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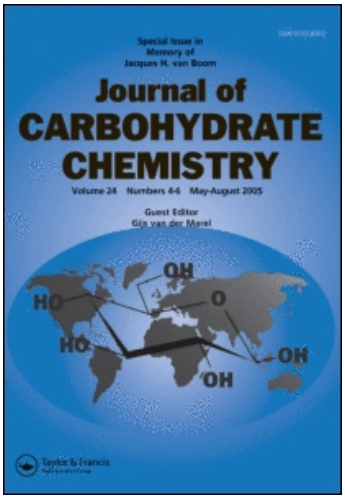

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Synthetic Studies on Sialoglycoconjugates 73: Synthesis of KDN-α-(2→6)-lactotetraosylceramide and KDN-α-(2→6)-neolactotetraosylceramide

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SYNTHETIC STUDIES ON SIALOGLYCOCONJUGATES 73: SYNTHESIS OF KDN-α-(2→6)-LACTOTETRAOSYLCERAMIDE AND KDN-α-(2→6)-NEOLACTOTETRAOSYLCERAMIDE

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ABSTRACT

Analogs of sialyl-α(2→6)-lactotetraosylceramide and sialyl-α(2→6)-neolactotetraosylceramide, in which the *N*-acetylneuraminic acid residue is replaced by a 3-deoxy-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid (KDN) unit, have been synthesized. Methyl *O*-(methyl 4,5,7,8,9-penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-2,4-di-*O*-benzoyl-3-*O*-benzyl-1-thio-β-D-galactopyranoside (**6**) was prepared from the phenyl β-thioglycoside derivative **1** of KDN and 2-(trimethylsilyl)ethyl 3-*O*-benzyl-β-D-galactopyranoside (**2**) in four steps. Each coupling of 2-(trimethylsilyl)ethyl *O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→3')-per-*O*-benzyl-β-lactoside (**7**) or 2-(trimethylsilyl)ethyl *O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3')-per-*O*-benzyl-β-D-lactoside (**8**), with **6** gave the pentasaccharides **9** and **13** in good yields. Compounds **9** and **13** were converted into the corresponding α-trichloroacetimidates **12** and **16** which on glycosylation with (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (**17**), gave the β-glycosides **18** and **21**, respectively. Finally, **18** and **21** were transformed, *via* selective reduction of the azido group, condensation with octadecanoic acid, *O*-deacylation, and saponification of the methyl ester group, into the target compounds **20** and **23**, respectively.

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INTRODUCTION

Sialic acids are known as the unique acidic component of glycolipids and glycoproteins, and play important roles in a variety of biological processes. More than 20 variant forms of sialic acid have been isolated from various gangliosides and glycoproteins, all derived, according to current knowledge, from two parent structures, namely *N*-acetyl- and *N*-glycosylneuraminic acid. In 1988, a novel type of sialic acid, 3-deoxy-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid (KDN), in which the acetamido group at C-5 of *N*-acetylneuraminic acid is replaced by a hydroxyl, was isolated[1] from the vitelline envelope of rainbow trout eggs. In 1991, KDN-ganglioside GM_3 was isolated[2] from rainbow trout sperm, and this first finding of a KDN-ganglioside indicated the possible wide occurrence[3] of this class of KDN-glycoconjugates in nature. However, the biological function of KDN-ganglioside has not been investigated, because only a limited quantity has been available.

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We have developed[4] a systematic synthesis of gangliosides and their analogs, based on a facile α-glycoside synthesis[5] of sialic acids, achieved by activation of their thioglycosides with dimethyl(methylthio)sulfonium triflate[5a,6] (DMTST) or *N*-iodosuccinimide-trifluoromethanesulfonic acid[7,8] (NIS-TfOH) in acetonitrile. Previously, we have reported the synthesis[9,10] of KDN-gangliosides by our procedure. As a part of our continuing studies on the synthesis and elucidation of the functions of sialoglycoconjugates, we now describe the synthesis of KDN-α(2→6)-lactotetraosylceramide and KDN-α(2→6)-neolactotetraosylceramide.

RESULTS AND DISCUSSION

For the synthesis of the desired KDN-gangliosides, we employed methyl *O*-(methyl 4,5,7,8,9-penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-2,4-di-*O*-benzoyl-3-*O*-benzyl-1-thio-β-D-galactopyranoside (**6**) as a glycosyl donor and 2-(trimethylsilyl)ethyl *O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-

2,3,6-tri-*O*-benzyl-β-D-glucopyranoside[12] (**7**) and 2-(trimethylsilyl)ethyl *O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside[10] (**8**) as the suitably protected glycosyl acceptors. The acceptor **7** or **8** was coupled with the donor **6** using DMTST as a promoter, to afford the corresponding pentasaccharides **9** and **13**. By further processing according to our usual procedures[13] the resulting pentasaccharide intermediates could be converted into the end products by introduction of a ceramide moiety.

