Glycoconjugate Journal 22, 95–108, 2005

6-*O*-Sulfo sialylparagloboside and sialyl Lewis X neo-glycolipids containing lactamized neuraminic acid: Synthesis and antigenic reactivity against G159 monoclonal antibody†

Masanori Yamaguchi[1,*], Hideharu Ishida[1], Akiko Kanamori[2], Reiji Kannagi[2,3] and Makoto Kiso[1,3]

[1] *Department of Applied Bio-Organic Chemistry, Gifu University, Gifu 501-1193, Japan;* [2] *Department of Molecular Pathology, Research Institute, Aichi Cancer Center, 1-1 Kanokoden, Chikusaku, Nagoya 464-8681, Japan;* [3] *CREST, Japan Science and Technology Corporation (JST), Tokyo, Japan*

Synthesis and antigenic reactivity of 6-*O*-sulfo sialylparagloboside (SPG) and sialyl Lewis X (sLe^X) neo-glycolipids containing lactamized neuraminic acid are described. The suitably protected GlcNAc-β(1→3)-Gal-β(1→4)-GlcOSE derivative was glycosylated with NeuTFAc-α(2→3)-Gal imidate to give NeuTFAc-α(2→3)-Galβ (1→4)-GlcNAc-β(1→3)-Gal-β(1→4)-GlcOSE pentasaccharide. The partial *N*,*O*-deacylation in the NeuTFAc-α(2→3)-Gal part afforded *N*-deacetylated SPG derivative which was converted to the desired oligosaccharide containing lactamized neuraminic acid. Similar treatment of the sLe^X hexasaccharide derivative, NeuTFAc-α(2→3)-Gal-β(1→4) [Fuc-α(1→3)]-GlcNAc-β(1→3)-Gal-β(1→4)-GlcOSE, gave the key hexasaccharide intermediate containing lactamized neuraminic acid. These suitably protected SPG and sLe^x oligosaccharides were converted stepwise into the desired neo-glycolipids (GSC-551 andGSC-552) by the coupling with 2-(tetradecyl)hexadecanol, 6-*O*-sulfation at C-6 of the GlcNAc residure, and complete deprotection.

Both lactamized-sialyl 6-*O*-sulfo SPG (GSC-551) and sLe^x (GSC-552) neo-glycolipids were clearly recognized with G159 monoclonal antibody showing that both the lactamized neuraminic acid and the 6-*O*-sulfate at C-6 of GlcNAc would be involved in the G159-defined determinant. However, the Fuc residue and the lipophilic (ceramide) part may not be critical for this recognition.
Published in 2005.

Keywords: **Selectin, glycolipid, sialic acid, sialyl Lewis X, sialylparagloboside, carbohydrate, antibody, sulfation**

Introduction

Selectins (L-, E- and P-selectin) are a family of carbohydrate-binding cell adhesion molecules which play important roles in homing of lymphocytes, recruitment of leukocytes to sites of inflammation, thrombosis, cancer metastasis, etc. [2,3]. It has been demonstrated that sialyl 6-*O*-sulfo Lewis X (**I**, Figure 1) is an endogenous L-selectin ligand on the human high endothelial venule (HEV) [4–8]. Recently, de-*N*-acetyl sialyl 6-*O*-sulfo Lewis X [9,10] was found to be a superior ligand for L-selectin, which may be inactivated (down regulation) by conversion into the cyclic structure detected with G159 monoclonal antibody (G159 mAb) [11].

In the previous study, we have shown that the structure defined by G159 mAb may be lactamized sialyl 6-*O*-sulfo Lewis X (**III**) suggesting the existence of a new immunity adjustment mechanism regulated by the structural change of sialic acid [4,11] (Figure 1).

Since this discovery, we have systematically synthesized a series of gangliosides [12] and neo-glycolipids [1] containing lactamized neuraminic acid for mapping the G159 mAb recognition sites in more detail. As described in a preceding paper [1], the lactamized- sialyl 6-*O*-sulfo Lewis X B_{30} neo-glycolipid (**GSC-534**) (Figure 2) was not recognized with G159 mAb to suggest that the lactose part and/or ceramide in lactamized-sialyl 6-*O*-sulfo Lewis X ganglioside (**GSC-535**) may be critically important for the recognition by G159 mAb.

† Synthetic studies on sialoglycoconjugates, Part 138. For part 136, see Ref [1], and for part 137, see Ref [19].

* Present address: Division of Life Science, Graduate School of Biostudies, Kyoto University, Sakyo-ku, Kyoto, 606-8502, Japan.
To whom correspondence should be addressed: H. Ishida/M. Kiso, Faculty of Applied Biological Sciences, Gifu University, Gifu 501-1193, Japan. Fax: +81-58-293-2840; E-mail: ishida@cc.gifu-u.ac.jp/kiso@cc.gifu-u.ac.jp.

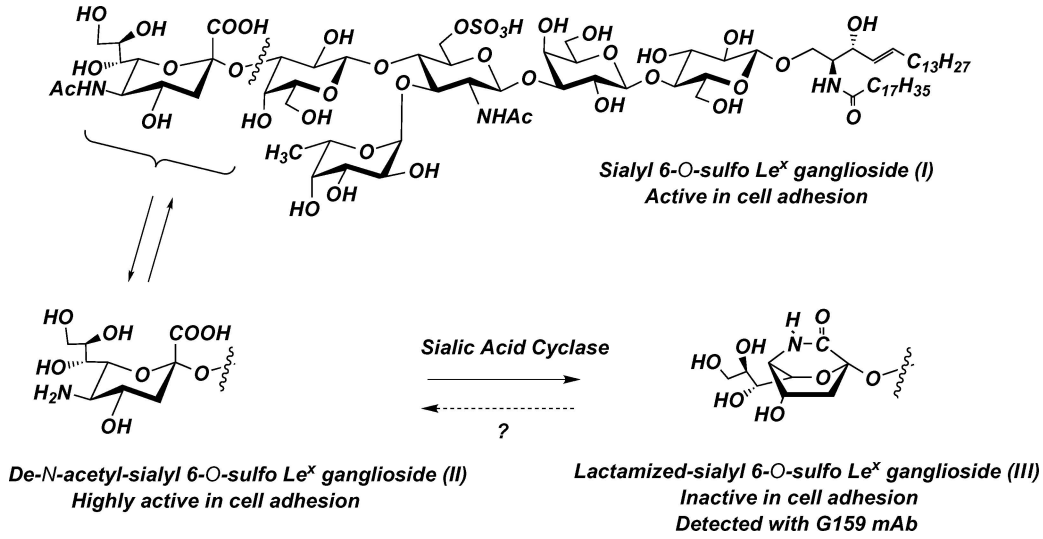


Figure 1. Proposed ligand processing pathway and binding reactivity for human L-selectin.

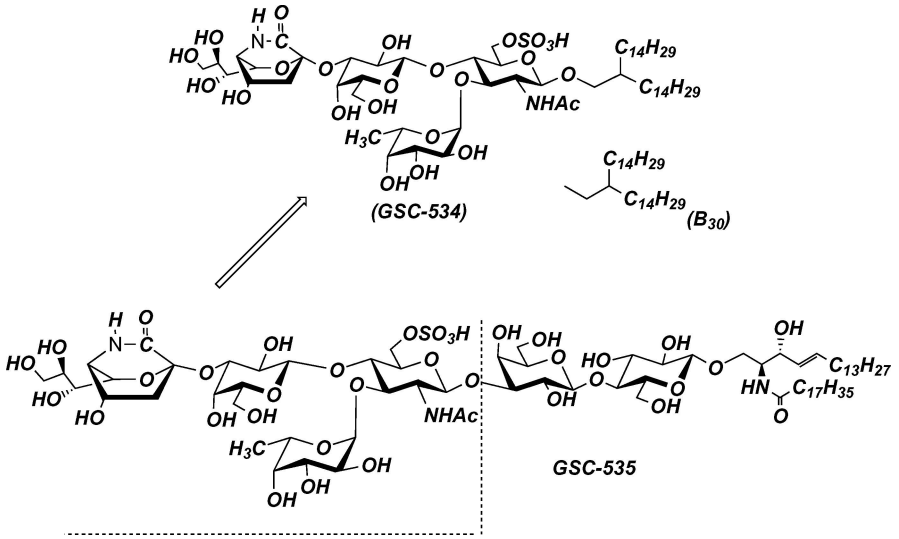


Figure 2. Lactamized sialyl 6-O-sulfo LewisX ganglioside (GSC-535) and the sLe^x tetrasaccha neo-glycolipid (GSC-534).

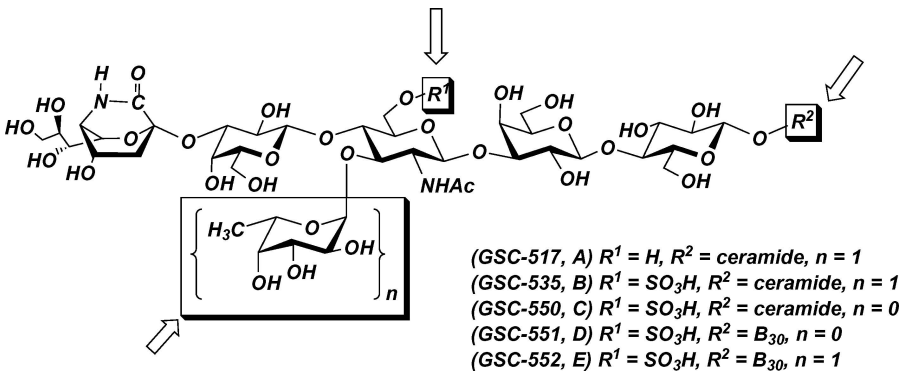
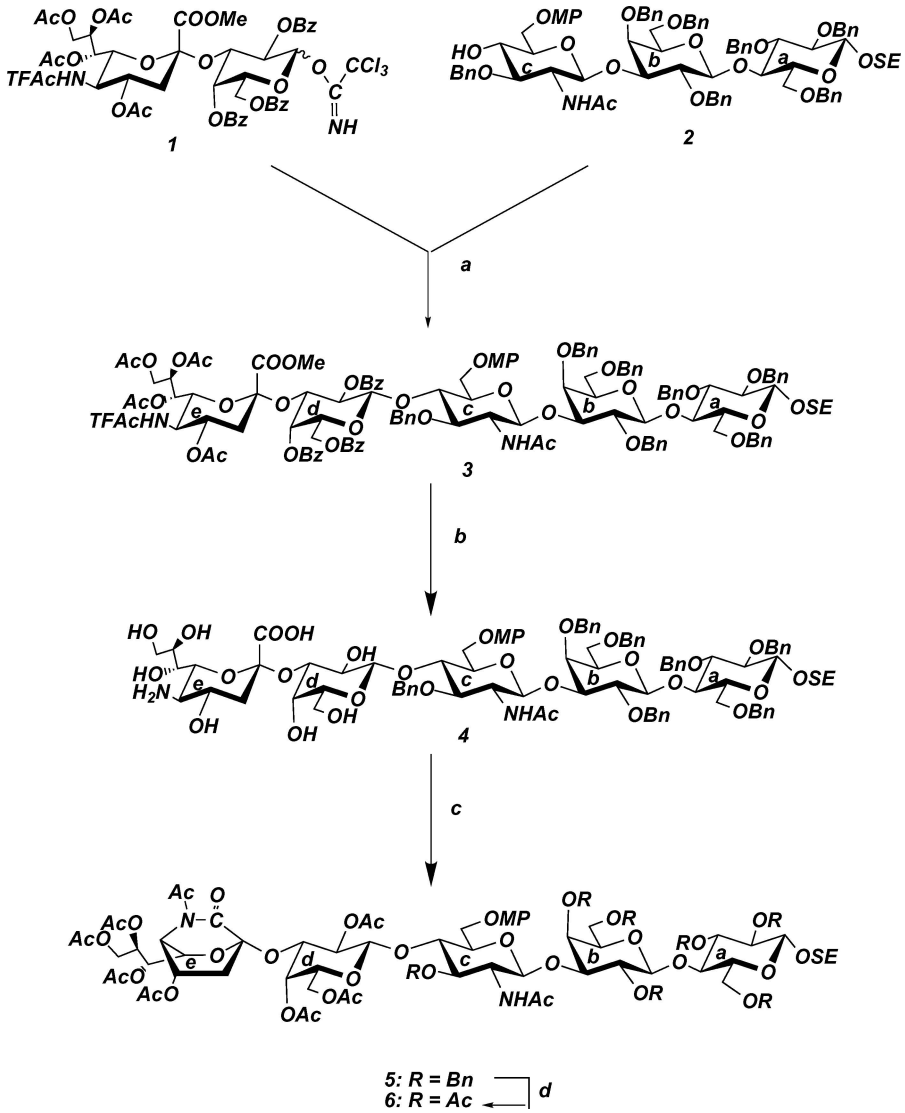


Figure 3. Three modification sites of Lactamized sialyl 6-O-sulfo Lewis X ganglioside fo clarifying the recognition specificity of G159 mAb.



