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Halogenium-Induced Cyclization of 5-Substituted 6,2′-*O*-Cyclouridine[1,2]

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Reaction of 5-chloro (or bromo)-6,2′-*O*-cyclouridine (IIa or IIb) with *N*-bromo(or chloro)-succinimide gave 5-bromo-5-chloro-5,6-dihydro-6,2′: 6,5′-di-*O*-cyclouridine (VI). A similar reaction of 5-nitro-6,2′-*O*-cyclouridine (VII) afforded the 5-chloro(or bromo)-5-nitro analog (VIII or IX). Both VI and VIII were mixtures of two diastereoisomers and each isomeric mixture was separated by preparative high-performance liquid chromatography. The steric configurations and mass spectra of VI, VIII and IX are discussed.

Keywords——5-substituted 6-*O*-cyclouridine; 5-halogeno-5-substituted 5,6-dihydro-6,6-di-*O*-cyclouridine; cyclization; *N*-halogenosuccinimide; steric configuration; MS

In a previous paper,[3] we reported halogenation of pyrimidine 6-*O*-cyclonucleosides to give the corresponding 5-monohalogeno derivatives, together with 5,5-dihalogeno-5,6-dihydro-6,6-di-*O*-cyclouridines. As a part of studies designed to define the scope and limitations of this novel reaction, we tried to halogenate 5-substituted 6,2′-*O*-cyclouridine with *N*-halogenosuccinimide to prepare the 5-halogeno-5-substituted analogs, having two different substituents at the 5-position.

5-Halogeno-6,2′-*O*-cyclouridine (II)

Treatment of 6,2′-*O*-cyclouridine (I) with *N*-halogenosuccinimide gave rise to II in an unsatisfactory yield, because of side reaction leading to the formation of the by-product, 5,5-dihalogeno-5,6-dihydro-6,2′: 6,5′-di-*O*-cyclouridine (III). This type of side reaction may take place *via* addition of the halogenium cation and the 5′-hydroxyl group to the 5,6-double bond of II. It was thus assumed that inhibition of the nucleophilic attack of the 5′-hydroxyl group would hinder the side reaction. This was found to be the case. The 5′-*O*-trityl derivative (IV) of I was treated with *N*-chlorosuccinimide (NCS) in dimethylformamide (DMF) at room temperature to give 5-chloro-5′-*O*-trityl-6,2′-*O*-cyclouridine (Va). Compound Va could not be isolated owing to its lability, but removal of the trityl group with 80% trifluoroacetic acid allowed us to isolate 5-chloro-6,2′-*O*-cyclouridine (IIa) as white plates in 65% yield from IV. A similar treatment of IV with *N*-bromosuccinimide (NBS), followed by removal of the protecting group, yielded the 5-bromo counterpart (IIb) in a comparable yield (Chart 1).

5-Bromo-5-chloro-5,6-dihydro-6,2′: 6,5′-di-*O*-cyclouridine (VI)

Treatment of IIa with 2 eq of NBS in DMF at room temperature for 30 min afforded white needles in 81% yield. This compound was identified as VI on the basis of elemental analysis and the mass spectrum (MS). A gross similarity exists between the proton magnetic resonance (^{1}H-NMR) spectrum of VI and that of III with the exception of the presence of two signals each due to the N^3-, 1′-, 2′- and 5′-protons. This observation suggests that VI must be a mixture of two diastereoisomers. The molar ratio of the isomers was estimated to be *ca.* 3 : 1,

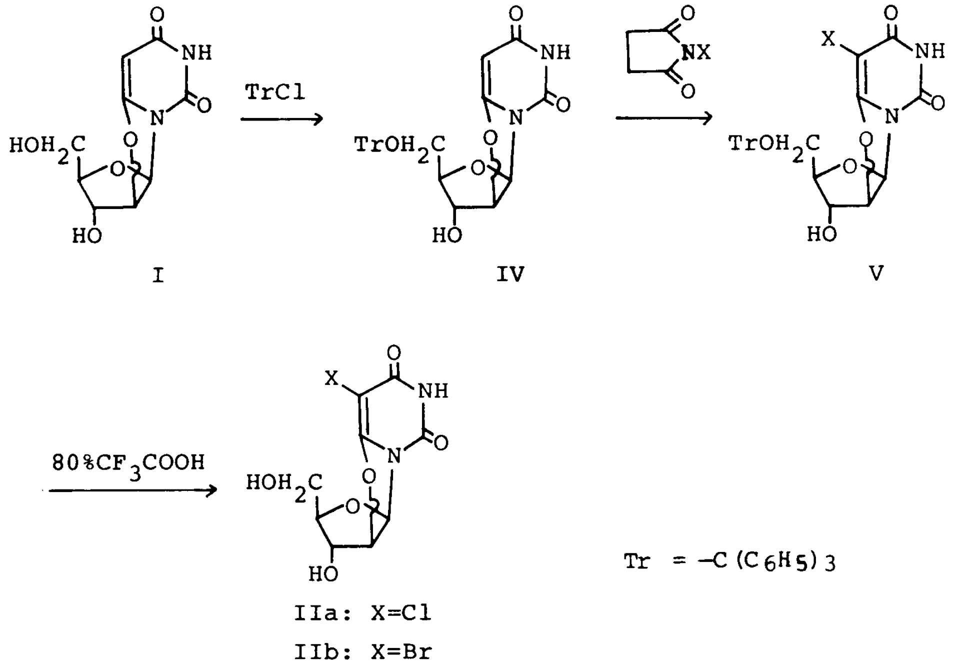

Chart 1

Table I. HPLC Separation[a] of 5-Chloro-5-Substituted 5,6-Dihydro-6,2′:6,5′-di-*O*-cyclouridines