The glycosylation[9] of 2-(trimethylsilyl)ethyl 3-*O*-benzyl-β-D-galactopyranoside[14] (**2**) with methyl (phenyl 4,5,7,8,9-penta-*O*-acetyl-3-deoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate[9] (**1**) in dry acetonitrile in the presence NIS-trimethylsilyl trifluoromethanesulfonate and powdered molecular sieves 3Å for 2 h at -40 °C, gave exclusively the α-glycoside **3** in 48% yield; significant signals of the KDN residue in the ^{1}H NMR spectrum were a one-proton doublet of doublets at δ 2.65 (J_{gem} = 13.2, $J_{3eq,4}$ = 4.6 Hz, H-3*eq*), at δ 4.90 (ddd, H-4), and a one-proton doublet of doublets at δ 5.37 ($J_{6,7}$ = 2.4, $J_{7,8}$ = 10.2 Hz, H-7), indicating the formed glycosidic linkage to be α, as anticipated. The remaining hydroxyls in **3** were benzoylated with benzoyl chloride to afford **4**; the ^{1}H NMR data for the Gal residue [δ 5.08 ($J_{1,2}$ = 7.9, $J_{2,3}$= 8.1 Hz, H-2) and 5.58 ($J_{3,4}$ = 3.1 Hz, H-4)] established the linkage position of KDN to be the 6-OH. Treatment[15] of **4** with boron trifluoride etherate in toluene in the presence of acetic anhydride gave the 1-β-acetate **5** in 92% yield. The replacement[12,16] of the anomeric acetoxy group in **5** with methylthio by stirring for 4 h at 0 °C with trimethyl(methylthio)silane in dry dichloromethane in the presence of boron trifluoride etherate gave the methyl β-1-thioglycoside derivative **6** in 98% yield. The glycosylation[11] of **7** or **8** with **6** in dry dichloromethane in the presence of DMTST and powdered molecular sieves 4Å gave the expected pentasaccharides **9** (77%) and **13** (81%), respectively. The ^{1}H NMR data for the Gal unit in **9** [δ 4.58 ($J_{1,2}$ = 8.3 Hz, H-1), 5.23 (t, $J_{2,3}$ = 8.3 Hz, H-2)] and in **13** [δ 4.55 ($J_{1,2}$ = 7.5 Hz, H-1), 5.34 (t, $J_{2,3}$ = 7.5 Hz, H-2)] established the newly formed glycosidic linkage to be β.

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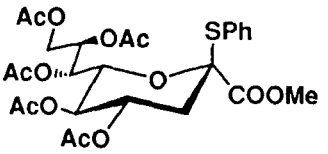

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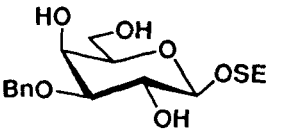

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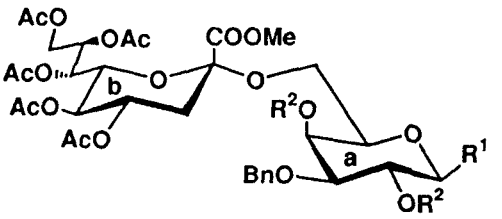
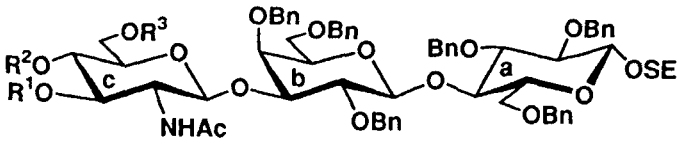


	R^1	R^2
3	OSE	H
4	OSE	Bz
5	OAc	Bz
6	SMe	Bz



	R^1	R^2	R^3
7	H	benzylidene	
8	Bn	H	Bn

SE = 2-(trimethylsilyl)ethyl
Bz = benzoyl
Bn = benzyl

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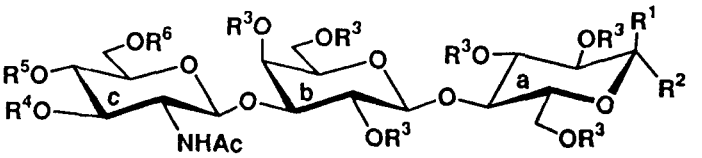
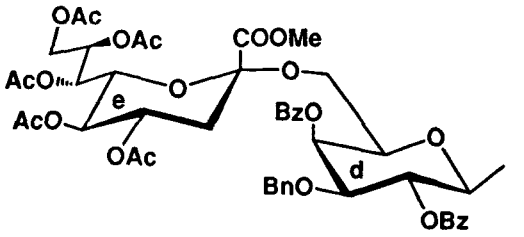


	R^1	R^2	R^3	R^4	R^5	R^6
9	H	OSE	Bn	X	benzylidene	
10	H	OSE	Ac	Y	Ac	Ac
11	H , OH		Ac	Y	Ac	Ac
12	$OC(=NH)CCl_3$	H	Ac	Y	Ac	Ac
13	H	OSE	Bn	Bn	X	Bn
14	H	OSE	Ac	Ac	Y	Ac
15	H , OH		Ac	Ac	Y	Ac
16	$OC(=NH)CCl_3$	H	Ac	Ac	Y	Ac

X =



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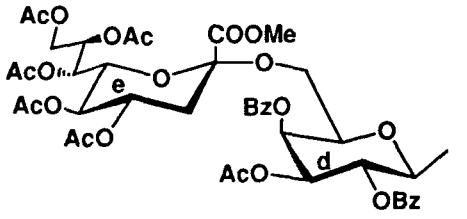

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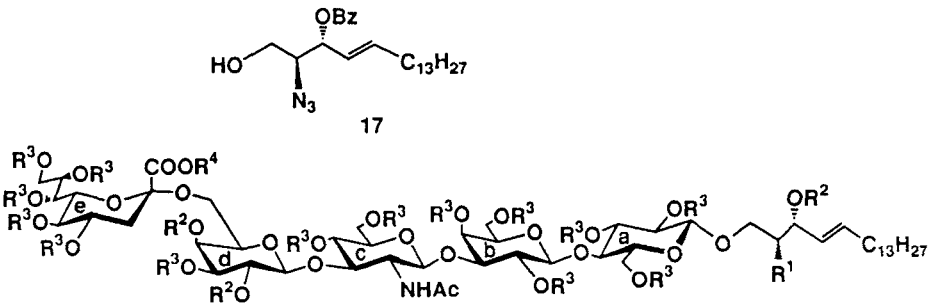
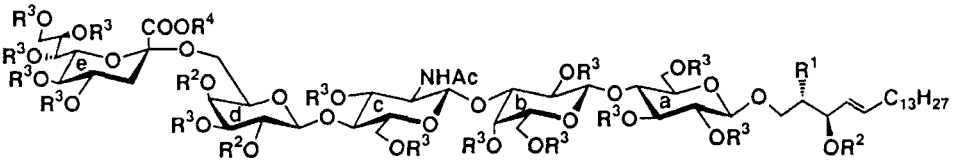


