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First total synthesis of α-(2→3)/α-(2→6)-disialyl lactotetraosyl ceramide and disialyl Lewis A ganglioside as cancer-associated carbohydrate antigens☆

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Abstract

The first total synthesis of α-(2→3)/α-(2→6)-disialyl lactotetraosyl ($DSLc_4$) ceramide and α-(2→3)/α-(2→6)-disialyl Lewis A ($DSLe^a$) ganglioside as cancer-associated antigens is described. The suitably protected lactotriose (Lc_3) derivatives were successively glycosylated with sialic acid, sialyl-α-(2→3)-D-galactose and/or L-fucose donors in a regio- and stereo-selective manner, to give the protected type I hexa- and hepta-saccharides, respectively, which were then converted to the target gangliosides by the introduction of ceramide and subsequent complete deprotection.

Keywords: Cancer-associated antigen; Disialyl Le^a ganglioside; Disialyl lactotetaraosyl ceramide

1. Introduction

The carbohydrate determinants, sialyl Lewis A and sialyl Lewis X, which are frequently expressed on human cancer cells, serve as ligands for selectin, a family of C-type cell-adhesion molecules involved in leukocyte recruitment to the site of inflammation, thrombosis, and in lymphocyte binding to high endothelial venules of lymph nodes during lymphocyte recirculation.[2,3] These carbohydrate determinants are also involved in adhesion of cancer cells to the vascular endothelium and thus contribute to the hematogenous metastasis of cancer.[4–6] We have systematically synthesized a series of the derivatives and the analogues of sialyl Lewis X[7] and sialyl Lewis A[8] gangliosides as versatile probes for elucidation of their biological functions.

α-(2→3)/α-(2→6)-Disialyl lactotetraosyl (DSLc4) ceramide **1** (Fig. 1) was isolated from human colonic adenocarcinoma by using a monoclonal antibody (FH-9).[9] Recently, it has also been shown that $DSLc_4$ ceramide **1** is a high-affinity ligand for siglec-7, which is expressed in natural killer (NK) cells and is able to affect NK activity through binding.[10]

Another target compound α-(2→3)/α-(2→6)-disialyl Lewis A ganglioside **2** (Fig. 1) was first isolated from human colonic adenocarcinoma by using a monoclonal antibody (FH-7),[11] and the determination of the (2→3)-sialylated Lewis A to α-(2→3)/α-(2→6)-disialylated Lewis A ratio in patients with pancreatic disease may be helpful for the differential diagnosis of pancreatic cancer and nonmalignant pancreatic disorders.[12–14]

We describe herein the first, efficient total synthesis of the two title compounds **1** and **2**, which contain the di- or tri-antennary structure at O-3, O-4, and O-6 of the GlcNAc residue as a structural feature. A part of this work has already been reported as a communication.[15]

The crucial point in the systematic synthesis of the target oligosaccharides is the successive α-stereo- and regio-selective sialylation at O-6, regio-selective introduction of the sialyl-α-(2→3)-D-galactose unit to O-3, and/or α-selective fucosylation of O-4 of the GlcNAc residue in 2-(trimethylsilyl)ethyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-gluco-

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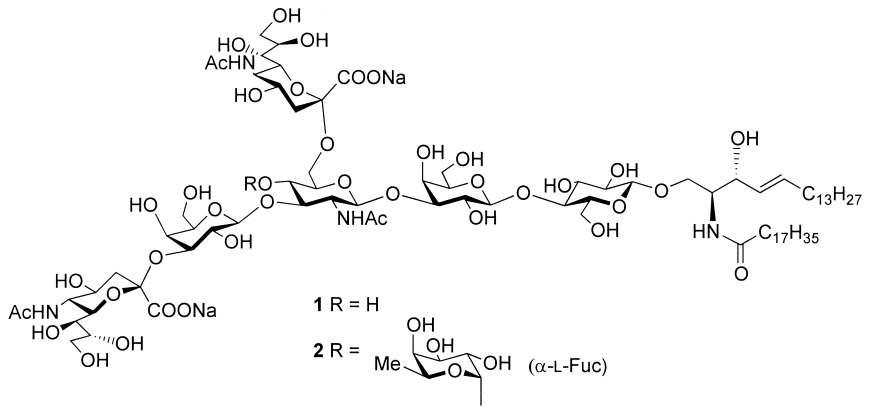


Fig. 1. Structure of target compounds.

pyranoside **3**[16] with the glycosyl donors **4**,[17] **5**[18] and **6**[19] (Fig. 2). In view of the systematic total synthesis of DSLc4 and DSLe[a], the fucosylation should be performed in the final stage of oligosaccharide synthesis. Introduction of the azidosphingosine derivative **18**,[20] followed by reduction of the azido group, N-acylation and deprotection, will lead to the desired target gangliosides **1** and **2**.

2. Results and discussion

The target compounds are shown in Fig. 1, and the retrosynthetic analysis, along with the glycosyl donors and acceptors to construct, are illustrated in Fig. 2. The two possible synthetic routes to construct DSLc4 oligosaccharide **10** are shown in Scheme 1. In the synthetic route A, the iodonium-ion promoted,[21,22] re-

