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## Studies on Sialic Acids. XI. Synthesis of 2-*O*-Glycosyl Derivatives of *N*-Acetylneuraminic Acid

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The reactions of methyl *N*-acetyl-4,7,8,9-tetra-*O*-acetyl-2-chloro-2-deoxy- $\beta$ -D-neuraminate (**2**) with 2',3'-di-*O*-acetylinoine (**1**) and with 2',3'-di-*O*-acetyl-*N*-benzoylcytidine (**8**) under Koenigs-Knorr-like reaction conditions gave the corresponding (2 $\rightarrow$ 5) linked disaccharide nucleoside analogues, in yields of 31% and 23%, respectively. These nucleoside 5'-*N*-acetylneuraminic acid analogues were converted *via* saponification or ammonolysis into the final target compounds. The stereochemistry of these compounds was confirmed by analysis of the proton nuclear magnetic resonance (<sup>1</sup>H-NMR) spectra and measurement of the rate of hydrolysis of the (2 $\rightarrow$ 5) glycosidic linkage.

**Keywords**—*N*-acetylneuraminic acid; Koenigs-Knorr-like reaction; nucleoside 5'-*N*-acetylneuraminic acid; stereochemistry; <sup>1</sup>H-NMR; hydrolysis

We have recently reported<sup>1,2,4-6</sup> the syntheses of several 2-*O*-glycosyl derivatives of *N*-acetylneuraminic acid and the confirmation of the stereochemistry at the anomeric position of the *N*-acetylneuraminic acid moiety of these compounds by <sup>1</sup>H-NMR spectroscopy, as well as the rate of hydrolysis, and the circular dichroism (CD) spectra. Furthermore, we have reported<sup>7,8</sup> that methyl [*N*-acetyl-4'',7'',8'',9''-tetra-*O*-acetyl(5-fluoro-2',3'-isopropylideneuridin-5'-yl)- $\alpha$ -D-neuraminosid]onate (**13**) and methyl [*N*-acetyl-4'',7'',8'',9''-tetra-*O*-acetyl(2',3'-di-*O*-acetylinoine-5'-yl)- $\alpha$ -D-neuraminosid]onate (**3**), among the synthesized compounds, induced suppressor T cells and inhibited the activities of sialyltransferases of murine lymphocytes. It was recently reported<sup>9</sup> that compound **13** also inhibited experimental pulmonary metastasis of mouse colon adenocarcinoma 26 sublines. Thus, the nucleoside 5'-*N*-acetylneuraminic acid analogues may show various inhibitory activities *in vivo*. Therefore, we tried to synthesize these nucleoside 5'-*N*-acetylneuraminic acid analogues in satisfactory yields.

We wish to report herein the synthesis of *N*-acetyl(inosin-5'-yl)- $\alpha$ - and - $\beta$ -D-neuraminosidoic acid (sialosylinoine  $\alpha$ - and  $\beta$ -anomer **6**, **7**) and *N*-acetyl(cytidin-5'-yl)- $\alpha$ - and - $\beta$ -D-neuraminosidoic acid (sialosylcytidine  $\alpha$ - and  $\beta$ -anomer **11**, **12**), as well as the determination of the configuration at the C-2'' position of these compounds by means of the <sup>1</sup>H-nuclear magnetic resonance (<sup>1</sup>H-NMR) spectral analysis and consideration of the rate of acid hydrolysis.

We examined the Koenigs-Knorr-like reaction, using methyl *N*-acetyl-4,7,8,9-tetra-*O*-acetyl-2-chloro-2-deoxy- $\beta$ -D-neuraminate (**2**) as a glycosyl donor, 2',3'-di-*O*-acetylinoine (**1**) as a glycosyl acceptor, and silver trifluoromethanesulfonate (AgOTf), which gave the highest yield in the Koenigs-Knorr-like reaction using **2**,<sup>6</sup> or Hg salt as a catalyst, as shown in Table I. The use of AgOTf as a catalyst and dry *N,N*-dimethylformamide (DMF) as a solvent gave methyl [*N*-acetyl-4'',7'',8'',9''-tetra-*O*-acetyl(2',3'-di-*O*-acetylinoine-5'-yl)- $\alpha$ - and - $\beta$ -D-neur-

aminosid]onate (3 and 4) in the highest yield. Under the same conditions, the Koenigs–Knorr-like reaction of 2',3'-di-*O*-acetylcytidine with 2 also gave methyl [*N*-acetyl-4'',7'',8'',9''-tetra-*O*-acetyl(2',3'-di-*O*-acetyl-*N*-benzoylcytidin-5'-yl)- $\alpha$ - and - $\beta$ -D-neuraminosid]onate (9 and 10) in 23% yield. In addition, methyl *N*-acetyl-4,7,8,9-tetra-*O*-acetyl-2,3-dehydro-2-deoxy-

