Antineoplastic Agents. 550. Synthesis of 10b(*S*)-Epipancratistatin from (+)-Narciclasine[⊥,1]

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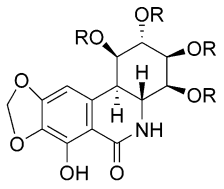
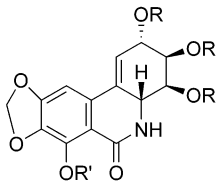
By means of a five-step reaction sequence, narciclasine (**2a**), isolated from *Narcissus* sp., was converted to 10b(*S*)-epipancratistatin (**3a**) in 5.7% overall yield. The key step entailed a radical-initiated 10b,1 C−O cleavage employing tributyltin hydride to yield a B/C *cis* ring juncture (**3b**). Biological evaluation of 10b(*S*)-epipancratistatin (**3a**) provided evidence that antineoplastic activity was reduced by a factor of 10 when the B/C *trans* juncture was replaced with a B/C *cis* ring juncture.

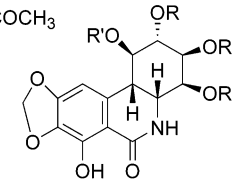
In 1984 we reported isolation of a new antineoplastic isocarbostyril, designated pancratistatin (**1**), from the Amaryllidaceae plant *Hymenocallis littorale*[2a] (a.k.a. *Pancratium littorale*). Initial *in vivo* evaluations of pancratistatin (**1a**) against the murine P388 lymphocytic leukemia and M5076 ovary sarcoma cell lines led to significant inhibition of these U.S. National Cancer Institute *in vivo* systems that exhibited 38−106% (0.75−12.5 mg/kg dose range) and 53−84% (0.38−3.0 mg/kg dose) life extensions, respectively. Because of the sparingly soluble properties and resistance to stable salt formation based on the phenol group, preclinical development of pancratistatin (**1a**) was delayed until we were able to synthesize promising phosphate prodrug modifications.[2b−e] Meanwhile, a variety of biological studies focused on pancratistatin have revealed an important selection of promising activities that include potent cancer vascular disruption (vascular shutdown complete within 2 h of treatment),[3] RNA antiviral,[4] antimicrosporidium,[5] and good stability (at least 15 days at rt) in human serum.[6] Such encouraging properties have led us and others to pursue efficient syntheses[7] and structural modifications[8] of pancratistatin (**1a**).



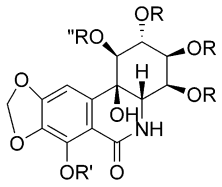
1a, R=H
1b, R= $COCH_3$



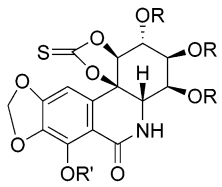
2a, R,R' = H
2b, R,R'=$COCH_3$
2c, R= $COCH_3$, R' = H



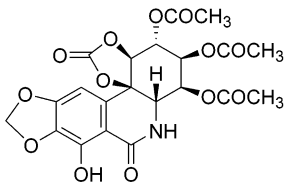
3a, R, R' = H
3b, R= $COCH_3$, R' = H
3c, R,R' = $COCH_3$



4a, R R' = $COCH_3$, R'' = H
4b, R = $COCH_3$, R', R'' = H
4c, R, R', R'' = $COCH_3$



5a, R R' = $COCH_3$
5b, R= $COCH_3$, R' = H



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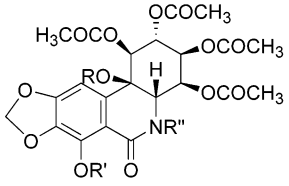
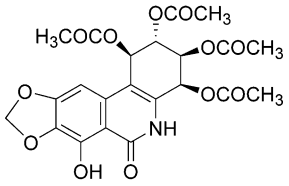


7a, R = $COCOOCH_3$, R', R'' =H
7b, R, R', R'' = $COCOOCH_3$



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Results and Discussion

As part of our research focused on utilizing the more readily available (from *Narcissus* sp.) narciclasine (**2a**) for a practical synthetic conversion to isocarbostyril **1a**[7] and for SAR studies, we, herein, summarize its conversion to the structural modification 10b-(*S*)-epipancratistatin (**3a**). Initially, the synthetic approach pursued was the deoxygenation of the tertiary benzylic alcohol group of 10b(*R*)-hydroxypancratistatin (**4**).[8c] Conventional synthetic approaches involving the dehydration of the 10b-tertiary alcohol followed by hydrogenation were expected to introduce the possibility of altering the configuration of the hydroxyl group at C-1. Other methods involving the reduction of a suitable alcohol derivative would be affected by steric hindrance and the possibility of elimination to the dehydro derivative. Therefore, radical-promoted deoxygenation of the 10b-tertiary alcohol was chosen as an attractive route to (+)-pancratistatin. Instead, this led to formation of the *cis* ring juncture isomer of pancratistatin (**3a**), which proved useful in our overall SAR evaluations.

