Biocatalytic approaches have yielded efficient total syntheses of the major Amaryllidaceae alkaloids, all based on the key enzymatic dioxygenation of suitable aromatic precursors. This paper discusses the logic of general synthetic design for lycoricidine, narciclasine, pancratistatin, and 7-deoxypancratistatin. Experimental details are provided for the recently accomplished syntheses of narciclasine, ent-7-deoxypancratistatin, and 10b-epi-deoxypancratistatin via a new and selective opening of a cyclic sulfate over aziridines followed by aza-Payne rearrangement. The structural core of 7-deoxypancratistatin has also been degraded to a series of intermediates in which the amino inositol unit is cleaved and deoxygenated in a homologous fashion. These truncated derivatives and the compounds from the synthesis of the unnatural derivatives have been tested against six important human cancer cell lines in an effort to further develop the understanding of the mode of action for the most active congener in this group, pancratistatin. The results of the biological activity testing as well as experimental, spectral, and analytical data are provided in this manuscript for all relevant compounds.

67 Total Synthesis and Biological Evaluation of Amaryllidaceae Alkaloids: Narciclasine, ent-7-Deoxypancratistatin, Regioisomer of 7-Deoxypancratistatin, 10b-epi-Deoxypancratistatin, and Truncated Derivatives

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References

(1) Preliminary disclosures for narciclasine, see ref 9l; for 7-deoxypancratistatin, see ref 10n, and for epi-7-deoxypancratistatin, see ref 50.
(2) Lycorine (1), the first alkaloid of this group to be isolated,4 was studied for its antitumor properties long before more oxygenated congeners were identified.5 Over the past two decades, lycoricidine (2), narciclasine (3), pancratistatin (5), and 7-deoxypancratistatin (4) have been screened for antitumor activity,7 and synthesized8-10 by a number of research groups. The history of the Amaryllidaceae alkaloids, their structure elucidation, and their biological profiles, as well as their syntheses, have been summarized on several occasions.3 These alkaloids are available only in minute quantities from natural sources,8 and their future as therapeutic agents depends on their availability. Because isolation in larger quantity is not
practical, there is a strong case for development of syntheses or semisyntheses of these alkaloids, their derivatives, and potential prodrugs.11

Some of these alkaloids also display antglycosidic (and hence antiviral) activity because of the similarity of their oxygenation pattern to that of natural sugars.12 This hence antiviral) activity because of the similarity of their oxygenation pattern to that of natural sugars. This additional biological spectrum of activities provides for even stronger justification of synthetic effort toward these alkaloids. The synthesis of Amaryllidaceae alkaloids is pursued by 10 or more research groups, and at least three of these groups have made substantial multigenerational improvement of synthetic protocols toward these compounds. This paper describes the details of our most recent syntheses of narciclasine (8 operations), ent-7-deoxypancratistatin (12 operations), and epi-7-deoxypancratistatin (12 operations) by different approaches. All are connected by a common motif: each synthesis begins with the biooxidation of an aromatic compound. Some truncated derivatives of 7-deoxypancratistatin have been prepared for biological evaluation, and the results are reported herein.

**Synthetic Strategy.** We have proposed that 15 steps be the limit for a practical synthesis for any desired compound.13 Arguments for acceptance of this limit are offered by basic algebraic considerations of "assumed" yields of 90% in each step—an optimistic projection at best. The success of inventing a short synthesis of the oxygenated phenanthridone nucleus is likely to be hampered by the need of protective and deprotective operations required to preserve the integrity of the oxygenated ring, which can be visualized as a C-substituted aminoisnitol.

The best strategy for an efficient synthesis of any of these alkaloids is to attach the aryl fragment to an electrophilic synthon that already contains most of the oxygenated centers. Because enantiomeric alkaloids may or may not be active, such a strategy should also accommodate the preparation of both enantiomers. Symmetry arguments similar to those already applied to the synthesis of inositols14–16 indicate that the enantiomeric series of pancratistatin is related by the "switch of the
trans-disposed functionalities" as indicated in Scheme 1. If one considers that the trans diol and the \( \beta \)-arylamine are interchangeable across the enantiotropic plane\(^{15a} \) shown, then a common strategy can be devised for both enantiomers by two identical routes from a single enantiomer of the material which contains the cis-diol unit "protected" from symmetrization by the presence of a removable group.

Such a strategy is implemented by performing two sequences of identical functionalizations in a different order, as shown in Scheme 1. Successful applications of this kind of strategy to sugar and inositol syntheses have been reported.\(^{13,15} \) The creation of either an electrophilic aziridine or an electrophilic oxirane at the more electron-rich olefin allows the rest of the synthesis to be completed by a series of identical chemical steps, executed in different order, as demonstrated for the first time in our enantiodivergent synthesis of \((-\) - and \((+)\) -pinitol.\(^{15a} \) Several reviews offer an expanded version of this argument.\(^{13,17} \)

Other solutions for the enantiodivergent synthesis of arene cis-diols have been reported. Boyd's strategy\(^{18} \) is based on the directing effects in the enzymatic oxidation and the greater rate of reduction of the directing group (iodine) by chemical means (\( \text{R}_2\text{SnH} \)). Because toluene dioxygenase-mediated oxidation of para-substituted dihalobenzenes produces mixtures of poor enantiomeric enrichment, Johnson's\(^{19} \) [ipas] resolution of intermediates derived from such mixtures must be used to enrich the optical purity of the desired ent-diols. We have used both strategies in the synthesis of ent-7-deoxypencratistatin.

**Scheme 1**

![Scheme 1](image)

Discussion

**Narciclasine.** Two possible disconnections were considered, both leading to amino enone 14, via a nitroso Diels–Alder reaction, Scheme 2. On the basis of our experience in the synthesis of lycoridine and various conduramines, we anticipated that the conduramine unit of narciclasine (3) would be formed by a regio- and stereospecific nitroso Diels–Alder addition to the diol derived from 1,3-dibromobenzene (7).

\(-\)Vanillin (11) serves to furnish the aromatic fragment of narciclasine in the form of borate 10,\(^{20,21} \) and 1,3-dibromobenzene (6) provides the asymmetric portion of the molecule by means of toluene dioxygenase oxidation to the corresponding cis-cyclohexadiene diol 7. The two bromine atoms are located in different proenantiotropic spaces by virtue of the particular symmetry present in the cis-cyclohexadiene diols, which we have exploited extensively in several preparations.\(^{15a,17a,21,22} \)

1,3-Dibromobenzene was subjected to whole-cell fermentation with E. coli M109 (pDTG601A), an organism developed by Gibson\(^{23} \) for the overexpression of toluene dioxygenase (TDO). Biooxidation yielded the new metabolite 7 (4 g/L, >99% ee), a compound that possesses unique latent symmetry and two chemically different vinylic bromine atoms. Diol 7 was transformed in a one-pot operation to bicyclic oxazine 9 in 70% yield (Scheme 3). The acetonide is prepared in neat 2,2-dimethoxypropane (DMP), which is also a suitable solvent for the Diels–Alder cycloaddition. Thus, after verification of complete conversion of diol 7 into acetonide 8, the periodate and the hydroxamic acid were added. In this way, we were able to shorten the preparation and avoid isolating acetonide 8 which tends to dimerize in its pure state.\(^{24} \)

Oxazines such as 9 were formed according to ample precedents for the reactions of cis-cyclohexadienediols with nitroso dienes.\(^{25,26} \) Our synthesis of cyclitol\(^{25,27} \) and the alkaloid lycoridine were based on these reactions.\(^{30,34} \) Reduction of 9 under Kek's conditions\(^{28} \) yielded the conduramine oxidation state as previously reported,\(^{29a} \) but gave predominantly the fully dehalogenated conduramine derivative \((-\) -15. This result, although unfavorable for the narciclasine synthesis, pro-


provided conclusive proof of the absolute stereochemistry of diol 7. Conduramine derivative (−)-15 was independently prepared from bromodiol (+)-16, whose absolute stereochemistry is well established (Scheme 3). This structure proof also confirmed the assumption that the polarized halodiene would undergo regiospecific nitroso Diels–Alder reaction.

