Antineoplastic Agents. 450. Synthesis of (+)-Pancratistatin from (+)-Narciclasine as Relay[1a]

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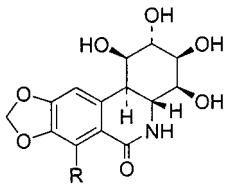
Received May 9, 2000 (Revised Manuscript Received December 14, 2000)

(+)-Narciclasine (**2**) available in quantity from certain Amaryllidaceae species or by total synthesis was employed as a precursor for a 10-step synthetic conversion (3.6% overall yield) to natural (+)-pancratistatin (**1a**). The key procedures involved epoxidation of natural (+)-narciclasine (**2**) to epoxide **6**, reduction to diol **8**, and formation of cyclic sulfate **12** and its ring opening with cesium benzoate followed by saponification of the benzoate to afford (+)-pancratistatin (**1a**).

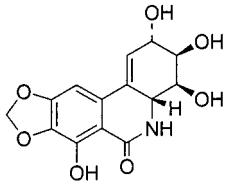
Introduction

Employing a 1980 Hawaii recollection of *Pancratium* (later reassigned *Hymenocallis*) *littorale* bulbs, we located a very potent anticancer constituent. In 1984, we reported[1b,c] the isolation (0.028% yield) and structure (by X-ray of the 7-methoxy derivative) of this important substance designated (+)-pancratistatin (**1a**). Subsequently, the U.S. National Cancer Institute initiated preclinical development owing to the high level of in vitro and in vivo cancer cell growth inhibitory (including antiviral)[2] activity. Unfortunately, the preclinical development of this potentially important anticancer drug has been slowed by supply constraints and the very low aqueous solubility (53 μg/mL) properties.[3] The latter problem was finally solved by conversion of (+)-pancratistatin (**1a**) to a water-soluble (>230 mg/mL) and comparably active phosphate (**1b**) prodrug.[4] While we have been able to gradually increase supplies of (+)-pancratistatin by cloning and growing the plant in Arizona,[5] a very efficient synthesis of this deceptively simple isocarbostyril would be especially useful.

Considerable research efforts[6] have been devoted to developing a very practical synthesis of pancratistatin (**1a**). Four of these have led to pancratistatin. The first synthesis (Danishefsky[7a]) provided racemic pancratistatin in 26 steps with an overall yield of 0.13%. The first enantioselective synthesis in 14 steps from bromobenzene (2% overall yield) of (+)-pancratistatin was reported by Hudlicky,[7b] in 1995. The same year, Trost[7c] summarized a synthesis with an impressive 11% overall yield for 13 steps. More recently, Haseltine[7d] and Magnus[7e] (22 steps, 1.2% yield) have presented a new synthesis of (+)-pancratistatin. In addition, a new synthesis of 7-deoxypancratistatin (**1c**) has been completed (13 steps, 21% overall yield),[8] and other new approaches to lactam **1a** are in progress.[9]



1a, R = OH
1b, R = OPO_3Na_2
1c, R = H



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From the beginning of our synthetic approaches to pancratistatin (**1a**), narciclasine[10] (**2**) has remained an attractive precursor, as it is available in practical quantities from the bulbs of certain Amaryllidaceae species. Lactam **2** has been studied in some detail,[11,12] leading to its recent synthesis in 12 steps by Hudlicky, starting from an enzymatic dihydroxylation of *m*-dibromobenzene.[13] Earlier, we attempted to develop a practical synthesis of pancratistatin from narciclasine (**2**) and easily obtained

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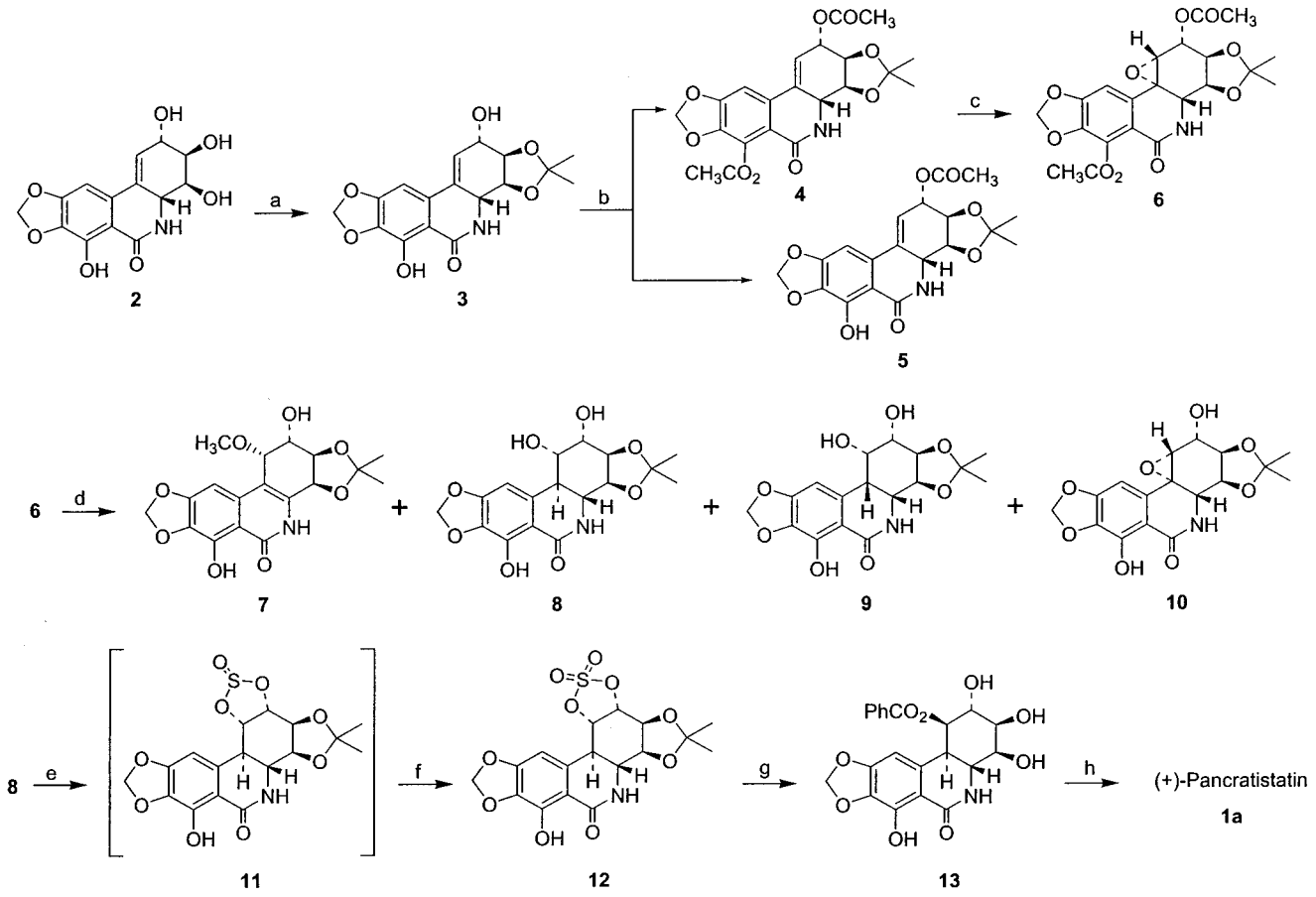
10.1021/jo000710n CCC: $20.00 © 2001 American Chemical Society
Published on Web 03/27/2001

