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EFFICIENT SYNTHESIS OF THREE TYPES OF SIALYL 6-*O*-SULFO LEWIS X: PROBES FOR THE COMPREHENSIVE SEARCH FOR THE INTERACTION BETWEEN CARBOHYDRATES AND OTHER BIOMOLECLUES[1]

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Abstract – Synthesis of three kinds of 6-*O*-sulfated sLe^x glyco probes with their relevance to ligand processing pathway of L-selectin has been described. The glycosylation of the suitably protected hexasaccharide acceptor **6** with the sialyl-α-(2→3)-D-galactopyranosyl SMe donors (**3** and **4**) using dimethyl(methylthio)sulfonium triflate (DMTST), afforded the protected tetrasaccharides **7** and **8**, respectively. Removal of all the benzyl protecting groups and the subsequent acetylation afforded the sLe^x derivatives **(9** and **10)**, which were converted to the target compounds **(15** and **16)** by the selective removal of 4-methoxyphenyl group, followed by 6-*O*-sulfation of the GlcNAc residue, and removal of all the protective groups under basic conditions. Finally **16** was lactamized with HBTU and HOBT, to give the desired target compound **17**.

INTRODUCTION

An efficient synthesis of carbohydrates and their derivatives as probes have many important roles in the development of glycobiology. We have achieved the total synthesis of sialyl Lewis X ganglioside, which is a common ligand for P, E and L-selectin.[2] In the process of synthesizing many sialyl Lewis X analogs, various synthetic methods were developed and their physiological roles were clarified.[3] In the search for L-selectin elucidated ligand, we have achieved synthesis of sialyl 6-*O*-sulfo Lewis X ganglioside **I**[4] and its variants **II**[5,6] and **III**[7], respectively. These synthetic compounds containing a ceramide or artificial

This paper is dedicated to Professor Emeritus Keiichiro Fukumoto on the occasion of his 75th birthday.

ceramide tail at the reducing end have played a very important role as probes in the biological research. In the previous study, by utilizing these compounds, we have clarified that the sialyl 6-*O*-sulfo Lewis X serves as the major endogenous ligand for L-selectin on HEV in human lymph nodes,[8,9] and proposed that the ligand processing pathway involving an "activation" by de-*N*-acetylation of sialic acid and "inactivation" by lactamization[10] (Figure 1).

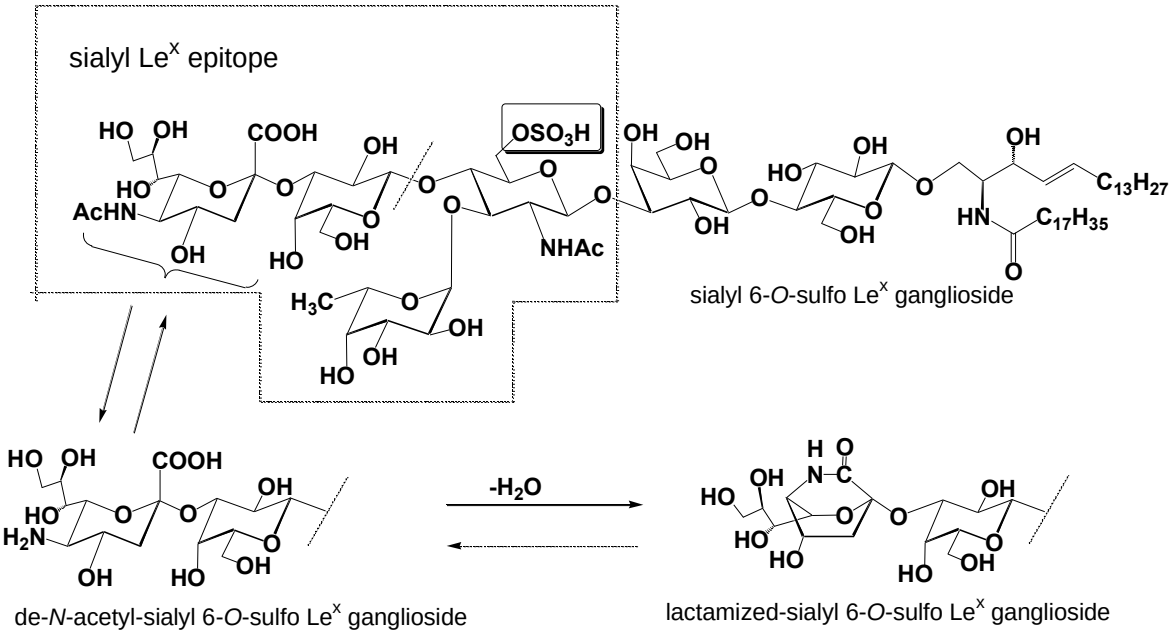


Figure 1. Proposed the ligand processing pathway for human L-selectin.

Recently, Kannagi's group has found that 6-*O*-sulfo sialyl Lewis X expressed on the subset of skin-homing helper memory T cells served as the ligand for peripheral skin-homing helper memory T cells in healthy persons.[11] Nabel's group clarified that hemagglutinin from H5N1 Influenza Virus has a high affinity for sulfate glycans, like sialyl 6-*O*-sulfo Lewis X.[12] So, sialyl 6-*O*-sulfo Lewis X is widely involved in various phenomena, such as maintenance of life and infection of virus, etc.

Taking into account these biological phenomena, sialyl 6-*O*-sulfo Lewis X analogs might play extremely important roles. To clarify these phenomena, large quantities of synthetic glyco probes are needed. However these probes are usually synthesized in multistep reactions and require extensive purification procedures after each synthetic step. The whole process is quite laborious and time consuming. Therefore, the improvement of the synthetic method would be a significant task not only for the synthetic carbohydrate chemistry but also for the elucidation of the unaddressed biological phenomena.

In our previous report,[4,5,6,7] sialyl Lewis X frame was constructed as follows; first, the trisaccharide acceptor containing protected GlcNAc residue (for sulfation at 6 position and fucosylation at 3 position) was coupled with sialyl galactose imidate donor to give the sialylparagloboside (SPG) intermediate. Next, the resulted SPG was converted into SPG acceptor by the selective removal of the protecting group at 3-*O*

of the GlcNAc residue. Lastly, the SPG acceptor was coupled with fucose donor to afford the sialyl Lewis X frame (Figure 2). In the present study, we have employed the new tetrasaccharide acceptor which contains fucose residue and this acceptor was coupled to sialyl galactose SMe donor, which can be prepared in fewer steps than that of imidate donor. The present synthetic route can construct the sialyl LewisX frame directly and effectively (Figure 2).

