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Studies on Sialic Acids. IV. Synthesis of *N*-Acetyl-D-neuraminic Acid *N*-Nucleoside Analogs[1,2]

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Some *N*-acetyl-D-neuraminic acid nucleoside analogs were synthesized. Methyl penta-*O*-acetyl-*N*-acetyl-D-neuraminate (**1**) was treated with trimethylsilyl-pyrimidine or -5-fluoropyrimidine and tin(IV) chloride to give a mixture of α- and β-anomers of *N*-nucleoside analogs. On the other hand, methyl tetra-*O*-acetyl-*N*-acetyl-2-chloro-β-D-neuraminate was allowed to react with trimethylsilyl-pyrimidine or -5-fluoropyrimidine under the Koenigs–Knorr reaction conditions to provide the β-anomer in a fair yield. The stereochemistry of these compounds was confirmed by measurements of the rate of hydrolysis of the glycosidic bond with water.

Keywords——*N*-acetylneuraminic acid; *N*-nucleoside; NMR; CD; hydrolysis rate; configuration

Recently, we reported the stereochemistry, syntheses, and some biological activities of several 2-*O*-glycosyl derivatives of *N*-acetyl-D-neuraminic acid.[3-7] The stereochemistry of these compounds was confirmed by comparing the chemical shifts of the H-3 (eq) double-doublets in the nuclear magnetic resonance (NMR) spectra.

In this paper we wish to report the synthesis of some *N*-glycosides of *N*-acetylneuraminic acid. Methyl 2,4,7,8,9-penta-*O*-acetyl-*N*-acetyl-β-D-neuraminate (**1**) was prepared by the method of Kuhn *et al.*[8] The reaction of the penta-acetate (**1**) with trimethylsilyluracil gave an anomeric mixture (1 : 1) of *N*-glycosides (**3**, **4**) in about 30—40% yield. The α- and β-anomers were separated by chromatography on a Lobar column. The reaction of **1** with 5-fluorotrimethylsilyluracil gave an anomeric mixture (1 : 1), as judged from the NMR spectrum, in 37% yield, but the mixture could not be separated by chromatography on a Lobar column or by thin layer chromatography.

When methyl 2-chloro-4,7,8,9-tetra-*O*-acetyl-β-D-neuraminate (**2**) was used instead of the penta-acetate (**1**) under the conditions of the Koenigs–Knorr reaction, the β-anomers (**4**, **5**) were obtained in about 50% yield. In this case, methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-2,3-dehydro-2-deoxyneuraminate (**6**) was formed in 20—45% yield.

Saponification of these acetates (**3**, **4**, **5**) with 1 N sodium hydroxide afforded the α- and β-anomers of 5-acetamido-2,3,5-trideoxy-2-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-D-*glycero*-D-*galacto*-nonulopyranosonic acid (**7**, **8**) and their 5-fluoro-derivatives (**9**).

The anomeric configuration of *N*-acetylneuraminic acid derivatives can usually be inferred from the chemical shifts of 3-H (eq) and 4-H. It has been shown that for α-anomers the chemical shift of 3-H (eq) ranges from δ 2.6 to 2.8 and that of 4-H is between δ 3.6 and 3.8. For β-anomers these values are δ 2.1—2.5 and 3.9—4.2, respectively.

The stereochemistry of the above products was therefore evaluated from the ^{1}H-NMR spectra. The chemical shifts (0.827 ppm low field shift) at the H-3 (eq) double-doublet of methyl 2,4,7,8,9-penta-*O*-acetyl-*N*-acetyl-α-D-neuraminate and the α- and β-anomer (**3**, **4**) are

