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Design and synthesis of versatile ganglioside probes for carbohydrate microarrays

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Abstract A series of ganglioside GM1-, GM2-, and GM3-type probes, in which the ceramide portion is replaced with a glucose residue, were systematically synthesized based on a convergent synthetic method.

Keywords Chemical synthesis · Gangliosides · Glycosylation · Carbohydrate probe

Introduction

Gangliosides, anionic glycosphingolipids with various sugar chains containing one or more residues of sialic acid, exist universally on cell surface. They participate in vital processes, such as immune or nervous systems, as molecules responsible for cell–cell and cell–ligand interactions [1, 2]. In particular, a series of gangliosides, such as GM1, GM2 and GM3, are important as regulatory factors for the differentiation of the central nervous system and serve as cell-attachment receptors for some viruses, bacteria and bacterial toxins [3, 4]. Moreover, many profound relationships between those gangliosides and a number of cancers and diseases have been demonstrated [5, 6]. However, the biological functions of gangliosides are not fully understood, due to their structural complexities and the low affinities of interaction with ligands, despite numerous studies conducted to date. To solve these issues, a considerable number of efforts have gone into the development of analytical techniques for sensitive detection of carbohydrate–ligand interactions. Consequently, many carbohydrate microarray technologies have been developed to facilitate glycomics research [7]. Coincidentally, many carbohydrate probes that incorporate specific functional groups such as azide [8], thiol [9] and maleimide [10] have been chemically synthesized for the fabrication of microarrays. Recently, oligosaccharide-immobilized chips (named Sugar_Chips), which provide real-time and high-throughput analysis of oligosaccharide–protein interaction without any labeling of the targeted protein, have been developed [11], in which chemically synthesized oligosaccharides having D-glucose, which provides a reactive aldehyde functionality, at the reducing end were used. The D-glucose residue also serves as a spacer between a targeted sugar chain and a scaffold for immobilization, because of its appropriate hydrophilicity and flexibility. Furthermore, it has been demonstrated that a reducing sugar directly participates in the noncovalent link to a scaffold [12, 13]. Accordingly, as exemplified in Fig. 1, the chemically synthesized oligo-

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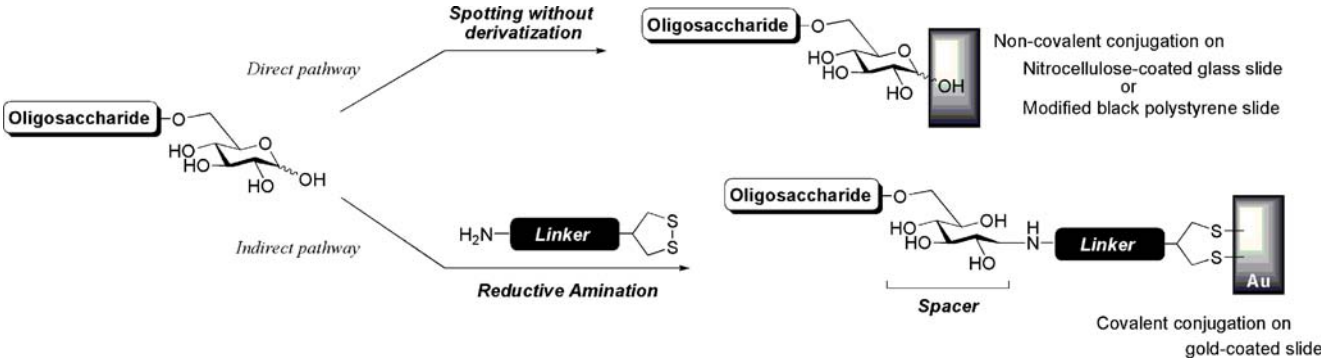


Fig. 1 Two examples for carbohydrate microarray fabrication

saccharide probes are expected to be immobilized by the direct and indirect attachment to scaffolds. We report here the facile synthesis of glucose-ended probes of ganglioside GM1, GM2, and GM3 for carbohydrate microarrays (Fig. 2).

Results and discussion

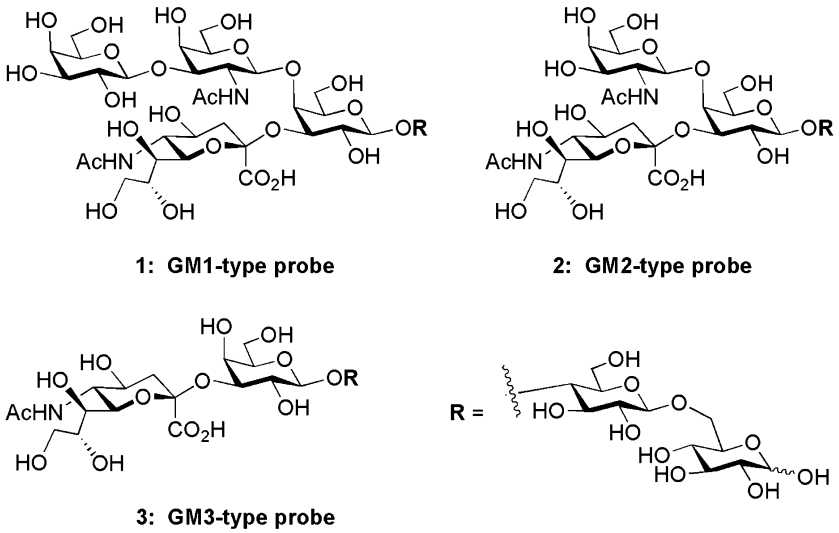
Taking a look at target molecules, we have hypothetically disconnected them into two parts: common sequence SAα(2→3)Galβ(1→4)Glcβ(1→6)Glc, and the other sugar parts. The common sequence was further disconnected at Galβ(1→4)Glc linkage, providing SAα(2→3)Gal and gentiobiose segments, based on the recently reported efficient syntheses of GM2 analogs [14]. Considering the difficulty to fashion a branch out from galactose residue, the incorporation of GalN parts into Gal residue was planned to be conducted earlier than that of gentiobiose as depicted in Fig. 3.

According to our previous report [14], 2,6-*O*-dibenzylated galactoside was efficiently sialylated at C-3 position with *N*-Troc-protected sialyl donor [15, 16], producing a key sialyl galactoside **4**, which can be obtained in a crystalline form after rough chromatographic purification of the reaction mixture (Fig. 4).

The disaccharide **4** was coupled with Galβ(1→3)GalN **6** [17] or GalN donor **5** in the presence of NIS and TfOH [18] to afford the GM2-core trisaccharide **7** in 97% yield and the GM1-core tetrasaccharide **8** in 89% yield, respectively, as depicted in Table 1.

