Synthesis of Biologically Active Sialyl Lewis X Mimetics

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Received September 28, 1994 (Revised Manuscript Received March 1, 1995⊗)

The design and synthesis of two sialyl Lewis X (SLe^x) mimetics are described. In the design of mimetic **1**, an ethylene glycol linkage is used to bridge the fucose and galactose moiety, and a carboxymethyl group is placed in the 3-OH position of the galactose residue to provide the negative charge which is believed to be essential for binding to (*E*)-selectin. In the design of mimetic **2**, a D-tartaric acid derivative is used to provide the *trans*-dihydroxyl groups originally from the glucosamine moiety for the linkage of the fucose and the carboxypentyl groups. At a concentration of 1.5 mM, **1** inhibits 50% of the binding of SLe^x glycoconjugate to immobilized recombinant (*E*)-selectin, while **2** has an IC_{50} of 10 mM. Mimetic **1** is also found to be stable toward α-L-fucosidase. Results from the ROESY and COSY experiments indicate that compound **1** is conformationally flexible, which may explain its relatively weak activity compared to SLe^x (IC_{50} = 0.8 mM).

Introduction

Sialyl Lewis X (SLe^x), a terminal tetrasaccharide fragment of membrane glycoproteins and glycolipids, has been identified as a ligand for the endothelial leukocyte adhesion molecule-1 ((*E*)-selectin), which mediates the early stage of adhesion of leukocytes to activated endothelial cells.[1] Though SLe^x has been considered to be potentially useful as an antiinflammatory agent, the search for novel SLe^x mimetics with simpler structure, higher affinity for the receptor, and better stability against glycosidases, especially fucosidase and sialidase, has been of great interest to chemists and biologists.[2]

The solution conformations of SLe^x and related molecules have been determined by this group and others.[3] It was further proposed[3b] that the binding domain of SLe^x is located on the hydrophilic surface composed of fucose, galactose, and the carboxyl group of the sialic acid residue, as shown in Figure 1. The (*E*)-selectin crystal structure in the absence of SLe^x has recently become available for use in modeling ligand binding[4] and in investigating the bound conformation of SLe^x based on NMR analysis and molecular modeling.[5] As part of our efforts directed toward the development of SLe^x mimetics, we have designed several molecules using model construction and computation. Here we report on the synthesis of two of these designed molecules (Figure 1).

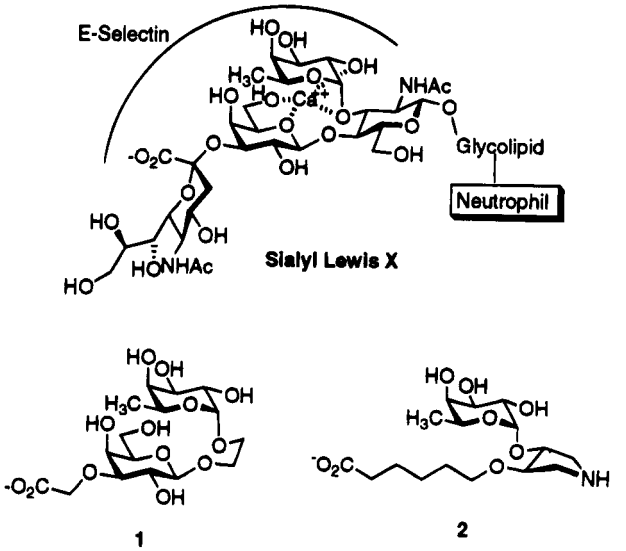

Figure 1. SLe^X and designed mimetics.

In the designed mimetic **1**, the fucose and galactose residues are tethered by a simple ethylene glycol linkage.[6] Previous studies have shown that (*E*)-selectin requires the hydroxyl groups at the 2-, 3-, and 4-positions of the fucose residue.[3b,7] The carboxylate group serves to mimic the negative charge of sialic acid in the natural

⊗ Abstract published in *Advance ACS Abstracts,* April 15, 1995.

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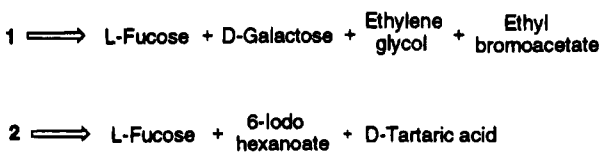
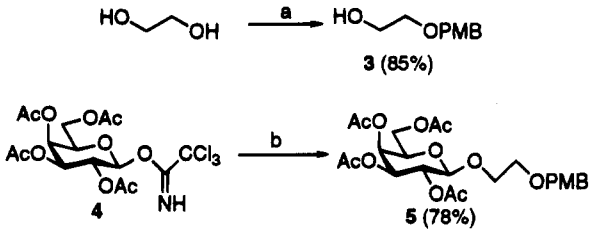


Figure 2.

Scheme 1[a]



[a] Key: (a) Bu_2SnO/benzene, then $MeOC_6H_5CH_2Br$; (b) **3**, $BF_3{\cdot}OEt_2/CH_2Cl_2$, 3 Å molecular sieves.

ligand, which is believed to be the most important feature of the sialic acid residue for recognition.[8] On the basis of our modeling study, the structure of **1** is an accessible conformation, though the ethylene glycol moiety may be too flexible to fix the glycosidic torsion angles.

Since the binding of SLe^x to (*E*)-selectin is a Ca^{2+} dependent process, the calcium binding site has also been incorporated into the designed molecule[9] (Figure 1). With all these elements together, we expect that compound **1**, in the presence of calcium ions and due to the influence of the *exo* anomeric effect, may adopt a conformation resembling the active form of SLe^x bound to (*E*)-selectin.

In the design of mimetic **2**, a D-tartaric acid derivative provides the (*R,R*)-*trans*-dihydroxyl groups originally from D-glucosamine in SLe^x. A simple linear five-carbon spacer is linked to one hydroxyl group of the *trans*-diol and a carboxylate group to replace the galactose and sialylic acid residues. This mimetic contains more rigid glycosidic torsional angles due to the use of a cyclic diol for linkage, but the corresponding 4- and 6-OH groups of the galactose residue are missing.

Results and Discussion

Synthesis of Compound 1. The synthesis of compound **1** started from L-fucose, D-galactose, and ethylene glycol (Figure 2). The direct coupling of unprotected ethylene glycol to various galactose donors gave very low yields due to the poor solubility of ethylene glycol in the reaction solvents and the high solubility of the desired product in water during workup extraction. The use of monoprotected ethylene glycol **3**, as shown in Scheme 1, solved the solubility problem. Furthermore, it allowed for the modification of the galactose residue before its coupling to the L-fucose derivatives. The PMB (4-methoxybenzyl) protecting group was cleaved when the monoprotected alcohol was treated with the galactosyl bromide and AgOTf. The addition of base to the reaction system avoided this cleavage but generated an ortho ester as the major product. Fortunately, the coupling of imidate **4** and alcohol **3** successfully gave the desired product **5** in a reasonable yield.[10]

Deacetylation followed by selective protection of the hydroxyl groups at C-3 and C-4 with an isopropylidene afforded galactose derivative **6** (Scheme 2). After benzylation of the remaining hydroxyl groups, removal of the isopropylidene freed the hydroxyl groups at C-3 and C-4 to give **7**. Refluxing of a solution of **7** with dibutyltin oxide in toluene gave the 3,4-*O*-dibutylstannylene complex, which was subsequently reacted with ethyl 2-bromoacetate to give lactone **8**. When benzene was used as the reaction solvent in the alkylation,[11] a mixture of **8** and the ethyl ester **8a** was obtained (Scheme 3). **8a** could however be converted to **8** by refluxing with a catalytic amount of TsOH in toluene. Compound **8** was deprotected by DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) to provide the primary alcohol **9**, which was ready to be coupled with the L-fucose derivative.

