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Synthesis of 6,5′-Cyclo-5′-deoxyuridines by Radical Cyclization (Nucleosides and Nucleotides. L)[1]

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6,5′-Cyclo-5′-deoxyuridine, a fixed *anti* form of uridine, was synthesized by a radical cyclization of 5′-bromo(or iodo)-5′-deoxy-2′,3′-*O*-isopropylidene-5-chloro(or bromo)-uridine with tri-*n*-butyltin hydride followed by dehydrohalogenation and deacetonation. The 5-bromo and 4-thio derivatives of the cyclouridine were also prepared and were converted to the 2′,3′-cyclic phosphates. These nucleotides were hydrolyzed by pancreatic ribonuclease. The result showed that the enzyme recognizes the pyrimidine nucleotides in the *anti* form. 6,5′-Cyclo-5′-deoxycytidine was also synthesized by two routes.

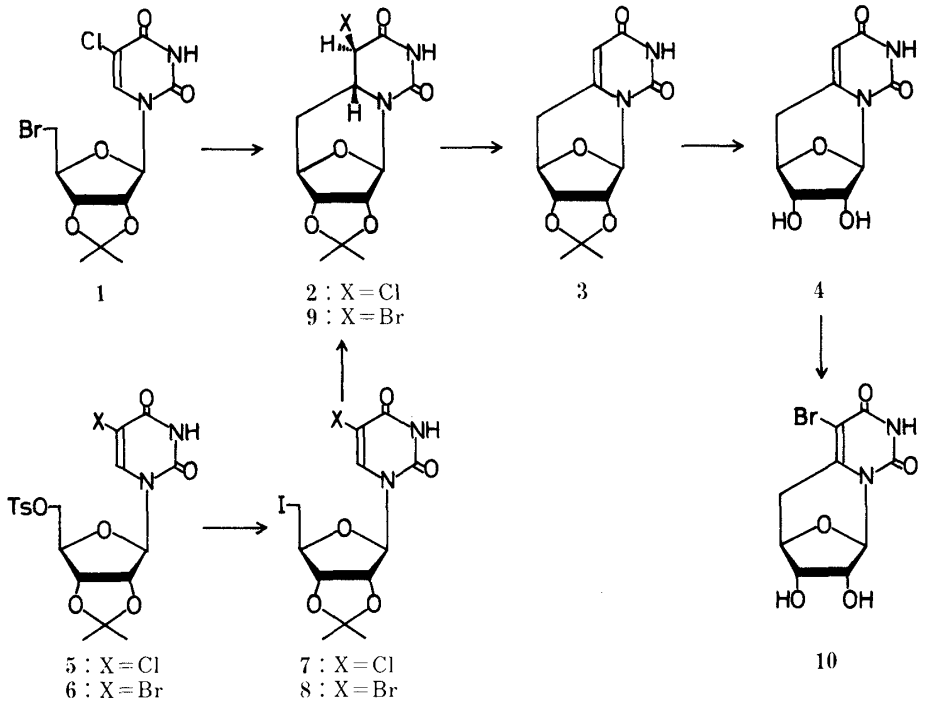
Keywords——cyclonucleosides; cyclouridine; radical cyclization; *n*-butyltin hydride; cyclocytidine; uridine; NMR; CD; ribonuclease A

It has been generally recognized that the *syn–anti* conformation around the N-glycosylic linkages of nucleosides is one of the important determinants in the interaction of nucleosides and nucleotides with various enzymes utilizing them. We have recently synthesized fixed *anti*-conformers of purine nucleosides and nucleotides, namely 8,5′-cyclo-5′-deoxyadenosine[2] and -inosine,[2] 8,5′-cycloadenosines,[3] and 8,5′-cyclo-5′-deoxyguanosine 2′,3′-cyclic phosphate.[4] The last compound was utilized in the stereochemical investigation of the interaction of guanylic acid and ribonuclease T_1.[4] A similar investigation would be possible for pancreatic ribonuclease (RNase A) if suitable conformationally fixed pyrimidine nucleosides and nucleotides were available. Fox and co-workers have presented a method of synthesis of 6,5′-cyclouridines involving an intramolecular aldol reaction of a 5-hydroxyuridine-5′-aldehyde as the key steps.[5] However, this procedure requires a rather laborious multistep conversion of uridine. We have recently found a facile 6,5′-cyclization of 5′-deoxy-5′-iodo-2′,3′-*O*-isopropylideneuridine by a radical reaction with tri-*n*-butyltin hydride, leading to 6,5′-cyclo-5′-deoxy-5,6(*R*)-dihydrouridine in high yield.[6]

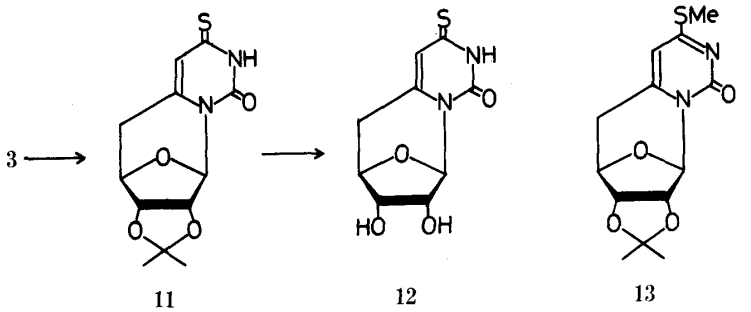
This paper describes the synthesis of 6,5′-cyclo-5′-deoxyuridine, a fixed *anti* form of uridine, and its derivatives by an extension of this radical cyclization procedure.[7]

