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SYNTHESIS OF 10b(*R*)-HYDROXYPANCRATISTATIN, 10b(*S*)-HYDROXY-1-EPIPANCRATISTATIN, 10b(*S*)-HYDROXY-1,2-DIEPIPANCRATISTATIN AND RELATED ISOCARBOSTYRILS[1]

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Abstract - Narciclasine (**2**) was transformed by a series of reactions where Sharpless asymmetric hydroxylations served as the stereochemical controlling step to 10b(*R*)-hydroxypancratistatin (**3**), 10b(*S*)-hydroxy-1-epipancratistatin (**13**) and 10b(*S*)-hydroxy-1,2-diepipancratistatin (**16**). Synthesis of 10b(*S*)-hydroxy-1,2-diepipancratistatin (**16**) proceeded from ·-triol (**11**) *via* cyclic sulfate (**14**) and inversion of C-2 with cesium benzoate followed by saponification and treatment with a catalytic amount of acid. Compared to pancratistatin (**1**), these structural modifications led to decreased cancer cell growth inhibition against a mini-panel of human cancer cell lines. Narciclasine (**2**) inhibited the pathogenic yeast *Cryptococcus neoformans*, and modifications (**4**, **14** and **15**) inhibited growth of the pathogenic bacterium *Neisseria gonorrhoeae*.

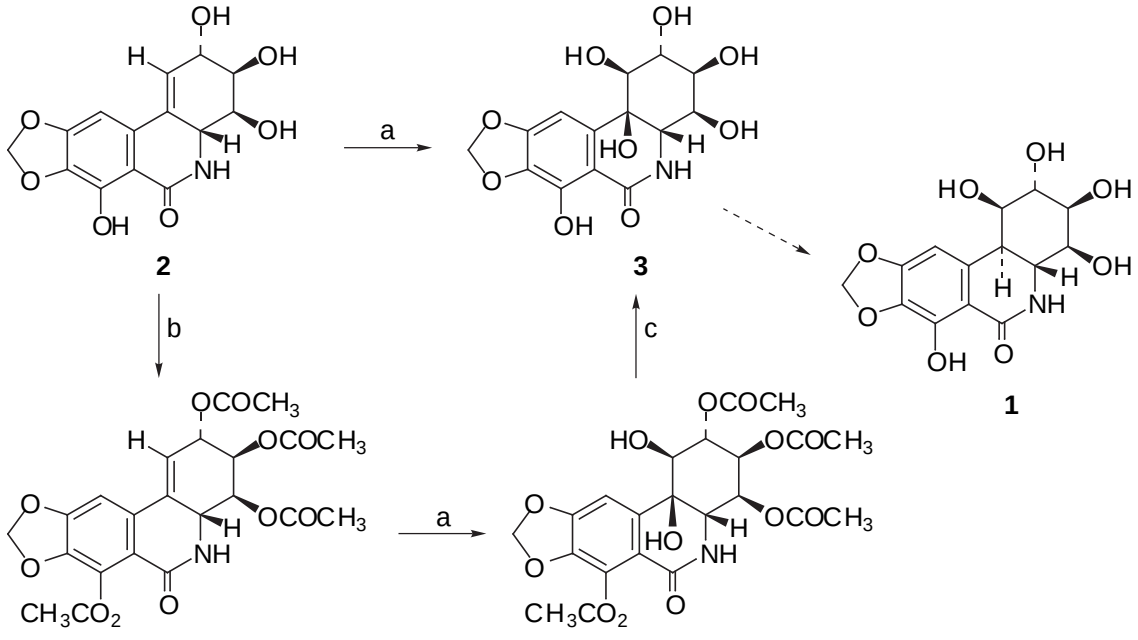
The terrestrial flora continues to be an exceptionally productive, albeit still largely unexplored, source of new and potentially useful antineoplastic substances. Recent examples of such biologically active constituents from plants include new combretastatins,[2] other stilbenes,[3] flavonoids,[3] podophyllotoxin-type lignans,[4] quinones,[5,6] tetrahydrofurans,[7] steroidal glycosides,[8] pentacyclic triterpenes,[9] ambewelamides A and B[10] from a lichen and the new anticancer drug hydroxymethylacylfulvene, a derivative of the mushroom constituent illudin S.[11] Our early research[12] directed at discovery of useful plant constituents for improving human cancer treatment has resulted in on-going clinical development of combretastatin A-4 prodrug[13] and phyllanthostatin 1/phyllanthoside,[14] and (+)-pancratistatin (**1**)[15] in advanced preclinical development.

In anticipation of meeting clinical supply requirements for pancratistatin (**1**), we developed a practical horticultural procedure[16] for producing this anticancer and antiviral isocarbostyril while in parallel investigating synthetic[17,18] approaches with narciclasine (**2**) as relay[19] that became successful.[20,21] In some *Narcissus* spp. (Amaryllidaceae),[22] narciclasine (**2**) is reasonably abundant,[16] and we have employed

(following isolation) this plant constituent as a very useful intermediate for synthetic conversion[20] to (+)-pancratistatin (**1**) and to conduct a series of SAR studies. The present investigation was concerned with further defining structural requirements for maintaining or enhancing the anticancer activity of (+)-pancratistatin (**1**).

As we reported in a preliminary communication,[19] 10b(*R*)-hydroxypancratistatin (**3**) and 10b(*R*)-hydroxy-2,3,4,7-tetra-*O*-acetylpancratistatin (**5**) were synthesized from narciclasine (**2**) and 2,3,4,7-tetra-*O*-acetylnarciclasine (**4**), respectively, using osmium tetroxide and the chiral directing ligand hydroquinine 4-chlorobenzoate (HQ)[23] (Scheme 1). Interestingly, 10b(*S*)-hydroxy-1-epipancratistatin was not obtained when HQ was replaced with its diastereoisomer, hydroquinidine 4-chlorobenzoate (HQD). Presumably, the stereochemistry of the C-2 allylic hydroxyl group of narciclasine (**2**) and its peracetate derivative was causing the osmium tetroxide to approach the olefinic bond from the opposite facial side.[24]

