Full Papers

Antineoplastic Agents. 545. Isolation and Structure of Turbostatins 1−4 from the Asian Marine Mollusk *Turbo stenogyrus*[†,1]

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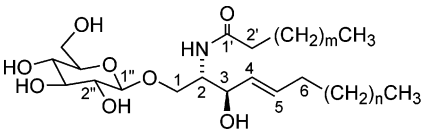
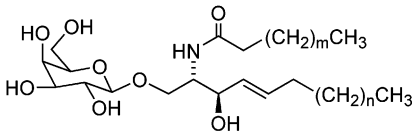
The cancer cell line bioassay-guided separation of an extract from the marine mollusk *Turbo stenogyrus* led to the isolation of four new cerebrosides designated turbostatins 1−4 (**1**−**4**). The structure of each glycolipid was determined by interpreting results of a series of HR-APCI-MS and NMR (1D and 2D) spectral analyses. All four turbostatins exhibited significant (GI_{50} 0.15−2.6 μg/mL) cancer cell growth inhibition against the murine P388 lymphocytic leukemia and a panel of human cancer cell lines.

In 1965−1966, we began the first systematic investigation of marine organisms[2] as potential sources of new, structurally unique, and very effective anticancer drugs.[3a] One of the early (1968) leads uncovered in our geographically far-reaching exploratory evaluation of various marine organisms was the Asian topshell snail *Turbo stenogyrus*[3a,4] (phylum Mollusca, gastropod family Trochidae) collected in Taiwan. In Asia *T. stenogyrus* is well known as the home shell[5] for hermit crabs.[6] More generally, topshells are known for the use of some species in the manufacture of pearl ornaments and as a seafood in the Caribbean and Central America. Some (e.g., *T. pica*) of these algae feeders have been reported to contain toxic tissue.[2]

We soon found the soft tissue (0.5 kg) from *T. stenogyrus* to give a 2-propanol extract that displayed strong activity (T/C 177 at 400 mg/kg) against the murine P388 lymphocytic leukemia (in vivo) cell line.[3a,4] By the summer of 1971 we were able to proceed with the 2-propanol extract from 22.7 kg of *T. stenogyrus*, and with separation techniques then available we isolated taurine as one of the anticancer constituents.[4] Evaluation of taurine using P388 in vivo at dose levels from 4.0 to 800 mg/kg led to T/C values that never exceeded 123 (i.e., 23% increase in median survival time). Furthermore, taurine was found inactive against the in vivo L-1210 lymphoid leukemia and human epidermoid carcinoma of the nasopharynx (KB)[7] cell lines. As noted at the time,[4] we planned to reinvestigate the *T. stenogyrus* lead when bioassay and chemical separation techniques had improved. We now report the isolation and structural elucidation of four new cancer cell growth inhibitory glycosphingolipids from *T. stenogyrus* designated turbostatins 1−4 (**1**−**4**).



1, m = 13, n = 9
2, m = 15, n = 9



3, m = 13, n = 9
4, m = 15, n = 9

Results and Discussion

The further evaluation of *T. stenogyrus* fractions was greatly assisted by the subsequent availability of the P388 cell line and human cancer cell lines for bioassay-directed separations, especially combined with advances in general separation procedures and HPLC equipment. Accordingly, another aliquot of the 1971 2-propanol extract of *Turbo stenogyrus* was first subjected to a 9:1 → 3:2 CH_3OH-H_2O/hexane → CH_2Cl_2 solvent partition sequence. The final active methylene chloride extract (P-388: ED_{50} 3.52 μg/mL) was carefully fractionated by an extensive series of separations involving column gel permeation (Sephadex LH-20), partition chromatography, and a final isolation by reversed-phase HPLC columns on Zorbax SB-C18 in 85:15 CH_3OH-H_2O. These procedures afforded colorless glycosphingolipids **1**−**4** as amorphous solids.

