Published in final edited form as:
J Org Chem. 2010 May 7; 75(9): 3069–3084. doi:10.1021/jo1003136.

Chemoenzymatic synthesis of Amaryllidaceae constituents and biological evaluation of their C-1 analogs. The next generation synthesis of 7-deoxypancratistatin and dihydrolycoricidine.[1]

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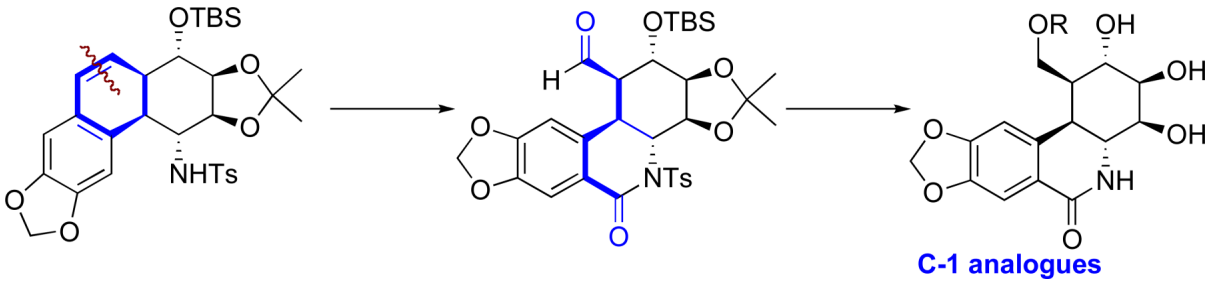
Abstract



An efficient synthesis of C-1 derivatives of 7-deoxypancratistatin is reported. The key steps include the following: selective opening of an epoxide with aluminum acetylide in the presence of an aziridine; solid-state silica-gel-catalyzed opening of an aziridine; oxidative cleavage of a phenanthrene core and its recyclization to phenanthridone to provide the key C-1 aldehyde **22**. The conversion of this aldehyde to C-1 acetoxymethyl and C-1 hydroxymethyl derivatives is described along with the evaluation of their biological activity against several cancer cell lines and in an apoptosis study. The C-1 acetoxymethyl derivative has shown promising activity comparable to that of the natural product. In addition, a total synthesis of *trans*-dihydrolycoricidine and a formal total synthesis of 7-deoxypancratistatin are reported from aldehyde **22**. Detailed experimental and spectral data are provided for all new compounds.

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Supporting Information Available: Copies of ^{1}H NMR and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

Introduction

More than 100 constituents have been isolated from various Amaryllidaceae species since the identification of lycorine (**1**) in 1877.[2] The medicinal value of the plants of the Amaryllidaceae family had been known for centuries but only in the past few decades were the active cytotoxic principles isolated and subjected to medicinal evaluation as promising anti-tumor agents. From such studies pancratistatin (**2**) and narciclasine (**4**), Figure 1, emerged as the most active compounds, with the corresponding 7-deoxy congeners, 7-deoxypancratistatin (**3**) and lycoricidine (**4**), less active.[3] The drop in activity was attributed to be the consequence of the missing donor-acceptor quality of the phenanthridone-phenol functionality in the latter group of compounds. The saturated constituents **6** and **7**, lack C-1 functionality and are also less active.

The synthetic community took active notice of these constituents starting in the 1970s with the first synthesis of lycoricidine[4] by Ohta in 1975. The total syntheses of the constituents followed: lycorine[5] in 1975 by Tsuda, 7-deoxypancratistatin[6] in 1976 by Ohta, trans-dihydrolycoricidine[7] in 1978 by Isobe, pancratistatin[8] in 1989 by Danishefsky, narciclasine[9] in 1997 by Rigby, and trans-dihydronarciclasine[10] in 2007 by Cho.

The activity in total synthesis has not diminished as evidenced by the number of creative approaches that continue to appear in the literature as well as the number of reviews published on the topic of total synthesis and biology.[11] Our research program in this area started with the chemoenzymatic total synthesis of lycoricidine in 1992 and continued with the first asymmetric synthesis of pancratistatin in 1995. Since that time we have focused our program on two major goals, the first being the provision of practical and scaleable synthesis of pancratistatin or narciclasine in order to solve the supply problem of these compounds. The second goal, pursued in collaboration with Pettit's group, aims at the design and synthesis of unnatural derivatives of Amaryllidaceae constituents that would have improved solubility and bioavailability with full retention or even enhancement of activities.

The pioneering research of Pettit on the investigation of biological activities of Amaryllidaceae constituents have eventually led to a proposal for the essential pharmacophore of pancratistatin as depicted in Figure 2.

The current efforts of our group, McNulty, and Kornienko have produced many unnatural derivatives of pancratistatin, some with modest activities. For example, McNulty investigated the importance of hydroxylation[12] and constitution[13] of the aminoinositol ring. Kornienko has also explored various truncations of the C ring.[14] 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.[8h] In an effort to improve the aqueous solubility of various Amaryllidaceae constituents, the Pettit group has synthesized 7-*O*-phosphate prodrug analogues of **2**,[15] 3,4-*O*-cyclic phosphate analogues of **2, 4**-**7**, and the C-1 benzoyl analog.[16] These phosphate prodrugs are currently being evaluated on a preclinical level. We have prepared an indole mimic of 7-deoxypancratistatin,[17] several truncated derivatives,[18] a cis-fused epimer of 7-deoxypancratistatin,[19] which was completely devoid of activity, and bis-trimethylsilyl analogues[20] attained by cobalt-catalyzed cyclotrimerization of acetylene-equipped scaffolds. Chapleur prepared lactone analogs of lycoricidine and found such compounds completely inactive.[21] From the results of biological testing of these derivatives it became evident that full hydroxylation and trans-fused ring junction are essential for activity. Furthermore, the donor-acceptor functionality of pancratistatin is also essential for activity, which is greatly diminished in the constituents lacking the C-7 phenol. Finally, the efforts of Gabrielsen[22] established that the Amaryllidaceae constituents also exhibit antiviral activity. It may very well be that the amino mannose configuration of the amino inositol ring is responsible

for such biological activity. To complete the activity testing of the Amaryllidaceae constituents will require supply of the compounds from sources other than isolation.

The first goal, the attainment of practicality would require a total synthesis of less than eight steps as well as serious optimization, and so far this requirement would seem out of reach as the shortest of our syntheses is 11 steps long. To reach the second goal we have to date prepared a number of truncated derivatives that did display moderate activities when tested against the standard human cancer cell lines. The quest for both of the defined goals is now in its 8th generation effort. In this paper we report a concise chemoenzymatic synthesis of several C-1 derivatives of 7-deoxypancratistatin by an efficient strategy involving the phenanthrene-phenanthridone transform via oxidative cleavage and recyclization. Two of the derivatives displayed high and promising activities, which are reported herein. In addition, formal total syntheses of 7-deoxypancratistatin and trans-dihydrolycoricidine have also been completed.

Results and Discussion

Almost twenty years ago we have implemented a general chemoenzymatic strategy toward the four major Amaryllidaceae constituents. The design for lycoricidine and narciclasine envisioned the generation of the aminocoduritol moiety via hetero Diels-Alder reaction of *cis*-dihydrodiols such as **8** and **10**, derived from bromobenzene and *m*-dibromobenzene, respectively. The introduction of the aryl fragment then relied on an appropriate Heck or Suzuki coupling, Figure 3. For the more highly functionalized pancratistatin and its 7-deoxy congener attachment of the aryl residue was performed by the nucleophilic opening of aziridine **14** or its *N*-Tosyl derivative. These two strategies yielded the total syntheses of these alkaloids in reasonably short sequences, further improved in subsequent generations. An additional strategy for 7-deoxypancratistatin was implemented via cobalt–catalyzed cyclotrimerization of a fully functionalized amino inositol scaffold **15** yielding the TMS derivative of 7-deoxypancratistatin **16**. Solid-phase silica-gel-catalyzed reaction of indole with aziridine **18** led to the preparation of the indole mimic of Amaryllidaceae constituent, namely **19**. It was the success of the solid-phase synthesis that provided for the latest design for 7-deoxypancratistatin via the intramolecular opening of aziridine **20** that provided phenanthrene **21** whose oxidative cleavage and recyclization yields, after full deprotection, C-1 derivatives, such as the aldehyde **22**, suitable for further functionalization to unnatural derivatives. The entire strategy aimed at the synthesis of aldehyde **22** was based on the expectation that extended functionalization of C-1 alcohol in 7-deoxypancratistatin would be arduous because of the dense substitution of the amino inositol ring.

Synthesis of 7-deoxypancratistatin C-1 carboxaldehyde

The synthesis of this key aldehyde begins with diol **8**, produced by fermentation of bromobenzene with *E. coli* JM 109 (pDTG601),[23] protected as its acetonide, and subjected to the Yamada-Jacobsen-Evans protocol of aziridination24 to yield the known *N*-tosyl aziridine **24**,[25] used in previous generations of pancratistatin synthesis, Scheme 1.

Dehalogenation of the vinyl bromide (*n*-Bu_3SnH, AIBN, 76%) produced cleanly vinylaziridine 18 whose epoxidation with *m*-CPBA furnished epoxy aziridines **25a** and **25b** in 96% yield as a 3:1 mixture of diastereomers. Successive recrystallizations from isopropyl alcohol allowed for enrichment of the ratio up to 10:1 (**25a:25b**) but in general two recrystallizations provided a workable ratio of 7:1. Addition of the mixture of epoxy aziridines to the alane derived from aryl acetylene **26** (*n*-BuLi, Me_2AlCl, -50 - 0 °C) produced an intermediate acetylenic alcohol which was immediately protected to give its silyl ether **27** (TBDMSOTf, Et_3N, -78 °C, 77%). Initial attempts at the epoxide opening provided the desired product in poor yield and further investigation revealed that strict control of temperature, both in the formation of the alane and in the addition of the epoxide, was necessary to attain reproducible yields. Deviation from this

protocol led to decreased yields, attributed to either the incomplete formation of the alane or various side reactions of the epoxy aziridine. 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. Silyl ether **27** was subjected to either the Lindlar hydrogenation protocol (Lindlar's catalyst, quinoline, 94%) or the treatment with dicyclohexylborane (BH_3/SMe_2, Cyclohexene, 0 °C, 76%) followed by protonolysis to afford the required cis-alkene **20**, Scheme 1. In the absence of quinoline, this hydrogenation protocol provided mixtures of alkene **20** and its fully reduced alkane **28**.

Adsorption of the aziridine on activated silica followed by heating at 120 °C under argon for 24 hours provided, after elution of the material with hexanes:ethyl acetate, 8:1 – 5:1, the key phenanthrene skeleton **21** in 74% yield, Scheme 2. Oxidative cleavage of this material was investigated in detail and several methods were compared for overall efficiency in the generation of the dialdehyde **31**.

Osmylation of **21** produced the required cis-diol **29** as a mixture with over-oxidized keto alcohol **30** (OsO_4, NMO, rt) This crude mixture was reduced completely to cis-diol **30** ($NaBH_4$, dioxane/EtOH, rt,) before the oxidative cleavage with periodate to dialdehyde **31** ($NaIO_4$, dioxane/H_2O, rt, 83% over 3 steps). The use of excess equivalents of co-oxidant (NMO) in the osmylation step allowed for isolation of hydroxy ketone **30** as the sole product in 89% yield.

The complete assignment of stereochemistry in **30** was determined by extensive NMR analysis of its acetate **37**, as shown in Figure 4. First, the complete assignments of the ^1H and ^{13}C chemical shifts were made based on the ^1H-^1H and ^1H-^{13}C (one-bond and long-range) couplings seen in the DQCOSY, gHMQC and gHMBC spectra, correspondingly. The connectivity in **37** was confirmed during this assignment.

The stereochemistry of **37** was inferred from ^1H-^1H coupling constants and confirmed by nOes. The coupling constant between 2.57 and 2.99 is 4.2 Hz, which means that the junction of rings B and C is *cis*. 4.77 and 2.57 have a coupling constant of 13.3 Hz, therefore they are both axial on ring B. The conformation of ring C is chair, as demonstrated by the nOe of 1.30 with 4.25, which is much larger than the nOe of 1.30 with 4.10. 1.37 has large nOes with both 3.76 and 4.77. 3.76 couples with 2.99 and 4.10 with coupling constants of 12.3 and 8.7 Hz, respectively, therefore they are all axial on ring C. Both 2.57 and 4.25 are equatorial on ring C, and, as expected, their coupling constants with 5.82 are small. The nOe of 5.82 with 4.77 demonstrates that 5.82 is *cis* to ring B.

Hydroxy ketone **30** was then converted to dialdehyde **31** by the same reduction-oxidative cleavage sequence as described above. At room temperature, dialdehyde **31** appeared to be a complex mixture of phenanthridol **32** and various atropoisomers of dialdehyde **31** (*vide* ^1H NMR). Oxidation of **32** allowed for ready conversion to the complete phenanthridone skeleton of 7-deoxypancratistatin, C-1 aldehyde **22** (IBX, DMF, rt, 61% from **30**). We had initially intended to oxidize aldehyde **22** with *m*-CPBA directly to formate **36**, a protected form of 7-deoxypancratistatin. This attempt was based on relatively few reports in the literature describing a Baeyer-Villiger oxidation of cyclohexane carboxaldehyde to the corresponding formate;[26] most other aliphatic aldehydes are known to yield carboxylic acids.[27] In this case, the oxidation with buffered *m*-CPBA in dichloromethane led in 85% yield to the C-1 acid **34**, which was converted to its methyl ester **35** with diazomethane in 83% yield.

A potentially more efficient route to acid **34** was envisioned via direct ozonolysis of phenanthrene **21** followed by oxidative workup. This transformation was indeed attempted by means of ozonolysis followed by an oxidative workup and a mixture of carboxylate derivatives **33** was obtained, composed of diacids, diesters, as well as mono-acid/mono-esters), as indicated in Scheme 2. Upon warming of the reaction mixture cyclization of the various derivatives **33** to phenanthridones **34** and **35** occurred in low yields, inferior to those obtained via dialdehyde **31**.

