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Note

Total synthesis of 6-*O*-sulfo-sialylparagloboside: a widely useful glycoprobe for biochemical research☆

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Abstract—The total synthesis of 6-*O*-sulfo-sialylparagloboside is described. A suitably protected β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p*OSE derivative was glycosylated with an α-D-Neu*p*5Ac-(2→3)-D-Gal*p* derived imidate to give the corresponding protected α-D-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p*OSE pentasaccharide derivative. Proper manipulation of the protecting groups of the pentasaccharide afforded the corresponding glycosyl imidate, which was coupled with (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol. Selective reduction of the azido group, N-acylation with octadecanoic acid, 6-O-sulfation of the Glc*p*NAc residue, and complete removal of the protecting groups gave the desired 6-*O*-sulfo-sialylparagloboside.

Keywords: Sialic acid; Sialylparagloboside; Oligosaccharide; Glycoprobe

Gangliosides are critical components of living systems and mediate a vast number of fundamental biological processes. These molecules have been classified into four series (hematoside-, ganglio-, lacto (neolacto)-, and globo (isoglobo)- series) depending on the structure of the oligosaccharide backbones. The sialic acids residues that are located on the non-reducing termini of these oligosaccharides play important roles in processes such as viral infection, immune defense, and inflammation.[1]

We have established a method for the efficient α-glycosylation of sialic acid, and have reported the synthesis of a wide range of gangliosides, which are sialylated glycosphingolipids.[2] The first completely stereocontrolled total synthesis of the sLe^x ganglioside, a member of the neolacto-series (Fig. 1), was achieved[3] by employing our regio- and α-stereoselective sialylation method.[4] This compound has been effectively utilized for the initial elucidation of the selectin ligands.[5] Furthermore, we synthesized 6-*O*-sulfo sLe^x ganglioside (6-*O*-sulfated Glc*p*NAc residue, Fig. 1), and its variants, the de-*N*-acetyl and lactamized 6-*O*-sulfo sLe^x derivatives. Utilizing these probes, we showed that 6-*O*-sulfo sLe^x serves as the major endogenous ligand for L-selectin and proposed a novel immune response controlled by a ligand processing pathway involving 'activation' by de-N-acetylation of sialic acid and 'inactivation' by lactamization.[6]

In a recent study, Nabel's group showed that hemagglutinin from H5N1 influenza virus has a high affinity for sulfated glycans, including α-D-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→4)-β-D-6OSO$_3$-Glc*p*NAc.[7] In view of these results, the 6-O-sulfation of Glc*p*NAc sialoglycans has important implications in various organisms, prompting us to establish the efficient synthesis of glycoprobes containing sialic acid and sulfate groups, which can be used to understand the mechanism of these phenomena at the molecular level. In the present paper, we describe the synthesis of α-D-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p*(1→1)ceramide, which can be used in various biochemical experiments to investigate the mechanism of increasing ligand affinity to hemagglutinin.

☆ Part 146 of the series: synthetic studies on sialoglycoconjugates. For part 145: see Ref. 15.

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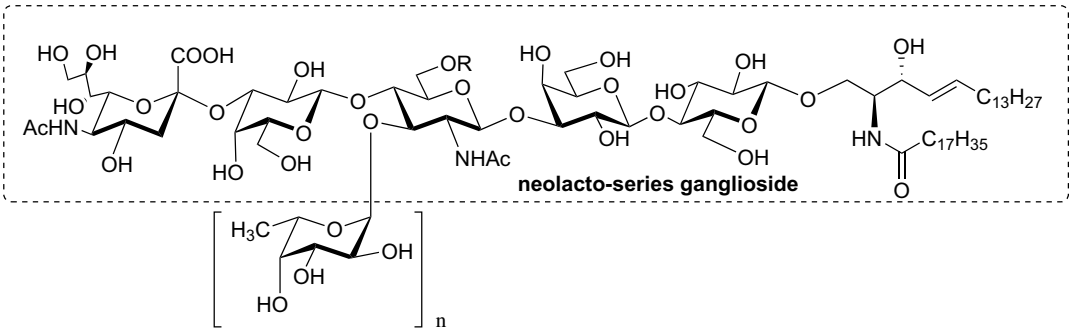
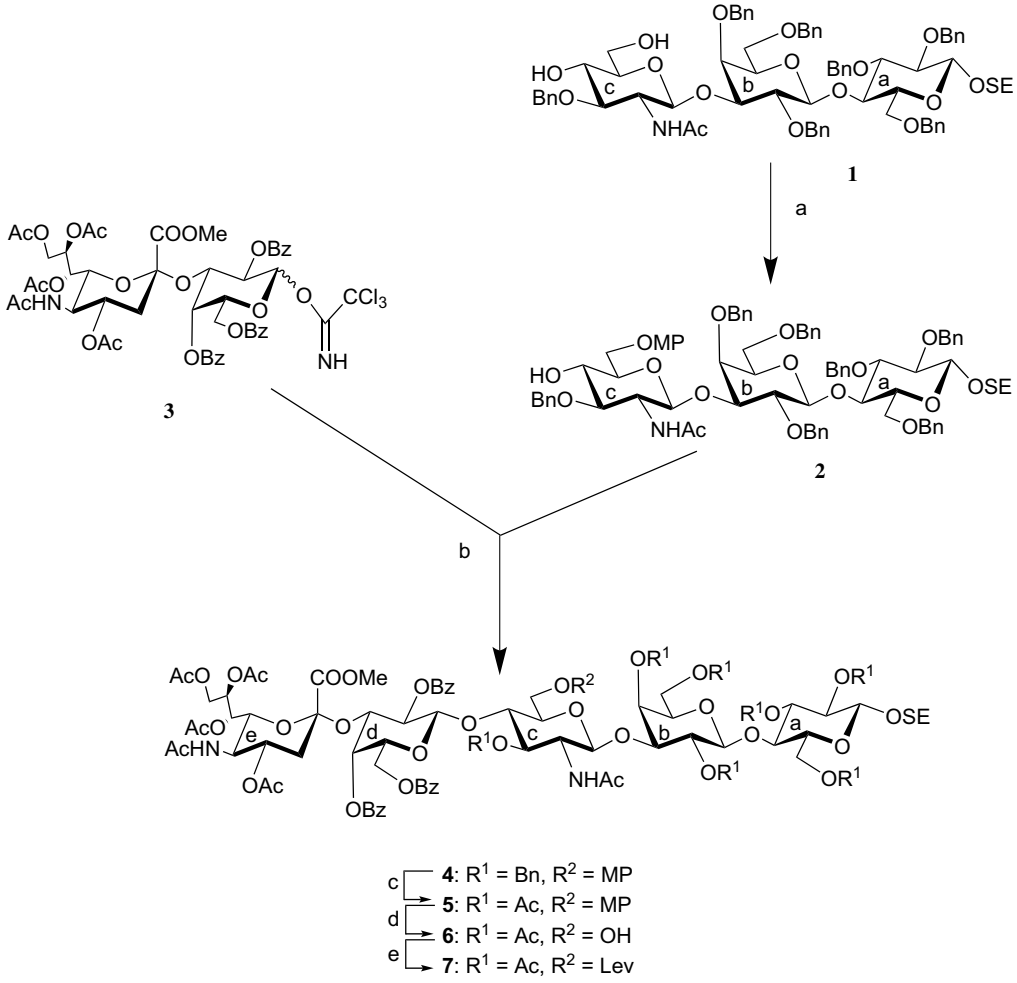
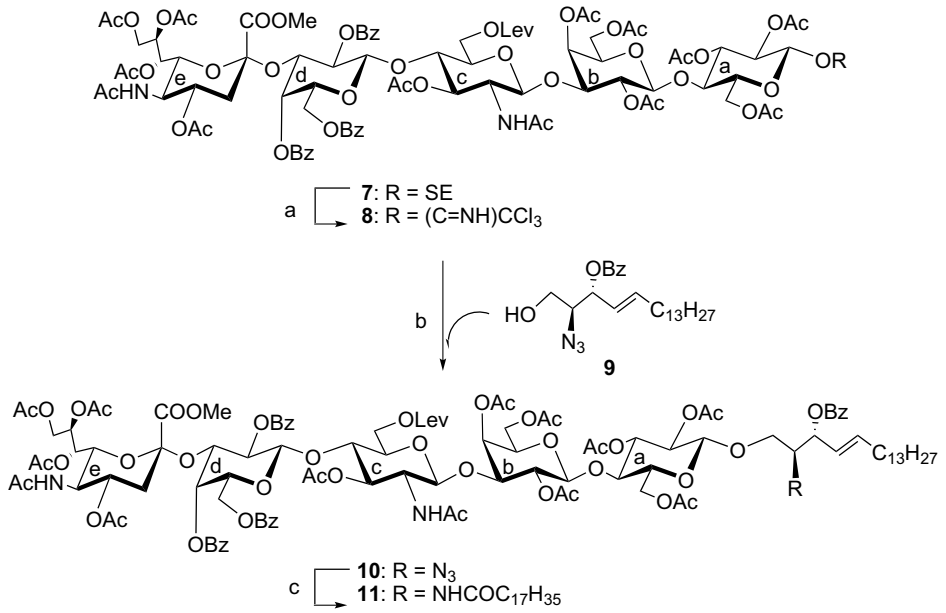


