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Carbohydrate Research 302 (1997) 223–227

CARBOHYDRATE RESEARCH

Note

Synthesis of 6′-GM2, a regioisomer of ganglioside GM2, for studying the mechanism of action of GM2 activator [1]

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Received 6 March 1997; accepted 31 March 1997

Keywords: Ganglioside; GM2 activator; Tay–Sachs disease

GM2 activator is a protein cofactor that assists β-hexosaminidase (HexA) in hydrolyzing the β-linked *N*-acetylgalactosamine (GalNAc) in ganglioside GM2. Tay–Sachs disease can be caused by a deficiency of HexA or GM2 activator [2,3]. Although the GM2 activator was discovered in 1973 [4], the mechanism of action of this activation protein is still not well understood. In this paper, we report a synthesis of the regioisomer of GM2, 6′-GM2, in which the GalNAc residue is linked, not to the 4-OH, but to the 6-OH of Gal to show that the resistance of GalNAc in GM2 to HexA is due to the specific structure of the GM2-epitope, β-D-GalNAc-(1 → 4)-α-Neu5Ac-(2 → 3)-β-D-Gal → .

Removal of the benzylidene group from 2-(trimethylsilyl)ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**1**) [5] with aqueous 80% acetic acid at 50 °C gave, after column chromatography, the lactose acceptor **2** in 93% yield. The glycosylation of **2** with the oxazoline **3**, which was prepared [6] from the peracetylated derivative of GalNAc, in the presence of pyridinium *p*-toluenesulfonate in dichloromethane for 24 h under reflux gave the desired β-glycoside **4** in 61% yield. Removal of the 4-methoxybenzyl group in **4** in acetonitrile–water in the presence of ceric ammonium nitrate (CAN) for 1 h at room temperature afforded the expected glycosyl acceptor **5** in 67% yield. Glycosylation [7] of **5** with the phenyl 2-thioglycoside of Neu5Ac (**6**) [8] in acetonitrile for 3 h at −25 °C in the presence of *N*-iodosuccinimide (NIS)-trifluoromethanesulfonic acid (TfOH) [9] gave the expected α-glycoside **7** in a low yield (23%), with the unreacted glycosyl acceptor being recovered in 70% yield. The observed chemical shift (δ 4.87) for H-4 in the Neu5Ac moiety is characteristic for the α-glycosidic linkage [10]. Removal of the benzyl groups from **7** by catalytic hydrogenolysis over 10% Pd–C in 1:1 ethanol–acetic acid for 24 h at 40 °C and subsequent acetylation gave the peracetylated tetrasaccharide **8** in 88% yield. The resulting tetrasaccharide was converted into the corresponding trichloroacetimidate **10** in good yield by selective removal of the 2-(trimethylsilyl)ethyl group with trifluoroacetic acid and subsequent imidate formation [11,12].

The final glycosylation [13] of (2*S*,3*R*,4*E*)-2-

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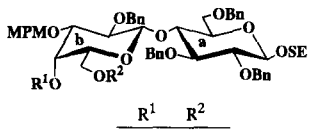
[1] Synthetic studies on sialoglyconjugates, Part 94. For Part 93, see ref. [1].

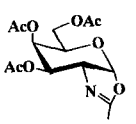
[2] Deceased 10 October 1996.

PII S0008-6215(97)00120-1

azido-3-*O*-(*tert*-butyldiphenylsilyl)-4-octadecene-1,3-diol (**11**) [14] with **10** in dichloromethane for 12 h at 0 °C in the presence of trimethylsilyl trifluoromethanesulfonate and 4 Å molecular sieves (AW-300) gave only the β-glycoside **12** in 80% yield. Selective reduction [15] of the azido group in **12** with triphenylphosphine in benzene–water gave the amine **13**, which on condensation [16] with octadecanoic acid using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC) in dichloromethane furnished the fully protected target compound **15** as follows: desilylation [17] of **14** by treatment with tetrabutylammonium fluoride in acetonitrile, and O-deacylation with sodium methoxide in methanol, and subsequent saponification of the methyl ester group, yielded the desired 6′-GM2 ganglioside **15** in a quantitative yield after a Sephadex LH-20 column chromatography.

