Synthesis of a Sialic Acid Dimer Derivative, 2′α-*O*-Benzyl Neu5Ac-α-(2→5)Neu5Gc

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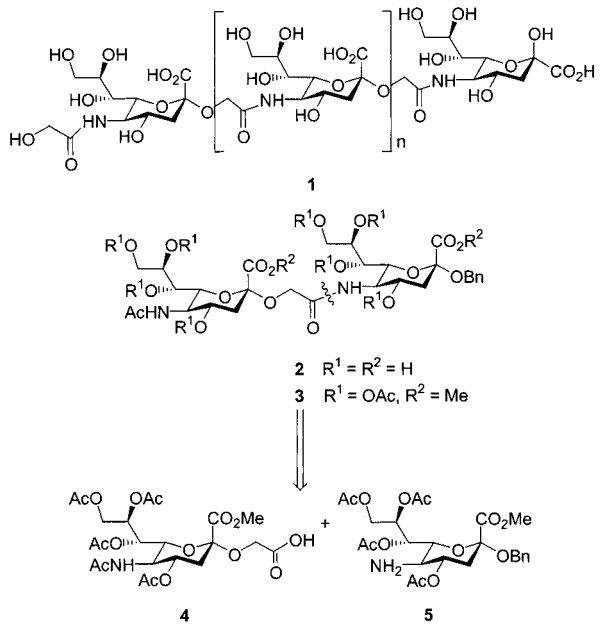
Abstract: The preparation of a disaccharide **2**, Neu5Ac-α-(2→5)Neu5Gc having a α-benzyl protecting group at the reducing end, by the coupling of the easily accessible building units **4** and **5** is described. Subsequent deprotection of the coupling adduct led to the isolation of the target compound **2** in high yield.

With respect to glycosidic linkages in polysialic acids (PSA), three kinds of PSA's have been isolated from mammalian cells and microorganisms: α(2→8)PSA (Neu5Ac, Neu5Gc, and KDN), α(2→9)PSA (Neu5Ac), and α(2→8)/α(2→9) alternating PSA (Neu5Ac).[1,2] Recently, a new PSA chain with Neu5Gc residues ketosidically linked to the glycolyl group of Neu5Gc ((→5-$O_{glycolyl}$Neu5Gc-α2→)$_n$, called poly-α-(2→5)-*N*-glycolylneuraminic acid (Neu5Gc)), **1**, has been isolated from the jelly coat of sea urchin egg and found to play an important role in the fertilization of eggs.[3−5] In an effort to learn more about its involvement in the biological processes, an efficient preparation of this class of compounds would provide easy access to various structural congeners required for our study. Here, we report the first synthesis of a sialic acid dimer derivative, 2′α-*O*-benzyl Neu5Ac-α-(2→5)Neu5Gc (**2**).

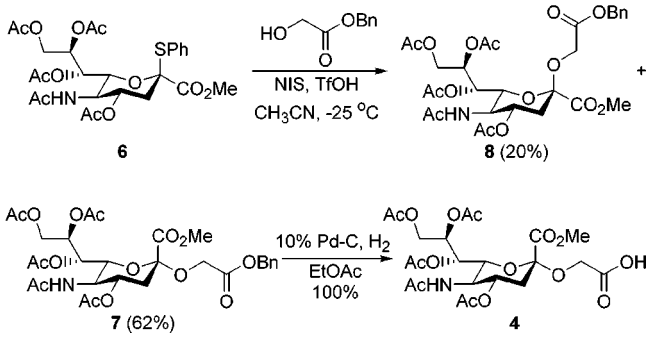
Our retrosynthetic analysis (Scheme 1) involves the separate preparation of the substituted glycolic acid **4** and the amine **5** fragments. The amide linkage of the target molecule would be assembled by the coupling reaction between compound **4** and **5**. This approach was straightforward and proved to be effective for the construction of protected disaccharides 2′α-*O*-benzyl Neu5Ac-α-(2→5)Neu5Gc (**2** and **3**) in good yield.

The synthesis of compounds **4** and **5** began from a common precursor, thioglycosides **6** (Scheme 2), prepared from *N*-acetylneuraminic acid by the reported procedure.[6] Treatment of thioglycosides **6** (α/*β* = 1:4) with benzyl glycolate in the presence of NIS and a catalytic amount of triflic acid (TfOH)[7] at −25 °C gave **7** (62%) and 20% of the *β*-anomer **8**, and both were separated by flash chromatography on silica gel as shown in Scheme 2. Hydrogenolytic removal of the benzyl group of **7** over 10% Pd on carbon in ethyl acetate cleanly afforded the desired substituted glycolic acid **4** in quantitative yield.

