Studies on Selectin Blocker. 1. Structure−Activity Relationships of Sialyl Lewis X Analogs

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As a part of our studies of selectin blockers, we prepared 1-deoxy-3′-*O*-sulfo Le^x analogs (**1**−**3**), 1-deoxy-3′-*O*-phosphono Le^x analogs (**4**), and 1-deoxy sLe^x analogs (**5**−**7**), and examined their inhibitory activities against natural ligand (sLe^x) binding to E-selectin, P-selectin, and L-selectin. The 1-deoxy sLe^x **5** was up to 20 times more potent an inhibitor than the sLe^x tetrasaccharide toward P- and L-selectin binding. This indicates that the modification of the 1 or 2 position of sLe^x is useful in the design of a more potent selectin blocker.

Cell adhesion molecules (CAMs) mediate the regulation of inflammation.[1] The selectins are a family of CAMs that plays an important role in the initial interactions of leukocyte homing, platelet binding, and neutrophil extravasation.[2]

Recent studies have indicated the involvement of selectin−oligosaccharide interactions in various inflammatory diseases.[3] It is known that E-selectin (ELAM-1), P-selectin (GMP-140), and L-selectin (LECAM-1) play important roles in the migration of inflammatory cells from the blood stream to inflammatory sites. The selectin family is expressed on a variety of cell surfaces. For example, ELAM-1 is an adhesion molecule that is expressed on vascular endothelial cells during inflammation.[4] GMP-140 is an adhesion molecule that is expressed on platelets and vascular endothelial cells,[5] and LECAM-1 is an adhesion molecule expressed on leukocytes.[6] These selectins are believed to be involved in the progression of the clinical manifestations of complicated diseases, such as chronic inflammation.[7] Attempts have been made, therefore, to find a selectin blocker that effectively inhibits their cell-adhesion activities at an early stage of inflammation.[8] To this end, it would be desirable for the blocker to exert its cell adhesion−inhibitory effects on all members of the selectin family.

Phillips et al. reported that the native ligand for ELAM-1 is the tetrasaccharide sialyl Lewis X (sLe^x).[9] Although the natural ligand for each selectin has not been completely characterized, it was reported recently that both P-selectin and L-selectin can also recognize the sLe^x structure.[10] Since then, a number of its derivatives have been reported.[11] These derivatives have activities similar to that of sLe^x against the natural ligand (sLe^x)−E-selectin binding. However, there are few reports regarding their inhibitory activities toward either sLe^x−P-selectin or −L-selectin binding. As a part of our studies of selectin blockers, we prepared 1-deoxy-3′-*O*-sulfo Le^x analogs (**1**−**3**), 1-deoxy-3′-*O*-phosphono Le^x analogs (**4**), and 1-deoxy sLe^x analogs (**5**−**7**) (see Chart 1) and examined their inhibitory activities toward the natural ligand (sLe^x) binding to each of the selectins.

Results and Discussion

Chemistry. Compounds **1**−**7** were synthesized according to published procedures.[12] For the synthesis of compound **1**, the key intermediate **8** was treated with a sulfur trioxide pyridine complex in DMF for 1 h at room temperature to afford the sulfated Le^x analog **9**, and this was transformed quantitatively by the removal of the protecting groups into the desired compound **1** (Scheme 1). Compounds **2** and **3** were synthesized from the corresponding intermediates **10** and **12** by a similar method.

For the synthesis of compound **4**, the key intermediate **12** was treated with diphenyl chlorophosphate in pyridine for 12 h at room temperature to give the 3′-*O*-diphenylphosphono derivative **14**, which upon hydrogenolysis of the phenyl groups in the presence of a prereduced Adams platinum catalyst, and subsequent treatment with sodium methoxide in methanol gave the 3′-*O*-phosphono Le^x analog **4** in good yield.

For the synthesis of compound **5**, hydrogenolysis of the benzyl groups in **15** and subsequent acetylation gave the protected sLe^x analog **16** in an 85% yield (Scheme 2). Compound **16** was deacylated with a sodium methoxide in methanol, and hydrolysis of the methyl group quantitatively yielded the desired sLe^x analog **5**. Compounds **6** and **7** were synthesized from the corresponding intermediates **17** and **19**, respectively.