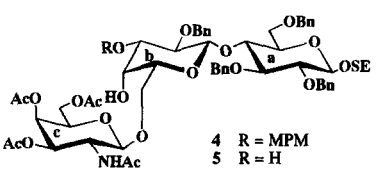


	R^1	R^2
1	benzylidene	
2	H	H

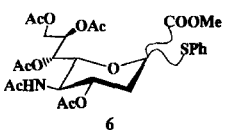


3

SE = 2-(trimethylsilyl)ethyl
Bn = benzyl
MPM = *p*-methoxybenzyl



4 R = MPM
5 R = H



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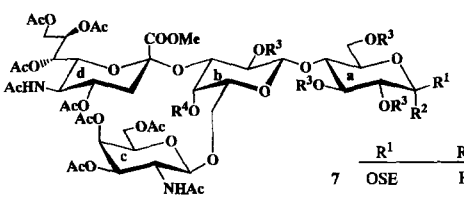
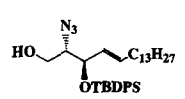
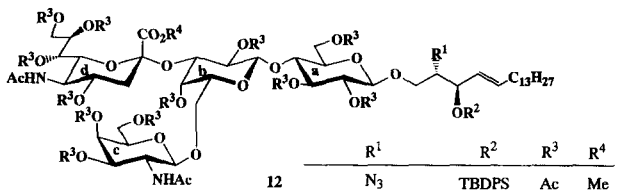


	R^1	R^2	R^3	R^4
7	OSE	H	Bn	H
8	OSE	H	Ac	Ac
9	H , OH		Ac	Ac
10	H	$OC(=NH)CCl_3$	Ac	Ac



11

TBDPS = *tert*-butyldiphenylsilyl



	R^1	R^2	R^3	R^4
12	N_3	TBDPS	Ac	Me
13	NH_2	TBDPS	Ac	Me
14	$NHCO(CH_2)_{16}CH_3$	TBDPS	Ac	Me
15	$NHCO(CH_2)_{16}CH_3$	H	H	H

In contrast to GM2, the β-D-GalNAc-(1 → 6)-Gal linkage in 6′-GM2 was readily hydrolyzed by HexA in the absence of the GM2 activator, indicating that the resistance of GM2 to HexA is due to the specific structure of the GM2-epitope and that the GM2 activator may interact with GM2 to make GalNAc accessible to HexA. [3]

1. Experimental

General procedures.—Specific rotations were determined with a Union PM-201 polarimeter at 25 °C, and ^{1}H NMR spectra were recorded at 270 MHz with a Jeol JNM-GX 270 spectrometer or at 200 MHz with a Varian Gemini-2000 spectrometer. Preparative TLC was performed on Silica Gel 60 (E. Merck), and column chromatography on silica gel (Fuji Silysia Co., 300 mesh) was accomplished with the solvent systems (v/v) specified. Concentrations and evaporations were conducted in vacuo.

2-(Trimethylsilyl)ethyl 2-O-benzyl-3-O-(4-methoxybenzyl)-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**2**).—A soln of **1** (1.01 g, 1 mmol) in aq 80% CH_3COOH (5 mL) was heated for 48 h at 40 °C and concd. Column chromatography (1:1 AcOEt–hexane) of the residue on silica gel gave **2** (0.74 g, 93%) as a syrup: $[\alpha]_D$ +21.7° (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 1.00 (m, 2 H, $Me_3SiCH_2CH_2$), 3.78 (s, 3 H, *Me*OPh), and 6.82–7.53 (m, 29 H, 6 Ph). Anal. Calcd for $C_{53}H_{66}O_{12}Si$ (923.19): C, 68.96; H, 7.21. Found: C, 68.8; H, 7.11.

