

Chemoenzymatic synthesis of complex natural and unnatural products: morphine, pancratistatin, and their analogs

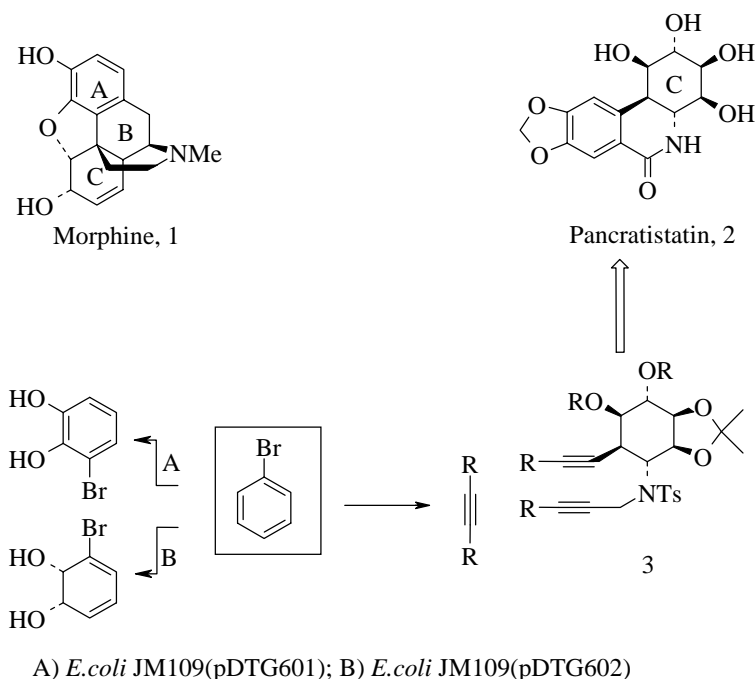
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Abstract

The synthesis of morphine (**1**) and pancratistatin (**2**) have been the subject of intense effort by the synthetic community for many years. Our focus on the total synthesis of these challenging targets resulted in several generations of approaches that combine enzymatic transformations with traditional synthetic protocols in order to provide for maximum efficiency and brevity in attaining the targets.



Recombinant strains that express toluene dioxygenase (TDO) are used to provide the homochiral diene *cis*-diol derived from bromobenzene and containing the structural features of both ring C of morphine and ring C of pancratistatin. Bromocatechol, representing ring A of morphine, is also derived from bromobenzene by fermentation with *E.coli* JM109 that expresses TDO as well as catechol dehydrogenase. In this fashion twelve of the carbons in morphine originate in the same starting material. One approach is based on the Kazmaier-Claisen

rearrangement of glycinate esters, the other on an intramolecular Heck reaction. The route to pancratistatin and to its unnatural analogs is based on the metal-mediated cyclotrimerization of acetylenic substrates of type **3**. The core of this Amaryllidaceae constituent has been attained in the initial stages of this project. The details of the synthetic endeavours toward these and other heterocyclic targets will be disclosed with emphasis on practicality, brevity, and efficiency as guiding principles of environmentally benign manufacturing of relevant compounds.

Keywords: Morphine, pancratistatin, total synthesis of alkaloids, cyclotrimerization, enzymatic oxidation of aromatics, toluene dioxygenase

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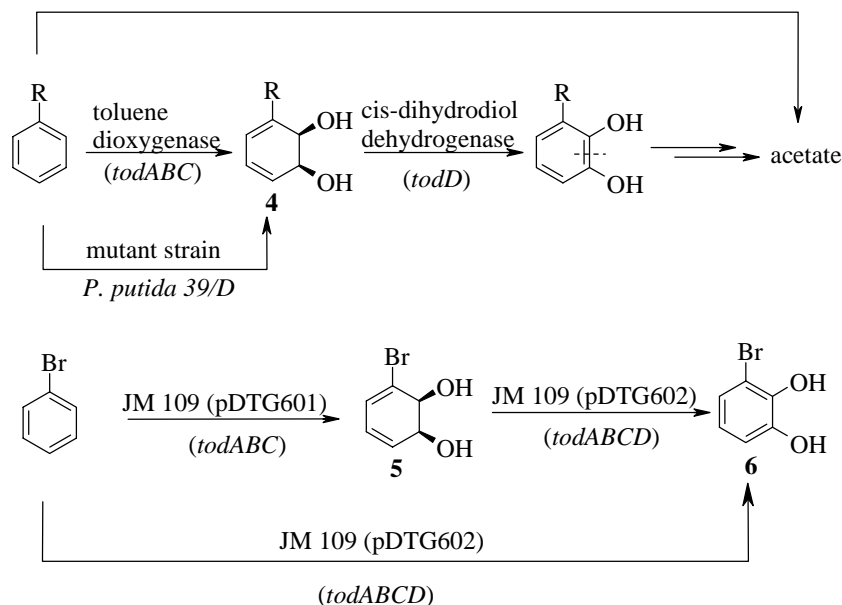
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Introduction

This lecture provides an overview of our long-term commitment toward practical and efficient syntheses of medicinally important compounds. Morphine, (**1**), an alkaloid originating in opium, is used as an anesthetic and an analgesic to the extent of more than 80,000 tons in the U.S. alone. Although its supply from the natural sources may be limited by political instabilities in those regions that produce it, no large-scale synthesis of this important molecule has yet materialized despite over 20 total syntheses published.¹⁻³ Pancratistatin, (**2**), is a constituent of the Amaryllidaceae groups and has a long history as an interesting synthetic target and because of its anti-cancer activity.⁴ Its bioavailability is poor, as is its solubility. A serious effort has been under way by several groups to provide better analogs of this important compound.

From a synthetic perspective both morphine and pancratistatin present challenges in design. Our mission is to provide, through multi-generational iterations, a short, practical synthesis of each compound and, in the case of pancratistatin, search for a more soluble derivative that would retain its potent biological activity. To achieve both briefly and practically we have turned to biocatalysis and its incorporation into traditional synthetic protocols in order to provide preparations that are efficient and environmentally benign. The core technology employed in the design of synthesis for both targets is the enzymatic oxidation of aromatic compounds, discovered by Gibson almost 40 years ago, portrayed in Scheme 1.⁵



Scheme 1. Metabolism of aromatics by soil bacteria.

