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Synthesis and Physicochemical Properties of 6-*O*-Cyclopyrimidine Nucleosides[1]

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Cyclization of 5-iodo-1-(β-D-xylofuranosyl)uracil and -cytosine with sodium methoxide afforded 6,3′-*O*-cyclouridine (IIa) and -cytidine (IIc), respectively. The rate of cyclization of 5-iodopyrimidine nucleosides is greatly dependent on the ring size formed on cyclization. Introduction of a trityl group at the 5′-position of the nucleoside brought about a decrease in the rate of cyclization. The ease with which ring-opening of 6-*O*-cyclouracil nucleoside isomers was effected with diluted sulfuric acid was in the opposite order to that of cyclization. The ultraviolet, circular dichroism and mass spectra of 6-*O*-cyclopyrimidine nucleosides and their isomers were compared.

Keywords——cyclonucleoside; pyrimidine nucleoside; cyclization; ring-opening; UV spectrum; CD; mass spectrum

A variety of pyrimidine cyclonucleosides is known. Some of these have been used to examine the relationship between the circular dichroism (CD) spectra and the *N*-glycosyl conformation, while others have proven to be useful synthetic intermediates.[2] In addition, the clinical application of 2-*O*-cyclocytidine[3] accelerated synthetic studies of cyclonucleosides in the pyrimidine series. As compared with 2-*O*-cyclopyrimidine nucleosides, the 6-*O*-cyclo counterparts have been less well explored; *e.g.* the synthesis of the 6,3′-*O*-cycloisomer (II) has not yet been reported. This paper deals with the synthesis and physicochemical properties of 6-*O*-cyclopyrimidine nucleosides including II.

Synthesis of 6-*O*-Cyclouridines

It has been reported that treatment of 5-bromo-UA[4] (IV) with sodium methoxide may give rise to 6,2′-*O*-cyclouridine (Ia).[5,6] We compared the rates of cyclization[7] of IV and 5-iodo-UA (Va) under the same conditions. The rate of cyclization of Va was *ca.* 10 times larger than that of IV (Fig. 1). Therefore, 5-iodopyrimidine nucleosides were used for the subsequent cyclization. The rates of cyclization of 5-iodo-UX[4] (VIa) and -uridine (VIIa) were *ca.* 1/130 and 1/400 times smaller than that of Va, respectively. This result indicates that the rate of cyclization is greatly dependent on the size of the ring formed, the relative rates of cyclization being in the order arabinoside≫xyloside>riboside, where five-, six- and seven-membered rings were formed, respectively. Treatment of 5-iodouracil nucleosides with sodium methoxide afforded 6,3′-*O*-cyclouridine (IIa), a new compound, and 6,5′-*O*-cyclouridine (IIIa)[8] in 62% and 44% yields, respectively.[10] The rate of cyclization of 5-iodo-5′-*O*-trityl-UA (Vb) was *ca.* 1/3 of that of Va, and the rate for 5-iodo-5′-*O*-trityl-UX (VIb) was also much smaller than that of VIa (Fig. 2). Introduction of the trityl group at the 5′-position thus resulted in a decrease in the rate of cyclization. This could be explained as follows: (1) the bulky 5′-*O*-trityl group might cover the "upward" hydroxyl group in the sugar moiety of Vb (or VIb), (2) Va (or VIa) would adopt the predominant *anti*-conformation favorable for the cyclization, because the two intramolecular anions at the N^3- and 5′-positions, which are generated by the dissociation with sodium methoxide, should suffer electrostatic repulsion. Such a repulsion could be excluded in Vb (or VIb) by blocking of the 5′-hydroxyl group, (3) the bulky 5-iodo and 5′-*O*-trityl groups of Vb (or VIb) might cause steric hindrance leading to the predominant *syn*-conformation. The rate of cyclization of the 2′,3′-*O*-isopropylidene derivative of VIIa was *ca.* 300 times larger than that of VIIa (Chart 1). Introduction of the isopropylidene group

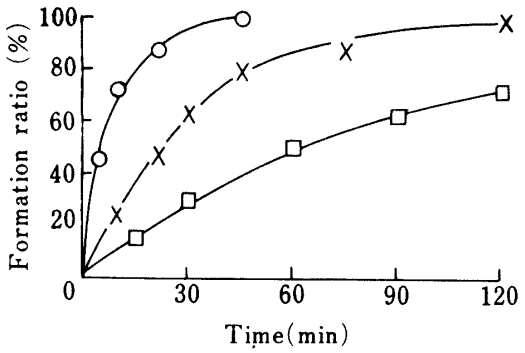


Fig. 1. Formation of Ia and Ib from 5-Halogeno-UA and Its 5′-*O*-Trityl Derivative by MeONa Treatment

○—○, Ia (from Va); □—□, Ia (from IV); ×—×, Ib.

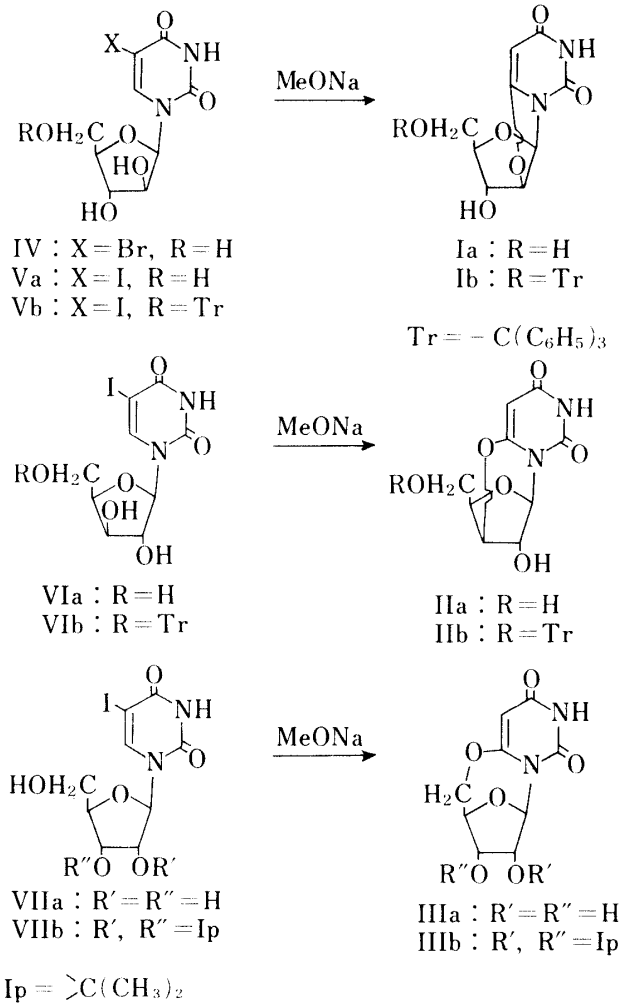
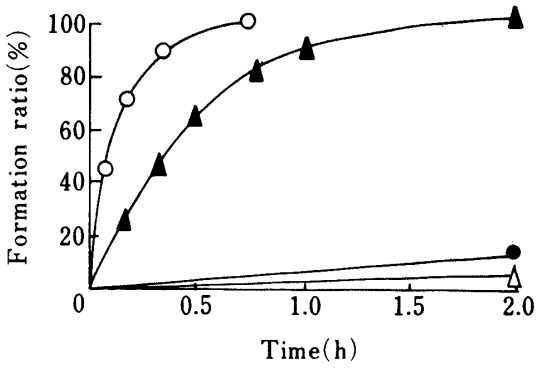