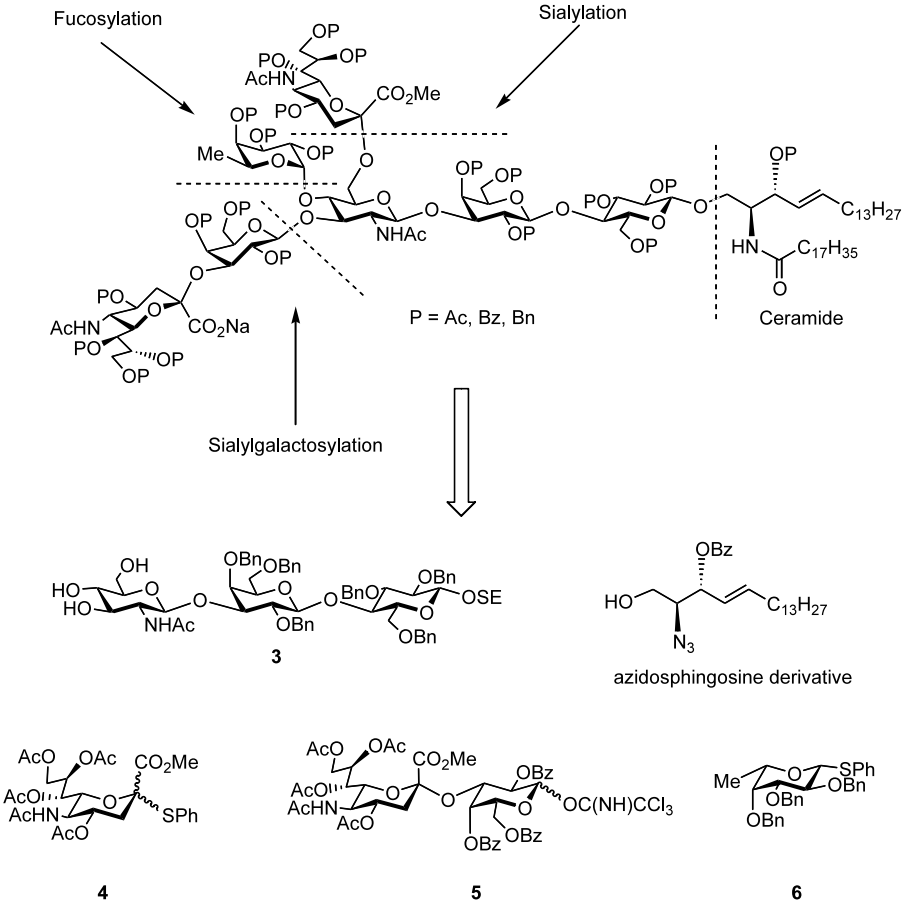
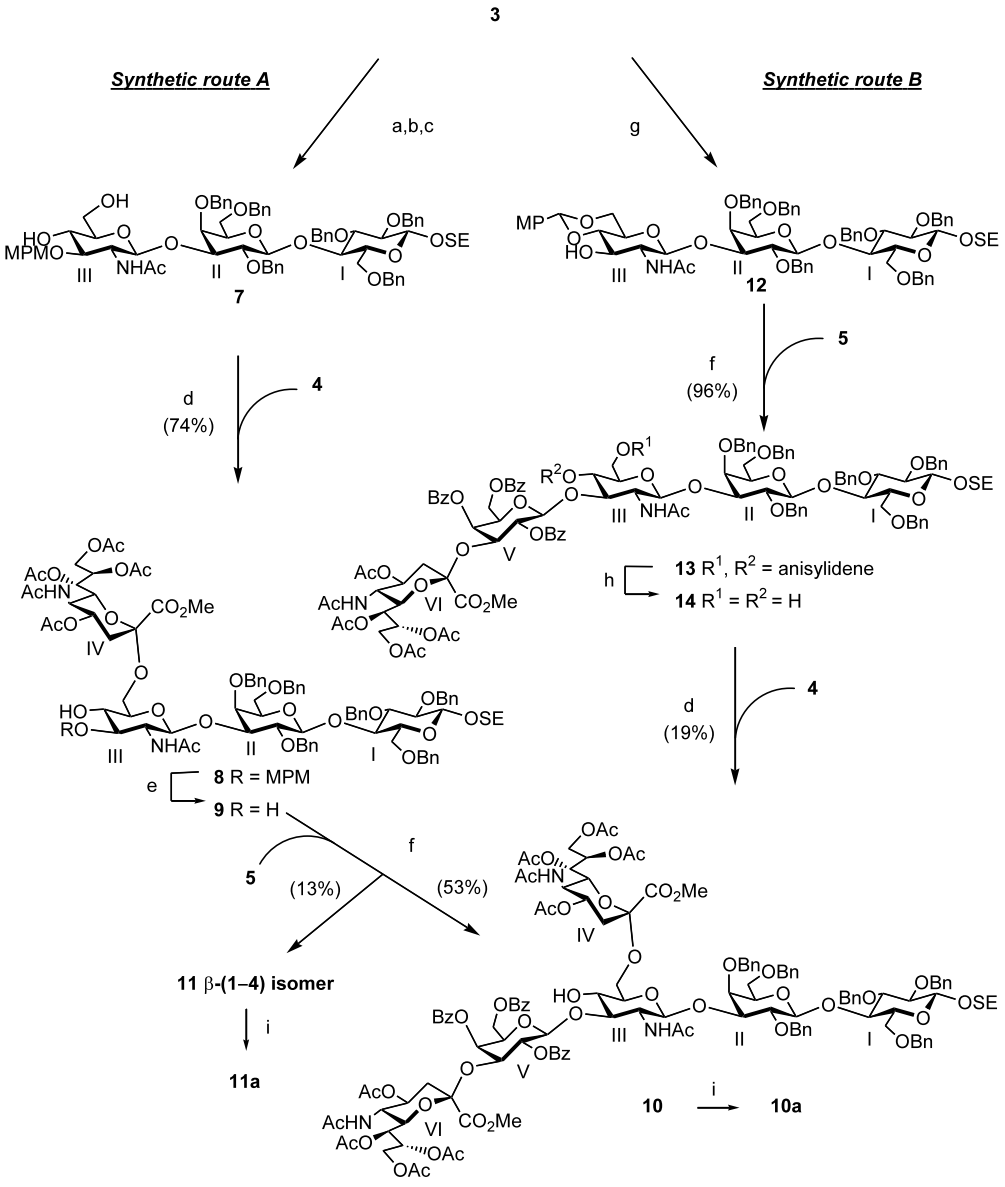


Fig. 2. Retrosynthetic analysis: gloycosyl acceptors (**3** and **18**) and glycosyl donors (**4**–**6**) as the building blocks.



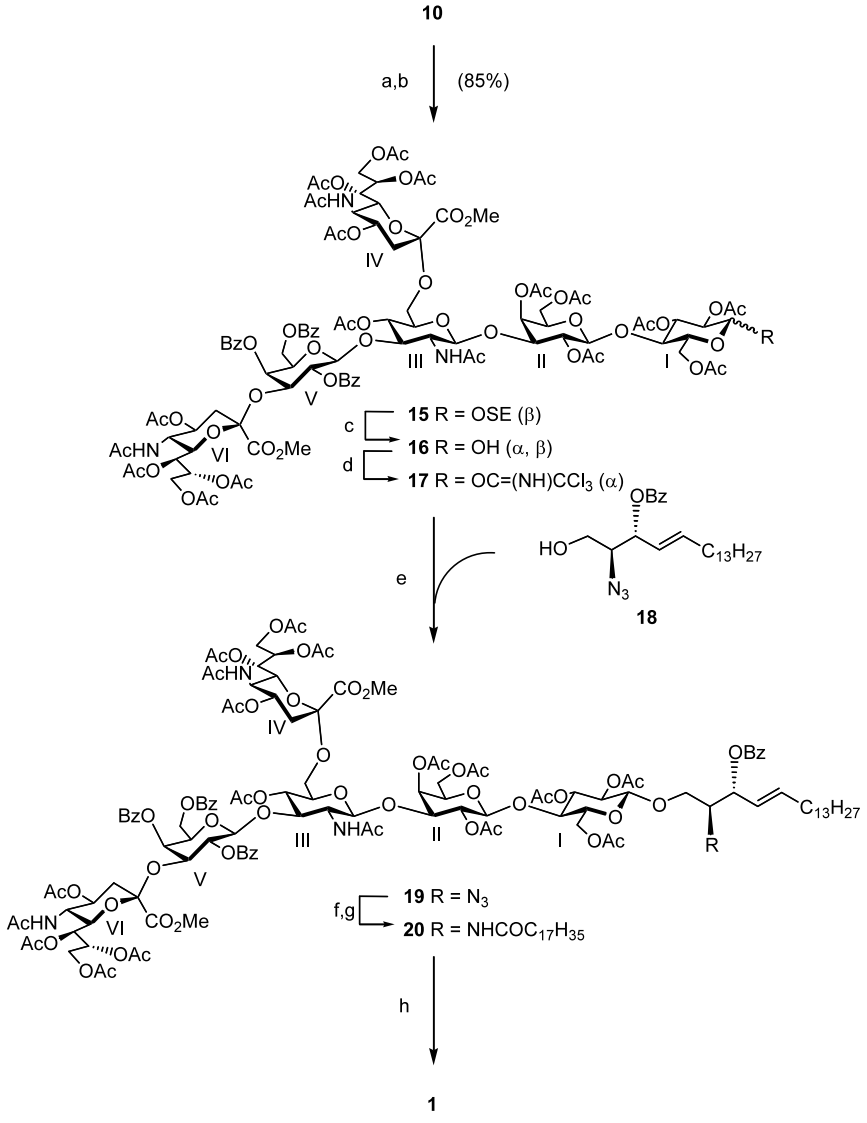
Scheme 1. *Reagents and conditions*: (a) BDA, CSA, DMF; (b) MPMCl, NaH, DMF; (c) 80% AcOH; (d) NIS, TfOH, CH_3CN, -35 °C; (e) CAN, CH_3CN–H_2O; (f) TMSOTf, CH_2Cl_2, -10 °C; (g) ADA, CSA, DMF; (h) $TsOH{\cdot}H_2O$, CH_3OH; (i) Ac_2O, pyridine.