17

	R^1	R^2	R^3	R^4
18	N_3	Bz	Ac	Me
19	$NHCO(CH_2)_{16}Me$	Bz	Ac	Me
20	$NHCO(CH_2)_{16}Me$	H	H	H



	R^1	R^2	R^3	R^4
21	N_3	Bz	Ac	Me
22	$NHCO(CH_2)_{16}Me$	Bz	Ac	Me
23	$NHCO(CH_2)_{16}Me$	H	H	H

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Catalytic hydrogenolysis (10% Pd-C) in ethanol-acetic acid of the benzyl and benzylidene groups in **9** or the benzyl groups in **13** and subsequent *O*-acetylation gave the corresponding per-*O*-acyl pentasaccharide derivatives **10** and **14** in good yields, respectively. Treatment[15a,17] of compound **10** or **14** with trifluoroacetic acid in dichloromethane gave the 1-hydroxy compounds **11** and **15**, which were reacted with trichloroacetonitrile in dichloromethane in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to give the corresponding α-trichloroacetimidates **12** and **16** in high yields. The ^{1}H NMR data for the Glc unit in **12** [δ 6.47 ($J_{1,2}$ = 3.9 Hz, H-1), 8.65 (C = NH)] and in **16** [δ 6.45 ($J_{1,2}$ = 3.7 Hz, H-1), 8.64 (C = NH)] established the anomeric configuration of the imidates.

The final glycosylation[13,18] of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[19] (**17**) with **12** or **16** thus obtained, in dichloromethane in the presence of boron trifluoride etherate and molecular sieves 4Å for 6 h at room temperature, gave the

desired β-glycosides **18** and **21**, in 64 and 62% yields, respectively. Selective reduction[13,20] of the azido group in **18** and **21** with hydrogen sulfide in aq 83% pyridine and subsequent condensation with octadecanoic acid, using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC) in dichloromethane furnished good yields of the corresponding acylated ganglioside analogs **19** and **22**, which were transformed *via O*-deacylation with sodium methoxide in methanol, with subsequent saponification of the sialate methyl ester group, into the desired KDN-α(2→6)-lactotetraosylceramide (**20**) and KDN-α(2→6)-neolactotetraosylceramide (**23**) in good yields.

EXPERIMENTAL

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General Procedures. Specific rotations were determined with a Union PM-201 polarimeter at 25 °C. ^{1}H NMR spectra were recorded with a JEOL JNM-GX 270 spectrometer. Preparative chromatography was performed on silica gel (Wako Chemical Co., 200 mesh) with the solvent systems specified. Concentrations were conducted *in vacuo*.

2-(Trimethylsilyl)ethyl *O*-(Methyl 4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-3-*O*-benzyl-β-D-galactopyranoside (3). To a solution of methyl (phenyl 4,5,7,8,9-penta-*O*-acetyl-3-deoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate[9] (**1**, 2.2 g, 3.8 mmol) and 2-(trimethylsilyl)ethyl 3-*O*-benzyl-β-D-galactopyranoside[11] (**2**) (**2**, 1.0 g, 2.7 mmol) in dry CH_3CN (15 mL) were added powdered molecular sieves 3Å (MS-3Å, 3 g) and the mixture was stirred for 12 h at room temperature then cooled to -40 °C. *N*-Iodosuccinimide (NIS, 1.38 g, 5.6 mmol) and trimethylsilyl trifluoromethanesulfonate (TMS•OTf, 0.07 mL, 0.37 mmol) were added to the mixture and it was stirred for 2 h at -40 °C. After dilution with CH_2Cl_2 (50 mL) the solids were collected and washed with CH_2Cl_2, and the combined filtrate and washings were washed with M Na_2CO_3, M $Na_2S_2O_3$ and water, dried (Na_2SO_4), and concentrated. Column chromatography (100:1 toluene-MeOH) of the residue on silica gel (100 g)

gave **3** (1.1 g, 48%) as an amorphous mass: $[\alpha]_D$ -14.3° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.99 (m, 2H, $Me_3SiCH_2CH_2$), 1.92, 1.97, 2.01, 2.06, 2.13 (5s, 15H, 5AcO), 2.65 (dd, 1H, J_{gem} = 13.2 Hz, $J_{3eq,4}$ = 4.6 Hz, H-3b*eq*), 3.79 (s, 3H, MeO), 4.84 (t, 1H, $J_{4,5} = J_{5,6}$ = 10.8 Hz, H-5b), 4.90 (ddd, 1H, $J_{3ax,4}$ = 12.7 Hz, H-4b), 5.37 (dd, 1H, $J_{6,7}$ = 2.4 Hz, $J_{7,8}$ = 10.2 Hz, H-7b), 5.38 (m, 1H, H-8b), and 7.21-8.27 (m, 5H, Ph).

Anal. Calcd for $C_{38}H_{56}O_{19}Si$ (844.9): C, 54.02; H, 6.68. Found: C, 53.97; H, 6.47.

