

Studies on Sialic Acids. XXX. Synthesis of 2,7-Anhydrosialic Acid

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N-Acetyl- and *N*-glycolyl-2,7-anhydroneuraminic acid were synthesized from methyl 5-acetamido-3,5-dideoxy-2-thio- α -D-glycero-D-galacto-2-nonulopyranosonate in high yield. The structures and stereochemistry of these glycosanic sialic acids were elucidated from $^1\text{H-NMR}$ spectra and X-ray crystal analysis. The ring systems of *N*-acetyl- and *N*-glycolyl-2,7-anhydroneuraminic acid (**1** and **10**) were determined to have the same $^2\text{C}_5(\text{D})$ conformation.

Keywords sialic acid; *N*-acetyl-2,7-anhydroneuraminic acid; *N*-glycolyl-2,7-anhydroneuraminic acid; methyl thioglycoside; dimethyl(methylthio)sulfonium triflate (DMTST); glycosan

Introduction

The glycosan of sialic acid, *N*-acetyl-2,7-anhydroneuraminic acid (**1**), was first isolated from cerumen of wet type by Suzuki *et al.*¹⁾ Moreover, Li *et al.*²⁾ recently found that leeches contains novel sialidases which release **1** almost quantitatively from α -sialosylglycoconjugate.

In the present study, we carried out the synthesis of 2,7-anhydrosialic acids for confirmation of the proposed structure as well as for elucidation of the biological significance of **1**.¹⁾ For the formation of the intramolecular glycoside, we employed Hasegawa's procedure using the methyl thioglycoside of a sialic acid derivative³⁾ as a starting material and dimethyl(methylthio)sulfonium triflate (DMTST) as a glycosyl promoter.⁴⁾

The structures of the synthesized derivatives were elucidated mainly on the basis of the 300 MHz proton nuclear magnetic resonance ($^1\text{H-NMR}$) spectra. The proton assignments were based on published data spin-decoupling experiments. Furthermore, the glycosan structure of methyl *N*-acetyl-2,7-anhydroneuraminic acid (**5**) was confirmed by an X-ray crystal analysis.

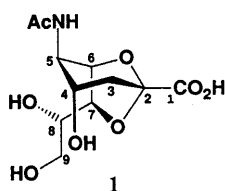


Fig. 1

Results and Discussion

The starting material, methyl 5-acetamido-3,5-dideoxy-2-thio- α -D-glycero-D-galacto-2-nonulopyranosonate (**2**),³⁾ was prepared by the described procedure. The highly regioselective benzylation of two hydroxy groups at the C-4 and C-9 positions in **2** was carried out by the treatment of **2** with benzoic anhydride in pyridine, to give the 4,9-di-*O*-benzoate of **2** (**3**) in 85% yield. The sterically hindered 7-OH and 8-OH were not benzyloylated owing to their low reactivity to benzoic anhydride.⁵⁾

The structure of **3** was confirmed by analysis of the $^1\text{H-NMR}$ spectra, the chemical shifts at δ 5.20 for 4-H, δ 4.51 for 9-H, and δ 4.71 for 9-H' being strongly indicative of the positions di-*O*-benzyloylated.

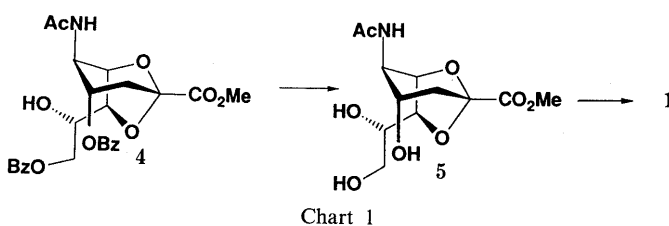
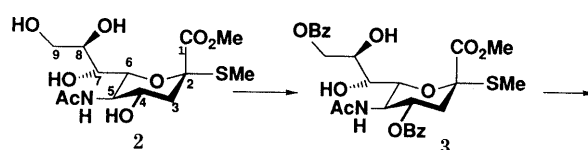


