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Synthesis of the starfish ganglioside LLG-3 tetrasaccharide

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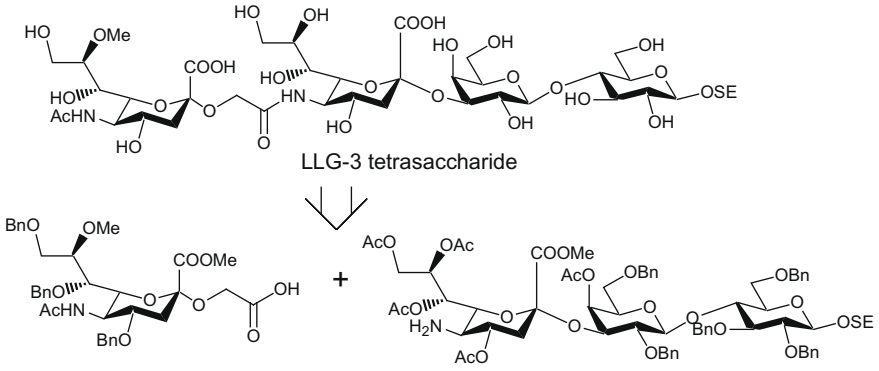
ABSTRACT

The first synthesis of the ganglioside LLG-3 tetrasaccharide, which has attractive biological activities as well as a unique structure, is described. A C8-methoxy decorated sialic acid building block was initially prepared and a glycolic acid moiety was then introduced by sialylation. Amide condensation between the sialyl glycolic acid and an amino group at C5 on the sialyllactoside unit afforded the fully protected LLG-3 tetrasaccharide. Finally, the desired tetrasaccharide part of LLG-3 was obtained after careful global deprotection.



1. Introduction

The structural diversity found in sialic acid and sialyl-glycolipids has great significance in biological systems and metabolic pathways of organisms.[1,2] Sialic acids identified in mammalian gangliosides mainly possess *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc) residues with α-(2→3/6) linkages to adjacent galactose, glucosamine, and galactosamine residues. Echinoderm gangliosides have more modifications of the sialic acid residues and exhibit various attachment linkages between the sialic acids and neighboring sugar residues. In some cases, distinctive sialic acids modified by acetylation and sulfation are known to be significant gateways for viral infections.[3] However, the actions of methylated sialic acids have remained unclear because of their presence in natural sources in small amount.

Another remarkable characteristic of this class of glycoconjugates is the connecting mode around the sialic acid residues.[4] One class of such gangliosides having di/tri-sialic acid residues with an α-(2→11) linkage has been found to possess nerve cell-stimulating and -protecting activities.[4,5] Due to the significant bioactivities of these unique sialic acid linkages, some pioneering studies toward the synthesis of these echinoderm gangliosides have been reported.[6]

The unique ganglioside LLG-3 (Scheme 1), isolated from the starfish *Linckia laevigata*,[5a] has potent nerve cell-stimulating activity for the neuron-like rat adrenal pheochromocytoma (PC-12) cell line through nerve cell growth factors (NGFs), which is of comparable magnitude to the mammalian ganglioside GM1.[5c,7] The distinctive tetrasaccharide sequence of LLG-3 contains a C8-methylated Neu5Ac (Neu8Me5Ac) terminus, which is connected to the *N*-glycolyl hydroxyl group (C11-OH) of the neighboring Neu5Gc residue. Because of its intriguing potent nerve cell-stimulating activities and its unique α-(2→11)-disialyl connection with

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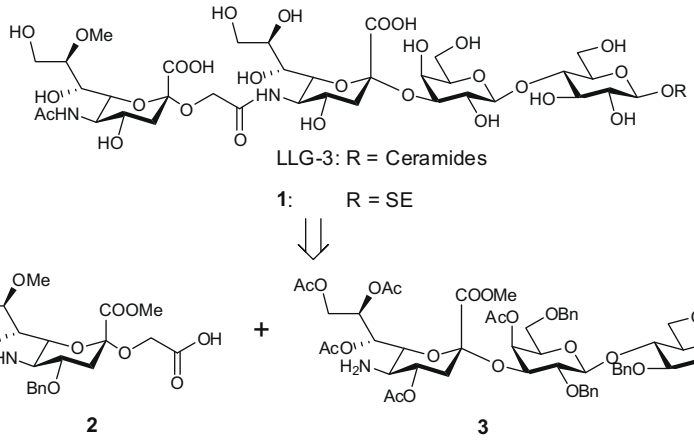
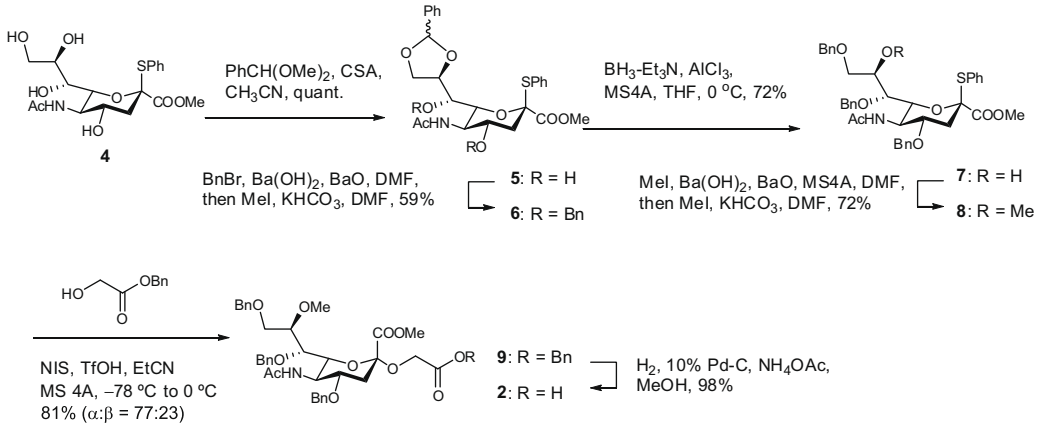


Scheme 1.



Scheme 2.

Neu8Me5Ac capping, we carried out the first synthesis of the LLG-3 tetrasaccharide, **1**.