Scheme 1. (a) TMSOTf/CH_2Cl_2, MS4Å, 3°C, 76%; (b) NaOMe, MeOH, then H_2O, 45°C, 80%; (c) 1,WSC·HCl, HOBt, DMF, 70°C, 2, Ac_2O, Pyr., 46% (two steps); (d) 1, H_2, $Pd(OH)_2$, EtOH, 2, Ac_2O Pyr., 93% (two steps).

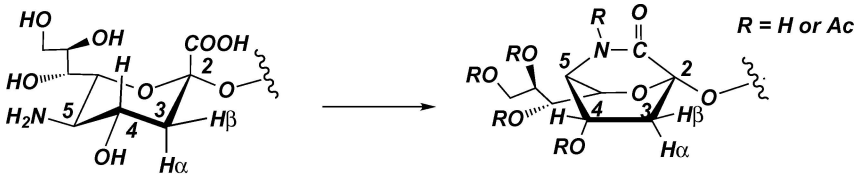
We here report the synthesis of novel sialylparagloboside (**D**, **GSC-551**) and sialyl Le^X (**E**, **GSC-552**) neo-glycolipids containing both lactamized neuraminic acid and lactose (Figure 3), and examine the structural requirements for recognition with G159 mAb by comparing the immunostaining reactivity of the related gangliosides (**A**, **GSC-517**; **B**, **GSC-535**; **C**, **GSC-550**) [12].

Results and discussion

Coupling of **1** [13] and suitably protected trisaccharide **2** [14] in the presence of trimethylsilyl trifluoromethanesufonate (TMSOTf) and powdered molecular sieves 4 Å (AW 300) gave the expected pentasaccharide **3** in 76% yield (Scheme 1).

The partial *N*,*O*-deacylation of **3** with sodium methoxide in methanol for 72 h at 45°C, and subsequent saponification of the methyl ester group afforded **4** in 80% yield. Lactamization of the neuraminic acid residure in **4** was achieved by treatment with WSCHCl and HOBt in DMF at 70°C, followed by acetylation, to give **5** (46% in 2 steps). In the ^{1}H NMR spectrum of **5**, the values of vicinal couplings ($J_{3,4}$) changed dramatically (Table 1) to indicate clearly that typical $^{5,2}B$ boat conformation was formed by lactamization of the neuraminic acid part of **4** ($^2C_5$ chair conformation). The lactamization reaction was greatly improved by using *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) and HOBt in DMF at 65°C as described for the sialyl Le^x derivative **13** (Scheme 3). Hydrogenolytic removal of the benzyl (Bn) groups in **5** and the following acetylation gave **6** (93% in 2 steps). Compound **6** was then transformed into the corresponding trichloroacetimidate **7** by selective removal of the 2-(trimethylsilyl)ethyl (SE) group with trifluoroacetic acid (TFA) and subsequent imidate formation (Scheme 2). Glycosylation of

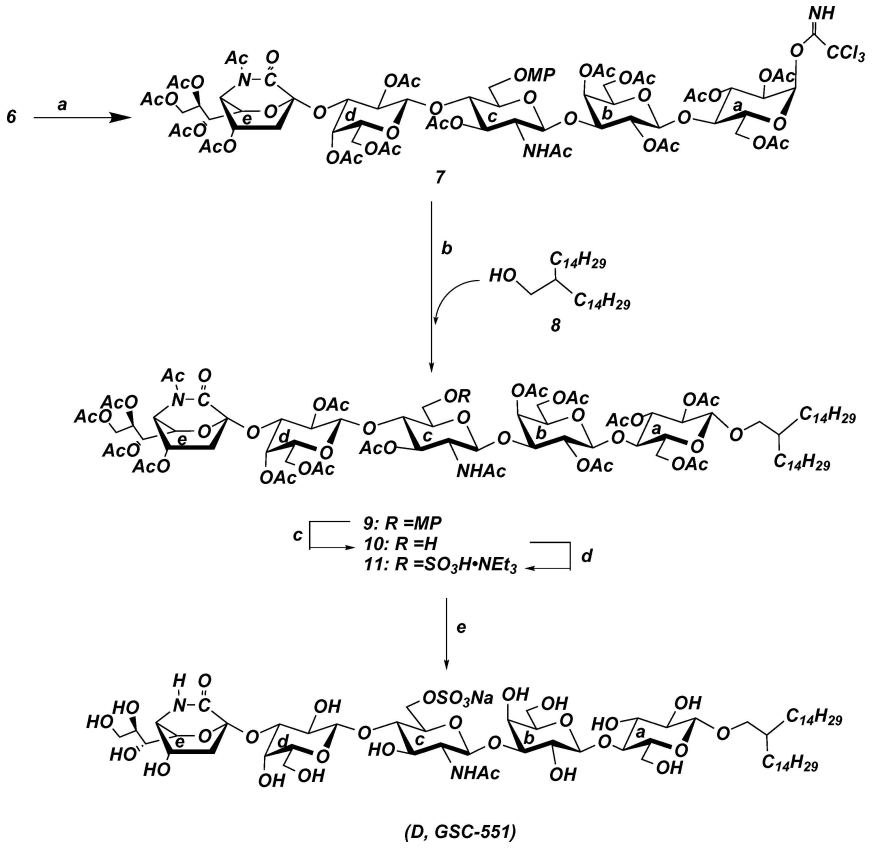
Table 1. Comparison of the selected ^{1}H NMR data[a] for H-3α and H-3β of the *N*-deacetylated ($^2C_5$) and lactamized (^{5,2}B) neuraminic acid $\alpha(2 \rightarrow 3)$-linked to the terminal galactose residue.



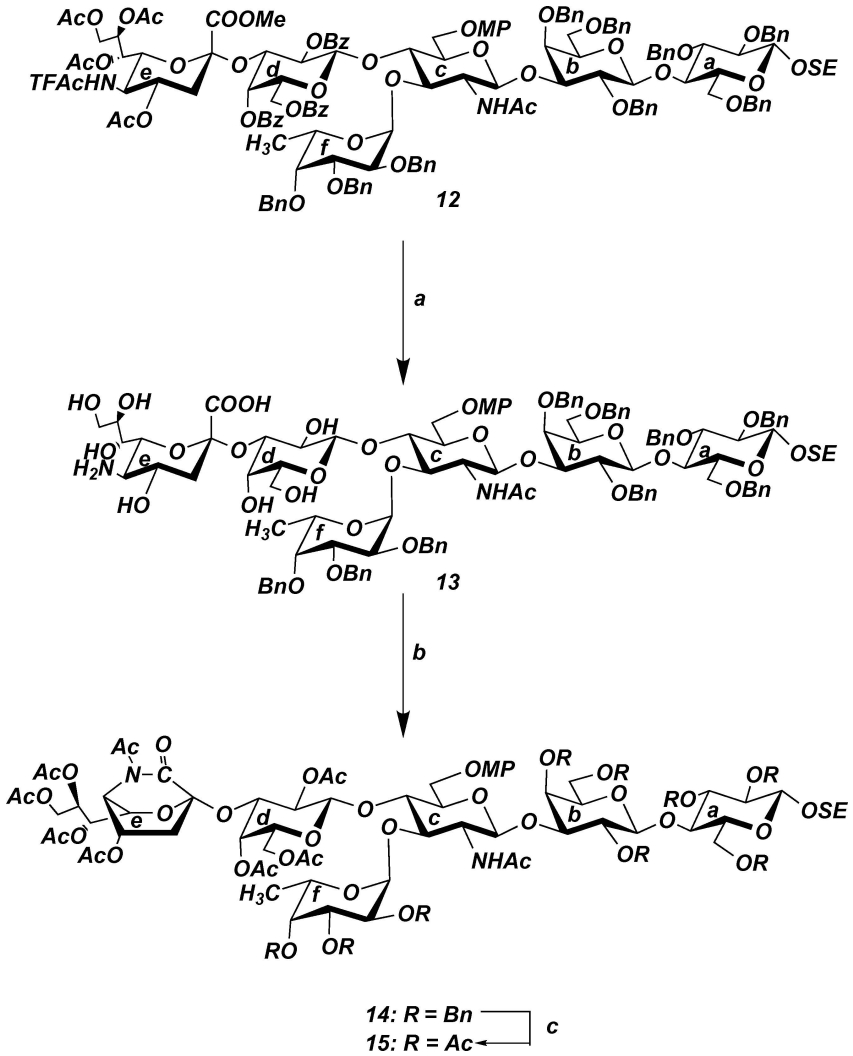
$^2C_5$ **(4, 13)** $^{5,2}B$ **(5-7, 9, 11, 15, 19, GSC-551, GSC-552)**

Compound No. (Conformation)	*H-3α*	*H-3β*
4($^2C_5$)	1.75 (*t*, $J_{gem} = J_{3\alpha,4} = 12.1$)	2.87 (dd, $J_{gem} = 12.1$, $J_{3\beta,4} = 5.0$)
5(^{5,2}B)	2.37 (dd, $J_{gem} = 14.4$, $J_{3\beta,4} = 5.5$)	2.29 (dd, $J_{gem} = 14.4$, $J_{3\beta,4} = 10.3$)
6(^{5,2}B)	2.31 (dd, $J_{gem} = 14.6$, $J_{3\alpha,4} = 5.7$)	2.22 (dd, $J_{gem} = 14.6$, $J_{3\beta,4} = 10.5$)
7(^{5,2}B)	2.31 (dd, $J_{gem} = 14.6$, $J_{3\alpha,4} = 5.7$)	2.23 (dd, $J_{gem} = 14.6$, $J_{3\beta,4} = 10.9$)
9(^{5,2}B)	2.32 (dd, $J_{gem} = 14.4$, $J_{3\alpha,4} = 5.7$)	2.23 (dd, $J_{gem} = 14.4$, $J_{3\beta,4} = 10.7$)
11(^{5,2}B)	2.45 (dd, $J_{gem} = 13.9$, $J_{3\alpha,4} = 5.7$)	2.24 (dd, $J_{gem} = 13.9$, $J_{3\beta,4} = 10.5$)
13($^2C_5$)	1.75 (*t*, $J_{gem} = J_{3\alpha,4} = 11.9$)	2.83 (dd, $J_{gem} = 11.9$, $J_{3\beta,4} = 5.0$)
15(^{5,2}B)	2.24 (dd, $J_{gem} = 13.9$, $J_{3\alpha,4} = 5.3$)	2.20 (dd, $J_{gem} = 13.9$, $J_{3\beta,4} = 10.5$)
19(^{5,2}B)	2.39 (dd, $J_{gem} = 13.7$, $J_{3\alpha,4} = 5.9$)	2.27 (dd, $J_{gem} = 13.7$, $J_{3\beta,4} = 10.3$)
D,GSC-551(^{5,2}B)	1.99 (dd, $J_{gem} = 14.4$, $J_{3\alpha,4} = 5.3$)	2.29 (dd, $J_{gem} = 14.4$, $J_{3\beta,4} = 9.8$)
D,GSC-552 (^{5,2}B)	2.02 (dd, $J_{gem} = 14.1$, $J_{3\alpha,4} = 4.8$)	2.32 (dd, $J_{gem} = 14.1$, $J_{3\beta,4} = 10.8$)

[a] δ (multiplicity, J(Hz)). Measured at 500 MHz in $CDCl_3$ or CD_3OD.



Scheme 2. (a) 1) TFA/CH_2Cl_2, 0°C, 93%, 2) CCl_3CN, DBU/CH_2Cl_2, 0°C, 83%; (b) TMSOTf/CH_2Cl_2, AW-300, 0°C, 43%; (c) CAN/CH_3CN/H_2O, 0°C, 73%; (d) SO_3· Pyr complex/DMF, then Et_3N, r.t., 89%; (e) NaOMe/MeOH, r.t., 92%.