Chart 1

TABLE I. $^1\text{H-NMR}$ Data for **3–5**, and **1**

Proton	3	4	5	1
3-H _{ax}	2.28 dd (12.5, 12.0)		2.14 dd (5.5, 15.5)	2.10 dd (5.5, 15.5)
3-H _{eq}	2.92 dd (4.8, 12.5)	2.30 br s	1.98 br d (15.5)	1.95 d (15.5)
4-H	5.20 ddd (4.8, 10.5, 12.0)	5.17 br s	3.87 br d (5.5)	3.87 d (5.5)
5-H	4.18 dt (7.5, 10.0)	4.38 d (8.5)	3.85 br s	3.84 br s
6-H	3.77 dd (1.6, 10.0)	4.82 br s	4.56 br s	4.51 br s
7-H	3.63 br dd (9.0, 1.6)	4.70 d (8.0)	4.43 d (7.5)	4.38 d (7.5)
8-H	4.27 ddd (9.0, 6.5, 2.5)	3.91 q (3.8)	3.44 ddd (2.5, 6.0, 7.5)	3.43 ddd (2.5, 6.0, 7.5)
9-H	4.51 dd (6.5, 11.5)	4.48 dd (4.5, 11.4)	3.47 dd (6.0, 11.0)	3.48 dd (7.5, 11.0)
9-H'	4.71 dd (2.5, 11.5)	4.61 dd (3.0, 11.5)	3.62 dd (2.5, 11.0)	3.63 dd (2.5, 11.0)
NH	6.17 d (7.5)	6.47 br d (8.5)		
NAc	1.93 s	2.03 s	1.92 s	1.91 s
SMe	2.21 s			
COOMe	3.86 s	3.80 s	3.74 s	
Phenyl group	7.42–8.05	7.13–8.01		

Spectra were measured in CDCl_3 for **3** and **4** and in D_2O for **1** and **5**. Chemical shifts are given in δ (ppm). Coupling constants (Hz) are given in parentheses.

Intramolecular glycosylation of **3** in acetonitrile was performed in the presence of DMTST and molecular sieves 3A to give methyl 5-acetamido-2,7-anhydro-4,9-di-*O*-benzoyl-3,5-dideoxy- α -D-glycero-D-galacto-2-nonulopyranonate (**4**) in 92% yield. The structure of **4** was deduced from a comparison of the spectral data of **3** and **4**. The $^1\text{H-NMR}$ spectra showed the signal due to 7-H at δ 4.70 for **4** and at δ 3.63 for **3**. Furthermore, the vicinal coupling constants on the pyranose ring in **4** were all small, reflecting the structural change in **3**, that is, the conversion of the pyranose ring in **4** from $^2\text{C}_5(\text{D})$ to $^5\text{C}_2(\text{D})$. These results indicated that the intramolecular glycosyl linkage was formed between C-2 and 7-OH.

Debenzoylation of **4** with sodium methoxide in methanol gave methyl 2,7-anhydro-*N*-acetylneuramate (**5**) as a crystalline compound. After saponification of **5** with 0.5 N sodium hydroxide, subsequent treatment with Dowex-50 (H^+) afforded *N*-acetyl-2,7-anhydroneuraminic acid (**1**) in 95% yield as a white powder, which was identical with an authentic sample of natural **1**.^{1,6}

In order to confirm the glycosan structure of **5**, X-ray crystallography was conducted. Figure 2 shows a perspective drawing of **5**. The vicinal coupling constants between the hydrogen atoms on the ring of **5** indicate that the ring has the same conformation both in the crystal and in solution.