2-(Trimethylsilyl)ethyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl-(2 → 6)-2-O-benzyl-3-O-4-(methoxybenzyl)-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**4**).—To a soln of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-glucopyranose (4.7 g, 12.0 mmol)

[3] Details of the studies on the enzymatic hydrolysis of 6′-GM2 will be published elsewhere.

was added $FeCl_3$ (4.7 g, 29.0 mmol), and the mixture was stirred for 1.5 h at 50 °C, at the end of which time it was cooled and neutralized with M $NaHCO_3$. The insoluble materials were removed by filtration and washed thoroughly with CH_2Cl_2. The filtrate and washings were combined and extracted with CH_2Cl_2. The extract was washed with M $NaHCO_3$ and brine, dried (Na_2SO_4), and concd to give the oxazoline **3** (3.85 g, 95%), which was used for the next reaction without further purification.

To a soln of **2** (3.6 g, 3.9 mmol) and **3** (3.85 g, 11.7 mmol) in CH_2Cl_2 (30 mL) was added pyridinium *p*-toluenesulfonate (0.59 g, 0.13 mmol), and the soln was stirred under reflux for 24 h. Dichloromethane (50 mL) was added, and the soln was successively washed with M $NaHCO_3$, water, and 2 M HCl, dried (Na_2SO_4), and concd. Column chromatography (1:1 AcOEt–hexane) of the residue on silica gel gave **5** (3.0 g, 61%) as an amorphous mass: $[\alpha]_D$ +3.6° (*c* 0.9, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 1.00 (m, 2 H, $Me_3SiCH_2CH_2$), 1.96, 2.02, 2.04, 2.09 (4 s, 12 H, 3 AcO and AcN), 2.66 (t, 1 H, $J_{1,2} = J_{2,3} = 7.6$ Hz, H-2a), 3.81 (s, 3 H, *Me*OPh), 5.02 (d, 1 H, $J_{NH,2}$ 8.8 Hz, NH), 6.83–7.55 (m, 24 H, 5 Ph). Anal. Calcd for $C_{67}H_{85}NO_{20}Si$ (1252.5): C, 64.25; H, 6.84; N, 1.12. Found: C, 64.20; H, 6.75; N, 0.83.

*2-(Trimethylsilyl)ethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-galactopyranosyl-(2 → 6)-2-*O*-benzyl-β-*D*-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-β-*D*-glucopyranoside* (**5**).—A soln of **4** (0.66 g, 0.53 mmol) and ceric ammonium nitrate (CAN; 1.45 g, 2.6 mmol) in MeOH (80 mL) was stirred for 1 h at room temperature. After completion of the reaction, $NaHCO_3$ was added, and the mixture was concd and extracted with CH_2Cl_2. The extract was washed with water, dried (Na_2SO_4), and concd. Column chromatography (45:1 CH_2Cl_2–MeOH) of the residue on silica gel gave **6** (0.4 g, 67%) as an amorphous mass: $[\alpha]_D$ +4.5° (*c* 0.44, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 1.00 (m, 2 H, $Me_3SiCH_2CH_2$), 1.96, 1.97, 2.04, 2.10 (4 s, 12 H, 3 AcO and AcN), 2.92 (t, 1 H, $J_{1,2} = J_{2,3} = 5.8$ Hz, H-2a), 5.05 (d, 1 H, $J_{1,2}$ 10.8 Hz, H-1c), 5.08 (s, 1 H, H-4c), 5.12 (d, 1 H, $J_{2,3}$ 8.8 Hz, H-3c), 5.52 (d, 1 H, $J_{NH,2}$ 8.8 Hz, NH), 7.26–7.53 (m, 20 H, 4 Ph). Anal. Calcd for $C_{59}H_{77}NO_{19}Si$ (1132.3): C, 62.58; H, 6.85; N, 1.24. Found: C, 62.56; H, 6.65; N, 1.21.