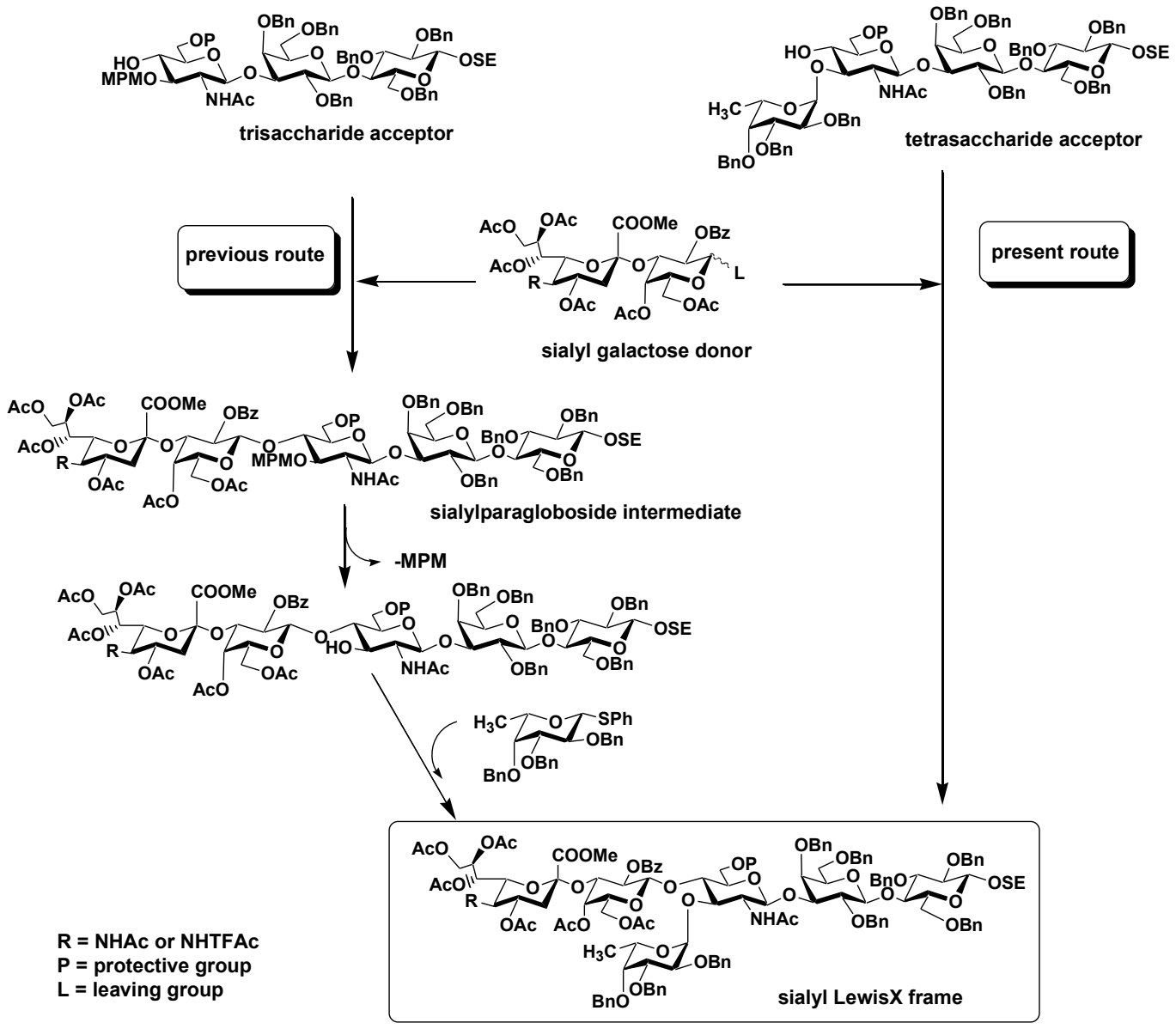
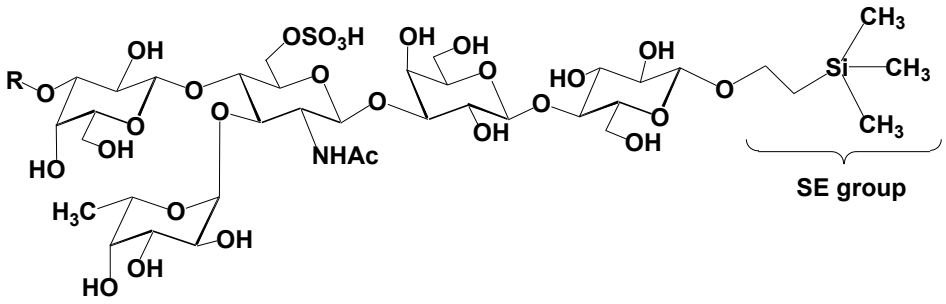


Figure 2. Synthetic strategy of sialyl LewisX frame.

Comprehensive search for the interaction between carbohydrates and other biomolecules such as proteins, these sialyl Lewis X like hexasaccharide probes can be desirable (Figure 3).



R = sialic acid, de-*N*-acetyl sialic acid or lactamized sialic acid

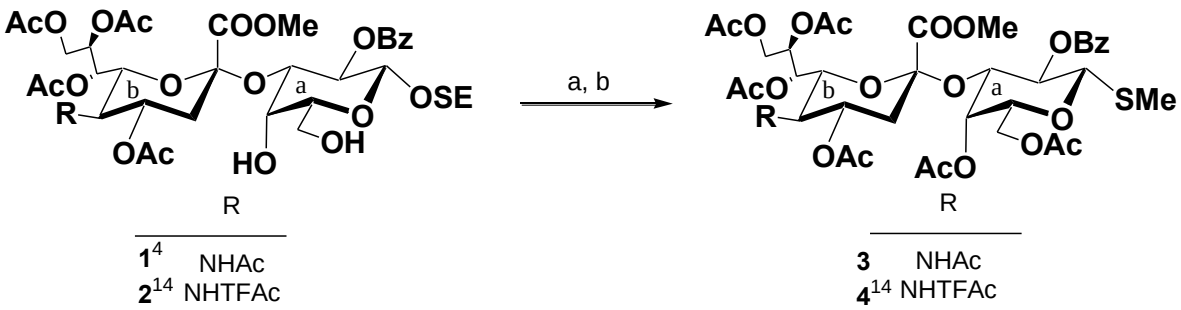
Figure 3. Three sialyl Lewis X like hexasaccharide probes.

The reducing terminal of these probes was protected by 2-(trimethylsilyl)ethyl (SE) group, as SE is already known for its stability in the organic synthesis and also does not get influenced by water solubility, measurement of binding assay and crystal structual analysis.[13] Futhermore, these glycoprobes can also be used for the latest high-throghput analysis *i.e.,* surface plasmon resonance analysis (SPR), quartz crystal microbalance (QCM) and isothermal titration calorimeter (ITC). In the present report, we describe an efficient synthesis of three hexasaccharide probes which can be widely applicable for various biochemical research.

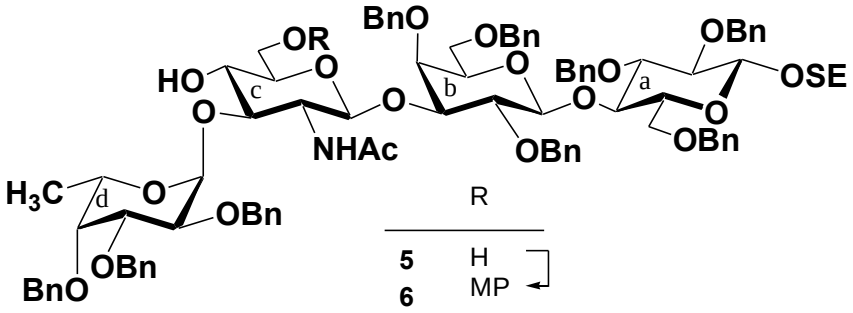
RESULTS AND DISCUSSION

The two key points for the construction of three target hexasaccharides are: i) de-*N*-acetylation and lactamization of sialic acid, ii) selective 6-*O*-sulfation of the GlcNAc residue. We have enabled the synthesis of these compounds to optimize the reactivity of donors and acceptor, and to select the suitable protecting groups. For the effective synthesis of three target compounds **15**, **16** and **17**, we employed the Neu5Ac / Neu5TFAc-α-(2→3)-Gal SMe (**3** and **4**) as glycosyl donors (Scheme 1) and suitably protected tetrasaccharide as an acceptor **6** (Scheme 2). The TFAc protected sialic acid can be easily converted into de-*N*-acetylated and lactamized sialic acid. Selective 6-*O*-sulfation of the GlcNAc residue was achieved by protecting the 6-OH with 4-methoxyphenyl (MP) group which can be chemoselectively cleaved by ceric ammonium nitrate (CAN).

The sialyl galactose donors **3** and **4** were efficiently prepared by the same procedure as reported in our previous paper[4,14] (Scheme 1). For the synthesis of acceptor **6**, compound **5** was synthesized by using the established method[15]. Then regioselective 4-methoxyphenylation at *O*-6 of **6** was carried out by treating with *p*-methoxyphenol (MPOH), PPh_3 and diethylazodicarboxylate (DEAD) in THF for 14 h at 80 °C,[16,17] to give **6** in 70% yield (Scheme 2).



Scheme 1. Synthesis of sialyl galactose donors. *Reagents and conditions*: (a) BF_3OEt_2, Ac_2O / CH_2Cl_2, rt. (b) TMSSMe, TMSOTf / CH_2ClCH_2Cl, 50 ℃.