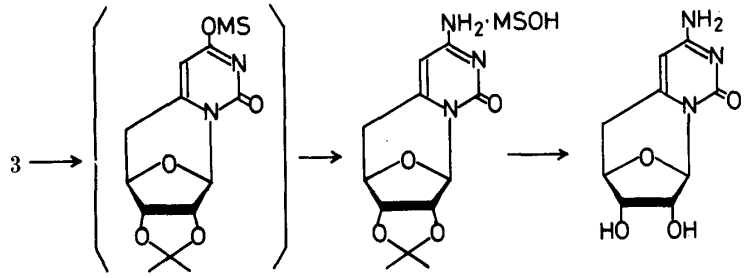
Treatment of 5′-bromo-5′-deoxy-2′,3′-*O*-isopropylidene-5-chlorouridine (**1**), prepared from 2′,3′-*O*-isopropylidene-5-chlorouridine, by a standard procedure (dropwise addition of a mixture of tri-*N*-butyltin hydride and azobisisobutyronitrile (AIBN) in benzene under reflux) afforded the product (**2**) as a crystalline precipitate. The structure of **2** was confirmed by instrumental analyses. The configuration of protons at C-5 and C-6 of **2** is *trans* as evidenced by their coupling constant ($J=11.2$ Hz) in the nuclear magnetic resonance (NMR) spectra. Assuming the stereochemistry of the radical addition to C-6 to be the same as in the case of 5′-deoxy-5′-iodo-2′,3′-*O*-isopropylideneuridine,[6] giving the C-6(*R*)-diastereomer, it is clear that stereospecific *cis*-addition had occurred in the present cyclization. No other diastereomers were detected in the reaction. Treatment of **2** with sodium ethoxide in ethanol gave the 6,5′-cyclouridine (**3**) in high yield. It is conceivable that epimerization at C-5 by ethoxide occurred

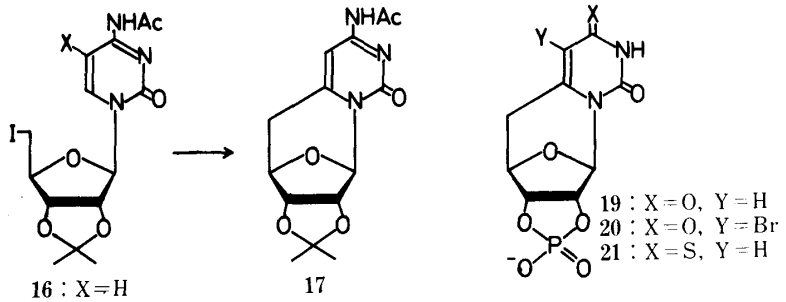
during dehydrochlorination of **2**, so that *trans* elimination became possible to give **3**. Deacetonation of **3** was achieved by treatment with 1 N hydrochloric acid in 50% aqueous methanol and crystalline 6,5′-cyclo-5′-deoxyuridine (**4**) was obtained from water in high yield. The physical data for **4** were consistent with the structure. A positive Cotton band with somewhat larger molar ellipticity in the circular dichroism (CD) spectra of **4** is characteristic of an *anti* form of uridine fixed in this manner. The same 6,5′-cyclouridine (**3**) was also obtained from the 5′-iodo derivatives (**7** and **8**) of 5-chlorouridine or 5-bromouridine, which were obtained from the corresponding 5′-*O*-*p*-toluenesulfonates (**5** and **6**). In the case of the 5-bromouridine derivative **8**, the yield of **3** was lower, probably due to over-reduction of the cyclodihydro 5-bromouridine intermediate (**9**) by tri-*n*-butyltin hydride. Compound **7** gave **3** in satisfactory yield.



Treatment of **4** with bromine in aqueous methanol gave 6,5′-cyclo-5′-deoxy-5-bromouridine (**10**) in high yield.[8] The physical properties of **10** are similar to those of **4** except for the red shift of the ultraviolet (UV) absorption maximum of **10** to 283 nm. In order to study the interaction of uridylate and RNase A by spectroscopy, the use of a substrate with an absorption maximum well separated from that of the enzyme is desirable, and 4-thiouridine phosphates meet this requirement (they are substrates, and have absorption maxima at around 330 nm.[9] Treatment of **3** with phosphorus pentasulfide in dioxane afforded the 4-thiocyclouridine (**11**) as yellow needles. Deprotection of **11** with 1 N hydrochloric acid gave 6,5′-cyclo-5′-deoxy-4-thiouridine (**12**) in high yield. It is noteworthy that 4-thiouridine itself is very labile to acid hydrolysis, giving uridine, while **12** is quite stable. An attempt to exchange the 4-thiono function with ammonia *via* the 4-methylthio intermediate (**13**) was unsuccessful. It is assumed that in the nucleophilic substitution at C-4 of the 2-pyrimidinone system the prior addition of the nucleophile to C-6 greatly enhances the C-4 substitution.[10] In the present case, such addition would have been prevented due to the presence of the 6,5′-cyclo linkage. On the other hand, treatment of **3** with mesitylenesulfonyl chloride followed by ammonia[11] gave the expected 6,5′-cyclocytidine derivative as the mesitylenesulfonate (**14**). It seems that the C-4 mesitylenesulfonyloxy leaving group is active enough for the ammonolysis to occur. Acid hydrolysis of **14** afforded 6,5′-cyclo-5′-deoxycytidine (**15**). However, the overall yield of the conversion was not satisfactory.







