Structure–Activity Relationships of Sialyl Lewis x-Containing Oligosaccharides. 1. Effect of Modifications of the Fucose Moiety

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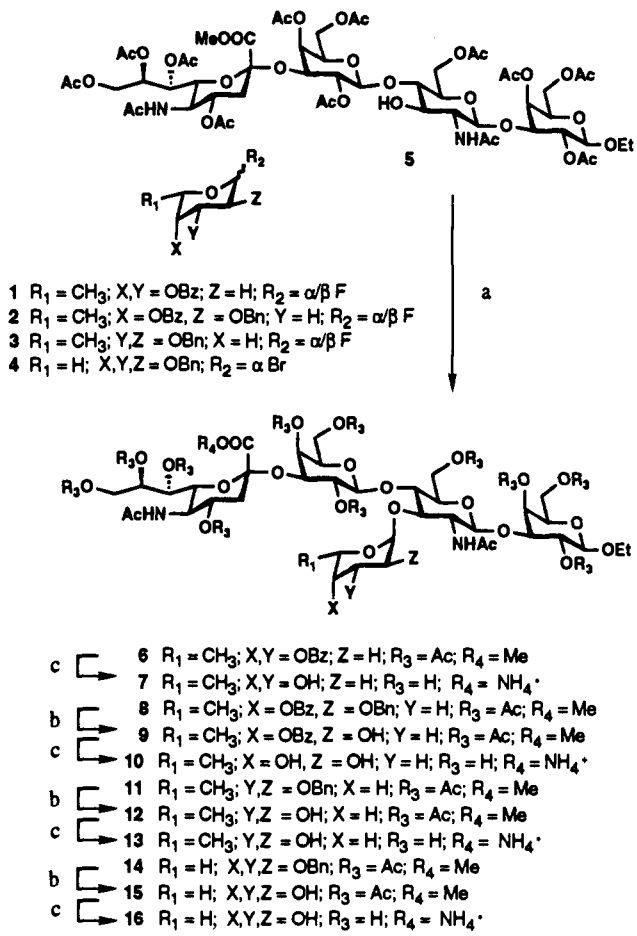
Leukocyte adhesion to the vasculature is mediated by E-, P-, and L-selectins. The natural ligands for E- and P-selectins have not been fully characterized but have been shown to contain the tetrasaccharide sialyl Lewis x structure (SLe^x). To determine the importance of the fucose moiety of SLe^x, various analogs of SLe^x containing modifications thereof were prepared and tested as inhibitors of E-selectin-mediated cell adhesion. Cellular experiments indicate that replacement of the hydroxyl groups of fucose by hydrogen abrogated E-selectin binding. However, the arabinose analog of fucose (CH_3 ΔH) inhibited cell adhesion but was 5-fold less potent than native SLe^x. This data suggests that modifications of fucose on SLe^x are generally deleterious toward E-selectin binding.

The selectins[1] are C-type Ca^{2+}-dependent animal lectins which play an important role in the recruitment of leukocytes to sites of inflammation.[2] Although the natural ligand for the selectins has not been fully characterized, the terminal oligosaccharide glycotope recognized by E-, P-, and L-selectin has been shown to be the tetrasaccharide sialyl Lewis x^3 (SLe^x), although, sialyl Lewis a^4 (SLe^a), Le^x 3′-*O*-sulfate,[5] and Le^a 3′-*O*-sulfate may also function as ligands for these receptors. Conformational analysis of SLe^x using 2-D NMR suggests that the carboxylate of sialic acid, fucose, and the galactose may be directly involved in binding to selectins.[3,4a,5] In addition, it has been proposed that one or more hydroxyl substituents on fucose play a role in binding to Ca^{2+} on the selectins.[6,7] Several analogs of SLe^x that maintain E- and P-selectin avidity, *in vitro*, have been reported and include substitutions of sialic acid with sulfate,[3b,8] other sialic acid analogs,[9] and N-substituted glucosamine derivatives.[9] As part of a comprehensive structure–activity relationship study of the requirements for SLe^x binding to selectins, we prepared a series of fucose analogs and examined their effect on human E-selectin-mediated cellular adhesion.

