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Total Syntheses of Symbioramide Derivatives from L-Serine and Their Antileukemic Activities

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Naturally occurring symbioramide, (2*S*,3*R*,2′*R*,3′*E*)-*N*-(2′-hydroxy-3′-octadecenoyl)-dihydrosphingosine **1a**, was synthesized from D-*erythro*-dihydrosphingosine (amino part, **2**) and (2*R*,3*E*)-2-hydroxy-3-octadecenoic acid (acid part, **3a**), both of which were prepared from L-serine. Its diastereomer, (2*S*,3*R*,2′*S*,3′*E*)-**1b**, having an enantiomer of the unnatural-type acid part that was prepared from D-mannitol, and its corresponding (*Z*)-isomers, (2*S*,3*R*,2′*R*,3′*Z*)-**1c** and (2*S*,3*R*,2′*S*,3′*Z*)-**1d**, were also prepared. The antileukemic activities of **1a**−**d** against HL-60 and L-1210 cells were appreciated by a MTT assay. None of the four symbioramide derivatives showed antileukemic activities in HL-60 cells. In L-1210 cells, all the symbioramide derivatives showed moderate antileukemic activities. Compound **1d** had the most effective activity against L-1210 cells among the four derivatives. The data suggest that unnatural types of (2′*S*)-isomers of acid parts are more active than those of (2′*R*)-isomers.

Introduction

Sphingolipids and glycosphingolipids are ubiquitous membrane components of essentially all eukaryotic cells and serve physiologically important roles in bioorganisms.[1] Sphingolipids are structurally formed from three units: a sphingoid base, a fatty acid, and a polar headgroup. Recent studies have shown that sphingolipids exert an important function as intracellular second messengers in the regulation of cell growth,[2,3] differentiation,[3,4] and programmed cell death (apoptosis).[3,5] Ceramide, a metabolite or a precursor of sphingolipids, is an important molecule in the second messenger role of sphingolipid signaling. Ceramide is generated by neutral or acidic sphingomyelinase via the so-called sphingomyelin cycle[6] in response to various extracellular agents and stress such as the antibody against FAS,[7] tumor necrosis factor α (TNF-α),[8] and ionizing radiation.[9] Although the downstream of ceramide signaling is still not known, ceramide-induced apoptosis has been well characterized.

Another recent advancement in sphingolipid research was the identification of lipid microdomains, so-called "rafts".[10] Membrane sphingolipids such as sphingomyelin and glycosphingolipids constitute the microdomains by clustering cholesterol, glycosylphosphatidylinositol-anchored proteins, and membrane-associated proteins.

In 1988, a new type of bioactive ceramide, symbioramide (**1a**) was isolated from the laboratory-cultured dinoflagellate *Symbiodium* sp. obtained from the insides of gill cells of an Okinawan bivalve *Fragum* sp. by Kobayashi et al.[11] They also reported that symbioramide increased the sarcoplasmic reticulum Ca^{2+}−ATPase activity, which was the first example from marine sources, and also exhibited antileukemic activity against L-1210 murine leukemia cells in vitro (IC_{50} = 9.5 μg/mL).[12] Symbioramide is composed of D-*erythro*-dihydrosphingosine (**2**) as the amino part and (2*R*,3*E*)-2-hydroxy-3-octadecenoic acid (**3a**) as the acid part. Recently, a fatty

(1) (a) Kanfer, J. N.; Hakamori, S. *Handbook of Lipid Research*; Hanahan, D. J., Ed.; Plenum Press: New York, 1983; Vol. 3. (b) Kolter, T.; Sandhoff, K. *Chem. Soc. Rev.* **1996**, 371. (c) Kolter, T.; Sandhoff, K. *Angew. Chem., Int. Ed.* **1999**, *38*, 1532.

(2) (a) Olivera, A.; Spiegel, S. *Nature* **1993**, *365*, 557. (b) Olivera, A.; Zhang, H.; Carlson, R. O.; Mattie, M. E.; Schmidt, R. R.; Spiegel, S. *J. Biol. Chem.* **1994**, *269*, 17924. (c) Van Brocklyn, J. R.; Lee, M.-J.; Menzeleev, R.; Olivera, A. Edsall, A.; Cuvillier, O.; Thomas, D. M.; Coopman, P. J. P.; Thangada, S.; Liu, C. H.; Hla, T.; Spiegel, S. *J. Cell Biol.* **1998**, *142*, 229.

(3) (a) Spiegel, S.; Cuvillier, O.; Edsall, L.; Kohama, T.; Menzeleev, R.; Olivera, A.; Thomas, D.; Tu, Z.; Van Brocklyn, J.; Wang, F. *Biochemistry. Biokhimiia* **1998**, *63*, 69−73. (b) Pyne, S.; Pyne, N. J. *Biochem. J.* **2000**, *349*, 385.

(4) Riboni, L.; Prinetti, A.; Bassi, R.; Caminiti, A.; Tettamanti, G. *J. Biol. Chem.* **1995**, *270*, 26868. Furuya, S.; Mitoma, J.; Makino, A.; Hirabayashi, Y. *J. Neurochemistry* **1998**, *71*, 366. Toman, R E.; Spiegel, S.; Faden, A. *J. Neurotrauma* **2000**, *17*, 891.

(5) Cuvillier, O.; Pirianov, G.; Kleuser, B.; Vanek, P. G.; Coso, O. A.; Gutkind, J. S.; Spiegel, S. *Nature* **1996**, *381*, 800. Okazaki, T.; Kondo, T.; Kitano, T.; Tashima, M. *Cell. Signalling* **1998**, *10*, 685. Manna, S. K.; Sah, N. K.; Aggarwal, B. B. *J. Biol. Chem.* **2000**, *275*, 13297. Birbes, H.; Bawab, S. E.; Obeid, L. M., Hannum, Y. A. *Adv. Enzyme Regul.* **2002**, *42*, 113−129. Tomassini, B.; Testi, R. *Biochimie* **2002**, *84*, 123.

(6) Hannum, Y. A. *J. Biol. Chem.* **1994**, *269*, 3125.

(7) Cifone, M. G.; Maria, R. D.; Roncaioli, P.; Rippo, M. R.; Azuma, M.; Lanier, L. L.; Santoni, A.; Testi, R. *J. Exp. Med.* **1994**, *177*, 1547.

(8) Kim, M.; Linardic, C.; Obeid, L.; Hannun, Y. *J. Biol. Chem.* **1991**, *266*, 484.

(9) Haimovitz-Friedman, A.; Kan, C.; Ehleiter, D.; Persaud, R. S.; McLoughlin, M.; Fuks, Z. Kolesnic, R. N. *J. Exp. Med.* **1994**, *180*, 525.

(10) Prieschl, E, E.; Baumruker, T. *Immunol. Today* **2000**, *21*, 555.

(11) Kobayashi, J.; Ishibashi, M.; Nakagawa, H.; Hirata, Y.; Yamasu, T.; Sasaki T.; Ohizumi, Y. *Experientia* **1988**, *44*, 800.

(12) Kobayashi, J. *J. Nat. Prod.* **1989**, *52*, 1036.

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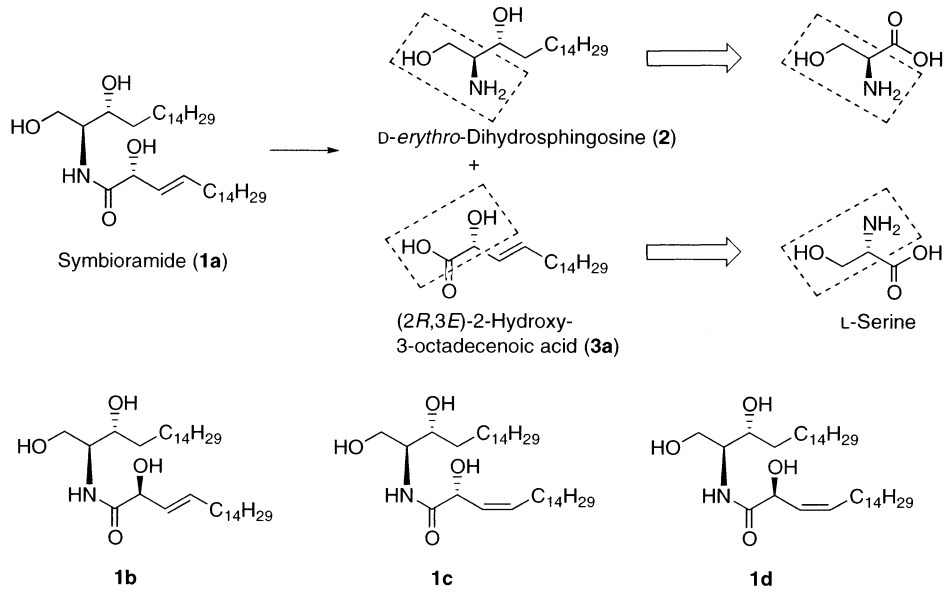
SCHEME 1. Synthetic Plan for the Preparation of Symbioramide (1a) and the Structures of Three Diastereomers (1b−d) of 1a



acid variant of symbioramide, symbioramide-C16, was isolated from a different *Symbiodinium* sp.[13] α-Hydroxy-*β*,*γ*-unsaturated carboxylic acids are a unique structure in the natural product, because this type of acid is not involved in biosynthesis and degradation of the usual fatty acids.