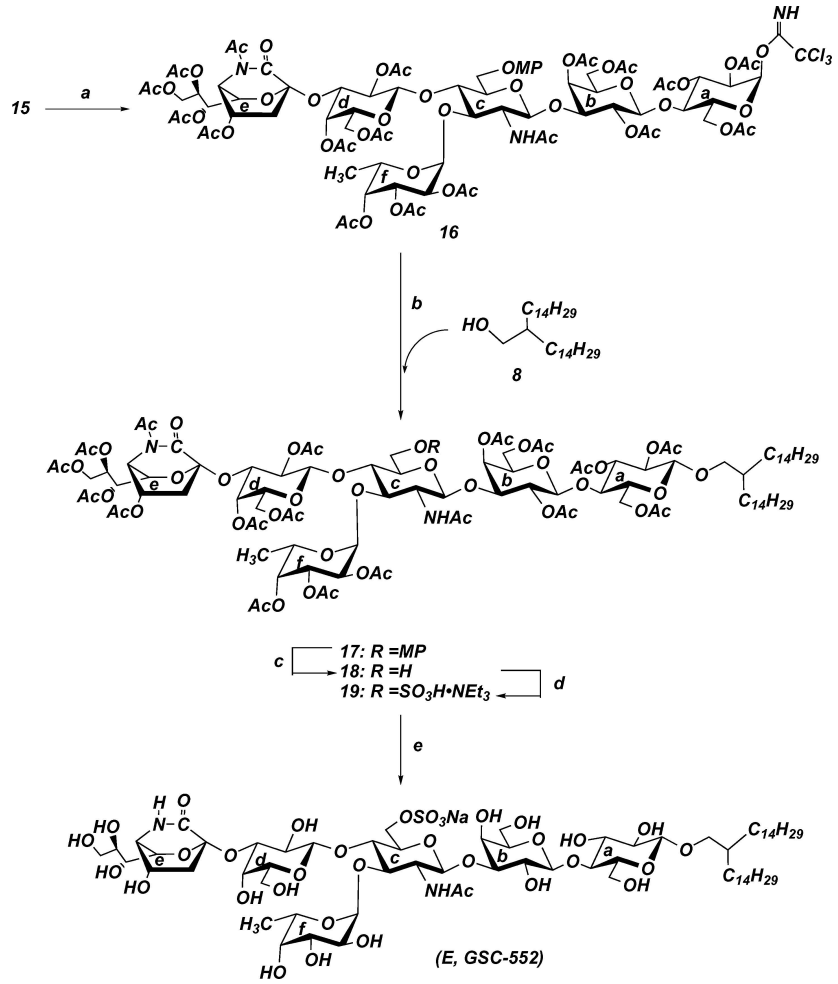
Scheme 3. (a) NaOMe, MeOh, then H_2O, 45°C, 85%; (b), 1, HBTU, HOBt, DMF, 65°C, 2, Ac_2O, Pyr., 70% (two steps); (c) 1, H_2, $Pd(OH)_2$, EtOH, 2, Ac_2O, Pyr., 90% (two steps).

2-(tetradecyl)hexadecanol **8** with **7** in the presence of TMSOTf and AW 300 gave the desired β-glycoside **9** (43%). Selective cleavage of the 4-methyoxyphenyl (MP) group in **9** and the subsequent 6-*O*-sulfation of **10** with the sulfur trioxide-pyridine complex in DMF, followed by an addition of triethylamine afforeded **11** in high yield. Removal of all protective groups in **11** under alkaline conditions furnished the target compound (**D**, **GSC-551**) in 92% yield (Scheme 2).

The conversion of **12** [12] into the lactam derivative **14** was accomplished by treatment of **13** with HBTU and HOBt in DMF for 3 h at 65°C and following complete acetylation (70% in 2 steps). The ^{1}H NMR spectrum of **14** showed signals at δ 2.37 (dd, 1H, $J_{3\alpha,4}$ 5.7, J_{gem} 13.8 Hz, H-3eα) and 2.30 (dd, 1H, $J_{3\beta,4}$ 10.1, J_{gem} 13.8 Hz, H-3eβ) characteristic of the $^{5,2}B$ boat conformation of sialic acid (Table 1).

Hydrogenolytic removal of the benzyl groups in **14** and the following complete acetylation gave **15** (90% in 2 steps) which was then converted to the trichloroacetimidate derivative **16** as described for **7**. The imidate **16** was coupled with **8**, and the resulting **17** was treated with ceric ammonium nitrate (CAN) in acetonitrile to give **18**, which was successively sulfated and stabilized by treatment with triethylamine. Complete deprotection of **19** under alkaline conditions and purification on a column of Sephadex LH-20 gave the desired lactamized-sialyl 6-*O*-sulfo Le^x neo-glycolipid (**E**, **GSC-552**) in high yield.

As shown in Figure 4, the synthesized compounds GSC-551 (**D**) and GSC-552 (**E**) were clearly stained with G159 mAb in TLC-immunostaining as well as GSC-535 (**B**) and GSC-550 (**C**). In contrast, GSC-517 (**A**) was not stained. These results suggest that the sulfate group at *O*-6 of GlcNAc could be essential for the recognition of G159 mAb, while the fucose residue not. The difference in the structures of the ceramide and the artificial ceramide (B30) may not be critical for the recognition with G159 mAb. In addition, the significance of the lactose moiety was also demonstrated. A further study to elucidate the details of the recognition mapping defined by G159 mAb is now under investigation.



Scheme 4. (a) 1) TFA/CH_2Cl_2, 0°C, 93%, 2) CCl_3CN, DBU/CH_2Cl_2, 0°C, 83%; (b) TMSOTf/CH_2Cl_2, AW-300, 0°C, 43%; (c) CAN/CH_3CN/H_2O, 0°C, 70.6%; (d) SO_3· Pyr complex/DMF, then Et_3N, r.t., 80%; (e) NaOMe/MeOH, r.t., quant.

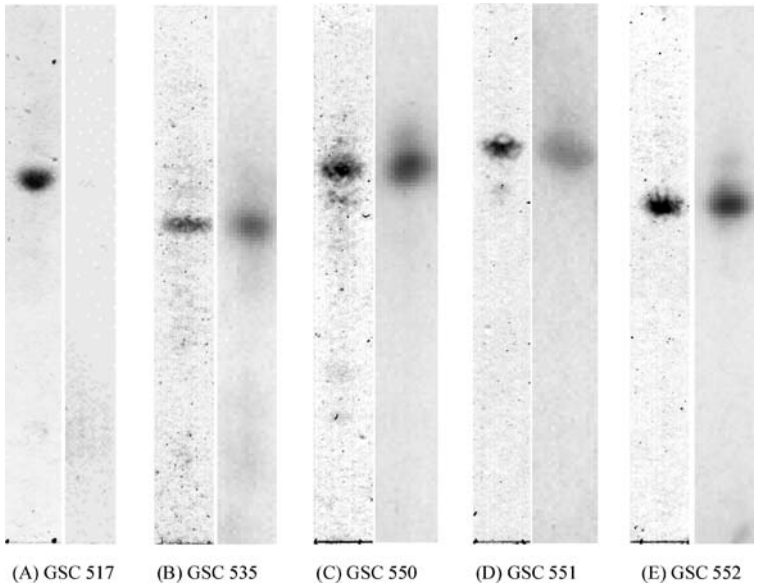


Figure 4. Reactivity of G159 mAB with four glycolipids. Each of left side plates show orcinol/H_2SO_4 staining which visualizes all glycolipids and right side plates show G159 immunostaining. All of them, except GSC-517, have been recognized with G159 mAb.

Conclusions

In summary, we have achieved the synthesis of novel glycolipids (GSC-551 and GSC-552) related to lactamized-sialyl 6-*O*-sulfo Lewis X ganglioside and sialylparagloboside. Utilizing the synthesized glycolipid probes, we demonstrated the recognition specificity of G159 mAb which was obtained during the course of study on the endogenous L-selectin ligand [11].

Taking it into consideration that modified sialic acids have also been found as the constituents of other sialoglycoconjugates [15–17], modification of sialic acids can be recognized as a general phenomenon.

Experimental section

General methods

TLC was conducted on E. Merck silica gel 60 F-254 aluminum plate. Compounds were visualized either 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 Unity Inova 500 (500 MHz) or Varian Unity Inova 400 (100.6 MHz) spectrometer, respectively. The values of δ (ppm) are given relative to Me_4Si as the internal standard. Dichloromethane, methanol, ethanol, benzene and DMF were kept dry over 4 Å MS, while pyridine and acetonitrile were kept dry over 3 Å MS.

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)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-(2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-4-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 **1** (703 mg, 0.63 mmol) and the trisaccharide acceptor **2** (650 mg, 0.47 mmol) in dry dichloromethane (5 mL) was added molecular sieves 4 Å (1.4 g), and the mixture was stirred for 5 h at room temperature, then cooled to 0°C. Trimethylsilyl trifluoromethanesulfonate (TMSOTf; 17 μL, 84.9 μmol) was added to the mixture and this was stirred for 24 h at 3°C, neutralized with Et_3N and filtered, and the residue was washed with chloroform. The combined filtrate and washings were concentrated. Column chromatography (100:1 $CHCl_3$-MeOH) of the residue on silica gel gave **3** (850 mg, 76%) as an amorphous mass; $[\alpha]_D^{24} = +14.5^\circ$ (c = 1.0, $CHCl_3$); ^{1}H NMR($CDCl_3$): δ = 8.16−6.73 (m, 54 H, MeO*Ph*, 10 Ph), 6.26 (d, 1 H, br-d, NHe), 5.56 (m, 1 H, H-8e), 5.50 (dd, 1 H, $J_{1,2}$7.8, $J_{2,3}$ 10.1 Hz, H-2d), 5.39 (d, 1H, H-4d), 5.32 (d, 1 H, $J_{2,NH}$8.9 Hz, NHc), 5.22 (dd, 1 H, $J_{6,7}$ 2.3, $J_{7,8}$ 9.4 Hz, H-7e), 5.12 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1d), 4.93 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 3.2 Hz, H-3d), 4.40 (d, 1 H, $J_{1,27.3}$ Hz, H-1c), 4.34 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1a), 3.86 (s, 3 H, COO*Me*) , 3.71 (s, 3 H, *Me*OPh), 3.57 (m, 2 H, $Me_3SiCH_2CH_2$), 2.54 (dd, 1 H, $J_{3eq,4}$ 4.8, J_{gem}12.8 Hz, H-3e*eq*), 2.18, 1.97, 1.92, 1.47 (4 s, 12 H, 4 AcO), 1.67 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.8 Hz, H-3e*ax*), 1.46 (s, 3 H, AcN), 1.03 (m, 2 H, $Me_3SiCH_2CH_2$).

^{13}C NMR($CDCl_3$): δ = 170.77 (C=O), 170.67 (2C=O), 170.16 (C=O), 169.65 (C=O), 167.77 (C=O), 166.08 (C=O), 165.54 (C=O), 165.10 (C=O), 153.73, 152.60 (MeO*Ph*), 139.12, 139.03, 138.98, 138.69, 138.26, 133.49, 133.36, 133.25, 130.04, 129.93, 129.81, 129.32, 129.15, 128.89, 128.53, 128.48, 128.44, 128.20, 128.15, 128.12, 128.07, 127.90, 127.83, 127.62, 127.46, 127.39, 127.26, 126.99, 126.96, 125.89 (arom-C), 115.54, 114.37 (MeO*Ph*), 102.98, 102.48, 102.22, 101.47, 96.86, 82.76, 82.26, 81.73, 79.67, 76.57, 75.99, 75.27, 74.92, 74.84, 74.54, 74.12, 73.49, 73.40, 73.10, 73.06, 72.87, 71.65, 71.37, 71.28, 70.81, 68.54, 68.29, 68.10, 67.97, 67.37, 67.21, 66.30, 62.70, 62.02, 55.75, 55.51, 53.37, 49.43, 37.23, 29.59, 22.84, 21.36, 20.73, 20.28, 20.12, 18.34; elemental analysis calcd (%) for $C_{128}H_{141}F_3N_2O_{36}Si$ (2366.90): C 64.91, H 6.00, N 1.18; found: C 64.80, H 5.89, N 1.06.