Chart 1



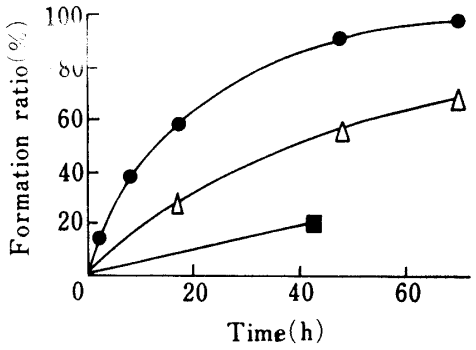


Fig. 2. Formation of 6-*O*-Cyclouridines from 5-Iodouracil Nucleosides by MeONa Treatment

○—○, Ia; ●—●, IIa; △—△, IIIa; ▲—▲, IIIb; ■—■, IIb.

presumably caused a change of sugar puckering to permit easy access of the 5′-hydroxyl group to the 6-position of the uracil base.[13]

Synthesis of 6-*O*-Cyclocytidines

Compound CX[4] has been prepared by the glycosylation of cytosine base.[14] We attempted to synthesize CX *via* 2′,5′-di-*O*-trityl-2,3′-*O*-cyclo-CX,[15] starting from the readily available cytidine. The route was, however, found to be unsatisfactory, owing to the lability of the key compound. Acetylation of 2′,5′-di-*O*-trityl-UX followed by treatments such as thiation, amination, deacetylation and detritylation afforded CX. These processes were thus useful for the preparation of a large amount of CX, because the overall yield was good and the intermediates could be isolated as stable crystalline products. The preparation of 5-iodo-CX was achieved in a better yield by replacing the solvent, acetic acid,[16] with trifluoroacetic acid, and similar iodination of cytidine yielded 5-iodocytidine (Chart 2).

6,2′- and 6,5′-*O*-Cyclocytidine (Ic and IIIc) have been prepared by treatment of 5-iodo-CA[4] and 5-iodocytidine, respectively, with *tert*-butoxide.[9,17] We applied sodium methoxide