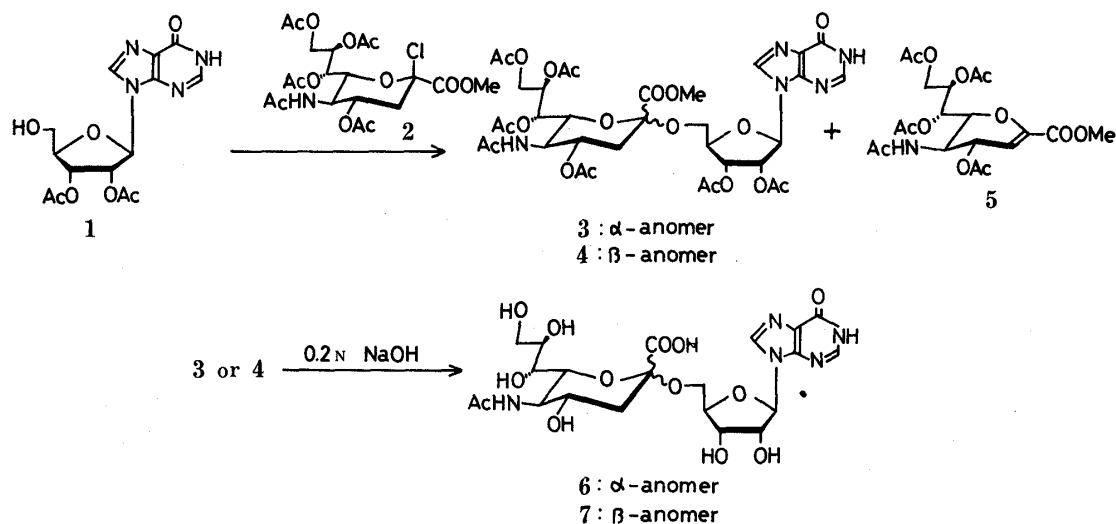


Chart 1

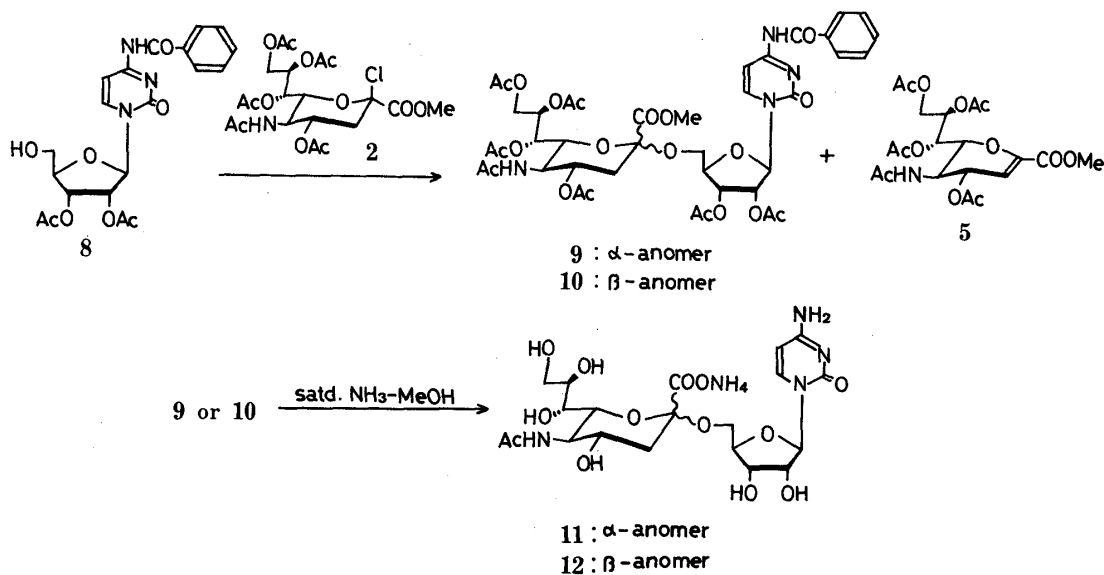


Chart 2

TABLE I. Koenigs–Knorr-like Reaction of 2',3'-Di-*O*-Acetyluridine (1) with 2

Entry	Catalyst	Solvent	Reaction temperature (°C)	Reaction time (d)	Total yield (%)	Ratio of $\alpha$ : $\beta$
1	Hg(CN) <sub>2</sub> /HgBr <sub>2</sub>	CH <sub>3</sub> NO <sub>2</sub> or CH <sub>3</sub> CN	20–25	1	5–6	1:1
2	AgOTf	THF	20–25	1	13	1:2
3		CH <sub>2</sub> Cl <sub>2</sub>	20–25	1	9	1:2
4		DMF	20–25	1	31	3:7
5			0	2	32	1:2
6			–20	2	26	3:10
7		DMSO	20–25	1	18	1:2

neuraminate (**5**) was obtained in 30–50% yield. The ratios of **3** to **4** and **9** to **10** were 1:2.

The stereochemistry of these compounds was elucidated by consideration of the  $^1\text{H}$ -NMR spectra, the rate of hydrolysis, and the CD spectra.

In the  $^1\text{H}$ -NMR spectra, the differences between the chemical shifts at the H-3'' (eq) double-doublet resonance of  $\alpha$ -anomers (**3**, **6**, **9**, and **11**) and those of  $\beta$ -anomers (**4**, **7**, **10**, and **12**) were +0.17, +0.38, +0.09, and +0.34 ppm, respectively, as shown in Table II. It is empirically known<sup>3,4</sup> that the chemical shift at H-3'' (eq) of the  $\alpha$ -anomer is usually observed at lower field than that of the  $\beta$ -anomer, and this result followed the empirical rule. In particular, the differences between the chemical shifts at H-3'' (eq) of deprotected compounds were larger than those of the protected compounds. This suggests that the  $\alpha$ -anomer has a greater deshielding effect at H-3'' (eq) owing to the carboxyl group, and the effect becomes less when the carboxyl group is esterified. In conclusion, the stereochemistry at the anomeric position could be assessed from the  $^1\text{H}$ -NMR data.

