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Stereoselective Synthesis of Neu5Acα(2→5)Neu5Gc: The Building Block of Oligo/Poly(→5-*O*$_{glycolyl}$Neu5Gcα2→) Chains in Sea Urchin Egg Cell Surface Glycoprotein

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Abstract: The synthesis of a sialic acid dimer derivative, Neu5Acα(2→5)Neu5Gc, is described. The synthetic strategy is based on the use of allyl alcohol to achieve an exclusive α-sialylation product. The allyloxy group is also a latent glycolic acid that provides the subsequent coupling with neuraminate with minimal protection−deprotection manipulations.

The species-specific interactions between sperm and the cell surface molecules of an egg play a central role in the fertilization of many organisms.[1] In the case of sea urchins, the binding of motile sperm with egg jelly coat will trigger an acrosomal reaction for fertilization.[2,3] The recent studies reveal that the egg jelly coat contains polysialylated glycoproteins, in which the polysialic acid chains, (→5-*O*$_{glycolyl}$Neu5Gcα2→)$_n$ (where *n* ranges from 4 to more than 40), incorporate the repeated units of *N*-glycolylneuraminic acid (Neu5Gc) with the novel 2,5-glycoside linkages (Figure 1).[4,5] For the short oligo (→5-Neu5Gcα-2→)$_3$ chain, the nonreducing termini is capped by 9-*O*-sulfated *N*-glycolyneuraminic acids.[3] This sulfated oligosialic acid is a component of a GalNAc-containing *O*-linked glycoprotein. Unlike α2−8- or α2−9-linked polysialic acid chains[6] on bacterial or mammalian cells, the (→5-*O*$_{glycolyl}$Neu5Gcα2→)$_n$ chains are resistant to exo- and endosialidases.[7]

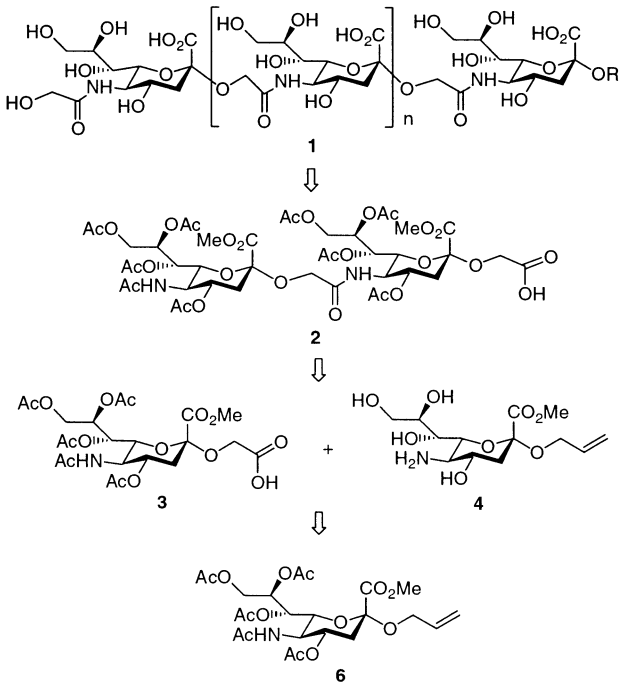

FIGURE 1. Synthetic plan of oligo/poly(→5-*O*$_{glycolyl}$Neu5Gcα2→) chains.

Successful synthesis of the novel (→5-*O*$_{glycolyl}$-Neu5Gcα2→)$_n$ polysialic acids in reasonable quantities would certainly facilitate their relevant biological studies and applications. In addition to the variants of (1→5)oligosialic acid,[8] there is so far only one report on the synthesis of a dimeric sialic acid derivative, Neu5Acα(2→5)-Neu5Gc.[9] The reported method requires many tedious protection−deprotection steps to prepare the thiosialic acid donor and glycolic acid acceptor. Moreover, the coupling reaction between the thiosialic acid derivative and benzyl glycolate affords a mixture of α- and *β*-anomers (3:1). Though the α-anomer can be isolated by column chromatography, it would be desirable to devise an efficient and stereoselective synthesis of glycolylsialic acid in the exclusive α-form. We describe herein an expedient method for the synthesis of Neu5Acα(2→5)-Neu5Gc derivative (**2**) as an approach to oligo/poly(→5-*O*$_{glycolyl}$-Neu5Gcα2→)$_n$.