A series of ganglioside-core frames **4**, **7**, and **8** were converted into the corresponding glycosyl donors **13**, **14**, and **15**, respectively. The selective removal of the Troc group of **4** by the action of zinc–copper couple [19, 20] in acetic acid/1,2-dichloroethane at 40°C proceeded smoothly to give a free amino derivative, which, on successive treatment with acetic anhydride in pyridine afforded the corresponding *N*-acetyl derivative **9**. The use of 1,2-dichloroethane (DCE) was critical for an efficient reduction of Troc group; otherwise the reaction was sluggish. Initially, we were afraid that DCE as solvent itself consumes zinc–copper couple as reductant. Though it is not clear whether DCE is advantageous for electron transfer from zinc–copper couple, we were intriguingly able to observe smooth proceeding of the reaction in a single liquid

Fig. 2 Structure of synthetic ganglioside probes



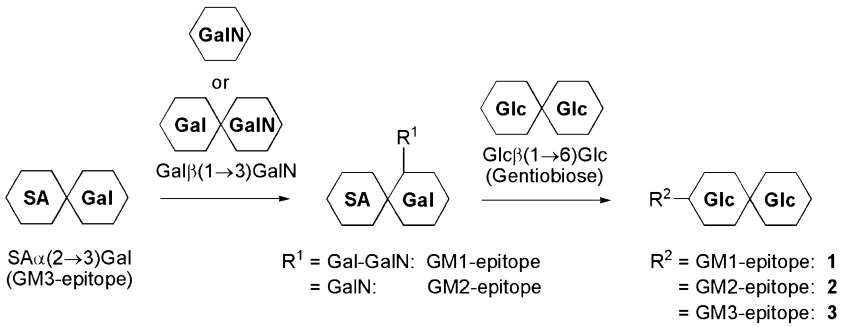


Fig. 3 Systematic reaction scheme for preparation of the reductive glucose-functionalized ganglioside probes

phase within a short time. The cleavage of benzyl groups was executed by hydrogenolysis and the following benzoylation of the resulting hydroxyl groups gave **11**. Libration of the anomeric hydroxyl group of **11** was achieved by treatment with ceric ammonium nitrate (CAN) in acetonitrile–toluene–water (6:5:3) [21]. The obtained hemiacetal was then converted into the β-trichloroacetimidate **13**, which was ready for the final glycosylation with the gentiobiose acceptor **21** as mentioned hereinafter. Interestingly, the use of less than a stoichiometric amount of DBU resulted in the predominant formation of the β-imidate derivative. The conversion of **7** and **8** into the corresponding donor **14** and **15** were also achieved by similar procedure, respectively. (Scheme 1)

Scheme 2 shows the preparation of the gentiobiose acceptor **21** as the common synthetic block, which was expected to have an enhanced reactivity at C-4 hydroxyl due to the effect of electron-donating benzyl groups. Coupling of the known glucose donor **16** [22] and acceptor **17** [23] was conducted in the presence of NIS and TfOH in CH_2Cl_2 at 0°C to give the disaccharide **18** in 90% yield. The β-configuration of the newly formed intersaccharide linkage between **16** and **17** is apparent from the relatively large coupling constant (8.2 Hz) between H-1′ and H-2′ in ^{1}H NMR spectra. Removal of the benzoyl groups under conventional conditions and benzylation of the hydroxyl groups gave **20** with a yield of 88% in two steps. Finally, reductive opening of the benzylidene group was achieved by a treatment with triethylsilane and $BF_3{\cdot}OEt_2$ in CH_2Cl_2 [24] to afford **21** with a yield of 85%.

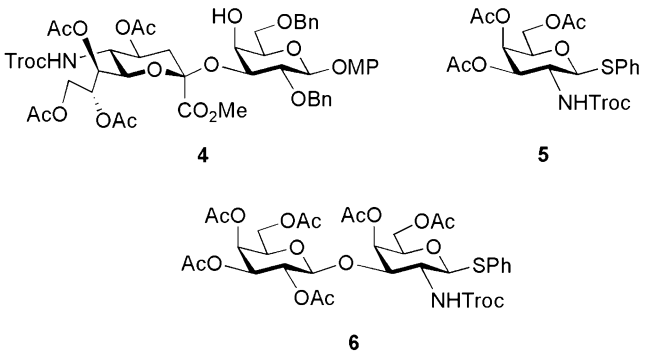


Fig. 4 Structure of glycosyl acceptor (**4**) and donors (**5**, **6**)

Scheme 3 incorporates final glycosylations of **21** with a series of ganglioside-core donors, **13**, **14**, and **15** in the presence of TMSOTf in CH_2Cl_2 at 0°C. The β-imidate **13** was coupled with the gentiobiose acceptor **21** by treatment with TMSOTf at 0°C to afford the desired β-glycoside **22** in an excellent yield. The α-imidate **14** and **15** were subjected to the glycosidation with **21** under essentially the same conditions for **13** to give **23** and **24** in good yields, respectively. Finally, global deprotection of the above-mentioned glycans was conducted. After de-acylation under Zemplén conditions and subsequent saponification of the fully protected oligosaccharides, **24**, **23**, and **22**, hydrogenolysis for each resultant compound was performed in the presence of $Pd(OH)_2/C$ under H_2 atmosphere to afford the target carbohydrate probes **1**, **2** and **3** in good to excellent yields, respectively.

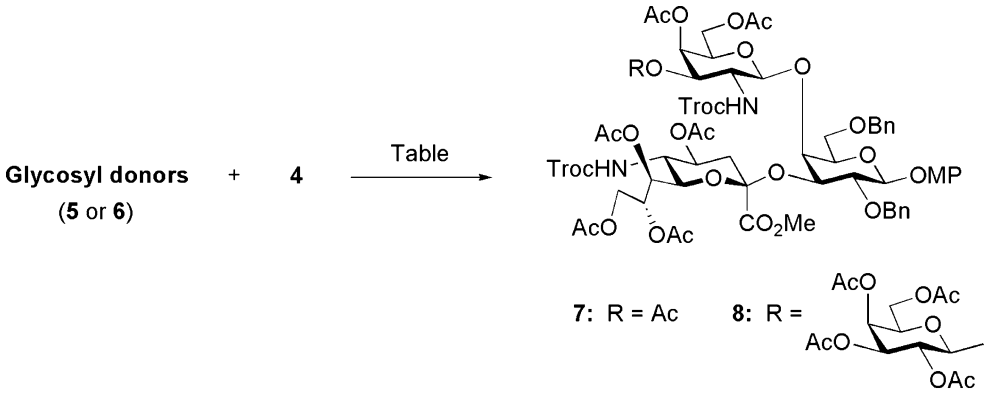
In conclusion, we have succeeded in the synthesis of ganglioside GM1-, GM2-, and GM3-type probes for carbohydrate microarray analyses. It was found that the convergent synthetic strategy between the defined ganglioside-core frame and the reducing end glucose can be used for the synthesis of complex ganglioside probes. In addition, synthesized ganglioside probes are currently used as one of the oligosaccharide probes on immobilized-chips by Suda's group. We are currently underway to expand the existing pool of functional carbohydrate probes containing more complex gangliosides.

