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Synthesis and Growth Inhibitory Activity of Chiral 5-Hydroxy-2-*N*-Acyl-(3*E*)-Sphingenines: Ceramides with an Unusual Sphingoid Backbone

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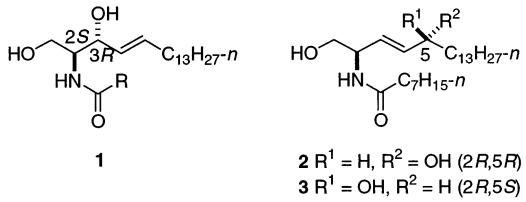
The unusual sphingoid base 5-hydroxy-3-sphingenine was identified in the hydrolysate of brain sphingolipids more than 40 years ago. We present here the first synthesis of the 5*R* and 5*S* diastereoisomers of the *N*-acyl derivatives of 5-hydroxy-3-sphingenine, **2** and **3**, respectively, which represent regioisomers of (2*S*,3*R*)-ceramide (**1**). The key steps include the synthesis of α,β-unsaturated ketone intermediates **4** and **5** from *N*-Cbz- and *N*-Boc-L-serine and diastereoselective reduction of the enones. The configuration at the new carbinol center was deduced by proton NMR analysis of (*R*)- and (*S*)-Mosher [methoxy(trifluoromethyl)phenylacetate] ester derivatives. Ceramide analogues **2** and **3** showed a markedly higher antiproliferative activity than **1** on MCF-7 cells.

Introduction

Sphingolipids are widely distributed in mammalian membranes, where they play a structural role and also participate in a plethora of cellular events. They all have, by definition, a "sphingoid base" backbone, the most common of which is (2*S*,3*R*,4*E*)-2-aminooctadec-4-ene-1,3-diol (C_{18}-sphingosine).[1] A variety of sphingosines exist that differ with respect to the lipid chain length and location of unsaturation, as well as the number of hydroxy groups. The sphingoid base 5-hydroxy-(3*E*)-sphingenine occurs naturally. It was isolated by TLC[2] and HPLC[3] from the acid hydrolysate of a human brain sphingolipid mixture; however, the configuration at C-5 was not established. As part of our interest in analyzing structure−function relationships of ceramides (*N*-acyl-sphingosines) that differ with respect to the location of the double bond and hydroxy groups in the sphingoid base,[4] we report here the synthesis of ceramides **2** and **3**

CHART 1



(see Chart 1). These diastereomers represent regioisomers of (2*S*,3*R*)-ceramide (**1**),[5] which occupies the "hub" of sphingolipid metabolism and serves as a coordinator of eukaryotic stress responses and other biological activities.[6] In view of the capacity of **1** to regulate various biological functions, the availability of some of its analogues, such as **2** and **3**, would contribute to our understanding of the complex structural biology of ceramide. We report here that **2** and **3** are significantly more effective than **1** in inhibiting the growth of a breast tumor cell line, although the mechanism by which they exert their antiproliferative action is unclear.

Results and Discussion

Retrosynthetic Analysis. Scheme 1 illustrates our strategy for the preparation of ceramides **2** and **3**. The stereochemistry of the C-5-hydroxy group is generated by diastereoselective reduction of the key enone intermediates **4** and **5**. The stereochemistry of the 3*E*-double bond results from the Horner−Wadsworth−Emmons (HWE) reaction of L-serine-derived aldehydes **6** and **7**

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(5) Note that the priority sequence at C-2 is reversed in compounds **2** and **3** compared with that in compound **1**.

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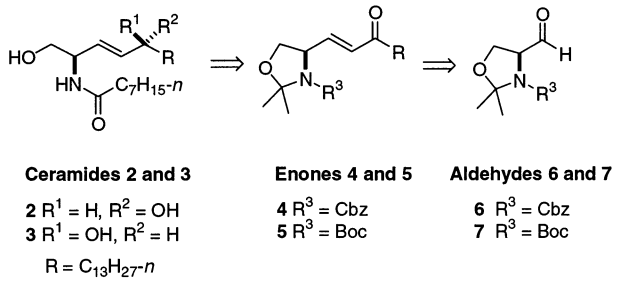
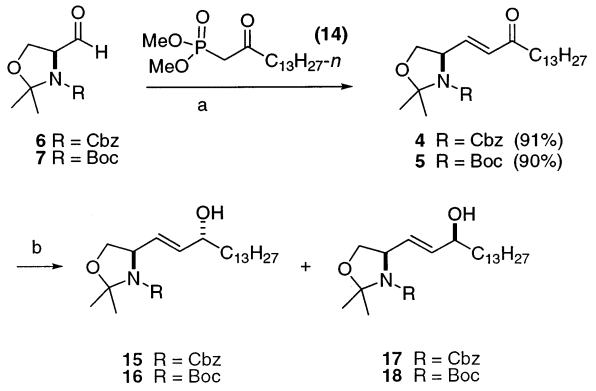
TABLE 1. Diastereoselective Reduction of Enones 4 and 5