*2-(Trimethylsilyl)ethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-galactopyranosyl-(2 → 6)-[(methyl 5-acetamido-4,7,8,9-*tetra-O*-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosylonate)-(2 → 3)]-2-O-benzyl-β-*D*-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-β-*D*-glucopyranoside* (**7**).—To a soln of **5** (100 mg, 0.09 mmol) and methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-thio-2-nonuropyranosid)onate (**6**; 103 mg, 0.1 mmol) in dry acetonitrile (1.7 mL) was added 3 Å molecular sieves (100 mg), and the mixture was stirred for 5 h at room temperature, then cooled to −25 °C. To this mixture were added, with stirring, *N*-iodosuccinimide (NIS; 80 mg, 0.36 mmol) and trifluoromethanesulfonic acid (TfOH; 3.1 μL, 0.036 mmol), and the stirring was continued for 3 h at −25 °C. The precipitate was removed by filtration and washed thoroughly with CH_2Cl_2. The filtrate and washings were combined, and the soln was successively washed with M $NaHCO_3$, M $Na_2S_2O_3$, and water, dried (Na_2SO_4), and concd. Column chromatography (40:1 CH_2Cl_2–MeOH) of the residue on silica gel gave **7** (32 mg, 23%) as an amorphous mass: $[\alpha]_D$ −8.1° (*c* 0.6, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 1.00 (m, 2 H, $Me_3SiCH_2CH_2$), 1.88, 1.96, 1.97, 2.00, 2.01, 2.03, 2.04, 2.09, 2.11 (9 s, 27 H, 7 AcO and 2 AcN), 2.42 (dd, 1 H, J_{gem} 12.6, $J_{3eq,4}$ 5.7 Hz, H-3d*eq*), 3.80 (s, 3 H, MeO), 4.66 (dd, 1 H, $J_{5,6}$ 3.8, J_{gem} 12.6 Hz, H-6a), 4.87 (m, 1 H, H-4d), 5.02 (d, 1 H, $J_{1,2}$ 9.5 Hz, H-1c), 5.04 (s, 1 H, H-3c), 5.12 (d, 1 H, $J_{2,3}$ 12.1 Hz, H-3c), 5.34 (dd, 1 H, $J_{6,7}$ 2.2, $J_{7,8}$ 9.5 Hz, H-7d), 5.35 (m, 1 H, H-8d), 5.63 (d, 1 H, $J_{NH,2}$ 9.5 Hz, NH), 7.24–7.55 (m, 20 H, 4 Ph). Anal. Calcd for $C_{79}H_{104}N_2O_{31}Si$ (1605.8): C, 59.09; H, 6.53; N, 1.74. Found: C, 58.93; H, 6.31; N, 1.45.

*2-(Trimethylsilyl)ethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-galactopyranosyl-(2 → 6)-[(methyl 5-acetamido-4,7,8,9-*tetra*-O-*acetyl*-3,5-*dideoxy*-D-glycero-α-D-galacto-2-*nonulopyranosylonate*)-(2 → 3)]-2,4-di-O-acetyl-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside* (**8**).—A soln of **7** (0.89 g, 0.55 mmol) in EtOH (23 mL) and CH_3COOH (23 mL) was hydrogenated in the presence of 10% Pd–C (0.9 g) for 24 h at 40 °C, then filtered and concd. The residue was acetylated with a mixture of Ac_2O (6 mL) and pyridine (9.8 mL) for 12 h at 40 °C. The product was purified by chromatography on a column of silica gel with 20:1 CH_2Cl_2–MeOH to give **8** (0.71 g, 88%) as an amorphous mass: $[\alpha]_D$ −19.0° (*c* 2.0, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 1.00 (m, 2 H, $Me_3SiCH_2CH_2$), 1.69 (t, 1 H, $J_{gem} = J_{3ax,4}$ = 12.3 Hz, H-3d*ax*), 1.85–2.19 (14 s, 42 H, 12 AcO and 2 AcN), 2.56 (dd, 1 H, $J_{3eq,4}$ 4.6 Hz, H-3d*eq*), 3.95 (d, 1 H, $J_{1,2}$ 9.7 Hz, H-1b), 4.50 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1a), 4.82 (d, 1 H, $J_{1,2}$ 6.6 Hz, H-1c), 4.97

