# Stereoselective Synthesis of Neu5Ac $\alpha(2 \rightarrow 5)$ Neu5Gc: The Building Block of Oligo/Poly( $\rightarrow$ 5-O glycoly Neu5Gc $\alpha \mathbf{2} \rightarrow$ ) Chains in Sea Urchin Egg Cell Surface Glycoprotein 

Gang-Ting F an, ${ }^{\text {,T, } \ddagger \text { Chen-Chang } \mathrm{Lee}, ~}{ }^{\dagger}$
Chun-Cheng Lin, ${ }^{*},+\S$ and J im-Min Fang ${ }^{\ddagger}$

Academia Sinica, Institute of Chemistry, Nankang, Taipei 115, Taiwan, Department of Chemistry, National Taiwan University, Taipei 106, Taiwan, and Department of Chemistry, National Changhua University of Education, Changhua 500, Taiwan

> cclin@chem.sinica.edu.tw

Received May 29, 2002


#### Abstract

The synthesis of a sialic acid dimer derivative, Neu5Ac $\alpha(2 \rightarrow 5)$ Neu5Gc, is described. The synthetic strategy is based on the use of allyl alcohol to achieve an exclusive $\alpha$-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 rol e 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 polysial ylated glycoproteins, in which the polysialic acid chains, $\left(\rightarrow 5-\mathrm{O}_{\text {glycoly }} \text { Neu5Gco } 2 \rightarrow\right)_{n}$ (where n ranges from 4 to more than 40), incorporate the repeated units of N -glycolylneuraminic acid ( $\mathrm{Neu5Gc}$ ) with the novel $2,5-$ glycoside linkages (Figure 1).4,5 For the short oligo ( $\rightarrow 5$ -Neu5Gca-2 $\rightarrow)_{3}$ chain, the nonreducing termini is capped by $9-\mathrm{O}$-sulfated N -glycol yneuraminic acids. ${ }^{3}$ This sulfated oligosialic acid is a component of a GalNAc-containing O-linked glycoprotein. Unlike $\alpha 2-8$ - or $\alpha 2-9$-linked polysialic acid chains ${ }^{6}$ on bacterial or mammalian cells, the $\left(\rightarrow 5-\mathrm{O}_{\text {glycoly }} \mathrm{Neu5Gc} \mathrm{\alpha} 2 \rightarrow\right)_{n}$ chains are resistant to exoand endosialidases. ${ }^{7}$

[^0]

FIGURE 1. Synthetic plan of oligo/poly $\left(\rightarrow 5-\mathrm{O}_{\text {glycoly }} \mathrm{Neu} 5 \mathrm{Gc} \alpha 2 \rightarrow\right.$ ) chains.

Successful synthesis of the novel $\left(\rightarrow 5-\mathrm{O}_{\text {glycoly }}-\mathrm{Neu5-}\right.$ $\mathrm{Gc} 2 \rightarrow)_{n}$ polysialic acids in reasonable quantities would certainly facilitate their relevant biological studies and applications. In addition to the variants of ( $1 \rightarrow 5$ )oligosialic acid, ${ }^{8}$ there is so far only one report on the synthesis of a dimeric sialic acid derivative, Neu5Aca(2 $\rightarrow 5$ )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 $\alpha$ - and $\beta$-anomers (3:1). Though the $\alpha$-anomer can be isolated by column chromatography, it would be desirable to devise an efficient and stereoselective synthesis of glycolylsialic acid in the exclusive $\alpha$-form. We describe herein an expedient method for the synthesis of $\mathrm{Neu5Ac} \mathrm{\alpha}(2 \rightarrow 5)$ Neu5Gc derivative (2) as an approach to oligo/poly $(\rightarrow 5$ $\mathrm{O}_{\text {glycaly }}$-Neu5Gca2 $\left.\rightarrow\right)_{n}$.

Our synthetic strategy (Figure 1) is to use 2-allylsialic acid derivative $\mathbf{6}$ as the pivotal compound leading to the sugar donor $\mathbf{3}$ and acceptor 4. Allyl al cohol is chosen for sialylation to ensure a high $\alpha$-selectivity, ${ }^{10}$ and transfor-