Figure 1. R = H, $n = 1$, sLeX ganglioside. R = SO_3H, $n = 1$, 6-*O*-sulfo sLeX ganglioside. R = SO_3H, $n = 0$, 6-*O*-sulfo-sialylparagloboside (target compound).

The critically important problems in the synthesis of the target compound are (a) the selective protection of the hydroxyl groups at C-6 of the Glc*p*NAc residue to allow regioselective sulfation, (b) the efficient construction of the terminal α-D-Neu*p*5Ac-(2→3)-β-D-Gal*p*-structure linked to O-4 of the Glc*p*NAc residue, and (c) the facile introduction of the ceramide moiety. The synthesis of sulfated sialylparagloboside was achieved in an efficient manner from the suitably protected pentasaccharide intermediate **4**, in which O-6 of the Glc*p*NAc residue is protected by a 4-methoxyphenyl (MP) group. The MP group can be chemoselectively cleaved by ceric



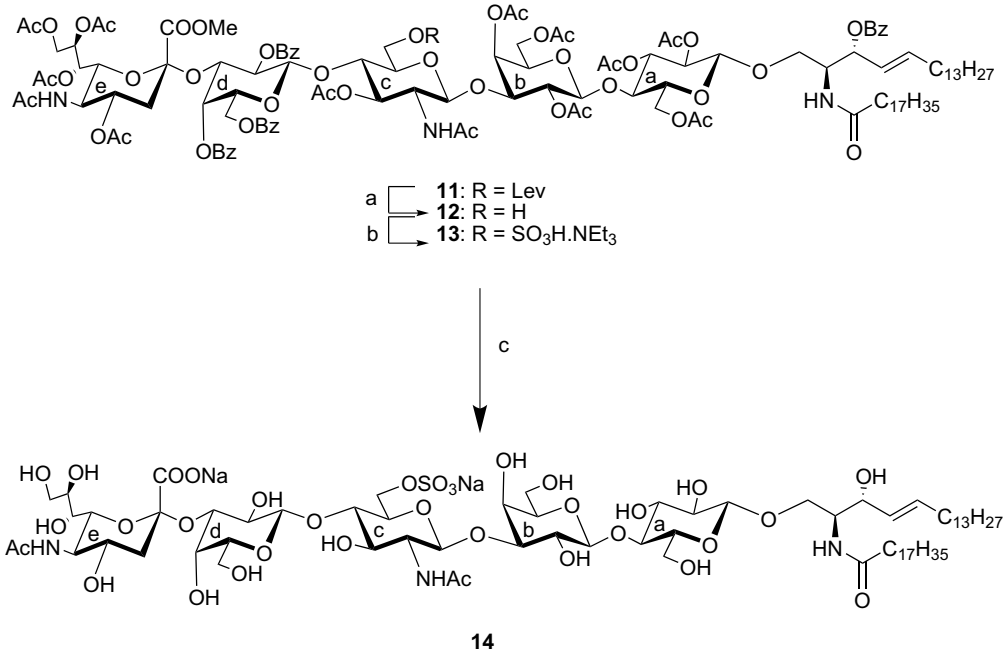
Scheme 1. Synthesis of the suitably protected sialylparagloboside intermediate. Reagents and conditions: (a) MPOH, PPh_3, DEAD, THF, 80 °C, 89%; (b) TMSOTf, CH_2Cl_2, AW300, 0 °C, 70%; (c) (1) $Pd(OH)_2$, H_2 gas, AcOH, EtOH; (2) Ac_2O, Pyr, rt, 2 steps, 84%; (d) CAN, CH_3CN, H_2O, 0 °C, 93%; (e) Lev_2O, DMAP, Pyr, 60 °C, 82%.



Scheme 2. Synthesis of the protected 6-*O*-sulfo-sialylparagloboside. Reagents and conditions: (a) (1) TFA, CH_2Cl_2, 0 °C, quant.; (2) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 89%; (b) TMSOTf, CH_2Cl_2, AW300, 0 °C, 49%; (c) (1) H_2S gas, Pyr, H_2O, 0 °C; (2) stearic acid, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, rt, 2 steps, 49%.

ammonium nitrate (CAN) (Scheme 1). Then, the pentasaccharide was converted into a suitably protected donor, and the introduction of ceramide and sulfate group led to the target compound **14**.

