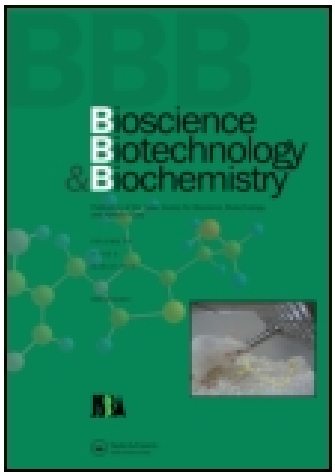

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Stereoselective Total Synthesis of Tri- and Tetrahexoside Wheat Flour Ceramide

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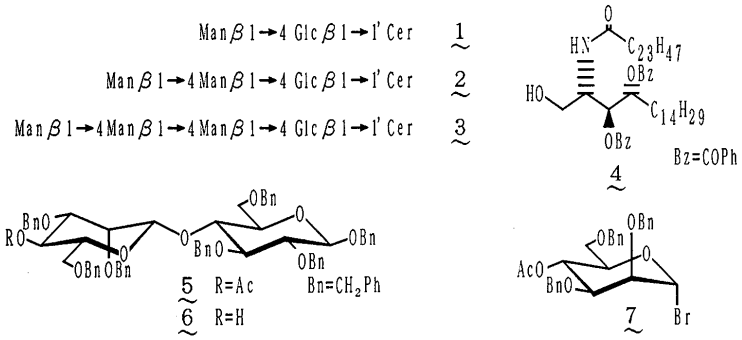
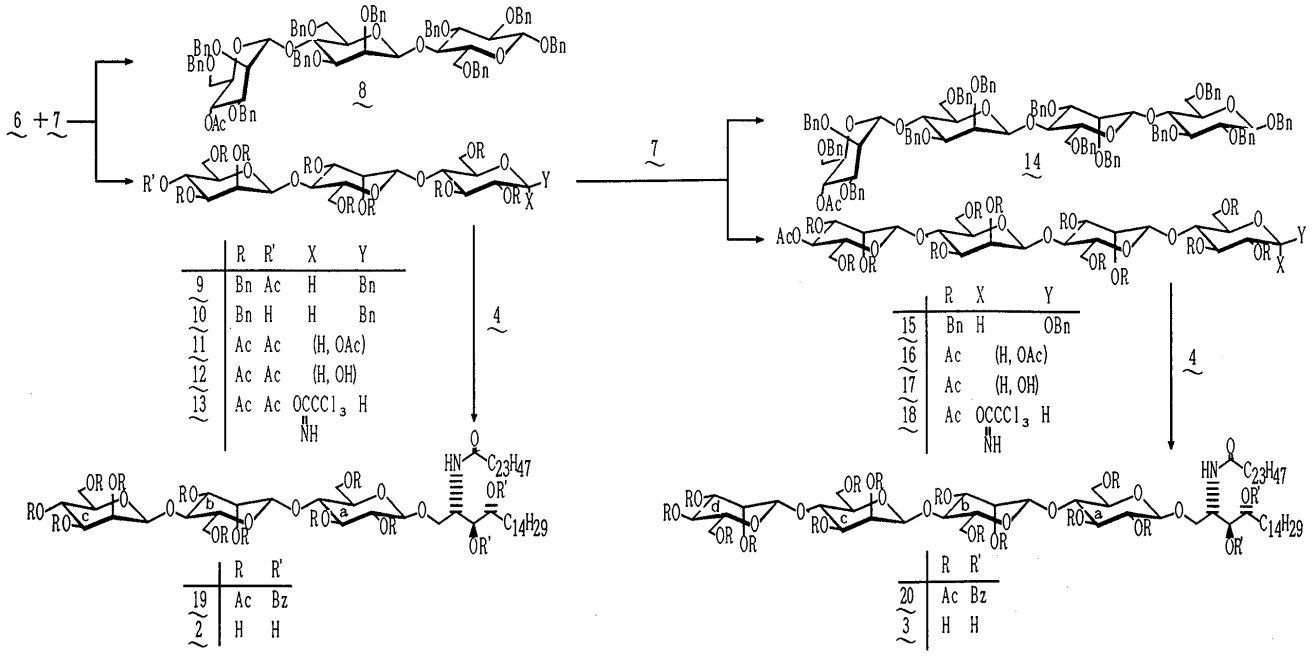
The wheat glycosphingolipids, *O*-(β-D-mannopyranosyl-[(1→4)-*O*-(β-D-mannopyranosyl)]$_n$-*O*-(β-D-glucopyranosyl)-(1→1)-(2*S*,3*S*,4*R*)-4-hydroxy-*N*-tetracosanoylsphinganine (n=1 and 2), were stereoselectively synthesized. Silver silicate promoted glycosylation when 4-*O*-acetyl-2,3,6-tri-*O*-benzyl-α-D-mannopyranosyl bromide was used for elongating the glycan chains, which were latter coupled with the ceramide derivative by the trichloroacetimidate method.

Since our successful synthesis[1] of the ceramide, dihexoside **1**, a prototype molecule for the D-mannose-containing wheat flour glycosphinogolipids,[2–6] our attention has been directed toward the development of a synthetic route to the multi-mannosylated molecules of this class. It is noteworthy that all the hexose residues (D-glucose and D-mannose) reported for wheat flour, wheat grain, and rice grain glycosphingolipids appear to be linked uniformly in β-1→4 fashion with each other, while the structures of the component ceramides are diverse in terms of their composition of fatty acids as well as the long-chain bases (sphinganine derivatives).[2–8]

The preferential formation of a β-D-mannopyranosidic linkage has rarely been achieved, in spite of the accumu-



Scheme 1.