The molecular formula of turbostatin 1 (**1**) was assigned $C_{38}H_{74}NO_8$ on the basis of high-resolution APCI mass ($[M + H]^+$ at *m/z* 672.53855) and ^1H and ^{13}C NMR spectral analyses (Table 1). An IR absorption band at 3393 cm^{-1} indicated the presence of hydroxyl groups. The typical IR absorptions at 1630 and 1537 cm^{-1} suggested an amide linkage, which was confirmed by a nitrogen-attached carbon signal at δ 55.05 and a carbonyl signal at δ 173.32 in the ^{13}C NMR spectrum. The ^1H NMR spectrum exhibited a doublet at δ 8.35 (J = 7.5 Hz) due to an NH proton, which was exchangeable with D_2O; a broad singlet at δ 1.21 (methylene protons); a triplet at δ 0.85 (two terminal methyls); an anomeric proton at δ 4.96 (J = 8.2 Hz); and carbinol protons appearing as multiplets between δ 3.90 and 4.82, suggesting a glycosphingolipid structure.[8−11] The

[†] Dedicated to the memory of Dr. Cecil R. Smith, Jr. (1924−2004), a productive pioneer in the discovery of naturally occurring anticancer drugs.

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Table 1. ^{1}H and ^{13}C NMR Spectral Assignments (δ/ppm) for Turbostatins **1** and **2** in Py-d_5

	δ_H		δ_C	
position	**1**	**2**	**1**	**2**
lipid base unit				
1a	4.82 (m)	4.82 (m)	70.46	70.42
1b	4.23 (m)	4.22 (m)		
2	4.81 (m)	4.80 (m)	55.05	55.02
3	4.75 (m)	4.75 (m)	72.58	72.57
4	5.98 (dd, 6.0, 15.0)	5.97 (dd, 6.0, 15.2)	132.20	132.21
5	5.85 (dt, 6.0, 15.0)	5.83 (dt, 6.0, 15.2)	132.51	132.49
6	2.03 (q, 7.0)	2.01(q, 7.1)	32.71	32.71
7−15	1.21 (brs)	1.21 (brs)	22.9−32.1	22.9−32.1
16	0.85 (t, 8.6)	0.85 (t, 8.6)	14.25	14.24
NH	8.35 (d, 7.5)	8.37 (d, 7.8)		
N-acyl unit				
1′			173.32	173.30
2′	2.41 (t, 7.0)	2.41 (t, 7.0)	32.71	32.70
3′	1.80 (m)	1.80 (m)	26.37	26.39
4′−15′ or 4′−17′	1.21 (brs)	1.21 (brs)	22.9−32.1	22.9−32.1
16′ or 18′	0.85 (t, 8.6)	0.85 (t, 8.6)	14.25	14.24
glycoside				
1″	4.96 (d, 8.2)	4.96 (d, 8.1)	105.46	105.44
2″	4.06 (dd, 8.4, 9.6)	4.04 (dd, 8.1, 9.3)	75.15	75.15
3″	4.24 (m)	4.24 (m)	78.48	78.48
4″	4.37 (m)	4.37 (m)	71.64	71.63
5″	3.90 (m)	3.91 (m)	78.47	78.46
6a″	4.51 (dd, 5.1, 11.7)	4.50 (dd, 5.0, 11.6)	62.74	62.74
6b″	4.20 (dd, 2.3, 11.7)	4.22 (dd, 2.3, 11.6)		

^{1}H NMR spectrum also showed two olefinic proton signals at δ 5.97 (^{1}H, dd, J = 15.0, 6.0 Hz, H-4) and 5.83 (^{1}H, dt, J = 15.2, 6.0 Hz, H-5), attributable to the presence of one disubstituted double bond. The amino alcohol fragment was identified as a sphingosine unit by the characteristic signals that appeared in the ^{1}H and ^{13}C NMR spectra, especially owing to the presence of a typical Δ^4 double bond.[8,9] The large vicinal coupling constants of H-4 and H-5 (J = 15.0 Hz) clearly indicated an *E*-geometry for the double bond.[9,10]

In the ^{13}C NMR spectrum the carbon resonances appeared at δ 62.74 (CH_2), 71.64 (CH), 75.15 (CH), 78.47 (CH), 78.48 (CH), and 105.46 (CH), revealing the presence of a β-glucopyranoside.[12] The anomeric proton at δ 4.96 (d, J = 8.2 Hz) correlated to the carbon signal at δ 105.46 in the HMQC spectrum, further confirming the β-configuration of the glucoside unit. The length of the lipid (sphingoid base) base and the lipid amide were determined by APCI-MS. In addition to the quasimolecular ion at *m/z* 672 $[M + H]^+$, the APCI spectrum of turbostatin 1 exhibited an intense fragment peak at *m/z* 510, which was produced by elimination of the glucosyl unit from the protonated molecular ion. The loss of palmitoylamide from the molecular ion gave rise to the fragment at *m/z* 254. The typical fragment ion at *m/z* 384 was formed by elimination of decene from that at *m/z* 510 through McLafferty rearrangement.[13,14] Therefore, the number of carbons in the lipid base and lipid amide were both determined to be 16.