Scheme 1



Scheme 2



N,O-deacetylation of the thioglycoside **6*β*** with methanesulfonic acid in methanol[8] was followed by selective protection of the resulting free amino functional group as *N*-2,2,2-trichloroethoxycarbonyl carbamate[9] to give **9** in 77% yield. (Scheme 3) Peracetylation of **9** by the acetic anhydride−pyridine protocol afforded **10** in 94% yield. Reaction of **10** with benzyl alcohol under NIS/TfOH conditions provided the α-benzyl glycoside **11** (66%) and the *β*-benzyl glycoside **12** (14%); both were separated by flash chromatography on silica gel. Treatment of **11** with zinc dust in glacial acetic acid at room temperature for 3 h afforded the desired amine **5** in 84% yield.[10] It is interesting to note that amine **5** could be purified by

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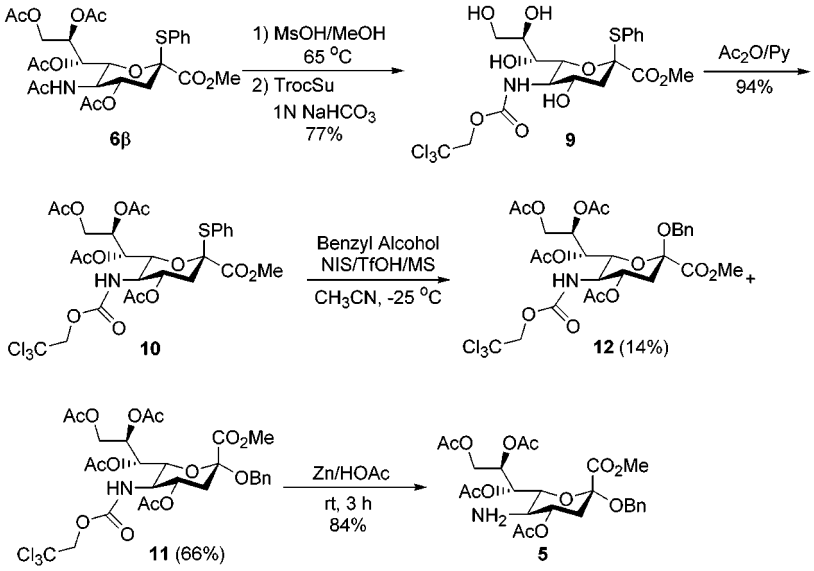
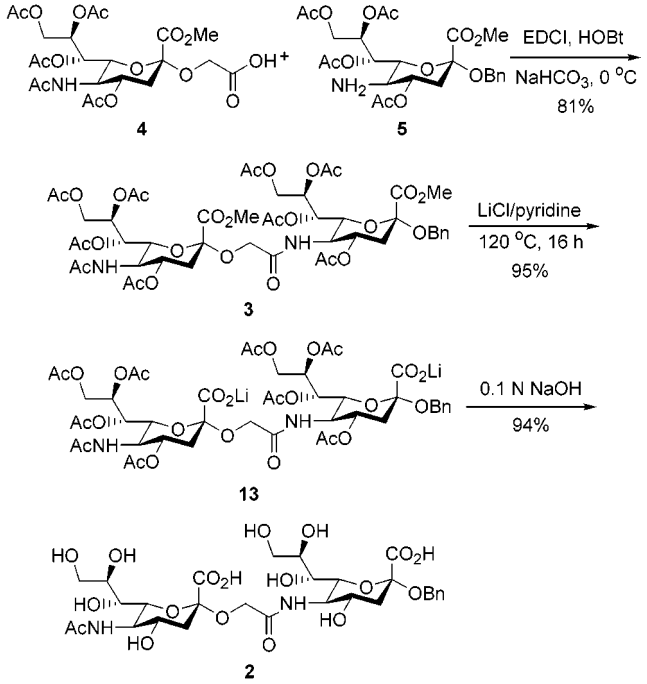
Scheme 3



Scheme 4



chromatography and stored at 0 °C for few days without decomposition.[11]

The coupling of **4** with **5** (Scheme 4) was conducted in the presence of EDC and HOBt[12] in acetonitrile to give the fully protected dimer **3** in 81% yield, as shown in Scheme 4. Finally, transformation of **3** to **2** was performed in two steps. Compound **3** was first converted to **13** by reaction with LiCl in pyridine in 95% yield.[13] Basic hydrolysis of **13** with 0.1 N NaOH cleanly provided the desired product **2** in 94% yield.

In conclusion, the synthetic disaccharide 2′α-*O*-benzyl Neu5Ac-α-(2→5)Neu5Gc (**2**) is more easily traced in enzymatic reactions as a substrate with a benzyl group in the reducing end. Application of this synthetic strategy for the preparation of 2′-α-*O*-benzyl-α(2→5)-*N*-glycolyl-neuraminic acid dimer and oligomer is currently underway, and these species will be used as acceptors for the biosynthetic study of α-(2→5)Neu5Gc polymer.

Experimental Section

General Considerations. NMR spectra were recorded with Bruker AM-400 (400 MHz) and Bruker Avance DMX-500 (500 MHz) spectrometers. Assignment of ^{1}H NMR spectra was achieved using 2D methods (COSY, NOESY, HETCOR). Chemical shifts are expressed in ppm using residual $CHCl_3$ and CHD_2-OD as references. High-resolution FAB-MS was recorded with a JEOL SX-120 mass spectrometer. Reactions were monitored by TLC using alumina or glass plates coated with silica gel 60 F_{254} (Merck) and visualized by using either UV light or by charring with a molybdate solution (a 0.02 M solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4). Column chromatography was performed on silica gel 60 (Merck, 70−230 mesh). Methanol was dried by refluxing with magnesium methoxide and distilled immediately before use. Dioxane, acetonitrile, and pyridine were freshly distilled under N_2 over CaH_2. Molecular sieves (3 Å) were activated in vacuo at 300 °C for 8 h and stored under N_2.

