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A FACILE, REGIO- AND STEREO-SELECTIVE SYNTHESIS OF GANGLIOSIDE GM_3*

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ABSTRACT

Gangliosides GM_3, containing three different fatty acyl groups at the ceramide moiety, have been synthesized. Coupling of 2-(trimethylsilyl)ethyl *O*-(6-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,6-di-*O*-benzoyl-β-D-glucopyranoside (**4**), prepared from 2-(trimethylsilyl)ethyl β-lactoside (**1**) by selective 3′-*O*-benzylation, *O*-benzoylation, and subsequent removal of the benzyl group, with methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (**5**) using dimethyl(methylthio)sulfonium triflate as a glycosyl promoter, gave 2-(trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(6-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,6-di-*O*-benzoyl-β-D-glucopyranoside (**6**), which was converted, *via* *O*-acetylation, selective removal of the 2-(trimethylsilyl)-ethyl group, and subsequent imidate formation, into the α-*N*-acetylneuraminyl-(2→3′)-lactose trichloroacetimidate **9**. Glycosylation of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (**10**) with **9** afforded the β-glycoside **11**, which was converted, *via* selective reduction of the azide group, coupling with fatty acids, *O*-deacetylation, and de-esterification, into the title compounds.

INTRODUCTION

Ganglioside GM_3 was first isolated from horse erythrocytes by Yamakawa *et al.*[1] in 1952, and is the major ganglioside component in erythrocytes of many animal species[2–5]. A total synthesis of ganglioside GM_3 was achieved by Ogawa *et al.*[6].

Recently, various types of important, biological functions of gangliosides have been reported by many groups[7–9]. In view of these facts, and in order to investigate the structure–function relationship of gangliosides, the synthesis of a variety of gangliosides and their various types of analogs is of critical importance. As a part of our continuing efforts[10–13] on the synthesis of sialoglycoconjugates, we

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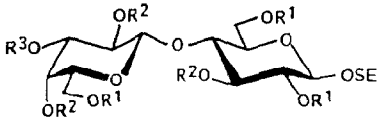
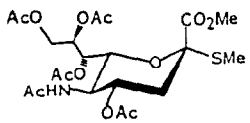
describe here a facile, regio- and stereoselective synthesis of ganglioside GM_3, in which fatty acyl groups at the ceramide moiety consist of tetradecanoyl, octadecanoyl, and tetracosanoyl groups.

RESULTS AND DISCUSSION

For the synthesis of ganglioside GM_3, we set out to synthesize 2-(trimethylsilyl)ethyl *O*-(6-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,6-di-*O*-benzoyl-β-D-glucopyranoside (**4**) as a suitably protected glycosyl acceptor, and then couple thus with methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate[10,13] (**5**) using dimethyl(methylthio)sulfonium triflate[10,13,14] (DMTST) as a glycosyl promoter, and finally convert the intermediate, by introduction of a ceramide moiety, into the end product.





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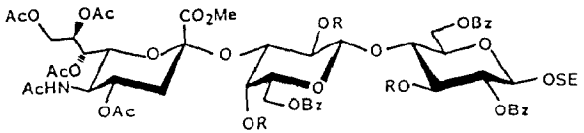
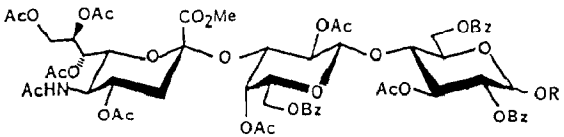
1 $R^1 = R^2 = R^3 = H$
2 $R^1 = R^2 = H$, $R^3 = Bn$
3 $R^1 = Bz$, $R^2 = H$, $R^3 = Bn$
4 $R^1 = Bz$, $R^2 = R^3 = H$
SE = 2-(trimethylsilyl)ethyl
Bn = benzyl
Bz = benzoyl



6 R = H
7 R = Ac



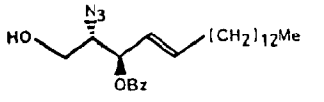
8 R = H
9 R = C(=NH)CCl$_3$

Dibutyltin oxide-mediated, selective etherification of 2-(trimethylsilyl)ethyl *O*-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside[15–17] (**1**) to give the 3′-*O*-benzyl derivative (**2**) was achieved in 75% yield. Regioselective benzoylation of **2**

with benzoyl chloride (3.3 equiv.) in pyridine–CH_2Cl_2 at −50° gave 2-(trimethylsilyl)ethyl *O*-(6-*O*-benzoyl-3-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,6-di-*O*-benzoyl-β-D-glucopyranoside (**3**) in 67% yield after column chromatography; significant signals in ^{1}H-n.m.r. spectrum were a one-proton doublet of doublets at δ 3.53 ($J_{2',3'}$ 9.5, $J_{3',4'}$ 3.3 Hz, H-3'), 5.33 (dd, 1 H, $J_{1,2}$ 8.1, $J_{2,3}$ 8.2 Hz, H-2), and a twenty-proton multiplet at δ 7.38–8.18 (4 Ph), indicating the structure assigned. Removal of the benzyl group in compound **3** by hydrogen-transfer reduction with 10% Pd–C catalyst in methanol in the presence of formic acid as the hydrogen donor, gave compound **4** in 70% yield.