Hydrolyses<sup>4,5</sup> of two pairs of anomers, **6** and **7**, and **11** and **12**, were carried out at the concentration of 500  $\mu\text{g}/\text{ml}$  in hydrochloric acid (HCl) solution at 60 °C and the results are shown in Fig. 1. The retention time of *N*-acetylneuraminic acid (Neu5Ac) was 8.8 min in high performance liquid chromatography (HPLC), as shown in Fig. 2.

In the case of sialosylinosine, the  $\alpha$ -anomer (**6**) was decomposed, whereas half of the  $\beta$ -anomer (**7**) remained, at 2 h. Sialosylcytidines (**11**, **12**) also gave a similar result at 5 h. Thus, the rates of hydrolyses of the  $\alpha$ -anomers (**6**, **11**) were remarkably high in comparison with those of the  $\beta$ -anomers (**7**, **12**). However, *N*-acetylneuraminic acid was decomposed in the acid

TABLE II.  $^1\text{H}$ -NMR Chemical Shifts ( $\delta$  ppm) of H-3'' (eq) for Nucleoside 5'-*N*-Acetylneuraminic Acid Derivatives

Compound	H-3'' (eq)	$\Delta$ ( $\alpha$ - $\beta$ )	Compound	H-3'' (eq)	$\Delta$ ( $\alpha$ - $\beta$ )
<b>3</b>	2.71	0.17	<b>9</b>	2.63	0.09
<b>4</b>	2.54		<b>10</b>	2.54	
<b>6</b>	2.66	0.38	<b>11</b>	2.67	0.34
<b>7</b>	2.28		<b>12</b>	2.33	

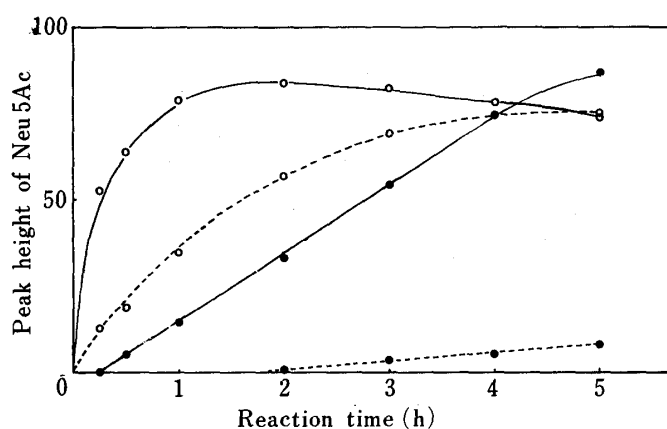


Fig. 1. Acid Hydrolysis of the (2→5)Glycosidic Linkage Sialosylinosine  $\alpha$ -Anomer (**6**) (—○—), Sialosylinosine  $\beta$ -Anomer (**7**) (---○---), Sialosylcytidine  $\alpha$ -Anomer (**11**) (—●—), and Sialosylcytidine  $\beta$ -Anomer (**12**) (---●---)

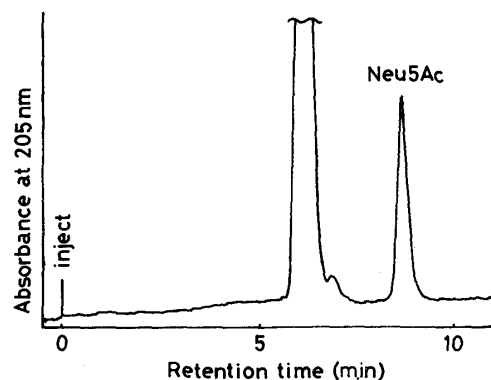


Fig. 2. HPLC Pattern of the 15 min Hydrolyzate of Sialosylinosine  $\alpha$ -Anomer (**6**)

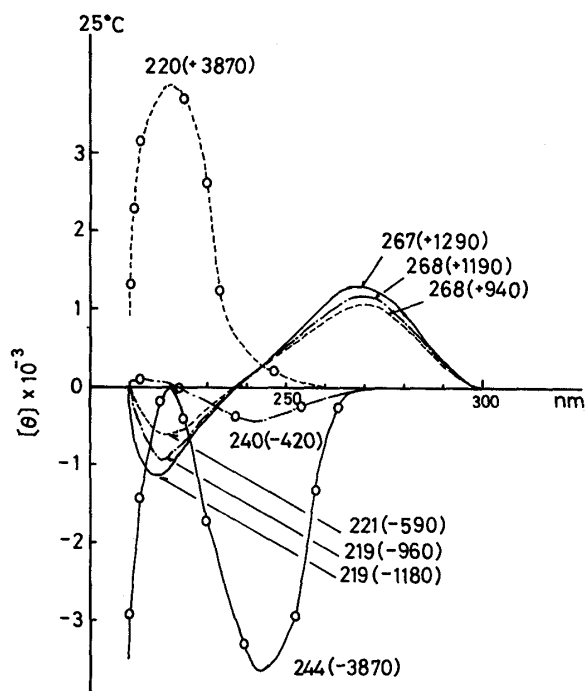


Fig. 3. CD Curves of Sialosylinosine  $\alpha$ -Anomer (6) (—○—), Sialosylinosine  $\beta$ -Anomer (7) (---○---), Inosine (---○---), Sialosylcytidine  $\alpha$ -Anomer (11) (—), Sialosylcytidine  $\beta$ -Anomer (12) (-----), and Cytidine (---) in Water (pH = 7)

solution can be seen from Fig. 1. It is clear that the measurement of the rate of hydrolysis is a useful method for confirmation of anomeric stereochemistry.

