Nucleosides. 101. Conformationally Restricted Analogues of Pyrimidine Nucleosides. 1. Synthesis of 6,5′(S)- and 6,5′(R)-Cyclouridine[1]

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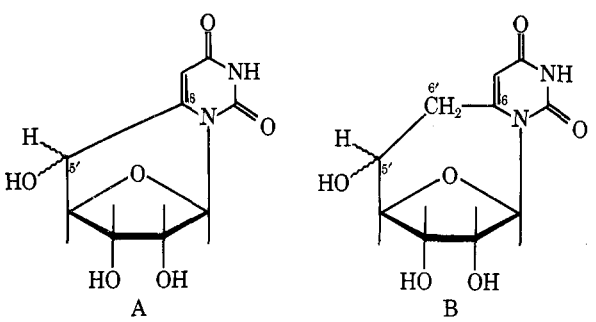
Received March 24, 1976

Methods are described for the synthesis of the 5′(S) and 5′(R) epimers of 6,5′-cyclouridine, conformationally restricted nucleosides that simulate the anti:gauche-trans and anti:trans-gauche conformers of uridine. The previously reported 2′,3′-O-isopropylidene-5-hydroxy-6,5′(S)-cyclouridine serves as starting material for both epimers. Mesylation of the phenolic 5-hydroxyl group, followed by desulfonyloxylation with hydrogen and palladium–charcoal in the presence of triethylamine, affords 2′,3′-O-isopropylidene-6,5′(S)-cyclouridine. Deblocking with 80% acetic acid then gives 6,5′(S)-cyclouridine. Both the 5′-mesyl and 5′-acetyl esters of 2′,3′-O-isopropylidene-6,5′(S)-cyclouridine undergo base-catalyzed epimerization at C-5′ to give equilibrium mixtures of the 5′(S) and 5′(R) esters. Separation of 5′-O-acetyl-2′,3′-O-isopropylidene-6,5′(R)-cyclouridine from its 5′(S) isomer, followed by removal of protecting groups under acidic conditions, affords a convenient route to 6,5′(R)-cyclouridine. NMR experiments in pyridine-d_6 containing D_2O indicate that the 5′-epimerization reactions involve carbanion intermediates.

A knowledge of the conformations of enzyme-bound nucleosides and nucleotides would be invaluable for gaining insight into enzyme mechanisms and the nature of active sites, and could serve eventually as a basis for the design of nucleoside antimetabolites having enhanced affinity and specificity for their target enzymes. In order to explore the relationship between conformation[2] and biological activity[3] in the pyrimidine nucleoside series, we have undertaken the synthesis of conformationally restricted compounds of the types illustrated by the uridine analogues A and B in Scheme I. These

Scheme I

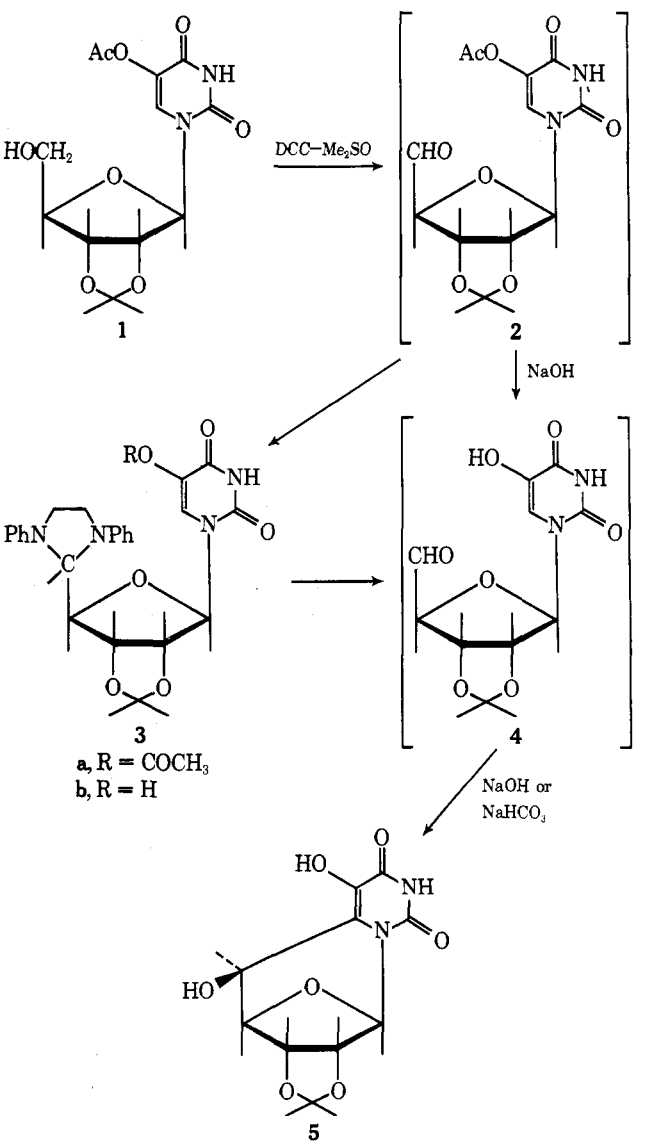


nucleosides, and the corresponding nucleotides, are suitable conformational probes for the following reasons. Both the 6,5′-cyclo (A) and methylene-bridged (B) types retain the full complement of hydrogen-bonding sites of their unrestricted analogues. Both types are constrained within the anti range, a desirable feature because previous studies have shown that conformationally abnormal, syn nucleosides do not, in general, substitute for their anti counterparts in enzyme-catalyzed reactions.[3a,b,4] Further, since nucleosides of types A and B are asymmetric at C-5′, each can exist as pairs of D-allo (5′R) and L-talo (5′S) isomers. The orientations of the 5′-hydroxyl (or 5′-phosphate) groups in these epimeric pairs correspond approximately to the gauche-trans and trans-gauche $C_{4'}$, $C_{5'}$ rotamers of ordinary nucleosides,[2,5] and the behavior of each epimer in enzyme-catalyzed reactions may allow a general assessment of the importance of this conformational feature.

