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TOTAL SYNTHESIS OF CEREBROSIDES: (2*S*, 3*R*, 4*E*)-1-*O*-β-D-GALACTOPYRANOSYL-*N*-(2′*R* AND 2′*S*)-2′-HYDROXYTETRACOSANOYLSPHINGENINE*

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ABSTRACT

Total syntheses of both (2*S*, 3*R*, 4*E*)-1-*O*-β-D-galactopyranosyl-*N*-(2′*R*)-2′-hydroxytetracosanoylsphingenine **23** and the (2′*S*) stereoisomer were performed in an unambiguous way by employing either (2*S*, 3*R*, 4*E*)-*N*-(2′*R*)-2′-(*tert*-butyldiphenylsilyloxy)tetracosanoylsphingenine or its (2′*S*) stereoisomer as the key glycosyl acceptors. The synthetic cerebroside **23** was shown to be identical with the natural product through comparison of their 400-MHz, ^{1}H-n.m.r. spectra, thus providing synthetic evidence for the 2′*R* configuration of the natural cerebroside.

INTRODUCTION

The cerebrosides were first isolated by Thudichum in 1874 from brain where they constitute a major lipid component of myelin sheaths. Their constitutions were established by Carter and his co-workers[2] in 1950. Their distribution in brain and maturational changes are known to coincide well with the myelination process[3].

In 1961, Shapiro and Flowers[4] reported the first total synthesis of a cerebroside, which they compared with the natural product by i.r. spectroscopy. In order to confirm the absolute configuration at C-2′ of the α-hydroxy fatty acid in natural cerebroside, we report here an unambiguous synthesis of both the (2′*R*) and (2′*S*) stereoisomers (**23** and **24**). The ^{1}H-n.m.r. spectrum of the synthetic (2′*R*) isomer **23** is completely identical with that of the natural cerebroside reported by Dabrowski *et al.*[5].

RESULTS AND DISCUSSION

Ethyl (2*S*)-2-acetoxytetracosanoate (**1**) was obtained from (*S*)-(−)-malic acid

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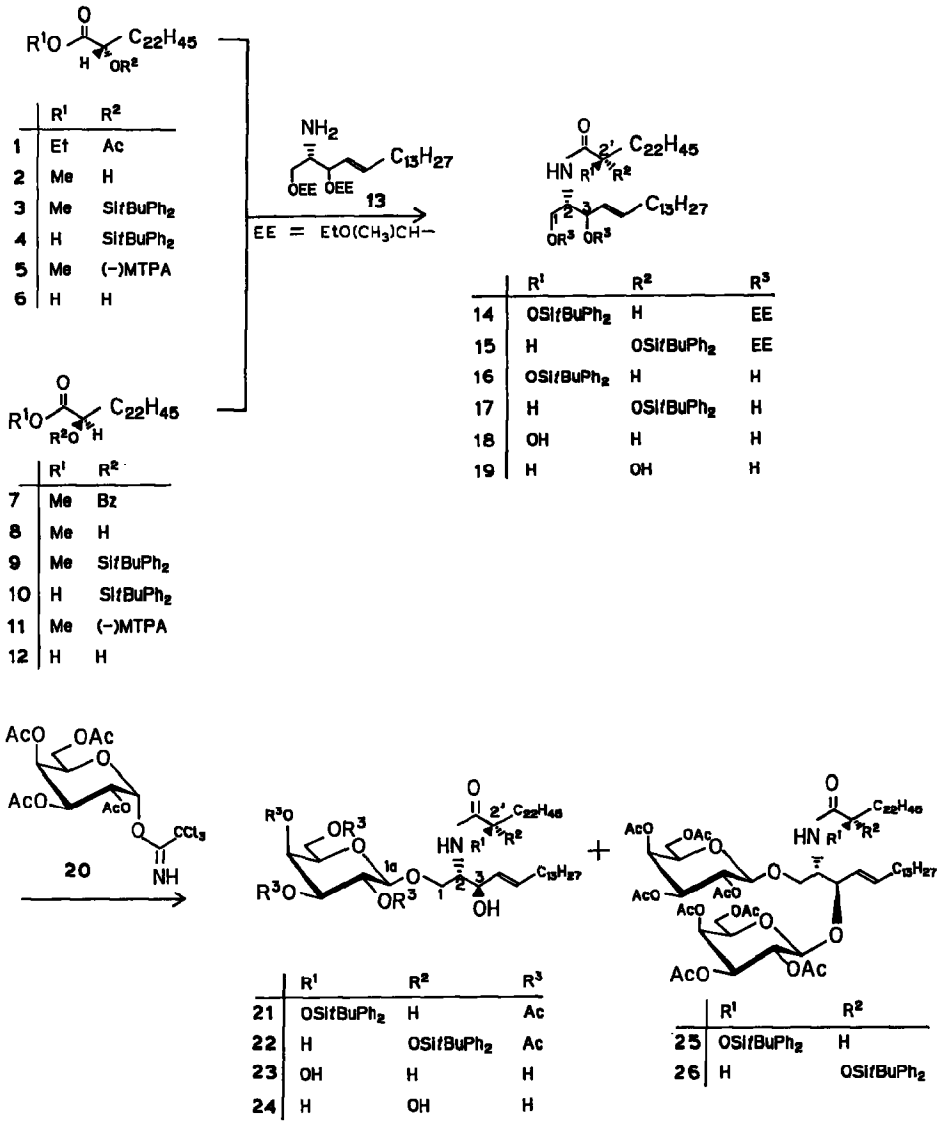
according to the procedure[6] of Horn and Pretorius[†]. Compound **1** was then transformed into (2*S*)-2-(*tert*-butyldiphenylsilyloxy) tetracosanoic acid (**4**) in 77% overall yield in three steps: (*i*) saponification of compound **1** with sodium methoxide in 1:1 methanol–tetrahydrofuran to give compound **2**, which was obtained in 96.2% ee, as judged from the ^{1}H-n.m.r. data for the (−)-MTPA[††] ester (**5**), (*ii*) treatment of compound **2** with *tert*-butyldiphenylsilyl chloride and imidazole in *N,N*-dimethylformamide, to give compound **3**, and (*iii*) saponifiation of compound **3** with sodium hydroxide in 1:1 methanol–tetrahydrofuran. In a similar way, (2*R*)-2-*tert*-butyldiphenylsilyloxytetracosanoic acid **10** was prepared in 50% overall yield from compound **2** in four steps: (*i*) treatment of compound **2** according to Mitsunobu and Eguchi[7] to give benzoate **7**, (*ii*) saponification of compound **7** with sodium methoxide in 1:1 methanol–tetrahydrofuran to give alcohol **8**, (*iii*) silylation of alcohol **8** to give compound **9**, and (*iv*) saponification of compound **9** to give acid **10**. Integration of the ^{1}H-n.m.r. spectrum of the (−)-MTPA ester **11** showed that alcohol **8** had been obtained in 95.4% ee.