Methyl [2-(2′-Benzyloxy-2′-oxoethyl)-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α- and -β-D-glycero-D-galacto-2-nonulopyranosid]onate (7 and 8). A mixture of thioglycoside **6** (4.0 g, 6.85 mmol), benzyl glycolate (1.71 g, 10.28 mmol), and 3 Å molecular sieves (6.0 g) in acetonitrile (40 mL) was stirred for 1 h under N_2. After the solution was cooled to −40 °C, NIS (1.85 g, 8.22 mmol) and TfOH (0.12 mL, 1.37 mmol) were added successively. The reaction mixture was stirred at −25 °C for 1 h, diluted with DCM (100 mL), and filtered through a pad of Celite. The Celite was washed with DCM (3 × 20 mL), and the combined filtrate was washed with saturated aqueous $Na_2S_2O_3$ (15 mL) and saturated aqueous $NaHCO_3$ (20 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (50−100% gradient EtOAc in hexane) to afford

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the α-anomer **7** (2.72 g, 62%) and the *β*-anomer **8** (0.88 g, 20%) as a white foam. **7**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33−7.28 (m, 5H), 5.33 (ddd, J = 8.3, 5.5, 2.7 Hz, 1H), 5.28 (d, J = 9.9 Hz, 1H), 5.27 (dd, J = 8.3, 2.2 Hz, 1H), 5.14 (dd, J = 25.2, 12.3 Hz, 2H), 4.90 (ddd, J = 12.0, 10.2, 4.8 Hz, 1H), 4.29 (dd, J = 32.1, 16.4 Hz, 2H), 4.24 (dd, J = 12.5, 3.0, 1H), 4.06−4.04 (m, 1H), 4.01 (t, J = 10.2 Hz, 1H), 3.91 (dd, J = 10.2, 2.2 Hz, 1H), 3.70 (s, 3H), 2.82 (dd, 1H, J = 12.0, 4.8 Hz, 1H), 2.09 (s, 3H), 2.08(s, 3H), 2.00 (s, 3H), 1.99−1.97 (m, 4H), 1.84 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.93 (C), 170.57 (C), 170.23 (C), 170.06 (C), 169.98 (C), 169.12 (C), 167.50 (C), 135.39 (C), 128.48 (CH), 128.30 (CH), 98.15 (C), 72.51 (CH), 68.93 (CH), 68.38 (CH), 67.14 (CH), 66.58 (CH_2), 62.28 (CH_2), 61.81 (CH_2), 52.90 (CH_3), 49.30 (CH), 37.53 (CH_2), 23.11 (CH_3), 21.04 (CH_3), 20.79 (CH_3), 20.74 (CH_3), 20.66 (CH_3); LRMS (FAB; *m*/*z* (%)) 663 (M + Na$^+$, 12), 640 (MH$^+$, 12), 580 (25), 474 (17), 414 (100), 196 (10), 154 (16), 91 (91), 58 (36); HRMS (FAB, MH$^+$) calcd for $C_{29}H_{38}O_{15}N$ 640.2241, found 640.2223. **8**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.30 (m, 5H), 5.35 (d, J = 10.4 Hz, 1H), 5.27 (dd, J = 3.9, 2.1 Hz, 1H), 5.23 (ddd, J = 10.9, 6.5, 4.9 Hz, 1H), 5.16 (dd, J = 20.3, 12.2 Hz, 2H), 5.15−5.11 (m, 1H), 4.73 (dd, J = 12.3, 2.5, 1H), 4.26 (d, J = 1.02 Hz, 2H), 4.16 (dd, J = 10.4, 2.1 Hz, 1H), 4.09 (q, J = 10.4 Hz, 1H), 3.95 (dd, J = 12.3, 8.0 Hz, 1H), 3.70 (s, 3H), 2.46 (dd, J = 13.1, 4.9 Hz, 1H), 2.09 (s, 3H), 2.00 (m, 4H), 1.97 (s, 3H), 1.93 (s, 3H), 1.82 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.76 (C), 170.54 (C), 170.48 (C), 170.21 (C), 169.99 (C), 169.22 (C), 166.69 (C), 135.22 (C), 128.60 (CH), 128.52 (CH), 128.48 (CH), 98.56 (C), 72.17 (CH), 71.71 (CH), 68.62 (CH), 68.38 (CH), 66.95 (CH_2), 62.51 (CH_2), 61.52 (CH_2), 52.84 (CH_3), 48.92 (CH), 36.86 (CH_2), 23.03 (CH_3), 20.87 (CH_3), 20.79 (CH_3), 20.68 (CH_3), 20.65 (CH_3); LRMS (FAB; *m*/*z* (%)) 663 (M + Na$^+$, 42), 640 (17), 580 (25), 474 (18), 414 (100), 154 (23), 91 (74), 58 (68); HRMS (FAB, MH$^+$) calcd for $C_{29}H_{38}O_{15}N$ 640.2241, found 640.2243.