gio- and α-stereocontrolled glycosylation of **7**, which was prepared from **3**[16] in three steps,[19] with Neu5Ac donor **4**[17] was performed at -35 °C in acetonitrile to give the sialyl-α-(2→6)-GlcNAc-β-(1→3)-Gal-β-(1→4)-Glc tetrasaccharide derivative **8** (74%), accompanied by the corresponding β-sialoside (16%). The most significant signal in the ^1H NMR spectrum of **8** was a one-proton doublet of doublets at δ 2.60 due to H-3eq of the newly introduced α-(2→6)-linked sialyl residue.[23] In the spectrum of the β-sialoside, the H-3eq was observed at δ 2.47. Treatment[24] of **8** with ceric(IV) ammonium nitrate (CAN) in 9:1 acetonitrile–H_2O gave the 3,4-diol tetrasaccharide acceptor **9**, which was glycosylated with the sialyl-α-(2→3)-galactosyl trichloroacetimidate donor **5**[18] in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) to afford the desired β-(1→3)-linked disialyl lactotetraose ($DSLc_4$) derivative **10** (53%), preferentially, accompanied by the corresponding β-(1→4)-linked hexasaccharide (13%). A significant signal in the ^1H NMR spectrum of **10** was a one-proton doublet of doublets at δ 5.48 ($J_{1,2}$ 8.0, $J_{2,3}$ 10.3 Hz, H-2 of Gal) indicating the newly formed glycosidic linkage to be β. The structure of compounds **10** and **11** was determined by the spectra of the corresponding acetylated derivatives **10a** and **11a**. The observed chemical shifts of the GlcNAc unit, which were assigned from the cross-peaks in the COSY spectra, for H-3^{III} (δ 4.60 for **10a**; δ 4.92 for **11a**) and for H-4^{III} (δ

4.55 for **10a**; δ 4.00 for **11a**) indicate the glycosylated position to be O-3 and O-4, respectively. The high regioselectivity at O-3 of the GlcNAc residue may be due to the steric hindrance at O-4 caused by the (2→6)-linked sialic acid.

In synthetic route B, the suitably protected trisaccharide **12**, which was prepared from **3** by introduction of the anisylidene group,[25] was first glycosyated with **5** in dichloromethane at −10 °C to give the sialyl lactotetraose derivative **13** (96%). A significant signal in the ^{1}H NMR spectrum of **13** was a one-proton doublet of doublets at δ 5.12 ($J_{1,2}$ 8.0, $J_{2,3}$ 10.3 Hz, H-2^V) indicating the newly formed glycosidic linkage to be β. Treatment of **13** with *p*-toluenesulfonic acid monohydrate in methanol gave the 4,6-diol pentasaccharide acceptor **14**. Iodonium ion-promoted, regio- and α-stereocontrolled sialylation of **14** with the Neu5Ac donor **4** was performed at −35 °C in acetonitrile to afford the desired hexasaccharide **10** (19%) accompanied by the corresponding β-sialoside (5%), which was not easily separated by silica gel chromatography. The unreacted acceptor was recovered in 70% yield. It is worth noting that the coupling of the sialyl donor with bulky acceptors tends to give lower yields in general. Therefore the α-(2→6)-sialylation should be carried out before introduction of the sialyl-α-(2→3)-D-galactose unit, showing that the synthetic route A is superior to route B.

Hydrogenolytic removal of the benzyl groups in **10** over $Pd(OH)_2$ in ethanol, followed by treatment with Ac_2O in pyridine, afforded the peracylated hexasaccharide **15** (85%) (Scheme 2). Selective removal of the



Scheme 2. *Reagents and conditions*: (a) H_2, $Pd(OH)_2$, EtOH; (b) Ac_2O, Pyr.; (c) TFA, CH_2Cl_2; (d) CCl_3CN, DBU, CH_2Cl_2; (e) TMSOTf, CH_2Cl_2, 0 °C; (f) H_2S, 83% aq pyridine, 0 °C; (g) octadecanoic acid, WSC·HCl, CH_2Cl_2; (h) $NaOCH_3$, CH_3OH, then H_2O.

2-(trimethylsilyl)ethyl (SE) group was achieved by treatment[26] of **15** with trifluoroacetic acid in dichloromethane to give the 1-hydroxy compound **16** (96%), which upon further treatment[27] with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dichloromethane, gave the trichloroacetimidate **17** (94%). Significant signals in the ^{1}H NMR spectrum of **17** were a one-proton doublet at δ 6.47 ($J_{1,2}$ 3.8 Hz, H-1^{I}) and a one-proton singlet at δ 8.64 (C=N*H*), indicating the α-trichloroacetimidate form.

Coupling of **17** with (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol **18** was carried out in the presence of boron trifluoride diethyletherate for 24 h at 0 °C to give **19** (36%). The low yield of the reaction could be due to a formation of the corresponding orthester as an undesired by-product. The observed chemical shift and coupling constants were a one-proton doublet of triplets at δ 5.91 ($J_{4,5}$ 13.9 Hz, $J_{5,6} = J_{5,6'} = 7.0$ Hz, H-5 of the sphingosine unit). Selective reduction of the azido group[28] in **19** afforded amine derivative, which, on condensation with octadecanoic acid, using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC·HCl) in dichloromethane, gave the peracylated α-(2→3)/α-(2→6)-disialyl lactotetraosyl ceramide **20** (79%). Finally, O-deacylation of **20** with sodium methoxide in methanol, with subsequent saponification of the methyl ester group, yielded the desired α-(2→3)/α-(2→6)-disialyl lactotetraosyl ceramide **1** after chromatography on a column of Sephadex LH-20 (90%) (Scheme 2). The ^{1}H NMR data and the mass spectrum data of the product thus obtained were consistent with the structure assigned.

The fucosylation of **10** was performed in benzene for 72 h at 7 °C in the presence of *N*-iodosuccunimide (NIS)–trifluoromethanesulfonic acid (TfOH) to give disialyl Lewis A heptasaccharide **21** in 75% yield only when the excess amount of fucose donor **6**[19] (15 mol equiv) was employed the reaction. The low yield could be explained by the apparent incompatibility of the highly reactive donor and the much less reactive acceptor (Scheme 3). Signals of the fucose unit in the ^{1}H NMR spectrum of **21** were a three-proton doublet at δ 1.34 ($J_{5,6}$ 6.4 Hz, H-6^{VI}) and a one-proton doublet at δ 4.86 ($J_{1,2}$ 3.0 Hz, H-1g), indicating the newly formed fucoside to be α. It has been reported that the fucosylation of the 4-OH in Galβ1→3GlcNAc to give Le^a in the type I chain is more difficult than that of the 3-OH in Galβ1→4GlcNAc to give Le^x in a type II chain, so that the present result might be acceptable.

The peracylated heptasaccharide **22** (77%) was prepared from **21** as described for **15**. Selective removal of the SE group (84%), followed by trichloroacetimidate formation (87%) afforded **24**, which was then coupled with **18** to give **25** (40%). Selective reduction of the azido group in **25** and successive N-acylation of the resulting amine by condensation with octadecanoic acid gave the peracylated α-(2→3)/α-(2→6)-disialyl Lewis A ganglioside **26** (76%). Finally, O-deacylation of **26**, followed by subsequent saponification of the methyl ester group, yielded the desired α-(2→3)/α-(2→6)-disialyl Lewis A ganglioside **2** (94%) (Scheme 3). The ^{1}H NMR data of the product thus obtained are consistent with the structure assigned.

In conclusion, a total synthesis of the two type I gangliosides containing the α-(2→6) linked sialic acid at O-6 of the GlcNAc residue was achieved by employing the successive glycosylation of the suitably protected lactotriose acceptor **3** with Neu5Ac donor **4**, sialyl-α-(2→3)-Gal donor **5** and/or fucose donor **6**.

