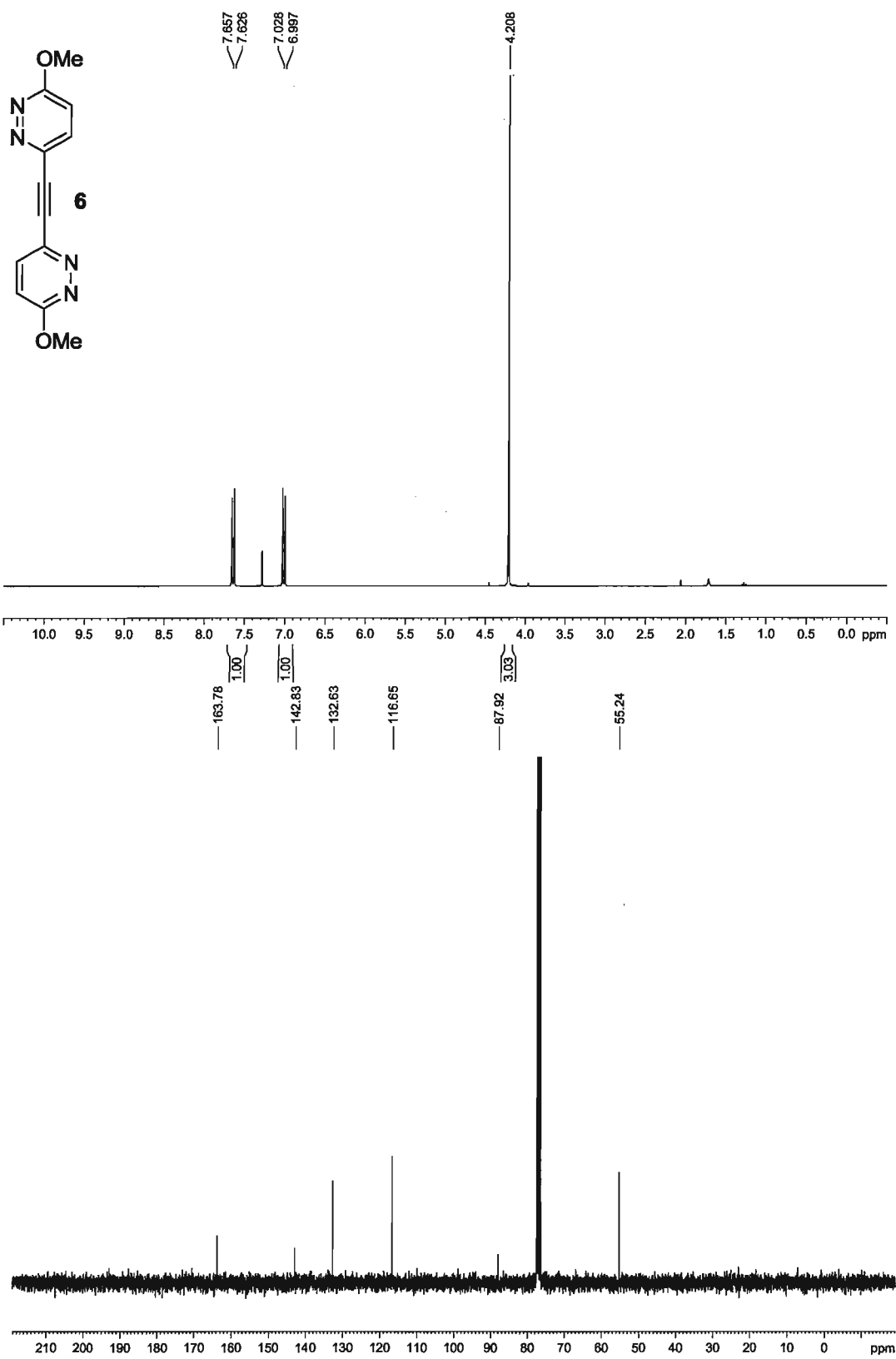
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- 7.283
- 7.251
- 7.237
- 6.829
- 6.815
- 6.751
- 6.030
- 5.226
- 5.200
- 4.922
- 4.897
- 4.404
- 4.394
- 4.392
- 4.381
- 4.230
- 4.222
- 4.132
- 4.123
- 4.114
- 3.830
- 3.786
- 3.727
- 3.714
- 3.704
- 3.691
- 3.212
- 3.204
- 3.191
- 3.183
- 3.171
- 3.162
- 2.344
- 2.333
- 2.324
- 2.315
- 2.309
- 2.301
- 2.292
- 2.037
- 2.029
- 2.017
- 2.014
- 2.009
- 2.006

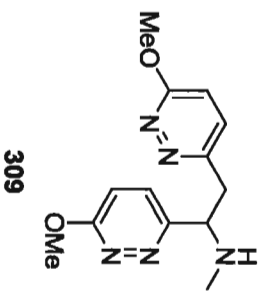






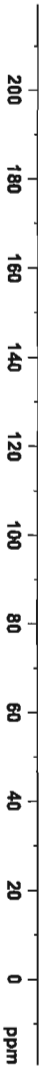
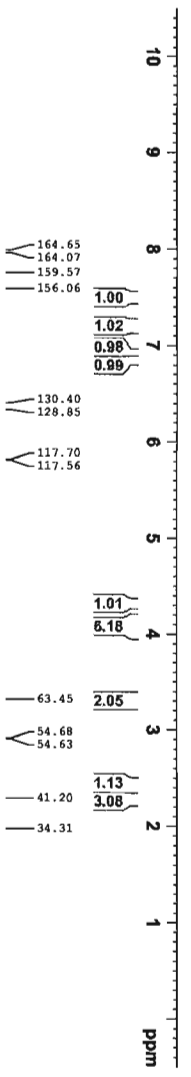






- 7.484
- 7.468
- 7.283
- 7.213
- 7.208
- 7.199
- 7.193
- 7.185
- 6.937
- 6.922
- 6.907
- 6.906
- 6.871
- 6.856
- 6.840

- 4.314
- 4.304
- 4.104
- 4.101
- 4.097
- 4.084
- 4.077
- 4.065
- 3.309
- 3.304
- 3.297
- 3.288
- 2.297
- 2.294
- 2.289
- 2.278
- 2.277



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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.