Articles

A Convenient and Efficient Synthesis of SLeX Analogs

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Received January 19, 1996[⊗]

Described is the preparation and use of tetrasaccharide **1**, which enables a rapid and preparative scale synthesis of sialyl Lewis X (SLeX) analogs having 1-*O*- and 2-*N*-disubstituted glucosamine (GlcN) moieties. Such modifications should bring a dramatic change of the physical and pharmacological properties of the SLeX analogs. Therefore, tetrasaccharide **1** is a convenient intermediate for the synthesis of various SLeX analogs, since it has convertible 2-(trimethylsilyl)-ethyl (SE) glycoside and the free amino group on GlcN moiety. The intermediate **1** was constructed from a glucosamine derivative by a highly efficient combined use of enzymatic galactosylation/sialylation and chemical fucosylation. Thus obtained **1** was converted into SLeX analogs by *N*-substitution followed by transformation of SE glycoside into other glycosides and deprotection. These synthesized analogs were found to inhibit cell adhesion of HL-60 cells to recombinant soluble human E-selectin.

The tetrasaccharide sialyl Lewis X antigenic determinant (SLeX, **2**) is known to be a ligand for E-selectin[1] and P-selectin.[2] SLeX exists at the nonreducing termini of several glycoproteins and glycolipids found on the surface of neutrophils and plays an important role in selectin-mediated cell adhesion involved in various aspects of immune cell trafficking.[3] E-selectin is synthesized and expressed on the lumenal surface of endothelial cells in areas of tissue injury upon activation by various cytokines.[3,4] Interaction of E-selectin and SLeX on the surface of leukocytes causes leukocytes to slowly roll along the surface of endothelial cells. Firm adhesion occurs as rolling leukocytes interact with integrin Arg-Gly-Asp (RGD)-containing ligands within intercellular adhesion molecule (ICAM-1).[5] This enables leukocytes to migrate into surrounding tissue and causes further damage. Blocking SLeX−E-selectin interactions and thereby neutrophil emigration should therefore be a novel therapeutic approach for treating inflammatory diseases.[6]

Recently, unnatural SLeX analogs with modified Gal,[7] Fuc,[8] NeuAc,[8] and GlcN residues[9,10] were prepared as inhibitors of the SLeX−selectin binding. The biological properties[7−10] and conformational analysis[9b,11] of these analogs indicate that Gal, Fuc, and NeuAc residues are essential for the binding to selectins. An important feature of the GlcN moiety is to fix the relative conformation between the NeuAc-Gal and Fuc residues. Although it is believed that the GlcN moiety is independent with respect to binding to selectins, modification of the GlcN residue could change the physical and pharmacological properties of the SLeX analogs. Until now, neither transformation of 2-*N*-substituents nor combination of substituents on 1-*O*- and 2-*N*-positions of GlcN moiety has been studied systematically, partly because of their structural complexity and difficulty in their synthesis.

Generally, oligosaccharides have been synthesized by enzymatic and/or chemical methods. In the field of enzymatic synthesis of SLeX and related oligosaccharides, galactosyltransferase (GalT),[12] α(2,3)-sialyltransferase (2,3-ST),[13] and cytidine 5′-monophospho-*N*-acetylneuraminic acid synthetase (CMP-NeuAc synthetase)[14] have been available to date, and schemes for their

[⊗] Abstract published in *Advance ACS Abstracts,* April 1, 1996.

(1) (a) Phillips, M. L.; Nudeman, E.; Gaeta, F. C. A.; Perez, M.; Singhal, A. K.; Hakomori, S.; Paulson, J. C. *Science* **1990**, *250*, 1130. (b) Walz, G.; Aruffo, A.; Kolanus, W.; Bevilacqua, M.; Seed, B. *Science* **1990**, *250*, 1132. (c) Lowe, J. B.; Stoolman, L. M.; Nair, R. P.; Larsen, R. D.; Berhend, T. L.; Marks, R. M. *Cell* **1990**, *63*, 475. (d) Tiemyer, M.; Swiedler, S. J.; Ishihara, M.; Moreland, M.; Schweingruber, H.; Hirtzer, P.; Brandley, B. K. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1138.

(2) (a) Polley, M. J.; Phillips, M. L.; Wayner, E.; Nudeman, E.; Singhal, A. K.; Hakomori, S.; Paulson, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6224. (b) Zhou, Q.; Moore, K. L.; Smith, D. F.; Varki, A.; McEver, R.; Cummings, R. D. *J. Cell. Biol.* **1991**, *115*, 557.

(3) (a) Paulson, J. C. In *Adhesion: its role in inflammatory disease*; Harlan, J., Liu, D., Eds.; W. H. Freeman: New York, 1992; Chapter 2, p 19. (b) Bevilapua, M. P.; Nelson, R. M. *J. Clin. Invest.* **1993**, *91*, 379. (c) Springer, T. A. *Cell* **1994**, *76*, 301 and reference therein.

(4) (a) Butcher, E. C. *Cell* **1991**, *67*, 1033. (b) Shimizu, Y.; Newman, W.; Tanaka, Y.; Shaw, S. *Immunol. Today* **1992**, *13*, 106.

(5) Pytela, R.; Pierschbacher, M. D.; Ginsberg, M. H.; Plow, E. F; Ruoslahti, E. *Science* **1986**, *231*, 1559.

(6) In preliminary *in vivo* studies, adminstration of SLeX showed a marked thrapeutic effect on P-selectin-dependent induced lung injuries (Mulligan, M. S.; Paulson, J. C.; DeFrees, S. A.; Zeng, Z.-L.; Lowe, J. B.; Ward, P. A. *Nature* **1993**, *364*, 149) and on selectin-dependent myocardial reperfusion injuries (Buerke, M.; Weyrich, A. S.; Zheng, Z.; Gaeta, F. C. A.; Forest, M. J.; Lefer, A. M. *J. Clin. Invest.* **1994**, *93*, 1140).

(7) Stahl, W.; Sprengard, U.; Kretzschmar, G.; Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2096.

(8) Brandley, B. K.; Kiso, M.; Abbas, S.; Nikrad, P.; Srivasatava, O.; Foxall, C.; Oda, Y.; Hasegawa, A. *Glycobiology* **1993**, *3*, 633.

(9) (a) Tyrrell, D.; James, P.; Rao, N., Foxall, C.; Abbas, S.; Dasgupta, F.; Hasegawa, A.; Kiso, M.; Asa, D.; Kidd, J.; Brandley, B. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10372. (b) DeFrees, S. A., Gaeta, F. C. A.; Lin, Y.-C.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 7549.

(10) Nelson, R. M.; Dolich, S.; Aruffo, A.; Cecconi, O.; Bevilacqua, M. P. *J. Clin. Invest.* **1993**, *91*, 1157.

(11) Ichikawa, Y.; Lin, Y.-C.; Dumas, D. P.; Shen, G.-J.; Garcia-Junceda, E.; Williams, M. A.; Bayer, R.; Ketcham, C.; Walker, L. E.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 9283.

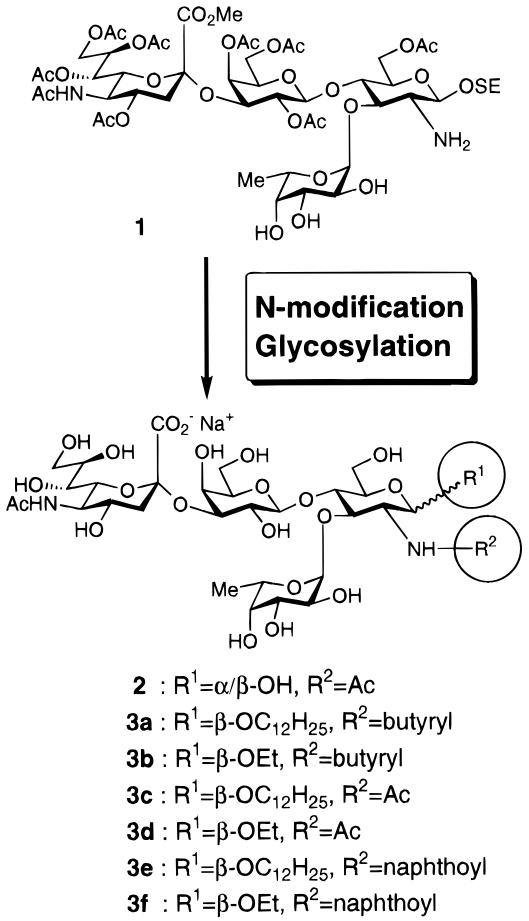
(12) Purchased from Sigma Chemical Co.

(13) Wen, D. X.; Livinigston, B. D.; Medrihdszky, K. F.; Kelm, S.; Burlingame, A. L.; Paulson, J. C. *J. Biol. Chem.* **1992**, *267*, 21011.

(14) (a) Vann, W. F.; Silver, R. P.; Abeijon, C.; Chang, K.; Aaronson, W.; Sutton, A.; Finn, C. W.; Lindner, W.; Kotsatos, M. *J. Biol. Chem.* **1987**, *262*, 17556. (b) Shames, S. L.; Simon, E. S.; Christpher, C. W.; Schmid, W.; Whitesides, G. M.; Yang, L.-L. *Glycobiology* **1991**, *1*, 187.

