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SYNTHESES OF 2-*O*-GLYCOSYL DERIVATIVES OF *N*-ACETYL-D-NEURAMINIC ACID*

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ABSTRACT

Syntheses of *N*-acetyl-D-neuraminic acid derivatives are reported. Methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-2-chloro-2-deoxy-β-D-neuraminate (**3**) was prepared directly from methyl *N*-acetyl-β-D-neuraminate (**2**) in good yield. Koenigs–Knorr reaction of **3** with an excess of methanol gave the methyl α-glycoside of methyl *N*-acetyl-D-neuraminate (**4**). 2,3-*O*-Isopropylidene-D-ribono-1,4-lactone, 2,3-*O*-isopropylideneuridine, and 5-fluoro-2,3-*O*-isopropylideneuridine reacted with **3** to give anomeric mixtures of methyl *N*-acetyl-D-neuraminate derivatives. The stereochemistry of these compounds was confirmed from n.m.r. and c.d. spectra, and measurements of the rate of hydrolysis of the glycosidic bond.

INTRODUCTION

N-Acetyl-D-neuraminic acid is widely distributed in membrane glycoproteins and glycolipids, and plays an important role in animal cells. The circular dichroism (c.d.) spectra of a number of *N*-acetylneuraminic acid derivatives have been studied by Brossmer *et al.*[2]; we reached similar conclusions using derivatives prepared from *N*-acetylneuraminic acid obtained from edible birds' nest[3].

We report here syntheses of the α and β glycosides of *N*-acetyl-D-neuraminic acid and some disaccharide nucleoside analogs that contain the *N*-acetyl-D-neuraminic acid moiety.

N-Acetyl-β-D-neuraminic acid (5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonic acid, **1**) was obtained[4] in 5–6% yield from edible birds' nest by heating with 25mM H_2SO_4.

Methyl *N*-acetyl-β-D-neuraminate (**2**) was prepared by the action of methanol and Dowex-50 (H^+) at room temperature. The methyl β-glycoside (**6**) of methyl

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N-acetyl-D-neuraminate was prepared by using methanol and Dowex-50 (H^+) under reflux[5,6]. Acetylation of **6** with acetic anhydride–pyridine gave the *O*-tetra-acetate in 60% yield. Reduction of **6** by sodiumn borohydride yielded methyl 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranoside (**8**).

To confirm the stereochemistry at C-2, methyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranoside (**7**) was prepared as shown in Chart 1.

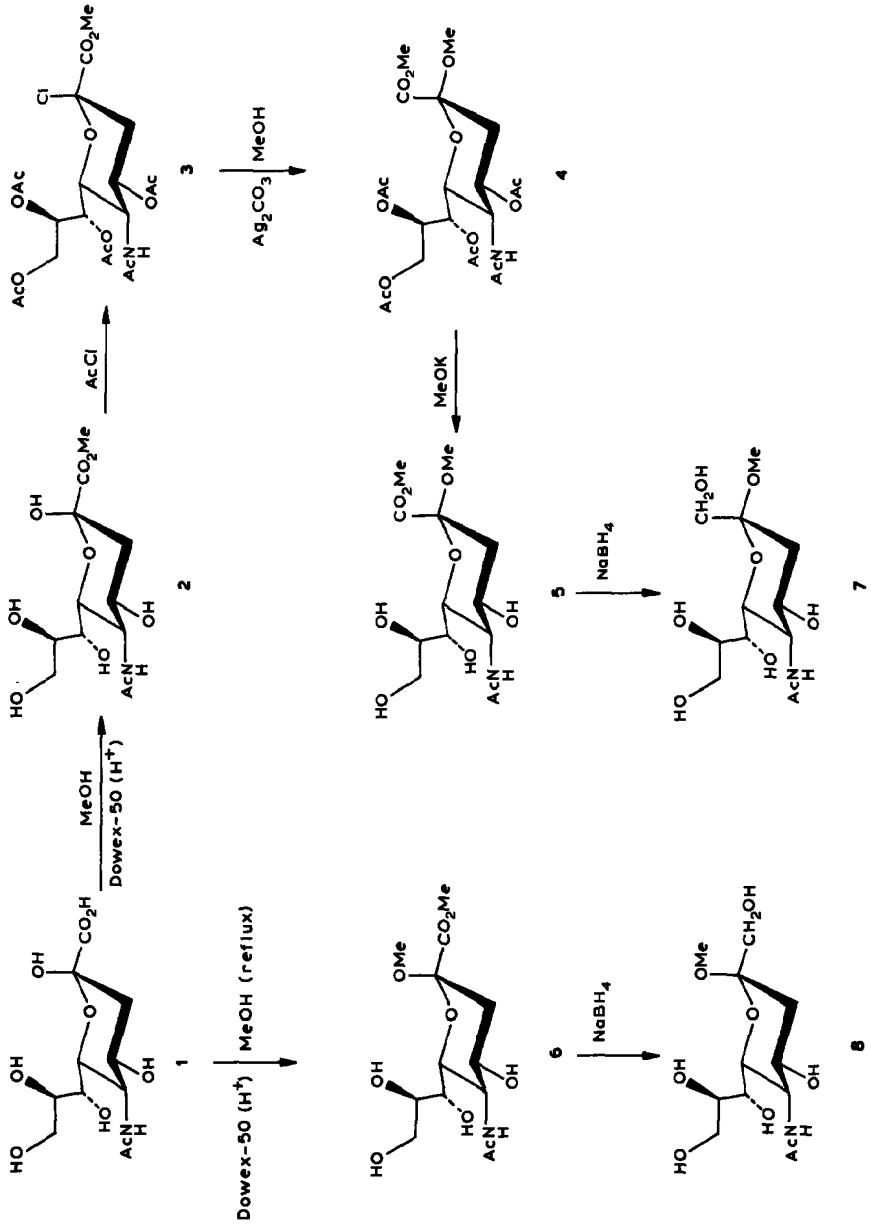
One-step treatment of methyl *N*-acetyl-β-D-neuraminate (**2**) with an excess of acetyl chloride at room temperature yielded methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-2-chloro-2-deoxy-β-D-neuraminate (**3**) in good yield as fine crystals, m.p. 116–118°. This compound has previously been prepared as a syrup from methyl 2,4,7,8,9-penta-*O*-acetyl-*N*-acetyl-β-D-neuraminate with acetyl chloride and hydrogen chloride in a sealed vessel by the method of Kuhn *et al.*[5].