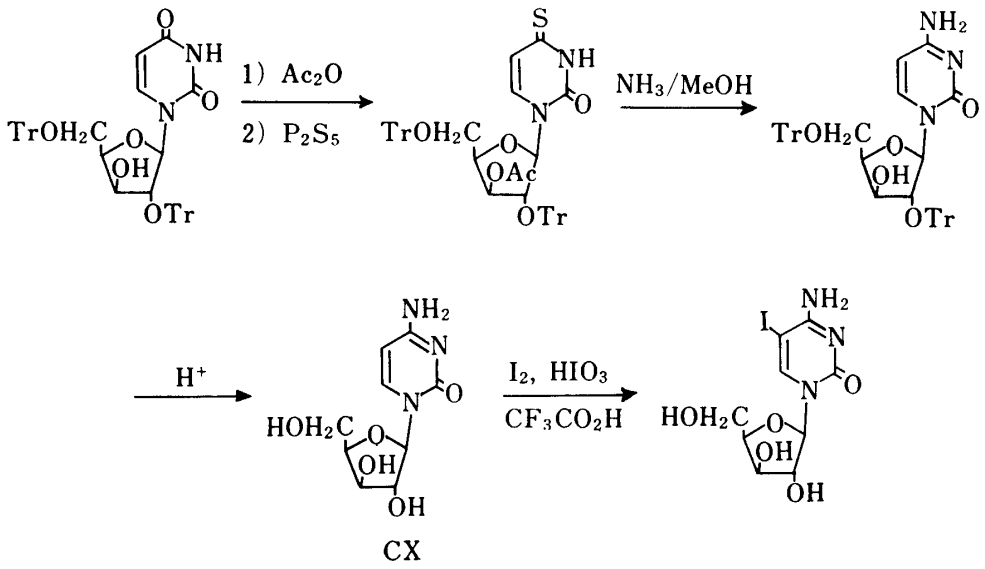


Chart 2

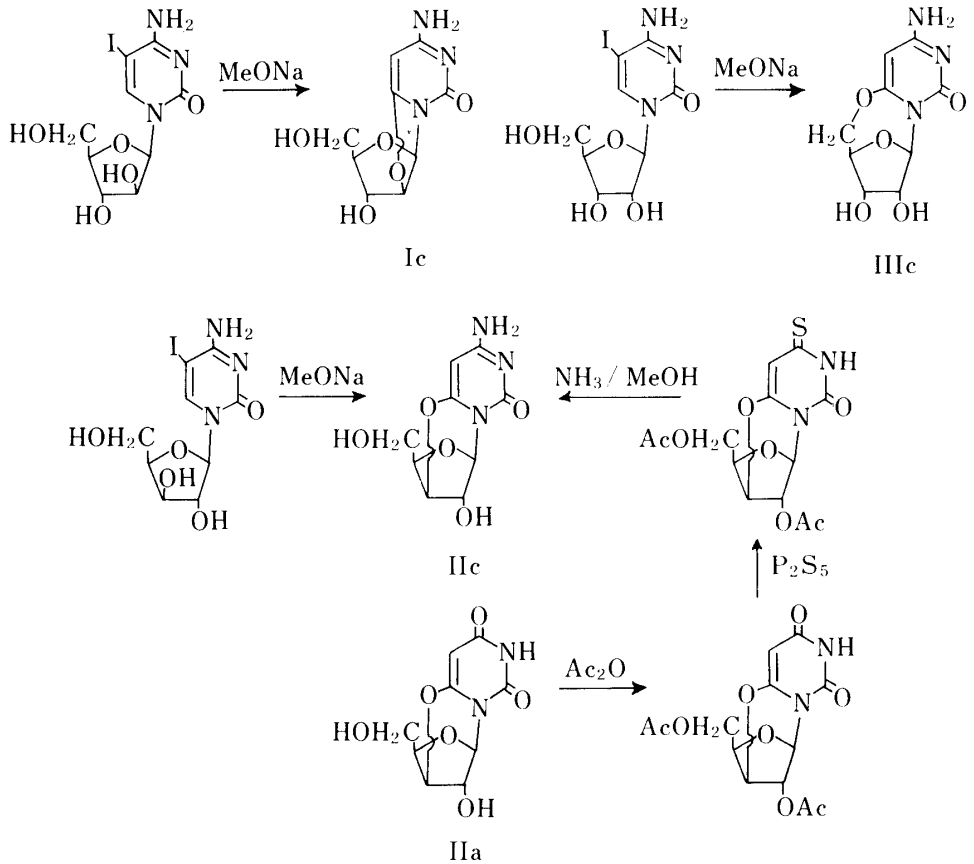


Chart 3

instead of *tert*-butoxide to cyclize the 5-iodocytosine nucleosides, as in the case of the 5-iodouracil nucleosides. The ease of cyclization was in the same order as that in the uracil series. However, the rate of cyclization in the cytosine series was much larger than that in the uracil series. The reason might be as follows: 5-iodocytosine nucleosides do not give rise to such a dissociation in the base moiety with sodium methoxide as 5-iodouracil nucleosides. This

would result in easier nucleophilic attack of the hydroxyl group of the sugar moiety on the 6-position of the cytosine base. The reaction of 5-iodo-CA and sodium methoxide was achieved in boiling methanol for 15 min, and treatment of the reaction mixture afforded Ic in 55% yield. A similar reaction of 5-iodo-CX yielded a novel 6,3′-*O*-cyclocytidine (IIc). The analogous reaction of 5-iodocytidine gave IIIc. Compound IIc was stable on heating over a long period with methanolic ammonia, and no other compound was detected by thin-layer chromatography. This result shows that IIc does not undergo such a rearrangement to the isomeric 2,2′-*O*-cyclopyrimidin-6-one nucleoside as in the case of Ic.[18] Acetylation of the 2′- and 5′-hydroxyl groups of IIa, followed by thiation and amination, afforded IIc. This route is an alternative method for the synthesis of IIc (Chart 3).

Ring-opening of 6-*O*-Cyclouridines

In order to compare the ease of hydrolytic ring-opening, compounds Ia—IIIa were allowed to react with 2 N sulfuric acid at 70 °C. Compound IIIa was completely hydrolyzed within 3 h, while Ia was stable even for 3 d.[19] The rate of hydrolysis of IIa is shown in Fig. 3. The ease of hydrolysis of the three isomers was in the following order: riboside >xyloside> arabinoside. This order is opposite to that in the cyclization described above.

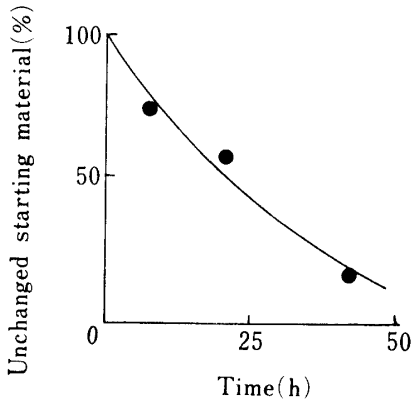


Fig. 3. Hydrolysis of IIa with 2 N H_2SO_4 at 70°C

Ultraviolet (UV) Spectra of 6-*O*-Cyclopyrimidine Nucleosides

The absorption maxima of 2-*O*-cyclouridines have been reported to shift to the long wavelength side on ring-opening to uridine.[20] The absorption maxima of 6-*O*-cyclouridines (or cytidines) decreased in the same order as that of 2-*O*-cyclouridines: uridine (or cytidine) ≒6,5′->6,3′-≫6,2′-*O*-cycloisomer. The values for the 6,5′- and 6,3′-*O*-cycloisomers in both the uracil and cytosine series were, however, close to those of uridine and cytidine, respectively. The molecular extinction coefficients were in the opposite order to those of the absorption maxima in both the uracil and cytosine series (Table I).

Table I. UV Data for 6-*O*-Cyclopyrimidine Nucleosides [$\lambda_{max}^{H_2O}$ nm (ε)]

	0.1 N HCl	pH *ca.* 7.0	0.1 N NaOH
Ia	251 (16700)	251.5(16400)	253.5(12100)
IIa	259.5(15300)	259.5(15100)	262 (12200)
IIIa	262 (12900)	261.5(13000)	264 (9900)
Ic	265 (22900)	261 (14500)	262 (15100)
IIc	273 (21000)	269.5(14000)	270 (14300)
IIIc	278 (17300)	271.5(11600)	272.5(11800)

CD Spectra of 6-*O*-Cyclopyrimidine Nucleosides

2,2′-*O*-Cyclouridine has been reported to show a positive Cotton effect of much higher amplitude than that of uridine in its optical rotatory dispersion (ORD) spectrum, while 2,3′-*O*-cyclouridine shows a small, positive Cotton effect.[21] The CD spectrum of 6,2′-*O*-cyclouridine, in which the sugar-base torsion angle differs by 180° from that of 2,2′-*O*-cyclouridine, has a large, negative Cotton effect, whereas the CD spectrum of 6,3′-*O*-cyclouridine, whose torsion angle differs by 180° from that of 2,3′-*O*-cyclouridine, has a small negative Cotton effect. The CD spectrum of 6,5′-*O*-cyclouridine showed a large, positive Cotton effect,