Scheme 2.

† To whom inquiries should be addressed.

Abbreviations: DMF, *N*,*N*-dimethylformamide; DBU, 1,8-diazabicyclo[5,4,0]-7-undecene; $BF_3 \cdot Et_2O$, boron trifluoride etherate; THF, tetrahydrofuran; DMAP, 4-dimethylaminopyridine.

lation of new techniques for glycosylation. The reaction with α-bromides as glycosyl donors in the presence of a heterogeneous promotor such as silver silicate has been the only effective way so far to achieve direct mannosylation in favor of β-D-linkage formation.[9,10] As described previously for the synthesis of **1**, we observed fairly good

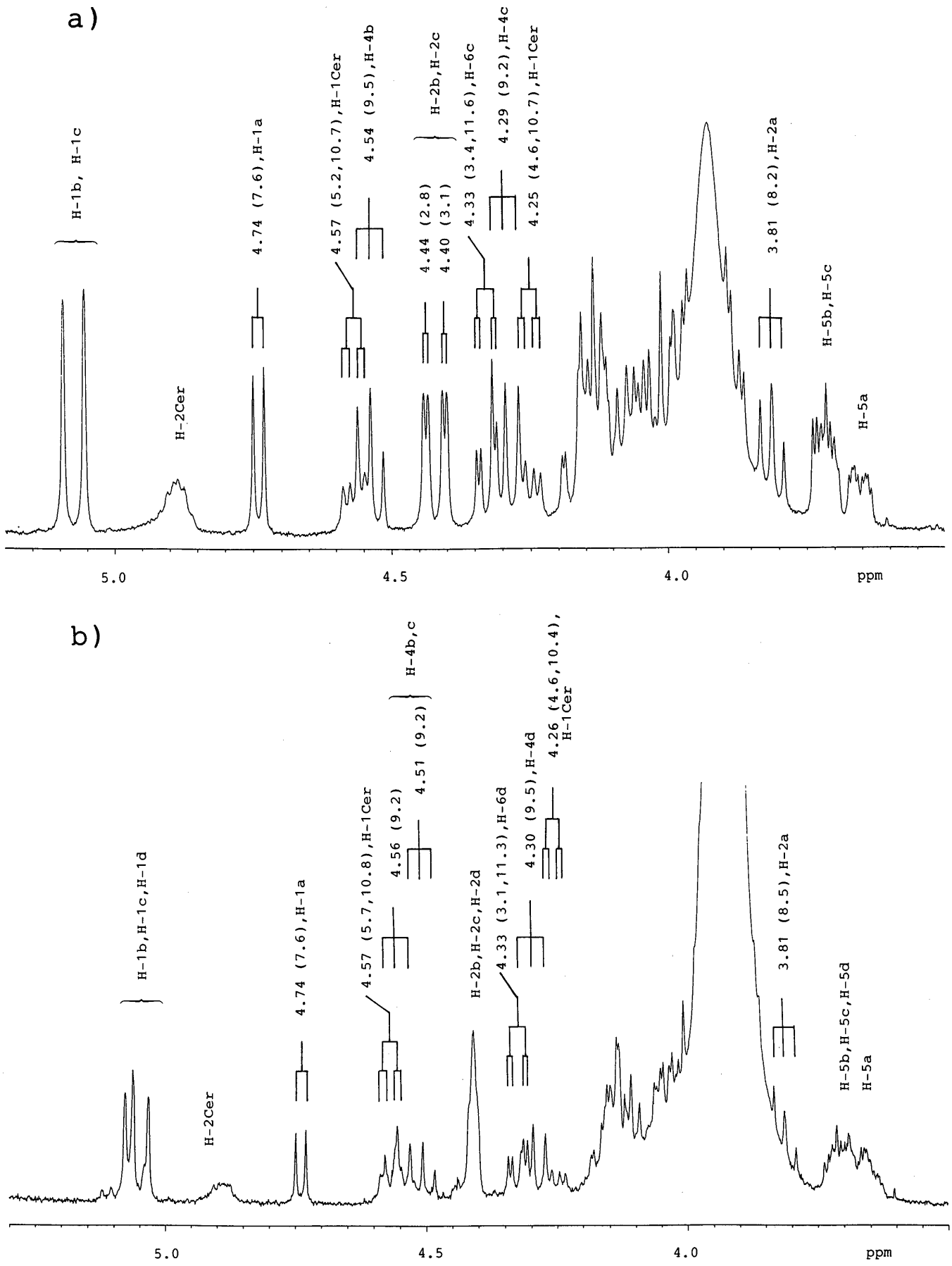


Fig. 400 MHz ^{1}H-NMR Data for Synthetic Ceramide Tri- and Tetrahexosides.

The spectra were recorded in pyridine-d_5 for each sample after exchanging several times with pyridine-d_5–D_2O. The values in parentheses are $^3J_{HH}$ values expressed in Hz. a) compound **2** at 90°C; b) compound **3** at 90°C.

selectivity ($\beta/\alpha = 1.9$) in the formation of the disaccharide, β-D-Man-(1→4)-D-Glc, using silver silicate. The same promotor would also be useful to produce β-D-Man-(1→4)-D-Man linkages present in the highly glycosylated plant sphingolipids.