Scheme 1



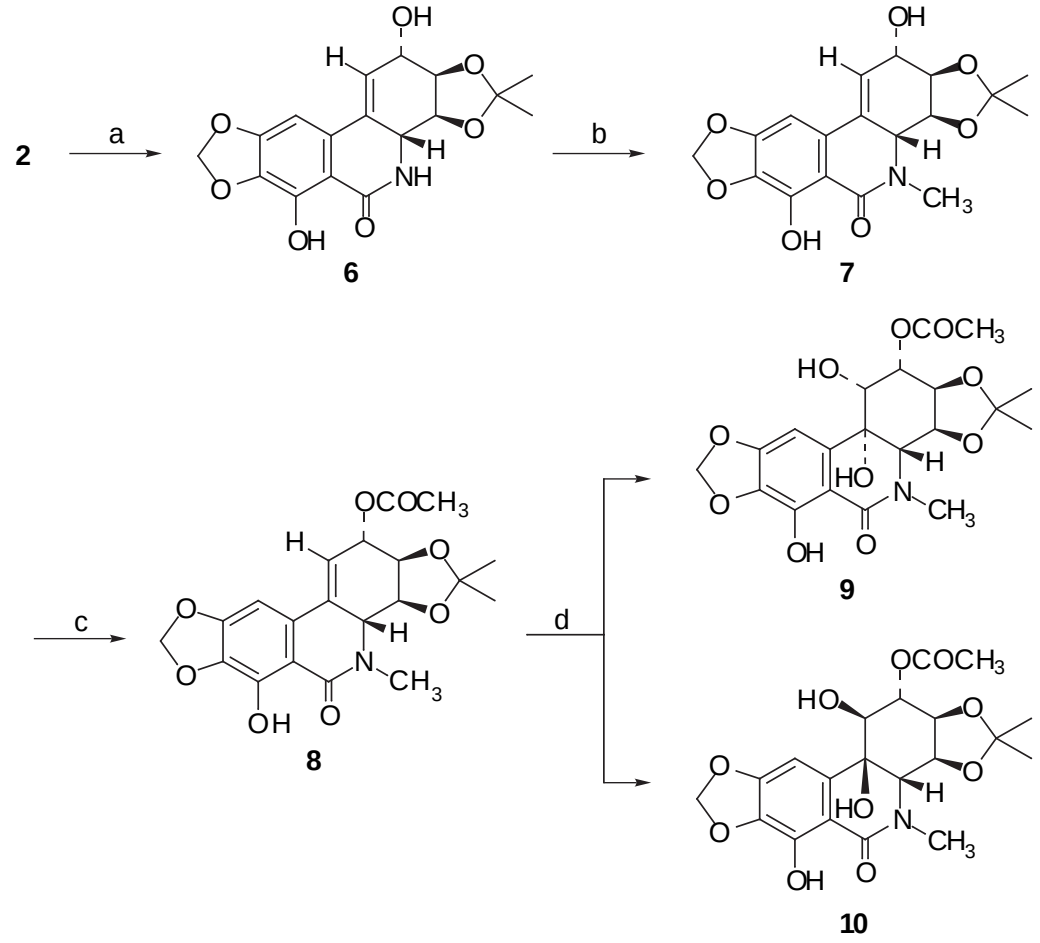
2 **3** **1** **4** **5**

(a) OsO_4 (cat.), NMO, HQ 4-chlorobenzoate, DMF-H_2O (9:1), 0 - 24 ^oC;
(b) Ac_2O, py, 24 h; (c) CH_2Cl_2, 2M NH_3 in CH_3OH

Accordingly, we proceeded to investigate the possibility of obtaining the 10b(S)-hydroxy-1-epipancratistatin by preparing narciclasine derivatives which might reverse this β-approach of the osmium tetroxide to the olefinic bond. We found that the narciclasine derivative *N*-methyl-2-*O*-acetylnarciclasine-3,4-acetonide (**8**), upon treatment with a catalytic amount of osmium tetroxide and the chiral directing ligand HQD, afforded *N*-methyl-2-*O*-acetyl-10b(*S*)-hydroxy-1-epipancratistatin-3,4-acetonide (**9**) in a 2:1 ratio with its 10b-(*R*)-diastereoisomer (**10**) (Scheme 2). When

the osmylation reaction was repeated under the same conditions using the HQ chiral directing ligand, the ratio of **9**:**10** was found to be 1:6. These results agree with the prediction that olefin (**8**) is positioned as suggested by the Sharpless and coworkers[25] mnemonic device with the smallest substituent (the hydrogen) in the southeast quadrant which is considered the most hindered space, and the aromatic group occupying the southwest quadrant. Osmium tetroxide is more likely to attack from the β-face in the case of hydroquinidine (HQD) derivatives, or from the α-face in the case of hydroquinine (HQ) derived ligands.

Scheme 2



(a) PTSA, DMP, DMF, 24 h; (b) CH_3I, K_2CO_3; (c) Ac_2O (1 equiv), py;
(d) OsO_4 (cat.), NMO, HQD 4-chlorobenzoate, acetone-H_2O (9:1)

The diols were separated by column chromatography and examined by NMR. Crystals grown from acetone-hexane and examined by X-Ray crystallography confirmed the structure and stereochemistry (Figure 1) of α-diol (**9**), and, as a consequence, β-diol (**10**) as well. Encouraged by the preceding results, synthesis of the 10b(*S*)-hydroxy-1-epipancratistatin (**13**) (Scheme 3) was undertaken. The 10b(*S*)-hydroxy-1-epipancratistatin-3,4-acetonide (**11**) was obtained in a 2:1 ratio with 10b(*R*)-hydroxypancratistatin-3,4-acetonide (**12**) when narciclasine-3,4-acetonide (**6**) was allowed to react with osmium tetroxide and either the chiral directing ligand

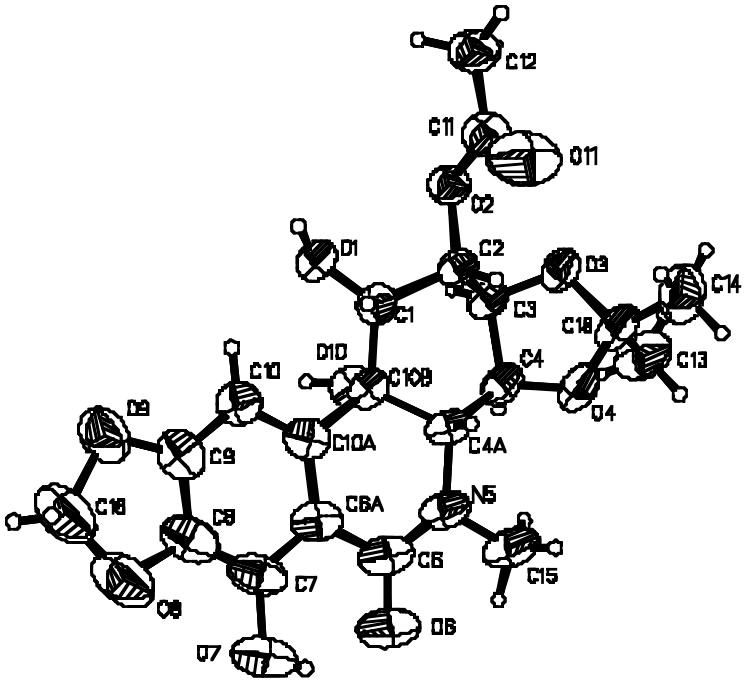
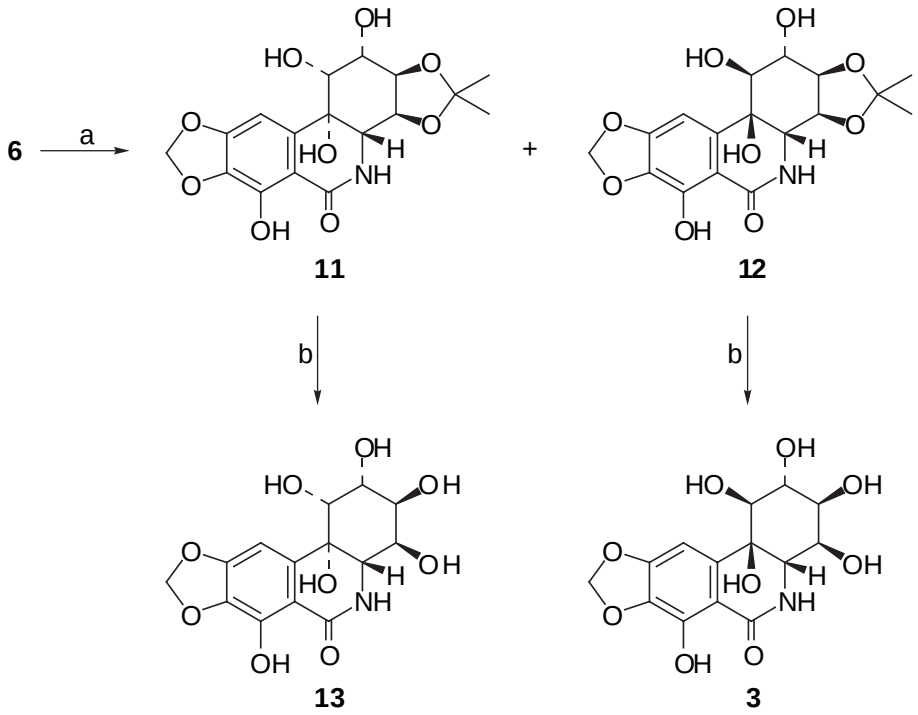


Figure 1. The crystal structure of α-diol (**9**) (50% thermal ellipsoid probability plot).