Figure 3 shows the CD spectra of sialosylinosines (6, 7) and inosine, and sialosylcytidines (11, 12) and cytidine. We have already reported<sup>1,4-6)</sup> the CD spectra of a number of *N*-acetylneuraminic acid derivatives and the peak around 220 nm was assigned to the  $n\text{-}\pi^*$  Cotton effect of the carboxyl group. The negative sign of the Cotton effect was assigned to  $\alpha$ -glycoside and the positive sign to  $\beta$ -glycoside.<sup>1)</sup> In sialosylinosines, the  $\beta$ -anomer (7) showed a positive sign and the  $\alpha$ -anomer (6) showed a negative sign at around 220 nm. In sialosylcytidines (11, 12), the difference of sign at around 220 nm was not clear. Thus, the difference of the  $n\text{-}\pi^*$  Cotton effect around 220 nm due to the carboxyl chromophore did not appear clearly in the nucleoside 5'-*N*-acetylneuraminic acid derivatives.<sup>4)</sup>

In conclusion, in the nucleoside 5'-*N*-acetylneuraminic acid derivatives, the stereochemistry at the anomeric position of the *N*-acetylneuraminic acid moiety could be confirmed on the basis of the <sup>1</sup>H-NMR spectra and the rate of acid hydrolysis.

### 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 GF254 (Merck) plates, and the spots were detected by ultraviolet (UV) irradiation and with 5% sulfuric acid solution. Field desorption mass spectra (FD-MS), fast atom bombardment mass spectra (FAB-MS), UV spectra, and infrared (IR) spectra were measured with JEOL JMS-DX300, JEOL JMS-3100, Hitachi 340, and JASCO IR-A2 instruments, respectively. CD spectra were measured in a 0.1 cm cell with a JASCO J-20 spectrometer. The NMR spectra were measured in chloroform-*d* (CDCl<sub>3</sub>) with tetramethylsilane (TMS) and in deuterium oxide (D<sub>2</sub>O) with sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) as an internal standard, with Varian 90 and Varian 400 spectrometers. Column chromatography was conducted on Silica gel 60 (70–230 mesh, Merck), and gel filtration column chromatography on TOYO PEARL HW-40 fine gel (Toyo Soda).

**HPLC**—*N*-Acetylneuraminic acid and its derivatives were analyzed by cation exclusion chromatography using an Aminex HPX-87H strong cation exchange resin column (7.8 × 300 mm, Bio-Rad Laboratories, Richmond, CA, U.S.A.) at 45 °C. A mobile phase of 0.006 N sulfuric acid was used at a flow rate of 0.58 ml/min. The column effluent was monitored with a UV detector at 205 nm (Nihon Seimitsu Kagaku, model NS-310).

**Methyl [*N*-Acetyl-4'',7'',8'',9''-tetra-*O*-acetyl(2',3'-di-*O*-acetylinosin-5'-yl)- $\alpha$ - and - $\beta$ -D-neuraminosid]onates (3, 4)**

—Compound **2** (2.4 mmol) and AgOTf (2.4 mmol) were added to a solution of 2',3'-di-*O*-acetylinosine (**1**, 2 mmol) and powdered molecular sieves 4A (1.0 g) in dry DMF (10 ml). The mixture was stirred at room temperature overnight in the dark under an argon atmosphere. The reaction mixture was filtered through Celite and washed with dichloromethane ( $\text{CH}_2\text{Cl}_2$ , 50 ml). The resulting solution was evaporated *in vacuo* to a syrup. The residue was extracted with  $\text{CH}_2\text{Cl}_2$  (30 ml) and the  $\text{CH}_2\text{Cl}_2$  extract was washed with a saturated sodium chloride (NaCl) solution (30 ml  $\times$  2). The  $\text{CH}_2\text{Cl}_2$  extract was dried with anhydrous magnesium sulfate, and evaporated *in vacuo*. The residue was dissolved in a small volume of chloroform ( $\text{CHCl}_3$ ), then separated and purified by silica gel column chromatography ( $\text{CHCl}_3$ -MeOH, 40:1—10:1) to give the  $\alpha$ -anomer (**3**), the  $\beta$ -anomer (**4**), and **5** each as an amorphous powder in 153 mg (9.3%), 360 mg (21.8%), and 520 mg yields, respectively.