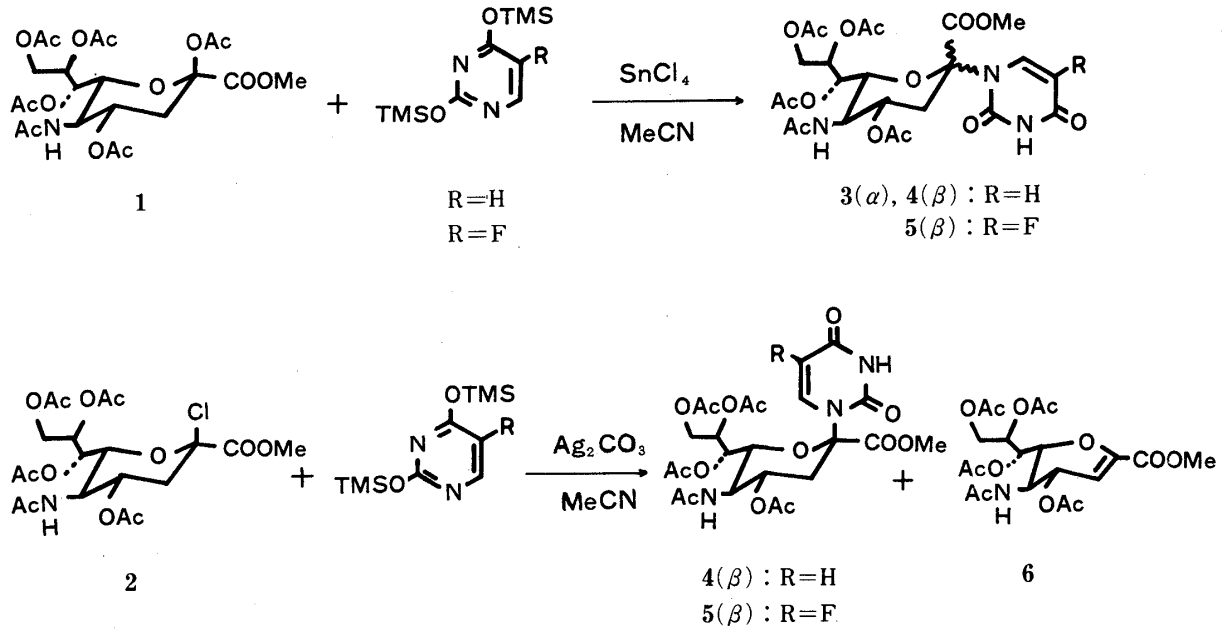


Chart 1

TABLE I. Chemical Shifts of Sialic Acid Derivatives ($CDCl_3$; δ ppm)

	Methyl 2,4,7,8,9-penta-*O*-acetyl-*N*-acetyl-D-neuraminate			**3**	**4**		**7**	**8**	
Anomeric configuration	$\alpha^{a)}$	$\beta^{b)}$	Δ ($\alpha-\beta$)	α	β	Δ ($\alpha-\beta$)	α	β	Δ ($\alpha-\beta$)
3-H_{ax}	1.930	—		2.385	1.830	+0.555	—	1.63	
3-H_{eq}	2.718	2.550	+0.168	3.545	3.300	+0.245	3.05	3.09	−0.04
4-H	4.924	5.258	−0.334	5.110	5.615	−0.505	—	—	

a) Ref. 9. *b*) 400 MHz.

summarized in Table I. As can be seen from the Table, the α-anomer (**3**) gives δ 3.545 for 3-H (eq) and δ 5.110 for 4-H; the β-anomers (**4**, **5**) give δ 3.300 (**4**), 3.26 (**5**) for 3-H (eq) and δ 5.615 (**4**) for 4-H. These values are quite different from those of **1**. The shifts to lower magnetic fields were observed in both α- and β-anomer. This can probably be explained by the anisotropic effect of the heterocyclic moiety at the 2′ position. The difference between the values of α- and β-anomer, [$\Delta(\alpha-\beta)$], was slightly higher than that of **1**, but in the case of the free acids (**7**, **8**) the Δ value became negative (−0.04 ppm), as shown in Table I. In conclusion, the stereochemistry at the anomeric position could not be assessed from the NMR data.

Figure 1 shows the circular dichroism (CD) spectra of the α- and β-anomers of 2-azido-*N*-acetylneuraminic acid, 5-acetamido-2,3,5-trideoxy-2-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-D-*glycero*-D-*galacto*-nonulopyranosonic acid (**7**, **8**), and its 5-fluoro derivative (**9**). The *n*-π^* Cotton effect of the α-anomer of 2-azido-*N*-acetylneuraminic acid had an opposite sign to and corresponded well with that of the β-anomer. However, the CD spectra of the other pair of anomers of the nucleoside did not provide any information about the configuration at the anomeric position of *N*-acetylneuraminic acid.