In this paper we describe the synthesis of both the 5′(R) and 5′(S) isomers of 6,5′-cyclouridine (A)[6] by procedures that we plan to extend to the synthesis of other nucleosides of type A, and to the methylene-bridged types B.

The basic procedure for the synthesis of 6,5′-cyclopyrimidine nucleosides was developed in this laboratory by Rabi and Fox.[7] This method (Scheme II) depends on the fact that 5-hydroxyuracils are susceptible to electrophilic substitution at C-6,[8] and can, for example, undergo base-catalyzed hydroxymethylation at this position.[9] When the 5′-aldehyde (4)

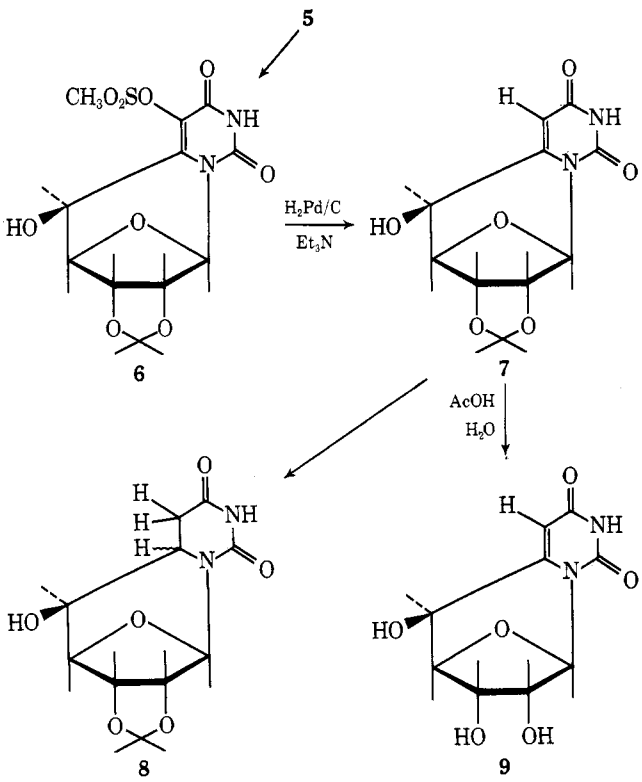
Scheme II



derived from 5-hydroxyuridine is treated with sodium bicarbonate, hydroxyalkylation proceeds in an intramolecular manner to afford the 6,5′(*S*)-cyclonucleoside **5**.[7] In the original work, the 5′-aldehydonucleoside **2** resulting from Me_2SO–DCC oxidation of 5-acetoxy-2′,3′-*O*-isopropylideneuridine (**1**) was converted into **5** via the 5′-imidazolidine derivatives **3a** and **3b**. We have simplified this procedure by treating reaction mixtures containing **2** with excess sodium hydroxide, thereby generating **4** which spontaneously cyclizes to give **5** directly in 40% yield. The ring closure **4** → **5**, whether catalyzed by sodium hydroxide or sodium bicarbonate, affords only the 5′-*S* isomer of **5**; none of the 5′-*R* isomer has been detected. Therefore, for conversion of **5** into the isomeric 6,5′-cyclouridines, methods were required for epimerization at C-5′, as well as for removal of the pyrimidine 5-hydroxyl group.