Glycosylation of **4** with methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate[10,13] (**5**; 2.0 equiv. to the glycosyl acceptor) in acetonitrile for 24 h at −15° in the presence of DMTST (4.0 equiv. to glycosyl donor) and 3Å molecular sieves afforded the desired α-glycoside of Neu5Ac, 2-(trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(6-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,6-di-*O*-benzoyl-β-D-glucopyranoside (**6**) in 47% yield, together with 45–50% unreacted glycosyl acceptor **4**; it is noteworthy that neither the β-glycoside of Neu5Ac nor a positional isomer of **6** was isolated in this reaction*. Acetylation of **6** with acetic anhydride in pyridine gave the acetate **7** in 94% yield. The structure of **7** was unambiguously proved by 270 MHz ^{1}H-n.m.r. spectroscopy. The observed chemical shifts and coupling constants of the Neu5Ac unit for H-3*e* (δ 2.68, $J_{3a,3e}$ 12.6, $J_{3e,4}$ 4.8 Hz), H-4 (δ 4.94, $J_{3a,4}$ 12.6 Hz), and H-7 (δ 5.45, $J_{6,7}$ 2.6, $J_{7,8}$ 8.8 Hz), are characteristic of the α-glycosidic linkages[10,18–22] of Neu5Ac. Other ^{1}H-n.m.r. data are given in the Experimental Section and are consistent with the structure assigned.

Selective removal of the 2-(trimethylsilyl)ethyl group[13,16] in **7** was performed by treatment of **7** with $BF_3 \cdot OEt_2$ in CH_2Cl_2 for 7 h at room temperature to give **8** in 81% yield. When treated with trichloroacetonitrile[23,24] in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) for 2 h at 0°, compound **8** afforded the trichloroacetimidate **9** as the α anomer in 94% yield after column chromatography.



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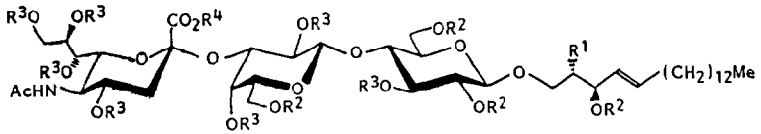

11 $R^1 = N_3$, R^2 = Bz, R^3 = Ac, R^4 = Me

12 $R^1 = NH_2$, R^2 = Bz, R^3 = Ac, R^4 = Me

13,14,15 $R^1 = NHCO(CH_2)_nMe$, R^2 = Bz, R^3 = Ac, R^4 = Me, n = 12,16,22

16,17,18 $R^1 = NHCO(CH_2)_nMe$, $R^2 = R^3 = R^4 = H$, n = 12,16,22

*Yields based on the weight of acceptor employed.

The glycosylation of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[12,25] (**10**) by **2** in the presence[23,24] of $BF_3 \cdot OEt_2$ for 4 h at 0°, yielded only the expected β-glycoside **11**, in 92% yield. Significant signals in the ^{1}H-n.m.r. spectrum of **11** were a one-proton doublets at δ 4.70 ($J_{1,2}$ 7.9 Hz, H-1) and a one-proton doublet of doublets at δ 5.26 ($J_{2,3}$ 9.5 Hz, H-2), showing the newly formed β-glycosidic linkage. Other ^{1}H-n.m.r. data are consistent with structure **11**.

Selective reduction[12,25] of the azide group in compound **11** with H_2S in 5:1 pyridine–water gave the amine **12**, which, on condensation with tetradecanoic, octadecanoic, and tetracosanoic acids, respectively, using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC) in CH_2Cl_2, gave the corresponding ganglioside GM_3 derivatives **13–15** in high yields. Finally, *O*-deacylation of **13–15** with sodium methoxide in methanol, and subsequent saponification of the methyl ester group, yielded almost quantitatively the corresponding three kinds (**16–18**) of ganglioside GM_3 containing different fatty acyl groups at the ceramide moiety.

In conclusion, regio- and α-stereo-selective glycosidation of Neu5Ac was achieved by using the methyl α-thioglycoside **5** of Neu5Ac as the glycosyl donor and the suitably protected 2-(trimethylsilyl)ethyl β-lactoside **4** as the acceptor with DMTST in acetonitrile under kinetically controlled conditions. The glycoside **11** thus obtained was readily converted into ganglioside GM_3, indicating that this procedure is useful for synthesis of sialoglycoconjugates.

EXPERIMENTAL

General methods. — Melting points are uncorrected. Optical rotations were determined with a Union PM-201 polarimeter at 25°, and i.r. spectra were recorded with a Jasco IRA-1 spectrophotometer. ^{1}H-N.m.r. spectra were recorded at 270 MHz with a Jeol JNM-GX270 spectrometer, and n.m.r. assignments were confirmed by use of decoupling techniques. Preparative chromatography was performed on silica gel (Waco Co.; 200 mesh) with the solvent systems specified. Concentrations and evaporations were conducted *in vacuo*.