Based on the pioneering investigations of Barton,[9] radical deoxygenation of the cyclic thiocarbonate (**5a**)[8c] was planned using Bu_3SnH. The more stable radical should be formed, followed by deoxygenation at the tertiary position. Attempts at synthesizing the cyclic thionocarbonate from the partially acetylated diol (**4a**) by treatment with thiocarbonyldiimidazole[10] in THF was challenging in that the 7-*O*-acetate proved to be unstable. Difficult-to-separate

⊥ Dedicated to the late Dr. Kenneth L. Rinehart of the University of Illinois at Urbana−Champaign for his pioneering work on bioactive natural products.

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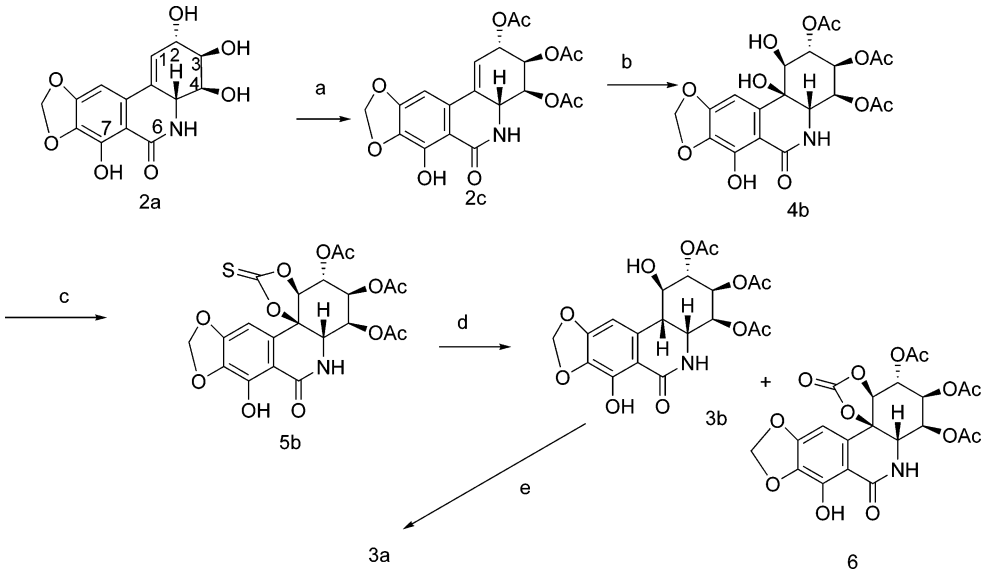
Table 1. Human Cancer Cell Line and Murine P-388 Lymphocytic Leukemia Inhibitory Activities of Structures **1**, **2a**−**c**, **3a**,**b**, **4a**−**c**, **5b**, **6**, and **7b**

	ED_{50} (μg/ml)			GI_{50} (μg/mL)			
compound	leukemia P-388	pancreas-a BXPC-3	breast MCF-7	CNS SF268	lung-NSC NCIH460	colon KM20L2	prostrate DU-145
1	0.017	0.02	0.023	0.014	0.032	0.025	0.015
2a	0.0012	0.0035	0.0032	0.0031	0.0084	0.0032	0.0032
2b	0.027	0.16	0.088	0.055	0.28	0.043	0.023
2c	0.013	0.011	0.060	0.16	0.033	0.051	0.028
3a	0.14	0.32	0.27	0.26	0.23	0.31	0.21
3b	>1	>1	>1	>1	>1	>1	>1
3c	0.20	1.8	2.0	3.0	1.3	1.8	1.4
4a	1.7	14.4	5.2	9.9	>10	3.6	4.3
4b	>10	>10	>10	>10	>10	>10	>10
4c	8.9	>10	>10	>10	>10	>10	>10
5b	>1	>1	>1	>1	>1	>1	>1
6	1.5	3.1	4.9	7.5	2.9	2.8	1.5
7b	>10	>10	>10	>10	>10	>10	>10

Scheme 1



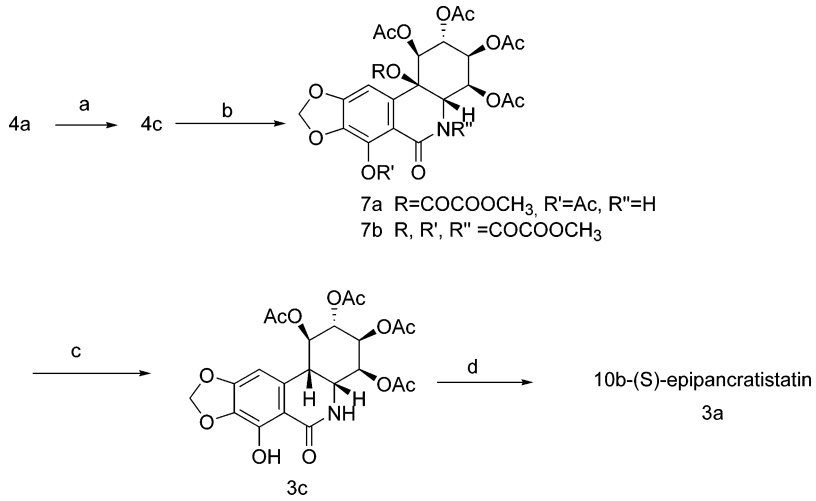
mixtures of the tetraacetate (**5a**) and triacetate (**5b**) were obtained. Because the tetraacetate of narciclasine (**2b**) was found to cause a contact dermatitis, it was decided to use narciclasine triacetate (**2c**) prepared by acetylating narciclasine employing 3.25 equiv of acetic anhydride and separating it from the resulting mixture of acetates using column chromatography. As part of the study, triacetate (**2c**) was also dihydroxylated using OsO_4 to yield diol **4b**.