Methyl [2-(2′-Oxido-2′-oxoethyl)-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosid]onate (4). To a solution of compound **7** (1.60 g, 2.50 mmol) in EtOAc (50 mL) was added 10% Pd on carbon (262 mg). The mixture was stirred under hydrogen at 1 atm for 20 h until TLC analysis indicated that the reaction had gone to completion. The catalyst was removed by filtration through a pad of Celite, and the cake was washed with EtOAc (3 × 20 mL). The filtrate was concentrated under reduced pressure to give **4** as a white foam (1.37 g, 100%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.81−5.56 (br, 1H), 5.49 (d, J = 9.1 Hz, 1H), 5.37 (ddd, J = 8.2, 5.8, 2.5 Hz, 1H), 5.30−5.28 (m, 1H), 4.96 (ddd, J = 12.3, 9.8, 4.5 Hz, 1H), 4.30 (dd, J = 44.3, 16.7 Hz, 2H), 4.29 (dd, J = 12.5, 2.5 Hz, 1H), 4.07 (dd, J = 12.5, 5.8 Hz, 1H), 4.06−4.00 (m, 2H), 3.81 (s, 3H), 2.70 (dd, J = 12.9, 4.5 Hz, 1H), 2.13 (s, 3H), 2.04−1.97 (m, 7H), 1.89 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.31 (C), 171.69 (C), 171.49 (C), 170.99 (C), 170.85 (C), 168.26 (C), 98.91 (C), 73.18 (CH), 69.59 (CH), 69.21 (CH), 67.87 (CH), 63.06 (CH_2), 62.29 (CH_2), 53.72 (CH_3), 50.07 (CH), 38.01 (CH_2), 23.68 (CH_3), 21.71 (CH_3), 21.46 (CH_3), 21.36 (CH_3). LRMS (FAB; *m*/*z* (%)): 572 (M + Na$^+$, 25), 550 (MH$^+$, 12), 490 (43), 414 (100), 372 (13), 154 (57), 136 (52), 55 (42). HRMS (FAB, MH$^+$): calcd for $C_{22}H_{32}O_{15}N$ 550.1722, found 550.1766.

Methyl [Phenyl 5-(2,2,2-trichloroethoxycarbonylamino)-3,5-dideoxy-2-thio-*β*-D-glycero-D-galacto-2-nonulopyranosid]onate (9). To a solution of thioglycoside **6*β*** (15.0 g, 25.70 mmol) in methanol (100 mL) was added methanesulfonic acid (2.0 mL, 30.83 mmol), and the resulting mixture was stirred at 65 °C for 24 h. After it was cooled to room temperature, the solution was neutralized with Dowex 1×8-50 ion-exchange resin. The resin was removed by filtration and washed with methanol (2 × 40 mL). The filtrate was concentrated to afford a pale brown foam. Without purification, the crude material was dissolved in 1 M sodium hydrogen carbonate aqueous solution (27 mL). A solution of succinimidyl 2,2,2-trichloroethyl carbonate (8.2 g, 28.28 mmol) in dioxane (30 mL) was added in one portion, and the resulting mixture was stirred vigorously for 1 h. Dioxane was removed under reduced pressure, and the remaining alkaline suspension was extracted with EtOAc (3 × 150 mL). The combined organic layer was washed with water (2 × 20 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash silica gel chromatography (0−10% gradient methanol in EtOAc) to provide **9** as a white foam (10.86 g, 77%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65−7.63 (m, 2H), 7.37−7.30 (m, 3H), 4.96 (d, J = 12.3 Hz, 2H), 4.70 (d, J = 12.3 Hz, 1H), 4.59 (d, J = 10.3 Hz, 1H), 4.25 (ddd, J = 15.8, 11.8, 4.7 Hz, 1H), 3.88−3.55 (m, 10H), 3.45 (s, 3H), 2.69 (dd, J = 13.7, 4.7 Hz, 1H), 2.00 (dd, J = 13.7, 12.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.51 (C), 156.91 (C), 137.33 (C), 130.27 (C), 129.79 (CH), 97.23 (C), 91.37 (C), 75.26 (CH_2), 73.54 (CH), 71.08 (CH), 70.96 (CH), 70.84 (CH), 69.55 (C), 67.41 (CH), 65.09 (CH_2), 55.98 (CH), 52.74 (CH_3), 42.63 (CH_2). LRMS (FAB; *m*/*z* (%)): 576 ((M + Na + 6)$^+$, 2), 574 ((M + Na + 4)$^+$, 8), 572 ((M + Na + 2)$^+$, 22), 570 ((M + Na)$^+$, 20), 554 ((MH + 6)$^+$, 1), 552 ((MH + 4)$^+$, 3), 550 ((MH + 2)$^+$, 8), 548 (MH$^+$, 7), 442 (5), 440 (18), 438 (19), 307 (14), 154 (100), 136 (77), 107 (25), 89 (27), 77 (26). HRMS (FAB, MH$^+$): calcd for $C_{22}H_{32}O_{15}N$ 548.0316, found 548.0326.