Biological Activities. The method using selectin−IgG chimeras reported by Foxall et al. was followed.[10c]

First, the sLe^x tetrasaccharide was found to inhibit the binding of the ligand (sLe^x pentasaccharide ceramide) to purified human E-, P-, and L-selectin−Ig, with IC_{50} values of 0.6, > 1.0, and > 1.0 mM, respectively.

As shown in Table 1, compounds **1**−**3**, the 1-deoxy-3′-*O*-sulfo Le^x analogs, were all less potent (IC_{50} > 1.0 mM) than sLe^x (IC_{50} 0.6 mM) in the ligand−E-selectin competitive binding assay. In contrast, compounds **2**

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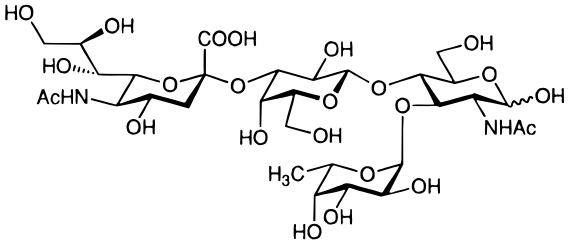
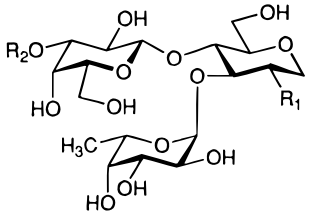
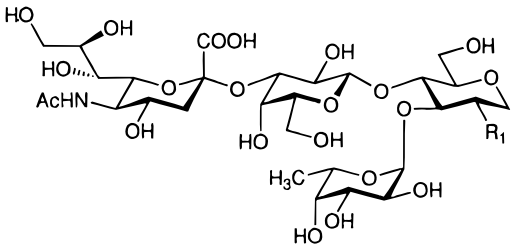
Chart 1



sLe^x

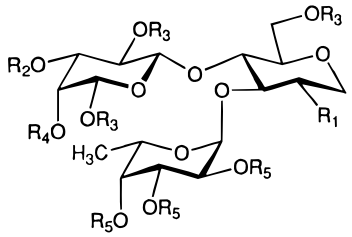


1: R_1=NHAc, R_2=SO_3Na
2: R_1=OH, R_2=SO_3Na
3: R_1=H, R_2=SO_3Na
4: R_1=H, R_2=PO_3Na



5: R_1=NHAc
6: R_1=OH
7: R_1=H

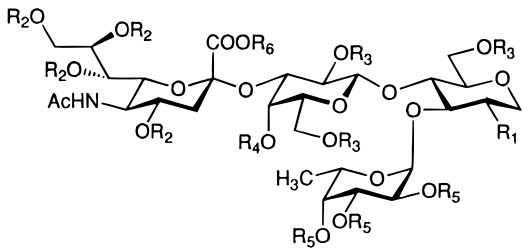
Scheme 1[a]



a → **8**: R_1=NHAc; R_2=H; R_3=R_4=Bz; R_5=Ac
b → **9**: R_1=NHAc; R_2=SO_3•Py; R_3=R_4=Bz; R_5=Ac
→ **1**: R_1=NHAc; R_2=SO_3Na; R_3=R_4=R_5=H
a → **10**: R_1=OBz; R_2=H; R_3=Bz; R_4=R_5=Ac
b → **11**: R_1=OBz; R_2=SO_3•Py; R_3=Bz; R_4=R_5=Ac
→ **2**: R1=OH; R_2=SO_3Na; R_3=R_4=R_5=H
a → **12**: R_1=R_2=H; R_3=Bz; R_4=R_5=Ac
b → **13**: R_1=H; R_2=SO_3•Py; R_3=Bz; R_4=R_5=Ac
→ **3**: R_1=R_3=R_4=R_5=H; R_2=SO_3Na
c
d → **14**: R_1=H; R_2=$PO(OPh)_2$; R_3=Bz; R_4=R_5=Ac
→ **4**: R_1=R_3=R_4=R_5=H; R_2=PO_3Na

[a] Reagents and conditions: (a) pyr·SO_3, DMF (**9**, 93%; **11**, 85%; **13**, 100%); (b) NaOMe, MeOH (**1**, 100%; **2**, 98%; **3**, 100%); (c) $ClPO(OPh)_2$, pyridine MeOH, (**14**, 100%); (d) PtO_2, EtOH, NaMe (**4**, 89%).

