Tetrahedron Vol. 45, No. 9, pp. 2557 to 2570, 1989
Printed in Great Britain.

0040-4020/89 $3.00+.00

SYNTHESES OF D-ERYTHRO-1-DEOXYDIHYDROCERAMIDE-1-SULFONIC ACID AND PHOSPHONOSPHINGOGLYCOLIPID FOUND IN MARINE ORGANISMS VIA A COMMON PRECURSOR†

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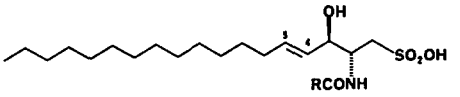
(*Received in Japan* 23 *January* 1989)

Abstract: D-Erythro-1-deoxydihydroceramide-1-sulfonic acid, isolated from alkali-stable hydrogenated lipids in a non-photosynthetic marine diatom, Nitzschia alba, and (2S,3R)-N-palmitoyl-1-O-[6'-O-{2"-(N-methylamino)ethylphosphonyl}-β-D-galacto-pyranosyl]-D-sphingosine, found in marine snail Turbo cornutus were synthesized via a common precursor (10) starting from galactose.

New sulfolipids were isolated by one of the authors (M.K.) from alkali-stable lipids in a non-photosynthetic marine diatom Nitzschia alba and their structures were detremined by spectrometry and identification of their hydrolysis products as 1-deoxyceramide-1-sulfonic acids (DCS) (1).[1]

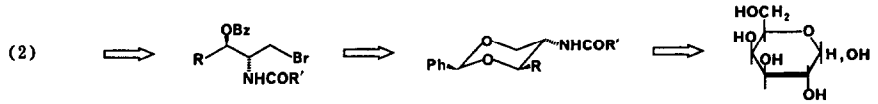


1a: R = $C_{13}H_{27}$
1b: R = $C_{13}H_{25}$
1c: R = $C_{15}H_{31}$
1d: R = $C_{15}H_{29}$ (3E)
2: R = $C_{15}H_{31}$; 4,5-dihydro

Although the structure of DCS, but not the stereochemistry, was clarified almost 10 years ago, only the synthesis of the rac-1-deoxydihydroceramide-1-sulfonic acid[2] has been reported. As part of our continuing studies synthesizing lipids, we undertook the synthesis of this family of ceramides. In designing our approach to DCS we set as our first target, a saturated D-erythro-DCS (2), partly because DCS has a stereochemistry (C-2 and -3) similar to that of sphingosine which could be derived enantioselectively from galactose and partly because the authentic sample for comparison could be obtained in relatively pure form by hydrogenation of a mixture of natural sulfolipids.[3] The first synthesis of (-)-dihydro-DCS is reported herein.

The synthetic plan involved the cleavage of acetal (10) by the Hanessian-

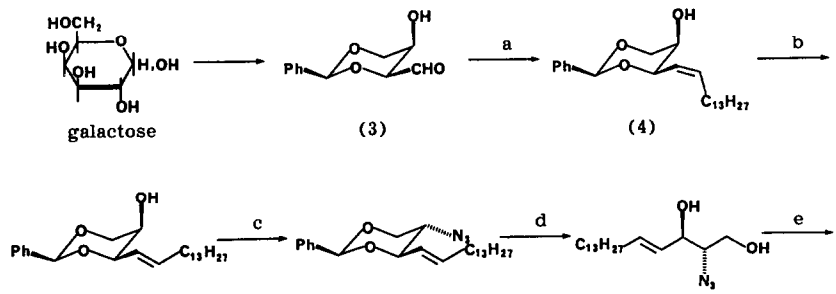
Hullar reaction as the key step in the reaction sequence (Scheme 1). Of the many methods for synthesizing sphingosine,[4] the required intermediate (10) could be prepared efficiently by modified Schmidt's procedure[5] using galactose as a starting material. Thus, the galactose was converted into hydroxy aldehyde (3)

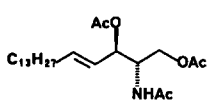


(10)

Scheme 1

by partial acetalization with benzaldehyde and anhydrous zinc chloride follwed by periodate oxidation. Schmidt et al. reported that Schlosser's modification of the Wittig reaction (3) exclusively gave the trans olefin (5). However, since we did not observe any selectivity for the trans isomer, we used the usual Wittig reaction and photo-isomerization[6] sequence. Treatment of (3) with tetradecanyl-triphenylphosphonium bromide and potassium t-butoxide in THF at 0°C gave a 4:1 mixture of cis and trans olefins (GLC). The mixture was then irradiated using a high-pressure mercury lamp in the presence of diphenyldisulfide to afford a trans olefin [(4):(5) = 1:10] in 56% yield from (3). Treatment of (5) with methanesulfonyl chloride and triethylamine afforded a mesylate, which without purification, was treated with sodium azide in DMF to obtain an azide (6) in 55% yield. To firmly establish the stereochemistry, the azide was transformed into the known triacetylsphingosine (8)[5] by the following reaction sequence: (i) hydrolysis (1 N HCl, THF), (7), (ii) reduction[7] (Ph_3P, THF-H_2O) and (iii)



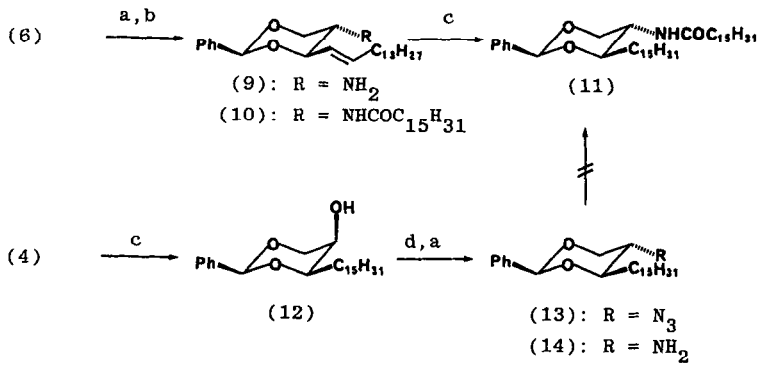


(8)

Scheme 2. (a) $C_{14}H_{29}PPh_3^+Br^-$/t-BuOK/THF; (b) hν/cyclohexane-dioxane/$(PhS)_2$; (c) MsCl/Et_3N; NaN_3/DMF; (d) 1 N HCl/THF; (e) Ph_3P/THF-H_2O; Ac_2O/py.

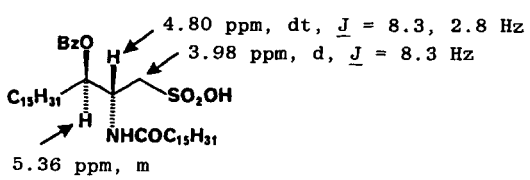
acetylation (Ac_2O, py) (Scheme 3).

Reduction of the azide (6) with triphenylphosphine afforded an amine (9) which was acylated with p-nitrophenylpalmitate in pyridine to obtain an amide (10) in 95% yield. The ^1H NMR spectrum of (10) showed a diequatorial arrangement of substituents on the 1,3-dioxane ring (H-3, δ 4.20, t, $J_{2,3} = J_{3,4} = 8$ Hz, numbering refers to those of ceramides). Catalytic hydrogenation of (10) using 5% rhodium on alumina at room temperature under atmospheric pressure of hydrogen gave (11) in 93% yield. Although this reaction sequence seems rather roundabout, the dihydro amine (14), which was obtained from the cis olefin (4) by reduction [H_2/Rh-Al_2O_3, (12)], mesylation followed by azidation [1. MsCl/Et_3N, 2. NaN_3/DMF, (13)] and reduction [Ph_3P/THF-H_2O, (14)], could not be acylated even under severe conditions. The inertness of the amine (14) was probably due to steric hindrance by a "floppy' large alkyl group. The geometry of double bonds in the olefin (4) and (5) are also important for S_N2 reactions. The mesylate from the trans olefin (5) reacted with sodium azide to afford the azide (6), whereas the mesylate from the cis olefin (4) did not react at all (Scheme 2 and 3). This was probably also



Scheme 3. (a) Ph_3P/THF-H_2O; (b) p-nitrophenylpalmitate/py; (c) H_2 Rh-Al_2O_3; (d) H_2/Rh-Al_2O_3; (e) MsCl/Et_3N; NaN_3/DMF.

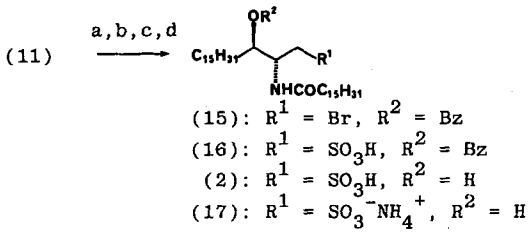
due to greater steric hindrance of the cis olefin chain towards attacking nucleophiles. Having obtained the requisite precursor, we turned our attention to the construction of the sulfonic acid moiety. One of the most useful synthetic reactions for introducing a bromine atom at C-1 of the benzylidene derivative (11) is the Hanessian-Hullar reaction.[8] Treatment of the acetal (11) with freshly recrystallized N-bromosuccinimide (NBS) in refluxing carbon tetrachloride in the presence of solid barium carbonate gave bromo benzoate (15) as an oil in 72% yield. Various attempts to convert the bromide (15) into a mercaptan were unsuccessful. To this end, the bromide (15) was directly converted to sulfonic acid (16) in 59% yield by sodium sulfite under a phase-transfer catalytic condition followed by acidification. The ^1H NMR spectrum of (16) supports the structure.