Scheme 3

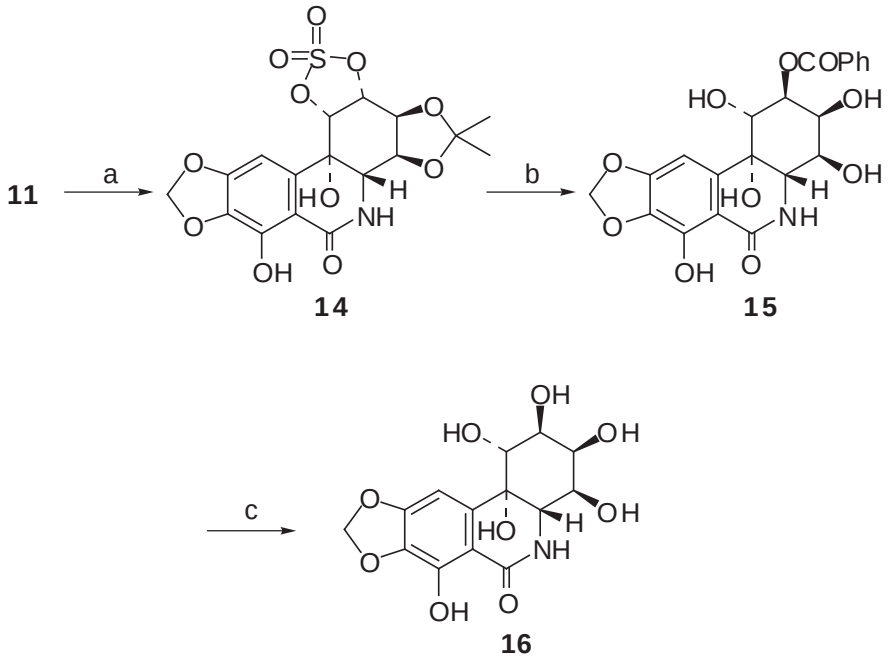


(a) OsO_4 (cat.), NMO, $(DHQD)_2PHAL$, DMF-H_2O (9:1), 0 - 15 °C;
(b) THF, H_2SO_4, H_2O, 24 h

hydroquinidine 4-chlorobenzoate (HQD) or dihydroquinidine phthalazine $(DHQD)_2PHAL$[26] in DMF-H_2O (overall yield of 79%).

It should be noted that we were unsuccessful in obtaining either diol using the AD-mix formulation.[26] The acetonide protecting group was removed from each isomer by treating with sulfuric acid in THF-H_2O to give 10b(*S*)-hydroxy-1-epipancratistatin (**13**) and 10b(*R*)-hydroxypancratistatin (**3**). In an effort to synthesize the 10b(*S*)-hydroxypancratistatin, the cyclic sulfate (**14**) was synthesized by reacting acetonide (**11**) with thionyl chloride. Here it was considered probable that the 10b(*S*)-hydroxy cyclic sulfate (**14**) would undergo inversion at C-1 when allowed to react with cesium benzoate.[18,20] Subsequent treatment with acid would give the desired 10b(*S*)-hydroxypancratistatin. However, the S_N2 substitution occurred at C-2 with inversion of configuration, to give only the C-2 benzoate (**15**) (Scheme 4).

Scheme 4



(a)(i) $SOCl_2$, $(C_2H_5)_3N$, THF; (ii) $RuCl_3.3H_2O/NaIO_4$, CH_3CN-CCl_4-H_2O; (b)(i) Cs_2CO_3, $C_6H_5CO_2H$, DMF; (ii) THF-H_2O/H_2SO_4 (cat.), 60 °C; (c) K_2CO_3, CH_3OH, rt

Presumably, the presence of the 10b(*S*)-hydroxyl group made substitution at the C-2 position more favorable electronically. Presence of the benzoate group at the C-2 carbon was discovered by careful examination of the ^1H NMR, COSY, and ROESY spectra. The signal assigned to the H-2 proton was down field (δ 5.02 ppm) relative to the H-1 signal (δ 4.29 ppm). A large coupling constant was observed between the two protons (J = 9 Hz), which confirmed the *trans* relationship. The small coupling constant between H-2 and H-3 (J = 2.5 Hz) implied that those protons were on the same side of the molecule in a *cis* relationship. The ROESY spectrum showed very clear correlation peaks between the H-1 signal and the H-4a signal indicating their positions on the same side of the molecule. Strong correlation peaks were also observed between H-3 and H-4, and between H-2 and the hydroxyl groups 1 and 10b, as expected. The C-2 benzoyl group was removed by saponification with potassium

carbonate to give pentaol (**16**). A crystal of alcohol (**16**) was grown from methanol and the structure deduced by X-Ray crystallography, which confirmed the structure (**16**) as shown in Figure 2.

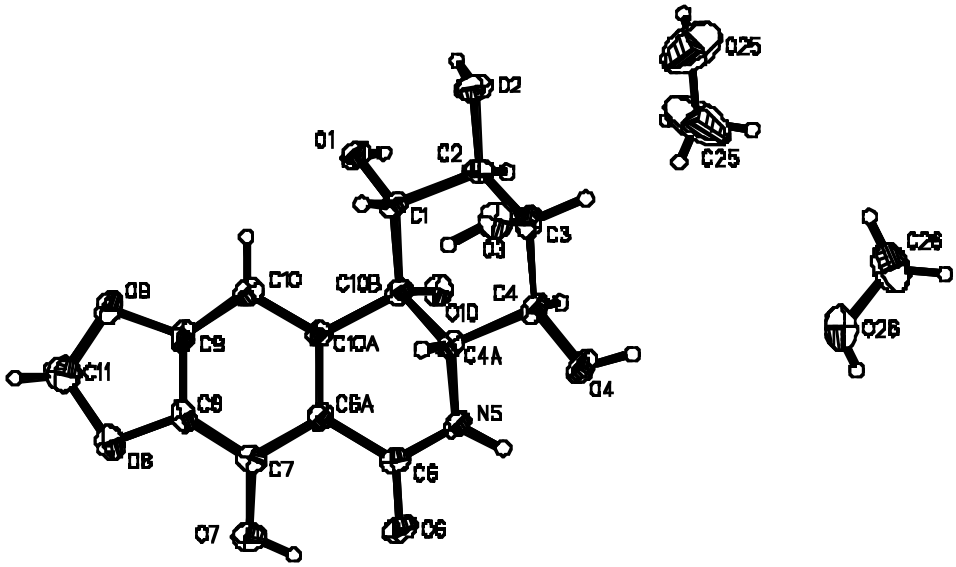


Figure 2. The crystal structure (50% thermal probability ellipsoids) of 10b(*S*)-hydroxy-1,2-diepipancratistatin (**16**), including the two associated solvate molecules of methanol.

Isocarbostyrils (**3**-**5**) and (**7**-**16**) were tested for potential activity against a mini-panel of human cancer lines and the P388 murine leukemia cell line (Table 1). The compounds all showed decreased cancer cell growth inhibitory activity. Interestingly, the presence of the C-2 benzoate group caused a considerable improvement in the activity of **15** compared with **16**. In broth microdilution assays (National Committee for Clinical Laboratory Standards, Documents M27-A and M7-A4, 1997), narciclasine (**2**) exhibited antifungal activity (MIC=8-16 μg/mL, *Cryptococcus neoformans*), and pancratistatin structural modifications **4**, **14** and **15** exhibited marginal antibacterial activity (MIC=32-64 μg/mL, *Neisseria gonorrhoeae*).