		reduction of enone **4**		reduction of enone **5**	
reducing agent	reduction conditions	ratio of **15** to **17**	overall yield (%)	ratio of **16** to **18**	overall yield (%)
$NaBH_4$, $CeCl_3$	MeOH, 0 °C, 3 h	1.0:1.0	88	1.0:1.8	90
DIBAL-H	THF, 0 °C, 1 h	1.0:1.0	90	1.0:1.1	89
$LiAlH_4$, Chirald	Et_2O, 78 °C, 6 h	2.0:1.0	86	5.2:1.0	87
BH_3·Me_2S, oxazaborolidine	THF, rt, 1 h	1.4:1.0	85	2.3:1.0	84
L-Selectride	THF, 0 °C-to rt, 1 h	8.0:1.0	91	18.0:1.0	90

SCHEME 1. Retrosynthetic Plan



SCHEME 2. Diastereoselective Reduction of Enones 4 and 5[a]



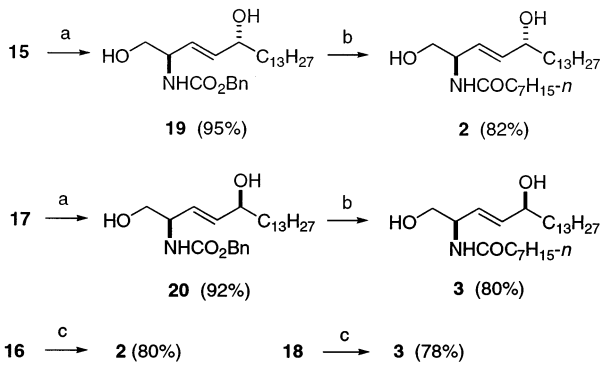
[a] Reagents and conditions: (a) Cs_2CO_3, 2-PrOH, rt; (b) see Table 1.

with ketophosphonate **14**. The configuration at C-2 is derived from L-serine as the chiral precursor.

Synthesis of Aldehydes 6 and 7. Aldehyde **6** was prepared by oxazolidine formation of *N*-Cbz-L-serine **8** (2,2-dimethoxypropane, catalytic *p*-TsOH, benzene),[7] followed by reduction of acetonide **10** with DIBAL-H in toluene at −78 °C. Similarly, (*S*)-Garner aldehyde **(7)** was prepared from *N*-Boc-L-serine **9** via acetonide **11**.[8]

Installation of the Lipid Chain and Diastereoselective Reduction of Enones 4 and 5 (Scheme 2). Treatment of dimethyl methanephosphonate (**12**) with *n*-BuLi in THF at −78 °C and reaction with methyl tetradecanoate (**13**) provided *β*-ketophosphonate **14** in 95% yield. As shown in Scheme 2, HWE reaction of **14** with L-(*N*-Cbz)- and L-(*N*-Boc)serinals **6** and **7** gave enones **4** and **5**, respectively, in high yield. Several reducing agents were screened for the attempted diastereoselective reduction of the ketone. Table 1 shows that reduction of *N*-Boc-protected enone **5** generally showed modestly higher selectivity with a variety of reducing agents than that of *N*-Cbz-protected enone **4**. The *tert*-butyl group appears to be more effective than the benzyl group with respect to blocking one face of the carbonyl group with all of the reducing agents shown in Table 1 except DIBAL-H. We found that reduction of **4** and **5** at 0 °C with $NaBH_4$ in the presence of $CeCl_3$ in methanol gave alcohols **15** and **16** in low stereoselectivity. Reduction with $LiAlH_4$ in the presence of a Chirald (a chiral ligand)[9] provided diastereomers **15** and **16** as the major products in ∼2:1 and 5.2:1 ratios of isomers, respectively. Similar results were obtained with oxazaborolidine-catalyzed reduction.[10] Fortunately, high stereoselectivity was achieved by using the bulky L-Selectride in THF (1 h, 0 °C to rt), and high ratios of **15** to **17** (8:1) and **16** to **18** (18:1) were obtained. The two diastereoisomers were readily separated by column chromatography. Thus, L-Selectride is the reducing agent of choice for the synthesis of ceramide **2** (via **16**), whereas $NaBH_4$/$CeCl_3$ is preferred for the preparation of ceramide **3** (via **18**).

SCHEME 3. Deprotection and *N*-Acylation[a]



[a] Reagents and conditions: (a) *p*-TsOH·H_2O, MeOH, rt; (b) (i) Li, NH_3, −78 °C, (ii) *p*-$O_2NC_6H_4CO_2C_7H_{15}$-*n*, THF, rt; (c) (i) 1 M HCl, dioxane, 100 °C, (ii) *p*-$O_2NC_6H_4CO_2C_7H_{15}$-*n*, THF, rt.

