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Design and synthesis of a novel neo-glycolipid containing sialyl Lewis X determinant carried on the mucin GlcNAcβ1-6GalNAcα core structure☆

Nobumasa Otsubo,[a] Hideharu Ishida,[a,*] Reiji Kannagi[b,c] and Makoto Kiso[a,c,*]

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

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Abstract—A novel neo-glycolipid containing sialyl Lewis X determinant carried on the mucin GlcNAcβ1-6GalNAcα core structure has been designed and synthesized. By employing this compound as a probe, the structure required for the recognition of anti-cancer antibodies, NCC-ST-439, has been elucidated.

1. Introduction

Sialyl Lewis X, known as a ligand for selectins, is suggested to be involved in the hematogeneous metastasis of many cancer cells.[2,3] Sialyl Lewis X is carried by various internal carbohydrate structures, which generate a considerable heterogeneity in molecular species of sialyl Lewis X determinants at the surface of cells and tissues, each of which might have distinct physiological significance.

The antibody NCC-ST-439 was initially raised against human gastric cancer cells[4] and later shown to recognize a tumor-associated carbohydrate antigen in breast, gastric, and colon cancers. This suggested that the antigen recognized by NCC-ST-439 is closely related to sialyl Lewis X.

As part of our continuing studies on a chemical approach to carbohydrate antigens of cancers, we report herein the design and synthesis of a novel neo-glycolipid containing the sialyl Lewis X determinant carried on the mucin GlcNAcβ1-6GalNAcα core structure, (GSC-384) **16** and its reactivity against the NCC-ST-439 antibody in comparison with the conventional sialyl Lewis X determinants on straight (GSC-64) or branched (GSC-154) carbohydrate skeleton (Fig. 1).[5,6]

2. Results and discussion

The most significant problems in the synthesis of the title compound are: (i) α-stereoselective glycoside formation of the GalNAc residue with the lactose moiety as a spacer, (ii) efficient construction of the sialyl Lewis X on the mucin GlcNAcβ1-6GalNAcα core structure, and (iii) introduction of the lipid into the complex oligosaccharide.

The first problem was solved by employing a 2-azido derivative of galactose, which is equivalent to galactosamine. Trisaccharide **1** synthesized according to the procedure previously described,[7] was coupled with glucosamine donor **2**[8] in the presence of *N*-iodosuccinimide (NIS)-trifluoromethanesulfonic acid (TfOH)[9,10] at −15 °C, to give tetrasaccharide **3** (85%) which has the core 6 structure (Scheme 1).

For the construction of the sialyl Lewis X structure, we employed the strategy, which begins with the introduction of the sialylgalactose moiety to *O*-4 of GlcNAc and is followed by the fucosylation at *O*-3 of GlcNAc. Removal of the phthaloyl and acetyl groups of **3** with H_2N–NH_2·H_2O in ethanol, and the selective *N*-acetylation with acetic anhydride in methanol gave the

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* Corresponding authors. Tel.: +81 58 293 2918/2916; fax: +81 58 293 2840; e-mail addresses: ishida@cc.gifu-u.ac.jp; kiso@cc.gifu-u.ac.jp