2-(Trimethylsilyl)ethyl O-(3-O-benzyl-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (**2**). — To a solution of 2-(trimethylsilyl)ethyl *O*-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside[17] (**1**; 6.59 g, 14.9 mmol) in MeOH (100 mL) was added dibutyltin oxide (4.92 g, 19.8 mmol). The mixture was refluxed with stirring for 3 h and evaporated to a residue that was dissolved in dry benzene (200 mL). To the solution were added Bu_4NBr (4.8 g) and $PhCH_2Br$ (14 mL), and the mixture was refluxed with stirring, the course of the reaction being monitored by t.l.c. After 3 h, the mixture was evaporated to a syrup that was chromatographed on a column of silica gel (300 g) using hexane and then 4:1 EtOAc–hexane as the eluents. The latter eluent gave compound **2** (5.96 g, 75.2%) as needles; m.p. 185–186°, $[\alpha]_D$ −0.14° (*c* 1.48, $CHCl_3$); ^{1}H-N.m.r. data (CD_3OD): δ 0.96 (m, 2 H, $Me_3SiCH_2CH_2O$), 3.21 (t, 1 H, $J_{1,2} = J_{2,3} = 8.1$ Hz, H-2), 3.35 (dd, 1 H, $J_{2',3'}$ 9.5,

$J_{3',4'}$ 3.3 Hz, H-3'), 3.97 (d, 1 H, H-4'), 4.26 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.34 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1'), 4.58, 4.72 (2d, 2 H, J_{gem} 11.9 Hz, benzyl methylene), and 7.21–7.41 (m, 5 H, Ph).

Anal. Calc. for $C_{24}H_{40}O_{11}Si$ (532.7): C, 54.12; H, 7.57. Found: C, 54.08; H, 7.64.

2-(Trimethylsilyl)ethyl O-*(6-O-benzoyl-3-O-benzyl-β-*D-*galactopyranosyl)-(1→4)-2,6-di-*O-*benzoyl-β-*D-*glucopyranoside* (**3**). — To a stirred solution of **2** (1.32 g, 2.48 mmol) in dry pyridine (8 mL) and CH_2Cl_2 (20 mL), cooled to −50°, was added dropwise a cooled solution (−50°) of BzCl (1.15 g, 8.2 mmol) in dry CH_2Cl_2 (15 mL) during 30 min, and the progress of the reaction was monitored by t.l.c. MeOH (1 mL) was added to the mixture, and the solution was evaporated to a syrup that was dissolved in CH_2Cl_2 (200 mL). The solution was successively washed with 2M HCl and water, dried (Na_2SO_4), and then evaporated. The residue was chromatographed on a column of silica gel (70 g) with CH_2Cl_2 and 200:1 CH_2Cl_2–MeOH. The latter eluent gave compound **3** (1.4 g, 67%) as a syrup; $[\alpha]_D$ +14.0° (*c* 0.85, $CHCl_3$); ν 3600–3500 (OH), 1730 and 1260 (ester), 860 and 840 (Me_3Si), and 710 cm^{-1} (Ph); ^{1}H-n.m.r. data (1:1 CD_3OD–$CDCl_3$): δ 0.96 (m, 2 H, $Me_3SiCH_2CH_2O$), 3.53 (dd, 1 H, $J_{2',3'}$ 9.5, $J_{3',4'}$ 3.3 Hz, H-3'), 4.51 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1'), 4.63 (dd, 1 H, $J_{5,6}$ 5.9, $J_{6,6'}$ 11.9 Hz, H-6), 4.73 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 5.04 (dd, 1 H, H-6), 5.33 (dd, 1 H, $J_{2,3}$ 8.2 Hz, H-2), and 7.38–8.18 (m, 20 H, 4 Ph).

Anal. Calc. for $C_{45}H_{52}O_{14}Si$ (845.0): C, 63.97; H, 6.20. Found: C, 63.91; H, 6.35.

2-(Trimethylsilyl)ethyl O-*(6-O-benzoyl-β-*D-*galactopyranosyl)-(1→4)-2,6-di-*O-*benzoyl-β-*D-*glucopyranoside* (**4**). — To a solution of **3** (1.4 g, 1.6 mmol) in MeOH (50 mL) was added 10% Pd–C catalyst (1.0 g) and HCO_2H (1.0 mL), and the mixture was heated, with stirring, for 2 h at 60°. The catalyst was filtered off and washed thoroughly with MeOH. The filtrate and washings were combined and evaporated to a syrup that was chromatographed on a column of silica gel (50 g) with 50:1 CH_2Cl_2–MeOH, to give compound **4** (880 mg, 70%) as needles; m.p. 106–108°, $[\alpha]_D$ +11.0° (*c* 0.6, $CHCl_3$); ν 3500 (OH), 1720 and 1260 (ester), 860 and 840 (Me_3Si), and 700 cm^{-1} (Ph); ^{1}H-n.m.r. data (1:1 CD_3OD–$CDCl_3$): δ 4.50 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1'), 4.64 (dd, 1 H, $J_{5,6}$ 5.9, J_{gem} 11.9 Hz, H-6), 4.74 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.81 (dd, 1 H, H-6), 4.99 (dd, 1 H, J_{gem} 10.3 Hz, H-6'), 5.33 (dd, 1 H, $J_{2,3}$ 9.6 Hz, H-2), and 7.40–8.17 (m, 15 H, 3 Ph).