We studied the reduction of the oxazine in some detail. Our initial plan was to follow the previous reports by Keck et al. by opening the oxazine bridge by reduction with aluminum amalgam in order to obtain a brominated amino conduritol derivative such as 17. However, we found the same type of overreduction problems that we had observed in the synthesis of lycoricidine. The vinylic bromine on C10a (narciclasine numbering) was reduced under these conditions, and we isolated the fully debrominated conduramine derivative (−)-15 and the desired 17 in a 99:1 ratio (HPLC).

Both tributyltin hydride and tris-trimethylsilylsilane (TTMSS), normally suited for reduction of oxazine 9 to unsaturated ketone 19 cannot be applied here as dehalogenation is unavoidable under such conditions. However, Mo(CO)₆ cleanly reduced dibrominated oxazine 9 to the corresponding bromo enone 19 with concomitant cleavage of the acetonide protecting group (Scheme 4). Because the mechanism of the cleavage with Mo(CO)₆ does not involve radical formation but is rather a metal insertion process, it can be performed successfully in the presence of vinylic halides.

We explored directed hydride reduction by means of Zn(BH₄)₂\(^{30,31}\) or NaB(ACO)₃H.\(^{32}\) Because of the strong chelating properties of the zinc cation, the reagent has been used to attain anti selectivity in the reduction of acyclic \(\alpha\)-hydroxy ketones\(^{33}\) and has been also exploited for the reduction of \(\beta\)-hydroxy ketones with high selectivity.\(^{32}\) However, in our hands only a disappointing 20% diastereomeric excess has been observed in these reductions (Scheme 4).

To circumvent the problem of overreduction\(^{34}\) of the bromine atom at C10a, we decided to couple the aromatic portion of the alkaloid (borate 10) directly to oxazine 9 and postpone the bridge opening to a later stage in the synthesis. The coupling was performed under the standard Suzuki–Miyaura conditions\(^{34}\) and proceeded only in fair yield (30%). Oxazine 13 was isolated along with 10–15% of ketone 14 (Scheme 5) and 20–25% of substituted biphenyls formed by homocoupling. Enone 14 was formed also through a palladium insertion mechanism similar to the regular cleavage of the nitrogen–oxygen bond in oxazine 9 by Mo(CO)₆ as discussed above. To the best of our knowledge, this is the first example of Suzuki coupling of a halo-oxazine and a phenyl borate.

Because 13 was resistant to aluminum amalgam reduction under Keck’s conditions, and stronger reducing agents (sodium amalgam or H₂/Pd) led to fully saturated products we transformed 13 into unsaturated ketone 14 instead with tris-trimethylsilyl silane (TTMSS). Further improvement was obtained by adding acetonitrile and Mo(CO)₆ directly to the Suzuki reaction mixture after the coupling of 9 and 10 was completed. Heating this mixture for 12 h afforded ketone 14 in 45% yield. In this fashion we were able to optimize a preparation of the advanced intermediate 14 in only three operations from 1,3-dibromo benzene (Scheme 5).

To set the stereochemistry at C2 (narciclasine numbering), we applied a Luche reduction followed by Mitsunobu inversion as reported by Chida in his preparation of lycoricidine. This procedure gave the desired \(\alpha\)-benzoate 23 cleanly in 60% yield from ketone 14 (Scheme 6).

A modification of the Bischler–Napieralski reaction reported by Banwell\(^{29}\) and applied with success in simplified models of phenanthridone alkaloids\(^{38}\) was chosen for the last steps of the synthesis. This interesting variation uses a 5:3 mixture of trifluoromethanesulfonic anhydride and DMAP instead of POCl₃ to attain cyclization. The reaction has been applied successfully to sensitive molecules not only by Banwell\(^{30}\) but also by us in the preparation of both enantiomers of 7-deoxypancratistatin, as described below and as previously reported.\(^{10b,e}\) The acetonide protecting group in 23 was removed by an acidic resin in methanol. This method is convenient because the diols are generally very soluble in methanol and simple filtration of the resin yields a solution of essentially pure product which was treated with acetic anhydride and pyridine. The resulting diacetate 24 was obtained in 90% yield over the two steps (performed as a single operation).

Compound 24 was subjected to Banwell’s conditions and afforded phenanthridone 25 in 40% yield. The particular ratio of Tf₂O and DMAP (5:3) was empirically determined by Banwell.\(^{35}\) With an equimolar mixture, no cyclization is observed. The application of this reaction to other substrates (including acetonides 22 and 23 and ketone 14) afforded only phenolic material. Although the closure could result in two isomers (phenanthridones 25 and 26), we never observed the formation of the latter product. A different result was obtained by Magnus in his synthesis of pancratistatin where a 3:1 ratio of isomers was detected in a related cyclization.\(^{10b}\)

The esters in 25 were removed with a basic Amberlyst resin in methanol. The reaction worked efficiently to form a polar fluorescent solid whose \(^1\)H NMR spectrum and optical rotation ([\(\alpha\)]\(^{26}\)_D = +204 (c 0.3, DMSO)) matched those of the compound prepared by methylation of natural narciclasine using Piozzi’s procedure\(^{36}\) (diazomethane in ethanol, 5 days, 50%) ([\(\alpha\)]\(^{26}\)_D = +219 (c 1.0, DMSO)).


Cleavage of the methyl ether on the C7–OH of 27 proved to be problematic. After several trials under the conditions reported by Trost and Pulley (LiI, DMF, 80 °C, several hours) for the deprotection of 7-O-methylpancratistatin,10c we observed only degradation products. An improved and updated procedure37 performed on 1.0 (0.1 mg of 7-O-methyl narciclasine (27)) afforded a polar compound with strong yellow-green fluorescence. Purification of this material afforded 0.3 (0.1 mg of a compound that showed an identical 1H NMR spectrum and a matching optical rotation with the literature data for narciclasine ([α]23D +130 (c 0.03, DMSO), lit.9+j +141.8). The TOCSY spectrum was fully consistent with structure 3.

The total synthesis of narciclasine was completed from 1,3-dibromobenzene in 12 steps (14 from o-vanillin) and only eight individual operations. This was the second synthesis of narciclasine to be published,1 and it is 11 steps shorter than the first preparation.9j Keck has reported the completion of the third total synthesis of this alkaloid in 12 steps.9k

ent-7-Deoxypancratistatin. In a preliminary communication,10m we reported a 12-step synthesis of this compound prepared for biological evaluation. The only other Amaryllidaceae alkaloid prepared in the ent-series is ent-lycoricidine, reported by Keck.9k For our approach to the ent-alkaloid, we chose the corresponding ent-conduramine A, which would be manipulated to the required vinylaziridine by the recently published protocol of Olivo,38 reporting an improved route to aziridine synthons of this type. The Mitsunobu protocol is greatly superior to the previously used aziridination by the Evans–Jacobsen–Yamada method,39 which we used during all of our previous syntheses of pancratistatin and 7-deoxypancratistatin.

Recent studies by Boyd18 have shown that the iodine atom present in dihalogenated cis-diols such as 29 (obtained by biooxidation with toluene dioxygenase expressed in the blocked mutant P. putida UV4) can be selectively removed by catalytic hydrogenolysis (H2, Pd/C), a procedure which leads to a mixture of (2S,3S) and (2R,3R) enantiomers of bromodiol 16. Boyd used a second fermentation step with a nonblocked strain of Pseudomonas (P. putida NCIB 8819) to metabolize the “normal” (2S,3S) isomer to increase its optical purity. Boyd’s method provides a route to (2R,3R) enantiomers of monosubstituted cis-dihydrodiols; however, the valuable (2S,3S) isomer is destroyed in this procedure. As we were interested in both enantiomers of amino alcohol 15, we developed an alternative route that would provide both enantiomers in high enantiomeric excess. In addition since the hydrogenolysis of 29 did not provide good results in our hands, the iodine atom was instead removed with Bu3SnH /AIBN21 to yield (−)-16 (55%, 20% ee).

