Synthesis of Disaccharide Nucleoside Derivatives of 3-Deoxy-D-*glycero*-D-*galacto*-2-nonulosonic Acid (KDN)[1,2]

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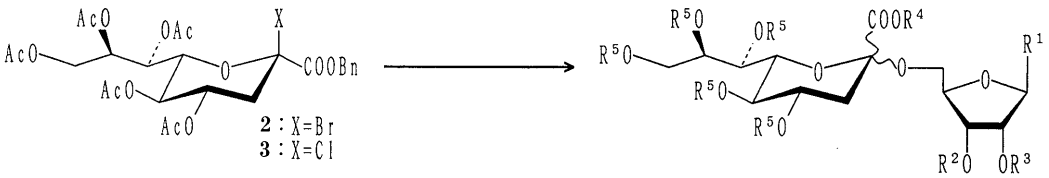
The reaction of benzyl 4,5,7,8,9-penta-*O*-acetyl-2-bromo- or -chloro-2,3-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate (2, 3) with uridine, 5-fluorouridine, and cytidine under Koenigs–Knorr reaction conditions gave the corresponding (2→5) linked disaccharide nucleoside derivatives, in yields of 32—47%. A similar reaction of 3 with inosine gave the (2→N^1) linked derivative. These nucleoside analogues were converted into the final target compounds. The configuration at the anomeric position of these compounds was elucidated by means of proton and carbon nuclear magnetic resonance (^{1}H-, ^{13}C-NMR) analysis, and consideration of the rate of hydrolysis of the (2→5) glycosidic linkage.

Keywords KDN; glycosylation; disaccharide nucleoside; NMR; hydrolysis

A deaminated sialic acid, 3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (KDN, **1**) was isolated from polysialoglycoprotein (PSGP) of rainbow trout egg.[3] KDN has the same configuration as *N*-acetylneuraminic acid,[4] so KDN derivatives might have some of the biological activities of *N*-acetylneuraminic acid derivatives. In studies on *N*-acetylneuraminic acid, several disaccharide nucleoside derivatives were synthesized,[5,6] and some biological activities of these compounds were reported.[7-10] We have synthesized the monosaccharide nucleoside derivatives of **1** under Vorbrüggen and Williamson reaction conditions,[11] and Achiwa *et al.* have synthesized the disaccharide derivatives of **1** under Koenigs–Knorr reaction conditions.[12] In this paper, we wish to report the synthesis of uridine, 5-fluorouridine, cytidine, and inosine derivatives of **1** under Koenigs–Knorr reaction conditions. The configuration at the anomeric position of these disaccharide nucleosides was elucidated by means of poton and carbon nuclear magnetic resonance (^{1}H-, ^{13}C-NMR) and circular dichroism (CD) spectral analyses and a consideration of the rate of acid-catalyzed hydrolysis.

Synthesis of Disaccharide Derivatives of KDN Koenigs–Knorr reaction of benzyl 4,5,7,8,9-penta-*O*-acetyl-2-bromo-2,3-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate (**2**) with 2′,3′-isopropylideneuridine under various conditions (Table I) gave *O*-[benzyl (4,5,7,8,9-penta-*O*-acetyl-3-deoxy-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosyl)-onate]-(2→5′)-2′,3′-*O*-isopropylideneuridine (**4a, b**). When silver triffuoromethanesulfonate was used as a promoter in dichloromethane, the yield was about 20%. When a mixture of mercury cyanide and mercury bromide was used as a promoter in dichloromethane, **4a** and **4b** were obtained in a total yield of 47%, though when the same promoter was used in acetonitrile, the yield was 21%. A similar



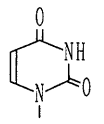
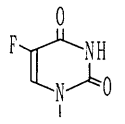
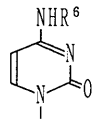
compound	anomer	R^1	R^2	R^3	R^4	R^5
4a	α		isopropylidene		benzyl	Ac
4b	β					
9a	α				H	H
9b	β					
5a	α		isopropylidene		benzyl	Ac
5b	β					
10a	α				H	H
10b	β					
6a	α	 R^6=COPh	Ac	Ac	benzyl	Ac
6b	β					
11a	α	R^6=H	H	H	H	H
11b	β					