Experimental

General procedures

All reactions were carried out under a positive pressure of argon, unless otherwise noted. All chemicals were purchased from commercial suppliers and used without further purification, unless otherwise noted. Molecular sieves were purchased from Wako Chemicals Inc. and dried at 300°C for 2 h in muffle furnace prior to use. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian Inova 400/500 spectrometer and a JEOL ECA 500/600 spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Data are presented as

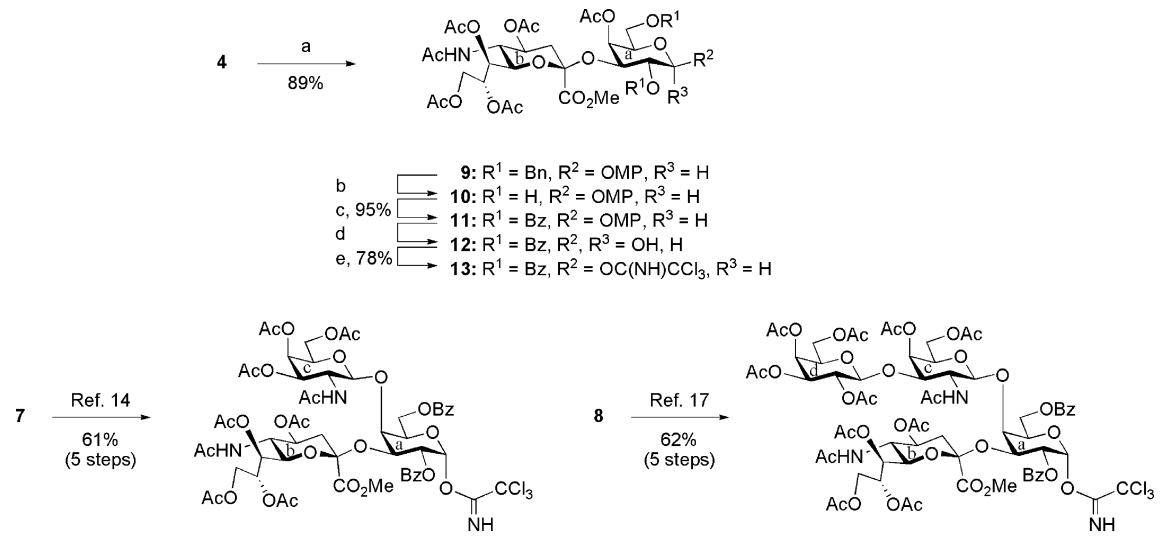
Table 1 Glycosylation of **4** with glycosyl donors **5** and **6**



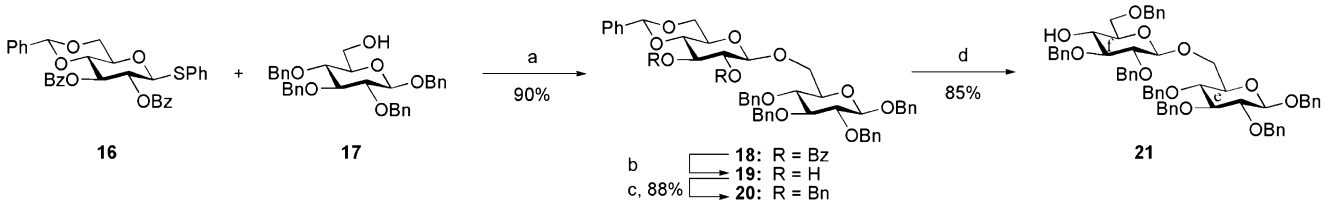
Entry	Donor	Condition	Temp.[°C]	Product	% Yield
1	**5**	NIS TfOH MS4Å CH_2Cl_2	0	**7**	97
2	**6**		-40	**8**	89

follows: Chemical shift, multiplicity (s=singlet, d=doublet, t=triplet, dd=double of doublet, m=multiplet and/or multiple resonances), integration, coupling constant in Hertz (Hz). MALDI-TOF MS spectra were recorded in the positive ion mode on a Bruker Autoflex with the use of α-cyano-4-hydroxy-cinnamic acid (CHCA) as a matrix. Optical rotations were measured with a 'Horiba SEPA-300' polarimeter. Column chromatography was performed on silica gel (Fuji Silysia Co., 80 and 300 mesh). Reactions were monitored by TLC on silica gel $60F_{254}$ (Merck, glass plate) and the compounds were detected by examination under UV light (2,536 Å) and visualized by dipping the plates in a 10% sulfuric acid–ethanol solution or 20% phosphomolybdic acid–ethanol solution followed by heating. Organic solutions were concentrated by rotary evaporation below 45°C under reduced pressure. Solvent systems in chromatography were specified in *v/v*.

4-Methoxyphenyl {methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate-(2→3)}-4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranoside (**9**) To a solution of compound **4** (500 mg, 465 μmol) in 1,2-dichloroethane (6.1 ml) were added acetic acid (18.3 ml) and zinc–copper couple (2.50 g). The mixture was stirred for 1.5 h at 40°C, as the proceeding of the reaction was monitored by TLC ($CHCl_3$/MeOH=15:1). The



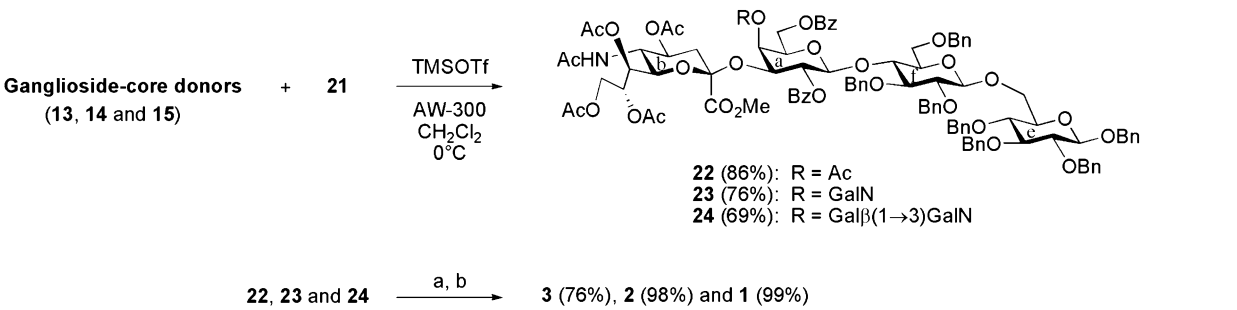
Scheme 1 Conversion of ganglioside-core frames to the corresponding glycosyl donors. Reagents and conditions: *a* Zn-Cu, AcOH, 1,2-DCE, 40°C then Ac_2O, Py; *b* $Pd(OH)_2/C$, H_2, EtOH; *c* Bz_2O, Py; *d* CAN, CH_3CN-PhMe-H_2O (6/5/3); *e* CCl_3CN, DBU, CH_2Cl_2, 0°C