The linkages of the three component units of turbostatin 1 were deduced from the HMBC spectrum. The carbon signal at δ 173.32 (C-1′) correlated with the proton signals at δ 4.81 (H-2) and 2.41 (H-2′). The proton signal at δ 4.81 (H-2) gave cross-peaks with the carbon signals at δ 72.58 (C-3) and 70.46 (C-1). In addition, the latter also correlated with the proton signal at δ 4.96 (C1″). The carbon signal at δ 72.58 (C-3) showed cross-peaks with the proton signals at δ 5.98 (H-4) and 5.85 (H-5). From these analyses, the structure of turbostatin 1 was elaborated and the overall assignments (Table 1) of ^{1}H NMR and ^{13}C NMR data were unambiguously made on the basis of the ^{1}H−^{1}H COSY, TOCSY, HMQC, and HMBC spectra. By considering biogenetic relationships,[15] steric factors, and the chemical shift of H-2, the chemical shifts of the carbon signals of C-1 to C-3 and C-1′ may be utilized to determine the absolute stereochemistry of glucosphingolipids and sphingolipids.[16−18] The proton signal at δ 4.81 (H-2) and the carbon signals at δ 70.46 (C-1), 55.05 (C-2), 72.58 (C-3), and 173.32 (C-1′) of turbostatin 1 were in good agreement with those reported for glycosphingonines (as model structures) with the 2*S*,3*R* configuration.[8−10,14,19] The optical rotation of turbostatin 1 ($[\alpha]^{23}_D$ +10.2°) was very close to that of 1-*O*-(β-D-glucopyranosyl)-D-(+)-(2*S*,3*R*)-2-(docosanoylamide)-1,3-eicosanediol ($[\alpha]^{27}_D$ +8.6).[8] All of these considerations were used to assign turbostatin 1 as 1-*O*-β-D-glucopyranosyl-2*S*-hexadecanoylamino-3*R*-hydroxy-4*E*-hexadecene.

As with turbostatin 1, the molecular formula of turbostatin 2 (**2**) was assigned as $C_{40}H_{78}NO_8$ on the basis of high-resolution APCI mass spectroscopy ($[M + H]^+$ at *m/z* 700.57498) and the results of ^{1}H and ^{13}C NMR spectral interpretations (Table 1). The NMR results were found to be essentially identical to those of amide **1**, which confirmed that turbostatin 2 (**2**) was also a glycosphingolipid and differed only in the length of the lipid base and lipid amide units. In addition to the quasimolecular ion at *m/z* 700 $[M + H]^+$, the APCI spectrum of ceramide **2** exhibited an intense fragment peak at *m/z* 538, which was produced by elimination of the glucosyl unit from the protonated molecular ion. The loss of octadecoylamide from the molecular ion gave rise to the fragment at *m/z* 254. The typical fragment ion at *m/z* 412 was formed by elimination of decene through McLafferty rearrangement.[13,14] Therefore, the number of carbons in the lipid base and lipid amide were determined to be 16 and 18, respectively. Thus, turbostatin 2 was assigned structure **2**.

The molecular formula of turbostatin 3 (**3**) was found to be $C_{38}H_{74}NO_8$ on the basis of high-resolution APCI mass spectroscopy ($[M + H]^+$ at *m/z* 672.54142) as well as ^{1}H and ^{13}C NMR spectral results (Table 2). Again, the ^{1}H and ^{13}C NMR spectra were found to be nearly identical to those of turbostatins 1 and 2 except for the glycoside signals and confirmed that turbostatin 3 (**3**) was also a glycosphingolipid. In the ^{13}C NMR spectrum the glycoside unit carbon

Table 2. ^{1}H and ^{13}C NMR Spectral Assignments (δ/ppm) for Turbostatins **3** and **4** in Py-d_5