Therefore, radical cyclization starting from the cytidine derivative was also attempted. 5′-Deoxy-5′-iodo-2′,3′-*O*-isopropylidene-N^4-acetylcytidine[12] (**16**) was treated with tri-*n*-butyltin hydride and AIBN. Among various products, the expected 6,5′-cyclocytidine (**17**) was isolated. The structure of **17** was confirmed by comparison of its physical data with those of an authentic sample prepared by the photolysis of 5′-deoxy-5′-phenylthio-2′,3′-*O*-isopropylidene-N^4-acetylcytidine.[13] The same product was obtained when the reaction was carried out with the 5-bromo derivative (**18**) of **16**. Therefore, in the case of the cytosine nucleus, the radical addition of the 5′-methylene group to C-6 would have been followed by deprotonation in the former case, or dehydrobromination *in situ* in the latter case. In any case, the yields of the product **17** were unsatisfactory and several by-products were formed. Further study for optimization of the reaction conditions will be required.

6,5′-Cyclo-5′-deoxyuridines prepared were then phosphorylated to the corresponding 2′,3′-cyclic phosphates of cyclouridine (**19**), 5-bromouridine (**20**) and 4-thiouridine (**21**), by treatment with polyphosphoric acid in dimethylformamide according to our reported procedure.[14] A preliminary experiment using RNase A and these cyclouridine cyclic phosphates showed that they were all substrates for this enzyme, affording the corresponding 3′-phosphates, although the rates of hydrolysis were slower than those of the natural substrates, uridine 2′,3′-cyclic phosphate and 4-thiouridine 2′,3′-cyclic phosphate (Table 1). On the other hand the 2′,3′-cyclic phosphates of 6-methyluridine and 6-butyluridine[15]

TABLE I. Hydrolysis of Cyclopyrimidine 2′,3′-Cyclic Phosphates with Pancreatic Ribonuclease

Nucleotides	Degree of hydrolysis (%)
U>P[a)]	>95
S^4U>P	>95
19	40
20	38
21	68
6-MeU>P	5
6-BuU>P	0

a) U>P, uridine 2′,3′-cyclic phosphate; S^4U>P, 4-thiouridine 2′,3′-cyclic phosphate; 6-MeU>P, 6-methyluridine 2′,3′-cyclic phosphate; 6-BuU>P, 6-butyluridine 2′,3′-cyclic phosphate. For reaction conditions, see the text.

strongly resisted hydrolysis. Since these nucleotides are expected to adopt the *syn* form as the preferred conformers, it is evident that the natural substrates should exist in the *anti* form, like the 6,5′-cyclouridine nucleotides, for the interaction with RNase A. The kinetic studies of the interaction of these cyclopyrimidine nucleoside 2′,3′-cyclic phosphates with RNase A will be reported elsewhere.

Experimental

Melting points were determined on a Yanagimoto Mp-3 micromelting point apparatus and are uncorrected. The ^{1}H-NMR spectra were recorded on a JEOL FX-100FT or FX-200FT spectrometer in $CDCl_3$ or DMSO-d_6 as the solvent with tetramethylsilane as an internal standard. Chemical shifts are reported in ppm (δ), and signals are described as s (singlet), d (doublet), t (triplet), m (multiplet), or br (broad). All exchangeable protons were confirmed by addition of D_2O. UV spectra were recorded with a Shimadzu UV-240 spectrophotometer. Mass spectra (MS) were measured on a JEOL D-300 spectrometer. CD spectra were recorded on a JASCO J-40 spectropolarimeter at room temperature. Thin layer chromatography (TLC) was carried out on Merck pre-coated plates $60F_{254}$. Silica gel for column chromatography was Wako-gel C-200. Uridine and other nucleosides used were from Yamasa Shoyu Co., Ltd.

