Pergamon

Bioorganic & Medicinal Chemistry, Vol. 4, No. 8, pp 1167–1176, 1996

PII: S0968-0896(96)00105-8
0968-0896/96 $15.00 + 0.00

A Highly Practical Synthesis of the Sialyl Lewis X Pentasaccharide and an Investigation of Binding to E-, P-, and L-Selectins

Takao Kiyoi,[a] Yoshiyuki Nakai,[a] Hirosato Kondo,[a,*] Hideharu Ishida,[b] Makoto Kiso[b] and Akira Hasegawa[b]

[a]*Department of Medicinal Chemistry, Kanebo Ltd, New Drug Research Laboratories, 5-90 Tomobuchi-Cho, Miyakojima-Ku, Osaka 534, Japan*

[b]*Department of Applied Bioorganic Chemistry, Gifu University, Gifu 501-11, Japan*

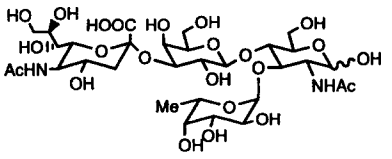
Abstract—A practical synthesis of the sialyl Lewis X (sLe^x) pentasaccharide, NeuAcα2–3Galβ1–4(Fucα1–3)GlcNAcβ1–3GalβOEt (**1**), as a potential blocker for E-selectin has been described. The glycosylation of a trisaccharide acceptor, Fucα(1–3)GlcNAcβ(1–3)GalβOEt, with a disaccharide donor, NeuAcα(2–3)GalβSMe, did not yield the desired sLe^x pentasaccharide **1** at all. However, the glycosylation of a disaccharide acceptor, GlcNAcβ(1–3)GalβOEt, with a disaccharide donor, NeuAcα(2–3)GalβSMe, quantitatively yielded the tetrasaccharide NeuAcα(2–3)Galβ(1–4)GlcNAcβ(1–3)GalβOEt. This tetrasaccharide is readily converted to the title compound in a high yield by fucosylation, followed by deprotection. The inhibitory activities of compound **1** toward the binding of the natural ligand (sLe^x) with the E-, P-, and L-selectins were stronger than those of the sLe^x tetrasaccharide. Copyright © 1996 Elsevier Science Ltd

Introduction

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 between leukocyte homing, platelet binding, and neutrophil extravasation.[2] Recent studies 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 an important role in the migration of inflammatory cells from the blood stream to inflammatory sites. Among the selectins, ELAM-1 is expressed in response to cytokines released by tissue injury.[4] GMP-140 is an adhesion molecule expressed on platelets and vascular endothelial cells, and LECAM-1 is an adhesion molecule expressed on leukocytes. These selectins are believed to be involved in a complicated manner in the progress of the clinical manifestations of disorders such as acute and chronic inflammation.[5]

Phillips et al. reported that the native ligand for ELAM-1 is the tetrasaccharide sialyl Lewis X antigenic determinant (sLe^x).[6] Recently, it has been reported that both P- and L-selectin also recognize the sLe^x structure.[7] Since then, the synthesis of sLe^x oligosaccharides has attracted the interest of organic chemists because of the low abundance of sLe^x from natural sources. The total synthesis of sLe^x has been reported by several groups.[8] However, chemical approaches toward sLe^x production were mainly limited to the synthesis of the tetrasaccharide NeuAcα2–3Galβ1–4(Fucα1–3)GlcNAc, except for our studies[9] of the synthesis of sLe^x penta- and hexasaccharide ceramides.

On the other hand, recent studies indicated that the sLe^x pentasaccharide (**1**, Chart 1) inhibited E-selectin-mediated inflammation in vivo[10] and could be a candidate for a new type of anti-inflammatory agent. Compound **1** has been synthesized by chemoenzymatic and enzymatic approaches,[11] however, it is not easy to prepare compound **1** because the key enzyme used for



sLe^x

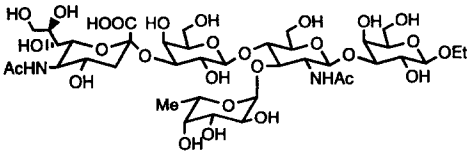


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Chart 1.

the above chemoenzymatic and enzymatic methods is not commercially available. Additionally, a major disadvantage of the enzymatic methods would be that the narrow substrate specificity of the glycosyltransferases impedes the synthesis of various modified oligosaccharides.[11] Therefore, we sought to develop a practical synthesis of sLe^x pentasaccharide by a chemical method. Our practical chemical synthesis of sLe^x has some advantages compared with the enzymatic method, in view of applications to the synthesis of several sLe^x derivatives, and so on. We describe herein a highly practical synthesis of the sLe^x pentasaccharide **1** and its inhibitory activities toward E-, P-, and L-selectin binding.