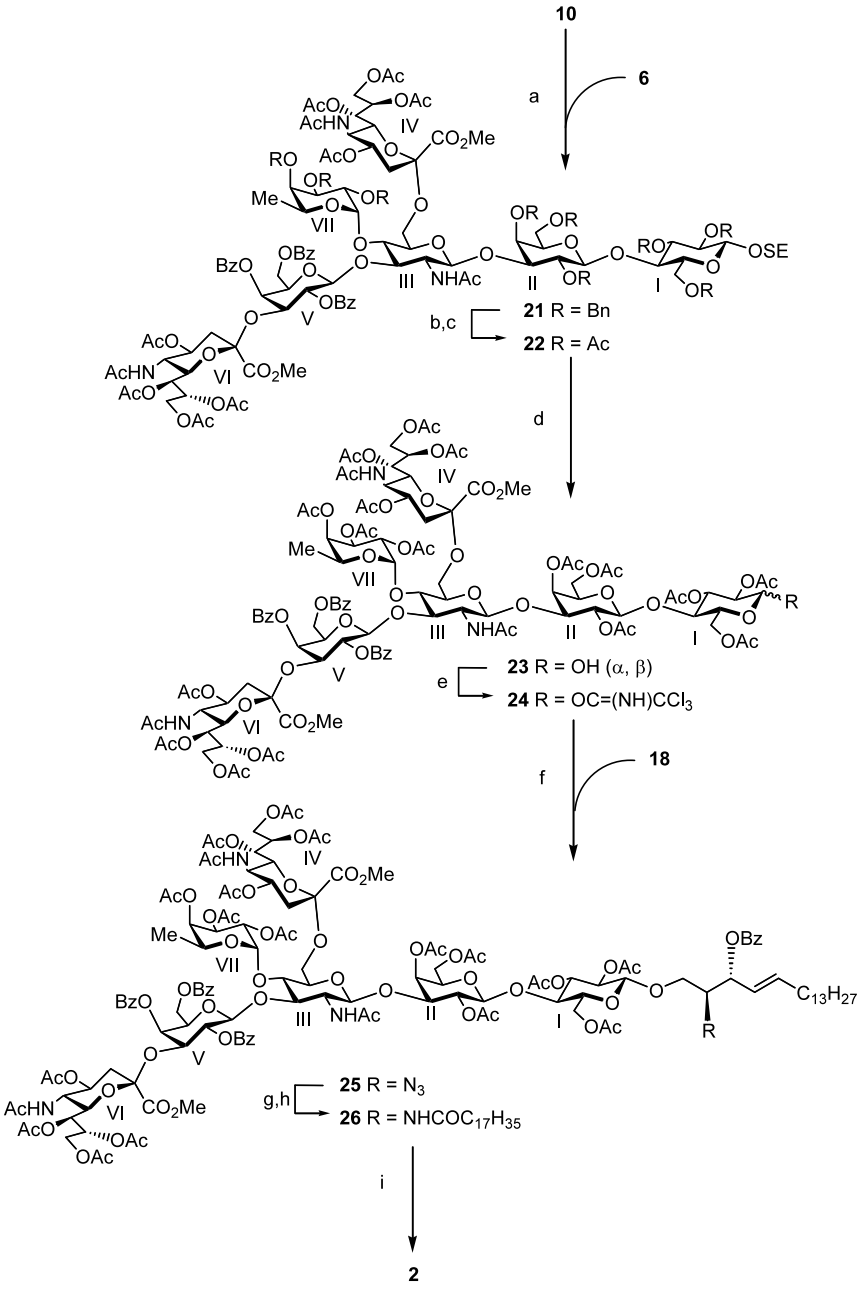
3. Experimental

3.1. General methods

Specific rotations were determined with a Horiba SEPA-300 high sensitive polarimeter at 25 °C, and ^{1}H NMR spectra were recorded on a Varian UNITY Inova (500 MHz) spectrometers with TMS as the internal standard. FABMS spectra were recorded on a JEOL JMS-SX 120A mass spectrometer/JMA-DA 7000 data system. All reactions were monitored by TLC (E. Merck silica gel glass plates $60F_{254}$), and preparative chromatography was performed on silica gel (Fuji Silysia Co. 300 mesh) with the solvent systems specified in v/v. Concentrations and evaporations were conducted *in vacuo*.

3.2. 2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-2-acetamido-2-deoxy-3-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (8)

To a stirred mixture of **7** (785 mg, 0.607 mmol), **4** (709 mg, 1.21 mmol) and 3 Å molecular sieves (1.7 g) in acetonitrile (18 mL) were added *N*-iodosuccinimide (NIS; 545 mg, 2.42 mmol) and trifluoromethanesulfonic acid (TfOH; 22 μL, 242 μmol) at −35 °C, and the stirring was continued overnight at −35 °C. The resulting precipitate was filtered off and washed with chloroform. The filtrate and washings were combined and successively washed with M sodium carbonate (Na_2CO_3) and sodium thiosulfate ($Na_2S_2O_3$), dried (Na_2SO_4) and concentrated. Column chromatography (AcOEt, then 25:1 toluene–methanol) of the residue on silica gel gave **8** (794 mg, 74%). $[\alpha]_D$ −4.0° (*c* 0.18, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.39–7.13 (m, 34 H, 6 Ph, MeO*Ph*), 5.38 (m, 1 H, H-8^{IV}), 5.29 (dd, 1 H, $J_{7,8}$ 8.4 Hz, H-7^{IV}), 3.79 (s, 3 H, *Me*OPh), 3.72 (s, 3 H, COO*Me*), 2.60 (dd, 1 H, J_{gem} 13.0, $J_{3eq,4}$ 4.8 Hz,



Scheme 3. *Reagents and conditions*: (a) NIS, TfOH, benzene, 7 °C; (b) H_2, $Pd(OH)_2$, EtOH; (c) Ac_2O, Pyr.; (d) TFA, CH_2Cl_2; (e) CCl_3CN, DBU, CH_2Cl_2; (f) TMSOTf, CH_2Cl_2, 0 °C; (g) H_2S, 83% aq pyridine, 0 °C; (h) octadecanoic acid, WSC·HCl, CH_2Cl_2; (i) $NaOCH_3$, CH_3OH, then H_2O.

H-3^{IV}*eq*), 2.14, 2.08, 2.04, 1.99 (4 s, 12 H, 4 AcO), 1.89, 1.44 (2 s, 6 H, 2 AcN), 1.01 (m, 2 H, $SiCH_2CH_2O$). Anal. Calcd for $C_{95}H_{118}N_2O_{29}Si$ (1780.04): C, 64.10; H, 6.68; N, 1.57. Found: C, 64.10; H, 6.67; N, 1.54.

3.3. 2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (9)

To a suspension of **8** (400 mg, 204 μmol) in acetonitrile (4.5 mL) and water (0.5 mL) was added ceric ammonium nitrate (CAN; 336 mg, 612 μmol), and the mixture was stirred for 1 h at room temperature and extracted with toluene. The extract was dried (Na_2SO_4) and concentrated. Column chromatography (20:1 chloroform–methanol) of the residue on silica gel gave **9** (357 mg, 95%). $[\alpha]_D$ −12.7° (*c* 0.12, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.38–7.12 (m, 30 H, 6 Ph), 5.42 (m, 1 H, H-8^{IV}), 5.31 (dd, 1 H, $J_{7,8}$ 8.1 Hz, H-7^{IV}), 4.44 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^{II}), 4.33 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^{I}), 3.73

(s, 3 H, COO*Me*), 2.59 (dd, 1 H, J_{gem} 13.2, $J_{3eq,4}$ 4.8 Hz, H-3^{IV}*eq*), 2.13, 2.12, 2.03, 1.96 (4 s, 12 H, 4 AcO), 1.89, 1.40 (2 s, 6 H, 2AcN), 1.01 (m, 2 H, SiCH_2CH$_2$O). Anal. Calcd for $C_{87}H_{110}N_2O_{28}Si$ (1659.9): C, 62.95; H, 6.68; N, 1.69. Found: C, 62.95; H, 6.68; N, 1.69.