Synthesis of C-1 derivatives of 7-deoxypancratistatin

In the course of Pettit's relay synthesis of pancratistatin the activity studies of a C-1 benzoate ester, derived from narciclasine, were reported.[28] This C-1 analogue was shown to possess potent activity, particularly against the murine leukemia cell line (ED_{50} 0.0017 μg/mL). This activity is comparable to that of narciclasine (ED_{50} 0.0044 μg/mL) and an order of magnitude greater than pancratistatin (ED_{50} 0.032 μg/mL) against the same cell line. We therefore set out to make several C-1 derivatives of 7-deoxypancratistatin that we could evaluate for biological activity. This anti-cancer activity could then be compared with the known data for 7-deoxypancratistatin, itself generally an order of magnitude less active than pancratistatin. Should such activities show promise a full scale lead optimization would then be undertaken. A reasonable first choice seemed to be the synthesis and evaluation of the C-1 aldehyde and the corresponding acid and ester, as well as the C-1 hydroxymethylene and its acetate. The synthesis of these compounds was relatively straightforward and is shown in Scheme 3. Thus ester **35** was subjected to detosylation to phenanthridone **38** (Na/naphthalene, -58 °C, 58%), whose deprotection (3% HCl in MeOH, 69%) provided the fully hydroxylated ester **39**. Base-catalyzed hydrolysis (LiOH, rt, 95%) furnished the corresponding acid **40**. The aldehyde **22** was reduced to alcohol **41** ($NaBH_4$, 0 °C, 85%). In this case, reduction reactions performed above 0 °C resulted in competitive reduction of the activated phenanthridone. The alcohol **41** was further converted to its acetate **42** (Ac_2O, pyridine, DMAP, 81%) prior to detosylation. Phenanthridone **43** was converted without isolation to alcohol **44** (TBAF, 0 °C, 74% over 2 steps). Exhaustive hydrolysis of this compound (K_2CO_3 then 3% HCl in MeOH, 75%) gave the completely hydroxylated derivative **45** while the acid-catalyzed deprotection (3% HCl in MeOH, 45%) furnished the C-1 acetoxymethylene derivative **46**. Four of these compounds, the C-1 acid **40**, its methyl ester 39, and the hydroxymethylene derivatives **45** and **46** were subjected to screening against the human cancer cell lines (see the last section for the summary of biological activities).

Formal synthesis of 7-deoxypancratistatin and total synthesis of dihydrolycoricidine

The failure to produce formate **36** directly from the protected aldehyde **22** by Baeyer-Villiger oxidaton was disappointing as that manouver would have led to the shortest synthesis of 7-deoxypancratistatin to date. We have attempted to convert the aldehyde and the silyl ether functionalities in **22** to an olefin, which would correspond to a formal synthesis of 7-deoxypancratistatin but a set of very unusual problems was encountered. First, the oxidative decarboxylation employed by Wender in his synthesis of retigeranic acid[29] failed to produce the olefin from the β-hydroxy acid derived from **34** by removal of the silyl protecting group. Second, we attempted the creation of the olefin via oxetane, as was done by Grieco in his synthesis of compactin,[30] and followed Nicolaou's three-step protocol,[31] utilized in his taxol synthesis, for oxetane generation from 1,3-diol derived from **34**. Neither strategy met with success and we attempted the conversion of acid **34** to the C-1 bromide via Hunsdiecker reaction, also to no avail. Conversion of the C-1 aldehyde in **22** to the methyl ketone and Baeyer-Villiger oxidation of the latter led cleanly to methyl ester **35**, accompanied by the lactone derived from the ester and C-3 hydroxyl as a result of the acetonide hydrolysis. Base-catalyzed elimination of the β-silyloxy group from either acid **34** or ester **35** did not lead to the

corresponding unsaturated carbonyl compound; the inertness of the C-1/C-2 functionalities to the above variety of conditions remains unexplained.

Having failed in the manipulations of functional groups in aldehyde **22** or the acid **34** we subjected **22** to Wilkinson decarbonylation ($RhCl(PH_3)_3$, toluene, 130 °C) to produce **47**, which was smoothly detosylated to phenanthridone **48** (Na/naphthalene, -78 °C, 74% over 2 steps). Treatment of **48** with acidic methanol (3% HCl, MeOH, 71%) allowed for hydrolysis of the remaining protecting groups and completed the total synthesis of trans-dihydrolycoricidine (**7**) ($[\alpha]_D^{22}$ 28.6 (*c* 0.25, DMSO), lit.[32] $[\alpha]_D^{25}$ 138 (*c* 0.96, DMSO). The formal total synthesis of 7-deoxypancratistatin was also completed by first protection of lactam **48** (NaH, PMBBr, 0 °C) as its *p*-methoxybenzyl derivative followed by cleavage of the silyl ether (TBAF, 0 °C, 64% over 2 steps) yielding alcohol **49**. Chugaev elimination of the C-2 alcohol (NaH, CS_2, MeI, xylenes, 165 °C, 35%) provided olefin **50**, an intermediate in Padwa's synthesis of 7-deoxypancratistatin (mp 172-174 °C (ethyl acetate/hexanes), lit. 171-173 °C).[4m] A more direct route to 7-deoxypancratistatin was envisioned by the oxidative cleavage of trimethylsilyl enol ether derived from **22** followed by deprotection of the C-3 silyl ether and a directed reduction of the C-1 carbonyl ($NaBH_4$, EtOH). This protocol, performed in a preliminary manner, yielded the C-1/C-2 diol leaving only the detosylation and hydrolysis to provide **3** in a shorter sequence.

Biological evaluation of C-1 derivatives

To date the search for a more active or more bioavailable synthetic analogue of the Amaryllidaceae constituents has yielded relatively few compounds that showed promise. In continuation of this effort the novel C-1 analogues of 7-deoxypancratistatin, synthesized in this work, were evaluated for antitumor activities in cancer cell lines in vitro. While the C-1 acid **40** and its methyl ester **39** were found inactive, the hydroxymethyl analogue **45** and its acetate **46** displayed useful levels of activities. Therefore, to assess their potential, these two compounds were further evaluated for antiproliferative activities in a panel of human cancer cell lines. In addition, their ability to induce apoptosis in human leukemia and neuroblastoma cells was also examined.

Cancer cell line growth inhibitory activities

Table I provides the antiproliferative potencies of several relevant Amaryllidaceae constituents, compounds **45** and **46**, and synthetic C-1 benzoyl derivatives **51** and **52**, previously synthesized by Pettit's group and found to be the most potent Amaryllidaceae isocarbostyril analogues identified to date. Although the C-1 analogues **45** and **46** provide antiproliferative potencies somewhat inferior to the natural isocarbostyrils pancratistatin and narciclasine, this fact is consistent with the overriding influence of the 7-hydroxyl phenolic functionality, present in **2** and **4**, but not in **45** and **46**. However, these compounds appear to be at least as good as or better than 7-deoxypancratistatin, pointing to the beneficial effect of the C-1 derivatization. Furthermore, it appears that large lipophilic C-1 substituents are preferred, whereas elevated polarity at C-1 is not tolerated. Indeed, the C-1 acid **40** and its methyl ester **39** (presumably undergoing intracellular hydrolysis to **40**) are inactive, while acetate **46** is somewhat more potent than the parent alcohol **45**. These findings are consistent with the beneficial effect of the C-1 benzoyl moiety in **51** as found by Pettit. It is not entirely clear at this point whether the benzoyl in **51** and the acetyl in **46** are part of the pharmacophore or if they merely assist the parent hydroxyl compounds in cell penetration and then undergo intracellular hydrolytic removal. The answer to this question will have to await further studies involving the preparation and testing of analogues with non-hydrolyzable large lipophilic substituents at C-1.

Apoptosis induction

Apoptotic morphology was observed in Jurkat and SH-SY5Y cells after 72 hours treatment with compound **45** or **46** (Figure 5) through Hoechst staining and Annexin-V binding. The effective dose at which 50% of cells were apoptotic (ED50) for compound **45** in Jurkat cells was 1 μM and in SH-SY5Y cells was 10 μM. Compound **46** had greater apoptotic efficacy than **45** in both Jurkat and SH-SY5Y cells, as the ED50 was determined to be 0.5 μM for both cell types (Figure 6). This indicates that **46**, much like **2**, has the ability to induce apoptosis specifically in cancerous cells. Most importantly, compound **46** did not induce apoptosis in non-cancerous normal human cells such as normal human fibroblasts (NHFs) and peripheral mono-nucleated blood cells (PMBC) prepared from blood obtained from healthy volunteers (Figure 7). These results indicate that compound **46** is selectively targeting cancer cells to induce apoptosis, and could be a safer alternative to toxic chemotherapy.

Conclusions

We have completed a short and potentially practical synthesis of several C-1 derivatives of 7-deoxypancratistatin, two of which (**45** and **46**) exhibited promising biological activities in several cell lines as well as in apoptosis screens. Given that these compounds, lacking the crucial 7-hydroxyl group, exhibited good activities it stands to reason that the next step in this program should be the evaluation of the corresponding C-1 acetoxymethyl and related compounds that contain the otherwise complete pharmacophore of pancratistatin. We would expect that such derivatives should equal the natural product in potency and would therefore provide the rationale for further lead optimization toward more soluble and bioavailable derivatives. As a side benefit, a total synthesis of trans-dihydrolycoricidine **7** was accomplished form aldehyde **22** along with a formal total synthesis of 7-deoxypancratistatin.

Experimental Section

Cell Culture

The Human neuroblastoma (SH-SY5Y) and B-cell leukemia (Jurkat) cell lines were purchased from ATCC (Manassas, VA). Cells were maintained and grown at 37°C, 95% humidity and 5% CO_2. Jurkat cells were grown with RPMI-1640 media (Sigma-Aldrich, Oakville, ON, Canada) supplemented with 10% fetal bovine serum (FBS) and 10 mg/ml Gentamycin (Gibco BRL, Mississauga, ON, Canada). SH-SY5Y cells were grown with Dulbecco's Modified Eagles Medium (DMEM) HAM F12 (Sigma-Aldrich), supplemented with 2 mM L-Glutamine, 10 mg/ml Gentamycin, and 10 % FBS. Normal Human Fibroblasts (NHFs) obtained from Coriell Institute for Medical Research (New Jersey, USA) were cultured in Earle's Minimum Essential Medium (MEME) (Sigma Chemical Company, Mississauga, Ontario, Canada) completed with 15% fetal bovine serum, 2mM L-Glutamine, 10 mg/ml Gentamycin, 1.5 g/l sodium bicarbonate, 1% vitamins, and essential (2%) and non-essential amino acids (1%) (Gibco BRL, VWR, Mississauga, ON, Canada).

Human non-small cell lung cancer line NCI-H460 (ATCC # HTB-177) and human pancreatic adenocarcinoma cancer cell line BxPC-3 (ATCC # CRL-1687) were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) (Gibco, Carlsbad, CA), 100 mg/L penicillin G and 100 mg/L streptomycin and (Cellgro, Manassas, VA). Human prostate carcinoma cells DU-145 (ATCC # HTB-81) were cultured in Dulbecco's modified Eagle Medium (Cellgro) supplemented with 10% FBS, 100 mg/L penicillin G and 100 mg/L streptomycin. Human mammary carcinoma cells MCF-7 (ATCC # HTB-22) were cultured using Dulbecco's modified Eagle Medium supplemented with 10% FBS, 100 mg/L penicillin G, 100 mg/L streptomycin, 1.0 mM Glutamax and 1.0 mM sodium pyruvate, (Gibco).

Peripheral blood was obtained from healthy non-smoking volunteers aged 25-50 y upon written and informed consent (University of Windsor REB #04-147). Whole blood samples were collected in BD Vacutainer™ Cell Preparation Tube and mononuclear cells were separated by density gradient centrifugation. The isolated cells were maintained in RPMI 1640 media supplemented and maintained as was the Jurkat culture.

MTT Assay

To evaluate the cytotoxic effects of the C-1 derivatives of 7-deoxypancratistatin, mitochondrial dehydrogenase activities were measured. Jurkat and SH-SY5Y cells were grown and treated for 24, 48, and 72 hrs. Cells were treated with concentrations ranging from 0.25 to 10 μM of each derivative dissolved in DMSO Similarly, ncPBMCs and NHFs were treated to assess the effects of the C-1 derivatives on non-cancerous cells. In addition, the BxPC-3, CRL-1687, DU-145 and MCF-7 lines were assessed by seeding 4×10^3 cells per well into microplates. The cells were grown for 24 hrs before treatment at concentrations ranging from 0.01 to 10 μM and incubated for 48 hrs. MTT reagent (5 mg/mL, MP Biomedical, Solon, OH) was added to each well and incubated further for 2 hrs. The resulting formazan crystals were dissolved in DMSO and the OD was determined at a wavelength of 490 nm. The experiments were repeated at least twice for each compound per cell line. Cells treated with 0.1% DMSO were used as a control.

Cellular Staining

Nuclear morphology was visualized using a final concentration of 10 μM of Hoechst 33342 dye (Molecular Probes, Eugene, OR, USA). Phosphatidyl serine flipping, (an apoptotic biochemical marker) was visualized with Annexin-V-FITC binding assay as per manufacturer's protocol (Molecular Probes, Eugene OR, USA). Cells were observed with a fluorescent microscope (Leica DM IRB, Germany); apoptotic indices were determined by counting brightly stained condensed nuclei (apoptotic cells) from at least 5 fields at 40X objective. Apoptotic cells were expressed as a percentage of the total number of cells counted. Standard error was calculated from at least 3 separate experiments.