The protected fucosyl fluoride **11** was synthesized in one step by treating 2,3,4-protected **10**[12] with DAST ((diethylamino)sulfur trifluoride) at room temperature for 10 min (Scheme 4).

The coupling reaction between alcohol **9** and fluoride **11** was carried out using silver perchlorate and tin(II) chloride as catalysts[13] to yield a 3.3:1 mixture of **12** (α anomer) and its diastereomer **12a** (β anomer) (Scheme 5). While the α and β anomers were inseparable by normal thin layer chromatography, they could be separated by HPLC using a silica gel column. Debenzylation and peracetylation also failed to give separable compounds on thin layer chromatography plates.

Hydrogenation of compound **12** in methanol catalyzed by $Pd(OH)_2$ on carbon gave a mixture of methyl ester **13** and free acid **14**. The mixture of **13** and **14** was further converted to **1** by base hydrolysis.

For comparison, another coupling reaction was carried out under conditions similar to those for the coupling of **9** and **11** (Scheme 6). The coupling between alcohol **15** and fucose derivative **11** gave **16** and its diastereomer with an α/β (at the fucosidic bond) ratio of 2.9:1. Alcohol **15** was synthesized from **5** by reacting with DDQ.

Synthesis of Compound 2. The retrosynthetic strategy for molecule **2** is shown in Figure 2. The synthesis (Scheme 7) started from the preparation of diol **17** from D-tartaric acid.[14] The amine in **17** protected by a benzyl group was still very basic and polar and would cause a lot of problems in further reactions and purifications. An alternative way was to change the protecting group on the amine. Hydrogenation of compound **17** required the presence of acetic acid. The free amine was then protected with a CBZ group to give diol **18**, which was converted via dibutylstannylene complex to afford the PMB derivative **19**.

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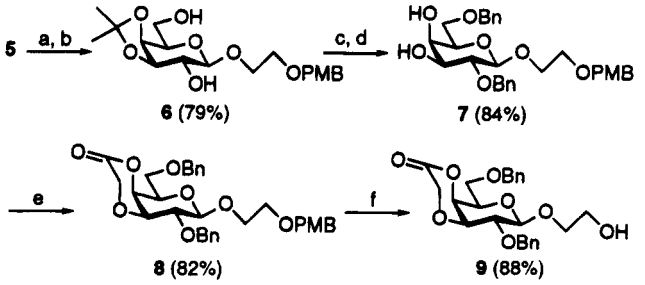
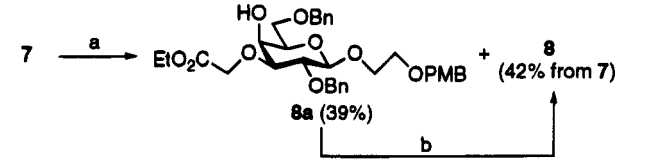
Scheme 2[a]



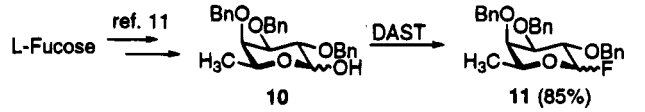
[a] Key: (a) (i) NaOMe, MeOH; (ii) Dowex 50W-X8; (b) (i) 2,2-dimethoxypropane, *p*-TsOH; (ii) HOAc/H_2O (3:1), rt, 1 h; (c) NaH/DMF, then BnBr, Bu_4NI; (d) HOAc/H_2O (3:1), 60 °C, 1 h; (e) Bu_2SnO/toluene, reflux, then $BrCH_2CO_2Et$, Bu_4NI, 130 °C, 2 h; (f) DDQ, CH_2Cl_2/H_2O (18:1).

Scheme 3[a]

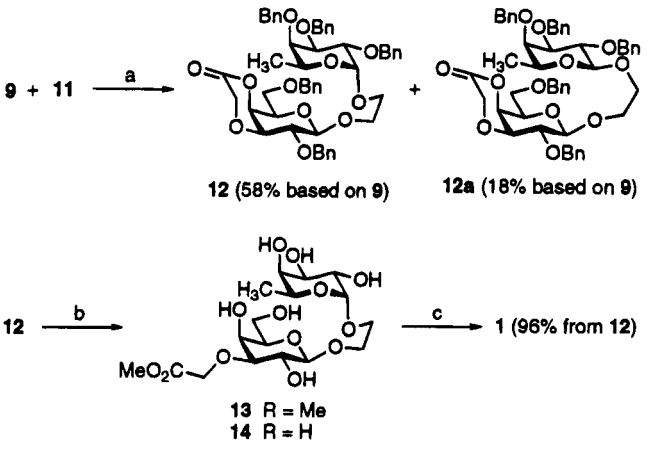


[a] Key: (a) Bu_2SnO/benzene, reflux, then $BrCH_2CO_2Et/Bu_4NI$, 80 °C; (b) *p*-TsOH/toluene, reflux, 60%.

Scheme 4



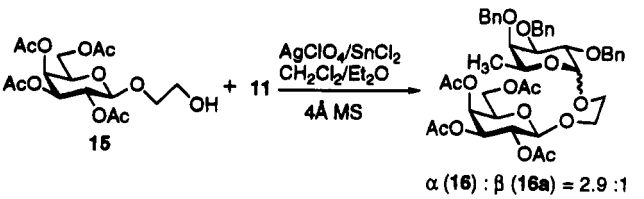
Scheme 5[a]



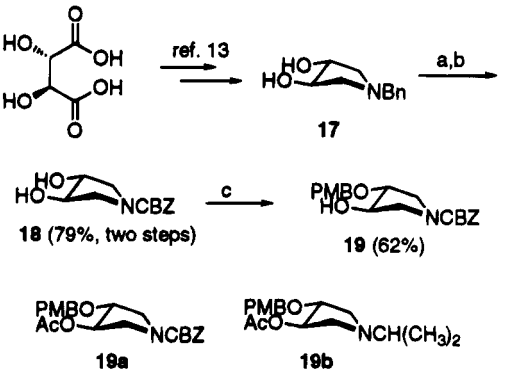
[a] Key: (a) $AgClO_4$, $SnCl_2$, CH_2Cl_2/Et_2O, 4 Å molecular sieves, −78 °C → rt, 12 h; (b) H_2/Pd(OH)$_2$C, MeOH, 1 h; (c) 0.1 N NaOH, 3 h.

Iodide **20** was freshly prepared from commercially available 6-bromohexanoic acid. This acid was first converted to a methyl ester through standard diazomethane procedures. The bromide was then replaced with iodide to increase the reactivity by reacting it with potassium iodide in acetone. Coupling of iodide **20** with alcohol **19** turned out to be more straightforward than expected (Scheme 8). Strongly basic reaction conditions (NaH/DMF) did not affect the methyl ester, when the reaction was quenched by the addition of drops of acetic acid prior to the extractions. The product **21** was then deprotected to give alcohol **22**, ready for coupling to fucosyl fluoride **11**. Under a similar condition as mentioned earlier for the synthesis of **1**, **23** was formed and was deprotected by hydrogenation (H_2/Pd(OH)$_2$−C, MeOH/EtOAc/HOAc), followed by base hydrolysis to afford the desired compound **2** (Scheme 9).