$\alpha$ -Anomer (**3**):  $[\alpha]_{\text{D}}^{24} -21.6^\circ$  ( $c=1.0$ , MeOH). FD-MS  $m/z$ : 826 ( $\text{M}^+ + 1$ ), 690 ( $\text{M}^+ - 135$ ), 414 ( $\text{M}^+ - 411$ ). UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm ( $\epsilon$ ): 245 (10460). IR  $\nu_{\text{max}}^{\text{film}}$   $\text{cm}^{-1}$ : 3300 (NH), 2950 (CH), 1740 (ester), 1690 (amide I), 1585 (amide II). Silica gel TLC ( $\text{CHCl}_3$ -MeOH, 10:1):  $R_f=0.09$ .  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : (inosine moiety) 3.662 (1H, dd,  $J=2.3$ ,  $-11.3$  Hz, H-5'b), 4.101 (1H, dd,  $J=2.3$ ,  $-11.3$  Hz, H-5'a), 4.405 (1H, dd,  $J=2.3$ , 2.5 Hz, H-4'), 5.661 (1H, dd,  $J=5.0$ , 2.3 Hz, H-3'), 5.754 (1H, dd,  $J=6.8$ , 5.0 Hz, H-2'), 6.262 (1H, d,  $J=6.8$  Hz, H-1'), 8.139 (1H, s, H-8), 8.225 (1H, s, H-2), 12.65 (1H, s, 1-NH).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : (sialic acid moiety) 1.884 (3H, s, NAc), 2.000 (1H, dd,  $J=9.0$ ,  $-13.0$  Hz, H-3''ax), 2.018, 2.031, 2.040, 2.138 (3H  $\times$  4,  $s \times 4$ , OAc  $\times$  4), 2.160 (6H,  $s \times 2$ , OAc  $\times$  2), 2.712 (1H, dd,  $J=4.6$ ,  $-13.0$  Hz, H-3''eq), 3.770 (3H, s, COOMe), 4.060 (1H, dd,  $J=10.5$ , 10.5 Hz, H-5''), 4.065 (1H, dd,  $J=5.5$ ,  $-12.5$  Hz, H-9''b), 4.167 (1H, dd,  $J=10.5$ , 2.0 Hz, H-6''), 4.260 (1H, dd,  $J=2.6$ ,  $-12.5$  Hz, H-9''a), 4.968 (1H, m,  $J=4.6$ , 10.5, 9.0 Hz, H-4''), 5.310 (1H, dd,  $J=9.0$ , 2.0 Hz, H-7''), 5.397 (1H, m, NH), 5.397 (1H, m, H-8''). *Anal.* Calcd for  $\text{C}_{34}\text{H}_{43}\text{N}_5\text{O}_{19}$ : C, 49.45; H, 5.21; N, 8.48. Found: C, 49.49; H, 5.40; N, 8.57.

$\beta$ -Anomer (**4**):  $[\alpha]_{\text{D}}^{24} -5.4^\circ$  ( $c=1.0$ , MeOH). FD-MS  $m/z$ : 826 ( $\text{M}^+ + 1$ ), 690 ( $\text{M}^+ - 135$ ), 414 ( $\text{M}^+ - 411$ ). UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm ( $\epsilon$ ): 245 (8940). IR  $\nu_{\text{max}}^{\text{film}}$   $\text{cm}^{-1}$ : 3250 (NH), 2950 (CH), 1740 (ester), 1690 (amide I), 1585 (amide II). Silica gel TLC ( $\text{CHCl}_3$ -MeOH, 10:1):  $R_f=0.22$ .  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : (inosine moiety) 3.665 (1H, dd,  $J=2.5$ ,  $-11.0$  Hz, H-5'b), 3.935 (1H, dd,  $J=3.5$ ,  $-11.0$  Hz, H-5'a), 4.376 (1H, ddd,  $J=4.2$ , 2.5, 3.5 Hz, H-4'), 5.788 (1H, dd,  $J=5.7$ , 4.2 Hz, H-3'), 6.000 (1H, t,  $J=5.7$  Hz, H-2'), 6.241 (1H, d,  $J=5.7$  Hz, H-1'), 8.190 (1H, s, H-8), 8.370 (1H, s, H-2), 12.25 (1H, s, 1-NH).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : (sialic acid moiety) 1.846 (1H, dd,  $J=11.5$ ,  $-13.0$  Hz, H-3''ax), 1.977 (3H, s, NAc), 1.995, 2.008, 2.030, 2.100, 2.122, 2.165 (3H  $\times$  6,  $s \times 6$ , OAc  $\times$  6), 2.540 (1H, dd,  $J=4.8$ ,  $-13.0$  Hz, H-3''eq), 3.802 (3H, s, COOMe), 3.839 (1H, dd,  $J=10.8$ , 2.4 Hz, H-6''), 3.989 (1H, dd,  $J=8.8$ ,  $-12.2$  Hz, H-9''b), 4.150 (1H, dd,  $J=10.3$ , 10.8 Hz, H-5''), 4.837 (1H, dd,  $J=2.8$ ,  $-12.2$  Hz, H-9''a), 4.86 (1H, ddd,  $J=4.8$  Hz, 11.5, 10.8 Hz, H-4''), 5.142 (1H, ddd,  $J=2.8$ , 8.8, 2.4 Hz, H-8''), 5.247 (1H, t,  $J=2.4$  Hz, H-7''), 6.184 (1H, d,  $J=10.3$  Hz, NH). *Anal.* Calcd for  $\text{C}_{34}\text{H}_{43}\text{N}_5\text{O}_{19}$ : C, 49.45; H, 5.21; N, 8.48. Found: C, 49.46; H, 5.26; N, 8.41.