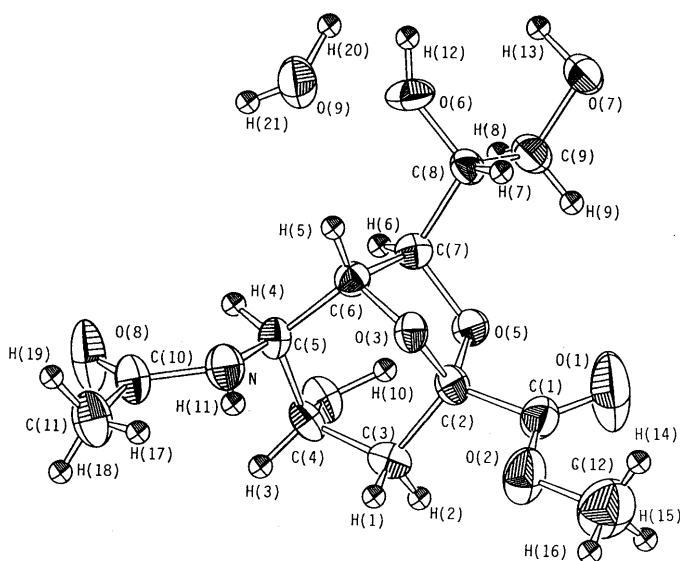


Fig. 2. Crystal Structure of **5**

As an extension of our synthetic studies of 2,7-anhydro-*N*-glycolylneuraminic acid (**10**). Several methods are available for preparing *N*-glycolylneuraminic acid,⁷⁻⁹ and furthermore, we have established the feasibility of synthesizing the partially protected *N*-glycolylneuraminic acid derivative using *N*-acetylneuraminic acid as a starting material.

The *N*-acetyl group in **2** was hydrolyzed with barium hydroxide to give methyl 5-amino-3,5-dideoxy-2-thio- α -D-glycero-D-galacto-2-nonulopyranosidonic acid (**6**) in 85% yield.¹⁰ Treatment of **6** with an activated ester, *N*-succinimidyl *O*-benzylglycolate, gave the *N*-(*O*-benzylglycolyl) derivative of **6**, which was submitted to the next esterification step without isolation. Benzyl esterification was carried out by treating the cesium salt with benzyl bromide in *N,N*-dimethylformamide (DMF) to give benzyl [methyl 5-*N*-(*O*-benzylglycolyl)-3,5-dideoxy-2-thio- α -D-glycero-D-galacto-2-nonulopyranosid]onate (**7**) in 70% yield. Benzoylation of **7** using benzoic anhydride in pyridine also gave regioselectively the 4,9-di-*O*-benzoyl derivative (**8**) in 85% yield. Their structures were elucidated on the basis of the $^1\text{H-NMR}$ spectra as shown in Table I. The *O*-benzoylated positions of **8** were elucidated to be at C-4 and C-9 on the basis of the fact that three deshielded signals due to 4-H, 9-H, and 9-H' appeared at δ 5.32, δ 4.50, and δ 4.69, respectively.

In the same manner as for the synthesis of **4**, treatment of **8** in acetonitrile with DMTST for 17 h at -10°C gave benzyl 2,7-anhydro-4,9-di-*O*-benzoyl-5-*N*-(*O*-benzylglycolyl)-3,5-dideoxy- α -D-glycero-D-galacto-2-nonulopyranosonate (**9**) in 96% yield. The structure of **9** was unambiguously assigned from the $^1\text{H-NMR}$ spectrum. The pyranose ring conformation of **9** was elucidated from the $^1\text{H-NMR}$ coupling constants, which indicated a $^5\text{C}_2(\text{D})$ structure, as shown in Table I. Comparison of the chemical shifts of 7-H in **8** and **9** reveals a significant shift ($\Delta\delta = -1.1$) to lower field for 7-H of **9**, indicating that the intramolecular glycosyl linkage was formed between 2-C and 7-OH.

After saponification of **9** with 1 N sodium hydroxide, the product was subjected to catalytic hydrogenation over palladium on charcoal to afford *N*-glycolyl-2,7-anhydroneuraminic acid (**10**) in good yield. The structure of **10** was assigned on the basis of the $^1\text{H-NMR}$ spectrum as shown in Table II, with characteristic signals of 3- H_{ax} at δ 2.16 (dd, $J=15.0, 5.5$ Hz) and 3- H_{eq} at δ 1.98 (s, $J=15.0$ Hz). The $^1\text{H-NMR}$ spectra show coupling constants consistent