This paper details the synthesis of ceramide tri- (**2**) and tetrahexosides (**3**) containing two and three D-mannose residues, respectively.[11]

Results and Discussion

Disaccharide **5** is a suitably protected key intermediate for the synthesis of **1**, **2**, and **3**. In the previous work[1] on ceramide dihexoside **1**, we converted **5** into hepta-*O*-acetylated dihexosyl trichloroacetimidate and condensed it with ceramide derivative **4**.[12]

For the synthesis of **2** and **3**, elongation of the saccharide chains with **5** was necessary. Deacetylation of **5** gave disaccharide glycosyl acceptor **6**, which was allowed to react with 4-*O*-acetyl-2,3,6-tri-*O*-benzyl-α-D-mannopyranosyl bromide (**7**)[1] in the presence of silver silicate and 4A molecular sieves in dichloromethane to afford a mixture of trisaccharides **8** and **9** in 15 and 54% yields, respectively. The structures of **8** and **9** were assigned from ^{1}H- and ^{13}C-NMR data. Minor product **8** exhibited the characteristic signals for an α-D configuration, a signal for H-1c at δ 5.32 ppm with a coupling constant of 1.7 Hz, and a signal for C-1c at δ 99.9 ppm with a $^1J_{CH}$ value of 170.9 Hz, while isomer **9** had three signals for anomeric carbons with β-D configuration at δ 101.0 ppm ($^1J_{CH}$ 155.0 Hz), 101.3 ppm ($^1J_{CH}$ 155.0 Hz) and 102.5 ppm ($^1J_{CH}$ 157.5 Hz).[13] For preparation of the tetrasaccharide, β-isomer **9** was deacetylated to **10** and then glycosylated with **7** under the same condition as those already mentioned to produce a mixture of **14** and **15** in a 69% yield. The reaction, however, did not proceed in favor of β-glycoside formation, and desired **15** was isolated in a 17% yield. The stereochemistry of compounds **14** and **15** was again elucidated by ^{13}C-NMR data.

With the tri- and tetrasaccharide fragments at hand, their conversion to the glycosyl donors for coupling with **4** was next examined.

Hydrogenolysis of **9** with 10% Pd–C in MeOH–THF–H_2O–HCO_2H and subsequent acetylation afforded a mixture of α and β anomers of peracetate **11** in a ratio of 1.2:1 and a 85% overall yield. The mixture **11** was chemoselectively deacetylated with hydrazinium acetate[14] in *N*, *N*-dimethylformamide (DMF) to give a 66% yield of hemiacetal **12**, which, after treating with trichloroacetonitrile and 1,8-diazabicyclo[5,4,0]-7-undecene (DBU),[15] was converted to α-D-trichloroacetimidate **13** in a 95% yield.

Similarly, tetrasaccharide **15** was transformed into corresponding trichloroacetimidate **18** by sequential hydrogenolysis, acetylation, selective deacetylation, and trichloroacetimidation in a 49% overall yield.

Crucial glycosylation[16] of ceramide derivative **4** with **13** and **18** was carried out by using boron trifluoride etherate ($BF_3 \cdot Et_2O$) as the promotor in dichloromethane, and fully protected ceramide tri- and tetrahexosides, **19** and **20** were stereoselectively produced in 39 and 20% yields, respectively. The ^{13}C-NMR spectrum of **19** contained three signals for anomeric carbons of β-D configuration at δ 97.5 ppm ($^1J_{CH}$ 159.9 Hz), 97.8 ppm ($^1J_{CH}$ 159.9 Hz), and 100.3 ppm ($^1J_{CH}$ 162.4 Hz). The stereochemistry of the newly formed glycosidic linkage was also assignable from the ^{1}H-NMR data, in which an anomeric proton (H-1a) signal was split with a coupling constant of 7.8 Hz at δ 4.37 ppm. Analogously, the ^{1}H-NMR spectrum of **20** containing a doublet signal for H-1a at δ 4.36 ppm with *J* 7.8 Hz proved the β-D configuration of the D-glucose residue.

Compounds **19** and **20** thus obtained were deprotected with sodium methoxide ($NaOCH_3$) in methanol to complete the total synthesis of glycosphingolipids **2** and **3**. The structures of the synthetic compounds were evident from the unambiguous synthetic route and was confirmed by the ^{1}H-NMR data depicted in Fig.

In conclusion, we succeeded in the first total synthesis of unique D-mannose-containing glycosphingolipids **2** and **3** which represent the tri- and tetrahexosyl members of the wheat flour glycosphingolipid family.

Experimental

General. Optical rotation values ($[\alpha]_D$) were measured with a Perkin-Elmer Model 241 MC polarimeter as a solution in $CHCl_3$ at 25°C, unless noted otherwise. Column chromatography was carried out in columns of silica gel (Merck, 70–230 mesh), and flash chromatography was done in columns of Wako gel C-300 (200–300 mesh). TLC and high-performance TLC were conducted on $60F_{254}$ silica gel (Merck, Darmstadt). 4A and AW300 molecular sieves were purchased from Nakarai Chemicals and Union Showa, respectively. NMR spectra were recorded with a JNM-GX400, JNM-FX100FT, or JNM-FX90Q NMR spectrometer as a solution in $CDCl_3$, unless noted otherwise. The values for δ_C and δ_H are expressed in ppm downwards from the signal for internal Me_4Si.