Chart 1. Synthesis of Uridine, 5-Fluorouridine, and Cytidine Derivatives

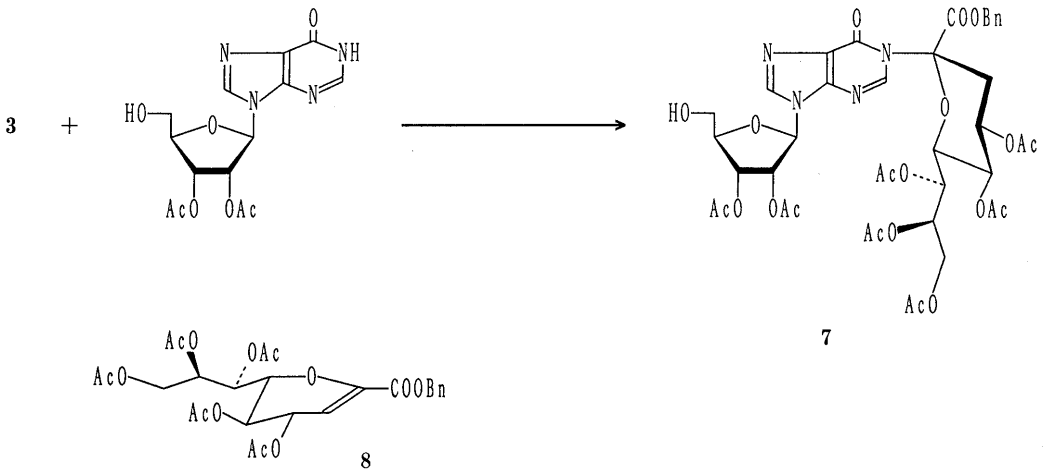


Chart 2. Synthesis of the Inosine Derivative

TABLE I. Reaction Conditions and Yields of Disaccharide Derivatives

Acceptor[a]	Donor	Promoter	Solvent	Temp.	Time (d)	Yield (%)			8
						α	β	(α:β)	
U	2	AgOTf (2.7 eq)	CH_2Cl_2	r.t.	10	12	10	(10: 8)	61
U	2	AgOTf (1.3 eq)	CH_2Cl_2	40 °C	7	11	10	(10: 9)	50
U	2	$Hg(CN)_2$ (0.72 eq)/$HgBr_2$ (0.96 eq)	CH_2Cl_2	r.t.	3	18	29	(10:16)	25
U	2	$Hg(CN)_2$ (0.72 eq)/$HgBr_2$ (0.96 eq)	CH_3CN	r.t.	4	8	13	(10:17)	34
FU	2	AgOTf (2.7 eq)	CH_2Cl_2	r.t.	10	6	17	(10:27)	65
FU	2	AgOTf (1.3 eq)	CH_2Cl_2	40 °C	7	3	12	(10:43)	68
FU	2	$Hg(CN)_2$ (0.72 eq)/$HgBr_2$ (0.96 eq)	CH_2Cl_2	r.t.	3	12	20	(10:17)	36
C	2	AgOTf (2.7 eq)	CH_2Cl_2	r.t.	7	5	5	(1: 1)	60
C	3	AgOTf (2.7 eq)	CH_2Cl_2	r.t.	6	8	8	(1: 1)	72
C	3	$AgClO_4$ (2.7 eq)	CH_2Cl_2	r.t.	7	5	5	(1: 1)	73
C	3	$Hg(CN)_2$ (0.6 eq)/$HgBr_2$ (0.82 eq)	CH_2Cl_2	r.t.	14	9	24	(10:25)	58
I	3	AgOTf (1.3 eq)	CH_2Cl_2	r.t.	4		44		35
I	3	$AgClO_4$ (1.3 eq)	CH_2Cl_2	r.t.	4		34		28

a) U, 2′,3′-*O*-isopropylideneuridine; FU, 5-fluoro-2′,3′-*O*-isopropylideneuridine; C, 2′, 3′-di-*O*-acetyl-*N*-benzoylcytidine; I, 2′,3′-di-*O*-acetylinosine. r.t. = room temperature.

reaction of **2** with 5-fluoro-2′,3′-*O*-isopropylideneuridine under various conditions (Table I) gave *O*-[benzyl(4,5,7,-8,9-penta-*O*-acetyl-3-deoxy-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosyl)onate]-(2→5′)-5-fluoro-2′,3′-*O*-isopropylideneuridine (**5a, b**). When a mixture of mercury cyanide and mercury bromide was used as a promoter in dichloromethane, the desired products were obtained in good yield (total 32%). A similar reaction of 2 or benzyl 4,5,7,8,9-penta-*O*-acetyl-2-chloro-2,3-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate (**3**) with 2′,3′-di-*O*-acetyl-*N*-benzoylcytidine under various conditions (Table I) gave *O*-[benzyl (4,5,7,8,9-penta-*O*-acetyl-3-deoxy-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosyl)onate]-(2→5′)-2′,3′-di-*O*-acetyl-*N*-benzoylcytidine (**6a, b**). When a mixture of mercury cyanide and mercury bromide was used as a promoter in dichloromethane, the desired products were obtained in good yield (33%), as in the reaction of uridine and the 5-fluorouridine derivative. In all trials, benzyl 4,5,7,8,9-penta-*O*-acetyl-2,6-anhydro-2,3-dideoxy-D-*glycero*-D-*galacto*-non-2-enonate (**8**) was obtained as a by-product in 25—73% yield. From these results, as shown in Table I, it was concluded that mercury cyanide and mercury bromide were the best promoters and dichloromethane was the best solvent for these reactions. These disaccharide derivatives of **1** were deprotected with 1 N sodium hydroxide solution or ammonia-saturated methanol to give *O*-[(3-deoxy-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosyl)onic acid]-(2→5′)-2′,3′-*O*-isopropylideneuridine (**9a, b**), *O*-[(3-deoxy-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosyl)onic acid]-(2→5′)-5-fluoro-2′,3′-*O*-isopropylideneuridine (**10a, b**), and *O*-[(3-deoxy-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosyl)-onic acid]-(2→5′)-cytidine (**11a, b**) in almost quantitative yields, respectively.

On the other hand, synthesis of the inosine derivative was attempted. The reaction of **3** with 2′,3′-di-*O*-acetylinosine gave *O*-[benzyl (4,5,7,8,9-penta-*O*-acetyl-3-deoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl)onate]-(2→N^1)-2′,3′-di-*O*-acetylinosine (**7**) under various conditions (Table I). A desired KDN (2→5′)-inosine derivative was not obtained.