Run No.	Substituent	Compd.	Retention time (min)
1	Br	VIa	23.6
		VIb	29.6
2	Cl	IIIa	20.0
		IIIb	32.0
	Br	VIa	22.8
		VIb	28.8
3	NO_2	VIIIa	6.8
		VIIIb	8.0

a) Column, Nucleosil ODS (5 μ), i.d. 4.0 × 150 mm; column temp., room temp.; mobile phase, 2.5 mM phosphate buffer (pH 6.9); flow rate, 1.0 ml/min; detector, UV (254 nm).

Table II. Molar Ratio of the Isomers VIa and VIb Prepared by the Reaction of IIa with NBS

Temp. (°C)	Time (min)	NBS (eq)	Molar ratio VIa : VIb
−20	Overnight	2	15 : 1
0	5	2	16 : 1
0	30	2	15 : 1
20	5	2	15 : 1
20	15	2	5 : 1
20	30	2	3 : 1
20	60	2	3 : 1
50	30	2	3 : 1
80	30	2	3 : 1

based on the relative integrated intensity of the N^3-proton signals, which were most readily resolvable (Chart 2). The mixture could not be separated even by thin-layer chromatography (TLC), but separation could be achieved by high-performance liquid chromatography (HPLC) (Table I). The molar ratio of the two components was also estimated by the half-width method in HPLC, and the result was in good accordance with that obtained by ^{1}H-NMR spectroscopy. The isomers were isolated as white needles in each case by the preparative HPLC, and had similar melting points and MS. Scarcely any depression of the melting point of the admixture was detected. The ^{1}H-NMR spectra showed that the N^3- and 1′-proton signals of the major isomer (VIa) were shifted upfield compared with the corresponding signals of the minor isomer (VIb).

A series of NBS treatment of IIa was carried out at various temperatures and for various times, and the molar ratio of the isomers formed was estimated by HPLC. In the reactions at

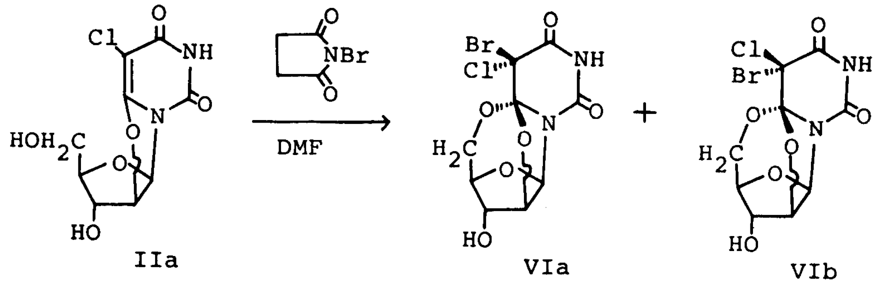

Chart 2

20 °C, practically stereospecific formation of VIa was effected as early as 5 min, and an equilibrium of VIa and VIb (3 : 1) was reached in 30 min or thereafter. In the reactions at 0 °C, stereospecific formation of VIa was maintained even after 30 min (Table II). These results indicate that VIa is initially formed, then isomerizes to and equilibrates with VIb.

Treatment of IIb with 2 eq of NCS in DMF at 50 °C overnight afforded a white powder. The compound showed a single spot on TLC, but was revealed to be a mixture of four components by HPLC (Table I). These components were isolated by preparative HPLC, and were identified as IIIa (5,5-dichloro analog),[3] VIa, VIb and IIIb (5,5-dibromo analog),[3] based on the melting points, MS and retention times. A plausible mechanism for the formation of IIIa and IIIb is as follows. Regeneration of aromaticity results in the release of halogen from VI. The lower ionization potential of the bromine than of the chlorine leads to the predominant elimination of the former from VI, and the resulting anion forms IIa, which reacts with NCS to give IIIa, while the released bromonium ion reacts with IIb to yield IIIb. This interpretation is supported by the facts that the reaction of VI with NCS gave rise to both IIIa and IIIb, while the reaction of IIIb with NCS afforded both VI and IIIa (Chart 3).