The procedure shown in Scheme III for the removal of the

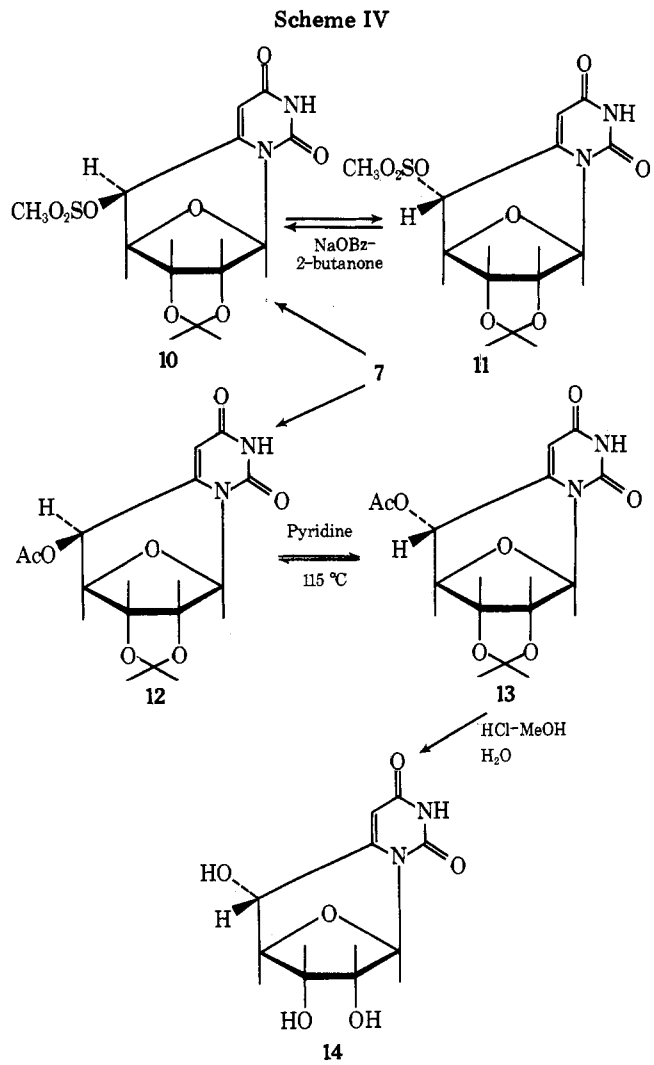
Scheme III



pyrimidine 5-hydroxyl group of **5** is an extension of the method developed by Clauss and Jensen[10] for the deoxygenation of phenols, namely the hydrogenolysis of phenol sulfonic esters in the presence of a base. In our case, the required 5-methanesulfonyl ester **6** was prepared by selective esterification of **5** in pyridine. The presence of a 5′-hydroxyl signal in the NMR spectrum of **6** (δ 6.29, $J_{5',5'OH}$ = 6.4 Hz), together with a uv spectrum appropriate for a 5-O-substituted 5-hydroxyuridine, confirms that esterification takes place at the 5 position of **5** as expected. Hydrogenation of **6** in the presence of palladium–carbon catalyst and an equivalent amount of triethylamine affords 2′,3′-*O*-isopropylidene-6,5′(*S*)-cyclouridine (**7**) in ~57% yield.[11] The structure of **7** was evident from the NMR spectrum, in which H-5 (δ 5.69) appears as a narrow doublet, coupled (1.7 Hz) to the 5′ proton appearing at δ 4.69. The chemical shift of the single 5′ proton and the 5′-hydroxyl signal at δ 6.54 confirm that reduction of the allylic 5′ position does not occur under these conditions. However, the desulfonyloxylation reaction **6** → **7** has to be monitored carefully because the product (**7**) undergoes further reduction to give the 5,6-dihydronucleoside **8**. The NMR spectrum of **8** (H-6, δ 3.40; H-5a, 2.96; H-5b, 2.65) shows a single isomer, although the various coupling constants do not allow an unequivocal assignment of the configuration at C-6. The relative rates of the reactions **6** → **7** and **7** → **8** are such that a clear-cut change in the rate of hydrogen uptake is not observed. Consequently, preparations of **7** invariably contained small amounts of **8**. This contaminant is not separable by chromatography, but can be removed by careful recrystallization of **7**. In practice, the use of impure **7** for further reactions posed no problems. For example, hydrolysis of **7** containing about 10% of **8** in refluxing 80% acetic acid affords the desired 6,5′(*S*)-cyclouridine (**9**), which is readily obtained in a high state of purity.

An obvious method for inverting the C-5′ configuration of these 6,5′-cyclonucleosides would be displacement of a 5′-sulfonyl ester by an oxygen nucleophile, under conditions that favor an SN2 mechanism. Accordingly, the 5′-mesyl ester **10** (Scheme IV) was prepared from **7** and refluxed in 2-butanone

Scheme IV



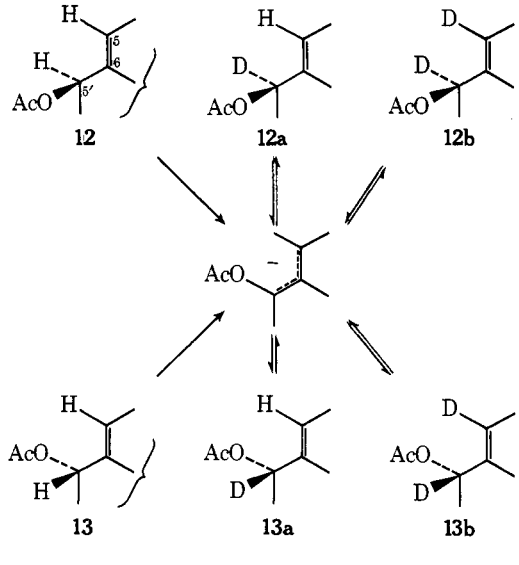
with sodium benzoate. This treatment results in the gradual appearance of material that migrates on TLC plates with a mobility very close to that of starting material **10**, although the reaction apparently does not go to completion. The NMR spectrum of this mixture, after removal of sodium benzoate, shows that the 5′-mesyl group is not displaced by benzoate ion under these rather mild conditions, but that epimerization at C-5′ had nevertheless taken place. This conclusion follows from the presence of NMR signals assignable to the 5′(*R*) mesyl ester **11**. The NMR spectrum of **11** obtained after fractional crystallization shows a value of <1 Hz for the H-4′, H-5′ coupling constant. This value is diagnostic of the 5′(*R*)

configuration because the 4′,5′ dihedral angle approaches 90°, and is quite different from the $J_{4',5'}$ value of 6.4 Hz noted for the 5′(S) isomer **10**, where the dihedral angle is ~30°. The 5′(S) mesyl ester **10** is itself stable in refluxing 2-butanone, but rapidly equilibrates with the 5′(R) isomer **11** on addition of triethylamine. This finding is consistent with benzoate ion acting as a base in the epimerization **10** → **11**, although it does not exclude the possibility of salt effects promoting a carbonium-ion mechanism. However, the results described below indicate that the epimerization most likely involves carbanionic intermediates.

We have not attempted to hydrolyze the 5′-mesyl group of **11** because the strongly basic conditions required would probably lead to equilibration of the S and R isomers, with the subsequent formation of both forms of 6,5′-cyclouridine. Instead, we have prepared the 5′-acetyl ester **12**, where all the blocking groups are acid labile, and studied the 5′ epimerization induced by treatment with refluxing pyridine. Compound **12** epimerizes slowly under these conditions, giving a **12** (S):**13** (R) ratio of ~2:1 at 24 h.[12] The isomers **12** and **13** are separable by silica gel chromatography, and again, assignment of the 5′(R) configuration to **13** rests on a $J_{4',5'}$ value of 0.8 Hz, as compared with a value of 6.5 Hz for **12**. Removal of the 5′-acetyl group of **13** by treatment with 30% hydrogen chloride in methanol, followed by the addition of water for the hydrolysis of the 2′,3′-*O*-isopropylidene group, affords 6,5′(R)-cyclouridine (**14**) in excellent yield. None of the 5′(S) isomer **9** was formed in this process, showing that **13** is not susceptible to acid-catalyzed isomerization to **12** prior to hydrolysis under these conditions.