Stereochemistry at the Anomeric Position Figure 1 shows the CD spectra of the deprotected α-anomers (**9a, 10a, 11a**) and β-anomers (**9b, 10b, 11b**) in methanol. Based on the CD spectra of the *O*-glycosyl derivatives,[4,13] the peak around 220—230 nm is due to the n–π* Cotton effect of the carboxyl group and the negative Cotton effect was assigned to the α-anomer and the positive one to the

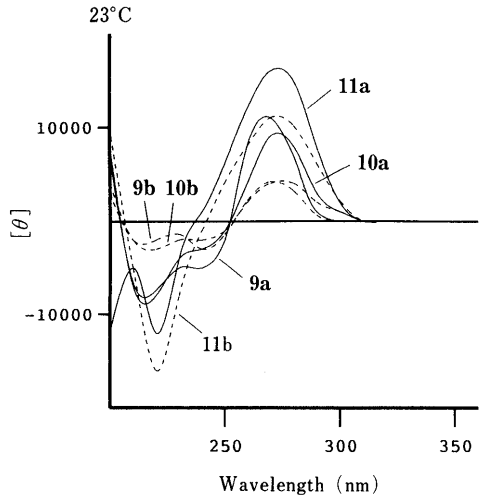


Fig. 1. CD Curves of **9a**, **9b**, **10a**, **10b**, **11a**, and **11b** in MeOH

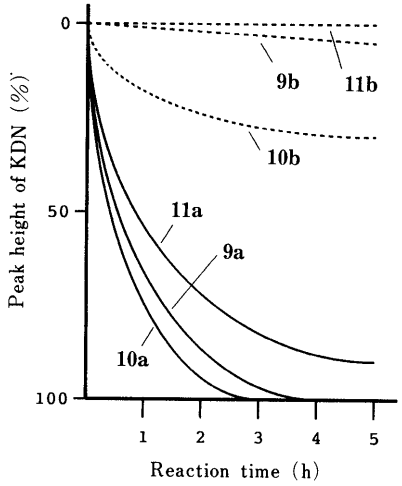


Fig. 2. Acid-Catalyzed Hydrolysis of **9a**, **9b**, **10a**, **10b**, **11a**, and **11b** (0.5 N H_2SO_4, 60 °C)

TABLE II. Proton Chemical Shifts and Spin-Coupling Data at 300 MHz

Compound	Chemical shift (ppm)				Spin coupling (Hz)
	H-3_{eq}	$\Delta(\alpha-\beta)$	H-4	$\Delta(\alpha-\beta)$	$J_{7,8}$
4a	2.64		4.93		9.2
4b	2.52	0.21	5.43	−0.50	4.0
9a	2.53		3.51		—
9b	2.28	0.25	3.86	−0.35	—
5a	2.69		4.95		9.5
5b	2.53	0.16	5.40	−0.45	3.8
10a	2.54		3.49		—
10b	2.29	0.25	3.83	−0.34	—
6a	2.71		4.98		9.5
6b	2.58	0.13	5.31	−0.33	6.0
11a	2.63		3.61		—
11b	2.33	0.30	3.89	−0.28	—

β-anomer. As shown in Fig. 1, the spectra are not in accordance with the above concept.

Table II shows the selected ^{1}H-NMR data of the uridine, 5-fluorouridine, and cytidine derivatives. Empirical studies[4,13-16] of **1** and *N*-acetylneuraminic acid indicated that the H-3(eq) signal of the α-anomer is usually observed at lower field than that of the β-anomer, the H-4 signal of the β-anomer is observed at lower field than that of the α-anomer, and the spin coupling of the α-anomer is larger than that of the β-anomer (α, 8—10 Hz; β, 3—6 Hz). The configuration at the anomeric position of these compounds was evaluated by applying this empirical rule. That of the inosine derivative (**7**) could not be elucidated by this approach, since only one anomer was obtained. So, it was elucidated on the basis of the coupling pattern of C-1 in gated proton-decoupled or selective proton-decoupled ^{13}C-NMR spectra, by analogy with that of the monosaccharide derivatives of **1**.[11] The value of $J_{C1,3ax}$ was observed as 1 Hz, and therefore, **7** was confirmed to be the β-anomer.

The previous study[5,16] of *N*-acetylneuraminic acid derivatives indicated that the rate of hydrolysis of the α-anomer was remarkably high in comparison with that of the β-anomer. Figure 2 shows the rate of acid catalyzed hydrolysis of the deprotected uridine, 5-fluorouridine, and cytidine derivatives (**9a, b**, **10a, b**, **11a, b**) in 0.5 N sulfuric acid at 60 °C. In the case of uridine derivatives (**9a, b**) and 5-fluorouridine derivatives (**10a, b**), the α-anomers were decomposed within 4 h, whereas half of the β-anomers remained at 5 h. In the case of the cytidine derivatives (**11a, b**), the α-anomer was decomposed within 5 h, whereas the β-anomer was not hydrolyzed within 5 h. It is clear that measurement of the rate of hydrolysis is a useful method for confirmation of the anomeric configuration of KDN derivatives, as well as *N*-acetylneuraminic acid derivatives.

Conclusion

We have synthesized the KDN (2→5′) linked disaccharide nucleoside derivatives of uridine, 5-fluorouridine, and cytidine under Koenigs–Knorr reaction conditions. However, a similar reaction of **3** with 2′,3′-di-*O*-acetylinosine gave the corresponding (2→N^1) linked derivative. The stereochemistry at the anomeric configuration of these compounds was elucidated by ^{1}H, ^{13}C-NMR spectral analysis and a consideration of the rate of hydrolysis. The biological activities of these disaccharide derivatives are under investigation.

Experimental

Melting points were measured with a Yamato melting point apparatus and the results are uncorrected. Optical rotations were measured with a JASCO DIP-4-polarimeter. Thin layer chromatoraphy (TLC) was performed on Silica gel (Merck) plates, and spots were detected by spraying with 5% sulfuric acid solution. Fast atom bombardment mass spectra (FAB-MS), and infrared (IR) spectra were measured with JEOL JMS-DX300 and JASCO IR-A2 instruments, respectively. CD spectra were measured in a 0.1 cm cell with a JASCO J-720 spectropolarimeter. The ^{1}H-NMR spectra were measured with a Varian VXR-300 spectrometer. Tetramethylsilane (TMS) in $CDCl_3$ or sodium 3-(trimethylsilyl)-1-propanesulfonate (DDS) in D_2O was used as an internal reference. Column chromatography was conducted on Silica gel 60 (70—230 mesh).