Scheme 2[a]



a → **15**: R_1=NHAc; R_2=Ac; R_3=R_4=Bz; R_5=Bn; R_6=Me
b → **16**: R_1=NHAc; R_2=Ac; R_3=R_4=Bz; R_5=Ac; R_6=Me
→ **5**: R_1=NHAc; R_2=R_3=R_4=R_5=R_6=H
a → **17**: R_1=OBz; R_2=Ac; R_3=Bz; R_4=H; R_5=Bn; R_6=Me
b → **18**: R_1=OBz; R_2=R_4=R_5=Ac; R_3=Bz; R_6=Me
→ **6**: R1=OH; R_2=R_3=R_4=R_5=R_6=H
c → **19**: R_1=R_4=H; R_2=Ac; R_3=Bz; R_5=Bn; R_6=Me
→ **7**: R_1=R_2=R_3=R_4=R_5=R_6=H

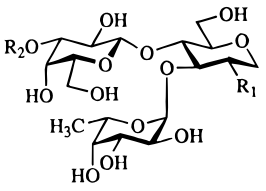
[a] Reagents and conditions: (a) 10% Pd−C, EtOH, AcOH, Ac_2O−pyridine (**16**, 85%; **18**, 73%); (b) NaOMe, MeOH, Amberlite IR-120(H^+) resin (**5**, 100%; **6**, 100%); (c) 10% Pd−C, EtOH, AcOH, NaOMe, MeOH, KOH, Amberlite IR-120(H^+) resin (**7**, 100%).

and **3** were more potent (IC_{50} 0.56 mM for **2**, 0.67 mM for **3**) than sLe^x (IC_{50} >1.0 mM) against the P-selectin in the competitive binding assay, which demonstrated that the substitution of the 2-position of 1-deoxy-3′-*O*-sulfo Le^x was not essential for binding to P-selectin.

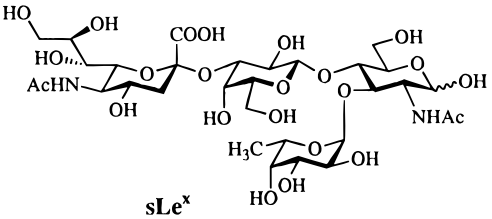
As shown in Table 1, compounds **5**−**7**, the 1-deoxy sLe^x analogs, were examined for their inhibitory activities toward ligand binding to each of the selectins. Compounds **6** and **7** inhibited the ligand−E-selectin binding at IC_{50} values of 0.36 and 0.24 mM, respectively. Interestingly, compound **5**, which lacks the 1-hydroxyl group from sLe^x, very strongly inhibited P-selectin and L-selectin−ligand binding, with IC_{50} values of 0.044 and 0.035 mM, respectively. In contrast, the inhibitory effect of **5** for E-selectin was less potent than that of sLe^x. These results indicate that the binding mode of the 1-deoxy sLe^x analogs (**5**−**7**) to each selectin is different from that of the 1-deoxy-3′-*O*-sulfo Le^x analogs (**1**−**3**).

The solution conformations of sLe^x have been reported by several groups.[13] It was further proposed that the fucose and galactose moieties, as well as the negatively charged sialic acid residues of sLe^x, would be critical for binding to E-selectin.[13b] The carboxylate group, therefore, serves to mimic the sialic acid in sLe^x as a type of negative charge, such as that within a sulfonate or phosphonate group. Our results of the blocking activities of compounds **3** (sulfonate), **4** (phosphonate), and **7** (sialic acid), shown in Table 1, indicate that the sialic acid residue was most favorable for the recognition by E-selectin; however, it was not necessarily optimal for the recognition by P-selectin and L-selectin. It was found that 1-deoxy-3′-*O*-sulfo Le^x **2**, **3**, and 1-deoxy sLe^x **5** inhibited the P-selectin−ligand binding more potently