2-(Trimethylsilyl)ethyl *O*-(Methyl 4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-2,4-di-*O*-benzoyl-3-*O*-benzyl-β-D-galactopyranoside (4). To a solution of **3** (1.0 g, 1.2 mmol) in pyridine (2 mL) was added a solution of benzoyl chloride (0.55 mL, 4.7 mmol) in CH_2Cl_2 (12 mL) and the mixture was stirred at room temperature. After completion of the reaction the mixture was diluted with CH_2Cl_2 and the solution was successively washed with 2M HCl, M Na_2CO_3 and water, dried (Na_2SO_4), and concentrated. Column chromatography (1:2 EtOAc-hexane) of the residue on silica gel (100 g) gave **4** (1.14 g, 92%) as an amorphous mass: $[\alpha]_D$ +42.5° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.98 (m, 2H, $Me_3SiCH_2CH_2$), 2.00, 2.01, 2.03, 2.06, 2.13 (5s, 15H, 5AcO), 2.66 (dd, 1H, J_{gem} = 12.8 Hz, $J_{3eq,4}$ = 4.0 Hz, H-3b*eq*), 3.82 (s, 3H, MeO), 4.40 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1a), 5.08 (dd, 1H, $J_{2,3}$ = 8.1 Hz, H-2a), 5.33 (dd, 1H, $J_{6,7}$ = 2.1 Hz, $J_{7,8}$ = 9.2 Hz, H-7b), 5.37 (m, 1H, H-8b), 5.58 (br d, 1H, $J_{3,4}$ = 3.1 Hz, H-4a), and 7.25-8.20 (m, 15H, 3Ph).

Anal. Calcd for $C_{52}H_{64}O_{21}Si$ (1053.2): C, 59.31; H, 6.13. Found: C, 59.10; H, 5.90.

***O*-(Methyl 4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-1-*O*-acetyl-2,4-di-*O*-benzoyl-3-*O*-benzyl-β-D-galactopyranose (5).** To a solution of **4** (1.41 g, 1.3 mmol) in dry toluene (8 mL) and Ac_2O (2 mL), cooled to 0 °C, was added BF_3•OEt_2 (0.15 mL, 1.2 mmol) and the mixture was stirred for 2 h at 0 °C then diluted with CH_2Cl_2 (50 mL). The solution was washed with M Na_2CO_3, dried (Na_2SO_4) and concentrated.

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Column chromatography (2:1 EtOAc-hexane) of the residue on silica gel (100 g) gave **5** (1.12 g, 83%) as an amorphous mass: $[\alpha]_D$ +60.0° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.00, 2.01, 2.04, 2.05, 2.09, 2.10 (6s, 18H, 6AcO), 2.63 (dd, 1H, J_{gem} = 13.4 Hz, $J_{3eq,4}$ = 4.4 Hz, H-3b*eq*), 3.38 (s, 3H, MeO), 3.87 (dd, 1H, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 3.5 Hz, H-3a), 4.85 (m, 1H, H-4b), 5.31 (dd, 1H, $J_{6,7}$ = 2.0 Hz, $J_{7,8}$ = 8.8 Hz, H-7b), 5.41 (m, 1H, H-8b), 5.56 (t, 1H, $J_{1,2}$ = 10.1 Hz, H-2a), 5.86 (d, 1H, H-1), and 7.02-8.17 (m, 15H, 3Ph).

Anal. Calcd for $C_{49}H_{54}O_{22}$ (995.0): C, 59.15; H, 5.47. Found: C, 59.05; H, 5.21.

Methyl *O*-(Methyl 4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-2,4-di-*O*-benzoyl-3-*O*-benzyl-1-thio-β-D-galactopyranoside (6). To a solution of **5** (920 mg, 0.91 mmol) in dry CH_2Cl_2 (6 mL) , cooled to 0 °C, were added Me_3SiSMe (0.5 mL, 2.3 mmol) and BF_3•OEt_2 (0.35 mL, 2.8 mmol) and the mixture was stirred for 4 h at 0 °C. The solution was diluted with CH_2Cl_2 (50 mL) and successively washed with M Na_2CO_3 and water, dried (Na_2SO_4) and concentrated. Column chromatography (1:3 EtOAc-hexane) of the residue on silica gel (80 g) gave **6** (890 mg, 98%) as an amorphous mass: $[\alpha]_D$ +55.6° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.99, 2.00, 2.03, 2.10, 2.11, 2.22 (6s, 18H, 5AcO, MeS), 2.63 (dd, 1H, J_{gem} = 13.2 Hz, $J_{3eq,4}$ = 4.2 Hz, H-3b*eq*), 3.38 (s, 3H, MeO), 4.56 (d, 1H, $J_{1,2}$ = 10.1 Hz, H-1a), 4.84 (t, 1H, $J_{4,5}$ = $J_{5,6}$ = 9.0 Hz, H-5b), 5.30 (dd, 1H, $J_{6,7}$ = 1.8 Hz, $J_{7,8}$ = 9.0 Hz, H-7b), 5.39 (m, 1H, H-8b), 5.55 (t, 1H, $J_{2,3}$ = 10.1 Hz, H-2a), and 7.04-8.12 (m, 15H, 3Ph).

Anal. Calcd for $C_{48}H_{54}O_{20}$ (983.0): C, 58.65; H, 5.54. Found: C, 58.47; H, 5.53.

2-(Trimethylsilyl)ethyl *O*-(Methyl 4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-*O*-(2,4-di-*O*-benzoyl-3-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (9). To a solution of **6** (496 mg, 0.5 mmol) and **7**[12] (500 mg, 0.39 mmol)

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in CH_2Cl_2 (4 mL) were added powdered molecular sieves 4Å (MS-4Å, 2 g) and the mixture was stirred for 12 h at room temperature then cooled to 0 °C. A mixture of DMTST (516 mg, 2.0 mmol) and MS-4Å (240 mg) was added, and the mixture was stirred for 48 h at 7 °C. The solids were collected and washed with CH_2Cl_2 and the combined filtrate and washings was washed with M Na_2CO_3 and water, dried (Na_2SO_4) and concentrated. Column chromatography (2:3 EtOAc-hexane) of the residue on silica gel (100 g) gave **9** (670 mg, 77%) as an amorphous mass: $[\alpha]_D$ +27.0° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.98 (m, 2H, $Me_3SiCH_2CH_2$), 1.90, 1.97, 1.99, 2.01, 2.03, 2.10 (6s, 18H, 5AcO, AcN), 2.64 (dd, 1H, J_{gem} = 13.2 Hz, $J_{3eq,4}$ = 4.8 Hz, H-3e*eq*), 3.27 (s, 3H, MeO), 4.58 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1d), 5.23 (t, 1H, $J_{2,3}$ = 8.3 Hz, H-2d), 5.33 (dd, 1H, $J_{6,7}$ = 2.4 Hz, $J_{7,8}$ = 9.4 Hz, H-7e), 5.44 (m, 1H, H-8e), and 6.93-8.15 (m, 50H, 10Ph).

