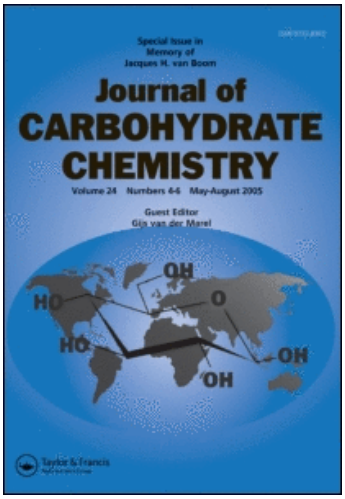

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Synthesis of Sialyl-α-(2→3)-Neolactotetraose Derivatives Containing Different Sialic Acids: Molecular Probes for Elucidation of Substrate Specificity of Human α1,3-Fucosyltransferases

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SYNTHESIS OF SIALYL-α-(2→3)-NEOLACTOTETRAOSE DERIVATIVES CONTAINING DIFFERENT SIALIC ACIDS: MOLECULAR PROBES FOR ELUCIDATION OF SUBSTRATE SPECIFICITY OF HUMAN α1,3-FUCOSYLTRANSFERASES[1]

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ABSTRACT

A series of sialyl-α-(2→3)-neolactotetraose derivatives containing *N*-acetyl- (NeuAc), *N*-glycolyl- (NeuGc) and *N*-butanoylneuraminic acid, and 3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (KDN) have systematically been synthesized as molecular probes for elucidation of substrate specificity of human α1,3-fucosyltransferases (Fuc-TVII and Fuc-TVI). 2-Methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2',1':4,5]-2-oxazoline (**1**) was coupled with 2-(trimethylsilyl)ethyl (2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**2**) to give a trisaccharide **3** which, upon successive *O*-deacetylation, benzylidenation and reductive opening of the benzylidene group, afforded a common glycosyl acceptor **5**. Glycosylation of **5** with sialyl-α-(2→3)-galactose donors **6-8**, **19** and **21** gave the corresponding pentasaccharides **22-25**, which were converted to a series of sialyl-α-(2→3)-neolactotetraose derivatives **30-33**. In the competitive enzyme

assay, the NeuGc derivative **32** showed the most potent activity for Fuc-TVII, while the KDN derivative **31** was less active than the standard NeuAc derivative **30**. In contrast, the *N*-butanoylation of neuraminic acid enhanced the activity for Fuc-TVI.

INTRODUCTION

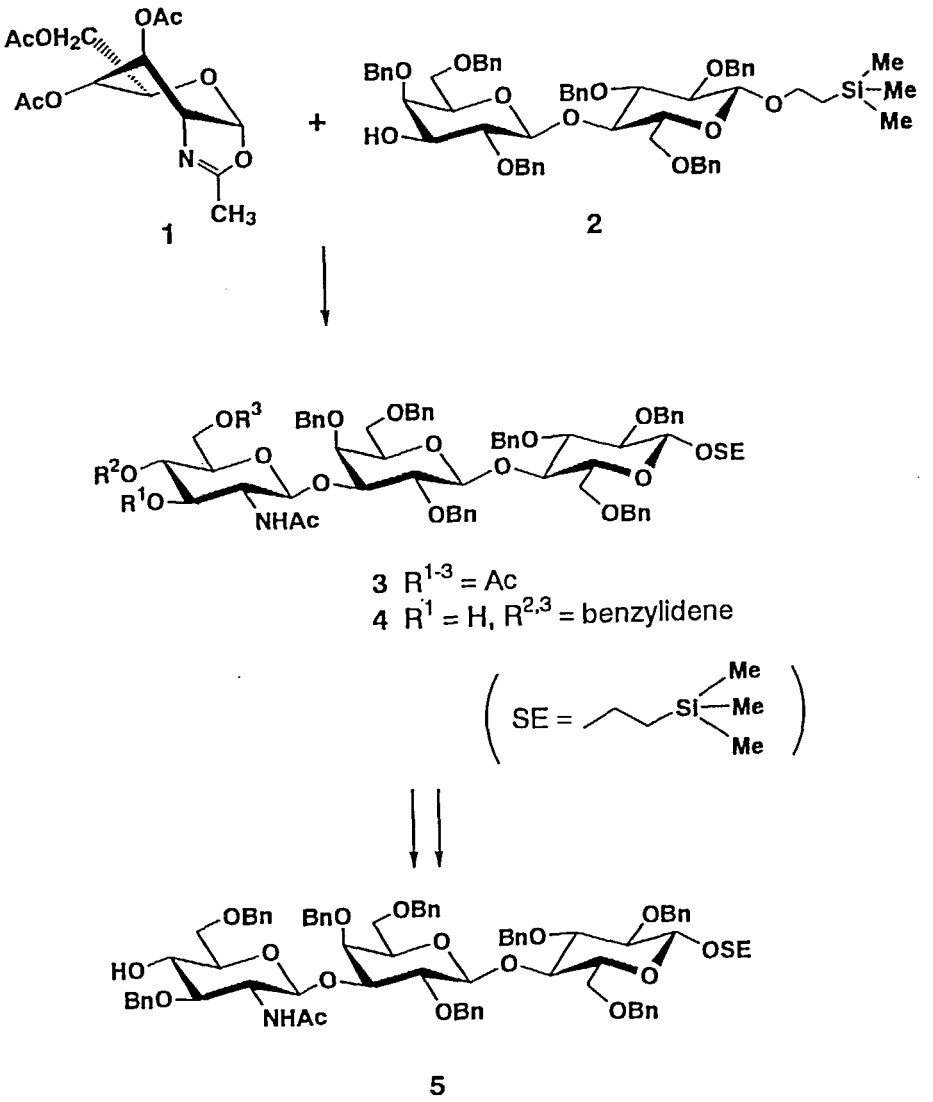
The sialyl Lewis x (sLe^x) tetrasaccharide determinant, NeuAc-α-(2→3)-Gal-β-(1→4)-[Fuc-α-(1→3)]-GlcNAc, has been identified not only as a tumor-associated antigen[2] but also as a common carbohydrate ligand for selectins[3] that are a family of cell adhesion molecules expressed on leukocytes, vascular endothelium and platelets, being implicated in leukocyte trafficking, thrombosis, inflammation, hematogenous metastasis of cancers, and so on.

