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Synthesis and selectin-binding activity of *N*-deacetylsialyl Lewis X ganglioside☆

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Dedicated to Professor Derek Horton on the occasion of his 70th birthday

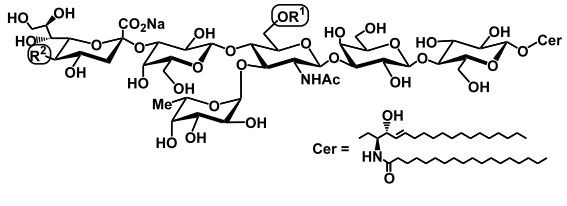
Abstract

A novel analogue of sialyl Lewis X ganglioside, *N*-deacetylsialyl Lewis X ganglioside, was synthesized. Methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-D-galactopyranosyl trichloroacetimidate was coupled with 2-(trimethylsilyl)ethyl [2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(4-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-galactopyranoside to give the desired pentasaccharide in high yield. The glycosylation of the pentasaccharide acceptor, which was derived from its precursor by removal of the 3-methoxybenzyl group, with the phenyl 1-thioglycoside derivative of L-fucose using *N*-iodosuccinimide–trifluoromethanesulfonic acid as promoter, produced the hexasaccharide. Proper manipulation of the protecting groups of the hexasaccharide 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, and the complete removal of the protecting groups gave the desired *N*-deacetylsialyl Lewis X ganglioside. L-Selectin bound more strongly to *N*-deacetylsialyl Lewis X ganglioside than to the sialyl Lewis X ganglioside, whereas E- and P-selectins bound equally well to the two gangliosides.

Keywords: Ganglioside; Sialyl Lewis X; Selectin; *N*-Deacetylsialic acid

1. Introduction

It has been shown[2,3] that selectin–carbohydrate interactions mediate cell adhesions involved in various biological processes. L-Selectin, a member of the selectin family that is expressed on leukocytes, binds to saccharide ligands on high endothelial cells in post-capillary venules of lymph nodes. It plays a key role in the initial stages of leukocyte extravasation into peripheral lymph nodes and areas of acute and chronic inflammation.[4,5] We have demonstrated with chemically synthesized gangliosides (Fig. 1) that the sialyl Lewis X (sLe^x) sequence with 6-*O*-sulfo functionality at *N*-acetylglucosamine (6-*O*-sulfo sLe^x)[6] constitutes a strong ligand



sLe^x : R^1 = H, R^2 = NHAc
6-*O*-Sulfo sLe^x : R^1 = SO_3Na, R^2 = NHAc
6-*O*-sulfo *N*-deacetyl sLe^x : R^1 = SO_3Na, R^2 = NH_2
N-deacetyl sLe^x : R^1 = H, R^2 = NH_2 (Target Compound)

Fig. 1. Synthetic analogs of sialyl Lewis X ganglioside.

☆ Synthetic studies on sialoglycoconjugates, Part 126. For Part 125, see Ref. 1.

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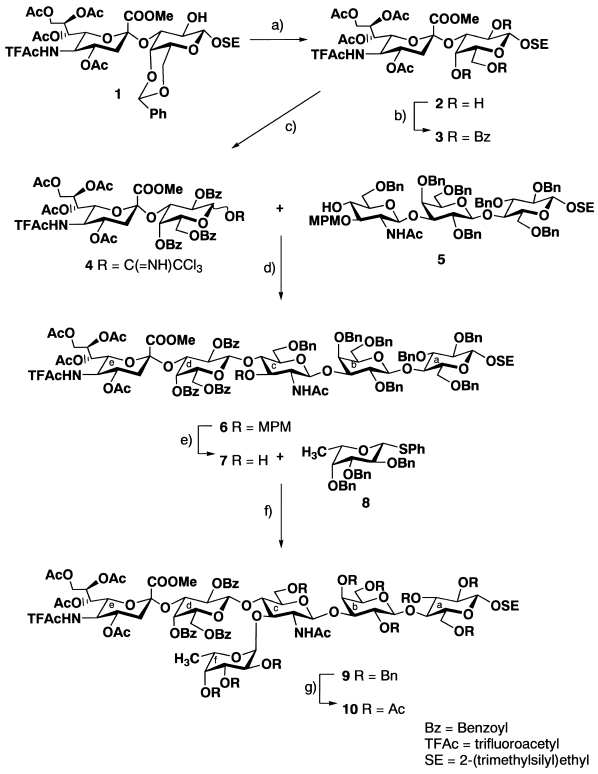
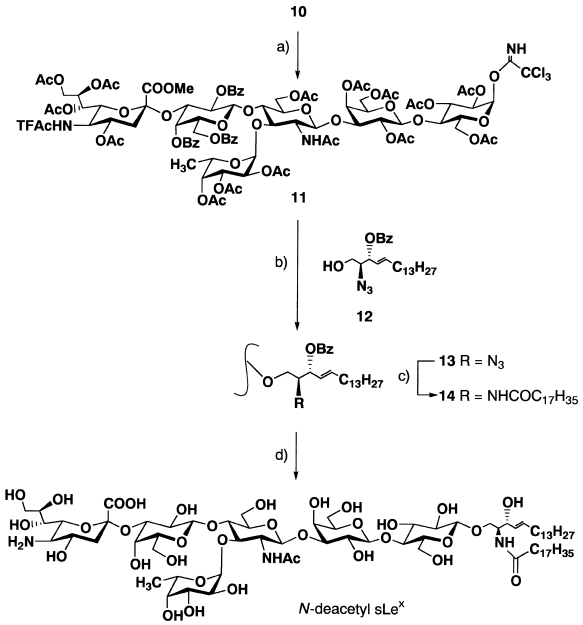