Our synthetic strategy (Figure 1) is to use 2-allylsialic acid derivative **6** as the pivotal compound leading to the sugar donor **3** and acceptor **4**. Allyl alcohol is chosen for sialylation to ensure a high α-selectivity,[10] and transfor-

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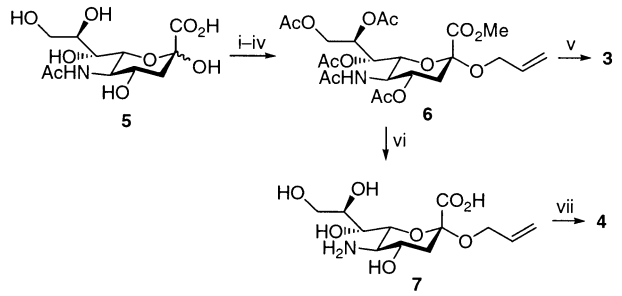
SCHEME 1[a]



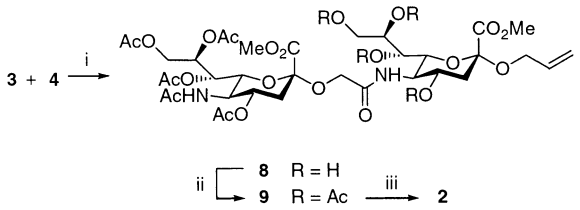
[a] Reagents and conditions: (i) MeOH, cat. TFA, rt, 48 h, 94% yield; (ii) Ac_2O, pyridine, rt, 48 h, 98%; (iii) AcCl, $HCl_{(g)}$, 0 °C, 36 h; (iv) allyl alcohol, silver salicylate, rt, 2 h, 88% overall yield for two steps; (v) $NaIO_4$, cat. $RuCl_3{\cdot}xH_2O$, $CCl_4/CH_3CN/H_2O$, rt, 2 h, 83%; (vi) Me_4NOH, BuOH, 105 °C, 24 h, 97%; (vii) Me_3SiCl, MeOH, rt, 20 h, 62%.

mation of the allyloxy group into glycolic acid moiety can be achieved by an oxidative cleavage of the double bond.[11] According to the known procedure,[12] sialic acid **5** was subjected to esterification and peracetylation. The fully protected sialic acid was then treated in AcCl solution saturated with HCl gas to give the chloro derivative,[13] which was replaced by allyl alcohol in the presence of silver salicylate to give an exclusive α-anomer of compound **6** (Scheme 1).[10] On treatment with $NaIO_4$ and ruthenium trichloride hydrate,[11] an oxidative cleavage of the olefinic double bond in **6** occurred to give acid **3** in 83% yield. On the other hand, hydrolysis of **6** with Bu_4NOH in refluxing butanol afforded the fully deprotected compound **7** (97%).[10b] By using chlorotrimethylsilane as both catalyst and dehydrating agent,[14] the selective esterification of **7** was realized to give **4** in 62% yield. Attempts to prepare ester **4** by direct N,O-deacetylations of **6** with methanesulfonic acid in MeOH[15] resulted in a complicated mixture.

With sialyl acid **3** and sialylamine **4** in hand, we proceeded to study their solution-phase coupling reaction. After meticulous investigations (such as activation of **3** as *N*-succinimidyl ester or by using EDC with/without HOBt and triethylamine), we found that benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) was the coupling reagent of choice. The sialic acid dimer **8** was thus obtained in 70% yield from the coupling reaction of **3** and **4** using PyBOP and Et_3N in DMF solution (Scheme 2). The hydroxyl groups of **8** were protected as acetates, giving **9**, and the subsequent oxidative cleavage of the olefinic double bond[11] afforded the target molecule **2** in 67% yield. The prolonged oxidation should be avoided as it caused further cleavage of the glycosidic bond.

As various C-2-modified mannosamine derivatives have been shown to be the substrates of sialic acid

SCHEME 2[a]



[a] Reagents and conditions: (i) PyBOP, Et_3N, DMF, 0 °C, 2 h, 70%; (ii) Ac_2O, pyridine, rt, 16 h, 80%; (iii) $NaIO_4$, cat. $RuCl_3{\cdot}xH_2O$, $CCl_4/CH_3CN/H_2O$, rt, 20 min, 67%.