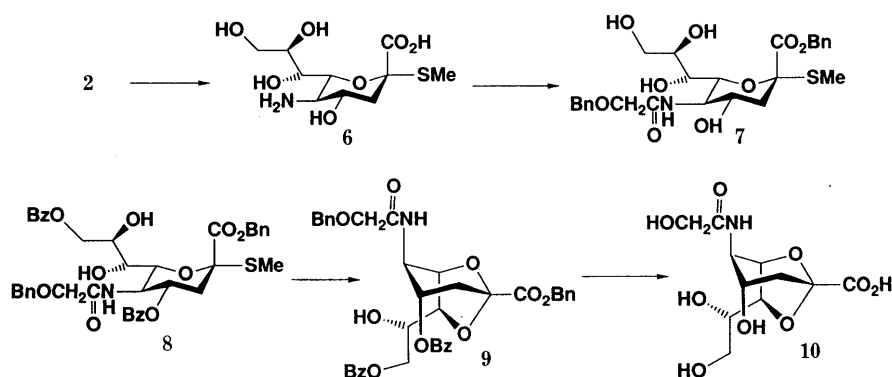


Chart 2

TABLE II. ¹H-NMR Data for 6–9, and 10

Proton	6	7	8	9	10
3-H _{ax}	1.65 dd (11.5, 12.5)	1.96 dd (11.5, 13.0)	2.25 dd (12.5, 11.5)	2.33 dd (15.5, 4.5)	2.16 dd (15.5, 5.5)
3-H _{eq}	2.68 dd (5.0, 12.5)	2.87 dd (4.7, 13.0)	3.00 dd (5.0, 12.5)	2.36 dd (2.0, 15.5)	1.98 br d (15.0)
4-H	3.64 ddd (10.0, 5.0, 11.5)	3.59 ddd (10.5, 4.7, 11.5)	3.52 dt (11.0, 5.0)	5.15 br s	3.91 dt (1.5, 5.5)
5-H	3.09 t (10.0)	3.92 br q (9.0)	4.24 br q (10.0)	4.39 br d (9.0)	3.94 br s
6-H	3.73 dd (10.0, 2.0)	3.37 dd (1.7, 10.5)	3.54 dd (2.0, 9.0)	4.81 br s	4.54 br s
7-H	3.65 dd (8.5, 2.0)	3.54 br d (8.5)	3.57 dd (2.0, 8.5)	4.67 d (7.5)	4.25 d (7.5)
8-H	3.81 ddd (2.5, 5.5, 8.5)	3.84–3.92	4.25 ddd (8.5, 6.5, 2.0)	3.94 m	3.48 ddd (7.5, 6.0, 2.5)
9-H	3.57 dd (12.0, 5.5)	3.71 dd (5.0, 11.5)	4.50 dd (6.5, 11.5)	4.47 dd (4.5, 12.0)	3.51 dd (11.5, 6.0)
9-H'	3.76 dd (12.0, 2.5)	3.84–3.92	4.65 dd (2.0, 11.5)	4.58 dd (12.0, 3.5)	3.68 dd (11.5, 2.5)
NH		6.65 d (8.5)	7.03 d (8.5)	7.21 d (9.0)	
SMe	2.00 s	2.02 s	2.06 s		
CO ₂ CH ₂ Ph		5.20 d (12.0), 5.27 d (12.0)	5.27 d (12.0), 5.36 d (12.0)	5.24 d (12.0), 5.30 d (12.0)	
COCH ₂ O		3.91 d (15.5), 3.98 s	3.83 d (15.5), 4.01 d (15.5)	3.99 d (15.5)	4.02 s
CH ₂ Ph		4.48 d (12.0), 4.53 s	4.45 d (12.0), 4.51 d (12.0)	4.61 d (12.0)	
Phenyl group		7.27–7.40	7.20–8.10	7.15–8.05	

Spectra were measured in CDCl₃ for 7, 8, and 9 and in D₂O for 6 and 10. Chemical shifts are given in δ (ppm). Coupling constants (Hz) are given in parentheses.

with a ⁵C₂(D) conformation of pyranose ring in 10.