Gibson elucidated the degradation pathway by which soil bacteria utilize arenes as carbon and energy source, and he provided key recombinant organisms for the whole-cell fermentation production of diols such as **4**.⁶ Toluene dioxygenase (TDO) is overexpressed in *E. coli* JM109(pDTG601) and provides, with good substrates such as bromobenzene, 20 g/L of the corresponding bromochiral diol **5**. The second enzyme in the pathway, dihydrodiol dehydrogenase (DHDH) is expressed by a similar organism, *E. coli* JM109 (pDTG602) and leads directly to functionalized catechols such as **6**.⁷

Hundreds of metabolites of aromatics are known and available in multigram quantities through whole-cell fermentation protocols.⁸ The application of these in asymmetric synthesis of complex molecules are numerous and have been reviewed on several occasions.⁹⁻¹¹

Examples of the diversity of metabolites produced substrates that are tolerated and metabolized by TDO are shown in Figure 1. Such diversity finds immediate application in the synthesis of complex molecules. We have incorporated dihydroarene cis-diols into the design of many natural products, some of which are shown in Figure 2. The utility of the enzymatic oxidation of aromatics in asymmetric synthesis is clearly well established and new applications continue to emerge. Through directed evolution it is now possible to improve yields and expand substrate specificity of many enzymes, including arene dioxygenases, as has recently been demonstrated on the biocatalytic synthesis of strawberry furanone.¹²

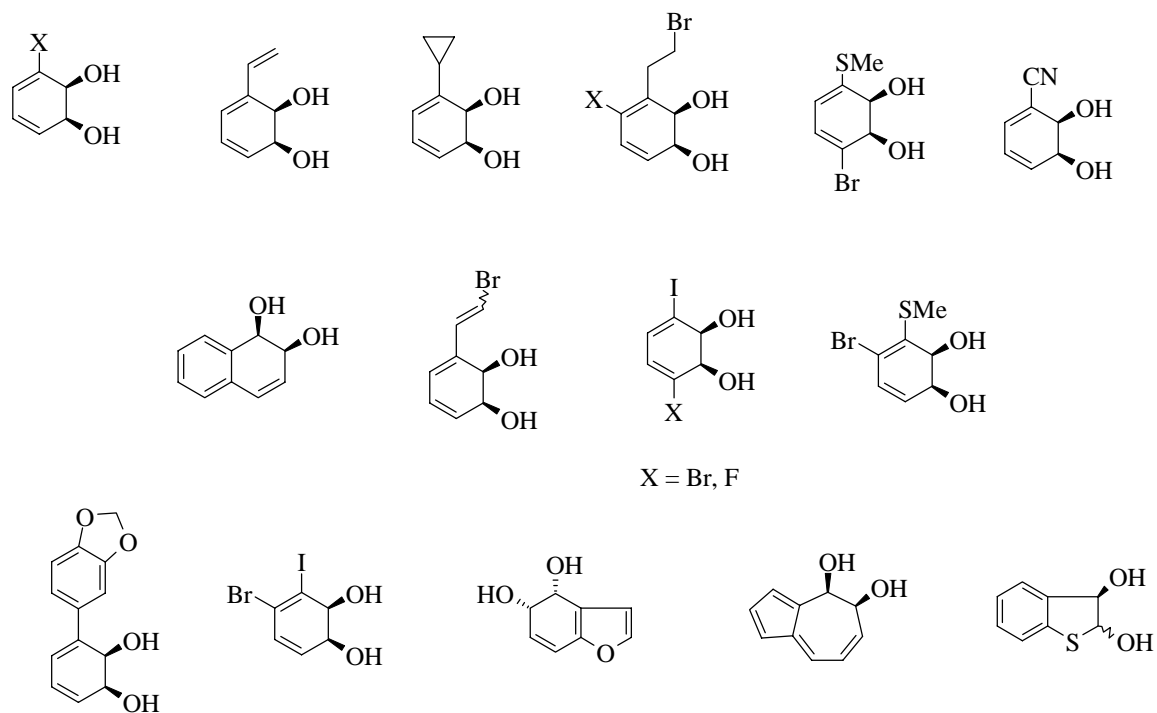


Figure 1. Examples of some metabolites of arenes.