Benzyl O-(2,3,6-tri-O-benzyl-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**6**). To a solution of compound **5**[1] (708 mg, 697 μmol) in MeOH-tetrahydrofuran (THF; 1:1, 20 ml) was added 0.1 N $NaOCH_3$ in MeOH (70 μl), and the mixture was stirred for 16 h at room temperature. The mixture was neutralized with Amberlist 15 (H^+ form) and filtered through Celite, before the filtrate was evaporated *in vacuo*. Chromatography of the residue (SiO_2, AcOEt–*n*-hexane = 1:3) gave compound **6** (558 mg, 82%), $[\alpha]_D$ −41.5° ($c = 0.50$), R_f 0.17 developed with AcOEt–*n*-hexane = 3:7. NMR data: δ_H 4.50 (1H, d, $J = 7.6$ Hz, H-1a), 4.49 (1H, br. s, H-1b), 3.95 (1H, t, $J = 9.3$ Hz, H-4a), 3.95 (1H, t, $J = 9.3$ Hz, H-4b), 3.71 (1H, d, $J = 2.4$ Hz, H-2b), 3.22 (1H, dt, $J = 5.4$ and 9.3 Hz, H-5b), and 3.13 (1H, dd, $J = 2.7$ and 9.3 Hz, H-3b): δ_C 102.5 ($J = 156.0$ Hz, C-1a) and 100.5 ($J = 156.3$ Hz, C-1b).

Anal. Found: C, 75.29; H, 6.51%. Calcd. for $C_{61}H_{64}O_{11}$: C, 75.29; H, 6.63%.

Benzyl O-(4-O-acetyl-2,3,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→4)-O-(2,3,6-tri-O-benzyl-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**8**) *and benzyl O-(4-O-acetyl-2,3,6-tri-O-benzyl-β-D-mannopyranosyl)-(1→4)-O-(2,3,6-tri-O-benzyl-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside* (**9**). To a mixture of compound **6** (584 mg, 600 μmol), silver silicate (1 g) and 4A molecular sieves (1 g) in dichloromethane (2 ml) was added dropwise a solution of bromide **7**, which had been freshly prepared from *p*-nitrophenyl 4-*O*-acetyl-2,3,6-tri-*O*-benzyl-D-mannopyranoside (770 mg, 1.20 mmol) and HBr,[1] in dichloromethane (1.5 ml) at −50°C. The mixture was stirred at −50°C up to room temperature for 16 h. The mixture was diluted with AcOEt (100 ml) and filtered through Celite. The filrate was successively washed with aq. 5% $NaHCO_3$, water, and satd. brine, dried ($MgSO_4$), and evaporated *in vacuo*. Purification of the product was performed by medium-pressure chromatography with LICHROPREP Si60 size C in AcOEt–toluene = 1:10 to give compound **8** (131 mg, 15%) and compound **9** (466 mg, 54%).

Compound **8**. $[\alpha]_D$ −18.5° ($c = 0.54$), R_f 0.22 developed with AcOEt–toluene = 1:10. NMR data: δ_H 5.36 (1H, t, $J = 9.5$ Hz, H-4c), 5.32 (1H, d, $J = 1.7$ Hz, H-1c), 4.50 (1H, d, $J = 7.8$ Hz, H-1a), 4.48 (1H, br. s, H-1b), 3.99 (1H, t, $J = 9.0$ Hz, H-4a), 3.96 (1H, t, $J = 8.8$ Hz, H-4b), 3.27–3.32

(1H, m, H-5b), 3.17 (1H, dd, J=2.9 and 9.5 Hz, H-3b), and 1.89 (3H, s, OAc); δ_C 169.7 (CO), 102.5 (J=157.5 Hz, C-1a), 100.2 (J=155.0 Hz, C-1b), 99.9 (J=170.9 Hz, C-1c), and 20.9 ($OCOCH_3$).

Anal. Found: C, 73.66; H, 6.42%. Calcd. for $C_{90}H_{94}O_{17}\cdot H_2O$: C, 73.75; H, 6.60%.

Compound **9**. $[\alpha]_D$ −38.0° (c=0.63), R_f 0.11 developed with AcOEt–toluene=1:10. NMR data: δ_H 5.23 (1H, t, J=9.8 Hz, H-4c), 4.54 (1H, br. s, H-1c), 4.51 (1H, br. s, H-1b), 4.48 (1H, d, J=7.8 Hz, H-1a), 4.20 (1H, t, J=9.4 Hz, H-4b), 3.91 (1H, t, J=9.3 Hz, H-4a), 3.74 (1H, d, J=2.7 Hz, H-2c), and 3.60 (1H, t, J=9.0 Hz, H-3a); δ_C 169.9 (CO), 102.5 (J=157.5 Hz, C-1a), 101.3, 101.0 (J=155.0 Hz, C-1b and C-1c), and 21.0 ($OCOCH_3$).

Anal. Found: C, 73.77; H, 6.47%. Calcd. for $C_{90}H_{94}O_{17}\cdot H_2O$: C, 73.75; H, 6.60%.