Evidence that the mechanism of the above 5′-epimerization reactions involves carbanion intermediates comes from an NMR study of the interconversion of **12** and **13** in pyridine-d_6 containing 5% D_2O (Scheme V). At 80 °C, both the C-5 and

Scheme V



C-5′ hydrogens of the S isomer **12** undergo exchange for deuterium, with the rate of exchange at the allylic C-5′ position exceeding that of the pyrimidine C-5 position. Thus H-5′ had undergone 80% exchange at 30 min whereas H-5 was exchanged to the extent of 40%; at 2 h, H-5′ was exchanged completely, as compared with 60% exchange for H-5. At this stage the NMR spectrum shows a mixture of **12a** and **12b**; that is, exchange of H-5′ for deuterium proceeds with retention of configuration and greatly exceeds the rate of racemization. With further heating, where the deuterium exchange reactions become invisible, increasing amounts of the R isomer **13b** are observed, together with traces of **13a**. The S:R ratio reaches an equilibrium value of 2:1 at 40 h. These results indicate that **12** forms a resonance stabilized carbanion that can undergo deuteration at C-5 and C-5′;[13] and that because of asymmetric ion solvation, or the steric effects promoted by the asymmetry of the rest of the molecule, deuteration from the rear side of C-5′ (retention) predominates over deuteration from the front side (inversion). When the R isomer **13** is heated at 80 °C in pyridine-d_6–D_2O, the initial formation of **13a** and **13b** (exchange with retention) was *not* observed. Instead, **13** is converted gradually into a mixture of S isomers containing **12b** with traces of **12a**. This result can be accounted for in two ways. The R isomer **13** could undergo isoinversion[14]—that is, inversion without exchange for deuterium—to give the S isomer **12** directly, which would then undergo exchange at C-5 and C-5′ with predominant retention of configuration, as seen above. Alternatively, the carbanion derived from **13**, being formally the same as that derived from **12**, would on the basis of the above results undergo deuteration preferentially from the rear side (inversion) to give **12a**, and eventually **12b**. In either case, **12b** would be expected to reequilibrate with **13b**, and indeed, a substantial decrease in the integration value of H-5 and H-5′ of **13** is seen at 40 h, reflecting the presence of **13b**. The equilibrium S:R ratio at 40 h is the same as that observed when starting from the S isomer **12**.

The extensive studies by Cram and associates[14] on the stereochemistry of carbanion reactions have shown that the extent of inversion, retention, or racemization is very sensitive to changes in the base–solvent combination. It is therefore quite likely that different reaction conditions would lead to increased amounts of the R isomer (**13**) in the equilibrium with **12**. Similar base-catalyzed epimerizations should greatly facilitate the synthesis of other 6,5′-cyclopyrimidine nucleosides, and may find application in the 8,5′-cyclopurine nucleoside series. Studies of the chemistry of the R and S cyclouridines, and their behavior with some of the enzymes of pyrimidine nucleoside metabolism, are currently under investigation.

Experimental Section

General Procedures. Melting points were determined on a Thomas-Hoover apparatus (capillary method) and are uncorrected. The nuclear magnetic resonance spectra were determined on a JEOL PFT-100 spectrometer operating in the Fourier transform mode (EC-100 computer), with internal deuterium field-frequency lock. Values given for coupling constants (hertz) and chemical shifts (δ) are first order, and the resolution resulting from various combinations of spectral widths and computer data points is noted for each spectrum. Tetramethylsilane was used as internal standard. Ultraviolet spectra were measured on a Cary Model 15 spectrometer. Thin layer chromatography was performed on microscope slides coated with silica gel GF_{254} (Merck); separated materials were detected with ultraviolet light and by spraying with 10% v/v sulfuric acid in ethanol followed by charring. Evaporations were carried out in vacuo with bath temperatures kept below 45 °C. Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich.

5-Hydroxy-2′,3′-*O*-isopropylidene-6,5′(*S*)-cyclouridine (5). Pyridine (5 ml) and trifluoroacetic acid (2.5 ml) were added to a solution of 5-acetoxy-2′,3′-*O*-isopropylideneuridine (**1**, 17.1 g, 50 mmol) in dry dimethyl sulfoxide (200 ml) containing dicyclohexylcarbodiimide (40 g). The mixture was stirred at room temperature for 15 h, and then diluted with 25 ml of water. The precipitated dicyclohexylurea was collected after cooling and washed with acetone. The filtrate was concentrated to dryness at 35–40 °C (bath) in a rotary evaporator (oil pump) equipped with a Dewar condenser cooled with 2-propanol–dry ice, and the resulting syrup was dissolved in dichloromethane. Residual dicyclohexylurea was removed and the syrup remaining after evaporation of solvent was dissolved and shaken in a mixture of 500 ml of 50% methanol and 110 ml of 1 N NaOH. The solution was kept at room temperature for 30 min and then neutralized with 1 N acetic acid (~60 ml) to pH ~6. Solids were removed by filtration through a pad of Celite and the filtrate was clarified, where necessary, by storage overnight. The solution was decanted from precipitated, oily material and evaporated to remove methanol. After a final filtration (where needed), the clear aqueous solution was extracted with ethyl

acetate (5 × 200 ml), and the combined extracts were dried (Na_2SO_4) and then concentrated to give syrupy **5** (5.45 g) that crystallized spontaneously. A further 600 mg of **5** was obtained from a second series of ethyl acetate extractions, and the combined mother liquors and washings afforded 490 mg, bringing the total yield to 6.54 g (44%). In similar runs the yields ranged from 5.0 g (33%) to 6.79 g (46%). This product on occasion contains traces of dicyclohexylurea but is suitable for further reactions. Material obtained after recrystallization from ethanol was identical (melting point, uv, NMR, TLC) with authentic[7] **5**.