In conclusion, we have developed a facile method for the synthesis of 1 and 10 by using the methyl thioglycoside of sialic acid derivatives (3 and 8) and DMTST. The stereochemistry of 2,7-anhydrosialic acid was confirmed by the ¹H-NMR spectra and an X-ray crystal analysis.

Experimental

Melting points were measured with a Yamato melting point apparatus and the results are uncorrected. Optical rotations were measured with a JASCO JIP-4 digital polarimeter. Thin-layer chromatography (TLC) was performed on silica gel (Merck) plates, and spots were detected by spraying with 5% sulfuric acid solution. Infrared (IR) spectra were recorded with a JASCO A-2 spectrometer and NMR spectra on a Varian VXR-300 spectrometer. Tetramethylsilane (TMS) in CDCl₃ or sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) in D₂O was used as an internal reference. Circular dichroism (CD) spectra were measured in a 0.02 cm cell with a JASCO J-720 spectropolarimeter. Column chromatography was conducted on Silica gel 60 (70–230 mesh, Merck).

Methyl (Methyl 5-Acetamido-4,9-di-O-benzoyl-3,5-dideoxy-2-thio-α-D-glycero-D-galacto-2-nonulopyranosid)onate (3) A solution of 2 (170 mg, 0.5 mmol) in dry pyridine (2 ml) containing benzoic anhydride (452 mg, 2 mmol) was kept for 16 h at room temperature. The mixture was poured into 3% sodium hydrogen carbonate solution and extracted with chloroform (10 ml × 2). The extract was evaporated to dryness. The residue was purified on a column of silica gel with chloroform–methanol (50:1) to give 2 (238 mg, 85%) as a colorless powder. $[\alpha]_D^{25} + 36.4^\circ$ (*c* = 1, MeOH). *Anal.* Calcd for C₂₇H₃₁NO₁₀S: C, 57.75; H, 5.56; N, 2.49. Found: C, 57.73; H, 5.59; N, 2.41. IR $\nu_{\max}^{\text{film}} \text{ cm}^{-1}$: 3500, 3360, 1740, 1675, 1605, 1550. ¹H-NMR data are given in Table I.

Methyl 5-Acetamido-2,7-anhydro-4,9-di-O-benzoyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosonate (4) Molecular sieves 3A (0.5 g) was added to a solution of 3 (150 mg, 0.29 mmol) in dry acetonitrile (5 ml), and the mixture was cooled to –40 °C. DMTST (120 mg) was added, and the mixture was kept for 17 h at –10 °C. Methanol (0.5 ml) was added, and the whole was neutralized with triethylamine (0.5 ml), then evaporated to dryness. The residue was dissolved in dichloromethane and the solution was successively washed with 3% sodium hydrogen carbonate solution and brine, dried and concentrated. The residue was purified on a column of silica gel with chloroform–methanol (100:1) to give 4 (126 mg, 92%) as a colorless powder. $[\alpha]_D^{25} + 91.6^\circ$ (*c* = 1, MeOH). *Anal.* Calcd for C₂₄H₂₇NO₁₀: C, 58.89; H, 5.55; N, 2.86. Found: C, 58.85; H, 5.56; N, 2.82. IR $\nu_{\max}^{\text{film}} \text{ cm}^{-1}$: 3350, 1755, 1710, 1670, 1605, 1590, 1540.

Methyl 5-Acetamido-2,7-anhydro-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosonate (5) A 0.1 M sodium methoxide solution (0.5 ml) was added to a solution of 4 (100 mg, 0.19 mmol) in dry methanol (10 ml), and the mixture was kept for 1 h at room temperature, then neutralized with Dowex-50 (H⁺) resin. The resin was filtered off and washed with methanol (20 ml). The filtrate and washings were combined and evaporated to dryness. The residue was crystallized from methanol–ethyl acetate (1:9) to give 5 (54 mg, 91%) as colorless prisms. mp 95–96 °C,

$[\alpha]_D^{16} + 77.8^\circ$ (*c* = 1, MeOH). *Anal.* Calcd for C₁₂H₁₉NO₈·H₂O: C, 44.58; H, 6.55; N, 4.33. Found: C, 44.51; H, 6.73; N, 4.15. IR $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$: 3500, 1745, 1670, 1555. ¹H-NMR data are given in Table I.