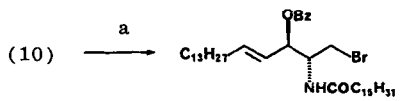
(16)

Base hydrolysis of (16) gave dihydro-DCS (2) in 68% yield. The sulfonic acid was characterized as its ammonium salt (17), obtained by neutralization with ammonium hydroxide in methanol (Scheme 4). The synthetic (2) and (17) were identical with those of authentic samples obtained from the hydrogenated alkali-stable fraction of lipids of the diatom cells.

Attempts to synthesize (1c) via (18) by an essentially similar reaction sequence were unsuccessful. When (18), which had been obtained from (10) with NBS, was made to react with sodium sulfite, the reaction was very slow and the double bond was partially reduced under the reaction conditions used.



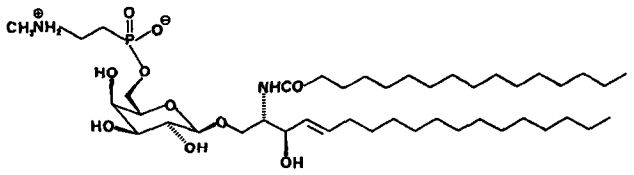
(15): R^1 = Br, R^2 = Bz
(16): R^1 = SO_3H, R^2 = Bz
(2): R^1 = SO_3H, R^2 = H
(17): R^1 = $SO_3^-NH_4^+$, R^2 = H



(18)

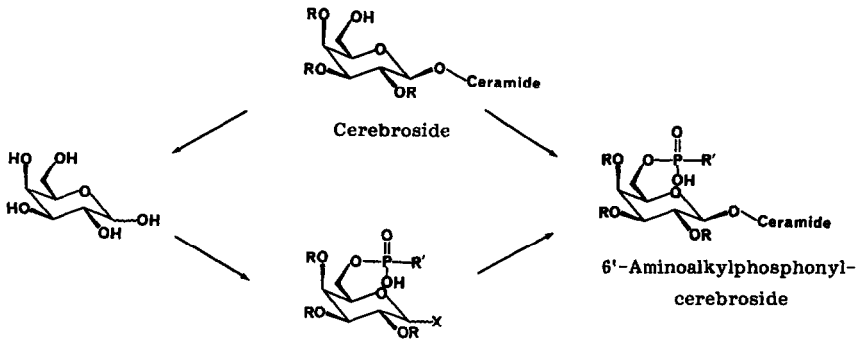
Scheme 4. (a) NBS/$BaCO_3$/CCl_4; (b) Na_2SO_3/n-Bu_4NF/$CHCl_3$-H_2O; H^+; (c) 1% NaOH/MeOH; (d) NH_4OH/MeOH

The second target molecule is a phosphonosphingoglycolipid. Recently, many phosphonosphingoglycolipids have been isolated from tissues of marine Mollusca and marine Protostomia.[9] Although their biological functions are not clear, they probably play important roles as receptors or transmitters of information. Hayashi and his colleagues isolated new phosphonosphingoglycolipids from muscle tissues of the marine snail Turbo cornutus.[9] For one of the simplest compounds, they proposed the structure (19) based on the results of degradative work and spectroscopic analysis.[10] The first synthesis of (19) via the common intermediate (10), which was used in the previous section, is reported herein.



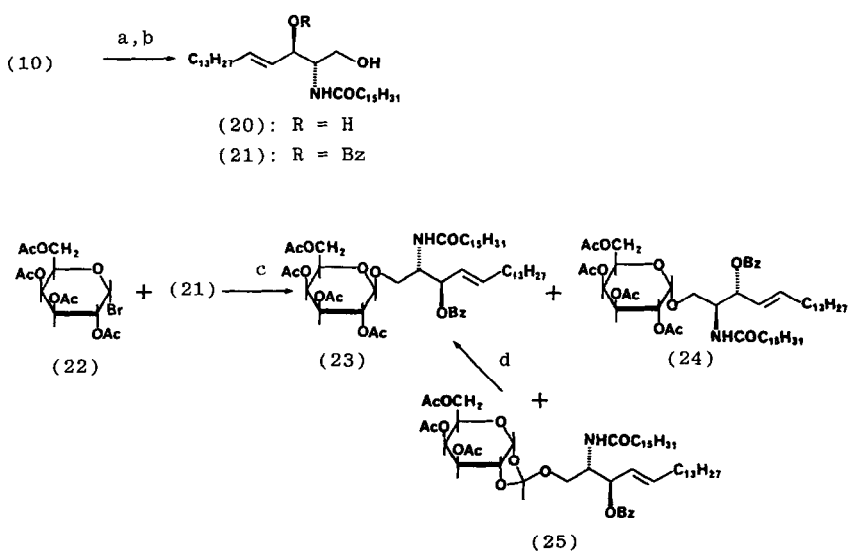
(19)

For the synthesis of (19), two routes are possible for the coupling of the components (Scheme 5). A convergent synthesis of (19) was assembled by the coupling of a cerebroside with a protected aminoethylphosphonic acid. Hydrolysis of the protected ceramide (10) with hydrochloric acid gave ceramide (20). Glycosidation of (20) at the C-1 hydroxyl group succeeded only when the allylic alcohol was protected.[11] Alcohol (21) was prepared from (20) in a three-stage



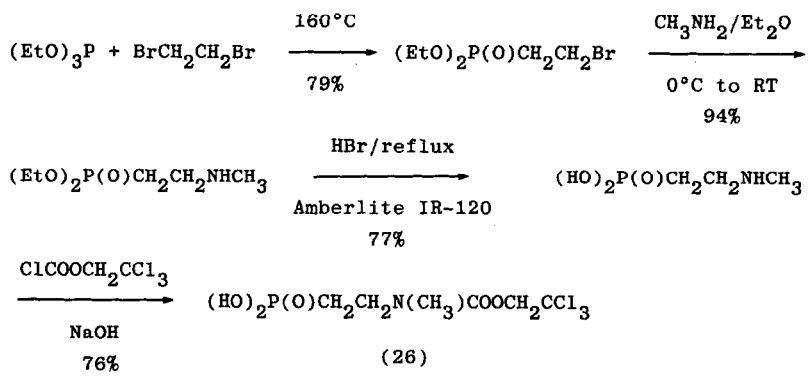
Scheme 5

reaction sequence: (i) silylation with t-butyldiphenylsilyl chloride and imidazole, (ii) benzoylation with benzoyl chloride and pyridine and (iii) desilylation with tetra-n-butylammonium fluoride in THF. Glycosidation with α-D-bromotetraacetyl-galactose (22) and (21) by Thornton's procedure[12] using anhydrous mercuric cyanide as a catalyst in nitromethane gave a mixture of three components[13] (23), (24) and (25), one of which was probably an orthoester (25) as suggested by Ogawa.[14] Thus, the mixture was treated with trimethylsilyltriflate in dichloromethane in the presence of 4A molecular sieves to rearrange the orthoester to a β-glycoside, and the desired 1-O-β-D-tetraacetylgalactosylceramide (23) was obtained in 42% yield from (21). In order to obtain (23) in pure form, we explored the Lubineau procedure.[15] Treatment of (21) with (22), stannous triflate and 1,1,3,3-tetramethylurea as a base in the presence of 4A molecular sieves gave pure (23) in 47% yield (Scheme 6). The stereochemistry and structure of (23) were fully confirmed by ^{1}H NMR, FAB-MS.



Scheme 6. (a) 2N HCl/THF; (b) t-$BuPh_2SiCl$/imidazole/DMF; PhCOCl/py; n-Bu_4NF/THF; (c) $Hg(CN)_2/CH_3NO_2$ or $Sn(OTf)_2/(Me_2N)_2CO$/4A molecular sieves/CH_2Cl_2; (d) TMSOTf/4A molecular sieves/$ClCH_2CH_2Cl$.

The protected 2-methylaminoethylphosphonic acid (26) was prepared in four steps using the method of Isbell[16] (Scheme 7).