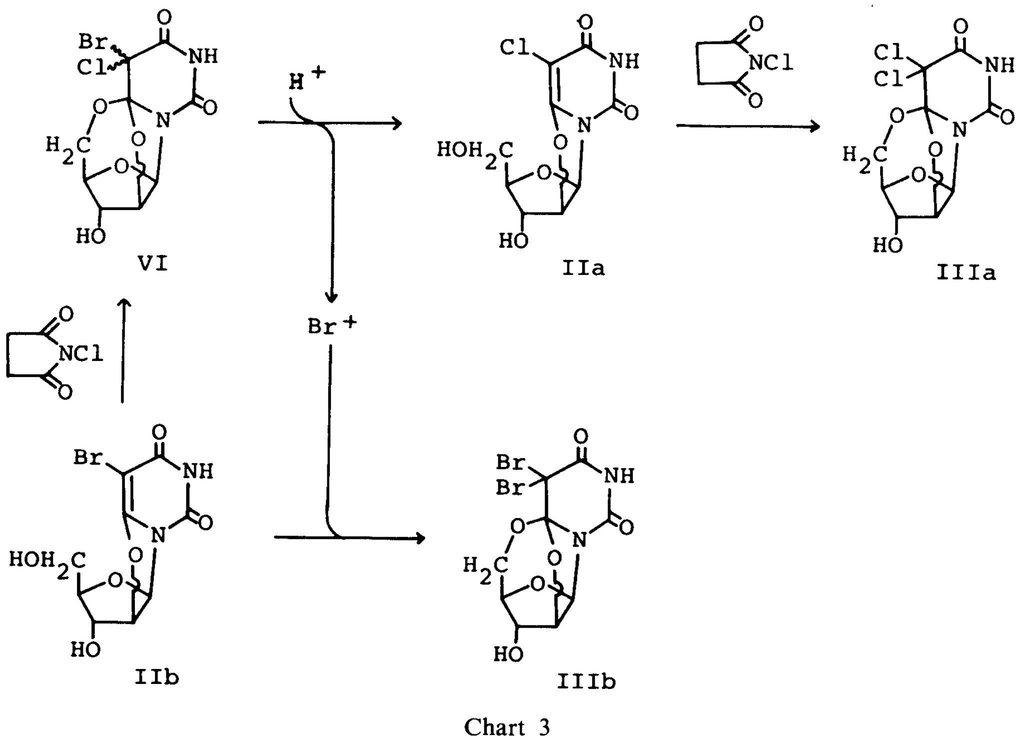

Chart 3

5-Chloro-5-nitro-5,6-dihydro-6,2′ : 6,5′-di-*O*-cyclouridine (VIII)

We next examined the reaction of 5-nitro-6,2′-*O*-cyclouridine (VII) with *N*-halogenosuccinimide. It has been reported that nitration of uridine with 50% nitric acid was accompanied by oxidation of the 5′-hydroxyl group to give 5-nitro-1-(β-D-ribosyluronic acid)uracil.[4)] In order to avoid such a side reaction, I was allowed to react with 2 eq of nitronium tetrafluoroborate in sulfolane[5)] at room temperature. The reaction mixture showed a bathochromic shift of the absorption maximum from 251 nm to *ca.* 290 nm and provided, after work-up, white prisms in 88% yield. Compound VII was extremely labile to alkali, and the absorption maximum was shifted from 290 nm to 318 nm. The lability may be attributed to nucleophilic attack of hydroxyl anion on the electron-deficient C-6, followed by alkyl-O-fission of the 6,2′-*O*-cyclic bond[6)] (Chart 4). An attempt to prepare the 5,5-di-nitro analog of III was unsuccessful.

Treatment of VII with 2 eq of NCS in DMF at 50 °C overnight afforded white needles, which were identified as VIII on the basis of elemental analysis and the characteristic relative abundance of chloro isotopes in the MS. The compound showed a single spot on TLC, but was found to be a mixture of two diastereoisomers by ^{1}H-NMR spectroscopy. The proton signals due to the sugar of one isomer showed such great differences in chemical shift from the corresponding signals of the other isomer that they could be assigned unequivocally by the decoupling technique. The molar ratio of the two isomers was *ca.* 1 : 1, based on the relative integrated intensity of the proton signals. This value was in good accordance with that obtained by the peak-height method in HPLC.

A series of NCS treatment of VII was carried out under various conditions and the molar ratio of the two isomers (VIII) formed was estimated by HPLC (Table I). In the reaction of VII with 5 eq of NCS at 21 °C, stereospecific formation of the major isomer (VIIIa) was effected as early as 5 min, and VIIIa was obtained in 75% yield. Equilibrium of the major

TABLE III. Molar Ratio of the Isomers VIIIa and VIIIb Prepared by the Reaction of VII with NCS

Temp. (°C)	Time (min)	NCS (eq)	Molar ratio VIIIa : VIIIb
19	30	2	—
19	60	2	—
19	120	2	10 : 1
19	Overnight	2	1 : 1
21	30	5	40 : 1
21	60	5	4 : 1
21	75	5	1 : 1
50	Overnight	2	1 : 1