[^1]
## SCHEME 1a


a Reagents and conditions: (i) MeOH , cat. TFA, rt, $48 \mathrm{~h}, 94 \%$ yield; (ii) $\mathrm{Ac}_{2} \mathrm{O}$, pyridine, $\mathrm{rt}, 48 \mathrm{~h}, 98 \%$; (iii) $\mathrm{AcCl}, \mathrm{HCl}_{(\mathrm{g})}, 0^{\circ} \mathrm{C}, 36$ h; (iv) allyl alcohol, silver salicylate, rt, $2 \mathrm{~h}, 88 \%$ overall yield for two steps; (v) $\mathrm{NaIO}_{4}$, cat. $\mathrm{RuCl}_{3} \cdot \mathrm{xH}_{2} \mathrm{O}, \mathrm{CCl}_{4} / \mathrm{CH}_{3} \mathrm{CN} / \mathrm{H}_{2} \mathrm{O}, \mathrm{rt}, 2 \mathrm{~h}$, $83 \%$; (vi) $\mathrm{Me} \mathrm{H}_{4} \mathrm{NOH}, \mathrm{BuOH}, 105^{\circ} \mathrm{C}, 24 \mathrm{~h}, 97 \%$; (vii) $\mathrm{Me} \mathrm{SiCl}_{3}, \mathrm{MeOH}$, 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 $\mathbf{5}$ 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 $\alpha$-anomer of compound 6 (Scheme 1). ${ }^{10}$ On treatment with $\mathrm{NaIO}_{4}$ and ruthenium trichloride hydrate, ${ }^{11}$ an oxidative cleavage of the ol efinic double bond in $\mathbf{6}$ occurred to give acid $\mathbf{3}$ in $83 \%$ yield. On the other hand, hydrolysis of 6 with $\mathrm{Bu}_{4}-$ NOH in refluxing butanol afforded the fully deprotected compound $\mathbf{7}$ (97\%). ${ }^{10 \mathrm{~b}}$ By using chlorotrimethylsilane as both catalyst and dehydrating agent, ${ }^{14}$ the selective esterification of $\mathbf{7}$ was realized to give $\mathbf{4}$ in $62 \%$ yield. Attempts to prepare ester $\mathbf{4}$ by direct N,O-deacetylations of $\mathbf{6}$ with methanesulfonic acid in $\mathrm{MeOH}{ }^{15}$ 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 $\mathbf{3}$ as N -succinimidyl ester or by using EDC with/without HOBt and triethylamine), we found that benzotriazol-1yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) was the coupling reagent of choice. The sialic acid dimer 8 was thus obtained in $70 \%$ yield from the coupling reaction of $\mathbf{3}$ and $\mathbf{4}$ using PyBOP and $\mathrm{Et}_{3} \mathrm{~N}$ in DMF solution (Scheme 2). The hydroxyl groups of $\mathbf{8}$ 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

[^2]
## SCHEME 2a


${ }^{\text {a }}$ Reagents and conditions: (i) PyBOP, $\mathrm{Et}_{3} \mathrm{~N}, \mathrm{DMF}, 0^{\circ} \mathrm{C}, 2 \mathrm{~h}$, $70 \%$; (ii) $\mathrm{Ac}_{2} \mathrm{O}$, pyridine, $\mathrm{rt}, 16 \mathrm{~h}, 80 \%$; (iii) $\mathrm{NaIO}_{4}$, cat. $\mathrm{RuCl}_{3} \cdot \mathrm{xH}_{2} \mathrm{O}$, $\mathrm{CCl}_{4} / \mathrm{CH}_{3} \mathrm{CN} / \mathrm{H}_{2} \mathrm{O}, \mathrm{rt}, 20 \mathrm{~min}, 67 \%$.


FIGURE 2. Attempted enzymatic synthesis of Neu5Ac $\alpha$ ( $2 \rightarrow 5$ )Neu5Gc. Reagents and conditions: (i) N-hydroxysuccinimide, EDC, $\mathrm{CH}_{2} \mathrm{Cl}_{2}, \mathrm{rt}, 24 \mathrm{~h}, 92 \%$; (ii) mannosamine hydrochloric salt, $\mathrm{Et}_{3} \mathrm{~N}, \mathrm{CH}_{2} \mathrm{Cl}_{2}, \mathrm{rt}, 20 \mathrm{~h}, 76 \%$; (iii) pyruvic acid, sialic acid aldolase, phosphate buffer ( $\mathrm{pH}=7$ ), $37^{\circ} \mathrm{C}, 36 \mathrm{~h}$.
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 $\mathbf{1 1}$ in Figure 2. Acid $\mathbf{3}$ was activated as the N -succinimidyl ester ( $92 \%$ ) and subjected to the coupling reaction with mannosamine to give 10 (76\%), Neu5Aca(2 $\rightarrow 2$ Man, under basic conditions. The corresponding acid $\mathbf{1 2}$ was obtained in $96 \%$ yield by hydrolysis of $\mathbf{1 0}$ using a catalytic amount of NaOMe in MeOH . Unfortunately, many attempts failed to effect the coupling reactions of $\mathbf{1 0}$ (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 sialatemannosamine conjugates $\mathbf{1 0}$ and $\mathbf{1 2}$ 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