Methyl [Phenyl 5-(2,2,2-trichloroethoxycarbonylamino)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-*β*-D-glycero-D-galacto-2-nonulopyranosid]onate (10). A solution of compound **9** (4.23 g, 7.71 mmol) in pyridine (6.0 mL), and acetic anhydride (6.0 mL) was stirred at room temperature for 24 h. After the mixture was cooled to 0 °C, methanol (3.0 mL) was added over 10 min, and the mixture was stirred at room temperature for 30 min. The volatile material was removed under reduced pressure, and the residue was purified by flash silica gel chromatography (20−50% gradient EtOAc in hexane) to give **10** as a white foam (5.2 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46−7.44 (m, 2H), 7.37−7.27 (m, 3H), 5.51 (t, J = 2.4 Hz, 1H), 5.46 (ddd, J = 10.7, 10.7, 4.9 Hz, 1H), 5.25 (br, 1H), 5.01 (ddd, J = 2.3, 2.4, 8.0 Hz, 1H), 4.91 (d, J = 12.1 Hz, 1H), 4.68 (dd, J = 10.7, 2.4 Hz, 1H), 4.50 (d, J = 12.3, 2.3 Hz, 1H), 4.47 (dd, J = 12.3, 2.3 Hz, 1H), 4.04 (dd, J = 12.3, 8.0 Hz, 1H), 3.77 (q, J = 10.7 Hz, 1H), 3.60 (s, 3H), 2.74 (dd, J = 13.8, 4.9 Hz, 1H), 2.12 (s, 3H), 2.11−2.03 (m, 4H), 1.98 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.53 (C), 171.02 (C), 170.94 (C), 170.70 (C), 168.79 (C), 154.94 (C), 136.78 (CH), 130.37 (CH), 129.75 (CH), 129.49 (C), 96.02 (C), 89.45 (C), 75.20 (CH_2), 73.47 (CH), 73.30 (CH), 69.45 (CH), 69.30 (CH), 63.13 (CH_2), 53.23 (CH_3), 52.44 (CH), 38.19 (CH_2), 21.64 (CH_3), 21.46 (CH_3), 21.39 (CH_3), 21.34 (CH_3). LRMS (FAB; *m*/*z* (%)): 742 ((M + Na + 4)$^+$, 1), 740 ((M + Na + 2)$^+$, 2), 738 ((M + Na)$^+$, 2), 718 ((MH + 2)$^+$, 1), 716 (MH$^+$, 1), 658 (4), 656 (4), 609 (4), 607 (5), 550 (30), 548 (100), 546 (99), 488 (15), 486 (15), 368 (31), 366 (31), 330 (35), 328 (36), 154 (43), 136 (45), 78 (20). HRMS (FAB, MH$^+$): calcd for $C_{28}H_{35}O_{14}NCl_3$ 716.0738, found 716.0729.

Methyl [Benzyl 5-(2,2,2-trichloroethoxycarbonylamino)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α- and *β*-D-glycero-D-galacto-2-nonulopyranosid]onate (11 and 12). A mixture of compound **10** (5.2 g, 7.25 mmol), benzyl alcohol (1.10 mL, 10.87 mmol), and 3 Å molecular sieves (4.8 g) in acetonitrile (40 mL) was stirred for 1 h under N_2. After the solution was cooled to −40 °C, NIS (1.96 g, 8.70 mmol) and TfOH (0.25 mL, 2.90 mmol) were added successively. The reaction mixture was stirred at −25 °C for 1 h, diluted with DCM (100 mL), and filtered through a pad of Celite. The Celite was washed with DCM (3 × 20 mL), and the combined filtrate was washed with saturated aqueous $Na_2S_2O_3$ (15 mL) and saturated aqueous $NaHCO_3$ (20 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (10−30% gradient EtOAc in hexane) to afford the α-anomer **11** (3.4 g, 66%) and the *β*-anomer **12** (0.7 g, 14%) as a white foam. **11**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−7.27 (m, 5H), 5.47 (ddd, J = 8.9, 4.9, 4.7 Hz, 1H), 5.41 (dd, J = 8.9, 1.8 Hz, 1H), 5.00 (ddd, J = 12.1, 9.9, 4.4 Hz, 1H), 4.93−4.90 (dm, J = 12.1 Hz, 2H), 4.82 (d, J = 12.0 Hz, 1H), 4.49 (d, J = 12.1 Hz, 1H), 4.43 (d, J = 12.0 Hz, 1H), 4.32 (dd, J = 12.5, 2.7 Hz, 1H), 4.24 (dd, J = 10.7, 1.8 Hz, 1H), 4.15 (dd, J = 12.5, 4.9 Hz, 1H), 3.74−3.66 (m, 4H), 2.73 (dd, J = 12.1, 4.4 Hz, 1H), 2.17 (s, 3H), 2.16 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 1.97−1.91 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.32 (C), 171.05 (C), 170.77 (C), 170.62 (C), 168.90 (C), 154.74 (C), 137.78 (C), 128.88 (CH), 128.47 (CH), 128.35 (CH), 99.05 (C), 96.05 (C), 75.17 (CH_2), 72.61 (CH), 69.17 (CH), 68.85 (CH), 68.06 (CH), 67.53 (CH_2), 62.85 (CH_2), 53.30 (CH_3), 52.36 (CH), 38.91 (CH_2), 21.74 (CH_3), 21.46 (CH_3), 21.40(CH_3); LRMS (FAB; *m*/*z* (%)) 738 ((M + Na + 2)$^+$, 1), 736 ((M + Na)$^+$, 1), 714 (MH$^+$, 1), 656 (5), 654 (5), 609 (5), 607 (5), 550 (12), 548 (38), 546 (40), 488 (8), 486 (8), 370 (5), 368 (13), 366 (15), 332 (17), 330 (17), 328 (5); HRMS (FAB, MH$^+$) calcd for $C_{28}H_{35}O_{14}NCl_3$ 714.1123, found 714.1113. **12**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29−7.19 (m, 5H), 5.37 (dd, J = 5.0, 1.7