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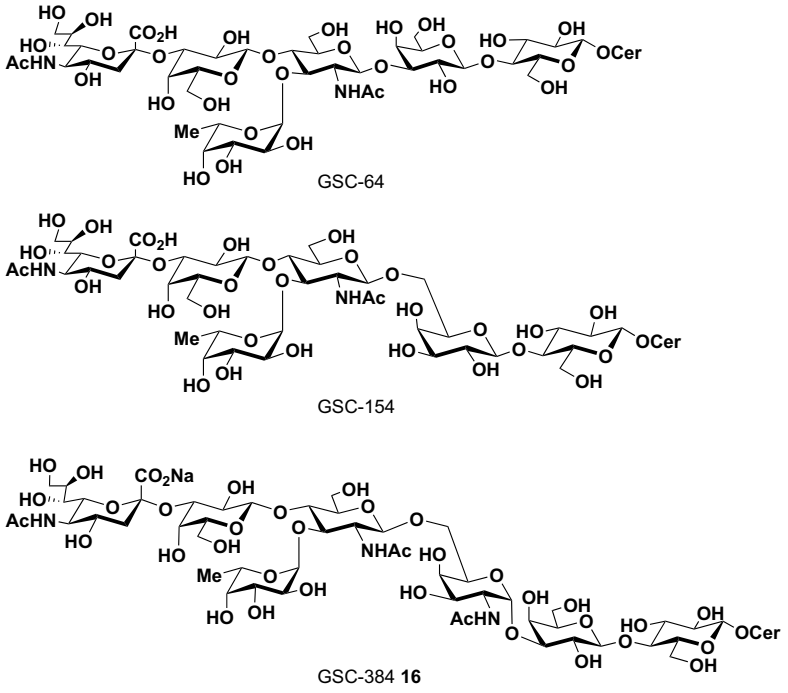
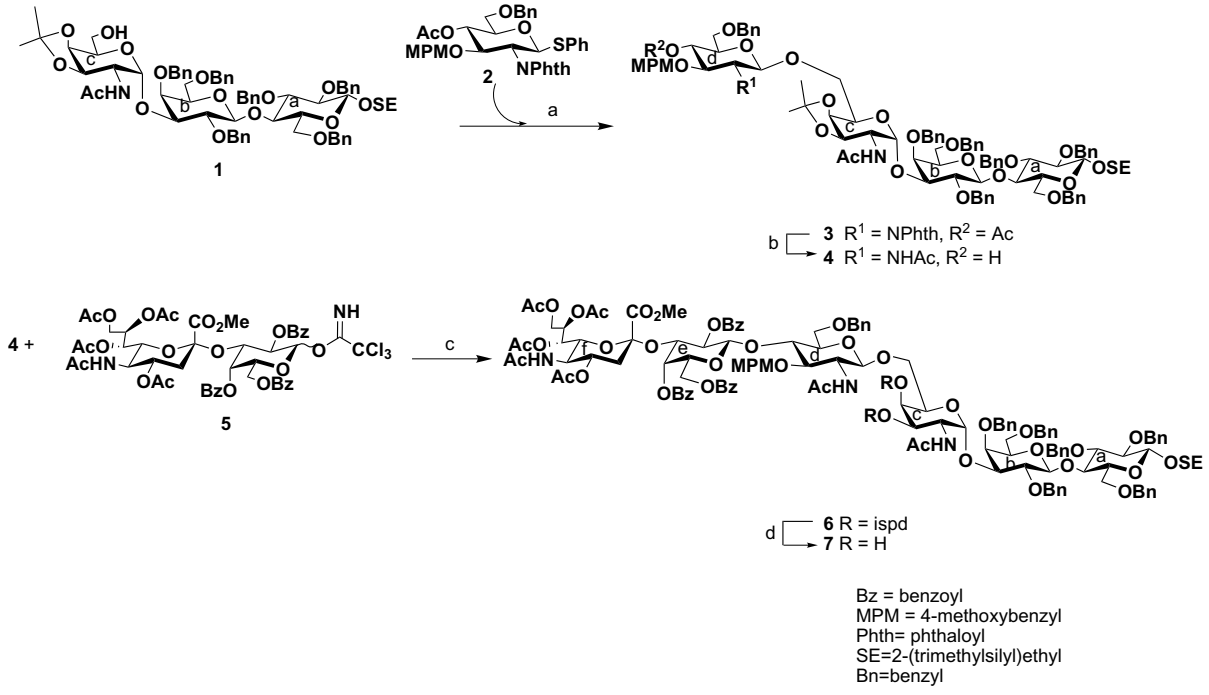
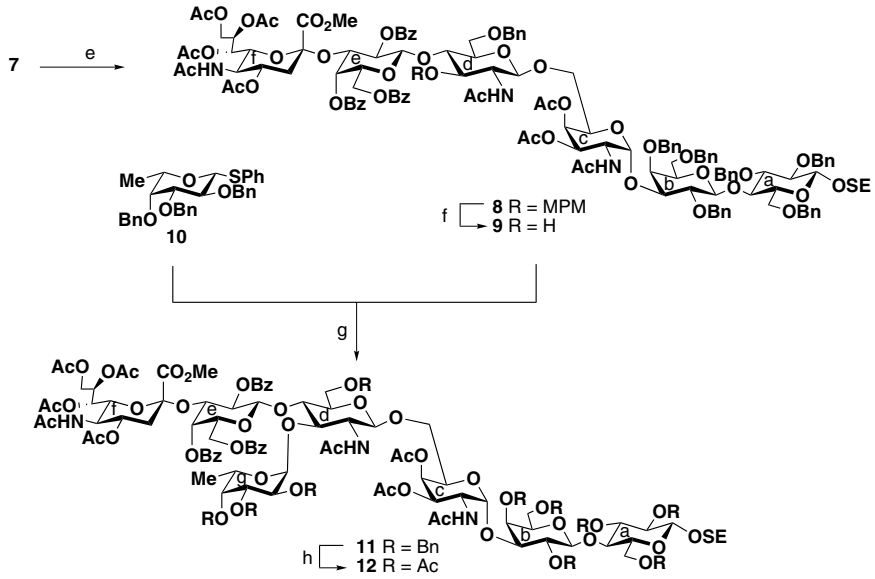


Figure 1. Structure of sLe^x-containing glycolipids.



Scheme 1. Reagents and conditions: (a) NIS-TfOH, molecular sieves 4 Å, CH_2Cl_2, −15 °C, 85%; (b) (1) $H_2NNH_2{\cdot}H_2O$, EtOH, reflux, (2) Ac_2O, MeOH, 80%; (c) TMSOTf, AW-300, CH_2Cl_2, 0 °C, 68%; (d) 80% aq AcOH, 40 °C, 90%.

tetrasaccharide acceptor **4** (80%). Acceptor **4** was glycosylated with the freshly prepared sialyl-α (2→3)-galactose trichloroacetimidate donor **5**[11] in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf), to give the desired hexasaccharide **6** (68%). The acid labile isopropylidene group at *O*-3 and 4 of GalNAc was replaced with an acetyl group before removal of the 4-methoxybenzyl (MPM) group in the GlcNAc residue. The hexasaccharide acceptor **9** was obtained in three steps: (i) removal of the isopropylidene group with aqueous 80% acetic acid (90%); (ii) acetylation with acetic anhydride/pyridine (quant.), and (iii) removal of the MPM group with ceric ammonium nitrate (93%).[12] This acceptor was fucosylated with donor **10**[13] and promoted by NIS-TfOH in benzene, to afford the desired heptasaccharide **11** in 80%. The stereochemistry of the newly formed glycosidic linkage was indicated to be α by the small coupling of the proton of H-1 ($J_{1,2}$ 3.4 Hz), which



Scheme 2. Reagents and conditions: (e) Ac_2O, Pyr., quant.; (f) CAN, MeCN–H_2O, 93%; (g) NIS, TfOH, MS4Å, C_6H_6, 7 °C, 80%; (h) (1) $Pd(OH)_2$, H_2 gas, EtOH, 40 °C, (2) Ac_2O, Pyr., 89%.

is a characteristic feature for 1,2-*cis* fucopyranosides (Scheme 2).