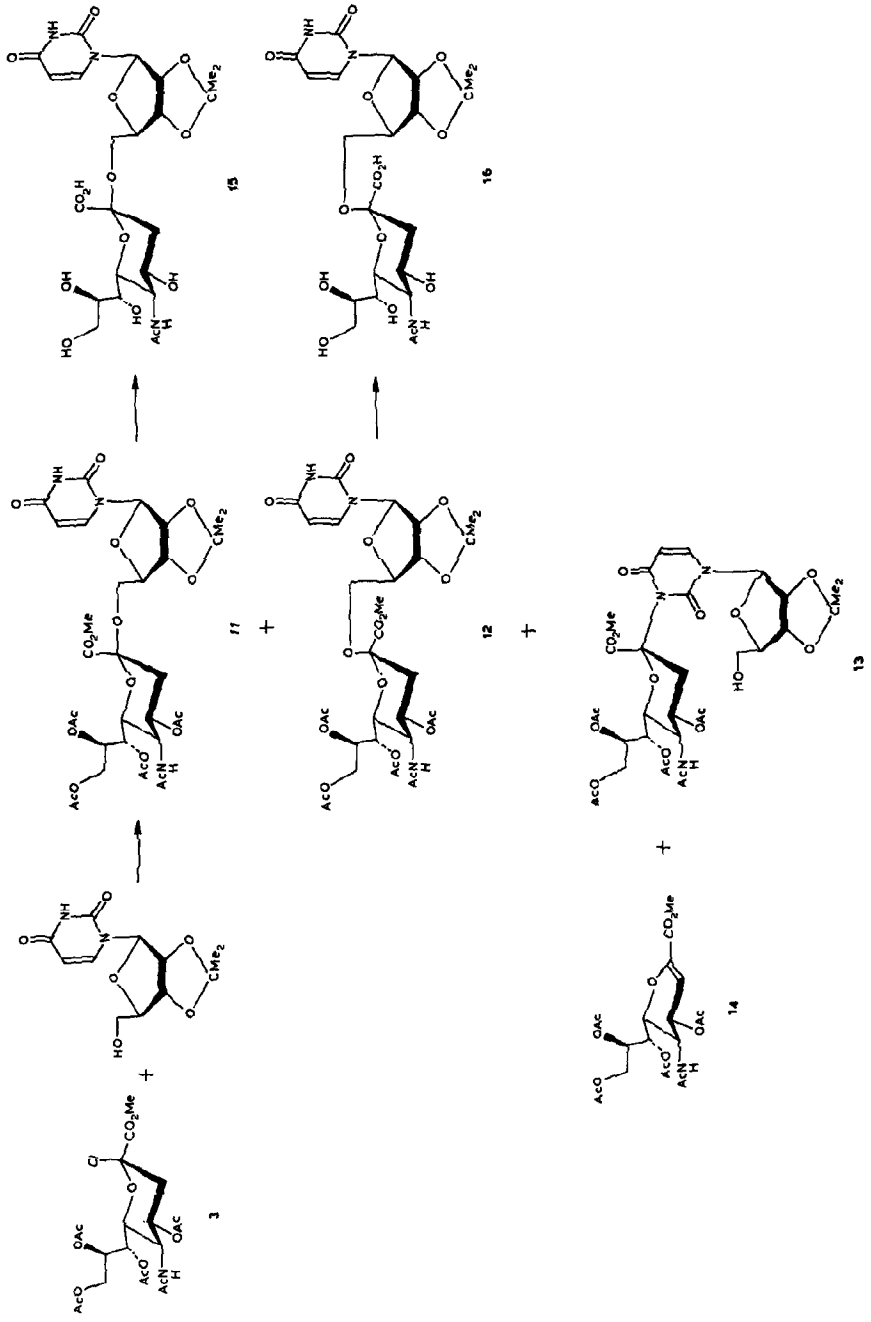
Koenigs–Knorr reaction of the chloride **3** with silver carbonate in methanol gave the methyl α-glycoside (**4**) of methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-D-neuraminate in high yield.

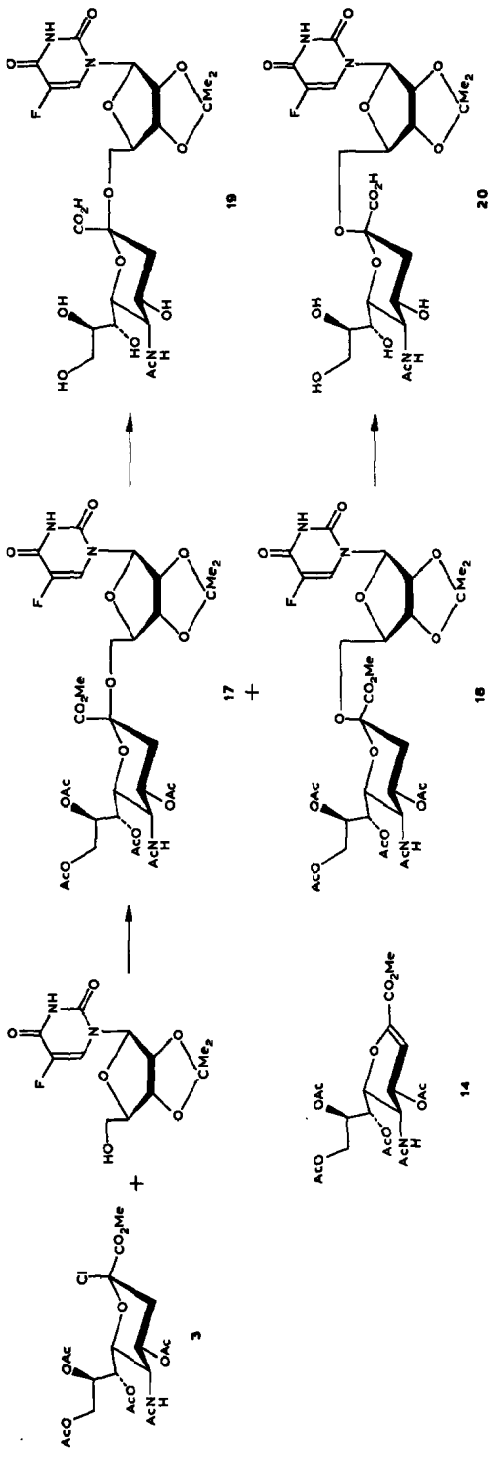
Saponification of **4** with potassium methoxide in methanol afforded the methyl α-glycoside (**5**) of methyl *N*-acetyl-D-neuraminate as colorless prisms in good yield. Reduction of this compound with sodium borohydride gave methyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranoside (**7**). The sign of the n-π^* Cotton effect at ~220 nm is positive for the β-glycoside, and negative for the α-glycoside. The α-glycoside changed into the β-glycoside by treatment with M HCl in methanol.

Disaccharide nucleosides were prepared by Koenigs–Knorr coupling under various conditions. *O*-[Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulopyranosyl)onate]-(2→5)-2,3-*O*-isopropylidene-D-ribono-1,4-lactone (**9**) was prepared from the chloride (**3**) and 2,3-*O*-isopropylidene-D-ribono-1,4-lactone with mercuric cyanide. *O*-Deacetylation with potassium methoxide in methanol afforded methyl (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulopyranosyl)onate-(2→5)-2,3-*O*-isopropylidene-D-ribono-1,4-lactone (**10**) in high yield. Koenigs–Knorr reaction of 2′,3′-*O*-isopropylideneneuridine with the chloride (**3**) in the presence of mercuric cyanide and mercuric bromide as catalyst gave *O*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulopyranosyl)onate]-(2→5′)-2′,3′-*O*-isopropylideneuridine (**11**) in ~30% yield and its β-glycoside (**12**) in ~10% yield. When silver perchlorate and silver carbonate were used as a catalyst, *O*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulopyranosyl)onate]-(2→N^3)-2′,3′-*O*-isopropylideneuridine (**13**) was obtained in 10% yield instead of the β-glycoside **12**. In each instance, methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-2-deoxy-2,3-dehydro-D-neuraminate (**14**) was formed in ~30% yield.