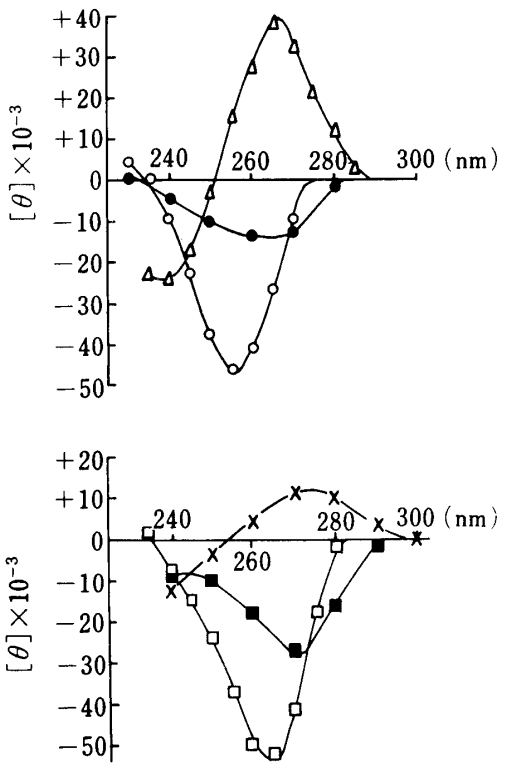


Fig. 4. CD Spectra of 6-*O*-Cyclopyrimidine Nucleosides (H_2O)

○—○, Ia; ●—●, IIa; △—△, IIIa; □—□, Ic; ■—■, IIc; ×—×, IIIc.

as would be expected from that of its 2′,3′-*O*-isopropylidene derivative.[22] These results are consistent with the reported dependence of the Cotton effect on the sugar-base torsion angle.[21]

The CD spectra of 6-*O*-cyclocytidines showed the same signs of Cotton effects as those of the corresponding 6-*O*-cyclouridines. Their $[\theta]$ values were, however, considerably different: the $[\theta]$ values of 6,2′-, 6,3′- and 6,5′-*O*-cyclocytidine were smaller than those of the corresponding 6-*O*-cyclouridine isomers (Fig. 4).

Mass Spectra (MS) of 6-*O*-Cyclopyrimidine Nucleosides

Some data have been published on the mass spectra of 6,2′- and 6,5′-*O*-cyclouridine,[23] and 6,5′-*O*-cyclocytidine.[24] We compared the mass spectra of all 6-*O*-cyclouridine and -cytidine isomers, including the new 6,3′-isomer. The spectra of these 6-*O*-cyclo compounds showed a high abundance of M^+ fragments, as is characteristic of those of other cyclonucleosides.[25] By analogy to previously reported data for 2-*O*-cyclouridines,[23] 6,2′- and 6,3′-*O*-cyclouridine could be readily distinguished from the 6,5′-isomer, but not from each other (Table II). The high-resolution mass spectrum of 6,3′-*O*-cyclouridine showed, besides M^+ ($C_9H_{10}N_2O_6$) ion peak, some main fragment ion peaks, such as M−31 ($C_8H_7N_2O_5$) formed by simple loss of 5′-CH_2OH,[23] M−113 ($C_4H_5N_2O_3$) which was derived from 6-hydroxyuracil,[23] and M−74 ($C_7H_6NO_4$) and M−103 ($C_6H_5NO_3$) which are presumed to arise by decomposition of the base moiety. The high-resolution mass spectrum of 6,3′-*O*-cyclocytidine gave, in addition to M^+ ($C_9H_{11}N_3O_5$), M−31 ($C_8H_8N_3O_4$), M−113 ($C_4H_6N_3O_2$) and M−114 ($C_4H_5N_3O_2$) fragment ion peaks, M−86 ($C_5H_5N_3O_3$) which could be related to the N^1-formyl derivative of 6-hydroxycytosine,[26] and M−130 ($C_4H_3N_2O_2$) which could be produced by release of the amino group from 6-hydroxycytosine.[24] It should be noted that in the cytosine series without exception the base peak was M−130.

The relative intensity of M−113 and M−114 ion peaks of 6-*O*-cycloisomers was in the order 6,5′->6,3′->6,2′-isomer in both the uracil and cytosine series. The result supports the view that the 6,5′-isomer is the most susceptable and the 6,2′-isomer is the most resistant to alkyl-*O*-fission. The order is in good agreement with that of instability of the 6-*O*-cyclo linkage

Table II. Selectd Ions from the Mass Spectra (70 eV) of 6-*O*-Cyclopyrimidine Nucleosides (% Relative Abundance)

Compd. No.	M	M−29	M−31	M−74	M−89	M−113	M−114	M−130
Ia	89.5	—	33.9	100.0	53.5	3.8	4.0	4.0
IIa	100.0	—	10.3	39.4	19.6	28.9	21.7	9.8
IIIa	53.9	43.5	—	17.7	10.5	100.0	44.2	9.3
Ic	51.0	—	39.4	2.0	50.1	7.9	5.8	100.0
IIc	74.0	—	18.1	—	41.1	19.2	34.2	100.0
IIIc	19.2	—	1.5	—	7.5	44.6	63.0	100.0

of each isomer with acid. The relative intensity of the M−89 ion peak, which might consist of the base plus its O atom link to the sugar C-1′ and -2′,[23] was in the order 6,2′->6,3′->6,5′-isomer in both the uracil and cytosine series. This supports the correctness of the structural assignment for the fragment ion (TableII).