(37) After a personal communication with Dr. Pulley, we obtained the correct procedure that used LiCl in DMF at 120 °C for 2 h, not LiI as reported in ref 10c.


Enantiomerically impure diol (−)-16 was converted to amino alcohol 15 via oxazine 30 as described in Scheme 7. Alcohol 15 was acetylated with acetic anhydride and pyridine to give 31. To obtain the optically pure enantiomers, a method similar to that employed by Johnson was used. Under carefully controlled conditions, crude porcine pancreatic lipase (PPL, Sigma type II crude) catalyzed the hydrolysis of optically impure 31 to afford (+)-amino alcohol (+)-15 and acetate 33 with high enantiomeric purity (99% ee) for a 35–45% conversion of 31. The optical purity of (+)-15 was determined by comparing the optical rotation value ([R]26D) + 29.1 (c 1.0, CHCl3) to the corresponding value of the enantiomer (−)-15 prepared from the “natural” bromocylohexadiene cis-diol: [R]25D = −30.1 (c 1.1, CHCl3).25a (see Scheme 7).

After the conversion of (+)-15 to the corresponding ent-aziridine (+)-32, the synthesis of ent-7-deoxypancratistatin was completed exactly as previously published for the natural isomer (Scheme 8), and the compound was submitted for biological evaluation (see the section on biological activity for a discussion of results).

Regioisomer of 7-Deoxypancratistatin. This alkaloid has served a number of investigators as a somewhat easier model on which to base approaches to the more complex pancratistatin, as the presence of the phenolic hydroxyl in pancratistatin makes for a more difficult synthesis.

After the first and second generation syntheses of the title compound, we turned to a completely new strategy inspired by two reports in the literature. The first was that of Gauthier and Bender, who reported successful intramolecular opening of an epoxide in 41 with a transmetalated arene as shown below. Their ultimate plan called for benzylc oxidation and recyclization of the “Danishefsky lactone” intermediate following the installation of an amine at C4a. However, the oxidation was not reported in the original disclosure, nor has a subsequent report appeared. The second report involved the intramolecular opening of aziridines, first reported by the Rapoport group with studies continued by Bergmeier.

In a model study directed at the synthesis of ent-7-deoxypancratistatin by a strategy similar to Bender’s, we were also able to cyclize aryl bromide 43 onto the

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aziridine with t-BuLi/Et₂O to produce conduramine 44, which possesses the ent-configuration required for the alkaloid. 44 However, extension of this model study to the piperonyl derivative of 43 was not successful.

With these precedents, we formulated the strategy outlined in Scheme 9. Protection of the diol in 16 as the acetonide followed by aziridination under Evans's and Jacobsen's protocol 36 generated the tosyl aziridine 45 in 63% yield. Dehalogenation of vinyl bromide 45 under radical conditions followed by epoxidation at elevated temperature produced an inseparable mixture of epoxides 47 (α:β = 2.6:1). 45 Because of the redundant outcome [for definitions of redundant operations see ref 13] of the nucleophilic opening of epoxide 47, we assumed that either trans diol 48 or trans piperonyl ethers 49 or 50 would be obtained from the isomeric mixture of epoxides 47 upon selective trans-diaxial opening of the oxirane with oxygen nucleophiles. Either transmetalation (50) or acid-catalyzed treatment (49) would then be used to open the aziridine.

As reported in a recent publication, 46a the outcome of this approach led to the synthesis of an isomer of 7-deoxypancratistatin, 53, via the initial opening of the aziridine with the oxygen nucleophile and the subsequent acid-catalyzed cyclization of epoxy ether 52.

**Scheme 9**

The inability to open the oxirane selectively in 47 ultimately led to our investigation of the selectivity of nucleophilic opening of cyclic sulfates or sulfites 47 over aziridines contained in the same molecule. Such investigations have not been reported in the literature, to our knowledge. To this end, the cyclic sulfate 55 was prepared as shown in Scheme 10. The tosyl aziridine 46 was synthesized as previously reported. 46 Dihydroxylation of 46 provided cis diol 54 in 85% yield. This material was converted to the cyclic sulfate 55 in 93% yield with sulfonyl chloride in CH₂Cl₂.

We were pleased to find that ammonium salts of several benzoic acid derivatives cleanly differentiated between the aziridine and the cyclic sulfate and led chemoselectively to the trans-disposed ester-alcohols 56a-c in 60−90% yield. 46 In contrast, when epoxides 47, sulfate 55 and the corresponding sulfite (prepared by reaction of diol 54 with thiyl chloride in CH₂Cl₂) were allowed to react with sodium or potassium salts of piperonol, decomposition or opening of the aziridine rather than the epoxide was observed. Only ammonium salts of carboxylic acids were found to differentiate clearly between these functional groups. Several benzoate derivatives were prepared by this method, namely piperonyl and o-bromopiperonyl esters, to study the possibility of intramolecular opening of the aziridine ring by either a

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46) (a) Schilling, S.; Rinner, U.; Chan, C.; Ghiviriga, I.; Hudlicky, T. Can J. Chem. 2001, 79, 1659. (b) We have reported the synthesis of 7-deoxypancratistatin by the intramolecular aziridine opening at many occasions at conferences. The proceedings of the Heterocyclic Congress in Vienna, August 1999, were published before the erroneous assignment was observed: Hudlicky, T. J. Heterocycl. Chem., 2000, 37, 535. The incorrectly assigned structure as well as reasons for not detecting this error has been reported as a full paper (ref 46a). The major lesson learned from these endeavor was the recognition that structure assignment of highly similar compounds is very difficult by NMR techniques alone.
Lewis acid-mediated process or by an organometallic species derived from 56c. Unfortunately all attempts to close the ring failed.

Therefore, we decided to return to our original idea of intramolecular aziridine opening in an ether such as 49. When ester 57 was treated with sodium methoxide in THF, migration of the TBS group took place and alcohols 59 and 60 were obtained, as shown in Scheme 11. We found that increasing the reaction time of the ester cleavage also increased the percentage of alcohol 60 in the reaction mixture. Eventually, we succeeded in preparing the free alcohol 59 without observing silyl migration when ester 57 was treated with excess sodium methoxide for a reaction time of less than 1 min.

Any attempts to alkylate the hydroxyl function in 59 to produce ether 65 failed, but instead gave the epoxide 64 via an aza-Payne rearrangement as shown in Scheme 11. Even deprotonation of the alcohol using tert-butyllithium at −30 °C followed by quenching of the alkoxide with excess piperidyl bromide only yielded compound 64. Interestingly, alklylation of alcohol 60 by means of the same procedure also afforded epoxy amide 64, which indicates that the reaction sequence involves silyl migration followed by an aza-Payne rearrangement yielding intermediate 63, which is subsequently alkylated by piperidyl bromide.

Epoxy amide 64 smoothly cyclized to 66 with Me2AlCl in CH2Cl2 in good yield (68%). Prior to RuCl3/NaIO4 oxidation of the benzylic position, the free hydroxyl group of alcohol 66 was protected as a methoxy methyl ether (67). Cleavage of the tosyl group in phenanthridine 69 under reductive conditions using Na/naphthalene in DME at −50 °C afforded amine 69 in 75% yield. Final deprotection of the acetamide, TBS- and MOM-ether with DIC in 60% and 45% overall yields. Several reported methods for removal of the tosyl group were attempted on diol 75, all of which were unsuccessful. To facilitate removal of the tosyl group, diol 75 was acetylated under conditions using excess base as well as excess di-tert-butyl dicarbonate which produced alcohol 77; nevertheless, deosylation attempts still failed. Finally, deprotection of carbamate 76 was achieved by base hydrolysis (10%aq KOH) furnishing the free amine, which was subsequently isolated as the hydrochloride salt 78 as shown in Scheme 12.