Deprotection and *N*-Acylation (Scheme 3). Acid hydrolysis of **15** (*p*-TsOH, MeOH) provided **19** in 95% yield; similarly, **20** was obtained from **17** in 92% yield. After the corresponding regioisomeric sphingosine analogues were obtained by removal of the Cbz group with lithium in liquid NH_3, *N*-acylation with *p*-nitrophenyl octanoate gave the diastereoisomeric ceramide analogues **2** and **3** in 82% and 80% yield, respectively. Ceramides **2** and **3** were also obtained in good yield from **16** and **18**, respectively, by removal of the *N*-Boc and isopropylidene

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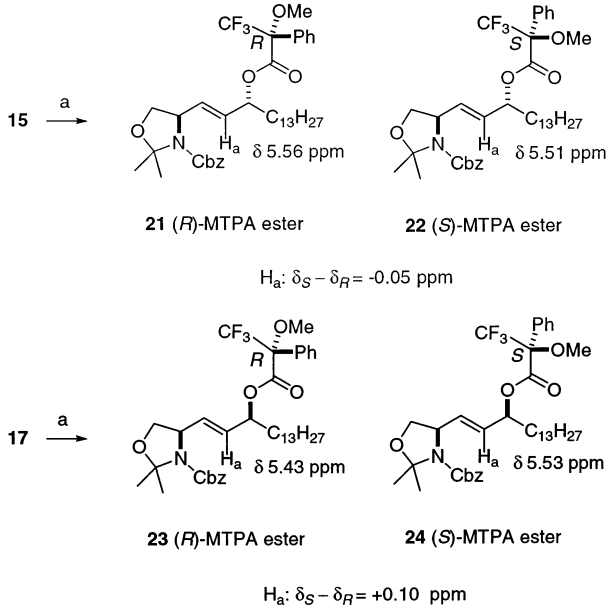
SCHEME 4. Configurational Assignment by ^{1}H NMR Analysis of the Diastereomeric (*S*)- and (*R*)-MTPA Esters[a]



[a] Reagents and conditions: (a) (*S*)-(+)- or (*R*)−(−)-MTPA chloride, CH_2Cl_2, DMAP, rt.

protecting groups (1 M HCl, dioxane, 100 °C) and *N*-acylation with *p*-nitrophenyl octanoate.

Configurational Assignment (Scheme 4). The assignment of the configuration at C-5 of **15** and **17** was made by ^{1}H NMR analysis[11] of the corresponding (*S*)- and (*R*)-Mosher esters, which were prepared by the reaction of (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) chloride or (*R*)-MTPA chloride with **15** and **17** in the presence of DMAP. The difference between the chemical shifts of the vinyl proton H_a in the (*R*)- and (*S*)-MTPA ester derivatives was used to determine the absolute configuration at C-5.[11,12] The upfield signal of H_a in the (*S*)-MTPA ester **22** (δ 5.51 ppm) compared to that in the (*R*)-MTPA ester **21** (δ 5.56 ppm) indicates that **15** has the *R* configuration ($\Delta\delta_{Ha} = \delta_S - \delta_R = -0.05$ ppm). Similarly, the *S* configuration was assigned to **17** by the downfield shift of H_a in **24** (δ 5.53 ppm) compared with **25** (δ 5.43 ppm) ($\Delta\delta_{Ha} = \delta_S - \delta_R = +0.10$ ppm).

Biological Evaluation of Compounds 2 and 3 (Figure 1). Synthetic ceramides with a short *N*-acyl chain (such as octyl) have been widely used for in vitro studies because they tend to be more cell permeable than the long-chain endogenous ceramides. To assess whether C8-ceramides having a 5-hydroxy-(3*E*)-sphingenine backbone show antiproliferative activity against epithelial tumor cells, we treated exponentially growing MCF-7 cells with varying concentrations of compounds **2** and **3** (0−20 μM) for 48 h. Figure 1 shows a comparison of the

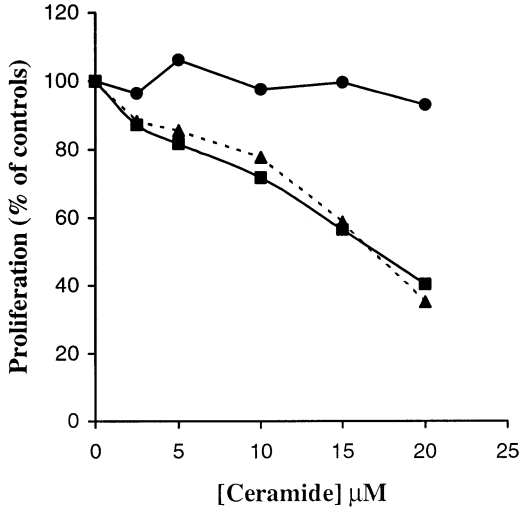


FIGURE 1. Effects of ceramides **1**−**3** on the proliferation of MCF-7 cells. Cells were grown in medium containing 5% serum and **1**−**3** (0−20 μM) for 48 h. The cell numbers were determined as described in the Experimental Section. Key: (●) **1**; (■) **2**; (▲) **3**.