5′-Bromo-5′-deoxy-2′,3′-*O*-isopropylidene-5-chlorouridine (1)——A mixture of 2′,3′-*O*-isopropylidene-5-chlorouridine[16] (1.28 g), triphenylphosphine (1.1 g), and carbon tetrabromide (1.7 g) in 10 ml of dimethylformamide was stirred overnight at room temperature.[17] After addition of MeOH (10 ml), the solvent was evaporated off *in vacuo* and the residue was dissolved in $CHCl_3$. The solution was applied to a column of silica gel (30 g). The product was eluted with $CHCl_3$, the eluate was concentrated, and the residue was extracted several times with hexane to remove triphenylphosphine oxide. The residue was dissolved in a small amount of $CHCl_3$ and hexane was added until precipitation occurred. The precipitate was crystallized from EtOH–$CHCl_3$ to give 0.63 g (41%) of **1**, mp 212.5—213.5 °C. *Anal.* Calcd for $C_{12}H_{14}BrClN_2O_5$: C, 27.77; H, 3.70; Br, 20.93; Cl, 9.29; N, 7.34. Found: C, 27.84; H, 3.62; Br, 21.21; Cl, 9.33; N, 7.41.

5′-*O*-*p*-Toluenesulfonyl-2′,3′-*O*-isopropylidene-5-chlorouridine (5)——2′,3′-*O*-Isopropylidene-5-chlorouridine (23 g) in 300 ml of pyridine was treated with *p*-toluenesulfonyl chloride (20.6 g, 1.5 eq) and the solution was stirred for 5 h. After addition of a small volume of EtOH the solvent was removed *in vacuo* and the residue was taken up in $CHCl_3$. The solution was washed with H_2O, dried with Na_2SO_4, and the solvent was evaporated off. The residue was crystallized from EtOH to give 19.2 g (56%) of **5**, mp 167—168.5 °C. MS *m/z*: 474, 472 (M), 459, 457 (M−15), 155 (Ts, base peak). *Anal.* Calcd for $C_{19}H_{21}ClN_2O_8S$: C, 48.16; H, 4.47; Cl, 7.69; N, 5.91; S, 6.77. Found: C, 48.13; H, 4.44; Cl, 7.68; N, 5.92; S, 6.73.

5′-Deoxy-5′-iodo-2′,3′-*O*-isopropylidene-5-chlorouridine (7)——A solution of **5** (3.6 g) and NaI (1.7 g) in 40 ml of 2-butanone was refluxed for 2 h, then cooled. The precipitate (TsONa) was removed and the solution was concentrated to about one-third of the initial volume, then poured into ice water. The separated solid was collected and crystallized from EtOH to give 2.6 g (80%) of **7**, mp 226—227 °C. MS *m/z*: 430, 428 (M), 415, 413 (M−15), 300 (M−HI), 283 (sugar, base peak). NMR ($CDCl_3$): 1.37, 1.58 (s each, 3+3, Me_2C), 3.37 (dd, 1, H-5′a), 3.53 (dd, 1, H-5′b), 4.31 (ddd, 1, H-4′), 4.78 (dd, 1, H-3′), 4.99 (dd, 1, H-2′), 5.71 (d, 1, H-1′), 7.67 (s, 1, H-6), 8.91 (br s, 1, H–N^3). $J_{4',5'a}=5.3$ Hz, $J_{5'a,b}=11$ Hz, $J_{4',5'b}=6$ Hz, $J_{3',4'}=4$ Hz, $J_{2',3'}=7$ Hz, $J_{1',2'}=3$ Hz. *Anal.* Calcd for $C_{12}H_{14}ClIN_2O_5$: C, 33.55; H, 3.28; Cl, 8.49; I, 29.54; N, 6.52. Found: C, 33.54; H, 3.30; Cl, 8.37; I, 29.59; N, 6.45.

5′-Deoxy-5′-iodo-2′,3′-*O*-isopropylidene-5-bromouridine (8)——5′-*O*-*p*-Toluenesulfonyl-2′,3′-*O*-isopropylidene-5-bromouridine[17] (**6**, 10.81 g) was refluxed overnight with NaI (4.7 g) in 100 ml of acetone. The precipitate (TsONa) was filtered off and the filtrate was concentrated to dryness. The residue was taken up in $CHCl_3$. This solution was washed with H_2O, and dried over Na_2SO_4, then the solvent was evaporated off. The residue was crystallized from EtOH to give 9.21 g (93%) of **8**, mp 277—279 °C. *Anal.* Calcd for $C_{12}H_{14}BrIN_2O_5$: C, 30.47; H, 2.98; Br, 16.89; I, 26.83; N, 5.92. Found: C, 30.38; H, 2.97; Br, 16.61; I, 26.83; N, 5.83.