2. Results and discussion

2.1. Synthetic plan

In our synthetic plan (Scheme 1), tetrasaccharide **1** is constructed by a [1+3] amide condensation between carboxylic acid **2** and the sialyllactoside **3**, which contains an amino group at C5. This approach avoids a poorly selective sialylation reaction later in the synthetic route.[8] To synthesize carboxylic acid unit **2**, the glycolic acid is liberated after introduction of the 8-methoxy sialic acid building block **8** by a glycosylation. The GM3-type trisaccharide **3** is synthesized by sialylation between the reactive *N*-Troc protected sialic acid building block **10** and lactose acceptor **11**,[9] followed by removal of the *N*-Troc group.

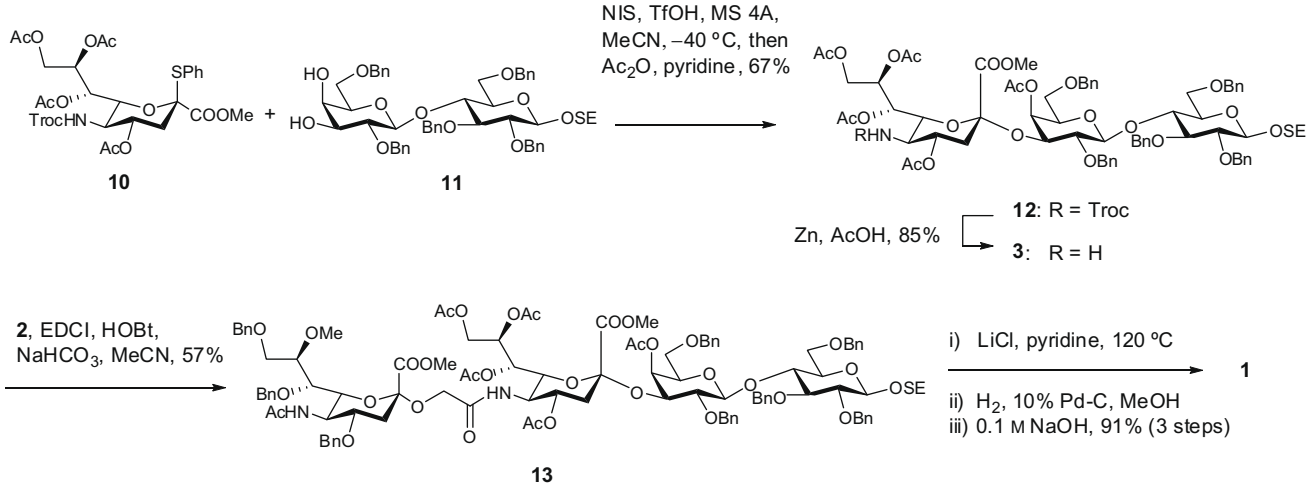
2.2. Synthesis of building block (2)

Synthesis of the Neu8Me5Ac carboxylic acid building block **2** began with β-phenyl thiosialoside **4**[10] (Scheme 2). The C8- and C9-hydroxyl groups were first protected as a benzylidene acetal by treatment with benzaldehyde dimethylacetal and camphorsulfonic acid to give **5** quantitatively. The C4/C7 di-*O*-benzyl intermediate was successfully produced by subsequent treatment with a combination of benzyl bromide, $Ba(OH)_2$ and BaO, conditions that were able to avoid undesirable N-benzylation.[11] Subsequent reprotection of the hydrolyzed carboxylic acid with a methyl group produced **6** in 59% yield. Reductive benzylidene ring opening with $BH_3{\cdot}Me_3N$ and $AlCl_3$ produced **7** in 72% yield.[12] To introduce a methyl group on the liberated C8-hydroxyl group, **7** was treated with MeI, $Ba(OH)_2$, and BaO in the presence of 4 Å molecular sieves; re-esterification produced the desired **8** in 72% yield. The obtained Neu8Me5Ac building block **8** was subjected to sialylation reactions with commercial benzyl glycolate in the presence of NIS and TfOH to afford **9** in 81% yield (α/β = 77/23, α-anomer: $^3J_{C1-H3ax}$ = 4.9 Hz).[13] The desired carboxylic acid **2** was produced by catalytic hydrogenation for **9α** with 10% Pd–C and excess NH_4OAc in 98% yield.[14]

2.3. Synthesis of LLG-3 tetrasaccharide (1)

The synthesis of the target LLG-3 tetrasaccharide **1**, involving formation of the full sequence through stepwise glycan chain elongations as well as subsequent global deprotections, is depicted in Scheme 3. To form the sialyllactoside **12** on a gram scale, initial sialylation between the 1-*O*-trimethylsilylethyl (SE) protected lactose acceptor **11**[15] and the potent *N*-Troc sialic acid building donor **10**,[16] which was activated by NIS–TfOH in CH_3CN, produced an inseparable crude mixture of trisaccharide. This mixture was acetylated[17] to give **12α** (α/β = 6:1, α-anomer: $^3J_{C1-H3ax}$ = 4.1 Hz)[18] in 67% yield. The desired trisaccharide was readily separated by silica gel chromatography after acetylation.

The *N*-Troc group of **12** was reductively removed using Zn powder in AcOH to afford the desired amine **3** in 85% yield. The liber-



Scheme 3.

ated amine **3** was coupled with the carboxylic acid **2** using EDCI, HOBt, and $NaHCO_3$ to provide the fully protected LLG-3 tetrasaccharide **13** in 57% yield.[19] In addition, acetyl migration to liberated amino group of the compound **3** was observed. As a final task, global deprotection of **13** was carefully undertaken to avoid undesired side reactions at the labile glycolyl moiety, which could involve amide migration[19b] and elimination.

To remove the methyl esters of **13** selectively, the tetrasaccharide **13** was treated with excess LiCl in pyridine under reflux conditions. Following acidic treatment readily provided biscarboxylic acid intermediate.[19b,20] All the benzyl protecting groups were reductively removed by hydrogenation with 10% Pd–C. Finally, careful basic treatment with 0.1 M aqueous NaOH for 2 h yielded the fully deprotected tetrasaccharide **1** (91% 3 steps). Purification (desalting) was achieved by direct application of the reaction mixture to a Sephadex G-15 size exclusion column with elution with water.