***N*-Acetyl(inosin-5'-yl)- $\alpha$ - and - $\beta$ -D-neuraminosidoic Acids (**6**, **7**)**—A 2N NaOH solution (2 ml) was added to a solution of **3** or **4** in  $\text{H}_2\text{O}$  (10 ml) and the mixture was stirred for 2 h at room temperature. Dowex 50W  $\times$  8 ( $\text{H}^+$ ) resin was added to the reaction mixture, which was adjusted to pH 3–4 in an ice bath. The whole was filtered and washed with water. The resulting solution was freeze-dried to yield 40 mg (82%) of the  $\alpha$ -anomer (**6**), or 48 mg (98%) of the  $\beta$ -anomer (**7**), as white powder.

$\alpha$ -Anomer (**6**):  $[\alpha]_{\text{D}}^{25} -21.4^\circ$  ( $c=1.0$ ,  $\text{H}_2\text{O}$ , pH=7). FD-MS  $m/z$ : 559 ( $\text{M}^+$ ), 423 ( $\text{M}^+ - 136$ ), 291 ( $\text{M}^+ - 268$ ), 136 ( $\text{M}^+ - 423$ ). UV  $\lambda_{\text{max}}^{\text{H}_2\text{O}(\text{pH}=7)}$  nm ( $\epsilon$ ): 248 (9170). IR  $\nu_{\text{max}}^{\text{film}}$   $\text{cm}^{-1}$ : 3350 (NH), 1690 (amide I), 1580 (amide II).  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )  $\delta$ : 1.94 (3H, s, NAc), 2.66 (1H, dd,  $J=5.0$ ,  $-12.0$  Hz, H-3''eq), 6.02 (1H, d,  $J=4.2$  Hz, H-1'), 8.12 (1H, s, 2-H), 8.33 (1H, s, 8-H).  $^{13}\text{C-NMR}$  ( $\text{D}_2\text{O}$ )  $\delta$ : 91.10 (C-1'), 103.14 (C-2'), 176.22, 177.96 (1''-COOH, 4''-NAc). *Anal.* Calcd for  $\text{C}_{21}\text{H}_{29}\text{N}_5\text{O}_{13} \cdot 2.5\text{H}_2\text{O}$ : C, 41.72; H, 5.63; N, 11.59. Found: C, 41.85; H, 5.69; N, 11.47.

$\beta$ -Anomer (**7**):  $[\alpha]_{\text{D}}^{25} -10.2^\circ$  ( $c=1.0$ ,  $\text{H}_2\text{O}$ , pH=7). FD-MS  $m/z$ : 559 ( $\text{M}^+$ ), 423 ( $\text{M}^+ - 136$ ), 291 ( $\text{M}^+ - 268$ ), 136 ( $\text{M}^+ - 423$ ). UV  $\lambda_{\text{max}}^{\text{H}_2\text{O}(\text{pH}=7)}$  nm ( $\epsilon$ ): 248 (11970). IR  $\nu_{\text{max}}^{\text{film}}$   $\text{cm}^{-1}$ : 3300 (NH), 1685 (amide I), 1610 (amide II).  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )  $\delta$ : 1.91 (3H, s, NAc), 2.28 (1H, dd,  $J=4.3$ ,  $-13.5$  Hz, H-3''eq), 5.97 (1H, d,  $J=4.2$  Hz, H-1'), 8.08 (1H, s, H-2), 8.25 (1H, s, H-8).  $^{13}\text{C-NMR}$  ( $\text{D}_2\text{O}$ )  $\delta$ : 91.02 (C-1'), 103.31 (C-2'), 177.73, 177.83 (4''-NAc, 1''-COOH). *Anal.* Calcd for  $\text{C}_{21}\text{H}_{29}\text{N}_5\text{O}_{13} \cdot 2.5\text{H}_2\text{O}$ : C, 41.72; H, 5.63; N, 11.59. Found: C, 41.45; H, 5.34; N, 11.46.

**Methyl [*N*-Acetyl-4'',7'',8'',9''-tetra-*O*-acetyl(2',3'-di-*O*-acetyl-*N*-benzoylcytidin-5'-yl)- $\alpha$ - and - $\beta$ -D-neuraminosid]jonates (**9**, **10**)**—Compound **2** (12 mmol) and AgOTf (12 mmol) were added to a solution of **8** (10 mmol) and powdered molecular sieves 4A (3.0 g) in dry DMF (40 ml). The mixture was stirred at room temperature overnight in the dark under an argon atmosphere. The reaction mixture was filtered through Celite and washed with  $\text{CH}_2\text{Cl}_2$  (200 ml). The resulting solution was evaporated to a syrup *in vacuo*. The residue was extracted with  $\text{CH}_2\text{Cl}_2$  (150 ml). The  $\text{CH}_2\text{Cl}_2$  extract was washed with a saturated NaCl solution (50 ml  $\times$  2), dried with anhydrous magnesium sulfate, and evaporated *in vacuo*. The residue was separated by silica gel column chromatography ( $\text{CHCl}_3$ -MeOH, 20:1). The eluate was separated and purified by silica gel column chromatography (benzene-acetone, 2:1), affording 0.68 g (7.5%) of **9** and 1.22 g (13.5%) of **10**, each as a white powder. **9** was recrystallized from ethyl acetate as colorless prisms. Furthermore, **5** was collected in 2.80 g yield.