Koenigs–Knorr reaction of 5-fluoro-2′,3′-*O*-isopropylideneuridine with chloride **3** in the presence of mercuric cyanide and mercuric bromide gave *O*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulopyranosyl)onate]-(2→5′)-5-fluoro-2′,3′-*O*-isopropylideneuridine (**17**) in


MeOH
Dowex-50 (H+)
AcCl
Ag2CO3
MeOH
MeOK
NaBH4
NaBH4
Dowex-50 (H+)
MeOH (reflux)





~20% yield, and its β anomer **18** in ~40% yield, together with 2,3-dehydro derivative **14** in 14% yield.

Deacetylation of the α-glycoside **11** and β-glycoside **12** afforded the α-D-*galacto* and β-D-*galacto* anomers of *O*-[methyl (5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulopyranosyl)onate]-(2→5′)-2′,3′-*O*-isopropylideneuridine in fair yields. When M sodium hydroxide was used, the α and β anomers of *N*-acetyl-D-neuraminyl-(2→5′)-2′,3′-*O*-isopropylideneuridine (**15** and **16**) were obtained.

Deacetylation of the α-glycoside **17** and β-glycoside **18** with M sodium hydroxide afforded the α and β anomers of *N*-acetyl-D-neuraminyl-(2→5′)-5-fluoro-2′,3′-*O*-isopropylideneuridine (**19, 20**).

The stereochemistry of the products was confirmed by ^{1}H-n.m.r. spectral comparison of the chemical shifts of the H-3*e* doublets of doublets [lower-field shift (δ 2.5–2.7) for the α-glycoside, higher field shift (δ 2.3–2.5) for the β-glycoside][8,9] of various neuraminic acid derivatives as summarized in Table I. The ^{13}C-n.m.r. data gave information concerning the configurational differences between α and β anomers as summarized in Table II. ^{13}C-N.m.r. data of a number of α- and β-glycosides of *N*-acetylneuraminic acid have been reported[10].

Fig. 1. shows the c.d. spectra of the α-D-*galacto* and β-D-*galacto* anomers of *O*-[methyl (5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulopyranosyl)onate]-

TABLE I

H-N.M.R. CHEMICAL SHIFTS OF H-3*e* FOR *N*-ACETYLNEURAMINIC ACID DERIVATIVES AND ANALOGS (δ_{CDCl_3}, p.p.m.)

Compound	*H-3*e	*Δ* (*α − β anomer*)
N-Acetyl-α-neuraminic acid	2.730[a,b,c]	
1	2.20[a,b,d]	0.522
2	2.31	
5	2.69[c]	
6	2.30[c]	0.39
7	2.29[a]	
8	2.28[a]	0.01
11	2.53	
12	2.46	0.07
13	2.77	
O-[Methyl (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulopyranosyl)onate]-(2→5′)-2′,3′-*O*-isopropylideneuridine	2.606[a]	
O-[Methyl (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulopyranosyl)onate]-(2→5′)-2′,3′-*O*-isopropylideneuridine	2.476[a]	0.130
15	2.62[a]	
16	2.43[a]	0.19
17	2.57	
18	2.50	0.07
19	2.61[a]	
20	2.48[a]	0.13

[a] δ_{D_2O} from DSS. [b] Ref. 8. [c] Ref. 9. [d] Ref. 16.

TABLE II

^{13}C-N.M.R. CHEMICAL SHIFTS FOR *N*-ACETYLNEURAMINIC ACID DERIVATIVES ($CDCl_3$, Me_4Si AT 25°)

		2′,3′-Isopropylideneuridine[a]	*N-Acetyl-D-neuraminic acid*			**11**	**12**	**13**
			α	*β*				
Uridine moiety	2	152.0				150.5	150.6	154.5
	4	165.7				163.8	163.8	166.9
	5	102.8				103.4	103.5	95.6
	6	143.5				142.5	142.6	147.7
	1	93.4				93.4	93.4	94.7
	2	82.0				79.8	79.8	80.4
	3	88.1				84.8	84.8	88.1
	4	85.5				84.1	84.2	85.1
	5	62.8				62.7	63.1	62.1
	6	114.8				114.9	115.0	114.2
	7	27.6[b]				27.3[b]	27.3[b]	27.3[b]
	8	27.6[b]				27.3[b]	27.3[b]	25.3[b]
N-Acetylneuraminic acid moiety	1		—[c]	177.87[c]	170.9[d]	171.6	171.6	171.5
	$NHCOCH_3$		—	175.98	174.4	171.3	171.4	171.3
	$OCOCH_3$					171.1	171.2	171.0×2
					170.9	171.0	170.6	
					170.5	170.6	168.6	
					167.6	167.8		
	2		98.42	97.61	95.4	99.2	99.2	99.0
	3		41.94	40.63	41.0	37.4	37.4	36.3
	4		69.38	68.51	65.5	69.1	69.3	68.0
	5		53.07	53.50	51.4	49.0	49.1	49.3
	6		72.75	71.45	71.4	72.2	72.3	71.5
	7		69.38	69.82	69.3	69.1	69.3	68.5
	8		73.77	71.59	71.4	72.7	72.7	72.9
	9		64.10	64.55	63.7	62.7	62.7	62.3
	CO_2CH_3					53.1	53.1	53.6
	$NHCOCH_3$		—	23.34	21.4	23.2	23.2	23.1
	$OCOCH_3$					21.1	20.8×4	20.8×4
						20.8×3		

[a]Recorded in Me_2SO-d_6. [b]Values may be interchanged. [c]Ref. 8, p. 156; recorded in ^2H_2O at p^{2}H 7.0. [d]Ref. 1; recorded for the solid state by c.p.-m.a.s.

(2→5′)-2′,3′-*O*-isopropylideneuridine in comparison with that of 2′,3′-*O*-isopropylideneuridine in methanol. The curves for the pair of anomers show Cotton effects due to the carbonyl chromophore, of unexpected sign (*α*-glycoside positive, *β*-glycoside negative) at 225 nm. This phenomenon probably indicates that the large 2-glycoside group exists in *anti* conformation to the 6-residue of the *N*-acetylneuraminic acid moiety, as determined from the sector rule[11] and the planar rule[12].