3.4. 2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (10) and 2-(trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (11)

A mixture of **9** (300 mg, 180 μmol), **5** (300 mg, 270 μmol) and 4 Å molecular sieves (AW-300, 600 mg) in dichloromethane (3 mL) was stirred for 4 h at room temperature, and then cooled to −10 °C. Trimethylsilyl trifluoromethanesulfonate (TMSOTf; 5 μL, 27 μmol) was added, and the stirring was continued overnight at −10 °C. The solids were filtered off and washed with chloroform. The filtrate and washings were combined and successively washed with M Na_2CO_3 and water, dried (Na_2SO_4), and concentrated. Column chromatography (45:1 chloroform–methanol) on silica gel gave **10** (250 mg, 53%) and **11** (61 mg, 13%). **10**: $[\alpha]_D$ −4.0° (*c* 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.17–7.11 (m, 45 H, 9 Ph), 5.62 (m, 1 H, H-8^{IV}), 5.48 (dd, 1 H, $J_{2,3}$ 10.3 Hz, H-2^{V}), 5.28 (m, 1 H, H-8^{VI}), 4.92 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{V}), 3.83, 3.58 (2 s, 6 H, 2 COO*Me*), 2.55 (dd, 1 H, J_{gem} 13.2, $J_{3eq,4}$ 4.8 Hz, H-3^{IV}*eq*), 2.41 (dd, 1 H, J_{gem} 12.8, $J_{3eq,4}$ 4.8 Hz, H-3^{VI}*eq*), 2.16–1.14 (11 s, 33 H, 8 AcO, 3 AcN), 1.01 (m, 2 H, SiCH_2CH$_2$O). Anal. Calcd for $C_{134}H_{159}N_3O_{48}Si$ (2607.8): C, 61.72; H, 6.15; N, 1.61. Found: C, 61.63; H, 6.11; N, 1.38.

11: $[\alpha]_D$ −4.3° (*c* 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.27–7.07 (m, 45 H, 9 Ph), 5.70 (m, 1 H, H-8^{IV}), 5.48 (dd, 1 H, $J_{2,3}$ 8.0 Hz, H-2^{V}), 5.40 (bd, 1 H, H-4^{V}), 4.92 (d, 1 H, $J_{1,2}$ 10.3 Hz, H-1^{V}), 3.83, 3.58 (2 s, 6 H, 2 COO*Me*), 2.63 (dd, 1 H, J_{gem} 13.2, $J_{3eq,4}$ 4.8 Hz, H-3^{IV}*eq*), 2.46 (dd, 1 H, J_{gem} 12.8, $J_{3eq,4}$ 4.8 Hz, H-3^{VI}*eq*), 2.16–1.14 (11 s, 33 H, 8 AcO, 3 AcN), 1.01 (m, 2 H, SiCH_2CH$_2$O). Anal. Calcd for $C_{134}H_{159}N_3O_{48}Si$ (2607.8): C, 61.72; H, 6.15; N, 1.61. Found: C, 61.63; H, 5.94; N, 1.49.

3.5. 2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)]-2-acetamido-4-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (10a)

To a solution of **10** (50 mg, 19 μmol) in pyridine (1 mL) was added acetic anhydride (1 mL), and the mixture was stirred for 16 h at room temperature. After completion of the reaction, methanol was added, and the mixture was stirred for 20 min at room temperature, concentrated, and extracted with dichloromethane. The extract was washed with 2 M hydrochloric acid, M Na_2CO_3, and water, dried (Na_2SO_4), and concentrated to give **10a** (48 mg, 94%). **10a**: $[\alpha]_D$ −12.0° (*c* 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.17–7.11 (m, 45 H, 9 Ph), 5.62 (m, 1 H, H-8^{IV}), 4.60 (t, 1 H, H-3^{III}), 4.56 (t, 1 H, H-4^{III}), 3.81, 3.52 (2 s, 6 H, 2 COO*Me*), 2.78 (bq, 1 H, H-2^{III}), 2.55 (dd, 1 H, J_{gem} 13.2, $J_{3eq,4}$ 4.8 Hz, H-3^{IV}*eq*), 2.41 (dd, 1 H, J_{gem} 12.8, $J_{3eq,4}$ 4.8 Hz, H-3^{VI}*eq*), 2.16–1.14 (12 s, 36 H, 9 AcO, 3 AcN), 1.01 (m, 2 H, SiCH_2CH$_2$O). Anal. Calcd for $C_{136}H_{161}N_3O_{49}Si$ (2649.81): C, 61.64; H, 6.12; N, 1.59. Found: C, 61.57; H, 6.12; N, 1.45.

3.6. 2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)]-2-acetamido-3-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (11a)

To a solution of **11** (50 mg, 19 μmol) in pyridine (1 mL) was added acetic anhydride (1 mL), and the mixture was stirred for 16 h at room temperature. After completion of the reaction, methanol was added, and the mixture was stirred for 20 min at room temperature, concentrated, and extracted with dichloromethane. The extract was washed with 2 M hydrochloric acid, M Na_2CO_3, and water, dried (Na_2SO_4), and concentrated to give **11a** (50 mg, 96%). **11a**: $[\alpha]_D$ −8.0° (*c* 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.17–7.11 (m, 45 H, 9 Ph), 5.62 (m, 1 H, H-8^{IV}), 5.07 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1^{V}), 4.92 (t, 1 H, H-3^{III}), 4.00 (t, 1 H, H-4^{III}), 3.83, 3.58 (2 s, 6 H, 2 COO*Me*), 2.55 (dd, 1 H, J_{gem} 13.2, $J_{3eq,4}$ 4.8 Hz, H-3^{IV}*eq*), 2.41 (dd, 1 H, J_{gem} 12.8, $J_{3eq,4}$ 4.8 Hz, H-3^{VI}*eq*), 2.16–1.14 (12 s, 36 H, 9 AcO, 3 AcN), 1.01 (m, 2 H, SiCH_2CH$_2$O). Anal. Calcd for

$C_{134}H_{159}N_3O_{48}Si$ (2607.8): C, 61.72; H, 6.15; N, 1.61. Found: C, 61.45; H, 5.93; N, 1.52.

3.7. 2-(Trimethylsilyl)ethyl 2-acetamido-4,6-*O*-anisylidene-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (12)

To a solution of **3** (1.0 g, 843 μmol) in *N*, *N*-dimethylformamide (10 mL) were added *p*-anisaldehyde dimethylacetal (286 μL, 1.68 mmol) and camphorsulfonic acid (19.0 mg, 8 μmol). The mixture was stirred at room temperature for 1 day, then neutralized with triethylamine and concentrated. Column chromatography (45:1 chloroform–methanol) of the residue on silica gel gave **12** (880 mg, 80%). $[\alpha]_D$ −25.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.43–6.87 (m, 34 H, 6 Ph, MeO*Ph*), 5.53 (s, 1 H, MeOPh*CH*), 4.70 (d, $J_{1,2}$ 1 H, 8.5 Hz, H-1^{III}), 4.47 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.35 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1^{I}), 3.79 (s, 3 H, *Me*OPh), 1.45 (s, 3 H, AcN), 1.00 (m, 2 H, Si*C*H_2CH_2O). Anal. Calcd for $C_{75}H_{89}NO_{17}Si$ (1304.6): C, 69.05; H, 6.88; N, 1.07. Found: C, 69.02; H, 6.60; N, 1.00.