EXPERIMENTAL

Solvents were purified by redistillation and in addition tetrahydrofuran was distilled from sodium benzophenone, dimethyformamide from phosphorous pentoxide and triethylamine from potassium hydroxide. TLC was performed on Merck kieselgel 60F 254 plates eluting with the solvents indicated. Visualization was provided using a 254 mm UV lamp and development with a ceric sulfate spray with heat. Flash column chromatography was performed with Merck silica gel 60 slurry packed in flash columns using the initiating solvent. Melting points are uncorrected and were observed using a Fisher-Johns melting point apparatus. NMR spectra were acquired at either 300 MHz, 400 or 500 MHz for ^{1}H and for ^{13}C employing Varian Gemini 300 and Inova-500

Table 1 Human Cancer Cell Line and Murine P388 Lymphocytic Leukemia Cell Line Inhibition Values for Pancratistatin (**1**) and Narciclasine (**2**) and Synthetic Intermediates (**3**-**5**) and (**7**-**16**)

	Cell Type	Cell-line	Isocarbostyril **1**	**2**	**3**	**4**	**5**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
GI_{50}	Pancreas-a	BXPC-3	$2.8x10^{-2}$	$2.6x10^{-2}$	>10	$1.6x10^{-1}$	14.4	>10	>10	>10	>10	>10	>10	4.4	>10	1.4	>10
(µg/mL)	Breast adn	MCF-7	$3.2x10^{-2}$	$1.9x10^{-2}$	>10	$8.8x10^{-2}$	5.2	>10	>10	>10	>10	>10	>10	2.8	13.7	$3.2x10^{-1}$	11.4
	CNS Gliobl	SF268	$1.7x10^{-2}$	$2.1x10^{-2}$	>10	$5.5x10^{-2}$	9.9	>10	>10	>10	>10	>10	>10	3.5	>10	$8.3x10^{-1}$	>10
	Lung-NSC	NCI-H460	$4.8x10^{-2}$	$3.2x10^{-2}$	>10	$2.8x10^{-1}$	>10	>10	>10	>10	>10	>10	>10	1.6	>10	$2.1x10^{-1}$	>10
	Colon	KM20L2	$2.6x10^{-2}$	$2.1x10^{-2}$	>10	$4.3x10^{-2}$	3.6	>10	>10	>10	>10	>10	>10	2.8	>10	$4.9x10^{-1}$	5.3
	Prostate	DU-145	$1.6X10^{-2}$	$1.1x10^{-2}$	>10	$2.3X10^{-2}$	4.3	>10	>10	>10	>10	>10	>10	2.4	>10	$2.8X10^{-1}$	14.4
TGI	Pancreas-a	BXPC-3	$1.4x10^{-1}$	$1.1x10^{-2}$	>10	>1	>10	>10	>10	>10	>10	>10	>10	>10	>10	6.9	>10
(µg/mL)	Breast adn	MCF-7	$1.1x10^{-1}$	$9.3x10^{-2}$	>10	>1	>10	>10	>10	>10	>10	>10	>10	9.4	>10	1.5	>10
	CNS Gliobl	SF268	$9.1x10^{-2}$	$9.9x10^{-2}$	>10	$10.0x10^{-1}$	>10	>10	>10	>10	>10	>10	>10	>10	>10	5.2	>10
	Lung-NSC	NCI-H460	$4.3x10^{-1}$	$1.6x10^{-1}$	>10	>1	>10	>10	>10	>10	>10	>10	>10	5.9	>10	$7.1x10^{-1}$	>10
	Colon	KM20L2	$2.3x10^{-1}$	$9.6x10^{-2}$	>10	$4.3x10^{-1}$	>10	>10	>10	>10	>10	>10	>10	>10	>10	4.9	>10
	Prostate	DU-145	$10.0x10^{-2}$	$5.6x10^{-2}$	>10	$3.2x10^{-1}$	>10	>10	>10	>10	>10	>10	>10	7.2	>10	$9.5x10^{-1}$	>10
ED50	Murine	P388	$3.9x10^{-2}$	$1.2x10^{-3}$	15.9	$2.7x10^{-2}$	1.7	>10	>10	>10	>10	69.7	5.4	$5.4x10^{-1}$	62.2	$3.8x10^{-1}$	40.4
(µg/mL)	Leukemia																

instruments. The EIMS spectra were determined using a Finnigan-MAT spectrometer (model 312) instrument. Analytical combustion analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee.

10b(*R*)-Hydroxypancratistatin (3).

Before adding narciclasine (**2**), (0.7 g, 2.28 mmol), a solution of DMF-water (9:1 mL) and hydroquinidine 4-chlorobenzoate (0.1 g, 0.21 mmol, 10.8 eq.), *N*-methyl-morpholine-*N*-oxide (NMO) (0.6 mL, 50% in water) and osmium tetroxide (0.1 mL, 4 mol%) was stirred at 0°C for 1 h. The reaction mixture was allowed to warm to rt and stirred for 24 h. Sodium metabisulfite (2 g) was added followed by an equal volume of acetone and sodium sulfate (2 g). The mixture was stirred at rt for a further 2 h. The solid was collected and the solvent concentrated (under vacuum). The crude product was separated by column chromatography on silica gel using dichloromethane-methanol (1:9) as eluent to give a white solid, (0.3 g, 40%): mp 269°C, $[\alpha]_D^{22}$ +70° (c 0.58, CH_3OH); EIMS *m/z* (%) 341 (M^+, 27), 250(15), 233(16), 222(100), 165(11, 135(6), 107(3), 73(26Z), 60(9); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.75 (s, 1H), 8.40 (s, 1H), 6.61 (s, 1H), 6.02 (m, 2H), 5.48 (d, *J* = 5.7 Hz, 1H), 5.40 (s, 1H), 4.75 (d, *J* = 6 Hz, 1H), 4.62 (d, *J* = 4.8 Hz, 1H), 4.55 (d, *J* = 7.2 Hz, 1H), 4.02 (m, 1H), 3.74 (d, *J* = 3 Hz, 1H), 3.6-3.5 (m, 2H), 3.15 (t, *J* = 8.0 Hz, 1H) ppm. ^{13}C NMR (300 MHz, DMSO-d_6) δ 169.9, 152.3, 145.0, 140.9, 132.8, 104.7, 102.5, 99.9, 75.6, 74.4, 71.8, 71.6, 71.3, 58.2 ppm. Anal. Calcd for $C_{14}H_{15}NO_9$. $1H_2O$; C, 46.83; H, 4.77; N, 3.90. Found: C, 46.95; H, 4.79; N, 3.96.

2,3,4,7-Tetra-*O*-acetylnarciclasine (4).[27]

To a solution of pyridine (5 mL) and acetic anhydride (5 mL) was added narciclasine (0.9 g, 3.25 mmol) and the mixture stirred at rt for 16 h. Iced water was added and the white precipitate was collected by filtration and recrystallized from dichloromethane: methanol to give a colorless crystalline compound (0.85 g, 61%), mp 249°C, $[\alpha]_D^{24}$ +229° (c 0.33, $CHCl_3$), EIMS *m/z* (%) 475 (M^+, 5) 433(33), 373(6), 313(29), 271(100), 242(11), 169(2), 140(3), 83(8), 60(3). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.92 (s, 1H), 6.15 (m, 1H), 6.10 (d, *J* = 1 Hz, 1H), 6.09 (d, *J* = 1 Hz, 1H), 5.86 (s, 1H), 5.43 (m, 1H), 5.34 (m, 1H), 5.2 (ddd, *J* = 1.2, 2.4, 9 Hz, 1H), 4.57 (br d, *J* = 9 Hz, 1H), 2.37 (s, 3H), 2.12 (s, 3H), 2.1 (s, 3H), 2.08 (s, 3H) ppm. ^{13}C NMR (500 MHz, $CDCl_3$) δ 170.5, 169.7, 169.5, 169.0, 162.2, 152.5, 141.6, 134.2, 133.8, 131.6, 118.2, 114.7, 103.1, 101.9, 71.4, 68.3, 68.04, 50.2, 20.8, 20.9, 20.7 ppm.