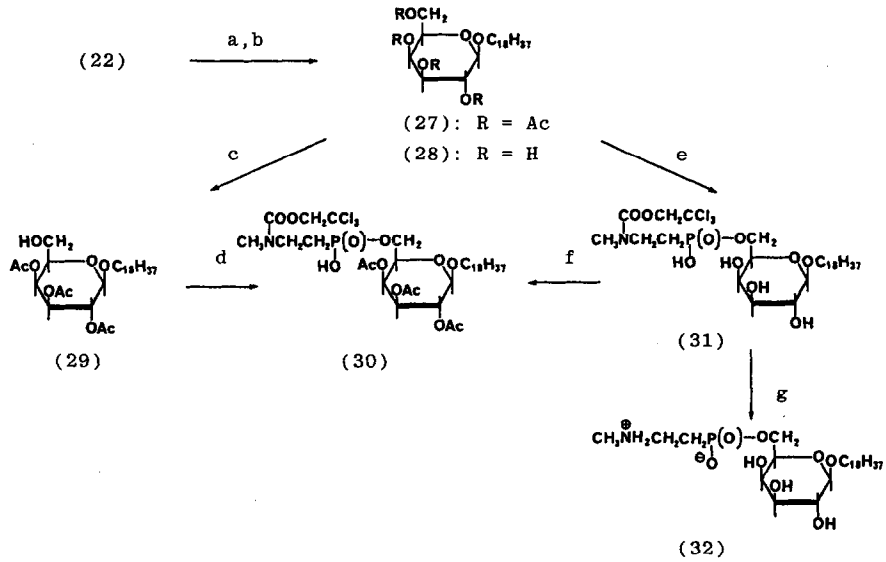
Scheme 7.

To test the feasibility of the coupling (26) and the C-6 hydroxyl group of galactose, model reactions were examined. Although many reactions involving the activation of phosphonic acid failed, condensation of an alcohol with (26) could be carried out most effectively using dicyclohexylcarbodiimide (DCC) as a dehydrating agent. Phosphonate (30) was prepared by treatment of 2,3,4-tri-O-acetyl-1-O-stearyl-β-D-galactopyranoside (29) with (26) and DCC in pyridine. Compound (29) had been obtained by a five-stage reaction sequence: (i) glycosidation of (22) and stearyl alcohol by the Lubineau procedure, (ii) hydrolysis of acetate, (iii) tritylation, (iv) acetylation and (v) partial hydrolysis. Eventually, the phosphonation was found to proceed selectively at the C-6' hydroxyl group of the galactose moiety without the need for special protection by other hydroxyl groups. Treatment of (26) and (28) with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (DEC)[17] and dimethylaminopyridine in pyridine under irradiation with ultra-sound gave (31). Deprotction of (31) with zinc and acetic acid afforded (32) in 55% yield (Scheme 8).

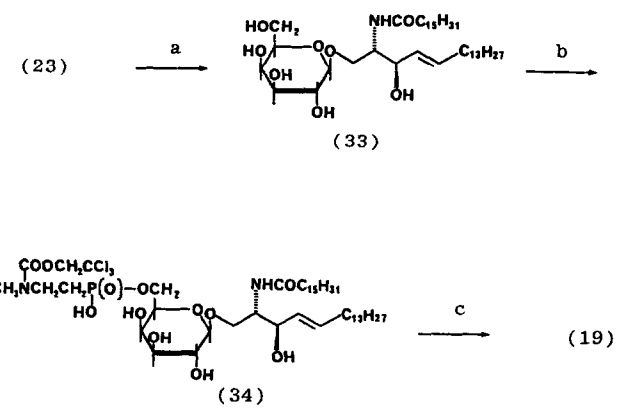
Base hydrolysis of (23) with sodium methoxide in methanol gave pentaol (33). Ultrasound-assisted coupling of (26) and (33) using DEC, DMAP in pyridine gave (34) in 72% yield. In the absence of ultrasound, such a coupling did not occur. Deprotection of (34) with zinc and 90% acetic acid followed by chromatography on Iatrobeads®[18] gave (19) in 81% yield as a colorless solid, which was identical with the natural lipid[19] (Scheme 9). The NMR spectrum was not diagnostic because of peak broadening, but the FAB-MS spectrum showed characteristic peaks at m/z 821 $(M + 1)^+$, 520 ($C_{34}H_{66}O_2N^+$: ceramide), and 140 ($C_3H_{11}NO_3P^+$: methyl aminoethylphosphonic acid moiety).

We have outlined a short and highly convergent synthesis of optically active 1-deoxydihydroceramide-1-sulfonic acid and phosphonosphingoglycolipid via the common intermediate (10). The overall yields of (2) and (19) based on galactose are 2.3% and 6.6%, respectively. The special features of the first section are:

(i) cleavage of the acetal (11) with NBS which gives the desired functionality and oxidation state and (ii) convenient conversion of the bromide into sulfonic acid under a phase-transfer condition. The unique chemistry features of the second section are: (i) phosphonation of a cerebroside using DEC as a condensing agent, (ii) facilitation of this condensation by ultrasound irradiation and (iii) highly selective phosphonation without special protection of the other hydroxyl groups. This strategy should be applicable to the synthesis of aminoethylphosphonylceramidepolyhexosides which are lipid constituents found in marine snail.



Scheme 8. (a) stearyl alcohol/$Sn(OTf)_2$/$(Me_2N)_2CO$/4A molecular sieves/CH_2Cl_2; (b) MeONa/MeOH; (c) TrCl/py; Ac_2O/py; 90%AcOH-H_2O; (d) (26)/DCC/THF; (e) (26)/DEC/py/THF; (f) Ac_2O/py; (g) Zn/90%AcOH-H_2O.



Scheme 9. (a) MeONa/MeOH; (b) (26)/DEC/DMAP/py; (c) Zn/90%AcOH-H_2O.

EXPERIMENTAL

THF, ether and benzene were distilled from benzophenone ketyl, dichloromethane, DMF and triethylamine from calcium hydride. All reactions were monitored by thin-layer chromatography carried out on 0.25 mm E. Merck silica gel plates (60F-254). UV light or 7% phosphomolybdic acid-ethanol and heat was used as developing agent. E. Merck silica gel (60, particle size 0.040-0.063 mm) and Fuji silica gel (KC-2, 100-200 mesh) were used for column chromatography. Melting and boiling points were uncorrected. IR spectra were recorded on a Nippon Bunko IRA-1 IR spectrometer. High-resolution electron-impact and FAB mass spectra were obtained on a JEOL JMS-HX 100 spectrometer. ^{1}H NMR spectra of 60 and 200 MHz were recorded on Hitachi R-600L and JEOL FX-200 spectrometers, respectively. Elemental analyses were performed by Mr. Goda, Osaka City University.

Preparation of Saturated-DCS (2). Total lipids of diatom cells (freeze-dried, 5 g) were extracted with $CHCl_3$-MeOH-H_2O (1:2:0.8, 190 mL) as described in ref. 1. The lipids obtained (140 mg) were then deacylated in 10 mL of 0.1 N NaOH in MeOH-$CHCl_3$ (4:1) at r.t. for 40 min; $CHCl_3$ (6 mL) and 1 N HCl (7.2 mL) were added, the biphasic mixture was centrifuged and the lower layer was removed, diluted with benzene and made alkaline with 1.5 N NH_4OH in MeOH and concentrated to a small volume (0.5 mL) under a stream of N_2. The ammonium salts of the acidic lipids (DCS and sterol sulfate) were then precipitated by ten-fold dilution with acetone at 4°C. The precipitate was centrifuged, washed with 1 mL of cold acetone and dried in vacuum; a second crop was obtained from the combined acetone supernatants.

The combined crops were then treated with 2 mL of 0.005 M HCl in THF for 3 h at r.t. Chloroform-methanol (1:1, 10 mL) and 0.2 N aqueous HCl (4.5 mL) were added, the biphasic system was centrifuged and the lower $CHCl_3$ phase was made alkaline with 1.5 N methanolic NH_4OH, diluted with benzene and brought to dryness in a stream of N_2. The residue was precipitated from $CHCl_3$-MeOH (1:1, 0.5 mL) by tenfold dilution with acetone at 0°C. The acetone insoluble material in methanol solution was hydrogenated with PtO_2 catalyst by bubbling H_2 for 15 min. The catalyst was removed by centrifugation washed with MeOH-$CHCl_3$ (2:1) and the combined supernatants were diluted with $CHCl_3$ and 0.1 N HCl to form two phases. The lower chloroform phase was made alkaline with 1.5 N NH_4OH in methanol and brought to dryness under a stream of N_2. The residue was dissolved in $CHCl_3$-MeOH (1:1), cleared by centrifugation and the supernatant was brought to dryness under a stream of N_2; yield of hydrogenated-DCS, 2.5 mg; TLC R_f in $CHCl_3$-MeOH-NH_4OH (65:35:5), 0.73 (slight contamination with sterol sulfate, R_f 0.65); FAB-MS (matrix: glycerol+thioglycerol): m/z 621 $[M+1]^+$, 604 $[M-17]^+$.