2',3'-*O*-Isopropylidene-5-mesyloxy-6,5'(*S*)-cyclouridine (6). A solution of methanesulfonyl chloride (5.73 ml, 74 mmol) in benzene (30 ml) was added dropwise (~1 h) to a stirred, ice-cold solution of 5 (4.4 g, 14.8 mmol) in pyridine (40 ml). Stirring was continued for an additional 1 h before ice was added to hydrolyze excess methanesulfonyl chloride. After a further 30 min, the volume was reduced to ~5 ml, water (20 ml) was added, and the mixture was extracted with ethyl acetate (3 × 150 ml). The combined extracts were dried (Na_2SO_4) and evaporated to dryness. The resulting syrup crystallized from ethanol (10 ml), affording 3.2 g of **6**. An additional 1 g of material (total yield 75%) was obtained on concentration of the mother liquor. A sample recrystallized from ethanol had mp 145 °C (sinters), 230–234 °C (effervescence), 257 °C dec; uv, pH 1 λ_{max} 265 nm, λ_{min} 228; pH 13 λ_{max} 264, λ_{min} 230; NMR (Me_2SO-d_6, res 0.3 Hz) exchangeable protons at δ 12.01 (1, broad s, N^3 H), 6.29 (1, d, 5'-OH, $J_{5',5'OH}$ = 6.4 Hz); Me_2SO-d_6 + D_2O (res 0.19 Hz), 5.88 (1, s, H-1'), 5.11 (1, d, H-2'), 5.06 (1, d, H-5'), 4.79 (1, d, H-3'), 4.52 (1, d, H-4'), 3.47 (3, s, mesyl CH_3), 1.41 (3, s) and 1.29 (3, s, isopropylidene methyls), $J_{1',2'} = 0$, $J_{2',3'} = 5.8$, $J_{3',4'} = 0$, $J_{4',5'} = 7.2$ Hz.

Anal. Calcd for $C_{13}H_{16}N_2O_9S \cdot 0.5H_2O$: C, 40.52; H, 4.45; N, 7.27. Found: C, 40.54; H, 4.27; N, 7.09.

2',3'-*O*-isopropylidene-6,5'(*S*)-cyclouridine (7). A suspension of 10% palladium on carbon (1 g) in water (10 ml) was added to a solution of **6** (3.0 g, 8 mmol) and triethylamine (1.09 ml, 8 mmol) in methanol (50 ml). The mixture was shaken under a hydrogen atmosphere in a Parr apparatus for ~170 min (variable), at which time hydrogen uptake reached ~12 mmol. The catalyst was removed and the filtrate concentrated to give a colorless syrup. Crystallization from ~20 ml of hot water afforded 1.28 g (57%) of **7** contaminated with small (and variable) amounts of **8**. A further recrystallization gave material with mp 290–291 °C dec; uv, pH 1 λ_{max} 268 nm, λ_{min} 234; pH 13 λ_{max} 269, λ_{min} 243; NMR (Me_2SO-d_6, res 0.3 Hz) exchangeable protons at δ 11.36 (1, broad s, N^3 H) and 6.54 (1, d, 5'-OH, $J_{5'5'OH}$ = 5.8 Hz); in Me_2SO-d_6 + D_2O res 0.09 Hz), 5.90 (1, s, H-1'), 5.69 (1, d, H-5), 4.90 (1, d, H-2'), 4.73 (1, d, H-3'), 4.43 (1, d, H-4'), 4.69 (1, dd, H-5'), 1.40 (3, s), and 1.26 (3, s, isopropylidene methyls), $J_{1',2'} = 0$, $J_{2',3'}$ = 5.9, $J_{3',4'} = 0$; $J_{4',5'} = 6.3$, $J_{5',5} = 1.7$ Hz.

Anal. Calcd for $C_{12}H_{14}N_2O_6$: C, 51.07; H, 5.00; N, 9.93. Found: C, 51.23; H, 5.22; N, 9.92.

The aqueous phase from the above reaction contains starting material (**6**), small amounts of **7**, and substantial amounts of **8**. Separation of **6** from **7** and **8** can be effected by chromatography on silica gel 60 (Merck, 70–230 mesh) using heptane–ethyl acetate (1:2 v/v), but this procedure does not separate **7** from **8**. Compound **7** containing small amounts of **8** can be purified by recrystallization from ethyl acetate–petroleum ether (bp 30–60 °C) mixtures.

5,6-Dihydro-2',3'-*O*-isopropylidene-6,5'(*S*)-cyclouridine (8). A mixture of **7** and **8** (500 mg), obtained from a reaction similar to that described above, was dissolved in 50% methanol (50 ml) containing triethylamine (0.16 ml). The mixture was reduced in the presence of 10% palladium on carbon catalyst (500 mg) on a Parr apparatus for 5 h. Catalyst and solvents were removed, and the resulting residue was recrystallized from 95% ethanol to give pure, non-uv-absorbing **8**: mp 238–239 °C; NMR (acetone-d_6, res 0.3 Hz), exchangeable protons at δ 9.25 (1, broad s, N^3 H) and 5.14 (1, broad d, 5'-OH, $J_{5',5'OH}$ ~ 4.6 Hz); acetone-d_6 + D_2O (res 0.15 Hz), 5.86 (1, s, H-1'), 4.97 and 4.79 (2, AB system, H-2' and H-3'), 4.28 (1, d, H-4'), 3.67 (1, dd, H-5'), 3.40 (1, eight-line m, H-6), 2.96 and 2.65 (2, two four-line m, H-5a and H-5b), 1.44 (3, s) and 1.34 (3, s, isopropylidene methyls), $J_{1',2'} = 0$, $J_{2',3'} = 5.7$, $J_{3',4'} = 0$, $J_{4',5'} = 4.3$, $J_{5',6} = 9.1$, $J_{5a,6} = 4.6$, $J_{5b,6} = 11.5$, $J_{5a,5b} = 16.8$ Hz.