10b(*R*)-Hydroxy-2,3,4,7-tetra-*O*-acetylpancratistatin (5).

Dimethylformamide (9 mL) and water (1 mL) were stirred together in a round-bottomed flask. Hydroquinine 4-chlorobenzoate (0.1 g, 0.2 mmol, 17 mol%) was added followed by osmium tetroxide (0.1 mL, 0.1 mmol, 8 mol%) and the reaction mixture was stirred and cooled to 0°C for 1 h before adding **3** (0.6 g. 1.26 mmol). The reaction mixture was allowed to warm to rt and stirred for a further 40 h. Sodium metabisulfite (2 g) was added followed by an equal volume of acetone. Sodium sulfate (2 g) was added and the reaction mixture stirred for 4 h. The solids were collected by filtration and the filtrate concentrated to a crude solid which crystallized from methanol at

0°C in a colorless crystalline solid (0.425 g, 66%). Further recrystallization from methanol gave crystals which were suitable for X-Ray crystallography. mp 266°C, $[\alpha]_D^{25}$ = +165° (c 0.55, DMSO), EIMS *m/z* (%) 509 (M^+, 9), 467(16), 371(12), 329(18), 287(100), 258(21), 222(63), 201(5), 165(5), 115(5), 60(98). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.12 (s, 1H), 6.13 (s, 1H), 6.11 (s, 1H), 5.72 (s, 1H), 5.44 (t, *J* = 10.5 Hz, 1H), 5.29-5.20 (m, 3H), 3.80 (d, *J* = 1.8 Hz, 1H), 3.50 (t, *J* = 8.5 Hz, 1H), 2.22 (s, 3H), 2.04 (s, 3H), 1.60 (s, 3H), 1.93 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO-d_6) δ 169.8, 169.6, 169.2, 168.4, 161.9, 150.8, 140.9, 139.2, 132.1, 112.9, 105.3, 102.8, 72.8, 71.9, 70.9, 69.4, 68.2, 55.9, 20.9, 20.7, 20.6, 20.4, ppm. Anal. Calcd for $C_{22}H_{23}NO_{13}.CH_3OH$: C, 51.06; H, 5.03; N, 2.59. Found: C, 51.27; H, 4.69; N, 2.61.

Narciclasine-3,4-acetonide (6).

2,2-Dimethoxypropane (25 mL) was added to a solution of narciclasine (**2**, 5 g, 16.3 mmol) in DMF (25 mL). *p*-Toluenesulfonic acid (0.83 g) was added to the solution and the reaction 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 using vacuum filtration, washed with water and dried at 64°C over P_2O_5 under high vacuum to give an amorphous powder of narciclasine-3,4-acetonide (5.49 g, 97%), mp 270-273°C (lit.[28] mp 275°C), IR (KBr) 3497, 3184, 1676, 1599, 1467, 1373, 1294, 1111, 1039 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.70 (s, 1H), 8.80 (s, 1H), 7.01 (s, 1H), 6.47 (br s, 1H), 6.06 (m, 2H), 5.73 (br s, 1H), 4.2-3.90 (m, 4H), 1.45 (s, 3H), 1.31 (s, 1H) ppm. ^{13}C NMR (300 MHz, DMSO-d_6) δ 167.6, 152.5, 145.2, 133.3, 128.8, 128.3, 125.98, 109.8, 104.3, 102.0, 94.2, 79.0 ppm. EIMS *m/z* (%) 347 (M^+, 28); 332(1), 289(2), 273(6), 260(8), 247(100), 242(22), 218(10), 85(2), 73(10).

***N*-Methylnarciclasine-3,4-acetonide (7).**

Compound (**6**) (1.3 g, 2.88 mmol) was dissolved in dry acetone (50 mL) and DMF (56 mL), and stirred under argon at rt. Potassium carbonate (1.0 g, 5.06 mmol) was added followed by methyl iodide (1 mL, 16 mmol) and the reaction mixture was heated at reflux for 18 h to give one product by TLC (chloroform:acetone 4:1). The mixture was cooled and chloroform (100 mL) was added. The organic layer was washed with water (2 x 100 mL) followed by brine and dried over sodium sulfate. The solvent was removed by concentration under vacuum to give a residue (1.10 g). Column chromatography on silica gel (eluent; chloroform) yielded compound (**7**, 1 g, 96%) following recrystallization from chloroform:acetone: mp 245-247°C, R_f 0.78, (CH_2Cl_2:MeOH 4%). EIMS *m/z* (%) 361 (M^+, 28), 343(1), 303(4), 286(6), 274(5), 260(100), 256(40), 232(14), 204(3), 174(3), 118(3), 85(5), 77(5). ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.42 (s, 1H), 6.65 (s, 1H), 6.35 (t, *J* = 3 Hz, 1H), 6.03 (d, *J* = 1 Hz, 1H), 6.02 (d, *J* = 1 Hz, 1H), 4.31-4.29 (m, 1H), 4.25 (t, *J* = 7.75, Hz, 1H), 4.13 (m, 1H), 3.97 (t, *J* = 7.75 Hz, 1H), 3.26 (s, 3H), 1.55 (s, 3H), 1.37 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 166.7, 152.6, 145.8, 134.5, 127.7, 127.6, 125.1, 11.7, 105.4, 102.3, 93.4, 79.6, 79.3, 72.6, 61.9, 33.3, 27.1, 24.7 ppm. HRMS (FAB^+): calcd for $C_{18}H_{20}NO_7$ ($M+H^+$) 362.3579. Found: 362.1242. Anal. Calcd for $C_{18}H_{19}NO_7$: C, 59.83; H,

5.30; N, 3.88. Found: C, 59.66; H, 5.40; N, 3.86.

2-*O*-Acetyl-*N*-methylnarciclasine-3,4-acetonide (8).

To a solution of **7** (1 g, 2.77 mmol) in pyridine (6 mL) was added acetic anhydride (0.3 mL, 3.17 mmol). The reaction mixture was stirred and monitored by TLC (hexane:acetone 6:4). The reaction was stopped after 24 h with the addition of iced water (100 mL). A white crystalline compound precipitated from solution. The compound was filtered and recrystallized from hot ethanol to yield **8** (0.95 g, 85%) mp 95°C, R_f 0.86 (CH_2Cl_2:MeOH 2%) $[\alpha]_D^{23}$ = +75° (c 0.48, $CHCl_3$) EIMS *m/z* (%) 403 (M^+, 46), 345(24), 303(50), 285(27), 274(53), 261(100), 257(64), 232(1), 191(6), 149(6), 115(5), 85(8), 60(4). ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.39 (s, 1H), 6.61 (s, 1H), 6.03 (d, *J* = 1.5 Hz, 1H), 6.01 (d, *J* = 1.5 Hz, 1H), 5.28 (m, 1H), 4.26-4.17 (m, 3H), 3.26 (s, 3H), 3.20 (s, 3H), 1.55 (s, 3H), 1.36 (s, 3H) ppm. ^{13}C NMR (500 MHz, $CDCl_3$) δ 170.5, 166.7, 152.6, 145.8, 134.8, 128.7, 127.4, 122.4, 111.8, 105.4, 102.3, 93.6, 79.02, 75.9, 73.9, 61.7, 33.4, 26.9, 24.7, 21.1 ppm. Anal. Calcd for $C_{20}H_{21}NO_8$: C, 59.55; H, 5.25; N, 3.47. Found: C, 59.18; H, 5.41; N, 3.47.