Several methods for the preparation of **2** have been published,[14] and more recently, we reported a synthetic method of preparing **2** from L-serine as a starting material using Wittig olefination followed by asymmetric epoxidation.[15]

The asymmetric total syntheses of **3a** and **3b** have been carried out starting from two kinds of the chiral glyceraldehydes by Nakagawa et al., and **1a** has been completely configured.[16,17] Their method of preparing **3a** is convenient, but the method of (*S*)-glyceraldehyde preparation as a chiral component gave a low yield.[18] Other preparation methods of **3a** and **3b** have been reported. Mori and co-worker[19] synthesized **3a** and **3b** using Sharpless asymmetric epoxidation of (*E*)-2-octadecen-1-ol, and Sugimura et al.[20] prepared **3a** by reducing *β*,*γ*-unsaturated α-oxo esters having a chiral auxiliary.

This report presents a new method for preparing the natural-type acid part **3a** from L-serine as the starting material and a method for preparing the natural-type amino part (**2**) (Scheme 1). The unnatural-type acid part **3b**, an enantiomer of **3a**, was also synthesized from D-mannitol. Structural isomers of both **3a** and **3b** with the (*Z*)-configuration, **3c** and **3d**, respectively, were also prepared. Four symbioramide derivatives (**1a**−**d**) containing these four acid parts were obtained, respectively, and the ability of these compounds to induce cell death in two leukemia cell lines (HL-60 and L-1210 cells) was assessed by MTT assay. We aim to elucidate the structure−activity relationships in ceramide-mediated apoptosis.

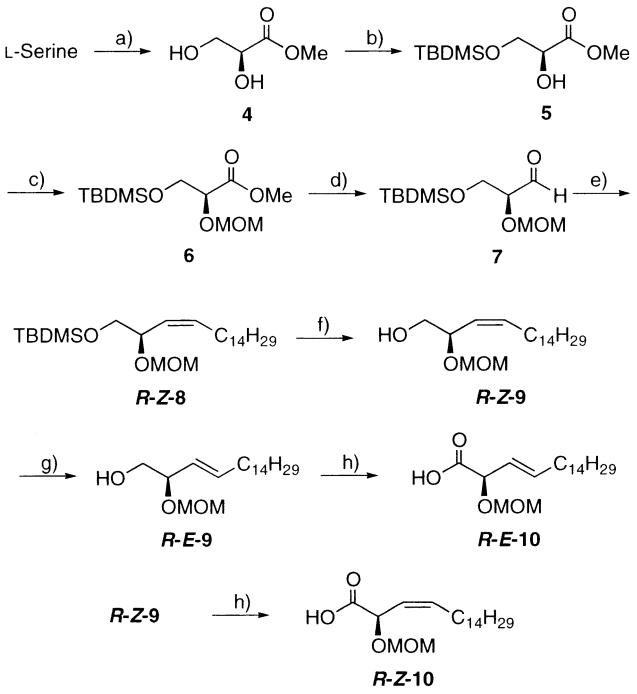
Results and Discussion

We focused our attention on protected (*Z*)-2-hydroxy-3-octadecen-1-ol species for acid parts since they are readily formed by the Wittig olefination of aldehydes from L-serine and D-mannitol with a Wittig reagent. And their related (*E*)-isomers could be prepared by photoisomerization of (*Z*)-derivatives in the presence of a sensitizer.

I. Natural-Type Acid Part. The synthetic approach to natural acid part **3a** is outlined in Scheme 2. The first approach is to convert the amino group of L-serine to the secondary hydroxy group without inversion at the center by the diazotization process. The preparation of chiral 1,2-dihydroxyester **4** was accomplished by a method reported in the literature[21] in 82% yield. Subsequent protections of the primary hydroxyl group of **4** with *tert*-butyldimethylsilyl chloride (TBDMSCl) and then the remaining secondary hydroxyl group with chloromethyl methyl ether (MOMCl) gave **6** in 67% yield (two steps), which was reduced with DIBALH at −78 °C in toluene to produce aldehyde **7** in 84% yield. The Wittig olefination of **7** with pentadecyltriphenylphosphonium bromide using *n*-BuLi as a base at −78 °C in THF gave the olefin ***R*-*Z*-8** (J = 11.2 Hz) as the sole product in 58% yield {-[α]$^{25}_{D}$ −60.6 (*c* 1.46, $CHCl_3$)}. The use of freshly prepared lithium hexamethyldisilazide (LHMDS, from *n*-BuLi and $NH(SiMe_3)_2$ or sodium hexamethyldisilazide (NaHMDS)) as the base resulted in a slightly lower yield (42−44%)

(13) Nakamura, H.; Kawase, Y.; Maruyama, K.; Murai, A. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 781.
(14) a) Reist, E. J.; Christie, P. H. *J. Org, Chem.* **1970**, *35*, 3521. (b) Roush, W. R.; Adam, M. A. *J. Org, Chem.* **1985**, *50*, 3752. (c) Sibi, M. P.; Li, B. *Tetrahedron Lett.* **1992**, *33*, 4115.
(15) Azuma, H.; Tamagaki, S.; Ogino, K. *J. Org. Chem.* **2000**, *65*, 3538.
(16) Nakagawa, M.; Yoshida, J.; Hino, T. *Chem. Lett.* **1990**, 1407.
(17) Yoshida, J.; Nakagawa, M.; Seki, H.; Hino, T. *J. Chem. Soc., Perkin Trans. 1* **1992**, 343.
(18) Takano, S.; Numata, H.; Ogasawara, K. *Heterocycles* **1982**, *19*, 327.
(19) Mori, K.; Uenishi, K. *Liebigs Ann. Chem.* **1994**, 41.
(20) Sugimura, H.; Yoshida, K. *J. Org. Chem.* **1993**, *58*, 4484.
(21) Mukaiyama, T.; Shiina, I.; Iwwadare, H.; Saitoh, M.; Nishimura, T.; Ohkawa, N.; Sakoh, H.; Nishimura, K.; Tani, Y.; Hasegawa, M.; Yamada, K.; Saitoh, K. *Chem. Eur. J.* **1999**, *5*, 121.

SCHEME 2[a]



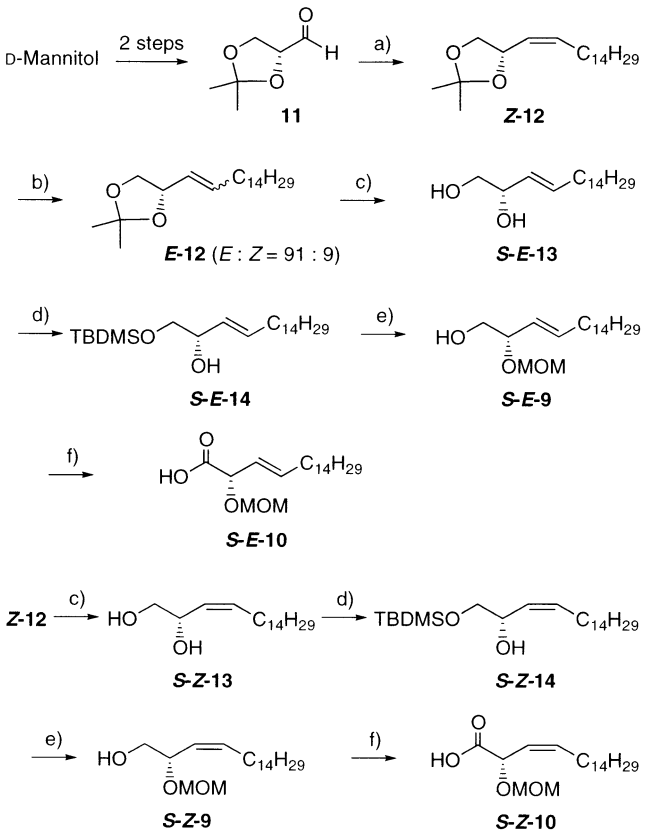
[a] Conditions: (a) $NaNO_2$, H_2SO_4, H_2O, rt; then $HC(OMe)_3$, H_2SO_4, MeOH, 60 °C. (b) TBDMSCl, Et_3N, cat. DMAP, CH_2Cl_2, rt. (c) MOMCl, $(i\text{-}Pr)_2NEt$, CH_2Cl_2, rt. (d) DIBALH, toluene, −78 °C. (e) $C_{15}H_{31}PPh_3Br$, *n*-BuLi, THF, −78 °C. (f) Bu_4NF, THF, 0 °C. (g) PhSSPh, *hv*, cyclohexane−1,4-dioxane (3:1). (h) PDC, DMF, 40−50 °C.

of olefin formation than the use of *n*-BuLi. The obtained olefin ***R*-*Z*-8** was photoisomerized using diphenyl disulfide (PhSSPh) as the sensitizer in a 1:3 v/v mixed solvent of 1,4-dioxane and cyclohexane. The ^{1}H NMR analysis of this reaction mixture showed the presence of (*E*)- and (*Z*)-olefins in a 74:26 ratio. Unfortunately, the separation of these products and PhSSPh by column chromatography was difficult; thus, the deprotection of ***R*-*Z*-8** was carried out before the photoisomerization. After removal of the silyl protection, the resulting unsaturated primary alcohol ***R*-*Z*-9** was photoisomerized using the above method. The ^{1}H NMR analysis of this reaction mixture showed the presence of (*E*)- and (*Z*)-olefins in a 76:24 ratio. Column chromatographic separation with *n*-hexane−EtOAc (3:1) as an eluent and then recrystallization of the obtained waxy solid with *n*-hexane gave pure ***R*-*E*-9** (J = 15.6 Hz) as a powdery solid in 55% yield {$[\alpha]^{25}_D$ −80.4 (*c* 1.24, $CHCl_3$)}.

Finally, oxidation of ***R*-*E*-9** with pyridinium dichromate (PDC) in DMF provided the carboxylic acid ***R*-*E*-10** in 78% yield. The protected MOM group of the obtained acid was easily decomposed; thus, this protected acid was immediately used in the next step of the amide formation reaction. The isomer ***R*-*Z*-10** was prepared using the same methodology without photoisomerization and obtained in 69% yield based on ***R*-*Z*-9** {$[\alpha]^{25}_D$ −116.1 (*c* 1.764, $CHCl_3$)}.