Anal. Calcd for $C_{121}H_{137}NO_{36}Si$ (2209.5): C, 65.78; H, 6.25; N, 0.63. Found: C, 65.56; H, 6.25; N, 0.61.

2-(Trimethylsilyl)ethyl *O*-(Methyl 4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-*O*-(3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (10). A solution of **9** (261 mg, 0.12 mmol) in EtOH (80 mL) and AcOH (8 mL) was hydrogenolyzed in the presence of 10% Pd-C (300 mg) for 48 h at 40 °C, then filtered and concentrated. The residue was acetylated with Ac_2O (3 mL) in pyridine (5 mL) for 24 h at 40 °C. The product was purified by chromatography on a column of silica gel (60 g) with 50:1 CH_2Cl_2-MeOH, affording **10** (170 mg, 77%) as an amorphous mass: $[\alpha]_D$ +10.5° (*c* 1.3, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.98 (m, 2H, $Me_3SiCH_2CH_2$), 1.84-2.11 (14s, 42H, 13AcO, AcN), 2.57 (dd, 1H, J_{gem} = 12.5 Hz, $J_{3eq,4}$ = 4.8 Hz, H-3e*eq*), 3.25 (s, 3H, MeO), 4.46 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1a), 4.66 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1d), 4.81 (d, 1H, $J_{2,3}$ = 7.9 Hz, H-2a), 5.14 (t, 1H, $J_{3,4}$ = 7.9 Hz, H-3a), 5.27 (br d, 1H, $J_{3,4}$ = 3.1 Hz, H-4b), 5.32 (dd, 1H, $J_{6,7}$ = 2.5 Hz, $J_{7,8}$ = 9.4 Hz, H-7e), 5.39 (t, 1H, $J_{2,3}$ = 7.5 Hz, H-2d), 5.71 (br d, 1H, $J_{3,4}$ = 3.1 Hz, H-4d), and 7.46-8.12 (m, 10H, 2Ph).

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Anal. Calcd for $C_{83}H_{109}NO_{45}Si$ (1868.8): C, 53.34; H, 5.88; N, 0.75. Found: C, 53.13; H, 5.59; N, 0.66.

***O*-(Methyl 4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-*O*-(3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (12).** To a solution of **10** (52.7 mg, 25 μmol) in CH_2Cl_2 (0.5 mL) at 0 °C was added CF_3CO_2H (1.0 mL), and the mixture was stirred for 2 h at room temperature and concentrated. The product was purified by chromatography on a column of silica gel (30 g) with 30:1 CH_2Cl_2-MeOH to give the 1-hydroxy compound (**11**, 49.2 mg, quantitative). To a solution of **11** (125 mg, 0.07 mmol) in CH_2Cl_2 (0.7 mL) cooled to -5 °C were added trichloroacetonitrile (0.35 mL), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 12 mg) and Drierite (200 mg), and the mixture was stirred for 1 h at 0 °C then directly applied to a column of silica gel (30 g) eluted with 30:1 CH_2Cl_2-MeOH, affording **12** (135 mg, quantitative) as an amorphous mass: $[\alpha]_D$ +39.5° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.25-2.16 (14s, 42H, 13AcO, AcN), 2.57 (dd, 1H, J_{gem} = 14.1 Hz, $J_{3eq,4}$ = 4.6 Hz, H-4e*eq*), 3.62 (s, 3H, MeO), 5.04 (dd, 1H, $J_{1,2}$ = 3.9 Hz, $J_{2,3}$ = 10.3 Hz, H-2a), 5.12 (d, 1H, NH), 5.71 (br d, 1H, $J_{3,4}$ = 2.8 Hz, H-4d), 6.47 (d, 1H, H-1a), 7.27-8.12 (m, 10H, 2Ph) and 8.65 (s, 1H, C=NH).

Anal. Calcd for $C_{80}H_{97}N_2O_{45}$ (1913.0): C, 50.23; H, 5.11; N, 1.46. Found: C, 50.09; H, 4.97; N, 1.33.

2-(Trimethylsilyl)ethyl *O*-(Methyl 4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-*O*-(2,4-di-*O*-benzoyl-3-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (13). Glycosylation of **8**[10] (507 mg, 0.37 mmol) with **6** (511 mg, 0.52 mmol) in dry CH_2Cl_2 (4 mL) in the presence of DMTST (533 mg, 2.06 mmol) and MS-4Å (2 g) for 48 h at 7 °C and workup as described for **9** gave **13** (700 mg, 81%)

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as an amorphous mass: $[\alpha]_D$ +10.5° (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.00 (m, 2H, $Me_3SiCH_2CH_2$), 1.50 (s, 3H, AcN), 1.95, 1.99, 2.00, 2.04, 2.05 (5s, 15H, 5AcO), 2.61 (dd, 1H, J_{gem} = 13.8 Hz, $J_{3eq,4}$ = 4.8 Hz, H-3e*eq*), 3.27 (s, 3H, MeO), 4.55 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1d), 5.32 (dd, 1H, $J_{6,7}$ = 2.0 Hz, $J_{7,8}$ = 9.2 Hz, H-7e), 5.34 (t, 1H, $J_{2,3}$ = 7.5 Hz, H-2d), 5.83 (br d, 1H, $J_{3,4}$ = 3.1 Hz, H-4d), and 7.05-8.09 (m, 55H, 11Ph).