Fig. 2 shows data for the rate of hydrolysis in water of the two pairs of anomers **15** and **16**, and **19** and **20**. When the hydrolysis was performed at 80°, the *α* anomers (**15** and **19**) were decomposed within 1 h, whereas the *β* anomers (**16**

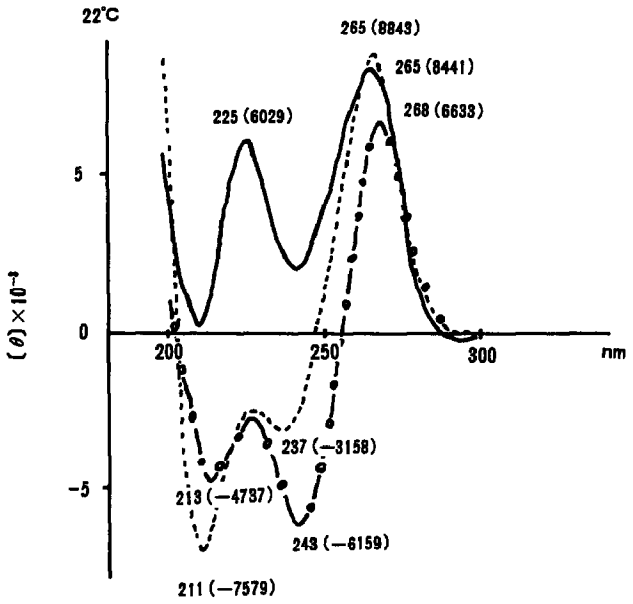


Fig. 1. Circular dichroism curves of 2′,3′-*O*-isopropylideneuridine (—○—), *O*-[methyl (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulopyranosyl)onate]-(2→5′)-2′,3′-*O*-isopropylideneuridine (——), and its β anomer (------) in methanol.

and **20**) decomposed after ~2 h. When the hydrolysis proceeded at 60°, the α anomers (**15** and **19**) decomposed after ~5 h, whereas the β anomers (**16** and **20**) were not hydrolyzed within 5 h. It is clear that measurement of the rate of hydrolysis is a useful method for confirmation of anomeric stereochemistry.

Biological activities of these compounds have been reported by Osawa *et al.*[13,14].

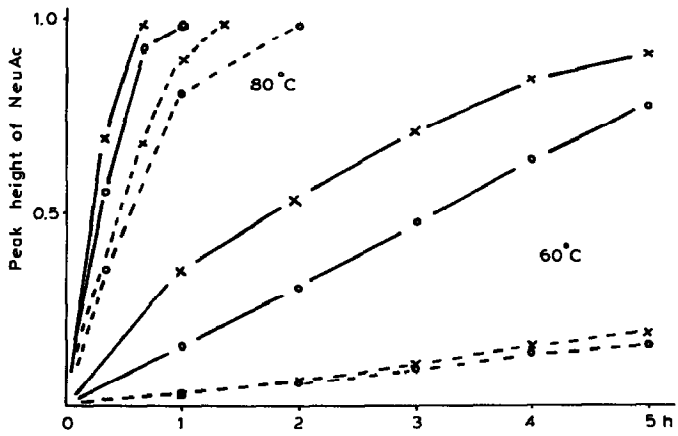


Fig. 2. Hydrolysis of the glycosidic bond of O-(*N*-acetyl-α-D-neuraminyl)-(2→5′)-2′,3′-O-*isopropylideneuridine* (**15**, —×—), its 5-fluoro analog (**19**, —○—),and the corresponding β anomers **20**, (---○---), and **16**, (---×---) in water. L.c. conditions: Aminex HPX-87H (300 × 7.8 mm); eluant, 3mM H_2SO_4; flow, 0.65 mL/min; temperature, 40°; detector, refractive index.

EXPERIMENTAL

General methods. — Melting points are uncorrected. Infrared (i.r.) spectra were recorded with a JASCO A-2 spectrometer and n.m.r. spectra with a Varian EM-390 spectrometer. Tetramethylsilane (Me_4Si in $CDCl_3$) or sodium 4,4-dimethyl-4-silapentane-1-sulfonate hydrate (DSS in D_2O) were used as internal references. Optical rotations were measured in a 50-mm cell with a JASCO DIP-181 automatic polarimeter. C.d. data were obtained with a Japan Spectroscopic Model J-20 recording polarimeter.

Glycoside-bond hydrolysis was performed in H_2O, with analysis by cation-exclusion chromatography by Aminex HPX-87H strong cation resin (Bio-Rad Laboratories, Richmond, CA, U.S.A.). A mobile phase of 3mM H_2SO_4 was used at flow rate of 0.66 mL/min.

N-*Acetyl-β-*D-*neuraminic acid* (**1**). — Edible birds' nest (100 g) in 25mM sulfuric acid (4.5 L) was stirred for 1.5 h at 55–60°. The portion positive to Roseman's periodate–resorcinol reagent[15] was freeze-dried to afford a crude white powder as described in literature[4]. Crystallization from 1:2 water–acetic acid yielded **1** as colorless needles, m.p. 186–187° dec; $[\alpha]_D^{19}$ −33.8° (*c* 1, H_2O).

Methyl N-*acetyl-β-*D-*neuraminate* (**2**). — The method reported by Kuhn *et al.*[5] from **1** (30.0 g) gave 28.5 g (86%) of **2** as colorless prisms after recrystallization from methanol; m.p. 180–182° dec (lit.[5]) m.p. 179–180° (dec), $[\alpha]_D^{20}$ −28.0° (*c* 1, H_2O) [lit.[5]) $[\alpha]_D^{20}$ −28° (*c* 1, H_2O)]; ν_{max}^{KBr} 3250 (OH), 1735 (CO), 1600, and 1560 (CONH) cm^{-1}; n.m.r. (D_2O): δ 1.97 (1 H, dd, *J* 11.5 and 13.0 Hz, H-3*a*), 2.31 (1 H, dd, *J* 5.0 and 13.0 Hz, H-3*e*), and 3.82 (3 H, s, CO_2Me).

Anal. Calc. for $C_{12}H_{21}NO_9 \cdot H_2O$: C, 42.2; H, 6.8; N, 4.1. Found: C, 42.2; H, 6.8; N, 4.1.

*Methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-2-chloro-2-deoxy-*D*-neuraminate* (**3**). — A solution of **2** (1.0 g) in an excess of acetyl chloride was stirred overnight at room temperature. Evaporation of the excess reagent yielded 1.5 g (95%) of **3** as a white powder. Crystallization from benzene–diethyl ether–petroleum ether afforded 0.95 g (60%) of colorless fine needles (lit.[5] syrup), m.p. 116–118°, $[\alpha]_D^{20}$ −68.0° (*c* 1, $CHCl_3$) [lit.[5] $[\alpha]_D^{20}$ −63° (*c* 1, $CHCl_3$)]; ν_{max}^{KBr} 1735 (CO), 1654, 1532 (CONH) cm^{-1}; n.m.r. ($CDCl_3$): δ 1.92–2.10 (15 H, s, NHAc, OAc), 2.78 (1 H, dd, *J* 5.0 and 12.0 Hz, H-3*e*), and 3.91 (3 H, s, CO_2Me).

*Methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-*glycero*-α-*D*-galacto-nonulopyranosid)onate* (**4**). — To a mixture of silver carbonate (2.0 g) and molecular sieves in methanol (25 mL), the chloride (**3**, 1.0 g) was added. The mixture was stirred for 30 min, filtered, and the filtrate was evaporated to dryness to give 0.79 g (85%) of **4** as a white powder (lit.[5] syrup); $[\alpha]_D^{20}$ −5.0° (*c* 1, MeOH) [lit.[5] $[\alpha]_D^{20}$ −18° (*c* 4, MeOH)]; ν_{max}^{KBr} 1735 (CO), 1655, and 1535 (CONH) cm^{-1}; n.m.r. ($CDCl_3$): δ 1.80–2.20 (15 H, s, NAc, OAc), and 3.72 (3 H, s, CO_2Me).