**Biological Activity Profile**

Synthesis of the truncated derivatives, and especially of 7-deoxypancratistatin (4) and ent-7-deoxypancratistatin (ent-4), as well as the positional regioisomer of 7-deoxypancratistatin, 53, and the 10b-epimer or cis-7-deoxypancratistatin 70 provided the basis for an important extension of prior SAR11,53–55 cancer cell growth inhibition studies of (+)-pancratistatin (5),52,61 Against a minipanel of six human cancer cell lines and the marine P388 lymphocytic leukemia cell line, the following results were obtained. Evaluation of 7-deoxypancratistatin (4) led to good cancer cell growth inhibition (GI50, μg/mL):


Scheme 10
CNS SF-295 (0.29), colon KM 20L2 (0.22), lung NCI-H460 (0.24), melanoma SK-MEL-5 (0.23), ovary OVCAR-3 (0.24), renal A498 (0.47), and leukemia P388 (0.44), but the enantiomer with the opposite absolute configuration, ent-7-deoxypancratistatin (ent-4), was about 10-fold less active exhibiting GI50 values of 2.0–3.4 μg/mL. Among the truncated substances, only alcohol 77 (shown in Scheme 12) gave any indication of cancer cell line inhibition with GI50 5.3 μg/mL against pancreas-a BX-PG-3 and 8.5 μg/mL with lung NCI-H460. The evaluation of the cis epimer 70 against the six major human cell lines found this compound to be inactive. This finding is interesting from the viewpoint of providing useful information about the precise stereochemical requirements for activity. Note for example that the opposite configuration (in 7-deoxypancratistatin) provides for moderate activity as does the sp2 hybridization found in lycoridine and narcidiline. Several derivatives related to the positional isomer 53 have been tested against the same cell lines. Of these compounds, two were found to be moderately active 79 and 80, which showed GI50 values of less than 10 μg/mL against the breast cancer cell line MCF-7. Of the compounds related to the cis-fused phenanthridine nucleus of cis-7-deoxypancratistatin 70 only 66 showed similar levels of activity against the cell line MCF-7. These results again emphasize the impor-
tance of a nearly intact pancratistatin (5) molecule including the phenolic hydroxyl for retaining maximum (e.g., P388 leukemia, GI50 0.03 μg/mL) cancer cell inhibitory properties.11,53,54

**Conclusion**

It appears that major improvements in the synthesis of important antitumor alkaloids of the Amaryllidaceae group have been attained. Our results provide for a synthesis of narciclasine that is only 12 steps and 8 operations. Certainly this brevity begins to support a case for total synthesis as a solution to supply for natural sources. On the other hand, the several generations of synthetic approaches to these fascinating compounds have been focused solely on further improvements in the attainment of the target. This strategy is at the core of all of our approaches to these compounds and is one of the major reasons for their brevity.

If major improvements in brevity and yields are to materialize, the synthetic strategy that is utilized must be taken as a starting point for the development of new methods for the regio- and stereoselective synthesis of narciclasine, a strategy attended to by many recent investigators55 with no simple solution in sight.

In the area of structure–activity relationships, we have provided additional results and evidences that a full structural core, with the natural stereochemical relationship is required for high level of activity. Our efforts will now be focused solely on further improvements in the brevity of synthetic approaches to these fascinating compounds. We look forward to reporting new results in due course.

**Experimental Section**

3,5-Dibromo-15,25)-3,5-cyclohexadiene-1,2-diol (7,56

Escherichia coli [EM109 (pDTG601A)] was grown overnight at 35 °C with continuous shaking (150 rpm) in an enriched medium (9.6 g of KH2PO4, 8.4 g of KH2PO4, 3.0 g of (NH4)2SO4, 9.0 g of yeast extract, 60 mg of ampicillin, dissolved in 600 mL of tap water). The preculture was then transferred to a 12-L fermentor containing 12 g of medium adjusted to pH 7.0 (60.0 g of KH2PO4, 16.0 g of citric acid, 40.0 g of MgSO4, 9.6 mL of concentrated H2SO4, 9.6 mL of a 270 g/L solution of ferric ammonium citrate, 16.0 mL of a trace metal solution, 0.7 mL of antifoam, 2.69 g of thiamine hydrochloride, and 880 mg of ampicillin), and the cells were grown for approximately 2 h to an OD = 70 (λ = 660 nm). 1,3-Dibromobenzene (50.0 g, 0.32 mol) was added in portions to the culture, and the diol production was checked every 20 min by measuring a characteristic absorbance peak in the UV region (λ = 282 nm). After all metabolic activity ceased (or no more diol formation was observed by UV), the fermentation was stopped, and the pH was adjusted to 7.5 with NH4OH. The cells were separated from the broth by centrifugation at 7000 rpm for 20 min, and the resulting clear solution was saturated with sodium chloride and extracted with ethyl acetate. The organic layer was dried over anhydrous MgSO4, and the solvent was removed under reduced pressure. The crude diol was purified by recrystallization (methylene chloride/pentane) to yield 7 as a yellowish solid.

Data for the intermediate are as follows: Rf 0.4 (hexanes/ethyl acetate, 1:1); mp 80–81 °C; [αp]D25 +21.3 (c 1.1, acetone); IR (KBr) ν 3255, 1558 cm−1; 1H NMR (CDCl3, 300 MHz) δ 6.43 (dd, J = 1.5, 0.9 Hz, 1 H), 6.25 (dd, J = 4.2, 1.5 Hz, 1 H), 4.41 (dd, J = 6.3, 4.2 Hz, 1 H), 4.29 (dd, J = 6.3, 0.9 Hz, 1 H), 2.80 (bs, 2 H); 13C NMR (acetone-d6, 75.4 MHz) δ 131.7, 130.3, 129.9, 114.9, 72.1, 71.0; MS (−)ESI CH3COO−m/z 271+Br−+81Br (M−H)− 269+Br−+81Br (M−H−1), 267+81Br−+81Br (M−H−2); Anal. Calcd for C16H12BrO2: H, 0.4.

1,8-Dibromo-11-carbomethoxy-4,4-dimethyl-(1R,25,6S,7S)-3,5,10,11-tetraoxaazatricyclo[5.2.2.02,6]-8-undecene (9). To a solution of diol 7 (1.5 g, 5.6 mmol) in 2,2-dimethoxypropane (72 mL) was added a catalytic amount of p-toluenesulfonic acid. After complete consumption of starting material (TLC analysis), the solution was cooled to 0 °C before water (6 mL) was added. On a preparative scale, the intermediate acetonide was not isolated (analytical samples of 4,6-dibromo-2,2-dimethyl-(3aS,7aS)-benzo[d][1,3]-dioxole (8) were purified by flash column chromatography). Data for the intermediate are as follows: Rf 0.5 (hexanes/ethyl acetate 4:1); [αp]D25 +23.3 (c 1.0, C6H12OH); 1H NMR (DMSO-d6, 500 MHz) δ 6.56 (m, 1 H), 6.40 (dd, J = 4.4, 1.2 Hz, 1 H), 4.80 (d, J = 8.8 Hz, 1 H), 4.76 (dd, J = 8.7, 4.4 Hz, 1 H), 1.33 (s, 3 H), 1.30 (s, 3 H); 13C NMR (DMSO-d6, 125 MHz) δ 129.3, 127.2, 125.8, 117.4, 106.3, 74.3, 73.5, 72.0, 25.2.