Anal. Calcd for $C_{128}H_{142}NO_{38}Si$ (2330.6): C, 65.97; H, 6.14; N, 0.60. Found: C, 65.75; H, 6.09; N, 0.54.

2-(Trimethylsilyl)ethyl *O*-(Methyl 4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-*O*-(3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (14). Hydrogenolysis of **13** (263 mg, 0.11 mmol) in EtOH (30 mL) and AcOH (6 mL) in the presence of Pd-C (300 mg) for 48 h at 40 °C, and subsequent acetylation with Ac_2O (2 mL) in pyridine (5 mL) as described for **10** gave **14** (178 mg, 85%) as an amorphous mass: $[\alpha]_D$ +1.3° (*c* 1.4, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.02 (m, 2H, $Me_3SiCH_2CH_2$), 1.85-2.15 (14s, 42H, 13AcO, AcN), 2.60 (dd, 1H, J_{gem} = 12.5 Hz, $J_{3eq,4}$ = 4.2 Hz, H-3e*eq*), 3.41 (s, 3H, MeO), 4.30 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1b), 4.46 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1a), 4.60 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1d), 4.83 (t, 1H, $J_{2,3}$ = 7.9 Hz, H-2a), 5.15 (t, 1H, $J_{3,4}$ = 7.9 Hz, H-3a), 5.46 (dd, 1H, $J_{2,3}$ = 9.6 Hz, H-2d), 5.66 (d, 1H, NH), 5.79 (br d, 1H, $J_{3,4}$ = 2.9 Hz, H-4d), and 7.42-8.11 (m, 10H, 2Ph).

Anal. Calcd for $C_{83}H_{109}NO_{45}Si$ (1868.3): C, 53.34; H, 5.88; N, 0.75. Found: C, 53.14; H, 5.70; N, 0.70.

***O*-(Methyl 4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-*O*-(3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl trichloroacetimi-**

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date (16). Selective removal of the 2-(trimethylsilyl)ethyl group in **14** (52.7 mg, 0.028 mmol) with CF_3CO_2H (0.5 mL) in CH_2Cl_2 (0.5 mL) for 4 h at 0 °C, and subsequent reaction with trichloroacetonitrile (0.2 mL) in CH_2Cl_2 (0.5 mL) in the presence of DBU (20 mg) and Drierite (50 mg) for 2 h at 0 °C as described for **12** gave **16** (51.5 mg, 94%) as an amorphous mass: $[\alpha]_D$ +22.5° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.70-2.15 (14s, 42H, 13AcO, AcN), 2.57 (dd, 1H, J_{gem} = 14.6 Hz, $J_{3eq,4}$ = 4.3 Hz, H-3e*eq*), 3.71 (s, 3H, MeO), 4.33 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1b), 4.60 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1d), 4.97 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 10.3 Hz, H-3a), 5.04 (dd, 1H, $J_{1,2}$ = 3.7 Hz, H-2a), 5.54 (t, 1H, $J_{2,3}$ = 7.8 Hz, H-2d), 5.77 (br d, 1H, $J_{3,4}$ = 2.9 Hz, H-4d), 6.45 (d, 1H, H-1a), 7.41-8.11 (m, 10H, 2Ph), and 8.64 (s, 1H, C=NH).

Anal. Calcd for $C_{80}H_{97}N_2O_{45}$ (1913.0): C, 50.23; H, 5.11; N, 1.46. Found: C, 50.11; H, 5.02; N, 1.45.

***O*-(Methyl 4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-*O*-(3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (18).** To a solution of **12** (137 mg, 0.072 mmol) and (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[19] (**17**, 61.6 mg, 0.172 mmol) in CH_2Cl_2 (0.8 mL) were added MS-4Å (700 mg) and the mixture was stirred for 5 h at room temperature then cooled to 0 °C. Boron trifluoride etherate (26 μL) was added and the mixture was stirred for a further 6 h at room temperature. The solids were filtered off and washed with CH_2Cl_2, and the combined filtrate and washings was washed with M Na_2CO_3 and water, dried (Na_2SO_4) and concentrated. Column chromatography (100:1 CH_2Cl_2-MeOH) of the residue on silica gel (50 g) gave **18** (101 mg, 64%) as an amorphous mass: $[\alpha]_D$ +10.0° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, CH_3CH_2), 1.24 (s, 22H, $11CH_2$), 2.58 (dd, 1H, J_{gem} = 11.1 Hz, $J_{3eq,4}$ = 4.7 Hz, H-3e*eq*), 3.28 (s, 3H, MeO), 4.34 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1b), 4.64 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1d),

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4.90 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1a), 5.14 (t, 1H, $J_{2,3}$ = 7.7 Hz, H-2a), 5.30 (t, 1H, $J_{2,3}$ = 7.9 Hz, H-2b), 5.71 (br d, 1H, $J_{3,4}$ = 2.8 Hz, H-4d), 5.94 (dt, 1H, $J_{4,5}$ = 14.3 Hz, $J_{5,6}$ = $J_{5,6'}$ = 7.3 Hz, H-5 of sphingosine), and 7.28-8.42 (m, 15H, 3Ph).

Anal. Calcd for $C_{103}H_{134}N_4O_{47}$ (2180.0): C, 56.74; H, 6.20; N, 2.57. Found: C, 56.71; H, 6.08; N, 2.43.