	δ_H		δ_C	
position	**3**	**4**	**3**	**4**
lipid base unit				
1a	4.81 (m)	4.82 (m)	70.44	70.45
1b	4.24 (m)	4.23 (m)		
2	4.80 (m)	4.81 (m)	55.04	55.03
3	4.75 (m)	4.75 (m)	72.60	72.59
4	5.99 (dd, 6.0, 15.2)	5.98 (dd, 6.0, 15.0)	132.15	132.18
5	5.85 (dt, 6.0, 15.2)	5.83 (dt, 6.0, 15.0)	132.50	132.49
6	2.03 (q, 7.2)	2.01(q, 7.0)	32.72	32.70
7−15	1.21 (brs)	1.21 (brs)	22.9−32.1	22.9−32.1
16	0.85 (t, 8.6)	0.85 (t, 8.6)	14.26	14.25
NH	8.37 (d, 7.7)	8.36 (d, 7.8)		
N-acyl unit				
1′			173.33	173.33
2′	2.41 (t, 7.2)	2.41 (t, 7.1)	32.71	32.70
3′	1.80 (m)	1.80 (m)	26.38	26.38
4′−15′ or 17′	1.21 (brs)	1.21 (brs)	22.9−32.1	22.9−32.1
16′ or 18′	0.85 (t, 8.6)	0.85 (t, 8.6)	14.26	14.25
glycoside				
1″	4.89 (d, 7.5)	4.88 (d, 7.3)	106.47	106.46
2″	4.52 (dd, 7.5, 9.5)	4.52 (dd, 7.5, 9.4)	72.71	72.71
3″	4.16 (dd, 3.0, 9.5)	4.14 (dd, 3.0, 9.4)	75.38	75.40
4″	4.56 (d, 3.0)	4.55 (3.0)	70.21	70.22
5″	4.07 (dd, 6.0, 9.0)	3.07 (dd, 6.2, 8.9)	77.07	77.06
6″	4.44 (m)	4.44 (m)	62.34	62.35

Table 3. Murine P388 Lymphocytic Leukemia Cell Line and Human Cancer Cell Line Inhibition Values (GI_{50} expressed in μg/mL) for Turbostatins **1**−**4**[a]

cancer cell line[b]	**1**	**2**	**3**	**4**
P388	0.27	0.15	0.25	0.29
MXPC-3	0.71	1.0	1.6	0.93
MCF-7	0.44	0.39	0.48	0.44
SF268	0.98	1.9	1.9	1.5
NCI-H460	0.34	0.53	0.55	0.49
KML20L2	0.35	0.35	0.48	0.41
DU-145	1.6	1.7	2.6	2.6

[a] In DMSO. [b] Cancer type: P388 (lymphocytic leukemia); BX-PC-3 (pancreas adenocarcinoma); MCF-7 (breast adenocarcinoma); SF268 (CNS glioblastoma); NCI-H460 (lung large cell); KM20L2 (colon adenocarcinoma); DU-145 (prostate carcinoma).

resonances appeared at δ 62.34 (CH_2), 70.21 (CH), 72.71 (CH), 75.38 (CH), 77.07 (CH), and 106.47 (CH), revealing the presence of a β-galactopyranoside.[12] The anomeric proton at δ 4.89 (d, J = 7.5 Hz) correlated to the carbon signal at δ 106.47 in the HMQC spectrum, further confirming the β-configuration of the galactose unit. The APCI-MS spectrum of cerebroside **3** also exhibited three fragment peaks at *m/z* 510, 384, and 254, which suggested the number of carbons in the lipid base and lipid amide were both 16. Therefore, structure **3** was determined to represent turbostatin 3.

The molecular formula of turbostatin 4 (**4**) was assigned as $C_{40}H_{78}NO_8$ on the basis of the spectral data sequence, $[M + H]^+$ at *m/z* 700.57537 and as recorded in Table 2, which was essentially identical with that of turbostatin 3, the structures differing only in the length of the lipid base and lipid amide. The APCI-MS spectrum of amide **4** also exhibited three fragment peaks at *m/z* 538, 412, and 254, as already found for amide **2**. This indicated the number of carbons in the lipid base and lipid amide were also 16 and 18, respectively, and allowed assignment of structure **4** to turbostatin 4.