The fluoro deoxy fucosides **1**, **2**, and **3** were prepared using DAST reaction conditions[10] starting with the respective reducing sugars[11] (Scheme 1). Glycosidation of the linear tetrasaccharide **5**[12] with **1**–**3** was performed using $AgClO_4/SnCl_2$[10] with tetramethylurea (TMU) providing the pentasaccharides **6**, **8**, and **11**.[13] If TMU was not present in the reaction medium, the acidic conditions caused a slow hydrolysis of the labile α-fucoside, furnishing primarily starting material **5** as the isolable product. Deprotection of **6**, **8**, and **11** by hydrogenation of the benzyl ethers and deacetylation then provided the free pentasaccharides **7**, **10**, and **13**.

The SLe^x arabinoside analog (CH_3 ΔH) was prepared by glycosidation of the tetrasaccharide **5** with D-arabinosyl bromide **4**[14] using the standard conditions[15] of Et_4NBr to produce the pentasaccharide **14** (Scheme 1). Hydrogenation and deacetylation then provided the free arabinoside **16**.

Scheme 1[a]



[a] Reagents and conditions: (a) (i) $AgClO_4$, $SnCl_2$, TMU, $ClCH_2CH_2Cl$, −20 °C (**6**, 90%; **8**, 84%; **11**, 85%) or (ii) Et_4NBr, CH_2Cl_2 (**14**, 85%); (b) $Pd(OH)_2/C$, H_2, ethanol (**9**, 91%; **12**, 56%; **15**, 88%); (c) NaOMe, MeOH then H_2O (**7**, 90%; **10**, 87%; **13**, 88%; **16**, 92%).

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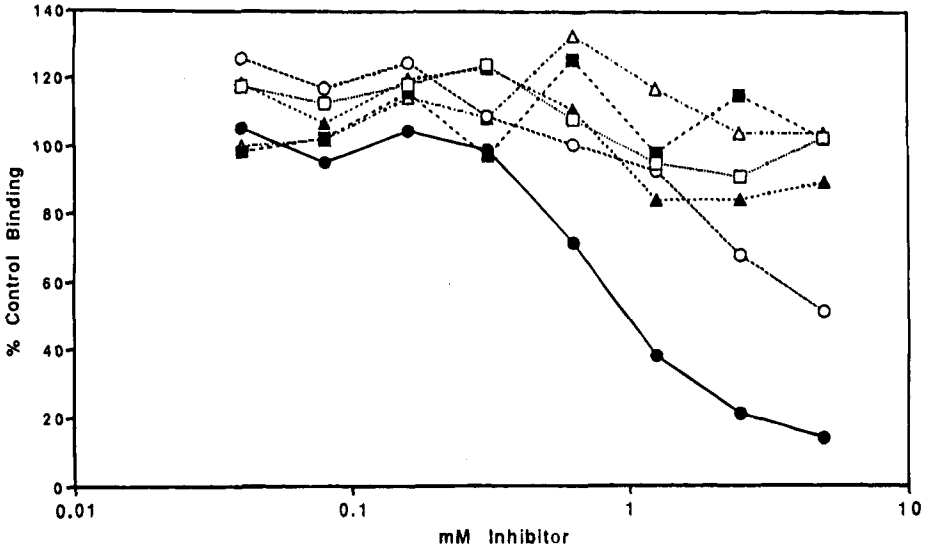

Figure 1. Inhibition of HL-60 cell binding to rsE-selectin by SLe^x fucose analogs. The assay is described in the supplementary material. Each data point is the average of duplicates. Binding is expressed as the percentage of rsE-selectin binding in the absence of inhibitor. The SLe^x analogs tested were (●) SLe^x pentasaccharide **17**; (□) linear tetrasaccharide **18**; (○) SLe^x arabinoside **16**; (△) SLe^x 2-deoxy fucoside **7**; (■) SLe^x 3-deoxy fucoside **10**; (▲) SLe^x 4-deoxy fucoside **13**.