Fuc-TVII,[4] a member of the α1,3-fucosyltransferase (Fuc-T) family, has been found to be a key enzyme in the biosynthesis of selectin ligands represented by the sLe^x tetrasaccharide or its structural variants.[5] Therefore, selective inhibitors for Fuc-TVII are expected to be therapeutics for the treatment of inflammatory diseases and cancer metastasis. We have recently reported the acceptor specificity of a cloned human Fuc-TVII by using a variety of sialyl-α-(2→3)-neolactotetraose probes as the biosynthetic precursors.[6] In the present paper, we describe the synthesis of a series of sialyl-α-(2→3)-neolactotetraose derivatives containing different sialic acids, and the acceptor specificity of Fuc-TVII in comparison with that of Fuc-TVI which shows activity toward both α2,3-sialylated and nonsialylated type-2 oligosaccharides.[7]

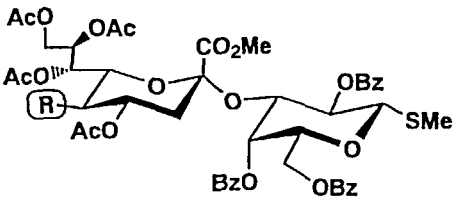
RESULTS AND DISCUSSION

For the systematic synthesis of the target pentasaccharides **30-33**, we selected the suitably protected GlcNAc-α-(1→3)-Gal-β-(1→4)-Glc trisaccharide **5**[8] as a common key glycosyl acceptor (Scheme 1), and a series of sialyl-α-(2→3)-galactose derivatives **6**,[9] **7**,[8] **8**,[10] **19** and **21** as the disaccharide glycosyl donors (Scheme 2).

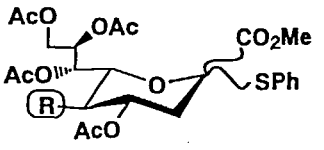
2-(Trimethylsilyl)ethyl (2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside[9] (**2**) was coupled with 2-methyl-(3,4,6-tri-*O*-

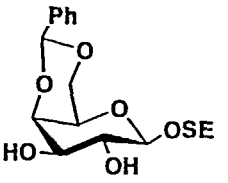


Scheme 1



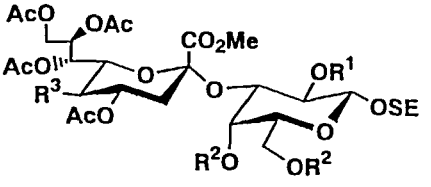
	R
6	NHAc
7	OAc
8	$NHCOCH_2OAc$



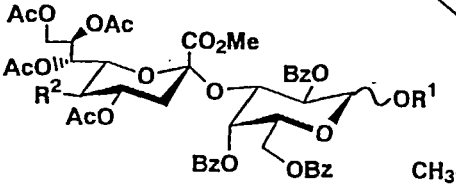


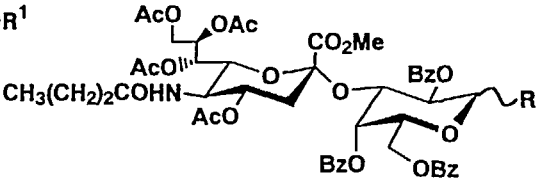
	R
9	$NHCOCH_2OAc$
10	$NHCO(CH_2)_2CH_3$

11



	R^1	R^2	R^3
12	H	benzylidene	$NHCOCH_2OAc$
13	H	benzylidene	$NHCO(CH_2)_2CH_3$
14	H	H	$NHCOCH_2OAc$
15	H	H	$NHCO(CH_2)_2CH_3$
16	Bz	Bz	$NHCOCH_2OAc$
17	Bz	Bz	$NHCO(CH_2)_2CH_3$





	R^1	R^2
18	H	$NHCOCH_2OAc$
19	$C(=NH)CCl_3$	$NHCOCH_2OAc$

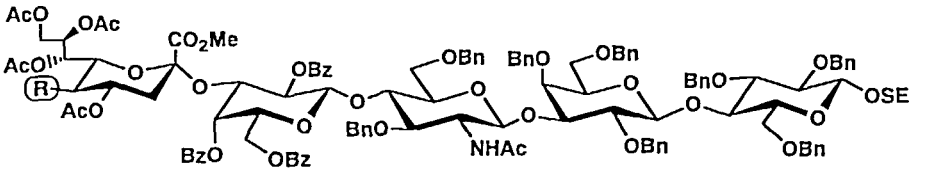
	R
20	OAc
21	SMe

Scheme 2

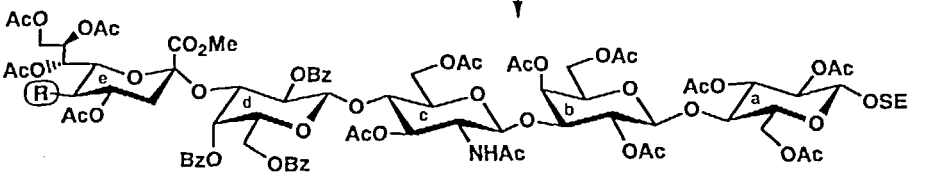
acetyl-1,2-dideoxy-α-D-glucopyrano)-[2',1':4,5]-2-oxazoline[11] (**1**) in dichloroethane in the presence of *p*-toluenesulfonic acid pyridinium salt at 70-80 °C to give the desired trisaccharide **3**[12] (66%), which was converted, by successive *O*-deacetylation, benzylidenation, benzylation and reductive opening of the benzylidene group, into a key glycosyl acceptor **5** (Scheme 1).

For the preparation of the *N*-glycolyl (**19**) and *N*-butanoyl (**21**) sialyl-α-(2→3)-galactose donors, the phenyl 2-thioglycosides of sialic acids (**9** and **10**) were each coupled[13] with **11** in the presence of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) in acetonitrile medium at -35 °C to give the desired α(2→3) glycosides **12** (63%) and **13** (55%), respectively (Scheme 2). Hydrogenolytic removal of the benzylidene group in **12** and **13**, and the subsequent benzoylation by use of benzoic anhydride and 4-dimethylaminopyridine (DMAP) in pyridine afforded **16** and **17** in high yields. The 2-(trimethylsilyl)ethyl group in **16** was selectively cleaved[14] by treatment with trifluoroacetic acid in dichloromethane to give the 1-hydroxy compound **18**, which upon further treatment[15] with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dichloromethane gave the trichloroacetimidate derivative **19** in 98% yield. On the other hand, treatment of **17** with acetic anhydride and BF_3 etherate in toluene afforded the 1-OAc derivative **20**, which was then converted to the methylthio glycoside **21** (Scheme 2).