Scheme 2 Preparation of the gentiobiosyl acceptor **21**. Reagents and conditions: *a* NIS, TfOH, MS4Å, CH_2Cl_2, 0°C; *b* NaOMe, MeOH-THF (2/1); *c* BnBr, NaH, DMF; *d* TESH, $BF_3{\cdot}OEt_2$, CH_2Cl_2

reaction mixture was filtered through Celite. The combined filtrate and washings was extracted with $CHCl_3$, and the organic layer was washed with H_2O, sat. Na_2CO_3, and brine, dried over Na_2SO_4 and concentrated. To a solution of the residue in pyridine (5.0 ml) was added acetic anhydride (2.5 ml). The mixture was stirred for 13 h at ambient temperature, as the proceeding of the reaction was monitored by TLC ($CHCl_3$/MeOH=15:1). The reaction mixture was coevaporated with toluene and extracted with $CHCl_3$. The organic layer was washed with 2 M HCl, H_2O, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. The residue was purified with column chromatography on silica gel (EtOAc/hexane=3:1) to give **9** (406 mg, 89%).; $[\alpha]_D$=−15.4° (*c* 0.9, $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.45–6.77 (m, 14 H, 2 Ph and 1 MP), 5.53 (m, 1 H, H-8b), 5.33 (dd, 1 H, H-7b), 5.24 (d, 1 H, $J_{5,NH}$=8.9 Hz, NH), 5.07 (m, 2 H, H-1a, 4a), 4.96–4.88 (m, 3 H, H-4b, 2 CH*H*Ph), 4.63 (dd, 1 H, H-3a), 4.53 (d, 1 H, CH*H*Ph), 4.46 (d, 1 H, CH*H*Ph) 4.36 (dd, 1 H, H-9'b), 4.13 (q, 1 H, $J_{5,NH}$= 8.9 Hz, H-5b), 3.96–3.94 (m, 2 H, H-6′a, 9b), 3.85 (s, 3 H, OMe), 3.76–3.73 (m, 5 H, H-2a, 6b, OMe), 3.56–3.52 (m, 2 H, H-5a, 6a), 2.63 (dd, 1 H, H-3b$_{eq}$), 2.12–1.83 (m, 19 H,6 Ac, H-3b$_{ax}$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.9, 170.6, 170.3, 170.2, 170.0, 168.1, 155.1, 151.7, 139.4, 138.0, 128.3, 128.1, 127.7, 127.6, 127.1, 118.2, 114.4, 102.4, 97.1, 78.1, 74.8, 73.5, 73.1, 72.3, 72.2, 69.5, 68.9, 68.7, 68.6, 67.2, 62.2, 55.6, 53.1, 49.2, 37.6, 23.2, 21.3, 20.8, 20.8, 20.5; MALDI MS: *m/z*: calcd for $C_{49}H_{59}O_{20}NNa$: 1,004.35; found: 1,004.35 $[M + Na]^+$.

*4-Methoxyphenyl {methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate-(2→3)}-4-O-acetyl-2,6-di-O-benzoyl-β-D-galactopyranoside (***11***)* To a solution of compound **9** (385 mg, 392 μmol) in EtOH (30 ml) was added palladium hydroxide [$Pd(OH)_2$] (20 wt% Pd on carbon; 400 mg). The mixture was vigorously stirred for 4 h at ambient temperature under hydrogen atmosphere, as the proceeding of the reaction was monitored by TLC ($CHCl_3$/MeOH=15:1). The reaction mixture was filtered through Celite. The combined filtrate and washings was concentrated. To a solution of the residue in pyridine (5.0 ml) was added benzoic anhydride (354 mg, 1.57 mmol). The mixture was stirred for 16 h at ambient temperature, as the proceeding of the reaction was monitored by TLC ($CHCl_3$/MeOH=15:1). The reaction mixture was coevaporated with toluene and extracted with $CHCl_3$. The organic layer was washed with 2 M HCl, H_2O, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. The residue was purified with column chromatography on silica gel (EtOAc/hexane=3:1) to give **11** (380 mg, 95%); $[\alpha]_D$=+ 27.9° (*c* 4.2, $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 8.17–6.67 (m, 14 H, 2 Ph and 1 MP), 5.59 (m, 1 H, H-8b), 5.55 (t, 1 H, $J_{1,2}$=8.3 Hz, $J_{2,3}$=10.3 Hz, H-2a), 5.26 (d, 1 H, $J_{1,2}$=8.3 Hz, H-1a), 5.20 (dd, 1 H, $J_{6,7}$=2.8 Hz, H-7b), 5.16 (d, 1 H, $J_{3,4}$=3.4 Hz, H-4a), 5.14 (d, 1 H, NH), 4.87 (dd, 1 H, $J_{2,3}$=10.3 Hz, $J_{3,4}$=3.4 Hz, H-3a), 4.85 (m, 1 H, H-4b), 4.46 (t, 1 H, H-6′a), 4.35 (dd, 1 H, H-6a), 4.27 (dd, 1 H, H-9'b), 4.19 (t, 1 H, H-5b), 3.91 (dd, 1 H, H-9b), 3.86–3.79 (m, 4 H, H-5b, OMe) 3.71 (s, 3 H, OMe), 3.61 (dd, 1 H, $J_{6,7}$=2.8 Hz,



Scheme 3 Coupling of the ganglioside-core donors (**13**, **14** and **15**) and the gentiobioside acceptor (**21**), and subsequent global deprotections. Reagents and conditions: *a* NaOMe, MeOH, 45°C or reflux, then H_2O; (b) $Pd(OH)_2$/C, H_2, H_2O or MeOH-H_2O (5/2), RT or 40°C

H-6b), 2.59 (dd, 1 H, H-3b$_{eq}$), 2.19–1.44 (m, 19 H, 6 Ac, H-3b$_{ax}$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.7, 170.6, 170.3, 170.2, 170.0, 168.0, 165.7, 165.3, 155.4, 151.3, 133.2, 133.0, 130.1, 130.0, 129.7, 128.3, 128.3, 118.9, 114.2, 101.1, 96.7, 71.6, 71.1, 70.8, 69.3, 67.6, 67.4, 66.4, 62.3, 62.0, 55.4, 53.0, 48.7, 37.2, 23.2, 21.3, 20.7, 20.1; MALDI MS: *m/z*: calcd for $C_{49}H_{55}O_{22}NNa$: 1,032.31; found: 1,032.38 [M + Na] $^+$.

*{Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate-(2→3)}-4-O-acetyl-2,6-di-O-benzoyl-β-D-galactopyranosyl Trichloroacetimidate (***13***)* To a solution of compound **11** (164 mg, 162 μmol) in mixed solvent (MeCN/PhMe/H_2O= 3.5:2.9:1.7 ml) was added diammonium cerium(IV) nitrate (CAN; 445 mg, 812 μmol). The mixture was stirred for 5 h at ambient temperature, as the proceeding of the reaction was monitored by TLC ($CHCl_3$/MeOH=20:1). The reaction mixture was extracted with $CHCl_3$, and the organic layer was washed with H_2O, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. The residue was purified with column chromatography on silica gel ($CHCl_3$/MeOH= 65:1) to give **12** (147 mg). To a solution of compound 12 in CH_2Cl_2 (5.0 ml) were added trichloroacetonitrile (410 μl, 407 μmol) and 1,8-diazabicyclo[5.4.0]-7-undecene (DBU; 4.9 μl, 33.0 μmol). The mixture was stirred for 2 h at 0°C, as the proceeding of the reaction was monitored by TLC ($CHCl_3$/MeOH=20:1). The reaction mixture was concentrated and the residue was purified with column chromatography on silica gel ($CHCl_3$/MeOH=75:1) to give **13** (132 mg, 78%).; $[\alpha]_D$=+ 18.6° (*c* 0.8, $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 8.67 (s, 1 H, C=NH), 8.10–7.41 (m, 10 H, 2 Ph), 6.20 (d, 1 H, $J_{1,2}$=8.3 Hz, H-1a), 5.60–5.56 (m, 2 H, H-2a, H-8b), 5.22–5.20 (m, 2 H, H-4a, H-7b), 4.98 (d, 1 H, $J_{5,NH}$=10.3 Hz, NH-b), 4.93 (dd, 1 H, H-3a), 4.87 (m, 1 H, H-4b), 4.49 (q, 1 H, H-6'a), 4.34–4.29 (m, 3 H, H-5a, 6a, 9'b), 3.93 (dd, 1 H, H-9b), 3.85–3.77 (m, 4 H, H-5b, OMe), 3.60 (dd, 1 H, H-6b), 2.58 (dd, 1 H, H-3b$_{eq}$), 2.19–1.43 (m, 19 H, 6 Ac, H-3b$_{ax}$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.8, 170.7, 170.6, 170.2, 170.2, 170.0, 168.0, 165.7, 165.1, 161.1, 133.2, 130.1, 129.9, 129.7, 129.7, 128.3, 128.3, 96.8, 96.4, 90.3, 77.2, 71.8, 71.5, 71.1, 70.0, 69.4, 67.6, 67.4, 66.5, 62.4, 61.5, 53.1, 48.8, 37.3, 29.7, 23.1, 21.4, 20.8, 20.7, 20.2; MALDI MS: *m/z*: calcd for $C_{44}H_{49}O_{21}N_2Cl_3Na$: 1,069.18; found: 1,069.41 [M + Na] $^+$.