Scheme 1[a]



[a] Key: (a) DMF, DMP, PTSA; (b) Ac_2O, pyr; (c) *m*-CPBA, phosphate buffer; (d) (i) H_2, 10% Pd/C; (ii) K_2CO_3, aq CH_3OH; (e) $SOCl_2$, Et_3N; (f) $RuCl_3$, $3H_2O/NaIO_4$, CH_3CN, CCl_4, H_2O; (g) (i) $PhCO_2H$, Cs_2CO_3; (ii) THF/H_2O, cat. H_2SO_4; (h) K_2CO_3, CH_3OH.

10b-*R*-hydroxypancratistatin.[14] But the last step, hydrogenolysis of the benzyl alcohol, did not lead to (+)-pancratistatin. We now report the first synthesis of (+)-pancratistatin (**1a**) from (+)-narciclasine (**2**) in 3.6% overall yield.

The 3,4-acetonide of narciclasine (**3**)[10c] was prepared in 97% yield (Scheme 1), and the C-2 and C-7 hydroxyls were protected by acetylation to afford diacetate **4** in quantitative yield. However, an attempt at further purification using silica gel column chromatography caused some hydrolysis of diacetate **4** to monoacetate **5** in a ratio of 6:1, respectively. Oxidation of olefin **4** using *m*-chloroperoxybenzoic acid in dichloromethane and a phosphate buffer gave α-epoxide **6** in 55% yield. The structure of epoxide **6** was confirmed by an X-ray structure determination using a crystal grown from acetone.[15] Hydrogenation of the epoxide in the presence of 10% palladium on carbon, followed by saponification, gave a mixture (by ^1H NMR) of four compounds. These were separated by column chromatography on silica gel, isolated and identified by NMR. The methyl ether **7**, trans B/C diol **8**, cis B/C diol **9**, and deprotected epoxide **10** were isolated in 19%, 28%, 27%, and 2% yields, respectively. Methylation of the C-1 hydroxyl group to provide ether **7** apparently occurred during the saponification step.

The overall yield of C-1 α-alcohol **8** from narciclasine was 15%. Detailed analysis of the NMR 2D COSY, $^1H-^1H$ correlation spectrum, allowed assignment of the hydrogen atoms. The NMR coupling constants (δ_a 2.93, δ_b 3.58, δ_c 4.31, $J_{ab} = 14.5$ Hz, $J_{ac} = 7.0$ Hz) for alcohol **8** allowed assignment of the stereochemistry. These assignments were confirmed by an X-ray crystal structure determination using a crystal of **8** grown from methanol solution.[15]

Treatment of alcohol **8** with thionyl chloride led to a high yield of the corresponding epimeric (sulfoxide) cyclic sulfites **11**. Oxidation[7c,16a−c] of the cyclic sulfite epimers to the corresponding cyclic sulfate **12** was achieved using 0.18 equiv of ruthenium trichloride with 3.5 equiv of sodium iodate. Such reactions are usually performed using a catalytic amount of ruthenium trichloride and sodium iodate, but that procedure gave incomplete oxidation with only one of the epimeric sulfur atoms oxidized. Attempts at separating the mixture were not productive.