(2R,3R)-cis-1,3-O-Benzylidene-1,2,3-octadec-4-enetriol (4). To a stirred solution of tetradecanylphosphonium bromide (12.85 g, 23.8 mmol) in THF (90 mL) was added a solution of potassium t-butoxide (4.69 g, 41.8 mmol) in THF (20 mL) at 0°C under an argon atmosphere. After the mixture had been stirred for 20 min, 2,4-O-benzylidene-D-threose (3) (3.67 g, 17.6 mmol) in THF (30 mL) was added at 0°C. The mixture was warmed gradually to room temperature and stirred overnight, quenched with sat NH_4Cl solution and the product was extracted with ethyl acetate. The organic layer was washed with brine, dried ($MgSO_4$) and evaporated. The residue was treated with hexane, filtered and the filtrate was evaporated. The residue was purified by chromatography (SiO_2, elution with C_6H_6-AcOEt 97:3) to afford (4) (4.25 g, 62%), mp 48.0-50.3°C. The product was shown to be a 4:1 mixture of cis and trans isomers by GLC (3% OV-101); IR (neat) 3400, 1660, 1100, 760, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3H), 1.0-1.5 (m, 22H), 1.9 (s, 1H), 2.0-2.3 (m, 2H), 3.42 (m, 1H), 4.10 (dd, J = 11.3, 1.8 Hz, 1H), 4.24 (dd, J = 11.3, 2.1 Hz, 1H), 4.72 (dd, J = 7, 1.8 Hz, 1H), 5.5-5.8 (m, 3H), 7.2-7.55 (m, 5H); EI-MS, calcd as $C_{25}H_{40}O_3$ m/z 388.3002, obsd 388.2977.

(2R,3R)-trans-1,3-O-Benzylidene-1,2,3-octadec-4-enetriol (5). A solution of the olefin (4) (1.20 g, 3.09 mmol) and diphenyldisulfide (0.346 g, 1.58 mmol) in a mixture of cyclohexane (120 mL) and dioxane (30 mL) was irradiated with a 100 W high-pressure mercury lamp for 3 h. After removal of the solvents, the residue was purified by chromatography (SiO_2, elution with C_6H_6-AcOEt 97:3) to yield (5) (1.08 g, 89.6%) as a pale yellow solid which was shown to be a 1:10 mixture of cis and trans isomers by GLC (3% OV-101): mp 50.0-52.6°C; IR (neat) 3400, 1100, 960, 760 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3H), 1.0-1.5 (m, 22H), 1.96-2.2 (m, 2H), 2.64 (br s, 1H), 3.42 (m, 1H), 4.06 (dd, J = 11.3, 1.8 Hz, 1H), 4.23 (dd, J = 11.3, 2.1 Hz, 1H), 4.40 (dd, J = 7.0, 1.8 Hz, 1H), 5.53 (dd, J = 15.1, 7.0 Hz, 1H), 5.84 (dt, J = 15.1, 8.0 Hz, 1H), 5.96 (s, 1H), 7.2-7.55 (m, 5H); EI-MS, calcd as $C_{25}H_{40}O_3$ m/z 388.2921, obsd 388.2899. Anal. calcd for $C_{25}H_{40}O_3$: C, 77.28; H, 10.38. found: C, 77.27; H, 10.37.

(2R,3R)-trans-2-Azide-1,3-O-benzylidene-1,3-octadec-4-enediol (6). A solution of (5) (1.05 g, 2.69 mmol) in methylene chloride (13 mL) and triethylamine (1.5 mL, 10.8 mmol) was cooled to0°C, and methanesulfonyl chloride (0.45 mL, 5.50 mmol) was added dropwise. The mixture was allowed to warm to room temperature with continued stirring for 1.5 h. The reaction mixture was partitioned between sat $NaHCO_3$ solution and CH_2Cl_2. The organic phase was washed with brine and evaporated and the residue was redissolved in ether. The organic layer was washed

with brine, dried ($MgSO_4$), and evaporated to give a pale yellow solid (1.25 g, 99.4%): mp 81.5-83.0°C; IR (Nujol) 1170, 770, 710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3H), 1.2-1.5 (m, 22H), 3.09 (s, 3H), 3.7-3.8 (m, 1H), 4.05 (dd, J = 11.3, 1.8 Hz, 1H), 4.36 (dd, J = 11.3, 2.1 Hz, 1H), 4.57 (dd, J = 7.0, 1.8 Hz, 1H), 5.48 (dd, J = 15.1, 7.0 Hz, 1H), 5.88 (dt, J = 15.1, 8.0 Hz, 1H), 5.96 (s, 1H), 7.2-7.9 (m, 5H); EI-MS, calcd for $C_{26}H_{42}O_5S$ m/z 466.2762, obsd 466.2753; Anal. calcd for $C_{26}H_{42}O_5S$: C, 66.92; H, 9.07. found: C, 66.55; H, 9.10.

To a suspension of sodium azide (1.01 g, 15.5 mmol) in dry DMF (2 mL) was added a solution of the crude mesylate (1.94 g, 4.01 mmol) in dry DMF (3 mL) and the reaction mixture was heated at 90°C for 3 h under an argon atmosphere. After removal of the solvent in vacuo, the residue was partitioned between ice-water and methylene chloride. The organic phase was washed with water and brine, successively, dried ($MgSO_4$) and evaporated. The crude product was purified by chromatography (SiO_2, elution with n-hexane-C_6H_6 2:1) to give the azide (6) (0.953 g, 57%) as a pale yellow oil: IR (neat) 2100, 1090, 780, 710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3H), 1.0-1.5 (m, 22H), 1.95-2.14 (m, 2H), 3.40 (dd, J = 11.3, 6.2 Hz, 1H), 3.52 (dd, J = 11.3, 4.2 Hz, 1H), 3.82-4.03 (ddd, J = 11.0, 6.2, 4.2 Hz, 1H), 4.1-4.42 (td, J = 11.0, 6.9 Hz, 1H), 5.53 (dd, J = 15.1, 7.0 Hz, 1H), 5.76 (dt, J = 15.1, 8.0 Hz, 1H), 5.96 (s, 1H), 7.2-7.5 (m, 5H); EI-MS, calcd as $C_{25}H_{39}O_2N_3$ m/z 413.2986, obsd 413.3042.

(2S,3R)-1,3-O-Benzylidene-D sphingosine (9). A mixture of (6) (0.438 g, 1.06 mmol) and triphenylphosphine (0.278 g, 1.06 mmol) in THF (3.5 mL) and H_2O (0.5 mL) was stirred overnight at room temperature. The mixture was diluted with methylene chloride and the layers were separated. The organic phase was dried and evaporated. The residue was extracted with n-hexane. Combined extracts were evaporated, leaving the amine (9) as a colorless oil (0.408 g, 99.5%): IR (neat) 3400, 1590, 1090, 690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3H), 1.0-1.5 (m, 22H), 1.95-2.3 (m, 2H), 2.15 (m, 2H), 2.88 (dd, J = 11.3, 6.2 Hz, 1H), 3.0 (dd, J = 11.3, 4.2 Hz, 1H), 3.80 (ddd, J = 11.0, 6.2, 4.2 Hz, 1H), 4.24 (dd, J = 11.0, 6.9 Hz, 1H), 5.53 (dd, J = 15.1, 7.0 Hz, 1H), 5.86 (dt, J = 15.1, 8.0 Hz, 1H), 5.96 (s, 1H), 7.1-7.5 (m, 5H); EI-MS, calcd as $C_{25}H_{41}O_2N$ m/z 387.3185, obsd 387.3137.

p-Nitrophenyl Palmitate. A mixture of palmitic acid (5.02 g, 18.9 mmol) and thionyl chloride (4.8 mL, 65.8 mmol) was heated at 50°C for 30 min. The excess thionyl chloride was removed azeotropically with dry benzene. To a solution of the palmitoyl chloride in dry benzene (5 mL) was added dropwise a solution of p-nitrophenol (2.99 g, 21.5 mmol) in pyridine (3.3 mL) and anhydrous ether (10 mL). After 1 h stirring at room temperature, ice-water was added to this mixture. The layers were separated and the aqueous layer was extracted with methylene chloride. The combined organic layers were washed successively with cold 1N hydrochloric acid, sat sodium bicarbonate solution and brine and dried ($MgSO_4$). The solvent was removed and the residue was purified by recrystallization from methylene chloride to give colorless needles (6.63 g, 93%): mp 58.5-60.3°C; IR (Nujol) 1750, 1540, 1350, 720cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3H), 1.0-2.0 (m, 26H), 2.6 (t, J = 7.0 Hz, 2H), 7.25 (d, J = 7.0 Hz, 2H), 8.26 (d, J = 7.0 Hz, 2H).