The structure of tetrasaccharide **1** was totally supported by HR-ESI-MS data and 2D NMR spectra. Direct comparison of the ^{13}C NMR spectra with natural LLG-3 ganglioside in pyridine-d_5, which was reported by Higuchi et al.,[5a] failed because the synthesized tetrasaccharide **1** was completely insoluble in pyridine-d_5. Additionally, a set of ^{1}H and ^{13}C NMR data derived from the terminal disialyl structure showed good agreement with the corresponding synthetic Neu5Ac-α-(2→11)-Neu5Gc spectrum reported by Ren.[20a] The precise 2D NMR spectra of compound **1** in D_2O are given in Supplementary data.

3. Conclusion

The synthesis of the LLG-3 tetrasaccharide **1**, which is terminated with Neu8Me5Ac and contains an α-(2→11) disialyl linkage, was achieved. For the synthesis of the building block **2**, the C8-hydroxyl group was selectively methylated with MeI, BaO, and $Ba(OH)_2$. Subsequent coupling with benzyl glyclolate and selective removal of its benzyl ester produced carboxylic acid **2**. The sialyllactose building block **3** was produced via a sialylation reaction between the known *N*-Troc sialic acid building block **10** and lactose derivative **11**. To form the complete tetrasaccharide **13**, carboxylic acid **2** and amino trisaccharide **3** were condensed under mild basic conditions. Finally, a sequence of global deprotection procedures involving removal of methyl esters, hydrogenolysis of benzyl ethers, and hydrolysis of acetyl and methyl esters provided the desired LLG-3 tetrasaccharide **1** in excellent yield.

4. Experimental procedures

4.1. General procedures

Optical rotations were measured in a 0.5 dm tube with a JASCO P-1020 polarimeter. IR spectra were recorded with Shimadzu Prestege-21 and JASCO FT/IR-4200 spectrometers. ^{1}H and ^{13}C NMR spectra were measured with JEOL ECX-400, ECA-500, and ECA-600 spectrometers and Bruker DRX-600 spectrometer referenced to tetramethylsilane (0.00 ppm). Column chromatography was performed on silica gel (Silica gel 60 N, spherical, neutral, 70–230 mesh, Kanto Kagaku Co.). Thin-layer chromatography (TLC) on Silica gel $60F_{254}$ (Merck) was used to monitor the reactions. High-resolution ESI-TOF mass spectra were measured with JEOL JMS-T100LC AccuTOF mass spectrometer.

4.2. Methyl (phenyl 5-acetamido-8,9-*O*-benzylidene-3,5-dideoxy-2-thio-D-*glycero*-β-D-*galacto*-2-nonulopyranosid)onate (5)

A solution of **4** (2.13 g, 5.14 mmol) in CH_3CN (50 mL) containing benzaldehyde dimethyl acetal (1.0 mL, 11 mmol), and camphorsulfonic acid (100 mg, 0.43 mmol) was stirred for 1 h. Then, the reaction mixture was neutralized with Et_3N, and concentrated. The resulting crude product was crystallized from diethyl ether to give **5** (2.58 g, 5.14 mmol, quant.). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.51–7.20 (m, 10H, Ar), 4.21 (m, 1H, $J_{7,8}$ = 7.0 Hz, $J_{8,9}$ = 7.0 Hz, $J_{8,9}$ = 5.1 Hz, H-8), 4.12 (dd, 1H, J = 8.4 Hz, H-9a), 3.91 (m, 1H, H-9b), 3.71 (m, 1H, H-5), 3.56–3.37 (m, 2H, H-4, H-7), 3.32–3.30 (m, 4H, H-6, OMe), 2.71 (dd, 1H, J_{gem} = 12.5 Hz, $J_{3eq,4}$ = 4.8 Hz, H-3eq), 1.85 (s, 3H, 3 × Ac), 1.69 (t, 1H, $J_{3ax,4}$ = 11.7 Hz, H-3ax); ^{13}C NMR (150 MHz, $CDCl_3$) δ 176.0, 175.7, 174.3, 174.1, 139.8, 139.2, 135.7, 135.5, 133.6, 130.4, 130.2, 129.5, 129.4, 129.33, 129.27, 128.94, 128.87, 127.8, 127.7, 105.6, 104.8, 93.4, 93.0, 76.22, 76.20, 74.0, 73.8, 71.8, 71.2, 69.8, 69.6, 69.2, 69.0, 54.0, 53.9, 43.6, 43.5, 23.0.

4.3. Methyl (phenyl 5-acetamido-8,9-*O*-benzylidene-4,7-di-*O*-benzyl-3,5-dideoxy-2-thio-D-*glycero*-β-D-*galacto*-2-nonulopyranosid)onate (6)

To a solution of **5** (2.59 g, 5.15 mmol) in dry DMF (40 mL) were added BaO (5.50 g, 35.9 mmol), $Ba(OH)_2$ (1.6 g, 5.1 mmol), and BnBr (6.0 mL, 8.6 mmol), and the mixture was stirred for 15 h at

room temperature. Then, the reaction mixture was filtered through a Celite pad. The filtrate was diluted with $NaHCO_3$ solution, and the aqueous phase was extracted twice with CH_2Cl_2. Next, the combined organic layers were washed with $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Then, the residue was re-dissolved in DMF (40 mL) before the addition of $KHCO_3$ (5.04 g, 50.3 mmol) and MeI (1.6 mL, 26 mmol). The mixture was stirred for 2 h at room temperature, and poured into $NaHCO_3$ solution. The aqueous phase was extracted with CH_2Cl_2, and the organic layers were washed with $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified with silica-gel flash column chromatography (hexane–EtOAc 2:1 → 1:1) to give **6** (1.5 g, 2.2 mmol, 59%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58–7.27 (m, 10H, 2Ph), 5.45 (s, 1H, C*H*Ph), 5.13 (d, 1H, $J_{NH,5}$ = 8.8 Hz, NH), 4.69 (dd, 1H, $J_{6,7}$ = 1.6 Hz, $J_{7,8}$ = 8.9 Hz, H-7), 4.68 (d, 1H, J_{AB} = 11.3 Hz, CH_2Ph), 4.58 (d, 1H, J_{AB} = 11.3 Hz, CH_2Ph), 4.54 (d, 1H, J_{AB} = 12.0 Hz, CH_2Ph), 4.23 (d, 1H, J_{AB} = 12.0 Hz, CH_2Ph), 4.36 (dd, 1H, $J_{9a,9b}$ = 10.6 Hz, $J_{8,9}$ = 5.0 Hz, H-9), 4.23 (m, 1H, H-5), 4.04–4.01 (m, 2H, H-4, H-8), 3.82 (dd, 1H, $J_{5,6}$ = 9.5 Hz, H-6), 3.60 (dd, 1H, H-9b), 3.60 (s, 3H, OMe), 2.75 (dd, 1H, $J_{3ax,3eq}$ = 14.0 Hz, $J_{3eq,4}$ = 4.5 Hz, H-3eq), 1.97 (m, 4H, H-3ax, Ac); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.2, 168.9, 138.1, 138.1, 137.6, 135.3, 135.2, 131.0, 129.2–126.2, 101.3, 89.5, 73.5, 72.1, 71.0, 70.4, 69.6, 67.8, 62.0, 52.6, 50.3, 36.6, 29.0, 23.7; HR-ESI-MS: *m*/*z* [M+Na]$^+$: calcd for $C_{39}H_{41}NO_8SNa$, 706.2450; found, 706.2425.