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, DME, and toluene were distilled from potassium/benzophenone. Methylene chloride and acetonitrile were distilled from calcium hydride. Flash column chromatography was performed using Kieselgel 60 (230-400 mesh). Analytical thin-layer chromatography was performed using silica gel 60-F_{254} plates. Melting points are reported uncorrected. IR spectra were recorded as neat samples or in KBr pellets. ^{1}H and ^{13}C NMR spectra were obtained on either a 300-MHz or 600 MHz instrument. Data are reported as (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad; coupling constants(s) in Hz, integration. Specific rotation measurements are given in deg cm^3 g^{-1} dm^{-1}. Mass spectra and high resolution mass spectra were performed by the analytical division at Brock University.;l

(1S, 2S, 4R, 5S, 6S, 7S)-5,6-(isopropylidenedioxy)-3-(4'-methylphenylsulfonyl)-8-oxa-3-aza-tricyclo[5.1.0.0]octane (25a), (1S, 2S, 4R, 5S, 6S, 7R)-5,6-(isopropylidenedioxy)-3-(4' methylphenylsulfonyl)-8-oxa-3-aza-tricyclo[5.1.0.0]octane (25b)

A solution of *m*-CPBA (10.5 g, 46.7 mmol) in 1,2-dichloroethane (150 mL) was dried over Na_2SO_4 and filtered into a flask containing aziridine **18** (5.0 g, 15.6 mmol). The resulting solution was heated to reflux until total consumption of starting material (6 hours). The reaction mixture was allowed to cool to room temperature at which time *m*-chlorobenzoic acid crystallized out as a white solid. The reaction mixture was filtered and concentrated. The

residue was taken up EtOAc (350 mL) and washed sequentially with $NaHSO_3$ (2 × 100 mL), carbonate (2 × 100 mL), and brine (35 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated to yield a 2.89:1 mixture of epoxides **25a** and **25b** (5.035g. 96 %) as slightly yellow solid. The epoxide could be used without further purification or enriched in favor of the major isomer by successive recrystalizations form 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 epoxide **25a** and **25b**.

Epoxide 25a—R_f 0.43 (hexanes/ethyl acetate, 2:1); mp 115-116 °C (isopropanol); $[\alpha]_D^{21}$ -79.36 (*c* 1.0, $CHCl_3$); IR (film) ν2988, 2936, 1597, 1383, 1373, 1329, 1251, 1218, 1158, 1091, 1055 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 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 (t, *J* = 3.7 Hz, 1H), 3.38 (dd, *J* = 6.8 Hz, *J* = 3.8 Hz, 1H), 3.13 (dd, *J* = 3.5 Hz, *J* = 0.9 Hz, 1H), 3.04 (dd, *J* = 6.8 Hz, *J* = 1.1 Hz, 1H), 2.46 (s, 3H), 1.46 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 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 $C_{16}H_{19}NO_5S$ (M^+-15): 322.0749, Found: 322.0744; Anal. calcd for $C_{16}H_{19}NO_5S$ C, 56.96; H, 5.68; found C, 56.83; H, 5.61

Epoxide 25b—R_f 0.43 (hexanes/ethyl acetate, 2:1); mp 179-180 °C (isopropanol); $[\alpha]_D^{21}$ – 62.82 (*c* 1.0, $CHCl_3$); IR (film) ν2985, 2929, 1602, 1384, 1366, 1324, 1245, 1222, 1163, 1153, 1041, 1001 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) 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, *J* = 2.3 Hz, 1H), 3.58 (dd, *J* = 3.9 Hz, *J* = 2.4 Hz, 1H), 3.44 (dd, *J* = 6.7 Hz, *J* = 2.0 Hz, 1H), 3.27 (d, *J* = 3.5 Hz, 1H), 3.18 (t, *J* = 6.8 Hz, 1H), 2.48 (s, 3H), 1.54 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) 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 $C_{16}H_{19}NO_5S$ (M^+-15): 322.0749, Found: 322.0753; Anal. calcd for $C_{16}H_{19}NO_5S$ C, 56.96; H, 5.68; found C, 56.23; H, 5.61

(1S,2R,3R,4R,5S,6R)-3,4-(Isopropylidenedioxy)-5-[(*tert*-butyldimethylsilyl)oxy]-6-2-Benzo [1.3]dioxol-5-ylethynyl-(4'-methylphenylsulfonyl)-7-azabicyclo[4.1.0]heptanes (27)

To a solution of acetylene **26** (0.43 g, 2.96 mmol) in 8 mL dry toluene at -50 °C was added 1.46 mL of a solution of nBuLi in hexanes (2.01 M, 2.96 mmol) over 5 min. The solution was stirred for 15 minutes before 2.97 mL of a solution of Me_2AlCl (1.0M in CH_2Cl_2, 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 **25a:25b** (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 mixture was cooled in an ice bath and quenched with 1 M HCl (1 ml). Ethyl acetate (50 mL) was added and the layers where separated. The aqueous phase was extracted with 3 × 50 mL EtOAc and the combined organic layers dried over Na_2SO_4. 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 as a clear and colorless oil; $[\alpha]^{22}_D$ -113.05 (*c* 0.5, $CHCl_3$); R_f 0.30 (hexanes:ethyl acetate, 2:1); IR (film) *v* 3491, 2988, 1163 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.78 (d, *J* = 8.1 Hz, 2H), 7.38 (d, *J* = 8.1 Hz, 2H), 6.91 (dd, *J* = 8.2 Hz, 1.8 Hz 1H), 6.83 (d, *J* = 1.5 Hz, 1H), 6.73 (d, *J* = 7.9 Hz, 1H), 5.97 (s, 2H), 4.47 (d, *J* = 6.4 Hz, 1H), 4.22 (dd, *J* = 6.1, 4.4 Hz, 1H), 3.98 (m, 1H), 3.40 (d, *J* = 6.4 Hz, 1H), 3.24 (m, 2H), 3.06 (d, *J* = 9.6 Hz, 1H), 2.47 (s, 3H), 1.49 (s, 3H), 1.32 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ: 148.1,

147.5, 145.7, 134.2, 130.4, 128.1, 126.4, 116.2, 111.8, 110.3, 108.6, 101.5, 84.2, 83.8, 75.4, 70.1, 68.7, 42.3, 40.5, 31.1, 27.4, 25.2, 21.9 ppm; HRMS (FAB M^+) calcd for $C_{25}H_{25}NO_7S$ 484.1430, found 484.1428.

The free alcohol intermediate (0.8 g, crude) was dissolved in 20 mL of CH_2Cl_2 and triethylamine (0.484 mL, 3.48 mmol) was added. The reaction flask was cooled to -78 °C and *t*-butyldimethylsilytriflate (0.457 mL, 0.1.99 mmol) was added dropwise to the stirring solution. After stirring for 30 minutes at -78 °C the reaction mixture was quenched with water (20 ml) and the two phases separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 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 to 2:1) affording **27** (0.677g, 77%) as a colorless oil.; $[\alpha]^{24}_D$ +57.7 (c 0.5, $CHCl_3$); R_f 0.49 (hexanes:ethyl acetate, 2:1); IR (film) *v* 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 $C_{27}H_{30}NO_7SSi$: 540.1481; Found, 540.1487.

(1S,2R,3R,4R,5S,6R)-3,4-(Isopropylidenedioxy)-5-[(*tert*-butyldimethylsilyl)oxy]-6-2-Benzo [1,3]dioxol-5-ylethenyl-(4'-methylphenylsulfonyl)-7-azabicyclo[4.1.0]heptane (20)

A) Alkyne **27** (500 mg, 0.837 mmol) was taken up in 45 mL MeOH and quinoline (34 mg, 0.168 mmol) added. Lindlar's catalyst (35 mg, 0.335 mmol) added. The reaction mixture was purged with aspirator vacuum and flushed with H_2 before being placed under H_2 using a balloon, and stirred for 3 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 (480 mg, 95%)

B) To a 1.0 M solution of BH_3.THF complex (2.5 mL, 2.5 mmol) was added cyclohexene (0.484 mL, 4.77 mmol) at 0 °C. After 10 minutes a heavy precipitate was formed. The reaction mixture was kept at 0 °C for 1 h before acetylene derivative **27** (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. 60 mL EtOAc were added and the reaction mixture was washed with saturated aq. $NaHCO_3$ (2 × 15 mL), H_2O (2 × 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 **20** (76%).; $[\alpha]^{23}_D$ -26.14 (c 1.0, $CHCl_3$; R_f 0.35 (hexanes:ethyl acetate, 4:1); IR (film) ν 2986, 2930, 2894, 2856, 1598,1489 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 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; ^{13}C NMR (150 MHz, $CDCl_3$) 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, 39.5, 30.1, 27.8, 25.8, 25.79. 25.75, 25.72, 25.51, 23.7, 21.7, 18.1, -4.3, -4.7 ppm; HRMS-EI Calcd for $C_{31}H_{41}NO_7SSi$: 599.2373; Found, 599.2376; Anal. calcd for $C_{31}H_{41}NO_7SSi$ C, 62.28; H, 6.58; found C, 61.30; H, 6.63. [Compound is a heavy oil, retaining residula solvent].

(1S,2R,3R,4R,5S,6R)-3,4-(Isopropylidenedioxy)-5-[(*tert*-butyldimethylsilyl)oxy]-6-2-Benzo [1,3]dioxol-5-ylethanyl-(4'-methylphenylsulfonyl)-7-azabicyclo[4.1.0]heptane (28)

Alkyne **27** (100 mg, 0.168 mmol) was taken up in 10 mL MeOH and Lindlar's catalyst (35 mg, 0.34 mmol) added. The reaction mixture was purged with aspirator vacuum and flushed with H_2 before being placed under H_2 using a balloon, and stirred for 24 hours. The reaction mixture was filtered through a short plug a celite and the solvent was removed under reduced pressure. The crude residue was purified by flash column chromatography (hexanes:ethyl acetate, 4:1) to give the title compound as a clear and colorless oil (95 mg, 94%).; $[\alpha^{23}_D]$ -477.2 (c 1.8, $CHCl_3$) 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, $CDCl_3$) δ: 7.87 (d, *J* = 7 Hz, 2H), 7.37 (d, *J* = 7 Hz, 2H), 6.70 (d, *J* = 6.5 Hz, 1H), 6.55 (s, 1H), 6.51 (d, *J* = 7 Hz, 1H), 5.93 (s, 2H), 4.41 (d, *J* = 5 Hz, 1H), 3.79 (t, *J* = 5 Hz, 1H), 3.43 (m, 1H), 3.18 (d, *J* = 6 Hz, 1H), 2.84 (m, 1H), 2.60 (m, 1H), 2.45 (s, 3H), 2.40 (m, 1H), 2.05 (m, 1H), 1.68 (s, 3H), 1.34 (s, 3H), 0.83 (s, 9H), 0.06 (s, 3H), 0.03 (s, 3H); ^{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, 1081, 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 $C_{31}H_{43}NO_7SSi$: 601.2530; Found, 601.2534;

N-[(1R,2aS,4aS,5S,5aR,12bR)-5-(*tert*-Butyl-dimethyl-silanyloxy)-3,3-dimethyl-1,2a,4a,5,5a, 12b-hexahydro-phenanthro[2,3-*d*][1,3]dioxol-1-yl]4-methyl-benzenesulfonamide (21)

A flame-dried 25-mL flask was charged with olefin **20** (336 mg, 0.561 mmol) and silica gel which has been activate by heating under vacuum at 120 °C overnight (1.5 g). The starting materials were suspended in 10 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 249 mg (74%) of olefin **21** as a clear and colorless oil.; $[\alpha]^{23}_D$ -123.7 (*c* 1.0, $CHCl_3$); R_f 0.35 (hexanes:ethyl acetate, 2:1); IR (film) *v* 3268, 2929, 2887, 2857, 1598,1503, 1485 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ: 7.43 (d, *J* = 7 Hz, 2H), 7.13 (d, *J* = 7 Hz, 2H), 6.49 (s, 2H), 6.34 (d, *J* = 8 Hz, 1H), 5.95 (s, 1H), 5.86 (s, 1H), 5.76 (d, *J* = 8 Hz, 1H), 4.51 (d, *J* = 7 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, *J* = 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 $C_{31}H_{41}NO_7SSi$: 599.2373; Found, 599.2370; Anal. calcd for $C_{31}H_{41}NO_7SSi$ 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 (30)

To a solution of olefin **21** (0.24 mg, 0.4 mmol) in methylene chloride (10 mL) was added 4-methylmorpholine *N*-oxide (96.7 mg, 0.8 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 mixture 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 × 30 mL). The organic phase was dried over sodium sulfate, filtered, and concentrated *in vacuo* to provide hydroxyketone **30** 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 (hexanes/ethyl acetate); IR (film) *v* 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, *J* = 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 $C_{27}H_{32}NO_9SSi$ (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 (22)

To a 10 mL round bottomed flask was added hydroxyl ketone **30** (0.4 g, 0.63 mmol) and 6 mL of a 1:1 mixture of ethanol:dioxane. The reaction flask was cooled externally in an ice bath and $NaBH_4$ (24 mg, 0.63 mmol) was added in one portion. The reaction mixture was removed from the bath and allowed to warm to room temperature over 1 h, and was then quenched with 1 N HCl (4 mL) and separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL) and the organic phase combined before drying over sodium sulfate. The crude mixture was concentrated in a 25 mL round bottomed flash and taken up in dioxane (8 mL). The reaction mixture was stirred while sodium periodate (0.332, 1.5 mmol) was added. The flask was covered to exclude light and H_2O (15 drops) added. Stirring was continued until total consumption of starting material (23h) as monitored by TLC. The reaction mixture was quenched with H_2O (10 mL) and separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL) and the combined organic phases dried over sodium sulfate. Concentration provided hemi-aminal (3a*S*,11*R*,12*S*,12a*S*)-12-{[*tert*-butyl(dimethyl)silyl]oxy}-5-hydroxy-2,2-dimethyl-4-[(4-methylphenyl)sulfonyl] 3a,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5-*c*:4',5'-*j*]phenanthridine-11-carbaldehyde **32**.