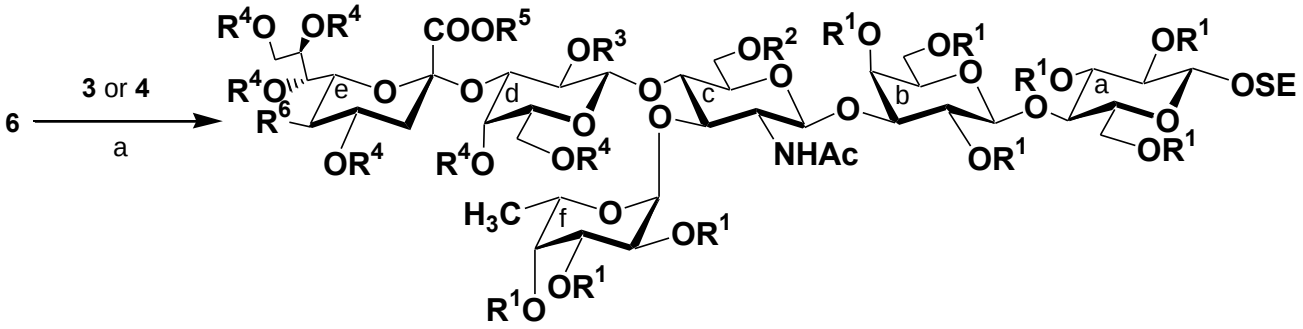


Scheme 2. Synthesis of tetrasaccharide acceptor. *Reagents and conditions:* MPOH, PPh_3, DEAD / THF, 80 ℃, 70%.

The glycosylation of **6** with **3** or **4** in dichloromethane in the presence of dimethyl(methylthio)sulfonium triflate (DMTST)[18, 19] and molecular sieves 4Å (MS 4Å) for 48 h at 0 °C, gave the desired hexasaccharide (**7**, 61%) and (**8**, 61%), respectively. The β-configuration of **7** and **8** was assigned from ^{1}H NMR data that showed the signals at δ 4.92 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1d of **7**) and 4.90 (d, 1 H, $J_{1,2}$ 8.0, Hz, H-1d of **8**) for the obtained hexasaccharides. Hydrogenolytic removal of the benzyl (Bn) groups in **7** and **8** by the catalytic hydrogenenolysis of $Pd(OH)_2$ in ethanol : acetic acid (5 : 1) for 12 h at room temperature, followed by acetylation, gave (**9**, 89% : 2 steps) and (**10**, 88% : 2 steps). Selective cleavage of the MP group in **9** and **10** in acetonitrile-water in the presence of CAN for 1.5 h at room temperature afforded the desired GlcNAc 6-OH derivative (**11**, 83%) and (**12**, 85%), respectively. The 6-*O*-sulfation of **11** and **12** with a sulfur trioxide-pyridine (SO_3•Pyr) complex in DMF, followed by an addition of triethylamine to stabilize the sulfate group during the column chromatography, gave (**13**, 80%) and (**14**, 83%). Removal of all the protective groups in **13** and **14** under alkaline conditions furnished the target compound sialyl 6-*O*-sulfo Lewis X (**15**: GSC-536) and de-*N*-acetyl sialyl 6-*O*-sulfo Lewis X (**16**: GSC-537) almost in a quantitative yield (Scheme 3).

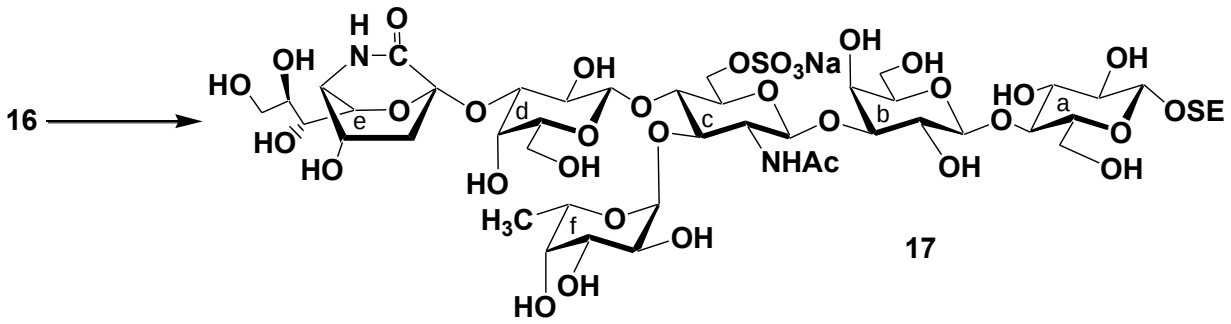
Compound **16** was finally lactamized with *O*-benzotriazol-1-yl-*N,N,N'N'*-tetramethyluronium hexafluorophosphate（HBTU)[20] and 1-Hydroxybenzotriazole (HOBT) in DMF for 2 h at 65 °C to afford a lactamized sialyl 6-*O*-sulfo Lewis X (**17**: GSC-549, Scheme 4) in 98% yield. The ^{1}H NMR spectrum of **17** showed signals at 2.41 (dd, 1H, $J_{3\beta,4}$ 10.5, J_{gem} 13.9 Hz, H-3eβ) and 2.16 (dd, 1H, $J_{3\alpha,4}$ 4.8, J_{gem} 13.9 Hz, H-3eα), respectively showed characteristics of the $^{5,2}B$ boat conformation of sialic acid (Figure 4).[7, 21]

In conclusion, we have achieved the synthesis of three variants of sialyl Lewis X glycoprobes. The synthetic route for the sialyl 6-*O*-sulfo Lewis X frame was the shortest (five synthetic stages reduced) and the most efficient than that of our previous reports. These probes can be used for the precise analysis of the interaction between carbohydrate and other biomolecules, and can also be used for the latest high-throughput analysis. Thus, we anticipate that the present report will surely accelerate the glycobiology.

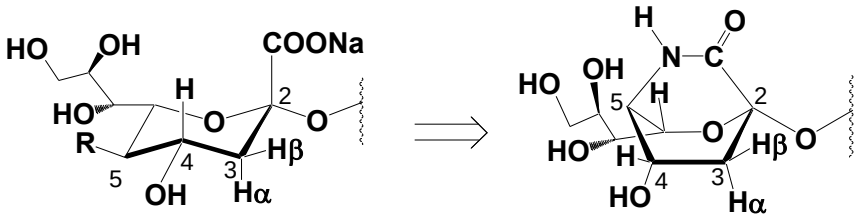


	R^1	R^2	R^3	R^4	R^5	R^6	
7	Bn	MP	Bz	Ac	Me	NHAc	
8	Bn	MP	Bz	Ac	Me	NHTFAc	b
9	Ac	MP	Bz	Ac	Me	NHAc	
10	Ac	MP	Bz	Ac	Me	NHTFAc	c
11	Ac	OH	Bz	Ac	Me	NHAc	
12	Ac	OH	Bz	Ac	Me	NHTFAc	d
13	Ac	SO_3HEt_3N	Bz	Ac	Me	NHAc	
14	Ac	SO_3HEt_3N	Bz	Ac	Me	NHTFAc	e
15	OH	SO_3Na	OH	OH	Na	NHAc	
16	OH	SO_3Na	OH	OH	Na	NH_2	

Scheme 3. Synthesis of sialyl 6-*O*-sulfo Lewis X and de-*N*-acetyl sialyl 6-*O*-sulfo Lewis X. *Reagents and conditions:* (a) DMTST / CH_2Cl_2, MS 4Å, 0 ℃. (b) 1, H_2, $Pd(OH)_2$, EtOH, 2, Ac_2O, Pyr. (c) CAN / CH_3CN/H_2O, 0 ℃. (d) SO_3Pyr complex / DMF, then Et_3N, rt. (e) NaOMe / MeOH, 45 ℃.