4.4. Methyl (phenyl 5-acetamido-4,7,9-tri-*O*-benzyl-3,5-di-deoxy-2-thio-D-*glycero*-β-D-*galacto*-2-nonulopyranosid)onate (7)

To a solution of **6** (1.5 g, 2.2 mmol) in dry THF (40 mL) were added $AlCl_3$ (1.8 g, 14 mmol), $BH_3{\cdot}Me_3$ N (973 mg, 13.3 mmol), and 4 Å molecular sieves (3.2 g) at 0 °C, and the mixture was gradually warmed up to room temperature over 1 day. Then, the reaction mixture was poured into 1 M aqueous H_2SO_4 solution and filtered through a Celite pad. The filtrate was extracted twice with Et_2O, and the combined organic layers were washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (hexane–EtOAc 2:1) to give **7** (1.08 g, 1.58 mmol, 72%). $[\alpha]_D^{26}$ −63 (*c* 0.4, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43–7.20 (m, 20H, Ar), 4.84 (d, 1H, $J_{5,6}$ = 10.3 Hz, H-6), 4.68 (s, 2H, CH_2Ph), 4.60 (d, 1H, $J_{NH,5}$ = 8.1 Hz, *H*N), 4.56 (d, 1H, $J_{A,B}$ = 12.0 Hz, CH_2Ph), 4.34 (d, 1H, $J_{A,B}$ = 12.0 Hz, CH_2Ph), 4.48 (d, 1H, $J_{A,B}$ = 11.7 Hz, CH_2Ph), 4.46 (d, 1H, $J_{A,B}$ = 11.7 Hz, CH_2Ph), 4.27 (ddd, 1H, $J_{3ax,4}$ = 6.0, $J_{3eq,4}$ = 4.8, $J_{4,5}$ = 9.1 Hz, H-4), 3.77 (dd, 1H, $J_{8,9a}$ = 4.6, $J_{9a,9b}$ = 9.5 Hz, H-9a), 4.08 (m, 1H, H-8), 3.75 (bs, 1H, H-7), 3.68 (dd, 1H, $J_{8,9a}$ = 6.5 Hz, H-9b), 3.60 (m, 1H, H-5), 3.48 (s, 3H, COOMe), 2.90 (br d, 1H, OH), 2.77 (dd, 1H, $J_{3ax,3eq}$ = 13.7 Hz, H-3eq), 1.96 (dd, 1H, H-3ax), 1.68 (s, 3H, HNAc); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.2, 168.6, 138.3, 138.2, 138.1, 136.1, 129.4–127.8, 125.3, 89.9, 75.0, 73.4, 72.9, 72.6, 72.3, 71.3, 71.1, 70.9, 52.8, 52.4, 37.8, 23.6; HR-ESI-MS: *m*/*z* [M+Na]$^+$: calcd for $C_{39}H_{43}NO_8SNa$, 708.2607; found, 708.2625.

4.5. Methyl (phenyl 5-acetamido-4,7,9-tri-*O*-benzyl-3,5-dideoxy-8-*O*-methyl-2-thio-D-*glycero*-β-D-*galacto*-2-nonulopyranosid)onate (8)

To a solution of **7** (776 mg, 1.13 mmol) in dry DMF (5.0 mL) were added 4 Å molecular sieves (500 mg), BaO (356 mg, 2.32 mmol), $Ba(OH)_2$ (362 mg, 1.15 mmol), and MeI (400 μL, 5.65 mmol). The mixture was stirred for 1.5 h at room temperature under an atmosphere of argon and then filtered through a pad of Celite. Then, the filtrate was diluted with $NaHCO_3$ solution, and the aqueous phase was extracted twice with CH_2Cl_2. The combined organic layers were washed with $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel flash column chromatography (hexane–EtOAc 3:2) to give **8** (570 mg, 0.81 mmol, 72%). $[\alpha]_D^{26}$ −62 (*c* 2.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43–7.20 (m, 20H, Ar), 4.80 (d, 1H, $J_{NH,5}$ = 8.1 Hz, *H*N), 4.69 (d, 1H, $J_{5,6}$ = 10.3 Hz, H-6), 4.72 (d, 1H, $J_{A,B}$ = 11.5 Hz, CH_2Ph), 4.65 (d, 1H, $J_{A,B}$ = 11.5 Hz, CH_2Ph), 4.56 (d, 1H, $J_{A,B}$ = 12.0 Hz, CH_2Ph), 4.35 (d, 1H, $J_{A,B}$ = 12.0 Hz, CH_2Ph), 4.49 (s, 2H, CH_2Ph), 4.21 (ddd, 1H, $J_{3ax,4}$ = 4.8, $J_{3eq,4}$ = 4.3, $J_{4,5}$ = 9.1 Hz, H-4), 4.01 (dd, 1H, $J_{8,9a}$ = 2.6, $J_{9a,9b}$ = 10.7 Hz, H-9a), 3.83 (dd, 1H, $J_{8,9b}$ = 5.3 Hz, H-8), 3.70 (dd, 1H, H-9b), 3.64 (ddd, 1H, H-5), 3.56 (dd, 1H, $J_{7,8}$ = 7.8 Hz, H-7), 3.48 (s, 3H, COOMe), 3.43 (s, 3H, OMe), 2.70 (dd, 1H, $J_{3ax,3eq}$ = 13.7 Hz, H-3eq), 2.04 (s, 3H, HNAc), 2.00 (dd, 1H, H-3ax); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.2, 168.9, 138.5, 138.4, 138.3, 135.6, 130.5, 129.1–127.6, 89.7, 81.1, 74.9, 73.4, 73.3, 73.2, 72.5, 71.0, 69.3, 57.9, 52.7, 52.3, 37.5, 23.6; HR-ESI-MS: *m*/*z* [M+Na]$^+$: calcd for $C_{40}H_{45}NO_8SNa$, 722.2763; found 722.2723.