2-*O*-Acetyl-*N*-methyl-10b(S)-hydroxy-1-epipancratistatin 3,4-acetonide (9) and 2-*O*-acetyl-*N*-methyl-10b(R)-hydroxypancratistatin-3,4-acetonide (10).

A 100 ml round-bottomed flask was charged with acetone (7 mL) and water (2 mL), NMO (60% aq soln, 0.59 mL, 3.3 mmol), and dihydroquinidine 4-chlorobenzoate (0.59 g, 0.11 mmol). The mixture was cooled in an ice-salt bath over a cold plate stirrer and OsO_4 (50 mL of a 1 M soln in toluene, 12.5 mmol) was added. The solution was stirred at 0°C for 1 h before adding **8** (0.9 g, 0.22 mmol). The reaction mixture was then stirred at 15°C for 20 h. Sodium metabisulfite (2 g) was added and the mixture was stirred for 1 h. Dichloromethane (equal volume) was next added and the mixture was stirred for a further 30 min. Sodium sulfate (2 g) was added and the mixture was stirred for 30 min. The pink solids were collected by filtration through a bed of celite and washed several times with dichloromethane. The filtrate and washings were concentrated to a yellow solid which was dissolved in ethyl acetate (350 mL) and washed with 2 N HC1 (3 x 50 mL) and water (100 mL), dried over Na_2SO_4, and concentrated to a yellow solid 0.7 g. Column chromatography on silica gel (eluent; chloroform:acetone 14%) gave **9** (0.275 g, 28%). It was recrystallized from acetone:hexane to give crystals which were examined by X-Ray crystallography. R_f 0.28 ($CHCl_3$:CH_3COCH_3 8:1), mp 255°C, $[\alpha]_D$ +95.8° (c 0.26, $CHCl_3$), EIMS *m/z* (%), 437 (M^+, 62), 377(4), 302(4), 252(87), 235(100), 206(9), 149(8), 85(13), 59(13). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.9 (s, 1H), 6.83 (s, 1H), 5.94 (s, 2H), 4.85 (dd, *J* = 6.2, 11 Hz, 1H), 4.62-4.47 (m, 3H), 3.68 (d, *J* = 6.8 Hz, 1H), 3.63 (s,s 1H), 3.20 (m, 4H), 2.15 (s, 3H), 1.43 (s, 3H), 1.35 (s, 3H) ppm. ^{13}C NMR (400 MHz, $CDCl_3$) δ 170.3, 167.9, 152.5, 145.5, 135.4, 134.4, 110.4, 106.2, 102.4, 97.6, 75.7, 72.6, 71.3, 70.4, 68.9, 61.4, 30.1, 27.7, 25.6, 20.9 ppm. Anal. Calcd for $C_{20}H_{23}NO_{10}$: C, 54.92; H, 5.30; N, 3.20. Found: C, 54.80; H, 5.40; N, 3.19.

Continuing elution gave **10** (0.139 g, 14%), which upon recrystallization (acetone:hexane) gave crystals which were examined by X-Ray crystallography, R_f 0.18

($CHCl_3$:CH_3COCH_3), mp 253°C, $[\alpha]_D^{23}$ -65.4° (c 0.46, $CHCl_3$) EIMS *m/z* (%) 437 (M^+, 62), 377(4), 302(4), 252(16), 234(87), 235(100), 206(9), 149(8), 85(13), 59(13). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.60 (s, 1H), 6.78 (s, 1H), 6.04 (m, 2H), 5.32 (dd, *J* = 7, 10 Hz, 1H), 4.18 (t, *J* = 7.2 Hz, 1H), 4.12-3.97 (m, 3H), 3.67 (d, *J* = 10 Hz, 1H), 3.59 (d, *J* = 8.8 Hz, 1H), 3.26 (s, 3H), 2.17 (s, 3H), 1.55 (s, 3H), 1.28 (s, 3H) ppm. ^{13}C NMR (400 MHz, $CDCl_3$) δ 171.3, 166.2, 152.7, 145.5, 134.4, 110.7, 106.6, 102.5, 98.3, 75.8, 75.5, 72.4, 71.9, 68.4, 66.5, 36.8, 27.1, 24.6, 21.1 ppm. Anal. Calcd for $C_{20}H_{23}NO_{10}$: C, 54.92; H, 5.30; N, 3.20. Found: C, 55.00; H, 5.71; N, 3.08.

The X-Ray crystal structure determination of 2-*O*-acetyl-*N*-methyl-10b(S)-hydroxy-1-epipancratistatin-3,4-acetonide (9).

A small, block-shaped crystal (~0.24 x 0.20 x 0.20 mm), grown from CH_3COCH_3:hexane solution, was mounted on the tip of a glass fiber. Cell parameter measurements and data collection were performed at 296 ± 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: $C_{20}H_{23}N_1O_{10}$, FW=437.39, orthorhombic, $P2_12_12_1$, a=11.443(2), b=12.354(3), c=14.356(3) Å, V=2029.5(7)) Å^3, Z=4, ρ_c=1.432 Mg/m^3, μ(CuKα)=0.991 mm^{-1}, λ=1.54178 Å.

All reflections corresponding to a complete octant (0<=h<=11, 0<=k<=13, 0<=1<=16) were collected over the range of 0<2θ<130° using the ω/2θ scan technique. Friedel reflections were also collected (whenever possible) immediately after each reflection. Three intensity control reflections were also measured for every 60 minutes of X-Ray exposure time and showed a maximum variation of -1.2% over the course of the collection. A total of 1599 reflections were collected. Subsequent statistical analysis of the complete reflection data set using the XPREP[29] program, verified that the space group as $P2_12_12_1$. A total of 1499 reflections were considered observed ($I_o > 2\sigma(I_o)$) and 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).[30] Structure determination and refinement was readily accomplished with the direct-methods program in SHELXTL-pc.[31] All non-hydrogen atom coordinates were located in a routine run using default values for that program. The remaining H atom coordinates were calculated at optimum positions. All non-hydrogen atoms were refined anisotropically in a full-matrix least-squares refinement procedure. The H atoms were included, their Uiso thermal parameters fixed at either 1.2 or 1.5 (depending on atom type) 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 **9** was 0.0511 for observed data and 0.0550 for all data. The goodness-of-fit on F^2 was 1.076. The corresponding Sheldrick R values were wR_2 of 0.1410 and 0.1455, respectively. A final difference Fourier map showed minimal residual electron density; the largest difference peak and hole being 0.234 and -0.277 e/Å^3, respectively. Final bond distances and angles were all within expected and acceptable limits. The absolute stereochemical structure of **9**, as shown in Figure

1, was assigned by relaying the known absolute stereochemistry of the unaffected chiral centers at C3 and C4 in the starting material (narciclasine). Thus, using the numbering shown in Figure 1, the chiral centers of **9** can be assigned as follows: 1R, 2S, 3S, 4S, 4aS, 10bS.

10b(*S*)-Hydroxy-1-epipancratistatin-3,4-acetonide (11) and 10b(*R*)-hydroxy-pancratistatin-3,4-acetonide (12).