Methyl acetate (1.2 g, 5.6 mmol) was added to the reaction vessel before methyl carbamate (0.59 g, 5.6 mmol, in 10 mL of methanol) was added dropwise. After addition, the solution was allowed to warm to room temperature and stirred for 16 h. Upon completion of the reaction (TLC analysis), an excess of saturated aqueous sodium bisulfite was added carefully until a light straw color was obtained. The mixture was extracted with Et2O (3 × 100 mL), the organic phase was washed with brine (2 × 50 mL) and dried over MgSO4, and the solvent was removed in vacuo. The product was isolated by flash column chromatography (hexanes/ethyl acetate 7:3) affording 1.3 g (90%) of 9 as a colorless solid. Rf 0.3 (hexanes/ethyl acetate 7:3); mp 150–152 °C; [αp]D25 +36.4 (c 1.1, CHCl3); IR (KBr) ν 1724, 1601 cm−1; 1H NMR (CDCl3, 500 MHz) δ 6.37 (dd, J = 2.3, 0.9 Hz, 1 H), 5.13 (dd, J = 4.4, 2.3 Hz, 1 H), 4.24 (dd, J = 6.9, 1.0 Hz, 1 H), 4.07 (dd, J = 6.9, 4.3 Hz, 1 H), 3.26 (s, 6, 500 MHz).
4-Methoxybenzo[d][1,3]dioxole-6-boronic Acid (10). To a solution of 6-bromo-4-methoxybenzod[d][1,3]dioxole (3.0 g, 13.0 mmol) in anhydrous THF (55 mL) cooled to −78 °C was added dropwise 0.7 M tert-butyl lithium in hexane (317 mL). After 15 min, triethyl borate (3.1 mL, 18.2 mmol) was added dropwise. During the addition the solution turned dark purple. After 15 min, the reaction mixture was allowed to cool to room temperature. The reaction was quenched with saturated aq NH₄Cl. Ethyl acetate (50 mL) and water (30 mL) were added, the layers were separated, and the aqueous phase was extracted with EtOAc (4 × 30 mL). The combined organic layer was washed with brine (2 × 15 mL), dried over MgSO₄, and concentrated to afford 2.5 g (97%) of product.

**Tandem Suzuki Coupling–Oxazine Reduction.** To a solution of Pd(PPh₃)₄ (100 mg, 0.26 mmol) in benzene (16 mL) were added bromide 9 (100 mg, 0.26 mmol) and Na₂CO₃ (1 mL). Borate 10 (64 mg, 0.30 mmol) in ethan (2 mL) was added, and the mixture was stirred at reflux until total consumption of the starting material (12 h). The product was extracted with EtOAc (3 × 25 mL), and the organic phase was washed with 5% hydrochloric acid (10 mL) and brine (3 × 10 mL) and then dried over MgSO₄ before the solvent was removed under reduced pressure. The residue was purified by flash column chromatography using gradient mixtures of ethyl acetate and hexanes affording 220 mg (50%) as colorless solid.

**Aryl Alcohol Oxidation.** A solution of 12 (220 mg, 0.51 mmol) in anhydrous THF (10 mL) was added tributylphosphine (0.26 mL, 1.02 mmol), benzaldehyde (125 mg, 1.02 mmol), and DEAD (0.1 mL, 1.02 mmol) at 25 °C, and the solution was stirred until total consumption of starting material (2 h). The mixture was concentrated, and the residue was purified by flash column chromatography using gradients of hexanes and ethyl acetate affording 23 as a beige solid. 23 was used as an acyl (133 mg, 52%), R₂ 0.2 (ethyl acetate); mp: 91–94 °C; [α]D²⁰ -14.4 (c 0.8, CHCl₃); IR (KBr) ν 2820, 2860, 1540, 1420 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 6.74 (s, 1 H), 6.53 (s, 1 H), 5.92 (s, 2 H), 4.65 (m, 4 H), 4.40 (d, J = 9 Hz, 1 H), 3.85 (s, 3 H), 3.64 (s, 3 H), 2.88 (d, J = 10 Hz, 1 H), 1.30 (s, 3 H), 1.27 (s, 3 H); ¹³C NMR (CDCl₃, 75 MHz) δ 156.5, 149.1, 143.5, 137.0, 135.2, 133.6, 130.6, 109.2, 106.5, 101.5, 99.8, 66.5, 56.8, 52.3, 51.0, 26.1, 24.5; MS (CI) 392 (M⁺); 334, 259, 173; HRMS Calcd for C₁₉H₁₃NO₈: 394.1502. Found: 394.1478. Anal. Calcd for C₁₉H₇O₈N: C, 58.01; H, 5.89. Found: C, 58.07; H, 6.29.

**Aryl Alcohol Oxidation.** A solution of alcohol 22 (200 mg, 0.51 mmol) in anhydrous THF (10 mL) was added tributylphosphine (0.26 mL, 1.02 mmol), benzaldehyde (125 mg, 1.02 mmol), and DEAD (0.1 mL, 1.02 mmol) at 25 °C, and the solution was stirred until total consumption of starting material (2 h). The mixture was concentrated, and the residue was purified by flash column chromatography using gradients of hexanes and ethyl acetate affording 23 as a beige solid. 23 was used as an acyl (133 mg, 52%), R₂ 0.2 (ethyl acetate); mp: 91–94 °C; [α]D²⁰ -14.4 (c 0.8, CHCl₃); IR (KBr) ν 2820, 2860, 1540, 1420 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 6.74 (s, 1 H), 6.53 (s, 1 H), 5.92 (s, 2 H), 4.65 (m, 4 H), 4.40 (d, J = 9 Hz, 1 H), 3.85 (s, 3 H), 3.64 (s, 3 H), 2.88 (d, J = 10 Hz, 1 H), 1.30 (s, 3 H), 1.27 (s, 3 H); ¹³C NMR (CDCl₃, 75 MHz) δ 156.5, 149.1, 143.5, 137.0, 135.2, 133.6, 130.6, 109.2, 106.5, 101.5, 99.8, 66.5, 56.8, 52.3, 51.0, 26.1, 24.5; MS (CI) 392 (M⁺); 334, 259, 173; HRMS Calcd for C₁₉H₁₃NO₈: 394.1502. Found: 394.1478. Anal. Calcd for C₁₉H₇O₈N: C, 58.01; H, 5.89. Found: C, 58.07; H, 6.29.
6-Aminocarbomethoxy-1,2-di-hydroxy-5-(7-methoxybenzoyl)[1,3]dioxolo[4,5]-4-yl benzoate (24). To a solution of benzose (120 mg, 0.24 mmol) in methanol (7 mL) was added a catalytic amount of Dowex 50 × 8–10 ion-exchange resin. After the mixture was stirred for 12 h at room temperature (until no more starting material could be detected by TLC), the resin was removed by filtration. The solvent was evaporated under reduced pressure to afford the intermediate diol. The crude product was dissolved in pyridine (1 mL, 12.5 mmol) and cooled to 0 °C. Acetic anhydride (0.5 mL, 5.3 mmol) and a catalytic amount of DMAP (28.4 mg, 0.23 mmol) in CH₂Cl₂ (2 mL) were added. The reaction mixture was stirred at room temperature until total consumption of the starting material (3 h). Ether (5 mL) and water (2 mL) were added, and the organic phase was washed with 2 mL aliquots of 10% aq copper(II) sulfate. The reaction mixture was stirred at room temperature until total consumption of the starting material (3 h). Ether (5 mL) and water (2 mL) were added, and the organic phase was washed with 2 mL aliquots of 10% aq copper(II) sulfate and then 2 mL of brine. The organic layer was dried over MgSO₄, the solvent removed under reduced pressure, and the residue purified by flash column chromatography (hexanes/ethyl acetate) affording benzoate (25). 