Anal. Calc. for $C_{21}H_{31} \cdot 0.5\ H_2O$: C, 49.0; H, 6.2; N, 2.7. Found: C, 49.2; H, 5.9; N, 2.6.

Methyl (methyl 5-acetamido-3,5-dideoxy-D-glycero-*α*-D-galacto-*nonulopyranosid)onate* (**5**). — To an ice-cooled solution of **4** (700 mg) in methanol (10 mL) was added an ice-cooled solution prepared from potassium metal (100 mg) and methanol (10 mL). The mixture was stirred for 20 min at 0°, and then cooled to −20°, made neutral with Dowex-50 (H^+), and filtered. The filtrate was evaporated and the resulting crystalline powder was recrystallized from methanol–diethyl ether to yield 320 mg (88%) of **5** as colorless needles, m.p. 162–163° (lit.[5] syrup); $[\alpha]_D^{20}$ −10.0° (*c* 1, H_2O) [lit.[5] $[\alpha]_D^{20}$ −6.3° (*c* 0.5, MeOH)]; ν_{max}^{KBr} 3320 (OH), 1735 (CO), 1625, and 1550 (CONH) cm^{-1}; n.m.r. (D_2O): *δ* 1.79 (1 H, dd, *J* 12.0 and 13.0 Hz, H-3*a*), 2.02 (3 H, s, NAc), 2.69 (1 H, dd, *J* 4.5 and 12.0 Hz, H-3*e*), 3.38 (3 H, s, OMe), and 3.78 (3 H, s, CO_2Me).

Anal. Calc. for $C_{13}H_{23}NO_4$: C, 46.3; H, 6.9; N, 4.2. Found: C, 46.3; H, 6.8; N, 4.1.

Methyl (methyl 5-acetamido-3,5-dideoxy-D-glycero-*β*-D-galacto-*nonulopyranosid)onate* (**6**). — This compound was prepared from **1** (20.0 g) by the method of Kuhn *et al.*[5], giving 14.7 g (58%) of **6** as colorless prisms after recrystallization from methanol; m.p. 115–120° (lit.[5] m.p. 115–130°), $[\alpha]_D^{25}$ −45.0° (*c* 1, MeOH) (lit.[5] $[\alpha]_D^{20}$ −46° (*c* 0.67, MeOH)]; ν_{max}^{KBr} 3280 (OH), 1720 (CO), 1610, and 1525 (CONH) cm^{-1}; n.m.r. (D_2O): *δ* 2.00 (1 H, dd, *J* 14.0 and 14.0 Hz, H-3*a*), 2.03 (3 H, s, NAc), 2.30 (1 H, dd, *J* 6.0 and 14.0 Hz, H-3*e*), 3.25 (3 H, s, OMe), and 3.83 (3 H, s, CO_2Me).

Methyl (methyl 5-acetamido-4,7,8,9-tetra-O-*acetyl-3,5-dideoxy*-D-glycero-*β*-D-galacto-*nonulopyranosid)onate.* — A solution of **6** (1.0 g) in acetic anhydride (10 mL) and pyridine (5 mL) was kept for 24 h at room temperature and then processed conventionally to give the crude product from the chloroform extract. Recrystallization from isopropyl ether yielded 0.85 g (60%) of the title compound as colorless prisms, m.p. 134–135°, $[\alpha]_D^{20}$ −19.9° (*c* 1, $CHCl_3$); ν_{max}^{KBr} 1740 (CO), 1665, and 1530 (CONH) cm^{-1}; n.m.r. ($CDCl_3$): *δ* 1.89–2.19 (15 H, s, NAc, OAc), 2.50 (1 H, dd, *J* 5.0 and 13.0 Hz, H-3*e*), 3.29 (3 H, s, OMe), and 3.83 (3 H, s, CO_2Me).

Anal. Calc. for $C_{21}H_{31}NO_{13}$: C, 49.9; H, 6.2; N, 2.8. Found: C, 49.9; H, 6.2; N, 2.7.

Methyl 5-acetamido-3,5-dideoxy-D-glycero-*α*-D-galacto-*2-nonulopyranoside* (**7**). — To an ice-cooled solution of **5** (500 mg) in methanol (20 mL) was added a solution of sodium borohydride (500 mg) in methanol (20 mL). The mixture was stirred at room temperature for 2 h, made neutral with Dowex 50 (H^+), and filtered. The filtrate was evaporated and the resulting crystalline powder was recrystallized from methanol–diethyl ether to yield 190 mg (40%) of **7** as colorless needles, m.p. 162–164°, $[\alpha]_D^{20}$ −21.0° (*c* 1, H_2O); ν_{max}^{KBr} 3320 (OH), 1610, and 1530 (CONH) cm^{-1}; n.m.r. (D_2O): *δ* 1.73 (1 H, dd, *J* 11.0 and 13.0 Hz, H-3*a*), 2.21 (3 H, s, NAc), 2.29 (1 H, dd, *J* 4.5 and 13.0 Hz, H-3*e*), and 3.32 (3 H, s, OMe).

Anal. Calc. for $C_{12}H_{23}NO_8$: C, 46.6; H, 7.6; N, 4.5. Found: C, 46.5; H, 7.6; N, 4.5.

Methyl 5-acetamido-3,5-dideoxy-D-glycero-*β*-D-galacto-*2-nonulopyranoside*

(**8**). — Treatment of **6** with $NaBH_4$ in methanol gave **8** in 90% yield as colorless prisms, m.p. 108–115° (dec.) from methanol–diethyl ether (lit.[7] m.p. 110–130° dec.); $[\alpha]_D^{25}$ −54.0° (*c* 1, MeOH) [lit.[7] $[\alpha]_D^{25}$ −54° (*c* 1, MeOH)]; ν_{max}^{KBr} 3300 (OH, NH), 1610, 1530 (CONH) cm^{-1}; n.m.r. (D_2O): δ 2.19 (3 H, s, NAc) and 3.42 (3 H, s, OMe).

O-[*Methyl (5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*nonulopyranosyl)onate*]-*(2→5)-2,3*-O-*isopropylidene*-D-*ribono-1,4-lactone* (**9**). — The chloride **3** (510 mg) was added to a stirred mixture of 2,3-*O*-isopropylidene-D-ribono-1,4-lactone (1.0 g), mercuric cyanide (150 mg), mercuric bromide (300 mg), and molecular sieves (1 g) in acetonitrile (30 mL). The mixture was stirred for 12 h at room temperature and filtered. The filtrate was evaporated and the residue dissolved in ethyl acetate (100 mL) and the solution was treated with 30% potassium iodide to remove mercury salts. The solvent was removed and the remaining substance was chromatographed on silica gel to afford 260 mg (40%) of **9** as a white powder; $[\alpha]_D^{24}$ −8.2° (*c* 1, MeOH); ν_{max}^{KBr} 1785, 1742 (CO), 1670, and 1535 (CONH) cm^{-1}; n.m.r. ($CDCl_3$): δ 1.36 (3 H, s, CMe_2), 1.44 (3 H, s, CMe_2), 1.96–2.10 (15 H, s, NAc, OAc), 2.76 (1 H, dd, *J* 3.5 and 12.0 Hz, H-3*e*), and 3.8 (3 H, s, CO_2Me); *m/z* calc. for $C_{28}H_{39}NO_{17}$: 661; found: 661, 646, 618, 602.