Measurements of the rate of hydrolysis confirmed the configuration at the anomeric position of *N*-acetylneuraminic acid. Figure 2 shows the rate data for hydrolysis of the three pairs of anomers in water at 80 °C. While the α-anomers decomposed in about 2 h, the β-anomers could not be hydrolyzed in 5 h.

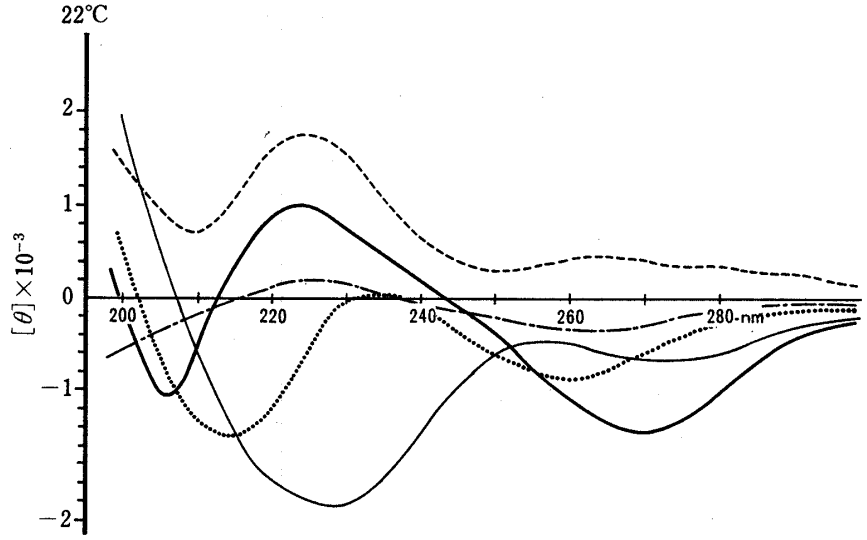


Fig. 1. CD Curves of 2-*N*-Glycoside Derivatives of *N*-Acetylneuraminic Acid

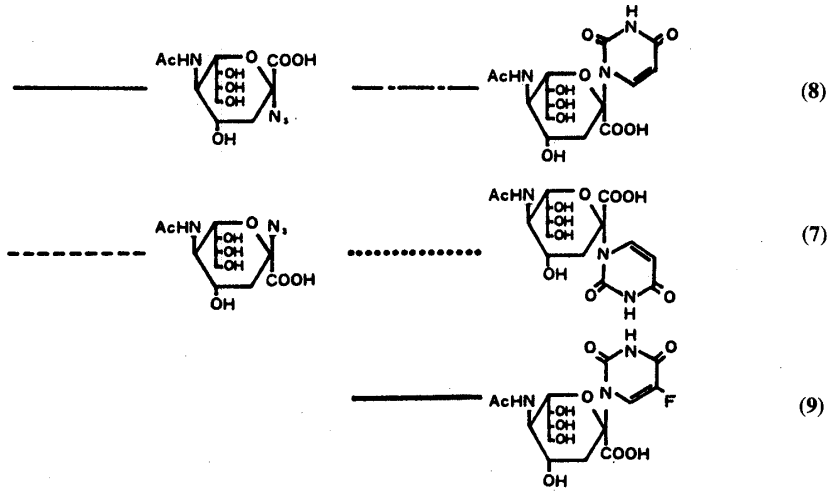
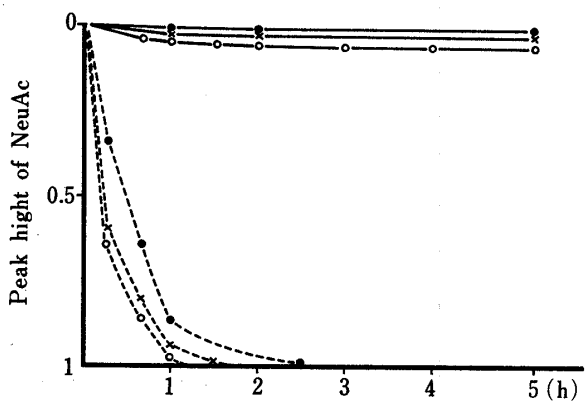
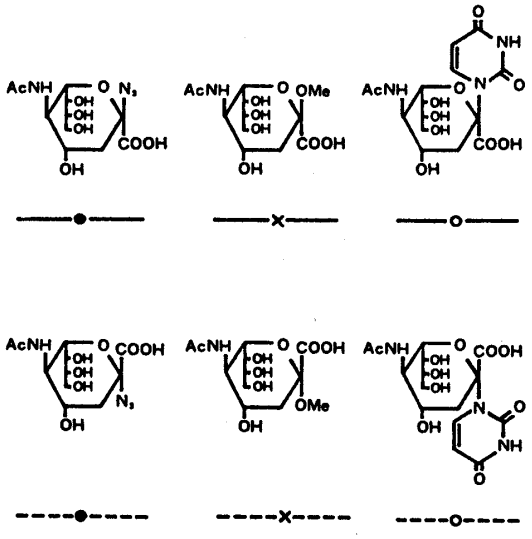