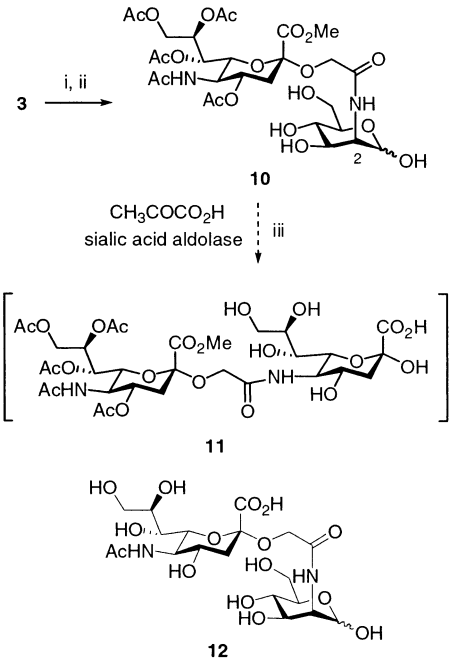


FIGURE 2. Attempted enzymatic synthesis of Neu5Acα-(2→5)Neu5Gc. Reagents and conditions: (i) *N*-hydroxysuccinimide, EDC, CH_2Cl_2, rt, 24 h, 92%; (ii) mannosamine hydrochloric salt, Et_3N, CH_2Cl_2, rt, 20 h, 76%; (iii) pyruvic acid, sialic acid aldolase, phosphate buffer (pH = 7), 37 °C, 36 h.

aldolase in formation of sialic acid derivatives,[16] we also considered the possibility of using the enzymatic method to synthesize Neu5Gc dimers such as compound **11** in Figure 2. Acid **3** was activated as the *N*-succinimidyl ester (92%) and subjected to the coupling reaction with mannosamine to give **10** (76%), Neu5Acα(2→2)Man, under basic conditions. The corresponding acid **12** was obtained in 96% yield by hydrolysis of **10** using a catalytic amount of NaOMe in MeOH. Unfortunately, many attempts failed to effect the coupling reactions of **10** (or **12**) with pyruvic acid by the catalysis of *N*-acetylneuraminic acid aldolase (EC 4.1.3.3, from microorganism). This outcome was presumably because the sialate−mannosamine conjugates **10** and **12** had bulky substituents at C-2 that could not fit into the active site of aldolase.[16, 17]

In conclusion, we have demonstrated in Schemes 1 and 2 a straightforward method for the synthesis of the sialic

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acid dimer derivative **2**, Neu5Acα(2→5)Neu5Gc. The α-anomer of 2-allyl sialate **6** serves as the common precursor of the sugar donor (acid **3**) and acceptor (amine **4**). The allyloxy group is used as a latent glycolic acid upon oxidative cleavage of the olefinic double bond. This method thus provides a route to oligo(→5-$O_{glycolyl}$-Neu5Gcα2→)$_n$ by iterative coupling of **2** with 2-allyl neuraminate and oxidative cleavage of the olefinic double bond. We are currently engaged in this endeavor.

Experimental Section

General Considerations. Chemicals used were reagent grade and were used as supplied except where noted. Ethers were distilled from sodium benzophenone ketyl; (chlorinated) hydrocarbons, and amines from CaH_2. Unless otherwise stated, all reactions requiring anhydrous conditions were performed under an atmosphere of argon. Analytical thin-layer chromatography was performed using silica gel 60 F254 glass plates (Merck); compound spots were visualized by UV light (254 nm) and/or by staining with a yellow solution containing $Ce(NH_4)_2(NO_3)_6$ (0.5 g) and $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (24.0 g) in 6% H_2SO_4 (500 mL) or a red solution containing *p*-anisaldehyde (3.7 mL), acetic acid (15 mL), and concentrated H_2SO_4 (50 mL) in ethanol (1350 mL). Flash column chromatography was performed on silica gel 60 (40−63 μm, Merck). Chemical shifts of ^{1}H and ^{13}C NMR spectra are reported relative to $CDCl_3$ [δ_H 7.24, δ_C (central line of t) 77.0]. Correlation spectroscopy (COSY) was often applied to the NMR peak assignments. Low- and high-resolution mass spectra were recorded under fast atom bombardment (FAB) conditions. Optical rotations were measured on a digital polarimeter with a cuvette of 10 cm length. $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}.