Scheme 1. *Reagents and conditions*: (a) H_2, Pd–C, AcOH; (b) Bz_2O, DMAP, pyr.; (c) (i) TFA, CH_2Cl_2, (ii) CCl_3CN, DBU, CH_2Cl_2; (d) TMSOTf, CH_2Cl_2, 7 °C; (e) CAN, CH_3CN–H_2O; (f) NIS–TfOH, benzene, 7°C; (g) (i) H_2, Pd–C, EtOH, HOAc, 40 °C, (ii) Ac_2O, pyr., 40 °C.



Scheme 2. *Reagents and conditions*: (a) (i) TFA, CH_2Cl_2, (ii) CCl_3CN, DBU, CH_2Cl_2, 0 °C; (b) TMSOTf, CH_2Cl_2, 0 °C; (c) (i) H_2S, pyr.–H_2O, 0 °C, (ii) WSC, CH_2Cl_2; (d) MeONa, MeOH, 45 °C, then H_2O.

for L-selectin.[7] Also, the *N*-deacetylated form of 6-*O*-sulfo sLe^x (6-*O*-sulfo-*N*-deacetyl sLe^x)[8,9] is a superior ligand of L-selectin compared with the parental 6-*O*-sulfo sLe^x.[7,9] As a component of our continuing studies on selectin ligands, in order to elucidate the structure required for the recognition by L-selectin, we describe the synthesis and selectin-binding activity of *N*-deacetylsialyl Lewis X ganglioside (*N*-deacetyl sLe^x).

2. Results and discussion

For the synthesis of the target molecule, we used methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-D-galactopyranosyltrichloroacetimidate (**4**) as a glycosyl donor, which was prepared from the known disaccharide **1**[8,9] in 70% yield (four steps). In the glycosyl donor **4**, the amino function at C-5 of neuraminic acid is protected as the trifluoroacetamide, which can be simultaneously converted into the amino function again at the final *O*-deacylation under the Zemplén conditions.

Coupling of **4** with the suitably protected trisaccharide acceptor **5**[10] gave the sialyl α-(2→3)-neolactotetraose derivative **6** in 78% yield. The *p*-methoxybenzyl (MPM) group at C-3 of GlcNAc in **6** was selectively removed (84%) by treatment with ceric ammonium nitrate (CAN)–H_2O, and the resulting **7** was fucosylated with **8**[6] in the presence of *N*-iodosuccinimide (NIS)–trifluoromethanesulfonic acid (TfOH) in benzene at 7 °C to produce the desired hexasaccharide **9** in 99% yield. Hydrogenolytic removal of the benzyl groups in the hexasaccharide and the following O-acetylation gave **10** in a quantitative yield. In the ^{1}H NMR spectrum of **10**, a significant one-proton doublet ($J_{1,2}$ 3.4 Hz, H-1 of fucose) appeared at δ 5.07, showing the newly formed glycoside to be an α-L-fucopyranoside. The hexasaccharide **10** was then converted to the imidate derivative **11** by the removal of the 2-(trimethylsilyl)ethyl group and the treatment of the hemiacetal thus obtained with trichloroacetonitrile and DBU (90% in two steps) (Schemes 1 and 2).

Glycosylation of the azidosphingosine derivative **12**[11,12] with **11**, and successive reduction of the azido function and N-acylation was carried out using the established method.[12,13] Finally, removal of all protective groups under the basic conditions furnished the target molecule, *N*-deacetylsialyl Lewis X (*N*-deacetyl sLe^x) in 96% yield (Scheme 2).

A negative-ion mass spectrum acquired from the target molecule gave an $[M-H]^-$ ion at m/z 1648 and fragment ions at m/z 1399, 1237, 888 and 726. The ion at m/z 1399 (-249 Da) corresponds to the fragment obtained by glycosidic cleavage of the terminal *N*-deacetylated neuraminic acid.

We investigated the binding of the selectins to the target molecule, *N*-deacetyl sLe^x, in comparison with sLe^x, 6-*O*-sulfo sLe^x, and 6-*O*-sulfo *N*-deacetyl sLe^x. The results shown in Fig. 2 are one of three data sets which were in overall agreement. L-Selectin consistently bound more strongly to the *N*-deacetyl sLe^x than to sLe^x, as in Fig. 2 panel B. The strength of binding to the *N*-deacetyl sLe^x was of the same order as to the 6-*O*-sulfo sLe^x, but not as strong as to 6-*O*-sulfo *N*-deacetyl sLe^x, which to date, is the most potent ligand for L-selectin.[7,9] Thus the hierarchy of L-selectin binding strengths was in the order 6-*O*-sulfo *N*-deacetyl sLe^x > 6-*O*-sulfo sLe^x = *N*-deacetyl sLe^x > sLe^x, showing that N-deacetylation as well as 6-*O*-sulfation potentiates the binding of L-selectin. With E-selectin, the binding signals for the target compound and the three other sLe^x analogs were consistently of the same order, as shown in Fig. 2(A). With P-selectin, the binding to the target compound was of the same order as that to sLe^x and to 6-*O*-sulfo sLe^x. This binding was consistently less than to the 6-*O*-sulfo *N*-deacetyl sLe^x.