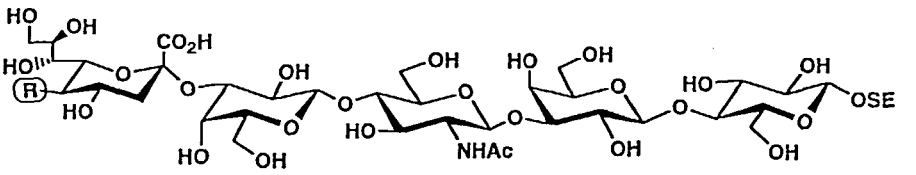
Couplings of the methylthio glycoside donors (**6-8**, **21**) with **5** were carried out[8,16] in the presence of dimethyl(methylthio)sulfonium triflate (DMTST) and molecular sieves 4Å (MS-4Å) in dichloromethane to give the corresponding pentasaccharides **22** (78%), **23**[8] (83%), **24** (55%) and **25** (60%), respectively. Coupling of the trichloroacetimidate donor **19** with **5** in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) in dichloromethane gave **24** in 77% yield (Scheme 3). Hydrogenolytic removal of the benzyl groups in **22-25** over $Pd(OH)_2$ in ethanol, followed by complete acetylation of the resulting free hydroxyl groups with Ac_2O-pyridine, afforded the fully acylated pentasaccharides **26-29** in high yields. *O*-Deacylation with sodium methoxide in methanol and subsequent saponification of the methyl ester group furnished the target sialyl-α(2→3)-neolactotetraose probes **30**



		R
5 + 6	→ 22	NHAc
5 + 7	→ 23	OAc
5 + 8 or 5 + 19	→ 24	$NHCOCH_2OAc$
5 + 21	→ 25	$NHCO(CH_2)_2CH_3$



	R
26	NHAc
27	OAc
28	$NHCOCH_2OAc$
29	$NHCO(CH_2)_2CH_3$



	R
30 (GSC-253)	NHAc
31 (GSC-304)	OH
32 (GSC-306)	$NHCOCH_2OH$
33 (GSC-391)	$NHCO(CH_2)_2CH_3$

Scheme 3

(GSC-253), **31** (GSC-304), **32** (GSC-306) and **33** (GSC-391) in almost quantitative yields after chromatography on a column of Sephadex LH-20.

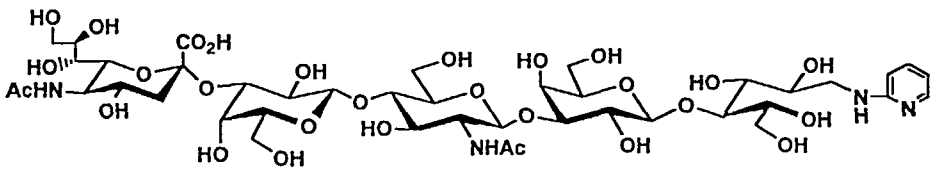
The competitive enzyme assay of the synthetic sialyl-α(2→3)-neolactotetraose probes **30-33** against the pyridylaminated sialyl-α(2→3)-neolactotetraose derivative (**34**) was performed[6] for human α1,3-fucosyltransferases, Fuc-TVII and Fuc-TVI (Scheme 4 and Table 1). The competition of **30-33** was measured and compared to that of the NeuAc derivative (**30**, GSC-253). Modification of the acetamido group at C-5 of *N*-acetylneuraminic acid (**30**, GSC-253) with the glycolylamino group (**32**, GSC-306) significantly increased the relative competition for Fuc-TVII (100→145.1%), while the degree for Fuc-TVI was almost comparable. Therefore, GSC-306 seems to be a good substrate for Fuc-TVII. In contrast, substitution at C-5 with the hydroxyl group (**31**, GSC-304) was not effective for the competition for either Fuc-TVII or Fuc-TVI. Compound **33** (GSC-391), in which the acetamido group at C-5 of **30** is replaced by the butanamido group, exhibited a significantly higher activity (100→139.6%) for Fuc-TVI than Fuc-TVII. Therefore, these analogs could be good candidates not only for designing selective inhibitors for Fuc-TVII or Fuc-TVI, but also for producing the corresponding sLe^x analogs enzymatically.

EXPERIMENTAL

1. Chemical synthesis

General methods. Optical rotations were determined with a Union PM-201 polarimeter at 25 °C, and ^{1}H NMR spectra were recorded on Varian UNITY Inova (400 and 500 MHz) spectrometers with TMS as the internal standard. All reactions were monitored by TLC (Merck silica gel aluminum plates 60F-254) and preparative chromatography was performed on silica gel (Fuji Silysia Co. 300 mesh) with the solvent systems specified. Concentrations were conducted *in vacuo*.

2-(Trimethylsilyl)ethyl (3,4,6-Tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyrano-



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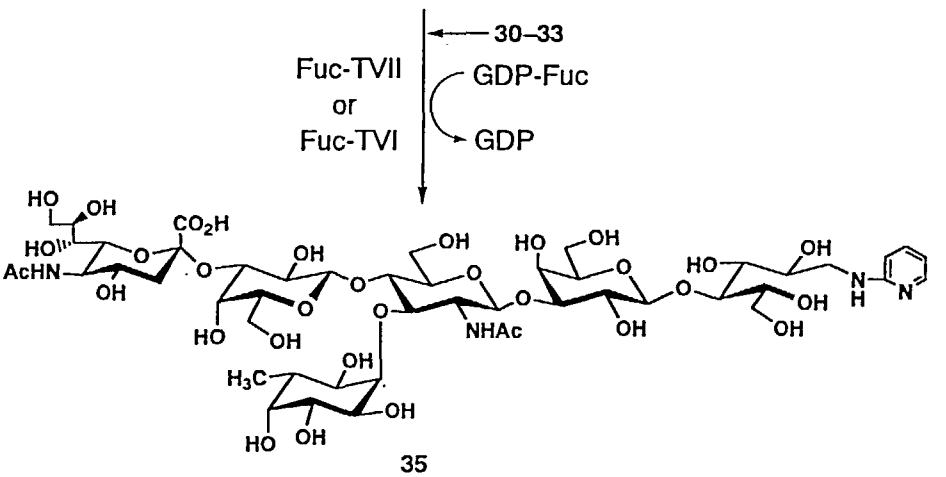

35

Scheme 4

Table 1. Relative Competition of **30-33** for Fuc-TVII and Fuc-TVI

Compound	Relative Competition[a] (%)	
	Fuc-TVII	Fuc-TVI
30 (GSC-253)	100	100
31 (GSC-304)	78.9	90.8
32 (GSC-306)	145.1	88.7
33 (GSC-391)	56.1	139.6

a. See Experimental section and ref.6.

syl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (3). To a solution of 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2',1':4,5]-2-oxazoline (**1**, 4.7 g, 14.3 mmol) and 2-(trimethylsilyl)ethyl (2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**2**, 3.5 g, 3.3 mmol) in 1,2-dichloroethane (30 mL) was added powdered *p*-toluenesulfonic acid pyridinium salt (675 mg, 2.68 mmol), and the mixture was stirred for 24 h at 70-80 °C. The mixture was diluted with chloroform, washed with water, dried (Na_2SO_4), and concentrated to a residue which was chromatographed on a column of silica gel with 5:1 and 2:1 hexane-ethyl acetate to give **2** (1.0 g, 29%) and **3** (3.1 g, 66%), respectively. The physicochemical properties and spectral data of **3** thus obtained were identical with those of **3** reported by Nashed et al.[12]

2-(Trimethylsilyl)ethyl (2-Acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (5). To a solution of **3** (2.4 g, 1.83 mmol) in dry methanol (20 mL) was added 5 drops of 28% methanolic sodium methoxide, and the mixture was stirred for 40 min at room temperature. The solution was neutralized with Amberlite IR-120 (H^+) resin and concentrated to a residue, which was treated with benzaldehyde dimethyl acetal (560 mg) and *p*-toluenesulfonic acid monohydrate (30 mg) in acetonitrile (15 mL) for 1.5 h at room temperature; it was neutralized with Amberlite IR-45 resin and concentrated. Column chromatography (2:1 ethyl acetate-hexane) of the residue on silica gel gave the 4,6-*O*-benzylidene derivative (**4**, 2.2 g, 94%).