S0022-3263(96)00125-9 CCC: $12.00

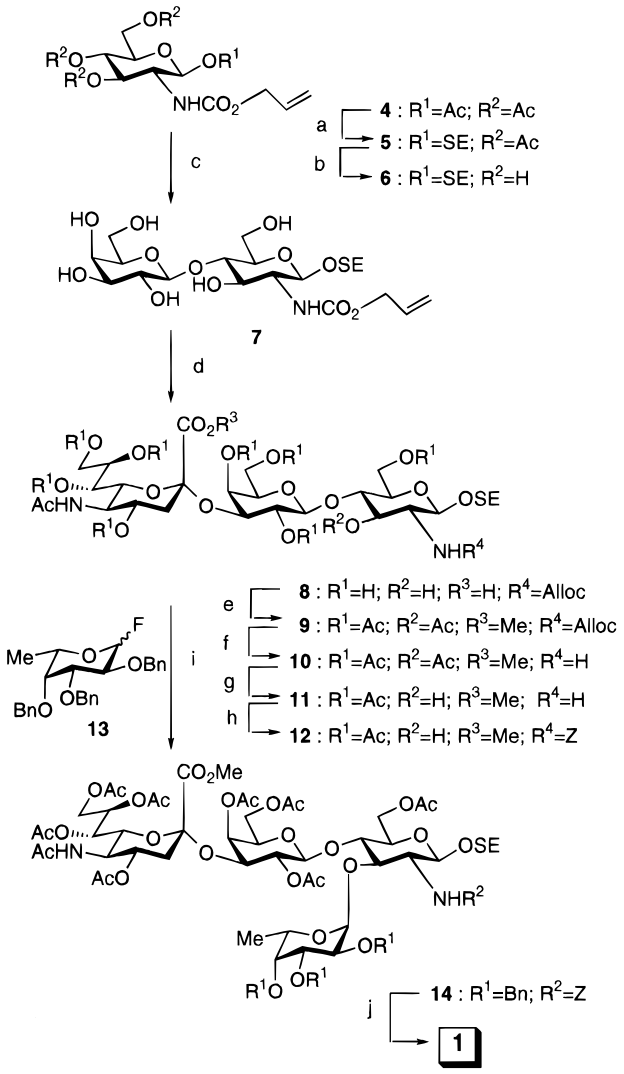
Scheme 1



utilization in multigram reactions have been developed.[11] Although enzymatic glycosylation proceeds regio- and stereoselectively, there can be major drawbacks with this enzymatic method which results from strict enzyme−substrate specificities or insoluble substrates in water. These limitations make it difficult to synthesize various kind of oligosaccharide analogs, especially those structures with bulky or hydrophilic residues.[11] Alternatively, chemical synthesis offers more flexibility in synthetic strategy and design but requires tedious multiple protection/deprotection steps.[11] In this paper, we describe the preparation and the application of tetrasaccharide **1** which enables a rapid and preparative scale synthesis of SLeX analogs having 1-*O*- and 2-*N*-disubstituted GlcN moieties. It was constructed by a highly efficient combined use of enzymatic galactosylation/sialylation and chemical fucosylation. Tetrasaccharide **1**, containing a 2-(trimethylsilyl)ethyl (SE) glycoside[15] and a free amino group on the GlcN moiety, has two attractive advantages for the synthesis of bioactive SLeX analogs: (a) SE glycosides can be smoothly transformed into other glycosides via the corresponding glycosyl donors such as 1-chloro sugars[15] and (b) desirable functional groups can be introduced on the amino group by treatment with desired electrophiles such as acyl halides. Scheme 1 summarizes the synthetic pathway for the preparation of SLeX analogs from the key intermediate **1**.

The preparative method for the intermediate **1** is shown in Scheme 2. A 1-*β*-*O*- and 2-*N*-diprotected

Scheme 2[a]



[a] Reagents and conditions: (a) (i) HBr in AcOH, CH_2Cl_2, −15 °C; (ii) Se-OH, Ag_2CO_3, MS-4Å, CH_2Cl_2, −5 °C, 78% overall; (b) NaOMe, MeOH, 25 °C, 79%; (c) UDP-Glc, UDPGE, BSA, $MnCl_2$, ClAP, GalT, sodium cacodylate buffer (0.05 mM, pH 7.5), 25 °C, 98%; (d) NeuAc, PEP, $MgCl_2$, $MnCl_2$, CMP, ATP, BSA, $HS(CH_2)_2$-OH, CMP-NeuAc synthetase, MK, PK, PPase, 2,3-ST, HEPES buffer (0.1 M, pH 7.5); (e) (i) Ac_2O, DMAP, pyridine, 25 °C; (ii) MeOH, 25 °C; (iii) Ac_2O, DMAP, pyridine, 25 °C, 88% yield from **7**; (f) $Pd(PPh_3)_4$, PMHS, THF, 25 °C, 89%; (g) AcOH, MeOH, H_2O, 50 °C; (h) Z-Cl, $NaHCO_3$, CH_2Cl_2, 25 °C, 77% yield from 10; (i) $AgClO_4$, $SnCl_2$, TMU, Ms-4Å, $ClCH_2CH_2Cl$, −20 °C → 25 °C, 85%; (j) 10% Pd−C, $HCOONH_4$, EtOH, reflux, 95%.

glucosamine derivative **6** was chosen as a starting material for the synthesis of 1-*β*-*O*-protected 2-amino tetrasaccharide **1**. It was prepared from tetraacetate **4**[16] by the Köenigs−Knorr-type glycosylation reaction with SE alcohol followed by treatment with NaOMe. A first crucial step in the synthesis would be the enzymatic galactosylation of monosaccharide **6**. On the basis of previous results,[17] however, we expected the glucosamine derivative **6** to be a suitable substrate for enzymatic galactosylation under the usual reaction conditions.[18,19]

(15) (a) Jansson, K.; Ahlfors, S.; Frejd, T.; Kihlberg, J.; Magnusson, G. *J. Org. Chem.* **1988**, *53*, 5629. (b) Jansson, K.; Noori, G.; Magnusson, G. *J. Org. Chem.* **1990**, *55*, 3181.

(16) Boullanger, P.; Banoub, J.; Descotes, G. *Can. J. Chem.* **1987**, *65*, 1343.

(17) DeFrees, S. A. et al. have already found that allyloxycarbonyl group on the amino group of glucosamine derivatives does not interfere with enzymatic galactosylation (private communication).

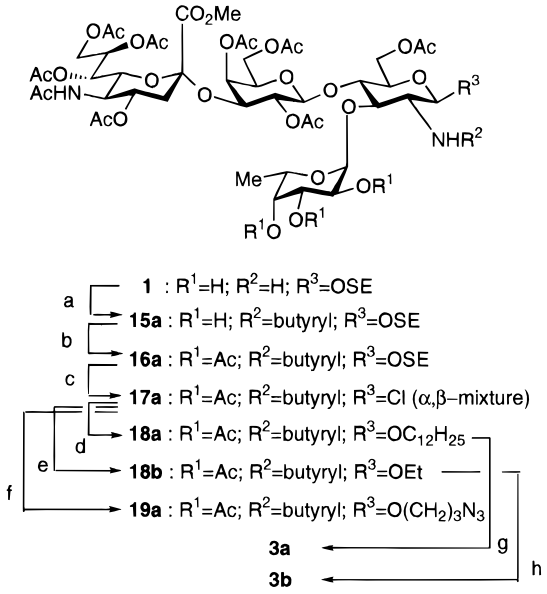
(18) Unverzagt, C.; Kunz, H.; Paulson, J. C. *J. Am. Chem. Soc.* **1990**, *112*, 9308.

Glucosamine derivative **6** was dissolved in 50 mM sodium cacodylate buffer (pH 7.5), UDP-glucose, bovine serum albumin (BSA), $MnCl_2$, UDP-galactose 4-epimerase (UDPGE), calf intestinal alkaline phosphatase (CIAP), and GalT were added, and the solution was incubated at 37 °C for 8 days. The reaction mixture was concentrated and purified by gel filtration to afford lactosamine derivative **7** in 98% yield. Moreover, it was found that disaccharide **7** was a well-recognized substrate for enzymatic sialylation.[20] The sialylation utilized the multienzyme system reported by Ichikawa et al.[11] The resulting sialylated product **8** was then converted into fully protected compound **9** in three steps consisting of acetylation, methanolysis of the lactone, and successive acetylation of a resulting hydroxyl group on the Gal moiety (88% overall yield from **7**).[21] The allyloxycarbonyl (Alloc) group of **9** was removed by reaction of carbamate **8** with $Pd(PPh_3)_4$ in the presence of poly(methylhydrosiloxane) (PMHS) affording amine **10** in 89% yield. Treatment of amine **10** in aqueous methanol with a catalytic amount of acetic acid provided the desired amino alcohol **11** as a single product.[22] The amino group on **11** was reprotected selectively by benzyloxycarbonyl chloride (Z-Cl) to give glycosyl acceptor **12** in 77% overall yield from **10**. Introduction of the Fuc group onto the hydroxyl group of **12** was achieved by using glycosyl fluoride **13**,[23] in the presence of $AgClO_4$, $SnCl_2$, and tetramethylurea (TMU)[23,24] to provide the tetrasaccharide **14** stereoselectively in 85% yield. Thus, the desired key intermediate **1** could be obtained by hydrogenalytic cleavage of the benzyl groups and Z group of **14** in 95% yield.