To a solution of hemi-aminal **32** (394 mg, 0.62 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 × 10 mL), H_2O (10 × 1mL), and brine (10 mL). The organic phase was dried over magnesium sulfate, filtered and concentrated. The final product **22** was isolated by column chromatography (hexanes:ethyl acetate, 4:1). Yield: 225 mg, 61%, 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) *v* 2929, 2857, 1725, 1689, 1619, 1505, 1484, 1386, 1361, 1287, 1255, 1220, 1172, 1077, 1036 cm^{-1}; ^{1}H 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, *J* = 5.2 Hz, 1H), 4.79 (m, 1H), 4.50 (dd, *J* = 12.7 Hz, *J* = 8.4 Hz, 1H), 4.27 (dd, *J* = 5.2 Hz, *J* = 2.7 Hz, 1H), 3.83 (dd, *J* = 12.6, *J* = 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 $C_{30}H_{36}NO_9SSi$ (M^+-15): 614.1879, Found: 614.1870; Anal. calcd for $C_{31}H_{39}NO_9SSi$ C, 59.12; H, 6.24; found C, 59.31; H, 6.29.

(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-carboxylic acid (34)

To a solution of aldehyde **22** (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 **34** as a white crystalline solid (0.125g, 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, $CHCl_3$); IR (KBr) *v* 3246, 2930, 2891, 2857, 1710, 1688, 1619, 1505, 1484, 1361, 1240, 1220, 1172, 1078, 1033 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 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, *J* = 8.4 Hz, 1H)), 4.84 (t, *J* = 4.7 Hz, 1H), 4.22 (dd *J* = 5.22, *J* = 2.8 Hz, 1H), 3.76 (dd, *J* = 12.4 Hz, *J* = 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; ^{13}C NMR (150 MHz, $CDCl_3$) 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 $C_{27}H_{30}NO_{10}SSi$ (M^+-57): 588.1359, Found: 588.1354; Anal. calcd for $C_{31}H_{39}NO_{10}SSi$ C, 57.65; H, 6.09; found C, 58.01; H, 6.37

(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-carboxylic acid methyl ester (35)

To a solution of carboxylic acid **34** (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 mixture 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 × 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 **35** that was used without further purification. Yield: 38mg, 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, $CHCl_3$); IR (KBr) *v* 2986, 2953, 2931, 2896, 2858, 1739, 1692, 1620, 1598, 1505, 1485, 1361, 1289, 1264, 1173 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 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, *J* = 5.4 Hz, 1H), 4.9 (dd, *J* = 12.5 Hz, *J* = 8.3 Hz, 1H), 4.78 (t, *J* = 3.0 Hz, 1H), 4.24 (dd *J* = 5.36 Hz, *J* = 2.9 Hz, 1H), 3.79 (dd, *J* = 12.4, *J* = 4.2 Hz, 1H), 3.56 (s, 3H), 3.40 (t, *J* = 3.7 Hz, 1H), 2.45 (s, 3H), 1.41 (s, 3H), 1.35 (s, 1H), 0.98 (s, 9H), 0.24 (s, 3H), 0.23 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) 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 $C_{28}H_{32}NO_{10}SSi$ (M^+-57): 602.1516, Found: 602.1516

(3aS,3bR,10bR,11R,12S,12aS)-12-(*tert*-Butyl-dimethyl-silanyloxy)-2,2-dimethyl-5-oxo-3a,3b,4,5,10b,11,12,12a-octahydro-1,3,7,9-tetraoxa-4-aza dicyclopenta[*a,h*]phenanthrene-11-carboxylic acid methyl ester (38)

To a solution of **35** (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 mixture was quenched with saturated aqueous ammonium chloride solution (1 mL), warmed to room temperature, and extracted with CH_2Cl_2 (6 × 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 to 2:1). Yield: 23 mg, 58%, clear oil; R_f 0.28 (hexanes:ethyl acetate, 1:1); $[\alpha]_D^{22}$ – 14.51 (*c* 0.50, $CHCl_3$); IR (film) ν3320, 2952, 2930, 2895, 2857, 1743, 1669, 1619, 1504, 1484, 14601385, 1369, 1321, 1288, 1260, 1222 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) 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, *J* = 8.2 Hz, 1H), 4.18 (dd, *J* = 8.25 Hz, *J* = 4.8 Hz, 1H), 4.11 (m, 1H), 3.66 (s, 3H), 3.40 (dd, *J* = 13.6 Hz, *J* = 3.7 Hz, 1H), 3.33 (m, 1H), 2.06 (s, 1H),

1.40 (s, 3H), 1.37 (s, 3H), 0.92 (s, 9H), 0.21 (s, 3H), 0.20 (s, 3H) ppm; ^{13}C NMR (150 MHz, CDCl3) δ:169.6, 165.4, 151.4, 146.6, 135.4, 122.6, 110.5, 108.6, 103.3, 101.7, 69.2, 53.1, 51.9, 45.9, 33.4, 27.6, 26.5, 25.7, 17.9, -4.9, -5.0 ppm; HRMS-EI Calcd for $C_{25}H_{35}NO_8Si$ (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 (39)

To a solution of the detosylated methyl ester **38** (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 **39** (11 mg, 69%) as a white crystalline solid.; mp >200 °C (methylene chloride/methanol); R_f 0.06 (methylene chloride/methanol, 20:1); $[\alpha]_D^{22}$ + 24.53 (*c* 0.25,MeOH); IR (KBr) *v*3311, 2913, 1732, 1648, 1609, 1497, 1462, 1349, 1259, 1037 cm^{-1}; ^{1}H NMR (300 MHz, MeOD) δ: 7.33 (s, 1H), 6.59 (s, 1H), 5.93 (d, *J* = 3.7, 2H), 4.50 (t, *J* = 3.12, 1H), 4.21 (dd, *J* = 13.1 Hz, *J* = 10.1 Hz, 1H), 3.86 (m, 1H), 3.79, (dd, *J* = 10.1, *J* = 3.0, 1H), 3.51 (s, 3H), 3.39 (m, 1H), 3.29 (dd, *J* = 13.1, *J* = 4.1, 1H) ppm; ^{13}C NMR (75 MHz, MeOD) δ: 170.8, 166.4, 151.7, 146.4, 137.3, 121.7, 106.9, 103.7, 101.8, 72.2, 71.9, 70.9, 51.4, 50.6, 44.8, 35.4 ppm; HRMS-FAB: (*m/z)* (M + H)$^+$: Calcd for $C_{16}H_{17}NO_8$: 352.0947, 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 (40)

To a solution of **39** (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 mixture was made slightly acidic with the addition of HCl (5 drops, 1M) and concentrated to provide acid **40** (5 mg, 95%) as a white crystalline solid.; mp >200 ^{o}C (methanol); R_f 0.06 (methylene chloride:methanol, 4:1); IR (KBr) *v* 3412, 2920, 2115, 1641, 1505, 1471, 1409, 1462, 1363, 1267 cm^{-1}; ^{1}H 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, *J* = 10.1 Hz, 1H), 3.99 (m, 1H), 3.89, (dd, *J* = 10.1, *J* = 3.0, 1H), 3.45 (m, 1H), 3.38 (m, 1H) ppm; ^{13}C NMR (75 MHz, MeOD) δ: 172.1, 166.4, 151.7, 146.4, 137.6, 121.7, 106.8, 103.8, 101.8, 72.4, 71.9, 71.1, 51.34, 45.03, 35.4 ppm;

(3aS,3bR,10bR,11S,12S,12aS)-12-(*tert*-Butyl-dimethyl-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-(41)

To a solution of aldehyde **22** (175 mg, 0.278 mmol) in EtOH/dioxane (1:1, 5 mL) at 0°C was added $NaBH_4$ (3 mg, 0.08 mmol). The reaction mixture was allowed to warm to room temperature over 1.5 hours before being quenched with a solution of saturated NH_4Cl (1 mL). The EtOH/dioxane mixture was removed under reduced pressure and the aqueous residue was extracted with CH_2Cl_2 (3 × 25 mL). The organic phases were combined, dried over sodium sulfate, filtered, and concentrated to provide alcohol **41** which was used without further purification. Yield: 150 mg, 85%, clear oil; ; R_f 0.44 (hexanes:ethyl acetate, 1:1); $[\alpha]_D^{22}$ – 47.72 (*c* 1.50, $CHCl_3$); IR (film) *v* 3547, 2986, 2932, 2586, 1692, 1616, 1594, 1508, 1481, 1360 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) 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, *J* = 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, *J* = 8.9 Hz, 1H), 3.78 (m 2H), 3.38 (dd, *J* = 11.3 Hz, *J* = 3.6 Hz, 1H), 2.55 (bs, 1H), 2.43 (s, 3H), 1.96 (bs, 1H), 1.43 (s, 3H), 1.35 (s, 3H), 0.96 (s, 9H), 0.20 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) 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.

Acetic acid (3aS,3bR,10bR,11S,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*]phenanthren-11-ylmethyl ester (42)

To a solution of **41** (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 μL, 0.475 mmol) was added and the reaction mixture was stirred for 1 hour before being quenched with saturated sodium bicarbonate (5 mL) and diluted with Et_2O (75 mL) and separated. The aqueous layer was extracted with Et_2O (2 × 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 oil; R_f 0.51 (hexanes/ethyl acetate, 1:1); $[\alpha]_D^{22}$ – 41.081 (*c* 3.0, $CHCl_3$); IR (film) ν2988, 2952, 2930, 2858, 1742, 1694, 1619, 1598, 1505, 1485, 1395, 1362, 1254; ^{1}H 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, CDCl3) 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 $C_{32}H_{40}NO_{10}SSi$ (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 (44)

To a solution of **42** (137 mg, 0.203 mmol) in dry DME (5 mL) at -78 °C was added a 0.5 M solution of Na/naphthalene in DME until a green color persisted and total consumption of starting material was observed (by TLC). The solution was stirred for 10 minutes then it was quenched with saturated aqueous ammonium chloride solution (2 mL). The reaction was warmed to room temperature, concentrated to remove DME, and extracted with CH_2Cl_2 (3 × 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 minutes. The reaction mixture was stirred until total consumption of starting material was observed (TLC) before the stirring bar was removed, silica (200 mg added), and the mixture 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); $[\alpha]_D^{22}$ – 38.301 (*c* 1.35, DMSO); IR (film) ν3303, 2982, 2922, 2901, 2853, 1734, 1655, 1652, 1612, 1483, 1459, 1364, 1246, 1235, 1215; ^{1}H NMR (300 MHz, DMSO) δ: 7.76 (s, 1H), 7.35 (s, 1H), 7.03 (s, 1H) 6.09 (d, *J* = 1.8, 2H), 5.48 (d, *J* = 4.2, 1H), 4.35, (s, 1H), 4.24 (d, *J* = 5.3, 1H), 4.19 – 4.10 (m, 3H), 3.46 (dd, *J* = 14.0 Hz, *J* = 8.2 Hz, 1H), 3.21 (dd, *J* = 13.9 Hz, *J* = 3.8 Hz, 1H), 2.80 (bs, 1H), 2.02 (s, 1H), 1.39 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ:170.9, 163.9, 151.3, 146.7, 134.5, 124.2, 108.9, 107.6, 105.4, 102.2, 77.9, 77.2, 65.3, 61.2, 53.5, 34.7, 28.3, 26.4, 21.2; HRMS-EI Calcd for $C_{20}H_{23}NO_8$ (M^+): 405.1424, Found: 405.1431

((1S,2S,3R,4S,4aR,11bR)-2,3,4-trihydroxy-6-oxo-1,2,3,4,4a,5,6,11b-octahydro-[1,3]dioxolo [4,5-j]phenanthridin-1-yl)methyl acetate (46)

To a solution of acetate **44** (21 mg, 0.052 mmol) in MeOH (1 mL) was added an HCl solution (3 % in MeOH, 3 mL). The reaction mixture 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 (methlene chloride: methanol, 5:1); $[\alpha]_D^{22}$ 97.32 (*c* 0.3, DMSO); ^{1}H 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, *J* = 2.9 Hz, 1H), 3.50 (dd *J* = 13.2 Hz, *J* = 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; ^{13}C 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 $C_{17}H_{20}NO_8$ (M + 1): 366.1082, Found: 366.1088.

(1S,2S,3R,4S,4aR,11bR)-2,3,4-trihydroxy-1-(hydroxymethyl)-1,2,3,4,4a,5-hexahydro-[1,3]dioxolo[4,5-j]phenanthridin-6(11bH)-one (45)

To a solution of acetate **44** (25 mg, 0.062 mmol) at 0 °C, in MeOH (5 mL) was added K_2CO_3 (40 mg, 0.62 mmol) and H_20 (1 mL). The suspension was stirred until total consumption of starting material (TLC) before being quenched with HCl (4 drops, 6N). The reaction mixture 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 × 5 mL) and the MeOH washes collected and concentrated. The final product was isolated by column chromatography (methylene chloride: methanol, 5:1). Yield: 15 mg, 75%, white solid; mp >200 °C (methylene chloride/methanol); R_f 0.20 (methlene chloride: methanol, 5:1); $[\alpha]_D^{22}$ 90.91 (*c* 0.25, DMSO); IR (film) ν3361, 2916, 1646, 1608, 1503, 1460, 1385, 1361, 1252; ^{1}H NMR (600 MHz, DMSO) δ: 7.34 (s, 1H), 6.97 (s, 1H), 6.66, (s, 1H), 6.09, (d, *J* = 0.78, 2H), 5.04 – 4.97, (m, 3H), 4.47 (dd *J* = 6.6 Hz, *J* = 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, *J* = 9.9 Hz, 1H), 3.39 – 3.32 (m, 1H), 3.15, (dd *J* = 13.3 Hz, *J* = 4.5 Hz, 1H), 2.41 (s, 1H) ppm; ^{13}C NMR (150 MHz, DMSO) δ: 164.2, 151.2, 146.3, 136.3, 123.7, 107.4, 105.6, 102.1, 73.3, 71.6, 69.7, 57.8, 51.8, 44.4, 37.3 ppm; HRMS-FAB Calcd for $C_{15}H_{18}NO_7$ (M + 1): 324.1085, Found: 324.1084.