Attempted synthesis of the cyclic thionocarbonate (**5b**) from **4b** was next carried out in various solvents, such as tetrahydrofuran, dimethylformamide, and dioxane, but the reaction did not go to completion and/or a number of products were obtained. The best results were realized with the method developed by Kutney and colleagues[11] using butan-2-one as the solvent and heating to 80 °C to afford thionocarbonate (**5b**) in 72% yield. Recrystallization from dichloromethane−ethanol yielded crystals suitable for X-ray analysis, and the resulting crystal structure was used for structure confirmation. When cyclic thionocarbonate (**5b**) was treated with Bu_3SnH and AIBN in toluene at reflux, the *cis* isomer (**3b**) was obtained in 50% yield, along with the cyclic carbonate (**6**) in 22% yield. The carbonate, recrystallized from dichloromethane−ethanol, yielded crystals suitable for X-ray crystallography, which was employed to confirm its structure and stereochemistry.

A potential synthetic route to natural (+)-pancratistatin (**1a)** was also evaluated and involved reduction of the tertiary methyloxalate[12] (**7a**) using Bu_3SnH (AIBN as radical initiator) in toluene. Here it was assumed that methyl oxalyl chloride would react with the hindered alcohol of **4c** to form methyl oxalate (**7a**). However, the product was a mixture of oxalates, and trioxalate (**7b**) was separated by crystallization (29% yield). When the methyl oxalate mixture was passed through a silica gel column using a mixture of 9:1 dichloromethane−methanol as eluent, an ^1H NMR of the fraction recovered revealed a mixture predominately consisting of the tertiary monomethyloxalate derivative (68% yield). Bu_3SnH reduction of the pure trioxalate or of the oxalate mixtures proceeded to yield the *cis* isomer (**3c**) as the major product. The dehydro product (**8**) and a trace amount of the pancratistatin tetraacetate derivative (**1b**) were also observed in the reaction product by ^1H NMR. In a final HPLC comparison experiment using pure pancratistatin tetraacetate as a reference compound, the presence of that compound in the reaction product was confirmed. When an excessive amount of AIBN was added to the reaction mixture, the yield of *cis* ring juncture increased, the amount of dehydro decreased considerably to a trace quantity, and no pancratistatin tetraacetate could be detected in the proton NMR of the reaction products. Further experiments directed at improving the yield of pancratistatin tetraacetate from the potentially very efficient conversion of (+)-narciclasine to natural pancratistatin should eventually be devised.

Evaluation of 10b(*S*)-epipancratistatin (**3a**) against the P388 lymphocytic leukemia cell line and a minipanel of human cancer cell lines (Table 1) showed that the epimerization at ring juncture position 10b reduced antineoplastic activity by a factor of 10.[13a] Preliminary evaluation[13b] of isomer **3a** and a selection of synthetic intermediates for antibacterial and/or antifungal activity did not provide any significant results.

Scheme 2



Experimental Section

General Experimental Procedures. Solvents were purified by redistillation, and in addition, tetrahydrofuran was distilled from sodium benzophenone and pyridine was dried over KOH pellets and redistilled prior to use. Organic extracts of aqueous solutions were dried using anhydrous Na_2SO_4. Thin-layer chromatography was performed on Analtech silica gel GHLF plates eluting with the solvents indicated. Visualization was provided by UV lamp and development with a ceric sulfate spray/heat. Flash column chromatography was conducted with Merck silica gel 60 slurry packed in columns with the initiating solvent.

Optical rotation values were recorded using a Perkin-Elmer 241 polarimeter. HPLC was performed with an analytical ZorbaxSB-C18 column (4.6 mm × 25 cm) and a semipreparative ZorbaxSB-C18 column (9.4 × 25 cm) using a Waters Co. Delta 600 HPLC with dual UV detectors. Melting points are uncorrected and were observed using an Electrothermal 9100 capillary and Fisher-Johns melting point apparatus. Nuclear magnetic resonance spectra were acquired at either 300 or 500 MHz for ^{1}H and 75 and 125 MHz for ^{13}C employing Varian Gemini 300 MHz and Varian Unity 500 MHz instruments. The mass spectra were determined using a JEOL LCmate magnetic sector in APCI mode with a polyethylene glycol reference. Analytical combustion analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. The X-ray crystal structure data were determined with a Bruker SMART 6000 diffractometer.