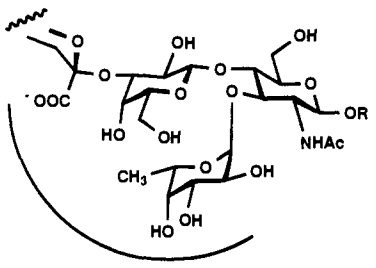

Figure 2. Sialyl Lewis x binding site interactions.

The SLe^x pentasaccharide **17**[12,16] was found to inhibit the cell adhesion of HL-60 cells containing SLe^x to purified recombinant human E-selectin[17] with an IC_{50} of 1.0 mM (Figure 1). In contrast, compound **18**,[12,16] a sialylated tetrasaccharide that lacks the fucose group, did not inhibit E-selectin-mediated cell adhesion up to concentrations of 6.5 mM, which clearly demonstrates the need for the fucose moiety. The role of the methyl group of fucose of SLe^x in E-selectin binding was examined by the arabinoside SLe^x analog **16** (CH_3 ΔH) which was found to be 5-fold less potent (IC_{50} = 5.0 mM) than the parent pentasaccharide **17** (IC_{50} = 1 mM). In addition, the pentasaccharides **7**, **10**, and **13**, each lacking one of the fucose hydroxyl groups, did not inhibit cell adhesion at concentrations up to 6.5 mM. These results are interesting since the solution conformations of SLe^x, SLe^a, bivalent SLe^x, and their similar predicted topostructures which include the fucose, carboxylate, and galactose residues suggest that the hydroxyl groups on fucose would be important for selectin interactions (Figure 2).[5b,c] The crystal structure of E-selectin and its structural homology with the mannose binding protein (MBP) has also suggested that the hydroxyl groups of fucose would be critical for SLe^x lectin interactions.[6] Although speculative, docking of SLe^x to the E-selectin surface utilizing a configuration of fucose similar to that of mannose bound to the MBP predicts that two of the fucose hydroxyls would bind directly to the calcium ion on E-selectin. Our results clearly indicate that all of the hydroxyl groups on fucose are essential for SLe^x binding to E-selectin although the actual points of interaction remain unresolved. It is interesting to note that SLe^x itself can coordinate directly with calcium via interactions with the 2-position hydroxyl group of fucose.[7]

In summary, this study supports the concept that the interaction between E-selectin and SLe^x involves the entire fucose moiety, and the fucose interaction with E-selectin may be similar to that of mannose binding to the mannose binding protein. Although the role of the E-selectin Ca^{2+} ion in ligand interactions has not been firmly established, further experiments employing additional fucose modifications should provide some insight into the process of E-selectin-mediated cell adhesion and may provide a basis for the development of other antiadhesion inhibitors.

Experimental Section

All reactions were monitored by thin-layer chromatography carried out on 0.25 mm Whatman silica gel plates (60F-254) using UV light and anisaldehyde reagent[1] as developing agent. E. Merck silica gel (60, particle size 0.040–0.063 mm) was used for flash column chromatography, and Biorad Biogel P-2 (500–2000 Da) resin was used for gel filtration chromatography.

All reactions were carried out under an argon atmosphere with anhydrous solvents from Aldrich unless otherwise noted. The L-fucose was purchased from Aldrich. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials unless otherwise stated. NMR spectra were recorded on a 300 MHz General Electric QE-300 NMR. The FAB mass spectra and exact mass calculations were acquired on a VG Fisons ZAB 2SE mass spectrometer. Analytical HPLC was performed on a Dionex LC 20 system with pulsed amperometric detection, a Dionex MA-1 column (4 × 250 mm), and an isocratic 0.1 M NaOH mobile phase.