Scheme 6

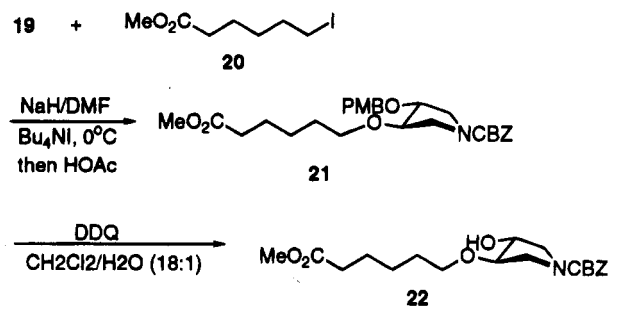


Scheme 7[a]

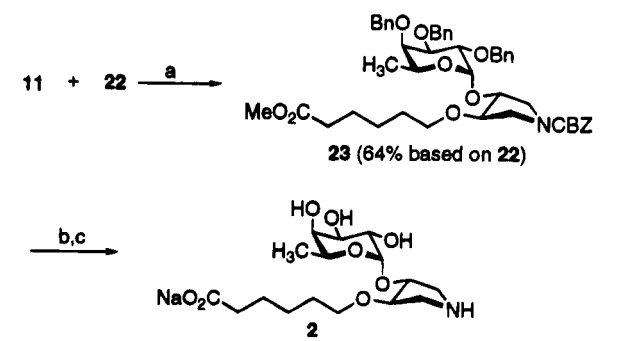


[a] Key: (a) H_2 (40 psi), Pd(OH)$_2$C, HOAc/MeOH (1:2); (b) THF, aqueous Na_2CO_3 (6%), then CBZCl, 0 °C → rt; (c) Bu_2SnO, Bn, reflux, 3 h, then PMBBr, *n*-Bu_4NI.

Scheme 8



Scheme 9[a]



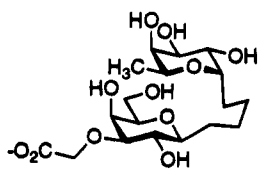
[a] Key: (a) $AgClO_4$, $SnCl_2$, TMU, 4 Å molecular sieves, ether, −78 °C → rt, 12 h; (b) H_2, Pd(OH)$_2$C, MeOH/EtOAc/HOAc (4:1:1); (c) 0.1 N NaOH.

Biological Activities and NMR Studies. Compound **1** was found stable at pH 5.5 and toward α-fucosidase. It was active in an assay system which measured the binding of SLe^x glycoconjugate to immobilized recombinant (*E*)-selectin. The activity was concentration dependent with a 50% inhibition at 1.5 mM. Compound

2 inhibited the binding with an IC_{50} of 10 mM. In comparison, SLe^x and Le^x-3′-*O*-sulfate inhibited the binding with an IC_{50} of about 0.8 mM and 2 mM, respectively, under the same conditions.[15]

COSY and ROESY experiments were also carried out to help in the assignment of the NMR spectra and the study of the relative structure of compound **1**. The NOE's observed in SLe^x, e.g., the methyl group of the fucose residue and the H-2 of the galactose residue,[3b] were, however, not observed in **1**. Calcium titration experiments were also carried out to see if calcium cation would induce the active conformation, but no NOE was observed between those protons even in the presence of 20 equiv of calcium cation. These results indicate that, in solution, **1** exhibits a flexible conformation.

For comparison, the activity of **1** is better than that of the corresponding C-linked analog **24** (23% inhibition at 10 mM) but similar to that of the C-linked fucopeptide **25** (IC_{50} = 1.3 mM)[16] which contains the five essential hydroxyl groups in space corresponding to the fucose and galactose residues and the carboxylate group from the sialic acid residue.



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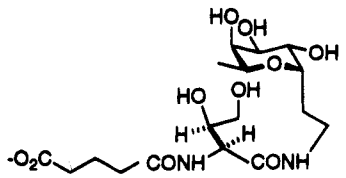

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In summary, this study confirms the structural elements of SLe^x required for (*E*)-selectin binding. Work is in progress to complete the synthesis of other designed molecules that possess either macrocyclic structures or conformationally restricted tethers, which are expected to have much better inhibition effects. Their synthesis and activities will be reported in the near future.

Experimental Section

1-Hydroxyl-2-[(4-methoxybenzyl)oxy]ethane (3). Ethylene glycol **2** (1.4 mL, 25.1 mmol) was refluxed overnight in benzene with dibutyltin oxide (6.87 g, 27.6 mmol) using a Dean–Stark apparatus. The freshly prepared PMBBr (6.1 g, 30.1 mmol) was then added dropwise at 50 °C, followed by addition of Bu_4NI (6.5 g, 17.5 mmol). The mixture was allowed to react for 6 h at 80 °C and then concentrated and purified by flash chromatography (2:1 hexane/EtOAc) to yield 3.9 g (85%) of the title compound as a colorless liquid. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.249–7.272 (2 H, m, Ph-H), 6.856–6.882 (2 H, m, Ph-H), 4.470 (2 H, s, PhC-H), 3.783 (3 H, OCH_3), 3.697–3.716 (2 H, m, ethylene), 3.529–3.548 (2 H, m, ethylene). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 159.0, 129.9, 129.4, 113.7, 72.78, 71.03, 61.65, 55.13. HRMS: calcd for $C_{10}H_{14}O_3Cs$ ($M + Na^+$) 205.0841, found 205.0840.

2-[(4-Methoxybenzyl)oxy]ethyl 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranoside (5). Imidate **4** (5.04 g, 10.0 mmol) and alcohol **3** (1.82 g, 1.0 equiv) were mixed and completely dried before being dissolved in anhydrous CH_2Cl_2 (80 mL). The mixture was stirred with 3 Å molecular sieves for 30 min. A solution of $BF_3{\cdot}EtO_2$ (0.1 equiv) in CH_2Cl_2 (5 mL) was then added dropwise at −43 °C. The reaction was conducted under argon until it was complete in 2 h and then quenched with Et_3N, followed by addition of saturated aqueous $NaHCO_3$ at −43 °C. The mixture was warmed up to rt, filtered, and diluted with $CHCl_3$. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, concentrated, and purified by flash chromatography (2:1 hexane/EtOAc) to yield 3.99 g (78%) of the title compound as a colorless syrup. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.248–7.279 (2 H, m, Ph-H), 6.883 (2 H, d, J = 8.5 Hz, Ph-H), 5.388 (1 H, d, J = 3.5 Hz, H-4), 5.233 (1 H, dd, J = 8, 10.5 Hz, H-3), 5.020 (1 H, dd, J = 3.5, 10.5 Hz, H-2), 4.586 (1 H, d, J = 8 Hz, H-1), 4.472 (2 H, AB, J = 11.5 Hz, $\Delta\nu$ = 14.5 Hz, PhC-H), 4.109–4.184 (2 H, m, H-6a,6b), 3.965–4.003 (1 H, m, ethylene), 3.899 (1 H, t, J = 13 Hz, H-5), 3.802 (3 H, s, OCH_3), 3.560–3.781 (3H, m, ethylene), 2.150 (3 H, s, OAc), 2.042 (3 H, s, OAc), 2.008 (3 H, s, OAc), 1.987 (3 H, s, OAc). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.3, 170.2, 170.1, 169.4, 159.1, 130.0, 129.3, 129.1, 113.7, 101.2, 72.79, 70.79, 70.48, 69.01, 68.98, 68.79, 68.67, 66.93, 61.17, 55.13, 20.63, 20.55, 20.48. HRMS: calcd for $C_{24}H_{33}O_{12}$ ($M + H^+$) 513.1972, found 513.1970.