Compound **1** could be converted into a SLeX analog **3a** having lipophilic *O*-dodecyl/*N*-butylyl substituents, as shown in Scheme 3. Utilizing this scheme, the amino group of **1** was selectively acylated with butyryl chloride in the presence of $NaHCO_3$ and was then acetylated to afford butanamide **16a** in 86% yield. The SE glycoside **16a** was then transformed into dodecyl glycoside **18** utilizing Magnusson's method[15b,25] in 52% yield. The peracetate **18a** was then treated with basic conditions to give the difunctionalized SLeX analog **3a** in 78% yield. The derivatives **3b**−**f** were synthesized in a similar manner.

These SLeX analogs were found to inhibit cell adhesion of human leukocyte (HL-60) to recombinant soluble human E-selectin.[26] The IC_{50} values of analogs **3a**, **3b**, **3c**, **3d**, **3e**, and **3f** are 2.0, 1.0, 1.6, 1.5, 0.1, and 0.1 mM, respectively, as shown in Figure 1. Herein, IC_{50} means the concentration that inhibited cell adhesion of HL-60 to human E-selectin by 50%. Butanamides **3a** and **3b** showed almost the same activity as acetamides **3c** and **3d** as expected from the result reported by Nelson et al.[10] In contrast, naphthamides **3e** and **3f** were indicated to be five times or more active than acetamides **3c** and **3d**, similar to the result reported by Ramphal et al.[22] These results suggest that modification of GlcN moiety on SLeX should be of great value for the search of effective inhibitor of cell adhesion.

Scheme 3[a]



[a] Reagents and conditions: (a) butyryl chloride, $NaHCO_3$, CH_2Cl_2, 25 °C; (b) Ac_2O, pyridine, DMAP, 25 °C, 86% yield from **1**; (c) 1,1-dichloromethyl methyl ether, $ZnCl_2$, $CHCl_3$, 25 °C; (d) 1-dodecanol, $Sn(OTf)_2$, TMU, MS-4Å, CH_2Cl_2, 25 °C, 52% yield from **16a**; (e) EtOH, $Sn(OTf)_2$, TMU, MS4A, CH_2Cl_2, 25 °C, 41% yield from **16a**; (f) 3-azido-1-propanol, $Sn(OTf)_2$, TMU, MS-4Å, CH_2Cl_2, 25 °C, 38% yield from **16a**; (g) NaOMe, MeOH, H_2O, 25 °C, 78% yield; (h) NaOMe, MeOH, H_2O, 25 °C, 75% yield.

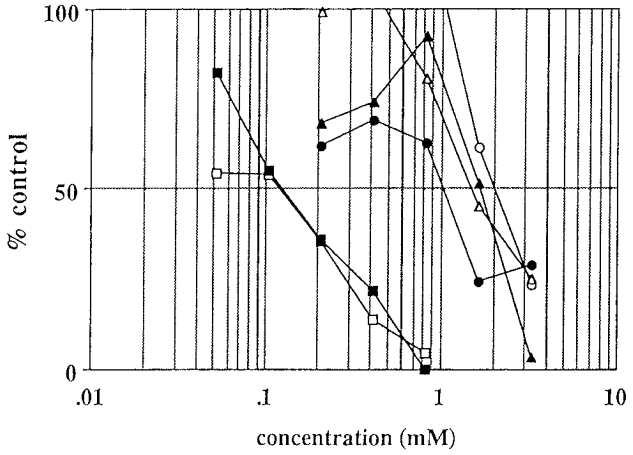


Figure 1. Inhibition of HL-60 adhesion to recombinant soluble E-selectin-coated plates: (○) **3a**; (●) **3b**; (△) **3c**; (▲) **3d**; (□) **3e**; (■) **3f**.

(19) For reviews of substrate specificity for GalT, see: (a) Wong, C.-H.; Ichikawa, Y.; Krach, T.; Narvor, C. G.-L.; Dumas, D. P.; Look, G. C. *J. Am. Chem. Soc.* **1991**, *113*, 8137. (b) Wong, C.-H.; Halcomb, R. L.; Ichikawa, Y.; Kajimoto, T. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 521.

(20) For reports on substrate specificities of 2,3-ST, see: (a) Kunz, H.; Harrews, A. *Liebigs Ann. Chem.* **1982**, 41. (b) Schmidt, R. R.; Zimmreman, P. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 725. (c) Sato, S.; Nunomura, S.; Nakano, T.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1988**, *29*, 4097. (d) Ito Y.; Paulson, J. C. *J. Am. Chem. Soc.* **1993**, *115*, 1603.

(21) This three-step procedure was developed by Ratcliffe, R. M. (private communication). See also: DeFrees, S. A.; Gaeta, F. C. A.; Gaudino, J. J.; Zheng, Z.; Hayashi, M. *Int. Pat.* 1994, WO 9426760; *Chem. Abstr.*, 1994.

(22) Ramphal, J. Y.; Hiroshige, M.; Lou, B.; Guadino, J.; Hayashi, M.; Chen, S. M.; Chiang, L. C.; Gaeta, F. C. A.; DeFrees, S. A. *J. Med. Chem.*, in press.

(23) Nicolaou, K. C.; Hummel, C. W.; Bockovich, N. J.; Wong, C.-H. *J. Chem. Soc., Chem. Commun.* **1991**, *15*, 872.

(24) Mukaiyama, T.; Murai, Y.; Shoda, S. *Chem. Lett.* **1981**, 431.

(25) Lubineau, A., Gallic, J. L.; Malleron, A. *Tetrahedron Lett.* **1987**, *28*, 5041.

(26) For the evaluation method of inhibition of HL-60 adhesion to recombinant soluble human E-selectin, see: DeFrees, S. A.; Kosch, W.; Way, W.; Paulson, J. C.; Sabesan, S.; Halcomb, R. L.; Huang, D.-H.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1995**, *117*, 66.

To further demonstrate utility of this synthetic approach, the above methodology was used to prepare the SLeX derivative **19**. Thus obtained compound **19** has been used as a precursor to allow coupling of the oligosaccharide to lipids.[27] Moreover, compound **19** can be used for coupling to proteins such as bovine serum albumin and provide neoglycoproteins which are useful as antigens.[28]

In conclusion, our synthetic approach to intermediate **1** provides ready access to SLeX analogs with modified GlcN residues. We continue to study the synthesis and structure−activity relationships of SLeX both *in vitro* and *in vivo* and will report the results in due course.

Experimental Section

^{1}H-NMR (270 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded in the indicated solvent. Melting points (mp) were uncorrected. All reactions involving nonaqueous solutions were carried out under a nitrogen atomosphere. All reactions were monitored by thin-layer chromatography carried out on silica gel glass-backed plates (0.25 mm). Silica gel column chromatography was performed on silica gel 60 (230−400 or 70−230 mesh) using the indicated solvents. Gel filtration chromatography was performed on Bio-Gel P-2 gel (65 ± 20 μm). Dichloromethane and 1,2-dichloroethane were dried over 4 Å molecular sieves (MS-4Å). Tetrahydrofuran (THF) was dried over MS-5Å.

2-(Trimethylsilyl)ethyl 2-*N*-(Allyloxycarbonyl)-2-amino-2-deoxy-3,4,6-tri-*O*-acetyl-*β*-D-glucopyranoside (5). Hydrogen bromide in acetic acid (25% assay, 388.4 g, 1.20 mmol) was added dropwise to a stirred solution of 2-*N*-(allyloxycarbonyl)-2-amino-2-deoxy-1,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranose (**4**) (172.56 g, 0.40 mmol) in dichloromethane (1035 mL) at −15 °C over a 1 h period, and the mixture was stirred at −15 °C for 2 h. Then, the mixture was washed with water, 5% aqueous sodium hydrogen carbonate, and water in that order. The organic layer was dried over MS-4Å and then filtered. The filtrate was added dropwise to the mixture of 2-(trimethylsilyl)ethanol (94.6 g, 0.80 mmol), silver carbonate (331 g, 1.20 mmol), and powdered MS-4Å (429 g) in dichloromethane (1035 mL) at −5 °C over a 90 min period. After being stirred for 1 h at −5 °C, the reaction mixture was filtered through a pad of Celite and the filtrate was washed with 5% aqueous sodium hydrogen carbonate and water in that order. The organic layer was concentrated under reduced pressure, and the resulting residue was dissolved in toluene (245 g). The solution was added dropwise into stirred hexane (817 g), and the resulting crystals were collected by filtration to afford **5** (153.0 g, 78% yield) as colorless crystals: $R_f = 0.57$ (developed with a 1:1 mixture of hexane and ethyl acetate); mp 70−72 °C; ^{1}H NMR ($CDCl_3$) δ 5.81 (1H, m), 5.27−5.08 (4H, m), 4.96 (1H, t, J = 9.6 Hz), 4.65−4.45 (2H, m), 4.47 (1H, d, J = 4.3 Hz), 4.20 (1H, dd, J = 4.6 and 11.9 Hz), 4.04 (1H, dd, J = 2.3 and 11.9 Hz), 3.89 (1H, m), 3.65 (1H, m), 3.63−3.45 (2H, m), 1.99 (3H, s), 1.94 (3H, s), 1.93 (3H, s), 0.94−0.80 (2H, m), and −0.08 (9H, s). Anal. Calcd for $C_{21}H_{35}NO_{10}Si\cdot H_2O$: C, 49.69; H, 7.35; N, 2.76. Found: C, 49.82; H, 6.98; N, 2.81.