Anal. Calc. for $C_{38}H_{46}O_{14}Si$ (754.9): C, 60.46; H, 6.14. Found: C, 60.41; H, 6.25.

2-(Trimethylsilyl)ethyl O-*(methyl 5-acetamido-4,7,8,9-tetra-*O-*acetyl-3,5-di-deoxy-*D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)-*O-*(6-O-benzoyl-β-*D-*galactopyranosyl)-(1→4)-2,6-di-*O-*benzoyl-β-*D-*glucopyranoside* (**6**). — To a solution of **4** (150 mg, 0.2 mmol) and methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate[10,13] (**5**; 210 mg, 0.4 mmol) in dry MeCN (2.0 mL) was added molecular sieves 3Å (MS-3Å; 300 mg). The mixture was stirred overnight at room temperature and then cooled

to −30°. To the cooled mixture was added, with stirring, a mixture (680 mg; 60% DMTST by weight) of dimethyl(methylthio)sulfonium triflate[14b] (DMTST) and MS-3Å, and the stirring was continued for 24 h at −15°. The precipitate was filtered off, and washed thoroughly with CH_2Cl_2. The filtrate and washings were combined, and the solution was successively washed with M Na_2CO_3 and water, dried (Na_2SO_4), and evaporated to a syrup that was chromatographed on a column of silica gel (50 g) with 4:1 EtOAc–hexane, to give compound **6** (114 mg, 47%) as an amorphous mass; $[\alpha]_D$ +10.9° (*c* 1.74, $CHCl_3$); ν 3600–3100 (OH, NH), 1730 and 1220 (ester), 1650 and 1540 (amide), 850 and 830 (Me_3Si), and 710 cm^{-1} (Ph); ^{1}H-n.m.r. data (1:1 CD_3OD–$CDCl_3$): lactose unit δ 0.98 (m, 2 H, $Me_3SiCH_2CH_2O$), 3.69 (ddd, 1 H, $Me_3SiCH_2CH_2O$), 4.62 (dd, 1 H, $J_{5,6}$ 5.9, J_{gem} 11.9 Hz, H-6), 4.72 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1'), 4.77 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.83 (dd, 1 H, H-6), 5.36 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-2), and 7.46–8.20 (m, 15 H, 3 Ph); Neu5Ac unit δ 1.99–2.31 (5s, 15 H, AcN, 4 AcO), 2.81 (dd, 1 H, $J_{3a,3e}$ 12.7, $J_{3e,4}$ 4.5 Hz, H-3*e*), 3.92 (s, 3 H, MeO), 4.97 (ddd, 1 H, H-4), 5.38 (dd, 1 H, $J_{7,8}$ 8.21 Hz, H-7), and 5.42 (m, 1 H, H-8).

Anal. Calc. for $C_{58}H_{73}NO_{26}Si$ (1228.3): C, 56.72; H, 5.99; N, 1.14. Found: C, 56.83; H, 6.15; N, 1.08.

*2-(Trimethylsilyl)ethyl O-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosylonate)-(2→3)-*O*-(2,4-di-*O*-acetyl-6-*O*-benzoyl-β-*D*-galactopyranosyl)-(1→4)-3-*O*-acetyl-2,6-di-*O*-benzoyl-β-*D*-glucopyranoside* (**7**). — Compound **6** (85 mg, 0.075 mmol) was acetylated with Ac_2O (3 mL) in pyridine (5 mL) overnight at room temperature. The product was purified by chromatography on silica gel (10 g) with 60:1 CH_2Cl_2–MeOH, to give **7** (87 mg, 94%) as an amorphous mass; $[\alpha]_D$ +5.75° (*c* 1.74, $CHCl_3$); ν 3300 (NH), 1750 and 1230 (ester), 1660 and 1540 (amide), 860 and 840 (Me_3Si), and 720 cm^{-1} (Ph); ^{1}H-n.m.r. data (1:1 CD_3OD–$CDCl_3$): lactose unit δ 0.98 (m, 2 H, $Me_3SiCH_2CH_2O$), 4.74 (dd, 1 H, $J_{2',3'}$ 10.2, $J_{3',4'}$ 3.3 Hz, H-3'), 4.81 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 5.00 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1'), 5.13 (dd, 1 H, H-2'), 5.14 (dd, 1 H, H-4'), 5.32 (dd, 1 H, H-2), 5.59 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.7 Hz, H-3), 7.50–8.18 (m, 15 H, 3 Ph); Neu5Ac unit δ 1.67 (t, 1 H, $J_{3a,3e} = J_{3a,4}$ = 12.6 Hz, H-3*a*), 1.84 (s, 3 H, AcN), 2.68 (dd, 1 H, $J_{3e,4}$ 4.8 Hz, H-3*e*), 3.83 (s, 3 H, MeO), 4.94 (ddd, 1 H, H-4), 5.45 (dd, 1 H, $J_{6,7}$ 2.6, $J_{7,8}$ 8.8 Hz, H-7), and 5.64 (m, 1 H, H-8); *O*-acyl groups δ 1.98, 1.99, 2.02, 2.03, 2.04, 2.12, and 2.21 (7s, 21 H, 7 AcO).