Table III. ^{1}H-NMR Data for 6-*O*-Cyclopyrimidine Nucleosides (DMSO-d_6)

Compd. No.	H1′	H2′	H3′	H4′	H5′	H5	N-H
Ia	6.18 (d, 1) $J_{H1'-H2'}=5.0$	5.17 (d, 1) $J_{H2'-H3'}=0$	4.30 (q, 1) $J_{H3'-H4'}=2.0$	4.00 (sextet, 1) $J_{H4'-H5'}=6.0$	3.28 (m, 2)	4.94 (d, 1)	10.86 (br s, 1)
IIa	5.90 (d, 1) $J_{H1'-H2'}=1.0$	4.50 (m, 1)	4.90 (m, 1) $J_{H3'-H4'}=2.0$	4.30 (sextet, 1) $J_{H4'-H5'}=6.0$	3.52 (m, 2)	4.95 (s, 1)	11.08 (br s, 1)
IIIa	6.15 (s, 1) $J_{H1'-H2'}=0$	4.29 (d-like, 2)		4.36 (s-like, 1) $J_{H4'-H5'a}=1.5$ $J_{H4'-H5'b}=0.5$	4.53(q, 1) 3.92(q, 1) $J_{H5'a-H5b'}=13$	5.23 (d, 1)	11.24 (br s, 1)
Ic	6.12 (d, 1) $J_{H1'-H2'}=5.0$	5.12 (d, 1) $J_{H2'-H3'}=0$	4.30 (m, 1) $J_{H3'-H4'}=2.0$	3.95 (sextet, 1) $J_{H4'-H5'}=6.0$	3.3 (m, 2)	5.09 (s, 1)	7.01 (br s, 2)
IIc	5.97 (s, 1) $J_{H1'-H2'}=0$	4.37 (br s, 1) $J_{H2'-H3'}=2.0$	4.79 (t, 1) $J_{H3'-H4'}=2.5$	4.25 (sextet, 1) $J_{H4'-H5'}=6.0$	3.48 (t, 2)	5.05 (s, 1)	7.00 (br s, 2)
IIIc	6.29 (s, 1) $J_{H1'-H2'}=0$	4.22 (m, 2)		4.30 (br s, 1) $J_{H4'-H5'a}=1.5$ $J_{H4'-H5'b}=0.5$	4.47(q, 1) 3.84(q, 1) $J_{H5'a-H5'b}=12$	5.31 (s, 1)	7.11 (br s, 2)

Experimental

All melting points were determined on a Yanagimoto micromelting point apparatus (hot stage type) and are uncorrected. The UV spectra were recorded with a Shimadzu UV-190 digital spectrometer. The ^{1}H-nuclear magnetic resonance (NMR) spectra were recorded with a Hitachi R-42 (90 MHz) spectrometer in DMSO-d_6 with tetramethylsilane as an internal standard. Mass spectra were measured with a Shimadzu-LKB 9000 B spectrometer and the CD spectra with a JASCO ORD/UV-5 spectropolarimeter in 10 mm path-length cells. Paper chromatography (PC) was carried out on Toyo filter paper No. 51 by the ascending method using the following solvents: A, BuOH–AcOH–H_2O (5: 2: 3); B, BuOH–H_2O (84: 16). Thin layer chromatography (TLC) was carried out on plates (2×10 cm) coated with Wakogel B-5 including fluorescent indicator F_{254} (Merck).

Rate of Cyclization of 5-Halogenopyrimidine Nucleoside with Sodium Methoxide——A solution of 5-halogenopyrimidine nucleoside (0.2 mmol) in 1 N MeONa (2 ml) was refluxed with exclusion of moisture. After a definite time, a part of the solution was diluted with water and adjusted to pH 2 with 1 N HCl. In the analysis of a binary mixture of components, three wavelength pairs, 289 (ε 7780) and 251 nm (ε 16700), 290 (ε 8000) and 260 nm (ε 15300), and 290 (ε 8000) and 262 nm (ε 12900) were used for the estimation of Va (or Vb) and Ia (or Ib), VIa (or VIb) and IIa (or IIb), and VIIa (or VIIb) and IIIa (or IIIb), respectively. The extent of cyclization was calculated from the optical densities[29] at each of the wavelength pairs, using molar absorptivities. The results are shown in Figs. 1 and 2.

6,2′-*O*-Cyclouridine (Ia)——A solution of Va (800 mg, 2.16 mmol) in 1 N MeONa (10.8 ml) was refluxed for 1 h with exclusion of moisture, then cooled. The solvent was removed *in vacuo*, and the residue was dissolved in water (25 ml). The aqueous solution was passed through a column of Amberlite IR 120 B (H^+) (2.6×12 cm) and the column was washed with water. The effluent and the washings were combined (700 ml) and concentrated to deposit colorless prisms (386 mg, 74%), which showed a single UV-absorbing spot on TLC [AcOEt–MeOH (5: 1), *Rf* 0.54] and PC (solvent A, *Rf* 0.52 and B, *Rf* 0.26). mp 249—251°C (lit.[6] 246—248°C). *Anal.* Calcd for $C_9H_{10}N_2O_6$: C, 44.63; H, 4.16; N, 11.57. Found: C, 44.69; H, 4.25; N, 11.48.

5-Iodo-5′-*O*-trityl-1-(β-D-arabinofuranosyl)uracil (Vb)——Va (200 mg, 0.54 mmol) was treated with trityl chloride (556 mg) in the same manner as described for VIb to give white crystals (177 mg, 54%), which showed a single UV-absorbing spot on TLC (AcOEt, *Rf* 0.65). UV λ_{max}^{MeOH} nm: 284.5.

5′-*O*-Trityl-6,2′-*O*-cyclouridine (Ib)——Vb (120 mg, 0.20 mmol) was dissolved in 1 N MeONa (6 ml), and the solution was refluxed for 2 h with exclusion of moisture. After cooling, the reaction mixture was carefully neutralized with AcOH and evaporated to dryness *in vacuo*. To the residue was added a small amount of water, and insoluble material was separated by filtration. The solid was recrystallized successively from H_2O–MeOH and EtOH to give white crystals (33 mg, 40%), which showed a single UV-absorbing spot on TLC [$CHCl_3$–EtOH (9: 1), *Rf* 0.55]. mp 217.5°C. UV λ_{max}^{MeOH} nm: 251, $\lambda_{max}^{H_2O\ (pH3)}$ nm: 252, $\lambda_{max}^{H_2O\ (pH13)}$ nm: 253.

6,3′-*O*-Cyclouridine (IIa)——VIa (1.43 g, 3.87 mmol) was dissolved in 1 N MeONa (77.3 ml), and the solution was refluxed for 3 d. The reaction mixture was treated in the same manner as described in the preceding section. The effluent and the washings were combined and evaporated to dryness. The syrup was triturated with EtOH (15 ml) to give crystals. Recrystallization of the product from EtOH (20 ml) yielded colorless needles (687 mg, 73%), which showed a single UV-absorbing spot on TLC [AcOEt–MeOH (10: 1), *Rf* 0.47] and PC (solvent A, *Rf* 0.55 and B, *Rf* 0.32). mp 250—253°C. *Anal.* Calcd for $C_9H_{10}N_2O_6$: C, 44.63; H, 4.16; N, 11.57. Found: C, 44.93; H, 4.08; N, 11.52.