2-(Trimethylsilyl)ethyl 2-*N*-(Allyloxycarbonyl)-2-amino-2-deoxy-*β*-D-glucopyranoside (6). Sodium methoxide in methanol (28% assay, 6.0 g, 0.02 mmol) was added to a stirred solution of **5** (49.0 g, 0.10 mmol) in methanol (150 mL) at room temperature. After 1 h, the mixture was concentrated under reduced pressure and diluted with dichloromethane (720 g). The solution was added to stirred water (480 g), and the mixture was neutrilized with 1 N hydrochloric acid. The organic layer was then separated and concentrated under reduced pressure. The residue was dissolved in a small amount of dichloromethane, and the solution was added dropwise to heptane (500 g) at 0 °C. The resulting precipitate was collected by filtration to afford **6** (28.6 g, 79% yield) as colorless crystals: $R_f = 0.70$ (developed with a 10:2:1 mixture of ethyl acetate, ethanol, and water); mp 134−138 °C; ^{1}H NMR ($CDCl_3$) δ 5.90 (1H, m), 5.75 (1H, d, J = 8.3 Hz), 5.30 (1H, dd, J = 1.3 and 17.2 Hz), 5.18 (1H, dd, J = 1.3 and 10.2 Hz), 5.05 (1H, s), 4.72 (1H, s), 4.55 (1H, d, J = 5.6 Hz), 4.48 (1H, m), 3.96 (1H, m), 3.85 (2H, m), 3.80−3.23 (6H, m), 2.41 (1H, s), 0.99−0.85 (2H, m), and 0.00 (9H, s). Anal. Calcd for $C_{15}H_{29}NO_7Si$: C, 49.57; H, 8.04; N, 3.85. Found: C, 49.40; H, 7.92; N, 4.03.

2-(Trimethylsilyl)ethyl *β*-D-Galactopyranosyl-(1→4)-*O*-(2-*N*-(allyloxycarbonyl)-2-amino-2-deoxy-*β*-D-glucopyranoside) (7). Galactosyltransferase (GalT, EC 2.4.1.22, 30 U) and uridine 5′-diphosphogalactose 4′-epimerase (UDPGE, EC 5.1.3.2, 240 U) were added to a solution containing 50 mM aqueous sodium cacodylate (pH 7.5, 120 mL), 5% aqueous bovine serum albumin (BSA, 2.46 mL), 0.35 M aqueous manganese(II) chloride (0.78 mL), 0.50 M aqueous sodium azide (1.68 mL), calf intestinal alkaline phosphatase (CIAP, EC 3.1.3.1, 1320 U), uridine 5′-diphosphoglucose disodium salt (UDP-Glc·2Na, 4.56 g, 8.05 mmol), and monosaccharide **6** (2.18 g, 6.00 mmol). The reaction mixture was inverted several times and allowed to stand at 37 °C for 4 days, and then additional GalT (10 U) was added to the mixture. After standing at 37 °C for an additional 4 days, the mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure, and the residue was purified by gel filtration chromatography. Disaccharide **7** was afforded as a white solid after lyophilization (3.01 g, 98% yield): $R_f = 0.38$ (developed with a 10:2:1 mixture of ethyl acetate, ethanol, and water); ^{1}H NMR (D_2O) δ 5.96 (1H, m), 5.33 (1H, d, J = 17.1 Hz), 5.25 (1H, d, J = 10.5 Hz), 4.74−4.50 (3H, m), 4.47 (1H, d, J = 7.6 Hz), 4.06−3.40 (14H, m), 1.08−0.93 (2H, m), and 0.00 (9H, s).

2-(Trimethylsilyl)ethyl (5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopylanosylonic acid)-(2→3)-*O*-(*β*-D-galactopyranosyl)-(1→4)-*O*-[2-*N*-(allyloxycarbonyl)-2-amino-2-deoxy-*β*-D-glucopyranoside] (8). (−)-*N*-Acetylneuraminic acid (6.30 g, 20.3 mmol), phospho(enol)pyruvate trisodium salt monohydrate (PEP·3Na·H_2O, 11.58 g, 49.5 mmol), 1.0 M aqueous magnesium chloride (20.3 mL), 1.0 M aqueous manganese(II) chloride (5.38 mL), 1.0 M aqueous potassium chloride (20.3 mL), cytidine 5′-monophosphate (CMP, 656 mg, 2.03 mmol), adenosine 5′-triphosphate (ATP, 112 mg, 0.203 mmol), 5% aqueous BSA (16.3 mL), and 2-mercaptethanol (64 μL) were added to a solution of **7** (8.30 g, 16.8 mmol) in HEPES buffer (pH 7.5, 820 mL). The pH of the solution was adjusted to 7.5 with 1.0 M aqueous sodium hydroxide, and the enzymes, inorganic pyrophosphatase (PPase, EC 3.6.1.1, 2444 U), myokinase (MK, EC 2.7.4.1, 32587 U), pyrvate kinase (PK, EC 2.7.1.40, 52956 U), cytidine 5′-monophospho-*N*-acetylneuraminic acid synthetase (CMP-NeuAc synthetase, EC 2.7.7.43, 62 U), and α(2,3)-sialyl transferase (2,3-ST, EC 2.4.99.4, 162 U) were added to the solution. After standing at room temperature for 5 days, the mixture was diluted with excess methanol and concentrated under reduced pressure to give **8**. The resulting amorphous solid was subjected to the next reaction without further purification: $R_f = 0.49$ (developed with a 4:2:1 mixture of ethyl acetate, ethanol, and water); ^{1}H NMR (D_2O) δ 5.96 (1H, m), 5.34 (1H, dd, J = 1.3 and 17.1 Hz), 5.25 (1H, dd, J = 1.3 and 10.5 Hz), 4.60−4.51 (4H, m), 4.14−3.37 (21H, m), 2.75 (1H, dd, J = 4.6 and 12.5 Hz), 2.03 (3H, s), 1.80 (1H, t, J = 12.2 Hz), 1.05−0.82 (2H, m), and 0.00 (9H, s).

One-Pot Preparation of 2-(Trimethylsilyl)ethyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-*O*-[2-*N*-(allyloxycarbonyl)-2-amino-2-deoxy-3,6-di-*O*-acetyl-*β*-D-glucopyranoside] (9). Acetic anhydride (227 mL) and 4-(dimethylamino)pyridine (0.50 g) were added to a stirred solution of crude **8** (obtained above) in pyridine (377 mL) at 0 °C. After stirring for 12 h, methanol (580 mL) was added dropwise to the mixture at 0 °C. The mixture was warmed to

(27) DeFrees, S. A. Private communication. See also: DeFrees, S. A.; Phillips, L.; Zalipsky, S.; Guo, L. Submitted.

(28) Rice, K. G. In *Neoglycoconjugates: Preparation and Applications*; Lee, Y. C., Lee, R. T., Eds.; Academic Press: San Diego, 1994; Chapter 9, pp 285−321.

room temperature, stirred for 1 day, and concentrated under reduced pressure. The residue was treated with pyridine (240 mL) and acetic anhydride (150 mL) at 0 °C, and the mixture was stirred at room temperature for an additional 3 h. Then, methanol (500 mL) was added dropwise to the mixture at 0 °C. After stirring at 0 °C for 30 min, the mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate and washed with saturated copper(II) sulfate, saturated sodium hydrogen carbonate, and brine in that order. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography eluting with hexane, ethyl acetate, and ethanol (10/10/2) to afford **9** (16.77 g, 88% yield from **6**) as a pale yellow crystalline powder: $R_f = 0.40$ (developed with a 10:10:3 mixture of hexane, ethyl acetate, and ethanol); mp >200 °C dec; IR (KBr) 2958, 1741, 1670, 1542, 1372, 1230, 1047 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.86 (1H, m), 5.55−3.50 (29H, m), 3.85 (3H, s), 2.59 (1H, dd, J = 4.3 and 12.5 Hz), 2.24 (3H, s), 2.15 (3H, s), 2.10 (3H, s), 2.08 (3H, s), 2.07 (3H, s), 2.06 (3H, s), 2.05 (3H, s), 2.04 (3H, s), 2.00 (3H, s), 1.85 (3H, s), 1.68 (1H, t, J = 12.5 Hz), 0.98−0.91 (2H, m), and −0.01 (9H, s). Anal. Calcd for $C_{51}H_{76}N_2O_{29}Si$: C, 50.66; H, 6.33; N, 2.32. Found: C, 50.30; H, 6.32; N, 2.29.