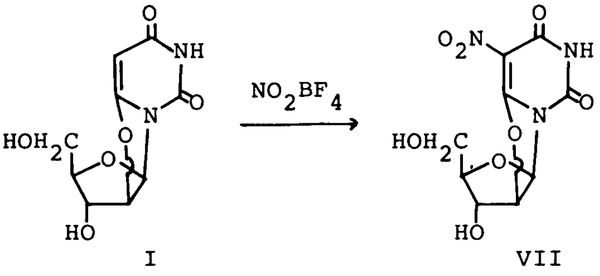

Chart 4

TABLE IV. Proton Chemical Shifts (ppm) in the 400 MHz Spectrum of 5-Halogeno-5-substituted 5,6-Dihydro-6,6-di-*O*-cyclouridine in DMSO-d_6

Compd.	H-1′	H-2′	H-3′	H-4′	H-5′a	H-5′b	N^3-H	Other
VIa	6.23	4.83	4.27	4.36	4.15	3.63	11.70	5.70
	(d)	(dd)	(s)	(m)	(dd)	(dd)	(s)	(br s, 3′-OH)
VIb	6.27	4.82	4.27	4.37	4.14	3.62	11.76	5.69
	(d)	(d)	(s)	(m)	(dd)	(d)	(s)	(d, 3′-OH)
VIIIa	6.26	4.74	4.25	4.40	4.22	4.17	12.51	5.72
	(d)	(dd)	(s)	(m)	(dd)	(dd)	(s)	(br s, 3′-OH)
VIIIb	6.31	4.87	4.22	4.38	4.17	3.80	12.33	5.72
	(d)	(dd)	(s)	(m)	(dd)	(dd)	(s)	(br s, 3′-OH)
IX	6.31	4.86	4.21	4.36	4.14	3.76	12.07	
	(d)	(dd)	(s)	(m)	(dd)	(dd)	(s)	

isomer (VIIIa) and the minor isomer (VIIIb) (1 : 1) was reached in 75 min (Table III). The melting point of VIIIb was 5 °C higher than that of the admixture of VIIIa and VIIIb.

5-Bromo-5-nitro-5,6-dihydro-6,2′ : 6,5′-di-*O*-cyclouridine (IX)

Treatment of VII with 2 eq of NBS in DMF at room temperature for 30 min afforded IX as white needles in 57% yield; this product was concluded to be stereochemically pure on the basis of chromatographic (TLC, HPLC) and spectroscopic (^{1}H-NMR spectra, MS) analyses (Chart 5, Tables IV and V).

TABLE V. First-Order Coupling Constants (Hz) of 5-Halogeno-5-substituted 5,6-Dihydro-6,6-di-*O*-cyclouridine

Compd.	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'a}$	$J_{4',5'b}$	$J_{5'a,5'b}$
VIa	4.4	1.3	a)	3.1	1.3	13.4
VIb	4.0	0	a)	2.8	0	13.5
VIIIa	4.4	1.3	a)	3.1	1.2	13.5
VIIIb	4.4	1.3	a)	2.8	1.5	13.3
IX	4.4	1.3	a)	2.7	1.2	12.8

a) Unresolved.