(dd, 1 H, $J_{1,2} = J_{2,3} = 10.2$ Hz, H-2a), 5.12 (t, 1 H, $J_{2,3} = J_{3,4} = 8.8$ Hz, H-3a), 5.19 (d, 1 H, $J_{NH,2}$ 8.6 Hz, NH), 5.33 (dd, 1 H, $J_{6,7}$ 2.7, $J_{7,8}$ 8.6 Hz, H-7d), 5.41 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-4b), 5.64 (m, 1 H, H-8d), 5.76 (dd, 1 H, $J_{2,3}$ 11.4, $J_{3,4}$ 3.3 Hz, H-3c), 6.71 (d, 1 H, $J_{NH,2}$ 7.1 Hz, NH). Anal. Calcd for $C_{61}H_{90}N_2O_{36}Si$ (1455.4): C, 50.34; H, 6.23; N, 1.92. Found: C, 50.23; H, 6.19; N, 1.80.

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl-(2 → 6)-[(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)]-2,4-di-O-acetyl-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-O-acetyl-D-glucopyranose (**9**).—To a soln of **8** (0.56 g, 0.38 mmol) in dry CH_2Cl_2 (2.7 mL) was added CH_3COOH (2.6 mL), and the soln was stirred for 2 h at room temperature and then concd. Column chromatography (15:1 CH_2Cl_2–MeOH) of the residue on silica gel gave **9** (0.52 g, quantitative) as an amorphous mass: ^{1}H NMR ($CDCl_3$): δ 1.70 (t, 1 H, $J_{gem} = J_{3ax,4} = 10.8$ Hz, H-3d*ax*), 1.86–2.28 (14 s, 42 H, 12 AcO and 2 AcN), 2.57 (dd, 1 H, $J_{3eq,4}$ 4.7 Hz, H-3d*eq*). Anal. Calcd for $C_{61}H_{90}N_2O_{36}$ (1355.2): C, 49.63; H, 5.8; N, 2.07. Found: C, 49.54; H, 5.63; N, 1.84.

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl-(2 → 6)-[(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)]-2,4-di-O-acetyl-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-O-acetyl-α-D-glucopyranosyl trichloroacetimidate (**10**).—To a soln of **9** (0.47 g, 0.35 mmol) in CH_2Cl_2 (8 mL) and trichloroacetonitrile (1.45 mL) cooled to 0 °C was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 72.5 μL), and the soln was stirred for 2 h at 0 °C. The reaction mixture was directly chromatographed on a column of silica gel (40:1 CH_2Cl_2–MeOH) to give **10** (0.5 g, quantitative) as an amorphous mass: $[\alpha]_D$ +18.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.70 (t, 1 H, $J_{gem} = J_{3ax,4} = 10.83$ Hz, H-3d*ax*), 1.98–2.31 (14 s, 42 H, 12 AcO and 2 AcN), 2.57 (dd, 1 H, $J_{3eq,4}$ 4.9 Hz, H-3d*eq*), 3.64 (dd, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 3.5 Hz, H-3b), 3.87 (s, 3 H, MeO), 3.97 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1b), 4.95 (d, 1 H, $J_{1,2}$ 9.1 Hz, H-1c), 5.33 (dd, 1 H, $J_{6,7}$ 3.6, $J_{7,8}$ 11.3 Hz, H-7d), 5.42 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4b), 5.66 (m, 1 H, H-8d), 5.86 (dd, 1 H, $J_{2,3}$ 10.8, $J_{3,4}$ 2.9 Hz, H-3c), 6.45 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1a), 6.71 (d, 1 H, $J_{NH,2}$ 8.2 Hz, NH), 8.63 (s, 1 H, C=NH). Anal. Calcd for $C_{58}H_{78}Cl_3N_3O_{36}$ (1499.6): C, 46.45; H, 5.24; N, 2.80. Found: C, 46.45; H, 5.15; N, 2.69.