Stepwise Preparation of 2-(Trimethylsilyl)ethyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-*O*-[2-*N*-(allyloxycarbonyl)-2-amino-2-deoxy-3,6-di-*O*-acetyl-*β*-D-glucopyranoside] (9). Acetic anhydride (3.5 mL) and 4-(dimethylamino)pyridine (10 mg) were added to a stirred solution of **8** (554 mg, 0.678 mmol) in pyridine (9.0 mL) at 0 °C. After stirring for 1 day, the mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate and washed with saturated sodium hydrogen carbonate and brine in that order. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography eluting with hexane, ethyl acetate, and acetone (1/1/0.5) to afford lactone **8a** (704 mg, 92% yield) as a colorless crystalline powder: $R_f = 0.45$ (developed with a 1:1:1 mixture of hexane, ethyl acetate, and acetone); mp 116−120 °C; IR (KBr) 2958, 1748, 1655, 1542, 1372, 1236, 1036 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.13 (1H, d, J = 10.2 Hz), 5.82 (1H, m), 5.38−5.02 (8H, m), 4.70−4.32 (7H, m), 4.25−3.79 (8H, m), 3.67−3.43 (4H, m), 2.47 (1H, br.), 2.37 (1H, dd, J = 5.3 and 13.2 Hz), 2.14 (3H, s), 2.04 (3H, s), 2.03 (3H, s), 2.02 (3H, s), 2.01 (3H, s), 2.00 (3H, s), 1.99 (3H, s), 1.95 (3H, s), 1.82 (3H, s), 1.77 (1H, t, J = 13.2 Hz), 0.87 (2H, m), and −0.06 (9H, s). Anal. Calcd for $C_{48}H_{70}N_2O_{27}Si{\cdot}H_2O$: C, 49.99; H, 6.29; N, 2.43. Found: C, 49.90; H, 6.23; N, 2.47.

4-(Dimethylamino)pyridine (5.2 mg, 0.043 mmol) was added to a solution of lactone **8a** (244 mg, 0.215 mmol) in methanol (3.0 mL), and the solution was stirred at room temperature for 18 h. The solution was then concentrated under reduced pressure, and the resulting residue was purified by silica gel column chromatography eluting with hexane, ethyl acetate, and acetone (1/1/0.5) to give ester **8b** (176 mg, 70% yield) as a colorless crystalline powder: $R_f = 0.37$ (developed with a 1:1:1 mixture of hexane, ethyl acetate, and acetone); mp 102−105 °C; ^1H NMR ($CDCl_3$) δ 5.87 (1H, m), 5.45−4.25 (17H, m), 4.10−3.40 (12H, m), 3.86 (3H, s), 2.83 (1H, br), 2.67 (1H, dd, J = 4.6 and 12.5 Hz), 2.15 (3H, s), 2.14 (3H, s), 2.06 (15H, s), 2.02 (3H, s), 1.89 (3H, s), 1.84 (1H, t, J = 12.5 Hz), 0.93 (2H, m), and −0.01 (9H, s). Anal. Calcd for $C_{49}H_{74}N_2O_{28}Si{\cdot}2\ H_2O$: C, 48.91; H, 6.53; N, 2.33. Found: C, 49.10; H, 6.33; N, 2.57.

Acetic anhydride (1.0 mL) and 4-(dimethylamino)pyridine (5.0 mg) were added to a stirred solution of ester **8b** (164 mg, 0.141 mmol) in pyridine (3.0 mL) at 0 °C. After stirring for 1 day, the mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate and washed with saturated sodium hydrogen carbonate and brine in that order. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography eluting with hexane, ethyl acetate, and ethanol (10/10/2) to afford **9** (156 mg, 92% yield) as a colorless crystalline powder. This sample showed identical physical and spectral properties with those recorded on **9** obtained above.

2-(Trimethylsilyl)ethyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-*O*-(2-amino-2-deoxy-3,6-di-*O*-acetyl-*β*-D-glucopyranoside) (10). Tetrakis(triphenylphosphine)-palladium(0) [$Pd(PPh_3)_4$] (3.30 g) and poly(methylhydrosiloxane) (PMHS, 1.6 mL) were added to a stirred solution of **9** (16.5 g, 13.7 mmol) in THF (165 mL) at room temperature. After stirring for 2.5 h, $Pd(PPh_3)_4$ (3.30 g) and PMHS (1.6 mL) were added again. After an additional 12 h, the mixture was diluted with dichloromethane and washed with water. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography eluting with hexane, ethyl acetate, and methanol (10/10/1) to afford **10** (13.61 g, 89% yield) as a pale yellow amorphous solid: $R_f = 0.19$ (developed with a 10:10:3 mixture of hexane, ethyl acetate, and ethanol); ^1H NMR ($CDCl_3$) δ 5.50 (1H, m), 5.38 (1H, m), 5.11−4.85 (4H, m), 4.65−3.45 (19H, m), 3.83 (3H, s), 2.76 (1H, t, J = 8.9 Hz), 2.60 (1H, dd, J = 4.6 and 12.9 Hz), 2.26 (3H, s), 2.15 (3H, s), 2.10 (3H, s), 2.08 (3H, s), 2.07 (3H, s), 2.06 (3H, s), 2.05 (3H, s), 2.04 (3H, s), 1.99 (3H, s), 1.84 (3H, s), 1.70 (1H, m), 1.05−0.93 (2H, m), and 0.01 (9H, s). Anal. Calcd for $C_{47}H_{72}N_2O_{27}Si{\cdot}2H_2O$: C, 48.62; H, 6.60; N, 2.41. Found: C, 48.85; H, 6.49; N, 2.45.

2-(Trimethylsilyl)ethyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-*O*-(6-*O*-acetyl-2-amino-2-deoxy-*β*-D-glucopyranoside) (11). Acetic acid (0.72 mL) was added to a solution of **10** (13.61 g, 12.1 mmol) in methanol (1089 mL) and water (272 mL). The mixture was warmed to 50 °C and stirred at the temperature for 1 day. Concentration under reduced pressure afforded crude **11** as a pale yellow amorphous solid which was subjected to the sequential reaction without further purification: $R_f = 0.76$ (developed with a 9:1 mixture of chloroform and methanol); ^1H NMR ($CDCl_3$) δ 5.50 (1H, m), 5.40 (1H, m), 5.12−4.85 (4H, m), 4.65−3.48 (20H, m), 3.83 (3H, s), 2.76 (1H, t, J = 8.7 Hz), 2.56 (1H, dd, J = 4.3 and 12.5 Hz), 2.27 (3H, s), 2.15 (3H, s), 2.10 (3H, s), 2.08 (3H, s), 2.07 (3H, s), 2.06 (3H, s), 2.05 (3H, s), 2.00 (3H, s), 1.85 (3H, s), 1.67 (1H, t, J = 12.5 Hz), 1.02−0.92 (2H, m), and 0.01 (9H, s). Anal. Calcd for $C_{45}H_{70}N_2O_{26}Si{\cdot}H_2O$: C, 49.08; H, 6.59; N, 2.54. Found: C, 48.75; H, 6.56; N, 2.60.

2-(Trimethylsilyl)ethyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-*O*-[6-*O*-acetyl-2-amino-2-*N*-(benzyloxycarbonyl)-2-deoxy-*β*-D-glucopyranoside] (12). Benzyl chloroformate (Z-Cl, 2.6 mL, 18.2 mmol) was added dropwise to a mixture of **11** and powdered sodium hydrogen carbonate (3.05 g, 36.3 mmol) in dichloromethane (262 mL) at room temperature. The mixture was stirred at room temperature for 12 h and then diluted with ethyl acetate. The solution was washed with water, dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography eluting with hexane, ethyl acetate, and methanol (10/10/1) to give **12** (11.30 g, 77% yield from **10**) as a colorless crystalline powder: $R_f = 0.71$ (developed with a 9:1 mixture of chloroform and methanol); mp 115−120 °C; ^1H NMR ($CDCl_3$) δ 7.34−7.27 (5H, m, Ph-H), 5.51 (1H, m), 5.41 (1H, m), 5.15−3.25 (26H, m), 3.82 (3H, s), 2.58 (1H, dd, J = 4.6 and 12.5 Hz), 2.28 (3H, s), 2.17 (3H, s), 2.11 (3H, s), 2.10 (3H, s), 2.09 (3H, s), 2.07 (3H, s), 2.02 (3H, s), 2.01 (3H, s), 1.86 (3H, s), 1.66 (1H, t, J = 12.5 Hz), 0.95−0.82 (2H, m), and −0.01 (9H, s). Anal. Calcd for $C_{53}H_{76}N_2O_{28}Si{\cdot}H_2O$: C, 51.53; H, 6.36; N, 2.27. Found: C, 51.56; H, 6.38; N, 2.26.