3. Experimental

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 Varian Unity Inova (400 and 500 MHz) spectrometers with TMS as the internal standard. Liquid secondary-ion mass spectrometry (LSIMS) was carried out on a VG Analytical ZAB2-E mass spectrometer, fitted with a cesium ion gun operated at 25 keV and an emission current of 0.5 μA. Spectra were acquired in the negative-ion mode directly from the surface of a silica gel TLC plate as described.[14] Preparative thin-layer chromatography (TLC) was performed on Silica Gel 60 (E. Merck), and column chromatography on Silica Gel (Fuji Silysia Co., 300 mesh) was performed with the specified solvent systems (v/v). Concentrations and evaporations were conducted in vacuo.

2-(Trimethylsilyl)ethyl (methyl 4,7,8,9-tetra-O-*acetyl-3,5-dideoxy-5-trifluoroacetamido*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)-β*-D-*galactopyranoside* (**2**).—A solution of **1**[8,9] (1.55 g, 1.73 mmol) in HOAc (100 mL) was vigorously stirred with 10% Pd–C (1.5 g) for 12 h at room temperature (rt) under a H_2 atmosphere. The catalyst was collected and washed with MeOH. (Caution! Extreme fire hazard.) The combined filtrate and washings were concentrated. Column chromatography (20:1 $CHCl_3$–MeOH) of the residue on silica gel gave **2** (1.39 g, 96%) as an amorphous mass: $[\alpha]_D$ −9.3° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.09 (m, 2 H, $Me_3SiCH_2CH_2$), 2.03, 2.04, 2.14, 2.15 (4 s, 12 H, 4 AcO), 1.99 (t, 1 H, $J_{gem} = J_{3ax,4}$ 13.4 Hz, H-3^{II}ax), 2.74 (dd, 1 H, $J_{3eq,4}$ 4.5 Hz, H-3^{II}eq), 3.85 (s, 3 H, COOMe), 4.89 (dd, 1 H, $J_{8,9}$ 1.8, J_{gem} 10.7 Hz, H-9^{II}), 4.43 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^{I}), 5.05 (m, 1 H, H-4^{II}), 5.28 (dd, 1 H, $J_{6,7}$ 1.8, $J_{7,8}$ 8.4 Hz, H-7^{II}), 5.43 (m, 1 H, H-8^{II}) 6.71 (d, 1 H, $J_{5,NH}$ 9.6 Hz, NH^{II}). Anal. Calcd for $C_{31}H_{48}F_3NO_{18}Si$ (807.79): C, 46.09; H, 5.99; N, 1.73. Found: C, 46.06; H, 5.85; N, 1.60.

2-(Trimethylsilyl)ethyl (methyl 4,7,8,9-tetra-O-*acetyl-3,5-dideoxy-5-trifluoroacetamido*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)-2,4,6-tri*-O-*benzoyl-β*-D-*galactopyranoside* (**3**).—A mixture of **2** (814 mg, 1.01 mmol), benzoic anhydride (1.2 g, 5.28 mmol) and 4-dimethylaminopyridine (DMAP, 53 mg, 0.43 mmol) was dissolved in pyridine (5 mL), and the mixture was stirred for 12 h at rt, then cooled to 0 °C. MeOH (5 mL) was added and the mixture was concentrated. The residue was extracted with $CHCl_3$ and successively washed with cold 2 M HCl and water, dried (Na_2SO_4) and concentrated. Column chromatography (1:4 EtOAc–hexane) of the residue on silica gel resulted in **3** (1.06 g, 92%) as an amorphous mass: $[\alpha]_D$ +26.4° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 0.93 (m, 2 H, $Me_3SiCH_2CH_2$), 1.44, 1.91, 2.06, 2.18 (4 s, 12 H, 4

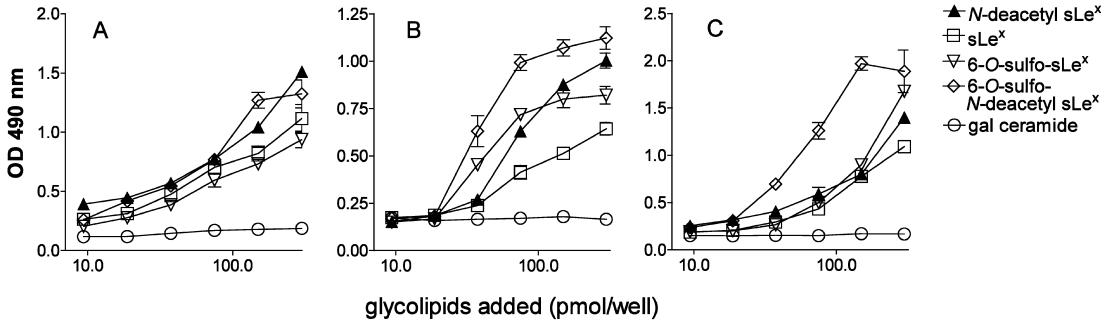


Fig. 2. Comparison of the selectin binding siganls elicited by the largest compound, *N*-deacetyl sLe^x, with those elicited by sLe^x, 6-*O*-sulfo sLe^x and 6-*O*-sulfo-*N*-deacetyl sLe^x. Binding of the recomboinant soluble E-, L- and P-selectins (panels A, B, and C, respectively) to the glycolipids immobialized in microwells was assayed as described in Section 2. Results are expressed as means in duplicate wells. The range, where visible, is indicated as error bars.