General Glycosidation Conditions for the Preparation of Compounds 6, 8, and 11: Ethyl (Methyl 4,7,8,9-tetra-*O*-acetyl-5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosonate)-(2–3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1–4)-*O*-[2-deoxy-3,4-di-*O*-benzoyl-α-L-fucopyranosyl-(1–3)-*O*]-(6-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1–3)-*O*-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (6). Ethyl (methyl 4,7,8,9-tetra-*O*-acetyl-5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosonate)-(2–3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1–4)-*O*-(6-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1–3)-*O*-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside[3] (**5**) (77 mg, 0.057 mmol), fluoride 1 (164 mg, 0.46 mmol), and TMU (0.63

mmol, 76 μL) were dissolved in 1,2-dichloroethane (2 mL), and activated 4 Å sieves (100 mg) were added. Stirring was continued for 6 h. The reaction flask was wrapped in aluminum foil and cooled to −20 °C, at which time $AgClO_4$ (42 mg, 0.20 mmol) and $SnCl_2$ (38 mg, 0.20 mmol) were added. Stirring was then continued at −20 °C for 1 h and the mixture warmed slowly to room temperature over 18 h. The reaction was diluted with ethyl acetate (500 mL), washed with H_2O (2 × 100 mL) and saturated NaCl (2 × 100 mL), and dried ($MgSO_4$). Concentration and chromatography (silica, 93% CH_2Cl_2/MeOH) afforded 81 mg (85%) of a waxy solid: R_f = 0.35 (93% CH_2Cl_2/MeOH); ^1H NMR (300 MHz, $CHCl_3$) δ 8.11−7.22 (m, 10H, aromatic), 6.15 (d, J = 5.3 Hz, 1H), 5.51−5.35 (m, 8H), 5.34 (d, J = 3 Hz, 1H), 5.19−4.82 (m, 7H), 4.79−3.39 (m, 20H), 3.82 (s, 3H, COOMe), 3.25 (m, 1H), 2.57 (dd, J = 3, 10 Hz, 1H, H-3_{eq}-sialic acid), 2.27 (s, 3H, OAc), 2.18 (s, 3H, OAc), 2.15 (s, 3H, OAc), 2.09 (s, 6H, 2 OAc), 2.05 (s, 6H, 2 OAc), 2.01 (s, 3H, OAc), 1.99 (s, 3H, NAc), 1.95 (s, 3H, NAc), 1.82 (s, 3H OAc), 1.67 (dd, J = 10, 10 Hz, 1H, H-3_{ax}-sialic acid), 1.21 (m, 6H, CH_2CH_3, H-6 fucose); HRMS (FAB) m/z calcd for $C_{76}H_{98}N_2O_{40}Cs^+$ 1811.4750, found 1811.4862.

Compounds **8** and **11** were similarly prepared. Yields and ^1H NMR and analytical data for the compounds are reported as follows.

Ethyl (methyl 5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyronosylonate)-(2−3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1,4)-*O*-[2-*O*-benzyl-3-deoxy-4-*O*-benzoyl-α-L-fucopyranosyl-(1−3)-*O*]-(6-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1−3)-*O*-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (8): yield 281 mg (84%); R_f = 0.39 (93% CH_2Cl_2/MeOH); ^1H NMR (300 MHz, $CHCl_3$) δ 8.01−7.21 (m, 10H, aromatic), 5.87 (d, J = 5 Hz, 1H), 5.51−5.31 (m, 6H), 5.38 (d, J = 4 Hz, 1H), 5.25 (d, J = 6.5 Hz, 1H), 5.19−5.04 (m, 2H), 4.95−4.76 (m, 9H), 4.61−3.42 (m, 20H), 3.81 (s, 3H, COOMe), 2.95 (m, 1H), 2.57 (dd, J = 3, 10 Hz, H-3_{eq}-sialic acid, 1H), 2.27 (s, 3H, OAc), 2.21 (s, 3H, OAc), 2.17 (s, 3H, OAc), 2.11 (s, 6H, 2 OAc), 2.09 (s, 6H, 2 OAc), 2.01 (s, 9H, 3 OAc), 1.99 (s, 3H, NAc), 1.91 (s, 3H, NAc), 1.82 (s, 3H, OAc), 1.67 (dd, J = 10, 10 Hz, H-3_{ax}-sialic acid, 1H), 1.19 (m, 6H, CH_2CH_3, H-6 fucose); HRMS (FAB) m/z calcd for $C_{76}H_{100}N_2O_{39}Cs^+$ 1797.4958, found 1797.5037.