(3aS,3bR,10bR,12S,12aS)-12-(tert-butyldimethylsilyloxy)-2,2-dimethyl-3b,4,10b,11,12,12a-hexahydrobis[1,3]dioxolo[4,5-c:4',5'-j]phenanthridin-5(3aH)-one (48)

To a solution of aldehyde **22** (192 mg, 0.305 mmol) in toluene (10 mL) was added $RhCl(PPh_3)_3$ (424 mg, 0.458 mmol). The reaction vessel was sealed, lowered into a preheated 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 mixture was quenched with saturated aqueous ammonium chloride solution (3 mL), warmed to room temperature, concentrated to remove DME, and extracted with CH_2Cl_2 (3 × 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); $[\alpha]_D^{22}$ – 20.929 (*c* 1.5, $CHCl_3$); IR (film) ν3360, 2952, 2929, 2894, 2857, 1670, 1615, 1505, 1482, 1459, 1384, 1345, 1270, 1256, 1243, 1219; ^{1}H NMR (600 MHz, $CDCl_3$) 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, *J* = 4.8 Hz, 1H), 4.11 (m, 1H),

3.43 (dd, *J* = 13.2 Hz, *J* = 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 $C_{23}H_{33}NO_6Si$ (M^+): 447.2077, Found: 447.2083

(3aS,3bR,10bR,12S,12aR)-12-Hydroxy-4-(4-methoxy-benzyl)-2,2-dimethyl-3b,4,10b, 11,12,12a-hexahydro-3a*H*-1,3,7,9-tetraoxa-4-aza-dicyclopenta[*a,h*]phenanthren-5-one (49)

To a solution of acetonide **48** (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 mixture was stirred at 0 °C for 10 min and then at rt for 20 min. The reaction mixture was cooled in ice again before *p*-methoxy benzylbromide (11 μL, 0.07 mmol) was added and then being allowed to warm to rt over 2 h. The mixture was taken up in Et_2O (5 mL) and quenched with H_2O (2 mL), transferred to a separatory funnel and diluted further with Et_2O (50 mL). The ether layer was wased with H_2O (6 × 0.5 mL), brine (1 × 1 mL), dried over magnesium sulfate, flitered, 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 mixture was stirred for 20 minutes. Silica (0.2g) added and the mixture 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 0.041 (hexanes:ethyl acetate, 2:1); $[\alpha]_D^{22}$ + 40.90 (*c* 0.50, $CHCl_3$); IR (film) *v* IR (film) *v* 3550, 2920, 1642, 1516, 1453 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) 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, *J* = 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, *J* = 7.9 Hz, 1H), 3.19 (td, *J* = 12.4, 5.0 Hz, 1H), 2.36-2.28 (m, 2H), 2.01 (qd, *J* = 13.6, 11.9, 4.9 Hz, 1H), 1.387 (s, 3H), 1.34 (s, 3H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ: 165.2,158.4, 151.1, 146.8, 135.4, 131.8, 128.2, 123.1, 113.7, 109.5, 108.6, 104.2, 101.6, 78.1, 75.9, 66.6, 61.6, 61.9, 55.3, 46.3, 32.3, 21.0, 28.0, 26.1 ppm; HRMS-EI Calcd for $C_{25}H_{27}NO_7$ (M+): 453.1788, Found: 453.1787.

4-(4methoxybenzyl)-2,2-dimethyl-3*b*,4,10*b*,12*a*-tetrahydro-3a*H*-1,3,7,9-tetraoxa-4-aza-dicyclopenta[*a,h*]phenanthren-5-one (50)

To a solution of alcohol **49** (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 mixture 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 mixture was concentrated to remove THF, the aq residue was extracted with EtOAc (3 × 30 mL), and the organic phases combined and dried over sodium sulfate. Following filtration, the EtOAc was removed under reduced pressure, the residue taken up in *o*-zylene (2 mL) and heated at reflux for 21 h. The reaction mixture 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); R_f 0.51 (hexanes:ethyl acetate, 1:1); $[\alpha]_D^{22}$ + 37.48 (*c* 0.20, $CHCl_3$); IR (film) *v* 2920, 1647, 1511, 1451 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) 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, *J* = 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, *J* = 7.1 Hz, 1H), 3.80 (s, 3H), 3.66 (dd, *J* = 11.9, *J* = 9.3 Hz, 1H), 3.49 (d, *J* = 12.1, 1H), 1.38 (s, 3H), 1.35 (s, 3H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) 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 $C_{25}H_{25}NO_6$ (M+): 435.1682, Found: 435.1683

7-deoxy-trans-dihydrolycoricidine (7)

To a solution of acetonide **48** (13 mg, 0.29 mmol) in MeOH (1 mL) was added a 10% solution of HCl in MeOH (10 mL). The reaction mixture 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); $[\alpha]_D^{22}$ 28.6 (*c* 0.25, DMSO); IR (KBr) *v* 3559, 3488, 3450, 3429, 1671, 1471, 1263 cm^{-1}; ^1H NMR (600 MHz, 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, *J* = 3.6 Hz, 1H), 2.15 (dt, *J* = 13.0, *J* = 3.0 Hz, 1H), 1.65 (td, *J* = 12.9, *J* = 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 $C_{14}H_{16}NO6$ (M_++1): 294.0978, Found: 294.1011.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors are grateful to the following agencies for financial support of this work: Natural Sciences and Engineering Research Council of Canada (NSERC), (Idea to Innovation and Discovery Grants); Canada Research Chair Program, Canada Foundation for Innovation (CFI), Research Corporation, TDC Research, Inc.; Brock University; and the Ontario Partnership for Innovation and Commercialization (OPIC) and Lotte and John Hecht Memorial Foundation grant to SP. AER and AK thank the National Institutes of Health (P20 RR016480) for financial support and Professor Snezna Rogelj for her kind assistance with the cell culture.

References

1. For a preliminary account see: Collins J, Drouin M, Sun X, Rinner U, Hudlicky T. Org. Lett 2008;10:361. [PubMed: 18163635]
2. Gerrard AW. Pharm. J 1877;8:214.
3. Evidente A, Kornienko A. Phytochem. Rev 2009;8:449. (b) For a recent summary of biological activities of narciclasine and congeners see reference [12k].
4. Lycoricidine: a Ohta S, Kimoto S. Tetrahedron Lett 1975;27:2279. b Ohta S, Kimoto S. Chem. Pharm. Bull 1976;24:2977. c Paulsen H, Stubbe M. Tetrahedron Lett 1982;23:3171. d Paulsen H, Stubbe M. Liebigs Ann. Chem 1983:535. e Chida N, Ohtsuka M, Ogawa S. Tetrahedron Lett 1991;32:4525. f Hudlicky T, Olivo HF. J. Am. Chem. Soc 1992;114:9694. g Chida N, Ohtsuka M, Ogawa S. J. Org. Chem 1993;58:4441. h Martin SF, Tso HH. Heterocycles 1993;35:85. i Hudlicky T, Olivo HF, McKibben B. J. Am. Chem. Soc 1994;116:5108. j Keck GE, Wager TT. J. Org. Chem 1996;61:8366. (*ent*-lycoricidine). k Elango S, Yan TH. Tetrahedron 2002;58:7375. l Padwa A, Zhang H. Org. Lett 2006;8:247. [PubMed: 16408886] m Padwa A, Zhang H. J. Org. Chem 2007;72:2570. [PubMed: 17338575] n Matveenko M, Kokas OJ, Banwell MG, Willis AC. Org. Lett 2007;9:3683. (*ent*-lycoricdine). [PubMed: 17685535]
5. Lycorine: a Tsuda Y, Sano T, Taga J, Isobe K, Toda J, Irie H, Tanaka H, Tagaki S, Yakami M, Murata M. Chem Commun 1975:333. b Yamaki M, Murata M, Takagi S, Tsuda Y, Sano T, Taga J, Isobe K, Tanaka H, Irie H, Uyeo S. Heterocycles 1976;5:163. c Moeller O, Steinberg EM, Torssell K. Acta Chem. Scand., Ser. B 1978;32:98. d Tsuda Y, Sano T, Taga JI, Isobe K, Toda J, Takagi S, Yamaki M, Murata M, Irie H, Tanaka H. J. Chem.. Soc., Perkin Trans. I 1979:1358. e Umezawa B, Hoshino O, Sawaki S, Sashida H, Mori K. Heterocycles 1979;12:1475. f Sano T, Kashiwaba N, Toda J, Tsuda Y, Irie H. Heterocycles 1980;14:1097. g Martin SF, Tu C-Y. J. Org. Chem 1981;46:3763. h Martin SF, Tu C-Y, Kimura M, Simonsen SH. J. Org. Chem 1982;47:3634. i Umezawa B, Hoshino O, Sawaki S,

Sashida H, Mori K, Hamada Y, Kotera K, Iitaka Y. Tetrahedron 1984;40:1783. j Boeckman RK, Goldstein SW, Walters MA. J. Am. Chem. Soc 1988;110:8250. k Hoshino O, Ishizaki M, Kamei K, Tauchi M, Nagao T, Iwaoka K, Sawaki S, Umezawa B, Iitaka Y. Chemistry Letters 1991;8:1365. l Schultz AG, Holoboski MA, Smyth MS. J. Am. Chem. Soc 1996;118:6210. (*ent*-lycorine). m Hoshino O, Ishizaki M, Kamei K, Tauchi M, Nagao T, Iwaoka K, Sawaki S, Umezawa B, Iitaka Y. J. Chem. Soc., Perkin Trans. I 1996:571. n Yamada K, Yamashita M, Sumivoshi T, Nishimura K, Tomioka K. Org. Lett 2009;11:1631. [PubMed: 19243131]

6. 7-Deoxypancratistatin: a Ohta S, Kimoto S. Chem. Pharm. Bull 1976;24:2969. b Ohta S, Kimoto S. Chem. Pharm. Bull 1976;24:2977. c Paulsen H, Stubbe M. Liebigs Ann. Chem 1983:535. d Tian X, Maurya R, Königsberger K, Hudlicky T. Synlett 1995:1125. e Keck GE, McHardy SF, Murry JA. J. Am. Chem. Soc 1995;117:7289. f Chida N, Jitsuoka M, Yamamoto Y. Heterocycles 1996;43:1385. g Keck GE, Wagner TT, McHardy SF. J. Org. Chem 1998;63:9164. h Keck GE, McHardy SF. J. Org. Chem 1999;64:4465. i Akgün H, Hudlicky T. Tetrahedron Lett 1999;40:3081. (*ent*-7-deoxypancratistatin). j Acena JL, Arjona O, Leon ML, Plumet J. Org. Lett 2000;2:3683. [PubMed: 11073675] k Padwa A, Zhang H. Tetrahedron Lett 2006;47:3905. l Hakansson AE, Palmelund A, Holm H, Madsen R. Chem. Eur. J 2006;12:3243.
7. trans-Dihydrolycoricidine: a Isobe K, Taga J-I, Tsuda Y. Heterocycles 1978;9:625. b Chida N, Jitsuoka M, Yamamoto Y, Ohtsuka M, Ogawa S. Heterocycles 1996;43:1385. c Fujimura T, Shibuya M, Ogasawara K, Iwabuchi Y. Heterocycles 2005;66:167. d Szanto G, Hegedus L, Mattyasovszky L, Simon A, Simon A, Kadas I. Tetrahedron Lett 2009;50:2857. e Szanto G, Hegedus L, Mattyasovszky L, Simon A, Simon A, Bitter I, Toth G, Toke L, Kadas I. Tetrahedron 2009;65:8412.; by hydrogenation of lycoricidine: f Gabrielsen B, Monath TP, Huggins JW, Kefauver DF, Pettit GR, Groszek G, Hollingshead M, Kirsi JJ, Shannon WM, Schubert EM, Dare J, Ugarkar B, Ussery MA, Phelan MJ. J. Nat. Prod 1992;55:1569. [PubMed: 1336040] g Pettit GR, Ducki S, Eastham SA, Melody N. J. Nat. Prod 2009;72:1279. [PubMed: 19522518]
8. Pancratistatin: a Danishefsky S, Lee JY. J. Am. Chem. Soc 1989;111:4829. b Tian X, Hudlicky T, Königsberger K. J. Am. Chem. Soc 1995;117:3643. c Trost BM, Pulley SR. J. Am. Chem. Soc 1995;117:10143. d Hudlicky T, Tian X, Königsberger K, Maurya R, Rouden J, Fan F. J. Am. Chem. Soc 1996;118:10752. e Doyle TJ, Hendrix M, VanDerveer D, Javanmard S, Haseltine J. Tetrahedron 1997;53:11153. f Magnus P, Sebhat IK. J. Am. Chem. Soc 1998;129:5341. g Rigby JH, Maharoof USM, Mateo ME. J. Am. Chem. Soc 2000;122:6624. h Pettit GR, Melody N, Herald DL. J. Org. Chem 2001;66:2583. [PubMed: 11304174] i Kim S, Ko H, Kim E, Kim D. Org. Lett 2002;4:1243. j Li M, Wu A, Zhou P. Tetrahedron Lett 2006;47:3707. k Madsen R, Dam JH. Eur. J. Org. Chem 2009;37:4666.
9. Narciclasine: a Rigby JH, Mateo ME. J. Am. Chem. Soc 1997;119:12655. b Keck GE, Wager TT, Rodriquez JFD. J. Am. Chem. Soc 1999;121:5176. c Gonzalez D, Martinot T, Hudlicky T. Tetrahedron Lett 1999;40:3077. d Rigby JH, Maharoof USM, Mateo ME. J. Am. Chem. Soc 2000;122:6624. e Hudlicky T, Rinner U, Gonzalez D, Akgun H, Schilling S, Siengalewicz P, Martinot TA, Pettit GR. J. Org. Chem 2002;67:8726. [PubMed: 12467383] f Elango S, Yan T-H. J. Org. Chem 2002;67:6954. [PubMed: 12353988] g Matveenko M, Banwell MG, Willis AC. Tetrahedron 2008;64:4817. (*ent*-narciclasine, *ent*-lycoricidine).
10. trans-Dihydronarciclasine: a Shin I-J, Choi E-S, Cho C-G. Angew. Chem., Int. Ed 2007;46:2303. b Jana CK, Studer A. Chem. Eur. J 2008;14:6326. c Choi E-S, Cho C-G. Tetrahedron 2008;64:2172.; by hydrogenation of narciclasine: d Mondon A, Krohn K. Chem. Ber 1975;108:445. e Pettit GR, Ducki S, Eastham SA, Melody N. J. Nat. Prod 2009;72:1279. [PubMed: 19522518]
11. Reviews: a Cook, JW.; Loudon, JD. Alkaloids of the Amaryllidaceae, The Alkaloids. Manske, RHF.; Holmes, HL., editors. Vol. II. Academic Press; New York: 1952. p. 331 b Hartwell JL. Lloydia 1967;30:379. c Wildman, WC. The Alkaloids. Manske, RHF., editor. Academic Press; New York: 1968. p. 307 d Fuganti, C. The Alkaloids. Manske, RHF., editor. Academic Press; New York: 1975. p. 83 e Martin, SF. The Alkaloids. Brossi, AR., editor. Vol. 40. Academic; New York: 1987. p. 251 f Polt, R. Organic Synthesis: Theory and Applications. Hudlicky, T., editor. Vol. 3. JAI Press; Greenwich, CT: 1997. p. 109 g Ruan L. Yaoxue Tongbae 1988;23:453. h Hoshino, O. The Alkaloids. Cordell, GA., editor. Vol. 51. Academic; New York: 1998. p. 323 i Rinner U, Hudlicky T. Synlett 2005:365. j Chapleur Y, Chretien F, Ahmed I, Khaldi M. Curr. Org. Synth 2006;3:331. k Kornienko A, Evidente A. Chem. Rev 2008;108:1982. [PubMed: 18489166] l Manpadi M, Kornienko A. Org. Prep. Proced. Int 2008;40:107. m Jin Z. Nat. Prod. Rep 2009;26:363. [PubMed: 19240946]