***O*-[Benzyl (4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosyl)onate]-(2→5′)-2′,3′-*O*-isopropylideneuridine (4a, b)** A typical glycosylation was carried out as follows. A solution of 2′,3′-*O*-isopropylideneuridine (340 mg, 1.20 mmol) in dried dichloromethane (25 ml) was stirred with molecular sieves 4A (1.2 g). After 1 h, **2** (652 mg, 1.00 mmol), $Hg(CN)_2$ (150 mg, 0.72 mmol) and $HgBr_2$ (300 mg, 0.96 mmol) were added to the solution, and the mixture was stirred for 3 d at room temperature. The whole was filtered through Celite, the filtrate was evaporated to dryness, and the residue was extracted with benzene. The extract was washed with potassium chloride solution and brine, dried

over anhydrous magnesium sulfate, and evaporated *in vacuo*. The residual syrup was chromatographed on a column of silica gel with $CHCl_3$–MeOH (400 : 1) to give the α-anomer (**4a**) (153 mg, 18%), β-anomer (**4b**) (243 mg, 29%), and benzyl 4,5,7,8,9-penta-*O*-acetyl-2,6-anhydro-2,3-dideoxy-D-*glycero*-D-*galacto*-non-2-enonate (**8**) (139 mg, 25%).

α-Anomer (**4a**): $[\alpha]_D^{28}$ +15.8° (c=0.53, $CHCl_3$). FAB-MS m/z: 835 (M^++1). *Anal.* Calcd for $C_{38}H_{46}N_2O_{19}$: C, 54.68; H, 5.55; N, 3.36. Found: C, 54.96; H, 5.64; N, 3.01. IR ν_{max}^{KBr} cm^{-1}: 2950, 1750, 1700. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: (KDN moiety) 1.88 (1H, dd, J=11.4, 12.5 Hz, H-3_{ax}), 2.00, 2.00, 2.04, 2.09, 2.16 (each 3H, s, OAc), 2.64 (1H, dd, J=4.2, 12.5 Hz, H-3_{eq}), 4.08 (1H, dd, J=5.3, 12.4 Hz, H-9a), 4.24 (1H, dd, J=2.1, 9.7 Hz, H-6), 4.25 (1H, dd, J=2.7, 12.4 Hz, H-9b), 4.90 (1H, t, J=9.7 Hz, H-5), 4.93 (1H, m, H-4), 5.17, 5.25 (each 1H, d, J=12.0 Hz, $-CH_2Ph$), 5.33 (1H, dd, J=2.1, 9.2 Hz, H-7), 5.39 (1H, ddd, J=2.7, 5.3, 9.2 Hz, H-8), 7.31—7.38 (5H, m, aromatic H), (uridine moiety) 1.34, 1.57 (each 3H, s, $-CH_3$), 3.41 (1H, dd, J=3.0, 10.8 Hz, H-5′a), 4.00 (1H, dd, J=3.5, 10.8 Hz, H-5′b), 4.34 (1H, q, J=4.0 Hz, H-4′), 4.63 (1H, dd, J=2.7, 6.1 Hz, H-2′), 4.74 (1H, dd, J=3.0, 6.1 Hz, H-3′), 5.41 (1H, dd, J=2.0, 7.2 Hz, H-5), 5.86 (1H, d, J=2.7 Hz, H-1′), 7.43 (1H, d, J=7.2 Hz, H-6), 8.61 (1H, br s, NH).

β-Anomer (**4b**): $[\alpha]_D^{28}$ −9.6° (c=0.35, $CHCl_3$). FAB-MS m/z: 835 (M^++1). *Anal.* Calcd for $C_{38}H_{46}N_2O_{19}$: C, 54.68; H, 5.55; N, 3.36. Found: C, 54.94; H, 5.54; N, 3.10. IR ν_{max}^{KBr} cm^{-1}: 2980, 1760, 1700. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: (KDN moiety): 1.91 (1H, dd, J=11.0, 13.1 Hz, H-3_{ax}), 1.97, 1.98, 1.99, 2.04, 2.08 (each 3H, s, OAc), 2.52 (1H, dd, J=5.0, 13.1 Hz, H-3_{eq}), 4.05 (1H, dd, J=8.0, 12.3 Hz, H-9a), 4.08 (1H, dd, J=2.0, 10.0 Hz, H-6), 4.74 (1H, dd, J=2.5, 12.3 Hz, H-9b), 4.81 (1H, t, J=10.0 Hz, H-5), 5.16, 5.25 (each 1H, d, J=12.0 Hz, $-CH_2Ph$), 5.29 (1H, dd, J=2.0, 4.0 Hz, H-7), 5.37 (1H, ddd, J=2.5, 4.0, 8.0 Hz, H-8), 5.43 (1H, ddd, J=5.0, 9.8, 11.0 Hz, H-4), 7.31—7.37 (5H, m, aromatic H); (uridine moiety): 1.34, 1.54 (each 3H, s, $-CH_3$), 3.56 (1H, dd, J=2.9, 10.9 Hz, H-5′a), 3.88 (1H, dd, J=3.9, 10.9 Hz, H-5′b), 5.05 (1H, dd, J=1.5, 6.3 Hz, H-2′), 5.12 (1H, dd, J=4.8, 6.3 Hz, H-3′), 5.61 (1H, d, J=1.5 Hz, H-1′), 5.78 (1H, dd, J=2.0, 8.0 Hz, H-5), 7.28 (1H, d, J=8.0 Hz, H-6), 9.10 (1H, br s, NH).