The introduction of a lipid into the complex oligosaccharide, a third problem, was accomplished by the glycosydation reaction via 1,2-orthoester formation. Removal of the benzyl groups from **11** by catalytic hydrogenation over palladium hydroxide in ethanol, and subsequent acetylation gave the per-*O*-acylated heptasaccharide **12** (89%). Selective removal[14] of the 2-(trimethylsilyl)ethyl (SE) group from **12** (quant.) with trifluoroacetic acid gave the corresponding 1-hydroxy compound **13**. Treatment[15] of **13** with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) gave the α-trichloroacetimidate **14** (94%). Treatment of a mixture of imidate **14** and 2-tetradecylhexadecanol with TMSOTf (0.08 equiv, 72 h) afforded the 1,2-orthoester, which was further converted into the desired β-glycoside (46%) by the addition of TMSOTf (0.16 equiv) and additional stirring (24 h). Finally, removal of all the protecting groups under basic conditions furnished the target molecule in 92%. Significant signals in the ^{1}H NMR spectrum of **16** were four one-proton doublets at 4.26, 4.43, 4.49 and 4.59 ($J_{1,2}$ 7.7–8.0 Hz, four β-anomeric-H), showing the finally formed glycosidic linkage to be β. In the fast-atom bombardment (FAB) mass spectrum of **16**, the molecular ion of $[M-H]^-$ was clearly detected at *m*/*z* 1767.1, providing evidence for the assigned structure (Scheme 3).

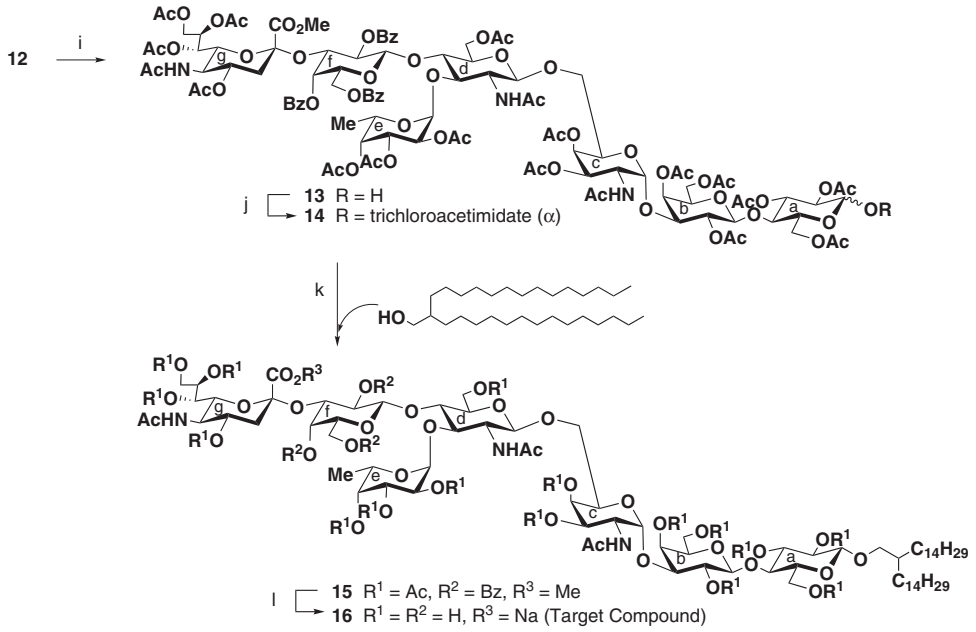
The reactivity of antibodies, NCC-ST-439, CSLEX-1 and GSC154-27, against the sialyl Lewis X determinant carried by various internal structures, was examined.[16] Antibody NCC-ST-439 was initially raised against human stomach cancer cell line ST-4,[4] and GSC154-27 against the conventional sialyl Lewis X determinant on branched polylactosamine structure.[17] CSLEX-1 is known to react with the sialyl Lewis X related-determinant independent from the inner structures. The specificity of the three antibodies is summarized in Table 1. It is clear that antibody NCC-ST-439 is specifically reactive to the sialyl Lewis X carried by the GlcNAcβ1-6GalNAcα mucin core. This core structure is known to be present in mucin glycoproteins, but not in glycolipids. Trials over a long period to find a glycolipid antigen recognized by NCC-ST-439 in tumor tissues have so far been unsuccessful. The chemical approach, design and synthesis of a novel neo-glycolipid could meet with success in determining the structures recognized by the antibody NCC-ST-439 as shown here.

3. Experimental

Optical rotations were determined with a Union PM-201 polarimeter at 25 °C. ^{1}H NMR spectra were recorded at 400 MHz with a Varian Inova 400, or 500 MHz with a Varian Inova 500 spectrometer. FAB-MS were recorded on a JEOL JMS-SX 120A mass spectrometer/JMA-DA 7000 data system. Preparative TLC was performed on Silica Gel 60 (E. Merck), and column chromatography on silica gel (Fuji Silysia Co., 300 mesh) was accomplished with the solvent system (v/v) specified. Concentrations and evaporations were conducted in vacuo.

3.1. 2-(Trimethylsilyl)ethyl *O*-[4-*O*-acetyl-6-*O*-benzyl-2-deoxy-3-*O*-(4-methoxybenzyl)-2-phthalimido-β-D-glucopyranosyl]-(1→6)-*O*-(2-acetamido-2-deoxy-3,4-*O*-isopropylidene-α-D-galactopyranosyl)-(1→3)-*O*-(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** (1.0 g, 0.82 mmol) and **2** (0.86 g, 1.31 mmol) in dry CH_2Cl_2 (25 mL) were added powdered molecular sieves 4 Å (1.5 g), and the mixture was stirred for 3 h at room temperature and then cooled to −10 °C. *N*-Iodosuccinimide (NIS; 0.6 g, 2.66 mmol) and trifluoromethanesulfonic acid (TfOH; 11 μL, 0.12 mmol) were



Scheme 3. Reagents and conditions: (i) TFA, CH_2Cl_2, quant.; (j) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 94%; (k) TMSOTf, AW-300, CH_2Cl_2, 46%; (l) NaOMe, H_2O, MeOH–THF, 92%.