Regioselective 4-O-methoxyphenylation of O-6 of **1**[8] was carried out by treatment with *p*-methoxyphenol (MPOH), PPh_3, and diethylazodicarboxylate (DEAD) in THF,[9] and the resulting product, **2**, was coupled with the suitably protected α-D-Neu*p*5Ac-(2→3)-β-D-Gal*p*- donor **3**,[10] in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) and molecular sieves (AW-300) in CH_2Cl_2 at 0 °C, to give the desired



Scheme 3. Synthesis of 6-*O*-sulfo-sialylparagloboside. Reagents and conditions: (a) NH_2NH_2·AcOH, EtOH, 90%; (b) SO_3·Pyr complex, DMF, then Et_3N, rt, 70%; (c) NaOMe, MeOH, then H_2O, rt, quant.

sialylparagloboside (SPG) pentasaccharide **4**, in 70% yield (Scheme 1). Hydrogenolytic removal of the benzyl groups in **4** and subsequent acetylation gave **5** in 84% yield. Because the ceramide moiety has been found to be labile to CAN, which is used to cleave the MP group, the MP group in **5** was replaced[11] by the levulinoyl (Lev) group to afford **7** in 76% yield in two steps (Scheme 1). The 2-(trimethylsilyl)ethyl (SE) group in **7** was then selectively cleaved[12] by treatment with TFA, and the resulting hemiacetal was converted to the trichloroacetimidate derivative **8** in 89% yield. Coupling of **8** and the azidosphingosine derivative **9**[13] in the presence of TMSOTf and AW-300 molecular sieves in CH_2Cl_2 at 0 °C gave **10** in 49% yield. The subsequent conversion of the azido group in **10** to the stearoylamino group was carried out by using the established method[14] to afford **11** in 49% yield over two steps (Scheme 2). The Lev group in **11** was selectively cleaved by treatment with hydrazine acetate to give **12** in 90% yield. Then, 6-O-sulfation of **12** with a sulfur trioxide–pyridine complex in DMF, followed by the addition of triethylamine to stabilize the sulfate group during column chromatography, gave **13** in 70% yield. Removal of all protecting groups in **13** under alkaline conditions afforded 6-*O*-sulfo paragloboside **14** (GSC-568), in quantitative yield (Scheme 3).

In summary, we have succeeded in the efficient and completely stereocontrolled total syntheses of the 6-*O*-sulfo sialylparagroboside. We expect that the utilization of the synthetic glycoprobe will help elucidating the bound structure of hemagglutinin with the carbohydrate at molecular level.

1. Experimental

1.1. General methods

TLC was conducted on E. Merck Silica Gel 60 F-254 aluminum plates. Compounds were visualized by exposure to UV light or by spraying with a solution of 10% H_2SO_4 in EtOH. Column chromatography on silica gel (Fuji Silysia Co., 300 mesh) was performed with the solvent systems (v/v) specified. Specific rotations were determined with a Horiba SEPA-300 high-sensitive polarimeter at 25 °C. ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 K with a Varian Inova 400/500 spectrometer, respectively. Chemical shifts (ppm) are given relative to Me_4Si as the internal standard. MALDI-TOFMS spectra were recorded in negative ion mode on a Brucker Autoflex with the use of α-cyano-4-hydroxy-cinnamic acid (CHCA) as a matrix. CH_2Cl_2, MeOH, EtOH, benzene, and DMF were kept dry over 4 Å MS, while pyridine and acetonitrile were kept dry over 3 Å MS.

1.2. 2-(Trimethylsilyl)ethyl 2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-4-methoxy-phenyl-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 (2)

To a solution of **1** (905 mg, 0.70 mmol) in THF (25 mL) were added PPh_3 (910 mg, 3.45 mmol), DEAD (1.05 mL, 2.39 mmol), and MPOH (523 mg, 4.18 mmol), and the mixture was stirred under reflux for 24 h. After completion of the reaction, the mixture was concentrated. Column chromatography (1:1 EtOAc–Hexane) of the residue on silica gel afforded **2** (860 mg, 89%) as an amorphous solid; $[\alpha]_D^{25}$ −1.8(*c* 1.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.36–6.75 (m, 39H, MeO*Ph*, 7Ph). 4.40 (d, 1H, $J_{1,2} = 7.6$ Hz, H-1b), 4.33 (d, 1H, $J_{1,2} = 8.3$ Hz, H-1c), 4.10 (dd, 1H, $J_{2,3} = 10.9$, $J_{3,4} = 4.8$ Hz, H-3b), 3.71 (s, 3H, *Me*OPh), 3.58–3.54 (m, 2H, $Me_3SiCH_2CH_2$), 3.50 (t, 1H, $J_{3,4} = 8.9$ Hz, H-3c), 3.36 (dd, 1H, $J_{1,2} = 8.3$, $J_{2,3} = 9.0$ Hz, H-2c), 1.45 (s, 3H, AcN), 1.00 (m, 2H, $Me_3SiCH_2CH_2$); ^{13}C NMR ($CDCl_3$): δ 170.12, 154.07, 152.72, 139.34, 139.15, 139.06, 138.76, 138.30, 138.21, 128.59, 128.53, 128.44, 128.31, 128.24, 128.22, 128.12, 127.97, 127.91, 127.68, 127.63, 127.53, 127.45, 127.05, 126.50, 115.69, 114.64, 103.06, 102.56, 101.56, 82.83, 81.79, 81.63, 81.57, 80.23, 76.60, 76.04, 75.31, 75.07, 74.92, 74.70, 74.49, 73.86, 73.80, 73.33, 73.22, 71.21, 68.62, 68.24, 67.28, 56.10, 55.66, 23.06, 18.42. Anal. Calcd for $C_{81}H_{95}NO_{17}Si$: C, 70.36; H, 6.93; N, 1.01. Found: C, 70.06; H, 6.88; N, 0.80.