Table 1. Blocking Activity of Compounds **1**−**7** and sLe^x



1-7



sLe^x

			IC$_{50}$, mM		
compd	R_1	R_2	E-selectin	P-selectin	L-selectin
1	NHAc	SO_3Na	>1.0	>1.0	>1.0
2	OH	SO_3Na	>1.0	0.67	>1.0
3	H	SO_3Na	>1.0	0.56	>1.0
4	H	PO_3Na	>1.0	>1.0	0.42
5	NHAc	NeuAc[a]	>1.0	0.044	0.035
6	OH	NeuAc	0.24	>1.0	0.22
7	H	NeuAc	0.36	>1.0	>1.0
sLe^x			0.60	>1.0	>1.0

[a] NeuAc, sialic acid.

than sLe^x. In addition, 1-deoxy-3′-*O*-phosphono Le^x **4** and 1-deoxy sLe^x **5** and **6** were more effective inhibitors of the L-selectin−ligand binding than sLe^x. These results suggest that the combination of the negative charge of the 3′-O position and the substituent at the 2 position of 1-deoxy Le^x skeleton is very important for the recognition of the P- and L-selectins by the ligand. Especially, 1-deoxy sLe^x **5** was up to the 20 times more potent than sLe^x tetrasaccharide toward P- and L-selectin binding.

This study supports the proposition that the interaction between the ligand and each selectin becomes favorable by the removal of the 1-hydroxyl group in sLe^x, and the hydrophobicity of the 1-position of GlcNAc may be important for tight binding to each selectin. Our data indicate that the modification of the 1- or 2-position of sLe^x could be useful for the design of more potent selectin blockers, which could be specific to each selectin or generally used toward all selectins.

Experimental Section

Construction, Expression, and Purification of Selectin−Immunoglobulin Fusion Protein. The construction of selectin−immunoglobulin was carried out according to the previous paper.[10c]

Selectin−immunogloblin fusion proteins (selectin−Ig) used in ELISA assays are recombinant chimeric molecules containing the lectin domain, epidermal growth factor domain, and two L-selectin−Ig, two P-selectin−Ig, or two E-selectin−Ig complement reguratory repeats coupled to the hinge, CH_2, and CH_3 regions of human IgG1. The corresponding E-selectin cDNA and P-selectin cDNA domain was amplified from HUVEC mRNA by RT-PCR. L-selectin domain was amplified from Jurkat cells mRNA by RT-PCR. Selectin cDNAs were fused to the hinge and Fc region of human IgG1 heavy chain. Selectin−Ig was expressed in COS7 (American Type Culture Collection CRL 1651) cells by transient transfection with selectin−Ig cDNA in pCDM8 vector (Funakoshi Co.) or expressed in CHO cells by stable transfection with selectin−Ig cDNA in pCIneo vector (Promega) by the lipofectamine (Gibco BRL). Selectin−Igs were affinity-purified from culture media using protein A silica gel (Nihon Gaishi Co.).

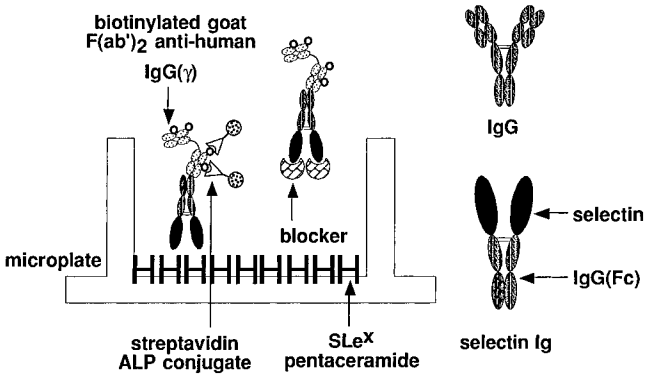


Figure 1. Schematic diagram of ELISA inhibition assay using selectin−Ig chimera. For details, see the Experimetal Section.

Inhibition Assay of E-, P-, and L-selectin−sLe^x Binding. A solution of sLe^x−pentaceramide in a 1:1 mixture of methanol and distilled water was pipetted into microtiter plate wells (96 wells, Falcon PRO-BIND) at 100 pmol/50 μL per well and adsorbed by evaporating the solvent. The wells were washed twice with distilled water, blocked with 5% BSA (bovine serum albumin)−PBS (phosphate buffered saline) for 1 h at room temperature, and washed with PBS three times.