Anal. Calc. for $C_{64}H_{79}NO_{29}Si$ (1354.4): C, 56.76; H, 5.88; N, 1.03. Found: C, 56.70; H, 5.87; N, 1.05.

*O-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosylonate)-(2→3)-*O*-(2,4-di-*O*-acetyl-6-*O*-benzoyl-β-*D*-galactopyranosyl)-(1→4)-3-*O*-acetyl-2,6-di-*O*-benzoyl-*D*-glucopyranose* (**8**). — To a stirred solution of **7** (660 mg, 0.49 mmol) in CH_2Cl_2 (10 mL), cooled to 0°, was added dropwise $BF_3 \cdot OEt_2$ (0.5 mL). The mixture was stirred for 7 h at 10–20°, the course of the reaction being monitored by t.l.c. Dichloromethane (50 mL) was added to the mixture, and the solution was successively washed with M $NaHCO_3$ and water,

dried (Na_2SO_4), and evaporated. The residue was chromatographed on a column of silica gel (20 g) with 40:1 CH_2Cl_2–MeOH, to give compound **8** (499 mg, 81%) as an amorphous mass; $[\alpha]_D$ +40° (*c* 1.0, $CHCl_3$); ν 3700–3150 (OH, NH), 1740 and 1230 (ester), 1660 and 1550 (amide), and 720 cm^{-1} (Ph).

Anal. Calc. for $C_{59}H_{67}NO_{29}$ (1254.2): C, 56.50; H, 5.38; N, 1.12. Found: C, 56.38; H, 5.49; N, 1.15.

O-*(Methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)*-O-*(2,4-di*-O-*acetyl-6*-O-*benzoyl-β*-D-*galacto-pyranosyl)-(1→4)-3*-O-*acetyl-2,6-di*-O-*benzoyl-α*-D-*glucopyranosyl trichloroacetimidate* (**9**). — To a stirred solution of **8** (499 mg, 0.4 mmol) in dry CH_2Cl_2 (3 mL), cooled to −5°, was added Cl_3CCN (1.2 mL) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 32 mg). The mixture was stirred for 2 h at 0° and then evaporated. The residue was chromatographed on a column of silica gel (20 g) with 70:1 CH_2Cl_2–MeOH, to give compound **9** (522 mg, 94%) as an amorphous mass; $[\alpha]_D$ +42° (*c* 0.76, $CHCl_3$): ^{1}H-n.m.r. data ($CDCl_3$): lactose unit δ 6.66 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 7.43–8.06 (m, 15 H, 3 Ph), and 8.55 (s, 1 H, C=NH); Neu5Ac unit δ 1.84 (s, 3 H, AcN), 2.59 (dd, 1 H, $J_{3a,3e}$ 13.4, $J_{3e,4}$ 4.8 Hz, H-3*e*), and 3.71 (s, 3 H, MeO); *O*-acetyl groups δ 1.97, 2.00, 2.01, 2.04, 2.06, 2.13, and 2.21 (7s, 21 H, 7 AcO).

Anal. Calc. for $C_{61}H_{67}N_2O_{29}Cl_3$ (1398.6): C, 52.39; H, 4.83; N, 2.00. Found: C, 52.42; H, 4.85; N, 1.90.