Ethyl (methyl 5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyronosylonate)-(2−3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1−4)-*O*-[2,3-di-*O*-benzyl-4-deoxy-α-L-fucopyranosyl-(1−3)-*O*]-(6-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1−3)-*O*-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (11): yield 435 mg (90%); R_f = 0.39 (93% CH_2Cl_2/MeOH); ^1H NMR (300 MHz, $CHCl_3$) δ 7.42−7.19 (m, 10H, aromatic), 5.91 (d, J = 6.8 Hz, 1H), 5.75 (d, J = 4.6 Hz, 1H), 5.55−5.35 (m, 4H), 5.25 (d, J = 3 Hz, 1H), 5.18 (d, J = 5.1 Hz, 1H), 5.15−4.39 (m, 17H), 4.37−3.25 (m, 16H), 3.82 (s, 3H, COOMe), 3.05 (m, 1H), 2.55 (dd, J = 3, 10 Hz, 1H, H-3_{eq}-sialic acid), 2.29 (s, 3H, OAc), 2.19 (s, 3H, OAc), 2.16 (s, 3H, OAc), 2.11 (s, 6H, 2 OAc), 2.07 (s, 6H, 2 OAc), 2.01 (s, 9H, 3 OAc), 1.99 (s, 3H, NAc), 1.91 (s, 3H, NAc), 1.85 (s, 3H, OAc), 1.58 (dd, J = 10, 10 Hz, 1H, H-3_{ax}-sialic acid), 1.19 (m, 6H, CH_2CH_3, H-6 fucose); HRMS (FAB) m/z calcd for $C_{76}H_{102}N_2O_{38}Cs^+$ 1783.5165, found 1783.5173.

Ethyl (Methyl 5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-(2−3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1−4)-*O*-[2,3,4-tri-*O*-benzyl-β-D-arabinopyranosyl-(1−3)-*O*]-(6-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1−3)-*O*-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (14). Ethyl (methyl 5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosonate)-(2−3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1−4)-*O*-(6-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1−3)-*O*-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside[3] (**5**) (100 mg, 0.075 mmol) and tetra-*n*-butylammonium bromide were dissolved in anhydrous dichloromethane (5 mL) and DMF (0.5 mL) under an argon atmosphere at 25 °C. Activated 4 Å sieves (500 mg) were added, and the mixture was stirred for 1 h. Bromide **4** (0.5 mmol, freshly prepared) was then added in anhydrous dichloromethane (1.0 mL). Stirring was then continued at room temperature for 3 days (72 h). The reaction mixture was filtered through Celite and the filtrate concentrated. The residue was chromatographed (silica, dichloromethane/methanol (15:1)) and afforded 100 mg (76%) of a waxy solid: R_f = 0.32 (15:1, CH_2Cl_2/MeOH); ^1H NMR (300 MHz, $CHCl_3$) δ 7.4−7.0 (m, 15H, aromatic), 5.75 (d, J = 5.2 Hz, 1H), 5.55 (d, J = 8 Hz, 1H), 5.5−5.2 (m, 4H), 5.1−4.4 (m, 7H), 4.4−3.3 (m, 20H), 3.80 (s, 3H, COOMe), 3.1 (m, 1H), 2.58 (dd, J = 3, 10 Hz, 1H, H-3_{eq}-sialic acid), 2.27−1.6 (13 acetyl groups), 1.15 (t, 3H, CH_2CH_3).