***O*-[Benzyl (4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosyl)onate]-(2→5′)-5-fluoro-2′,3′-*O*-isopropylideneuridine (5a, b)** A solution of 5-fluoro-2′,3′-*O*-isopropylideneuridine (362 mg, 1.20 mmol) in dried dichloromethane (25 ml) was stirred with molecular sieves 4A (1.2 g). After 1 h, **2** (652 mg, 1.00 mmol), $Hg(CN)_2$ (150 mg, 0.72 mmol) and $HgBr_2$ (300 mg, 0.96 mmol) were added to the solution, and the mixture was stirred for 3 d at room temperature. The solution was processed as described for **4a** and **4b** to give the α-anomer (**5a**) (102 mg, 12%), β-anomer (**5b**) (171 mg, 20%), and **8** (200 mg, 36%).

α-Anomer (**5a**): $[\alpha]_D^{28}$ +18.0° (c=0.33, $CHCl_3$). FAB-MS m/z: 853 (M^++1). *Anal.* Calcd for $C_{38}H_{45}FN_2O_{19}$: C, 53.52; H, 5.32; N, 3.28. Found: C, 53.32; H, 5.36; N, 3.30. IR ν_{max}^{KBr} cm^{-1}: 2960, 1760, 1725. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: (KDN moiety): 1.92 (1H, dd, J=11.2, 13.0 Hz, H-3_{ax}), 1.99, 2.00, 2.04, 2.09, 2.16 (each 3H, s, OAc), 2.69 (1H, dd, J=4.7, 13.0 Hz, H-3_{eq}), 4.08 (1H, dd, J=5.0, 12.4 Hz, H-9a), 4.24 (1H, dd, J=2.4, 12.4 Hz, H-9b), 4.30 (1H, dd, J=2.4, 10.0 Hz, H-6), 4.90 (1H, t, J=9.5 Hz, H-5), 4.95 (1H, ddd, J=4.7, 9.5, 11.2 Hz, H-4), 5.11, 5.31 (each 1H, d, J=12.0 Hz, $-CH_2Ph$), 5.34 (1H, dd, J=2.4, 9.5 Hz, H-7), 5.40 (1H, ddd, J=2.4, 5.0, 9.5 Hz, H-8), 7.29—7.38 (5H, m, aromatic H); (5-fluorouridine moiety): 1.35, 1.57 (each 3H, s, $-CH_3$), 3.43 (1H, dd, J=2.4, 11.0 Hz, H-5′a), 4.03 (1H, dd, J=2.9, 11.0 Hz, H-5′b), 4.34 (1H, m, H-4′), 4.57 (1H, dd, J=2.8, 6.1 Hz, H-2′), 4.79 (1H, dd, J=3.0, 6.1 Hz, H-3′), 5.95 (1H, dd, J=1.1, 2.8 Hz, H-1′), 7.69 (1H, d, J=6.5 Hz, H-6), 8.90 (1H, br s, NH).

β-Anomer (**5b**): $[\alpha]_D^{28}$ +0.1° (c=0.35, $CHCl_3$). FAB-MS m/z: 853 (M^++1). *Anal.* Calcd for $C_{38}H_{45}FN_2O_{19}$: C, 53.52; H, 5.32; N, 3.28. Found: C, 53.50; H, 5.46; N, 3.16. IR ν_{max}^{KBr} cm^{-1}: 2950, 1725. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: (KDN moiety): 1.90 (1H, dd, J=11.7, 13.1 Hz, H-3_{ax}), 1.97, 1.98, 1.98, 2.05, 2.09 (each 3H, s, OAc), 2.53 (1H, dd, J=5.1, 13.1 Hz, H-3_{eq}), 4.04 (1H, dd, J=8.2, 12.0 Hz, H-9a), 4.08 (1H, dd, J=2.1, 10.0 Hz, H-6), 4.78 (1H, dd, J=2.8, 12.0 Hz, H-9b), 4.82 (1H, t, J=10.0 Hz, H-5), 5.16, 5.25 (each 1H, d, J=12.0 Hz, $-CH_2Ph$), 5.30 (1H, dd, J=2.1, 3.8 Hz, H-7), 5.38 (1H, ddd, J=2.8, 3.8, 8.2 Hz, H-8), 5.40 (1H, ddd, J=5.1, 10.0, 11.7 Hz, H-4), 7.31—7.38 (5H, m, aromatic H); (5-fluorouridine moiety): 1.35, 1.54 (each 3H, s, $-CH_3$), 3.56 (1H, d, J=3.3, 11.0 Hz, H-5′a), 3.89 (1H, dd, J=4.3, 11.0 Hz, H-5′b), 5.11 (1H, d, J=2.0 Hz, H-3′), 5.11 (1H, s, H-2′), 5.47 (1H, s, H-1′), 7.40 (1H, d, J=5.5 Hz, H-6), 9.38 (1H, bs, NH).

***O*-[Benzyl (4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosyl)onate]-(2→5′)-2′,3′-di-*O*-acetyl-*N*-benzoylcytidine (6a, b)** A solution of 2′,3′-di-*O*-acetyl-*N*-benzoylcytidine (503 mg, 1.30 mmol) in dried dichloromethane (25 ml) was stirred with molecular sieves 4A (1.2 g). After 1 h, **3** (586 mg, 1.00 mmol), $Hg(CN)_2$ (150 mg, 0.60 mmol) and $HgBr_2$ (300 mg, 0.82 mmol) were added to the solution, and the mixture was stirred for 14 d at room temperature. The solution was processed as described for **4a** and **4b** to give the α-anomer (**6a**) (88 mg, 9%), β-anomer (**6b**) (236 mg, 24%), and **8** (320 mg, 58%).