To a solution of benzoate (25) (10 mg, 0.024 mmol) in methanol (2 mL) was added a catalytic amount of Amberlyst A-21 weakly basic ion-exchange resin. The mixture was stirred for 2 h at room temperature until total consumption of starting material (TLC analysis). The resin was removed by filtration and the solvent removed under reduced pressure affording 6 mg (80%) of the known derivative of narciclasine (27).[27] Re: 0.40 (4:1 CH₂Cl₂/Methanol); [α]D₂⁰ +204 (c 0.3, DMSO); IR (KBr) ν 3423 (br), 2952, 2366, 1631, 1465 cm⁻¹; ¹H NMR (CD₂OD, 300 MHz) δ 6.91 (1 H, 6.17 (m, 1 H), 6.08 (d, J = 1.1 Hz, 1 H), 6.02 (d, J = 1.1 Hz, 1 H), 4.24 (m, 2 H), 3.98 (s, 3 H), 3.90 (m, 2 H); ¹³C NMR (CD₂OD, 75 MHz) δ 154.1, 145.5, 140.2, 135.2, 133.7, 123.7, 115.0, 103.6, 100.5, 74.2, 71.0, 70.8, 61.1, 53.6; MS (ESI MS): 653.5 (M + H⁺); 322 (M + H⁺).

2,3,4-Trihydroxy-7-methoxy-(25,3R,4S,4aR)-2,3,4,6-tetrahydro-[1,3]dioxolo[4,5]-jphenanthridin-6-one (7-Methyl narciclasine) (27). This derivative of (+)-narciclasine was prepared as described in the literature.[28] A pure sample of natural narciclasine (3 mg) was dissolved in excess freshly prepared diazomethane in ethanol/acetonitrile. The reaction mixture was stirred for several hours until the yellow color disappeared. The extent of the reaction was determined by TLC, and the residue was purified by flash column chromatography using a mixture of methylene chloride and methanol. All data obtained for this compound matched the synthetic product, including ¹H NMR and optical rotation. [α]D₂⁰ +219 (c 1.0, DMSO).

2,3,4,7-Tetrahydroxy-(25,3R,4S,4aR)-2,3,4,6-tetrahydro-[1,3]dioxolo[4,5]-jphenanthridin-6-one (Narciclasine) (3). To a solution of crude triol (27) (15 mg, 0.047 mmol) in anhydrous DMF (2 mL) was added anhydrous LiCl (10 mg, 0.24 mmol) under a stream of argon. The mixture was heated to 120 °C until total consumption of starting material (4 h). After the solvent was removed under reduced pressure, the residue was adsorbed on silica gel and purified by flash column chromatography (methylene chloride/methanol 4:1) affording 3 mg of narciclasine (20%). For a detailed study of the NMR spectra of narciclasine see ref 6g. [α]D₂⁰ +28 (CH₂Cl₂/Methanol, 4:1); ¹H NMR (DMSO-d₆, 500 MHz) δ 13.25 (1 H, s), 7.88 (1 H, s), 6.85 (1 H, s), 6.15 (dd, J = 4.5, 2.8 Hz, 1 H), 6.08 (m, 2 H), 5.19 (d, J = 6.3 Hz, 1 H), 5.16 (d, J = 5.6 Hz, 1 H), 5.01 (d, J = 3.8 Hz, 1 H), 4.18 (dd, J = 8.6, 2.4, 1.4 Hz, 1 H), 4.01 (m, 1 H), 3.79 (dd, J = 8.0, 5.5, 2.2 Hz, 1 H), 3.69 (m, 1 H). Signals at 13.25, 7.88, 5.19, 5.16, and 5.01 can exchange with deuterium oxide. A TOCSY experiment confirmed the assigned structure. Optical rotation of this compound matched that obtained by Rigby as well as the value for the natural product.

3-[Methoxy carbonyl]-1-bromo-5,6,6-isopropylidene-2-oxa-3-azobicyclo[2.2.2]oct-7-ene-5,6-diol (30). To a solution of the acetonide of (−)-16 (9.6 g, 0.042 mol) in MeOH/H₂O (164, 150 mL) at 0 °C were added NaOH (8.9 g, 42 mmol) and N-hydroxymethyl carbamate (3.6 g, 42 mmol). The reaction mixture was allowed to warm to room temperature, and the solution was stirred until total consumption of the starting material (18 h). Water (100 mL) and concentrated aqueous NaH₂SO₄ (100 mL) were then added, and the resulting mixture was extracted with CH₂Cl₂ (2 × 75 mL). The combined organic layer was washed with brine and dried over MgSO₄, and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (hexane/ethyl acetate 4:1) to yield 30 as colorless solid (7.7 g, 70%). [α]D₂⁰ +23 (hexane/ethyl acetate, 4:1); [α]D₂⁰ +8.3 (c 1.19 CH₂Cl₂); ¹H NMR (300 MHz, CDCl₃) δ 6.51 (dd, J = 9.0, 1.5 Hz, 1H), 6.41 (m, 1H), 5.05 (m, 1H), 4.60 (dd, J = 2.0 Hz, 2H), 3.77 (s, 3H), 1.35 (s, 3H), 1.32 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 158.0, 134.0, 131.7, 111.5, 81.2, 74.1, 54.0, 53.0, 25.6, 25.4.

6-[Methoxycarbonyl]amino)-1,2,6-isopropylidenedioxo-4-en-1,2,3-triol (15). To a solution of 30 (7 g, 22 mmol) in a mixture of THF (500 mL) and H₂O (50 mL) was added 4.15 g of Al (H₃) at 0 °C. The reaction mixture was stirred at for 3 h 0 °C and then at room temperature until total consumption of the starting material (12 h). The reaction mixture was diluted with THF (250 mL) and stirred for 10 min, filtered through Celite, and concentrated in vacuo. The
residue was purified by flash chromatography (hexanes/ethyl acetate, 1:2) to yield alcohol 15 (3.7 g, 65%); Rf: 0.57 (hexanes/ethyl acetate, 1:2); 1H NMR (300 MHz, CDCl3): δ 8.56 (dd, J = 10.0, 2.7 Hz, 1H), 7.54 (dd, J = 10.0, 1.6 Hz, 1H), 5.50 (d, J = 8.0 Hz, 1H), 4.15 (m, 3H), 4.02 (m, 1H), 3.60 (m, 4H), 1.38 (s, 3H), 1.28 (s, 3H); 13C NMR (75 MHz, CDCl3): δ 157.1, 131.6, 130.0, 109.4, 79.60, 80.0, 69.2, 52.6, 51.4, 27.2, 25.0; HRMS (Cl) calc for C31H39NO8: 524.2448; Found: 524.2445. Anal. Calcld for C31H39NO8: C 52.17; H, 7.11; N, 5.53. Found: C, 52.10; H, 6.78; N, 5.33.

**Methyl N(15,25,35,3R,6R)-6-(1,3-Benzodioxol-5-yl)-5,6-dihydroxy-3,4-epoxycholest-1-ylcarbamate (37).** To a solution of defin 36 (250 mg, 0.81 mmol) in benzene (20 mL) were added VO(acac)2 (18 mg, 0.065 mmol) and 5 M t-BuOH (1.0 mL). The reaction mixture was heated at 70 °C for 5 h (total consumption of starting material). After the solution was cooled to room temperature, the solvent was removed under reduced pressure and the residue purified by flash chromatography (chloroform/methanol, 8:1) to afford epoxyde 37 (175 mg, 67%) as white solid. Rf: 0.28 (chloroform/methanol, 8:1); mp 193–195 °C (chloroform/methanol); [α]D28 = -65.8 (c 0.8, MeOH); 1H NMR (300 MHz, CDCl3): 6.96 (d, J = 8.0 Hz, 1H), 6.80 (d, J = 8.1 Hz, 1H), 6.72 (d, J = 8.0 Hz, 1H), 5.90 (s, 2H), 4.25 (t, J = 5.0 Hz, 1H), 3.78 (t, J = 10.0 Hz), 3.46 (s, 3H), 3.38 (m, 3H), 3.08 (d, J = 10.9 Hz, 1H); 13C NMR (75 MHz, CDCl3) δ 198.0, 150.0, 143.8, 135.2, 123.5, 109.0, 108.7, 73.2, 68.0, 60.0, 54.4, 52.3, 51.9, 48.3.