1.3. 2-(Trimethylsilyl)ethyl-methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-4-methoxyphenyl-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 (4)

To a solution of **3** (347 mg, 0.31 mmol) and the trisaccharide acceptor **2** (350 mg, 0.25 mmol) in dry CH_2Cl_2 (4 mL) were added 4 Å molecular sieves (2 g), and the mixture was stirred for 3 h at room temperature, then cooled to 0 °C. Trimethylsilyl trifluoromethanesulfonate (9 μL, 45 μmol) was added to the mixture and this was stirred for 24 h at 0 °C, before being neutralized with Et_3N and filtered. The residue was washed with chloroform, and the combined filtrate and washings were concentrated. Column chromatography (100:1 $CHCl_3$–MeOH) of the residue on silica gel gave **4** (410 mg, 70%) as an amorphous solid; $[\alpha]_D^{25}$ +18.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.14–6.70 (m, 54H, MeO*Ph*, 10Ph), 5.57 (m, 1H, H-8e), 5.48 (dd, 1H, $J_{1,2} = 8.0$, $J_{2,3} = 10.0$ Hz, H-2d), 5.36 (d, 1H, H-4d), 5.33 (d, 1H, $J_{2,NH} = 11.9$ Hz, NHc), 5.22 (dd, 1H, $J_{6,7} = 2.5$, $J_{7,8} = 9.6$ Hz, H-7e), 5.08 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1d),

4.93 (dd, 1H, $J_{2,3} = 10.0$, $J_{3,4} = 3.2$ Hz, H-3d), 4.81 (m, 1H, H-4e), 4.60 (d, 1H, $J_{1,2} = 7.3$ Hz, H-1c), 4.37 (d, 1H, $J_{1,2} = 7.5$ Hz, H-1b), 4.33 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1a), 3.88 (dd, 1H, $J_{8,9} = 5.2$, $J_{gem} = 13.5$ Hz, H-9e), 3.82 (s, 3H, COO*Me*), 3.68 (s, 3H, *Me*OPh), 3.55 (m, 2H, $Me_3SiCH_2CH_2$), 2.46 (dd, 1H, $J_{3eq,4} = 4.3$, $J_{gem} = 12.3$ Hz, H-3e*eq*), 2.15, 1.93, 1.90, 1.76 (4s, 12H, 3AcO and AcN), 1.64 (t, 1H, $J_{gem} = J_{3ax,4} = 12.3$ Hz, H-3e*ax*), 1.46 (s, 3H, AcN), 1.00 (m, 2H, $Me_3SiCH_2CH_2$); ^{13}C NMR ($CDCl_3$): δ 172.16, 172.13, 172.06, 171.38, 171.14, 169.25, 167.22, 167.17, 166.98, 155.34, 154.07, 140.71, 140.58, 140.55, 140.13, 139.82, 139.75, 134.95, 134.82, 134.58, 131.76, 131.56, 131.24, 131.07, 130.96, 130.64, 130.56, 130.15, 129.94, 129.71, 129.66, 129.61, 129.53, 129.49, 129.44, 129.36, 129.32, 129.14, 128.97, 128.93, 128.87, 128.81, 128.75, 128.64, 128.42, 116.87, 115.93, 104.45, 103.88, 103.87, 101.45, 98.21, 84.35, 84.08, 83.33, 80.93, 80.92, 77.36, 76.64, 76.54, 76.33, 76.05, 75.88, 74.65, 74.52, 73.94, 73.14, 72.82, 72.68, 72.02, 70.35, 69.86, 69.75, 69.53, 69.46, 69.11, 68.68, 67.72, 63.34, 57.05, 56.82, 55.36, 54.75, 51.00, 38.67, 24.26, 22.75, 21.95, 21.79, 21.45, 19.83. Anal. Calcd for $C_{128}H_{144}N_2O_{37}Si$: C, 65.96; H, 6.23; N, 1.20. Found: C, 65.86; H, 6.11; N 1.16.

1.4. 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→4)-2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-4-methoxyphenyl-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 (5)

A solution of **4** (410 mg, 0.17 mmol) in EtOH (40 mL) and acetic acid (8 mL) was vigorously stirred with $Pd(OH)_2$ (410 mg) for 12 h at room temperature under hydrogen. The catalyst was collected and washed with MeOH. The combined filtrate and washings were concentrated, and the residue was treated with acetic anhydride (9 mL) and pyridine (15 mL) for 12 h at room temperature, then cooled to 0 °C. MeOH (5 mL) was added and the mixture was concentrated, and the residue was extracted with chloroform and successively washed with cold 2 M hydrochloric acid and water, dried (Na_2SO_4), and concentrated. Column chromatography (50:1 $CHCl_3$–MeOH) of the residue on silica gel gave **5** (295 mg, 84%) as an amorphous solid; $[\alpha]_D^{25}$ +16.2 (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.13–6.64 (m, 19H, MeO*Ph*, 3Ph), 5.56 (m, 1H, H-8e), 5.38 (dd, 1H, $J_{1,2} = 7.8$, $J_{2,3} = 10.2$ Hz, H-2d), 5.34 (d, 1H, H-4d), 5.20 (dd, 1H, $J_{6,7} = 3.2$, $J_{7,8} = 9.3$ Hz, H-7e), 5.14 (t, 1H, $J_{2,3} = 9.3$ Hz, H-3a), 5.07 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1d), 4.98 (dd, 1H, $J_{1,2} = 7.8$, $J_{2,3} = 9.6$ Hz, H-2b), 4.86 (dd, 1H, $J_{1,2} = 8.2$, $J_{2,3} = 9.3$ Hz, H-2a), 4.82 (dd, 1H, $J_{2,3} = 10.2$, $J_{3,4} = 2.9$ Hz, H-3d), 4.78 (m, 1H, H-4e), 4.52 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1c), 4.46 (d, 1H, $J_{1,2} = 8.2$ Hz, H-1a), 4.29 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1b), 3.84 (dd, 1H, $J_{8,9} = 5.5$, $J_{gem} = 11.4$ Hz, H-9e), 3.79 (s, 3H, COO*Me*), 3.75 (s, 3H, *Me*OPh), 3.64 (d, 1H, $J_{2,3} = 9.6$, $J_{3,4} = 3.6$ Hz, H-3b), 3.55 (m, 2H, $Me_3SiCH_2CH_2$), 2.46 (dd, 1H, $J_{3eq,4} = 4.5$, $J_{gem} = 12.5$ Hz, H-3e*eq*), 2.12, 2.10, 2.064, 2.061, 2.05, 2.04, 2.01, 1.98, 1.93, 1.91, 1.90, 1.76 (12s, 36H, 11AcO and AcN), 1.60 (t, 1H, $J_{gem} = J_{3ax,4} = 12.5$ Hz, H-3e*ax*), 1.44 (s, 3H, AcN), 0.91 (m, 2H, $Me_3SiCH_2CH_2$); ^{13}C NMR ($CDCl_3$): δ 171.17, 170.78, 170.72, 170.66, 170.57, 170.47, 170.42, 170.39, 170.15, 169.97, 169.95, 169.74, 169.60, 169.25, 167.97, 165.21, 154.06, 153.04, 135.04, 134.85, 133.34, 131.67, 131.34, 131.06, 130.73, 130.55, 130.19, 130.02, 129.75, 128.66, 115.67, 114.65, 102.57, 102.25, 101.28, 100.63, 96.74, 76.44, 74.07, 73.59, 72.13, 71.76, 71.73, 71.28, 71.22, 71.07, 70.97, 70.78, 69.95, 69.00, 68.43, 67.57, 67.48, 67.37, 66.34, 64.69, 62.26, 61.89, 61.97, 55.68, 53.24, 48.44, 37.18, 29.72, 23.72, 23.51, 21.42, 21.07, 20.86, 20.78, 20.71, 20.52, 20.34, 19.58. Anal. Calcd for $C_{93}H_{116}N_2O_{44}Si$: C, 56.02; H, 5.86; N, 1.40. Found: C, 56.01; H, 5.78; N, 1.25.