O-*(Methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)*-O-*(2,4-di*-O-*acetyl-6*-O-*benzoyl-β*-D-*galacto-pyranosyl)-(1→4)*-O-*(3*-O-*acetyl-2,6-di*-O-*benzoyl-β*-D-*glucopyranosyl)-(1→1)-(2S,3*R*,4*E*)-2-azido-3*-O-*benzoyl-4-octadecene-1,3-diol* (**11**). — To a solution of **9** (504 mg, 0.36 mmol) and (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[13,25] (**10**; 310 mg, 0.72 mmol) in dry CH_2Cl_2 (10 mL) was added MS-4Å (6.0 g), and the mixture was stirred for 30 min at room temperatur and then cooled to 0°. Boron trifluoride etherate (104 mg) was added to the mixture, and this was stirred for 4 h at 0°, the progress of the reaction being monitored by t.l.c. The precipitate was filtered off and washed thoroughly with CH_2Cl_2. The solution was washed successively with M $NaHCO_3$ and water, dried (Na_2SO_4), and evaporated to a syrup that was chromatographed on a column of silica gel (60 g) with 3:2 EtOAc–hexane to give compound **11** (552 mg, 92%) as an amorphous mass; $[\alpha]_D$ −2.8° (*c* 0.57, $CHCl_3$); ν 3300 (NH), 2100 (N_3), 1740 and 1230 (ester), 1650 and 1540 (amide), and 710 cm^{-1} (Ph); ^{1}H-n.m.r. data ($CDCl_3$): lactose unit δ 4.61 (dd, 1 H, $J_{2',3'}$ 10.1, $J_{3',4'}$ 3.3 Hz, H-3′), 4.70 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.89 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 5.00 (broad d, 1 H, H-4′), 5.04 (dd, 1 H, H-2′), and 5.26 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-2); Neu5Ac unit δ 1.68 (t, 1 H, $J_{3a,3e} = J_{3a,4}$ = 12.6 Hz, H-3*a*), 1.84 (s, 3 H, AcN), 2.58 (dd, 1 H, $J_{3e,4}$ 4.6 Hz, H-3*e*), 3.71 (s, 3 H, MeO), 4.86 (m, 1 H, H-4), and 5.26 (d, 1 H, $J_{NH,5}$ 10.3 Hz, NH); Cer unit δ 5.68 (dt, 1 H, $J_{4,5}$ 14.1, $J_{5,6} = J_{5,6'}$ = 7.1 Hz, H-5); *O*-acyl groups δ 1.99, 2.00, 2.02, 2.03, 2.04, 2.12, and 2.21 (7s, 21 H, 7 AcO), and 7.35–8.07 (m, 20 H, 4 Ph).

Anal. Calc. for $C_{84}H_{104}N_4O_{31}$ (1665.8): C, 60.57; H, 6.29; N, 3.36. Found: C, 60.43; H, 6.34; N, 3.35.

O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4-di-O-acetyl-6-O-benzoyl-β-D-galactopyranosyl)-(1→4)-O-(3-O-acetyl-2,6-di-O-benzoyl-β-D-glucopyranosyl)-(1→1)-(2S,3R,4E)-3-O-benzoyl-2-tetradecanamido-4-octadecene-1,3-diol (**13**). — Hydrogen sulfide was bubbled through a solution of **11** (100 mg, 0.06 mmol) in pyridine (10 mL) and water (2 mL) for 36 h while the solution was stirred at room temperature, the course of the reaction being monitored by t.l.c. The mixture was evaporated to give the syrupy amine **12**, which was used for the next reaction without further purification. To a solution of **12** in dry CH_2Cl_2 (5 mL) were added tetradecanoic acid (28 mg, 0.12 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC; 35 mg), and the mixture was stirred overnight at room temperature. After completion of the reaction, CH_2Cl_2 (30 mL) was added to the mixture, and the solution was washed with water, dried (Na_2SO_4), and evaporated to a syrup that was chromatographed on a column of silica gel (20 g) with 60:1 CH_2Cl_2–MeOH, to give compound **13** (96 mg, 86.5%) as an amorphous mass; $[\alpha]_D$ +10° (*c* 1.9, $CHCl_3$); ν 3300 (NH), 2940 and 2980 (Me, methylene), 1740 and 1220 (ester), 1660 and 1530 (amide), and 710 cm^{-1} (Ph); ^{1}H-n.m.r. data ($CDCl_3$): lactose unit δ 4.60 (dd, 1 H, $J_{2',3'}$ 10.4, $J_{3',4'}$ 3.7 Hz, H-3′), 4.61 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.84 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1′), 5.00 (broad d, 1 H, H-4′), 5.01 (dd, 1 H, H-2′), and 5.19 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2); Neu5Ac unit δ 1.67 (t, 1 H, $J_{3a,3e} = J_{3a,4}$ = 12.5 Hz, H-3*a*), 1.84 (s, 3 H, AcN), 2.57 (dd, 1 H, $J_{3e,4}$ 4.5 Hz, H-3*e*), 3.71 (s, 3 H, MeO), and 5.16 (d, 1 H, $J_{NH,5}$ 10.3 Hz, NH); Cer unit δ 5.66 (d, 1 H, $J_{NH,2}$ 9.2 Hz, NH), and 5.77 (dt, 1 H, $J_{5,6} = J_{5,6'}$ = 7.1 Hz, H-5); *O*-acyl groups δ 2.00 (2), 2.02 (2), 2.03, 2.11, and 2.18 (7s, 21 H, 7 AcO), and 7.25–8.06 (m, 20 H, 4 Ph).

Anal. Calc. for $C_{98}H_{132}N_2O_{32}$ (1850.1): C, 63.62; H, 7.19; N, 1.51. Found: C, 63.62; H, 7.23; N, 1.55.