Anal. Calcd for $C_{12}H_{16}N_2O_6$: C, 50.70; H, 5.67; N, 9.85. Found: C, 50.55; H, 5.49; N, 9.84.

6,5'(*S*)-Cyclouridine (9). A solution of **7** (800 mg, 2.8 mmol) in 80% acetic acid (25 ml) was refluxed for 5 h, at which time TLC (ethyl acetate) indicated that the hydrolysis was complete. The solution was evaporated to dryness and two 15-ml portions of ethanol were evaporated from the residue. Recrystallization from 20% ethanol afforded pure **9** (590 mg, 87%): mp 293–294 °C; uv pH 1 λ_{max} 268 nm (ϵ 10 750), λ_{min} 233 (1700); pH 10.8 λ_{max} 268 (8530), λ_{min} 243 (4190); NMR (Me_2SO-d_6, res 0.09 Hz) δ 11.27 (1, broad s, NH, exchanges), 6.44 (1, d, 5'-OH, exchanges), 5.74 (1, s, H-1'), 5.64 (1, d, broadened by unresolved N^3 H coupling, H-5), 5.35 (1, d, 3'-OH, exchanges), 5.23 (1, d, 2'-OH, exchanges), 4.61 (1, six-line m, H-5'), 4.31 and 4.22 (2, m, H-2' and H-4'), 4.02 (1, three lines, H-3'), $J_{1',2'} = 0$, $J_{2',3'} = 6.1$, $J_{3',4'} = 0$, $J_{4',5'} = 6.0$, $J_{5'5} = 1.6$, $J_{5',5'OH} = 6.1$, $J_{3',3'OH} = 5.3$, $J_{2',2'OH} = 6.8$ Hz.

Anal. Calcd for $C_9H_{10}N_2O_6$: C, 44.63; H, 4.16; N, 11.57. Found: C, 44.45; H, 4.19; N, 11.55.

2',3'-*O*-Isopropylidene-5'-*O*-mesyl-6,5'(*S*)-cyclouridine (10). A solution of **7** (500 mg, 2.07 mmol) and methanesulfonyl chloride (1.35 ml) in pyridine (20 ml) was stirred at room temperature until TLC (ethyl acetate) indicated disappearance of starting material (~4 h). Water (10 ml) was added to the mixture, and solvents were removed by evaporation. The residue was partitioned between water (10 ml) and ethyl acetate (3 × 50 ml), and the organic phase was dried (Na_2SO_4) and then concentrated to dryness. Two recrystallizations from ethanol afforded **10** (495 mg, 66%): mp 260 °C (sinters), 264–265 °C dec; uv λ_{max} pH 1 268 nm, λ_{min} 233, pH 10.8 λ_{max} 268, λ_{min} 243; NMR (Me_2SO-d_6, res 0.3 Hz), exchangeable proton at 11.6 (1, broad s, N^3 H), Me_2SO-d_6 + D_2O (res 0.3 Hz), 5.95 (1, s, H-1'), 5.87 (1, dd, H-5'), 5.74 (1, d, H-5), 4.91 (1, d, H-2'), 4.83 (1, d, H-3'), 4.71 (1, d, H-4'), 3.56 (3, s, mesyl CH_3), 1.41 (3, s), and 1.28 (3, s, isopropylidene methyls); $J_{1',2'} = 0$, $J_{2',3'} = 5.5$, $J_{3',4'} = 0$, $J_{4',5'} = 6.4$, $J_{5',5} = 1.5$ Hz.

Anal. Calcd for $C_{13}H_{16}N_2O_8S$: C, 43.33; H, 4.48; N, 7.77. Found: C, 43.16; H, 4.68, N, 7.75.

5'-*O*-Acetyl-2',3'-*O*-isopropylidene-6,5'(*S*)-cyclouridine (12). A solution of **7** (760 mg, 2.7 mmol) and acetic anhydride (0.51 ml, 5.4 mmol) in pyridine (10 ml) was stirred at room temperature for 4 h. Water (10 ml) was added and stirring continued for an additional 1 h before concentration to dryness. The residue was recrystallized from ethanol, and then from ethyl acetate–petroleum ether, to give pure **12** (610 mg, 70%): mp 220 °C (sinters), 227–228 °C; uv, pH 1 λ_{max} 267 nm, λ_{min} 235; NMR (Me_2SO-d_6, res 0.15 Hz) δ 11.49 (1, d, N^3 H, exchanges), 5.95 (1, s, H-1'), 5.79 (1, dd, H-5'), 5.67 (1, three lines, H-5), 4.86 (2, s, H-2' and H-3'), 4.60 (d, H-4'), 2.19 (3, s, OAc), 1.40 (3, s), and 1.27 (3, s, isopropylidene methyls), $J_{1',2'} = J_{3',4'} = 0$, $J_{4',5'} = 6.5$, $J_{5,N^3\text{-}H}$ = 1.5, $J_{5,5'} = 1.5$ Hz. In Me_2SO-d_6 + D_2O, H-2' and H-3' give an AB system at δ 4.90 and 4.82, with $J_{2',3'} = 5.8$ Hz.

Anal. Calcd for $C_{14}H_{16}N_2O_7$: C, 51.85; H, 4.97; N, 8.64. Found: C, 51.92; H, 5.03; N, 8.61.