2-(Trimethylsilyl)ethyl (5-amino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-(β-D-galactopyranosyl)-(1→4)-(2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-4-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 (4)

To a solution of **3** (603.6 mg, 0.29 mmol) in methanol (12 mL) was added a catalytic amount of 28% sodium methoxide in MeOH, and the mixture was stirred for 72 h at 45°C. Water (1 mL) was added and the mixture was stirred for 24 h at 45°C, neutralized with Amberlite IR-120 (H^+) resin, and filtered. The resin was washed with MeOH, and the combined filtrate and washings was concentrated. Column chromatography (MeOH) of the residue on Sephadex LH-20 gave **4** (425 mg, 80.3%) as an amorphous mass; $[\alpha]_D^{24} = -1.05^\circ$ (c = 0.9, $CHCl_3$−MeOH); ^{1}H NMR (CD_3OD): δ = 7.41 − 6.82 (m, 39 H, MeO*Ph*, 7 Ph), 4.51 (1 H, $J_{1,2}$ 7.5 Hz, H-1c), 4.35 (d, 1 H, $J_{1,2}$7.8 Hz, H-1b), 4.05 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1a), 3.70 (s, 3 H, *Me*OPh), 3.55 (m, 2 H, $Me_3SiCH_2CH_2$), 3.09 (t, 1 H, H-5e), 2.87 (dd, 1 H, $J_{3eq,4}$ 5.0, J_{gem}12.1 Hz, H-3e*eq*), 1.75 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.1 Hz, H-3e*ax*), 1.58 (s, 3 H, AcN), 0.97 (m, 2 H, $Me_3SiCH_2CH_2$).

^{13}C NMR (CD_3OD): δ = 176.67 (C=O), 174.42 (C=O), 156.88, 155.44 (MeO*Ph*), 141.79, 141.01, 131.14, 130.94, 130.82, 130.77, 130.62, 130.53, 130.50, 130.41, 130.27, 130.18, 129.83, 129.54 (arom-C), 118.46, 117.29 (MeO*Ph*), 105.50, 105.17, 104.81, 99.08, 97.12, 86.24, 85.28, 84.31, 81.38, 79.07, 77.73, 77.23, 77.11, 76.13, 75.66, 75.40, 72.81, 71.18, 69.60, 65.60, 64.46, 57.48, 24.73, 20.61; elemental analysis calcd (%) for $C_{96}H_{120}N_2O_{28}Si$ (1776.78): C 64.85, H 6.80, N 1.58; found: C 64.76, H 6.66, N 1.37

2-(Trimethylsilyl)ethyl (4,7,8,9-tetra-*O*-acetyl-5-acetylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl 1,5-lactam)-(2→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-4-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 (5)

To a solution of **4** (386.4 mg, 0.21 mmol) in DMF (15 mL) was added WSCHCl (103 mg, 0.55 mmol) and HOBt (73.3 mg, 0.54 mmol), and the mixture was stirred for 5 h at 70°C, and then concentrated. Column chromatography (MeOH) of the residue on Sephadex LH-20 gave the lactamized sLe^x derivative. The residue was treated with acetic anhydride (8 mL) and pyridine (16 mL) for 12 h, then cooled to 0°C. Methanol (3 mL) was added and the mixture was concentrated, and the residue was extracted with chloroform and successively washed with cold 2M hydrochloric acid and water, dried (Na_2SO_4) and concentrated. Column chromatography (100:1 $CHCl_3$:MeOH) of the residue on silica gel gave **5** (203 mg, 46%, 2 steps) as an amorphous mass; $[\alpha]_D^{24} = +5.6^\circ$ ($c = 0.9$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 7.34 - 6.76$ (m, 39 H, MeO*Ph*, 7 Ph), 5.75 (dd, 1H, $J_{6,7}$ 3.9, $J_{7,8}$9.8 Hz, H-7e), 5.72 (1H, $J_{2,NH}$9.4 Hz, NHc), 5.42 (m, 1H, H-8e), 5.20 (d, 1H, H-4d), 5.13 (dd, 1H, $J_{1,2}$ 8.2, $J_{2,3}$ 10.6 Hz, H-2d), 4.84 (m, 1H, H-4e), 4.57 (1H, $J_{1,2}$8.0 Hz, H-1d), 4.33 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1c), 3.71 (s, 3H, *Me*OPh), 3.59 (m, 2H, $Me_3SiCH_2CH_2$), 2.56 (s, 3H, AcNe), 2.37 (dd, 1H, $J_{3\alpha,4}$ 5.5, J_{gem} 14.4 Hz, H-3eα), 2.29 (dd, 1H, $J_{3\beta,4}$ 10.3, J_{gem} 14.4 Hz, H-3eβ), 2.17, 2.14, 2.12, 2.09, 2.05, 1.97, 1.94 (7 s, 21 H, 7 AcO), 1.59 (s, 3H, AcNc), 0.99 (m, 2H, $Me_3SiCH_2CH_2$).

^{13}C NMR ($CDCl_3$): $\delta = 170.51$ (C═O), 170.36 (2C═O), 170.25 (C═O), 169.72 (C═O), 169.65 (C═O), 169.06 (2C═O), 164.87 (C═O), 157.09, 154.26, (MeO*Ph*), 142.20, 138.83, 138.60, 138.48, 138.22, 128.46, 128.42, 128.36, 128.29, 128.19, 128.03, 127.96, 127.58, 127.54, 127.46, 127.11 (arom-C), 115.85, 114.78 (MeO*Ph*), 103.14, 102.66, 102.13, 99.93, 95.88, 82.90, 81.90, 81.71, 79.95, 78.41, 76.09, 75.40, 74.99, 74.63, 73.23, 73.04, 72.53, 71.44, 71.23, 70.33, 69.49, 68.64, 68.30, 67.60, 67.38, 61.76, 61.35, 55.69, 47.99, 35.68, 29.75, 26.41, 22.92, 21.05, 20.83, 20.75, 20.76, 18.51; elemental analysis calcd (%) for $C_{112}H_{134}N_2O_{35}Si$ (2094.85): C 64.17, H 6.44, N 1.34; found: C 64.00, H 6.30, N 1.18.

2-(Trimethylsilyl)ethyl (4,7,8,9-tetra-*O*-acetyl-5-acetylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl 1,5-lactam)-(2→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-4-methoxyphenyl-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 (6)

A solution of **5** (193.1 mg, 95 μmol) in ethanol (15 mL) was vigorously stirred with $Pd(OH)_2$ (200 mg) for 24 h at room temperature under hydrogen. The catalyst was collected and washed with methanol. The combined filtrate and washings was concentrated, and the residue was treated with acetic anhydride (5 mL) and pyridine (7 mL) for 12 h, then cooled to 0°C. Methanol (2 mL) was added and the mixture was concentrated. The residue was extracted with chloroform and successively washed with cold 2M hydrochloric acid and water, dried (Na_2SO_4) and concentrated. Column chromatography (80:1 $CHCl_3$:MeOH) of the residue on silica gel gave **6** (155.5 mg, 93%) as an amorphous mass; $[\alpha]^{24}{}_D = +14.9^\circ$ ($c = 0.5$, $CHCl_3$)^{1}H NMR ($CDCl_3$): $\delta = 6.95-6.84$ (m, 4H, MeO*Ph*), 5.72 (dd, 1H, $J_{6,7}$ 3.9, $J_{7,8}$ 9.6 Hz, H-7e), 5.60 (d, 1H, $J_{NH,2}$ 8.7 Hz, NHc), 5.42 (m, 1H, H-8e), 5.20 (d, 1H, H-4d), 5.06 (dd, 1H, $J_{1,2}$ 8.0, $J_{2,3}$10.0 Hz, H-2d), 5.01 (dd, 1H, $J_{1,2}$ 8.0, $J_{2,3}$9.6 Hz, H-2a), 4.86 (dd, 1H, $J_{1,2}$8.0, $J_{2,3}$9.6 Hz, H-2b), 4.82 (m, 1H, H-4e), 4.60 (d, 1H, $J_{1,2}$7.1 Hz, H-1c), 4.51 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1d), 4.45 (d, 1H,$J_{1,2}$ 7.8 Hz, H-1b), 4.31 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1a), 4.25 (dd, 1H, $J_{8,9}$, 5.3, J_{gem}11.9 Hz, H-9'e), 4.10 (dd, 1H, $J_{8,9}$ 5.5, J_{gem}11.9 Hz, H-9e), 4.04 (dd, 1H, $J_{2,3}$ 10.3, $J_{3,4}$ 3.4 Hz, H-3d), 3.77 (s, 3 H, *Me*OPh), 3.57 (m, 2H, $Me_3SiCH_2CH_2$), 2.55 (s, 3 H, AcNe), 2.31 (dd, 1H, $J_{3\alpha,4}$ 5.7, J_{gem}14.6 Hz, H-3eα), 2.22 (dd, 1H, $J_{3\beta,4}$ 10.5, J_{gem}14.6 Hz, H-3eβ), 2.17, 2.14, 2.13, 2.11, 2.10, 2.08, 2.073, 2.07, 2.05, 2.04, 2.02, 2.01, 1.99, 1.93, 1.89 (15 s, 45 H, 14 AcO and AcN), 0.98 (m, 2H, $Me_3SiCH_2CH_2$).

^{13}C NMR ($CDCl_3$): $\delta = 170.70$ (C═O), 170.57 (2C═O), 170.44 (2C═O), 170.32 (C═O), 170.15 (2C═O), 170.08 (2C═O), 169.85 (C═O), 169.65 (C═O), 169.58 (C═O), 169.37 (C═O), 168.95 (C═O), 167.65 (C═O), 164.73 (C═O), 154.10, 152.97, 116.33, 115.99 (MeO*Ph*),100.62, 99.93 (2C), 96.81, 95.80, 75.90, 75.82, 75.40, 74.15, 73.12, 72.72, 72.62, 71.66, 71.39, 71.11, 70.40, 70.20, 68.80, 68.58, 68.45, 68.26, 67.46, 67.13, 66.91, 62.25, 61.78, 61.54, 61.25, 55.60, 54.25, 53.67, 53.34, 49.85, 35.56, 29.67, 26.30, 23.16, 22.67, 21.38, 20.88, 20.83, 20.74, 20.69, 20.65, 20.51, 20.42, 17.84; elemental analysis calcd (%) for $C_{77}H_{106}N_2O_{42}Si$ (1758.60): C 52.55, H 6.07, N 1.59; found: C 52.50, H 5.83, N 1.44.

(4,7,8,9-Tetra-*O*-acetyl-5-acetylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl 1,5-lactam)-(2→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-4-methoxyphenyl-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (7)

The 2-(trimethylsilyl)ethyl group of **6** (173 mg, 97 μmol) was removed by treatment with trifluoroacetic acid (1.7 mL) in dichloromethane (4 mL) for 3 h at room temperature. Ethyl acetate (2 mL) was added and the mixture was concentrated. Column chromatography (50:1 $CHCl_3$:MeOH) of the residue on silica gel gave the 1-OH free derivative (155 mg, 95%). This compound was treated with trichloroacetonitrile (278 μL, 22.2 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 14.2 μL, 85.2 mmol) in dichloromethane (5 mL) for 2 h at 0°C. The mixture was concentrated and the residue was chromatographed (50:1 $CHCl_3$:MeOH) on a column of silica gel

to give the trichloroacetimidate **16** (108.9 mg, 65%) as an amorphous mass; $[\alpha]^{24}{}_{D} = +10.7^{\circ}$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 8.65$ (s, 1H, NH of imidate), 6.95-6.84 (m, 4H, MeO*Ph*), 6.47 (d, 1H, $J_{1,2}$ 3.4 Hz, H-1a), 5.74 (dd, 1H, $J_{6,7}$ 3.9, $J_{7,8}$ 9.6 Hz, H-7e), 5.65 (d, 1H, $J_{NH,2}$ 8.7 Hz, NHc), 5.13 (t, 1H, $J_{2,3} = J_{3,4} = 9.4$ Hz, H-3a), 5.41 (m, 1H, H-8e), 5.11 (dd, 1H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.2 Hz, H-2d), 5.07–5.03 (m, 2H, H-2a and H-2b), 4.83 (m, H, H-4e), 4.61 (d, 1H, $J_{1,2}$ 7.1 Hz, H-1c), 4.51 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1d), 4.38 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1b), 4.25 (dd, 1H, $J_{8,9}$, 5.3, J_{gem}11.9 Hz, H-9'e), 4.11 (dd, 1H, $J_{8,9}$ 5.7, J_{gem}11.9 Hz, H-9e), 4.05 (dd, 1H, $J_{2,3}$ 10.3, $J_{3,4}$ 3.4 Hz, H-3d), 3.76 (s, 3H, *Me*OPh), 2.55 (s, 3H, AcNe), 2.31 (dd, 1H, $J_{3\alpha,4}$ 5.7, J_{gem}14.6 Hz, H-3eα), 2.23 (dd, 1H, $J_{3\beta,4}$ 10.9, J_{gem}14.6 Hz, H-3eβ), 2.17, 2.14, 2.13, 2.10, 2.07 (×2), 2.06 (×2), 2.05, 2.04, 2.02, 2.01, 2.00, 1.94, 1.89 (15 s, 45 H, 14 AcO and AcN). elemental analysis calcd (%) for $C_{74}H_{94}Cl_3N_3O_{43}$ (1817.43): C 48.84, H 5.21, N 2.31; found: C 48.66, H 4.99, N, 2.23.