6,5′-Cyclo-5′-deoxy-2′,3′-*O*-isopropylidene-5(*R*),6(*R*)-dihydro-5-chlorouridine (2)——a) A mixture of Bu_3SnH (0.41 ml) and AIBN (15 mg) in 0.5 ml of benzene was added dropwise to a refluxing solution of **1** (470 mg) in 15 ml of benzene over a period of 20 min. After 30 min, further Bu_3SnH (0.2 ml) and AIBN (4 mg) mixture in 0.2 ml of benzene was added dropwise and heating was continued for a further 1 h. After cooling of the mixture, the separated crystals were collected and washed with benzene to give 197 mg (53.2%) of **2**, mp 278 °C (dec.). *Rf* on TLC ($CHCl_3$–MeOH, 20 : 1): 0.27 (visualized on heating after H_2SO_4 spray). *Rf* of **1**: 0.44. MS *m/z*: 304, 302 (M), 289, 287 (M − 15), 149, 147 (base peak). NMR ($CDCl_3$): 1.34, 1.51 (s each, 3 + 3, Me_2C), 1.8—1.95 (ddd, 1, H-5′a), 2.10 (dd, 1, H-5′b), 3.50 (dt, 1, H-6), 4.27 (d, 1, H-5), 4.55 (m, 1, H-4′), 4.58 (d, 1, H-3′), 4.66 (d, 1, H-2′), 6.03 (s, 1, H-1′), 7.65 (br s, 1, H–N^3). $J_{5'a,b}=16$ Hz, $J_{5,6}=11.2$ Hz, $J_{5'a,6}=10$ Hz, $J_{5'b,6}=4$ Hz, $J_{2',3'}=5.4$ Hz. An analytically pure sample was obtained by recrystallization from MeOH. *Anal.* Calcd for $C_{12}H_{15}ClN_2O_5$: C, 47.61; H, 4.99; Cl, 11.71; N, 9.25. Found: C, 47.64; H, 4.83; Cl, 11.65; N, 9.09.

b) Compound **7** (4.0 g) was suspended in 100 ml of benzene and argon gas was passed through the flask. A mixture of Bu_3SnH (3.17 ml) and AIBN (50 mg) in 8 ml of benzene was added dropwise over a period of 40 min under reflux. At 30 min after the end of the addition, the mixture was cooled and the crystalline precipitate was collected to give 2.2 g (78%) of **2**. This was identical with the product obtained above.

6,5′-Cyclo-5′-deoxy-2′-3′-*O*-isopropylidene-5-bromo-5,6-dihydrouridine (9)——Compound **8** (2.64 g) was treated with 12 ml of Bu_3SnH and 200 mg of AIBN in 80 ml of refluxing toluene by the same procedure as described above. The precipitated crystals were collected and recrystallized from EtOH to give 760 mg (39.7%) of **9**, mp 285—290 °C (dec.). *Anal.* Calcd for $C_{12}H_{15}BrN_2O_5$: C, 41.52; H, 4.36; Br, 23.02; N, 8.07. Found: C, 41.64, H, 4.35; Br, 22.82; N, 8.17.

6,5′-Cyclo-5′-deoxy-2′,3′-*O*-isopropylideneuridine (3)——a) Compound **2** (1.50 g) was suspended in 20 ml of abs. EtOH and 9.9 ml of 1 N NaOEt (2 eq) was added to the mixture. After refluxing for 10 min the solution was neutralized with 1 N HCl, and poured into $CHCl_3$–H_2O (100 ml each). The oraganic layer was separated, dried by passing it through a Whatman 1 PS filter paper, and evaporated to dryness. The residue was crystallized from EtOH to give 1.16 g (88%) of **3** as colorless plates, mp 288 °C (dec.). MS *m/z*: 266 (M), 151 (M − 15), 151 (base peak). UV λ_{max}^{MeOH}: 270 nm (ε, 8900), λ_{min}^{MeOH}: 234 nm (ε, 1500). NMR ($CDCl_3$): 1.32, 1.50 (s each, 3 + 3, Me_2C), 2.54 (dd, 1, H-5′a, *endo*), 3.20 (ddd, 1, H-5′b, *exo*), 4.59 (br d, 1, H-4′), 4.64 (s, 2, H-2′, 3′), 5.46 (br s, 1, H-5), 6.20 (s, 1, H-1′), 8.40 (br s, 1, H–N^3). $J_{5'a,b}=20$ Hz, $J_{4',5'a}=1.5$ Hz, $J_{4',5'b}=6.5$ Hz. *Anal.* Calcd for $C_{12}H_{14}N_2O_5$: C, 54.21; H, 5.30; N, 10.52. Found: C, 54.04; H, 5.22, N, 10.34.

b) Compound **9** (1.22 g) in 20 ml of EtOH was refluxed with NaOEt (2 eq) for 30 min. The mixture was neutralized with AcOH–EtOH. The separated crystals were collected and recrystallized from EtOH to give 320 mg (34%) of **3**. This was identical with the product obtained above.

6,5′-Cyclo-5′-deoxyuridine (4)——Compound **3** (1.0 g) was suspended in 40 ml of 1 N HCl in 50% aqueous MeOH and heated for 2 h at 90 °C. After cooling, the precipitate was collected and the filtrate was concentrated to dryness. The precipitate and residue were combined and recrystallized from H_2O to give 740 mg (87%) of **4** as colorless needles, mp 298 °C (dec.). MS *m/z*: 226 (M). UV $\lambda_{max}^{H_2O}$: 271 nm (ε, 11200), 210 nm (ε, 8200); $\lambda_{max}^{1\,N\,NaOH}$: 271.5 nm (ε, 9350), 223 nm (ε, 8500). CD in H_2O: 263 nm, $[\theta]=+10600$. NMR (DMSO-d_6): 2.70 (d, 1, H-5′a), 3.09 (ddd, 1, H-5′b), 4.10 (s, 2, H-2′,3′), 4.41 (d, 1, H-4′), 5.44 (s, 1, H-5), 5.81 (s, 1, H-1′), $J_{5'a,b}=19$ Hz, $J_{4',5'a}=1.7$ Hz, $J_{4',5'b}=6.4$ Hz. *Anal.* Calcd for $C_9H_{10}N_2O_5$: C, 47.79; H, 4.46; N, 12.38. Found: C, 47.75; H, 4.47; N, 12.26.