Benzyl O-(2,3,6-tri-O-benzyl-β-D-mannopyranosyl)-(1→4)-O-(2,3,6-tri-O-benzyl-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**10**). Compound **9** (230 mg, 159 μmol) was treated in the same way as that described for compound **6**. Chromatography of the product in AcOEt–*n*-hexane=3:7 gave compound **10** (214 mg, 95%), $[\alpha]_D$ −47.5° (c=0.59), R_f 0.19 developed with AcOEt–*n*-hexane=3:7. NMR data: δ_H 4.53 (2H, br. s, H-1b and H-1c), 4.49 (1H, d, J=7.8 Hz, H-1a), 4.18 (1H, t, J=9.0 Hz, H-4b), 3.94 (1H, t, J=9.3 Hz, H-4a), 3.91 (1H, t, J=9.3 Hz, H-4c), 3.38–3.43 (1H, m, H-5a), 3.32 (1H, dd, J=2.9 and 9.0 Hz, H-3b), 3.24–3.29 (1H, m, H-5b), 3.18–3.23 (1H, m, H-5c) and 3.13 (1H, dd, J=2.7 and 9.3 Hz, H-3c); δ_C 102.4 (J=158.7 Hz, C-1a), 101.5, and 100.8 (J=155.0 Hz, C-1b and C-1c).

Anal. Found: C, 72.95; H, 6.38%. Calcd. for $C_{88}H_{92}O_{17}\cdot 3/2H_2O$: C, 72.96; H, 6.61%.

O-(2,3,4,6-Tetra-O-acetyl-β-D-mannopyranosyl)-(1→4)-O-(2,3,6-tri-O-acetyl-β-D-mannopyranosyl)-(1→4)-1,2,3,6-tetra-O-acetyl-D-glucopyranose (**11**). To a mixture of compound **9** (158 mg, 109 μmol) and 10% Pd–C (150 mg) in H_2O–MeOH–THF (1:4:4, 10 ml) was added HCO_2H (0.5 ml), before stirring for 1 h at 50°C under an atmosphere of N_2. The mixture was filtered through Celite, and the filtrate was evaporated *in vacuo*. To a solution of the residue in pyridine (5 ml) was added a catalytic amount of 4-dimethylaminopyridine (DMAP) and Ac_2O (5 ml), and the mixture was stirred for 16 h at room temperature. The mixture was evaporated *in vacuo*, and chromatography of the residue (SiO_2, THF–*n*-hexane=2:3) gave compound **11** (90 mg, 85%), R_f 0.41 developed with *n*-hexane–THF=1:1. NMR data: δ_C 97.7, 97.4 (J=159.9 Hz, C-1b and C-1c), 91.5 (J=168.4 Hz, C-1aβ), and 88.8 (J=177.0 Hz, C-1aα).

Anal. Found: C, 52.39; H, 6.03%. Calcd. for $C_{40}H_{54}O_{27}\cdot 3/4C_6H_5CH_3$: C, 52.46; H, 5.84%.

O-(2,3,4,6-Tetra-O-acetyl-β-D-mannopyranosyl)-(1→4)-O-(2,3,6-tri-O-acetyl-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-D-glucopyranose (**12**). A mixture of compound **11** (106 mg, 110 μmol) and hydrazinium acetate[14] (12 mg, 130 μmol) in DMF (1 ml) was stirred for 1 h at room temperature. The mixture was diluted with AcOEt (50 ml), successively washed with water and satd. brine, dried ($MgSO_4$), and evaporated *in vacuo*. Chromatography of the residue (SiO_2, THF–*n*-hexane=9:11) gave compound **12** (67 mg, 66%), R_f 0.23 developed with *n*-hexane–THF=1:1. NMR data: δ_C 97.8, 97.2 (J=159.9 Hz, C-1b and C-1c), 95.3 (J=167.2 Hz, C-1aβ), and 90.0 (J=173.3 Hz, C-1aα).

Anal. Found: C, 50.41; H, 5.81%. Calcd. for $C_{38}H_{52}O_{26}\cdot 1/2C_4H_8O$: C, 50.00; H, 5.87%.

O-(2,3,4,6-Tetra-O-acetyl-β-D-mannopyranosyl)-(1→4)-O-(2,3,6-tri-O-acetyl-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-α-D-glucopyranosyl trichloroacetimidate (**13**). To a mixture of compound **12** (72 mg, 78 μmol) and trichloroacetonitrile (155 μl, 1.54 mmol) in dichloromethane (1 ml) was added DBU (12 μl, 80 μmol) at 0°C, and the mixture was stirred for 2 h at 0°C. The mixture was chromatographed (SiO_2, THF–*n*-hexane=9:11) to give compound **13** (79 mg, 95%), R_f 0.39 developed with *n*-hexane–THF=1:1. NMR data: δ_H 8.65 (1H, s, NH), 6.49 (1H, d, J=3.7 Hz, H-1a), 5.55 (1H, t, J=9.8 Hz, H-4c), 5.41 (1H, d, J=2.4 Hz, H-2c), 5.35 (1H, d, J=2.7 Hz, H-2b), 5.20 (1H, t, J=9.8 Hz, H-3a), 5.11 (1H, dd, J=3.4 and 9.8 Hz, H-3b), 4.69, 4.71 (1H×2, br. s×2, H-1b and H-1c), 3.93 (1H, t, J=9.3 Hz, H-4a), 3.90 (1H, t, J=9.5 Hz, H-4b), 2.17, 2.16, 2.13, 2.11, 2.09, 2.08, 2.01, and 1.99 (3H×8, s×8, OAc×8), and 2.05 (6H, s, OAc×2).