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Table 1. Human Cancer and Murine Lymphocytic Leukemia Cell Line Inhibition Values for Pancratistatin (1a) and Narciclasine (2) and Synthetic Intermediates (3−13)

			isocarbostyril											
	cell type	cell line	**1a**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**12**	**13**
GI_{50} (μg/mL)	pancreas-a	BXPC-3	2.8×10^{-2}	1.6×10^{-2}	>10	>10	>10	>10	>10	8.8	>10	>10	>10	9.4×10^{-4}
	ovarian	Ovcar-3	3.2×10^{-2}	1.6×10^{-2}	>10	>10	>10	>10	>10	5.0	>10	>10	>10	$<1.0 \times 10^{-3}$
	CNS	SF-295	1.7×10^{-2}	1.2×10^{-2}	>10	>10	>10	>10	>10	7.2	>10	>10	>10	1.3×10^{-3}
	lung-NSC	NCI-H460	4.8×10^{-2}	2.2×10^{-2}	>10	>10	>10	>10	>10	>10	>10	>10	>10	$<1.0 \times 10^{-3}$
	colon	KM20L2	2.6×10^{-2}	1.8×10^{-2}	>10	>10	>10	>10	>10	6.9	>10	>10	>10	$<1.0 \times 10^{-3}$
	prostate	DU-145	1.6×10^{-2}	7.1×10^{-3}	>10	>10	>10	>10	>10	3.8	>10	>10	>10	$<1.0 \times 10^{-3}$
TGI (μg/mL)	pancreas-a	BXPC-3	1.4×10^{-1}	4.5×10^{-2}	>10	>10	>10	>10	>10	>10	>10	>10	>10	5.3×10^{-3}
	ovarian	Ovcar-3	1.1×10^{-1}	4.4×10^{-2}	>10	>10	>10	>10	>10	>10	>10	>10	>10	1.9×10^{-3}
	CNS	SF-295	9.1×10^{-2}	4.9×10^{-2}	>10	>10	>10	>10	>10	>10	>10	>10	>10	4.9×10^{-3}
	lung-NSC	NCI-H460	4.3×10^{-1}	1.0×10^{-1}	>10	>10	>10	>10	>10	>10	>10	>10	>10	1.1×10^{-3}
	colon	KM20L2	2.3×10^{-1}	1.2×10^{-2}	>10	>10	>10	>10	>10	>10	>10	>10	>10	3.4×10^{-3}
	prostate	DU-145	10.0×10^{-2}	3.6×10^{-2}	>10	>10	>10	>10	>10	>10	>10	>10	>10	2.6×10^{-3}
ED_{50} (μg/mL)	murine leukemia	P388	0.032	0.0044	>10	>10	>10	>10	>10	2.6	>10	>10	>10	0.0017

Using the excess oxidant method, cyclic sulfate **12** was obtained in 47% yield from alcohol **8**.

Nucleophilic attack by cesium benzoate[7c,16a] on the cyclic sulfate **12** in dimethylforamide occurred over 4 h. Hydrolysis of the alkyl sulfate in tetrahydrofuran using sulfuric acid allowed simultaneous cleavage of the acetonide to afford, following column chromatography, benzoate **13** in 74% yield. The benzoyl group was easily removed to give (+)-pancratistatin (**1a**) identical with the natural product.[1] (+)-Pancratistatin (**1a**) was obtained in 10 steps with an overall yield of 3.6% from narciclasine (**2**).

Synthetic conversion of (+)-narciclasine (**2**) to (+)-pancratistatin (**1a**) provided a useful opportunity to further study structure/activity relationships, and the result (Table 1) was a clear demonstration that even minor structural modifications of pancratistatin (**1a**) led to decreased cancer cell growth inhibitory activity in all but the case of **13**, where the activity was greatly enhanced by the presence of a C-1 benzoyl group.

Experimental Section

Narciclasine 3,4-Acetonide (3).[10c] A solution prepared from natural (+)-narciclasine (**2**, 5 g, 16.3 mmol), 2,2 dimethoxypropane (25 mL), and *p*-toluenesulfonic acid (0.83 g) in DMF (25 mL) was stirred at rt for 16 h. Pyridine (8.5 mL) followed by water (150 mL) was added to the suspension and the mixture stirred for 1 h at rt. The precipitated product was collected, washed with water, and dried (at 64 °C over P_2O_5 under high vacuum) to give narciclasine 3,4-acetonide (5.5 g, 97%) as an amorphous powder: mp 270−273 °C (lit.[10c] mp 275 °C); IR (KBr) 3497, 3184, 1676, 1599, 1467, 1373, 1294, 1111, 1039 cm^{-1}; ^{1}HNMR δ (DMSO, 300 MHz) 13.7 (s, 1H), 8.8 (s, 1H), 7.01 (s, 1H), 6.47 (bs, 1H), 6.06 (m, 2H), 5.73 (bs, 1H), 4.2−3.9 (m, 4H), 1.45 (s, 3H), 1.31 (s, 1H); ^{13}CNMR (DMSO, 300 MHz) δ 167.6, 152.5, 145.2, 133.3, 128.8, 128.3, 125.9, 109.8, 104.3, 102.0, 94.2, 79.0; EIMS *m/z* 347 (M^+, 28), 332 (1), 289 (2), 273 (6), 260 (8), 247 (100), 242 (22), 218 (10), 85 (2), 73 (10).