α-Anomer (**6a**): $[\alpha]_D^{28}$ +29.4° (c=0.37, $CHCl_3$). FAB-MS m/z: 982 (M^++1). *Anal.* Calcd for $C_{46}H_{51}N_3O_{21}$: C, 56.27; H, 5.23; N, 4.28. Found: C, 55.97; H, 5.38; N, 4.02. IR ν_{max}^{KBr} cm^{-1}: 2950, 1760. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: (KDN moiety): 2.01 (1H, dd, J=12.0, 12.8 Hz, H-3_{ax}), 2.01, 2.03, 2.04, 2.05, 2.11 (each 3H, s, OAc), 2.71 (1H, dd, J=4.5, 12.8 Hz, H-3_{eq}), 4.11 (1H, dd, J=4.8, 12.3 Hz, H-9a), 4.21 (1H, dd, J=2.5, 12.3 Hz, H-9b), 4.26 (1H, dd, J=2.0, 10.0 Hz, H-6), 4.91 (1H, t, J=9.5 Hz, H-5), 4.98 (1H, ddd, J=4.5, 9.5, 12.0 Hz, H-4), 5.19, 5.30 (each 1H, d, J=12.0 Hz, $-CH_2Ph$), 5.34 (1H, dd, J=2.0, 9.5 Hz, H-7), 5.41 (1H, ddd, J=2.5, 4.8, 9.5 Hz, H-8), 7.27—8.05 (5H, m, aromatic H); (cytidine moiety): 2.12, 2.16 (each 3H, s, OAc), 3.52 (1H, dd, J=2.3, 11.0 Hz, H-5′a), 4.03 (1H, dd, J=1.8, 11.0 Hz, H-5′b), 4.32 (1H, m, H-4′), 5.31 (1H, dd, J=5.1, 6.2 Hz, H-2′), 5.47 (1H, dd, J=3.2, 5.1 Hz, H-3′), 6.36 (1H, d, J=6.2 Hz, H-1′), 7.53 (1H, d, J=7.3 Hz, H-6), 7.89 (1H, d, J=7.3 Hz, H-5), 7.27—8.05 (5H, m, aromatic H), 8.70 (1H, br s, NH).

β-Anomer (**6b**): $[\alpha]_D^{28}$ +20.7° (c=0.30, $CHCl_3$). FAB-MS m/z: 982 (M^++1). *Anal.* Calcd for $C_{46}H_{51}N_3O_{21}$: C, 56.27; H, 5.23; N, 4.28. Found: C, 56.01; H, 5.35; N, 4.14. IR ν_{max}^{KBr} cm^{-1}: 2960, 1750. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: (KDN moiety): 1.96 (1H, dd, J=12.0, 13.4 Hz, H-3_{ax}), 2.00, 2.04, 2.05, 2.07, 2.09 (each 3H, s, OAc), 2.58 (1H, dd, J=5.0, 13.4 Hz, H-3_{eq}), 4.04 (1H, dd, J=6.5, 12.5 Hz, H-9a), 4.06 (1H, dd, J=1.8, 10.0 Hz, H-6), 4.64 (1H, dd, J=2.2, 12.5 Hz, H-9b), 4.93 (1H, t, J=9.8 Hz, H-5), 5.19, 5.28 (each 1H, d, J=12.0 Hz, $-CH_2Ph$), 5.31 (1H, m, H-4), 5.32 (1H, m, H-8), 5.37 (1H, dd, J=1.8, 6.0 Hz, H-7), 7.33—8.06 (5H, m, aromatic H); (cytidine moiety): 2.09, 2.11 (each 3H, s, OAc), 3.75 (1H, dd, J=2.8, 11.3 Hz, H-5′a), 3.85 (1H, dd, J=2.8, 11.3 Hz, H-5′b), 4.28 (1H, m, H-4′), 5.38 (1H, t, J=5.2 Hz, H-2′), 5.43 (1H, t, J=5.2 Hz, H-3′), 6.34 (1H, d, J=5.2 Hz, H-1′), 7.52 (1H, d, J=7.0 Hz, H-6), 7.89 (1H, d, J=7.0 Hz, H-5), 7.33—8.06 (5H, m, aromatic H), 8.74 (1H, br s, NH).

***O*-[Benzyl (4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl)onate]-(2→N^1)-2′,3′-di-*O*-acetylinosine (7)** A solution of 2′,3′-di-*O*-acetylinosine (586 mg, 1.20 mmol) in dried dichloromethane (25 ml) was stirred with molecular sieves 4A (1.2 g). After 1 h, **3** (586 mg, 1.00 mmol) and silver trifluoromethanesulfonate (334 mg, 1.30 mmol) were added to the solution, and the mixture was stirred for 96 h at room temperature. The solution was processed as described for **4a** and **4b** to give the β-anomer (**7**) (392 mg, 44%), and **8** (193 mg, 35%).

7: $[\alpha]_D^{28}$ −69.7° (c=0.35, $CHCl_3$). FAB-MS m/z: 903 (M^++1). *Anal.* Calcd for $C_{40}H_{46}N_4O_{20}$: C, 53.22; H, 5.14; N, 6.21. Found: C, 53.18; H, 5.34; N, 5.98. IR ν_{max}^{KBr} cm^{-1}: 2940, 1750. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: (KDN moietyl): 1.65, 1.98, 1.98, 2.00, 2.02 (each 3H, s, OAc), 2.30 (1H, dd, J=11.6, 13.7 Hz, H-3_{ax}), 2.97 (1H, dd, J=5.3, 13.7 Hz, H-3_{eq}), 4.11 (1H, dd, J=4.8, 12.9 Hz, H-9a), 4.30 (1H, dd, J=2.3, 12.9 Hz, H-9b), 4.40 (1H, dd, J=2.0, 10.0 Hz, H-6), 4.99 (1H, ddd, J=2.3, 4.8, 8.0 Hz, H-8), 5.02 (1H, t, J=10.0 Hz, H-5), 5.13, 5.20 (each 1H, d, J=12.0 Hz, $-CH_2Ph$), 5.38 (1H, dd, J=2.0, 8.0Hz, H-7), 5.46 (1H, dd, J=5.3, 9.6, 11.6 Hz, H-4), 7.12—7.31 (5H, m, aromatic H); (inosine moiety): 2.10, 2.16 (each 3H, s, OAc), 3.88 (1H, d, J=12.0 Hz, H-5′a), 3.97 (1H, dd, J=2.3, 12.5 Hz, H-5′b), 4.36 (1H, d, J=1.0 Hz, H-4′), 5.55 (1H, dd, J=2.3, 11.0 Hz, 5-OH), 5.69 (1H, dd, J=1.0, 5.0 Hz, H-3′), 6.00 (1H, dd, J=5.0, 7.8 Hz, H-2′), 6.07 (1H, d, J=7.8 Hz, H-1′), 7.12—7.31 (5H, m, aromatic H), 7.96 (1H, s, H-2), 8.02 (1H, s, H-8).