Turbostatins 1−4 were evaluated against the murine P388 lymphocytic leukemia cell line and a minipanel of human cancer cell lines (see Table 3) and were found to exhibit significant cancer cell growth inhibition against each cell line. That was a promising result since some ceramides (parent is an 18-carbon lipid base, 14-carbon lipid amide) and derived hexose glycosides such as glucocerebrosides (e.g., **1** and **2**) and galactocerebrosides (e.g., **3** and **4**) function as a cellular second messenger and intermediary in a variety of important cell functions such as apoptosis, cell senescence, and terminal cell differentiation.[20] Ceramide is also known to stimulate mitogen-activated protein kinase[21] through binding to protein kinase c-Raf,[22] and some cerebrosides are known to possess anticancer, antiviral, antifungal, antimicrobial, Cox-2 inhibitory, immunostimulative, and immunosuppressive activities. Some of these properties would appear promising for the treatment of Alzheimer's disease.[23] Interestingly, α-alactosylceramides have been shown earlier to have anticancer activity.[24−27] As an illustration, KRN7000 has been shown to display remarkable activity against a disparate group of diseases, such as cancer, including melanoma, pancreatic, and colon cancer, as well as malaria, juvenile diabetes, hepatitis B, and autoimmune encephalomyelitis, using in vivo versions of these diseases.[27] We intend to proceed with further biological evaluation of turbostatins 1−4, as these new glycocerebrosides may have a useful role in inhibiting the sphingolipid biochemical pathways of the cancer cell.

Experimental Section

General Experimental Procedures. Melting points were measured using an Olympus electrothermal melting point apparatus and are uncorrected. IR spectra were recorded with a Thermo Nicolet Avatar 360 infrared spectrometer. NMR spectra were obtained with a Varian XL-300 or a Varian UNITY INOVA-500 spectrometer with tetramethylsilane (TMS) as an internal reference. High-resolution mass spectra were obtained using a JEOL LCMate magnetic sector instrument in the APCI positive mode, with a poly(ethylene glycol) reference.

All chromatographic solvents were redistilled. Sephadex LH-20 used for partition column chromatography was obtained from Pharmacia Fine Chemicals AB. Analytical HPLC was conducted with a Hewlett-Packard Model 1050 HPLC coupled with a Hewlett-Packard diode-array detector. Semipreparative HPLC was performed on a Waters Deltaprep-600 instrument on 9.4 × 250 mm Zorbax SB-C18 columns.

Turbo stenogyrus. The topshell *T. stenogyrus* (phylum Mollusca, class Gastropoda, subclass Prosobronchia) is a member of the Turbinidae family in the order Archaeogastropoda. A summer of 1971 re-collection (22.7 kg; from along the coast of Taiwan) of *T. stenogyrus* was employed in the present study and supplied by Mr. Elliot Glanz, The Butterfly Company, Brooklyn, New York, NY, in 1968. The voucher specimen is maintained in our Institute, and the taxonomic authority was Dr. I. E. Wallen, Smithsonian Oceanographic Sorting Center, Smithsonian Institution, Washington, D.C., 20560.

Extraction and Initial Separation of *T. Stenogyrus.* The snail portion of the 1971 re-collection of *T. stenogyrus* was extracted with 2-propanol. The extract (the long period of storage was in a tightly sealed glass container, maintained in the dark at ca. 20 °C) was dissolved in CH_3OH-H_2O (9:1) and the solution filtered to remove insoluble material. The resulting solution was partitioned four times between hexane and 9:1 CH_3OH-H_2O. The hexane layer was removed and concentrated to yield 13.3 g (P388 ED_{50} 60 μg/mL) of black-brown material. The CH_3OH-H_2O phase was diluted to give a ratio of 3:2 (by addition of H_2O) and extracted four times with CH_2Cl_2. The CH_2Cl_2 layer was concentrated to afford a black oily P388-active (14.7 g, ED_{50} 3.52 μg/mL) fraction. The remaining CH_3OH-H_2O solution was P388 cell line inactive.

Isolation of Turbostatins 1−4 (1−4). A 14.6 g aliquot of the P388-active CH_2Cl_2 fraction was partially dissolved in CH_3OH, and the solution was filtered and separated on a Sephadex LH-20 column with CH_3OH as eluent. Ten fractions were obtained. One of the fractions (2.5 g, ED_{50} 1.25 μg/mL) was further separated on a Sephadex LH-20 column in $CH_3OH-CH_2Cl_2$ (3:2) to yield seven fractions. A 1.4 g fraction with ED_{50} 0.30 μg/mL was rechromatographed on a Sephadex LH-20 column in hexane−CH_3OH−2-propanol (8:1:1). Two fractions obtained from this step showed P388 activity, and a 180 mg fraction with ED_{50} 0.76 μg/mL was further separated on a Sephadex LH-20 column in hexane−toluene−acetone−CH_3OH (1:4:3:4). Six active fractions were combined and rechromatographed on a Sephadex LH-20 column using hexane−ethanol−toluene−CH_2Cl_2 (17:1:1:1) as eluent. Four active fractions were obtained, recombined, and rechromatographed on a Sephadex LH-20 column with hexane−EtOAc−CH_3OH (4:5:1) as eluent. All five fractions obtained from this step showed P388 activity, and a 56 mg fraction with ED_{50} 0.21 μg/mL, a dark brown material, was separated on a semipreparative reversed-phase HPLC Zorbax SB C_{18} column with 85:15 CH_3OH-H_2O (a flow rate of 4 mL/min and the UV detector set at 208 nm). Turbostatins 1 (**1**) and 2 (**2**) were obtained in the following order: **1** (10.1 mg) at 20.5 min and **2** (11.2 mg) at 25.1 min. A 103 mg fraction with ED_{50} 0.56 μg/mL was separated on a semipreparative reversed-phase HPLC Zorbax SB C_{18} column with 85:15 CH_3OH-H_2O (a flow rate of 4 mL/min and the UV detector set at 208 nm). The result was that turbostatins 3 (**3**) and 4 (**4**) were obtained in the following order: **3** (11.6 mg) at 23.7 min and **4** (8.3 mg) at 28.5 min. All four new compounds were colorless and had very limited solubility in CH_3OH, CH_2Cl_2, CH_3CN, and H_2O.