[^3]acid dimer derivative 2, Neu5Aca(2 $\rightarrow 5$ )Neu5Gc. The $\alpha$-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 $\rightarrow 5-\mathrm{O}_{\text {glycolyl }}-\mathrm{Neu5-}$ Gca2 $\rightarrow)_{n}$ 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 $\mathrm{CaH}_{2}$. 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 F 254 glass plates (Merck); compound spots were visualized by UV light (254 nm) and/or by staining with a yellow solution containing $\mathrm{Ce}\left(\mathrm{NH}_{4}\right)_{2^{-}}$ $\left(\mathrm{NO}_{3}\right)_{6}(0.5 \mathrm{~g})$ and $\left(\mathrm{NH}_{4}\right)_{6} \mathrm{M} \mathrm{O}_{7} \mathrm{O}_{24} \cdot 4 \mathrm{H}_{2} \mathrm{O}(24.0 \mathrm{~g})$ in $6 \% \mathrm{H}_{2} \mathrm{SO}_{4}(500$ mL ) or a red solution containing p-anisaldehyde ( 3.7 mL ), acetic acid ( 15 mL ), and concentrated $\mathrm{H}_{2} \mathrm{SO}_{4}(50 \mathrm{~mL}$ ) in ethanol (1350 mL ). Flash column chromatography was performed on silica gel 60 (40-63 $\mu \mathrm{m}$, Merck). Chemical shifts of ${ }^{1 \mathrm{H}}$ and ${ }^{13} \mathrm{C}$ NMR spectra are reported relative to $\mathrm{CDCl}_{3}\left[\delta_{\mathrm{H}} 7.24, \delta_{\mathrm{C}}\right.$ (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 $\mathrm{cm}^{2} \mathrm{~g}^{-1}$.

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- $\alpha$-D-glycero-D-galacto-2-nonulopyranosid)onate (3). To a biphasic solution of compound 6 ( $0.50 \mathrm{~g}, 0.94$ $\mathrm{mmol})$ and $\mathrm{NaIO}_{4}(0.82 \mathrm{~g}, 3.85 \mathrm{mmol})$ in $\mathrm{CCl}_{4}(2 \mathrm{~mL}) / \mathrm{CH}_{3} \mathrm{CN}(2$ $\mathrm{mL}) / \mathrm{H}_{2} \mathrm{O}(3 \mathrm{~mL})$ was added ruthenium(III) chloridehydrate (0.01 $\mathrm{g}, 0.05 \mathrm{mmol})$. The reaction mixture was stirred vigorously at room temperature for 2 h and then diluted with $\mathrm{CH}_{2} \mathrm{Cl}_{2}(10 \mathrm{~mL})$. The aqueous layer was separated and washed with $\mathrm{CH}_{2} \mathrm{Cl}_{2}$ (20 $\mathrm{mL} \times 3$ ). The combined organic phase was dried over anhydrous $\mathrm{MgSO}_{4}$, filtered over a short pad of Celite, concentrated, and purified by silica gel column chromatography $\left(\mathrm{CHCl}_{3} / \mathrm{MeOH}\right.$, $9: 1-2: 1, \mathrm{v} / \mathrm{v}$ ) to give product 3 as a white foam ( $0.43 \mathrm{~g}, 83 \%$ ): $\mathrm{TLC}\left(\mathrm{MeOH} / \mathrm{CHCl}_{3}(1: 4)\right) \mathrm{R}_{\mathrm{f}}=0.21 ;[\alpha]^{23} \mathrm{D}-6.2$ (c 1.2, MeOH ); ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{DMSO}_{6}$ ) $\delta 1.66$ (3 H, s), 1.69 (1 H, dd, J $=12.4,12.4 \mathrm{~Hz}), 1.92(3 \mathrm{H}, \mathrm{s}), 1.97(3 \mathrm{H}, \mathrm{s}), 2.00(3 \mathrm{H}, \mathrm{s}), 2.07$ $(3 \mathrm{H}, \mathrm{s}), 2.57(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=4.8,12.4 \mathrm{~Hz}), 3.67-3.73(1 \mathrm{H}, \mathrm{m})$, $3.73(3 \mathrm{H}, \mathrm{s}), 3.80-3.95(3 \mathrm{H}, \mathrm{m}), 4.01(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=6.0,12.4$ $\mathrm{Hz}), 4.17(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=3.2,12.4 \mathrm{~Hz}), 4.72(1 \mathrm{H}, \mathrm{ddd}, \mathrm{J}=4.8,9.6$, $12.4 \mathrm{~Hz}), 5.13(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=1.6,8.4 \mathrm{~Hz}), 5.24(1 \mathrm{H}, \mathrm{ddd}, \mathrm{J}=3.2$, $6.0,8.4 \mathrm{~Hz}$ ), $7.69(1 \mathrm{H}, \mathrm{d}, \mathrm{J}=9.2 \mathrm{~Hz}) ;{ }^{13} \mathrm{C} \mathrm{NMR}(100 \mathrm{MHz}$, $\left.\mathrm{CDCl}_{3}\right) \delta 20.66,20.66,20.73,21.05,22.94,37.24,49.16,53.23$, 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 $\mathrm{C}_{22} \mathrm{H}_{32} \mathrm{NO}_{15}\left(\mathrm{M}+\mathrm{H}^{+}\right) 550.1772$, found 550.1779.