2,7-Diacetoxynarciclasine 3,4-Acetonide (4) and 2-Acetoxynarciclasine 3,4-Acetonide (5). Narciclasine 3,4-acetonide (**3**, 3.18 g, 9.15 mmol) was dissolved in acetic anhydride (20 mL)−pyridine (20 mL). The mixture was heated and stirred at 60 °C (under argon) for 24 h. The suspension slowly dissolved. Ethyl acetate (300 mL) was added, and the organic layer was washed with water (2 × 40 mL), dried (Na_2SO_4), and concentrated. The product was dried under high vacuum for 24 h to remove traces of pyridine. The amorphous powder was examined by ^{1}H NMR ($CDCl_3$) and found to be the diacetate (**4**) with a trace of pyridine still present. Column chromatographic purification was conducted on silica gel using a gradient elution system 1:99 → 1:9 (CH_2Cl_2−CH_3OH) to give the monoacetate (**5**, 0.15 g, 4.2%): R_f 0.79 (98:2, CH_2Cl_2−CH_3-OH); $[\alpha]^{27}_D$ +84 (*c* 1.01, $CHCl_3$); mp 250−253 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.85 (s, 1H), 6.65 (s, 1H), 6.4 (s, 1H), 6.09 (m, 2H), 5.36 (m, 1H), 4.3 (dd, J = 5.4, 6.3 Hz, 1H), 4.12 (m, 2H), 2.18 (s, 3H), 1.51 (s, 3H), 1.37 (s, 3H); ^{13}C NMR ($CDCl_3$ 300 MHz) δ 170.3, 167.6, 153, 146, 134.7, 128, 127.9, 121.5, 111.6, 104.9, 102.4, 94.5, 78.5, 75.3, 74, 55.2, 27.1, 24.9, 21.1; EIMS *m/z* 389 (M^+, 37), 331 (33), 289 (74), 271 (29), 260 (42), 247 (100), 242 (43), 218 (10), 85 (7), 44 (59). Anal. Calcd for $C_{19}H_{19}O_9N$: C, 58.6; H, 4.9; N, 3.59. Found: C, 58.2, H, 5.0, N, 3.4.

The diacetate (**4**, 3.2 g, 81%) exhibited: R_f 0.6 (98:2 CH_2Cl_2−CH_3OH); $[\alpha]^{24}_D$ = +60° (*c* 0.91, MeOH); mp 130−132 °C; ^{1}H NMR ($CDCl_3$ 300 MHz) δ 6.97 (s, 1H), 6.12 (s, 1H), 6.08 (s, 2H), 6.01 (s, 1H), 5.37 (m, 1H), 4.40 (dd, J = 5.7, 6.6 Hz, 1H), 4.12 (m, 2H), 2.38 (s, 3H), 2.21 (s, 3H), 1.51 (s, 3H), 1.38 (s, 3H); ^{13}C NMR ($CDCl_3$ 300 MHz) 170.3, 169.2, 160.9, 152.4, 141.4, 134.0, 129.5, 128.5, 121.9, 113.4, 111.4, 103.0, 100.1, 78.7, 75.3, 73.8, 55.1, 27.11, 24.9, 21.1, 20.9; EIMS *m/z* 431 (M^+, 14), 389 (60), 373 (28), 331 (75), 314 (9), 289 (100), 271 (35), 260 (42), 247 (99), 242 (39), 85 (9), 44 (95). Anal. Calcd for $C_{21}H_{21}O_9N{\cdot}CH_3OH$; C, 57.02; H, 5.43; N, 3.25. Found: C, 57.16, H, 4.99, N, 3.11.

1,10b-α-Epoxy-2,7-diacetoxynarciclasine 3,4-acetonide (6). Diacetate **4** (3.0 g, 6.96 mmol) was dissolved in CH_2Cl_2 (180 mL), and phosphate buffer (pH 8) (180 mL) was added followed by *m*-chloroperoxybenzoic acid (4.5 g, 26 mmol, 3.7 equiv). The biphasic mixture was stirred for 16 h, whereby all the starting material had reacted (TLC). Dichloromethane (300 mL) was added, and the organic layer was separated, thoroughly washed with 5% $Na_2S_2O_3$ (3 × 300 mL), followed by 5% Na_2CO_3 (3 × 300 mL) and water (3 × 300 mL), dried ($MgSO_4$), and concentrated. The crude product was washed with acetone and the white precipitate collected to give an amorphous solid (1.6 g, 52% yield). Recrystallization from hot acetone gave crystals which were examined (see below) by X-ray crystallography: mp 224−225 °C; $[\alpha]^{24}_D$ = +138° (*c* 1.06, CH_2Cl_2); ^{1}H NMR (300 MHz $CDCl_3$) δ 6.46 (s, 1H), 6.1 (m, 2H), 5.86 (bs, 1H), 5.3 (d, J = 6 Hz, 1H), 4.4 (dd, J = 6.3, 6.9 Hz, 1H), 4.3 (t, J = 8.7 Hz, 1H), 4.06 (s, 1H), 4.03 (d, J = 8.4 Hz), 2.38 (s, 3H), 2.24 (s, 3H), 1.47 (s, 3H), 1.34 (s, 3H); ^{13}C NMR (300 MHz, $CDCl_3$) 170.5, 168.8, 161.3, 152.4, 142, 134, 128.4, 118.6, 109.38, 103.2, 101.1, 75.7, 75.4, 74.0, 58.1, 55.1, 53.4, 26.8, 24.2, 21.0, 20.8; EIMS M^+ 447 (M^+, 10), 405 (84), 345 (10), 287 (33), 258 (16), 234 (54), 44 (100). Anal. Calcd for $C_{21}H_{21}O_{10}N$: C, 56.37; H, 4.73; N, 3.13. Found: C, 56.72; H, 4.83; N, 3.09.

X-ray Crystal Structure Determination. Epoxide 6. A thick, plate-shaped X-ray sample (~0.70 × 0.60 × 0.40 mm) was obtained by crystallization from acetone. Data collection was performed at 293 ± 1 K. Accurate cell dimensions were determined by least-squares fitting of 25 carefully centered reflections in the range of 35° < θ < 40° using Cu Kα radiation.

Crystal Data: $C_{21}H_{21}O_{10}N_1$, FW = 447.39, monoclinic, *C*2, a = 18.200(4) Å, b = 9.2435(18) Å, c = 12.864(3) Å, β = 105.73

(3)°, $V = 2083.2(7)$ Å^3, $Z = 4$, $\rho_c = 1.426$ Mg/m^3, μ(Cu Kα) = 0.982 mm^{-1}, $\lambda = 1.541\,78$ Å, $F(000)$ 936.