Fig. 2. Hydrolysis of the *N*-Glycosidic Bond of *N*-Acetylneuraminic Acid Derivatives





In conclusion, it is clear that measurement of the rate of hydrolysis is an important means of confirmation of the anomeric configuration.

Experimental

All temperatures are uncorrected. Infrared (IR) spectra were recorded with a JASCO A-2 spectrometer and NMR spectra on Varian EM-390 and XL-300 spectrometers. Tetramethylsilane (TMS) in $CDCl_3$ or sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) in ^2H_2O was used as an internal reference. Optical rotations were measured in a 50 mm cell with a JASCO DIP-181 automatic polarimeter. CD data were obtained with a JASCO J-20 recording polarimeter.

High-Performance Liquid Chromatography (HPLC)——Hydrolysis of the glycoside bond was carried out in water. *N*-Acetylneuraminic acid and its derivatives were analyzed by cation exclusion chromatography using an Aminex HPX-87H strong cation exchange resin column designed for organic acid analysis, 300 × 7.8 mm, at 50 °C (Bio-Rad Laboratories, Richmond, CA, U.S.A.). A mobile phase of 0.006 N sulfuric acid was used at a flow rate of 0.66 ml/min. The column effluent was monitored by an ultraviolet (UV) detector at 206 nm (Nihon Seimitsu Kagaku, model NS-310).

Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-2-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-D-*glycero*-α- and β-D-*galacto*-nonulopyranosonate (3, 4)——a) Tin(IV) chloride (1.04 g, 4 mmol) was added to a solution of methyl penta-*O*-acetyl-*N*-acetyl-D-neuraminate (**1**) (1.067 g, 2 mmol) and trimethylsilylpyrimidine (1.026 g, 4 mmol) in acetonitrile (10 ml). This solution was stirred for 4 d at room temperature, and then sodium hydrogen carbonate (1 g) in water (2 ml) was added under stirring. After evaporation of the reaction mixture under reduced pressure, the residue was extracted with chloroform to yield 0.93 g of white powder. After purification on a Lobar column, the α-anomer (0.12 g; 10%) and the β-anomer (0.15 g; 13%) were each obtained as a fine powder.

α-Anomer (**3**): IR ν_{max}^{KBr} cm^{-1}: 1710, 1530, 1435, 1370. $[\alpha]_D^{22}$ −64.5° (c = 0.63, MeOH). NMR (300 MHz; $CDCl_3$) data are summarized in Table II. MS (FD) m/z: 586 ($M^+ + 1$). *Anal.* Calcd for $C_{24}H_{31}N_3O_{14}$: C, 49.23; H, 5.34; N, 7.18. Found: C, 49.48; H, 5.26; N, 7.13.