5-Acetamido-2,7-anhydro-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosonic Acid (1) A solution of 5 (20 mg, 0.06 mmol) in 0.5 N sodium hydroxide (0.5 ml) was stirred at 35 °C for 3 h. This solution was diluted with water (10 ml), and washings were combined and lyophilized to give a white powder. This was dissolved in a little water, and precipitated with dioxane to give 1 (13 mg, 70%) as a colorless powder. CD (MeOH): $[\theta]_{235}^{23} - 350$, $[\theta]_{213}^{23} + 3550$. *Anal.* Calcd for C₁₁H₁₇NO₈: C, 45.36; H, 5.88; N, 4.81. Found: C, 45.35; H, 5.89; N, 4.83. IR $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$: 3320, 1730, 1620, 1540. ¹H-NMR data are given in Table I.

Methyl 5-Amino-3,5-dideoxy-2-thio-α-D-glycero-D-galacto-2-nonulopyranosidonic Acid (6) A solution of 2 (5 g, 14 mmol) in 5% barium hydroxide (20 ml) was heated for 15 h at 90 °C, and then water (20 ml) was added. The mixture was neutralized with solid carbon dioxide and filtered through Celite. The filtrate was filtered through IRC-50 (H⁺) resin (20 ml) which was washed with water (20 ml), then the filtrate and washings were combined and lyophilized. The residue was purified on a column of Diaion HP20 with methanol–water (4:1) to give 6 (3.57 g, 85%) as a white powder. $[\alpha]_D^{20} + 18.0^\circ$ (*c* = 1, H₂O). *Anal.* Calcd for C₁₀H₁₉NO₇: C, 40.39; H, 6.44; N, 4.71. Found: C, 40.30; H, 6.52; N, 4.65. IR $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$: 3360, 1620. ¹H-NMR data are given in Table II.

Benzyl [Methyl 5-N-(O-Benzylglycolyl)-3,5-dideoxy-2-thio-α-D-glycero-D-galacto-2-nonulopyranosid]onate (7) *N*-Succinimidyl *O*-benzylglycolate (3.16 g, 12 mmol) and triethylamine (3.0 g) were added to a solution of 6 (2.97 g, 10 mmol) in acetonitrile–water (4:1, 20 ml), and the mixture was kept for 16 h at room temperature, and then evaporated to dryness. The residue was dissolved in ethyl acetate (100 ml) and the solution was filtered. The filtrate was evaporated to dryness. This residue was dissolved in DMF (20 ml), and anhydrous cesium carbonate (4.9 g, 15 mmol) and benzyl bromide (5.0 g, 29 mmol) were added. The whole was stirred for 5 h at room temperature and evaporated at 60 °C under a reduced pressure. The residual syrup was purified on a column of silica gel with chloroform–methanol (50:1) to give 7 (3.75 g, 70%) as a colorless powder. $[\alpha]_D^{16} + 14.6^\circ$ (*c* = 1, MeOH). *Anal.* Calcd for C₂₆H₃₃NO₉S: C, 58.31; H, 6.21; N, 2.62. Found: C, 58.28; H, 6.21; N, 2.59. IR $\nu_{\max}^{\text{film}} \text{ cm}^{-1}$: 3400, 1715, 1660, 1540. ¹H-NMR data are given in Table I.

Benzyl [Methyl 4,9-Di-O-benzoyl-5-N-(O-benzylglycolyl)-3,5-dideoxy-2-thio-α-D-glycero-D-galacto-2-nonulopyranosid]onate (8) A solution of 7 (214 mg, 0.4 mmol) in dry pyridine (2 ml) containing benzoic anhydride (0.3 g, 1.3 mmol) was stirred for 16 h at room temperature. The mixture was poured into 3% sodium hydrogen carbonate and extracted with chloroform. The extract was evaporated to dryness. The residue was purified on a column of silica gel with chloroform–methanol (200:1) to give 8 (255 mg, 85%) as a colorless powder. $[\alpha]_D^{16} + 25.0^\circ$ (*c* = 1, MeOH). *Anal.* Calcd for C₄₀H₄₁NO₁₁S: C, 64.59; H, 5.56; N, 1.88. Found: C, 64.57; H, 5.53; N, 1.84. IR $\nu_{\max}^{\text{film}} \text{ cm}^{-1}$: 3500, 3370, 1710, 1665, 1605, 1590, 1535. ¹H-NMR data are given in Table II.