Benzyl O-(4-O-acetyl-2,3,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→4)-O-(2,3,6-tri-O-benzyl-β-D-mannopyranosyl)-(1→4)-O-(2,3,6-tri-O-benzyl-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**14**) *and benzyl O-(4-O-acetyl-2,3,6-tri-O-benzyl-β-D-mannopyranosyl)-(1→4)-O-(2,3,6-tri-O-benzyl-β-D-mannopyranosyl)-(1→4)-O-(2,3,6-tri-O-benzyl-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside* (**15**). To a mixture of compound **10** (223 mg, 157 μmol), silver silicate (500 mg) and 4A molecular sieves (500 mg) in dichloromethane (1.5 ml) was added dropwise crude bromide **7**, which had been freshly prepared from the corresponding *p*-nitrobenzoate (305 mg, 476 μmol), as described for compound **9**, in dichloromethane (1 ml) at −50°C. The mixture was stirred at −50°C up to room temperature for 16 h, before being diluted with AcOEt (50 ml) and filtered through Celite. The filtrate was successively washed with aq. 5% $NaHCO_3$, water and satd. brine, dried ($MgSO_4$), and evaporated *in vacuo*. Chromatography of the residue (SiO_2 AcOEt–toluene=1:9) gave compound **14** (154 mg, 52%), compound **15** (50 mg, 17%), and unconverted compound **10** (38 mg).

Compound **14**. $[\alpha]_D$ −44.2° (c=0.66), R_f 0.17 developed with AcOEt–toluene=1:9. NMR data: δ_H 5.34 (1H, t, J=9.5 Hz, H-4d), and 5.31 (1H, d, J=1.7 Hz, H-1d); δ_C 169.7 (C=O), 99.6 (J=170.9 Hz, C-1d), 97.3 (J=159.9 Hz, C-1a, C-1b, and C-1c), and 20.6 (OAc).

Anal. Found: C, 72.61; H, 6.33%. Calcd. for $C_{117}H_{122}O_{22}\cdot 3H_2O$: C, 72.65; H, 6.67%.

Compound **15**. $[\alpha]_D$ +2.9° (c=0.53), R_f 0.08 developed with AcOEt–toluene=1:9. NMR data: δ_H 5.23 (1H, t, J=9.8 Hz, H-4d), 4.49, 4.50 4.55 (1H×3, br. s×3, H-1b, H-1c, and H-1d), 4.49 (1H, d, J=7.6 Hz, H-1a), 3.92 (1H, t, J=9.5 Hz, H-4a), 3.68, 3.69, 3.75 (1H×3, d×3, J=3.2 Hz, H-2b, H-2c, and H-2d), 3.62 (1H, t, J=8.8 Hz, H-3a), and 1.85 (3H, s, OAc); δ_C 169.9 (CO), 102.5 (J=161.1 Hz, C-1a), 101.6, 101.2 (J=156.3 Hz, C-1b, C-1c or C-1d), 100.9 (J=159.9 Hz, C-1b, C-1c or C-1d), and 21.0 ($OCOCH_3$).

Anal. Found: C, 70.37; H, 6.55%. Calcd. for $C_{117}H_{122}O_{22}\cdot 6H_2O$: C, 70.68; H, 6.79%.

O-(2,3,4,6-Tetra-O-acetyl-β-D-mannopyranosyl)-(1→4)-O-(2,3,6-tri-O-acetyl-β-D-mannopyranosyl)-(1→4)-O-(2;3,6-tri-O-acetyl-β-D-mannopyranosyl)-(1→4)-1,2,3,6-tetra-O-acetyl-D-glucopyranose (**16**). Compound **15** (46 mg, 24 μmol) was hydrogenated and acetylated as described for compound **11** to give compound **16** (24 mg, 79%), R_f 0.32 developed with *n*-hexane–THF=1:1. NMR data: δ_C 97.8, 97.3, and 97.1 (J=158.7 Hz, C-1b, C-1c, and C-1d), 91.5 (J=170.0 Hz, C-1aβ), and 88.8 (J=177.0 Hz, C-1aα).

Anal. Found: C, 51.88; H, 5.73%. Calcd. for $C_{52}H_{70}O_{35}\cdot 2C_4H_8O$: C, 51.50; H, 6.20%.

O-(2,3,4,6-Tetra-O-acetyl-β-D-mannopyranosyl)-(1→4)-O-(2,3,6-tri-O-Acetyl-β-D-mannopyranosyl)-(1→4)-O-(2,3,6-tri-O-acetyl-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-D-glucopyranose (**17**). Compound **16** (24 mg, 19 μmol) was treated with hydrazinium acetate (2 mg, 22 μmol) as described for compound **12**. Chromatography of the product in *n*-hexane–THF=1:1 gave compound **17** (15 mg, 65%), R_f 0.16 developed with *n*-hexane–THF=1:1.

Anal. Found: C, 54.01; H, 6.41%. Calcd. for $C_{50}H_{68}O_{34}\cdot 2C_6H_{14}$: C, 53.75; H, 6.98%.