2-(Tetradecyl)hexadecyl (4,7,8,9-tetra-*O*-acetyl-5-acetylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl 1,5-lactam)-(2→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-4-methoxyphenyl-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 **7** (108.9 mg, 60.2 μmol) and 2-(tetradecyl)hexadecanol (**8**; 121 mg, 0.27 μmol) in dry dichloromethane (1.5 mL) was added MS4Å (type AW300; 2.9 g) and the mixture was stirred for 2 h at room temperature, and then cooled to 0°C. Trimethylsilyl trifluoromethanesulfonate (TMSOTf; 0.93 μL, 4.74 μmol) was added to the mixture, and this was stirred for 5.5 h at room temperature, neutralized with Et_3N and filtered. Chromatography (60:1 $CHCl_3$:MeOH) of the residue on silica gel afforded **9** (53 mg, 42%) as an amorphous mass; $[\alpha]^{24}{}_{D} = +23.2^{\circ}$ ($c = 0.56$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 6.95$-6.84 (m, 4H, MeO*Ph*), 5.73 (dd, 1H, $J_{6,7}$ 3.9, $J_{7,8}$ 9.8 Hz, H-7e), 5.42 (m, 1H, H-8e), 5.34 (m, 1H, H-5e), 5.20 (d, 1H, H-4d), 5.17 (t, 1H, $J_{2,3} = J_{3,4} = 10.3$ Hz, H-3a), 5.02 (dd, 1H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, H-2a), 4.88 (dd, 1H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.8 Hz, H-2b), 4.81 (m, 1H, H-4e), 4.64 (d, 1 H, $J_{1,2}$ 7.1 Hz, H-1c), 4.50 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1d), 4.40 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1b), 4.31 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1a), 4.25 (dd, 1H, $J_{8,9}$, 5.5, J_{gem}11.4 Hz, H-9'e), 3.76 (s, 3H, *Me*OPh), 3.58 (dd, 1 H, O*CH*$_2$ of alkyl part), 3.34 (dd, 1H, O*CH*$_2$ of alkyl part), 2.55 (s, 3 H, AcNe), 2.32 (dd, 1H, $J_{3\alpha,4}$ 5.7, J_{gem}14.4 Hz, H-3eα), 2.23 (dd, 1H, $J_{3\beta,4}$ 10.7, J_{gem}14.4 Hz, H-3eβ), 2.17, 2.14, 2.13, 2.12, 2.11, 2.10, 2.09, 2.08, 2.07, 2.05, 2.04, 1.98, 1.93, 1.89 (14 s, 42 H, 14 AcO), 1.69 (s, 3H, AcNc), 1.25 (m, 52 H, 26 CH_2), 0.89 (t, 6 H, *J* 6.9 Hz, 2 *Me*CH_2).

^{13}C NMR ($CDCl_3$): δ = 170.81 (C=O), 170.64 (C=O), 170.50 (3C=O), 170.40 (2C=O), 170.38 (C=O), 170.32 (2C=O), 169.72 (C=O), 169.57 (C=O), 169.40 (C=O), 169.07 (C=O), 168.95 (C=O), 168.84 (C=O), 164.81 (C=O), 154.35, 152.52, 116.39, 114.73 (MeO*Ph*), 101.84, 100.85, 100.70, 96.77, 95.86, 75.92, 74.12, 74.05, 72.65, 72.29, 72.05, 71.73, 71.52, 71.25, 71.02, 70.90, 70.03, 69.94, 69.36, 68.64, 67.51, 66.97, 61.82, 61.31, 55.64, 47.96, 38.08, 35.61, 31.95, 31.21, 30.08, 29.73, 29.39, 26.80, 26.80, 26.34, 23.20, 22.72, 20.80, 20.70, 20.26, 14.15; elemental analysis calcd (%) for $C_{102}H_{154}N_2O_{43}$ (2094.99): C 58.44, H 7.40, N 1.34; found: C 58.22, H 7.38, N 1.24.

2-(Tetradecyl)hexadecyl (4,7,8,9-tetra-*O*-acetyl-5-acetylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl 1,5-lactam)-(2→3)-(2,4,6-tri-*O*-acetyl-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 **9** (28.4 mg, 13.5 μmol) in acetonitrile (1.8 mL) and water (0.2 mL) was added ceric ammonium nitrate (CAN; 23 mg, 41.4 μmol), and the mixture was stirred for 2 h at 0° C and extracted with chloroform. The extract was successively washed with 1M sodium carbonate and water, dried (Na_2SO_4) and concentrated. Column chromatography (60:1 $CHCl_3$:MeOH) of the residue on silica gel gave **10** (19.7 mg, 73%) as an amorphous mass; $[\alpha]^{24}_{D} = -19.2^{\circ}$ ($c = 0.5$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 5.73$ (dd, 1H, $J_{6,7}$3.7, $J_{7,8}$ 9.6 Hz, H-7e), 5.44 (m, 1H, H-8e), 5.41 (d, 1H,$J_{NH,2}$ 8.9 Hz, NHc), 5.36 (m, 1H, H-5e), 5.20 (d, 1H, H-4d), 5.16 (t, 1H, $J_{2,3} = J_{3,4} =$ 9.4 Hz, H-3a), 5.08 (dd, 1H, $J_{1,2}$ 8.2, $J_{2,3}$ 10.9 Hz, H-2d), 5.00 (dd, 1H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.8 Hz, H-2a), 4.88 (dd, 1H, $J_{1,2}$ 8.2, $J_{2,3}$ 9.6 Hz, H-2b), 4.85 (m, 1H, H-4e), 4.69 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1c), 4.52 (d, 1H, $J_{1,2}$ 8.2 Hz, H-1d), 4.41 (d, 1H, $J_{1,2}$ 8.2 Hz, H-1b), 4.37 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1a), 4.24 (dd, 1H, $J_{8,9}$, 5.5, J_{gem} 11.9 Hz, H-9'e), 4.12 (dd, 1H, $J_{2,3}$ 10.3, $J_{3,4}$ 3.2 Hz, H-3b), 3.64 (m, 1H, O*CH*$_2$ of alkyl part), 3.35 (br-d, 1H, H-6c), 3.26 (dd, 1H, O*CH*$_2$ of alkyl part), 2.56 (s, 3H, AcNe), 2.37 (dd, 1H, $J_{3\alpha,4}$ 5.3, J_{gem} 14.6 Hz, H-3eα), 2.27 (dd, 1H, $J_{3\beta,4}$ 10.3, J_{gem}14.6 Hz, H-3eβ), 2.184, 2.182, 2.17, 2.14, 2.11, 2.109, 2.101, 2.08, 2.07, 2.06, 2.05, 2.04, 2.03, 2.01, 1.90 (15 s, 45H, 14 AcO and AcN), 1.25 (m, 52H, 26 CH_2), 0.88 (t, 6H, *J* 6.9 Hz, 2 *Me*CH_2); elemental analysis calcd (%) for $C_{95}H_{148}N_2O_{42}$ (1988.95): C 57.33, H 7.50, N 1.41; found C 57.13, H 7.35, N, 1.31.

2-(Tetradecyl)hexadecyl (4,7,8,9-tetra-*O*-acetyl-5-acetylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl 1,5-lactam)-(2→3)-(2,4,6-tri-*O*-acetyl-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 triethylammonium salt (11)

To a solution of **10** (10 mg, 5 μmol) in DMF (0.5 mL) was added sulfur trioxide pyridine complex (7.9 mg, 50 μmol), and

the mixture was stirred for 2 h at room temperature. 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 (30:1 $CHCl_3$:MeOH) on silica gel to afford **11** (9.6 mg, 88.7%) as an amorphous mass; $[\alpha]_D^{24} = +5.2^\circ$ ($c = 0.2$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 5.76$ (dd, 1H, $J_{6,7}$3.7, $J_{7,8}$ 9.2 Hz, H-7e), 5.58 (d, 1H, $J_{NH,2}$ 8.5 Hz, NHc), 5.37 (m, 1H, H-8e), 5.30 (m, 1H, H-5e), 5.15 (t, 1H, $J_{2,3} = J_{3,4} = 9.2$ Hz, H-3a), 5.04 (dd, 1H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.6 Hz, H-2d), 5.01 (dd, 1H, $J_{1,2}$ 7.6 Hz, H-2b), 4.89 (dd, 1H, $J_{1,2}$ 7.6 Hz, H-2a), 4.87 (m, 1H, H-4e), 4.67 (d, 1 H,$J_{1,2}$ 8.0 Hz, H-1d), 4.46 (d, 1H, $J_{1,2}$ 7.6 Hz, H-1c), 4.41 (d, 1H,$J_{1,2}$ 7.6 Hz, H-1a), 4.35 (d, 1H,$J_{1,2}$ 7.6 Hz, H-1b), 4.18 (m, 1H, H-6e), 3.58 (m, 1 H, OCH_2 of alkyl part), 3.26 (dd, 1H, OCH_2 of alkyl part), 3.17 (q, 6 H, $3CH_3CH_2N$), 2.55 (s, 3H, AcNe), 2.45 (dd, 1H, $J_{3\alpha,4}$ 5.7, J_{gem} 13.9 Hz, H-3eα), 2.24 (dd, 1H, $J_{3\beta,4}$ 10.5, J_{gem} 13.9 Hz, H-3eβ), 2.20, 2.16, 2.14, 2.13, 2.11, 2.10, 2.095, 2.090, 2.06, 2.05, 2.03, 2.01, 2.00, 1.99, 1.94 (15 s, 45 H, 14 AcO and AcN), 1.39 (t, 9 H, $3CH_3CH_2N$), 1.25 (m, 52 H, 26 CH_2), 0.86 (t, 6 H, J 6.6 Hz, 2 $MeCH_2$); elemental analysis calcd (%) for $C_{101}H_{163}N_3O_{45}S$ (2170.03): C 55.87, H 7.57, N 1.94; found C 55.61, H 7.52, N 1.64.

2-(tetradecyl)hexadecyl (5-amino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl 1,5-lactam)-(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 (D, GSC-551)

To a solution of **11** (9.6 mg, 4.5 μmol) in methanol (1.5 mL) and dioxane (0.4 mL) was added catalytic amount of 28% sodium methoxide in methanol, and the mixture was stirred for 24 h at room temperature. After completion of the reaction the mixture was concentrated. Column chromatography (MeOH) of the residue on Sephadex LH-20 gave the target molecule **(D, GSC-551)** (6 mg, 92%) as an amorphous mass; $[\alpha]_D^{24} = +5.8^\circ$ $c = 0.1$, 1:1 $CHCl_3$:MeOH);^{1}H NMR (CD_3OD):$\delta = 4.58$ (d, 1H, $J_{1,2}$ 8.5 Hz, H-1c), 4.42 (d, 1H, $J_{1,2}$ 7.6 Hz, H-1d), 4.35 (br-d, 1H, H-6e), 4.28 (d, 1H, $J_{1,2}$ 7.3 Hz, H-1b), 4.15 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1a), 3.99 (m, 1H, H-4e), 3.96 (dd, 1H, $J_{2,3}$ 9.8, $J_{3,4}$ 3.4 Hz, H-3d), 3.75 (dd, 1H, $J_{6,7}$ 3.4 Hz, H-7e), 3.69 (dd, 1H, $J_{1,2}$ 8.5, $J_{2,3}$ 7.8 Hz, H-2c), 3.51-3.48 (m, 2H, H-2d and H-2b), 3.40 (t, 1H, $J_{2,3} = J_{3,4} = 9.4$ Hz, H-3a), 3.29 (m, 1H, H-8e), 3.14 (dd, 1 H, H-2a), 2.29 (dd, 1H, $J_{3\beta,4}$ 9.8, J_{gem} 14.4 Hz, H-3eβ), 1.99 (dd, 1H, $J_{3\alpha,4}$ 5.3, J_{gem} 14.4 Hz, H-3e α), 1.88 (s, 3H, AcNc), 1.50-1.19 (m, 53H, 26 CH_2 and CH), 0.80 (t, 6H, J 6.4 Hz, 2 $MeCH_2$); FAB (−) MS: m/z: calcd for $C_{65}H_{117}N_2NaO_{30}S$:1460.7310; found: 1438.7054 [M-Na]$^-$, 1206 [M-Lactamized Neu-Na]$^-$, 761 [lactosyl 2-(tetradecyl)hexadecyl]$^-$, 599 [glucosyl 2-(tetradecyl) hexadecyl]$^-$.