Hz, 1H), 5.35 (ddd, J = 11.0, 11.0, 5.0 Hz, 1H), 5.23 (ddd, J = 7.0, 5.0, 2.4 Hz, 1H), 5.10 (d, J = 9.9, 1H), 4.80 (d, J = 12.1 Hz, 1H), 4.64 (dd, J = 12.5, 2.4 Hz, 1H), 4.42 (quin, J = 11.8 Hz, 3H), 4.06 (dd, J = 12.5, 7.0 Hz, 1H), 4.01 (dd, J = 10.7, 1.7 Hz, 1H), 3.70−3.58 (m, 4H), 2.55 (dd, J = 12.9, 5.0 Hz, 1H), 2.08 (s, 3H), 1.95 (s, 3H), 1.91 (s, 3H), 1.83−1.76 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.63 (C), 170.45 (C), 170.08 (C), 167.29 (C), 154.21 (C), 136.53 (C), 128.52 (CH), 127.91 (CH), 127.28 (CH), 98.36 (C), 95.41 (C), 74.48 (CH_2), 71.17 (CH), 68.45 (CH), 68.30 (CH), 68.67 (CH_2), 62.15 (CH_2), 52.67 (CH_3), 51.71 (CH), 37.48 (CH_2), 20.87 (CH_3), 20.78 (CH_3), 20.73 (CH_3); LRMS (FAB; *m*/*z* (%)) 738 ((M + Na + 2)$^+$, 1), 736 ((M + Na)$^+$, 1), 716 ((MH + 2)$^+$, 1), 714 (MH$^+$, 1), 674 (2), 672 (2), 656 (23), 654 (24), 552 (1), 550 (8), 548 (22), 546 (23), 506 (4), 504 (4), 488 (4), 486 (4), 386 (3), 384 (3), 368 (7), 366 (6), 330 (7), 328 (7); HRMS (FAB, MH$^+$) calcd for $C_{28}H_{35}O_{14}NCl_3$ 714.1123, found 714.1114.

Methyl [Benzyl 5-amino-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosid]onate (5). To a solution of compound **11** (0.235 g, 0.329 mmol) in glacial acetic acid (5.0 mL) was added freshly activated zinc dust (1.5 g). The mixture was stirred at room temperature for 3 h, diluted with DCM (80 mL), and filtered. The filtrate was washed with saturated aqueous $NaHCO_3$ solution, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash silica gel chromatography (50−100% gradient EtOAc in hexane) to afford **5** (0.151 g, 84%) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33−7.25 (m, 6H), 5.50 (dd, J = 3.5, 2.0 Hz, 1H), 5.46 (dd, J = 10.0, 2.0 Hz, 1H), 4.80−4.77 (m, 1H), 4.79 (d, J = 8.0 Hz, 1H), 4.44 (d, J = 8.8 Hz, 1H), 4.34 (dd, J = 12.6, 2.0 Hz, 1H), 4.29 (dd, J = 12.6, 3.5 Hz, 1H), 3.97 (d, J = 10.0 Hz, 1H), 3.70 (s, 3H), 2.77 (dd, J = 12.5, 4.6 Hz, 1H), 2.64 (t, J = 10.0 Hz, 1H), 2.19 (s, 3H), 2.18 (s, 3H), 2.11 (s, 3H), 2.07 (s, 3H), 1.78 (t, J = 12.5 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.09 (C), 170.68 (C), 170.42 (C), 170.08 (C), 168.05 (C), 137.34 (C), 128.17 (CH), 127.75 (CH), 127.59 (CH), 98.35 (C), 74.12 (CH), 71.21 (CH), 67.99 (CH), 66.83 (CH), 66.60 (CH_2), 61.98 (CH_2), 52.54 (CH_3), 51.17 (CH), 37.74 (CH_2), 21.11 (CH_3), 20.97 (CH_3), 20.93 (CH_3), 20.77 (CH_3), 20.71 (CH_3). LRMS (FAB; *m*/*z* (%)): 580 ((M + 41)$^+$, 33), 540 (MH$^+$, 35), 480 (8), 432 (5), 372 (5), 192 (8), 126 (8), 91 (100). HRMS (FAB, MH$^+$): calcd for $C_{25}H_{34}O_{12}N_2$ 540.2081, found 540.2090.