All reflections corresponding to a complete quadrant ($0 \le h \le 13$, $0 \le k \le 9$, $-19 \le l \le 19$) were collected over the range of $0 < 2\theta < 110°$ using the $\omega/2\theta$ scan technique. Friedel reflections were also collected (whenever possible) immediately after each reflection. Three intensity control reflections were also measured for every 60 min of X-ray exposure time and showed a maximum variation of 0.9% over the course of the collection. A total of 6050 reflections were collected. Subsequent statistical analysis of the complete reflection data set using the XPREP[17] program verified that the space group as *C*2. After Lorentz and polarization corrections, merging of equivalent reflections and rejection of systematic absences, 2605 unique reflections (R(int) = 0.0524) remained, of which 2583 were considered observed ($I_o > 2\sigma(I_o)$) and were used in the subsequent structure determination and refinement. Linear and anisotropic decay corrections were applied to the intensity data as well as an empirical absorption correction (based on a series of psi-scans).[18] Structure determination was readily accomplished with the direct-methods program SHELXS.[19] All non-hydrogen atom coordinates were located in a routine run using default values in that program. The remaining H atom coordinates for the parent molecule were calculated at optimum positions. All non-hydrogen atoms were refined anisotropically in a full-matrix least-squares refinement using SHELXL.[19] The H atoms were included, their Uiso thermal parameters fixed at either 1.2 or 1.5 (depending upon their atomic environment) of the value of the Uiso of the atom to which they were attached and forced to ride that atom. The final standard residual R_1 value for **6** was 0.066 for observed data and 0.0663 for all data. The corresponding Sheldrick R values were wR_2 of 0.1551 and 0.1555, respectively. The goodness-of-fit on F^2 was 1.101. The structure of epoxide **6** is shown in Figure S1 (Supporting Information). The absolute stereochemistry of the epoxide[15] could be assigned with certainty on the basis of the value of the Flack absolute structure parameter,[20] i.e., −0.1(3). A final difference Fourier map showed minimal residual electron density; the largest difference peak and hole being +0.474 and −0.417 e/Å^3. Final bond distances and angles were all within expected and acceptable limits.

1-Methoxyisonarciclasine 3,4-Acetonide (7), 1-Isopancratistatin 3,4-Acetonide (8), B/C *cis*-1-Isopancratistatin 3,4-Acetonide (9), and 1,10b-α-Epoxynarciclasine 3,4-Acetonide (10). The epoxide **6** was hydrogenated and the resulting mixture saponified in 3 × 1 g batches as follows. To a solution of epoxide **6** (1 g) in ethyl acetate (150 mL) was added 10% palladium-on-carbon (1 g). The flask was evacuated, flushed with hydrogen (5×), and hydrogenated at rt for 2.5 h using a hydrogen-filled balloon. The palladium-on-carbon was collected and the filtrate concentrated to dryness to give a white solid (0.94 g). The ^{1}H NMR ($CDCl_3$) indicated a mixture of products. The solid was dissolved in 10% aq CH_2OH (20 mL) and DCM (10 mL). Potassium carbonate (0.6 g, 4.4 mmol) was added, and the reaction mixture stirred overnight at rt. A precipitate slowly developed. The reaction mixture was neutralized using IR-120 H^+ Amberlite resin. The resin was collected and the filtrate concentrated to a yellow solid (0.91 g). The results of three such reactions were combined to give a yellow solid (2.65 g) which was purified by silica gel flash chromatography using 98:2 DCM/MeOH to give alcohol **7** (0.46 g, 18.7%), diol **8** (0.68 g, 27.7%), diol **9** (0.31 g), and a mixture of acetate **9** and epoxide **10** (0.78 g). The mixture of **9** and **10** was separated by dissolution in hot methanol and collection of the insoluble material (**10**, 0.051 g, 2%). Recrystallization of the mother liquor gave alcohol **9** (0.38 g). The total amount of alcohol **9** recovered from the column was 0.68 g (27%). Recrystallization of diol **8** from hot methanol gave crystals that were used for X-ray crystal structure elucidation (see below). Methyl ether **7** corresponded to: R_f 0.53 (CH_2Cl_2/CH_3OH, 4%); $[\alpha]^{24}_D = 15°$ (*c* 1.1, CH_3OH); mp 239−240 °C; ^{1}H NMR (DMSO, 500 MHz) δ 12.8 (s, 1H), 9.39 (s, 1H), 6.77 (s, 1H), 6.1 (dd, J = 3.5, 9.0 Hz, 2H), 5.17 (d, J = 7.5 Hz, 1H), 4.68 (d, J = 3 Hz, 1H), 4.63 (t, J = 7 Hz, 1H), 3.86 (m, 1H), 3.42 (s, 3H), 2.58 (d, J = 8 Hz, 1H), 1.48 (s, 3H), 1.46 (s, 3H); ^{13}C NMR (DMSO, 500 MHz) 165.6, 154.4, 144.8, 134.3, 132.3, 111.5, 110.5, 108.5, 102.5, 92.9, 77.7, 74.8, 73.2, 71.5, 58.0, 27.2, 25.2; EIMS *m*/*z* 377 (M^+, 100), 362 (3), 349 (5), 319 (8), 288 (14), 270 (14), 258 (35), 247 (17), 292 (8), 218 (13), 101 (24), 56 (14), 44 (24), 28 (34). Anal. Calcd for $C_{18}H_{19}O_8N\cdot CH_3OH$: C, 55.89; H, 5.39; N, 3.43. Found: C, 56.06; H, 5.39; N, 3.46.