Methyl (2-Allyl-5-amino-3,5-dideoxy- $\alpha-D-g l y c e r o-D-g a l a c t o-$ 2-nonulopyranosid)onate (4). To a solution of compound 7 ( $0.30 \mathrm{~g}, 1.0 \mathrm{mmol}$ ) in dry methanol ( 5 mL ) under an atmosphere of argon was added chlorotrimethylsilane ( $0.28 \mathrm{~mL}, 2.2 \mathrm{mmol}$ ). 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 $\left(\mathrm{CHCl}_{3} / \mathrm{MeOH}, 29: 1-9\right.$ : 1, v/v) to give product 4 as a pale yellow foam (194 mg, 62\%): $\mathrm{TLC}\left(\mathrm{MeOH} / \mathrm{CHCl}_{3} / \mathrm{H}_{2} \mathrm{O}(5: 5: 1)\right) \mathrm{R}_{\mathrm{f}}=0.57 ;[\alpha]^{23} \mathrm{D}-12.5$ (c 2.4, MeOH ); ${ }^{1} \mathrm{H}$ NMR (400 MHz, CD ${ }_{3} \mathrm{OD}$ ) $\delta 1.75$ ( $1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=12.4$, $12.4 \mathrm{~Hz}), 2.70(\mathrm{dd}, 1 \mathrm{H}, \mathrm{J}=4.8,12.4 \mathrm{~Hz}), 2.96(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=$ $10.0,10.0 \mathrm{~Hz}), 3.55(1 \mathrm{H}$, ddd, J $=4.8,10.0,12.4 \mathrm{~Hz}), 3.71-$ $3.79(3 \mathrm{H}, \mathrm{m}), 3.84(3 \mathrm{H}, \mathrm{s}), 3.86-3.93(2 \mathrm{H}, \mathrm{m}), 3.97(1 \mathrm{H}$, dddd,
$\mathrm{J}=1.2,2.8,5.6,12.8 \mathrm{~Hz}), 4.28(1 \mathrm{H}$, dddd, $\mathrm{J}=1.2,2.8,5.6$, 12.8 Hz ), $5.12(1 \mathrm{H}$, dddd, $\mathrm{J}=1.2,1.2,3.2,10.8 \mathrm{~Hz}), 5.24(1 \mathrm{H}$, dddd, J = 1.2, 1.2, 3.2, 17.2 Hz ), $5.86(1 \mathrm{H}$, dddd, J $=5.6,5.6$, 10.8, 17.2 Hz ); ${ }^{13} \mathrm{C}$ NMR (100 MHz, DMSO-d ${ }_{6}$ ) $\delta$ 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 $\mathrm{C}_{13} \mathrm{H}_{24} \mathrm{NO}_{8}\left(\mathrm{M}+\mathrm{H}^{+}\right)$ 322.1502, found 322.1503.