To a stirred solution of **4** (2.0 g, 1.57 mmol) in DMF (10 mL) was added 60% sodium hydride (83 mg, 1.88 mmol) at -15 °C, and the stirring was continued for 2 h. Benzyl bromide (354 mg, 1.88 mmol) was added, and the mixture was cooled to 0 °C and a small amount of methanol was added to decompose the excess reagents. The product was extracted with toluene and the extract was successively washed with 2M HCl, sat. $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. Column chromatography (1:2 ethyl acetate-hexane) of the residue on silica gel afforded 2-(trimethylsilyl)ethyl (2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-

(1 → 3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (2.04 g, 95%), which was converted to the title compound **5** (1.65 g, 77%) as described in ref. 8.

Methyl (Phenyl 4,7,8,9-Tetra-*O*-acetyl-5-butanoylamino-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (10). A mixture of methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate[17] (3.0g, 5.14 mmol) and methanesulfonic acid (0.5 mL, 7.71 mmol) in methanol (20 mL) was heated for 24 h under reflux (bath temp., 60 °C); the reaction being monitored by TLC (3:2 chloroform-methanol). The pH of the reaction mixture was adjusted to 9-10 with triethylamine at 0 °C, and the mixture was further treated with butyric anhydride (3.4 mL, 20.78 mmol) overnight at room temperature, and then concentrated. To the solution of the residue in acetic anhydride (20 mL) was added pyridine (5 mL) dropwise at 0 °C, and the mixture was stirred overnight at room temperature. Methanol was added at 0 °C and the mixture was concentrated. The residue was taken-up in chloroform and washed with cold M hydrochloric acid and water, dried (Na_2SO_4) and concentrated. Column chromatography (2:1 ethyl acetate-hexane) of the residue on silica gel gave **10** (2.53 g, 81%) as an amorphous mass: ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3H, *Me*CH_2-), 1.59 (m, 2H, MeCH_2-), 1.97, 2.03, 2.08, 2.11 (4s, 12H, 4AcO), 2.13 (t, 2H, $MeCH_2CH_2CO$-), 2.68 (dd, 1H, J_{gem} = 13.7 Hz, $J_{3eq,4}$ = 4.9 Hz, H-3*eq*), 3.60 (s, 3H, *Me*O), 4.00, 4.48 (2dd, 2H, J_{gem} = 12.1 Hz, $J_{8,9}$ = 8.8 Hz, $J_{8,9'}$ = 2.2 Hz, H-9,9'), 4.16 (~q, 1H, J = 10.5 Hz, H-5), 4.63 (dd, 1H, $J_{5,6}$ = 10.5 Hz, $J_{6,7}$ = 2.2 Hz, H-6), 4.94 (m, 1H, $J_{7,8}$ = 2.2 Hz, H-8), 5.41 (m, 1H, H-4), 5.45 (~t, 1H, J = 2.2 Hz, H-7), 5.63 (d, 1H, $J_{NH,5}$ = 10.3 Hz, NH), and 7.33-7.47 (m, 5H, Ph).

Anal. Calcd for $C_{28}H_{37}NO_{12}S$ (611.67): C, 54.98; H, 6.10; N, 2.29. Found: C, 54.91; H, 5.85; N, 2.03.

2-(Trimethylsilyl)ethyl (Methyl 5-Acetoxyacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-4,6-*O*-benzylidene-β-D-galactopyranoside (12). To a stirred mixture of **9** (1.08 g, 1.77 mmol), 2-(trimethylsilyl)ethyl 4,6-*O*-benzylidene-β-D-

galactopyranoside (**11**, 500 mg, 1.36 mmol) and molecular sieves 3Å (MS-3Å, 800 mg) in acetonitrile (9 mL) were added *N*-iodosuccinimide (NIS, 713 mg, 3.18 mmol) and trifluoromethanesulfonic acid (TfOH, 28 μL, 0.32 mmol) at -35 °C; the stirring was continued overnight at -35 °C. The precipitate was filtered off and washed with chloroform. The filtrate and washings were combined and successively washed with M sodium carbonate and sodium thiosulfate, dried (Na_2SO_4) and concentrated. Column chromatography (80:1 chloroform-methanol, then 40:1 toluene-methanol) of the residue on silica gel gave **12** (740 mg, 63%): $[\alpha]_D$ +2.12° (*c* 0.6, $CHCl_3$): ^1H NMR ($CDCl_3$) δ 1.00 (m, 2H, $Me_3SiCH_2CH_2$-), 1.98, 2.02, 2.13, 2.16, 2.19 (5s, 15H, 5AcO), 2.74 (dd, 1H, J_{gem} = 13.3 Hz, $J_{3eq,4}$ = 4.4 Hz, H-3b*eq*), 3.59 (s, 3H, *Me*O), 3.84 (dd, 1H, $J_{1,2}$ = 7.7 Hz, $J_{2,3}$ = 9.5 Hz, H-2a), 4.19 (dd, 1H, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 3.7 Hz, H-3a), 4.28, 4.58 (2dd, 2H, J_{gem} = 15.4 Hz, $AcOCH_2CONH$-), 4.44 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1a), 5.23 (dd, 1H, $J_{6,7}$ = 1.8 Hz, $J_{7,8}$ = 9.2 Hz, H-7b), 5.36 (s, 1H, C*H*Ph), 5.42 (m, 1H, H-8b), 5.87 (d, 1H, NH), and 7.24-7.49 (m, 5H, Ph).

Anal. Calcd for $C_{40}H_{57}NO_{20}Si$ (899.97): C, 53.38; H, 6.38; N, 1.56. Found: C, 53.35; H, 6.20; N, 1.35.

2-(Trimethylsilyl)ethyl (Methyl 4,7,8,9-Tetra-*O*-acetyl-5-butanoylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-4,6-*O*-benzylidene-β-D-galactopyranoside (13). To a stirred mixture of **10** (1.08 g, 1.76 mmol), **11** (500 mg, 1.36 mmol) and MS-3Å (800 mg) in acetonitrile (6 mL) were added NIS (714 mg) and TfOH (28 μL) at -35 °C, and the stirring was continued overnight at -35 °C. Work-up and column chromatography (100:1 chloroform-methanol) on silica gel as described for **12** afforded **13** (624 mg, 55%): $[\alpha]_D$ +6.6° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.89 (t, 3H, *Me*CH_2-), 1.02 (m, 2H, $Me_3SiCH_2CH_2$-), 1.56 (m, 2H, $MeCH_2$-), 1.98, 2.02, 2.14, 2.17 (4s, 12H, 4AcO), 2.72 (dd, 1H, J_{gem} = 13.7 Hz, $J_{3eq,4}$ = 4.9 Hz, H-3b*eq*), 3.58 (s, 3H, *Me*O), 4.45 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1a), 5.26 (dd, 1H, $J_{6,7}$ = 1.8 Hz, $J_{7,8}$ = 9.2 Hz, H-7b), 5.31 (d, 1H, $J_{NH,5}$ = 9.5 Hz, NH), 5.36 (s, 1H, C*H*Ph), 5.40 (m, 1H, H-8b), and 7.29-7.48 (m, 5H, Ph).