Anal. Calc. for $C_{28}H_{39}NO_{17} \cdot 0.5\ H_2O$: C, 50.2; H, 6.0; N, 2.1. Found: C, 50.1; H, 5.8; N, 2.2.

O-*Methyl (5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*nonulopyranosyl)onate*]-*(2→5)-2,3*-O-*isopropylidene*-D-*ribono-1,4-lactone* (**10**). — To an ice-cooled solution of **9** (1.0 g) in methanol (10 mL) was added a solution prepared from potassium metal (100 mg) and methanol (10 mL). The mixture was stirred for 20 min at 0° and then treated with Dowex 50 (H^+) at −20°. The filtrate was evaporated to yield 595 mg (80%) of **10** as a white powder; $[\alpha]_D^{23}$ −21.8° (*c* 1, MeOH); ν_{max}^{KBr} 3300 (OH), 1775, 1730 (CO), 1650, and 1532 (CONH) cm^{-1}; n.m.r. (D_2O): δ 1.38 (3 H, s, CMe_2), 1.45 (3 H, CMe_2), 2.15 (3 H, s, NAc), and 3.80 (3 H, s, CO_2Me).

Anal. Calc. for $C_{20}H_{31}NO_{13}$: C, 48.7; H, 6.3; N, 2.8. Found: C, 48.21; H, 6.0; N, 2.5.

Koenigs–Knorr reaction of 2,3-O-*isopropylideneuridine with methyl 4,7,8,9-tetra*-O-*acetyl*-N-*acetyl-2-chloro-2-deoxy-β*-D-*neuraminate* (**3**). — To a stirred mixture of 2,3-*O*-isopropylideneuridine (1.0 g), mercuric cyanide (150 mg), mercuric bromide (300 mg), and molecular sieves (1 g) in acetonitrile (50 mL), was added the chloride **3** (510 mg). After stirring at room temperature for 24 h the filtrate was evaporated, the residue was dissolved in ethyl acetate, and the product chromatographed on a column of alumina that was eluted with ethyl acetate–ethanol to give successively methyl 2,3-dehydroneuraminate (**14**), 2′,3′-*O*-isopropylideneuridine, the α anomeric product **11**, and the β anomer **12**.

Methyl 4,7,8,9-tetra-O-*acetyl*-N-*acetyl-2,3-dehydro-2-deoxy*-D-*neuraminate* (**14**) was obtained as a white powder, 95 mg (20%)–140 mg (30%); $[\alpha]_D^{20}$ +23.5° (*c* 1, MeOH); ν_{max}^{KBr} 1738 (CO), 1630, and 1542 (CONH) cm^{-1}; n.m.r. ($CDCl_3$): δ 1.87

(3 H, s, NAc), 2.04, 2.08, 2.10, 2.15 (12 H, s, OAc), 3.74 (1 H, s, CO_2Me), 3.98 (1 H, dd, *J* 6.0 and 12.0 Hz, H-9), 4.26 (1 H, dd, *J* 12.0 and 3.1 Hz, H-9), 4.51 (1 H, ddd, *J* 0.9, 9.9, and 11.0 Hz, H-5), 4.80 (1 H, t, *J* 1.9 Hz, H-4), 5.09 (1 H, m, H-8), 5.41 (1 H, dd, *J* 2.0 and 10.0 Hz, H-7), 5.51 (1 H, dd, *J* 11.0 and 2.0 Hz, H-6), and 5.98 (1 H, d, *J* 1.9 Hz, H-3).

Anal. Calc. for $C_{20}H_{27}NO_{12}$: C, 50.7; H, 5.7; N, 3.0. Found: C, 50.7; H, 5.9; N, 2.8.

O-[*Methyl (5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*nonulopyranosyl)onate*]-*(2→5′)-2′,3′*-O-*isopropylideneuridine* (**11**) was obtained as a white powder, 230 mg (30%)–300 mg (40%); $[\alpha]_D^{22}$ −2.1° (*c* 1, MeOH); ν_{max}^{KBr} 1735 (CO), 1678, 1530 (CONH) cm^{-1}; n.m.r. ($CDCl_3$): δ 1.37 (3 H, s, CMe_2), 1.58 (3 H, s, CMe_2), 1.88 (3 H, s, NAc), 2.01 (6 H, s, OAc), 2.12 (6 H, s, OAc), 2.53 (1 H, dd, *J* 4.8 and 12.6 Hz, H-3*e*″), 3.78 (3 H, s, CO_2Me), 5.64 (1 H, d, *J* 7.8 Hz, H-5), 5.87 (1 H, d, *J* 2.4 Hz, H-1′), 7.53 (1 H, d, *J* 7.8 Hz, H-6), 8.93 (1 H, br s, 3-NH); *m/z* calc. for $C_{32}H_{43}N_3O_{18}$: 757; found 757, 742, 714, 698.

Anal. Calc. for $C_{32}H_{43}N_3O_{18}$: C, 50.7; H, 5.7; N, 5.6. Found: C, 50.6; H, 5.9; N, 5.2.

O-[*Methyl (5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-β-D-galacto-*nonulopyranosyl)onate*]-*(2→5′)-2′,3′*-O-*isopropylideneuridine* (**12**) was obtained as white powder, 78 mg (10%); $[\alpha]_D^{22}$ +3.4° (*c* 1, MeOH); ν_{max}^{KBr} 1735 (CO), 1680, and 1535 (CONH) cm^{-1}; n.m.r. ($CDCl_3$): δ 1.37 (3 H, s, CMe_2), 1.56 (3 H, s, CMe_2), 1.88 (3 H, s, NAc), 1.99, 2.01, 2.05, 2.12 (12 H, s, OAc), 2.46 (1 H, dd, *J* 4.8 and 12.9 Hz, H-3*e*″), 3.82 (3 H, s, CO_2Me), 5.72 (1 H, d, *J* 2.1 Hz, H-1′), 5.83 (1 H, d, *J* 8.4 Hz, H-5), 7.35 (1 H, d, *J* 8.4 Hz, H-6), and 9.83 (1 H, br s, 3-NH); *m/z* calc for $C_{32}H_{43}N_3O_{18}$: 757; found 757, 742, 714, 698.

Anal. Calc. for $C_{32}H_{43}N_3O_{18}$: C, 50.7; H, 5.7; N, 5.5. Found: C, 50.4; H, 5.8; N, 5.4.

(*b*) To a stirred mixture of 2′,3′-*O*-isopropylideneuridine (0.70 g), the chloride **3** (1.58 g), and molecular sieves (0.7 g) in acetonitrile (30 mL), was added silver carbonate (0.42 g) and silver perchlorate (0.51 g). After stirring at room temperature for 4 h, the filtrate was evaporated. A solution of the residue in ethyl acetate was chromatographed on a column of alumina that was eluted with ethyl acetate–ethanol. Methyl 2-deoxy-2,3-dehydroneuraminate (**14**), **11**, and **13** were obtained in 30, 30, and 10% yields, respectively.