Methyl [2-Allyl-5-glycolylamido-3,5-dideoxy-5-O glycolyl $^{-}$ [methyl (5-acetamido-4,7,8,9-tetra-0-acetyl-3,5-dideoxy- $\alpha$ -D-glycero-D-galacto-2-nonulopyranosyl)onate]- $\alpha$-D-glycero-D-galacto-2-nonulopyranosid]onate (8). Under an atmosphere of argon, benzotriazole-1-yloxytripyrrol idinophosphonium hexafluorophosphate (PyBOP, $374 \mathrm{mg}, 0.72 \mathrm{mmol}$ ) was added in one portion to a solution of acid $\mathbf{3}(200 \mathrm{mg}, 0.36 \mathrm{mmol})$ in anhydrous DMF $(0.8 \mathrm{~mL})$ at $0^{\circ} \mathrm{C}$. The resulting solution was stirred at 0 ${ }^{\circ} \mathrm{C}$ for 10 min , and a solution of amine 4 ( $152 \mathrm{mg}, 0.47 \mathrm{mmol}$ ) and triethylamine ( $75 \mu \mathrm{~L}, 0.54 \mathrm{mmol}$ ) in DMF ( 1.2 mL ) was added slowly. The reaction was stirred at $0^{\circ} \mathrm{C}$ for 2 h and then partitioned between EtOAc and water. The organic layer was washed with brine, dried over anhydrous $\mathrm{MgSO}_{4}$, concentrated, and purified by silica gel column chromatography ( $\mathrm{CHCl}_{3} / \mathrm{MeOH}$, 29:1-9:1, v/v) to give compound 8 as a colorless oil ( 217 mg , $70 \%$ ): $\mathrm{TLC}\left(\mathrm{MeOH} / \mathrm{CHCl}_{3}, 1: 4\right) \mathrm{R}_{\mathrm{f}}=0.63 ;[\alpha]^{23} \mathrm{D}+1.3\left(\mathrm{c} 0.3, \mathrm{CH}_{2^{-}}\right.$ $\mathrm{Cl}_{2}$ ); ${ }^{1} \mathrm{H}$ NMR ( $400 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 1.88(3 \mathrm{H}, \mathrm{s}), 1.92(1 \mathrm{H}, \mathrm{dd}$, J $=11.6,13.2 \mathrm{~Hz}), 2.01(3 \mathrm{H}, \mathrm{s}), 2.02(3 \mathrm{H}, \mathrm{s}), 2.04(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=$ $9.6,13.6 \mathrm{~Hz}), 2.09(3 \mathrm{H}, \mathrm{s}, \mathrm{OAc}), 2.12(3 \mathrm{H}, \mathrm{s}), 2.61(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}$ $=5.2,13.6 \mathrm{~Hz}), 2.83(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=4.8,13.6 \mathrm{~Hz}), 3.45(1 \mathrm{H}, \mathrm{br})$, $3.47(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=1.6,10.8 \mathrm{~Hz}), 3.49-3.53(1 \mathrm{H}, \mathrm{m}), 3.69-3.78$ ( $3 \mathrm{H}, \mathrm{m}$ ), $3.80(3 \mathrm{H}, \mathrm{s}), 3.81(3 \mathrm{H}, \mathrm{s}), 3.84-3.96$ ( $3 \mathrm{H}, \mathrm{m}, \mathrm{H}-5$, $\mathrm{H}-8$ ), 3.92 ( 1 H , dddd, J = 1.2, 1.2, 5.6, 12.8 Hz ), $4.00(1 \mathrm{H}, \mathrm{dd}$, $\mathrm{J}=5.6,12.8 \mathrm{~Hz}), 4.03(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=1.6,10.8 \mathrm{~Hz}), 4.11-4.18(1$ $\mathrm{H}, \mathrm{m}$ ), 4.22-4.30 ( $2 \mathrm{H}, \mathrm{m}$ ), $4.28(1 \mathrm{H}$, dddd, J = 1.2, 1.2, 5.6, 12.8 Hz ), $4.36(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=5.6,12.8 \mathrm{~Hz}), 4.53(1 \mathrm{H}, \mathrm{d}, \mathrm{J}=4.4$ Hz ), $4.98(1 \mathrm{H}$, ddd, J $=5.2,9.6,9.6 \mathrm{~Hz}), 5.14(1 \mathrm{H}$, dddd, J $=$ 1.2, 1.2, 3.2, 10.4 Hz ), 5.23 ( 1 H , dddd, J $=1.2,1.2,3.2,17.2$ $\mathrm{Hz}), 5.24-5.30(2 \mathrm{H}, \mathrm{m}), 5.33(1 \mathrm{H}, \mathrm{d}, \mathrm{J}=10.0 \mathrm{~Hz}), 5.83(1 \mathrm{H}$, dddd, $\mathrm{J}=5.6,5.6,10.4,17.2 \mathrm{~Hz}), 6.53(1 \mathrm{H}, \mathrm{d}, \mathrm{J}=8.0 \mathrm{~Hz}) ;{ }^{13} \mathrm{C}$ NMR ( $100 \mathrm{MHz}, \mathrm{CDCl}_{3} / \mathrm{CD}_{3} \mathrm{OD}$ ) $\delta 19.70,19.70,19.79,20.15$, $21.58,36.17,48.76,51.88,52.27,52.40,61.74,62.57,63.09,64.66$, $66.32,66.49,67.58,68.50,69.01,70.55,71.63,71.71,72.83,98.01$, 98.09, 116.22, 133.14, 167.13, 169.25, 169.73, 170.25 ( $2 \times$ ), 171.00, 171.50, 171.64; HRMS (FAB) calcd for $\mathrm{C}_{35} \mathrm{H}_{53} \mathrm{~N}_{2} \mathrm{O}_{22}$ (M $+\mathrm{H}^{+}$) 853.3090, found 853.3102.