4.6. Methyl (benzyloxycarbonylmethyl 5-acetamido-4,7,9-tri-*O*-benzyl-3,5-dideoxy-8-*O*-methyl-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (9)

A solution of **8** (532 mg, 0.76 mmol) in dry EtCN (5 mL) containing benzyl glycolate (170 μL, 1.20 mmol) and 4 Å molecular sieves (250 mg) was stirred for 30 min under an atmosphere of argon. Then, the reaction mixture was cooled to −78 °C, and NIS (284 mg, 1.24 mmol) and TfOH (6 μL, 0.07 mmol) were added. The reaction mixture was gradually warmed up from −78 °C to 0 °C and was then neutralized with Et_3 N. The mixture was then filtered through a Celite pad, and the filtrate was diluted with CH_2Cl_2. The organic layer was washed with 3% $Na_2S_2O_3$ solution and brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel flash column chromatography (hexane–EtOAc 2:1 → 1:1) to give **9α** (373 mg, 0.49 mmol, 62%) and **9β** (116 mg, 0.150 mmol, 19%). Compound **9α**: $[\alpha]_D^{25}$ −8.5 (*c* 1.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.51–7.25 (m, 20H, Ar), 5.09 (d, 1H, *J* = 12.2 Hz, CO_2CH_2Ph), 5.03 (d, 1H, *J* = 12.2 Hz, CO_2CH_2Ph), 4.67 (d, 1H, $J_{A,B}$ = 12.0 Hz, CH_2Ph), 4.63 (d, 1H, $J_{A,B}$ = 12.0 Hz, CH_2Ph), 4.58 (d, 1H, $J_{NH,5}$ = 8.8 Hz, *H*N), 4.56 (s, 2H, CH_2Ph), 4.54 (d, 1H, $J_{A,B}$ = 12.0 Hz, CH_2Ph), 4.39 (d, 1H, $J_{A,B}$ = 12.0 Hz, CH_2Ph), 4.37 (d, 1H, J_{gem} = 16.5 Hz, CH_2CO_2Bn), 4.28 (d, 1H, J_{gem} = 16.5 Hz, CH_2CO_2Bn), 3.87 (m, 2H, H-6, H-9a), 3.80 (ddd, 1H, $J_{4,5}$ = 8.7 Hz, H-5), 3.68 (m, 1H, H-4), 3.66 (s, 3H, COOMe), 3.58 (m, 1H, H-8), 3.66 (m, 2H, H-9b, H-7), 3.42 (s, 3H, OMe), 2.87 (dd, 1H, $J_{3eq,4}$ = 4.5 Hz, $J_{3ax,3eq}$ = 12.6 Hz, H-3eq), 1.77 (s, 3H, Ac), 1.75 (dd, 1H, $J_{3ax,4}$ = 7.3 Hz, H-3ax); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.1, 169.6, 167.9, 138.4, 138.1, 135.4, 128.9–127.7, 98.4, 79.2, 74.3, 73.7, 73.5, 73.5, 73.5, 72.8, 70.6, 67.4, 66.6, 66.6, 66.6, 61.4, 57.7, 57.7, 52.5, 52.5, 51.2, 37.0, 29.5, 23.7, 14.2; HR-ESI-MS: *m*/*z* [M+Na]$^+$: calcd for $C_{43}H_{49}NO_{11}SNa$, 778.3203; found, 778.3193.

4.7. Methyl (carboxymethyl 5-acetamido-4,7,9-tri-*O*-benzyl-3,5-dideoxy-8-*O*-methyl-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (2)

A solution of compound **9α** (357 mg, 0.472 mmol) in MeOH (3 mL) containing 10% Pd–C (382 mg) and NH_4OAc (96 mg, 1.3 mmol) was stirred for 1 h at room temperature under an atmosphere of hydrogen. Then, the Pd catalyst was removed by filtration through a pad of Celite, and filtrate was concentrated. The residue was purified by silica gel flash column chromatography (MeOH–chloroform 1:20 with 1% AcOH) to give **2** (308 mg, 0.463 mmol, 98%). $[\alpha]_D^{23}$ −21 (*c* 0.7, $CHCl_3$); ^{1}H NMR (CD_3OD, 600 MHz) δ 7.36–7.25 (m, 10H), 4.67 (d, 1H, $J_{A,B}$ = 11.0 Hz, CH_2Ph), 4.65 (d, 1H, $J_{A,B}$ = 12.4 Hz, CH_2Ph), 4.48 (d, 1H, $J_{A,B}$ = 12.4 Hz,

CH_2Ph), 4.58 (d, 1H, $J_{A,B}$ = 11.7 Hz, CH_2Ph), 4.54 (d, 1H, $J_{A,B}$ = 11.7 Hz, CH_2Ph), 4.32 (d, 1H, $J_{A,B}$ = 16.5 Hz, OCH_2CO), 4.23 (d, 1H, $J_{A,B}$ = 16.5 Hz, OCH_2CO), 4.16 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 10.3 Hz, H-4), 3.90 (dd, 1H, $J_{5,6}$ = 11.0 Hz, $J_{6,7}$ = 1.2 Hz, H-6), 3.77 (d, 1H, $J_{9a,9b}$ = 11.0 Hz, H-9a), 3.74 (s, 3H, OMe), 3.67–3.64 (m, 2H, H-5, H-9b), 3.58 (m, 1H, H-7), 3.50 (m, 1H, H-8), 3.45 (s, 3H, OMe), 2.86 (dd, 1H, $J_{3eq,4}$ = 4.1 Hz, $J_{3,3ax}$ = 12.4 Hz, H-3eq), 1.94 (s, 3H, Ac), 1.68 (dd, 1H, $J_{3ax,4}$ = 12.4 Hz, H-3ax); ^{13}C NMR (CD_3OD, 150 MHz) δ 173.4, 169.3, 139.7, 139.5, 129.5–128.6, 99.7, 80.4, 76.8, 75.9, 74.5, 74.3, 72.0, 68.3, 62.1, 58.1, 53.0, 51.4, 38.1, 23.1; HR-ESI-MS: m/z [M+Na]$^+$: calcd for $C_{36}H_{43}NO_{11}Na$, 688.2734; found, 688.2743.

