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Carbohydrate Research 328 (2000) 85–94

CARBOHYDRATE RESEARCH

Systematic synthesis of sulfated sialyl-α-(2→3)-neolactotetraose derivatives and their acceptor specificity for an α-(1→3)-fucosyltransferase (Fuc-TVII) involved in the biosynthesis of L-selectin ligand☆

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Received 8 February 2000; accepted 13 March 2000

Abstract

Sulfated sialyl-α-(2→3)-neolactotetraose ($IV^3NeuAcnLcOse_4$) derivatives at C-6 of GlcNAc (6-*O*-sulfo), terminal Gal (6′-*O*-sulfo), and both GlcNAc and Gal (6,6′-di-*O*-sulfo) residues have systematically been synthesized. (Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4-di-*O*-benzoyl-6-*O*-levulinoyl-D-galactopyranosyl trichloroacetimidate was coupled with 2-(trimethylsilyl)ethyl (2-acetamido-2-deoxy-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-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 to give the suitably protected pentasaccharide which, upon selective removal of the *p*-methoxyphenyl and/or levulinoyl groups at C-6 of the GlcNAc and the terminal Gal residues, successive O-sulfation(s) and deprotection, afforded the desired three sulfated $IV^3NeuAcnLcOse_4$ derivatives. Acceptor specificity of the synthetic $IV^3NeuAcnLcOse_4$ probes for a human α-(1→3)-fucosyltransferase (Fuc-TVII) was examined to study the biosynthetic pathway of L-selectin ligand. Only the 6-sulfated derivative at C-6 of GlcNAc was recognized by Fuc-TVII to give 6-*O*-sulfo sialyl Le^X.

Keywords: Fuc-TVII; L-selectin; 6-*O*-Sulfo sialyl Le^X, sulfated sialyl-α-(2→3)-neolactotetraose

1. Introduction

L-selectin is a cell-adhesion molecule involved in lymphocyte binding to high endothelial venules (HEV) of lymph nodes during lymphocyte recirculation and in leukocyte recruitment to sites of inflammation [2–4]. The molecular species of sulfated sialyl Lewis X (sLe^X) determinants, 6-*O*-sulfo, 6′-*O*-sulfo, and 6,6′-di-*O*-sulfo sLe^X determinants have been investigated to identify the putative L-selectin ligand [5,6]. In the previous study, we indicated that 6-sulfo sLe^X and related structures were high-affinity endogenous L-selectin ligand on HEV [7,8]. Fuc-TVII, an α-(1→3)-fucosyltransferase, has been sug-

☆ Part 115 of the series: Synthetic studies on sialoglycoconjugates. For Part 114, see Ref. [1].

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0008-6215/00/$ - see front matter
PII: S0008-6215(00)00088-4

gested to be mainly involved in the biosynthesis of L-selectin ligand as well as E- and P-selectin [9–12], and it has an ability to transfer L-fucose residue only to α-(2→3)-sialylated type 2 oligosaccharides such as sialyl-α-(2→3)-neolactotetraose ($IV^3NeuAcnLcOse_4$) derivatives. In our continuing study to elucidate the enzymatic properties of Fuc-TVII [13–15], we describe herein the systematic synthesis of three sulfated $IV^3NeuAcnLcOse_4$ derivatives **15**–**17** and their acceptor specificity for a recombinant human Fuc-TVII [13].

2. Results and discussion

For the systematic synthesis of three sulfated (6-*O*-sulfo, 6′-*O*-sulfo and 6,6′-di-*O*-sulfo) $IV^3NeuAcnLcOse_4$ probes, we selected the *p*-methoxyphenyl (MP) and levulinoyl (Lev) groups as the selective protecting groups at C-6 of GlcNAc and the terminal Gal, respectively. The key trisaccharide acceptor, 2-(trimethylsilyl)ethyl (2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxyphenyl-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 (**3**) was readily prepared (83%) by treatment of **2** [16] with *p*-methoxyphenol, PPh_3 and dimethyl azodicarboxylate (Mitsunobu reaction [17]). Coupling of **3** with **4** [18] was carried out in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) in CH_2Cl_2 gave **5** (86%) which, upon hydrogenolytic removal of the benzyl groups over $Pd(OH)_2$ on carbon and acetylation with acetic anhydride–pyridine, afforded the suitably protected pentasaccharide **6** (Scheme 1).

The MP group in **6** was then selectively removed (88%) by treatment with ceric ammonium nitrate (CAN) in acetonitrile, and the resulting **7** was sulfated with sulfur trioxide–pyridine complex (SO_3·pyr) in *N*,*N*-dimethylformamide to give **11** in 79% yield. For the synthesis of **12**, the 6-OH of GlcNAc in **7** was first acetylated, and the Lev group in **8** was then selectively removed with hydrazine monoacetate in EtOH to afford **9** (76%). Sulfation of **9** with SO_3·pyr in *N*,*N*-dimethylformamide gave **12** in 77% yield. Under the same reaction conditions, the Lev group in **7** was selectively cleaved, and the resulting 6,6′-hydroxyls in **10** were simultaneously sulfated to give **13** (64%).

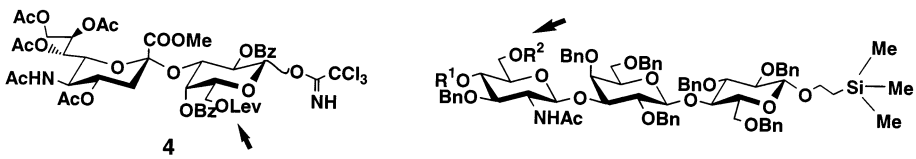
Finally, treatment of **11**–**13** with an excess amount of NaOMe in methanol, followed by saponification of the methyl ester group by addition of water, gave the desired sodium salts of 6-*O*-sulfo, 6′-*O*-sulfo and 6,6′-di-*O*-sulfo sialyl-α-(2→3)-neolactotetraose derivatives **15**–**17**, which were each purified by chromatography on a column of Sephadex LH-20. The structures of **15**–**17** were characterized by ^1H NMR (500 MHz) spectroscopy and FABMS (see Section 3, Scheme 2).