Separately, a 1:1 volumetric mixture of a 1:500 dilution in 1% BSA−PBS of biotin−anti-human IgG (Fc) (BioSource International Inc., Lot 1201)/streptavidin−alkaline phosphatase (Zymed Lab Inc., Lot 50424702) and a E-selectin−immunoglobulin fusion protein (E-selectin−Ig) was incubated at room temperature for 30 min to form a complex. The test compounds were dissolved in distilled water at 1.0 mM and finally diluted to final concentrations of 100, 25, 6.25, and 1.56 μM, respectively. Reactant solutions were prepared by incubating 30 μL of this solution at each concentration with 30 μL of the above complex solution for 30 min at room temperature. This reactant solution was then added to the above microtiter wells at 50 μL/well and incubated at 37 °C for 45 min. The wells were washed three times with PBS and distilled water, and the mixture was added to the solution of *p*-nitrophenyl phosphate (1 mg/mL) and 0.01% of $MgCl_2$ in 1 M diethanolamine (pH 9.8) at 50 μL/well. The reactant mixture was developed for 120 min at 23 °C, and the absorbance at 405 nm was measured. Percent binding was calculated by the following equation:

$$\% \text{ binding} = (X - C/A - C) \times 100$$

where X is the absorbance of wells containing the test compounds at each concentration, C is the absorbance of wells not containing the selectin−Ig and test compounds, and A is the absorbance of control wells not containing the test compounds. Inhibition of P- or L-selectin−sLe^x binding was repeated except that P-selectin−Ig or L-selectin−Ig was replaced for E-selectin−Ig. The results of inhibitory activities are presented in Table 1 as IC$_{50}$ values, the concentrations of blockers necessary to cause 50% inhibition of the ligand binding to each of the selectins by the compounds **1**−**7**. The number of replicates is two. For a schematic representation of the ELISA blocking assay, see Figure 1.

***O*-(2,4,6-Tri-*O*-benzoyl-3-*O*-sulfo-*β*-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-2-acetamido-1,5-anhydro-6-*O*-benzoyl-D-glucitol Pyridine Salt (9).** To a solution of **8**[12a] (33 mg, 0.031 mM) in DMF (0.3 mL) was added sulfur trioxide pyridine complex (25 mg), and the mixture was stirred for 1 h. MeOH (0.1 mL) was added to the mixture and concentrated at 25 °C. Column chromatography (15:1 CH_2Cl_2−MeOH) of the residue on silica gel (10 g) gave **9** (35 mg, 93%) as an amorphous mass: $[\alpha]_D$ −5.5° (*c* 1.2, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.01 (d, J = 6.2 Hz, 3H, H-6b), 1.88−2.08 (4s, 12H, 3AcO, AcN), 7.10−8.10 (m, 25H, 4Ph, pyridine). Anal. Calcd ($C_{59}H_{62}N_2O_{24}S$) C, H, N.

***O*-(3-*O*-Sulfo-*β*-D-galactopyranosyl)-(1−4)-*O*-[α-L-fucopyranosyl-(1−3)]-2-acetamido-1,5-anhydro-D-glucitol Sodium Salt (1).** To a solution of **9**[12a] (50 mg, 0.041 mM) in MeOH (1 mL) was added NaOMe (5 mg), and the mixture was stirred overnight at room temperature then concentrated at 30 °C. Column chromatography (4:1 MeOH−H_2O) of the residue on Sephadex LH-20 (30 g) gave **1** (25.5 mg, 100%) as an amorphous mass: $[\alpha]_D$ −24.0° (*c* 0.6, 1:1 MeOH−H_2O); ^{1}H NMR (270 MHz, D_2O) *δ* 1.20 (d, *J* = 6.6 Hz, 3H, H-6b), 2.25 (s, 3H, AcN), 3.33 (t, *J* = 11.2 Hz, 1H, H-1a), 4.58 (d, *J* = 7.9 Hz, 1H, H-1c). The mass spectrum of 1 (negative ion mode) showed the base peak at *m*/*z* 592.5 $(M - H)^-$.