AcO), 1.67 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.8 Hz, H-3^{II}ax), 2.53 (dd, 1 H, $J_{3eq,4}$ 4.5 Hz, H-3^{II}eq), 3.86 (s, 3 H, COOMe), 3.96 (dd, 1 H, J_{gem} 12.3, $J_{8,9}$ 4.3 Hz, H-9^{II}), 4.33 (dd, 1 H, $J_{8,9'}$ 2.9 Hz, H-9′^{II}), 4.35 (dd, 1 H, $J_{5,6'}$ 5.0, J_{gem} 9.8 Hz, H-6′^{I}), 4.51 (dd, 1 H, $J_{5,6}$ 6.8 Hz, H-6^{I}) 4.83 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{I}), 4.95 (m, 1 H, H-4^{II}), 5.18 (dd, 1 H, $J_{6,7}$ 2.0, $J_{7,8}$ 9.3 Hz, H-7^{II}), 5.42 (dd, 1 H, $J_{2,3}$ 10.1 Hz, H-2^{I}), 5.62 (m, 1 H, H-8^{II}), 6.26 (d, 1 H, $J_{5,NH}$ 9.1 Hz, NH^{II}), 7.26–8.18 (m, 15 H, 3 Ph). Anal. Calcd for $C_{52}H_{60}F_3NO_{21}Si$ (1120.12): C, 55.76; H, 5.40; N, 1.25. Found: C, 55.67; H, 5.20; N, 1.07.

Methyl 4,7,8,9-tetra-O-*acetyl-3,5-dideoxy-5-trifluoroacetamido*-D-glycero-α-D-galacto-*2-nonulopyranosylonate-(2→3)-2,4,6-tri*-O-*benzoyl*-D-*galactopyranosyltrichloroacetimidate* (**4**).—Compound **3** (1.06 g, 0.99 mmol) was treated with trifluoroacetic acid (4.4 mL) in CH_2Cl_2 (7 mL) for 1 h at rt. EtOAc (5 mL) was added, and the mixture was concentrated. Column chromatography (1:2 EtOAc–hexane) of the residue on silica gel gave the free 1-OH derivative (898 mg). This compound (898 mg, 0.91 mmol) was treated with Cl_3CCN (2.8 mL, 28 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 144 μL, 0.96 mmol) in CH_2Cl_2 (9 mL) for 2 h at 0 °C. The mixture was concentrated, and the residue was chromatographed (100:1 $CHCl_3$–MeOH) on a column of silica gel to give the trichloroacetimidate **4** as the mixture of α and β isomers (α:β = 2:1) (1.02 g, 92% two steps). ^{1}H NMR ($CDCl_3$) of α isomer: δ 1.91, 1.95, 2.07, 2.10 (4 s, 12 H, 4 AcO), 1.63 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.8 Hz, H-3^{V}ax), 2.56 (dd, 1 H, J_{gem} 12.8, $J_{3eq,4}$ 4.4 Hz, H-3^{V}eq), 3.81 (s, 3 H, COOMe), 5.01 (m, 1 H, H-4^{II}), 5.43 (dd, 1 H, $J_{6,7}$ 1.5, $J_{7,8}$ 9.2 Hz, H-7^{II}), 5.60 (m, 1 H, H-8^{II}), 6.54 (d, 1 H, $J_{5,NH}$ 9.5 Hz, NH^{II}), 6.87 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^{I}), 7.27–8.19 (m, 15 H, 3 Ph), 8.64 (s, 1 H, NH of imidate). Anal. Calcd for $C_{49}H_{48}Cl_3F_3N_2O_{21}$ (1164.27): C, 50.55; H, 4.16; N, 2.41. Found: C, 50.49; H, 3.96; N, 2.25.