*Benzyl 2,3-di-O-benzoyl-4,6-O-benzylidene-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (***18***)* To a solution of compound **16** (970 mg, 1.70 mmol) and **17** (762 mg, 1.41 mmol) in CH_2Cl_2 (31 ml) was added molecular sieves 4 Å (1.70 g). The suspension was stirred for 2 h and cooled to 0°C. To the mixture were added *N*-iodosuccinimide (NIS; 765 mg, 3.40 mmol) and trifluoromethanesulfonic acid (TfOH) (30 μl, 0.34 mmol) and stirring was continued for 1.5 h. Completion of the reaction was confirmed by TLC (EtOAc/hexane=1:3). The reaction mixture was filtered through Celite. The combined filtrate and washings was extracted with $CHCl_3$, and the organic layer was washed with sat. Na_2CO_3, sat. $Na_2S_2O_3$ and brine, dried over Na_2SO_4 and concentrated. The residue was purified with column chromatography on silica gel (EtOAc/hexane=1:5) to give **18** (1.26 g, 90%).; $[\alpha]_D$=-9.3° (*c* 1.0, $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.95–7.13 (m, 35 H, 7 Ph), 5.75 (t, 1 H, $J_{2,3}$=8.8 Hz, $J_{3,4}$=8.6 Hz, H-3f), 5.55 (s, 1 H, >C*H*Ph), 5.52 (t, 1 H, $J_{1,2}$=8.2 Hz, $J_{2,3}$=8.8 Hz, H-2f), 4.91–4.86 (m, 3 H, H-1f, 2 CH*H*Ph), 4.77–4.65 (m, 4 H, 4 CH*H*Ph), 4.49–4.39 (m, 4 H, H-1e, 6f, 2 CH*H*Ph), 4.14 (d, 1 H, J_{gem}= 11.0 Hz, H-6e), 3.99 (t, 1 H, $J_{3,4}$=8.6 Hz, $J_{4,5}$=9.6 Hz, H-4f), 3.89 (br t, 1 H, J_{gem}=10.3 Hz, $J_{5,6}$=9.3 Hz, H-6′f), 3.73–3.63 (m, 2 H, H-6′e, 5f), 3.57 (t, 1 H, $J_{2,3}$=8.4 Hz, H-3e), 3.45–3.40 (m, 3 H, H-2e, 4e, 5e); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 165.7, 165.2, 138.6, 138.5, 138.1, 137.5, 137.0, 133.3, 133.2, 129.9, 129.8, 129.5, 129.3, 129.1, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 126.3, 102.5, 101.6, 101.4, 84.7, 82.2, 78.8, 77.8, 75.7, 75.0, 74.9, 74.7, 72.7, 72.3, 71.1, 68.8, 68.4, 67.2, 66.6, 29.8; MALDI MS: *m/z*: calcd for $C_{61}H_{58}O_{13}Na$: 1,021.38; found: 1,021.49 [M + Na] $^+$.

*Benzyl 2,3-di-O-benzyl-4,6-O-benzylidene-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (***20***)* To a solution of compound **18** (1.25 g, 1.25 mmol) in mixed solvent (MeOH/THF=15:7.5 ml) was added sodium methoxide (28% in MeOH; 24 mg). The mixture was stirred for 7.5 h at ambient temperature, as the proceeding of the reaction was monitored by TLC ($CHCl_3$/MeOH= 50:1). The reaction mixture was neutralized with Dowex (H^+) and filtered through cotton. The combined filtrate and washings was concentrated under diminished pressure. To a solution of the residue in DMF (12.5 ml) were added sodium hydride 60% (200 mg, 5.00 mmol) and benzyl bromide (594 μl, 5.00 mmol). The mixture was stirred for 3 h at ambient temperature, as the proceeding of the reaction was monitored by TLC (toluene/EtOAc= 12:1). Triethylamine and ammonium chloride were added to the reaction mixture. The reaction mixture was washed with H_2O and brine, dried over Na_2SO_4 and concentrated. The residue was purified with column chromatography on silica gel (toluene/EtOAc=40:1) to give **20** (1.07 g, 88%).; $[\alpha]_D$=−21.7° (*c* 1.1, $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.49–7.21 (m, 35 H, 7 Ph), 5.56 (s, 1 H, >C*H*Ph), 4.96–4.69 (m, 10 H, 10 CH*H*Ph), 4.59 (d, 1 H, $J_{1,2}$=8.2 Hz, H-1f), 4.54–4.45 (m, 3 H, H-1e, 2 CH*H*Ph), 4.33 (dd, 1 H, J_{gem}=9.3 Hz, $J_{5,6}$=4.8 Hz, H-6f), 4.16 (d, 1 H, J_{gem}=11.0 Hz, H-6e), 3.79–3.72 (m, 2 H, H-6′e, 6′f), 3.68–3.64 (m, 3 H, H-2f, 4f, 5f), 3.57 (t, 1 H, $J_{2,3}$=8.5 Hz, $J_{3,4}$=9.0 Hz, H-3e), 3.50–3.47 (m, 2 H, H-2e, 2f), 3.44 (t,

1 H, $J_{3,4}$=9.6 Hz, $J_{4,5}$=9.6 Hz, H-4e), 3.35 (m, 1 H, H-5e); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 138.6, 138.6, 138.8, 138.1, 137.6, 137.5, 129.1, 128.7, 128.5, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 126.1, 104.3, 102.7, 101.3, 84.8, 82.4, 82.1, 81.6, 81.0, 78.3, 77.3, 75.8, 75.4, 75.2, 75.1, 74.9, 71.3, 68.9, 66.1, 29.8; MALDI MS: *m/z*: calcd for $C_{61}H_{62}O_{11}Na$: 993.42; found: 993.50 [M + Na] $^+$.