β-Anomer (**4**): IR ν_{max}^{KBr} cm^{-1}: 1735, 1540, 1440, 1375. $[\alpha]_D^{18}$ −37.8° (c = 0.87, MeOH). NMR (300 MHz; $CDCl_3$) data are summarized in Table II. MS m/z: 586 ($M^+ + 1$). *Anal.* Calcd for $C_{24}H_{31}N_3O_{14}$: C, 49.23; H, 5.34; N, 7.18. Found: C, 49.11; H, 5.51; N, 7.09.

b) Silver carbonate (4.27 g, 15.47 mmol) was added to a solution of **2** (7.89 g, 15.47 mmol) and trimethylsilylpyrimidine (7.93 g, 30.94 mmol) in acetonitrile (100 ml). This solution was stirred for 48 h at room temperature, then sodium hydrogen carbonate (7.5 g) in water (15 ml) was added under stirring. The whole was filtered, the filtrate was evaporated to dryness under reduced pressure and the residue was extracted with chloroform. The extract yielded 7.96 g of white powder. Purification on a Lobar column with chloroform–methanol gave 2.74 g (30.2%) of the β-anomer (**4**), which was identical with the compound described in a). In this case, the α-anomer (**3**) could not be

Table II. ^{1}H-NMR Data for **3** and **4** in $CDCl_3$ (300 MHz, δ ppm)

Compound	**3**		**4**	
3-H_{ax}	2.385	1H, dd, J = 14.4, 13.2 Hz	1.830	1H, dd, J = 13.5, 11.1 Hz
3-H_{eq}	3.545	1H, dd, J = 4.5, 14.4 Hz	3.300	1H, dd, J = 5.8, 13.5 Hz
4-H	5.110	1H, ddd, J = 4.5, 13.2, 10.5 Hz	5.615	1H, ddd, J = 5.8, 11.1, 10.8 Hz
5-H	4.270	1H, ddd, J = 9.6, 10.5, 11.1 Hz	4.150	1H, ddd, J = 10.5, 10.8, 10.8 Hz
6-H	4.030	1H, dd, J = 11.1, 2.0 Hz	3.920	1H, dd, J = 10.8, 2.2 Hz
7-H	5.370	1H, dd, J = 2.0, 6.2 Hz	5.360	1H, dd, J = 8.4, 2.2 Hz
8-H	5.275	1H, ddd, J = 6.2, 7.2, 2.4 Hz	5.215	1H, ddd, J = 8.4, 6.6, 3.0 Hz
9-H	4.130	1H, dd, J = 7.2, 12.0 Hz	4.050	1H, dd, J = 6.6, 12.9 Hz
9-H	4.565	1H, dd, J = 2.4, 12.0 Hz	4.310	1H, dd, J = 12.9, 3.0 Hz
$NHCOCH_3$	1.900	3H, s	1.920	3H, s
$NHCOCH_3$	6.560	1H, d, J = 9.6 Hz	5.705	1H, d, J = 10.5 Hz
$(COCH_3)_4$	2.060, 2.070 2.110, 2.210	12H	2.020, 2.040 2.070, 2.180	12H
Pyrimidine ring				
$COOCH_3$	3.855	3H, s	3.820	3H, s
5-H	5.740	1H, br d, J = 9.0 Hz	5.850	1H, dd, J = 8.4, 1.5 Hz
6-H	7.665	1H, d, J = 9.0 Hz	7.710	1H, d, J = 8.4 Hz
3-NH	9.580	1H, br s	8.980	1H, d, J = 1.5 Hz

obtained.

Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,4-trideoxy-2-(2,4-dioxo-5-fluoro-1,2,3,4-tetrahydropyrimidin-1-yl)-D-*glycero*-α- and -β-D-*glycero*-nonulopyranosonate (5)——a) Tin(IV) chloride (10 g, 3.8 mmol) was added to a solution of **1** (2.67 g, 5 mmol) and trimethylsilylpyrimidine (2.75 g, 10 mmol) in acetonitrile (20 ml). This solution was stirred for 24 h at room temperature, then sodium hydrogen carbonate (2.5 g) in water (5 ml) was added under stirring. The whole was filtered, the filtrate was evaporated under reduced pressure, and the residue was extracted with chloroform. The extract yielded 2.48 g of pale yellow powder. Purification by silica gel column chromatography gave 1.12 g (37.1%) of white powder. This compound was found to be a 1 : 1 mixture of the α- and β-anomers from the ^{1}H-NMR spectrum. The mixture could not be separated by thin layer chromatography (Merck Kieselgel 60 F254) or Lobar column using a variety of sovent combinations. NMR (90 MHz; $CDCl_3$) δ: α-Anomer: 3.35 (1H, dd, J=13.0, 5.5 Hz, 3′-H), 3.80 (3H, s, $COOCH_3$), 6.38 (1H, d, J=8.7 Hz, $NHCOCH_3$), 7.66 (1H, d, J=6.2 Hz, 6-H); β-Anomer: 3.26 (1H, dd, J=13.0, 5.5 Hz, 3′-H), 3.76 (3H, s, $COOCH_3$), 6.03 (1H, d, J=8.7 Hz, $NHCOCH_3$), 7.80 (1H, d, J=6.2 Hz, 6-H).

b) Silver carbonate (0.55 g, 2 mmol) was added to a solution of **2** (1.02 g, 2 mmol) and 5-fluorotrimethylsilylpyrimidine (1.10 g, 4 mmol) in acetonitrile (20 ml). This solution was stirred for 48 h at room temperature, then sodium hydrogen carbonate (1 g) in water (2 ml) was added under stirring. The whole was filtered, the filtrate was evaporated to dryness under reduced pressure, and the redidue was extracted with chloroform. The extract yielded 1.00 g of white powder. Purification on a Lobar column with chloroform–methanol gave 0.33 g (27.3%) of the β-anomer (**5**) as a fine powder. In this reaction, no α-anomer could be separated. IR ν_{max}^{KBr} cm^{-1}: 1730, 1660, 1550, 1440, 1375. $[\alpha]_D^{25}$ −33.4° (c=1, MeOH).NMR ($CDCl_3$) δ: 1.91 (3H, s, $COCH_3$), 1.95—2.18 (12H, $(COCH_3)_4$), 3.26 (1H, dd, J=13,0, 5.5 Hz, 3′-H_{eq}), 3.76 (3H, s, $COOCH_3$), 6.03 (1H, d, J=8.7 Hz, $NHCOCH_3$), 7.80 (1H, dd, J=6.2 Hz, 6-H), 9.86 (1H, br s, 3-NH). MS (FD) m/z: 603 (M^+), 544 (M^+ − 59). *Anal.* Calcd for $C_{24}H_{30}FN_3O_{14}\cdot 2H_2O$: C, 45.07; H, 5.35; N, 6.57. Found: C, 44.98; H, 5.25; N, 6.42.

Methyl 4.7,8,9-Tetra-*O*-acetyl-*N*-acetyl-2,3-dehydro-2-deoxyneuraminate (**6**) was crystallized from ethanol as needles, mp 132—135 °C (0.43 g; 45%). $[\alpha]_D^{20}$ +40.0° (c=1, MeOH). NMR ($CDCl_3$: C_5D_5N=9 : 1) δ: 1.90 (3H, s, $NCOCH_3$), 2.03 (3H, s, $OCOCH_3$), 2.08 (3H, s, $OCOCH_3$), 2.11 (3H, s, $OCOCH_3$), 2.24 (3H, s, $OCOCH_3$), 3.74 (3H, s, $COOCH_3$), 4.25 (1H, dd, J=12.5, 7.3 Hz, 9-H), 4.42 (1H, dd, J=9.6, 3.2 Hz, 6-H), 4.50 (1H, ddd, J=9.6, 7.9, 8.2 Hz, 5-H), 4.72 (1H, dd, J=12.5, 2.6 Hz, 9-H), 5.39 (1H, ddd, J=4.8, 7.3, 2.8 Hz, 8-H), 5.64 (1H, dd, J=3.1, 7.9 Hz, 4-H), 5.67 (1H, dd, J=3.2, 4.8 Hz, 7-H), 5.96 (1H, d, J=3.1 Hz, 3-H), 8.59 (1H, d, J=8.2 Hz, NH). *Anal.* Calcd for $C_{20}H_{27}NO_{12}$: C, 50.74; H, 5.71; N, 2.96. Found: C, 50.65; H, 5.80; N, 2.84.