The non-sulfated probe **14** (GSC-253) [15] and its three sulfated derivatives **15** (GSC-386), **16** (GSC-385) and **17** (GSC-387) were subjected to competitive enzyme assay using Fuc-TVII. In this assay system, the addition of compounds that compete with a labeled acceptor **18**, leads to a reduction in the generation of labeled sLe^X hexasaccharide **19** (Scheme 3). Therefore, competition of **14**–**17** with the labeled acceptor **18** can be considered to be a guidepost of their affinity for Fuc-TVII. The 6-*O*-sulfo derivative **15**, in which the hydroxyl group at C-6 of GlcNAc is sulfated, competed with the labeled acceptor more potently than **14**, while the 6′-*O*-sulfo derivative **16**, in which the hydroxyl group at C-6 of Gal is sulfated, or the 6,6′-di-*O*-sulfo derivative **17**, in which two hydroxyl groups at C-6 of both GlcNAc and Gal are sulfated, did not exhibit competition up to 100 mM (Fig. 1).

The K_i values determined for the 6-*O*-sulfo derivative **15** (22.6 μM) was lower than that for non-sulfated derivative **14** (71.9 μM), indicating that Fuc-TVII had a higher affinity toward the 6-*O*-sulfo derivative **15** than the non-sulfated form **14** (Table 1). To confirm whether the 6-*O*-sulfo derivative **15** was an acceptor or an inhibitor for Fuc-TVII, the reaction products were directly analyzed by negative-ion fast-atom bombardment mass spectrometry (FABMS) (Fig. 2). After incubation of **15** with Fuc-TVII, an ion peak corresponding to the 6-*O*-sulfo sLe^X structure [18] was observed at m/z 1345 $(M-Na)^-$ as well as another peak corresponding to the unreacted **15** at m/z 1199 $(M-Na)^-$ (Fig. 2(B)).

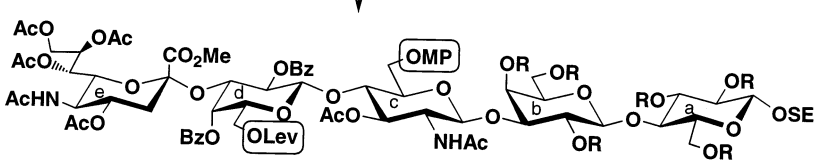
On the other hand, the incubation without Fuc-TVII gave only the parent ion peak for **15** at m/z 1199 $(M-Na)^-$ (Fig. 2(A)). Therefore, the 6-*O*-sulfo derivative **15** was confirmed to be an acceptor for Fuc-TVII to generate the 6-*O*-sulfo sLe^X structure (see Scheme 4).

The 6-*O*-sulfo sLe^X [18] has been identified as a major capping group of L-selectin ligand on HEV in human lymph nodes [7,8], while its biosynthetic pathway is not yet completely clear. Fuc-TVII, which has been demonstrated to participate in the biosynthesis of L-selectin ligand, shows activity only toward α-(2→3)-sialylated type 2 oligosaccharides. The relative order of fucosylation and sulfation is a key point to address this issue. Maly et al. [12] examined the enzyme activity of Fuc-TVII toward sulfated or non-sulfated acceptors and reported the several biosynthetic pathways for the L-selectin ligand. In the present study, we have demonstrated that only the 6-*O*-sulfo



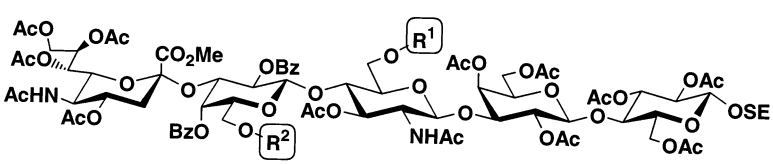
	R^1	R^2
1	benzylidene	
2	H	H
3	H	MP

(MP = *p*-methoxyphenyl)



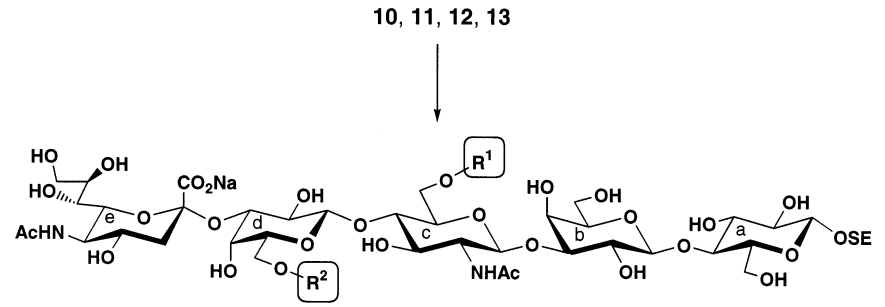
	R
5	Bn
6	Ac

(SE = Si(Me)(Me)Me)