Methyl [2-Allyl-5-glycolylamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-O ${ }_{\text {glycolyl }}$-[methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy- $\alpha$-D-glycero-D-galacto-2-nonulopyran-osyl)onate]- $\alpha$-D-glycero-D-galacto-2-nonulopyranosid]onate (9). To a solution of compound 8 ( $217 \mathrm{mg}, 0.25 \mathrm{mmol}$ ) in pyridine ( 2 mL ) was added neat acetic anhydride ( 3 mL ) dropwise of over a period of 1 min at $0^{\circ} \mathrm{C}$. 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 $\mathrm{MgSO}_{4}$, concentrated, and purified by silica gel column chromatography ( $\mathrm{CHCl}_{3} / \mathrm{MeOH}, 29: 1-9: 1, \mathrm{v} / \mathrm{v}$ ) to give product 9 as a pale yellow oil ( $208 \mathrm{mg}, 80 \%$ ): TLC ( $\mathrm{MeOH} / \mathrm{CHCl}_{3}$, $1: 9) \mathrm{R}_{\mathrm{f}}=0.68 ;[\alpha]^{23} \mathrm{D}-8.5\left(\mathrm{c} 0.5, \mathrm{CH}_{2} \mathrm{Cl}_{2}\right)$; ${ }^{1} \mathrm{H}$ NMR ( 400 MHz , $\left.\mathrm{CDCl}_{3}\right) \delta 1.84(3 \mathrm{H}, \mathrm{s}), 1.93(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=12.4,12.4 \mathrm{~Hz}$ ), 1.98 ( 1 $\mathrm{H}, \mathrm{dd}, \mathrm{J}=12.8,12.8 \mathrm{~Hz}), 1.98(3 \mathrm{H}, \mathrm{s}), 2.00(9 \mathrm{H}, \mathrm{s}), 2.07(3 \mathrm{H}$, s), $2.08(3 \mathrm{H}, \mathrm{s}), 2.09(3 \mathrm{H}, \mathrm{s}), 2.11(3 \mathrm{H}, \mathrm{s}), 2.61(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=$ $4.8,12.8 \mathrm{~Hz}), 2.62(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=4.8,12.4 \mathrm{~Hz}), 3.75(3 \mathrm{H}, \mathrm{s})$, $3.73-3.77(1 \mathrm{H}, \mathrm{m}), 3.81(3 \mathrm{H}, \mathrm{s}), 3.80-3.85(1 \mathrm{H}, \mathrm{m}), 4.02-4.13$ $(7 \mathrm{H}, \mathrm{m}), 4.21-4.26(2 \mathrm{H}, \mathrm{m}), 4.27(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=2.8,9.6 \mathrm{~Hz})$, 4.82-4.89 ( $2 \mathrm{H}, \mathrm{m}$ ), $5.12(1 \mathrm{H}$, ddd, J $=1.6,1.6,2.8,10.8 \mathrm{~Hz}$ ), $5.19(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=1.6,8.0 \mathrm{~Hz}), 5.24(1 \mathrm{H}, \operatorname{dddd}, \mathrm{J}=1.6,1.6,3.2$, 17.2 Hz ), $5.28(2 \mathrm{H}, \mathrm{br}), 5.36-5.40(2 \mathrm{H}, \mathrm{m}), 5.81(1 \mathrm{H}, \mathrm{dddd}$, J $=5.6,5.6,10.8,17.2 \mathrm{~Hz}), 6.12(1 \mathrm{H}, \mathrm{d}, \mathrm{J}=10.0 \mathrm{~Hz}) ;{ }^{13} \mathrm{C}$ NMR ( $100 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 20.66,20.68,20.71,20.75,20.77,20.80$, 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) cal cd for $\mathrm{C}_{43} \mathrm{H}_{61} \mathrm{~N}_{2} \mathrm{O}_{26}\left(\mathrm{M}+\mathrm{H}^{+}\right)$1021.3513, found 1021.3526.