2-(Trimethylsilyl)ethyl (methyl 4,7,8,9-tetra-O-*acetyl-3,5-dideoxy-5-trifluoroacetamido*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)-2,4,6-tri*-O-*benzoyl*-β-D-*galactopyranosyl-(1→4)-2-acetamido-6*-O-*benzyl-2-deoxy-3*-O-p-*methoxybenzyl*-β-D-*glucopyranosyl-(3)-2,4,6-tri*-O-*benzyl*-β-D-*galactopyranosyl-(1→4)-2,3,6-tri*-O-*benzyl*-β-D-*glucopyranoside* (**6**).—To a solution of **4** (850 mg, 0.77 mmol) and the trisaccharide acceptor **5**[10] (540 mg, 0.39 mmol) in dry CH_2Cl_2 (8 mL) was added 4 Å molecular sieves (800 mg), and the mixture was stirred for 3 h at rt, then cooled to 0 °C. Trimethylsilyl trifluoromethanesulfonate (TMSOTf; 13.5 μL, 67.5 μmol) was added to the mixture, and stirring was continued for 12 h at 7 °C. The reaction mixture was neutralized with Et_3N and filtered, and the residue was washed with $CHCl_3$. The combined filtrate and washings were concentrated. Column chromatography (100:1 $CHCl_3$–MeOH) of the residue on silica gel gave **6** (622 mg, 78%) as an amorphous mass: $[\alpha]_D$ + 7.3° (*c* 0.38, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.02 (m, 2 H, $Me_3SiCH_2CH_2$), 1.44 (s, 3 H, AcN), 1.48, 1.90, 1.96, 2.16 (4 s, 12 H, 4 AcO), 1.67 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.0 Hz, H-3^{V}ax), 2.49 (dd, 1 H, $J_{3eq,4}$ 4.3 Hz, H-3^{V}eq), 3.64 (s, 3 H, MeOPh), 3.84 (s, 3 H, COOMe), 4.89 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1^{IV}), 5.67 (m, 1 H, H-8^{V}), 6.23 (d, 1 H, $J_{5,NH}$ 9.1 Hz, NH^{V}), 6.60–8.23 (m, 54 H, aromatic protons). Anal. Calcd for $C_{128}H_{141}F_3N_2O_{37}Si$ (2382.89): C, 64.47; H, 5.96; N, 1.17. Found: C, 64.47; H, 5.89; N, 1.02.

2-(Trimethylsilyl)ethyl (methyl 4,7,8,9-tetra-O-*acetyl-3,5-dideoxy-5-trifluoroacetamido*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)-2,4,6-tri*-O-*benzoyl*-β-D-*galactopyranosyl-(1→4)-2-acetamido-6*-O-*benzyl-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* (**7**).—To a solution of **6** (331 mg, 0.14 mmol) in CH_3CN (3.6 mL) and water (0.4 mL) was added ceric ammonium nitrate (CAN, 300 mg, 0.55 mmol), and the mixture was stirred for 1 h at rt and extracted with $CHCl_3$. The extract was successively washed with 1 M Na_2CO_3 and water, dried (Na_2SO_4) and concentrated. Column chromatography (80:1 $CHCl_3$–MeOH) of the residue on silica gel gave **7** (263 mg, 84%) as an amorphous mass: $[\alpha]_D$ −7.0° (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.02 (m, 2 H, $Me_3SiCH_2CH_2$), 1.44 (s, 3 H, AcN), 1.48, 1.90, 1.96, 2.16 (4 s, 12 H, 4 AcO), 1.70 (dd, 1 H, J_{gem} 12.4, $J_{3ax,4}$ 12.0 Hz, H-3^{V}ax), 2.49 (dd, 1 H, $J_{3eq,4}$ 4.3 Hz, H-3^{V}eq), 3.84 (s, 3 H, COOMe), 5.67 (m, 1 H, H-8^{V}), 7.08–8.23 (m, 50 H, 10 Ph). Anal. Calcd for $C_{120}H_{133}F_3N_2O_{36}Si$ (2264.44): C, 63.65; H, 5.92; N, 1.24. Found: C, 63.56; H, 5.81; N, 1.23.

2-(Trimethylsilyl)ethyl (methyl 4,7,8,9-tetra-O-*acetyl-3,5-dideoxy-5-trifluoroacetamido*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)-2,4,6-tri*-O-*benzoyl*-β-D-*galactopyranosyl-(1→4)-[2,3,4-tri*-O-*benzyl*-α-L-*fucopyranosyl-(1→3)]-2-acetamido-6*-O-*benzyl-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 solution of **7** (263 mg, 0.12 mmol) and **8**[6] (74 mg, 0.20 mmol) in dry benzene (6 mL) was added 4 Å molecular sieves (400 mg), and the mixture was stirred for 2 h at rt, then cooled to 0 °C. *N*-Iodosuccinimide (NIS; 136 mg, 0.6 mmol) and trifluoromethanesulfonic acid (TfOH, 8.9 μL, 0.1 mmol) were added to the mixture, and the resultant mixture was stirred for 2 h at 7 °C and neutralized with Et_3N. After dilution with $CHCl_3$, the precipitate was filtered off and washed with $CHCl_3$. The filtrate and washings were combined, and successively washed with 1 M aq Na_2CO_3 and satd aq $Na_2S_2O_3$, dried (Na_2SO_4) and concentrated. Column chromatography (80:1 $CHCl_3$–MeOH) of the residue on silica gel afforded **9** (298 mg, 99%) as an amorphous mass: $[\alpha]_D$ −8.1° (*c* 1.2,