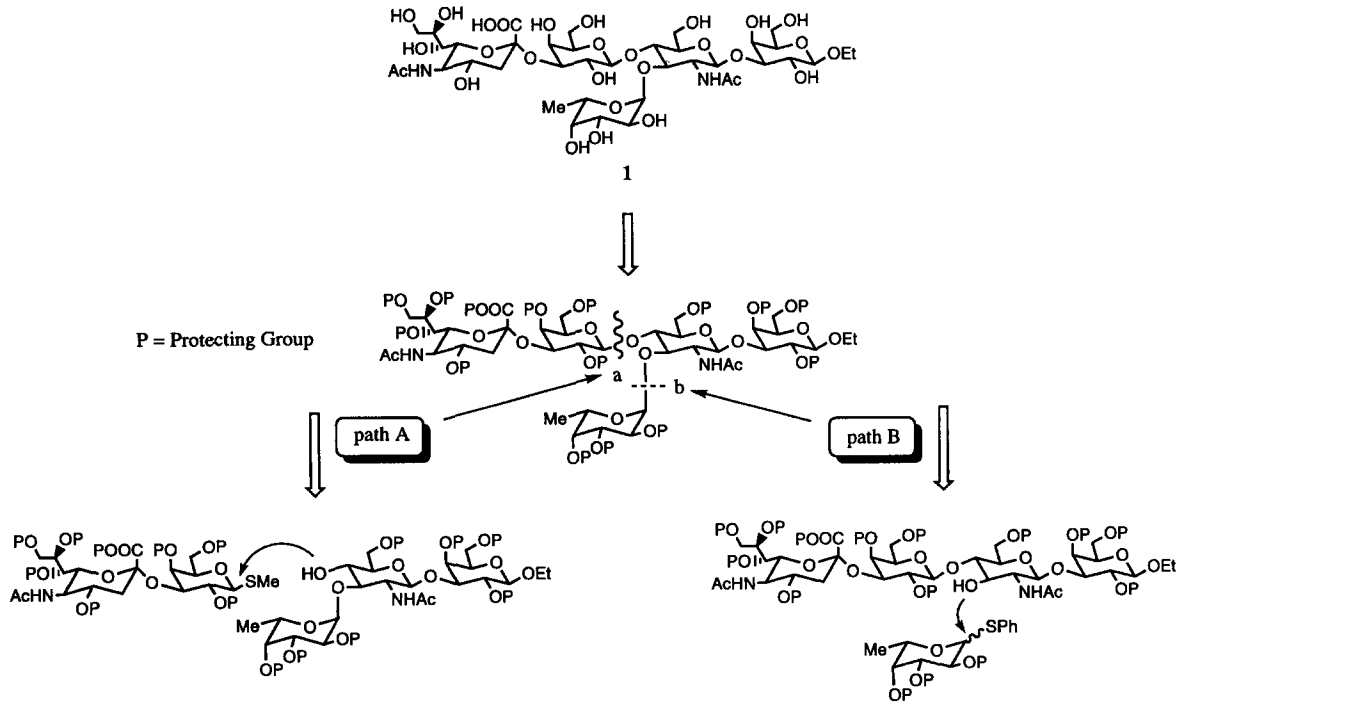
Synthetic Strategy and Results

Our interest was to establish a rational approach toward the sLe^x pentasaccharide synthesis. Our objective is to construct a portion of the sLe^x pentasaccharide by the glycosylation of a trisaccharide acceptor with a disaccharide donor (path A), and a key tetrasaccharide precursor of the sLe^x pentasaccharide is produced by the glycosylation of a disaccharide acceptor with a disaccharide donor, followed by incorporation of fucose at the C-3 hydroxyl group of GlcNAc (path B). Our synthetic strategy for the sLe^x pentasaccharide is illustrated in Scheme 1.

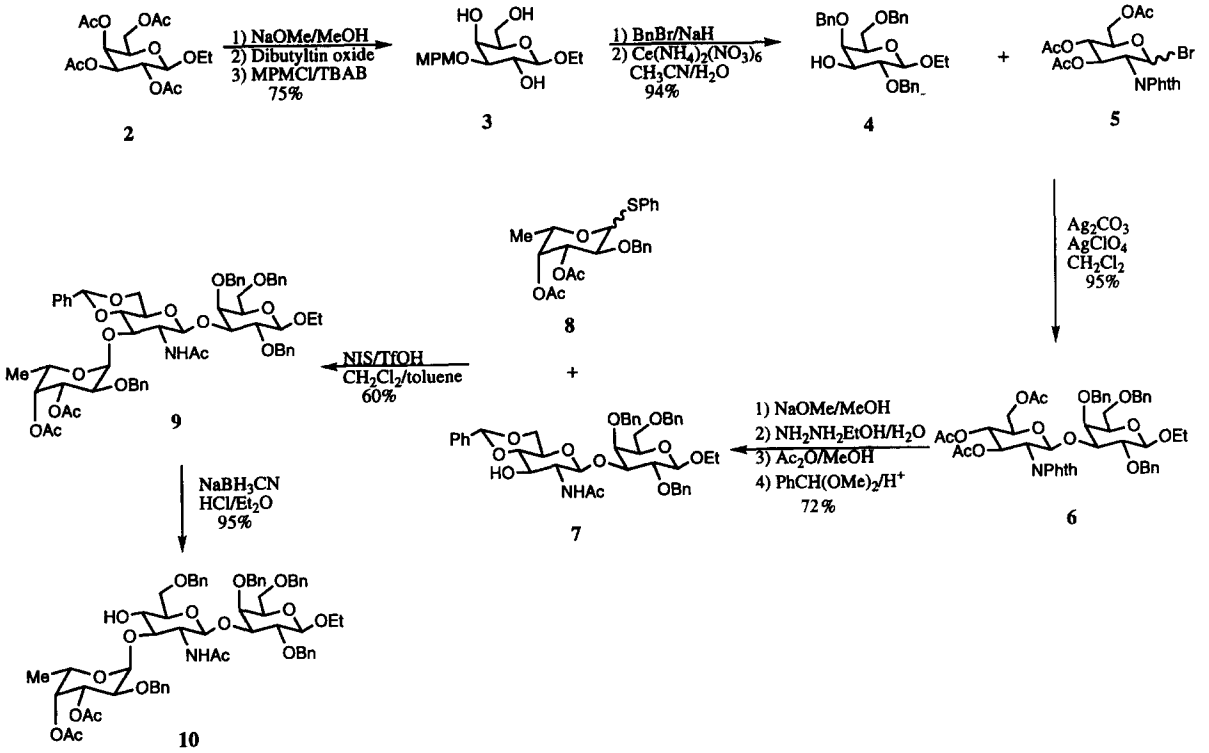
Path A: glycosylation of the NeuAcα(2–3)Gal donor and the Fucα(1–3)GlcNAcβ(1–3)GalβOEt acceptor for the sLe^x pentasaccharide

Deacetylation of compound **2**, which was easily derived from commercially available tetra-*O*-acetylgalactosyl bromide, followed by regioselective monoalkylation at the C-3 hydroxyl group by the Bu_2SnO method,[12] provided **3** in a 75% yield. Perbenzylation of **3**, followed by deprotection of the MPM group, afforded **4** in a 86% yield. Glycosylation of **4** with **5**, using $Ag_2CO_3/AgClO_4$ as the promoter system in CH_2Cl_2, exclusively afforded the β-linked disaccharide **6** in a 95% yield. Deacetylation of **6** with sodium methoxide in MeOH and dephthaloylation with NH_2NH_2, followed by N-acetylation with Ac_2O in MeOH, and benzylidenation at the C-4 and C-6 hydroxyl groups with $PhCH(OMe)_2$ under acidic conditions, provided the disaccharide acceptor **7** in a 72% yield. The reaction of **7** with the fucose donor **8** in CH_2Cl_2, with equimolar amounts of NIS/TfOH, exclusively yielded the α-linked trisaccharide **9** in a 60% yield. Reductive ring opening of the benzylidene group of **9** with $NaBH_3CN$ in $HCl–Et_2O$ gave the trisaccharide acceptor **10** in a 95% yield (Scheme 2).

We next investigated the glycosylation of the disaccharide donor **11**[13] and the trisaccharide acceptor **10** using DMTST as the promoter system in several solvents. Unfortunately, the desired sLe^x pentasaccharide was



Scheme 1.