1.5. 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→4)-2-acetamido-3-*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 (6)

To a solution of **5** (295 mg, 0.15 mmol) in acetonitrile (8.1 mL) and water (0.9 mL) was added ceric ammonium nitrate (255 mg, 0.43 mmol). The mixture was stirred for 2 h at 0 °C before being extracted with EtOAc. The extract was successively washed with 1 M sodium carbonate and water, dried (Na_2SO_4), and concentrated. Column chromatography (50:1 $CHCl_3$–MeOH) of the residue on silica gel gave **6** (260 mg, 93%) as an amorphous solid; $[\alpha]_D^{25}$ +13.7 (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.22–7.43 (m, 15H, 3Ph), 5.71 (m, 1H, H-8e), 5.41 (d, 1H, H-4d), 5.37 (dd, 1H, $J_{1,2} = 8.0$, $J_{2,3} = 10.0$ Hz, H-2d), 5.34 (dd, 1H, $J_{6,7} = 3.5$, $J_{7,8} = 7.5$ Hz, H-7e), 4.98 (t, 1H, $J_{2,3} = 9.3$ Hz, H-2a), 4.88 (dd, 1H, $J_{2,3} = 10.0$, $J_{3,4} = 4.0$ Hz, H-3d), 4.86 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1d), 4.60 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1c), 4.46 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1b), 4.32 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1a), 3.82 (s, 3H, COO*Me*), 3.59 (m, 2H, $Me_3SiCH_2CH_2$), 3.21 (br d, H-6c), 2.47 (dd, 1H, $J_{3eq,4} = 4.3$, $J_{gem} = 12.3$ Hz, H-3e*eq*), 2.16, 2.13, 2.12, 2.11, 2.10, 2.08, 2.07, 2.03, 2.02, 1.96, 1.91, 1.88 (12s, 36H, 11AcO and AcN), 1.63 (t, 1H, $J_{gem} = J_{3ax,4} = 12.3$ Hz, H-3e*ax*), 1.46 (s, 3H, AcN), 0.91 (m, 2H, $Me_3SiCH_2CH_2$); ^{13}C NMR ($CDCl_3$): δ 173.35, 172.19, 172.14, 172.05, 172.03, 171.88, 171.64, 171.57, 171.43, 171.37, 171.26, 171.14, 170.42, 169.60, 167.25, 166.83, 134.68, 134.61, 134.64, 131.73, 131.67, 130.94,

130.59, 130.06, 129.56, 102.14, 102.02, 101.57, 101.28, 98.21, 77.38, 76.84, 76.53, 74.30, 74.10, 74.03, 73.52, 73.19, 72.84, 72.62, 72.59, 71.63, 70.81, 70.67, 69.32, 68.78, 67.83, 63.68, 63.64, 63.54, 62.97, 62.29, 56.21, 54.53, 50.16, 39.11, 38.64, 31.38, 29.20, 24.56, 22.73, 22.31, 22.26, 22.23, 22.14, 21.82, 19.25. Anal. Calcd for $C_{86}H_{110}N_2O_{43}Si$: C, 54.71; H, 5.87; N, 1.48. Found: C, 54.57; H, 5.65; N, 1.40.

1.6. 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)-2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-levulinoyl-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 (7)

To a solution of **6** (260 mg, 0.14 mmol) in pyridine (8 mL) were added levulinic anhydride (209 mg, 0.96 mmol) and DMAP (26 mg, 0.20 mmol), and the mixture was stirred for 36 h at 60 °C, then cooled to 0 °C. MeOH (3 mL) was added and the mixture was concentrated, and the residue was extracted with chloroform and successively washed with cold 2 M hydrochloric acid and water, dried (Na_2SO_4), and concentrated. Column chromatography (60:1 $CHCl_3$–MeOH) of the residue on silica gel gave **7** (223.2 mg, 82%) as an amorphous solid; $[\alpha]_D^{25}$ +8.5 (*c* 1.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.10–7.45 (m, 15H, 3Ph), 5.62 (m, 1H, H-8e), 5.37 (dd, 1H, $J_{1,2} = 7.8$, $J_{2,3} = 10.1$ Hz, H-2d), 5.23 (dd, 1H, $J_{6,7} = 2.7$, $J_{7,8} = 9.3$ Hz, H-7e), 4.98 (dd, 1H, $J_{1,2} = 8.0$, $J_{2,3} = 9.8$ Hz, H-2a), 4.92 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1d), 4.87 (dd, 1H, $J_{1,2} = 7.8$, $J_{2,3} = 9.6$ Hz, H-2b), 4.83 (dd, 1H, $J_{2,3} = 10.1$, $J_{3,4} = 3.2$ Hz, H-3d), 4.54 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1c), 4.46 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1b), 4.37 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1a), 4.00 (dd, 1H, $J_{8,9'} = 5.0$, $J_{gem} = 12.4$ Hz, H-9′e), 3.80 (s, 3H, COO*Me*), 3.60 (m, 2H, $Me_3SiCH_2CH_2$), 3.53 (dd, 1H, $J_{2,3} = 9.4$ Hz, H-2c), 2.70–2.42 (m, 5H, MeOCH_2CH_2 and H-3e*eq*), 2.15, 2.14, 2.10, 2.09, 2.083, 2.080, 2.05, 2.02, 2.00, 1.99, 1.90, 1.89, 1.77 (13s, 39H, 11AcO, AcN, and *Me*OCH_2CH_2), 1.61 (t, 1 H, $J_{gem} = J_{3ax,4} = 12.4$ Hz, H-3e*ax*), 1.50 (s, 3H, AcN), 0.89 (m, 2H, $Me_3SiCH_2CH_2$); ^{13}C NMR ($CDCl_3$): δ 173.30, 172.20, 172.13, 172.07, 172.03, 171.88, 171.69, 171.59, 171.47, 171.31, 171.22, 171.03, 170.41, 169.57, 167.19, 166.90, 166.26, 134.85, 134.76, 134.65, 131.74, 131.40, 131.16, 130.98, 130.65, 130.01, 129.87, 102.19, 102.04, 101.83, 101.37, 98.25, 77.23, 76.82, 76.55, 74.30, 74.21, 74.03, 73.51, 73.16, 73.10, 72.80, 72.64, 72.51, 71.97, 70.83, 70.60, 69.34, 68.87, 68.79, 67.84, 63.67, 63.60, 63.37, 62.94, 62.78, 56.23, 54.57, 50.12, 39.15, 38.63, 31.25, 29.20, 24.61, 24.55, 22.83, 22.31, 22.24, 22.21, 22.14, 21.82, 19.26. Anal. Calcd for $C_{91}H_{116}N_2O_{45}Si$: C, 55.03; H, 5.89; N, 1.41. Found: C, 55.01; H, 5.75; N, 1.36.