Table 1. Summary of the monoclonal antibody reactivities

Glycolipid	Antibody reactivities		
	NCC-ST-439	GSC-154-27	CSLEX-1
GSC-64	–	+	+
GSC-154	–	+	+
GSC-384	+	–	+

added to the mixture, which was stirred for 12 h at −10 °C. The solids were filtered off and washed with $CHCl_3$. The combined filtrate and washings was successively washed with 1 M $NaHCO_3$ and 1 M $Na_2S_2O_3$, dried over Na_2SO_4, and concentrated. Column chromatography (1:1 EtOAc–hexane) of the residue on silica gel gave **3** (1.22 g, 85%) as a white solid: $[\alpha]_D$ = +53.4 (*c* 2.3, $CHCl_3$); IR (film) 3350, 2950, 1750, 1720, 860, 840, 700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.02 (m, 2H, $Me_3SiCH_2CH_2$), 1.05 (s, 3H, AcN), 1.30, 1.32 (2s, 6H, Me_2C), 1.91 (s, 3H, AcO), 3.51 (s, 3H, MeO), 3.83 (m, 1H, H-2c), 4.93 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1c), 4.96 (t, 1H, $J_{3,4} = J_{4,5}$ = 8.4 Hz, H-4d), 5.25 (d, 1H, $J_{2,NH}$ = 9.5 Hz, NH-c), 6.38–7.65 (m, 43H, 7Ph, MeO*Ph*, phthaloyl-H). Anal. Calcd for $C_{100}H_{116}N_2O_{24}Si$ (1758.10): C, 68.32; H, 6.65; N, 1.59. Found: C, 68.24; H, 6.41; N, 1.32.

3.2. 2-(Trimethylsilyl)ethyl *O*-[2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(4-methoxybenzyl)-β-D-glucopyranosyl]-(1→6)-*O*-(2-acetamido-2-deoxy-3,4-*O*-isopropylidene-α-D-galactopyranosyl)-(1→3)-*O*-(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** (4.9 g, 2.79 mmol) in EtOH (40 mL) was added $NH_2NH_2{\cdot}H_2O$ (3.9 mL, 0.12 mmol), and the mixture was stirred under reflux for 14 h. The solids were filtered off and washed with $CHCl_3$ and then concentrated. The obtained residue was dissolved in methanol (30 mL) and treated with Ac_2O (2.6 mL, 27.6 mmol) for 8 h at room temperature. After the completion of the reaction, the mixture was concentrated. Column chromatography (2:1 EtOAc–hexane) of the residue on silica gel gave **4** (3.6 g, 80%): $[\alpha]_D$ = +22.5 (*c* 1.8, $CHCl_3$); IR (film) 3550, 3350, 2950, 1680, 1520, 860, 840, 700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.02 (m, 2H, $Me_3SiCH_2CH_2$), 1.15, 1.43, 1.69 (3s, 12H, 2AcN, Me_2C), 3.56 (m, 1H, H-2d), 3.76 (s, 3H, MeO), 4.17 (m, 1H, H-2c), 4.38 (d, 1H, $J_{1,2}$ = 10.9 Hz, H-1d), 4.91 (d, 1H, $J_{1,2}$ = 4.3 Hz, H-1c), 5.21 (d, 1H, $J_{2,NH}$ = 8.2 Hz, NH-d), 5.39 (d, 1H, $J_{2,NH}$ = 9.6 Hz, NH-c), 6.82–7.37 (m, 39H, 7Ph, MeO*Ph*). Anal. Calcd for $C_{93}H_{114}N_2O_{22}Si$ (1639.99): C, 68.11; H, 7.01; N, 1.71. Found: C, 67.85; H, 6.97; N, 1.50.

3.3. 2-(Trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(4-methoxybenzyl)-β-D-glucopyranosyl]-(1→6)-*O*-(2-acetamido-2-deoxy-3,4-*O*-isopropylidene-α-D-galactopyranosyl)-(1→3)-*O*-(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 **4** (2.4 g, 2.16 mmol) and **5** (1.6 g, 0.98 mmol) in dry CH_2Cl_2 (10 mL) were added molecular sieves 4 Å (AW-300, 2.0 g), and the mixture was stirred for 3.5 h at room temperature. It was then cooled to −15 °C. TMSOTf (16.5 μL, 85.3 μmol) was added to the mixture, which was stirred for 30 h at −15 °C. After completion of the reaction, the solids were filtered off and washed with $CHCl_3$. The combined filtrate and washings was washed with 1 M $NaHCO_3$ and water, dried over Na_2SO_4, and concentrated. Column chromatography (EtOAc) of the residue on silica gel gave **6** (1.72 g, 68%): $[\alpha]_D$ = +27.7 (*c* 1.6, $CHCl_3$); IR (film) 3350,