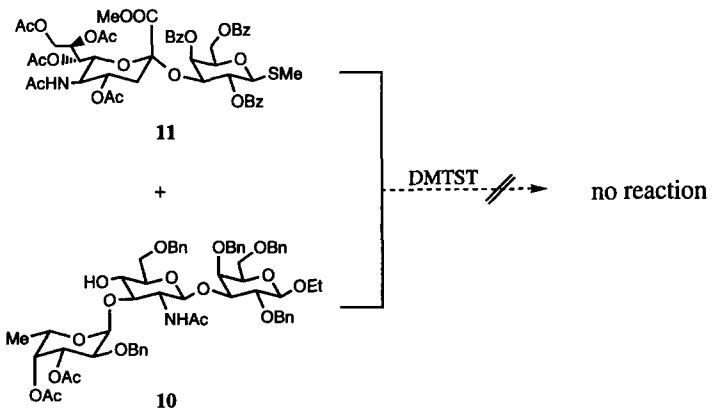
Scheme 2.

not obtained (Scheme 3). While it is not obvious why the glycosylation of **10** and **11** was unsuccessful, a possible reason is that the steric hindrance around the hydroxyl group of the acceptor **10** might have prevented the glycosylation.

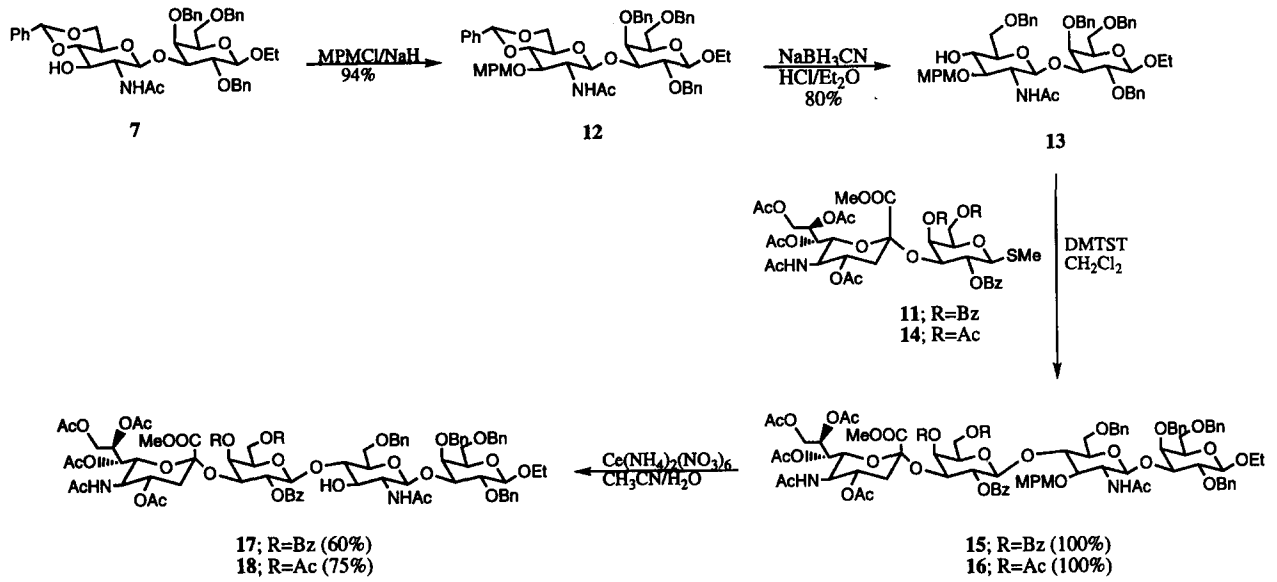
Path B: glycosylation of the NeuAcα(2–3)Galβ(1–4)GlcNAcβ(1–3)GalβOEt acceptor and the fucose donor for the sLe^x pentasaccharide

Our next synthetic plan, based on the results of the path A described above, includes at first glycosylation of the GlcNAcβ(1–3)GalβOEt acceptor with the NeuAcα(2–3)GalβSMe donor, and then an incorporation of fucose at the C-3 hydroxyl group of the GlcNAc. Protection of **7** with an MPM group, followed by the reductive deprotection of the benzylidene group with $NaBH_3CN$ in HCl–Et_2O, afforded **13** in a 75% overall yield. Surprisingly, the glycosylation of **13** with donors **11** and **14**[14] in CH_2Cl_2 with DMTST provided the tetrasaccharides **15** and **16** in quantitative yields. Deprotection of the MPM groups of **15** and **16** under oxidative conditions, with $Ce(NH_4)_2(NO_3)_6$ in CH_3CN–H_2O, gave **17** and **18** in 60% and 75% yields, respectively (Scheme 4).

We next investigated the glycosylation of the tetrasaccharide acceptors **17** and **18** with the fucose donors **8** and **19** using NIS/TfOH as the promoter system in several solvents. Fortunately, the desired sLe^x pentasaccharides **20** and **21** were obtained in 80% and 85%



Scheme 3.



Scheme 4.

yields, respectively (Scheme 5). As compared with the case of path A, the dramatic improvement of the glycosylation yield suggested that a significant decrease in the steric hindrance around the hydroxyl group of acceptor occurred.

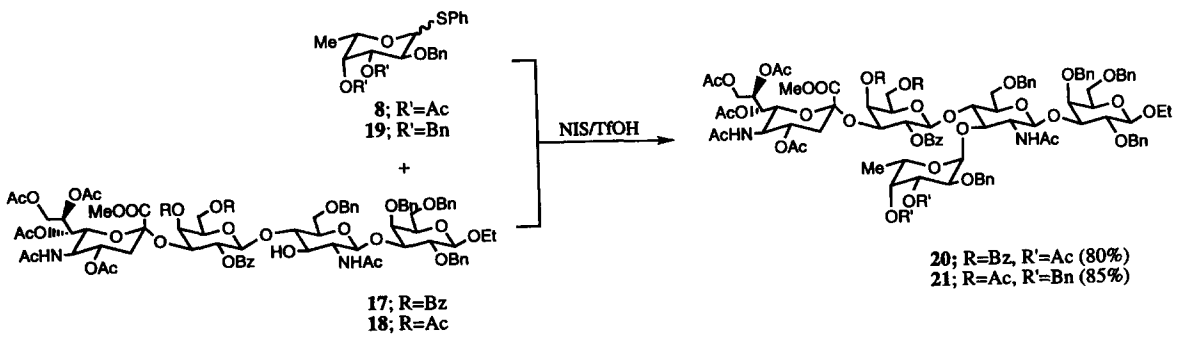
We next studied the transformation of **21** to the title sLe^x pentasaccharide **1**. Hydrogenolysis of **21** with a catalytic amount of Pd/C, followed by treatment with acetic anhydride, gave **23** in a quantitative yield. Finally, deprotection of **23** with sodium methoxide in MeOH provided the title compound **1** in a 90% yield (Scheme 6). The ^{1}H NMR data of the sLe^x pentasaccharide **1** were identical to those of **1** reported by Wong et al.[11a]

Inhibitory activity of the sLe^x pentasaccharide 1 toward E-, P-, and L-selectin–sLe^x binding