II. Unnatural-Type Acid Part. The synthetic approach to unnatural acid part **3b** is outlined in Scheme 3. The chiral acetonide *Z*-**12** was readily prepared from

SCHEME 3



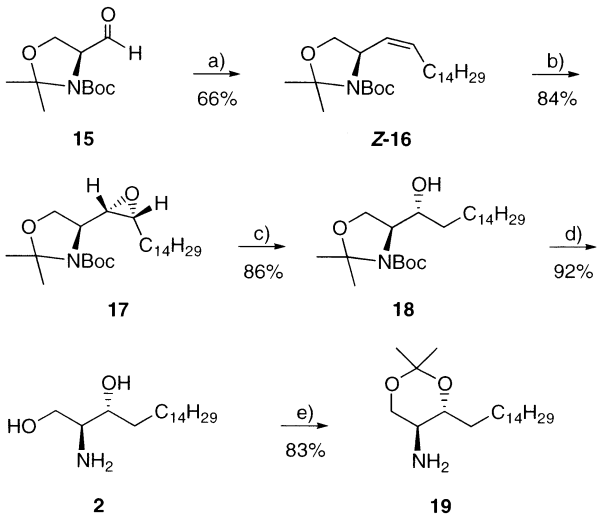
[a] Conditions: (a) $C_{15}H_{31}PPh_3Br$, *n*-BuLi, −78 °C. (b) PhSSPh, *hv*, cyclohexane-dioxane (3:1). (c) H^+, rt. (d) TBDMSCl, Et_3N, cat. DMAP, CH_2Cl_2, rt. (e) MOMCl, $(i\text{-}Pr)_2NEt$, CH_2Cl_2, rt then Bu_4NF, THF, 0 °C. (f) PDC, DMF, 40−50 °C.

D-mannitol by a literature method.[22] The photoisomerization of *Z*-**12** was carried out using the same method described above. The ^{1}H NMR analysis of this reaction mixture showed the presence of (*E*)- and (*Z*)-olefins in a 91:9 ratio. The separation of (*E*)- and (*Z*)-isomers was difficult by column chromatography; thus, the acetonide groups of these isomers were deprotected by acid before the purification. The obtained mixture of (*E*)- and (*Z*)-1,2-diol isomers could not be separated, but after recrystallization of the obtained crude solid, the mixture gave pure ***S*-*E*-13** in 69% yield based on *Z*-**12**. Two protections of the primary hydroxy group with TBDMSCl and the secondary hydroxy group with MOMCl followed by desilylation produced the primary alcohol ***S*-*E*-9** {$[\alpha]^{25}_D$ −80.9 (*c* 1.01, $CHCl_3$)} in 65% yield based on ***S*-*E*-13**. This alcohol was converted to the acid ***S*-*E*-10** using the same methodology for ***R*-*E*-10**. The isomer ***S*-*Z*-10** was also prepared without photoisomerization and obtained in 45% yield based on ***S*-*Z*-13** {$[\alpha]^{25}_D$ +111.1 (*c* 1.36, $CHCl_3$)}.

III. Natural-Type Amino Part (D-*erythro*-Dihydrosphingosine, 2). A synthetic method for preparing D-*erythro*-dihydrosphingosine **2** from L-serine has been reported[15] (eight steps, 21% yield based on L-serine, Scheme 4). The diol group of **2** was protected as an acetonide to facilitate the next amide formation reaction. Treatment of **2** with excess 2,2-dimethoxypropane and

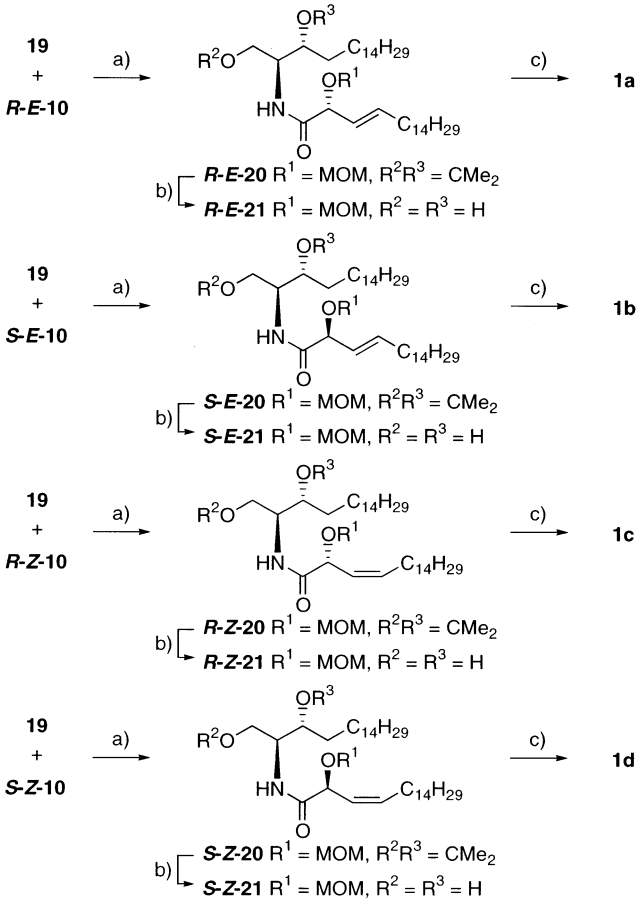
(22) Mulzer, J.; Brand, C. *Tetrahedron* **1986**, *42*, 5961.

SCHEME 4



[a] Conditions: (a) $C_{15}H_{31}PPh_3Br$, LiHMDS, THF, −78 °C. (b) *m*-CPBA, THF, rt. (c) Excess $LiAlH_4$, Et_2O, 0 °C. (d) TFA−H_2O, rt. (e) PPTS, $(Me)_2C(OMe)_2$, benzene, reflux.

SCHEME 5[a]



[a] Conditions: (a) DCC, HOBt, CH_2Cl_2, rt. (b) TsOH, CH_2Cl_2−MeOH (1:1), rt. (c) BF_3−Et_2O, EtSH, rt.

equivalent pyridinium *p*-toluenesulfonate monohydrate in benzene at reflux for 4 h gave acetonide **19** in 72% yield as a waxy solid: $[\alpha]^{25}_D$ +31.5 (*c* 2, $CHCl_3$) {lit.[17] $[\alpha]^{22}_D$ +29.5 (*c* 1.178, $CHCl_3$), as a brown oil; lit.[19] $[\alpha]^{21}_D$ +32.4 (*c* 1.08, $CHCl_3$), as a waxy solid}.

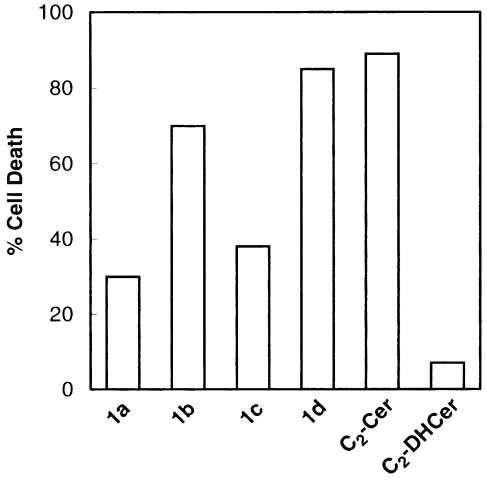


FIGURE 1. MTT assay of L-1210 cells after treatment with 10 μM symbioramide derivative for 6 h.

IV. Natural-Type Symbioramide (1a). Acid part ***R-E*-10** and amino part **19** were condensed to obtain a natural type of symbioramide according to the method of Nakagawa et al.[17] This amide formation was successfully accomplished by the conventional method using dicyclohexylcarbodiimide (DCC) in the presence of *N*-hydroxybenzotriazole (HOBt) to give the full-protected amide **20a** as a solid in 79% yield. Two deprotection reactions of the acetonide moiety with *p*-toluenesulfonic acid and of the MOM group with BF_3·Et_2O in EtSH followed by recrystallization from acetone−benzene gave a natural type of symbioramide **1a** as a powdery solid (two steps, 55%). The specific rotation value of our synthetic **1a** was $[\alpha]^{25}_D$ +1.19 (*c* 0.5, $CHCl_3$) {natural **1a**, $[\alpha]^{19}_D$ +5.8 (*c* 1, $CHCl_3$);[12] Nakagawa's synthetic **1a**, $[\alpha]^{19}_D$ +2.65 (*c* 1, $CHCl_3$)}.[17] Mori et al. exhibited the temperature dependence of the specific rotation value of **1a**: $[\alpha]^{19}_D$ +3.6, $[\alpha]^{23}_D$ +0.76, $[\alpha]^{28}_D$ −1.5, $[\alpha]^{35}_D$ −5.5 (*c* 0.31, $CHCl_3$).[19] Accordingly, our value seems appropriate.

V. Unnatural Types of Symbioramides (1b−d). We also synthesized three other diastereomers of symbioramide (**1b−d**). **1b**, **1c**, and **1d** were prepared as described above using ***S-E*-10**, ***R-Z*-10**, and ***S-Z*-10**, respectively. The specific rotation values of **1b**, **1c**, and **1d** were $[\alpha]^{25}_D$ +17.2 (*c* 0.502, $CHCl_3$), $[\alpha]^{25}_D$ +49.9 (*c* 0.5, $CHCl_3$), and $[\alpha]^{25}_D$ +63.3 (*c* 0.5, $CHCl_3$), respectively.