Turbostatin 1 (1) 1-*O*-*β*-D-glucopyranosyl-2*S*-hexadecanoylamino-3*R*-hydroxy-4*E*-hexadecene: colorless amorphous solid; mp 207−209 °C; $[\alpha]^{23}_D$ +10.2° (*c* 0.10, pyridine); IR (KBr) ν_{max} 3393 (OH), 2950, 1630 (C=O), 1537, 1450, 1083, 1032, and 720 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; APCI-MS (positive) *m/z* 672 $[M + H]^+$, 654, 510, 492, 384, 254; APCI-HRMS (positive) *m/z* 672.53855 $[M + H]^+$ (calcd for $C_{38}H_{74}NO_8$, 672.54142).

Turbostatin 2 (2) 1-*O*-*β*-D-glucopyranosyl-2*S*-octadecanoylamino-3*R*-hydroxy-4*E*-hexadecene: colorless amorphous solid; mp 209−210 °C; $[\alpha]^{23}_D$ +10.7° (*c* 0.10, pyridine); IR (KBr) ν_{max} 3394 (OH), 2950, 1631 (C=O), 1538, 1450, 1084, 1030, and 720 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; APCI-MS (positive) *m/z* 700 $[M + H]^+$, 682, 538, 520, 412, 254; APCI-HRMS (positive) *m/z* 700.57498 $[M + H]^+$ (calcd for $C_{40}H_{78}NO_8$, 700.57272).

Turbostatin 3 (3) 1-*O*-*β*-D-galactopyranosyl-2*S*-hexadecanoylamino-3*R*-hydroxy-4*E*-hexadecene: colorless amorphous solid; mp 213−214 °C; $[\alpha]^{23}_D$ −6.3° (*c* 0.10, pyridine); IR (KBr) ν_{max} 3395 (OH), 2951, 1633 (C=O), 1537, 1450, 1085, 1031, and 720 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; APCI-MS (positive) *m/z* 672 $[M + H]^+$, 654, 510, 492, 384, 254; APCI-HRMS (positive) *m/z* 672.53979 $[M + H]^+$ (calcd for $C_{38}H_{74}NO_8$, 672.54142).

Turbostatin 4 (4) 1-*O*-*β*-D-galactopyranosyl-2*S*-octadecanoylamino-3*R*-hydroxy-4*E*-hexadecene: colorless amorphous solid; mp 214−215 °C; $[\alpha]^{23}_D$ −6.5° (*c* 0.10, pyridine); IR (KBr) ν_{max} 3394 (OH), 2950, 1632 (C=O), 1538, 1451, 1084, 1030, and 720 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; APCI-MS (positive) *m/z* 700 $[M + H]^+$, 682, 538, 520, 412, 254; APCI-HRMS (positive) *m/z* 700.57537 $[M + H]^+$ (calcd for $C_{40}H_{78}NO_8$, 700.57272).

Cancer Cell Line Methods. The National Cancer Institute's standard sulforhodamine B assay was used to assess inhibition of human cancer cell growth as previously described.[28] The murine P388 lymphocytic leukemia cell line results were obtained using 10% horse serum/Fisher medium with incubation for 24 h. Serial dilutions of the compounds were added, and after 48 h, cell growth inhibition (ED_{50}) was calculated using a Z1 Coulter particle counter.

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References and Notes

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