(2S,3R)-N-Palmitoyl-1,3-O-benzylidene-D-sphingosine (10). A solution of (9) (1.37 g, 3.53 mmol) in pyridine (5 mL) was treated with p-nitrophenylpalmitate (1.33 g, 3.52 mmol) and the mixture was stirred overnight at room temperature. After removal of the solvent in vacuo, the residue was dissolved in methylene chloride, washed successively with cold 1N hydrochloric acid, sat sodium bicarbonate solution and brine, dried and evaporated. The product was purified by chromatography (SiO_2, elution with C_6H_6-AcOEt 95:5 then 9:2) to yield (10) (2.097 g, 95%) as a colorless needles: mp 77.3-78.9°C; IR (Nujol) 3420, 1670, 1100, 670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 6H), 1.0-1.42 (m, 48H), 1.59 (q, J = 7.0 Hz, 2H), 2.15 (t, J = 7 Hz, 2H), 3.40 (dd, J = 11.3, 6.2 Hz, 1H), 3.64 (dd, J = 11.3, 4.2 Hz, 1H), 3.8-4.0 (m, 1H), 4.20 (t, J = 8.0 Hz, 1H), 5.53 (dd, J = 15.1, 7.0 Hz, 1H), 5.76 (dt, J = 15.1, 8.0 Hz, 1H), 5.92 (d, J = 8.0 Hz, 1H), 5.95 (s, 1H), 7.1-7.5 (m, 5H); EI-MS, calcd as $C_{41}H_{71}O_3N$ m/z 625.5426, obsd 625.5434.

(2S,3R)-trans-2-Azide-1,3-octadec-4-enediol (7). To a solution of (6) (0.05 g, 0.12 mmol) in THF (2.5 mL) was added 1N hydrochloric acid (1 mL). The reaction mixture was heated under reflux for 1 h. After cooling, the reaction mixture was taken up in ethyl acetate and the organic layer was dreid ($MgSO_4$) and evaporated. The residue was purified by chromatography (SiO_2, elution with C_6H_6-AcOEt 95:5 then 2:1) to give (7) (0.028 g, 72%) as a colorless oil: IR (neat) 3400, 2100, 970 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3H), 1.0-1.4 (m, 22H), 1.4-1.8 (m, 2H), 2.05 (q, J = 7.0 Hz, 2H), 3.30 (dd, J = 12.0, 6.0 Hz, 1H), 3.42 (dd, J = 12.0, 4.0 Hz, 1H), 3.5-3.7 (m, 1H), 4.04 (t, J = 7.0 Hz, 1H), 5.42 (dd, J = 15.1, 7.0 Hz, 1H), 5.80 (dt, J = 15.1, 8.0 Hz, 1H).

(2S,3R)-1,3-O-2-N-Triacetyl-D-sphingosine (8). A mixture of (7) (0.095 g, 0.29 mmol) and triphenylphosphine (0.077 g, 0.29 mmol) in THF (2 mL) and H_2O (0.3 mL) was stirred overnight at room temperature. The reaction mixture was diluted with chloroform and the layers were separated. The organic phase was dried and evaporated. The residue was dissolved in pyridine (2 mL) and treated with acetic anhydride (0.1 mL, 1.11 mmol). After 4 h at room temperature, the mixture was poured into ice-water and methylene chloride was added. The layers were separated, and the organic layer was washed successively with sat sodium bicarbonate

solution and brine. After drying ($MgSO_4$), the solvent was removed and the residue was purified by chromatography (SiO_2, elution with C_6H_6-AcOEt 6:1 then 4:1) to give 88 mg (71%) of (8) as colorless needles: mp 104.6-106.0°C (lit[5] mp 106-107°C); IR (Nujol) 3400, 1720, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3H), 1.0-1.6 (m, 22H), 1.9-2.2 (m, 1H), 4.25-4.35 (m, 1H), 4.4-4.5 (m, 2H), 5.36 (dd, J = 15.1, 7.0 Hz, 1H), 5.71 (dt, J = 15.1, 8.0 Hz, 1H), 5.82 (d, J = 8.2 Hz, 1H); $[\alpha]_D^{22}$ -21.6° (c = 0.88, HOAc), (lit[5] $[\alpha]_D^{22}$ -22.3° (c = 2, HOAc)).

(2R,3R)-1,3-O-Benzylidene-1,2,3-octadecanetriol (12). A mixture of (4) (1.132 g, 2.91 mmol) and 5% Rh-Al_2O_3 (0.53 g) in cyclohexane (22 mL) was hydrogenated under a hydrogen atmosphere. The reaction mixture was filtered and the solvent was removed in vacuo. The residue was purified by chromatography (SiO_2, elution with C_6H_6-AcOEt 99:1) to obtain (12) (0.95 g, 83%) as colorless needles: mp 66.5-68.0°C; IR (Nujol) 3400, 1100, 710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3H), 1.1-1.5 (m, 26H), 1.6-1.8 (m,1H), 1.8-2.0 (m, 2H), 3.47 (br s, 1H), 3.86 (t, J = 7.0 Hz, 1H), 4.06 (dd, J = 11.0, 2.1 Hz, 1H), 4.23 (dd, J = 11.0, 1.8 Hz, 1H), 5.56 (s, 1H), 7.2-7.5 (m, 5H).

(2R,3R)-2-Azide-1,3-O-benzylidene-1,3-octadecanediol (13). To a solution of (12) (0.945 g, 2.42 mmol) in methylene chloride (9 mL) and triethylamine (1.5 mL, 10.8 mmol) at 0°C was added methanesulfonyl chloride (0.8 mL, 10.2 mmol). After stirring 3h at room temperature, the mixture was partitioned between ice-water and methylene chloride. The organic phase was washed with brine, dried ($MgSO_4$) and evaporated. The residue was purified by chromatography (SiO_2, elution with n-hexane-C_6H_6 1:4 then C_6H_6) to obtain (2R,3R)-1,3-O-benzylidene-2-O-methanesulfonyl-1,2,3-octadecanetriol (1.10 g, 97%) as colorless crystalls: mp 110-111.3°C; IR (Nujol) 1450, 1090, 710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3H), 1.0-1.4 (m, 26H), 1.8-2.0 (m, 2H), 3.13 (s, 3H), 3.96 (td, J = 7.0, 2.1 Hz,1H), 4.06 (dd, J = 11.0, 1.8 Hz, 1H), 4.56 (dd, J = 11.0, 2.1 Hz, 1H), 4.9 (m, 1H), 5.56 (s, 1H), 7.2-7.5 (m, 5H).

To a suspension of sodium azide (0.281 g, 3.35 mmol) in dry DMF (1 mL) was added a solution of the above mesylate (0.510 g, 1.09 mmol) in dry DMF (4 mL) and the reaction mixture was heated at 125°C for 8 h under an argon atmosphere. After removal of the solvent in vacuo, the residue was partitioned between ice-water and methylene chloride. The organic phase was washed successively with water and brine, dried ($MgSO_4$) and evaporated. The crude product was purified by chromatography (SiO_2, elution with n-hexane-C_6H_6 2:1) to afford (13) (0.225 g, 50%) as colorless needles: mp 54-56.3°C; IR (Nujol) 2100, 1090, 780, 710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3H), 1.1-1.4 (m, 26H), 1.8-2.0 (m, 2H), 3.42 (dt, J = 11.0, 6.0 Hz, 1H), 3.48-3.52 (m, 1H), 3.67 (t, J = 11.2 Hz, 1H), 4.36 (dd, J = 11.2, 5.4 Hz, 1H), 5.45 (s, 1H), 7.2-7.5 (m, 5H).

(2S,3R)-2-Amino-1,3-O-benzylidene-1,3-octadecanediol (14). A mixture of (13) (0.142 g, 0.34 mmol) and triphenylphosphine (0.089 g, 0.33 mmol) in THF (3.5 mL) and water (0.5 mL) was stirred overnight at room temperature. The mixture was diluted with methylene chloride and the layers were separated. The organic phase was dried and evaporated. The residue was extracted with n-hexane. Combined extracts were evaporated, leaving (14) as an oil (0.129 g, 90%): IR (neat) 3400, 1590, 1090, 690 cm^{-1}.

(2S,3R)-N-Palmitoyl-1,3-O-benzylidene-4,5-dihydro-D-sphingosine (11). A mixture of (10) (1.09 g, 1.74 mmol) and 5% Rh-Al_2O_3 (0.305 g) in ethyl acetate (15 mL) was hydrogenated under a hydrogen atmosphere. The reaction mixture was filtered and the solvent was removed in vacuo. The residue was purified by chromatography (SiO_2, elution with C_6H_6-AcOEt 95:5) to obtain (11) (1.02 g, 93%) as colorless crystals: mp 101.6-103.5°C; IR (Nujol) 3260, 1640, 1090, 780 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 6H), 1.0-1.8 (m, 52H), 2.16 (q, J = 7.0 Hz, 2H), 2.33 (t, J = 7.0 Hz, 2H), 3.23 (t, J = 11.0 Hz, 1H), 3.52 (t, J = 11.2 Hz, 1H), 4.0-4.2 (m, 1H), 4.26 (dd, J = 11.2, 5.4 Hz, 1H), 5.14 (d, J = 8.0 Hz, 1H), 5.44 (s, 1H), 5.2-7.5 (m, 5H); EI-MS, calcd as $C_{41}H_{73}O_3N$ m/z 627.5571, obsd 627.5591.