$CHCl_3$). ^1H NMR ($CDCl_3$): δ 0.99 (m, 2 H, $Me_3SiCH_2CH_2$), 1.11 (d, 3 H, $J_{5,6}$ 6.4 Hz, H-6^{VI}), 1.49 (s, 3 H, AcN), 1.52, 1.94, 1.99, 2.18 (4 s, 12 H, 4 AcO), 1.71 (t, 1 H, $J_{gem} = J_{3eq,4}$ 12.3 Hz, H-3^Vax), 2.51 (dd, 1 H, $J_{3eq,4}$ 4.3 Hz, H-3^Veq), 3.83 (s, 3 H, COOMe), 4.32 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^I), 4.43 (m, 1 H, H-5^{VI}), 4.81 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1^{IV}), 5.04 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1^{VI}), 5.25 (dd, 1 H, $J_{6,7}$ 2.0, $J_{7,8}$ 9.6 Hz, H-7^V), 5.27 (d, 1 H, $J_{2,NH}$ 9.6 Hz, NH^{III}), 5.69 (m, 1 H, H-8^V), 6.17 (d, 1 H, $J_{5,NH}$ 8.9 Hz, NH^V), 7.17–8.23 (m, 65 H, 13 Ph). Anal. Calcd for $C_{147}H_{161}F_3N_2O_{40}Si$ (2680.96): C, 65.86; H, 6.05; N, 1.04. Found: C, 65.64; H, 6.03; N, 1.01.

2-(Trimethylsilyl)ethyl (methyl 4,7,8,9-tetra-O-*acetyl-3,5-dideoxy-5-trifluoroacetamido*-D-glycero-*α*-D-galacto-*2-nonulopyranosylonate)-(2→3)-2,4,6-tri*-O-*benzoyl-β*-D-*galactopyranosyl-(1→4)-[2,3,4-tri*-O-*acetyl-α*-L-*fucopyranosyl-(1→3)]-2-acetamido-6*-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* (**10**).—A solution of **9** (298 mg, 0.12 mmol) in EtOH (40 mL) and AcOH (8 mL) was vigorously stirred in the presence of 10% Pd–C (300 mg) for 48 h at 40 °C under an H_2 atmosphere. The catalyst was collected and washed with MeOH. (Caution! Extreme fire hazard.) The combined filtrate and washings were concentrated, and the residue was treated with Ac_2O (5.5 mL) and pyridine (9 mL) for 24 h at 40 °C. MeOH (10 mL) was added at 0 °C, and the mixture was concentrated. The residue was extracted with $CHCl_3$ and successively washed with cold 2 M HCl and water, dried (Na_2SO_4) and concentrated. Column chromatography (50:1 $CHCl_3$–MeOH) of the residue on silica gel gave **10** (250 mg, quant) as an amorphous mass: $[\alpha]_D$ −13.3°(*c* 0.2, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 0.98 (m, 2 H, $Me_3SiCH_2CH_2$), 1.22 (d, 3 H, $J_{5,6}$ 6.4 Hz, H-6^{VI}), 1.56 (s, 3 H, AcN), 1.65 (dd, 1 H, J_{gem} 12.4, $J_{3ax,4}$ 12.0 Hz, H-3^Vax), 1.84–2.13 (14 s, 42 H, 14 AcO), 2.45 (dd, 1 H, $J_{3eq,4}$ 4.3 Hz, H-3^Veq), 3.83 (s, 3 H, COOMe), 4.44 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^I), 4.68 (dd, $J_{1,2}$ 3.4, $J_{2,3}$ 6.3 Hz, H-2^{VI}), 4.81 (m, 1 H, H-5^{VI}), 4.84 (dd, $J_{1,2}$ 7.7, $J_{2,3}$ 9.6 Hz, H-2^I), 5.06 (d, 1 H, H-1^{VI}), 5.22 (d, 1 H, $J_{2,NH}$ 9.8 Hz, NH^{III}), 5.27 (dd, 1 H, $J_{6,7}$ 3.4, $J_{7,8}$ 7.5 Hz, H-7^V), 5.59 (m, 1 H, H-8^V), 6.45 (d, 1 H, $J_{5,NH}$ 9.1 Hz, NH^V), 7.49–8.18 (m, 15 H, 3 Ph). Anal. Calcd for $C_{98}H_{123}F_3N_2O_{50}Si$ (2214.11): C, 53.16; H, 5.60; N, 1.27. Found: C, 52.98; H, 5.42; N, 1.17.