2-(Trimethylsilyl)ethyl (5-amino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl)-(1→4)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-(2-acetamido-2-deoxy-6-*O*-4-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 (13)

To a solution of **12** (505 mg, 0.19 mmol) in methanol (10 mL) was added a catalytic amount of 28% sodium methoxide in methanol, 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 45°C, neutralized with Amberlite IR-120 (H^+) resin, and filtered. The resin was washed with MeOH, and the combined filtrate and washings was concentrated. Column chromatography (MeOH) of the residue on Sephadex LH-20 gave **13** (355.3 mg, 85%) as an amorphous mass; $[\alpha]_D^{24} = -31.9^\circ$ ($c = 1.2$, MeOH); ^{1}H NMR (CD_3OD): $\delta = 7.38$-6.80 (m, 49 H, MeO*Ph*, 9 Ph), 5.32 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1f), 4.42 (1 H, $J_{1,2}$ 7.6 Hz, H-1c), 4.31 (d, 1 H, $J_{1,2}$7.6 Hz, H-1b), 3.95 (dd, 1 H, $J_{1,2}$ 3.7 Hz, H-2f), 3.65 (s, 3 H, *Me*OPh), 3.56 (m, 2 H, $Me_3SiCH_2CH_2$), 3.06 (t, 1 H, H-5e), 2.83 (dd, 1 H, $J_{3eq,4}$ 5.0, J_{gem} 11.9 Hz, H-3e*eq*), 1.75 (t, 1 H, $J_{gem} = J_{3ax,4}$11.9 Hz, H-3e*ax*), 1.66 (s, 3 H, AcN), 1.14 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6f), 0.95 (m, 2 H, $Me_3SiCH_2CH_2$).

^{13}C NMR (CD_3OD): δ = 176.33 (C=O), 173.95 (C=O), 156.77, 155.32 (MeO*Ph*), 141.96, 141.69, 141.58, 141.41, 141.38, 141.29, 140.87, 140.76, 140.70, 130.91, 130.84, 130.82, 130.73, 130.58, 130.52, 130.50, 130.47, 130.42, 130.40, 130.20, 130.16, 130.05, 129.87, 129.78, 129.72, 129.63 (arom-C), 118.51, 117.23 (MeO*Ph*), 105.38, 104.79, 104.77, 102.22, 98.72, 93.93, 85.16, 84.81, 84.19, 81.70, 81.29, 80.99, 79.13, 78.27, 76.32, 76.07, 75,87, 75.58, 75.31, 74.97, 74.75, 74.49, 72.42, 71.17, 70.22, 69.48, 68.97, 64.96, 64.50, 57.47, 55.40, 43.18, 24.73, 20.51, 18.18; elemental analysis calcd (%) for $C_{116}H_{142}N_2O_{33}Si$ (2118.93): C 65.71, H 6.75, N 1.32; found C 65.55, H 6.49, N 1.29.

2-(Trimethylsilyl)ethyl (4,7,8,9-tetra-*O*-acetyl-5-acetylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl 1,5-lactam)-(2→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-(2-acetamido-2-deoxy-6-*O*-4-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 (14)

To a solution of **13** (285.1 mg, 0.13 mmol) in DMF (5 mL) was added HBTU (262.8 mg, 0.69 mmol) and HOBt (73.4 mg, 0.54 mmol), and the mixture was stirred for 3 h at 65°C, and then concentrated. Column chromatography (MeOH) of the residue on Sephadex LH-20 gave the lactamized sLe^x derivative. The residue was treated with acetic anhydride (6 mL) and pyridine (10 mL) for 24 h, then cooled to 0°C. MeOH (2 mL) was added and the mixture was concentrated, and the residue was extracted with chloroform and successively washed with cold

2M hydrochloric acid and water, dried (Na_2SO_4) and concentrated. Column chromatography (100:1 $CHCl_3$:MeOH) of the residue on silica gel gave **14** (229.6 mg, 70 %, 2 steps) as an amorphous mass; $[\alpha]_D^{24} = -7.6^\circ$ ($c = 0.46$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 7.33 - 6.73$ (m, 49 H, MeO*Ph*, 9 Ph), 5.93 (1 H, $J_{2,NH}$ 9.2 Hz, NHc), 5.75 (dd, 1 H, $J_{6,7}$ 3.9, $J_{7,8}$ 9.8 Hz, H-7e), 5.43 (m, 1 H, H-8e), 5.23 (d, 1 H, H-4d), 5.17 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1f), 5.11 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.8 Hz, H-2d), 4.85 (m, 1H, H-4e), 4.55 (1 H, $J_{1,2}$ 7.8 Hz, H-1d), 4.33 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1c), 4.11 (dd, 1 H, $J_{3,4}$ 3.4 Hz, H-3d), 4.00 (dd, 1 H, $J_{1,2}$ 3.4, $J_{2,3}$ 9.8 Hz, H-2f), 3.69 (s, 3 H, *Me*OPh), 3.51 (m, 2 H, $Me_3SiCH_2CH_2$), 3.32 (t, 1 H, $J_{2,3}$ 10.3 Hz, H-2 c), 2.57 (s, 3 H, AcNe), 2.37 (dd, 1H, $J_{3\alpha,4}$ 5.7, J_{gem} 13.8 Hz, H-3eα), 2.30 (dd, 1H, $J_{3\beta,4}$ 10.1, J_{gem} 13.8 Hz, H-3eβ), 2.18, 2.12, 2.10, 2.07, 2.05, 2.01, 1.94 (7 s, 21 H, 7 AcO), 1.56 (s, 3 H, AcNc), 1.02 (d, 3 H, $J_{5,6}$ 6.6 Hz, H-6f), 0.84 (m, 2 H, $Me_3SiCH_2CH_2$).

^{13}C NMR ($CDCl_3$): δ = 171.90 (C=O), 171.82 (2C=O), 171.61 (C=O), 171.54 (C=O), 171.13 (C=O), 171.03 (C=O), 170.72 (C=O), 170.39 (C=O), 166.22 (C=O), 155.74, 153.77 (MeO*Ph*), 140.74, 140.55, 140.36, 140.23, 139.94, 139.82, 134.34, 131.14, 129.84, 129.72, 129.62, 129.58, 129.52, 129.38, 129.31, 128.89, 128.83, 128.78, 128.67, 128.61, 128.41 (arom-C), 117.09, 116.21 (MeO*Ph*), 104.50, 104.11, 103.97, 101.08, 98.82, 97.24, 84.80, 84.31, 83.30, 80.70, 80.56, 78.02, 77.77, 77.36, 77.04, 76.74, 76.33, 76.20, 75.89, 75.83, 75.03, 74.66, 74.53, 74.33, 74.28, 73.88, 73.59, 72.74, 72.65, 71.50, 70.87, 70.03, 69.88, 69.68, 69.43, 68.97, 68.68, 68.15, 63.06, 62.71, 57.00, 49.38, 37.04, 27.72, 25.81, 24.20, 23.83, 23.54, 23.38, 22.38, 22.34, 22.15, 22.06, 19.86, 17.95; elemental analysis calcd (%) for $C_{132}H_{156}N_2O_{40}Si$ (2437.00): C 65.01, H 6.45, N 1.15; found C 64.71, H 6.29, N 1.10.

2-(Trimethylsilyl)ethyl (4,7,8,9-tetra-*O*-acetyl-5-acetylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl 1,5-lactam)-(2→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-(2-acetamido-2-deoxy-6-*O*-4-methoxyphenyl-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 (15)

A solution of **14** (210 mg, 86 μmol) in ethanol (15 mL) was vigorously stirred with $Pd(OH)_2$ (210 mg) for 48 h at room temperature under hydrogen. The catalyst was collected and washed with methanol. The combined filtrate and washings was concentrated, and the residue was treated with acetic anhydride (4 mL) and pyridine (5 mL) for 48 h, then cooled to 0°C. MeOH (2 mL) was added and the mixture was concentrated. The residue was extracted with chloroform and successively washed with cold 2M hydrochloric acid and water, dried (Na_2SO_4) and concentrated. Column chromatography (80:1 $CHCl_3$:MeOH) of the residue on silica gel gave **15** (155.5 mg, 90 %) as an amorphous mass; $[\alpha]_D^{24} = -14.1^\circ$ ($c = 0.12$, $CHCl_3$) ^{1}H NMR ($CDCl_3$): δ = 6.98-6.86 (m, 4 H, MeO*Ph*), 5.71 (dd, 1H, $J_{6,7}$ 4.1, $J_{7,8}$ 9.4 Hz, H-7e), 5.42 (m, 1H, H-8e), 5.36 (d, 1H, $J_{1,2}$ 4.1 Hz, H-1f), 5.33 (m, 1H, H-5e), 5.30 (d, 1H, H-4d), 5.18 (dd, 1H, $J_{2,3}$ 10.9, $J_{3,4}$ 3.2 Hz, H-3f), 5.03 (dd, 1H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.4 Hz, H-2d), 4.96 (dd, 1H, $J_{1,2}$ 4.1, $J_{2,3}$7.8 Hz, H-2f), 4.87 (dd, 1H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.2 Hz, H-2b), 4.82-4.78 (m, 2H, H-5f and H-4e), 4.48 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1d), 4.47 (d, 1 H,$J_{1,2}$ 8.0 Hz, H-1b), 4.25 (dd, 1 H, $J_{8,9}$, 5.0, J_{gem}11.4 Hz, H-9'e), 4.17 (dd, 1 H, $J_{8,9}$ 6.4, J_{gem} 11.4 Hz, H-9e), 3.75 (s, 3 H, *Me*OPh), 3.54 (m, 2 H, $Me_3SiCH_2CH_2$), 2.54 (s, 3 H, AcNe), 2.24 (dd, 1H, $J_{3\alpha,4}$ 5.3, J_{gem}13.9 Hz, H-3eα), 2.20 (dd, 1H, $J_{3\beta,4}$ 10.5, J_{gem}13.9 Hz, H-3eβ), 2.17, 2.16, 2.15, 2.14, 2.13, 2.11, 2.109, 2.102, 2.09, 2.07, 2.05, 2.02, 2.01, 1.99, 1.956, 1.955 (16 s, 48 H, 16 AcO), 1.59 (s, 3 H, AcNc), 1.81 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6f), 0.88 (m, 2 H, $Me_3SiCH_2CH_2$).

^{13}C NMR ($CDCl_3$): δ = 172.60 (C=O), 172.22 (C=O), 172.12 (C=O), 171.97 (2C=O), 171.92 (C=O), 171.88 (C=O), 171.81 (C=O), 171.59 (C=O), 171.34 (C=O), 171.24 (C=O), 171.10 (C=O), 171.01 (C=O), 170.81 (2C=O), 170.74 (C=O), 170.46 (C=O), 169.87 (C=O), 166.26 (C=O), 155.82, 153.93, 117.77, 116.26 (MeO*Ph*), 102.13, 101.72, 101.39, 101.15, 97.24, 96.73, 77.36, 76.95, 76.13, 75.68, 74.74, 74.14, 74.08, 73.37, 73.25, 73.10, 72.93, 72.80, 72.74, 71.45, 70.74, 70.27, 70.11, 69.98, 69.49, 69.07, 68.94, 68.59, 65.70, 63.77, 63.45, 63.27, 62.76, 58.59, 56.98, 49.33, 36.95, 27.72, 24.80, 22.44, 22.37, 22.34, 22.27, 22.24, 22.16, 22.10, 22.09, 22.05, 19.30, 17.19; elemental analysis calcd (%) for $C_{87}H_{120}N_2O_{49}Si$ (2004.67): C 52.09, H 6.03, N 1.40; found C 51.98, H 5.89, N 1.21.