Compounds **6** and **7** were prepared from sialic acid according to the published procedures.[10,12]

Methyl (2-Carboxylmethyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosid)onate (3). To a biphasic solution of compound **6** (0.50 g, 0.94 mmol) and $NaIO_4$ (0.82 g, 3.85 mmol) in CCl_4 (2 mL)/CH_3CN (2 mL)/H_2O (3 mL) was added ruthenium(III) chloride hydrate (0.01 g, 0.05 mmol). The reaction mixture was stirred vigorously at room temperature for 2 h and then diluted with CH_2Cl_2 (10 mL). The aqueous layer was separated and washed with CH_2Cl_2 (20 mL × 3). The combined organic phase was dried over anhydrous $MgSO_4$, filtered over a short pad of Celite, concentrated, and purified by silica gel column chromatography ($CHCl_3$/MeOH, 9:1−2:1, v/v) to give product **3** as a white foam (0.43 g, 83%): TLC (MeOH/$CHCl_3$ (1:4)) R_f = 0.21; $[\alpha]^{23}_D$ −6.2 (*c* 1.2, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.66 (3 H, s), 1.69 (1 H, dd, *J* = 12.4, 12.4 Hz), 1.92 (3 H, s), 1.97 (3 H, s), 2.00 (3 H, s), 2.07 (3 H, s), 2.57 (1 H, dd, *J* = 4.8, 12.4 Hz), 3.67−3.73 (1 H, m), 3.73 (3 H, s), 3.80−3.95 (3 H, m), 4.01 (1 H, dd, *J* = 6.0, 12.4 Hz), 4.17 (1 H, dd, *J* = 3.2, 12.4 Hz), 4.72 (1 H, ddd, *J* = 4.8, 9.6, 12.4 Hz), 5.13 (1 H, dd, *J* = 1.6, 8.4 Hz), 5.24 (1 H, ddd, *J* = 3.2, 6.0, 8.4 Hz), 7.69 (1 H, d, *J* = 9.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.66, 20.66, 20.73, 21.05, 22.94, 37.24, 49.16, 53.23, 62.27, 62.78, 67.03, 68.52, 69.04, 72.18, 98.24, 168.01, 170.03, 170.80, 170.93, 174.05, 171.25, 174.76; HRMS (FAB) calcd for $C_{22}H_{32}NO_{15}$ (M + H$^+$) 550.1772, found 550.1779.

Methyl (2-Allyl-5-amino-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosid)onate (4). To a solution of compound **7** (0.30 g, 1.0 mmol) in dry methanol (5 mL) under an atmosphere of argon was added chlorotrimethylsilane (0.28 mL, 2.2 mmol). The mixture was stirred at room temperature for 20 h. The volatiles were removed under reduced pressure, and the residue was subjected to flash chromatography ($CHCl_3$/MeOH, 29:1−9:1, v/v) to give product **4** as a pale yellow foam (194 mg, 62%): TLC (MeOH/$CHCl_3$/H_2O (5:5:1)) R_f = 0.57; $[\alpha]^{23}_D$ −12.5 (*c* 2.4, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 1.75 (1 H, dd, *J* = 12.4, 12.4 Hz), 2.70 (dd, 1 H, *J* = 4.8, 12.4 Hz), 2.96 (1 H, dd, *J* = 10.0, 10.0 Hz), 3.55 (1 H, ddd, *J* = 4.8, 10.0, 12.4 Hz), 3.71−3.79 (3 H, m), 3.84 (3 H, s), 3.86−3.93 (2 H, m), 3.97 (1 H, dddd, *J* = 1.2, 2.8, 5.6, 12.8 Hz), 4.28 (1 H, dddd, *J* = 1.2, 2.8, 5.6, 12.8 Hz), 5.12 (1 H, dddd, *J* = 1.2, 1.2, 3.2, 10.8 Hz), 5.24 (1 H, dddd, *J* = 1.2, 1.2, 3.2, 17.2 Hz), 5.86 (1 H, dddd, *J* = 5.6, 5.6, 10.8, 17.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 52.70, 53.05, 63.60, 64.21, 68.19, 68.30, 68.45, 71.49, 74.94, 98.35, 116.55, 134.52, 169.38; HRMS (FAB) calcd for $C_{13}H_{24}NO_8$ (M + H$^+$) 322.1502, found 322.1503.