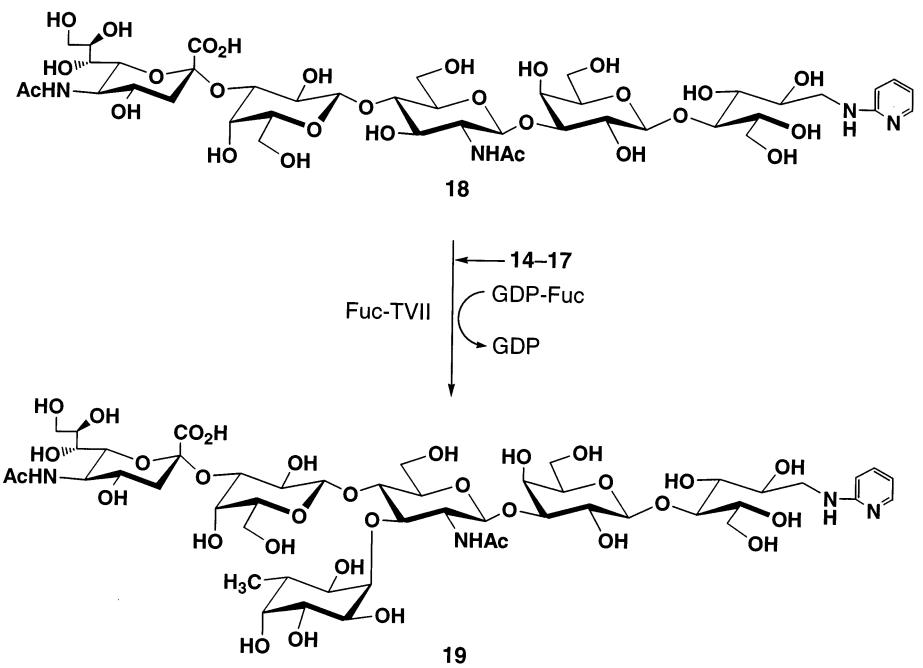
	R^1	R^2
7	H	Lev
8	Ac	Lev
9	Ac	H
10	H	H
11	SO_3H	Lev
12	Ac	SO_3H
13	SO_3H	SO_3H

Scheme 1.



	R^1	R^2
14 (GSC-253)	H	H
15 (GSC-386)	SO_3Na	H
16 (GSC-385)		SO_3Na
17 (GSC-387)	SO_3Na	SO_3Na

Scheme 2.



Scheme 3.

derivative **15** was recognized by Fuc-TVII with much higher affinity than that for non-sulfated **14** (Fig. 1 and Scheme 4). Therefore, it seems more plausible that the 6-sulfation of GlcNAc catalyzed by 6-sulfotransferases [19–22] occurs prior to Fuc-TVII-catalyzed fucosylation. This result has also been supported by the reconstitution study of functional L-selectin ligands on a cultured human endothelial cell by cotransfection of Fuc-TVII and a newly cloned *N*-acetylglycosamine-6-sulfotransferase [23]. Very recently, we have also demonstrated [24] that the 6-*O*-sulfo *N*-deacetylsialyl Lewis X is a superior L-selectin ligand, suggesting a new molecular mechanism in the regulation of selectin-dependent cell adhesion [25].

In conclusion, three sulfated sialyl-α-(2→3)-neolactoteraose probes were systematically synthesized. Only the 6-*O*-sulfo derivative (**15**, GSC-386) showed superior recognition by Fuc-TVII, supporting the idea

that 6-sulfation of the GlcNAc residue occurs prior to fucosylation.

3. Experimental

General procedures.—Specific 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. FAB mass spectra were recorded on a JEOL JMS-SX 120A mass spectrometer/JMA-DA 7000 data system. Preparative thin-layer chromatography (TLC) was performed on Silica Gel 60 (E. Merck), and column chromatography on Silica Gel (Fuji Silysia Co., 300 mesh) was accomplished with the solvent systems (v/v) specified. Concentrations and evaporations were conducted in vacuo.

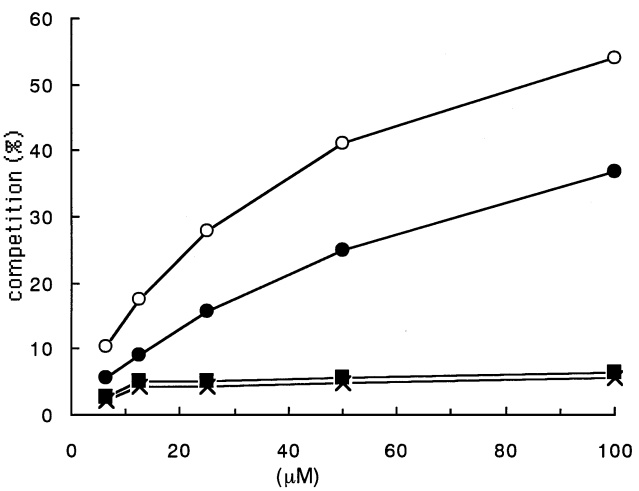


Fig. 1. Competition of the non-sulfated (**14**) and sulfated (**15**–**17**) sialyl-α-(2→3)-neolactotetraose derivatives against the pyridylaminated derivative **18** (Scheme 3) for Fuc-TVII. Non-sulfated **14** (●), 6-sulfo **15** (○), 6′-sulfo **16** (×), and 6,6′-bissulfo **17** (■).

Table 1
Relative competition activity and K_i values for the sialyl-α-(2→3)-neolactotetraose probes (**14**–**17**)

Compound	Relative competition (%) [a]	K_i (μM)
14 (GSC-253)	100	71.9
15 (GSC-386)	147	22.6
16 (GSC-385)	17.7	nd [b]
17 (GSC-387)	15.8	nd [b]

[a] See Section 3 and Ref. [14].
[b] nd, not determined.