2-[(4-Methoxybenzyl)oxy]ethyl 3,4-*O*-Isopropylidene-β-D-galactopyranoside (6). To a solution of compound **5** (3.7 g, 7.2 mmol) in anhydrous methanol (50 mL) was added a catalytic amount of NaOMe, and the mixture was stirred under argon. After 2 h, the mixture was stirred with Dowex 50W-X8 for 15 min until the supernatant became clear. The mixture was filtered, concentrated, and completely dried, and 2,2-dimethoxypropane (60 mL) was added. The solution was stirred with a catalytic amount of *p*-TsOH for 2 h under argon before the reaction was quenched with triethylamine. The mixture was concentrated in vacuo, followed by the addition of a mixture of HOAc/H_2O (3:1, 50 mL). The suspension was stirred for 1 h to remove the protecting group at the 6- position of galactose and then concentrated. Flash chromatography (EtOAc) gave 2.18 g of the title compound (79% from **5**). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.256–7.273 (2 H, m, Ph-H), 6.872–6.889 (2 H, m, Ph-H), 4.497 (2 H, s, CH_2 of PMB), 4.258 (1 H, d, J = 8 Hz, H-1 of Gal), 4.151 (1 H, dd, J = 2, 5.5 Hz, H-3 of Gal), 4.103 (1 H, dd, J = 5.5, 8 Hz, H-2 of Gal), 4.029–4.058 (1 H, m, ethylene), 3.963–3.997 (1 H, m, ethylene), 3.743–3.871 (6 H, m, H-4,5 of Gal, ethylene, CH_3 of PMB), 3.569–3.647 (3 H, m, H-6a,6b of Gal, ethylene), 1.523 (3 H, s, CH_3 of isopropylidene), 1.348 (3 H, s, CH_3 of isopropylidene). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 129.5, 113.8, 110.5, 102.7, 78.8, 73.9, 73.6, 73.5, 72.9, 68.9, 68.8, 62.5, 55.3, 28.1, 26.4. HRMS: calcd for $C_{19}H_{28}O_8Cs$ ($M + Cs^+$) 517.0839, found 517.0852.

2-[(4-Methoxybenzyl)oxy]ethyl 2,6-Di-*O*-benzyl-β-D-galactopyranoside (7). To a solution of compound **6** (1.84 g, 4.79 mmol) in DMF (50 mL) under argon were added portions of NaH (80% suspension in mineral oil, 850 mg) at 0 °C. The mixture was stirred at 0 °C for 1 h and at rt for 30 min and then cooled to 0 °C, and BnBr (1.8 mL) and Bu_4NI (0.5 equiv) were added. The mixture was allowed to warm to rt gradually. After 2 h at rt, the reaction was quenched with water at 0 °C and then diluted with EtOAc at rt. The organic layer was washed successively with water and brine and dried over $MgSO_4$. The concentrated residue was then purified by flash chromatography (2:1 hexane/EtOAc), and the product was dissolved in a mixture of HOAc and H_2O (3:1, 30 mL), stirred at 60 °C for 1 h, and then concentrated in vacuo again. Flash chromatography (3:1 hexane/EtOAc) afforded the title product (2.11 g, 84% from **6**). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.210–7.318 (12 H, m, Ph-H), 6.706 (2 H, d, J = 8 Hz, Ph-H of PMB), 4.817 (2 H, AB, J = 11 Hz, $\Delta\nu$ = 168 Hz, PhC-H), 4.552 (2 H, s, PhC-H), 4.467 (2 H, s, PhC-H), 4.410 (1 H, d, J = 7.5 Hz, H-4), 4.030–4.093 (1 H, m, ethylene), 3.917 (1 H, brs), 3.512–3.747 (9 H, m), 2.856 (2 H, brs). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 138.4, 137.7, 130.1, 129.2, 128.3, 128.1, 17.7, 127.6, 113.6, 103.7, 78.9, 74.4, 73.5, 73.2, 73.0, 72.7, 69.2, 68.9, 68.8, 55.12. HRMS: calcd for $C_{22}H_{28}O_7Cs$ ($M + Cs^+$) 537.0889, found 537.0875.

Compound 8. Compound **7** (2 g, 3.8 mmol) was refluxed in toluene with dibutyltin oxide (1.13 g, 1.2 equiv) in a Dean–Stark apparatus for 5 h. Ethyl 2-bromoacetate (1.9 equiv) and

(15) DeFrees, S. A.; Kosch, W.; Way, W.; Paulson, J. C.; Sabesan, S.; Halcomb, R.; Huang, D.-H.; Ichikawa, Y.; Wong, C. H. *J. Am. Chem. Soc.* **1995**, *117*, 66. The assay for cell adhesion inhibition was conducted at Sandoz. Details will be published elsewhere. Le^x-3′-sulfate was reported to be as active as SLe^x (see ref 8). It is, however, less active on the basis of this assay.

(16) Uchiyama, T.; Vassilev, V. P.; Kajimoto, T.; Wong, W.; Huang, H.; Lin, C.-C.; Wong, C.-H. *J. Am. Chem. Soc.*, in press.

Bu_4NI (0.5 equiv) were then added at 40 °C. The mixture was allowed to react for 2 h at 130 °C and then concentrated and purified by flash chromatography (5:1 to 3:1 toluene/EtOAc) to yield **8** (1.75 g, 81.6%) as a white powder. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.266–7.354 (12 H, m, Ph-H), 6.842 (2 H, d, J = 8.5 Hz, Ph-H), 4.821 (2 H, AB, J = 12 Hz, $\Delta\nu$ = 6.5 Hz, PhC-H), 4.717 (1 H, d, J = 4 Hz, H-4), 4.543 (2 H, AB, J = 12 Hz, $\Delta\nu$ = 13 Hz, PhC-H), 4.506 (2 H, s, PhC-H), 4.485 (1 H, d, J = 7.5 Hz, H-1), 3.860 (2 H, AB, J = 18 Hz, $\Delta\nu$ = 285 Hz, OCH_2CO_2), 4.027–4.067 (1 H, m, ethylene), 3.875 (1 H, dd, J = 4, 10 Hz, H-3), 3.770 (3 H, s, OCH_3), 3.556–3.814 (7 H, m, ethylene, H-2,5,6a,6b). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 166.5, 159.1, 137.6, 130.1, 129.3, 128.7, 128.4, 128.1, 127.8, 127.7, 113.7, 104.0, 74.3, 73.7, 73.6, 72.9, 71.8, 71.7, 71.5, 69.1, 68.9, 67.0, 60.1, 55.2. HRMS: calcd for $C_{32}H_{36}O_9Cs$ (M + Cs^+) 697.1414, found 697.1431.