Scheme 4. Synthesis of lactamized sialyl 6-*O*-sulfo Lewis X. *Reagents and conditions:* HBTU, HOBT, DMF, 65 ℃, 98%.



Compound No. (Conformation)	H-3α δ (multiplicity, *J* [Hz])	H-3β δ (multiplicity, *J* [Hz])
15 ($^2C_5$)	1.55 (t, $J_{gem} = J_{3\alpha,4} = 12.3$)	2.43 (dd, $J_{gem} = 12.3$, $J_{3\beta,4} = 4.1$)
16 ($^2C_5$)	1.76 (t, $J_{gem} = J_{3\alpha,4} = 12.5$)	2.75 (dd, $J_{gem} = 12.5$, $J_{3\beta,4} = 4.3$)
17 ($^{5,2}B$)	2.41 (dd, $J_{gem} = 13.9$, $J_{3\alpha,4} = 4.8$)	2.25 (dd, $J_{gem} = 13.9$, $J_{3\beta,4} = 10.5$)

Measured at 500 MHz in D_2O

Figure 4. Comparison of the selected ^{1}H NMR data for H-3α and H-3β of the *N*-acetyl, de-*N*-acetyl and lactamized sialic acid.

EXPERIMENTAL

General methods: TLC was conducted on E.Merck silica gel 60 F-254 aluminum plate. Compounds were visualized by exposure to UV light or by spraying with a solution of 10% H_2SO_4 in ethanol. Column chromatography on silica gel (Fuji Silysia Co., 300 mesh) was performed with the solvent systems (v/v) specified. Specific rotations were determined with a Horiba SEPA-300 high-sensitive polarimeter at 25 °C. ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 K with a Varian Inova 500/400 spectrometer, respectively. The values of (ppm) are given relative to Me_4Si (in $CDCl_3$) or HOD (in D_2O, δ = 4.80) as the internal standard. Dichloromethane, methanol, ethanol, benzene and DMF were kept dry over MS 4Å, while pyridine and acetonitrile were kept dry over MS 3Å.

Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-1-thio-β-D-galactopyranoside (3): To a solution of **1** (368 mg, 0.43 mmol) in Ac_2O (0.1 mL) and dry CH_2Cl_2 (2 mL) was added BF_3OEt_2 (476 μL, 1.72 mmol), and the mixture was stirred for 24 h at rt and extracted with $CHCl_3$. The extract was successively washed with aq. $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated. Column chromatography (70 :1 $CHCl_3$-MeOH) of the residue on silica gel gave the acetate derivative (360 mg, 95%) as an amorphous mass (α/β=5/1). To a solution of the acetate derivative (360 mg, 0.4 mmol) in dry CH_2ClCH_2Cl (2 mL) were added TMSSMe (190 μL, 1.86 mmol) and TMSOTf (104 μL, 0.41 mmol). The mixture was stirred for 72 h at 50 °C. After dilution with chloroform, and the extract was successively washed with 1 M Na_2CO_3 and water, dried (Na_2SO_4) and concentrated. Column chromatography (100:1 $CHCl_3$-MeOH) of the residue on silica gel afforded **3** (302 mg, 85%) as an amorphous mass; $[\alpha]_D$ +32.0° (*c* 1.6, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.42 (s, 3 H, MeS), 1.70 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.3 Hz, H-3b*ax*), 1.75, 1.94, 2.03, 2.05, 2.11, 2.15, 2.18 (7 s, 21 H, AcN, 6 AcO), 2.59 (dd, 1 H, $J_{3eq,4}$ 4.5, J_{gem} 12.3 Hz, H-3b*eq*), 3.82 (s, 3 H, COO*Me*), 4.28 (dd, 1H, $J_{8,9}$ 2.0, J_{gem} 12.5 Hz, H-9b), 4.67 (d, 1 H, $J_{1,2}$ 9.8 Hz, H-1a), 4.73 (dd, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 3.2 Hz, H-3a), 4.81 (m, 1 H, H-4b), 5.01 (d, 1 H, H-4a), 5.19 (dd, 1 H, $J_{6,7}$ 2.7, $J_{7,8}$ 9.3 Hz, H-7b), 5.33 (t, 1 H, $J_{2,3}$ 10.0 Hz, H-2a), 5.54 (m, 1 H, H-8b), 7.44-8.12 (m, 5 H, Ph). ^{13}C NMR ($CDCl_3$): δ 11.4, 20.26, 20.74, 20.77, 20.83, 21.52, 23.14, 37.35, 48.81, 53.17, 62.24, 62.28, 66.42, 67.51, 68.07, 68.43, 69.36, 71.76, 71.98, 74.40, 83.28, 96.80, 128.42, 130.05, 130.29, 133.10, 165.51, 168.07, 170.03, 170.30, 170.43, 170.64, 170.72, 170.81. Anal. Calcd for $C_{38}H_{49}NO_{20}S$ (871.26): C, 52.35; H, 5.66; N, 1.61. Found: C, 52.33; H, 5.56; N, 1.32.

2-(Trimethylsilyl)ethyl (2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-(2-acetamido-6-*O*-4-methoxyphenyl-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 (6): To a solution of **5** (272 mg, 0.16 mmol) in THF (8 mL) was added PPh_3 (274 mg, 1.04 mmol), DEAD (284 μL, 0.65 mmol), and MPOH (121 mg, 0.97 mmol), and the mixture was stirred under reflux for 14 h at 80 °C. After completion of the reaction, the mixture was concentrated. Column chromatography (1:4 AcOEt-Hexane) of the residue on silica gel afforded **6** (194 mg, 70%) as an amorphous mass; $[\alpha]_D$ –19.3° (*c* 0.9, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.02 (m, 2 H, $Me_3Si\mathit{CH_2}CH_2$), 1.15 (d, 3 H, $J_{5,6}$ 6.4 Hz, H-6d), 1.32 (s, 3 H, AcN), 3.55 (m, 2 H, $Me_3SiCH_2\mathit{CH_2}$), 3.74 (s, 3H, *Me*OPh), 4.13 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1a), 4.34 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1b), 4.42 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1c), 6.78-7.41 (m, 49 H, MeO*Ph*, 9 Ph). ^{13}C NMR ($CDCl_3$): δ 17.97, 19.86, 24.24, 31.11, 56.34, 57.11, 68.71, 69.60, 69.68, 69.82, 71.75, 74.59, 74.79, 75.16, 75.39, 75.75, 76.04, 76.36, 76.41, 76.77, 77.25, 77.55, 80.35, 81.16, 83.22, 83.81, 84.33, 86.85, 100.93, 103.42, 103.88, 104.47, 116.02, 117.20, 127.82,

128.45, 128.79, 128.87, 128.96, 129.01, 129.16, 129.33, 129.39, 129.42, 129.56, 129.65, 129.67, 129.74, 129.79, 129.83, 129.89, 139.64, 139.69, 139.73, 139.76, 139.97, 140.20, 140.52, 140.70, 140.88, 154.46, 155.33, 171.98. Anal. Calcd for $C_{101}H_{117}NO_{21}Si$ (1707.79): C, 70.98; H, 6.90; N, 0.82. Found: C, 70.72; H, 6.71; N, 0.69.