General Procedure for the Deprotection of the Fucose Benzyl Groups of Compounds 8, 12, and 15: Ethyl (Methyl 5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyronosylonate)-(2−3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1−4)-*O*-[3-deoxy-4-*O*-benzoyl-α-L-fucopyranosyl-(1−3)-*O*]-(6-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1−3)-*O*-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (9). Palladium hydroxide on carbon (32 mg, 50% by weight palladium) was added to a solution of **8** (65 mg, 39 μmol) in 5 mL of methanol. The suspension was purged three times with hydrogen gas and then placed under hydrogen atmosphere (1 atm) for 72 h. The mixture was then degassed, filtered through Celite, and concentrated. The residue was chromatographed (silica, 92% CH_2Cl_2/methanol) to afford **9** (55 mg, 89%) as a white waxy solid: R_f = 0.41 (silica, 92% CH_2Cl_2/methanol); ^1H NMR (300 MHz, $CHCl_3$) δ 8.01−7.39 (m, 10H, aromatic), 5.97 (d, J = 5 Hz, 1H), 5.57−5.31 (m, 7H), 5.19−5.04 (m, 6H), 4.95−4.61 (m, 18H), 4.58 (dd, J = 3, 10 Hz, 1H), 4.38 (d, J = 6 Hz, 1H), 4.37−3.82 (m, 7H), 3.81 (s, 3H, COOMe), 3.79−3.33 (m, 8H), 2.57 (dd, J = 3, 10 Hz, 1H, H-3_{eq}-sialic acid), 2.27 (s, 3H, OAc), 2.24 (s, 3H, OAc), 2.15 (s, 3H OAc), 2.11 (s, 6H, 2 OAc), 2.09 (s, 6H, 2 OAc), 2.01 (s, 9H, 3 OAc), 1.99 (s, 3H, NAc), 1.91 (s, 3H, NAc), 1.87 (s, 3H, OAc), 1.77 (dd, J = 10, 10 Hz, 1H, H-3_{ax}-sialic acid), 1.19 (m, 6H, CH_2CH_3, H-6 fucose); HRMS (FAB) m/z calcd for $C_{69}H_{94}N_2O_{39}Cs^+$ 1707.4488, found 1707.4496.

Compounds **12** and **15** were similarly prepared. Yields and ^1H NMR and analytical data for the compounds are reported as follows.

Ethyl (methyl 5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyronosylonate)-(2−3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1−4)-*O*-[4-deoxy-α-L-fucopyranosyl-(1−3)-*O*]-(6-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1−3)-*O*-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (12): yield 78 mg (56%); R_f = 0.41 (92% CH_2Cl_2/MeOH); ^1H NMR (300 MHz, $CHCl_3$) δ (d, J = 6.5 Hz, 1H), 5.58−5.35 (m, 5H), 5.24 (d, J = 7.2 Hz, 1H), 5.15 (m, 1H), 5.09 (d, J = 3.5 Hz, 1H), 4.91 (m, 4H), 4.78−4.21 (m, 12H), 4.19−3.39 (m, 15H), 3.79 (s, 3H, COOMe), 3.35 (m, 1H), 2.59 (dd, J = 3, 10 Hz, 1H, H-3_{eq}-sialic acid), 2.29 (s, 3H, OAc), 2.19 (s, 3H, OAc), 2.15 (s, 3H, OAc), 2.11 (s, 6H, 2 OAc), 2.04 (s, 6H, 2 OAc), 2.01 (s, 9H, 3 OAc), 1.99 (s, 3H, NAc), 1.91 (s, 3H, NAc), 1.85 (s, 3H, OAc), 1.77 (dd, J = 10, 10 Hz, 1H, H-3_{ax}-sialic acid), 1.21 (m, 6H, CH_2CH_3 and H-6 fucose); HRMS (FAB) m/z calcd for $C_{62}H_{90}N_2O_{38}Cs^+$ 1603.4226, found 1603.4319.