The obtained yields of three diastereomers **1b**, **1c** and **1d** based on the corresponding acids were 36, 45, and 41%, respectively. **1c** and **1d** are newly synthesized compounds.

VI. Antileukemic Activity of Symbioramide Derivatives against L-1210 and HL-60 Cells. To study the influence of the different structures of the acid parts, the cell death activity of the natural-type symbioramide **1a** was compared with that of three unnatural-type derivatives **1b−d** by MTT assay. Two leukemia cell lines (HL-60 cells and L-1210 cells) were treated with 10 μM of a symbioramide derivative for 6 h. C_2-Ceramide (*N*-acetyl-D-*erythro*-sphingosine, **C_2-Cer**) and C_2-dihydroceramide (*N*-acetyl-D-*erythro*-dihydrosphingosine, **C_2-DH-Cer**) were used as positive and negative controls, respectively. None of the four symbioramide derivatives showed antileukemic activities in HL-60 cells (data not shown). In L-1210 cells, however, all symbioramide

derivatives showed moderate antileukemic activities (Figure 1). Compound **1d** was the most effective among the symbioramide derivatives, and the antileukemic activity is in the order **C_2-Cer** > **1d** > **1b** > **1c** > **1a** > **C_2-DHCer**. It is interesting that unnatural types of (2′*S*)-isomers of acid parts, **1d** and **1b**, are more active than those of (2′*R*)-isomers, **1c** and **1a**.

Conclusion

Naturally occurring symbioramide **1a** and its three diastereomers (**1b**−**d**) having different acid part structures were synthesized. The proposed methods of preparing the (*E*)- and (*Z*)-isomers are useful because α-hydroxy-*β*,*γ*-unsaturated fatty acid-containing symbioramide is seldom found in natural sources.

All symbioramide derivatives showed antileukemic activity against L-1210 cells, although these activities were less than that of **C_2-Cer**, which is known to induce apoptosis in various cell types.[23] Dihydroceramides are considered to be physiologically inactive. In fact, **C_2-Cer** showed strong activity but **C_2-DHCer** showed no cell death (Figure 1). Nevertheless, dihydroceramide-types **1a**−**d** had antileukemic activity against L-1210 cells. It is possible that no activities of these derivatives against HL-60 cells resulted from low cell-permeability. In the future, we intend to prepare symbioramide analogues having short-chain acid parts (a chain length less than eight carbon atoms) to enhance the cell-permeability.

Experimental Section

All materials were obtained commercially (guaranteed reagent grade) and used without further purification. All solvents were freshly distilled under nitrogen before use. THF and diethyl ether were distilled from $LiAlH_4$; CH_2Cl_2 and benzene were distilled from P_2O_5. Column chromatography was performed on silica gel.

Methyl (*S*)-3-(*tert*-Butyldimethylsiloxy)-2-(methoxymethoxy)propionate (6). **5**[21] (7.03 g, 30 mmol) and *N*,*N*-diisopropylethylamine (19.4 g, 150 mmol) were dissolved in dry CH_2Cl_2 (150 mL). The solution was stirred at room temperature, and then chloromethyl methyl ether (MOMCl) (9.66 g, 120 mmol) was added dropwise, and the solution was stirred for 2 h. The mixture was subsequently poured into water, and the organic layer was separated and washed with water, dried with Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography with chloroform to give **6** as a colorless oil (6.91 g, 84%): $[\alpha]^{25}_D$ −26.0 (*c* 2.37, $CHCl_3$); IR (NaCl) 2955, 2858, 1755, 1463, 1437, 1362, 1258, 1205, 1155, 1126, 1047, 922, 839, 779 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.06 (s, 6 H), 0.88 (s, 9 H), 3.40 (s, 3 H), 3.75 (s, 3 H), 3.91 (d, 2 H, J = 4.9 Hz), 4.25 (t, 1 H, J = 4.9 Hz), 4.73 (s, 2 H); HRMS (FAB, direct) calcd for $C_{12}H_{26}O_5Si$, $[M + H]^+$ 279.1628; found, 279.1624 (22%). Anal. Calcd: C, 51.77; H, 9.41. Found: C, 51.64; H, 9.64.

Methyl (*S*)-3-(*tert*-Butyldimethylsiloxy)-2-(methoxymethoxy)propanal (7). To a solution of **6** (6.0 g, 21.6 mmol) in dry toluene (100 mL) at −78 °C was added DIBAL in toluene (1.5 M, 21.6 mL). The reaction mixture was stirred for 1 h at −78 °C, and then methanol (30 mL) and saturated phosphorus buffer (pH 7.0, 200 mL) were added. After the reaction mixture had been stirred for 30 min, the mixture was treated with brine and extracted with CH_2Cl_2. The organic layer was dried with Na_2SO_4 and concentrated to give a crude product. Purification by column chromatography with *n*-hexane−EtOAc (6:1) gave **7** (4.52 g, 84%) as a colorless oil: $[\alpha]^{25}_D$ −4.4 (*c* 2.859, $CHCl_3$); IR (NaCl) 2954, 2889, 1736, 1474, 1256, 1111, 1040, 920, 837, 779 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.07 (s, 6 H), 0.88 (s, 9 H), 3.43 (s, 3 H), 3.93 (d, 2 H, J = 4.9 Hz), 4.04 (dt, 1 H, J = 1, 4.9 Hz), 4.78 (dd, 2 H, J = 6.8, 13.7 Hz), 9.72 (s, 1 H); HRMS (FAB, direct) calcd for $C_{12}H_{26}O_5Si$, $[M + H]^+$ 249.1522; found, 249.1521 (25%).

(2*R*,3*Z*)-1-(*tert*-Butyldimethylsiloxy)-2-(methoxymethoxy)-3-octadecene (*R*-*Z*-8). To a solution of pentadecyltriphenylphosphonium bromide (4.15 g, 7.5 mmol) in dry THF (10 mL) was added *n*-BuLi (1.6 M solution in hexane, 4.78 mL) slowly over 10 min at 0 °C. The resulting dark red solution was added dropwise to a solution of aldehyde **7** (1.69 g, 6.8 mmol) in dry THF (50 mL) at −78 °C and stirred for 1 h. After stirring overnight at room temperature, the mixture was poured into ice-cooled 1 M HCl and extracted with EtOAc. After drying with Na_2SO_4, the solvent was concentrated, and cold ether (100 mL) was added to deposit the resulting triphenylphosphine oxide. After the precipitate was separated and the solvent was removed, the residue was purified by column chromatography with *n*-hexane-AcOEt (20:1) to give ***R*-*Z*-8** (1.75 g, 58%) as a colorless oil. The (*E*)-isomer was not found (by ^1H NMR analysis): $[\alpha]^{25}_D$ −46.3 (*c* 3.443, $CHCl_3$); IR (NaCl) 2926, 2855, 1473, 1256, 1213, 1126, 1101, 1034, 920, 837, 777 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.07 (s, 6 H), 0.88 (t, 3 H, J = 6.8 Hz), 0.90 (s, 9 H), 1.25 (brs, 24 H), 2.10 (q, 2 H, J = 7.3 Hz), 3.37 (s, 3 H), 3.57 (dd, 1 H, J = 4.4, 10.7 Hz), 3.68 (dd, 1 H, J = 7.3, 10.7 Hz), 4.43−4.49 (m, 1 H), 4.59 (d, 1 H, J = 6.8 Hz), 4.68 (d, 1 H, J = 6.3 Hz), 5.21 (dd, 1 H, J = 9.5, 11.2 Hz), 5.65 (dt, 1 H, J = 11.2, 7.3 Hz); HRMS (FAB, direct) calcd for $C_{26}H_{53}O_3Si$, $[M - H]^+$ 441.3764; found, 441.3744 (30%).

(2*R*,3*Z*)-2-(Methoxymethoxy)-3-octadecen-1-ol (*R*-*Z*-9). To a solution of ***R*-*Z*-8** (2 g, 4.52 mmol) in dry THF (10 mL) was added tetrabutylammonium fluoride (1 M solution in THF, 6 mL) dropwise at 0 °C, and the solution was stirred at room temperature for 1 h. The solution was subsequently poured into water and extracted with diethyl ether. The ether layer was washed with brine and dried with Na_2SO_4. The solvent was concentrated, and the residue was purified by column chromatography with *n*-hexane−EtOAc (3:1) to give ***R*-*Z*-9** (1.29 g, 87%) as a colorless oil: $[\alpha]^{25}_D$ −60.6 (*c* 1.46, $CHCl_3$); IR (NaCl) 3447, 2922, 2853, 1466, 1209, 1151, 1101, 1039, 920, 721 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, 3 H, J = 6.8 Hz), 1.26 (brs, 24 H), 2.05−2.14 (m, 2 H), 2.44 (dd, 1 H, J = 4.9, 7.8 Hz), 3.41 (s, 3 H), 3.53−3.60 (m, 2 H), 4.46−4.52 (m, 1 H), 4.60 (d, 1 H, J = 6.3 Hz), 4.73 (d, 1 H, J = 6.8 Hz), 5.25 (dd, 1 H, J = 9.0, 10.7 Hz), 5.67 (dt, 1 H, J = 10.7, 7.6 Hz); HRMS (FAB, direct) calcd for $C_{20}H_{40}O_3Na$, $[M + Na]^+$ 351.2875; found, 351.2867 (52%).