In order to confirm that no significant racemization had occurred either by saponification of ester **3** to **4** or by desilylation of **3** to **2**, compound (2*S*)-**4** was successively treated with (*i*) diazomethane, (*ii*) tetrabutylammonium fluoride in tetrahydrofuran, and (*iii*) (−)-MTPA chloride in pyridine[8], to give (2*S*)-**5** in 96.0% ee.

(2*S*, 3*R*, 4*E*)-1,3-Di-*O*-ethoxyethylsphingenine **13**, reported previously[9], was acylated with (2*R*)-acid **10** and (2*S*)-acid **4** in the presence of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole, to give the protected (2*S*, 3*R*, 4*E*, 2′*R*)-ceramide **14** and (2*S*, 3*R*, 4*E*, 2′*S*)-ceramide **15** in 91 and 90% yield, respectively. Removal of the ethoxyethyl groups of **14** and **15** was effected by solvolysis in 1:1 methanol–dichloromethane in the presence of Amberlyst 15, to afford (2*S*, 3*R*, 4*E*, 2′*R*)-glycosyl acceptor **16** and (2*S*, 3*R*, 4*E*, 2′*S*)-glycosyl acceptor **17** in 67 and 61% yield, respectively. Treatment of (2′*R*)-silyl alcohol **16** and the (2′*S*)-isomer **17** with tetrabutylammonium fluoride in tetrahydrofuran afforded deblocked (2*S*, 3*R*, 4*E*, 2′*R*)-ceramide **18** and the (2*S*, 3*R*, 4*E*, 2′*S*)-isomer **19** in 91 and 79% yield, respectively.

The crucial glycosylation of (2′*R*)-ceramide **16** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (**20**) in chloroform in the presence of boron trifluoride etherate and molecular sieves 4A according to the method of Schmidt and Michel[10] afforded a 33% yield of protected (2′*R*)-cerebroside **21**. In addition to the desired product **21**, diglycosylated product **25** was isolated in 11% yield, of which the structure was assigned from ^{1}H-n.m.r. data. The anomeric configuration of the product was assigned as β-D- from the ^{1}H-n.m.r. data for **21**, which revealed a signal for H-1a at δ 4.488 as a doublet with *J* 8.1 Hz. Complete deprotection of compound **21** by successive treatments with tetrabutylammonium fluoride and sodium methoxide in methanol afforded a 62% yield of (2′*R*)-cerebroside **23**. Similarly, (2*S*, 3*R*, 4*E*)-1-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-*N*-(2′*S*)-2′-*tert*-

[†]A synthetic approach to α-hydroxy acids by the use of enzymes has been reported[6a].
[††]MTPA = α-methoxy-α-trifluoromethylphenylacetyl.