2,3,4,-Tri-*O*-acetylnarciclasine (2c). To a solution of narciclasine (**2a**) (5 g, 0.016 mol)[8c] in pyridine (38 mL) was added acetic anhydride (5 mL, 0.053 mol, 3.25 equiv), and the reaction mixture was stirred at rt under argon for 24 h. Ethyl acetate (400 mL) was added to the reaction mixture and the solution washed with water (4 × 100 mL), dried, and concentrated to a brown foam. The triacetate was isolated as a colorless, crystalline powder (2.05 g, 29%) following column chromatography on silica gel (eluent: DCM−CH_3OH 3%). Recrystallization from CH_2Cl_2−MeOH gave needles: mp 221 °C; $[\alpha]_D^{25}$ +152 (*c* 0.45, $CHCl_3$) [lit.[14a] mp 200−201 °C, lit.[14b] $[\alpha]_D^{23}$ +202 (*c* 0.44, $CHCl_3$)]; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.44 (1H, s, OH-7), 6.65 (1H, s, H-10), 6.22 (1H, s, NH), 6.15 (1H, nm, H-1), 6.08−6.06 (2H, m, O_2CH_3), 5.45 (1H, m), 5.35−5.32 (1H, m), 5.22 (1H, dd, J = 2.1, 9.6 Hz, H-4), 4.62 (1H, m, H-4a), 2.13 (3H, s, $OCOCH_3$), 2.11 (3H, s, $OCOCH_3$), 2.01 (3H, s, $OCOCH_3$); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 169.8, 169.1, 169.0, 168.6, 152.5, 145.3, 134.7, 132.7, 129.9, 117.7, 105.5, 102.1, 96.0, 70.7, 67.9, 67.4, 49.8, 20.3 (2 20.2).

10b(*R*)-Hydroxy-2,3,4-tri-*O*-acetylepipancratistatin (4b). To a 250 mL round-bottom flask were added dimethylformamide (9 mL) and water (1 mL) followed in succession by $(DHQ)_2PHAL$ (0.1 g, 0.128 mmol), 4-*N*-methylmorpholine *N*-oxide (0.6 mL, 60 wt % in H_2O), and osmium tetroxide (4 wt % in H_2O, 0.1 mL, 0.0157 mmol). The pale green solution was stirred for 2 h, and narciclasine triacetate (**2c**, 1 g, 2.3 mmol) was then added. The reaction was stopped following 22 h of stirring by the addition of sodium metabisulfite (4 g) followed by an equal volume of acetone and Na_2SO_4 (2 g). The mixture was stirred for 3 h, the precipitate collected, and the solvent concentrated to a yellow residue. Separation by flash column chromatography on silica gel (eluent: gradient 97:3 → 9:1 DCM−CH_3OH) yielded a white solid (0.92 g, 85% yield). Recrystallization from MeOH gave colorless crystals: mp 183 °C; $[\alpha]_D^{23}$ +94.2 (*c* 1.24, CH_3OH); HRMS (APCI) calcd for $C_{20}H_{22}NO_{12}$ $[M + H]^+$, 468.1142; found $[M + H]^+$ 468.1142; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.65 (1H, s, OH-7), 8.76 (1H, s, NH), 6.75 (1H, s, H-10), 6.06−6.05 (2H, m, $-O_2CH_2$), 5.72 (1H, OH-10b), 5.43 (1H, t, J = 12 Hz, H-2), 5.34 (1H, m), 5.23 (1H, dd, J = 3.7, 10.3 Hz), 5.09 (1H, d, J = 8.1 Hz, OH-1), 3.88 (1H, J = 3 Hz, H-4a), 3.46 (1H, t, J = 8.4 Hz, H-1), acetates; ^{13}C NMR (DMSO, 75 MHz) δ 170.5, 170.2, 162.9, 152.5, 145.1, 144.5, 140.7, 133.2, 104.9, 102.7, 100.6, 73.2 72.8, 71.2, 69.9, 68.9, 56.8, 21.5, 21.3, 21.1; *anal*. C 49.46%, H 4.82%, N 2.70%, calcd for $C_{20}H_{21}NO_{12}\cdot 2CH_3OH$, C 49.77%, H 5.46%, N 2.64%.

10b,1-Cyclic-thionocarbonate-2,3,4-tri-*O*-acetylepipancratistatin (5b). A solution of 10b(*R*)-hydroxy-2,3,4-tri-*O*-acetylpancratistatin (**4b**) (0.3 g, 0.64 mmol) and *N*,*N*′-thiocarbonydiimidazole (90%, 0.25 g, 1.26 mmol, 1.97 equiv) in 2-butanone (16 mL) was heated to 80 °C under argon for 24 h. An ^{1}H NMR of the crude reaction mixture showed approximately 40% conversion to product at this point. Thus, *N*,*N*′-thiocarbonyldiimidazole (90%, 0.27 g, 1.36 mmol, 2 equiv) in 2-butanone (20 mL) was added dropwise (addition funnel) to the reaction mixture. After 48 h, the reaction was approximately 75% complete. The mixture was concentrated to minimum volume, and ethyl acetate (50 mL) was added. The solution was washed with 1 N HC1 (2 × 25 mL), saturated NaCl (1 × 25 mL), and $NaHCO_3$ (saturated 25 mL), dried, and evaporated to dryness. Flash chromatography on silica gel (eluent 96:4 DCM−CH_3OH) gave 0.236 g (72%), which recrystallized from DCM−EtOH as needles, which were examined by X-ray crystallography: mp 151−153 °C; $[\alpha]_D^{23}$ −124.8 (*c* 1.09, CH_2Cl_2); HRMS $(APCI)^+$ calcd for $C_{21}H_{20}O_{12}NS$, 510.0651 $[M + H]^+$; found 510.0706 $[M + H]^+$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.42 (1H, s, OH-7), 6.49 (1H, s, H-10), 6.27 (1H, m, NH), 6.16, 6.14 (each 1H, d, J = 1.2 Hz, OCH_2O), 5.56 (1H, t, J = 3.3 Hz, H-3), 5.36 (1H, t, J = 3 Hz, H-2), 5.31 (1H, d, J = 1.8 Hz, H-1), 5.04 (1H, dd, J = 2.7, 10.8 Hz, H-4), 4.35 (1H, dd, J = 4.1, 10.7, H-4a), 2.19 (3H, s, $OCOCH_3$), 2.10 (3H, s, $OCOCH_3$), 2.03 (3H, s, $OCOCH_3$); ^{13}CNMR ($CDCl_3$, 100 MHz) δ 187.2, 169.6, 169.4, 168.4, 167.5, 153.2, 146.9, 136.8, 123.9, 107.4, 103.3, 98.5, 84.1, 80.2, 67.6, 67.3, 66.4, 52.4, 20.6, 20.54, 20.48; *anal*. C 49.21%, H 4.46%, N 2.36%, calcd for $C_{21}H_{19}NO_{12}S\cdot CH_3CH_2OH$, C 49.73%, H 4.54%, N 2.52%.