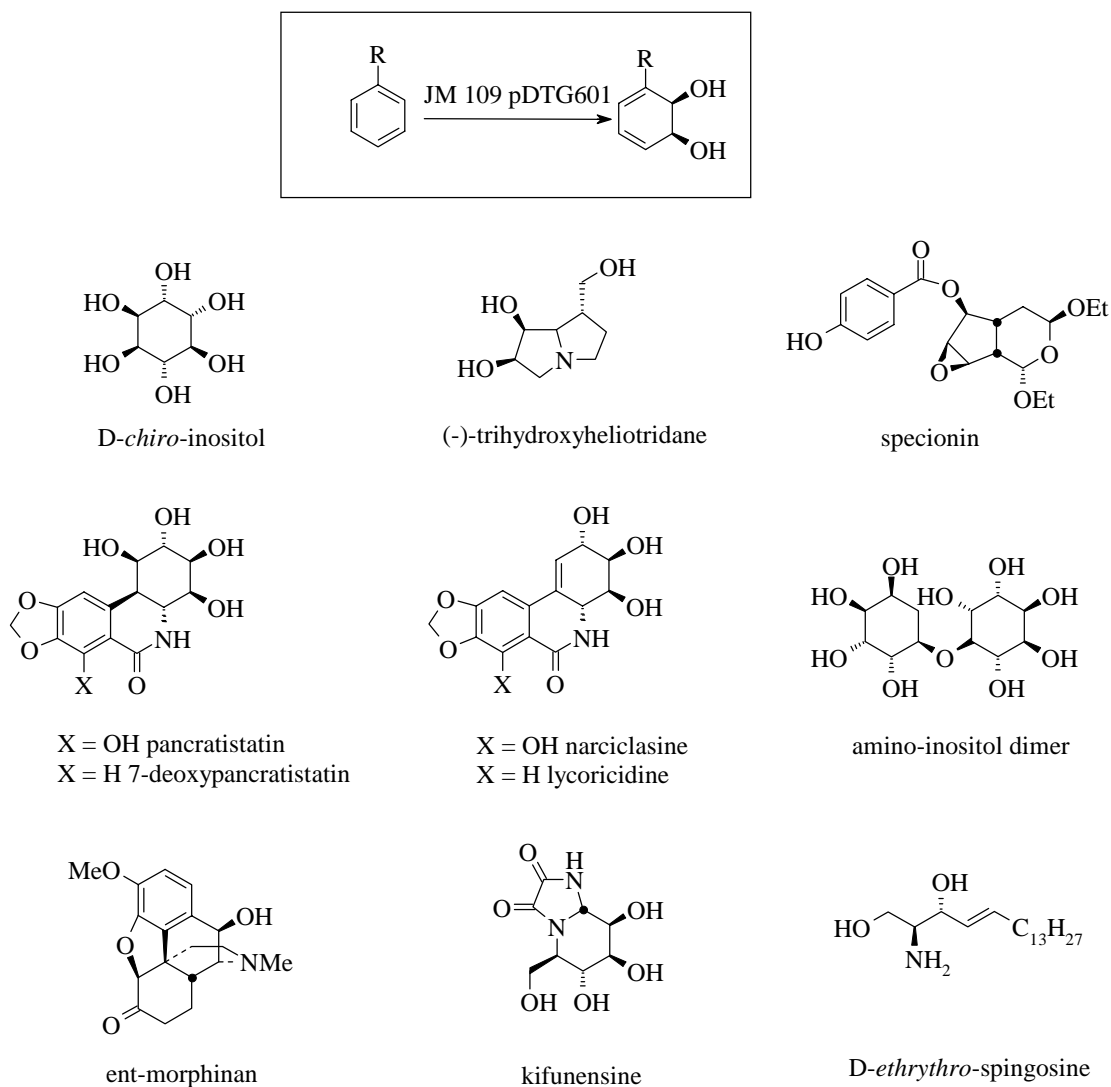


Figure 2. Examples of targets synthesized from arene-cis-diols.

1. Results and Discussion

To approach the synthesis of highly oxygenated targets such as pancratistatin and morphine, we designed a general strategy based on the recognition of common elements shared by the structures. Figure 3 shows morphine, pancratistatin, and narciclasine rendered as biphenyl core structures that have been oxygenated. One strategy that relies on aromatic dioxygenases starts with biphenyl and its oxygenation with an organism that expresses both TDO and DHDH, yielding directly a catechol. Exposure of such catechol to the clone expressing TDO provides the homochiral diol **7**, a core common to the targets. It is known that as the oxygen content of aromatic compounds increases the TDO processing to diols diminishes and the yields of diols of type **7** with two or more alkoxy groups would not be expected to be high. Therefore, a

convergent strategy has been implemented in which bromobenzene provides the starting carbon content for both bromocatechol **6** when subjected to the pDTG602 strain and diol **5** from fermentation with the pDTG601 strain. Simple procedures based on Suzuki coupling then provide **7**, which contains the oxygenation pattern of rings A and C of morphine. With the fundamentals of the general design in place we can turn to an overview of the total syntheses for both targets.

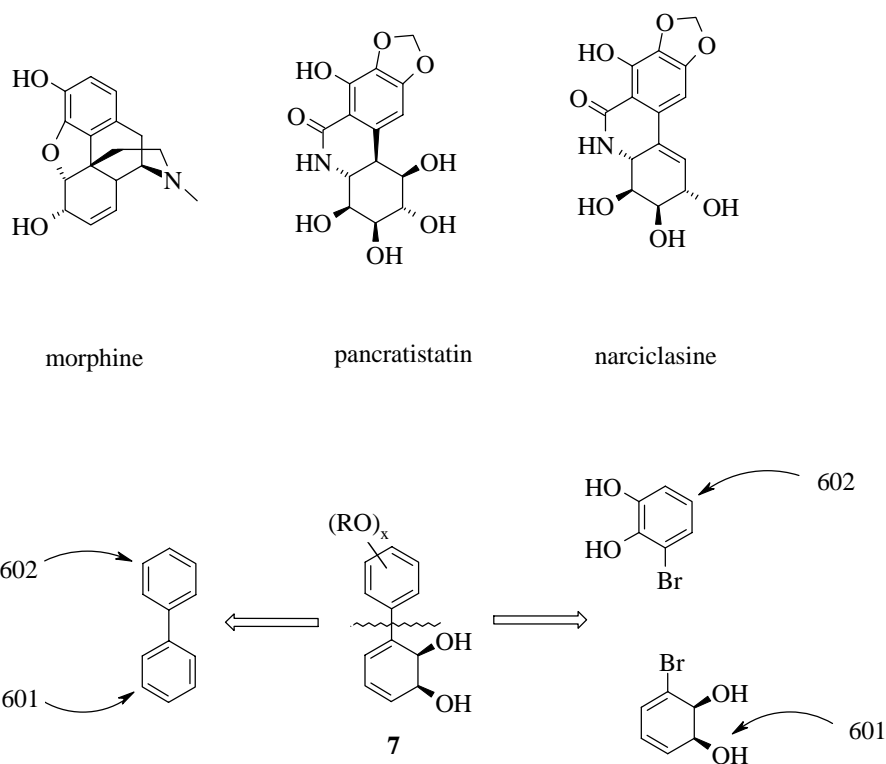
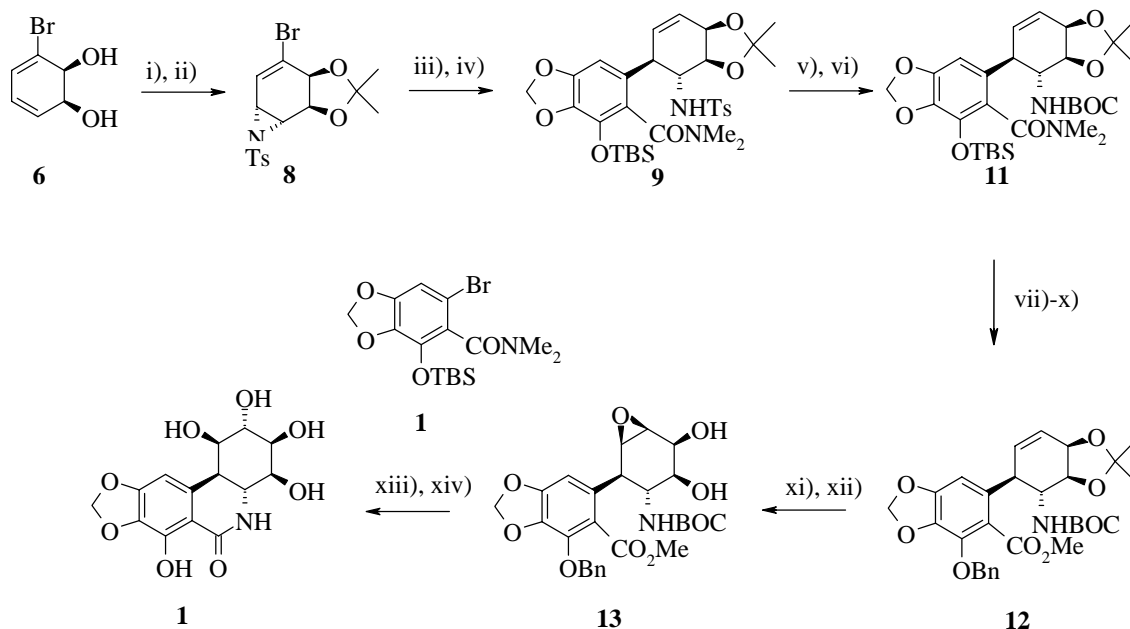