2-[(4-Methoxybenzyl)oxy]ethyl 2,6-Di-*O*-benzyl-3-[(ethoxycarbonyl)methyl]-β-D-galactopyranoside (8a) and Compound 8. Compound **7** (1.1605 g, 2.215 mmol) was refluxed overnight in benzene with dibutyltin oxide (660 mg, 1.2 equiv) in a Dean–Stark apparatus. Ethyl 2-bromoacetate (485 μL, 2 equiv) and Bu_4NI (803 mg, 1 equiv) were then added at 40 °C. The reaction was allowed to proceed for 6 h at 90 °C, and the mixture was concentrated and purified by flash chromatography (4:1 to 3:1 toluene/EtOAc) to yield pure **8a** (527 mg, 39%) and **8** (567 mg, 42%).

8a. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.219–7.338 (12 H, m, Ph-H), 6.810–6.827 (2H, m, Ph-H), 4.807 (2 H, AB, J = 11 Hz, $\Delta\nu$ = 140.5 Hz, PhC-H), 4.577 (2 H, AB, J = 14 Hz, $\Delta\nu$ = 8.4 Hz, PhC-H), 4.480 (2 H, AB, J = 12 Hz, $\Delta\nu$ = 8.1 Hz, PhC-H), 4.407 (1 H, d, J = 6.5Hz, H-1), 4.308 (2 H, AB, J = 15.7 Hz, $\Delta\nu$ = 122 Hz, OCH_2CO_2), 4.166–4.200 (2H, m, OCH_2Me), 4.044–4.051 (2H, m, H-4, ethylene), 3.770 (3 H, s, OCH_3), 3.658–3.826 (6 H, m, ethylene, H-2,6a,6b), 3.381 (1 H, dd, J = 3, 9 Hz, H-3), 3.318–3.323 (1 H, m, H-5), 1.257 (3 H, t, J = 7 Hz, CH_3 of ethyl). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 171.5, 158.9, 138.5, 138.1, 130.7, 129.3, 128.4, 128.2, 128.1, 127.8, 127.7, 127.5, 113.7, 103.7, 83.4, 78.8, 75.0, 73.7, 73.1, 72.8, 69.3, 69.0, 68.8, 67.2, 61.2, 55.2. HRMS: calcd for $C_{34}H_{42}O_{10}Cs$ (M + Cs^+) 743.1832, found 743.1839.

Compound 9. Compound **8** (300 mg, 0.53 mmol) was dissolved in a mixture of CH_2Cl_2 (4.5 mL) and water (0.25 mL), and DDQ (144.8 mg, 1.2 equiv) was added. After the mixture stirred for 2.5 h at rt, the reaction was quenched with saturated aqueous $NaHCO_3$. The mixture was then diluted with CH_2Cl_2, and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine and then dried over $MgSO_4$. The concentrated crude product was purified by flash chromatography (2:1 to 1:1 hexane/EtOAc) to yield pure **9** (207 mg, 88%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.296–7.375 (10 H, m, Ph-H), 4.810 (2 H, AB, J = 12 Hz, $\Delta\nu$ = 49 Hz, PhC-H), 4.698 (1 H, dd, J = 1, 4 Hz, H-4), 4.558 (2 H, AB, J = 12 Hz, $\Delta\nu$ = 18.5 Hz, PhC-H), 4.557 (1 H, d, J = 7.5Hz, H-1), 3.894 (2 H, AB, J = 18 Hz, $\Delta\nu$ = 272 Hz, OCH_2CO_2), 3.603–3.972 (8 H, m, ethylene, H-2,3,5,6a,6b). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 166.4, 137.3, 128.6, 128.5, 128.5, 128.3, 128.0, 127.9, 104.4, 74.3, 74.1, 73.7, 73.5, 72.3, 72.0, 71.7, 67.1, 62.3, 60.1. HRMS: calcd for $C_{24}H_{28}O_8Cs$ (M + Cs^+) 577.0839, found 577.0839.

2,3,4-Tri-*O*-benzyl-5-deoxy-L-galactopyranosyl Fluoride (11). Compound **10** (1.788 g, 4.12 mmol) was dissolved in 30 mL of CH_2Cl_2, followed by the dropwise addition of a solution of DAST (600 μL, 1.1 equiv) in CH_2Cl_2 (10 mL). The solution was stirred at rt for 30 min before the reaction was quenched by the addition of water. The mixture was then diluted with CH_2Cl_2 and washed successively with water and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Flash chromatography (8:1 hexane/EtOAc) of the residue afforded the title compound (1.55 g) as a mixture of α (51.2%) and β (34.1%) anomers. The NMR and HRMS data agree with those reported in literature.[12]

Compound 12. In a reaction flask, **9** (163 mg, 0.367 mmol) and **11** (293.5 mg, 0.519 mmol) were mixed, completely dried, and dissolved in anhydrous ether (5 mL) and CH_2Cl_2 (0.9 mL). The mixture was then stirred with 4 Å molecular sieves and 1,1,3,3-tetramethylurea (1 equiv) under argon at rt for 15 min. The temperature was lowered to −78 °C, and $AgClO_4$ (3 equiv) and $SnCl_2$ (3 equiv) were quickly added. The reaction bath temperature was then allowed to warm up to rt gradually overnight. The mixture was then filtered through Celite 545 and diluted with chloroform. The organic layer was successively washed with 0.1 N H_2SO_4, saturated aqueous $NaHCO_3$, water, and brine and dried over $MgSO_4$. The crude mixture was concentrated and purified by flash chromatography (5:1 toluene/EtOAc) to afford the title compound (239 mg, 76% based on **9**) as a 3.26:1 mixture of the α and β anomers. This mixture of anomers was further separated by HPLC (silica gel; 4:1 hexane/EtOAc) to afford pure α anomer **12**. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.245–7.355 (25 H, m, Ph-H), 4.951 (1 H, d, J = 11.5 Hz, PhC-H), 4.847 (1 H, d, J = 4 Hz, H-1 of Fuc), 4.822 (1 H, d, J = 11.5 Hz, PhC-H), 4.781 (1 H, d, J = 12 Hz, PhC-H), 4.773 (1 H, d, J = 11.5 Hz, PhC-H), 4.686 (1 H, d, J = 12 Hz, PhC-H), 4.604–4.666 (3 H, m, PhC-H), 4.609 (1 H, d, J = 4.5 Hz, H-4 of Gal), 4.541 (2 H, s, PhC-H), 4.519 (1 H, d, J = 7.5 Hz, H-1 of Gal), 4.111 (1 H, d, J = 18 Hz, C(O)-CH_2O), 4.021–4.063 (1 H, m, ethylene), 4.017 (1 H, dd, J = 4, 10 Hz, H-2 of Fuc), 3.859–3.922 (2 H, m, H-3,5 of Fuc), 3.808–3.872 (2 H, m, ethylene), 3.666–3.773 (5 H, m, H-3,5,6a,6b of Gal, ethylene), 3.555 (1 H, brd, J = 2 Hz, H-4 of Fuc), 3.512 (1 H, dd, J = 7.5, 10 Hz, H-2 of Gal), 3.508 (1 H, d, J = 18 Hz, $C(O)CH_2O$), 1.082 (3 H, d, J = 6.5 Hz, CH_3 of Fuc). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 166.6, 138.9, 138.6, 138.5, 137.7, 128.6, 128.4, 128.3, 128.2, 128.1, 127.9, 127.9, 127.8, 127.6, 127.6, 127.4, 127.3, 127.2, 103.3, 98.10, 79.28, 77.49, 76.35, 74.80, 74.43, 73.70, 73.29, 73.03, 71.83, 71.42, 68.26, 67.48, 67.14, 66.30, 60.04, 16.70. HRMS: calcd for $C_{51}H_{56}O_{12}Cs$ (M + Cs^+) 993.2826, found 993.2850.