A solution of DMF (4.5 mL) and H_2O (0.5 mL) was stirred at 15°C on a cold plate stirrer; NMO (60% by wt/H_2O) (0.3 mL) was added followed by $(DHQD)_2$ PHAL (0.045 g, 0.053 mmol) and OsO_4 [100 μL of a 0.65 M biphasic solution toluene (4 mL) and H_2O (2 mL). The solution turned orange upon addition of the OsO_4 and was stirred for 1 h before adding **6** (0.7 g, 2.02 mmol). The reaction mixture was stirred at 17°C for 24 h. Sodium metabisulfite (2 g) was added followed by acetone (5 mL), and the mixture was stirred for 30 min before adding Na_2SO_4(2 g). The mixture was stirred for a further 2 h before collecting the salts by filtration. The filtrate was concentrated under vacuum followed by high vacuum to remove the DMF. The brown residue was separated using flash chromatography on silica gel and a gradient elution of CH_2Cl_2:CH_3OH 2%-CH_2Cl_2:CH_3OH 5% to give diol (**11**) (0.38 g, 54%) and diol (**12**) (0.17 g, 24%). Diol (**11**) was recrystallized from CH_2Cl_2:CH_3OH 1:1 (minimum volume with heating), needlelike crystals were obtained R_f 0.28 (CH_2Cl_2:CH_3OH 4%), mp 225°C, $[\alpha]_D^{25}$ +15.1° (c 0.93, DMSO), EIMS *m/z* (%) 381 (M^+), 366, 323, 306, 288, 263, 234, 221, 73, 60. IR (thin film) 3381, 3055, 2945, 2833, 1674, 1643, 1602, 1348, 1267, 1028, 738 cm^{-1}. ^1H NMR (500 MHz DMSO-d_6) δ 13.30 (br s, 1H), 8.63 (s, 1H), 7.20 (s, 1H), 6.06 (s, 1H), 6.02 (s, 1H), 5.30 (br s, 1H), 5.23 (s, 1H), 5.10 (s, 1H), 4.30 (t, *J* = 7.2 Hz, 1H), 4.10 (m, 1H), 3.80 (m, 1H), 3.50 (d, *J* = 8.5 Hz, 1H), 1.31 (s, 3H), 1.39 (s, 1H) ppm. ^{13}C NMR (500 MHz DMSO-d_6) δ 168.6, 151.5, 145.0, 138.8, 132.7, 108.6, 106.4, 101.8, 98.3, 76.0, 74.9, 69.7, 69.4, 69.3, 56.3, 27.9, 25.5 ppm. Anal Calcd for $C_{17}H_{19}NO_9$• 1/2 H_2O: C, 52.35; ;H, 5.12; N, 3.59. Found: C, 52.39; H, 5.02; N, 3.67.

Diol (**12**) R_f 0.20 (CH_2Cl_2:CH_3OH 4%) mp 229-230°C, $[\alpha]_D^{25}$ = + 31.3° (c 0.81, DMSO), EIMS *m/z* (%) 381 (M^+) 366, 323, 234, 221, 165, 85, 73, 60, 44. IR (thin film) 3396, 2918, 1672, 1624, 1467, 1354, 1221, 1078, 1016 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ 6.85 (s, 1H), 6.00 (s, 2H), 4.25 (dd, *J* = 6, 4 Hz, 1H), 4.13 (t, *J* = 6.7 Hz, 1H), 4.05 (d, *J* = 4 Hz, 1H), 3.97 (dd, *J* = 8, 10 Hz, 1H), 3.55 (d, *J* = 10 Hz, 1H), 1.53 (s, 1H), 1.35 (s, 3H) ppm. ^{13}C NMR (500 MHz, CD_3OD) δ 170.8, 154.2, 146.4, 141.0, 134.7, 110.7, 106.1, 76.9, 73.8, 73.7, 73.1, 58.2, 28.3, 26.1 ppm. Anal Calcd for $C_{17}H_{19}NO_9$: C, 53.55; H, 5.02; N, 3.67. Found: C, 53.23; H, 5.23; N, 3.38.

10b(*S*)-Hydroxy-1-epipancratistatin (13).

To a solution of THF (2 mL) was added **7** (0.086 g, 0.225 mmol). The solution was stirred at rt, water (3 drops from a pipette) and conc. H_2SO_4 (2 drops from a pipette) was added, and the mixture was stirred for 22 h at rt. THF (10 mL) was added and ethyl acetate (5 mL) was added. The solution was neutralized by washing with 5% $NaHCO_3$. The organic layer was separated and washed with water (10 mL) dried

Na_2SO_4, and concentrated to a white solid (0.04 g, 52%), which was recrystallized from CH_2Cl_2 CH_3OH (1:1), mp 185° $[\alpha]_D$= -54.5° (c 0.2, DMSO-d_6). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.03 (s, 1H), 7.48 (s, 1H), 7.44 (s, 1H), 6.06 (s, 1H), 6.02 (s, 1H), 5.44 (br s, 1H), 5.03-4.99 (m, 3H), 3.98-3.92 (m, 2H), 3.83 (br s, 2H), 3.49 (d, *J* = 10.8 Hz, 1H) ppm. ^{13}C (500 MHz, DMSO-d_6) δ 169.1, 151.2, 144.9, 138.9, 132.6, 107, 101.8, 98.6, 74.9, 71.9, 71.5, 67.4, 64.9, 55.9 ppm. Anal. Calcd for $C_{14}H_{15}NO_9 \cdot CH_3OH$; C, 48.26; H, 5.13; N, 3.75. Found: C, 48.9; H, 5.31; N, 3.38.

Cyclic sulfate (14).

Diol (**7**) (0.13 g, 0.34 mmol) was dissolved in dry THF (3.5 mL), triethylamine (0.19 mL, 1.36 mmol) was added and the reaction mixture cooled to 0°C using an ice bath before adding $SOCl_2$ (0.027 mL, 0.4 mmol). The reaction mixture was stirred at 0°C for 10 min and TLC (CH_2Cl_2:CH_3OH 4%) showed complete conversion to product. The reaction was stopped after 15 min by the addition of ethyl acetate (20 mL). The organic layer was washed with water (2 x 10 mL) followed by brine (10 mL), dried ($MgSO_4$), filtered and concentrated to a glassy solid. The solid was purified by passing it through a silica gel column using flash chromatography and ethyl acetate as the eluent before proceeding to the next step. The clear oil (0.12 g) was taken up in CCl_4:CH_3CN 1.2:1.5, and stirred at rt until dissolved. $RuCl_3.3H_2O$ (0.0054 g, 0.027 mmol) and $NaIO_4$ (0.096 g, 0.447 mmol) were added together to the reaction solution quickly followed by H_2O (1.2 mL). The black-green mixture was stirred for 50 min before adding ethyl acetate (20 mL). The organic layer was washed with water (3 x 5 mL), brine (1 x 5 mL) and dried ($MgSO_4$), filtered through a pad of silica gel, and concentrated to a crystalline solid (0.079 g, 63% yield), R_f 0.8 (DCM:MeOH 4%), mp 169-170°C, $[\alpha]_D^{25}$ = -3.04° (c 0.82, CH_3CN), EIMS *m/z* (%) 443(31), 355(16), 287(26), 267(14), 249(16), 221(15), 207(100), 191(21), 133(37), 96(17), 73(19), 64(66), 59(37), 49(32), 44(91). IR (thin film) 3213, 2976, 2872, 1676, 1464, 1400, 1356, 1213, 1068, 898, 856 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.24 (s, 1H), 9.12 (s, 1H), 6.72 (s, 1H), 6.55 (s, 1H), 6.14 (s, 1H), 6.10 (s, 1H), 5.59 (d, *J* = 10 Hz, 1H), 5.19 (t, *J* = 8.25 Hz, 1H), 4.73 (t, *J* = 7.75 Hz, 1H), 4.50 (t, *J* = 7.5 Hz, 1H), 3.74 (d, *J* = 7 Hz, 1H), 1.46 (s, 3H), 1.35 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO-d_6) δ 167.8, 152.0, 145.2, 134.4, 133.8, 110.5, 105.9, 102.4, 98.0, 84.4, 82.2, 75.8, 73.9, 54.9, 27.3, 24.9 ppm. Anal. Calcd for $C_{17}H_{17}NO_{11}S$; C, 46.05; H, 3.86; N, 3.16. Found: C, 45.76; H, 4.50; N, 3.07.