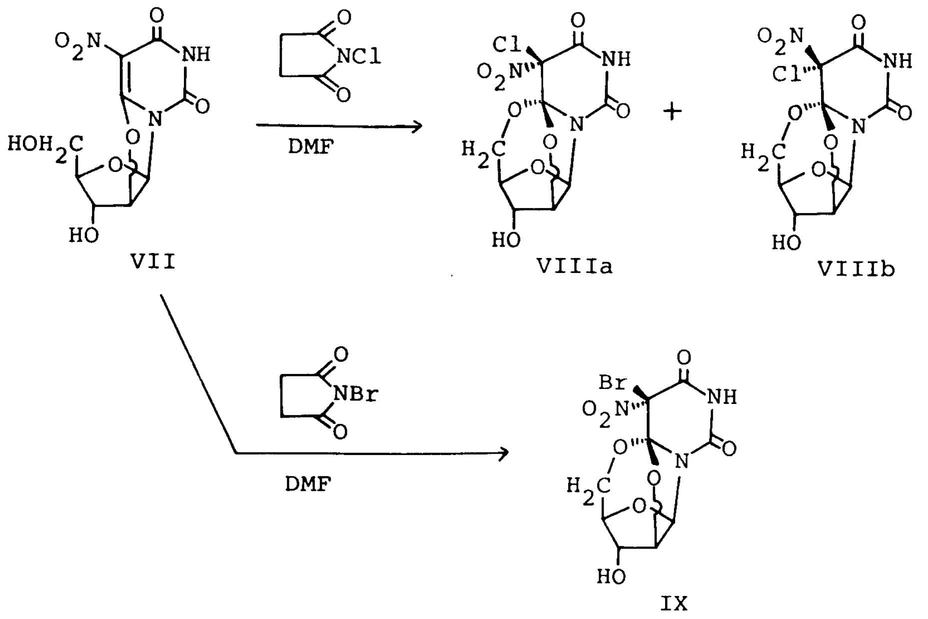

Chart 5

Steric Configuration of VI, VIII and IX

A number of papers have been published dealing with the stereochemistry of 5,5-disubstituted 5,6-dihydrouracil. The bromine (or chlorine) and the alkoxy group add in a *trans* manner across the 5,6-double bond of 5-fluorouracil, while the fluorine and the alkoxy group add in a *cis* manner to the corresponding double bond of 5-bromouracil.[7] The iodine and the 5′-hydroxyl group of thymidine add in a *trans* manner across the 5,6-double bond of thymidine.[8] It has also been reported for the electrophilic addition of XY to simple olefins that as the sum of the electronegativities of X and Y increases, there is an increasing tendency to form *cis* rather than *trans* adducts.[9] Since the torsion angle around the *N*-glycosidic bond of IIa is fixed by the 6,2′-*O*-cyclic bond, the 5′-hydroxyl group of IIa undergoes a one-way addition to the 5,6-double bond to adopt *S*-configuration at the 6-position. The bromine and the 5′-hydroxyl group of IIa, as noted above, add in a predominantly *trans* manner across the 5,6-double bond. Accordingly, the major isomer (VIa) and the minor one (VIb) should take *S*- and *R*-configurations at the 5-position, respectively (Chart 2). This assumption was proved to be correct by X-ray crystallography of VIa.[10] The chlorine and the 5′-hydroxyl group of VII add in a preferentially *trans* manner across the 5,6-double bond of VII. The major isomer (VIIIa) should take *S*-configuration, and the minor one (VIIIb) *R*-configuration. The bromine and the 5′-hydroxyl group of VII should also add in a *trans* manner across the 5,6-double bond, and X should take *S*-configuration (Chart 5).

MS of VI, VIII and IX

The spectrum of VIa affords useful information for identification, because of the characteristic relative abundance of the chloro and (or) bromo isotope(s). In the previous paper,[3] we reported that the spectrum of IIIa had a different type of fragmentation pattern from that of IIIb, and the difference might be ascribed to a difference in the ease of elimination of the halogeno ion. Compound VIb showed no substantial isomeric difference in fragmentation pattern, and each isomer had two types of fragmentation. The major type proceeds through the pathway A (a→b→c), which involves elimination of bromine followed by cleavage of the bond between C-6 and O-5′. The minor type consists of the pathway B (a→d→e→f), where the fragments retain the two halogens and the bond between C-6 and O-5′ (Chart 6). Compounds VIIIa and VIIIb did not show any practical isomeric difference, and each isomer showed only the major type of fragmentation pattern, with a characteristic relative abundance of the chloro isotope, proceeding through the pathway (g→h($M^+ + H - NO_2$) → i($M^+ - CH_2NO_3$) → j($M^+ - C_2H_3N_2$)). Compound IX showed a similar major type of fragmentation pattern (Chart 7). These results indicate that the nitro groups of VIII and IX are eliminated very easily.