***O*-(Methyl 4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-*O*-(3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (19).** Hydrogen sulfide was bubbled through a stirred solution of **18** (100 mg, 0.022 mmol) in aq 83% pyridine (10 mL) for 3 days at 0 °C, with the progress of the reaction monitored by TLC. The mixture was concentrated and the residue was stirred with octadecanoic acid (26.2 mg, 0.092 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC, 17.6 mg, 0.092 mmol) in dry CH_2Cl_2 (2 mL) overnight at room temperature. After completion of the reaction, CH_2Cl_2 (20 mL) was added to the mixture, and the solution was washed with water, dried (Na_2SO_4) and concentrated. Column chromatography (60:1 CH_2Cl_2-MeOH) of the residue on silica gel (30 g) afforded **19** (70 mg, 63%) as an amorphous mass: $[\alpha]_D$ +19.0° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.90 (t, 6H, $2CH_3CH_2$), 1.25 (s, 52H, $26CH_2$), 2.56 (dd, 1H, J_{gem} = 10.2 Hz, $J_{3eq,4}$ = 4.5 Hz, H-3e*eq*), 3.73 (s, 3H, MeO), 4.28 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1b), 4.42 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1a), 4.62 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1d), 4.84 (t, 1H, $J_{2,3}$ = 7.6 Hz, H-2a), 5.12 (t, 1H, $J_{2,3}$ = 7.7 Hz, H-2b), 5.70 (br d, 1H, $J_{3,4}$ = 2.9 Hz, H-4d), 5.94 (dt, 1H, $J_{4,5}$ = 14.8 Hz, $J_{5,6}$ = $J_{5,6'}$ = 7.0 Hz, H-5 of sphingosine), and 7.40-8.12 (m, 15H, 3Ph).

Anal. Calcd for $C_{121}H_{170}N_2O_{48}$ (2420.7): C, 60.04; H, 7.08; N, 1.16. Found: C, 59.94; H, 6.90; N, 1.08.

***O*-(3-Deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glu-**

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copyranosyl)-(1→3)-*O*-β-D-galactopyranosyl)-(1→4)-*O*-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol (20). To a solution of **19** (50 mg, 0.03 mmol) in MeOH (5 mL) was added NaOMe (30 mg), and the mixture was stirred for 12 h at room temperature. Potassium hydroxide (0.2 M, 5 mL) was added and the mixture was stirred an additional 6 h at room temperature, then neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered off and washed with 1:1 $CHCl_3$-MeOH. The filtrate and washings were combined and concentrated. Column chromatography (5:4:0.7 $CHCl_3$-MeOH-H_2O) of the residue on a column of Sephadex LH-20 (50 g) with 5:4:0.7 $CHCl_3$-MeOH-H_2O gave **20** (21 mg, 96%) as an amorphous mass: $[\alpha]_D$ -8.2° (*c* 0.2, 1:1 $CHCl_3$-MeOH); ^1H NMR [49:1 $(CD_3)_2SO$-D_2O, at 55 °C] δ 0.86 (t, 6H, $2CH_3CH_2$), 1.25 (s, 52H, $26CH_2$), 2.64 (dd, 1H, J_{gem} = 12.8 Hz, $J_{3eq,4}$ = 4.6 Hz, H-3e*eq*), 4.72 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1c), 5.40, 5.60 (2m, 2H, H-4 and H-5 of sphingosine).

Anal. Calcd for $C_{71}H_{128}N_2O_{31}$ (1505.8): C, 56.63; H, 8.57; N, 1.86. Found: C, 56.53; H, 8.71; N, 1.91.

***O*-(Methyl 4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-*O*-(3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (21).** Coupling of **16** (209 mg, 0.11 mmol) with **17** (94 mg, 0.22 mmol) in CH_2Cl_2 (0.8 mL) in the presence of BF_3•OEt_2 (0.04 mL) and MS-4Å (600 mg) as described for **18** gave **21** (148 mg, 62%) as an amorphous mass: $[\alpha]_D$ -3.6° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87 (t, 3H, CH_3CH_2), 1.24 (s, 22H, $11CH_2$), 1.84-2.15 (14s, 42H, 13AcO, AcN), 3.42 (s, 3H, MeO), 4.31 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1b), 4.49 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1a), 4.66 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1d), 4.83 (t, 1H, $J_{2,3}$ = 7.9 Hz, H-2a), 4.96 (dd, 1H, $J_{2,3}$ = 9.9 Hz, H-2b), 5.15 (t, 1H, $J_{3,4}$ = 7.9 Hz, H-3a), 5.20 (dd, 1H, $J_{6,7}$ = 2.0 Hz, $J_{7,8}$ = 9.5 Hz, H-7e), 5.57 (t, 1H, $J_{2,3}$ = 8.1 Hz, H-2d), 5.77 (br d, 1H, $J_{3,4}$ = 3.1 Hz, H-4d), 5.91 (dt, 1H, $J_{4,5}$ = 14.3 Hz, $J_{5,6}$ = $J_{5,6'}$ = 6.8 Hz, H-5 of sphingosine), and 7.41-8.11 (m, 15H, 3Ph).

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Anal. Calcd for $C_{103}H_{134}N_4O_{47}$ (2180.2): C, 56.74; H, 6.20; N, 2.57. Found: C, 56.64; H, 6.09; N, 2.52.

***O*-(Methyl 4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-*O*-(3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (22).** Selective reduction of the azido group in **21** (150 mg, 0.068 mmol) with H_2S in aq 83% pyridine (10 mL), and subsequent coupling with octadecanoic acid (39 mg, 0.14 mmol) in CH_2Cl_2 (2 mL) in the presence of WSC (26.7 mg, 0.14 mmol) as described for **19** gave **22** (112 mg, 68%) as an amorphous mass: $[\alpha]_D$ +5.8° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, 2C*H*$_3$CH$_2$), 1.25 (s, 52H, 26CH$_2$), 1.84-2.15 (14s, 42H, 13AcO, AcN), 2.59 (dd, 1H, J_{gem} = 12.6 Hz, $J_{3eq,4}$ = 4.0 Hz, H-3e*eq*), 3.42 (s, 3H, MeO), 4.25 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1b), 4.42 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1a), 4.59 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1d), 4.84 (t, 1H, $J_{2,3}$ = 7.7 Hz, H-2a), 4.94 (dd, 1H, $J_{2,3}$ = 7.9 Hz, H-2b), 5.52 (t, 1H, $J_{2,3}$ = 7.7 Hz, H-2d), 5.76 (br d, 1H, $J_{3,4}$ = 3.1 Hz, H-4d), 5.86 (dt, 1H, $J_{4,5}$ = 14.1 Hz, $J_{5,6}$ = $J_{5,6'}$ = 6.8 Hz, H-5 of sphingosine), and 7.41-8.11 (m, 15H, 3Ph).