6,5′-Cyclo-5′-deoxy-5-bromouridine (10)——Br_2 (0.08 ml in 2 ml of MeOH) was added to a suspension of **4** (300 mg) in 8 ml of H_2O with stirring at room temperarure. After 6 h, a few drops of the bromine solution were added and the mixture was kept for 2 h while the color persisted. The solvent was evaporated off and coevaporation of the residue with H_2O was carried out several times. The residue was taken up in 20 ml of EtOH and refluxed for 2 h, then cooled. The precipitate was collected and crystallized from H_2O to give 364 mg (92%) of **10**, mp 228 °C (dec.). MS *m/z*: 306, 304 (M). UV λ_{max}^{MeOH}: 283 nm (ε, 12100), 213 nm (ε, 9100). NMR (DMSO-d_6): 2.70 (d, 1, H-5′a), 3.00 (dd, 1, H-5′b), 4.14 (m, 2, H-2′, 3′), 4.48 (d, 1, H-4′), 5.17, 5.41 (d each, 1 + 1, HO-2′, 3′), 5.80 (s, 1, H-1′), 11.80 (s, 1, H–N^3). $J_{5'a,b}=18.8$ Hz, $J_{4',5'b}=6.8$ Hz. *Anal.* Calcd for $C_9H_9BrN_2O_5$: C, 35.43; H, 2.97; Br, 26.19; N, 9.18. Found: C, 35.47; H, 2.95; Br, 26.04; N, 9.03.

6,5′-Cyclo-5′-deoxy-2′,3′-*O*-isopropylidene-4-thiouridine (11)——A mixture of **3** (820 mg) and P_2S_5 (685 mg) in 40 ml of dioxane was refluxed for 40 min, then cooled. The solution was concentrated to about one-fourth of the initial volume and the concentrate was poured into ice water. The separated solid was collected and the filtrate was extracted with $CHCl_3$. The solid and the $CHCl_3$ layer were combined, washed with sat. $NaHCO_3$ solution and dried over Na_2SO_4. The solvent was evaporated off and the residue was crystallized from EtOH to give 480 mg (55%) of **11** as yellow needles, mp 285 °C (dec.). MS *m/z*: 282 (M), 267 (M − 15). NMR ($CDCl_3$): 1.32, 1.51 (s each, 3 + 3, Me_2C),

2.48 (d, 1, H-5′a), 3.16 (ddd, 1, H-5′b), 4.61—4.72 (m, 3, H-2′, 3′, 4′), 6.17 (s, 1, H-1′), 6.22 (d, 1, H-5), 9.26 (br s, 1, H–N^3). $J_{5'a,b}=17.8$ Hz, $J_{4',5'b}=6.6$ Hz, $J_{5,5'b}=1.7$ Hz. *Anal.* Calcd for $C_{12}H_{14}N_2O_4S$: C, 51.05; H, 5.01; N, 9.93; S, 11.36. Found: C, 51.06; H, 5.08; N, 10.03; S, 11.14.

6,5′-Cyclo-5′-deoxy-4-thiouridine (12)——A mixture of **11** (400 mg) in 8 ml of MeOH and 8 ml of 2 N HCl was heated at 90 °C for 50 min. After cooling, a precipitate was collected. Additional precipitate was obtained from the mother liquor by concentration. The combined precipitate was crystallized from H_2O to give 304 mg (89%) of **12**, mp 275—288 °C (darkened). MS *m/z*: 242 (M). UV $\lambda_{max}^{H_2O}$: 337 nm (ε, 19900), 249 nm (ε, 3870). NMR (DMSO-d_6): 2.68 (d, 1, H-5′a), 3.05 (ddd, 1, H-5′b), 4.13 (m, 2, H-2′,3′), 4.41 (d, 1, H-4′), 5.24, 5.46 (d each, 1+1, HO-2′, 3′), 5.78 (s, 1, H-1′), 6.15 (d, 1, H-5), 12.58 (br s, 1, H–N^3). $J_{5'a,b}=19$ Hz, $J_{4',5'b}=6.3$ Hz, $J_{5,5'b}=1.5$ Hz. CD in H_2O: 331 nm $[\theta]=+9500$. *Anal.* Calcd for $C_9H_{10}N_2O_4S$: C, 44.63; H, 4.16; N, 11.57; S, 13.24. Found: C, 44.54; H, 4.11; N, 11.48; S, 13.06.