(4,7,8,9-Tetra-*O*-acetyl-5-acetylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl 1,5-lactam)-(2→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-(2-acetamido-2-deoxy-6-*O*-4-methoxyphenyl-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (16)

The 2-(trimethylsilyl)ethyl group of **15** (112.8 mg, 0.056 mmol) was removed by treatment with trifluoroacetic acid (1.2 mL) in dichloromethane (2 mL) for 3 h at room temperature. Ethyl acetate (2 mL) was added and the mixture was concentrated. Column chromatography (40:1 $CHCl_3$:MeOH) of the residue on silica gel gave the 1-OH free derivative (100 mg 93%). This compound was treated with trichloroacetonitrile (150 μL, 12 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 4.0 μL, 24 μmol) in dichloromethane (5 mL) for 2 h at 0°C. The mixture was concentrated and the residue was chromatographed (30:1 $CHCl_3$:MeOH) on a column of silica gel to give the trichloroacetimidate **16** (89 mg, 83%) as an amorphous mass; $[\alpha]_D^{24} = +13.8^\circ$ ($c = 0.89$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 8.56 (s, 1 H, NH of imidate), 6.97-6.85 (m, 4 H, MeO*Ph*), 6.47 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1a), 5.72 (dd, 1 H, $J_{6,7}$ 3.9, $J_{7,8}$ 9.6 Hz, H-7e), 5.50 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.6 Hz, H-3a), 5.41 (m, 1 H, H-8e), 5.36 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1f), 5.32 (m, 1 H, H-5e), 5.24 (d, 1 H, H-4d), 5.18 (dd, 1 H, $J_{2,3}$ 10.9, $J_{3,4}$ 3.4 Hz, H-3f), 5.05 (dd, 1 H, $J_{1,2}$ 3.9, $J_{2,3}$ 8.7 Hz, H-2a), 5.02-4.98 (m, 2 H,

H-2d and H-2b), 4.95 (dd, 1 H, $J_{1,2}$ 3.9, $J_{2,3}$ 10.9 Hz, H-2f), 4.84-4.77 (m, 2 H, H-4e and H-5f), 4.46 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1d), 4.34 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1b), 4.25 (dd, 1 H, $J_{8,9}$, 5.0, J_{gem}11.7 Hz, H-9'e), 4.18-4.15 (H-6e and H-9e), 4.10 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1c), 3.89 (dd, 1 H, $J_{2,3}$ 9.2, $J_{3,4}$ 3.4 Hz, H-3d), 3.80 (t, 1 H, $J_{2,3}$ 9.6 H-2c), 3.69 (s, 3 H, *Me*OPh), 2.54 (s, 3 H, AcNe), 2.23 (dd, 1H, $J_{3\alpha,4}$ 5.0, J_{gem}15.3 Hz, H-3eα), 2.16, 2.15, 2.14, 2.12, 2.11, 2.10, 2.08, 2.07, 2.06, 2.04, 2.03, 2.02, 2.016, 2.008, 2.00, 1.96, 1.95 (17 s, 51 H, 16 AcO and AcN), 1.19 (d, 3 H, $J_{5,6}$ 6.4 Hz, H-6f); elemental analysis calcd (%) for $C_{84}H_{108}Cl_3N_3O_{49}$ (2047.51): C 49.21, H 5.31, N 2.05; found: C 48.94, H 5.21, N, 1.95.

2-(Tetradecyl)hexadecyl (4,7,8,9-tetra-*O*-acetyl-5-acetylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl 1,5-lactam)-(2→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-(2-acetamido-2-deoxy-6-*O*-4-methoxyphenyl-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 (17)

To a solution of **16** (44.5 mg, 21.8 μmol) and 2-(tetradecyl)hexadecanol (**8**; 47 mg, 107 μmol) in dry dichloromethane (1 mL) was added MS4Å (type AW300; 2 g) and the mixture was stirred for 2 h at room temperature, and then cooled to 0°C. Trimethylsilyl trifluoromethanesulfonate (TMSOTf; 0.42 μL, 2.14 μmol) was added to the mixture, and this was stirred for 12 h at 7°C, neutralized with Et_3N and filtered. Chromatography (60:1 $CHCl_3$:MeOH) of the residue on silica gel afforded **17** (21.8 mg, 43%) as an amorphous mass; $[\alpha]_D^{24} = +4.1$ ($c = 0.43$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 6.99$-6.86 (m, 4H, MeO*Ph*), 5.72 (dd, 1H, $J_{6,7}$ 4.1, $J_{7,8}$ 9.8 Hz, H-7e), 5.46 (d, 1H, $J_{NH,2}$8.7 Hz, NHc), 5.42 (m, 1H, H-8e), 5.37 (d, 1H, $J_{1,2}$ 3.4 Hz, H-1f), 5.33 (m, 1H, H-5e), 5.26 (d, 1H, H-4d), 5.19 (dd, 1H, $J_{3,4}$ 3.4 Hz, H-3f), 5.15 (t, 1H, $J_{2,3} = J_{3,4}$ 9.6 Hz, H-3a), 5.03 (dd, 1H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.1 Hz, H-2d), 4.99 (dd, 1H, $J_{1,2}$ 8.2 Hz, H-2b), 4.96 (dd, 1H, $J_{1,2}$ 3.9, $J_{2,3}$ 10.9 Hz, H-2f), 4.89 (dd, 1H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.6 Hz, H-2a), 4.84 (m, 1H, H-4e), 4.81 (m, 1H, H-5f), 4.47 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1d), 4.41 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1a), 4.33 (d, 1H, $J_{1,2}$ 8.2 Hz, H-1b), 4.24 (dd, 1 H, $J_{8,9}$, 4.8, J_{gem} 11.7 Hz, H-9'e), 4.17 (m, 1H, H-6e), 4.15 (dd, 1H, $J_{8,9}$ 6.2 Hz, H-9e), 3.90 (dd, 1H, $J_{2,3}$ 10.1, $J_{3,4}$ 3.7 Hz, H-3d), 3.76 (s, 3H, *Me*OPh), 3.61 (dd, 1H, O*CH*$_2$ of alkyl part), 3.30 (dd, 1H, O*CH*$_2$ of alkyl part), 2.55 (s, 3H, AcNe), 2.24 (dd, 1H, $J_{3\beta,4}$ 9.8 Hz, H-3eβ), 2.17, 2.16, 2.15, 2.13, 2.12, 2.11, 2.109, 2.102, 2.09, 2.08, 2.07, 2.05, 2.03, 2.019, 2.017, 1.96, 1.95 (17 s, 51H, 16 AcO and AcN), 1.26 (m, 52H, 26 CH_2), 1.20 (d, 3H, $J_{5,6}$ 6.4 Hz, H-6f), 0.88 (t, 6 H, *J* 6.9 Hz, 2 *Me*CH_2).

^{13}C NMR ($CDCl_3$): δ = 171.18 (C=O), 170.87 (C=O), 170.76 (C=O), 170.70 (C=O), 170.53 (C=O), 170.46 (2C=O), 170.40 (2C=O), 170.15 (C=O), 169.91 (C=O), 169.82 (C=O), 169.67 (C=O), 169.38 (C=O), 169.31 (C=O), 169.05 (C=O), 168.38 (C=O), 164.81 (C=O), 154.37, 152.52, 116.33, 114.82 (MeOPh), 100.91, 100.30, 99.65, 98.13, 95.80, 95.31, 75.88, 75.66, 74.69, 74.28, 73.49, 73.24, 72.65, 72.52, 71.97, 71.83, 71.50, 71.32, 71.17, 70.00, 69.31, 68.81, 68.68, 68.08, 67.66, 67.15, 65.76, 64.24, 62.40, 61.96, 61.84, 61.33, 57.28, 55.55, 47.90, 37.87, 37.09, 35.54, 32.74, 31.92, 31.23, 31.10, 30.92, 30.02, 29.70, 29.37, 27.08, 26.89, 26.81, 26.64, 26.71, 26.62, 26.30, 23.40, 22.69, 21.03, 20.92, 20.86, 20.81, 20.74, 20.62, 19.73, 15.77, 14.13; elemental analysis calcd (%) for $C_{112}H_{168}N_2O_{49}$ (2325.07): C 57.82, H 7.28, N 1.20; found: C 57.54, H 7.03, N 1.09.

2-(Tetradecyl)hexadecyl (4,7,8,9-tetra-*O*-acetyl-5-acetylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl 1,5-lactam)-(2→3)-(2,4,6-tri-*O*-acetyl-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 (18)

To a solution of **17** (21.8 mg, 9.4 μmol) in acetonitrile (1.8 mL) and water (0.2 mL) was added ceric ammonium nitrate (CAN; 20 mg, 36 μmol), and the mixture was stirred for 3 h at 0°C and extracted with chloroform. The extract was successively washed with 1M sodium carbonate and water, dried (Na_2SO_4) and concentrated. Column chromatography (50:1 $CHCl_3$:MeOH) of the residue on silica gel gave **18** (14.8 mg, 70.6%) as an amorphous mass; $[\alpha]_D^{24} = -7.6°$ ($c = 0.06$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 5.75 (dd, 1H, $J_{6,7}$ 3.9, $J_{7,8}$ 9.8 Hz, H-7e), 5.55 (d, 1H, $J_{1,2}$ 3.4 Hz, H-1f), 5.44 (m, 1H, H-8e), 5.36 (m, 1H, H-5e), 5.28 (d, 1H, H-4d), 5.21 (dd, 1H, $J_{2,3}$ 10.8, $J_{3,4}$ 3.4 Hz, H-3f), 5.17 (t, 1H, $J_{2,3}$ $J_{3,4}$ 9.4 Hz, H-3a), 5.04 (dd, 1H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.8 Hz, H-2d), 4.96 (dd, 1H, $J_{1,2}$ 3.4, $J_{2,3}$ 10.8 Hz, H-2f), 4.89 (dd, 1H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.8 Hz, H-2a), 4.82 (m, 1H, H-4e), 4.62 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1d), 4.41 (d, 1H,$J_{1,2}$ 8.0 Hz, H-1a), 4.35 (d, 1H,$J_{1,2}$ 7.6 Hz, H-1b), 4.03 (dd, 1H, $J_{8,9}$ 6.9 Hz, H-9e), 3.67 (m, 1H, O*CH*$_2$ of alkyl part), 3.31 (br-d, 1 H, H-6c), 3.27 (dd, 1H, O*CH*$_2$ of alkyl part), 2.56 (s, 3H, AcNe), 2.39 (dd, 1H, $J_{3\alpha,4}$ 5.9, J_{gem} 13.7 Hz, H-3eα), 2.27 (dd, 1H, $J_{3\beta,4}$ 10.3, J_{gem} 13.7 Hz, H-3eβ), 2.20, 2.187, 2.183, 2.17, 2.15, 2.14, 2.13, 2.12, 2.11, 2.10, 2.09, 2.088, 2.084, 2.07, 2.06, 2.02, 1.96 (17 s, 51 H, 16 AcO and AcN), 1.26 (m, 52 H, 26 CH_2), 0.86 (t, 6 H, *J* 6.2 Hz, 2 *Me*CH_2).

^{13}C NMR ($CDCl_3$): δ = 171.54 (C=O), 170.95 (2C=O), 170.83 (C=O), 170.77 (C=O), 170.71 (3C=O), 170.65 (C=O), 170.44 (C=O), 170.41 (C=O), 170.21 (2C=O), 170.11 (C=O), 169.89 (C=O), 169.73 (C=O), 169.64 (C=O), 169.72 (C=O), 164.53 (C=O), 101.87, 100.48, 97.00 (2C), 96.84, 95.12, 74.45, 73.13, 72.81, 71.80, 71.61, 71.44, 71.04, 70.79, 69.82, 69.34, 68.62, 68.31, 67.98, 67.17, 66.27, 64.64, 63.70, 63.53, 63.03, 62.18, 61.93, 61.71, 61.52, 53.36, 49.65, 37.76, 37.11, 32.36, 31.92, 29.75, 29.65, 29.58, 29.40, 29.34, 29.15, 28.66, 27.80, 23.31, 22.68, 21.40, 20.87, 20.83, 20.70, 20.64, 20.58, 20.41, 20.77, 20.06, 15.91, 14.12; elemental analysis calcd (%) for

$C_{105}H_{162}N_2O_{48}$ (2219.03): C 56.80, H 7.35, N 1.26; found: C 56.76, H 7.20, N, 1.21.