X-ray Crystal Structure Determination of 10b(*R*)-1-Cyclic-thiocarbonate-2,3,4-tri-*O*-acetylepipancratistatin (5b). A thin, colorless, needle-shaped crystal (~0.39 × 0.03 × 0.02 mm), grown from a dichloromethane−methanol solution, was mounted on the tip of a glass fiber. Cell parameter measurements and data collection were performed at 123 ± 2 K on a Bruker SMART 6000 diffractometer. Final cell constants were calculated from a set of 2237 reflections from the actual data collection. Frames of data were collected in the θ range of 6.47−65.86° ($-14 \le h \le 14$, $-8 \le k \le 8$, $-16 \le l \le 16$) using 0.396° steps in ω such that a comprehensive coverage of the sphere of reflections was performed. After data collection, an empirical absorption correction was applied with the program SADABS.[15] Subsequent

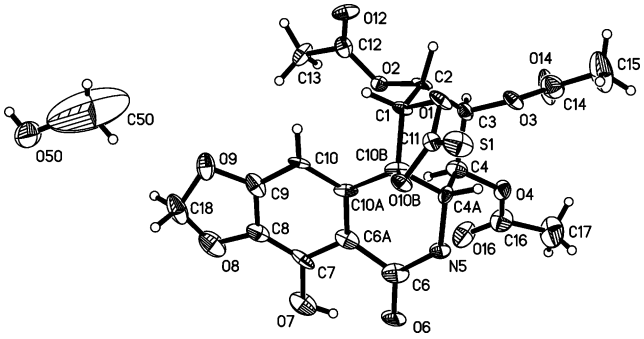


Figure 1. X-ray asymmetric unit content of the cyclic thiocarbonate compound **5b**. Thermal ellipsoids are displayed at the 50% probability level.

statistical analysis of the complete reflection set using the XPREP[15] program indicated the monoclinic space group $P2_1$.

Crystal data: $C_{21}H_{19}NO_{12}S\cdot CH_3OH$, $a = 12.2768(8)$ Å, $b = 7.1383(5)$ Å, $c = 13.6850(11)$ Å, $\beta = 90.219(4)°$, $V = 1199.28(15)$ Å^3, $\lambda =$ (Cu Kα) $= 1.54178$ Å, $\mu = 1.849$ mm^{-1}, $\rho_c = 1.499$ g cm^{-3} for $Z = 2$ and fw $= 541.47$, $F(000) = 564$. A total of 3648 reflections were collected, of which 2674 were unique ($R_{int} = 0.0886$) and considered observed ($I_o > 2\sigma(I_o)$). These were used in the subsequent structure solution and refinement with SHELXTL-V5.1.[15] All non-hydrogen atoms for **5b** were located using the default settings of that program. Hydrogen atoms were placed in calculated positions, assigned thermal parameters equal to either 1.2 or 1.5 (depending upon chemical type) times the U_{iso} value of the atom to which they were attached, then both coordinates and thermal values were forced to ride that atom during final cycles of refinement. All non-hydrogen atoms were refined anisotropically in a full-matrix least-squares refinement process. The final standard residual R_1 value for the model shown in Figure 1, consisting of a single molecule of the thionocarbonate and single molecule of disordered MeOH solvent in the asymmetric unit, converged to 0.0834 (for observed data) and 0.1062 (for all data). The corresponding Sheldrick R values were $wR_2 = 0.2018$ and 0.2124, respectively, and the GOF $= 0.969$ for all data. The difference Fourier map showed small residual electron density; the largest difference peak and hole were $+0.880$ and -0.415 e/Å^3, respectively. Final bond distances and angles were all within acceptable limits.