6,5′-*O*-Cyclouridine (IIIa)——A solution of VIIa (10.74 g, 29 mmol) in 1 N MeONa (290 ml) was refluxed for 7 d with exclusion of moisture and then concentrated (70 ml). The reaction mixture was added to a stirred mixture of Amberlite IR 120 B (H^+ form, 200 ml) and water (500 ml). The resin was filtered off and the filtrate was concentrated (50 ml) to give crystals. Recrystallization of the product from water yielded colorless prisms (3.12 g, 44%). mp 288—289°C (dec.) (lit.[9] 283—285°C).

2′,3′-*O*-Isopropylidene-6,5′-*O*-cyclouridine (IIIb)——A solution of VIIb (1 g, 2.44 mmol) in 1 N MeONa (25 ml) was refluxed for 2 h with exclusion of moisture. After cooling, the reaction mixture was carefully neutralized with AcOH and evaporated to dryness *in vacuo*. Addition of a small amount of water to the residue afforded crystals, which were recrystallized from water to yield white crystals (0.47 g, 68%). mp 248—250°C (lit.[27] 251—253°C). UV λ_{max}^{MeOH} nm: 260.

5-Iodo-5′-*O*-trityl-1-(β-D-xylofuranosyl)uracil (VIb)——A solution of VIa (200 mg, 0.54 mmol) in pyridine (4 ml) was concentrated (2 ml) *in vacuo*, then trityl chloride (556 mg, 2 mmol) was added. The reaction mixture was left at room temperature for 48 h, then water (1 ml) was added. Evaporation of the solvent left a syrup, which was dissolved in a mixture of $CHCl_3$ (10 ml) and water (5 ml). The organic layer was washed with water (5 ml), dried over $MgSO_4$ and evaporated to dryness *in vacuo* to give a syrup. The residue was dissolved in toluene (5 ml) and the solution was evaporated to dryness. This procedure was repeated three times and the toluene solution was passed through a column of silica gel (1.7 × 6 cm). The column was eluted successively with benzene (70 ml) and AcOEt–benzene (66: 34) (70 ml). The desired fractions were combined and evaporated to dryness to yield a syrup. The residue was triturated with AcOEt (2 ml) to afford white crystals (248 mg, 75%), which showed a single UV-absorbing spot on TLC (AcOEt, *Rf* 0.68). mp 234—235°C. *Anal.* Calcd for $C_{28}H_{25}IN_2O_6$: C, 54.91; H, 4.11; N, 4.57. Found: C, 54.70; H, 4.02; N, 4.60. UV λ_{max}^{MeOH} nm: 284.

6,2′-*O*-Cyclocytidine (Ic)——A solution of 5-iodo-1-(β-D-arabinofuranosyl)cytosine (244 mg, 0.66 mmol) in 1 N MeONa (6.6 ml) was refluxed for 30 min, then cooled. The solvent was removed *in vacuo*, and the syrup was dissolved in water (3 ml). The aqueous solution was passed through a column of Amberlite IR 120 B (H^+) (1.7 × 7 cm) and the column was washed with water (200 ml) and eluted with 1.5 N NH_4OH (500 ml). Concentration of the eluate afforded pale-brown crystals, which were recrystallized from H_2O–EtOH to yield colorless plates (87 mg, 55%). mp 273—274°C (dec.) (lit.[18] 278—279°C). *Anal.* Calcd for $C_9H_{11}N_3O_5$: C, 44.81; H, 4.61; N, 17.42. Found: C, 44.72; H, 4.80; N, 17.26.

3′-*O*-Acetyl-2′,5′-di-*O*-trityl-1-(β-D-xylofuranosyl)uracil——2′,5′-Di-*O*-trityl-1-(β-D-xylofuranosyl)uracil (12.83 g, 17.60 mmol) was dissolved in pyridine (80 ml) and the solution was evaporated to dryness *in vacuo*. The residue was dissolved in pyridine (120 ml) and then Ac_2O (26 ml) was added. The mixture was sealed and warmed at 50°C for 2 d, then water (20 ml) was added. The solution was kept at 4°C overnight and concentrated (70 ml) *in vacuo*. Addition of MeOH (200 ml) to the concentrate gave white prisms (12.33 g, 91%), which showed a single UV-absorbing spot on TLC [$CHCl_3$–EtOH (40: 1), *Rf* 0.22]. mp 280—283°C.

3′-*O*-Acetyl-2′,5′-di-*O*-trityl-1-(β-D-xylofuranosyl)-4-thiouracil——A suspension of 3′-*O*-acetyl-2′,5′-di-*O*-trityl-1-(β-D-xylofuranosyl)uracil (12.00 g, 15.56 mmol) and P_2S_5 (11.58 g) in a mixture of toluene (169 ml) and pyridine (72 ml) was refluxed with stirring for 4 h, then cooled. The solvent was decanted off and the residue was washed twice with benzene (15 ml). The solvent and the washings were combined and evaporated to dryness *in vacuo* to give a syrup. The residue was dissolved in a mixture of benzene (300 ml) and AcOEt (100 ml), and the solution was washed three times with water (100 ml), then dried over $MgSO_4$. Evaporation of the solvent afforded a syrup, which was triturated with EtOH to yield pale yellowish needles (10.58 g, 86%). The product was contaminated with the starting material (*ca.* 2%) and purified by silica gel column chromatography ($CHCl_3$) to show a single UV-absorbing spot on TLC ($CHCl_3$, *Rf* 0.45). mp 220—223°C. UV λ_{max}^{MeOH} nm: 330, 255 (sh). *Anal.* Calcd for $C_{49}H_{42}N_2O_6S$: C, 74.79; H, 5.38; N, 3.56. Found: C, 74.69; H, 5.29; N, 3.50.