$\alpha$ -Anomer (**9**): mp 207.5–209°C.  $[\alpha]_{\text{D}}^{24} +24.1^\circ$  ( $c=1.08$ ,  $\text{CHCl}_3$ ). FD-MS  $m/z$ : 905 ( $\text{M}^+ - 1$ ). IR  $\nu_{\text{max}}^{\text{film}}$   $\text{cm}^{-1}$ : 3270 (NH), 1740 (ester), 1650 (amide I), 1550 (amide II). Silica gel TLC (benzene-acetone, 1:1),  $R_f=0.23$ .  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : (cytidine moiety) 3.627 (1H, dd,  $J=2.0$ ,  $-11.2$  Hz, H-5''b), 4.145 (1H, dd,  $J=2.0$ ,  $-11.2$  Hz, H-5'a), 4.377 (1H, dd,  $J=2.8$ , 2.0 Hz, H-4'), 5.395 (1H,  $J=6.5$ , 5.0 Hz, H-2'), 5.522 (1H, dd,  $J=5.0$ , 2.8 Hz, H-3'), 6.410 (1H,

d,  $J=6.5$  Hz, H-1'), 7.52 (3H, m, Ph), 7.62 (2H, m, Ph), 7.89 (1H, d,  $J=7.6$  Hz, H-5), 8.22 (1H, d,  $J=7.6$  Hz, H-6).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : (sialic acid moiety) 1.890 (3H, s, NAc), 2.036, 2.056, 2.072, 2.136, 2.152, 2.162 (18H, s  $\times 6$ , OAc  $\times 6$ ), 2.63 (1H, dd,  $J=4.8$ ,  $-12.5$  Hz, H-3''eq), 3.84 (3H, s, COOMe), 4.064 (1H, dd,  $J=4.5$ ,  $-12.2$  Hz, H-9''b), 4.065 (1H, dd,  $J=10.5$ ,  $10.5$  Hz, H-5''), 4.09 (1H, dd,  $J=10.5$ ,  $2.2$  Hz, H-6''), 4.260 (1H, dd,  $J=2.6$ ,  $-12.2$  Hz, H-9''a), 5.005 (1H, ddd,  $J=4.8$ ,  $12.0$ ,  $10.5$  Hz, H-4''), 5.327 (1H, dd,  $J=9.0$ ,  $2.2$  Hz, H-7''), 5.395 (1H, ddd,  $J=2.6$ ,  $4.5$ ,  $9.0$  Hz, H-8), 8.22 (1H, d,  $J=10.5$  Hz, 5''-NH). *Anal.* Calcd for  $\text{C}_{40}\text{H}_{48}\text{N}_4\text{O}_{20}$ : C, 53.10; H, 5.31; N, 6.19. Found: C, 53.26; H, 5.28; N, 5.85.

$\beta$ -Anomer (**10**):  $[\alpha]_{\text{D}}^{24} +25.4^\circ$  ( $c=0.93$ ,  $\text{CHCl}_3$ ). FD-MS  $m/z$ : 905 ( $\text{M}^+ + 1$ ). IR  $\nu_{\text{max}}^{\text{film}} \text{cm}^{-1}$ : 3280 (NH), 1740 (ester), 1660 (amide I), 1550 (amide II). Silica gel TLC (benzene-acetone, 1:1),  $R_f=0.29$ .  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : (cytidine moiety) 3.732 (1H, dd,  $J=2.5$ ,  $-11.8$  Hz, H-5'b), 4.167 (1H, dd,  $J=1.9$ ,  $-11.8$  Hz, H-5'a), 4.263 (1H, ddd,  $J=6.5$ ,  $2.5$ ,  $1.9$  Hz, H-4'), 5.57 (1H, dd,  $J=3.0$ ,  $6.5$  Hz, H-2'), 5.83 (1H, d,  $J=3.0$  Hz, H-1'), 5.597 (1H, t,  $J=6.5$  Hz, H-3'), 7.43 (1H, d,  $J=7.5$  Hz, H-5), 7.43 (1H, m, Ph), 7.54 (2H, m, Ph), 7.85 (1H, d,  $J=7.5$  Hz, H-6), 7.85 (2H, m, Ph).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : (sialic acid moiety) 1.891 (1H, dd,  $J=11.0$ ,  $-13.0$  Hz, H-3''ax), 1.95 (3H, s, NAc), 1.959, 1.998, 2.048, 2.141 (12H, s  $\times 4$ , OAc  $\times 4$ ), 2.113  $\times 2$  (6H, s, OAc  $\times 2$ ), 2.541 (1H, dd,  $J=5.0$ ,  $-13.0$  Hz, H-3''eq), 3.80 (3H, s, COOMe), 4.002 (1H, dd,  $J=9.0$ ,  $-12.0$  Hz, H-9''b), 4.167 (1H, dd,  $J=10.5$  Hz, H-5''), 4.472 (1H, dd,  $J=10.5$ ,  $2.0$  Hz, H-6''), 4.992 (1H, dd,  $J=2.2$ ,  $-12.0$  Hz, H-9''a), 5.32 (1H, m, H-8''), 5.355 (1H, m, H-7''), 7.14 (1H, d,  $J=10.5$  Hz, 5''-NH). *Anal.* Calcd for  $\text{C}_{40}\text{H}_{48}\text{N}_4\text{O}_{20}$ : C, 53.10; H, 5.31; N, 6.19. Found: C, 53.58; H, 5.24; N, 6.15.