Compound 1. Compound **12** (18 mg, 20.9 μmol) was dissolved in methanol (5 mL), and then a catalytic amount of $Pd(OH)_2$ on carbon was added. Hydrogen was supplied to the reaction system through a balloon. After the reaction was complete in 1 h, the mixture was filtered through Celite 545 and concentrated in vacuo to give a mixture of ester **13** and **14**, which was then dissolved in 0.1 N NaOH (4 mL) and stirred for 3 h. The crude products were purified on biogel P-2 chromatography using water as eluent. The collected fractions were combined and lyophilized to afford 6 mg (96% from **12**) of the title compound as a white powder. ^{1}H-NMR (500 MHz, D_2O): δ 4.774 (1 H, d, J = 3.5 Hz, H-1 of Fuc), 4.323 (1 H, d, J = 8 Hz, H-1 of Gal), 3.992 (1 H, t, J = 6.5 Hz, H-5 of Fuc), 3.935 (1 H, d, J = 3 Hz, H-4 of Gal), 3.913–4.011 (3 H, m, OCH_2CO_2, ethylene), 3.710–3.760 (3 H, m, H-3 of Fuc, ethylene), 3.645 (1 H, d, J = 3.5 Hz, H-4 of Fuc), 3.513–3.664 (5 H, m, H-2 of Fuc, H-5,6a,6b of Gal, ethylene), 3.478 (1 H, dd, J = 8, 10 Hz, H-2 of Gal), 3.332 (1 H, dd, J = 3, 10 Hz, H-3 of Gal), 1.057 (3 H, d, J = 6.5 Hz, CH_3 of Fuc). ^{13}C-NMR (125 MHz, D_2O): δ 173.9, 112.5, 99.4, 82.8, 75.7, 72.6, 70.3, 70.3, 69.5, 69.1, 68.8, 67.8, 67.4, 66.0, 61.8, 16.0. HRMS: calcd for $C_{16}H_{28}O_{13}Na$ (M + H^+) 451.1428, found 451.1440.

Compound 16. In a reaction flask, **15** (286 mg, 0.730 mmol) and **11** (336 mg, 0.771 mmol) were mixed, completely dried, and dissolved in anhydrous ether (10 mL). The mixture was then stirred with 4 Å molecular sieves and 1,1,3,3-tetramethylurea (1 equiv) under argon at rt for 30 min. The mixture was cooled to −43 °C, and $AgClO_4$ (3 equiv) and $SnCl_2$ (3 equiv) were quickly added. The reaction bath temperature was then allowed to warm up to rt gradually overnight, and the mixture was filtered through Celite 545 and diluted with chloroform. The organic layer was successively washed with 0.1 N H_2SO_4, saturated aqueous $NaHCO_3$, water, and brine and dried over $MgSO_4$. The crude mixture was concentrated and purified by flash chromatography (2:1 hexane/EtOAc) to afford the title compound (420 mg, 71.2% based on **15**) as a 2.9:1 mixture of the α and β anomers. α anomer. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.263–7.406 (15 H, m, Ph-H), 5.343 (1 H, d, J = 3 Hz, H-4 of Gal), 5.324 (1 H, d, J = 2.5 Hz, H-4 of Gal), 5.183 (1 H, dd, J = 8, 10.5 Hz, H-2 of Gal), 4.967 (1 H, dd, J = 3, 10.5 Hz, H-3 of Gal), 4.798 (1 H, d, J = 4 Hz, H-1 of Fuc), 4.640–4.990 (6 H, m, PhC-H), 4.076–4.160 (2 H, m, H-6a,6b of Gal), 3.963–4.043 (2 H, m, H-2 of Fuc, ethylene), 3.924 (1 H, dd, J = 3, 10 Hz, H-3 of Fuc), 3.875 (1 H, q, J = 6.5 Hz, H-5 of Fuc), 3.805–3.831 (1 H, m, H-5 of Gal), 3.711–3.794 (2 H, m, ethylene), 3.641–3.674 (2 H, m, H-4 of Fuc,

ethylene), 2.126 (3 H, s, CH_3 of acetyl), 2.035 (3 H, s, CH_3 of acetyl), 2.013 (3 H, s, CH_3 of acetyl), 1.978 (3 H, s, CH_3 of acetyl), 1.103 (3 H, d, J = 6.5 Hz, CH_3 of Fuc). The 2.9:1 mixture of the α and β anomers. ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.3, 169.4, 138.6, 138.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 127.7, 127.6, 127.5, 127.5, 127.4, 101.5, 101.0, 97.8, 82.4, 79.4, 79.3, 77.6, 77.3, 77.3, 77.2, 77.1, 77.1, 76.2, 74.8, 74.6, 73.3, 73.1, 70.9, 70.6, 70.3, 68.8, 68.3, 67.0, 66.8, 66.3, 61.2, 20.7, 20.7, 20.6, 16.6. HRMS: calcd for $C_{43}H_{52}O_{15}Cs$ (M + Cs^+) 941.2361, found 941.2365.

Compound 18. Benzyl-protected amine **17** (800 mg, 4.15 mmol) was dissolved in methanol (7 mL), followed by addition of HOAc (5 mL) and $Pd(OH)_2C$ (300 mg). The mixture was then exposed to H_2 (40 psi) for 24 h until the debenzylation was complete as observed by ^{1}H-NMR analysis. The mixture was then removed from the hydrogenator and filtered through Celite 545. The filtrate was concentrated and then dissolved in dioxane (10 mL). The solution was cooled to 0 °, and aqueous Na_2CO_3 (6%, 10 mL) was added dropwise to give a solution of pH 10. CBZCl (280 mL) was added portionwise to the reaction mixture. More aqueous Na_2CO_3 (ca. 5 mL) was added during the addition of CBZCl to maintain the solution around pH 9. The mixture was stirred for 30 min at 0 °C and then warmed up to rt and stirred for another 30 min. The solution was concentrated and then diluted with EtOAc, dried over $MgSO_4$, filtered, and concentrated again. Flash chromatography (1:1 hexane/EtOAc → 100%, EtOAc) afforded the title compound **18** (777 mg, 79%) as a colorless syrup. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.27–7.34 (5 H, m, Ph-H), 5.11 (2 H, s, PhC-H), 4.07–4.16 (2 H, brm, C*H*(OH) × 2), 3.68 (2 H, dd, J = 4.5, 12 Hz, C*H*(H)N × 2), 3.40 (2 H, dd, J = 14, 12 Hz, C*H*(H)N × 2), 3.06 (1 H, brs, OH), 2.86 (1 H, brs, OH). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 155.4, 136.5, 128.5, 128.1, 127.9, 75.4, 74.8, 67.1, 51.9, 51.6. HRMS: calcd for $C_{12}H_{16}NO_4$ (M + H^+) 238.1079, found 238.1085.

Compound 19. Diol **18** (267 mg, 1.13 mmol) was refluxed in toluene (ca. 30 mL) with dibutyltin oxide (310 mg, 1.1 equiv) in a Dean–Stark apparatus for 5 h. Freshly prepared PMBBr (ca. 1.5 equiv) and Bu_4NI (0.5 equiv) were then added at 35 °C. The mixture was allowed to react for 12 h at 80 °C and then the reaction was stopped by evaporation of the solvent in vacuo. The concentrated mixture was diluted with chloroform, washed successively with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated. The product was purified by flash chromatography (3:1 to 1:1 hexane/EtOAc) to yield **19** (62%) as colorless oil. A small amount of **19** was acetylated (Ac_2O/pyridine/DMAP) to give **19a** for further characterization. According to the NMR spectra, compound **19** contained rotamers resulting from the CBZ group. This was confirmed by converting **19a** to **19b**, which was shown to be a single compound.