Figure 3. General strategy toward oxygenated alkaloids.

1.1 Pancratistatin and 7-deoxypancratistatin

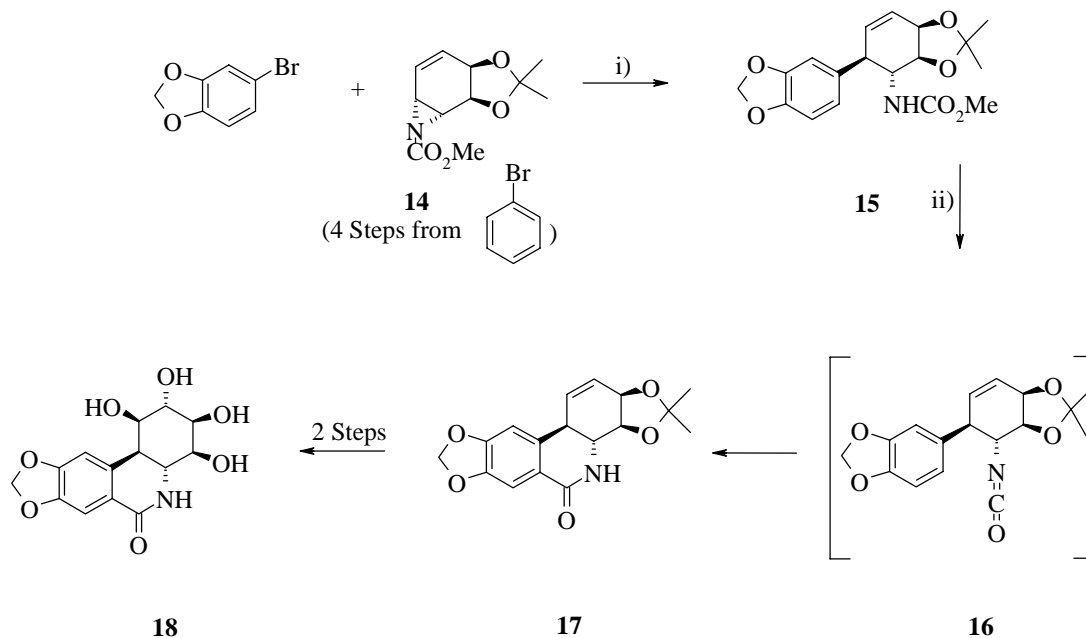
In 1995 we published the first enantioselective synthesis¹³ of this compound, previously prepared in racemic form by Danishefsky.¹⁴ The diol derived from bromobenzene was converted to aziridine **8** and combined with the functionalized benzamide **10** to yield, in four steps, the core of the target with four of its six centers correctly installed, Scheme 2.



Reagents and Conditions: i) DMP, *p*-TSA, (cat.), acetone, r.t.; ii) PhI=NTs, Cu(acac)₂, CH₂CN, r.t., 45% (over 2 steps); iii) Bu₃SnH, AIBN, THF, reflux, 78%; iv) **1**, *s*-BuLi, TMEDA, THF, CuCN, r.t., -78 °C; v) *s*-BuLi, THF, (BOC)₂O; vi) Na/anthracene, DME, -78 °C; vii) SMEAH/morpholine, THF, -45 °C; viii) BnBr, K₂CO₃, DMF; ix) NaClO₂, KH₂PO₄; x) CH₂N₂; xi) HOAc, THF, H₂O, 60 °C; xii) *t*-BuOOH, VO(acac)₂, C₆H₆, 60 °C; xiii) H₂O, BzONa (cat.), 100 °C; xiv) H₂, Pd(OH)₂/C, EtOAc.

Scheme 2. First enantioselective total synthesis of pancratistatin.