Benzyl 2,7-Anhydro-4,9-di-O-benzoyl-5-N-(O-benzylglycolyl)-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosonate (9) Molecular sieves 3A (0.5 g) was added to a solution of 8 (140 mg, 0.19 mmol) in dry acetonitrile (5 ml), and the mixture was cooled to –40 °C, DMTST (120 mg) was

TABLE III. Positional Parameters and B_{eq} for **5**

Atom	x	y	z	B_{eq}
O(1)	1.0125 (8)	0.9519	1.1006 (6)	8.4 (5)
O(2)	1.0302 (5)	1.209 (2)	1.2364 (5)	4.7 (3)
O(3)	0.7905 (4)	1.039 (2)	1.2497 (4)	2.5 (2)
O(4)	0.8530 (5)	0.462 (2)	1.4130 (4)	3.1 (2)
O(5)	0.8578 (4)	0.677 (2)	1.2028 (4)	2.6 (2)
O(6)	0.5391 (4)	0.647 (2)	1.0899 (4)	3.5 (2)
O(7)	0.6564 (4)	0.441 (2)	0.9130 (4)	3.6 (2)
O(8)	0.6052 (7)	0.854 (2)	1.5906 (5)	5.3 (3)
O(9)	0.4738 (5)	0.282 (2)	1.2073 (4)	4.3 (3)
N	0.6993 (5)	1.034 (2)	1.4587 (5)	3.0 (3)
C(1)	0.9869 (7)	1.018 (2)	1.1866 (6)	3.3 (3)
C(2)	0.8947 (6)	0.893 (2)	1.2541 (5)	2.5 (3)
C(3)	0.9423 (6)	0.849 (2)	1.3745 (6)	2.9 (3)
C(4)	0.8464 (7)	0.716 (2)	1.4323 (5)	2.6 (3)
C(5)	0.7175 (6)	0.816 (2)	1.3988 (5)	2.5 (3)
C(6)	0.6976 (6)	0.872 (2)	1.2753 (5)	2.3 (3)
C(7)	0.7262 (6)	0.660 (2)	1.2028 (5)	2.4 (3)
C(8)	0.6648 (6)	0.662 (2)	1.0862 (5)	2.4 (3)
C(9)	0.7127 (7)	0.458 (2)	1.0221 (5)	3.2 (3)
C(10)	0.6490 (7)	1.034 (2)	1.5570 (6)	3.2 (3)
C(11)	0.6479 (9)	1.269 (2)	1.6156 (7)	4.1 (4)
C(12)	1.116 (1)	1.352 (3)	1.174 (1)	6.4 (6)
H(1)	1.0163	0.7572	1.3771	3.5
H(2)	0.9617	0.9978	1.4103	3.5
H(3)	0.8651	0.7394	1.5107	3.1
H(4)	0.6582	0.7023	1.4178	3.0
H(5)	0.6183	0.9320	1.2533	2.8
H(6)	0.7062	0.5171	1.2389	2.8
H(7)	0.6841	0.8068	1.0499	3.0
H(8)	0.7983	0.4785	1.0183	4.0
H(9)	0.7004	0.3132	1.0606	4.0
H(10)	0.8647	0.4445	1.3297	3.9
H(11)	0.7464	1.0446	1.4770	3.2
H(12)	0.4719	0.6155	1.0508	4.3
H(13)	0.5749	0.3648	0.9260	4.4
H(14)	1.1869	1.2561	1.1628	8.4
H(15)	1.0791	1.4009	1.1053	8.4
H(16)	1.1446	1.4879	1.2165	8.4
H(17)	0.6860	1.3873	1.5755	5.2
H(18)	0.6888	1.2533	1.6870	5.2
H(19)	0.5651	1.3168	1.6220	5.2
H(20)	0.4637	0.2667	1.2718	5.1
H(21)	0.4368	0.1819	1.1379	5.1