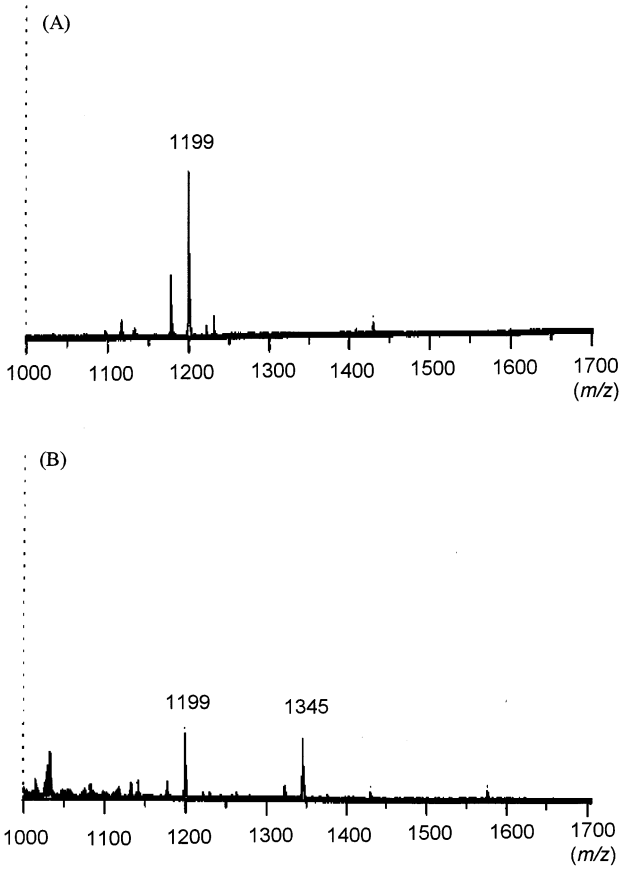
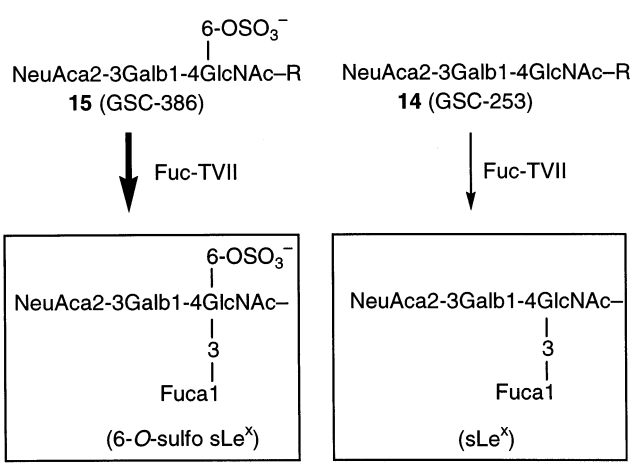


Fig. 2. FABMS (negative-ion) spectra of the reaction mixture for the 6-*O*-sulfo derivative **15** (GSC-386) after incubation without Fuc-TVII (A) and with Fuc-TVII (B).



Scheme 4.

*2-(Trimethylsilyl)ethyl (2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxyphenyl-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* (**3**).—To a solution of **2** (500 mg, 0.39 mmol) prepared from **1** [16] in THF (9 mL) were added triphenylphosphine (205 mg, 0.78 mmol), diethyl azodicarboxylate (DEAD; 134 μL, 0.78 mmol) and *p*-methoxyphenol (243 mg, 1.95 mmol), and the mixture was heated for 4 h at 80 °C under reflux, then concentrated. Column chromatography (1:4 hexane–AcOEt) of the residue on silica gel gave **3** (447 mg, 83%) as an amorphous mass: $[\alpha]_D$ −1.8° (*c* 1.8, $CHCl_3$); ^1H NMR ($CDCl_3$): *δ* 0.99 (m, 2 H, Me_3SiCH_2-CH_2), 1.45 (s, 3 H, AcN), 2.75 (br s, 1 H, 4-OH), 3.70 (s, 3 H, MeO), 6.76, 6.82 (2 d, 4 H, *Ph*OMe), 7.11–7.36 (m, 35 H, 7 Ph). H-4 of the GlcNAc residue in the 4-*O*-benzoyl derivative of **3** appeared at *δ* 5.32 (t, 1 H, *J* 9.2 Hz). Anal. Calcd for $C_{81}H_{95}NO_{17}Si$ (1382.72): C, 70.36; H, 6.93; N, 1.01. Found: C, 70.28; H, 6.64; N, 0.72.

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-di*-O-*benzoyl-6*-O-*levulinoyl-β*-D-*galacto-pyranosyl)-(1→4)-(2-acetamido-3*-O-*benzyl-2-deoxy-6*-O-p-*methoxyphenyl-β*-D-*glucopyranosyl)-(1→3)-(2,4,6-tri*-O-*benzyl-β*-D-*galacto-pyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-β*-D-*glucopyranoside* (**5**).—To a solution of **3** (110 mg, 79.4 μmol) and **4** [18] (114 mg, 103.2 μL) in CH_2Cl_2 (0.7 mL) was added powdered 4 Å MS (AW-300, 200 mg), and the mixture was stirred for 5 h at room temperature (rt), then cooled to 0 °C. TMSOTf (1.6 μL, 8.3 μmol) was added, and the reaction mixture was stirred overnight at 0 °C. The solids were filtered off and washed with $CHCl_3$. The combined filtrate and washings were washed with M Na_2CO_3 and water, dried (Na_2SO_4) and concentrated. Column chromatography (40:1 $CHCl_3$–MeOH) of the residue on silica gel gave **5** (158.4 mg, 86%) as an amorphous mass: $[\alpha]_D$ +17.3° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): *δ* 1.00 (m, 2 H, $Me_3SiCH_2CH_2$), 1.44, 1.45 (2 s, 6 H, 2 AcN), 1.65 (t, 1 H, J_{gem} 12.6 Hz, H-3e_{ax}), 1.77, 1.90, 1.97, 2.10, 2.22 (5 s, 15 H, 4 AcO and $MeCOCH_2CH_2CO$), 2.44, 2.63 (m, 5 H, H-3e_{eq} and $MeCOCH_2CH_2CO$), 3.71 (s, 3 H, *Me*OPh), 3.89 (s, 3 H, COO*Me*), 4.96 (d, 1 H, NH), 5.06 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1d), 5.22 (dd, 1 H, $J_{6,7}$ 2.7, $J_{7,8}$ 8.9 Hz, H-7e), 5.23 (d, 1 H, $J_{3,4}$ 3.0 Hz, H-4d), 5.31 (d, 1 H, NH), 5.44 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.8 Hz, H-2d), 5.55 (m, 1 H, H-8e), 6.75, 6.79 (2 d, 4 H, *Me*OPh), 7.33–8.14 (m, 45 H, 9 Ph). Anal. Calcd for $C_{126}H_{146}N_2O_{38}Si$ (2324.62): C, 65.10; H, 6.33; N, 1.21. Found: C, 65.04; H, 6.11; N, 1.17.