5-Acetamido-2,3,5-trideoxy-2-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-D-*glycero*-α- and -β-D-galacto-nonulopyranosonic Acid (7, 8)——A solution of **3** or **4** (0.10 g, 0.17 mmol) in 1 N NaOH (4 ml) was stirred for 2 h at room temperature. This solution was neutralized with Dowex 50 (H^+) and the filtrate was evaporated to dryness under reduced pressure. The α-anomer (94.5%) or β-anomer (96.0%) was obtained as a white powder.

α-Anomer (**7**): IR ν_{max}^{KBr} cm^{-1}: 1680, 1555, 1380. UV $\lambda_{max}^{H_2O}$ nm (log ε): 263 (3.86). $[\alpha]_D^{22}$ −49.2° (c=0.74, MeOH). NMR (D_2O; DSS) δ: 2.06 (3H, s, $NHCOCH_3$), 3.05 (1H, dd, J=5.5, 13.0 Hz, 3-H_{eq}), 5.90 (1H, d, J=7.8 Hz, 5-H), 7.83 (1, d, J=7.8 Hz, 6-H). *Anal.* Calcd for $C_{15}H_{21}N_3O_{10}$: C, 44.67; H, 5.25; N, 10.42. Found: C, 43.30; H, 5.68; N, 9.81.

β-Anomer (**8**): IR ν_{max}^{KBr} cm^{-1}: 1660, 1555, 1390. UV $\lambda_{max}^{H_2O}$ nm (log ε): 263 (3.89). $[\alpha]_D^{19}$ −52.2° (c=1.11, MeOH). NMR (D_2O; DSS) δ: 1.63 (1H, dd, J=11.0, 13.0 Hz, 3′-H_{ax}), 1.98 (3H, s, $NHCOCH_3$), 3.09 (1H, dd, J=5.5, 13.0 Hz, 3′-H_{eq}), 5.87 (1H, d, J=7.8 Hz, 5-H), 8.15 (1H, d, J=7.8 Hz, 6-H). *Anal.* Calcd for $C_{15}H_{21}H_3O_{10}$: C, 44.67; H, 5.25; N, 10.42. Found: C, 44.57; H, 5.43; N, 10.15.

5-Acetamido-2,3,5-trideoxy-2-(5-fluoro-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-D-*glycero*-β-D-*galacto*-nonulopyranosonic Acid (9)——A solution of **6** (0.10 g, 0.17 mmol) in 1 N NaOH (4 ml) was stirred for 2 h at room temperature. The solution was neutralized with Dowex 50 (H^+), then filtered, and the filtrate was evaporated to dryness under reduced pressure to give 0.068 g (97.4%) of **9** as a white powder. IR ν_{max}^{KBr} cm^{-1}: 1660, 1560, 1380. UV $\lambda_{max}^{H_2O}$ nm (log ε): 271 (3.92). $[\alpha]_D^{20}$ −61.8° (c=1.2, MeOH). NMR (D_2O; DSS) δ: 1.61 (1H, dd, J=11.0, 13.0 Hz, 3′-H_{ax}), 2.00 (3H, s, $NHCOCH_3$), 3.08 (1H, dd, J=5.5, 13.0 Hz, 3′-H_{eq}), 8.32 (1H, d, J=6.9 Hz, 6-H). *Anal.* Calcd for $C_{15}H_{20}FN_3O_{10}\cdot H_2O$: C, 38.96; H, 4.79; N, 9.08. Found: C, 38.75; H, 4.83; N, 8.97.

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References and Notes

1) This paper is dedicated to Professor Morio Ikehara for the occasion of his retirement from Osaka University on March 1986.
2) This constitutes Part XXVIII in the series entitled "Studies on Nucleoside Analogs."
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