O-[*Methyl (methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*nonulopyranosyl)onate*]-*(2→N³)-2′,3′*-O-*isopropylideneuridine* (**13**) had m.p. 132–134°; $[\alpha]_d^{27}$ −32.0° (*c* 1, MeOH); ν_{max}^{KBr} 1735 (CO), 1678, and 1535 (CONH) cm^{-1}; n.m.r. ($CDCl_3$): δ 1.36 (3 H, s, CMe_2), 1.59 (3 H, s, CMe_2), 1.88 (3 H, s, NAc), 2.05 (6 H, s, OAc), 2.10 (6 H, s, OAc), 2.77 (1 H, dd, *J* 4.8 and 13.0 Hz), H-3*e*″), 3.82 (3 H, s, CO_2Me), 5.68 (1 H, d, *J* 2.1 Hz, H-1′), 5.90 (1 H, d, *J* 8.4 Hz, H-5), and 7.85 (1 H, d, *J* 8.4 Hz, H-6); *m/z* calc. for $C_{32}H_{43}N_3O_{18}$: 757; found 757, 742, 714, 698.

Anal. Calc. for $C_{32}H_{43}N_3O_{18}$: C, 50.7; H, 5.7; N, 5.5. Found: C, 50.7; H, 5.7; N, 5.5.

O-[*Methyl (5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*nonulopyranosyl)onate*]-*(2→5')-2',3'-isopropylideneuridine*. — This compound was prepared from **11** (500 mg) by the method described for **10**; 230 mg of the product was obtained as a white powder; $[\alpha]_D^{20}$ −9.5° (*c* 1, MeOH); ν_{max}^{KBr} 3300 (OH), 1735 (CO), 1645, and 1530 (CONH) cm^{-1}; n.m.r. (400 MHz, D_2O): δ 1.405 (3 H, s, CMe_2), 1.593 (3 H, s, CMe_2), 2.026 (3 H, s, NAc), 2.606 (1 H, dd, *J* 3.91 and 11.72 Hz, H-3*e*"), 3.865 (3 H, s, OAc), 5.884 (1 H, d, *J* 7.81 Hz, H-5), and 7.796 (1 H, d, *J* 7.81 Hz, H-6).

Anal. Calc. for $C_{24}H_{35}N_3O_{14}\cdot 0.5\ H_2O$: C, 48.16; H, 6.02; N, 7.02. Found: C, 48.25; H, 6.03; N, 7.05.

O-[*Methyl (5-acetamido-3,5-dideoxy*-D-glycero-β-D-galacto-*nonulopyranosyl)onate*]-*(2→5')-2',3'*-O-*isopropylideneuridine*. — This compound was prepared from **12** (500 mg) by the method described for **10**; 290 mg (75%) of the product was obtained as a white powder; $[\alpha]_D^{20}$ −3.4° (*c* 1, MeOH); ν_{max}^{KBr} 3300 (OH), 1720 (CO), 1540 (CONH) cm^{-1}; n.m.r. (400 MHz, D_2O): δ 1.405 (3 H, s, CMe_2), 1.593 (3 H, s, CMe_2), 2.051 (3 H, s, NAc), 2.476 (1 H, dd, *J* 3.42 and 10.74 Hz, H-3*e*"), 3.800 (3 H, s, CO_2Me), 5.892 (1 H, d, *J* 7.81 Hz, H-5), and 7.747 (1 H, d, *J* 7.81 Hz, H-6).

Anal. Calc. for $C_{24}H_{35}N_3O_{14}\cdot 3\ H_2O$: C, 44.79; H, 6.42; N, 6.53. Found: C, 45.23; H, 5.51; N, 6.38.

O-*(*N*-Acetyl-α-*D*-neuraminyl)-(2→5')-2',3'*-O-*isopropylideneuridine* (**15**). — A solution of **11** (230 mg) in M sodium hydroxide was stirred for 2 h at room temperature. This solution was cooled to −20°, adjusted to pH 3 with Dowex-50 (H^+) resin, and the resulting filtrate was freeze-dried to give a crude powder that was treated with charcoal to afford **15** as a white powder; 122 mg (70%); $[\alpha]_D^{25}$ −14.0° (*c* 1, MeOH); λ_{max}^{MeOH} (log ε) 260 nm (3.98); ν_{max}^{film} 1680 (CO), 1630, 1550 (CONH) cm^{-1}; n.m.r. (D_2O): δ (3 H, s, CMe_2), 1.57 (3 H, s, CMe_2), 1.67 (1 H, t, *J* 12.0 Hz, H-3*a*"), 2.02 (3 H, s, NAc), 2.62 (1 H, dd, *J* 3.2 and 12.0 Hz, H-3*e*"), 5.83 (1 H, br s, H-1'), 5.87 (1 H, d, *J* 7.5 Hz, H-5), and 7.80 (1 H, d, *J* 7.5 Hz, H-6).

Anal. Calc. for $C_{23}H_{33}N_3O_{14}$: C, 48.00; H, 5.78; N, 7.30. Found: C, 47.88; H, 5.91; N, 7.12.

O-*(*N*-Acetyl-β-*D*-neuraminyl)-(2→5')-2',3'-isopropylideneuridine* (**16**). — A solution of **12** in M sodium hydroxide was treated as in the preceding experiment to give **16** as a white powder in 82% yield; $[\alpha]_D^{25}$ −12.0° (*c* 1, MeOH); λ_{max}^{MeOH} (log ε) 260 nm (3.96); ν_{max}^{film} 1660 (CO), 1530 (CONH) cm^{-1}; n.m.r. (D_2O): δ 1.40 (3 H, s, CMe_2), 1.58 (3 H, s, CMe_2), 1.70 (1 H, dd, *J* 11.5 and 12.8 Hz, H-3*a*"), 2.03 (3 H, s, NAc), 2.43 (1 H, dd, *J* 3.8 and 12.8 Hz, H-3*e*"), 5.82 (1 H, br s, H-1'), 5.85 (1 H, d, *J* 8.2 Hz, H-5), and 7.72 (1 H, d, *J* 8.1 Hz, H-6).

Anal. Calc. for $C_{23}H_{33}N_3O_{14}$: C, 48.00; H, 5.78; N, 7.30. Found: C, 47.91; H, 5.84; N, 7.25.

Koenigs–Knorr reaction of 5-fluoro-2',3'-O-*isopropylideneuridine with methyl 4,7,8,9-tetra*-O-*acetyl*-N-*acetyl-2-chloro-2-deoxy-β*-D-*neuraminate* (**3**). (*a*) 5-Fluoro-2',3'-*O*-isopropylideneuridine was treated with **3**, plus mercuric cyanide, and mercuric bromide as catalysts as described for the reaction with 2',3'-*O*-isopropyl-

ideneuridine. Chromatography on Lobar (type C) with chloroform–methanol gave successively the β anomer **18**, the α anomer **17**, and starting material.