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-di*-O-*benzoyl-6*-O-*levulinoyl-β*-D-*galacto-pyranosyl)-(1→4)-(2-acetamido-3*-O-*acetyl-2-deoxy-6*-O-p-*methoxyphenyl-β*-D-*glucopyranosyl)-(1→3)-(2,4,6-tri*-O-*acetyl-β*-D-*galacto-pyranosyl)-(1→4)-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranoside* (**6**).—A solution of **5** (158.4 mg, 68.0 μmol) in EtOH (10 mL) was hydrogenated over 20% $Pd(OH)_2$–C (158 mg) overnight at 40 °C, then filtered and concentrated. The residue was acetylated by treatment with Ac_2O (0.18 mL) and pyridine (3 mL). The product was purified by chromatography (50:1 $CHCl_3$–MeOH) on a column of silica gel to give **6** (115.3 mg, 86%) as an amorphous mass: $[\alpha]_D$ +17.9° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): *δ* 0.92 (m, 2 H, $Me_3SiCH_2CH_2$), 1.44, 1.77 (2 s, 6 H, 2 AcN), 1.61 (t, 1 H, J_{gem} 12.5 Hz, H-3e_{ax}), 1.90, 1.91, 1.94, 1.98, 2.02, 2.04, 2.05, 2.06, 2.11, 2.19, 2.22 (12 s, 36 H, 11 AcO and $MeCOCH_2$-CH_2CO), 2.44 (dd, 1 H, J_{gem} 12.5, $J_{3eq,4}$ 4.4 Hz, H-3e_{eq}), 2.61, 2.76 (m, 4 H, $MeCOCH_2$-CH_2CO), 3.76 (s, 3 H, *Me*OPh), 3.91 (s, 3 H, COO*Me*), 5.03 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1d), 5.32 (dd, 1 H, $J_{1,2}$ 7.7 Hz, $J_{2,3}$ 9.9 Hz, H-2d), 5.53 (d, 1 H, NH), 5.56 (m, 1 H, H-8e), 6.68, 6.77 (2 d, 4 H, *Ph*MeO), 7.37–8.15 (m, 10 H, 2 Ph). Anal. Calcd for $C_{91}H_{118}N_2O_{45}Si$ (1988.01): C, 54.98; H, 5.98; N, 1.41. Found: C, 54.79; H, 5.81; 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-di*-O-*benzoyl-6*-O-*levulinoyl-β*-D-*galactopyranosyl)-(1→4)-(2-acetamido-3*-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* (**7**).—To a solution of **6** (200 mg, 101.3 μmol) in MeCN (2 mL) and water (0.5 mL) was added ceric ammonium nitrate (CAN, 277.6

mg, 0.51 mmol), and the mixture was stirred for 15 min at rt, then extracted with $CHCl_3$. The extract was successively washed with M Na_2CO_3 and water, dried (Na_2SO_4) and concentrated. Column chromatography (40:1 $CHCl_3$–MeOH) of the residue on silica gel gave **7** (166.7 mg, 88%) as an amorphous mass: $[\alpha]_D$ +18.1° (*c* 1.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.92 (m, 2 H, $Me_3SiCH_2CH_2$), 1.49, 1.77 (2 s, 6 H, 2 AcN), 1.62 (t, 1 H, J_{gem} 12.6 Hz, H-3e$_{ax}$), 1.88, 1.91, 1.94, 2.00, 2.02, 2.03, 2.07, 2.10, 2.13, 2.14, 2.19, 2.23 (12 s, 36 H, 11 AcO and $MeCOCH_2CH_2CO$), 2.45 (dd, 1 H, J_{gem} 12.6, $J_{3eq,4}$ 4.3 Hz, H-3e$_{eq}$), 2.60, 2.75 (m, 4 H, $MeCOCH_2CH_2CO$), 3.22 (br s, 1 H, 6-OH), 3.90 (s, 3 H, COO*Me*), 4.32, 4.46, 4.60 (3 d, 3 H, $J_{1,2}$ 8.0 Hz, H-1a–c), 4.93 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1d), 5.32 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.8 Hz, H-2d), 5.41 (d, 1 H, $J_{3,4}$ 3.4 Hz, H-4d), 5.48 (d, 1 H, NH), 5.70 (m, 1 H, H-8e), 7.48–8.22 (m, 10 H, 2 Ph). Anal. Calcd for $C_{84}H_{112}N_2O_{44}Si$ (1881.88): C, 53.61; H, 6.00; N, 1.49. Found: C, 53.42; H, 5.93; N, 1.19.