3.8. 2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4,6-*O*-anisylidene-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (13)

To a solution of **12** (700 mg, 54 μmol) and **5** (899 mg, 810 μmol) in dichloromethane (20 mL) were added 4 Å molecular sieves (AW-300, 1500 mg), and the mixture was stirred for 3 h at room temperature, then cooled to 0 °C. TMSOTf (1 μL, 5.5 μmol) was added, and the mixture was stirred for 24 h at −10 °C. The solids were filtered off and washed with chloroform. The filtrate and washings were combined and successively washed with M Na_2CO_3 and water, dried (Na_2SO_4), and concentrated. Column chromatography (50:1 toluene–methanol) of the residue on silica gel gave **13** (1.09 g, 90%). $[\alpha]_D$ −0.6° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.43–6.87 (m, 49 H, 9 Ph, MeO*Ph*), 5.53 (s, 1 H, MeOPh*CH*), 5.37 (dd, 1 H, H-2^{V}), 5.29 (bd, 1 H, $J_{4,5}$ 2.9 Hz, H-4^{V}), 5.20 (dd, 1 H, $J_{6,7}$ 2.6, $J_{7,8}$ 9.7 Hz, H-7^{VI}), 5.12 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{V}), 3.80 (s, 3 H, COO*Me*), 3.79 (s, 3 H, *Me*OPh), 2.43 (dd, 1 H, J_{gem} 12.4, $J_{3eq,4}$ 4.6 Hz, H-3^{VI}*eq*), 2.14–1.79 (4 s, 12 H, 4 AcO), 1.45, 0.88 (2 s, 6 H, 2 AcN), 1.00 (m, 2 H, Si*C*H_2CH_2O). Anal. Calcd for $C_{122}H_{138}N_2O_{37}Si$ (2252.5): C, 65.05; H, 6.18; N, 1.24. Found: C, 64.93; H, 6.02; N, 0.97.

3.9. 2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (14)

To a solution of **13** (600 mg, 226 μmol) in methanol (60 mL) was added *p*-toluensulfonic acid monohydrate (6 mg, 30 μmol) and the mixture was stirred for 30 min at room temperature, then neutralized with triethylamine and concentrated. Column chromatography (40:1 chloroform–methanol) of the residue on silica gel gave **14** (550 mg, 97%). $[\alpha]_D$ +2.5° (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.43–7.11 (m, 45 H, 9 Ph), 5.37 (dd, 1 H, H-2^{V}), 5.29 (bd, 1 H, $J_{4,5}$ 2.9 Hz, H-4^{V}), 5.20 (dd, 1 H, $J_{6,7}$ 2.6, $J_{7,8}$ 9.7 Hz, H-7^{VI}), 5.12 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{V}), 3.80 (s, 3 H, COO*Me*), 3.79 (s, 3 H, *Me*OPh), 2.43 (dd, 1 H, J_{gem} 12.4 Hz, $J_{3eq,4}$ 4.6 Hz, H-3^{VI}*eq*), 2.14–1.79 (4 s, 12 H, 4 AcO), 1.45, 0.88 (2 s, 6 H, 2AcN), 1.00 (m, 2H, Si*C*H_2CH_2O). Anal. Calcd for $C_{114}H_{132}N_2O_{36}Si$ (2134.4): C, 64.15; H, 6.23; N, 1.31. Found: C, 63.99; H, 6.18; N, 1.21.

3.10. 2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)]-2-acetamido-4-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (15)

Compound **10** (110 mg, 42 μmol) in ethanol (5 mL) was hydrogenated in the presence of 20% $Pd(OH)_2$ on carbon (110 mg) for 48 h at room temperature, and the reaction mixture was filtered through Celite, washing with MeOH. The filtrate was concentrated, and then the residue that was obtained was acetylated with acetic anhydride (1 mL) and pyridine (1 mL) for 24 h at room temperature. The mixture was concentrated, and a solution of the residue in chloroform was successively washed with 2 M HCl and M Na_2CO_3, dried (Na_2SO_4) and concentrated. Column chromatography (20:1 chloroform–methanol) of the residue on silica gel gave **15** (95 mg, 85%). $[\alpha]_D$ +5.0° (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.19–7.43 (m, 15 H, 3 Ph), 5.64 (m, 1 H, H-8^{IV}), 3.81, 3.79 (2 s, 6 H, 2 COO*Me*), 2.53 (dd, 1 H, J_{gem} 12.8, $J_{3eq,4}$ 4.4 Hz, H-3^{IV}*eq*), 2.44 (dd, 1 H, J_{gem} 12.4, $J_{3eq,4}$ 4.4 Hz, H-3^{VI}*eq*), 2.16–1.54 (18 s, 54 H, 3 AcN, 15 AcO), 0.94 (m, 2 H, Si*C*H_2CH_2O). Anal. Calcd for $C_{106}H_{137}N_3O_{55}Si$ (2361.3): C, 53.92; H, 5.85; N, 1.78. Found: C, 53.79; H, 5.72; N, 1.67.

3.11. (Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)]-2-acetamido-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranose (16)

To a solution of **15** (120 mg, 51 μmol) in dichloromethane (2.4 mL) was added trifluoroacetic acid (1.2 mL) at 0 °C, and the mixture was stirred for 3 h at room temperature and concentrated. Column chromatography (10:1 chloroform–methanol) of the residue on silica gel gave **16** (110 mg, 96%). $[\alpha]_D$ +29.5° (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.19–7.43 (m, 15 H, 3 Ph), 5.62 (m, 1 H, H-8^{IV}), 3.81, 3.79 (2 s, 6 H, 2 COO*Me*), 2.52 (dd, 1 H, J_{gem} 12.8, $J_{3eq,4}$ 4.4 Hz, 3-^{IV}*eq*), 2.43 (dd, 1 H, J_{gem} 12.4, $J_{3eq,4}$ 4.4 Hz, H-3^{VI}*eq*), 2.16–1.54 (18 s, 54 H, 3 AcN, 15 AcO). Anal. Calcd for $C_{101}H_{125}N_3O_{55}$ (2261.1): C, 53.65; H, 5.57; N, 1.86. Found: C, 53.59; H, 5.36; N, 1.75.

3.12. (Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)]-2-acetamido-4-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl trichloroacetimidate (17)

To a solution of **16** (240 mg, 106 μmol) in dichloromethane (3 mL) and trichloroacetonitrile (310 μL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 31 μL, 210 μmol) at 0 °C, and the mixture was stirred for 1 h at room temperature and concentrated. Column chromatography (50:1 chloroform–methanol) of the residue on silica gel gave **17** (240 mg, 94%). $[\alpha]_D$ −18.5° (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.64 (s, 1 H, C=NH), 8.19–7.42 (m, 15 H, 3 Ph), 6.47 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1^I), 5.62 (m, 1 H, H-8^{IV}), 5.50 (t, 1 H, $J_{1,2} = J_{2,3}$ 9.5 Hz, H-2^{VI}), 3.81, 3.79 (2 s, 6 H, 2 COO*Me*), 2.53 (dd, 1 H, J_{gem} 12.8, $J_{3eq,4}$ 4.4 Hz, H-3^{IV}*eq*), 2.44 (dd, 1 H, J_{gem} 12.4, $J_{3eq,4}$ 4.4 Hz, H-3^{VI}*eq*), 2.15–1.53 (18 s, 54 H, 3 AcN, 15 AcO). Anal. Calcd for $C_{103}H_{125}Cl_3N_4O_{55}$ (2405.5): C, 51.43; H, 5.24; N, 2.33. Found: C, 51.40; H, 5.00; N, 2.10.