Synthesis of 10b(*S*)-2,3,4-Tri-*O*-acetylpancratistatin (3b) and 10b-(*R*)-1-Cyclic-carbonate-2,3,4-tri-*O*-acetylepipancratistatin (6). To a three-necked round-bottom flask under argon was added toluene (dry) (9 mL). The toluene was heated to reflux before Bu_3SnH (0.09 mL, 0.087 g, 0.3 mmol, 2 equiv) was added followed by AIBN. A solution of **5b** (0.075, 0.15 mmol) in toluene (5 mL) was then added dropwise slowly over time (1.5 h), and the reaction was monitored by TLC (97:3 DCM−MeOH). The reaction mixture was stirred at 80 °C for 16 h, then cooled and concentrated to remove toluene. Acetonitrile (10 mL) was added and extracted with hexane (3 × 15 mL). The acetonitrile fraction was then concentrated to a glass, which was separated by column chromatography to yield crude carbonate **6** (16 mg) and alcohol **3b (**33 mg, 49.6%). The products from several experiments were combined for characterization purposes. Recrystallization of **6** from CH_2Cl_2−EtOH yielded needles, which were examined by X-ray crystallography: mp 253−255 °C, $[\alpha]^{25}_D$ −108.8 (*c* 0.33, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.4 (1H, s, OH-7), 6.52 (1H, s, H-10), 6.14−6.13 (2H, m, OCH_2O), 5.5 (1H, t, $J = 3.3$ Hz, H-3), 5.33 (1H, t, $J = 3.3$ Hz, H-2), 5.18 (1, bd, $J = 2.4$ Hz, H-1), 5.08 (1H, dd, $J = 10.4$, 3.3 Hz, H-4), 4.32 (1H, dd, $J = 10.5$, 4.2 Hz, H-4a), 2.17 (3H, s, $OCOCH_3$), 2.08 (3H, s, $OCOCH_3$), 2.01 (3H, s, $OCOCH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.7, 169.4, 168.5, 167.6, 153.2, 151.0, 146.9, 140.3, 136.6, 107.4, 103.3, 98.3, 79.6, 76.7, 67.5, 67.3, 66.7, 52.9, 20.6, 20.5 (2); HRAPCI$^+$ *m/z* 494.0926 (calcd for $C_{21}H_{20}NO_{13}$ $(M + H)^+$, 494.0934); *anal*. C 50.65%, H 3.93%, N 2.79%, calcd for $C_{21}H_{19}NO_{13}$, C 51.12%, H 3.88%, N 2.84%.

The alcohol **3b** was crystallized from CH_2Cl_2−EtOH: mp 216 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.02 (1H, s), 6.87 (s, 1H), 6.45 (s, 1H), 6.01 (m, 2H), 5.53 (t, $J = 3$ Hz, H-4), 5.39 (t, $J = 10$ Hz, H-2), 5.29 (s, 1H, OH-1), 5.23 (dd, $J = 3$, 10 Hz, H-3), 3.96 (t, $J = 3$ Hz, H-4a), 3.84 (t, $J = 10$ Hz, H-1), 3.02 (1H, dd, $J = 3$, 10 Hz, H-10b); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.9, 170.5, 170.1, 169.5, 152.4, 146.4, 133.9, 133.6, 106.1, 102.4, 102.3, 72.0, 71.9, 69.2, 68.6, 53.0, 42.2, 20.9, 20.8, 20.6; HRMS (APCI+) calcd for $C_{20}H_{22}NO_{11}$ $(M + H)^+$, 452.1193; found 452.1193; *anal*. C 51.09%, H 5.34%, N 2.80%, calcd for $C_{20}H_{21}NO_{11}\cdot H_2O$, C 51.17%, H 4.93%, N 2.98%.

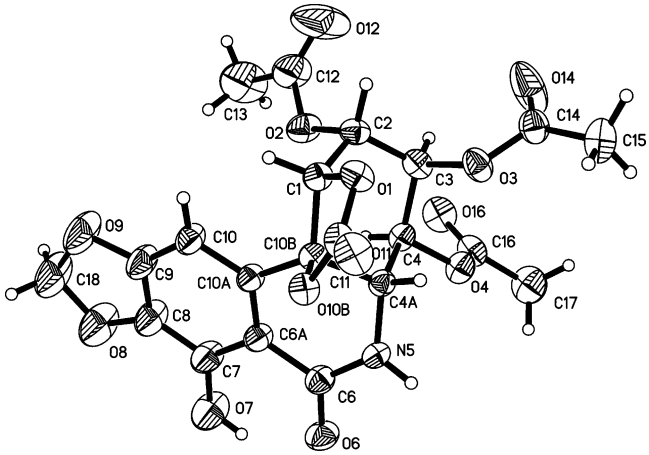


Figure 2. X-ray structure of the cyclic carbonate **6**. Atoms are displayed as 20% thermal probability ellipsoids.

X-ray Crystal Structure Determination of 10b(*R*)-1-Cyclic-carbonate-2,3,4-tri-*O*-acetylepipancratistatin (6). In a procedure analogous to that described for the X-ray data collection of the cyclic thiocarbonate compound **5b**, a colorless, plate-shaped crystal (~0.36 × 0.13 × 0.10 mm), grown from a CH_2Cl_2−MeOH, was used in the structure determination of **6**. Frames of data were collected in the θ range of 6.47−65.86° ($-15 \le h \le 15$, $-15 \le k \le 15$, $-32 \le l \le 30$). The XPREP[16] program indicated the rather unusual tetragonal space group $P4_32_12$ (#96).