Diol **8** was characterized with R_f 0.39 (96:4 $CH_2Cl_2-CH_3OH$); $[\alpha]^{24}_D = -26°$ (*c* 0.48, CH_3OH); mp 231−232 °C; ^{1}H NMR ($CDCl_3$ 500 MHz) δ 12.45 (s, 1H), 6.9 (s, 1H), 6.18 (s, 1H), 6.03 (narrow m, 1H), 4.38 (t, J = 8 Hz, 1H), 4.31 (m, 2H), 3.92 (dd, J = 5, 7.25 Hz, 1H), 3.57 (dd, J = 14.5, 8.5 Hz, 1H), 3.05 (bs, 1H), 2.93 (dd, J = 14.5, 7.5 Hz), 2.68 (bs, 1H), 1.48 (s, 3H), 1.38 (s, 3H); ^{13}C NMR ($CDCl_3$, 500 MHz) 169.8, 153.2, 146.2, 135.4, 133.1, 110.9, 106.3, 102.3, 98.8, 77.6, 75.5, 70.8, 70.6, 52.9, 41.0, 27.3, 24.9; EIMS *m*/*z* 365 (M^+, 100), 350 (7), 290 (3), 272 (4), 260 (4), 234 (9), 205 (35), 190 (4), 176 (5), 147 (5), 85 (5), 73 (7), 60 (10), 44 (9), 28 (1). Anal. Calcd for $C_{17}H_{19}O_8N$: 55.89; H, 5.24; N, 3.83. Found: C, 55.5; H, 5.42; N, 3.85.

B/C-*cis*-diol 9 showed: R_f 0.13 (96:4 $CH_2Cl_2-CH_3OH$); $[\alpha]_D = +27°$ (*c* 0.86, CH_3OH); mp 245 °C; ^{1}H NMR (DMSO, 500 MHz) δ 13.06 (s, 1H), 8.08 (s, 1H), 6.55 (s, 1H), 6.01 (s, 2H), 4.94 (d, J = 6.5 Hz, 1H), 4.53 (d, J = 4 Hz, 1H), 4.38 (d, J = 4.5 Hz, 1H), 4.1 (d, J = 5.5 Hz, 1H), 4.05 (dd, J = 8.5, 5.5 Hz, 1H), 3.58 (m, 1H), 3.52 (m, 1H), 3.01 (m, 1H), 1.42 (s, 3H), 1.29 (s, 3H); ^{13}C NMR (DMSO, 500 MHz) 169.5, 151.5, 144.4, 135.7, 131.9, 108.6, 107.1, 101.6, 99.5, 76.1, 75.5, 74.9, 71.9, 49.6, 38.7, 28.3, 26.5; EIMS *m*/*z* 365 (M^+, 100), 350 (6), 305 (3.5), 258 (7), 234 (31.6), 205 (77), 176 (7), 147 (10), 119 (5), 100 (9), 85 (12), 73 (17.5), 60 (24), 44 (22), 28 (22). Anal. Calcd for $C_{17}H_{19}O_8N\cdot H_2O$: C, 53.3; H, 5.5; N, 3.6. Found: C, 53.6; H, 5.5; N, 3.6.

Epoxide 10: R_f 0.15 (96:4 $CH_2Cl_2-CH_3OH$); $[\alpha]^{22}_D = -4.2°$ (*c* 0.57, THF); mp 242−244 °C; ^{1}H NMR (DMSO 300 MHz) δ 11.9 (s, 1H), 6.83 (s, 1H), 6.12 (s, 1H), 5.09 (dd, J = 9, 11 Hz, 1H), 4.96 (d, J = 10 Hz, 1H), 4.66 (dd, J = 3.6, 6 Hz, 1H), 4.33 (dd, J = 6.6, 3 Hz, 1H), 3.53 (m, 1H), 1.38 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (DMSO, 300 MHz) δ 166.4, 154.2, 144.3, 132.0, 131.3, 114.9, 109.9, 108.9, 102.9, 100.2, 94.5, 76.2, 72.4, 71.2, 66.3, 28.7, 26.7; EIMS *m*/*z* 363 (M^+, 100), 345 (5), 305 (22), 288 (10), 276 (13), 258 (39), 233 (21), 205 (11), 60 (14), 44 (33), 28 (34).

X-ray Crystal Structure Determination. **Diol acetonide 8:** A plate-shaped crystal (∼0.35 × 0.20 × 0.10 mm) was obtained by crystallization from methanol and mounted in a sealed capillary with the specimen immersed in mother liquor. Data collection was performed at 293 ± 1 K. Accurate cell dimensions were determined by least-squares fitting of 25 carefully centered reflections in the range of $35° < \theta < 40°$ using Cu Kα radiation.

Crystal Data: $C_{17}H_{19}O_8N_1\cdot CH_3OH$, FW = 397.37, triclinic, $P1$, $a = 7.9890(12)$ Å, $b = 9.072(2)$ Å, $c = 13.564(2)$ Å, $\alpha = 87.805(16)°$, $\beta = 82.317(13)°$, $\gamma = 71.734(16)°$, $V = 925.1(3)$ Å^3, $Z = 2$, $\rho_c = 1.426$ Mg/m^3, μ(Cu Kα) = 0.982 mm^{-1}, $\lambda = 1.541\,78$ Å, $F(000)$ 420.