added, and the solution was stirred for 17 h at -10°C . Methanol (0.5 ml) was added, and the mixture was neutralized with triethylamine (0.2 ml), evaporated to dryness. The residue was dissolved in dichloromethane and the solution was washed successively with 3% sodium hydrogen carbonate solution and brine, then dried and concentrated. The residue was purified on a column of silica gel with chloroform-methanol (50:1) to give **9** (124 mg, 96%) as a colorless powder. $[\alpha]_D^{16} +51.4^\circ$ ($c=1$, MeOH). *Anal.* Calcd for $\text{C}_{39}\text{H}_{37}\text{NO}_{11}$: C, 67.33; H, 5.36; N, 2.01. Found: C, 67.29; H, 5.40; N, 1.98. IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 3400, 1720, 1680, 1600, 1585, 1525. $^1\text{H-NMR}$ data are given in Table II.

2,7-Anhydro-N-glycolyl-3,5-dideoxy- α -D-glycero-D-galacto-2-nonulopyranosonic Acid (10) A solution of **9** (100 mg, 0.14 mmol) in 1 N sodium hydroxide (0.5 ml) was kept at room temperature for 2 h, then diluted with water (10 ml), and acidified with Dowex-50 (H^+) resin at 0°C . The

resin was filtered off and washed with water (10 ml). The filtrate and washing were combined and lyophilized. The residue was dissolved in water (2 ml) and the solution was washed with ether to remove benzoic acid. The aqueous solution was lyophilized. The residue was dissolved in methanol (10 ml) and treated with hydrogen over 5% palladium on charcoal (0.1 g) for 5 h at room temperature. The solution was filtered through Celite and evaporated to dryness at 20°C . The residue was dissolved in a little water, and precipitated with dioxane to give **10** (31 mg, 70%) as a colorless powder. CD (H_2O): $[\theta]_{220}^{20} +930$, $[\theta]_{248}^{20} +676$. *Anal.* Calcd for $\text{C}_{11}\text{H}_{17}\text{NO}_8$: C, 43.00; H, 5.58; N, 4.56. Found: C, 43.11; H, 5.62; N, 4.48. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3330, 1620, 1550. $^1\text{H-NMR}$ data are given in Table II.

Crystal Data for 5 A crystal with the dimensions of $0.5 \times 0.3 \times 0.3 \text{ mm}^3$ was used for the structure determination. The cell dimensions and diffraction intensities were measured on a Rigaku four-circle diffractometer (AFC-5R), using graphite monochromated MoK_α radiation.

Crystal Data: $\text{C}_{12}\text{H}_{19}\text{NO}_8 \cdot \text{H}_2\text{O}$, monoclinic, space group $P2_1$, $a = 11.034(3)$, $b = 5.622(2)$, $c = 12.190(3) \text{ \AA}$, $\beta = 95.67(2)^\circ$, $V = 752.6(4) \text{ \AA}^3$, $Z = 2$, $D_{\text{calcd}} = 1.427 \text{ g/cm}^3$. In total, 2008 independent reflections with the range of $2\theta < 55.0^\circ$ were collected by use of the $2\theta-\omega$ scan mode with a scanning rate of $16^\circ \text{ min}^{-1}(\omega)$. In total, 1355 independent reflections with $I > 3.0\sigma(I)$ were obtained and corrected for Lorentz and polarization factors, but not for absorption. The structure was solved by direct methods using the program MITHRIL.¹¹ The positions of all hydrogen atoms were located in the difference Fourier map except those of the two methyl groups. Atomic scattering factors were taken from the International Table for X-Ray Crystallography.¹² All calculations were performed using the TEXSAN¹³ crystallographic software package of Molecular Structure Corporation. The final R value was 5.5%. The final atomic parameters are listed in Table III.

As a result, **5** was confirmed to be the glycosan retaining the anomeric configuration of **3** and the ring system was determined to have $^3\text{C}_2$ conformation.

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