*Benzyl 2,3,6-tri-O-benzyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (***21***)* To a solution of compound **20** (82 mg, 84.5 μmol) in CH_2Cl_2 (845 μl) were added triethylsilane (162 μl, 1.01 mmol) and boron trifluoride diethyl etherate (BF_3·OEt_2; 21.4 μl, 169 μmol). The mixture was stirred for 1.5 h at ambient temperature, as the proceeding of the reaction was monitored by TLC (toluene/EtOAc=12:1). The reaction mixture was diluted with $CHCl_3$ and washed with sat. $NaHCO_3$, H_2O and brine, dried over Na_2SO_4 and concentrated. The residue was purified with column chromatography on silica gel (toluene/EtOAc=20:1) to give **21** (70 mg, 85%).; $[\alpha]_D$=−12.9° (*c* 1.0, $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.35–7.21 (m, 35 H, 7 Ph), 5.01–4.69 (m, 10 H, 10 CH*H*Ph), 4.59–4.51 (m, 3 H, H-1f, 2 CH*H*Ph), 4.46 (d, 1 H, $J_{1,2}$=9.6 Hz, H-1e), 4.19 (d, 1 H, J_{gem}=11.0 Hz, H-6e), 3.74–3.58 (m, 6 H, H-6′e, 3f, 4f, 5f, 6f, 6′f), 3.50–3.39 (m, 5 H, H-2f, 2e, 3e, 4e, 5e), 2.54 (s, 1 H, -OH); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 138.9, 138.7, 138.5, 138.5, 138.2, 138.0, 137.6, 128.6, 128.5, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 104.1, 102.7, 84.8, 84.2, 82.4, 81.6, 78.4, 77.3, 75.8, 75.4, 75.3, 75.1, 74.9, 74.8, 74.1, 73.8, 71.8, 71.3, 68.8, 29.8; MALDI MS: *m/z*: calcd for $C_{61}H_{64}O_{11}Na$: 995.43; found: 995.38 [M + Na] $^+$.

*Benzyl {methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate-(2→3)}-4-O-acetyl-2,6-di-O-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (***22***)* To a solution of compound **13** (107 mg, 102 μmol) and **21** (200 mg, 206 μmol) in CH_2Cl_2 (5.0 ml) was added molecular sieves 4 Å (1.00 g). The suspension was stirred for 1 h and cooled to 0°C. To the mixture was added trimethylsilyl trifluoromethanesulfonate (TMSOTf; 3.7 μl, 20 μmol) and stirring was continued for 1 h. Completion of the reaction was confirmed by TLC ($CHCl_3$/MeOH=20:1). The reaction mixture was filtered through Celite. The combined filtrate and washings was extracted with $CHCl_3$, and the organic layer was washed with sat. Na_2CO_3 and brine, dried over Na_2SO_4 and concentrated. The residue was purified with column chromatography on silica gel ($CHCl_3$/MeOH=75:1) to give **22** (170 mg, 86%).; $[\alpha]_D$=+ 2.0° (*c* 0.5, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.24–7.15 (m, 45 H, 9 Ph), 5.66 (m, 1 H, H-8b), 5.31 (t, 1 H, $J_{1,2}$=8.0 Hz, $J_{2,3}$= 9.7 Hz, H-2a), 5.18 (dd, 1 H, H-7b), 5.13 (d, 1 H, $J_{1,2}$=8.0 Hz, H-1a), 5.06 (d, 1 H, $J_{3,4}$=3.4 Hz, H-4a), 4.99 (d, 1 H, CH*H*Ph), 4.92–4.66 (m, 12 H, H-3a, 4b, NH, 9 CH*H*Ph), 4.49–4.36 (m, 7 H, H-6'a, 1e, 1f, 4 CH*H*Ph), 4.29 (d, 1 H, H-9′b), 4.13–3.88 (m, 6 H, H-5a, 6a, 5b, 9b, H-6′ of Glc units), 3.77 (q, 1 H, H-5b), 3.71 (s, 1 H, OMe), 3.67–3.35 (m, 10 H, H-6b, Glc units), 3.22 (m, 1 H, H-5 of Glc units), 2.52 (dd, 1 H, J_{gem}=12.6 Hz, $J_{3eq,4}$=4.6 Hz H-3b$_{eq}$), 2.13–1.43 (m, 19 H, 6 Ac, H-3b$_{ax}$) ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.8, 170.7, 170.3, 170.2, 170.1, 168.0, 165.4, 165.1, 139.1, 138.6, 138.4, 138.0, 137.6, 133.3, 133.0, 130.3, 130.0, 129.8, 129.7, 128.6, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 127.9, 127.9, 127.7, 127.6, 127.4, 127.3, 127.2, 127.1, 103.7, 102.7, 100.4, 96.9, 84.7, 82.9, 82.3, 81.6, 78.2, 76.3, 75.7, 75.2, 75.1, 74.9, 74.8, 74.8, 74.4, 72.8, 71.7, 71.5, 71.2, 70.4, 69.4, 69.0, 68.5, 67.4, 67.0, 66.5, 62.5, 61.2, 53.0, 48.8, 37.3, 29.7, 23.2, 21.3, 20.8, 20.7, 20.7, 20.3; MALDI MS: *m/z*: calcd for $C_{103}H_{111}O_{31}NNa$: 1,880.70; found: 1,880.96 [M + Na] $^+$.

*Benzyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl-(1→4)-{methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate-(2→3)}-2,6-di-O-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (***23***)* To a solution of compound **14** (58 mg, 43 μmol) and **21** (84 mg, 86 μmol) in CH_2Cl_2 (2.0 ml) was added molecular sieves 4 Å (165 mg). The suspension was stirred for 1 h at ambient temperature and cooled to 0°C. To the mixture was added TMSOTf (1.6 μL, 8.6 μmol) and stirring was continued for 3.5 h. Completion of the reaction was confirmed by TLC ($CHCl_3$/MeOH=15:1). Triethylamine was then added to quench the reaction. The reaction mixture was filtered through Celite. The combined filtrate and washings was extracted with $CHCl_3$, and the organic layer was washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. The residue was purified with column chromatography on silica gel ($CHCl_3$/MeOH=50:1) to give **23** (70 mg, 76%).; $[\alpha]_D$=−11.0° (*c* 0.76, $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 8.01–7.15 (m, 45 H, 9 Ph), 5.97 (d, 1 H, NH-c), 5.49 (dd, 1 H, $J_{3,4}$=2.7 Hz, H-3c), 5.40 (m, 1 H, H-8b), 5.37 (d, 1 H, $J_{3,4}$=2.7 Hz, H-4c), 5.34 (t, 1 H, $J_{1,2}$= 10.2 Hz, H-2a), 5.25 (d, 1 H, H-7b), 5.14 (br d, 1 H, NH-b), 5.06 (d, 1 H, $J_{1,2}$=8.9 Hz, H-1c), 5.00 (dt, 1 H, $J_{3eq,4}$= 4.8 Hz, H-4b), 4.95–4.88 (m, 4 H, 4 CH*H*Ph), 4.82–4.80 (m, 3 H, 3 CH*H*Ph), 4.79 (d, 1 H, $J_{1,2}$=10.2 Hz, H-1a), 4.72 (t, 2 H, 2 CH*H*Ph), 4.67 (d, 1 H, CH*H*Ph), 4.62 (q, 1 H, H-6c), 4.51 (d, 1 H, CH*H*Ph), 4.47 (d, 1 H, CH*H*Ph), 4.43 (d, 1 H, CH*H*Ph), 4.40 (d, 1 H, $J_{1,2}$=8.2 Hz, H-1f), 4.37 (d, 1 H, $J_{1,2}$= 8.2 Hz, H-1e), 4.28 (d, 1 H, CH*H*Ph), 4.19 (t, 1 H, H-5a), 4.15–3.96 (m, 10 H, H-3a, 4a, 6a, 6'a, 5b, 9b, 9'b, 2c, 6'c, 5e), 3.95 (t, 1 H, H-4f), 3.83–3.81 (m, 4 H, OMe, H-6b), 3.64