	R¹	R²
1	Et	Ac
2	Me	H
3	Me	SitBuPh₂
4	H	SitBuPh₂
5	Me	(–)MTPA
6	H	H

	R¹	R²
7	Me	Bz
8	Me	H
9	Me	SitBuPh₂
10	H	SitBuPh₂
11	Me	(–)MTPA
12	H	H

	R¹	R²	R³
14	OSitBuPh₂	H	EE
15	H	OSitBuPh₂	EE
16	OSitBuPh₂	H	H
17	H	OSitBuPh₂	H
18	OH	H	H
19	H	OH	H

	R¹	R²	R³
21	OSitBuPh₂	H	Ac
22	H	OSitBuPh₂	Ac
23	OH	H	H
24	H	OH	H

	R¹	R²
25	OSitBuPh₂	H
26	H	OSitBuPh₂

butyldiphenylsilyloxytetracosanoylsphingenine (**22**) was obtained from (2′*S*)-glycosyl acceptor **17** in 31% yield. In this glycosylation, the diglycosylated product **26** also was isolated, in 29% yield. The ^{1}H-n.m.r. spectrum of the desired product **22** contains a signal for H-1a at δ 4.483 as a doublet with J 8.1 Hz, while that of compound **26** contains two doublets, at δ 4.519 and 4.512, with J values of 8.1 and 7.8 Hz, respectively, for H-1a and H-1b. Removal of the protecting groups from compound **22** was achieved as in the case of **21**, to afford (2*S*, 3*R*, 4*E*, 2′*S*)-cerebroside **24** in 78% yield.

In conclusion, the desired (2*S*, 3*R*, 4*E*, 2′*R*)- and (2*S*, 3*R*, 4*E*, 2′*S*)-cerebrosides **23** and **24** were synthesized in an unambiguous way. Comparison of the 400-MHz, ^{1}H-n.m.r. spectra of the synthetic cerebrosides **23** and **24** with that of the natural product[5] showed that the configuration of the natural product is (2*S*, 3*R*, 4*E*, 2′*R*).

EXPERIMENTAL

General. — Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Optical rotations were determined with a Perkin–Elmer Model 241 MC polarimeter, for solutions in $CHCl_3$ at 25°, unless noted otherwise. Column chromatography was performed on columns of silica gel (Merck, 70–230 mesh). Flash chromatography was conducted on columns of Wako-gel C-300 (200–300 mesh). T.l.c. and high-performance t.l.c. were performed on Silica Gel 60 F_{254} (Merck, Darmstadt). Molecular sieves were purchased from Nakarai Chemicals, Ltd. I.r. spectra were recorded with an EPI-G2 Hitachi spectrophotometer, using KBr pellets for the crystalline samples, and films for the liquid samples. ^{1}H-N.m.r. spectra were recorded with either a JNM-GX400 or a JNM-FX90Q n.m.r. spectrometer. ^{13}C-N.m.r. spectra were recorded with a JNM-FX 100FT n.m.r. spectrometer operated at 25.05 MHz. The values of δ_C and δ_H are expressed in p.p.m. downwards from the signal for internal Me_4Si, for solutions in $CDCl_3$, unless noted otherwise. Values of δ_H (D_2O) and δ_C (D_2O) are expressed in p.p.m. downward from Me_4Si, by reference to internal standards of Me_2CO (2.225) or Me_3COH (1.230), and 1,4-dioxane (67.4) or MeOH (49.8), respectively.

Ethyl (2S)-2-acetoxytetracosanoate (**1**). — A mixture of ethyl hydrogen (2*S*)-2-acetoxysuccinate (21.0 g, 103 mmol) and docosanoic acid (10.2 g, 30 mmol) in EtOH (150 mL) containing NaOEt prepared from Na (0.1 g) was transformed into **1** (1.65 g, 14%) by anodic coupling according to the procedure of Horn and Pretorius[6].

Compound **1**: m.p. 42–43°, $[\alpha]_D$ −13° (*c* 1.6); R_F 0.74 in 4:1 hexane–EtOAc; n.m.r. data: δ_H 4.96 (t, 1 H, *J* 5.9 Hz, H-2), 4.20 (q, 2 H, *J* 7.2 Hz, OCH_2CH_3), and 2.13 (s, 3 H, Ac); δ_C 169.9 (C=O), 72.2 (C-2), and 60.7 (OCH_2CH_3).

Anal. Calc. for $C_{28}H_{54}O_4$: C, 73.96; H, 11.97. Found: C, 74.08; H, 11.91.

Methyl (2S)-2-hydroxytetracosanoate (**2**) *and methyl (2S)-2-(−)-α-methoxy-α-trifluoromethylphenylacetyloxytetracosanoate* (**5**). — A solution of compound **1** (1.023 g, 2.25 mmol) in a mixture of THF (5 mL)–5% MeONa–MeOH (5 mL) was stirred for 16 h at 5°, and then diluted with $CHCl_3$ (500 mL), and de-ionized with Amberlyst 15. Evaporation and chromatography on SiO_2 in 9:1 hexane–EtOAc afforded **2** (844 mg, 94%): m.p. 67–68°, $[\alpha]_D$ +5.2° (*c* 0.62); R_F 0.54 in 4:1 hexane–EtOAc; n.m.r. data: δ_H 4.200 (dt, 1 H, *J* 4.5 and 7.5 Hz, H-2), 3.791 (s, 3 H, OMe), 2.701 (d, 1 H, *J* 4.5 Hz, C_2-O*H*), and 0.880 (t, 3 H, *J* 6.6 Hz, CH_2CH_3); δ_C 70.6 (C-2), 52.4 (OMe), and 14.1 (CH_2CH_3).

Anal. Calc. for $C_{25}H_{50}O_3$: C, 75.32; H, 12.64. Found: C, 75.10; H, 12.50.

Compound **2** was saponified to the acid **6**, $[\alpha]_D$ −3.4° (*c* 0.64, pyridine).