2-Benzoyl-10b(*S*)-1,2-diepipancratistatin (15).

A DMF (4 mL) solution of **10** (0.35 g, 0.79 mmol) was stirred at 65°C under reflux and a drying tube. Benzoic acid (0.145 g, 1.06 mmol) was added, followed by Cs_2CO_3 (0.41 g, 1.26 mmol). The reaction mixture was stirred for 23 h at 65°C, and all the product was on the base line by TLC (CH_2Cl_2:CH_3OH 4%). The reaction mixture was concentrated under high vacuum to remove the DMF. The brown residue was taken up in THF (10 mL) and water (12 drops from a pipette) was added to the suspension followed by H_2SO_4 (8 drops from a pipette). The reaction mixture was heated under reflux to 65-75°C for 2 h. The crude reaction mixture was purified on a column of

silica gel using flash chromatography and CH_2Cl_2:CH_3OH 10% as the eluent. The C-1-benzoyl product was isolated as a crystalline solid (0.215 g, 61.2%) R_f 0.41 (CH_2Cl_2:CH_3OH 10%), mp 185°C, $[\alpha]_D^{21}$ +39.5° (c 0.56, EtOH); IR (thin film) 3367, 2362, 1703, 1670, 1620, 1465, 1342, 1280, 1122, 1085, 1028, 9378 cm^{-1}. EIMS *m/z* (%), 445 (M^+, 34), 341(11), 287(5), 263(10), 233(18), 221(16), 205(7), 122(45), 105(100), 77(53), 45(62). ^1H NMR (500 MHz, DMSO-d_6) δ 13.08 (br s, 1H), 8.06 (d, *J* = 7.5 Hz, 2H), 7.66 (t, *J* = 7.5 Hz, 1H), 7.54 (t, *J* = 7.5 Hz, 2H), 7.47 (s, 1H), 7.41 (s, 1H), 6.06 (s, 1H), 6.04 (s, 1H), 5.43 (s, 1H), 5.39 (d, *J* = 7.5 Hz, 1H), 5.28 (d, *J* = 4 Hz, 1H), 5.15 (d, *J* = 5.5 Hz, 1H), 5.02 (dd, *J* = 10.0, 2.5 Hz, 1H), 4.29 (t, *J* = 8.5 Hz, 1H), 4.08 (br s, 1H), 3.80 (m, 1H), 3.60 (d, *J* = 10.5 Hz, 1H) ppm. ^{13}C NMR (500 MHz, DMSO-d_6) δ 169.2, 165.8, 151.2, 145.1, 139.0, 133.2, 132.7, 130.3, 129.5(2C), 128.5(2C), 107.4, 101.9, 98.8, 75.6, 69.5, 68.8, 68.9, 66.7, 55.7 ppm. HRMS (FAB): calcd for $C_{21}H_{19}NO_{10}$ (M^+) 445.1009. Found: 445.0992.

10b(*S*)-Hydroxy-1,2-diepipancratistatin (16).

Compound (**15**) (0.172 g, 0.386 mmol) was dissolved in CH_3OH (5 mL) and stirred at rt, and K_2CO_3 (0.01 g, 0.06 mmol) was added. A white precipitate developed. TLC (CH_2Cl_2:CH_3OH 10%) showed that no starting material remained after 7 h stirring. The reaction mixture was concentrated to a white solid which was dissolved in THF and the insoluble salts were filtered off. The solution was concentrated to a white solid (0.07 g, 53%), which crystallized from hot methanol in crystals that were examined by X-Ray crystallography, R_f 0.07 (CH_2Cl_2:CH_3OH 10%), mp 199.4-200°C, $[\alpha]_D^{25}$ = -30.2° (c 0.57, MeOH), EIMS *m/z* (%), 341 (M^+, 78), 287(3), 247(11), 233(64), 221(35), 205(21), 175(18), 165(16), 122(20), 105(32), 91(16), 77(26), 45(100). ^1H NMR (500 MHz, DMSO-d_6) δ 13.07 (s, 1H), 7.41 (s, 1H), 7.35 (s, 1H), 6.05 (d, *J* = 0.5 Hz, 1H), 6.01 (d, *J* = 0.5 Hz, 1H), 5.03 (s, 1H), 4.90 (d, *J* = 7.0 Hz, 1H), 4.86 (d, *J* = 6.5 Hz, 1H), 4.80 (d, *J* = 3.5 Hz, 1H), 4.64 (d, *J* = 6.5 Hz, 1H), 3.84 (m, 2H), 3.68 (m, 1H), 3.50 (m, 2H) ppm. ^{13}C NMR (500 MHz, DMSO-d_6) δ 169.2, 151.2, 145.0, 139.7, 132.5, 107.2, 101.8, 98.9, 71.9, 71.6, 71.3, 68.9, 67.1, 55.9 ppm. Anal. Calcd for $C_{14}H_{15}NO_9 \cdot CH_3OH$. C, 48.26; H, 5.13; N, 3.75. Found: C, 48.02; H, 4.50; N, 3.90.

X-Ray crystal structure determination of 16.

A thin plate (~0.04 x 0.20 x 0.24 mm), grown from hot methanol solution, was mounted on the tip of a glass fiber. Cell parameter measurements and data collection were performed at 298 ± 1°K with a Bruker SMART 6000 diffractometer system using CuKα radiation. A sphere of reciprocal space was covered using the multirun technique.[31] Thus, six sets of frames of data were collected with 0.40° steps in ω, and a last set of frames with 0.40° steps in ω such that 97.5% coverage of all unique reflections to a resolution of 0.84Å was accomplished. Crystal Data: $C_{16}H_{23}NO_{11} \cdot 2\ CH_3OH$ (methanol solvate), FW=405.35 monoclinic, $P2_1$, a=7.8697(4), b=7.4195(4), c=14.9035(8) Å, β=95.131(3)°, V=866.72(8) $Å^3$, Z=2, ρ_c=1.553 Mg/m^3, μ(CuKα)=1.147 mm^{-1}, λ=1.54178 Å.

A total of 6046 reflections were collected, of which 2366 reflections were

independent reflections (R(int)=0.0870). Subsequent statistical analysis of the data set with the XPREP[29] program indicated the spacegroup was $P2_1$. Final cell constants were determined from the set of the 2094 observed (>2σ (I)) reflections which were used in structure solution and refinement. An absorption correction was applied to the data with SADBS.[33] Structure determination and refinement was readily accomplished with the direct-methods program SHELXTL.[31] All non-hydrogen atom coordinates were located in a routine run using default values for that program. The remaining H atom coordinates were calculated at optimum positions. All non-hydrogen atoms were refined anisotropically in a full-matrix least-squares refinement procedure. The H atoms were included, their Uiso thermal parameters fixed at either 1.2 or 1.5 (depending on atom type) 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 **16** was 0.0594 for observed data and 0.0632 for all data. The goodness-of-fit on F^2 was 1.025. The corresponding Sheldrick R values were wR_2 of 0.1451 and 0.1475, respectively. A final difference Fourier map showed minimal residual electron density; the largest difference peak and hole being 0.307 and –0.322 e/Å^3, respectively. Final bond distances and angles were all within expected and acceptable limits. The Flack absolute structure parameter [8] for the model shown in Figure 2 is 0.0(4), indicating that the absolute stereochemical structure shown for **16** is correct. Consequently, the stereochemistry at the chiral centers in **16** can be assigned as follows: 1*R*, 2*S*, 3*S*, 4*S*, 4a*S*, 10b*S*.

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