(2S,3R)-3-O-Benzoyl-4,5-dihydro-1-deoxyceramide-1-bromide (15). A mixture of (11) (0.254 g, 0.40 mmol), NBS (0.295 g, 1.66 mmol) and barium carbonate (0.803 g, 4.07 mmol) in carbon tetrachloride (11 mL) was refluxed for 5 h under an argon atmosphere. The mixture was filtered and solids were washed with carbon tetrachloride and the filtrate was evaporated under reduced pressure. The residue was dissolved in ether, washed with sat sodium bicarbonate solution, dried ($MgSO_4$) and evaporated. The crude product was purified by chromatography (SiO_2, elution with n-hexane-C_6H_6 3:2) to obtain (15) (0.207 g, 72%) as a pale yellow oil: IR (neat) 3450, 1720, 1650, 780 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 6H), 1.0-1.4 (m, 52H), 1.4-1.7 (m, 4H), 4.09 (d, J = 8.0 Hz, 2H), 4.8-5.0 (m, 1H), 5.3-5.5 (m, 2H), 7.2-7.6 (m, 5H).

(2S,3R)-3-O-Benzoyl-4,5-dihydro-1-deoxyceramide-1-sulfonic Acid (16). A mixture of (15) (0.095 g, 0.13 mmol), tetra-n-butylammonium bromide (0.006g, 0.03 mmol), sat aqueous sodium sulfite solution (2 mL) and chloroform (2 mL) was refluxed for 4 days. After acidification with 1N hydrochloric acid, the mixture was extracted with chloroform and methanol (87:13), dried ($MgSO_4$) and evaporated. The residue was purified by chromatography (SiO_2, elution with C_6H_6-$CHCl_3$ 99:1) to obtain (16) (0.054 g, 57%) as a colorless oil: IR ($CHCl_3$) 3400, 1720, 1660, 1210, 1090 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 6H), 1.1-1.8 (m, 56H), 3.95 (d, J = 8.0 Hz, 2H), 3.5-4.0 (m, 1H), 5.2-5.5 (m, 2H), 7.2-7.9 (m, 5H).

(2S,3R)-4,5-Dihydro-1-deoxyceramide-1-sulfonic Acid (2). A solution of (16) (0.091 g, 0.13 mmol) in 1% methanolic sodium hydroxide solution (4 mL) was stirred at room temperature for 2 h. After removal of the solvent under reduced pressure, the residue was acidified with cold 1N hydrochloric acid and extracted with chloroform and methanol (87:13). The extract was dried ($MgSO_4$) and evaporated. The crude product was purified by chromatography (SiO_2, elution with $CHCl_3$-MeOH 9:1 then 85:15) to afford (2) (0.053 g, 68%) as colorless plates: mp 104.5-106.2°C; IR (Nujol) 3300, 1640, 1460, 1260, 1060 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 6H), 1.2-1.6 (m, 52H), 1.7-2.2 (m, 5H), 3.4-3.7 (m, 4H), 4.25-4.3 (m, 1H), 6.7-6.9 (m, 1H); FAB-MS (positive, matrix: glycerol + thioglycerol) m/z 604 $[M + 1]^+$; $[\alpha]_D^{28}$ -3.13° (c = 0.15, $CHCl_3$-MeOH).

Ammonium (2S,3R)-4,5-Dihydro-1-deoxyceramide-1-sulfonate (17). A solution of (2) (13 mg, 0.02 mmol) in 0.2 N methanolic ammonium hydroxide solution (2 mL) was stirred at room temperature for 3 h. After removal of the solvent under reduced pressure, the residue was extracted with benzene. The extract was evaporated to dryness and the residue was re-dissolved in chloroform and methanol (87:13), dried ($MgSO_4$) and evaporated to obtain (17) (12 mg, 92%) as crystalline solid: mp 171-173°C, (authentic sample; mp 169.5-174.3°C); TLC (SiO_2) R_f = 0.35 ($CHCl_3$-MeOH-H_2O 65:25:4), (authentic sample; R_f = 0.52, 0.35, 0.02, the same solvent system); FAB-MS (positive, matrix: glycerol + thioglycerol) m/z 621 $[M]^+$, 604 $[M - 17]^+$.

(2S,3R)-N-Palmitoyl-D-sphingosine (20). A mixture of (10) (1.12 g, 1.8 mmol), THF (30 mL) and 2N hydrochloric acid (20 mL) was heated under reflux for 1 h. The mixture was extracted with a mixture of chloroform and methanol (87:13). The organic layer was dried ($MgSO_4$) and evaporated. The crude product was purified by chromatography (SiO_2, elution with C_6H_6-AcOEt 4:1 then $CHCl_3$-MeOH 99:1) to afford (10) (0.690 g, 72%): mp 95.0-96.0°C; IR (Nujol) 3370, 1050, 970 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 6H), 1.1-1.4 (m, 48H), 2.06 (q, J = 7.0 Hz, 2H), 2.19 (t, J = 7.0 Hz, 2H), 2.4-3.1 (br s, 2H), 3.6-4.3 (m, 4H), 5.53 (dd, J = 15.1, 7.0 Hz, 1H), 5.76 (dt, J = 15.1, 8.0 Hz, 1H), 5.92 (d, J = 8.0 Hz, 1H); FAB-MS (positive, matrix: glycerol) m/z 538 $[M + 1]^+$; $[\alpha]_D^{28}$ -5.82° (c = 0.98, $CHCl_3$-MeOH 9:1); Anal. calcd for $C_{34}H_{67}O_3N$: C, 75.92; H, 12.55; N, 2.60. found: C, 75.79; H, 12.57; N, 2.48.

(2S,3R)-N-Palmitoyl-3-O-benzoyl-D-sphingosine (21). To a suspension of (20) (0.80 g, 1.49 mmol) and imidazole (0.202 g, 2.97 mmol) in 1.5 mL of dry DMF was added tert-butyldiphenylsilyl chloride (0.58 mL, 2.23 mmol). After 13 h of heating at 60°C, the mixture was quenched with ice-water, and extracted with chloroform. The organic layer was dried and concentrated in vacuo to leave a yellow oil, which was purified by chromatography (SiO_2, elution with C_6H_6-AcOEt 95:5) to afford a silyl ether (0.765 g, 66%) as a colorless oil: IR (neat) 3400, 3300, 1640, 1110 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 6H), 1.03 (s, 9H), 1.2-1.4 (m, 48H), 1.83 (q, J = 7.0 Hz, 2H), 2.09 (t, J = 7 Hz, 2H), 3.3-3.6 (m, 4H), 3.98 (t, J = 7.0 Hz, 1H), 5.28 (dd, J = 15.1, 7.0 Hz, 1H), 5.44 (dt, J = 15.1, 8.0 Hz, 1H), 5.69 (d, J = 8.0 Hz, 1H), 7.3-7.7 (m, 10H); FAB-MS (positive, matrix: thioglycerol) m/z 776 $[M + 1]^+$, 758.

The silyl ether (0.695 g, 0.90 mmol) was dissolved in pyridine (4 mL) and treated with benzoyl chloride (0.2 mL, 1.72 mmol) at 0°C. The mixture was stirred for 30 min at room temperature. After removal of the solvent under reduced pressure, the reaction mixture was treated with ice-water, and extracted with chloroform. The organic layer was washed successively with 1N hydrochloric acid, a sat sodium bicarbonate solution and brine, dried and evaporated to leave a yellow oil. The crude product was purified by chromatography(SiO_2, elution with n-hexane-benzene 2:1 then benzeneonly) to give a silyloxy benzoate (0.701 g, 89%) as a colorless oil: IR (neat) 3300, 1720, 1640, 1262, 1110 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 6H), 1.03 (s, 9H), 1.2-1.4 (m, 48H), 1.84 (q, J = 7.0 Hz, 2H), 2.04 (t, J = 7.0 Hz, 2H), 3.5-3.8 (m, 3H), 4.37 (t, J = 8.0 Hz, 1H), 5.33 (dd, J = 15.1, 7.0 Hz, 1H), 5.52 (dt, J = 15.8, 8.0 Hz, 1H), 5.88 (d, J = 8.0 Hz, 1H), 7.2-7.7 (m, 15H); FAB-MS (positive, matrix: thioglycerol) m/z 880 $[M + 1]^+$.

The silyloxy benzoate was hydrolyzed over a 30 min period at room temperature in a mixture of THF (3 mL) and 1M tetra-n-butylammonium fluoride THF solution (0.9 mL, 0.9 mmol). The mixture was dried ($MgSO_4$) and evaporated. The crude product was purified by chromatography (SiO_2, elution with C_6H_6-AcOEt 95:5 then 9:1) to give (21) (0.240 g, 90%) as colorless crystals: mp 51.8-53.0°C; IR (Nujol) 3300, 1720, 1640, 1110 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3H), 1.1-1.7 (m, 49H), 2.03 (q, J = 7.0 Hz, 2H), 2.17 (t, J = 7.0 Hz, 2H), 3.2-3.6 (m, 3H), 3.7-4.0 (m, 1H), 5.58 (dd, J = 15.1, 8.0 Hz, 1H), 6.05 (m, 1H), 7.3-8.1 (m, 5H); FAB-MS (positive, matrix: glycerol) m/z 642 $[M + 1]^+$.