4.8. 2-(Trimethylsilyl)ethyl (methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trichloroethoxycarbonylamino-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (12)

A solution of **10** (1.20 g, 1.67 mmol) and **11** (1.06 g, 1.13 mmol) in dry CH_3CN (30 mL) containing 4 Å molecular sieves (2.5 g) was stirred for 20 min at room temperature under an atmosphere of argon. Then, the mixture was cooled to −40 °C, and NIS (578 mg, 2.57 mmol) and TfOH (35 μL, 0.40 mmol) were added. After stirring for 2 h at −40 °C, the mixture was neutralized with Et_3N, filtered through a pad of Celite, and the filtrate was diluted with EtOAc. The organic layer was then washed with 3% $Na_2S_2O_3$ solution and brine, dried over $MgSO_4$, and concentrated. The residue was re-dissolved into dry pyridine (15 mL), and Ac_2O (7.5 mL) was added at 0 °C. After stirring overnight at room temperature, the mixture was concentrated and re-dissolved in EtOAc. Purification by silica gel flash column chromatography (hexane–EtOAc 4:1 → 2:1) gave **12α** (1.17 g, 0.760 mmol, 67%, α/β = 6:1). Compound **12α**: $[\alpha]_D^{26}$ +1.0 (c 2.3, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40–7.20 (m, 25H, Ar), 5.59–5.57 (m, 1H, H-8$_{Neu}$), 5.35 (dd, 1H, J = 1.7, 8.4 Hz, H-7$_{Neu}$), 5.03 (d, 1H, J = 2.7 Hz, H-4$_{Gal}$), 4.97 (d, 1H, J = 10.7 Hz, Bn), 4.75 (d, 1H, J = 10.7 Hz, Bn), 5.00 (ddd, 1H, J = 11.9, 4.6, 10.9 Hz, H-4$_{Neu}$), 4.90 (d, 1H, J = 11.7 Hz, Bn), 4.67 (d, 1H, J = 11.7 Hz, Bn), 4.80 (d, 1H, J = 9.9 Hz, N*H*), 4.77 (d, 1H, J = 12.0 Hz, Bn), 4.71 (d, 1H, J = 12.0 Hz, Bn), 4.74 (d, 1H, J = 7.6 Hz, H-1$_{Gal}$), 4.48 (d, 1H, J = 12.9 Hz, Troc), 4.47 (d, 1H, J = 12.9 Hz, Troc), 4.41 (d, 1H, J = 11.9 Hz, Bn), 4.33 (d, 1H, J = 11.9 Hz, Bn), 4.36 (d, 1H, J = 7.7 Hz, H-1$_{Glc}$), 4.24 (dd, 1H, J = 2.3, 12.5 Hz, H-9a$_{Neu}$), 4.08 (dd, 1H, J = 10.8 Hz, H-6$_{Neu}$), 4.05−3.97 (m, 2H, SE, H-9b$_{Neu}$), 3.91 (t, 1H, J = 9.2 Hz, H-3$_{Glc}$), 3.71–3.65 (m, 3H, H-6a$_{Glc}$, H-6b$_{Glc}$, H-5$_{Neu}$), 3.61–3.55 (m, 1H, SE), 3.52 (dd, 1H, J = 7.7, 7.7 Hz, H-4$_{Glc}$), 3.48–3.46 (m, 3H, H-2$_{Gal}$, H-5$_{Glc}$, H-6a$_{Gal}$), 3.39–3.37 (m, 3H, H-2$_{Glc}$, H-5$_{Gal}$, H-6b$_{Gal}$), 2.63 (dd, 1H, J = 4.6, 11.6 Hz, H-3eq$_{Neu}$), 2.08 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.75 (s, 3H, Ac), 1.76 (dd, 1H, J = 11.6, 11.6 Hz, H-3ax$_{Neu}$), 1.03–0.88 (m, 2H, SE), 0.02 (s, 9H, $SiMe_3$); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.6, 170.2, 169.9, 169.8, 169.8, 167.7, 154.2, 139.5, 139.3, 138.8, 138.7, 138.2, 128.3–127.0, 103.0, 102.1, 97.2, 95.3, 82.8, 82.0, 79.4, 76.7, 75.0, 75.0, 74.7, 74.5, 73.9, 73.2, 72.8, 71.7, 71.4, 69.0, 69.0, 68.7, 68.1, 67.6, 67.3, 67.1, 62.0, 53.1, 51.4, 37.6, 21.2–20.4, 18.5, –1.4; HR-ESI-MS: m/z [M+Na]$^+$: calcd for $C_{75}H_{92}Cl_3NO_{25}SiNa$, 1562.4691; found, 1562.4646; Compound **12β**: $[\alpha]_D^{24}$ −2 (c 0.2, chloroform); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52–7.10 (m, 25H), 5.40 (m, 1H, H-7$_{Neu}$), 5.32–5.29 (m, 2H, H-8$_{Neu}$, H-4$_{Gal}$), 5.16 (d, 1H, J = 11 Hz, N*H*), 5.07 (ddd, 1H, J = 5.0, 10.5, 11.9 Hz, H-4$_{Neu}$), 4.95–4.89 (m, 4H, H-9a$_{Neu}$, Bn), 4.71 (d, 1H, J = 11.0 Hz, Bn), 4.70 (d, 1H, J = 11.0 Hz, Bn), 4.67 (dd, 1H, J = 1.8, 8.7 Hz, H-6$_{Neu}$), 4.62 (s, 2H, Bn), 4.58–4.49 (m, 5H, H-1$_{Gal}$, H-3$_{Gal}$, Bn, Troc), 4.33 (d, 1H, J = 7.8 Hz, H-1$_{Glc}$), 4.27 (d, 1H, J = 12.4 Hz, Bn), 4.02–3.90 (m, 3H, H-9b$_{Neu}$, H-4$_{Glc}$, SE), 3.83 (m, H-5$_{Gal}$), 3.81–3.66 (m, 3H, H-5$_{Neu}$, H-6ab$_{Glc}$), 3.59 (m, 1H, SE), 3.51 (dd, 1H, J = 9.2, 9.2 Hz, H-3$_{Glc}$), 3.48 (dd, 1H, J = 7.8, 9.3 Hz, H-2$_{Gal}$), 3.43 (s, 3H, OMe), 3.41–3.32 (m, 3H, H-2$_{Glc}$, H-6ab$_{Gal}$), 3.26 (m, 1H, H-5$_{Glc}$), 2.62 (dd, 1H, J = 5.0, 13.8 Hz, H-3eq$_{Neu}$), 2.13 (s, 3H), 2.08 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H), 1.84 (s, 3H), 1.78 (dd, 1H, J = 13.8, 13.8 Hz, H-3ax$_{Neu}$), 1.02 (m, 2H, SE), 0.02 (s, 9H, $SiMe_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.6, 171.9, 170.7, 170.5, 170.4, 169.9, 166.5, 154.6, 139.1, 138.9, 138.5, 138.2, 137.9, 129.3–127.4, 103.2, 102.1, 99.2, 95.6, 82.6, 82.0, 78.4, 75.8, 75.7, 75.3, 75.11, 75.05, 74.7, 73.3, 72.6, 72.2, 71.8, 71.7, 70.7, 69.4, 68.5, 67.4, 67.3, 62.8, 52.4, 50.8, 37.3, 21.1, 20.9, 20.8, 18.6, -1.3; HR-ESI-MS: m/z [M+Na]$^+$: calcd for $C_{75}H_{92}Cl_3NO_{25}SiNa$, 1562.4691; found, 1562.4652.