3.13. (Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-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 (19)

To a solution of **17** (250 mg, 104 μmol) and (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (**18**) (90 mg, 208 μmol) in dichloromethane (4 mL) were added 4 Å molecular sieves (AW-300, 250 mg), and the mixture was stirred for 3 h at room temperature, then cooled to 0 °C. Boron trifluoride diethyl etherate (75 μL) was added, and the mixture was stirred for 24 h at 0 °C, then filtered. The residue was washed with chloroform. The combined filtrate and washings were concentrated. Column chromatography (30:1 chloroform–methanol) of the residue on silica gel gave **19** (99 mg, 36%). $[\alpha]_D$ −12.0° (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.18–7.42 (m, 20 H, 4 Ph), 5.91 (m, $J_{4,5}$ 13.9, $J_{5,6} = J_{5,6'}$ 7.0 Hz, H-5 sphingosine unit), 5.62 (m, 1 H, H-8^{VI}), 5.50 (t, 1 H, H-2 sphingosine unit), 4.59 (t, 1 H, H-3^{III}), 3.81, 3.79 (2 s, 6 H, 2 COO*Me*), 2.53 (dd, 1 H, J_{gem} 12.6, $J_{3eq,4}$ 4.6 Hz, H-3^{IV}*eq*), 2.44 (dd, 1 H, J_{gem} 12.6, $J_{3eq,4}$ 4.4 Hz, H-3^{VI}*eq*), 2.15–1.53 (18 s, 54 H, 3 AcN, 15 AcO), 1.24 (s, 22 H, 11 CH_2 sphingosine unit), 0.89 (t, 3 H, CH_3 sphingosine unit). Anal. Calcd for $C_{126}H_{162}N_6O_{57}$ (2672.7): C, 56.62; H, 6.11; N, 3.14. Found: C, 56.62; H, 5.94; N, 3.02.

3.14. (Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-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 (20)

Hydrogen sulfide was bubbled through a stirred solution of **19** (90 mg, 34 μmol) in aqueous 83% pyridine (10 mL) for 2 days at 0 °C. The reaction mixture was stirred and concentrated. To a solution of the residue in dichloromethane (2 mL) were added octadecanoic acid (20 mg, 68 μmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (13 mg, 100 μmol), and the mixture was diluted with chloroform, and the solution was washed with water, dried (Na_2SO_4) and concentrated. Column chromatography (30:1 chloroform–methanol) of the residue on silica gel gave **20** (80 mg, 79%). $[\alpha]_D$ −12.0° (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.18–7.42 (m, 20 H, 4 Ph), 5.86 (m, 1 H, H-5 ceramide unit), 5.62 (m, 1 H, H-8^{VI}), 5.53 (t, 1 H, $J_{3,4} = J_{4,5}$ 7.3 Hz, H-3 ceramide unit), 5.45 (dd, 1 H, $J_{3,4}$ 7.6, $J_{4,5}$ 14.9 Hz, H-4, ceramide unit), 4.59 (t, 1 H, H-3^{III}), 3.81, 3.78 (2 s, 6 H, 2 COO*Me*), 2.53 (dd, 1 H, J_{gem} 12.8 Hz, $J_{3eq,4}$ 4.8 Hz, H-3^{IV}*eq*), 2.44 (dd, 1 H,

J_{gem} 12.8, $J_{3eq,4}$ 4.6 Hz, H-3^{VI}*eq*), 2.15–1.53 (18 s, 54 H, 3 AcN, 15 AcO). 1.24 (s, 52 H, 26 CH_2 ceramide unit), 0.94 (t, 6 H, 2 CH_3 ceramide unit). Anal. Calcd for $C_{144}H_{198}N_4O_{58}$ (2913.1): C, 59.37; H, 6.85; N, 1.92. Found: C, 59.21; H, 6.70; N, 1.74.

3.15. α-(2→3)/α-(2→6)-Disialyl lactotetraosyl ceramide (1)

To a solution of **20** (63 mg, 22 μmol) in methanol (2 mL) was added sodium methoxide (10 mg, 28% methanol solution), and the mixture was stirred for 48 h at room temperature, and then water (0.5 mL) was added. The solution was stirred for 3 days at room temperature, neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered off and washed with 1:1 chloroform–methanol, and the combined filtrate and washings were concentrated. Column chromatography (5:5:1 chloroform–methanol–water) of the residue on Sephadex LH-20 gave **1** (35 mg, 90%). $[\alpha]_D$ −24.0° (*c* 0.1, $CHCl_3$–MeOH–H_2O); ^{1}H NMR (96:4 $(CD_3)_2SO$–D_2O, 40 °C): δ 5.55 (dt, 1 H, H-5, ceramide unit), 5.36 (dd, 1 H, $J_{3,4}$ 7.3, $J_{4,5}$ 15.1 Hz, H-4, ceramide unit), 4.60 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{III}), 4.26 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.17 (2 d, 2 H, H-1^{I}, H-1^{IV}), 3.05 (t, 1 H, H-2^{I}), 2.69, 2,64 (2 dd, 2 H, H-3^{IV}*eq*, H-3^{VI}*eq*), 2.04 (t, 2 H, COCH_2CH$_2$, ceramide unit), 1.89, 1.88 (2 s, 6 H, AcN-^{IV,VI}), 1.79 (s, 3 H, AcN-^{III}), 1.24 (s, 52 H, 26 CH_2 ceramide unit), 0.86 (t, 6 H, 2 CH_3, ceramide unit). FABMS (negative ion): Calcd for $C_{84}H_{146}N_4Na_2O_{39}$ 1882.04; found *m*/*z* 1858.0 $(M-Na)^-$.

3.16. 2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→4)]-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (21)

To a stirred mixture of **10** (200 mg, 76 μmol) and **6** (198 mg, 0.38 mmol) and 3 Å molecular sieves (400 mg) in benzene (8 mL) were added NIS (86 mg, 0.38 mmol) and TfOH (2 μL, 20 μmol,) at 7 °C, and the stirring was continued for 72 h at 7 °C. During the reaction, compound **7** (192 mg) and NIS (86 mg, 0.38 mmol) were added twice every 24 h. The precipitate was filtered off and washed with chloroform. The filtrate and washings were combined and successively washed with M Na_2CO_3 and $Na_2S_2O_3$, dried (Na_2SO_4) and concentrated. Column chromatography (40:1 chloroform–methanol) of the residue on silica gel gave **21** (175 mg, 75%). $[\alpha]_D$ −40.2° (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.17–7.06 (m, 60 H, 12 Ph), 5.56 (m, 1 H, H-8^{IV}), 5.47 (t, 1 H, H-2^{V}), 4.86 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1^{VII}), 3.66, 3.40 (2 s, 6 H, 2 COO*Me*), 2.60 (dd, 1 H, J_{gem} 13.0, $J_{3eq,4}$ 4.6 Hz, H-3^{IV}*eq*), 2.36 (dd, 1 H, J_{gem} 12.6, $J_{3eq,4}$ 4.6 Hz, H-3^{VI}*eq*), 2.13–1.65 (11 s, 33 H, 3 AcN, 8 AcO). 1.34 (d, 3 H, $J_{5,6}$ 6.4 Hz, H-6^{VII}), 1.03 (m, 2 H, SiCH_2CH$_2$). FABMS (negative ion): Calcd for $C_{161}H_{187}N_3O_{52}Si$ 3024.28; found *m*/*z* 3023.3 $(M-H)^-$.