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)-(4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-(2-acetamido-6-*O*-4-methoxyphenyl-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 (7): To a solution of **3** (152 mg, 0.17 mmol) and **6** (142 mg, 0.08 mmol) in dry CH_2Cl_2 (2 mL) was added molecular sieves 4Å (130 mg), and the mixture was stirred for 2 h at rt, then cooled to 0 °C. DMTST (234 mg, 0.49 mmol) was added to the mixture, and the resultant mixture was stirred for 2 days at 0 °C and neutralized with Et_3N. After dilution with $CHCl_3$, the precipitate was filtered off, and washed with $CHCl_3$. The filtrate and washings were combined, and successively washed with 1 M Na_2CO_3 and water, dried (Na_2SO_4) and concentrated. Column chromatography (80:1 $CHCl_3$-MeOH) of the residue on silica gel afforded **7** (128 mg, 61%) as an amorphous mass; $[\alpha]_D$ +0.9° (*c* 0.6, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 0.89 (m, 2 H, $Me_3SiCH_2CH_2$), 1.01 (d, 3 H, $J_{5,6}$ 6.4 Hz, H-6f), 1.44, 1.46 (2 s, 6 H, 2 AcN), 1.71 (t, 1 H, $J_{gem} = J_{3eq,4}$ 12.5 Hz, H-3e*ax*), 1.81, 1.93, 1.96, 1.98, 2.06, 2.19 (6 s, 18 H, 6 AcO), 2.54 (dd, 1 H, $J_{3eq,4}$ 4.3, J_{gem} 12.5 Hz, H-3e*eq*), 3.53 (m, 2 H, $Me_3SiCH_2CH_2$), 3.60 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.8 Hz, H-2c), 3.70 (s, 3H, *Me*OPh), 3.87 (s, 3 H, COO*Me*) , 4.30 (dd, 1H, H-9e), 4.38 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1c) , 4.82 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 3.4 Hz, H-3d), 4.92 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1d), 5.05 (d, 1H, $J_{1,2}$ 3.4 Hz, H-1f), 5.23 (dd, 1 H, $J_{6,7}$ 2.1, $J_{7,8}$ 8.0 Hz, H-7e), 5.25 (t, 1 H, $J_{2,3}$ 10.0 Hz, H-2d), 5.57 (m, 1 H, H-8e), 5.78 (d, 1 H, $J_{5,NH}$ 8.9 Hz, NHe), 6.72-8.16 (m, 54 H, MeO*Ph*, 10 Ph). ^{13}C NMR ($CDCl_3$): δ 17.49, 19.60, 20.15, 21.28, 21.51, 21.65, 22.47, 23.75, 38.37, 50.83, 54.54, 56.72, 67.48, 68.01, 68.39, 69.52, 69.66, 69.90, 72.32, 72.34, 73.45, 73.98, 74.25, 74.38, 75.01, 75.97, 76.28, 79.24, 79.82, 80.01, 83.22, 84.28, 85.53, 95.48, 97.74, 97.94, 100.24, 103.28, 104.13, 115.75, 116.43, 127.78, 128.27, 128.33, 128.52, 128.63, 128.66, 128.94, 129.06, 129.11, 129.63, 130.39, 130.61, 130.77, 131.05, 131.28, 139.48, 139.53, 139.68, 139.73, 139.85, 140.22, 140.33, 140.58, 153.73, 155.18, 167.08, 168.93, 170.65, 170.68, 170.82, 170.89, 171.60, 171.68, 171.72. Anal. Calcd for $C_{138}H_{162}N_2O_{41}Si$ (2531.04): C, 65.44; H, 6.45; N, 1.11. Found: C, 65.28; H, 6.40; N, 0.94.

2-(Trimethylsilyl)ethyl (methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-(2-acetamido-6-*O*-4-methoxyphenyl-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 (8): To a solution of **4** (51 mg, 0.06 mmol) and **6** (47.1 mg, 0.03 mmol) in dry CH_2Cl_2 (0.7 mL) was added molecular sieves 4Å (43 mg), and the mixture was stirred for 2 h at rt, then cooled to 0 °C. DMTST (78 mg, 0.16 mmol) was added to the mixture, and the resultant mixture was stirred for 2 days at 0 °C and neutralized with Et_3N. After dilution with $CHCl_3$, the precipitate was filtered off, and washed with $CHCl_3$. The filtrate and washings were combined, and successively washed with 1M Na_2CO_3 and water, dried (Na_2SO_4) and concentrated. Column chromatography (100:1 $CHCl_3$-MeOH) of the residue on silica gel afforded **8** (43.3 mg, 61%) as an amorphous mass; $[\alpha]_D$ –5.7° (*c* 0.8, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 0.88 (m, 2 H, $Me_3SiCH_2CH_2$), 1.03 (d, 3 H, $J_{5,6}$ 6.6 Hz, H-6f), 1.42 (s, 6 H, AcO and AcN), 1.70 (t, 1 H, $J_{gem} = J_{3eq,4}$ 12.5 Hz, H-3e*ax*), 1.90, 1.941, 1.948, 2.02, 2.16 (5 s, 15 H, 5 AcO), 2.57 (dd, 1 H, $J_{3eq,4}$ 4.5, J_{gem} 12.5 Hz, H-3e*eq*), 3.56 (m, 2 H, $Me_3SiCH_2CH_2$), 3.67 (s, 3 H, *Me*OPh), 3.72 (t, 1 H, $J_{2,3}$ 9.0 Hz, H-2c), 3.86 (s, 3 H, COO*Me*), 4.24 (dd, 1 H, $J_{8,9}$ 2.0, J_{gem} 12.5 Hz, H-9e), 4.37 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1c), 4.77 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 3.4 Hz, H-3d), 4.90 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1d), 4.99 (m, 1 H, H-4e), 5.01 (d, 1H, $J_{1,2}$ 3.4 Hz, H-1f), 5.18 (dd, 1 H, $J_{6,7}$ 2.0, $J_{7,8}$ 8.5 Hz, H-7e), 5.24 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, H-2d), 5.53 (m, 1 H, H-8e), 5.70 (d, 1 H, $J_{5,NH}$ 9.1 Hz, NHe), 6.12 (d, 1 H, $J_{2,NH}$ 8.9 Hz, NH), 6.69-8.13 (m, 54 H, MeO*Ph*, 10 Ph). ^{13}C NMR ($CDCl_3$): δ 17.97, 20.77, 21.51, 21.84, 21.93, 21.97, 22.07, 22.81, 24.09, 31.10, 38.70, 51.18, 54.77, 57.04, 62.97, 63.28, 67.72, 68.16, 68.67, 68.84, 69.00, 69.34, 69.89, 72.33, 72.50, 73.93, 74.44, 74.57, 74.62, 75.55, 75.91, 76.29, 76.61, 76.73, 77.17, 77.27, 80.40, 80.51, 83.49, 84.51, 85.31, 98.29, 98.56, 101.03, 103.67, 104.15, 104.47, 116.10, 116.78, 128.12, 128.41, 128.46, 128.53, 128.57, 128.64, 128.78, 128.88, 128.98, 129.14, 129.29, 129.34, 129.50, 129.54, 129.60, 129.64, 129.75, 129.76, 130.29, 131.16, 131.59, 135.12, 139.09, 139.82, 139.96, 140.04, 140.22, 140.74, 140.69, 140.89, 154.11, 155.53, 167.44, 169.12, 171.05, 171.15, 171.45, 171.65, 171.95. Anal. Calcd for $C_{138}H_{159}F_3N_2O_{41}Si$ (2585.01): C, 64.07; H, 6.20; N, 1.08. Found: C, 63.91; H, 6.19; N, 0.84.

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)-(4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-(2-acetamido-6-*O*-4-methoxyphenyl-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): A solution of **7** (128 mg, 0.05 mmol) in EtOH (10 mL) and acetic acid (2 mL) was vigorously stirred with $Pd(OH)_2$ (130 mg) for 12 h at rt under hydrogen. The catalyst was collected and washed with MeOH. The combined filtrate and washings were concentrated, and the residue was treated with acetic anhydride (2.2 mL) and pyridine (4 mL) for 12 h at rt, then cooled to 0 °C. MeOH (3 mL) was added and the mixture was concentrated, and the residue was extracted with $CHCl_3$ and successively washed with cold 2 M HCl and water, dried (Na_2SO_4) and concentrated. Column chromatography (50 : 1