O-[*Methyl (5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*nonulopyranosyl)onate*]-*(2→5′)-5-fluoro-2′,3′*-O-*isopropylideneuridine* (**17**) was obtained as a white powder in 11% yield; $[\alpha]_D^{25}$ +11.0° (*c* 1, MeOH); ν_{max}^{KBr} 1735 (CO), 1680, and 1530 (CONH) cm^{-1}; n.m.r. ($CDCl_3$): δ 1.37 (3 H, s, CMe_2), 1.58 (3 H, s, CMe_2), 1.87 (3 H, s, NAc), 2.02 (6 H, s, OAc), 2.16, (6 H, s, OAc), 2.57 (1 H, dd, *J* 3.5 and 13.5 Hz, H-3*e*″), 3.76 (3 H, s, CO_2Me), 5.92 (1 H, br s, H-1′), and 7.89 (1 H, d, *J* 8.0 Hz, H-6); *m/z* calc. for $C_{32}H_{42}FN_3O_{18}$: 775; found 775, 760, 714, 698.

Anal. Calc. for $C_{32}H_{42}FN_3O_{18}$: C, 49.55; H, 5.42; N, 5.45. Found: C, 49.24; H, 5.80; N, 5.12.

O-[*Methyl (5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*nonulopyranosyl)onate*]-*(2→5′)-5-fluoro-2′,3′*-O-*isopropylideneuridine* (**18**) was obtained as a white powder in 12% yield; $[\alpha]_D^{25}$ +11.0° (*c* 1, MeOH); ν_{max}^{KBr} 1735 (CO), 1680, and 1530 (CONH) cm^{-1}; n.m.r. ($CDCl_3$): δ 1.37 (3 H, s, CMe_2), 1.56 (3 H, s, CMe_2), 1.87 (3 H, s, NAc), 1.99 (6 H, s, OAc), 2.02, 2.13 (6 H, s, OAc), 2.50 (1 H, dd, *J* 3.5 and 14 Hz, H-3*e*″), 3.77 (3 H, s, CO_2Me), 5.54 (1 H, br s, H-1′), and 7.44 (1 H, d, *J* 8.0 Hz, H-6); *m/z* calc. for $C_{32}H_{42}FN_3O_{18}$: 775; found 775, 760, 714, 698.

Anal. Calc. for $C_{32}H_{42}FN_3O_{18}$: C, 49.55; H, 5.42; N, 5.45. Found: C, 49.34; H, 5.65; N, 5.22.

O-*(N-Acetyl-α-D-neuraminyl)-(2→5′)-5-fluoro-2′,3′*-O-*isopropylideneuridine* (**19**). Treatment of a solution of **17** in M sodium hydroxide as in the preceding experiment gave **19** as a white powder in 80% yield; $[\alpha]_D^{25}$ −6.0° (*c* 1, MeOH); ν_{max}^{KBr} 3400 (OH), 1690, and 1580 (CO) cm^{-1}; n.m.r. (D_2O): δ 1.38 (3 H, s, CMe_2), 1.58 (3 H, s, CMe_2), 1.72 (1 H, t, *J* 3.5 Hz, H-3*a*″), 2.03 (3 H, s, NAc), 2.61 (1 H, dd, *J* 3.4 and 13.5 Hz, H-3*e*″), 5.81 (1 H, br s, H-1′), and 7.96 (1 H, d, *J* 8 Hz, H-6).

Anal. Calc. for $C_{23}H_{32}FN_3O_{14}$: C, 46.54; H, 5.40; N, 7.08. Found: C, 46.25; H, 5.42; N, 7.11.

O-*(N-Acetyl-β-D-neuraminyl)-(2→5′)-5-fluoro-2′,3′*-O-*isopropylideneuridine* (**19**). Treatment of a solution of **18** in M sodium hydroxide as in the preceding experiment gave **20** as a white powder in 80% yield; $[\alpha]_D^{25}$ −9.0° (*c* 1, MeOH); ν_{max}^{KBr} 3400 (OH), 1688, and 1580 (CO) cm^{-1}; n.m.r. (D_2O): δ 1.40 (3 H, s, CMe_2), 1.58 (3 H, s, CMe_2), 1.74 (1 H, t, *J* 14 Hz, H-3*a*″), 2.04 (3 H, s, NAc), 2.48 (1 H, dd, *J* 3.5 and 14.0 Hz, H-3*e*″), 5.86 (1 H, br s, H-1′), and 7.95 (1 H, d, *J* 8 Hz, H-6).

Anal. Calc. for $C_{23}H_{32}FN_3O_{14}$: C, 46.54; H, 5.40; N, 7.08. Found: C, 46.89; H, 5.40; N, 6.96.

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REFERENCES

1 H. OGURA, K. FURUHATA, H. SAITO, G. IZUMI, M. ITOH, AND Y. SHITORI, *Chem. Lett.*, (1984) 1003–1006.
2 G. KEILICH, R. BROSSMER, V. ESCHENFELDER, AND L. HOLMQUIST, *Carbohydr. Res.*, 40 (1975) 255–262.
3 H. OGURA AND K. FURUHATA, *Tetrahedron Lett.*, 22 (1981) 4265–4268.
4 M. F. CZARNIECKI AND E. R. THORNTON, *J. Am. Chem. Soc.*, 99 (1977) 8273–8278.
5 R. KUHN, P. LUTZ, AND D. L. MACDONALD, *Chem. Ber.*, 99 (1966) 611–617.
6 R. K. YU AND R. J. LEDEEN, *J. Biol. Chem.*, 244 (1969) 1306–1313.
7 R. BROSSMER AND L. HOLMQUIST, *Z. Physiol. Chem.*, 352 (1971) 1715–1719.
8 R. SCHAUER, *Sialic Acids, Chemistry, Metabolism, and Function*, Springer-Verlag, Vienna, New York, 1982.
9 U. DABROWSKI, H. FRIEBOLIN, R. BROSSMER, AND M. SUPP, *Tetrahedron Lett.*, (1979) 4637–4640.
10 V. ESCHENFELDER, R. BROSSMER, AND H. FRIEBOLIN, *Tetrahedron Lett.*, (1975) 3069–3072.
11 J. P. JENNINGS, W. KLYNE, AND P. M. SCOPES, *J. Chem. Soc.*, (1965) 7211–7229.
12 L. D. MELTON, E. R. MORRIS, D. A. REES, AND D. THOM, *J. Chem. Soc. Perkin Trans. II*, (1979) 10–17.
13 I. KIJIMA, K. EZAWA, S. TOYOSHIMA, K. FURUHATA, H. OGURA, AND T. OSAWA, *Chem. Pharm. Bull.*, 30 (1982) 3278–3283.
14 I. KIJIMA-SUDA, S. TOYOSHIMA, M. ITOH, K. FURUHATA, H. OGURA, AND T. OSAWA, *Chem. Pharm. Bull.*, 33 (1985) 730–739.
15 G. W. JOURDIAN, L. DEAN, AND S. ROSEMAN, *J. Biol. Chem.*, 246 (1971) 430–435.
16 R. RENSCH, W. BAUMANN, AND H. FRIEBOLIN, *J. Carbohydr. Chem.*, 2 (1983) 415–425.