1.7. 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)-2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-levulinoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (8)

The 2-(trimethylsilyl)ethyl group of **7** (223.2 mg, 0.11 mmol) was removed by treatment with trifluoroacetic acid (2.1 mL) in CH_2Cl_2 (4 mL) for 4 h at room temperature. EtOAc (4 mL) was added and the mixture was concentrated. Column chromatography (20:1 $CHCl_3$–MeOH) of the residue on silica gel gave the corresponding reducing sugar (211.6 mg, quant.). This compound was treated with trichloroacetonitrile (296 μL, 23.6 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 15.4 μL, 0.094 mmol) in CH_2Cl_2 (6 mL) for 2 h at 0 °C. The mixture was concentrated and the residue was purified by chromatography (30:1 $CHCl_3$–MeOH) on a column of silica gel to give trichloroacetimidate **8** (202.7 mg, 89%) as an amorphous solid; $[\alpha]_D^{25}$ +4.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.65 (s, 1H, NH of imidate), 8.12–7.42 (m, 15H, 3Ph), 6.45 (d, 1H, $J_{1,2} = 3.6$ Hz, H-a), 5.63 (m, 1H, H-8e), 5.49 (t, 1H, $J_{2,3} = 9.6$, Hz, H-3a), 5.39 (dd, 1H, $J_{1,2} = 7.8$, $J_{2,3} = 10.3$ Hz, H-2d), 5.21 (dd, 1H, $J_{6,7} = 2.3$, $J_{7,8} = 9.3$ Hz, H-7e), 5.04 (dd, 1H, $J_{1,2} = 3.6$, $J_{2,3} = 9.6$ Hz, H-2a), 4.92 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1d), 4.87 (dd, 1H, $J_{1,2} = 8.0$, $J_{2,3} = 9.8$ Hz, H-2b), 4.52 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1c), 4.30 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1b), 3.82 (s, 3H, COO*Me*), 2.75–2.43 (m, 5H, MeCOCH_2CH_2 and H-3e*eq*), 2.15, 2.14, 2.10, 2.09, 2.08, 2.06, 2.04, 2.02, 2.01, 1.99, 1.90, 1.89, 1.76 (13s, 39H, 11AcO, AcN, and *Me*COCH_2CH_2), 1.60 (t, 1H, $J_{gem} = J_{3ax,4} = 12.5$ Hz, H-3e*ax*), 1.42 (s, 3H, AcN). Anal. Calcd for $C_{88}H_{104}Cl_3N_3O_{45}$: C, 52.06; H, 5.16; N, 2.07. Found: C, 52.05; H, 4.97; N, 1.79.

1.8. 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)-2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-levulinoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (10)

To a solution of **8** (202.7 mg, 0.10 mmol) and (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzol-4-octadecene-1,3-diol **9** (79 mg, 0.18 mmol) in dry CH_2Cl_2 (2 mL) were added 4 Å molecular sieves (type AW300; 4 g), and the mixture was stirred for 6 h at room temperature, and then cooled

to 0 °C. Trimethylsilyl trifluoromethanesulfonate (1.74 μL, 8.89 μmol) was added to the mixture, and this was stirred for 36 h at 0 °C, neutralized with Et_3N, and filtered. Chromatography (60:1 $CHCl_3$–MeOH) of the residue on silica gel afforded **10** (113.7 mg, 49%) as an amorphous solid; $[\alpha]_D^{25}$ +2.1 (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.18–7.43 (m, 20H, 4Ph), 5.90 (dt, 1H, $J_{4,5} = 14.8$, $J_{5,6} = J_{5,6'} = 6.6$ Hz, H-5 of sphingosine), 5.64 (m, 1H, H-8e), 5.59 (m, 1H, H-4 of sphingosine), 5.37 (dd, 1H, $J_{1,2} = 7.8$, $J_{2,3} = 10.2$ Hz, H-2d), 5.35 (d, 1H, H-4d), 5.22 (dd, 1H, $J_{6,7} = 2.3$ Hz, H-7e), 5.17 (dd, 1H, $J_{1,2} = 8.0$, $J_{2,3} = 9.8$ Hz H-2a), 4.97 (dd, 1H, $J_{1,2} = 7.8$, $J_{2,3} = 9.8$ Hz, H-2b), 4.90 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1d), 4.85 (dd, 1H, $J_{2,3} = 10.2$, $J_{3,4} = 3.2$ Hz, H-3d), 4.52 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1c), 4.49 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1b), 4.36 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1a), 3.80 (s, 3H, COO*Me*), 2.69–2.43 (m, 5H, MeCOCH_2CH_2 and H-3e*eq*), 2.14, 2.13, 2.09, 2.08, 2.07, 2.06, 2.03, 2.01, 2.00, 1.99, 1.90, 1.88, 1.77 (13s, 39H, 11AcO, AcN, and *Me*COCH_2CH_2), 1.58 (t, 1H, $J_{gem} = J_{3ax,4} = 12.1$ Hz, H-3e*ax*), 1.50 (s, 3H, AcN), 1.23 (s, 22H, 11 CH_2), 0.87 (t, 3H, J_{vic} 6.6 Hz, *Me*CH_2); ^{13}C NMR ($CDCl_3$): δ 171.90, 170.76, 170.64, 170.35, 170.24, 170.15, 170.05, 169.86, 169.74, 169.55, 168.97, 168.14, 165.76, 165.49, 165.07, 164.84, 139.05, 133.41, 133.32, 133.22, 130.32, 129.99, 129.89, 129.75, 129.58, 129.23, 128.56, 128.44, 122.56, 100.77 (2C), 100.40 (2C), 96.84, 75.59, 75.38, 75.09, 74.67, 72.89, 72.82, 72.60, 72.11, 71.74, 71.40, 71.22, 71.13, 70.54, 69.38, 69.16, 68.34, 67.90, 67.30, 66.38, 63.49, 62.14, 61.96, 61.51, 61.33, 54.82, 53.16, 48.77, 37.74, 37.20, 32.38, 31.92, 29.84, 29.66, 29.58, 29.40, 29.35, 29.16, 28.72, 27.77, 23.21, 23.16, 22.69, 21.42, 20.89, 20.80, 20.72, 20.39, 14.14. Anal. Calcd for $C_{111}H_{141}N_5O_{47}$: C, 58.03; H, 6.19; N, 3.05. Found: C, 57.91; H, 6.16; N, 2.79.