$CHCl_3$-MeOH) of the residue on silica gel gave **9** (94 mg, 89 %) as an amorphous mass; $[\alpha]_D$ –6.6° (*c* 0.1, $CHCl_3$) ^{1}H NMR ($CDCl_3$): δ 0.89 (m, 2 H, $Me_3SiCH_2CH_2$), 1.16 (d, 3 H, $J_{5,6}$ 6.6 Hz, H-6f), 1.42 (s, 3 H, AcN), 1.67 (t, 1H, $J_{gem} = J_{3ax,4}$ 12.3 Hz, H-3e*ax*), 1.76 (s, 3 H, AcN), 1.92-2.17 (15 s, 45 H, 15 AcO), 2.49 (dd, 1H, $J_{3eq,4}$ 4.6, J_{gem} 12.3 Hz, H-3e*eq*), 3.54 (m, 2 H, $Me_3SiCH_2CH_2$), 3.73 (s, 3 H, *Me*OPh), 3.76 (t, 1 H, $J_{2,3}$ 10.3 Hz, H-2c), 3.85 (s, 3 H, COO*Me*), 4.20 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1a), 4.43 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1b), 4.71 (dd, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 3.4 Hz, H-3d), 4.84 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.3 Hz, H-2b), 4.91 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1d), 4.97 (dd, 1 H, $J_{2,3}$ 9.3, $J_{3,4}$ 3.6 Hz, H-3b), 5.04 (d, 1 H, H-4d), 5.17 (d, 1 H, $J_{1,2}$ 2.9, H-1f), 5.19 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.6 Hz, H-2d), 5.49 (m, 1 H, H-8e), 6.81-8.15 (m, 9 H, MeO*Ph*, Ph). ^{13}C NMR ($CDCl_3$): δ 17.88, 20.37, 20.51, 20.59, 20.69, 20.68, 20.78, 20.86, 21.03, 21.44, 23.15, 23.27, 29.71, 37.28, 48.74, 53.26, 55.63, 61.69, 61.96, 62.32, 64.37, 66.27, 67.38, 67.51, 67.67, 67.95, 68.24, 69.00, 69.49, 70.87, 70.96, 71.09, 71.21, 71.28, 71.46, 71.71, 71.77, 72.16, 72.67, 73.87, 74.70, 76.06, 95.42, 96.95, 99.64, 99.73, 99.93, 100.69, 114.67, 115.64, 128.68, 129.70, 130.22, 133.34, 153.02, 154.05, 165.25, 167.96, 169.20, 169.63, 169.78, 169.94, 169.97, 170.10, 170.36, 170.44, 170.48, 170.52, 170.61, 170.81, 171.14. Anal. Calcd for $C_{93}H_{126}N_2O_{50}Si$ (2098.71): C, 53.19; H, 6.05; N, 1.33. Found: C, 52.99; H, 6.01; N, 1.28.

2-(Trimethylsilyl)ethyl (methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galacto-pyranosyl)-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-(2-acetamido-6-*O*-4-methoxy-phenyl-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): A solution of **8** (43.3 mg, 0.02 mmol) in EtOH (5 mL) and acetic acid (1 mL) was vigorously stirred with $Pd(OH)_2$ (45 mg) for 12 h at rt under hydrogen. The catalyst was collected and washed with MeOH. The combined filtrate and washings were concentrated, and the residue was treated with acetic anhydride (2 mL) and pyridine (3 mL) for 12 h at rt, then cooled to 0 °C. MeOH (3 mL) was added and the mixture was concentrated, and the residue was extracted with $CHCl_3$ and successively washed with cold 2 M HCl and water, dried (Na_2SO_4) and concentrated. Column chromatography (50 :1 $CHCl_3$-MeOH) of the residue on silica gel gave **10** (33.5 mg, 88%) as an amorphous mass; $[\alpha]_D$ –9.5° (*c* 0.2, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 0.84 (m, 2 H, $Me_3SiCH_2CH_2$), 1.20 (d, 3 H, $J_{5,6}$ 6.4 Hz, H-6f), 1.41 (s, 3 H, AcN), 1.71 (t, 1H, $J_{gem} = J_{3ax,4}$ 12.3 Hz, H-3e*ax*), 1.92-2.18 (15 s, 45 H, 15 AcO), 2.56 (dd, 1 H, $J_{3eq,4}$ 4.5, J_{gem} 12.5 Hz, H-3e*eq*), 3.55 (m, 2 H, $Me_3SiCH_2CH_2$), 3.74 (s, 3 H, *Me*OPh), 3.89 (s, 3 H, COO*Me*), 4.24 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1a), 4.44 (d, 1H, $J_{1,2}$ 7.7 Hz, H-1b), 4.69 (dd, 1 H, $J_{2,3}$ 9.8, $J_{3,4}$ 3.6 Hz, H-3d), 4.85 (dd, 1 H, $J_{1,2}$ 7.7, $J_{2,3}$ 9.5 Hz, H-2b), 4.91 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1d), 4.97 (dd, 1 H, $J_{2,3}$ 9.5, $J_{3,4}$ 3.8 Hz, H-3b), 5.05 (d, 1 H, H-4d), 5.16 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1f), 5.20 (dd, 1 H, $J_{1,2}$ 8.2, $J_{2,3}$ 9.8 Hz, H-2d), 5.49 (m, 1 H, H-8e), 6.04 (d, 1 H, $J_{NH,2}$ 9.1Hz, NHc), 6.83-8.10 (m, 9 H,

MeO*Ph*, Ph). ^{13}C NMR ($CDCl_3$): δ 17.34, 19.30, 21.52, 21.87, 21.96, 22.17, 22.20, 22.46, 22.82, 24.73, 31.13, 38.65, 51.07, 54.83, 57.05, 63.03, 65.72, 68.59, 68.93, 69.39, 69.97, 72.37, 72.63, 72.90, 73.12, 74.10, 75.13, 76.07, 77.45, 78.13, 78.45, 78.77, 96.80, 98.34, 101.30, 101.36, 102.13, 102.45, 116.09, 117.05, 130.10, 131.58, 169.08, 169.70, 170.63, 171.18, 171.53, 171.88, 172.42, 172.56. Anal. Calcd for $C_{93}H_{123}F_3N_2O_{50}Si$ (2152.69): C, 51.86; H, 5.76; N, 1.30. Found: C, 51.82; H, 5.52; N, 1.22.

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)-(4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-(2-acetamido-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 (11): To a solution of **9** (94 mg, 0.04 mmol) in MeCN (5.4 mL) and water (0.6 mL) was added ceric ammonium nitrate (CAN, 98.2 mg, 0.18 mmol), and the mixture was stirred for 1.5 h at 0 °C and extracted with $CHCl_3$. The extract was successively washed with 1M Na_2CO_3 and water, dried (Na_2SO_4) and concentrated. Column chromatography (50:1 $CHCl_3$-MeOH) of the residue on silica gel gave **11** (74.3 mg, 83.2%) as an amorphous mass; $[\alpha]_D$ −16.4° (*c* 0.3, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 0.89 (m, 2 H, Me_3Si*CH*$_2$$CH_2$), 1.24 (d, 3 H, $J_{5,6}$ 6.6 Hz, H-6f), 1.48 (s, 3 H, AcN), 1.75 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.3 Hz, H-3e*ax*), 1.77 (s, 3 H, AcN), 1.95-2.21 (15 s, 45 H, 15 AcO), 2.52 (dd, 1 H, $J_{3eq,4}$ 4.5, J_{gem} 12.3 Hz, H-3e*eq*), 3.06 (br-d, H-6c), 3.56 (m, 2 H, Me_3SiCH_2*CH*$_2$), 3.86 (s, 3 H, COO*Me*), 4.29 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1a), 4.45 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1b), 4.48 (dd, 1 H, $J_{8,9}$ 2.5, J_{gem} 12.1 Hz, H-9e), 4.72 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 3.4 Hz, H-3d), 4.90 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1d), 4.92 (dd, 1 H, $J_{1,2}$ 7.7, $J_{2,3}$ 9.8 Hz, H-2a), 5.06 (d, 1 H, H-4d), 5.19 (dd, 1 H, $J_{1,2}$ 8.2, $J_{2,3}$ 10.7 Hz, H-2d), 5.66 (m, 1 H, H-8e), 7.51-8.17 (m, 5 H, Ph). ^{13}C NMR ($CDCl_3$): δ 15.93, 17.89, 20.41, 20.67, 20.78, 20.87, 20.92, 21.13, 21.24, 21.53, 23.14, 23.45, 37.32, 48.69, 53.24, 61.35, 61.49, 62.14, 63.08, 64.19, 67.11, 67.34, 67.51, 68.01, 69.48, 70.93, 71.25, 71.71, 71.87, 72.60, 72.83, 73.28, 75.44, 75.84, 95.31, 96.98, 98.85, 99.99, 100.19, 100.70, 128.58, 129.60, 130.35, 133.23, 164.91, 167.97, 169.25, 169.63, 169.88, 170.28, 170.47, 170.55, 170.82, 171.23, 171.42. Anal. Calcd for $C_{86}H_{120}N_2O_{49}Si$ (1992.67): C, 51.80; H, 6.07; N, 1.40. Found: C, 51.68; H, 5.97; N, 1.19.