***O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-sulfo-*β*-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-1,5-anhydro-2,6-di-*O*-benzoyl-D-glucitol Pyridine Salt (11).** To a solution of **10**[12b](126 mg, 0.12 mM) in DMF (1 mL) was added sulfur trioxide pyridine complex (95 mg), and the mixture was stirred for 1 h. MeOH (0.2 mL) was added to the mixture and concentrated at 25 °C. Column chromatography (20:1 CH_2Cl_2−MeOH) of the residue on silica gel (60 g) gave **11** (121 mg, 85%) as an amorphous mass: $[\alpha]_D$ −2.0° (*c* 0.9, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) *δ* 1.29 (d, *J* = 6.4 Hz, 3H, H-6b), 1.85−2.15 (4s, 12H, 4AcO), 3.24 (t, *J* = 10.6 Hz, 1H, H-1a), 5.14 (dd, *J* = 11.1 Hz, 4.0 Hz, 1H, H-3b), 5.45 (d, *J* = 3.4 Hz, 1H, H-4c), 7.13−8.06 (m, 25H, 4Ph, pyridine). Anal. Calcd ($C_{59}H_{61}NO_{25}S$) C, H, N.

***O*-(3-*O*-Sulfo-*β*-D-galactopyranosyl)-(1−4)-*O*-[α-L-fucopyranosyl-(1−3)]-1,5-anhydro-D-glucitol Sodium Salt (2).** To a solution of **11** (120 mg, 0.1 mM) in MeOH (2 mL) was added NaOMe (20 mg), and the mixture was stirred overnight at room temperature and then concentrated at 25 °C. Column chromatography (4:1 MeOH−H_2O) of the residue on Sephadex LH-20 (60 g) gave **2** (56 mg, 98%) as an amorphous mass: $[\alpha]_D$ −9.7° (*c* 1.1, 1:1 MeOH−H_2O); ^{1}H NMR (270 MHz, D2O) *δ* 1.53 (d, *J* = 6.6 Hz, 3H, H-6b), 3.63 (t, *J* = 10.6 Hz, 1H, H-1a). The mass spectrum of **2** (negative ion mode) showed the base peak at *m*/*z* 550.5 $(M - H)^-$.

***O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-sulfo-*β*-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-1,5-anhydro-6-*O*-benzoyl-2-deoxy-D-*arabino*-hexitol Pyridine Salt (13).** To a solution of **12**[12b] (150 mg, 0.16 mM) in DMF (0.3 mL) was added sulfur trioxide pyridine complex (127 mg), and the mixture was stirred for 1 h. MeOH (0.2 mL) was added to the mixture and concentrated at 25 °C. Column chromatography (20:1 CH_2Cl_2−MeOH) of the residue on silica gel (60 g) gave **13** (173 mg, 100%) as an amorphous mass: $[\alpha]_D$ −23.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) *δ* 1.29 (d, *J* = 6.4 Hz, 3H, H-6b), 1.85−2.15 (4s, 12H, 4AcO), 7.23−8.57 (m, 20H, 3Ph, pyridine). Anal. Calcd ($C_{52}H_{57}NO_{23}S$) C, H, N.

***O*-(3-*O*-Sulfo-*β*-D-galactopyranosyl)-(1−4)-*O*-[α-L-fucopyranosyl-(1−3)]-1,5-anhydro-2-deoxy-D-*arabino*-hexitol Sodium Salt (3).** To a solution of **13** (173 mg, 0.16 mM) in MeOH (4 mL) and THF (2 mL) was added NaOMe (10 mg), and the mixture was stirred overnight at room temperature and then concentrated at 25 °C. Column chromatography (4:1 MeOH−H_2O) of the residue on Sephadex LH-20 (60 g) gave **3** (89.7 mg, 100%) as an amorphous mass: $[\alpha]_D$ −92.5° (*c* 0.8, MeOH); ^{1}H NMR (270 MHz, D_2O) *δ* 1.03 (d, *J* = 6.4 Hz, 3H, H-6b), 4.41 (d, *J* = 7.5 Hz, 1H, H-1c), 4.44 (m, 1H, H-5b), 4.71 (d, *J* = 3.4 Hz, 1H, H-1b). The mass spectrum of **3** (negative ion mode) showed the base peak at *m*/*z* 534.9 $(M - H)^-$.