Crystal data: $C_{21}H_{19}NO_{13}$, $a = 12.8186(7)$ Å, $b = 12.8186(7)$ Å, $c = 27.0417(19)$ Å, $V = 4443.4(5)$ Å^3, $\lambda =$ (Cu Kα) $= 1.54178$ Å, $\mu = 1.085$ mm^{-1}, $\rho_c = 1.475$ g cm^{-3} for $Z = 8$ and fw $= 493.37$, $F(000) = 2048$. A total of 4127 reflections were collected, of which 2367 were unique ($R_{int} = 0.0486$) and considered observed ($I_o > 2\sigma(I_o)$). The final standard residual R_1 value for the model shown in Figure 2, consisting of a single molecule of the cyclic carbonate **6** in the asymmetric unit, converged to 0.0611 (for observed data) and 0.0905 (for all data). The corresponding Sheldrick R values were $wR_2 = 0.1622$ and 0.1759, respectively, and the GOF $= 0.894$ for all data. The difference Fourier map showed minimal residual electron density; the largest difference peak and hole were 0.349 and -0.181 e/Å^3, respectively. Final bond distances and angles were all within acceptable limits.

10b(*S*)-Epipancratistatin (3a). To a 10% aqueous MeOH−CH_2Cl_2 mixture (1:1, 6 mL) were added acetate **3b** (0.05 g, 0.11mmol) and K_2CO_3 (0.015 g, 0.1 mmol, 1 equiv). The yellow solution was stirred at rt and monitored by TLC (96:4 DCM−CH_3OH 4%) for 16 h. The mixture was neutralized with acetic acid and concentrated to an off-white residue, which was dissolved in 4:1 DCM−CH_3OH and filtered through a silica gel plug. The filtrate was concentrated to a white, amorphous solid (23 mg, 64%) and recrystallized from MeOH to afford a colorless, crystalline solid, which was characterized as follows: mp >300 °C; $[\alpha]_D^{25}$ +72 (*c* 0.2, CH_3OH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.85 (s, 1H), 8.32 (s, 1H), 6.4 (s, 1H), 6.10 (s, 1H), 6.01−6.00 (m, 2H), 5.06 (s, 1H), 4.62 (m, 2H), 3.92 (s, 1H), 3.66 (s, 1H), 3.41 (m, 2H), 3.04 (m, 1H), 2.73−2.70 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.8, 151.0, 144.9, 137.0, 132.1, 106.1, 102.4, 101.7, 73.5, 73.2, 70.6, 69.4, 55.1, 40.7; HRFAB calcd for $C_{14}H_{16}NO_8$ $(M + H)^+$, 326.0876; found $(M + H)^+$, 326.0887.

10b(*R*)-Hydroxy-1,2,3,4,7-penta-*O*-acetylpancratistatin (4c). To a solution of 10b(*R*)-hydroxy-2,3,4,7-tetra-*O*-acetylnarciclasine (**4a**, ref 8c) (1.55 g, 3.04 mmol) in pyridine (7 mL) was added acetic anhydride (7 mL) and the solution stirred at rt under anhydrous conditions for 6 h. Ice−water was added, and the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The dried extract was concentrated (under vacuum) to a foam, which was dissolved in toluene with heating. Crystallization occurred overnight at 0 °C to yield colorless crystals (1.38 g, 82%): mp 236 °C; R_f 0.53 (hexane−acetone, 1:1); $[\alpha]_D$ +109 (*c* 0.41, $CHCl_3$); EIMS *m/z* (%) 551 [M + 2.8], 509 (100), 347 (12.9),

287 (49.3), 258 (30), 222 (84), 165 (5), 115 (2.8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95 (s, 1H), 6.08 (m, 2H), 5.69 (m, 1H), 5.5 (t, $J = 3.3$ Hz, 1H), 5.38 (m, 2H), 4.0 (d, 1H), 3.3 (s, 1H), 2.33 (s, 3H), 2.16 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 169.8, 169.1, 168.9, 168.7, 162.3, 152.5, 140.6, 137.5, 133.8, 112.9, 103.1(2C), 73.2, 72.9, 69.5, 69.0, 67.7, 56.9, 20.8, 20.7, 20.6, 20.5, 20.3; HRFAB calcd for $C_{24}H_{26}NO_{14}$ $[M + H]^+$, 552.1353; found $[M + H]^+$, 552.1365; *anal*. C 50.77%, H 4.80%, N 2.46%, calcd for $C_{24}H_{25}NO_{14}\cdot H_2O$, C 50.62%, H 4.78%, N 2.46%.