2′,5′-Di-*O*-trityl-1-(β-D-xylofuranosyl)cytosine——A suspension of 3′-*O*-acetyl-2′,5′-di-*O*-trityl-1-(β-D-xylofuranosyl)-4-thiouracil (10.31 g, 13.10 mmol) in MeOH (80 ml) was saturated with NH_3 at −15°C to yield a homogeneous solution. The solution was heated at 100°C in a sealed tube (200 ml) for 1 d. After

cooling to 0°C, the reaction mixture was evaporated to dryness *in vacuo*, then the residue was dissolved in $CHCl_3$ (100 ml), and the solution was filtered to remove insoluble materials. Evaporation of the solvent gave a residue, which was triturated with MeOH (100 ml) to yield white crystals (7.27 g, 76%). The product was contaminated with a small amount of the starting material and purified by silica gel column chromatography (AcOEt) to give a single UV-absorbing spot on TLC (AcOEt, *Rf* 0.15). mp 248—251°C (lit.[15b] 235—240°C). *Anal.* Calcd for $C_{47}H_{41}N_3O_5 \cdot 1/2H_2O$: C, 76.61; H, 5.75; N, 5.70. Found: C, 76.49; H, 5.78; N, 5.49.

1-(β-D-Xylofuranosyl)cytosine Hydrochloride——Water (24 ml) was added to a solution of 2′,5′-di-*O*-trityl-1-(β-D-xylofuranosyl)cytosine (6.77 g, 9.30 mmol) in AcOH (96 ml). The mixture was heated at 75—80°C for 10 h, then cooled and evaporated to dryness *in vacuo*. The residue was dissolved in a mixture of $CHCl_3$ (50 ml) and water (100 ml). The aqueous layer was washed twice with $CHCl_3$ (25 ml), concentrated (50 ml) *in vacuo*, and filtered to remove insoluble materials. The filtrate was passed through a column of Amberlite IR 120 B (H^+) (2.5×30 cm). The column was washed with water (1 l) and eluted with 1.5 N NH_4OH (1.5 l). The eluate was evaporated to dryness *in vacuo* to give a syrup, which was dissolved in 1 N HCl (15 ml). Evaporation of the solution to dryness gave a residue, which was triturated with EtOH (35 ml) to yield white prisms (1.98 g, 76%). TLC [AcOEt–MeOH (3: 2)]: a single UV-absorbing spot, *Rf* 0.43. mp 204—205.5°C.

1-(β-D-Xylofuranosyl)cytosine——A solution of the above-described hydrochloride (100 mg) in water (10 ml) was passed through a column of Amberlite IRA 400 (AcO^-) (1.5×9 cm), and the column was washed with water. The effluent and the washings were combined and evaporated to dryness *in vacuo* to give a syrup. The residue was triturated with EtOH (2 ml) to deposit colorless needles (70 mg, 80%). mp 236—238°C (lit.[14] 237—238°C). UV $\lambda_{max}^{0.05\ N\ HCl}$ nm: 280; $\lambda_{max}^{H_2O}$ nm: 271, 230 (sh); $\lambda_{max}^{0.05\ N\ NaOH}$ nm: 272.

5-Iodo-1-(β-D-xylofuranosyl)cytosine——To a solution of 1-(β-D-xylofuranosyl)cytosine hydrochloride (558 mg, 2 mmol) in a mixture of CF_3CO_2H (2 ml) and water (2 ml) were added I_2 (300 mg), HIO_3 (180 mg) and CCl_4 (2 ml). The mixture was stirred at room temperature for 15 h, then the aqueous layer was separated and evaporated to dryness *in vacuo*. The syrup was dissolved in water (15 ml) and the solution was evaporated to dryness; this procedure was repeated three times. The residue was dissolved in water (10 ml) and solution was applied to a column of Amberlite IRA 400 (AcO^-) (1.7×12.5 cm). The column was washed with water. The effluent and the washings were combined and concentrated *in vacuo* to give white needles (358 mg, 48%), which showed a single UV-absorbing spot on TLC [AcOEt–MeOH (4: 1), *Rf* 0.34]. mp 223—224°C (lit.[28] 205.8—206.2°C). UV $\lambda_{max}^{0.1\ N\ HCl}$ nm (ε): 308 (10200); $\lambda_{max}^{H_2O}$ nm (ε): 282 (6000); $\lambda_{max}^{0.1\ N\ NaOH}$ nm (ε): 284 (6300).

2′,5′-Di-*O*-acetyl-6,3′-*O*-cyclouridine——A solution of IIa (607 mg, 2.51 mmol) in a mixture of pyridine (2 ml) and Ac_2O (1 ml) was kept at room temperature for 3 h. The reaction was stopped by the addition of water (3 ml). The mixture was evaporated to dryness *in vacuo* to give a syrup. The desidue was dissolved in water (3 ml) and the solution was evaporated to dryness. This procedure was repeated three times. The residue was triturated with EtOH (3 ml) to deposit white prisms (740 mg, 90%), which showed a single UV-absorbing spot on TLC [benzene–AcOEt (1: 1), *Rf* 0.36]. mp 188—189°C. MS *m/e*: 326 (M^+). *Anal.* Calcd for $C_{13}H_{14}N_2O_8$: C, 47.85; H, 4.33; N, 8.59. Found: C, 47.87; H, 4.17; N, 8.21.

2′,5′-Di-*O*-acetyl-6,3′-*O*-cyclo-4-thiouridine——A solution of 2′,5′-di-*O*-acetyl-6,3′-*O*-cyclouridine (640 mg, 1.96 mmol) in a mixture of pyridine (9 ml) and toluene (21 ml) was treated with P_2S_5 (1.45 g). The mixture was refluxed for 4 h, then P_2S_5 (3 g) was added. The reaction was continued for a further 6 h. The mixture was cooled, and insoluble materials were removed by decantation. The organic layer was evaporated to dryness *in vacuo*. Pyridine was removed twice as the toluene (30 ml) azeotrope. The residue was dissolved in AcOEt (50 ml) and purified by silica gel column (1.7×8 cm) chromatography. The desired fractions were combined and evaporated to dryness to give a viscous gum. The residue was triturated with 50% EtOH (10 ml) to deposit pale yellowish needles (357 mg, 53%), which showed a single UV-absoring spot on TLC [benzene–AcOEt (1: 1), *Rf* 0.64]. mp 115—117°C. MS *m/e*: 342 (M^+). UV $\lambda_{max}^{0.05\ N\ HCl}$ nm: 327, λ_{max}^{MeOH} nm: 327, $\lambda_{max}^{0.05\ N\ NaOH}$ nm: 313. *Anal.* Calcd for $C_{13}H_{14}N_2O_7S$: C, 45.61; H, 4.12; N, 8.18. Found: C, 45.56; H, 4.55; N, 7.92.