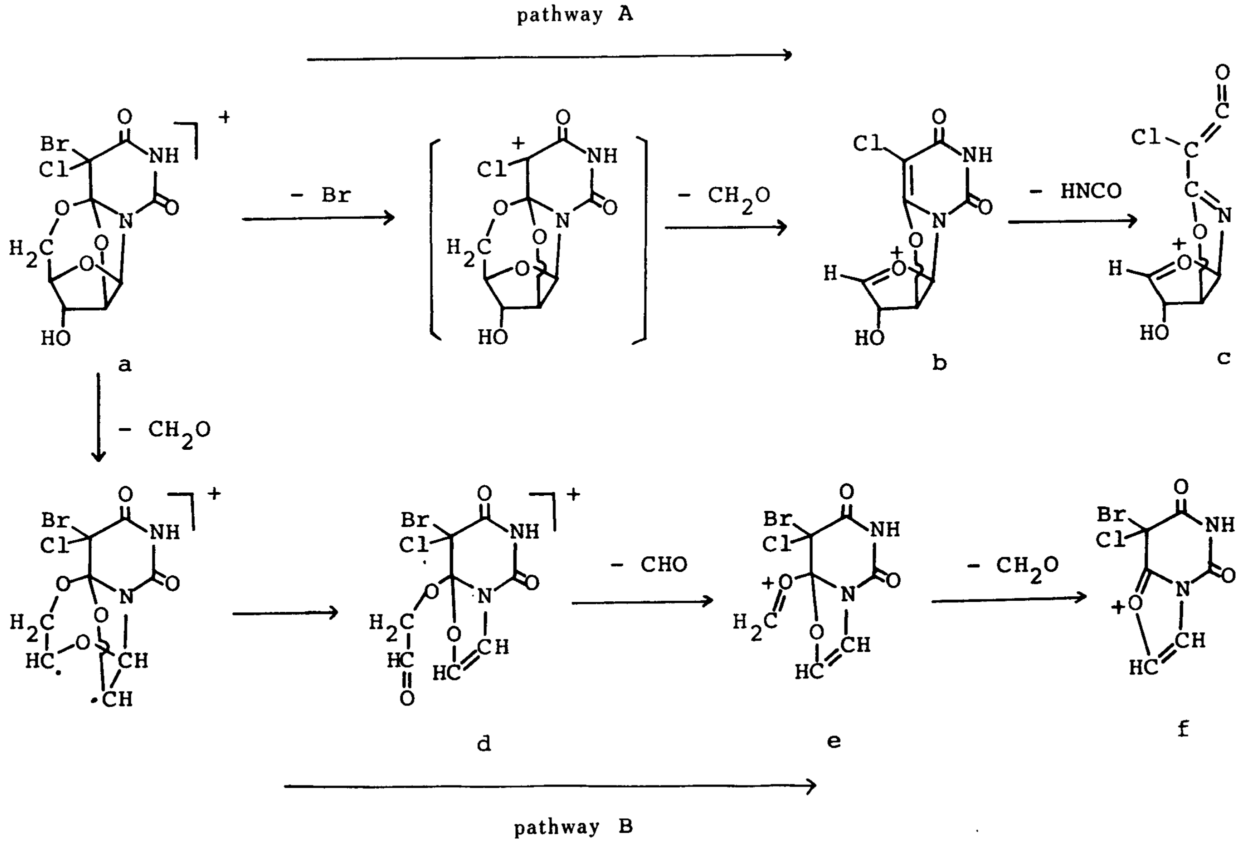

Chart 6

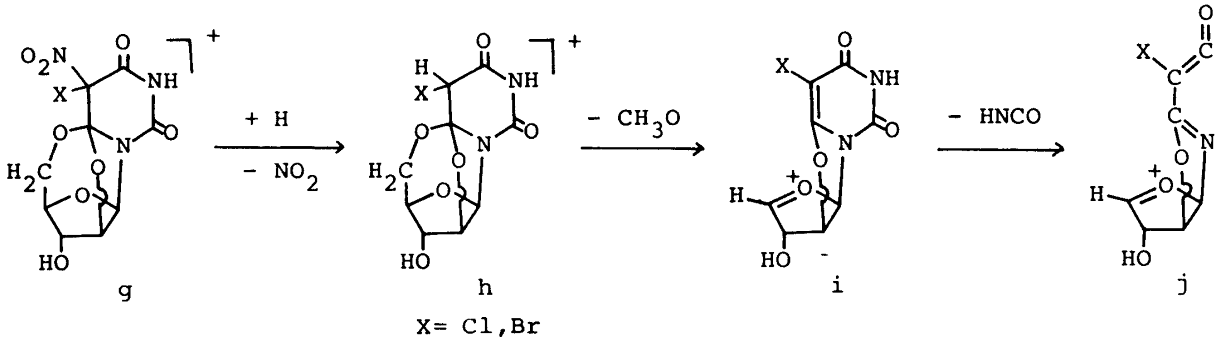

Chart 7

Experimental

All melting points were determined on a Yanagimoto micromelting point apparatus (hot stage type) and are uncorrected. TLC was carried out on Merck precoated Silica gel 60 F_{254} aluminum sheets (Art. 5554). *Rf* values refer to the following solvent systems (v/v): Rf_1 $CHCl_3$–EtOH (10 : 1), Rf_2 $CHCl_3$–EtOH (4 : 1). HPLC was conducted with a Shimadzu LC-2 model. Ultraviolet (UV) spectra were recorded with a Shimazdu UV-190 digital spectrometer. ^1H-NMR spectra were recorded with a Varian EM 360 spectrometer (60 MHz) and a JEOL GX-400 spectrometer (400 MHz) with tetramethylsilane as an internal standard in both cases. MS were measured with a Shimadzu-LKB 9000 B spectrometer.

5′-*O*-Trityl-6,2′-*O*-cyclouridine (IV)——A mixture of 6,2′-*O*-cyclouridine (I)[11] (1.20 g, 4.96 mmol) and trityl chloride (4.28 g, 17.71 mmol) in pyridine (20 ml) was stirred at room temperature for 2 d. After addition of water (5 ml), the reaction mixture was evaporated to dryness *in vacuo*. Toluene was added to the residue, and the azeotropic mixture was distilled off. The residue was dissolved in a mixture of water (50 ml) and AcOEt (50 ml). The organic layer was washed with water (50 ml), dried over $MgSO_4$ and concentrated to deposit a solid. Recrystallization of the product from $CHCl_3$ yielded white crystals (1.60 g, 66%), which showed a single UV-absorbing spot (Rf_1 0.50). mp 214—216 °C (lit.[11] 217.5 °C). UV λ_{max}^{MeOH} nm (ε): 250 (12800). *Anal.* Calcd for $C_{28}H_{24}N_2O_6$: C, 69.41; H, 4.99; N, 5.78. Found: C, 69.00; H, 4.86; N, 5.69.