2950, 1750, 1680, 1520, 860, 840, 700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.01 (m, 2H, $Me_3SiCH_2CH_2$), 1.42, 1.44, 1.55, 1.69, 1.79, 1.84, 1.92, 1.99, 2.16 (9s, 27H, 3AcN, 4AcO, Me_2C), 1.67 (t, 1H, $J_{gem} = J_{3ax,4} = 12.5$ Hz, H-3f*ax*), 2.46 (dd, 1H, $J_{3eq,4} = 4.5$ Hz, H-3f*eq*), 3.67 (s, 3H, MeO), 3.83 (s, 3H, COOMe), 4.84 (m, 1H, H-4f), 4.89 (d, 1H, $J_{1,2} = 8.7$ Hz, H-1e), 4.94 (d, 1H, $J_{1,2} = 2.7$ Hz, H-1c), 5.04 (dd, 1H, $J_{2,3} = 10.7$, $J_{3,4} = 3.4$ Hz, H-3e), 5.24 (dd, 1H, $J_{6,7} = 2.7$, $J_{7,8} = 9.8$ Hz, H-7f), 5.36 (d, 1H, $J_{2,NH} = 9.6$ Hz, NH-c), 5.39 (d, 1H, H-4e), 5.48 (dd, 1H, H-2e), 5.70 (m, 1H, H-8f), 6.65–8.23 (m, 54H, 10Ph, MeO*Ph*). Anal. Calcd for $C_{140}H_{163}N_3O_{42}Si$ (2587.87): C, 64.98; H, 6.35; N, 1.62. Found: C, 64.77; H, 6.28; N, 1.40.

3.4. 2-(Trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(4-methoxybenzyl)-β-D-glucopyranosyl]-(1→6)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-*O*-(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 **6** (330 mg, 0.13 mmol) in AcOH (12 mL) was added water (3 mL), which was stirred for 10 h at 40 °C and then concentrated. Column chromatography (30:1 $CHCl_3$–CH_3OH) of the residue on silica gel gave **7** (294 mg, 90%): $[\alpha]_D = +22.9$ (*c* 1.7, $CHCl_3$); IR (film) 3550, 3350, 2950, 1750, 1680, 1520, 860, 840, 700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.02 (m, 2H, $Me_3SiCH_2CH_2$), 1.36, 1.52, 1.76, 1.82, 1.88, 1.96, 2.14 (7s, 21H, 3AcN, 4AcO), 1.64 (t, 1H, $J_{gem} = J_{3ax,4} = 12.5$ Hz, H-3f*ax*), 2.46 (dd, 1H, $J_{3eq,4} = 4.4$ Hz, H-3f*eq*), 3.67 (s, 3H, MeO), 3.81 (s, 3H, COOMe), 4.84 (m, 1H, H-4f), 4.72 (dd, 1H, $J_{gem} = 11.0$, $J_{8,9} = 3.3$ Hz, H-9f), 4.93 (d, 1H, $J_{1,2} = 3.3$ Hz, H-1c), 5.15 (d, 1H, $J_{5,NH} = 9.9$ Hz, NH-f), 5.21 (dd, 1H, $J_{6,7} = 2.6$, $J_{7,8} = 9.9$ Hz, H-7f), 5.38 (d, 1H, $J_{3,4} = 3.3$ Hz, H-4e), 5.46 (dd, 1H, $J_{1,2} = 8.8$, $J_{2,3} = 9.9$ Hz, H-2e), 5.52 (d, 1H, $J_{2,NH} = 8.7$ Hz, NH-c), 5.69 (m, 1H, H-8f), 5.82 (d, 1H, $J_{2,NH} = 8.1$ Hz, NH-d), 6.73–8.22 (m, 54H, 10Ph, MeO*Ph*). Anal. Calcd for $C_{137}H_{159}N_3O_{42}Si$ (2547.84): C, 64.58; H, 6.29; N, 1.65. Found: C, 64.52; H, 6.29; N, 1.46.

3.5. 2-(Trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*–D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(4-methoxybenzyl)-β-D-glucopyranosyl]-(1→6)-*O*-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 8

Compound **7** (660 mg, 0.26 mmol) was dissolved in pyridine (3 mL) and treated with Ac_2O (1 mL) for 12 h at room temperature. After the completion of the reaction, MeOH was added to the reaction mixture to decompose excess reagent and then concentrated. The residue was diluted with $CHCl_3$, which was washed with 2 M HCl and water, dried over Na_2SO_4, and concentrated. Column chromatography (40:1 $CHCl_3$–CH_3OH) of the residue on silica gel gave **8** (670 mg, quant.): $[\alpha]_D = +27.3$ (*c* 1.0, $CHCl_3$); IR (film) 3350, 2950, 1750, 1680, 1520, 860, 840, 700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.01 (m, 2H, $Me_3SiCH_2CH_2$), 1.32, 1.50, 1.61, 1.77, 1.91, 1.93, 1.97, 1.99, 2.13 (9s, 27H, 3AcN, 6AcO), 1.65 (t, 1H, $J_{gem} = J_{3ax,4} = 12.5$ Hz, H-3f*ax*), 2.44 (dd, 1H, $J_{3eq,4} = 4.3$ Hz, H-3f*eq*), 3.64 (s, 3H, MeO), 3.81 (s, 3H, COOMe), 4.83 (d, 1H, $J_{1,2} = 9.8$ Hz, H-1e), 4.92 (d, 1H, $J_{1,2} = 3.2$ Hz, H-1c), 5.22 (dd, 1H, $J_{6,7} = 2.2$, $J_{7,8} = 9.6$ Hz, H-7f), 5.37 (d, 1H, $J_{3,4} = 2.5$ Hz, H-4e), 5.46 (t, 1H, $J_{2,3} = 9.8$ Hz, H-2e), 5.69 (m, 1H, H-8f), 5.15 (d, 1H, $J_{5,NH} = 9.9$ Hz, NH-f), 6.63-8.24 (m, 54H, 10Ph, MeO*Ph*). Anal. Calcd for $C_{141}H_{163}N_3O_{44}Si$ (2631.92): C, 64.35; H, 6.24; N, 1.60. Found: C, 64.34; H, 6.18; N, 1.53.