O-(2,3,4,6-Tetra-O-acetyl-β-D-mannopyranosyl)-(1→4)-O-(2,3,6-tri-O-acetyl-β-D-mannopyranosyl)-(1→4)-O-(2,3,6-tri-O-acetyl-β-D-mannopyranosyl)-(1→4)-2,3-6-tri-O-acetyl-α-D-glucopyranosyl trichloroacetimidate (**18**). Compound **17** (14 mg, 12 μmol) was transformed, as described for compound **13**, into compound **18** (15 mg, 96%), R_f 0.27 developed with *n*-hexane–THF=1:1. NMR data: δ_H 8.65 (1H, s, NH), 6.49 (1H, d, J=3.7 Hz, H-1a), 5.54 (1H, t, J=9.8 Hz, H-4d), 5.34, 5.35, 5.41 (1H×3, d×3, J=3.4 Hz, H-2b, H-2c, and H-2d), 5.21 (1H, t, J=9.8 Hz, H-3a), 4.72 (1H, br. s, H-1b, or H-1c, or H-1d), 4.67 (2H, br. s, H-1b, or H-1c, or H-1b), 3.52–3.70 (3H, m, H-5b, H-5c, and H-5d), 2.16, 2.15, 2.13, 2.11, 2.11, 2.09, 2.07, 2.02, 2.00, and 1.99 (3H×10, s×10, OAc×10), and 2.05 (9H, s, OAc×3).

O-(2,3,4,6-Tetra-O-acetyl-β-D-mannopyranosyl)-(1→4)-O-(2,3,6-tri-O-acetyl-β-D-mannopyranosyl)-(1→4)-O-(2,3,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→1)-(2S,3S,4R)-3,4-di-O-benzoyl-N-tetracosanoylsphinganine (**19**). To a mixture of compound **13** (79 mg, 74 μmol), compound **4** (77 mg, 88 μmol) and AW300 molecular sieves (300 mg) in

dichloromethane (0.7 ml) was added 10% $BF_3 \cdot Et_2O$ in dichloromethane (208 μl, 166 μmol) under an atmosphere of Ar, and the mixture was stirred for 16 h at room temperature. The mixture was diluted with AcOEt (50 ml) and filtered through Celite. The filtrate was successively washed with aq. 5% $NaHCO_3$, water, and satd. brine, dried ($MgSO_4$), and evaporated *in vacuo*. Chromatography of the residue (SiO_2, THF–*n*-hexane = 7 : 13) gave compound **19** (52 mg, 39%) and unconverted compound **4** (49 mg), $[\alpha]_D$ −6.6° (c = 1.1), R_f 0.27 developed with THF–*n*-hexane = 2 : 3. NMR data: δ_H 7.40–8.02 (10H, m, aromatic-H), 6.22 (1H, d, J = 9.0 Hz, NH), 5.57 (1H, dd, J = 3.4 and 8.6 Hz, H-3Cer), 5.40 (1H, d, J = 2.5 Hz, H-2c), 5.34 (1H, dt, J = 3.5 and 9.5 Hz, H-4Cer), 5.29 (1H, d, J = 3.0 Hz, H-2b), 5.20 (1H, t, J = 10.0 Hz, H-4c), 5.08 (1H, t, J = 9.5 Hz, H-3a), 5.05 (1H, dd, J = 3.2 and 9.8 Hz, H-3b), 5.00 (1H, dd, J = 3, 4 and 10.0 Hz, H-3c), 4.80 (1H, dd, J = 7.8 and 9.5 Hz, H-2a), 4.69 (1H, br. s, H-1c), 4.57 (1H, ddt, J = 3.7, 8.5 and 12.0 Hz, H-2Cer), 4.54 (1H, br. s, H-1b), 4.37 (1H, d, J = 7.8 Hz, H-1a), 4.29 (1H, dd, J = 6.1 and 12.7 Hz, H-6c), 4.27 (1H, dd, J = 3.7 and 7.5 Hz, H-6b), 4.13 (1H, dd, J = 2.2 and 12.4 Hz, H-6a), 3.72 (1H, t, J = 9.5 Hz, H-4a), 3.68 (1H, dd, J = 3.9 and 10.6 Hz, H′-1Cer), 3.58–3.63 (1H, m, H-5c), 3.50–3.55 (1H, m, H-5b), 3.44–3.48 (1H, m, H-5a), 2.16, 2.12, 2.11, 2.08, 2.05, 2.03, 2.02, 1.99, 1.97, and 1.95 (3H × 10, s × 10, OAc × 10), 0.88 (3H, t, J = 7.1 Hz, $CH_2C\underline{H}_3$), and 0.87 (3H, t, J = 7.1 Hz, $CH_2C\underline{H}_3$); δ_C 100.3 (J = 162.4 Hz, C-1a), 97.8, and 97.5 (J = 159.9 Hz, C-1b and C-1c).

Anal. Found: C, 62.47; H, 7.80; N, 0.78%. Calcd. for $C_{94}H_{143}NO_{31} \cdot H_2O$: C, 62.68; H, 8.11; N, 0.78%.