Anal. Calcd for $C_{121}H_{170}N_2O_{48}$ (2420.7): C, 60.04; H, 7.08; N, 1.16. Found: C, 59.97; H, 6.90; N, 1.14.

***O*-(3-Deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl)-(1→4)-*O*-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol (23).** *O*-Deacylation and saponification of the methyl ester group in **22** (111 mg, 0.046 mmol) as described for **20** yielded **23** (60.5 mg, 90%) as an amorphous mass: $[\alpha]_D$ -6.1° (*c* 0.7, 1:1 $CHCl_3$-MeOH); ^1H NMR [49:1 $(CD_3)_2SO$-D_2O, at 55 °C] δ 0.88 (t, 6H, 2C*H*$_3$CH$_2$), 1.26 (s, 52H, 26CH$_2$), 2.60 (dd, 1H, J_{gem} = 13.0 Hz, $J_{3eq,4}$ = 4.8 Hz, H-3e*eq*), 4.26, 4.28, 4.31 (3d, 3H, $J_{1,2}$ = 7.5-7.7 Hz, H-1a,b,d), 4.72 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1c), 5.40, 5.59 (m, 2H, H-4,5 of sphingosine).

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Anal. Calcd for $C_{71}H_{128}N_2O_{31}$ (1505.8): C, 56.63; H, 8.57; N, 1.86. Found: C, 56.41; H, 8.85; N, 1.90.

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REFERENCES

1. S. Inoue, A. Kanamori, K. Kitajima and Y. Inoue, *Biochem. Biophys. Res. Commun.*, **153**, 172 (1988).
2. S. Yu, K. Kitajima, S. Inoue and Y. Inoue, *J. Biol. Chem.*, **266**, 21929 (1991).
3. T. Terada, S. Kitazume, K. Kitajima, S. Inoue, F. Ito, F. A. Troy and Y. Inoue, *J. Biol. Chem.*, **268**, 2640 (1993).
4. H. K. Ishida, Y. Ohta, Y. Tsukada, Y. Isogai, H. Ishida, M. Kiso and A. Hasegawa, *Carbohydr. Res.*, **252**, 283 (1994), and references therein.
5. a) T. Murase, H. Ishida, M. Kiso and A. Hasegawa, *Carbohydr. Res.*, **184**, c1 (1988); b) A. Hasegawa, H. Ohki, T. Nagahama, H. Ishida and M. Kiso, *Carbohydr. Res.*, **212**, 277 (1991); c) A. Hasegawa, T. Nagahama, H. Ohki, K. Hotta, H. Ishida and M. Kiso, *J. Carbohydr. Chem.*, **10**, 493 (1991); d) A. Hasegawa, In *Oligosaccharide Synthesis*-Indispensable Probes for the Life Sciences, P. Kovác Ed.; ACS Symposium Series 560, American Chemical Society, Washington, DC, p 184 (1994).
6. P. Fügedi and P. J. Garegg, *Carbohydr. Res.*, **149**, c9 (1986).
7. a) P. Konradsson, U. E. Udodong and B. Fraser-Reid, *Tetrahedron Lett.*, **31**, 4313 (1990); b) G. H. Veeneman, S. H. van Leeuwen and J. H. van Boom, *Tetrahedron Lett.*, **31**, 1331 (1990).
8. A. Hasegawa, H. K. Ishida and M. Kiso, *J. Carbohydr. Chem.*, **12**, 371 (1993).
9. T. Terada, M. Kiso and A. Hasegawa, *J. Carbohydr. Chem.*, **12**, 425 (1993).
10. T. Terada, M. Kiso and A. Hasegawa, *Carbohydr. Res.*, **259**, 201 (1994).
11. A. Hasegawa, K. Hotta, A. Kameyama, H. Ishida and M. Kiso, *J. Carbohydr. Chem.*, **10**, 439 (1991).
12. A. Kameyama, H. Ishida, M. Kiso and A. Hasegawa, *Carbohydr. Res.*, **200**, 269 (1990).
13. T. Murase, H. Ishida, M. Kiso and A. Hasegawa, *Carbohydr. Res.*, **188**, 71 (1989).
14. T. Murase, A. Kameyama, K. P. R. Kartha, H. Ishida, M. Kiso and A. Hasegawa, *J. Carbohydr. Chem.*, **8**, 265 (1989).
15. a) K. Jansson, S. Ahlfors, T. Frejd, J. Kihlberg, G. Magnusson, D. Dahmén, G. Noori and K. Stenvall, *J. Org. Chem.*, **53**, 5629 (1988); b) K. P. R. Kartha, M. Kiso and A. Hasegawa, *J. Carbohydr. Chem.*, **8**, 675 (1989).

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16. P. Pozsgay and H. J. Jennings, *Tetrahedron Lett.*, **28**, 1375 (1987).
17. A. Kameyama, H. Ishida, M. Kiso and A. Hasegawa, *J. Carbohydr. Chem.*, **10**, 729 (1991).
18. R. R. Schmidt and G. Grundler, *Synthesis*, 885 (1981).
19. a) R. R. Schmidt and P. Zimmermann, *Angew. Chem. Int. Ed. Engl.*, **25**, 46 (1986); b) Y. Ito, M. Kiso and A. Hasegawa, *J. Carbohydr. Chem.*, **8**, 285 (1989).
20. T. Adachi, Y, Yamada, I, Inoue and M. Saneyoshi, *Synthesis*, 45 (1977).

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