(2*R*,3*E*)-2-(Methoxymethoxy)-3-octadecen-1-ol (*R*-*E*-9). A solution of ***R*-*Z*-9** (720 mg, 2.19 mmol) and diphenyl disulfide (158 mg, 0.72 mmol) in dry cyclohexane−dioxane (3:1, 20 mL) in a Pyrex test tube (25 mL) was degassed under N_2. The solution was irradiated by a water-cooled high-pressure mercury lamp for 1 h. The slightly yellow solution was treated twice with diphenyl disulfide (2 × 158 mg, 2 × 0.72 mmol) and irradiated over a total period of 3 h. The reaction mixture was then concentrated to give the residue containing the (*E*)- and (*Z*)-olefins **9** in a 74:26 ratio (by ^1H NMR analysis). The residue was purified by column chromatography with *n*-hexane−EtOAc (3:1) and recrystallized from *n*-hexane to give ***R*-*E*-9** (370 mg, 51%) as a powdery solid: mp 44 °C (lit.[19] mp 39−40 °C); $[\alpha]^{25}_D$ −80.4 (*c* 1.24, $CHCl_3$) {lit.[19] $[\alpha]^{23}_D$ −77.5 (*c* 0.9, $CHCl_3$)}; IR (KBr) 3320, 2918, 2851, 1468, 1152, 1098, 1036, 966, 914, 721 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, 3 H, J = 6.8 Hz), 1.25 (brs, 22 H), 1.34−1.41 (m, 2 H), 2.05 (q, 2 H, J = 6.8 Hz), 2.37 (t, 3 H, J = 6.83 Hz), 3.40 (s, 3 H), 3.56−3.60 (m, 2 H), 4.10 (dt, 1 H, J = 4.9, 6.8 Hz), 4.61 (d, 1

(23) (a) Ito, A.; Uehara, T.; Tokumitsu, A.; Okuma, Y.; Nomura, Y. *Biochim. Biophys. Acta* **1999**, *1452*, 263. (b) Monti, B.; Zanghellini, P.; Contestabile, A. *Neurochem. Int.* **2001**, *39*, 11. (c) Itakura, A.; Tanaka, A.; Aioi, A.; Tonogaito, H.; Matsuda, H. *Exp. Hematology* **2002**, *30*, 272.

H, J = 6.8 Hz), 4.75 (d, 1 H, J = 6.8 Hz), 5.31 (dd, 1 H, J = 7.8, 15.6 Hz), 5.76 (dt, 1 H, J = 15.6, 6.8 Hz); MS (FAB, direct) calcd for $C_{20}H_{40}O_3Na$, $[M + Na]^+$ 351.3; found, 351.4 (24%).

(2*R*,3*E*)-2-(Methoxymethoxy)-3-octadecenoic Acid (*R*-*E*-10). A mixture of ***R*-*E*-9** (400 mg, 1.22 mmol) and PDC (2.74 g, 7.27 mmol) in *N*,*N*-dimethylformamide (DMF, 8 mL) was stirred for 3 h at 40−50 °C under N_2.The resulting mixture was diluted with water and extracted with ethyl acetate. The extract was washed with saturated brine, dried with Na_2SO_4, and concentrated to give the crude acid as a brown oil. The residue was purified by column chromatography with *n*-hexane−EtOAc (1:1) to give ***R*-*E*-10** (330 mg, 78%) as a solid. Since the MOM group of ***R*-*E*-10** could be easily decomposed, this acid was used immediately in the next step: mp 57−58 °C; $[\alpha]^{25}_D$ −64.5 (*c* 0.882, $CHCl_3$); IR (KBr) 2918, 2851, 1736, 1464, 1202, 1111, 1026, 970, 897, 719 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, 3 H, J = 6.8 Hz), 1.25 (brs, 22 H), 1.36−1.42 (m, 2 H), 2.09 (q, 2 H, J = 6.8 Hz), 3.41 (s, 1 H), 4.64 (d, 1 H, J = 7.3 Hz), 4.69 (d, 1 H, J = 6.8 Hz), 4.77 (d, 1 H, J = 6.8 Hz), 5.47 (dd, 1 H, J = 7.3, 15.6 Hz), 5.94 (dt, 1 H, J = 15.6, 6.8 Hz); HRMS (FAB, direct) calcd for $C_{20}H_{37}O_4$, $[M - H]^+$ 341.2692; found, 341.2690 (100%).

(2*R*,3*Z*)-2-(Methoxymethoxy)-3-octadecenoic Acid (*R*-*Z*-10). The reaction was carried out as described above, using ***R*-*Z*-9** (400 mg, 1.22 mmol) to give ***R*-*Z*-10** (290 mg, 69%) as a colorless oil: $[\alpha]^{25}_D$ −116.1 (*c* 1.764, $CHCl_3$); IR (NaCl) 2910, 2890, 1729, 1466, 1215, 1151, 1107, 1040, 920, 768, 721 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, 3 H, J = 6.8 Hz), 1.26 (brs, 22 H), 1.36−1.42 (m, 2 H), 2.05 (s, 1 H), 2.19 (q, 1 H, J = 7.3 Hz), 3.41 (s, 1 H), 4.12 (q, 1 H, J = 7.3 Hz), 4.67 (d, 1 H, J = 6.8 Hz), 4.74 (d, 1 H, J = 6.8 Hz), 5.05 (d, 1 H, J = 9.3 Hz), 5.39 (dd, 1 H, J = 9.3, 10.7 Hz), 5.85 (dt, 1 H, J = 10.7, 7.3 Hz); HRMS (FAB, direct) calcd for $C_{20}H_{37}O_4$, $[M - H]^+$ 341.2692; found, 341.2689 (100%).

(2*S*,3*E*)-3-Octadecen-1,2-diol (*S*-*E*-13). A solution of *Z*-**12**[22] (4.58 g, 14.1 mmol) and diphenyl disulfide (340 mg, 1.55 mmol) in dry cyclohexane−dioxane (3:1, 100 mL) in a Pyrex flask (100 mL) was degassed under N_2. The solution was irradiated by a water-cooled high-pressure mercury lamp for 1 h. The slightly yellow solution was treated twice with diphenyl disulfide (2 × 340 mg, 2 × 1.55 mmol) and irradiated over a total period of 3 h. The reaction mixture was then concentrated to give the residue containing the (*E*)- and (*Z*)-olefins **13** in a 91:9 ratio (by ^1H NMR analysis). The residue was dissolved in a mixture of acetic acid (25 mL), water (15 mL), THF (4.5 mL), and 1 M sulfuric acid (1 mL) and stirred for 24 h at room temperature. After neutralization with potassium carbonate and extraction with ethyl acetate, the organic phase was dried with Na_2SO_4 and concentrated. The residue was purified by column chromatography with chloroform−methanol (10:1) and recrystallized from *n*-hexane to give ***S*-*E*-13** (2.77 g, 69%) as a solid: mp 60.5−61.5 °C (lit.[17] mp 60−60.5 °C); $[\alpha]^{25}_D$ +9.07 (*c* 1.534, $CHCl_3$) {lit.[17] $[\alpha]^{29}_D$ +9.05 (*c* 0.398, $CHCl_3$)}; IR (KBr) 3342, 2920, 2849, 1470, 1082, 1022, 976, 719 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$); δ 0.88 (t, 3 H, J = 6.8 Hz), 1.26 (brs, 22 H), 1.34−1.42 (m, 2 H), 2.02 (q, 2 H, J = 6.8 Hz), 2.10 (brs, 1 H), 2.19 (brs, 1 H), 3.49 (dd, 1 H, J = 7.8, 10.7 Hz), 3.63 (d, 1 H, J = 11.2 Hz), 4.19 (m, 1 H), 5.44 (dd,1 H, J = 6.8, 15.6 Hz), 5.78 (dt, 1 H, J = 15.6, 6.8 Hz); MS (EI, direct) calcd for $C_{18}H_{35}O_2$, $[M]^+$ 284; found, 284 (0.4%), $[M - CH_2OH]^+$ 253; found, 253 (87%).

(2*S*,3*E*)-1-(*tert*-Butyldimethylsiloxy)-3-octadecen-2-ol (*S*-*E*-14). To a solution of ***S*-*E*-13** (1.54 g, 5.41 mmol), triethylamine (0.66 g, 6.49 mmol), and 4-(dimethylamino)pyridine (27 mg, 0.22 mmol) in dry CH_2Cl_2 (50 mL) was added a solution of *tert*-butylchlorodimethylsilane (0.9 g, 6 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C. The reaction mixture was stirred overnight at room temperature, and then phosphate buffer (pH 7.0) was added. The mixture was extracted with diethyl ether, and the organic layer was washed with brine and dried with Na_2SO_4. After concentration of the solvent, the residue was purified by column chromatography with chloroform to give ***S*-*E*-14** (1.74 g, 81%) as a colorless oil: $[\alpha]^{25}_D$ +10.9 (*c* 1.451, $CHCl_3$) {lit.[19] $[\alpha]^{23}_D$ −11.4 (*c* 1.33, $CHCl_3$)}; IR (NaCl) 2936, 2855, 1464, 1254, 1111, 968, 837, 777 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.08 (s, 6 H), 0.89 (t, 3 H, J = 7.2 Hz), 0.91 (s, 9 H), 1.26 (brs, 22 H), 1.34−1.42 (m, 2 H), 2.03 (q, 2 H, J = 6.8 Hz), 2.57 (d, 1 H, J = 2.8 Hz), 3.42 (dd, 1 H, J = 8.4, 10 Hz), 3.62 (dd, 1 H, J = 3.6, 10 Hz), 4.08−4.18 (m, 1 H), 5.39 (dd, 1 H, J = 3, 15.6 Hz), 5.76 (dt, 1 H, J = 15.6, 6.8 Hz); HRMS (FAB, direct) calcd for $C_{24}H_{49}O_2Si$, $[M - H]^+$ 397.3502; found, 397.3492 (100%).