Methyl [2-Carboxymethyl-5-glycolylamido-4,7,8,9-tetra-0-acetyl-3,5-dideoxy-5-O ${ }_{\text {glycolyl }}$ [methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy- $\alpha$-D-glycero-D-galacto-2-non-ulopyranosyl)onate]- $\alpha-D$-glycero-D-galacto-2-nonulopyranosid]onate (2). By a procedure similar to that for compound 3, a mixture of compound $9(30 \mathrm{mg}, 0.03 \mathrm{mmol})$ with $\mathrm{NaIO}_{4}(25.8$ $\mathrm{mg}, 0.12 \mathrm{mmol})$ and $\mathrm{RuCl}_{3} \cdot \times \mathrm{H}_{2} \mathrm{O}(0.3 \mathrm{mg}, 1.5 \mu \mathrm{~mol})$ in $\mathrm{CCl}_{4}(0.1$ $\mathrm{mL}) / \mathrm{CH}_{3} \mathrm{CN}(0.1 \mathrm{~mL}) / \mathrm{H}_{2} \mathrm{O}(0.15 \mathrm{~mL})$ was stirred vigorously at room temperature for 20 min . The reaction mixture was extracted with $\mathrm{CH}_{2} \mathrm{Cl}_{2}$ twice. The combined organic phase was dried over $\mathrm{MgSO}_{4}$ and subjected to silica gel column chromatography ( $\mathrm{CHCl}_{3} / \mathrm{MeOH}, 19: 1-9: 1, \mathrm{v} / \mathrm{v}$ ) to give the product 2 as a white foam ( $20.4 \mathrm{mg}, 67 \%$ ): TLC ( $\left.\mathrm{MeOH} / \mathrm{CHCl}_{3}(1: 9)\right) \mathrm{R}_{\mathrm{f}}=$ $0.45 ;[\alpha]^{23} \mathrm{D}-10.0\left(\mathrm{c} 1.0, \mathrm{CH}_{2} \mathrm{Cl}_{2}\right)$; ${ }^{1 \mathrm{H}} \mathrm{NMR}\left(400 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta$ $1.89(3 \mathrm{H}, \mathrm{s}), 1.99-2.08(2 \mathrm{H}, \mathrm{m}), 2.03(3 \mathrm{H}, \mathrm{s}), 2.04(3 \mathrm{H}, \mathrm{s})$, $2.05(3 \mathrm{H}, \mathrm{s}), 2.05(3 \mathrm{H}, \mathrm{s}), 2.13(3 \mathrm{H}, \mathrm{s}), 2.13(3 \mathrm{H}, \mathrm{s}), 2.14(3 \mathrm{H}$, s), $2.14(3 \mathrm{H}, \mathrm{s}), 2.67(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=4.8,12.8 \mathrm{~Hz}), 2.74(1 \mathrm{H}, \mathrm{dd}$, $\mathrm{J}=4.8,12.8 \mathrm{~Hz}), 3.81-3.84(1 \mathrm{H}, \mathrm{m}), 3.83(3 \mathrm{H}, \mathrm{s}), 3.87(3 \mathrm{H}$, s), $4.04(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=6.4,12.4 \mathrm{~Hz}), 4.05-4.18(7 \mathrm{H}, \mathrm{m}), 4.25(1$ $\mathrm{H}, \mathrm{dd}, \mathrm{J}=2.8,12.4 \mathrm{~Hz}$ ), $4.28(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=2.0,8.4 \mathrm{~Hz}), 4.37(1$ $\mathrm{H}, \mathrm{d}, \mathrm{J}=18 \mathrm{~Hz}$ ), $4.91(1 \mathrm{H}$, ddd, $\mathrm{J}=4.8,9.6,12.0 \mathrm{~Hz}), 4.98(1$ H , ddd, J = 4.8, 9.6, 12.0 Hz ), $5.22(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=2.0,8.8 \mathrm{~Hz}$ ), $5.25(1 \mathrm{H}, \mathrm{d}, \mathrm{J}=9.6,9.6 \mathrm{~Hz}), 5.33-5.34(2 \mathrm{H}, \mathrm{m}), 5.36(1 \mathrm{H}$, ddd, J = 2.8, 6.4, 8.8 Hz ), $6.17(1 \mathrm{H}, \mathrm{d}, \mathrm{J}=9.6 \mathrm{~Hz})$; ${ }^{13} \mathrm{C}$ NMR $\left(100 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta 20.82,20.86(2 \times), 20.93,20.96,21.14,21.24$, 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 $\mathrm{C}_{42} \mathrm{H}_{59} \mathrm{~N}_{2} \mathrm{O}_{28}\left(\mathrm{M}+\mathrm{Na}^{+}\right)$1061.3073, found 1061.3072.

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

2-Deoxy-2-[methyl (5-acetamido-3,5-dideoxy- $\alpha$-D-glycero-D-galacto-2-nonulopyranosyl )onate]mannopyranose (12). To a solution of compound $\mathbf{1 0}$ ( $42 \mathrm{mg}, 0.06 \mathrm{mmol}$ ) in anhydrous $\mathrm{MeOH}(1 \mathrm{~mL})$ under Ar at room temperature was added sodium methoxide ( $9 \mathrm{mg}, 0.18 \mathrm{mmol}$ ). The reaction was kept at room temperature for 48 h and purified by gel filtration ( $\mathrm{P}-2, \mathrm{H}_{2} \mathrm{O}$ ) to give the product as white foam ( $30.5 \mathrm{mg}, 96 \%$ ): ${ }^{1} \mathrm{H}$ NMR ( 400 $\mathrm{MHz}, \mathrm{MeOH}) \delta 1.90(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=4.8,12.0 \mathrm{~Hz}), 1.96(1 \mathrm{H}, \mathrm{dd}$, $\mathrm{J}=9.6,12.4 \mathrm{~Hz}), 2.14(3 \mathrm{H}, \mathrm{s}), 2.86(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=4.8,12.8 \mathrm{~Hz})$, $2.92(1 \mathrm{H}, \mathrm{dd}, \mathrm{J}=4.8,12.8 \mathrm{~Hz}), 3.50-4.30(30 \mathrm{H}, \mathrm{m}), 4.80-5.10$ ( $2 \mathrm{H}, \mathrm{m}$ ); LRMS (ESI) calcd for $\mathrm{C}_{19} \mathrm{H}_{32} \mathrm{~N}_{2} \mathrm{NaO}_{15}\left(\mathrm{M}+\mathrm{Na}^{+}\right)$ 551.17, found 551.59.