O-(β-D-Mannopyranosyl)-(1→4)-O-(β-D-mannopyranosyl)-(1→4)-O-(β-D-glucopyranosyl)-(1→1)-(2S,3S,4R)-4-hydroxy-N-tetracosanoylsphinganine (**2**). To a solution of compound **19** (45 mg, 25 μmol) in MeOH–THF (1 : 1, 2 ml) was added dropwise 0.5*N* $NaOCH_3$ in MeOH (45 μl), and the mixture was stirred for 16 h at room temperature. The mixture was neutralized with Amberlist 15 (H^+ form) and filtered through Celite. The filtrate was evaporated *in vacuo*, and chromatography of the residue (Sephadex LH-20, pyridine) gave compound **2** (27 mg, 94%), $[\alpha]_D$ −15.8° (c = 0.54, in pyridine), R_f 0.45 developed with H_2O–MeOH–$CHCl_3$ = 2.3 : 15 : 30. NMR data: δ_H (pyridine-d_5–D_2O, 90°C) 5.09, 5.06 (1H × 2, br. s × 2, H-1b and H-1c), 4.88 (1H, m, H-2Cer), 4.74 (1H, d, J = 7.6 Hz, H-1a), 4.57 (1H, dd, J = 5.2 and 10.7 Hz, H-1Cer), 4.54 (1H, t, J = 9.5 Hz, H-4b), 4.44 (1H, d, J = 2.8 Hz, H-2b, or H-2c), 4.40 (1H, d, J = 3.1 Hz, H-2b, or H-2c), 4.33 (1H, dd, J = 3.4 and 11.6 Hz, H-6c), 4.29 (1H, t, J = 9.2 Hz, H-4c), 4.25 (1H, dd, J = 4.6 and 10.7 Hz, H′-1Cer), 3.81 (1H, t, J = 8.2 Hz, H-2a), 3.67–3.75 (2H, m, H-5b and H-5c), 3.63–3.66 (1H, m, H-5a), and 0.88 (6H, t, J = 7.0 Hz, $CH_2C\underline{H}_3$ × 2).

O-(2,3,4,6-Tetra-O-acetyl-β-D-mannopyranosyl)-(1→4)-O-(2,3,6-tri-O-acetyl-β-D-mannopyranosyl)-(1→4)-O-(2,3,6-tri-O-acetyl-β-D-mannopyranosyl)-(1→4)-O-(2,3,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→1)-(2S,3S,4R)-3,4-di-O-benzoyl-N-tetracosanoylsphinganine (**20**). Compound **18** (14 mg, 10 μmol) was coupled with compound **4** (11 mg, 13 μmol) as described for compound **19**. Chromatography of the product in THF–*n*-hexane = 2 : 3 gave compound **20** (4 mg, 20%), $[\alpha]_D$ −13.6° (c = 0.22), R_f 0.62 developed with *n*-hexane–THF = 1 : 1. NMR data: δ_H 6.19 (1H, d, J = 9.3 Hz, NH), 5.56 (1H, dd, J = 3.4 and 8.5 Hz, H-3Cer), 5.35, 5.41 (1H × 2, d × 2, J = 3.2 Hz, H-2b, or H-2c, or H-2d), 5.32–5.37 (1H, m, H-4Cer), 5.28 (1H, d, J = 3.7 Hz, H-2b, or H-2c, or H-2d), 5.20 (1H, t, J = 9.8 Hz, H-4d), 4.79 (1H, dd, J = 7.8 and 9.5 Hz, H-2a), 4.52, 4.64, 4.71 (1H × 3, br. s × 3, H-1b, H-1c, and H-1d), 4.57 (1H, ddt, J = 3.4, 8.3 and 9.0 Hz, H-2Cer), 4.36 (1H, d, J = 7.8 H, H-1a), 3.70 (1H, t, J = 9.5 Hz, H-4a), 3.40–3.65 (4H, m, H-5a, H-5b, H-5c, and H-5d), 2.23 (2H, t, J = 7.1 Hz, H-2′Cer and H′-2′Cer), 2.16, 2.15, 2.12, 2.11, 2.10, 2.09, 2.05, 2.05, 2.01, 2.00, 1.99, 1.96, and 1.95 (3H × 13, s × 13, OAc × 13), 0.88 (3H, t, J = 7.1 Hz, $CH_2C\underline{H}_3$), and 0.87 (1H, t, J = 7.1 Hz, $CH_2C\underline{H}_3$).

Anal. Found: C, 60.89; H, 7.46; N, 0.55%. Calcd. for $C_{106}H_{159}NO_{39} \cdot H_2O$: C, 60.93; H, 7.77; N, 0.67%.

O-(β-D-Mannopyranosyl)-(1−4)-O-(β-D-mannopyranosyl)-(1→4)-O-(β-D-mannopyranosyl)-(1→4)-O-(β-D-glucopyranosyl)-(1→1)-(2S,3S,4R)-4-hydroxy-N-tetracosanoylsphinganine (**3**). Compound **20** (3 mg, 1.5 μmol) was deacylated, as described for compound **2**, to give compound **3** (2 mg, quantitative yield), $[\alpha]_D$ −18.2° (c = 0.1, in pyridine), R_f 0.28 developed with H_2O–MeOH–$CHCl_3$ = 2.3 : 15 : 30. NMR data: δ_H (pyridine-d_5–D_2O, 90°C), 5.08, 5.07, 5.04 (1H × 3, br. s × 3, H-1b, H-1c, and H-1d), 4.88 (1H, m, H-2Cer), 4.74 (1H, d, J = 7.6 Hz, H-1a), 4.57 (1H, dd, J = 5.7 and 10.8 Hz, H-1Cer), 4.56, 4.51 (1H × 2, t × 2, J = 9.2 Hz, H-4b and H-4c), 4.41 (3H, m, H-2b, H-2c, and H-2d), 4.33 (1H, dd, J = 3.1 and 11.3 Hz, H-6d), 4.30 (1H, t, J = 9.5 Hz, H-4d), 4.26 (1H, dd, J = 4.6 and 10.4 Hz, H′-1Cer), 3.81 (1H, t, J = 8.5 Hz, H-2a), 3.63–3.75 (4H, m, H-5a, H-5b, H-5c, and H-5d), and 0.88 (6H, t, J = 7.0 Hz, $CH_2C\underline{H}_3$ × 2).

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