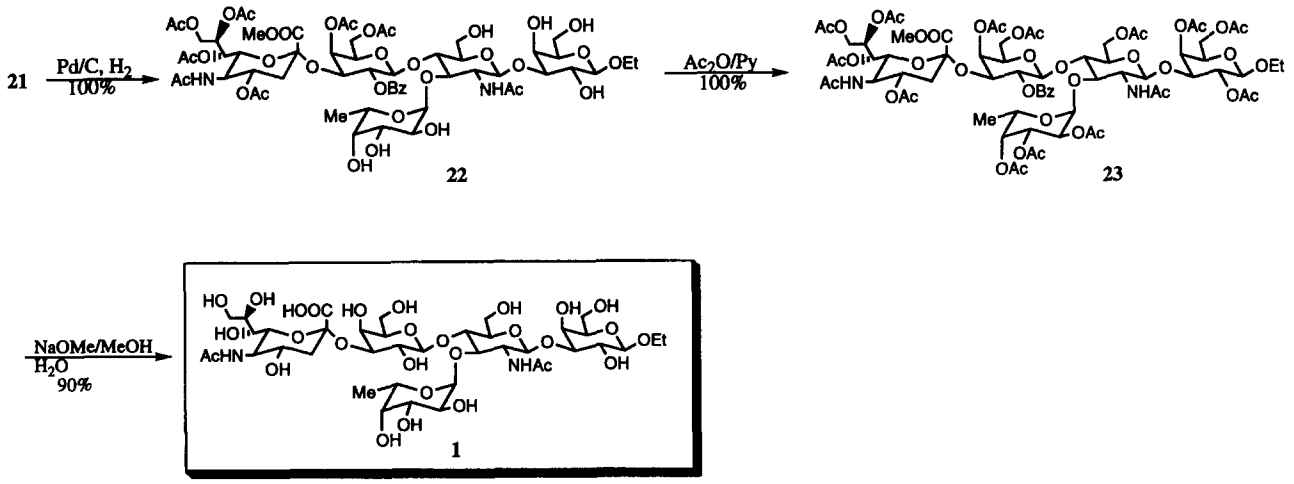
The method for the use of selectin–IgG chimeras reported by Foxall et al. was followed.[15] The construction of the selectin–immunoglobulin was carried out according to the previous paper.[16] The selectin–immunogloblin fusion proteins (selectin–Ig) used in the ELISA assays are recombinant chimeric molecules containing the lectin domain, epidermal growth factor domain, and two (L-selectin–Ig), two (P-selectin–Ig), or two (E-selectin–Ig) complement regulatory repeats coupled to the hinge, CH_2, and CH_3 regions of human IgG1.

We investigated the inhibitory activities of the sLe^x pentasaccharide **1** and the sLe^x tetrasaccharide toward E-, P-, and L-selectin–sLe^x binding. The inhibitory activities of compound **1** toward the binding of the natural ligand (sLe^x ceramide) with the E-, P-, and L-selectins were stronger than those of the sLe^x tetrasaccharide (sLe^x, Fig. 1).

In conclusion, a highly practical synthesis of the sLe^x pentasaccharide **1**, which could be a candidate for a new type of anti-inflammatory agent, was accomplished. Our successful route for the chemical synthesis involves the glycosylation of the NeuAcα(2–3)GalβSMe donor and the GlcNAcβ(1–3)GalβOEt acceptor, and then the incorporation of fucose. This synthetic strategy is expected to be a useful method for the production of sLe^x pentasaccharide mimetics.



Scheme 5.



Scheme 6.

Experimental

Inhibition assay of E-, P-, and L-selectin–sLe^x binding

A solution of sLe^x–pentasaccharide ceramide, in 1:1 methanol:distilled water, was pipetted into microtiter plate wells (96 wells) at 200 pmol/well and was adsorbed by evaporating the solvent. The wells were washed with distilled water, blocked with 5% BSA–PBS (bovine serum albumin–phosphate buffered saline) for 1 h, and were washed again with distilled water after discarding the blocking solution.

Separately, a 1:1 volumetric mixture of a 1:1000 dilution of horseradish peroxidase-labelled anti-human IgG Fc in 1% BSA–PBS and a culture supernatant containing the P-selectin–IgG chimera was incubated at room temperature for 30 min to form a complex. The test compounds were dissolved in distilled water at 10 mM and finally diluted to final concentrations at 1000 and 100 μM for compound **1**, 1000 μM for sLe^x, respectively. Reactant solutions were prepared by incubating this solution at each concentration with the above complex solution for 30 min at room temperature. This reactant solution was then added to the above microtiter wells at a 50 μL/well and allowed to react at room temperature for 2 h. The wells were washed three times with PBS and distilled water, respectively, and developed for 10 min by adding a 0.2 mg/mL of *o*-phenylenediamine and 0.015% H_2O_2 in 0.05 M citrate–phosphate buffer (pH 9.5) at 50 μL/well. The reaction was stopped by the addition of 2 N sulfuric acid at 50 μL/well and absorbance at 490 nm was measured. Percent binding was calculated by the following equation: % binding $= (X/A) \times 100$, wherein X is the absorbance of wells containing the test compounds at each concentration, and A is the absorbance of control wells not containing the test compounds. Inhibitions of the P- and L-selectin–sLe^x binding were repeated except that the P-selectin–IgG chimera and the L-selectin–IgG chimera were replaced for the E-selectin–IgG chimera.

Ethyl 3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (3). A suspension of ethyl β-D-galactopyranoside (**2a**)[11a] (5.30 g, 25.5 mmol) and dibutyltin oxide (9.50 g, 38.2 mmol) in methanol (50 mL) was stirred and heated for 6 h at 70 °C, then concentrated. To the solution of the residue in benzene (80 mL) were added 4-methoxybenzyl chloride (10.4 mL, 76.5 mmol), tetrabutylammonium bromide (4.11 g, 12.8 mmol), and 4 Å molecular sieves (powder, 1.00 g), and the mixture was stirred at 80 °C for 1.5 h. To the reaction mixture, 5.60 g of diethylamine was added at 0 °C, then the mixture was filtered and concentrated. Column chromatography (3:1 hexane:ethyl acetate, gradient elution to ethyl acetate) of the residue on silica gel gave **3** (6.23 g, 75%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.26 (t, $J=7$ Hz, 3H, $OCH_2\underline{CH}_3$), 3.81 (s, 3H, OCH_3), 4.27 (d, $J=8$ Hz, 1H, H-1), 4.68 (s, 2H, CH_2Ar), 6.90 (d, $J=9$ Hz, 2H, aromatic H), 7.31 (d, $J=9$ Hz, 2H, aromatic H).

Ethyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (4). To a solution of **3** (5.12 g, 15.6 mmol) in DMF (40 mL) was added a suspension of sodium hydride in oil (2.81 g, 60% of sodium hydride by weight). The mixture was stirred for 30 min at 0 °C, benzyl bromide (8.35 mL, 70.2 mmol) was added dropwise, and stirring was continued for 1 h at room temperature. The reaction mixture was cooled to 0 °C, and 5 mL of methanol was added, then the mixture was washed with water, and extracted with ethyl acetate. The extract was washed with brine, dried (Na_2SO_4), and concd. Column chromatography (10:1 hexane:ethyl acetate, gradient elution to 3:1) of the residue on silica gel gave ethyl 2,4,6-tri-*O*-benzyl-3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (9.89 g, 100%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.26 (t, $J=7$ Hz, 3H, $OCH_2\underline{CH}_3$), 3.80 (s, 3H, OCH_3), 4.35 (d, $J=8$ Hz, 1H, H-1), 4.65 (s, 2H, CH_2Ar), 6.85 (d, $J=9$ Hz, 2H, aromatic H), 7.25–7.36 (m, 17H, aromatic H).

To a solution of ethyl 2,4,6-tri-*O*-benzyl-3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (4.08 g, 6.75 mmol) in 37 mL of acetonitrile and 4 mL of water was added

cerium(IV) diammonium nitrate (7.40 g, 13.5 mmol) at 5–10 °C. The solution was stirred at room temperature for 2 h, then 100 mL of ethyl acetate was added and washed with saturated aqueous Na_2CO_3. The aqueous layer was extracted with ethyl acetate and the organic layers were combined, washed with water, dried over $MgSO_4$, and concentrated in vacuo. Column chromatography (10:1 hexane:ethyl acetate, gradient elution to 3:1) of the residue on silica gel gave **4** (3.03 g, 94%). ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.26 (t, J=7 Hz, 3H, OCH_2CH_3), 3.45–3.65 (m, 6H), 4.35 (d, J=7 Hz, 1H, H-1), 7.10–7.40 (m, 15H, aromatic H).

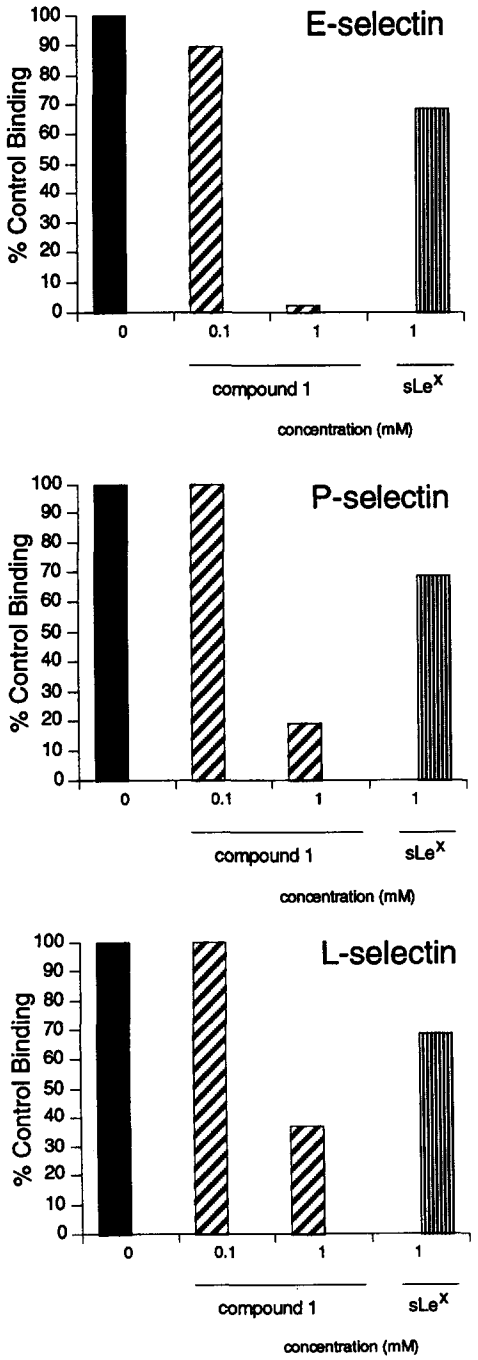


Figure 1. Inhibition of E-, P-, and L-selectin–Ig binding to immobilized sLe^x pentasaccharide ceramide by compound **1** and sLe^x. E-, P-, and L-selectin–Ig were incubated with 0, 0.1 and 1 mM of compound **1** in a competitive binding ELISA.

Ethyl *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (6). To a solution of **4** (2.63 g, 5.50 mmol) in dichloromethane (9.0 mL) were added silver carbonate (1.52 g, 5.50 mmol), silver perchlorate (1.27 g, 5.50 mmol), and 4 Å molecular sieves (powder, 1.0 g) and the mixture stirred for 18 h at room temperature in the dark (mixture A). A solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranosyl bromide **5**[13] (3.95 g, 8.25 mmol) in dichloromethane (9.0 mL) was treated with 4 Å molecular sieves (powder, 1.0 g) as above and then added to a mixture A at 0 °C. After stirring for 24 h at room temperature in the dark, the precipitate was collected and washed with dichloromethane, and the combined filtrate and washings were concentrated. Column chromatography (5:1 hexane:ethyl acetate, gradient elution to 2:1) of the residue on silica gel gave **6** (4.67 g, 95%). ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.06 (t, J=7 Hz, 3H, OCH_2CH_3), 1.85 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.03 (s, 3H, OAc), 3.40–4.60 (m, 20H), 4.24 (d, J=8 Hz, 1H, Gal, H-1), 5.16 (dd, J=9, 10 Hz, 1H, GlcNAc, H-3), 5.71 (d, J=8 Hz, 1H, GlcNAc, H-1), 5.84 (dd, J=9, 11 Hz, 1H, GlcNAc, H-4), 7.00–7.60 (m, 19H, aromatic H).

Ethyl *O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (7). A solution of **6** (5.84 g, 6.52 mmol) in methanol (100 mL) was stirred with sodium methoxide (200 mg) for 40 min at room temperature. The mixture was treated with Amberlite IR-120 (H^+) resin and concentrated, and a solution of the residue in aqueous 95% ethanol (50 mL) was treated with hydrazine hydrate (2.18 g) for 1 h under reflux. The precipitate was collected and washed with methanol, and the combined filtrate and washings were concentrated. The residue was treated with acetic anhydride (12.3 mL) in methanol (100 mL) for 30 min at room temperature, pyridine (10.5 mL) was added, the mixture was concentrated, and a solution of the residue in ethyl acetate was successively washed with 2 M hydrochloric acid, water, saturated sodium carbonate solution, and brine, dried (Na_2SO_4), and concentrated. Column chromatography (1:2 hexane:ethyl acetate, gradient elution to 20:1 ethyl acetate:methanol) of the residue on silica gel gave ethyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (3.82 g, 86%). ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.22 (t, J=7.0 Hz, 3H OCH_2CH_3), 1.50 (s, 3H, NAc), 4.39 (d, J=7 Hz, 1H, Gal, H-1), 4.76 (d, J=8 Hz, 1H, GlcNAc, H-1), 4.82 (d, J=12 Hz, 1H, CHHPh), 5.20 (d, J=12 Hz, 1H, CHHPh), 7.26–7.37 (m, 15H, Ph).