10b(*R*),*N*,7-*O*-Methyloxalyl-1,2,3,4-tetra-*O*-acetylpancratistatin (7b). To a solution of alcohol **4c** (0.3 g, 0.5 mmol) in pyridine (3 mL) was added methyl oxalyl chloride (0.5 mL, 5.4 mmol, 10.8 equiv). The reaction mixture turned brown, and an immediate precipitate separated. Pyridine (4 mL) was added, and the reaction mixture was stirred at rt under argon for 6 h, whereupon TLC examination (9:1 DCM−CH_3OH) indicated starting material had been consumed. The reaction mixture was cooled and placed in an ice−water bath, ice−water was added, and the resultant precipitate was collected. The precipitate was washed with H_2O, which removed the brown color. The remaining white precipitate was dissolved in CH_2Cl_2, washed with H_2O (20 mL), and dried, and the solution was filtered and concentrated to a white residue. The residue was dissolved in hexane (10 mL) and acetone added until the product dissolved with heating. Crystallization occurred overnight at 0 °C: 120.6 mg, 28.9%, mp 265 °C; $[\alpha]_D$ −11.3 (*c* 1.04, CH_2Cl_2); R_f 0.81 (19:1 DCM−CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (s, 1H), 6.57 (d, 1H), 6.20 (m, 2H), 5.74 (d, $J = 6.9$ Hz, 1H), 5.30 (t, $J = 1.5$ Hz, 1H), 5.22 (t, $J = 1.5$ Hz, 1H), 5.05 (dd, $J = 6.9$ Hz, 1.8 Hz, 1H), 3.99 (s, 3H) 3.88 (s, 3H), 3.19 (s, 3H), 2.19 (s, 3H), 2.17 (s, 3H), 1.99 (s, 3H), 1.94 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.6, 169.0, 168.4, 167.7, 162.0, 161.0, 158.9, 156.8, 156.4, 155.3, 154.1, 152.8, 142.5, 133.3, 128.5, 114.9, 109.2, 102.3 (2C), 79.4, 67.8, 67.4, 67.1, 67.1, 54.0, 53.7, 53.0, 51.7, 30.9, 20.6, 20.5, 20.2; EIMS *m*/*z* (%) 767 (M^+, 13.3), 695 (18.9), 636 (25.2), 622 (100), 594 (28.7), 550 (44.8), 374 (27.9), 287 (35.7), 248 (41.9); *anal*. C 49.20%, H 4.25%, N 2.02%, calcd for $C_{31}H_{29}O_{22}N$, C 48.51%, H 3.81%, N 1.82%.

10b(*S*)-1,2,3,4-Tetra-*O*-acetylepipancratistatin (3c). To a solution of methyloxalate (**7b**) (0.05 g, 0.065 mmol) in dry toluene (20 mL), under argon at reflux, were added tributyltin hydride (150 mL, 8.57 equiv) and AIBN (20 mg). The reaction mixture was heated at reflux for 2.5 h, and TLC (19:1, DCM−CH_3OH) showed complete consumption of starting material. The solution was concentrated to a white, gelatinous solid, which crystallized from toluene−hexane to yield an amorphous solid upon standing at 0 °C for 16 h. After washing with hexane to remove tributyltin hydride and drying, the solid obtained (32 mg) was found to consist mainly of acetate (**3c**), with four minor impurities (one being pancratistatin tetraacetate by ^{1}H NMR). The purification of acetate (**3c**) proved difficult using standard silica gel column chromatography techniques. However, purification of a small quantity (7 mg) was accomplished via separation of the mixture using C_{18} reversed-phase HPLC (ZorbaxSB-C18, 3:2 H_2O−CH_3OH to CH_3OH): mp 169 °C; EIMS *m*/*z* (%) 493 (M^+, 15.5], 433 (5), 313 (18.9), 271 (100), 206 (17.2); HRFAB calcd for $C_{22}H_{24}O_{12}N$ $[M + H]^+$, 494.1298; found $[M + H]^+$, 494.1296; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.97 (s, 1H), 6.27 (s, 1H), 6.03 (m, 2H), 5.52−5.47 (m, H-4, H-2), 5.30 (dd, $J = 11$,3 Hz, H-3), 5.25 (t, $J = 11$ Hz, H-1), 4.01 (m, H-4a), 3.15 (m, H-10b), 2.19 (s, 3H), 2.04 (S, 3H), 2.03 (s, 3H), 1.95 (s, 3H); ^{13}C NMR (125 Hz, $CDCl_3$) δ 170.2, 170.2, 170.1, 169.4, 169.0, 152.4, 146.6, 134.1, 132.4, 106.4, 102.5, 101.0, 72.0, 69.4, 69.2, 68.3, 52.9, 40.0, 20.7, 20.6, 20.6, 20.5.

Cancer Cell Line Procedures. Inhibition of human cancer cell growth was assessed using the National Cancer Institute's standard sulforhodamine B assay as previously described.[17] Briefly, cells in a 5% fetal bovine serum/RPMI1640 medium solution were inoculated in 96-well plates and incubated for 24 h. Serial dilutions of the compounds were then added. After 48 h, the plates were fixed with trichloroacetic acid, stained with sulforhodamine B, and read with an automated microplate reader. A growth inhibition of 50% (GI_{50} or the drug concentration causing a 50% reduction in the net protein increase) was calculated from optical density data with Immunosoft software.

Mouse leukemia P388 cells[18] were incubated 24 h in a 10% horse serum/Fisher medium solution followed by a 48 h incubation with serial dilutions of the compounds. Cell growth inhibition (ED_{50}) was then calculated using a Z1 Beckman/Coulter particle counter.

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