3.6. 2-(Trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-*O*-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 9

To a solution of **8** (600 mg, 0.23 mmol) in MeCN (4.5 mL) and water (0.5 mL) was added ceric ammonium nitrate (CAN; 380 mg, 0.69 mmol), and the mixture stirred for 1 h at room temperature, and extracted with $CHCl_3$. The extract was successively washed with water and 1 M $NaHCO_3$, dried over Na_2SO_4 and concentrated. Column chromatography (50:1 $CHCl_3$–CH_3OH) of the residue on silica gel gave **9** (530 mg, 93%): $[\alpha]_D = +44.0$ (*c* 1.2, $CHCl_3$); IR (film) 3550, 3350, 2950, 1750, 1680, 1520, 860, 840, 700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.03 (m, 2H, $Me_3SiCH_2CH_2$), 1.34, 1.56, 1.69, 1.75, 1.78, 1.91, 1.94, 2.01, 2.21 (9s, 27H, 3AcN, 6AcO), 1.62 (t, 1H, $J_{gem} = J_{3ax,4} = 12.8$ Hz, H-3f*ax*), 2.44 (dd, 1H, $J_{3eq,4} = 4.4$ Hz, H-3f*eq*), 3.86 (s, 3H, COOMe), 4.73 (dd, $J_{gem} = 10.1$, $J_{8,9} = 3.3$ Hz, H-9f), 4.82 (m, 1H, H-4f), 4.90 (d, 1H, $J_{1,2} = 9.8$ Hz, H-1e), 4.93 (d, 1H, $J_{1,2} = 2.6$ Hz, H-1c), 5.04 (dd, 1H, $J_{2,3} = 10.2$, $J_{3,4} = 2.6$ Hz, H-3c), 5.08 (d, 1H, H-4c), 5.24 (dd, 1H, $J_{6,7} = 2.9$, $J_{7,8} = 9.9$ Hz, H-7f), 5.34 (d, 1H, $J_{2,NH} = 9.9$ Hz, NH-c), 5.36 (d, 1H, $J_{3,4} = 3.3$ Hz, H-4e), 5.49 (t, 1H, $J_{2,3} = 9.8$ Hz, H-2e), 5.18 (m, 1H, H-8), 7.00–8.23 (m, 50H, 10Ph). Anal. Calcd for $C_{133}H_{155}N_3O_{43}Si$ (2511.77): C, 63.60; H, 6.22; N, 1.67. Found: C, 63.50; H, 6.08; N, 1.60.

3.7. 2-(Trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-*O*-(2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-*O*-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 11

To a solution of **9** (470 mg, 0.23 mmol) and **10** (198 mg, 0.37 mmol) in dry benzene (5 mL) were added powdered

molecular sieves 4 Å (0.5 g), and the mixture was stirred for 5 h at room temperature, then cooled to 0 °C. NIS (253 mg, 1.12 mmol) and TfOH (27.0 μL, 0.30 μmol) were added to the mixture, which was stirred for 2 h at 7 °C, then neutralized with Et_3N. The solids were filtered off and washed with $CHCl_3$. The combined filtrate and washings was successively washed with 1 M $NaHCO_3$ and water, dried over Na_2SO_4 and concentrated. Column chromatography (60:1 $CHCl_3$–CH_3OH) of the residue on silica gel gave **11** (438 mg, 80%): $[\alpha]_D = -2.9$ (*c* 0.8, $CHCl_3$); IR (film) 3350, 2950, 1750, 1680, 1520, 860, 840, 700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.03 (m, 2H, $Me_3SiCH_2CH_2$), 1.21 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6g), 1.25, 1.31, 1.53, 1.64, 1.78, 1.91, 1.95, 1.98, 2.13 (9s, 27H, 3AcN, 6AcO), 1.70 (t, 1H, $J_{gem} = J_{3ax,4}$ = 12.4 Hz, H-3f*ax*), 2.41 (dd, 1H, $J_{3eq,4}$ = 4.5 Hz, H-3f*eq*), 3.73 (s, 3H, COOMe), 4.87 (dd, 1H, $J_{2,3}$ = 9.8, $J_{3,4}$ = 3.6 Hz, H-3e), 4.94 (m, 1H, H-4f), 5.03 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1e), 5.06 (d, 1H, $J_{1,2}$ = 2.1 Hz, H-1c), 5.18 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1g), 5.23 (dd, 1H, $J_{6,7}$ = 2.7, $J_{7,8}$ = 9.8 Hz, H-7f), 5.28 (d, 1H, H-4e), 5.45 (t, 1H, H-2e), 5.47 (d, 1H, $J_{2,NH}$ = 8.2 Hz, NH-c), 5.68 (m, 1H, H-8f), 5.73 (d, 1H, $J_{2,NH}$ = 8.0 Hz, NH-d), 6.94–8.22 (m, 65H, 13Ph). Anal. Calcd for $C_{160}H_{183}N_3O_{47}Si$ (2928.28): C, 65.63; H, 6.30; N, 1.43. Found: C, 65.44; H, 6.19; N, 1.30.