To a solution of ethyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (640 mg, 0.938 mmol) in acetonitrile (10 mL) was added Drierite (1.5 g), and after stirring for 1 h, benzaldehyde dimethyl acetal (0.282 mL, 1.88 mmol) was added, and the mixture was stirred for 15 h at

room temperature. Anhydrous (*R*)-(−)-camphor-10-sulfonic acid (20 mg) was added, and the mixture was stirred for 5 h at room temperature, then neutralized with triethylamine and concentrated. Column chromatography (5:1 hexane:ethyl acetate, gradient elution to 1:1 ethyl acetate:dichloromethane) of the residue on silica gel gave **7** (607 mg, 84%). ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.22 (t, J=7 Hz, 3H, $OCH_2\underline{CH}_3$), 1.54 (s, 3H, NAc), 5.58 (s, 1H, C<u>H</u>Ph), 7.10–7.60 (m, 20H, Ar).

Ethyl *O*-(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (9). To a solution of **7** (200 mg, 0.260 mmol) and phenyl 3,4-di-*O*-acetyl-2-*O*-benzyl-1-thio-L-fucopyranoside **8** (145 mg, 0.338 mmol) in toluene (2.0 mL) and dichloromethane (1.0 mL), were added 4 Å molecular sieves (powder, 100 mg), and the mixture was stirred for 5 h at room temperature. *N*-Iodosuccinimide (131 mg, 0.585 mmol) and trifluoromethanesulfonic acid (4 μL, 0.039 mmol) were added to the mixture at −30 °C, and the stirring was continued for 18 h at −30 °C. Moreover, *N*-iodosuccinimide (131 mg, 0.585 mmol) and trifluoromethanesulfonic acid (4 μL, 0.039 mmol) were added to the mixture at −10 °C, and the mixture was stirred for 1 h at −10 °C . Ethyl acetate was added to the mixture, and the precipitates were filtered off, and washed with saturated sodium carbonate solution, saturated sodium thiosulfate solution, water, and brine, dried (Na_2SO_4), and concentrated. Column chromatography (3:1 hexane:ethyl acetate, gradient elution to 2:1) of the residue on silica gel gave **9** (171 mg, 60%). ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.55 (d, J=7 Hz, 3H, H-6, fucose unit), 1.26 (t, J=7 Hz, 3H, $OCH_2\underline{CH}_3$), 1.43 (s, 3H, NAc), 1.93 (s, 6H, $(OAc)_2$), 5.01 (d, J=4 Hz, 1H, H-1, fucose unit), 5.24 (d, J=8 Hz, 1H, H-1, GlcNAc unit), 5.51 (s, 1H, C<u>H</u>Ph), 7.10–7.50 (m, 25H, Ph).

Ethyl *O*-(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-*O*-(2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (10). To a solution of **9** (510 mg, 0.468 mmol) in THF (15 mL) were added 4 Å molecular sieves (powder, 700 mg), and the mixture stirred for 2.5 h at room temperature, and sodium cyanoborohydride (441 mg) was gradually added. After the reagent had dissolved, hydrogen chloride in ether was added dropwise at 0 °C until the evolution of gas ceased. The mixture was neutralized with triethylamine and filtered, the residue was washed with methanol and the combined filtrate and washings were concentrated. Column chromatography (2:1 hexane:ethyl acetate, gradient elution to 1:1) of the residue on silica gel gave **10** (486 mg, 95%). ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.11 (d, J=7 Hz, 3H, H-6, fucose unit), 1.19 (t, J=7 Hz, 3H, $OCH_2\underline{CH}_3$), 1.36 (s, 3H, NAc), 1.96 (s, 3H, OAc), 2.11 (s, 3H, OAc), 4.62 (d, J=3 Hz, 1H, H-1, fucose unit), 5.11 (d, J=8 Hz, 1H, H-1, GlcNAc unit), 7.10–7.40 (m, 25H, Ph).

Ethyl *O*-[2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(4-methoxybenzyl)-β-D-glucopyranosyl]-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (12). To a solution of **7** (800 mg, 1.04 mmol) in DMF (4 mL) was added a suspension of sodium hydride in oil (42 mg, 60% of sodium hydride by weight). After stirring of the mixture for 10 min at 0 °C, 4-methoxybenzyl chloride (183 μL, 1.35 mmol) was added dropwise, and stirring was continued for 2 h at room temperature. The reaction mixture was cooled to 0 °C, and 5 mL of methanol was added, then the mixture was washed with water, and extracted with ethyl acetate. The extract was washed with brine, dried (Na_2SO_4), and concentrated. The obtained crystalline solid was washed with diethyl ether to afford **12** (867 mg, 94%). ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.21 (t, J=7 Hz, 3H, $OCH_2\underline{CH}_3$), 1.63 (s, 3H, NAc), 3.30–5.10 (m, 24H), 3.80 (s, 3H, OMe), 5.58 (s, 1H, C<u>H</u>Ph), 6.83 (d, J=9 Hz, 2H, aromatic H), 7.18 (d, J=9 Hz, 2H, aromatic H), 7.20–7.60 (m, 20H, Ph).

Ethyl *O*-[2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(4-methoxybenzyl)-β-D-glucopyranosyl]-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (13). To a solution of **12** (680 mg, 0.764 mmol) in THF (35 mL) were added 4 Å molecular sieves (powder, 1.00 g), and the mixture was stirred for 1.5 h at room temperature, and sodium cyanoborohydrate (720 mg) was gradually added. After the reagent had dissolved, hydrogen chloride in ether was added dropwise at 0 °C until the evolution of gas ceased. The mixture was neutralized with triethylamine and filtered, the residue was washed with methanol and the combined filtrate and washings were concentrated. Column chromatography (2:1 hexane:ethyl acetate, gradient elution to 1:1) of the residue on silica gel gave **13** (545 mg, 80%). ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.21 (t, J=7 Hz, 3H, $OCH_2\underline{CH}_3$), 1.60 (s, 3H, NAc), 3.79 (s, 3H, OMe), 3.96 (dd, J=7, 9 Hz, 1H, H-2, galactose unit), 4.33 (dd, J=4, 8 Hz, 1H, H-4, galactose unit), 4.72 (d, J=9 Hz, 1H, H-1, galactose unit), 4.86 (d, J=8 Hz, 1H, H-1, GlcNAc unit), 4.90 (d, J=12 Hz, 1H, C<u>H</u>HPh), 5.03 (d, J=12 Hz, 1H, C<u>H</u>HPh), 6.86 (d, J=9 Hz, 2H, aromatic H), 7.10–7.40 (m, 22H, aromatic H).