A mixture of compound **2** (10 mg, 25 μmol) and (−)-MTPA chloride (20 mg, 79 μmol) in pyridine (0.5 mL) was stirred for 16 h at 20°. Processing and chromatography on SiO_2 in 50:1 hexane–EtOAc afforded **5** (12.5 mg, 81%); R_F 0.54 in 9:1 hexane–EtOAc; n.m.r. data: δ_H 5.163 (t, 1 H, *J* 6.6 Hz, H-2), 3.787 (s, 3 H, COOMe), 3.659 (q, 3 H, $^5J_{HF}$ 1.2 Hz, $COCH_3$), and 0.880 (t, 3 H, *J* 6.6 Hz, CH_2CH_3); δ_C 74.0 (C-2), 55.7 ($COCH_3$), and 52.3 (CO_2CH_3). Proton signals for CO_2Me of **5** and **11** were observed, by enlargement of the spectrum at δ 3.787 and 3.745, respectively, in the ratio of 98.1:1.9.

*Methyl (2S)-2-*tert*-butyldiphenylsilyloxytetracosanoate* (**3**). — A mixture of compound **2** (128 mg, 320 μmol), *t*$BuPh_2SiCl$ (148 μL, 512 μmol), and imidazole (44 mg, 640 μmol) in DMF (2.7 mL) was stirred for 24 h at 20°, and diluted with $CHCl_3$ (100 mL). The solution was successively washed with aq. $NaHCO_3$, H_2O, and satd. saline, dried ($MgSO_4$), and evaporated *in vacuo*. Chromatography of the residue on SiO_2 in 19:1 hexane–EtOAc afforded **3** (184 mg, 90%); m.p. 44–45°, $[\alpha]_D$ −9.5° (*c* 1.9); R_F 0.41 in 19:1 hexane–EtOAc; n.m.r. data: δ_H 4.218 (t, 1 H, *J* 5.4 Hz, H-2), 3.470 (s, 3 H, OMe), 1.094 (s, 9 H, CMe_3), and 0.879 (t, 3 H, *J* 6.6 Hz, CH_2CH_3); δ_C 173.6 (C=O), 72.9 (C-2), and 51.2 (OMe).

Anal. Calc. for $C_{41}H_{68}O_3Si$: C, 77.30; H, 10.76. Found: C, 77.45; H, 10.66.

*(2S)-2-*tert*-Butyldiphenylsilyloxytetracosanoic acid* (**4**) *and methyl (2S)-2-(−)-α-methoxy-α-trifluoromethylphenylacetyloxytetracosanoate* (**5**). — A solution of compound **3** (304 mg, 477 μmol) in THF (5.4 mL) and 1:19.5 NaOH–MeOH (5.4 mL) was stirred for 16 h at 20°, diluted with THF (200 mL), and de-ionized with Amberlyst 15. Evaporation and chromatography on SiO_2 in 9:1 hexane–EtOAc afforded **4** (272 mg, 91%); m.p. 49–50°, $[\alpha]_D$ +10.3° (*c* 0.91); R_F 0.50 in 4:1 hexane–EtOAc; n.m.r. data: δ_H 4.296 (t, 1 H, *J* 5.2 Hz, H-2), 1.116 (s, 9 H, CMe_3), and 0.878 (t, 3 H, *J* 6.6 Hz, CH_2CH_3); δ_C 177.1 (C=O) and 72.6 (C-2).

Anal. Calc. for $C_{40}H_{66}O_3Si$: C, 77.11; H, 10.68. Found: C, 77.09; H, 10.68.

Compound **4** (28 mg, 45 μmol) was treated with an excess of diazomethane in ether, and the crude product was chromatographed on SiO_2 in 17:1 hexane–EtOAc, to give **3** (22 mg, 75%). To a solution of compound **3** (22 mg) in tetrahydrofuran (0.3 mL) was added M Bu_4NF in THF (0.5 mL), and the mixture was stirred for 1.5 h at 20°. The usual processing and chromatography of the crude product afforded **3** (7.7 mg, 57%). A mixture of **3** (7.7 mg) and (−)-MTPA chloride (20 mg) in pyridine (0.5 mL) was stirred for 16 h at 20° and processed, to give **5** (5.0 mg). Integration of the ^{1}H-n.m.r. signals at 3.787 and 3.745 indicated the ratio of diastereomers to be 98.0:2.0.

Methyl (2R)-2-benzoyloxytetracosanoate (**7**). — To a mixture of **2** (56 mg, 140 μmol), Ph_3P (82 mg, 311 μmol), and benzoic acid (36 mg, 292 μmol) in THF (2 mL) was added a solution of diethyl azodicarboxylate (44 μL) in THF (0.2 mL). The mixture was stirred for 1 h at 20°, and evaporated *in vacuo*. Chromatography of the residue on SiO_2 in 20:1 hexane–EtOAc gave **7** (65 mg, 92%); m.p. 49–50°, $[\alpha]_D$ +3.0° (*c* 1.7); R_F 0.47 in 9:1 hexane–EtOAc; n.m.r. data: δ_H 5.24 (t, 1 H, *J*

6.2 Hz, H-2) and 3.75 (s, 3 H, OMe); δ_C 72.9 (C-2) and 52.2 (OMe).

Anal. Calc. for $C_{32}H_{54}O_4$: C, 76.45; H, 10.83;. Found: C, 76.22; H, 10.78.