1.9. 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)-2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-levulinoyl-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 (11)

Hydrogen sulfide was bubbled through a stirred solution of **10** (36 mg, 0.016 mmol) in pyridine (5 mL) and water (1 mL) for 72 h at 0 °C. The mixture was concentrated and the residual syrup was treated with octadecanoic acid (12 mg, 0.042 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (8 mg, 0.042 mmol) in CH_2Cl_2 (1 mL) for 48 h at room temperature. The mixture was extracted with chloroform and the extract was successively washed with 1 M hydrochloric acid and water, dried (Na_2SO_4), and concentrated. Column chromatography (60:1 $CHCl_3$–MeOH) of the residue on silica gel gave **11** (19.5 mg, 49%) as an amorphous solid; $[\alpha]_D^{25}$ +13.0 (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.18–7.42 (m, 20H, 4Ph), 5.84 (dt, 1H, $J_{4,5} = 14.6$, $J_{5,6} = J_{5,6'} = 6.6$ Hz, H-5 of sphingosine), 5.74 (d, 1H, $J_{NH,2} = 9.4$ Hz, NH of ceramide), 5.63 (m, 1H, H-8e), 5.47 (m, 1H, H-4 of sphingosine), 5.37 (dd, 1H, $J_{1,2} = 7.8$, $J_{2,3} = 10.3$ Hz, H-2d), 5.35 (d, 1H, H-4d), 5.22 (dd, 1H, $J_{6,7} = 2.9$, $J_{7,8} = 9.8$ Hz, H-7e), 5.12 (t, 1H, $J_{2,3} = 9.6$ Hz, H-3a), 4.93 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1d), 4.87 (dd, 1H, $J_{1,2} = 7.8$, $J_{2,3} = 9.6$ Hz, H-2a), 4.85 (dd, 1H, $J_{2,3} = 10.3$, $J_{3,4} = 3.0$ Hz, H-3d), 4.54 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1c), 4.51 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1b), 4.42 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1a), 3.80 (s, 3H, COO*Me*), 2.69–2.51 (m, 4H, MeCOCH_2CH_2), 2.47 (dd, 1H, $J_{3eq,4} = 4.8$, $J_{gem} = 12.4$ Hz, H-3e*eq*), 2.14, 2.13, 2.09, 2.088, 2.085, 2.082, 2.07, 2.01, 2.009, 2.00, 1.99, 1.91. 1.90 (13s, 39H, 11 AcO, AcN, and *Me*COCH_2CH_2), 1.61 (t, 1H, $J_{gem} = J_{3ax,4} = 12.4$ Hz, H-3e*ax*), 1.50 (s, 3H, AcN), 1.25 (s, 52H, 26 CH_2), 0.88 (t, 6H, $J_{vic} = 6.6$ Hz, 2*Me*CH_2); ^{13}C NMR ($CDCl_3$): δ 206.82, 172.67, 171.89, 170.72, 170.64, 170.21, 170.11, 170.05, 169.77, 169.73, 169.65, 168.93, 168.18, 165.78, 165.53, 165.20, 164.87, 137.53, 133.40, 133.29, 133.21, 133.02, 130.33, 130.01, 129.78, 129.67, 129.63, 129.34, 128.60, 128.44, 128.41, 124.69, 100.82, 100.65, 100.51, 100.42, 96.91, 75.48, 75.13, 74.13, 72.98, 72.90, 72.39, 72.18, 71.83, 71.76, 71.46, 71.29, 70.63, 69.40, 69.22, 68.01, 67.46, 66.52, 62.24, 61.99, 61.58, 61.40, 54.87, 53.14, 50.71, 48.90, 37.80, 37.29, 37.12 36.87, 33.75, 32.78, 32.35, 31.94, 30.05, 29.80, 29.71, 29.63, 29.54, 29.50, 29.44, 29.37, 29.26, 28.97, 27.84, 27.43, 27.10, 26.76, 25.74, 23.19, 23.15, 22.70, 21.40, 20.78, 20.73, 20.70, 20.57, 20.39, 19.74, 14.11. Anal. Calcd for $C_{129}H_{177}N_3O_{48}$: C, 61.05; H, 7.03; N, 1.66. Found: C, 60.96; H, 6.92; N, 1.55.

1.10. 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)-2-acetamido-3-*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-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (12)

To a solution of **11** (19.5 mg, 0.0077 mmol) in EtOH (1 mL) was added hydrazine acetate (3.6 mg, 0.038 mmol), and the mixture was stirred for 3 h at room temperature and then concentrated. Column chromatography (50:1 $CHCl_3$–MeOH) of the residue on silica gel gave **12** (16.9 mg, 90%) as an amorphous solid; $[\alpha]_D^{25}$ −13.2 (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.22–7.42 (m, 20H, 4Ph), 5.86 (dt, 1H, $J_{4,5} = 14.0$, $J_{5,6} = J_{5,6'}$ 6.4 Hz, H-5 of sphingosine), 5.74 (d, 1H,

$J_{NH,2} = 9.5$ Hz, NH of ceramide), 5.70 (m, 1H, H-8e), 5.46 (m, 1H, H-4 of sphingosine), 5.41 (d, 1H, H-4d), 5.37 (dd, 1H, $J_{1,2} = 8.0$, $J_{2,3} = 10.0$ Hz, H-2d), 4.98 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1d), 4.87 (dd, 1H, $J_{1,2} = 8.0$, $J_{2,3} = 9.5$ Hz, H-2a), 4.84 (dd, 1H, $J_{2,3} = 10.0$, $J_{3,4} = 3.0$ Hz, H-3d), 4.59 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1c), 4.43 (d, 1H, $J_{1,2} = 7.5$ Hz, H-1b), 4.28 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1a), 3.82 (s, 3H, COO*Me*), 3.22 (br d, 1H, H-6c), 2.47 (dd, 1H, $J_{3eq,4} = 4.5$, $J_{gem} = 12.5$ Hz, H-3e*eq*), 2.16, 2.15, 2.12, 2.08, 2.07, 2.01, 2.009, 2.00, 1.96, 1.93, 1.91, 1.87, 1.77 (13s, 39H, 11AcO and 2AcN), 1.66 (t, 1H, $J_{gem} = J_{3ax,4}$ 12.5 Hz, H-3e*ax*), 1.26 (s, 52H, $26CH_2$), 0.88 (t, 6H, J_{vic} 7.0 Hz, $2MeCH_2$); ^{13}C NMR($CDCl_3$):δ 172.65, 171.78, 170.62, 170.48, 170.09, 170.04, 169.63, 169.26, 168.61, 168.48, 167.84, 166.39, 165.73, 165.55, 165.50, 165.22, 164.93, 137.56, 133.31, 130.28, 129.94, 129.69, 129.60, 128.56, 128.43, 124.61, 100.98, 100.73, 100.65, 99.94, 98.77, 75.60, 75.22, 74.24, 74.11, 72.78, 72.33, 72.14, 71.68, 71.38, 71.05, 70.58, 69.07, 68.42, 67.96, 67.42, 67.05, 66.71, 62.82, 61.96, 61.50, 60.44, 54.82, 53.37, 50.62, 49.69, 37.33, 36.85, 32.35, 31.93, 29.70, 29.52, 29.49, 29.42, 29.35, 29.23, 28.95, 25.72, 23.24, 22.70, 21.36, 20.82, 20.73, 20.37, 20.13, 14.13. Anal. Calcd for $C_{124}H_{171}N_3O_{46}$: C, 61.05; H, 7.06; N, 1.72. Found: C, 61.03; H, 6.86; N, 1.52.