effects of **1**−**3** on MCF-7 cell growth. Surprisingly, we found that ceramides **2** and **3** possessed significantly higher antiproliferative activity than **1**, which is known to induce apoptosis in many cells.[13] The IC_{50} value (the drug concentration required to inhibit growth by 50%) for **2** and **3** was ∼15 μM, indicating that the configuration at C-5 did not affect the activity, whereas the IC_{50} value of **1** was ≫20 μM. Thus, MCF-7 cells are significantly more resistant to C8-ceramides with the prevalent 3-hydroxy-(4*E*)-sphingenine backbone than with a 5-hydroxy-(3*E*)-sphingoid backbone. No information is available concerning the metabolism and intracellular localization of lipids containing the unusual 5-hydroxy-(3*E*)-sphingenine core. Further studies are planned to clarify the mechanisms by which ceramides with an altered alkenyl sphingoid chain such as **2** and **3** exert their antiproliferative action.

Conclusion

In summary, the first synthesis of 5*R* and 5*S* diastereoisomeric ceramides **2** and **3**, which have the naturally occurring but unusual 5-hydroxy-(3*E*)-sphingenine long-chain base and an *N*-octanoyl residue, was achieved in several steps from serinal derivatives **6** and **7**. A higher degree of diastereoselectivity was achieved in the L-Selectride reduction of *N*-Boc-enone **5** than in that of *N*-Cbz-enone **4**. The in vitro antiproliferative activity of 3-alkenylceramides **2** and **3** in the breast tumor cell line MCF-7 was much higher than that of D-*erythro*-*N*-C8-ceramide **1**.

Experimental Section[14]

General Information. Chirald (Darvon alcohol), L-Selectride, L-(*N*-Cbz)serine methyl ester (**8**), L-(*N*-Boc)serine methyl ester (**9**), and (*R*)-(−)- and (*S*)-(+)-α-methoxy-α-(tri-

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(12) H_a is affected by the anisotropic magnetic field around the phenyl ring (see ref 11). The chemical shifts reported here for H_a are for the central line of the double doublet.

(13) For a recent review of apoptosis induced by **1**, see: Andrieu-Abadie, N.; Gouaze, V.; Salvayre, R.; Levade, T. *Free Radical Biol. Med.* **2001**, *31*, 717−728.

(14) General experimental methods have been described; see, for example, ref 4.

fluoromethyl)phenylacetic acid (MTPA) chloride were used directly as obtained commercially. NMR spectra (400 MHz for ^{1}H, 100 MHz for ^{13}C) were recorded in $CDCl_3$ unless otherwise noted. IR spectra were recorded in chloroform. The preparation of starting materials *N*-Cbz-L-serine oxazolidine **10**, *N*-Cbz-L-serinaldehyde **6**, *N*-Boc-L-serine oxazolidine **11**, and (*S*)-Garner aldehyde (**7**) is described in the Supporting Information.

Dimethyl 2-Oxopentadecanephosphonate (14). To a solution of 1.5 g (12.0 mmol) of dimethyl methanephosphonate (**12**) in 30 mL of dry THF was added 4.8 mL (12.0 mmol) of *n*-BuLi (a 2.5 M solution in hexanes) at −78 °C under N_2. After the mixture was stirred for 30 min at −78 °C, a solution of 2.4 g (10.0 mmol) of methyl tetradecanoate (**13**) in 10 mL of THF was added dropwise with stirring. The mixture was kept at −78 °C for 1 h and then allowed to warm to 0 °C for 1 h. The reaction was quenched with saturated aqueous NH_4Cl solution, extracted with $CHCl_3$ (3 × 30 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was recrystallized (EtOAc/hexane) to give 2.8 g (92%) of ketophosphonate **14** as a white solid: mp 40.2−41.5 °C; ^{1}H NMR δ 0.88 (t, 3H, J = 6.6 Hz), 1.25 (m, 20H), 1.57 (t, 2H, J = 7.0 Hz), 2.61 (t, 2H, J = 7.3 Hz), 3.10 (d, 2H, J = 22.7 Hz), 3.76 (d, 2H, J = 10.8 Hz); ^{13}C NMR δ 14.1, 22.7, 23.4, 29.0, 29.4, 29.6, 29.7, 31.9, 40.6 (J = 128.3 Hz), 44.2, 53.0, 202.1; HR-MS [DEI, M^+] m/z calcd for $C_{17}H_{35}PO_4$ 334.2273, found 334.2271.