2',3'-*O*-Isopropylidene-5'-*O*-mesyl-6,5'(*R*)-cyclouridine (11). **A.** Sodium benzoate (75 mg, 0.52 mmol) was added to a solution of **10** (150 mg, 0.42 mmol) in 2-butanone (10 ml), and the mixture was heated to reflux with stirring. TLC (EtOAc–petroleum ether, 3:1 v/v) showed the formation of a slower moving component (**11**) that did not increase in concentration after ~5 h. The mixture was filtered and the filtrate was concentrated to dryness. The residue was partitioned between water and chloroform, and the organic phase was dried and concentrated to afford a crystalline mixture containing approximately equal amounts (NMR) of **10** and **11**. Attempts to separate these isomers by thick layer chromatography were unsuccessful because the mixture crystallized at the origin when applied to the plates. Recrystallization of the mixture from ethanol afforded several crops of **10**, crops containing both components, and finally moderately pure **11**. Recrystallization (EtOH) afforded ~40 mg of chromatographically pure **11**: mp 255–258 °C dec; uv, pH 1 λ_{max} 271 nm, λ_{min} 235.5; pH 10.8 λ_{max} 271, λ_{min} 241; NMR (Me_2SO-d_6, res 0.15 Hz) δ 11.64 (1, broad s, N^3 H, exchanges), 5.98 (1, s, H-1'), 5.78 (1, s, broadened by unresolved $J_{5,5'}$, H-5), 5.56 (1, s, broadened by unresolved $J_{4',5'}$, H-5'), 4.87 (1, d, H-2'), 4.76 and 4.73 (2, H-3' d overlapped by H-4's), 3.46 (3, s, mesyl CH_3), 1.41 (3, s), and 1.26 (3, s, isopropylidene methyls); $J_{1',2'} = J_{3',4'} = 0$, $J_{2',3'} = 5.6$, $J_{4',5'} \simeq J_{5',5} < 1$ Hz.

Anal. Calcd for $C_{13}H_{16}N_2O_8S$: C, 43.33; H, 4.48; N, 7.77. Found: C, 43.52; H, 4.48; N, 7.71.

B. A solution of **10** (200 mg) in 2-butanone (10 ml) containing triethylamine (0.2 ml) was refluxed for 4 h. Removal of solvent afforded a solid residue with an NMR spectrum identical with that of the **10**:**11** mixture obtained above.

5'-*O*-Acetyl-2',3'-*O*-isopropylidene-6,5'(*R*)-cyclouridine (13). A solution of **12** (900 mg) in pyridine (20 ml) was protected from moisture and refluxed for 24 h. The pale brown solution was then evaporated to dryness and residual pyridine was removed by codistillation with aqeous ethanol and then ethanol. The NMR spectrum of the residue in Me_2SO-d_6 showed a **12**:**13** ratio of 2:1. A solution of the residue in chloroform was applied to a column of silica gel G (Merck, 200 g, 4.5 × 40 cm) that had been packed under air pressure in benzene–ether[15] (1:1). The column was eluted with the same solvent pair, using air pressure to achieve a reasonable flow rate. Combination of the appropriate fractions afforded 402 mg of **12** (eluted from the

column first) and 226 mg of **13**. Attempts to recover **13** by fractional crystallization of fractions containing both **12** and **13** were unsuccessful. Chromatographically pure **13** (multiple development in ethyl acetate–benzene, 2:3) showed mp 240 °C (sinters), 248–251 °C; uv, pH 1 λ_{max} 271 nm, λ_{min} 236; NMR (Me_2SO-d_6, res 0.07 Hz) δ 11.55 (1, broad s, N^3 H, exchanges), 5.96 (1, s, H-1′), 5.70 (1, d, broadened by unresolved J_{5,N^3H}, H-5), 5.54 (1, three lines, H-5′), 4.92 (1, d, H-2′), 4.71 (1, d, H-3′), 4.54 (1, broadened s, H-4′), 2.10 (3, s, OAc), 1.40 (3, s), and 1.27 (3, s, isopropylidene methyls); $J_{1',2'} = 0$, $J_{2',3'} = 5.6$, $J_{3',4'} = 0$, $J_{4',5'} = J_{5',5} = 0.8$ Hz.

Anal. Calcd for $C_{14}H_{16}N_2O_7$: C, 51.85; H, 4.97; N, 8.64. Found: C, 51.64; H, 4.94; N, 8.48.

The NMR study of the interconversions of the 5′(S)-acetyl compound **12** and 5′(R)-acetyl compound **13** was performed as follows.

Each isomer (5 mg) was dissolved in 0.4 ml of pyridine-d_6 containing 5% D_2O. Spectra were recorded immediately and at various intervals after heating at 80 °C in an oil bath. Each spectrum was determined at 1250 Hz width using five 90 ° (23 μs) pulses with 15-s repetition time. The results are described in the text.