6,5′-Cyclo-5′-deoxy-2′,3′-*O*-isopropylidene-4-methylthiouridine (13)——Compound **11** (400 mg) in 40 ml of MeOH was treated with 0.97 ml of MeI and 1.42 ml of 1 N NaOH under stirring at room temperature. After 1.5 h, the solvent was evaporated off and the residue was partitioned between $CHCl_3$ and H_2O. The organic layer was dried by passing it through a Whatman 1 PS paper and the fitrate was centrated. The residue was crystallized from EtOH to give 352 mg (84%) of **13**, mp 205—206 °C. MS *m/z*: 296 (M), 281 (M−15). UV λ_{max}^{MeOH}: 304 nm. NMR ($CDCl_3$): 1.31, 1.51 (s each, 3+3, Me_2C), 2.52 (d, 1, H-5′a), 2.53 (s, 3, MeS), 3.25 (ddd, 1, H-5′b), 4.56—4.72 (m, 3, H-2′, 3′, 4′), 6.00 (s, 1, H-5), 6.29 (s, 1, H-1′). $J_{5'a,b}=18.6$ Hz, $J_{4',5'b}=6.8$ Hz. *Anal.* Calcd for $C_{13}H_{16}N_2O_4S$: C, 52.69; H, 5.44; N, 9.45; S, 10.82. Found: C, 52.44; H, 5.59; N, 9.29; S, 10.80.

6,5′-Cyclo-5′-deoxy-2′,3′-*O*-isopropylidenecytidine Mesitylenesulfonate (14)——Mesitylenesulfonyl chloride (822 mg, 2 eq) and triethylamine (0.79 ml, 3 eq) were added to a solution of **3** (500 mg) in 15 ml of CH_2Cl_2, and the mixture was stirred at room temperature for 6 h. Acetonitrile (15 ml) was added to the mixture and NH_3 gas was bubbled into the solution in an ice-bath. After 6 h, the solvent was evaporated off and the residue was partitioned with $CHCl_3$–H_2O. The aqueous layer was concentrated and the residue was dissolved in MeOH. This solution was applied to a column of silica gel (50 g, 2.2 × 36 cm) and eluted with $CHCl_3$–MeOH (10 : 1). The eluate was evaporated, and the residue was crystallized from EtOH to give **14** (256 mg, 29%), mp 267 °C (dec.). MS *m/z*: 265 (M), 250 (M−15), 200 (mesitylenesulfonyl). NMR (DMSO-d_6): 1.27, 1.40 (s each, 3+3, Me_2C), 2.17 (s, 3, *p*-Me–MS), 2.50 (s, 6, *o*-Me–MS), 2.87 (d, 1, H-5′a), 3.29 (ddd, 1, H-5′b), 4.62 (d, 1, H-4′), 4.70 (d, 1, H-3′), 4.87 (d, 1, H-2′), 5.84 (s, 1, H-5), 5.95 (s, 1, H-1′), 6.76 (s, 2, *m*-H–MS), 8.26, 9.31 (br s each, 1+1, NH_2). $J_{5'a,b}=19$ Hz, $J_{4',5'b}=5.9$ Hz, $J_{2',3'}=5.6$ Hz. *Anal.* Calcd for $C_{21}H_{27}N_3O_7S$: C, 54.18; H, 5.85; N, 9.03; S, 6.89. Found: C, 54.27; H, 5.80; N, 9.07; S, 6.70.

6,5′-Cyclo-5′-deoxycytidine (15)——Compound **14** (200 mg) was dissolved in MeOH (2 ml) and 1 N HCl (2 ml) and the solution was heated at 85—90 °C for 2 h, then neutralized with sat. $NaHCO_3$. The solvent was evaporated off, during which time crystalline **15** precipitated (42 mg, 44%), mp 216 °C (dec.). UV $\lambda_{max}^{H_2O}$: 276.5 nm (ε, 10200), 229 (sh, ε, 8500), 218 (sh, ε, 10100); $\lambda_{max}^{0.1\,N\,HCl}$: 285.5 nm (ε, 14000), 215 nm (ε, 8900). MS *m/z*: 225 (M). CD in H_2O: 269 nm $[\theta]=+16000$. NMR (DMSO-d_6) 2.63 (d, 1, H-5′a), 3.06 (ddd, 1, H-5′b), 4.02 (m, 2, H-2′, 3′), 4.33 (d, 1-H-4′), 5.10 (d, 1, HO-3′), 5.41 (d, 1, HO-2′), 5.44 (s, 1, H-5), 5.81 (s, 1, H-1′), 6.97 (br s, 2, NH_2). $J_{5'a,b}=18.8$ Hz, $J_{4',5'b}=5.9$ Hz, $J_{3',3'OH}=5.9$ Hz. *Anal.* Calcd for $C_9H_{11}N_3O_4$: C, 48.00; H, 4.92; N, 18.66. Found: C, 47.79; H, 4.84; N, 18.56.

5′-Deoxy-5′-iodo-2′,3′-isopropylidene-N^4-acetyl-5-bromocytidine (18)——A mixture of 2′,3′-*O*-isopropylidene-N^4-acetyl-5-bromocytidine[17] (3.0 g) and methyltriphenoxyphosphonium iodide (5.92 g) in 80 ml of dimethylformamide was stirred for 1.5 h at room temperature. After addition of 2.5 ml of MeOH to the solution, the whole was evaporated *in vacuo* and the residue was dissolved in 70 ml of $CHCl_3$. The solution was washed successively with 1 N $Na_2S_2O_3$ (60 ml × 2), sat. $NaHCO_3$ (60 ml × 2), and H_2O (60 ml × 2), and dried over Na_2SO_4. The $CHCl_3$ solution was applied to a column of silica gel (2.5 × 28 cm) and the product was eluted with $CHCl_3$–MeOH (50 : 1). The combined eluate was evaporated, then the residue was dissolved in a small volume of $CHCl_3$. This solution was dropped into *n*-hexane to give **18** as a white solid (1.1 g). This was used for the next step without further purification.