(2*S*,3*Z*)-1-(*tert*-Butyldimethylsiloxy)-3-octadecen-2-ol (*S*-*Z*-14). The reaction was carried out as described above, using ***S*-*Z*-13**[22] (500 mg, 1.76 mmol) to give ***S*-*Z*-14** (500 mg, 71%) as a colorless oil: $[\alpha]^{25}_D$ +25.2 (*c* 1.608, $CHCl_3$); IR (NaCl) 3463, 2955, 2855, 1464, 1312, 1256, 1107, 1069, 968, 837, 779, 721 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.09 (s, 6 H), 0.88 (t, 3 H, J = 6.8 Hz), 0.92 (s, 9 H), 1.26 (brs, 24 H), 2.02−2.15 (m, 2 H), 2.54 (d, 1 H, J = 2.4 Hz), 3.41 (dd, 1 H, J = 8.4, 10 Hz), 3.57 (dd, 1 H, J = 3.6, 10 Hz), 4.43−4.50 (m, 1 H), 5.32 (dd,1 H, J = 8.5, 11.2 Hz), 5.57 (dt, 1 H, J = 11.2, 6.4 Hz); HRMS (FAB, direct) calcd for $C_{24}H_{49}O_2Si$ $[M - H]^+$ 397.3502; found, 397.3496 (100%).

(2*S*,3*E*)-2-(Methoxymethoxy)-3-octadecen-1-ol (*S*-*E*-9). To a solution of ***S*-*E*-14** (1.74 g, 4.36 mmol) and *N*,*N*-diisopropylethylamine (2.32 g, 18 mmol) in dry CH_2Cl_2 (50 mL) was added MOMCl (1.74 g, 21.6 mmol) dropwise at 0 °C. The reaction mixture was stirred for 2 h at room temperature. The mixture was subsequently poured into water, and the organic layer was separated and washed with brine, dried with Na_2SO_4, and concentrated in vacuo. The residue was dissolved in THF (50 mL), and the solution was stirred in an ice bath. Then, tetrabutylammonium fluoride (1 M solution in THF, 5.8 mL) was added dropwise at 0 °C, and the mixture was stirred at room temperature for 1 h. The mixture was subsequently poured into water and extracted with diethyl ether. The ether layer was washed with brine, dried with Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography with *n*-hexane−EtOAc (3:1) and recrystallized from *n*-hexane to give ***S*-*E*-9** (1.15 g, 80%) as a powdery solid; mp 44 °C; $[\alpha]^{25}_D$ +80.9 (*c* 1.01, $CHCl_3$); MS (FAB, direct) calcd for $C_{20}H_{40}O_3Na$, $[M + Na]^+$ 351.3; found 351.4 (5%).

(2*S*,3*Z*)-2-(Methoxymethoxy)-3-octadecen-1-ol (*S*-*Z*-9). The reaction was carried out as described above, using ***S*-*Z*-14** (800 mg, 2.01 mmol) to give ***S*-*Z*-9** (500 mg, 76%) as a colorless oil: $[\alpha]^{25}_D$ +59.6 (*c* 1.74, $CHCl_3$); HRMS (FAB, direct) calcd for $C_{20}H_{40}O_3Na$ $[M + Na]^+$ 351.2875; found 351.2870 (28%).

(2*S*,3*E*)-2-(Methoxymethoxy)-3-octadecenoic Acid (*S*-*E*-10). The reaction was carried out as described above, using ***S*-*E*-9** (400 mg, 1.22 mmol) to give ***S*-*E*-10** (330 mg, 79%) as a solid: mp 56−57 °C; $[\alpha]^{25}_D$ +64.8 (*c* 2.09, $CHCl_3$); HRMS (FAB, direct) calcd for $C_{20}H_{37}O_4$, $[M - H]^+$ 341.2692; found, 341.2673 (100%).

(2*S*,3*Z*)-2-(Methoxymethoxy)-3-octadecenoic Acid (*S*-*Z*-10). The reaction was carried out as described above, using ***S*-*Z*-9** (300 mg, 0.91 mmol) to give ***S*-*Z*-10** (260 mg, 83%) as a colorless oil; $[\alpha]^{25}_D$ +111.1 (*c* 1.36, $CHCl_3$); HRMS (FAB, direct) calcd for $C_{20}H_{37}O_4$, $[M - H]^+$ 341.2692; found 341.2681 (100%).

1,3-Isopropylidene-D-*erythro*-dihydrosphingosine (19). A solution of **2**[15] (1.12 g, 3.71 mmol), pyridinium *p*-toluenesulfonate (0.94 g, 3.72 mmol), and 2,2-dimethoxypropane (9 mL) in dry benzene (20 mL) was heated under reflux for 4 h. The mixture was cooled and washed with a saturated $NaHCO_3$ solution. The organic layer was dried with Na_2SO_4 and concentrated. The residue was purified by column chromatography with chloroform−methanol (40:1) and crystallized with *n*-hexane to give **19** (910 mg, 72%) as a waxy solid: mp 36−37 °C (lit.[17] brown oil and lit.[19] mp 61−62 °C); $[\alpha]^{25}_D$ +31.7 (*c* 1.03, $CHCl_3$) {lit.[17] $[\alpha]^{22}_D$ +29.5 (*c* 1.178, $CHCl_3$) and lit.[19] $[\alpha]^{21}_D$ +32.4 (*c* 1.08, $CHCl_3$); IR (KBr) 2914, 2851, 1468, 1375, 1202, 1167, 891, 719 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, 3 H, J = 6.8 Hz), 1.01 (brs, 2 H), 1.26 (brs, 26 H), 1.39 (s, 3 H), 1.44 (s, 3 H), 1.45−1.55 (m, 1 H), 1.68−1.78 (m, 1 H), 2.64 (dt,

1 H, J = 4.9, 9.5 Hz), 3.40 (dt, 1 H, J = 2, 9 Hz), 3.45 (dd, 1 H, J = 11.2, 9.8 Hz), 3.81 (dd, 1 H, J = 11.2, 5.4 Hz); MS (FAB, direct) calcd for $C_{21}H_{43}NO_2$, $[M + H]^+$ 342.3; found 342.5 (73%).

(2*S*,3*R*,2′*R*,3′*E*)-2-*N*-(2′-Methoxymethoxy-3′-octadecenoyl)-1,3-*O*-isopropyliden-[2-amino-octadecan-1,3-diol] (*R*-*E*-20). To a suspension of acid ***R*-*E*-10** (169 mg, 0.49 mmol), DCC (104 mg, 0.49 mmol), and HOBt (67 mg, 0.49 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise a solution of primary amine **19** (167 mg, 0.49 mmol) in dry CH_2Cl_2 (2 mL), and the mixture was stirred overnight at room temperature. The resulting suspension was filtered and concentrated. The obtained residue was purified by column chromatography with *n*-hexane−EtOAc (3:1) to give ***R*-*E*-20** (260 mg, 79%) as a waxy solid: mp 58−60 °C; $[\alpha]^{25}_D$ −16.7 (*c* 2.07, $CHCl_3$); IR (KBr) 3320, 2920, 2849, 1647, 1520, 1464, 1375, 1205, 1153,-1024, 986, 920, 719 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 50 °C) δ 0.88 (t, 6 H, J = 6.8 Hz), 1.25 (brs, 50 H), 1.39 (s, 3 H), 1.45 (s, 3 H), 1.37−1.54 (m, 2 H), 2.07 (q, 2 H, J = 6.8 Hz), 3.38 (s, 3 H), 3.54−3.62 (m, 2 H), 3.85−3.94 (m, 2 H), 4.48 (d, 1 H, J = 7.3 Hz), 4.62 (d, 1 H, J = 6.3 Hz), 4.74 (d, 1 H, J = 6.3 Hz), 5.36 (dd, 1 H, J = 7.3, 15.6 Hz), 5.86 (dt, 1 H, J = 15.6, 6.8 Hz), 6.44 (d, 1 H, J = 9.3 Hz); MS (FAB, direct) calcd for $C_{41}H_{80}NO_5$, $[M + H]^+$ 666.60; found, 666.75 (17%).

(2*S*,3*R*,2′*S*,3′*E*)-2-*N*-(2′-Methoxymethoxy-3′-octadecenoyl)-1,3-*O*-isopropyliden-[2-amino-octadecan-1,3-diol] (*S*-*E*-20). The reaction was carried out as described above, using ***S*-*E*-10** (360 mg, 1.05 mmol) to give ***S*-*E*-20** (440 mg, 63%) as a waxy solid: mp 51−52 °C; $[\alpha]^{25}_D$ −50.3 (*c* 1.114, $CHCl_3$); IR (KBr) 3280, 2916, 2849, 1651, 38 1468, 1379, 1202, 1153,1083, 1030, 966, 918, 721 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 50 °C) δ 0.88 (t, 6 H, J = 6.8 Hz), 1.26 (brs, 50 H), 1.38 (s, 3 H), 1.43 (s, 3 H), 1.35−1.58 (m, 2 H), 2.07 (q, 2 H, J = 7.3 Hz), 3.37 (s, 3 H), 3.54 (dd, 1 H, J = 7.8, 11.2 Hz), 3.60 (dd, 1 H, J = 3.4, 8.3 Hz), 3.78−3.88 (m, 1 H), 3.90 (dd, 1 H, J = 5.4, 11.2 Hz), 4.45 (d, 1 H, J = 7.3 Hz), 4.61 (d, 1 H, J = 6.8 Hz), 4.71 (d, 1 H, J = 6.4 Hz), 5.37 (dd, 1 H, J = 7.1, 15.6 Hz), 5.84 (dt, 1 H, J = 15.6, 7.8 Hz), 6.41 (d, 1 H, J = 9.3 Hz); HRMS (FAB, direct) calcd for $C_{41}H_{79}NO_5$, $[M]^+$ 665.5958; found, 665.5962 (2.6%).