Methyl {2-Benzyl-5-glycolylamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-*O*$_{glycolyl}$-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosyl)onate]-α-D-glycero-D-galacto-2-nonulopyranosid}onate (3). To a solution of the compound **4** (192 mg, 0.349 mmol) and compound **5** (200 mg, 0.371 mmol) in acetonitrile (4.0 mL) was added sequentially $NaHCO_3$ (84 mg, 1.0 mmol), HOBt (10 mg, 0.084 mmol), and EDC (100 mg, 0.525 mmol). The resulting mixture was stirred at room temperature for 18 h. The solvent was removed under reduced pressure, and the residue was purified by flash silica gel chromatography (0−2% gradient MeOH in EtOAc) to give compound **3** (0.302 g, 81%) as a white foam. ^{1}H NMR (400 MHz, d_6-benzene): δ 7.43 (d, J = 7.4 Hz, 2H), 7.15−7.12 (m, 2H), 7.04 (t, J = 7.4 Hz, 1H), 6.46 (d, J = 10.1 Hz, 1H), 5.94 (ddd, J = 10.0, 7.4, 2.7 Hz, 1H), 5.72 (dd, J = 7.4, 2.2 Hz, 1H), 5.68 (ddd, J = 7.9, 6.0, 2.5 Hz, 1H), 5.49 (dd, J = 7.9, 2.2 Hz, 1H), 5.30 (ddd, J = 12.3, 10.3, 4.7 Hz, 1H), 5.13 (d, J = 12.2 Hz, 1H), 4.82 (ddd, J = 12.3, 10.7, 4.7 Hz, 1H), 4.78−4.63 (m, 4H), 4.50 (dd, J = 10.7, 2.2 Hz, 1H), 4.41−4.32 (m, 3H), 4.11 (dd, J = 10.7, 2.4 Hz, 1H, 3.47 (s, 3H), 3.18 (s, 3H), 2.94 (dd, J = 12.3, 4.5 Hz, 1H), 2.64 (dd, J = 12.3, 4.5 Hz, 1H), 2.30 (t, J = 12.3 Hz, 1H), 2.18 (s, 3H), 2.16 (s, 3H), 2.14 (s, 3H), 2.07 (s, 3H), 1.98 (t, J = 12.3 Hz, 1H), 1.94 (s, 3H), 1.85 (s, 3H), 1.84 (s, 3H), 1.72 (s, 3H), 1.68 (s, 3H). ^{13}C NMR (100 MHz, d_6-benzene): δ 170.81 (C), 170.69 (C), 170.46 (C), 170.41 (C), 169.73 (C), 169.17 (C), 169.05 (C), 168.76 (C), 138.35 (C), 128.81(CH), 128.68 (CH), 128.56 (CH), 128.14 (C), 99.68 (C), 99.36 (C), 74.01 (CH), 70.26 (CH), 69.41 (CH), 69.36 (CH), 68.66 (CH), 67.83 (CH), 67.61 (CH_2), 64.88 (CH_2), 63.56 (CH_2), 63.26 (CH_2), 53.16 (CH_3), 52.45 (CH_3), 50.07 (CH), 49.29 (CH), 39.29 (CH_2), 37.73 (CH_2), 23.31 (CH_3), 21.51 (CH_3), 31.37 (CH_3), 31.14 (CH_3), 21.09 (CH_3), 20.86 (CH_3), 20.77 (CH_3), 20.67 (CH_3). LRMS (FAB; *m*/*z* (%)): 1093 ((M + Na)$^+$, 3), 1071 (MH$^+$, 4), 1011 (12), 903 (61), 474 (24), 430 (25), 414 (97), 252 (18), 196 (26), 136 (29), 91 (100). HRMS (FAB, MH$^+$): calcd for $C_{47}H_{63}O_{26}N_2$ 1071.3669, found 1071.3677.

Lithium {2-Benzyl-5-glycolylamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-*O*$_{glycolyl}$-[lithium (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosyl)onate]-α-D-glycerol-D-galacto-2-nonulopyranosid}onate (13). To a solution of **3** (180 mg, 0.168 mmol) in pyridine (4.0 mL) was added LiCl (61 mg, 1.44 mmol). The solution was heated at 120 °C for 16 h, and the solvent was removed under reduced pressure. The dark brown residue was purified by flash silica gel chromatography (5−20% gradient MeOH in DCM) to afford the dilithium salt **13** as a white foam (168.5 mg, 95%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 8.20 (d, J = 5.3 Hz, 1H), 7.74 (d, J = 9.8 Hz, 1H), 7.34−7.22 (m, 5H), 5.36 (ddd, J = 7.1, 7.1, 3.0 Hz, 1H), 5.20 (ddd, J = 7.0, 7.0, 2.8 Hz, 1H), 5.11−5.07 (m, 2H), 4.97 (ddd, J = 11.4, 11.4, 4.7 Hz, 1H), 4.87 (ddd, J = 11.4, 11.4, 4.7 Hz, 1H), 4.75 (d, J = 11.3 Hz, 1H), 4.65 (d, J = 12.0 Hz, 1H), 4.47 (d, J = 12.0 Hz, 1H), 4.43−4.37 (m, 2H), 4.19 (dd, J = 12.0, 2.8 Hz, 1H), 4.03 (dd, J = 12.0, 7.0 Hz, 1H), 3.82 (q, J = 11.4 Hz, 1H), 3.78−3.60 (m, 3H), 2.70 (dd, J = 11.4, 4.7 Hz, 1H), 2.44 (dd, J = 11.4, 4.7 Hz, 1H), 2.05 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H), 1.96 (s, 3H), 1.95 (s, 6H), 1.91 (s, 3H), 1.88 (s, 3H), 1.63 (s, 3H), 1.43 (t, J = 11.4 Hz, 1H), 1.39 (t, J = 11.4 Hz, 1H). ^{13}C NMR (100 MHz, d_6-DMSO): δ 170.60 (C), 170.09 (C), 169.68 (C), 169.50 (C), 169.41 (C), 169.15 (C), 139.06 (C), 127.97 (CH), 127.57 (CH), 126.96 (CH), 100.459 (C), 71.14 (CH), 70.78 (CH), 70.25 (CH), 68.79 (CH), 68.32 (CH), 67.98 (CH), 65.44 (CH_2), 62.59 (CH_2), 62.42 (CH_2), 48.97 (CH), 48.66 (CH), 39.13 (CH_2), 38.46 (CH_2), 22.46 (CH_3), 21.10 (CH_3), 20.92 (CH_3), 20.81 (CH_3), 20.67 (CH_3), 20.53 (CH_3). LRMS (FAB; *m*/*z* (%)): 1087 ((M+2Na)$^+$, 0.5), 1061 ((M + 18)$^+$, 2.3), 1055 ((M + 2Li)$^+$, 1), 313 (21), 160 (78), 154 (70), 136 (62), 91 (100). HRMS (FAB, MH$^+$): calcd for $C_{45}H_{61}O_{27}N_2$ 1061.3462, found 1061.3485.