2-(Tetradecyl)hexadecyl (4,7,8,9-tetra-*O*-acetyl-5-acetylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl 1,5-lactam)-(2→3)-(2,4,6-tri-*O*-acetyl-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*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside triethylammonium salt (19)

To a solution of **18** (6.38 mg, 2.8 μmol) in DMF (1 mL) was added sulfur trioxide pyridine complex (4.4 mg, 28 μmol), and the mixture was stirred for 2 h at room temperature. 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 (30:1 $CHCl_3$:MeOH) on silica gel to afford **19** (5.53 mg, 80%) as an amorphous mass; $[\alpha]_D^{24} = +6.9^\circ$ ($c = 0.1$, $CHCl_3$); ^{1}H NMR ($CDCl_3$):gδ = 5.77 (dd, 1H, $J_{6,7}$ 4.1, $J_{7,8}$ 8.7 Hz, H-7e), 5.65 (d, 1H, NHc), 5.44 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1f), 5.34 (m, 1H, H-8e), 5.28 (m, 1H, H-5e), 5.19 (dd, 1H, $J_{2,3}$ 10.9 Hz, H-3f), 5.15 (t, 1H, $J_{2,3} = J_{3,4} =$ 9.6 Hz, H-3a), 5.03 (dd, 1H, $J_{2,3}$ 9.2 Hz, H-2d), 4.97 (dd, 1H, $J_{2,3}$ 9.3 Hz, H-2b), 4.90 (dd, 1H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.6 Hz, H-2a), 4.86 (m, 1H, H-4e), 4.41 (d, 1H, $J_{1,2}$ 8.2 Hz, H-1a), 4.33 (d, 1H, $J_{1,2}$ 7.1 Hz, H-1d), 4.23 (d, 1H, $J_{1,2}$ 8.0, H-1b), 4.13 (dd, 1H, $J_{8,9}$, 5.3, J_{gem} 11.8 Hz, H-9'e), 4.02 (dd, 1H, $J_{8,9}$ 6.9, J_{gem} 11.8 Hz, H-9e), 3.76 (dd, 1H, $J_{2,3}$ 9.2, $J_{3,4}$ 3.2 Hz, H-3d), 3.65 (m, 1H, OCH_2 of alkyl part), 3.27 (dd, 1H, OCH_2 of alkyl part), 3.15 (q, 6H, $3CH_3CH_2N$), 2.56 (s, 3H, AcNe), 2.41 (dd, 1H, $J_{3\alpha,4}$ 5.5, J_{gem} 14.8 Hz, H-3eα), 2.28 (dd, 1H, $J_{3\beta,4}$ 10.9, J_{gem} 14.8 Hz, H-3eβ), 2.23, 2.20, 2.17, 2.15, 2.14, 2.13, 2.12, 2.11, 2.09, 2.09, 2.08, 2.06, 2.05, 2.01, 2.00, 1.95, 1.94 (17 s, 51 H, 16 AcO and AcN), 1.40 (t, 9 H, $3CH_3CH_2N$), 1.25 (m, 52 H, 26 CH_2), 0.88 (t, 6 H, *J* 6.6 Hz, 2 *Me*CH_2); elemental analysis calcd (%) for $C_{111}H_{177}N_3O_{51}S$ (2400.11): C 55.51, H 7.43, N 1.75; found: C 55.43, H 7.18, N 1.67.

2-(Tetradecyl)hexadecyl (5-amino-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 (E, GSC-552)

To a solution of **19** (5.5 mg, 2.1 μmol) in methanol (0.8 mL) and dioxane (0.2 mL) was added a catalytic amount of 28% sodium methoxide in methanol, and the mixture was stirred for 24 h at room temperature. After completion of the reaction the mixture was concentrated. Column chromatography (MeOH) of the residue on Sephadex LH-20 gave the target molecule **(E, GSC-552)** (3.7 mg, quant) as an amorphous mass; $[\alpha]_D^{24} = -5.4^\circ$ (c = 0.07, 1:1 $CHCl_3$:MeOH);^{1}H NMR (CD_3OD): δ = 4.96 (d, 1H, $J_{1,2}$ 3.9 Hz, H-1f), 4.66 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1c), 4.49 (d, 1H, $J_{1,2}$ 7.6 Hz, H-1d), 4.36 (br-d, 1H, H-6e), 4.28 (d, 1H, $J_{1,2}$ 7.3 Hz, H-1b), 4.15 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1a), 4.00 (m, 1H, H-4e), 3.98 (dd, 1H, $J_{2,3}$ 9.8, $J_{3,4}$ 2.9 Hz, H-3d), 3.85 (dd, 1H, $J_{2,3}$ 7.8 Hz, H-2c), 3.80 (dd, 1H, $J_{2,3}$ 10.1, $J_{3,4}$ 3.4 Hz, H-3f), 3.75 (dd, 1H, $J_{6,7}$ 4.1 Hz, H-7e), 3.56 (dd, 1H, $J_{2,3}$ 10.8 Hz, H-2b), 3.51 (dd, 1H, $J_{1,2}$ 3.9, $J_{2,3}$ 10.1 Hz, H-2f), 3.48 (dd, 1H, $J_{2,3}$ 9.2 Hz, H-2d), 3.40 (t, 1H, $J_{2,3} = J_{3,4} =$ 9.2 Hz, H-3a), 3.29 (m, 1H, H-8e), 3.14 (dd, 1H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.2 Hz, H-2a), 2.32 (dd, 1H, $J_{3\beta,4}$ 10.8, J_{gem} 14.1 Hz, H-3eβ), 2.02 (dd, 1H, $J_{3\alpha,4}$ 4.8, J_{gem} 14.1 Hz, H-3eα), 1.87 (s, 3H, AcNc), 1.49-1.19 (m, 53 H, 26 CH_2 and CH), 1.08 (d, 3 H, $J_{5,6}$ 6.6 Hz, H-6f), 0.80 (t, 6 H, *J* 6.9 Hz, 2 *Me*CH_2); FAB (−) MS: m/z: calcd for $C_{71}H_{127}N_2NaO_{34}S$:1606.7889; found: 1584.8484 [M-Na]$^-$, 1352 [M-Lactamized Neu-Na]$^-$, 1218 [M-Lactamized Neu-Fuc-Na]$^-$, 1190 [M-Lactamized Neu-Gal-Na]$^-$, 761 [lactosyl 2-(tetradecyl)hexadecyl]$^-$, 599 [glucosyl 2-(tetradecyl)hexadecyl]$^-$

Acknowledgments

This work was partly supported by Japan Society for the Promotion of Science (Grant-in-Aid for Scientific Research No. 12306007 to M.K.) and CREST of Japan Science and Technology Agency (JST). We thank Sanwa Kagaku Kenkyusho Co. for the mass spectrometric analyses, and Ms. Kiyoko Ito for the technical assistance.

References

1 Yamaguchi M, Ishida H, Kanamori A, Kannagi R, Kiso M, Synthesis and antigenic property of a novel sialyl 6-*O*-sulfo Lewis X neo-glycolipid containing lactamized neuraminic acid, *J Carbohydr Chem* **23**, 201–15, 2004.

2 Lasky LA, Selectins: Interpreters of cell-specific carbohydrate information during inflammation, *Science* **258**, 964–9 (1992).

3 Cummings RD, Smith DF, The selectin family of carbohydrate-binding proteins: Structure and importance of carbohydrate ligands for cell adhesion, *BioEssays* **14**, 849–56 (1992).

4 Kannagi R, Mitsuoka C, Ohmori K, Kanamori A, Kiso M, Khoo K.-H, Inoue Y, Cyclic sialic acid: A new regulatory mechanism of selectin ligand activity. In *Sialobiology and Other Novel Forms of Glycosylation,*edited by Inoue Y, Lee YC, Troy FA, (Gakushin Publisher, Osaka, Japan, 1999) pp. 37–43.

5 Kannagi R, Regulatory roles of carbohydrate ligands for selectins in the homing of lymphocytes, *Curr Opin Struct Biol* **12**, 599–608 (2002).

6 Mitsuoka C, Kawakami-Kimura N, Kasugai-Sawada M, Hiraiwa N, Toda K, Ishida H, Kiso M, Hasegawa A, Kannagi R, Sulfated sialyl Lewis X, the putative L-selectin ligand, detected on endothelial cells of high endothelial venules by a distinct set of anti-sialyl Lewis X antibodies, *Biochem Biophys Res Commun* **230**, 546–51 (1997).

7 Galustian C, Lawson AM, Komba S, Ishida H, Kiso M, Feizi T, Sialyl-Lewisx sequence 6-O-sulfated at N-acetylglucosamine rather than at galactose is the preferred ligand for L-selectin and

de-N-acetylation of the sialic acid enhances the binding strength, *Biochem Biophys Res Commun* **240**, 748–51 (1997).

8 Mitsuoka C, Sawada-Kasugai M, Ando-Furui K, Izawa M, Nakanishi H, Nakamura S, Ishida H, Kiso M, Kannagi R, Identification of a major carbohydrate capping group of the L-selectin ligand on high endothelial venules in human lymph nodes as 6-sulfo sialyl Lewis X, *J Biol Chem* **273**, 11225–33 (1998).

9 Komba S, Galustian C, Ishida H, Feizi T, Kannagi R, Kiso M, The first total synthesis of 6-sulfo de-*N*-acetylsialyl Lewis x ganglioside: A superior ligand for human L-selectin, *Angew Chem Int Ed* **38**, 1131–3 (1999).

10 Komba S, Yamaguchi M, Ishida H, Kiso M, 6-*O*-Sulfo de-*N*-acetylsialyl Lewis x as a novel high-affinity ligand for human L-selectin: Total synthesis and structural characterization, *Biol Chem* **382**, 233–40 (2001).

11 Mitsuoka C, Ohmori K, Kimura N, Kanamori A, Komba S, Ishida H, Kiso M, Kannagi R, Regulation of selectin binding activity by cyclization of sialic acid moiety of carbohydrate ligands on human leukocytes, *Proc Natl Acad Sci USA* **96**, 1597–1602 (1999).

12 Yamaguchi M, Ishida H, Kanamori A, Kannagi R, Kiso M, Studies on the endogenous L-selectin ligands: Systematic and highly efficient total synthetic routes to lactamized-sialyl 6-*O*-sulfo Lewis x and other novel gangliosides containing lactamized neuraminic acid, *Carbohydr Res* **338**, 2793–812 (2003).

13 Yamaguchi M, Ishida H, Galustian C, Feizi T, Kiso M, Synthesis and selectin-binding activity of *N*-deacetylsialyl Lewis X ganglioside, *Carbohydr Res* **337**, 2111–7 (2002).

14 Fukunaga K, Shinoda K, Ishida H, Kiso M, Systematic synthesis of sulfated sialyl-$\alpha(2\rightarrow3)$-neolactotetraose derivatives and their acceptor specificity for a $\alpha(1\rightarrow3)$-fucosyltransferase (Fuc-TVII) involved in the biosynthesis of L-selectin ligand, *Carbohydr Res* **328**, 85–94 (2000).

15 Hanai N, Dohi T, Nores GA, Hakomori S, A novel ganglioside, de-*N*-acetyl-GM3 ($II^3NeuNH_2LacCer$), acting as a strong promoter for epidermal growth factor receptor kinase and as a stimulator for cell growth, *J Biol Chem* **263**, 6296–301 (1988).

16 Sjoberg ER, Chammas R, Ozawa H, Kawashima I, Khoo KH, Morris HR, Dell A, Tai T, Varki A, Expression of de-*N*-acetylgangliosides in human melanoma cells is induced by genistein or nocodazole, *J Biol Chem* **270**, 2921–30 (1995).

17 Manzi AE, Sjoberg ER, Diaz S, Varki A, Biosynthesis and turnover of *O*-acetyl and *N*-acetyl groups in the gangliosides of human melanoma cells, *J Biol Chem* **265**, 13091–103 (1990).

18 Kannagi R, Monoclonal anti-glycosphingolipid antibodies. Methods in enzymology, *Methods Enzymol* **312**, 160–79 (2000).

19 Otsubo N, Ishida H, Kannagi R, Kiso M, Design and synthesis of a novel neo-glycolipid containing sialyl Lewis X determinant carried on the mucin GlcNAcβ1-6GalNAcα core structure, *Tetrahedron: Asymmetry* **16**, 1321–1327 (2005).

Received 9 July 2004; revised 10 January 2005; accepted 18 January 2005