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-di*-O-*benzoyl-6*-O-*levulinoyl-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* (**8**).—Compound **7** (100 mg, 53.3 μmol) was treated with Ac_2O (10 μL) and pyridine (0.8 mL) overnight at rt. Workup and column chromatography (30:1 $CHCl_3$–MeOH) on silica gel gave **8** (98%) as an amorphous mass: $[\alpha]_D$ +23.9° (*c* 1.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.92 (m, 2 H, $Me_3SiCH_2CH_2$), 1.53, 1.78 (2 s, 6 H, 2 AcN), 1.60 (t, 1 H, J_{gem} 12.6 Hz, H-3e$_{ax}$), 1.88, 1.91, 1.98, 1.99, 2.00, 2.02, 2.04, 2.08, 2.09, 2.10, 2.10, 2.20, 2.23 (13 s, 39 H, 12 AcO and $MeCOCH_2CH_2CO$), 2.44 (dd, 1 H, J_{gem} 12.6, $J_{3eq,4}$ 4.58 Hz, H-3e$_{eq}$), 2.61, 2.76 (m, 4 H, $MeCOCH_2CH_2CO$), 3.91 (s, 3 H, COO*Me*), 4.30, 4.46, 4.54 (3 d, 3 H, $J_{1,2}$ 8.0 Hz, H-1a–c), 4.92 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1d), 5.34 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.8 Hz, H-2d), 5.51 (d, 1 H, NH), 5.62 (m, 1 H, H-8e), 7.30–8.18 (m, 10 H, 2 Ph). Anal. Calcd for $C_{86}H_{114}N_2O_{45}Si$ (1923.92): C, 53.69; H, 5.97; N, 1.46. Found: C, 53.48; H, 5.77; N, 1.16.

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-di*-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* (**9**).—To a solution of **8** (77 mg, 40.8 μmol) in EtOH (1 mL) was added hydrazine monoacetate (4.1 mg, 44.8 μmol), and the mixture was stirred for 1 h at 0 °C. Column chromatography (45:1 $CHCl_3$–MeOH) of the mixture on silica gel gave **9** (55.2 mg, 76%) as an amorphous mass: $[\alpha]_D$ +34.2° (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.92 (m, 2 H, $Me_3SiCH_2CH_2$), 1.55, 1.78 (2 s, 6 H, 2 AcN), 1.64 (t, 1 H, J_{gem} 12.6 Hz, H-3e$_{ax}$), 1.88–2.22 (12 s, 36 H, 12 AcO), 2.44 (dd, 1 H, J_{gem} 12.6, $J_{3eq,4}$ 4.4 Hz, H-3e$_{eq}$), 3.81 (s, 3 H, COO*Me*), 4.30, 4.46, 4.56 (3 d, 3 H, $J_{1,2}$ 8.0 Hz, H-1a–c), 4.72 (m, 1 H, H-4e), 4.93 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1d), 5.11, 5.23 (2 d, 2 H, $J_{3,4}$ 3.0, 3.7 Hz, H-4b, H-4d), 5.40 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.1 Hz, H-2d), 5.56 (d, 1 H, NH), 5.63 (m, 1 H, H-8e), 7.29–8.19 (m, 10 H, 2 Ph). Anal. Calcd for $C_{81}H_{108}N_2O_{43}Si$ (1825.82): C, 53.29; H, 5.96; N, 1.53. Found: C, 53.12; H, 5.88; N, 1.38.

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-di*-O-*benzoyl-β*-D-*galactopyranosyl)-(1→4)-(2-acetamido-3*-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* (**10**).—To a solution of **7** (83.7 mg, 44.6 μmol) in EtOH (1 mL) was added hydrazine monoacetate (4.5 mg, 49.1 μmol), and the mixture was stirred for 1 h at 0 °C. Column chromatography (45:1 $CHCl_3$–MeOH) of the mixture on silica gel gave **10** (58.3 mg, 74%) as an amorphous mass: $[\alpha]_D$ +27.1° (*c* 0.85, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.92 (m, 2 H, $Me_3SiCH_2CH_2$), 1.51, 1.79 (2 s, 6 H, 2 AcN), 1.67 (t, 1 H, J_{gem} 12.6 Hz, H-3e$_{ax}$), 1.88, 1.910, 1.913, 1.92, 2.00, 2.02, 2.03, 2.07, 2.10, 2.13, 2.23 (11 s, 33 H, 11 AcO), 2.45 (dd, 1 H, J_{gem} 12.6, $J_{3eq,4}$ 4.3 Hz, H-3e$_{eq}$), 3.82 (s, 3 H, COO*Me*), 4.31, 4.46, 4.59 (3 d, 3 H, $J_{1,2}$ 8.0 Hz, H-1a–c), 4.71 (m, 1 H, H-4e), 4.94 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1d),