(2S,3R)-N-Palmitoyl-3-O-benzoyl-1-O-(2',3',4',6'-tetra-O-acetyl-β-D-galactopyranosyl)-D-sphingosine (23). (i) The Königs-Knorr procedure: A mixture of (21) (0.165 g, 0.26 mmol), tetra-O-acetyl-α-D-galactopyranosyl bromide (22) (0.319 g, 0.78 mmol) and mercury(II) cyanide (0.266 g, 1.05 mmol) in anhydrous nitromethane (3 mL) was stirred for 2 h at 80°C under an argon atmosphere. After dilution with chloroform, the organic layer was washed successively with water and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by chromatography (SiO_2, elution with C_6H_6-AcOEt 95:5 then 4:1) to give a mixture of (23), an α-anomer of (23) [(24)] and (25) (0.206 g). The mixture was dissolved

in anhydrous 1,2-dichloroethane (5 mL) and treated with trimethylsilyltriflate (60 mg, 0.2 mmol) and 4A molecular sieves at 0°C under an argon atmosphere. After stirring for 3 h at 0°C, a sat sodium bicarbonate solution was added to this mixture and filtered. The organic layer was dried and concentrated to leave an oil. The crude product was purified by chromatography (SiO_2, elution with C_6H_6-AcOEt 95:5 then 4:1) to give (23) (0.100 g, 43%).

(ii) The Lubineau procedure: A solution of (21) (0.396 g, 0.62 mmol) and 1,1,3,3-tetramethylurea (0.3 mL, 2.48 mmol) in 5 mL of methylene chloride was added to a suspension of (22) (0.761 g, 1.85 mmol), stannous triflate (1.03 g, 2.47 mmol) and molecular sieves (0.732 g) in 6 mL of methylene chloride at room temperature under an argon atmosphere. After stirring for 16 h, the mixture was filtered and a filtrate was treated with a 5% sodium bicarbonate solution and extracted with a mixture of chloroform and methanol (87:13). The organic phase was successively washed with water and brine and dried. The crude product was purified by chromatography (SiO_2, elution with C_6H_6-AcOEt 95:5 then 9:1) to give (23) (0.280 g, 47%) as a colorless oil: IR (neat) 3400, 1750, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 6H), 1.1-1.7 (m, 48H), 1.97-2.2 (m, 16H), 3.65 (t, J = 6.2 Hz, 2H), 3.86 (dt, J = 6.5, 4.0 Hz, 1H), 4.1-4.22 (m, 2H), 4.4-4.46 (m, 1H), 4.60 (d, J = 8.0 Hz, 1H), 5.02 (dd, J = 10.5, 3.6 Hz, 1H), 5.22 (dd, J = 10.5, 8.0 Hz, 1H), 5.25-5.3 (m, 1H), 5.29 (dd, J = 15.1, 7.8 Hz, 1H), 5.39 (dd, J = 3.7, 1.2 Hz, 1H), 6.0-6.1 (m, 1H), 7.3-8.1 (m, 5H); FAB-MS (positive, matrix: triethanolamine) m/z 972 $[M + 1]^+$.

Diethyl 2-Bromoethylphosphonate. A mixture of triethylphosphite (10.3 mL, 86.1 mmol) and 1,2-dibromoethane (44.5 mL, 516.5 mmol) was heated at 160°C for 4 h. The reaction mixture was purified by distillation under reduced pressure to give diethyl 2-bromoethylphosphonate as a clear colorless liquid: bp 105-115°C/3.0-4.0 mmHg; IR (neat) 1280, 1240, 1160, 1050, 1020 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35 (t, J = 7.0 Hz, 6H), 2.09-2.68 (m, 2H), 3.35 (m, 2H), 4.01-4.38 (m, 4H).

Diethyl 2-(N-Methylamino)ethylphosphonate. Anhydrous methylamine (ca. 10 mL), which was generated from 40% aqueous methylamine solution, was distilled directly below the surface of an ice-cold solution of diethyl 2-bromoethylphosphonate (15.0 g) in dry ether (75 mL). After stirring for 1 h at 0°C and then for 20 h at room temperature, the mixture was extracted with water. The aqueous phase was re-extracted with methylene chloride. The organic layer was dried ($MgSO_4$) and evaporated to give diethyl 2-(N-methylamino)ethylphosphonate as a clear colorless liquid (11.59 g, 97%), which was used for the next step without further purification: IR (neat) 3320, 1480, 1230, 1160, 1050, 1020 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34 (t, J = 7.0 Hz, 6H), 1.7-2.42 (m, 3H), 2.44 (s, 3H), 2.66-3.15 (m, 2H), 3.90-4.39 (m, 4H).

2-(N-Methylamino)ethylphosphonic Acid. A solution of diethyl 2-(N-methylamino)-ethylphosphonate (1.45 g, 7.44 mmol) and 47% hydrobromic acid (3 mL, 26 mmol) was heated at 170°C for 20 h. After cooling. the hydrobromic acid distilled off under reduced pressure. The resulting hydrobromide was dissolved in a small amount of water and charged on an Amberlite CG-120 column (H^+ form). The column was washed with water until the eluent became neutral and further eluted with water. The neutral eluent was evaporated under reduced pressure. The crude product was recrystallized from methanol to give 2-(N-methylamino)ethylphosphonic acid (0.802 g, 79%) as colorless needles: mp 290-291°C; IR (Nujol) 3350, 2700, 1260, 1160 cm^{-1}.

2-[N-Methyl-N-(2',2',2'-trichloroethoxycarbonyl)amino]ethylphosphonic Acid (26). To a solution of 2-(N-methylamino)ethylphosphonic acid (0.801 g, 5.84 mmol) in 4N aqueous sodium hydroxide (2 mL) was added 2',2',2'-trichloroethyl chloroformate (2.4 mL, 17.53 mmol) and 4N aqueous sodium hydroxide solution separately at such a rates that the solution was always alkaline (pH 8-10). After stirring for 20 h at room temperature, the mixture was extracted with ethyl acetate. The aqueous layer was acidified with conc hydrochloric acid to pH 2, and extracted with ethyl acetate. The combined organic extracts were successively with sat sodium bicarbonate solution and brine. The organic phase was dried and evaporated to leave a crystalline residue, recrystallization of which from n-hexane gave (26) as colorless crystals (1.57 g, 86%): mp 106°C; IR (Nujol) 2750, 1700, 1270, 1080 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.00 (m, 2H), 3.04 (s, 3H), 3.60 (m, 2H), 4.76 (s, 2H), 6.70 (s, 2H), 6.70 (br s, 2H); FAB-MS (positive, matrix: glycerol) m/z 314 $[M + 1]^+$.

2,3,4,6-Tetra-O-acetyl-1-O-stearyl-β-D-galactopyranoside (27). A solution of stearyl alcohol (0.792 g, 2.93 mmol) and 1,1,3,3-tetramethylurea (0.340 g, 2.93 mmol) in methylene chloride (10 mL) was added in one portion to a stirred suspension of aceto-α-bromogalactose (22) (1.20 g, 2.93 mmol), stannous triflate (0.785 g, 2.93 mmol) and 4A molecular sieves (1 g) in 10 mL of methylene chloride at room temperature. After stirring for 30 min, the mixture was poured onto a stirred mixture of 5% aqueous sodium bicarbonate solution. To the resulting emulsion, a mixture of chloroform and methanol (87:13) was added and filtered through a layer of Celite. The organic layer was washed with brine, dried and evaporated. The crude product was purified by chromatography (SiO_2, elution with C_6H_6-AcOEt 9:1) to give (27) (492 mg, 28%): ^1H NMR ($CDCl_3$) δ 0.88 (3H, J = 7 Hz, 3H), 1.1-1.7 (m, 32H), 1.98 (s, 3H), 2.04 (s, 3H), 2.13 (s, 3H), 2.16 (s, 3H), 3.47 (m, 2H), 3.89 (m, 1H), 4.15 (m, 1H), 4.45 (d, J = 8 Hz, 1H), 5.01 (dd, J = 11, 3 Hz, 1H), 5.20 (dd, J = 11, 8 Hz, 1H), 5.38 (dd, J = 3, 1 Hz, 1H).

2,3,4-Tri-O-acetyl-6-O-[2'-{N-methyl-N'-(2",2",2"-trichloroethoxycarbonylamino)-ethylphosphonyl}]-1-O-stearyl-β-D-galactopyranoside (30). (i) To a solution of (27) (0.336 g, 0.56 mmol) in 3 mL of abs methanol was added a 0.25M methanolic sodium methoxide solution (1 mL). After being stirred for 30 min at room temperature, the reaction mixture was treated with Amberlyst 15 (500 mg) until the solution became neutral. The resin was filtered and the solid was extracted twice with a mixture of chloroform and methanol (87:13). The combined filtrate and extracts were concentrated to a colorless liquid (28) (0.238 g, 98%). The product was dissolved in a mixture of chloroform and methanol (87:13) and dried by an azeotropic distillation with benzene. The residue was dissolved in 1 mL of pyridine and treated with trityl chloride (160 mg, 0.55 mmol), and dimethylaminopyridine (7 mg, 0.055 mmol). The mixture was stirred overnight at room temperature and then heated at 60°C for 4 h.