***O*$_{glycolyl}$-(5-Acetamido-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosylonic acid)-(2→5)-2-benzyl-5-glycolylamido-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosonic acid (2).** Compound **13** (168.5 mg, 0.160 mmol) was treated with 0.1 M aqueous NaOH (15 mL) at room temperature for 3 h. The reaction mixture was cooled to 0 °C, neutralized with Amberlite IR-120 (H$^+$) ion-exchange resin, and filtered, and the resin was washed with water (2 × 5 mL). The filtrate was lyophilized to give the crude product as a light brown solid. The crude product was purified by flash silica gel chromatography (20−60% gradient MeOH in DCM) to yield compound **2** as a colorless powder (104 mg, 94%). ^{1}H NMR (500 MHz, D_2O): δ 7.48−7.43 (m, 5H), 4.82 (d, J = 10.9 Hz, 1H), 4.58 (d, J = 10.9 Hz, 1H), 4.36 (d, J = 15.4 Hz, 1H), 4.17 (d, J = 15.4 Hz, 1H), 2.84 (dd, J = 12.4, 4.9 Hz, 1H), 2.80 (dd, J = 12.4, 4.9 Hz, 1H), 2.08 (s, 3H), 1.85 (t, J = 12.4 Hz, 1H), 1.74 (t, J = 12.4 Hz, 1H). ^{13}C NMR (100 MHz, D_2O): δ 175.46 (C), 174.05 (C), 173.62 (C), 173.30 (C), 137.46 (C), 129.19 (CH), 129.12 (CH), 128.74 (CH), 101.38 (C), 100.79 (C), 73.17 (CH), 72.75 (CH), 72.29 (CH), 71.94 (CH), 68.64 (CH), 68.54 (CH), 68.50 (CH), 68.35 (CH), 67.52 (CH_2), 63.49 (CH_2), 63.02 (CH_2), 62.98 (CH_2), 52.23 (CH), 52.16 (CH), 40.92 (CH_2), 39.88 (CH_2), 22.46 (CH_3). ^{1}H NMR (500 MHz, d_6-DMSO): δ 8.50 (d, J = 8.4 Hz, 1H), 8.01 (s, 1H), 7.36−7.27 (m, 5H), 4.74 (d, J = 11.6 Hz, 1H), 4.45 (d, J = 15.8 Hz, 1H), 4.17 (d, J = 15.8 Hz, 1H), 3.98 (d, J = 15.8 Hz, 1H), 2.71 (d, J = 10.8 Hz, 1H), 2.58 (dd, J = 12.3, 4.3 Hz, 1H), 1.89 (s, 3H), 1.62 (t, J = 12.3 Hz, 1H), 1.47 (t, J = 10.8 Hz, 1H). ^{13}C NMR (100 MHz, d_6-DMSO): δ 172.80 (C), 170.90 (C), 170.85 (C), 169.39 (C), 138.16 (C), 128.22 (CH), 127.61 (CH), 127.51 (CH), 99.38 (C), 98.80 (C), 73.93 (CH), 72.79 (CH), 71.81 (CH), 70.98 (CH), 69.61 (CH), 68.46 (CH), 66.38 (CH), 66.05 (CH), 65.57 (CH_2), 63.95 (CH_2), 63.67 (CH_2), 60.83 (CH_2), 53.30 (CH), 52.86 (CH), 40.77 (CH_2), 39.18 (CH_2), 22.52 (CH_3). LRMS (FAB; *m*/*z* (%)): 729 ((M + Na)$^+$, 0.6), 707 (MH$^+$, 0.5), 523 (1.5), 505 (1.2), 154 (100), 136 (70). HRMS (FAB, MH$^+$): calcd for $C_{29}H_{43}O_{18}N_2$ 707.2511, found 707.2515.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectral data for selective compounds **2**, **3**, **7**, **8**, **11**, **12,** and **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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