Methyl (2R)-2-hydroxytetracosanoate (**8**) *and methyl 2(R)-2-(−)-α-methoxy-α-trifluoromethylphenylacetyloxytetracosanoate* (**11**). — Compound **7** (1.246 g, 2.5 mmol) was treated as described for compound **1**, to give **8** (730 mg, 74%); m.p. 67–68°, $[\alpha]_D$ −4.5° (*c* 1.4); R_F 0.54 in 4:1 hexane–EtOAc. The n.m.r. data were found to be identical with those of compound **2**.

Anal. Calc. for $C_{25}H_{50}O_{30}$: C, 75.32; H, 12.64. Found: C, 75.14; H, 12.70.

Compound **8** was saponified to the acid **12**, $[\alpha]_D$ +3.6° (*c* 0.5, pyridine).

Compound **8** (10 mg, 25 μmol) was treated as described for the conversion of compound **2** into compound **5**, to afford **11** (11 mg, 71%); R_F 0.70 in 4:1 hexane–EtOAc; n.m.r. data: δ_H 5.175 (t, H, *J* 6.6 Hz, H-2), 3.749 (s, 3 H, COOMe), 3.569 (q, 3 H, *J* 1.0 Hz, COMe), and 0.879 (t, 3 H, *J* 6.8 Hz, CH_2CH_3); δ_C 74.2 (C-2), 55.5 ($COCH_3$), and 52.3 ($COOCH_3$). The molar ratio of compounds **11** and **5** was determined as 97.7:2.3 by integration of the ^{1}H-n.m.r. signals at 3.745 and 3.786 p.p.m. (CO_2CH_3).

*Methyl (2R)-2-*tert*-butyldiphenylsilyloxytetracosanoate* (**9**) *and (2R)-2-*tert*-butyldiphenylsilyloxytetracosanoic acid* (**10**). — Compound **8** (680 mg, 1.71 mmol) was treated as described for compound **2**, to give **9** (quantitative); m.p. 46–47°, $[\alpha]_D$ +9.4° (*c* 5.1); R_F 0.41 in 19:1 hexane–EtOAc. The n.m.r. data were found to be identical with those of compound **3**.

Anal. Calc. for $C_{41}H_{68}O_3Si$: C, 77.30; H, 10.76. Found: C, 77.18; H, 10.74.

Compound **9** (528 mg, 828 μmol) was treated as described for compound **3**, to give **10** (380 mg, 74%); m.p. 48–49°, $[\alpha]_D$ −11.6° (*c* 0.3); R_F 0.50 in 4:1 hexane–EtOAc; n.m.r. data identical with those of compound **4**.

*(2S, 3R, 4E)-*N*-(2'R)-2'-*tert*-Butyldiphenylsilyloxytetracosanoyl-1,3-di-*O*-(1-ethoxyethyl)-sphingenine* (**14**). — A mixture of compound **10** (194 mg, 312 μmol), compound **13** (142 mg, 319 μmol), 1-hydroxybenzotriazole (51 mg, 374 μmol), and dicyclohexylcarbodiimide (80 mg, 392 μmol) in CH_2Cl_2 (2.5 mL) was stirred for 16 h at 20°, and filtered through a cotton plug. Chromatography on SiO_2 in 95:5:1 hexane–EtOAc–Et_3N afforded **14** (299 mg, 91%); $[\alpha]_D$ −3.6° (*c* 0.7); R_F 0.45 and 0.52 in 4:1 hexane–EtOAc; ν_{max} 3450, 1680, 1590, and 1510 cm^{-1}.

Anal. Calc. for $C_{66}H_{117}NO_6Si$: C, 75.59; H, 11.24. Found: C, 75.95; H, 11.32.

*(2S, 3R, 4E)-*N*-(2'S)-2'-*tert*-Butyldiphenylsilyloxytetracosanoyl-1,3-di-*O*-(1-ethoxyethyl)-sphingenine* (**15**). — Compound **4** (268 mg, 430 μmol) was treated as described for the conversion of compound **10** into compound **14** to give **15** (406 mg, 90%), $[\alpha]_D$ −0.5° (*c* 1.8), R_F 0.62 and 0.55 in 4:1 hexane–EtOAc, ν_{max} 3400, 1650, 1580, and 1570 cm^{-1}.

Anal. Calc. for $C_{66}H_{117}NO_6Si$: C, 75.59; H, 11.24. Found: C, 75.93; H, 11.36.