5.04 (d, 1 H, NH), 5.37 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.1 Hz, H-2d), 5.40 (d, 1 H, $J_{3,4}$ 3.9 Hz, H-4d), 5.47 (d, 1 H, NH), 5.69 (m, 1 H, H-8e), 7.50–8.23 (m, 10 H, 2 Ph). Anal. Calcd for $C_{79}H_{106}N_2O_{42}Si$ (1783.78): C, 53.19; H, 5.99; N, 1.51. Found: C, 53.19; H, 5.73; 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-di*-O-*benzoyl-6*-O-*levulinoyl-β*-D-*galactopyranosyl)-(1→4)-(2-acetamido-3*-O-*acetyl-2-deoxy-6*-O-*sulfo-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* (**11**) *and 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-6*-O-*sulfo-β*-D-*glucopyranosyl)-(1→3)-(β*-D-*galactopyranosyl)-(1→4)-β*-D-*glucopyranoside sodium salt (***15**, *GSC-386).*—To a solution of **7** (50 mg, 26.6 μmol) in DMF (0.2 mL) was added sulfur trioxide-pyridine complex (SO_3·pyr; 21.2 mg, 133 μmol), and the mixture was stirred for 4 h at rt, then cooled to 0 °C. The excess reagent was decomposed by adding MeOH (0.5 mL), and the mixture was concentrated. Column chromatography (1:1 $CHCl_3$–MeOH) of the residue on Sephadex LH-20 gave the crude product which was purified by column chromatography on silica gel with 30:1 $CHCl_3$–MeOH afford **11** (43.0 mg, 79%) as an amorphous mass: $[\alpha]_D$ +14.6° (*c* 0.85, 1:1 $CHCl_3$–MeOH); ^{1}H NMR (CD_3OD): δ 0.92 (m, 2 H, $Me_3SiCH_2CH_2$), 1.55 (t, 1 H, J_{gem} 12.6 Hz, H-3e$_{ax}$), 1.67, 1.74 (2 s, 6 H, 2 AcN), 1.858, 1.862, 1.95, 1.98, 1.99, 2.06, 2.07, 2.08, 2.09, 2.11, 2.16, 2.19 (12 s, 36 H, 11 AcO and $MeCOCH_2CH_2CO$), 2.33 (dd, 1 H, J_{gem} 12.6, $J_{3eq,4}$ 4.6 Hz, H-3e$_{eq}$), 2.57, 2.81 (m, 4 H, $MeCOCH_2CH_2CO$), 3.79 (s, 3 H, COO*Me*), 4.50, 4.56, 4.59 (3 d, 3 H, $J_{1,2}$ 8.0 Hz, H-1a–c), 5.28 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.8 Hz, H-2d), 5.41, 5.45 (2 d, 2 H, $J_{3,4}$ 3.2 Hz, H-4b, H-4d), 5.63 (m, 1 H, H-8e), 7.53–8.26 (m, 10 H, 2 Ph).

To a solution of **11** (68.5 mg, 33.7 μmol) in MeOH (1 mL) was added an excess of NaOMe (pH 10–11), and the mixture was stirred for 24 h at rt, and overnight after addition of water (0.5 mL). It was then concentrated at 30 °C to a residue which was chromatographyed (1:1 MeOH–water) on a column of Sephadex LH-20 to afford **15** (35 mg, 87%) as an amorphous mass: $[\alpha]_D$ −7.4° (*c* 0.54, 1:1 MeOH–water); ^{1}H NMR (CD_3OD): δ 0.95 (m, 2 H, $Me_3SiCH_2CH_2$), 1.71 (t, 1 H, J_{gem} 12.1 Hz, H-3e$_{ax}$), 1.97, 1.98 (2 s, 6 H, 2 AcN), 2.82 (dd, 1 H, J_{gem} 12.1, $J_{3eq,4}$ 3.9 Hz, H-3e$_{eq}$), 4.27, 4.49, 4.64 (3 d, 3 H, $J_{1,2}$ 7.8, 7.8, 8.2 Hz, 3 H-1), 4.3–4.4 (m, 3 H, H-1, H-6c,6′c); FABMS (negative-ion mode, glycerol matrix): *m*/*z* 1199.47 [M − Na]$^-$ ($C_{42}H_{72}N_2NaO_{32}SSi$ MW, Exact 1199.3456, Ave. 1200.1691), 1177.49 [M − 2Na]$^-$, 886.4 [M − 2Na − NeuAc]$^-$, 724.4 [M − 2Na − NeuAc − Gal]$^-$, 441.3 [Lac − OSE]$^-$.

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-di*-O-*benzoyl-6*-O-*sulfo-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* (**12**) *and 2-(trimethylsilyl)ethyl (5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid)-(2→3)-(6*-O-*sulfo-β*-D-*galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1→3)-(β*-D-*galactopyranosyl)-(1→4)-β*-D-*glucopyranoside disodium salt (***16**, *GSC-385).*—To a solution of **9** (55 mg, 30.8 μmol) in DMF (0.4 mL) was added SO_3·pyr (24.5 mg, 154 μmol), and the mixture was stirred for 5 h at rt. Workup and purification by column chromatography as described for **11** gave **12** (46 mg, 77%) as an amorphous mass: $[\alpha]_D$ +25.4° (*c* 0.92, 1:1 $CHCl_3$–MeOH); ^{1}H NMR (CD_3OD): δ 0.92 (m, 2 H, $Me_3SiCH_2CH_2$), 1.42 (t, 1 H, J_{gem} 12.5 Hz, H-3e$_{ax}$), 1.46, 1.71 (2 s, 6 H, 2 AcN), 1.86, 1.87, 1.98, 1.99, 2.02, 2.057, 2.06 × 2, 2.07, 2.08, 2.09, 2.25 (12 s, 36 H, 12 AcO), 2.42 (dd, 1 H, J_{gem} 12.5, $J_{3eq,4}$ 4.6 Hz, H-3e$_{eq}$), 3.95 (s, 3 H, COO*Me*), 4.47, 4.59, 4.69 (3 d, 3 H, $J_{1,2}$ 8.0, 7.8, 8.2 Hz, H-1a–c), 4.96 (dd, 1 H, $J_{1,2}$ 7.8 Hz, H-1d), 5.11 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.3 Hz, H-2d), 5.35, 5.36 (2 d, 2 H, $J_{3,4}$ 3.4, 3.2 Hz, H-4b, H-4d), 5.65 (m, 1 H, H-8e), 7.52–8.19 (m, 10 H, 2 Ph).