Anal. Calcd for $C_{40}H_{59}NO_{18}Si$ (869.99): C, 55.22; H, 6.84; N, 1.61. Found: C, 55.15; H, 6.77; N, 1.52.

2-(Trimethylsilyl)ethyl (Methyl 5-Acetoxyacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-β-D-galactopyranoside (14). Compound **12** (1.2 g) was hydrogenolyzed in the presence of 10% Pd-C (1.2 g) in acetic acid (15 mL). Work-up and column chromatography (50:1 toluene-methanol) on silica gel gave **14** (0.79 g, 74%): $[\alpha]_D$ -12.3° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.97 (m, 2H, $Me_3SiCH_2CH_2$-), 2.00, 2.02, 2.11, 2.13, 2.16 (5s, 15H, 5AcO), 2.69 (dd, 1H, J_{gem} = 12.8 Hz, $J_{3eq,4}$ = 4.4 Hz, H-3b*eq*), 3.83 (s, 3H, *Me*O), 4.30, 4.57 (2dd, 2H, J_{gem} = 15.4 Hz, $AcOCH_2CO$-), 4.39 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1a), 5.00 (m, 1H, H-4b), 5.23 (dd, 1H, $J_{6,7}$ = 2.2 Hz, $J_{7,8}$ = 8.8 Hz, H-7b), 5.42 (m, 1H, H-8b), and 5.98 (d, 1H, NH).

Anal. Calcd for $C_{33}H_{53}NO_{20}Si$ (811.86): C, 48.82; H, 6.58; N, 1.73. Found: C, 48.60; H, 6.32; N, 1.60.

2-(Trimethylsilyl)ethyl (Methyl 4,7,8,9-Tetra-*O*-acetyl-5-butanoylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-β-D-galactopyranoside (15). Compound **13** (532 mg) was hydrogenolyzed as described for **12**. The product was purified by chromatography (15:1 toluene-methanol) on a column of silica gel to give **15** (392 mg, 82%) as an amorphous mass: $[\alpha]_D$ -10.6° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.89 (t, 3H, $MeCH_2$-), 1.09 (m, 2H, $Me_3SiCH_2CH_2$-), 1.56 (m, 2H, $MeCH_2$-), 1.68, 2.00, 2.02, 2.12 (4s, 12H, 4AcO), 2.67 (dd, 1H, J_{gem} = 12.8 Hz, $J_{3eq,4}$ = 4.4 Hz, H-3b*eq*), 3.81 (s, 3H, *Me*O), 4.40 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1a), 4.97 (m, 1H, H-4b), 5.22 (d, 1H, $J_{NH,5}$ = 9.5 Hz, NH), 5.25 (dd, 1H, $J_{6,7}$ = 1.6 Hz, $J_{7,8}$ = 9.0 Hz, H-7b), and 5.40 (m, 1H, H-8b).

Anal. Calcd for $C_{33}H_{55}NO_{18}Si$ (781.88): C, 50.69; H, 7.09; N, 1.79. Found: C, 50.52; H, 7.06; N, 1.64.

2-(Trimethylsilyl)ethyl (Methyl 5-Acetoxyacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (16). A mixture of **14** (790 mg, 1.0 mmol), benzoic anhydride (1.03 g, 4.5 mmol) and 4-dimethylaminopyridine (DMAP; 12 mg, 0.1 mmol) in pyridine (5 mL) was stirred for 2

days at room temperature. Methanol was added and the product was extracted with chloroform. The extract was successively washed with ice-cooled 2M hydrogen chloride and water, dried (Na_2SO_4), and concentrated. Column chromatography (50:1 chloroform-methanol) of the residue on silica gel gave **16** (958 mg, 87%) as an amorphous mass. The physiochemical properties and spectral date of **16** were identical with those reported in ref. 10.

2-(Trimethylsilyl)ethyl (Methyl 4,7,8,9-Tetra-*O*-acetyl-5-butanoylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (17). Compound **15** (292 mg) was benzoylated as described for **16**, and the product was purified by chromatography (100:1 chloroform-methanol) on a column of silica gel to give **17** (345 mg, 85%) as an amorphous mass: $[\alpha]_D$ +28.2° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.93 (t, 3H, *Me*CH_2-), 1.02 (m, 2H, $Me_3SiCH_2CH_2$-), 1.56 (m, 2H, MeCH_2-), 1.71, 2.00, 2.16, 2.27 (4s, 12H, 4AcO), 2.55 (dd, 1H, J_{gem} = 12.5 Hz, $J_{3eq,4}$ = 4.8 Hz, H-3b*eq*), 3.94 (s, 3H, *Me*O), 4.94 (m, 1H, H-4b), 5.03 (d, 1H, $J_{NH,5}$ = 10.3 Hz, NH), 5.26 (dd, 1H, $J_{6,7}$ = 2.4 Hz, $J_{7,8}$ = 9.7 Hz, H-7b), 5.46 (~d, 1H, H-4a), 5.52 (dd, 1H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 9.9 Hz, H-2a), 5.72 (m, 1H, H-8b), and 7.50-8.26 (m, 15H, Ph).

Anal. Calcd for $C_{54}H_{67}NO_{21}Si$ (1094.20): C, 59.28; H, 6.17; N, 1.28. Found: C, 59.19; H, 6.13; N, 1.06.

(Methyl 5-Acetoxyacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate (19). To a solution of **16** (400 mg, 0.36 mmol) in dichloromethane (2 mL) was added trifluoroacetic acid (TFA, 2 mL) at 0 °C, and the mixture was stirred for 45 min at room temperature. Toluene was added and the mixture was concentrated to dryness. Column chromatography (50:1 chloroform-methanol) of the residue on silica gel afford **18** (360 mg, quant.). To a stirred solution of **18** (363 mg, 0.36 mmol) in dichloromethane (3 mL) were added trichloroacetonitrile (1.1. mL, 10.8 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 54 μL, 0.36 mmol) at 0 °C; the stirring was continued for 1 h at 0 °C. The

mixture was concentrated (bath temp., 35 °C) to a syrup which was chromatographed (50:1 chloroform-methanol) on a column of silica gel to give **19** (408 mg, 98%) as an amorphous mass: $[\alpha]_D$ +15.2° (*c* 2.4, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.89, 1.97, 2.07, 2.10, 2.17 (5s, 15H, 5AcO), 2.49 (dd, 1H, J_{gem} = 12.8 Hz, $J_{3eq,4}$ = 4.4 Hz, H-3b*eq*), 3.80 (s, 3H, *Me*O), 4.28, 4.59 (2dd, 2H, J_{gem} = 15.4 Hz, AcOC*H*$_2$CO-), 4.90 (m, 1H, H-4b), 5.40 (dd, 1H, $J_{6,7}$ = 1.8 Hz, $J_{7,8}$ = 9.5 Hz, H-7b), 5.51 (dd, 1H, $J_{2,3}$ = 10.6 Hz, $J_{3,4}$ = 2.9 Hz, H-3a), 5.64 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.6 Hz, H-2a), 5.72 (d, 1H, $J_{3,4}$ = 2.9 Hz, H-4a), 5.87 (d, 1H, NH), 6.88 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1a), 7.42-8.19 (m, 15H, 3Ph), and 8.63 (s, 1H, C=NH).