Methyl [2-Allyl-5-glycolylamido-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 (8). Under an atmosphere of argon, benzotriazole-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP, 374 mg, 0.72 mmol) was added in one portion to a solution of acid **3** (200 mg, 0.36 mmol) in anhydrous DMF (0.8 mL) at 0 °C. The resulting solution was stirred at 0 °C for 10 min, and a solution of amine **4** (152 mg, 0.47 mmol) and triethylamine (75 μL, 0.54 mmol) in DMF (1.2 mL) was added slowly. The reaction was stirred at 0 °C for 2 h and then partitioned between EtOAc and water. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, concentrated, and purified by silica gel column chromatography ($CHCl_3$/MeOH, 29:1−9:1, v/v) to give compound **8** as a colorless oil (217 mg, 70%): TLC (MeOH/$CHCl_3$, 1:4) R_f = 0.63; $[\alpha]^{23}_D$ +1.3 (*c* 0.3, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.88 (3 H, s), 1.92 (1 H, dd, *J* = 11.6, 13.2 Hz), 2.01 (3 H, s), 2.02 (3 H, s), 2.04 (1 H, dd, *J* = 9.6, 13.6 Hz), 2.09 (3 H, s, OAc), 2.12 (3 H, s), 2.61 (1 H, dd, *J* = 5.2, 13.6 Hz), 2.83 (1 H, dd, *J* = 4.8, 13.6 Hz), 3.45 (1 H, br), 3.47 (1 H, dd, *J* = 1.6, 10.8 Hz), 3.49−3.53 (1 H, m), 3.69−3.78 (3 H, m), 3.80 (3 H, s), 3.81 (3 H, s), 3.84−3.96 (3 H, m, H-5, H-8), 3.92 (1 H, dddd, *J* = 1.2, 1.2, 5.6, 12.8 Hz), 4.00 (1 H, dd, *J* = 5.6, 12.8 Hz), 4.03 (1 H, dd, *J* = 1.6, 10.8 Hz), 4.11−4.18 (1 H, m), 4.22−4.30 (2 H, m), 4.28 (1 H, dddd, *J* = 1.2, 1.2, 5.6, 12.8 Hz), 4.36 (1 H, dd, *J* = 5.6, 12.8 Hz), 4.53 (1 H, d, *J* = 4.4 Hz), 4.98 (1 H, ddd, *J* = 5.2, 9.6, 9.6 Hz), 5.14 (1 H, dddd, *J* = 1.2, 1.2, 3.2, 10.4 Hz), 5.23 (1 H, dddd, *J* = 1.2, 1.2, 3.2, 17.2 Hz), 5.24−5.30 (2 H, m), 5.33 (1 H, d, *J* = 10.0 Hz), 5.83 (1 H, dddd, *J* = 5.6, 5.6, 10.4, 17.2 Hz), 6.53 (1 H, d, *J* = 8.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD) δ 19.70, 19.70, 19.79, 20.15, 21.58, 36.17, 48.76, 51.88, 52.27, 52.40, 61.74, 62.57, 63.09, 64.66, 66.32, 66.49, 67.58, 68.50, 69.01, 70.55, 71.63, 71.71, 72.83, 98.01, 98.09, 116.22, 133.14, 167.13, 169.25, 169.73, 170.25 (2×), 171.00, 171.50, 171.64; HRMS (FAB) calcd for $C_{35}H_{53}N_2O_{22}$ (M + H$^+$) 853.3090, found 853.3102.