To a solution of **12** (46 mg, 24.1 μmol) in MeOH (0.8 mL) was added an excess of NaOMe (pH 10–11), and the mixture was

stirred overnight at rt, and overnight after addition of water (0.5 mL). Workup and column chromatography (1:1 MeOH–water) on a column of Sephadex LH-20 gave **16** (28.2 mg, 92%) as an amorphous mass: $[\alpha]_D$ +11.2° (*c* 0.75, MeOH); ^{1}H NMR (CD_3OD): δ 0.95 (m, 2 H, $Me_3SiCH_2CH_2$), 1.71 (t, 1 H, J_{gem} 12.5 Hz, H-3e$_{ax}$), 1.97, 1.98 (2 s, 6 H, 2 AcN), 2.82 (dd, 1 H, J_{gem} 12.5, $J_{3eq,4}$ 4.6 Hz, H-3e$_{eq}$), 4.1–4.2 (m, 2 H, H-6d,6′d), 4.27, 4.36, 4.46, 4.66 (4 d, 4 H, $J_{1,2}$ 7.8, 6.9, 7.1, 8.2 Hz, 4 H-1), FABMS (negative-ion mode, glycerol matrix): *m/z* 1199.47 $[M-Na]^-$ ($C_{42}H_{72}N_2NaO_{32}SSi$ MW, Exact 1199.3456, Ave. 1200.1691), 1177.49 $[M-2Na]^-$, 886.4 $[M-2Na-$ NeuAc$]^-$, 644.4 $[M-2Na-NeuAc-6$-sulfo-Gal$]^-$, 441.3 $[Lac-OSE]^-$.

*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-di-*O*-benzoyl-6-*O*-sulfo-β-*D*-galactopyranosyl)-(1→4)-(2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-sulfo-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* (**13**) *and 2-(trimethylsilyl)ethyl (5-acetamido-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosylonic acid)-(2→3)-(6-*O*-sulfo-β-*D*-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-6-*O*-sulfo-β-*D*-glucopyranosyl)-(1→3)-(β-*D*-galactopyranosyl)-(1→4)-β-*D*-glucopyranoside trisodium salt (**17**, GSC-387).*—To a solution of **10** (42.1 mg, 23.7 μmol) in DMF (0.3 mL) was added SO_3·pyr (37.8 mg, 237 μmol), and the mixture was stirred for 2 h at rt. Workup and purification by column chromatography as described for **11** gave **13** (31.8 mg, 64%) as an amorphous mass: $[\alpha]_D$ +14.0° (*c* 0.63, 1:1 $CHCl_3$–MeOH); ^{1}H NMR (CD_3OD): δ 0.92 (m, 2 H, $Me_3SiCH_2CH_2$), 1.51 (t, 1 H, J_{gem} 12.3 Hz, H-3e$_{ax}$), 1.56, 1.73 (2 s, 6 H, 2 AcN), 1.86, 1.87, 1.97, 1.98, 2.00, 2.05, 2.07, 2.08, 2.09, 2.12, 2.18 (11 s, 33 H, 11 AcO), 2.37 (dd, 1 H, J_{gem} 12.4, $J_{3eq,4}$ 4.6 Hz, H-3e$_{eq}$), 3.86 (s, 3 H, COO*Me*), 5.26 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.1 Hz, H-2d), 5.43, 5.49 (2 d, 2 H, $J_{3,4}$ 3.2, 3.0 Hz, H-4b, H-4d), 5.63 (m, 1 H, H-8e), 7.52–8.25 (m, 10 H, 2 Ph).

O-Deacylation of **13** (31 mg, 14.8 μmol) with an excess amount of NaOMe in MeOH (1.5 mL), and successive saponification of the methyl ester group of NeuAc were performed as described for **15**. Workup and purification by chromatography (1:1 MeOH–water) on a column of Sephadex LH-20 afforded **17** (19 mg, 90%) as an amorphous mass: $[\alpha]_D$ +2.3° (*c* 0.44, 1:1 MeOH–water); ^{1}H NMR (D_2O): δ 1.00 (m, 2 H, $Me_3SiCH_2CH_2$), 1.78 (t, 1 H, J_{gem} 12.3 Hz, H-3e$_{ax}$), 2.00, 2.01 (2 s, 6 H, 2 AcN), 2.72 (dd, 1 H, J_{gem} 12.3, $J_{3eq,4}$ 4.6 Hz, H-3e$_{eq}$), 4.15 (m, 2 H, H-6d,6′d), 4.27 (dd, 1 H, $J_{5,6}$ 5.5, J_{gem} 11.2 Hz, H-6c), 4.41 (br d, 1 H, J_{gem} 11.2 Hz, H-6′c), 4.42, 4.47, 4.59, 4.68 (4 d, 4 H, $J_{1,2}$ 7.8, 8.0, 8.0, 8.2 Hz, 4 H-1), FABMS (negative-ion mode, glycerol matrix): *m/z* 1301.47 $[M-Na]^-$ ($C_{42}H_{71}N_2Na_2O_{35}S_2Si$ MW, Exact 1301.2843, Ave. 1302.2152), 1279.48 $[M-2Na]^-$, 988.4 $[M-2Na-$ NeuAc$]^-$, 724.4 $[M-2Na-NeuAc-6$-sulfo-Gal$]^-$, 441.3 $[Lac-OSE]^-$.

Enzyme assay.—Preparation of recombinant soluble human Fuc-TVII and competitive enzyme assay were carried out as described previously [13,14]. Briefly, test compounds were incubated at 37 °C for 2 h in a total volume of 30 μL of 100 mM cacodylate buffer (pH 7.5) with 25 mM $MnCl_2$, 0.05 mM GDP-fucose, 0.025 mM pyridylaminated sialyl-α-(2→3)-neolactotetraose (IV^{3}NeuAcnLc$_4$-PA) derivative **18**, and the recombinant enzyme (Scheme 3). Measurement of K_i value for each compound and MS analysis of the reaction mixture were also performed as described previously [14].

Acknowledgements

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan. The authors are grateful to Dr Takao Ikami for the FABMS measurements, and to Drs Katsutoshi Sasaki and Hirofumi Nakano for multiform support.

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