Anal. Calcd for $C_{51}H_{53}Cl_3N_2O_{23}$ (1168.34): C, 52.43; H, 4.57; N, 2.40. Found: C, 52.31; H, 4.38; N, 2.30.

(Methyl 4,7,8,9-Tetra-*O*-acetyl-5-butanoylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2 → 3)-1-*O*-acetyl-2,4,6-tri-*O*-benzoyl-D-galactopyranose (20). To a solution of **17** (300 mg, 0.27 mmol) in toluene (1.5 mL) were added acetic anhydride (0.36 mL, 4.14 mmol) and BF_3 etherate (66 μL, 0.25 mmol), and the mixture was stirred for 4 h at room temperature. The product was extracted with toluene, and the extract was successively washed with M Na_2CO_3 and water, dried (Na_2SO_4), and concentrated. Column chromatography (80:1 toluene-methanol) of the residue on silica gel afforded **20** (252 mg, 89%): as a mixture of (α:β = 1:7): $[\alpha]_D$ +40.7° (*c* 1.1, $CHCl_3$); ^1H NMR for β acetate ($CDCl_3$) δ 0.84 (t, 3H, *Me*CH_2-), 1.47 (m, 2H, Me*CH*$_2$-), 1.62, 1.90, 2.02, 2.10, 2.18 (5s, 15H, 5AcO) 2.48 (dd, 1H, J_{gem} = 12.6 Hz, $J_{3eq,4}$ = 4.8 Hz, H-3b*eq*), 3.86 (s, 3H, *Me*O), 4.82 (m, 1H, H-4b), 4.96 (d, 1H, $J_{NH,5}$ = 10.3 Hz, NH), 5.44 (~d, 1H, J = 2.7 Hz, H-4a), 5.57 (dd, 1H, H-2a), 5.59 (m, 1H, H-8b), 6.16 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1a), and 7.39-8.14 (m, 15H, 3Ph). The anomeric proton of α-isomer appeared at δ 6.57 (d, $J_{1,2}$ = 3.7 Hz).

Anal. Calcd for $C_{51}H_{57}NO_{22}$ (1036.00): C, 59.13; H, 5.55; N, 1.35. Found: C, 59.02; H, 5.27; N, 1.20.

Methyl (Methyl 4,7,8,9-Tetra-*O*-acetyl-5-butanoylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2 → 3)-2,4,6-

tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (21). A mixture of **20** (158 mg, 0.18 mmol), methylthiotrimethylsilane (TMSSMe; 52 μL, 0.37 mmol) and trimethylsilyl trifluoromethanesulfonate (TMSOTf; 35 μL, 0.18 mmol) in dichloroethane (2 mL) was stirred for 6.5 h at 50 °C. The product was extracted with chloroform, and the extract was successively washed with M Na_2CO_3 and water, dried (Na_2SO_4), and concentrated. Column chromatography (100:1 chloroform-methanol) of the residue on silica gel gave **21** (152 mg, 97%) as an amorphous mass: $[\alpha]_D$ +29.7° (*c* 0.89, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3H, *Me*CH_2-), 1.60 (m, 2H, MeCH_2-), 1.49, 1.91, 2.08, 2.17, 2.27 (5s, 15H, 4AcO, MeS), 2.48 (dd, 1H, J_{gem} = 12.7 Hz, $J_{3eq,4}$ = 4.5 Hz, H-3b*eq*), 3.83 (s, 3H, *Me*O), 4.84 (m, 1H, H-4b), 4.95 (d, 1H, $J_{NH,5}$ = 10.6 Hz, NH), 5.18 (dd, 1H, $J_{6,7}$ = 2.5 Hz, $J_{7,8}$ = 9.6 Hz, H-7b), 5.44 (~d, 1H, J = 3.2 Hz, H-4a), 5.56 (dd, 1H, $J_{1,2}$ = 9.6 Hz, $J_{2,3}$ = 10.3 Hz, H-2a), 5.60 (m, 1H, H-8b), and 7.27-8.14 (m, 15H, 3Ph).

Anal. Calcd for $C_{50}H_{57}NO_{20}S$ (1024.06): C, 58.64; H, 5.61; N, 1.37. Found: C, 58.47; H, 5.41; N, 1.36.

2-(Trimethylsilyl)ethyl (Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (22). A mixture of **5** (683 mg, 0.5 mmol), **6** (996 mg, 1.0 mmol) and MS-4Å (2 g) in dichloromethane (10 mL) was stirred 6 h at room temperature, and then cooled to 0 °C. Dimethyl(methylthio)sulfonium triflate (DMTST; 780 mg, 3 mmol) was added, and the stirring was continued for 24 h at 7 °C. The solids were filtered off and washed with chloroform. The filtrate and washings were combined, and successively washed with sat. $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. Column chromatography (2:1 hexane-ethyl acetate) of the residue on silica gel gave **22** (0.9 g, 78%): $[\alpha]_D$ +10.9° (*c* 1.69, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.00 (m, 2H, $Me_3SiCH_2CH_2$-), 1.45, 1.50 (2s, 6H, 2AcN), 1.88, 1.93, 1.98, 2.13 (4s, 12H, 4AcO), 2.44 (dd, 1H, J_{gem} = 12.3 Hz, $J_{3eq,4}$ = 4.4 Hz, H-3*eq* of NeuAc), 3.79 (s, 3H, *Me*O), 5.08 (d, 1H,

$J_{1,2}$ = 7.8 Hz, H-1 of terminal Gal), 5.24 (dd, 1H, $J_{6,7}$ = 2.5 Hz, $J_{7,8}$ = 9.6 Hz, H-7 of NeuAc), and 7.07-8.23 (m, 55H, 11Ph).

Anal. Calcd for $C_{128}H_{144}N_2O_{36}Si$ (2314.62): C, 66.42; H, 6.27; N, 1.21. Found: C, 66.41; H, 6.18; N, 0.97.