5-Chloro-6,2′-*O*-cyclouridine (IIa)——A mixture of IV (1.30 g, 2.69 mmol) and NCS (718 mg, 5.38 mmol) in DMF (20 ml) was stirred under argon at 50 °C overnight and evaporated to dryness *in vacuo*. The residue was triturated with water to yield a crystalline powder (Va), which was immediately dissolved in 80% trifluoroacetic acid (6 ml). The solution was left at room temperature for 5 min, and evaporated to dryness. Water was added to the residue, and the azeotropic mixture was distilled off. The residue was dissolved in a mixture of water (10 ml) and $CHCl_3$ (10 ml). The aqueous layer was evaporated to dryness. The syrup was triturated with EtOH to afford white plates (479 mg, 64%). mp 275—278 °C.[3] Rf_1 0.26. UV $\lambda_{max}^{0.05\ N HCl}$ nm: 264, $\lambda_{max}^{H_2O}$ nm: 264, $\lambda_{max}^{0.05\ N NaOH}$ nm: 264. *Anal.* Calcd for $C_9H_9ClN_2O_6$: C, 39.08; H, 3.28; N, 10.13. Found: C, 39.08; H, 3.21; N, 9.92.

5-Bromo-6,2′-*O*-cyclouridine (IIb)——A mixture of IV (1.04 g, 2.15 mmol) and NBS (428 mg, 2.40 mmol) in DMF (8 ml) was stirred at room temperature for 30 min. The reaction mixture was worked up in a similar manner to that described in the preceding section to give white plates (413 mg, 58%). mp 259—261 °C.[2] Rf_2 0.39. UV $\lambda_{max}^{0.05\ N HCl}$ nm: 266.5, $\lambda_{max}^{H_2O}$ nm: 266, $\lambda_{max}^{0.05\ N NaOH}$ nm: 265. *Anal.* Calcd for $C_9H_9BrN_2O_6 \cdot 1/2H_2O$: C, 32.75; H, 3.05; N, 8.49. Found: C, 32.35; H, 2.73; N, 8.17.

5-Bromo-5-chloro-5,6-dihydro-6,2′ : 6,5′-di-*O*-cyclouridine (VI)——A mixture of IIa (553 mg, 2.0 mmol) and NBS (712 mg, 4.0 mmol) in DMF (20 ml) was stirred at room temperature for 30 min and then evaporated to dryness. The residue was triturated with water to deposit white needles (575 mg, 81%), which showed a single UV-absorbing spot (Rf_1 0.32). mp 222—225 °C. *Anal.* Calcd for $C_9H_8BrClN_2O_6$: C, 30.42; H, 2.27; N, 7.88. Found: C, 30.62, H, 2.24; N, 8.03. A small volume of solution of the product in hydrated DMF was analyzed by HPLC; the molar ratio of VIa : VIb was 2.8 : 1 (the half-width method). A solution of the product (10 mg) in DMF was subjected to preparative HPLC (Nucleosil ODS (10 μ), i.d. 20 × 250 mm)) to obtain two fractions. Each fraction was evaporated to dryness and the residue was extracted with AcOEt. Each extract was evaporated to dryness and the residue was triturated with a small volume of water to yield white needles. VIa (3 mg). mp 225—227 °C. MS *m*/*z*: 328, 326, 324 (1 : 4 : 3, $M^+ - CH_2O$). VIb (0.5 mg). mp 226—227 °C. MS *m*/*z*: 328, 326, 324 (1 : 4 : 3, $M^+ - CH_2O$).

Stereospecific Preparation of VIa——A mixture of IIa (28 mg, 0.1 mmol) and NBS (36 mg, 0.2 mmol) in DMF (1.0 ml) was stirred at 0 °C for 30 min. Water (10 ml) was added to the reaction mixture, and the whole was left at 4 °C for 1 d to yield white needles (25 mg, 70%). mp 222—225 °C. *Anal.* Calcd for $C_9H_8BrClN_2O_6$: C, 30.42; H, 2.27; N, 7.88. Found: 30.45; H, 2.02; N, 7.79. The sample was shown to contain a small amount (6%) of VIb by HPLC analysis, which was performed in the same way as described in Table I.

Chlorination of IIb——A mixture of IIb (640 mg, 2.0 mmol) and NCS (543 mg, 4.0 mmol) in DMF (20 ml) was stirred under argon at 50 °C overnight, then worked up in a similar manner to that described for VI to give a white crystalline powder (599 mg), which showed a single UV-absorbing spot on TLC ($CHCl_3$–EtOH (10 : 1)). The sample was analyzed by HPLC (Table I), which showed the product to be a mixture of four components; the molar ratio of IIIa : VIa : VIb : IIIb was 2.06 : 1.18 : 1.65 : 1.00 (the half-width method). The product (60 mg) was thus separated into each component by preparative HPLC (Nucleosil ODS (10 μ), i.d. 20 × 250 mm), which was carried out in a similar way to that described for VI to afford the respective products as white needles. IIIa (9 mg). mp > 300 °C.2) MS *m/z*: 284, 282, 280 (1 : 7 : 10, $M^+ - CH_2O$). VIa (3 mg). mp 225—227 °C. MS *m/z*: 328, 326, 324 (1 : 4 : 3, $M^+ - CH_2O$). VIb (12 mg). mp 226—227 °C. MS *m/z*: 328, 326, 324 (1 : 4 : 3, $M^+ - CH_2O$). IIIb (6 mg). mp 222—224 °C (lit.2) 224—226 °C). MS *m/z*: 322, 320 (1 : 1, $M^+ - Br + H$).