3.8. 2-(Trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-*O*-(2-acetamido-6-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-*O*-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside 12

A solution of **11** (420 mg, 0.14 μmol) in EtOH (7 mL) was hydrogenated over $Pd(OH)_2$ (450 mg) for 14 h at 40 °C, then filtered and concentrated. The residue was acetylated with Ac_2O (2 mL) and pyridine (2 mL) for 12 h at room temperature. The solution was diluted with $CHCl_3$, and the solution washed with 2 M HCl and water, dried over Na_2SO_4, and concentrated. After work-up as described in the synthesis of **8**, column chromatography (30:1 $CHCl_3$–CH_3OH) of the residue on silica gel gave **12** (314 mg, 89%): $[\alpha]_D = -4.9$ (*c* 1.3, $CHCl_3$); IR (film) 3350, 2950, 1750, 1680, 1520, 860, 840, 700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.96 (m, 2H, $Me_3SiCH_2CH_2$), 1.19 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6g), 1.25-2.15 (19s, 57H, 3AcN, 16AcO), 1.61 (t, 1H, $J_{gem} = J_{3ax,4}$ = 12.3 Hz, H-3f*ax*), 2.41 (dd, 1H, $J_{3eq,4}$ = 4.6 Hz, H-3f*eq*), 3.81 (s, 3H, COOMe), 4.17 (dd, 1H, J_{gem} = 12.1, $J_{8,9}$ = 6.1 Hz, H-9f), 4.33 (dd, 1H, $J_{8,9'}$ = 6.1 Hz, H-9′f), 4.68 (m, 1H, H-4f), 5.04 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1c), 5.44 (t, 1H, $J_{1,2} = J_{2,3}$ = 8.5 Hz, H-2e), 5.66 (m, 1H, H-8f), 5.87 (d, 1H, $J_{2,NH}$ = 9.1 Hz, NH-d), 6.39 (d, 1H, $J_{2,NH}$ = 9.6 Hz, NH-c), 5.03 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1e), 7.42–8.19 (m, 15H, 3Ph). Anal. Calcd for $C_{110}H_{143}N_3O_{57}Si$ (2447.41): C, 53.98; H, 5.89; N, 1.72. Found: C, 53.91; H, 5.73; N, 1.49.

3.9. 2-(Trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-*O*-(2-acetamido-6-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-*O*-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)]-*O*-(2-acetamido-4-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α-β-D-glucopyranose 13

To a solution of **12** (225 mg, 91.9 μmol) in CH_2Cl_2 (2 mL), cooled to 0 °C, was added CF_3COOH (2 mL), and the mixture was stirred for 1 h at room temperature and concentrated. The product was applied for the next reaction without further purification.

3.10. *O*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-*O*-(2-acetamido-6-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-*O*-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate 14

To a solution of the product obtained above in CH_2Cl_2 (2 mL), cooled to 0 °C, was added trichloroacetonitrile (270 μL, 2.69 mmol) and DBU (13.0 μL, 86.9 μmol). The mixture was stirred for 1 h at 0 °C, and the progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was concentrated. Column chromatography (35:1 CH_2Cl_2–CH_3OH) of the residue on silica gel gave **14** (175 mg, 89%): $[\alpha]_D = +14.3$ (*c* 1.8, $CHCl_3$); IR (film) 3350, 2950, 1750, 1680, 1520, 700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.22 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6g), 1.60–2.16 (19s, 57H, 3AcN, 16AcO), 1.62 (t, 1H, $J_{gem} = J_{3ax,4}$ = 12.6 Hz, H-3f*ax*), 2.42 (dd, 1H, $J_{3eq,4}$ = 4.6 Hz, H-3f*eq*), 3.81 (s, 3H, COOMe), 4.52 (m, 1H, H-2c), 4.71 (m, 1H, H-4f), 5.08 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-1e), 5.44 (d, 1H, $J_{3,4}$ = 3.8 Hz, H-4e), 5.57 (t, 1H, $J_{2,3}$ = 9.7 Hz, H-2e), 5.67 (m, 1H, H-8f), 5.72 (d, 1H, $J_{2,NH}$ = 8.9 Hz, NH-d), 6.06 (d, 1H, $J_{2,NH}$ = 9.6 Hz, NH-c), 6.51 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1a), 7.43–8.18 (m, 15H, 3Ph), 8.68 (s, 1H, C=NH). Anal. Calcd for $C_{107}H_{131}N_4O_{57}Cl_3$ (2491.56): C, 51.58; H, 5.30; N, 2.25. Found: C, 51.48; H, 5.19; N, 1.96.

3.11. 2-(Tetradecyl)hexadecyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-*O*-(2-acetamido-6-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-*O*-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside 15

To a solution of **14** (217 mg, 87.1 μmol) and 2-(tetradecyl)hexadecanol (115 mg, 0.26 μmol) in dry CH_2Cl_2 (7 mL) were added molecular sieves 4 Å (AW-300,

0.5 g), and the mixture was stirred for 4.5 h at room temperature and then cooled to 0 °C. TMSOTf (1.3 μL, 6.72 μmol) was added to the mixture, which was stirred for 72 h at 0 °C and for another 24 h after the addition of TMSOTf (2.6 μL, 13.4 μmol). After completion of the reaction, the solids were filtered off and washed with $CHCl_3$. The combined filtrate and washings was washed with 1 M $NaHCO_3$ and water, dried over Na_2SO_4, and concentrated. Column chromatography (30:1 $CHCl_3$–CH_3OH) of the residue on silica gel gave **14** (111 mg, 46%): $[\alpha]_D$ = +9.2 (*c* 1.1, $CHCl_3$); IR (film) 3350, 2950, 1750, 1680, 1520, 700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (t, 6H, J = 6.6 Hz, $2CH_3$), 1.22 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6g), 1.24–1.43 (m, 53H, $26CH_2$ and CH), 1.58–2.15 (19s, 57H, 3AcN, 16AcO), 2.42 (dd, 1H, J_{gem} = 12.8, $J_{3eq,4}$ = 4.3 Hz, H-3f*eq*), 3.81 (s, 3H, COOMe), 4.54 (m, 1H, H-2c), 4.72 (m, 1H, H-4f), 5.01 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1c), 5.66 (m, 1H, H-8f), 5.69 (d, 1H, $J_{2,NH}$ = 8.4 Hz, NH-d), 6.02 (d, 1H, $J_{2,NH}$ = 9.6 Hz, NH-c), 7.43–8.16 (m, 15H, 3Ph). Anal. Calcd for $C_{135}H_{191}N_3O_{57}$ (2767.98): C, 58.58; H, 6.95; N, 1.52. Found: C, 58.54; H, 6.82; N, 1.44.