2-(Trimethylsilyl)ethyl (Methyl 5-Acetoxyacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2 → 3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (24). (Route A: **5** + **8**) A mixture of **5** (720 mg, 0.53 mmol), **8** (830 mg, 0.79 mmol) and MS-4Å (1.6 g) in dichloromethane (12 mL) was stirred for 5 h at room temperature. DMTST (mg, 3.1 mmol) was added, and the stirring was continued for 48 h at 7 °C. Work-up and column chromatography (160:1 dichloromethane-methanol) on silica gel afforded **24** (687 mg, 55%). (Route B: **5** + **19**) A mixture of **5** 265 mg, 0.20 mmol), **19** (194 mg, 0.17 mmol) and MS-4Å (AW-300, 450 mg) in dichloromethane (0.8 mL) was stirred for 4 h at room temperature, and then cooled to 0 °C. TMSOTf (3.29 μL, 17 μmol) was added and the stirring was continued overnight at 0 °C. The solids were filtered off and washed with chloroform. The filtrate and washings were combined and successively washed with M Na_2CO_3 and water, dried (Na_2SO_4), and concentrated. Column chromatography (80:1 chloroform-methanol) on silica gel gave **24** (304 mg, 77%) as an amorphous mass: $[\alpha]_D$ +7.3° (*c* 0.71, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.00 (m, 2H, $Me_3SiCH_2CH_2$-), 1.44 (s, 3H, AcN), 1.67 (t, 1H, J = 12.6 Hz, H-3e*ax*), 1.49, 1.89, 1.96, 2.13. 2.16 (5s, 15H, 5AcO), 2.47 (dd, 1H, J_{gem} = 12.6 Hz, $J_{3eq,4}$ = 4.4 Hz, H-3*eq*), 3.84 (s, 3H, *Me*O), 4.20, 4.49 (dd, 2H, J_{gem} = 15.3 Hz, AcOCH_2CO-), 5.07 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1d), 5.36 (d, 1H, $J_{3,4}$ = 3.4 Hz, H-4d), 5.49 (dd, 1H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 9.8 Hz, H-2d), 5.67 (m, 1H, H-8e), and 7.06-8.23 (m, 55H, 11Ph).

Anal. Calcd for $C_{130}H_{146}N_2O_{38}Si$ (2372.66): C, 65.81; H, 6.20; N, 1.18. Found: C, 65.60; H, 5.94; N, 0.91.

2-(Trimethylsilyl)ethyl (Methyl 4,7,8,9-Tetra-*O*-acetyl-5-butanoylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (25). A mixture of **5** (156 mg, 0.11 mmol), **21** (152 mg, 0.15 mmol) and MS-4Å (215 mg) in dichloromethane (2 mL) was stirred for 6 h at room temperature, and then cooled to 0 °C. DMTST (287 mg, 0.68 mmol) was added and the stirring was continued for 48 h at 7 °C. Work-up as described for **22** and column chromatography (1:1 ethyl acetate-hexane) on silica gel gave **25** (160 mg, 60%) as an amorphous mass: $[\alpha]_D$ +11.3° (*c* 0.48, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.85 (t, 3H, *Me*CH_2-), 1.01 (m, 2H, $Me_3SiCH_2CH_2$-), 1.50 (m, 2H, $MeCH_2$-), 1.43-2.14 (4s, 12H, 4AcO), 1.95 (t, 2H, $MeCH_2CH_2CO$-), 2.44 (dd, 1H, J_{gem} = 12.6 Hz, $J_{3eq,4}$ = 4.6 Hz, H-3e*eq*), 3.85 (s, 3H, *Me*O), 4.84 (m, 1H, H-4e), 5.14 (d, 1H, $J_{NH,5}$ = 9.2 Hz, NH), 5.35 (d, 1H, $J_{3,4}$ = 2.7 Hz, H-4d), 5.47 (dd, 1H, H-2d), 5.66 (m, 1H, H-8e), and 7.06-8.23 (m, 15H, 3Ph).

Anal. Calcd for $C_{130}H_{148}N_2O_{37}Si$ (2358.68): C, 66.20; H, 6.32; N, 1.19. Found: C, 66.17; H, 6.04; N, 0.95.

2-(Trimethylsilyl)ethyl (Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (26). Compound **22** (0.9 g) was hydrogenolyzed in the presence of 20% $Pd(OH)_2$-C (50% wet, 1.0 g) in ethanol (30 mL) at 30 °C. The catalyst was filtered off and washed with methanol. The filtrate and washings were combined, and concentrated to a residue which was treated with acetic anhydride (2 mL) in pyridine (5 mL) for 48 h at 40 °C, and then the mixture was concentrated. The product was extracted with chloroform and the extract was successively washed with 2M hydrogen chloride, sat. $NaHCO_3$, and water, dried (Na_2SO_4), and concentrated. Column chromatography (50:1 chloroform-

methanol) on silica gel gave **26** (670 mg, 89%). The physicochemical properties and spectral data of **26** were identical with those reported in ref. 9.

2-(Trimethylsilyl)ethyl (Methyl 5-Acetoxyacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (28). Compound **24** (304 mg) was hydrogenolyzed in the presence of 20% $Pd(OH)_2$-C (340 mg) in ethanol (10 mL). Work-up and concentration gave a syrup which was acetylated by treatment with acetic anhydride (0.15 mL) in pyridine (1 mL). The product was purified by chromatography (40:1 chloroform-methanol) on a column of silica gel to give **28** (222.3 mg, 88%): $[\alpha]_D$ +12.5° (*c* 1.4, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.92 (m, 2H, Me_3SiCH_2-), 1.52 (s, 3H, AcN), 1.59 (t, 1H, J_{gem} = 12.5 Hz, H-3e*ax*), 1.75-2.15 (13s, 39H, 13AcO), 2.48 (dd, 1H, J_{gem} = 12.5 Hz, $J_{3eq,4}$ = 4.0 Hz, H-3e*eq*), 3.83 (s, 3H, *Me*O), 4.21, 4.48 (dd, 2H, J_{gem} = 15.3 Hz, $AcOCH_2CO$-), 5.36 (d, 1H, $J_{3,4}$ = 3.3 Hz, H-4d), 5.41 (dd, 1H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 10.6 Hz, H-2d), 5.63 (m, 1H, H-8e), 5.71 (d, 1H, NH), and 7.45-8.19 (m, 15H, 3Ph).

Anal. Calcd for $C_{90}H_{114}N_2O_{46}Si$ (1987.96): C, 54.38; H, 5.78; N, 1.41. Found: C, 54.20; H, 5.67; N, 1.15.

2-(Trimethylsilyl)ethyl (Methyl 4,7,8,9-Tetra-*O*-acetyl-5-butanoylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (29). Compound **25** (159 mg) was hydrogenolyzed in the presence of 20% $Pd(OH)_2$-C (160 mg) in ethanol (5 mL) as described for **28**, and the product acetylated. Column chromatography (50:1 chloroform-methanol) on silica gel gave **29** (100 mg, 76%): $[\alpha]_D$ +11.1° (*c* 0.56, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.85 (t, 3H, *Me*CH_2-), 0.92 (m, 2H, Me_3SiCH_2-), 1.54 (s, 3H, AcN), 1.89-2.13 (12s, 36H, 12AcO), 2.46 (dd, 1H, J_{gem} = 12.7 Hz, $J_{3eq,4}$ = 4.7 Hz, H-3e*eq*), 3.82 (s, 3H, *Me*O),

4.83 (m, 1H, H-4e), 5.62 (m, 1H, H-8c); and 7.43-7.63, 8.05-8.20 (m, 15H, 3Ph).