All reflections corresponding to a complete hemisphere ($0 \le h \le 9$, $-10 \le k \le 10$, $-15 \le l \le 15$) were collected over the range of $0 < 2\theta < 120°$ using the $\omega/2\theta$ scan technique. Friedel reflections were also collected (whenever possible) immediately after each reflection. Three intensity control reflections were also measured for every 60 min of X-ray exposure time and showed a maximum variation of −1.5% over the course of the

(17) XPREP: The automatic space group determination program in the SHELXTL. (see ref 19).

(18) North, A. C.; Phillips, D. C.; Mathews, F. S. *Acta Crystallogr.* **1968** A2, 351.

(19) SHELXTL-PC Version 5.101, 1997, an integrated software system for the determination of crystal structures from diffraction data, Bruker Analytical X-ray Systems, Inc., Madison, WI 53719. This package includes, among others: XPREP, an automatic space group determination program; XS, the Bruker SHELXS module for the solution of X-ray crystal structures from diffraction data; XL, the Bruker SHELXL module for structure refinement; XP, the Bruker interactive graphics display module for crystal structures.

(20) Flack, H. D. *Acta Crystallogr.* **1983**, 876−881.

collection. A total of 6235 reflections were collected. Subsequent statistical analysis of the complete reflection data set using the XPREP[17] program verified that the space group as *P*1. After Lorentz and polarization corrections, merging of equivalent reflections and rejection of systematic reflections, a total of 5550 were considered observed ($I_o > 2\sigma(I_o)$) and were used in the subsequent structure determination and refinement. Linear and anisotropic decay corrections were applied to the intensity data as well as an empirical absorption correction (based on a series of ψ-scans).[18] The structure was solved with the direct-methods program SHELXS.[19] All non-hydrogen atom coordinates were located in a routine run using default values in that program. The asymmetric unit (i.e., unit cell) was found to contain two independent molecules of the parent compound, as well as two molecules of disordered methanol solvent. The remaining H atom coordinates for the parent molecules were calculated at optimum positions. All non-hydrogen atoms were refined anisotropically in a full-matrix least-squares refinement using SHELXL.[19] The H atoms were included, their U_{iso} thermal parameters fixed at either 1.2 or 1.5 (depending upon their atomic environment) of the value of the Uiso of the atom to which they were attached and forced to ride that atom. The final standard residual R_1 value for diol acetonide **8** was 0.0661 for observed data and 0.0766 for all data. The corresponding Sheldrick *R* values were wR_2 of 0.1863 and 0.2048, respectively. The goodness-of-fit on F^2 was 1.065. The structure of one of the molecules of the diol acetonide **8**, present in the unit cell, is shown in Figure S2 (Supporting Information). The Flack absolute structure parameter[20] was −0.4(3). A final difference Fourier map showed minimal residual electron density; the largest difference peak and hole being +0.585 and −0.398 e/Å^3. Final bond distances and angles were all within expected and acceptable limits.

1-Isopancratistatin 1,2-Cyclic Sulfate-3,4-acetonide (12). To a solution of diol **8** (0.25 g, 0.67 mmol) in THF (10 mL)−triethylamine (0.4 mL, 2.9 mmol) was added thionyl chloride (0.1 mL, 1.37 mmol, 2 equiv). The reaction was carried out at rt under argon for 40 min. Ethyl acetate (50 mL) was added, and the organic layer was washed with water (2 × 10 mL) followed by brine (10 mL). After drying and concentration, an off-white solid (0.26 g, 93%) was obtained. The ^{1}H NMR ($CDCl_3$, 300 MHz) indicated the sulfite product (**11**) was obtained as a mixture of epimers. The solid (0.26 g, 0.63 mmol) was dissolved in CH_3CN (15 mL)−CCl_4 (12 mL), and $RuCl_3 \cdot H_2O$ (0.024 g, 0.115 mmol, 0.18 equiv) and $NaIO_4$ (0.48 g, 2.24 mmol, 3.5 equiv) were added together as a solid, followed by water (12 mL). The mixture was stirred at rt for 19 h. Ethyl acetate (80 mL) was added and the organic layer separated, washed with water (2 × 15 mL), dried ($MgSO_4$), filtered through a pad of silica gel, and concentrated to a brown solid that was dried under high vacuum for 16 h (0.13 g, 45%). Recrystallization from THF/hexane provided **12** as a white amorphous solid: R_f 0.7 (96:4 CH_2Cl_2−CH_3OH); $[\alpha]^{24}_D = +7.3$ (*c* 0.15, THF); mp 223 °C; ^{1}H NMR (DMSO, 500 MHz) δ 13.1 (s, 1H) 9.06 (s, 1H), 6.39 (s, 1H), 6.11 (d, $J = 1$ Hz, 1H), 6.09 (d, $J = 1$ Hz, 1H), 5.58 (t, $J = 7.5$ Hz, 1H), 5.26 (dd, $J = 7.0$, 9.0 Hz, 1H), 4.71 (dd, $J = 7.8$, 9.3 Hz, 1H), 4.6 (t, $J = 8$ Hz, 1H), 3.74 (dd, $J = 7.8$, 14.8 Hz, 1H), 3.66 (dd, $J = 8$, 14 Hz, 1H), 1.47 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (DMSO, 500 MHz) δ 168.3, 152.5, 145.5, 133.0, 132.5, 110.8, 102.3, 97.5, 84.4, 82.5, 77.0, 73.2, 56.0, 50.2, 37.2, 27.1, 25.0; EIMS *m*/*z* 427 (M^+, 100), 412 (10), 396 (1.7), 370 (2.6), 352 (3.5), 327 (10.5), 272 (21), 242 (36), 218 (10.5), 205 (29), 147 (17), 119 (9.6), 85 (8.7), 60 (1.7), 44 (42). Anal. Calcd for $C_{17}H_{17}O_{16}NS$: C, 47.8; H, 4.01; N, 3.27. Found: C, 48.08; H, 4.16; N, 3.13.