***N*-Benzyloxycarbonyl-4(*R*)-[3′-oxo-(1′*E*)-hexadecenyl]-2,2-dimethyl-1,3-oxazolidine [(−)-4].** To a suspension of ketophosphonate **14** (3.70 g, 11.1 mmol) and Cs_2CO_3 (3.58 g, 11.0 mmol) in 40 mL of 2-propanol was added a solution of aldehyde **6** (2.63 g, 10.0 mmol) in 10 mL of 2-propanol at 0 °C. After being stirred at rt overnight, the mixture was diluted with 200 mL of EtOAc and washed with water and brine. The organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by chromatography (hexane/EtOAc, 4:1) to give 4.30 g (91%) of enone **4** as a colorless oil: $[\alpha]^{25}_D$ −35.6° (c 5.0, $CHCl_3$); IR 1702, 1631, 1467, 1408, 1349, 1256, 1094 cm^{-1}; ^{1}H NMR δ 0.88 (t, 3H, J = 6.6 Hz), 1.26 (s, 20H), 1.49−1.60 (m, 2H), 1.57 (s, 3H) 1.67 (s, 3H), 2.54 and 2.40 (two sets of t, 2H, J = 7.2 Hz), 3.83 (dd, 1H, J = 9.2, 2.2 Hz), 4.12 (dd, 1H, J = 9.2, 6.4 Hz), 4.45−4.55 and 4.55−4.65 (two sets of m, 1H), 5.00−5.16 (m, 2H), 6.06 (d, 0.68H, J = 15.7 Hz), 6.24 (d, 0.32H, J = 15.7 Hz), 6.68 and 6.64 (two sets of dd, 1H, J = 15.7, 7.0 Hz), 7.26−7.36 (m, 5H); ^{13}C NMR δ 14.1, 22.6 (23.9),[15] 24.4 (24.7), 26.3 (27.3), 28.9, 29.1, 29.2, 29.3, 29.36, 29.4, 29.56, 29.58 (29.60), 31.9 (33.8), 58.0 (58.6), 66.8, (67.4) 67.7, 76.69 (77.00), 77.3, (94.3) 94.9, 128.0, 128.4, 130.5, 136.1, (142.3) 142.7, 152.1 (152.5), 200.0; HR-MS [DCI/NH_3, MH^+] m/z calcd for $C_{29}H_{46}NO_4$ 472.3426, found 472.3421.

***N*-*tert*-Butoxycarbonyl-4(*R*)-[3′-oxo-(1′*E*)-hexadecenyl]-2,2-dimethyl-1,3-oxazolidine [(−)-5].** This compound was prepared from aldehyde **7** in 90% yield by the same procedure as described for **4**: mp 38.0−39.0 °C; $[\alpha]^{25}_D$ −43.7° (c 1.0, $CHCl_3$); IR 1693, 1633, 1456, 1391, 1255, 1172, 1098 cm^{-1}; ^{1}H NMR (C_6D_6, 70 °C)[16] δ 0.88 (t, 3H, J = 6.7 Hz), 1.0−1.3 (m, 20H), 1.37 (s, 9H), 1.48 (s, 3H), 1.67 (m, 5H), 2.29 (t, 2H, J = 7.2 Hz), 3.43 (dd, 1H, J = 9.0, 2.7 Hz), 3.67 (dd, 1H, J = 9.0, 6.4 Hz), 4.15 (br s, 1H), 6.13 (d, 1H, J = 15.8 Hz), 6.60 (dd, 1H, J = 15.8, 7.0 Hz); ^{13}C NMR (C_6D_6) δ 14.3, 23.1, 23.7, 24.4, 25.8, 26.8, 27.6, 28.4, 29.6, 29.8, 29.9, 30.1, 32.3, 40.5, 58.5, (67.2) 67.5, 79.6 (79.9), (93.8) 94.6, 130.7, (142.7) 143.4, 151.7, 198.6; HR-MS [DCI/NH_3, MH^+] m/z calcd for $C_{26}H_{48}NO_4$ 438.3583, found 438.3588.