Ethyl (methyl 5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-(2−3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1−4)-*O*-[β-D-arabinopyranosyl-(1−3)-*O*]-(6-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1−3)-*O*-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (15): crude yield 85 mg (100%); R_f = 0.45 (92% CH_2Cl_2/MeOH). The ^1H NMR of this crude compound showed complete disappearance of all aromatic protons. This compound was not purified but was instead converted directly to **16**.

General Procedure for the Deprotection of the Acyl and Methyl Ester Groups of Compounds 7, 10, 13, and 16: Ethyl (Ammonium 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosonate)-(2−3)-*O*-(β-D-galactopyranosyl)-(1−4)-*O*-(2-deoxy-α-L-fucopyranosyl-(1−3)-*O*)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1−3)-*O*-β-D-galactopyranoside (7). To a stirred solution of compound **6** (39 mg, 23 μmol) in 5 mL of 4:1 MeOH/H_2O at room temperature was added a methanolic solution of sodium methoxide (1.25 mg, 23 μmol, 1 μL). After 72 h, the mixture was concentrated and chromatographed (Biogel P-2, 0.1 M

NH_4HCO_3) to afford after lyophylization 21 mg (90%) of a white solid: R_f = 0.54 (70% 2-propanol/1 M NH_4OAc); ^1H NMR (300 MHz, D_2O) δ 5.09 (d, J = 4 Hz, 1H, H-1 fucose), 4.63 (d, 1H, H-1 glucosamine), 4.43 (d, J = 8 Hz, 1H, H-1 galactose), 4.35 (d, J = 8 Hz, 1H, H-1 galactose), 4.05 (d, J = 2 Hz, 1H), 3.99–3.39 (m, 30H), 2.65 (dd, J = 3, 11 Hz, 1H, H-3_{eq}-sialic acid), 1.95 (s, 3H, NAc), 1.91 (s, 3H, NAc), 1.78–1.57 (m, 3H, H-3_{ax}-sialic acid, H-2 and H-2′ fucose), 1.14 (t, J = 6 Hz, 3H, CH_2CH_3), 1.05 (d, J = 6 Hz, 3H, H-6 fucose); HRMS (FAB) m/z calcd for $C_{39}H_{66}N_2O_{27}Cs^+$ 1127.2907, found 1127.2850; HPLC (0.1 M NaOH, 1 mL/min) t_R 20.2 min (98%).

Compounds **10**, **13**, and **16** were similarly prepared. Yields and ^1H NMR and analytical data for the compounds are reported as follows.

Ethyl (ammonium 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosonate)-(2–3)-*O*-(β-D-galactopyranosyl)-(1–4)-*O*-[3-deoxy-α-L-fucopyranosyl-(1–3)-*O*]-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1–3)-*O*-β-D-galactopyranoside (10): yield 32 mg (87%) of a white solid; R_f = 0.54 (70% i-PrOH/1 M NH_4OAc); ^1H NMR (300 MHz, D_2O) δ 5.09 (d, J = 4 Hz, 1H, H-1 fucose), 4.63 (m, 2H), 4.49 (d, J = 8 Hz, 1H, H-1 galactose), 4.39 (d, J = 8 Hz, 1H, H-1 galactose), 4.09 (d, J = 2 Hz, 1H), 4.03 (dd, J = 3, 11 Hz, 1H), 3.99–3.41 (m, 28H), 2.65 (dd, J = 3, 11 Hz, 1H, H-3_{eq}-sialic acid), 1.98 (s, 3H, NAc), 1.95 (s, 3H, NAc), 1.85–1.77 (m, 3H, H-3_{ax}-sialic acid, H-3 and H-3′ fucose), 1.19 (t, J = 6 Hz, 3H, CH_2CH_3), 1.05 (d, J = 6 Hz, 3H, H-6 fucose); HRMS (FAB) m/z calcd for $C_{39}H_{66}N_2O_{27}Cs^+$ 1127.2907, found 1127.2881; HPLC (0.1 M NaOH, 1 mL/min) t_R 16.5 min, (99%).