12. a McNulty J, Mao J, Give R, Mo R, Wolf S, Pettit GR, Herald DL, Boyd MR. Bioorg. Med. Chem. Lett 2001;11:169. [PubMed: 11206451] b McNulty J, Larichev V, Pandey S. Bioorg. Med. Chem. Lett 2005;15:5315. [PubMed: 16183280]
13. McNulty J, Nair JJ, Griffin C, Pandey S. J. Nat. Prod 2008;71:357. [PubMed: 18225861]
14. Manpadi M, Kireev AS, Magedov IV, Altig J, Tongwa P, Antipin MY, Evidente A, van Otterlo WAL, Kornienko A. J. Org. Chem 2009;74:7122. [PubMed: 19743883]
15. a Pettit GR, Freeman S, Simpson MJ, Thompson MA, Boyd MR, Williams MD, Pettit GR III, Doubek DL. Anti-Cancer Drug Des 1995;10:243, 250. b Pettit GR, Orr B, Ducki S. Anti-Cancer Drug Des 2000;15:389–395.
16. a Pettit GR, Melody N, Simpson M, Thompson M, Herald DL, Knight J. J. Nat. Prod 2003;66:92. [PubMed: 12542352] b Pettit GR, Melody N, Herald DL. J. Nat. Prod 2004;67:322. [PubMed: 15043403] c Pettit GR, Melody N. J. Nat. Prod 2005;68:207. [PubMed: 15730244] d Shnyder SD, Cooper PA, Millignton NJ, Gill JH, Bibby MC. J. Nat. Prod 2008;71:321. [PubMed: 18154271]
17. a Hudlicky T, Rinner U, Finn KJ, Ghiviriga I. J. Org. Chem 2004;70:3494. b Rinner U, Hudlicky T, Gordon H, Pettit GR. Angew. Chem., Int. Ed 2004;43:5342.
18. Rinner U, Hillebrenner HL, Adams DR, Hudlicky T, Pettit g. R. Bioorg. Med. Chem. Lett 2004;14:2911. [PubMed: 15125958]
19. a Rinner U, Siengalewicz P, Hudlicky T. Org. Lett 2002;4:115. [PubMed: 11772104] b Hudlicky T, Rinner U, Gonzalez D, Akgun H, Schilling S, Siengalewicz P, Martinot TA, Pettit GR. J. Org. Chem 2002;67:8726. [PubMed: 12467383]
20. a Moser M, Sun X, Hudlicky T. Org Lett 2005;7:5669. [PubMed: 16321018] b Moser M, Banfield SC, Rinner U, Chapuis J-C, Pettit GR, Hudlicky T. Can. J. Chem 2006;84:1313.
21. Ibn-Ahmed S, Khaldi M, Chetien F, Chapleur Y. J. Org. Chem 2004;69:6722. [PubMed: 15387596]
22. Gabrielsen B, Monath TP, Huggins JW, Kefauver DF, Pettit GR, Groszek G, Hollingshead M, Kirsi JJ, Shannon WM, Schubert EM, Dare J, Ugarkar B, Ussery MA, Phelan MJ. J. Nat. Prod 1992;55:1569. [PubMed: 1336040]
23. Zylstra GJ, Gibson GT. J. Biol. Chem 1989;264:14940. [PubMed: 2670929]
24. a Yamada Y, Yamamoto T, Okawara M. Chem. Lett 1975:361. PhI=NTs is prepared according the procedure of Yamada. b Evans DA, Faul MM, Bilodeau MT. J. Org. Chem 1991;56:6744.
25. See references [6d,] 8b,d.
26. a Royer J, Beugelmans-Verrier M. C. R. Hebd. Seances. Acad. Sci. Ser. C 1971;272:1818.b ibid. 1974. p. 1049 c Buchbauer G, Heneis VM, Krejci V, Talsky C, Wunderer H. Monatsh. Chem 1985;116:1345. d Hagiwara H, Akama T, Uda H. Chem. Lett 1989;11:2067. e Margot C, Simmons DP, Reichin D, Skuy D. Helv. Chim. Acta 2004;87:2662.
27. For a review of Baeyer-Villiger oxidations see: Krow GR. Org. React 1993;43:251.
28. Pettit GR, Melody N, Herald D. J. Org. Chem 2001;66:2583. [PubMed: 11304174]
29. a Wender PA, Singh SK. Tetrahedron Lett 1990;31:2517. b Tauchi H, Yamamoto H, Nozaki H. Tetrahedron Lett 1975;19:1545.
30. Grieco PA, Zelle RE, Lis R, Finn J. J. Am. Chem. Soc 1983;105:1403.
31. a Nicolaou KC, Yang Z, Liu JJ, Ueno H, Nantermet PG, Guy RK, Clairborne CF, Renaud J, Couladouros EA, Paulvannan K, Sorensen EJ. Nature 1994;367:630. [PubMed: 7906395] b Nicolaou KC, Ueno H, Liu J-J, Nantermet PG, Yang Z, Renaud J, Paulvannan K, Chadha R. J. Am. Chem. Soc 1995;117:653.
32. Pettit GR, Pettit GR III, Backhaus RA. J. Nat. Prod 1993;56:1682. [PubMed: 8277308]

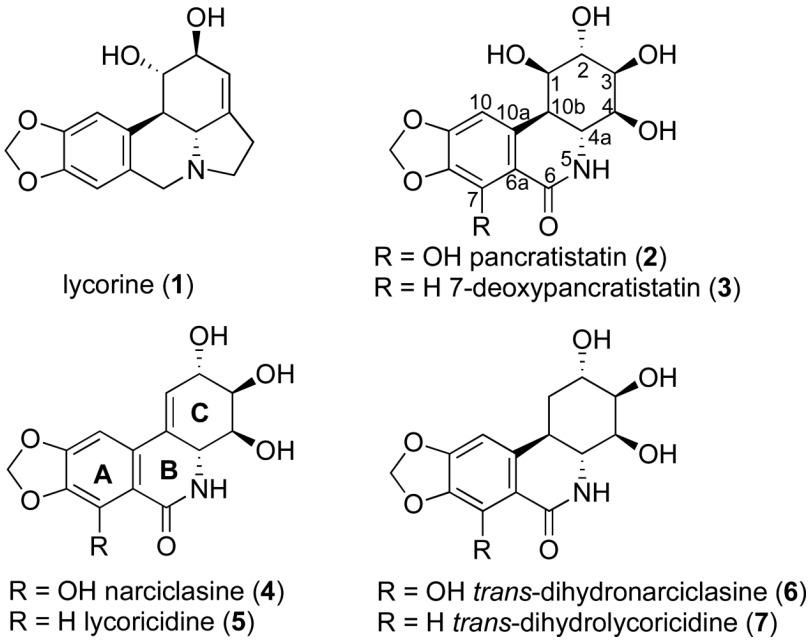


Figure 1.
Representative members of the Amaryllidaceae constituents.

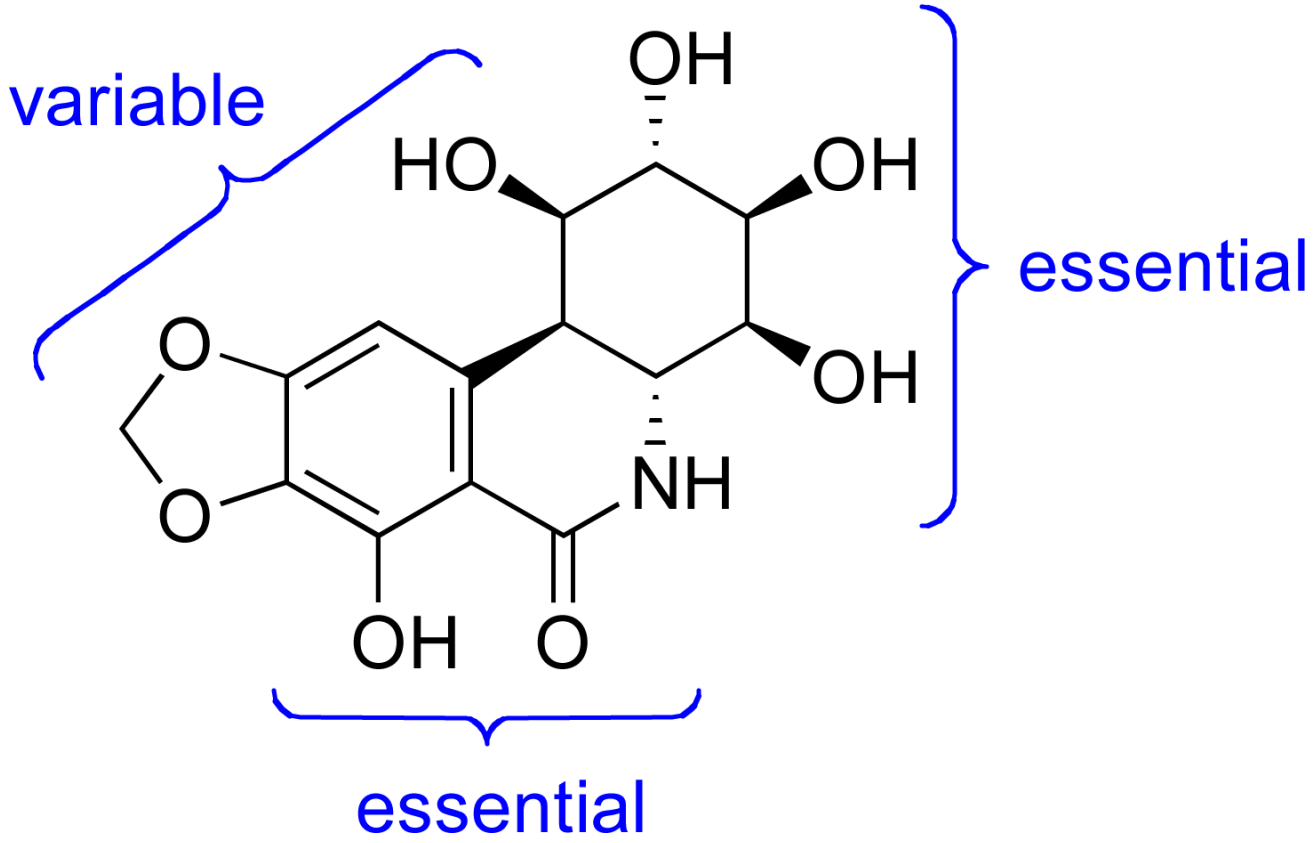


Figure 2.
Proposed pharmacophore of pancratistatin.