***N*-Benzyloxycarbonyl-4(*R*)-[3′-hydroxy-(1′*E*)-hexadecenyl]-2,2-dimethyl-1,3-oxazolidine (15, 17).** To a solution of $CeCl_3$ (0.81 g, 3.29 mmol) and $NaBH_4$ (0.12 g, 3.71 mmol) in 30 mL of MeOH was added a solution of enone **4** (1.18 g, 2.50 mmol) in 10 mL of MeOH at 0 °C. The mixture was stirred for 3 h at 0 °C, then diluted with 100 mL of EtOAc, and filtered through a pad of silica gel, which was rinsed with 100 mL of EtOAc. The filtrate was concentrated under reduced pressure. The residue was purified by chromatography (hexane/EtOAc, 3:1) to give diastereoisomers **15** (0.53 g, 45%) and **17** (0.51 g, 43%) as colorless oils. Data for **15**: $[\alpha]^{25}_D$ −9.8° (c 1.3, $CHCl_3$); IR 1698, 1467, 1410, 1350, 1253, 1095 cm^{-1}; ^{1}H NMR δ 0.88 (t, 3H, J = 6.6 Hz); 1.25 (s, 22H), 1.40−1.60 (m, 2H), 1.55 (s, 3H), 1.65 (s, 3H), 1.84 (br s, 2H), 3.75 (dd, 1H, J = 2.2, 8.9 Hz), 3.99 (br s, 1H), 4.05 (dd, 1H, J = 6.1, 8.9 Hz), 4.30−4.40 and 4.40−4.50 (two sets of m, 1H), 4.97−5.19 (m, 2H), 5.30−5.70 (m, 2H), 7.29−7.39 (m, 5H); ^{13}C NMR δ 14.1, 22.7 (23.6), 24.9 (25.4), 26.4, 27.27, (29.33) 29.5, 29.56, 29.59, 29.63, 29.65, 29.66 (30.9), 31.9, 37.0 (37.1), 58.5, (66.5) 66.9, 68.3 (68.6), (94.0) 94.4, 128.0, 128.1, (128.3) 128.5, 129.1, 135.3 (135.4), 136.4, 136.6, 152.4; HR-MS [DCI/NH_3, MH^+] m/z calcd for $C_{29}H_{48}NO_4$ 474.3583, found 474.3561. Data for **17**: $[\alpha]^{25}_D$ −18.9° (c 2.0, $CHCl_3$); IR 1698, 1466, 1410, 1350, 1254, 968 cm^{-1}; ^{1}H NMR δ 0.88 (t, 3H, J = 6.6 Hz), 1.25 (s, 22H) 1.43−1.50 (m, 2H), 1.55 (s, 3H), 1.65 (s, 3H), 1.73 (br s, 1H), 3.77 (dd, 1H, J = 2.1, 8.9 Hz), 4.01 (br s, 1H), 4.06 (dd, 1H, J = 6.0, 8.9 Hz), 4.34−4.43 and 4.45−4.51 (two sets of m, 1H), 4.95−5.35 (m, 2H), 5.50−5.90 (m, 2H), 7.28−7.40 (m, 5H); ^{13}C NMR δ 14.1, 22.7 (23.6), 25.3 (26.4), 29.5, 29.58, 29.60, 29.63, 29.65, 29.7, 30.9, 31.9, 37.1 (37.2), 58.5 (59.1), 66.5 (66.9), (68.3) 68.6, 72.0, 94.4 (94.5), 127.95 (128.02), 128.4 (128.5), 129.0, 135.5 (135.7), (136.4) 136.5, 153.4; HR-MS [DCI/NH_3, MH^+] m/z calcd for $C_{29}H_{48}NO_4$ 474.3583, found 474.3583.

***N*-*tert*-Butoxycarbonyl-4(*R*)-[3′-hydroxy-(1′*E*)-hexadecenyl]-2,2-dimethyl-1,3-oxazolidine (16, 18).** Compounds **16** and **18** was prepared in 90% overall yield by the same procedure as described for **15** and **17** ($NaBH_4$ reduction). Purification by chromatography (hexane/EtOAc, 2:1) afforded diastereoisomers **16** (32%) and **18** (58%) as colorless oils. Data for **16**: $[\alpha]^{25}_D$ −22.5° (c 1.0, $CHCl_3$); IR 1690, 1601, 1392, 1171, 1099 cm^{-1}; ^{1}H NMR δ (C_6D_6, 70 °C) 0.88 (t, 3H, J = 6.1 Hz), 1.20−1.80 (m, 39H), 3.54 (dd, 1H, J = 8.7, 2.4 Hz), 3.75 (dd, 1H, J = 8.7, 6.2 Hz), 3.98 (d, 1H, J = 5.2 Hz), 4.19 (br s, 1H), 5.62 (m, 2H); ^{13}C NMR δ (C_6D_6) 14.3, 23.1, 23.8, 25.2, 26.0, 27.0, 27.5, 28.5, 29.8, 30.1, 32.3, 37.8, 38.0, 59.0 (59.3), 68.4, 72.0 (72.2), 79.2 (79.8), (93.6) 94.2, (129.0) 129.3, 135.7 (136.7), 152.0; HR-MS [FAB, MNa^+] m/z calcd for $C_{26}H_{49}NO_4Na$ 462.3559, found 462.3579. Data for **18**: $[\alpha]^{25}_D$ −40.0° (c 1.0, $CHCl_3$); IR 1689, 1602, 1392, 1253, 1171 cm^{-1}; ^{1}H NMR (C_6D_6, 70 °C) δ 0.88 (t, 3H, J = 6.6 Hz), 1.20−1.80 (m, 39H), 3.54 (dd, 1H, J = 6.3, 2.3 Hz), 3.75 (dd, 1H, J = 8.7, 6.1 Hz), 4.00 (dd, 1H, J = 11.3, 5.8 Hz), 4.19 (br s, 1H), 5.62 (m, 2H); ^{13}C NMR (C_6D_6) δ 14.3, 23.1, 23.8, 25.0, 26.0, 27.1, 27.7, 28.5, 29.8, 30.1, 30.2, 32.3, 37.7, 38.0, 59.0, (68.2) 68.5, 72.0 (72.2), 79.1, 79.1 (79.7), (93.6) 94.2, (128.6) 129.6, 135.8 (136.2), 152.0; HR-MS [FAB, MNa^+] m/z calcd for $C_{26}H_{49}NO_4Na$ 462.3559, found 462.3566.