As we described in our 1995 communication, we did not anticipate the problems encountered upon attempts to provide the phenanthridone core directly from **9** by transamidation. The benzamide **9** existed as a mixture of atropisomers that were found to equilibrate toward the one in which the dimethylamide and tosylamide were 180° apart thus precluding cyclization. To circumvent this problem six steps were required to manipulate the functionality to the stage of ester **13**, which, upon deprotection cyclized to the amide and yielded pancratistatin in a total of 13 steps. In order to solve the problems encountered with robust amides, we changed the strategy of phenanthridone synthesis to cyclization of the isocyanate, as reported by Banwell.¹⁵ Thus 7-deoxypancratistatin was prepared from N-carbomethoxyaziridine¹⁴ via the in situ generated isocyanate which smoothly cyclized to phenanthridone **17**, Scheme 3.^{16,17}

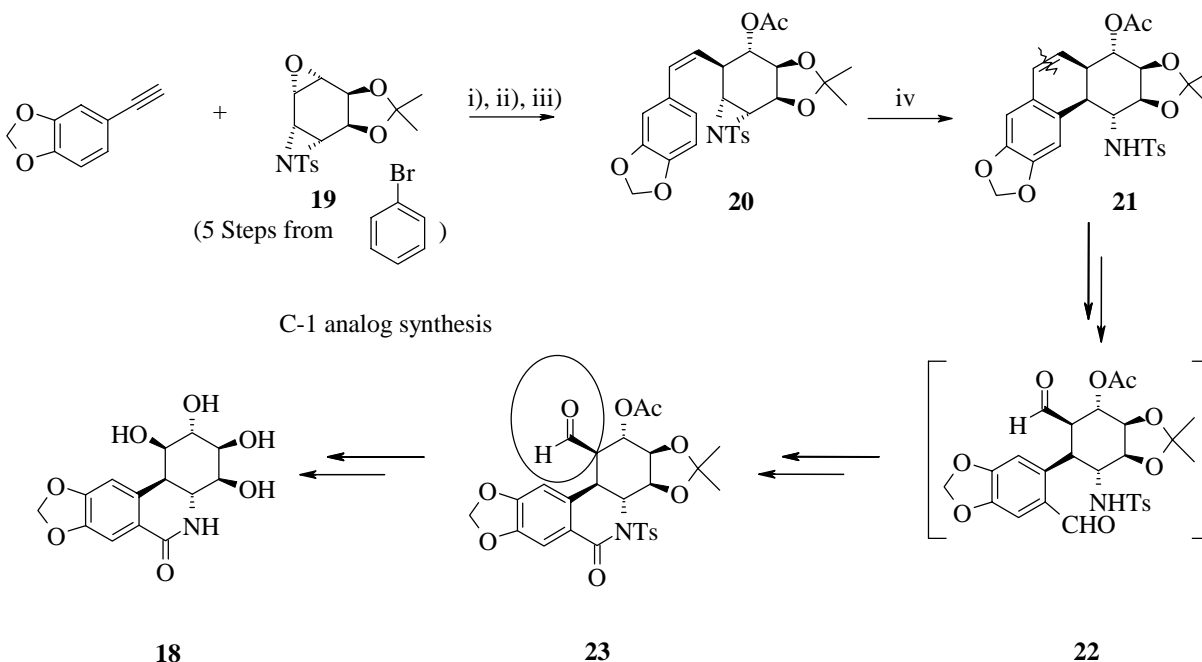


Reagents and Conditions: i) $\text{BF}_3 \cdot \text{Et}_2\text{O}$, THF, $-78\text{ }^\circ\text{C}$ to $-30\text{ }^\circ\text{C}$.; ii) Tf_2O , DMAP, CH_2Cl_2 , $5\text{ }^\circ\text{C}$

Scheme 3. 2nd-5th generation synthesis: 7-deoxypancratistatin

These improvements furnished the pancratistatin core in 8-9 steps and showed promise toward larger-scale preparations of these important compounds. Several other approaches have been developed; these have been reviewed recently.⁴

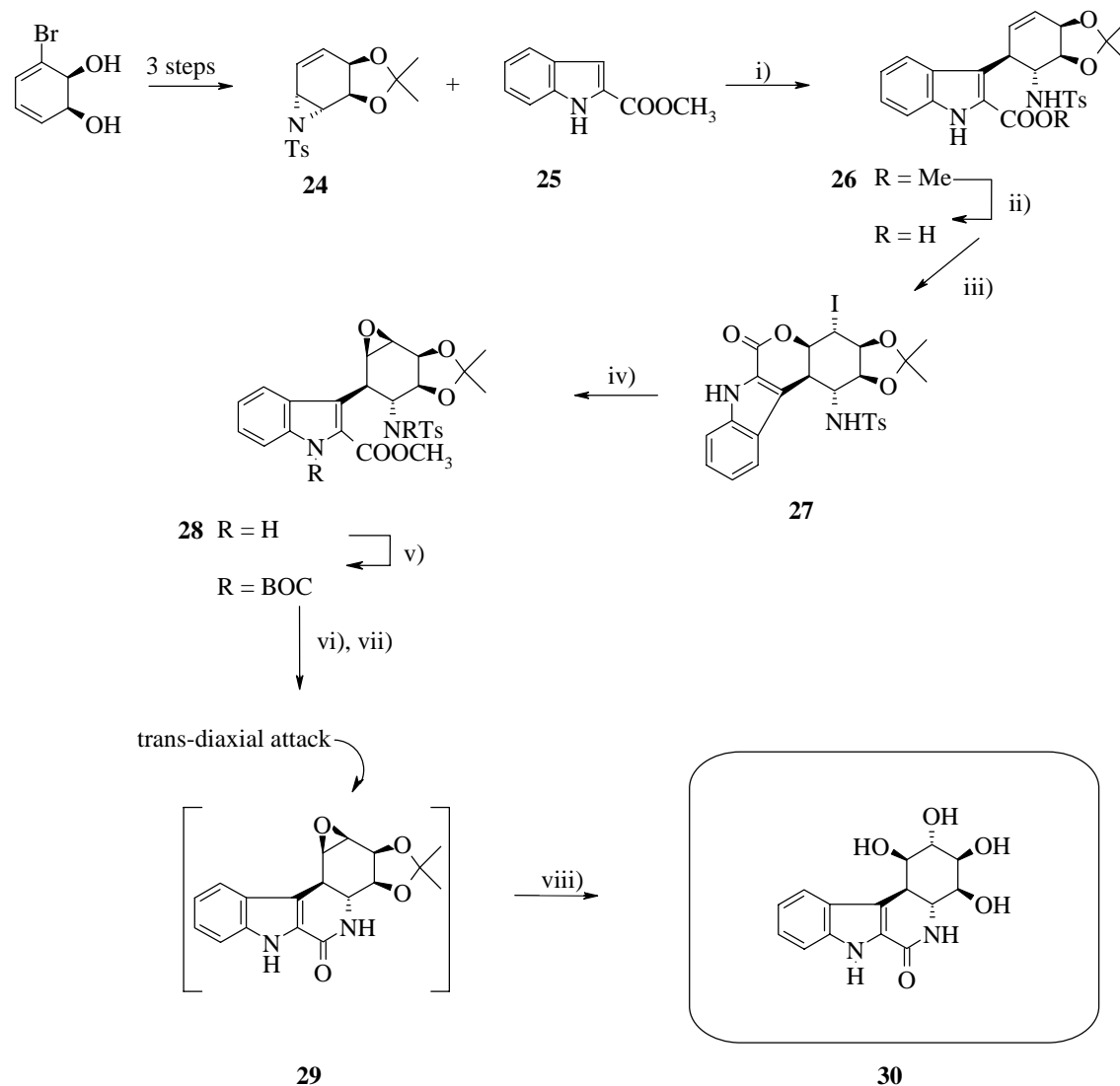
Two new strategies are currently being pursued. One approach involves the intramolecular opening of aziridine **20** derived from controlled opening of the epoxide in **19** with aluminum complex of piperonylacetylene as shown in Scheme 4. Cyclization, catalyzed by silica gel in solid phase, proceeds smoothly to **21**, which is subjected to oxidative cleavage to dialdehyde **22**. We anticipate that recyclization and conversion of **23** to 7-deoxypancratistatin will be accomplished soon.