(t, 1 H, H-5c), 3.63–3.59 (m, 2 H, H-3e, 6e), 3.53 (t, 1 H, H-6'e), 3.50–3.49 (m, 2 H, H-6f, 6'f), 3.47 (t, 1 H, H-3f), 3.44 (t, 1 H, H-4e), 3.39 (t, 1 H, H-2f), 3.36 (t, 1 H, H-2e), 3.14 (m, 1 H, H-5f), 2.22 (dd, 1 H, J_{gem}=13.7 Hz, $J_{3eq,4}$=4.8 Hz, H-3b$_{eq}$), 1.93 (t, 1 H, H-3b$_{ax}$), 2.19–1.75 (9 s, 27 H, 9 Ac); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.7, 170.4, 170.3, 170.2, 169.7, 169.6, 168.0, 165.8, 164.1, 138.8, 138.6, 138.5, 138.4, 138.3, 137.9, 137.5, 133.2, 133.1, 129.9, 129.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128.0, 127.8, 127.8, 127.6, 127.5, 127.5, 127.3, 127.0, 103.7, 102.6, 101.1, 100.0, 98.6, 84.6, 82.6, 82.2, 81.6, 78.2, 77.1, 76.2, 76.2, 75.6, 75.1, 74.8, 74.7, 74.3, 73.9, 73.1, 72.0, 72.0, 71.2, 71.1, 70.3, 70.0, 68.9, 68.3, 68.2, 67.2, 67.1, 66.3, 63.3, 62.1, 61.4, 53.1, 51.5, 49.1, 35.8, 29.6, 23.2, 23.1, 21.0, 20.8, 20.7, 20.7, 20.5, 20.4, 20.3; MALDI MS: *m*/*z*: calcd for $C_{115}H_{128}N_2O_{38}Na$: 2,167.80; found: 2,167.91 $[M + Na]^+$.

*Benzyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4,6-di-O-acetyl-2-deoxy-β-D-galactopyranosyl-(1→4)-{methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate-(2→3)}-2,6-di-O-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (***24***)* To a solution of compound **15** (105 mg, 64.5 μmol) and **21** (137 mg, 129 μmol) in CH_2Cl_2 (1.9 ml) was added molecular sieves 4 Å (300 mg). The suspension was stirred for 30 min and cooled to 0°C. To the mixture was added TMSOTf (1.2 μl, 6.5 μmol) and stirring was continued for 45 min. Completion of the reaction was confirmed by TLC (toluene/EtOAc=7:1). The reaction mixture was filtered through Celite. The combined filtrate and washings was extracted with $CHCl_3$, and the organic layer was washed with sat. Na_2CO_3 and brine, dried over Na_2SO_4 and concentrated. The residue was purified with column chromatography on silica gel ($CHCl_3$/MeOH=200:3) to give **24** (110 mg, 69%).; $[\alpha]_D$=+ 0.0° (*c* 0.8, $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 8.18–7.09 (m, 45 H, 9 Ph), 5.88 (d, 1 H, $J_{5,NH}$=6.3 Hz, NH-c), 5.66 (m, 1 H, H-8b), 5.38–5.31 (m, 3 H, H-2a, 4c, 4d), 5.19 (dd, 1 H, $J_{6,7}$= 2.3 Hz, $J_{7,8}$=9.7 Hz, H-7b), 5.15 (d, 1 H, $J_{1,2}$=8.0 Hz, H-1c), 5.11–5.07 (m, 2 H, H-3a, 2d), 5.01–4.58 (m, 17 H, H-6a, 4b, 3c, 1d, 3d, 1f, NH-b, 10 CH*H*Ph), 4.48–4.37 (m, 5 H, $J_{1,2}$=7.4 Hz, H-1a, $J_{1,2}$=8.1 Hz, H-1e, 6e, 2 CH*H*Ph), 4.29–4.22 (m, 3 H, H-9b, 2 CH*H*Ph), 4.12–3.25 (m, 28 H, H-4a, 5a, 6a, 6′a, 5b, 6b, 9′b, 2c, 5c, 6c, 6′c, 5d, 6d, 6′d, 2e, 3e, 4e, 5e, 6′e, 2f, 3f, 4f, 5f, 6f, 6′f, -OMe), 2.73 (dd, 1 H, J_{gem}= 12.6 Hz, $J_{3eq,4}$=4.3 Hz, H-3b$_{eq}$), 2.19–1.49 (m, 37 H, H-3b$_{ax}$, 12 Ac); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 172.1, 170.9, 170.7, 170.5, 170.3, 170.2, 170.2, 170.0, 169.3, 168.4, 165.5, 165.1, 138.9, 138.6, 138.6, 138.5, 138.1, 137.6, 133.4, 133.1, 130.3, 130.1, 130.0, 129.6, 128.7, 128.4, 128.2, 128.0, 128.0, 127.9, 127.8, 127.7, 127.7, 127.6, 127.3, 127.3, 103.8, 102.7, 101.1, 100.7, 99.0, 97.9, 84.7, 83.2, 82.4, 81.8, 78.3, 75.7, 75.2, 75.0, 75.0, 74.8, 74.5, 73.9, 73.6, 72.9, 72.2, 71.9, 71.6, 71.3, 71.0, 70.6, 69.2, 69.1, 69.0, 68.6, 67.1, 66.9, 66.6, 63.2, 62.8, 62.6, 61.0, 55.3, 52.8, 49.3, 36.9, 29.8, 24.0, 23.2, 22.8, 21.4, 20.9, 20.8, 20.8, 20.8, 20.7, 20.7, 20.4, 20.3, MALDI MS: *m*/*z*: calcd for $C_{127}H_{144}N_2O_{46}Na$: 2,455.89; found: 2,455.52 $[M + Na]^+$.