2-(Trimethylsilyl)ethyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-*O*-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→3)-*O*]-[6-*O*-acetyl-2-amino-2-*N*-(benzyloxy-

carbonyl)-2-deoxy-β-D-glucopyranoside] (14). Powdered MS-4Å (2.6 g), 1,1,3,3-tetramethylurea (TMU, 3.30 mL, 27.6 mmol), and 2,3,4-tri-*O*-benzyl-L-fucosyl fluoride (**13**) (12.0 g, 27.5 mmol) were added to a stirred solution of **12** (5.60 g, 4.60 mmol) in 1,2-dichloroethane (25 mL). After stirring for 90 min, the mixture was shielded from light, cooled to −20 °C, and treated with tin(II) chloride (3.49 g, 18.4 mmol) and silver perchlorate (3.85 g, 18.4 mmol). The reaction mixture was allowed to warm to room temperature over a 90 min period and stirred for 24 h at room temperature. The suspension was filtered through a pad of Celite, and the filtrate was washed with water. The organic layer was separated, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography eluting with hexane, ethyl acetate, and methanol (10/10/1) to give **14** (6.37 g, 85% yield) as a colorless crystalline powder: $R_f = 0.41$ (developed with a 10:10:3 mixture of hexane, ethyl acetate, and methanol); mp 102−108 °C; ^{1}H NMR ($CDCl_3$) δ 7.46−7.24 (20H, m), 5.43 (1H, d, J = 9.5 Hz), 5.46 (1H, d, J = 9.5 Hz), 5.20−3.50 (36H, m), 3.94 (3H, s), 2.60 (1H, dd, J = 4.6 and 12.5 Hz), 2.24 (3H, s), 2.18 (3H, s), 2.10 (3H, s), 2.08 (3H, s), 2.07 (3H, s), 2.04 (6H, s), 1.88 (3H, s), 1.84 (3H, s), 1.70 (1H, t, J = 12.5 Hz), 1.26 (3H, d, J = 6.3 Hz), 0.94−0.84 (2H, m), and 0.00 (9H, s). Anal. Calcd for $C_{80}H_{104}N_2O_{32}Si\cdot 3H_2O$: C, 56.93; H, 6.57; N, 1.66. Found: C, 56.72; H, 6.22; N, 1.71.

2-(Trimethylsilyl)ethyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)-*O*-[6-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside] (1). A mixture of **14** (6.25 g, 3.83 mmol), ammonium formate (10 g), and 10% palladium on activated carbon (Pd−C, wet, 10 g) in ethanol (125 mL) was refluxed with stirring for 8 h. Additional ammonium formate (10 g) and 10% Pd−C (wet, 10 g) were added to the mixture, and the mixture was refluxed for an additional 8 h. The mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure to give amine **1** (4.45 g, 95% yield) as a colorless crystalline powder: $R_f = 0.44$ (developed with a 10:2:1 mixture of ethyl acetate, ethanol, and water); mp 149−152 °C; IR (KBr) 3420, 2960, 1745, 1655, 1373, 1232, 1042 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.48−5.40 (2H, m), 5.34 (1H, d, J = 3.3 Hz), 5.13 (1H, d, J = 10.6 Hz), 4.96−4.84 (3H, m), 4.68−4.53 (4H, m), 4.35−3.50 (22H, m), 3.83 (3H, s), 2.75 (1H, t, J = 9.4Hz), 2.57 (1H, dd, J = 4.6 and 12.5 Hz), 2.20 (3H, s), 2.15 (3H, s), 2.12 (3H, s), 2.09 (3H, s), 2.06 (9H, s), 1.99 (3H, s), 1.84 (3H, s), 1.67 (1H, t, J = 12.5 Hz), 1.33 (3H, d, J = 6.6 Hz), 0.99−0.91 (2H, m), and 0.01 (9H, s); ^{13}C NMR ($CDCl_3$) δ 170.9, 170.8, 170.6, 170.5, 170.4, 170.1, 169.4, 169.3, 167.9, 103.1, 100.0, 99.6, 96.8, 79.0, 75.2, 73.3, 72.3, 72.0, 71.3, 71.0, 70.9, 69.7, 69.5, 69.4, 68.0, 67.7, 67.4, 66.6, 66.0, 62.3, 61.7, 61.6, 58.9, 53.2, 49.1, 37.4, 23.1, 21.5, 20.9, 20.8, 20.8, 20.7, 20.7, 20.6, 18.1, 16.3, and −1.4; MS m/z (FAB+) calcd for $C_{51}H_{80}O_{30}N_2Si$ 1228, found 1229 (base peak; M + H$^+$). Anal. Calcd for $C_{51}H_{80}N_2O_{30}Si\cdot 3H_2O$: C, 47.73; H, 6.75; N, 2.18. Found: C, 47.92; H, 6.66; N, 2.41.

General Procedure for *N*-Acylation. Synthesis of 2-(Trimethylsilyl)ethyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)-*O*]-[6-*O*-acetyl-2-(acylamino)-2-deoxy-β-D-glucopyranoside] (15). Powdered sodium hydrogen carbonate (171 mg, 2.04 mmol) and acyl chloride (1.02 mmol) were added to a solution of amine **1** (500 mg, 0.406 mmol) in dichloromethane (40 mL) at room temperature. After the mixture was stirred for 20 h, methanol (10 mL) and pyridine (10 mL) were added to the reaction mixture. After 15 min, the mixture was concentrated under reduced pressure to afford amide **15**, which was then used in the next reaction without further purification.

General Procedure for Fucose Acetylation. Synthesis of 2-(Trimethylsilyl)ethyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→3)-*O*]-[6-*O*-acetyl-2-(acylamino)-2-deoxy-β-D-glucopyranoside] (16). Triol **15** was treated with acetic anhydride (20 mL) and 4-(dimethylamino)pyridine (80 mg) in pyridine (40 mL) at room temperature. After the mixture was stirred for 2 h, methanol (25 mL) was added dropwise to the mixture at 0 °C. The mixture was stirred at room temperature for 0.5 h and then concentrated in vacuo. The resulting residue was diluted with ethyl acetate and washed with saturated copper(II) sulfate, saturated sodium hydrogen carbonate and brine in that order. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford **16**.

General Procedure for Conversion of the 1-*O*-SE Group to Other Glycosides. Synthesis of Alkyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→3)-*O*]-[6-*O*-acetyl-2-(acylamino)-2-deoxy-β-D-glucopyranoside] (18). 1,1-Dichloromethyl methyl ether (32.0 μL, 0.355 mmol) and zinc chloride (2.4 mg, 17.6 μmol) were added to a stirred solution of **16** (0.071 mmol) in chloroform (5.0 mL) at room temperature. After stirring for 6 h, the mixture was concentrated under reduced pressure to give crude **17**.

A solution of alcohol (0.178 mmol) and TMU (17.0 μL, 0.142 mmol) in dichrolomethane (1.0 mL) was added dropwise to the stirred suspension of **17** obtained above, tin(II) trifluoromethanesulfonate [$Sn(OTf)_2$] (59.0 mg, 0.142 mmol), and powdered MS-4Å (95.0 mg) in dichloromethane (6.0 mL) at room temperature. After the mixture was stirred for 8 h, additional $Sn(OTf)_2$ (59.0 mg, 0.142 mmol), alcohol (0.178 mmol) and TMU (17.0 μL, 0.142 mmol) were added to the mixture. The mixture was stirred for 16 h at room temperature and then filtered through a pad of Celite. The filtrate was washed with saturated sodium hydrogen carbonate and brine in that order, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The resulting residue was purified by preparative thin layer chromatography (silica gel 60, 0.5 mm, 20 cm × 20 cm, E. Merck) developed with a mixture of chloroform and methanol to give **18**.

General Procedure for Deprotection. Synthesis of Alkyl (5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)-*O*]-[2-(acylamino)-2-deoxy-β-D-glucopyranoside] (3). To a stirred solution of **18** (36.8 μmol) in methanol (5.0 mL) was added sodium methoxide in methanol (28% assay, 50 μL) at room temperature. After stirring for 7 h, water (2.5 mL) was added to the mixture and the mixture was stirred for 2 days. The mixture was then concentrated under reduced pressure, purified by gel filtration chromatography, and lyophilized to afford **3**.

2-(Trimethylsilyl)ethyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)-*O*]-(6-*O*-acetyl-2-amino-2-*N*-butyryl-2-deoxy-β-D-glucopyranoside) (15a). The title compound was obtained as a crude residue from **1**, which was then used in the next reaction without further purification: $R_f = 0.63$ (developed with a 10:2:1 mixture of ethyl acetate, ethanol, and water).

2-(Trimethylsilyl)ethyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→3)-*O*]-(6-*O*-acetyl-2-amino-2-*N*-butyryl-2-deoxy-β-D-glucopyranoside) (16a). The title compound was obtained as colorless crystals in 86% yield from **1**: $R_f = 0.56$ (developed with a 9:1 mixture of chloroform and methanol); mp 152−155 °C; ^{1}H NMR ($CDCl_3$) δ 5.50−3.45 (30H, m), 3.85 (3H, s), 2.59 (1H, dd, J = 4.3 and 12.5 Hz), 2.20 (3H, s), 2.15 (3H, s), 2.13 (3H, s), 2.12 (3H, s), 2.09 (3H, s), 2.08 (3H, s), 2.07 (3H, s), 2.06 (3H, s), 2.04 (3H, s), 2.00 (3H, s), 2.00 (2H, t, J = 5.2 Hz), 1.94 (3H, s), 1.85 (3H, s), 1.70−1.56 (3H, m), 1.18 (3H, d, J = 6.6 Hz), 0.97−0.85 (2H, m), 0.88 (3H, t, J =

7.6 Hz), and 0.00 (9H, s). Anal. Calcd for $C_{61}H_{92}N_2O_{34}Si\cdot 2H_2O$: C, 50.13; H, 6.62; N, 1.92. Found: C, 50.27; H, 6.57; N, 1.99.

Dodecyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→3)-*O*]-(6-*O*-acetyl-2-amino-2-*N*-butyryl-2-deoxy-β-D-glucopyranoside) (18a). The title compound was obtained as a pale yellow amorphous solid in 52% yield from **16a**: $R_f = 0.47$ (developed with a 9:1 mixture of chloroform and methanol); ^{1}H NMR ($CDCl_3$) δ 5.50−3.35 (30H, m), 3.84 (3H, s), 2.56 (1H, dd, J = 4.3 and 12.5 Hz), 2.19 (3H, s), 2.14 (3H, s), 2.12 (3H, s), 2.11 (3H, s), 2.08 (3H, s), 2.07 (3H, s), 2.06 (3H, s), 2.05 (6H, s), 1.98 (3H, s), 1.93 (3H, s), 2.10−1.90 (2H, m),1.83 (3H, s), 1.75−1.45 (5H, m), 1.35−1.05 (18H, m), 1.17 (3H, d, J = 6.3 Hz), 0.93 (3H, t, J = 7.2 Hz), and 0.86 (3H, t, J = 7.2 Hz).

Dodecyl (5-Acetamide-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)-*O*]-(2-amino-2-*N*-butyryl-2-deoxy-β-D-glucopyranoside) (3a). The title compound was obtained as a colorless lyophilized solid in 78% yield from **18a**: $R_f = 0.48$ (developed with a 12:10:3 mixture of chloroform, methanol, and 15 mM aqueous calcium chloride); ^{1}H NMR (CD_3OD) δ 4.92 (1H, d, J = 4.0 Hz) 4.39 (1H, d, J = 7.6 Hz), 4.30 (1H, d, J = 7.6 Hz), 3.95−3.30 (25H, m), 2.75 (1H, dd, J = 4.3 and 12.5 Hz), 2.06 (2H, m), 1.89 (3H, s), 1.65−1.35 (5H, m), 1.17 (18H, br.s), 1.03 (3H, d, J = 6.6 Hz), 0.85 (3H, t, J = 7.4 Hz), and 0.78 (3H, t, J = 6.6 Hz); MS m/z (FAB+) calcd for $C_{45}H_{79}N_2O_{23}Na$ 1038, found 1039 ($M + H^+$). Anal. Calcd for $C_{45}H_{79}N_2O_{23}Na\cdot 5H_2O$: C, 47.86; H, 7.94; N, 2.48. Found: C, 47.75; H, 7.82; N, 2.46.

Ethyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→3)-*O*]-(6-*O*-acetyl-2-amino-2-*N*-butyryl-2-deoxy-β-D-glucopyranoside) (18b). The title compound was obtained as a pale yellow amorphous solid in 41% yield from **16a**: $R_f = 0.43$ (developed with a 9:1 mixture of chloroform and methanol); ^{1}H NMR ($CDCl_3$) δ 5.50−3.40 (30H, m), 3.85 (3H, s), 2.59 (1H, dd, J = 4.3 and 12.5 Hz), 2.19 (3H, s), 2.17 (3H, s), 2.15 (3H, s), 2.13 (3H, s), 2.11 (3H, s), 2.09 (3H, s), 2.07 (3H, s), 2.06 (3H, s), 2.05 (3H, s), 1.99 (3H, s), 1.94 (3H, s), 2.10−1.90 (2H, m), 1.84 (3H, s), 1.68−1.55 (3H, m), 1.17 (3H, d, J = 6.3 Hz), 1.15 (3H, t, J = 6.9 Hz), and 0.93 (3H, t, J = 7.4 Hz).

Ethyl (5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)-*O*]-(2-amino-2-*N*-butyryl-2-deoxy-β-D-glucopyranoside) (3b). The title compound was obtained as a colorless lyophilized solid in 75% yield from **18b**: $R_f = 0.48$ (developed with a 12:10:3 mixture of chloroform, methanol, and 15 mM aqueous calcium chloride); ^{1}H NMR (D_2O) δ 5.00 (1H, d, J = 4.0 Hz), 4.48−4.40 (2H, m), 4.00−3.39 (25H, m), 2.66 (1H, dd, J = 4.3 and 12.2 Hz), 2.15 (2H, t, J = 5.6 Hz), 1.92 (3H, s), 1.69 (1H, t, J = 12.2 Hz), 1.51 (2H, m), 1.06 (3H, d, J = 6.3 Hz), 1.05 (3H, t, J = 6.9 Hz), and 0.83 (3H, t, J = 7.4 Hz); MS m/z (FAB+) calcd for $C_{35}H_{59}N_2O_{23}Na$ 898, found 899 (base peak; $M + H^+$). Anal. Calcd for $C_{35}H_{59}N_2O_{23}Na\cdot 5H_2O$: C, 42.51; H, 7.03; N, 2.83. Found: C, 42.26; H, 6.67; N, 2.79.

2-(Trimethylsilyl)ethyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)-*O*]-(6-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranoside) (16c). Acetic anhydride (3.5 mL) and 4-(dimethylamino)pyridine (20 mg) were added to a solution of **1** (500 mg, 0.406 mmol) in pyridine (6.0 mL) at room temperature. After stirring for 18 h, methanol (6.0 mL) was added dropwise to the mixture at 0 °C. The mixture was stirred at room temperature for 1 h and then concentrated in vacuo. The resulting residue was diluted with ethyl acetate and washed with saturated sodium hydrogen carbonate and brine in that order. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography eluting with hexane, ethyl acetate, and acetone (1/1/1) to afford **16c** (489 mg, 86% yield) as pale yellow crystals: $R_f = 0.58$ (developed with a 1:1:2 mixture of hexane, ethyl acetate, and acetone); IR (KBr) 2967, 1745, 1670, 1542, 1438, 1373, 1240, 1048 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.55−3.45 (30H, m), 3.86 (3H, s), 2.59 (1H, dd, J = 4.3 and 12.6 Hz), 2.21 (3H, s), 2.16 (3H, s), 2.14 (3H, s), 2.12 (3H, s), 2.10 (3H, s), 2.08 (3H, s), 2.07 (3H, s), 2.06 (3H, s), 2.04 (3H, s), 2.00 (3H, s), 1.96 (3H, s), 1.95 (3H, s), 1.85 (3H, s), 1.68 (1H, t, J = 12.6 Hz), 1.19 (3H, d, J = 6.6 Hz), 0.94−0.84 (2H, m), and −0.01 (9H, s). Anal. Calcd for $C_{59}H_{88}N_2O_{34}Si\cdot 3H_2O$: C, 48.82; H, 6.53; N, 1.93. Found: C, 48.71; H, 6.33; N, 2.00.

Dodecyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→3)-*O*]-(6-*O*-acetyl-2-acetamide-2-deoxy-β-D-glucopyranoside) (18c). The title compound was obtained as a pale yellow amorphous solid in 46% yield from **16c**: ^{1}H NMR ($CDCl_3$) δ 5.69 (1H, d, J = 7.5 Hz), 5.55−3.30 (29H, m), 3.83 (3H, s), 2.56 (1H, dd, J = 4.3 and 12.5 Hz), 2.19 (3H, s), 2.14 (3H, s), 2.12 (3H, s), 2.11 (3H, s), 2.09 (3H, s), 2.06 (6H, s), 2.04 (6H, s), 1.98 (3H, s), 1.93 (6H, s), 1.84 (3H, s), 1.67 (1H, t, J = 12.5 Hz), 1.51 (2H, m), 1.35−1.00 (18H, m), 1.17 (3H, d, J = 6.3 Hz), and 0.86 (3H, t, J = 6.4 Hz).

Dodecyl (5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)-*O*]-(2-acetamido-2-deoxy-β-D-glucopyranoside) (3c). The title compound was obtained as a colorless lyophilized solid in 43% yield from **18c**: $R_f = 0.57$ (developed with a 12:10:3 mixture of chloroform, methanol, and 15 mM aqueous calcium chloride); ^{1}H NMR (D_2O) δ 5.01 (1H, d, J = 4.0 Hz), 4.50−4.40 (2H, m), 4.00−3.30 (25H, m), 2.68 (1H, dd, J = 4.3 and 12.5 Hz), 1.95 (6H, s), 1.71 (1H, t, J = 12.5 Hz), 1.45 (2H, m), 1.25−1.10 (18H, m), 1.08 (3H, d, J = 6.3 Hz), and 0.77 (3H, t, J = 6.6 Hz); MS m/z (FAB+) calcd for $C_{43}H_{76}N_2O_{23}Si$ 988, found 989 ($M + H^+$), 1011 ($M + Na^+$).

Ethyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→3)-*O*]-(6-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranoside) (18d). The title compound was obtained as a pale yellow amorphous solid in 72% yield from **16c**: ^{1}H NMR (CD_3OD) δ 5.55−3.40 (30H, m), 3.87 (3H, s), 2.58 (1H, d, J = 4.3 and 12.5 Hz), 2.18 (3H, s), 2.14 (3H, s), 2.11 (3H, s), 2.10 (3H, s), 2.08 (3H, s), 2.06 (3H, s), 2.05 (3H, s), 2.04 (3H, s), 2.03 (3H, s), 1.97 (3H, s), 1.94 (3H, s), 1.91 (3H, s), 1.81 (3H, s), 1.51 (1H, t, J = 12.5 Hz), 1.14 (3H, d, J = 6.9 Hz), and 1.13 (3H, t, J = 7.2 Hz).