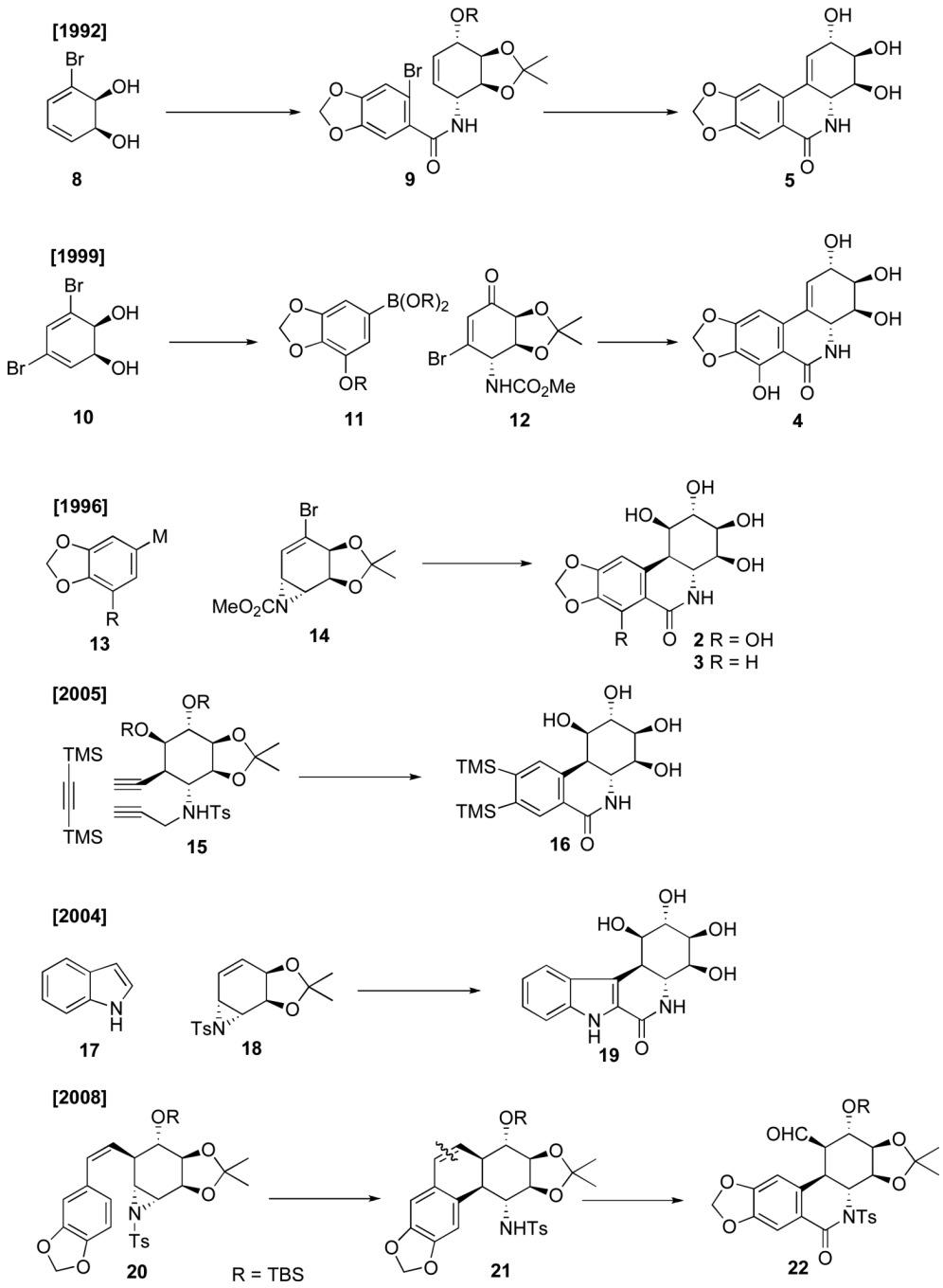
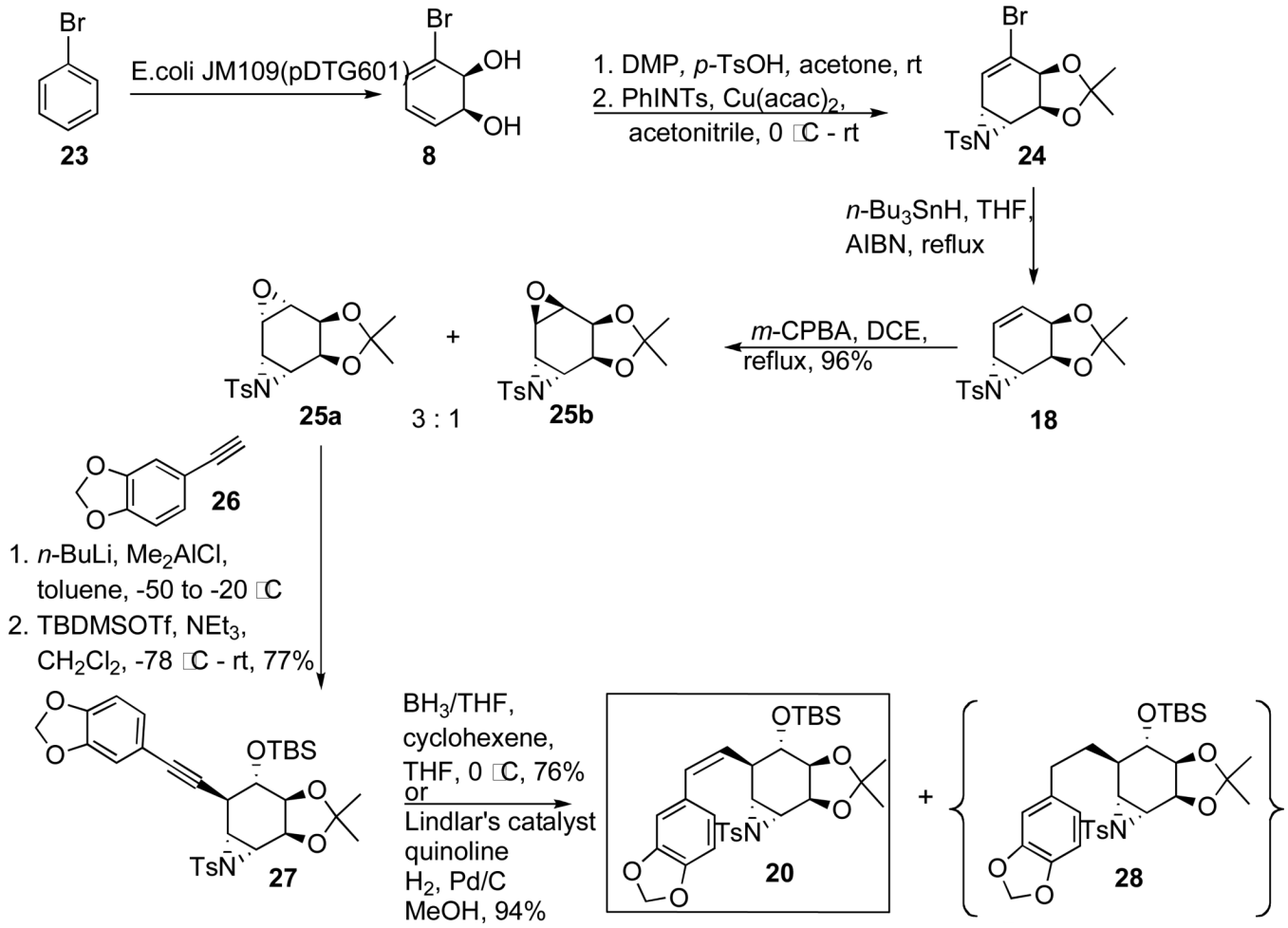


Figure 3.
Evolution of diverse strategies toward Amaryllidaceae constituents.



Scheme 1.

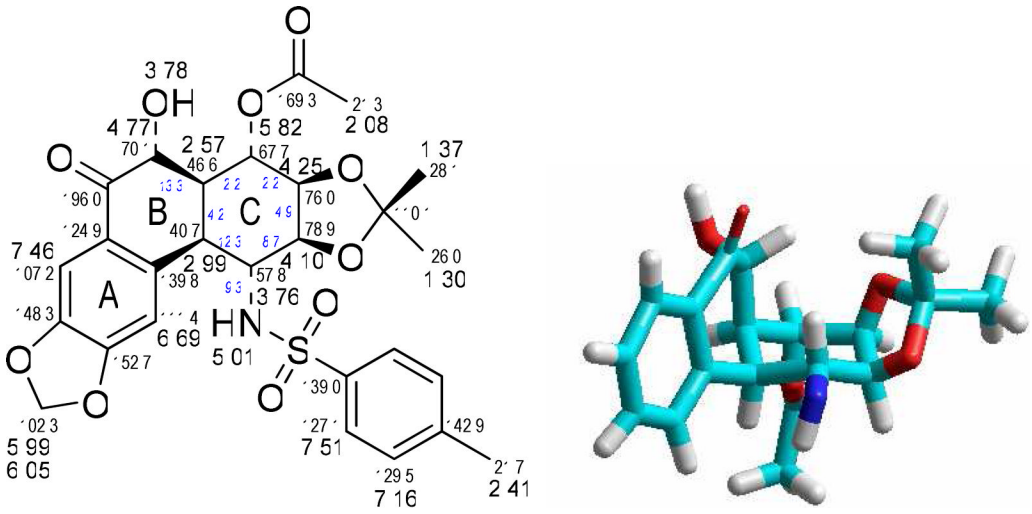
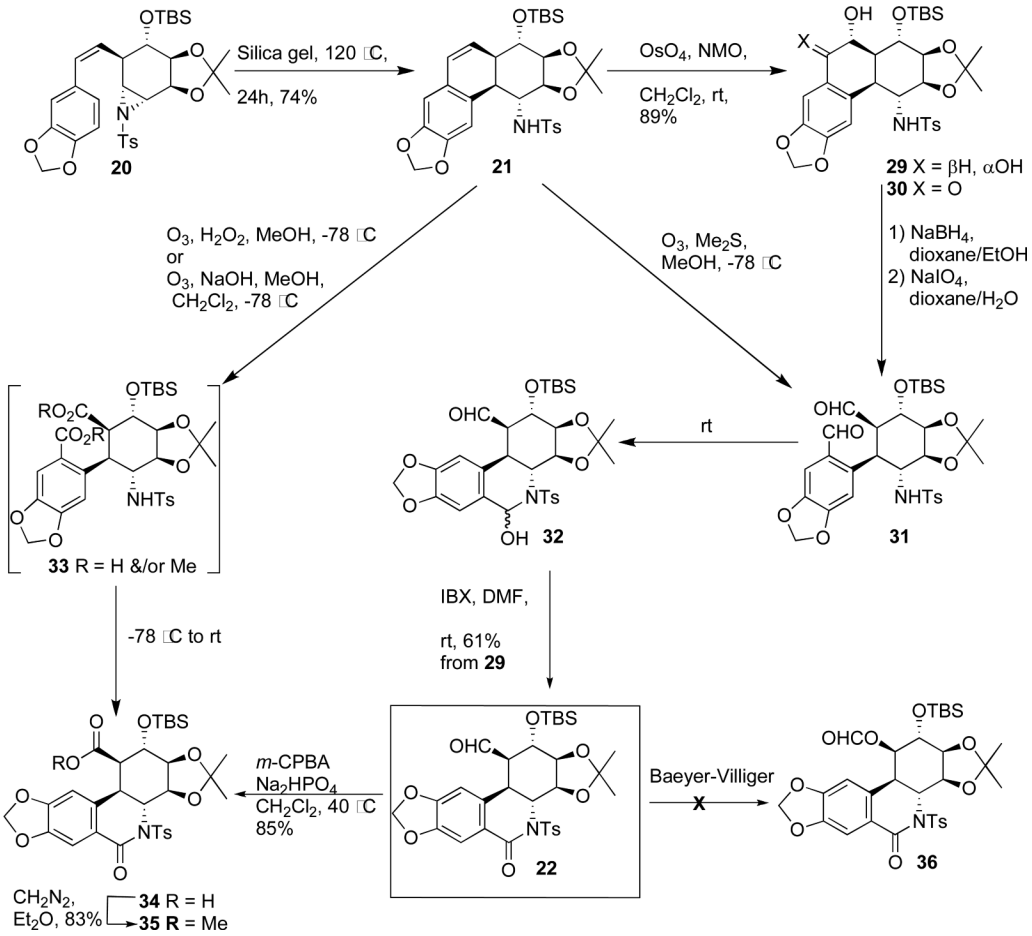
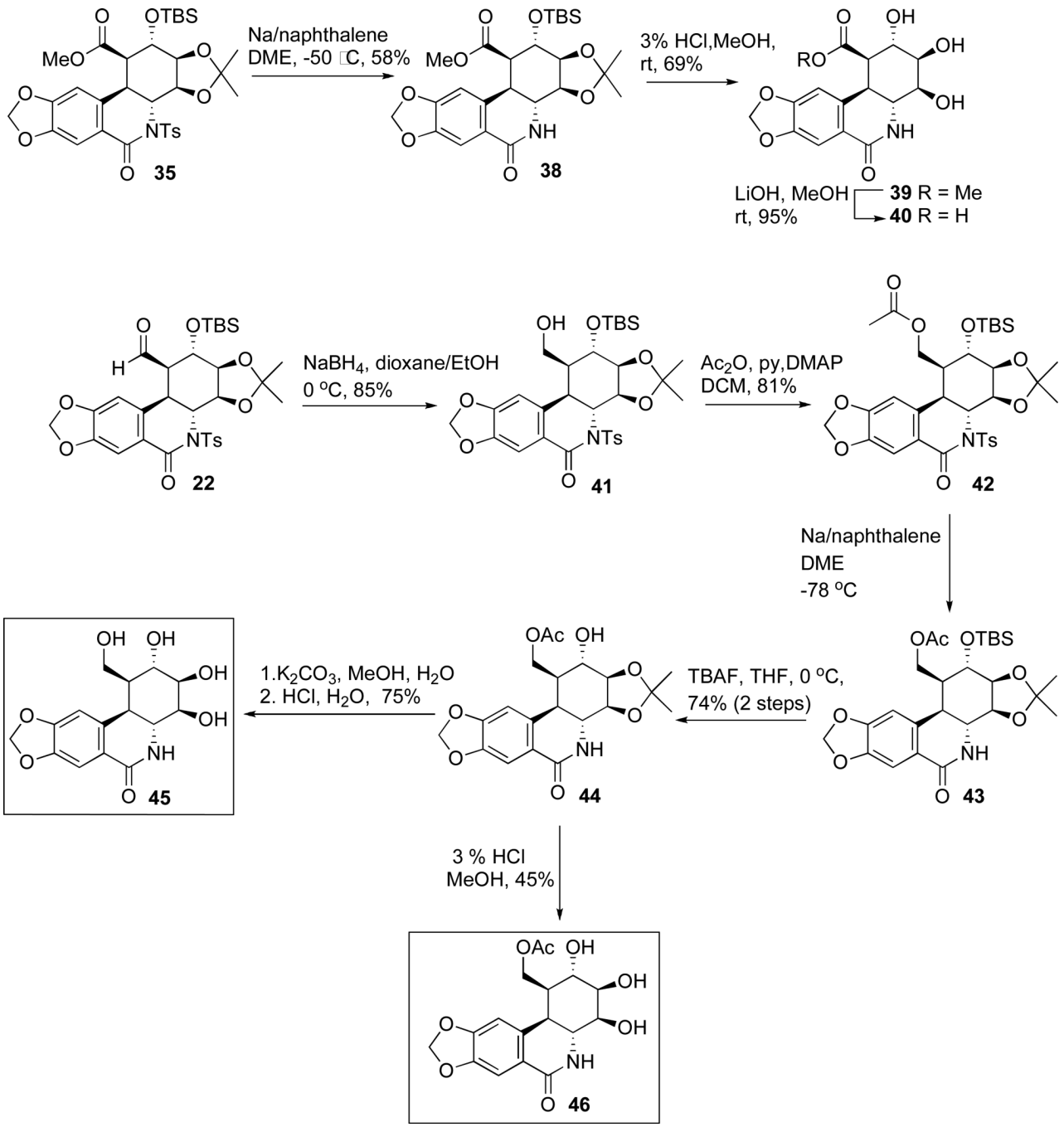


Figure 4.