6,5′(R)-Cyclouridine (14). Compound **13** (100 mg, 0.31 mmol) was suspended in 30% hydrogen chloride in methanol (5 ml) and the mixture was stirred at room temperature. Solution was complete within 5 min, and TLC (EtOAc) showed complete loss of the 5′-acetyl group of **13** at 2 h. Water (0.5 ml) was added and the solution was stored until TLC showed hydrolysis of the 2′,3′-*O*-isopropylidene group to be complete (~5 h, total). The clear solution was concentrated to dryness and the residue was dried by repeated coevaporation of ethanol. The crystalline residue was suspended in ether, collected, and washed liberally with ether. This material (69 mg, 92%) is chromatographically pure and can be recrystallized with good recovery from water: mp 265 °C (sinters), 284–285 °C dec; uv, pH 1 λ_{max} 272 nm (ϵ 10 250), λ_{min} 235 (1450); pH 10.8 λ_{max} 272 (8100), λ_{min} 244 (4000); NMR (Me_2SO-d_6, res 0.15 Hz) δ 11.33 (1, broad s N^3 H exchanges), 6.17 (1, d, 5′-OH, exchanges), 5.76 (1, s, H-1′), 5.59 (1, d, H-5), 5.36 (1, d, 2′(3′)-OH exchanges), 5.18 (1, d, 3′(2′)-OH, exchanges), 4.24 (1, d, broadened by unresolved couplings, H-5′), 4.18 (1, s, broadened by unresolved couplings, H-4′), 4.0 (2, six-line m, H-2′ and H-3′), $J_{1',2'} = 0$, $J_{5',5'\text{-OH}} = 6.1$, $J_{2'(3'),2'(3')\text{OH}} = 5.5$, $J_{3'(2'),3'(2')\text{-OH}} = 5.8$, $J_{5,N^3\text{-H}} = 1.7$ Hz, $J_{4',5'}$ unresolved, $J_{5'5}$ unresolved. In Me_2SO-d_6 + D_2O, H-2′ and H-3′ given an AB system at δ 4.05 and 3.97 with $J_{2',3'} = 6.1$ Hz.

Anal. Calcd for $C_9H_{10}N_2O_6$: C, 44.63; H, 4.16; N, 11.57. Found: C, 44.58; H, 4.23; N, 11.52.

Registry No.—**1**, 36507-00-3; **5**, 36507-05-8; **6**, 59686-57-6; **7**, 59686-58-7; **8**, 59686-59-8; **9**, 59686-60-1; **10**, 59686-61-2; **11**, 59728-00-6; **12**, 59686-62-3; **13**, 59728-01-7; **14**, 59728-02-8; methanesulfonyl chloride, 124-63-0; acetic anhydride 108-24-7.

References and Notes

(1) This investigation was supported in part by funds from the American Cancer Society (Grant CH-38) and from the National Institutes of Health, U.S. Public Health Service (Grant 17085).

(2) For recent reviews on nucleoside and nucleotide conformation see (a) The Jerusalem Symposia on Quantum Chemistry and Biology, "Conformation of Biological Molecules and Polymers", Vol. V, E. D. Bergman and B. Pullman, Ed., Academic Press, New York, N.Y., 1973; (b) "Basic Principles in Nucleic Acid Chemistry", P. O. P. T'so, Academic Press, New York, N.Y., 1974; (c) M. Sundaralingham, *Ann. N.Y. Acad. Sci.*, **255**, 3 (1975).

(3) Reviews that stress the relationship between nucleoside–nucleotide conformation and biological activity include (a) D. C. Ward and E. Reich, *Annu. Rep. Med. Chem.* (1969); (b) W. Saenger, *Angew. Chem., Int. Ed. Engl.*, **12**, 591 (1972). Recent papers suggesting a relationship between conformation and the biological activities of 8-azaadenylic acid,[3c] 6-thiopurine ribonucleoside 5′-phosphate,[3d] and poly(formycin B)–poly(C) complexes[3e] are (c) C-H. Lee, F. E. Evans, and R. H. Sarma, *J. Biol. Chem.*, **250**, 1290 (1975); (d) F. E. Evans and R. H. Sarma, *J. Am. Chem. Soc.*, **97**, 3215 (1975); (e) P. T. Torrence, E. DeClercq, J. A. Waters, and B. Witkop, *Biochem. Biophys. Res. Commun.*, **62**, 658 (1975).

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(5) The D-allo (5′*R*) isomers simulate the gauche-trans conformation—that is, the 5′-OH group is gauche with respect to the sugar-ring oxygen and trans with respect to C-3′ in a Newman projection along the $C_{4'}$–$C_{5'}$ bond. The opposite case, namely trans-gauche, is simulated by the L-talo (5′*S*) isomers. In most nucleotides, both in solution and in the solid state, the gauche-gauche conformation predominates (5′-OH group gauche with respect to *both* C-3′ and the ring oxygen), but interestingly, the available low-resolution x-ray structures of nucleotide–enzyme complexes show gauche-trans and trans-gauche conformers. See ref 2c and references cited therein.

(6) These compounds are the pyrimidine counterparts of 8,5′-cycloadenosine, an anti purine cyclonucleoside with a similarly restricted range of sugar conformations. One of the 5′ epimers (unseparable) of the corresponding 8,5′-cycloadenylic acid participated efficiently in a variety of enzyme-catalyzed reactions that normally require adenylic acid. See A. Hampton, P. J. Harper, and T. Sakai, *Biochemistry*, **11**, 4965 (1972).

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(11) We assume that the desulfonyloxylation of **6** involves C–O bond cleavage to give **7** directly, but it is possible that the reaction proceeds by reduction of the 5,6 double bond of **6**, followed by base-catalyzed elimination of methanesulfonic acid from the resulting 5-mesyloxy-5,6-dihydrouracil nucleoside. Indeed, we have previously used a similar reduction–elimination procedure to prepare 1,3-dimethyl-6-propyluracil from 1,3-dimethyl-5-mesyloxy-6-propyluracil.[9] The only other example of reductive removal of a pyrimidine-5-hydroxyl group of which we are aware involves hydrogenolysis of uracil-5-(1-phenyltetrazoyl) ether.[9]

(12) Although the 5′(*S*)-acetyl compound **12** and the 5′(*S*)-mesyl ester **10** are prepared in pyridine, the conditions used are too mild (4 h, room temperature) to result in any appreciable 5′ epimerization. For similar reasons, the 6,5′(*S*)-cyclonucleoside **7** does not epimerize during its preparation in the presence of triethylamine; in addition, ionization of the 5′-hydroxyl group would probably inhibit epimerization.

(13) N_3 H will also be ionized under these conditions.

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(15) Mallinckrodt USP grade ether, containing a maximum ethanol content of 3.5%.