19. ^{1}H-NMR (500 MHz, $CDCl_3$, 20 °C): δ 7.260–7.351 (5 H, m, Ph-H), 7.227 (2 H, dd, J = 2.5, 8.5 Hz, Ph-H), 6.872 (2 H, d, J = 8.5 Hz, Ph-H), 5.093–5.126 (2 H, m, PhC-H), 4.419–4.514 (2 H, m, PhC-H), 4.254 (1 H, brs, C*H*(OH)), 3.895 (1 H, dt, J = 13, 2 Hz, C*H*(OPMB)), 3.784 (3 H, s, CH_3 of PMB), 3.413–3.657 (4 H, m, CH_2N × 2). ^{13}C-NMR (125 MHz, $CDCl_3$, 20 °C): δ 159.2, 155.2, 136.5, 136.5, 129.6, 129.5, 129.3, 129.3, 128.4, 127.9, 127.7, 113.7, 81.84, 81.19, 73.44, 72.40, 71.02, 66.94, 66.87, 55.20, 52.22, 51.81, 49.36, 49.26. HRMS: calcd for $C_{20}H_{23}NO_5Na$ (M + Na^+) 380.1474, found 380.1463.

19a. ^{1}H-NMR (500 MHz, DMSO, 80 °C): δ 7.276–7.357 (5 H, m, Ph-H), 7.213–7.257 (2 H, m, Ph-H), 6.883–6.901 (2 H, m, Ph-H), 5.130 (1 H, d, J = 5 Hz, C*H*(OAc)), 5.086 (2 H, s, PhC-H), 4.053 (1 H, brm, C*H*(OPMB)), 3.752 (3 H, s, CH_3), 3.665 (1 H, dd, J = 5, 12.5 Hz, C*H*(H)N), 3.533 (1 H, dd, J = 4.5, 12 Hz, C*H*(H)N), 3.460 (1 H, d, J = 12 Hz, C*H*(H)N), 3.401 (1 H, d, J = 12.5 Hz, C*H*(H)N), 2.011 (3 H, s, Ac). ^{13}C-NMR (125 MHz, DMSO, 80 °C): δ 169.1, 158.7, 153.7, 136.6, 129.5, 128.7, 127.9, 127.3, 127.0, 113.5, 69.90, 65.70, 54.80, 49.42, 49.18, 20.17.

19b (prepared from **19a** by treating with H_2 catalyzed by $Pd(OH)_2$–C in acetone). ^{1}H-NMR (500 MHz, $CDCl_3$, 20 °C): δ 7.248–7.265 (2 H, m, Ph-H), 6.859–6.877 (2 H, m, Ph-H), 5.108 (1 H, dt, J = 6, 2 Hz, CH(OAc)), 4.514 (2 H, AB, J = 12.5 Hz, Δν = 55 Hz, PhC-H), 4.050 (1 H, dt, J = 2, 6 Hz, C*H*(OPMB)), 3.799 (3 H, s, CH_3 of PMB), 3.194 (1 H, dd, J = 6.5, 10 Hz, C*H*(H)N), 2.874 (1 H, dd, J = 6.5, 11 Hz, C*H*(H)N), 2.783 (1 H, dd, J = 2, 11 Hz, C*H*(H)N), 2.370–2.416 (2 H, m, C*H*(H)N and $CH(CH_3)_2$), 1.086 (3 H, d, J = 6 Hz, $CHCH_3$), 1.058 (3 H, d, J = 6 Hz, $CHCH_3$). ^{13}C-NMR (125 MHz, $CDCl_3$, 20 °C): δ 170.7, 159.2, 129.9, 129.5, 113.7, 82.31, 78.27, 77.25, 76.99, 76.74, 71.33, 56.72, 56.66, 55.28, 55.24, 54.63, 21.23, 21.13, 20.88.

Compound 21. To alcohol **19** (344 mg, 0.96 mmol) in DMF (10 mL) was added NaH (120 mg, 60% suspension in mineral oil) at 0 °C. The reaction mixture was gradually warmed up to rt over a period of 2 h under argon. Methyl 6-iodohexanoate **20** (500 μL) and Bu_4NI (1.1 equiv) were then added at 0 °C, and then the reaction temperature was raised to rt. After 3 h, the reaction was quenched by HOAc at 0 °C. The mixture was diluted with EtOAc, washed successively with water, saturated aqueous $NaHCO_3$, and brine, and then dried over $MgSO_4$. The solution was concentrated and purified by flash chromatography (3:1 hexane/EtOAc) to yield compound **21** (273 mg). ^{1}H-NMR (500 MHz, DMSO, 80 °C): δ 7.29–7.36 (5 H, m, Ph of CBZ), 7.225–7.242 (2 H, m, Ph-H of PMB), 6.883–6.901 (2 H, m, Ph-H of PMB), 5.074 (2 H, s, CH_2 of CBZ), 4.486 (2 H, s, CH_2 of PMB), 3.978–3.983 (1 H, m, pyrrolidine ring), 3.928–3.937 (1 H, m, pyrrolidine ring), 3.752 (3 H, s, $CH_3OC(O)CH_2$), 3.581 (3 H, s, CH_3 of PMB), 3.355–3.485 (6 H, m, 4 H of pyrrolidine ring and 2 H of OCH_2CH_2), 2.265 (2 H, t, J = 7.5 Hz, $OC(O)CH_2CH_2$), 1.444–1.555 (4 H, m, $OC(O)CH_2CH_2CH_2$ and $CH_2CH_2CH_2O$), 1.279–1.325 (2 H, m, $OC(O)CH_2CH_2CH_2CH_2CH_2CH_2$). ^{13}C-NMR (125 MHz, DMSO, 80 °C): δ 172.6, 159.6, 153.9, 136.7, 129.8, 128.6, 127.8, 127.2, 126.9, 113.4, 69.77, 68.07, 65.49, 54.76, 50.44, 49.26, 49.26, 32.88, 28.41, 24.67, 23.73. HRMS: calcd for $C_{27}H_{35}NO_7Cs$ (M + Cs^+) 618.1468, found 618.1460.