2-(Trimethylsilyl)ethyl (methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-(2-acetamido-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): To a solution of **10** (22 mg, 0.01 mmol) in MeCN (1.8 mL) and water (0.2 mL) was added ceric ammonium nitrate (CAN, 17 mg, 0.31 mmol), and the mixture was stirred for 1.5 h at 0 °C and extracted with $CHCl_3$. The extract was successively washed with 1 M Na_2CO_3 and water, dried (Na_2SO_4) and

concentrated. Column chromatography (50:1 $CHCl_3$-MeOH) of the residue on silica gel gave **12** (17.8 mg, 85%) as an amorphous mass; $[\alpha]_D$ –21.1° (*c* 0.4, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 0.88 (m, 2 H, $Me_3SiCH_2CH_2$), 1.20 (d, 3H, $J_{5,6}$ 6.4 Hz, H-6f), 1.54 (s, 3 H, AcN), 1.64 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.5 Hz, H-3e*ax*), 1.90-2.15 (15 s, 45 H, 15 AcO), 2.46 (dd, 1 H, $J_{3eq,4}$ 4.3, J_{gem} 12.5 Hz, H-3e*eq*), 3.20 (br-d, H-6c), 3.57 (m, 2 H, $Me_3SiCH_2CH_2$), 3.83 (s, 3 H, COO*Me*), 4.30 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1a), 4.46 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1b), 4.86 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 3.2 Hz, H-3d), 4.97 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1d), 5.11 (d, 1 H, $J_{1,2}$ 2.7 Hz, H-1f), 5.20 (dd, 1 H, $J_{1,2}$ 8.4, $J_{2,3}$ 10.0 Hz, H-2d), 5.60 (m, 1 H, H-8e), 6.06 (d, 1 H, $J_{NH,5}$ 9.1 Hz, NHc), 7.43-8.18 (m, 5 H, Ph). ^{13}C NMR ($CDCl_3$): δ 17.55, 19.58, 21.33, 21.51, 21.83, 21.89, 22.15, 22.19, 22.35, 22.40, 22.48, 22.78, 31.15, 38.52, 50.12, 53.82, 62.25, 63.87, 64.61, 65.85, 67.54, 67.84, 68.43, 69.85, 70.48, 70.63, 71.33, 71.65, 72.48, 75.81, 76.32, 95.82, 97.03, 100.92, 101.42, 101.84, 102.13, 128.51, 129.73, 130.56, 133.88, 165.66, 167.79, 169.54, 169.58, 169.73, 169.77, 169.88, 170.16, 170.32, 170.45, 170.69, 170.98, 171.34, 171.59. Anal. Calcd for $C_{86}H_{117}F_3N_2O_{49}Si$ (2046.64): C, 50.44; H, 5.76; N, 1.37. Found: C, 50.42; H, 5.70; N, 1.32.

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)-(4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-(2-acetamido-2-deoxy-6-*O*-sulfo-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 triethylammonium salt (13): To a solution of **11** (74.3 mg, 0.04 mmol) in DMF (3 mL) was added sulfur trioxide pyridine complex (36.3 mg, 0.22 mmol), and the mixture was stirred for 6 h at rt. Triethylamine (0.1 mL) was added and the mixture was concentrated. Column chromatography (1:1 $CHCl_3$-MeOH) of the residue on Sephadex LH-20 gave the crude sulfated product, and this was purified by column chromatography (10:1 $CHCl_3$-MeOH) on silica gel to afford **13** (61.2 mg, 80%) as an amorphous mass; $[\alpha]_D$ –19.5° (*c* 0.1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 0.88 (m, 2 H, $Me_3SiCH_2CH_2$), 1.23 (d, 3 H, $J_{5,6}$ 6.6 Hz, H-6f), 1.36 (t, 9 H, NCH_2CH_3), 1.66 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.9 Hz, H-3e*ax*), 171-2.14 (17 s, 51 H, 15 AcO, 2 AcN), 2.44 (dd, 1 H, $J_{3eq,4}$ 4.5, J_{gem} 12.9 Hz, H-3e*eq*), 3.18 (q, 6 H, NCH_2CH_3), 3.54 (m, 2 H, $Me_3SiCH_2CH_2$), 3.86 (s, 3 H, COO*Me*), 4.07 (dd, 1 H, $J_{8,9'}$ 5.4, J_{gem} 11.9 Hz, H-9'e), 4.30 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1a), 4.44 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1b), 4.77 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 3.4 Hz, H-2f), 4.85 (dd, 1 H, $J_{1,2}$ 7.7, $J_{2,3}$ 9.6 Hz, H-2b), 4.88 (m, 1 H, H-4e), 4.92 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.8 Hz, H-2a), 4.98 (dd, 1 H, $J_{2,3}$ 10.9, $J_{3,4}$ 3.8 Hz, H-3d), 5.01 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1d), 5.17 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 3.4 Hz, H-3b), 5.20 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 10.9 Hz, H-2d), 5.57 (m, 1 H, H-8e), 7.58-8.21 (m, 5 H, Ph). Anal. Calcd for $C_{92}H_{135}N_3O_{52}SSi$ (2173.75): C, 50.80; H, 6.26; N, 1.93. Found: C, 50.74; H, 6.12; N, 1.80.

2-(Trimethylsilyl)ethyl (methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-

α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-(2-acetamido-2-deoxy-6-*O*-sulfo-β-D-gluco-pyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-gluco-pyranoside triethylammonium salt (14): To a solution of **12** (17.8 mg, 8.1 μmol) in DMF (1.5 mL) was added sulfur trioxide pyridine complex (8 mg, 48.4 μmol), and the mixture was stirred for 6 h at rt. Triethylamine (0.1 mL) was added and the mixture was concentrated. Column chromatography (1:1 $CHCl_3$-MeOH) of the residue on Sephadex LH-20 gave the crude sulfated product, and this was purified by column chromatography (10:1 $CHCl_3$-MeOH) on silica gel to afford **14** (15.2 mg, 83%) as an amorphous mass; $[\alpha]_D$ –27.8° (*c* 0.3, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 0.88 (m, 2 H, $Me_3SiCH_2CH_2$), 1.18 (d, 3 H, $J_{5,6}$ 6.4 Hz, H-6f), 1.31 (t, 9 H, NCH_2CH_3), 1.87 (s, 3 H, AcN), 190-2.11 (15 s, 45 H, 15 AcO), 2.33 (dd, 1 H, $J_{3eq,4}$ 4.5, J_{gem} 12.5 Hz, H-3e*eq*), 3.10 (q, 6 H, NCH_2CH_3), 3.55 (m, 2 H, $Me_3SiCH_2CH_2$), 3.63 (s, 3 H, COO*Me*), 3.71 (t, 1H, $J_{2,3}$ 9.4 Hz, H-2c), 4.18 (dd, 1H, $J_{8,9}$ 2.7, J_{gem} 10.2 Hz, H-9e), 4.25 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1a), 4.39 (dd, 1 H, $J_{8,9'}$ 3.8, J_{gem} 10.6 Hz, H-9'e), 4.45 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1b), 4.73 (m, 1 H, H-4e), 4.85 (dd, 1H, $J_{1,2}$ 7.7, $J_{2,3}$ 9.3 Hz, H-2b), 4.90 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.4 Hz, H-2a), 4.95 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 3.2 Hz, H-3d), 5.20 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1f), 5.33 (d, 1 H, H-4b), 5.41 (dd, 1 H, $J_{1,2}$ 8.2, $J_{2,3}$ 10.0 Hz H-2d), 5.60 (m, 1 H, H-8e), 6.18 (d, 1 H, $J_{NH,5}$ 9.3 Hz, NHc), 7.44-8.27 (m, 5 H, Ph). Anal. Calcd for $C_{92}H_{132}F_3N_3O_{52}SSi$ (2227.72): C, 49.57; H, 5.97; N, 1.89. Found: C, 49.56; H, 5.83; N, 1.73.