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl-(2 → 6)-[(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)]-2,4-di-O-acetyl-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1 → 1)-(2S,3R,4E)-2-azido-3-O-(tert-butyldiphenylsilyl)-4-octadecene-1,3-diol (**12**).—To a soln of **10** (0.5 g, 0.33 mmol) and (2*S*,3*R*,4*E*)-2-azido-3-*O*-(*tert*-butyldiphenylsilyl)-4-octadecene-1,3-diol (**11**; 0.37 g, 0.66 mmol) in dry CH_2Cl_2 (7.0 mL) was added molecular sieves (AW-300; 5 g), and the mixture was stirred for 5 h at room temperature, then cooled to 0 °C. To the cooled mixture was added trimethylsilyl trifluoromethanesulfonate (Me_3SiOTf; 130 μL, 0.66 mmol), and the mixture was stirred for 12 h at 0 °C and then filtered. The insoluble materials were washed with CH_2Cl_2, and the combined filtrate and washings was successively washed with M $NaHCO_3$ and water, dried (Na_2SO_4), and concd. Column chromatography (20:1 CH_2Cl_2–MeOH) of the residue on silica gel gave **12** (50 mg, 80%) as an amorphous mass: $[\alpha]_D$ −18.5° (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3 H, $J_{Me,CH2}$ 6.6 Hz, *Me*CH_2), 1.22 (s, 22 H, 11 CH_2), 1.66 (t, 1 H, $J_{gem} = J_{3ax,4} = 12.6$ Hz, H-3d*ax*), 1.88–2.29 (14 s, 42 H, 12 AcO and 2 AcN), 2.57 (dd, 1 H, $J_{3eq,4}$ 4.4 Hz, H-3d*eq*), 3.84 (s, 3 H, MeO), 3.96 (d, 1 H, $J_{1,2}$ 6.4 Hz, H-1b), 4.83 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1c), 4.95 (dd, 1 H, $J_{1,2}$ 8.4, $J_{2,3}$ 9.5 Hz, H-2a), 5.08 (t, 1 H, $J_{2,3} = J_{3,4} = 9.3$ Hz, H-3a), 5.17 (d, 1 H, $J_{NH,2}$ 5.3 Hz, NH), 5.33 (dd, 1 H, $J_{6,7}$ 3.6, $J_{7,8}$ 11.2 Hz, H-7d), 5.42 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4b), 5.66 (m, 1 H, H-8d), 5.73 (dd, 1 H, $J_{2,3}$ 8.6, $J_{3,4}$ 3.8 Hz, H-3c), 6.58 (d, 1 H, $J_{NH,2}$ 7.7 Hz, NH), 7.27–7.67 (m, 10 H, 2 Ph). Anal. Calcd for $C_{90}H_{129}N_5O_{37}Si$ (1901.1): C, 56.86; H, 6.84; N, 3.68. Found: C, 56.57; H, 6.83; N, 3.57.

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl-(2 → 6)-[(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)]-2,4-di-O-acetyl-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1 → 1)-(2S,3R,4E)-3-O-(tert-butyldiphenylsilyl)-2-octadecanamido-4-octadecene-1,3-diol (**14**).—To a soln of **12** (300 mg, 0.16 mmol) in benzene (13.5 mL) and water (0.6 mL) was added triphenylphosphine (84 mg, 0.32 mmol), and the mixture was stirred for 24 h at 30 °C and concd to give the crude amine **13**. A soln of the residue in dry CH_2Cl_2 (9 mL) was stirred with octadecanoic acid (136 mg, 0.48 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC; 92 mg, 0.48 mmol) for 24 h at room temperature.