Acknowledgment. We thank the Academia Sinica, National Science Council in Taiwan (NSC 90-2323-B-001-005 and NSC 90-2113-M-001-056), and National Taiwan University for their financial support.

Supporting Information Available: ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ spectra of compounds $\mathbf{2}, \mathbf{3}, \mathbf{4}, \mathbf{8}$, and $\mathbf{9}$. This material is available free of charge via the Internet at http://pubs.acs.org.
J O025988P


[^0]:    * To whom correspondence should be addressed. Fax: +886-227835007.
    ${ }^{\dagger}$ Academia Sinica.
    $\ddagger$ National Taiwan University.
    § National Changhua University of Education.
    (1) Mengerink, K. J.; Vacquier, V. D. Glycobiology 2001, 11, 37R43R.
    (2) Vacquier, V. D.; Moy, G. W. Proc. Natl. Acad. Sci. U.S.A. 1997, 74, 2456-2460.
    (3) Kitazume-Kawaguchi, S.; Inoue, S.; Inoue, Y.; Lennarz, W. J . Proc. Natl. Acad. Sci. U.S.A. 1997, 94, 3650-3655.
    (4) (a) Lennarz, W. J. In Sialobiology and Other Novel Forms of Glycosylation; Inoue, Y., Lee, Y. C., Troy, F. A., II, Eds.; Gakushin Publishing Co.: Osaka, 1999; pp 1-5. (b) Kitazume, S.; Kitajima, K.; I noue, S.; Haslam, S. M.; M orris, H. R.; Dell, A.; Lennarz, W. J .; Inoue, Y. J. Biol. Chem. 1996, 271, 6694-6701.
    (5) Kitazume, S.; Kitajima, K.; Inoue, S.; Troy, F. A., II; Cho, J .-W.; Lennarz, W. J.; Inoue, Y. J. Biol. Chem. 1994, 269, 22712-22718.
    (6) Troy, F. A., II. In Biology of the Sialic Acids; Rosenberg, A., Ed.; Plenum Press: New York, 1995; pp 95-144.

[^1]:    (7) Kitazume, S.; Kitajima, K.; Inoue, S.; Troy, F. A., II; Lennarz, W. J.; Inoue, Y. Biochem. Biophys. Res. Commun. 1994, 205, 893898.
    (8) (a) Szabo, L.; Smith, B. L.; McReynolds, K. D.; Parrill, A. L.; Morris, E. R.; Gervay, J. J. Org. Chem. 1998, 63, 1074-1078. (b) Ramamoorthy, P. S.; Gervay, J. J. Org. Chem. 1997, 62, 7801-7805. (c) Gervay, J .; Flaherty, T. M. Nguyen, C. Tetrahedron Lett. 1997, 38, 1493-1496.
    (9) Ren, C.-T.; Chen, C.-S.; Wu, S.-H. J . Org. Chem. 2002, 67, 13761379.
    (10) (a) van der Vleugel, D. J. M.; van Heeswijk, W. A. R.; VliegentHart, J. F. G. Carbohydr. Res. 1982, 102, 121-130. (b) Roy, R.; Laferriére, C. A. Can. J. Chem. 1990, 68, 2045-2054.

[^2]:    (11) Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. J . Org. Chem. 1981, 46, 3936-3938. For a recent method, see: Travis, B. R.; Narayan, R. S.; Borhan, B. J . Am. Chem. Soc. 2002, 124, 38243825.
    (12) (a) Marra, A.; Sinaÿ, P. Carbohydr. Res. 1989, 190, 317-322. (b) Marra, A.; Sinay, P. Carbohydr. Res. 1989, 187, 35-42.
    (13) Chappell, M. D.; Halcomb, R. L. Tetrahedron 1997, 53, 1110911120.
    (14) Brook, M. A.; Chan, T. H.Synthesis 1983, 201-203.
    (15) Sugata, T.; Kan, Y.; Nagaregawa, Y.; Miyamoto, T.; Higuchi, R. J. Carbohydr. Chem. 1997, 16, 917-925.

[^3]:    (16) Lin, C.-C.; Lin, C.-H.; Wong, C.-H. Tetrahedron Lett. 1997, 38, 2649-2652.
    (17) Fitz, W.; Schwark, J .-R.; Wong, C.-H. J. Org. Chem. 1995, 60, 3663-3670.