6,5′-Cyclo-5′-deoxy-2′,3′-*O*-isopropylidene-N^4-acetylcytidine (17)——a) 5′-Deoxy-5′-iodo-2′,3′-*O*-isopropylidene-N^4-acetylcytidine (**16**,[12] 400 mg) was suspended in 15 ml of benzene, and a solution of Bu_3SnH (0.32 ml) and AIBN (50 mg) in 5 ml of benzene was added through an injector over a period of 40 min under reflux. After cooling, the solution was concentrated and the concentrate was added dropwise to *n*-hexane. The precipitate was collected and dissolved in $CHCl_3$, and the solution was applied to a column of silica gel (2.4 × 50 cm). The eluate with $CHCl_3$–MeOH (30 : 1) was concentrated and the residue was crystallized from EtOH to give **17** (110 mg, 40%), mp 247 °C (dec.). MS *m/z*: 307 (M), 292 (M−15), 249 (M−58). NMR ($CDCl_3$): 1.31, 1.52 (s each, 3+3, Me_2C), 2.24 (s, 3, Ac), 2.67 (d, 1, H-5′a), 3.35 (ddd, 1, H-5′b), 4.62, 4.69 (d each, 1+1, H-2′, 3′), 4.65 (d, 1, H-4′), 6.30 (s, 1, H-1′), 7.21 (d, 1, H-5), 9.16 (s, 1, H–N^4). $J_{5'a,b}=19$ Hz, $J_{5,5'b}=1.5$ Hz, $J_{4',5'b}=7$ Hz, $J_{2',3'}=5.6$ Hz. *Anal.* Calcd for $C_{14}H_{17}N_3O_5$: C, 54.69; H, 5.59; N, 13.67. Found: C, 54.43; H, 5.71; N, 13.38.

b) Compound **18** (680 mg) in 20 ml of benzene was treated with 0.45 ml of Bu_3SnH and AIBN (50 mg) by the procedure described above. After removal of the solvent, the residue was taken up in CH_3CN, and the solution was poured into *n*-hexane. The CH_3CN layer was concentrated, the residue was taken up in $CHCl_3$, and the solution was applied to a column of silica gel (2.4 × 40 cm). The eluate with $CHCl_3$–MeOH (30 : 1) was concentrated and the residue was crystallized from EtOH to give **17** (155 mg, 38%). The physical properties were identical with those of the

product obtained by method a).

6,5′-Cyclo-5′-deoxyuridine 2′,3′-Cyclic Phosphate (19)——A mixture of **4** (113 mg), polyphosphoric acid (2.5 mmol as H_3PO_4) and tri-*n*-butylamine (1 mmol) in 50 ml of dimethylformamide was heated to reflux for 1 h. After evaporation of the solvent *in vacuo*, the residue was dissolved in aqueous ammonia. The solution was extracted with ether (15 ml × 2) and the aqueous layer was concentrated. The residue was redissolved in 20 ml of H_2O, and the solution was applied to a column of DEAE- cellulose (1.9 × 40 cm, bicarbonate form). Elution was performed with 500 ml of H_2O followed by a linear gradient system (1000 ml each of H_2O and 0.1 M $Et_3NH^+CO_3^-$); 18 ml fractions were collected. Fractions No. 55—65 were combined and concentrated to leave 4180 OD units (75%) of **19** as the triethylammonium salt. Migration on paper electrophoresis (0.05 M $Et_3NH^+HCO_3^-$, 700 V, 50 min): +8, 5 cm. Migrations of UMP, uridine, and **4** were +11.5 cm, +4 cm, and +4 cm, respectively.

6,5′-Cyclo-5′-deoxy-5-bromouridine 2′,3′-Cyclic Phosphate (20)——Compound **10** (100 mg) was phosphorylated by a procedure similar to that described above, and **20** was obtained in 55% yield.

6,5′-Cyclo-5′-deoxy-4-thiouridine 2′,3′-Cyclic Phosphate (21)——Compound **12** (50 mg) was phosphorylated similarly, except that the heating period was 30 min, to give a 52% yield of **21**.

Hydrolysis of 19, 20, and 21 with RNase A——A cyclic phosphate and RNase A were dissolved in 10 mM Tris-HCl buffer (pH 7.2) containing 1 mM EDTA at concentrations of 10 mM and 73 μM, respectively. The reaction mixture was kept at 37 °C for 16 h. The rate of hydrolysis was determined by separating the hydrolysis product (3′-phosphate) and unreacted 2′,3′-cyclic phosphate by paper electrophoresis, followed by measurement of the absorptivities. The results are summarized in Table I.

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