***O*-(3-*O*-Phosphono-*β*-D-galactopyranosyl)-(1−4)-*O*-[α-L-fucopyranosyl-(1−3)]-1,5-anhydro-2-deoxy-D-*arabino*-hexitol Sodium Salt (4).** A solution of **14**[12b] (187 mg, 0.16 mM) in EtOH (8 mL) was stirred for 6 h at room temperature in the presence of prereduced Adams platinum catalyst (200 mg) under hydrogen. The catalyst was collected, and the solution was concentrated. To a solution of the residue in dry MeOH (5 mL) was added NaOMe (20 mg), and the mixture was stirred for 24 h at room temperature then concentrated at 25 °C. Column chromatography (1:1 MeOH−H_2O) of the residue on Sephadex LH-20 (80 g) gave **4** (80.5 mg, 89%) as an amorphous mass: $[\alpha]_D$ −23.5° (*c* 1.1, MeOH−H_2O); ^{1}H NMR (270 MHz, D_2O) *δ* 1.37 (d, *J* = 6.6 Hz, 3H, H-6b), 4.67 (d, *J* = 7.7 Hz, 1H, H-1c), 4.80 (m, 1H, H-5b), 5.19 (d, *J* = 3.9 Hz, 1H, H-1b). The mass spectrum of **4** (negative ion mode) showed the base peak at *m*/*z* 534.9 $(M - 2H)2^-$.

***O*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate)-(2−3)-*O*-(2,4,6-tri-*O*-benzoyl-*β*-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-2-acetamido-1,5-anhydro-6-*O*-benzoyl-2-deoxy-D-glucitol (16).** A solution of **15**[12a] (158 mg, 0.095 mM) in EtOH (20 mL) and AcOH (5 mL) was stirred with 10% Pd−C (160 mg) overnight at room temperature under hydrogen, then filtered, and concentrated. The residue was treated with Ac_2O (1 mL) and pyridine (2 mL) overnight at room temperature and concentrated. Column chromatography (50:1 CH_2Cl_2−MeOH) of the product on silica gel (30 g) gave **16** (120 mg, 85%) as an amorphous mass: $[\alpha]_D$ +0.5° (*c* 1.5, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) *δ* 1.12 (d, *J* = 6.4 Hz, 3H, H-6b), 1.49−2.06 (9s, 27H, 7AcO, 2AcN), 2.40 (dd, *J* = 12.5 Hz, 4.5 Hz), 3.78 (s, 3H, MeO), 4.77 (m, 1H, H-4d), 5.35 (d, *J* = 3.8 Hz,1H, H-1b), 5.48 (dd, *J* = 8.1, 10.1 Hz, 1H, H-2c), 5.65 (m, 1H, H-8d), 7.26−8.15 (m, 20H, 4Ph). Anal. Calcd $C_{74}H_{83}N_2O_{32}$) C, H, N.

***O*-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosylonic acid)-(2−3)-*O*-*β*-D-galactopyranosyl-(1−4)-*O*-[α-L-fucopyranosyl-(1−3)]-2-acetamido-1,5-anhydro-2-deoxy-D-glucitol (5).** To a solution of **16** (80 mg, 0.054 mM) in MeOH (2 mL) was added NaOMe (6 mg), and the mixture was stirred overnight at room temperature. Water (1 mL) was added to the mixture, and this was stirred for 5 h at room temperature, neutralized with Amberlite IR-120 (H^+) resin, and filtered, the resin was washed with MeOH, and the combined filtrate and washings were concentrated. Column chromatography (MeOH) of the residue on Sephadex LH-20 (30 g) gave **5** (42 mg, 100%) as an amorphous mass: $[\alpha]_D$ −25.0° (*c* 0.7, MeOH); ^{1}H NMR (270 MHz, DMSO-d_6) *δ* 1.12 (d, *J* = 6.2 Hz, 3H, H-6b), 1.89, 1.98 (2s, 6H, 2AcN), 3.19 (t, 1H, H-1a), 4.42 (d, *J* = 7.6 Hz, 1H, H-1c), 5.02 (d, *J* = 2.8 Hz, 1H, H-1b). Anal. Calcd ($C_{31}H_{52}N_2O_{22}$) C, H, N.