Ethyl *O*-(methyl 5-acetamido-4,7,8-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-*O*-(4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(4-methoxybenzyl)-β-D-glucopyranosyl]-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (16). Compound **13** (278 mg, 0.312 mmol) and methyl *O*-(methyl 5-acetamido-4,7,8-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-*O*-(4,6-di-*O*-acetyl-2-*O*-benzoyl-1-thio-β-D-galactopyranoside) **14**[14] (421 mg, 0.483 mmol) were dissolved in 4.0 mL of dry CH_2Cl_2 and stirred over 4 Å molecular sieves (powder, 400 mg) at room temperature for 17 h. The mixture was then cooled to 0 °C, and a mixture of DMTST (908 mg, 3.385 mmol) and 4 Å molecular sieves (powder, 743 mg) was added. The mixture was stirred for 96 h during which time the temperature was kept at 7 °C. To the reaction mixture, 0.5 mL of

methanol, 0.5 mL of triethylamine and 10 mL of ethyl acetate were added, then the mixture was filtered and washed with water. The aqueous layer was extracted with ethyl acetate and the organic layer was washed with brine, and dried over anhydrous Na_2SO_4, then the solvent was removed in vacuo. The residue was subjected to flash chromatography (10:1 hexane:ethyl acetate, gradient elution to 1:2) to afford 560 mg (100% yield) of **16** as a white solid: ^{1}H NMR (270 MHz, $CDCl_3$): δ1.20 (t, J=7 Hz, 3H, $OCH_2\underline{CH_3}$), 1.49 (s, 3H, NAc), 1.57 (s, 3H, NAc), 1.94 (s, 3H, OAc), 1.97 (s, 6H, 2 OAc), 2.09 (s, 6H, 2 OAc), 2.17 (s, 3H, OAc), 2.53 (dd, J=4, 12 Hz, 1H, H-3e, Neu5Ac unit), 3.76 (s, 3H, $PhOC\underline{H_3}$), 3.86 (s, 3H, CO_2CH_3), 4.21 (d, J=8 Hz, 1H, H-1, galactose unit), 5.22 (dd, J=2, 12 Hz, 1H, H-7, Neu5Ac unit), 5.55–5.70 (m, 1H, H-8, Neu5Ac unit), 6.77 (d, J=8 Hz, 2H, aromatic H), 7.05–8.20 (m, 27H, aromatic H).

Ethyl *O*-(methyl 5-acetamido-4,7,8-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(4-methoxybenzyl)-β-D-glucopyranosyl]-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (15). Compound **15** was synthesized similarly to the method described for the synthesis of **16** in 100% yield. $[\alpha]_D$ +7.6° (*c* 0.5, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.20 (t, J=7 Hz, 3H, $OCH_2\underline{CH_3}$), 1.52 (s, 3H, NAc), 1.56 (s, 3H, NAc), 1.91 (s, 3H, OAc), 1.92 (s, 6H, 2 OAc), 1.94 (s, 3H, OAc), 2.15 (s, 3H, OAc), 2.40–2.50 (m, 1H, H-3e, Neu5Ac unit), 3.65 (s, 3H, $PhOC\underline{H_3}$), 3.82 (s, 3H, CO_2CH_3), 5.08 (d, J=8 Hz, 1H, H-1″, galactose unit), 6.63 (d, J=9 Hz, 2H, aromatic H), 7.10–8.30 (m, 37H, aromatic H). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 171.1, 171.0, 170.6, 170.5, 168.6, 166.1, 139.8, 139.4, 138.8, 138.6, 133.6, 133.4, 131.2, 130.7, 130.4, 130.3, 130.2, 130.1, 129.9, 129.8, 129.7, 129.1, 129.0, 128.9, 128.7, 128.6, 128.3, 128.1, 128.0, 127.8, 127.6, 114.0, 103.9, 102.5, 100.4, 97.4, 81.8, 79.7, 79.2, 74.8, 74.8, 74.0, 73.8, 73.3, 73.1, 72.3, 71.9, 71.3, 69.8, 68.8, 67.7, 67.1, 65.6, 62.9, 62.2, 60.7, 55.5, 53.5, 49.4, 23.5, 21.7, 21.4, 21.1 20.8, 15.6, 14.6.

Ethyl *O*-(methyl 5-acetamido-4,7,8-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (17). Compound **17** was synthesized similarly to the method described for the synthesis of **4** in 88%. $[\alpha]_D$ +39.1° (*c* 0.5, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.18 (t, J=7 Hz, 3H, $OCH_2\underline{CH_3}$), 1.53 (s, 3H, NAc), 1.56 (s, 3H, NAc), 1.93 (s, 3H, OAc), 1.94 (s, 6H, 2 OAc), 2.03 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.40–2.50 (m, 1H, H-3e, Neu5Ac unit), 3.85 (s, 3H, CO_2CH_3), 5.03 (d, J=7 Hz, 1H, H-1″, galactose unit), 7.10–8.30 (m, 35H, aromatic H). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 171.1, 171.0, 170.6, 170.5, 168.6, 166.6, 166.1, 165.5, 139.8, 139.3, 138.8, 138.6, 133.9, 133.8, 133.6, 133.4, 130.8, 130.4, 130.34, 130.26, 130.20, 130.15, 129.8, 129.6, 129.1, 129.0, 128.9, 128.7, 128.6, 128.4, 128.1, 127.9, 127.74, 127.65, 127.4, 127.0, 104.0, 102.5, 102.2, 97.4, 82.8, 81.5, 74.8, 74.6, 74.2, 73.8, 72.5, 72.2, 71.8, 71.4, 69.9, 69.8, 68.8, 67.8, 67.5, 67.2, 65.6, 63.1, 56.2, 53.7, 53.5, 49.3, 37.9, 23.5, 21.8, 21.2, 21.0, 20.9, 15.6.

Ethyl *O*-(methyl 5-acetamido-4,7,8-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-*O*-(4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[2-acetamido-6-*O*-benzyl-2-deoxy-D-glucopyranosyl]-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (18). Compound **18** was synthesized similarly to the method described for the synthesis of **4** in 75% yield. ^{1}H NMR ($CDCl_3$, 270 MHz): δ 1.18 (t, J=7 Hz, 3H, $OCH_2\underline{CH_3}$), 1.52 (s, 3H, NHAc), 1.56 (s, 3H, NHAc), 1.96 (s, 3H, OAc), 1.97 (s, 3H, OAc), 2.036 (s, 3H, OAc), 2.040 (s, 3H, OAc), 2.15 (s, 3H, OAc), 2.19 (s, 3H, OAc), 2.53 (dd, J=5, 12 Hz, 1H, H-3e, Neu5Ac unit), 3.84 (s, 3H, COOMe), 5.04 (d, J=8 Hz, 1H, H-1″, galactose unit), 5.20 (dd, J=3, 9 Hz, 1H, H-7, Neu5Ac unit), 5.55–5.70 (m, 1H, H-8, Neu5Ac unit), 7.00–8.30 (m, 25H, Ar).

Ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-*O*-(4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-*O*-(2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (21). To a solution of **18** (414 mg, 0.259 mmol) and phenyl 2,3,4-tri-*O*-benzyl-1-thio-L-fucopyranoside **19** (273 mg, 0.519 mmol) in toluene (2.0 mL) and dichloromethane (2.0 mL), were added 4 Å molecular sieves (powder, 400 mg), and the mixture was stirred for 13 h at room temperature. *N*-Iodosuccinimide (199 mg, 0.882 mmol) and trifluoromethanesulfonic acid (4 μL, 0.039 mmol) were added to the mixture at −20 °C, and the stirring was continued for 2 h at −20 °C. Moreover, *N*-iodosuccinimide (199 mg, 0.882 mmol) and trifluoromethanesulfonic acid (4 μL, 0.039 mmol) were added to the mixture at −10 °C, and the mixture was stirred for 2 h at −10 °C . Ethyl acetate was added to the mixture, and the precipitates were filtered off, and washed with saturated sodium carbonate solution, saturated sodium thiosulfate solution, water, and brine, dried (Na_2SO_4), and concentrated. Column chromatography (10:1 hexane:ethyl acetate, gradient elution to 1:2) of the residue on silica gel gave **21** (471 mg, 85%). ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.15–1.25 (m, 6H), 1.51 (s, 3H), 1.56 (s, 3H), 1.95 (s, 3H), 1.97 (s, 3H), 2.04 (s, 3H), 2.15 (s, 3H), 2.51 (dd, J=13 Hz, 5 Hz, 1H), 3.84 (s, 3H), 5.55–5.65 (m, 1H), 6.95–8.25 (m, 4H).

Ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[*O*-(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-(2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (20). Compound **20** was synthesized similarly to the method described for the synthesis of

21 in 80% yield. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.06 (d, J=6 Hz, 3H, H-6, fucose unit), 1.18 (t, J=7 Hz, 3H, OCH_2CH_3), 1.49 (s, 3H, NAc), 1.54 (s, 3H, NAc), 1.92 (s, 3H, OAc), 1.93 (s, 6H, 2 OAc), 2.03 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.13 (s, 3H, OAc), 2.19 (s, 3H, OAc), 2.35–2.50 (m, 1H, H-3e, Neu5Ac unit), 3.86 (s, 3H, CO_2CH_3), 7.00–8.30 (m, 40H, aromatic H).

Ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nulopyranosylonate)-(2→3)-*O*-(4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[*O*-(α-L-fucopyranosyl)-(1→3)]-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside (22). A solution of **21** (270 mg, 0.126 mmol) in ethanol (20 mL) was hydrogenated at ambient pressure with 10% Pd/C as a catalyst. After stirring at 50 °C for 44 h, the suspension was filtered through Celite, concentrated and diluted in ethyl acetate to afford **22** (174 mg, 100%). ^{1}H NMR (270 MHz, $CDCl_3/CD_3OD$): δ 1.23 (t, J=7 Hz, 3H), 1.34 (d, J = 7 Hz, 3H), 1.55 (s, 3H), 1.65 (t, J=13 Hz, 1H), 1.76 (s, 3H), 1.96 (s, 3H), 2.09 (s, 3H), 2.10 (s, 3H), 2.15 (s, 3H), 2.21 (s, 3H), 2.52 (dd, J=4, 13 Hz, 1H), 3.85 (s, 3H), 4.17 (d, J=8 Hz, 1H), 4.48 (dd, J=2, 12 Hz, 1H), 4.54 (d, J=7 Hz, 1H), 5.08 (d, J=3 Hz, 1H), 5.60–5.70 (m, 1H), 7.45–8.25 (m, 5H).

Ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-*O*-(4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-(2-acetamido-6-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (23). To **22** (72 mg, 0.0521 mmol) were added pyridine (2 mL) and acetic anhydride (1 mL), and the mixture was stirred at 40 °C for 20 h. The mixture was diluted with methanol (5 mL) and concentrated in vacuo, and the residue was purified with column chromatography (1:2 hexane:ethyl acetate, gradient elution to only ethyl acetate) to afford **23** (87 mg, 100%). ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.17 (t, J=7 Hz, 3H), 1.25 (d, J=7 Hz, 3H), 1.66 (t, J=12 Hz, 1H), 1.78 (s, 3H), 1.90 (s, 3H), 1.95 (s, 3H), 1.96 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 2.05 (s, 3H, OAc), 2.07 (s, 3H), 2.09 (s, 3H), 2.10 (s, 6H), 2.15 (s, 6H), 2.16 (s, 3H), 2.19 (s, 3H), 2.52 (dd, J=5, 13 Hz, 1H), 3.63 (dd, J=4, 10 Hz, 1H), 3.87 (s, 3H), 4.30 (d, J=8 Hz, 1H), 5.06 (d, J=4 Hz, 1H), 5.19 (t, J=9 Hz, 1H), 5.25 (dd, J=3, 11 Hz, 1H), 5.55–5.65 (m, 1H), 7.30–8.10 (m, 5H).

Ethyl *O*-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-[*O*-(α-L-fucopyranosyl)-(1→3)]-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside (1). To **23** (9.2 mg, 0.0055 mmol) was added 28% NaOMe/methanol (20 mg). The mixture was stirred at 40 °C for 4 h. To this water (0.5 mL) was then added, and the mixture was stirred for an additional 16 h at 40 °C. The reaction mixture was cooled to 5 °C, and the pH was adjusted to 7.0 with ion-exchange resin (Dowex 50X8). The resin was removed by filtration and the filtrate concentrated. The residue was chromatographed with Sephadex LH-20 (chloroform:methanol:water, 5:4:0.7) to afford **1** (5.0 mg, 90%). ^{1}H NMR (250 MHz, D_2O): δ 1.03 (d, J=7 Hz, 3H, H-6, fucose unit), 1.09 (t, J=8 Hz, 3H, OEt), 1.66 (t, J=13 Hz, 1H, H-3a, Sia unit), 1.88 (s, 3H, NAc), 1.90 (s, 3H, NAc), 2.63 (dd, J=4.5, 13 Hz, 1H, H-3e, Sia unit), 3.95 (dd, J=4, 11 Hz, 1H), 4.01 (d, J=3 Hz, 1H), 4.25 (d, J=9 Hz, 1H,), 4.98 (d, J=4 Hz, 1H).

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(*Received in Japan* 26 *February* 1996)