2-(Trimethylsilyl)ethyl (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic-acid)-(2→3)-(β-D-galactopyranosyl)-(1→4)-[(α-L-fucopyranosyl)-(1→3)]-(2-acetamido-2-deoxy-6-*O*-sulfo-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside disodiumsalt (15): To a solution of **13** (61.2 mg, 12 mmol) in MeOH (4 mL) and dioxane (1mL) was added 0.5 mL of 28% sodium methoxide in MeOH, and the mixture was stirred for 72 h at 45 °C. Water (0.5 mL) was added and the mixture was stirred for 24 h at rt and then concentrated. Column chromatography (1:1 $CHCl_3$-MeOH) of the residue on Sephadex LH-20 gave the target molecule **15** (39.9 mg, 99%) as an amorphous mass; $[\alpha]_D$ –17.7° (*c* 0.07, 1 : 1 $CHCl_3$: MeOH). ^{1}H NMR (D_2O): δ 0.98 (m, 2 H, $Me_3SiCH_2CH_2$), 1.16 (d, 3 H, $J_{5,6}$ 6.6 Hz, H-6f), 1.82 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.0 Hz, H-3e*ax*), 2.01, 2.02 (2 s, 6 H, 2AcN), 2.75 (dd, 1 H, $J_{3eq,4}$ 4.0, J_{gem} 12.0 Hz, H-3e*eq*), 3.27 (t, 1 H, $J_{1,2}$ 8.0 Hz, H-2b), 3.50 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 9.0 Hz, H-2d), 3.58 (m, 2 H, H-2a and H-4f), 3.65 (dd, 1 H, $J_{1,2}$ 3.6, $J_{2,3}$ 7.0 Hz, H-2f), 3.79 (m, 1 H, H-4e), 3.94 (d, 1 H, $J_{3,4}$ 3.0 Hz, H-4d), 4.11 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 3.0 Hz, H-3d), 4.17 (m, 1 H, H-5e), 4.43 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1a), 4.49 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1b), 4.59 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1d), 4.73 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1c), 5.11 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1f). Anal. Calcd for $C_{48}H_{82}N_2Na_2O_{36}SSi$ (1368.39) C, 42.10; H, 6.04; N, 2.05. Found: C, 41.95; H, 5.99; N, 1.88. FAB(-)MS: m/z: calcd for $C_{48}H_{82}N_2Na_2O_{36}SSi$: 1368.39; found: 1345.60 $[M-Na]^-$, 870.4 [M-H-NeuAc-Gal]$^-$, 690.3 [870.4-Fuc]$^-$,

441.2 [disaccharide of reducing terminal]$^-$.

2-(Trimethylsilyl)ethyl (5-amino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonicacid)-(2→3)-(β-D-galactopyranosyl)-(1→4)-[(α-L-fucopyranosyl)-(1→3)]-(2-acetamido-2-deoxy-6-*O*-sulfo-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside disodiumsalt (16): To a solution of **14** (15.2 mg, 6.8 μmol) in MeOH (5 mL) and dioxane (0.4 mL) was added 0.5 mL of 28% sodium methoxide in MeOH, and the mixture was stirred for 72 h at 45 °C. Water (0.3 mL) was added and the mixture was stirred for 24 h at rt, and then concentrated. Column chromatography (1:1 $CHCl_3$-MeOH) of the residue on Sephadex LH-20 gave the target molecule **16** (7.8 mg, quant) as an amorphous mass; $[\alpha]_D$ –1.4° (*c* 0.01, 1:2 $CHCl_3$:MeOH). ^{1}H NMR (D_2O): δ 0.98 (m, 2 H, $Me_3SiCH_2CH_2$), 1.13 (d, 3 H, $J_{5,6}$ 5.9 Hz, H-6f), 1.77 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.5 Hz, H-3e*ax*), 1.99 (s, 3 H, AcN), 2.75 (dd, 1H, $J_{3eq,4}$ 4.3, J_{gem} = 12.5 Hz, H-3e*eq*), 3.09 (t, 1 H, H-5e), 3.27 (t, 1 H, $J_{1,2}$ 7.8 Hz, H-2b), 3.49 (t, 1 H, $J_{2,3}$ 9.6 Hz, H-2d), 3.53-3.57 (m, 2 H, H-7e and H-2a), 3.64 (br-dd, 1 H, H-2f), 3.66 (m, 1 H, H-8e), 3.68 (m, 1 H, H-4e), 3.74-3.77 (m, 2 H, H-3a and H-6e), 3.91 (m, 1 H, H-9e), 4.08 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 3.0 Hz, H-3d), 4.40 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1a), 4.48 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1b), 4.57 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1d), 5.08 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1f). Anal. Calcd for $C_{46}H_{80}N_2Na_2O_{35}SSi$ (1326.38) C, 41.63; H, 6.08; N, 2.11. Found: C, 41.40; H, 6.05; N, 1.96. FAB(-)MS: m/z: calcd for $C_{46}H_{80}N_2Na_2O_{35}SSi$: 1326.38; found: 1303.4 [M-Na]$^-$, 870.4 [M-H-de-*N*-acetyl Neu-Gal]$^-$, 690.3 [870.4-Fuc]$^-$, 441.2 [disaccharide of reducing terminal]$^-$.

2-(Trimethylsilyl)ethyl (5-acetylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl-1,5-lactam)-(2→3)-(β-D-galactopyranosyl)-(1→4)-[(α-L-fucopyranosyl)-(1→3)]-(2-acetamido-2-deoxy-6-*O*-sulfo-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside sodiumsalt (17): To a solution of **16** (1.8 mg, 1.3 μmol) in DMF (1 mL) was added HBTU (3 mg, 8 μmol) and HOBT (1 mg, 7.4 μmol), and the mixture was stirred for 2 h at 65 °C, and then concentrated. Column chromatography (MeOH) of the residue on Sephadex LH-20 gave the target molecule **17** (1.7 mg, 98%) as an amorphous mass; $[\alpha]_D$ –23.5° (*c* 0.03, MeOH); ^{1}H NMR (D_2O): δ 0.98 (m, 2 H, $Me_3SiCH_2CH_2$), 1.16 (d, 3 H, $J_{5,6}$ 6.6 Hz, H-6f), 1.99 (s, 3 H, AcN), 2.16 (dd, 1H, $J_{3\alpha,4}$ 4.8, J_{gem} 13.9 Hz, H-3eα), 2.41 (dd, 1H, $J_{3\beta,4}$ 10.5, J_{gem} 13.9 Hz, H-3eβ), 3.24-3.27 (m, 2 H, H-2d and H-2b), 3.56 (m, 2 H, $Me_3SiCH_2CH_2$), 3.73 (dd, 1 H, $J_{1,2}$ 7.3, $J_{2,3}$ 9.6 Hz, H-2c), 3.89 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 3.2 Hz, H-3f), 4.11 (dd, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 3.2 Hz, H-3d), 4.13-4.15 (m, 2 H, H-4d and H-4e), 4.35 (br-d, 1 H, H-6e), 4.42 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1a), 4.48 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1b), 4.58 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1d), 5.10 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1f); FAB(-)MS: m/z: calcd for $C_{46}H_{79}N_2NaO_{34}SSi$: 1286.3902; found: 1263.4004 [M-Na]$^-$, 870.4

[M-Na-Lactamized Neu-Gal]$^-$, 690.4 [M-Na-Lactamized Neu-Gal-Fuc]$^-$, 441.3 [disaccharide of reducing terminal]$^-$.

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