***O*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate)-(2−3)-*O*-(4-*O*-acetyl-2,6-di-*O*-benzoyl-*β*-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-1,5-anhydro-2,6-di-*O*-benzoyl-D-glucitol (18).** A solution of **17**[12b] (98 mg, 0.06 mM) in EtOH (10 mL) and AcOH (2 mL) was stirred with 10% Pd−C (100 mg) for 2 days at 40 °C under hydrogen. The precipitate was collected, and the solution was concentrated. Treatment of the residue with Ac_2O (1 mL) and pyridine (2 mL) in the presence of 4-dimethylaminopyridine (5 mg) occurred overnight at 45 °C. MeOH was added to the mixture, and the mixture was concentrated. Column chromatography (200:1 CH_2Cl_2−MeOH) of the residue on silica gel gave **18** (66 mg, 73%) as an amorphous mass: $[\alpha]_D$ +14.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) *δ* 1.44 (d, *J* = 6.6 Hz, 3H, H-6b), 1.45−2.13 (9s, 27H, 8AcO, AcN), 2.52 (dd, *J* = 12.4 Hz, 4.4 Hz), 3.19 (t, *J* = 10.6 Hz, 1H, H-1a), 3.68 (s, 3H, MeO), 7.27−8.17 (m, 20H, 4Ph). Anal. Calcd ($C_{74}H_{83}NO_{34}$) C, H, N.

***O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2−3)-*O*-*β*-D-galactopyranosyl-(1−4)-*O*-[α-L-fucopyranosyl-(1−3)]-1,5-anhydro-D-glucitol (6).** To a solution of **18** (53 mg, 0.033 mM) in MeOH (2 mL) was added NaOMe (10 mg), and the mixture was stirred overnight at room temperature. Water (0.5 mL) was added to the mixture, and this was stirred for a further 12 h at room temperature, neutralized with Amberlite IR-120 (H^+) resin, and filtered, the resin was washed with MeOH, and the combined filtrate and washings were concentrated. Column chromatography (MeOH) of the residue on Sephadex LH-20 (30 g) gave **6** (26 mg, 100%) as an amorphous mass: $[\alpha]_D$ +20.5° (*c* 0.5, MeOH); ^{1}H NMR (270 MHz, DMSO-d_6) *δ* 0.97 (d, *J* = 6.4 Hz, 3H, H-6b), 1.84 (s, 3H, AcN), 2.45 (dd, *J* = 13.2, 3.2 Hz, H-3d), 4.25 (d, *J* = 7.7 Hz, 1H, H-1c), 5.14 (d, *J* = 3.2 Hz, 1H, H-1b). Anal. Calcd ($C_{29}H_{49}NO_{22}$) C, H, N.

***O*-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosylonic acid)-(2−3)-*O*-*β*-D-galactopyranosyl-(1−4)-*O*-[α-L-fucopyranosyl-(1−3)]-1,5-anhydro-2-deoxy-D-glucitol (7).** A solution of **19**[12b] (130 mg, 0.086 mM) in EtOH (15 mL) and AcOH (5 mL) was hydrogenolyzed in the presence of 10% Pd−C (130 mg) for 48 h at 40 °C, then

filtered and concentrated. To a solution of the residue in MeOH (5 mL) was added NaOMe (20 mg), and the mixture was stirred for 24 h at room temperature. Potassium hydroxide (0.2 M, 3 mL) was added the mixture, and this was stirred for 6 h at room temperature, neutralized with Amberlite IR-120 (H^+) resin, and filtered, the resin was washed with MeOH, and the combined filtrate and washings were concentrated. Column chromatography (MeOH) of the residue on Sephadex LH-20 (30 g) gave **7** (64 mg, 100%) as an amorphous mass: $[\alpha]_D$ −23.0° (*c* 1.5, MeOH); ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.03 (d, J = 6.4 Hz, 3H, H-6b), 1.87 (s, 3H, AcN), 2.57 (dd, J = 13.2, 3.2 Hz, H-3d), 4.37 (d, J = 7.2 Hz, 1H, H-1c), 4.80 (d, J = 3.2 Hz, 1H, H-1b). Anal. Calcd ($C_{29}H_{49}NO_{21}$) C, H, N.

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