(Methyl 4,7,8,9-tetra-O-*acetyl-3,5-dideoxy-5-trifluoroacetamido*-D-glycero-*α*-D-galacto-*2-nonulopyranosylonate)-(2→3)-2,4,6-tri*-O-*benzoyl-β*-D-*galactopyranosyl-(1→4)-[2,3,4-tri*-O-*acetyl-α*-L-*fucopyranosyl-(1→3)]-2-acetamido-6*-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-α*-D-*glucopyranosyl trichloroacetimidate* (**11**).—Compound **10** (250 mg, 0.12 mmol) was treated with trifluoroacetic acid (2 mL) in CH_2Cl_2 (4 mL) for 3 h at rt. EtOAc (1 mL) was added and the mixture was concentrated. Column chromatography (50:1 $CHCl_3$–MeOH) of the residue on silica gel gave the 1-OH free derivative. This compound was treated with Cl_3CCN (195 μL, 15.6 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 9.5 μL, 0.059 mmol) in CH_2Cl_2 (4 mL) for 2 h at 0 °C. The mixture was concentrated, and the residue was chromatographed (40:1 $CHCl_3$–MeOH) on a column of silica gel to give the trichloroacetimidate **11** (246 mg, 90% two steps) as an amorphous mass: $[\alpha]_D$ −10.8° (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.23 (d, 3 H, $J_{5,6}$ 6.4 Hz, H-6^{VI}), 1.55 (s, 3 H, AcN), 1.63 (dd, 1 H, J_{gem} 12.8, $J_{3ax,4}$ 12.0 Hz, H-3^Vax), 1.83–2.11 (s, 42 H, 14 AcO), 2.56 (dd, 1 H, J_{gem} 12.8, $J_{3eq,4}$ 4.3 Hz, H-3^Veq), 3.84 (s, 3 H, COOMe), 4.68 (dd, $J_{1,2}$ 3.4, $J_{2,3}$ 6.3 Hz, H-2^{VI}), 4.81 (m, 1 H, H-5^{VI}), 5.06 (d, 1 H, H-1^{VI}), 5.66 (m, 1 H, H-8^V), 6.08 (d, 1 H, $J_{5,NH}$ 9.1 Hz, NH^V), 6.47 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^I), 7.45–8.19 (m, 15 H, 3 Ph), 8.53 (s, 1 H, C=NH). Anal. Calcd for $C_{95}H_{111}Cl_3F_3N_3O_{50}$ (2258.26): C, 50.53; H, 4.95; N, 1.86. Found: C, 50.42; H, 4.95; N, 1.84.

(Methyl 4,7,8,9-tetra-O-*acetyl-3,5-dideoxy-5-trifluoroacetamido*-D-glycero-*α*-D-galacto-*2-nonulopyranosylonate)-(2→3)-2,4,6-tri*-O-*benzoyl-β*-D-*galactopyranosyl-(1→4)-[2,3,4-tri*-O-*acetyl-α*-L-*fucopyranosyl-(1→3)]-2-acetamido-6*-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*)-2-azido-3*-O-*benzoyl-4-octadecene-1,3-diol* (**13**).—To a solution of **11** (98 mg, 46 μmol) and (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzol-4-octadecene-1,3-diol (**12**; 29 mg, 67 μmol) in dry dichloromethane (0.5 mL) was added 4 Å molecular sieves (type AW300; 300 mg), and the mixture was stirred for 2 h at rt, and then cooled to 0 °C. Trimethylsilyl trifluoromethanesulfonate (TMSOTf; 50 mM in CH_2Cl_2, 86 μL, 4.3 μmol) was added to the mixture, and this was stirred for 24 h at 0 °C, neutralized with Et_3N filtered and concentrated. The residue was dissolved in 80% aq HOAc (3 mL), stirred for 2 h at rt and then concentrated. Chromatography (60:1 $CHCl_3$–MeOH) of the residue on silica gel afforded **13** (24 mg, 21%) as an amorphous mass: $[\alpha]_D$ −15.2° (*c* 0.4, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 0.87 (t, 3 H, $J_{Me,CH2}$ 7.0 Hz, $MeCH_2$), 1.26 (s, 22 H, 11 CH_2), 1.61 (s, 3 H, AcN), 1.67 (dd, 1 H, J_{gem} 12.6, $J_{3ax,4}$ 12.3 Hz, H-3^Vax), 1.83–2.12 (14 s, 42 H, 14 AcO), 2.51 (dd, 1 H, $J_{3eq,4}$ 4.6 Hz, H-3^Veq), 3.84 (s, 3 H, COOMe), 4.47 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^I), 4.72 (dd, $J_{1,2}$ 3.4, $J_{2,3}$ 6.3 Hz, H-2^{VI}), 4.82 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1^{IV}), 5.07 (d, 1 H, H-1^{VI}), 5.24 (dd, 1 H, $J_{6,7}$ 3.8, $J_{7,8}$ 9.6 Hz, H-7^V), 5.66 (m, 1 H, H-8^V), 5.93 (dt, 1 H, $J_{4,5}$ 14.2, $J_{5,6} = J_{5',6}$ 7.3 Hz, H-5 of sphingosine), 6.12 (d, 1 H, $J_{5,NH}$ 8.6 Hz, NH^V), 7.45–8.17 (m, 20 H, 4 Ph). Anal. Calcd for $C_{118}H_{148}F_3N_5O_{52}$ (2525.46): C, 56.12; H, 5.91; N, 2.77. Found: C, 55.86; H, 5.79; N, 2.54.