Ethyl (ammonium 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-(2–3)-*O*-(β-D-galactopyranosyl)-(1–4)-*O*-[4-deoxy-α-L-fucopyranosyl-(1–3)-*O*]-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1–3)-*O*-β-D-galactopyranoside (13): yield 47 mg (88%) of a white solid; ^1H NMR (300 MHz, D_2O) δ 5.05 (d, J = 4 Hz, 1H, H-1 fucose), 4.63 (d, J = 7 Hz, 1H, H-1 glucosamine), 4.49 (d, J = 8 Hz, 1H, H-1 galactose), 4.39 (d, J = 8 Hz, 1H, H-1 galactose), 4.01 (d, J = 2 Hz, 1H), 3.99 (dd, J = 3, 11 Hz, 1H), 3.95–3.37 (m, 28H), 3.25 (m, 1H), 2.62 (dd, J = 3, 11 Hz, 1H, H-3_{eq}-sialic acid), 1.98 (s, 3H, NAc), 1.95 (s, 3H, NAc), 1.85–1.77 (m, 3H, H-3_{ax}-sialic acid, H-4 and H-4′ fucose), 1.19 (t, J = 6 Hz, 3H, CH_2CH_3), 1.05 (d, J = 6 Hz, 3H, H-6 fucose); HRMS (FAB) m/z calcd for $C_{39}H_{66}N_2O_{27}Cs^+$ 1127.2907, found 1127.2881; HPLC (0.1 M NaOH, 1 mL/min) t_R 20.2 min (99%).

Ethyl (ammonium 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-(2–3)-*O*-(β-D-galactopyranosyl)-(1–4)-*O*-[β-D-arabinopyranosyl-(1–3)-*O*]-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1–3)-*O*-β-D-galactopyranoside (16): yield 50 mg (88%) of a white solid; ^1H NMR (300 MHz, D_2O) δ 5.15 (d, J = 4 Hz, 1H, H-1 fucose), 4.63 (d, J = 7 Hz, 1H), 4.51 (d, J = 8 Hz, 1H), 4.37 (d, J = 8 Hz, 1H), 4.08 (d, J = 3 Hz, 1H), 4.05 (dd, J = 3, 10 Hz, 1H), 3.95–3.4 (m, 37H), 2.72 (dd, J = 3, 12 Hz, 1H, H-3_{eq}-sialic acid), 1.98 (s, 3H, NAc), 1.97 (s, 3H, NAc), 1.77 (t, J = 12 Hz, 1H, H-3_{ax}-sialic acid), 1.19 (t, J = 6 Hz, 3H, CH_2CH_3); HRMS (FAB) m/z calcd for $C_{35}H_{64}N_2O_{28}Cs^+$ 1129.2700, found 1129.2719; HPLC (0.1 M NaOH, 1 mL/min) t_R 24.1 min (86%).

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Supplementary Material Available: Preparation procedures and data for compounds 1, **2**, and **3** as well as a description of the E-selectin cell adhesion assay (16 pages). Ordering information is given on any current masthead page.

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(16) Compound **17**, NANAα(2,3)Galβ(1,4)[Fucα(1,3)]GlcNAcβ(1,3)-GalβOEt (Scheme 1, $R_1 = CH_3$; X, Y, Z = OH; R_3 = H; $R_4 = NH_4^+$); compound **18** (SLN), NANAα(2,3)Galβ(1,4)GlcNAcβ(1,3)-GalβOEt.
(17) The recombinant human E-selectin is coated on ELISA plates and HL-60 cells are added with carbohydrate. After washing, the amount of cell adhesion is determined by a myloperoxidase assay. See the supplementary material for a complete description of the assay.