L-Selectride Reduction of Enone 5. To a solution of enone **5** (101 mg, 0.23 mmol) in 10 mL of dry THF was added 0.46 mL (0.46 mmol) of L-Selectride (lithium tri-*sec*-butylborohydride; a 1.0 M solution in THF) dropwise at 0 °C. The reaction mixture was stirred for 0.5 h at 0 °C and then allowed to warm to rt for another 0.5 h. The mixture was then diluted with 100 mL of EtOAc and filtered through a pad of silica gel, which was rinsed with 100 mL of EtOAc. The filtrate was concentrated under reduced pressure. The residue was purified by chromatography (hexane/EtOAc, 3:1) to give diastereoisomers **16** (86 mg, 85%) and **18** (4.8 mg, 4.7%) as colorless oils (the ratio of **16** to **18** was 18:1).

(2*R*,5*R*)-2-[(Benzyloxycarbonyl)amino]-(3*E*)-octadecene-1,5-diol [(+)-19]. A solution of oxazolidine **15** (0.45 g, 0.95 mmol) and *p*-TsOH·H_2O (0.18 g, 10 μmol) in 20 mL of MeOH was stirred overnight at rt. The reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography ($CHCl_3$/MeOH, 25:1) to give 0.39 g (95%) of **19** as a white solid: mp 92.0−93.0 °C; $[\alpha]^{25}_D$ +8.6° (c

(15) The ^{13}C NMR chemical shifts in parentheses indicate the small peaks arising from the minor rotamers in the dynamic equilibrium of the oxazolidine system, which is slow at ambient temperature.

(16) Proton NMR spectra of *N*-Boc-containing compounds were recorded at elevated temperature to facilitate the interconversion of the rotamers and thus simplify the spectra.

2.5, $CHCl_3$); IR 3435, 1720, 1601, 1503, 1467, 1232 cm^{-1}; ^1H NMR δ 0.88 (t, 3H, J = 6.6 Hz), 1.25 (s, 22H), 1.45−1.50 (m, 2H), 2.85 (br s, 2H), 3.60 (dd, 1H, J = 11.0, 4.0 Hz), 3.66 (dd, 1H, J = 11.0, 3.8 Hz), 4.06 (q, 1H, J = 6.1 Hz), 4.27 (br s, 1H), 5.09 (s, 2H), 5.42 (br s, 1H), 5.59 (dd, 1H, J = 15.7, 4.5 Hz), 5.66 (dd, 1H, J = 15.7, 5.8 Hz), 7.27−7.36 (m, 5H); ^{13}C NMR δ 14.1, 22.7, 25.4, 29.3, 29.5, 29.6, 29.7, 31.9, 37.1, 53.9, 64.9, 66.9, 72.3, 128.1, 128.2, 128.5, 135.2, 136.3, 158.0; HR-MS [FAB, MNa^+] m/z calcd for $C_{26}H_{43}NO_4Na$ 456.3090, found 456.3089.

(2*R*,5*S*)-2-[(Benzyloxycarbonyl)amino]-(3*E*)-octadecene-1,5-diol [(−)-20]. This compound was prepared from **17** in 92% yield by the procedure described above: mp 93.5−95.0 °C; $[\alpha]^{25}_D$ −2.5° (*c* 2.5, $CHCl_3$); IR 3437, 1719, 1602, 1503, 1467, 1232 cm^{-1}; ^1H NMR ($CDCl_3$/MeOD) δ 0.88 (t, 3H, J = 6.5 Hz), 1.10−1.70 (m, 24H), 3.59 (d, 2H, J = 4.0 Hz), 4.02 (m, 1H), 4.20 (m, 1H), 5.09 (br s, 1H), 5.62 (m, 2H); ^{13}C NMR ($CDCl_3$/MeOD) δ 14.2, 22.9, 25.7, 29.6, 29.8, 29.9, 32.1, 37.1, 54.5, 64.5, 67.0, 72.1, 127.8, 128.1, 128.3, 128.7, 135.7, 136.7, 157.0; HR-MS [FAB, MNa^+] m/z calcd for $C_{26}H_{43}NO_4Na$ 456.3090, found 456.3090.