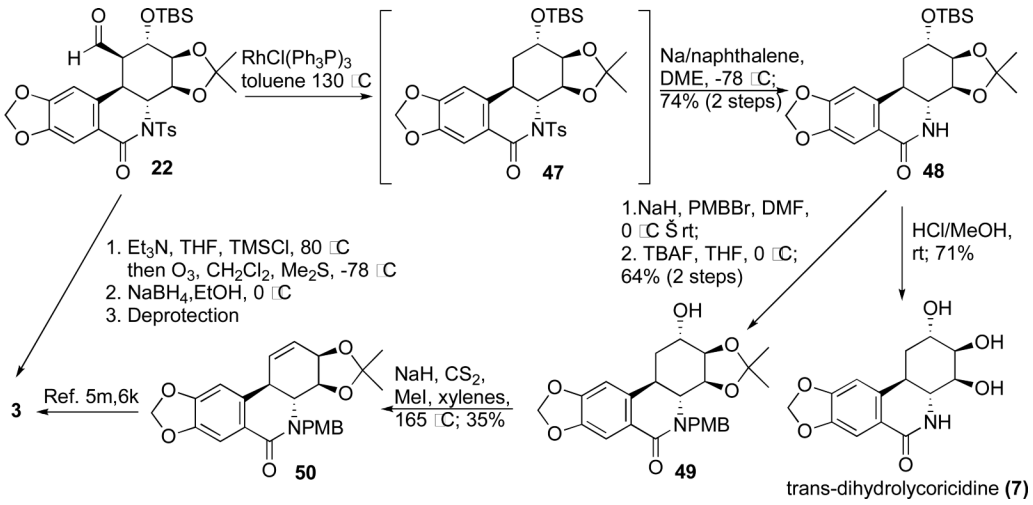
Full assignment of stereochemistry for the acetate **37**, derived from hydroxy ketone **30** (For clarity, the tosyl group was removed in the 3D model).



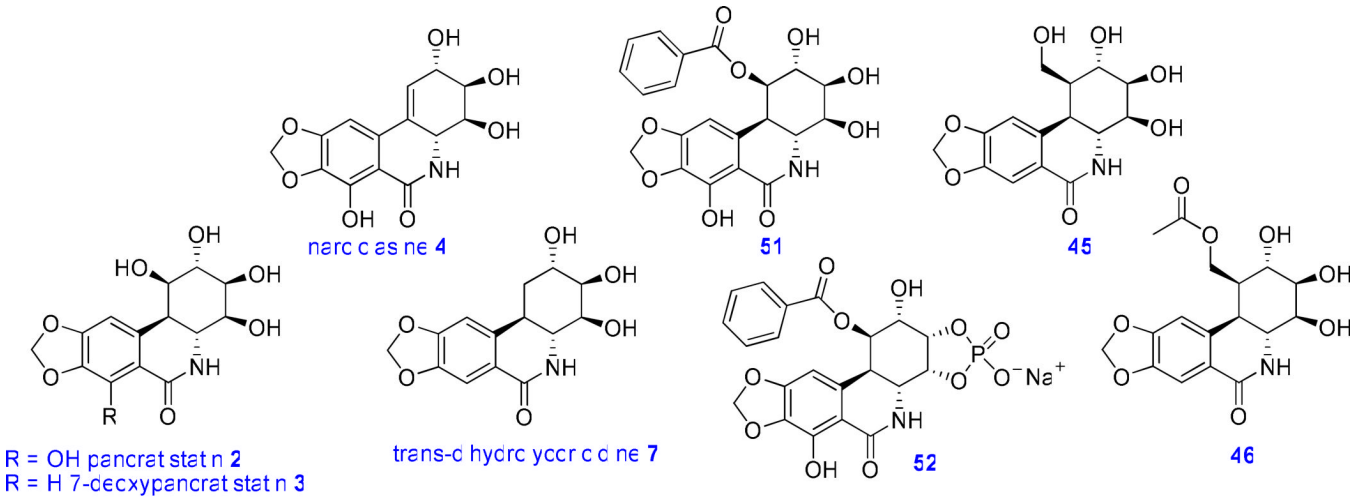
Scheme 2.

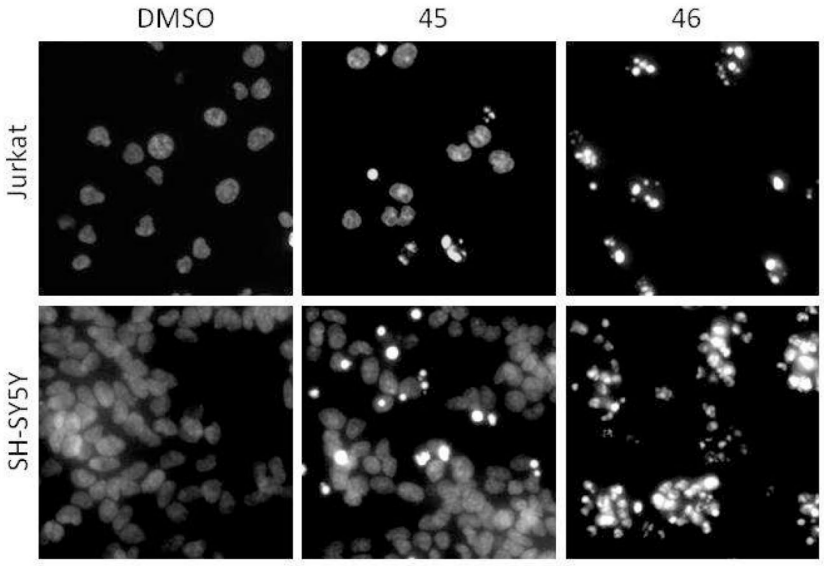


Scheme 3.

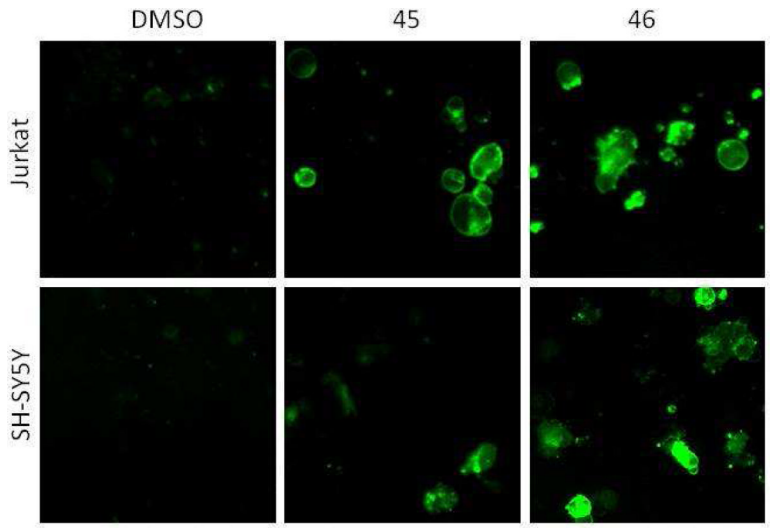


Scheme 4.





a.



b

Figure 5.

Hoechst cell permeable dye and Annexin-V binding were used to observe nuclear morphology after exposure to compound **45** or **46**. Cells with brightly stained, condensed nuclei are considered to be apoptotic (a). Apoptosis was confirmed by Annexin-V binding after 48hr exposure to either **45** or **46** at 0.5 μM concentration (b).

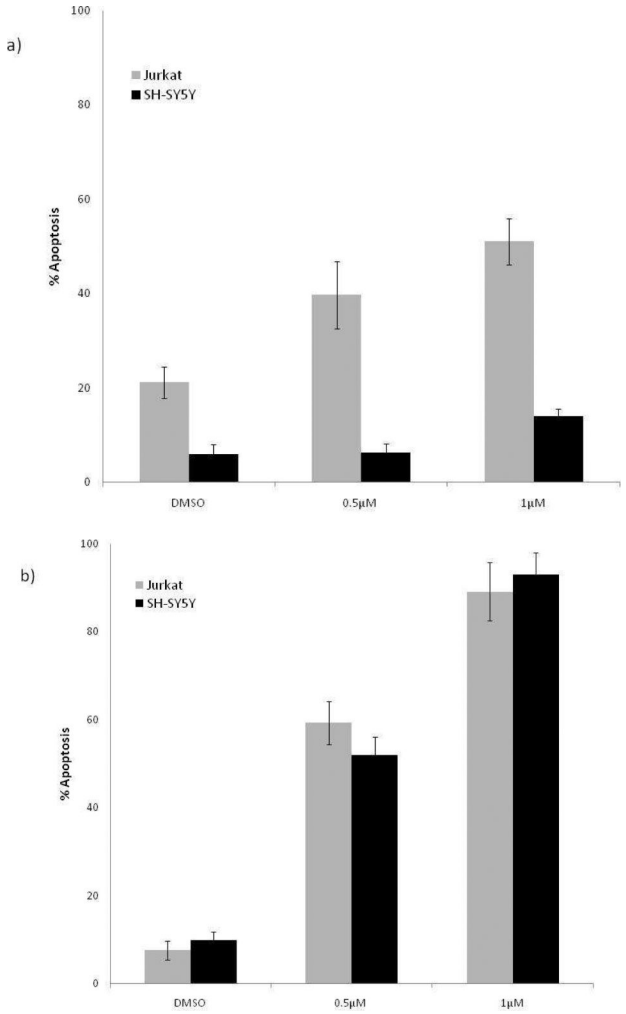


Figure 6.

Apoptosis is induced by compounds **45** (a) and **46** (b) in Jurkat and SH-SY5Y cells after 72hrs exposure. Cells were stained with Hoechst dye and counted to determine the percentage of apoptotic cells; a minimum of 5 fields containing at least 100 cells per field was counted.

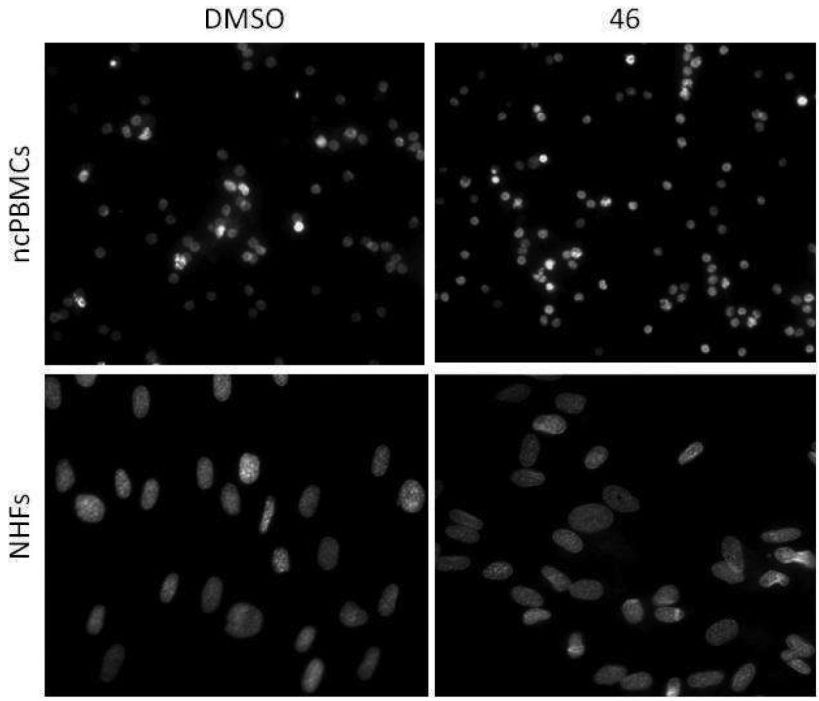


Figure 7.
Compound **46** does not induce apoptosis in non-cancerous human cells. Normal Human Fibroblasts (NHFs) and Non-cancerous peripheral blood mononuclear cells (ncPBMC) were treated with compound **46** and stained with Hoechst as described in materials and methods. Apoptotic nuclear morphology is not visible in normal human fibroblasts (NHFs) or peripheral mono-nucleated blood cells (PMBC) prepared from blood obtained from healthy volunteers treatment with **46**.

Table 1

Human Cancer Cell Line and Murine P-388 Lymphocytic Inhibitory Activities. P388 Murine Lymphoceutic Leukemia and Human Cancer Cell Results [μg/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
2	0.039	0.028	0.032	0.017	0.048	0.062	0.016	0.163	0.163
3	0.44	–	–	–	0.29	0.22	–	–	–
4	0.001	0.026	0.019	0.021	0.032	0.021	0.011	–	–
7	0.029	0.046	0.034	0.059	0.043	0.051	0.040	–	–
51	0.0016	0.0019	0.00031	0.00055	0.0001	0.00037	0.00021		
52	0.061	0.25	0.041	0.17	0.029	0.13	0.13		
45		0.19	0.65		0.09		0.26	1.615	1.615
46		0.11	0.29		0.11		0.37	0.183	0.183