4.9. 2-(Trimethylsilyl)ethyl (methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-amino-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (3)

A solution of **12** (208 mg, 0.135 mmol) in acetic acid (5 mL) containing zinc powder (2.13 g) was stirred for 1 h. Then, the mixture was filtered through a pad of Celite, and filtrate was concentrated under reduced pressure at 30 °C. The residue was purified with silica gel flash column chromatography (toluene–acetone 4:1) to give **3** (157 mg, 0.115 mmol, 85%). $[\alpha]_D^{23}$ −3.5 (c 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.37–7.18 (m, 25H), 5.66 (m, 1H), 5.42 (dd, 1H, J = 1.4, 8.9 Hz), 5.09 (d, 1H, J = 3.4 Hz), 4.97 (d, 1H, J = 10.3 Hz), 4.94 (d, 1H, J = 12.4 Hz), 4.88 (d, 1H, J = 11.0 Hz), 4.75 (d, 1H, J = 12.4 Hz), 4.74 (d, 1H, J = 9.6 Hz), 4.71 (m, 1H), 4.69 (d, 1H, J = 11.0 Hz), 4.62 (d, 1H, J = 12.4 Hz), 4.50 (d, 1H, J = 11.7 Hz), 4.41 (d, 1H, J = 11.7 Hz), 4.37–4.33 (m, 3H), 4.30 (dd, 1H, J = 2.1, 13.1 Hz), 4.20 (d, 1H, J = 11.7 Hz), 4.17 (dd, 1H, J = 4.1, 13.1 Hz), 3.99 (m, 1H), 3.92 (dd, 1H, J = 9.6, 9.6 Hz), 3.80 (s, 3H), 3.78 (d, 1H, J = 9.6 Hz), 3.62 (dd, 1H, J = 6.9, 6.9 Hz), 3.61–3.56 (m, 2H), 3.54 (dd, 1H, J = 8.9, 8.9 Hz), 3.49 (dd, 1H, J = 1.4, 10.3 Hz), 3.44 (dd, 1H, J = 8.2, 9.6 Hz), 3.39–3.34 (m, 2H), 3.28 (d, 2H, J = 6.2 Hz), 2.66 (dd, 1H, J = 4.8, 13.1 Hz), 2.53 (dd, 1H, J = 9.6, 10.3 Hz), 2.10 (s, 3H), 2.07 (s, 3H), 2.00 (s, 3H), 2.00 (s, 3H), 1.70 (s, 3H) 1.03 (m, 2H), 0.01 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.8, 170.6, 170.2, 169.80, 169.78, 167.9, 139.5, 139.3, 138.8, 138.7, 138.2, 128.2–127.1, 103.0, 102.2, 97.5, 82.8, 82.0, 79.3, 76.7, 75.05, 75.02, 74.9, 74.64, 74.62, 73.8, 73.2, 72.9, 72.3, 71.7, 69.0, 67.9, 67.8, 67.3, 52.9, 51.1, 36.8, 21.2, 21.0, 20.7, 20.3, −1.4; HR-ESI-MS: m/z [M+Na]$^+$: calcd for $C_{72}H_{91}NO_{23}SiNa$, 1388.5649; found, 1388.5629.

4.10. 2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,9-tri-*O*-benzyl-8-*O*-methyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→11)-(methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-glycolylamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (13)

A solution of **3** (92 mg, 0.15 mmol) and crude **2** (157 mg, 0.115 mmol) in CH_3CN (2 mL) containing $NaHCO_3$ (61 mg, 0.72 mmol), HOBt (40 mg, 0.29 mmol), and EDCI (59 mg, 0.31 mmol) was stirred at room temperature for 18 h under an atmosphere of argon. Then, the mixture was diluted with H_2O and extracted three times with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and filtered. The remaining residue was purified by silica gel flash column chromatography (toluene–acetone 4:1) to give **13** (132 mg, 0.065 mmol, 57%). $[\alpha]_D^{26}$ −6.2 (c 0.9, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39–7.18 (m, 40H), 6.23 (d, 1H, J = 10.3 Hz), 5.60 (m, 1H), 5.21 (dd, 1H, J = 2.5, 8.2 Hz), 4.98 (d, 1H, J = 11.7 Hz), 4.89 (m, 1H), 4.88 (d,