Reagents and Conditions: i) *n*-BuLi, Me₂AlCl, Toluene, -78 °C; ii) Ac₂O, Pyridine, DMAP; iii) H₂ Lindlar catalyst, MeOH, 40 psi; iv) Silica gel, heat.

Scheme 4. Intramolecular aziridine opening approach to 7-deoxypancratistatin.

The silica-gel-mediated opening of aziridine **20** deserves brief mention. We found that aziridines and epoxides are opened by a variety of nucleophiles in solid phase and moderate temperatures. The synthesis of the indole mimic of pancratistatin, shown in Scheme 5, was accomplished by the condensation of indole derivative **25** with tosylaziridine **24** at 70°C when both reagents were adsorbed on flash silica surface and heated. Indole **26** was isolated in 68% yield and converted in a brief sequence to the indole mimic **30**, which was found to have mild antitumor activity against several cell lines.



Reagents and Conditions: i) silica gel, 70 °C, 68%; ii) LiOH / H₂O, 95%; iii) I₂ / NaHCO₃, THF, 71%; iv) LiOMe / MeOH, 85%; v) (BOC)₂O, 40 °C, 88%; vi) Na/naphthalene; vii) silica gel, 1 hr, H₂O, 170 °C, 67%; viii) silica gel, H₂O, 170 °C, 16 hrs, 31%.

Scheme 5. Synthesis of indole mimic of pancratistatin.

During the synthesis of **30**, silica-catalyzed deprotection of acetone and BOC groups in water was also observed.¹⁹ The preparation of **30** represents to date the most efficient method of synthesis for pancratistatin-like compounds containing the amininositol moiety. Nevertheless pancratistatin despite its potency, is not an ideal medicinal agent because of the poor bioavailability. In collaboration with Professor Pettit's group at Arizona State University we have embarked on a program to synthesize diverse unnatural derivatives of **2** and test all the compounds against common mammalian cell lines in search for an active agent with improved solubility. Many unnatural derivatives have been tested²⁰ and in order to increase the proof of

compounds we designed a library-type approach via cyclotrimerization of acetylenes as shown in Figure 4.

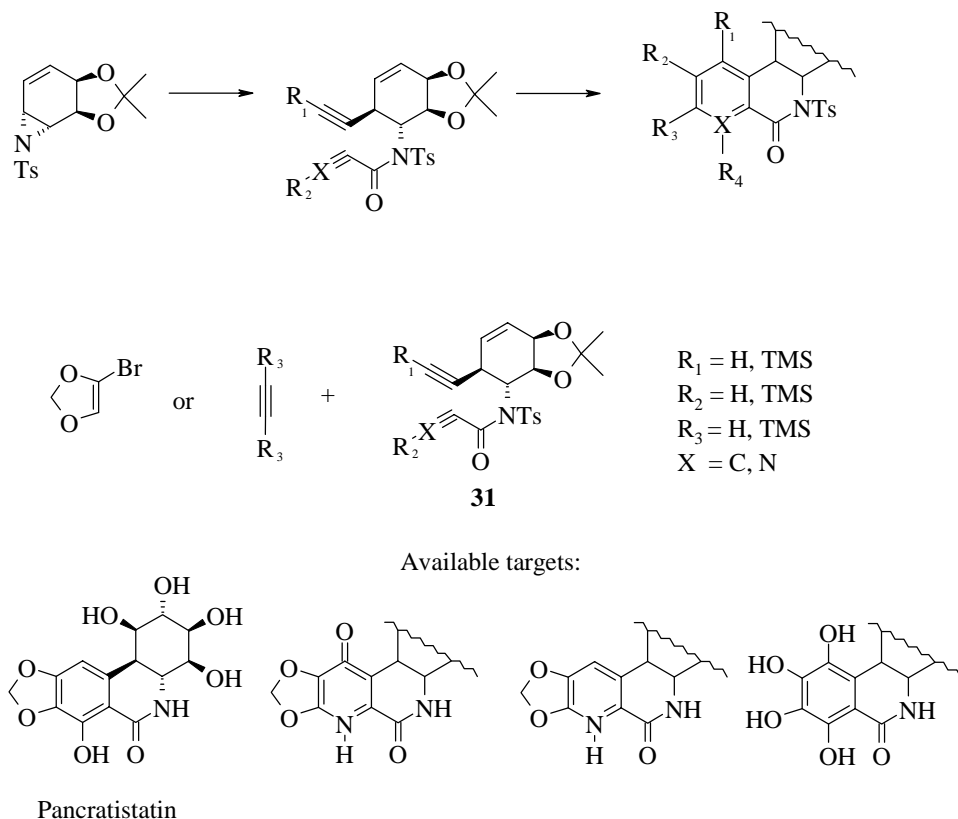
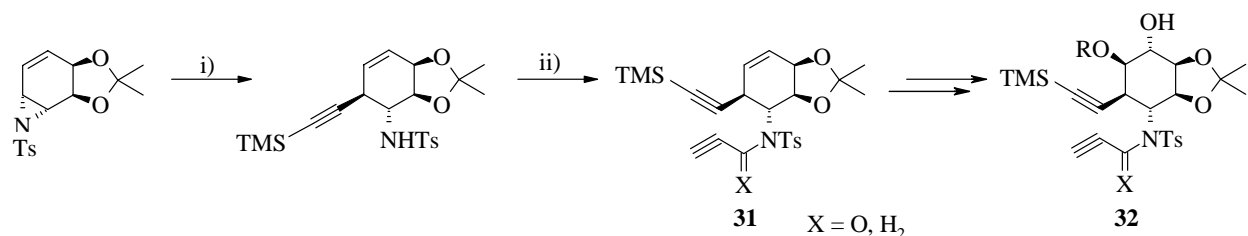