Methyl [2-Allyl-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 (9). To a solution of compound **8** (217 mg, 0.25 mmol) in pyridine (2 mL) was added neat acetic anhydride (3 mL) dropwise of over a period of 1 min at 0 °C. The mixture was stirred at room temperature for 16 h, concentrated under reduced pressure, and then partitioned between EtOAc and water. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, concentrated, and purified by silica gel column chromatography ($CHCl_3$/MeOH, 29:1−9:1, v/v) to give product **9** as a pale yellow oil (208 mg, 80%): TLC (MeOH/$CHCl_3$, 1:9) R_f = 0.68; $[\alpha]^{23}_D$ −8.5 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.84 (3 H, s), 1.93 (1 H, dd, *J* = 12.4, 12.4 Hz), 1.98 (1 H, dd, *J* = 12.8, 12.8 Hz), 1.98 (3 H, s), 2.00 (9 H, s), 2.07 (3 H, s), 2.08 (3 H, s), 2.09 (3 H, s), 2.11 (3 H, s), 2.61 (1 H, dd, *J* = 4.8, 12.8 Hz), 2.62 (1 H, dd, *J* = 4.8, 12.4 Hz), 3.75 (3 H, s), 3.73−3.77 (1 H, m), 3.81 (3 H, s), 3.80−3.85 (1 H, m), 4.02−4.13 (7 H, m), 4.21−4.26 (2 H, m), 4.27 (1 H, dd, *J* = 2.8, 9.6 Hz), 4.82−4.89 (2 H, m), 5.12 (1 H, ddd, *J* = 1.6, 1.6, 2.8, 10.8 Hz), 5.19 (1 H, dd, *J* = 1.6, 8.0 Hz), 5.24 (1 H, dddd, *J* = 1.6, 1.6, 3.2, 17.2 Hz), 5.28 (2 H, br), 5.36−5.40 (2 H, m), 5.81 (1 H, dddd, *J* = 5.6, 5.6, 10.8, 17.2 Hz), 6.12 (1 H, d, *J* = 10.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.66, 20.68, 20.71, 20.75, 20.77, 20.80, 20.94, 21.05, 23.07, 37.43, 38.06, 48.73, 49.02, 52.61, 53.16, 62.21, 62.55, 63.72, 65.85, 67.14, 67.45, 68.42, 68.55, 68.59, 68.80, 72.55, 72.87, 98.34, 98.45, 117.23, 113.45, 167.58, 168.35, 168.51, 169.85, 169.94, 169.99, 170.17, 170.27, 170.48, 170.53, 170.58, 170.83; HRMS (FAB) calcd for $C_{43}H_{61}N_2O_{26}$ (M + H$^+$) 1021.3513, found 1021.3526.

Methyl [2-Carboxymethyl-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 (2). By a procedure similar to that for compound **3**, a mixture of compound **9** (30 mg, 0.03 mmol) with $NaIO_4$ (25.8 mg, 0.12 mmol) and $RuCl_3 \cdot xH_2O$ (0.3 mg, 1.5 μmol) in CCl_4 (0.1 mL)/CH_3CN (0.1 mL)/H_2O (0.15 mL) was stirred vigorously at room temperature for 20 min. The reaction mixture was extracted with CH_2Cl_2 twice. The combined organic phase was dried over $MgSO_4$ and subjected to silica gel column chromatography ($CHCl_3$/MeOH, 19:1−9:1, v/v) to give the product **2** as a white foam (20.4 mg, 67%): TLC (MeOH/$CHCl_3$ (1:9)) R_f = 0.45; $[\alpha]^{23}_D$ −10.0 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.89 (3 H, s), 1.99−2.08 (2 H, m), 2.03 (3 H, s), 2.04 (3 H, s), 2.05 (3 H, s), 2.05 (3 H, s), 2.13 (3 H, s), 2.13 (3 H, s), 2.14 (3 H, s), 2.14 (3 H, s), 2.67 (1 H, dd, *J* = 4.8, 12.8 Hz), 2.74 (1 H, dd, *J* = 4.8, 12.8 Hz), 3.81−3.84 (1 H, m), 3.83 (3 H, s), 3.87 (3 H, s), 4.04 (1 H, dd, *J* = 6.4, 12.4 Hz), 4.05−4.18 (7 H, m), 4.25 (1 H, dd, *J* = 2.8, 12.4 Hz), 4.28 (1 H, dd, *J* = 2.0, 8.4 Hz), 4.37 (1 H, d, *J* = 18 Hz), 4.91 (1 H, ddd, *J* = 4.8, 9.6, 12.0 Hz), 4.98 (1 H, ddd, *J* = 4.8, 9.6, 12.0 Hz), 5.22 (1 H, dd, *J* = 2.0, 8.8 Hz), 5.25 (1 H, d, *J* = 9.6, 9.6 Hz), 5.33−5.34 (2 H, m), 5.36 (1 H, ddd, *J* = 2.8, 6.4, 8.8 Hz), 6.17 (1 H, d, *J* = 9.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.82, 20.86 (2×), 20.93, 20.96, 21.14, 21.24, 23.29, 37.56, 37.80, 45.38, 48.85, 49.35, 53.22, 53.40, 62.35, 62.72, 63.90, 67.28, 67.32, 68.18, 68.41, 68.64, 68.90, 70.11, 72.84, 73.04, 95.56, 95.57, 167.76, 167.95, 168.78, 170.05, 170.09, 170.12, 170.28, 170.43, 170.62, 170.67, 170.18, 171.00, 199.80; HRMS (MALDI) calcd for $C_{42}H_{59}N_2O_{28}$ ($M + Na^+$) 1061.3073, found 1061.3072.