*β-D-Galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-{5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid-(2→3)}-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranose (***1***)* To a solution of compound **24** (95 mg, 39 μmol) in MeOH (1.6 ml) was added sodium methoxide (28% in MeOH; 14 mg). The mixture was stirred for 74 h under reflux condition, as the proceeding of the reaction was monitored by TLC ($CHCl_3$/MeOH/H_2O=3:2:0.3). H_2O (1.6 ml) was then added and stirring was continued for 14 h at ambient temperature. The reaction mixture was neutralized with Dowex (H^+) and filtered through cotton. The combined filtrate and washings was concentrated under diminished pressure to give a syrup compound. To a solution of the residue in H_2O (1.4 ml) was added palladium hydroxide [$Pd(OH)_2$] (20 wt% Pd on carbon; 345 mg). The mixture was vigorously stirred for 4 h at 40°C under hydrogen atmosphere, as the proceeding of the reaction was monitored by TLC (1-BuOH/MeOH/H_2O= 2:1:1). The reaction mixture was filtered through Celite, and the combined filtrate and washings was concentrated. The residue was purified with gel filtration column chromatography (Sephadex LH-20, H_2O as eluent) to give **1** (43 mg, 99%).; $[\alpha]_D$=+ 0.1° (*c* 1.0, H_2O); ^{1}H-NMR (600 MHz, CD_3OD): δ 5.16 (d, 1 H, $J_{1,2}$=3.7 Hz, H-1e), 4.79 (d, 1 H, H-1c), 4.57 (d, 1 H, $J_{1,2}$=8.0 Hz, H-1d), 4.49–4.45 (m, 3 H,H-1a, 1b, 1f), 4.15–3.19 (m, 39 H, ring H), 2.62 (dd, 1 H, H-3b$_{eq}$), 1.99 and 1.96 (2 s, 6 H, 2 Ac), 1.87 (m, 1 H, H-3b$_{ax}$), ^{13}C-NMR (150 MHz, CD_3OD) δ 175.0, 174.8, 174.1, 106.1, 105.7, 105.5, 104.1, 104.0, 103.4, 101.8, 97.0, 95.3, 94.2, 93.0, 91.5, 84.4, 81.2, 78.1, 77.6, 76.5, 75.1, 74.8, 74.5, 74.3, 73.9, 73.5, 73.4, 72.6, 72.1, 71.5, 70.8, 70.2, 68.9, 68.0, 67.1, 61.2, 61.0, 60.7, 59.7, 59.3, 58.8, 52.5, 51.6, 48.8, 47.5, 28.7, 25.9, 23.5; MALDI MS: *m*/*z*: calcd for $C_{43}H_{72}N_2O_{34}$: 1160.40; found: 1159.75 $[M\text{-}H]^-$.

*2-Acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-{5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid-(2→3)}-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-D-glucopyranose (***2***)* To a solution of compound **23** (38 mg, 18 μmol) in MeOH (2.0 ml) was added catalytic amounts of sodium methoxide (10 mg). The mixture was stirred for 96 h under reflux conditions, as

the proceeding of the reaction was monitored by TLC (1-BuOH/MeOH/H_2O=4:1:1). H_2O was then added and stirring was continued for 10 h at ambient temperature. The reaction mixture was neutralized with Dowex (H^+) and filtered through cotton. The combined filtrate and washings was concentrated under diminished pressure to give a syrupy compound. The residue was purified by gel filtration column chromatography on Sephadex LH-20 (MeOH) to give a white solid. To a solution of the solid in MeOH/H_2O (2.5/1 ml) was added palladium hydroxide [$Pd(OH)_2$] (20 wt% Pd on carbon; 40 mg). The mixture was vigorously stirred overnight at 40°C under hydrogen atmosphere, as the proceeding of the reaction was monitored by TLC (1-BuOH/MeOH/H_2O=2:1:1). The reaction mixture was filtered through Celite. The combined filtrate and washings was concentrated. The residue was purified with gel filtration column chromatography (Sephadex LH-20, MeOH/H_2O=1:1 as eluent) using MeOH as eluent, to give **2** (18 mg, 98%).; $[\alpha]_D$=+19.4° (*c* 1.7, MeOH:H_2O=1:1); ^{1}H-NMR (500 MHz, CD_3OD/D_2O=1:1): δ 2.69 (dd, 1 H, J_{gem}=11.4 Hz, $J_{3eq,4}$=4.6 Hz, H-3b_{eq}), 2.04 and 2.02 (2 s, 6 H, 2 NAc), 1.91 (t, 1 H, H-3b_{ax}); ^{13}C-NMR (125 MHz, CD_3OD/D_2O=1:1) δ 176.0, 175.4, 175.0, 103.9, 103.9, 103.7, 102.9, 97.3, 93.4, 80.0, 78.5, 77.0, 76.1, 75.8, 75.6, 75.4, 75.1, 71.0, 70.8, 69.8, 69.6, 69.5, 69.1, 64.2, 62.3, 61.5, 61.2, 53.5, 53.0, 49.5, 49.4, 48.4, 38.0, 23.6, 22.8; MALDI MS: *m/z*: calcd for $C_{37}H_{61}N_2O_{29}$: 997.33; found: 997.25 [*M*-H]$^-$.

*{5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid-(2→3)}-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-D-glucopyranose (**3**)* To a solution of compound **22** (45 mg, 24 μmol) in MeOH (3.0 ml) was added sodium methoxide (28% in MeOH; 11 mg). The mixture was stirred for 48 h at 45°C, as the proceeding of the reaction was monitored by TLC ($CHCl_3$/MeOH=5:1). H_2O (1.0 ml) was then added and stirring was continued for 18 h at 45°C. The reaction mixture was neutralized with Dowex (H^+) and filtered through cotton. The combined filtrate and washings was concentrated under diminished pressure to give a syrupy compound. To a solution of the residue in H_2O (2.0 ml) was added palladium hydroxide [$Pd(OH)_2$] (20 wt% Pd on carbon; 100 mg). The mixture was stirred for 8 h at ambient temperature under hydrogen atmosphere, as the proceeding of the reaction was monitored by TLC ($CHCl_3$/MeOH/H_2O=3:1:0.1). The reaction mixture was filtered through Celite. The combined filtrate and washings was concentrated. The residue was purified with gel filtration column chromatography (Sephadex LH-20, H_2O as eluent) to give **3** (14 mg, 76%).; $[\alpha]_D$=+ 8.3° (*c* 0.6, H_2O); ^{1}H-NMR (400 MHz, D_2O): δ 5.21 (d, 1 H, $J_{1,2}$=3.7 Hz, H-1e), 4.64–4.51 (m, 2 H, H-1a, 1f), 4.21–2.87 (m, 25 H, ring H), 2.75 (dd, 1 H, J_{gem}=12.0 Hz, $J_{3eq,4}$=4.6 Hz, H-3b_{eq}), 2.02 (s, 3 H, Ac), 1.77 (m, 1 H, H-3b_{ax}), ^{13}C-NMR (100 MHz, D_2O) δ 177.7, 176.6, 105.4, 105.2, 102.5, 98.7, 94.8, 80.9, 78.4, 77.9, 77.6, 77.5, 77.5, 77.0, 76.7, 75.6, 75.5, 75.4, 74.5, 74.1, 73.1, 72.2, 72.1, 71.5, 71.4, 71.1, 70.8, 70.2, 65.3, 65.2, 63.7, 62.7, 57.1, 54.4, 42.4, 24.8, 21.8, 17.7; MALDI MS: *m/z*: calcd for $C_{29}H_{24}NO_{24}$: 795.26; found: 794.24 [*M*-H]$^-$.

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