(2*S*,3*R*,2′*R*,3′*Z*)-2-*N*-(2′-Methoxymethoxy-3′-octadecenoyl)-1,3-*O*-isopropyliden-[2-amino-octadecan-1,3-diol] (*R*-*Z*-20). The reaction was carried out as described above, using ***R*-*Z*-10** (270 mg, 0.784 mmol) to give ***R*-*Z*-20** (380 mg, 73%) as a colorless oil; $[\alpha]^{25}_D$ −54.5 (*c* 0.618, $CHCl_3$); IR (NaCl) 3323, 2916, 2849, 1647, 1529, 1468, 1375, 1207, 1157,1105, 1051, 976, 922, 721 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 50 °C) δ 0.88 (t, 6 H, J = 6.8 Hz), 1.25 (brs, 50 H), 1.37−1.54 (m, 2 H), 1.39 (s, 3 H), 1.45 (s, 3 H), 2.21 (q, 2 H, J = 7.3 Hz), 3.37 (s, 3 H), 3.58 (dd, 1 H, J = 7.3, 11.2 Hz), 3.57−3.62 (m, 1 H), 3.84−3.96 (m, 2 H), 4.59 (d, 1 H, J = 6.8 Hz), 4.71 (d, 1 H, J = 6.4 Hz), 4.87 (d, 1 H, J = 9.3 Hz), 5.19 (dd, 1 H, J = 9.3, 10.7 Hz), 5.85 (dt, 1 H, J = 10.7, 7.3 Hz), 6.49 (d, 1 H, J = 9.3 Hz); HRMS (FAB, direct) calcd for $C_{41}H_{80}NO_5$, $[M + H]^+$ 666.6036; found, 666.6059 (84%).

(2*S*,3*R*,2′*S*,3′*Z*)-2-*N*-(2′-Methoxymethoxy-3′-octadecenoyl)-1,3-*O*-isopropyliden-[2-amino-octadecan-1,3-diol] (*S*-*Z*-20). The reaction was carried out as described above, using ***S*-*Z*-9** (250 mg, 0.73 mmol) to give ***S*-*Z*-20** (330 mg, 68%) as a waxy solid: mp 44−45 °C; $[\alpha]^{25}_D$ +81.5 (*c* 1.525, $CHCl_3$); IR (KBr) 3422, 2920, 2853, 1680, 1506, 1472, 1387, 1159, 1109, 1057, 920, 799, 718 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 50 °C) δ 0.88 (t, 6 H, J = 6.8 Hz), 1.26 (brs, 50 H), 1.38 (s, 3 H), 1.43 (s, 3 H), 1.38−1.61 (m, 2 H), 2.21 (q, 2 H, J = 6.8 Hz), 3.37 (s, 3 H), 3.55 (dd, 1 H, J = 7.6, 11.2 Hz), 3.60 (dt, 1 H, J = 3.4, 8.3 Hz), 3.78−3.87 (m, 1 H), 3.91 (dd, 1 H, J = 5.4, 11.2 Hz), 4.59 (d, 1 H, J = 6.8 Hz), 4.69 (d, 1 H, J = 6.8 Hz), 4.85 (d, 1 H, J = 9.3 Hz), 5.21 (dd, 1 H, J = 9.3, 10.7 Hz), 5.81 (dt, 1 H, J = 10.7, 7.5 Hz), 6.44 (d, 1 H, J = 8.8 Hz); HRMS (FAB, direct) calcd for $C_{41}H_{80}NO_5$, $[M + H]^+$ 666.6036; found, 666.6040 (100%).

(2*S*,3*R*,3′*R*,3′*E*)-2-*N*-(2′-Methoxymethoxy-3′-octadecenoyl)-[2-amino-octadecan-1,3-diol] (*R*-*E*-21). A solution of ***R*-*E*-20** (510 mg, 0.763 mmol) and *p*-toluenesulfonic acid (20 mg) in CH_2Cl_2 (10 mL) was stirred for 1 h at room temperature. The reaction mixture was washed with saturated $NaHCO_3$, dried with Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography with CH_2Cl_2−MeOH (40:1) to give ***R*-*E*-21** (380 mg, 79%) as a solid: mp 100−104 °C; $[\alpha]^{25}_D$ −24.2 (*c* 0.84, $CHCl_3$) {lit.[17] $[\alpha]^{23}_D$ −27.1 (*c* 0.981, $CHCl_3$)}; IR (KBr) 3289, 2918, 2849, 1653, 1541, 1466, 1155, 1072, 1036, 982, 918, 719 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 50 °C) δ 0.88 (t, 6 H, J = 6.8 Hz), 1.25 (brs, 48 H), 1.35−1.41 (m, 2 H), 1.45−1.58 (m, 2 H), 2.07 (q, 2 H, J = 7.3 Hz), 2.71 (d, 1 H, J = 5.9 Hz), 2.91 (brs, 1 H), 3.40 (s, 3 H), 3.79 (m, 3 H), 4.02 (d, 1 H, J = 11.7 Hz), 4.50 (d, 1 H, J = 7.3 Hz), 4.67 (d, 1 H, J = 6.8 Hz), 4.75 (d, 1 H, J = 6.8 Hz), 5.42 (dd, 1 H, J = 7.3, 15.1 Hz), 5.88 (dt, 1 H, J = 15.1, 6.8 Hz), 7.38 (d, 1 H, J = 7.8 Hz); HRMS (FAB, direct) calcd for $C_{38}H_{75}NO_5$, $[M + H]^+$ 626.5723; found, 626.5724 (53%).

(2*S*,3*R*,2′*S*,3′*E*)-2-*N*-(2′-Methoxymethoxy-3′-octadecenoyl)-[2-amino-octadecan-1,3-diol] (*S*-*E*-21). The reaction was carried out as described above, using ***S*-*E*-20** (240 mg, 0.359 mmol) to give ***S*-*E*-21** (180 mg, 80%) as a solid: mp 97−98 °C; $[\alpha]^{25}_D$ +37.8 (*c* 0.943, $CHCl_3$); IR (KBr) 3240, 2918, 2851, 1661, 1553, 1470, 1153, 1109, 1043, 974, 918, 719 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 50 °C) δ 0.88 (t, 6 H, J = 6.8 Hz), 1.25 (brs, 48 H), 1.36−1.42 (m, 2 H), 1.51−1.59 (m, 2 H), 2.07 (q, 2 H, J = 7.3 Hz), 2.56 (d, 1 H, J = 5.9 Hz), 2.60 (brs, 1 H), 3.39 (s, 3 H), 3.73−3.84 (m, 3 H), 4.02 (d, 1 H, J = 11.7 Hz), 4.49 (d, 1 H, J = 7.3 Hz), 4.66 (d, 1 H, J = 6.8 Hz), 4.73 (d, 1 H, J = 6.3 Hz), 5.44 (dd, 1 H, J = 7.3, 15.1 Hz), 5.87 (dt, 1 H, J = 15.1, 6.8 Hz), 7.27 (d, 1 H, J = 7.8 Hz); HRMS (FAB, direct) calcd for $C_{38}H_{75}NO_5$, $[M + H]^+$ 626.5723; found, 626.5717 (76%).

(2*S*,3*R*,2′*R*,3′*Z*)-2-*N*-(2′-Methoxymethoxy-3′-octadecenoyl)-[2-amino-octadecan-1,3-diol] (*R*-*Z*-21). The reaction was carried out as described above, using ***R*-*Z*-20** (350 mg, 0.524 mmol) to give ***R*-*Z*-21** (280 mg, 85%) as a solid: mp 62−63 °C; $[\alpha]^{25}_D$ −73.2 (*c* 0.22, $CHCl_3$); IR (KBr) 3289, 2918, 2851, 1655, 1539, 1470, 1153, 1049, 980, 918, 719 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 50 °C) δ 0.88 (t, 6 H, J = 6.8 Hz), 1.25 (brs, 48 H), 1.38−1.48 (m, 2 H), 1.48−1.58 (m, 2 H), 2.22 (q, 2 H, J = 7.3 Hz), 2.44 (d, 1 H, J = 5.4 Hz), 2.58 (brs, 1 H), 3.39 (s, 3 H), 3.76−3.82 (m, 3 H), 4.01 (d, 1 H, J = 11.7 Hz), 4.64 (d, 1 H, J = 6.3 Hz), 4.71 (d, 1 H, J = 6.3 Hz), 4.88 (d, 1 H, J = 9.3 Hz), 5.29 (dd, 1 H, J = 9.0, 10.7 Hz), 5.82 (dt, 1 H, J = 10.7, 7.3 Hz), 7.29 (d, 1 H, J = 6.4 Hz); MS (FAB, direct) calcd for $C_{38}H_{75}NO_5$, $[M + H]^+$ 626.6; found, 626.7 (35%).

(2*S*,3*R*,2′*S*,3′*Z*)-2-*N*-(2′-Methoxymethoxy-3′-octadecenoyl)-[2-amino-octadecan-1,3-diol] (*S*-*Z*-21). The reaction was carried out as described above, using ***S*-*Z*-20** (300 mg, 0.449 mmol) to give ***S*-*Z*-21** (210 mg, 74%) as a solid; mp 70.5−72.5 °C; $[\alpha]^{25}_D$ −71.3 (*c* 1.1, $CHCl_3$); IR (KBr) 3280, 2918, 2849, 1655, 1553, 1468, 1239, 1153, 1101, 1057, 980, 949, 907, 799, 721 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 50 °C) δ 0.88 (t, 6 H, J = 6.8 Hz), 1.25 (brs, 50 H), 1.47−1.58 (m, 2 H), 2.22 (q, 2 H, J = 6.8 Hz), 2.55 (d, 1 H, J = 5.9 Hz), 2.61 (brs, 1 H), 3.39 (s, 3 H), 3.72−3.84 (m, 3 H), 4.01 (d, 1 H, J = 11.2 Hz), 4.63 (d, 1 H, J = 6.8 Hz), 4.71 (d, 1 H, J = 6.3 Hz), 4.88 (d, 1 H, J = 9.3 Hz), 5.29 (dd, 1 H, J = 8.9, 10.7 Hz), 5.82 (dt, 1 H, J = 10.7, 7.3 Hz), 7.29 (d, 1 H, J = 6.8 Hz); HRMS (FAB, direct) calcd for $C_{38}H_{75}NO_5$, $[M + H]^+$ 626.5723; found, 626.5714 (100%).