Figure 4. Seventh generation design for pancratistatin. A library approach?

Such strategy allows, in principle, the synthesis of carbocyclic and heterocyclic variants of **2** with structural and functional changes in the aromatic core, a region of the molecule that has not yet been investigated in serious SAR studies. The preparations of scaffolds for cyclization is shown in Scheme 6.

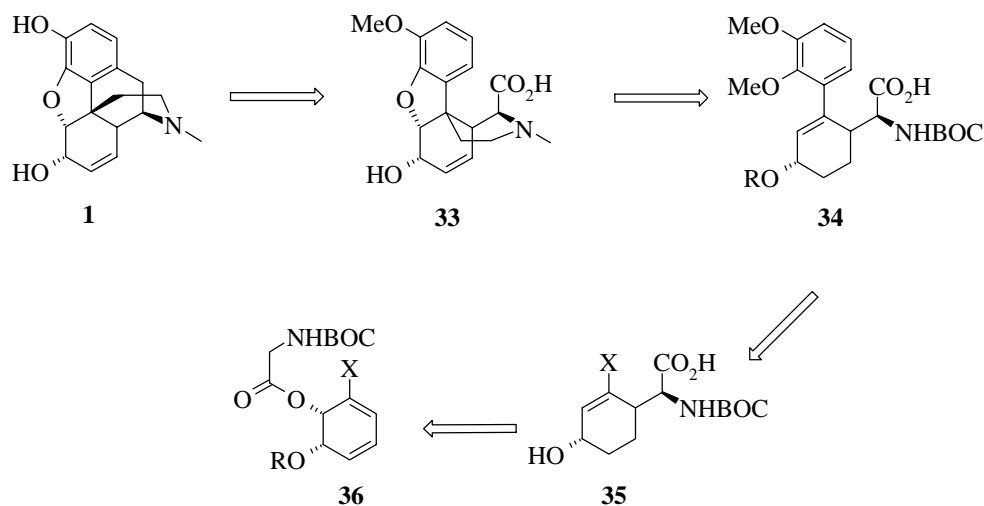


Reaction Conditions: i) TMS-acetylene, *n*-BuLi, AlCl₃, toluene, 0 °C to rt then DMP, *p*-TSA, acetone, rt, 67%; ii) NaHDMS, propionic acid anhydride, 54%, or NaHDMS, propargyl bromide, BuNI, 94%.

Scheme 6. Synthesis of scaffolds for cyclotrimerization.

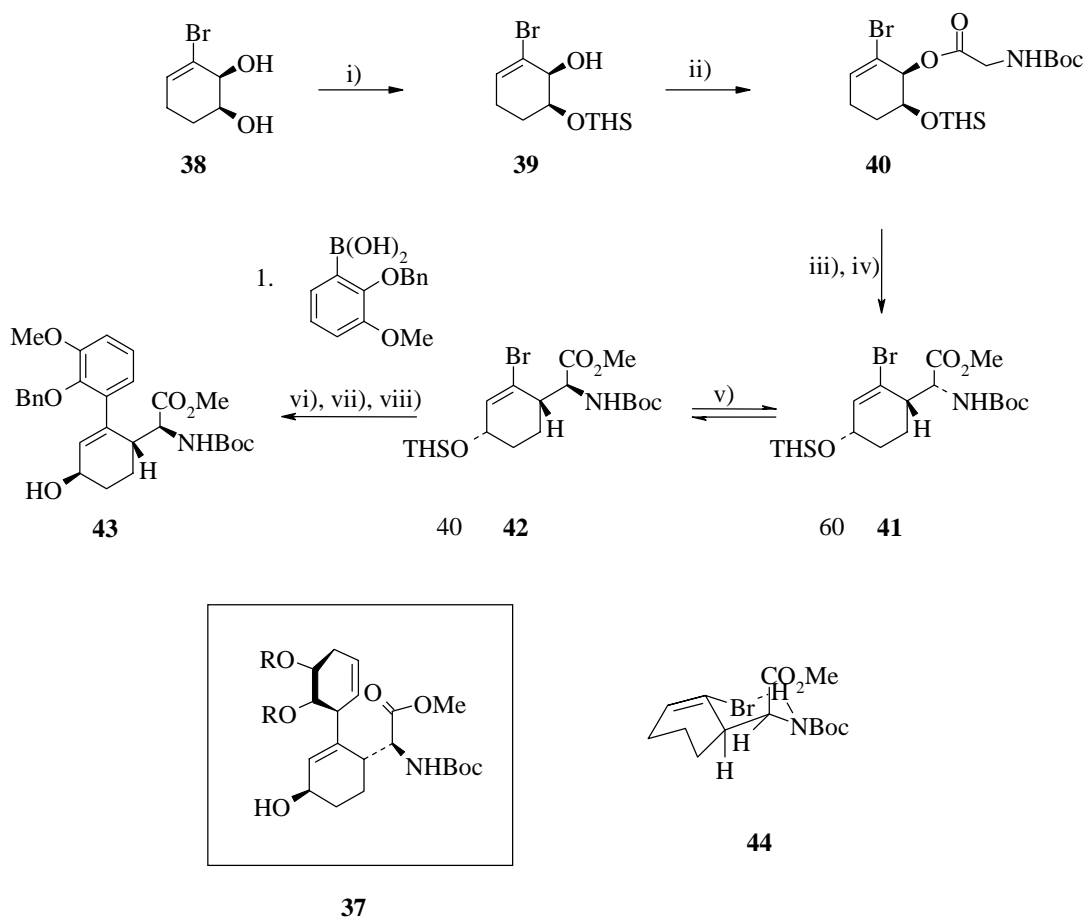
Preliminary experiments in cyclotrimerizations of scaffolds **31** and **32** provided the core of pancratistatin substituted with trimethylsilyl groups and further functionalization to the fully oxygenated natural products is in progress.