To the cooled reaction mixture was added acetic anhydride (1 mL) and dimethylaminopyridine (10 mg). The reaction mixture was stirred at room temperature for 19 h, poured into ice-water and extracted with chloroform. The combined organic layers were successively washed with dil hydrochloric acid, an aqueous sodium bicarbonate solution and brine, dried and concentrated in vacuo. The crude product was purified by chromatography (SiO_2, elution with C_6H_6-AcOEt 9:1) to give a trityl ether (0.307 g, 77%): ^1H NMR ($CDCl_3$) δ 0.89 (s, 3H), 1.26 (m, 32H), 1.87 (s, 3H), 1.97 (s, 3H), 2.03 (s, 3H), 3.08 (t, J = 8.0 Hz, 1H), 3.4-3.5 (m, 2H), 3.7-3.9 (m, 2H), 4.40 (d, J = 7.0 Hz, 1H), 5.0-5.2 (m, 2H), 5.54 (d, J = 3 Hz, 1H), 7.2-7.4 (m, 15H).

A solution of the trityl ether (0.290 g, 0.384 mmol) in 90% acetic acid (1.5 mL) was heated at 100°C for 1 h. After removal of the solvent in vacuo, the residue was dissolved in a mixture of chloroform and methanol (87:13) and washed with an aqueous sodium bicarbonate solution and brine. The organic phase was dried and concentrated in vacuo to leave a crystalline product which was purified by chromatography (SiO_2, elution with $CHCl_3$-MeOH 97:3) to afford (29) (0.130 g, 61%): IR (neat) 3500, 1750, 1230, 1080, 1060 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J =6 Hz, 3H), 1.24 (m, 32H), 2.0 (s, 3H), 2.04 (s, 3H), 2.16 (s, 3H), 3.45-3.62 (m, 2H), 3.70-3.84 (m, 1H), 3.84-3.98 (m, 1H), 4.34 (d, J = 6 Hz, 1H), 4.49 (t, J = 8 Hz, 2H), 4.96 (dd, J = 10, 3 Hz, 1H), 5.07 (dd, J = 10, 8 Hz, 1H), 5.30 (d, J = 3 Hz, 1H).

A solution containing 100 mg (0.179 mmol) of (29), 145 mg (0.461 mmol) of (26) and dimethylaminopyridine (5 mg, 0.023 mmol) in 2 mL of pyridine was treated with DCC (190 mg, 0.925 mmol). After stirring for 4 days at room temperature, the mixture was poured into water and extracted with a mixture of chloroform and methanol (87:13). The organic phase was washed with brine, dried and evaporated to leave a crude product which was purified by chromatography (SiO_2, elution with $CHCl_3$-MeOH 97:13) to afford (30) (23 mg, 15%): IR ($CHCl_3$) 1730, 1700, 1650, 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.87 (s, 3H), 1.24 (m, 2H), other signals became broad coalescence peaks and could not be identified; FAB-MS (negative, matrix: triethanolamine) m/z 818 [M - 35]$^-$, 776, 742.

(ii) To a solution of (28) (100 mg, 0.231 mmol) and (26) (145 mg, 0.462 mmol) in 2 mL of pyridine was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (DEC) (221 mg, 1.16 mmol) and DMAP (2 mg, 0.02 mmol). The mixture was stirred for 16 h and then 7 h under ultrasonic irradiation. After addition of excess acetic anhydride, the mixture was stirred overnight. Usual work-up gave (30).

6-O-(2'-N-Methylaminoethylphosphonyl)-1-O-stearyl-β-D-galactopyranoside (32). To a solution of (28) (10 mg, 0.231 mmol) and (26) (145 mg, 0.462 mmol) in 2 mL of pyridine was added DEC (221 mg, 1.16 mmol) and DMAP (2 mg, 0.02 mmol). The mixture was stirred for 16 h and then for 7 h under ultrasonic irradiation. After work-up as before, the crude product (31) was then dissolved in 3 mL of 90% acetic acid and treated with zinc (300 mg, 4.6 mg-atom) at 100°C for 20 min. The solid was filtered and the filtrate was made neutral with sat sodium bicarbonate solution and extracted with a mixture of chloroform and methanol (87:13). Combined extracts were dried and evaporated. The crude material was purified by chromatography (Iatrobeads, elution with $CHCl_3$-MeOH-H_2O 56:38:10) to afford (32) (70 mg, 55%): FAB-MS (positive, matrix: glycerol) m/z 554 [M + 1]$^+$, 536.

(2S,3R)-N-Palmitoyl-1-O-β-D-galactopyranosyl-D-sphingosine (33). To a stirred solution of sodium methoxide (prepared from 0.102 g of sodium and 5 mL of abs methanol) was added a solution of (23) (0.184 g, 0.19 mmol) in 10 mL of abs methanol under a nitrogen atmosphere. After stirring 1 h at room temperature, the reaction mixture was acidified with dil hydrochloric acid and extracted with a mixture of chloroform and methanol (87:13). The organic phase was washed with brine, dried and evaporated. The residue was purified by chromatography (SiO_2, elution with $CHCl_3$-MeOH 95:5 then 9:1) to give (33) (0.125 g, 95%) as a colorless plates: mp 124.5-125.8°C; IR ($CHCl_3$) 3400, 3350, 1650 cm^{-1}; ^1H NMR ($CDCl_3$-CD_3OD) δ 0.88 (t, J = 6.9 Hz, 6H), 1.0-1.7 (m, 48H), 2.08 (q, J = 7.0 Hz, 2H), 2.21 (t, J = 7.6 Hz, 2H), 3.52 (dd, J = 10.5, 3.6 Hz, 1H), 3.64 (dd, J = 10.5, 8.0 Hz, 1H), 3.69 (dd, J = 3.7, 1.2 Hz, 1H), 3.91 (dt, J = 6.5, 3.5 Hz, 1H), 4.13-4.2 (m, 10H), 5.47 (dd, J = 15.1, 7.8 Hz, 1H), 5.78 (dt, J = 15.1, 7.5 Hz, 1H); FAB-MS (positive, matrix: glycerol) m/z 700 [M + 1]$^+$.

(2S,3R)-N-Palmitoyl-1-O-[6'-O-{2"-(N-methyl-N-<2'",2'",2'"-trichloroethoxycarbonyl>amino)ethylphosphonyl}-β-D-galactopyranosyl]-D-sphingosine (34). To a solution of (33) (0.100 g, 0.14 mmol) and (26) (0.180 g, 0.57 mmol) in 3 mL of anhydrous pyridine was added DEC (0.219 g, 1.14 mmol) under an argon atmosphere. The mixture was heated for 18 h at 60°C under ultrasonic irradiation. The solvent was removed azeotropically with toluene. The residue was purified by chromatography (Iatrobeads, elution successively with $CHCl_3$-MeOH 94:4, 9:1 and 4:1) to afford (34) (0.105 g, 74%) as a colorless oil: IR ($CHCl_3$) 3450, 3320, 1710, 1660, 1120, 1090, 1040 cm^{-1}; FAB-MS (positive, matrix: triethanolamine) m/z 997 $[M + 1]^+$.

(2S,3R)-N-Palmitoyl-1-O-[6'-O-{2"-(N-methylamino)ethylphosphonyl}-β-D-galactopyranosyl]-D-sphingosine (19). To a stirred solution of (34) (0.040 g, 0.04 mmol) in 4 mL of 90% acetic acid was added zinc powder (0.052 g, 0.08 mg-atom). After 15 h stirring at room temperature, the mixture was filtered and the filtrate was neutralized with a sodium hydrogen carbonate solution. The aqueous solution was extracted with a mixture of chloroform and methanol (87:13), the combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by chromatography (Iatrobeads, elution successively with $CHCl_3$-MeOH-H_2O 9:1:0 then 65:25:4) to afford (19) (0.027 g, 82%) as colorless crystals: mp 154.5-155.0°C (natural lipid: mp 150.5-155.5°C); IR ($CHCl_3$) 3450, 3300, 1640, 1070, 1040 cm^{-1}; TLC R_f = 0.23 ($CHCl_3$-MeOH-28% NH_4OH 56:38:10), natural lipid: R_f = 0.21; $[\alpha]_D^{19}$ -2.5° (c = 0.16, $CHCl_3$-MeOH 9:1); FAB-MS (positive, matrix: glycerol) m/z 821 $[M + 1]^+$.

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19. We thank Professor T. Matsubara, Kinki University, for supplying us with the natural lipid.
20. Private communication with Professor A. Hayashi, Kinki University. We would like to thank Professor Hayashi for valuable suggestions.