O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4-di-O-acetyl-6-O-benzoyl-β-D-galactopyranosyl)-(1→4)-O-(3-O-acetyl-2,6-di-O-benzoyl-β-D-glucopyranosyl)-(1→1)-(2S,3R,4E)-3-O-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (**14**). — The azide group in **11** (100 mg, 0.06 mmol) was converted into amine as described for **13**, which was then condensed with octadecanoic acid (34 mg, 0.12 mmol) in the presence of WSC (36 mg), to give **14** (102 mg, 89.5%) as an amorphous mass; $[\alpha]_D$ +9.6° (*c* 2.04, $CHCl_3$); ^{1}H-n.m.r. data ($CDCl_3$): lactose unit δ 4.60 (dd, 1 H, $J_{2',3'}$ 10.4, $J_{3',4'}$ 3.7 Hz, H-3′), 4.61 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.84 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 5.00 (broad d, 1 H, H-4′), 5.01 (dd, 1 H, H-2′), and 5.19 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2); Neu5Ac unit δ 1.69 (t, 1 H, $J_{3a,3e} = J_{3a,4}$ = 12.5 Hz, H-3*a*), 1.84 (s, 3 H, AcN), 2.56 (dd, 1 H, $J_{3e,4}$ 4.8 Hz, H-3*e*), 3.71 (s, 3 H, MeO), and 5.30 (d, 1 H, $J_{NH,5}$ 9.8 Hz, NH); Cer unit δ 5.66 (d, 1 H, $J_{NH,2}$ 9.0 Hz, NH), and 5.77 (dt, 1 H, $J_{4,5}$ 14.2, $J_{5,6} = J_{5,6'}$ = 7.1 Hz, H-5); *O*-acyl groups δ 2.00, 2.01, 2.02, 2.03, 2.04, 2.11, and 2.18 (7s, 21 H, 7 AcO), and 7.25–8.10 (m, 20 H, 4 Ph).

Anal. Calc. for $C_{102}H_{140}N_2O_{32}$ (1906.2): C, 64.27; H, 7.40; N, 1.47. Found: C, 64.10; H, 7.58; N, 1.52.

O-*(Methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)*-O-*(2,4-di*-O-*acetyl-6*-O-*benzoyl-β*-D-*galactopyranosyl)-(1→4)*-O-*(3*-O-*acetyl-2,6-di*-O-*benzoyl-β*-D-*glucopyranosyl)-(1→1)-(2S,3R,4E)-3*-O-*benzoyl-2-tetracosanamido-4-octadecene-1,3-diol* (**15**). — Reduction of the azide group in **11** (100 mg, 0.06 mmol) and subsequent coupling with tetracosanoic acid (44 mg, 0.12 mmol) using WSC (35 mg), according to the procedure described for **13**, gave compound **15** (102 mg, 85.7%) as an amorphous mass; $[\alpha]_D$ +9.3° (*c* 2.04, $CHCl_3$); ^{1}H-n.m.r. data ($CDCl_3$): lactose unit δ 4.61 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.84 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1'), 5.19 (dd, 1 H, $J_{2,3}$ 9.8 Hz, H-2); Neu5Ac unit δ 1.67 (t, 1 H, $J_{3a,3e} = J_{3a,4}$ = 12.5 Hz, H-3*a*), 1.84 (s, 3 H, AcN), 2.57 (dd, 1 H, $J_{3e,4}$ 4.7 Hz, H-3*e*), 3.71 (s, 3 H, MeO), and 5.13 (d, 1 H, $J_{NH,5}$ 10.3 Hz, NH); Cer unit δ 5.65 (d, 1 H, $J_{NH,2}$ 9.2 Hz, NH), and 5.77 (dt, 1 H, $J_{4,5}$ 15.0, $J_{5,6} = J_{5,6'}$ = 7.2 Hz, H-5); *O*-acyl unit δ 2.00 (3), 2.02, 2.03, 2.11, and 2.18 (7s, 21 H, 7 AcO), and 7.30–8.05 (m, 20 H, 4 Ph).

Anal. Calc. for $C_{108}H_{152}N_2O_{32}$ (1990.3): C, 65.17; H, 7.70; N, 1.41. Found: C, 65.15; H, 7.83; N, 1.40.