Morphine. Several approaches to this alkaloid have been pursued in the groups over the last 15 years and have been published²¹⁻²⁶ and reviewed.¹⁻³ In 1997 we reported an approach to morphine based on the recognition that the biphenyl-like compound **33** could be derived from the aminoacid **34** by combination of Suzuki coupling and Kazmaier-Claisen rearrangement of glycinate ester **36** to acid **35** as shown in Scheme 7.



Scheme 7. Kazmaier-Claisen approach to morphine.

Preliminary results of the successful synthesis of axial acid **34** have been reported.²⁷ Second generation improvements yielded a medium-scale synthesis (~100 g) of intermediate **37** with correct stereochemistry at C-9 and C-14, Scheme 8.



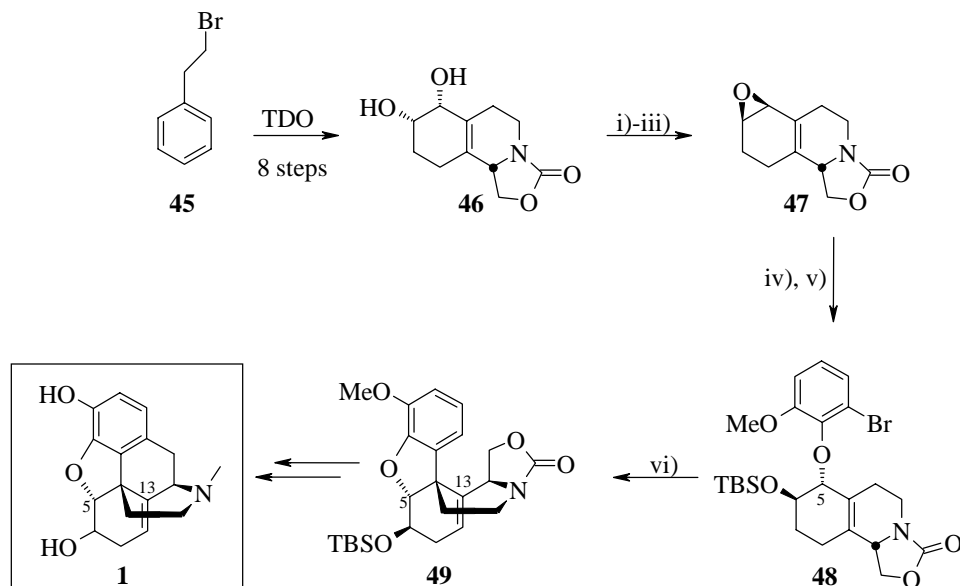
Reagents: i) THS-Cl, imidazole, DMF, -8 °C; ii) Gly -Boc, DCC, CH₂Cl₂; iii) 2 eq LDA, 1.2 eq ZnCl₂; iv) CH₂N₂; v) DBU; vi) boronic acid; vii) TBAF; viii) Mitsunobu

Scheme 8. Second generation synthesis.

The glycinate **40** provides a 40:60 mixture of diastereomeric acids, which can be epimerized upon conversion to their esters to **42**, presumably because of a hydrogen-bonded species **44** in which the ester occupies an axial position in major product **41** and is therefore subject to equilibration to the more stable equatorial configuration found in **42**. Experiments are underway to perform intramolecular radical cyclizations from a free phenol derived from **37** in order to establish the C-13 quaternary center and eventually close the phenanthrene core by Friedel-Crafts acylation.

Another approach to morphine has been designed based on the intramolecular Heck cyclization, β -bromoethylbenzene **45** is converted to the corresponding arene cis-diol and transformed to isoquinoline derivative **46** as shown in Scheme 9. Generation of epoxide **47** and its opening to **48** provides the key precursor for the intramolecular Heck cyclization to **49** in which all stereogenic centers of morphine except C-9 have been established. The oxidation state in **49** is that of neopine-type alkaloids and will provide codeinone upon C-10/C-11 closure and

oxidation of C-6 alcohol followed by isomerization of the olefin. Compounds of the type **49** have been previously converted to morphinans in the *ent* series and therefore adequate precedent exists for the completion of the total synthesis by this route.



Reaction conditions: i) TsCl/Py, ii) BzOH/nBu₃P/DEAD, iii) MeONa/MeOH/THF, iv) potassium 2-bromo-6-methoxyphenoxide/DME/18-crown-6; v) TBSOTf/(iPr)₂NEt/CH₂Cl₂; vi) Pd(PPh₃)₄/Proton Sponge/PhCh₃/D.

Scheme 9. Intramolecular Heck cyclization approach to morphine.

2. Conclusions

We have demonstrated that incorporation of biocatalysis into traditional synthetic protocols greatly increases the brevity synthesis of medicinally important agents. Furthermore, mutigenerational approaches to the targets discussed in this lecture frequently yield shorter and more efficient routes. Further progress will be reported in due course.

Acknowledgements

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