Compound 22. Compound **21** (126 mg, 0.26 mmol) was dissolved in a mixture of CH_2Cl_2 (3.6 mL) and water (0.2 mL), and DDQ (80 mg, 1.2 equiv) was added. After stirring for 3 h at rt, the reaction was quenched by saturated aqueous $NaHCO_3$. The mixture was then diluted with CH_2Cl_2, and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine and then dried over $MgSO_4$. The solution was concentrated and purified by flash chromatography (2:1 to 1:1 hexane/EtOAc) to yield the title compound (98.5 mg, 83%). ^{1}H-NMR (500 MHz, DMSO, 80 °C): δ 7.276–7.357 (5 H, m, Ph-H), 5.073 (2 H, s, PhC-H), 4.948 (1 H, d, J = 3.5 Hz, OH), 4.068 (1 H, brs, C*H*(OH)), 3.731 (1 H, brt, $CH(OCH_2)$), 3.583 (3 H, s, CH_3), 3.499 (1 H, dd, J = 4.5, 11.5 Hz, $NCH(H)CH(OCH_2)$), 3.399–3.474 (3 H, m, $NCH(H)CH(OH)$ and OCH_2CH_2), 3.319 (1 H, d, J = 11.5 Hz, $NCH(H)CH(OCH_2)$), 3.247 (1 H, d, J = 11.5 Hz, $NCH(H)CH(OH)$), 2.270 (2 h, t, d = 7.5 Hz, $CH_3O_2CCH_2CH_2$), 1.516–1.576 (2 H, m, $CH_3O_2CCH_2CH_2CH_2$), 1.453–1.509 (2 H, m, CH_2CH_2O), 1.276–1.336 (2 H, m, $CH_2CH_2CH_2CH_2CH_2$). ^{13}C-NMR (125 MHz, DMSO, 80 °C): δ 172.9, 153.9, 136.8, 127.8, 127.2, 126.9, 67.95, 65.36, 51.72, 50.44, 49.04, 32.87, 28.46, 24.68, 23.74. HRMS: calcd for $C_{19}H_{27}NO_6Na$ (M + Na^+) 388.1735, found 388.1739.

Compound 23. Compound **22** (113 mg, 0.31 mmol) and **11** (300 mg, 0.53 mmol) were mixed, completely dried, and dissolved in anhydrous ether (10 mL). The mixture was then stirred with 4 Å molecular sieves and 1,1,3,3-tetramethylurea (1 equiv) under argon at rt for 1 h. The temperature was lowered to −78 °C, and $AgClO_4$ (3 equiv) and $SnCl_2$ (3 equiv) were quickly added. The reaction bath temperature was then allowed to warm up to rt gradually overnight. The mixture was then filtered through Celite 545 and diluted with CH_2Cl_2. The organic layer was successively washed with 0.1 N HCl, saturated aqueous $NaHCO_3$, water, and brine and dried over $MgSO_4$. The crude mixture was concentrated and purified by flash chromatography (5:1 to 2:1 hexane/EtOAc) to afford the title compound **23** (155 mg of 64% based on **22**) as the α anomer only. ^{1}H-NMR (500 MHz, DMSO, 90 °C): δ 7.248–7.359 (20 H, m, Ph-H), 5.067 (2 H, s, CH_2 of CBZ), 5.024 (1 H, d, J = 3 Hz, H-1 of Fuc), 4.711 (2 H, AB, J = 11.5 Hz, Δν = 130.2 Hz, CH_2 of Bn), 4.717 (2 H, AB, J = 12 Hz, Δν = 9 Hz, CH_2 of Bn), 4.619 (2 H, AB, J = 11.4 Hz, Δν = 11.8 Hz, CH_2 of Bn), 4.131–4.140 (1 H, m, pyrrolidine ring), 3.919–3.932 (2H, m, H-5 of Fuc, and H-1 of pyrrolidine ring), 3.828–3.851

(3 H, brm, H-2,3,4 of Fuc), 3.580 (3 H, s, $CH_3OC(O)CH_2$), 3.363–3.542 (6 H, m, 4 H of pyrrolidine ring and 2 H of OCH_2CH_2), 2.270 (2 H, t, J = 7.5 Hz, $OC(O)CH_2CH_2$), 1.482–1.568 (4 H, m, $OC(O)CH_2CH_2CH_2$ and $CH_2CH_2CH_2O$), 1.298–1.329 (2 H, m, $OC(O)CH_2CH_2CH_2CH_2CH_2$), 1.135 (2 H, d, J = 6.5 Hz, CH_3 of Fuc). ^{13}C-NMR (125 MHz, DMSO, 90 °C): δ 170.0, 153.8, 138.5, 127.7, 127.5, 127.5, 127.1, 127.0, 126.8, 126.6, 126.6, 126.6, 95.9, 77.77, 77.47, 75.14, 73.85, 71.37, 71.03, 68.11, 66.09, 65.48, 49.34, 49.08, 32.82, 28.31, 24.59, 23.65, 15.79. HRMS: calcd for $C_{46}H_{55}NO_{10}Cs$ ($M + Cs^+$) 914.2880, found 914.2841.

Compound 2. Compound **23** (119 mg, 0,15 mmol) was dissolved in MeOH (2 mL) and HOAc (1 mL), and a catalytic amount of $Pd(OH)_2$–C was added before the solution was exposed to H_2 (45 psi) for 15 h. The mixture was then filtered through Celite 545 to remove $Pd(OH)_2$–C. The filtrate was concentrated and treated with 0.1 N NaOH (10 mL) for 5 h. The solution was lyophilized and purified by biogel P-2 chromatography (H_2O) to give the title compound. ^{1}H NMR (500 MHz, D_2O): δ 4.955 (1 H, d, J = 3 Hz, H-1 Fuc), 4.39–4.45 (1 H, brs, pyrrolidine ring), 4.16–4.21 (1 H, brm, pyrrolidine ring), 4.002 (1 H, q, J = 7 Hz, H-5 of Fuc), 3.70–3.79 (3 H, m, H-2,3,4 of Fuc), 3.35–3.60 (4 H, m, 2 H of pyrrolidine ring and 2 H of OCH_2CH_2), 2.85–2.91 (2 H, m, pyrrolidine ring), 2.108 (2 H, t, J = 7.5 Hz, $OC(O)CH_2CH_2$), 1.481–1.550 (4 H, m, $OC(O)CH_2CH_2CH_2$ and $CH_2CH_2CH_2O$), 1.258–1.289 (2 H, m, $OC(O)CH_2CH_2CH_2CH_2CH_2$), 1.161 (2 H, d, J = 6.5 Hz, CH_3 of Fuc). ^{13}C-NMR (125 MHz, D_2O): δ 169.9, 84.2, 67.4, 64.4, 57.6, 55.8, 55.2, 53.6, 53.5, 45.2, 44.9, 28.2, 23.4, 14.4, 11.5, 11.1. HRMS: calcd for $C_{16}H_{29}NO_8Na$ ($M + H^+$) 386.1791, found 386.1783.

Biological Activity Assay. The activity of **1** and **2** were evaluated using an ELISA assay as described previously.[14] The activity of compound **1** was concentration dependent with a 50% inhibition at 1.5 mM, while the IC_{50} for **2** is 10 mM. In comparison, SLe^x and Le^x-3′-*O*-sulfate inhibit the binding with IC_{50}'s of 0.8 and 2 mM, respectively, under the same conditions.

To test the stability of compounds **1** against bovine kidney α-L-fucosidase (EC 3.2.1.51, from Sigma), the compound (400 μL, 1 mM) was incubated with the enzyme (0.5 unit) in a 50 mM sodium acetate buffer solution (pH 5.5) at 25 °C for 12 h. No L-fucose was detected by thin layer chromatography (solvent system: 4:2:1, EtOAc/HOAc/H_2O). In another study, compound **1** (2 mM) in a sodium acetate buffer (50 mM, pH 5.5) was stored at 4 °C for a month. No decomposition was detected by thin layer chromatography.

Acknowledgment. This research was partially supported by the NSF (CHE-9310081). We thank Dr. R. L. Halcomb for his many helpful suggestions. We also acknowledge Dr. D.-H. Huang for his help with the NMR analysis and Dr. G. Siuzdak for the high-resolution mass analysis.

Supplementary Material Available: NMR spectra for compounds **1–3**, **5–9**, **12**, **16**, **18**, **19**, **19a,b**, and **21–23** (17 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO941876P