Dichloromethane (120 mL) was added, and the soln was washed with water, dried (Na_2SO_4), and concd. Column chromatography (30:1 CH_2Cl_2–MeOH) of the residue on silica gel gave **15** (236 mg, 70%) as an amorphous mass: $[\alpha]_D$ −14.7° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (t, 6 H, $J_{Me,CH2}$ 6.4 Hz, 2 *Me*CH_2), 1.22 (s, 52 H, 26 CH_2), 1.85–2.30 (14 s, 42 H, 12 AcO and 2 AcN), 2.57 (dd, 1 H, $J_{3eq,4}$ 4.8, J_{gem} 12.8 Hz, H-3d*eq*), 4.97 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 8.2 Hz, H-2a), 5.39 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-4b), 5.66 (m, 1 H, H-8d), 5.73 (dd, 1 H, $J_{2,3}$ 11.5, $J_{3,4}$ 4.1 Hz, H-3c), 6.53 (d, 1 H, $J_{NH,2}$ 6.7 Hz, NH), 7.27–7.73 (m, 10 H, 2 Ph). Anal. Calcd for $C_{108}H_{165}N_3O_{38}Si$ (2141.6): C, 60.57; H, 7.77; N, 1.96. Found: C, 60.42; H, 7.57; N, 1.88.

*2-Acetamido-2-deoxy-β-*D*-galactopyranosyl-[(5-acetamido-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosylonic acid)-(2 → 3)]-O-(2 → 6)-β-*D*-galactopyranosyl-(1 → 4)-β-*D*-glucopyranosyl-(1 → 1)-(2S,3R,4E)-2-octadecanamido-4-octadecene-1,3-diol* (**15**).—To a soln of **14** (300 mg, 0.16 mmol) in acetonitrile (5 mL) was added tetrabutylammonium fluoride (TBAF), and the soln was stirred for 24 h at 30 °C, and water (0.5 mL) was added. The soln was stirred for an additional 24 h at room temperature, then it was treated with Amberlite IR-120 (H^+) resin and filtered. The resin was washed with MeOH, and the combined filtrate and washings was concd. Column chromatography (5:5:1 $CHCl_3$–MeOH–H_2O) of the residue on Sephadex LH-20 gave **15** (87 mg, quantitative) as an amorphous mass: $[\alpha]_D$ +30.0° (*c* 1.0, 5:5:1 $CHCl_3$–MeOH–H_2O); ^1H NMR [$(CD_3)_2SO$]: δ 0.88 (t, 6 H, $J_{Me,CH2}$ 6.4 Hz, 2 *Me*CH_2), 1.22 (s, 52 H, 26 CH_2), 1.85 and 2.30 (2 s, 6 H, 2 AcN), 2.71 (dd, 1 H, J_{gem} 7.2, $J_{3eq,4}$ 4.8 Hz, H-d*eq*), 4.83 (d, 1 H, $J_{1,2}$ 6.0 Hz, H-1a), 4.89 (d, 1 H, $J_{1,2}$ 6.1 Hz, H-1c), 5.31, 5.51 (2 m, 2 H, H-4,5 for Cer unit). Anal. Calcd for $C_{66}H_{120}N_3O_{24}$ (1339.68): C, 59.17; H, 9.03; N, 3.14. Found: C, 58.90; H, 8.93; N, 3.12.

Acknowledgements

This work was supported in part by Grants-in-Aid for Scientific Research (B) (No. 07456162) and Scientific Research on Priority Areas (No. 05274102) from the Ministry of Education, Science and Culture of Japan, and a grant (NS 09626) from the United States National Institutes of Health.

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