Pancratistatin 1-Benzoate (13). To a solution of cyclic sulfate **12** (0.09 g, 0.21 mmol) in DMF (2 mL) was added benzoic acid (0.042 g, 0.36 mmol, 1.7 equiv) followed by Cs_2CO_3 (0.1 g, 0.31 mmol, 1.5 equiv). The mixture was stirred under argon at 60 °C for 4 h. The DMF was removed (high vacuum), and the residue was suspended in THF (2 mL). Water (three drops from a pipet) followed by H_2SO_4 (concentrated two drops from a pipet)[7c] was added, and the suspension became a solution. The solution was stirred at 70 °C for 24 h. Additional THF (2 mL), water (two drops), and H_2SO_4 (two drops) were added, and the reaction was allowed to continue for 2 h. The crude reaction mixture was separated by passing through a silica gel flash column (9:1 CH_2Cl_2−CH_3OH). The product was collected and recrystallized by dissolving in 9:1 CH_2Cl_2−CH_3OH, concentrating to a viscous oil, followed by solution in chloroform and addition of hexane to turbidity. The benzoate (**13**) recrystallized at rt as a colorless amorphous solid weighing 0.066 g, 74%: $[\alpha]^{24}_D = -21°$ (c 0.19 CH_3OH); mp 180−185 °C; ^{1}H NMR (DMSO, 500 MHz) δ 13.2 (s, 1H), 7.98 (m, 3H), 767 (t, $J = 9$ Hz, 1H), 7.52 (t, $J = 10$ Hz, 2H), 6.25 (s, 1H), 6.07 (d, $J = 1.5$ Hz, 1H), 6.02 (d, $J = 1.5$ Hz, 1H), 5.8 (bs, 1H), 5.68 (s, 1H), 4.96 (bs, 1H), 4.14−4.09 (m, 2H), 3.93−3.86 (m, 2H), 3.37 (m, 1H); ^{13}C NMR (DMSO, 500 MHz) δ 169.3, 165.4, 152.1, 145.7, 134.0, 133.4, 132.2, 129.6, 129.5, 129.1, 128.6, 128.3, 107.3, 101.9, 95.7, 71.7, 70.0, 69.9, 68.8, 50.6, 38.0; EIMS *m*/*z* 429 (M^+, 12.5), 325 (3.5), 307 (9.8), 271 (15.2), 247 (50), 218 (9.8), 205 (7), 122 (69.6), 105 (100), 77 (80), 52 (46), 28 (53). Anal. Calcd for $C_{21}H_{19}O_9N \cdot H_2O$: C, 56.38; H, 4.73; N, 3.13. Found: C,56.82; H,4.61; N, 2.95.

(+)-Pancratistatin (1a). A methanol (0.5 mL) solution of benzoate **13** (0.025 g, 0.058 mmol) was treated with K_2CO_3 for 16 h at rt. TLC (5:1 CH_2Cl_2−CH_3OH) showed product development with starting material still present. The reaction mixture was heated to 55−60 °C for 3 h, whereby TLC showed no starting material present. The mixture was concentrated to a brown solid, and the residue was dissolved using a mixture of DMF (0.4 mL) and 9:1 CH_2Cl_2−CH_3OH. The crude material was purified by passage through a column of silica gel with 4:1 CH_2Cl_2−CH_3OH as the eluent, to afford (+)-pancratistatin (**1a**) as an off-white solid (0.014 g, 75%): R_f 0.38 (4:1 CH_2Cl_2−CH_3OH); mp 265−270 °C dec; ^{1}H NMR (DMSO, 500 MHz) δ 13.05 (s, 1H), 7.49 (s, 1H), 6.48 (s, 1H), 6.05 (s, 1H), 6.02 (s, 1H), 5.36 (bs, 1H), 5.07 (m, 2H), 4.82 (d, $J = 7$ Hz, 1H), 4.27 (s, 1H), 3.95 (bs, 1H), 3.84 (bs, 1H), 3.72 (m, 2H), 2.96 (d, $J = 12.5$ Hz, 1H); ^{13}C NMR (DMSO, 500 MHz) d 169.5, 152.0, 145.4, 135.6, 131.7, 107.5, 101.7, 97.7, 73.3, 70.2, 69.9, 68.5, 50.5. Comparison (NMR, IR, TLC) with an authentic specimen[1b,c] of natural (+)-pancratistatin confirmed the mutual identity of the synthetic and natural samples.

Acknowledgment. The very necessary financial support for this investigation was provided by Outstanding Investigator Grant CA-44344-01A1-01-12 from the Division of Cancer Treatment and Diagnosis, National Cancer Institute, DHHS; the Arizona Disease Control Research Commission; the Fannie E. Rippel Foundation; Robert B. Dalton Endowment Fund; Gary L. and Diane R. Tooker; Sally Schloegel; Billie Jane Baguley; the Fraternal Order of Eagles Art Ehrmann Cancer Fund; and the Ladies Auxiliary to the Veterans of Foreign Wars. We are also very pleased to thank for other assistance Professor Barry M. Trost (Stanford University), Drs. Gordon M. Cragg, Ralph A. Backhaus, and Dennis L. Doubek, Professors Cherry L. Herald and Fiona Hogan, Dr. Sheo Bux Singh, George R. Pettit III, and the U. S. National Science Foundation (Grant No. CHE-9808678).

Supporting Information Available: Tables of crystallographic data, bond lengths and angles, atomic coordinates, and anisotropic thermal parameters are available for structures **6** and **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO000710N