O-*(5-Acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid)-(2→3)*-O-*β*-D-*galactopyranosyl-(1→4)*-O-*β*-D-*glucopyranosyl-(1→1)-(2S,3R,4E)-2-tetradecanamido-4-octadecene-1,3-diol* (**16**). — To a solution of **13** (100 mg, 0.054 mmol) in MeOH (5 mL) was added NaOMe (20 mg) and the mixture was stirred for 8 h at room temperature, the course of the reaction being monitored by t.l.c. Water (0.5 mL) was added to the mixture at 0°, and this was stirred for 4.5 h, and then treated with Amberlite IR-120 (H^+) resin to remove the base. The solution was evaporated, and the residue was thoroughly washed with ether, to give compound **16** (quantitative) as an amorphous mass, which showed a single spot in t.l.c.; $[\alpha]_D$ +1.8° (*c* 0.42, 1:1 MeOH–$CHCl_3$); ν 3700–2800 (OH, NH), 2940 and 2840 (Me, methylene), 1710 (C=O), and 1630, and 1550 cm^{-1} (amide); ^{1}H-n.m.r. data (2:1 CD_3OD–$CDCl_3$): lactose unit δ 4.31 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.43 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1'); Neu5Ac unit δ 2.02 (s, 3 H, AcN), 2.86 (dd, 1 H, H-3*e*); Cer unit δ 0.89 (t, 6 H, J_{Me,CH_2} 6.6 Hz, *Me*CH_2), 2.18 (t, 2 H, J_{CH_2,CH_2} 7.4 Hz, CH_2CH_2CO), 4.20 (dd, 1 H, $J_{1,2}$ 4.6, $J_{1,1'}$ 9.7 Hz, H-1), 5.44 (dd, 1 H, $J_{3,4}$ 7.5, $J_{4,5}$ 15.4 Hz, H-4), and 5.69 (dt, 1 H, $J_{5,6} = J_{5,6'}$ = 7.2 Hz, H-5).

Anal. Calc. for $C_{55}H_{100}N_2O_{21}$ (1125.7): C, 58.70; H, 8.96; N, 2.49. Found: C, 58.73; H, 9.05; N, 2.45.

O-*(5-Acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid)-(2→3)*-O-*β*-D-*galactopyranosyl-(1→4)*-O-*β*-D-*glucopyranosyl-(1→1)-(2S,3R,4E)-2-octadecanamido-4-octadecene-1,3-diol* (**17**). — The *O*-acetyl and methyl ester groups in **14** (100 mg, 0.052 mmol) were removed, as described for **16**, to give compound **17** (60 mg, 96%) as an amorphous mass; $[\alpha]_D$ +1.8° (*c* 0.2, 1:1 MeOH–$CHCl_3$); ν 3700–2800 (OH, NH), 2940 and 2840 (Me, methylene), 1710 (C=O), and 1630 and 1550 cm^{-1} (amide); ^{1}H-n.m.r. data (2:1 CD_3OD–$CDCl_3$): lactose unit $[\delta]_D$ 4.31 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.44 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1'); Neu5Ac unit δ 2.02 (s, 3 H, AcN), 2.85 (dd, 1 H, H-3*e*); Cer unit δ 0.89 (t, 6 H, J_{Me,CH_2} 6.6

Hz, *Me*CH_2), 2.20 (t, 2 H, J_{CH_2,CH_2} 6.7 Hz, CH_2CH_2CO), 4.20 (dd, 1 H, $J_{1,1'}$ 10.0, $J_{1,2}$ 4.3 Hz, H-1), 5.44 (dd, 1 H, $J_{3,4}$ 7.5, $J_{4,5}$ 15.4 Hz, H-4), and 5.69 (dt, 1 H, $J_{5,6} = J_{5,6'} = 7.2$ Hz, H-5).

Anal. Calc. for $C_{59}H_{108}N_2O_{21}$ (1181.5): C, 59.98; H, 9.21; N, 2.37. Found: C, 59.86; H, 9.35; N, 2.35.

O-*(5-Acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid)*-*(2→3)*-O-β-D-*galactopyranosyl-(1→4)*-O-β-D-*glucopyranosyl-(1→1)*-*(2S,3R,4E)-2-tetracosanamido-4-octadecene-1,3-diol* (**18**). — Treatment of **15** (120 mg, 0.06 mmol), as described for the preparation of **16**, gave **18** (74 mg, 96%) as an amorphous mass; $[\alpha]_D$ +0.8° (*c* 0.5, 1:1 MeOH–$CHCl_3$); *ν* 3600–2800 (OH, NH), 2930 and 2840 (Me, methylene), 1710 (C=O), and 1630, and 1550 cm^{-1} (amide); ^{1}H-n.m.r. data (1:1 CD_3OD–$CDCl_3$): lactose unit δ 4.31 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.43 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1'); Neu5Ac unit δ 2.04 (s, 3 H, AcN), 2.86 (dd, 1 H, H-3*e*); Cer unit δ 0.89 (t, 6 H, J_{Me,CH_2} 6.5 Hz, *Me*CH_2), 2.18 (t, 2 H, J_{CH_2,CH_2} 7.7 Hz, CH_2CH_2CO), 4.19 (dd, 1 H, $J_{1,1'}$ 9.9, $J_{1,2}$ 4.3 Hz, H-1), 5.45 (dd, 1 H, $J_{3,4}$ 7.5, $J_{4,5}$ 15.8 Hz, H-4), and 5.70 (dt, 1 H, $J_{5,6} = J_{5,6'} = 7.1$ Hz, H-5).

Anal. Calc. for $C_{65}H_{120}N_2O_{21}$ (1277.6): C, 62.04; H, 9.47; N, 2.19. Found: C, 62.11; H, 9.53; N, 2.15.

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