Anal. Calcd for $C_{90}H_{116}N_2O_{44}Si$ (1957.97): C, 55.21; H, 5.97; N, 1.43. Found: C, 55.14; H, 5.95; N, 1.29.

2-(Trimethylsilyl)ethyl (5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2 → 3)-(β-D-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (30). To a solution of **26** (160 mg) in methanol (10 mL) was added five drops of 28% sodium methoxide in methanol, and the mixture was stirred for 24 h at room temperature. Water (0.5 mL) was added and the stirring was continued overnight, being monitored by TLC (butanol:ethanol:H_2O = 5:5:1). The mixture was neutralized with Amberlite IR-120B (H^+) ion-exchange resin and filtered. The filtrate was concentrated to a residue which was chromatographed (methanol) on a column of Sephadex LH-20 to afford **30** (88 mg, 97%) as an amorphous mass: $[\alpha]_D$ -12.2° (*c* 0.36, MeOH); ^1H NMR ($CDCl_3$) δ 0.98 (m, 2H, Me_3SiCH_2-), 1.26, 1.97 (2s, 6H, 2AcN), 2.76 (dd, 1H, J_{gem} = 12.5 Hz, $J_{3eq,4e}$ = 4.2 Hz, H-3e*eq*), 4.28 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1a), 4.34 (d, 1H, $J_{1,2}$ = 6.3 Hz, H-1d), 4.42 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1b), 4.63 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1c).

Anal. Calcd for $C_{130}H_{146}N_2O_{38}Si$ (2372.66): C, 65.81; H, 6.20; N, 1.18. Found: C, 65.60; H, 5.94; N, 0.91.

2-(Trimethylsilyl)ethyl (3-Deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-(β-D-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (31). *O*-Deacylation of **27** (100 mg) and saponification of the methyl ester were performed as described for **30**. Work-up and column chromatography (1:1 methanol-water) on Sephadex LH-20 gave **31** (53.3 mg, 99%) as an amorphous mass: $[\alpha]_D$ -23.8° (*c* 1.1, MeOH); ^1H NMR (CD_3OD) δ 0.97 (m, 2H, Me_3SiCH_2-), 1.68 (t, 1H, J_{gem} = 11.7 Hz, H-3e*ax*), 1.97 (s, 3H, AcN), 2.73 (dd, 1H, J_{gem} = 11.7 Hz, $J_{3eq,4}$ = 3.5 Hz, H-3e*eq*).

Anal. Calcd for $C_{40}H_{71}NO_{29}Si$ (1058.08): C, 45.41; H, 6.76; N, 1.32. Found: C, 45.11; H, 6.70; N, 1.08.

2-(Trimethylsilyl)ethyl (3,5-Dideoxy-5-glycolylamino-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-(β-D-galactopyranosyl)-(1 → 4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (32). Deprotection of **28** (80 mg) and column chromatography were carried out as described for **31** to give **32** (42.1 mg, 95%) as an amorphous mass: $[\alpha]_D$ +12.5° (*c* 1.4, MeOH); ^{1}H NMR (CD_3OD) δ 1.01 (m, 2H, Me_3SiCH_2-), 1.80 (t, 1H, J_{gem} = 12.4 Hz, H-3e*ax*), 2.01 (s, 3H, AcN), 2.75 (dd, 1H, J_{gem} = 12.4 Hz, $J_{3eq,4}$ = 4.6 Hz, H-3e*eq*).

Anal. Calcd for $C_{42}H_{74}N_2O_{30}Si$ (1115.13): C, 45.24; H, 6.69; N, 2.51. Found: C, 45.21; H, 6.55; N, 2.42.

2-(Trimethylsilyl)ethyl(5-Butanoylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-(β-D-galactopyranosyl)-(1 → 4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (33). The title compound **33** (27.2 mg, 94%) was obtained as an amorphous mass from **29** (50 mg) as described for **31**: $[\alpha]_D$ -8.3° (*c* 0.39, MeOH); ^{1}H NMR (CD_3OD) δ 0.88 (t, 3H, *Me*CH_2-), 0.95, 1.05 (2m, 2H, Me_3SiCH_2-), 1.58 (m, 2H, J = 7.3 Hz, $MeCH_2$-), 1.78 (t, 1H, J_{gem} = $J_{3ax,4}$ = 12.3 Hz, H-3e*ax*), 2.01 (s, 3H, AcN), 2.24 (t, 2H, J = 7.3 Hz, $MeCH_2CH_2CO$-), 2.73 (dd, 1H, J_{gem} = 12.3 Hz, $J_{3eq,4}$ = 4.6 Hz, H-3e*eq*), 4.41, 4.47, 4.53, 4.67 (4d, 4H, J = 8.0, 8.0, 8.0, 8.2 Hz, anomeric protons).

Anal. Calcd for $C_{44}H_{78}N_2O_{29}Si$ (1127.18): C, 46.89; H, 6.98; N, 2.49. Found: C, 46.85; H, 6.74; N, 2.45.

2. Enzyme assay

Soluble recombinant Fuc-TVII and Fuc-TVI were prepared as reported by Shinoda et al.[18] Standard Fuc-T assays were performed,[6] in a total volume of 30 μL of 100 mM cacodylate buffer (pH 7.5), 25 mM $MnCl_2$, 0.05 mM GDP-fucose, 0.025 mM pyridylaminated sialyl-α-(2→3)-neolactotetraose derivative (**34** in Scheme 4), and one of the recombinant enzyme (1.0 μg each). In the competitive enzyme assay, the synthetic probes (**30-33**) were added to the reaction mixture at 100 μM. After

incubation at 37 °C for 2 h, the reaction was stopped by boiling for 5 min. After centrifugation, each reaction mixture was subjected to HPLC analysis on a YMC ODS AQ column (6×150 mm). The reaction product was eluted with 20 mM NH_4OAc (pH 4.0) at the flow rate (1.0-1.5 mL/min) and monitored with a fluorescence spectrometer (320 and 400 nm). The structures of the products were identified by use of the corresponding authentic sLe^x compounds and FABMS.

The addition of **30-33** which compete with a labeled-acceptor **34** leads to a reduction in the generation of pyridylaminated sLe^x hexasaccharide **35** accompanied by the production of each sLe^x analog (Scheme 4). In fact, the standard NeuAc derivative **30** reduced the generation of **35** to 63.0% (Fuc-TVII) and 52.7% (Fuc-TVI), respectively, compared to without **30**. Therefore, the competition of **30** against **34** was 37.0% and 47.3% for Fuc-TVII and Fuc-TVI, respectively. The relative competition % was calculated based on the competition of **30** (GSC-253) as a standard (Table 1).

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