3.17. 2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→4)]-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (22)

Compound **22** was prepared from **21** as described from **15** in 77% after chromatography (25:1 chloroform–methanol). $[\alpha]_D$ −36.8° (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.20–7.41 (m, 15 H, 3 Ph), 5.62 (m, 1 H, H-8^{VI}), 5.01 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1^{V}), 4.54 (t, 1 H, H-3^{III}), 3.77, 3.73 (2 s, 6 H, 2 COO*Me*), 2.84 (bq, 1 H, H-2^{III}), 2.64 (dd, 1 H, J_{gem} 12.8, $J_{3eq,4}$ 4.8 Hz, H-3^{IV}*eq*), 2.39 (dd, 1 H, J_{gem} 12.8, $J_{3eq,4}$ 4.4 Hz, H-3^{VI}*eq*), 2.22–1.57 (20 s, 60 H, 3 AcN, 17 Ac). 1.25 (d, 3 H, $J_{5,6}$ 6.6 Hz, H-6^{VII}), 0.91 (m, 2 H, SiCH_2CH$_2$). Anal. Calcd for $C_{116}H_{151}N_3O_{61}Si$ (2591.5): C, 54.35; H, 5.94; N, 1.64. Found: C, 54.33; H, 5.75; N, 1.45.

3.18. (Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→4)]-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranose (23)

Compound **23** was prepared for compound **22** as described for **16** in 84% yield after chromatography (20:1 chloroform–methanol). $[\alpha]_D$ −7.5° (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.20–7.41 (m, 15 H, 3 Ph), 5.62 (m, 1 H, H-8^{IV}), 5.01 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1^{V}), 4.54 (t, 1 H, H-3^{III}), 3.77, 3.73 (2 s, 6 H, 2 COO*Me*), 2.84 (bq, 1 H, H-2^{III}), 2.64 (dd, 1 H, J_{gem} 12.8, $J_{3eq,4}$ 4.8 Hz, H-3^{IV}*eq*), 2.39 (dd, 1 H, J_{gem} 12.8, $J_{3eq,4}$ 4.4 Hz, H-3^{VI}*eq*), 2.22–1.57 (20 s, 60 H, 3 AcN, 17 AcO). 1.25 (d, 3 H, $J_{5,6}$ 6.6 Hz, H-6^{VII}). Anal. Calcd for $C_{111}H_{139}N_3O_{61}$ (2491.3): C, 53.51; H, 5.62; N, 1.69. Found: C, 53.40; H, 5.34; N, 1.66.

3.19. (Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→4)]-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl trichloroacetimidate (24)

Compound **24** was prepared for compound **23** as described from **17** in 87% yield after chromatography (30:1 chloroform–methanol). $[\alpha]_D$ +2.5° (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.64 (s, 1 H, C=NH), 8.20–7.41 (m, 15 H, 3 Ph), 6.47(d, 1 H, $J_{1,2}$ 3.8 Hz, H-1^{I}), 5.62 (m, 1 H, H-8^{VI}), 5.01 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1^{V}), 4.54 (t, 1 H, H-3^{III}), 3.77, 3.73 (2 s, 6 H, 2 COO*Me*), 2.84 (bq, 1 H, H-2^{III}), 2.64 (dd, 1 H, J_{gem} 12.8, $J_{3eq,4}$ 4.8 Hz, H-3^{IV}*eq*), 2.39 (dd, 1H, J_{gem} 12.8, $J_{3eq,4}$ 4.4 Hz, H-3^{VI}*eq*), 2.22–1.57 (20 s, 60 H, 3 AcN, 17 AcO). 1.25 (d, 3 H, $J_{5,6}$ 6.6 Hz, H-6^{VII}). Anal. Calcd for $C_{113}H_{139}Cl_3N_4O_{61}$ (2635.7): C, 51.49; H, 5.32; N, 2.13. Found: C, 51.43; H, 5.08; N, 1.95.

3.20. (Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→4)]-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-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 (25)

Compound **25** was prepared for compound **24** as described from **19** in 25% yield after chromatography (30:1 chloroform–methanol). $[\alpha]_D$ −21.5° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.21–7.41 (m, 20 H, 4 Ph), 5.93 (m, 1 H, H-5 sphingosine unit), 4.59 (t, 1 H, H-3, sphingosine unit), 377, 3.75 (2 s, 6 H, 2 COO*Me*), 2.84 (bq, 1 H, H-2^{III}), 2.65 (dd, 1 H, J_{gem} 13.0, $J_{3eq,4}$ 4.6 Hz, H-3^{IV}*eq*), 2.38 (dd, 1 H, J_{gem} 12.6, $J_{3eq,4}$ 4.4 Hz, H-3^{VI}*eq*), 2.15–1.43 (20 s, 6 H, 3 AcN, 17 AcO). 1.24 (s, 22 H, 11 CH_2 sphingosine unit), 0.89 (t, 3 H, CH_3, sphingosine unit). Anal. Calcd for $C_{136}H_{176}N_6O_{63}$ (2902.9) C, 56.27; H, 6.11; N, 2.90. Found: C, 56.12; H, 5.98; N, 2.70.

3.21. (Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→4)]-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-benzoyl-2-octadecenamido-4-octadecene-1,3-diol (26)

Compound **26** was prepared for compound **25** as described from **20** in 76% yield after chromatography (40:1 chloroform–methanol). $[\alpha]_D$ −16.2° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.20–7.40 (m, 20 H, 4 Ph), 5.86 (m, 1 H, H-5 ceramide unit), 5.60 (m, 1 H, H-8^{VI}), 5.53 (t, 1 H, $J_{3,4} = J_{4,5}$ 7.3 Hz, H-3 ceramide unit), 5.45 (dd, 1 H, $J_{3,4}$ 7.7, $J_{4,5}$ 15.0 Hz, H-4 ceramide unit), 4.59 (t, 1 H, H-3^{III}), 3.77, 3.73 (2 s, 6 H, 2 COO*Me*), 2.82 (bd, 1 H, H-2^{III}), 2.64 (dd, 1 H, J_{gem} 12.8, $J_{3eq,4}$ 4.8 Hz, H-3^{IV}*eq*), 2.39 (dd, 1 H, J_{gem} 12.8, $J_{3eq,4}$ 4.6 Hz, H-3^{VI}*eq*), 2.20–1.57 (20 s, 60 H, 3 AcN, 17 AcO), 1.25 (s, 52 H, 26 CH_2 ceramide unit), 0.94 (t, 6 H, $2CH_3$ ceramide unit). Anal. Calcd for $C_{154}H_{212}N_4O_{64}$ (3143.3) C, 58.84; H, 6.80; N, 1.78. Found: C, 58.59; H, 6.64; N, 1.69.

3.22. α-(2→3)/α-(2→6)-Disialyl Lewis A ganglioside (2)

Compound **2** was prepared for compound **26** as described from **1** in 76% yield after chromatography (5:5:1 chloroform–methanol–water). $[\alpha]_D$ −16.2° (*c* 0.2, $CHCl_3$–MeOH–H_2O); ^1H NMR (96:4 $(CD_3)_2SO$–D_2O, 40 °C): δ 5.55 (dt, 1 H, H-5, ceramide unit), 5.36 (dd, 1 H, $J_{3,4}$ 7.3, $J_{4,5}$ 15.1 Hz, H-4 ceramide unit), 4.88 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1^{VII}), 4.61 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{III}), 4.33 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1^{V}), 4.27 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.17 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{I}), 3.05 (t, 1 H, H-2^{I}), 2.72, 2.64 (2 dd, 2 H, H-3^{IV}*eq*, H-3^{VI}*eq*), 2.04 (t, 2 H, COCH_2CH$_2$, ceramide unit), 1.89, 1.88 (2 s, 6 H, AcN-^{IV,VI}), 1.86 (s, 3 H, AcN-^{III}), 1.24 (s, 52 H, 26 CH_2 ceramide unit), 0.86 (t, 6 H, 2 CH_3, ceramide unit). FABMS (negative ion): Calcd for $C_{90}H_{156}N_4Na_2O_{43}$ found *m*/*z* 2005 $(M - Na)^-$.

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