6,3′-*O*-Cyclocytidine (IIc)——i) A solution of 5-iodo-1-(β-D-xylofuranosyl)cytosine (358 mg, 0.97 mmol) in 1 N MeONa (9.7 ml) was refluxed for 15 h with exclusion of moisture. After cooling, the reaction mixture was evaporated to dryness *in vacuo* to give a syrup. The residue was dissolved in water (20 ml) and the solution was passed through a column of Amberlite IR 120 B (H^+) (1.7×9 cm). The column was washed with water (70 ml), and then eluted with 1.5 N NH_4OH (550 ml). The eluate was concentrated (2 ml) to afford pale brown fibrous crystals (129 mg, 55%), which showed a single UV-absorbing spot on PC (solvent A, *Rf* 0.51 and solvent B, *Rf* 0.15). mp 246—248°C (dec.). *Anal.* Calcd for $C_9H_{11}N_3O_5$: C, 44.81; H, 4.60; N, 17.42. Found: C, 44.36; H, 4.67; N, 17.17.

ii) A solution of 2′,5′-di-*O*-acetyl-6,3′-*O*-cyclo-4-thiouridine (152 mg, 0.44 mmol) in MeOH (20 ml) saturated at 0°C with NH_3 was heated in a sealed tube at 100°C for 36 h. After cooling to 0°C, the reaction mixture was evaporated to dryness *in vacuo*. The residue was dissolved in EtOH (5 ml) and treated with charcoal to deposit pale brown needles (53 mg, 50%). mp 247—249°C (dec.).

Stability of IIc to Methanolic Ammonia——A solution of IIc (10 mg) in MeOH (10 ml) saturated at 0°C with NH_3 was heated in a sealed tube (50 ml) at 100°C for 18 h. After cooling, the solution was evaporated

to dryness *in vacuo*. The residue showed a single UV-absorbing spot with the same mobility as that of the starting material on TLC and gave a UV spectrum superimposable on that of the starting material.

Formation of 5′-*O*-Trityl-6,3′-*O*-cyclouridine (IIb)——A solution of VIb (122 mg, 0.2 mmol) in 1 N MeONa (2 ml) was refluxed for 42 h with exclusion of moisture. After cooling, the reaction mixture was carefully neutralized with AcOH and evaporated to dryness *in vacuo*. The residue was proved by TLC [$CHCl_3$–EtOH (10: 1)] to contain a UV-absorbing substance (IIb) (UV λ_{max}^{MeOH} nm: 262, *Rf*, 0.44) in *ca.* 10% yield in addition to VIb (*Rf* 0.68).

5-Iodocytidine——To a solution of cytidine (3.88 g, 16 mmol) in a mixture of CF_3CO_2H (5 ml) and water (8 ml) were added HIO_3 (1.44 g), I_2 (2.4 g) and CCl_4 (8 ml). The mixture was stirred at room temperature for 8 h and insoluble materials were removed by filtration. The aqueous layer was separated and evaporated to dryness *in vacuo*. The syrup was dissolved in water (20 ml) and the solution was evaporated to dryness; this procedure was repeated three times. The residue was dissolved in water (30 ml) and the solution was passed through a column of Amberlite IRA 400 (AcO^-) (2.7 × 8 cm). The column was washed with water. The effluent and the washings were combined (500 ml) and evaporated to dryness *in vacuo* to give a syrup. The residue was dissolved in 1 N HCl (20 ml) and the solution was evaporated to dryness. The solid was dissolved in MeOH (40 ml) and the solution was, after addition of EtOH (20 ml), concentrated (15 ml) to deposit yellow crystals (HCl salt, 3.64 g). The product was dissolved in water (50 ml) and the solution was applied to a column of Amberlite IRA 400 (AcO^-) (1.7 × 15 cm). The effluent and the washings were combined and evaporated to dryness *in vacuo* to afford a viscous gum (2.90 g, 49%), which showed a single UV-absorbing spot on TLC [AcOEt–MeOH (3: 1), *Rf* 0.36]. UV $\lambda_{max}^{0.05\ N\ HCl}$ nm: 309, $\lambda_{max}^{H_2O}$ nm: 290.

Formation of 6,5′-*O*-Cyclocytidine (IIIc)——A solution of 5-iodocytidine (150 mg, 0.41 mmol) in 1 N MeONa (10 ml) was refluxed for 16 h with exclusion of moisture, then cooled. AcOH (0.7 ml) was added to the reaction mixture, which was evaporated to dryness *in vacuo*. The residue was dissolved in water (10 ml) and the solution was applied to a column of activated charcoal (2 g). The column was washed with water (100 ml) and then eluted with EtOH–pyridine (4: 1) (150 ml). The eluate was evaporated to dryness to give a syrup. Pyridine was removed as the water (20 ml) azeotrope. The residue was dissolved in aqueous MeOH and analyzed by TLC [$CHCl_3$–EtOH (1: 1)] and PC (solvents A, B), which showed that it contained four UV-absorbing substances. The compound (*ca.* 7.4% yield) which showed third-greatest mobility on TLC (*Rf* 0.27) and ran the same distance as the authentic sample of IIIc, had a UV spectrum superimposable on that of IIIc.

Ring-opening of 6-*O*-Cyclouridines with Sulfuric Acid——A suspension of Ia, IIa or IIIa (5 mg) in 2 N H_2SO_4 (0.2 ml) was heated at 70°C with stirring. After a suitable time, an aliquot was analyzed by PC [iso-PrOH–H_2O (7: 3), descending method] to estimate the contents of the starting material and the hydrolysate.

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References and Notes

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4) Abbreviations used are: UA = 1-(β-D-arabinofuranosyl)uracil, UX = 1-(β-D-xylofuranosyl)uracil, CX = 1-(β-D-xylofuranosyl)cytosine, CA = 1-(β-D-arabinofuranosyl)cytosine.
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