***O*-[(3-Deoxy-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosyl)onic acid]-(2→5′)-2′,3′-isopropylideneuridine (9a, b)** A 1 N sodium hydroxide solution (2 ml) was added to **4a** or **4b** (40 mg, 0.048 mmol) and the mixture was stirred for 2 h at room temperature. Dowex-50(H^+) resin was added to the reaction mixture, which was adjusted to pH 3—4 in an ice bath. The whole was filtered and the filtrate was freeze-dried to give the α-anomer (9a) (26 mg, 100%), or β-anomer (**9b**) (26 mg, 100%), each as a white powder.

α-Anomer (**9a**): $[\alpha]_D^{28}$ +0.2° (c=0.17, MeOH). FAB-MS m/z: 535 (M^++1). *Anal.* Calcd for $C_{21}H_{30}N_2O_{14}$: C, 47.19; H, 5.66; N, 5.24. Found: C, 47.35; H, 5.45; N, 5.51. IR ν_{max}^{KBr} cm^{-1}: 3370, 1750. ^{1}H-NMR (300 MHz, D_2O) δ: (KDN moiety): 1.52 (1H, dd, J=12.0, 12.5 Hz, H-3_{ax}), 2.53 (1H, dd, J=4.5, 12.0 Hz, H-3_{eq}), 3.51 (1H, ddd, J=4.5, 8.5, 12.5 Hz, H-4); (uridine moiety): 1.32, 1.52 (each 3H, s, $-CH_3$), 3.63 (1H, dd, J=3.0, 11.0 Hz, H-5′a), 3.90 (1H, dd, J=4.5, 11.0 Hz, H-5′b), 4.47 (1H, m, H-4′),

4.91 (1H, dd, J=2.5, 6.0 Hz, H-3′), 4.96 (1H, dd, J=2.5, 6.0 Hz, H-2′), 5.79 (1H, d, J=2.5 Hz, H-1′), 5.82 (1H, d, J=8.0 Hz, H-5), 7.74 (1H, d, J=8.0 Hz, H-6).

β-Anomer (**9b**): $[\alpha]_D^{28}$ −13.5° (c=0.33, MeOH). FAB-MS m/z: 535 (M^++1). *Anal.* Calcd for $C_{21}H_{30}N_2O_{14}$: C, 47.19; H, 5.66; N, 5.24. Found: C, 47.42; H, 5.56; N, 5.05. IR ν_{max}^{KBr} cm^{-1}: 3350, 1730. ^{1}H-NMR (300 MHz, D_2O) δ: (KDN moiety): 1.56 (1H, dd, J=12.0, 13.0 Hz, H-3_{ax}), 2.28 (1H, dd, J=5.0, 12.0 Hz, H-3_{eq}), 3.49 (1H, t, J=10.0 Hz, H-5), 3.86 (1H, m, 4-H); (uridine moiety): 1.34, 1.53 (each 3H, s, $-CH_3$), 3.41 (1H, dd, J=5.3, 10.5 Hz, H-5′a), 3.79 (1H, dd, J=6.5, 10.5 Hz, H-5′b), 4.43 (1H, m, H-4′), 4.97 (1H, dd, J=3.0, 6.3 Hz, H-3′), 5.03 (1H, dd, J=2.5, 6.3 Hz, H-2′), 5.79 (1H, d, J=2.5 Hz, H-1′), 5.83 (1H, d, J=8.0 Hz, H-5), 7.69 (1H, d, J=8.0 Hz, H-6).

***O*-[(3-Deoxy-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosyl)onic acid]-(2→5′)-5′-fluoro-2′,3′-*O*-isopropylideneuridine (10a, b)** A 1 N sodium hydroxide solution (2 ml) was added to **5a** or **5b** (40 mg, 0.047 mmol) and the mixture was stirred for 2 h at room temperature. The solution was processed as described for **9a** and **9b** to give the α-anomer (**10a**) (26 mg, 100%), or β-anomer (**10b**) (26 mg, 100%), each as a white powder.

α-Anomer (**10a**): $[\alpha]_D^{28}$ +20.7° (c=0.21, MeOH). FAB-MS m/z: 553 (M^++1). *Anal.* Calcd for $C_{21}H_{29}FN_2O_{14}$: C, 45.66; H, 5.29; N, 5.07. Found: C, 45.94; H, 5.05; N, 4.89. IR ν_{max}^{KBr} cm^{-1}: 3400, 1760. ^{1}H-NMR (300 MHz, D_2O) δ: (KDN moiety): 1.54 (1H, t, J=12.0 Hz, H-3_{ax}), 2.54 (1H, dd, J=4.5, 12.0 Hz, H-3_{eq}), 3.49 (1H, m, H-4); (5-fluorouridine moiety): 1.33, 1.52 (each 3H, s, $-CH_3$), 3.64 (1H, dd, J=2.5, 11.0 Hz, H-5′a), 3.91 (1H, dd, J=4.2, 11.0 Hz, H-5′b), 4.51 (1H, m, H-4′), 4.93 (2H, m, H-2′ and H-3′), 5.77 (1H, s, H-1′), 7.69 (1H, d, J=6.3 Hz, H-6).