3.12. 2-(Tetradecyl)hexadecyl *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-[(α-L-fucopyranosyl)-(1→3)]-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside 16

To a solution of **15** (63.8 mg, 23.0 μmol) in MeOH (3 mL) and THF (1 mL) was added a catalytic amount of sodium methoxide, and the mixture was stirred for 40 h at room temperature, then water (0.1 mL) was added. After completion of the reaction (24 h), the mixture was 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 (5:4:0.7 $CHCl_3$–CH_3OH–H_2O) of the residue on Sephadex LH-20 (40 g) gave **16** (37 mg, 92%) as an amorphous mass: $[\alpha]_D$ = +16.3 (*c* 0.7, 2:3 $CHCl_3$–CH_3OH); ^1H NMR (CD_3OD): δ 0.89 (t, 6H, J = 6.8 Hz, $2CH_3$), 1.16 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6g), 1.23–1.38 (m, 53H, $26CH_2$ and CH), 1.71 (t, 1H, $J_{gem} = J_{3ax,4}$ = 11.9 Hz, H-3f*ax*), 1.96, 1.99, 2.01 (3s, 9H, 3AcN), 2.88 (dd, 1H, $J_{3eq,4}$ = 3.2 Hz, H-3f*eq*), 4.04 (dd, 1H, $J_{2,3}$ = 9.8, $J_{3,4}$ = 2.9 Hz, H-3e) 4.33 (dd, 1H, $J_{1,2}$ = 3.4, $J_{2,3}$ = 10.9 Hz, H-2c), 4.26, 4.43, 4.49, 4.59 (4d, 4H, $J_{1,2}$ = 7.7–8.0 Hz, four β-anomeric-H), 4.94 (d, 1H, H-1c), 5.05 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1g). FAB-MS (negative ion mode, triethanolamine matrix) *m/z*: 1767.1 $[M-H]^-$ ($C_{81}H_{144}N_3O_{38}$ MW, Exact 1766.9428, Ave. 1768.0317), 1476.0 $[M-H-NeuAc]^-$, 1313.9 [1476.0–Gal]$^-$, 964.7 [1313.9–GlcNAc–Fuc]$^-$, 761.6 [964.7–GalNAc]$^-$.

4. Reactivity of antibodies

Reactivity of antibodies was measured by ELISA, which was performed using glycolipid antigens immobilized at the bottom of 96-well culture plates by a standard method described previously.[18] Peroxidase-conjugated goat anti-mouse IgM (μ-chain specific) was obtained from Cappel Inc. (Malvern, PA).

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References

1. Yamaguchi, M.; Ishida, H.; Kanamori, A.; Kannagi, R.; Kiso, M. *J. Carbohydr. Chem.* **2004**, *23*, 201–215.
2. Hakomori, S. *Cancer Res.* **1996**, *56*, 5309–5318.
3. Kannagi, R. *Glycoconjugate J.* **1997**, *14*, 577–584.
4. Hirohashi, S.; Watanabe, M.; Shimosato, Y.; Sekine, T. *Gann* **1984**, *75*, 485–488.
5. Kameyama, A.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1991**, *209*, c1–c4.
6. Kameyama, A.; Ishida, H.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1991**, *10*, 549–560.
7. Otsubo, N.; Ishida, H.; Kiso, M. *Aust. J. Chem.* **2002**, *55*, 105–112.
8. Kameyama, A.; Ehara, T.; Yamada, Y.; Ishida, H.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1995**, *14*, 507–523.
9. Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331–1334.
10. Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313–4316.
11. Hasegawa, A.; Suzuki, N.; Ishida, H.; Kiso, M. *J. Carbohydr. Chem.* **1996**, *15*, 623–637.
12. Nilsson, M.; Norberg, T. *J. Carbohydr. Chem.* **1990**, *9*, 1–14.
13. Komba, S.; Ishida, H.; Kiso, M.; Hasegawa, A. *Bioorg. Med. Chem.* **1996**, *4*, 1833–1847.
14. Jansson, K.; Ahlfors, S.; Frejd, T.; Kihlberg, J.; Magnusson, G.; Dahmen, J.; Noori, G.; Stenvall, K. *J. Org. Chem.* **1988**, *53*, 5629–5647.
15. Numata, M.; Sugimoto, M.; Koike, K.; Ogawa, T. *Carbohydr. Res.* **1987**, *163*, 209–225.
16. Kumamoto, K.; Mitsuoka, C.; Izawa, M.; Kimura, N.; Otsubo, N.; Ishida, H.; Kiso, M.; Yamada, T.; Hirohashi, S.; Kannagi, R. *Biochem. Biophys. Res. Commun.* **1998**, *247*, 514–517.
17. Mitsuoka, C.; Sawada-Kasugai, M.; Ando-Furui, K.; Izawa, M.; Nakanishi, H.; Nakamura, S.; Ishida, H.; Kiso, M.; Kannagi, R. *J. Biol. Chem.* **1998**, *273*, 11225–11233.
18. Hakomori, S.; Kannagi, R. In *Handbook of Experimental Immunology*; Weir, D. M., Herzenberg, L., Blackwell, C., Herzenberg, L. A., Eds.; Immunochemistry; Blackwell: Boston, 1986; Vol. 1, pp 9.1–9.39.