**Ammonium [*N*-Acetyl(cytidin-5'-yl)- $\alpha$ - and - $\beta$ -D-neuraminosid]onate (**11**, **12**)**—Saturated ammonia gas was passed through a solution of **9**, or **10** (0.066 mmol) in methanol (15 ml) and the reaction mixture was left in a refrigerator overnight. It was evaporated *in vacuo* to remove methanol and purified by gel filtration column chromatography (2.2  $\times$  30 cm; mobile phase, methanol). The eluate was evaporated *in vacuo* to give **11** (35 mg, 98%), or **12** (35 mg, 98%), each as an amorphous solid.

$\alpha$ -Anomer (**11**):  $[\alpha]_{\text{D}}^{23} +15.1^\circ$  ( $c=0.97$ ,  $\text{H}_2\text{O}$ , pH=7). FAB-MS (Xe, glycerol)  $m/z$ : 534 ( $\text{M}^+ - \text{NH}_3$ ), 291 ( $\text{M}^+ - 260$ ), 244 ( $\text{M}^+ - 307$ ). UV  $\lambda_{\text{max}}^{\text{H}_2\text{O}(\text{pH}=7)} \text{nm}$  ( $\epsilon$ ): 269 (7200). IR  $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$ : 3350 (NH), 1660 (amide I), 1490 (amide II).  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )  $\delta$ : (cytidine moiety) 4.178 (1H, dd,  $J=3.7$ ,  $5.0$  Hz, H-2'), 4.221 (1H, t,  $J=5.0$  Hz, H-3'), 5.806 (1H, d,  $J=3.7$  Hz, H-1'), 5.969 (1H, d,  $J=7.3$  Hz, H-6), 7.801 (1H, d,  $J=7.3$  Hz, H-5). (sialic acid moiety) 1.753 (1H, dd,  $J=11.6$ ,  $-12.5$  Hz, H-3''ax), 1.900 (3H, s, NHAc), 2.670 (1H, dd,  $J=4.5$ ,  $-12.5$  Hz, H-3''eq), 3.665 (1H, ddd,  $J=4.5$ ,  $11.6$ ,  $10.3$  Hz, H-4''), 3.83 (1H, t,  $J=10.3$  Hz, H-5''), 3.88 (1H, dd,  $J=3.4$ ,  $-11.0$  Hz, H-9''b), 3.915 (1H, dd,  $J=3.1$ ,  $-11.0$  Hz, H-9''a), 4.144 (1H, m, H-8'').  $^{13}\text{C-NMR}$  ( $\text{D}_2\text{O}$ )  $\delta$ : 37.80 (C-3''), 62.42 (C-5''), 63.87 (C-9''), 81.57 (C-9'), 98.35 (C-2''). *Anal.* Calcd for  $\text{C}_{30}\text{H}_{29}\text{N}_4\text{O}_{13} \cdot \text{NH}_4$ : C, 43.56; H, 5.99; N, 12.70. Found: C, 43.87; H, 6.46; N, 14.31.

$\beta$ -Anomer (**12**):  $[\alpha]_{\text{D}}^{23} +15.6^\circ$  ( $c=1.03$ ,  $\text{H}_2\text{O}$ , pH=7). FAB-MS (Xe, glycerol)  $m/z$ : 534 ( $\text{M}^+ - \text{NH}_3$ ), 291 ( $\text{M}^+ - 260$ ), 244 ( $\text{M}^+ - 307$ ). UV  $\lambda_{\text{max}}^{\text{H}_2\text{O}(\text{pH}=7)} \text{nm}$  ( $\epsilon$ ): 269 (7800). IR  $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$ : 3300 (NH), 1650 (amide I), 1490 (amide II).  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )  $\delta$ : (cytidine moiety) 3.411 (1H, d,  $J=5.0$ ,  $-12.0$  Hz, H-5'a), 4.176 (1H, t,  $J=5.0$  Hz, H-3'), 4.212 (1H, dd,  $J=3.4$ ,  $5.0$  Hz, H-2'), 5.787 (1H, d,  $J=3.4$  Hz, H-1'), 5.957 (1H, d,  $J=7.5$  Hz, H-6), 7.757 (1H, d,  $J=7.5$  Hz, H-5).  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )  $\delta$ : (sialic acid moiety) 1.608 (1H, dd,  $J=11.2$ ,  $-13.0$  Hz, H-3''ax), 1.950 (3H, s, NHAc), 2.332 (1H, dd,  $J=5.0$ ,  $-13.0$  Hz, H-3''eq), 3.547 (1H, dd,  $J=6.0$ ,  $-12.0$  Hz, H-9''b), 3.712 (1H, dd,  $J=3.0$ ,  $-12.0$  Hz, H-9''a), 3.937 (1H, m, H-4''), 4.115 (1H, m, H-8'').  $^{13}\text{C-NMR}$  ( $\text{D}_2\text{O}$ )  $\delta$ : 36.92 (C-3''), 61.39 (C-5''), 62.82 (C-9''), 79.79 (C-1'), 98.78 (C-2''). *Anal.* Calcd for  $\text{C}_{20}\text{H}_{29}\text{N}_4\text{O}_{13} \cdot \text{NH}_4$ : C, 43.56; H, 5.99; N, 12.70. Found: C, 44.27; H, 6.13; N, 12.42.

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