(2*R*,5*R*)-2-[Octanoylamido]-(3*E*)-octadecene-1,5-diol [(+)-2]. Method A. To the blue solution prepared by addition of 0.10 g (14.4 mmol) of Li metal to 20 mL of liquid NH_3 was added a solution of **19** (0.26 g, 0.60 mmol) in 10 mL of dry THF at −78 °C. After the mixture was stirred for 30 min, the reaction was quenched by addition of NH_4Cl (0.78 g, 14.6 mmol). After removal of NH_3 by a stream of N_2, the mixture was diluted with 100 mL of Et_2O and washed with brine. The organic layer was dried over Na_2SO_4 and concentrated. The residue was dissolved in 20 mL of THF, and *p*-nitrophenyl octanoate (0.16 g, 0.60 mmol) was added. After the mixture was stirred overnight, it was concentrated, and the product was purified by column chromatography ($CHCl_3$/MeOH, 9:1) to give 0.21 g (82%) of **2** as a white solid.

Method B. A solution of 44 mg (0.1 mmol) of **16** in 5 mL of 1 M HCl and 5 mL of dioxane was heated at 100 °C with stirring for 1 h under N_2. The reaction mixture was cooled to rt and neutralized with 1 M NaOH (5 mL). The product was extracted with EtOAc (3 × 20 mL), and the combined organic layers were washed with brine and dried (Na_2SO_4). Removal of the solvent provided a crude sphingosine analogue as a white solid, which was dissolved in 6 mL of dry THF. After 54 mg (0.20 mmol) of *p*-nitrophenyl octanoate was added at rt, the reaction mixture was stirred for 24 h and concentrated. Purification by flash chromatography ($CHCl_3$/MeOH, 9:1) afforded 34 mg (80%, two steps) of ceramide **2** as a white solid: mp 69.0−70.0 °C; $[\alpha]^{25}_D$ +4.3° (*c* 1.0, $CHCl_3$); IR 3437, 1659, 1602, 1503, 1466, 1219 cm^{-1}; ^1H NMR δ 0.88 (t, 6H, J = 6.5 Hz), 1.30 (s, 32H), 1.48−1.52 (m, 2H), 1.62−1.65 (m, 2H), 2.31 (t, 2H, J = 7.5 Hz), 3.41 (br s, 2H), 3.64 (dd, 1H, J = 11.5, 4.3 Hz), 3.68 (dd, 1H, J = 11.5, 3.7 Hz), 4.08 (q, 1H, J = 6.0 Hz), 4.30−4.53 (m, 1H), 5.62 (dd, 1H, J = 15.8, 4.1 Hz), 5.67 (dd, 1H, J = 15.8, 4.9 Hz), 6.35 (d, 1H, J = 7.5 Hz); ^{13}C NMR δ 14.0, 14.1, 22.6, 22.7, 24.9, 25.5, 25.7, 25.8, 29.0, 29.1, 29.25, 29.3, 29.59, 29.61, 29.63, 29.65, 29.67, 31.7, 33.9, 36.8, 37.1, 52.6, 65.0, 72.3, 127.8, 135.4, 173.5; HR-MS [DCI/NH_3, MH^+] m/z calcd for $C_{26}H_{52}NO_3$ 426.3947, found 426.3938.

(2*R*,5*S*)-2-[Octanoylamido]-(3*E*)-octadecene-1,5-diol [(−)-3]. This compound was prepared from **18** and **20** by the procedure described above (methods A and B): mp 78.5−79.5 °C; $[\alpha]^{25}_D$ −11.0° (*c* 1.0, $CHCl_3$); IR 3435, 1657, 1503, 1466, 1218 cm^{-1}; ^1H NMR δ 0.85 (t, 6H, J = 6.5 Hz), 1.10−1.70 (m, 34H), 2.19 (t, 2H, J = 7.4 Hz), 3.63 (d, 2H, J = 4.5 Hz), 4.06 (dd, 1H, J = 12.2, 5.9 Hz), 4.49 (m, 1H), 5.62 (m, 2H), 6.04 (d, 1H, J = 7.7 Hz); ^{13}C NMR δ 14.0, 22.6, 22.7, 25.5, 25.7, 29.0, 29.2, 29.3, 29.6, 29.7, 31.7, 31.9, 36.8, 37.1, 40.4, 52.6, 65.1, 72.1, 127.3, 135.7, 173.7; HR-MS [DCI/NH_3, MH^+] m/z calcd for $C_{26}H_{52}NO_3$ 426.3947, found 426.3955.

Cell Cultures. MCF-7 (breast cancer) cells, originally obtained from the American Type Culture Collection, were grown to the exponential phase in medium supplemented with 5% fetal bovine serum and antibiotics, as described previously.[17] The cells were treated with compounds **1**−**3** (0−20 μM) for 48 h, and the increase in cell numbers after 48 h was determined[17] and expressed as a percentage of the controls, which received no drug. The results are the means of experiments made with quadruplicate wells. The standard deviations from the means were <10%. Stock solutions of the ceramides were made in EtOH. The final concentration of EtOH was <0.1%.

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Supporting Information Available: Preparation of starting materials (**6**, **7**, **10**, **11**) and ^1H and ^{13}C NMR spectra for compounds **2**−**5** and **14**−**20**. This material is available free of charge via the Internet at http://pubs.acs.org.

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