1.11. 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→4)-2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-sulfo-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 (13)

To a solution of **12** (15 mg, 0.0062 mmol) in DMF (1.5 mL) was added sulfur trioxide pyridine complex (9 mg, 0.056 mmol), and the mixture was stirred for 8 h at room temperature. Triethylamine (0.1 mL) was added and the mixture was concentrated. Column chromatography (1:1 $CHCl_3$–MeOH) of the residue on Sephadex LH-20 gave the crude sulfated product, and this was purified by column chromatography (30:1, $CHCl_3$–MeOH) on silica gel to afford **13** (11.3 mg, 70%) as an amorphous solid; $[\alpha]_D^{25}$ +2.30 (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.28–7.44 (m, 20H, 4Ph), 5.86 (dt, 1H, $J_{4,5} = 15.0$, $J_{5,6} = J_{5,6'} = 7.0$ Hz, H-5 of sphingosine), 5.73 (d, 1H, $J_{NH,2} = 9.0$ Hz, NH of cermide), 5.63 (m, 1H, H-8e), 5.46 (m, 1H, H-4 of sphingosine), 5.43 (d, 1H, H-4d), 5.37 (dd, 1H, $J_{1,2} = 8.0$, $J_{2,3} = 10.0$ Hz, H-2d), 5.29 (dd, 1H, $J_{6,7} = 2.0$, $J_{7,8} = 9.0$ Hz, H-7e), 4.97 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1d), 4.87 (dd, 1H, $J_{1,2} = 8.0$, $J_{2,3} = 9.5$ Hz, H-2a), 4.42 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1b), 4.27 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1a), 3.75 (s, 3H, COO*Me*), 3.11 (q, 6H, $3NCH_2CH_3$), 2.41 (dd, 1H, $J_{3eq,4} = 4.5$, $J_{gem} = 12.5$ Hz, H-3e*eq*), 2.10, 2.08, 2.07, 2.05, 2.04, 2.02, 2.00, 1.99, 1.97, 1.96, 1.94, 1.89, 1.78 (13s, 39H, 11AcO and 2AcN), 1.56 (t, 1H, $J_{gem} = J_{3ax,4} = 12.0$ Hz, H-3e*ax*), 1.34 (t, 9H, $3NCH_2CH_3$), 1.25 (s, 52H, $26CH_2$), 0.87 (t, 6H, $J_{vic} = 7.0$ Hz, $2MeCH_2$); ^{13}C NMR($CDCl_3$): δ 172.63, 171.83, 170.70, 170.48, 170.15, 170.03, 169.62, 169.31, 168.64, 168.39, 167.82, 166.45, 165.79, 165.58, 165.49, 165.14, 164.88, 137.56, 133.41, 130.32, 129.95, 129.71, 129.55, 128.62, 128.38, 124.56, 101.22, 100.68, 100.32, 99.92, 96.95, 75.58, 75.19, 74.27, 74.12, 72.76, 72.41, 72.14, 71.71, 71.36, 71.07, 70.51, 69.02, 68.42, 67.93, 67.45, 67.08, 66.69, 62.82, 61.91, 61.51, 60.42, 54.81, 53.40, 50.62, 49.64, 46.48, 37.81, 37.25, 32.32, 31.94, 29.80, 29.53, 29.40, 29.34, 29.26, 28.86, 25.75, 23.24, 22.70, 21.42, 20.75, 20.70, 20.39, 14.15, 8.73. Anal. Calcd for $C_{130}H_{186}N_4O_{49}S$: C, 59.57; H, 7.15; N, 2.14. Found: C, 59.39; H, 7.10; N, 1.91.

1.12. 5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-6-*O*-sulfo-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol disodium salt; 6-*O*-sulfo-sialylparagloboside (14)

To a solution of **13** (10.0 mg, 0.0038 mmol) in MeOH (1 mL) and dioxane (0.2 mL) was added a catalytic amount of 28% sodium methoxide in MeOH, and the mixture was stirred for 96 h at room temperature. Water (0.1 mL) was added and the mixture was stirred for 24 h at room temperature, and then concentrated. Column chromatography (1:3:1 $CHCl_3$–MeOH–H_2O) of the residue on Sephadex LH-20 gave the target molecule **14** (6.3 mg, quant.) as an amorphous solid; $[\alpha]_D^{25}$ +67.5 (*c* 0.2, 1:3:1 $CHCl_3$–MeOH–H_2O); ^{1}H NMR (CD_3OD): δ 5.89 (dt, 1H, $J_{4,5} = 13.5$, $J_{5,6} = J_{5,6'} = 6.5$ Hz, H-5 of sphingosine), 5.35 (m, 1H, H-4 of sphingosine), 4.57 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1c), 4.43 (dd, 1H, $J_{1,2} = 8.0$ Hz, H-1d), 4.27 (d, 1H, $J_{1,2} = 7.5$ Hz, H-1b), 4.20 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1a), 4.11 (dd, 1H, $J_{2,3} = 10.5$, $J_{3,4} = 4.5$ Hz, H-3d), 2.74 (br d, 1H, H-3e*eq*), 2.07 (t, 1H, H-1' of stearoyl), 1.93 (dd, 1H, H-6 of sphingosine), 1.90 (s, 3H, AcN), 1.88 (s, 3H, AcN), 1.69 (t, 1H, H-3e*ax*), 1.48 (m, 2H, H-2′ of stearoyl), 1.28 (m, 1H, H-6' of sphingosine), 1.19 (s, 52H, $26CH_2$), 0.81 (t, 6H, J_{vic} 7.0 Hz, $2MeCH_2$); ^{13}C NMR(CD_3OD): δ 175.03, 174.58, 170.01, 121.11, 105.34, 105.20, 104.83, 103.92, 101.65, 83.98, 82.39, 81.15, 77.03, 76.75, 76.65, 75.90, 74.95, 74.62, 74.40, 74.31, 73.42, 72.81, 71.78, 70.75, 70.49, 68.07, 64.89, 62.80, 62.24, 57.54, 39.66, 33.29, 32.32, 31.28, 30.98, 30.91, 30.70, 23.95, 23.59, 22.93, 14.66; MALDI-TOFMS calcd for $C_{73}H_{130}N_3Na_2O_{34}S$: *m*/*z* 1669.80; found: 1646.95: $[M-Na]^-$.

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