**Methyl N(15,25,35,3R,4R,SR,6R)-2-(1,3-Benzodioxol-5-yl)-3,4,5,6-tetrahydroxycholesterol[1-2H7] (38).** To a solution of epoxide 37 (170 mg, 0.49 mmol) in 5 mL of water was added sodium benzoate (5 mg, 0.034 mmol). The mixture was kept at 100 °C until total consumption of the starting material (8 d). The solution was cooled to room temperature, the water was removed in vacuo, and the residue purified by flash column chromatography (chloroform/methanol, 9:1) to afford arylaminocarbamate 38 (130 mg, 86%) as white solid; Rf: 0.25 (chloroform/methanol, 1:1); mp 190–202 °C (ethyl acetate/methanol); [α]D28 = -1.69 (c 0.95, MeOH); 1H NMR (300 MHz, CDCl3): δ 6.90 (s, 1H), 6.77 (dd, J = 8.0, 1.8 Hz, 1H), 6.70 (d, J = 8.0 Hz, 1H), 5.85 (m, 2H) 4.30 (m, 1H), 4.00 (m, 2H) 3.75 (m, 2H), 3.50 (s, 3H), 3.30 (s, 3H), 3.18 (dd, J = 12.0, 2.0 Hz, 1H); 13C NMR (75 MHz, CDCl3) δ 159.9, 148.6, 147.5, 134.9, 129.3, 107.9, 106.8, 73.0, 73.5, 74.8, 52.3, 51.3, 48.3.

**Methyl N(15,25,35,3R,4R,SR,6R)-2-(1,3-Benzodioxol-5-yl)-3,4,5,6-tetrahydroxycholesterol[1-2H7] (38).** To a solution of arylaminocarbamate 38 (50 mg, 0.15 mmol) in pyridine (1.0 mL) was added acetic anhydride (1.0 mL). The reaction mixture was stirred at room temperature for 16 h (total consumption of starting material). The solvent was removed in vacuo and the residue purified by flash column chromatography (hexanes/ethyl acetate, 1:1) to afford tetraacetate 39 (60 mg, 82%) as white solid; Rf: 0.41 (hexanes/ethyl acetate 2:3); mp 108–111 °C (hexanes/ethyl acetate); [α]D28 = -13.9 (c 0.95, CHCl3); 1H NMR (300 MHz, CDCl3) δ 6.72 (m, 3H), 5.93 (s, 2H), 5.59 (s, 1H), 5.09 (m, 2H), 4.70 (m, 1H), 4.40 (bd, J = 8.0 Hz, 1H), 3.54 (s, 3H), 3.22 (t, J = 11.4 Hz, 1H), 2.02 (s, 6H); 13C NMR (75 MHz, CDCl3) δ 170.5, 169.3, 168.8, 168.3, 156.6, 147.7, 147.0, 129.6, 122.2, 109.1, 108.2, 101.0, 71.1, 68.7, 68.1, 52.2, 48.1, 47.2, 20.8, 20.6.

**Methyl N(15,25,35,3R,4R,SR,6R)-2-(1,3-Benzodioxol-5-yl)-3,4,5,6-tetrahydroxycholesterol[1-2H7] (38).** To a solution of tetraacetate 39 (33 mg, 0.65 mmol) in CH2Cl2 (3 mL) were added trifluoromethanesulfonyl anhydride (60 mg, 0.216 mmol) and DMAP (24 mg, 0.195 mmol). The reaction mixture was stirred at 5 °C for 18 h (total consumption of starting material). After the solvent was removed in vacuo, THF (2 mL) and 2 N aq HCl (0.2 mL) were added, and the mixture was stirred at room temperature for 2 h.
To a solution of aziridine \(+\) temperature until total consumption of the starting material (2 h). The reaction mixture was stirred at room temperature for 10 min (total consumption of the starting material) before it was quenched with aqueous NH\(_4\)-Cl (50 mg) in methanol (5 mL) was added compound \(\text{Na}_2\text{SO}_4\) and filtered. The combined organic phase was dried over \(\text{Na}_2\text{SO}_4\), and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (hexanes/ethyl acetate, 1:1) to afford the desired compound \(40\) (20 mg, 61%) as white solid. The reaction mixture was stirred at room temperature until total consumption of the starting material (6 h). The mixture was quenched with saturated NaHCO\(_3\) and the aqueous layer was extracted with ethyl acetate (3 \(\times\) 5 mL). The combined organic layer was dried over MgSO\(_4\), the solvent was removed under reduced pressure, and the residue purified by flash column chromatography (hexanes/ethyl acetate, 1:1) to afford the desired compound \(40\) (20 mg, 61%) as white solid. The reaction mixture was stirred at room temperature until total consumption of the starting material (2 h). The reaction mixture was stirred at room temperature for 10 min (total consumption of the starting material) before it was quenched with aqueous NH\(_4\)-Cl (50 mg) in methanol (5 mL) was added compound \(\text{Na}_2\text{SO}_4\) and filtered. The combined organic phase was dried over \(\text{Na}_2\text{SO}_4\), and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (hexanes/ethyl acetate, 1:1) to afford the desired compound \(40\) (20 mg, 61%) as white solid.
Amaryllidaceae Alkaloids

(1R,2R,3S,4S,5S,6R)-4,5-[(isopropylidenedioxy)-3-[tert-butyldimethylsilyloxy]-6-N-(3,4-dimethoxybenzylidene)-N-(4-methylphenylsulfonyl)-7-oxabicyclo[4.1.0]heptane (64). To a solution of alcohol 59 (100 mg, 0.21 mmol) in dry THF (15 mL) at −78 °C was added tert-BuLi (1.6 M in hexanes; 60.0 mL, 100 mmol, 52%) as pale yellow oil. After 30 min before the reaction was quenched with saturated aq NH4Cl, the combined organic phase was dried over Na2SO4, then filtered. The residue was purified by flash chromatography (ethanol/CH2Cl2 2:1) as colorless oil (4.69 mg, 50%) as pale yellow oil.

(1R,2R,3S,4R,10Rb)-3,4,4a,11b-Hexahydro-1-methoxy-2-(tert-butyldimethylsiloxy)-3,4-isopropylidenedioxy-5-N-(4-methylphenylsulfonyl)-1,3-dioxolo[4,5-c]phenanthridin-6(2H)-one (68). To a suspension of 67 (25 mg, 0.093 mmol) in CH2Cl2/CHCl3/H2O (2:2:3) were added Na/naphthalene (66 mg,0.31 mmol) and a catalytic amount of RuCl3·3H2O. The reaction mixture was stirred at room temperature until total consumption of the starting material (30 min). The heterogeneous mixture was diluted with CH2Cl2 (40 mL) and filtered through a plug of silica gel before it was extracted with water (30 mL). The combined organic phase was dried over Na2SO4, the slightly greenish solution was filtered through silica, and the solvent was removed under reduced pressure. Flash column chromatography (hexanes/ethyl acetate, 5:1) of the residue provided 68 (14 mg, 50%) as pale yellow oil.