2-Deoxy-2-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosyl)onate]-mannopyranose (10). To a suspended mixture of D-mannose amine hydrochlorate (50 mg, 0.23 mmol) and triethylamine (64 μL, 0.46 mmol) in anhydrous DMF (0.7 mL) was added the *N*-hydroxysuccinimide-linked compound **3** (150 mg, 0.23 mmol) under Ar at 0 °C. The reaction was stirred at 0 °C to room temperature for 20 h and isolated by silica gel chromatography ($CHCl_3$/MeOH, 49:1−4:1). The product was further purified by gel filtration (LH20, $CHCl_3$/MeOH 1:1) to give the product as colorless foam (125.4 mg, 76%): ^{1}H NMR (400 MHz, $CDCl_3$/MeOH) for α-anomer δ 1.35 (1 H, t, *J* = 7.2 Hz), 1.87 (3 H, s), 1.98 (1 H, t, *J* = 12.6 Hz), 2.03 (3 H, s), 2.04 (3 H, s), 2.15 (3 H, s), 2.16 (3 H, s), 2.73 (1 H, dd, *J* = 4.8, 12.6 Hz), 3.17 (1 H, dd, *J* = 7.2, 7.2 Hz), 3.19 (1 H, dd, *J* = 7.2, 7.2 Hz), 3.54 (1 H, t, *J* = 9.2 Hz), 3.80−3.85 (2 H, m), 3.86 (3 H, s), 3.98−4.18 (5 H, m), 4.23−4.42 (4 H, m), 4.83−4.95 (1 H, m), 5.13 (1 H, d, *J* = 1.6 Hz), 5.32 (1 H, dd, *J* = 2.0, 8.8 Hz), 5.36−5.45 (1 H, m); for β-anomer δ 1.35 (1 H, t, *J* = 7.2 Hz), 1.87 (3 H, s), 1.98 (1 H, t, *J* = 12.6 Hz), 2.03 (3 H, s), 2.04 (3 H, s), 2.15 (3 H, s), 2.16 (3 H, s), 2.73 (1 H, dd, *J* = 4.8, 12.6 Hz), 3.28−3.36 (1 H, m), 3.46 (1 H, t, *J* = 9.2 Hz), 3.70−3.75 (1 H, m), 3.80−3.85 (2 H, m), 3.86 (3 H, s), 3.98−4.18 (5 H, m), 4.23−4.42 (5 H, m), 4.83−4.95 (1 H, m), 5.32 (1 H, dd, *J* = 2.0, 8.8 Hz), 5.36−5.45 (1 H, m, H-8); LRMS (ESI) calcd for $C_{28}H_{43}N_2O_9$ ($M + H^+$) 711.25, found 711.07.

2-Deoxy-2-[methyl (5-acetamido-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosyl)onate]mannopyranose (12). To a solution of compound **10** (42 mg, 0.06 mmol) in anhydrous MeOH (1 mL) under Ar at room temperature was added sodium methoxide (9 mg, 0.18 mmol). The reaction was kept at room temperature for 48 h and purified by gel filtration (P-2, H_2O) to give the product as white foam (30.5 mg, 96%): ^{1}H NMR (400 MHz, MeOH) δ 1.90 (1 H, dd, *J* = 4.8, 12.0 Hz), 1.96 (1 H, dd, *J* = 9.6, 12.4 Hz), 2.14 (3 H, s), 2.86 (1 H, dd, *J* = 4.8, 12.8 Hz), 2.92 (1 H, dd, *J* = 4.8, 12.8 Hz), 3.50−4.30 (30 H, m), 4.80−5.10 (2 H, m); LRMS (ESI) calcd for $C_{19}H_{32}N_2NaO_{15}$ ($M + Na^+$) 551.17, found 551.59.

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Supporting Information Available: ^{1}H and ^{13}C spectra of compounds **2**, **3**, **4**, **8**, and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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