5-Nitro-6,2′-*O*-cyclouridine (VII)——Compound I11) (1.00 g, 4.13 mmol) was added to a solution of NO_2BF_4 (1.33 g) in sulfolane (20 ml), and the mixture was stirred at room temperature until the UV absorption maximum had shifted to 290 nm. $CHCl_3$ (20 ml) and H_2O (40 ml) added to the reaction mixture, and the whole was stirred vigorously to obtain white prisms (837 mg). The filtrate was concentrated *in vacuo*, toluene was added to the concentrate, and the azeotropic mixture was distilled off. Addition of EtOH deposited a second crop of crystals (211 mg). Total yield, 88%. mp 225—227 °C. Rf_2 0.20. UV $\lambda_{max}^{0.1\,N\,HCl}$ nm (ε): 290 (9100), $\lambda_{max}^{H_2O}$ nm (ε): 290 (9400), $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 335 (10200). ^1H-NMR (DMSO-d_6) δ: 11.70 (1H, s, N_3-H), 6.40 (1H, d, $J = 5.5$ Hz, $C_{1'}$-H), 5.45 (1H, d, $J = 5.5$ Hz, $C_{2'}$-H), 4.47 (1H, m, $C_{3'}$-H), 4.12 (1H, m, $C_{4'}$-H), 3.43 (2H, m, $C_{5'}$-H). MS *m/z*: 287 (M^+). *Anal.* Calcd for $C_9H_9N_3O_8 \cdot H_2O$: C, 35.41; H, 3.60; N, 13.77. Found: C, 35.14; H, 3.32; N, 13.75.

5-Chloro-5-nitro-5,6-dihydro-6,2′ : 6,5′-di-*O*-cyclouridine (VIII)——A mixture of VII (285 mg, 1.0 mmol) and NCS (270 mg, 2.0 mmol) was dissolved in DMF (5 ml). The solution was stirred under argon at 50 °C overnight and evaporated to dryness *in vacuo*. The residue was triturated with a small amount of water to give white needles (96 mg, 30%), which showed a single UV-absorbing spot (Rf_2 0.70). mp 219—220 °C. MS *m/z*: 278, 276 (1 : 3, $M - NO_2 + H$). *Anal.* Calcd for $C_9H_8ClN_3O_8$: C, 33.61; H, 2.51; N, 13.06. Found: C, 33.65; H, 2.47; N, 12.93. HPLC revealed the sample to be a mixture of VIIIa and VIIIb (Table I).

Stereospecific Preparation of VIIIa——NCS (928 mg, 6.80 mmol) was added to a solution of VII (400 mg, 1.36 mmol) in DMF (10 ml), and the mixture was stirred under argon at 21 °C for 5 min. Water (70 ml) was added, and the whole was left in a refrigerator for 1 d to afford white needles (330 mg, 76%). mp 227—230 °C. MS *m/z*: 278, 276 (1 : 1, $M^+ - NO_2 + H$). *Anal.* Calcd for $C_9H_8ClN_3O_8$: C, 33.61; H, 2.51; N, 13.06. Found: C, 33.66; H, 2.23; N, 12.70. HPLC and ^1H-NMR showed the sample to be pure.

5-Bromo-5-nitro-5,6-dihydro-6,2′ : 6,5′-di-*O*-cyclouridine (IX)——A mixture of VII (285 mg, 1.0 mmol) and NBS (356 mg, 2.0 mmol) was dissolved in DMF (10 ml). The solution was stirred at room temperature for 30 min, then evaporated to dryness. The residue was triturated with water to deposit white needles (208 mg, 57%). mp 222—224 °C. Rf_2 0.60. MS *m/z*: 322, 320 (1 : 1, $M^+ - NO_2 + H$). *Anal.* Calcd for $C_9H_8BrN_3O_8 \cdot H_2O$: C, 28.14; H, 2.62; N, 10.90. Found: C, 28.23; H, 2.43; N, 10.75. The product gave a single UV-absorbing peak (retention time, 11.2 min) on HPLC, which was performed in a similar manner to that described in Table I.

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References and Notes

1) A part of this work was presented at the 12th Symposium on Nucleic Acids Chemistry, Kanazawa, Oct. 1984. This paper is dedicated to Professor M. Ikehara on the occasion of his retirement from Osaka University (March, 1986).
2) 6,2′-*O*-Cyclouridine is the abbreviation for 6,2′-anhydro-1-(β-D-arabinofuranosyl)-6-hydroxyuracil. Other similar abbreviations are used in this paper.
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