β-Anomer (**10b**): $[\alpha]_D^{28}$ −22.9° (c=0.37, MeOH). FAB-MS m/z: 553 (M^++1). *Anal.* Calcd for $C_{21}H_{29}FN_2O_{14}$: C, 45.66; H, 5.29; N, 5.07. Found: C, 45.35; H, 5.02; N, 4.78. IR ν_{max}^{KBr} cm^{-1}: 3350, 1730. ^{1}H-NMR (300 MHz, D_2O) δ: 1.57 (1H, dd, J=11.8, 13.0 Hz, H-3_{ax}), 2.29 (1H, dd, J=5.0, 13.0 Hz, H-3_{eq}), 3.49 (1H, t, J=9.5 Hz, H-5); (5-fluorouridine moiety): 1.34, 1.52 (each 3H, s, $-CH_3$), 3.41 (1H, dd, J=5.0, 10.7 Hz, H-5′), 4.43 (1H, m, H-4′), 4.98 (2H, m, H-2′ and H-3′), 5.79 (1H, s, H-1′), 7.89 (1H, d, J=6.0 Hz, H-6).

***O*-[(3-Deoxy-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosyl)onic acid]-(2→5′)-cytidine (11a, b)** A solution of **6a** or **6b** (80 mg, 0.081 mmol) in ammonia saturated methanol (4 ml) was stirred for 3 d at room temperature. The solution was evaporated and the residues was dissolved in water (10 ml). Dowex-50 (H^+) was added to the solution, which was adjusted to pH 3—4 in an ice bath. The whole was filtered, and the filtrate was freeze-dried to give the α-anomer (**11a**) (39 mg, 98%) and β-anomer (**11b**) (37 mg, 94%), each as a white powder.

α-Anomer (**11a**): $[\alpha]_D^{28}$ +48.5° (c=0.23, MeOH). FAB-MS m/z: 493 (M^+). *Anal.* Calcd for $C_{18}H_{27}N_3O_{13}$: C, 43.82; H, 5.52; N, 8.52. Found: C, 43.69; H, 5.33; N, 8.44. IR ν_{max}^{KBr} cm^{-1}: 3400, 1720. ^{1}H-NMR (300 MHz, D_2O) δ: (KDN moiety): 1.71 (1H, dd, J=11.5, 13.0 Hz, H-3_{ax}), 2.63 (1H, dd, J=4.0, 13.0 Hz, H-3_{eq}), 3.52 (1H, t, J=9.5 Hz, H-5), 3.61 (1H, ddd, J=4.0, 9.5, 11.5 Hz, H-4); (cytidine moiety): 3.82 (1H, dd, J=2.5, 10.5 Hz, H-5′a), 3.91 (1H, dd, J=3.0, 10.5 Hz, H-5′b), 4.51 (1H, m, H-4′), 4.19 (1H, t, J=4.5 Hz, H-3′), 4.22 (1H, dd, J=3.0, 4.5 Hz, H-2′), 5.81 (1H, d, J=3.0 Hz, H-1′), 6.02 (1H, d, J=7.5 Hz, H-5), 7.88 (1H, d, J=7.5 Hz, H-6).

β-Anomer (**11b**): $[\alpha]_D^{28}$ +26.7° (c=0.31, MeOH). FAB-MS m/z: 493 (M^+). *Anal.* Calcd for $C_{18}H_{27}N_3O_{13}$: C, 43.82; H, 5.52; N, 8.52. Found: C, 43.57; H, 5.80; N, 8.46. IR ν_{max}^{KBr} cm^{-1}: 3500, 1750. ^{1}H-NMR (300 MHz, D_2O) δ: (KDN moiety): 1.62 (1H, dd, J=12.0, 13.0 Hz, H-3_{ax}), 2.33 (1H, dd, J=5.0, 13.0 Hz, H-3_{eq}), 3.53 (1H, t, J=9.5 Hz, H-5), 3.62 (1H, dd, J=5.5, 12.0 Hz, H-9a), 3.76 (1H, dd, J=2.5, 12.0 Hz, H-9b), 3.89 (1H, ddd, J=5.0, 9.5, 12.0 Hz, H-4); (cytidine moiety): 3.43 (1H, dd, J=4.5, 11.0 Hz, H-5′a), 3.93 (1H, dd, J=3.0, 11.0 Hz, H-5′b), 4.14 (1H, m, H-4′), 4.19 (1H, t, J=5.0 Hz, H-3′), 4.22 (1H, dd, J=3.0. 5.0 Hz, H-2′), 5.81 (1H, d, J=3.0 Hz, H-1′), 6.03 (1H, d, J=7.5 Hz, H-5), 7.77 (1H, d, J=7.5 Hz, H-6).

The Hydrolysis of 9a, 9b, 10a, 10b, 11a, and 11b KDN was determined by HPLC after hydrolysis of each sample at he concentration of 500 μg/ml in 0.5 N sulfuric acid solution at 60 °C (Fig. 2).

HPLC Method KDN was analyzed by anion exclusion chromatography[13] using a Hitachi GEL 3013-N strongly basic anion-exchange resin column (4.6 × 150 mm) at 70 °C. A mobile phase of 30 mM sodium sulfate was used at a flow rate of 0.8 ml/min. The column effluent was monitored with a UV detector at 205 nm (Nihon Seimitsu Kagaku, model NS-310).

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