(2*S*,3*R*,2′*R*,3′*E*)-2-*N*-(2′-Hydroxy-3′-octadecenoyl)-[2-amino-octadecan-1,3-diol] (Symbioramide, 1a). To a stirred suspension of ***R*-*E*-22** (300 mg, 0.449 mmol) in ethanethiol (20 mL) was added a few drops of boron trifluoride-diethyl ether under N_2, and the mixture was stirred for 1 h at room temperature. The resulting clear solution was poured into saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$. The extract was dried with Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography with $CHCl_3$−MeOH (20:1) and recrystallized from acetone−benzene to give **1a** (180 mg, 69%) as a powdery solid: mp 114−115 °C (lit.[11]

mp 105−107 °C, lit.[17] mp 112−113 °C, and lit.[19] mp 115−116.5 °C); $[\alpha]^{25}_D$ +1.19 (*c* 0.5, $CHCl_3$) {lit.[11] $[\alpha]^{22}_D$ +5.8 (*c* 1, $CHCl_3$), lit.[17] $[\alpha]^{19}_D$ +2.65 (*c* 0.378, $CHCl_3$), and lit.[19] $[\alpha]^{19}_D$ +3.6, $[\alpha]^{23}_D$ +0.76, $[\alpha]^{28}_D$ −1.5, $[\alpha]^{35}_D$ −5.5 (*c* 0.31, $CHCl_3$)}; IR (KBr) 3280, 2918, 2849, 1651, 1626, 1541, 1468, 1069, 978, 721 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 50 °C) δ 0.88 (t, 6 H, *J* = 6.8 Hz), 1.26 (brs, 50 H), 1.38 (m, 1 H), 1.53 (m, 1 H), 2.08 (q, 2 H, *J* = 7.0 Hz), 2.60 (d, 1 H, *J* = 6.4 Hz), 2.77 (brs, 1 H), 3.26 (d, 1 H, *J* = 3.6 Hz), 3.77−3.84 (m, 3 H), 3.99 (d, 1 H, *J* = 11.2 Hz), 4.53 (dd, 1 H, *J* = 3.4, 6.8 Hz), 5.57 (dd, 1 H, *J* = 6.8, 15.4 Hz), 5.89 (dt, 1 H, *J* = 14.2, 6.8 Hz), 7.01 (d, 1 H, *J* = 7.8 Hz); HRMS (FAB, direct) calcd for $C_{36}H_{71}NO_4$, $[M + H]^+$ 582.5461; found, 582.5455 (100%). Anal. Calcd: C, 74.30; H, 12.30; N, 2.41. Found: C, 74.30; H, 12.44; N, 2.35.

(2*S*,3*R*,2′*S*,3′*E*)-2-*N*-(2′-Hydroxy-3′-octadecenoyl)-[2-amino-octadecan-1,3-diol] (1b). The reaction was carried out as described above, using ***S*-*E*-22** (180 mg, 0.287 mmol) to give **1b** (120 mg, 72%) as a powdery solid; mp 104 °C (lit.[19] mp 99.5−100.5 °C); $[\alpha]^{25}_D$ +17.2 (*c* 0.502, $CHCl_3$) {lit.[19] $[\alpha]^{18}_D$ +42.9, $[\alpha]^{23}_D$ +37.2, $[\alpha]^{27}_D$ +30.6, $[\alpha]^{39}_D$ +27.5, $[\alpha]^{35}_D$ +22.3 (*c* 0.15, $CHCl_3$)}; IR (KBr) 3320, 2916, 2849, 1649, 1553, 1472, 1074, 1037, 964, 718 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 50 °C) δ 0.88 (t, 6 H, *J* = 6.8 Hz), 1.26 (brs, 50 H), 1.38−1.46 (m, 1 H), 1.48−1.56 (m, 1 H), 2.08 (q, 2 H, *J* = 7.3 Hz), 2.32 (d, 1 H, *J* = 5.9 Hz), 2.41 (brs, 1 H), 2.95 (d, 1 H, *J* = 3.4 Hz), 3.72−3.85 (m, 3 H), 4.02 (d, 1 H, *J* = 11.2 Hz), 4.51 (d, 1 H, *J* = 4.4 Hz), 5.56 (dd, 1 H, *J* = 7, 15.6 Hz), 5.90 (dt, 1 H, *J* = 15.1, 5.9 Hz), 6.89 (d, 1 H, *J* = 6.8 Hz); HRMS (FAB, direct) calcd for $C_{36}H_{71}NO_4$, $[M + H]^+$ 582.5461; found 582.5441 (100%). Anal. Calcd: C, 74.30; H, 12.30; N, 2.41. Found: C, 74.15; H, 12.30; N, 2.40.

(2*S*,3*R*,2′*R*,3′*Z*)-2-*N*-(2′-Hydroxy-3′-octadecenoyl)-[2-amino-octadecan-1,3-diol] (1c). The reaction was carried out as described above, using ***R*-*Z*-22** (220 mg, 0.351 mmol) to give **1c** (150 mg, 73%) as a powdery solid; mp 92−93 °C; $[\alpha]^{25}_D$ −49.9 (*c* 0.5, $CHCl_3$); IR (KBr) 3289, 2918, 2851, 1655, 1539, 1470, 1153, 1049, 980, 918, 719 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 50 °C) δ 0.88 (t, 6 H, *J* = 6.8 Hz), 1.26 (brs, 48 H), 1.38−1.46 (m, 1 H), 1.48−1.56 (m, 1 H), 2.22 (q, 2 H, *J* = 8 Hz), 2.56 (d, 1 H, *J* = 5.9 Hz), 2.71 (brs, 1 H), 3.18 (brs, 1 H), 3.72−3.84 (m, 3 H), 3.98 (d, 1 H, *J* = 11.1 Hz), 4.87 (d, 1 H, *J* = 8.8 Hz), 5.44 (dd, 1 H, *J* = 9, 10.7 Hz), 5.77 (dt, 1 H, *J* = 10.7, 7.8 Hz), 7.25 (d, 1 H, *J* = 2 Hz); HRMS (FAB, direct) calcd for $C_{36}H_{71}NO_4$, $[M + H]^+$ 582.5461; found, 582.5463 (100%). Anal. Calcd: C, 74.30; H, 12.30; N, 2.41. Found: C, 74.28; H, 12.41; N, 2.42.

(2*S*,3*R*,2′*S*,3′*Z*)-2-*N*-(2′-Hydroxy-3′-octadecenoyl)-[2-amino-octadecan-1,3-diol] (1d). The reaction was carried out as described above, using ***S*-*Z*-22** (210 mg, 0.334 mmol) to give **1d** (160 mg, 82%) as a powdery solid; mp 87−88 °C; $[\alpha]^{25}_D$ +63.3 (*c* 0.5, $CHCl_3$); IR (KBr) 3290, 2955, 2851, 1663, 1549, 1470, 1259, 1140, 1053, 926, 822, 719 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 50 °C) δ 0.88 (t, 6 H, *J* = 6.8 Hz), 1.26 (brs, 50 H), 1.38−1.46 (m, 1 H), 1.48−1.56 (m, 1 H), 2.19−2.26 (m, 2 H), 2.42 (d, 1 H, *J* = 4.8 Hz), 2.51 (brs, 1 H), 3.01 (brs, 1 H), 3.72−3.84 (m, 3 H), 4.01 (d, 1 H, *J* = 10.7 Hz), 4.87 (d, 1 H, *J* = 8.8 Hz), 5.44 (dd, 1 H, *J* = 9.3, 10.7 Hz), 5.77 (dt, 1 H, *J* = 10.7, 7.6 Hz), 6.93 (d, 1 H, *J* = 7.3 Hz); HRMS (FAB, direct) calcd for $C_{36}H_{71}NO_4$, $[M + H]^+$ 582.5461; found, 582.5463 (100%). Anal. Calcd: C, 74.30; H, 12.30; N, 2.41. Found: C, 74.33; H, 12.32; N, 2.40.

MTT Assay. After washing with serum-free RPMI-1640 medium, cells were suspended at 4×10^5/mL with 10 μM of symbioramide derivatives (stock solution, 10 mM in 98% EtOH−2% dodecane) and plated in 96 well plates. Cells (4×10^4/well) were cultured for 6 h. MTT (10 mL of 5 mg/mL) was added to each well 2 h before the end of the culture, and reactions were stopped by the addition of 100 μL of 0.04 M HCl/2-propanol; two optical densities of the mixture were measured at 570 and 650 nm. Final values were obtained by subtracting 650 nm from 570 nm lectures.[23c]

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Supporting Information Available: ^1H NMR spectra of all new compounds **6**, **7**, ***R*-*Z*-8**, ***R*-*Z*-9**, ***R*-*Z*-10**, ***S*-*Z*-14**, ***R*-*Z*-20**, ***S*-*Z*-20**, ***R*-*Z*-21**, ***S*-*Z*-21**, **1c**, and **1d** and of the eight known compounds ***R*-*E*-9**, ***R*-*E*-10**, ***S*-*E*-13**, **19**, ***R*-*E*-20**, ***S*-*E*-20**, **1a**, and **1b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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