2H, J = 11.0 Hz), 4.77 (d, 1H, J = 7.6 Hz), 4.76 (d, 1H, J = 11.0 Hz), 4.68 (d, 1H, J = 11.0 Hz), 4.67 (d, 1H, J = 10.6 Hz), 4.64 (d, 1H, J = 13.1 Hz), 4.61 (d, 1H, J = 11.0 Hz), 4.58–4.55 (m, 3H), 4.54 (d, 1H, J = 11.0 Hz), 4.48 (d, 1H, J = 12.4 Hz), 4.47 (dd, 1H, J = 2.7, 9.9 Hz), 4.40 (d, 1H, J = 12.4 Hz), 4.36 (d, 2H, J = 12.4 Hz), 4.34 (d, 1H, J = 8.2 Hz), 4.28 (dd, 1H, J = 2.8, 12.4 Hz), 4.20 (d, 1H, J = 15.1 Hz), 4.19 (d, 1H, J = 11.7 Hz), 4.10 (ddd, 1H, J = 10.3, 10.3, 11.0 Hz), 4.03 (d, 1H, J = 11.0 Hz), 3.98 (m, 1H), 3.95 (dd, 1H, J = 5.5, 12.4 Hz), 3.91 (dd, 1H, J = 9.6, 9.6 Hz), 3,86–3.75 (m, 4H), 3.82 (s, 3H), 3.79 (s, 3H), 3.69–3.62 (m, 4H), 3.59–3.54 (m, 3H), 3.54 (dd, 1H, J = 9.6, 9.6 Hz), 3.44 (dd, 1H, J = 7.6, 9.6 Hz), 3.41 (s, 3H), 3.37 (m, 1H), 3.37 (dd, 1H, J = 7.8, 8.9 Hz), 3.60 (d, 2H, J = 6.2 Hz), 2.82 (dd, 1H, J = 4.8, 13.1 Hz), 2.63 (dd, 1H, J = 4.1, 12.4 Hz), 2.08 (s, 3H), 1.99 (s, 3H), 1.94 (s, 3H), 2.82 (dd, 2H, J = 12.4, 12.4 Hz), 1.78 (s, 3H), 1.76 (s, 3H), 1.69 (s, 3H), 1,04 (m, 2H), 0.01 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.5, 170.4, 170.1, 170.0, 169.9, 169.4, 168.2, 168.0, 139.5, 139.3, 138.8, 138.7, 138.2, 138.2, 138.1, 138.0, 129.1–127.0, 103.0, 102.1, 98.7, 97.4, 82.9, 82.0, 79.5, 79.0, 76.7, 75.0, 75.0, 74.9, 74.7, 74.3, 73.8, 73.7, 73.6, 73.5, 73.2, 72.9, 72.8, 72.4, 71.4, 70.6, 69.0, 68.8, 68.7, 68.3, 67.6, 67.3, 67.2, 67.1, 63.2, 62.2, 57.6, 53.2, 52.7, 51.3, 48.5, 37.7, 37.0, 29.7, 23.7, 21.2–20.5, 18.5, 1.1, –1.4; HR-ESI-MS: m/z $[M+Na]^+$: calcd for $C_{108}H_{132}N_2O_{33}SiNa$, 2035.8379; found, 2035.8399.

4.11. 2-(Trimethylsilyl)ethyl 5-acetamido-8-*O*-methyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid-(2→11)-3,5-dideoxy-5-glycolylamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (1)

A solution of **13** (63 mg, 0.032 mmol) in pyridine (2 mL) containing LiCl (59 mg, 1.4 mmol) was stirred for 19 h at 120 °C under an atmosphere of argon. Then, the mixture was cooled to room temperature and concentrated. The residue was acidified with 10% citric acid, and the aqueous phase was extracted three times with $CHCl_3$. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was then dissolved in MeOH (3 mL) and 10% Pd–C (83 mg) was added. After stirring for 20 h at room temperature under an atmosphere of hydrogen gas, the mixture was filtered through Celite pad, and the filtrate was concentrated. Then, the residue was dissolved in 0.1 M NaOH (4 mL), and the reaction mixture was stirred for 2 h at room temperature. The reaction mixture was then directly applied to a size exclusion column chromatography (Sephadex G-15, H_2O) to give **1** (31 mg, 0.029 mmol, 91%). $[\alpha]_D^{24}$ −16 (c 1.4, H_2O); ^{1}H NMR (600 MHz, with CH_3OH as internal standard) δ 4.40 (d, 1H, J = 7.6 Hz, H-1$_{Gal}$), 4.36 (d, 1H, J = 8.3 Hz, H-1$_{Glc}$), 4.15 (d, 1H, J = 15.3 Hz, OCH_2CO), 3.98 (m, 1H, H-3$_{Gal}$), 3.97 (d, 1H, J = 15.6 Hz, OCH_2CO), 3.92–3.21 (m, 27H), 3.34 (s, 3H, OMe), 3.15 (dd, 1H, J = 8.2, 8.9 Hz, H-2$_{Glc}$), 2.63 (dd, 1H, J = 4.1, 12.4 Hz, H-3eq$_{Neu5Ac}$), 2.50 (dd, 1H, J = 4.1, 12.4 Hz, H-3eq$_{Neu5Gc}$), 1.89 (s, 3H), 1.67 (dd, 1H, J = 11.7, 12.4 Hz, H-3ax$_{Neu5Ac}$), 1.60 (dd, 1H, 11.7, 12.4 Hz, H-3ax$_{Neu5Gc}$), 0.94 (ddd, 1H, J = 5.3, 12.4, 13.1 Hz, SE), 0.84 (ddd, 1H, J = 5.4, 12.4, 13.1 Hz, SE), –0.11 (s, 9H, SE); ^{13}C NMR (D_2O with CH_3OH as internal standard, 150 MHz) δ 175.6 (N-Ac), 174.5 (C-1$_{Neu5Ac}$), 174.0 (C-1$_{Neu5Gc}$), 173.4 (N-glycolyl), 103.3 (C-1$_{Gal}$), 102.0 (C-1$_{Glc}$), 101.3 (C-2$_{Neu5Ac}$), 100.5 (C-2$_{Neu5Gc}$), 80.7 (C-8$_{Neu5Ac}$), 78.8 (C-4$_{Glc}$), 76.1 (C-3$_{Gal}$), 75.8, 75.4, 75.2, 73.5, 73.3, 73.2, 72.6, 70.0, 69.1, 68.7, 68.6, 68.1, 67.7, 63.8, 63.3, 61.7, 60.7 (SE), 60.1 (C-9$_{Neu5Ac}$), 57.2 (OMe), 52.7 (C-5$_{Neu5Gc}$), 52.2 (C-5$_{Neu5Ac}$), 40.3 (C-3$_{Neu5Ac}$), 40.1 (C-3$_{Neu5Gc}$), 22.7 (Ac), 18.2 (SE), –1.9 (SE); HR-ESI-MS: m/z $[M-H]^-$: calcd for $C_{40}H_{69}N_2O_{28}Si$, 1053.3806; found, 1053.3810.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2009.02.003.

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