*(2S, 3R, 4E)-*N*-(2'R)-2'-*tert*-Butyldiphenylsilyloxytetracosanoylsphingenine* (**16**). — A mixture of compound **14** (572 mg, 545 μmol) and Amberlyst 15 (1.0 g) in 1:1 CH_2Cl_2–MeOH (5 mL) was stirred for 16 h at 20°, and filtered. Evaporation of the filtrate *in vacuo* and chromatography of the residue on SiO_2 in 4:1 benzene–

EtOAc afforded **16** (330 mg, 67%); m.p. 44–45°, $[\alpha]_D$ +6.4° (*c* 1.1); R_F 0.30 in 7:3 hexane–EtOAc; n.m.r. data: δ_H 5.789 (dt, 1 H, *J* 15.4 and 6.6 Hz, H-5), 5.503 (dd, 1 H, *J* 6.6 and 15.4 Hz, H-4), 4.324 (t, 1 H, *J* 3.9 Hz, H-2'), 4.255 (m, 1 H, H-3), 3.88–3.84 (m, 2 H, H-1A and H-2), 3.584 (m, 1 H, H-1B), 2.683 (d, 1 H, *J* 4.9 Hz, C_3–O*H*), 2.360 (m, 1 H, C_1–O*H*), 2.039 (q, 2 H, *J* 7.0 Hz, H-6A and H-6B), 1.31 (s, 9 H, CMe_3), and 0.880 (t, 6 H, *J* 6.6 Hz, CH_2CH_3); δ_C 134.4 (C-4), 128.9 (C-5), 74.4 (C-3*), 74.2 (C-2'*), 62.6 (C-1), and 54.7 (C-2); ν_{max} 3420, 1660, 1590, and 1540 cm^{-1}.

Anal. Calc. for $C_{58}H_{101}NO_4Si$: C, 77.02; H, 11.25; N, 1.55. Found: C, 77.27; H, 11.33; N, 1.50.

*(2*S*, 3*R*, 4*E*)-*N*-(2'*S*)-2'-*tert*-Butyldiphenylsilyloxytetracosanoylsphingenine* (**17**). — Compound **15** (100 mg, 95 μmol) was treated as described for the conversion of compound **14** into compound **16**, to afford **17** (53 mg, 61%); $[\alpha]_D$ −6.4° (*c* 1.9); R_F 0.46 in 7:3 hexane–EtOAc; n.m.r. data: δ_H 5.756 (dt, 1 H, *J* 15.6 and 8.6 Hz, H-5), 5.503 (dd, 1 H, *J* 6.4 and 15.6 Hz, H-4), 4.324 (t, 1 H, *J* 3.7 Hz, H-2'), 4.255 (m, 1 H, H-3), 3.940 (bd, 1 H, *J* 11 Hz, H-1A), 3.827 (m, 1 H, H-2), 3.673 (m, 1 H, H-1B), 2.810 (bs, 1 H, C_1–O*H*), 2.350 (d, 1 H, *J* 4.6 Hz, C_3–O*H*), 2.631 (q, 2 H, *J* 7.0 Hz, H-6A and H-6B), 1.131 (s, 9 H, CMe_3), and 0.880 (t, 6 H, *J* 6.7 Hz, CH_2CH_3); δ_C 134.3 (C-4), 128.6 (C-5), 74.4, 74.2 (C-3 and C-2'), 62.5 (C-1), and 54.6 (C-2); ν_{max} 3400, 1650, 1580, and 1510 cm^{-1}.

Anal. Calc. for $C_{58}H_{101}NO_4Si$: C, 77.02; H, 11.25; N, 1.55. Found: C, 76.92; H, 11.23; N, 1.56.

*(2*S*, 3*R*, 4*E*)-*N*-(2'*R*)-2'-Hydroxytetracosanoylsphingenine* (**18**). — To a solution of compound **16** (51 mg, 56 μmol) in THF (0.5 mL) was added M solution of Bu_4NF in THF (0.5 mL). The mixture was stirred for 10 min at 20°, diluted with $CHCl_3$ (30 mL), washed with water, dried ($MgSO_4$), and evaporated *in vacuo*. Chromatography of the residue on SiO_2 in 19:1 $CHCl_3$–MeOH afforded **18** (34 mg, 91%); m.p. 98–99°, $[\alpha]_D$ +8.7° (*c* 1.1, 9:1 $CHCl_3$–MeOH); R_F 0.45 in 9:1 $CHCl_3$–MeOH; n.m.r. data: δ_H (9:1 $CDCl_3$–CD_3OD), 5.745 (dt, 1 H, *J* 15.3 and 7.2 Hz, H-5), 5.461 (dd, 1 H, *J* 6.7 and 15.3 Hz, H-4), 4.124 (t, 1 H, *J* 6.1 Hz, H-3), 4.031 (dd, 1 H, *J* 3.7 and 8.2 Hz, H-2'), 3.843 (dt, 1 H, *J* 8.9 and 3.7 Hz, H-2), 3.797 (dd, 1 H, *J* 4.9 and 11.3 Hz, H-1A), 3.687 (dd, 1 H, *J* 3.7 and 11.3 Hz, H-1B), and 2.033 (q, 2 H, *J* 7.0 Hz, H-6A and H-6B); δ_C (9:1 $CDCl_3$–CD_3OD), 176.1 (*C*=O), 134.4 (C-4), 129.0 (C-5), 73.4 (C-3), 72.3 (C-2'), 61.7 (C-1), and 54.7 (C-2).

Anal. Calc. for $C_{42}H_{83}NO_4$: C, 75.73; H, 12.56; N, 2.10. Found: C, 76.10; H, 12.63; N, 2.18.