Ethyl (5-Acetamide-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)-*O*]-(2-acetoamido-2-deoxy-β-D-glucopyranoside) (3d). The title compound was obtained as a colorless lyophilized solid in 87% yield from **18d**: ^{1}H NMR (D_2O) δ 5.00 (1H, d, J = 4.2 Hz), 4.48−4.40 (2H, m), 4.00−3.40 (25H, m), 2.72 (1H, dd, J = 4.3 and 12.5 Hz), 1.94 (6H, s), 1.71 (1H, t, J = 12.5 Hz), 1.07 (3H, d, J = 6.6 Hz), and 1.06 (3H, t, J = 6.9 Hz); MS m/z (FAB+) calcd for $C_{33}H_{56}N_2O_{23}Si$ 848, found 871 ($M + Na^+$).

2-(Trimethylsilyl)ethyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)-*O*]-[6-*O*-acetyl-2-amino-2-deoxy-2-*N*-(2-naphthoyl)-β-D-glucopyranoside] (15e). The title compound was obtained as a crude residue from **1**, which was then used in the next reaction without further purification: $R_f = 0.70$ (developed with a 10:2:1 mixture of ethyl acetate, ethanol, and water).

2-(Trimethylsilyl)ethyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→3)-*O*]-[6-*O*-acetyl-2-amino-2-deoxy-2-*N*-(2-naphthoyl)-β-D-glucopyranoside] (16e). The title com-

pound was obtained as colorless crystals in 86% yield from **1**: R_f = 0.38 (developed with a 1:1:1 mixture of hexane, ethyl acetate, and acetone); mp 156−158 °C; ^{1}H NMR ($CDCl_3$) δ 8.28 (1H, s), 7.92−7.82 (4H, m), 7.58−7.53 (2H, m), 6.37 (1H, d, J = 9.6 Hz), 5.60−4.65 (143H, m), 4.56 (1H, dd, J = 4.0 and 9.9 Hz), 4.45−3.80 (11H, m), 3.86 (3H, s), 3.70−3.45 (3H, m), 2.59 (1H, dd, J = 4.6 and 12.5 Hz), 2.23 (3H, s), 2.17 (3H, s), 2.11 (3H, s), 2.08 (9H, s), 2.07 (9H, s), 2.00 (3H, s), 1.93 (3H, s), 1.85 (3H, s), 1.70 (1H, t, J = 12.5 Hz), 1.18 (3H, d, J = 6.6 Hz), 0.90−0.82 (2H, m), and −0.08 (9H, s). Anal. Calcd for $C_{68}H_{92}N_2O_{34}Si\cdot 2H_2O$: C, 52.84; H, 6.26; N, 1.81. Found: C, 52.86; H, 6.28; N, 1.84.

Dodecyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-*O*-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→3)-*O*]-[6-*O*-acetyl-2-amino-2-deoxy-2-*N*-(2-naphthoyl)-*β*-D-glucopyranoside] (18e). The title compound was obtained as a pale yellow amorphous solid in 74% yield from **16e**: R_f = 0.54 (developed with a 19:1 mixture of chloroform and methanol); ^{1}H NMR ($CDCl_3$) δ 8.27 (1H, s), 7.92−7.81 (4H, m), 7.58−7.53 (2H, m), 6.28 (1H, d, J = 8.9 Hz), 5.56−3.75 (26H, m), 3.86 (3H, s), 3.63 (2H, m), 3.40 (1H, m), 2.59 (1H, dd, J = 4.5 and 12.5 Hz), 2.22 (3H, s), 2.17 (3H, s), 2.11 (3H, s), 2.08 (6H, s), 2.07 (9H, s), 2.04 (3H, s), 2.00 (3H, s), 1.93 (3H, s), 1.85 (3H, s), 1.75−1.55 (3H, m), 1.35−1.00 (18H, m), 1.19 (3H, d, J = 6.3 Hz), 0.87 (3H, t, J = 7.2 Hz).

Dodecyl (5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-(*β*-D-galactopyranosyl)-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)-*O*]-[2-amino-2-deoxy-2-*N*-(2-naphthoyl)-*β*-D-glucopyranoside] (3e). The title compound was obtained as a colorless lyophilized solid in 89% yield from **18e**: R_f = 0.56 (developed with a 12:10:3 mixture of chloroform, methanol, and 15 mM aqueous calcium chloride); ^{1}H NMR (CD_3OD) δ 8.28 (1H, s), 7.87−7.80 (4H, m), 7.50−7.45 (2H, m), 5.04 (1H, d, J = 4.0 Hz), 4.72 (1H, m), 4.46 (1H, d, J = 7.6 Hz), 4.10−2.90 (25H, m), 2.78 (1H, dd, J = 4.5 and 12.5 Hz), 1.91 (3H, s), 1.63 (1H, t, J = 12.5 Hz), 1.38 (2H, m), 1.20−0.90 (18H, m), 1.06 (3H, d, J = 6.6 Hz), 0.79 (3H, t, J = 7.2 Hz); MS m/z (FAB+) calcd for $C_{52}H_{79}N_2O_{23}Na$ 1122, found 1123 ($M + H^+$). Anal. Calcd for $C_{52}H_{79}N_2O_{23}Na\cdot 5.5H_2O$: C, 51.10; H, 7.42; N, 2.29. Found: C, 51.07; H, 7.54; N, 2.50.

Ethyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-*O*-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→3)-*O*]-[6-*O*-acetyl-2-amino-2-deoxy-2-*N*-(2-naphthoyl)-*β*-D-glucopyranoside] (18f). The title compound was obtained as a pale yellow amorphous solid in 77% yield from **16e**: R_f = 0.30 (developed with a 1:1:1 mixture of hexane, ethyl acetate, and acetone); ^{1}H NMR ($CDCl_3$) δ 8.29 (1H, s), 7.99−7.81 (4H, m), 7.59−7.52 (2H, m), 6.37 (1H, d, J = 8.9 Hz), 5.60−3.50 (26H, m), 3.86 (3H, s), 3.67−3.45 (3H, m), 2.59 (1H, dd, J = 4.3 and 12.5 Hz), 2.23 (3H, s), 2.17 (3H, s), 2.12 (3H, s), 2.11 (3H, s), 2.09 (6H, s), 2.08 (6H, s), 2.05 (3H, s), 2.01 (3H, s), 1.94 (3H, s), 1.86 (3H, s), 1.71 (1H, t, J = 12.5 Hz), 1.19 (3H, d, J = 6.6 Hz), 1.12 (3H, t, J = 7.2 Hz).

Ethyl (5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-(*β*-D-galactopyranosyl)-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)-*O*]-[2-amino-2-deoxy-2-*N*-(2-naphthoyl)-*β*-D-glucopyranoside] (3f). The title compound was obtained as a colorless lyophilized solid in 98% yield from **18f**: R_f = 0.51 (developed with a 12:10:3 mixture of chloroform, methanol, and 15 mM aqueous calcium chloride); ^{1}H NMR (D_2O) δ 8.23 (1H, s), 7.97−7.87 (3H, m), 7.72 (1H, dd, J = 2.0 and 8.9 Hz), 7.60−7.52 (2H, m), 5.09 (1H, d, J = 4.0 Hz), 4.64 (1H, m), 4.46 (1H, d, J = 7.9 Hz), 4.10−3.35 (25H, m), 2.66 (1H, dd, J = 4.6 and 12.5 Hz), 1.92 (3H, s), 1.70 (1H, t, J = 12.5 Hz), 1.06 (3H, d, J = 6.3 Hz), 0.99 (3H, t, J = 7.2 Hz); MS m/z (FAB+) calcd for $C_{42}H_{59}N_2O_{23}Na$ 982, found 983 (base peak; $M + H^+$). Anal. Calcd for $C_{42}H_{59}N_2O_{23}Na\cdot 8H_2O$: C, 44.76; H, 6.71; N, 2.49. Found: C, 44.45; H, 6.51; N, 2.54.

3-Azidopropyl [Methyl (5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-*O*-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→3)-*O*]-[6-*O*-acetyl-2-amino-2-*N*-butyryl-2-deoxy-*β*-D-glucopyranoside] (19). The title compound was obtained as a pale yellow amorphous solid in 39% yield from **16a**: R_f = 0.47 (developed with a 9:1 mixture of chloroform and methanol); IR (KBr) 2958, 2102, 1746, 1656, 1546, 1373, 1232, 1047 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.50−3.50 (28H, m), 3.85 (3H, s), 3.62 (2H, t, J = 5.4 Hz), 3.37 (2H, m), 2.58 (1H, dd, J = 4.3 and 12.5 Hz), 2.19 (3H, s), 2.15 (3H, s), 2.13 (3H, s), 2.12 (3H, s), 2.09 (3H, s), 2.08 (3H, s), 2.06 (9H, s), 2.00 (3H, s), 1.94 (3H, s), 1.84 (3H, s), 1.75−1.60 (5H, m), 1.17 (3H, d, J = 6.6 Hz), and 0.94 (3H, t, J = 7.3 Hz).

Acknowledgment. We are grateful to Drs. J. C. Paulson and R. Bayer in Cytel Corporation (La Jolla, CA) for providing us with enzymes (2,3-ST and CMP-NeuAc synthetase). We appreciate Drs. R. M. Ratcliffe and S. A. DeFrees in Cytel Corporation for their helpful discussions. We also thank Miss N. Kawamura for evaluation of the biological activity.

JO960125F