(Methyl 4,7,8,9-tetra-O-*acetyl-3,5-dideoxy-5-trifluoroacetamido*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)-2,4,6-tri*-O-*benzoyl-β*-D-*galactopyranosyl-(1→4)-[2,3,4-tri*-O-*acetyl-α*-L-*fucopyranosyl-(1→3)]-2-acetamido-6*-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* (**14**).—H_2S was bubbled through a stirred solution of **13** (71 mg, 28 μmol) in pyridine (12.4 mL) and water (2.5 mL) for 72 h at 0 °C. The mixture was concentrated, and the residual syrup was treated with octadecanoic acid (24 mg, 84 μmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrogenchloride (WSC; 23 mg, 0.12 mmol) in CH_2Cl_2 (3 mL) 12 h at rt. The mixture was extracted with $CHCl_3$ and the extract was successively washed with 1 M HCl and water, dried (Na_2SO_4) and concentrated. Column chromatography (50:1 $CHCl_3$–MeOH) of the residue on silica gel gave **13** (33 mg, 43%) as an amorphous mass: $[\alpha]_D$ −13.1° (*c* 6.6, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 0.89 (t, 6 H, $J_{Me,CH2}$ 6.9 Hz, 2 $MeCH_2$), 1.27 (s, 52 H, 26 CH_2), 1.57 (s, 3 H, AcN), 1.67 (dd, 1 H, J_{gem} 12.5, $J_{3ax,4}$ 12.3 Hz, H-3^{V}ax), 1.84–2.14 (14 s, 42 H, 14 AcO), 2.50 (dd, 1 H, $J_{3eq,4}$ 4.6 Hz, H-3^{V}eq), 3.84 (s, 3 H, COOMe), 4.40 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^I), 4.70 (dd, $J_{1,2}$ 2.9, $J_{2,3}$ 7.3 Hz, H-2^{VI}), 4.94 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1^{IV}), 5.07 (d, 1 H, H-1^{VI}), 5.26 (dd, 1 H, $J_{6,7}$ 3.8, $J_{7,8}$ 9.8 Hz, H-7^V), 5.45 (dd, 1 H, $J_{2,3}$ 7.7 Hz, H-2^{IV}), 5.66 (m, 1 H, H-8^V), 5.88 (dt, 1 H, $J_{4,5}$ 14.7, $J_{5,6} = J_{5',6}$ 7.3 Hz, H-5 of sphingosine), 6.25 (d, 1 H, $J_{5,NH}$ 8.6 Hz, NH^V), 7.45–8.18 (m, 20 H, 4 Ph). Anal. Calcd for $C_{136}H_{184}F_3N_3O_{53}$ (2765.93): C, 59.06; H, 6.71; N, 1.52. Found: C, 59.02; H, 6.51; N, 1.48.

5-Amino-3,5-dideoxy-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid-(2→3)-β*-D-*galactopyranosyl-(1→4)-[α*-L-*fucopyranosyl-(1→3)]-2-acetamido-6*-O-*acetyl-2-deoxy-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 (*N-*deacetyl sLe^x).*—To a solution of **14** (33 mg, 12 μmol) in MeOH (3 mL) was added a catalytic amount of 28% NaOMe in MeOH, and the mixture was stirred for 72 h at 45 °C. Water (1 mL) was added, and the mixture was stirred for 24 h at rt. The mixture was then neutralized with Amberlite IR-120 (H^+) resin and filtered, and the residue was washed with MeOH. The combined filtrate and washings were concentrated. Column chromatography (5:4:0.7 $CHCl_3$–MeOH–H_2O) of the residue on Sephadex LH-20 gave the target molecule (19 mg, 96%) as an amorphous mass: $[\alpha]_D$ −11.4°(*c* 1.4, 5:5:1 $CHCl_3$–MeOH–H_2O); ^{1}H NMR (CD_3OD): δ 0.91 (t, 6 H, $J_{Me,CH2}$ 7.0 Hz, $MeCH_2$), 1.17 (d, 3 H, $J_{5,Me}$ 6.6 Hz, H-6^{VI}), 1.29 (s, 52 H, 26 CH_2), 1.72 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.1 Hz, H-3^{V}ax), 1.98 (s, 3 H, AcN), 2.05 (m, 1 H, H-6 sphingosine), 2.85 (dd, 1 H, $J_{3eq,4}$ 3.2 Hz, H-3^{V}eq), 4.29, 4.35, 4.86 (3 d, 3 H, $J_{1,2}$ 7.3, 7.5, 7.3 Hz, for each three β-anomeric-H), 5.05 (d, 1 H, $J_{1,2}$ 4.1 Hz, H-1^{VI}), 5.44 (m, 1 H, H-4 of sphingosine), 5.67 (m, 1 H, H-5 of sphingosine). Anal. Calcd for $C_{77}H_{139}F_3N_3O_{34}$ (1650.95) C, 56.02; H, 8.49; N, 2.55. Found: C, 55.94; H, 8.30; N, 2.32.

LSIMS (negative-ion mode, 2:2:1 diethanolamine/tetramethlyurea/*m*-nitrobenzyl alcohol as the liquid matrix): 1648 $[M-H]^-$, 1399 $[M-H-Neu]^-$, 1237 $[1399-Gal]^-$, 888 [lactosyl ceramide]$^-$, 726 [glucosyl ceramide]$^-$.

Selectin binding assays.—Binding of the recombinant E-, L- and P-selectins (Fcμ chimeras)[15] to glycolipids immobilized in microwells was assayed as described previously[16] after harvesting the glycolipids by preparative TLC.[7] Binding to the target molecule, *N*-deacetyl sLe^x, was compared with binding to the gangliosides sLe^x (GSC 64)[17] 6-*O*-sulfo-sLe^x (GSC 269)[6] and 6-*O*-sulfo-*N*-deacetyl sLe^x (GSC 406).[9] Galactosyl ceramide (Sigma) was included as a negative control.

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