*(2*S*, 3*R*, 4*E*)-*N*-(2'*S*)-2'-Hydroxytetracosanoylsphingenine* (**19**). — Compound **17** (50 mg, 55 μmol) was treated as described for the conversion of compound **16** into compound **18**, to afford **19** (29 mg, 79%); m.p. 100–101°, $[\alpha]_D$ −11.1° (*c* 1.4, 9:1 $CHCl_3$–MeOH); R_F 0.51 in 9:1 $CHCl_3$–MeOH; n.m.r. data: δ_H (9:1 $CDCl_3$–CD_3OD) 5.775 (ddt, 1 H, *J* 1.2, 15.4, and 7.4 Hz, H-5), 5.486 (dd, 1 H, *J* 6.4 and 15.4 Hz, H-4), 4.257 (t, 1 H, *J* 4.9 Hz, H-3), 4.024 (dd, 1 H, *J* 3.4 and 8.3

*These assignments may have to be interchanged.

Hz, H-2'), 2.050 (q, 2 H, *J* 6.8 Hz, H-6A and H-6B), and 0.882 (t, 6 H, *J* 6.8 Hz, CH_2CH_3); δ_C (9:1 $CDCl_3$–CD_3OD) 134.0 (C-4), 128.0 (C-5), 72.7, 71.8 (C-3 and C-2'), 61.4 (C-1), and 54.4 (C-2).

Anal. Calc. for $C_{42}H_{83}NO_4$: C, 75.73; H, 12.56; N, 2.10. Found: C, 75.39; H, 12.14; N. 2.06.

(2S,3R,4E)-N-*(2'R)-2'*-tert-*Butyldiphenylsilyloxytetracosanoyl-1*-O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)sphingenine* (**21**). — To a mixture of compound **20** (73 mg, 150 μmol), compound **16** (90 mg, 100 μmol), and powdered molecular sieves 4A (500 mg) in $CHCl_3$ (2 mL) was added BF_3·ether (18 μL) at −5°. The mixture was stirred for 5 h at −5° and then for 16 h at 20°, and filtered through Celite. The filtrate was successively washed with aq. $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated. Chromatography of the residue on SiO_2 in 200:3 $CHCl_3$–MeOH afforded first **25** (9 mg, 11%), then **21** (21 mg, 33%), and finally, unreacted **16** (43 mg).

Compound **21**: $[\alpha]_D$ +1.6° (*c* 1.1), R_F 0.48 in 50:1 $CHCl_3$–MeOH; n.m.r. data: δ_H 5.779 (dt, 1 H, *J* 14.7 and 6.5 Hz, H-5), 5.474 (dd, 1 H, *J* 14.7 and 6.2 Hz, H-4), 5.376 (d, 1 H, *J* 3.4 Hz, H-4a), 5.184 (dd, 1 H, *J* 8.0 and 10.5 Hz, H-2a), 4.997 (dd, 1 H, *J* 3.4 and 10.5 Hz, H-3a), 4.488 (d, 1 H, *J* 8.1 Hz, H-1a), 2.123 (Ac), 2.047 (Ac), 1.979 (Ac), 1.964 (Ac), and 0.880 (t, 6 H, *J* 6.6 Hz, CH_2CH_3).

Anal. Calc. for $C_{72}H_{119}NO_{13}Si$: C, 70.03; H, 9.71; N, 1.13. Found: C, 70.14; H, 9.72; N, 1.19.

Compound **25**: R_F 0.57 in 50:1 $CHCl_3$–MeOH; n.m.r. data: δ_H (90 MHz) 7.75–7.30 (m, 10 H, aromatic protons), and 2.10–1.90 (m, 26 H, 8 Ac and $=CHCH_2$).

(2S, 3R, 4E)-N-*(2'S)-2'*-tert-*Butyldiphenylsilyloxytetracosanoyl-1*-O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)sphingenine* (**22**). — Compound **17** (75 mg, 83 μmol) was glycosylated with **20** (61 mg, 125 μmol) as described for the conversion of compound **16** into compound **21**, to give **22** (32 mg, 31%) and **26** (38 mg, 29%).

Compound **22**: $[\alpha]_D$ −2.8° (*c* 1.1); R_F 0.56 in 200:3 $CHCl_3$–MeOH; n.m.r. data: δ_H 5.640 (dt, 1 H, *J* 15.4 and 6.6 Hz, H-5), 5.370 (d, 1 H, *J* 3.4 Hz, H-4a), 5.318 (dd, 1 H, *J* 8.3 and 15.4 Hz, H-4), 5.175 (dd, 1 H, *J* 8.0 and 10.5 Hz, H-2a), 5.016 (dd, 1 H, *J* 3.4 and 10.5 Hz, H-3a), 4.483 (d, 1 H, *J* 8.1 Hz, H-1a), 2.075 (Ac), 2.067 (Ac), 2.059 (Ac), 1.987 (Ac), 1.146 (CMe_3), and 0.879 (t, 6 H, *J* 6.6 Hz, CH_2CH_3).

Anal. Calc. for $C_{72}H_{119}NO_{13}Si$: C, 70.03; H, 9.71; N, 1.13. Found: C, 70.14; H, 9.72; N, 1.19.