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\text{IR (CHCl}_3\text{); } \nu \text{ IR } 3600, 3400, 2900, 1700, 1600, 1500, 1400, 1300, 1200, 1100, 1000, 900, 800, 700, 600, 500, 400 \text{ cm}^{-1}; \text{HRMS (FAB) calcd for } C_{32}H_{46}O_{9}N_{S}S_{i} 648.2663, \text{ found } 648.2668 \text{.}
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(1R,2S,3S,4S,5R,6S)-4,5-[(isopropylidenedioxy)-3-[tert-butyldimethylsiloxy]-3,4-isopropylidenedioxy-5-N-(4-methylphenylsulfonyl)-1,3-dioxolo[4,5-c]phenanthridin-6(2H)-one (69). To a solution of 68 (70 mg, 0.045 mmol) in dry DME (5 mL) at −50 °C was added a 0.5 M Na/naphthalene in DME until a green color persisted (total consumption of starting material according to TLC). The solution was stirred for 10 min before the reaction was quenched with saturated aq NH4Cl. The mixture was warmed to room temperature, diluted with water, and extracted with EtOAc (3 × 30 mL). The combined organic phase was dried over Na2SO4, the solvent was removed in vacuo, and the residue was subjected to flash column chromatography (hexanes/ethyl acetate, 9:1), affording 69 (40.2 mg, 68%) as colorless oil.

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\text{IR (CHCl}_3\text{); } \nu \text{ IR } 2002, 1874, 1494, 1383, 1330, 1200, 1170, 1130, 1050, 1000, 900, 800, 700, 600, 500, 400 \text{ cm}^{-1}; \text{HRMS (FAB) calcd for } C_{25}H_{32}O_{N}_{S}S_{i} 508.2367, \text{ found } 508.2368 \text{.}
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**epi-7-Deoxypancratinstatin (70).** To a solution of 69 (15 mg, 0.03 mmol) in methanol (1.5 mL) was added 3% HCl in methanol (0.5 mL). The reaction mixture was stirred until total consumption of the starting material (2 d). The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography affording 7 mg of the epimer (70%) of the natural product (70%). Rf: 0.1 (chloroform/methanol, 4:1); [α]D25 +5.9 (0.49, MeOH); IR ν = 3328, 1648, 1468, 1254 cm⁻¹; 1H NMR (500 MHz, CD3OD) δ 0.90 (s, 1H), 7.05 (s, 1H), 7.24 (s, 1H), 5.95 (s, 1H), 5.86 (s, 1H), 4.95 (dt, J = 2.6 Hz, 1H), 4.74 (t, J = 9.3 Hz, 1H), 4.64 (dd, J = 9.6, 2.8 Hz, 1H), 4.52 (t, J = 9.3 Hz, 1H), 4.30 (t, J = 9.6 Hz, 1H), 3.65 (dd, J = 10.2, 3.7 Hz, 1H); 13C NMR (125 MHz, CD3OD-δ) δ 164.6, 149.1, 146.3, 136.8, 122.8, 109.9, 106.6, 100.4, 74.2, 73.9, 71.6, 70.9, 55.6, 41.7; HRMS (EI pos) calcd for C21H19NO8S 478.1166, found 478.1166.

**N-(1R,2R,3S,4S,5S,6S)-2-(1,3-Benzodioxol-5-yl)-3,4-dihydroxy-5,6-(isopropylidenediyloxy)cyclohex-1-yl]carbamate (76).** A solution of diol 74 (1.97 g, 5.17 mmol) in THF/H2O/TFA (4:1:1, 45 mL) was stirred at room temperature for 16 h. The residue after removal of the solvents via Kugelrohr distillation was dissolved in acetonitrile (20 mL) and the stirred mixture treated with a solution of NaIO4 (3.73 g, 17.4 mmol) in H2O (20 mL). After 4 h, the reaction mixture was diluted with H2O (5 mL), and excess acetonitrile was removed under reduced pressure. The concentrate was extracted with EtOAc (3 × 60 mL), dried over Na2SO4, and concentrated in vacuo. The residue was purified by flash column chromatography (methylene chloride/diisopropyl ether, 1:4) to afford diol 74 (1.7 g). The residue was dissolved in acetonitrile (2 × 70 mL) and then dried under reduced pressure and purification of the residue by flash column chromatography (methylene chloride/diisopropyl ether, 1:4) to afford diol 74 (1.7 g). The residue was dissolved in acetonitrile (2 × 70 mL) and then dried under reduced pressure and purification of the residue by flash column chromatography (methylene chloride/diisopropyl ether, 1:4) to afford diol 74 (1.7 g). The residue was dissolved in acetonitrile (2 × 70 mL) and then dried under reduced pressure and purification of the residue by flash column chromatography (methylene chloride/diisopropyl ether, 1:4) to afford diol 74 (1.7 g). The residue was dissolved in acetonitrile (2 × 70 mL) and then dried under reduced pressure and purification of the residue by flash column chromatography (methylene chloride/diisopropyl ether, 1:4) to afford diol 74 (1.7 g).
organic extracts were dried over MgSO4, and solvent removed under reduced pressure. The remaining residue was purified via flash column chromatography (hexanes/ethyl acetate, 1:1) to afford 77 (540 mg, 83%) as a white solid: Rf 0.62 (hexanes/ethyl acetate, 1:1); mp 67–69 °C; [α]D20 +18.1 (c 1.0, CHCl3); IR (KBr) ν 3284, 1744, 1492 cm⁻¹; ¹H NMR (500 MHz, CDCl3) δ 7.67 (d, J = 8.2 Hz, 2 H), 7.25 (d, J = 8.5 Hz, 2 H), 6.68 (d, J = 7.7 Hz, 1 H); 6.55–6.56 (m, 2 H), 5.90 (s, 2 H), 4.52 (bs, 1 H), 4.21 (dd, J = 11.0, 7.6 Hz, 1 H), 4.15 (dd, J = 11.0, 6.0 Hz, 1 H), 3.93–3.81 (m, 3 H); 3.13 (dd, J = 10.7, 7.1 Hz, 1 H); 2.40 (s, 3 H); 1.42 (s, 18 H); 13C NMR (125 MHz, CDCl3) δ 153.0, 152.8, 147.9, 147.1, 143.4, 137.4, 129.6, 129.5, 127.0, 121.9, 108.8, 108.5, 101.1, 82.6, 82.2, 66.4, 66.2, 52.7, 44.9, 27.7, 27.6, 21.5; HRMS (EI) calcd. for C28H37NO10S 579.2138, found 579.2128. Anal. Calcd for C28H37NO10S: C, 58.02; H, 6.43; N, 2.42. Found: C, 57.75; H, 6.43; N, 2.33.

(2R,3R)-2-Amino-3-(1,3-Benzodioxol-5-yl)-butane-1,4-diol Hydrochloride (78). To a solution of diol 76 (198 mg, 0.70 mmol) in CH3OH (6 mL) was added 10% aq KOH (4.5 mL), and the mixture was heated at reflux for 14 h. The reaction mixture was extracted with Et2O (3 × 20 mL). The combined organic extract was dried over Na2SO4 and concentrated in vacuo. A solution of the remaining residue in CH3OH (5 mL) was added to CH3OH saturated with HCl at 0 °C, with stirring. After 5 min, the solvent was removed under reduced pressure. The residue was dissolved in 2-propanol and filtered into chilled Et2O to precipitate amine hydrochloride 78 (149 mg, 82%) as a pale tan solid: [α]D20 +59.0 (c 1.0, CH3OH); IR (neat) ν 3416, 1631, 1504, 1490, cm⁻¹; ¹H NMR (300 MHz, CD3OD) δ 6.79 (s, 1 H), 6.73 (m, 2 H), 5.86 (s, 2 H), 4.24 (t, J = 8.5 Hz, 1 H), 4.13 (dd, J = 10.4, 6.3 Hz, 1 H), 3.85–3.78 (m, 2 H), 3.31 (td, J = 7.4, 4.6 Hz, 1 H); ¹³C NMR (75 MHz, CD3OD) δ 149.8, 148.6, 133.7, 122.1, 109.5, 108.7, 102.6, 75.8, 72.2, 59.8, 51.5; HRMS (EI) calcd for C11H16NO4 (M + H – Cl) 226.1079, found 226.1082.

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