Compound **26**: R_F 0.50 in 200:3 $CHCl_3$–MeOH; n.m.r. data: δ_H 7.7–7.3 (m, 10 H, 2 Ph), 5.694 (dt, 1 H, *J* 15.4 and 6.6 Hz, H-5), 5.390 (d, 1 H, *J* 3.0 Hz, H-4a*), 5.355 (d, 1 H, *J* 3.6 Hz, H-4b*), 5.191 (dd, 1 H, *J* 8.0 and 10.0 Hz, H-2a*), 5.186 (dd, 1 H, *J* 8.0 and 10.0 Hz, H-2b*), 5.007 (dd, 1 H, *J* 3.7 and 10.5 Hz, H-3a*), 4.998 (dd, 1 H, *J* 3.7 and 10.5 Hz, H-3b*), 4.519 (d, 1 H, *J* 8.1 Hz, H-1a*), 4.512 (d, 1 H, *J* 7.8 Hz, H-1b*), 2.127, 2.059, 2.054, 2.051, 2.046, 2.040, 1.987, 1.982 (8 s, 24 H, 8 Ac), and 0.879 (t, 6 H, *J* 6.6 Hz, 2 CH_2CH_3).

(2S, 3R, 4E)-1-O-β-D-Galactopyranosyl-N-(2'R)-2'-hydroxytetracosanoylsphingenine (**23**) *and (2S, 3R, 4E)-1-O-β-D-galactopyranosyl-N-(2'S)-2'-hydroxytetracosanoylsphingenine* (**24**). — To a solution of compound **21** (16 mg, 13 μmol) in THF (1.0 mL) was added M solution of Bu_4NF in THF (14 μL). The mixture was stirred for 30 min at 20°, diluted with $CHCl_3$, washed with water, dried ($MgSO_4$), and evaporated *in vacuo*. To a solution of the residue in MeOH (1.0 mL) was added M NaOMe in MeOH (63 μL). The mixture was stirred for 4 h at 20°, made neutral with Amberlyst 15, and filtered. Evaporation of the filtrate and precipitation of the residue from MeOH afforded **23** (6.5 mg, 62%); $[\alpha]_D$ +8.5° (*c* 0.4, 1:1 $CHCl_3$–MeOH); R_F 0.37 in 17:3 $CHCl_3$–MeOH; n.m.r. data: δ_H (49:1 Me_2SO-d_6–D_2O, 65°) 5.587 (dt, 1 H, *J* 17.0 and 7.3 Hz, H-5), 5.336 (dd, 1 H, *J* 7.3 and 16.7 Hz, H-4), 4.095 (d, 1 H, *J* 7.3 Hz, H-1a), 4.005 (t, 1 H, *J* 7.0 Hz, H-3), 3.910 (dd, 1 H, *J* 5.9 and 10.3 Hz, H-1A), 3.83–3.78 (m, 2 H, H-2,2'), 3.652 (d, 1 H, *J* 1.5 Hz, H-4a), 3.555 (dd, 1 H, *J* 5.9 and 11.0 Hz, H-6aA), 3.552 (dd, 1 H, *J* 5.9 and 9.1 Hz, H-1B), 3.500 (dd, 1 H, *J* 5.9 and 11.7 Hz, H-6aB), and 0.854 (t, 6 H, *J* 6.6 Hz, CH_2CH_3); δ_H lit.[5] (Me_2SO-d_6, 65°), 5.583 (H-5), 5.383 (H-4), 4.093 (H-1a), 4.001 (H-3), 3.909 (H-1A), and 3.651 (H-4a).

To a solution of **22** (22 mg, 18 μmol) in THF (1.0 mL) was added M solution of Bu_4NF in THF (20 μL), and the mixture was stirred for 30 min at 20°, diluted with $CHCl_3$, washed with water, dried ($MgSO_4$), and evaporated *in vacuo*. To a solution of the residue in 1:1 MeOH–THF (2 mL) was added 2.14M NaOMe in MeOH (38 μL), and the mixture was stirred for 2 h at 20°. Neutralization with Amberlyst 15, and evaporation of the filtrate, afforded a residual solid which was washed with CH_2Cl_2 to give **2** (11.5 mg, 78%); $[\alpha]_D$ −25.2° (*c* 0.48, 1:1 $CHCl_3$–MeOH); R_F 0.48 in 17:3 $CHCl_3$–MeOH; n.m.r. data: δ_H (49:1 Me_2SO-d_6–D_2O, 65°) 5.712 (dt, 1 H, *J* 5.9 and 15.4 Hz, H-5), 5.321 (dd, 1 H, *J* 7.8 and 15.4 Hz, H-4), 4.209 (t, 1 H, *J* 7.4 Hz, H-3), 4.085 (d, 1 H, *J* 7.3 Hz, H-1a), 3.82–3.77 (m, 2 H, H-2,2'), 3.640 (d, 1 H, *J* 2.7 Hz, H-4a), 3.596 (dd, 1 H, *J* 5.3 and 11.0 Hz, H-1A), 3.544 (dd, 1 H, *J* 5.9 and 11.0 Hz, H-6aA), 3.505 (dd, 1 H, *J* 5.7 and 10.7 Hz, H-6aB), 3.440 (dd, 1 H, *J* 4.8 and 11.0 Hz, H-1B), 1.980 (q, 2 H, *J* 6.6 Hz, H-6A,6B), and 0.855 (t, 6 H, *J* 6.6 Hz, CH_2CH_3).

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